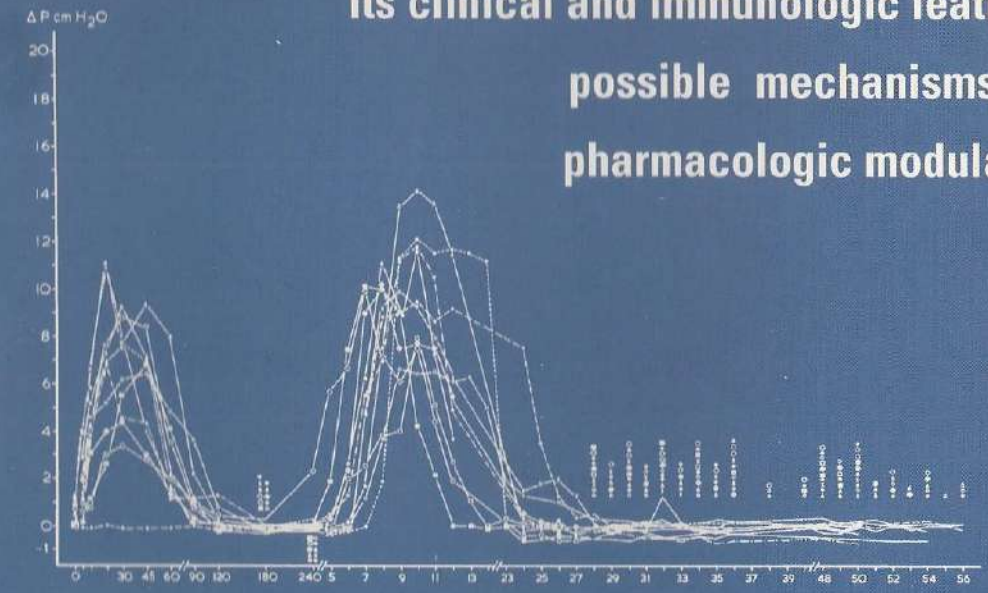
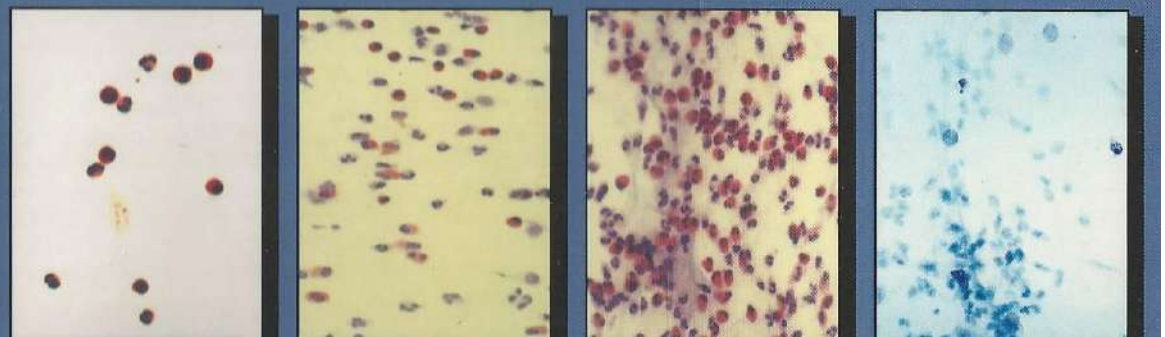
Z. Pelikan, M.D. THE LATE NASAL RESPONSE

Z. Pelikan

[LNR] THE LATE NASAL RESPONSE

Its clinical and immunologic features, possible mechanisms and pharmacologic modulation





VRIJE UNIVERSITEIT

THE LATE NASAL RESPONSE

**Its clinical and immunologic features,
possible mechanisms
and pharmacologic modulation**

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor aan
de Vrije Universiteit te Amsterdam,
op gezag van de rector magnificus
prof. dr E. Boeker,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de faculteit der geneeskunde
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door

Zdenek Pelikan

geboren te Pressburg

Promotoren: prof. dr L. Feenstra
prof. dr H.M. Jansen

Referenten: prof. dr P. van Cauwenberg
prof. dr T. Sminia

To my wife and colleague Marta
To my children Denise and Harold
To my mother and in memory of my father

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[LNR]

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Z. Pelikan

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CONTENTS

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I. INTRODUCTION

Allergic rhinitis is a non-infectious disorder, characterized by nasal symptoms, i.e. obstruction due to swelling of the nasal mucosa, hypersecretion, sneezing and itching.[1,2] These symptoms may be caused by two different basic mechanisms: an allergy component and a non-specific hyperreactivity component. They may both participate to various degrees in the same patient.[1-5]

A. THE ALLERGIC COMPONENT

The allergy component is due to the immunologic mechanism(s), initiated by an antigen-antibody (or antigen-T-cell) interaction, influencing the immuno-competent or target cell(s) (which can be changed, damaged or stimulated for selective secretion), a process which leads to the release of the mediators acting then either directly on the various effector organs (e.g. smooth muscles, mucosal glands, goblet cells, epithelial cells, endothelial cells, capillary network, neurosynapses, receptors etc.) or indirectly through the effects on the other cell types. The combined response of the effector organs results in a variety of the clinical symptoms representing the particular allergic disorder. [1,2]

The allergy mechanism may be of a seasonal or non-seasonal (perennial) character, depending on the kind of allergens involved. Of the four basic types of hypersensitivity (= allergy) reaction, as proposed by Coombs and Gell[6], three (Type I-immediate, Type III-late and Type IV-delayed) can be involved in the production of symptoms in the rhinitis patient.[2,3,7-11,11a-11i,12,13,13a-13c,14,14a-14g,15-25] The particular types of the nasal response, the immediate (INR), late (LNR) and delayed (DYNR) nasal response can be demonstrated by the nasal provocation tests with allergen (Figures 1,2 pages 371, 372).[2-5,7-11,11a-11i,12,13,13a-13c,14,14a-14g,15-25,25a,25b,26,27,27a-27b,28-34,35b,35c,35e,35f,37-40,40a-40g,41,41a-41i]

The immediate hypersensitivity (Type I) reaction, causing the so-called "immediate nasal response" (INR) in the rhinitis patients, has, however, been most frequently studied and described.[1-4,8-11,11a-11j,14,14a-14g,17,18,20-24,25b,28-31,34,35b,35c,35e,36-40,40a,40b,40e,41,41a-41c,41f]

B. THE NON-SPECIFIC HYPERREACTIVITY COMPONENT

The non-specific hyperreactivity component may lead to a spectrum of nasal symptoms which can be partly similar to that caused by an immunologic mechanism, however, without any initial antigen-antibody interaction. The non-specific agents, mainly small molecular chemical compounds, physical factors such as temperature differences, vapours, smoke, perfumes etc, or mechanical factors such as non-organic dust, either (1) influence the immuno-competent or target cell directly, causing a non-specific release of mediators, or indirectly, e.g. through the stimulation of the nasal mucosal sensory nerves and/or a variety of mucosal receptors, resulting in the activation and release of various neuropeptides which then affect the immuno-competent cells; or (2) act via the stimulation of mediator precursors, firstly leading to the stimulation of the mediator production, which then acts directly on the effector organs and secondly leading to the feedback-inhibition of these mediators or immuno-competent cells; or

(3) act on the effector organs and their receptors directly, thus causing the clinical effects (Figure 2). Nasal challenge with histamine (or serotonin, acetylcholine, methacholine, cold air etc.) may simulate and confirm the involvement of the non-specific hyperreactivity and its degree in the nasal complaints of rhinitis patients.[3-5,35,35a-35f]

The positive nasal response (NR) of any of the basic types to the allergen challenge is an indicator for the involvement of the allergy component (ALL) in the the nasal symptoms, whereas the decreased nasal threshold of histamine (methacholine Br or Cl, cold air, etc) is an indicator for the involvement of the non-specific hyperreactivity component (N-SH) in the nasal complaints.[4,5,35a-35c]

Both the components (ALL and N-SH) may be considered as two independent processes, due to principally different mechanisms.[35a-35c] Both the components can participate to various and variable degrees in the nasal complaints of the rhinitis patients.[35a-35c] They both can exist one beside the other in the same patient, but neither can be regarded as a necessary condition for the other.[35a-35c] In approximately 39% of patients with chronic rhinitis both the components have been confirmed; whereas in 47% of the rhinitis patients only the allergy component and in 14% only the non-specific hyperreactivity component has been found (Figure 3).[35a-35c]

C. NASAL PROVOCATION TEST (NPT)

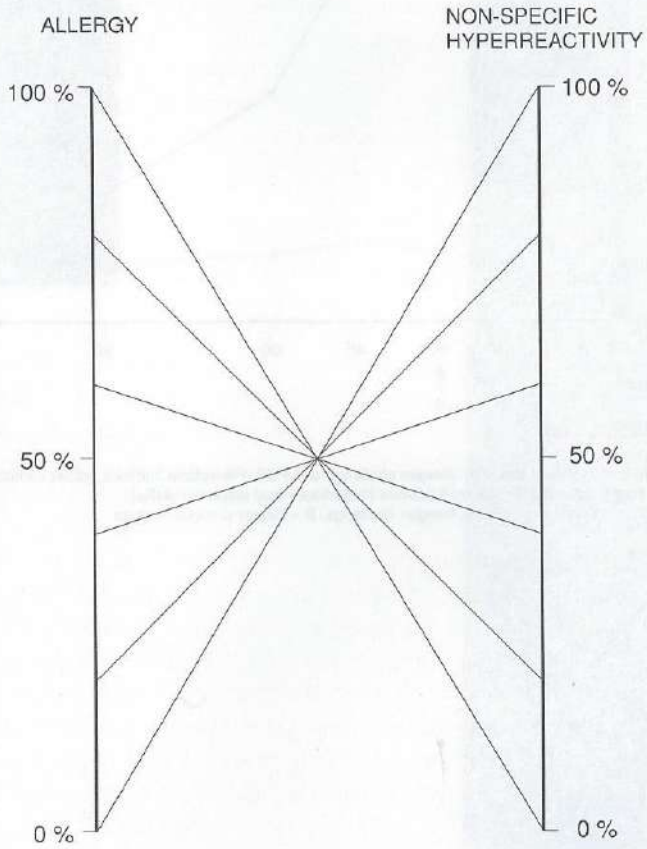
The nasal provocation tests with allergen (NPT), also called nasal challenges, are widely accepted as an important research technique and model for the investigation of the pathophysiological and pharmacological aspects of the nasal mucosa. They are also considered as one of the most important "*in vivo*" diagnostic tests for the detection of the allergic component, for the confirmation of the role of a particular allergen in the nasal complaints of patients with rhinitis and for the confirmation of the causal role of the nasal mucosa (allergy) and/or nasal response in the disorders and/or responses of the related organs.[2-5,7-11,11a-11h,12,13,13a-13c,14, 14a-14g,15-25,25a,25b,26,27,27a-27b,28-34, 37-40,40a-40n,41,41a-41j,46-48,48a-48l,49]

Nasal challenges with histamine (or serotonin, methacholine, cold air etc.) may then confirm the role of the non-specific hyperreactivity in these patients.[3-5,35,35a-35f]

The NPT should be regarded as a model technique and as a simulated reproduction of the patient's complaints and symptoms caused by an exposure to a certain allergen or non-specific agent.[3-5] Preference should be given to the nasal challenge as a model exposure to an allergen or non-specific agent, rather than the natural exposure, where the dose and concentration of the particular agent cannot be satisfactorily monitored and isolated from the simultaneously acting factors, and the nasal parameters cannot always be quantitatively recorded.[3-5] The nasal provocation test is the only technique for the demonstration of the particular type of nasal response caused by a certain allergen. [2-5] Moreover, the nasal provocation test is the only technique for the confirmation of the participation of the allergy component and non-specific hyperreactivity component in a certain patient and this is the only technique able to discriminate both the components.[2-5,11h,41h-41j]

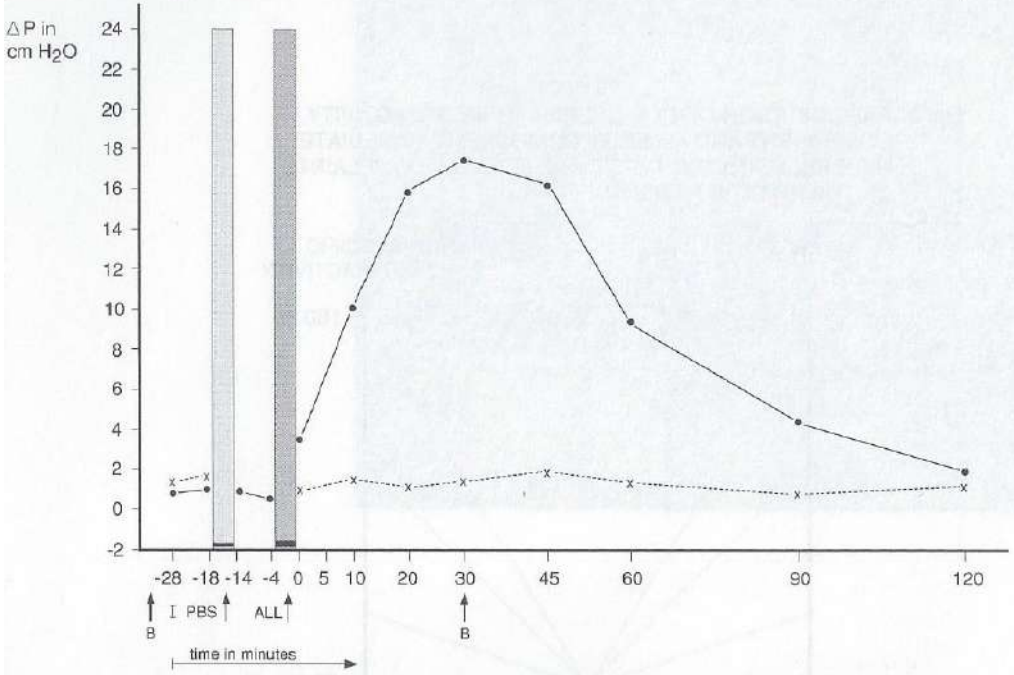
Patients with nasal allergy who are challenged with an allergen during the NPT, may develop different types of nasal response: immediate response (INR) or the so-called "non-immediate types", a late (LNR) and a delayed response (DYNR) (Figures 4,5,6).[2, 7-11,11a-11i,12,13,13a-13c,14,14a-14g,15-25,25a,28-34,37-40,40a-40n,41a-41j]

Fig. 3. PARTICIPATION OF NON-SPECIFIC HYPERREACTIVITY COMPONENT AND ALLERGY COMPONENT (IMMEDIATE HYPERSENSITIVITY, TYPE I) IN THE NASAL COMPLAINTS OF THE RHINITIS PATIENTS



References: 3-5,35a-35f,41b,41i

Fig. 4. IMMEDIATE NASAL RESPONSE TO ALLERGEN CHALLENGE (INR)

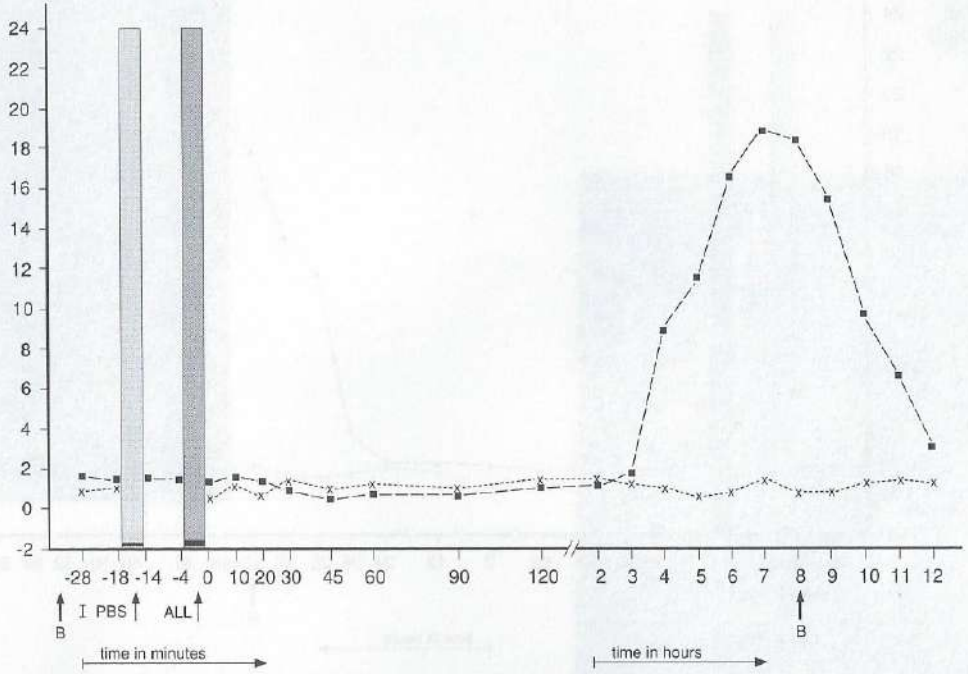


The mean NPG values recorded before and after allergen challenge and PBS (Phosphate buffered saline) controle challenge, were always calculated from 6 patients developing 6 positive Immediate nasal response (INRs).
I = Initial values; PBS = Control challenge; ALL = Allergen challenge; B = Biopsy of nasal mucosa.

• ——— • = INR (n=6)
x ····· x = PBS control challenge (n=6)

References: 2,8,9-11,11a-11g,11i-11j,14,14e,14f,18,20,23,25b,35c,35f,40e,41f,41i

Fig. 5. LATE NASAL RESPONSE TO ALLERGEN CHALLENGE (LNR)

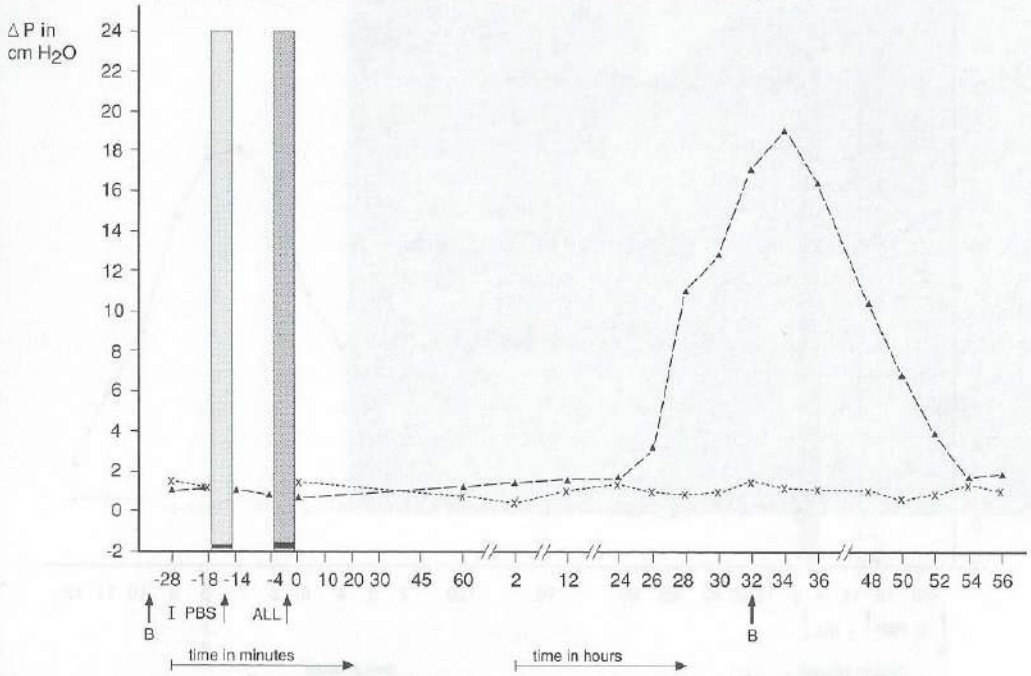


The mean NPG values recorded before and after allergen challenge and PBS (Phosphate buffered saline) control challenge, were always calculated from 6 patients developing 6 positive late nasal response (LNRs).
I = Initial values; PBS = Control challenge; ALL = Allergen challenge; B = Biopsy of nasal mucosa.

■ ——— ■ = LNR (n=6)
x ····· x = PBS control challenge (n=6)

References: 2,7,11c,11f-11h,12,14,14e,14f,16,19,25,25a,29,32,33,35c,40c-40f,41b,41c,41f,41i

Fig. 6. DELAYED NASAL RESPONSE TO ALLERGEN CHALLENGE (DNR)



The mean NPG values recorded before and after allergen challenge and PBS (Phosphate buffered saline) controle challenge, were always calculated from 6 patients developing 6 positive delayed nasal response (DYNRs).
I = Initial values; PBS = Control challenge; ALL = Allergen challenge; B = Biopsy of nasal mucosa.

▲------▲ = DYNR (n=6)
x---------- x = PBS control challenge (n=6)

References: 2,7,11f,13,13a-13c,35c,41f,41i

II. LATE NASAL RESPONSE [LNR]

The immediate hypersensitivity (Type I allergy) reaction causing the so-called "immediate nasal response" (INR) has been most frequently studied and described in the literature (Figure 4).[2,3,30,34,36,40b,40h-40n]

The existence of the so-called "non-immediate nasal responses" was first suggested by Taylor and colleagues.[28,29] They have described this phenomenon as an "Arthus-type reaction" in the nasal airways and skin in 37% of the pollen-sensitive patients.

The first controlled investigation of the "non-immediate types" of nasal response has been carried out by us (author of this thesis and his colleagues).[7] We have studied 600 patients with allergic rhinitis and have found, besides an immediate nasal response (INR), also two types of the "non-immediate response", a late nasal response (LNR) and a delayed nasal response (DYNR) (Figures 7,8). We described the occurrence, clinical features, characteristics and forms of both these types of "non-immediate nasal response".[2,7]

Since then the LNR has been studied extensively by us from various points of view,[2,11c,11f,11g,11h,12,14,14a-14g,15-17,19,24,25,25a,26, 34,37-40,40a-40g,41,41a-41i] in relationship to other organs,[14a-14g,17,21,22,26,27,27a,27b,41b, 41g,41i] with respect to various antigens, [7,15,16,19,24,26,37-40,41b] and its pharmacologic modulation has also been investigated. [12,13,14,26,38,40,40a-40f,41b,41g,41i]

After some initial reluctance LNR is becoming recognized as an important clinical phenomenon in patients with allergic rhinitis.[31,32-34,42-48,48a-48e,49-51,51a-51d,52-57,57a] This response may regularly play an important role in the patients with chronic allergic rhinitis, is often overlooked in practice and may be responsible for the failure of the usual treatment in these subjects.[2,7,12,26,41b,41g,51a, 51d,57]

A. OCCURRENCE

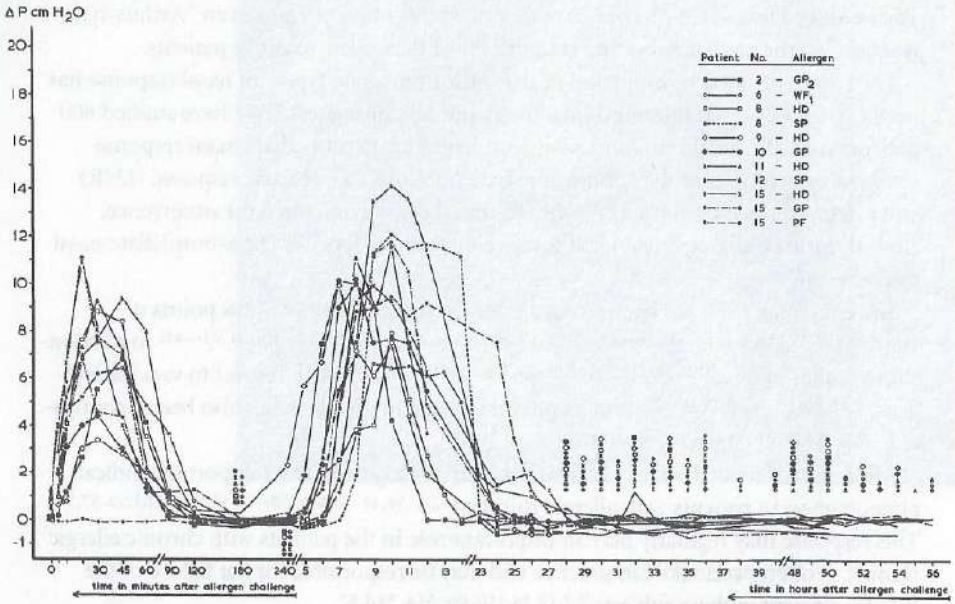
Data in the literature concerning the occurrence of LNR vary greatly. Some authors have reported a rather low occurrence, e.g. 17%,[31,57a] while others are convinced that the LNR occurs more frequently, e.g. 37%,[28,29] 30 to 50%.[51,51a-51c] In our previous studies we have found LNR in 5%, respectively in 11% of the patients with allergic rhinitis.[7,12] Recently, since the long-term NPT have become a part of the routine diagnostic procedure at our department, the LNR is recorded in approximately 41% of the rhinitis patients, in one third (14%) in its isolated form (ILNR) and in two thirds (27%) in a combination with the INR (Tables 1a,1b).[41b,41i]

B. CLINICAL FEATURES, CHARACTERISTICS, AND FORMS

The clinical course of the LNR, recorded by rhinomanometry (see page 250), is as follows: onset within 4 to 8 hrs, maximum within 6 to 12 hrs and resolving within 24 hrs after the allergen challenge (Table 2).[2,7,11c-11h,12,14,14a-14d,15,16,19,24,25,25a,40b-40f, 41,41a-41g,51a-51c,52-55,57a]

The LNR occurs in two sub-forms, either as an "isolated late response" (ILNR) or as a so-called "dual late response" (DLNR) (Figure 7) in which the immediate response appears firstly (within 2 hrs), followed, after a symptom-free period of 3 to 7 hrs, by the

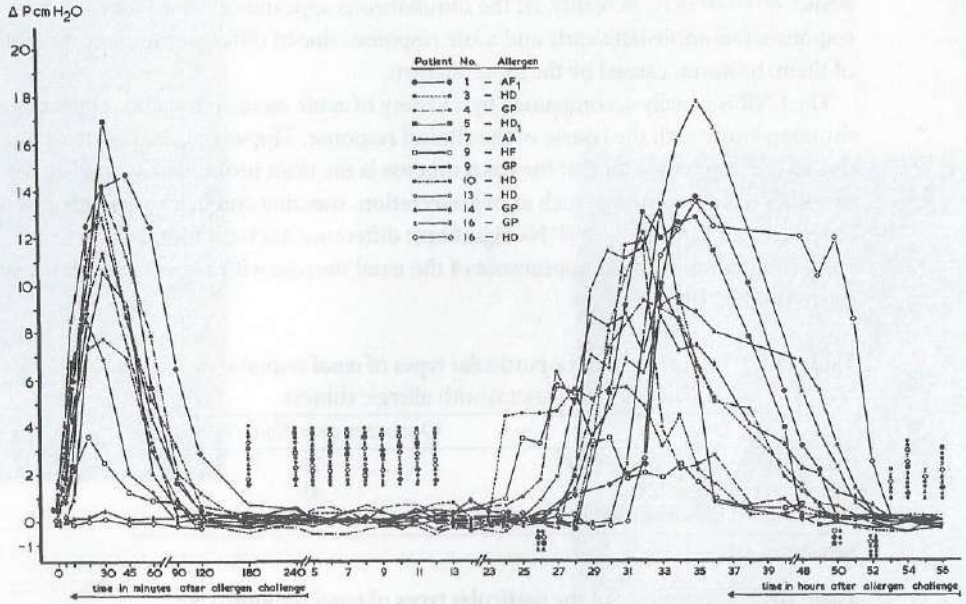
Fig. 7. THE LATE NASAL RESPONSE TO ALLERGEN CHALLENGE [LNR]



The mean NPG values recorded after the allergen challenge, with respect to the mean NPG values after the coca's solution challenge (= control), in patients who demonstrated the "late nasal response" to allergen challenge [LNR]. HD = house dust, GP = grass pollen, SP = spring pollen, WF = wheat flour, PF = pigeon feathers.

References: 7

Fig. 8. THE DELAYED NASAL RESPONSE TO ALLERGEN CHALLENGE [DYNR]



The mean NPG values recorded after the allergen challenge, with respect to the mean NPG values after the coca's solution challenge (= control), in patients who demonstrated the "delayed nasal response" to allergen challenge [DYNR]. HD = house dust, GP = grass pollen, HF = hairs and feathers (= animal danders), AF = Aspergillus fumigatus, AA = acidum acetylsalicylicum.

References: 7

late response.[2,7,11h,12,14,14a-14d,15,16,19,24,40b-40f,41b,41g, 41h,41i]

However, with respect to our data generated from a series of clinical trials concerning the pharmacologic modulation of the so-called "dual late asthmatic response" (DLAR),[68b,68c,68g,121g,121h,121k,178e-178g] the existence of the genuine DLAR[68g,121k, 178e-178g] as a compact clinical event consisting of two phases should be seriously doubted. Our results suggest that the DLAR and analogically also the "dual late nasal response"[121b,121j] may, in reality, be the simultaneous appearance of two independent responses (an immediate/early and a late response) due to different mechanisms, both of them, however, caused by the same allergen.

The LNR is usually accompanied by a variety of acute nasal complaints, appearing simultaneously with the course of the clinical response. The severe nasal obstruction due to the distinct swelling of the nasal mucosa is the most prominent symptom, while the other nasal symptoms, such as hypersecretion, sneezing and itching, are present to a lesser degree (Table 3).[7,12,41b] No significant difference has been found either in the nasal complaints or in the appearance of the nasal mucosa with respect to both the subforms of LNR.[41b]

Table 1a Occurrence of the particular types of nasal response to allergen challange in 785 patients with allergic rhinitis.

	Occurrence in % (n=785)
Immediate nasal response (INR)	73
Late nasal response (LNR)	41
Delayed nasal response (DYNR)	14

Reference: 41i

Table 1b Occurrence of the particular types of nasal response (NR) in 785 patients with allergic rhinitis

	Occurrence in % (n=785)
Isolated immediate NR	50
Dual late (= immediate + late) NR	19
Isolated late NR	32
Dual delayed (= immediate+ delayed) NR	4
Isolated delayed NR	10

Reference: 41i

Table 2 Time course of the particular clinical types of nasal response (NR) to allergen challenge.

Nasal response	Onset	Maximum	Resolving
Immediate (INR)	< 10	20-45	< 90-120 minutes
Late (LNR)	4-6	6-10	< 24 hours
Delayed (DYNR)	24-30	30-40	< 60 hours

References: 2,7,11,11a-11h,12,13,13a-13c,18,25,26,40c,40d,40f,41a-41d,41f,41i, 57a,71,72, 72a,72c,72d,96,97,97a,121j

Table 3 A survey of nasal complaints accompanying the particular types of nasal response

Nasal complaints	Nasal response		
	Immediate (INR)	Late (LNR)	Delayed (DYNR)
Obstruction	++	+++	+++
Hypersecretion	+++	+	±
Sneezing	+++	+	–
Itching	+++	±	–

– = Absent; ± = Very slight; + = Slight; ++ = Moderate; +++ = Severe
References: 2,7,11,11a-11h,12,13,13a-13c,18,25,26,40c,40d,40f,41a-41d,41f,41i, 57a,71,72, 72a,72c,72d,96,97,97a,121j

C. DETECTION OF "LNR"

1. NASAL PROVOCATION TESTS (NPT)

The definite confirmation of the existence of particular types of the nasal response (immediate, late, delayed) due to the exposure with a certain allergen, and their participation in the nasal complaints of the individual patient, can only be provided by the nasal provocation test (= nasal challenge) with allergen (NPT).[2, 7-11,12,13,13a-13c,14,14a-14d,15-25,25b,26,27,27a, 27b,29-34,40c-40n,41b,41d,41i]

The most important aspect of the provocation test is the comparison of the objective parameters and simultaneously also subjective complaints before and, repeatedly, after the challenge with a particular allergen (or non-specific agent).[3,8]

The NPT can be supplemented by a recording of various *"in vivo"* as well as *"in vitro"* diagnostic parameters and functions, such as clinical symptoms (pulse rate, blood pressure, body temperature), other organs' functions (tympanometry, conjunctival appearance, sinuses X-ray or echography, lung functions etc.)[14a-14d,17,21,22,23,27,27a, 27b,39,41b,41e] or other parameters (nasal biopsy, biochemical, cytologic, and immunologic investigation of the nasal secretions and nasal mucosa, estimation of mediators, immunoglobulins and other compounds in the nasal secretions and/or serum, physical and chemical properties of the nasal secretions, such as consistency, pH, viscosity etc.).[7-11, 11a-11h,12,13,13a-13c,14,14a-14d,15-25,25a, 26,27,27a,27b,32-35,35e, 37-40,40a-40n,41,41a-41g,44,46-48,48c-48d,49,51a-51c,52-57,58-60,71-72,72a-72d,73-82,82a-82j,83-85,85a-85d,86-95,95a, 95b,96,97, 97a-97y,117h,121j]

The nasal response to allergen challenge can be recorded and assessed by various methods and techniques. There are two basic methods, (1) recording of the subjective complaints (obstruction, hypersecretion, sneezing, itching) by means of various scores; and (2) recording of the objective parameters. The objective parameters are related to the changed aerodynamics in the nose due to the increased nasal obstruction caused by swelling of the nasal mucosa and hypersecretion, both of them due either to the antigen-antibody (antigen-T-cell) interaction respectively or to the direct effects of non-specific agents.[3,7,8,30,41b, 48,48a,57,57b,58-60,85a]

Recording of the objective parameters, mostly nasal airway resistance (NAR), by means of which the nasal mucosa response can be assessed, deserves preference.[3,4] The

NAR can be measured by means of recording of the "air-pressure" and either "air-flow" or "air-volume" parameters, or their derivatives, such as "air-passage" or "conductance" (which is the reciprocal value of the nasal resistance).[2,3,4,41j]

2. BASIC TECHNIQUES OF NPT

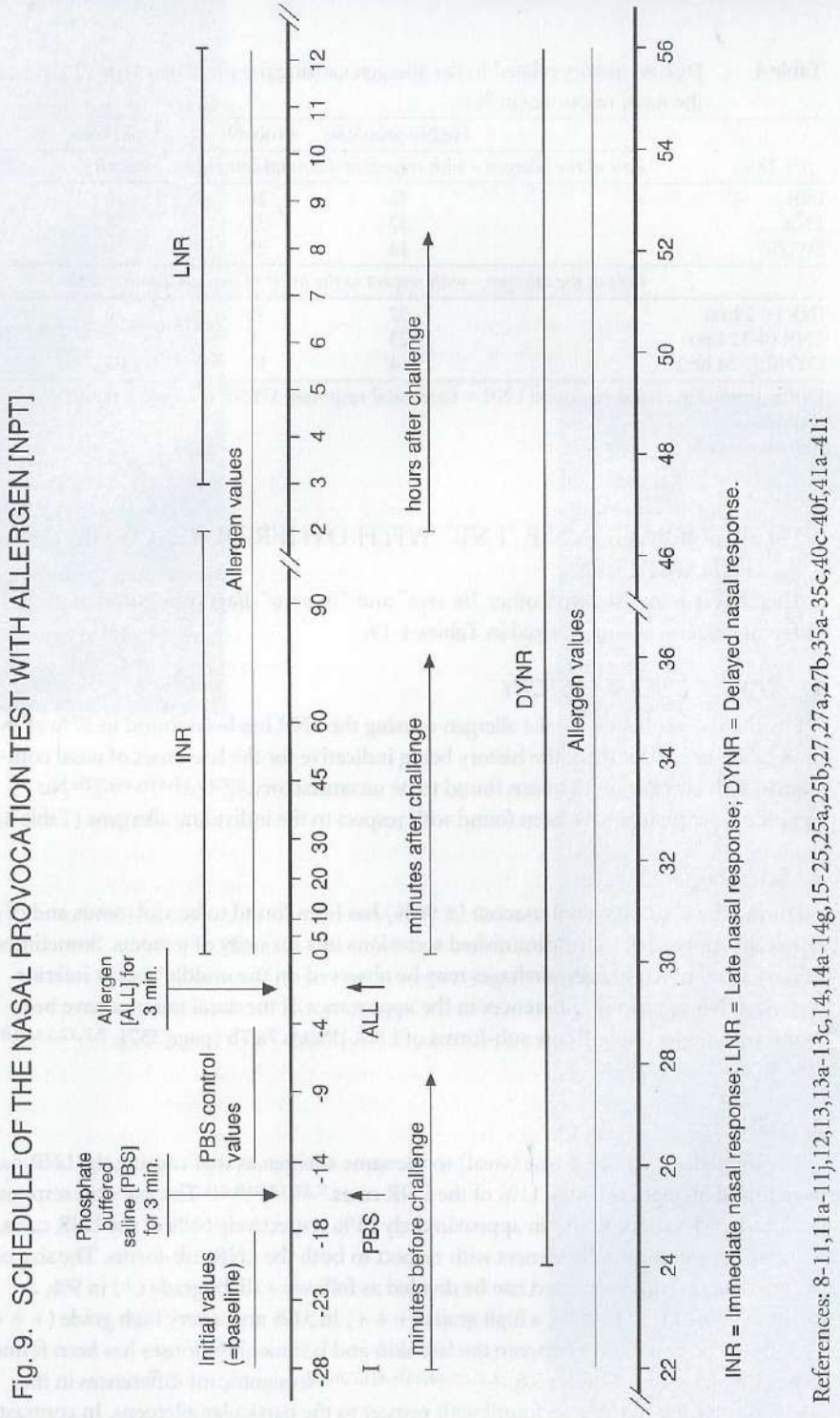
These techniques can be divided into 5 groups: (1) nasal peak-flow measurement, (2) plethysmography, (3) rhinomanometry (anterior, posterior, combined and/or modified techniques, performed either in an active or a passive manner), (4) acoustic rhinometry (5) non-rhinomanometry techniques, e.g. recording of nasal blood flow using Doppler velocimetry, the ^{133} Xenon washout method, or nasometry (Plates 1-6) .[7-11,11a-11h,12, 13,13a-13c,14,14a-14d,15-25,25b,28-33,35e,40h-40n,41j,48a,48f-48j,57a,58-60, 85a,91,92]

3. BALLOON TECHNIQUE

We use the "balloon method", one of the combined rhinomanometry techniques (= recording of NPG = nasopharynx-nostril-pressure gradients, expressed in cm of H_2O), as described in detail in our previous papers[3,4,7-11,11a-11h,12,13,13a-13c,14,14a-14d,15-25, 25b] as a standard method [Plates 1a,1b, (page 353)]. The passive anterior rhinomanometry is used by us for children [Plates 2a,2b,5 (pages 354, 356)], while the volume-flow as well as the volume-pressure diagram (active posterior rhinomanometry and active anterior rhinomanometry) are used by us for research purposes or as an arbitrary test (Plates 3,4 [(page 355)].

4. BASIC SCHEDULE OF NPT

The basic schedule of NPT used by us is as follows: (1) "Initial test" or "baseline" – the NPG values are recorded at 0, 5, and 10 min; (2) "Control values" – after a 3 minute application of the control solution (PBS, Coca's solution or Saline), the values are recorded at 0, 5, and 10 min. If no significant changes in the NPG control values, with respect to the initial NPG values, appear, the test may be continued; (3) Post-challenge values - after an allergen challenge (usually for 3 min), the NPG values are recorded at 0,5,10,20,30,45,60,90, and 120 min, and then every hour up to the 11(12)th hour and, if necessary (the response has not resolved or a delayed response is expected), every hour on the second or third day respectively (Figure 9). A control challenge with control solution is performed in the same way on another day and the NPG parameters are recorded during the same period of time as those after the allergen challenge. The protection tests, performed at our department as a part of the routine diagnostic procedure are, in principle, nasal challenges carried out after the pretreatment or during the treatment with the particular drug.[10,11,11d-11e,12,13,13b,14,38,40,40a-40f,41b,48l,72a,72b,72d]



References: 8-11,11a-11j,12,13,13a-13c,14,14a-14g,15-25,25a,25b,27,27a,27b,35a-35c,40c-40f,41a-41i

Table 4 Disease history related to the allergen causing the particular type of the nasal response (in %).

n = 785	Highly probable	Probable	Unknown
	Role of the allergen - with respect to the nasal complaints generally		
INR	42	28	30
LNR	37	25	38
DYNR	18	23	59
	Role of the allergen - with respect to the onset of the nasal complaints		
INR (< 2 hrs)	37	33	30
LNR (4-12 hrs)	23	8	69
DYNR (>24 hrs)	4	11	85

INR = Immediate nasal response; LNR = Late nasal response; DYNR = Delayed nasal response.
Reference: 41 i

D. ASSOCIATION OF "LNR" WITH OTHER DIAGNOSTIC PARAMETERS

The LNR is associated with other "*in vivo*" and "*in vitro*" diagnostic parameters, to different degrees, as summarized in Tables 4-19:

1. POSITIVE DISEASE HISTORY

Positive disease history to the allergen causing the LNR has been found in 37% of the LNR cases, in 23% of them the history being indicative for the late onset of nasal complaints. This correlation has been found to be unsatisfactory.[2,7,12,14,41b,41i,72b] No significant differences have been found with respect to the individual allergens (Table 4).

2. RHINOSCOPY

During the LNR, the nasal mucosa (± 90%) has been found to be violaceous and of a rather dry aspect due to the diminished secretions in a majority of patients. Sometimes solitary small mucosal haemorrhages may be observed on the middle and/or inferior turbinate. No significant differences in the appearance of the nasal mucosa have been found with respect to both the sub-forms of LNR [Plates 7a,7b (page 357]. [2,7,12,14,41b, 41i,72b]

3. SKIN TEST

The immediate skin response (weal) to the same allergen as that causing the LNR has been found in approximately 11% of the LNR cases.[7,12,14,41b,41i] The late skin response (induration) has been found in approximately 51% respectively 65% of the LNR cases, without any significant differences with respect to both the LNR sub-forms. The size of the late skin response recorded can be divided as follows: a slight grade (+) in 9%, a medium grade (+ +) in 20%, a high grade (+ + +) in 31% and a very high grade (+ + + +) in 5%. The correlation between the late skin and late nasal responses has been found to be non-significant (Tables 5,6).[2,7,12,14,41b,41i,72b] No significant differences in the late skin responses have been found with respect to the particular allergens. In contrast,

Iliopoulos et al.[51c] have found the positive late skin response only in 47% of patients developing the LNR [Plates 8-11 (pages 358-361)].

Table 5 Survey of the particular types of the skin responses recorded after the intracutaneous skin tests (i.c.) with the same allergen as that causing the particular type of the nasal response (in %).

	Skin response			
Nasal response	immediate (<20 min)	late (4 - 24 hrs)	delayed (> 36 hrs)	negative
Immediate [INR] (n=246)	63	8	0	29
Late [LNR] (n =225)	11	51	1	37
Delayed [DYNR] (n=93)	15	2	42	41
Negative [NNR] (n=182)	18	12	2	68

INR = Immediate nasal response; LNR = Late nasal response; DYNR = Delayed nasal response; NNR = Negative nasal response (= no nasal response).
Reference: 41 i

4. TOTAL IgE IN THE SERUM

The concentration of total IgE antibodies in the serum (PRIST) has been found by us to be significantly increased (>500 IU/ml) in only 6% of LNR cases, most of them being a part of the dual late nasal response (Table 6). [14,25a,41b,41i,72b]

5. SPECIFIC IgE IN THE SERUM

The specific IgE antibodies in the serum (RAST) to the same allergen as that causing the clinical LNR has been significantly positive (score grade 3 or 4) in approximately 9% of the LNR cases, most of them being a part of the dual late nasal response. [14,25a,41b,41i, 72b] No significant differences in the RAST have been found by us with respect to the particular allergens. These results are in agreement with other investigators' observation of the non-significant correlation between the *specific IgE* antibodies in the serum and the positive LNR (Table 6).[51c]

6. TOTAL IgG, IgG SUB-CLASSES, TOTAL IgA, TOTAL IgM IN THE SERUM

The particular immunoglobulin classes and sub-classes were semi-quantitatively determined by single radial immunodiffusion (Mancini technique).[92b] The serum concentration of total IgG immunoglobulins has been found by us to be increased in approximately 51% of the LNR cases, of total IgM immunoglobulins in 8% and that of total IgA immunoglobulins in approximately 1%. The serum concentration of the individual *IgG sub-classes* during the LNR has been recorded as follows: elevation of IgG_1 in 2%, IgG_3 in 19%, IgG_4 in 16%, while decrease in IgG_2 in 11%.[7,12,16,41b,41f, 41i,72b] These findings may be suggestive of the possible involvement of IgG antibodies, at least in some of the LNR cases. However, because the IgG antibodies and IgG sub-classes were not antigen-specific, such a presumption will be of a limited validity until more convincing evidence can be provided (Table 6).

Table 6 The association of the particular types of nasal response with other diagnostic parameters (in %)

	Nasal mucosa response to allergen challenge			
Response-related parameters	Immediate (n=148)	Late (n=131)	Delayed (n=63)	Negative (n=205)
Positive skin response				
Immediate	70			31
Late		65		9
Delayed			67	3
Increased total IgE in the serum (PRIST)	17	6	5	9
Positive specific IgE in the serum (RAST)	27	9	2	11
Total IgG	19	51	1	3
IgG1	0	2	0	1
IgG2	0	0[a]	0	4
IgG3	2	19	0	1
IgG4	0	16	1	2
Total IgM	0	8	0	0
Total IgA	1	1	0	1
Increase in blood leucocytes	4	20	11	3
Increase in blood eosinophils	5	43	0	1
Increase in body temperature (more than 37°C = 98.6°F)	0	2	2	0
General malaise complaints	0	6	12	2
Aspects of the nasal mucosa				
hyperemia	34	10	0	18
violaceous aspect	59	90	100	1
Nasal mucosa hemorrhages	0	24	43	0
Patient-related parameters				
Increased reactivity of the nasal mucosa to histamine	31	23	2	89

[a] IgG_2 in the serum decreased in 16% of the LNR cases.
References: 2,7,11,11a-11h,12,13,13a-13c,18,25,26,40c,40d,40f,41a-41d,41f,41i,57a,71,72, 72a,72c,72d,96,97,97a,121j

7. EOSINOPHIL COUNT IN THE BLOOD

The *increased blood eosinophil count* (more than 300 x 10^6/L) has been recorded during 23% of the positive dual late nasal responses and during 20% of the isolated late nasal responses (Table 6). [14,41b,41i,72b]

8. LEUKOCYTE COUNT IN THE BLOOD

The *increased blood leukocyte count* (more than 10 x 10^9/L) has been recorded during 13% of the isolated, and during 7% of the dual late nasal response (Table 6).[14, 41b,41i,72b]

9. BODY TEMPERATURE

The *increased body temperature* (> 37°C => 98.6° F axillary) has been recorded during 2% of the LNRs (Table 6).[14, 15-16,24,41b,41i,72b]

10. NASAL HISTAMINE THRESHOLD (NHT)

The *nasal histamine threshold* [NHT] - normal value is > 4.0 mg/ml (= > 12 mmol/ml). The increased nasal mucosa responsiveness to histamine (PD_{20}) or its contra-value, the so-called decreased *nasal histamine threshold*, has been recorded by us in only 15-20% of the patients developing the LNR. The NHT varied mostly between 2.0 and 4.0 mg/ml (= 6.0 - 12.0 mmol/ml) [Tables 6,11,14,16; Figures 10,11 (pages 37,38,373)].[35b,35c,35f, 41b,41i]

In conrast, the decreased *nasal histamine threshold* has been recorded by us in 31-69% of the patients developing only the positive INR, in 0 - 2% of those developing only the positive DYNR, and finally in 86-100% of the patients with chronic rhinitis, who did not demonstrate any positive type of nasal response to allergen challenge (= negative nasal responses; NNR) (Tables 6-13,15,16; Figures 12-18).[35a-35c,35f,41b,41i]

11. NASAL METHACHOLINE THRESHOLD (NMT=NMCLT, NMBRT)

The *nasal methacholine chloride threshold* [NMCT] - normal value is >8.0 mg/ml, and the *nasal methacholine bromide threshold* [NMBT] - normal value is > 4.0 mg/ml. The increased nasal mucosa responsiveness to methacholine chloride and/or bromide, or its contra-value, the so-called decreased NMCT, has been recorded by us in 11% and the decreased NMBT in 9% of the patients developing the LNR (Tables 14,16; Figures 10,11).[35a-35c,35f] In contrast, in patients developing only the positive INR, the decreased NMCT has been recorded by us in 30% and the decreased NMBT in 15% of the cases. In patients developing only the positive DYNR, the decreased NMCT has been measured in 6% and the decreased NMBT in 0% of the cases. However, in patients with chronic rhinitis, who did not develop any positive type of nasal response to allergen challenge (= negative nasal responses; NNR), the decreased NMCT has been recorded in 75% and the decreased NMBT in 57% of the cases [Tables 15,16; Figures 13-18 (pages 41-49, 374, 375)].[35a, 35c,35f]

Table 7 Survey of allergy and non-specific hyperreactivity component in 132 rhinitis patients.

Immediate nasal response to allergen challenge	Non-specific hyperreactivity in the nose (=nasal responsiveness to histamine)=counter-value is the nasal histamine threshold	
	increased	non-increased (normal)
Positive (n=113)	78	35
Negative (n=19)	19	0

Reference: 35b

Table 8 Distribution of the nasal histamine threshold in 132 rhinitis patients.

Nasal response to allergen challenge (Immediate nasal response)	Nasal histamine threshold (mg/ml)						
	0.125	0.25	0.5	1.0	2.0	4.0	>4.0
Positive [+INR](n=113)	6	5	8	15	21	23	35
negative [-INR](n=19)	3	7	5	2	2	0	0

Reference: 35b

Table 9 Allergy and non-specific hyperreactivity components in 154 patients with chronic rhinitis.

Nasal response to allergen challenge (Immediate nasal response)	Non-specific hyperreactivity in the nose (= nasal responsiveness to histamine) = counter-value is the nasal histamine threshold	
	increased	non-increased (normal)
Positive (n=127)	83	44
Negative (n=27)	27	0

Reference: 35f

Table 10 Distribution of the nasal histamine threshold in 154 rhinitis patients

Nasal allergy (= response to allergen challenge)	Nasal histamine threshold (mg/ml)						
	0.125	0.25	0.5	1.0	2.0	4.0	>4.0
Positive (n=127)	3	7	5	20	27	21	44
Negative (n=27)	4	10	7	3	3	0	0

Reference: 35f

Table 11 Survey of allergy and non-specific hyperreactivity components in 166 patients with chronic rhinitis.

Patients with nasal response to allergen challenge [NR] (n=166)	Non-specific hyperreactivity in the nose = nasal responsiveness to histamine [counter-value is the nasal histamine threshold]	
	increased	non-increased (normal)
Positive NR (n=142)	45	97
- isolated immediate NR (n=42)	23	19
- dual late NR (n=41)	15	26
- isolated late NR (n=37)	6	31
- dual delayed NR (n=12)	1	11
- isolated delayed NR (n=10)	0	10
Negative NR (n=24)	23	1

Reference: 35c

Table 12 Distribution of the nasal histamine thresholds in 166 rhinitis patients.

Patients with nasal response to allergen challenge [NR] (n=166)	Nasal histamine threshold (mg/ml)						
	0.125	0.25	0.5	1.0	2.0	4.0	>4.0
Positive NR (n=42)							
- isolated immediate NR(n=42)	2	1	4	5	5	6	19
- dual late NR (n=41)	0	0	3	7	2	3	26
- isolated late NR (n=37)	0	0	1	0	3	1	31
- dual delayed NR (n=12)	0	0	0	1	0	0	11
- isolated delayed NR (n=10)	0	0	0	0	0	0	10
Negative NR (n=24)	5	6	8	3	1	0	1*

* This patient demonstrated decreased nasal threshold to methacholine HCl.

Reference: 35c

Table 13 Survey of the nasal thresholds of histamine and methacholines (Cl, Br) in patients with allergic rhinitis (n=46), developing only the Immediate nasal response to allergen challenge [INR] with various "inhalant" allergens (in %).

Immediate nasal response (n=46)	Histamine		Methacholine Cl		Methacholine Br	
	decreased	normal	decreased	normal	decreased	normal
Histamine						
- decreased	46 (21)	x	20 (9)	26 (12)	9 (4)	37 (17)
- normal	x	54 (25)	10 (5)	44 (20)	6 (3)	48 (22)
Methacholine Cl						
- decreased	20 (9)	10 (5)	30 (14)	x	4 (2)	26 (12)
- normal	26 (12)	44 (20)	x	70 (32)	11 (5)	59 (27)
Methacholine Br						
- decreased	9 (4)	6 (3)	4 (2)	11 (5)	15 (7)	x
- normal	37 (17)	48 (22)	26 (12)	59 (27)	x	85 (39)

The genuine numbers of cases are presented in parenthesis.

References: 35a-35c, 35f

Table 14 Survey of the nasal thresholds of histamine and methacholines (Cl, Br) in patients with allergic rhinitis (n=35), developing only the Late nasal response to allergen challenge [LNR] with various "inhalant" allergens (in %).

Late nasal response (n=35)	Histamine		Methacholine Cl		Methacholine Br	
	decreased	normal	decreased	normal	decreased	normal
Histamine						
- decreased	20 (7)	x	11 (4)	9 (3)	6 (2)	14 (5)
- normal	x	80 (28)	0 (0)	80 (28)	3 (1)	77 (27)
Methacholine Cl						
- decreased	11(4)	0 (0)	11 (4)	x	3(1)	8 (3)
- normal	9 (3)	80 (28)	x	89 (31)	6 (2)	83 (29)
Methacholine Br						
- decreased	6 (2)	3 (1)	3 (1)	6 (2)	9 (3)	x
- normal	14 (5)	77 (27)	8 (3)	83 (29)	x	91 (32)

The genuine numbers of cases are presented in parenthesis.
References: 35a-35c, 35f

Table 15 Survey of the nasal thresholds of histamine and methacholines (Cl, Br) in patients with chronic rhinitis (n=28) who did not develop any positive nasal response to allergen challenge [NNR] (in %).

Negative nasal response (n=28)	Histamine		Methacholine Cl		Methacholine Br	
	decreased	normal	decreased	normal	decreased	normal
Histamine						
- decreased	86 (24)	x	64 (18)	22 (6)	50 (14)	36 (10)
- normal	x	14(4)	11 (3)	3 (1)	7 (2)	7 (2)
Methacholine Cl						
- decreased	64 (18)	11 (3)	75 (21)	x	36 (10)	39 (11)
- normal	22 (6)	3 (1)	x	25 (7)	21 (6)	4 (1)
Methacholine Br						
- decreased	50 (14)	7 (2)	36 (10)	21 (6)	57 (16)	x
- normal	36 (10)	7 (2)	39 (11)	4 (1)	x	43 (12)

The genuine numbers of cases are presented in parenthesis.
References: 35a-35c, 35f

TABLE 16 Survey of the nasal thresholds of histamine and methacholines (Cl, Br) in patients with chronic rhinitis demonstrating only one type of the nasal response to nasal challenge with various "inhalant" allergens, expressed in %.

Patients developing	Histamine		Methacholine chloride		Methacoline bromide	
	decreased	normal	decreased	normal	decreased	normal
INR (n=46)	46	54	30	70	15	85
LNR (n=35)	20	80	11	89	9	91
DYNR (n=17)	0	100	6	96	0	100
NNR (n =28)	86	14	75	25	57	43

INR = Immediate nasal response; LNR = Late nasal response; DYNR = Delayed nasal response; NNR = Negative nasal response (= no nasal response to various inhalant allergens)
Reference: 35c

12. HISTAMINE IN THE BLOOD

Histamine has been detected in the blood plasma of the patients developing the positive LNR only sporadically and without any significant changes in its concentration (Table 17).[97v] Similar results have been recorded by us in the patients demonstrating the positive INR and negative nasal responses (NNR). No histamine has been detected in patients developing the positive DYNR (Table 17).[97v]

13. HISTAMINE IN THE NASAL SECRETIONS

Histamine has been detected in the nasal secretions (NS) during the LNR only sporadically and without any significant changes in its concentration (Table 19; Figure 20).[11f] In contrast, histamine has been detected and significant changes in its concentration have been recorded during the majority of the positive INR cases. No histamine has been detected in the NS during the positive DYNR (Tables 18,19; Figures 19,21).[11f,11j]

TABLE 17 Histamine (HS) in the blood plasma and the changes in its concentration during the basic types of nasal response (NR) to allergen challenge.

Patients (n=90)	Histamine in the blood plasma		
Nasal response (n=90)	present(detected)		absent (not detected)
	changes	no changes	
Immediate nasal response (n=32)	5	4	27
	1		
Late nasal response (n=30)	1	1	29
	0		
Delayed nasal response (n=16)	0	0	16
	0		
Negative nasal response (n=12)	1	1	11
	0		
Phosphate buffered saline- control challenge: (n=90)	0	0	90
	0		

Reference : 97 v

Table 18 Histamine (HS) in the nasal secretions (NS) and the changes in its concentration during the Immediate nasal response (INR) and Negative nasal response (NNR) to allergen challenge and Phosphate buffered saline (PBS) control challenge.

Patients n=30	Histamine in nasal secretions		
	Detected		Not detected
	changes	no changes	
INR (n=42)	31		11
	29	2	
NNR (n=11)	1		10
	1	0	
PBS control (n=30)	0		30
	0	0	

INR = Immediate nasal response; NNR = Negative nasal response; PBS = Phosphate buffered saline.
Reference: 11j

Table 19 Histamine (HS) in the nasal secretions (NS) and the changes in its concentrations during the basic types of nasal response (NR) to allergen challenge

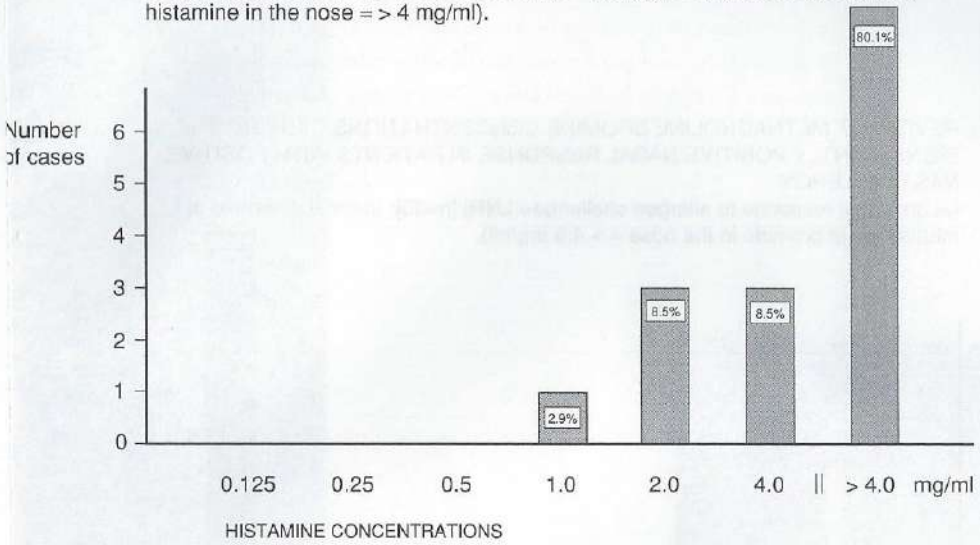
Patients n=52	Histamine in nasal secretions		
	Detected		Not detected
	changes	no changes	
Immediate nasal response (n=18)	15		3
	14	1	
Late nasal response (n=15)	1		14
	0	1	
Delayed nasal response (n=8)	0		8
	0	0	
Negative nasal response (n=11)	1		10
	1	0	
Phosphate buffered saline-control challenge (n=52)	0		52
	0	0	

Reference: 11j

14. PRECIPITATING ANTIBODIES IN THE SERUM

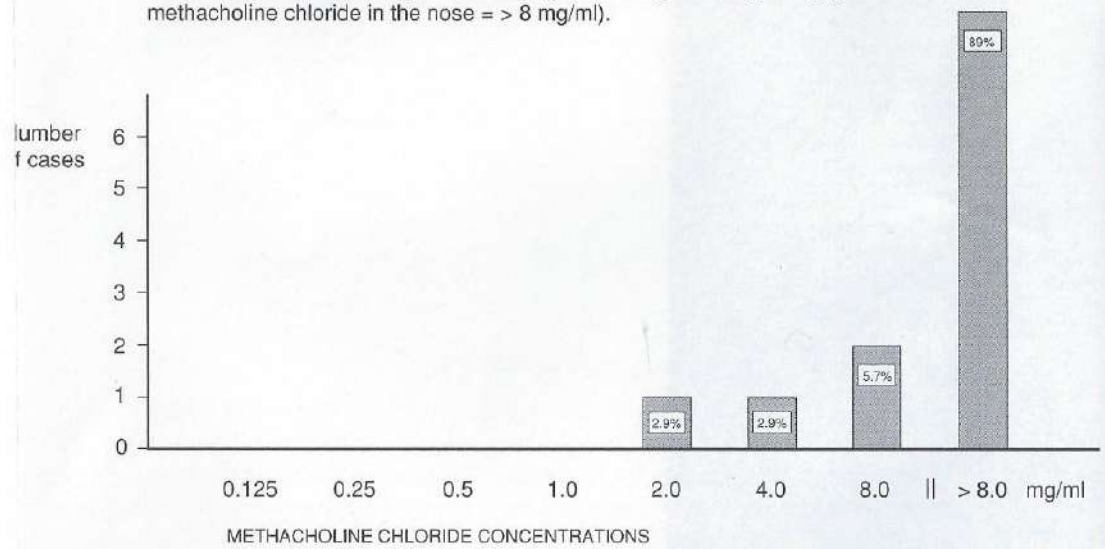
Precipitating antibodies, usually of the IgG class, can be determined by double immuno-diffusion in gel (Ouchterlony & Nilsson technique).[92a,92b] The LNR due to some kinds of antigens, such as bird faeces and serum (pigeon faeces and serum),[15,16] wool,[14,41b] old paper,[19,24,41b] cardboard,[24,41b] flour,[7,12,41b] moulds,[7,12,41b,41f] has been found to be significantly associated with the increased concentration of the circulating precipitating antibodies in the blood serum, being probably of IgG and/or IgM classes [Figures 28-31; Plates 12a,12b (page 36)].

Fig. 10a. REVIEW OF HISTAMINE CONCENTRATIONS CAUSING THE SIGNIFICANTLY POSITIVE NASAL RESPONSE IN PATIENTS WITH POSITIVE NASAL ALLERGY [=Late nasal response to allergen challenge= LNR] [n=35]; (normal threshold of histamine in the nose = > 4 mg/ml).



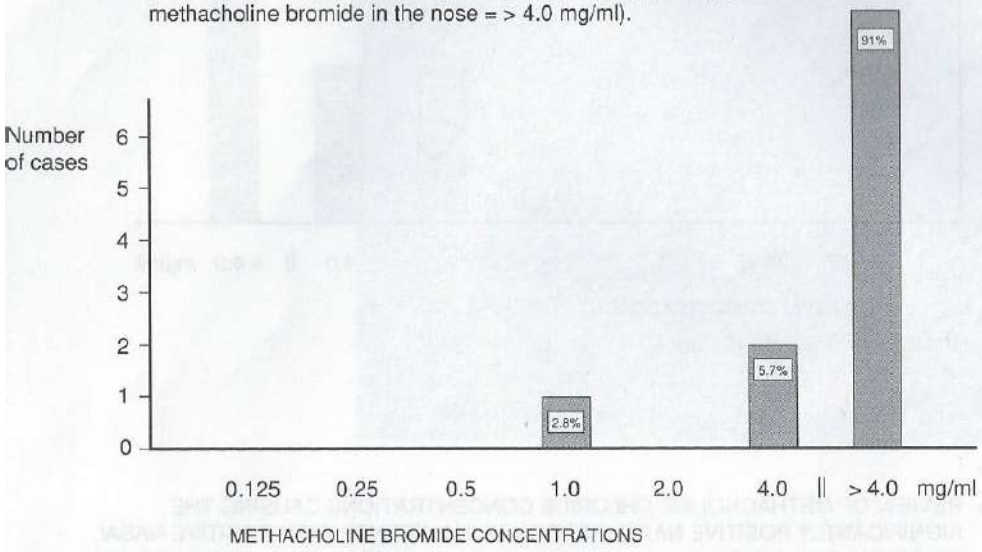
References: 35c,35f,41b,72b

Fig. 10b. REVIEW OF METHACHOLINE CHLORIDE CONCENTRATIONS CAUSING THE SIGNIFICANTLY POSITIVE NASAL RESPONSE IN PATIENTS WITH POSITIVE NASAL ALLERGY [=Late nasal response to allergen challenge= LNR] [n=35]; (normal threshold of methacholine chloride in the nose = > 8 mg/ml).



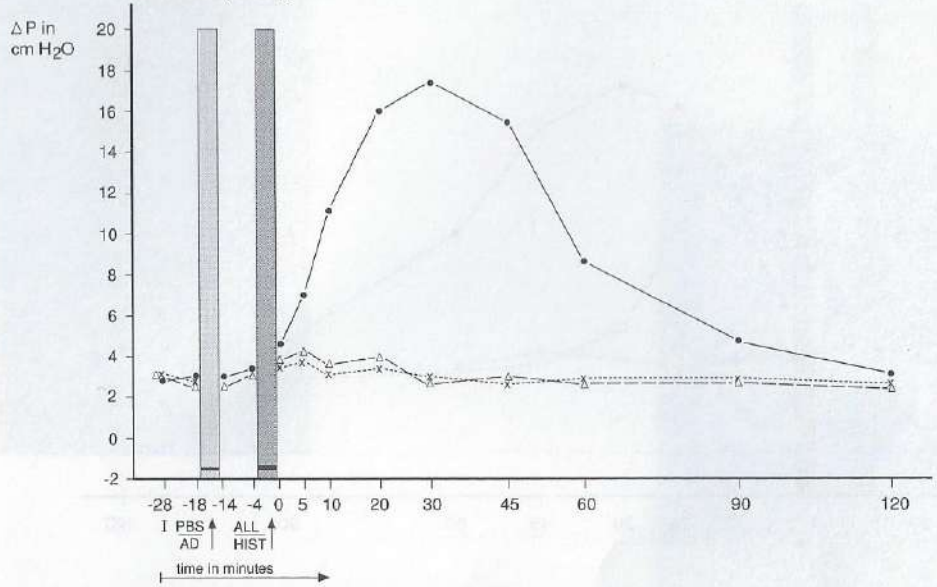
References: 35c,35f,41b,72b

Fig. 10c. REVIEW OF METHACHOLINE BROMIDE CONCENTRATIONS CAUSING THE SIGNIFICANTLY POSITIVE NASAL RESPONSE IN PATIENTS WITH POSITIVE NASAL ALLERGY
[=Late nasal response to allergen challenge= LNR] [n=35]; (normal threshold of methacholine bromide in the nose = > 4.0 mg/ml).



References: 35c,35f,41b,72b

Fig. 12. ALLERGIC COMPONENT (ALL) AND NON-SPECIFIC HYPERREACTIVITY COMPONENT (N-SH) IN PATIENTS WITH RHINITIS [positive immediate nasal response to allergen challenge (+INR) and normal (non-decreased) nasal histamine threshold (-NHT)]

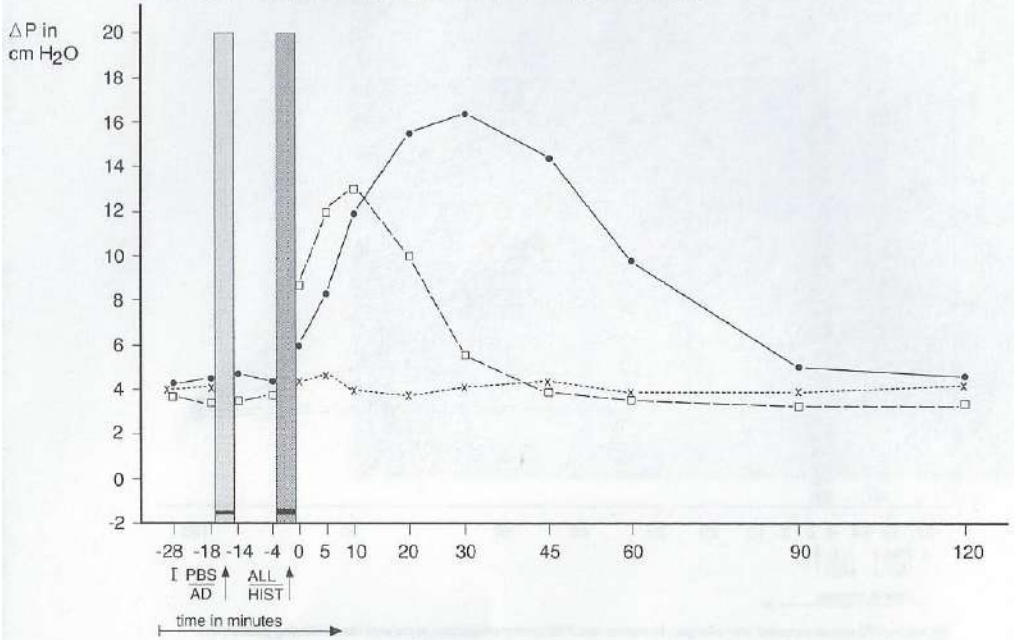


The mean NPG values recorded after allergen, histamine and PBS control challenges in patients demonstrating positive INR and normal (non-increased) nasal responsiveness to histamine (NHT > 4.0 mg/ml or 12 mmol/ml).
I = Initial values; PBS = Phosphate buffered saline; ALL= Allergen; AD = Distilled water; HIST = Histamine phosphate; NHT = nasal histamine threshold.

• ——— • = INR (n=35)
Δ ——— Δ = Histamine 4.0 mg/ml (n=35)
x ····· x = PBS control challenge (n=35)

References: 35a.35b.35c.35f.72b

Fig. 13a. ALLERGIC COMPONENT (ALL) AND NON-SPECIFIC HYPERREACTIVITY COMPONENT (N-SH) IN PATIENTS WITH RHINITIS [positive immediate nasal response to allergen challenge (+INR) and decreased nasal histamine threshold (+NHT)]

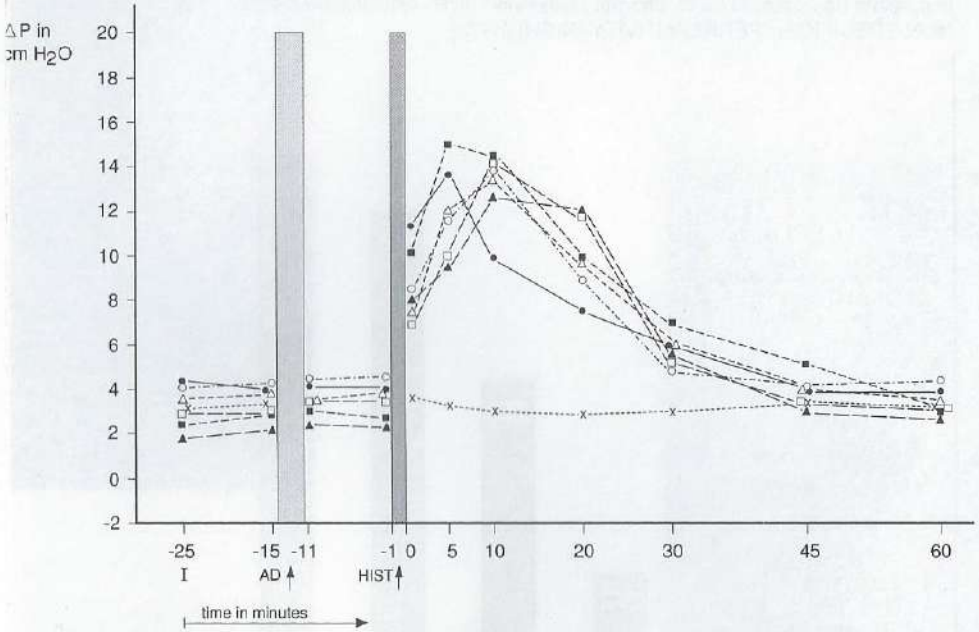


The mean NPG values recorded after allergen, histamine and PBS control challenges in patients demonstrating positive INR and increased nasal responsiveness to histamine (= decreased histamine threshold in the nose = > 4.0 mg/ml or 12 mmol/ml).
I = Initial values; PBS = Phosphate buffered saline; ALL= Allergen; AD = Distilled water; HIST = Histamine diphosphate; NHT = Nasal histamine threshold.

- = positive INR (n=78)
- = Histamine ≤ 4.0 mg/ml (n=78)
- = PBS control challenge (n=78)

References: 35a,35b,35c,35f,72b

]. 13b. SURVEY OF THE NASAL HISTAMINE THRESHOLDS (NHT) IN RHINITIS PATIENTS WITH POSITIVE ALLERGY AND INCREASED NON-SPECIFIC HYPERREACTIVITY (N-SH)

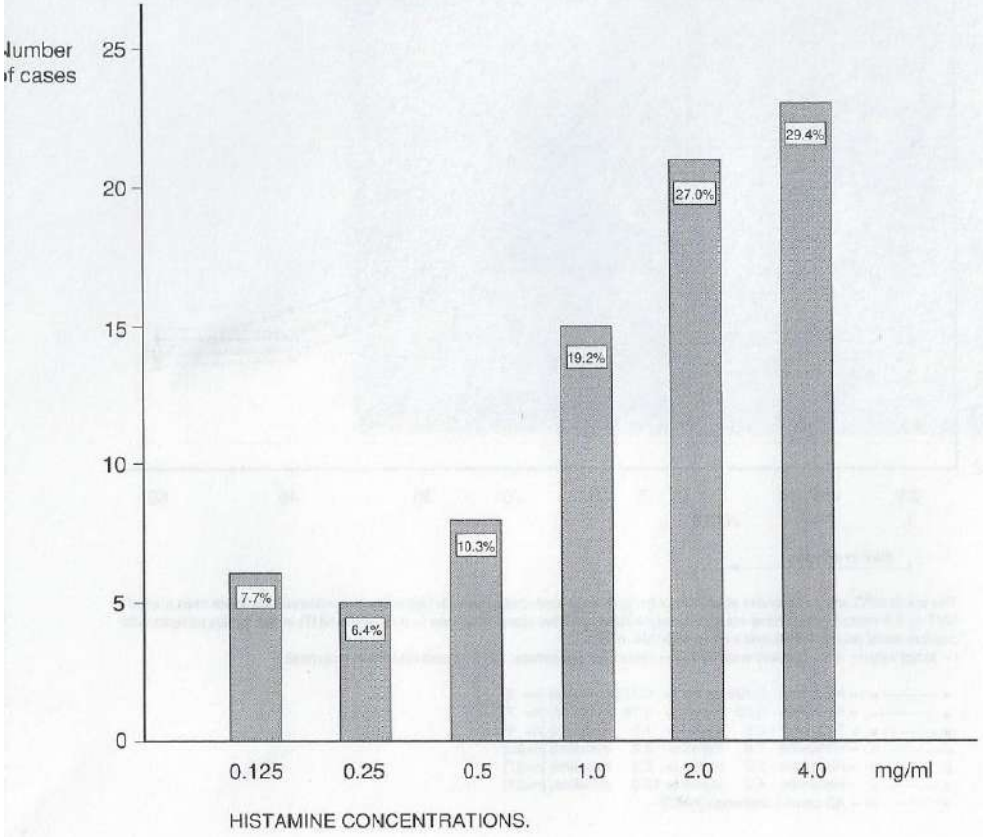


The mean NPG values recorded after AD control challenge and challenge with histamine in concentrations lower than normal NHT (≤ 4.0 mg/ml), which have caused the significantly positive nasal response (= decreased NHT) in the rhinitis patients with positive nasal allergy (+NR) and increased N-SH (n=83).
I = Initial values; AD = Distilled water; HIST = Histamine phosphate; NHT = nasal histamine threshold.

- = Histamine - 0.125 mg/ml (= 0.375 mmol/ml) [n= 3]
- = Histamine - 0.25 mg/ml (= 0.75 mmol/ml) [n= 7]
- = Histamine - 0.5 mg/ml (= 1.5 mmol.ml) [n= 5]
- = Histamine - 1.0 mg/ml (= 3.0 mmol/ml) [n=20]
- = Histamine - 2.0 mg/ml (= 6.0 mmol/ml) [n=27]
- = Histamine - 4.0 mg/ml (= 12.0 mmol/ml) [n=21]
- = AD control challenge [n=83]

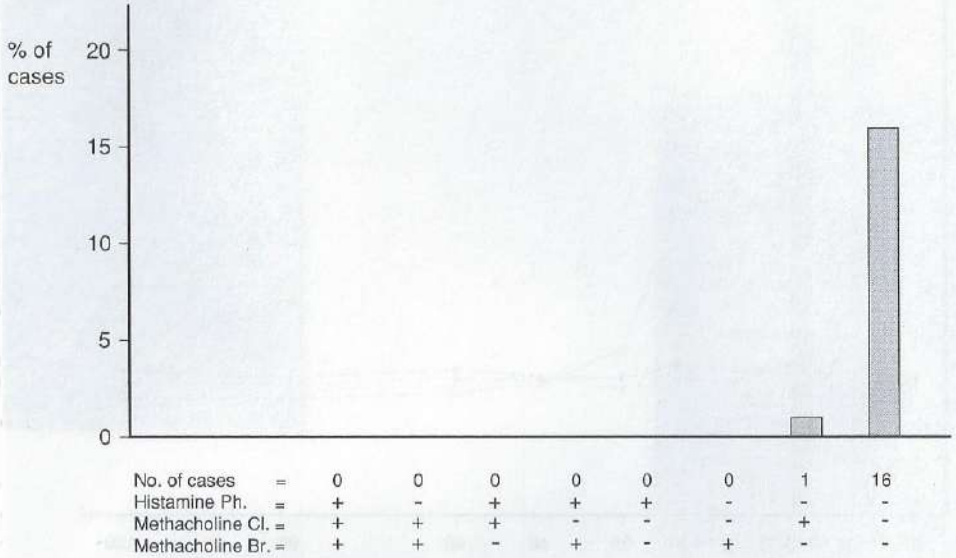
References: 35a,35b,35c,35f,72b

Fig. 13c. DISTRIBUTION OF NASAL HISTAMINE THRESHOLDS (NHT) IN RHINITIS PATIENTS WITH POSITIVE NASAL ALLERGY (positive immediate nasal response to allergen challenge = INR) AND INCREASED NON-SPECIFIC HYPERREACTIVITY (N-SH) [n=78]



References: 35a,35b,35c,35f,72b

Fig. 15. RELATIONSHIP OF CHANGED AND UN-CHANGED RESPONSIVENESS OF NASAL MUCOSA TO HISTAMINE, METHACHOLINE CHLORIDE AND BROMIDE IN PATIENTS DEVELOPING DELAYED NASAL RESPONSE [DYNR] (n=17)

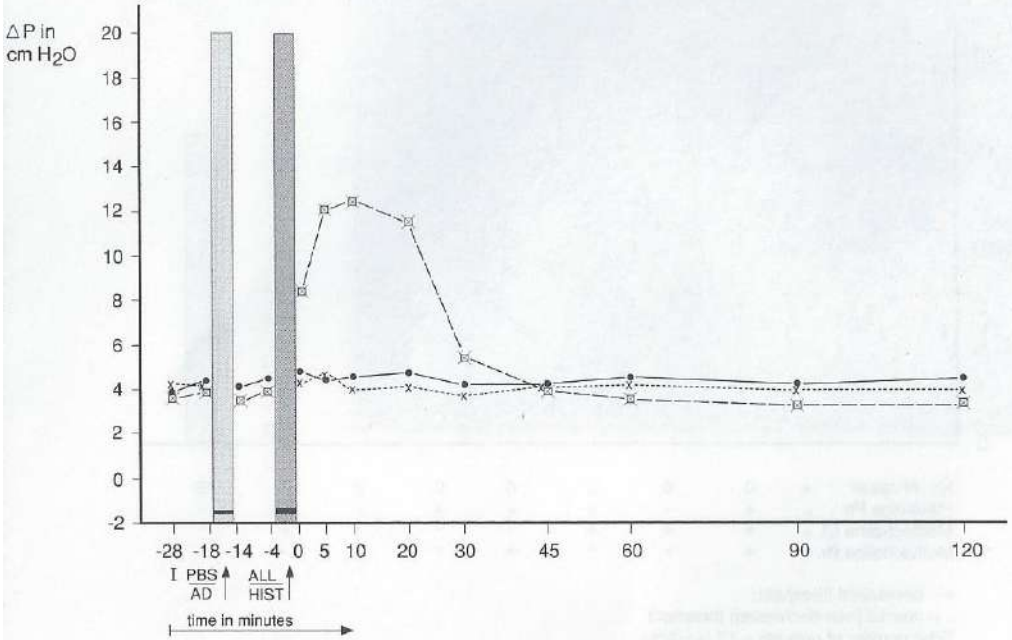


No. of cases =	0	0	0	0	0	0	1	16
Histamine Ph. =	+	-	+	+	+	-	-	-
Methacholine Cl. =	+	+	+	-	-	-	+	-
Methacholine Br. =	+	+	-	+	-	+	-	-

+ = decreased threshold;
- = normal (non-decreased) threshold
Total number of patients = 17 (=100%)

References: 35c,35f

Fig. 16a. NON-SPECIFIC HYPERREACTIVITY COMPONENT (N-SH) RHINITIS PATIENTS WITH NEGATIVE NASAL ALLERGY [negative immediate nasal response to allergen challenge (NNR) and decreased nasal histamine threshold (+NHT)] (n=19)

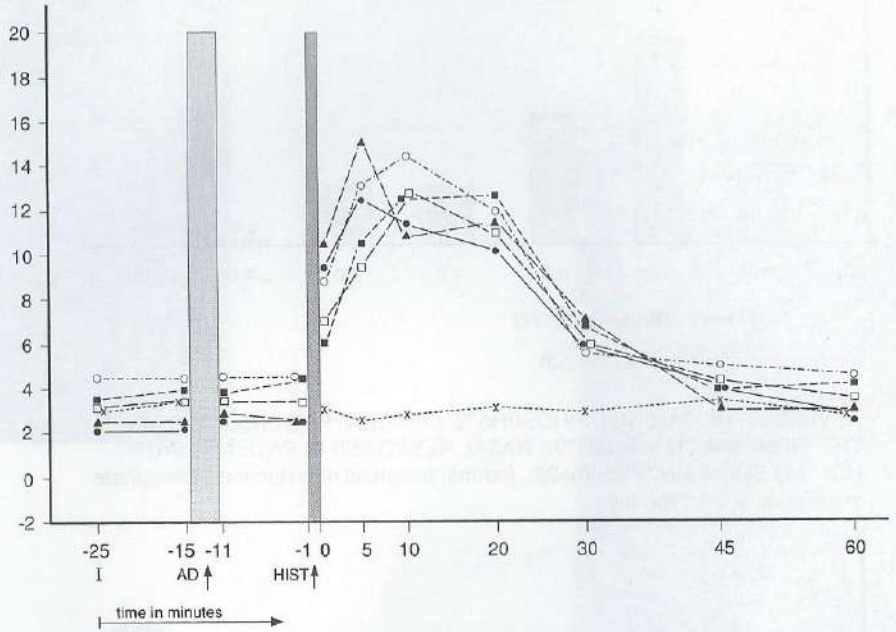


The mean NPG values recorded after allergen, histamine and PBS control challenge in patients demonstrating negative INR and increased nasal responsiveness to histamine (= decreased histamine threshold in the nose = ≤ 4.0 mg/ml or 12 mmol/ml).
I = Initial values; PBS = Phosphate buffered saline; ALL= Allergen; AD = Distilled water; HIST = Histamine diphosphate; NHT = nasal histamine threshold.

• ——— • = negative NNR (n=19)
⊠ ——— ⊠ = Histamine ≤ 4.0 mg/ml (n=19)
x ····· x = PBS control challenge (n=19)

References: 35a,35b,35c,35f,72b

Fig. 16b. POSITIVE NASAL RESPONSE TO VARIOUS CONCENTRATIONS OF HISTAMINE (= NASAL HISTAMINE THRESHOLDS = NHT) IN RHINITIS PATIENTS WITH NEGATIVE NASAL ALLERGY (negative immediate nasal response to allergen challenge = NNR) AND INCREASED NON-SPECIFIC HYPERREACTIVITY (N-SH) [n=19]

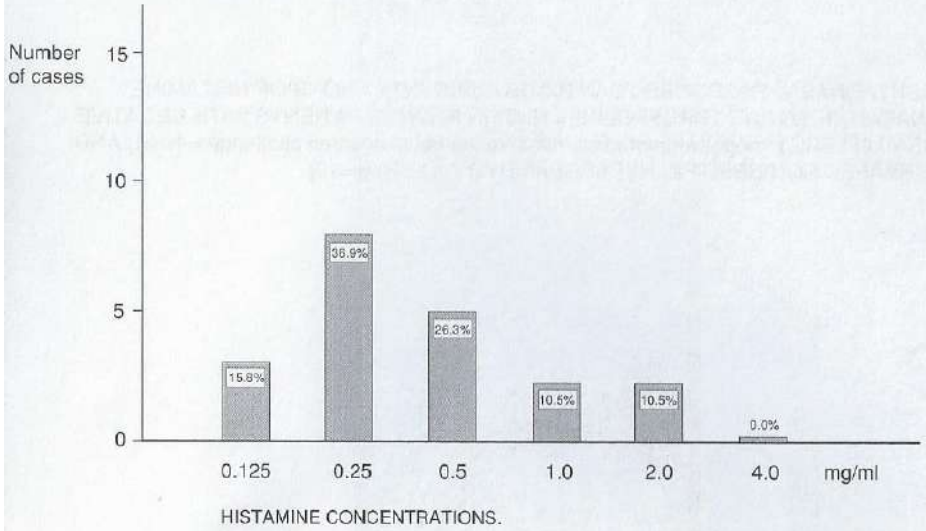


The mean NPG values recorded after AD control challenge and challenge with histamine in concentrations lower than normal NHT (≤ 4.0 mg/ml), which have caused the significantly positive nasal response (= decreased NHT) in the rhinitis patients with negative nasal allergy (-INR) and increased N-SH (n=19).
I = Initial values; AD = Distilled water; HIST = Histamine diphosphate; INR = Immediate nasal response to allergen challenge; NHT = nasal histamine threshold.

• ——— • = Histamine - 0.125 mg/ml (= 0.375 mmol/ml) [n=3]
▲ ——— ▲ = Histamine - 0.25 mg/ml (= 0.75 mmol/ml) [n=7]
■ ——— ■ = Histamine - 0.5 mg/ml (= 1.5 mmol/ml) [n=5]
○ ——— ○ = Histamine - 1.0 mg/ml (= 3.0 mmol/ml) [n=2]
□ ——— □ = Histamine - 2.0 mg/ml (= 6.0 mmol/ml) [n=2]
x ····· x = AD control challenge [n=19]

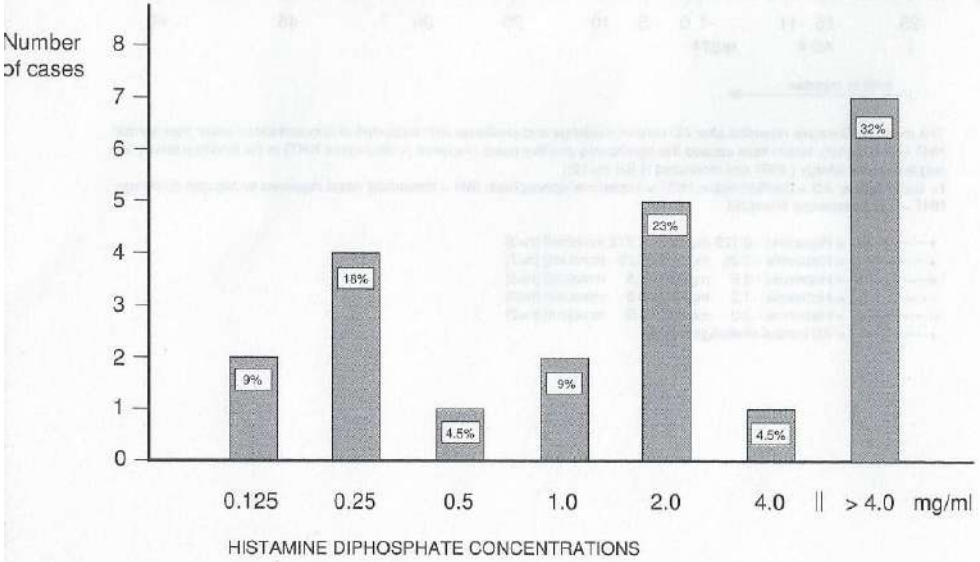
References: 35a,35b,35c,35f,72b

Fig. 16c. DISTRIBUTION OF NASAL HISTAMINE THRESHOLDS (NHT) IN RHINITIS PATIENTS WITH NEGATIVE NASAL ALLERGY (negative immediate nasal response to allergen challenge = NNR) AND INCREASED NON-SPECIFIC HYPERREACTIVITY (N-SH) [n=19]



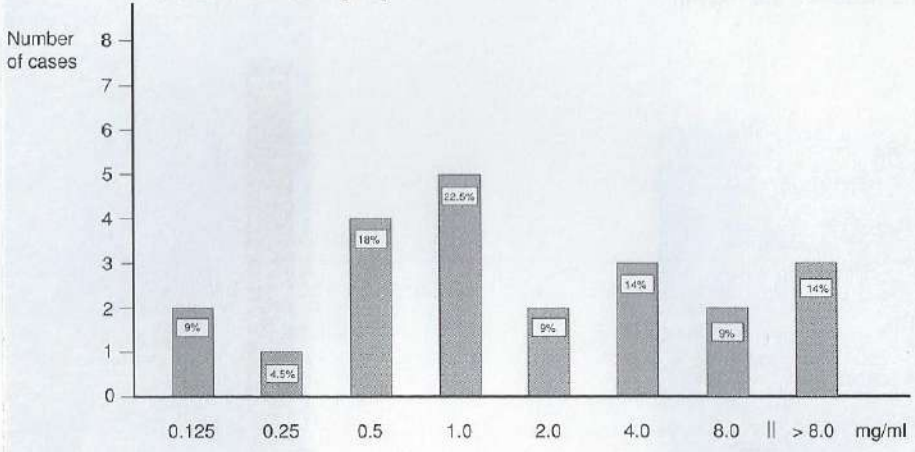
References: 35a,35b,35c,35f,72b

Fig. 17a. REVIEW OF HISTAMINE DIPHOSPHATE CONCENTRATIONS CAUSING THE SIGNIFICANTLY POSITIVE NASAL RESPONSE IN PATIENTS WITH NON-ALLERGIC RHINITIS (n=22). [normal threshold of histamine diphosphate in the nose = > 4.0 mg/ml]



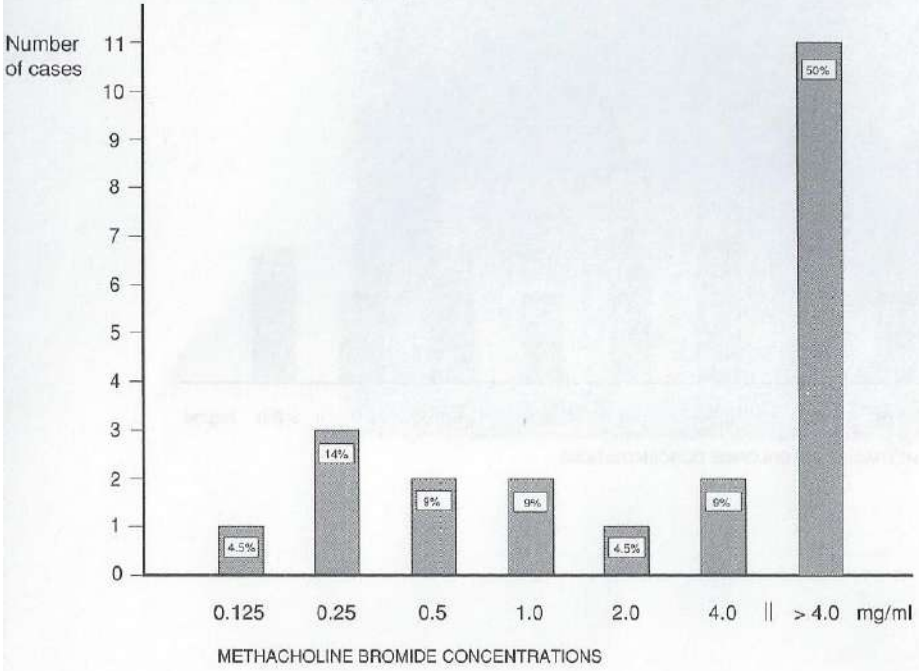
References: 35a,35b,35c,35f,72b

Fig. 17b. REVIEW OF METHACHOLINE CHLORIDE CONCENTRATIONS CAUSING THE SIGNIFICANTLY POSITIVE NASAL RESPONSE IN PATIENTS WITH NON-ALLERGIC RHINITIS (n=22). [normal threshold of methacholine chloride in the nose = > 8.0 mg/ml]



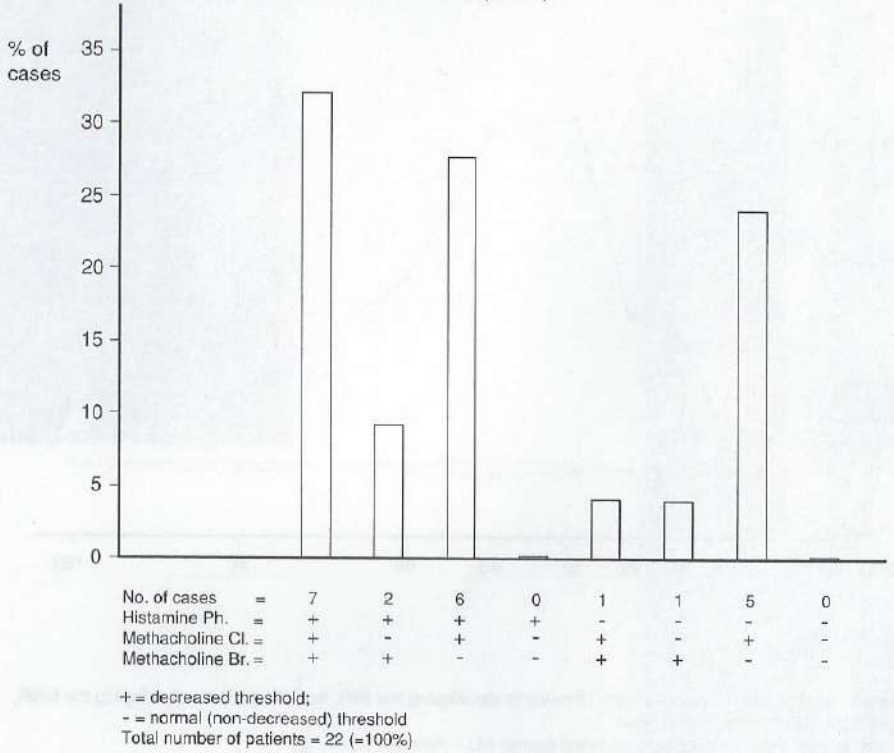
References: 35a,35b,35c,35f,72b

Fig. 17c. REVIEW OF METHACHOLINE BROMIDE CONCENTRATIONS CAUSING THE SIGNIFICANTLY POSITIVE NASAL RESPONSE IN PATIENTS WITH NON-ALLERGIC RHINITIS (n=22). [normal threshold of methacholine bromide in the nose = > 4.0 mg/ml]



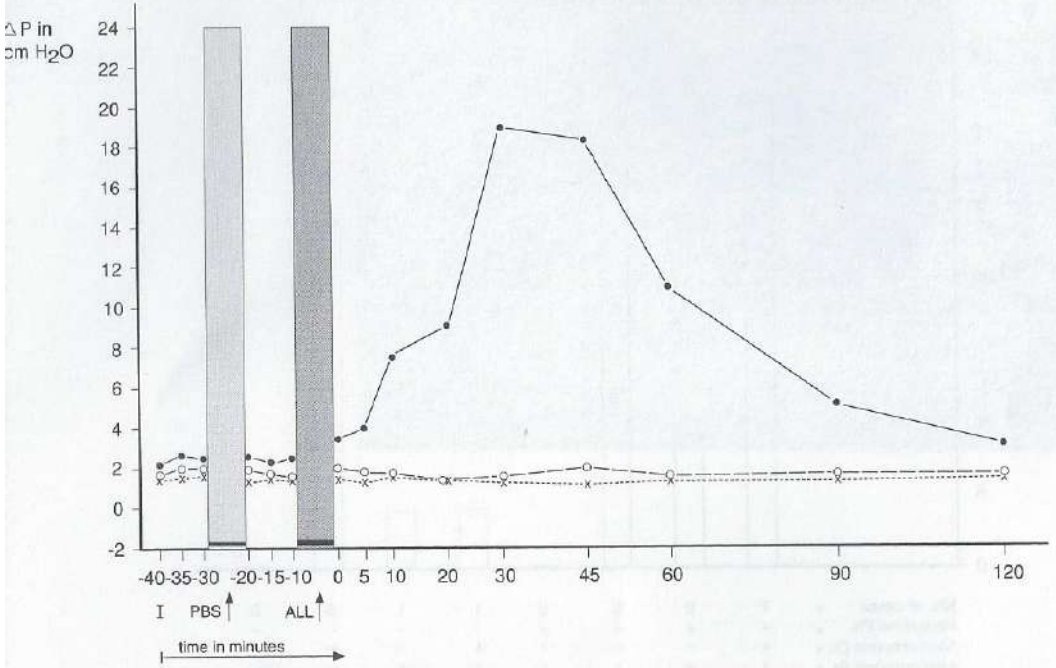
References: 35a,35b,35c,35f,72b

Fig. 17d. RELATIONSHIP OF CHANGED AND UN-CHANGED RESPONSIVENESS OF NASAL MUCOSA TO HISTAMINE, METHACHOLINE CHLORIDE AND BROMIDE IN PATIENTS WITH NON-ALLERGIC RHINITIS (n=22)



References: 35a,35b,35c,35f,72b

Fig. 19a. THE IMMEDIATE NASAL RESPONSE TO ALLERGEN CHALLENGE (INR), NEGATIVE NASAL RESPONSE (NNR) AND CONTROL CHALLENGES WITH PHOSPHATE BUFFERED SALINE (PBS)

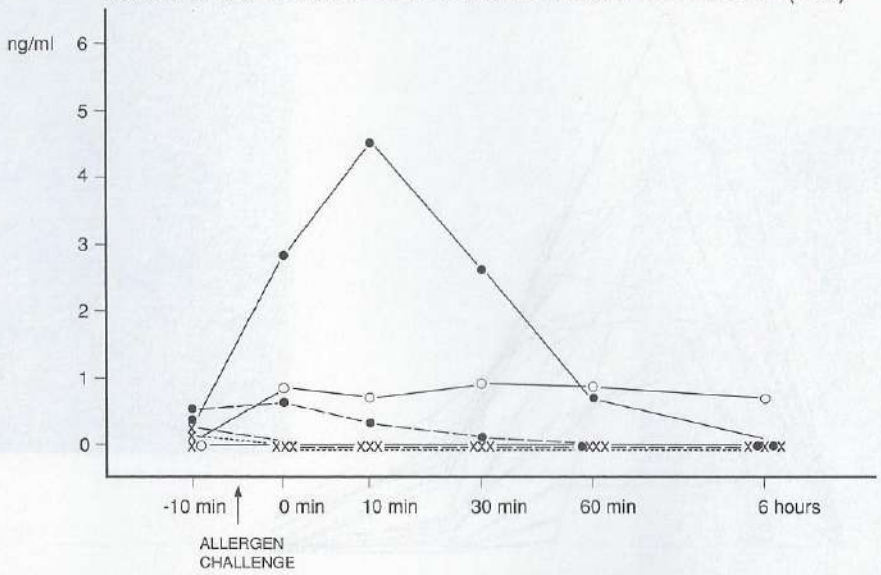


The mean NPG values calculated from 18 patients developing the INR, from 11 patients developing the NNR, and from 29 PBS control challenges
I = Initial values; PBS = Phosphate buffered saline; ALL= Allergen challenge

●——— ● = INR (n=18)
○– – – ○ = NNR (n=11)
x····· x = PBS control challenges (n=29)

References: 11f,11j

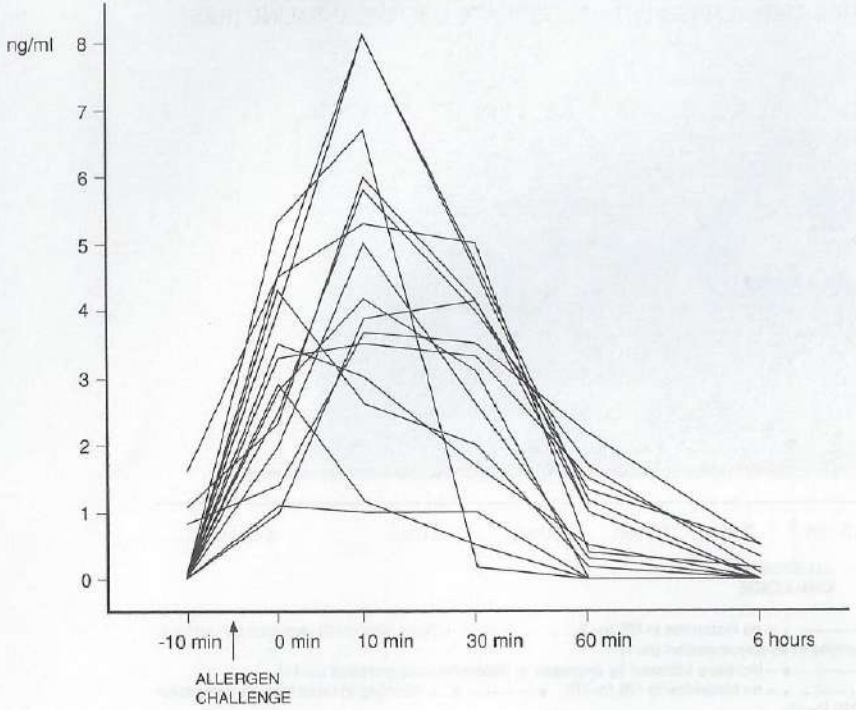
Fig. 19b. THE MEAN VALUES OF HISTAMINE CONCENTRATION IN NASAL SECRETIONS (NS) DURING THE 18 POSITIVE IMMEDIATE NASAL RESPONSES (INR), 11 NEGATIVE NASAL RESPONSES (NNR) AND 29 CONTROL CHALLENGES WITH PHOSPHATE BUFFERED SALINE (PBS)



INR: x——— x = no histamine in NS (n=3); ○——— ○ = histamine in NS detected but without changes in its concentration (n=1)
●——— ● = increase followed by decrease in histamine concentration (n=14)
NNR: x– – – – x = no histamine in NS (n=10); ●– – – – ● = changes in histamine concentration in NS (n=1)
PBS: x····· x = no histamine in NS (n=29)

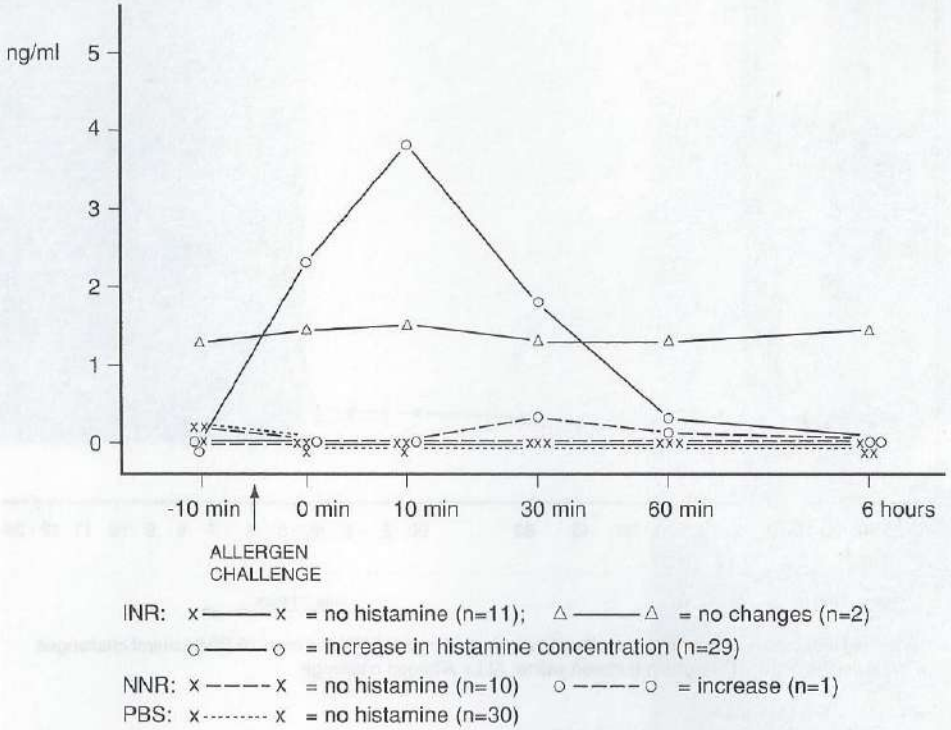
References: 11f,11j

Fig. 19c. SURVEY OF THE CHANGES IN HISTAMINE CONCENTRATION IN NASAL SECRETIONS (NS) DURING THE 15 OF THE 18 POSITIVE IMMEDIATE NASAL RESPONSES (INR)



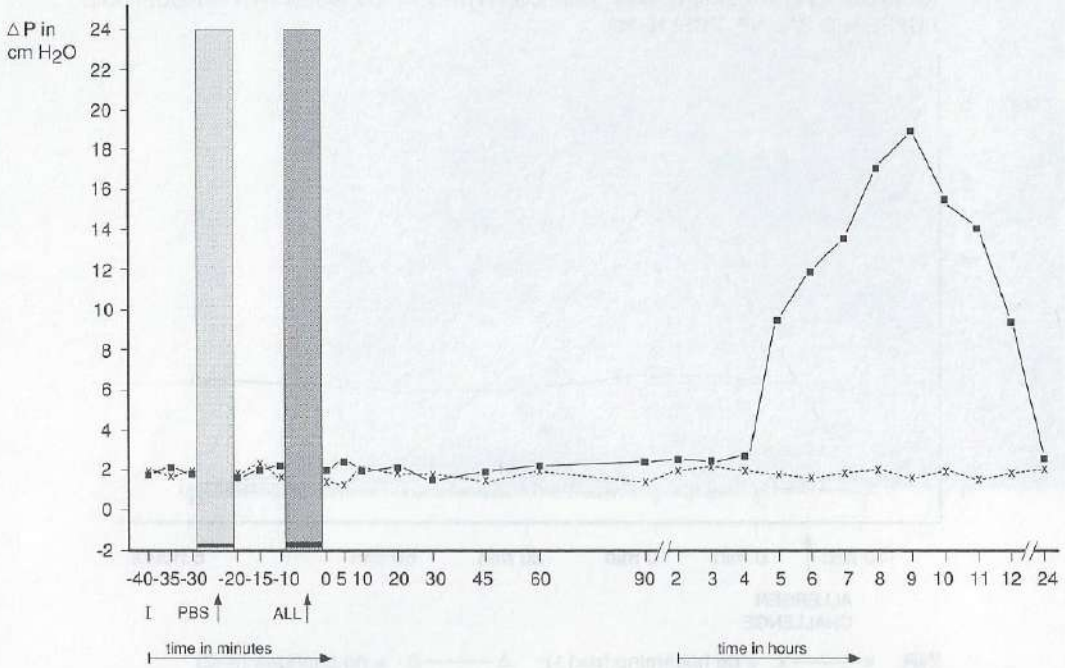
References: 11f,11j

Fig. 19d. THE MEAN VALUES OF HISTAMINE CONCENTRATIONS IN NASAL SECRETIONS (NS) DURING THE POSITIVE IMMEDIATE NASAL RESPONSE (INR; N=42), NEGATIVE NASAL RESPONSE (NNR; N=11) AND CONTROL CHALLENGES WITH PHOSPHATE BUFFERED SALINE (PBS; N=30)



References: 11f,11j

Fig. 20a. THE LATE NASAL RESPONSE TO ALLERGEN CHALLENGE (LNR) AND CONTROL CHALLENGES WITH PHOSPHATE BUFFERED SALINE (PBS)

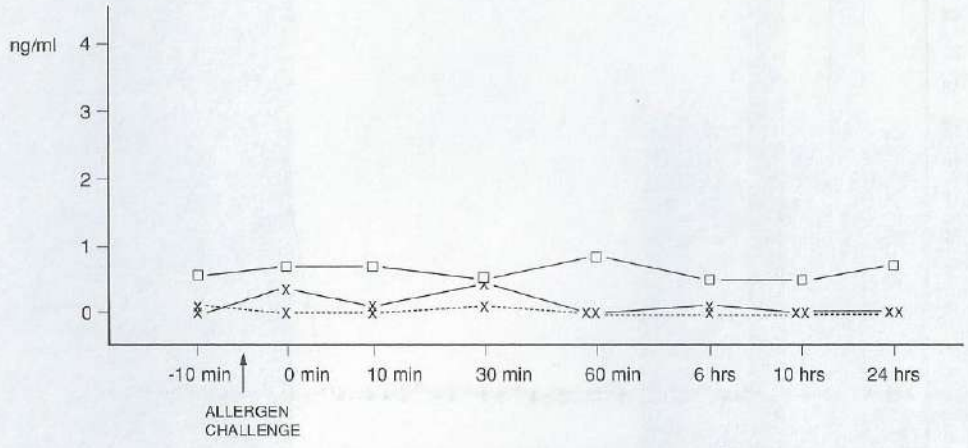


The mean NPG values calculated from 15 patients developing the LNR and from 15 PBS control challenges
I = Initial values; PBS = Phosphate buffered saline; ALL= Allergen challenge

■ ——— ■ = LNR (n=15)
x ····· x = PBS control challenge (n=15)

References: 11f

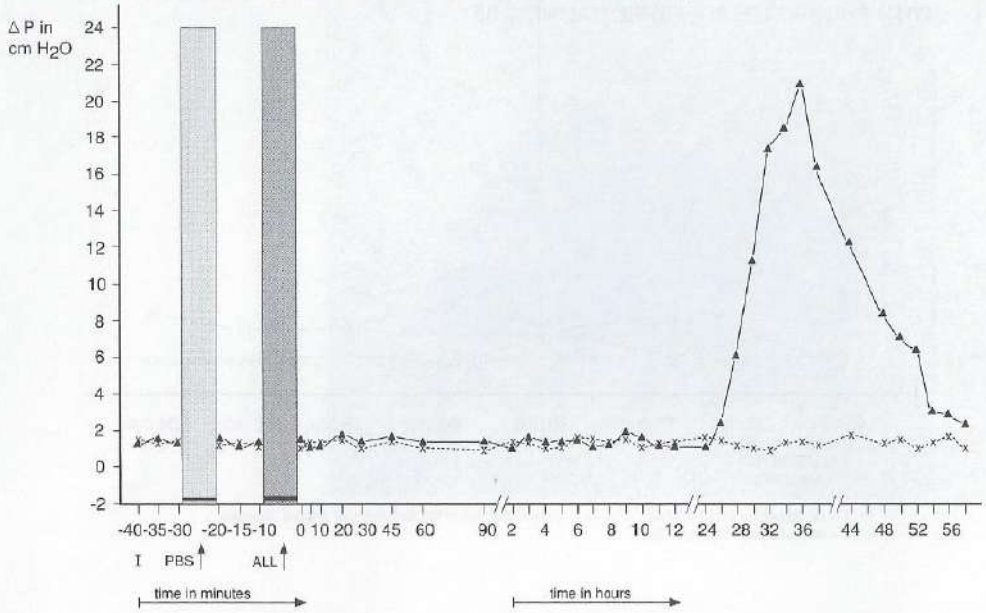
Fig. 20b. THE MEAN VALUES OF HISTAMINE CONCENTRATION IN NASAL SECRETIONS (NS) DURING THE 15 LATE NASAL RESPONSES (LNR) AND 15 CONTROL CHALLENGES WITH PHOSPHATE BUFFERED SALINE (PBS)



LNR: x ——— x = no histamine in NS (n=14); □ ——— □ = histamine in NS detected but without changes
PBS: x ····· x = no histamine in NS (n=15)

References: 11f

Fig. 21a. THE DELAYED NASAL RESPONSE TO ALLERGEN CHALLENGE (DYNR) AND CONTROL CHALLENGES WITH PHOSPHATE BUFFERED SALINE (PBS)

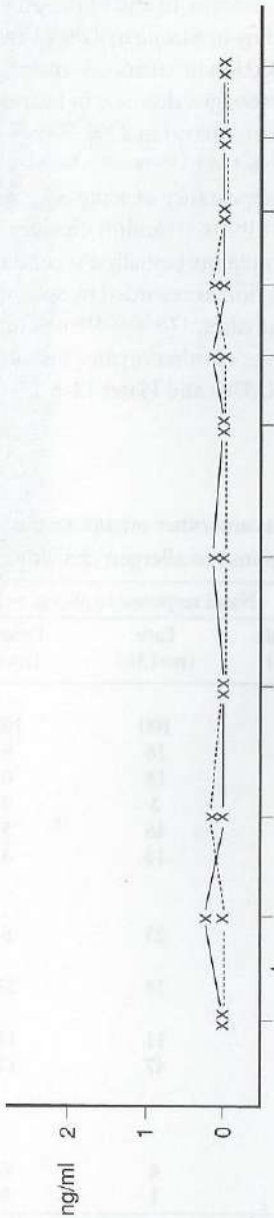


The mean NPG values calculated from 8 patients developing the DYNR and from 8 PBS control challenges
I = Initial values; PBS = Phosphate buffered saline; ALL= Allergen challenge

▲——— ▲ = DYNR (n=8)
x ····· x = PBS control challenge (n=8)

References: 11f

Fig. 21b. THE MEAN VALUES OF HISTAMINE CONCENTRATION IN NASAL SECRETIONS (NS) DURING THE 8 DELAYED NASAL RESPONSES (DYNR) AND 8 CONTROL CHALLENGES WITH PHOSPHATE BUFFERED SALINE (PBS)



References: 11f

E. ASSOCIATION OF "LNR" WITH OTHER ORGANS' RESPONSES

The LNR can also induce a secondary response in and of the other, related, organs. The LNR can, therefore, be accompanied by headache in 47% of cases,[14,26,40a, 41,41b] by conjunctival symptoms (conjunctival injection or chemosis) in 46% and/or palpebral edema in 13%,[17,21] middle ear response (otalgia, decrease in hearing, changes of the middle ear pressure as recorded by tympanometry) in 23%,[22,26,27,27a,27b, 41b] pressure in the maxillary and frontal sinuses in 18%,[12,14,14a-14g,41b,41g,41i] changes on the sinus X-ray (increase in mucosal thickness or appearance of acute edema of the sinus mucosa) in 11% respectively 18% [12,14,14a-14g,41b,41g,41i] and/or changes on the echogram of the sinuses,[14,a,14b,14d] and bronchial complaints (usually a secondary dyspnea due to the secondary induced bronchial constriction as recorded by spirometry, sometimes also wheezing and/or cough) in 4% of the cases, [12,40a,41,41b] or more, [41h,41i] and general malaise in 6% of cases.[12,41,41b] A survey of other organs' responses is presented in Tables 20-29, Figures 22-27 (pages 67-74, 376) and Plates 13-I, 13-II, 14, 15, 16a, 16b, 17 (pages 262-265).

Table 20 Survey of the nasal complaints and other organs' response accompanying the particular types of nasal response to allergen challenge (in %)

	Nasal response to allergen challenge			
	Immediate (n=148)	Late (n=131)	Delayed (n=63)	Negative (n=205)
Nasal complaints				
obstruction	100	100	100	0
sneezing	69	16	9	8
hypersecretion	93	18	0	10
itching	52	3	0	0
Conjunctival injection/chemosis	27	46	5	1
Palpebral edema	12	13	3	0
Middle ear response (otalgia, decrease in hearing, changes in middle ear pressure)	30	23	6	7
Pressure in the sinuses (maxillary and frontal)	37	18	22	7
Acute edema of sinus mucosa (X-ray)	3	11	14	1
Cephalgia	26	47	11	2
Bronchial complaints (mostly secondary bronchoconstriction, sometimes also wheezing and/or cough)	5	4	6	2
General malaise complaints	3	1	0	0

References: 2,7,11,11a-11h,12,13,13a-13c,18,25,26,40c,40d,40f,41a-41d,41f,41i,57a,71,72, 72a,72c,72d,96,97,97a,121j

The association of LNR with other "*in vivo*" and "*in vitro*" diagnostic parameters as well as with other organs' response showed some similarities to those observed by us in the case of the late asthmatic response (LAR).[61-68,68a-68g,96a-96r] However the relationship between the LNR and the other "*in vivo*" and "*in vitro*" parameters differed distinctly from those recorded during the immediate nasal response (INR),[11,11a-11g,11i,11j, 14,18,20,25b,41a-41c,41f,41i] the immediate asthmatic response (IAR), [63,69,70,96f,96i,96k,96l, 96p] the delayed nasal response (DYNR)[13,13a-13c] and the delayed asthmatic response (DYAR). [70a-70e]

Table 21a Allergic conjunctivitis - relationship to allergic rhinitis. A review of secondary conjunctival responses induced by the nasal provocation tests with allergens (NPT) in 23 patients with allergic conjunctivitis resistant to the usual topical ophthalmologic therapy.

	Positive NPT		Negative NPT	
Patients n=23	ocular +	complaints -	ocular +	complaints -
43 positive INRs in 17 patients	39	4	–	–
28 negative NR (NNR) in 6 patients–	–	-	18 (in 3 patients)	10 (in 3 patients)

NR = Nasal response; INR = Immediate nasal response; NNR = Negative nasal response; NPT = Nasal provocation tests with allergen; Ocular complaints = conjunctival hyperemia, injection, itching, tearing, burning, watery discharge (= hyperlacrimation), photophobia, palpebral edema.
Reference: 17

Table 21b Allergic conjunctivitis - relationship to allergic rhinitis. The results of the treatment in the 23 patients with allergic conjunctivitis, for 8 months.

	Treatment - 8 months		Improvement of ocular complaints after 8 months		Improvement of ocular complaints after addition of DSCG - IN
Patients n=23	DSCG IC only	DSCG IC + IN	+	–	
17 patients with positive INR	5	–	0	5	5
	–	12	12	0	–
6 patients with negative NR	6	–	2	4	2

INR = Immediate nasal response; NR = Nasal response; DSCG = Disodium cromoglycate = Cromolyn: IC = intraconjunctival application=eye drops; IN = intranasal application=nasal spray
Reference: 17

Table 22a The role of the nasal mucosa in the allergic conjunctivitis. A survey of the clinical features of the patients studied.

Patients n=31	n	Conjunctival complaints				Ophthalmologic examination					
		IT	IR	PP	HL	CI	HY	CH	PO	PH	LP
Conjunctivitis (allergic + vernal)	24	19	24	13	23	11	8	6	11	5	2
Kerato-conjunctivitis	4	3	4	3	3	1	4	3	1	3	4
Blepharo-conjunctivitis	3	3	3	0	1	2	1	1	3	2	0

IT = itching; IR = irritation; PP = photophobia; HL = hyperlacrimation; CI = conjunctival injection; HY = hyperemia; CH = chemosis; PO = palpebral edema; PH = papillary hypertrophy; LP = limbus phenomenon.
Reference: 21

Table 22b Survey of the nasal and conjunctival responses after the nasal challenges with allergens

Patients n=31	Positive INR		Negative NR (= NNR)	
	conjunctival response		conjunctival response	
	+	–	+	–
19 patients with 48 positive INR	42	6	–	–
12 patients with 43 negative NR (= NNR)	–	–	23 (in 7 patients)	20 (in 5 patients)

INR = Immediate nasal response; NR = Nasal response; NNR = Negative nasal response; conjunctival response = conjunctival hyperemia, injection, chemosis, itching, hyperlacrimation, palpebral edema.
Reference: 21

Table 22c Results of the treatment with DSCG (Disodium chromoglycate, Cromolyn) in the form of the eye drops only (DSCG - ocular) or in the combination with the intranasal DSCG (DSCG -nasal), for 6 months.

Patients n=31	Treatment for 6 months		Improvement of ocular complaints after 6 months			Improvement of ocular complaints after addition of DSCG - nasal
	DSCG - ocular	DSCG – ocular + DSCG - nasal	+	±	-	
19 patient with positive INR	5	–	0	0	5	5
	–	14	14	0	0	0
12 patients with negative NR (NNR)	12	–	0	3	9	8

NR = Nasal response; INR = Immediate nasal response; NNR = Negative nasal response.
Reference: 21

Table 23 Particular types of the conjunctival response induced by the nasal response to allergen challenge.

Nasal responses = patients n=52	Secondarily induced conjunctival response			
	Early [ECR]	Late [LCR]	Delayed [DYCR]	Negative[NCR]
Immediate nasal response [INR] (n=25)	24	1	0	0
Late nasal response [LNR] (n=18)	1	16	1	0
Delayed nasal response [DYNR] (n=9)	1	0	8	0
Negative nasal response [NNR] (n=11)	0	0	1	10

Reference: 41i

Table 24a Survey of the nasal and ear responses after the nasal challenges with allergens.

Patients n=31	Ear response	
	Positive	Negative
22 patients with		
- 85 positive NR	73	12
- 30 negative NR	3	27
9 patients with		
- 39 negative NR	25 (in 6 patients)	14 (in 3 patients)
31 control challenges with PBS	0	31

NR= Nasal response
Reference: 27

Table 24b Survey of the otological changes and complaints during the particular types of the nasal response (NR).

Patients n=31	n	Changes in MEP* accompanied by			Otalgia only
		Otalgia	Decrease in hearing	Secretions**	
85 positive NR	73	51	43	11	4
- 29 isolated immediate NR	24	13	15	4	4
- 38 dual late nasal NR	33	27	20	3	0
- 18 isolated late NR	16	11	8	4	0
69 negative NR	28	9	5	0	1

NR = Nasal response; * MEP = Middle ear pressure (the normally slightly negative MEP increased in negativity); ** Secretions = rapid increase in the middle ear effusions through the monolateral ventilation tube (in 3 patients). Reference: 27

Table 25a Survey of the patients with secretory otitis media (SOM).

SOM patients n=38			Skin test +	Skin test −	RAST +	RAST −
History - of nasal allergy -	suggestive	23	19	4	5	18
	unknown	15	12	3	1	14

Reference: 27b

Table 25b Middle ear response to the nasal allergen challenge (NPT) in patients with Secretory otitis media (SOM). Survey of the nasal (rhinomanometry) and ear (tympanometry) responses after the nasal challenge with allergens.

Patients n=38	Positive NR: Ear response +	Positive NR: Ear response −	Negative NR: Ear response +	Negative NR: Ear response −
31 patients with				
76 positive NR	65	11	–	–
21 negative NR	–	–	6	15
7 patients with				
12 negative NR	0	0	8 (in 5 patients)	4 (in 2 patients)
38 PBS control challenges	0	0	0	38

NR = Nasal response; PBS = Phosphate buffered saline.
Reference: 27b

Table 25c Middle car response to the nasal allergen challenge (NPT) in patients with Secretory otitis media (SOM). Survey of the otological complaints during the particular types of the nasal response (NR).

Patients n=38	n	Changes in MEP* accompanied by: Otalgia	Decrease in hearing	Secretions**	Otalgia only
76 positive NRs	61	56	35	13	4
21 isolated immediate (IINR)	19	18	6	4	2
24 isolated late (ILNR)	17	17	13	5	1
15 dual late (DLNR)	12	10	9	1	0
11 isolated delayed (IDYNR)	9	9	4	2	0
5 dual delayed (DDYNR)	4	2	3	1	1
33 negative NR (NNR)	13	6	2	1	1

NR = Nasal response; * MEP = middle ear pressure recorded by tympanometry; ** secretions = rapid increase in the middle ear effusions through the mono- or bilateral ventilation tube(s).
Reference: 27b

Table 26a Nasal and paranasal sinus responses after the nasal challenge with allergen

Patients n=78 / Nasal challenges n=193	Sinus response: maxillary	frontal	maxillary + frontal	negative
69 patients				
149 positive NR	121	3	14	11
15 negative NR	4*	1*	1*	9
9 patients				
29 negative NR	6*	0	2*	21
78 PBS controls	0	0	0	78

NR = Nasal response; SR = Sinus response; * = primary or "non-associated" form of the sinus response; the remaining responses are secondary or "associated" form of the sinus response. The agreement between positive NR and SR as well as negative NR and SR was statistically distinctly significant ($p<0.01$).
References: 14a-14e,41g

Table 26b Particular types of the nasal and paranasal sinus responses induced by the nasal challenge with allergen and their relationship (see also table 26a).

Nasal response n=193	Sinus response: maxillary (n=135)[a] ESR	LSR	DYSR	frontal (n=17)[b] ESR	LSR	DYSR
149 positive NR						
51 immediate/early	44	3	1	3	3	0
15 immediate + late	6	4	0	1	2	0
67 late	0	61	3	0	5	0
7 immediate+delayed	1	0	4	1	0	1
9 delayed	0	0	8	0	0	1
	maxillary (n=13)[c]			frontal (n=4)[d]		
44 negative NR	5*	7*	1*	3*	1*	0

NR = Nasal response; ESR = Early sinus response; LSR = Late sinus response; DYSR = Delayed sinus response; a: 135=121+14; b: 17=3+14; c: 13=4+6+1+2; d: 4=1+1+2 (see table 26a); *=primary or "non-associated" form of sinus response; the remaining responses are of the secondary or so-called "associated" form.
References : 14a-14e,41g

Table 26c The other organs' symptoms and general complaints accompanying the particular types of the paranasal sinus response (in %), induced by the nasal challenge with allergen(see also tables 26a and 26b).

Patients n=78	Sinus response to nasal challenge with allergen					
	maxillary sinuses			frontal sinuses		
Nasal challenges n=193	ESR n=56	LSR n=75	DYSR n=17	ESR n=8	LSR n=11	DYSR n=2
Nasal obstruction	92	91	94	62	91	100
Conjunctival injection or chemosis	7	13	12	0	9	0
Palpebral edema	2	5	6	12	9	50
Middle ear response (otalgia, hypacusia, changes in middle ear pressure)	13	15	12	0	9	0
Pressure in the sinuses	91	100	100	75	100	100
Bronchial complaints (mostly secondary bronchoconstriction, sometimes also wheezing and/or cough	9	11	6	0	0	0
Headache	2	12	12	75	91	100
Pharyngeal irritation	0	3	6	0	0	0
General malaise complaints	0	13	12	0	9	0

ESR = Early sinus response; LSR = Late sinus response; DYSR = Delayed sinus response.
References: 14a-14e,41g

Table 26d Radiographic and echographic changes recorded during particular types of the sinus response (=increased thickening of the mucosal membrane in the sinuses, increased opacification and/or decreased aeration) induced by the nasal challenge with allergen (see also tables 26a-26c).

	Changes on		
	radiographs only	echographs only	radiographs + echographs
Maxillary sinuses			
early SR (n=56)	3	1	52
late SR (n=75)	4	2	69
delayed SR (n=17)	1	0	16
Frontal sinuses			
early SR (n=8)	2	0	6
late SR (n=11)	0	1	10
delayed SR (n=2)	0	0	2
Total n=169 (=100 %)	10 (=6%)	4 (=2%)	155 (92%)

SR = Sinus response. The agreement between the radiographs and the echographs was highly significant both for the total comparison ($p<0.01$) and for the particular types of SR ($p<0.02$).
References: 14a-14e,41g

Table 27a The role of the nasal allergy in chronic sinusitis maxillaris. Survey of the radiographic (X-ray) and echographic changes in the maxillary sinuses after the nasal challenge with allergen.

Patients n=46	Changes	
	Radiographs	Echographs
37 patients		
- 37 positive NR	31	27
9 patients		
- 9 negative NR	3	2

Reference: 14d

Table 27b The role of the nasal allergy in chronic sinusitis maxillaris. Survey of the radiographic (X-ray) and echographic changes during the particular types of nasal response (NR).

Patients n=46	Changes	
	Radiographs	Echographs
Immediate NR (n=7)	6	4
Isolated late NR (n=14)	13	12
Dual late NR (n=13)	10	9
Isolated delayed NR (n=3)	2	2
Negative NR (n=9)	3	2

Reference: 14d

Table 28 Survey of radiographic and echographic changes in the maxillary sinuses (=increase in the thickening of the mucosal membrane) in 24 patients suffering from CMS (chronic maxillary sinusitis) after the nasal challenge with allergen.

Patients n=24	Changes on	
	Radiographs	Echographs
21 patients		
- 29 positive NR	26	22
- 4 negative NR	2	1
3 patients		
- 5 negative NR	1	0

NR = Nasal response. The agreement between the radiographs and the echographs was statistically significant ($p<0.05$).
References : 14f

Table 29 The review of the asthmatic responses (AR) induced by the nasal provocation tests (NPT) with allergens in 27 patients suffering from bronchial asthma with a low compliance to the usual "anti-asthmatic" treatment.

NPT (n=133)	Secondary induced asthmatic responses (AR)					
Nasal response (NR) positive (n=119)	Immediate (IAR)	Dual late (DLAR)	Late (LAR)	Dual delayed (DDYAR)	Delayed (DYAR)	Negative (NAR)
Immediate (INR) (n=28)	20	1	2	0	0	5
Dual late (DLNR) (n=19)	1	13	1	0	0	4
Late (LNR) (n=46)	0	0	36	0	0	10
Dual delayed (DDYNR) (n=9)	0	0	0	7	0	2
Delayed (DYNR) (n=17)	0	0	0	0	11	6
Negative (NNR)(n=14)	2	0	0	0	0	12
Total	23	14	39	7	11	39

Reference: 41h

F. ALLERGENS

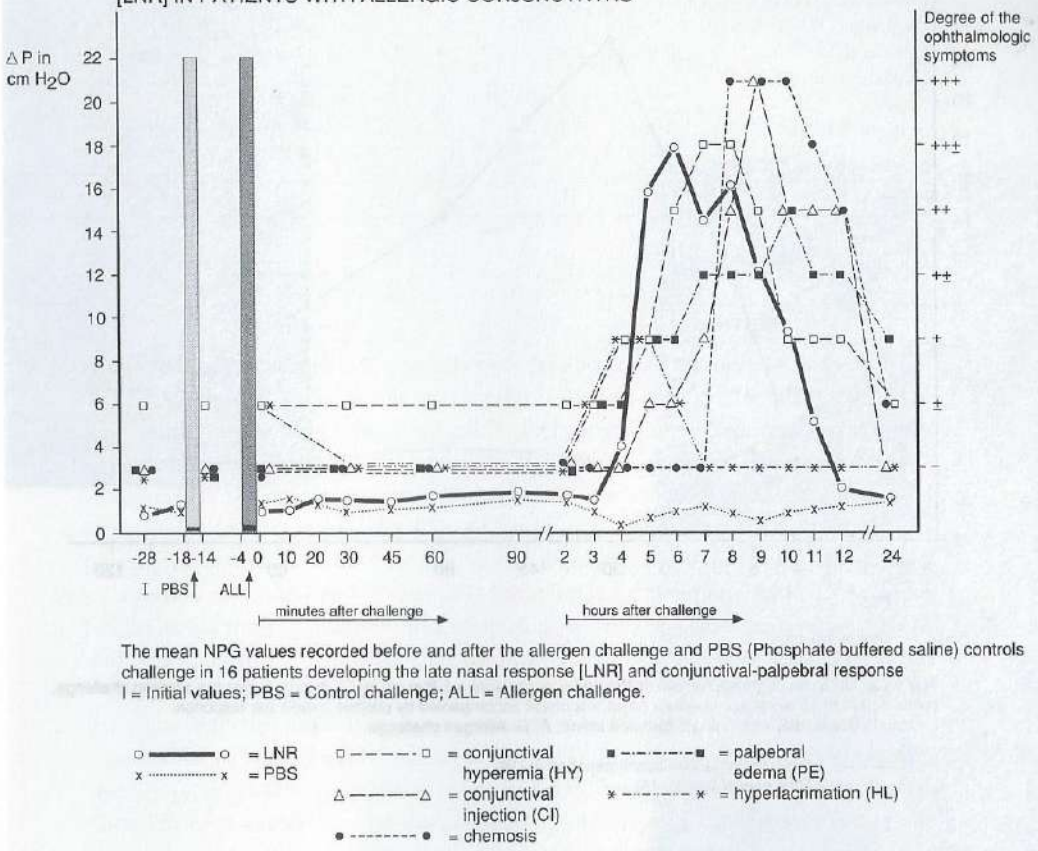
The LNR can be caused by various inhalant allergens. No differences in the occurrence of the LAR have been found by us with respect to the particular allergens.[41b] LNR was regularly recorded by us for a variety of allergens, such as house dust, mites (*Dermatophagoides pteronyssinus, farinae*), various animal danders such as cat, dog, horse, cow, pig, goat, sheep, rabbit, guinea pig, hamster, canary, parrot, turkey, goose, chicken, mouse, rat, parakeet, hen, pigeon,[7,12,14,41b] bird faeces (among others pigeon, parrot, canary and parakeet droppings), faeces of other animals, such as cow, pig and sheep,[15,16] wool,[14] old paper and cardboard antigens,[19,24] various kinds of moulds, e.g., *Aspergillus fumigatus etc.*,[7,12] various kinds of inhalant foods, e.g., flour kinds, (wheat, rye, oats, maize) [7,12,41b] cocoa powder, various spice powders,[41e] various grass-, tree-, flower-, weed pollen[7,12,25,41b] both in mixtures and as particular pollen species,[7,14,25, 41b,72b] such as timothy grass *(Phleum pratense)*, ryegrass *(Lolium perenne)*, sage *(Artemisia vulgaris)*, buckhorn plantain *(Plantago lanceolata)*, blue grass *(Poa pratensis)*, orchard grass *(Dactylis glomerata)*, ragweed short *(Ambrosia artemisiifolia)*, ragweed giant *(Ambrosia trifida)*, pine *(Pinus)*, poplar *(Populus)*, birch *(Betula pendula)*, and some drugs, e.g., *Acidum acetylsalicylicum* (Figures 7,28a, 28b,29,30,31).[7]

G. NASAL SECRETIONS (NS)

The nasal secretions (NS) are a very interesting and useful medium for supplementary investigations, from cytologic [1-3,11a-11e,11g,11h,13a-13c,18,20, 25,34, 40c-40f,41a-41d,41i,48a-48e,51a,51c,54-56,71,72,72a,-72d,73-82,82a,82i,83-85,85a,94] immunologic,[1-3,11f,11j,23,25a,34,41b, 41f,41i,44,46-48,49,51a,51c,52-56,72b,82a-82j,83-85,85a-85d,86-95,95a,95b,96,97,97a-97f,97i,97v-97y] as well as biochemical and biophysical [1,41b,41i,73,82a-82c,85,97g-97i] points of view.

The hypersensitivity reactions in the nasal mucosa, leading to the development of the particular types of the nasal response to allergen exposure (= challenge), are dynamic processes caused by the specific allergens, where various types of cells, mediators, com-

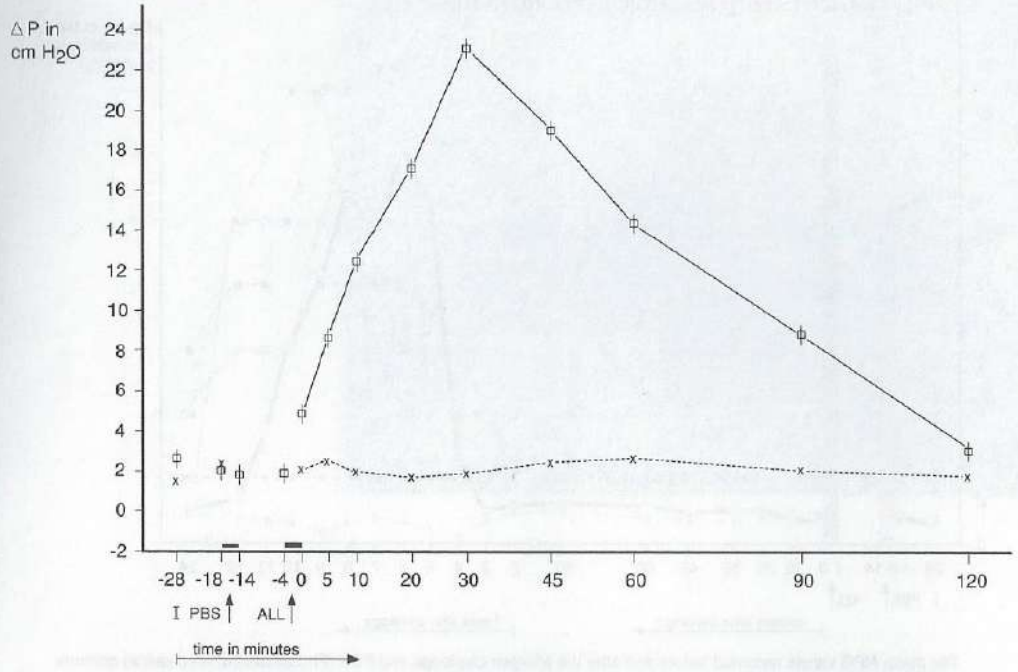
Fig. 23. THE LATE TYPE OF CONJUNCTIVAL-PALPEBRAL RESPONSE INDUCED BY THE LATE NASAL RESPONSE DUE TO THE ALLERGEN CHALLENGE [LNR] IN PATIENTS WITH ALLERGIC CONJUNCTIVITIS



The mean NPG values recorded before and after the allergen challenge and PBS (Phosphate buffered saline) controls challenge in 16 patients developing the late nasal response [LNR] and conjunctival-palpebral response
I = Initial values; PBS = Control challenge; ALL = Allergen challenge.

References: 17,21,41b,97w

Fig. 24a. THE IMMEDIATE (EARLY) NASAL RESPONSE TO ALLERGEN CHALLENGE (IINR) IN PATIENTS WITH SECRETORY OTITIS MEDIA (SOM)

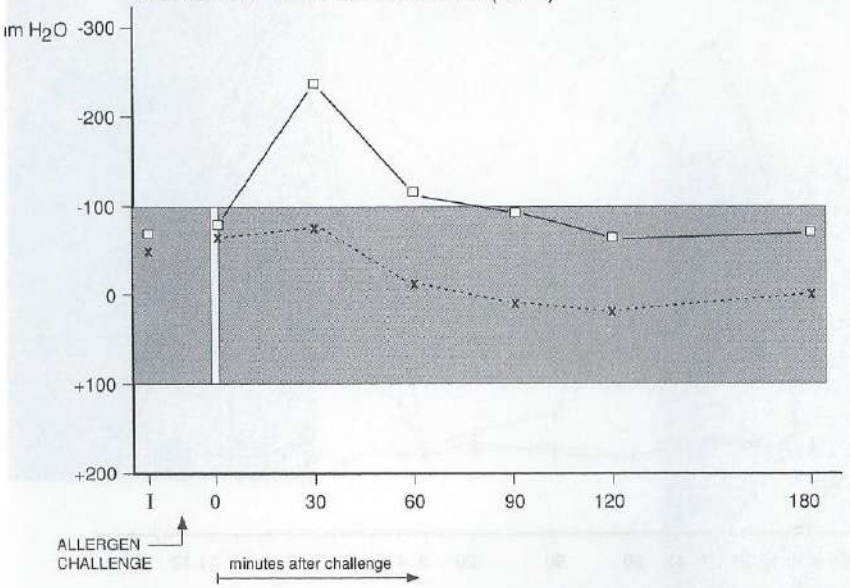


The mean NPG values (nasopharynx-nostril pressure gradient = △ P in cm H_2O) recorded after the allergen challenge, calculated from 19 Isolated immediate nasal responses accompanied by positive middle ear response.
I = Initial values; PBS = Phosphate buffered saline; ALL= Allergen challenge.

ϕ ——— ϕ = Isolated immediate nasal response (n=19)
x ····· x = PBS control test (n=19)

References: 22,26,27,27a,27b,41b

ig. 24b. THE MIDDLE EAR RESPONSE RECORDED DURING THE IMMEDIATE NASAL RESPONSE TO ALLERGEN CHALLENGE (IINR) IN PATIENTS WITH SECRETORY OTITIS MEDIA (SOM)

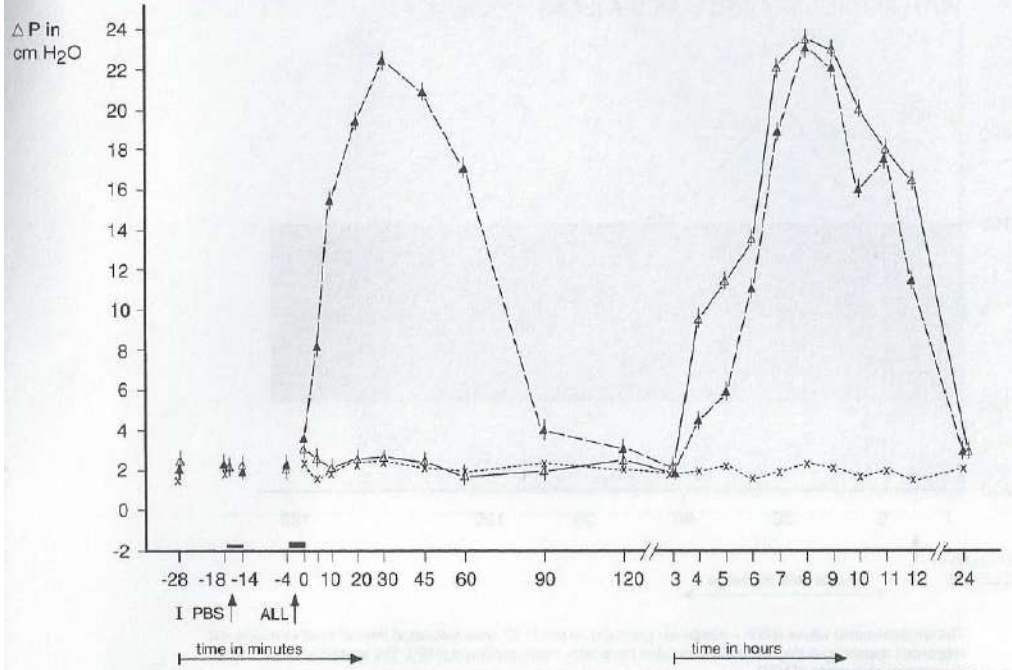


The tympanometric values (MEP = middle ear pressure), in mm H O, were calculated from all positive middle ear responses appearing during the positive Isolated immediate nasal responses (IINR). The spotted area represents the normal value range of MEP.

□ ——— □ = MEP values during the Isolated immediate nasal response (n=19)
x ····· x = MEP values during the PBS control test (n=19)

References: 22,26,27,27a,27b,41b

Fig. 25a. THE LATE NASAL RESPONSE TO ALLERGEN CHALLENGE (LNR) IN PATIENTS WITH SECRETORY OTITIS MEDIA (SOM)

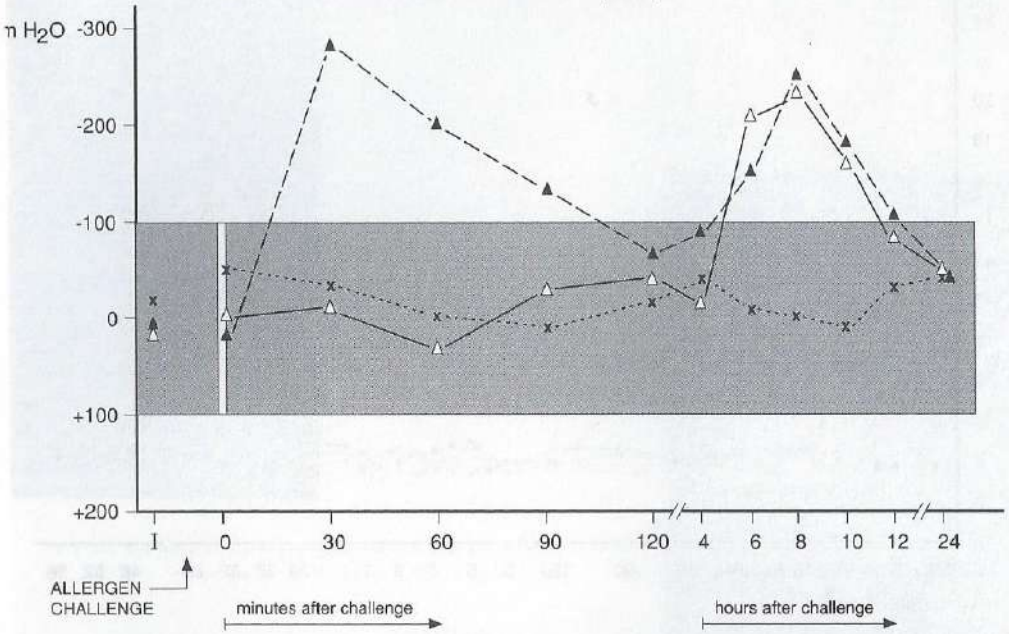


The mean NPG values (nasopharynx-nostril pressure gradient = Δ P in cm H_2O) recorded after the allergen challenge, calculated from 29 late nasal responses, of 2 sub-types, accompanied by positive ear response.
I = Initial values; PBS = Phosphate buffered saline; ALL= Allergen challenge.

Δ——Δ = Isolated late nasal response (n=17)
▲– – –▲ = Dual late nasal response (n=12)
x····· x = PBS control test (n=29)

References: 22.26.27.27a.27b.41b

g. 25b. THE MIDDLE EAR RESPONSE RECORDED DURING THE LATE NASAL RESPONSE TO ALLERGEN CHALLENGE (LNR)

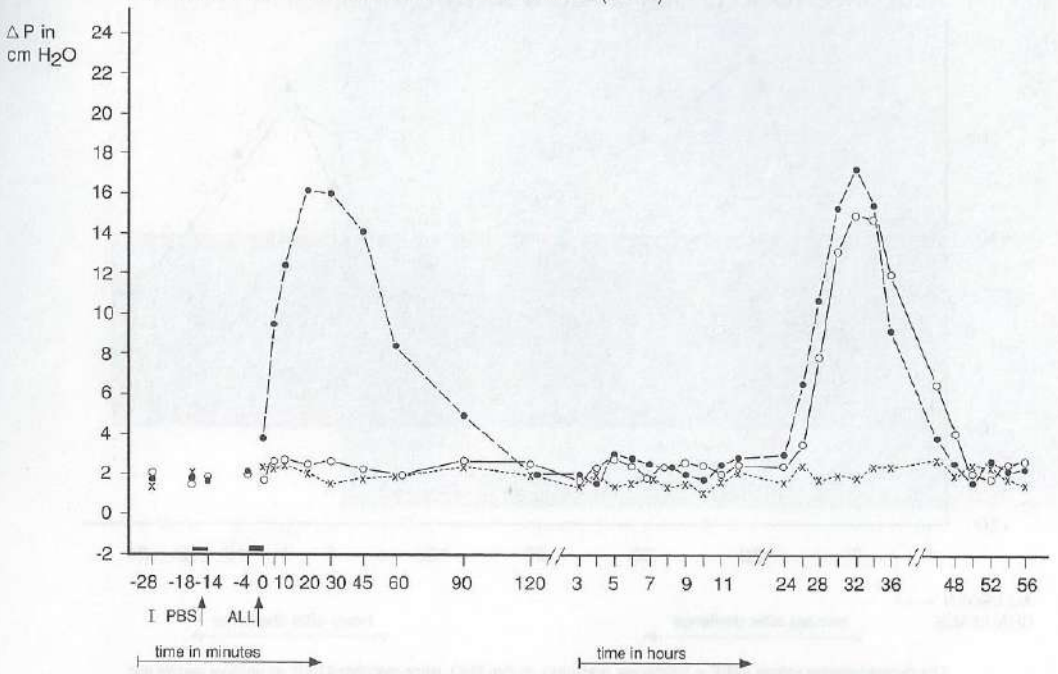


The tympanometric values (MEP = middle ear pressure), in mm H_2O, were calculated from all positive middle ear responses appearing during the both sub-types of the late nasal response, the Isolated late (ILNR) as well as dual late responses (DLNR) (=immediate + late). The spotted area represents the normal value range of MEP.

Δ——Δ = MEP values during the Isolated late nasal response (n=17)
▲– – –▲ = MEP values during the Dual late nasal response (n=12)
x····· x = MEP values during the PBS control test (n=29)

References: 22.26.27.27a.27b.41b

Fig. 26a. THE DELAYED NASAL RESPONSE TO ALLERGEN CHALLENGE (DNR) IN PATIENTS WITH SECRETORY OTITIS MEDIA (SOM)

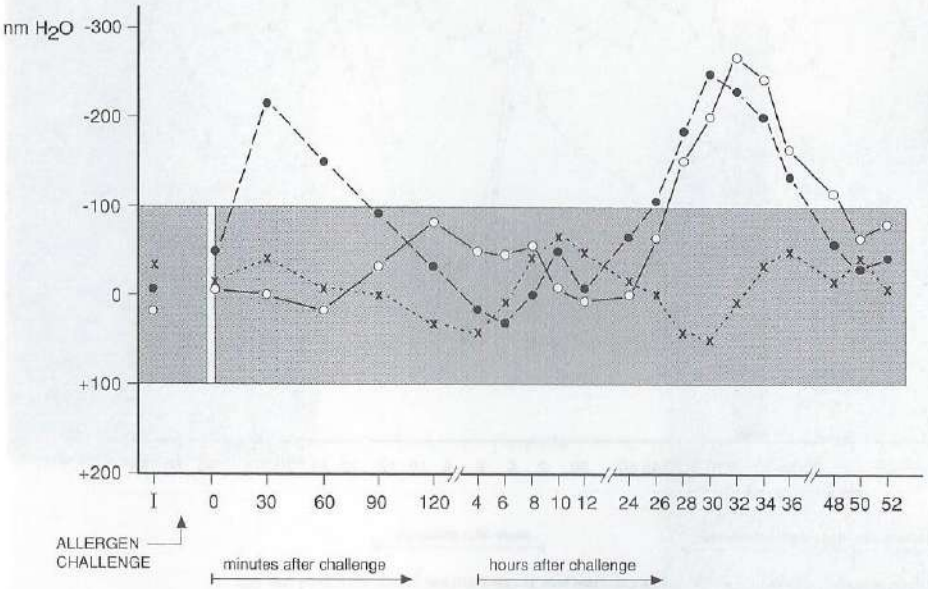


The mean NPG values (nasopharynx-nostril pressure gradient =Δ P in cm H_2O) recorded after allergen challenge, calculated from 13 delayed nasal responses, of 2 sub-types, accompanied by positive middle ear response.
I = Initial values; PBS = Phosphate buffered saline; ALL= Allergen challenge.

○———○ = Isolated delayed nasal response (n=9)
●– – – –● = Dual delayed nasal response (n=4)
x····· x = PBS control test (n=13)

References: 27,27a,27b

Fig. 26b. THE MIDDLE EAR RESPONSE RECORDED DURING THE DELAYED NASAL RESPONSE TO ALLERGEN CHALLENGE (DNR) IN PATIENTS WITH SECRETORY OTITIS MEDIA (SOM)

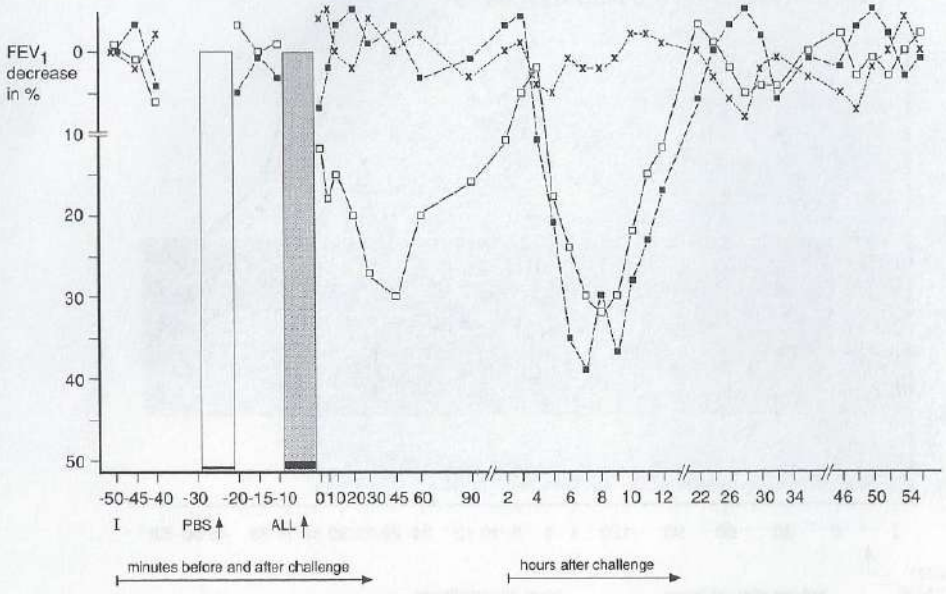


The tympanometric values (MEP = middle ear pressure), in mm H_2O, were calculated from all positive middle ear responses appearing during the both sub-types of the delayed nasal response, the Isolated delayed (IDNR) as well as dual delayed responses (DDNR) (=immediate + delayed). The spotted area represents the normal value range of MEP.

○———○ = MEP values during the Isolated delayed nasal response (n=9)
●– – – –● = MEP values during the Dual delayed nasal response (n=4)
x····· x = MEP values during the PBS control test (n=13)

References: 22,26,27,27a,27b,41b

Fig. 27. LATE AND DUAL LATE ASTHMATIC RESPONSES [LAR; DLAR] INDUCED BY THE ALLERGIC REACTION ORIGINATING PRIMARILY IN THE NASAL MUCOSA (n=39; 15)

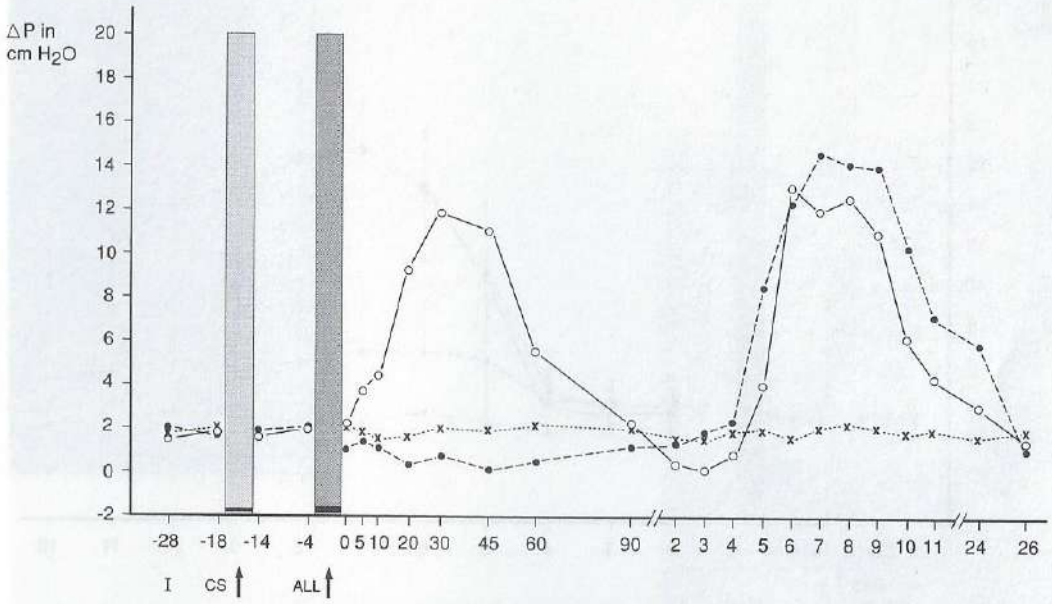


The mean percentage changes in the FEV_1 values calculated from 39 Late [LAR] and 15 Dual late [DLAR] asthmatic responses and 54 PBS control challenges
I = Initial values; ALL = Nasal challenge with allergen

■ ——— ■ = LAR (n=39)
□ — — — □ = DLAR (n=15)
x ····· x = PBS (n=54)

References: 12,40a,41,41b,41h,41i

Fig. 28a. DUAL (IMMEDIATE+LATE) [DLNR] AND ISOLATED LATE NASAL RESPONSE [ILNR] TO THE NASAL CHALLENGE WITH PIGEON DROPPING EXTRACT

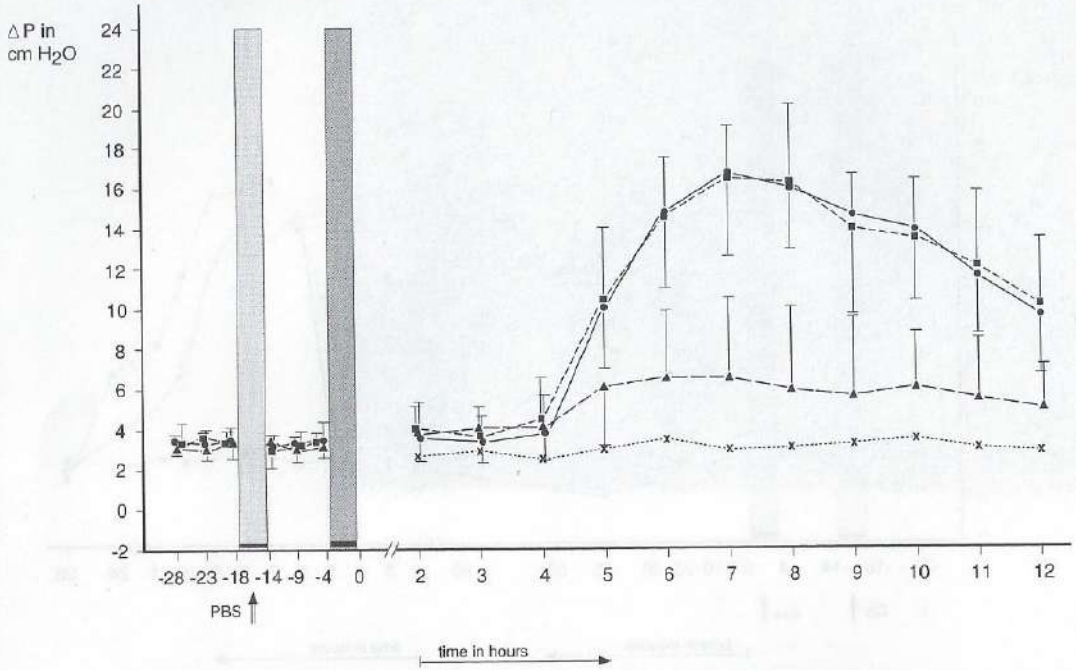


The mean NPG values recorded before and after the allergen challenge and the coca's solution control challenge in 25 patients developing both the types of the late nasal response.
I = Initial values; CS = Coca's solution; ALL = Allergen.

○ ——— ○ = Dual nasal response [DLNR] (n=19)
• ——— • = Isolated late nasal response [ILNR] (n=6)
x ····· x = Coca's solution control challenge [CS] (n=25)

References: 15,16,41b

Fig. 28b. EFFECTS OF BUDESONIDE ON THE LATE NASAL RESPONSE [LNR] TO CHALLENGE WITH BIRD FAECES EXTRACTS

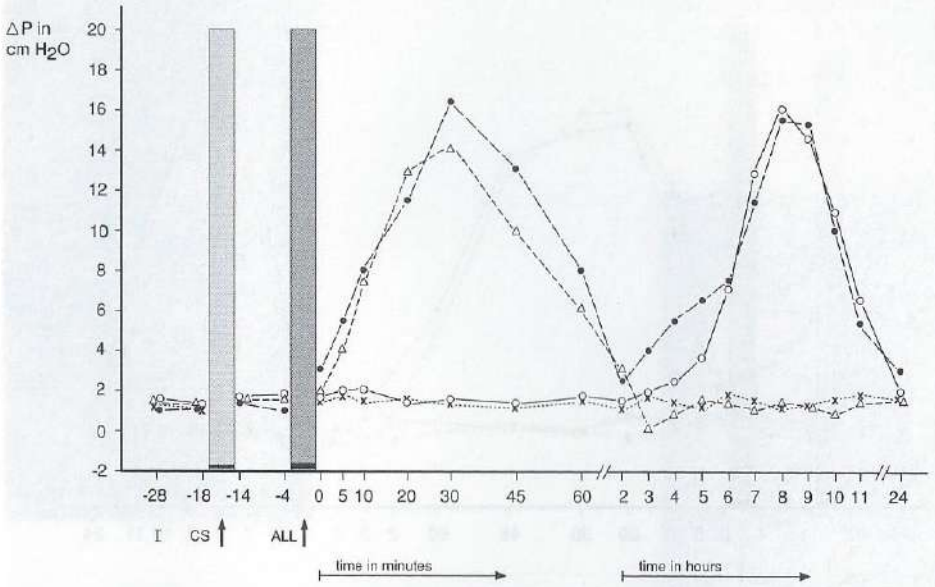


The mean NPG values and SD calculated from the non-pretreated nasal responses, nasal responses pretreated with BUD and PL, and PBS control challenges from 19 patients developing the LNR.
I = Initial values; PBS = Phosphate buffered saline; ALL = Allergen challenge; LNR = Late nasal response; BUD = Budesonide nasal spray; PL = Placebo nasal spray

- ●——● = Non-pretreated LNR (n=19)
- ▲– – –▲ = LNR pretreated with BUD (n=19)
- ■– – – –■ = LNR pretreated with Placebo (n=19)
- x ····· x = PBS control challenge (n=19)

References: 15,16,41b

Fig. 29. THE NASAL RESPONSES TO CHALLENGE WITH OLD PAPER EXTRACTS

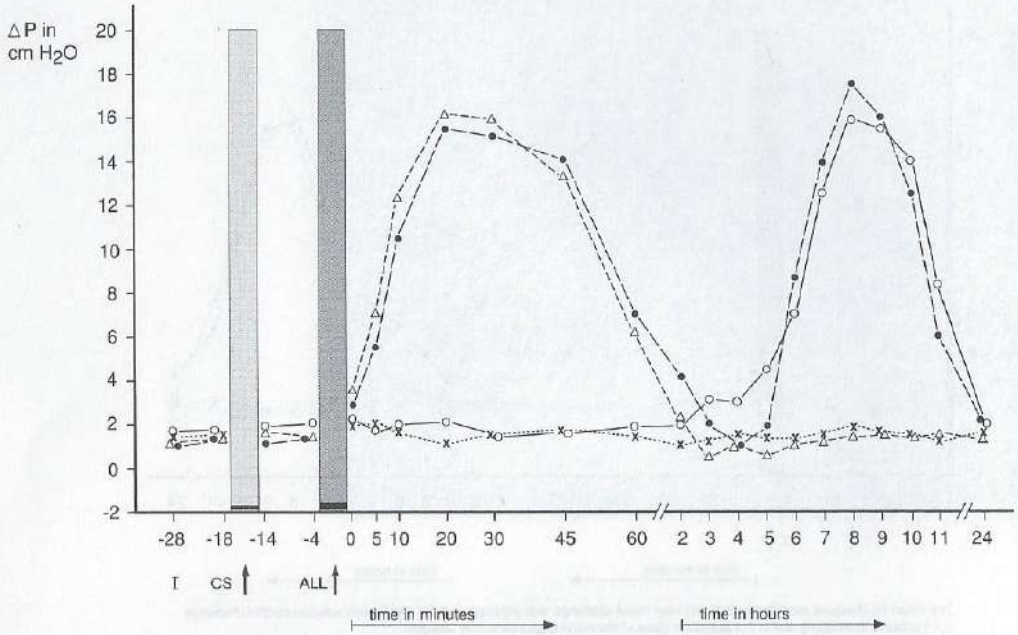


The mean NPG values recorded before and after nasal challenge with old paper extract and Coca's solution control challenge in 24 patients developing one of the particular types of the nasal response to this allergen.
I = Initial values; CS = Coca's solution; ALL = Allergen.

- △– – –△ = Isolated immediate nasal response [IINR] (n=5)
- ●——● = Dual late nasal response [DLNR] (n=8)
- ○——○ = Isolated late nasal response [ILNR] (n=11)
- x ····· x = Coca's solution control challenge [CS] (n=24)

References: 9,19,24

Fig. 30. THE NASAL RESPONSES TO CHALLENGE WITH CARDBOARD EXTRACTS

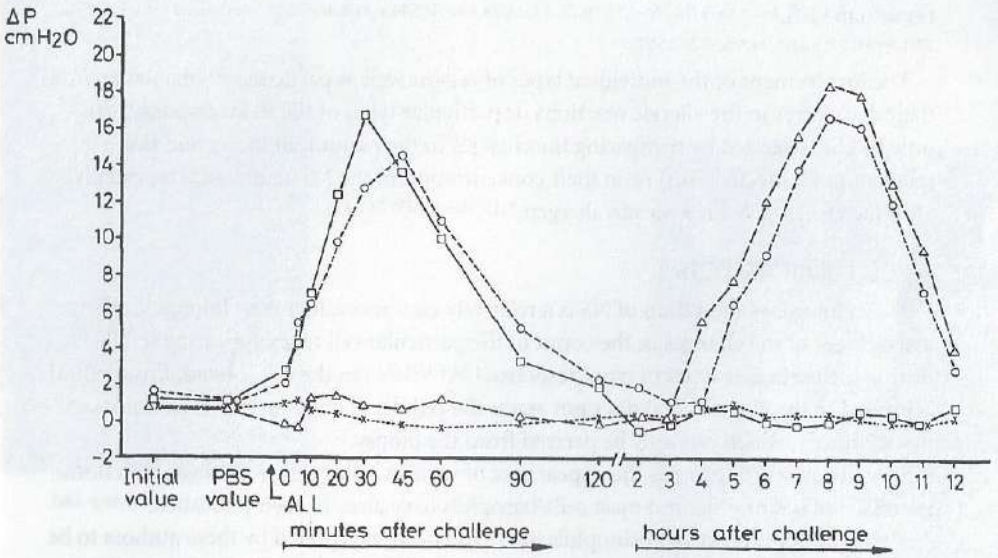


The mean NPG values recorded before and after nasal challenge with cardboard extract and Coca's solution control challenge in 27 patients reacting positively with the same type of nasal response.
I = Initial values; CS = Coca's solution; ALL = Allergen.

Δ-------Δ = Isolated immediate nasal response [IINR] (n=9)
•——— • = Dual late nasal response [DLNR] (n=4)
o——— o = Isolated late nasal response [ILNR] (n=14)
x·····x = Coca's solution control challenge [CS] (n=27)

References: 9,24

Fig. 31. TYPES OF NASAL RESPONSE TO CHALLENGE WITH FLOUR KINDS (WHEAT, OATS, RYE, CORN-MAIZE,BARLEY



The mean NPG values recorded after allergen challenge with respect to the appropriate "Phosphate Buffered Saline" NPG values, were calculated from all positively reacting patients with the same type of response.

□——□ = Isolated immediate nasal response (n= 7)
Δ——Δ = Isolated late nasal response (n=11)
o-----o = Dual late nasal response (n=21)
x·····x = Control challenge with PBS (n=39)

References: 7,12,41b

pounds and mechanisms may be involved in various steps and at various levels.[1,2,4, 11a-11h,13b,13c,41b-41d,41i,71,72,72a-72d] The non-specific hyperreactivity reactions in the nasal mucosa, caused by the non-specific agents, may also be considered to be dynamic processes upon involvement of the various cell types, receptors, compounds and mechanisms. [13a-13c,13e,13f]

The hypersensitivity reactions are also exfoliative processes, leading to the influx and release of various cell types, as well as various factors, compounds and chemical derivates into the NS.[1-3,11a-11h,13a-13c,18,20,23,25,34,40c-40f,41a-41d,48a-48d,51a-51c,53-56,71,72,72a-72d,79,80,82,83,85,94,96,97,97a-97p]

The involvement of the individual types of cells as well as particular mediators and/or their derivatives in the allergic reactions or particular types of the nasal response, can only be characterized by comparing the changes in their count, in their condition (activation vs. inactivation) or in their concentration in the NS before and, repeatedly, after the challenge with a certain allergen.[3,18,20,25,41b,71,72]

1. CELLULAR ASPECTS

The cytologic examination of NS is a relatively easy and valuable technique for the assessement of the changes in the count of the particular cell types appearing in NS during the particular types of nasal response.[13a,18,71,72] On the other hand, this method is limited to the NS only and does not assess the cellular changes in the nasal mucosa tissue directly, which can only be derived from the biopsy. [71,72,96,97,97a]

Several papers concerning the appearance of various cell types in the nasal secretions, especially of eosinophils and mast cells/basophils have already been published.[48,48a-48d, 74-80,82] The appearance of eosinophils in NS has been interpreted by these authors to be an indicator of nasal allergy. However, this presumption has been derived from a single sample of the patient's NS and, in most cases, it has not been directly related to a certain allergen.[48b,48d,76,78-80]

Other authors have suggested that the appearance of mast cells/basophils in NS should be considered to be a diagnostic parameter for the nasal allergy.[48b-48d,75,77,79, 82]

Papers concerning the appearance of other cell types in NS are not numerous.[80,81] There is a dearth of detailed knowledge concerning the appearance and exact role of individual cell types in NS during the individual hypersensitivity reactions in the nasal mucosa and especially during the particular types of nasal response to allergen.[2,71,72]

Cytologic examination of the NS during the particular types of the nasal response to allergen challenge (INR, LNR, DYNR) has been extensively studied by us (Tables 30-34, Figures 32-35).[2,11a-11e,11g,13a-13c,18,20,25,26,37,38,40,40c-40f,41,41a-41d,71,72,72a-72d,97,97a]

The cellular changes in NS during the LNR have been studied by us for the first time (Figure 7, 32b).[2,25,72]

The positive LNR has been accompanied by significant changes in the count of neutrophils in 84% of the cases (increase immediately before, and decrease during the appearance of LNR, followed by increase again during the resolving of LNR), eosinophils in 58% (increase immediately before, and decrease during appearance of LNR), epithelial cells in 73% (increase followed by decrease, running parallel with the clinical course of the LNR), goblet cells in 63% of the cases (increase followed by decrease),

basophils in 8% and lymphocytes in 6% (both the cell types demonstrated a slight increase in their count during the LNR), in the NS. No significant changes in the counts of other types of cells in the NS (monocytes, plasma cells, mast cells) have been recorded during most of the LNR cases [Table 31, Figure 32b, Plates 18-I,18-II (page 365)].

No significant changes in the count of any cell type in the NS have been recorded either during the PBS control challenge in any patient or during most of the negative responses.

We have concluded that the clinical LNR may be associated with changes in the count of neutrophils, eosinophils, epithelial cells and goblet cells, indicating their possible dynamic involvement in the mechanisms of this type of response. Moreover, the results of our studies have distinctly demonstrated that only a repeated examination of the NS samples before and after an allergen challenge can be accepted as a supplementary diagnostic and research parameter, rather than an examination of a single NS sample.[18, 20,25,71,72]

The cytologic changes in the NS, recorded during the LNR, are clearly different from those found by us during both the immediate nasal response [INR] (Tables 30a,30b; Figure 33b) [2,3,11a-11e,11g,40e,41a,41i,71,72a,72c,72d,82,83] and the delayed nasal response [DYNR] (Tables 32a,32b; Figure 34b).[13a-13c,41i,72a]

These results and conclusions may be supported by another aspect. The cells appearing in the NS before the allergen challenge, during the PBS control and during the negative nasal response, have been mostly intact, whereas those migrating and/or being expelled in the NS during the positive LNR and shortly after its resolving, demonstrated distinct intracellular changes, including changed cytoplasmic granules (degranulation) (Tables 33a, 33b).[11g,11h,40c,40d,40f,41d,41i,72,72c]

Besides the changes in the count of individual cell types in NS during the particular types of nasal response, we have also studied the condition, especially intracellular changes and cellular membranes, of the particular cell types (e.g. eosinophils, neutrophils, basophils, mast cells), appearing in NS during the immediate (INR)[11a,11b,11g,72b, 72d] late (LNR)[11h,40f,41d,72b] and delayed (DYNR) nasal response (Tables 33,34).[13a,13c] Moreover we have also investigated the effects of several drugs not only on the particular nasal response types (Figures 32-34),[10,11,12,13,13b,14,17,21,26,38,40, 40a,41i,72a,97w] but also on the accompanying changes in the cellular counts in the NS,[11d,11e,13b,40c-40f,41i,72a,72d] and on the intracellular changes displayed by the individual cell types (Tables 35-37, Figures 32-34).[13b,40f,72d]

In most of the cells appearing in NS during the positive LNR, various cellular, intracellular and other changes, such as degranulation, disappearance of the cytoplasmic granules, vacuolization, diminished intake of the stain, wrinkling of the cellular membrane, sometimes cellular disruption, have been recorded.[40f,41d,72c] The neutrophils (NE) degranulated during 94% of the positive LNR cases, eosinophils (EO) in 49% and basophils (BA) in 3%, while during the negative nasal response, the NE degranulated in 7% of the cases, EO in 7% and BA in 0% (Tables 33a, 33b).[40f,41d,72c]

In contrast, during the INR, degranulation of EO has been found in 63%, of NE in 74% and of BA in 16% of the cases [Tables 33, 33b, Plates 19-I,II,III,IV,V,VI (pages 366, 367)],[11a,11b,11h,72c,72d] whereas the DYNR has been accompanied by degranulation of NE

in 74% and intracellular changes of lymphocytes in 82% and of monocytes in 61% of the cases (Table 34).[13c,72a]

Bascom and colleagues [82] and Togias and co-workers [83], using the nasal lavage technique, have observed a significant increase in the count of what they called "alcian-blue-stained positive cells", most probably basophils, but also of eosinophils and neutrophils, a slight increase in the mononuclear cells, and a decrease in the count of the epithelial cells in NS during the "late nasal response" to allergen challenge. Walden and colleagues [94] have recorded increases in total cell counts, particularly of eosinophils, neutrophils and "alcian blue cells" (being probably basophils), in the NS during the LNR, correlating with increased release of some mediators (kinins, TAME-esterase activity, histamine, sulfidopeptide leukotrienes) and albumin into the nasal secretions. Iliopoulos et al[51c] have also observed an increased count of eosinophils and neutrophils in NS during LNR. However, they have found the significant increase only in the eosinophil count. The significant differences in the cell influx into the NS accompanying the LNR and those recorded during the INR, described by these authors[51c,82,83,94] are generally consistent with our results.[20,25,41c,41d,71,72,72a,72c]

Table 30a Presence of individual cell types in the nasal secretions and changes in their count during the Immediate nasal response [INR] in %.

INR (n=117) NNR (n= 83) PBS (n=200)	Presence of the cells			Changes in the cell count between before and after the challenge		
	INR	NNR	PBS	INR	NNR	PBS
Eosinophils	85	19	48	68*	5	3
Neutrophils	71	17	40	47*	3	0
Basophils	16	91	31	13*	0	0
Epithelial cells	68	23	25	9	7	4
Goblet cells	57	13	11	16*	4	2
Lymphocytes	11	4	7	2	3	0
Mast cells	4	2	3	0	0	0
Plasma cells	7	2	3	0	0	0
Monocytes	1	0	0	0	0	0

INR = Immediate nasal response; NNR = Negative nasal response; PBS = Phosphate buffered saline (=control); *= statistically significant changes ($p<0.05$). References : 11c,71,72a,97a

Table 30b Statistically significant magnitude of changes in the count of individual cell types in NS ($p < 0.05$).

Cell type	Mean ± SE
Eosinophils	7 (7.17 ± 0.91)
Basophils	2 (2.26 ± 0.71)
Mast cells	1 (1.21 ± 0.49)
Neutrophils	8 (8.33 ± 0.56)
Lymphocytes	2 (2.00 ± 0.25)
Monocytes	1 (1.26 ± 0.21)
Plasma cells	1 (1.27 ± 0.21)
Epithelial cells	5 (5.00 ± 0.63)
Goblet cells	4 (4.15 ± 0.65)

NS = Nasal secretions. References: 11c, 71, 72

Table 31 Presence of particular cell types in the nasal secretions and changes in their count during the late nasal response [LNR] in %.

LNR(n=104) NNR(n= 83) PBS(n=187)	Presence of the cells			Changes in the cell count between before and after the challenge		
	LNR	NNR	PBS	LNR	NNR	PBS
Eosinophils	61	19	49	58*	5	1
Neutrophils	96	17	45	84*	3	2
Basophils	15	9	10	8*	0	0
Epithelial cells	100	23	41	73*	4	1
Goblet cells	82	13	35	63*	3	0
Lymphocytes	18	4	9	6*	0	0
Mast cells	3	2	1	0	0	0
Plasma cells	4	2	1	0	0	0
Monocytes	1	0	0	0	0	0

LNR = Late nasal response; NNR = Negative nasal response; PBS = Phosphate buffered saline (=control); * = statistically significant changes ($0<0.05$).
References : 11c,11h,25,41b,41c,72,72a,97

Table 32a Presence of particular cell types in the nasal secretions and changes in their count during the delayed nasal response [DYNR] in %.

DYNR (n=83) NNR (n=71) PBS (n=83)	Presence of the cells			Changes in the cell count between before and after the challenge		
	DYNR	NNR	PBS	DYNR	NNR	PBS
Eosinophils	26	10	12	12	3	1
Neutrophils	59	13	11	53*	7	2
Basophils	2	1	0	1	0	0
Epithelial cells	73	14	10	37*	4	2
Goblet cells	60	11	7	18	0	0
Lymphocytes	86	13	14	77*	8	4
Mast cells	2	1	1	0	0	0
Plasma cells	10	4	2	0	0	0
Monocytes	16	3	1	6	1	0

DYNR = Delayed nasal response; NNR = Negative nasal response; PBS= Phosphate buffered saline (=control); * = statistically significant changes($p<0.05$). References : 13a-13c,72a

Table 32b The changes in the count of particular cell types in the nasal secretions (NS) accompanying the positive delayed nasal response to allergen challenge (DYNR).

Neutrophils	in 50% (increase-decrease-increase)*
Eosinophils	in 14% (slight increase-decrease)
Lymphocytes	in 71% (increase-decrease-increase)**
Epithelial cells	in 31% (increase after resolving of DYNR)
Goblet cells	in 5% (increase after resolving of DYNR)
Monocytes	in 11% (increase-decrease before the DYNR)
Plasma cells	in 6% (increase-before the onset of the DYNR)
Basophils	in 0% (no pattern)
Mast cells	in 0% (no pattern)

* Running almost parallel with the course of the DYNR. ** The lymphocyte count increased immediately before the onset of the DYNR, decreased gradually during the response and increased again after the resolving of the DYNR.
References: 13a-13c, 72a

Table 33a Review of the cellular and intracellular changes recorded in the individual cell types in nasal scretions (NS) before and during particular types of nasal response (in % of responses).

	INR (n=117)		LNR (n=38)		NNR (n=82)		PBS (n=154)	
	Before	During	Before	During	Before	During	Before	During
Eosinophils	4	81*	13	65*	0	6	0	3
Basophils	0	16*	5	11*	0	0	0	0
Mast cells	0	9+	0	0	0	0	0	0
Neutrophils	0	49*	7	87*	0	4	0	0

Cellular and intracellular changes: - disappearance of cytoplasmatic granules; - vacuolization; wrinkling of cellular membrane; diminished stain intake; cellular disruption. * = $p < 0.05$; + = $p = 0.05$. NS = Nasal secretions; Nasal response: INR = immediate, LNR = late, NNR = negative, PBS = Phosphate buffered saline control challenge.
References: 11a, 11b, 11g, 41d, 72c

Table 33b Cellular and intracellular changes recorded in the individual cell types in NS before and during a particular type of the nasal response (in % of responses).

	INR (n=117)		LNR (n=38)		NNR (n=82)		PBS (n=154)	
	Before	During	Before	During	Before	During	Before	During
Eosinophils	4	81	13	65	0	6	0	3
- disappearance of cytoplasmic granules	4	63	5	49	0	6	0	3
- vacuolization	2	61	5	65	0	4	0	0
- wrinkling of cellular membrane	2	71	2	65	0	4	0	1
- diminished stain intake	0	26	1	49	0	0	0	0
- cellular disruption	0	77	0	53	0	1	0	0
Basophils	0	16	5	11	0	0	0	0
- disappearance of cytoplasmic granules	0	16	3	3	0	0	0	0
- vacuolization	0	16	1	5	0	0	0	0
- wrinkling of cellular membrane	0	13	0	3	0	0	0	1
- diminished stain intake	0	5	0	2	0	0	1	0
- cellular disruption	0	10	1	3	0	1	0	0
Mast cells	0	9	0	0	0	0	0	0
- disappearance of cytoplasmic granules	0	9	0	0	0	0	0	0
- vacuolization	0	9	0	0	0	0	0	0
- wrinkling of cellular membrane	0	8	0	0	0	0	0	1
- diminished stain intake	0	2	0	0	0	1	0	0
- cellular disruption	0	6	0	0	0	0	0	0
Neutrophils	0	49	7	87	0	4	0	1
- disappearance of cytoplasmic granules	0	49	7	87	0	4	0	1
- vacuolization	0	22	4	75	0	2	0	1
- wrinkling of cellular membrane	0	30	3	78	0	1	0	0
- diminished stain intake	0	15	0	57	0	0	0	0
- cellular disruption	0	12	2	69	0	0	0	0

NS = Nasal secretions; Nasal response: INR = immediate, LNR = late, NNR = negative, PBS = control challenge.
References: 11a, 11b, 11g, 41d, 72c

Table 34a Cellular and intracellular changes in the particular cell types recorded during the delayed nasal response to allergen challenge [DYNR], negative nasal response [NNR] and phosphate buffered saline control challenges [PBS] (in % of responses).

Patients n=23	DYNR (n=23)		NNR (n=20)		PBS (n=23)	
	Before	During	Before	During	Before	During
Eosinophils	4	7	10	0	4	4
Neutrophils	4	78*	5	10	0	4
Basophils	0	0	0	0	0	4
Mast cells	4	0	0	5	0	0
Lymphocytes	10	83*	5	15	0	10
Monocytes	0	61+	0	0	0	4

NS = Nasal secretions; statistical significance of the cellular changes: * = $p < 0.05$; + = $p = 0.05$. References: 13c, 72a

Table 34b Cellular and intracellular changes recorded in the particular cell types in the nasal secretions (NS) during the delayed nasal response to allergen challenge [DYNR], negative nasal response [NNR] and control challenge with phosphate buffered saline [PBS] (in % of responses).

Patients n=23	DYNR (n=23)		NNR (n=20)		PBS (n=23)	
	Before	During	Before	During	Before	During
Neutrophils	4	78*	5	10	0	4
- disappearance of cytoplasmic granules	0	74*	0	0	0	0
- vacuolization	0	60+	0	5	0	0
- wrinkling of cellular membrane	0	65+	0	0	0	0
- diminished stain intake	4	70*	5	5	0	4
- cellular disruption	0	4	0	0	0	0
Lymphocytes	8	82*	5	15	0	4
- vacuolization	4	78*	0	5	0	0
- wrinkling of cellular membrane	0	78*	5	5	0	4
- diminished stain intake	4	82*	0	5	0	0
- diminished compactness of the nucleus	0	70*	0	0	0	0
- wrinkling of the nucleus	0	60+	0	0	0	0
- cellular disruption	0	4	0	0	0	0
Monocytes	0	61+	0	0	0	4
- increase in the size	0	39+	0	0	0	4
- vacuolization	0	48+	0	0	0	0
- wrinkling of cellular membrane	0	48+	0	0	0	0
- diminished stain intake	0	56*	0	0	0	0
- wrinkling of the nucleus	0	35	0	0	0	0
- cellular disruption	0	0	0	0	0	0

Statistical significance of the changes: * = $p < 0.05$; + = $p = 0.05$
References: 13c, 72a

Table 35a Presence of the particular cell types in the nasal secretions (NS) during the non-pretreated INRs and INRs pretreated with intranasal DSCG and BSA/BDA (in % of cases).

INR n=18	non-pretreated INRs	INRs pretreated with	
		DSCG	BSA/BDA
Eosinophils	89	22*	72
Neutrophils	72	39*	61
Basophils	17	0*	11
Mast cells	6	0	6
Epithelial cells	67	17*	72
Goblet cells	61	11*	49
Lymphocytes	11	10	5*
Plasma cells	0	0	0
Monocytes	0	0	0

* The count of particular cell types, which was significantly lower than that recorded during the non-pretreated INRs ($p < 0.05$). INR = Immediate nasal response; DSCG = Disodium cromoglycate (Cromolyn); BSA = Budesonide; BDA = Beclomethasone dipropionate.
References: 11d, 11e, 72a, 72d

Table 35b Changes in the count of particular cell types in the nasal secretions during the non-pretreated INRs and INRs pretreated with intranasal DSCG and BSA/BDA, with respect to the pre-challenge count (in % of cases).

INR n=18	non-pretreated INRs	INRs pretreated with	
		DSCG	BSA/BDA
Eosinophils	67*	11	61*
Neutrophils	55*	6	44*
Basophils	17*	0	11*
Mast cells	0	0	0
Epithelial cells	28+	0	22+
Goblet cells	33*	6	22+
Lymphocytes	0	0	0
Plasma cells	0	0	0
Monocytes	0	0	0

* statistically significant ($p < 0.05$); + = statistical borderline ($p = 0.05$). INR = Immediate nasal response; DSCG = Disodium cromoglycate (Cromolyn); BSA = Budesonide; BDA = Beclomethasone dipropionate.
References: 11d, 11e, 72a, 72d

Table 35c Degranulation and other intracellular changes recorded in the individual cell types in the nasal secretions (NS) during the non-pretreated INRs and the INRs pretreated with intranasal DSCG and BSA/BDA (in % of cases).

INR n=18	Non-pretreated INR		INR pretreated with			
			DSCG		BSA/BDA	
	D	ICH	D	ICH	D	ICH
Eosinophils	78*	67*	11	6	45*	39*
Neutrophils	67*	28	39*	17	6	0
Basophils	17*	11*	0	0	11*	6+
Mast cells	6	6	0	0	0	0

INR = Immediate nasal response; D = Degranulation; ICH = Intracellular changes (vacuolization, diminished stain intake, wrinkling of cellular membrane); DSCG = Disodium cromoglycate; BSA = Budesonide; BDA = Beclomethasone dipropionate.
Changes recorded after the allergen challenge as compared with the pre-challenge changes: * statistically significant ($p < 0.05$), + statistical borderline ($p = 0.05$)
References: 72a, 72d

Table 36a Presence of individual cell types in the nasal secretions (NS) during the non-pretreated LNRs and LNRs pretreated with intranasal DSCG and BSA/BDA (in % of cases).

LNR n=16	non-pretreated LNRs	LNRs pretreated with	
		DSCG	BSA/BDA
Eosinophils	69	19*	25*
Neutrophils	94	38*	31*
Basophils	19	0*	13
Mast cells	6	0	6
Epithelial cells	100	31*	37*
Goblet cells	81	6*	13*
Lymphocytes	13	0	0
Plasma cells	0	0	0
Monocytes	0	0	0

*The count of particular cell types, which was significantly lower than that recorded during the non-pretreated LNRs ($p < 0.05$)

LNR = Late nasal response; DSCG = Disodium cromoglycate (Cromolyn); BSA = Budesonide; BDA = Beclomethasone dipropionate.
References: 11c, 40d, 40c, 40f, 72a, 97

Table 36b Changes in the count of particular cell types in the nasal secretions during the non-pretreated LNRs and LNRs pretreated with intranasal DSCG and BSA/BDA, with respect to the pre-challenge count (in % of cases).

LNR n=16	non-pretreated LNRs	LNRs pretreated with	
		DSCG	BSA/BDA
Eosinophils	50*	6	19
Neutrophils	75*	19	0
Basophils	13*	0	6
Mast cells	0	0	0
Epithelial cells	88*	25	31+
Goblet cells	69*	6	13
Lymphocytes	0	0	0
Plasma cells	0	0	0
Monocytes	0	0	0

Changes: *statistically significant ($p < 0.05$); + = statistical borderline ($p = 0.05$).
LNR = Late nasal response; DSCG = Disodium cromoglycate (Cromolyn); BSA = Budesonide; BDA = Beclomethasone dipropionate.
References: 11c, 40d, 40c, 40f, 72a, 97

Table 36c Degranulation and other intracellular changes recorded in the individual cell types in the nasal secretions (NS) during the non-pretreated LNRs and the LNRs pretreated with DSCG and BSA/BDA (in % of cases).

LNR N=16	Non-pretreated LNR		LNR pretreated with			
			DSCG		BSA/BDA	
	D	ICH	D	ICH	D	ICH
Eosinophils	56*	50*	6	6	25+	25+
Neutrophils	75*	63*	38+	31+	13	6
Basophils	13	6*	0	0	6+	6
Mast cells	6*	6	0	0	6+	6

LNR = Late nasal response; D = Degranulation; ICH = Intracellular changes (vacuolization, diminished stain intake, wrinkling of cellular membrane); DSCG = Disodium cromoglycate; BSA = Budesonide; BDA = Beclomethasone dipropionate.
Changes recorded after the allergen challenge as compared with the pre-challenge changes: * statistically significant ($p < 0.05$), + statistical borderline ($p = 0.05$)
Reference: 40f

Table 37a Presence of individual cell types in the nasal secretions (NS) during the non-pretreated DYNRs and DYNRs pretreated with intranasal DSCG and BSA/BDA (in % of cases).

DYNR n=12	non-pretreated DYNRs	DYNRs pretreated with	
		DSCG	BSA/BDA
Eosinophils	25	17	25
Neutrophils	66	75	25*
Basophils	8	17	8
Mast cells	8	0	8
Epithelial cells	75	75	17*
Goblet cells	33	42	8*
Lymphocytes	100	91	8*
Plasma cells	0	0	0
Monocytes	8	0	0

DYNR = Delayed nasal response; DSCG = Disodium cromoglycate (Cromolyn); BSA = Budesonide; BDA = Beclomethasone dipropionate. * Statistically significant differences ($p < 0.05$), with respect to the non-pretreated DYNRs.
References: 13a-13c, 72a

Table 37b Changes in the count of particular cell types in the nasal secretions (NS) compared before and after allergen, during the non-pretreated DYNRs and DYNRs pretreated with intranasal DSCG and BSA/BDA (in % of cases).

DYNR n =12	non-pretreated DYNRs	DYNRs pretreated with	
		DSCG	BSA/BDA
Eosinophils	17+	8+	8
Neutrophils	42*	50*	17
Basophils	0	0	0
Mast cells	0	0	0
Epithelial cells	42*	42*	17
Goblet cells	17*	25*	0
Lymphocytes	92*	100*	25
Plasma cells	0	0	0
Monocytes	0	0	0

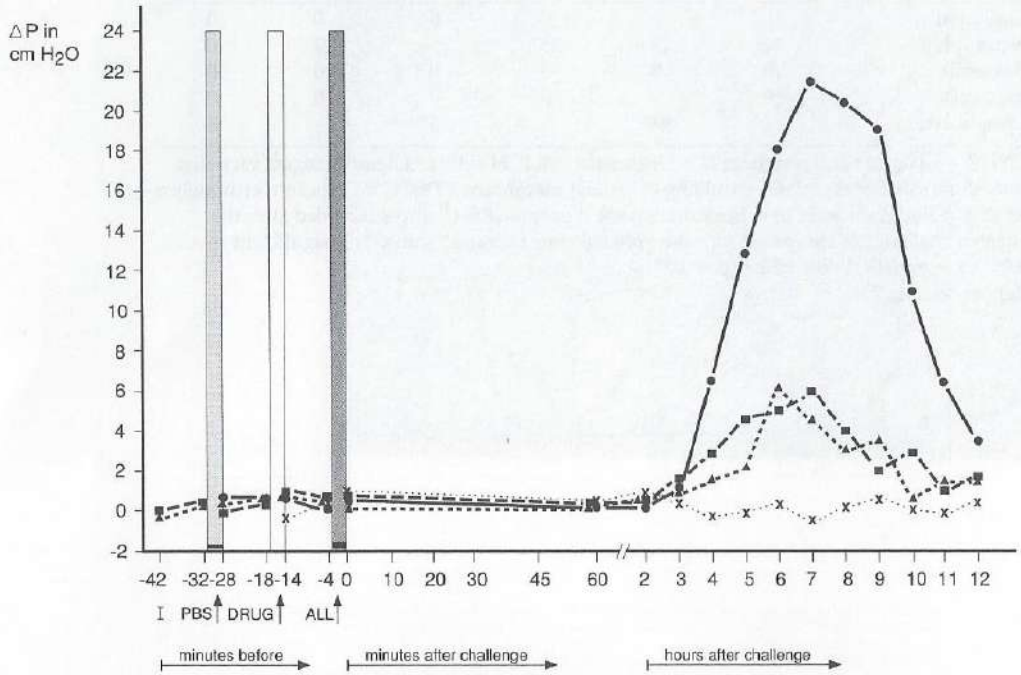
DYNR = Delayed nasal response; DSCG = Disodium cromoglycate (Cromolyn); BSA = Budesonide; BDA = Beclomethasone dipropionate. Changes in the count of particular cell types:* Statistically significant ($p < 0.05$); + = statistical borderline ($p = 0.05$)
References: 13a-13c, 72a

Table 37c Degranulation and other intracellular changes recorded in the individual cell types in the nasal secretions (NS) during the non-pretreated DYNRs and DYNRs pretreated with intranasal DSCG and BSA/BDA (in % of cases).

DYNR N=12	Non-pretreated DYNR		DYNR pretreated with			
			DSCG		BSA/BDA	
	D	ICH	D	ICH	D	ICH
Eosinophils	0	0	0	0	0	0
Neutrophils	42*	25+	33*	25+	17	8
Basophils	0	0	0	0	0	0
Mast cells	0	0	0	0	0	0
Lymphocytes	–	83*	–	75*	–	8

DYNR = Delayed nasal response; D = Degranulation; ICH = Intracellular changes (vacuolization, diminished stain intake, wrinkling of cellular membrane); DSCG = Disodium cromoglycate; BSA = Budesonide; BDA = Beclomethasone dipropionate. Changes recorded after the allergen challenge as compared with the pre-challenge changes:* statistically significant ($p < 0.05$) or + statistical borderline ($p = 0.05$)
Reference: 13b, 72a

Fig. 32a. THE LATE NASAL RESPONSE (LNR) TO ALLERGEN CHALLENGE AND PROTECTIVE EFFECTS OF DISODIUM CROMOGLYCATE (DSCG) AND BUDESONIDE (BSA)

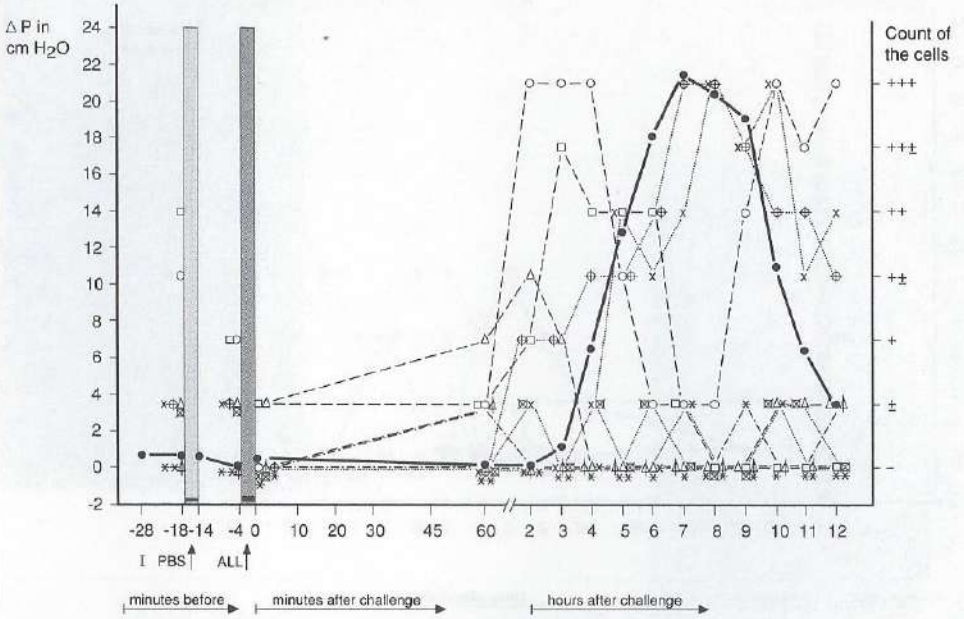


The mean NPG values recorded after the non-pretreated and pretreated nasal challenges, with respect to the appropriate control PBS (Phosphate Buffered Saline) NPG values, were always calculated from 16 patients developing 16 positive late nasal responses (LNR).
I = Initial values; PBS = Control challenge; ALL = Allergen challenge.

- = Non-pretreated LNR
- = LNR pretreated with DSCG
- = LNR pretreated with BSA
- = PBS control challenge

References: 11g,11h,12,25,40a,40c,40d,40f,41a,41b,41c,41d,41i,72,72a,72c,121b,121j

Fig. 32b. CYTOLOGIC CHANGES IN NASAL SECRETIONS (NS) DURING THE NON-PRETREATED LATE NASAL RESPONSE (LNR)

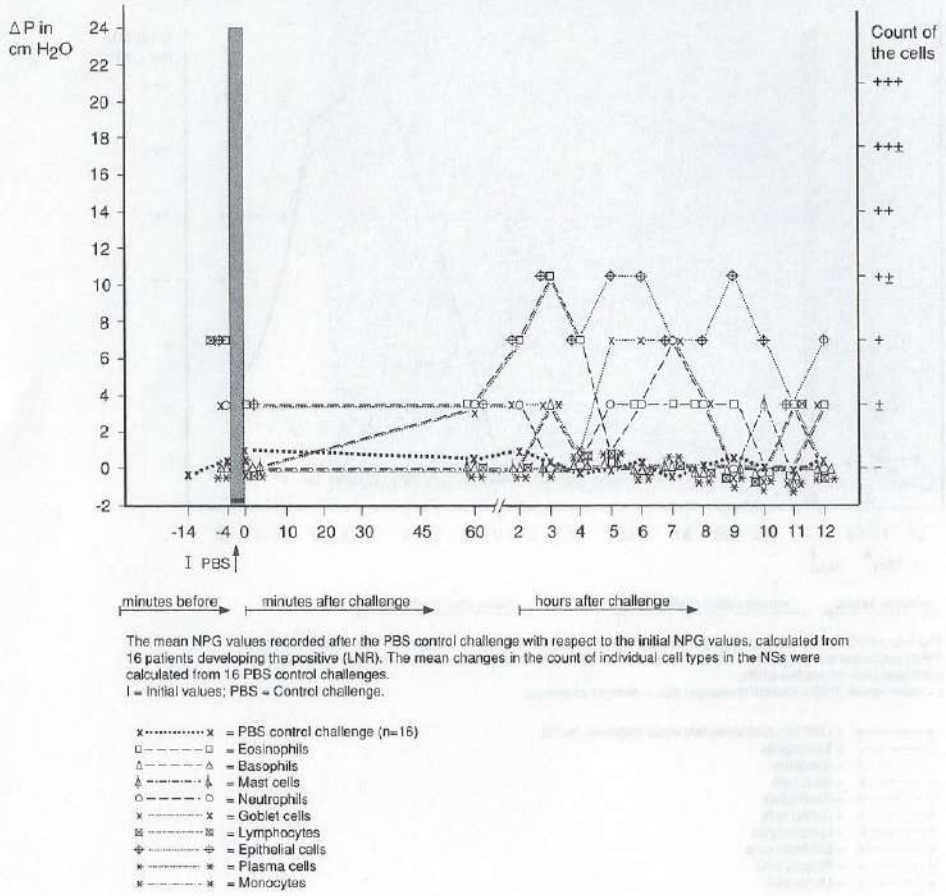


The mean NPG values recorded after the non-pretreated allergen challenge with respect to the control NPG values (PBS) calculated from 16 positive LNRs. The mean changes in the count of the individual cell types in the NSs were calculated from 16 positive LNRs.
I = Initial values; PBS = Control challenge; ALL = Allergen challenge.

- = LNR non-pretreated late nasal response (n=16)
- = Eosinophils
- = Basophils
- = Mast cells
- = Neutrophils
- = Goblet cells
- = Lymphocytes
- = Epithelial cells
- = Plasma cells
- = Monocytes

References: 11g,11h,12,25,40a,40c,40d,40f,41a,41b,41c,41d,41i,72,72a,72c,121b,121j

Fig. 32c. CYTOLOGIC CHANGES IN NASAL SECRETIONS (NS) DURING THE CONTROL CHALLENGE WITH PHOSPHATE BUFFERED SALINE (BPS)

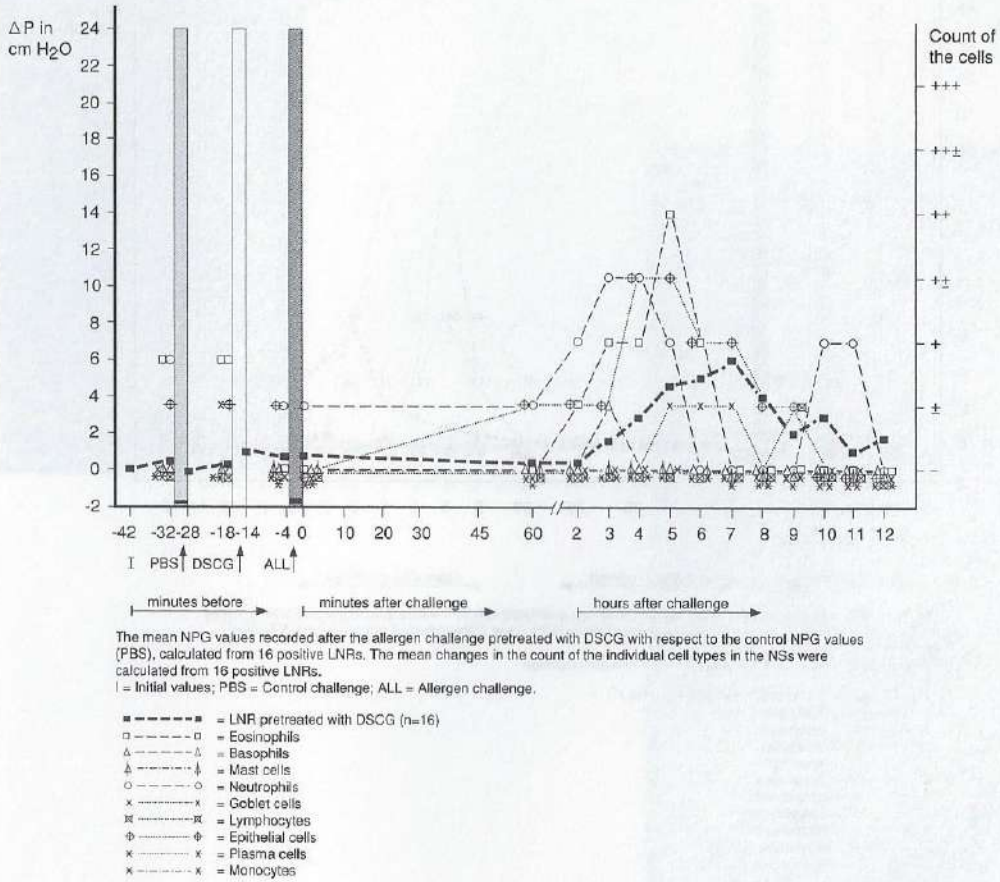


The mean NPG values recorded after the PBS control challenge with respect to the initial NPG values, calculated from 16 patients developing the positive (LNR). The mean changes in the count of individual cell types in the NSs were calculated from 16 PBS control challenges.
I = Initial values; PBS = Control challenge.

- = PBS control challenge (n=16)
- = Eosinophils
- = Basophils
- = Mast cells
- = Neutrophils
- = Goblet cells
- = Lymphocytes
- = Epithelial cells
- = Plasma cells
- = Monocytes

References: 11g,11h,12,25,40a,40c,40d,40f,41a,41b,41c,41d,41i,72,72a,72c,121b,121j

Fig. 32d. CYTOLOGIC CHANGES IN NASAL SECRETIONS (NS) DURING THE LATE NASAL RESPONSE (LNR) PRETREATED WITH DISODIUM CROMOGLYCATE (DSCG)

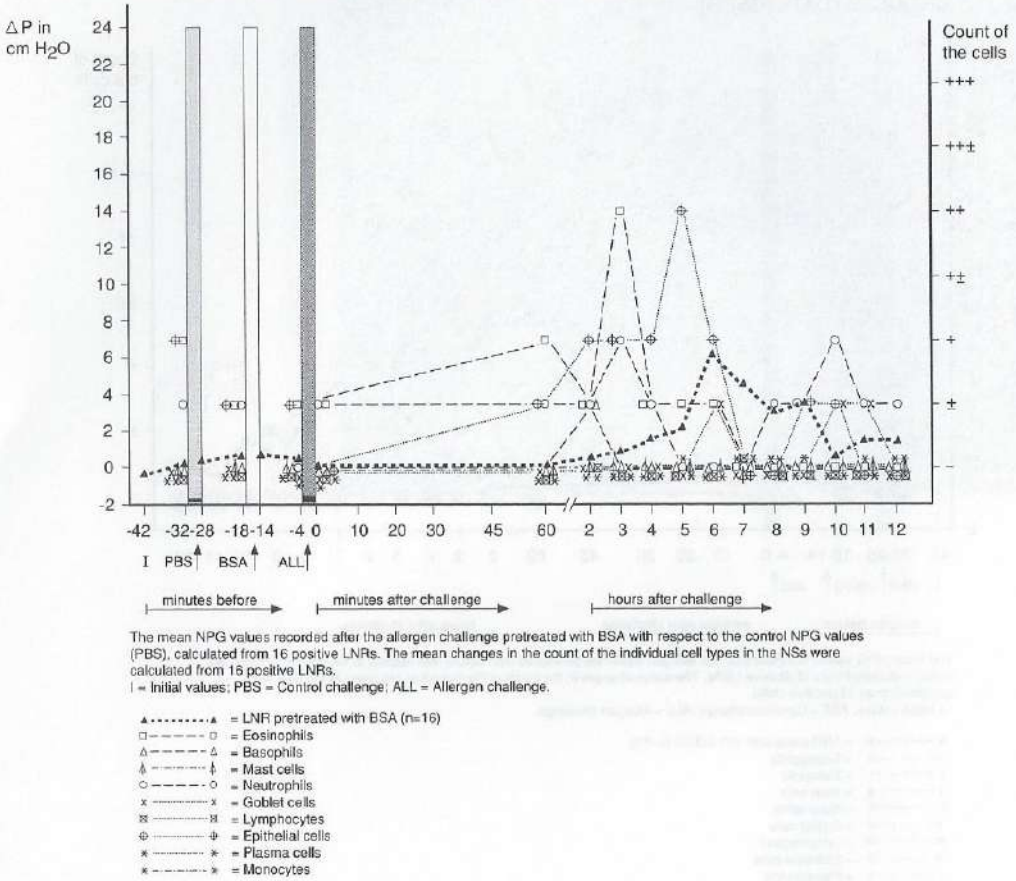


The mean NPG values recorded after the allergen challenge pretreated with DSCG with respect to the control NPG values (PBS), calculated from 16 positive LNRs. The mean changes in the count of the individual cell types in the NSs were calculated from 16 positive LNRs.
I = Initial values; PBS = Control challenge; ALL = Allergen challenge.

- = LNR pretreated with DSCG (n=16)
- = Eosinophils
- = Basophils
- = Mast cells
- = Neutrophils
- = Goblet cells
- = Lymphocytes
- = Epithelial cells
- = Plasma cells
- = Monocytes

References: 11g,11h,12,25,40a,40c,40d,40f,41a,41b,41c,41d,41i,72,72a,72c,121b,121j

Fig. 32e. CYTOLOGIC CHANGES IN NASAL SECRETIONS (NS) DURING THE LATE NASAL RESPONSE (LNR) PRETREATED WITH BUDESONIDE (BSA)

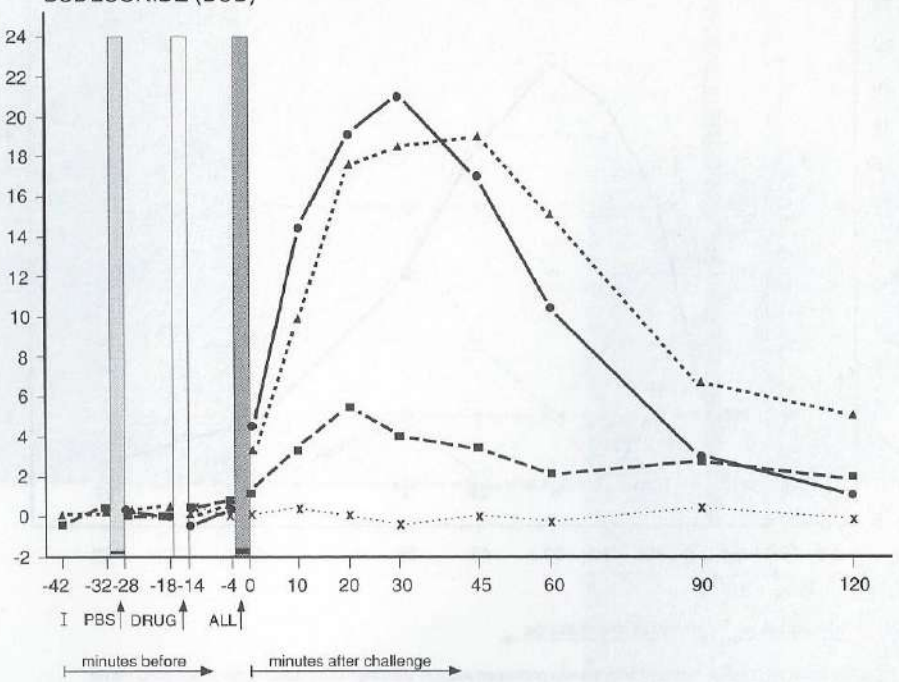


The mean NPG values recorded after the allergen challenge pretreated with BSA with respect to the control NPG values (PBS), calculated from 16 positive LNRs. The mean changes in the count of the individual cell types in the NSs were calculated from 16 positive LNRs.
I = Initial values; PBS = Control challenge; ALL = Allergen challenge.

▲·····▲ = LNR pretreated with BSA (n=16)
□——□ = Eosinophils
△——△ = Basophils
⋔——⋔ = Mast cells
○——○ = Neutrophils
x·····x = Goblet cells
⊠·····⊠ = Lymphocytes
⊕·····⊕ = Epithelial cells
✳·····✳ = Plasma cells
✳——✳ = Monocytes

References: 11g,11h,12,25,40a,40c,40d,40f,41a,41b,41c,41d,41i,72,72a,72c,121b,121j

ig. 33a. THE IMMEDIATE NASAL RESPONSE (INR) TO ALLERGEN CHALLENGE AND PROTECTIVE EFFECTS OF DISODIUM CROMOGLYCATE (DSCG) AND BUDESONIDE (BUD)

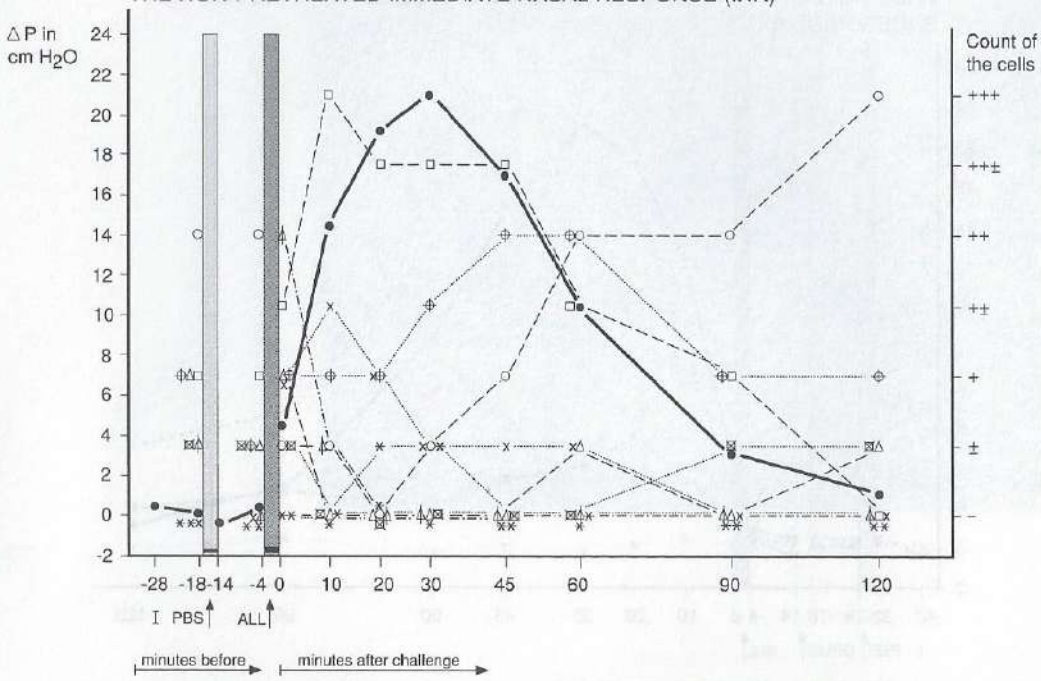


The mean NPG values recorded after the non-pretreated and pretreated nasal responses, with respect to the appropriate PBS control (Phosphate Buffered Saline) NPG values, were always calculated from 18 patients developing 18 positive immediate nasal responses (INR).
I = Initial values; PBS = Control challenge; ALL = Allergen challenge.

●——● = Non-pretreated INR
■– – –■ = INR pretreated with DSCG
▲·····▲ = INR pretreated with BUD
x·····x = PBS control challenge

References: 10,11,11a-11e,11g,14,20,40a,41a,41c,41i,71,72a,72c,72d

Fig. 33b. CYTOLOGIC CHANGES IN NASAL SECRETIONS (NS) DURING THE NON-PRETREATED IMMEDIATE NASAL RESPONSE (INR)

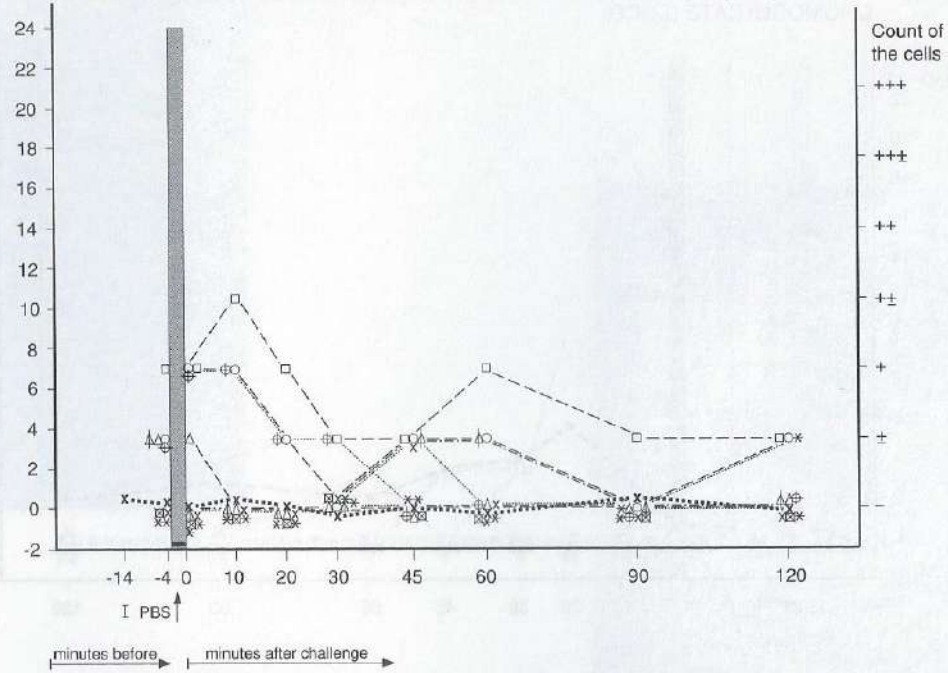


The mean NPG values recorded after the non-pretreated allergen challenge with respect to the control NPG values (PBS) calculated from 18 positive INRs. The mean changes in the count of the individual cell types in the NSs were calculated from 18 positive INRs.
I = Initial values; PBS = Control challenge; ALL = Allergen challenge.

- = INR positive immediate nasal response (n=18)
- = Eosinophils
- = Basophils
- = Mast cells
- = Neutrophils
- = Goblet cells
- = Lymphocytes
- = Epithelial cells
- = Plasma cells
- = Monocytes

References: 10,11,11a-11e,11g,14,20,40a,41a,41c,41i,71,72a,72c,72d

Fig. 33c. CYTOLOGIC CHANGES IN NASAL SECRETIONS (NS) DURING THE CONTROL CHALLENGE WITH PHOSPHATE BUFFERED SALINE (BPS)

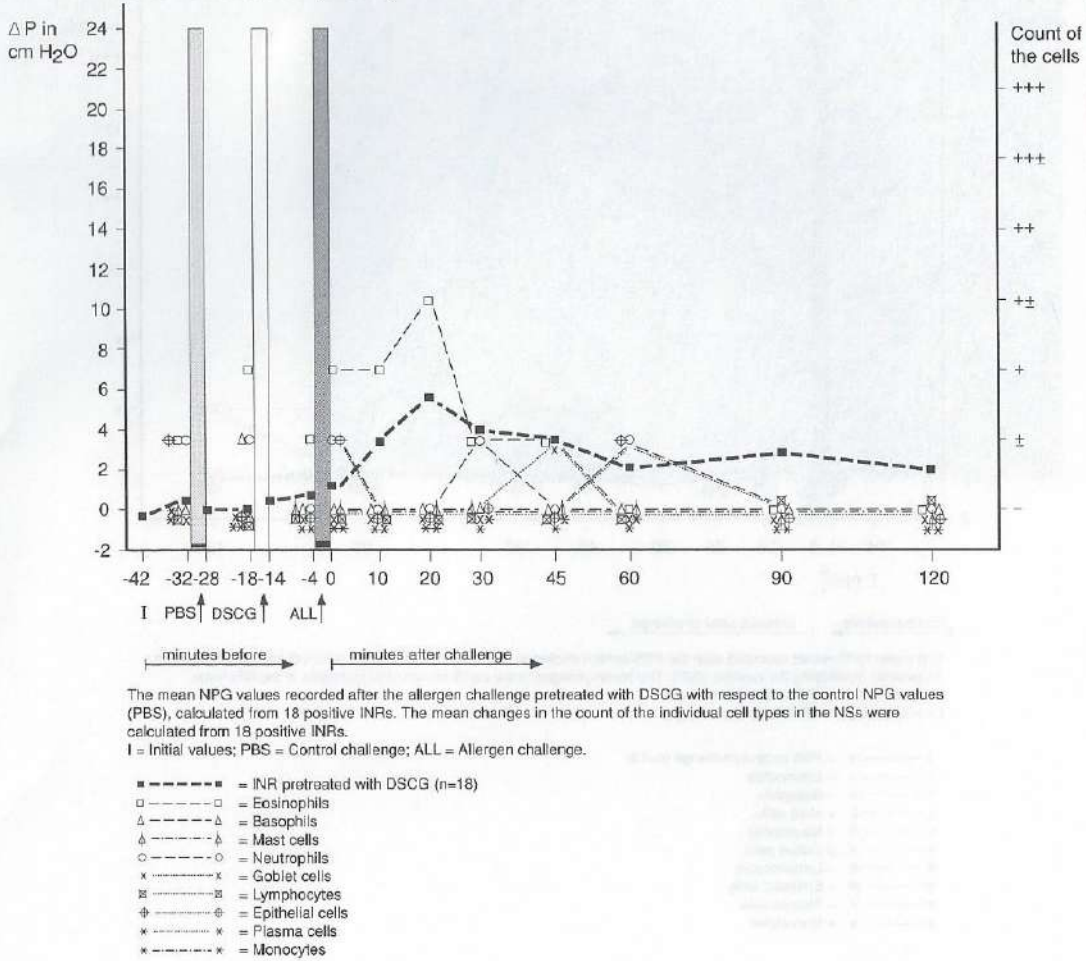


The mean NPG values recorded after the PBS control challenge with respect to the initial NPG values, calculated from 18 patients developing the positive (INR). The mean changes in the count of individual cell types in the NSs were calculated from 18 PBS control challenges.
I = Initial values; PBS = Control challenge.

- = PBS control challenge (n=18)
- = Eosinophils
- = Basophils
- = Mast cells
- = Neutrophils
- = Goblet cells
- = Lymphocytes
- = Epithelial cells
- = Plasma cells
- = Monocytes

References: 10,11,11a-11e,11g,14,20,40a,41a,41c,41i,71,72a,72c,72d

Fig. 33d. CYTOLOGIC CHANGES IN NASAL SECRETIONS (NS) DURING THE IMMEDIATE NASAL RESPONSE (INR) PRETREATED WITH DISODIUM CROMOGLYCATE (DSCG)

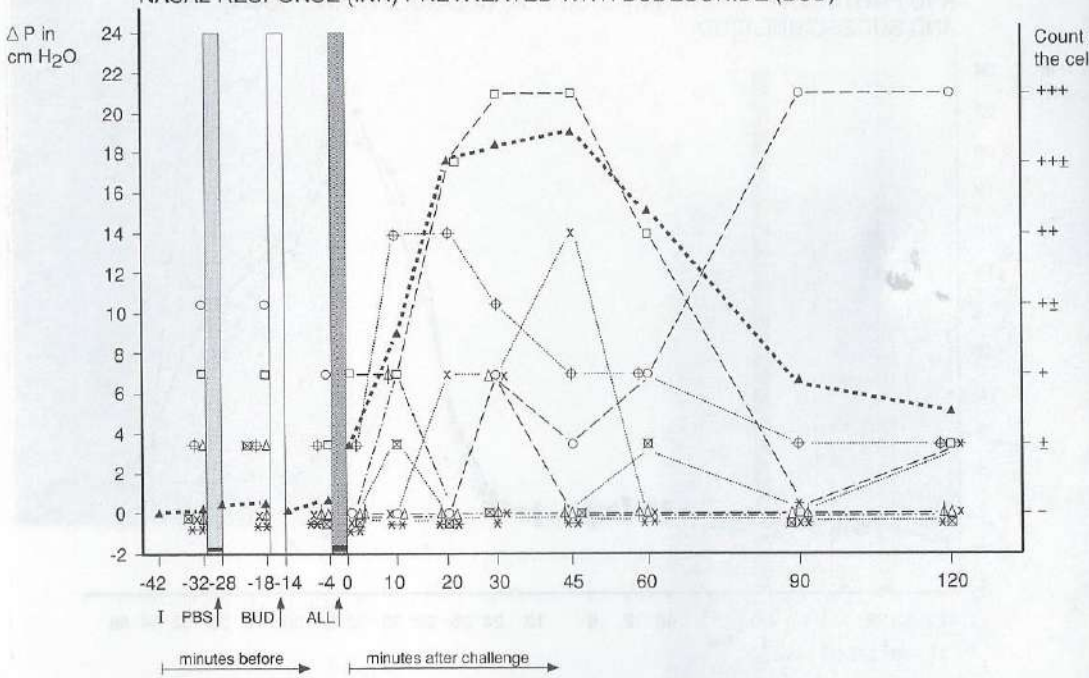


The mean NPG values recorded after the allergen challenge pretreated with DSCG with respect to the control NPG values (PBS), calculated from 18 positive INRs. The mean changes in the count of the individual cell types in the NSs were calculated from 18 positive INRs.
I = Initial values; PBS = Control challenge; ALL = Allergen challenge.

= INR pretreated with DSCG (n=18)
= Eosinophils
= Basophils
= Mast cells
= Neutrophils
= Goblet cells
= Lymphocytes
= Epithelial cells
= Plasma cells
= Monocytes

References: 10,11,11a-11e,11g,14,20,40a,41a,41c,41i,71,72a,72c,72d

Fig. 33e. CYTOLOGIC CHANGES IN NASAL SECRETIONS (NS) DURING THE IMMEDIATE NASAL RESPONSE (INR) PRETREATED WITH BUDESONIDE (BUD)

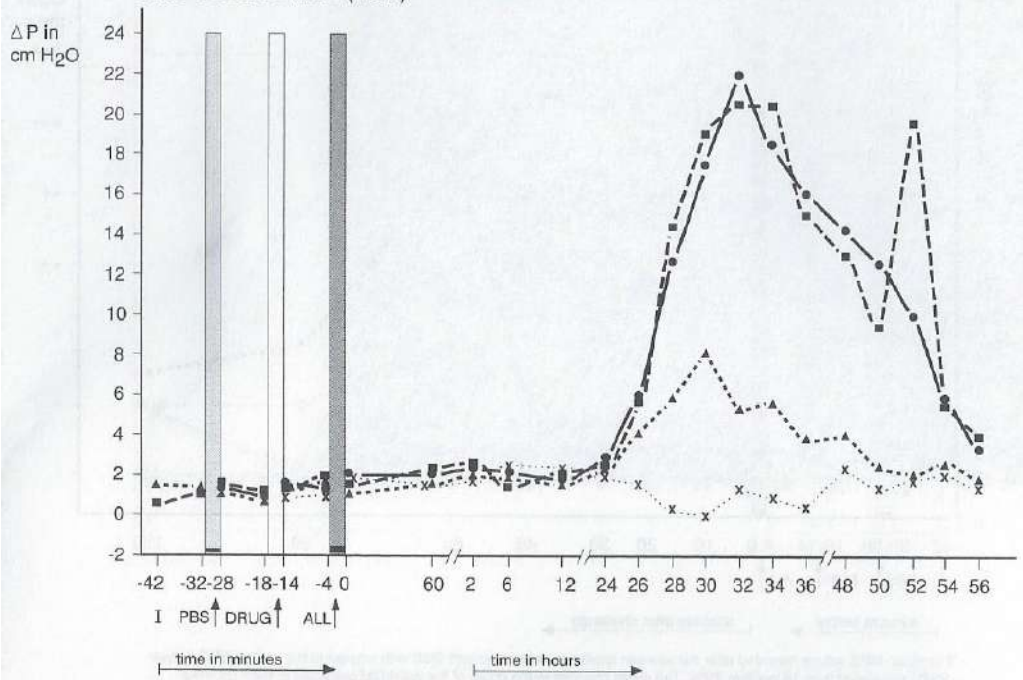


The mean NPG values recorded after the allergen challenge pretreated with BUD with respect to the control NPG values (PBS), calculated from 18 positive INRs. The mean changes in the count of the individual cell types in the NSs were calculated from 18 positive INRs.
I = Initial values; PBS = Control challenge; ALL = Allergen challenge.

= INR pretreated with BUD (n=18)
= Eosinophils
= Basophils
= Mast cells
= Neutrophils
= Goblet cells
= Lymphocytes
= Epithelial cells
= Plasma cells
= Monocytes

References: 10,11,11a-11e,11g,14,20,40a,41a,41c,41i,71,72a,72c,72d

Fig. 34a. THE DELAYED NASAL RESPONSE (DYNR) TO ALLERGEN CHALLENGE AND PROTECTIVE EFFECTS OF DISODIUM CROMOGLYCATE (DSCG) AND BUDESONIDE (BUD)

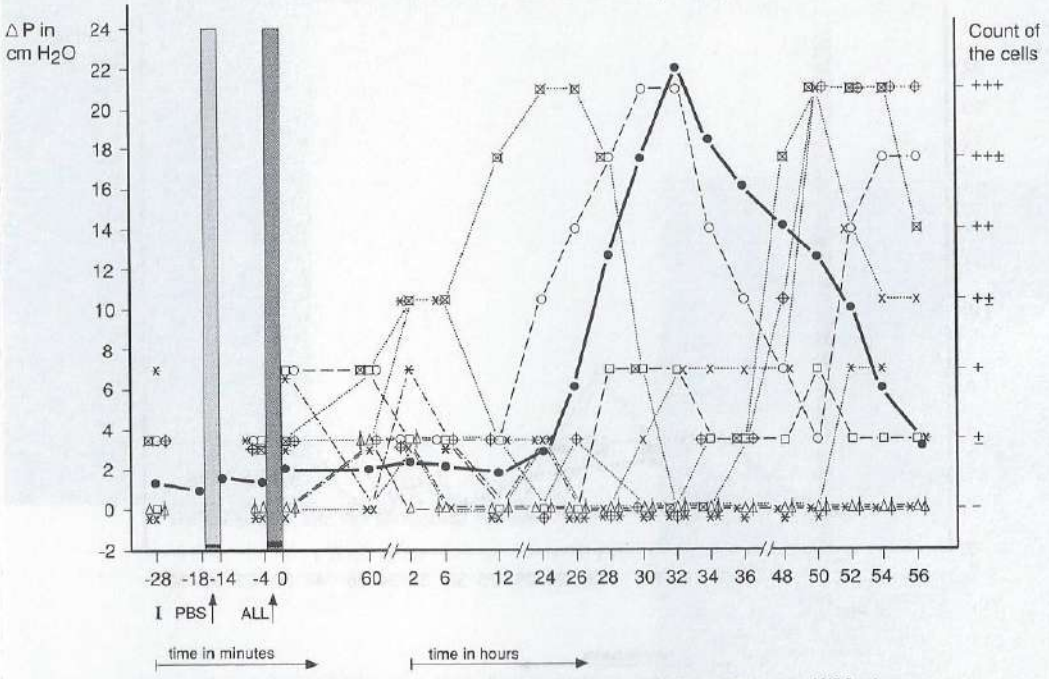


The mean NPG values recorded after the non-pretreated and pretreated nasal responses, with respect to the appropriate PBS (Phosphate Buffered Saline) control values, were always calculated from 12 patients developing 12 positive DYNRs.
I = Initial values; PBS = Control challenge; ALL = Allergen challenge.

- = Non-pretreated DYNR
- = DNR pretreated with DSCG
- = DNR pretreated with BUD
- = PBS control challenge

References: 13,13a-13c,40a,41i,72a

Fig. 34b. CYTOLOGIC CHANGES IN NASAL SECRETIONS (NS) DURING THE NON-PRETREATED DELAYED NASAL RESPONSE (DYNR)

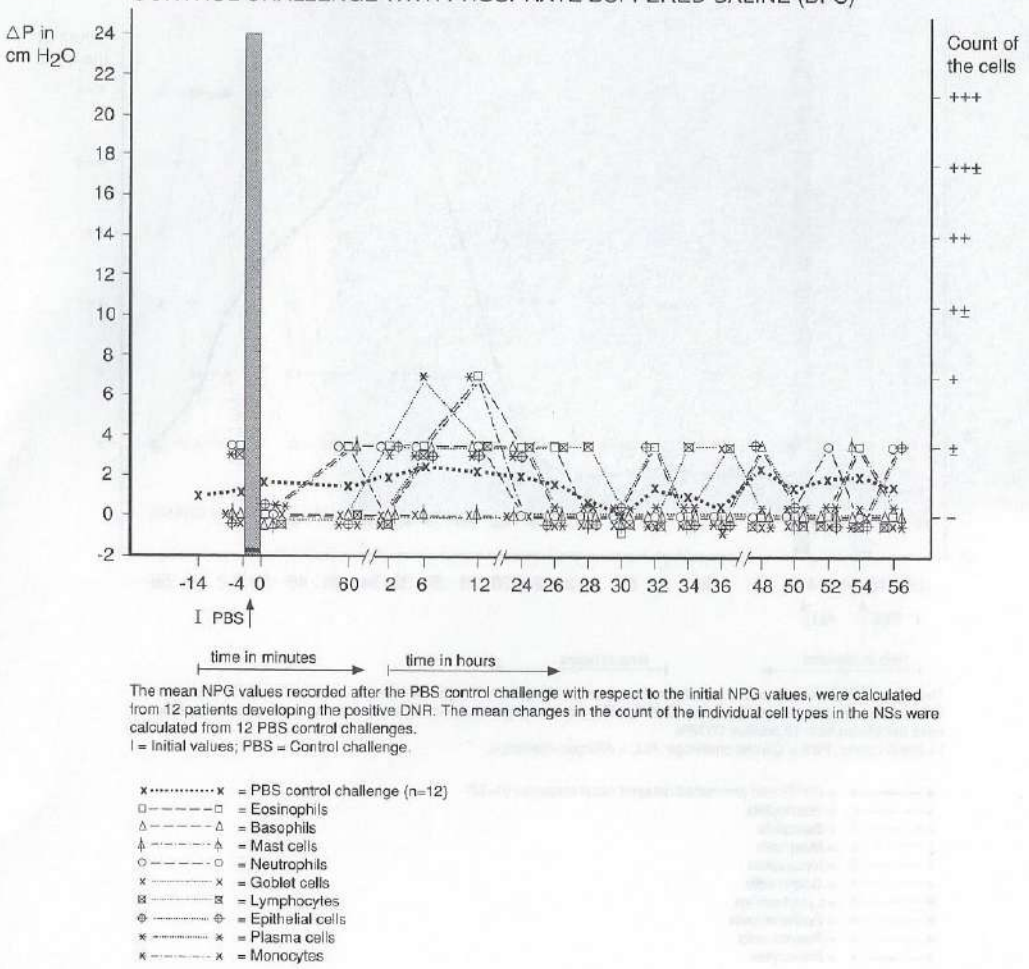


The mean NPG values recorded after the non-pretreated allergen challenge with respect to the control NPG values (PBS), were calculated from 12 positive DYNRs. The mean changes in the count of the individual cell types in the NSs were calculated from 12 positive DYNRs.
I = Initial values; PBS = Control challenge; ALL = Allergen challenge.

- = DYNR non-pretreated delayed nasal response (n=12)
- = Eosinophils
- = Basophils
- = Mast cells
- = Neutrophils
- = Goblet cells
- = Lymphocytes
- = Epithelial cells
- = Plasma cells
- = Monocytes

References: 13,13a-13c,40a,41i,72a

Fig. 34c. CYTOLOGIC CHANGES IN NASAL SECRETIONS (NS) DURING THE CONTROL CHALLENGE WITH PHOSPHATE BUFFERED SALINE (BPS)

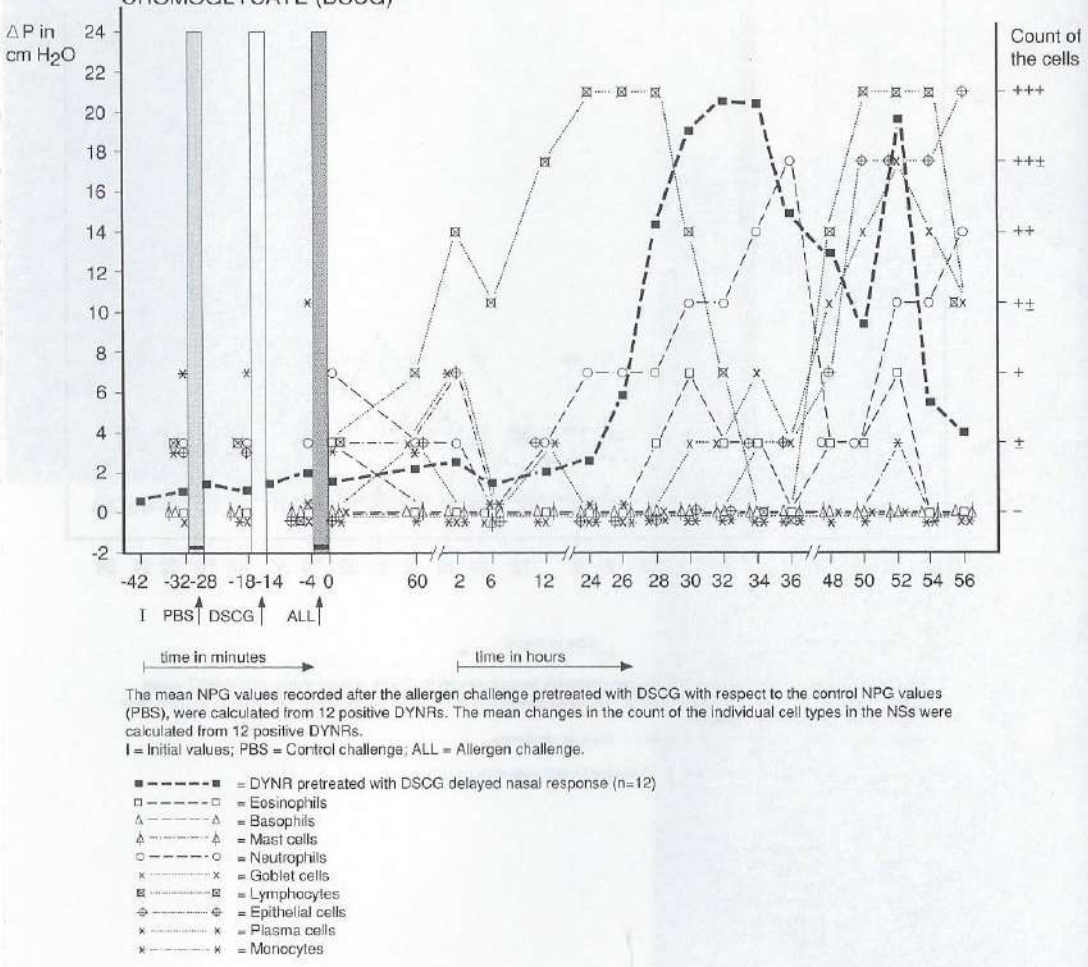


The mean NPG values recorded after the PBS control challenge with respect to the initial NPG values, were calculated from 12 patients developing the positive DNR. The mean changes in the count of the individual cell types in the NSs were calculated from 12 PBS control challenges.
I = Initial values; PBS = Control challenge.

- = PBS control challenge (n=12)
- = Eosinophils
- = Basophils
- = Mast cells
- = Neutrophils
- = Goblet cells
- = Lymphocytes
- = Epithelial cells
- = Plasma cells
- = Monocytes

References: 13,13a-13c,40a,41i,72a

Fig. 34d. CYTOLOGIC CHANGES IN NASAL SECRETIONS (NS) DURING THE DELAYED NASAL RESPONSE (DYNR) PRETREATED WITH DISODIUM CROMOGLYCATE (DSCG)

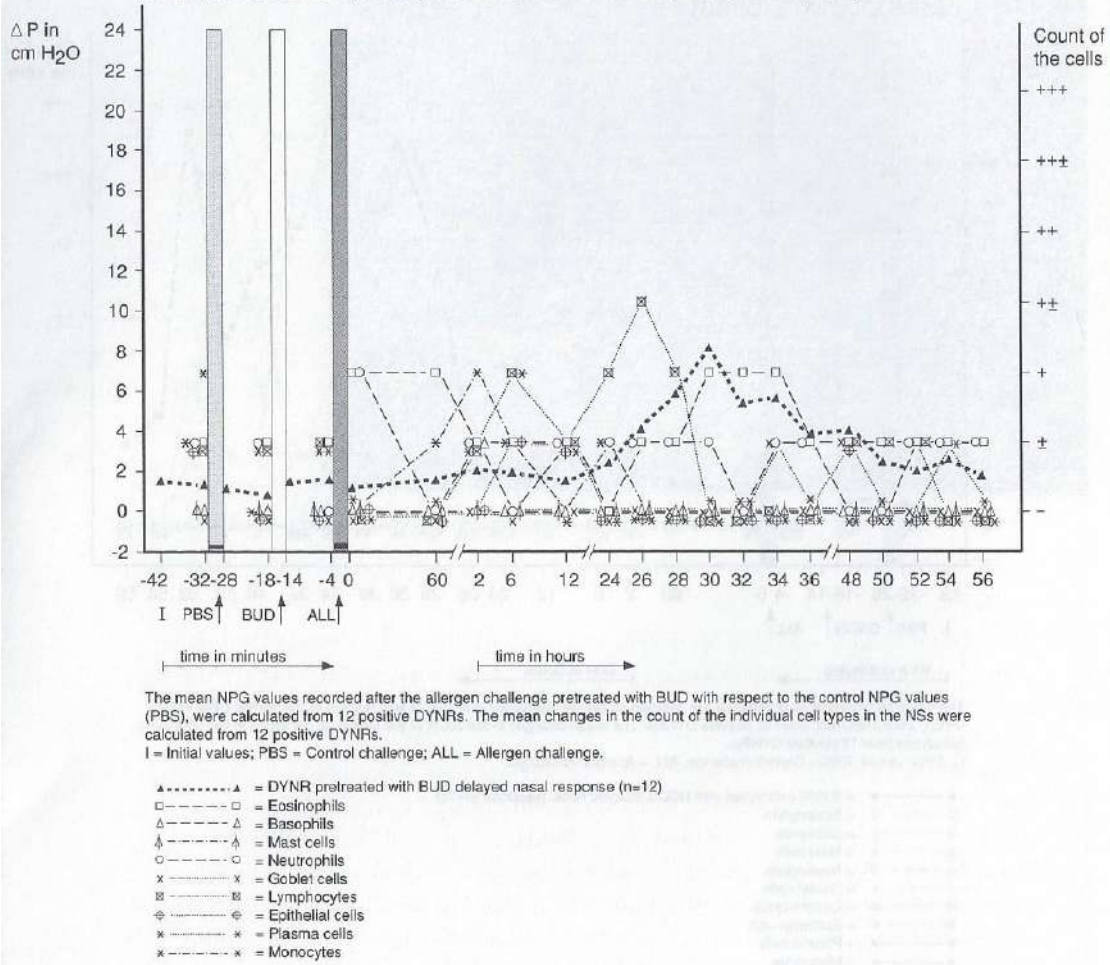


The mean NPG values recorded after the allergen challenge pretreated with DSCG with respect to the control NPG values (PBS), were calculated from 12 positive DYNRs. The mean changes in the count of the individual cell types in the NSs were calculated from 12 positive DYNRs.
I = Initial values; PBS = Control challenge; ALL = Allergen challenge.

- = DYNR pretreated with DSCG delayed nasal response (n=12)
- = Eosinophils
- = Basophils
- = Mast cells
- = Neutrophils
- = Goblet cells
- = Lymphocytes
- = Epithelial cells
- = Plasma cells
- = Monocytes

References: 13,13a-13c,40a,41i,72a

Fig. 34e. CYTOLOGIC CHANGES IN NASAL SECRETIONS (NS) DURING THE DELAYED NASAL RESPONSE (DYNR) PRETREATED WITH BUDESONIDE (BUD)

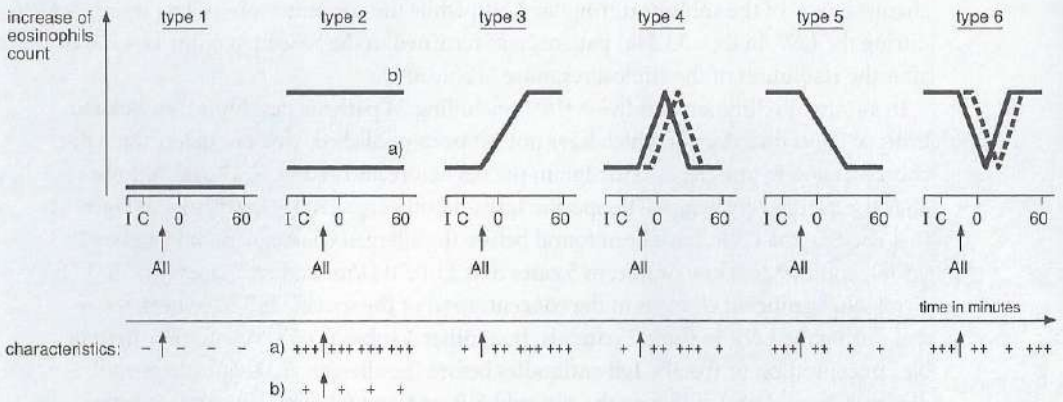


The mean NPG values recorded after the allergen challenge pretreated with BUD with respect to the control NPG values (PBS), were calculated from 12 positive DYNRs. The mean changes in the count of the individual cell types in the NSs were calculated from 12 positive DYNRs.
I = Initial values; PBS = Control challenge; ALL = Allergen challenge.

▲·····▲ = DYNR pretreated with BUD delayed nasal response (n=12)
□——□ = Eosinophils
△——△ = Basophils
⅄—·—·—⅄ = Mast cells
○——○ = Neutrophils
x ····· x = Goblet cells
⊠ ····· ⊠ = Lymphocytes
⊕ ····· ⊕ = Epithelial cells
✳ ····· ✳ = Plasma cells
✳—·—·— ✳ = Monocytes

References: 13,13a-13c,40a,41i,72a

Fig. 35. SURVEY OF THE BASIC PATTERNS (TYPES) OF THE CHANGES IN THE EOSINOPHILS' COUNT IN THE NASAL SECRETIONS DURING THE NASAL PROVOCATION TESTS.



LEGENDS: I = initial value, C = coca's value, All = allergen challenge.

2. IMMUNOLOGIC ASPECTS

The immunologic aspects of NS during the LNR predominantly comprise the immunoglobulins and other compounds, such as chemotactic factors, mediators and a variety of other products, synthesized and released during the mechanism(s) underlying the LNR.[1,11f,11h,25a,34,41b,41f,41i,48,53,54,73,78,82d-82j,84,85,86-91,93,94,95a,97b,97c,97v]

The immunoglobulins, total IgE, antigen-specific IgE, total IgG, and IgA (sometimes IgM), have already been repeatedly demonstrated in the nasal secretions of patients suffering from allergic rhinitis in both the perennial and seasonal forms.[36,84,85,86-92] However, most of these studies have been carried out as a single determination of these immunoglobulins in NS and they have not been related to a specific allergen. Some investigators have provided evidence for a local production of IgE, IgA, and IgG antibodies in the nasal mucosa.[36,46,84,86, 87, 90,91] Moreover, the local production of immunoglobulins was significantly higher in subjects with allergic rhinitis.[36,90,91]

Studies dealing with the determination of the particular immunoglobulin classes in the NS during the immediate nasal response to allergen challenge are only very few.[2,92]

The changes in concentration of the particular immunoglobulins in the NS during the LNR, in a sufficiently large group of patients, have to our knowledge, not yet been investigated.

In some patients (n = 24), developing an isolated form of LNR, in whom the cytologic changes in the NS were studied,[25] we also have determined the total and specific IgE, total IgG, total IgM and total IgA immunoglobulins in the NS before and, repeatedly, after the allergen challenge, as a preliminary investigation.[41b] The total IgE immunoglobulins were found in 2 patients (= 8.4%), allergen-specific IgE in another 3 (= 12.5%), IgG in 11 (= 45.8%), IgM in 1 (= 4,2%) and IgA immunoglobulins in 1 patient (= 4.2%). The concentration of total IgE, specific IgE, and total IgA immunoglobulins did not change in any of the subjects during the LNR, while the concentration of IgG decreased during the LNR in 8 (= 33,3%) patients and returned to the baseline within 12 to 24 h after the resolution of the clinical response (Table 38).

In another preliminary study,[25a,41f,41i] including 74 patients developing an isolated form of LNR, final data of which have not yet been published, we have determined the concentration of antigen-specific IgE in the NS before and at 3, 6, 9, 12 and 24 hours after the allergen challenge. The specific IgE antibodies against the particular allergen as that eliciting the LNR, have been found before the allergen challenge in only 7 cases (= 9.5%), and that to a low degree; in 5 cases of 0.35-0.70 U/ml and in 2 cases of 0.70-3.50 U/ml. No significant changes in the concentration of the specific IgE have been recorded during the LNR in these 7 patients. In another 4 subjects (=5%) with non-detectable concentration of specific IgE antibodies before the allergen challenge, these antibodies have been detected during the clinical LNR, at 6 and 9 hours, however, only to a slight degree (class 1) (Table 39).

Table 38 Presence and changes in the concentrations of particular immunoglobulins in the nasal secretions (NS) during the late nasal response (LNR), negative nasal response (NNR) and PBS controls.

	Presence of immunoglobulins			Changes in the immunoglobulin concentrations during the appropiate nasal response		
	LNR (n=24)	NNR (n=20)	PBS (n=24)	LNR	NNR	PBS
Total IgE	2 (8.4%)	0	1 (4.2%)	0	0	0
Antigen-specific IgE	3 (12.5%)	1 (5.0%)	1 (4.2%)	0	0	0
Total IgG	11(45.0%)	1 (5.0%)	6(25.0%)	8 (33.3%)*	0	0
- IgG1	0	0	0	0	0	0
- IgG2	7(29.1%)	0	2 (8.4%)	0	0	0
- IgG3	5(20.8%)	0	1 (4.2%)	0	0	0
- IgG4	0	0	0	0	0	0
Total IgM	1 (4.2%)	0	1 (4.2%)	0	0	0
Total IgA	1 (4.2%)	1 (5.0%)	1 (4.2%)	0	0	0

PBS = Phosphate buffered saline; * = increase
References: 25a, 41b, 41i

The mediators, also called "inflammatory mediators", chemotactic factors and other compounds (products or constituents of various types of cells), appearing in the NS during the clinical LNR, have attracted the attention of many investigators, especially during recent years, and many interesting studies have been published on these topics.[34,35e,47,48,48b,48d,48e,49,51c,52-57,82,82b,82d,82e,82h,82j, 83,91-95, 97b-97e]

Naclerio,[53,54] Bascom,[34] Togias,[56,83] Iliopoulos,[51c] Walden,[94] MacGlashan[97e] and their colleagues, using the nasal lavage technique, have repeatedly recorded increased concentrations of histamine, kinins and TAME-esterase (N-α-tosyl-L-arginine methyl-ester) in NS during the LNR, whereas during the INR they have found increased concentrations not only of histamine, kinins and TAME-esterase, but also of prostaglandin D_2 (PGD_2). Freeland and colleagues[49,97c] have detected an increased concentration of leukotriene B_4 (LTB_4) in NS during the LNR as well as during the INR.

Gert van Wijk[35e] has recorded an increased concentration of histamine, LTC_4/D_4 and albumin in the nasal lavage fluid 3-10 hours after the nasal challenge with allergen. However, in most of his patients the late appearance of the nasal obstruction (clinical LNR) has not been confirmed by rhinomanometry.

Table 39 Presence of the antigen-specific IgE antibodies (RAST) in the nasal secretions (NS) before and their changes during the late nasal response to allergen challenge (LNR).

LNR n=74	Presence of specific IgE before the allergen challenge	Changes in the concentration during the LNR: increase (appearance)	decrease (disappearance)	no changes
Group I (n=7)	7 (= 9.5%)	-	-	7
Group II (n=4)	0	4 (= 5.4%)	-	-
Group III (n=63)	0	-	-	0

0 = no case; - = not relevant
References: 41f, 41i

Togias and co-workers,[83] Pipkorn and colleagues [95] and Naclerio and colleagues [53] have reported the recording also of other leukotrienes (LTC_4, LTD_4, LTE_4) in NS during the LNR. However, there is a dearth of more detailed information concerning this topic. Bascom and co-workers[48,97b] have found a significant increase in the concentration of major basic protein (MBP) in NS during both the LNR and the INR, correlating with the eosinophil influx into the NS. Moreover, they also have measured a significant increase in the concentration of eosinophil-derived neurotoxin (EDN) in NS during the LNR. Togias and colleagues[56] have detected bradykinin and lysylbradykinin in the NS during both the LNR and the INR. Bisgaard and colleagues [82j] and Davies and co-workers [97f] have recorded a significant increase in concentration of the eosinophil cationic protein (ECP) in nasal secretions during the LNR (6-24 hours after the nasal challenge with grasspollen in the grasspollen-sensitive subjects), while they did not detect any significant ECP changes during the INR. Most of these studies, however,

concern a small number of subjects and no clinical course of the LNR (=nasal obstruction) has probably ever been recorded.

We have also investigated the concentration of histamine and its changes in NS during the immediate, late and delayed nasal response to allergen challenge (Tables 18,19).[11f] In contrast to other investigators' results,[34,51c,53,54] we have not detected histamine in the NS during most cases of LNR, whereas we have recorded mainly increases in the histamine concentration during 67% of INR cases (Tables 18,19; Figures 19-21).[11f, 11j,41b,41i]

Moreover, no increase or changes in the concentrations of histamine in the blood plasma have been detected by us during the INR, LNR or DYNR to allergen challenge (Table 17).[97v]

Finally, Skoner and colleagues[91] have measured an increased concentration of neutrophil chemotactic factor (NCF), histamine and prostaglandin ($PGF_{2\alpha}$) metabolites in the serum, during the LNR.

3. BIOCHEMICAL AND BIOPHYSICAL ASPECTS

Biochemical and biophysical characteristics of the NS have been summarized very comprehensibly by Mygind and colleagues[73,85] They comprise not only the composition of NS, such as ratio and participation of products of various secretory elements in the nasal mucosa (serous and sero-mucous glands, goblet cells etc.) and additional fluids, e.g. condensed expiratory water, plasma transudation, secretions of paranasal sinuses and tears; but also other properties of the NS, such as color, transparency, consistency, viscosity, dilution, density, pH, and a variety of chemical components (electrolytes, proteins, sulphates, sugars etc.).

Regarding the preliminary results of these investigators, the origin of nasal secretions may partly be due to the glandular secretions, partly due to the transudation from plasma, and partly due to the additional fluids.[73,97g,97h] Brofeldt and colleagues[97g,97h], studying the NS in a group of pollen-sensitive rhinitis patients, have measured an increased viscosity of nasal secretions after an allergen challenge, as compared with the baseline, whereas the viscosity of nasal secretions had decreased after the histamine and even more after the methacholine challenge. They have also found qualitative similarities between the nasal secretions and sputum, except for the dry weight sugar and protein content, being considerably lower in the NS. The albumin has been found in similar levels in NS and bronchial secretions, a fact which may indicate a comparable degree of transudation from the plasma. Raphael and co-workers,[97i] investigating the NS after an allergen challenge, have measured an increased concentration of the total protein in both ipsilateral and contralateral nasal secretions, an increased proportion of albumin relative to the total protein (the albumin percent) on the ipsilateral side, and increased relative proportions of lactoferrin and lysozyme (the lactoferrin percent and lysozyme percent) on the contralateral side. However, most of the investigations concerning the biochemical and biophysical aspects and properties of the NS have been performed either during the allergen-induced INR[97g-97i], or after the challenge with histamine or methacholine[97h,97i] or after other non-specific stimuli.[85] Similar biochemical and biophysical properties of NS and their changes during the LNR have, however, not yet been investigated to our knowledge.

In some patients developing the LNR(n = 12) as well as the INR (n = 14) we also have examined the biochemical and biophysical properties of NS.[12,41i] During the LNR, the viscosity of the NS increased approximately 7 times with respect to that measured during the INR, the density increased approximately 3 times, pH showed a slight increase in acidity, albumin concentration increased about twice, whereas the total protein concentration did not demonstrate any significant changes.

Our results should, however, be regarded as preliminary only, and more systematic attention should be paid to the biochemical and biophysical properties of NS during LNR.

H. BIOPSY OF THE NASAL MUCOSA

The biopsy of the nasal mucosa during the particular types of nasal response and its histological examination, seems to us to be a very important diagnostic parameter for the evaluation of the pathological changes in the nasal mucosa tissue, accompanying the appropriate type of nasal response, and also a very interesting research technique for studying the immunologic mechanisms underlying the particular types of the nasal response to allergen challenge. However, like several other very useful techniques, the biopsy of the nasal mucosa has some disadvantages concerning both the technical aspects, such as use of the topical anaesthetic drugs and their influence on the histologic changes in the tissue, the choice of the biopsy instruments and the location where the tissue samples should be taken, the technique of the processing of the tissue, and the interpretation aspects. Finally this technique is not totally without risk for the patient, e.g. bleeding.

Several papers dealing with the biopsy and histological changes of the allergic nasal mucosa have already been published.[40n,48c,51b,74,75,85,97f,97j-97u,97z]

The studies concerning the biopsy of the nasal mucosa related to a certain allergen are not numerous.[48c,51b,97j-97p,97s] Only very few studies include the biopsy performed before and repeatedly after the nasal challenge with an allergen, during the registration of the nasal mucosa response,[97t] and moreover, they predominantly concern the INR.[97t]

However, there is a dearth of information concerning the histological changes in the nasal mucosa during the LNR. We have been unable to find any paper on this topic in the available literature.

According to our preliminary findings[2] and some later studies, results of which have been recently published,[41i,96,97] the following histologic changes have been found in the biopsies of nasal mucosa during the LNR, as compared with the "pre-challenge" baseline: (1) the edematic epithelium showed diminished compactness, enlarged intercellular spaces and some breaches filled with fluid; some epithelial and goblet cells have been expelled and left empty holes in the epithelium; (2) the compactness of the basement membrane has been found to be irregular and with single breaches; (3) the edematic sub-epithelial layer of the lamina propria contained mixed eosinophil-neutrophil infiltrates and single mast cells, basophils, monocytes and lymphocytes; (4) the lamina propria showed a perivascular edema, dilatation of the terminal parts of the capillaries and sporadical rupture of the small capillaries with erythrocyte expulsion [Table 40; Figures 36a, 36b; Plate 20 (page 368)].

The histological changes having been recorded in the nasal mucosa during the LNR differed distinctly from those observed by us during both the INR (Table 41; Figure 37)[2,96,97a] and the DYNR (Table 42; Figure 38).[96] The changes in the nasal mucosa during the INR have been pronounced to a slight degree only and can be qualified to be of a "functional" and/or "transient" character, while those found during the LNR have been largely pronounced and included slight, however reversible, tissue damage of the nasal mucosa with some inflammatory components.

In contrast, the histological changes in the nasal mucosa having having been recorded during the positive DYNR represented damage of the nasal mucosa upon involvement of the distinct inflammatory component. Nevertheless, this damage of the nasal mucosa tissue may also be considered to be of a reversible character.

Table 40 Histological changes in the nasal mucosa (= biopsy) during the late nasal response (LNR) - as compared with the "pre-challenge" findings

- edematic epithelium with diminished compactness
- enlarged intercellular spaces and breaches in the epithelium
- expelled epithelial and goblet cells
- the epithelial surface shows empty holes
- irregular compactness of the basement membrane with single breaches
- edematic sub-epithelial layer of the lamina propria containing mixed eosinophil-, neutrophil infiltrates and single basophils, tissue mast cells, monocytes and lymphocytes
- perivascular edema in lamina propria with dilated, sometimes disrupted, capillaries and sporadical erythrocyte expulsion

These changes represent a slight damage of the tissue

Table 41 Histological changes in the nasal mucosa (= biopsy) during the Immediate nasal response (INR) - as compared with the "pre-challenge" findings

- increased amount of thin serous secretions on the epithelial surface
- enlarged ducti of mucosal glands
- enlarged intercellular spaces in the epithelium
- eosinophil and tissue mast cell accumulation, but not infiltrate forming, in the upper layer of the lamina propria (approximately 30% of eosinophils and 80% of mast cells were degranulated)
- dilated, but not disrupted, capillaries and a slight perivascular edema in the lamina propria (as far as this could be evaluated)
- the basement membrane was intact (not affected)

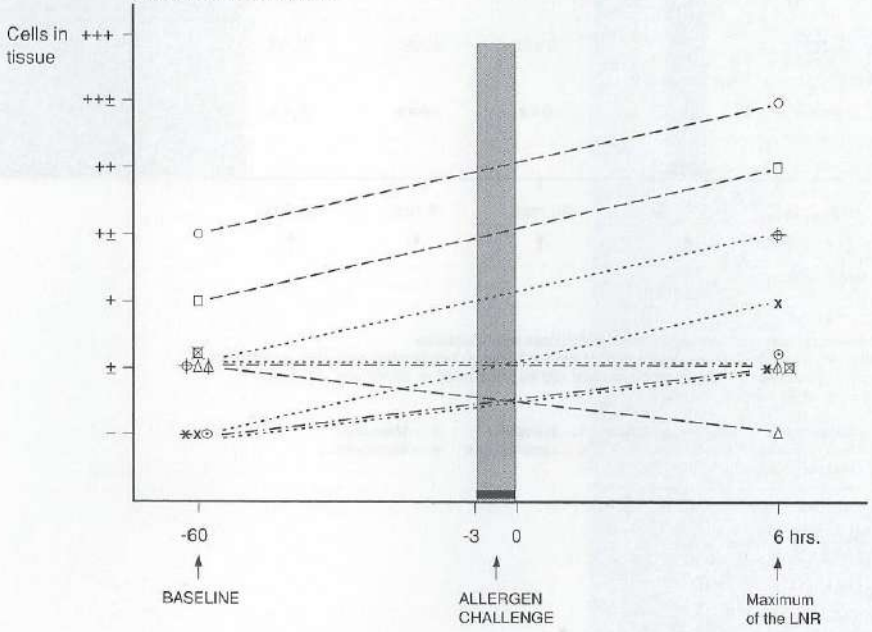
These changes are of a "functional" and transient character

Table 42 Histological changes in the nasal mucosa (= biopsy) during the delayed nasal response (DYNR) - as compared with the "pre-challenge" findings.

- edematic epithelium showing decreased compactness
- enlarged intercellular spaces, breaches and empty holes in the epithelium
- expelled epithelial and goblet cells
- diffuse hemorrhages in the epithelial layer
- distinct edema of the sub-epithelial layer
- several breaches in the basement membrane
- perivascular edema and infiltrates in the upper layer of the lamina propria formed by polymorphonuclear leukocytes, predominantly neutrophils and small lymphocytes, sometimes also plasma cells
- disrupted wall of several capillaries accompanied sometimes by expulsion of erythrocytes into the perivascular tissue.

These changes represent a distinct damage of the nasal mucosa due to a distinct inflammatory component.

Fig. 36a. HISTOLOGY OF NASAL MUCOSA DURING THE LATE NASAL RESPONSE

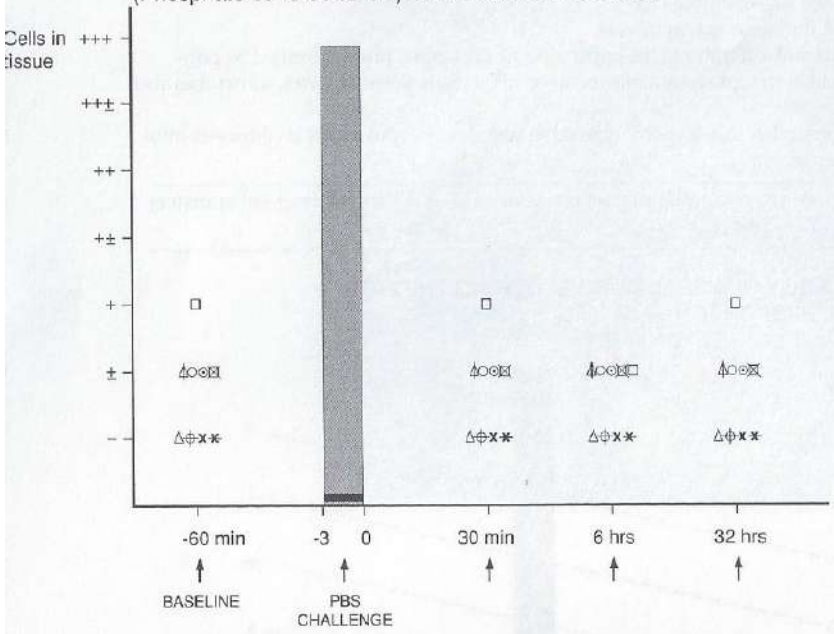


Appearance of particular cell types in the nasal mucosa before and during the "Isolated late nasal response to allergen challenge" (ILNR; n=6).

□ – – – – □ = Eosinophils
○ – – – – ○ = Neutrophils
△ – – – – △ = Basophils
▲ – · – · – ▲ = Mast cells
⊕ ····· ⊕ = Epithelial cells
x ····· x = Goblet cells
⊠ ····· ⊠ = Lymphocytes
✱ – · – · – ✱ = Monocytes
⊙ ····· ⊙ = Plasma cells

References: 96,97

Fig. 36b. HISTOLOGY OF NASAL MUCOSA DURING THE "PBS" (Phosphate buffered saline) CONTROL CHALLENGE

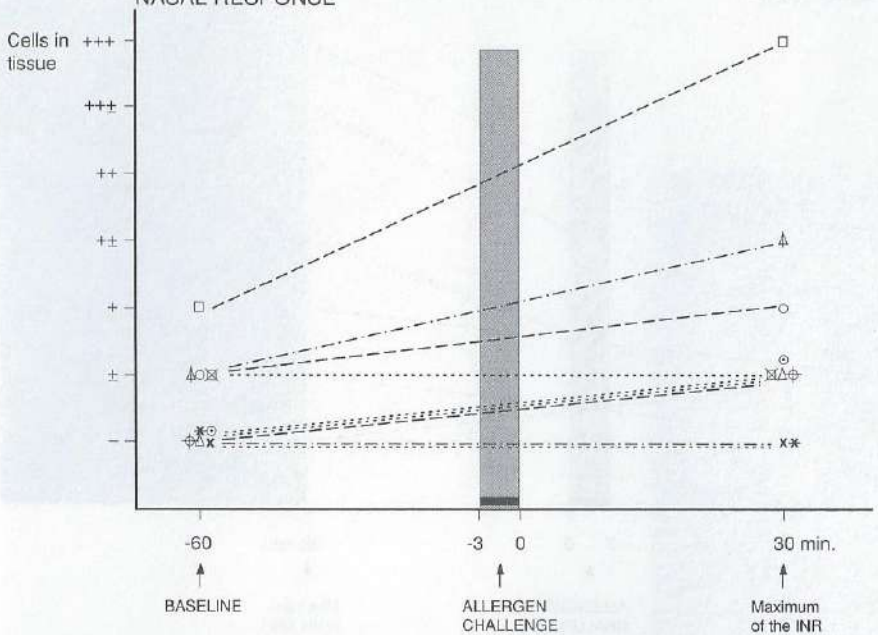


Appearance of particular cell types in the nasal mucosa before and after "PBS control challenge". Patients were divided into the 3 groups. Two biopsies were always performed in each of the patients; Gr I (n=8) at -30 min and +30 min, Gr II (n=5) at -30 min and +6 hrs and Gr III (n=5) at -30 min and +32 hrs.

□ = Eosinophils; ○ = Neutrophils; △ = Basophils; ⋔ = Mast cells;
⊕ = Epithelial cells; x = Goblet cells; ⊠ = Lymphocytes; ✳ = Monocytes;
⊙ = Plasma cells

References: 96,97,97a

Fig. 37. HISTOLOGY OF NASAL MUCOSA DURING THE IMMEDIATE NASAL RESPONSE

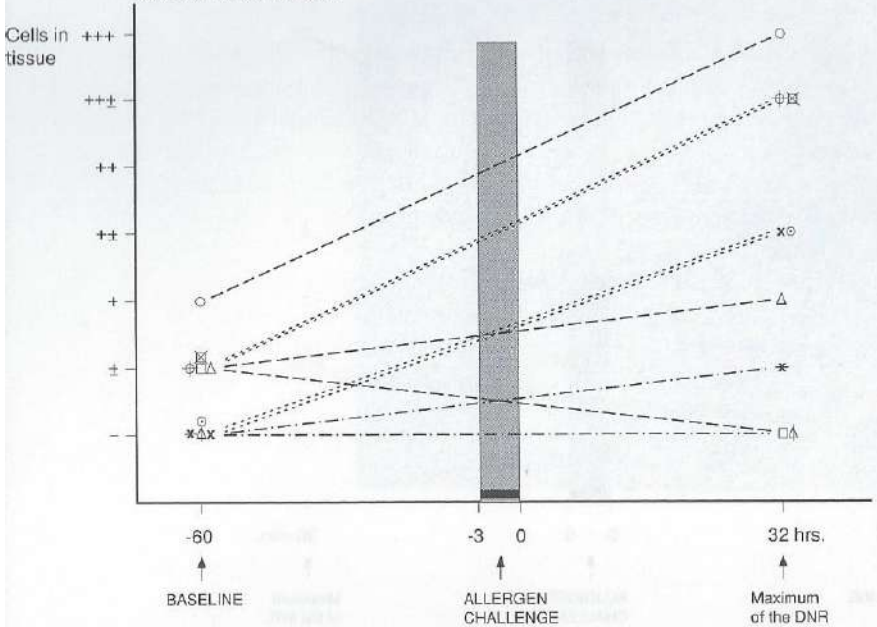


Appearance of particular cell types in the nasal mucosa before and during the "Isolated immediate nasal response to allergen challenge" (IINR; n=6).

□ – – – – □ = Eosinophils
○ – – – – ○ = Neutrophils
△ – – – – △ = Basophils
⋔ – · – · – ⋔ = Mast cells
⊕ ····· ⊕ = Epithelial cells
x ····· x = Goblet cells
⊠ ····· ⊠ = Lymphocytes
✳ – · – · – ✳ = Monocytes
⊙ ····· ⊙ = Plasma cells

References: 96,97a

Fig. 38. HISTOLOGY OF NASAL MUCOSA DURING THE DELAYED NASAL RESPONSE



Appearance of particular cell types in the nasal mucosa before and during the "Isolated delayed nasal response to allergen challenge" (IDYNR; n=6).

□ ---- □ = Eosinophils
○ ---- ○ = Neutrophils
△ ---- △ = Basophils
⋔ -·-·- ⋔ = Mast cells
⊕ ····· ⊕ = Epithelial cells
x ····· x = Goblet cells
⊠ ····· ⊠ = Lymphocytes
✱ -·-·- ✱ = Monocytes
⊙ ····· ⊙ = Plasma cells

References: 96

III. LATE NASAL RESPONSE TO FOOD

The LNR caused by "adverse reactions to foods" (the food allergy being one of the suspect mechanisms), should be regarded as a special form of the late-phase reaction (LPR) in the nose (Table 43; Figures 39,40).[26,27a,37,40a,41,41b,41e,41g,41i]

1. THE ROLE OF FOODS AND FOOD ALLERGY IN ALLERGIC DISORDERS

The role of food allergy and of food in general, in subjects with various allergic disorders, especially in patients with nasal symptoms and complaints, is still underestimated by the clinicians.[26] The participation of foods in patients with allergic rhinitis and the involvement through the hypersensitivity as one of the possible mechanisms, has already been discussed in the literature.[98-104] However, until now only few papers dealing with the nasal response due to food ingestion challenge have been published. [26,27a,37, 40a,41,41e,41i,101,104]

The role of foods in various allergic disorders has been a topic of our research and clinical interest for several years. We have studied the involvement of foods in patients with allergic rhinitis,[26,37-40,40a,40b,41,41b,41e] secretory otitis media, [26,27a] bronchial asthma,[39,105-111,111a-111c] urticaria,[112-114,114a] atopic eczema,[39,115,116,116a-116c,117] migraine,[39,117b-117e] and other disorders,[117a-117h] as well as the pharmacologic modulation of these responses.[26,37,38,40,40a, 40b,106,108,111,111a-111c,112-114,114a,116b,116c,117, 117a,117c-117e,117h, 117h]

2. BASIC TYPES OF NASAL RESPONSE TO FOOD INGESTION

The existence of various clinical types of nasal mucosa response due to the food ingestion challenge has probably been reported for the first time by us,[26,37,41,117] and is extensively described in our previous and recent papers.[38,40,40a,40b,41,41b,41e]

Three basic types of nasal response, following the food ingestion challenge, have been recorded by us: (1) an immediate response [INR], occurring within 70 min, with a peak within 105 min and resolving within 180 min; (2) a late response [LNR], appearing within 6 hrs, with a peak within 10 hrs and resolving within 24 hrs; (3) a delayed response [DYNR], beginning within 24 to 28 hrs, with a peak within 32 to 36 hrs and resolving within 48 to 52 hrs after the food ingestion challenge (Table 44; Figures 39,40, 41,42).[26, 37,41,117]

LNR to food has been recorded either as an isolated late response (ILNR) or as a dual response (DLNR), being a combination of an immediate and a late nasal response (Figure 40).[26,37,40,41]

The LNR occurs in approximately 47% of the patients with allergic rhinitis, in whom the foods participate in the nasal complaints.[41]

The clinical description of LNR to food in detail, its relationships and correlation with particular "*in vivo*" and "*in vitro*" diagnostic parameters, symptoms and other organs' reactions, the diagnostic procedures, including oral provocation with foods and the pharmacologic modulation and treatment, have already been extensively described in our previous papers.[26 41,41b]

Table 43 Survey of the disorders caused by foods, their ingredients or factors relating to them, which can lead to symptoms similar to those due to the food allergy mechanism.

1. Idiosyncrasy
2. Intolerance (e.g. enzymatic)
3. Non-specific hyperreactivity (e.g. histamine or other mediator liberators, food additives)
4. Toxicity
   (a) by non-controlled chemical compounds (e.g. insecticides, contaminants)
   (b) by micro-organisms
   (c) by products of micro-organisms
   – bacterial toxins
   – mycotoxins
   (d) by controlled chemical compounds exceeding their permitted threshold; or by permitted amounts of these compounds in individual subjects having increased susceptibility to these compounds (e.g. disinfectants) due to other metabolic disorders
5. Adverse non-immunological reaction to additives (controlled chemical compounds)
   (a) preservation and conservation compounds
   (b) colouring compounds
   (c) flavouring compounds
   (d) consistency correcting compounds, emulsifiers and stabilizers
   (e) antioxidants
   (f) adjuvants
6. Psychological disorders

References : 11h,26,27a,40a,41,109,111b,111c

Table 44 The time-course of the individual clinical types of nasal response to the food ingestion challenge.

	Onset	Maximum	Resolving
Immediate	10-20 minutes	30-45 minutes	90-120 minutes
Late	4-6 hours	6-10 hours	12-24 hours
Delayed	24-28 hours	32-36 hours	48-52 hours

The time is expressed in minutes or hours after a 60-minute waiting interval following the food ingestion challenge.
References: 26,40,40a,41,117f

3. "LNR" TO FOOD AND OTHER DIAGNOSTIC PARAMETERS

The LNR to food has been associated with other "*in vivo*" and "*in vitro*" diagnostic parameters and factors as follows (Table 45):[26,41] (1) positive disease history in 29%; (2) positive late skin response in 48%; (3) increased total serum IgE (PRIST) in 0.5%; (4) positive specific serum IgE in 1.5%; (5) increased total IgG in serum in 24%, total IgM in 10% and total IgA in 1%; (6) increased blood eosinophil count in 8% and leukocyte count in 9%; (7) appearance of nasal mucosa: hyperemic aspect in 23%, violaceous aspect in 76%; (8) nasal mucosa haemorrhages in 21%; (9) nasal symptoms: obstruction in 96%, hypersecretion in 14%, sneezing in 0%, itching in 51%; (10), changes in the nasal secretions count of eosinophils in 63%, of neutrophils in 70%, goblet cells in 51% and epithelial cells in 46%.

Table 45 The association of the particular types of nasal response to food ingestion challenge with other "*in vivo*" and "*in vitro*" diagnostic parameters.

	Nasal mucosa response to food ingestion			
	Immediate (n=267)	Late (n=203)	Delayed (n=164)	Negative (n=309)
Positive skin response - immediate	146			48
- late		98		11
- delayed			69	3
Increase in total serum IgE (PRIST)	11	1	0	3
Increase in specific serum IgE(RAST)	38	3	1	4
Increase in blood eosinophils	14	17	2	2
Increase in blood leukocytes	10	38	11	3
Aspects of the nasal mucosa:				
hyperemia	197	48	1	5
violaceous aspect	70	155	163	0
Nasal mucosa haemorrhages	0	42	72	0
Nasal secretions –				
Changes in count of:				
eosinophils	201	128	53	21
mast cells/basophils	53	30	6	0
neutrophils	108	142	151	15
goblet cells	39	103	114	2
lymphocytes	9	5	146	0
epithelial cells	17	93	158	3

References : 11h,26,40,40a,41,109,117f

4. "LNR" TO FOOD AND OTHER ORGANS'SYMPTOMS

The LNR to food has also been regularly accompanied by other organ symptoms, such as conjunctival injection, otalgia, pressure in the sinuses, general malaise complaints, fatigue syndrome, cephalgia, gastro-intestinal complaints (nausea, vomiting, diarrhea),[14g,26,40a,41,41g] and sometimes also bronchial obstruction,[26,41] migraine,[39, 117e] or other symptoms or complaints [Table 46; Figures 41b,43; Plates 21a-21c, 22a-22c (page 369)]. [117a-117h]

5. PHARMACOLOGIC MODULATION OF THE "LNR" TO FOOD

The pharmacologic modulation of LNR due to food ingestion challenge has also been studied by us.[26,38,40,40a,40b,117h,117i] Disodium cromoglycate (DSCG, Nalcrom®), in a daily oral dose of 4 x 200 mg, has demonstrated highly significant protective effects both on the LNR ($p < 0.01$) and on the INR ($p < 0.01$), whereas it has protected the DYNR to a slight degree only ($p \leq 0.05$) (Figures 40b,41c,42c).[26,38,40,40a,40b]

In contrast, we have found that the oral antihistamines (H_1-, H_2-receptor antagonists) as well as the nasal topical glucocorticosteroids have not been able to prevent significantly either LNR or INR to the food ingested.[118] Furthermore, the oral glucocorticosteroids (GCS) have prevented the food-related LNR ($p < 0.02$), but not the INR ($p > 0.05$), whereas the intravenously administered glucocorticosteroids (GCS) have prevented significantly LNR ($p < 0.02$) and have also partly influenced INR ($p = 0.05$) (unpublished data of the author).

Table 46 The nasal complaints and the other organs' responses accompanying the particular types of the nasal response to food ingestion challenge.

	Nasal mucosa response to food ingested			
	Immediate (n=267)	Late (n=203)	Delayed (n=164)	Negative (n=309)
Nasal complaints				
obstruction	267	203	164	0
sneezing	19	1	0	1
hypersecretion	193	166	39	16
itching	181	75	145	13
General malaise complaints	22	54	49	1
Conjunctival irritation	35	18	6	0
Middle ear response (otalgia, decrease in hearing, changes in middle ear pressure)	31	19	13	10
Pressure in the sinuses (maxillary and frontal, acute edema of sinus mucosa)	45	32	33	7
Cephalgia	56	91	125	42
Urticaria	4	7	8	5
Angio-neurotic edema (labial, palpebral or elsewhere)	9	6	3	3
Increase in body temperature	4	21	1	0
Bronchial complaints	13	15	12	8
Other complaints	2	1	2	0

References: 11h,26,40,40a,41,117f

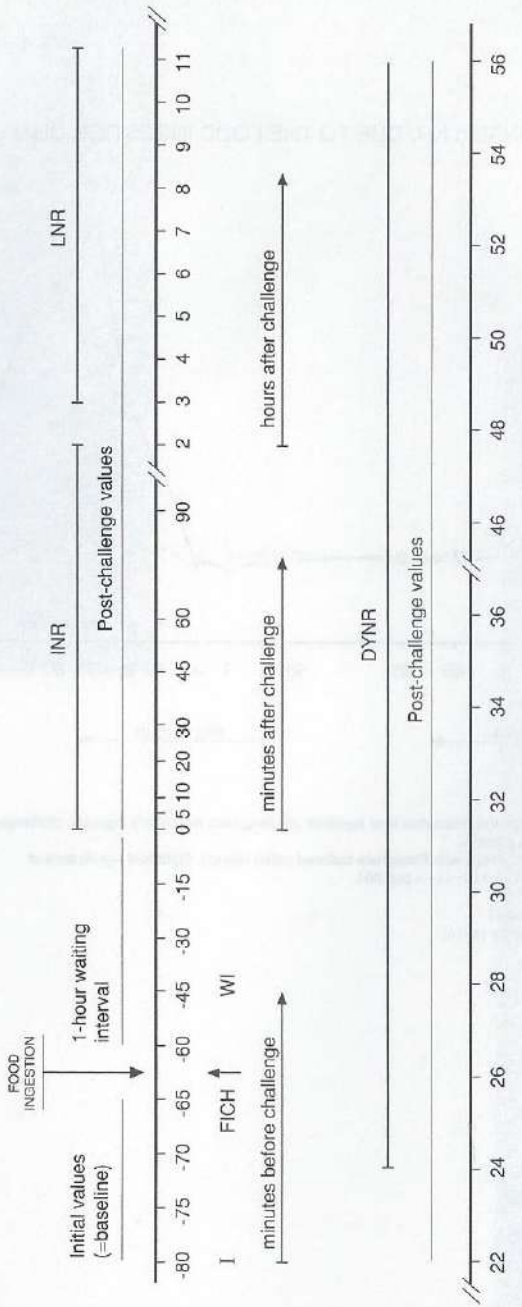
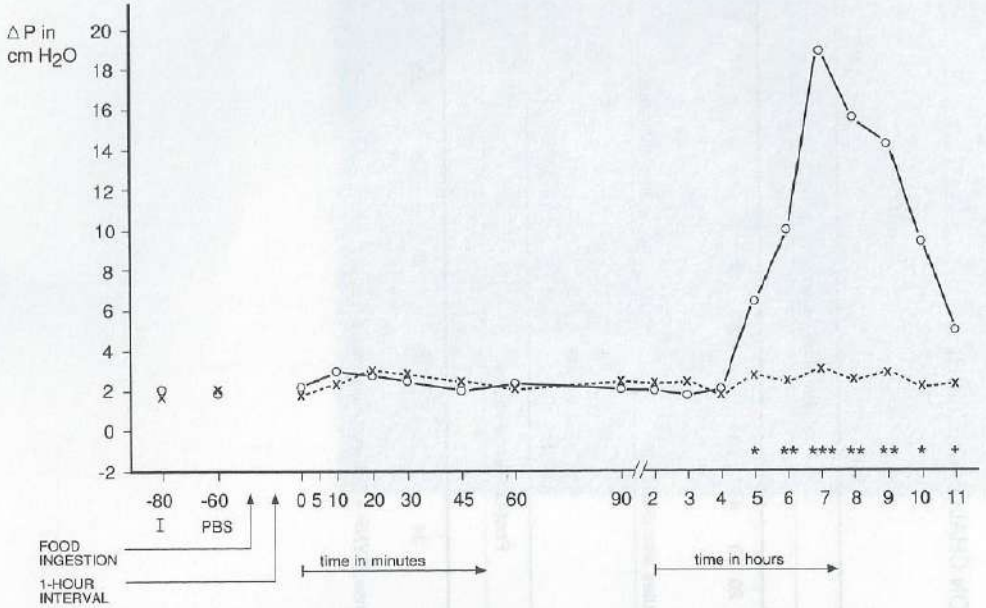


Fig. 39. SCHEDULE OF THE FOOD INGESTION CHALLENGE [FICH]

INR = Immediate nasal response; LNR = Late nasal response; DYNR = Delayed nasal response.

References: 26,27a,37,40a,41,41b,41e,41g,41i

Fig. 40a. THE LATE NASAL RESPONSE (LNR) DUE TO THE FOOD INGESTION CHALLENGE

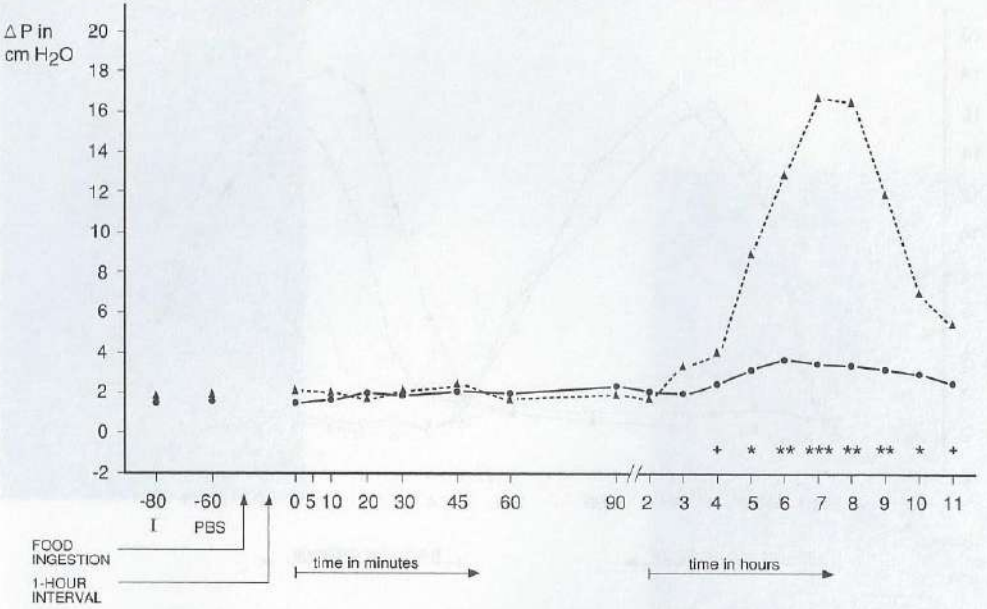


The mean NPG values, recorded after the non-pretreated food ingestion challenge and the control ingestion challenge with indifferent food, were calculated from 14 patients.
I = Initial value (mean); PBS = Control challenge with Phosphate buffered saline (mean), Statistical significance of differences: + = p=0.05; * = p<0.05; ** = p<0.01; *** = p<0.001.

o———— o = Non-pretreated LNR (n=14)
x ---------- x = Control ingestion challenge (n=14)

References: 11g,11h,14g,26,27a,37,38,40,40a,41,41b,41e,41g,41i,117e-117h

Fig. 40b. PROTECTIVE EFFECTS OF ORAL CROMOLYN (DSCG) ON THE LATE NASAL RESPONSE (LNR) DUE TO THE FOOD INGESTION CHALLENGE

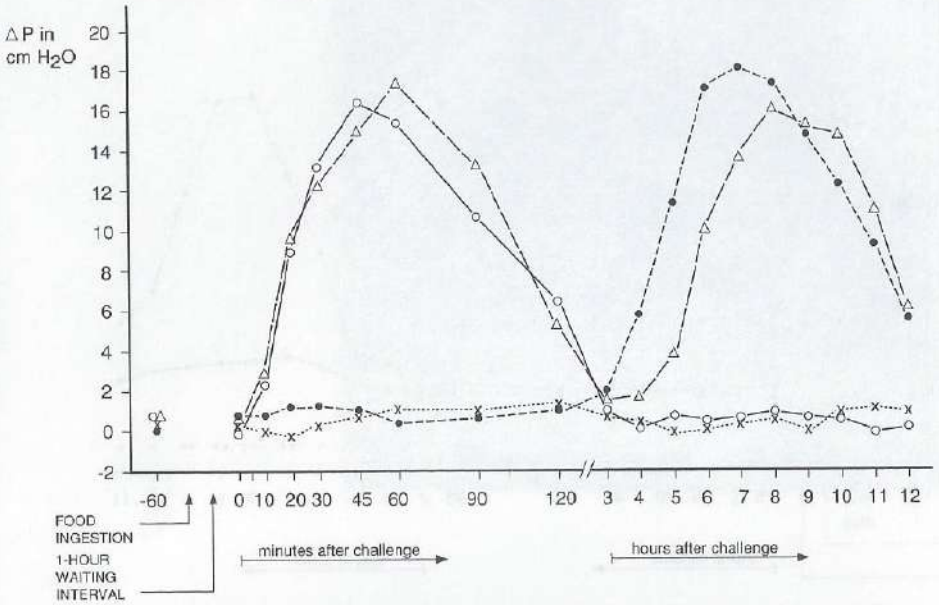


The mean NPG values, recorded after the food ingestion challenges pretreated with oral Disodium cromoglycate (Cromolyn, DSCG) and with Placebo, were calculated from 14 patients.
I = Initial value (mean); PBS = Phosphate buffered saline (mean), Statistical significance of the differences (protective effects of DSCG with respect to the Placebo): + = p=0.05; * = p<0.05; ** = p<0.01; *** = p<0.001.

•———— • = LNR pretreated with oral DSCG (n=14)
▲ ---------- ▲ = LNR pretreated with oral Placebo (n=14)

References: 11h,14g,26,27a,37,38,40,40a,41,41b,41e,41g,41i,117e-117h

Fig. 41a. IMMEDIATE, LATE AND DUAL LATE NASAL RESPONSE TO THE FOOD INGESTION CHALLENGE

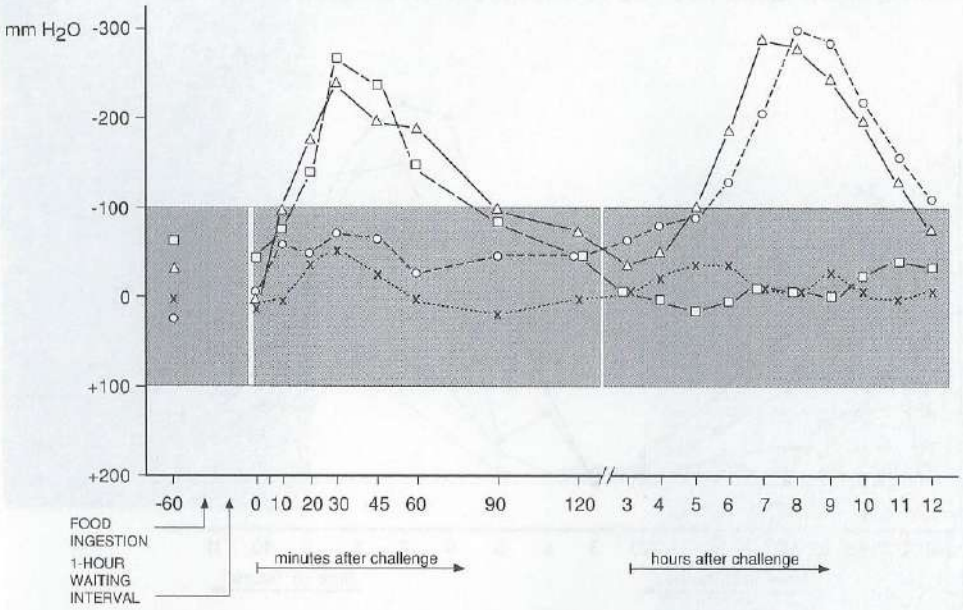


Mean nasopharynx-nostril pressure gradient (NPG) values recorded after the food ingestion challenge were calculated from all patients developing the same type of the nasal response (NR).

= Isolated immediate NR (n=10)
= Isolated late NR (n=15)
= Dual late NR (n=6)
= Control food challenge (n=28)

References: 27a,27b,41g

Fig. 41b. MIDDLE EAR RESPONSE TO THE FOOD INGESTION CHALLENGES

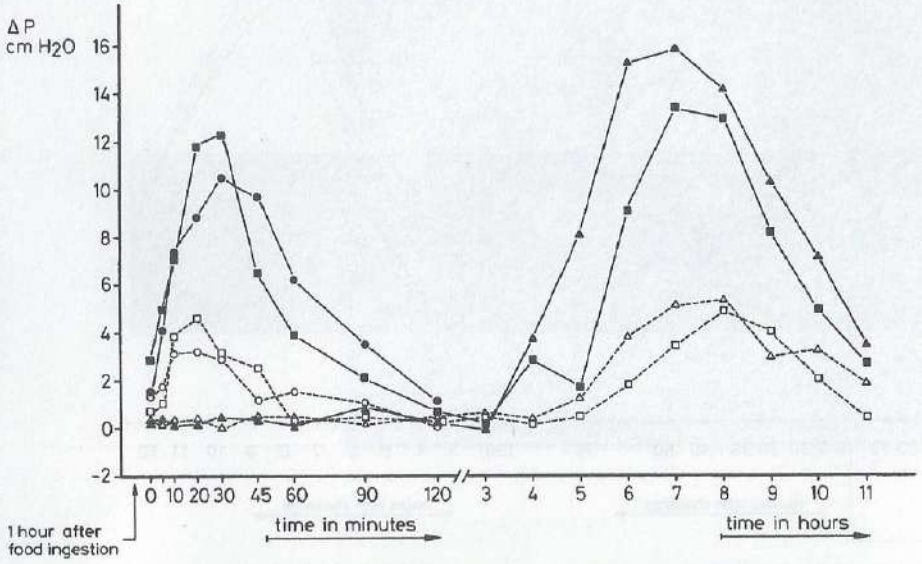


The tympanometric values (MEP=middle ear pressure in mmH_2O) were calculated from all patients with te same type of the middle ear response (MER). The spotted area represents the normal value range of MEP.

□ — — — □ = Isolated immediate MER (n=17)
△ ——— △ = Dual late MER (n=10)
○ — — — ○ = Isolated late MER (n=8)
x ------- x = Control food challenge (n=28)

References: 11h,26,41g,41i

Fig. 41c. THE PROTECTIVE EFFECTS OF ORAL DISODIUM CROMOGLYCATE (DSCG) ON THE NASAL RESPONSE DUE TO THE INGESTION CHALLENGE WITH FOOD



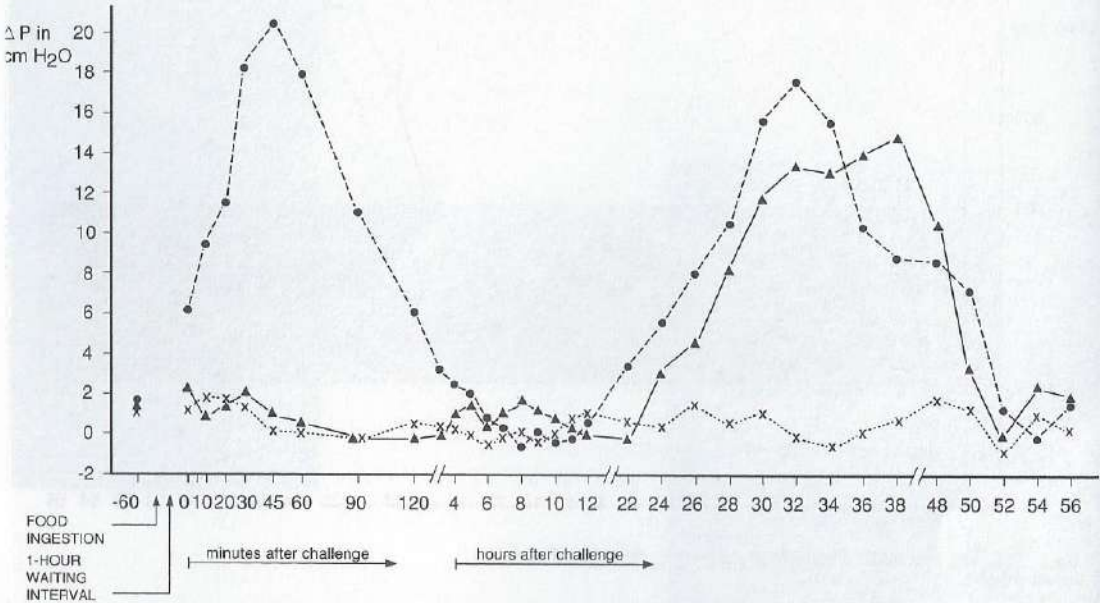
The mean NPG values after non-pretreated and pretreated nasal mucosa responses due to the food ingestion challenge, with respect to the appropriate "Coca's solution" NPG values, were always calculated from all patients developing the same type of nasal response.

DSCG = Disodium cromoglycate-orally.

ISOLATED IMMEDIATE RESPONSE (n=1): ●——● = non-pretreated, o-------o = pretreated with DSCG;
ISOLATED LATE RESPONSE (n=2): ▲——▲ = non-pretreated, △-------△ = pretreated with DSCG;
DUAL RESPONSE (immediate + late)(n=22): ■——■ = non-pretreated, □-------□ = pretreated with DSCG.

References: 27b,38,40,40a,41

Fig. 42a. DELAYED NASAL RESPONSE TO THE FOOD INGESTION CHALLENGE

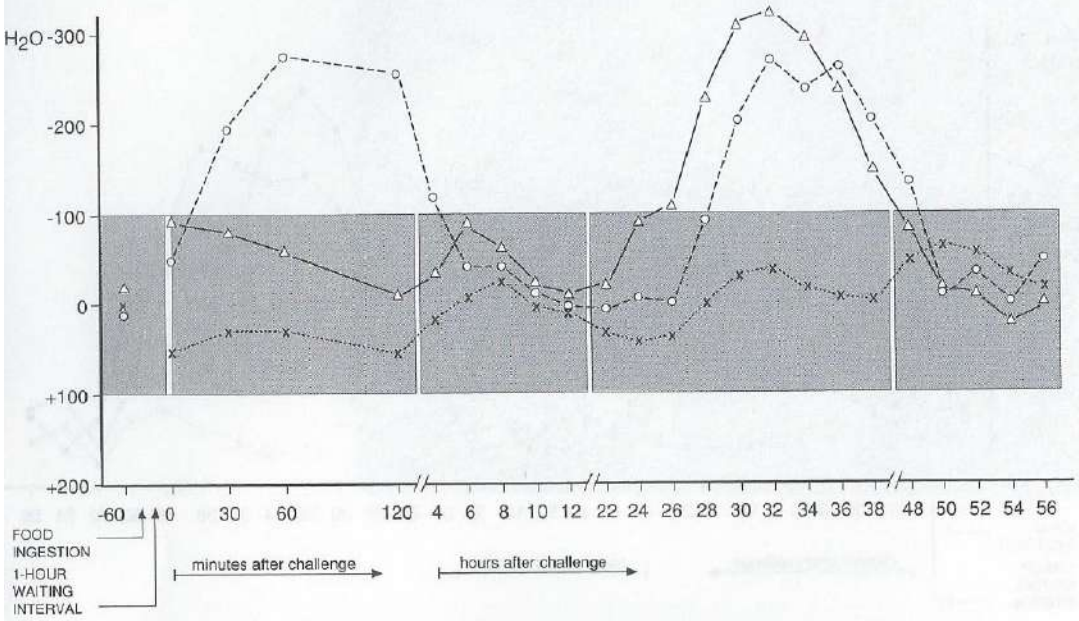


Mean nasopharynx-nostril-pressure gradient (NPG) values recorded after the food ingestion challenge, were calculated from all patients developing the same type of nasal response (NR).

▲——▲ = Isolated delayed NR (n=18)
●– – –● = Dual delayed NR (n=15)
x·····x = Control food challenge (n=35)

References: 27b,41

42b. MIDDLE EAR RESPONSE TO THE FOOD INGESTION CHALLENGE

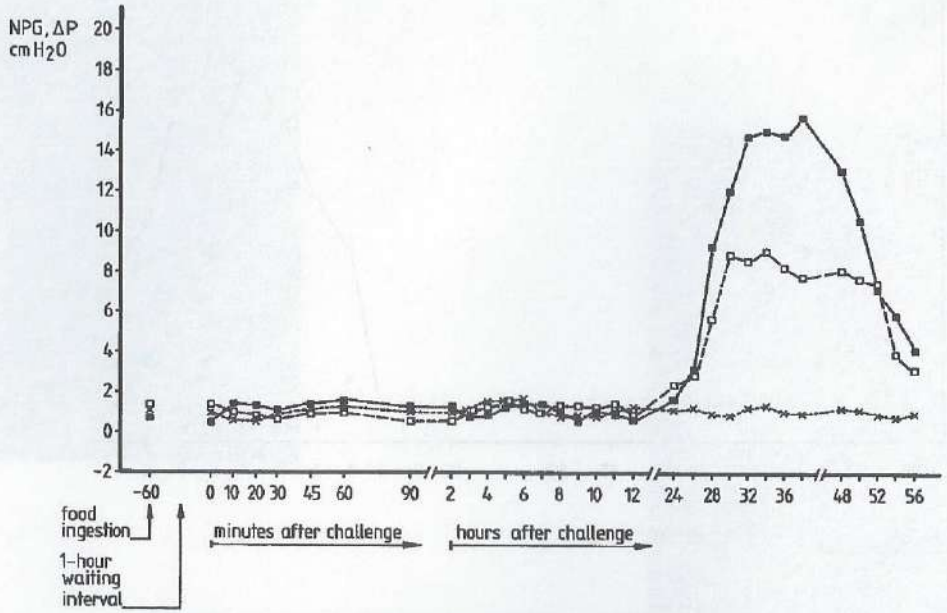


The tympanometric values (MEP=middle ear pressure in mmH_2O) were calculated from all patients with te same type of the middle ear response (MER). The spotted area indicates the normal value range of MEP.

△———△ = Isolated delayed MER (n=21)
o- - - - - o = Dual delayed MER (n=13)
x·····x = Control food challenge (n=34)

References: 27b,38,40,40a

Fig. 42c. THE PROTECTIVE EFFECTS OF ORAL DISODIUM CROMOGLYCATE (DSCG) ON THE DELAYED NASAL RESPONSE DUE TO THE INGESTION CHALLENGE WITH FOOD

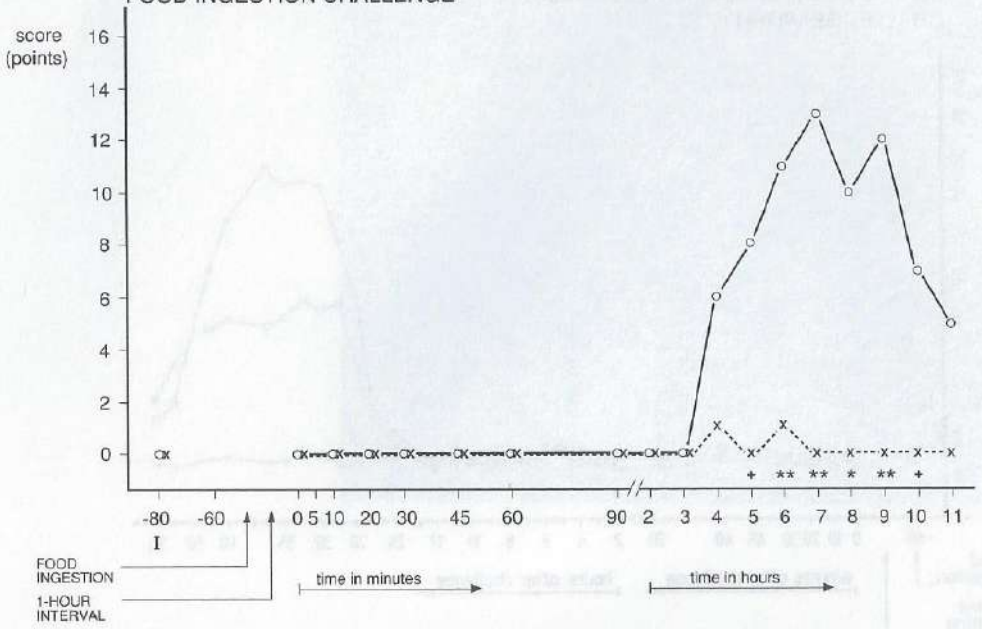


The mean NPG values after non-pretreated and pretreated nasal mucosa responses due to the food ingestion challenge, with respect to the appropriate control NPG values, were calculated from all patients developing the same type of nasal response.

DSCG = Disodium cromoglycate orally.
DELAYED NASAL RESPONSE (n=6):
■———■ = non-pretreated
□———□ = pretreated with DSCG
CONTROL TEST (n=6): x———x

References:

Fig. 43a. THE LATE MIGRAINE RESPONSE (LMR) [=MIGRAINE, HEADACHE] DUE TO THE FOOD INGESTION CHALLENGE

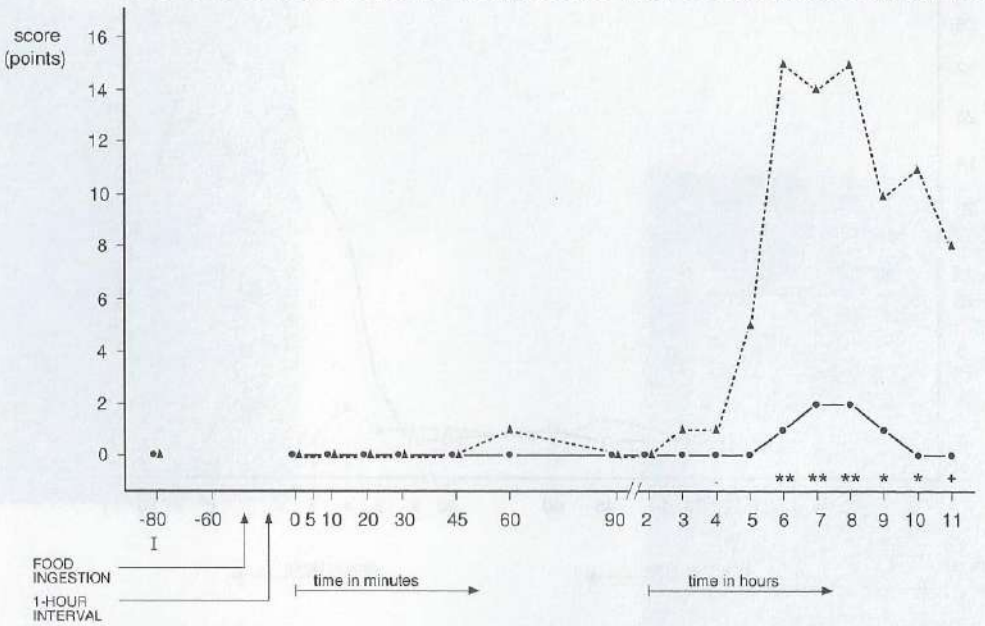


The mean score values of the "migraine headache", recorded after the non-pretreated food ingestion challenge and the control ingestion challenge with indifferent food, were calculated from 14 patients.
I = Initial value (mean). Statistical significance of the differences: + = p=0.05; * = p<0.05; ** = p<0.01.

o ——— o = Non-pretreated LMR (n=14)
x ---------- x = Control ingestion challenge (n=14)

References: 14g,26,39,40a,41,41g,117a-117h

Fig. 43b. PROTECTIVE EFFECTS OF ORAL CROMOLYN (DSCG) ON THE LATE MIGRAINE RESPONSE [=MIGRAINE, HEADACHE] DUE TO THE FOOD INGESTION CHALLENGE

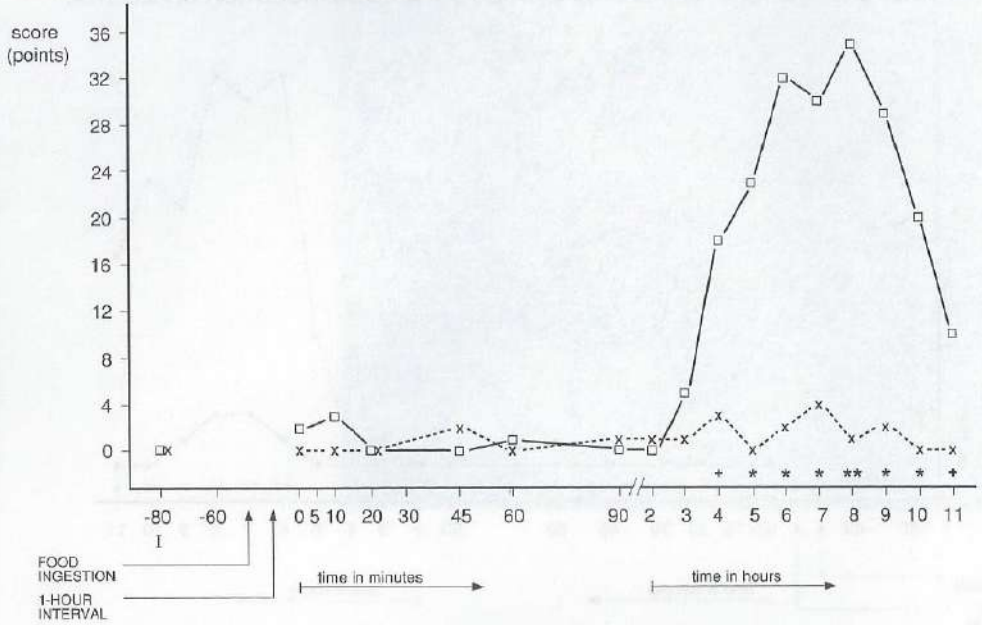


The mean score of the "migraine headache", recorded after the food ingestion challenges pretreated with oral Disodium cromoglycate (Cromolyn, DSCG) and with Placebo (PL), were calculated from 14 patients.
I = Initial value (mean), Statistical significance of the differences (=protective effects of DSCG with respect to the Placebo):
+ = p=0.05; * = p<0.05; ** = p<0.01.

• ——— • = LMR pretreated with oral DSCG (n=14)
▲ ---------- ▲ = LMR pretreated with oral Placebo (n=14)

References: 14g,26,39,40a,41,41g,117a-117h

Fig. 43c. LATE MIGRAINE RESPONSE (LMR) DUE TO THE FOOD INGESTION CHALLENGE

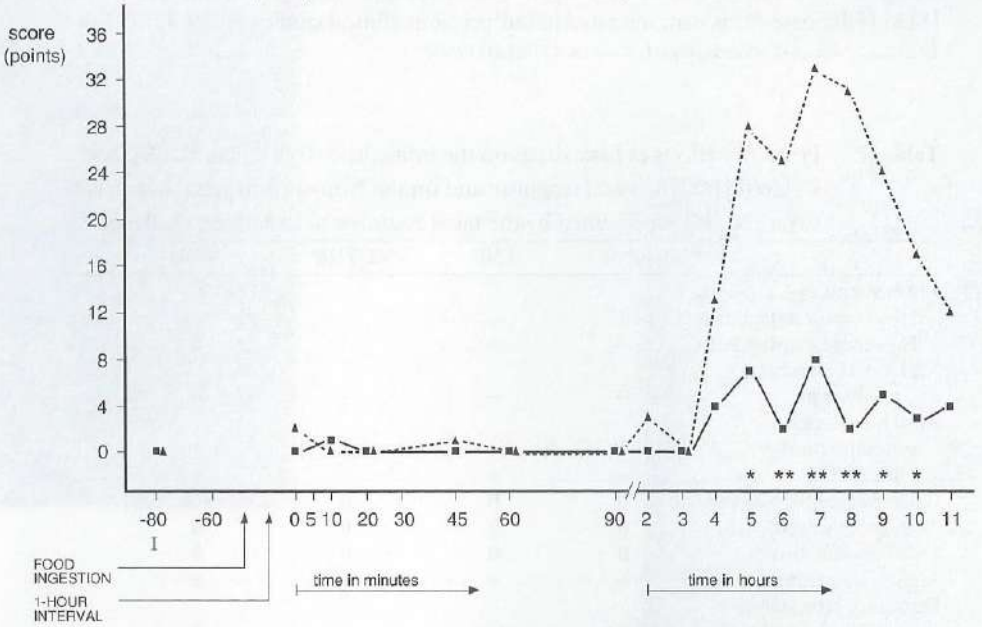


The mean score values of all symptoms (headache and accompanying symptoms: photophobia, nausea, visual aura, vomiting, dizziness, hypersalivation), recorded after the non-pretreated food ingestion challenge and the control ingestion challenge with indifferent food, were calculated from 14 patients.
I = Initial value (mean), Statistical significance of the differences: + = p=0.05; * = p<0.05; ** = p<0.01.

□———□ = Non-pretreated LMR (n=14)
x ---------- x = Control ingestion challenge (n=14)

References: 14g,26,39,40a,41,41g,117a-117h

Fig. 43d. PROTECTIVE EFFECTS OF ORAL CROMOLYN (DSCG) ON THE LATE MIGRAINE RESPONSE (LMR) DUE TO THE FOOD INGESTION CHALLENGE



The mean score values (headache and accompanying symptoms), recorded after the food ingestion challenges pretreated with oral Disodium cromoglycate (Cromolyn, DSCG) and with Placebo (PL), were calculated from 14 patients.
I = Initial value (mean), Statistical significance of the differences (=protective effects of DSCG with respect to the Placebo):
+ = p=0.05; * = p<0.05; ** = p<0.01.

■———■ = LMR pretreated with oral DSCG (n=14)
▲ ---------- ▲ = LMR pretreated with oral Placebo (n=14)

References: 14g,26,39,40a,41,41g,117a-117h

IV. PHARMACOLOGIC MODULATION OF "LNR"

The LNR can be prevented significantly (almost completely) by intranasal Disodium cromoglycate [DSCG] (Rynacrom®, Lomusol®, Intal®, Nasalcrom®) as well as by topical glucocorticosteroids such as Beclomethasone dipropionate [BDA] (Beconase®, Viarin®, Aldecin®, Vancenase®), Budesonide [BSA/BUD]* (Rhinocort®) or Fluticasone [FLS] (Flixonase ®), as demonstrated in our previous clinical studies (Table 47).[2,12,14,17,21,22,27,40c-40d,40f,41b,,41g,41i,72a,118, 121b,121i,121j]

Table 47 Protective effects of basic drugs on the Immediate [INR], Late [LNR], and Delayed [DYNR] nasal response and on the Non-specific nasal hyperreactivity [N-SH] represented by the nasal response to histamine challenge.

	INR	LNR	DYNR	N-SH
Antihistamines				
H_1-receptor antagonists	±*	–	–	+
H_2-receptor antagonists	–	–	–	0
H_1- + H_2- receptor antagonists	–	–	–	0
Anticholinergics				
systemic (oral)(a)	–	–	–	+
topical(b)	–	–	–	+
Calcium channel blockers	0	0	0	0
Acetylsalicylic acid	0	0	0	0
cAMP modulators	0	0	0	0
Alpha$_2$-sympathomimetics	–	–	–	±
Disodium cromoglycate (DSCG, Cromolyn)	+++	++	–	±
Nedocromil sodium (NDS)	+++	+++	±**	0
Corticosteroids				
systemic - oral	–	–	–	–
- injection (i.v., i.m.)	±	±	±	–
topical	–	+++	+++	+
Immunotherapy	±	–	–	–

–= No effect; ±= slight or partial effects (without significance); += positive effects ($p<0.05$); ++= distinctly significant effects ($p<0.01$); +++= highly significant effects ($p<0.001$); 0= lack of data. * Some of these drugs demonstrated significant protective effects on the INR (such as Cetirizine, Clemastine, Chlorphenamine, Mebhydroline and recently also Loratadine), whereas other did not (such as Ketotifen, Astemizole, Terfenadine, Levocabastine). ** Recent preliminary data may suggest some protective effects of this drug on the DYNR. (a) = Thiazinamium hydrochloride, Oxyphenonium. (b) = Ipratropium bromide

References: 10,11,11d,11e,11h,11i,12,13,13b,14,40c-40f,41b,41i,48l,72d,121i,121j,122a-122d

* Budesonide has been abbreviated as BSA in our earlier studies, whereas it has been designated as BUD in our later studies

1. DISODIUM CROMOGLYCATE (CROMOLYN)[DSCG] AND GLUCOCORTICOSTEROIDS [GCS]

However, some differences have been observed by us with respect to the two subforms of LNR.[12] The LNR being a part of the "dual response" has been prevented by DSCG to a slightly higher degree than the isolated LNR, while the isolated LNR has been prevented to a slightly higher degree by BDA or BSA/BUD than the LNR, being a part of the "dual response" (Tables 48,49,50; Figure 32a, 44).[12,14,41b] In contrast, the INR has been prevented highly significantly by DSCG, while it has not been affected by BDA or BSA/BUD at all (Figures 33a, 45, 46).[10,11,11e,11i,14,40b,72d,119,121d] The DYNR has been prevented highly significantly by BDA and BSA/BUD, while it has not been affected significantly by DSCG (Figure 34a).[13,13b]

No other comparative studies of the protective effects of DSCG and topical corticosteroids on the immediate and the late nasal response to allergen challenge have been found by us in the available literature.

Recently, we have studied the possible protective effects of intranasal BSA/BUD in 20 patients developing a LNR to nasal challenge with pigeon or tropical bird dropping extracts.[121b,121j] These twenty patients, being regularly exposed to pigeons or tropical birds, suffering from perennial nasal complaints, demonstrating suspect history, positive skin tests with the particular pigeon or tropical bird faeces extracts and positive precipitating antibodies to pigeon or tropical bird droppings in the serum, have developed 14 cases of an isolated form of LNR and 6 cases of a dual late nasal response, being a combination of an immediate (INR) and a late response (LNR) as compared with placebo, Budesonide, after a 3-week administration in a daily dose of 400 mcg, has protected highly significantly the LNR (differences BSA-placebo: 3.72; $p=0.001$), whereas it has not affected the INR in any of the 6 cases ($p>0.05$) (Figure 28b).

Interestingly, the effects of DSCG and topical corticosteroids (aerosols) on the LNR are comparable with their effects on the late asthmatic response to allergen challenge (LAR), as reported both in our previous papers [63,64,67,68,68a-68c,68e-68g,121e-121h,121k] and by other investigators. [96a,96b,96k,121l,124b,161c,178m] Moreover, the differences in the effects of DSCG and topical corticosteroids on INR are similar to those observed by us in the case of the immediate asthmatic response to allergen challenge (IAR).[63,64,70,121e,121h]

Our findings of significant protective effects of DSCG on the INR are consistent with the results reported by other authors,[28,29,120,121] while the protective effects of DSCG on the LNR have not yet been sufficiently investigated by other investigators.[121a]

Besides the effects of intranasal DSCG and BDA or BSA/BUD on the particular clinical types of nasal response to allergen challenge,[2,10,11,12,13,14,,40f,41b,41i, 72a,72d,118] we have also investigated the effects of both the drugs on the appearance of individual cell types in NS, on the changes in their counts, accompanying the particular types of nasal response,[11d,11e,13b,20,25,40c,40d,40f,41b-41d, 41i,72a,72d] and on the intracellular changes recorded in individual cell types (Tables 35a,35b,36a,36b,37a,37b; Figures 32a-32d,33a-33d,34a-34d).[11a,11b,40f,72d]

The LNR pretreated with DSCG has been accompanied by distinctly decreased counts of all cell types in NS, as compared with the non-pretreated LNR, and by non-significant

changes in the counts of particular cell types, as compared before and after the allergen challenge.[40c,40d,40f] The LNR pretreated with BDAor BSA/BUD has also been accompanied by distinctly lower counts of all cell types in the NS than those recorded during the non-pretreated LNR, and no significant changes have even been found by us in the counts of particular cell types found. [40c,40d,40f] However, small differences have been observed in the counts of particular cell types after the treatment with the individual drugs. These results might indicate possible involvement of different mechanisms or, at least, different modifications of the basic mechanism in LNR (Tables 35a,35b,36a,36b,37a,37b; Figures 32b,32d,32e).

The intracellular changes (degranulation, vacuolization, diminished intake of stain, wrinkling of cellular membrane, cell disruption) recorded by us in most eosinophils (ES), neutrophils (NE), basophils (BA) and mast cells (MC), appearing in NS during the non-pretreated LNR [41d], have been significantly reduced by both DSCG and BSA, however with some differences.[40f] The DSCG treatment has reduced significantly the degranulation and other intracellular changes in ES, BS and MC, and partly also in NE. The BSA treatment has reduced highly significantly the degranulation and other intracellular changes in NE, partly in ES and BS, but not in MC (Tables 35c,36c,37c). These results may suggest again the possible involvement of different mechanisms or, at least, different sub-mechanisms in LNR.

Furthermore, the above described effects of DSCG as well as BSA on the LNR and the LNR-related cellular and intracellular changes differ distinctly from the effects of both drugs on the INR[10,11,11d,11e,72a,72d] and on the DYNR (Figures 33b,33d,33e,34b,34d, 34e).[13,13b]

Table 48 Distribution of the protective effects of Disodium cromoglycate (DSCG), Beclomethasone dipropionate (BDA) and Budesonide (BSA/BUD) on the Late nasal response to allergen challenge [LNR]

LNR n=28	Protective effects on LNR			
	p<0.001	p<0.01	p<0.05	p>0.05
DSCG (n=28)	9 (32%)	13 (46%)	5 (18%)	1 (4%)
BDA (n=14)	6 (43%)	8 (57%)	0	0
BSA (n=14)	5 (36%)	8 (57%)	1 (7%)	0

References: 41b, 41i, 72a

Table 49 Distribution of the protective effects of Disodium cromoglycate (DSCG), Beclomethasone dipropionate (BDA) and Budesonide (BSA/BUD) on the Immediate nasal response to allergen challenge [INR].

INR n=35	Protective effects on INR			
	p<0.001	p<0.01	p<0.05	p>0.05
DSCG (n=35)	21 (60%)	11 (31%)	3 (9%)	0
BDA (n=16)	0	0	0	16 (100%)
BSA (n=19)	0	0	1 (5%)	18 (95%)

References: 41i, 72a

Table 50 Distribution of the protective effects of Disodium cromoglycate (DSCG), Beclomethasone dipropionate (BDA) and Budesonide (BSA/BUD) on the Delayed nasal response to allergen challenge [DYNR].

DYNR n=16	Protective effects on the DYNR			
	p < 0.001	p < 0.01	p < 0.05	p > 0.05
DSCG (n=16)	0	0	1 (6%)	15 (94%)
BDA (n=8)	4 (50%)	3 (38%)	1 (12%)	0
BSA (n=8)	5 (63%)	2 (25%)	1 (12%)	0

References: 41i, 72a

The significant protective effects of topical corticosteroids on the LNR, as reported by us, are in agreement with other authors' results,[94,119] and may be supported by the findings of some investigators studying changes in the concentrations of some mediators and/or other factors[82,82j,83,94,121c] and also cellular changes[82,83,94] in NS during the LNR.

Bisgaard and colleagues[82j], studying timothy grasspollen-sensitive subjects who had developed a dual late nasal response to challenge with this allergen, have recorded an increase in the concentration of the eosinophil cationic protein (ECP) in the nasal lavage fluid during the late-phase nasal response (LNR), but not during the early-phase (INR). The ECP increase has been completely inhibited by pretreatment with intranasal Budesonide, in a daily dose of 400 mcg for 2 weeks prior to the nasal allergen challenge.

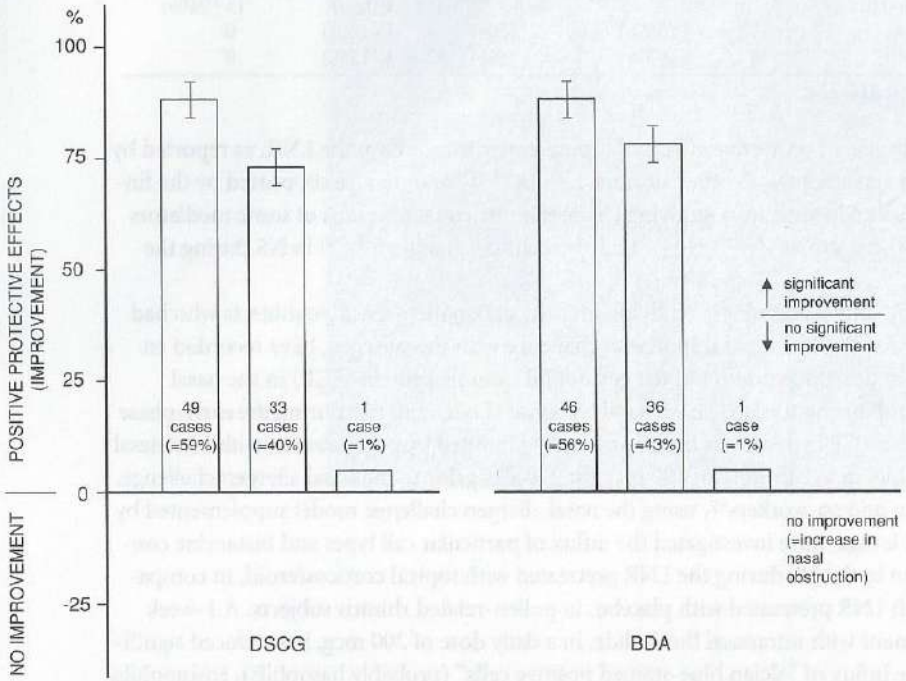
Bascom and co-workers[82], using the nasal allergen challenge model supplemented by the nasal lavage, have investigated the influx of particular cell types and histamine concentration in the NS during the LNR pretreated with topical corticosteroid, in comparison with LNR pretreated with placebo, in pollen-related rhinitis subjects. A 1-week pretreatment with intranasal flunisolide, in a daily dose of 200 mcg, has reduced significantly the influx of "alcian blue-stained positive cells" (probably basophils), eosinophils, neutrophils and mononuclear cells into NS. The histamine release into NS and late nasal symptoms have also been blocked by this topical corticosteroid treatment.

Other investigators studying predominantly the mediators and other factors in the nasal lavage fluid, have found that pretreatment with topical glucocorticosteroids (flunisolide) significantly reduced both the late nasal symptoms and the levels of histamine, TAME-esterase activity and kinins in NS, during the allergen-induced LNR.[83,121c]

The data generated by Bisgaard et al[82j] and Bascom et al[82] are very similar to our above described results concerning the cellular traffic in NS during LNR and its pharmacologic modulation by topical glucocorticosteroids. [11c,12,13,25,40c, 40d,40f,41b-41d,41i, 72a,72d]

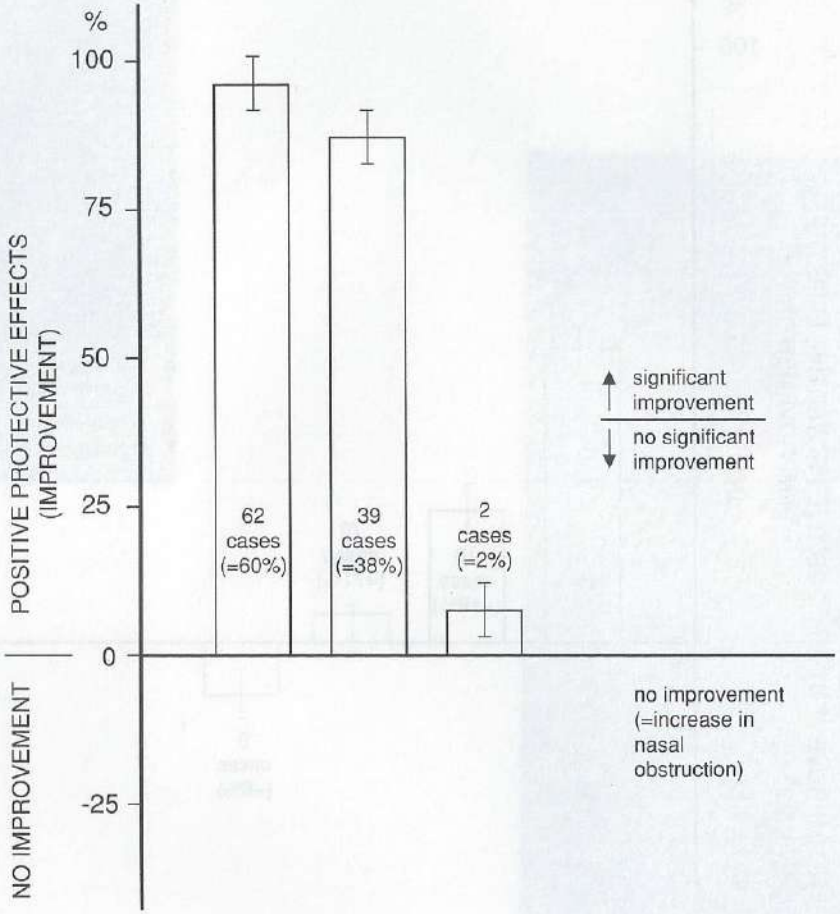
The lack of protective effects of topical corticosteroids on the INR, repeatedly observed by us and reported in our papers[10,11,11d,11e,72a,72d] is in agreement with results found by Houri et al[92] and Mygind et al[119], while in disagreement with data presented by Pipkorn et al[121c], Walden et al[94] and Togias et al.[83] More recently, Small and Barrett [121d] have demonstrated that topical corticosteroids even in high doses (Beclomethasone dipropionate - 400 mcg daily, Budesonide - 1200 mcg daily) administered for 2 weeks, did not affect the INR to nasal challenge with ragweed pollen, while they prevented significantly the nasal response to histamine.

Fig. 44. Distribution of the protective effects of DSCG and BDA (BSA) on the LNR (n=83).



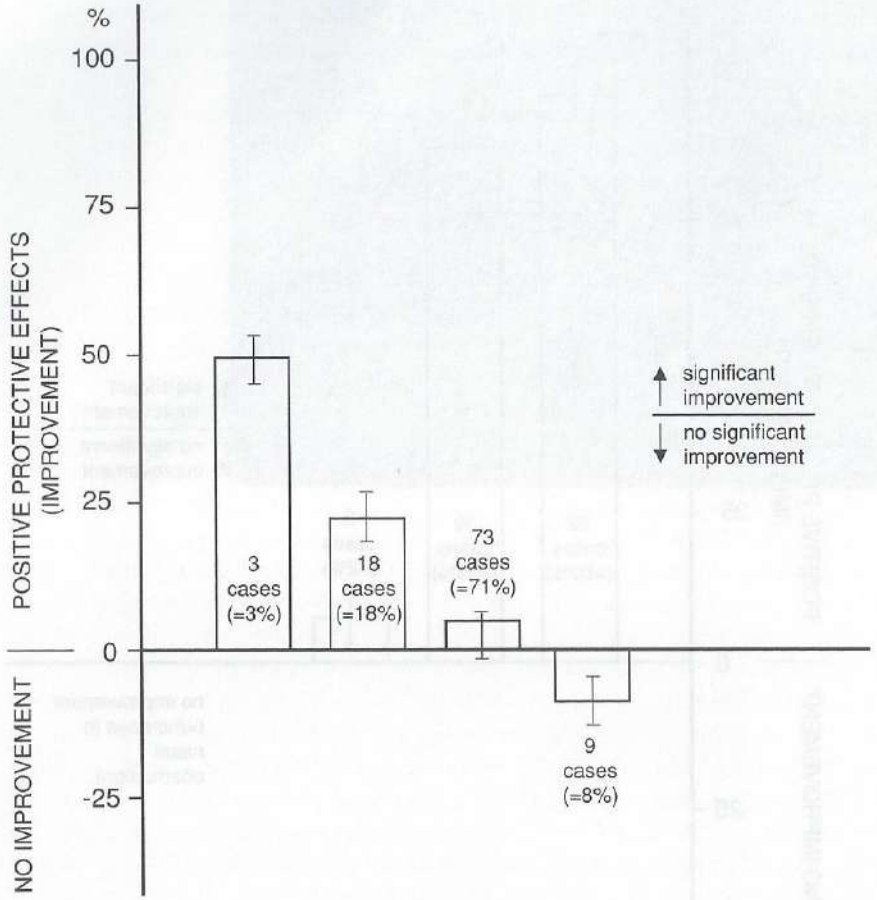
References: 14,41b

Fig. 45. Distribution of the protective effects of DSCG on the INR (n=103)



References: 10,11,11i,14

Fig. 46. Distribution of the protective effects of BDA (BSA) on the INR (n=103).



References: 10,11,14

Systemic corticosteroids have been reported to reduce significantly both the concentration of some mediators in NS[48,94,95,97b,121c] and the influx of various cell types into the NS,[48d,82,94,97b,97c] during LNR, as well as to prevent the development of the clinical LNR to allergen challenge.[48,48d,57,83,94,95,121c] In contrast to their excellent effects on LNR, the oral corticosteroids did not demonstrate any effects on INR or early-phase nasal response.[48d,94,95,97b] Pipkorn and colleagues[95] have observed a distinct inhibition of allergen-induced LNR as well as significant reduction of the concentrations of certain mediators (histamine, TAME-esterase, kinins) and albumin in the nasal lavage fluid after the pretreatment with systemic glucocorticosteroid (prednisone in a daily dose of 3 x 20 mg, for 2 days). In contrast, these authors have not found any significant effects of the oral corticosteroid treatment on the the early-phase of the nasal response symptoms or on the mediators released into the nasal lavage fluid, except for a slight reduction in kinin concentration.

Walden and co-workers[94] have studied subjects with pollen-related rhinitis, developing a dual LNR to allergen challenge, consisting of an early- and a late phase. The early-phase was accompanied by an increase in concentration of histamine, TAME-esterase, kinins and PGD_2 in NS and by the influx of "alcian blue-stained positive cells", probably basophils, into the nasal lavage fluid, whereas the late phase was accompanied by an increase in the level of histamine, TAME-esterase, kinins, sulfidopeptide leukotrienes and albumin, but not PGD_2, in NS, and by an influx of "alcian blue-stained positive cells", eosinophils and neutrophils into nasal lavage fluid. The 2-day pretreatment with oral corticosteroid (prednisone in a daily dose of 3 x 20 mg) has prevented the symptoms of LNR, decreased significantly the concentration of histamine, TAME-esterase, kinins and albumin in the nasal lavage fluid and reduced significantly the influx of eosinophils, but not of neutrophils or mononuclear cells. In contrast, the 2-day oral corticosteroid pretreatment has not been able to reduce either the symptoms of the allergen-induced early nasal response or the concentrations of mediators accompanying the cell influx into the nasal lavage fluid. On the other hand, this group of investigators has reported significant effects of topical corticosteroids in reducing the symptoms as well as the levels of histamine, TAME-esterase activity and kinins during both the early- and the late-phase nasal responses. However, the topical corticosteroids did not alter either the total number of cells or the differential distribution of cells present in pre-challenge nasal lavage fluid, or even the influx of particular cells during the early-phase nasal response, whereas the influx of eosinophils, neutrophils and "alcian blue-stained positive cells" has been significantly reduced for "several hours after the antigen provocation", meaning during the late-phase nasal response.

Bascom and colleagues[48d] have reported similar suppressive effects of oral corticosteroids on the influx of eosinophils, but not of neutrophils or mononuclear cells into the nasal-lavage fluid during the LNR. In another study, Bascom et al[97b] have recorded a significant increase in the concentration of major basic protein (MBP) in the nasal lavage fluid during both the early phase (INR) and the late-phase (LNR) nasal response to allergen challenge, a significant increase in the concentration of eosinophil-derived neurotoxin (EDN) during the late phase, and finally a significant increase in the influx of eosinophils into NS during the late phase. Pretreatment with oral prednisone has

reduced significantly not only the eosinophil influx, but also the concentration of both MBP and EDN in the nasal lavage fluid.

Freeland and co-workers[97c] have found an increase in concentration of leukotriene B_4 (LTB_4) in the nasal lavage fluid during both the early phase (INR) and the late phase (LNR) nasal response to allergen challenge, in patients with pollen-related rhinitis. Moreover, LNR was also associated with significant increase in the influx of neutrophils into NS. Pretreatment with oral corticosteroid (prednisone in a daily dose of 3 x 20 mg for 2 days) did not demonstrate any effect either on the early or late increase in LTB_4 levels or on the neutrophil influx accompanying LNR.

It can be concluded that systemic (oral) corticosteroids are able to prevent significantly the LNR. However, in practice, the systemic corticosteroids should be regarded as drugs which are too heavy and exaggerated for the treatment of LNR.

2. H_1-RECEPTOR ANTAGONISTS AND ANTICHOLINERGIC DRUGS

The pharmacologic effects of antihistamines (H_1-receptor antagonists, H_2-receptor antagonists, or a combination of both) on the LNR have not yet been satisfactorily investigated.[50,51,57,122] However, with respect to the known pharmacologic effects of H_1-receptor antagonists (to block the action of histamine on the H_1-receptors, and which do not affect other mediators or their effects), as well as to their clinical effects (to diminish the hypersecretion, itching, and vasodilatation in the nasal mucosa, but not the nasal blockage), they would not be expected to be effective on the LNR, since in this type of nasal response a variety of mediators, other than histamine only, are also supposed to be involved,[11f,11h,23,25a,34,40c,40d,41b,41i,48d,49,51a,52-56,72b,82b,82d,82j,83,85,91,94, 95,97a,97b,97c,97u] and the severe nasal obstruction has been shown to be the most prominent clinical symptom accompanying the LNR (Table 3).[7,12,16,19,40f,41b,41c,72b]

In the past we have carried out a series of pilot studies comparing the protective effects of DSCG with effects of the various antihistamines (Promethazine hydrochloride, Chlorphenamine maleate, Clemastine, Ketotifen, Cinnarizine, Astemizole, Terfenadine) and of the anticholinergic compounds (Thiazinamium hydrochloride Oxyphenonium, Ipratropium bromide) both on the INR and on the LNR. Some of these results have already been published,[41b,72a,122a] whereas the remaining data have still to be published.[122b,122d] Despite a slight subjective relief, neither antihistamines nor anticholinergics have demonstrated significant protective effects on the LNR, while DSCG has prevented this type of nasal response to a highly significant degree ($p < 0.001$) (Tables 47,51).[41b]

Recently, we have studied the effects of Cetirizine (CZ), a member of the third generation of the non-sedating H_1-receptor antagonists, on the immediate (INR) and the late nasal response (LNR) to allergen challenge, and on the accompanying eosinophil influx into the nasal secretions (NS).[40e,122a-122e] Surprisingly, the 9-day pretreatment with CZ, in a daily dose of 10 mg, has prevented most of the INR cases and it has also partially prevented some of the LNR cases and decreased the eosinophil influx into the NS during both types of nasal response. We were not able to find any report concerning the effects of CZ on the LNR in the available literature (Table 51; Figures 47a,47b, 48a,48b).

We have also studied and compared the effects of terfenadine (TN), a member of the

second generation of the H_1-receptor antagonists and cetirizine (CZ) and loratadine (LD), members of the third generation of the H_1-receptor antagonists on the immediate (INR) and the late nasal response (LNR) to allergen challenge and on the accompanying influx of eosinophils into the nasal secretions.[122a,122e] Cetirizine, in the therapeutical oral daily doses, has been able to decrease significantly both the INR and the accompanying nasal secretion eosinophilia, loratadine has demonstrated a slight effect both on the INR and on the nasal secretion eosinophilia, whereas terfenadine did not affect either the INR or the nasal secretion eosinophilia.[122a,122c,122e]

On the other hand, neither cetirizine, loratadine, nor terfenadine, administered in therapeutical oral daily doses (CZ = 1 x 10 mg, LD = 1 x 10 mg, TN = 2 x 60 mg), has been able to prevent significantly the LNR. However, the influx of eosinophils into the nasal secretions accompanying the LNR has been decreased to a moderate degree by cetirizine and loratadine, whereas terfenadine has been found to be totally ineffective (Table 51; Figures 49a,49b,50a,50b).[122e]

Moreover, our recent data suggest that the INR to allergen challenge may also be prevented to a significant degree by loratadine, in a daily oral dose of 2 x 5 mg, however, after a longer pretreatment, e.g. 20-21 days.[122c]

3. NEDOCROMIL SODIUM [NDS/NS]*

Recently, a new compound, Nedocromil Sodium (NDS = disodium salt of a novel pyranoquinoline dicarboxylic acid), has been found by some investigators to be a promising drug for the treatment of LNR[123,123a,124, 124a-124b,124f-124n,125,125a-125c and also by us.[41b,41i,72a,124c-124e,124p]

This drug has demonstrated a number of unique pharmacologic properties, including a variety of the anti-allergic and anti-inflammatory effects. [123,124,124a-124k] Moreover, nedocromil sodium has been shown to prevent significantly both the immediate (IAR) and the late (LAR) asthmatic response to allergen challenge.[121g,124a,124b,124l-124n,178e, 178f,178h] More recently, our preliminary data suggest that NDS may also be able to decrease significantly the delayed asthmatic response (DYAR).[124p] In three preliminary studies, data of which have not yet been finally published,[124c-124e] we have investigated the possible protective effects of Nedocromil Sodium in the nasal spray on the LNR (n = 14) and the INR (n=18) as well as on the cellular changes in NS accompanying both the nasal response types, after a 2-week pretreatment in a daily dose of 16 mg, divided equally between both the nasal cavities (4 x daily 1 puff containing 2 mg of NDS in each nostril = 4 x 2 mg x 2 = 16 mg).

The NDS has prevented significantly the INR ($p < 0.01$) and highly significantly the LNR ($p < 0.001$) (Figures 51a,52a). Moreover, NDS has reduced significantly the influx of eosinophils, neutrophils, mast cells and basophils into the NS, during the INR, whereas it has almost completely prevented the influx of neutrophils, eosinophils, and basophils and decreased significantly the count of epithelial and goblet cells in NS during the LNR (Table 52; Figures 51a-51d,52a-52d).[124d,124e]

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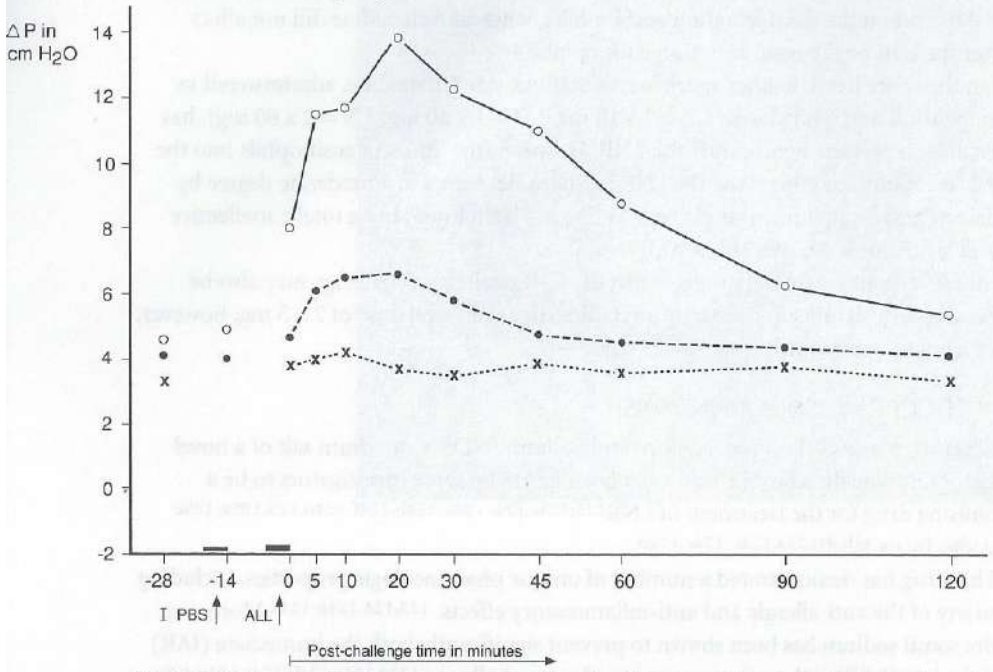
* Nedocromil Sodium has been abbreviated as NDS in some of our studies, whereas as NS in other of our studies.

Fig. 47a. THE PROTECTIVE EFFECTS OF ORAL CETIRIZINE (CZ) ON THE IMMEDIATE NASAL RESPONSE [INR]

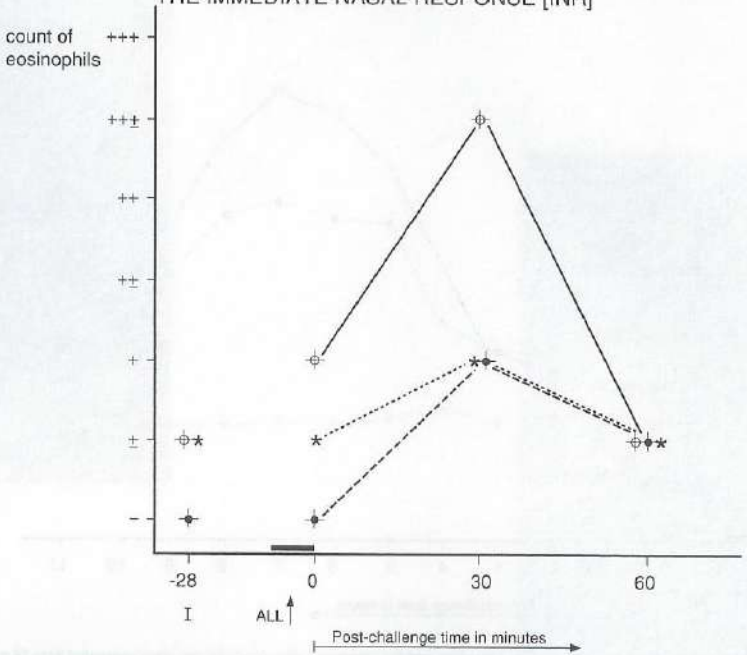


The mean NPG values, recorded after the non-pretreated and the pretreated allergen challenge, were calculated from 8 patients.
I = Initial value (=baseline); PBS= Phosphate buffered saline; ALL= Allergen challenge.

o ——— o = Non-pretreated INR (n=8)
• - - - - - • = INR pretreated with Cetirizine (n=8)
x ····· x = PBS control challenge (n=8)

References: 40e,122b

Fig. 47b. THE EFFECTS OF ORAL CETIRIZINE (CZ) ON THE EOSINOPHILS IN NASAL SECRETIONS (NS) DURING THE IMMEDIATE NASAL RESPONSE [INR]

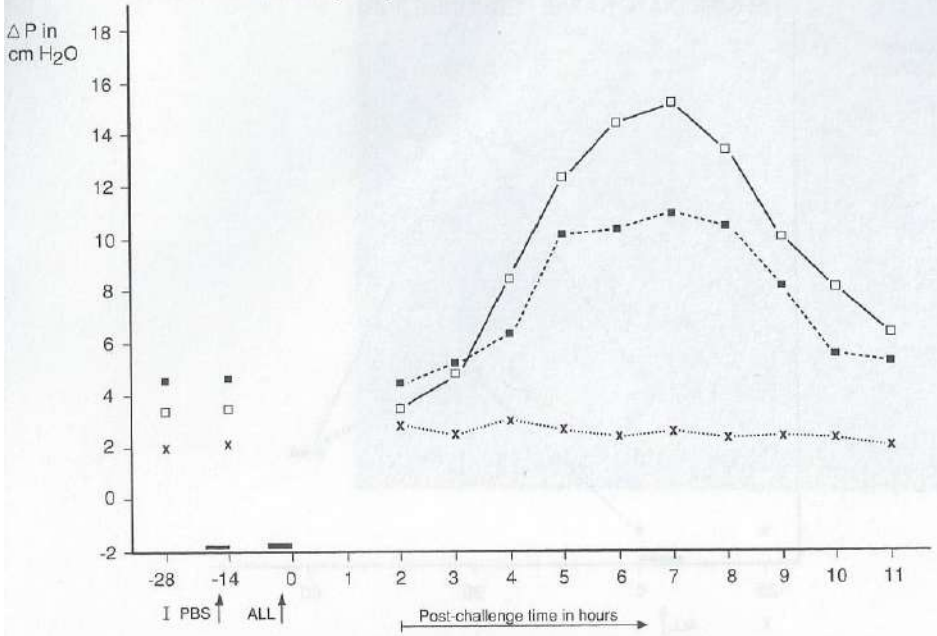


The mean changes in the count of eosinophils in the nasal secretions (NS), during the non-pretreated and the pretreated INR, were calculated from 8 patients.

⊕ ——— ⊕ = Eosinophils in NS-non-pretreated INR (n=8)
• - - - - - • = Eosinophils in NS-INR pretreated with Cetirizine (n=8)
* ····· * = PBS control challenge (n=8)

References: 40e,122b

Fig. 48a. THE PROTECTIVE EFFECTS OF ORAL CETIRIZINE (CZ) ON THE LATE NASAL RESPONSE [LNR]

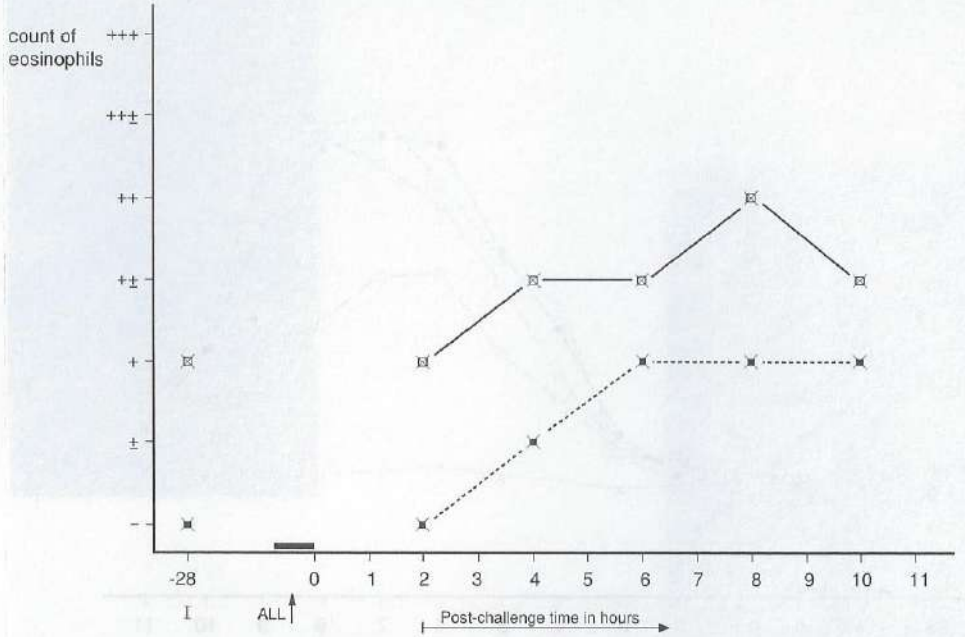


The mean NPG values, recorded after the non-pretreated and the pretreated allergen challenge, were calculated from 14 patients.
I= Initial value (=baseline); PBS= Phosphate buffered saline; ALL= Allergen challenge.

□ ——— □ = Non-pretreated LNR (n=14)
▪ ---------- ▪ = LNR pretreated with Cetirizine (n=14)
x ---------- x = PBS (Phosphate buffered saline) (n=14)

References: 40e,122b

Fig. 48b. EOSINOPHILS IN NASAL SECRETIONS (NS) DURING THE LATE NASAL RESPONSE [LNR]

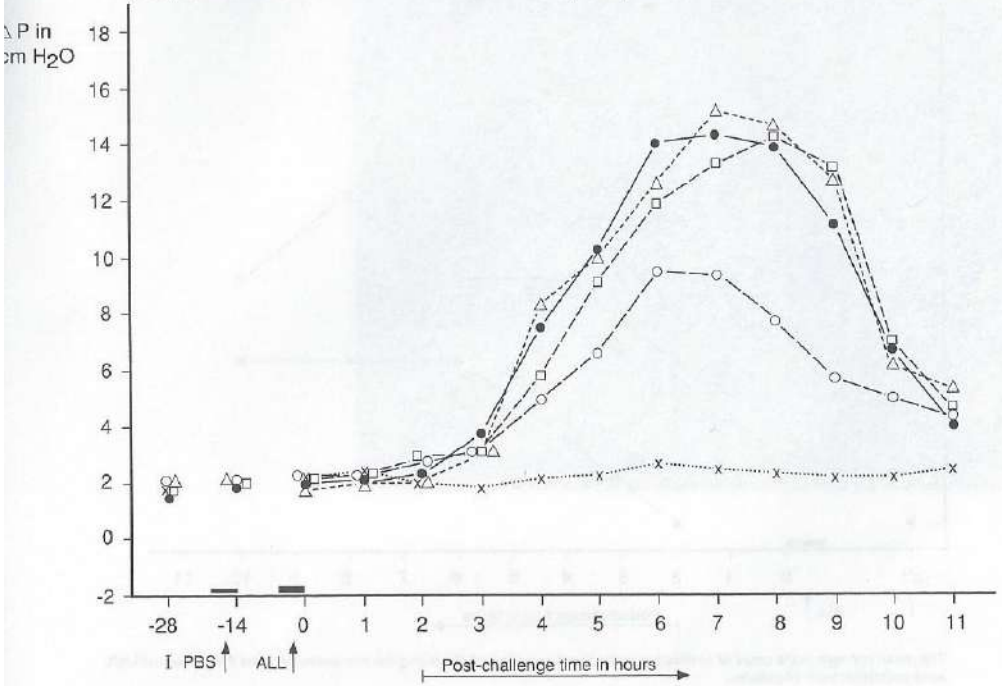


The mean changes in the count of eosinophils in the nasal secretions (NS), during the non-pretreated and the pretreated LNR, were calculated from 14 patients.

⊠ ——— ⊠ = Eosinophils in NS-non-pretreated LNR (n=14)
⊠ ---------- ⊠ = Eosinophils in NS-LNR pretreated with Cetirizine (n=14)

References: 40e,122b

Fig. 49a. EFFECTS OF ORAL CETIRIZINE (CZ), LORATADINE (LD) AND TERFENADINE (TN) ON THE LATE NASAL RESPONSE [LNR]

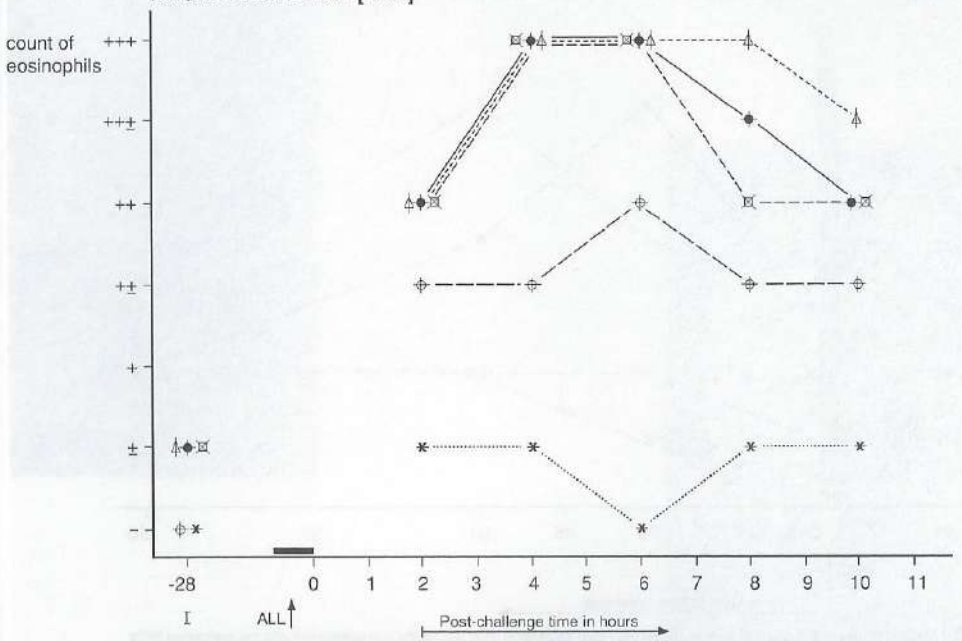


The mean NPG values recorded after the non-pretreated and the pretreated challenges.
I= Initial values (=baseline); PBS= Phosphate buffered saline; ALL= Allergen challenge.

- ●——● = Non-pretreated LNR (n=12)
- x·····x = PBS control challenge (n=12)
- ○——○ = LNR pretreated with Cetirizine (n=12)
- □------□ = LNR pretreated with Loratadine (n=12)
- △·····△ = LNR pretreated with Terfenadine (n=12)

References: 122a-122c

Fig. 49b. EFFECTS OF ORAL CETIRIZINE (CZ), LORATADINE (LD) AND TERFENADINE (TN) ON THE EOSINOPHILS IN NASAL SECRETIONS (NS) DURING THE LATE NASAL RESPONSE [LNR]

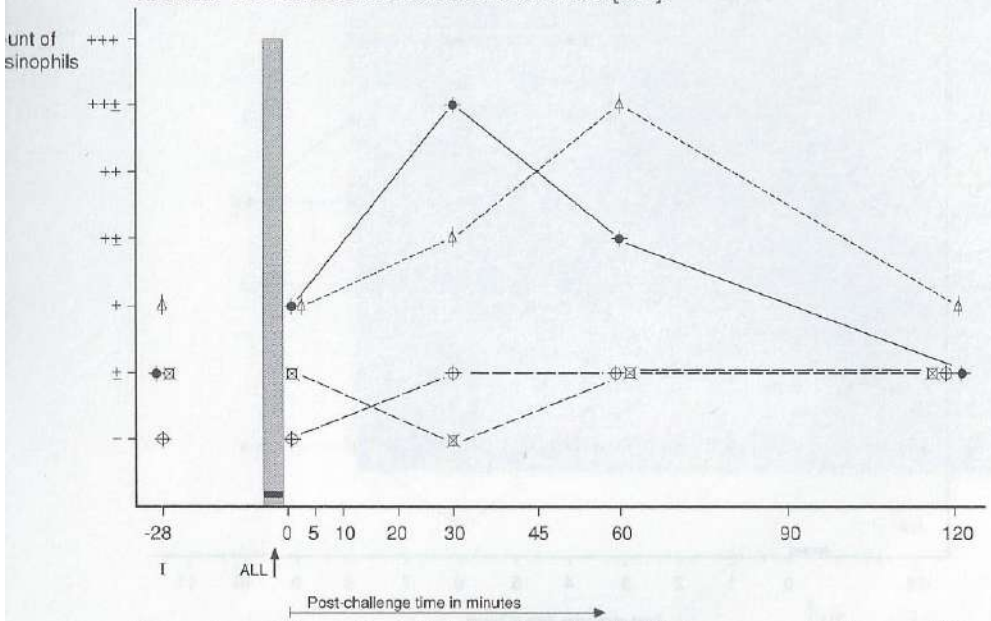


The mean changes in the count of eosinophils in the nasal secretions (NS), during the non-pretreated and the pretreated LNRs

- ●——● = Eosinophils in NS-non-pretreated LNRs (n=12)
- x·····x = Eosinophils in NS-PBS control challenges (n=12)
- ⊕——⊕ = Eosinophils in NS-LNRs pretreated with Cetirizine (n=12)
- ⊠------⊠ = Eosinophils in NS-LNRs pretreated with Loratadine (n=12)
- ⍋·····⍋ = Eosinophils in NS-LNRs pretreated with Terfenadine (n=12)

References: 122a-122c

g. 50a. THE EFFECTS OF ORAL CETIRIZINE (CZ), LORATADINE (LD) AND TERFENADINE (TN) ON THE EOSINOPHILS IN NASAL SECRETIONS (NS) DURING THE IMMEDIATE NASAL RESPONSE [INR].

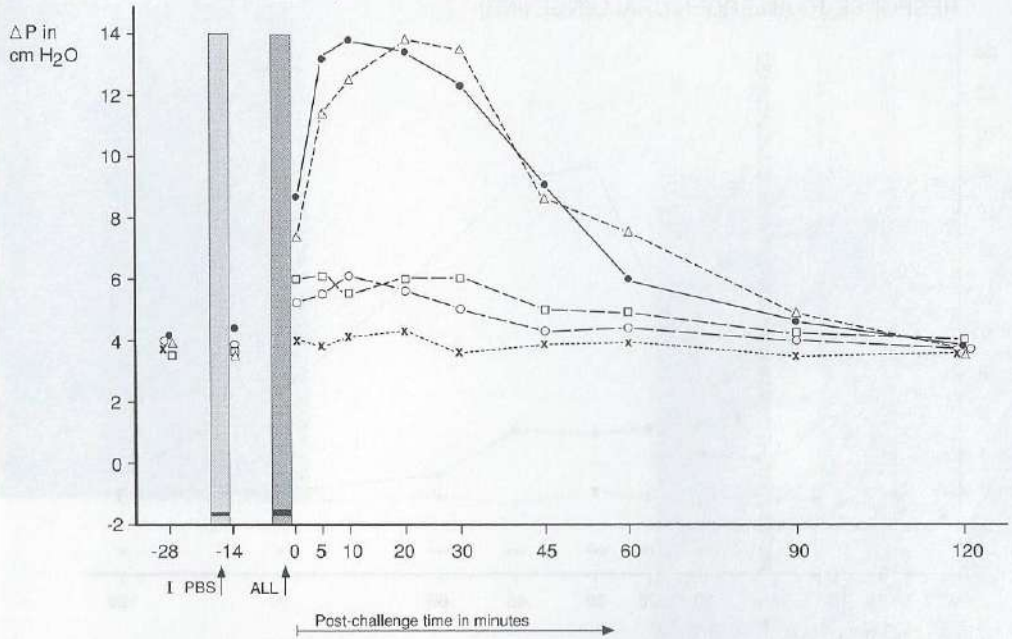


The mean changes in the count of eosinophils in the nasal secretions (NS) during the non-pretreated and the pretreated INRs.
I = Initial values (=baseline); ALL = Allergen challenge.

= Eosinophils in NS-non-pretreated INR (n=24)
= Eosinophils in NS-INR pretreated with Cetirizine (n=24)
= Eosinophils in NS-INR pretreated with Loratadine (n=12)
= Eosinophils in NS-INR pretreated with Terfenadine (n=12)

References: 122a-122c

Fig. 50b. THE EFFECTS OF ORAL CETIRIZINE (CZ), LORATADINE (LD) AND TERFENADINE (TN) ON THE IMMEDIATE NASAL RESPONSE [INR]

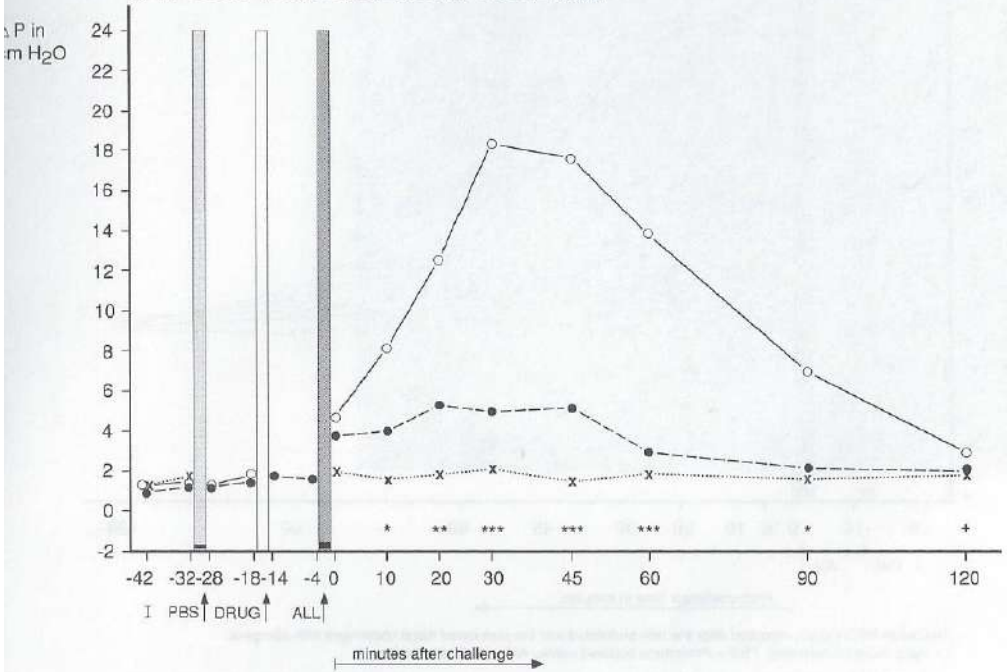


The mean NPG values, recorded after the non-pretreated and the pretreated nasal challenges with allergens.
I = Initial values (=baseline); PBS = Phosphate buffered saline; ALL = Allergen challenge.

= Non-pretreated INR (n=24)
= PBS control challenge (n=24)
= INR pretreated with Cetirizine (n=24)
= INR pretreated with Loratadine (n=12)
= INR pretreated with Terfenadine (n=12)

References: 122a-122c

Fig. 51a. THE PROTECTIVE EFFECTS OF TOPICALLY ADMINISTERED NEDOCROMIL SODIUM (NDS) ON THE IMMEDIATE NASAL RESPONSE TO ALLERGEN CHALLENGE [INR]

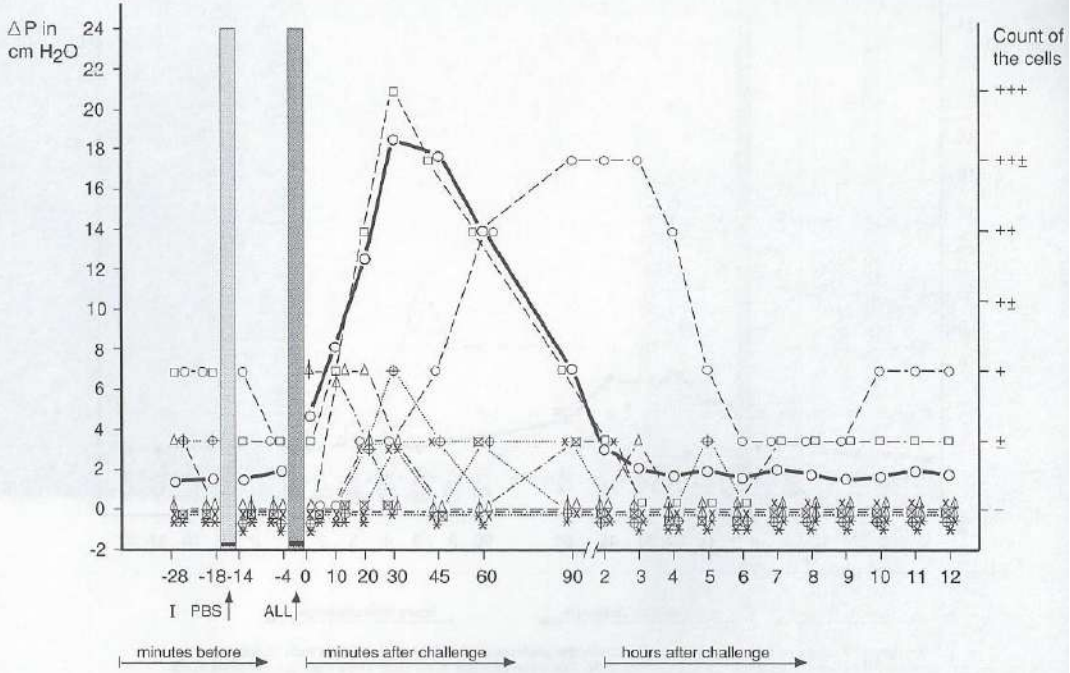


The mean NPG values, recorded after the non-pretreated and pretreated nasal challenges, were always calculated from 18 patients developing 18 positive immediate nasal responses (INR).
I = Initial values (=baseline); PBS = Phosphate buffered saline control challenge; ALL = Allergen challenge;
NDS = Nedocromil sodium.
Statistical significance of differences: + = p = 0.05; * = p < 0.05; ** = p < 0.01; *** = p < 0.001

○——○ = Non-pretreated INR (n=18)
●– – –● = INR pretreated with NDS (n=18)
x ····· x = PBS control challenge (n=18)

References: 41i,124c,124d

Fig. 51b. CYTOLOGIC CHANGES IN NASAL SECRETIONS (NS) DURING THE IMMEDIATE NASAL RESPONSE [INR]

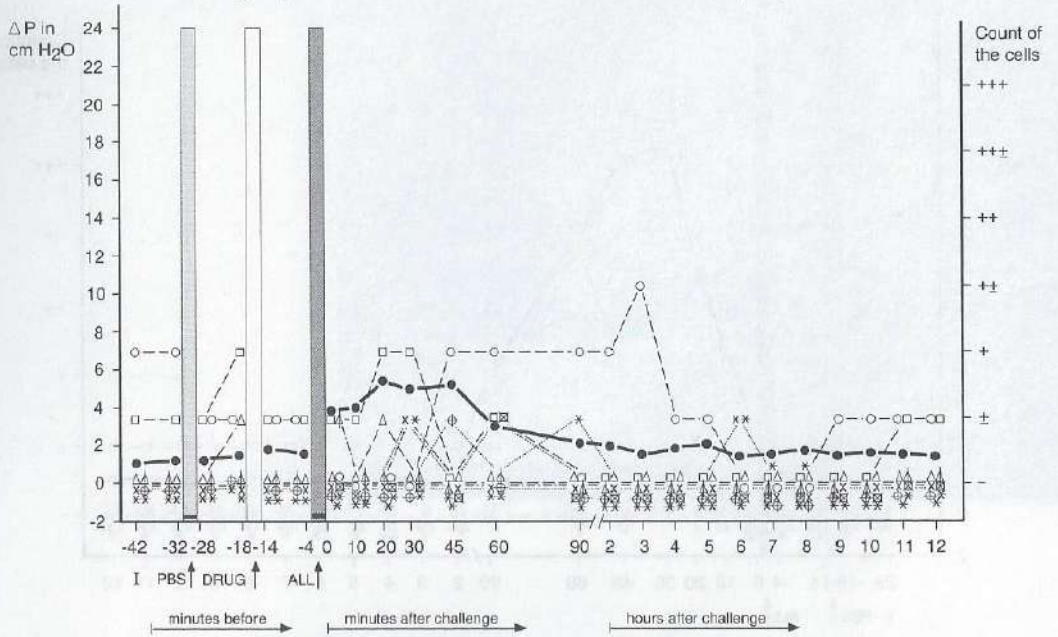


The mean NPG values recorded after the non-pretreated nasal challenges were calculated from 18 patients developing 18 positive immediate nasal responses (INR). The mean changes in the count of the individual cell types in the NS were calculated from 18 positive INRs.
I = Initial values (=baseline); PBS = Phosphate buffered saline (=control challenge); ALL = Allergen challenge;
NS = Nasal secretions.

○——○ = Non pretreated INR (n=18)
□– – –□ = Eosinophils
△– – –△ = Basophils
⋔–·–·–⋔ = Mast cells
○– – –○ = Neutrophils
x ····· x = Goblet cells
⊠ ····· ⊠ = Lymphocytes
⊕ ····· ⊕ = Epithelial cells
x ····· x = Plasma cells
x –·–·– x = Monocytes

References: 41i,124c,124d

Fig. 51c. CYTOLOGIC CHANGES IN NASAL SECRETIONS (NS) DURING THE IMMEDIATE RESPONSE [INR] PRETREATED WITH NEDOCROMIL SODIUM (NDS)

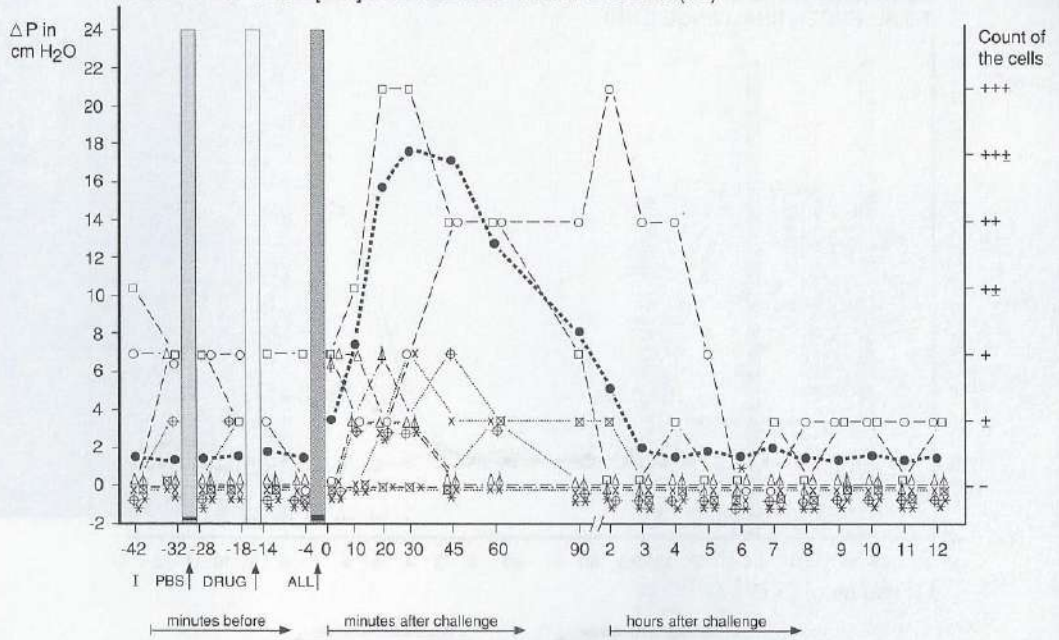


The mean NPG values recorded after the allergen challenges pretreated with NDS were calculated from 18 patients developing 18 positive immediate nasal responses (INR). The mean changes in the count of the individual cell types in the NS were calculated from 18 positive INRs.
I = Initial values (=baseline); PBS = Phosphate buffered saline (=control challenge); ALL = Allergen challenge; NS = Nasal secretions; NDS = Nedocromil sodium aerosol

- = INR pretreated with NDS (n=18)
- = Eosinophils
- = Basophils
- = Mast cells
- = Neutrophils
- = Goblet cells
- = Lymphocytes
- = Epithelial cells
- = Plasma cells
- = Monocytes

References: 41i,124c,124d

Fig. 51d. CYTOLOGIC CHANGES IN NASAL SECRETIONS (NS) DURING THE IMMEDIATE NASAL RESPONSE [INR] PRETREATED WITH PLACEBO (PL)

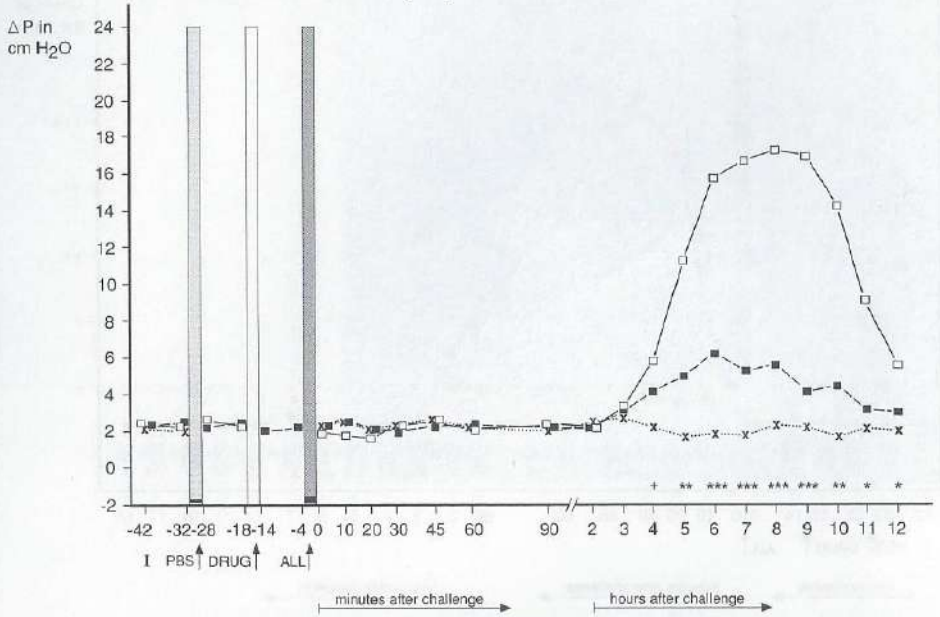


The mean NPG values recorded after the allergen challenges pretreated with PL were calculated from 18 patients developing 18 positive immediate nasal responses (INR). The mean changes in the count of the individual cell types in the NS were calculated from 18 positive INRs.
I = Initial values (=baseline); PBS = Phosphate buffered saline (=control challenge); ALL = Allergen challenge; NS = Nasal secretions; PL = Placebo aerosol.

- = INR pretreated with PL (n=18)
- = Eosinophils
- = Basophils
- = Mast cells
- = Neutrophils
- = Goblet cells
- = Lymphocytes
- = Epithelial cells
- = Plasma cells
- = Monocytes

References: 41i,124c,124d

Fig. 52a. THE PROTECTIVE EFFECTS OF TOPICALLY ADMINISTERED NEDOCROMIL SODIUM (NDS) ON THE LATE NASAL RESPONSE TO ALLERGEN CHALLENGE [LNR]

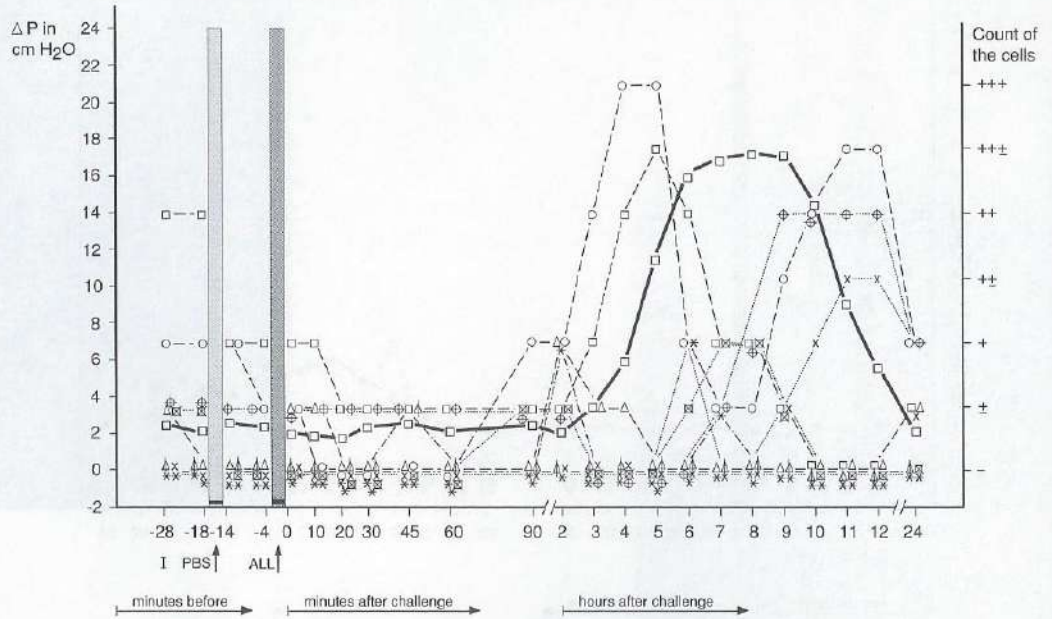


The mean NPG values, recorded after the non-pretreated and pretreated nasal challenges, were always calculated from 14 patients developing 14 positive late nasal responses (LNR).
I = Initial values (=baseline); PBS = Phosphate buffered saline control challenge; ALL = Allergen challenge; NDS = Nedocromil sodium.
Statistical significance of differences: + = p = 0.05; * = p < 0.05; ** = p < 0.01; *** = p < 0.001

□——□ = Non-pretreated LNR (n=14)
■------■ = LNR pretreated with NDS (n=14)
x·····x = PBS control challenge (n=14)

References: 41i,124c,124d

Fig. 52b. CYTOLOGIC CHANGES IN NASAL SECRETIONS (NS) DURING THE LATE NASAL RESPONSE [LNR]

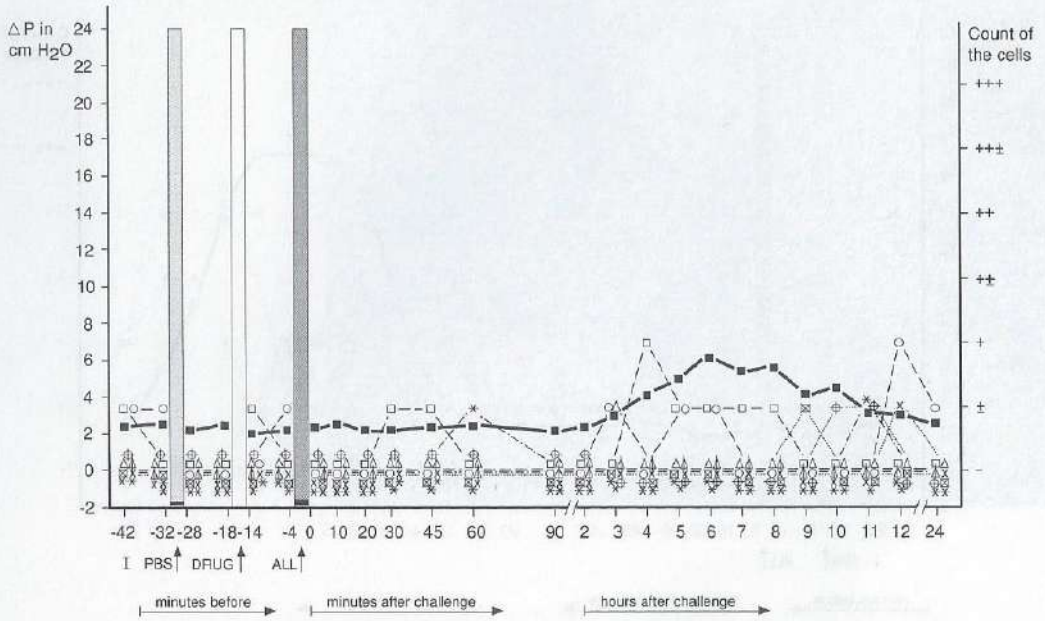


The mean NPG values recorded after the non-pretreated nasal challenges were calculated from 14 patients developing 14 positive late nasal responses (LNR). The mean changes in the count of the individual cell types in the NS were calculated from 14 positive LNRs.
I = Initial values (=baseline); PBS = Phosphate buffered saline (=control challenge); ALL = Allergen challenge; NS = Nasal secretions.

□——□ = Non-pretreated LNR (n=14)
□------□ = Eosinophils
△------△ = Basophils
▲·-·-·△ = Mast cells
○------○ = Neutrophils
x·····x = Goblet cells
⊠·····⊠ = Lymphocytes
⊕·····⊕ = Epithelial cells
x·····x = Plasma cells
x·-·-·x = Monocytes

References: 41i,124c,124d

Fig. 52c. CYTOLOGIC CHANGES IN NASAL SECRETIONS (NS) DURING THE LATE NASAL RESPONSE [LNR] PRETREATED WITH NEDOCROMIL SODIUM (NDS)

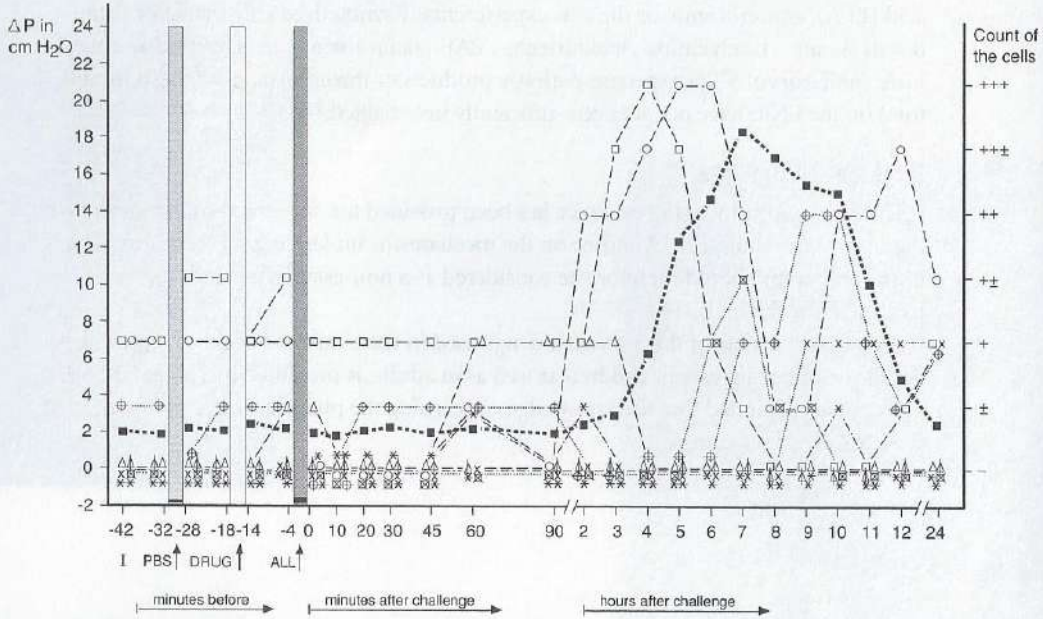


The mean NPG values recorded after the allergen challenges pretreated with NDS were calculated from 14 patients developing 14 positive late nasal responses (LNR). The mean changes in the count of the individual cell types in the NS were calculated from 14 positive LNRs.
I = Initial values (=baseline); PBS = Phosphate buffered saline (=control challenge); ALL = Allergen challenge; NS = Nasal secretions; NDS = Nedocromil sodium aerosol.

= LNR pretreated with NDS (n=14)
= Eosinophils
= Basophils
= Mast cells
= Neutrophils
= Goblet cells
= Lymphocytes
= Epithelial cells
= Plasma cells
= Monocytes

References: 41i,124c,124d

Fig. 52d. CYTOLOGIC CHANGES IN NASAL SECRETIONS (NS) DURING THE LATE NASAL RESPONSE [LNR] PRETREATED WITH PLACEBO (PL)



The mean NPG values recorded after the allergen challenges pretreated with PL were calculated from 14 patients developing 14 positive late nasal responses (LNR). The mean changes in the count of the individual cell types in the NS were calculated from 14 positive LNRs.
I = Initial values (=baseline); PBS = Phosphate buffered saline (=control challenge); ALL = Allergen challenge; NS = Nasal secretions; PL = Placebo aerosol.

= LNR pretreated with PL (n=14)
= Eosinophils
= Basophils
= Mast cells
= Neutrophils
= Goblet cells
= Lymphocytes
= Epithelial cells
= Plasma cells
= Monocytes

References: 41i,124c,124d

4. OTHER DRUGS

The possible protective effects of other drugs, such as beta$_2$-sympathomimetics, anticholinergic drugs (Ipratropium bromide), calcium channel blockers (Nifedipine, Verapamil), non-steroidal anti-inflammatory agents [NSAID](acetylsalicylic acid and its derivatives, indomethacin, flurbiprofen, ibuprofen, prostaglandin-suppressing compounds), cAMP modulators, H_2-receptor antagonists (Cimetidine, Ranitidine), eicosapentaenoic acid (EPA), antiserotonin, or the new experimentally synthetized anti-mediator compounds (kinins-, bradykinins-, leukotrienes-, PAF-, neurotoxins-, neuropeptides- antagonists, inhibitors of 5-lipoxygenase-pathway products or thromboxane-synthesis inhibitors) on the LNR, have not yet been sufficiently investigated.[11h,41i,57,72a,72b]

5. IMMUNOTHERAPY

Until now no convincing evidence has been provided for any effects of the immunotherapy on the clinical LNR and/or on the mechanisms underlying this response. The immunotherapy should therefore be considered as a non-established and unproved treatment for the LNR.[11h,41i,57,72a]

A complete review of the particular drugs used in the treatment of the allergic disorder of the upper airways in children as well as in adults is presented in Tables 53-56.[41g]

It can be concluded that the knowledge concerning the pharmacologic modulation of the LNR is not yet complete.

Table 51 Survey of possible protective effects of various H_1-receptor antagonists on the immediate (INR), late (LNR) and delayed (DYNR) nasal response to the allergen challenge, and on the nasal response to the nasal challenge with histamine, representing the nasal non-specific hyperreactivity (N-SH).

	Nasal response to the allergen challenge			Nasal response to the challenge with histamine
	INR	LNR	DYNR	
The "first generation"				
Promethazine	– [n=28]	– [n=21]	– [n=17]	– [n=16]
Chlorphenamine	+ [n=22]	± [n=16]	– [n=11]	+ [n=15]
Clemastine	++ [n=20]	± [n=12]	– [n=14]	+ [n=17]
Ketotifen	– [n=18]	– [n=15]	– [n=12]	– [n=15]
Cinnarizine	± [n=15]	– [n=11]	– [n=10]	+ [n=22]
Triprolidine	± [n=10]	0	0	0
Oxatomide	– [n=12]	0	0	0
Mebhydroline	± [n=16]	± [n=10]	0	+ [n=20]
The " second generation"				
Astemizole	– [n=24]	– [n=14]	0	– [n=24]
Terfenadine	– [n=12]	– [n=12]	– [n=10]	– [n=20]
Azelastine	– [n=14]	– [n=10]	0	0
The "third generation"				
Loratadine	± [n=12] – [n=15]	± [n=11]	– [n=10]	+ [n=15]
Cetirizine	+ [n=24]	± [n=14]	– [n=12]	– [n=18]
Levocabastine	± [n=15]	– [n=15]	– [n=13]	– [n=12]

Protective effects: – = no effects ($p > 0.05$); ± = very slight or partial effects ($p \leq 0.05$); + = protective effects ($p < 0.05$); ++ = significantly protective effects ($p < 0.01$); +++ = highly significant protective effects ($p < 0.001$); 0 = lack of data. Numbers in the brackets = number of cases.

References: 40e, 41b, 41i, 72a, 122a-122

Table 52 Protective effects of Nedocromil sodium (NDS/NS) in comparison to those of Disodium cromoglycate (DSCG) on the immediate (INR), late (LNR) and delayed (DYNR) nasal response to allergen challenge.

	Nasal response to allergen challenge		
	INR	LNR	DYNR
Nedocromil sodium (NDS)	$p < 0.05$ [n=18]	$p < 0.001$ [n=14]	$p \leq 0.05$ [n=11]
Disodium cromoglycate	$p < 0.01$ [n=26]	$p < 0.05$ [n=34]	$p > 0.05$ [n=18]
Placebo	$p > 0.2$ [n=18]	$p > 0.1$ [n=14]	$p > 0.1$ [n=11]

Reference: 72a

Table 53 Daily doses of some anti-allergic and supplementary drugs recommended for adults.

Drug		Daily dose
H1-receptor antagonists		
- *the "first generation*		
Promethazine	OR	1-3 x 12,5 mg or 1 x 25 mg
Chlorphenamine	OR	1-2 x 12 mg
Clemastine	OR	1-2 x 1 mg
Ketotifen	OR	1-2 x 1 mg
Cinnarizine	OR	1-2 x 25 mg
Triprolidine	OR	1 x 10 mg
Chlorpheniramine	OR	1-2 x 6 mg
Oxatomide	OR	1-2 x 30 mg
Azatidine	OR	1-2 x 1 mg
Mebhydroline	OR	2-4 x 50 mg
Hydroxyzine	OR	2 x 25 mg
Brompheniramine	OR	3 x 4 mg
Dexchlorpheniramine	OR	3-4 x 1-2 mg
Dimethindene	OR	3 x 1-2 mg
- *the "second generation"*		
Astemizole	OR	1 x 10 mg
Terfenadine	OR	1-2 x 60 mg
Azelastine	OR	2 x 2 mg
Azelastine	TOP	2-3 x 1 puff (0.14 mg) each nostril
Acrivastine	OR	3 x 8 mg
- *the "third generation"*		
Cetirizine	OR	1 x 10 mg
Loratadine	OR	1 x 10 mg
Levocabastine	TOP	2 puffs (0.5 mg/ml) each nostril 2 x daily
Ebastine	OR	1 x 5 mg
H_2-receptor antagonists		
Cimetidine	OR	1-3 x 300 mg
Ranitidine	OR	2 x 150 mg
Anticholinergic agents		
Thiazinamium	OR	1-3 x 100-200mg
Oxyphenonium	OR	2-4 x daily x 5-10 mg
Ipratropium	TOP*	4 x 40-80mcg
Disodium cromoglycate		
Nasal drops 2% (20 mg/ml)	TOP	4 x 2 drops - each nostril;
Nasal spray 2% (20 mg/ml)	TOP	6 x 2 sprays - each nostril;
Nasal spray 4% (40 mg/ml)	TOP	4-5 x 1-2 sprays - each nostril;
Powder (1 caps=10 mg)	TOP	4 x 1 capsule divided between both the nostrils;
Oral formulation (powder in capsules or solution)		4 x 100-200mg

Administration: OR=oral; TOP=topical (intranasal); *=commercial formulation is not yet available.
References: 41g

Table 54 Daily doses of some anti-allergic and supplementary drugs recommended for adults

Drug		Daily dose
Nedocromil sodium	TOP*	4 x daily 2 sprays of 1% each nostril
Glucocorticosteroids		
- systemic		
Prednison	OR	0.5 - 1 mg/kg daily divided into 2-4 doses
Prednisolon	OR	0.5 - 0.8 mg/kg daily divided into 2-4 doses
Dexamethasone	OR	0.5 - 9 mg daily (up to 2 x 16 mg) divided in 2 doses
- topical		
Beclomethasone dipropionate	TOP	2-3 x 100 mcg (= 2 x 50 mcg) - each nostril
Budesonide	TOP	2-3 x 100 mcg (= 2 x 50 mcg) - each nostril
Flunisolide	TOP	2-3 x 50 mcg (= 2 x 25 mcg) - each nostril
Fluticasone	TOP	2 x 50 mcg - each nostril
Triamcinolone	TOP	4 x 25-50 mcg - each nostril
Decongestants		
Xylometazoline	TOP	2-3 x 1-2 drops of 0.1% - each nostril
Oxymetazoline	TOP	1-3 x 1-3 drops of 0.05%- each nostril
Naphazoline	TOP	2-3 x 1-2 sprays each nostril
Tetrahydrozoline	TOP	2-3 x 1-2 sprays each nostril
Phenylephrine	TOP	4 x 1-2 sprays each nostril
Varia		
Acetylsalicyl acid	OR	1-3 x 500 mg
Indomethacine	OR	1 mg/kg/24 hrs divided into 2-3 doses
Ibuprofen	OR	2-6 x 200 mg

Administration: OR=oral; TOP=topical (intranasal); *= commercial formulation is not yet available.
References: 41g

Table 55 Daily doses of some anti-allergic and supplementary drugs recommended for children

Drug		< 12 years	12-16 years
H_1-receptor antagonists			
The "first generation"			
Promethazine	OR	1-3y = 2-4 x 1-2 mg; 3-12y= 2-4 x 2-4 mg	2-4 x 4 mg
Chlorphenamine	OR	X	1 x 12 mg
Clemastine	OR	1-3y = 1-2 x 0.25 mg; 3-12y= 1-2 x 0.5 mg	1-2 x 1 mg
Ketotifen	OR	> 3 years; 0.025 mg/kg/24 hrs	1-2 x 1 mg
Cinnarizine	OR	> 3 years = 1 x 12,5 mg	1-2 x 12,5 mg
Triprolidine	OR	X/NE	1 x 10 mg
Chlorpheniramine	OR	1-2 x 2.5 mg or 0.35mg/kg/24 hrs	1 x 6 mg
Oxatomide	OR	1 mg/kg/24hrs divided into 2 doses	1-2 x 15-30 mg
Azatidine	OR	1-2 x 0.5 mg	1-2 x 1 mg
Mebhydroline	OR	1-2 x 50 mg	2-3 x 50 mg
Hydroxyzine	OR	2-12 yrs=2 mg/kg/24 hrs divided into 2 doses	2 mg/kg/24 hrs divided into 2 doses
Brompheniramine	OR	X/NE	X/NE
Dexchlorpheniramine	OR	2-12yrs = 2 x 1 mg repetabs	2-3 x 1 mg
Dimethindene	OR	1-3 yrs=3 x 0.5mg; 3-12yrs = 3 x 0.75mg	3 x 1 mg
The "second generation"			
Astemizole	OR	< 6 years = 0.2 mg/kg/24 hrs 6-12 yrs = 1 x 5 mg divided into 2 doses	1 x 10 mg
Terfenadine	OR	3-6 yrs = 2 x 15 mg; 6-12 yrs = 1-2 x 30 mg	1-2 x 30 mg
Azelastine	OR	X/NE	1 x 2 mg
Azelastine	TOP	> 6 yrs 2 x 1 puff (0.14 mg) each nostril	2-3 x 1 puff (0.14 mg) each nostril
Acrivastine	OR	X/NE	1-3 x 8 mg
The "third generation"			
Cetirizine	OR	NE	1-2 x 5 mg
Loratadine	OR	<30 kg= 1 x 5mg; >30 kg= 1-2 x 5mg	1-2 x 5 mg
Levocabastine	TOP	> 3 yrs=1 puff (0.5mg/ml) each nostril 2 x daily	2 puffs (0.5 mg/ml) each nostril 2 x daily
Ebastine	OR	X/NE	1 x 5 mg (NE)
H2-receptor antagonists			
Cimetidine	OR	X/NE	2-3x100mg or 20mg/kg/24hrs divided into 3 doses
Ranitidine	OR	UK/*	*
Anticholinergic agents			
Thiazinamium	OR	6-12yrs=2 x 100 mg	2-3 x 100 mg
Oxyphenonium	OR	6-12 yrs=1-2 x 2.5 mg	2-3 x 2.5 mg
Ipratropium	TOP	UK/*	UK/*

Administration: OR=oral; TOP=topical (intranasal); X = not used; UK=unknown; NE = the dose has not yet been definitively established; * = not yet commercially available.
References: 41g

Table 56 Daily doses of some anti-allergic and supplementary drugs recomended for children.

Drug		< 12 yaers	12-16 years
Disodium cromoglycate			
– Nasal drops 2% (20 mg/ml)	TOP	4 x daily 2 drops-each nostril	2 drops - each nostril 4-5 x daily
– Nasal spray 2% (20 mg/ml)	TOP	4 x daily 2 sprays - each nostril	2 sprays - each nostril 6 x daily
– Nasal spray 4% (40 mg/ml)	TOP	4 x daily 1 sprays - each nostril	1-2 sprays - each nostril 4 x daily
– Powder (1 caps=10mg)	TOP	> 3 years = 1 capsule divided between both the nostrils - 4 x daily	1 capsule - divided between both the nostrils 4 x daily
Oral formulation		4 x 50-100 mg	4 x 100-200 mg
Nedrocromil sodium	TOP	NE/ *	NE/*
Glucocorticosteroids			
- Systemic			
Prednison	OR	0.1-0.2 mg/kg/24 hrs	0.5 mg/kg/24 hrs divided in 2 doses
Prednisolon	OR	0.1-0.2 mg/kg/24 hrs divided in 2 doses	0.5 mg/kg/24 hrs divided in 2 doses
Dexamethasone	OR	2-4 yrs = 4 x 1 mg; 5-7 yrs = 4 x 1.6 mg; 8-11 yrs = 4 x 2.5 mg daily divided into 2 doses	4 x 3 mg daily divided into 2 doses
Topical			
Beclomethasone dipropionate	TOP	2 x 50 mcg each nostril 2 x daily	2 x 50 mcg each nostril 2 x daily
Budesonide	TOP	2 x 50 mcg each nostril 2 x daily	2 x 50 mg each nostril 2 x daily
Flunisolide	TOP	6-12 yrs = 1 x 25 mcg each nostril 2 x daily	2 x 25 mcg each nostril 2 x daily
Fluticasone	TOP	X/NE	1 x 100 mcg each nostril 1 x daily
Triamcinolone	TOP	X/NE	X/NE
Decongestants			
Xylometazoline	TOP	> 2 yrs 1-2 drops of 0.05% each nostril 1-3 x daily	1-2 drops of 0.1% each nostril 2-3 x daily
Oxymetazoline	TOP	> 2 yrs 1 drop of 0.025% each nostril 2-3 x daily	1-3 drops of 0.05% each nostril 1-3 x daily
Naphazoline	TOP	X/NE	1-2 sprays each nostril 1-3 x daily
Tetrahydrozoline	TOP	UK/X/NE	X/NE
Phenylephrine	TOP	UK/X/NE	X/NE
Varia			
Acetylsalicyl acid	OR	> 3 yrs 1-3 x 50 mg daily	1-3 x 200 mg daily
Indomethacine	OR	> 3 yrs 1 mg/kg/24 hrs divided into 2 doses	1 mg/kg/24 hrs divided into 2 doses
Ibuprofen	OR	X/NE	1-3 x 200 mg

Administration: OR=oral; TOP=topical (intranasal)l X = not used; UK=unknown; NE = the dose has not yet been definitively established; * = not yet commercially available.
References: 41g

V DIFFERENTIAL DIAGNOSIS

A. LATE NASAL RESPONSE [LNR] VERSUS IMMEDIATE (EARLY) NASAL RESPONSE [INR/ENR]
B. LATE NASAL RESPONSE [LNR] VERSUS DELAYED NASAL RESPONSE [DYNR]

The LNR differs substantially from INR and DYNR in several ways as well as with regard to various "*in vivo*" and "*in vitro*" diagnostic parameters.

1. CLINICAL CHARACTERISTICS

[A] Time-course

The time-course of LNR, recorded by rhinomanometry, the onset within 4 to 8 hrs, maximum within 6 to 12 hrs and the resolving within 24 to 26 hrs, differs distinctly from that of INR and of DYNR (Table 5; Figures 4-6).[2,7,11,11b-11d,12,13,13a-13c,14,41a-41d]

[B] Symptomatology

In most of the patients the symptomatology of the LNR may be characterized by the appearance of a distinct nasal blockage due to the swelling of the nasal mucosa, while the other symptoms appear to a slighter degree, in contrast to INR and DYNR (Table 3).[7,12,41a-41d]

[C] Nasal mucosa appearance

The appearance of the nasal mucosa, evaluated by anterior rhinoscopy, is usually violaceous and dry with very limited amount of secretions in the majority of LNR cases. Sometimes small mucosal hemorrhages may be seen (Table 6).[2,7,12,14,41b,72b]

[D] Association with other diagnostic parameters

The association of LNR with other "*in vivo*" and "*in vitro*" diagnostic parameters and the differences in those accompanying the other types of nasal response, are summarized in table 6.[7,12,14,41b,72b]

[E] Association with other organs' response

The appearance of other organs' symptoms accompanying the LNR, and the differences with respect to the other nasal response types are presented in Table 20.

2. MORPHOLOGIC CHARACTERISTICS

[A] Cytology of the nasal secretions (NS)

The LNR has been accompanied by significant changes in the count of neutrophils (increase before, decrease during, and increase throughout the resolving of LNR), eosinophils (increase before, decrease during, increase during a period of hours to days after disappearance of LNR), epithelial cells (increase, followed by decrease running

parallel with LNR), goblet cells (similar pattern to that of epithelial cells) .[2,11c,11g,12,25,40b-40f,41a-41d, 41i,72,72a,72c,96,97] Some authors have observed increased numbers of basophils during the LNR.[82,83] In contrast, the INR has been associated with different changes in the count of particular cell types, such as eosinophils (increase followed by decrease), neutrophils (decrease followed by increase), goblet cells (increase followed by decrease) and basophils (decrease).[2,11a-11d,18,20,41a,41c,41i,71, 72a,72c,72d,97a] Furthermore, the DYNR has been accompanied by influx and significant changes in the count of lymphocytes (increase immediately before the onset, decrease during the development and increase after resolving of the response), neutrophils (increase, decrease, increase, running almost parallel with the course of the response), and epithelial cells (increase after resolving of the response) into the NS (Tables 30-37; Figures 32-34; Plates 18,19).[13a-13c]

[B] Biopsy of the nasal mucosa

Biopsy of the nasal mucosa during LNR. The nasal mucosa showed the following histologic changes during the LNR: an edematic and damaged epithelium, enlarged intercellular spaces and breaches, expelled epithelial and goblet cells, breaches in the basement membrane, edematic sub-epithelial layer containing mixed eosinophil and neutrophil infiltrates, and perivascular edema in lamina propria with dilated, sometimes also disrupted, capillaries and erythrocyte excavation.[2,96,97] In contrast, the nasal mucosa during the INR could be characterized by enlarged intercellular spaces, enlarged ducti of mucosal glands, increased amount of thin secretions on the epithelial surface; eosinophils and tissue mast cell accumulation, but no infiltrate forming in the sub-epithelial layer; dilated, but not disrupted capillaries and a slight perivascular edema in the lamina propria.[2,96,97a] The DYNR has usually been accompanied by distinct histologic changes in the nasal mucosa, such as distinctly decreased compactness of the epithelial layer, including large breaches and hemorrhages; breaches in basement membrane; distinctly edematic lamina propria with perivascular infiltrates, formed by small lymphocytes, neutrophils and plasma cells; numerous disrupted capillaries and excavation of erythrocytes [Tables 40-42; Figures 36-38; Plates 20a, 20b (page 368)].[96]

3. IMMUNOLOGIC FEATURES

[A] Immunoglobulins in the serum

In some subjects, an increased concentration of the total IgG has been recorded during the LNR, while the concentration of other antibody classes did not demonstrate any significant appearance or changes.[2,14,25a,41a,41b,41f,41i,96] In contrast, an increase in the total and/or specific IgE antibodies in the serum may be measured during the INR in some of the patients.[2,9,11,14,41b,41f,41i] No increased concentrations of antibodies of any class have been recorded in the serum during the DYNR (Table 6).[41f,41i]

[B] Immunoglobulins in the nasal secretions (NS)

A very small number of the LNR cases (7%) has been accompanied by an increased concentration of total IgG antibodies in the NS, while antibodies of other classes, inclu-

ding the total and allergen-specific IgE, have not been detected.[25a,41b,41f,41i] In contrast, in some patients developing the INR (27%), the positive antigen-specific IgE, but not the total IgE antibodies, have been recorded in the NS during this response type.[41f,41i] The antibodies of classes other than IgE, have not been recorded in the NS during the INR.[41f,41i] No antibodies of any class have been detected in the NR during the DYNR.[13a,41f,41i]

[C] Mediators and other factors in the nasal secretions (NS)

The LNR may be accompanied by an increase in concentration of histamine, kinins, TAME-esterases, neutrophil chemotactic factor, major basic protein (MBP) and LTB_4 in NS, while bradykinin, lysylbradykinin and PGF2α may also be detected in NS.[34,49,51c,53,54, 56,82,82c,82j,83,94,97f] The INR may be associated with an increased concentration of histamine, PGD_2, TAME-esterases and kinins, while bradykinin, lysylbradykinin, high-molecular-weight neutrophil chemotactic factor of anaphylaxis, PGE_1, PGF2α, TXB_2 and leukotrienes (LTB_4, LTC_4, LTD_4, LTE_4) may also be found in NS.[34,48,51c,53,54,56,82j,83, 94,97b] More specific data concerning the appearance of mediators and other factors in NS during DYNR are not yet available. In contrast to other investigators' findings, we have recorded histamine in the NS only during 67% of INR, 7% of LNR and in 0% of DYNR cases (Tables 18,19; Figures 19-21).[11f,11j,41i]

4. PHARMACOLOGIC MODULATION

The effects of basic drugs on the particular types of nasal response to allergen challenge are summarized in Table 47.

VI. REPRODUCIBILITY AND CREDIBILITY OF THE "APPLICATION METHOD" AND THE "BALLOON TECHNIQUE" OF RHINOMANOMETRY

We have also compared the results of the "Balloon method" with those of the "Active posterior rhinomanometry" (Flow/Pressure diagram = "nasal loop")[8,21] as well as with those of the "Passive anterior rhinomanometry" (PAR) (data are not yet published).[25b]

In supplementary studies, we have investigated a possible influence of the "applicaton method" on the nasal mucosa and also on the NPT results (to be published). From these studies we have concluded that the probe saturated by PBS did not cause any artefacts or mechanical irritation of the nasal mucosa. This technique does not influence the results of the NPT, not even in patients with an increased non-specific hyperreactivity.

A. EVALUATION OF OUR "APPLICATION METHOD"

Three groups of patients between 20 to 50 years of age, have randomly been selected for this study. These patients had never used oral corticosteroids or undergone immunotherapy. No disodium cromoglycate, aerosolized corticosteroids, anticholinergics, long-term acting antihistamines or other therapy had been used by them for at least 6 weeks prior to the study. The short-term acting antihistamines were withdrawn for 72 hours and alpha sympathomimetics for 24 hours prior to the study. The first group consisting of 12 patients with atopic eczema or wasp-bee reactions but without nasal complaints, has been considered to be a control group (Gr I). The second group (Gr. II) included 24 patients with allergic rhinitis, demonstrating a positive immediate nasal response to inhalant allergens, but with normal nasal responsiveness to histamine (the non-decreased nasal histamine threshold; $\geq$ 4.0 mg/ml). The third group (Gr. III) consisted of 20 patients with rhinitis complaints due solely to the non-specific hyperreactivity (the distinctly decreased nasal histamine threshold; < 2.0 mg/ ml), whereas all nasal challenges with allergens performed in these patients remained negative.

In patients of all 3 groups the following tests have been performed and the nasal parameters (NPG values) have been recorded by means of the balloon technique up to 60 minutes and then every hour up to 12 hours. (1) The first test represents the recording of the baseline; (2) During the second test, after the baseline, the dry wad of cotton wool on the nasal probe has been introduced into the non-connected nostril under the middle turbinate for 3 minutes, followed by recording of the NPG parameters; (3) During the third test the wad of cotton wool on the nasal probe saturated with Phosphate buffered saline (PBS) has been applied for 3 minutes; (4) During the fourth test the nasal histamine threshold has been determined again and the parameters were registered up to 60 minutes after the histamine challenge.

The NPG values and their changes recorded during the individual tests in parti-

cular patients were statistically evaluated by means of Wilcoxon matched -paired signed rank test (A p value < 0.05 was considered to be statistically significant.) The NPG values and their changes recorded during the individual tests in patients within the group were compared and statistically evaluated by means of Mann-Whitney-U test (A p value < 0.05 was considered to be statistically significant.)

The results can be summarized as follows:

(1) -Differences of the NPG baseline values recorded in the patients of all 3 groups, being within 1.25 cm H_2O, have been considered to be statistically not significant ($p > 0.05$) as compared in individual patients as well as among the groups.

(2) -Group I. No statistically significant differences have been found between the baseline and the dry wad of cotton wool test ($p > 0.05$) or between the baseline and the wad of cotton wool saturated with PBS ($p > 0.05$) at each of the time-points up to 60 minutes and 12 hours. All patients demonstrated a non-decreased nasal histamine threshold (≥ 4.0 mg/ml). The NPG values recorded after the challenge with histamine in concentrations higher than 4.0 mg/ml were significantly positive at 5 and 10 minutes as compared with the baseline values ($p < 0.05$).

(3) -Group II. No statistically significant differences of the NPG values were found between the baseline and PBS test (($p > 0.05$). The NPG values recorded after the dry wad of cotton wool differed significantly from the baseline NPG values ($p < 0.05$). The NPG values recorded after the challenge with histamine in a concentration of 4.0 mg/ml (= non-decreased nasal histamine threshold) differed significantly from the baseline ($p < 0.05$).

Table 57 Survey of the statistical significance of the differences of NPG values recorded during the individual tests in patients within the particular groups.

	Baseline values mean NPG values in cm H_2O ±SD	±SE	Baseline test	Dry wad of cotton wool	PBS test	Histamine threshold dose	significance
Group I [no rhinitis] (n=12)	0.8±0.4	0.8±0.2	$p > 0.05$	$p > 0.05$	$p > 0.05$	> 4.0 mg/ml	$p < 0.05$
Group II [only allergy] (n=24)	1.2±0.5	1.2±0.3	$p > 0.05$	$p < 0.05$	$p > 0.05$	> 4.0 mg/ml	$p < 0.05$
Group III [only N-SH *] (n=20)	1.1±0.5	1.1±0.3	$p > 0.05$	$p < 0.01$	$p \geq 0.05$	< 2.0 mg/ml	$p < 0.001$

* N-SH = non-specific hyperreactivity

Table 58 Survey of the statistical significance of the differences in the nasal parameters (= NPG values), recorded during the individual tests between the particular groups of patients.

Group	Baseline test	Dry wad of cotton wool	PBS test	Histamine threshold
I - II	$p > 0.05$	$p < 0.05$	$p > 0.05$	$p > 0.05$
I - III	$p > 0.05$	$p < 0.01$	$p > 0.05$	$p < 0.01$
II -III	$p > 0.05$	$p \geq 0.05$	$p > 0.05$	$p < 0.01$

(4) -Group III. The NPG values measured after the dry wad of cotton wool differed statistically significantly from those recorded during the baseline ($p < 0.01$). The differences between the NPG values recorded after the PBS test and those during the baseline were statistically not significant ($p > 0.05$). The NPG values recorded after the challenge with histamine in concentration < 2.0 mg/ml differed significantly from those of the baseline ($p < 0.001$).

(5) The statistical analysis of the particular tests within the groups is shown in Table 57, while that between the particular groups of patients is presented in Table 58.

With respect to the above presented data, it can be concluded that the application technique using the wad of a cotton wool on a nasal probe saturated by PBS has not caused any significant artefacts or mechanical irritation of the nasal mucosa. This technique of application cannot, therefore, influence the results of the NPT either with allergen or with non-specific agent, even in the patients with an increased non-specific hyperreactivity.

B. COMPARISON OF THE "BALLOON TECHNIQUE" [BT/BM] WITH THE "ACTIVE POSTERIOR RHINOMANOMETRY" [APR] AND WITH THE "PASSIVE ANTERIOR RHINOMANOMETRY" [PAR]

1. BALLOON TECHNIQUE versus ACTIVE POSTERIOR TECHNIQUE

In a group of 12 patients with allergic rhinitis and known immediate nasal response (INR) due either to house dust or grasspollen, two nasal challenges with the same allergen have been performed. One of the nasal challenges has been recorded by the "Balloon method", whereas the other by the "Flow-Pressure technique" (APR). Both the tests have been performed by different investigators according to the double-blind cross-over schedule. An interval of 4-14 days has always been inserted between the two tests.

The nasal response (= obstruction, resistance) recorded by the "Balloon method" for each nasal cavity separately has been expressed in pressure differences, the so-called NPG values (nasopharynx-nostril-pressure gradients in cm of $H_2O = \Delta P$), whereas the nasal response measured by the "Flow-pressure technique" has been expressed as a total nasal resistance (R_N in cm H_2O/liter/sec), calculated from the simultaneously recorded values of the airflow and air-pressure differences by means of the

formula:

$$R_N = \frac{\text{Pressure}(=\Delta P)}{\text{Flow}} \times \text{Coefficient (apparatus)}$$

The immediate nasal responses recorded by the particular techniques in the individual patients have firstly been compared with the appropriate Coca's solution control values and their positivity has been statistically evaluated by Wilcoxon matched-paired signed-rank test. The two INRs, recorded by means of both the techniques in the same subject, have then been compared and statistically analyzed by the variance analysis (generalized multivariate analysis of variance model = MANOVA). A $p<0.05$ has been considered to be statistically significant for both the statistical methods. The immediate nasal response to allergen challenge (INR) recorded by both the techniques has been found to be significantly positive ($p < 0.01$). No statistically significant differences have been found either between the INR recorded by the BT and the INR recorded by the APR ($p > 0.05$) or between both the individual values at each of the time intervals during the 120 minutes after the allergen challenge (Table 59).[8]

2 BALLOON TECHNIQUE versus PASSIVE ANTERIOR TECHNIQUE

In another group consisting of 24 patients with allergic rhinitis, the NPTs have been performed with various "inhalant" allergens and the nasal responses have been recorded by the "Balloon technique" (BT) and by the "Anterior passive rhinomanometry" (PAR) and the parameters were recorded up to 12 hours after the challenge (these data have not yet been published). Two PBS control challenges have also been performed in each of the patients and recorded by each of these techniques. In the BT only the non-intubated nasal cavity has been challenged with allergen, while during the PAR method both the nasal cavities have been challenged simultaneously. An interval of 5 days has always been inserted between the two tests. The NPG values recorded by the "Balloon technique" have been expressed in "cm H_2O", while those measured by the PAR methods in cm H_2O/L/sec. The results were statistically analyzed by means of the Wilcoxon matched-paired signed-rank test ($p < 0.05$ was considered to be statistically significant). The immediate nasal response to allergen challenge (INR), recorded by the "BT", as compared with the PBS control response, has been found to be highly significantly positive ($p < 0.001$). The INR detected by the PAR method, as compared with the PBS control response, has also been found to be significantly positive for both the sides, left and right, ($p < 0.05$). No statistically significant differences have been found between the left and the right INR recorded by means of the PAR method ($p > 0.05$). The INRs recorded by the BT did not differ significantly from both the INRs (left and right) recorded by the PAR method ($p > 0.05$). The statistical significance of the INR recorded by the "BT" was slightly higher than that detected by PAR method. No significant changes have been recorded during the PBS control tests measured either by the BT ($p > 0.02$) or by the PAR method ($p > 0.05$), or between the left and right sides of the PAR technique ($p > 0.05$) (Table 59).[25b]

C. EVALUATION OF THE "BASE-LINE" (INITIAL VALUES)

We also have compared the base-line values recorded by the "Balloon technique" (mean NPG values in cm H_20) in 12 patients with the pollen-related allergic rhinitis and in 10 non-allergic subjects during a 12-hour period. No statistically significant differences in the variations of the NPG base-line values have been found either in the subjects within the particular groups ($p>0.05$;$p>0.05$) or between the two subject groups ($p>0.01$) (Table 60) (not yet published data).

From these studies we have concluded that all three rhinomanometry methods, "active posterior", "passive anterior" and "balloon", are in principle suitable and reliable techniques for the nasal provocation tests, with sufficient and acceptable reproducibility. Similar conclusions have been drawn by other investigators studying the reproducibility of the nasal parameters recorded by the "passive anterior", "active anterior", and "active posterior" rhinomanometry and "oscillometry", by means of comparison of the coefficients of data variation (Table 60).[48j,57a]

Table 59 Survey of the statistical significance of the nasal resistance values (INR) recorded by various rhinomanometry techniques

	INR	Comparison of INR recorded by the particular techniques		
		BM	APR	PAR
BM	$p<0.01$ (n=12) $p<0.001$ (n=24)	-	$p>0.05$	$p>0.05$
APR	$p<0.01$ (n=12)	$p>0.05$	-	-
PAR	$p<0.05$ (n=24)	$p>0.05$	-	-

BM = Balloon technique (NPG in cm H_20); APR = Active posterior rhinomanometry (R_n in cm H_20/L/sec);PAR = Passive anterior rhinomanometry (R_R, R_L in cm H_20/L/sec); INR = Immediate nasal response to allergen challenge (=the statistically significant increase in the nasal resistance parameters within 60 minutes after the allergen challenge); Interpretation of the "p values":$p>0.05$ = non-significant, $p<0.05$ = significant, $p<0.01$ = clearly significant, $p<0.001$ = highly significant

Table 60 Summary of some studies concerning the reproducibility of rhinomanometry techniques. The parameters were recorded in rhinitis and non-rhinitis subjects at baseline.

	Shelton et al.[48j]		Ferguson and Thomas[57a]		
	Coefficient of variation of NAR values in %		non-rhinitis subjects		
Rhinomanometry technique	control subjects(n=15)	rhinitis subjects(n=12)	Mean NAR	CV	SD
Passive anterior	10	12	0.219	27.9	±15.6
Active anterior	11	18	0.252	30.6	±14.1
Active posterior	14	19	0.271	23.2	±15.2
Oscillation	9	9	–	–	–

	Baseline - mean NPG values in cm H_2O								
	Control subjects (CS;n=10)	Rhinitis subjects (ARS;n=12)	Control vs Rhinitis (n=10/12)	SE		SD		2SD	
				CS	ARS	CS	ARS	CS	ARS
Balloon technique	0.8 (p>0.05)	1.2 (p>0.05)	1.1 (p>0.01)	0.2	0.3	0.3	0.5	0.6	1.0

NAR = Nasal airway resistance; Mean NAR = in Pa $ml^{-1}s$;CV = Coefficient of variation; CS = Control subjects; ARS = Allergic rhinitis subjects; SD = Standard deviation;SE = Standard error.
References: 11i,12,25b,184,185

VII. POSSIBLE MECHANISM(S) UNDERLYING THE "LNR"

There is no doubt of the existence of LNR, as it has been repeatedly demonstrated and confirmed by several investigators 2,7,11c,11f,12,14,14a-14c,15-17,19,24, 25,26,27a,27b,28,29,32-34,35c,40a,40c-40f,41,41a-41d,41f,41i,42,43,48,48d,48e,51, 51a-51c,53-56,57a,72,72a-72c,82,82c,83,94, 95a,96,97,97b,97c,97u,121b

The views on the pathogenetic and immunologic mechanism(s) presumably underlying the clinical LNR, however, vary.2,7,12,14,14a-14c,15-17,25,25a,26, 28, 29,32-34,35c,40a, 40c-40g,41a-41d,41f,41i,42,43,45,48, 48d,48e,51,51a-51c,53,54, 56,72,72a-72c,82,82c,83,94,95a,96,97, 97b,97c,97u,121b

There is a variety of controversial information, results, and hypotheses in the literature, trying to explain the clinical phenomenon "late-phase allergic reactions". Therefore we have decided to summarize the known facts and hypotheses concerning the possible mechanisms that may be involved in the late type of allergic responses, using the "late nasal response" (LNR) as an example, and to combine them with our own and other investigators' results. The summary is presented in Figures 53a,b and Table 61. However, the effects and possible role(s) of all particular mediators, intermediators, precursors, factors, chemotactic factors, derivatives, and recently reported cytokines, adhesion molecules and other new compounds and molecules in the presumed mechanism(s) underlying the LNR are not included in detail in this schedule.

A. THE "LNR" AND THE "LATE TYPE HYPERSENSITIVITY" [LH]

1. DEFINITION OF "LNR" AND "LH"

A distinction should be made between the LNR and the late type of hypersensitivity (Type III allergy, Arthus reaction, immune complex state). The late type of hypersensitivity is a well-defined immunologic mechanism, characterized by involvement of IgG and possibly also IgM antibodies, forming the immune complexes and resulting in the complex inflammatory reactions leading to the tissue damage.126-128, 128a,128b

The LNR should be regarded as a clinical phenomenon, defined by the appearance of nasal symptoms and complaints, predominantly obstruction, accompanied by other symptoms and changes, within 4 to 12 hrs after the allergen challenge or exposure (antigen-antibody interaction), which may be induced by complex mechanisms.2,7,12, 34,42-44,82,90,94,124, 129,130,130a,131,131a-131f Although the pathogenetic and immunologic mechanisms leading to the LNR can be different, the late type of hypersensitivity should be regarded as one of the possible mechanisms involved in the clinical LNR, but is far from being the only one [Figures 53a, 53b (pages 377,177), Table 61].7,41b,72b

2. SYSTEMIC LATE HYPERSENSITIVITY

Generally, in systemic late hypersensitivity (Type III allergy, Arthus reaction, immune-complex state), circulating antibodies of the IgG and IgM classes, being presumed to play a major role, react with circulating antigens in the blood stream or in the vascular wall, and in this way form the immune-complexes.[126-128,128a, 128b,129-131,132,132a] The immune-complexes then activate the complement system cascade,[126] especially C3a, C5a, C5b, C6, C7,[132a] with subsequent activation of the blood-clotting mechanisms, liberation of kinin,[129] release of lysosomal enzymes, vascular permeability factors and other factors[126-128] from the polymorphonuclear neutrophil leucocytes,[126,127,128a, 128b,129,131b,131c, 133-136,136a-136d] and release of vasoactive amines, lysosomal enzymes and other factors from the platelets, [126,128,129,136,137-139,139a-139d] and activation of the eosinophils.[140-146]

Besides various chemotactic and other factors, some cytokines may also participate in the activation and stimulation of neutrophils, as it has recently been shown.[131f] The IL-1 may enhance the blood neutrophil count and local neutrophil infiltration in the tissue; TNF ($\alpha + \beta$) may contribute to the local neutrophil infiltration in the tissue, but they also can act as the chemoattractans for the neutrophils, and finally IL-8 may also contribute to the neutrophil chemoattraction.[131f,146a,146b]

Data concerning the possible participation of cytokines in the immunoregulation and activation of platelets are not yet available in the literature.[139a-139d]

These factors as well as platelets and neutrophils themselves, may then be involved directly or indirectly in the tissue damage, which is a complex of various inflammatory reactions typical for the Type III hypersensitivity.[126-128,128a,128b, 129,131e,131f,132,134-136,136b,136c,138,139,146a]

The involvement of neutrophils, platelets and other inflammatory cells in the tissue damage necessitates firstly their activation, secondly their attraction to the appropriate tissue, and thirdly their adhesion to and interaction with the endothelial cells.[128b] The chemotaxis of neutrophils into the airways requires the expression of the cell surface proteins that recognize and adhere to the natural ligands on the vascular endothelium. One of such adhesion protein, expressed on the cellular membrane of neutrophils is the MAC-1 (membrane attack complex =CD11b/CD18 complex).[136c]

Increasing evidence also indicates that endothelial cells in the blood vessels may play an active role in the development of the immunologically mediated inflammation and tissue damage due to the immune complexes.[146d]

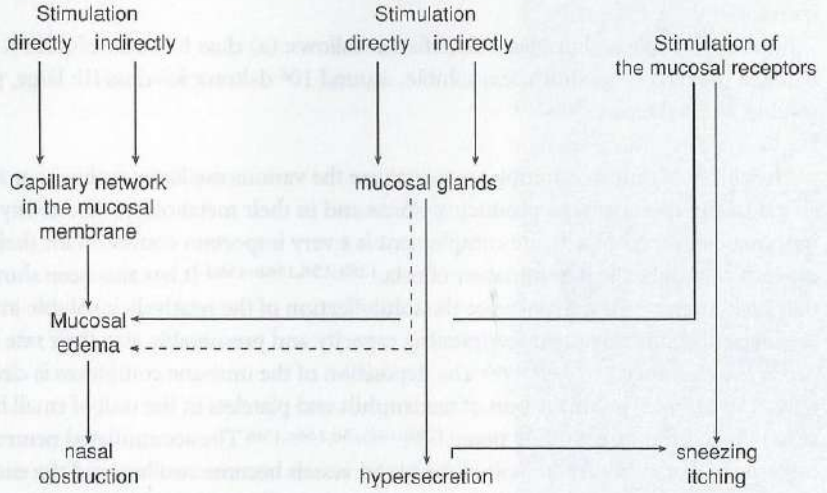
The participation of the neutrophils in the inflammatory processes, among others in the late hypersensitivity mechanisms, also through some other additional systems, processes and pathways cannot be excluded.[146e] The kinin system, including its components, such as Hageman factor, clotting factor XI, prekallikrein and high-molecular-weight kininogen (plasma-tissue interaction), should be considered as one of such additional pathways, since the neutrophils have been shown to contain neutral proteases capable of generating kinin and cathepsin D, leading to the liberation of the pharmacologically active peptides of 21-25 amino acids, called leukokinins.[146f, 146g,146h]

The IgG and possibly also IgM are presumed to play a major role in the formation of the immune-complexes after their interaction with an antigen,[126-128,128b,132,147]

Table 61 Survey of the compounds, being products of the particular cell types, involved in the intercellular traffic, signal transduction and in the cell-to-cell communication, in both directions, both up- and down-regulation, processes which may lead to the appearance of the clinical "LNR"

- Mediators, such as histamine, serotonin, acelylcholine, bradykinin	- Various enzymes	Superoxide anion O_2 and other oxygen radicals
- Platelet activating factors	- Cyclic nucleotides	Various protein molecules
- Leukotrienes	- Kinin system parts	lysosomal enzymes
- Prostaglandins	- Clothing system parts	Heparin
- Cytokines	- Properdin system parts	G-protein-linked receptors
- Chemotactic factors	- Thromboxanes	Phospholipids
- Neuropeptides	- Particular electrolytes	Adenylate cyclase
- Histamine releasing factors	- MBP (major basic protein)	Tryptase
- Complement parts	- ECP (eosinophil cationic protein)	Chymase
- Membrane-associated receptors	- EDN (eosinophil-derived neurotoxin)	
- Adhesion molecules	- EPO (eosinophil peroxidase)	
- Calcium channel factors	- Membrane-associated molecules	
- Chloride channel factors	- Parts of cyclooxygenase pathway	
	- Parts of lipoxygenase pathway	
	- Adenosine	

Fig. 53b.. THE POSSIBLE PATHWAYS UNDERLYING THE PARTICULAR NASAL SYMPTOMS RESULTING IN LNR.



References: 11h,41b

occurring in the bloodstream, in the vascular wall or, sometimes, in the tissue. The IgG antibodies can also directly activate the eosinophils through their membrane IgG receptors,[140-142,146,147-149] and stimulate the macrophages,[150,151] neutrophils[141,146,146e, 147,149-152] and platelets. [147, 151,153,153a,154,155] With regard to these facts, the IgG and possibly also IgM antibodies might be suspected of participating in the late hypersensitivity mechanism.[128b]

3. IMMUNE COMPLEXES [IC]

In the classical model of the immune complex-mediated inflammation, the antigen-antibody complexes, cross-linked in a lattice fashion, are formed in the circulation and are secondarily deposited in the tissue. The complexes, formed at the equivalence of antigen and antibody, or at a moderate antigen excess, are of the most effective size for the activation of the complement, and they also have the most prolonged residence in the circulation. Complexes formed at the antigen or antibody excess are small, because the saturation of all antibody - or antigen-binding sites respectively, prevents the multiple cross-linking required for the lattice formation. [128a,128b,147,156]

The soluble antigen-antibody complexes formed in the circulation are deposited in various tissues. If the immune complexes are of a large size, they become trapped, particularly on the basement membrane and in the blood vessels, where they are deposited on the internal elastic lamina.[156a] In addition to their size, the localization of the immune complexes in the particular tissue may also depend on some secondary factors, such as blood stream speed, blood flow turbulence, the ionic charge of the particular immune complex and other factors.[128b,156a]

In contrast, some other investigators have presumed that the antibody excess may lead to a formation of larger immune complexes with extensive lattice formation, which would rapidly be cleared from the circulation, rather than their deposition in the vascular wall.[126]

Immune complexes have been classified as follows: (a) class I: small, soluble, $< 10^6$ daltons; (b) class II: medium, less soluble, around 10^6 daltons; (c) class III: large, poorly soluble, $> 10^6$ daltons.[126,128b]

The ability of immune complexes to activate the various mediator pathways may play a vital role in their damage-producing effects and in their metabolism. The ability of the immune complexes to activate complement is a very important condition for their capacity to induce the inflammatory effects.[128b,156,156b-156d] It has also been shown that such interactions may increase the solubilization of the relatively insoluble immune complexes by affecting their precipitating capacity and presumably also their rate and site of the clearance.[155a,156e-156h] The deposition of the immune complexes is clearly related to the local accumulation of neutrophils and platelets in the wall of small blood vessels and in the surrounding tissue.[128b,148,156,156c,156i] The accumulated neutrophils undergo leukocytoclasis, the wall of the blood vessels become swollen, and the endothelial cells appear to be damaged.[128b] The endothelial cells are then able to synthesize some pro-inflammatory cytokines, such as IL-1, IL-6 and IL-8,[128b] as well as some proteolytic enzymes,[128b] vasoactive compounds including prostacyclin, "endothelial

derived relaxing factor" (EDRF) and some lipid-based mediators, such as platelet activating factors (PAF),[156j] indicating their active participation in the inflammatory responses. Finally, the endothelial cells constitute a major barrier against the fluid being lost from the vessels and their damage results in the increased permeability at the sites of the inflammation.[156j]

The endothelial cells are also able to synthesize and express a series of adhesion molecules on their surface, which are glycoproteins mediating the cell-cell and the cell-matrix interactions, playing an important role in the inflammation processes.[128b156j] The endothelial cells can express various cell adhesion molecules from the most families, such as integrins, selectins, adherins, immunoglobuline supergene family and cartilage-link proteins.[128b,156j] Some of these cell adhesion molecules on the endothelial cells are able to mediate the attachment of the leukocytes, among others neutrophils, and platelets to the endothelium, by binding with the corresponding cell adhesion molecules present on the surface of the leukocytes.[128b,156j,156k] The intercellular adhesion molecules-1 and -2 (ICAM-1, ICAM-2), presenting on the surface of the endothelial cells, bind to the cell adhesion molecule LFA-1 (= CD11a/CD18) expressed on the leukocytes.[156j] The ICAM-1 expression can be increased by IL-1, TNFα, IFNγ and LPS (= lipopolysaccharides), triggering the production of some cytokines, e.g. IL-1, IL-6, IL-8, TNFα, IFNα;[128b,156l] this in turn leads to the enhanced leukocyte binding and subsequent migration into the tissue.[128b,156d,156j]

Moreover, the "endothelial cell leukocyte adhesion molecule" (ELAM-1) or so-called "E-selectin", "vascular cell adhesion molecule-1" (VCAM-1), "inducible cell adhesion molecule-1" (INCAM-110), and "granular membrane protein-140" (GMP-140) or so-called "P-selection", are usually not expressed by endothelial cells, but their expression can be induced rapidly by some cytokines, such as IL-1 and TNFα (ELAM-1, VCAM-1) or histamine (GMP-140).[146d,156j,156m] The ELAM-1 and GMP-140 preferentially bind the neutrophils, whereas VCAM-1 may have more affinity for monocytes and lymphocytes.[128b,146d,156j-156n] The GMP-140 and the so-called "platelet activation dependent granule-external membrane protein" (PADGEM), belonging to the P-selectins, express also the chemotactic activity for platelets.[156j] Recently, a new endothelial cell surface adhesion molecule has been reported, the "vascular adhesion protein-1 (VAP-1)".[156p] Platelets also express various membrane glycoproteins, being involved in their adhesion, such as "integrin superfamily of adhesion receptors ($\alpha + \beta$), a subgroup of them are "very late activation factors 2,5,6" (VLA-2, -5, -6), "selectins", such as GMP-140, and "platelet-endothelial cell adhesion molecule-1" (PECAM-1).[139c]

The ability of immune complexes to activate the complement system represents one of the pivotal conditions for their capacity to induce the inflammatory effects.[128b,156, 156b-156e,156r] The activated complement then plays an important feed-back role in the clearance of the immune complexes.[128b,156] The complement activation can be initiated either through the classical (CCP) or the alternative pathway (ACP), depending on the immunoglobulin class. [128a,128b,146h] The IgG, IgG subclasses 1,2 and 3, and IgM-containing immune complexes may activate the complement via the classical pathway, whereas IgA-containing immune complexes may activate the complement probably via the alternative pathway. The IgD and IgE do not activate the complement sufficiently

and efficiently. The valence of the antibody as well as of the antigen affects the size and composition of the immune complexes.[128b]

The monovalent antigens combine with only one antibody binding site and are not able to cross-link the antibody molecules, whereas the multivalent antigens are able to bind the multiple antibodies with different specificities and to form the large immune complex lattices.[128b] The principal inflammatory factor derived from the complement cascade appears to be C5a, which also express the chemotactic activity for the neutrophils.[126,128a,132a,156b]

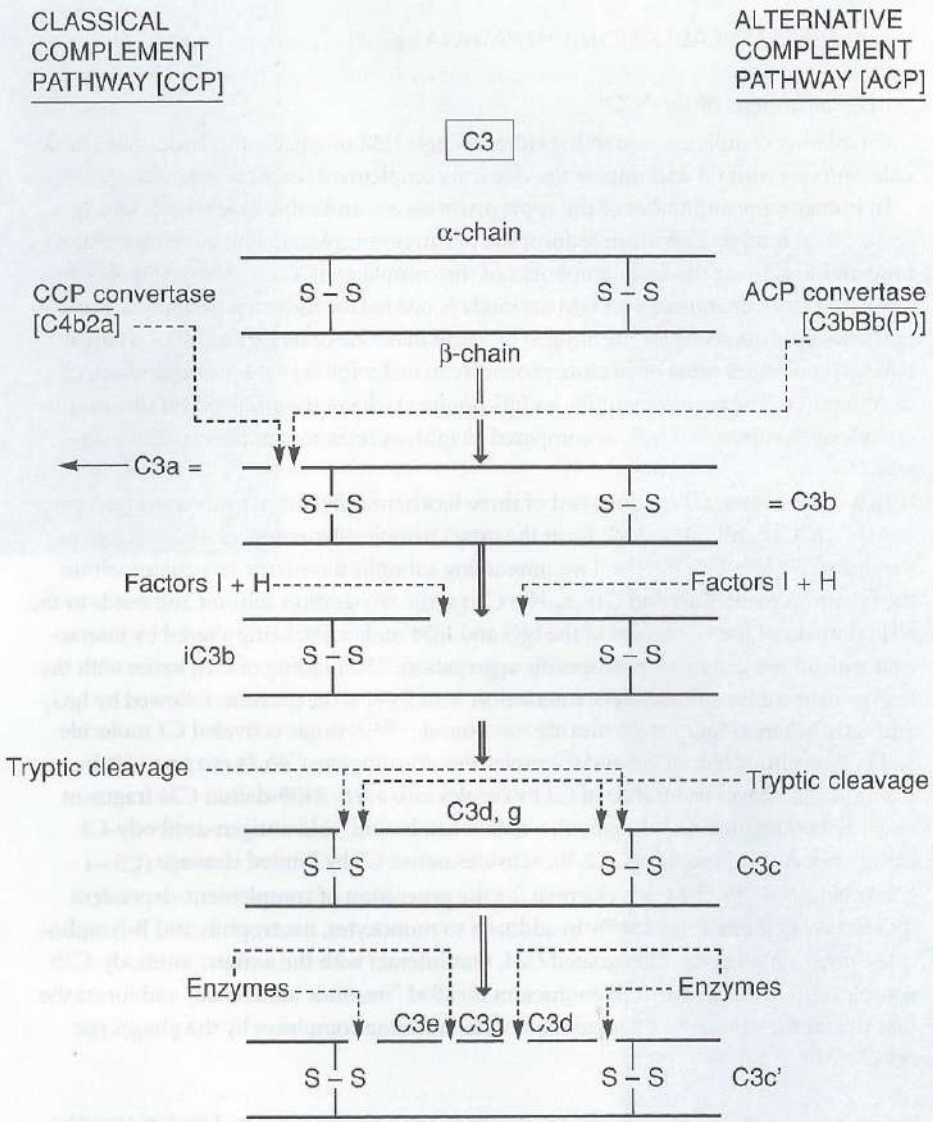
4. THE COMPLEMENT SYSTEM

The complement system includes approximately 20 distinct proteins which can be divided into four main groups, the classical pathway (CCP) components, the alternative pathway (ACP) components, the terminal components and the control proteins.[156s]

Two major pathways of complement activation operate in plasma, a classic and an alternative pathway.[128a,128b,156,156e,156r] The classical complement pathway is usually initiated by antigen-antibody complexes, whereas the more recently discovered alternative complement pathway does not necessarily need the presence of an antibody for its activation.[156,156b-156d] Both pathways operate through the interaction of specific proteins, called components. Both the pathways proceed by means of sequential activation and assembly of a series of proteins, leading to the formation of a complex enzyme capable of binding and cleaving a key protein, C3, which is common to both pathways.[128a,128b,156r] Thereafter, both pathways proceed together through the binding of the terminal components to form a membrane attack complex (MAC), which ultimately causes the cell lysis.[128a, 128b,146h] The proteins of each pathway interact in a precise sequence, called cascades.[156,156b] At several points in the activation sequence an inactive precursor molecule or zymogen is activated, with the zymogen acquiring the proteolytic activity specific for its substrate. This substrate is in turn activated ready to carry out its role in the cascade. One of the most important consequences of such a cascade system is that the stepwise amplification can occur at each activation step, since the newly generated proteolytic enzyme is able to activate a number of substrate molecules.[156s] When a protein is missing, e.g. in the case of some deficiences, the sequence is interrupted at that point.[146h] The early steps in the activation process are associated with the assembly of complement cleavage fragments to form enzymes that bind the next proteins in the sequence to continue the reaction cascade (these enzymes are designated with a bar above the symbol of the component to indicate the active enzymatic activity).[128a,128b,156,156b,156r]

The classical complement pathway includes the following components: $C1_{(q,r,s)}$, C_2 and C_4, whereas the alternative pathway includes components, such as properdin, factor B, factor D. The component C3 as well as the terminal components C5-C9 are common to both pathways.[128a,128b,146h,156,156b-156e,156r] Recently, other proteins have also been identified as participating in the classical pathway, such as C_1-in and C4bp.[156] The complement system is under the control of the so-called "control proteins" present either in the plasma or on the cell membranes, such as C_1-inhibitor, C_4-binding protein, factor H, factor I, S protein, anaphylatoxin inactivator and SP 40,40, which execute

Fig. 55. SCHEDULE OF C3 CONVERSION



the whole regulatory role of the complement system, including the particular stimulatory as well as inhibitory activities [Figures 54, 55 (pages 181, 378)].[128a,128b,156,156b-156e,156r]

The complement system modulates and amplifies a variety of biologic effects, including also those of immune complexes.

[A] THE CLASSICAL COMPLEMENT PATHWAY [CCP]

(1) Initial steps of the "CCP"

Circulating complexes containing either a single IgM or adjacent IgG (doublet) molecules interact with C1 and initiate the classical complement reaction sequence.[132a]

In humans, the antibodies of the appropriate classes and sub-classes (IgM, IgG, IgG_1, IgG_2, IgG_3) bind to an antigen to form the immune complexes, which are then able to bind and to activate the first component of the complement, C1.[156, 156b-156g,156i,156p, 156t-156y] Only one molecule of IgM antibody is needed for the activation of C1, but the IgM molecule must engage the antigen by more than one of its Fab arms. In contrast, two IgG molecules must be in close proximity in order for the C1 binding and activation to occur. The requirement for an IgG doublet reduces the efficiency of the immune complexes composed of IgG, as compared to IgM, as activators of the classical pathway.[132a]

In its native state, C1 is composed of three biochemically distinct sub-units (glycoproteins) C1q, C1r and C1s, which form the intact trimolecular complex with calcium as the ligand.[126,132a,156,156c,156s] Two interacting subunits have been recognized within the C1 component, C1q and $C1r_2s_2$.[156s] C1q is the recognition subunit and binds to the CH_2 domain of the Fc regions of the IgG and IgM molecules, being altered by interaction with an antigen or by non-specific aggregation. The binding of C1q varies with the heavy chain composition of IgG: interaction with IgG_3 is the greatest, followed by IgG_1 and IgG_2, whereas IgG_4 molecules are not bound. [156t] A single activated C1 molecule ($\overline{C1}$) cleaves hundreds of C4 and C2 molecules, forming the C4b,2a enzyme.[132a] In turn, $\overline{C4b,2a}$ cleaves thousands of C3 molecules into a free 8400-dalton C3a fragment and a 171,000-dalton C3b fragment, which when bound yield antigen-antibody-C3 complexes. A third enzyme, C4,2,3b, activates native C5 by limited cleavage (C5 → C5a+C5b)[132a, 156t] The C3 is essential for the generation of complement-dependent inflammatory mediators.[156,156c] In addition to monocytes, neutrophils and B-lymphocytes have C3b receptors, designated CRI, that interact with the antigen-antibody-C3b complexes. This attachment phenomenon is called "immune adherence" and forms the first step in the uptake and degradation of the immune complexes by the phagocytic cells.[126,156]

The binding of C1q to the antibody results in the activation of C1r.[156a,156i,156y] The activated C1r ($\overline{C1r}$) possesses serine protease activity and is capable of converting C1s to its enzymatically active form, $\overline{C1s}$. [132a,156]

The activated $\overline{C1s}$ then activates the C4 component.[157] Native C4 is composed of 3 chains, α, β, γ linked by disulfide bridges.[157a] The activation of C4 by C1 is accomplished by the cleavage of a small peptide (C4a) from the alpha chain of the molecule.[157]

This exposes an interchain reactive thiolester in the remaining portion of the alpha chain of the larger cleavage product, C4b.[157,157b] The nascent C4b then binds covalently to the cell surface or to the immunoglubin by means of either amidation of the amino groups of proteins by the acyl group of the thiolester, or esterification of the hydroxyl groups of the polysaccharides.[132a]

The activation of C4 by C1 is an amplification step in the complement cascade, since one enzymatically active C1 can cleave multiple C4 molecules.[132a] The C1 then activates the C2. The cleavage of the C2 produces a small peptide, C2b, which is released into the fluid phase. The larger fragment C2a, remains complexed with the C4b to form a bimolecular enzyme $\overline{C4b,2a}$ also called C3-convertase of the classical pathway,[157] which is capable of cleaving the C3, resulting in the production of C3a and C3b, and thereby initiating the assembly of the terminal components C5-C9, into the membrane attack complex.[132a,157] The generation and expression of the C3 cleaving enzyme, $\overline{C4b,2a}$ is controlled by a variety of factors, such as (a) the high lability of this enzyme causing its spontaneous decay with release of the C2a and loss of enzymatic activity,[132a] (b) the C1 esterase inhibitor, binding covalently to $\overline{C1r}$ and $\overline{C1s}$, dissects the C1 macromolecular complex, (c) the C4-binding protein (C4BP), by binding to C4a, increases the rate of dissociation of C2a from the $\overline{C4b,2a}$ enzyme and increases the susceptibility of the C4b to proteolysis caused by C3b/C4b, also called factor I, and (d) the decay accelerating factor (DAF), an integral membrane protein, which also contributes to the release of C2a from the $\overline{C4b,2a}$ complex.[157,157y]

(2) Activation of the C3, also including the C3 conversion

The activation of C3 leads to the rise of cleavage products C3a, having anaphylatoxic activity, and C3b, expressing opsonic activity. Moreover, C3b participates both through the classical and through the alternative pathways in the activation of the C5, and probably also of the other terminal components (Figure 55).[132a]

The C3 activation, both through the classical and through the alternative pathways, leads anyway to a rise of two fragments, C3a and C3b. However, in the C3 convertase participating in the classical pathway (=$\overline{C4b,2a}$), the C2a represents the active site, while in the C3 convertase taking part in the alternative pathway (=$\overline{C3b,Bb}$), the Bb is the active component (Figure 55).[132a]

The activation of C3 forms an important amplification step in the complement cascade, since each C3-cleaving enzyme can cleave hundreds of C3 molecules. The cleavage of native C3 by the C3 convertase leads not only to a release of a small peptide, C3a, from the alpha chain into the fluid-phase, where it can act as an anaphylatoxin, but also to the creation of nascent C3b. The internal triolester in the C3 alpha chain, similarly to the C4, then becomes disposable for amino- as well as transalyte hydroxyl groups.[157b] Some nascent C3b molecules are released into the fluid phase and are there inactivated by hydrolysis, whereas others may bind covalently to the immunoglobulins or cell surface by means of transamidation or transesterification.[157c] The C3b bound to the cell surface can act, at this moment, as an opsonin and/or it can combine either with the classical or with the alternative pathway C3-cleaving enzymes to create two new enzymes, the classical pathway C5-convertase and the alternative pathway C5-convertase.[132a]

The C3 bound to the cell surface may then be degraded by factor I and/or various proteases. This C3b degradation gives rise to the appearance of a number of different cleavage products, such as: iC3b, C3c, C3d, C3g, C3e, C3dk etc.[132a]

(3) Activation of the terminal components

The cleavage of the C5 into two fragments, C5a and C5b, represents the first step of the activation of the terminal complement components.[132a] The C5-cleaving enzyme of the classical pathway consists of C4b,2a,3b, whereas that of the alternative pathway is composed of C3b,Bb,3b.[157,157d] The two C3b molecules in the alternative pathway C5-cleaving enzyme accomplish various functions within the trimolecular complex, such as binding of the Bb sub-fragment to the cell surface in the proper configuration for expression of its enzymatic activity, or binding of the native C5 in the suitable location for cleavage by Bb sub-fragment.[157d]

Analogically to the C3, the activation of C5 by C5-convertase leads to cleavage of the alpha chain in the same location followed by a rise of the small peptide, C5a, and the large peptide, C5b.[132a] The C5a after release into the fluid phase, can play a role as an anaphylatoxin and chemotactic factor.[132a] On the other hand, the C5b interacting with the C6 component, becomes stabilized by the C6, and initiates the formation of the final target of the whole cascade, the membrane attack complex (MAC), a multimolecular complex consisting of C5b, C6, C7, C8 and C9, which accomplishes the cytolytic and destructive activity.[157e,157f] The particular complement components perform specific funtions within the MAC.[157,157f,157g] The C6 stabilizes the C5b,[157h] the C7 mediates the insertion of hydrophilic domains of the C5b,6,7 complex into the cell membrane, and the C8 not only amplifies the insertion of the complex, but also contributes to the formation of small transit gates in the cellular membrane for the passage of the small molecules. The interaction C5b and C8 is capable of initiating the polymerization of C9,[157f] a process leading to enlargement of the small membrane gates and to a formation of the large transmembrane channels through which the lipid molecules and other constituents of the target cell are displaced, a process resulting in final lysis of the target cell.[157e,157f,157g,157i]

The formation of the MAC is regulated by two proteins, the C8-binding protein, also called "homologous restriction factor"[132a], and the more recently described CD59 or HRF 20 factor.[132a,157j,157k]

The CD-binding protein, a widely distributed protein on the cell membrane of the peripheral blood cells, has significant sequence homology with both C8 and C9, interferes with the interaction of C8 and C9 on the normal cell membranes, and in this way it impedes the formation and insertion of the MAC. The CD59 of HRF 20 protein has no homology with either C8 or C9, but may inhibit the unfolding of the C9 required for its polymerization and membrane insertion.[157j-157m]

[B] THE ALTERNATIVE COMPLEMENT PATHWAY [ACP]

(1) Initial steps of "ACP"

This pathway can be initiated by antigen-antibody complexes. However, under certain conditions, it also can be initiated in absence of the antibody.[157b,157n,157p] The

appropriate agent, either antigen-antibody complex or antigen alone, activates the C3 component. Even in the absence of the activation of the classic complement pathway, a degree of the C3 conversion occurs due to the spontaneous hydrolysis and this is enhanced by other proteases, e.g. plasmin, or by other inflammatory products. This "low-level" or "tick-over" C3 conversion makes it possible for the alternative complement pathway to operate without the activation of earlier components of the classical pathway.[156s] The native C3, like the C4, contains an internal thiolester that spontaneously hydrolyzes.[157,157r,157s] The hydrolyzed C3, also called C3(H_2O), has some properties and is capable of executing some functions similar to those of the C3b, including the ability to bind the factor B and to mediate its cleavage by a serine-protease, also called factor D. The factor B is then cleaved into a small (Ba) and a large (Bb) fragment. The Bb fragment associates with the hydrolyzed C3 molecule to form a low-grade C3-cleaving enzyme, C3(H_2O)Bb, called the "priming" C3-convertase.[132a] This enzyme produces a continuous low rate cleavage of C3, and in this way also the continuous generation of nascent C3b. Analogically to the C4 component in the classical pathway, the cleavage of the native C3 component in the alternative pathway exposes the intrachain reactive thiolester, followed by transacylation with the particular acceptor groups on the surface of the appropriate cells.

The covalent binding of the nascent C3b to a cell surface leads to a formation of the reversible complexes with factor B, and is then cleaved by factor D, a process resulting in the generation of a very potent C3-cleaving enzyme, C3b,Bb, called the "amplification" C3-convertase.

It should be noted that the nascent C3b is not only a product of the alternative pathway C3-cleaving enzyme, but it is also a part of the enzyme itself. Therefore the activation of C3 within the alternative pathway can also act as a positive feedback amplification loop.[132a,157]

Furthermore, since the activation of the classical pathway leads to the generation of the nascent C3b, the classical pathway would also be able, under some circumstances, to activate the alternative pathway.[132a]

(2) Activation of the C3

The alternative pathway is regulated and controlled by a variety of factors, such as: (a) the properdin (-system) which stabilizes the binding of Bb to the C3b and decreases the intrinsic decay of the rather labile C3b,Bb enzyme;[157,157t] (b) factor H offers an alternative element to factor B for binding to C3b in the assembly of the C3b,Bb enzyme and additionally it is capable of displacing the Bb factor from the already formed enzyme complex.[157,157u,157v] Moreover, factor H is also capable of increasing and/or accelerating the inactivation of the C3b caused by factor I;[132a] (c) factor I inhibits the C3 cleaving enzyme of the alternative pathway by means of inactivating the cell bound C3b through a proteolytic cleavage resulting in the generation of iC3b.[157]

However, the binding of factor H to a particle, and thus its inhibitory effects on the alternative pathway C3-cleaving enzyme (C3b,Bb), is favored by the presence of sialic acid residues on the particles. The particles rich in sialic acid are therefore poor activators of the alternative pathway.[157x,157y]

(3) Activation of the terminal components

These steps of the ACP are similar and common to those of the CCP.

[C] CONTROL OF THE MEMBRANE ATTACK COMPLEX [MAC]

The formation of the MAC is regulated both in the fluid phase and on the cells.[156u, 157,157f,157g,157i] The C5b-7 complex can insert into the biologic membranes. The control in the fluid phase prevents diffusion from the activation site or target. The S-protein (Vitronectin), a 84-kD glycoprotein, is the major fluid-phase inhibitor of the MAC. By means of its attachment to the MAC, the S-protein blocks the ability of the C5b-7 to attach to a cellular membrane of the target cell.[156r,157m] The recently described SP-40,40 protein (Clusterin) may inhibit the MAC formation in a similar manner to that of the S-protein.[158] The CD59 glycolipid-anchored protein, also called "membrane inhibitor of reactive lysis" or "protectin", having binding sites for both the C8 and the C9, is capable of inhibiting the MAC assembly after the stage of the C7 binding.[156r, 157k,157m,158a]

[D] RECEPTORS FOR COMPLEMENT COMPONENTS ON THE CELL SURFACE (MEMBRANE)

The nine distinct types of receptors for the complement components or their cleavage products existing on the membrane of various cells have been recognized until now. These receptors, being in reality surface molecules that specifically bind with the particular components, may be divided into two groups: (a) receptors that bind the small diffusible and often labile products of the complement cleavage process, such as C3a and C5a; and (b) receptors that bind the larger cleavage products, such as C3b, being stably bound to the immune complexes or various particles.[132a,157,158b,158c]

The complement receptors can also be divided according to their ligands into: (a) The fluid phase activation peptides of C3, C4 and C5 components, such as anaphylatoxins C3a, C4a and C5a, which bind to either C3a/C4a or C5a receptors. These receptors initiate the important events of inflammation; (b) The C proteins being deposited on the immune complexes or surfaces that activate the alternative pathway, ligands including fixed C3 and C4 fragments (C3b, iC3b, C3d, C3dg and C4b), C1q and factor H. The receptors for these ligands have a major function in promoting the enhanced recognition of pathogenic substances, and particularly when present on the surface of the phagocytic cells, to initiate and operate the clearance and destruction of the solubilized immune complexes and opsonized microbial agents.[158c] The complement part receptors have been demonstrated on the membrane of various cell types in both animals and humans.[132a,146h,156r,157,157b,157e-157h,157l,157r,157s,157u,157v,158,158a, 158d-158l] However, the finding of the complement receptors also on the membrane surface of T-lymphocytes and antigen-presenting cells, provides evidence for some role of these receptors in the lymphocyte activation and antigen presentation, and in enhancing the primary response to certain T-cell-dependent antigens.[157c,158c,158m-158p] Regarding this evidence, neither the role of complement and/or its parts in the delayed (cell-mediated) hypersensitivity mechanisms, nor the contribution of the T-lymphocytes and their related factors to the other hypersensitivity types, can be excluded. Furthermore,

the CR2 (C3dg, C3d) receptor ligands have also been shown to stimulate the B-lymphocytes in a similar manner to the B cell growth factors.[158r]

(1) C1q receptors

The C1 component binds to the aggregated immunoglobulin, a process which leads to the activation of the C1 protease activity. The activated C1r and C1s sub-units are inactivated and dissociated by C1-inhibitor, whereas the C1q remains untouched at the site of the C1 binding.The C1q receptor can augment the binding of the immune complexes to the cells bearing both C1q and Fc receptors. The C1q receptor binds the immune complex-associated C1q preferentially.[158c] Various cell types express specific receptors for C1q, mostly through the collagen-like parts of the molecule.[157c,158c,158m-158u] The C1q binding to its receptors on the surface of the polymorphonuclear leukocytes (eosinophils, neutrophils)[157m, 158c,158m,158s-158u] and mononuclear phagocytes (monocytes, macrophages)[157c,158c,158m-158u] enhances their capacity to phagocytize the targets having been previously opsonized with IgG and C3 fragments, increases the respiratory burst and supports the intracellular and extracellular killing capacity.[132a, 156,156c,157g,158c]

The C1q receptors have also been found on the surface of the endothelial cells, platelets, vascular smooth muscle cells and fibroblasts.[132a,157g]

Since the C1q has chemotactic activity for fibroblasts, it may contribute to the platelet as well as fibroblast adhesion at the sites of the inflammatory processes and tissue damage.[132a]

The C1q also binds to the B lymphocytes. This binding may be effective only under simultaneous stimulation of the B cells by either polyclonal activators or soluble products of T-lymphocytes, both being able to cross-link the products of B cell and C1q receptors, resulting in the enhancement of the IgM and/or IgG production.[156,156b,156l, 157,158c]

The C1q has also been shown: (a) to inhibit the collagen-induced platelet aggregation and serotonin release;[158v] (b) to enhance the FcR-mediated uptake of soluble IgG-bearing immune complexes by lymphocytes;[158w] (c) to potentiate the FcR-dependent killing of IgG-coated target cells;[156,156c] (d) to inhibit lymphoblastoid cell expression of interleukin-1 (IL-1)[146b,158z] and (e) to act synergistically with the FcR on neutrophils, eosinophils, monocytes and macrophages in enhancing the production of superoxide, phagocytosis and cytotoxicity of the non-phagocytizable particles or organisms.[158t,158x, 158y] C1q receptor seems therefore to have a certain function in promoting the enhanced recognition of the IgG-bearing particles via FcR.[158c,158t-158y]

(2) Anaphylatoxin receptors (C3a, C4a, C5a)

The receptors for C3a have been found on the surface of mast cells, basophils, eosinophils, neutrophils, platelets and smooth muscle cells.[158c,158d,158e,158k, 159,159a-159c] These receptors bind the C3a with a high affinity.[158c]

The cellular receptors for C5a have been demonstrated on the membrane of mast cells, basophils, neutrophils, eosinophils, mononuclear phagocytes (monocytes, macrophages), endothelial cells and smooth muscle cells.[158g,158h]

The C5a receptors bind the C5a with a very high affinity and even extremely low C5a concentrations can elicit a distinct clinical reaction.[158c]

A variety of other complement components, proteins or their fragments, such as factor H and cleavage products of factor B (fragments C2b and C3b), have been reported to have the same effects on various cell types, presumably through the membrane receptors. These effects include increased vascular permeability, increase in the leucocyte count in the blood circulation, degranulation of the phagocytic cells and the up – as well as down – regulation of the production of immunoglobulins by B-lymphocytes/plasma cells.[132a,156,156c,156r,156t,157m,158c,158d,158i, 158k,158l,158m]

(3) Receptors for C3 and C4 components and fragments

The 4 membrane receptors for the C3 fragments, CR1, CR2, CR3 and CR4 with multiple and overlapping ligand specificity have been demonstrated on the membranes of various human cell types.[156r,158c,158h] These receptors may play a role not only in the phagocytosis processes, but also in the deposition of the circulating immune complexes, immuno-regulation and activation of the particular types of cells.[156u,158c] The CR1 should be considered a primary mechanism reducing the pathogenesis of circulating immune complexes present in both infections and auto-immune processes.[157,158b]

The CR1 receptor, also called "C3b receptor", CD35 molecule, or "immune adherence receptor" has been found on the membrane of mast cells, basophils, eosinophils, neutrophils, monocytes, macrophages, glomerular podocytes, B-lymphocytes, some T-lymphocytes (CD4+) and erythrocytes.[132a,156r,158b, 158c,158h,159d-159i] Besides the C3b fragment, the CR1 receptor probably also binds some degradation products of C4, such as C4b.[156r] The CR1 receptor present on the membrane of the phagocytic cells in low numbers can be, however, upregulated by factors such as INF-γ, C5a, INF, or even some bacterial products. [132a,156r,158c]

The CR2 receptor, also called "C3d or C3dg receptor" or CD21 molecule, has been demonstrated on the membrane surface of B-lymphocytes, immature epithelial cells, lymphnode follicular dendritic cells and in a few thymocytes, pharyngeal epithelial cells and some sub-types of T-lymphocytes. [132a,156r,158c, 159j,159k] The CR2 receptor binds the degradation products of C3, such as C3d and C3dg.[156r,158c,158r] This receptor is used by some virus types to promote virus penetration into the particular cell types.[156r, 158c]

The CR3 receptor, also called "iC3b receptor" or CD11b/18 molecule, and the CR4 receptor also designated CD 11c/18 or p150,95 molecule, belong to the "integrins family" of glycoproteins expressing adhesive functions.[156r,159e] The CR3 receptor has been found on the surface of neutrophils, monocytes, macrophages, eosinophils, some cytotoxic T-cells, follicular dendritic cells and LGL cells ($CD3^-$, TCR^-, Ig^-, $CD16^+$, $CD25^+$, $CD56^+$, $CD57^+$), called large granular lymphocytes with natural killer function.[132a,156k,158c,159k,159l,159m,159w] This receptor binds not only some C3 degradation products, such as iC3b, but also other factors and proteins including lipopolysaccharides, β-glucans, fibrinogen and factor X.[132a,156r,158c,159m]

The CR4 receptor is prominent on the cellular membrane of macrophages, but it has also been demonstrated on the surface of monocytes, neutrophils, large granular lymphocytes and follicular dendritic cells.[156k,158c,159l,159p] This receptor binds the iC3b fragment, lipopolysaccharides and some other cellular adhesion ligands.[132a,156k,156r, 158c,159w] The CR3 and CR4 receptors participate in promoting the uptake of some microorganisms by the phagocytic cells.[158c]

[E] ACTIVITIES EXECUTED BY THE ACTIVATED COMPLEMENT SYSTEM AND/OR ITS PARTS

(1) Anaphylatoxic activity

The anaphylatoxic activity is carried out by anaphylatoxins, C3a and C5a fragments, being the smaller cleavage products from the N-terminal portion of the alpha chains of the C3 respectively C5 components.[157,158c,158d,158e,158i]

The production of anaphylatoxins follows not only from the complement system activation, but also from the activation of other enzyme systems capable of cleaving directly the C3, C4 and C5 components, such as kallikrein, plasmin, tissue and cellular elements (leukocytes, especially neutrophils) releasing lysosomal enzymes and microorganisms producing bacterial proteases.[158k] The inflammation in the tissues stimulates the local production of the anaphylatoxins, which, by increasing local blood flow and vascular permeability and by their chemotactic effects, recruit various cell types and proteins to the site of the inflammation.[157]

Both anaphylatoxins are degraded by carboxypeptidase N in plasma, to the $C3a_{des\ Arg}$ and $C5a_{des\ Arg}$ products respectively.[157] The C5a is more potent than C3a and also than its derivate $C5a_{des\ Arg}$.[157,158c-158e] However, the $C5a_{des\ Arg}$ is supposed to be the major complement-derived inflammatory peptide "*in vivo*".[158c-158e] The anaphylatoxins have various biologic effects leading to the triggering of a variety of processes that may participate in the generating of the hypersensitivity and inflammatory mechanisms.[158d,158e,158i,158k,159n] The C3a and C5a may induce the contraction of smooth muscles, increase the vascular permeability and by means of ligation to their receptors on the cellular membrane of mast cells and basophils they may induce the degranulation of these cell types, followed by the release of histamine and probably also other mediators.[157,158c-158i,158k,159,159n]

The systemic effects of anaphylatoxins are usually well regulated by the activity of carboxy-peptidase N and by the rapid influx of the neutrophils and monocytes to the sites of complement activation. The receptors of neutrophils and monocytes act as a sink for C5a, which is internalized and inactivated by proteolysis. However, under certain circumstances, when complement is extensively activated, the mechanisms preventing the C5a effects may fail and serious consequences of the C5a activation occur. Such consequences include the release of toxic neutrophil products, such as oxygen radicals and/or granule constituents, such as neutrophil elastase.[157,159r]

(2) Chemotactic activity

The chemotactic activity is exercised by C5a, which is a smaller product of the cleaved C5 component.[157] The C5a fragment is a highly potent chemoattractant for neutrophils, eosinophils, basophils and monocytes,[156k,157,158c] and it also promotes the aggre-

gation and adherence of phagocytic cells to vascular endothelium.[132a,157] Possessing these effects, the C5a is capable of participating in the attraction of migrating phagocytes to the lung and peritoneal cavity tissue, being a site of the immune complex deposition or bacterial attack.[157] The activity of C5a is accompanied by degranulation of the neutrophils and lysosomal enzyme release from neutrophils, enhanced oxidative activity leading to the production of bactericidal oxygen derivates, and stimulation of lipoxygenase activity resulting in the LTB_4 production by neutrophils.[157,159n,159r,159s]

(3) Opsonic activity

The opsonic activity (which means "to prepare for ingestion") is executed by the C3b fragment, being a cleavage product of the activated C3 component.[132a,158c] The C3b fragment, after it has been fixed to the surface of the particles, such as immune-complexes or a micro-organism, is capable of acting as an opsonin.[132a, 156r,157,157n] The C3b promotes the attachment of the particles to the neutrophils and non-activated monocytes and when a small amount of IgG antibodies is bound to the particle, its processing by these cells is also stimulated by the C3b fragment.[132a,156c] The binding of IgG antibodies to the Fc receptors, expressed on the surface of phagocytic cells, leads to the distinct amplification of such phagocytic response when the target-bound C3b also simultaneously interacts with the CR1 receptor on the surface membrane of these phagocytic cells.[132a,156c,156r, 159t,159v] In contrast, in the already activated macrophages, the interaction C3b-CR1 triggering the processing and the phagocytosis of the particles, is not conditioned by the presence of the IgG antibodies.[132a,156c,159u]

Analogically to the C3b fragment, particles carrying the C3b degradation product, iC3b, on their surface, are capable of interacting with the CR3 receptor.[132a, 156c]

Similarly to the C3b-CR1 interaction, the iC3b-CR3 complex also needs the presence of IgG antibody to initiate the phagocytosis of the appropriate particles.[132a,156c,156r,159l]

The CR3 is probably the most prominent opsonic complement receptor "*in vivo*", since the iC3b is the most dominant fragment appearing on the surface of various particles.[132a,156r,159l]

Convincing evidence has been provided for the prominent role of the C3-related activity in the mechanism(s) underlying the complement-mediated host defence.[132a] However, our knowledge of these mechanisms in humans still remains incomplete.[156t]

(4) Bactericidal activity

Bactericidal activity of the complement system is generated by the activation of the terminal components C5, C6, C7, C8 and C9, and by assembly of the MAC.[132a] This activity can also be generated in the absence of the C9 complement component, which then seems to be slower than in the presence of the C9 component.[157h,157m] The outer lipid membrane of the gram-negative bacteria seems to be the site of the C5-C9 action.[132a]

(5) The role of the complement in the antibody formation

The role of the complement in the antibody formation includes various aspects.[132a, 156,156b-156d,156r] The macrophages, monocytes, B-lymphocytes and some subsets of the

T-lymphocytes have receptors for the C3 and C5 cleavage products.[158c] The C3 and C5 cleavage products can influence the antibody formation and production through affecting the functions of some cells participating in the antibody generation.[158b,158m-158p]

(6) The role of the complement in the processing of immune complexes

The role of the complement system in the processing of immune complexes may be manifold, as it has been repeatedly suggested by various investigators.[128,128a,128b,132a, 146h,156,156a-156g,156i,156p,156t-156y,158b] The activated C3b fragment carrying the opsonic activity can enhance the uptake of immune complexes by macrophages "*in vitro*".[157] The activation of C3 via the classical pathway may partly inhibit the precipitation of the immune complexes from the serum,[158j] whereas the activated C3 via the alternative pathway supports the solubility of the already formed immune complexes.[155a,156,156f, 156g,156u,156v,157] Both the prevention of precipitation and the dissolution of the immune complexes may be realized through the covalent binding of the C3b fragment to these complexes.[156r,157] The extent of the immune complex inflammation could be limited by a possible control system, including C3 convertase.[156f] It has been shown that immune complexes become soluble by the C3 convertase.[156f,156g,156u] This enzyme is formed by the activation of C3 by the factor B of the alternative pathway. When the C3 convertase is bound to immune complexes at equivalence, C3 fragments dissociate antigen-antibody binding, thereby breaking up the immune complexes.[128a,156] Small quantities of immune complexes are formed regularly in the absence of any disease. Normally, they are present transiently in small amounts, but they do not localize along filtering membranes and do not cause any injury. These small immune-complex amounts are eliminated through the phagocytosis by macrophages of the mononuclear phagocyte system (formerly called reticulo-endothelial system), which contain the membrane receptors for the Fc region of the IgG heavy chains and for C3, especially for C3b and C3d in animals, such as mice.[126,128a,128b,156v]

However, our knowledge of these mechanisms in man is still incomplete.[156t]

(7) "Acute-phase reactants"

Several complement proteins are so-called "acute-phase reactants", whose plasma concentration increases during the host response to injury or inflammation.[156d] The expression of the complement genes is regulated by a complex system, including various factors such as endotoxin, cytokines (IL-1, TNF-α, IL-6, IFN-γ and some growth factors), and sex steroid hormones, having been reviewed in detail by Perlmutter, Colten and colleagues.[156d,156w,156x]

[F] POSSIBLE PARTICIPATION OF THE COMPLEMENT SYSTEM IN THE IMMUNOLOGIC MECHANISMS

Despite our still limited knowledge, the complement system, is presumed to play a manifold role in various immunologic mechanisms at different biochemical levels.

This role includes a number of effects and interactions, such as increase in the blood vessel and capilary permeability, stimulation of the vasodilation, promoting the edema formation, increase in neutrophil adhesiveness, stimulation of chemotaxis of

various cell types, inducing the release of toxic oxygen species and lysosomal enzymes from phagocytic cell types, and a stimulation of the MAC formation, including the MAC-induced ion fluxes.[132a,156,156b,156c,156s,159t-159v,160,160a-160d]

(1) Vascular changes

The vascular changes include the vascular permeability, vasodilation and the edema formation.[159t-159v,160] The vascular changes are mediated predominantly by anaphylatoxins C4a, C3a and C5a.[156b,160] The vascular permeability and vasodilation are two separately controlled components of the inflammatory response with a certain synergism, which is a necessary condition for the production of the significant and prolonged tissue edema.[156e,159t] Another essential condition for the tissue edema is an increased accumulation and adherence of neutrophils to the endothelium of the small blood vessels at the C5a site.[156,159u] It has been proposed that the neutrophils may induce the increase in blood vessel permeability by direct production of, as yet poorly understood, " permeability factors", or else induce the stimulation of the endothelial cells to which they are attached to subsequently elicit factors such as; leukotrienes, D series prostaglandins and toxic oxygen radicals.[155a] Vasodilation appears to be mediated by prostaglandins which are produced by the neutrophils and/or endothelial cells, one of which is prostaglandin E_2.[155a,156e,159u]

The mode of action of prostaglandin synthetase and lipoxygenase inhibitors would support the role of prostaglandins as chemical mediators in the complement-induced vascular responses.[160e] Moreover, certain leukotrienes, such as LTB_4,[160e] cytokines, e.g. IL-1 and adhesion molecules, including E-selectines (ELAM-1) and P-selectins (PADGEM, GMP-140) have been suggested to participate in the adherence of the neutrophils to the vascular endothelium.[146c,146d,156j,156m,156n]

In most of the experimental systems, already very low doses of C5a, approximately of 1 ng, have been demonstrated to be able to produce a marked response.[160f] This amount is presumed to be physiologically attainable at sites of the complement activation, and therefore gains "*in vivo*" credibility. Moreover, the repeated exposure to it did not appear to produce significant desensitization. The relative effectiveness of C3a and particularly C4a needs more concurrent study.[160f]

The anaphylatoxins can be degraded by "*in vivo*" plasma carboxypeptidase N, previously termed "anaphylatoxin inactivator"[158d,160g], which removes the C-terminal arginine residue from each of the anaphylatoxins. This degradation leads to an appearance of $C4_{des\text{-}Arg}$, $C3a_{des\text{-}Arg}$ and $C5a_{des\text{-}Arg}$.[157,160g,160h] The relevance of this process is that it proceeds very rapidly to completion, particularly for C3a, with the newly generated "des-Arg" products actually mediating the "*in vivo*" biological effects.[157,160i] More recently, it has been accepted that the "des-Arg" derivatives of all the anaphylatoxins are not able to produce the histamine release from the mast cells.[160h] However, when injected into the skin, they trigger an inflammatory wheal-and-flare response, which cannot be distinguished either macroscopically or microscopically from that caused by the native anaphylatoxins.[160f] These facts may then support the hypothesis that histamine would not play any principal role in the anaphylatoxin-induced inflammatory reactions "*in vivo*".[160]

(2) Neutrophil adhesiveness

The "*in vitro*" studies using purified C5a showed that the binding to the neutrophil C5a receptor promotes the ability of neutrophils to bind to the endothelial cells but not to the smooth muscle cells or fibroblasts.[132a,146d,160j] Both C5a and $C5a_{des\text{-}Arg}$ seem to be equipotent in this respect.[158h,160e] Moreover, the neutrophil aggregation and up-regulation of cell surface C3 receptor numbers is induced by both these components.[146d,158h,160e]

The neutrophil surface adhesion molecules MAC 1 and p 150,95 have been found to be identical to CR3 and CR4 receptors respectively. Both the molecules are types of the neutrophil cell surface C3 receptors whose production is enhanced by C5a/$C5a_{des\text{-}Arg}$.[158h,160m] Some cytokines, such as IL-1 and TNF may also participate in the neutrophil adhesiveness.[131f,146b]

(3) Chemotaxis

The complement components, such as C5a, C3a and $C5a_{des\text{-}Arg}$ have been demonstrated to possess chemotactic activity for various cell types, including neutrophils, basophils, eosinophils and mononuclear phogocytes (monocytes and macrophages).[160n] The C5a seems to be the most potent chemotactic agent for neutrophils, followed by C3a and $C5a_{des\ Arg}$.[160i] However, the full expression of the human $C5a_{des\text{-}Arg}$ chemotactic activity requires the presence of a plasma co-factor (co-chemotaxin), a factor which is not necessary for the C5a chemotactic activity.[157,160p] Moreover, some of the cytokines have also been demonstrated to possess a distinct chemotactic activity for various cell types, such as IL-1, IL-2, IL-6, IL-8 IFN-γ and TNF for neutrophils, IL-1 and TNF for monocytes, interferons for macrophages[131f,146b,146d,156j,156m]

(4) Release of toxic oxygen radicals and lysosomal enzymes from neutrophils and phagocytic cells

Various biological stimuli, including C5a, are able to induce the production and release of significant amounts of the toxic oxygen species, such as superoxide anion and hydrogen peroxide from the neutrophils.[158s,160r] These oxygen species represent the group of the neutrophil-derived permeability factors causing the damage of the endothelial cells, followed by increased permeability and leakage of a protein-rich edema fluid.[160s]

Furthermore, the release of lysosomal enzymes by phagocytic cells also contributes to the tissue damage.[160t] The lysosomal enzymes are also known to be able to activate C1 and factor B "*in vitro*".[160t] The release of lysosomal enzymes can also be stimulated by C5a, $C5a_{des\text{-}Arg}$, C3e and by cross-linking of CR 1 on the surface of the phagocytic cells.[158g,160u] This cross-linking appears at the sites of the complement activation where C3b becomes bound to the activated surface of some cell types, such as microorganisms or antigen-antibody complexes, and is then able to crosslink the CR 1 receptors. The IgG-containing immune complexes with incorporated C3b have been demonstrated to be much more effective in inducing the lysosomal enzyme release from the neutrophils, than the IgM-containing complexes.[160v]

(5) Stimulation of the formation of the membrane attack complex (MAC) system

The MAC formation on and in the cell membranes at the site of the complement activation has been suggested to lead to the influx of calcium ions through the transmembrane pores. This ion influx may then trigger the arachidonate cascade.[157,157e, 157f,160w]

The human neutrophils display the phenomenon of "microvesicle shedding". Human neutrophils treated with purified MAC are able to protect themselves by aggregating the MACs and to release them on the surface of the microvesicles.[132a,159l, 160y] A variation in the degree of the specific MAC protein degradation by the neutrophils has also been reported.[160y]

Moreover, the treatment of platelets with C5b-9 led to the alpha granule and dense body release reactions.[160x] The platelets are able to release microvesicles which are coated with C5b-9 and which have membrane receptor sites for the coagulation factor Va.[160x] The C5b-9 effects on the platelets are probably mediated by the influx of the calcium ions through the activation of platelet protein kinase.[153,153a,160x] The C5b-9 is, therefore, generating the platelet-derived pro-coagulant activity, and is possibly helping to explain the question why the activation of the coagulation system regularly accompanies certain acute inflammatory processes.[160x]

The terminal sequence activation with the MAC formation and the membrane insertion can therefore represent the lethal cell lytic event, or else it may result in the specific alterations of the cell functions, some of which may be of the pro-inflammatory character.[132a,157] The "*in vivo*" MAC control measures and the C8 and C9 binding glycoproteins may act to ensure the maintenance of a balanced state, not only by minimizing the potentially damaging bystander cell lysis, but also at the same time by facilitating the more biologically beneficial effects.[132a,157,157f] It could be presumed that this subtle balance will be disturbed in certain disorders or pathologic states or during the stages of the intense complement activation.

[G] POSSIBLE ROLE(S) OF SOME COMPLEMENT PARTS, "IgG" AND "IgM" ANTIBODIES IN THE "LNR"

Regarding the possible role of the complement in the inflammatory processes,[136d, 156b, 156t] we have measured the concentration of C3 and C5 in the serum of patients developing the late asthmatic response [LAR] (n = 231) and the late nasal response [LNR] (n = 165) to the allergen challenge. The LAR has been accompanied by increase in the serum concentration of C3 in 76% of cases and of C5 in 69% of cases, whereas during the LNR an increase in the serum concentration of C3 has been found in 41% of cases and of C5 in 30% of cases. These preliminary, not yet published, data would not, at least, exclude any form of possible contribution of the complement system to the mechanisms underlying the LAR and LNR. However, our preliminary data have a screening character only. More concurrent studies of the complement system, including all the important parts participating in both the classical and the alternative pathways, would be needed to explore and confirm this hypothesis.

In other groups of patients developing either the LAR (n = 82) or LNR (n = 55) we have studied the possible existence and changes in the concentration of the immune complexes in the serum by means of the ^{125}I-Cl_q binding assay and Cl_q solid-phase assay.[156] In most of the subjects demonstrating the LAR as well as those with LNR, no significant amounts of the immune complexes have been detected. In 17% of the patients developing the LAR and 3% of those developing the LNR, the IgG containing immune complexes (IgG, IgG_1, IgG_2, IgG_3) have been detected in the serum to a slight degree, however, without any significant changes during the particular late phase responses. These preliminary data would not support the role of immune complexes in the late phase reactions, among others in LNR, at least at the present time. On the other hand, the available techniques for the detection of soluble as well as insoluble immune complexes still have some shortcomings regarding the specificity as well as sensitivity.

Some investigators reported the findings of immune-complexes in the patients with allergic bronchial asthma.[130,130a,147,161,161a,161b] However, we were unable to find any satisfactory investigation concerning the immune complexes in the patients demonstrating the LAR, in the available literature. The changes in the concentration of the IgG and IgM antibodies and the IgG subclasses in the serum, sputum, bronchial lavage fluid and in the bronchial mucosal membrane, the changes in the concentration of the chemical mediators, their precursors, derivatives and metabolites, the changes of the complement system parts, and the cellular changes within the mucosal membrane as well as their influx across the mucosal membrane have, in our opinion, not yet been sufficiently investigated in patients with LAR as well as with LNR and would require further studies. The IgG and possibly also the IgM antibodies are presumed to play a major role in the forming of immune complexes, after their interaction with the antigen,[126-128,129,132, 147,149,156y,161c,161d] occuring in the blood stream or in the vascular wall, and sometimes also in the tissue. The IgG antibodies can also directly activate the eosinophils through their membrane IgG receptors,[140,142,146,149] stimulate macrophages,[151,153] neutrophils,[141,146,146e,147,149,151,152] and platelets.[147, 150,151,153,154] The IgG and possibly also IgM antibodies are suspected to be responsible for the late hypersensitivity mechanism(s).[65,66,68,68c,148,161c]

The suggested involvement of the IgG and possibly also IgM antibodies in the "late phase responses" may also be supported by results from our previous studies concerning the "late asthmatic response to allergen challenge" (LAR).[63-66,68,68a-68f] The LAR has been accompanied by an increase in the serum concentration of IgG in 66% and of IgM in 49% of the cases, while positive specific IgE antibodies in the serum were recorded in only 29% of the LAR cases.[63-66,68a,68e] With respect to the IgG sub-classes, we have found a significant increase in the serum concentration of IgG_4 during 52%, of IgG_3 during 25% and of IgG_1 during 8% of the LARs, while IgG_2 concentration increased in only 2%, but decreased during 54% of the LAR cases.[63,65,68a] These results are partly in agreement with the findings of other investigators, who have performed bronchial challenges with allergen and concluded that IgG_4 antibodies could be involved in the late onset bronchial reactions in patients with bronchial asthma with negative prick tests and without specific IgE antibodies in the serum.[161,161a-161i] Homburger and colleagues[161i] have found a significantly higher concentration of IgG_4 antibodies in the

serum of patients with bronchial asthma or with what they called "inflammation of the airways" than in normal control subjects. Stanworth and coworkers,[161h,161k] Nakagawa et al,[161,161l] Goodwin,[161m] Gwynn et al[161e,161i] and Perelmutter[161g] have suggested the possible involvement of IgG_4 antibodies in the hypersensitivity states. The exact mode of this involvement is, however, not yet fully clarified.

The question of the role of the individual IgG subclasses in "late phase allergic reactions" would become more complicated if some investigators' suggestion of the possible existence of the allergen-specific IgG sub-classes were to be confirmed.[126-128,128b,161e, 161h,161i,161n,161p] The decrease in the IgG_2 antibodies during 54% of the LAR cases was a surprising result of this study.[65,66] We do not have any clear explanation either for the exact role of the IgG_2 antibodies in the LAR, or for their decrease during the LAR. The exact involvement and significance of the individual IgG-subclasses for the hypersensitivity mechanisms, particularly those underlying the "late phase reactions", is still uncertain.[161f] The changes in the IgG subclasses during the LAR, recorded in our studies, could implicate the possibility of a differentiated involvement of individual IgG subclasses in the interaction with the antigen, and in the "late phase allergic reaction", at least in LAR. Our results would not confirm the involvement of IgG as a uniform complex antibody.

Such a presumption of the possible participation of IgG, and possibly also of IgM antibodies in the LNR, can probably be made, or at least not be fully excluded, with regard to our results demonstrating an increased serum concentration of total IgG during 51% of the LNR cases.[12,14] In another preliminary study we have also found detectable amounts of IgG antibodies in NS in 45% of LNR cases, which concentration showed changes during 33% of LNR cases.[14]

Besides this classical conception of presumed involvement of IgG antibody (and possibly also IgM) in the late hypersensitivity mechanism by forming immune-complexes through the classical pathway, other possible pathways and other roles of the IgG antibodies in the late-phase reactions might also be considered.[68e,126-128,128b,130,130a, 131,146e,153,154,158j,161h,161l,161r-161t]

5. THE ROLE OF THE PARTICULAR CELL TYPES [Figure 56 (page 379)]

[A] NEUTROPHILS

Some authors have found evidence for the existence of membrane receptors for the IgG antibody (Fcγ RI, Fcγ RII and Fcγ RIII) on the neutrophils[146e,147,151,152,154,161r-161w] and FcγRII receptors on the platelets.[151,153a,154,161v,161w]

Other investigators have reported that eosinophils and neutrophils possess membrane receptors for IgG subclasses (IgG 1,2,3,4) and certain complement components, including C3b/C4 and C5a.[128a,141,146,148,149,154,158j,161x] They have suggested that "IgG (Fc) and C3b/C4 receptors can be enhanced following prior exposure to the chemotactic factors, suggesting that even as phagocytic cells are migrating in response to chemotactic factors, including those released from the mast cells, they are becoming more effective participants in the inflammatory reaction." The principal inflammatory factor

derived from the complement cascade appears to be C5a, which is chemotactic for neutrophils from the circulation and also from the surrounding tissues.[128a] They also have provided evidence for the activation of inflammatory cells by antigen challenge in asthmatic subjects, leading to an increase in the number of neutrophil C3b and IgG(Fc) rosettes. Welsh and Kay[148] have also found evidence for the binding of homologous IgG subclasses to human neutrophils and eosinophils, which could implicate a hypothesis that IgG interacting with an antigen can directly activate neutrophils and platelets, without forming any immune-complexes.

Most recently, three basic classes of the human Fc receptor for IgG, one high affinity receptor, Fc γ RI, and two low-affinity receptors, Fc γ RII and Fc γ RIII, have been identified.[161u,161v,161w] The Fc γ RI (CD 64) is a 72kDa glycoprotein present on monocytes, macrophages and neutrophils. The Fc γ RI is the only Fc receptor that binds monovalent IgG, predominantly IgG_1 and IgG_3, with high affinity. This Fc receptor is a member of the so-called "immunoglobulin superfamily", being a subgroup of the cell adhesion molecules. Moreover, the interferon-gamma (INF-γ) increases the expression of the Fc γ RI not only on the monocytes and macrophages, but also on the neutrophils. The Fc γ RII (CDw 32) is a 40kDa glycoprotein present on all hematopoietic cells except erythrocytes. The Fc γ RII is the major IgG-Fc receptor expressed on the surface of the platelets.[161v,161w] The Fc γ RIII (CD 16) receptor, a 50-70 kDa glycoprotein, is also expressed on the large granular lymphocytes (natural killer cells), eosinophils, neutrophils and macrophages.[161v-161z] The Fc γ RIII on the neutrophils is bound to the cell membrane through a phosphatidylinositol glycan linkage, while the Fc γ RIII on the macrophages and natural killer cells is a transmembrane protein.[161v,161w]

The results of our previous studies demonstrating significant changes in the count of neutrophils in nasal secretions (NS) during the 84% of LNR cases (increase immediately before, decrease during the appearance of LNR, and increase during the resolving of the response) are strongly suggestive of an active and dynamic role of neutrophils in the LNR.[2,11c,12,25,40c,40d,40f,41a-41d,72,72a,72c,96, 97] This hypothesis may be supported by other investigators' findings of significant changes in the count of neutrophils, mostly an increase, either in the nasal secretions[48d,51c,82,83,94,97c] or in the nasal mucosa biopsies,[51b] accompanying the clinical LNR.

Finally, other investigators have recorded increased concentrations of some mediators or factors directly related to the neutrophils and therefore considered to be indicators for neutrophil activation, in the nasal lavage fluid during the clinical LNR.[97c]

Another interesting aspect which may support the active involvement of neutrophils in the late phase reactions, including LNR, is their ability to degranulate.[131e] Two types of neutrophil degranulation have been recognized, an "intracellular" and an "extracellular" degranulation.[131e] The degranulation and other intracellular changes in neutrophils appearing in the nasal secretions (NR) during the immediate (INR) and the late (LNR) nasal response to allergen challenge has been observed and reported by us for the first time.[11a,11b,41b,41d,71,72,72a,72c] The degranulation and other intracellular changes recorded in neutrophils appearing in NR during the LNR were to a significantly higher degree (88%)[40f,41d,97] than those observed during the INR[11a, 11b,97a] Moreover, the degranulation and other intracellular changes in the neutrophils were prevented by

intranasal budesonide to a distinctly higher degree than by intranasal cromolyn[40c,40f, 41b,72d] Taken together, our results may support the important, if not key, role of neutrophils in the LNR, rather than in the INR.

Other evidence of the active role of neutrophils in the mechanisms leading to the development of the late phase allergic reactions, has been provided by investigators studying the antigen-induced late asthmatic response.[96g,162,162a-162c] Zweiman and colleagues[96g,162b] and Nagy et al[162c] have found a significant increase in neutrophil chemotactic activity in the serum of patients developing a positive late asthmatic response (LAR) to allergen challenge. Durham and co-workers[162d] have recorded a significant increase in neutrophil chemotactic activity, monocyte and neutrophil complement rosettes during the LAR to allergen challenge , but not during the methacholine-induced bronchoconstriction. Atkins[162e] and Zweiman et al[162b] have recorded a distinct increase in the blood neutrophil count 2 hours after the bronchial challenge with allergen, but not after the methacholine inhalation. Kay[162f] has concluded that the pathogenesis of the late phase reactions is incompletely understood. He has discussed three possible mechanisms which could be involved in the LAR:"(1) The LAR may be an example of the type III or Arthus response with neutrophil infiltration resulting from the generation of chemotactic factors, following activation of complement by immune complexes; (2) LAR may be associated with re-activation of mast cells, since elevated serum concentration of neutrophil chemotactic factor was recorded during LAR; and (3) the leukotrienes, prostaglandins and thromboxanes may play a role in the LAR as these mediators tend to have sustained biologic effects and might, for instance, cause prolonged contraction of the bronchial smooth muscles together with an edema of the submucosa as a result of their effects on the microvasculature".

Results of the bronchoalveolar lavage (BAL) during the LAR may also support the evidence of active involvement of the neutrophils as well as the eosinophils, in the hypersensitivity mechanisms leading to the development of clinical LAR.[162, 162a,162g-162j] Metzger and colleagues[162a,162h] have found significant increase in the count of neutrophils and eosinophils in the BAL-fluid at 4 hours after the bronchial challenge with allergen. However, after a local (intrabronchial) allergen challenge, a significant increase in the numbers of neutrophils, eosinophils, macrophages and lymphocytes has been recorded at 48 hours after the challenge.[162a,162g] Diaz and colleagues have also found a significant increase in the count of neutrophils, lymphocytes, eosinophils and lung mast cells in the BAL fluid at 6 hours after the bronchial challenge with allergen in subjects developing LAR.[162i]

[B] PLATELETS

The role of platelets and neutrophils in mediating the hypersensitivity reactions, seems to us to be still underestimated.[139a-139d,146e,153,153a,154] The platelets and neutrophils can be involved during the late hypersensitivity reactions in various manners and they may stimulate and induce other steps through various pathways.[139a-139d,146e, 153,153a,154] Platelets may probably play a double-role in hypersensitivity reactions, at one time as inflammatory cells, being an active participant in these mechanisms, and another time as a target cell of other processes and cells.[138,139,139a-139d,153, 154,162k,162l

The platelets contain intracellular granules similar to the classical lysosomes of the polymorphonuclear leukocytes, which can degranulate during the formation of the platelet plugs.[153,162k] Platelets accumulate in vessels adjacent to inflammatory foci and interact with antigen-antibody complexes.[139c] The platelet response to the surface stimulation by release and secretion of various factors is also accompanied by formation of prostaglandins and thromboxanes.[153] Platelets contribute to the inflammatory processes accompanying the tissue injury by releasing various highly potent intracellular constituents and factors that, besides other effects, also increase the vascular permeability in two phases: the acute phase, appearing within 15 minutes and the secondary (late) phase, reaching its maximum in 3 hours. The secondary phase is usually accompanied by polymorphonuclear infiltration.[153, 162l] The functional activation or activation followed by aggregation of the platelets is stimulated by a variety of factors,[129,139c,162k] such as "platelet activating factors" (PAF) generated and/or released directly from the activated mast cells and basophils[129,134,135, 138,139,139c] and sometimes also from other cell types, e.g. eosinophils, neutrophils or monocytes, by decreasing exogenous cAMP and/or increasing exogenous cGMP with subsequent decrease of intracellular cAMP in the platelets,[147] by some complement parts (C3a, C5a),[148] and probably also by other pathways.[153] The platelets may also be stimulated directly by IgG antibodies and/or immune complexes, since the Fc receptors for all four subclasses of IgG (Fcγ R) and complement parts (C3a, C5a), as well as the already known receptors for IgE (Fcε RI), have been demonstrated on their membranes.[139c,146e,153,154,162m]

As already mentioned, the platelets also express various membrane glycoprotein molecules, called "surface receptors", being involved in their adhesion to the other cell types and to the endothelial cells, such as "integrins", "superfamily of adhesion receptors ($\alpha + \beta$)", subgroup of them is formed by factors, called "very late activation -2, -5, -6 (VLA-2, VLA-5, VLA-6)", and "selectins", such as GMP-140, and "Platelet-endothelial cell adhesion molecule-1" (PECAM-1).[139a-139c,153]

Lysosomal enzymes are involved in the further stages of the immune complex-mediated tissue injury.[126,128,128b,137] Neutrophils are also involved in immune complex-mediated injury by their influx into the site of the immune-complexes as well as platelets which then adhere to and interact with the damaged epithelium and immune-complexes through various adhesion molecules upon regulation by various factors, chemotactic factors, and some cytokines.[126,128,138,139,139c, 153a,162k]

Some evidence has been gathered for the active role of platelets in the antigen-induced asthma [139c,162n] and in the antigen-induced late asthmatic response (LAR).[139c,162a] However, there is still a dearth of detailed information concerning the possible involvement of platelets in the LAR. Data concerning the possible participation of platelets in LNR are not yet available.

[C] EOSINOPHILS

Another cell type which may also play an important role in the late hypersensitivity mechanisms is the eosinophil.[2,11a-11e,13a-13c,18,20,25,34,40c-40f,41b-41d,48,65,66,68e,72,72a-72d,80,95a,96,97,97a-97c,97n,97t,97u,134,135,136a,140-146,148,149,156c,158j,161x,161z,162,162f, 162p-162z,163,163a-163z] The eosinophil bears and expresses various receptors and molecu-

les on its membrane, such as low affinity receptor for IgE (Fc_ε RII; CD23),[161z,162s,162v] receptors for IgG (Fc γ RII; CD32),[146,148,161y,161z] IgG subclasses, IgG_{1-4}(FCγRII; CD32 and FcγRIII; CD16),[148,161w,161y,161z,162r, 163b] IgA (Fc α R),[161y] complement parts (C1q, C3b = CR1, iC3b = CR3, C4b, high and low affinity receptors for C5a),[146,156t,161y,161z,162p,162v] adhesion molecules (LFA-1α = CD 11a, CR3-α = CD 11 b, P 15095-α = CD11c) [161z], VLA-4,[156m,162u] Fc γ RII (CDw 32),[161z] integrins (CD11/18)[162u] glucocorticosteroids,[161y] PAF-acether,[161y] histamine,[161y] oestradiol,[161y] and cytokines (high affinity receptors for IL-3, GM-CSF, IL-5 and for CD 25 molecule).[162v,163,163c,163v,163w]

Recently, it has been suggested that both the major histocompatibility complex class II proteins[163d] and CD4 molecule may be expressed on the surface of eosinophils stimulated by GM-CSF.[163e] The receptors for IgM antibodies on the eosinophil surface have not yet been confirmed.[162v,163b]

The eosinophils can be activated and stimulated by a variety of factors, including IgG, IgG-subclasses, complement parts and immune complexes.[140,142, 161z,163r]

The eosinophil also fulfils a dual role, acting as an immunoregulatory cell in some steps of hypersensitivity mechanisms (= integrated part of some processes), but also as an effector and target cell in other steps (= consequences of some processes).[161z,162t,162v,163,163h-163t]

The increased counts of eosinophils in the blood (blood eosinophilia), in the tissue (tissue eosinophilia), in the nasal secretions, sputum, tears as well as in the nasal and bronchoalveolar lavages during the late phase responses to allergen challenge, have repeatedly been reported [1,2,7,11c,12,16,19,25,40c-40f,41a-41d,48,48d,51a,51c,55,61,62-66,68a,68b, 68e,68f,70,72,72a,72c,82,82j,83,94,95a, 96,97,97b,97n,121b,121f,124b,128a,131a,131e,140-146,148,149, 158j,161f,161h,161x-161z,162,162a,162g,162h,162i,162n,162p-162v,163,163b,163c,163f-163t,163v,163w]

However, in contrast to other investigators' findings, referred to above, Venge and Dahl[163p] have reported a decrease in the count of eosinophils in peripheral blood during the LAR to a allergen challenge.

Interestingly, some classical immune complex disorders, such as serum sickness and vasculitis may also be associated with peripheral blood eosinophilia.[126, 128b,163t] There is a dearth of information in the literature concerning the possible occurrence of peripheral blood eosinophilia during the particular types of the nasal response to allergen challenge, especially the LNR. Varney and colleagues[51b] did not find any increased count of eosinophils in the peripheral blood, whereas we have previously recorded a slightly increased count of peripheral blood eosinophils during a minority of LNR cases.[14,41b]

However, our recent not yet published data concerning a relatively high number of the isolated LNRs (n = 272), show a slight, but not significant, increase in the count of eosinophils in the peripheral blood accompanying only 7% of the LNRs. These data may therefore support the hypothesis of the topical character of the nasal responses to allergen challenge, being limited predominantly to the nasal mucosal membrane.[11h,41i]

In contrast, the increased influx and significant changes in the count of various cell types, including eosinophils, in the nasal secretions (NS)[2,18,25,40c-40f, 41a-41d,72,72a, 72c,97,121b] or in the nasal lavage fluid,[48,48d,51c,55,82,83, 94,97b] accompanying the LNR, have repeatedly been demonstrated and reported.

Nevertheless, some small differences in the patterns of changes of eosinophil count in the NS during the LNR have been reported by various investigators, which may be related mainly to the methods used.[11c,40c-40f,41b,41c,48d,49,51b, 51c,53,97,97b,97c]

The accumulation of eosinophils, besides the other cell types, in the nasal mucosa tissue, during the LNR has also been reported.[2,96,97,97n]

The active role of eosinophils in the mechanisms underlying the LNR may also be supported by our previous findings of significant degranulation and other intracellular changes in the eosinophils and neutrophils migrating into the NS[2,11c,40d,40f,41b,41d,72a, 72c] and into the nasal mucosa tissue (biopsy)[2,96,97] during the LNR, as compared with their numbers and state both before the allergen, at baseline, and during the PBS controls.

The eosinophil traffic in the nasal mucosa and in the NS during the particular types of nasal response to allergen challenge, differs slightly from the results of the the bronchoalveolar lavage (BAL)[124b,162,162a,162g-162i,162w,162x,163g-163k] and bronchial mucosa biopsies [162n,163g] performed during the late asthmatic response to allergen challenge (LAR).

Unfortunately, the complex mechanisms leading to the increase in the count of eosinophils in the blood and the process of recruitment of eosinophils into various tissues, including the nasal mucosa, have not yet been fully clarified.[128a,131b-131f,136b,142-146, 156m,156n,158j,161x-161z,162,162c,162f,162n-162v,163,163a-163k,163m-163u]

The mobility and attraction of eosinophils due to various stimuli may be realized through chemokinesis, being a non-directed movement, or through chemotaxis, being a directed migration across a stimulus concentration gradient.[162v]

The eosinophil recruitment into the tissue initially involves the adhesion of eosinophils to the vascular endothelial cells in the tissue, their migration into the submucosa, and finally their subsequent activation.[128a,131c,131e,156j,156m,156n,161x-161z,162p,162v]

The process leading to the increased eosinophilia in the blood as well as to the eosinophil accumulation in the tissues may involve complex interactions of chemotactic factors, adhesion molecules, integrins, cytokines,[162v] and probably also other factors, such as neurotransmitters, neuropeptides,[131c,163u] signal transduction processes,[131b] clothing system,[146g] and factors which have not yet been recognized.

Chemotactic factors, such as LTB_4, histamine, PAFs, ECF-A, ESP (eosinophil stimulation promotor), neutrophilic ECF-A etc.,[162v] influencing the blood eosinophilia and modulating the influx of eosinophils into the tissues and secretions, originate from various sources and are generated by various cell types and systems.[161y,161z,162v,163r,163t] Some complement parts, such as C5a are also chemotactic for eosinophils.[146,161x,161z,163a]

However, there is still a lack of detailed knowledge concerning the eosinophil chemotaxis.[161z]

The cytokines, IL-3, IL-5 and GM-CSF have also been demonstrated to possess the chemotactic capability for eosinophils, inducing the blood eosinophilia as well as the eosinophil accumulation in the tissues.[161z,162t,162u,162v,163c,163v-163z]

The adhesion molecules probably involved in the eosinophil recruitment may include ICAM-1 (intercellular cell adhesion molecule-1), ELAM-1(endothelial leukocyte adhesion molecule-1), VCAM-1 (vascular cell adhesion molecule-1), and integrins, such as CD11/18 and VLA-4 (very late activation antigen-4).[146d, 156j,161z,162t,162v]

The eosinophil recruitment is probably also promoted by LCF (lymphocyte chemo-attractant factor) and by IL-2, since the eosinophils express the 55 unit of the IL-2 receptor (CD28) on their surface.[163r]

The eosinophils, besides the impressive number of the constistuents, granule proteins [MBP = major basic protein, ECP = eosinophil cationic protein, EDN = eosinophil-derived neurotoxin, EPO = eosinophil peroxidase] and other products,[11a,23,48,55,65,66,97b,131e,131f,135,136a,140,142,144,149,158j,161x, 162,162s,162t,163,163n,163r,163u] may also be capable of producing some cytokines, such as GM-CSF,[162p,162t,163c] IL-3,[162t,163c] IL-1α,[161z] and transforming growth factors, including TGFα[162p,163r] and TGF-β.[163r]

Recently, some evidence has been provided for the role of activated eosinophils as MHC-restricted, HLA - DR-dependent antigen-presenting cells to stimulate proliferative responses of $CD4^+$ lymphocytes.[161z,163d,163e,163r]

Another very important property of the eosinophils is their ability to participate, with or without the neutrophils, in the forming of eosinophil or eosinophil-neutrophil infiltrates in various tissues.[2,51a,51b,95a,96,97,97a,97f,97n,97s,97t, 131e,135,140-144,162,162d,162f,162n,162v,163,163i,163n]

The eosinophils can also contribute to the tissue damage, especially that of respiratory epithelium, through their granule proteins, e.g. MBP.[161z,162v,163n] The MBP causes ciliostasis and exfoliation of the respiratory epithelium cells,[161z, 162v] and incidentally it may also participate in the tissue damage through its effects on the C3 convertases of the classical and partly also of the alternative complement pathway.[156,156c,161z,162v]

Interestingly, Bascom et al[48] have recorded increased concentrations of MBP and EDN, whereas Bisgaard et al[82j] have found an increase in concentration of ECP in the nasal lavage fluid during the LNR. These findings would confirm an active role of eosinophils in the LNR mechanism.

Taken together, it can be concluded that eosinophil being a versatile cell, can participate in a manifold manner not only in the immediate-, but also in the late-phase reactions, among others in LNR, and its role cannot be excluded even in the classical late hypersensitivity mechanisms (immune complexes, Arthus phenomenon).

[D] MAST CELLS AND BASOPHILS

Other versatile cell types, participating directly as well as indirectly in various steps of various hypersensitivity mechanisms are basophils and mast cells. [162m, 162z,164,164a-164l]

Classically, the mast cells and basophils have primarily been understood in their role in the patho-mechanism of the immediate hypersensitivity.[2,4,34,41f,42-45,48d, 51a,51c,68e,82,85b,85c,128a,128g,131c,131e,163j,163s,163u,164a,164d,164e,164m-164u] Later, an evidence has been gathered, suggesting that mast cells, and probably also basophils, may also be important cytokine-producing cells.[131f,146b,164b,164c, 164v,164w] This fact would then fortify the possible contribution, at least indirectly, of the mast cells and probably also basophils, to the mechanisms underlying the non-immediate types of hypersensitivity, the late type being one of them.[51a,51c, 68e,82,164e] The mast cells and basophils have not only several features in common, but they also differ each from the other in several aspects.[162m, 164b-164d,164x,164y,164z]

A number of investigators have provided evidence for the existence of IgG receptors

FcγRII on the membrane of mast cells and possibly also of basophils, [140,153,161h,161l, 161s,161t,161v,161w,164,165,165a-165d] as well as the already repeatedly demonstrated IgE receptors Fcε RI on these cell types.[165e] Others have reported the presence of the specific receptors for IgG sub-classes on the membrane of both human mast cells and basophils.[161l,161u,165a,165b,165d,165f] Regarding this evidence, other authors proposed that IgG or some of the IgG sub-classes, interacting with antigen, might act directly on the mast cell and/or basophil, resulting in the release of their mediators.[165b] The unique membrane receptors for IgE[165e] on both the mast cells and the basophils can be characterized as a high affinity IgE receptor ($Fc_\varepsilon RI$), being a multimeric complex consisting of one α-chain, one β-chain and two disulfide linked γ–chains.[128a,131e,161p, 162m]

The antigen-specific IgE antibodies binding to this high affinity Fc_ε RI receptor, and being cross-linked by specific antigens, cause the aggregation of these receptors, a process which represents the specific signal leading then to the activation of these cells.[131b, 156a,161p,164b,164c,164k]

The receptors for IgG displayed on the cellular membrane of basophils as well as of mast cells, can be defined as a medium affinity IgG receptor (Fcγ RII).[131e,161v,161w, 162m,165c]

Basophils and mast cells can also be activated through the Fc γ RII receptors having been previously bound by the IgG-antigen complexes, a process which then leads to the release of their mediators, especially histamine. [161v,162m,165c]

Moreover, both the mast cells and the basophils possess the receptors for some complement parts, such as C5a, C3a and C3b, on their membrane.[156,156c,156t,156x,162m,164, 164a-164c,164f,164h] However, the particular complement parts, the so-called complement-derived anaphylatoxins, can release mediators from the mast cells and basophils to different degrees. The C5a is much more potent than C3a in stimulating these cells for mediator release (e.g. histamine), whereas the C3b effect results in the enhancement of the IgE-mediated mediator release from the basophils, but not from the mast cells.[162m,164]

The mast cells and basophils can release their mediators in two different manners, either by a semi-selective secretion, without any principal morphologic changes,[164w, 165g] or by degranulation, which is however accompanied by a variety of cellular and intracellular changes.[162m,164b,164c,164g]

The release of mediators from the mast cells and basophils can also be induced, besides the already mentioned mechanisms such as IgE receptor-mediated (Fcε RI), IgG receptor-mediated (Fcγ RII), and complement part receptor mediated (C5a, C3a), by a variety of other factors, molecules and compounds, including "histamine releasing factors", cytokines, "Stem cell factor", neuropeptides, and varia.[162m, 164a-164p,164v,165h-165z,166, 166a-166p]

(1) Histamine releasing factors (HRF)

Histamine releasing factors (HRF), are compounds capable of inducing the release of histamine from basophils and some of them also from mast cells.[162m,164a-164p,165h-165t,165v-165z,166,166a-166d, 166n,166p,166r] Several human cell types, including neutrophils,[164j,164p,165r,165s,166a,166p] eosinophils,[164j,165r,165s] monocytes,[164j,165i,165l,165r,165s]

macrophages,[164j,165r,165s,165v-165y] endothelial cells, [156j,165r,165s] platelets, [164j,165r,165s,165z] fibroblasts,[165k] and lymphocytes (B and T)[164j, 165r,165s] can synthesize and secrete some of these factors.[165r,165s]

The group of HFRs includes factors such as "neutrophil-derived histamine releasing activity" (HRA-N),[164i,164p,166a] "neutrophil activating factor"(NAF),[165t] "neutrophil activating peptide-1 and -2" (NAP-1, NAP-2),[164j,165y] "mononuclear cell-derived factor",[165l,166b] "connective-tissue-activating peptide III" (CTAP III),[164n,165j,165s,165v, 165w] "macrophage-derived HFR",[165y] "macrophage inflammatory peptide" (MIP 2), [165s] "monocyte chemotactic and activating factor" (MCAF),[164c,164j,165s,165w] also known as MCP-1,[164j,166r,166t] RANTES - a product of T-lymphocytes,[164j] "monocyte chemotactic factor",[165i,165l] and HFR from platelets.[165z]

Most of the histamine-releasing factors belong to the so-called "intercrine" or "chemokine" group of cytokine-like molecules.[164j,166u]

Some of the HRFs derived from the endothelial cells, platelets, and macrophages can bind IgE and are therefore dependent on the IgE activation for their function, whereas some other HRFs, derived from the neutrophils and mononuclear cells can act independently on the IgE.[164k,164l,165n,165y]

Recently, a group of substances with an inhibitory effect on histamine release, has been discoverd.[162m,164d,165m] These factors, being in reality antagonists to the HRFs, and called "histamine-release inhibitory factors" (HRIF), inhibit the HRF-induced histamine release from the basophils but not the histamine release induced by other secretagogues. Recent data suggest that NAP-1/IL-8 may be one of the HRIFs.[164j,165u] The NAP-1/IL-8 may also act as a histamine releasing factor, because it induces histamine release from IL-3-primed human basophils.[165m,165t] Some evidence suggests that one of the most important HRIF would be actually IL-8, although this has not been definitely confirmed.[164j,165s,166c]

However, there is a certain dissension with respect to the classification of the histamine-releasing factors (HRFs). Some authors consider the HRF as cytokines,[164n,165r,165s] others describe them as cytokine-like factors,[164j,165v] whereas another group of investigators call them simply HRFs.[165m]

We prefer to qualify these compounds as HRF, because (a) this group consists of very different compounds and so far this group is not homogenic; (b) most of them are, in reality, not genuine cytokines according to the classical conception; (c) and lastly the definition of cytokines is still discussed.

(2) Cytokines

An increased evidence has been gathered for the enhancement of histamine release from human basophils by some classical cytokines, such as interleukin 3 (IL-3)[166e-166g,166v] granulocyte-macrophage colony stimulating factor (GM-CSF)[164g,164n,164v, 166g,166v] interleukin 1α (IL-1α)[166h], interleukin 1β (IL-1β)[166h], and interleukin-5 (IL-5).[166j,166k]

The results concerning the possible increase in histamine release from human basophils by interferon-γ (INF-γ), are, however, contradictory. Some investigators have reported such effects of INF-γ,[166m] whereas others were unable to confirm them.[166i]

Interleukin-8 (IL-8) has previously been reported to cause the histamine release from human basophils,[165m,165t,166p,166w] whereas later reports suggested quite reverse effects of IL-8, the inhibition of the histamine release from the human basophils.[164j,165j,165s,166c]

In contrast to human basophils, there is a dearth of information concerning the possible effects of particular cytokines on the mediator release from the human mast cells and the significance of such processes for the particular allergic disorders. Surprisingly, studies concerning this topic are not numerous, and even their results and conclusions vary. Some investigators have reported the activation and enhancement of the mediators release (e.g. histamine) also from human mast cells by some classical cytokines, a process which may be comparable with their effects on mediator release from human basophils,[164g,164r,166u,166v,166x,166y] whereas other authors have been unable to confirm such effects of the cytokines on the human mast cells.[164n,164x,166f,166j,166k, 166z,167,167a]

In contrast to human mast cells, the rodent mast cells, e.g. from rat and mouse, can be activated by some of the classical cytokines, such as IL-3, IL-5, GM-CSF.[164n,164r, 166f,166k,166l,167b]

(3) The "stem cell factor" (SCF)

The "stem cell factor" (SCF) is another factor which can activate, prime and modulate mast cells, and that not only from rodents (mouse, rat),[164b,164c,167c-167k] but probably also the human mast cells.[164b,164c,164j,164n,166k,167a,167d, 167l-167n]

The mast cell development in mice is critically regulated by the "stem cell factor", [167d,167f] a product of fibroblasts[165k,167c,167d] and probably also of other cell types, which is a ligand for the tyrosine kinase receptor protein.[164b,165k,167d,167f,167i,167j, 167k,167n] The SCF has been shown to be an encoding product of Sl locus in the mouse and the ligand for the proto- oncogene c-kit tyrosine kinase receptor (allelic with the W locus).[164k,167d,167i,167k,167p,167t]

The "stem cell factor"[167f,167i] has also been termed "mast cell growth factor" (MGF), [167d,167n,167r] "kit ligand" (KL),[166k,167r] and "steel factor" (SF).[164i,167p]

The ligand for the c-kit receptor, SCF, is produced as a transmembrane protein, of which three alternatively spliced forms have been described.[167d,167r]

In mice, rats and non-human primates, recombinant SCF influences the migration, proliferation and maturation of cells in the mast cell lineage.[165k,167f,167g, 167j] Recombinant SCF can also induce c-kit receptor-dependent activation and mediator release from some populations of the mouse mast cells.[167d,167f,167g]

In rodents, both the mast cells and the stem cells express the "Kit" and the high affinity IL-3 receptor on their membrane.[164c,167j] The ligand for c-kit receptor (= SCF) and the IL-3 have both originally been defined as mast cell growth factors, but were later redefined as growth factors capable of promoting self-renewal of the stem cells.[164c] The mast cell also expresses the nuclear transcription factors GATA-1, GATA-2 and GATA-3.[164c,167p,167u] GATA-1 is a cis-acting DNA-binding protein that uses two novel zinc-finger domains to activate transcription messages for cell-specific products, such as globin and granule proteases.[164c, 167h,167p,167u] The SCF may be considered as a multicell growth factor similar to IL-3, with action primarily on the pluripotential progenitor and

probably also stem cells.[164b,164c,166k,167j] Such proposed function of SCF may be supported by data of other investigators showing that human mast cells have arisen from CD34+ progenitor cells.[164b,167d,167v] Originally, the SCF has been found in mice and rats and the existence of similar factors in humans has been doubted.[164x,166k,167d,167f,167w] However later, several investigators have provided evidence for existence of the human SCF.[164j,167l,167n,167w]

In later studies, the human homologue of SCF has been cloned and the effects of the rhSCF (recombinant human SCF) on the human bone-marrow-derived mast cells and basophils,[164b,164x,167v] on isolated human skin mast cells,[167d,167e,167l,167w] and human lung mast cells[166k,167a,167m,167n] have been examined.[166k, 167a,167m,167n]

In the isolated human skin mast cells, recombinant SCF has been shown to be able to directly induce the release of both the performed (e.g. histamine) and the "de novo synthesized" (e.g. PGD_2) mediators.[167e,167l,167w]

The SCF can also augment the ability of the isolated human skin mast cells to release their mediators in response to the stimulation through the Fc_ε RI receptors, which means the IgE-dependent mediators release.[167e] The SCF has also been reported to induce the mediator release from the human peripheral blood basophils.[167e,167w]

In studies using the isolated human lung mast cells, the recombinant human SCF has been shown to be able to activate these cells and to distinctly enhance the release of performed mediators (histamine) as well as of " de novo synthesized" mediators (PGD_2) from these cells.[166k,167x] However, a brief incubation and/or lower concentration of rhSCF has enhanced only the anti-IgE-induced histamine release, but not the release of PDG_2 from the human lung mast cells.[167x] Since the effects of rhSCF on the mast cell mediator release "*in vitro*" occur at concentrations of this factor similar to those found in the serum of normal subjects, it has been speculated about the possible contribution of SCF to the modulation of mast cell functions even under physiological conditions.[167w]

The rodent and the human mast cells differ in their response to cytokines. The murine and rat mast cells responded to the stimulation with several cytokines including IL-3, IL-4, Il-9, GM-SCF by proliferation and release of their mediators, whereas the human mast cells did not.[164n,166k,166z,167a,167d] However, the data generated from mice and rats are not easily transferable to the human system.[166k] Interestingly, some of the cytokines acting on the murine mast cells (IL-3, GM-SCF) may also affect some functions of the human basophil, [166k,166z,167a] but not those of the human mast cells.[166k, 166z] Furthermore, human basophils and human lung mast cells differ also in their responsiveness towards the IgE-independent triggering agents.[166k] Regarding these facts, the murine mucosal mast cells seem to have more functional resemblance to human basophils than to human mucosal mast cells.[166k] By contrast, the effects of SCF on the mast cell functions are not restricted to the human or rodent system.[166k] Galli and coworkers have demonstrated that the rat SCF may induce proliferation, maturation and functional activation of the mouse mast cells both "*in vitro*" and "*in vivo*".[167d, 167e-167g,167j,167l,167w] These parallels may emphasize the importance of SCF, acting through the activation of the c-kit receptors, as one of the important regulators of the mast cells and the mast cell-associated disorders, such as some forms of bronchial asthma and/or urticaria.[166k]

In contrast to the human skin and lung mast cells and the circulating blood basophils, no data are available concerning the possible effects of SCF on the nasal mucosal mast cells and basophils in the nasal secretions in general, and its effects on the appropiate types of nasal response to allergen challenge, in particular. Moreover, there is a dearth of detailed information concerning the effects of individual cytokines on the nasal mucosa, its parts, and individual cell types appearing in the mucosal membrane and in the nasal secretions during the particular types of nasal response. Furthermore, few data are available concerning the possible appearance of individual cytokines and changes in their concentrations both in the nasal secretions and in the nasal mucosa, especially during the particular nasal response types.

Sim and colleagues[164v] have reported increased concentrations of IL-1 in the nasal secretions during the INR and of IL-1 and GM-CSF during the LNR. Unfortunately, the number of patients studied by these investigators has been very limited and they did not report the simultaneous recording of the particular types of the nasal response to allergen challenge by means of rhinomanometric parameters.

Nevertheless, these aspects may also be very important for the explanation and understanding of the mechanisms underlying the particular types of nasal response to allergen challenge and those involved in the nasal response to the non-specific agents.[11c,11e,35a-35c,40c,40d,40f,72c,72d,96,97,97a] The importance of such data may be further stressed by previous findings of Naclerio and co-workers[53], Bascom and colleagues[34,48d,82] and Walden and co-workers[94] suggesting the role and activation of mast cell during the immediate, whereas the role and activation of basophil, during the late nasal response to allergen challenge. However, the results of our cytologic studies of the nasal secretions and histologic studies of the nasal mucosa during the particular types of nasal response to allergen challenge, may suggest an involvement of basophils, besides other cell types, in the mechanisms leading both to the immediate and to the late nasal response, rather than the involvement of the mucosal mast cells.[2,11a,11c,11d,13b,13c,40c,40d,41b-41d,71,72,72c,72d,96,97,97a]

In contrast to the nasal mucosa, the bronchoalveolar lavage studies have provided some evidence for the more prominent role of the mucosal mast cells, rather than that of basophils, in the lower allergic airways.[162,162a,162g,162i,162n,163j, 164c]

However, other investigators did not find increased numbers of mast cells in BAL or biopsies of the bronchial mucosa.[164s,164t,167y,167z]

Finally, the studies of the SCF and cytokines have contributed to the explanation not only of the differentation but also of the origin of mast cells and basophils. Recent evidence of high levels of cytokine production by mast cells and of the presence of macrophage-associated surface markers on the human mast cells suggest that the human mast cell lineage may have more in common with the macrophage, another cytokinin-producing cell, than with the myeloid cells such as basophils. The myeloid-associated surface markers on the human basophils may indicate their distinct and different developmental pathway.[164c]

(4) Neuropeptides

Another group of compounds being capable, under certain circumstances, of influencing the release of histamine, and probably also other mediators, from the human mast cells, are "*neuropeptides*" [97x,131c,163u,164g,164u,164y,165d,168, 168a-168z,169,169a-169z,170, 170a-170z,171,171a-171v]

Neuropeptides, small amino acid compounds, being localized near an in the neurons, are present throughout the body, in various organs and tissues.[163u,168f-168j]

Stimulation of the non-myelinated C-fiber sensory nerve in the respiratory tract leads to the antidromic release of neuropeptides. The released neuropeptides then cause dilatation of the blood vessels, increased vascular permeability, activation of various cell types, increased production and secretion of the mucus, effects which are termed by some authors as "neurogenic inflammation".[168h,168i,169m]

In the respiratory tract, the neuropeptides are located in neurons, neuro-endocrine cells and inflammatory cells.[163u,168f-168j,169d,169e] The neuro-endocrine cells are granulated epithelial cells distributed throughout the conducting airways that contain several peptides including "calcitonin", "katacalcin", "calcitonin-gene-related peptide" (CGRP), bombesin etc.[163u,169d] Other neuropeptides, such as "vasoactive intestinal peptide" (VIP) have been identified in various cell-types, including mast cells,[169f] eosinophils,[169g] mononuclear cells (monocytes),[168f, 169h,169i] and polymorphonuclear cells.[163u,168f] Once released, the neuropeptides may act either as neurotransmitters,[163u] or as mediators.[163u] In the respiratory tract, various neuropeptides serve as neurotransmitters modulating airway caliber, vascular tone, mucus secretion, and vascular permeability.[168g-168j, 168t,168u,168z,169a,169b,169j-169m] Some of the neuropeptides are also capable of affecting the functions of various cell types by modulating their mediator release [168f,168k,168l-168n] and chemotactic responses.[163u, 168f]

The following neuropeptides have been identified in the respiratory tract: (a) the tachykinins, such as substance P (SP), neurokinin A (NKA), neurokinin B (NKB), neuropeptide K (NPK); (b) neuropeptide Y (NPY); (c) vasoactive intestinal peptide (VIP) and two related peptides, peptide histidine isoleucine (PHI) and peptide histidine methionine (PHM); (d) calcitonin; (e) calcitonin gene-related peptide (CGRP); (f) somatostatin; (g) katacalcin; (h) galanin; (i) enkephalins; (j) cholecystokinin (CCK); (k) bombesin/gastrin releasing peptide (GRP); (l) atrial natriuretic factor; (m) neurotensin; (n) thymosins; (p)β-endorphin.[163u,168f-168i,168x,169,169b,169c-169e,169m,169n, 169z,170,171m,171n,171r]

The tachykinins form a group of peptides with a common c-terminal sequence of PHE-X-GLY-LEU-MET-NH_2 but different amino-terminal sequences that convey receptor specifics.[163u,168h,168i,169c] These neuropeptides are called tachykinins with respect to their rapid spasmogenic effects on the smooth muscles. [97x,163u,168h,168i,169c]

The neuropeptides have various biologic effects, termed "neurogenic inflammation", the most important of them are related to the vascular tone, vascular permeability, airway tone and direct influence on the functions of various cell types. [163u,168f-168m, 169b] The SP, CGRP, VIP, PHM and PHI are very effective vasodilators,[168g] whereas NPY may cause increase in vascular tone resulting in vasoconstriction.[163u,169p] NPY is likely to be a co-transmitter with norepinephrine and is predominantly localized

around the blood vessels in sympathetic fibers.[168y,169p] The tachykinins, SP, NKA and NKB can also increase vascular permeability,[169r, 169s] and their effects can be supported by CGRP.[169s] In contrast, the NPY may reduce the vascular permeability induced by SP.[169s]

The neuropeptides, such as SP, NKA and CGRP are potent bronchoconstrictors, whereas VIP, PHM and PHI are bronchodilators.[163u,168h,168i,169e] The neuropeptides can also directly modulate the functions of various cell types, the so-called pro- and anti-inflammatory effects.[163u,168h,168i,168x,169t]

The neuropeptides in the respiratory tract belong to the neurotransmitters of the so-called "non-adrenergic, non-cholinergic nervous system" (NANC).[163u, 168f,168h-168j, 168s] This system includes excitatory and inhibitory pathways that are neither adrenergic nor cholinergic, and this system exists besides the already established cholinergic and adrenergic nervous systems, where acetylcholine and norepinephrine act as intrinsic neurotransmitters.[163u,168,168b,168f,168h-168j,168s-168u,169d,169e,169l,169m,169x,169y]

The neural control of the airways is determined not only by the balance between the cholinergic and adrenergic nervous systems, according to the classical hypothesis, but also by the NANC system, as it has been recently confirmed.[168f, 168s,169d,169e,169g,169h]

The cholinergic (parasympathetic) nervous system in the respiratory tract is considered to be "excitatory" in nature, and it plays an important role in maintaining bronchial smooth muscle tone and in mediating acute bronchoconstriction.[163u,169g,169l] The cholinergic nervous system acts through the vagal afferent fibers in and around the airway lumen, which travel to the central nervous system and then terminate in the efferent fibers innervating airway smooth muscles. Vagal stimulation induces a diffuse constriction of the airways to the whole extent.[163u,169l] This effect may be antagonized by atropine and potentiated by acetylcholine-esterase inhibitors indicating that the effect is mediated by acetylcholine acting on the muscarinic receptors.[163u,170x] The muscarinic receptors are present in high density in the airways, however their distribution is greater in the large airways.[163u,170b,170c,170x]

In the human lung, three muscarinic receptor subtypes have already been pharmacologically identified (M1, M2, M3). However, five different muscarinic receptor subtypes are presumed.[170b,170c,170x]

The muscarinic receptor subtypes have been identified by means of specific antagonists, such as pirenzepine (M_1-selective), methoctramine (M_2-selective) and 4-diphenylacetoxy-N-methylpiperidine [4-DAMP](M_3-selective). The muscarinic receptors of human airway smooth muscles are mainly of the M_3-subtype, whereas human alveolar wall contains the M_1-subtype entirely.[170c] The human bronchial submucosal glands contain both the M_1- and the M_3-subtypes in 1:2 ratio. The M_1 receptors are located on the parasympathetic ganglia and submucosal glands, and facilitate vagal transmission and mucus secretion.[170b,170x] The M2 receptors may be present on the cholinergic nerves and act as autoreceptors inhibiting acetylcholine release. The M_3-receptors are located in airways as well as in submucosal glands and are responsible for the contraction of the airway smooth muscles and mucus secretion.[163u,170b,170c,170x]

The adrenergic (sympathetic) nervous system in the respiratory tract is considered as

"inhibitory" system, since the stimulation of the beta-receptors results in the relaxation of the bronchial smooth muscles and dilatation of the airways.[163u, 168s] The sympathetic nerve supply to the lung originates from the second to fourth thoracic preganglionic fibers that end in the extrapulmonary ganglia stellate. The postganglionic fibers enter the lungs at the hila accompanying the vagus nerves.[163u] The terminal parts of the adrenergic system in the human airways are formed by the alpha- and beta-adrenergic receptors that are important in regulation of the smooth muscle tone. Both types of receptors have been localized on various cell types, including alveolar and epithelial cells, and on the airway smooth muscles, in higher numbers in the peripheral airways. [170d] The beta-receptors appear, however, in higher density than the alpha-receptors.[170d]

The beta-receptors can be devided into $beta_1$- and $beta_2$-receptors, whose ratio is approximately 1:3 in the human lungs. The $beta_2$-receptor stimulation results in the relaxation of the bronchial smooth muscles, inhibition of the antigen-induced mast cell mediator release, release of the surfactant, fluid and protein exchange, ion transport, and decrease in the cholinergic neurotransmission.[163u,168h,168i,168s] The alpha-adrenergic receptors also appear in two subtypes, $alpha_1$- and $alpha_2$-receptors, whose exact role is, however, not yet fully understood. The $alpha_1$-receptor stimulation induces mucus secretion[170e] and may augment mediator release from the mast cells.[163u] Stimulation of the $alpha_2$-receptors has been shown to inhibit both the cholinergic and the non-cholinergic excitatory transmission.[163u,168h,168i]

The non-adrenergic non-cholinergic system (NANC) consists of: (a) the non-adrenergic inhibitory system pathways and (b) non-cholinergic excitatory system pathways.[168s] The non-adrenergic inhibitory system appears to be the predominant neural bronchodilatory system in the human lung.[168h,168i,169l] Potential neurotransmitters of this system in the humans include "vasoactive intestinal peptide " (VIP), peptide histidine methionine (PHM), and probably also nitric oxide.[163u,168h,168i] The VIP is not only a very potent endogenous smooth muscle relaxant, but it also inhibits both the mucus glycoprotein secretion from the human respiratory epithelial cells[163u,168u,170e] and a number of cellular functions, such as degranulation and mediator release from the mast cells,[168f,168k,168x, 169b] as well as the activation of macrophages, platelets, monocytes and T-lymphocytes. [163u,168h,168i,168m,168u,168w,168x,169i,171e] PHM is also a potent smooth muscle relaxant of the human bronchi.[168h,168i,169k]

The VIP inhibits the human natural killer cell activity, lymphocyte traffic and proliferation, release of IL-2, and the antigen-induced release of histamine from the guinea pig lung.[168r,168w,169v] The VIP also antagonizes the bronchoconstriction effects of various mediators, including histamine, $PGF_{2\alpha}$, LTD_4 and neurokinin A.[168h,168i,168u]

The VIP immuno-reactive nerves have been localized in the airway smooth muscle, bronchial glands, and pulmonary and bronchial blood vessels. The density of VIP immuno-reactive nerves is highest in the upper respiratory tract (e.g. nose) and is lowest in the bronchioles.[170f,170g] The VIP receptors have been found in the high density in pulmonary vascular smooth muscles, smooth muscles of the large but not in the small airways, bronchial epithelium, submucosal glands and in the alveolar walls.[170g]

The non-cholinergic excitatory system in the human lungs induces a bronchoconstriction which is not inhibited by atropine but it is blocked by antagonists to substance P.[169r] The same bronchoconstriction can be caused by capsaicin, a compound inducing the release of substance P or other neuropeptides from unmyelinated sensory nerves.[169t,170h] Potential neurotransmitters of this system include substance P, neurokinin A (NKA), neurokinin B (NKB), and calcitonin gene-related peptide (CGRP).[163u,168f, 168g,168h,168t,168w,169d,169j,169m,169p,169w,169y] Substance P immuno-reactive nerve fibers may be found in the neighbourhood of the blood vessels, airway epithelium and airway smooth muscle cells.[168h-168j,168r,168z,169l,170j]

The substance P, which is found in increased concentrations at the sites of local inflammatory processes,[163u,168f,169e,169m,169w,169x] may cause the T lymphocyte proliferation,[168f,168x,169b,169u,169v,171l] release of mediators from certain mast cells,[168f,168j, 168k,168n,168p,169b,169w,170] but not from basophils,[169b] release of proinflammatory and immunoregulatory substances from macrophages,[168f,169b] enhancement of phagocytosis by macrophages and polymorphonuclear leukocytes,[168f,169m] enhancement of the production of immunoglobulins by B lymphocytes (plasma cells),[169b,169v] and chemotaxis of mononuclear cells and polymorphonuclear leukocytes.[163u,168f,168j,168k,168l, 169b,169i] Both SP and NKA have also been shown to stimulate the release of interleukin-1, tumor necrosis factor-α and interleukin-6 from the human blood monocytes. [169h]

Recently, SP has been shown to induce the eosinophil infiltrates through the degranulation of mast cells in mice.[169w]

Substance P (SP) induces bronchoconstriction, stimulates the mucus glycoprotein secretion from the human tracheal glands and has distinct cardiovascular effects, especially vasodilatation.[168z,170e,170h,170k]

SP has also been shown to increase cholinergic-induced neural responses in animal airways, probably through the release of acetylcholine.[170l] However, a comparable effect of substance P in human airways has not yet been confirmed. The effects of substance P on individual cell types, also in man, have already been discussed in this section.

Somatostatin, in contrast to SP, has been shown to be able to induce degranulation both of the mast cells and of the basophils.[169,169b,170]

Somastostatin may also inhibit the proliferation of T-lymphocytes, besides its blocking effect on the release of substance P and neurotensin from the primary sensory neurones.[168x,169b,169z,171l,171n]

Another neuropeptide, neurotensin, released from the bipolar sensory neurones, causes not only distinct vasodilatation allowing influx of various inflammatory cells, but also induces the mast cell degranulation followed by the release of histamine and other mediators, and stimulates neutrophil chemotaxis and phagocytosis.[168x,169b, 171n]

NKA may also play an important role inside the non-cholinergic excitatory nervous system.[163u,168t] NKA is co-localized with substance P in sensory airway nerves.[168t] NKA immuno-reactive nerves are prominent in bronchial smooth muscles and pulmonary vessels, and they are also present in trachea, bronchioles and alveoli.[168t] NKA also induces the bronchoconstriction of human airways, however, to a higher degree than substance P.[169l] The effects of NKB on the human bronchi have not yet been confirmed.[169l]

CGRP is also co-localized with substance P in the airway nerves.[168f,168j] CGRP immuno-reactive nerves have been localized in the neighbourhood of ganglia, blood

vessels within the tracheo-bronchial smooth muscle layers, and underneath and within the respiratory epithelium.[170m,170n] CGRP has been reported to stimulate basal serous cell secretion and epithelium albumin transport, to modulate acetylcholine and probably to cause the constriction of human bronchi due to the contraction of the human bronchial smooth muscles.[163u,170n]

The possible participation of the non-cholinergic excitatory system in the mechanism of asthma has been proposed by Barnes, formulating the "axon-reflex" hypothesis.[131c, 168i] The antigen-antibody interaction or other mechanisms may result in the release of various mediators from various cell types, which then stimulate the vagal afferent nerve endings in the airway epithelium. This process may trigger not only the reflex increase in afferent cholinergic activity but also the antidromic release of neuropeptides such as substance P, NKA and CGRP, via a "short-circuited" local reflex loop. These neuropeptides, in turn, may cause airway smooth muscle contraction, edema, production of mucus, and release of other mediators, factors and constituents from various cell types.[168h,168i]

The knowledge concerning the other neuropeptides and their possible effects, especially in the human respiratory tract, is still limited.[168g-168i] The neuropeptide Y (NPY) has been found to be a potent vasoconstrictor. However, it does not appear to have a direct effect on the human airway smooth muscle cells.[168y,169l, 169p] NPY has been found in human lung and is localized primarily to the blood vessels.[170i] NPY is probably a co-transmitter of norepinephrine.[169p,170p] The "gastrin releasing peptide" (GRP) is the mammalian equivalent of the amphibian peptide bombesin. GRP has also been found in the human lung[170r] and may have a number of biologic effects, such as mitogenesis of the bronchial epithelial cells, contraction of bronchial smooth muscles and stimulation of the mucus secretions.[168h,168i] Cholecystokinin (CCK) has also been found in the lung tissue[168h,168i] and this neuropeptide has been shown to mediate human airway bronchoconstriction "*in vitro*".[170s]

Several studies "*in vitro*" as well as "*in vivo*" have demonstrated that the neuropeptides realize their effects through the activation of specific cellular receptors.[163u,168u, 170a,170g,170t,170u,170v]

For example, three high affinity receptor subtypes have been found for tachykinins.[163u] Substance P interacts with receptors of the NK-1 subclass (SP-P), NKA interacts preferentially with the NK-2 subclass (SP-E), whereas NKB interacts with the NK-3 subclass (SP-N) receptor.[168h,168i,169l]

SP, NKA and NKB are the endogenous mammalian agonists for the NK-1, NK-2 and NK-3 receptor subclasses. The binding of agonists to the neuropeptide receptors leads to a series of specific intracellular events.[168h,168i] The receptors for the putative peptide neurotransmitters of the NANC nervous system have been identified both in the human and in the animal lungs.[168h,168i]

Further description of particular neuropeptide receptors in detail would exceed the scope of this text. This topic is reviewed in an excellent manner elsewhere.[168h,168i,169l]

An interesting property of the NANC system is its ability for "co-transmission" and "co-localization" of the neuropeptides. It means that one type of nerve can release various types of neuropeptides simultaneously and these neuropeptides can appear at the same site, which is also called "co-release".[168t,169m,170m] Moreover, various neuropeptides can be co-localized with the classic neurotransmitters of the autonomic nervous system.[168f,168t,168y,169m,169p,171a]

According to the recently gathered evidence, the parasympathetic nerves may contain, besides acethylcholine, also VIP; the sympathetic nerves may contain both noradrenaline and neuropeptide Y; and the capsaicin-sensitive sensory C-fibers may contain SP, NKA and CGRP[168f,171a]. The stimulation of a particular type of nerve can then result in the release of various different neuropeptides and classic neurotransmitters with different and manifold effects. The final results of such co-release depends on the quantities of the particular transmitters released, the location of the release and the environment into which such release occurs.[168f, 168h,168i]

As in other biological systems, there is a basic functional balance inside the NANC system upon the participation of the antagonists to the particular neuropeptides[168f,171b] and various enzymes (peptidases) that can either inactivate the neuropeptides or change them into biologically different active forms.[168f,171b-171e] The peptidases are produced by various cell types of the so-called "neuroendocrine" as well as "inflammatory" cell groups and they may be associated with the cellular membranes or be soluble in the tissue fluids.[171b,171e]

Most of the neuropeptides have a short life in the blood stream.[171b,171e] The physical distance between the point of peptide release, the target cells, and the peptidases type, are the factors determining the biological actions and effects of the particular neuropeptides.[163u,171e]

There is a variety of peptidases capable of degrading the neuropeptides. These include neutral endopeptidase (NEP, CD 10) called also "enkephalinase", peptidyl dipeptidase, called also "angiotensin converting enzyme" (ACE), various carboxy-peptidases and aminopeptidases.[171e]

Most of the peptidases are not specific for the particular neuropeptides.[171e] NEP, a membrane-associated metallopeptidase, widely distributed in the body, is, outside the nervous system, predominantly localized in the epithelial cells, fibroblasts, and neutrophils.[171b-171e] NEP hydrolyzes peptides at the amino-side of the hydrophobic amino-acids. The peptides cleaved by NEP include the enkephalins, bradykinin, neurokinins, substance P, neurotensin, cholecystokinin (CCK), gastrin, gastrin-releasing peptide (GRP), and natriuretic factor.[171b-171e]

In contrast, NEP and its effects are inhibited by compounds, such as thiorphan and phosphoramidon.[163u,171f]

ACE, being an exopeptidase removing dipeptides sequentially from the C-terminus, is also widely located in the body. However, the predominant location of ACE is the luminal surface of vascular endothelium,[171b,171c] and epithelial and neuroepithelial cell surfaces.[171b,171g] ACE hydrolyzes the neuropeptides especially in the blood stream.[171e] ACE converts the angiotensin I to angiotensin II, inactivates the bradykinin and degrades enkephalins, substance P and neurotensin.[171b,171e,171g,171h] ACE and its effects are inhibited by captopril and enalapril, which have also been shown to augment both the

spontaneous and the antigen-induced histamine release from the lung and skin mast cells and probably also from the basophils.[171p]

Finally, the mast cell proteases, tryptases and chymase have also been shown to degrade some of the neuropeptides.[171i-171k] The mast cell tryptases and chymase are capable of degrading the VIP,[171k] while chymase, but not tryptase can degrade the substance P.[171i]

(5) Various compounds activating the mast cells and basophils

Finally, there are various other compounds and factors which have been shown to be capable of stimulating the mast cells and/or basophils to secrete and/or to degranulate and to release their mediators, such as: (a) Formyl methionine, being a part of some tripeptides, can bind to specific receptors and induce histamine release from the human basophils;[162m] (b) Arginine-rich major basic protein, stored in eosinophil intracellular granules ,may activate the basophils and mast cells for non-cytotoxic histamine release;[162m] (c) Ionophores, lipophilic compounds, insert themselves into the cell membranes and transport ions across the membrane. The Ca++ ionophore A23187 triggers the mediator release from the basophils and mast cells in the presence of Ca++ ions in the medium;[162m,174a] (d) Other compounds and factors, such as proteins released by neutrophils and diagnostic and therapeutic drugs, such as polymyxin B, compound 48/80, polylysine, polyarginine, morphine, tubocurarine, dextran, adenosine triphosphate, chymotrypsin, opioids, iodinated contrast media and lastly some physical stimuli, such as heat, cold, sunlight and mechanical pressure.[162m,162z,164z,165g]

6. INNERVATION, NEUROPEPTIDES AND NEUROGENIC CONTROL OF THE NASAL MUCOSA

Various neuropeptides are involved in the neurogenic control of the nasal mucosa and participate also in various steps of the hypersensitivity mechanisms and processes arising in that tissue.[168g-168j,169l,170e,170w,171r] The knowledge concerning the role of individual neuropeptides in the mechanisms underlying the particular types of the human nasal mucosa response to allergen exposure as well as to other stimuli is, however, still incomplete.[168f]

[A] INNERVATION OF THE NASAL MUCOSA

The innervation of the nasal mucosa includes not only the classical nerve systems, the sensory nerve system and the autonomic nerve systems consisting of the sympathetic (adrenergic) part containing noradrenaline as neurotransmitter and of the parasympathetic (cholinergic) part with acetylcholine as neurotransmitter, but also other neurogenic mechanisms.[168f-168i,169x,171s]

Recently, evidence has been provided for existence of such neurogenic mechanisms also in the human nasal mucosa.[97x,168s,171t,171u,171v] These mechanisms are neither adrenergic nor cholinergic and may form the so-called "third nervous system", being probably comparable with the "non-adrenergic non-cholinergic nervous system (NANC)" described in the lower airways, where various neuropeptides play the role of neurotransmitters.[168h,168i,168s,169m,171b,171e, 171r-171v]

(1) Sensory nerve

The sensory nerve supply of the nasal mucosa originates from the trigeminal nerve (*nervus trigeminus*), through its branches the maxillary (*n. maxillaris*) and the ophthalmic (*n. ophthalmicus*) nerves, and from the pterygopalatine ganglion, also called the sphenopalatine ganglion, located in the fossa pterygopalatina.

One of the ophthalmic nerve branches, the nasocilial nerve (*n. nasociliaris*) divides into 4 sub-branches, and two of them are related to the nasal mucosa; the posterior ethmoidal nerve (*n. ethmoideus posterior*) supplies the posterior ethmoidal sinus, and the anterior ethmoidal nerve (*n. ethmoideus anterior*) supplies the anterior part of the lateral nasal wall by means of its "*rami nasales anterior laterales*" and the anterior part of the septum through its "*rami nasales anterior septi*".

The branches of the maxillary nerve supply among others the maxillary sinuses ("*rami alveolares maxillares posterior*" and branches of the "*n. infraorbitalis*", "*ramus alveolaris maxillaris medius*" and "*rami alveolares maxillares anterior*", with adjacent "*plexus dentalis maxillaris*") and the nasal mucosa through its branches "*nervi pterygopalatini*" entering the pterygopalatine ganglion, and leaving this ganglion as: (a) "*rami nasales posterior*" (dividing then into "*lateralis*" sub-branches reaching the posterior part of the lateral nasal wall and "*septi*" sub-branches supplying the posterior part of the septum); (b) "*n. nasopalatinus*" divided into 3 branches, the great palatine nerve (*n. palatinus major*) innervating the hard palate, the middle palatine nerve (*n. palatinus medius*) and the small palatine nerve (*n. palatinus minor*) supplying the soft palate; and finally (c) "*rami orbitales*" reaching the ethmoidal and sphenoidal sinuses.

(2) Parasymphatetic fibers

The parasympathetic fibers arise from the superior salivary nucleus (*nucleus originis salivatorius pontis*), located in the lower pons, enter the facial nerve (*n. facialis*) and transfer then to its branch, the greater superficial petrosal nerve (*n. petrosus superficialis major*) at the level of the geniculate ganglion. This nerve emerges through the anterior surface of the petrous temporal bone in the midline cranial fossa, forming the bottom of the Meckel's cave (*cavum semilunare*) housing the ganglion Gasseri (=*ganglion-semilunare*). The greater superficial petrosal nerve joins then with the deep petrosal nerve (*n. petrosus profundus*) from the carotico-tympanic plexus on the internal carotid artery, housing the sympathetic fibers, and both the nerves together form the nerve of the pterygoid canal (*n. canalis pterygoidei*) , also called the "Vidian nerve". The nerve of the pterygoid canal transverses the canal in the sphenoid at the root of the pterygoid processus and emerges in the fossa pterygopalatina, where it enters the pterygopalatine ganglion(= sphenopalatine ganglion). The parasympathetic fibers have synapses here, and the postganglionic fibers pass via the branches of the pterygopalatine ganglion (*rami nasales posteriores, n. nasopalatinus, nervi palatini*) to the glandular, vascular and mucosal tissues of the nose and sinuses.

Recently, an additional parasympathetic pathway has been described, having the accessory postganglion neurons also in the mucosal "microganglia", located in the nasal mucosa.[170z]

(3) Sympathetic fibers

The sympathetic fibers are derived from the superior cervical ganglion (also called *ganglion cervicale craniale*), located on the front of the processus costotransversarius of the second and third cervical vertebra. The postganglionic sympathetic fibers enter, through the internal carotid nerve (*n. caroticus internus*) the *plexus caroticus internus*, continued as *plexus cavernosus*, and leaving this plexus as deep petrosal nerve (*n. petrosus profundus*) joining then the greater superficial petrous nerve and form together the nerve of the pterygoid canal, which enters the pterygopalatine ganglion. The sympathetic fibers are then distributed to the glandular tissues, blood vessels and nasal mucosal tissue by means of the above described branches of the pterygopalatine ganglion, parallel to the parasympathetic fibers.

Concerning the structure of the sympathetic and the parasympathetic system, this shows not only some similarities, such as the existence of two neurons, one supplying the preganglion fibers, whereas the other supplies the postganglion fibers; construction of the fibers, the preganglion fibers having a myelin sheath, whereas the postganglionic fibers do not; but also some differences, such as the non-existence of secondary and tertial sympathetic synapses, in contrast to the existence of such parasympathetic synapses and ganglions.[171y,171z] The sympathetic fibers do not relay and after arising from the superior cervical sympathetic ganglion they reach the appropriate sympathetic plexus directly.[171w]

(4) Basic types of neuroreceptors in the human nasal mucosa

The basic types of neuroreceptors, cholinergic (muscarinic) as well as adrenergic, have also been demonstrated in the human nasal mucosa.[168h,168i,170,171r, 171s,172a-172c]

Cholinergic muscarinic receptors have been localized in the nasal glands after "*in vivo*" administration of 3H-1-QNB (Quinuclidinylbenzilate), followed by dissection and autoradiography.[171r]

In patients with allergic rhinitis, a significantly decreased number of cholinergic muscarinic receptors in the nasal mucosa has been found, in comparison with the control subjects.[171r,172b,172c] However, no differences in the agonist binding or coupling of the muscarinic receptors to the effector system via the G-protein have been observed in patients with allergic rhinitis and the controls.[171r] These results may also confirm the parasympathetic control of the glycoprotein production by the nasal mucosal glands.[172d] Moreover, histochemical studies have demonstrated some nerve fibers containing acetylcholine-esterase, in the sub-epithelial plexus[172e] and blood vessels.[172f]

In contrast to the lower airways, no data concerning the existence of particular muscarinic receptors (e.g. M_1, M_2, M_3) in the nasal mucosa have been available until now. Recently, the investigator group from NIH in Bethesda[172g] have reported the autoradiographic localization and existence of muscarinic receptor sub-types also in the human nasal mucosa. They have found M_1 and M_3 sub-types in the submucosal glands and the M_1 to a slight degree also in the wall of the blood vessels in the human nasal mucosa. The localization of the M_1 and M_3 receptors on the submucosal glands may suggest that both the receptor sub-types, especially the latter, may contribute to glandular secretion.

The adrenergic receptors of the α-class (α_1 and α_2) as well as the β-class have also been repeatedly demonstrated in the human nasal mucosa.[171r,171s, 172a,172b] Moreover, the existence of a β_2-subtype adrenergic receptor has also been confirmed in the human nasal mucosa.[171r] In general, the α-stimulation of the nasal mucosa is mostly excitatory, whereas the β-stimulation is usually inhibitory.[172a]

No significant differences in the affinity or density of both the α_1 and α_2-receptors have been found in patients with allergic rhinitis in comparison with the control subjects, whereas the density of the β adrenergic receptor has significantly been reduced to a great degree in the nasal mucosa of the patients with allergic rhinitis in comparison to the control subjects.[171r,172b,172c] No differences in agonist binding, coupling to G-proteins or localization of the β-adrenergic receptors have been demonstrated in the allergic rhinitis patients and control subjects.[171r]

Afferent impulses from the nasal mucosa are propagated via the sensory fibers to the central nervous system and give rise to tickling, burning or pain. The afferent impulses result in sneezing, increased secretion, changes in blood flow and volume. Sensory intra-epithelial nerves have been found only in the extra-pulmonary airways. Sensory fibers in the neighbourhood of the blood vessels may probably be involved in the local axon reflexes.[172a,172f,172h]

Efferent impulses are propagated via the autonomic, vasomotor and secretomotor nerve fibers. The vasomotor fibers are both sympathetic and parasympathetic, whereas the secretomotor fibers are only parasympathetic.[172f]

The stimulation of parasympathetic fibers leads to dilatation of the blood vessels in the nasal mucosa, whereas the stimulation of the sympathetic fibers results in the contraction of blood vessels.[172a,172f,172h-172j] However, some investigators have reported the possibility of the so-called "dual sympathetic innervation" of the nasal blood vessels, where the α-adrenergic agonists induce a vasoconstriction, whereas the β-adrenergic agonists induce a vasodilatation.[171r,172k] Later, the importance of the α-receptors in the control of the nasal blood circulation (volume as well as flow) has been confirmed, whereas the importance of the β-receptors has weakened.[172l]

In contrast to the parasympathetic, the sympathetic fibers to the blood vessels possess the tone. These fibers are numerous in the wall of the sinusoids, which are permanently in a state of partial constriction due to the continous sympathetic stimulation. Since the sinusoids regulate the main part of the nasal blood volume in the turbinates, the changes in the relative balance of the autonomic system may rapidly cause considerable changes in the thickness of the nasal mucosa, especially edematic changes, followed by changes in the degree of the nasal patency and nasal resistance.[172a,172f]

In contrast to the blood vessels, the secretory as well as the contractile (myoepithelial) functions of the human nasal glands are regulated by the parasympathetic fibers.[172a, 172i] However, since the secretion of the nasal mucosal glands depends, among others, on the blood supply, the sympathetic nerve fibers may also affect the mucosal glands indirectly through the innervation of their blood vessels.[172a]

[B] NEUROPEPTIDES AS NEUROTRANSMITTERS IN THE HUMAN NASAL MUCOSA

As already mentioned, evidence has been provided for the existence of a non-adrenergic non-cholinergic nervous system, including various neuropeptides as neurotransmitters, also in the human nasal mucosa.[162z,168h,168i,168y,169l, 169m,169y,170i,171r,171s,171u, 171v,172c,172g,172k-172z,173,173a-173y,174]

Some of the neuropeptides have already been identified in the human nasal mucosa (inferior turbinate tissue) and measured in the following concentrations (mean ± SEM) :(1) Substance P [SP] = 5.91 ± 2.14 pmol/g tissue,[172m] 1.03 ± 0.2 pmol/g tissue,[172p] (2) Neurokinin A [NKA] = 0.76 ± 0.23 pmol/g tissue;[172p] (3) Neuropeptide Y [NPY] = 3.13 ± 0.79 pmol/g tissue;[168y,172r,173] (4) Vasoactive intestinal peptide [VIP] = 2.84 ± 0.47 pmol/g tissue;[172t] (5) Calcitonin gene-related peptide [CGRP] = 0.45 ± 0.3 pmol/g tissue;[172u] (6) and Bombesin/Gastrin releasing peptide [GRP] = 0.60 ± 0.09 pmol/g tissue.[172v]

Unfortunately, there is a dearth of information concerning the possible presence of other neuropeptides in the human nasal mucosa tissue as well as the appearance of neuropeptides in the nasal secretions, and their possible changes during the individual types of nasal response to allergen challenge.

Moreover, very few data are available concerning the appearance and concentrations of particular neuropeptides in the nasal secretions of healthy human subjects and patients with allergic rhinitis as well as those with vasomotor rhinitis (= non-specific hyperreactivity component) only.

Tonnesen and co-workers[173v] performing the nasal challenges with timothy grass pollen and methacholine bromide in patients with pollen-related allergic rhinitis, have recorded substance P in the nasal secretions in concentrations of 4.0 (0-17.5) pmol/L immediately after the allergen challenge and of 10.1 (4.0-15) pmol/L immediately after the methacholine challenge. Unfortunately, they have failed to include data concerning the baseline concentrations of substance P (= before the challenges) as well as the description of the nasal response both to the allergen and to the methacholine in the time-course.

Walker and colleagues[173l] have also carried out the nasal challenges with rye grass pollen, both in patients with pollen-related rhinitis and in control subjects, supplemented by measurement of the concentrations of substance P (SP), somatostatin 14 (SOM), calcitonin gene-related peptide (CGRP) and histamine in the nasal lavage fluid before and after the allergen challenge. They have recorded the following pre-challenge concentrations in the nasal secretions: (a) histamine: 25-35 ng/5 ml in control subjects and 25 ng/5 ml in allergic patients; (b) CGRP: 200 - 400 pg/5 ml in controls and 250-400 pg/5 ml in allergic patients; (c) SOM: 5-400 pg/5 ml in controls and 600-1000 pg/5 ml in allergic patients; (d) SP: 140-180 pg/5 ml in control subjects and 80-130 pg/5 ml in allergic rhinitis patients. After the allergen challenge in allergic patients, but not in control subjects, the significant increase has been recorded in concentration of histamine (3-fold) at 15-60 minutes, of CGRP (1.5-4-fold) at 15 minutes to 24 hours, and of SOM 14 (2-fold) at 6 hours, whereas the concentration of SP had not changed significantly. They concluded that "CGRP may mediate the nasal congestion directly and SOM may be one of the factors regulating the late involvement of basophils and mast cells in allergic rhinitis". However, these investigators have failed to present both the absolute values of the concentrations of neuropeptides in the nasal secretions (only the approximate values are readable from the figures), and the description of the nasal response to allergen challenge.

Furthermore, several nerve fibers containing various neuropeptides have been found in the human nasal mucosa and some of the neuropeptide -reactive neurons have been identified in the nerve tracti and/or ganglions related to the nasal mucosa.[169m,171v,173d-173f]

(1) Substance P (SP)

Substance P is present in the type C nociceptive sensorimotor neurons.[170l-170p,170v, 171r,171v,172b,172l-172p,172w] In the same neurons, SP is co-localized with the neurokinin A (NKA) and the "calcitonin gene-related peptide" (CGRP).[172p,173f] SP, NKA and CGRP can be released simultaneously from the same neuron and their co-release may then cause either synergistic or antagonistic effects in the target tissue.[173f,173g] In the human nasal mucosa, the SP-immuno-reactive nerve fibers are present in high density on the walls of arterioles, venules and venous sinusoids, and as individual fibers also in gland acini, near the basement membrane and in the epithelium.[172p,172z] SP-immuno-reactive nerve fibers have been found in the walls of arterioles at the junction of the adventitia and muscular layers and between the smooth muscle cells. [172p] SP-fibers form a plexus around the mucosal arterioles and venules.[172p] SP appears to be localized predominantly to the capsaicin-sensitive unmyelinated nerves in the airways.[168h]

Tachykinin effects on the target cells are mediated via specific receptors and each tachykinin activates selectively an appropiate receptor.[168h,170v] The neurokinin receptors, NK-1, are activated preferentially by SP, NK-2 by NKA, and NK-3 receptors by neurokinin B (NKB).[168h,168i,170v] In the human nasal mucosa, the SP-binding sites have been found in the epithelium, mucosal glands and on the blood vessels (arterioles as well as venules), whereas the NKA-binding sites only on the arterioles.[172p]

The NKA binding may therefore indicate the distribution of the NK-2 receptors on the arterioles, whereas the SP-binding may demonstrate the presence of the NK-1 receptors on the arterioles, venules and mucosal glands in the human nasal mucosa.[172p] No differences have been found in the SP-binding sites between the serous or mucous cells of the glands.[172p]

Nociceptive sensory nerves play an important role in the protection of the nasal mucosa.[172p,173f-173h] Mucosal injury, mechanical or thermal stimuli, or allergen exposure in sensitized subjects, may lead to the generation and release of a variety of factors, such as bradykinin, histamin, K^+, H^+, prostaglandins, leukotrienes, PAFs, which can then affect the sensory nerve receptors resulting in the sensory nerve depolarization and nerve impulse generation.[169t, 171v, 172p, 173g, 173h] Depolarization of a single peripheral axon branch leads to the depolarization of the entire extensively arborized sensory neuron, and central depolarization along the thin, unmyelinated central axon to the central nervous system.[173g,173h] These type C fibers transmit the nociceptive stimuli due to pain, itching and burning.[173f] The peripheral, branched axons pass near submucosal gland cells, myoepithelial cells, the walls of arterioles, arterio-venous anastomoses, venules and venous sinusoids.[171v,172p,173g,173h] In these locations, the axons are thickened into the so-called "neurosecretory varicosities", representing the neural switch. [173f] Depolarization results in the release of multiple neurotransmitters from these varicosi-

ties.[172p,173f] The neurotransmitters act upon local structures and induce a variety of changes such as vascular permeability, arteriolar vasodilatation, smooth muscle contraction, and submucosal gland secretion.[169t, 171v, 172p, 173g, 173h] This local "efferent" action of the "afferent" peripheral sensory nerves constitutes the axon response.[172p] The dual sensory and motor functions of the nociceptive nerves led some investigators to use the term "sensorimotor" neurons.[172p]

(2) Neurokinin A (NKA)

The neurokinin A - immuno-reactive nerve fibers have also been found in the walls of the arterioles at the junction of the adventitia, in the muscular layers and between the vascular smooth muscle cells. [172p,172z]. A dense plexus of fibers appeared to be present in the human nasal mucosa. Single free NKA fibers may also be occasionally found near venules, in the loose connective tissue beneath the basement membrane and between the epithelial cells. [172 p]. NKA nerve fibers have also been found in the nerve bundles deep in the nasal mucosa [172p]. NKA is capable of inducing a distinct constriction of the smooth muscles in the human airways.[168h]

(3) Neuropeptide Y (NPY)

The neuropeptide Y is usually located in a subpopulation of the postganglionic sympathetic fibers.[169p] In these fibers, NPY is stored, together with epinephrine, in large, dense core vesicles.[172r,172y] The NPY containing nerve fibers are mostly concentrated in the walls of small arteries and arterioles at the adventitial-medial junction or within the vascular muscular layer.[171v,172r] Moreover, the venous portions and venous sinusoids are also supplied by these nerves, to a lesser degree. Some free fibers may also be found in the adventitia of the small venules between the gland acini.[172r]

The NPY-immuno-reactive fibers present near the arteriolar vessels form a plexus around these vessels. [171v, 172r]. The release of the immuno-reactive NPY can be inhibited by phosphoramidon or thiorphan, inhibitors of the neutral endopeptidase ("enkephalinase"), being a member of the metallopetidases.[171b,171c,171e]

(4) Vasoactive intestinal peptide (VIP)

The "vasoactive intestinal peptide", being a neurotransmitter in the postganglionic parasympathetic neurons, is present along with acetylcholine in the parasympathetic nerve fibers.[169m,171v]

Postganglionic cholinergic neurons contain VIP, peptide histidine methionine (PHM), acetylcholine, choline acetyltransferase and acetylcholinesterase.[168h, 168i] The neurons enter the nasal mucosa via the posterior nasal nerves and innervate the submucosal glands, arterioles and venules.[169m,171u,171v,172s]

VIP is released along with acetylcholine by parasympathetic nerves and it may play an important role in the regulation of various functions of the nasal mucosa.[172t,173b] The VIP-immuno-reactive nerve fibers are located predominantly around the submucosal glands. [170f,172s,172t] The fibers contact the acinar glands directly.[168t,172h,172t] However, some fibers may also be found in the walls of the mucosal blood vessels, venules and

arterioles, especially in relation to the vascular smooth muscles of arterioles.[172t] Moreover, the binding of single VIP nerve fibers to the vascular endothelium cannot be excluded, although a precise differentiation between the smooth muscle and endothelium binding of the radio-labelled VIP has not yet been possible.[172t] The VIP-containing neurons also contain a closely related PHM peptide.[169m] Although VIP, PHM and acetylcholine are all present in the peripheral neurosecretory varicosities of the postganglionic parasympathetic neurons, the amounts of each released during the neural transmission appears to depend on the nerve impulse frequency.[173c] At low rates, only acetylcholine is released, whereas at high rates, acetylcholine together with VIP and PHM are released. VIP may augment the postsynaptic acetylcholine - induced secretory response in the glands, but it may also have presynaptic inhibitory effects on the further neuropeptide release. This mechanism would prevent the total release of stored neuropeptides since there are no re-uptake mechanisms, and the VIP and PHM can only be resupplied by axonal transport from the cell body.[173c] This process may play a very important role in the function of the parasympathetic neurons.[173c]

(5) Calcitonin gene-related peptide (CGRP)

The "calcitonin gene-related peptide" acts as a neurotransmitter in type C nociceptive sensimotor neurons and sensory nerve (C-fibers)[168h,168i 172u,173f,173g]. These neurons have a dual function, acting both as afferent sensimotor neurons and as efferent regulators of vasomotor function.[168t,173h] The afferent role involves the transmission of the message of mechanical and thermal stimuli to the central nervous system.[173h]

Peripherally, these branched dendritic fibers are widely dispersed near the blood vessels and act as the efferent mediators of the axon reflex.[173f-173h] CGRP being a potent vasodilator, is present in nerve fibers representing the nociceptive sensimotor nerves that innervate vascular structures (muscular arteries, arterioles, veins and venous sinusoids).[168t,171v,172u] The CGRP-immuno-reactive fibers form a plexus in the walls of the small, deeper in the submucosa localized, muscular artioles at the junction of the adventitia and the muscular layers and between the vascular smooth muscle cells.[168t, 172u] In contrast, the thin walls of venules and venous sinusoids are innervated by individual fibers. The single CGRP-containing fibers may also be found between the gland acini [172u] contacting the myeloepithelial and/or submucosal gland cells in the loose connective tissue beneath the basement membrane, between the epithelial cells, and finally in the nerve bundles deep in the mucosa.[172u]

There are several types of the CGRP-containing sensory neurons, including types thought to contain both CGRP and tachykinins, such as substance P and neurokinin A.[168t,172u,173c,173f-173h] CGRP is also co-stored and co-localized with substance P in afferent nerves.[168h,168i,168t,170m,170n,170r,173f,173g] CGRP may also be found in the trigeminal, nodose-jugular and dorsal root ganglia. [173i] CGRP binds to the specific surface receptors that are linked via a stimulating G-protein (Gs) to adenyl cyclase, thus increasing intracellular cyclic AMP concentration in the vascular tissue.[168h,168i,170u]

Although the single CGRP-containing nerve fibers have been identified adjacent to the submucosal gland acini and between the epithelial cells, there is no evidence for a role of the CGRP in the glandular secretion.[170r,172u]

Initial studies of the sensory nerves have been performed using capsaicin.[168t, 169t, 170m, 172m, 173f-173h, 173j, 173x] Capsaicin, the pungent agent has been used as an experimental tool in studies on the peptide-containing sensory nerves in the airways.[168t] This compound is known to activate selectively a population of the chemosensitive C-fiber afferent pathways in the airways.[173k] This initiates a cascade of both the central reflexes and the local release of bioactive peptides from the peripheral branches of sensory nerves. After exposure to a high dose of capsaicin, the largest portions of tachykinins and CGRP disappear from the nerves in the airways, whereas the VIP-containing nerves and sympathetic nerves remain unchanged.[168z, 169c, 170m, 172m, 173g] Because of the selective action of capsaicin treatment on the tachykinin - immuno-reactive and CGRP-immuno-reactive nerves, it is likely that these peptides are mainly present in the chemosensitive C-fiber afferents, which represent only a sub-population of the sensory nerves in the airways. [172m, 173h] The tachykinin - and CGRP-immuno-reactive nerves in the human nasal mucosa are very abundant, whereas they are comparatively more sparse in the human trachea and bronchi.[168t]

(6) Gastrin-releasing peptide (GRP)

The GRP is a mammalian equivalent of the amphibian neuropeptide "bombesin".[173m, 173n] A group of resembling neuropeptides, sharing the active C-terminal sequence, the so-called "GRP-related peptides", has also recently been identified,[173p, 173r] GRP is located in the nerve fibers and pulmonary neuroendocrine cells, and can also be detected in plasma.[173n] The GRP can act as a neurotransmitter, a neuroregulatory agent, and as a growth factor in fetal, normal and neoplastic respiratory tissue. [173f, 173n, 173 r] The GRP-containing nerve fibers have been demonstrated around the blood vessels and submucosal glands in the airways of several species including man.[170r] GRP has also been identified in the trigeminal sensory nerves (trigeminal nociceptive sensorimotor type C nerve fibers) that innervate the human nasal mucosa.[172v] The distribution of the GRP-containing nerves in the nasal arterial and venous vessels and glands is very similar, of not identical, to that of substance P(SP), neurokinin A (NKA) and calcitonin gene-related protein (CGRP).[172v] The GRP binding sites in the nasal and bronchial epithelium are present on the epithelial cells and submucosal glands.[172v, 172z] The arterioles are densely innervated by a plexus of GRP-immuno-reactive nerves, which fibers are mostly concentrated in the neighbourhood of the adventitial border and between the vascular smooth muscle cells, although some of them may penetrate up to the intima.[172v] The walls of the venous sinusoids and venules are innervated by individual fibers.[172v] Individual fibers can also be found in submucosal glands in close apposition to both the mucous and serous secretory cells and in connective tissue and beneath the epithelial basement membrane.[172v] The deep mucosal nerve bundles containing a population of intensely stained GRP-immuno-reactive neurons are usually also located in the human nasal mucosa. No GRP-containing epithelial or neuroendocrine cells were identified in the human nasal mucosa.[172r, 172v]

The GRP-immuno-reactive nerves are present between the gland acini of the nasal mucosa and the GRP binding sites are present on the submucosal glands and epithelial cells in the human nasal mucosa.[172v]

The GRP-immuno-reactive material has also been found in the arteriolar vessels in the nasal mucosa, but no GRP-binding sites were identified on the blood vessels.[171v, 172v] This finding may suggest that the presence of GRP in a nerve at a given location does not necessarily indicate that the peptide is active at that site.[172v] Rather, the presence of the receptors and their distribution probably determines the actions of neurally released neuropeptides. The response pattern may even be more complex, since GRP may be co-stored and co-localized with other neuropeptides.[173f] The origin of the GRP-containing neurons is not yet clearly known.[172v]

The co-distribution of the GRP, CGRP, NKA and SP may suggest that GRP is localized in the trigeminal nociceptive sensorimotor type C nerve fibers.[172v] In the case of GRP localization to the sensory neurons, the activation of the sensory neuron axon reflex would lead to the release of GRP near the submucosal glands and the epithelium.[173g, 173h]. Moreover, the HPLC results indicate that GRP and human nasal mucosal GRP-immuno-reactive material co-elute, and that there is only a single peak of extractable GRP-immuno-reactive material.[172v]

[C] EFFECTS OF NEUROPEPTIDES ON THE NASAL MUCOSA

The neuropeptides have manifold effects on the human nasal mucosa and its parts.

(1) Substance P (SP)

The SP induces distinct vasodilatation and vascular permeability with plasma extravasation, and increases the secretion of the submucosal glands in the human nasal mucosa.[163j, 168g-168i, 168n, 170i, 172p, 173f-173h, 173s]

SP stimulates glycoconjugate secretion without serous cell secretion from the human nasal mucosa.[172p] SP does not affect the lactoferrin release.[172p] SP is capable of causing a contraction of smooth muscles in the human airways, however, to a distinctly lesser degree than NKA.[169l]

Substance P also stimulates, to a higher degree than NKA, ion transport in the airway epithelium, it contributes to the release of PGE_2 from the airway epithelial cells and increases the mucociliary clearance in the maxillary sinuses and in the airways.[168h]

SP increases the nasal blood flow, with less effect on the nasal airflow, suggesting an effect on the resistance vessels, such as arteriovenous anastomoses rather than on the capacitance vessels.[173j] This effect is mimicked by neural stimulation and by capsaicin, indicating that tachykinin release may be an important endogenous mechanism. The nasal challenge with SP in subjects with allergic rhinitis produces a limited reduction in nasal airflow.[173u]

(2) Neurokinin A (NKA)

The NKA induces constriction of the smooth muscles and also has vascular effects, but to a lesser degree than those caused by substance P.[172p] However, NKA participates in regulation of the vasomotor tone.[168g-168i, 173f-173h]

NKA also stimulates the lactoferrin release.[172p] NKA may also stimulate the ion transport in the airway epithelium to a slight degree and it may also participate in the increase in mucociliary clearance in the maxillary sinuses and in the airways.[168h]

(3) Neuropeptide Y (NPY)

The NPY can induce long-term contraction of the arterial smooth muscles this prolonged vasoconstriction is not affected either by alpha-1 or alpha-2 or beta-adrenergic blockade.[172r,172x,173,173e] NPY may play an important role in the regulation of the tissue blood flow, especially the blood flow into venous sinusoids.[172r] The increased blood flow leads to the filling of venous sinusoids, vascular congestion, swelling of the turbinates, and obstructed nasal airflow that is perceived as nasal congestion. NPY has several actions as a sympathetic neurotransmitter.[172x] It has direct stimulatory effects on the post-junctional end organs. These effects are slow in onset, but of long duration. NPY acts through two types of receptors: Y1, which are postjunctional and are linked to phosphatidyl-inositol hydrolysis, and Y2, which are prejunctional and are coupled to adenyl cyclase inhibition.[173a] NPY potentiates the post-junctional effects of norepinephrine.[172x] It may also bind to the presynaptic receptors to inhibit sympathetic neurotransmitter release and to prevent the depletion of transmitters.[172x] NPY also inhibits the post-ganglionic cholinergic transmission at an unresolved presynaptic site.[169p,172r] NPY may also antagonize the vasodilatory effects of the neuropeptides released from the sensory and parasympathetic nerves.[168h,168i,169l,172r]

The arteriolar vasoconstriction appears to be mediated by a combination of rapid norepinephrine effects, acting on the alpha receptors, and the slower NPY effects.[172x] NPY may therefore have potential as a topical mucosal vasoconstrictive agent.[172r,173e]

(4) Vasoactive intestinal peptide (VIP)

The VIP may play an important role in the regulation of the serous cell secretion in the human nasal mucosa.[172t] VIP augments the effects of cholinergic stimulation by causing selective enrichment of serous cell products in the nasal secretions.[172t] The activation of VIP receptors on serous cells of submucosal glands represents an additional component participating in the parasympathetic reflexes in the human nasal mucosa.[173d] VIP also exerts a potent vasodilator activity independently on the cholinergic and adrenergic receptors in the nasal mucosa. VIP seems to influence both the blood flow (arterioles) and the volume in the nasal mucosa(venous sinusoids).[172j,173b] Stimulation of the preganglionic cholinergic neurons in the nervus canalis pterygoidei is associated with activation of the nicotinic receptors and the release of VIP-like immuno-reactivity into the nasal venous effluent parallel to the atropine-resistant vasodilatation.[168t]

Increased release of VIP and other neurotransmitters by the parasympathetic mechanism may contribute to the vascular congestion and hypersecretion in the nose.[173d]

(5) Calcitonigene-related peptide (CGRP)

The CGRP is a potent vasodilator, which has long-lasting effects.[168g,168i, 172u,173f-173i] In human nasal tissue, CGRP binding sites are most dense on the arterial vessels.[172u] It is possible that CGRP may be the predominant mediator of the arterial vasodilatation in response to the stimulation of the sensory nerves in the nose and bronchi, and in this way it increases mucosal blood flow by acting on the resistance vessels. CGRP may also be an important mediator of the airway hyperemia.[168i] In

contrast, CGRP has no direct effect on the airway microvascular leak.[168i]

No direct effects of CGRP on the human nasal mucosal secretion have been demonstrated.[172u] Although CGRP does not appear to be chemotactic for eosinophils, its proteolytic fragments may contribute to the eosinophil tissue infiltration.[168i] CGRP also inhibits the proliferative response of T lymphocytes to mitogens and antagonizes the macrophage secretion and the ability of macrophages to activate the T lymphocytes.[168i]

CGRP immuno-reactive material has been demonstrated in the peripheral branched axons present in the walls of arterioles, arterial-venous anastomoses, venules, venous sinusoids, near the submuscular gland cells, myoepithelial cells, and in the epithelium.[168g,171v,173f,173h] In these locations the axons are thickened into the neurosecretory varicosities.[173c,173f,173g] The stimulation of the sensory nerves (mechanical and thermic stimuli, histamine etc.) induces depolarization, which results in the release of neurotransmitters such as CGRP and others from these varicosities.[172u,173f,173h] The combinations of the co-released neurotransmitters act upon local structures and induce vascular permeability, arteriolar vasodilatation and submucosal gland secretion.[172u, 173f,173g,173h] This local effector action of the peripheral sensory nerves constitutes the so-called "axon reflex response".[172u]

The increased secretion of human nasal submucosal glands is probably induced by other, co-located, neuropeptides, different from CGRP, e.g. by substance P,[168h,168i, 168t,170m,170n,170r,173f,173g] since no evidence has been provided for a role of CGRP in the nasal glandular secretion.[172u]

CGRP is probably also released during the allergic responses, because a nasal challenge with allergen, in atopic subjects, leads to a significant increase in the CGRP and somatostatin concentrations in the nasal lavage fluids, whereas the concentration of the substance P does not change.[173l]

(6) Gastrin-releasing peptide (GRP)

The "gastrin-releasing peptide" (GRP) is a potent stimulant of mucus secretion in human as well as in animal airways. GRP stimulates both the serous cell lactoferrin and the mucous glycoconjugate secretion from the human nasal mucosa.[172v] Lactoferrin is synthesized and secreted by the submucosal serous gland cells. The mucous glycoconjugates are complex mixtures of mucous glycoproteins, proteoglycans and other glycoconjugates that are derived from the epithelial goblet cells and submucosal serous and mucous gland cells.[172d,172v,173t]

GRP may stimulate secretion not only from the submucosal serous and mucous gland cells, but probably also from the epithelial goblet cells.[172v, 173s]

CGP added exogenously is also capable of stimulating the gland secretion "*in vitro*".[172v] On the other hand, the possible effects of GRP on the blood vessels in the human nasal mucosa, such as vasomotor activity or vascular permeability, have not yet been demonstrated.[172v]

Based upon these facts, GRP appears to be a neurotransmitter which stimulates the secretion from the mucous and serous cells of the submucosal glands of the human nasal mucosa.[172v]

(7) Other neuropeptides

There is a dearth of information concerning the possible presence of other neuropeptides in the human nasal mucosa and their effects on this tissue.

[D] EFFECTS OF NEUROPEPTIDES ON THE PARTICULAR CELL TYPES

The neuropeptides (NP) have also demonstrated a variety of effects on the various cell types, such as mast cells, basophils, eosinophils, neutrophils, lymphocytes, monocytes, macrophages and epithelial cells, particularly on their metabolism and functions, some of which have a stimulatory character, whereas others have an inhibitory and protective character.[163u,168h-168j,169m,170w,171r,173s]

(1) *Mast cells.* Some of the neuropeptides belong to the mast cell secretagogues, causing either the semi-selective secretion of histamine and/or other mast cell mediators, [162z,163u,168f,169,169b,174,174a-174d] or the mast cell degranulation resulting in the release of histamine and/or other mediators.[162z,163u,168f,168x, 169m,170w,171p,174b]

However, there are differences and high variances, not only among the particular neuropeptides with respect to their effects on the mast cells,[162z,163u,168f,168h-168k,168x, 169b,169w,170j,173j,173s,174a] but also in the ability of the particular mast cell types to degranulate in response to an appropriate neuropeptide.[162z,163u, 168f,168k,170w,173s,174a]

The different ability of various mast cell populations to degranulate in response to the particular neuropeptides has been reported in the case of the mast cells from various animals,[162z,168a,168f-168j,169,169b,169k,170,170i,171n,171t,174a, 174d] between animal and human mast cells,[162z,163u,164y,168f,168h-168l,168z,169,169a,169b,169k,169l,169x,169y,170f, 170g,170r,170s,170u,170v,172x,173i,173k, 173m,173s,174a,174c,174e] and even among the human mast cells located in different tissues (skin, nasal mucosa, bronchial and alveolar tissue).[162z,163u,164y,168e,168f,168h,168i,168m-168p,168z,169b,169x,170,170a,170k,170m,172s,173k,173n, 173p-173s,174c-174h]

The detailed descriptions of the different responses of particular mast cell populations of various origins to individual neuropeptides are reported elsewhere.[162z,163u,164c,168f, 169b,170w,173k,174d]

The following neuropeptides have been reported to be capable of inducing a degranulation of the human skin mast cell: (a) substance P;[162z,168a,168d,168f,168l,168n,168w,169b, 174a-174c,174h,174f] (b) Neurokinin A;[174c] (c) Neurokinin B;[174c] (d) Neurotensin;[169b] (e) Somatostatin;[168d,169b,170] (f) Gastrin-releasing peptide;[169b] (g) Vasoactive intestinal peptide.[168d] In the case of the human lung mast cells, some investigators have reported the ability of substance P to cause their degranulation,[162z,174f,174h] while others did not observe such effects.[174e] However, the latter investigators have used removed human lung tissue containing carcinoma cells, which cannot be considered as suitable material for the experiments with neuropeptides from our point of view, with regard to various immunological processes and changes accompanying malignancy. Other investigators have observed the inhibitory effects of vasoactive intestinal peptide (VIP) on the human lung cells.[168l] In the human nasal mucosa, only the substance P has been reported to induce degranulation of the tissue mast cells.[169b]

Generally, there is a dearth of information concerning the effects of particular neuropeptides on the tissue mast cells in the human lung as well as in the nasal mucosa, especially with respect to their stimulation and degranulation.

(2) *Basophils.* Some of the neuropeptides have also been found to be capable of inducing the degranulation and histamine release from the human basophils, such as substance P,[162z,174f,174h] and somatostatin-14 and -18.[169z]

(3) *Eosinophils.* Substance P (SP) has been reported of inducing a degranulation of eosinophils in guinea pigs.[168h,174i] In mice, the subcutaneons injection of SP has led to the degranulation of the mast cells, followed by the forming of distinct eosinophil and neutrophil infiltrates.[169w] The injection of "calcitonin gene-related peptide (CGRP) into the human skin has induced eosinophil infiltration.[168i]

(4) *Neutrophils.* Besides the already mentioned forming of eosinophil and neutrophil infiltrates after the subcutaneous injection in the mouse, the substance P has also been found to increase the adherence of human neutrophils to the vascular endothelium "*in vitro*".[174j]

(5) *Lymphocytes.* In the mouse, CGRP inhibits the proliferative response of T-lymphocytes to mitogens and moreover the specific receptors for CGRP have been demonstrated on these cells.[174k] In contrast, the substance P may stimulate the human T-lymphocytes,[168u,168x,169u] whereas somatostatin and vasoactive intestinal peptide (VIP) have been shown to inhibit various functions not only of the murine T lymphocytes,[174l] but also of the human T lymphocytes, especially mitogen-induced proliferation, release of cytokines, a.o. interleukin-2 (IL-2) and natural killer function.[168f,168u,171l,174m]

(6) *Monocytes.* Tachykinins, especially substance P, may also be capable of stimulating the human monocytes to release some of the cytokines, such as interleukin 6 (IL-6).[168h,168w,169h] VIP has been shown to inhibit the respiratory burst in human monocytes and probably also other functions,[174p] since the VIP receptors have been demonstrated on their surface.[168u,169i,174n] Somatostatin and CGRP are also presumed to affect the function of human monocytes, probably by participation in their stimulation, since the somatostatin receptors have been found on the surface of these cells.[168x,171l] However, these effects have not yet been definitely confirmed.[168f]

(7) *Macrophages.* Tachykinins, especially substance P activate the guinea-pig alveolar macrophages upon involvement of NK2 and NK1 receptors.[168h,174r] Substance P stimulates various functions of the guinea-pig macrophages, such as generation of thromboxanes, synthesis and release of lysosomal enzymes, release of leukotrine C_4 and prostaglandine E_2, cytokines etc.[174s] VIP inhibits phagocytosis and superoxide radical production by rat alveolar macrophages, a process which is associated with stimulated cyclic AMP production.[168u] CGRP also inhibits the macrophage secretion and their capacity to activate the T lymphocytes.[168h, 174t]

CGRP has been shown to prevent the the H_2O_2 production by human macrophages activated by INF-gamma.[174t]

In contrast to the animal studies, there is a dearth of information concerning the effects of particular neuropeptides on the human macrophages.[131c,165v, 168h,168i,174u]

(8) *Epithelial cells, goblet cells, epithelium.* VIP is a potent stimulant of chloride ion transport and therefore also water secretion in the tracheal epithelium in the dog, suggesting that VIP may also regulate the mucociliary clearance,[168h] The high density of VIP-receptors on the epithelial cells of the human airways suggest that VIP may

regulate the ion transport and other epithelial functions also in the human airways and in the nasal mucosa. [170g, 172s] Substance P, and to a lesser degree also neurokinin A, are able to stimulate ion transport in the canine tracheal epithelium, indicating that NK1 receptors may be involved in this process.[174v] SP also stimulates the release of PGE_2 and possibly "epithelium-derived relaxant factor (Ep DRF) from the airway epithelial cells. [168h] The Ep DRF suppresses the bronchoconstrictor effects of various spasmogens, also including the tachykinins.[168h] Moreover, the tachykinins also increase the mucociliary clearance in the maxillary sinus and in the airways.[168h] Gastrin releasing peptide (GPR)/Bombesin is an important factor in the epithelial growth, since its binding sites are present on the epithelial cells and submucosal glands of the human bronchial as well as the nasal epithelium.[168i, 172v, 172z, 173r]

[E] NEUROGENIC CONTROL OF THE HUMAN NASAL MUCOSA

The neural control of the human nasal secretion, edema of the nasal mucosa and nasal perception can be summarized as follows. Trigeminal type C nociceptive sensory nerve fibers contain co-localized substance P (SP), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP). [168t, 171v, 173f-173h.]

The stimuli and factors, such as increased temperature, mechanical irritation, cigarette smoke and some chemical compounds such as histamine, bradykinin, capsaicin, serotonin, formaldehyde, nicotine, hydrogen and potassium ions lead to the neural depolarization.[173g, 173h] Sensations ranging from prickling itch to severe burning pain are registered centrally. No single subset of fibers or neurotransmitters may play a role in a single sensation. Central appreciation of pain is accompanied by the parasympathetic reflexes within seconds. Also the axon responses may be co-stimulated.[168t, 173g] Nociceptive, neuropeptide containing fibers densely innervate arterial and venous vessels. They are also present in the submucosal gland acini, in the interstitium, and extend up to the epithelium.[171v, 172p,172u] These nerves play a major role in the control of the nasal secretions. They form the afferent limb of the central, bilateral, parasympathetic, cholinergic and secretory reflexes.[173g,173h.] Substance P fibers may also contact the parasympathetic postganglionic cell bodies.[173g,173h.] This connection may produce a direct reflex between the afferent nociceptive fibers and the parasympathetic neurons. Upon stimulation, the nociceptive C fibers may also locally release their combinations of neuropeptides by axon reflex mechanism in the neighbourhood of the blood vessels, glands and other structures to induce the vasodilation and gland secretion.[173g,173h] In the human nasal mucosa, substance P may induce distinct vascular permeability and gland secretion, NKA may have little or no effect, and CGRP may induce arteriolar dilation and increased blood flow.[172p,172u.] A variety of antagonistic or synergistic effects may occur, since the co-localized neuropeptides are released together.[168t,169s]

The parasympathetic nerve synapses are localized in the pterygopalatine (sphenopalatine)ganglion. [171x,171y] These neurons release acetylcholine, VIP and PHM.[168h,168i,172t] The VIP containing nerves are present near glands and in the walls of the blood vessels. [171u,172t.] Parasympathetic nerves control the glandular secretion.[172a,172i,172t] VIP stimulates secretion of the serous cells to a higher degree, than that of the mucous cells from the human nasal mucosal explants.[172t.] VIP has also vasodilatory effects.[172t] The

role of VIP in parasympathetic reflexes in the human nasal mucosa is limited, as atropine treatment inhibits the reflex-mediated secretion "*in vivo*".[174w]

The sympathetic neurons contain the vasoconstrictors norepinephrine and neuropeptide Y (NPY).[171t,172x] NPY fibers are present in the walls of arterioles, arteriovenous anastomoses and other vessels.[168y,169p,172r] NPY binding sites are located on the arterioles and arteriovenous anastomoses, which in fact may support the important role of NPY in the regulation of the vascular tone. [171v,172r] Sympathetic impulses contribute to the nasal cycle by a periodic increase in the blood volume in the local mucosal capillary network, combined with unilateral nasal obstruction accompanied by the vascular collapse, macromolecule secretion, and nasal patency.[172a,172f] Removal of the sympathetic innervation, local anesthetics, and vasoconstriction disrupt the nasal cycle. Release of NPY from the sympathetic nerve endings near the arterial vessels usually leads to a long-lasting vasoconstriction with collapse of the venous sinusoids.[172r] These vascular effects in combination with passive elastic recoil forces may result in the thinning of the nasal mucosa accompanied by a transudation of the interstitial fluid into the nasal cavity.[171v,173s] The mucosal thinning would increase the nasal patency and reduce the airflow obstruction.

The subsequent parasympathetic input and a decay in the NPY-induced vasoconstriction would lead to arterial vasodilation, filling of the venous structures, plasma extravasation and glandular secretion. Increased vascular filling would thicken the nasal mucosa and in this way lead to a nasal obstruction, [171v] The extravasation of the plasma water and proteins, glandular secretion, and the increased vascular permeability would then replenish the surface macromolecules and lead to increased protein secretion being observed during the obstructive phase of the nasal cycle. The goblet cells of the nasal epithelium may function continuously, but they may also respond to various stimuli. [172l,173s] Such coordinated cycling of the sympathetic and parasympathetic discharges could lead to the synchronous, bilateral nasal cycle.[173s]

[F] NASAL PROVOCATION TESTS (NPT) WITH NEUROPEPTIDES AND RELEVANT/RELATED COMPOUNDS AND AGENTS

(1) Neuropeptides and capsaicin

The effects of some neuropeptides and relevant compounds, such as capsaicin, on the human nasal mucosa have also been investigated by means of the nasal provocation tests in control subjects and patients with allergic rhinitis.[171,173u,175,175a-175c] However, the papers concerning this topic are not numerous.

Miadonna and colleagues,[175b] studying the effects of substance P after intranasal challenge in 5 patients with pollen-related rhinitis and 5 control subjects, did not record any clinical symptoms or changes of the nasal resistance in any of the subjects tested, up to 20 minutes after the challenge. In contrast, all these subjects, the patients as well as the control subjects, developed a wheal and flare reaction after the intradermal injection with substance P. The investigators have explained the different responses of the skin and of the nasal mucosa by possible heterogeneity of the mast cells located at these different sites.

Petersson and co-workers,[175a] have also performed the nasal challenge with substance P in 14 healthy volunteers. These investigators have also been unable to record any response of the human nasal mucosa to SP, or even increased nasal secretion. In contrast, Devillier and colleagues,[173u] investigating the effects of substance P on the nasal mucosa in 17 rhinitis patients and 14 control subjects, have recorded a significant increase in the nasal airway resistance (NAR) after the intranasal challenge with substance P in rhinitis patients, but not in the control subjects. The increase in NAR was dose-dependent and on a molar basis.Substance P was 375-500-fold more potent than methacholine hydrochloride. The pretreatment with oxytropium bromide in a dose of 200 mcg topically has prevented the nasal response to methacholine bromide but not that caused by substance P. They have concluded that substance P is absorbed across the human nasal mucosa and causes local vasodilatation; - substance P is approximately 500-fold more potent than methacholine bromide in increasing the NAR; - substance P causes greater increase in NAR in rhinitis than in control subjects; and finally that - the increase in NAR caused by substance P is not mediated by the postganglionic parasympathetic mechanisms.

Geppetti and co-workers,[173x] performing the nasal challenge with capsaicin, substance P (SP) and CGRP in man, have not observed any effects of SP or CGRP on the human nasal mucosa, especially on its secretory function. However, they have recorded a dose-dependent increase in the nasal secretion volume by capsaicin, in doses of 1-100 mcg. Capsaicin seemed to be 200-times more potent than methacholine. Additionally, capsaicin also induced sneezing, intense nasal burning and pain in the subjects studied. Moreover, they have also reported that ipratropium bromide, indomethacin, dexchlorpheniramine and lidocaine did not alter the nasal secretion volume. They have proposed that capsaicin may induce the secretion in the human nasal mucosa primarily through the local release of neuropeptides from the peripheral terminals of the primary sensory neurons, whereas they have also reported that the local application of the SP and CGRP did not reveal any secretory response.

Stjärne and colleagues[173j], have carried out nasal challenges with capsaicin, nicotine and methacholine bromide in three groups of subjects: healthy subjects, patients with rhinitis due to the non-specific hyperreactivity [NS-H] to a moderate degree (sneezing, hypersecretion and/or nasal obstruction)and patients with a very pronounced non-specific hyperreactivity, all of them being without atopy or allergic disease history. Capsaicin applied unilaterally in doses of 3.3×10^{-6} to 3.3×10^{-3} M, in a volume of 50 µl of saline, has induced the dose-dependent increase in the nasal secretion. The control subjects and patients with mild non-specific hyperreactivity responded similarily, whereas patients with a pronounced NS-H responded with a larger nasal secretion volume. The ipsilateral capsaicin effects could be blocked by simultaneous administration of both intramuscular atropine plus intranasal ipratropium bromide and intranasal lignocaine chloride plus naphazoline chloride.

These results suggest that the secretory effect of capsaicin in the human nasal mucosa is realized through a parasympathetic reflex arc with an afferent limb (sensory nerve depolarization) and an efferent limb (cholinergic discharge).[173s]

Unfortunately, the role and effects of particular neuropeptides, and the neurons, fibers and nerves expressing them, on and in the physiologic and still more in the pathologic processes in the human nasal mucosa have not yet been satisfactorily clarified. [168h,168i,169m,171r,171v,173s,175c]

Furthermore, there is a dearth of information concerning the possible role of the nervous system and particular neuropeptides in the allergic reactions (ALL) and in the non-specific hyperreactivity reactions (NS-H), in the human nasal mucosa. [35e,171r, 171v,173s,175c]

(2) Relevant/related agents

However, some evidence has been found of the possibly predominant role of neuropeptides in the nonspecific hyperreactivity mechanisms in the human nasal mucosa, rather than in the nasal allergy.[35e,173s,175d-175g,175v] This evidence has been gathered from the nasal challenge studies with histamine,[35e,97g,175c,175f-175t] methacholines, [35e,97g,173u,173x,175g,175j-175l,175p-175s,175u] bradykinin,[85d,169t,175s,175w,175y,175z,176, 176a-176c] serotonin,[176c] and cold air.[175e, 175m,175x]

(a) Histamine

Histamine applied onto the nasal mucosa of the patients with NS-H in the nose may induce a dose-dependent nasal response consisting predominantly of nasal secretions, itching, sneezing, and to a lesser degree nasal obstruction due to its direct action on the mucosal vascular bed.[173s,175k,175p-175s] The effects of histamine on the nasal secretion and its composition have been studied by several investigators.[97g,97h,175l,175p,175t]

Histamine stimulation of the human nasal mucosa has produced an increase in ipsilateral secretions of the total protein, albumin and non-secretory IgA.[175p] The ratio albumin-total protein is an indicator of vascular permeability.[173s] Unilateral histamine provocation did not induce albumin secretion from the contralateral nostril.[175p]

Histamine can also produce limited contralateral protein secretions, containing elevated concentrations of the glandular sIgA.[175p] Topical pretreatment with an antihistamine (H_1-receptor antagonist) has completely abrogated the ipsilateral nasal secretory response to histamine.[175m,175p,175t] Topical pretreatment with atropine (a muscarinic receptor antagonist) did not affect significantly either the ipsilateral nasal secretions or the capacity of histamine to stimulate the contralateral secretions.[173s,175p,175t] These results suggest that histamine may stimulate the nasal secretions through two mechanisms; by a direct action that increases the extravasation of the plasma proteins from the capillaries in the nasal mucosa and by an indirect reflex mechanism that stimulates the glandular secretions.[173s,174w, 175k, 175n,175t]

These data also indicate that histamine may directly induce ipsilateral vascular permeability and indirectly stimulate the ipsilateral gland secretions and the contralateral gland secretions.[173s,175p] The vascular permeability may predominantly be a result of the direct action of histamine on the vascular H_1 receptors.[175n] The sensory nerves may be stimulated by H1 receptor activation and the neuropeptides which may then be released, partly due to the direct H1-receptor stimulating activity and partly via the axon response mechanism, could contribute to the induction of vascular permeabi-

lity.[173s,174w, 175g,175k,175n,175p,175r,175t] However, the axon responses have not been detected with respect to the overwhelming vascular effects of the H_1-receptor stimulation in the human nasal mucosa.[173s,175n]

The H_1-receptors probably mediate the stimulation of the sensory nerves resulting in the bilateral parasympathetic reflexes.[173s,175f,175h,175n,175t] The central, cholinergic reflexes probably dominate in the mediation of the glandular secretions, since histamine did not directly induce significant gland secretions in the human nasal mucosa "*in vitro*".[173s,175k]

On the other hand, the role of H_1-receptors in the vascular effects of histamine on the human nasal mucosa is probably not exclusive with respect to the observations of other investigators,[175i] who reported the significant protective effects of the H_1-receptor antagonist on the histamine-induced nasal symptoms and nasal secretions (increased protein and albumin concentrations), but with no effects on the blood flow or other micro-circulatory aspects of the nasal mucosa.[175i] They have concluded that the vascular effects of histamine on the human nasal mucosa are not mediated exclusively by the H_1-receptors. This observation might support the additional direct participation of the sensory nerves, besides the already mentioned stimulation through the H_1-receptors, in the vascular effects of histamine on the nasal mucosa. Furthermore, the role of some neuropeptides in mediating the effects of histamine on the human nasal mucosa may be supported by the observation of Majchel and colleagues,[175f] who reported a histamine-induced nasal response without generation of prostaglandins and/or leukotrienes in the nasal lavage fluid. These results may indicate that histamine does not necessarily influence the mucosal mast cells and that histamine may probably act through the stimulation of some neuropeptides, especially those which express direct vascular and glandular effects in the human nasal mucosa, but do not affect the mucosal mast cells.

(b) Methacholine

Another compound, frequently used for the demonstration of the "non-specific hyperreactivity" of the human nasal mucosa, is methacholine.[35d,35e,97g,173s,173u,175d, 175g,175j-175l,175n-175u] Methacholine induces, through the parasympathetic (= cholinergic) stimulation, a dose-dependent increase in the human nasal secretion, and sneezing, however, to a lesser degree than histamine.[173u,173x, 175d] The capability of methacholine to induce the nasal obstruction in humans seems to be a controversial issue. Some investigators did not observe any nasal obstruction in humans due to the methacholine challenge,[175g,175s] whereas others have reported the appearance of a limited nasal obstruction caused by methacholine.[175j,175r] Our results are somewhat contradictory. We have regularly observed a nasal obstruction due to the nasal challenge with methacholine bromide and/or chloride, in lower doses than histamine,[35a-35c] and even in patients with rhinitis complaints without any participation of an allergy component and with non-altered reactivity to histamine.[35a-35c] Our results are in agreement with other investigators' observations.[173u,175s]

Methacholine directly stimulates the muscarinic receptors.[175k] Methacholine has been shown to increase significantly not only the total protein and albumin, but also the

lysozyme and lactoferrin concentrations and their ratio to the total protein, in human nasal secretions. These methacholine effects can be inhibited by atropine (= a muscarinic receptor-antagonist),[97g,173d,173s, 175k] or by ipratropium bromide.[175r]

These studies suggest that stimulation of the muscarinic receptors on the submucosal nasal glands induces glandular secretion both from the serous and from the mucous cells of the submucosal glands and possibly also from the epithelial and/or goblet cells.[173s] The vascular processes, such as vascular permeability were not significantly affected.[173s] The binding sites of the muscarinic receptors of type M1 and M3 have been demonstrated on the submucosal glands and epithelium of the human nasal mucosa.[172g]

(c) Bradykinin

Another compound which is frequently used (mainly for research purposes), for the non-allergic stimulation of the nasal mucosa is bradykinin.[85d,169t,175w,175y, 175z,176,176a,176b] Bradykinin applied topically on the human nasal mucosa causes an increase in the production of nasal secretions containing a higher concentration of proteins including albumin,[175w] and also conjunctival injection, discharge and throat dryness and itching.[175y,176] The latter symptoms may probably be caused by bradykinin stimulation of the sensory nerves.[176] Bradykinin may act upon receptors on the smooth muscles of the nasal mucosa capillaries (arterioles, veins, venous sinusoids) to induce their vasodilatation followed by the increase in vascular permeability, since the binding sites for bradykinin have been identified at these sites.[85d] "*In vitro*", bradykinin stimulated glandular secretion from the cultured human nasal tissue.[173s] This stimulation was inhibited by the bradykinin antagonists and inhibitors of the arachidonic acid derivatives.[173s] Stimulation of the sensory nerves by bradykinin can lead to the local release of substance P and other sensory neuropeptides and it also induces the cholinergic reflexes.[175z,176b] The above mentioned data suggest that bradykinin may act upon the vascular walls to induce the capillary dilatation and vascular permeability, that bradykinin may stimulate the sensory nerves, a process leading to the parasympathetic reflexes, and finally that bradykinin may probably stimulate the production and release of some arachidonic acid metabolites which then affect the mucosal gland secretion. However, there is still a dearth of knowledge concerning the latter part of this hypothesis.[173s,175w]

(d) Serotonin

Sometimes serotonin is also used for confirmation of the "non-specific hyperreactivity" component in the human nasal mucosa. Serotonin, stimulating the sensory nerves,[176c] induces increased nasal secretion, which is dose-dependent. The sensory nerves stimulated by serotonin lead, through the axon reflex, to an increased release of substance P, which concentrations in the nasal secretions also significantly increase in a dose-dependent manner.[173s] Interestingly, the nasal secretion induced by serotonin can be inhibited by atropine, which effect may indicate the participation of cholinergic reflexes and mechanisms.[173s]

(e) Cold air

Finally, the challenge of the human nasal mucosa by cold air also causes a nasal res-

ponse. In the development of this nasal response, besides the already reported direct effects of cold air on the mucosal mast cells and/or basophils, the stimulated mucosal nervous system and neuropeptides may probably also participate.[175e, 175m,175x] The same suggestion may also be applicable for the human nasal response to challenge with the hyperosmolar environmental factors.[175e]

[G] STIMULATION OF SYMPATHETIC AND PARASYMPATHETIC NERVES

Stimulation of sympathetic (adrenergic) nerve fibers leads to the release of various neuropeptides which then affect alpha - as well as beta - adrenergic receptors in the human nasal mucosa.[173s] These receptors participate in the regulation of the blood flow in the nasal mucosa.[173s]

Stimulation of the parasympathetic (cholinergic) nerves leads to the activation of the muscarinic receptors located predominantly on the nasal mucosal glands.[171r, 172g] The muscarinic receptor stimulation by methacholine induces significant glandular secretion both "*in vitro*" and "*in vivo*", confirming the hypothesis that the muscarinic receptors are stimulated directly.[175k] Histamine induces predominantly vascular permeability "*in vivo*", but causes some glandular secretion as well.[175k] However, "*in vitro*", histamine has no effect on the glandular secretion, which may suggest that histamine acts predominantly on the nasal vascular bed and only affects the glandular secretion through the reflex actions.[175k] On the other hand, it has also been demonstrated that glandular secretion is directly stimulated by alpha-adrenergic and cholinergic agonists, but not by beta-adrenergic agonists. [175k.]

Stimulation of the H_1 receptors on sensory nerves in the human nasal mucosa produces a prominent reflex-mediated sneezing, itching and glandular hypersecretion.[175t] Moreover, some investigators have demonstrated that the reflex-stimulated glandular hypersecretion also involves a cholinergic stimulation of the submucosal glands.[174w]

[H] NERVOUS SYSTEM, NEUROPEPTIDES AND THE "NON-SPECIFIC HYPERREACTIVITY" OF THE HUMAN NASAL MUCOSA

The above discussed results and facts are highly suggestive of the important role of the nervous system and neuropeptides in mediating the so-called "non-specific hyperreactivity" of the human nasal mucosa.[3-5,35a-35c]

The non-specific agents, of mechanical, physical or chemical type, stimulate the mucosal sensory nerves, a process which then leads to the development of the reflexes, such as axon reflex and parasympathetic reflexes, resulting in the rapid release of various neuropeptides.[173s]

The neuropeptides may then affect: (a) the mucosal mast cells and/or basophils, inducing the mediator secretion directly or through the previous degranulation of these cells,[162z,169m,171v,174d,174e] and/or (b) the mucosal capillary network inducing the vasodilatation and vascular permeability,[171v,173s] and/or (c) the mucosal glands, inducing the increased secretion.[171v,173s,173x,175k]

However, no detailed data are available as yet to demonstrate the ratio of participation of the individual reflexes, their sequence or simultaneousness, and the role of particular neuropeptides in the mechanism(s) underlying the "non-specific hyperreactivity" (NS-H). The suggested role of neuropeptides in the NS-H may be supported by some of our findings and results.[35a-35c,72b]

The nasal response to the challenge with histamine, and/or methacholines, in patients with rhinitis due to the non-specific hyperreactivity component, develops very rapidly.[35a,72b] It appears within seconds and disappears within maximally 30 minutes.[35a,72b] In contrast, the immediate nasal response to the allergen challenge (INR), due to the IgE-antigen interaction followed by release of various mediators from mast cells and/or basophils, usually demonstrates much slower development and a longer time-course (the onset within 10, maximum within 20-45 and resolving within 90 minutes after the allergen challenge).[35c,72b]

The nasal response to the non-specific agents, respectively to histamine or methacholine, is usually characterized by rapid pronounced hypersecretion and sneezing, whereas the nasal obstruction appears to a slighter degree.[35a,35c,35e] In contrast, the INR to allergen challenge is accompanied by edema of the nasal mucosa producing the nasal obstruction, whereas the other nasal symptoms, such as hypersecretion, sneezing, and itching vary to different degrees and ratios.[35b,35c,72b] The aspect of the nasal mucosa is also different. During the NS-H response the nasal mucosa is usually hyperaemic and covered by abundant transparent watery secretions with a low viscosity and a low density weight containing only very few cells, particularly epithelial cells and sporadically eosinophils and/or (rarely) neutrophils. In contrast, during the INR to allergen challenge, the nasal mucosa is more violaceous and covered by a moderate amount of less transparent and tougher nasal secretions, containing various cell types, predominantly eosinophils, neutrophils and epithelial cells.[35b, 35c, 72b.]

Alpha-sympathomimetics (e.g. xylomethazoline hydrochloride) are capable of partially inhibiting the human nasal response due to the NS-H, whereas they are fully ineffective in the case of the INR.[72b]

We have also observed differences with respect to the various kinds of the non-specific agents, such as chemical, thermal or mechanical irritation of the nasal mucosa in patients with different participation of the NS-H component and the allergy component in their nasal complaints.[3-5] Moreover, the existence of the nasal response types, different from the INR, such as late and delayed nasal responses, would not support a primary role of the neuropeptides in the hypersensitivity mechanisms in the human nasal mucosa.[35c]

Regarding the above discussed facts, mechanisms and hypotheses, the secretion of some factors as well as the degranulation of the basophils and mast cells followed by release of their mediators and other factors, and the further steps induced by them, may follow different pathways and modifications.[4,34,36,41b,42-44, 48d, 51,51a,51b,82,83,85b,85c, 94,96e,96f,128a,131a-131c,131e,136a,136b,156,156i,156m,156t,162d,162m,162z,163u, 164a-164c,164e, 164h, 164r,164u,164w,164y,165b, 165d,165e,165g,166c,171v, 173s,174,174a,174g,176d-176y,177,177a-177z,178y, 178z]

7. CONCLUSIONS AND PERSPECTIVES

Should these mechanisms, or at least some of them, be confirmed later, then the sig-

nificance of the basophil and/or the mast cell for the late reactions will evidently increase.[4,11c,25,35e,41b,42,43,54,72,72a,72c,97f,131a,164n,164r,164w,165g,166m-166t,177g] Such a role of basophils and/or mast cells will be a very important contribution to the understanding not only of the mechanisms underlying the late type reactions but also of the pharmacologic modulation and control of these reactions by various drugs, and of the pharmacologic effects of particular drugs especially those of disodium cromoglycate, corticosteroids and nedocromil sodium.[11,12,14, 35e,40c-40f,41b,48d,72a,97b,97c,121c,121d, 123,124,124a-124d,125, 125a-125e,176a,176j,176r,176u]

These pathways and mechanisms would then probably also contribute to the explanation of why increased serum concentrations of IgG sub-classes but no positive specific IgE antibodies have been found in most of the patients suffering from allergic rhinitis due predominantly to the LNR, or why no specific IgE but increased IgG and IgG sub-classes have been found in the patient's serum during the positive LNR. [2,41b,86,90]

Nevertheless, our findings of increased serum concentration of total IgG antibodies and changes in the particular IgG sub-classes during the large number of LAR[64,65] and during certain cases of LNR[41b] (while the positive specific IgE antibodies in the serum were found in only a small number of LARs and LNRs), might suggest the possible involvement of IgG antibodies in the LAR and probably also in LNR. From this point of view our results are in agreement with the findings and conclusions of Pepys and co-workers[161c] and other authors as well as with results of our previous studies.[12,16,61,68a, 68e,68f,72b,121f,176k]

The suggested role of the IgG antibodies, which might activate the basophils and/or probably mast cells either directly, e.g. IgG interacting with an antigen and forming the IgG-antigen complexes binding then to the medium-affinity FcγR surface receptors, [128b,131e,132a,156,156c,161h,161l,162m,164,164a-164c,165a,165b,165d ,165f] or indirectly through the alternative mechanisms discussed above, would also be helpful in explaining the findings of increased IgG antibodies in the serum of some patients with bronchial asthma and developing an immediate asthmatic response to allergen challenge, described by some investigators[130a,131,161r] as well as by us,[25a,63-65,68e,68f,69] and subjects with allergic rhinitis, developing an immediate nasal response.[2,4,12-14,41b] Such mechanisms would also explain the results of our previous studies, in which in only 30% of the patients developing an immediate asthmatic response[68e,68f,69,176k] and in only 24% of subjects demonstrating an immediate nasal response to allergen challenge,[2,9,41b] positive specific IgE antibodies to the same allergen (positive RAST) have also been found.

B. "LNR" AND "IMMEDIATE HYPERSENSITIVITY" [IH]

1. DEFINITION OF "LNR" AND "IH"

The classically understood immediate hypersensitivity (Type I allergy) is mediated by IgE antibodies and the mast cells and/or basophils are considered to play the main role in these mechanisms.[1,2,6,36,85,136b,145,162m]

Moreover, various investigators have assumed the involvement of the "immediate hypersensitivity", including the IgE antibodies, mast cells and/or basophils, also in the "late type reactions", such as late skin response (LSR),[177,177a-177c,177i,178] late asthmatic

response (LAR)[42-44,51,51a,51d,96e,96f,162d,176n,176p,177d-177f,177j,177r,177t,178a] and late nasal response (LNR)[33,44,97s,97u,176s, 177g,177h] This assumption has been based predominantly on the earlier results of Solley and co-workers,[177,177i] Dolovich and colleagues[177j] and de Shazo and co-workers,[177k,178c] who have studied the late skin response and suggested the pivotal role of the IgE antibodies and the mast cells in both the immediate and the late skin response. In contrast, no similar changes have been observed either in the bronchial mucosal membrane during the LAR[162n,163k,163y] or in the nasal mucosa during the LNR.[41i,96,97,97f,97l,97n,97u] Furthermore, other investigators, studying the skin biopsy during the allergen-induced late phase cutaneous reactions, have described changes differing distinctly from those reported by the above referred authors.[177a]

There is, however, no unequivocal evidence that the late reactions on the skin can be fully comparable with those in the bronchial tree or in the nasal mucosa.[51a,65,66,72b] Moreover, there is some evidence against such a comparison.[11c,14b,41f,41i,45,46,51a,61-68,68a-68g,71,72,72a-72c,96g,161i,162b]

Furthermore, despite a certain relationship and correlation between the skin tests and the skin response on the one hand and either the bronchial allergy and the bronchial response or the nasal allergy and the nasal response on the other hand, the results generated on the skin cannot be applied to the bronchial tree or to the nasal mucosa without limitations.[3,4,12,41b,46,51a,61,65,66,68,68a,68e,69] The limitations are determined not only by the distinct anatomic, pathophysiologic and immunologic differences among these organs, but also by the differences in their response patterns.[51a,65,66,69,131a,162f,177g,178b]

In our previous studies the possible correlation between the late skin response (LSR) and either late nasal response (LNR)[2,7,12,16,41a-41d,41i,72,72a-72c] or late asthmatic response (LAR)[61,64-68,68a-68g,121e,121f] as well as between the immediate skin response (ISR) and either immediate nasal response (INR)[2,3,9,11,11d,11e,41i,71,72a,72b] or immediate asthmatic response (IAR)[63,69,70,176k] has been analyzed. The positive ISR has been found in 70% of INR cases, whereas the positive LSR in 65% of LNR. The positive ISR has been recorded in 68% of IAR cases, while the positive LSR in only 60% of LAR cases. However, none of these correlations has achieved statistical significance. The histologic findings in the nasal mucosa biopsies, having been performed by us during the basic types of the nasal response to allergen challenge,[96,97,97a] among others during the LNR[96,97], represented principally different processes. Skin biopsies taken from the site of the isolated immediate or isolated late skin reaction to inhalant allergens, in other subjects, had demonstrated changes which were not comparable with any of the findings in the nasal mucosa (unpublished data). With respect to our data, the comparability and compatibility of the late skin reactions with the late asthmatic or late nasal responses should be seriously doubted, if not excluded. Furthermore, our results would not support the predictability value of the LSR for the LAR or LNR.

The evidence against the presumed main role of the classical immediate hypersensitivity mechanism in the late type reactions, including LAR and LNR, is, nevertheless, growing.[11c-11h,11j,12,16,25a,40c-40f,41a,41c,41f,41i,45,51a-51c,61-68, 68a-68g,70,71,72,72a, 72b,92,95a,96,96g,96h,97,97a,121f,124a-124d,131,131a,131f,133,136d,148,161c,162b,162t,163k,163s, 168h,168i,177g,178b-178h]

Atkins[45] has concluded that responsibility of IgE-mast cell interaction for the late-phase bronchial responses seems unlikely. Walsh et al[148] have demonstrated that neutrophils and eosinophils were able to bind only the IgG antibodies but not the IgE antibodies. Moreover, this observation partly contradicts the proposed action of the IgE antibodies on neutrophils or eosinophils.[163b] Other investigators did not record any correlation between the antigen-specific IgE and the late asthmatic response.[61-66,68,96g, 162b]

Lemanske and Kaliner[51a] as well as Sheth and Lemanske[51d] have concluded that "taken together, these observations suggest that immediate and dual responses are initiated immunologically by the various components involved in immediate hypersensitivity reactions (IgE, mast cells/basophils, mediators); the mechanisms underlying the isolated late response to antigen challenge are unknown but may involve a variety of immunologic pathways including immediate hypersensitivity, Arthus or delayed hypersensitivity reactions."

2. IgE ANTIBODIES

[A] "IgE" AND "IH"

The antigen-specific IgE antibodies play, without any doubt, a key role in the immediate hypersensitivity (Type I allergy) mechanism(s) and in clinical disorders due to this hypersensitivity type, as it has already been repeatedly confirmed.[129,136b,145,161s,162m]

The IgE antibodies also play a main role in the mechanisms underlying the so-called "immediate type of organ responses" to challenge with inhalant allergens, such as "immediate asthmatic response (IAR)[36,96k,96p,129,145,161c,162f,177r] "immediate nasal response" (INR)[35e,36,55,78,79,83,85,97s,97t] "immediate skin response" (ISR),[36,50,129] having been described by various investigators and also analyzed extensively in our previous papers.[2-4,8-11,11c,14,68a,68e, 68f,69, 71,72b,72d,121h,176k] The antigen-specific IgE antibodies also play a central role in the immediate type responses in the bronchial tree, [105-111,111a] in the nose,[26,37,38,40,40a,41,41e] and on the skin[117b,117f] due to the food ingestion challenge, as we have previously reported.

[B] "IgE" and "LH"

On the other hand, the antigen-specific IgE antibodies have also been suggested to play a main role in the mechanism(s) underlying the so-called "late type responses", such as "late asthmatic response" (LAR)[42,51,96e,96j,162d,177d,177j, 177t] "late nasal response" (LNR)[32,33,42,51,55] and "late skin response" (LSR)[42,51,177,177a,177i] due to the allergen challenge.

Some investigators have presumed the participation of IgE antibodies in the LAR,[42, 43,51,96e,96j,96p,162d,177d,177j] LNR,[32,33,42,51,55] and LSR[42,51,51a, 176a,177a,177i] through the classically understood immediate hypersensitivity pathways, whereas others have proposed that various combinations and/or modifications of the immediate hypersensitivity mechanisms(s) may be involved in the "late type responses".[42,43,48,49,51,51a, 53,54,91,91y,96f,127,128,138,139,146e,149,152,153,162a-162d,162f,162x,165e,174,177d,177u] Cochrane and colleagues,[127,128] Henson,[146e,152] and Froese[165e] have proposed that IgE antibodies in animals may act as the essential trigger for the increased vascular permeability, which then promotes the vascular localization of the immune complexes. A similar role has also been suggested for human IgE.[126,127,128] This proposed pathway may, however, signify the existence of the immune complexes being affected by IgE, either directly or by means of their triggering effects for increased vascular permeability through the neutrophils and platelets, as well as by some mediators (PAF, NAF, NCA) released from the basophils after the IgE-antigen interaction has occured on their membranes.[179l]

In addition, the results of some investigators would not support the previously suggested unequivocal role of the IgE antibodies in the mechanism(s) underlying the "late type responses".[45,96g,96h,162b,178b,178c,178i,178j] Atkins[45] has concluded that responsibility of IgE-mast cell interaction for the late phase bronchial responses seems unlikely. Zweiman and co-workers[96g,162b] have not recorded an increased titre of the antigen-specific IgE antibodies in the serum of patients during the LAR. Lam and colleagues[96h] did not find a correlation between the specific IgE and LAR. Zetterström,[178b] regarding his data gathered from the late cutaneous responses, has concluded that activation of mast cells and/or basophils by IgE-independent mechanisms, upon involvement of inflammatory mechanisms, such as releasing factors, neuropeptides, kallikrein system, PAF and prostaglandins may probably play a more important role in the development of the "late type responses" than the IgE antibodies. Lemanske and Kaliner,[51a] comparing various research data, have concluded that the mechanisms underlying the isolated late pulmonary response to antigen challenge are unknown but may involve a variety of immunologic pathways including immediate hypersensitivity, Arthus or delayed hypersensitivity reactions.

[C] RESULTS OF OUR STUDIES

Finally, the results of our studies would also support the evidence against the main role of the antigen-specific IgE antibodies in the mechanisms underlying the "late type responses". The antigen-specific IgE antibodies in the serum (positive RAST or CAP) to the same allergen as that causing the clinical late nasal response (LNR) have been found by us in only 9%, whereas the total IgG antibodies in the serum had increased in 51% of the subjects developing the LNR.[12,14,25a,41b,41i,72b] No significant changes in the serum concentration of the antigen-specific IgE were recorded during the LNR. In addition, the antigen-specific IgE antibodies in the nasal secretions (NS) during the clinical LNR have been recorded by us in only a small number of patients (12.5%) and without any changes in their concentration, while the total IgG antibodies have been recorded in NS in 45.8% of these patients and in 33 % of them the initially increased total IgG antibodies decreased during the LAR and then recovered within 12 to 24 hours after the LNR resolution.[41b,41c,41f,41i,72b]

The positive antigen-specific IgE antibodies to foods have been demonstrated in only 1.5 % of patients developing a positive LNR to food ingestion challange.[26,40,40a,41]

In patients developing a "late asthmatic response" (LAR) to allergen challenge, the positive antigen-specific IgE antibodies to the same allergen as that causing the clinical LAR, have been recorded by us only in 2.8%, whereas the concentration of total IgG antibodies had increased in 71%, and of IgM in 54% of these subjects. In addition, va-

rious changes in the concentrations of the particular IgG-subclasses have been recorded, IgG1 had increased in 11%, IgG3 in 23% and IgG4 in 71% while IgG2 had decreased in 86% of these subjects.[61-68,68a,68b,68f,121f, 121h]

[D] ISOLATED FORMS OF LATE TYPE RESPONSES

Moreover, some investigators have even postulated that the "late reactions" do not exist as a single event, but may always be preceded by an "immediate reaction", together then forming the so-called "dual reactions", consisting of an early and a late-phase.[42, 173w,176m-176p,177j] This is the reason why these investigators have introduced the terms "early-phase" and "late-phase" of the allergic reactions, implicating that both phases belong to and are parts of one process. Furthermore, they have also presumed that both the early and the late phase are mediated by antigen-specific IgE antibodies.

Our results [7,16,19,40,41,61-68,97,109,121f,121h,178e] in agreement with other investigators' findings,[96h,161c,178k-178n] would not confirm this hypothesis, on the contrary, they would exclude it. We have repeatedly recorded and reported the isolated forms not only of the late nasal response,[7,12,14b-14g,15,16,19,40d-40f,41b,41i,72a,97] late asthmatic response, [61-66,68,68a-68g,121e,121h,178e] and late skin response,[12,14,41b,41i,65,66,72a,97,121g,178e] but also of the immediate nasal,[8-11, 18,71, 72a, 72d, 97a] asthmatic,[68b,68f,69,70,176k] and skin responses,[9,11,69,72d, 97a] and even isolated forms of the delayed nasal,[7,13,13a-13c,41i,96] asthmatic,[70b-70d] and skin responses,[7,13,13a,13b,70b-70d] due to the challenge with various inhalant allergens. The isolated forms of the nasal,[26,40,40a,41,41e,117b,117h] asthmatic[105-111,117b] and skin responses[26,41] have also been observed by us after the ingestion challenge with various foods.

[E] PHARMACOLOGIC MODULATION OF THE SO-CALLED "DUAL LATE ASTHMATIC RESPONSES"

Additionally, our data concerning the differences in the pharmacologic modulation of the early (IAR) as well as the late (LAR) phase of the so-called "dual asthmatic responses", by Disodium cromoglycate (Cromolyn, DSCG), Beclomethasone dipropionate (BDA), Budesonide (BUD, BSA)* and Nedocromil sodium (NS, NDS)**, administered at various time intervals with respect to the allergen challenge, would suggest involvement of different mechanisms in the IAR and the LAR.

In our previous studies, the LAR has been prevented highly significantly both by DSCG ($p<0.001$) and by BDA ($p<0.001$), whereas the IAR has been protected significantly only by DSCG ($p<0.01$), but it has not been affected by BDA ($p>0.05$).[63,64,67,68, 68a,68f,70,121f]

In patients developing the so-called "dual late asthmatic response" to allergen challenge, being a combination of the immediate (IAR) and the late (LAR) response, various studies concerning the pharmacological modulation and manipulation with topical inhalation corticosteroids (BDA, BSA), disodium cromoglycate (DSCG), nedocromil sodium (NDS) and salbutamol (SBT) have been performed by us.[63,64,67,68,68a-68d,68f, 68g,70,121e-121h,121k,178e-178g] (Some of these data have not yet been published).

* Nedocromil Sodium has been abbreviated as NDS in our earlier studies, whereas it has been designated as NS in some of our later studies.

** Budesonide has been abbreviated as BSA in our earlier studies, whereas it has been

In patients pretreated with DSCG and BDA, in whom the drug administration had been started 48 hours before and continued up to 48 hours after the allergen challenge, the IAR had been prevented significantly by DSCG ($p < 0.001$), but it had not been affected by BDA ($p > 0.05$), whereas the LAR had been protected significantly both by DSCG ($p < 0.01$) and by BDA ($p<0.001$).[63,64,67,68,68a,68f,70,121f]

Another group of patients with the "dual late asthmatic response" had also been pretreated with DSCG and BDA/BSA for 48 hours before the allergen challenge. DSCG administration which had been finished 10 minutes before the allergen challenge, had prevented significantly only the IAR ($p<0.05$), but not the LAR ($p>0.05$). However, if the administration of DSCG has been continued up to 12 hours after the allergen challenge, it has prevented significantly both the IAR ($p<0.01$) and the LAR ($p<0.05$). In contast, BDA/BSA demonstrated significant protective effects on the LAR ($p<0.01$) without any differences between the two treatment schedules (one of them had been finished before the allergen challenge, while the other had been continued up to 12 hours after the challenge), but they did not affect the IAR at all ($p>0.05$).[64,121g,178e-178g]

We have also compared the effects of a single dose of DSCG or budesonide (BSA) on the LAR, administered either before or after the allergen challenge (before the onset of LAR), in patients with the "dual late asthmatic response". DSCG administered 30 minutes before the allergen challenge, prevented the IAR significantly ($p<0.05$), but did not affect the LAR ($p>0.05$). DSCG given 1, 2, 3, or 4 hours after the allergen challenge did not prevent the LAR ($p>0.05$). BSA administered 30 minutes before the allergen challenge prevented the LAR significantly ($p<0.05$), but did not affect the IAR ($p>0.05$). Budesonide administered 1, 2 or 3 hours after the allergen challenge prevented the LAR significantly ($p<0.05$).[68b,68c,68f,68g,121g,121h]

In another group of patients we have investigated the possible protective effects of DSCG, BSA and NDS on the "dual late asthmatic response", administered in a single dose either 30 minutes before or 2 hours after the allergen challenge. Administered before the allergen challenge, NDS prevented significantly the IAR ($p<0.05$) and LAR ($p<0.01$), DSCG prevented only the IAR ($p<0.001$) but not the LAR ($p>0.1$), while BSA did not affect the IAR ($p>0.05$) but has prevented highly significantly the LAR ($p<0.001$). Administered 2 hrs after the allergen challenge, NDS ($p<0.01$) as well as BSA ($p<0.001$) prevented the LAR highly significantly, while DSCG did not affect the LAR ($p> 0.05$). [121g,121h]

In another of our studies we have analyzed the possible protective effects of a single dose of BSA administered either 30 minutes before or 1, 2 or 4 hours after the allergen challenge in patients with the "dual late athmatic response". The IAR had not been affected by the BSA when given 30 minutes before the allergen challenge ($p>0.05$), while the LAR had been prevented significantly by BSA administered either 30 minutes before ($p<0.01$) or 1 hour ($p<0.01$), or 2 hours ($p<0.05$), or 4 hours ($p<0.05$) after the allergen challenge.[68c,68g]

We have also investigated the effects of a single dose of NDS on the LAR, administered more than two hours after the allergen challenge, which means 90 to 30 minutes before the onset of LAR. Compared with the placebo, NDS having been administered in a single dose, spread out over three time-intervals, 90, 60 and 30 minutes before the

onset of LAR, has inhibited significantly the LAR ($p<0.01$ to $p<0.05$) at each time point measured from 6 to 10 hours after the allergen challenge.[68d,178e]

Other patients developing the "dual late asthmatic response" have been treated with a single dose of DSCG, NDS, and BSA/BUD, administered either 30 minutes before or 2 or 3 hours after the allergen challenge. Given before the allergen challenge, DSCG significantly prevented the IAR ($p<0.001$), but not the LAR ($p>0.05$), NDS protected significantly both the IAR ($p<0.01$) and the LAR ($p<0.05$), and BSA/BUD did not affect the IAR ($p>0.05$) but it prevented highly significantly the LAR ($p<0.001$). Given 2 hours after the allergen challenge, DSCG did not affect the LAR ($p>0.05$), whereas both NDS and BSA/BUD have prevented significantly the LAR ($p<0.05$ respectively $p<0.001$). Given 3 hours after the allergen challenge, DSCG did not show any effects on the LAR ($p>0.05$), whereas both the NDS and the BSA/BUD prevented the LAR significantly ($p<0.02$ respectively $p<0.01$).[121k,178f]

In one of our preliminary studies, the patients demonstrating the "dual late asthmatic response" had been pretreated with a single dose of DSCG, NDS, BSA/BUD and salbutamol (SBT), administered either 30 minutes before or 2 hours after the allergen challenge. After the pre-challenge administration, NDS prevented significantly both the IAR ($p<0.01$) and the LAR ($p<0.01$), DSCG has prevented only the IAR ($p<0.001$), but not the LAR ($p>0.05$), BSA/BUD did not affect the IAR ($p>0.05$), but demonstrated significant protective effects on the LAR ($p<0.001$), whereas SBT did not affect either the IAR ($p>0.05$) or the LAR ($p>0.1$). After the post-challenge administration, NDS as well as BSA/BUD prevented the LAR highly significantly ($p<0.001$), while DSCG as well as SBT did not affect the LAR ($p>0.05$ respectively $p \geq 0.1$).[121k,178h]

We also have investigated the possible influence of the duration of the drug administration on the protective effects of DSCG and topical corticosteroids (BDA and BSA/BUD) on the IAR and the LAR in patients developing the "dual late asthmatic response" to allergen challenge. Neither DSCG nor BDA nor BSA/BUD demonstrated any significant differences in their effects either on the IAR or on the LAR after a short-term administration, for 48 hours, and after a long-term administration, for respectively 2 to 6 weeks. DSCG prevented significantly both the IAR after 48-hour ($p<0.01$), 2-week ($p < 0.01$) and 6-week ($p<0.001$) administration, and the LAR after 48-hour ($p < 0.05$), 2-week ($p < 0.01$) and 6-week ($p<0.01$) treatment. BDA as well as BSA/BUD did not demonstrate any significant protective effects on the IAR after the 48-hour ($p > 0.1$), 2-week ($p > 0.05$) or 6-week ($p > 0.05$) administration, whereas both of them prevented highly significantly the LAR after the 48-hour ($p <0.01$), 2-week ($p < 0.001$) and 6-week ($p < 0.001$) administration.[68c,178g]

[F] PHARMACOLOGIC MODULATION OF THE SO-CALLED "DUAL LATE NASAL RESPONSE"

In our previous studies we have also investigated the protective effects of various drugs, among others of DSCG and topical corticosteroids (BDA) on the basic types of nasal response to allergen challenge, INR, LNR and DYNR.[2,10,11, 11c-11e,12,13,14,40c,-40d,40f,41b,41i,72b,72d,121b,121j] Similarly to the asthmatic response, INR has been prevented highly significantly by DSCG ($p<0.01$), but it has not been affected by BDA ($p>0.1$). The LNR has been prevented significantly both by DSCG ($p<0.05$) and by BDA ($p<0.05$). The DYNR has been protected significantly by BDA ($p<0.01$), whereas DSCG did not influence it at all ($p>0.1$).[2, 10,11,11c-11e, 12,13,13b,14,40c,40d,40f,41i,72a,72d]

We have also studied the pharmacologic modulation of the "dual late nasal response" by a single dose of DSCG and BDA/BUD-BSA administered either 30 minutes before or 2 or 3 hours after the allergen challenge, which means 1 or 2 hours before the onset of the LNR. DSCG in a single dose administered 30 minutes before the allergen challenge prevented highly significantly the INR ($p<0.001$), whereas it was not able to affect the LNR ($p>0.05$). In contrast to the late asthmatic response, DSCG having been administered at 2 or 3 hours after the allergen challenge (meaning 1 or 2 hours before the onset of the LNR) prevented significantly the LNR ($p<0.05$ respectively $p<0.02$). BDA administered 30 minutes before the allergen challenge, did not affect the INR ($p>0.05$), while it has prevented significantly the LNR ($p<0.05$). The BDA administered 2 or 3 hours after the allergen challenge, similarly to the LAR, prevented significantly the LNR ($p<0.01$ respectively $p<0.001$).[40b,40d,40f,72a,72b]

In another of our preliminary studies, BDA/BUD-BSA administered for 48 hours, 3 and 12 weeks prior to the allergen challenge, did not show any differences or changes in their protective effects either on the INR or on the LNR. Both the topical corticosteroids demonstrated significant protective effects on the LNR ($p<0.01$ respectively $p<0.01$), whereas they did not affect the INR, even after the long-term administration, 3 and 12 weeks ($p>0.1$ respectively $p>0.05$).[40d,72a,72b] Recently, we have investigated the protective effects of BUD-BSA on the LNR and "dual late nasal response" due to the nasal challenge with pigeon or tropical bird faeces extract in subjects with perennial nasal complaints, being exposed regularly to these birds. Even after a 3-week administration in a daily dose of 400 mcg, BUD-BSA has not been able to prevent the INR ($p>0.05$), whereas it has prevented highly significantly the LNR ($p = 0.001$).[121b]

[G] INTERPRETATION OF OUR EXPERIMENTAL DATA

These results and differences in the pharmacologic modulation of the immediate/early (IAR) as well as the late (LAR) asthmatic responses, and of the immediate/early (INR) as well as late (LNR) nasal responses would also increase the doubt about the existence of the "dual asthmatic response" and the "dual nasal response"as a compact event consisting of two phases. These results would suggest that the so-called "dual asthmatic response" as well as "dual nasal response" may, in reality, be the simultaneous appearance of two independent responses, both of them caused by one allergen, however due to the different mechanisms.[68g,178e]

3. MAST CELLS [MC] AND BASOPHILS [BS]

Another fundamental part of the immediate hypersensitivity [IH] (Type I allergy) mechanism is the mast cell and/or basophil.[6,35,35c,36,42-44,48c-48e,51,55, 63, 68c,70,77,78, 85,94,97y,128a,129,131e,136a,136b,145,147,162,162f-162n, 164,164a-164d,165g,166x,176w,176x,177r

[A] THE SUGGESTED/PRESUMED ROLE OF "MC" AND "BS" IN "IH"

The key role and central function of the mast cells and/or basophils in the immediate hypersensitivity mechanism(s) underlying the "immediate organ responses" to allergen challenge, such as "immediate asthmatic response" (IAR),[4,11,11a-11c,42-44,51a,63,69,70,124,161c,162g,162h] "immediate nasal response" (INR),[11a-11e,34,35,42-44,47,48,51a,54,56] "immediate skin response" (ISR),[42-44,51a,177i] etc., has already been repeatedly and unequivocally confirmed.

However, all circumstances, factors and conditions determining the activation and/or participation of either mast cells or basophils, even in the mechanisms leading to the development of the "immediate type responses" in different organs e.g. IAR, INR, ISR etc, have not yet been fully clarified.[4,51a,136b,177g] Despite recent data generated from the cytokine research,[131f,146d,156m,163y,164d,164v,165r,165w,166g, 166z,177a] cytologic investigations of the nasal secretions,[11a-11f,14,34,40c-40f, 41b,48,48d,49,54,71,72,72a-72d,77,82g,177g] biochemical and immunologic studies on and in the nasal mucosa and nasal secretions [11j,48,49,53,54,73,82d,82e, 83,85d,86-95,95b,97,97b-97d,97i,166d,171r,171v,175k,175l,175p,175s,175x, 176a, 177g] and nasal mucosa biopsies[41b,51b,85b,85c,96,97,97a,97f,97n-97u,97u,171r] as well as bronchoalveolar lavages,[96n,96p,162a,162g-162j,163g,163j, 163s,164t,167z] bronchial mucosa biopsies,[162k,162n,163k,163y,164t] and skin biopsies,[163i,177a] which have contributed distinctly to our knowledge of the role of both these cell types, there is still a great need for more information on this topic.

[B] THE SUGGESTED/PRESUMED ROLE OF "MC" AND "BS" IN "LH"

On the other hand, the possible role and participation of the mast cells and/or basophils in the mechanisms underlying the "late type responses" developed by various organs following the allergen challenge, such as LAR, LNR, LSR etc, as suggested by some authors, still remains not fully clear.[51a]

Regarding their results, some investigators[42-44,51,51a,55,96e,96f,96i,162a, 162d,177,177d,177i,177j,177t,178p] have presumed the direct involvement of the mast cells in the "late type organ responses" through the classically understood immediate hypersensitivity mechanism(s). Moreover, they have concluded that both the "immediate" (IR) and the "late type responses" (LR) are IgE-mediated and in both response types the mast cells may play the pivotal role. Other investigators have assigned a similar role to the basophils and have concluded that both the IR and the LR are IgE-mediated and in both response types the basophils play the central role.[156m,163f,164l,164n,166h,177b,178,178a,178p-178u]

However, both investigator groups have failed to present convincing data concerning the different involvement of both the cell types in the particular response types, IR or LR. Anyway, the important question still remains unanswered; why the mast cells and not the basophils, or vice versa, may be involved once in the isolated form of the IR, another time in the isolated form of LR, and finally in both the response types together.

[C] THE SUGGESTED DIFFERENTIATED ROLE OF THE "MC" AND "BS" IN THE "INR" AND "LNR"

Finally, other investigators,[34,52-54,82,97y] studying the mediators in the nasal lavage fluid during the INR and the LNR, have found an increased concentration of histamine, TAME-esterases, kinins, but not of PGD_2 in the nasal secretion during the LNR. With respect to these findings, they have concluded that the mast cells may play the main role in the INR, whereas basophils in the LNR, since PGD_2 is only produced by mast cells and not by basophils. A similar participation of mast cells in the IAR and not in LAR (where basophils would probably play the prominent role) has been proposed by other investigators studying the IAR and LAR after a segmental bronchial challenge with antigen.[163s]

Interestingly, several other authors have proposed various modified and alternative functions of the mast cells and/or basophils, by means of which these cells will be able to participate in the mechanisms leading to the development of the "late type responses",[34,44,45,51,51a,54,56,82,83,94,136a,162b-162d,162f,176l, 176v,177c,177i,177t,177v,178v]

[D] DIFFERENTIATED ROLE OF "MC" AND "BS" IN VARIOUS TYPES OF HYPERSENSITIVITY WITH RESPECT TO THE PARTICULAR PHASES OF THEIR ACTIVATION, DEGRANULATION AND MEDIATOR RELEASE.

Wasserman,[176l] Lewis and co-workers,[176v] Terral et al[178v] and Charlesworth and colleagues[178] have suggested the so-called "bi-phasic degranulation" of mast cells and basophils. The bi-phasic response of airways to inhaled allergen may then be comparable with the proposed bi-phasic cutaneous response to the IgE-dependent activation of mast cells.[177i] This could be the process by which the released mediators might provoke a bi-phasic inflammatory response. Casale and Kaliner[177t] have described three groups of mast cell-derived mediators: (1) preformed or primary mediators (e.g. histamine); (2) secondary or newly generated mediators (e.g. prostaglandins); (3) granule matrix mediators (e.g. peroxidases, heparin). They have presumed that the preformed and partly newly generated mediators are released rapidly and cause "the immediate allergic reactions", whereas the granule matrix mediators and some of the newly generated mediators might lead to the polymorphonuclear leukocyte infiltration, followed by mononuclear/macrophage infiltration and resulting in the "late allergic reactions". Kay,[162f] Nagy and colleagues[162c] and Durham and co-workers[162d] have formulated the existence of three phases of airway obstruction in bronchial asthma: - rapid (spasmogenic), - late (sustained), and - subacute inflammatory phase. All of these phases may be caused by the mast cell-derived mediators". The "rapid" phase (within 10-15 minutes) may be mediated predominantly by histamine; the "late" phase (4 to 8 hours) may be associated with what they called "re-activation of the mast cells" and with an increase in the circulating serum neutrophil chemotactic factor, and in which phase also leukotrienes, prostaglandins and thromboboxanes may presumably play a role; and finally, the subacute inflammatory phase, which is characterized by infiltration of eosinophils, neutrophils and mononuclear cells, and which is probably mediated by chemotactic factors from mast cells (NCF, LTB4, ECF-A, etc). Schleimer and co-workers[44,176s] have suggested the release of two groups of mediators during the pulmonary IgE-dependent

hypersensitivity mechanism, the primary mediators from mast cells, leading to immediate response and the secondary mediators from lung tissue, due probably to the basophils emigrating from the blood into the lung tissue site and causing the "late response". The release of the two mediator groups seems to them to correspond with the two phases of the IgE-dependent hypersensitivity, namely the early and the late phase. They have postulated that "after activation of mast cells and other cells that may be triggered during the early phase response, a late phase response occurs that involves migration of cells such as eosinophils, basophils, neutrophils and some mononuclear cells into the tissue site". The suggested participation of the mast cells in the immediate asthmatic response and that of basophils in the late asthmatic response is similar to the role of mast cells in the immediate and the basophils in the late nasal responses proposed by Naclerio and his group[34,54,56] and being also shared with other investigators.[163f,163s,164n,177g, 178,178t,178u] Such different roles of these cells in different types of allergic response is also supported by other investigators' findings that basophils, but not mast cells, are sensitive to steroids "*in vitro*",[121l,177v, 178w,179h,179i] and "*in vivo*".[177w] whereas the late type responses, but not the early type responses to allergen challenge on the skin, in the nose and in the bronchial tree, are inhibited by steroids.[12,40c-40f,41i,63,68,68a-68g,69,70,72d,164b,164l, 164n,177g,177s,178r,178t, 178u,178x,179j,179k] Recently, evidence has been provided for some inhibitory effects of topical glucocorticosteroids on some of the functions of mucosal mast cells in animals.[179,179a] In humans, the topical corticosteroids can, under certain circumstances, reduce the number and distribution of the mucosal mast cells (MCT), probably by inhibiting the production of IL-3 or possibly of other mast cell growth factors.[178x] Also a possible inhibition of mast cell-derived cytokines has been suggested.[178x] However, there is no evidence that topical glucocorticosteroids can inhibit the production and release of preformed and newly generated mediators by human mast cells,[178x] either "*in vitro*" or "*in vivo*".[178x]

[E] Corticosteroids and the immediate type of the nasal/asthmatic response

Moreover, neither systemic corticosteroids nor topical glucocorticosteroids have been shown to inhibit the immediate reactions to allergen challenge, such as "immediate asthmatic response"[40b,63,68f,68g,70,96k,121h,161c,178f,178g,178j, 178m,178n] or "immediate nasal response",[2,10,11,11a, 11d,11e,14,41i,48d,72b ,72d,92,94,95,97a-97c,97p,119-121,121b,121d,177h] even after a long-term administration, e.g. 2 weeks[121d], 3 weeks[121b], 6 weeks[72a,121h] or 12 weeks.[72d,121f] The lack of protective effects of topical glucocorticosteroids on the "immediate type reactions", repeatedly demonstrated by us as well as by several other investigators, would contradict the findings of some other investigators' concerning the possible inhibitory effects of topical glucocorticosteroids on the immediate asthmatic response [179b,179c] or on the immediate nasal response[121c] generated, however, from a limited number of subjects and sometimes poorly documented.

[F] The possible role of "MC" and "BS" in the hypersensitivity mechanisms with respect to their specific features and to the possible involvement of cytokines

Atkins and Zweiman[162e] and Zweiman and colleagues[162b] have observed an increase in the count of blood neutrophils 2 hours after an allergen, but not methacholine, inhalation. They have suggested that the immediate phase of asthmatic response may be caused by the mediators released from the degranulated mast cells, followed by the late neutrophil activation, which may be involved in the late phase asthmatic response. Talbot and co-workers[177c] have suggested the possibility of a "prolonged release" of mediators from the mast cells. Lemanske and Kaliner[51a] have formulated the lack of evidence for the contribution, even indirect, of mast cells for late asthmatic response to allergen challenge. Furthermore, they have concluded that "the contribution of the mast cell to the biologic and physiologic events surrounding the late response are less well established. The precise cell source of NCF is unknown. Thus while mast cell activation may initiate the allergic reaction, it is quite possible that a second or third immunologic or biologic signal is necessary for the allergen within the airway to lead to the development of the late obstructive reaction." Recent data, indicating that the activation of the animal basophils and mast cells, both of the IL-3-dependent and of the IL-3 independent cell lines, may result in an increased RNA expression and the release of various cytokines, has expanded the role of these cells. The following cytokines have been suggested to be released by the activated mast cells and/or basophils: IL-1, IL-2, IL-3, IL-4, IL-5, IL-6,IL-10, INF-gamma, granulocyte-monocyte colony stimulating factor, macrophage inflammatory peptides α and β, T cell activation gene 3, and tumor necrosis factor-α.[162m,164c,179d, 179e] However, virtually all the studies reporting the cytokine production by the mast cells and/or basophils have been carried out on animals, mainly murine or rodent cell lines or primary cell cultures.[162m,164c,179d,179e] Some investigators have reported a possible evidence for the production of some cytokines also by human mast cells and basophils. Data of these studies, having been summarized by Schwartz and Huff,[164c] suggest that the activated human mast cells might express mRNA for TNF-α and IL-4, whereas human mast cells as well as basophils might generate both TNF-α and IL-4 proteins. In spite of this somewhat weak suggestion of the production of cytokines also by the human mast cells and basophils, the participation of such cytokines in the hypersensitivity mechanisms in humans in general, and in the late type responses to allergen challenge in particular, has not yet been convincingly confirmed.[179e]

Furthermore, with respect to the specific features of the mast cells and basophils, such as "releasability"[131e,162m,164c,164h,164n,167z,178r] and "heterogeneity",[162m,164c,164d, 164n,164u,164y,167k,176t,178r,178y,178z,179f,179g] the question of the possible production of some cytokines by the human mast cells and/or human basophils, is becoming a more complex and topical issue.

The "releasability" means the variations in the extent of the histamine release not only between the basophils on the one hand and the mast cells on the other hand, but also with respect to the origin of these cells, e.g. from the cells of different subjects, from the cells of different locations of one subject, and finally with respect to different secretagogues eliciting different degrees of the histamine release.[131e,162m,164c,164h,164n,167z,178r]

Heterogeneity of the mast cells and basophils represents the histologic, histochemical and morphologic differences between the basophils and the mast cells, and even between the two basic mast cell populations, the "mucosal type" mast cells in rodents, being equivalent to the human mast cells containing tryptase only (MC_T), and the "connective type" mast cells in rodents, being equivalent to the human mast cells containing tryptase and chymase (MC_{TC}).[162m,164c,164d, 164n,164u,164y,167k,177v,177x, 178r,178y,178z,179f,179g]

Despite the proposed modified functions of the mast cells and/or basophils, and even the suggested alternative pathways, the exact involvement and detailed role of both these cell types in the mechanism(s) underlying the " late type responses" in particular organs, especially their relationships to other cell types, such as stimulation versus inhibition at various levels, sequence of the intercellular and transduction signals, and the degree of their stimulation and activation in various steps of these processes, are not yet fully known.[68e,131a-131c,162d,162m,162z, 164b,164c,164h,168f,168h,168i,168n,,170r,171v, 177g,178d,178g,178r,178u,179d]

Moreover, the existence of the possible stimulation and activation of the mast cells and basophils by various factors and compounds sometimes operating outside the structures of the established hypersensitivity types, as has already been discussed in the previous sub-sections, would then amplify the importance of the alternative pathways and extend the variance of the functions of these cell types.[68e,162z,164b,164c,164h]

VIII. GENERAL DISCUSSION

The purpose of this thesis was to define and establish the nature, clinical and immunologic features, clinical correlates and possible pharmacologic modulation and control of the clinical phenomenon, the "late nasal response" (LNR). The LNR has been compared with and differentiated from the other types of the nasal response, such as immediate/early nasal response (INR/ENR) and the delayed nasal response (DYNR). Furthermore, the possible mechanisms which may be presumed to contribute to and/or to participate directly in the development of the LNR have also been described.

This thesis is based on our own original studies, data and results of which have already been discussed in the previous sections.[2-5,7-11,11a-11j,12,13,13a-13c,14,14a-14g,15, 25,25a,26,27,27a,27b,35a-35c,37-40,40a-40f,41,41a-41g,71,72, 72a-72d,96,97,97a,97v, 97w,110,117e, 117f,117h,117i,118,121b,121i,121j,122a-122e,124e-124d]

A selection of the most relevant papers concerning the main target of this thesis has been included and presented in the section "Supplements".[7,9,11,11i,12,13,14c,16,18,41, 71,72]

In this chapter our attention will be focused on the folowing aspects:

[1] The detection of the particular types of the nasal response in general and of the LNR in particular, which means the "nasal provocation tests" (NPT), not only from the technical points of view, such as principles of the NPTs, basic conditions for the NPTs, basic indications, basic techniques, their advantages and disadvantages, reproducibility, but also the interpretation of the data generated by these tests;

[2] The position of the LNR in the hypersensitivity events occuring in the nasal mucosa, the significance and consequences of its existence for the practical diagnostic procedures and treatment of the allergic disorders of the upper airways and related organs;

[3] The importance of the existence of the LNR also for research purposes, as an interesting and easily accessible model for studying the hypersensitivity mechanisms from various points of view;

[4] The various clinical and immunologic aspects concerning the LNR, which still remain unknown, and which may be the target for the research activity on this field in the future, such as detailed mechanism(s) underlying this clinical phenomenon, the participation and role of the particular cell types and their interactions, the participation of the particular mediators, factors, constituents, molecules, compounds and biochemical processes in the development of the LNR. This might improve our understanding of the LNR, the integration of this phenomenon into the more general hypothesis of the hypersensitivity states of the upper airways and to optimize its pharmacologic control.

A. NASAL PROVOCATION TESTS [NPT]

The nasal provocation tests (= nasal challenge, NPT) might be considered not only as an interesting and important research technique and model for the investigation of the pathophysiological and pharmacological aspects of the nasal mucosa, [2,7,8,11,11i,12,13,14c,18,21,22,26,27,35d,35e,40a,41j,53,71,72,179,180-197] but also as an important part of the routine diagnostic procedure of the allergic disorders in the nose and of the role of the nose in the related organs.[2,3,7,9,11h,14c,21,22,26,27,30,35e,41,41j,53,180,186,187] The usefulness of nasal provocation tests for the clinical diagnosis is gradually becoming recognized.[7,11h,14c,21,26,27,30,35e,40a,41j,48f,53,85,180,186-192]

The NPT may be seen as a model technique and as a simulated reproduction of the patient's complaints and symptoms caused by an exposure to a certain allergen or non-specific agent.[3,180] Preference should be given to the nasal challenge as a model exposure to an allergen or non-specific agent, rather than the natural exposure, where the dose and concentration of the particular agent cannot be always monitored and nasal parameters cannot be always quantitatively recorded.[3,35b, 41b,180] The nasal provocation test is so far the only technique to demonstrate the particular types of the nasal response (immediate, late, delayed) due to a specific allergen.[2-4,7,11h,13,41b,41j,191]

1. PRINCIPLES OF THE "NPT"

The basic principle of the nasal challenge is repeated recording and comparison of parameters before and after the specific stimulus (= comparison of relative values).[3,7,8,11h,30,41j]

The principle of the provocation tests has two aspects: (a) The qualitative aspect: The NPT demonstrates that the nasal mucosa indeed reacts by the typical complaints and develops a certain type of nasal response to the challenge with a certain allergen or non-specific agent. The nasal challenge may confirm definitely the role of a certain allergen or non-specific agent in the patient's nasal complaints; (b) The quantitative aspect: The NPT demonstrates that the suspected allergen or non-specific agent, in a certain dose, during a certain time of exposure, is able to cause a certain nasal response, which can then be quantified and recorded (= dose-response and time-response curves).

2. BASIC TYPES OF THE "NPT"

There are 3 basic types of nasal challenges:[3,7,10,12,13,30,35b,35e,41b,41j,72a,72b]

(a) Nasal provocation tests with allergen, confirming the allergy component in the nose by the manifestation of a certain type of the nasal mucosal response due to a certain allergen.[2,3,7,9,41b,41j,72b,186,192-194]

(b) Nasal provocation tests with non-specific agents, their simulators or representatives (e.g. histamine, methacholine, cold air, etc), demonstrating the non-specific hyperreactivity component in the nose, and its participation in the nasal complaints.[4,35,35a-35d,35f,72b,195,196]

(c) Nasal provocation tests in combination with pharmacologic agents (=nasal protection tests). These tests being in principle a modification of the NPT, may confirm the effects of a certain drug on the nasal symptoms and on the nasal response of a certain type due to a certain allergen or non-specific agent.[9,11,11h,11i,12,13,40d,41b,48k,48l,72d,184,185,191-194,196]

The basic types of NPT are not alternatives to each other, they supplement one another. They discriminate participation of the allergy component on the one hand and that of the non-specific hyperreactivity component on the other hand in the patient's nasal symptoms. The protection tests may demonstrate the pharmacologic efficacy of a certain drug and its effects on the particular type of the nasal response.

3. BASIC CONDITIONS FOR THE "NPT"

(1) The nasal provocation tests should also meet some necessary requirements,[3,4,30,41j,187,191,197] the test should be safe, reproducible, sufficiently sensitive and free of artefacts. The allergen (non-specific hyperreactivity agent) should be applied in a sufficient and adequate but not dangerous dose during a suitable period of time by a reliable technique. The test should be as comfortable as possible for the patient as well as for the technician;

(2) The NPT should be performed by means of a reliable method and using reliable apparatus corresponding with up to date technical development.[3]

(3) The NPT should be performed in an adequately equipped room under standard conditions, e.g. room-temperature, humidity, ventilation, optimal furniture etc.[3]

(4) Adequate and sufficient facilities for reanimation and management of emergency or unexpected complications should be present, with respect to rare, but possible appearance of an acute dyspnea due to the broncho-constriction, epiglottis edema, anaphylactic reactions etc.[3]

(5) The benefit of the NPT depends on the technique, equipment and experience (quality) of the operating personnel;[3]

(6) The NPT cannot substitute the bronchial provocation tests in general, only in a limited number of exceptional indications.[3,4,30,191]

4. BASIC INDICATION FOR THE "NPT"

[1] The confirmation of the role of a certain allergen in the nasal complaints, especially in the case of discorrelation of skin tests and/or RAST and/or history [3,4,7,9,11b,11h,30,41b,41j,90,180,184,186,190,198]

[2] The discrimination of the role and involvement of an allergy component (nasal challenge with allergens) and a non-specific hyperreactivity component (nasal challenge with histamine or methacholine) in the nasal complaints of the patient [2-4,35,35b-35e,41b,48f,57a,72b,195]

[3] The discovery and the confirmation of the existence of the particular types of the nasal response; immediate, late or delayed [2-4,7,10,11-13,11h,13a,41b,96,124c-124e]

[4] The establishment of the dose-response curve (= allergen titration)[8]
[5] The confirmation of a possible role of a certain food in the nasal complaints (food allergy) [26,38,40a,41,41e]
[6] The confirmation of a possible involvement and association of the nasal mucosa and nasal allergy in the other organs and their responses, e.g. conjunctivae, middle ear, sinusses, bronchial tree etc.[14c,14d,21,22,26,27,30,91,187]
[7] The examination of the protective and/or therapeutical effects of a certain drug on the nasal response due to the particular allergen or non-specific agent (=protection tests) [10,11,11d,11e,11i,12,13,13b,13c,21,26,28,40a,40c,41b,41g,72a,95,121j,122a-122c,185,188,194,196]
[8] The confirmation of the occupational allergy [3-5,16]
[9] The assessment of various therapies and immunotherapy[191,192]
[10] The substitution of the bronchial allergen challenge by the nasal challenge in some exceptional cases (in patients in whom the nasal allergy may participate in broncho-constriction, or in whom the bronchial challenge cannot be performed e.g. in small children, in patients with high risk, or in patients with limited or unstable lung functions) [3,4,11h,30,41b]
[11] For research purposes - to study various pathophysiological, immunological 53,180,183 and pharmacological aspects of the nose and nasal allergy, nasal mucosa and nasal secretions.[11f,23,33,34,49,71,72,72c,82,83,86,88,94,124,184,191,192,198,199]

5. COMBINATION OF "NPT" WITH OTHER DIAGNOSTIC PARAMETERS

The NPT can be supplemented by various "*in vivo*" as well as "*in vitro*" diagnostic parameters, such as clinical symptoms (pulse rate, blood pressure, body temperature, etc)[32] and other organ functions (e.g. tympanometry, conjunctival appearance and lacrimation, sinuses X-ray and/or echography, lung functions, etc).[14c,21,26,41,59,91,187] to demonstrate the possible role (direct or indirect) of the nasal mucosa and/or nasal allergy in the response of the other organs. The NPT can also be combined with other parameters, such as rhinoscopy, endoscopy, biochemical, cytological and immunological examination of the nasal secretions and nasal lavage,[18,71,72] biopsy of the nasal mucosal membrane, estimation of immunoglobulins, mediators and other compounds in the nasal secretions [49,53,83,86,92,124] and in the blood,[9,11,11h,12,13] recording of physical-chemical properties of nasal secretions,[11h] and recording of changes in the count of blood cell types.[11h]

6. NASAL PROVOCATION TESTS [NPT]-TECHNIQUES AND METHODS

[A] APPLICATION OF THE ALLERGEN AND/OR NON-SPECIFIC AGENT

Several techniques have been used to apply allergens or non-specific agents or their representatives into the nose. Dropping of allergenic extracts onto the nasal mucosa using syringes[200] or bottle droppers[201] is a potentially hazardous method since the fluid may pass down to the larynx which occasionally causes laryngeal oedema or bronchospasm.[181] A similar disadvantage, however to a lower degree, also concerns

the aerosolization technique using either the pressurized air or the electronic devices or the spray-bottle.[35e,41j] Moreover, the distribution of the allergen applied by these methods cannot always be predicted. Some investigators use a micropipette to deliver small and exact volumes of allergen onto the interior turbinate[202,203], which seems to be a much more reliable technique than the previous techniques. However, the distribution of allergenic extract is difficult to control. Impregnated paper discs[35,204] inserted into the nose as well as the administration of the allergen via nebulized solutions [85,195,202] have also been used. Philips and co-workers[194] have applied the allergen extract by means of a cuvette placed directly onto the anterior section of the inferior turbinate.

The challenge procedure should be considered to be a model technique for the simulation of the natural exposure. All the above described techniques are capable of delivering allergen to the nose and initiating a response. However, some of them deliver allergen in a higher dose than that occuring naturally. Studies in which pollen grains have been delivered by an insufflating device are simple to perform, and the number of pollen grains delivered can be controlled.[97y] However, this method is applicable only for pollen and not for other allergens. Another disadvantage of this method is the possibility of penetration of the insufflated pollen grains into the larynx or even into the bronchial three, causing a laryngeal oedema or broncho-constriction[181].

We apply the allergen extract as well as the non-specific agent (e.g. histamine, methacholine etc.) to the nasal mucosa by means of a wad of cotton wool on a nasal probe introduced under the medial concha (middle turbinate), as it has been described in detail in our previous papers.[7-9,11,11h,11i,12,13,21,22,26,27,35a-35c,40a,48k,48l,71,72,184,185] The standard application time of the allergenic extracts is 3 minutes (sometimes shorter), while that of histamine and methacholines is 30 seconds.

[B] RECORDING OF THE NASAL RESPONSE [= RELEVANT PARAMETERS]

The nasal response to challenge with an allergen or non-specific agent may be recorded using various techniques.[7,11,11h,11i,12,13,30,31,41j,48h,48k,48l,57a,60,85,85a,120,121,180,182,184,185-220]

[B1] The most simple method is the anterior rhinoscopy[85], which, however, is only a qualitative method;

[B2] Recording of subjective complaints (obstruction, hypersecretion, sneezing, itching) by means of a score.[35,35e,85,205] This technique allows only semi-quantitative recording of the response, because: - not every nasal response in every patient may necessarily be accompanied by significant increase in all these complaints (especially sneezing, hypersecretion and itching), - there is a high degree of standard deviation, personal influence and a lack of reliable quantification. Therefore, the single recording of the subjective complaints is no longer an acceptable technique. It has been suggested to use these parameters only in conjunction with the recording of objective parameters. [3,4,7,8,11b,11h]

[B3] Recording of objective parameters, mostly nasal airway resistance (NAR). This can be measured using an air-pressure and either air-volume or air-flow, or their deri-

vatives, such as air-passage or conductance (which is the reciprocal value of nasal resistance). These techniques can be divided into 4 groups: (1) nasal peak-flow measurement, (2) plethysmography; (3) rhinomanometry (anterior, posterior, active, passive, combined and/or modified techniques), (4) Non-rhinomanometry techniques, e.g. recording of the nasal blood flow using Doppler velocimetry,[216-218] or the ^{133}Xenon washout clearance method.[182,218]

(1) Nasal peak-flow technique

A modified Wright's peak-flow meter to measure the nasal expiratory peak-flow has been used by some investigators.[206] This method is very easy to perform. However, following allergen or histamine challenge it is impossible to avoid the contamination of the equipment by nasal secretions. A modified mini peak-flow meter has also been developed. This is also simple to perform, however, the forced breathing through the nose can result in the reflex changes in nasal patency, which may significantly influence the challenge results.[30,207]

However, due to some distinct disadvantages, such as contamination of the equipment by nasal secretions, reflex changes in nasal patency, high deviations of the parameters recorded and the inability of this technique to record the whole course of the nasal respiration cycle (inspiration and expiration in their time-course), such techniques are not suitable for the nasal provocation tests.[3,8,30,41b,41j,180]

(2) Plethysmography

The body-plethysmography has been modified for nasal purposes by Cole et al[48g, 208] and described as "head-out" bodyplethysmography. This seems to be a very sensitive and accurate technique. However, it requires highly qualified and experienced personnel, is very laborious, and it is not always suitable for children because of anxiety. The reliable equipment is also very expensive. This technique is therefore suitable only for special research purposes.

(3) Rhinomanometry

The measurement of nasal airway resistance (NAR) using rhinomanometry can be divided into various types. In each type an air-pressure and either air-volume or airflow, or their derivatives [e.g. air-passage, conductance (= reciprocal value of the nasal resistance = R_N) etc.] are recorded. In passive rhinomanometry, the pressure and the airflow are recorded during breath hold, whilst active rhinomanometry involves their recording during the tidal nasal breathing. Active rhinomanometry can be further subdivided into an active anterior, active posterior, and modified techniques.[3,30,41b, 41j,197] The basic principle of the use of every rhinomanometry technique for the NPT purposes is the comparison of the relative values before and after the challenge with an allergen (or non-specific agent) and not a comparison of absolute values (parameters). From such a point of view (principle of comparison of relative values), the absolute values of the baseline would not be of decisive importance.

[a] Passive anterior rhinomanometry (PAR)

The principle of this procedure is the measurement of the passage of the air through one nostril at a fixed flow rate (e.g. 0.25 L/sec) while the breath is held with the mouth open. The air pressure is recorded and the resistance (mm H_2O/L/sec or cm H_2O/L/sec) can be calculated for each nostril independently (R_R,R_L).[25b,35e,41b, 41j,188-190,193-195,197] The usual equipment for PAR is PAR-Heyer (Germany, Netherlands), Cottle (USA), Elema (Sweden), Massing-Siemens (Germany), Connell's apparatus (USA), Mercury NR4 (UK). The total nasal resistance (R_{TOT}) can be calculated by the following formula:

$$R_{TOT} = \frac{R_R \times R_L}{R_R + R_L}$$

[R_{TOT} = total resistance; R_R = resistance - right side; R_L = resistance - left side]

This technique is simple from a practical point of view, but the results are not always exactly reproducible with respect to a higher standard deviation of the mean value (SD) and standard error (SE). The results can sometimes be influenced by anatomical abnormalities in the nose (e.g. distinct deviation of the septum) or by slight deformation of the nostril by the adapter-piece in order to prevent leakage of the air. The changes in the PAR parameters (cm H_2O/L/sec) of 30% or more with respect to the baseline are statistically significant. The SD for this technique is 10%, while SE is 6% (our not yet published data).

This technique is quick, easy and suitable, even for small children (with respect to their anxiety for sophisticated equipment)[3,209], for patients with vomiting reflex or for subjects having a problem with a dental prothesis.[3,41b] No high degree of cooperation by the patient and no highly qualified personnel is required for this technique.[3,41b]

[b] Active anterior rhinomanometry (AAR)

The principle of this method is the simultaneous measurement and recording of the airflow and transnasal pressure during the active (tidal) breathing [41j,180,189]. Nasal airway resistance (NAR) is then calculated from the nasal airflow and transnasal pressure. There is one pressure gradient across the nose which generates the airflow through the two nasal passages. The relationship between the pressure and flow, being a complex function due to the changing turbulent airflow and a plot of pressure against flow, is curvilinear. It is, therefore, necessary to define the point on the curve (loop) where the resistance is measured and this is usually at a pressure of 150 Pa. One nostril is sealed with a pressure sensor, by means of surgical tape, in order to measure the nares pressures. The nasal airflow is then recorded from the other nostril. The transnasal pressure is recorded across the nasal passage from the posterior nares to the mask. The resistance to the airflow of each nasal passage is recorded separately by changing the pressure sensor containing cannula alternately from one nostril to the other. The active anterior rhinomanometry involves measuring of the pressure-flow values of each nasal passage separately and then calculating the total nasal resistance.

The resistance is expressed as a pressure differences/unit of flow, in cm H_2O/L/sec (or Pascal units per cm^3 per second = k Pal-1s). There are two basic modifications of this method, one using the face mask, the other using the "nasal olives" (containing the sensors). The total nasal resistance (R_N) may than be calculated from the left (R_L) and the right (R_R) side, according to the following formula:

$$\frac{1}{R_N} = \frac{1}{R_R} + \frac{1}{R_L} \qquad R_N = \frac{R_R \times R_L}{R_R + R_L}$$

Resistance is calculated by analysis of the pressure/flow or alternatively of the pressure/volume curve, for the nostril which has not been occluded.[41j,180,189,190,197]

This technique is moderately laborious, sufficiently sensitive, it produces exact data with a good reproducibility and it does not require a high degree of co-operation by the patient but it does require well-trained personnel. The active anterior rhinomanometry can be performed in almost all adults and many children.[209] On the other hand, this technique has some weak points, such as the tube (or nasal olives) must seal the nostril perfectly to prevent the air-leakage, whereas they should not contact any part of the nasal mucosa; the "nasal olives" or the face mask must closely fit the airways, however, without any deformation or too great compression of the external nose. This technique cannot be used in patients having an unilateral nasal obstruction of more than 80%.[41j]

However, when used for allergen provocation, problems arise if one nostril becomes very occluded at which point the measurement of resistance falls below the limitations of the recording equipment. Moreover, the development of hypersecretion due to the allergen can diminish the tape adherence, resulting in a leakage in the system, then giving false data. This technique has a low standard deviation (SD) and standard error (SE), but it is not suitable for patients with fragile and bleeding nasal mucosa, with frequent sneezing, with vomiting problems [3,41b] or in children with anxiety problems for complicated equipment or even for the face mask. However, the equipment is expensive. The commercially available equipment is for example: OEM Medical Inc, (Fleischmann # 1 Pneumotachograph), Hewlett-Packard 8805 B (tranducers, amplifier and X-Y recorder), Tektronix (oscilloscope) in USA; NART (Nasal airway resistance tester), P.K. Horgan Ltd. in UK, or Mercury System (NRG) in UK.

[c] Active posterior rhinomanometry (APR)

The principle of this technique is the simultaneous recording of flow/pressure ($\dot{V}$-P) or volume/pressure (V-P) parameters during a normal (tidal) breathing. This is performed by use of a face mask fitted with a Fleischmann # 1 pneumotachograph head for monitoring either the nasal air-flow directly or the nasal volume indirectly (= parameter derived from the air-flow value) through both the nasal cavities, and an oral tube connected with p-amplifier and pressure transducer, which is also connected with the mask for monitoring of the transnasal pressure, being definied as ΔP between the pressure in the mask and the pharyngeal pressure.[8,30,41j,186,189,207]

A short oral tube is usually adequate for monitoring posterior pharyngeal pressure.

Firmly gripping the tube between the lips, the patient can keep the soft palate elevated and the upper surface of the tongue well down, allowing undisturbed communication between the oropharynx and the mouth. The most suitable mask for this technique is that covering mouth, nose and eyes, because it does not distort the soft and the cartilaginous parts of the nose. A supplementary oscilloscope for detection of the possible artefacts caused by the malposition of the pharynx and the tongue, and for the air-leaks as well as obstruction of the oral tube may improve this technique substantially. Both the parameters ($\dot{V}$/P or V/P) can also be recorded simultaneously by the X-Y plotter-recorder as an integrated "loop". The other electronic equipment as well as calibration procedure are similar to those of the active anterior technique. The nasal resistance calculated from the parameters recorded by this technique is a total nasal resistance (R_N), expressed in cm H_2O/L/sec. The total resitance R_N of the nasal-airways is calculated by means of the following formula:

$$R_N = \frac{dP}{\dot{V}}$$

The "R_N" is the total nasal resistance expressed in k Pal-/s,"dP" is the pressure difference (kPa) and "$\dot{V}$" is the flow rate (in 1 second). The equipment is produced by Mercury Comp (UK), and WE Collins Comp (USA). The advantage of this method is the possibility to obtain the total nasal resistance even when a distinct obstruction of one of the nostrils or hypersecretion is present, or when the patient underwent a partial resection or extraction of the nasal septum. There are, however, also some disadvantages to this technique. Some of normal subjects do not have sufficient control of their soft palate to allow the pressure-flow recording consistently free of artefacts.[30,41j,180] Other individuals cannot perform this test adequately despite the repeated training[210].

This technique is very sensitive, but also laborious, requires highly trained personnel, is not suitable for children (with regard to the anxiety aspects), for patients suffering from bronchial asthma (in an acute stage), respiratory insufficiency, vomiting problems, hyperventilation syndrome, hypersalivation and for persons wearing a dental prothesis. Additionally, the apparatus necessary for this technique is rather expensive. This technique is, therefore, suitable for research purposes or as an arbitrary test in a situation when the other techniques fail to produce reliable results.[41b,41j,186]

The general recommendation of the European Committee on Standardization of Rhinomanometry has suggested that resistance should be recorded and calculated at the fixed pressure of 150 Pa for the active anterior technique, and at 75 Pa for the active posterior technique[197].

[d] Computerized rhinomanometry

The active anterior rhinomanometry as well as the active posterior rhinomanometry, with respect to the complicated processing of data (analysis of the loop) and time-consuming calculation, can be distinctly improved by means of the combination of this equipment with a computer system, including a storage oscilloscope, X-Y recor-

der, processing unit (at least 64 K of memory), I/0 ports, a 12-bit analogue - to - digital converter, a 12-bit digital - to - analogue converter, a TV display, a terminal and a printer.[30,31,41j,48h] This equipment may also be supplemented by a ready-reading manual sensor, by means of which the loop, recorded by the X-Y recorder, could be directly analyzed, re-calculated and re-checked and in this manner the possible artefacts can be eliminated. The advantages of such a system include: (1) data recording is very accurate and quick; (2) artefacts can be discovered very easily and continuously; (3) the calculation time of the data can be spared; (4) a large number of date can be stored within a short period of time; (5) a high number of measurements can be performed by a parallel schedule and systematically stored[48h]. The computerization of the rhinomanometry represents a distinct improvement, however, it is also very expensive improvement of these techniques.

[e] Modified techniques of rhinomanometry

- The "balloon technique"* [3,7-9,11,11i,12,13,21,25b,48k,48l]

(1) Principle of this method. The nasal mucosa of the patient with rhinitis, when challenged topically with an allergen to which he is sensitive, c.q. with non-specific agent (e.g. histamine, methacholine) reacts with swelling, hypersecretion, sneezing and itching. These changes, especially the appearance or increase in the mucosal edema, due to an allergen or non-specific agent, interfere with the passage of the air through the nose, resulting in an increased pressure difference (Δ P) between the nasopharyngeal cavity and the outside air, while the airflow is constant. These increased pressure differences, the so-called NPG (nasopharynx-nostril-pressure gradients, expressed in cm of H_2O), are recorded and considered to be a parameter of the assessment of the nasal response (= obstruction). The mean NPG values are always recorded and calculated during the regular breathing for 90 to 120 seconds;

(2) The apparatus and equipment consists of a one-channel recorder, an electrical differential pressure transducer (p-amplifier), a water manometer, a small rubber balloon of 5 mm diameter and 25 mm length, and a polyethylene tube connecting system;

(3) Evaluation criteria. The statistically significant increase in the NPG values is 2.0 cm H_2O or more (1.2 ± 0.3 cm H_2O=mean $\pm$ SE; 1.2 ± 0.5 = mean $\pm$ SD);

(4) Procedure. After a calibration of the equipment, the small rubber balloon (25 mm in length and 5 mm in width) is introduced into one of the nasal cavities and placed under the posterior part of the middle turbinate, then connected with the pressure transducer and recorder through a water manometer and filled with 2 ml of air. The polyethylene tube is then fixed to the alae nasi by means of a surgical tape (Micropore). The patient breathes through the non-intubated nasal cavity, the mouth being closed and the intubated cavity also being closed by the patient's fingers placed on the alae nasi. Five to 15 minutes later, when the patient's breathing is stabilized and regular, the test can begin. The application of the diluent solution [= control tests, in the past Coca's solution, recently Phosphate buffered saline (PBS)] as well as of the allergen extract c.q. non-specific agent (e.g. histamine or methacholine) is carried out

* Balloon technique (BT) or method (BM)

by means of a saturated wad of cotton wool on a nasal probe introduced under the middle turbinate of the non-intubated nasal cavity.

(5) This technique, belonging to the modified posterior rhinomanometry, is a contra-lateral method, where one nasal cavity is challenged and the NPG parameters are recorded through the other nasal cavity. This technique is moderately laborious, requires experienced personnel, is highly sensitive, is not influenced by anatomical disproportions or deviations and has an acceptable SE (1.2 ± 0.3 cm H_2O = mean $\pm$ SE) as well as SD (1.2 ± 0.5 cm H_2O = mean $\pm$SD). This technique also demonstrates statistically significant reproducibility. However this method is not suitable for small children, for subjects with fragile or bleeding nasal mucosa and for the patients with the resected or extracted nasal septum.

We use the "balloon technique" as a standard method in adults and older children, the "passive anterior rhinomanometry" in small children and adults with above mentioned disadvantages, while the "volume-pressure method" (active posterior rhinomanometry) and the "flow-pressure method" (active anterior or posterior rhinomanometry) for research purposes or as an arbitrary parameter.

(4) Acoustic rhinometry [48f,48i,48j,211-215]

This technique utilizes the reflection of the sound waves from the nasal cavities to assess the cross-sectional area. The normal curve, measured by an acoustic click, shows the minimal cross-sectional area (I-notch) located at the isthmus nasi, while the second narrowest segment (C-notch) is located at the head of the inferior concha. Any change in the cross-sectional area of the nasal cavities results in a change in the acoustic impedance. In the acoustic rhinometry sound in the range 2-10000 Hz is pulsed into the nasal cavity at a fixed interval whilst the subject breathes in a tidal manner. By the accurate recording of the incident and reflected sound waves within a given time interval, the distance from source to the reflecting body can be determined. This can then be related to the cross-sectional area. The recording of the pressure in the mouth and the flow rates in the nose can then be combined with the acoustic measurements to give the measurement of the nasal airways resistance (NAR). This technique, modification of which is also called oscillometry,[48j, 212,213] has been compared with the more common methods of rhinomanometry and it has been shown to be very reproducible.[48j] The practical use of this technique for the routine nasal provocation has not yet been fully explored. However, this technique seems to be very promising, even for the NPT purposes. In view of the rather high price of the equipment, this technique remains limited to research purposes. Commertially available equipment is for example: Otodynamics, (UK) and Rhinoclack-Stimotron (Germany).

(5) Non-rhinomanometry techniques

Other techniques based on principles differing from those of rhinomanometry, are sometimes used for the assessment of the nasal response, mostly for research purposes:

(a) *Laser Doppler velocimetry* measuring the capillary blood flow in the nasal mucosa, alternating during the nasal response to allergen or non-specific agent.[215-218]

(b) *^{133}Xenon washout clearance* recording the capillary blood flow in the nasal mucosa,

being a result of different components of the nasal vasculature.[182,218]

(c) *Nasometry*, measuring the resonance of the subject's voice within the upper airways (nasal cavities, post-nasal space and sinuses).[219]

(6) Reproducibility of rhinomanometry

There is some criticism concerning the rhinomanometry techniques, especially a number of factors which could alter the recorded parameters, e.g. compression of the soft exterior nasal tissue by a tight fitting of the face mask or air leakage through or along the tape used for nostril occlusion, possible local irritation of the nasal mucosa by the rubber balloon etc.

However, according to our experiences, under standard and well defined conditions (e.g. well-trained personnel, suitable protocol, well-calibrated equipment, standardized environment, allergenic material and procedures), the rhinomanometry is a very useful and reproducible tool and an important part of the routine diagnostic procedure of the nasal allergy disorders.

B. PURPOSE OF THE PROVOCATION TEST

1. WHY THE PROVOCATION TESTS ARE PERFORMED IF THE SKIN TESTS ARE ALREADY POSITIVE?

Although the skin tests are an important diagnostic tool, they should be considered as the first screening method only.[11h,92,198] It is not entirely possible to draw full conclusions from the results obtained on one organ (in this case the skin) for another organ (nose or bronchial tree). Such interpretation and transformation of the results is not unlimited.[11h,25a,26,41f] Apart from the state of the skin itself (hypo-versus hyper-reactive skin, changed metabolism), appearance of skin disease with possible allergic component, e.g. urticaria, eczema, contact allergy, or other skin disorders, the positive skin tests demonstrate only the presence of antibodies (e.g. IgE) to a certain allergen in the skin. There is no sufficient certainty that: (a) the allergen causing a certain response on the skin would also be able to play the same or another role in another organ (e.g. nasal mucosa) and to elicit a response there which would be comparable with that on the skin; (b) the target organ would indeed develop a response to the specific allergen, which can be predicted from the skin response to the same allergen.[3,11h,25a,26,41f]

The skin tests could be influenced by stimuli coming from various other organs and therefore they could also be related to various other organs[11h].

The allergic reaction, in the wide sense of the word, has two basic parts, one central and one local[11h]. The local and central parts can participate to various degrees and in various ratios in the mechanisms underlying the allergic reaction and finally leading to the specific symptoms of the patient. With respect to the variable participation of both the parts, the individual diagnostic tests do not always generate fully relevant results. It is, therefore, necessary to include both the parts (the local as well as the central one) in the diagnostic procedure. For example: the allergic reaction in the nasal mucosa could

be solely a local event with or without changes in the local level of antibodies (specific IgE) and with or without changes in the concentration of the specific IgE antibodies in the serum, and also with or without positive skin tests (=skin reaction to the same allergen).[3,9,11h,41b,41i] The same question arises for the serum RAST or CAP (specific IgE) and PRIST (total IgE), which are also representatives of the central part.[9,11h,41b,41i] The concentration of total IgE in the serum can be influenced by antigen-antibody interactions occuring in various organs and systems. On the other hand, the level of specific IgE in the serum does not necessarily have to be related to an allergic reaction in a certain organ, where the local IgE antibodies may play the main role. Such a reaction would be limited to the organ itself.[3,4,9,11h,41i,88,92,198]

This problem is more complicated if the same patient has a combination of disorders with allergic component (e.g. bronchial asthma, allergic rhinitis and urticaria), where the skin tests and possibly specific IgE in the serum are positive for various allergens. How can we then indicate which allergen would play which role in which organ?

It is, therefore, necessary with respect to the local part of the allergic reaction, to perform provocation tests with the particular allergen on the target organ. Moreover, the provocation test is the only technique for the detection of the particular types of organ response and for the confirmation of the relationships between various organs (e.g. nose-maxillary sinuses, -conjunctivae, -middle ear or -bronchial tree).[11h, 14,14a-14g,17,21,22,26,27,27b,41g,41h]

2. WHY THE PROVOCATION TEST IS PERFORMED ON THE "SICK ORGAN"?

As a model investigation, this test demonstrates the antigen-antibody interaction followed by the consecutive steps in the appropriate (shock) organ. Moreover, this test demonstrates and confirms that this organ indeed responds to the stimulation with the particular allergen by the appropriate, and expected changes and symptoms (response).[11h,26]

Sometimes, an allergen challenge performed on one organ leads to a response in another organ (e.g. cephalgia, conjunctival injection, middle ear response, sinus response or bronchospasm can appear during the nasal provocation tests). This response may correspond with the patient's real complaints. Furthermore, in some patients even the nasal mucosa itself does not develop any response to the certain allergen, while some other organs do. Sometimes a patient is able to develop a bronchospasm after the nasal challenge with allergen, whereas the bronchial challenge with the same allergen does not result in any bronchial response.[4,11h,14a-14g,17,21,22,26,27,27b,41g,41h] In such cases, the administration of a certain drug, e.g. Disodium cromoglycate into the organ where the primary antigen-antibody interaction occurs (e.g. in the nose) may be highly effective in prevention of the secondary symptoms displayed by the other organ (e.g. bronchial tree).[21,26,27,40a,41g,41h]

C. POSITION OF THE "LNR" IN THE HYPERSENSITIVITY EVENTS OCCURING IN THE NASAL MUCOSA - ITS SIGNIFICANCE FOR THE DIAGNOSTIC PROCEDURE AND TREATMENT OF ALLERGIC DISORDERS OF THE UPPER AIRWAYS AND RELATED ORGANS

The allergic component involved in patients with a variety of disorders, such as allergic rhinitis, bronchial asthma, allergic conjunctivitis, urticaria, etc, has classically been attributed to the immediate hypersensitivity (Type I allergy) mechanisms.

This concept has undergone a principal revision upon the discovery and appreciation of the late phase allergic reactions (LPRs), which have been demonstrated in a variety of organs, including skin, lungs, nose, eyes etc.[2,7,11c,11g,11h,14e-14g,16,25,25a,26,27,27a,27b,29,33,34,40a-40f,41a-41d,41f-41i,43,48d,51a,51c,53-56,57a,61-68,68a-68g,72,72a-72c,94,96,96a-96p,97,97c,97u,97v,97w,105-109,121e-121k,124c,124d,131a,133,162y,163l] It has been recognized that the late reactions undoubtedly contribute substantially to the clinical symptomatology of various allergic disorders, including allergic rhinitis, bronchial asthma, urticaria, atopic eczema, allergic conjunctivitis and various forms of the food allergy.

Moreover, the recognition of the late responses has conceptually influenced and finally changed the therapeutic approaches to the allergic diseases.[2,11h,12,14,14d,17,21,26,27b,28,38,40,40a,40c,40d,40f,41b,41g,41h,41i,49,51,51a,65,68a,72a,72b,72d,96a,96c,96k,97w,106,111,111a-111c,112-114,116b,116c,117,117a,117h,121b,121e-121h,121j,121k,121l,124a-124f,124l,161c]

The LNR is regularly involved in the nasal complaints of allergic rhinitis patients, is often overlooked in the practice and may be responsible for the failure of the usual treatments in these patients.[11h,41b]

The LNR participates in the nasal complaints of the patients with allergic rhinitis more frequently than has previously been expected. In our earlier studies we have found the LNR in 5% respectively 11% of the patients with allergic rhinitis.[11h,41b] In our later studies, after the long-term nasal challenges (NPT) had become a part of the routine diagnostic procedure at our department, the LNR has been recorded in approximately 41% of the patients suffering from allergic rhinitis.[41b]

The frequent missing and/or overlooking of the LNR in practice is probably caused by the simple fact, that in majority of the departments and clinics where the patients with nasal allergy are examined, the standard routine diagnostic procedure performed in these patients does not include the nasal provocation tests with allergens, especially those by means of which the LNR may be recorded and confirmed.

Finally, the lack of knowledge of the existence of LNR and its participation in the nasal symptomatology can lead to insufficient therapeutical measures. In this way, the LNR may be responsible for the failure of the usual (first choice) treatment, such as H_1-receptor antagonists, in the patients with allergic rhinitis.[11h,41b]

Although a lot of knowledge concerning the clinical, pathogenetic and immunologic features of the LNR has already been gathered, the immunologic (hypersensitivity) mechanisms underlying this clinical phenomenon are not yet fully clarified.

However, there is growing evidence for a presumption that the mechanisms underlying the LNR may be different from those involved in the immediate nasal response

(INR) as well as from those leading to the delayed nasal response (DYNR).[2,7,11h,13a-13c,41b,48d,51a,51b,53,94,192]

Moreover, the clinical picture and symptoms associated with the LNR differ distinctly from those accompanying both the INR and the DYNR (Tables 2-6).[11h,41b] The LNR can be characterized by a distinct nasal obstruction due to the clear swelling of the nasal mucosa, while the other symptoms, such as hypersecretion and sneezing are present to a lesser degree and the itching is almost absent.

In contrast, the INR is usually associated with the hypersecretion, sneezing, itching and nasal obstruction almost to equal degrees.[2,4,8-11,11a-11e,14,41b, 41c,41f,41g,41i] Finally, the DYNR is associated with severe nasal obstruction, whereas all other symptoms are absent.[2,7,11h,13,13a-13c,41b,41g,41i]

The clinical features supplemented by the other "*in vivo*" and "*in vitro*" diagnostic and/or immunologic parameters, such as concentration of particular immunoglobulin classes and sub-classes in nasal secretions and blood, particular mediators, factors, constituents and compunds in the nasal secretions and blood, cytologic examination of the nasal secretions and blood, and nasal mucosa biopsy, would allow us to formulate the following hypothesis.[2,7,9,11c,11f,11g,11h,12,14b,14c,16,22,25,25a,34,35c,40c,40d,41,41a-41d,41g,48d,48e,49,51a-51c,53,72,72a-72c,94,95a,96,97,97c,97n,97u,121b,121j]

The INR predominantly represents a short-time, transient, "functional" and fully reversible immunologic event in the nasal mucosa, being limited to the nasal epithelium and upper layers of the nasal mucosa, upon involvement of a limited numbers of the cell types, such as mast cells, eosinophils, epithelial cells, and sometimes neutrophils and upon the participation of the specific IgE, sometimes IgG (IgM) antibodies and cell-derived mediators, factors and cytokines.

The LNR represents a largely pronounced, however reversible, and "in the time" gradually developing immunologic event in the nasal mucosa, where lower layers of the nasal mucosa, other mucosal structures, such as blood capillary network, a variety of the cell types, including basophils, eosinophils, neutrophils, epithelial cells, goblet cells, and monocytes, various mediators, chemotactic factors, cytokines, other compounds, cell consistuents and IgG (IgM) antibodies may also participate. The LNR is regularly accompanied by the formation of the tissue eosinophil-neutrophil infiltrates in the deeper mucosal layers (lamina propria), and by a slight, however reversible, epithelium damage upon the involvement of some inflammatory components.

The DYNR is a distinctly pronounced, long-period gradually increasing immunologic event, including almost all layers of the nasal mucosal membrane, upon the involvement of various cell types, such as T-lymphocytes, neutrophils, and monocytes, various cell-derived as well as extracellular (tissue) compound, cytokines, and a distinct inflammatory component. Moreover, the perivascular areas in the profound layers of the nasal mucosa are the predominant location of the numerous infiltrates consisting of neutrophils, lymphocytes, monocytes and plasma cells, accompanied by diffuse haemorhages and disrupted blood capillaries. The DYNR is usually accompanied by the pronounced epithelium and tissue damage, which is, however, mostly reversible. The DYNR represents a serious, however, reversible, immunologic injury of the nasal mucosa.

The LNR can also be characterized by its other special clinical feature, which means it does not correlate satisfactorily with other routinely performed diagnostic "*in vivo*" as well as "*in vitro*" tests and parameters (Table 6).[11h,41b]

The LNR has been found to associate with other diagnostic parameters as follows: (1) positive disease history in 23% of the cases, (2) late skin reaction in 65%, (3) increased serum concentration of total IgE in 6%, (4) positive specific IgE in the serum in 9%, (5) increased serum concentration of IgG in 51%, IgM in 8%, IgG subclasses in 2-19%, (6) increased blood eosinophil count in 23%, (7) increased blood leukocyte count in 13%[11h,41b]. Moreover, the late appearance of the LNR and the accompanying nasal complaints makes it almost impossible to trace this response type and to relate it to an exposure by a certain allergen, by means of the disease history (=medical anamnesis). The majority of the allergic rhinitis patients have no knowledge of the possible participation of the LNR in their nasal complaints.

The insufficient correlation of the LNR with the other diagnostic parameters does not allow the prediction of the existence of this nasal response type from these parameters.

The existence of the LNR can only be demonstrated and confirmed by means of the nasal challenge with allergen [NPT] (= recording of the nasal resistance parameters), supplemented by a number of other "*in vivo*" and "*in vitro*" diagnostic parameters.[3-7,9,11c,11h,12,14,16,25,33, 40c,40d,40f,41,41a-41d,41g,72,72a,72b,72c,96,97]

Moreover, the long-term NPT, supplemented by recording of the other parameters, such as echography, X-ray of the sinuses, lung functions, tympanography, body temperature, blood pressure, pulsus rate, conjunctival appearance etc., may be considered to be the sole technique to confirm the causal role of the LNR in the secondary response of the other (related) organs.[11h,14a-14g,16,17,21,22,26,27,27a,27b,41,41b,41g,41h, 72b,91,96,97]

Furthermore, the differences in the pharmaco-modulation and pharmacologic control of the LNR, in comparison to the INR and DYNR, would be of eminent and of a critical importance for the treatment of the allergic rhinitis in the individual subjects in the practice.[10,11,11d,11e,11i,12,13,13b,14,17,21,26,38,40a-40f,41b,41g,48d,49,57,72a, 72d,82, 97b,97c,121b-121d,121i,121j,125a]

The LNR has also an important significance for some other organs and their responses. The LNR is regularly associated with/or plays a causal role in a secondary response of other, related, organs, such as conjunctival response (in 46%), palpebral edema (in 13%), middle ear response (in 23%) maxillary and frontal sinuses (in 18%), headache (in 47%), migraine (in 18%), bronchial complaints, especially secondary broncho-constriction (in 4-11%) and general malaise complaints (in 6%). From a practical point of view, it is, therefore, very useful to combine the NPT with the monitoring of the other diagnostic parameters and in this way to demonstrate and confirm the causal role of the LNR in the other organ responses. In a limited number of cases, the NPT resulting in the LNR can replace the BPT leading to the LAR.

D. IMPORTANCE OF THE "LNR" FOR RESEARCH PURPOSES

The LNR represents an easibly accessible model for studying the hypersensitivity mechanisms from various points of view, such as immunological, cytological, biochemical and biophysical, which can be related not only to the specific allergen, but also to the clinical course of the LNR and to various changes in the nasal secretions and/or in the nasal mucosal membrane. The LNR can be easily supplemented by a variety of other research parameters. The LNR is an easily producible and reproducible phenomenon. This clinical phenomenon is associated with a very limited number of risks and/or life-threatening factors, and therefore it may be considered as a fairly safe research model. Moreover, the collection of the nasal secretions for the research purposes by means of our technique of blowing the nose onto a polyethylene sheet or the nasal lavage used by other investigators, may be considered to be a much easier and safer method than the broncho-alveolar lavage (BAL) technique. In contrast to the BAL, the collection of the nasal secretions can be repeated without any limitation. Furthermore, the LNR can serve as an interesting and easy model for the pharmacologic research, to investigate the therapeutic and protective effects of various drugs, upon monitoring of a variety of the "*in vivo*" as well as "*in vitro*" parameters. Finally, regarding the frequent occurrence of the LNR, approximately in 41% of patients with allergic rhinitis, it is possible to recruit a large number of subjects in whom the LNR occurs, within a short period of time.[11h,41b]

E. "LNR" AS A RESEARCH TARGET IN THE FUTURE

Despite a great deal of knowledge of the various clinical and immunologic features of the LNR having been already gathered, especially during the last decade, the LNR remains an important, attractive and interesting clinical phenomenon, which still needs more clinical and immunological investigation to clarify the mechanisms underlying this phenomenon and to improve the understanding of its clinical significance. Further studies of the clinical, immunologic, biochemical, biophysical, cytologic and therapeutic control aspects concerning the LNR may contribute to our understanding of this clinical phenomenon. These studies may include following aspects and targets:

(1) The mechanisms, in detail, underlying the LNR;
(2) The participation of the particular cell types and their sequential traffic, cooperation and interaction (stimulation vs inhibition) in the mechanisms leading to the development of the clinical LNR.
(3) The role and significance of a variety of the mediators, constituents, factors and compounds for and in the development of the clinical LNR, including: histamine, heparine, chemotactic factors for eosinophils, neutrophils and other cell types, exoglycosidases, neutral proteases (tryptase, chymase, etc), components of the kinin system, components of the clotting system, cyclooxygenase pathway products (prostaglandins, prostacyclins, thromboxanes), lipooxygenase pathway products (leukotrienes, lipoxines), platelet activating factors (PAFs), bradykinin, serotonin, acetylcholine, cyclic nucleotides (cAMP, cGMP), kallikrein system factors, various primary and secondary enzymes, lysosomal enzymes, hydrolytic

enzymes, neutral hydrolases, lipoprotein lipase, vasoactive amines, growing factors, major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), eosinophil peroxidose (EPO), oxygen-derived free radicals, vascular permeability factors, lipid mediators, complement parts, particular neuropeptides and neurotransmitters, histamine releasing factors (HRF), particular cytokines (lymphokines, monokines, interleukins, colony stimulating factors) and other factors.

(4) The role of particular classes and subclasses of immunoglobulins and their ligands (receptors) on the surface of the particular cells and capillary endothelium, in the individual types of the hypersensitivity mechanisms and processes, including the LNR. Moreover, the exact role of particular circulating and local immunoglobulins should be clarified.

(5) The exact role of the particular electrolytes and the corresponding ions, such as Ca^{++}, CL^{-}, N^{+}, K^{+}, in the various steps of various hypersensitivity mechanisms.

(6) The expression and participation of the various types of the surface receptors (surface determinants, CD molecules), including the adhesion molecules, on the particular types of the circulating as well as non-circulating cells, such as epithelial, endothelial and tissue cells, in the particular types of the hypersensitivity mechanisms and nasal response, among others in the LNR.

(7) The role, in detail, of the particular H-receptors (H_1, H_2, H_3), muscarinic receptors and other tissue receptor classes.

(8) The biochemistry of the cellular membranes, including the transmembrane traffic, membrane-related and transmembrane processes of and in the particular cell types participating in the development of the LNR, such as phosphorylation, hydrolysis of phosphoinositides, activation of protein kinases, activation of phospholipase D.

(9) The mechanism(s) of the release as well as secretion of various mediators, factors and other compounds by the particular cell types participating in the development of the LNR, such as classical degranulation, piecemeal degranulation, semiselective secretion, secretion and surface synthesis.

(10) The mechanism, role and significance of chemotaxis and phagocytosis in the development of the LNR and for the LNR.

(11) The role and significance of the particular subsets of T lymphocytes, especially CD^{+4} (Th_1 and Th_2), CD^{+8}, and natural killers in and for the LNR.

(12) The role of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) as well as their recombinant products in the intercellular traffic involved in the development of the LNR.

(13) The participation and the role of the neurogenic system and its balance, including its adrenergic (sympathetic) and cholinergic (parasympathetic) and the "third nervous system" components in and for the LNR.

(14) Intracellular, intercellular and extracellular signal transduction.

(15) The antigen-processing and antigen-presenting system in the nasal mucosa and the participation of its parts and components in the development of the LNR.

(16) The role of the connective tissue cells, epithelial and endothelial cells in the LNR.

(17) The participation of the nasal mucosal immunity system and nasal associated-lymphatic tissue (NALT), including the secretory IgA (monomer, dimer), particular population and subsets of T lymphocytes and their homing mechanisms, and the interaction of the NALT with bronchial (BALT), intestinal (GALT), skin (SALT) and ear (EALT) systems, in the development of the LNR.

(18) The possible influence of the immunogenicity, antigen specificity, immunologic balance and immunologic tolerance on the LNR.

(19) The pharmacologic modulation, manipulation and control of the LNR as well as of its particular steps and components, which means the effects of the particular drugs and pharmacologic agents, not only of those which are already known and used in practice, but also of those which will be synthetized in the future (specific anti-mediators and specific antagonists of particular factors and participants of the immunologic cascades).

IX. LIST OF ABBREVIATIONS

AAR	=	Active anterior rhinomanometry
AB	=	Asthma bronchiale
Ab	=	Antibody
AC	=	Allergic component
AC	=	Adenyl cyclase
ACE	=	Angiotensin converting enzyme = peptidyl dipeptidase
ACP	=	Alternative complement pathway
AD	=	Distilled water
AE	=	Atopic eczema
Ag	=	Antigen
AGEPC	=	Alkyl glycerol etherphosphoryl-choline
ALL	=	Allergy/allergen
APC	=	Antigen presenting cell
APR	=	Active posterior rhinomanometry
AR	=	Allergic rhinitis
AR	=	Asthmatic response [IAR = Immediate, LAR = Late, DYAR = Delayed Asthmatic Response]
AUC	=	Area under the (dose-response) curve
BA	=	Bronchial asthma
BAL	=	Broncho-alveolar lavage
BALT	=	Bronchus-associated lymphatic tissue
BDA	=	Beclomethasone dipropionate aerosol/nasal spray
BM	=	Balloon method (= BT = Balloon technique)
BPT	=	Bronchial provocation test(s) = Bronchial challenge
Br	=	Bromide
BT	=	Balloon technique (= BM = Balloon method)
BUD/BSA	=	Budesonide
C_3--->$_7$	=	Complement component 3--->7
CAM	=	Cell adhesion molecule
cAMP	=	Cyclic adenosine monophosphate (=adenosine 3',5'-phosphate)
CAP	=	Capacity system (=measurement of specific IgE antibodies)
CCK	=	Cholecystokinin
CCP	=	Classical complement pathway
cGMP	=	Cyclic guanosin monophosphate (=guanosine 3',5'-phosphate)
CGRP	=	Calcitonin gene-related peptide
CD	=	Cluster determinant or cluster differentiation (= receptors = molecules on the cell surface = cell membrane)
Cl	=	Chloride
cPALT	=	Central part-associated lymphatic tissue
CR 1,2,3,4,	=	Complement receptor 1,2,3,4
CTAP III	=	Connective-tissue-activating peptide III
Da	=	Dalton(s) [the unit of relative molecular mass]
DAF	=	Decay accelerating factor
DC	=	Dendritic cell
DSCG	=	Disodium cromoglycate
DYAR	=	Delayed asthmatic response
DYNR	=	Delayed nasal response
DYSR	=	Delayed skin response

EAF	=	Eosinophil activating factor
EALT	=	Ear-associated lymphatic tissue
ECF-A	=	Eosinophil chemotactic factor of anaphylaxis
E-CEF	=	Eosinophil cytotoxicity enhancing factor
Echo	=	Echography
ECP	=	Eosinophil cationic protein
EDF	=	Eosinophil differentiation factor
EDN	=	Eosinophil-derived neurotoxin
EDRF	=	Endothelium-derived relaxant factor (= endothelial relaxing factor)
EDTA	=	Ethylene diamine tetraacetic acid
ELAM - 1	=	Endothelial cell leukocyte adhesion molecule-1
Endo CAM	=	Endothelial cell adhesion molecule
EPA	=	Eicosapentaenoic acid
EpDRF	=	Epithelium-derived relaxant factor
EPO	=	Eosinophil peroxidae
ESP	=	Eosinophil stimulating promoter
Fc	=	Cristallizable fraction of the immunoglobulin molecule
FcR	=	Membrane receptor for Fc
FEV_1	=	Forced expiratory volume in 1 second
FICH	=	Food ingestion challenge
FMLP (=fMLP)	=	N-Formyl-Methionyl-Leucyl-Phenylalanine
G-protein(s)	=	Guanine nucleotide-binding protein
GABA	=	Gamma aminobutyric acid
GALT	=	Gut-associated lymphatic tissue
GATA -1,-2,-3	=	Nuclear transcription factor -1, -2, -3
GC	=	Guanylate cyclase
GCS	=	Glucocorticosteroid
G-CSF	=	Granulocyte colony - stimulating factor(s)
GM-CSF	=	Granulocyte - macrophage colony stimulating factor(s)
GMP-140	=	Granular membrane protein-140
GRP	=	Gastrin releasing peptide
GTP	=	Guanosine triphosphate
H-receptors	=	Histamine-receptors (H_1-,H_2-,H_3-)
HETE -5,-15	=	Hydroxyeicosatetraenoic acid
HHT	=	12-hydroxy -5,8,10-heptadecatrienoic acid
HLA	=	Human leukocyte antigen
HMVEC	=	Human microvascular endothelial cell(s)
HMW	=	High molecular weight
HRA-N	=	Neutrophil-derived histamine releasing activity
HRF	=	Histamine releasing factor(s)
HRIF	=	Histamine release inhibiting factor(s)
5-HT	=	5-Hydroxytryptamine=Serotonin
HUVEC	=	Human umbilical vein endothelial cell(s)
IAR	=	Immediate(Early)asthmatic response
IC	=	Immune complex(es)
i.c.	=	intracutaneous (=intradermal)
ICAM-1,-2	=	Intracellular adhesion molecule -1,-2
IFN-α,β,γ	=	Interferon (α,b,γ)
Ig	=	Immunoglobulin
IH	=	Immediate hypersensitivity

IL_{1-12}	=	Interleukin 1-12
i.m.	=	intramuscular
INCAM-110	=	Inducible cell adhesion mollecule-110
INR	=	Immediate(Early)nasal response
IP_3	=	Inositol triphosphate
ISR	=	Immediate(Early)skin response
IT	=	Immunotherapy
i.v.	=	intravenous
KL	=	Kit ligand = SCF (Stem cell factor)
l/L	=	Liter
LAD	=	Leukocyte adhesion deficiency
LAM-1	=	Leukocyte adhesion molecule-1
LAR	=	Late asthmatic response
LCF	=	Lymphocyte chemoattractant factor
LECAM-1	=	Lectin adhesion molecule-1
LFA-1	=	Leukocyte function-associated antigen-1
LGL	=	Large granular lymphocyte
LH	=	Late hypersensitivity
LMW	=	Low molecular weight
LNR	=	Late nasal response
LPS	=	Lipopolysaccharides
LSR	=	Late skin response
LTA_4	=	Leukotriene A_4
LTB_4	=	Leukotriene B_4
LTC_4	=	Leukotriene C_4
LTD_4	=	Leukotriene D_4
LTE_4	=	Leukotriene E_4
LXA_4	=	Lipoxin A_4
LXB_4	=	Lipoxin B_4
LXC_4	=	Lipoxin C_4
LXB_4	=	Lipoxin D_4
LXE_4	=	Lipoxin E_4
$M_{1,2,3}$	=	Muscarinic receptor 1,2,3
MAC	=	Membrane attack complex
MBP	=	Major basic protein
McAb	=	Monoclonal antibody
MCAF	=	Monocyte chemotactic and activating factor
mcg	=	microgram = mg
MCP-1	=	Monocyte chemotactic and activating factor
M-CSF	=	Macrophage colony stimulating factor(s)
MC_T	=	Mast cell- subtype containing tryptase (=mucosal type)
MC_{TC}	=	Mast cell - subtype containing tryptase and chymase
MDGF	=	Macrophage-derived growth factor
MEP	=	Middle ear pressure
mg	=	milligram
MGF	=	Mast cell frowth factor = SCF (Stem cell factor)
MGG	=	May-Grünwald Giemsa
MHC I,II	=	Major histocompatibility complex I, II
MIP 2	=	Macrophage inflammatory peptide
ML/ml	=	Milliliter

MPO	=	Myeloperoxidase
mRNA	=	Messenger ribonucleic acid
NAF	=	Neutrophil activating factor
NALT	=	Nose-associated lymphatic tissue
NANC	=	Non-adrenergic, non-cholinergic nervous system
NAP -1,-2	=	Neutrophil activating peptide -1,-2
NAR	=	Nasal airway resistance
NCA	=	Neutrophil chemotactic activity
NCF	=	Neutrophil chemotactic factor
NCF-A	=	Neutrophil chemotactic factor of anaphylaxis
NDA/NDS/NS	=	Nedocromil Sodium
NEP	=	Neutral endopeptidase = enkephalinase
NHT	=	Nasal histamine threshold
NK	=	Natural killer
$NK_{1,2,3}$	=	Neurokinin receptor -1,-2,-3
NKA	=	Neurokinin A
NKB	=	Neurokinin B
NMBrT	=	Nasal methacholine bromide threshold
NMChT	=	Nasal methacholine chloride nasal threshold
NPG	=	Nasopharynx-nostril-pressure gradient (in cm H_2O)
NPT	=	Nasal provocation test
NPY	=	Neuropeptide Y
NR	=	Nasal response
NS	=	Nasal secretions
NSAID	=	Non-steroidal anti-inflammatory drugs
N-SH	=	Non-specific hyperreactivity
OMS	=	Otitis media secretory = Secretory otitis media (=SOM)
"p"	=	a "p" value (statistic parameter)
P	=	Pressure
ΔP	=	Delta-pressure = pressure difference(s)
PAF	=	Platelet activating factor(s)
PADGEM	=	Platelet activation dependent granule-external membrane protein
PAR	=	Passive anterior rhinomanometry
PBS	=	Phosphate buffered saline
PC_{20}	=	Provocative concentration, causing 20% fall in FEV
PD_{20}	=	Provocative dose, causing 20% fall in FEV
PECAM-1	=	Platelet-endothelial cell adhesion molecule-1
PG	=	Prostaglandin
PGD_2	=	Prostaglandin D_2
PGE_2	=	Prostaglandin E_2
PGF_{2a}	=	Prostaglandin F_{2a}
PGG_2	=	Prostaglandin G_2
PGH_2	=	Prostaglandin H_2
PGI_2	=	Prostaglandin I_2
PHI	=	Peptide histidine isoleucine
PHM	=	Peptide histidine methionine
PI	=	Phosphoinositide (3-kinase)
PKC	=	Protein kinase C
PLA_2	=	Phospholipase A_2
PLC	=	Phospholipase C

PLD	=	Phospholipase D
PRIST	=	Paper-radio-immuno-sorbent test (estimation of the total IgE antibodies concentration)
RANTES	=	Protein (regulated upon activation, normal T-cell expressed and secreted) from human
RAST	=	Radio-allergo-sorbent test (=estimation of the allergen-specific IgE antibodies concentration)
rh	=	Recombinant variant of a compound/factor
rhSCF	=	Recombinant human stem cell factor
R_L (RL)	=	Resistance of the left side of the nose
R_N (Rn)	=	Total nasal resistance
RNA	=	Ribonucleic acid
R_R (RR)	=	Resistance of the right side of the nose
SALT	=	Skin-associated lymphatic tissue
SBT	=	Salbutamol
SCF	=	Stem cell factor
SD	=	Standard deviation
SE	=	Standard error
SEM	=	Standard error of the mean
SOM	=	Somatostatin
SOM	=	Secretory otitis media (=OMS)
SP	=	Substance P
TAME-esterase	=	N-a-tosyl-L-arginine methyl esther
TCR	=	T-cell receptor (α/β or γ/δ)
TGF	=	Transforming growth factor (α,β,β_1)
Th_1	=	T-lymphocyte of the helper cell subset, type 1 (IL-2, IFN-γ, TNF-β, IL-3, GM-CSF)
Th_2	=	T-lymphocyte of the helper cell subset, type 2 (IL-4, IL-5, IL-6, IL-10, IL-3, CG-CSF)
TNF (a, β)	=	Tumor necrosis factor (α,β)
TPA	=	Tissue plasminogen activator
TXA_2	=	Thromboxane A_2
TXB_2	=	Thromboxane B_2
VAP-1	=	Vascular adhesion protein-1
VC	=	Vital capacity
VCAM-1	=	Vascular cell adhesion molecule-1
VIP	=	Vasoactive intestinal peptide
VLA	=	Very late activation factor (-2,-5,-6)
$\dot{V}$	=	Flow
$\dot{V}$-P	=	Flow-Pressure
V-P	=	Volume-Presure
X-ray	=	Roentgenogram

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XI. SUMMARY

The aim of this thesis is the comprehensive presentation of the clinical phenomenon "late nasal response" [LNR], including results of our own studies, some of them belonging to the fundamental and "first hour" work on this field, and of other investigators' results; to review the present knowledge of this phenomenon and finally to discuss the possible immunologic mechanism(s) underlying this clinical phenomenon.

CHAPTER I. INTRODUCTION

Allergic rhinitis, a non-infectious disorder, can be characterized by a variety of symptoms and complaints, such as nasal obstruction (blockage) due to the swelling of the nasal mucosa, hypersecretion, sneezing and itching. The nasal symptoms can be caused by two different basic mechanisms, an allergy component and a non-specific hyperreactivity component, both of which participate in the same patient to various degrees and ratios. Both the components can exist one beside the other in the same patient, but neither can be regarded as a necessary condition for the other.

The allergy component is due to a variety of immunologic mechanism(s), beginning with an antigen-antibody/T-cell interaction, influencing the immuno-competent/target cell, a process leading to the release of the mediators and other factors acting then on the various effector organs (e.g. smooth muscles, mucosal glands, goblet cells, epithelial cells, endothelial cells, receptors, neurosynapses, capillary network etc.). The combined response of the effector organs results in a variety of the clinical symptoms being characteristic for the particular allergy disorder(s). The allergy component can be of a seasonal or non-seasonal (perennial) character, depending on the kind of allergens involved. Three of the four basic types of hypersensitivity (Type I-immediate, Type III-late and Type IV-delayed) can be involved in the production of the rhinitis symptoms. The particular types of the nasal response (immediate (INR) late (LNR), delayed (DYNR) can be demonstrated by the nasal provocation (challenge) with allergen.

The non-specific hyperreactivity component may lead to a partly comparable spectrum of nasal symptoms with that caused by the allergy component, however, without any initial antigen-antibody/T-cell interaction. The non-specific agents (small molecular chemical compounds, physical factors, temperature differences, vapours, smoke, perfumes, etc, affect either (1) the immuno-competent/target cell directly, this causing the non-specific mediator release, or indirectly, through the stimulation of the nasal mucosal sensory nerves, resulting in the activation and/or release of various neuropeptides which then affect the immuno-competent/target cell; or (2) they act via stimulation of mediator precursors leading firstly to the stimulation of the mediator production, which then act directly on the effector organs and secondly leading to the feed-back inhibition of these mediators and/or immuno-competent cells; or (3) they act directly on the effector organs and their receptors. Nasal challenge with histamine (serotonin, acetylcholine, methacholine, cold air etc.) may simulate and confirm the involvement of the non-specific hyperreactivity and its degree in the nasal complaints of the rhinitis patient.

The nasal provocation tests with allergen (NPT) became widely accepted not only as an important research model for studying the pathophysiologic and pharmacologic aspects of the nasal mucosa, but also as one of the important "in vivo" diagnostic tests for the detection and definite confirmation of the allergic component, the role of the particular allergen in the nasal complaints, the existence of the particular type of the nasal response to a certain allergen and finally the role of nasal mucosa allergy in the responses of the related organs, e.g. paranasal sinuses, middle ear, bronchial tree etc.

CHAPTER II. LATE NASAL RESPONSE [LNR]

The "late nasal response" [LNR] occurring in approximately 41% of the rhinitis patients, appears within 4-8 hours, reaches its maximum within 6-12 hours and resolves within 12-24 hours after the allergen exposure (challenge).

The LNR is characterized by a variety of nasal complaints, the severe nasal blockage (obstruction) being the most prominent one, while the other symptoms (hypersecretion, itching, sneezing) are present to a lesser degree.

The definite confirmation of the existence of the particular types of the nasal response due to a certain allergen, among others the LNR, and their participation in the nasal complaints of the individual patient, can only be provided by the nasal provocation test (=nasal challenge) with allergen [NPT]. The most important aspect of the NPT is the comparison of the objective parameters and subjective complaints before, and repeatedly after the challenge with a certain allergen. The nasal response to an allergen (non-specific hyperreactivity agent) can be recorded and assessed by various methods and techniques, such as (1) recording of subjective complaints by means of a symptom score; and (2) recording of the objective parameters, related to the changed aerodynamics in the nose due to the increased nasal obstruction (=blockage) caused by the swelling (edema) of the nasal mucosa, which is a result of the antigen-antibody) T-cell interaction. The techniques recording objective nasal parameters can be divided into: (1) nasal peak-flow measurement; (2) plethysmography; (3) rhinomanometry (anterior, posterior, combined and/or modified techniques, performed either an active or a passive manner); (4) non-rhinomanometry techniques, such as recording of the nasal blood flow by Doppler velocimetry, the ^{133}Xenon wash out technique, acoustic rhinometry, nasometry, etc.

The NPT can be supplemented by a variety of other "*in vivo*" as well as "*in vitro*" diagnostic parameters and functions, which can supplement the measurement of the LNR or which are able to confirm the causal role of the LNR in the secondary response of the other related organs.

The LNR can regularly be associated with other diagnostic parameters, such as: - positive disease history (in 23%), - rhinoscopic changes (in 90%), - late skin response (in 65%), increased serum concentration of total IgG (in 51%), increased blood eosinophil count (in 23%), increased blood leukocyte count (in 13%), increased nasal responsiveness to histamine (in 15%), methacholine chloride (in 11%), methacholine bromide (in 9%), and precipitating antibodies in the serum, mostly IgG, sometimes IgM.

The LNR can also be accompanied by symptoms in and of other organs, in which it

may play a (causal) role, such as headache, conjunctival symptoms, palpebral edema, middle ear response, paranasal sinuses response, bronchial obstruction and general malaise symptoms.

The LNR can be caused by a large variety of inhalant allergens. The nasal secretions (NS) represent a very interesting and useful medium for the supplementary aspects of the LNR from cytologic, immunologic, biochemical, biophysical and other points of view.

The positive LNR is usually accompanied by significant changes in the count of neutrophils in 84% of the cases (increase immediately before, decrease during the development and increase during the resolving of the LNR), eosinophils in 58% (increase immediately before, and decrease during the appearance of the LNR), epithelial cells in 73% (increase followed by decrease, running parallel with the course of the LNR), goblet cells in 63% (increase followed by decrease), basophils in 8% and lymphocytes in 6% of the cases (both the cell types demonstrate a slight increase in their count during the LNR). No significant changes are usually recorded in the counts of other cell types in NS during the positive LNR.

In most neutrophils (94%) eosinophils (49%) and basophils (3%) appearing in the nasal secretions during the positive LNR, various cellular and intracellular changes, such as degranulation or disappearance of the cytoplasmic granules, vacuolization, diminished intake of stain, wrinkling of the cellular membrane, sometimes also cell disruption, have been recorded.

The positive LNR may be accompanied by appearance of the total IgG antibodies in the nasal secretions in 46% of the cases and by a decrease in the IgG concentration in the NS during the LNR in 33% of the cases. Neither significant concentrations nor significant changes in the concentrations of the immunoglobulins of other classes have been recorded in the nasal secretions and blood serum during the positive LNR.

The LNR may be accompanied by increased concentration of some mediators and compounds in NS, such as kinins, TAME-esterase (N-a-tosyl-L-arginine methylester), leukotrienes B_4 (LTB_4), C_4 (LTC_4), D_4 (LTD_4), E_4 (LTE_4), major basic protein (MBP), eosinophil derived neurotoxin (EDN), bradykinin, lysylbradykinin, eosinophil cationic protein (ECP), neutrophil chemotactic factor (NCF), prostaglandin ($PGF_{2\alpha}$) and histamine. However, controversial results have been reported concerning the detectable amounts of histamine and the significant changes in its concentration in the NS during the positive LNR.

The biochemical and biophysical aspects of the nasal secretions during the positive LNR have not yet been studied sufficiently. There is a great dearth of knowledge on this topic.

The positive LNR may be accompanied by the following histologic changes in the nasal mucosa (=biopsy) as compared with the "pre-challenge" baseline: (1) The edematic epithelium with damaged compactness, enlarged intercellular spaces, sporadic breaches filled with fluid, sporadic empty holes on the epithelial surface due to the release of some epithelial and goblet cells from the epithelial context; (2) The basement membrane with irregular compactness and single breaches; (3) The edematic sub-epithelial layer of lamina propria containing mixed eosinophil-neutrophil infiltrates and

sporadically single mast cells, basophils, monocytes and lymphocytes; (4) A perivascular edema, dilatation of the terminal parts of the capillaries and sporadic rupture of the small capillaries with erythrocyte expulsion. The histologic changes recorded in the nasal mucosa during the positive LNR may be qualified as a slight reversible tissue damage of the nasal mucosa with some inflammatory components.

CHAPTER III. THE "LATE NASAL RESPONSE" TO FOOD.

The role of food allergy, and of food in general, in subjects with various allergic disorders, among others in patients with nasal symptoms and complaints, has already been repeatedly reported. Three basic types of nasal response, following the food ingestion challenge, have been recorded: (1) an immediate/early response [INR/ENR], appearing within 70 minutes, peaking within 105 minutes and resolving within 180 minutes; (2) a late response [LNR], beginning within 6 hours with a peak within 10 hours and resolving within 24 hours; (3) a delayed response [DYNR], starting within 24-28 hours, peaking within 32-36 hours and resolving within 48-52 hours after the food ingestion.

The LNR caused by "adverse reactions to foods" (the food allergy being one of the suspect mechanisms), should be regarded as a special form of the LNR.

The LNR occurs in approximately 47% of the patients with allergic rhinitis and is associated with a variety of other "*in vivo*" and "*in vitro*" diagnostic parameters, such as: positive disease history in 29%, positive late skin response in 48%, increased total IgG antibodies in the serum in 24%, increased blood eosinophil count in 8%, increased blood leukocyte count in 9%, nasal symptoms, predominantly nasal obstruction in 96%, followed by itching in 51% and hypersecretion in 14%, aspect of the nasal mucosa: hyperemic in 23%, violaceous in 76%, changes in the count of eosinophils in 63%, of neutrophils in 89%, of epithelial cells in 46%, goblet cells in 51% and basophils in 15% in the nasal secretions.

Similarly to the LNR due to the "inhalant" allergens, the LNR due to the food ingested can regularly be accompanied by symptoms and responses in other organs, such as headache, migraine, conjunctival symptoms, middle ear response, bronchial obstruction, paranasal sinus response, gastro-intestinal symptoms, and general malaise complaints.

The positive LNR due to the food ingested can be prevented highly significantly by oral Disodium cromoglycate (DSCG, Cromolyn, Nalcrom®), oral glucocorticosteroids (GCS) and partly intravenous GCS, whereas oral H_1- as well as H_2-receptor antagonists and nasal topical GCS have not been able to affect this form of LNR significantly.

CHAPTER IV. PHARMACOLOGIC MODULATION OF "LNR".

The positive LNR due to the "inhalant" allergens can be prevented significantly by topical (intranasal) Disodium cromoglycate (DSCG, Cromolyn), topical glucocorticosteroids (GCS), oral corticosteroids and topical Nedocromil Sodium (NDS/NS), whereas most of the H_1- and H_2-receptor antagonists (antihistamines) did not demonstrate any significant effects on the LNR.

DSCG, GCS and NDS have also demonstrated significant protective effects on the

particular cell types appearing in the nasal secretions during the LNR by decreasing their counts (or preventing the increase in their count) and by preventing the development of various cellular and/or intracellular changes in these cell types.

CHAPTER V. DIFFERENTIAL DIAGNOSIS.

The LNR differs substantially from the immediate/early nasal response [INR/ENR] and the delayed nasal response [DYNR] with respect to (1) the clinical characteristics, such as the time-course, symptomatology, appearance of the nasal mucosa, association with other "*in vivo*" and "*in vitro*" diagnostic parameters and appearance of symptoms in other organs accompanying the particular response type; (2) the morphologic characteristics, such as cytologic changes in the nasal secretions, and histologic changes in the nasal mucosa, associated with the particular type of nasal response; (3) the immunologic features, such as appearance of the particular classes and sub-classes of the immunoglobulins in the nasal secretions as well as in the blood serum and/or blood plasma and the changes in their concentrations, and the appearance of particular mediators and/or other compounds and factors in the nasal secretions and/or in the blood and the changes in their concentrations during the particular types of nasal response; (4) the pharmacologic modulation and control of the particular types of nasal response as well as the effects of individual drugs (=pharmacologic agents) on the particular nasal response types.

CHAPTER VI. REPRODUCIBILITY AND CREDIBILITY OF THE "APPLICATION METHOD" AND THE "BALLOON TECHNIQUE".

The "application method", this means the application of the allergen on the nasal mucosa by means of a wad of cotton wool on a nasal probe placed under the medial concha for a certain interval of time (mostly for 3 minutes), and the "balloon method" of rhinomanometry by means of which the nasal parameters have been recorded and the degree of the nasal obstruction (=nasal blockage) assessed, used by us, have been investigated with respect to their reproducibility and credibility (appearance of artefacts, mechanical irritation of the nasal mucosa, stability of the base-line, comparison with other techniques of rhinomanometry).

In three groups of patients [non-rhinitis subject; subjects with allergic rhinitis demonstrating only immediate nasal response to "inhalant" allergens, but with normal (non-decreased) nasal histamine threshold; subjects with nasal complaints due solely to the non-specific hyperreactivity represented by the decreased nasal histamine threshold, but without participation of any form of allergy component], the following tests have been performed:(1) The recording of the baseline NPG parameters during a sufficiently long interval of time; (2) After the repeated baseline, the dry wad of cotton wool on the nasal probe has been introduced into the non-connected nostril under the middle turbinate for 3 minutes, followed by the recording of the NPG parameters; (3) The wad of cotton wool on the nasal probe saturated with Phosphate buffered saline (PBS) has been applied on the same location for 3 minutes, followed by the recording of the NPG parameters; (4) The nasal threshold for histamine has been determined again. With respect

to the results, it has been concluded that the "application method" of the allergen used by us has not produced any significant artefacts, mechanical irritation of the nasal mucosa, or any significant influence on the nasal provocation tests and their results.

The "balloon technique" has been compared with the "flow-pressure" technique (active posterior rhinomanometry) in one group of patients with allergic rhinitis, before and after the allergen challenge, and with "PAR" (passive anterior rhinomanometry) in another group of patients with allergic rhinitis before and after the allergen challenge (=before and during the positive immediate nasal response to allergen challenge = INR). No statistically significant differences have been found either between the INR recorded by the "balloon technique and the INR recorded by the "flow-pressure" technique or the INR recorded by the "PAR". Moreover, the baselines recorded by the "balloon technique" in pollen-related allergic rhinitis patients and the non-allergic subjects did not differ significantly during a 12-hour period.

CHAPTER VII. POSSIBLE MECHANISM(S) UNDERLYING THE "LNR".

The various pathogenetic and immunologic mechanisms, which may presumably underlie the clinical phenomenon "LNR" have been discussed from various points of view and our own data concerning this topic have been compared with other investigators'data and general knowledge on this field.

Furthermore, the involvement of the late type hypersensitivity mechanism(s) on one hand and of the immediate hypersensitivity mechanism(s) and their parts and components on the other hand in the clinical LNR have been considered, compared and discussed.

The LNR should be regarded as a clinical phenomenon, defined by the appearance of nasal symptoms and complaints, predominantly nasal obstruction, accompanied by other symptoms and changes, within 4 to 12 hrs after the allergen exposure (antigen-antibody interaction or challenge), which can be induced by a complex mechanism. Although the pathogenetic and immunologic mechanisms leading to the LNR can be different, the late type hypersensitivity should be regarded as one of the possible mechanisms involved in the clinical LNR, but is far from being the only one.

The possible role and involvement of various components of the late hypersensitivity mechanism(s), either through the complex pathways or their participation as individual and single components in the patho-mechanism leading to the development of the clinical LNR, cannot be excluded. The presumed components are as follows: (1) immune complexes; (2) complement system and its parts, such as classical complement pathway, alternative complement pathway, the membrane attack complex, receptors for complement components on the membrane of various cell types; (3) IgG and/or IgM antibodies; (4) particular cell types, such as neutrophils, platelets, eosinophils, mast cells and basophils (including histamine releasing factors, cytokines, stem cell factors, neuropeptides and various compounds activating the mast cells and basophils).

Moreover the innervation and neurogenic control of the nasal mucosa, including neuropeptides as neurotransmitters in the human nasal mucosa, could, under certain circumstances, be involved in the development of the general conditions of the nasal mucosa then allowing expression of some steps of the immunologic pathways in the

human nasal mucosa.

On the other hand the possible role and involvement of various components of the immediate hypersensitivity mechanism(s), either through the classical pathways or as single components in the development of the late-phase reactions, among others in LNR, has already been suggested by some investigators. The presumed components are as follows: (1) The antigen-specific IgE antibodies; (2) The mast cells and basophils. However, our results would not support the proposed unequivocal role of the IgE antibodies as well as mast cells and/or basophils in the development of the clinical LNR.

CHAPTER VIII. GENERAL DISCUSSION

The detection and definite confirmation of the existence of particular types of the nasal response, among others the LNR, due to a certain allergen, and their participation in the nasal symptoms of the individual patient, can only be provided by the nasal provocation tests [NPT] (=nasal challenge) with allergen. The NPT is also the only technique to discriminate the allergy component due to the allergen from the non-specific hyperreactivity due to the non-specific agents, being simulated by the nasal challenge with histamine, methacholines or cold air.

The NPT can be considered not only as an important research technique and model for the investigation of the pathophysiological and pharmacological aspects of the nasal mucosa, but also as an important part of the routine diagnostic procedure of the allergic disorders in the nose and of the possible role of the nasal allergy in the related organs. The most important aspect of the NPT is the comparison of the objective parameters and simultaneously also subjective complaints before and repeatedly after the challenge with a particular allergen (or non-specific agent).

There are three basic types of the NPT; (1) NPT with allergen, confirming the allergy component in the nose and the existence of a certain type of nasal response due to a certain allergen; (2) NPT with non-specific agents or the simulators, such as histamine, methacholines, cold air, confirming the non-specific hyperreactivity in the nose and its participation in the nasal complaints; (3) NPT in combination with pharmacologic agents, so-called "nasal protection tests", which may conform or exclude the effects of a certain drug on the nasal symptoms and the nasal response of a certain type due to a certain allergen or non-specific agent.

The NPT should meet some necessary requirements and conditions, these being that they should be safe, reproducible, sufficiently sensitive, free of artefacts, as comfortable as possible for the patient as well as for the technician, reliable with respect to the application of the allergen/non-specific agent (e.g. effective but not dangerous dose).

The basic indications for the NPT include the conformation of the role of a certain allergen/non-specific agent in the nasal complaints of the particular patient, the confirmation of the existence of the particular type of the nasal response, the discrimination of the allergy component and the non-specific hyperreactivity component, the establishment of the dose-response and time-response curves, the demonstration of the involvement of the nasal mucosa and nasal allergy in the response of the other (related) organs, the investigation of possible effects of certain drugs on the nasal allergy and/or the particular types of nasal response, the monitoring of immunotherapy, and finally va-

rious research purposes.

The application of the allergen or non-specific agent to the nasal mucosa can be performed by various methods, each of them having specific advantages and disadvantages, such as dropping of the particular extracts or dilutions, aerosolization method, use of micropipettes, by means of impregnated paper discs or cuvettes inserted into the nose, or by means of a wad of cotton wool on a nasal probe saturated with the appropriate extract and placed in the nose.

The nasal response due to the challenge with an allergen or non-specific agent may be recorded by means of various techniques, such as anterior rhinoscopy, recording of the subjective complaints or recording of the objective parameters, mostly nasal airway resistance (NAR). The latter techniques can be divided into 5 groups: (1) nasal peak-flow measurement; (2) plethysmography; (3) rhinomanometry (anterior, posterior, active, passive, combined and/or modified techniques); (4) acoustic rhinometry; (5) non-rhinomanometry techniques, such as recording of nasal blood flow using Doppler velocimetry, or ^{133}Xenon washout clearance technique, acoustic rhinometry and nasometry measuring the resonance of the subject's voice.

The NPT and their diagnostic value cannot be replaced by other diagnostic tests and procedures, such as skin tests, PRIST/RAST, etc, with respect to their limitations and to the limited transfer of the conclusions drawn on one organ, for another organ.

The LNR represents a largely pronounced, however reversible, and "within the time" gradually developing immunologic event in the nasal mucosa, where also the lower layer of the nasal mucosa, other mucosal structures, such as blood capillary network, a variety of the cell types, including basophils, eosinophils, neutrophils, epithelial and goblet cells, and monocytes, and IgG (IgM) antibodies may participate. The LNR is regularly accompanied by the formation of the eosinophil-neutrophil infiltrates in the deeper mucosal layers, and by a slight, however reversible, epithelium damage with involvement of some inflammatory components.

The LNR represents an easily accessible model for studying the hypersensitivity mechanisms from various points of view, such as immunological, cytological, biochemical, biophysical, pharmacological and therapeutic, which can be related not only to the certain allergen, but also to the clinical course of the LNR, and to various changes in the nasal mucosal membrane. Moreover, the LNR can easily be produced and reproduced, and is associated with a very limited number of risks, and therefore may be considered as a safe clinical research model.

Despite the fact that a lot of data has already been gathered and generated, the LNR still remains an important and interesting clinical phenomenon, which needs more concurrent clinical and immunological studies to clarify the mechanisms underlying this clinical phenomenon and to improve our understanding of its clinical significance.

SAMENVATTING

Het doel van dit proefschrift is om een complete presentatie te geven van de huidige kennis van het klinische fenomeen "late nasale respons" [LNR]. Om dit doel te bereiken zijn naast eigen studies ook onderzoeksresultaten van anderen gebruikt, waarvan sommige fundamenteel en van het "eerste uur" zijn. De immunologische mechanismen die mogelijk een rol spelen bij de LNR worden hier besproken.

Hoofdstuk I.
INTRODUCTIE

Allergische rhinitis is een niet-infectieuze aandoening, die gekenmerkt wordt door een aantal typische symptomen, zoals neusobstructie, hypersecretie, niezen en jeuk in de neus. Deze neussymptomen kunnen in principe veroorzaakt worden door twee volstrekt verschillende basismechanismen: een allergie en een aspecifieke hyperreactiviteit. Beide mechanismen kunnen bij de neusklachten van dezelfde patiënt in diverse gradaties voorkomen. Beide kunnen naast elkaar bestaan bij dezelfde patiënt, maar geen van beide functioneert als een noodzakelijke voorwaarde voor de ander.

Diverse immunologische mechanismen kunnen ten grondslag liggen aan de allergische reactie. Het basisschema is als volgt: de allergische reactie begint met een antigeen (allergeen)-antilichaam/T-cel-interactie, die invloed uitoefent op de immunocompetente doelcel. Dit proces leidt tot het vrijkomen, eventueel na nieuwe synthese, van een aantal mediatoren en andere factoren, die hun invloed uitoefenen op zogenoemde effectororganen. Dit kunnen b.v. gladde spiercellen zijn in organen of in de capillaire bloedwand, slijmvliesklieren, epitheel-, endotheel- en bekercellen, diverse weefsel- en cellulaire membraangebonden receptoren, neurosynapsen, capillaire netwerken, evenals andere celtypen, die zich in het weefsel of in de bloedcirculatie bevinden. De gecombineerde respons, gevormd door de individuele responsen van de effectororganen, manifesteert zich door middel van klinische symptomen, die karakteristiek zijn voor de betreffende allergische aandoening.

De allergie kan seizoensgebonden of niet-seizoensgebonden ("perennial") klachten en symptomen geven. Dit is afhankelijk van het soort allergeen. Er bestaan 4 basistypen van allergische (hypersensitiviteit)reacties: (1) de type I- ofwel "directe" reactie; (2) de type II- ofwel "cytotoxische" reactie; (3) de type III- ofwel "late" reactie (Arthus fenomeen, vorming van immuuncomplexen); (4) de type IV- ofwel "vertraagde" ("delayed", celgemedieerde) reactie. Drie van deze vier basis allergische reactietypen, de typen I, III en IV, kunnen betrokken zijn bij de allergische rhinitis. De individuele neusresponstypen, met name de directe (INR), late (LNR) en vertraagde (DYNR) respons, kunnen gedemonstreerd worden door middel van de neusprovocatietest met allergenen.

Het aspecifieke hyperreactiviteitmechanisme wordt niet geïnitieerd door een antigeen-antilichaaminteractie, maar door aspecifieke prikkels: klein-moleculaire chemische en fysische factoren, zoals temperatuursveranderingen, dampen, tabaksrook, parfums etc. Dit gebeurt op 3 mogelijke manieren: (1) de aspecifieke prikkels beïnvloeden de immunocompetente doel-cellen ofwel (a) op directe wijze, waardoor mediatoren

vrijkomen ("non-specific release of mediators"), ofwel (b) op indirecte wijze via de stimulatie van "sensory nerves", wat resulteert in de activering en/of het vrijmaken van diverse neuropeptiden, die daarna de immunocompetente doel-cellen secundair beïnvloeden; (2) de aspecifieke prikkels stimuleren in eerste instantie de precursoren van de mediatoren, wat leidt tot (de stimulatie van) de aanmaak van de mediatoren, die vervolgens de effectororganen direct beïnvloeden. In tweede instantie leidt deze stimulatie tot de feed-back inhibitie van deze mediatoren en/of de immunocompetente doelcellen; (3) de aspecifieke prikkels beïnvloeden direct de effectororganen en hun receptoren.

De aspecifieke hyperreactiviteit leidt via deze mechanismen tot de ontwikkeling van de neussymptomen, die gedeeltelijk kunnen overeenkomen met de neussymptomen veroorzaakt door de allergie.

De neusprovocatietests met histamine, serotonine, acetylcholine, methacholines, koude lucht, etc., simuleren en bevestigen de participatie van de aspecifieke hyperreactiviteitcomponent en het aandeel van deze component in de nasale symptomen van de rhinitispatiënt.

De neusprovocatietests met allergeen alsook met aspecifieke prikkels worden steeds meer geaccepteerd en gewaardeerd, niet alleen als een belangrijk onderzoeksmodel voor het bestuderen van de pathofysiologische en farmacologische aspecten van het neusslijmvlies, maar ook als een van de meest belangrijke "*in vivo*"-diagnostische tests voor de detectie en definitieve bevestiging van de rol van de allergie en de aspecifieke hyperreactiviteit bij de rhinitisklachten van de individuele patiënt. De neusprovocatie met allergenen is de enige onderzoeksmethode waarmee men het bestaan van een bepaald type van neusrespons, veroorzaakt door een bepaald allergeen, kan aantonen en onderscheiden van de andere neusresponstypen. De neusprovocatietest is bovendien de enige methode waarmee de mogelijk causale rol van het neusslijmvlies of van de nasale allergie bij de respons van andere organen kan worden aangetoond, bijvoorbeeld bij hoofdpijn, migraine, otitis media, conjunctivitis, of een astmatische respons waar de nasale allergie een primaire rol kan spelen.

Hoofdstuk II.
LATE NEUSRESPONS [LNR]

De "late neusrespons" [LNR] manifesteert zich bij ongeveer 41% van de patiënten met allergische rhinitis. Deze respons begint na 4-8 uren, bereikt een maximum binnen 6-12 uren en eindigt 12-24 uren na de allergeen- expositie.

De LNR kan gekarakteriseerd worden door diverse neussymptomen, waarvan de neusobstructie het prominenste symptoom is. Andere neussymptomen, zoals hypersecretie, niezen en jeuk in de neus, treden duidelijk minder frequent op.

Het belangrijkste aspect van de neusprovocatietests is de vergelijking van de subjectieve klachten en de objectieve nasale parameters voor en herhaaldelijk na de neusprovocatie met een bepaald allergeen. De neusrespons, die wordt veroorzaakt door een bepaald allergeen of door een aspecifieke prikkel, kan worden gemeten en geregistreerd door tal van methoden en technieken, zoals: (1) de beoordeling van subjectieve klachten door middel van een score, (2) de meting en registratie van objectieve parameters, die

gerelateerd worden aan de veranderingen van de aërodynamiek in de neus, die worden veroorzaakt door de toenemende neusobstructie als gevolg van het oedeem van de nasale mucosa. De meettechnieken waarmee de neusslijmvliesreacties beoordeeld kunnen worden zijn: (1) nasale "peak-flow"-meting; (2) plethysmografie; (3) rhinomanometrie in diverse uitvoeringen, b.v. in actieve of passieve vorm ten opzichte van de patiënt of als anterieure, posterieure, gecombineerde of gemodificeerde techniek, afhankelijk van de meetlocatie in de neus; (4) "akoestische rhinometrie"; (5) niet-rhinomanometrietechnieken, zoals "nasale blood-flow"-meting door middel van "Doppler velocimetrie" of de "^{133}Xenon wash-out techniek".

De neusprovocaties kunnen worden aangevuld met de bepaling van tal van andere "*in vivo*" alsook "*in vitro*" diagnostische parameters en functies om de resultaten nauwkeuriger te definiëren of om de primaire rol van de neusrespons (LNR), b.v. bij de secundaire respons van andere (verwante of aangrenzende) organen te bevestigen.

Procentueel gezien is het gecombineerd voorkomen van LNR en andere diagnostische parameters als volgt: positieve anamnese in 23% van de gevallen, rhinoscopische afwijkingen (90%), late huidrespons (65%), verhoogde concentratie van totaal IgE in het serum (6%), positieve specifiek IgE in het serum (9%), verhoogde concentratie van totaal IgG in het serum (51%), verhoogd aantal bloedeosinofielen (23%), verhoogd aantal bloedleukocyten in (13%), verlaagde drempel in de neus voor histamine (15%), methacholine-chloride (11%) en methacholine-bromide (9%), positieve precipiterende antilichamen in het serum, meestal van de IgG-, soms van de IgM-klasse bij sommige specifieke LNR typen, b.v. voor extracten van vogelmest, tot 80% van de gevallen.

De LNR kan ook een (primaire) rol spelen bij het ontstaan van een secundaire respons van andere organen, waardoor parallel aan de LNR de volgende klinische verschijnselen kunnen optreden: cefalgie, migraine, conjunctivale symptomen, palpebraal oedeem, otitis media (secretoir), reactie van de paranasale sinussen, bronchusobstructie, algemene malaise etc.

De LNR kan ontstaan na de expositie aan verschillende "inhalatieallergenen". Deze zijn alle even geschikt om een LNR te veroorzaken.

Het neussecreet (NS) kan men beschouwen als een zeer interessant en bruikbaar medium voor het bestuderen van diverse cytologische, immunologische, biochemische en biofysische parameters tijdens de LNR.

De positieve LNR is meestal verbonden met significante veranderingen in de aantallen van individuele celtypen, zoals van de neutrofielen in 84% van de gevallen (verhoging onmiddellijk voor het verschijnen van de LNR, daling tijdens het maximum van de LNR, en weer verhoging tijdens de aflopende fase van de LNR), eosinofielen in 58% (verhoging onmiddellijk voor het verschijnen van de LNR en daling tijdens de ontwikkeling van de LNR), epitheelcellen in 73% (verhoging gevolgd door daling, parallel verlopend aan de LNR), bekercellen in 63% (verhoging gevolgd door daling), basofielen in 8% en lymfocyten in 6% van de gevallen (beide celtypen nemen in aantal toe tijdens de LNR).

De meeste neutrofielen (94%), eosinofielen (49%) en basofielen (3%), die in het neussecreet verschijnen tijdens de positieve LNR, vertonen diverse cellulaire en intracellulaire veranderingen, zoals degranulatie van hun cytoplasmatische granulae, vacuolisatie, verminderde kleurstofopname, krimping van de cellulaire membraan en soms ook

een cellulaire disruptie.

De positieve LNR kan gepaard gaan met de verschijning van totaal-IgG- antilichamen in het neussecreet in 46% van de gevallen en met een daling van de concentratie van totaal IgG tijdens de LNR in 33% van de gevallen. Tijdens de positieve LNR worden geen significante veranderingen in de concentratie van de andere klassen van immunoglobulinen in het neussecreet of in het bloedserum waargenomen.

Tijdens de positieve LNR worden verhoogde concentraties van diverse mediatoren en andere factoren in het neussecreet gemeten, zoals: kininen, TAME-esterase (N-a-tosyl-L-arginine-methylester), de leukotriënen LTB_4, LTC_4, LTD_4, "major basic protein" (MBP), "eosinophil-derived neurotoxin" (EDN), "bradykinin", "lysylbradykinin", "eosinophil cationic protein" (ECP), "neutrophil chemotactic factor" (NCF), prostaglandinen ($PGF_{2\alpha}$) en histamine. Over de meetbare concentraties van histamine in het neussecreet en veranderingen ervan tijdens de LNR bestaan controversiële gegevens.

De biochemische en biofysische aspecten van het neussecreet tijdens de LNR werden tot nu toe onvoldoende onderzocht. Hierover is derhalve weinig bekend.

De positieve LNR gaat gepaard met de volgende histologische veranderingen in het neusslijmvlies (biopsie), ten opzichte van het histologisch beeld vóór de allergeenprovocatie (= "baseline"): (1) het epitheel is oedemateus en vertoont een beschadigde compactheid, vergrote intercellulaire spaties, sporadische scheuren opgevuld met vocht, sporadische lege holtes aan de oppervlakte, die zijn ontstaan door het uitvallen van sommige epitheel- en bekercellen; (2) de basale membraan vertoont een onregelmatige compactheid en sporadische scheurtjes; (3) de oedematische subepitheliale laag van de lamina propria bevat gemengde eosinofiel-neutrofiele infiltraten en sporadisch ook mestcellen, basofielen, monocyten en lymfocyten; (4) er bestaat een perivasculair oedeem, meestal met dilatatie van de terminale delen van de capillairen. Soms ziet men een ruptuur van de kleine capillairen met uitgestoten erytrocyten.

Deze histologische veranderingen van het neusslijmvlies die optreden tijdens de positieve LNR, kan men kwalificeren als een lichte reversibele slijmvliesbeschadiging met enige inflammatoire componenten.

Hoofdstuk III.
LATE NEUSRESPONS VEROORZAAKT DOOR VOEDINGSMIDDELEN

De rol van de voedingsmiddelenallergie en van voedingsmiddelen in het algemeen bij patiënten met verschillende allergische aandoeningen, b.v. met nasale klachten en symptomen, is in de literatuur al herhaaldelijk vermeld. Er bestaan 3 basistypen van neusrespons bij de orale consumptieprovocatietests met levensmiddelen: (1) de directe neusrespons [INR/ENR]. Deze verschijnt binnen 70 minuten, bereikt een maximum binnen ongeveer 105 minuten en verdwijnt binnen 180 minuten; (2) de late neusrespons [LNR]. Deze begint binnen 6 uren, bereikt een maximum binnen 10 uren en verdwijnt binnen 24 uren; (3) de vertraagde neusrespons [= "delayed"; DYNR]. Deze ontstaat binnen 24-28 uren, bereikt een maximum binnen 32-36 uren en verdwijnt binnen 48-52 uren na de consumptietest met een bepaald voedingsmiddel.

De LNR die wordt veroorzaakt door een zogenaamd ongewenste reactie op voedingsmiddelen (voedingsmiddelenallergie) behoort tot de speciale vormen van de LNR.

De LNR op voedingsmiddelen komt voor bij ongeveer 47% van de patiënten met allergische rhinitis en gaat gepaard met tal van andere "*in vivo*" alsook "*in vitro*" diagnostische parameters, zoals: positieve anamnese in 29%van de gevallen, positieve late huidrespons (48%), verhoogde concentratie van totaal-IgG- antilichamen in het serum (24%), verhoogd eosinofielengetal in het bloed (8%), verhoogd leukocytengetal in het bloed (9%); nasale symptomen, vooral neusobstructie (99%), gevolgd door jeuk in de neus (51%) en hypersecretie (14%), hyperemisch aspect van het neusslijmvlies (23%) en livide aspect (76%), veranderingen in het neussecreet van het aantal eosinofielen (63%), neutrofielen (89%) epitheelcellen (46%) bekercellen (51%) en basofielen (15%).

Analoog aan de LNR veroorzaakt door inhalatieallergenen wordt ook de LNR voor de voedingsmiddelen vaak begeleid door parallel verschijnende symptomen en secundaire reacties van andere organen. Dit manifesteerd zich klinisch als b.v.: hoofdpijn, migraine, conjunctivale symptomen, oorklachten, bronchusobstructie, sinusopathie, gastro-intestinale symptomen en algemene-malaisesymptomen, etc.

De LNR op levensmiddelen kan zeer goed (significante effecten) preventief worden behandeld met oraal cromoglycinezuur (DSCG, Nalcrom®), orale glucocorticosteroïden en soms met intraveneuze toediening van glucocorticosteroïden. Er werden geen significant preventieve effecten van de oraal of lokaal toegediende H_1- en H_2-receptorantagonisten, evenals van de nasaal toegediende glucocorticosteroïden op deze vorm van LNR aangetoond.

Hoofdstuk IV.
FARMACOLOGISCHE MODULATIE VAN DE "LNR"

Er is aangetoond dat cromoglycinezuur (DSCG, Cromolyn), lokaal toegediende glucocorticosteroïden (GCS), orale glucocorticosteroïden (orale GCS) en nedocromil sodium (NDS/NS) in staat zijn, om de ontwikkeling van de LNR op inhalatie-allergenen op een significante manier te voorkomen.

De H_1- alsook H_2-receptorantagonisten kunnen een ontwikkeling van de LNR niet beletten.

Bovendien is gebleken dat DSCG, GCS en NDS in staat zijn om de cellulaire veranderingen in het neussecreet, die optreden tijdens de LNR, op een significante wijze te voorkomen.

Hoofdstuk V.
DIFFERENTIAALDIAGNOSE

De LNR en de andere neusresponstypen, de directe neusrespons [INR] en de vertraagde neusrespons [DYNR], onderscheiden zich van elkaar in verschillende opzichten, zoals; (1) klinisch: de symptomen en het beloop ervan, evenals de samenhang met de andere "*in vivo*" en "*in vitro*" diagnostische parameters en secundaire symtomen van andere organen; (2) morfologisch: de cytologische veranderingen in het neussecreet en histologische veranderingen in het neusslijmvlies hangen samen met de individuele typen van neusrespons; (3) immunologisch: de aanwezigheid en veranderingen in de

concentraties van de immunoglobulinen van individuele klassen, van de individuele mediatoren en andere factoren in het neussecreet en in het bloed c.q. bloedserum tijdens de individuele typen van neusrespons; (4) farmacologisch: de invloeden en effecten van diverse, relevante geneesmiddelen en farmacologische agentia op de individuele typen van de neusrespons verschillen per responstype.

Hoofdstuk VI.
REPRODUCEERBAARHEID EN BETROUWBAARHEID VAN DE "ALLERGEENAPPLICATIE METHODIEK" EN DE "BALLONMEET TECHNIEK"

Bij de neusprovocatie wordt het allergeen door middel van een gesatureerde wattenknoop, opgerold op een wattendrager, onder de concha media van de niet-geïntubeerde neusholte geplaatst, meestal gedurende 3 minuten. De mate van neusobstructie wordt vervolgens bepaald met de "ballontechniek", die een modificatie is van de actieve postereure rhinomanometrie, waarmee de nasale parameters (NPG = "nostril-nasopharynx-pressure-gradient" in cm H_20 = ΔP = reciproque derivaat van de neusweerstand) worden geregistreerd. Hierbij wordt een ballon op een kunststof canule (= slang) in een van beide neusholten, in de meatus medius, geplaatst. De NPG-waarden zijn een maat voor de neusobstructie (neusblokkade).

Bovenstaande wijze van allergeenapplicatie en de ballonmethode werden door ons aanvullend onderzocht, ter evaluatie van het mogelijk optreden van artefacten, mechanische irritatie van het slijmvlies en van de stabiliteit van de "base-line". Bovendien hebben wij de ballontechniek en de door deze techniek geregistreerde neusobstructie vergeleken met de resultaten geproduceerd door andere soorten van de rhinomanometrie. De standaard-deviatie (SD), de standaard-error (SE) en de variatie-coëfficiënt (VC) van de ballontechniek zijn reeds lange tijd bekend.

Drie groepen van patiënten werden onderzocht: (a) patiënten zonder rhinitisklachten (met urticaria of atopisch eczeem); (b) patienten met rhinitisklachten die uitsluitend door een allergische component veroorzaakt werden. Deze patiënten vertoonden een positieve neusrespons van het directe type en een niet-verlaagde neusdrempel voor histamine, en (c) patiënten met rhinitisklachten die uitsluitend veroorzaakt werden door aspecifieke hyperreactiviteit. Deze patiënten hadden een duidelijk verlaagde histaminedrempel in de neus, geen voedselallergie en de neusprovocaties met diverse inhalatie-allergenen waren alle negatief.

De volgende tests werden verricht bij de patiënten van de drie bovenstaande groepen: (1) de meting en registratie van de base-line-NPG-parameters gedurende 12 uren; (2) na een base-line-registratie van 10 minuten werd een droge wattenstok ingebracht in de niet-geïntubeerde neusholte onder de concha media gedurende 3 minuten. Vervolgens werden de NPG-parameters gemeten en geregistreerd gedurende 60 minuten en daarna elk uur tot 12 uur; (3) na een base-line-registratie van 10 minuten werd een wattenstok gesatureerd met controlevloeistof (fysiologische zoutoplossing gebufferd met fosfaat) in de neus geplaatst, in de niet-geïntubeerde neusholte onder de concha media gedurende 3 minuten; de NPG-parameters werden geregistreerd gedurende 60 minuten en daarna elk uur tot 12 uur; (4) de nasale histaminedrempel werd bepaald.

Op grond van de resultaten, onderlinge vergelijkingen en een statistische analyse konden wij concluderen, dat bij de door ons gebruikte "applicatiemethode" geen significante artefacten en mechanische irritatie van het slijmvlies optraden. De meting van de base-line en de uitslag van de neusprovocatie met het allergeen (= de directe neusrespons) werden door deze methode niet beïnvloed.

Bij twee andere groepen patiënten met uitsluitend allergische rhinitis, waarbij de nasale histaminedrempel dus niet was verlaagd, hebben wij de directe neusrespons (INR) op het inhalatie-allergeen (huisstof, huisstofmijt, graspollen, voorjaarspollen, katte- of hondeharen) gemeten met de ballontechniek en vergeleken met de directe respons [INR] gemeten met de actieve posterieure rhinomanometrie ("flow-pressure"-techniek) en met de passieve anterieure rhinomanometry (PAR). Er werden geen statistisch significante verschillen gevonden tussen INR gemeten met de ballontechniek en die gemeten met de PAR of de flow-pressure-techniek. Base-line-registratie met de ballontechniek bij de patiënten met de pollen-gerelateerde rhinitis en de patiënten zonder rhinitis toonde geen significante verschillen.

Hoofdstuk VII.
MECHANISMEN DIE MOGELIJKERWIJZE TEN GRONDSLAG LIGGEN AAN DE LATE NEUSRESPONS [LNR]

Diverse pathogenetische en immunologische mechanismen die ten grondslag zouden kunnen liggen aan het klinisch fenomeen LNR, werden vanuit verschillende kanten belicht. De eigen onderzoeksresultaten betreffende dit onderwerp werden vergeleken met en getoetst aan de gegevens van andere onderzoekers en aan de algemene kennis op dit gebied.

De LNR kan gedefinieerd worden als een klinisch fenomeen, waarbij een reeks van neussymptomen, vooral obstructie, tussen 4 tot 12 uren na de expositie met een allergeen (antigeen) optreden en waarbij complexe immunologische mechanismen zouden betrokken zijn.

Verschillende pathogenetische en immunologische mechanismen kunnen ten grondslag liggen aan de LNR. Het late type hypersensitiviteit (type III allergische reactie, immuuncomplexen, Arthus-fenomeen) kan worden beschouwd als een van de mogelijke belangrijke mechanismen die betrokken zijn bij de LNR.

De mogelijk causale rol van de verschillende componenten van het late basistype van allergische hypersensitiviteitsreactie kan niet worden uitgesloten. Deze zouden ofwel via de klassieke complexe pathogenetische banen ofwel door hun participatie als individuele componenten tot de ontwikkeling van de LNR kunnen leiden. Aan de volgende componenten zou hierbij gedacht moeten worden: (1) immuuncomplexen; (2) complementsysteem en zijn onderdelen, zoals de "klassieke complement-pathway", de "alternatieve complement-pathway", het "membrane attack complex", receptoren voor de complementcomponenten op de cellulaire membraan van diverse celtypen; (3) IgG- en IgM-antilichamen; (4) de individuele celtypen, zoals neutrofielen, eosinofielen, trombocyten, mestcellen en basofielen (inclusief "histamine releasing factors", cytokinen, "Stem cellfactors", neuropeptiden en diverse mestcel en basofielactiverende factoren).

Ook de innervatie en neurologische controle van het neusslijmvlies, evenals verschillende neuropeptiden functionerend als "neurotransmitters" in het humane neusslijmvlies, zouden een voorwaarde kunnen zijn voor de realisatie van sommige stappen van de immunologische processen in het humane neusslijmvlies.

Diverse componenten van het vroege basistype allergiereactie (= directe hypersensitiviteit, type I-allergie) zouden bij de late-faseallergiereacties, zoals de LNR, een rol kunnen spelen, wat reeds door sommige onderzoekers werd gesuggereerd. Deze rol zou uitgevoerd kunnen worden via het klassieke mechanisme of door een aparte participatie van de individuele componenten. De volgende componenten werden in de literatuur voorgesteld: (1) de antigeenspecifieke IgE-antilichamen; (2) de mestcellen en basofielen.

Onze resultaten konden de voorgestelde hypothese van de "absolute rol" van IgE-antilichamen alsook van de mestcellen en de basofielen bij de ontwikkeling van de klinische LNR niet ondersteunen en bevestigen.

Hoofdstuk VIII.
ALGEMENE DISCUSSIE

De detectie en definitieve bevestiging van de individuele typen van de neusrespons bijvoorbeeld de LNR, op een bepaald allergeen, en hun betrokkenheid bij de neussymptomen van een bepaalde patiënt, kunnen alleen plaatsvinden door middel van de neusprovocatietest [NPT] met allergeen. De NPT is ook de exclusieve techniek om onderscheid te maken tussen het aandeel van de allergiecomponent en het aandeel van de aspecifieke hyperreactiviteit in de neussymptomen van een bepaalde patiënt. De aspecifieke hyperreactiviteit wordt daarna getest door middel van een neusprovocatie met histamine, methacholine of koude lucht.

De NPT kan niet alleen beschouwd worden als een belangrijke onderzoekstechniek waarmee diverse pathofysiologische en farmacologische aspecten van het neusslijmvlies kunnen worden bestudeerd, maar ook als een zeer belangrijk onderdeel van het klinisch routineonderzoek van de allergie-aandoeningen in de neus en in andere organen - waarbij de neusallergie mogelijk een primaire rol speelt.

Het belangrijkste aspect van de NPT is de vergelijking van de objectieve parameters en simultaan ook van de subjectieve parameters voor en herhaaldelijk na de neusprovocatie met een bepaald allergeen of aspecifieke prikkel.

Er bestaan drie basissoorten van NPT: (1) de NPT met allergeen, die de allergiecomponent en het bestaan van een bepaald type van neusrespons bevestigt; (2) de NPT met een van de representanten (= simulatoren) van aspecifieke hyperreactiviteit, zoals histamine, methacholine en koude lucht, die het bestaan van de aspecifieke hyperreactiviteitcomponent en zijn participatie in de neusklachten van een bepaalde patiënt tracht te bevestigen; (3) de NPT in combinatie met een bepaald geneesmiddel of therapeuticum, zogenoemde neusprotectietest, die de farmacologische effecten van dat middel op de door het allergeen of door de aspecifieke prikkel uitgelokte neusrespons bevestigt of uitsluit.

De NPT moet ook aan een aantal noodzakelijke voorwaarden voldoen: hij moet voldoende veilig, reproduceerbaar, gevoelig en betrouwbaar zijn en hij moet comfortabel zijn voor de patiënt en voor de uitvoerende personen. De toediening van het allergeen en de registratie moeten voldoende effectief en betrouwbaar zijn; de toediening van het allergeen mag geen gevaar opleveren voor de patiënt. Dit moet verricht worden in een professioneel ingerichte ruimte waarin acute behandeling, zoals beademing en reanimatie, mogelijk is. De neusprovocatietests moeten worden verricht door goed opgeleid personeel, onder de professionele begeleiding van de medische staf.

Met behulp van de NPT kunnen de volgende gegevens bepaald worden: (1) de rol van een bepaald allergeen of aspecifieke prikkel bij de neusklachten van een bepaalde patiënt; (2) het bestaan van een bepaald type van neusrespons; (3) het aandeel van de allergie en de aspecifieke hyperreactiviteit in de neussymptomen van een bepaalde patiënt; (4) de dosis- en tijdcurven; (5) de primaire rol van het neusslijmvlies en de nasale allergie bij de respons van andere organen; (6) de beschermende en/of therapeutische effecten van een bepaald geneesmiddel op de neusallergie en op een bepaald type van de neusrespons; (7) effecten van immunotherapie; (8) diverse onderzoeksaspecten en -hypothesen.

Bij de applicatie van het allergeen en/of representant (simulator) van de aspecifieke hyperreactiviteit op het neusslijmvlies kunnen verschillende technieken gebruikt worden, die ieder hun voor- en nadelen hebben. Tot deze technieken behoren: (a) het druppelen van de extracten op het neusslijmvlies met behulp van een druppelaar of een micropipet, (b) de aërosolisatie van de extracten; (c) papier-disks, cuvetten of wattenstaven, die gesatureerd worden door de bepaalde extracten en daarna geplaatst worden in de neus, op een bepaalde locatie, gedurende een bepaald tijdsinterval.

De neusrespons op een allergeen of aspecifieke prikkel kan gemeten en geregistreerd worden door middel van diverse technieken/methoden. Hiertoe behoren: (1) anterieure rhinoscopie, (2) registratie van subjectieve klachten; (3) registratie van objectieve parameters, waarmee de ernst van de individuele neussymptomen, meestal neusobstructie, wordt geregistreerd en objectief wordt beoordeeld. De meeste van deze objectieve parameters hebben betrekking op de neusweerstand ("nasal airway resistence" = NAR). Ze kunnen op directe en op indirecte wijze via een derivaat of analoge parameter worden bepaald. Deze meet- en registratietechnieken van de objectieve neusparameters en -functies kunnen worden verdeeld in 5 groepen: (1) de nasale peak-flow; (2) de plethysmografie; (3) de rhinomanometrie: anterieure versus posterieure, actieve versus passieve; combinatie van de technieken; (4) akoestische rhinometrie; (5) niet-rhinomanometrische technieken, zoals meting van de doorbloeding van de neus met behulp van Doppler velocimetrie, meting van penetratie en uitscheiding van ^{133}Xenon door het neusslijmvlies, en meting van de resonantie van de stem met behulp van nasometrie.

De NPT heeft zijn eigen diagnostische waarde. Vervanging door andere diagnostische tests, zoals huidtests, RIST/RAST, die ook hun beperkingen hebben, is niet zomaar mogelijk. Conclusies die getrokken worden op grond van de resultaten die zijn verkregen bij onderzoek van een orgaan zoals de huid en het bloed, kunnen niet zonder bepaalde beperkingen en niet zonder voorbehoud toepasbaar zijn op een ander orgaan, zoals het neusslijmvlies.

De LNR representeert een duidelijk manifeste, maar reversibele, immunologische gebeurtenis in het neusslijmvlies. Dit proces ontwikkelt zich stapsgewijs. Bij dit proces

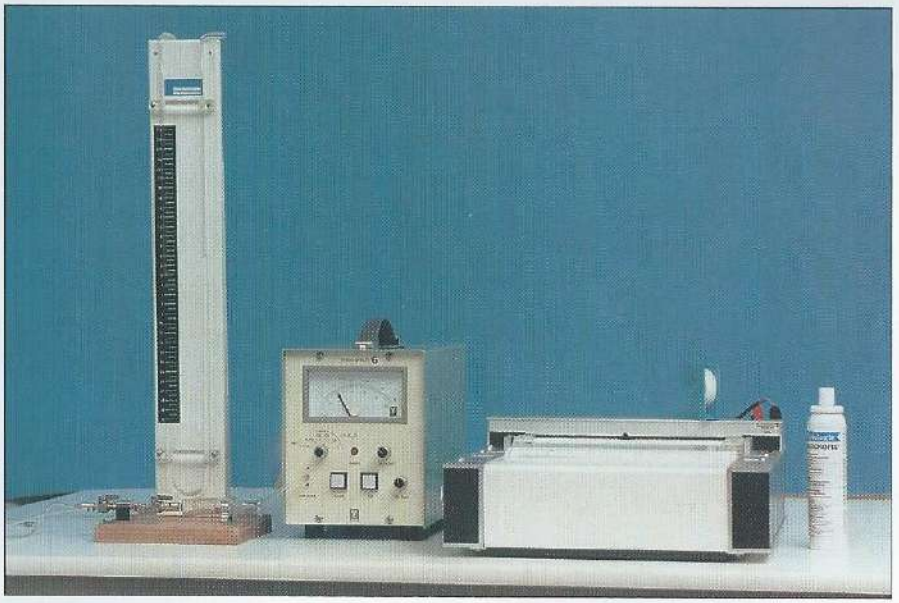
zijn ook de diepere lagen van het neusslijmvlies en andere mucosale structuren betrokken, zoals bloedcapillairnetwerk, diverse celtypen, b.v. basofielen, eosinofielen, neutrofielen, epithelialecellen en bekercellen, monocyten en precipiterende IgG (IgM)-antilichamen.

De LNR is regelmatig in verband gebracht met de vorming van de eosinofiel-neutrofiele infiltraten in de diepere lagen van het neusslijmvlies (lamina propria), met lichte, reversibele defecten van het epitheel en met participatie van enige "inflammatiecomponenten".

De LNR representeert ook een aantrekkelijk en gemakkelijk toegankelijk model voor het bestuderen van de hypersensitiviteitsmechanismen, c.q. immunologische, cytologische, biochemische, biofysische, farmacologische en therapeutische aspecten. Deze kunnen namelijk direct gerelateerd worden aan een bepaald allergeen, aan het klinisch verloop van de LNR, aan de diverse cytologische veranderingen in het neussecreet en aan histologische veranderingen in het neusslijmvlies. De LNR is ook makkelijk te produceren en te reproduceren. Er zijn praktisch geen risico's aan de LNR verbonden. Daarom kan de LNR beschouwd worden als een veilig klinisch onderzoeksmodel.

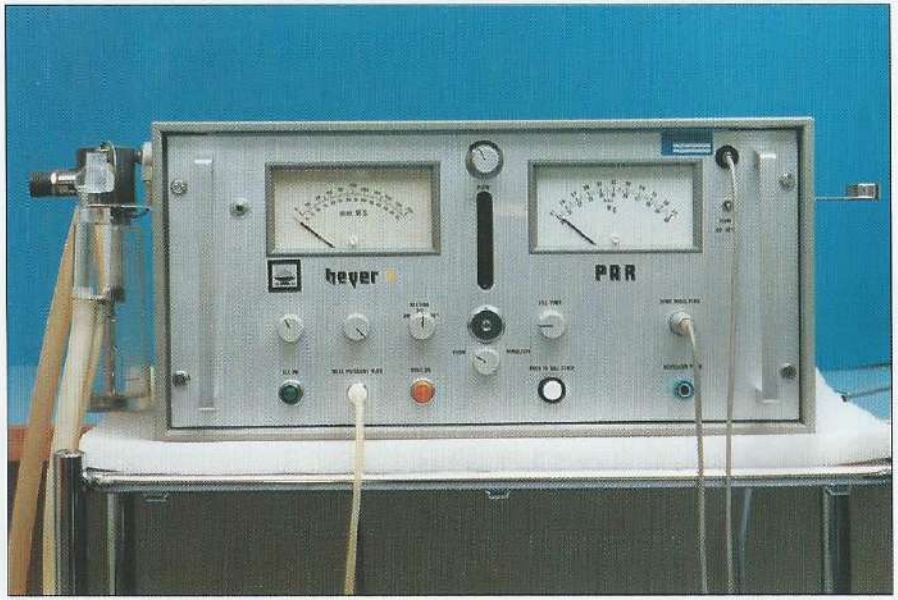
De LNR is een belangrijk en zeer interessant klinisch fenomeen, waarover steeds meer feiten bekend zijn geworden gedurende de laatste twee decenia. Ondanks deze kennis, zijn er verdere studies nodig om het belang van de LNR en de eraan ten grondslag liggende mechanismen beter te kunnen interpreteren en te beïnvloeden.

XII. PLATES

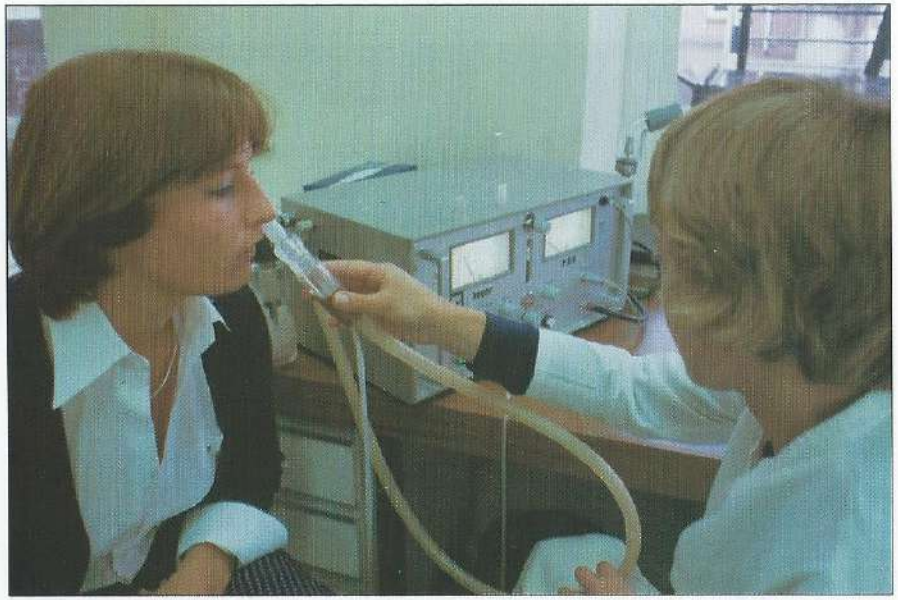


1a The "balloon" being a modified posterior rhinomanometry (the equipment was assembled by the author).
References: 7,8,9,9-11,25b

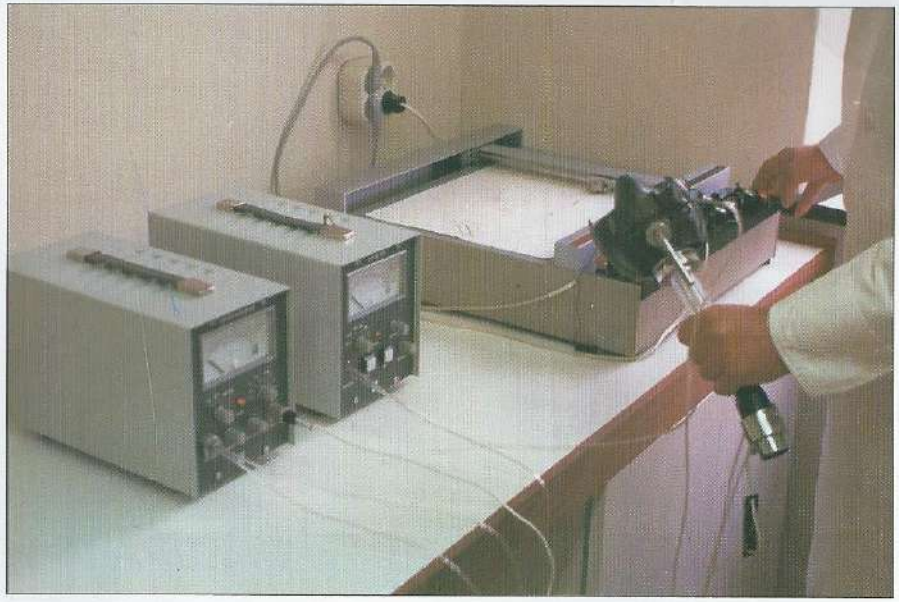
1b The "Balloon-method".
References: 7,8,9,9-11,25b



2a Passive anterior rhinomanometry (PAR - Heyer rhinomanometer)
Reference: 25b



2b Passive anterior rhinomanometer.
Reference: 25b

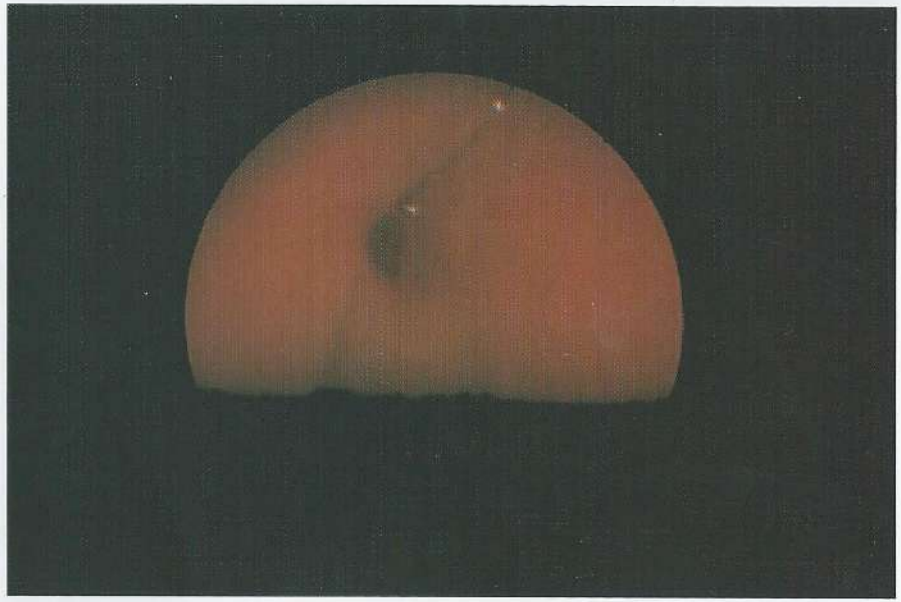


3 Active posterior rhinomanometry (The equipment was assembled by the author).
Reference: 8

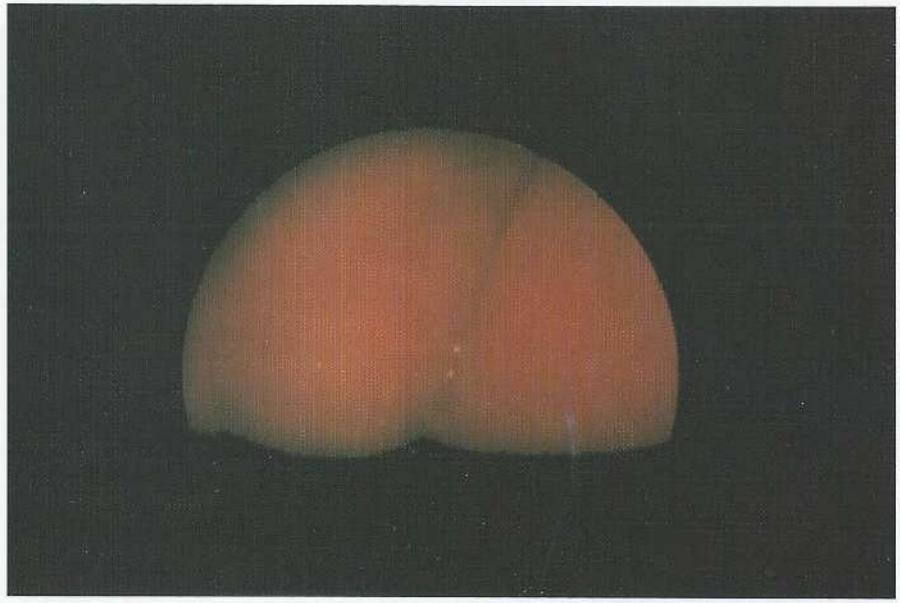
4 Active anterior/posterior rhinomanometry equipment (NRG Mercury system).
References: 41i,48j

5 Active anterior/posterior rhinomanometry equipment (Rhinoscreen Jäger).
References: 41i,48j

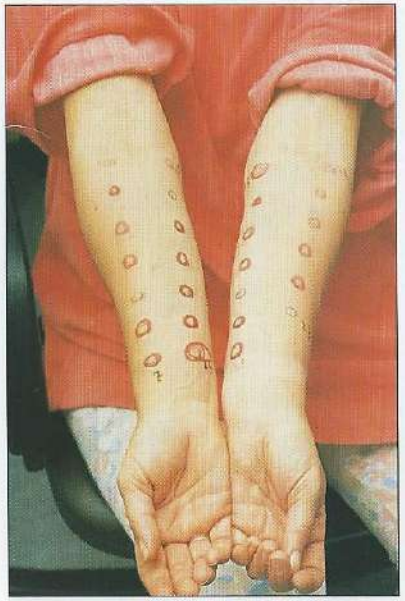
6 Acoustic rhinometry equipment (Rhinoclock Stimotron).
References: 41i,48f-48i



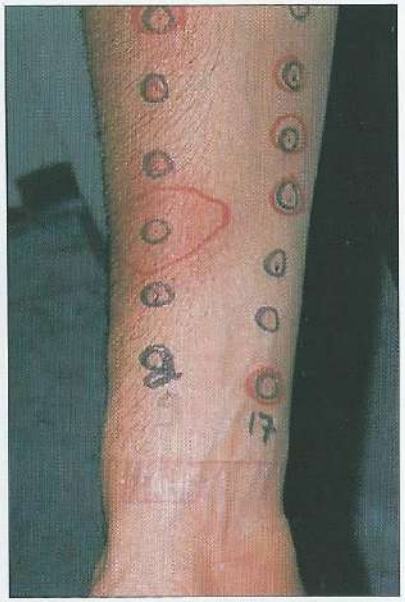
7a Rhinoscopy - before the allergen challenge.
Reference: 41i



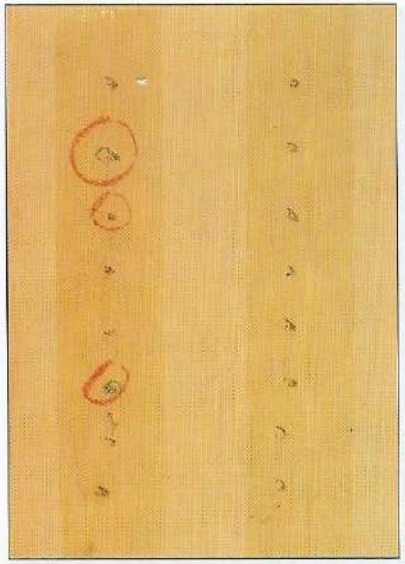
7b Rhinoscopy - at 6 hours after the nasal challenge during the positive "late nasal response" to grasspollen.
Reference: 41i



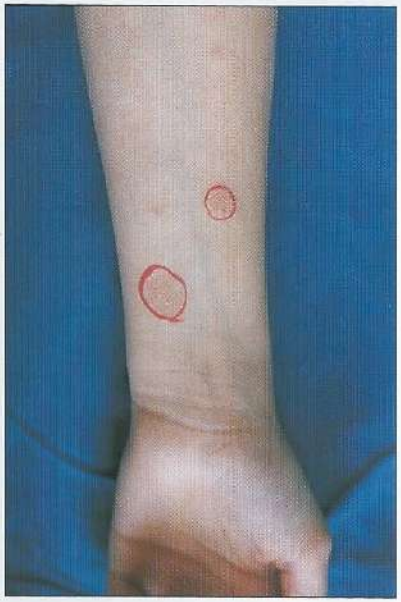
8a The immediate skin responses to various "inhalant allergens".
Reference: 41i



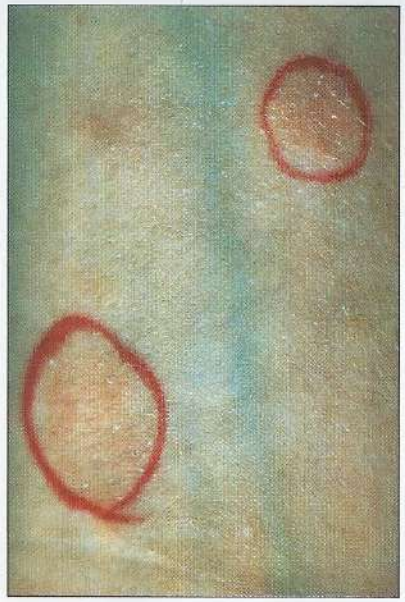
8b The immediate skin response to horse danders.
Reference: 41i



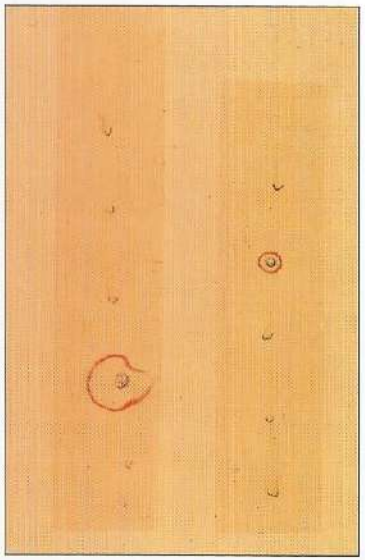
8c The immediate responses to house dust [No 3], grasspollen [No 6] and grasspollen P [No 7] (read from right to left).
Reference: 41i



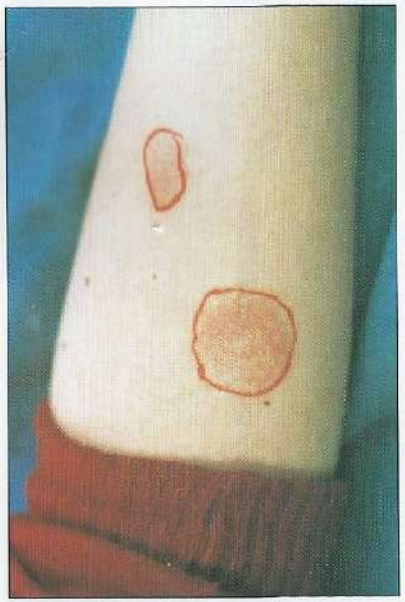
9a The late skin responses to the pigeon feathers (upper reaction) and to the pigeon feaces extract (lower reaction).
References: 11h,41b,41i



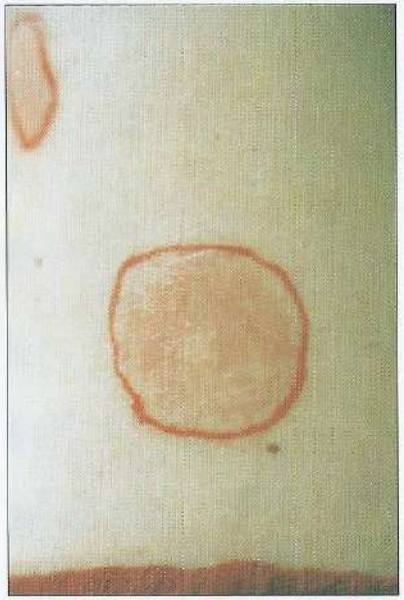
9b The late skin responses to the pigeon feathers (upper reaction) and to the pigeon feaces extract (lower reaction) in detail.
References: 11h,41b,41i



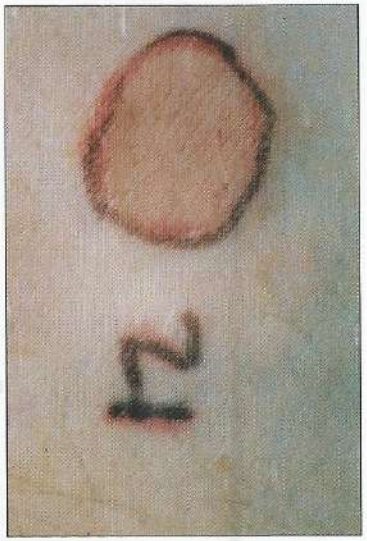
9c The late skin responses to the cat hairs (upper reaction) and to the hamster hairs (lower reaction).
References: 11h,41b,41i



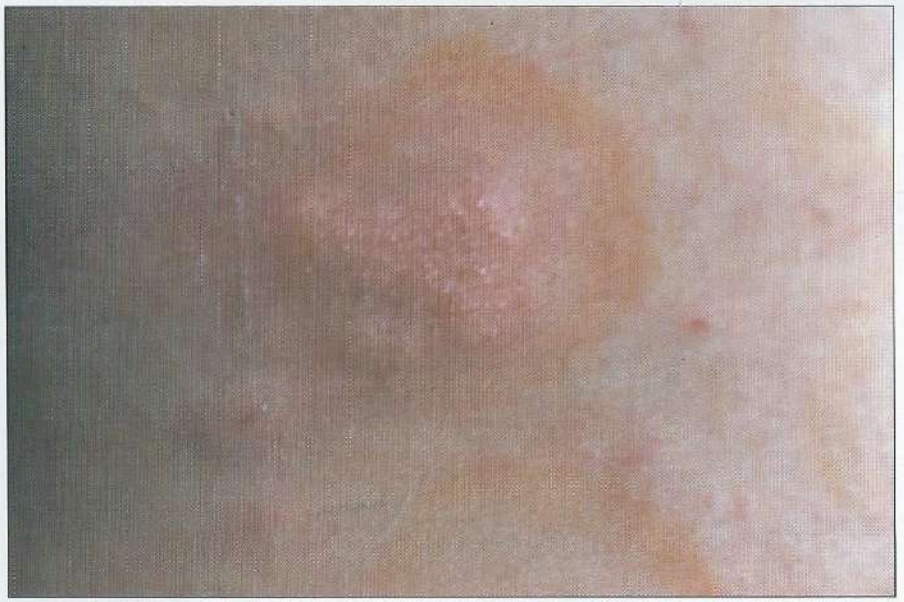
10a The delayed skin responses to the *Dermatophagoides pteronyssinus* [house-dust mites] (upper reaction) and to the *betula pendula* [birch pollen] (lower reaction) in detail.
Reference: 41i



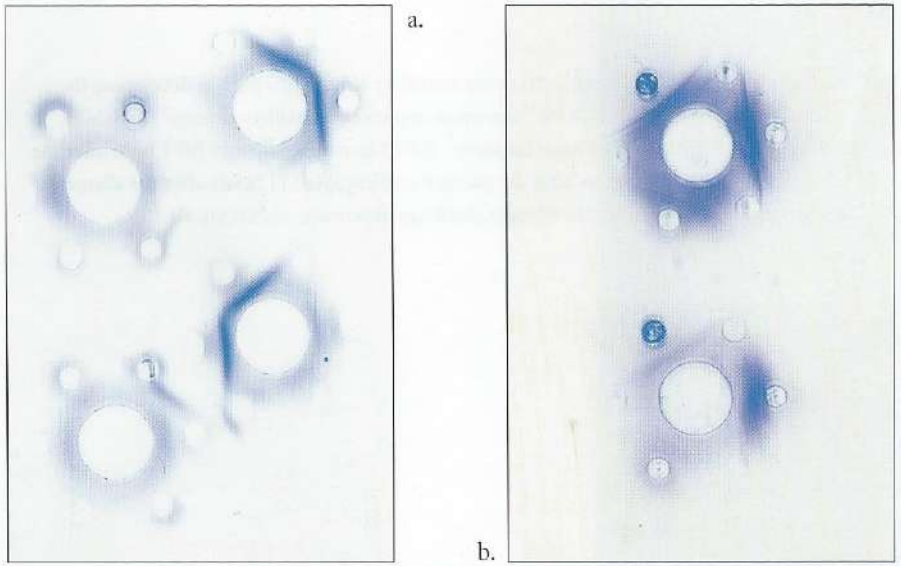
10b The delayed skin response to the birch pollen.
Reference: 41i



10c The delayed skin response to the canary dropping extract. Reference: 41i



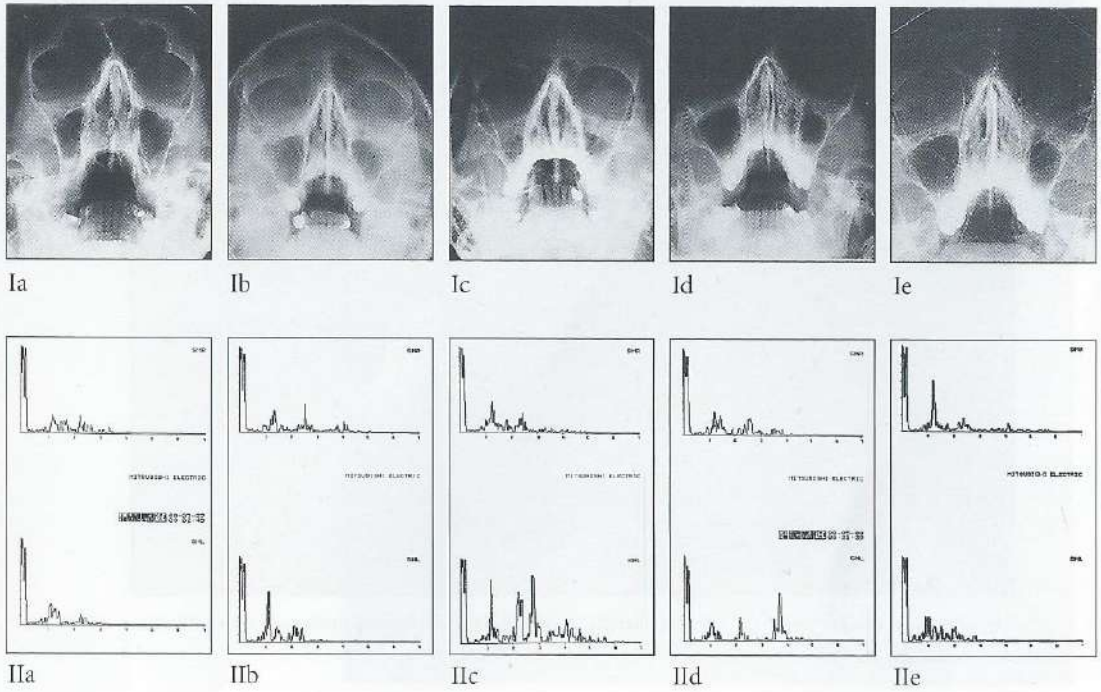
11 The delayed skin response (contact allergy) to the cat hairs applied by means of the patch test.
Reference: 41i



a.

b.

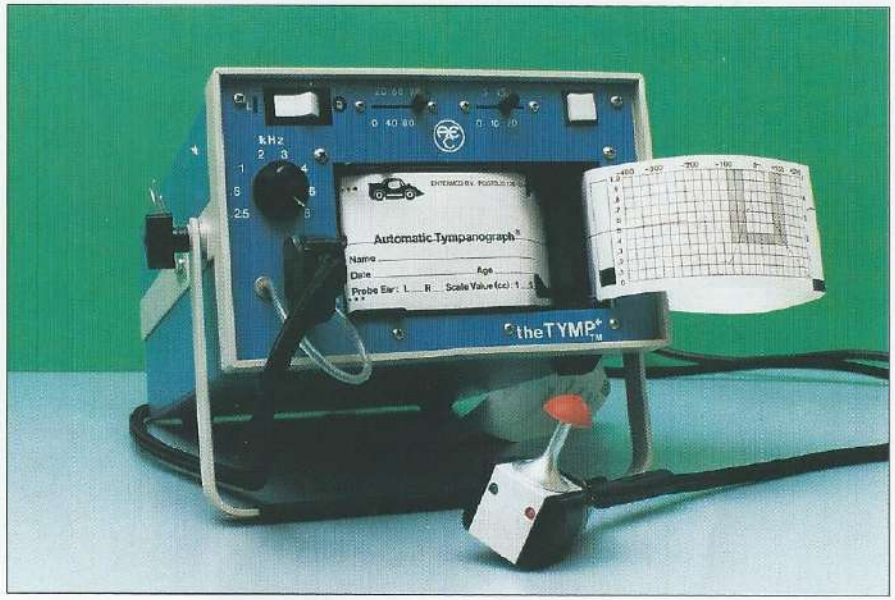
12 Positive double immuno-diffusion (Ouchterlony test) in the serum, demonstrating the presence of precipitating antibodies for pigeon dropping and feather antigen (a) and old paper and cardboard antigens (b) in patients developing the LNR to the nasal challenge with these antigens. References: 15,16,19,24,41i,121b,121j



13 Radiographs (I) and echographs (II) of the maxillary sinuses of a patient developing the secondary (associated) form of the "late sinus response of maxillary sinuses" (LSR-MS), induced by the primary "late nasal response" (LNR) to nasal challenge; (b) 1 hour after the allergen challenge; (c) 6 hours after the allergen challenge; (d) 11 hours after the allergen challenge; (e) 24 hours after the allergen challenge. References: 14a-14f,41g,41i

14 Wet spirograph (Lode Co, Groningen, The Netherlands, model D-75)
References: 41h,41i

15 Endoscopy equipment (Wolf, Germany).
Reference: 41i

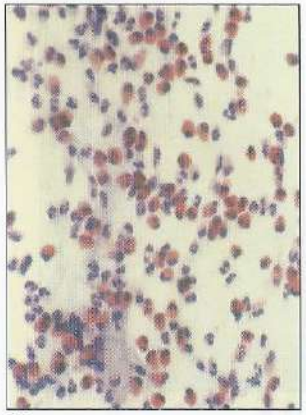


16a Tympanograph (American, USA).
References: 22,27,27a,27b,41i

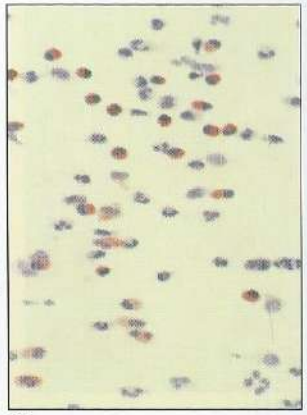
16b Tympanograph.
References: 22,27,27a,27b,41i

17 Echograph and printer.
References: 14a-14f,41g,41i

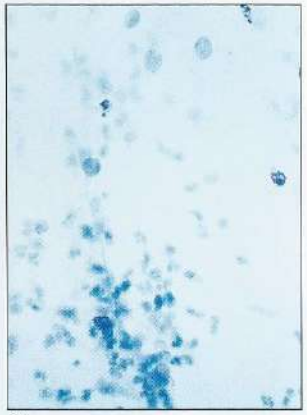
Ia



IIa

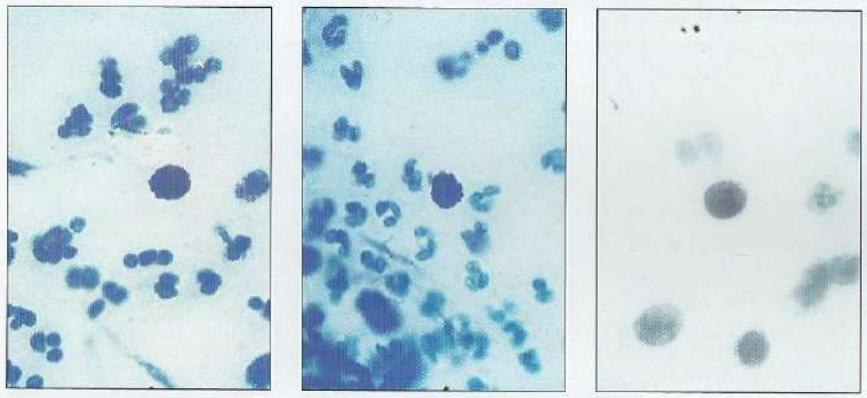


IIb



IIc

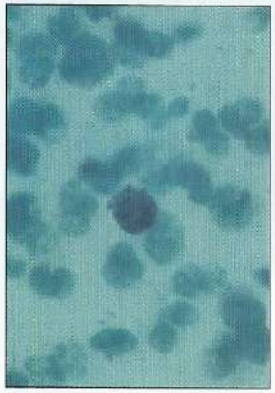
18 Cellular changes in the nasal secretions (NS) accompanying the LNR: [I] NS before the allergen challenge; [II] NS after the allergen challenge: (a) at the 4th hour = one hour before the onset of LNR; (b) at the 7th hour = at the peak of LNR; (c) at the 12th hour = one hour after the dissappearance of LNR. References: 11c,11e,11h,25,40c,41a,41b,41c,72,72a,72d,97



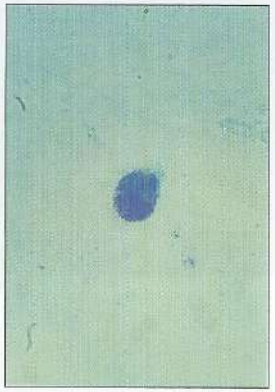
I a

I b1

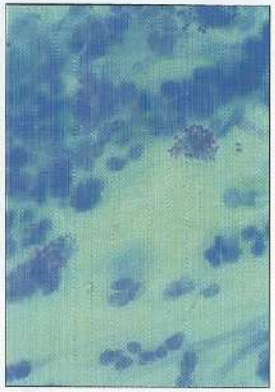
I b2



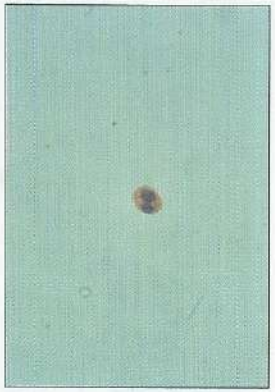
I b3



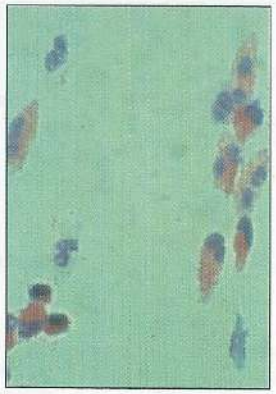
I b4



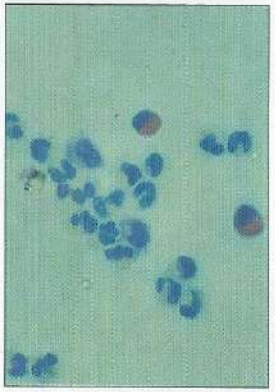
II



III a

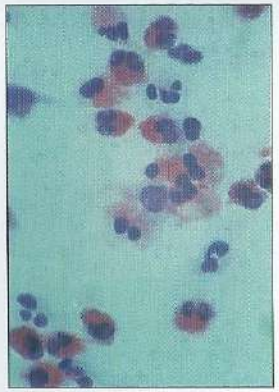


III b

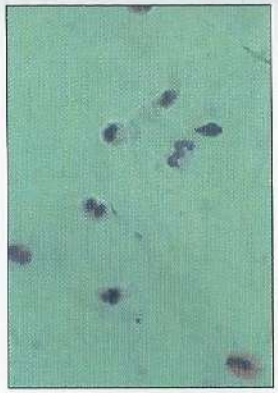


IV a

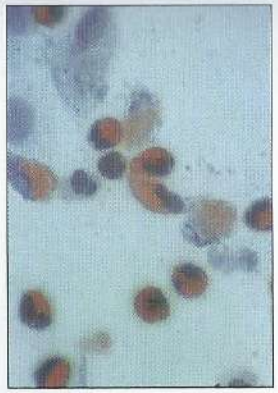
19 Intracellular changes, including changed granules (degranulation) in particular cell types in nasal secretions: I. Basophils - (a) before the allergen challenge; ($b_{1,2,3,4}$) sequential stages of degranulation during the positive INR; II. Mast cells - final stage of degranulation during the positive INR; III. Eosinophils - (a) before the allergen challenge; (b) degranulation during the positive INR; IV. Eosinophils - (a) before the allergen challenge; ($b_{1,2,3}$) various stages of degranulation during the positive LNR; V. Neutrophils - (a) before the allergen challenge; ($b_{1,2}$) final stage of degranulation during the positive LNR; VI. Expelled goblet and epithelial cells in the NS after the resolved LNR.



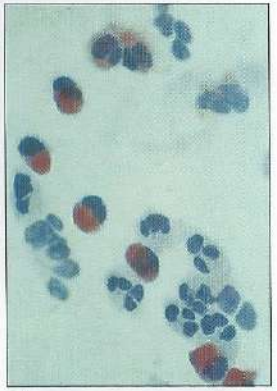
IV b1



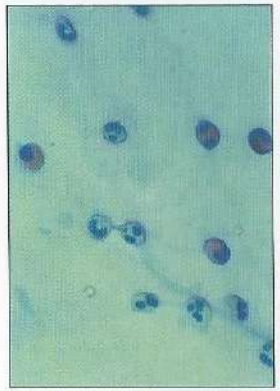
IV b2



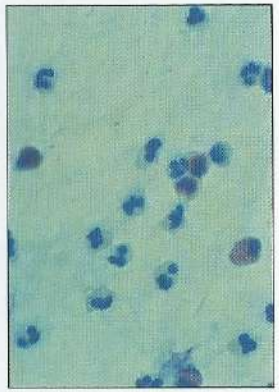
IV b3



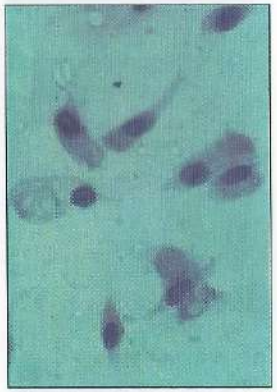
V a



V b1

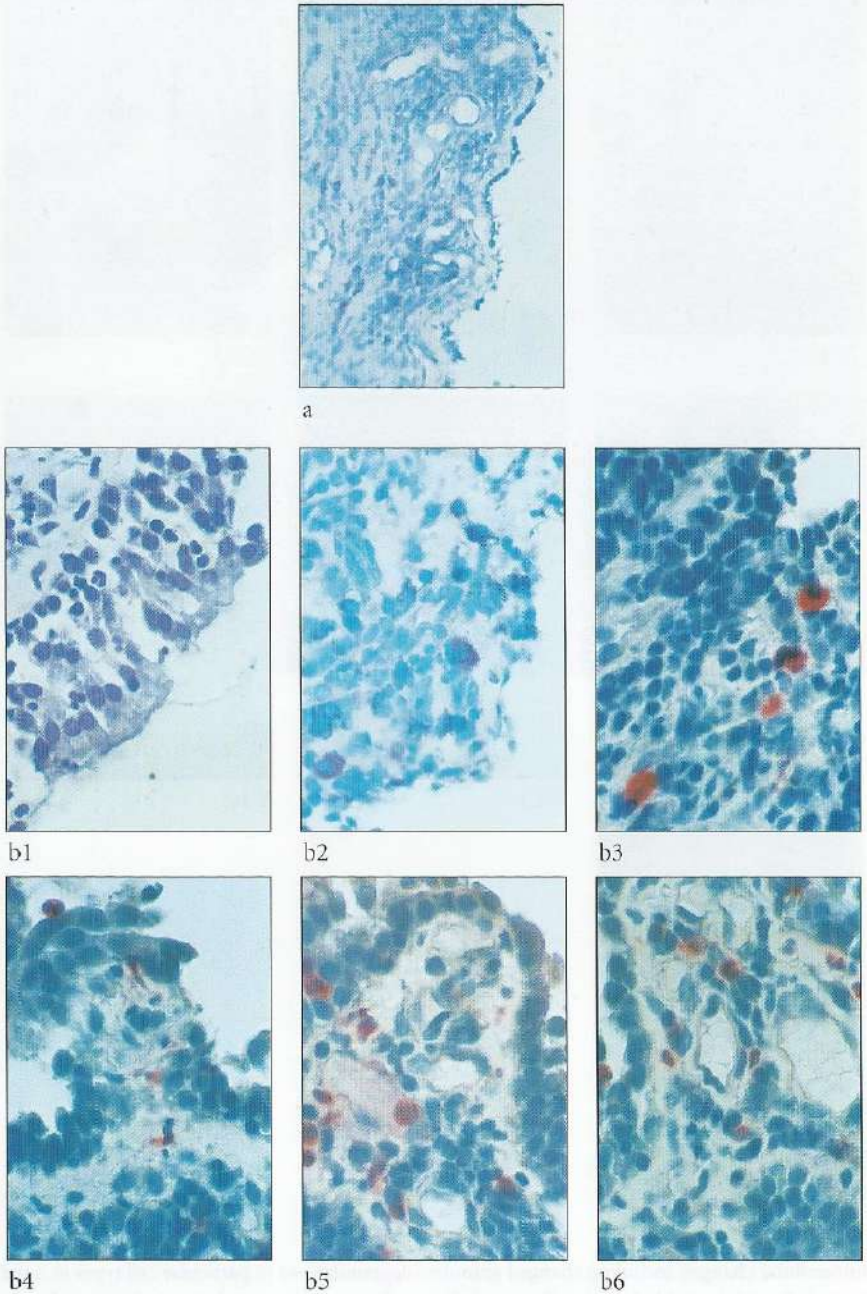


V b2



VI

19 Intracellular changes, including changed granules (degranulation) in particular cell types in nasal secretions: I. Basophils - (a) before the allergen challenge; ($b_{1,2,3,4}$) sequential stages of degranulation during the positive INR; II. Mast cells - final stage of degranulation during the positive INR; III. Eosinophils - (a) before the allergen challenge; (b) degranulation during the positive INR; IV. Eosinophils - (a) before the allergen challenge; ($b_{1,2,3}$) various stages of degranulation during the positive LNR; V. Neutrophils - (a) before the allergen challenge; ($b_{1,2}$) final stage of degranulation during the positive LNR; VI. Expelled goblet and epithelial cells in the NS after the resolved LNR.

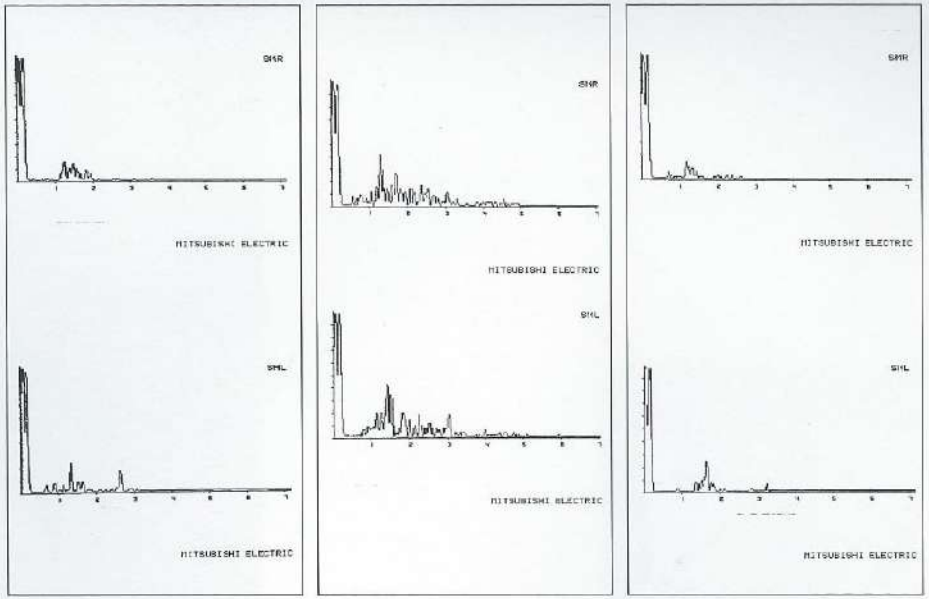


a

b1 b2 b3

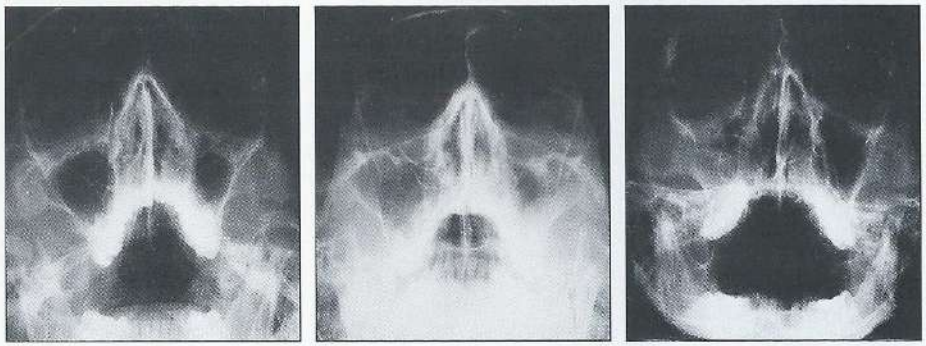
b4 b5 b6

20 Histologic changes of the nasal mucosa (= nasal mucosa biopsy) accompanying the LNR: (a) before the allergen challenge; (b_{1-6}) after the allergen challenge - during the LNR.
References: 41i,96,96,97



a b c

21 Echographs of the paranasal sinuses of a patient developing the associated form of the "late sinus response" (LSR-MS), induced by the late nasal response (LNR) to ingestion challenge with cow's milk: (a) before the ingestion challenge; (b) at 6 hours after the challenge - at a peak of the LNR; (c) at 24 hours after the challenge - after the resolving of LNR.
References: 14g,41g,117h

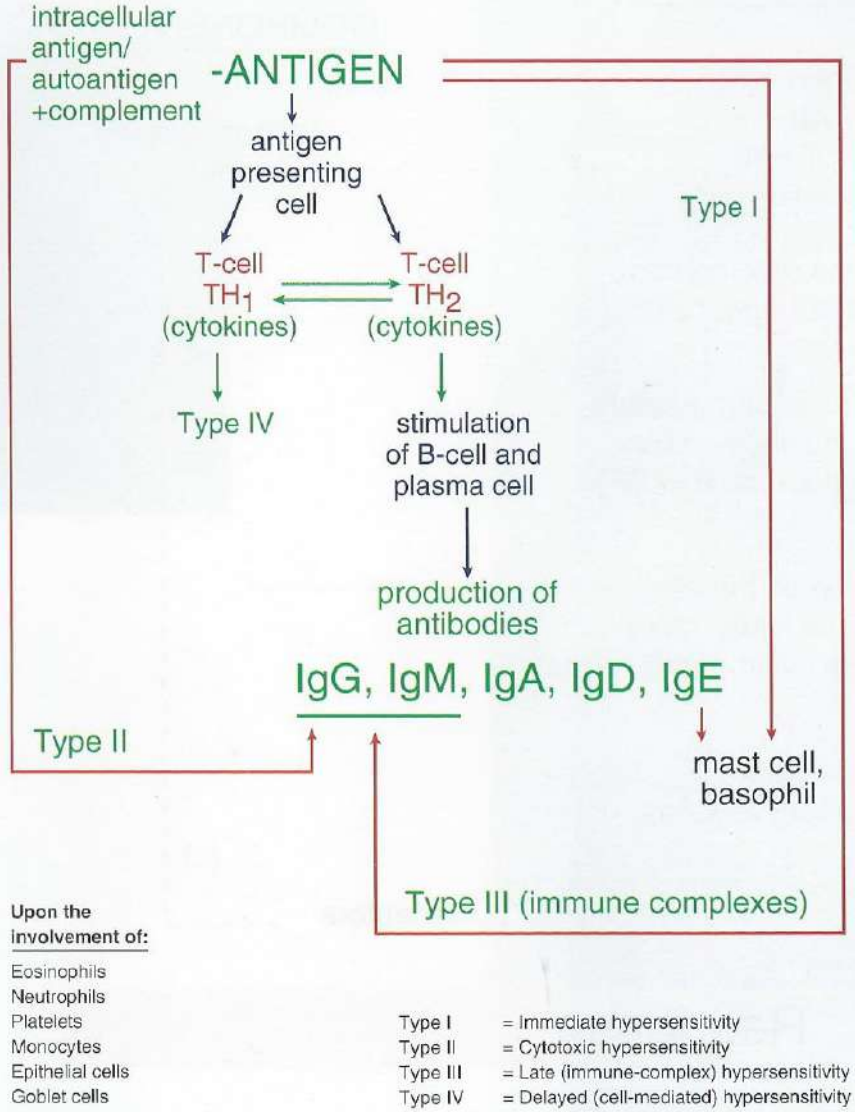


a b c

22 Radiographs of the paranasal sinuses of a patient developing the associated form of the "late sinus response" (LSR-MS), induced by the late nasal response (LNR) to ingestion challenge with cow's milk: (a) before the ingestion challenge; (b) at 6 hours after the challenge - at a peak of the LNR; (c) at 24 hours after the challenge - after the resolving of LNR.
References: 14g,41g

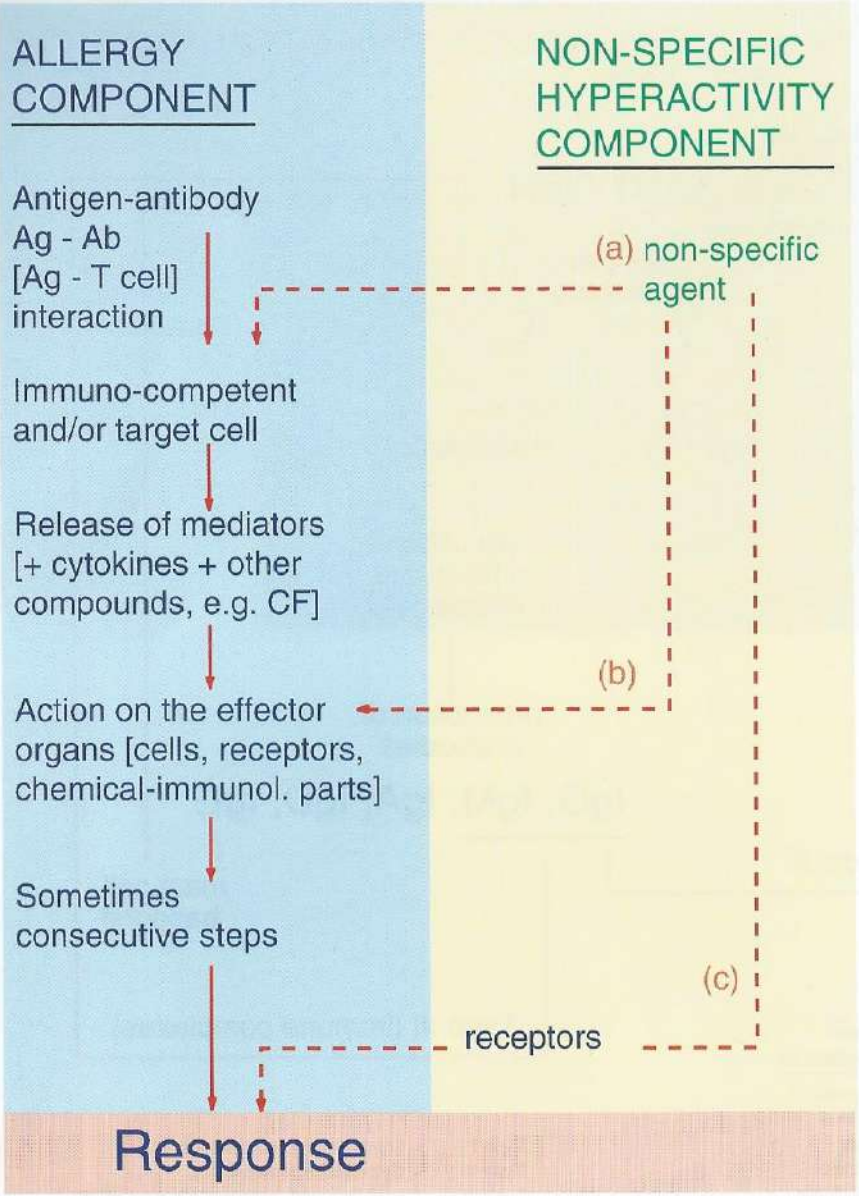
X. COLOR FIGURES

Fig.1. BASIC TYPES OF HYPERSENSITIVITY (ALLERGY) REACTIONS.



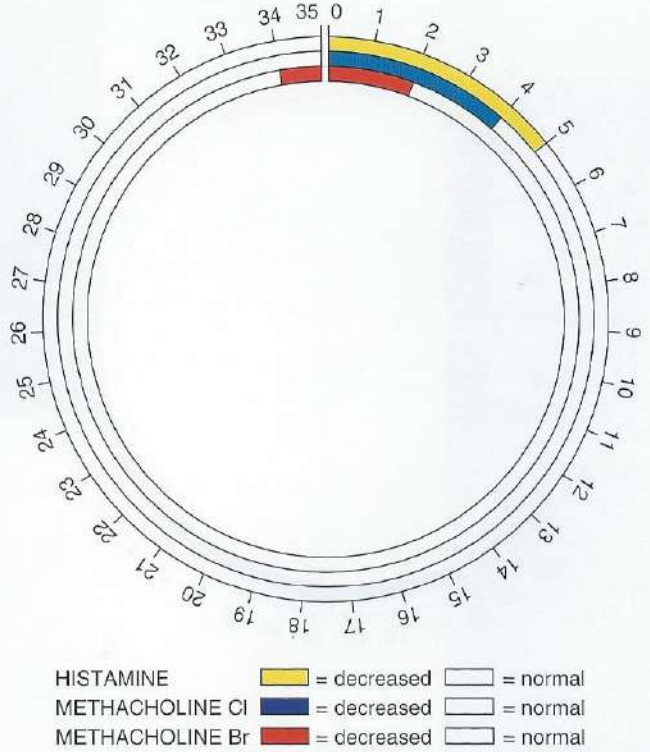
References: 1-3,6,11h

Fig. 2. SCHEDULE OF THE ALLERGY AND NON-SPECIFIC HYPERREACTIVITY PATHWAYS



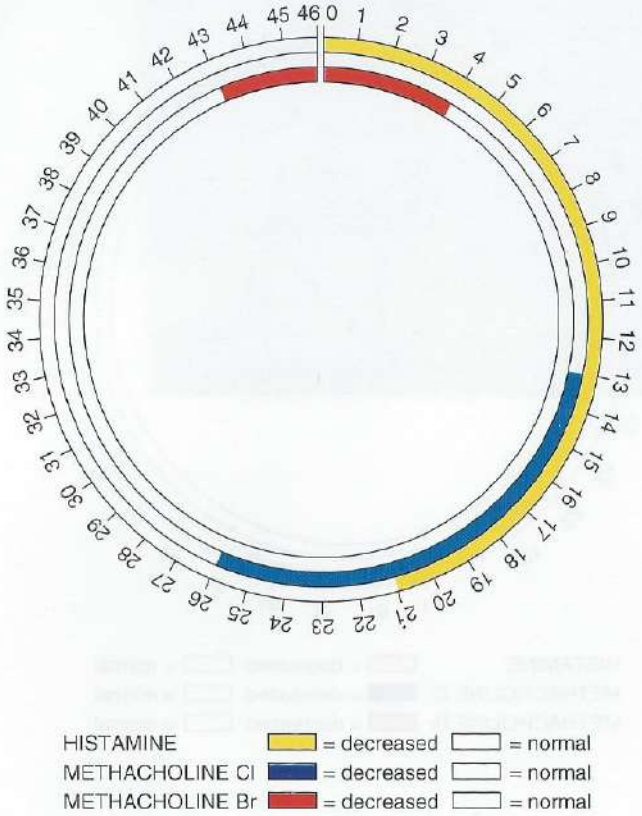
References: 1-3,6,11h

Fig. 11. REVIEW OF NASAL THRESHOLDS OF HISTAMINE, METHACHOLINE CHLORIDE AND METHACHOLINE BROMIDE IN PATIENTS WITH ALLERGIC RHINITIS [Late Nasal response] (n=35)



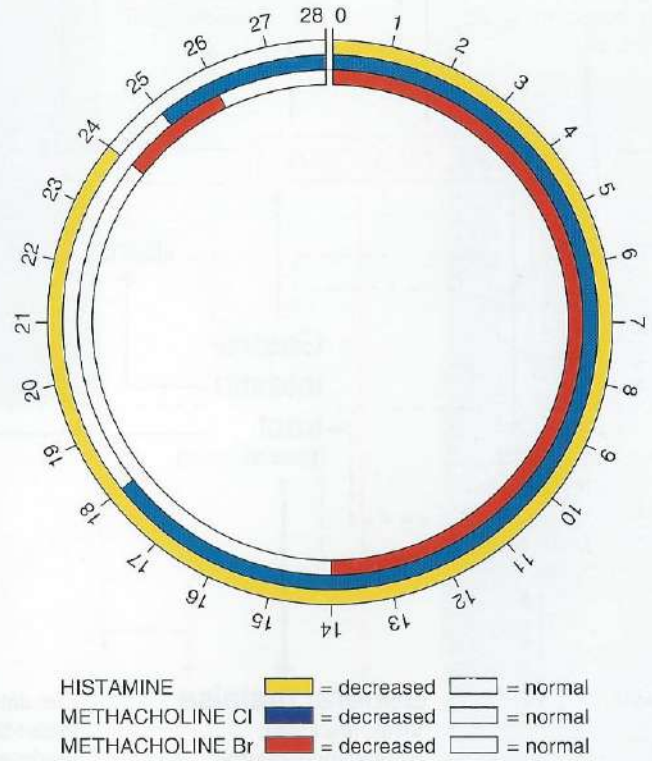
References: 35c,35f,41b

Fig. 14. REVIEW OF NASAL THRESHOLDS OF HISTAMINE, METHACHOLINE CHLORIDE AND METHACHOLINE BROMIDE IN PATIENTS WITH ALLERGIC RHINITIS [immediate nasal response] (n=46)



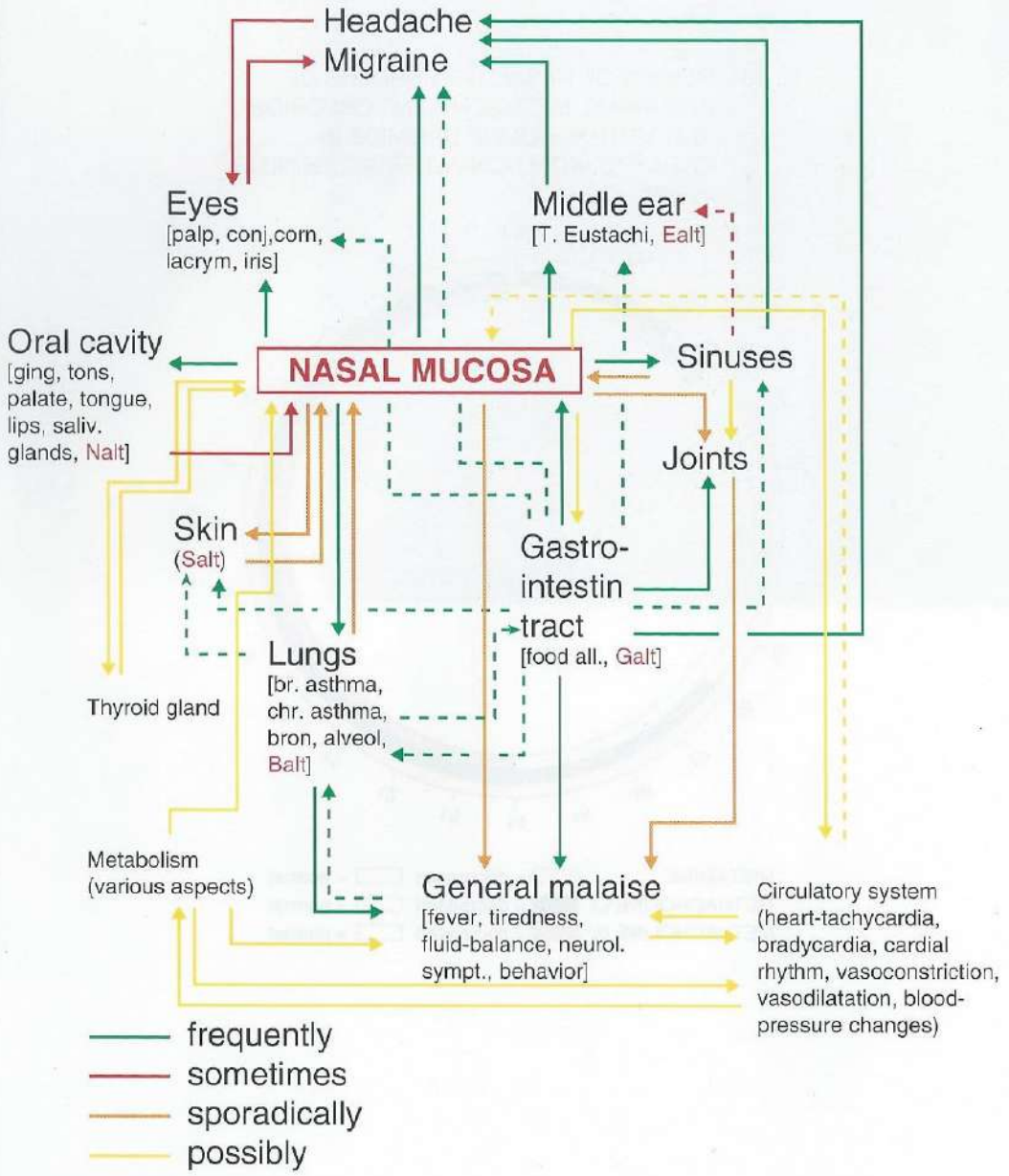
References: 35a,35b,35c,35f

Fig. 18. REVIEW OF NASAL THRESHOLDS OF HISTAMINE, METHACHOLINE CHLORIDE AND METHACHOLINE BROMIDE IN PATIENTS WITH NON-ALLERGIC RHINITIS (n=28)



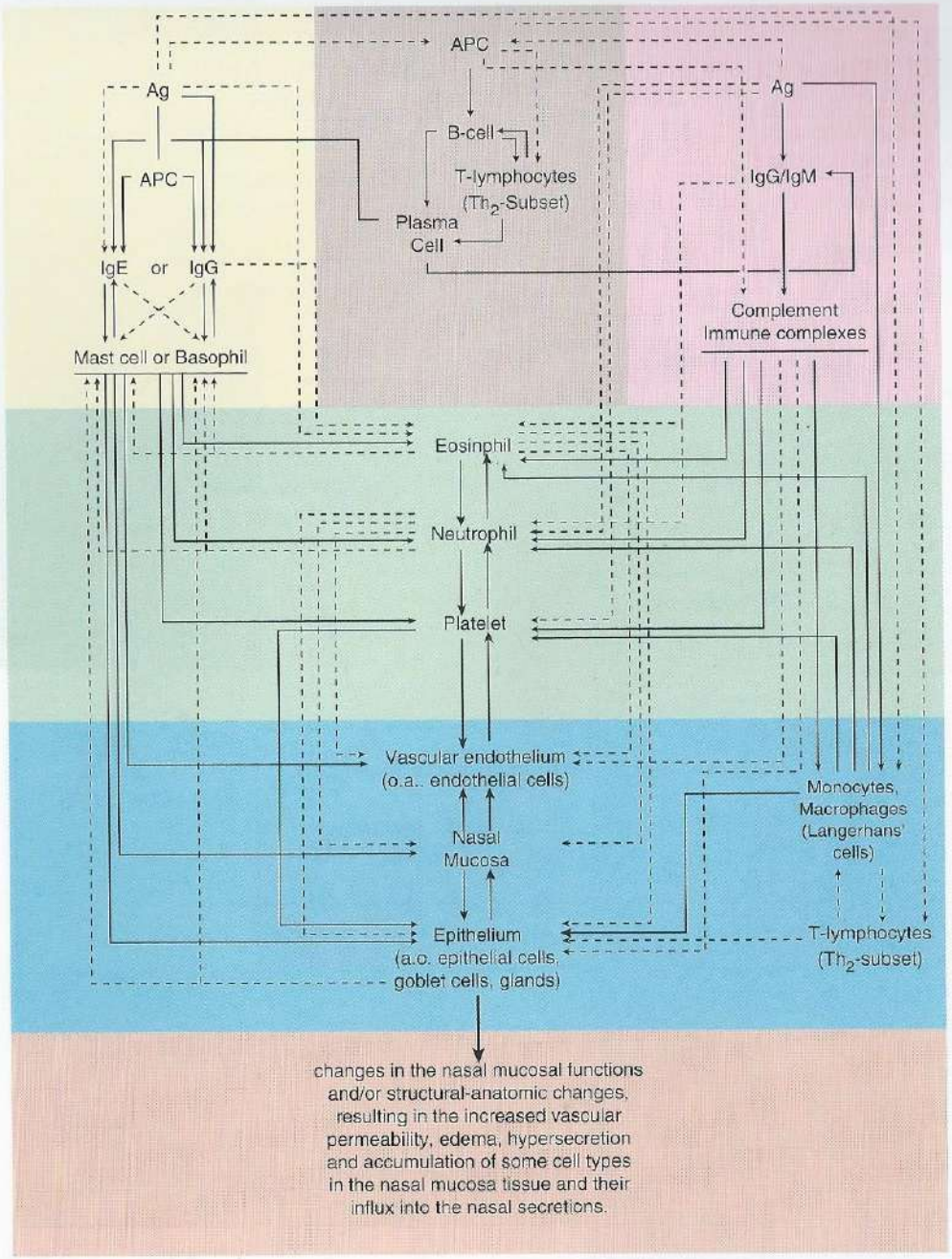
References: 35a,35b,35c,35f

Fig 22. POSSIBLE RELATIONSHIPS BETWEEN THE NASAL MUCOSA [ALLERGY, RESPONSE] AND OTHER ORGANS



References: 11h,14,14a-14g,15-19,21,22,26,27,27a,27b,37,38,40,40a,40c,40d, 40f,41,41b,41e,41g,41h,41i,72b,97w,117h,121b

Fig. 53a. Possible pathways involved in the "LNR"



Ag = Antigen, APC = Antigen presenting cell; B-cells = B-lymphocytes; T-cells = T-lymphocytes.

References: 11h,41b

Fig. 54. THE COMPLEMENT SYSTEM

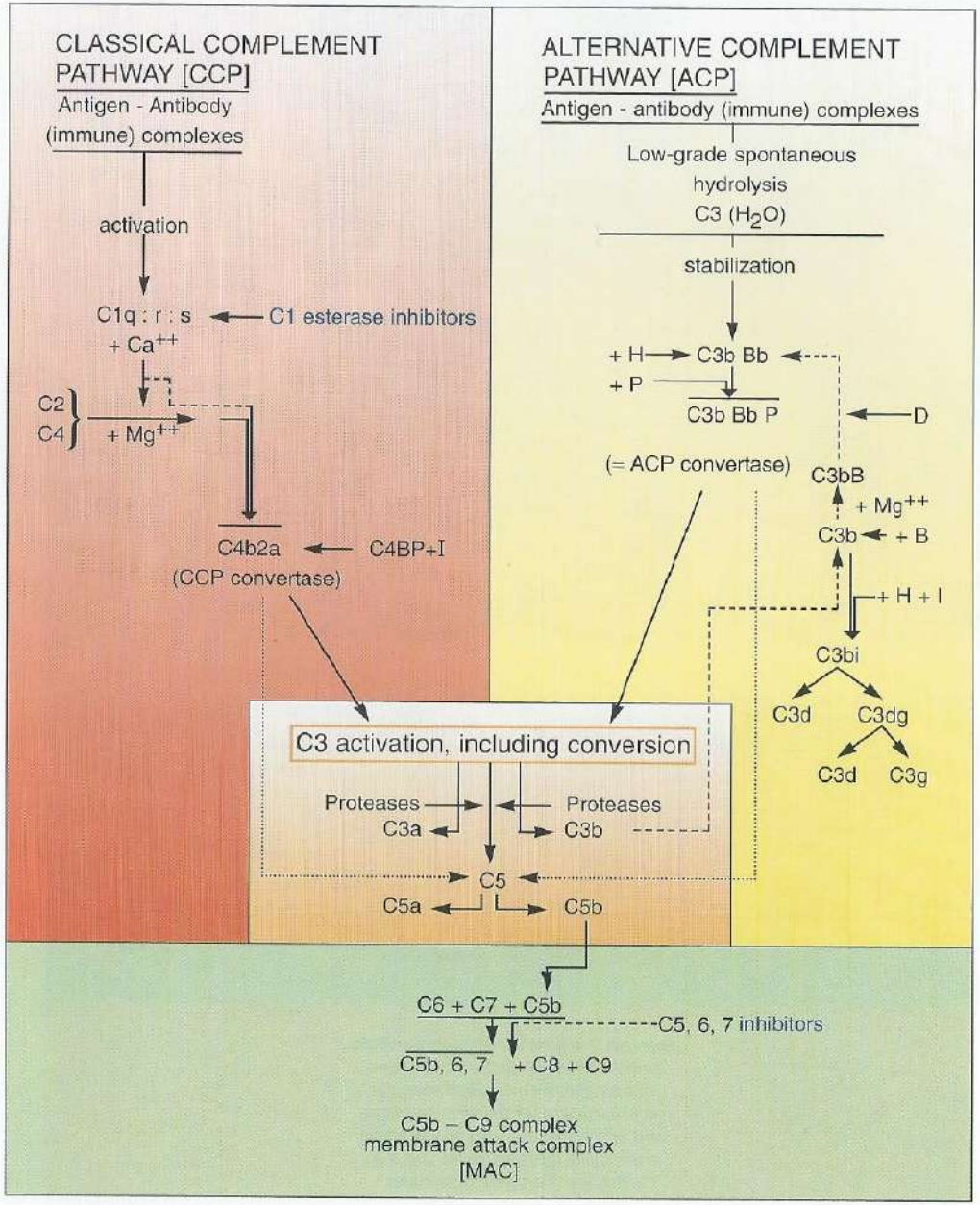
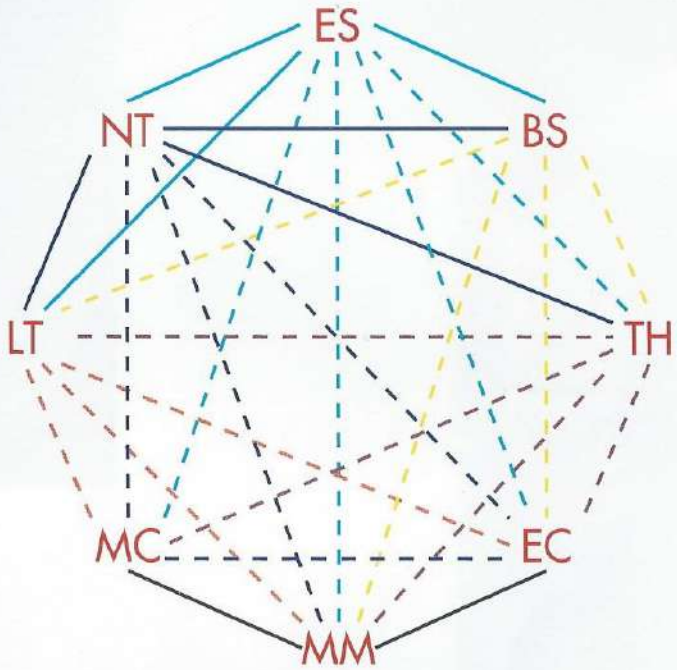


Fig. 56. Possible intercellular traffic during the "late-phase reactions", e.g. LNR



ES = Eosinophil
NT = Neutrophil
BS = Basophil
LT = Lymphocyte
MC = Mast cell
TH = Thrombocyte
EC = Epithelial cell
MM = Monocyte/Macrophage

XIV. SUPPLEMENTS

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A protective action of Disodium cromoglycate (code number "FPL 670"; trade name "Intal", "Lomudal") on the allergen challenge with house dust (5 mg/ml) or grass pollen mix. (10,000 NU/ml) on the nasal mucosa was demonstrated in 15 patients with pollinosis or vasomotor rhinitis. A good reproducibility of the nasal mucosa response was found after the allergen challenge. The mechanism of the protective action of DSCG was also shortly discussed.

PROTECTIVE EFFECT OF DISODIUM CROMOGLYCATE ON THE ALLERGEN PROVOCATION OF THE NASAL MUCOSA

Z. PELIKAN, M.D., W. J. SNOEK, H. BOOIJ-NOORD, M.D., N. G. M. ORIE, M.D., and K. de VRIES, M.D.

Introduction

DISODIUM CROMOGLYCATE (DSCG), a sodium salt of 1,3-bis-(2-carboxy-chromon-5-yloxy)-2-hydroxy-propane, is a recently developed drug, which may prevent allergic responses of the immediate type.[1,2,3]

This compound has, in pharmacological studies, neither an antihistaminic, anticholinergic, antiserotonic, antibradykininic, or anti-SRS-A activity, nor a bronchodilating activity.[3]

It has been suggested that DSCG inhibits the release, initiated by an antigen-antibody reaction, of mediators from the mast cells (type I) or possibly other cells (type III).[3,4,5,6,7]

Clinical experiments have shown that DSCG may prevent bronchial obstruction induced by experimental allergen provocation.[1,7,8,9,10]

The therapeutic value of this drug has been demonstrated by various clinical trials in allergic patients suffering either from "bronchial asthma" or "asthmatic bronchitis."[2,11-24]

No reports dealing with the effect of DSCG on the allergic responses, localized in the nasal mucosa (pollinosis, vasomotor rhinitis), were found in the available literature. The purpose of this communication is to present the results of a clinical experiment dealing with the DSCG protective activity of the allergic reaction of the nasal mucosa.

Materials and Methods

A. *Materials*

I. SUBSTANCES

House dust* (in concentration 5.0 mg dry weight of dialyzed and lyophilyzed extract per 1 ml of Coca solution) and Grass pollen mix* (10,000 NU per 1 ml of Coca solution) were used as allergens. 1 percent Disodium cromoglycate (DSCG)** in distilled water.

II. THE APPARATUS EQUIPMENT

A one channel recorder (Heath Built; model EUW-20A; USA); an electric differential pressure transducer (p-amplifier; Godart NV; The Netherlands); a water manometer; a small rubber balloon of a 5 mm diameter and of a 25 mm length (Lode NV; The Netherlands); and a polyethylene tubes connecting system were used.

B. *Methods*

I. PRINCIPLE OF METHOD

Changes of the nasal mucosa (e.g. swelling) influence the passage of air through the nose, which results in changes in the pressure differences between the nasopharyngeal cavity and open (outside) air. These pressure differences were recorded and considered as an index of the nasal flow.

A calibration curve of the apparatus used was made before each test. A small rubber balloon was introduced through one of the nasal cavities into the nasopharynx and the connecting tube was fixed to the alae nasi. The balloon was filled with 2 ml of air and it was then connected to the pressure transducer by a polyethylene tube. During the investigation the patient breathed through the free nasal cavity with a closed mouth.[25,26]

The changes in the nasopharynx-nostril pressure gradient (NPG) during the respiration were recorded. The NPG was expressed in cm of H_2O. The mean values of the NPG values were calculated over 90 to 120 seconds time intervals.

The control, allergen and DSCG solutions were applied on the nasal mucosa in the free nasal cavity (under the lowest concha) by means of a wad of cotton wool on a nasal probe.

II. PROCEDURE OF THE PROVOCATION AND PROTECTION TESTS

Provocation test: ± 10 minutes after the introduction of the balloon, when the patient demonstrated regular breathing, the experiment was performed in the following steps:

1. The recording of the NPG during three minutes, to obtain the so called "initial value" (basic value).
2. Control test with the application of the Coca solution during three minutes. The NPG was recorded during two minutes. If no significant changes were observed in respect to the initial value, the allergen challenge of the nasal mucosa followed.
3. Three minutes after application of the allergen (house dust 5.0 mg/ml or grass pollen mixture 10,000 NU/ml), the NPG was recorded in the following time intervals: 0, 10, 20, 30, 45, 60 minutes. The procedure of provocation was performed in eight patients with one allergen challenge and in seven patients the allergen challenge was repeated immediately after the first.
4. If the patient had some nasal complaints after the provocation test, an epinephrine (adrenaline) aqueous solution (1:1000) was applied on his nasal mucosa for one minute.

The provocation test was considered as positive, when the NPG value increased within 20 minutes after the end of allergen challenge by at least 1.5 cm of H_2O with respect to the initial NPG value.

Protection test: In all patients with a positive provocation test, the protection test with DSCG was carried out on another day. In the protection test, the same method was used as in the provo-

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*Prepared by Pharmacy "Diephuis" (Groningen).

**This compound was obtained from "Fisons Pharmaceuticals Ltd." (England) and it is produced under code number FPL 670 and trade mark "Intal", "Lomudal".

TABLE I. CHARACTERISTICS OF THE INVESTIGATED PATIENTS

Patient	single challenge of allergen							repeated challenge of allergen							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Sex	m	f	m	m	m	m	f	f	m	f	f	m	f	m	m
Age	25	29	14	21	18	22	27	19	23	13	18	28	16	26	22
Diagnosis	AB	PR	AB	AB	AB	PR	AB	AB	AB	AB	PR	AB	PR	AB	AB
Allergen	HD	HD	HD	HD	HD	GP	HD	HD	HD	HD	GP	HD	GP	HD	HD
State of nasal mucosa	n	n	h	h	h	n	h	h	h	h	h	h	h	h	h
Nasal complaints:															
obstruction		+	+	+	+	+	+	+	+		+	+	+	+	+
hypersecretion		+		+	+	+	+	+	+		+	+			+
sneezing		+	+	+	+	+	+	+	+		+	+	+		+
Blood eosinophilic cells per mm³	407	88	748	77	374	121	55	55	363	1023	451	132	x	264	858
Skin tests (routine)	±	±	+	±	+	+	+	+	+	±	±	±	+	+	+

m = male, f = female; AB = bronchial asthma or asthmatic bronchitis, PR = pollinosis or vasomotor rhinitis; HD = house dust, GP = grass pollen mixture; n = normal, h = hyperemic; X = unknown.

cation test. The DSCG was applied on the nasal mucosa in the non-connected nasal cavity for three minutes, just after the end of the Coca solution application. After seven minutes the NPG was recorded and then the allergen was applied.

The design of protection tests was performed in the same way as the provocation tests: in eight of them the single and in seven of them the repeated. In all patients, both with the single and with a repeated allergen challenge, the DSCG was applied only once.

III. PATIENTS

Fifteen patients selected for this clinical experiment were outpatients of the Pulmonary Division of the Department of Internal Medicine (University Hospital, Groningen). The selection was performed in two steps: first patients with symptoms of rhinitis vasomotoria or pollinosis, mostly combined with chronic obstructive lung disease and positive skin tests and second when there was a positive provocation test.

The patients were investigated in a period without manifest nasal complaints. Twenty-four hours before each test no antihistaminics or sympaticomimetica were given. Characteristics of the patients are summarized in Table I.

The investigated group of 15 patients was selected from 27 successively investigated patients, of which 12 had no reaction of the nasal mucosa after the allergen challenge.

Results

The values of the nasopharynx-nostril pressure gradient (NPG), which were measured during the provocation and the protection tests in the 15 patients, are given in the Table II. This table also shows a distinct difference in the mean values of NPG between the provocation test on the one hand and the protection test on the other hand.

TABLE II. SURVEY OF THE NASOPHARYNX-NOSTRIL PRESSURE GRADIENTS (IN CM H_2O) IN THE INDIVIDUAL PATIENTS AND THE MEAN AT DIFFERENT TIME INTERVALS; IN. VL. = INITIAL VALUE.

	Provocation Test							Protection Test						
	Time in minutes after the first allergen challenge							Time in minutes after first allergen challenge						
Patient	In. Vl.	0′	10′	20′	30′	45′	60′	In Vl.	0′	10′	20′	30′	45′	60′
1	1.3	5.3	12.1	7.8	6.5	6.1	2.9	2.0	2.9	2.9	3.3	2.8	3.3	2.6
2	1.5	4.2	5.0	4.8	4.8	4.4	3.2	1.7	2.1	1.8	1.6	1.2	1.1	0.9
3	4.1	7.5	8.9	11.0	10.1	15.1	7.7	4.4	4.0	4.2	3.6	3.6	3.0	2.1
4	1.3	3.6	7.6	4.4	2.8	2.4	2.3	1.9	2.5	2.9	2.6	2.3	1.9	2.1
5	2.6	16.1	17.4	14.4	13.1	7.2	2.3	2.4	3.8	3.8	3.3	2.6	1.9	1.8
6	1.9	8.9	9.2	7.6	5.0	4.0	3.3	2.1	1.9	2.6	2.1	2.1	2.0	1.9
7	1.9	4.0	3.6	4.1	3.4	2.9	2.2	1.8	2.3	2.7	2.6	2.2	1.6	1.5
8	0.9	9.3	20.4	13.0	14.8	19.2	8.1	0.9	2.1	6.3	4.5	3.2	3.4	2.0
9	2.0	4.6	5.7	6.7	5.4	5.6	3.2	1.8	1.2	1.6	2.0	2.1	2.1	1.7
10	2.0	4.0	6.5	7.2	6.7	6.7	3.0	3.3	3.0	5.7	3.3	3.1	2.9	2.2
11	0.7	1.8	2.2	2.4	3.4	2.9	1.0	1.5	2.4	1.2	1.2	0.9	1.2	0.9
12	1.2	2.5	3.3	4.8	3.7	4.1	1.5	1.4	2.0	2.1	2.4	2.1	1.9	1.9
13	0.6	2.3	3.1	1.8	1.6	1.4	0.7	0.9	1.4	1.6	1.1	1.3	1.0	0.8
14	1.5	7.4	6.0	6.1	6.6	3.8	2.9	2.3	3.1	3.2	3.1	3.6	2.6	2.3
15	1.4	7.4	10.6	12.3	12.3	4.5	1.8	1.6	2.3	2.9	2.3	2.6	1.8	1.6
Mean value	1.6	5.9	8.1	7.2	6.7	6.0	3.1	2.0	2.5	3.0	2.6	2.4	2.1	1.7

An analysis of variance was performed in respect to the differences between the values found in the same person at identical time intervals. The mean differences in respect to the time differ significantly ($p = 0.005$; $F\ (14,84) = 8.000$). It can be therefore concluded that DSCG has a significant protective effect on the reaction of the nasal mucosa after the allergen challenge.

From the data found in the provocation tests it may be concluded that the maximum of the allergen provocation effect is reached within 10 to 20 minutes after the end of allergen challenge and that this effect does not disappear completely within 60 minutes after the allergen challenge.

In seven of the patients, the allergen was applied twice, both in the provocation and in the protection test. The second challenge was carried out immediately after the first one. The results in those patients are shown in the Table III.

The same kind of analysis, described above, was applied to the differences of the values of the NPG found in the first and second allergen challenge in the provocation tests. No significant difference was observed between the first and the second allergen challenge ($p > 0.05$; $F\ [6,36] = 1.935$), indicating a good reproducibility of the response of the allergen challenge on the nasal mucosa.

In the protection tests using DSCG, no significant differences were found between the NPG values of the first and NPG values of the second allergen provocation ($p > 0.05$; $F\ [6,36] = 1.152$). This shows that the protective action of the DSCG on the allergen provocation effect of the nasal mucosa lasts at least two hours. The protective effect of DSCG was observed both after the challenge with the house dust (5 mg/ml) and after the challenge with the grass pollen (10.000 NU/ml).

In all 15 patients nasal complaints were observed after the allergen application in the provocation test. Nasal complaints were not observed during the protection test using the DSCG.

TABLE III. SURVEY OF THE NASOPHARYNX-NOSTRIL PRESSURE GRADIENTS (IN CM H_2O) IN INDIVIDUAL PATIENTS AND THE MEANS AT DIFFERENT TIME INTERVALS BY THE TWICE CHALLENGED GROUP.

	Provocation Test						Protection Test					
	Time in minutes after second allergen challenge						Time in minutes after second allergen challenge					
Patient	0′	10′	20′	30′	45′	60′	0′	10′	20′	30′	45′	60′
9	6.8	6.9	6.2	6.8	4.1	3.2	1.6	1.2	2.0	2.0	2.0	1.4
10	14.0	16.1	9.4	7.5	5.4	3.8	2.8	3.0	1.9	1.2	0.8	0.8
11	2.0	3.0	4.4	2.0	1.6	0.8	1.4	1.9	1.9	1.5	1.3	1.1
12	4.2	6.1	5.1	2.1	2.2	1.4	1.7	2.3	2.6	1.5	1.3	1.6
13	1.6	2.1	3.4	2.8	1.3	0.6	0.8	1.4	1.6	0.9	0.9	0.6
14	2.7	5.7	5.8	6.9	2.5	2.3	2.5	2.5	2.9	2.2	1.7	1.4
15	6.5	11.4	7.9	5.1	3.7	2.2	1.4	2.5	2.5	3.3	1.4	1.0
Mean value—2nd challenge	5.4	7.3	6.0	4.7	3.0	2.0	1.7	2.1	2.2	1.8	1.3	1.1
Mean value—1st challenge	4.3	5.3	5.9	5.7	4.1	2.0	2.2	2.6	2.2	2.2	1.9	1.6

Discussion

In respect to our results, it could be concluded that 1 percent solution of DSCG in distilled water, applied on the nasal mucosa before the allergen, has a clear protective action on the provocation effect of the house dust (5 mg/ml) or grass pollen mixture (10,000 NU/ml), also applied on the nasal mucosa. The protective action of DSCG lasts 2 hours at least as follows from the protection of the first as well as the second allergen challenge.

The results of our clinical experiment on the nasal mucosa are comparable with those of the clinical experiments on the bronchial tree.[1,7,8,9,10] This does not imply that the allergic process is identical in the bronchial tree and the nasal mucosa. In respect to the effector organ there are marked differences: in the bronchial tree the smooth muscles and in the nasal mucosa the blood vessels play a predominant role. The results, however, suggest that the mechanism of release of mediators in both organs plays an important role in triggering a reaction. This is in contrast to the lack of protective activity of DSCG on the skin tests. The allergic process in the skin (immediate type) differs from the process in the nasal mucosa and bronchial tree in respect to the release of mediators. Probably the action of DSCG is linked with the inhibition of the release of mediators from the mast cells.

These results may have consequences in the treatment of allergic vasomotor rhinitis and pollinosis. Some patients suffering from pollinosis with nasal complaints were, in spite of a course of desensitization, treated with nasal instillation of DSCG (4 × 10 mg per day), with good results. No controlled clinical trial was performed.

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In 600 patients suffering from allergic rhinitis the possible existence of another type of allergic reaction of the nasal mucosa other than Type I (immediate) was investigated by means of nasal provocation tests with allergens. Late response of the nasal mucosa to allergen challenge indicated that Type III hypersensitivity might be involved. Delayed reactions also made Type IV suspect.

LATE AND DELAYED RESPONSES OF THE NASAL MUCOSA TO ALLERGEN CHALLENGE

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Introduction

IMMEDIATE ALLERGIC REACTIONS (Type I) of the lower[1-6] as well as upper [7-12] respiratory tract to allergen challenge have been observed frequently and have been studied extensively.

Various authors have also described the "late" asthmatic reaction caused by the Type III allergy reaction (precipitin-mediated allergy).[3,4,13-15] There is as yet no direct evidence for Type IV (cell-mediated) allergic reaction in asthma but Pepys and co-workers have suggested that Type IV allergy may play a role in certain pulmonary diseases in man.[3,4,16] The possible role of the Type IV hypersensitivity reactions in the lungs of guinea pigs has been experimentally studied by Miyamoto and Kabe.[17] The Type IV response has been clearly defined as one of the allergic mechanisms that can occur in the skin following appropriate challenge.[4,16,18-22]

Most of the work on allergic response in the nose has been concerned with the immediate allergic reaction (Type I), although a few investigators have suggested the existence of a so-called "late" allergic reaction (Type III).[7,15,23]

The Type IV allergic reaction in the nose has not yet been clinically observed; however, some investigators have reported evidence of Type IV allergy in patients suffering from allergic rhinitis on the basis of either immunological tests *in vitro*[18,19,22,24-28] or skin tests[18-20,28] or intranasal antigen administration.[19,22] The delayed hypersensitivity mechanism was investigated by these authors as a possible factor in the production of rhinitis.

Nasal provocation tests have been used for several years by the author as a standard part of the clinical diagnostic procedure for the detection of the Type I allergic reaction in the nasal mucosa.[9-12]

The purpose of this clinical investigation was to determine whether other types of allergic reaction, different from Type I, could also occur in the nasal mucosa of the patients suffering from allergic rhinitis, after the allergen challenge, and the positive cases to observe and record their full clinical course.

Materials and Methods

I. *Allergens**
   a. *House Dust* in a concentration of 5.0 mg of dry weight of dialyzed and lyophilyzed extract per 1 ml of Coca's Solution.
   b. *Hairs and Feathers Mixture*** in a concentration of 2.5 mg of dry weight of dialyzed and lyophilyzed extract per 1 ml Coca's Solution.
   c. *Pigeon Feathers* in a concentration of 2.5 mg of dry weight of dialyzed and lyophilyzed extract per 1 ml Coca's Solution.
   d. *Aspergillus Fumigatus* in a concentration of 2.0 mg of dry weight of dialyzed and lyophilyzed

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* All allergen extracts were prepared by Pharmacy "Diephuis," Groningen, The Netherlands.

** Cat, dog, cattle, goat, hog, horse, rabbit, rat, mouse, hamster, guinea pig, canary, goose, duck, turkey, hen, pigeon, parrot, in equal proportions by weight.

extract per 1 ml of Coca's Solution.

e. *Moulds Mixture** in a concentration of 2.0 mg of dry weight of dialyzed and lyophilyzed extract per 1 ml of Coca's Solution.

f. *Flour Mixture*** in a concentration of 2.5 mg of dry weight of dialyzed and lyophilyzed extract per 1 ml of Coca's Solution.

g. *Wheat Flour* in a concentration of 2.5 mg of dry weight of dialyzed and lyophilyzed extract per 1 ml of Coca's Solution.

h. *Grass Pollen Mixture*† in a concentration of 10,000 Noon Units (NU) per 1 ml of Coca's Solution.††

i. *Spring Pollen Mixture*††† in a concentration of 10,000 Noon Units (NU) per 1 ml of Coca's Solution.

j. *Acetylsalicylic Acid* in a concentration of 0.5 mg per 1 ml of physiologic Saline (0.9% NaCl).

II. *Apparatus and Equipment consisted of*:[9-12]

One-channel recorder, an electric differential pressure transducer, a water manometer, a small rubber balloon of 5 mm diameter and 25 mm length, a polyethylene tubes connecting system.

III. *Principle of the Method*[9-12]

The nasal mucosa of the patient suffering from allergic rhinitis or pollinosis, when challenged topically by the appropriate allergen, reacts with swelling and hypersecretion. These changes influence the passage of air through the nose, resulting in changes in pressure differences between the nasopharyngeal cavity and the outside air. These pressure-differences, so-called NPG (nasopharynx-nostril pressure gradient), expressed in cm of H_2O, were recorded and considered as a parameter for the assessment of the reactivity of the nasal mucosa. The mean values of the NPG were always calculated during regular breathing over a time-period of 90 to 120 seconds.

IV. *Patients*

Six hundred patients, consisting of out-patients or hospitalized patients, were investigated by means of routine nasal provocation tests with various allergens to confirm or to exclude the Type I allergic reaction of the nasal mucosa (perennial rhinitis or pollinosis).

All patients investigated showed:

a. Nasal complaints (obstruction, hypersecretion, sneezing) probably due to the Type I allergic reaction of nasal mucosa.

b. Positive intracutaneous reactions to one or more allergens. Each of the patients was tested with a standard group of 32 inhalant allergens. Some of them were also tested with some additional allergens according to disease-history. In all patients the immediate reaction on the skin (Type I) was observed, in some of them a late (Type III) or delayed (Type IV) also occurred.

Of the 600 patients 17 complained of nasal symptoms from four to 48 hours after the end of the routine nasal provocation tests with allergen (that is from 6 to 50 hours after allergen challenge) and these were selected for further investigation. Their characteristics are presented in Table I. From the patients who did not complain of acute nasal symptoms from 4 to 48 hours after the nasal provocation tests, 30 were selected randomly for the control investigation. Of these 30 patients, 15 demonstrated a positive immediate nasal mucosa response after allergen challenge; the other 15 did not respond. These patients were investigated in the same manner and during the same period as those in the experimental group.

The patients were always investigated in a period without manifest nasal complaints. No anti-allergic therapy was allowed during 96 hours prior to the investigation. They were not treated with Disodium cromoglycate (Rynacrom, Lomudal, Intal), with corticosteroids or with immunotherapy.

Procedure

Before each test, after calibration of the equipment, a rubber balloon was introduced into the nasopharynx through one of the nasal cavities, filled with 2 ml of air and then connected to the pressure transducer. The patient breathed only through the non-intubated nasal cavity, the mouth being closed and the intubated cavity also being closed by means of the patient's finger. Five to fifteen minutes after the balloon introduction, when the patient demonstrated regular breathing, the test was started.

I. *The routine part* was performed by the following steps:[9-12]

The mean NPG values were recorded at 0, 5 and 10 minutes, to obtain so-called "initial values."

Coca's solution was applied for three minutes to the nasal mucosa under the lowest concha of the non-intubated cavity by means of a wad of cotton wool on a nasal probe. The mean NPG values were then recorded at 0, 5 and 10 minutes after the end of Coca application. If the patient showed no changes of the mean NPG values with respect to the "initial values," the investigation was continued.

The allergen was challenged on the nasal mucosa for three minutes in the same way (and at the same site) as the Coca's solution. The mean NPG values were then recorded at 0, 5, 10, 20, 30, 45, 60, 90 and 120 minutes after the end of the challenge.

There was a time interval of several days between the routine nasal provocation and the experimental portion of the investigation of the selected patients.

Cladosporium cladosporioides-elatum-herbarum, Penicillium brevi compactum-expansum-notatum-frequentans-commune, Aspergillus versicolor-niger-fumigatus, Mucor sphinosus-mucedo-racemosus, Pullularia pullulans, Botryris cinerea, Merculius domesticus, Epicoccum purpurascens, Alternaria tenuis, Stemphylium botryosum, Rhizopus nigricans, Fusarium culmorum and Trichoderma viride — in equal proportions by weight.

** Wheat flour, rye flour, rice flour, oatmeal and barley flour—in equal proportions by weigh.

† Dry weight percentage: *Secale cereale* 15%, *Dactylis glomerata* 15%, *Lolium perenne* 10%, *Anthoxanthum odoratum* 10%, *Agrostis alba* 10%, *Holcus lanatus* 10%, *Phleum pratense* 10%, *Cynosurus cristatus* 5%, *Alopecurus pratensis* 15%.

†† 1 Noon Unit (NU)=0.001 mg of dry pollen (powder)=0.5 PNU=1.3 TNU.

††† Dry weight percentage: *Corylus avellana* 20%, *Alnus species* 30%, *Salix species* 20%, *Betula species* 20%, *Myrica* species 10%.

Table I. Clinical features of the patients investigated.

Patient	Sex	Age	Nasal Complaints: Obstruction	Hyper-secretion	Sneezing	Lividity of nasal mucosa	Blood eosino-philic cells per mm3	Skin Tests (i.c.) — immediate response: HD	HF	GP	SP	Others	Allergen used for experimental nasal provo-cation tests	Type of nasal mucosa response
1	F	23	+	+ +	+	+	1155	+	-	-	-	+ Mm	AF	IV
2	F	14	+ + +	+ +		+	990	+	+	+	-	+ H	GP	III
3	M	34	+ + +			+	220	+	+	+	+	+ W	HD	IV
4	F	21	+	+	+	+	176	+	+	+	+	+ Mm	GP	IV
5	F	22	+	+		+	880	+	+	+	+	+ C	HD	IV
6	M	22	+ + +			+	363	+	+	+	+	+ RF	WF	III
7	F	15	+ + +			+	165	+	+	+	+	+	AA	IV
8	F	12	+ + +	+		+	495	+	+	+	+	+ AA scratch	HD, SP	III, I + III
9	F	36	+ + +	+ +	+	+	451	+	+	+	+	+ Mm	HD, HF, GP	III, I + IV, I + IV
10	M	45	+ + +		+	+	583	+	+	+	+	+ FP	HD, GP	IV, I + +
11	M	48	+ + +		+	+	495	+	+	+	+	+ FP	HD	III
12	F	48	+ + +		+	+	242	+	+	+	+	+ Mm	SP	III
13	M	30	+ + +	+ +	+	+	297	+	+	+	+	+ FP	HD	IV
14	F	27	+ + +	+ +	+	+	902	+	+	+	+	+ FP	GP	O + IV
15	M	37	+ + +	+ +	+	+	1309	+	+	+	-	- PF	HD, GP, PF	I + III, I + III, O + III
16	F	13	+ + +	+	+	+	561	+	+	+	-	- TP	HD, GP	O + IV, I + IV
17	M	17	+ + +	+	+	+	330	+	+	+	-	- Mm	HD	I only

Legends: M = male; F = female; HD = House Dust; GP = Grass Pollen; AF = Aspergillus Fumigatus; WF = Wheat Flour; AA = Acidum Acetylsalicylicum; SP = Spring Pollen; HF = Hairs and Feathers; PF = Pigeon Feathers; TP = Tree Pollen; FP = Flower Pollen; D = Dog Hair; RF = Rye Flower; Mm = Molds mix; W = Wool; C = Cat Hair.

O = immediate negative response; I = immediate positive response; III = late response; IV = delayed response.

Patients No. 1-16 were "non-immediate" positive. Patient No. 17 was "non-immediate" negative.

The evaluation of the intracutaneous tests:

- = normal skin appearance.
+ = wheal not greater than original injected papule.
++ = wheal increase up to 7.5 mm in diameter.
+++ = wheal increase up to 10.0 mm in diameter.
++++ = wheal increase up to 12.5 mm in diameter.
+++++ = wheal increase up to 15.0 mm in diameter.
++++ = wheal increase greater than 15 mm, with surrounding erythema and sometimes with "pseudopodia."

II. *The experimental part* consisted of the following tests, each of them performed on a separate day with a time interval of minimally 72 hours between them.

a. *The Provocation Test*

The mean NPG "initial" values were recorded at 0, 5 and 10 minutes.

Coca's solution was applied on the nasal mucosa for three minutes in the same manner as the routine tests. The mean NPG values were then recorded at 0, 5 and 10 minutes.

The allergen was challenged for three minutes on the nasal mucosa in the same manner and on the same sites as the routine tests. The mean NPG values were then recorded at 0, 5, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210 and 240 minutes, then every hour up to 12-respectively 14 hours after the end of the allergen challenge. From the 23rd (24th) hour to the 56th hour after the end of the allergen challenge the mean NPG values were recorded every second hour (when necessary every hour). The patients were not investigated during the 8-10 hours sleep interval in the night (between 12-23rd-respectively 14-24th hours and 36-48th-respectively 40-48th hours).

b. *The Control Test with Coca's Solution*

The mean NPG "initial" values were recorded at 0, 5 and 10 minutes.

Coca's Solution was applied to the nasal mucosa for three minutes in the same way as described above and the mean NPG values were recorded at the same time intervals as during the provocation tests with allergen (0 minutes - 56 hours).

The provocation tests were considered positive when the mean NPG values after the allergen challenge increased by at least 1.5 cm H_2O with respect to the "initial" and "Coca" values.

The patients were challenged during the clinical investigation by the same allergen as during the routine tests.

In some patients more allergens were tested to investigate whether the "non-immediate" response of the nasal mucosa could be caused by only one allergen or by additional allergens. The choice of the other allergens was based on positive intracutaneous tests and on the clinical or disease history. In other patients the provocation tests with the same allergen were repeated to investigate reproducibility.

Results

Of the group of 600 patients who were examined by means of the routine nasal provocation tests with allergens, 497 gave a positive immediate response of the nasal mucosa to the allergen challenged (Type I allergic reaction).

Sixteen of the 497 patients demonstrating positive immediate reaction of the nasal mucosa and one patient of the remaining 103 who did not demonstrate immediate response (Patient No. 14), reported acute nasal complaints between four and 48 hours after the end of the routine nasal provocation tests (that is 6-50 hours after the allergen challenge). These 17 cases were additionally studied by further nasal provocation tests, which were continued for up to 56 hours.

Sixteen of the 17 patients investigated also developed other types of the nasal mucosa response. These reactions, clinically different from Type I allergic reaction, occurred 6-50 hours after the allergen challenge. These patients are henceforth called "non-immediate positive."

In the one remaining patient from the group immediate positive who reported later nasal complaints no other type of nasal mucosa response was detected other than Type I. This patient is henceforth called "non-immediate negative" (Patient No. 17).

A survey of the mean NPG values recorded during the individual tests of this investigation in each of the 16 "non-immediate positive" patients is given in Table II.

The mean NPG values recorded after the allergen challenge with respect to the mean values measured after the Coca challenge in individual tests in "non-immediate positive" patients are graphically presented in Figures 1 and 2. Figure 1 shows the responses which were considered as late responses, while Figure 2 shows those which were considered as delayed.

In 16 "non-immediate positive" patients three types of the nasal mucosa response were observed.

1. Fifteen patients developed a nasal mucosa response within two hours after the allergen challenge. This response started within 10 minutes, reached its maximum within 30 minutes and disappeared at least 90-120 minutes after the allergen challenge. This response of the nasal mucosa was considered by us as Type I allergic reaction (immediate hypersensitivity).

2. Eight patients developed a nasal mucosa response within 6-14 hours after the allergen challenge (that is 4-12 hours after the end of the immediate reaction). This reaction began within six hours, reached its maximum within 8-10 hours and disappeared in most of the patients within approximately 24 hours after the allergen challenge. This type was considered by us with respect to its clinical course as a late response (possibly due to the Type III allergic reaction).

3. In 10 patients a nasal mucosa response appeared within 26-50 hours after the allergen challenge (that is 24-48 hours after the end of the immediate response). This response began within 26-30 hours, reached its maximum within 30-40 hours and in most of the patients resolved within 50 hours after the allergen challenge. This type was interpreted by us, in view of its clinical course, as a delayed response (possibly due to the Type IV allergic reaction).

In most patients investigated the late as well as the delayed nasal mucosa response were preceded by the

Table II. Survey of the mean nasopharynx-nostril-pressure gradient (NPG) values in cm H_2O recorded during the experimental nasal provocation tests with allergen in 16 "non-immediate positive" patients.

Patient	Allergen	Initial value	Coca value	Time in minutes after challenge								Time in hours after challenge											
				0'	5'	10'	20'	30'	45'	60'	90'	2h	3h	4h	5h	6	7h	8h	9h	10h	11h	12h	13h
1	AF	1.8	1.6	2.3	2.5	3.7	14.1	15.4	16.3	14.1	8.1	4.5	2.3	2.1	1.6	1.6	1.6	1.8	1.6	1.9	2.0	2.1	2.0
	AF	2.5	2.3	2.5	2.5	4.8	12.4	19.5	15.4	12.9	6.1	2.7	2.5	2.4	2.4	2.4	2.4	2.4	2.3	2.7	2.5	2.5	2.6
2	GP	1.5	1.5	1.7	2.7	3.9	5.2	6.9	5.7	4.3	1.8	1.5	1.3	4.4	8.9	11.6	8.2	7.1	3.4	2.0	1.7	1.7	1.5
	GP	1.8	2.0	2.2	2.2	3.2	4.6	7.5	8.8	6.1	3.0	2.0	1.9	1.7	3.9	9.4	12.1	12.0	10.9	6.2	4.1	2.0	2.0
3	HD	3.7	3.3	4.5	6.5	9.0	14.5	17.2	11.9	8.5	6.5	4.0	3.6	3.6	3.7	3.7	3.7	4.0	3.7	3.5	3.8	4.1	4.1
4	GP	3.4	3.5	4.4	6.3	10.3	12.4	14.8	11.4	5.6	4.8	3.3	3.3	3.3	3.0	3.0	3.2	3.3	3.3	3.3	3.3	3.0	3.3
5	HD	3.7	3.8	4.4	5.7	9.8	14.3	20.8	16.2	9.5	5.5	4.3	3.8	3.7	3.6	3.8	3.8	3.7	3.8	3.8	3.8	3.5	3.4
	HD	3.4	3.4	3.7	5.2	10.3	15.1	18.9	17.3	12.1	7.7	4.6	3.3	3.4	3.5	3.5	3.5	3.2	3.3	3.7	3.5	3.5	3.4
6	WF	1.5	1.5	2.5	3.6	6.1	6.7	7.7	7.9	6.3	5.1	2.3	1.3	1.5	1.4	1.5	2.7	5.2	6.1	7.9	8.0	7.6	7.8
	WF	2.0	2.0	2.8	4.6	5.2	5.9	7.3	8.3	6.7	5.0	3.0	2.0	2.1	1.7	1.9	2.0	2.1	4.7	7.5	9.2	8.7	9.0
7	AA	3.0	3.1	3.2	3.2	4.1	10.6	13.7	12.3	5.2	3.8	3.2	2.8	3.1	3.0	3.1	3.1	3.0	3.1	3.1	3.1	3.1	3.2
8	HD	4.6	4.6	4.7	5.6	8.7	12.2	13.4	13.0	8.1	5.4	4.6	4.6	4.5	4.6	6.6	7.1	8.4	7.6	12.5	9.7	7.3	5.7
	SP	4.0	4.0	4.2	7.9	9.8	14.1	11.7	11.0	5.5	4.1	4.1	3.9	4.3	3.7	3.8	4.0	5.9	15.3	16.1	15.5	15.7	15.5
9	HD	2.5	2.7	3.4	3.3	3.5	5.7	6.1	5.5	4.7	3.7	3.1	3.0	5.0	8.4	9.4	12.7	9.8	8.8	10.2	7.1	4.6	
	HF	2.2	2.4	3.1	3.4	4.5	6.0	4.9	3.7	3.3	3.2	2.8	2.5	2.5	2.5	2.5	2.2	2.7	2.2	2.9	2.2	2.4	
	GP	2.2	2.2	3.0	6.3	8.8	11.6	13.6	9.6	5.6	3.5	2.5	2.6	2.9	2.6	2.6	2.2	2.3	2.8	2.9	2.9	2.6	
10	HD	3.1	3.2	3.2	4.9	7.5	10.6	11.0	10.1	6.2	4.1	3.2	2.8	3.2	3.2	3.3	3.2	2.3	3.2	2.9	3.0	2.9	
	GP	2.1	2.1	3.0	4.1	5.2	6.1	6.5	5.1	3.5	2.3	2.1	2.2	2.2	2.5	4.4	11.1	12.3	9.6	9.8	9.6	7.5	
11	HD	2.5	2.6	3.7	5.5	7.8	9.9	11.9	9.7	4.8	3.6	2.8	2.6	2.5	2.5	2.6	4.3	10.1	16.1	16.8	16.2	14.1	
12	SP	2.1	2.3	2.9	3.5	6.5	9.2	10.0	11.7	10.3	4.5	2.4	2.3	2.4	2.4	4.9	8.6	13.4	11.2	13.9	9.7	6.0	
13	HD	2.4	2.5	4.1	5.4	6.8	11.0	9.4	4.6	3.8	2.5	2.7	2.6	2.5	2.5	2.5	2.6	2.6	2.5	2.7	2.6	2.8	
14	GP	1.7	1.7	1.7	1.8	1.9	1.9	2.2	1.8	1.9	1.9	1.9	2.3	1.6	1.8	2.1	2.2	2.1	2.2	2.1	1.8	2.2	1.7
15	HD	2.0	2.0	2.3	3.4	8.6	12.9	10.4	8.0	4.7	3.4	2.1	1.8	1.8	1.7	1.9	6.8	11.2	11.9	11.3	9.9	8.3	
	GP	3.4	3.5	4.6	7.5	7.3	10.4	8.0	7.8	4.7	4.7	3.8	3.5	3.5	3.9	4.8	9.0	10.5	14.8	15.1	14.0	8.4	
	PF	3.3	3.2	3.4	3.1	3.1	3.2	3.1	3.1	3.3	3.2	3.3	3.3	3.2	3.1	7.9	12.0	12.1	12.1	12.6	11.8	12.4	
16	GP	2.6	2.6	4.1	6.6	11.1	14.8	15.5	11.3	7.0	4.6	2.7	2.6	2.9	2.9	2.7	2.7	2.7	2.7	2.7	2.9	2.5	
	HD	2.0	2.2	2.2	2.2	2.1	2.3	2.4	2.2	2.2	2.1	2.1	2.1	2.4	2.3	2.2	2.4	2.3	2.3	2.1	2.2	2.2	

Legends: AF = Aspergillus Fumigatus; GP = Grass Pollen; HD = House Dust; WF = Wheat Flour; AA = Acidum Acetylsalicylicum; SP = Spring Pollen; HF = Hairs and Feathers; PF = Pigeon Feathers; - = not recorded.
The "initial value" as well as the "Coca value" are the mean NPG values, always calculated from 3 values, as recorded at 0, 5, and 10 minutes.

(continued on next page)

Table II. Survey of the mean nasopharynx-nostril-pressure gradient (NPG) values in cm H_2O recorded during the experimental nasal provocation tests with allergen in 16 "non-immediate positive" patients.

Time in hours after challenge

14h	23h	24h	25h	26h	27h	28h	29h	30h	31h	32h	33h	34h	35h	36h	38h	40	48h	49h	50h	51	52h	53h	54h	55h	56h
1.9	2.0	1.9	—	1.6	—	2.8	—	3.2	3.6	4.1	11.7	14.9	15.1	15.0	11.7	7.1	4.8	3.7	1.6	—	1.9	—	1.8	—	1.6
2.4	2.3	2.4	—	2.5	—	2.5	—	2.7	12.7	15.4	16.3	17.0	15.4	14.3	14.3	9.1	3.2	2.5	2.3	—	2.1	—	2.5	—	2.5
1.5	1.9	1.3	—	1.5	—	1.3	—	1.5	—	1.4	—	1.5	—	1.4	—	1.4	1.6	1.5	1.4	—	—	—	—	—	—
1.9	2.0	1.5	—	1.6	—	1.5	—	2.0	—	1.5	—	1.7	—	1.9	—	2.0	2.0	2.0	1.9	—	—	—	—	—	—
3.5	3.3	7.8	7.9	7.9	8.0	8.9	8.5	8.9	9.0	8.2	6.5	7.8	5.5	4.9	3.6	3.6	3.9	3.6	3.5	—	3.4	—	3.5	—	3.5
3.3	3.4	3.4	3.5	3.4	3.2	5.7	—	10.9	—	12.9	—	12.1	—	8.6	8.3	5.4	4.8	4.2	4.1	—	3.3	—	3.0	—	3.3
3.5	3.8	3.8	—	3.8	—	3.6	—	9.4	13.0	16.9	15.8	16.4	16.7	15.3	11.6	7.8	4.0	3.6	3.7	—	3.5	—	3.7	—	3.5
3.5	3.6	3.4	—	5.4	—	6.9	—	10.1	—	11.7	—	11.9	—	12.4	11.3	11.0	4.7	3.2	3.5	—	3.3	—	3.4	—	3.3
6.5	3.4	2.8	—	2.5	—	1.8	—	1.7	—	1.5	—	1.5	—	1.4	1.5	1.5	1.2	1.2	1.7	—	1.5	—	1.6	—	1.6
7.3	5.1	3.6	—	3.1	—	2.4	—	1.6	—	1.9	—	1.9	—	2.1	2.0	2.1	2.0	2.3	2.0	—	1.7	—	2.0	—	2.1
3.2	3.3	3.2	—	3.4	—	4.5	6.6	7.9	10.0	9.4	11.8	10.4	10.6	9.8	7.3	6.4	5.4	5.1	4.4	4.1	3.8	3.2	3.1	3.1	3.2
4.6	4.9	4.6	—	4.6	—	4.6	—	4.4	—	4.5	—	4.5	—	4.3	4.4	4.5	4.5	4.4	4.3	—	—	—	—	—	—
15.2	4.1	4.0	—	3.8	—	3.8	—	4.0	—	4.0	—	4.1	—	4.1	4.1	3.5	4.0	3.8	4.1	—	—	—	—	—	—
—	—	2.3	2.3	2.2	2.2	2.2	2.2	2.1	2.3	2.2	2.2	2.3	2.2	2.4	—	—	2.3	—	2.2	—	2.1	—	2.1	—	—
—	—	3.4	5.9	5.7	8.2	7.9	5.8	5.9	4.3	4.5	4.3	4.8	4.1	3.3	2.9	2.9	3.0	—	2.5	—	2.3	—	1.9	—	—
—	—	2.9	3.3	4.0	8.5	7.2	9.3	8.7	13.9	14.1	6.2	4.4	4.6	5.0	2.9	3.1	2.5	—	2.4	—	2.4	—	2.4	—	—
—	—	2.8	3.2	3.2	3.1	3.2	6.4	11.3	13.7	15.4	12.4	12.8	9.8	8.4	—	—	4.7	3.8	3.2	2.9	2.8	2.8	2.8	3.1	3.0
—	—	2.6	2.2	2.0	2.0	2.1	2.2	2.1	2.2	2.1	2.1	2.1	2.0	2.3	—	—	2.1	1.9	2.2	1.9	2.2	2.2	1.9	—	—
—	—	2.5	2.6	2.5	2.5	2.6	2.6	2.6	2.7	2.9	2.6	2.6	2.9	2.8	—	—	3.0	2.7	2.8	2.9	2.6	2.4	2.6	2.6	2.7
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	2.7	2.7	3.4	4.4	7.6	11.2	10.8	11.3	9.7	12.4	11.1	8.6	3.4	—	—	2.7	2.8	2.6	2.5	2.6	2.6	2.6	2.5	2.6
—	—	1.8	1.8	2.1	2.2	3.7	11.5	13.2	12.9	13.3	15.1	11.0	10.6	9.7	—	—	8.5	7.2	4.9	3.1	1.9	1.9	1.6	1.9	1.7
—	—	3.5	3.8	4.0	3.3	2.1	1.7	1.9	1.8	3.2	2.2	1.8	1.7	1.8	—	—	2.1	—	2.0	—	—	—	—	—	—
—	—	3.9	3.6	3.6	3.2	3.3	3.3	3.2	3.0	3.0	2.9	3.1	2.8	2.9	—	—	3.0	—	2.8	—	—	—	—	—	—
—	—	10.7	6.7	4.5	3.6	3.7	3.3	3.2	3.4	3.2	3.2	3.0	3.0	3.2	—	—	3.3	3.4	3.2	3.1	3.2	—	3.4	—	3.0
—	—	2.6	2.4	2.5	2.5	2.5	2.5	2.6	5.0	10.6	15.6	18.7	20.4	19.3	—	—	8.1	6.2	4.9	3.5	2.7	2.5	2.5	2.3	2.5
—	—	2.4	2.4	2.2	2.5	2.4	2.0	2.1	2.2	4.9	13.5	14.6	15.7	14.7	—	—	14.3	12.6	14.2	10.7	4.7	2.2	2.2	.20	2.1

Legends: AF = Aspergillus Fumigatus; GP = Grass Pollen; HD = House Dust; WF = Wheat Flour; AA = Acidum Acetylsalicylicum; SP = Spring Pollen; HF = Hairs and Feathers; PF = Pigeon Feathers; — = not recorded.
The "Initial value" as well as the "Coca value" are the mean NPG values, always calculated from 3 values, as recorded at 0, 5, and 10 minutes.

immediate response, a so-called "dual-response." However, one patient (No. 15 — pigeon feathers) demonstrated late response and two patients (No. 14 — grass pollen, No. 16 — house dust) showed delayed response which were not preceded by the immediate response.

Most of the late as well as delayed responses of the nasal mucosa observed were related to a single allergen in each patient. However, one patient (No. 15) demonstrated late response to three different allergens, one patient (No. 8) showed late response to two different allergens, one patient (No. 16) developed delayed response to two different allergens and in two patients (No's. 9, 10) both the late and delayed responses were recorded, caused by different allergens in the same person.

In two patients demonstrating late (No's. 2, 6) and in two others demonstrating delayed response (No's. 1, 5) the nasal provocation tests were repeated with the same allergen to assess reproducibility. A high similarity was observed between the first and the second test in each of the four patients (Table II).

It may therefore be concluded that in 16 "non-immediate positive" patients, totally, 11 late and 12 delayed responses of the nasal mucosa were observed.

A survey of all nasal provocation tests performed in each of the patients investigated is presented in Table III.

The mean NPG values recorded after the Coca challenge did not differ significantly both with respect to the "initial values", and during the whole "Coca control test" in any patient investigated. These changes varied within 1.2 cm H_2O.

None of the patients from the control group demonstrated any significant changes of the mean NPG values during the time interval 2-54 hours after the allergen challenge. All control patients were therefore "non-immediate" negative.

All types of the nasal mucosa responses (immediate, late, delayed) in all positively reacting patients, were accompanied by the appearance of acute nasal complaints. The course of the nasal complaints ran parallel to the course of the NPG changes. The nasal complaints first appeared during the immediate response and then disappeared. After a symptom-free period of several hours the acute nasal symptoms returned and lasted during the whole

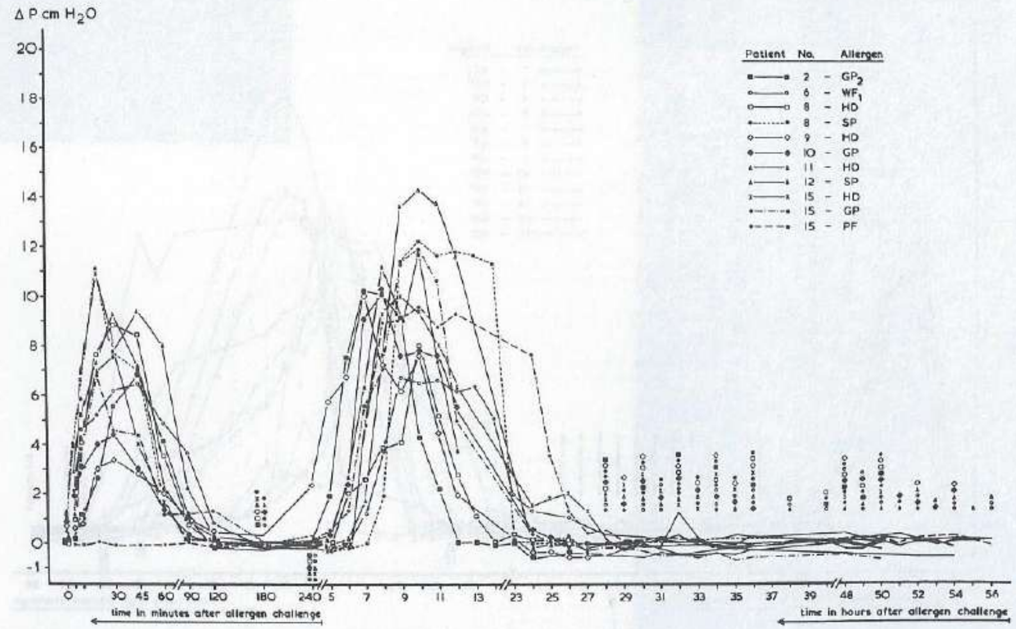

Figure 1. The mean NPG values after allergen challenge with respect to the mean NPG values after Coca solution challenge, recorded in those patients investigated who demonstrated the presumed Type III hypersensitivity of the nasal mucosa.
Legend: GP = Grass Pollen, WF = Wheat Flour, HD = House Dust, SP = Spring Pollen, PF = Pigeon Feathers.

course of the responses (late or delayed). The relative severity of the individual nasal complaints differed, however, in the various types of responses (Table IV).

The appearance of the nasal mucosa was also different in the different types of nasal mucosa response. The lividity of the nasal mucosa increased during the immediate and late response but was most pronounced during the delayed response. Hemorrhages occasionally occurred in the nasal mucosa during the delayed response (four patients).

A good correlation was found between the immediate nasal response and the intracutaneous tests in the same patient with the same allergen. No significant correlation was observed between the skin tests and nasal provocation tests with respect to the late or delayed responses.

Discussion

Allergic rhinitis (perennial rhinitis and pollinosis) has classically been attributed to the mechanism of immediate hypersensitivity (Type I allergic reaction).[29]

Lately, however, some authors have provided evidence for another type of allergic reaction of the nasal mucosa, different from Type I. This reaction has been suggested to be a Type III allergic reaction and called "late nasal response." The recognition of the Type III allergic reaction of the nasal mucosa was based partly on recording of subjective nasal complaints, partly on the measurement of nasal airways resistance, however within a limited time interval, and partly on some immunological parameters.[7,15,23]

The Type III reaction of nasal mucosa has been observed in two modifications — either as "late" only or as a part of the so-called "dual response" where the Type I preceded the Type III. The dual reaction has been found more frequently than the late response alone.[7,23]

Other investigators have found some evidence for a possible role of the Type IV allergic reaction in allergic rhinitis.[18,19,22,24-28,30]

Slavin and co-workers[19,20] have provided some evidence for delayed hypersensitivity to pollen occurring in the allergic nasal mucosa. Slavin suggested the possibility that symptoms of rhinitis might result also from delayed hypersensitivity.[20] He showed that a

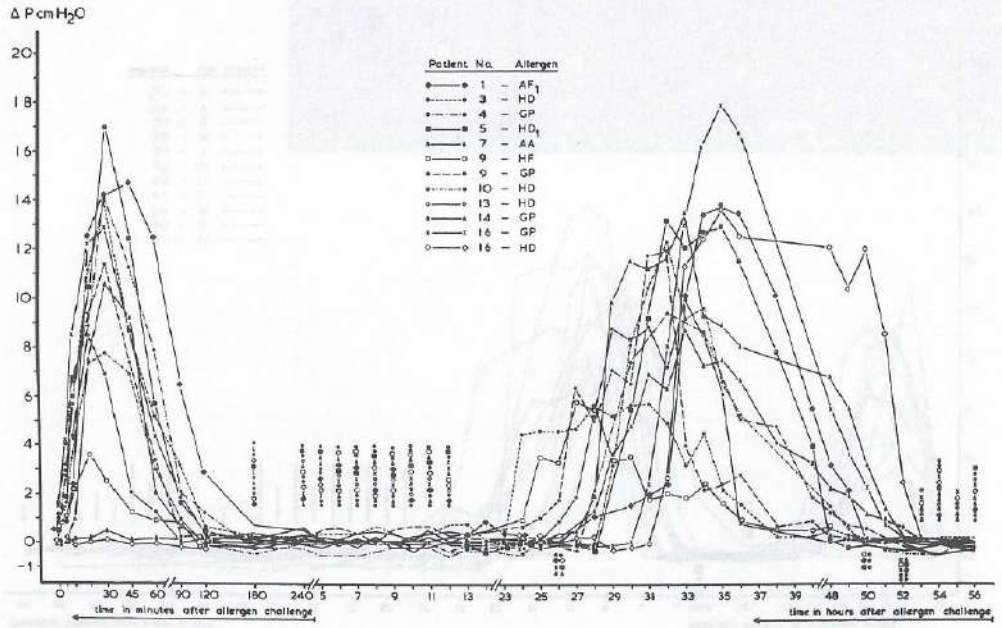

Figure 2. The mean NPG values after allergen challenge with respect to the mean NGP values after Coca solution challenge, recorded in those patients investigated who demonstrated the presumed Type IV hypersensitivity of the nasal mucosa.
Legend: AF = Aspergillus Fumigatus, HD = House Dust, GP = Grass Pollen, AA = Acidum Acetylsalicylicum, HF = Hairs and Feathers.

state of delayed hypersensitivity might be present even in the absence of the delayed skin reactions.[19] He observed that induction of delayed hypersensitivity by means of nasal administration of antigen was considerably different in two groups of patients, the atopic group having a far greater number of reactions.[20]

Brostoff and Roitt[18,28] have also found evidence for delayed hypersensitivity in some patients suffering from hay fever from tests on the patients' lymphocytes and leucocytes.

Rocklin and co-workers,[27] Richter and Naspitz,[26] Maini and co-workers,[30] Norman and Lichtenstein,[25] Evans and his co-workers[24] have concluded from immunological tests *in vitro* in patients with hay fever that a cellular immune response to some antigens may also be detected.

We have not yet found a study in the literature describing clinical recognition of the Type III and especially of the Type IV allergic reaction in the nose after allergen challenge. To record this was one of the purposes of our investigation.

The classification of the different types of allergic reaction is based on clinical, immunological and histopathological features.[29] One of the important clinical features is the time-course of the allergic reaction, i.e., the time-course of objective changes as well as of subjective complaints.

Several papers describe the time-course of the clinical manifestations of different types of allergic reaction of various organs or systems. Most of them, however, concern the skin and lower respiratory tract. The allergic responses of the skin were characterized, with respect to their time-course, as follows.[4,16,18-23,31,32]

Immediate — onset within 5-10, maximum within 10-20, resolving within 60-90 min.

Late — onset within 7-8 hours, resolving within 24 hours.

Delayed — onset within 24 hours, maximum at 48 (72) hours, resolving within a few days.

The allergic responses observed in the lower respiratory tract during the inhalation provocation tests with allergen were defined with respect to the

Table III. Survey of all nasal provocation tests (routine and experimental) performed in each of the patients investigated.

	Allergen																	
	Type allergic reaction																	
	HD			HF			GP			SP			Other			Other		
Patient	I	III	IV	I	III	IV	I	III	IV	I	III	IV	I	III	IV	I	III	IV
1	-	-	-	-	-	-	-			-			AF	+	-	+	Mm	+	-	-
2	+	-	-	-			+	+	-	-	-	-	TP	-	-	-	H	-		
3	+	-	+	-			-			-			FP	-			W	+		
4	-	-	-	-			+	-	+	-			FP	+			Mm	-		
5	+	-	+	+	-	-	-	-	-	-	-	-	D	+	-	-	C	-		
6	+	-	-	-			-			-			WF	+	+	-	Fm	+	-	-
7	-	-	-	-	-	-	+	-	-	-	-	-	AA	+	-	+	D	+	-	-
8	+	+	-	-			-	-	-	+	+	-	Mm	-						
9	+	+	-	+	-	+	+	-	+	+	-	-	Mm	+	-	-	FP	-		
10	+	-	+	+			+	+	-	-			FP	-			TP	-		
11	+	+	-	-	-	-	-	-	-	-			Mm	-			TP	-		
12	-			-	-	-	-	-	-	+	+	-	FP	-			TP	-		
13	+	-	+	+			-			-			FP	-						
14	-	-	-	-			-	-	+	-										
15	+	+	-	-			+	+	-	-			PF	-	+	-	Mm	+	-	-
16	-	-	+	-			+	-	+	-			TP	+						
17	+	-	-	+	-	-	-			-			Mm	-						

Legends: AF = Aspergillus Fumigatus; AA = Acidum Acetylsalicylicum; FP = Flower pollen; TP = Tree pollen; Mm = Molds mix; WF = Wheat flour; Fm = Flour mixed; PF = Pigeon feathers; H = Hay dust; W = Wool; D = Dog hair; C = Cat hair; HD = House dust; HF = Hairs and feathers; GP = Grass pollen; SP = Spring pollen.
I = Type I allergy reaction; III = Type III allergy reaction; IV = Type IV allergy reaction. + = positive; - = negative.
Patients No. 1-16 = "non-immediate" positive; Patient No. 17 = "non-immediate" negative.

Table IV. A survey of nasal complaints accompanying the different types of nasal mucosa response, as observed in the 16 "non-immediate" positive patients.

Nasal complaints	Type I (immediate)	Type III (late)	Type IV (delayed)
Obstruction	++ (moderate)	+++ (severe)	+++ (severe)
Hypersecretion	+++ (severe	+ (slight)	(±)- (very slight to absent)
Sneezing	+++ (severe)	+ (slight)	- (absent)

time-course as follows.[1-6,13-16]

Immediate — onset within 10, maximum within 30, disappearance within 120 min.

Late — onset within 4-8, maximum within 8-12, resolving within 16-24 hours.

Delayed — has not yet been precisely described; the presumed onset is 24 hours or later, duration and disappearance within several days.

There are not many papers describing the time-course of allergic reactions of the nasal mucosa. Most of them deal with the immediate response (caused by the Type I allergic reaction).[7,8,9-12,23]

The time-course of the nasal mucosa response to allergen challenge could be characterized as follows.

Immediate — onset within 10, maximum within 30, resolving within 90-120 minutes.

Late — onset within 6-8, maximum within 8-10, resolving within 24 hours.

Delayed — has not yet been described in precise terms; presumed maximum is later than 24 hours (Slavin[20]).

The classification of the non-immediate responses of the nasal mucosa recorded in this clinical investigation was based on their clinical time-course.

From the results of our present study it might therefore be concluded that the time-course of the late nasal mucosa response is the following: onset within 4-8 hours, maximum within 6-10 hours with resolution within 24 hours, while that of the delayed nasal mucosa response runs as follows: onset within 26-36 hours, maximum within 30-40 hours and resolution within 50-56 hours after the allergen challenge.

The observed late response of the nasal mucosa to allergen challenge might possibly be caused by the Type III hypersensitivity, while the delayed response might possibly be caused by the Type IV hypersensitivity. Our hypothesis is supported by preliminary results of another study, which is not yet completed and fully evaluated statistically. The results will be reported later.

In this study the possible effects of some drugs against the nasal mucosa responses were investigated in the patients who demonstrated the late and the delayed nasal mucosa response. Preliminary results indicate that the late nasal mucosa response was protected partially by Beclamethasone dipropionate* (by nasal spray, six times per day); the delayed response was fully protected. The preceding immediate response was not inhibited by this drug. The protective effects of another drug Disodium cromoglycate (Rynacrom, Intal) were also investigated.

This finding is in accordance with our previous experiments,[11] which have demonstrated the lack of protective effects of corticosteroids against the Type I allergic reaction.

For technical reasons the present work was not accompanied by immunological tests or histopathological examination of biopsies. It is conceivable that concurrent immunological investigations will further clarify the mechanisms underlying the late and delayed response we have recorded.

In most of the investigations providing evidence for the role of the Type III or the Type IV hypersensitivity in the nasal mucosa pollen extracts were used as allergens. In this investigation clinical evidence was obtained for non-immediate responses (late and delayed) of the nasal mucosa to various other allergens as well, such as house dust, spring pollen, grass pollen and *aspergillus fumigatus*. This statement might confirm Slavin's observation on the skin[20]: " . . . delayed skin reactivity to inhalant allergens such as molds, house dust, insects, etc. is not uncommon."

In some patients investigated the nasal provocation tests were repeated with the same allergen to ensure that the measured types of the non-immediate nasal mucosa responses were (a) not an artefact; (b) not caused by a second complementary natural exposition to the allergen and (c) were reproducible. By comparing the repeated tests in the same person good reproducibility and correlation were obtained.

*Aldecin-nasal spray (Schering USA — represented in the Netherlands by Essex Corp.), 50μg per dosis; or Beconase-nasal spray (Allen & Hanburys Ltd., Glaxo Holdings Ltd., England), 50μg per dosis.

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THE EFFECTS OF DISODIUM CROMOGLYCATE AND BECLOMETHASONE DIPROPIONATE ON THE IMMEDIATE RESPONSE OF THE NASAL MUCOSA TO ALLERGEN CHALLENGE

Z. PELIKAN, M.D., F.A.C.A., and M. PELIKAN-FILIPEK, M.D.

This study deals with the comparative investigation of the protective effects of disodium cromoglycate (DSCG) Rynacrom,® Inial® and beclomethasone dipropionate aerosol (BDA); Aldecin® Beconase® on the immediate nasal mucosa response to allergen challenge due to the immediate hypersensitivity (Type I allergy) in 50 patients suffering from allergic rhinitis. DSCG demonstrated distinct protective effects on the immediate nasal mucosa response to allergen challenge in all patients investigated, while BDA failed to demonstrate any protective effects on the immediate nasal mucosa response to allergen challenge in any of the patients studied.

Introduction

THE EFFECTS OF disodium cromoglycate (DSCG) on the nasal mucosa have been studied extensively during nasal provocation challenges with allergens[1–4] as well as during clinical trials.[5–9] The effects of beclomethasone dipropionate aerosol (BDA) on patients with nasal complaints have also been the subject of numerous clinical studies.[10–21] Most of these investigators reported favorable effects with both these drugs.

We have not yet found any report in the literature dealing with a comparison of the effects of DSCG and BDA aerosol on the nasal mucosa response to allergen challenge caused by an immediate hypersensitivity (Type I allergy), during provocation tests, in a group of well defined and diagnosed patients with allergic rhinitis.

A comparative investigation of both these drugs performed on the same group of patients would appear to be of clinical importance for several reasons. First, although these drugs are used very frequently in practice, there is a lack of well defined indications for their use. Further, both these drugs are often considered in practice to be equivalent alternatives, although they are completely different in their pharmacological effects and actions.

This investigation is part of a large clinical study dealing with a comparison of the effects of DSCG and BDA aerosol on the various types of nasal mucosa response to allergen challenge.

The aim of the present study is (a) to investigate the possible effects of DSCG and BDA on the nasal mucosa response to allergen challenge due to immediate hypersensitivity and (b) if such effects did exist to compare them and to use this information in defining the indications for these drugs in the treatment of allergic rhinitis due to immediate hypersensitivity.

Materials and Methods

*Allergens**

The dialyzed and lyophilyzed extracts were diluted in Coca's Solution (dry weight of allergen in mg per 1 ml of Coca's Solution) and used in the following concentrations.

For nasal challenges: (a) house dust, 5 mg/ml; (b) hairs and feathers mixture,** pigeon feathers, cat danders, dog danders and wheat flour—each of them, 2.5 mg/ml; (c) Aspergillus fumigatus and moulds mixture,***—each of

Portions of the results reported in this paper were presented in a preliminary paper at the Annual Meeting of the American Academy of Allergy in San Francisco, 1981.[30]

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* All allergen extracts were prepared by Pharmacy "Diephuis," Groningen, the Netherlands.

** Cat, dog, cattle, goat, hog, horse, rabbit, rat, mouse, hamster, guinea pig, canary, goose, duck, turkey, hen, pigeon, parrot in equal proportions by weight.

them, 2.0 mg/ml; (d) grass pollen mixture,† spring pollen mixture,‡ and weed pollen mixture§—each of them, 10,000 Noon Units/ml.×

For skin testing: Allergen extracts identical to those for the nasal challenges were used for skin testing, only these were diluted in a 1:10 ratio.

Drugs

(a) Disodium cromoglycate (DSCG)* was used in powder form kept in capsules. DSCG was applied topically to the nasal mucosa by means of a special nasal applicator. One capsule containing 20 mg of DSCG powder was placed into the special nasal applicator, then perforated and blown into the nose by an air flow. A single dose was always just one capsule, contents of which (20 mg DSCG) were divided equally into both nostrils.

(b) Beclomethasone dipropionate aerosol (BDA)** was used in the form of a spray equipped with a special nasal adaptor. It was administered topically to the nasal mucosa as an aerosol. Two puffs were always given in each nostril (1 puff = 50 μg; 1 dose = 2 puffs in each nostril = 2 × 2 puffs = 4 × 50 μg = 200 μg).

Apparatus and Equipment

This consisted of a one-channel recorder†, an electrical differential transducer‡, a water manometer§, a small rubber balloon of 5 mm diameter and 25 mm length and a polyethylene tubes connecting system.

Principle of the Method

The nasal mucosa of the patient suffering from allergic rhinitis, when challenged topically by an allergen to which he is sensitive, reacts with swelling, hypersecretion, sneezing and itching. These changes influence the passage of air through the nose, resulting in changes in pressure differences between the nasopharyngeal cavity and the outside air while the air-flow is constant. These pressure differences, the so-called NPG (nasopharynx-nostril-pressure gradient), expressed in cm H_2O, were recorded and considered as a parameter for assessment of the nasal mucosa reactivity. The mean values of the NPG were calculated during regular breathing over 90 to 120 seconds. This "balloon technique," being a combination of rhinomanometry anterior and posterior, has been used and described in our previous papers.[1,22-30]

Patients

In 50 patients from the Department of Allergology and Immunology suffering from rhinitis with perennial or seasonal allergic component or a combination of both, the 66 most positive nasal mucosa responses to allergen challenges due to immediate hypersensitivity (Type I allergy) were randomly selected.

All these patients showed (1) nasal complaints (obstruction, hypersecretion, sneezing, itching), (2) positive history to one or more allergens, (3) positive immediate intracutaneous tests, usually to more than one allergen, (4) increased blood eosinophilia and (5) positive short-term nasal provocation tests with various allergens.

These patients were investigated in a period while symptom-free. None had used DSCG, corticosteroids or had received previous immunotherapy. No antihistaminics or topical decongestants were prescribed during the five-day interval prior to the study.

The clinical characteristics of the patients investigated are presented in Table I.

Procedure

In each patient the trial with a single allergen consisted of one provocation test and two protection tests, using DSCG or BDA and one control test. There was always an interval of five to six days between the individual tests. Before each test, after calibration of the apparatus, a rubber balloon was introduced into the nasopharynx through one of the nasal cavities, filled with 2 ml of air and then connected to the pressure transducer. During the recording of NPG the patient breathed only through the non-intubated cavity, the mouth being closed and the intubated cavity also being closed by means of the patient's finger placed on the alae nasi. (The patient's finger closing the nostril did not influence the air-pressure inside the tubing and the balloon because the finger was placed beside the tubing and because the tubing material, being a PVC with a thick wall and low elasticity, does not allow such an influence.) Five to 15 minutes after the introduction of the balloon when the patient was breathing regularly the test was begun.

Provocation Test With an Allergen

This consisted of the following steps.

"Initial test." The NPG values were recorded at 0, 5 and 10 minutes to obtain the so-called "initial values."

"Coca's test." The Coca's Solution was applied for three minutes to the nasal mucosa of the non-intubated nasal cavity by means of a saturated wad of cotton wool on a nasal probe. The NPG values were then recorded at 0, 5 and 10 minutes. If no significant change of the mean NPG "Coca's values" with respect to the mean "initial values" was measured, the investigation was continued.

Allergen challenge. The nasal mucosa was challenged with the allergen for three minutes in the same way and on the same site as with Coca's Solution. The NPG values were then recorded at 0, 5, 10, 20, 30, 45, 60, (90) and 120 minutes after the end of the challenge.

The provocation tests were considered to be positive when the mean NPG values after the allergen challenge increased by at least 2.0 cm of H_2O with respect to the "initial" and "Coca's Solution" values.

*** Cladosporium cladosporioides-elatum-herbarum, Penicillium brevi-compactum-expansum-notatum-frequentans-commune, Aspergillus versicolor-niger-fumigatus, Mucor spinosus-mucedo-racemosus, Fullularia pullulans, Botryris cinerea, Merculius domesticus, Epicoccum purpurascens, Alternaris tenuis, Stemphylum botryosum, Rhizopus nigricans, Fusarium culmorum and Trichoderma viride – in equal proportions by weight.

† Dry weight percentage: Secale cereale 15%, Dactylis glomerata 15%, Lolium perenne 10%, Anthoxanthum odoratum 10%, Agrostis alba 10%, Holcus lanatus 10%, Phleum pratense 10%, Cynosurus cristatus 5%, Alopecurus pratensis 15%.

‡ Dry weight percentage: Corylus avellana 20%, Alnus species 30%, Salis species 20%, Betula species 20%, Myrica species 10%.

§ Dry weight percentage: Artemisia vulgaris 33%, Rumex acetosa 33%, Plantago lanceolata 33%.

× 1 Noon Unit (NU) = 0.001 mg of dry pollen (powder) = 0.5 PNU = 1.3 TNU.

* Henceforth abbreviated as DSCG.

** Henceforth abbreviated as BDA.

† A product of Kipp and Zonen, The Netherlands.

‡ A product of Gould, Godard B.V., The Netherlands.

§ A product of Lode, B.V., The Netherlands.

Table I. Clinical Characteristics of the Patients Investigated.

	Nasal complaints					Skin tests (i.c.)—immediate response							
Patient	Obstruction	Hypersecretion	Sneezing	Lividity of nasal mucosa	Blood eosinophilic cells per mm³	HD	HF	GP	SP	Others			Allergen used for experimental nasal provocation and protection
1	+	+	+	–	451	+	+	+±	±	±	C	+	D	HD, GP
2	+	+	+	+++	1309	+++	++±	+++	++	++±	C	±	D	HG, GP
3	+++	+++	+++	–	440	+	+	–	–	+	C	±	D	GP, HD
4	–	–	+++	–	649	++	–	+++	++	+±	TP	+	WP	SP
5	++	++	++	–	551	++	++	+++	++	+	C	+	D	GP
6	+	+	+	+++	770	+	±	+	+	±	C	±	D	GP, HD
7	+	+	+	+	451	+	–	+±	–	–	C	–	D	HD
8	+	+	+	+++	1045	+±	+	+++	+±	+	C	+±	D	HD
9	++	++	++	–	517	+±	+±	+++	+±	+±	C	+±	D	GP
10	++	++	++	–	616	++	++	+±	++	++	C	+	D	GP, HD
11	+	+	+	–	1441	++	+±	+++	+±	+	C	+	D	GP
12	+++	+++	+++	+++	539	+±	++	+	+	+	C	+	D	GP
13	+++	+++	+++	+	385	+	+±	+±	+±	++	C	++	D	GP
14	+++	+++	+++	–	220	±	–	±	+	+	TP	+	WP	HD
15	+	+	+	++	1341	++	+±	+++	+±	±	C	++	D	GP
16	++	++	++	+++	561	+++	++±	+	–	+±	C	±	D	GP
17	+++	+++	+++	–	506	+	+	++	±	+±	C	±	D	GP
18	++	++	++	+++	241	+	±	–	–	–	C	–	D	HD, SP
19	+++	+++	+++	+++	781	+	+±	+±	–	±	C	+	D	HD, GP
20	++	++	–	++	583	++	+±	+±	++	+±±	WP	+	C	WP
21	+++	+++	+++	+++	737	+±	+±	+	–	+++	C	+++	D	HD, GP
22	+++	+++	+++	+	682	+	–	–	–	–	D	+	Mm	HD, GP, SP, HF
23	+++	+	+++	–	682	+	+	++	+±	+	C	+±	D	GP
24	+++	++	+	–	440	++	+	±	–	+±	Mm	–	AF	GP, AF, Mm
25	+++	+++	+++	+	1210	+±±	++	++	+±	+±	C	+	D	HD
26	+	+	+	+++	1462	±	+	–	–	+	C	–	D	HD
27	+++	–	–	+	495	+±	+	–	–	+	D	+	Mm	Mm, HD
28	+	+	+	+	363	+±	+	+++	+	–	C	+	D	GP
29	+	–	–	+	517	+	+±	+	+	±	C	±	D	GP
30	+++	+++	+++	+++	363	+±	+	+	±	±	C	–	D	GP
31	+	+	+	+	1540	+±	+++	+++	+	++	C	+±	D	GP
32	+	+	+	–	649	+±	+±	++	+	+	Mm	+	TP	GP
33	+	+	+	+++	913	++±	+±	+±	+	+	C	+±	D	HF
34	–	+	–	++	715	+	±	–	–	–	C	–	D	GP
35	++	++	++	+++	671	+±	+±	+	+	+±	C	++	D	C
36	+++	+++	+++	+++	704	++±	++	++	++	++	C	++	D	SP
37	+	++	+	–	495	+++	++	+++	–	+++	C	+	D	C
38	+++	+	+	++	1045	++	+++	+	±	+	C	+++	D	D
39	++	++	++	–	484	+	–	–	–	–	C	–	D	HD
40	++	++	+	+	508	+±	–	–	±	±	C	–	Mm	Mm
41	+++	+++	+++	–	1177	+++	+++	+++	+	+	C	++	D	HD, GP
42	++	+	+	+	1485	++	+±	+	+	++	D	±	Mm	Mm
43	++	++	++	–	297	+	–	–	–	–	C	–	D	GP
44	+++	+++	+++	++	308	+	–	–	–	±	WP	±	FP	HD
45	+++	+++	+++	+	1144	+	–	+	–	+	C	–	D	HD
46	++	++	+	+	363	++	++±	–	–	+++	WF	++	FM	WF
47	++	++	++	+++	1100	+±	++	+	–	+±	D	+	PF	PF, GP
48	++	++	++	+	990	+	–	+++	++	+	TP	–	C	SP
49	+++	+++	+	+	1155	+	–	–	–	+	Mm	+	AF	AF
50	+	+	+	–	495	+	–	+++	–	–	C	–	D	HD

HD = house dust; HF = hairs and feathers mixture; GP = grass pollen mixture; SP = springpollen mixture; C = cat hair; D = dog hair; TP = tree pollen mixture; WP = weed pollen mixture; Mm = moulds mixture; AF = Aspergillus fumigatus; FP = flower pollen mixture; WF = wheat flour; FM = flour mix; PF = pigeon feathers.

The evaluation of the intracutaneous tests:

– = normal skin appearance.
± = wheal not greater than original injected papule.
\+ = wheal increase up to 7.5 mm in diameter.
+± = wheal increase up to 10.0 mm in diameter.
++ = wheal increase up to 12.5 mm in diameter.
++± = wheal increase up to 15.0 mm in diameter.
+++ = wheal increase greater than 15 mm, with surrounding erythema and sometimes with "pseudopodia".

Protection Test With Disodium Cromoglycate (DSCG) Protection Test with Beclomethasone Dipropionate (BDA)

Both the protection tests were performed in the same manner and according to the same schedule. The patients were always pre-treated with the appropriate drug of 4 × 1 dose daily, starting two days before allergen challenge. On the "challenge day" the drug was given only twice; at 120 and at 10 minutes prior to challenge.

The basic schedule of the protection tests is similar to that of the provocation test.

The protection tests were performed as "single blind," where the patient did not know which of the drugs was being used.

The protective effects of the drug were considered to be significant when the NPG values after drug followed by allergen challenge decreased by at least 50% with respect to the NPG values after allergen challenge only.

The Control Test with Coca's Solution

This test was performed three to four days after allergen challenge. After the "initial NPG values" at 0, 5 and 10 minutes, Coca's Solution was applied to the nasal mucosa in the same way and on the same site as previously and the mean NPG values were then recorded from 0 to 120 minutes.

*Statistical evaluation**

The results were analyzed and statistically evaluated by means of polynomials which were fitted and the hypotheses were tested by the Potthoff and Roy's[31] generalized multivariate analysis of variance model (generalized Manova Model) presented by Timm.[32]

Results

The results of this study are given in Tables II, III and IV and Figure 1.

Provocation Tests

A survey of the NPG values recorded during the provocation tests in individual patients is given in Table II.

As can be seen in Table II, the 50 patients investigated demonstrated a total of 66 positive nasal mucosa responses to allergen challenges. This type of nasal mucosa response, considered by us to be due to the immediate hypersensitivity (Type I allergy), began within 10 minutes, reached its maximum within 30 minutes and disappeared within 90–120 minutes after the end of allergen challenge in most cases.

Protection Test with DSCG

The NPG values recorded during the protection tests with DSCG in individual patients are presented in Table III.

Comparing the results given in Table III with those shown in Table II, it can be concluded that DSCG, when applied topically in a sufficient dose before allergen challenge, was able to decrease significantly or to prevent fully the development of the nasal mucosa response to allergen challenge due to immediate hypersensitivity. These effects last at least two hours.

Protection Test with BDA

The NPG values recorded during the protection tests with BDA in individual patients are shown in Table IV.

Comparing the results presented in Table IV with those shown in Table II, it could be concluded that BDA, when applied topically in a sufficient dose before allergen challenge, was not able to decrease significantly the development of the nasal mucosa response to allergen challenge due to immediate hypersensitivity in any of the patients investigated.

The mean NPG values recorded in all patients during all three tests with respect to the mean NPG values after the Coca's Solution challenge are summarized in Figure 1.

Control Test With Coca's Solution

The mean NPG values recorded after Coca's Solution application did not differ significantly during the whole test (up to 120 minutes). These changes varied within 1.2 cm H_2O.

Accompanying Findings

a. In all cases a positive nasal mucosa response to allergen challenge, recorded during the provocation tests (Table II) was accompanied by acute nasal complaints (obstruction, hypersecretion, sneezing, itching) whose course ran parallel to the changes in the NPG values.

b. During the protection tests and also, to a lesser degree, during the protection tests with BDA, lividity of the nasal mucosa appeared in most of the patients studied. This lividity was not observed during the protection tests with DSCG in any of the patients.

The Statistical Analysis of the Results

Hypothesis No. 1. All three curves (ALL, BDA, DSCG) coincide; this hypothesis is rejected ($p < 0.01$). *Hypothesis No. 2.* The ALL and BDA curves coincide; this hypothesis is not rejected ($p > 0.10$). *Hypothesis No. 3.* the DSCG curve is horizontal; this hypothesis is not rejected ($p > 0.15$).

Explanation of the abbreviations: ALL = NPG values recorded during the provocation tests; DSCG = NPG values recorded during the protection tests with DSCG; BDA = NPG values recorded during the protection tests with BDA.

Discussion

Nasal provocation tests have become a very important part of the diagnostic regimen in patients with allergic rhinitis.[1–3, 23–27, 30] These tests, which are performed directly at the site of the antigen-antibody interaction, i.e., the nasal mucosa, should confirm or exclude the role of individual allergens in the production of nasal complaints where allergic reactions are suspected.

* The calculations were performed by Dr. M.P.F. Berger (Dept. Psychology, University of Tilburg, Tilburg, The Netherlands), using PDP 11/45 computer.

Table II. Survey of the Mean Nasopharynx-nostril-pressure Gradient (NPG) Values in cm H_2O Recorded During the Nasal Provocation Tests with Allergen in Individual Patients.

		Allergen challenge		Time in minutes after challenge								
Patient	Allergen	Initial value	Coca value ↓	0′	5′	10′	20′	30′	45′	60′	90′	120′
1	HD	2.5	2.7	3.4	3.3	3.5	5.7	6.1	5.5	4.7	3.7	3.1
	GP	2.2	2.2	3.0	6.3	8.8	11.6	13.6	9.6	5.6	2.5	2.6
2	HD	2.0	2.0	2.3	3.4	8.6	12.9	10.0	6.0	3.4	2.1	2.1
	GP	3.4	3.5	4.6	7.5	7.3	10.4	8.0	7.8	4.7	4.7	3.8
3	GP	2.1	2.1	3.0	4.1	5.2	6.1	6.5	5.1	3.5	2.3	2.1
	HD	3.1	3.2	3.2	4.9	7.5	10.6	11.0	10.1	6.2	4.1	3.2
4	SP	1.8	2.1	2.3	3.3	2.7	6.4	9.0	9.9	10.2	—	6.4
5	GP	3.1	3.0	7.2	7.4	8.2	24.0	24.7	21.0	7.3	7.0	3.4
6	GP	4.7	4.8	13.7	22.2	24.8	26.0	21.7	20.1	20.0	11.3	4.5
	HD	4.0	4.1	19.6	24.6	27.7	30.9	30.7	11.9	11.4	—	3.9
7	HD	2.1	2.1	1.8	4.4	3.8	8.2	4.3	5.1	5.2	1.7	1.8
8	HD	4.4	4.5	9.3	10.4	15.4	22.6	13.9	14.5	9.3	—	4.9
9	GP	2.1	1.7	4.5	6.3	6.8	7.9	7.2	6.5	8.9	2.9	3.0
10	GP	1.4	1.6	3.0	3.7	4.6	7.1	8.1	9.4	7.0	3.3	3.2
	HD	3.2	3.4	6.2	24.0	24.4	23.4	26.7	26.7	26.8	10.3	8.5
11	GP	1.7	1.7	3.4	4.5	5.9	5.2	8.0	3.0	4.7	—	2.5
12	GP	1.8	1.8	3.4	3.0	5.7	4.7	6.2	3.7	3.7	—	2.2
13	GP	5.0	5.1	19.0	24.1	28.6	27.4	20.6	29.0	28.9	17.4	6.7
14	HD	2.2	3.2	5.0	5.3	8.3	12.1	3.8	3.2	3.7	—	2.9
15	GP	1.5	1.7	14.2	18.4	17.5	15.3	17.9	22.1	14.3	—	6.7
16	GP	2.1	2.4	7.9	8.0	11.0	9.6	9.8	6.7	6.2	4.0	3.3
17	GP	4.7	5.2	14.0	16.0	22.9	18.2	16.8	12.3	14.6	2.9	3.2
18	HD	6.0	6.4	6.9	7.5	8.3	8.8	10.9	10.9	11.8	—	4.8
	SP	5.2	5.6	8.5	8.5	8.8	11.7	9.2	9.3	9.8	—	5.6
19	HD	1.3	1.7	12.7	21.2	20.4	23.4	24.9	21.8	20.7	—	2.8
	GP	3.2	4.8	30.6	30.7	30.7	30.7	30.7	30.7	30.7	—	8.0
20	WP	2.8	3.3	7.7	7.8	10.2	13.7	23.3	19.0	15.9	—	6.9
21	HD	2.8	2.9	6.8	8.3	10.6	12.9	15.2	14.6	12.9	—	2.4
	GP	2.6	2.6	5.5	12.6	11.9	10.3	9.4	3.4	3.4	—	3.2
22	HD	3.1	3.2	3.5	5.0	5.7	5.5	7.7	6.6	7.6	—	3.5
	GP	3.0	3.1	5.8	11.7	12.6	14.2	14.8	11.4	11.6	—	2.7
	SP	1.8	2.1	4.3	3.2	5.1	7.0	7.3	4.0	4.2	—	3.3
	HF	3.7	3.7	5.8	6.8	7.0	9.7	14.1	13.7	10.4	—	4.8
23	GP	2.3	2.7	10.9	23.3	24.8	26.1	18.2	8.1	9.8	2.6	2.8
24	GP	3.2	3.2	3.7	4.6	5.5	6.1	6.9	9.6	8.9	—	4.2
	AF	2.8	2.9	6.2	15.7	28.1	25.0	23.3	10.6	10.6	—	3.1
	Mm	3.2	3.8	6.4	12.4	12.1	12.7	11.0	5.6	3.8	—	3.1
25	HD	1.0	1.1	1.9	4.6	5.5	8.6	9.3	9.6	6.0	—	1.8
26	HD	4.0	4.1	4.8	4.9	6.3	7.3	11.1	8.8	7.3	—	5.5
27	Mm	1.2	1.2	2.6	2.4	7.3	10.2	12.1	3.3	3.2	—	2.1
	HD	1.4	1.5	3.6	3.1	4.7	5.7	5.1	9.4	8.3	4.4	5.4
28	GP	3.6	4.1	6.4	5.9	12.1	9.9	8.2	8.0	7.6	3.7	4.8
29	GP	0.9	0.9	2.4	4.0	3.8	3.8	4.0	2.5	2.1	—	1.8
30	GP	1.8	1.8	2.1	7.2	6.2	7.7	8.9	4.6	6.2	—	2.4
31	GP	2.2	2.2	2.5	6.0	5.5	5.3	5.6	4.8	4.5	—	1.7
32	GP	4.5	4.7	4.2	7.0	6.2	6.8	15.8	8.5	11.5	9.6	7.3
33	HF	1.5	2.0	3.6	3.7	11.6	10.2	6.9	6.2	5.6	—	2.5
34	GP	3.7	3.5	5.2	5.2	5.3	12.3	10.6	12.0	10.1	7.1	4.7
35	Cat	2.1	2.6	5.2	8.0	10.3	14.2	16.9	11.2	8.7	—	6.5
36	SP	2.9	2.8	4.1	5.2	6.7	7.2	7.2	5.7	6.0	3.1	3.2
37	Cat	1.3	1.4	7.7	13.8	21.4	21.7	23.8	13.8	7.4	7.2	6.3
38	Dog	1.1	1.0	1.6	7.0	8.9	8.4	9.1	7.2	7.1	2.2	1.8
39	HD	2.4	2.3	3.2	2.8	3.3	9.0	7.0	4.8	5.8	—	5.2
40	Mm	3.1	2.6	2.7	3.4	2.9	5.3	5.3	6.6	11.8	10.2	6.7
41	HD	2.7	2.9	4.5	6.4	7.4	9.6	10.5	11.3	10.1	—	3.3
	GP	1.0	1.3	3.5	4.4	5.1	5.3	5.6	5.4	5.2	—	—
42	Mm	2.0	2.2	3.6	6.8	6.5	8.1	7.8	7.1	7.3	4.7	5.0
43	GP	1.4	1.3	1.2	1.2	3.7	15.2	15.8	6.8	5.2	—	1.5
44	HD	2.4	2.5	4.1	5.4	6.8	11.0	9.4	4.6	3.8	2.5	2.7
45	HD	3.6	3.2	4.9	5.7	6.0	7.8	11.7	15.8	13.9	6.7	2.5
46	WF	2.0	2.0	2.8	4.6	5.2	5.9	7.3	8.3	6.7	5.0	3.0
47	PF	2.1	2.1	2.1	2.7	6.7	6.6	6.5	8.0	6.2	—	2.3
	GP	1.1	1.3	2.4	3.1	3.2	4.3	5.2	6.2	2.6	—	—
48	SP	4.0	4.0	4.2	7.9	9.8	14.1	11.7	11.0	5.5	4.1	4.1
49	AF	2.5	2.3	2.5	2.5	4.8	12.4	19.5	15.4	12.9	6.1	2.7
50	HD	1.3	1.4	1.5	1.6	1.4	1.6	12.0	16.1	15.2	4.5	2.8

HD = house dust; GP = grass pollen; SP = spring pollen; WP = weed pollen; HF = hairs and feathers; AF = Aspergillus fumigatus; Mm = moulds mix; Cat = cat hair; Dog = dog hair; WF = wheat flour; PF = pigeon feathers.
The "Initial value" as well as the "Coca value" are the mean NPG values, always calculated from three values, as recorded at 0, 5, and 10 minutes.

Table III. Survey of the Mean Nasopharynx-nostril-pressure Gradient (NPG) Values in cm H_2O Recorded During the Nasal Protection Tests with Disodium Cromoglycate (DSCG) in Individual Patients.

Patient	Allergen	Initial value	Coca value	DSCG value	Allergen challenge ↓	Time in minutes after challenge 0′	5′	10′	20′	30′	45′	60′	90′	120′
1	HD	1.7	1.7	1.8		1.8	1.9	1.4	1.4	1.4	1.4	1.7	—	1.7
	GP	2.3	2.7	3.0		3.8	3.6	4.1	2.8	2.8	2.7	3.7	3.1	3.6
2	HD	1.2	1.5	1.0		1.1	1.1	1.0	1.0	1.2	1.0	1.0	1.3	1.4
	GP	3.8	3.7	3.7		3.5	3.9	4.1	5.2	4.1	3.5	3.5	4.2	3.7
3	GP	3.8	3.8	5.0		4.2	4.2	4.8	4.8	4.8	4.2	4.1	—	4.1
	HD	3.2	3.5	3.5		3.8	4.6	4.8	5.3	5.8	4.6	5.1	3.9	3.8
4	SP	2.1	1.9	1.9		1.6	1.6	1.8	1.6	1.5	2.1	2.1	—	1.7
5	GP	4.0	4.4	4.8		3.5	4.4	3.9	4.4	6.2	6.1	3.8	—	4.0
6	GP	2.5	2.6	2.6		3.4	3.6	4.1	3.4	3.5	3.8	3.9	—	2.9
	HD	3.4	3.8	3.5		5.7	5.1	5.0	4.8	4.4	4.0	4.0	—	3.4
7	HD	0.9	1.0	0.8		0.8	0.7	0.8	0.9	0.8	1.0	0.9	—	1.3
8	HD	2.5	3.0	2.8		2.9	2.9	3.0	2.4	2.8	2.8	3.2	—	2.6
9	GP	2.1	2.4	2.2		1.6	1.6	1.7	4.1	3.5	3.3	2.3	—	2.1
10	GP	1.0	1.0	1.0		1.8	2.7	2.6	1.7	1.1	1.1	1.2	—	1.6
	HD	0.9	0.9	0.9		1.8	1.7	2.0	2.0	1.4	1.6	1.9	—	1.5
11	GP	2.1	2.1	2.1		3.5	3.9	4.3	3.9	4.6	4.6	4.1	—	3.3
12	GP	2.8	3.5	3.5		3.5	3.7	3.7	3.2	4.3	3.9	3.5	—	2.5
13	GP	1.4	1.4	1.5		1.9	2.3	1.9	2.0	3.2	2.9	2.3	—	2.0
14	HD	1.4	1.5	1.9		2.9	3.9	2.4	3.2	3.1	1.7	1.4	—	1.3
15	GP	3.1	3.1	3.7		11.9	6.8	9.5	7.5	9.2	9.6	9.5	—	3.2
16	GP	2.7	2.5	1.7		1.7	1.9	2.1	2.3	2.3	2.3	1.5	—	2.9
17	GP	1.3	1.1	1.3		3.1	3.9	4.2	6.5	6.1	6.3	7.6	—	2.6
18	HD	6.2	6.5	6.1		5.8	6.1	5.1	5.8	4.0	5.1	4.8	—	4.0
	SP	2.1	3.0	2.6		2.7	2.4	2.0	2.1	2.8	2.2	2.6	—	2.9
19	HD	1.3	1.3	1.5		1.9	2.3	2.7	2.3	2.6	2.6	2.6	—	1.6
	GP	1.1	1.2	1.0		4.3	3.2	3.3	2.9	3.8	1.1	1.2	—	1.8
20	WP	2.7	2.3	2.2		2.2	2.0	2.0	2.0	1.9	2.0	2.2	—	2.4
21	HD	2.2	2.5	2.1		2.8	3.0	1.8	1.8	1.8	9.3	2.0	—	1.7
	GP	2.9	3.1	2.6		4.9	4.1	5.1	4.9	3.1	3.9	3.0	—	2.4
22	HD	3.4	4.3	3.5		4.7	5.7	4.9	4.7	5.0	4.3	4.8	4.9	4.7
	GP	1.1	1.5	1.5		1.5	1.5	1.5	1.6	1.4	1.8	1.9	—	1.7
	SP	3.7	3.7	3.7		4.8	5.2	5.7	5.2	3.8	4.0	4.3	—	3.2
	HF	1.9	1.8	1.8		1.1	1.6	2.0	2.6	1.7	2.2	2.2	—	2.0
23	GP	2.1	2.5	2.1		2.4	3.5	3.8	3.2	2.8	2.3	2.9	—	3.4
24	GP	2.8	2.5	2.5		3.2	3.3	3.0	3.1	2.3	2.0	2.1	—	3.2
	AF	2.9	2.9	2.9		4.5	7.6	8.8	7.9	9.2	9.9	8.3	—	3.3
	Mm	2.6	2.1	1.4		2.0	2.4	2.9	2.2	2.5	5.0	4.4	—	2.2
25	HD	2.6	2.5	2.7		2.2	2.0	2.3	2.1	2.1	2.4	2.1	—	2.9
26	HD	3.1	3.2	2.9		3.1	3.7	2.8	2.4	2.7	2.3	2.6	—	2.2
27	Mm	2.1	2.6	2.7		3.8	4.4	2.2	2.4	2.4	2.4	2.8	—	2.6
	HD	1.8	2.1	2.8		2.7	2.9	2.4	2.7	2.4	2.3	2.3	—	2.0
28	GP	4.2	3.9	3.5		4.0	3.9	4.2	4.0	4.9	5.1	4.4	4.3	3.9
29	GP	1.5	1.6	1.6		2.5	2.9	2.6	2.7	3.2	2.9	1.9	—	2.1
30	GP	3.9	4.2	4.2		5.5	4.2	4.2	3.6	3.7	4.2	3.7	—	2.9
31	GP	1.7	2.2	2.2		2.3	2.5	2.4	4.0	3.9	2.1	2.1	—	2.0
32	GP	4.9	4.7	4.7		5.3	6.4	6.9	4.4	4.5	5.3	4.4	—	5.8
33	HF	1.5	1.5	1.5		2.1	1.9	3.9	2.5	3.2	1.9	2.3	—	2.2
34	GP	0.9	0.9	1.3		1.3	1.6	1.1	1.1	0.9	0.9	1.3	—	0.9
35	Cat	3.9	4.1	4.3		3.6	3.8	4.5	4.3	4.3	4.3	3.7	3.9	4.4
36	SP	3.5	3.5	3.5		3.1	3.3	3.4	4.0	3.8	3.6	3.7	—	3.9
37	Cat	1.7	1.6	1.6		1.5	1.5	1.5	1.4	1.6	1.8	1.9	1.9	2.1
38	Dog	0.7	1.2	1.5		1.5	1.8	2.0	1.4	1.8	2.0	2.3	2.1	2.0
39	HD	2.7	2.9	3.2		3.0	2.9	2.7	2.4	2.7	2.5	2.4	—	3.0
40	Mm	1.6	1.6	1.6		1.3	1.7	1.5	1.7	1.4	1.4	1.5	—	1.4
41	HD	3.3	3.3	3.9		4.2	4.0	4.6	4.0	4.3	4.6	4.1	—	2.3
	GP	2.2	2.5	2.1		2.4	2.4	2.3	2.1	2.0	2.3	2.3	2.3	1.8
42	Mm	1.1	1.2	1.3		1.3	1.6	1.7	1.7	1.5	1.7	1.7	1.4	1.5
43	GP	2.4	2.7	2.5		1.9	2.6	3.1	3.7	4.0	3.6	2.3	—	3.0
44	HD	3.1	3.2	3.2		—	3.0	3.1	3.5	3.9	3.1	3.0	—	3.2
45	HD	1.0	1.1	1.4		1.5	1.1	1.4	1.3	1.7	1.6	1.3	—	—
46	WF	2.9	3.1	3.0		3.3	3.2	3.5	3.1	3.2	3.1	3.2	3.4	2.9
47	PF	1.6	1.8	1.9		3.4	2.4	2.3	2.4	2.3	1.6	1.7	—	2.1
	GP	2.5	3.1	2.8		2.4	3.4	3.9	3.5	2.6	3.0	2.6	2.4	2.9
48	SP	3.4	3.4	3.4		3.7	3.9	4.0	4.6	4.0	3.5	3.4	3.3	3.3
49	AF	2.4	2.6	2.7		3.1	3.4	3.3	4.8	4.4	4.3	4.2	3.2	3.0
50	HD	3.2	3.0	2.7		2.6	2.4	2.4	2.7	2.3	2.8	3.1	—	2.3

HD = house dust; GP = grass pollen; SP = spring pollen; WP = weed pollen; HF = hairs and feathers; AF = Aspergillus fumigatus; Mm = moulds mix; Cat = cat hair; Dog = dog hair; WF = wheat flour; PF = pigeon feathers.
The "Initial value" as well as the "Coca value" are the mean NPG values, always calculated from three values, as recorded at 0, 5 and 10 minutes.

Table IV. Survey of the Mean Nasopharynx-nostril-pressure Gradient (NPG) Values in cm H_2O Recorded During the Nasal Protection Tests with Beclomethasone Dipropionate (BDA) in Individual Patients.

Patient	Allergen	Initial value	Coca value	BDA value	Allergen challenge ↓	Time in minutes after challenge 0′	5′	10′	20′	30′	45′	60′	90′	120′
1	HD	2.7	2.8	2.7		2.8	5.5	5.8	6.4	6.4	7.9	6.1	—	3.2
	GP	1.8	1.9	1.7		3.3	3.5	4.9	8.3	12.0	10.4	5.3	2.2	2.3
2	HD	0.8	0.9	0.8		4.4	5.4	5.9	5.8	7.4	9.0	9.4	—	1.3
	GP	1.8	2.1	2.2		1.9	5.7	6.5	7.6	9.3	9.1	9.3	1.6	—
3	GP	1.5	2.3	2.0		2.4	9.9	14.1	9.0	10.8	8.2	6.9	4.5	4.0
	HD	2.2	2.8	2.6		6.6	7.3	12.3	14.4	18.3	11.0	9.9	—	3.6
4	SP	2.4	2.5	2.6		5.8	6.7	7.9	9.1	8.7	10.0	7.2	—	3.3
5	GP	3.8	3.7	3.7		13.1	13.5	28.0	28.0	15.4	15.5	16.2	—	6.2
6	GP	1.0	1.0	1.0		13.7	14.7	15.0	13.7	13.7	14.7	17.8	—	2.5
	HD	3.1	3.3	3.3		27.1	27.2	27.2	27.2	27.2	27.3	26.5	—	5.3
7	HD	2.8	2.6	2.6		6.1	7.1	9.6	9.9	10.2	11.4	6.3	—	2.7
8	HD	2.0	2.6	2.6		8.4	14.8	19.3	10.6	17.8	20.1	10.1	—	2.9
9	GP	3.5	4.5	4.1		25.4	24.0	25.3	28.5	22.0	26.1	28.6	—	6.6
10	GP	0.8	1.3	0.8		1.9	4.8	8.8	7.0	3.7	1.9	1.9	—	0.7
	HD	0.6	0.8	0.7		12.4	15.7	19.4	25.2	21.5	16.0	11.7	—	8.3
11	GP	1.8	1.9	2.3		5.4	8.8	8.3	8.8	7.2	3.3	3.0	—	1.8
12	GP	1.2	1.2	1.2		5.9	5.8	4.4	5.0	5.5	7.0	5.8	—	5.4
13	GP	2.8	3.1	2.7		12.0	19.2	21.0	19.3	20.6	17.9	17.6	—	3.7
14	HD	2.3	2.6	2.5		4.6	13.0	13.9	14.9	14.5	7.0	5.4	—	1.9
15	GP	1.9	2.2	2.1		14.0	27.3	27.0	22.0	23.5	13.5	13.9	—	4.2
16	GP	2.5	2.5	2.3		6.0	7.9	9.1	8.6	8.9	8.7	9.4	—	4.3
17	GP	3.8	3.8	3.7		14.0	18.7	19.1	23.0	25.0	23.4	20.5	—	4.8
18	HD	4.3	5.1	4.9		10.7	12.4	12.8	13.9	15.9	7.8	7.1	—	5.8
	SP	2.6	2.7	2.1		7.2	9.8	10.7	11.4	7.7	9.2	9.9	—	—
19	HD	1.0	1.0	1.0		9.4	21.2	18.2	19.0	28.7	10.3	8.1	—	2.9
	GP	1.1	1.1	1.1		4.3	20.5	20.8	28.1	30.1	30.1	27.2	—	20.6
20	WP	1.9	2.1	1.9		8.9	9.0	9.4	12.0	10.5	16.0	14.8	—	—
21	HD	1.5	1.2	1.3		5.3	4.5	10.1	8.1	7.1	9.5	7.6	—	3.3
	GP	2.9	3.9	4.2		7.5	10.5	11.6	13.8	14.0	10.4	10.5	—	—
22	HD	2.7	3.2	3.2		7.6	8.5	9.0	9.4	11.4	9.5	7.9	—	4.0
	GP	0.9	0.9	0.9		4.8	14.4	11.2	17.5	10.7	12.5	10.1	3.0	0.8
	SP	4.1	4.4	4.5		6.2	6.2	5.9	14.6	11.1	11.5	9.2	7.0	4.5
	HF	1.4	1.6	1.7		7.2	9.5	13.1	12.0	12.6	5.6	3.8	—	6.9
23	GP	1.4	1.4	1.5		10.0	11.7	15.0	13.6	23.1	24.5	8.9	—	1.6
24	GP	2.6	2.8	2.5		3.2	5.6	6.4	7.0	5.6	5.5	7.0	2.6	4.9
	AF	3.0	3.1	2.8		8.7	14.4	22.8	22.5	19.2	24.2	21.3	—	7.3
	Mm	1.5	1.5	1.6		2.6	6.4	11.6	11.1	10.2	9.4	8.7	—	2.4
25	HD	3.2	3.1	3.1		21.5	25.9	25.9	25.9	25.9	22.4	22.4	25.9	11.8
26	HD	2.5	2.5	2.6		4.8	4.9	6.0	9.4	8.8	7.4	3.0	1.7	2.0
27	Mm	2.0	2.5	2.3		2.4	5.2	10.2	10.1	10.1	11.1	9.8	—	5.0
	HD	2.1	2.5	2.4		4.5	8.4	8.6	7.0	8.1	10.3	10.6	4.6	3.0
28	GP	4.4	4.1	4.3		7.8	8.6	9.0	10.6	9.2	11.6	11.0	—	4.8
29	GP	2.1	2.2	2.2		4.0	13.9	12.6	14.5	13.8	18.6	8.7	—	3.1
30	GP	2.2	2.2	2.2		3.4	6.3	7.0	5.9	10.4	10.9	7.4	—	3.3
31	GP	1.9	1.8	1.8		10.3	13.0	17.3	13.1	11.1	11.1	3.9	—	1.8
32	GP	4.4	4.7	4.7		9.7	8.5	14.0	19.1	15.2	10.1	7.3	—	—
33	HF	1.2	1.2	1.2		3.6	4.3	8.4	2.6	7.1	4.1	2.4	1.8	3.1
34	GP	0.7	0.8	0.9		4.6	5.1	4.9	6.0	5.0	4.6	4.8	4.4	0.8
35	Cat	0.7	1.0	1.0		5.3	5.9	5.9	5.1	7.9	11.8	7.7	—	1.9
36	SP	3.1	3.3	3.5		6.9	7.0	16.1	17.7	12.4	12.1	9.6	—	3.5
37	Cat	2.5	2.6	2.6		7.5	7.8	9.9	22.9	19.6	18.3	16.0	—	3.1
38	Dog	0.8	0.9	0.7		3.2	3.9	5.9	7.5	6.4	4.6	5.8	5.5	2.7
39	HD	3.1	2.7	2.2		5.1	6.0	6.0	7.2	7.0	6.6	7.6	6.8	6.0
40	Mm	1.8	1.7	1.6		2.7	3.1	4.5	5.2	5.9	9.4	10.1	—	—
41	HD	2.2	2.7	2.5		8.0	7.7	8.8	13.9	13.3	9.0	—	—	3.5
	GP	2.5	2.8	2.4		9.7	13.7	13.0	14.2	9.5	11.0	15.2	2.3	2.4
42	Mm	2.1	2.7	3.1		9.0	7.8	6.8	11.2	7.9	6.8	6.5	—	—
43	GP	2.4	2.1	2.1		18.4	21.5	17.5	18.6	24.6	12.6	14.7	—	4.5
44	HD	2.5	2.7	2.8		3.0	2.8	14.3	10.2	10.6	10.4	10.6	3.4	2.5
45	HD	0.9	0.8	0.7		3.0	5.0	8.8	7.8	7.2	8.3	8.4	—	0.6
46	WF	3.1	3.3	2.7		4.3	5.2	7.9	9.4	10.9	10.1	5.8	6.5	3.7
47	PF	1.9	2.3	2.4		2.8	9.1	8.5	7.7	16.7	13.8	13.1	9.7	2.3
	GP	1.5	1.7	1.7		5.7	6.9	8.9	8.0	9.1	10.7	11.9	2.1	2.2
48	SP	1.8	2.1	2.3		6.5	7.1	7.8	13.4	15.5	15.2	9.1	6.6	3.1
49	AF	3.7	3.8	3.6		5.2	6.3	9.9	13.1	17.8	17.6	14.5	11.1	6.6
50	HD	2.6	2.2	2.2		2.5	6.1	8.1	12.0	19.2	13.1	7.6	7.9	—

HD = house dust; GP = grass pollen; SP = spring pollen; WP = weed pollen; HF = hairs and feathers; AF = Aspergillus fumigatus; Mm = moulds mix; Cat = cat hair; Dog = dog hair; WF = wheat flour; PF = pigeon feathers.
The "Initial value" as well as the "Coca value" are the mean NPG values, always calculated from three values, as recorded at 0, 5, and 10 minutes.

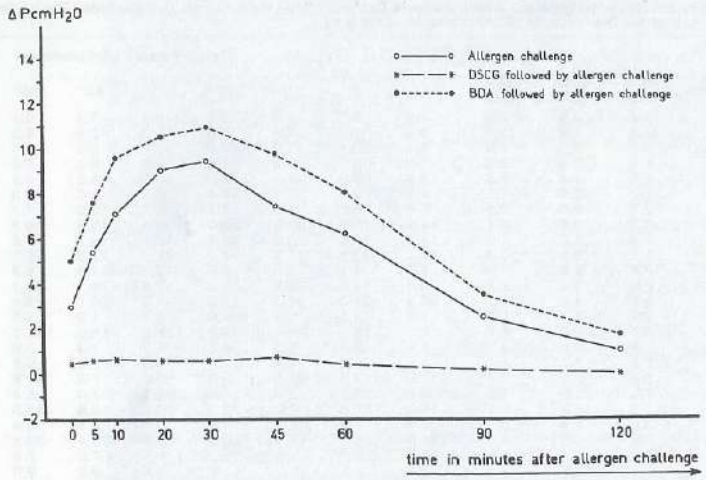

Figure 1. Provocation test and protection tests with DSCG and BDA. Mean NPG values recorded from all patients during these tests with respect to the appropriate Coca's Solution mean NPG values. DSCG = Disodium cromoglycate; BDA = Beclomethasone dipropionate.

We have used these tests as a standard part of the diagnostic approach for detection of the nasal mucosa response to allergen challenge due to immediate hypersensitivity (Type I allergy)[1,22–25,27,30] and recently we have also used the technique for the detection of other types of response in the nose, i.e., the late response (possibly Type III allergy) and the delayed response (possibly Type IV allergy.)[26,29] We have recently provided evidence that nasal provocation tests with allergens are more important for the diagnosis of immediate hypersensitivity in patients with rhinitis than the serum RAST.[27]

We also use these tests in another modification, the so-called protection tests, using an anti-allergic drug prior to allergen challenge. The "protective effects" of a drug commonly refer to its ability to prevent or decrease significantly the development of the changes in the nasal mucosa (swelling, hypersecretion, sneezing, itching) caused by an antigen-antibody interaction.[1,23,30]

The method and apparatus used in the present study were similar to those used in our previous investigations[1] and in our preliminary study.[30]

In these studies DSCG demonstrated distinct protective effects on the nasal mucosa response to allergen challenge due to immediate hypersensitivity (Type I allergy) in patients with pollinosis as well as with perennial rhinitis.[1,30] Our results have been confirmed by other studies dealing not only with clinical trials[5–9] but also with nasal provocation tests.[2–4]

The effects of BDA have also been studied both in patients with allergic rhinitis, either with a seasonal component (hay fever) or with a non-seasonal component (perennial rhinitis)[10–21,33–35] and in patients with allergic bronchial asthma.[36–42]

Most authors have reported a beneficial therapeutic effect of BDA in the treatment of rhinitis symptoms. They evaluated the effects of BDA by a simple clinical screening system (patient's subjective complaints score). They did not analyze exactly the different pathophysiological mechanisms (e.g., antigen-antibody interaction due to immediate hypersensitivity or the possible role of other types of allergic reactions, non-specific hyperreactivity of the nasal mucosa, possible reflex-reaction, etc.) playing a role on the nasal mucosa and all resulting in a similar spectrum of nasal complaints. Also they did not confirm the allergic component in these patients by nasal provocation tests with allergen.

In the literature we found only a few reports dealing with the investigation of the effects of BDA on the nasal mucosa using allergen challenge.[24,25,33–35] Vilswik et al[33] demonstrated significant inhibitory effects of BDA in a daily dose of 400 μg for one week on the "allergen-induced immediate nasal blockage" as measured by rhinomanometry. Haahtela[34] found beneficial protective effects of BDA (in a dose of 200 μg in each nostril) on the nasal mucosa response to allergen challenge. They did not find differences between the positive effects of DSCG and BDA on the nasal mucosa response. On the other hand, Mygind et al[35] could not demonstrate any significant effects of BDA in a daily dose of 800 μg on the nasal mucosa response to allergen challenge due to the Type I allergy.

The methods used by Vilswik et al[33] and by Haahtela[34] seem to us to have some disadvantages, e.g., the technique of only peak-expiratory flow, the nasal resistance was recorded during too short an interval after allergen challenge, insufficient analysis of the patients. This could explain the differences in the results of their and our studies. On the other hand, although only a single time point of measurement was used by Mygind et al,[35] their observation of a lack of BDA protective effects on the nasal mucosa response to allergen challenge due to Type I allergy is in agreement with our results. The lack of any protective effects by another topical corticosteroid (dexamethasone 21-isonicotine) against Type I allergy in the nose has been reported in our previous study.[23]

The results of our present study, demonstrating a lack of protective effects by BDA and showing the distinct protective effects of DSCG on the nasal mucosa response to allergen challenge due to immediate hypersensitivity (Type I allergy), are similar to those described by Pepys et al[36,37,39] and Booij-Noord et al[41] during bronchial challenges in asthmatics.

The results of the present study could be explained by the known pharmacological qualities of these drugs and their influence on the mediator cells such as mast cells (basophil leucocytes). These cells play a pivotal role in immediate hypersensitivity (Type I allergy). DSCG can protect the mast cells (basophil leucocytes) from their degranulation and subsequent release of mediators.[1,46] DSCG is, therefore, pharmacologically indicated for the treatment of allergic rhinitis, most cases of which are due to immediate hypersensitivity.

BDA, being a glucocorticosteroid, has a high topical anti-inflammatory activity but its direct protective effect on the mast cell and basophils has not yet been demonstrated. Recently some reports suggested possible effects of corticosteroids, including BDA, on the metabolism and action of cyclic AMP and cyclic GMP[44] which may be involved in the later stages of various types of hypersensitivity producing bronchial asthma symptoms.[42]

The role of cyclic AMP and cyclic GMP in immediate hypersensitivity of the nasal mucosa, being a typical topical event, has not yet been described. The absence of smooth muscles in the nasal mucosa makes such a role for cAMP and/or cGMP in allergic reactions of the nasal mucosa improbable. Glucocorticosteroids, however, inhibit other types of allergic reactions, different from Type I e.g. Type III[29,36,37,39] and perhaps Type IV.[29,43–45] Corticosteroids, including BDA, are more successful in forms of rhinitis, where a distinct inflammatory component also occurs, or where the nasal complaints (swelling of the mucosa, hypersecretion) are caused by mechanisms other than Type I allergy.

DSCG as well as BDA was used in the present study in doses which correspond to those reported by the literature.[1–9,10–21,33–35,40,46] The daily dose of DSCG recommended by these investigators was 4 × 20 mg, while the recommended daily dose of BDA was between 300–800 μg, usually 400 μg. It can, therefore, be concluded that the lack of protective effects of BDA observed in our study could not have been caused by under-dosage.

The present study confirms the distinct protective effects of DSCG on Type I allergy in the nose reported in several previous studies. However, our study did not confirm the beneficial therapeutic effects of BDA in patients with allergic rhinitis caused by Type I allergy, as reported by numerous authors.

The so-called beneficial effects of BDA in patients with rhinitis symptoms as observed by other authors can perhaps be explained by the following facts.

(a) The nasal complaints, at least in some of the rhinitis patients, were not caused by the immediate hypersensitivity mechanism but were caused by nonspecific hyperreactivity (vasomotor rhinitis) without any direct involvement of an antigen-antibody interaction.[15]

(b) Most of the trials did not demonstrate that the so-called positive action of BDA, evaluated by a recording of the simple daily nasal complaints' score, was really due to the positive effects of BDA on the antigen-antibody interaction and its consequent effect on the nasal mucosa (= due to immediate hypersensitivity).

(c) The relief of nasal symptoms could have been caused by the anti-inflammatory action of BDA (possibly also by its decongestant side effects).

(d) The analysis of patients investigated and evaluation parameters recorded in these studies are questionable.

The present study suggests that immediate hypersensitivity (Type I allergy) in the nasal mucosa can be successfully protected by DSCG, while BDA is ineffective.

DSCG is indicated for the treatment of most cases of allergic rhinitis caused by immediate hypersensitivity (Type I allergy).

BDA is indicated for the treatment of the forms of allergic rhinitis: (a) where types of allergic reactions, different from the Type I, may play a role, (b) as adjunct therapy to DSCG in patients where nasal mucosa edema cannot be sufficiently decreased, (c) where rhinitis is known precursor to bronchial asthma and (d) in cases where the inflammatory component is a predominant cause of nasal complaints.

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THE EFFECTS OF DISODIUM CROMOGLYCATE AND BECLOMETHASONE DIPROPIONATE ON THE LATE NASAL MUCOSA RESPONSE TO ALLERGEN CHALLENGE

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The protective effects of disodium cromoglycate (DSCG) and beclomethasone dipropionate (BDA) on the late nasal mucosa response to allergen challenge (LNR) were investigated in 42 patients with allergic rhinitis. The 42 patients, selected from a group of 370 patients, developed a total of 52 late nasal responses (LNR), 13 of which were isolated late responses (ILNR) and 39 of which were dual responses (DNR), i.e., where the late response (DLNR) has been preceded by an immediate response (INR). Significant protective effects on the immediate and on the late nasal mucosa responses were seen following the use of DSCG. The late response, being a part of the dual response, was decreased by DSCG to a higher degree than the isolated late response. Although BDA also decreased the late response, the isolated late response was decreased to a greater degree than that demonstrated as a part of the dual response. The immediate response was not influenced by BDA at all. It is suggested that DSCG should be used as a drug of first choice to control the allergic rhinitis symptoms. However, in the presence of the late nasal response, BDA should be added, at least at the beginning of the treatment and during the period of peak exposure to allergen, i.e., the pollen season.

Introduction

THE EFFECTS OF DISODIUM cromoglycate (DSCG) as well as beclomethasone dipropionate (BDA) in patients with allergic rhinitis have been investigated extensively. In most of these studies the patients were treated with these drugs at specific times and the only measures of effectiveness included clinical scores.[1-4] There are only a few reports of the effects of both drugs on the nasal mucosa using nasal provocation tests with allergens.[5-12]

We are unaware of any literature dealing with a comparison of the effects of DSCG and BDA on the late nasal mucosa response during nasal provocation tests in the same group of rhinitis patients. The late nasal responses (which could possibly be caused by late hypersensitivity, Type III allergy) have been described by the author in previous reports.[13,14]

This study is a continuation of our previous papers dealing with the protective effects of DSCG and BDA on the nasal mucosa response to allergen challenge due to immediate hypersensitivity (Type I allergy).[5,7]

The purpose of this investigation was to study the possible existence of protective effects of DSCG and BDA on the "late nasal mucosa response" during nasal provocation tests, to compare them and to determine the indication of both drugs for the practical treatment of allergic rhinitis. The late nasal mucosa response may indeed play an important role in producing symptoms of rhinitis in many patients.[18-22] This response is frequently overlooked in practice and could be responsible for the treatment failure in some rhinitis patients.

Materials and Methods

*Allergens**

Dialyzed and lyophilyzed water extracts were diluted in Coca's Solution (dry weight of allergen in mg per 1 ml of Coca's Solution) and used in the following concentrations;

For nasal challenges: (a) house dust, 5.0 mg/ml; (b) hairs and feathers mixture,** pigeon feathers, dog danders, cat danders, wheat flour, each of them of 2.5 mg/ml; (c) moulds mixture,*** Aspergillus fumigatus, 2.0 mg/ml; (d) grass pollen mixture,† spring pollen mixture,†† weed pollen mixture,††† tree pollen mixture,‡ each of them of 10,000 Noon Units (NU)/ml.◇

For intracutaneous tests: allergens, being identical to those used for nasal challenges, were diluted in a 1:10 ratio.

Evaluation of the Skin Tests

The intracutaneous tests were evaluated at 20 minutes, 6–8 hours, 12, 24, 48 and 60 hours after intracutaneous injection of allergen in a dose of 0.05 ml. These tests were considered to be immediate positive within 20 minutes and late positive after six or more hours (usually within 24 hours). The evaluation parameters are presented in the legends of Table I.

Drugs

a. Disodium cromoglycate (DSCG)* in powder form kept in capsules, was applied topically to the nasal mucosa by means of a special nasal applicator. One dose of DSCG was always one capsule containing 20 mg of DSCG powder, which was put into the nasal applicator, then perforated and blown into the nose by an air flow and in this way divided equally between both nostrils.

b. Beclomethasone dipropionate (BDA),** equipped with a special nasal adaptor, was administered to the nasal mucosa as an aerosol. One dose was always 2 puffs in each nostril (= 200 μg).

Apparatus and Methods

These have been described in previous papers.[5-7,14,17-20] The method could be briefly summarized as follows:

In a patient with allergic rhinitis who is challenged topically with an appropriate allergen to which he is sensitive the nasal mucosa reacts with swelling, hypersecretion, sneezing and itching. These changes then influence the passage of air through the nose, resulting in changes in pressure-differences between the nasopharyngeal cavity and the outside air, while the air flow remains constant. These pressure-differences, the so-called NPG (nasopharynx-nostril-pressure gradient), expressed in cm of H_2O, were recorded and considered as a parameter for the reactivity of the nasal mucosa.

Patients

Of a group of 370 patients with allergic rhinitis examined in our department by a routine diagnostic procedure including short-term nasal challenges with one or more allergens, 46 complained of acute nasal symptoms within 6–14 hours after allergen challenge. This late appearance of nasal symptoms was then confirmed by long-term nasal provocation tests, as a late nasal response to allergen challenge, in 42 patients. These 42 patients selected for the present study demonstrated (1) nasal complaints (obstruction, hypersecretion, sneezing, itching), (2) positive histories to one or more allergens, (3) positive intracutaneous tests to one or more allergens (immediate skin response), (4) increased blood eosinophilia, (5) positive nasal responses to allergen challenge due to immediate hypersensitivity (Type I allergy), (6) late nasal mucosa response to one or more allergens, which appeared after some short-term nasal provocation tests.

The patients were always studied during a period without manifest nasal complaints. They were not receiving anti-allergic therapy during the 10 days prior to this investigation. None of these patients had used disodium cromoglycate, corticosteroids (topical or systemic) or immunotherapy in the past.

The clinical characteristics of the patients investigated are given in Table I.

Procedure

This study consists of three experimental parts (provocation test, protection test with DSCG, protection test with BDA) and one control test with Coca's Solution, performed in each of the patients investigated. An interval of 3–5 days was allowed between the individual phases.

Each test began with calibration of the apparatus. A rubber balloon was then introduced into the nasopharynx through one of the nasal cavities, filled with 2 ml of air and connected to the pressure transducer. The patient breathed through the non-intubated nasal cavity only, the mouth being closed and the intubated cavity also being closed by the patient's fingers placed on the alae nasi. Five to 15 minutes later, when the patient's breathing was regular, the test was started.

Provocation Test with Allergen

The "initial test." The NPG values were recorded at 0, 5 and 10 minutes to obtain the so-called "initial values" (base line).

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* All allergen extracts were prepared by Pharmacy "Diephuis," Groningen, The Netherlands.

** Cat, dog, cattle, goat, hog, horse, rabbit, rat, mouse, hamster, guinea pig, canary, goose, duck, turkey, hen, pigeon, parrot, in equal proportions by weight.

*** Cladosporium cladosporiodes-elatum-herbarum, Penicillium brevi-compactum-expansum-notatum-frequentans-commune, Aspergillus versicolor-nigerfumigatus, Mucor spinosus-mucedo-racemosus, Pullularia pullulans, Botryis cinerea, Merculius domesticus, Epicoccum purpurascens, Alternaris tenuis, Stemphylium botryosum, Rhizopus nigricans, Fusarium culmorum and Trichoderma viride—in equal proportions by weight.

† Dry weight percentage: Secale cereale 15%, Dactylis glomerata 15%, Lolium perenne 10%, Anthoxanthum odoratum 10%, Agrostis alba 10%, Holcus lanatus 10%, Phleum pratense 10%, Cynosurus cristatus 5%, Alopecurus pratensis 15%.

†† Dry weight percentage: Corylus avellana 20%, Alnus species 30%, Salix species 20%, Betula species 20%, Myrica species 10%.

††† Dry weight percentage: Artemisia vulgaris 33%, Rumex species 33%, Plantago lanceolata 33%.

‡ Dry weight percentage: Tilia species 20%, Sambucus nigra 20%, Syringa vulgaris 20%, Philadelphus coronarius 20%, Robinia pseudo-acacia 20%.

◇ 1 Noon Unit (NU) = 0.001 mg of dry pollen (powder) = 0.5 PNU = 1.3 TNU.

* Disodium cromoglycate = henceforth abbreviated as DSCG.

** Beclomethasone dipropionate = henceforth abbreviated as BDA.

Table I. Clinical Features of the Patients Investigated.

Patient	Nasal complaints: Obstruction	Hypersecretion	Sneezing	Lividity of nasal mucosa	Blood eosinophils per mm³	Skin tests (i.c.)—immediate response Allergens: Basic HD	HF	GP	SP	Complementary	Allergen investigated and type of nasal response observed: I + III	O + III
1	+	+	+	–	451	+	+	+±	±	+ TP	HD	
2	+	+	+	+++	1309	+++	+++	+++	++	+++ PF	HD, GP	PF
3	+++	+++	+++	–	440	+	+	–	–	+ WP	GP	
4	+	+	+	+	451	±	–	+±	–	+++ WP	HD	
5	+	+	+	+++	1045	+±	+	+++	+±	+± D	HD	
6	++	++	++	–	517	++	+±	+++	++	++ D	GP	
7	+	+	+	–	1441	++	+±	+++	+±	+ C	GP	
8	+++	+++	+++	+++	539	++	+±	+	+	+ D	GP	
9	+++	+++	+++	+	385	+	++	+±	+±	++ D	GP	
10	+++	+++	+++	–	220	+	–	±	+	+ FP	HD	
11	+	+	+	++	1341	++	+±	+++	+±	++± TP	GP	
12	++	++	++	+++	561	+++	+++	+	–	++ C	GP	HD
13	+++	+++	+++	–	506	+	+	++	+	++ C	GP	
14	++	++	++	+++	241	+	+	–	–	+ Ca	SP	
15	+++	+++	+++	+++	781	+	+±	+±	–	+± FP	HD	
16	+++	+++	+++	+++	737	+±	+±	+	–	+++ C	HD	
17	+++	+++	+++	+	682	+	–	–	–	+ Mm	GP, SP, HF	
18	+++	++	+	–	440	++	+	+	–	++ Mm – AF	GP, AF, Mm	
19	+++	+++	+++	+	1210	+++	++	+±	+±	+± Mm	HD	
20	+	+	+	–	495	+	–	++±	–	+ CaF	HD	GP
21	+++	–	–	+	495	++	+	–	–	+ Mm	Mm	GP
22	+++	+++	+++	+++	363	+±	+	±	±	+ Mm	GP	
23	+	–	–	+	517	+	+±	+	+	++ Mm	GP	
24	+	+	+	+++	913	+++	++	+±	+	++ W	HF	
25	+++	+++	+++	+++	704	+++	++	++	++	+++ GPH	SP	
26	+++	+++	+++	–	1177	+++	+++	+++	+	++ D	HD, GP	
27	+++	+++	+++	–	495	+	–	–	–	+ CaF	HD	
28	++	++	+	+	508	+±	–	–	±	++ Mm	Mm	
29	+++	+	+	++	1045	++	+++	+	±	+++ D	D	
30	+	+	+	+++	770	+	±	+	+++	+ Mm	HD	
31	++	++	++	+++	1100	++	++	+	–	+ PF	PF	
32	+++	++	++	+	990	+	–	++±	++	+± FP	SP	
33	++	++	+	+	363	++	+++	–	–	+++ WF	WF	
34	–	–	+++	–	649	++	–	+++	++	+ WP		GP
35	+++	+++	+++	–	561	++	+±	++±	+++	+± WP		GP
36	+++	+++	+++	+	1144	+	–	±	–	+ Mm		HF
37	+++	+++	+++	+	429	–	+±	+	+	+± Mm		HD
38	+	+	+	+++	1462	+±	+	–	–	+ C		GP
39	+++	+++	+++	+	495	+±	++	+±	++±	+± TP		TP
40	++	++	+	+	209	++	++	++±	+++	+±	CaF	GP
41	+++	–	+++	–	2200	+	+±	–	+	+	Mm	GP
42	+	–	+	–	776	+±	+	–	+	+±	HeF	HD

HD = house dust; HF = hairs and feathers mixture; GP = grass pollen mixture; SP = spring pollen mixture; C = cat hair; D = dog hair; GPH = guinea pig hair; HeF = hen feathers; CaF = canary feathers; PR = pigeon feathers; TP = tree pollen mixture; FP = flower pollen mixture; WP = weed pollen mixture; WF = wheat flour; Mm = moulds mixture; AF = Aspergillus fumigatus; W = wool; Ca = capok.

Nasal mucosa responses:

0 = immediate response negative; I = immediate response positive; III = late response positive.

The evaluation of the intracutaneous tests:

– = normal skin appearance.
± = wheal not greater than original injected papule.
+ = wheal increase up to 7.5 mm in diameter.
+± = wheal increase up to 10.0 mm in diameter.
++ = wheal increase up to 12.5 mm in diameter.
++± = wheal increase up to 15.0 mm in diameter.
+++ = wheal increase greater than 15.0 mm, with surrounding erythema and sometimes with "pseudopodia."

The "Coca's Solution test." Coca's Solution was applied to the nasal mucosa under the medial concha of the non-intubated cavity by means of a wad of cotton wool on a nasal probe, for three minutes. The NPG values were recorded at 0, 5 and 10 minutes. If no significant changes in the NPG values after Coca's Solution with respect to the "initial NPG values" appeared, the investigation was continued.

The allergen challenge. The nasal mucosa was challenged by the allergen for three minutes in the same way on the same site as by the Coca's Solution. The NPG values were then recorded at 0, 5, 10, 20, 30, 45, 60, (90), 120, 150, 180, 210 and 240 minutes and then every hour up to the 11th hour and every second hour during the 24–36th-hour and 47–50th-hour intervals. The provocation tests were considered to be positive when the NPG values after allergen challenge increased by at least 2.0 cm of H_2O with respect to the "initial" and "Coca's Solution values."

Protection Test with Disodium Cromoglycate (DSCG)

Protection Test with Beclomethasone Dipropionate (BDA)

Both protection tests were performed in the same manner and according to the same schedule.

The patients were always pretreated with the appropriate drug in a daily dose of 4×1, starting two days before allergen challenge and continuing through the "challenge day" (daily dose of 5×1) up to two days after the "challenge day" (in a daily dose of 4×1). The drug was always administered at 7 a.m., 12 a.m., 6 p.m. and 11 p.m. With the exception of an extra drug dose, administered after "Coca's Solution values" and 10 minutes prior to the allergen challenge, the schedule of the protection tests is similar to that of the provocation tests. The protection tests were performed as "single-blind."

The protective effects of the drugs were considered to be significant, when the NPG values recorded after pretreated allergen challenge decreased by at least 50% with respect to the NPG values recorded after the non-pretreated allergen challenge.

The control test with Coca's Solution was performed three days after the provocation test. After the recording of the "initial NPG values" the Coca's Solution was applied to the nasal mucosa for three minutes in the same way as described above. The mean NPG values were recorded up to a 50 hours' interval.

*Statistical analysis.** The results were analyzed and statistically evaluated by means of polynomials, which were fitted and the hypotheses were tested by the Potthoff and Roy's[21] generalized multivariate analysis of variance model (generalized Manova Model) presented by Timm.[22] A p value < 0.01 was considered as highly significant, a p value < 0.05 as significant and a p value > 0.05 as non-significant.

Results

Provocation Tests

A survey of the NPG values recorded during the nasal provocation tests in individual patients is given in abbreviated form in Table II.

The 42 patients developed a total of 52 nasal mucosa responses within 4–24 hours after allergen challenge. This response began within 4–8 hours, reached its maximum within 6–12 hours and disappeared within 24–26 hours after allergen challenge in most cases. This type was considered to be the "late nasal mucosa response"* to allergen challenge which could possibly be due to the late hypersensitivity (Type III allergy).

The "late nasal mucosa response" was observed in two modifications; in 13 cases as an "isolated late response"** only and in 39 cases as a "dual response,"*** where first the immediate response† appeared and then after a symptom-free period of 3–7 hours the late response occurred.

Control Tests with Coca's Solution

The mean NPG values recorded after the Coca's Solution application did not differ significantly during the whole test (50 hours). The changes varied within 1.3 cm H_2O.

Protection Test with DSCG

The NPG values recorded during the protection tests with DSCG in individual patients are given in abbreviated form in Table III. By comparing the results given in Table III with those in Table II, the following conclusions could be drawn.

DSCG, when applied topically in a sufficient dose before and after allergen challenge, was able to decrease significantly or to prevent fully the development of the "late nasal mucosa response" to allergen challenge in all patients studied.

The "late nasal mucosa response," being a part of the "dual response," was decreased by DSCG to a slightly higher degree than the "isolated late nasal response."

The "immediate nasal response," recorded during the "dual response," was also decreased by DSCG significantly.

Protection Test with BDA

The NPG values recorded during the protection tests with BDA are presented in abbreviated form in Table IV. By comparing the results shown in Table IV with those presented in Table II the following conclusions could be drawn.

BDA, when applied topically in a sufficient dose before and after allergen challenge, was able to decrease the "isolated late nasal response" significantly.

The "late nasal mucosa response," being a part of the "dual response," was decreased by BDA, however, to a lesser degree than the "isolated late response."

The "immediate nasal response," being a part of the "dual response," was not decreased significantly by BDA at all.

The NPG values recorded during all three experimental parts, with respect to the "Coca's Solution NPG values," are summarized in Figure 1 for the "isolated late nasal mucosa response"and in Figure 2 for the "dual nasal mucosa response."

Accompanying Findings

In all cases the positive nasal mucosa responses of the immediate as well as of the late type were accompanied

* The calculations were performed by Dr. M. P. F. Berger (Dept. Psychology, Univ. of Tilburg, Tilburg, The Netherlands) using a PDP 11/45 computer.
* Late Nasal Mucosa Response = henceforth abbreviated as LNR.

** Isolated Late Nasal Mucosa Response = henceforth abbreviated as ILNR.
*** Late Nasal Mucosa Response as a part of the dual response = henceforth abbreviated as DLNR.
† Immediate Nasal Mucosa Response = henceforth abbreviated as INR.

Table II. Abbreviated Survey of the Mean Nasopharynx-nostril-pressure-gradient (NPG) Values in cm H_2O Recorded During the Nasal Provocation Tests with Allergen in Individual Patients.

Patient	Coca value	Allergen	Time in minutes after challenge						Time in hours after challenge							
			0′	10′	20′	30′	45′	60′	3h	4h	5h	6h	7h	8h	9h	10h	24h
DNR																	
1	2.7	HD	3.4	3.5	5.7	6.1	5.5	4.7	3.0	5.0	8.4	9.4	12.7	9.8	8.8	10.2	2.3
2	2.0	HD	2.3	8.6	12.9	10.0	6.0	3.4	1.8	1.8	1.7	1.9	6.8	11.2	11.9	11.3	3.5
	3.5	GP	4.6	7.3	10.4	8.0	7.8	4.7	3.5	3.5	3.9	4.8	9.0	10.5	14.8	15.1	3.9
3	2.1	GP	3.0	5.2	6.1	6.5	5.1	3.5	2.2	2.2	2.5	4.4	11.1	12.3	9.6	9.8	2.6
4	2.1	HD	1.8	3.8	8.2	4.3	5.1	5.2	1.8	1.4	5.4	6.7	7.2	5.9	5.4	4.5	4.5
5	4.5	HD	9.3	15.4	22.6	13.9	14.5	9.3	5.2	10.6	11.4	8.5	9.5	10.6	7.9	4.3	4.7
6	1.7	GP	4.5	6.8	7.9	7.2	6.5	8.9	3.0	3.5	3.2	5.4	7.5	7.7	6.1	5.6	2.7
7	1.7	GP	3.4	5.9	5.2	8.0	3.0	4.7	2.6	5.2	8.5	7.3	10.5	6.0	6.8	3.9	1.7
8	1.8	GP	3.4	5.7	4.7	6.2	3.7	3.7	2.2	2.3	9.5	7.2	5.3	15.2	17.6	15.4	1.5
9	5.1	GP	19.0	28.6	27.4	20.6	29.0	26.9	6.1	6.3	4.9	4.9	4.9	11.0	12.3	15.1	13.2
10	3.2	HD	5.0	8.3	12.1	3.8	3.2	3.7	2.2	4.6	4.2	5.1	14.8	12.4	7.0	5.4	1.6
11	1.7	GP	14.2	17.5	15.3	17.9	22.1	14.3	4.2	5.8	4.5	2.5	2.1	8.8	12.1	8.2	1.4
12	2.4	GP	7.9	11.0	9.6	9.8	6.7	6.2	3.8	4.1	5.6	7.3	9.2	8.1	13.7	12.6	10.3
13	5.2	GP	14.0	22.9	18.2	16.8	12.3	14.6	5.2	14.2	22.2	25.2	25.2	28.5	22.2	23.9	5.1
14	5.6	SP	8.5	8.8	11.7	9.2	9.3	9.8	5.5	6.5	5.9	5.4	15.1	8.9	9.9	9.8	7.8
15	1.9	HD	12.7	20.4	23.4	24.9	21.8	20.7	2.1	2.4	3.3	6.3	21.4	21.2	23.7	21.2	2.8
16	2.9	HD	6.8	10.6	12.9	15.2	14.6	12.9	3.2	7.5	6.1	6.3	6.2	6.5	7.3	4.8	4.4
17	3.1	GP	5.8	12.6	14.2	14.8	11.4	11.8	9.8	17.1	16.1	16.8	10.5	15.5	9.4	8.4	3.3
	2.1	SP	4.3	5.1	7.0	7.3	4.0	4.2	2.8	3.6	8.4	5.9	7.9	8.7	9.1	6.2	5.8
	3.7	HF	5.8	7.0	9.7	14.1	13.7	10.4	5.3	5.2	8.1	8.3	9.4	8.6	8.6	10.6	6.9
18	3.2	GP	3.7	5.5	6.1	6.9	9.6	8.9	3.9	5.7	24.1	13.9	14.7	23.3	14.4	25.5	24.0
	2.9	AF	6.2	28.1	25.0	23.3	10.6	10.6	3.5	3.0	16.3	22.1	22.1	17.0	17.6	15.1	10.5
	3.9	Mm	6.4	12.1	12.7	11.0	5.6	3.8	3.8	7.4	8.5	13.4	9.8	7.4	6.4	5.7	3.4
19	1.1	HD	1.9	5.5	8.6	9.3	9.6	6.0	1.9	1.7	8.8	5.5	5.6	5.7	3.6	4.2	2.3
20	1.4	HD	1.5	1.4	1.6	12.0	16.1	15.2	2.6	2.8	2.4	5.2	7.2	7.5	10.2	14.3	4.8
21	1.1	Mm	2.6	7.3	10.2	12.1	3.3	3.2	2.0	1.7	2.4	3.4	4.2	7.9	11.4	9.7	1.5
22	1.8	GP	2.1	6.2	7.7	8.9	4.6	6.2	3.3	4.1	4.9	5.8	9.9	11.7	7.5	8.1	4.9
23	0.9	GP	2.4	3.8	3.8	4.0	2.5	2.1	1.8	2.1	7.6	9.1	11.9	18.2	13.0	8.3	1.6
24	2.0	HF	3.6	11.6	10.2	6.9	6.2	5.6	2.5	4.6	4.8	5.6	5.3	7.0	6.7	5.7	4.2
25	2.8	SP	4.1	6.7	7.2	7.2	5.7	6.0	2.9	5.8	4.7	4.6	4.6	6.4	7.0	5.8	2.6
26	2.9	HD	4.5	7.4	9.6	10.5	11.3	10.1	3.4	2.9	3.2	6.2	7.4	11.4	6.5	4.5	4.1
	1.3	GP	3.5	5.1	5.3	5.6	5.4	5.2	4.6	12.9	3.1	2.5	3.1	7.9	12.6	14.6	2.8
27	2.3	HD	3.2	3.3	9.0	7.0	4.9	5.8	2.4	2.9	2.8	5.2	4.2	6.8	9.4	9.6	2.7
28	2.6	Mm	2.7	2.9	5.3	5.3	6.6	11.8	—	4.7	3.3	3.3	6.6	13.6	11.1	7.9	1.6
29	1.0	Dog	1.6	8.9	8.4	9.1	7.2	7.1	1.5	1.3	4.1	12.8	13.2	14.3	14.0	8.9	7.3
30	4.1	HD	19.6	27.7	30.9	30.7	11.9	11.4	3.9	6.8	11.9	10.7	20.8	21.4	8.5	5.8	5.6
31	2.1	PF	2.1	6.7	6.6	6.5	8.0	6.2	3.3	3.0	8.6	10.1	5.2	5.5	9.5	8.0	1.8
32	4.0	SP	4.2	9.8	14.1	11.7	11.0	5.5	3.9	4.3	3.7	3.8	4.0	5.9	15.3	16.1	4.0
33	2.0	WF	2.8	5.2	5.9	7.3	8.3	6.7	2.0	2.1	1.7	1.9	2.0	2.1	4.7	7.5	3.6
ILNR																	
2	3.2	PF	3.4	3.1	3.2	3.1	3.1	3.1	3.3	3.2	3.2	3.1	7.9	12.0	12.1	12.6	10.7
12	1.8	HD	2.7	2.3	2.2	1.9	2.3	2.9	4.6	4.4	8.3	7.5	16.4	15.0	13.9	14.0	1.7
20	1.5	GP	1.7	1.5	1.4	1.3	1.8	2.0	1.2	2.7	6.6	15.7	9.6	7.2	5.7	4.2	0.9
21	2.1	GP	2.1	1.7	2.4	3.1	2.4	2.5	3.5	8.0	6.1	6.8	4.7	5.8	6.5	6.4	5.4
34	1.2	GP	1.8	1.8	1.1	1.1	1.4	1.2	1.7	4.0	3.7	4.8	4.2	2.9	2.8	3.0	2.6
35	1.3	GP	2.1	2.3	2.1	2.7	2.0	2.7	1.8	1.6	2.3	1.6	5.8	5.6	7.1	9.8	0.6
36	2.5	HF	2.9	2.9	3.3	2.9	3.3	3.2	4.3	11.3	12.5	6.8	8.7	11.3	10.8	12.0	3.6
37	3.3	HD	2.9	3.3	3.4	3.2	3.7	4.2	7.1	7.3	7.0	8.2	11.4	8.3	10.2	7.6	7.5
38	3.1	GP	3.2	4.0	3.6	3.5	4.4	3.8	3.6	2.8	4.4	8.3	7.8	7.3	7.3	7.0	3.4
39	0.8	TP	1.0	0.9	0.9	1.0	1.0	1.0	2.0	1.9	3.3	7.0	8.6	8.8	4.9	5.8	1.4
40	5.1	GP	5.8	5.7	5.5	5.7	4.7	5.4	4.7	5.1	4.8	9.7	15.3	17.1	13.0	9.6	6.9
41	2.1	GP	2.6	2.2	2.9	3.1	2.9	2.8	3.0	2.9	5.5	7.0	8.3	20.2	9.0	9.5	4.4
42	1.5	HD	1.5	1.5	1.9	1.9	2.1	1.8	1.6	2.4	2.3	3.6	6.6	9.3	10.6	9.5	1.4

HD = house dust; GP = grass pollen mixture; SP = spring pollen mixture; HF = hairs and feathers mixture; Mm = moulds mixture; dog = dog hair; PF = pigeon feathers; WF = wheat flour; AF = aspergillus fumigatus; TP = tree pollen mixture.
The Coca's value is the mean NPG value, calculated always from three values as recorded at 0, 5 and 10 minutes after the application of Coca's Solution.
DNR = Dual Nasal Mucosa Response; ILNR = Isolated Late Nasal Mucosa Response.

by the appearance of acute nasal complaints (obstruction, hypersecretion, sneezing, itching). The course of the nasal complaints ran parallel to the course of the NPG changes.

Lividity of the nasal mucosa was observed in most of the patients during the immediate as well as during the late nasal mucosa response; however, it was more pronounced during the late response. No difference in lividity was found between both the modifications of the late response. During the protection tests with DSCG no lividity of the nasal mucosa was observed in any of the nasal mucosa responses. During the protection tests with BDA, nasal mucosa lividity was observed in most of the patients during the immediate response but not during both modifications of the late response.

The results of the intracutaneous tests are summarized in Table V. In 77% of the cases of immediate nasal mucosa response positive immediate skin responses to

Table III. Abbreviated Survey of the Mean Nasopharynx-nostril-pressure-gradient (NPG) Values in cm H_2O Recorded During the Nasal Protection Tests with Disodium Cromoglycate (DSCG) in Individual Patients.

Patient	Coca value	DSCG	Aller-gen	Time in minutes after challenge						Time in hours after challenge						
				0′	10′	20′	30′	45′	60′	3h	4h	5h	6h	7h	8h	9h	10h	24h
DNR																		
1	1.7	1.8	HD	1.8	1.4	1.4	1.4	1.4	1.7	1.8	2.4	3.4	3.3	2.5	3.4	3.7	3.4	2.3
2	1.5	1.0	HD	1.1	1.0	1.0	1.2	1.0	1.0	2.1	2.2	2.1	1.6	3.4	3.1	2.8	2.4	1.8
	3.7	3.7	GP	3.5	4.1	5.2	4.1	3.5	3.5	5.8	5.5	5.0	4.6	2.9	5.5	5.5	2.6	2.6
3	3.8	5.0	GP	—	4.8	4.8	4.8	4.2	4.1	3.4	3.2	6.6	3.4	3.8	3.7	2.3	2.3	2.6
4	1.0	0.8	HD	0.8	0.8	0.9	0.8	1.0	0.9	1.2	1.4	1.5	1.2	1.2	1.7	1.0	1.0	1.0
5	3.0	2.8	HD	2.9	3.0	2.4	2.8	2.8	3.2	2.8	2.9	2.4	2.9	2.8	3.0	2.6	2.6	3.6
6	2.4	2.2	GP	—	1.7	4.1	3.5	3.3	2.3	2.1	2.7	3.1	2.2	2.7	2.5	2.7	3.0	2.8
7	2.1	2.1	GP	3.5	4.3	3.9	4.6	4.6	4.1	4.2	2.6	3.0	3.2	1.4	2.2	3.1	1.4	1.8
8	3.5	3.5	GP	3.5	3.7	3.2	4.3	3.9	3.5	2.7	2.5	3.3	3.3	2.6	3.7	2.8	2.9	2.6
9	1.4	1.5	GP	1.9	1.9	2.0	3.2	2.9	2.3	3.6	5.6	3.4	4.5	3.9	2.8	2.3	2.9	—
10	1.5	1.9	HD	2.9	2.4	3.2	3.1	1.7	1.4	1.3	1.4	1.4	1.3	1.5	1.5	2.0	2.0	1.8
11	3.1	3.7	GP	11.9	9.5	7.5	9.2	9.6	9.5	3.1	4.1	3.1	4.2	3.1	2.8	2.2	3.0	2.0
12	2.5	1.7	GP	1.7	2.1	2.3	2.3	2.3	1.5	3.9	3.3	6.4	3.6	3.6	4.2	3.6	2.7	1.8
13	1.1	1.3	GP	3.1	4.2	6.5	6.1	6.3	7.6	3.7	3.1	5.1	6.3	6.1	6.0	4.4	5.6	1.7
14	3.0	2.6	SP	2.7	2.0	2.1	2.8	2.2	2.8	2.6	2.9	4.0	3.8	4.4	5.0	4.1	3.3	1.7
15	1.3	1.5	HD	1.9	2.7	2.3	2.6	2.6	2.6	2.3	3.0	3.7	4.4	6.1	4.3	3.3	3.4	1.9
16	2.5	2.1	HD	2.8	1.8	1.8	1.8	9.3	2.0	1.3	1.8	2.0	2.0	2.1	2.1	3.4	2.9	3.0
17	1.5	1.5	GP	1.5	1.5	1.8	1.4	1.8	1.9	2.4	2.2	1.6	1.7	1.8	1.7	1.3	1.5	1.2
	3.7	3.7	SP	4.8	5.7	5.2	3.8	4.0	4.3	3.2	3.6	3.0	2.8	3.0	3.2	5.7	4.8	2.7
	1.8	1.8	HF	1.1	2.0	2.6	1.7	2.2	2.2	2.9	3.2	2.5	2.7	3.3	4.9	2.9	3.3	1.8
18	2.5	2.5	GP	3.2	3.0	3.1	2.3	2.0	2.1	2.0	2.9	4.5	2.8	3.8	3.3	3.2	2.6	3.0
	2.9	2.9	AF	4.5	8.8	7.9	9.2	9.9	8.3	3.5	4.9	4.2	5.5	5.0	5.2	5.3	5.1	3.8
	2.2	1.4	Mm	2.0	2.9	2.2	2.5	5.0	4.4	2.3	2.6	2.4	3.0	3.2	4.0	3.3	3.8	3.1
19	2.5	2.7	HD	2.2	2.3	2.1	2.1	2.4	2.1	3.2	3.2	1.9	3.1	4.7	2.2	2.1	2.9	2.0
20	3.0	2.7	HD	2.6	2.4	2.7	2.3	2.8	3.1	3.2	3.0	3.2	2.7	3.4	2.4	2.7	2.2	1.9
21	2.8	2.7	Mm	3.8	2.2	2.4	2.4	2.4	2.8	1.7	3.3	4.8	3.2	3.0	4.1	3.9	4.4	1.9
22	4.2	—	GP	5.5	4.2	3.6	3.7	4.2	3.7	3.1	5.1	4.6	4.8	5.4	5.6	5.5	5.5	4.1
23	1.6	1.6	GP	2.5	2.6	2.7	3.2	2.9	1.9	2.2	5.9	3.9	2.9	2.3	2.8	3.3	6.0	1.3
24	1.5	—	HF	2.1	3.9	2.5	3.2	2.9	2.3	2.3	2.3	1.9	2.3	2.3	2.1	1.9	1.8	1.6
25	3.5	3.5	SP	3.1	3.4	4.0	3.8	3.6	3.7	3.5	2.9	3.5	3.7	2.8	3.6	3.9	2.9	2.4
26	3.3	3.9	HD	4.2	4.6	4.0	4.3	4.6	4.1	2.5	5.1	3.8	1.5	5.8	3.9	4.2	4.2	2.9
	2.5	2.1	GP	2.4	2.3	2.1	2.0	2.3	2.3	3.0	2.8	2.5	3.7	2.4	3.0	4.2	3.5	2.2
27	3.0	3.3	HD	3.0	2.7	2.4	2.7	2.5	2.4	2.8	2.6	3.2	3.0	4.6	3.7	2.9	2.3	2.4
28	1.7	1.5	Mn	1.7	1.7	1.4	1.4	1.5	1.4	3.2	2.4	2.6	3.7	3.0	2.4	3.0	2.6	1.5
29	1.2	1.5	Dog	1.5	2.0	1.4	1.8	2.0	2.3	1.0	1.6	1.5	1.4	1.4	1.1	1.8	1.9	1.0
30	3.8	3.5	HD	5.7	5.0	4.8	4.4	4.0	4.0	3.5	3.6	3.5	3.4	3.7	3.6	4.1	4.2	3.2
31	1.6	1.9	PF	3.4	2.3	2.4	2.3	1.6	1.7	4.8	2.7	4.2	6.0	6.9	3.2	6.4	8.4	3.9
32	3.4	3.4	SP	3.7	4.0	4.6	4.0	3.5	3.4	1.8	2.2	2.1	2.3	2.6	1.6	1.5	1.5	2.5
33	3.1	3.0	WF	3.3	3.5	3.1	3.2	3.1	3.2	3.0	3.0	2.8	3.4	3.7	3.5	2.7	3.6	3.1
ILNR																		
2	1.5	1.3	PF	1.2	1.1	1.1	1.1	1.3	1.3	1.7	1.6	1.4	1.3	1.3	1.3	2.2	1.9	2.1
12	1.6	1.7	HD	1.6	2.0	1.9	1.7	1.6	1.9	1.4	1.9	2.3	3.7	3.3	2.4	2.3	2.9	2.5
20	3.7	3.5	GP	3.5	3.8	3.5	3.7	3.8	3.5	4.1	3.6	3.3	3.7	4.3	4.0	4.8	4.4	3.9
21	2.1	2.1	GP	2.9	2.3	2.3	3.0	2.9	2.2	1.7	1.8	2.3	1.9	2.3	3.8	2.5	4.4	1.8
34	1.1	1.0	GP	1.1	1.3	1.1	1.2	1.2	1.4	1.5	1.4	1.3	1.2	1.0	1.2	1.2	1.2	0.9
35	1.0	0.9	GP	0.8	1.2	1.2	1.1	1.1	1.0	0.8	1.2	1.3	1.2	1.4	1.1	1.1	1.1	1.1
36	2.0	1.8	HF	2.0	1.8	2.0	1.4	2.2	2.2	2.2	2.2	1.8	2.8	2.3	1.7	3.0	2.1	1.5
37	4.5	2.3	HD	4.0	4.3	4.3	4.0	4.6	4.9	4.1	4.4	4.2	4.8	4.3	5.4	3.9	3.7	3.3
38	3.5	3.3	GP	3.6	3.3	3.3	3.1	3.2	3.6	3.5	2.6	3.0	4.0	3.7	2.6	3.1	3.3	2.6
39	0.9	1.0	TP	1.1	0.9	0.9	0.9	1.1	1.0	1.1	1.1	1.0	1.1	1.1	1.0	1.0	1.1	1.0
40	3.5	3.9	GP	3.3	4.0	4.8	4.7	4.2	4.3	3.5	4.0	4.7	6.5	3.9	3.8	3.9	3.5	3.3
41	2.1	2.5	GP	2.3	2.4	2.3	2.7	2.0	1.8	2.6	2.3	3.2	3.2	3.8	2.6	2.1	2.3	3.5
42	5.5	5.0	HD	5.3	4.8	4.8	5.4	5.3	5.0	5.5	5.1	5.5	6.3	5.8	6.6	6.2	5.6	4.5

HD = house dust; GP = grass pollen mixture; SP = spring pollen mixture; HF = hairs and feathers mixture; Mm = moulds mixture; Dog = dog hair; PF = pigeon feathers; WF = wheat flour; AF = Aspergillus fumigatus; TP = tree pollen mixture. DSCG = Disodium cromoglycate.
The Coca's value as well as the DSCG values are the mean NPG values calculated always from three values as recorded at 0, 5 and 10 minutes after the Coca's Solution, respectively DSCG administration.
DNR = Dual Nasal Mucosa Response; ILNR = Isolated Late Nasal Mucosa Response.

the same allergen were also found. Positive late skin responses were observed in 54% of the isolated late nasal responses and in 69% of the late responses being a part of the dual response.

Statistical Analysis

Isolated late nasal mucosa response: Hypothesis No. 1 = all three curves (ALL, DSCG, BDA) coincide; this hypothesis is rejected ($p < 0.01$). *Hypothesis No. 2* = the ALL and BDA curves coincide; this hypothesis is rejected for ($p < 0.05$), but not rejected for ($p > 0.01$). *Hypothesis No. 3* = the DSCG curve runs horizontally; this hypothesis is not rejected ($p > 0.05$).

Dual nasal mucosa response: (a) *Immediate response: Hypothesis No. 1* = all three curves (ALL, DSCG, BDA) coincide; this hypothesis is rejected ($p < 0.01$). *Hypothesis No. 2* = the ALL and BDA curves coincide; this hypothesis is not rejected ($p > 0.05$). *Hypothesis No. 3* = the

Table IV. Abbreviated Survey of the Mean Nasopharynx-nostril-pressure-gradient (NPG) Values in cm H_2O Recorded During the Nasal Protection Tests with Beclomethasone Dipropionate (BDA) in Individual Patients.

Patient	Coca value	BDA	Allergen	Time in minutes after challenge						Time in hours after challenge							
				0'	10'	20'	30'	45'	60'	3h	4h	5h	6h	7h	8h	9h	10h	24h
DNR																		
1	2.8	2.7	HD	2.8	5.8	6.4	6.4	7.9	6.1	2.9	3.1	3.0	2.9	3.1	2.9	2.8	3.4	2.8
2	0.9	0.8	HD	4.4	5.9	5.8	7.4	9.0	9.4	1.8	1.6	2.1	4.6	6.2	4.0	4.8	5.1	1.6
	2.1	2.2	GP	1.9	6.5	7.6	9.3	9.1	9.3	2.2	1.9	1.6	5.6	7.7	7.4	9.4	10.9	1.9
3	2.3	2.0	GP	2.4	14.1	9.0	10.8	8.2	6.9	3.8	3.6	7.3	13.4	12.2	5.9	6.7	5.3	2.4
4	2.6	2.6	HD	6.1	9.6	9.9	10.2	11.4	6.3	2.5	4.5	12.2	16.4	10.4	8.0	14.6	10.7	5.2
5	2.6	2.6	HD	8.4	10.3	10.6	17.8	20.1	10.0	3.0	2.6	8.0	6.8	7.8	9.6	12.9	21.6	4.1
6	4.5	4.1	GP	25.4	25.3	28.5	22.0	26.1	28.6	6.0	6.0	25.6	7.2	6.6	6.7	4.4	4.2	1.9
7	1.9	2.3	GP	5.4	8.3	8.8	7.2	3.3	3.0	3.3	2.1	4.1	4.3	3.2	4.4	4.6	2.8	—
8	1.2	1.2	GP	5.9	4.4	5.0	5.5	7.0	5.8	5.5	10.0	7.2	6.3	5.9	7.8	5.5	—	1.9
9	3.1	2.7	GP	12.0	21.0	19.3	20.6	17.9	17.6	3.5	3.7	8.0	6.6	5.9	4.7	3.6	4.8	2.9
10	2.6	2.5	HD	4.6	13.9	14.9	14.5	7.0	5.4	1.6	2.1	1.9	2.1	3.2	2.0	2.1	2.0	1.7
11	2.2	2.1	GP	14.0	27.0	22.0	23.5	13.5	13.9	5.7	3.4	9.7	6.6	2.1	2.9	2.8	6.7	2.0
12	2.5	2.3	GP	6.0	9.1	8.6	8.9	8.7	9.4	5.1	7.2	13.7	12.8	13.9	8.9	16.5	10.4	7.7
13	3.8	3.7	GP	14.0	19.1	23.0	25.0	23.4	20.5	5.1	7.4	16.7	17.5	17.2	23.7	24.9	26.3	2.9
14	2.7	2.1	SP	7.2	10.7	11.4	7.7	9.2	9.9	4.6	4.5	4.6	10.4	8.8	7.5	16.3	10.8	5.6
15	1.0	1.0	HD	9.4	18.2	19.0	28.7	10.3	8.1	2.9	9.8	9.9	9.7	15.4	14.8	—	16.7	5.6
16	1.2	1.3	HD	5.3	10.1	8.1	7.1	9.5	7.6	2.2	5.1	3.7	4.8	4.0	5.2	3.8	4.1	1.9
17	0.9	0.9	GP	4.8	11.2	17.5	10.7	12.9	10.1	0.8	6.6	8.6	13.1	11.4	15.7	24.2	12.0	2.5
	4.4	4.5	SP	6.2	5.9	14.8	11.1	11.5	9.2	4.5	5.8	9.6	9.7	9.2	8.0	11.2	6.5	4.1
	1.6	1.7	HF	7.2	13.1	12.0	12.6	5.6	3.8	6.9	5.2	5.6	8.8	7.2	7.0	9.9	6.0	1.5
18	2.8	2.5	GP	3.2	6.4	7.0	5.6	5.5	7.0	2.6	8.0	12.2	13.2	13.2	17.6	17.1	21.6	2.5
	3.1	2.8	AF	8.7	22.8	22.5	19.2	24.2	21.3	8.1	7.6	1.8	12.4	12.8	11.3	12.1	10.5	3.0
	1.5	1.6	Mm	2.6	11.6	11.1	10.2	9.4	8.7	2.4	2.4	2.6	15.1	12.8	8.1	6.4	5.5	2.0
19	3.1	3.1	HD	21.5	25.9	25.9	25.9	22.4	25.9	8.7	16.9	14.0	10.6	11.4	12.8	18.6	8.5	2.2
20	2.2	2.2	HD	2.5	8.1	12.0	19.2	13.1	7.6	—	5.1	2.5	2.4	5.2	5.3	4.7	5.8	2.9
21	2.5	2.3	Mm	2.4	10.2	10.1	10.1	11.1	9.8	4.3	2.9	2.8	16.6	9.7	8.6	11.9	13.2	3.6
22	2.2	—	GP	3.4	7.0	5.9	10.4	10.9	7.4	2.8	5.6	7.0	9.7	12.0	8.1	7.8	6.6	2.5
23	2.2	2.2	GP	4.0	12.6	14.5	13.8	18.6	8.7	3.4	1.7	2.3	6.7	6.7	9.0	13.2	12.0	3.8
24	1.2	—	HF	3.6	8.4	2.6	7.1	4.1	2.4	2.4	2.7	3.8	5.1	3.4	6.0	1.2	3.7	3.1
25	3.3	3.5	SP	6.9	16.1	17.7	12.4	12.1	9.6	3.2	3.7	2.7	2.4	2.7	4.8	3.5	3.7	2.0
26	2.7	2.5	HD	8.0	8.8	13.9	13.3	9.0	—	3.6	2.9	3.9	5.2	5.0	6.1	4.6	3.8	4.3
	2.8	2.4	GP	9.7	13.0	4.2	9.5	11.0	15.2	2.4	4.4	2.5	2.6	3.3	3.4	4.6	3.9	2.0
27	2.7	2.1	HD	5.1	6.0	7.2	7.0	6.6	7.6	5.4	4.3	3.4	3.9	5.9	3.5	2.6	3.6	2.4
28	1.7	1.6	Mm	2.7	4.5	5.2	5.9	9.4	10.1	7.3	3.0	2.9	2.4	3.1	2.6	2.8	2.1	1.6
29	0.9	0.7	Dog	3.2	5.9	7.5	6.4	4.6	5.8	2.7	1.5	3.8	10.1	10.1	8.4	12.9	13.3	3.6
30	3.3	3.3	HD	27.1	27.2	27.2	27.2	27.3	26.5	2.1	2.3	2.2	2.2	3.5	4.3	3.8	3.9	3.0
31	2.3	2.4	PF	2.8	6.5	7.7	16.7	13.8	13.1	4.5	4.5	6.9	6.5	6.6	7.1	6.1	6.1	1.3
32	2.1	2.3	SP	6.5	7.8	13.4	15.5	15.2	9.1	2.2	2.2	1.9	2.1	2.2	6.1	8.5	1.9	3.1
33	3.3	2.7	WF	4.3	7.9	9.4	10.9	10.1	5.8	3.4	3.0	3.1	4.7	5.3	6.9	7.1	6.3	4.0
ILNR																		
2	0.8	0.8	PF	0.8	1.0	0.7	0.8	1.1	0.9	3.2	2.7	4.5	4.4	4.5	6.7	2.1	2.6	1.5
12	1.4	1.7	HD	1.7	1.7	1.6	1.3	2.0	1.5	5.0	5.0	4.9	5.0	10.8	5.2	5.1	4.9	7.0
20	2.2	2.4	GP	3.2	3.0	2.8	2.8	3.0	2.5	2.0	2.1	2.3	4.3	4.4	4.9	5.1	4.9	2.3
21	2.4	2.5	GP	2.5	2.8	2.3	2.6	3.0	2.0	3.3	7.3	5.3	7.0	8.0	8.0	9.3	9.9	2.3
34	1.2	1.0	GP	1.2	2.6	1.4	1.4	1.2	1.0	1.4	1.2	1.0	2.4	5.3	5.5	3.8	4.2	1.7
35	3.3	3.0	GP	3.4	3.9	3.6	3.0	4.3	3.9	3.9	3.2	3.2	4.5	9.7	10.1	6.1	5.9	2.0
36	2.0	2.6	HF	2.5	2.7	2.7	2.7	2.1	2.5	3.4	11.6	9.3	6.1	7.3	7.6	8.0	6.2	2.7
37	4.2	4.1	HD	4.2	5.0	4.8	—	4.2	4.2	3.9	5.1	8.8	7.3	8.6	8.6	9.0	9.0	5.2
38	1.5	1.5	GP	1.7	1.7	1.6	1.6	2.8	1.6	2.6	3.0	2.7	5.1	6.7	6.6	5.6	5.4	2.4
39	2.1	1.9	TP	1.9	1.9	2.0	2.0	1.9	1.9	1.9	2.6	2.0	2.2	7.6	7.2	7.3	5.1	1.2
40	2.4	—	GP	3.0	2.5	2.4	2.2	2.4	2.6	5.9	9.0	8.7	7.7	9.3	8.9	9.7	10.3	3.2
41	0.9	0.9	GP	1.3	2.1	1.5	1.6	1.6	1.6	1.9	1.4	1.8	1.3	1.8	1.9	2.7	1.9	1.3
42	4.9	4.8	HD	5.1	4.8	5.6	5.1	6.1	6.0	5.5	5.9	4.6	5.5	5.1	7.7	7.2	7.1	4.9

HD = house dust; GP = grass pollen mixture; SP = spring pollen mixture; HF = hairs and feathers mixture; Mm = moulds mixture; Dog = dog hair; PF = pigeon feathers; WF = wheat flour; AF = Aspergillus fumigatus; TP = tree pollen mixture. BDA = Beclomethasone dipropionate.

The Coca's value as well as the BDA values are the mean NPG values calcualted always from three values as recorded at 0, 5 and 10 minutes after the Coca's Solution, respectively BDA administration.

DNR = Dual Nasal Mucosa Response; ILNR = Isolated Late Nasal Mucosa Response.

DSCG curve runs horizontally; this hypothesis is not rejected ($p > 0.05$).

Late response: Hypothesis No. 1 = all three curves (ALL, DSCG, BDA) coincide; this hypothesis is rejected ($p < 0.01$). *Hypothesis No. 2* = the ALL and BDA curves coincide; this hypothesis is not rejected ($p > 0.05$). *Hypothesis No. 3* = the DSCG curve runs horizontally; this hypothesis is not rejected ($p > 0.05$).

Explanation of abbreviations: ALL = NPG values recorded during the provocation tests; DSCG = NPG values recorded during the protection tests with DSCG; BDA = NPG values recorded during the protection tests with BDA.

Discussion

We have used nasal challenges for several years as a standard part of the diagnostic procedure in allergic rhinitis patients for detection of immediate hypersensitivity (Type I allergic component).[5–7,13–14,17–20] They have been used in two ways: as provocation tests with an allergen and as protection tests using an anti-allergic drug prior to the allergen challenge.[5–7,13,18] The protective effects of the drug refer to its ability to prevent or decrease significantly the development of the changes in the nasal mucosa caused by the antigen-antibody interaction with its subsequent steps. This interaction is represented and caused by the challenge of a known allergen under standard conditions.[5–7,14,18]

We have also used nasal provocation tests for detection of other types of allergic reaction in the nose, the so-called "non-immediate nasal mucosa responses." One of these is the "late nasal mucosa response."[13,14] Although the existence of this type of nasal mucosa response has earlier been suggested by some investigators,[10,15,16,23] there are not numerous papers describing this type extensively.[13–15,23]

Some investigators[25,38] observed that the immediate asthmatic reaction (Type I hypersensitivity) as well as the late asthmatic reaction (Type III hypersensitivity) to allergen challenge were inhibited by pretreatment with inhaled DSCG. They also found that inhaled BDA prevented the late asthmatic reaction (Type III hypersensitivity) to allergen challenge, in both its modifications but did not decrease the immediate asthmatic reaction. Similar conclusions comparing the effects of DSCG and systemic corticosteroids (prednisolone) were reported by Booij-Noord et al.[41]

The results of our present study differ slightly from the above mentioned observations in the bronchial tree. Although DSCG decreased significantly the LNR, the ILNR was inhibited to a lesser degree than the DLNR. BDA showed an adverse effect, by inhibiting the ILNR to a higher degree than the DLNR. This observation is compatible with the possibility that in some cases of the "dual response" the immediate allergic reaction and its parts may play a certain role (introductory role) in the following late allergic reaction.

The subjective nasal complaints accompanying the INR were increased by BDA, which corresponds to the objective NPG changes recorded. The subjective nasal complaints accompanying the LNR were decreased during the DSCG as well as the BDA protection tests to a similar degree. With respect to the differences observed between the subjective and objective parameters, it would be concluded that the recording of the objective parameters (NPG changes) provides more exact information than only a single recording of the subjective complaints score used in a number of the above-mentioned studies.[1–4]

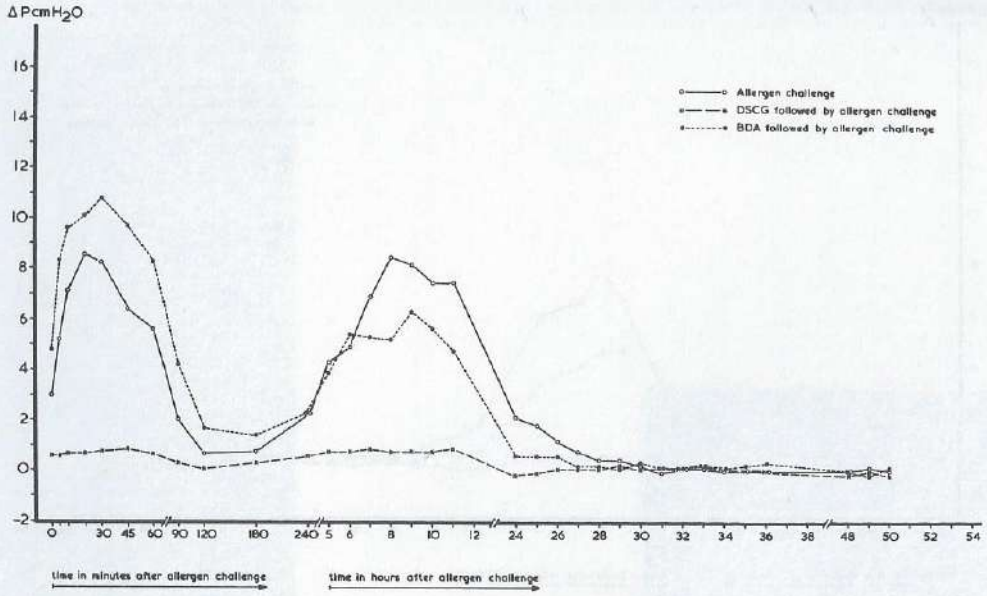

Figure 1. Groups I + III (positive immediate and late responses). Provocation tests and protection tests with DSCG and BDA. The mean NPG values recorded from all patients demonstrating "dual nasal mucosa response" (due to Type I and the Type III hypersensitivity) during these tests, with respect to the appropriate Coca's Solution mean NPG values. DSCG = disodium cromoglycate; BDA = beclomethasone dipropionate.

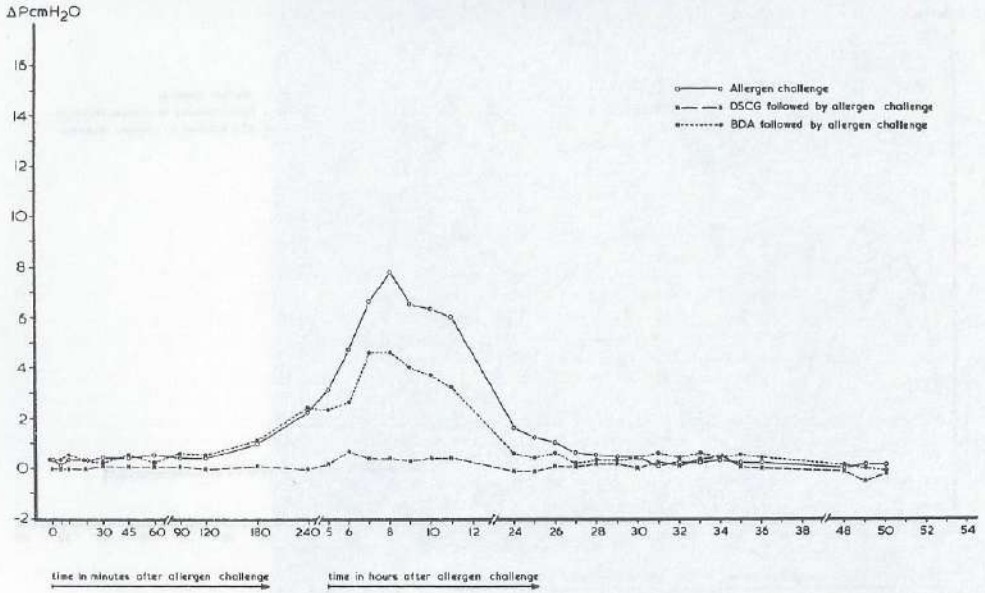

Figure 2. Groups O + III (positive isolated late responses). Provocation tests and protection tests with DSCG and BDA. The mean NPG values recorded from all patients demonstrating "isolated late nasal mucosa response" (due to Type III hypersensitivity) during these tests, with respect to the appropriate Coca's Solution mean NPG values. DSCG = disodium cromoglycate; BDA = beclomethasone dipropionate.

Generally, in systemic late hypersensitivity (Type III allergy, Arthus reaction, immuno-complex state), circulating antibodies of the IgG and IgM classes, being presumed to play a major role, react with circulating antigens in the blood stream or in the vascular wall and, in this way, form immuno-complexes.[24-27] Immuno-complexes then activate the complement cascade,[27] especially C3a, C5a, C5b, C6, C7, with subsequent activation of the blood clotting mechanism, liberation of kinin[28] and release of lysosomal enzymes, vascular permeability factors and other factors[27,28] from the polymorphonuclear neutrophil leucocytes,[27-32] and the release of vasoactive amines, lysosomal enzymes and other proteins from the platelets.[27,28,32-36] These factors as well as platelets and neutrophils may then be involved directly or indirectly in the tissue damage which is a complex of various inflammatory reactions typical of the Type III hypersensitivity.[24,27-35,34-36]

The changes in the concentration of the IgG and IgM antibodies, changes of the above-mentioned chemical mediators and of the complement system parts, which occur in systemic type late hypersensitivity, have not yet been sufficiently investigated in patients with the late nasal mucosa response[14] and requires further study.

On the other hand, the possibility also exists that IgE antibodies may not only play a role in immediate hypersensitivity (Type I allergy) but also in late response which may be triggered by late hypersensitivity (Type III allergy).[25,27,29,37-38] Apart from this, the possibility can not be excluded that antibodies of the IgG class which are suspected to be responsible for the late hypersensitivity could also be involved in the immediate hypersensitivity (Type I allergy).[5,14,20,25,26,38-41]

The results of this study, demonstrating the positive protective effects of both DSCG and BDA on the late nasal mucosa response to allergen challenge, could probably be best explained by the following hypothesis.

In the case of late hypersensitivity, neutrophil leucocytes may play a pivotal role. Neutrophils contain and can release a number of factors, such as kinin and kinin system factors,[42] lysosomal enzymes, vascular permeability factors, complement activating factor generating C5a and some other factors.[24,27-33] The neutrophils could probably also produce some of the mediators like those released by the mast cells (basophils) during immediate hypersensitivity, e.g., SRS-A and neutrophilic ECF-A.[27,30,42]

The platelets contain and can also release a number of factors, including vasoactive amines, lysosomal enzymes and probably vascular permeability factors.[24,27-29,33-36]

Table V. Survey of the Skin Responses (i.c. Tests) with Respect to the Different Types of Nasal Mucosa Response.

Nasal mucosa response	Skin responses (i.c. tests)				
	Immediate			Late	
	skin + IR −	skin + IR +	skin − IR +	skin + LR +	skin − LR +
Dual (= immediate + late response) N = 39	−	30	9	27	12
Isolated late N = 13	8	−	−	7	6
N total = 52		30 (77%)	9 (23%)	34 (65%)	18 (35%)

IR = Immediate nasal mucosa response; LR = late nasal mucosa response.

The lysosomal enzymes are involved in the further stages of the immuno-complex-mediated tissue injury.[27,28,33,36] The platelets as well as the neutrophils, in addition to their ability to release several factors, are also directly involved in the immuno-complex-mediated tissue injury[27-30,34-36] and the neutrophils by their influx into the site of the immuno-complexes and platelets by their interaction with damaged epithelium and adherence to immuno-complexes. The neutrophils are activated by immuno-complexes through the complement system.[24,27,28]

The activation and aggregation of platelets is stimulated by a variety of factors,[28] among others by PAF's (platelet-activating factors) generated and/or released directly from the activated mast cells and basophils[27,29,31,34-37,43-45] by the decreasing of exogenous cAMP and/or the increasing of cGMP[37] with subsequent decreasing of intracellular cAMP in the platelets, and probably also by other pathways. The PAF's could be generated and/or released, besides from activated mast cells and basophils, also from other cells, e.g., polymorphonuclear leucocytes[44-46] or monocytes.[44,45]

The neutrophils could be activated not only by immune-complexes[27-32] but also by complement parts,[27] by neutrophil chemotactic factor generated by mast cells and basophils,[28] by prostaglandins (PGE_1 enhances, while $PGF_{\alpha 2}$ inhibits the neutrophil chemotaxis)[44] and by increasing cGMP and decreasing cAMP. The neutrophils could probably also be directly activated by antigen. Zweiman et al[47] found a significant increase of neutrophil chemotactic activity in the serum, after bronchial challenge with allergen, in patients with a bronchus-obstructive response.

According to some suggestions, the IgE, basophils and mast cells might also play an important role in late hypersensitivity. Human IgE may play a role in triggering off increased vascular permeability and in the deposition of immuno-complexes.[27-29,36] Basophils and mast cells with membrane-bound IgE can generate and/or release a soluble PAF[27,29,34-36,44] and also a factor chemotactic for neutrophils.[28]

Another possible role of mast cells and basophils in the late hypersensitivity, especially in that being a part of the dual response, could be related to some authors' suggestions of the biphasic degranulation of mast cells and basophils.[44-46,48] The biphasic response of airways to inhaled antigen may be comparable to the biphasic cutaneous response to the IgE-dependent activation of mast cells.[49] This could be the process by which mast cells' mediators might provoke a biphasic inflammatory response.[50]

The protective effects of DSCG on the immediate as well as the late nasal mucosa response to allergen challenge could be explained by the ability of DSCG to prevent degranulation of mast cells and basophils with subsequent release of the mediators.[13,14,25,26,38,44,50]

However, the protective effects of DSCG on the DLNR were more pronounced than on the ILNR. These differences could probably depend on the participation of various of the above mentioned basic mechanisms and the role of the mast cells and basophils in the development of both these modifications of the INR.

The different ways in which these mechanisms participate could then probably result in the two different modifications of the late nasal mucosa response.

In the case of the DLNR the mast cells and/or basophils could be involved as the γ predominant mechanism. However, these could be in different ways—either directly, with respect to the suspected biphasic degranulation,[44-46,48] or indirectly, with respect to the generating of PAF, to the triggering off of increased vascular permeability or to the generating of the factor chemotactic for neutrophils[27,29,34-36,45]—while the other mechanisms play a less important role. Therefore, DSCG protecting the mast cells and basophils demonstrated good protective effects on this kind of late nasal response.

In the case of the ILNR, probably the other mechanisms (activation of neutrophils,[27-32] activation of complement,[27] activation of platelet by PAF from sources different from mast cells and basophils,[44-46] decreasing of exogeneous cAMP and/or increasing of cGMP,[37] etc) play a predominant role, while the mast cells and/or basophils are involved to a lesser degree. Therefore, DSCG, protecting mainly the mast cells and basophils but not influencing distinctly the other mechanisms, demonstrated significant protective effects on the ILNR but to a lesser degree than on the DLNR. However, there is some evidence that DSCG could block the neutrophils' chemotactic activity in the serum, increased after allergen inhalation in patients with positive bronchial responses.[47]

The corticosteroids, including BDA, increase and potentiate the action of cAMP, decrease and inhibit the

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THE EFFECTS OF DISODIUM CROMOGLYCATE (DSCG) AND BECLOMETHASONE DIPROPIONATE (BDA) ON THE DELAYED NASAL MUCOSA RESPONSE TO ALLERGEN CHALLENGE

Z. PELIKAN, M.D., F.A.C.A.

The protective effects of disodium cromoglycate (DSCG) and beclomethasone dipropionate (BDA) on the "delayed nasal response" to allergen challenge (DNR) were investigated in 37 patients with allergic rhinitis. These thirty-seven patients, from a group of 268, developed 43 "delayed nasal responses" (DNR), 16 cases of "isolated delayed responses" (IDNR) and 27 cases of "dual delayed responses" (DDNR), where the delayed response (DLDNR) was preceded by an immediate response (INR). BDA demonstrated significant protective effects on the DNR in both its modifications; however, to a higher degree in the case of the IDNR. DSCG significantly decreased only the INR, being a part of the DDNR, while in the case of the DNR in both its modifications, DSCG was completely ineffective. It is suggested that BDA should be the drug of first choice in allergic rhinitis patients demonstrating the DNR. When immediate responses to the same or other allergens are also present, DSCG should be added at the beginning of the treatment for a temporary period of a few months.

Introduction

ALLERGIC RHINITIS classically has been attributed to immediate hypersensitivity (Type I allergy). Recently some investigators have observed other types of nasal reactions — different from the immediate response — the so-called "non-immediate responses,"[1,2,3] such as the "late nasal mucosa response" and the "delayed nasal mucosa response." The clinical features have been described in detail in our previous papers.[2,3]

Other investigators have found evidence for possible involvement of delayed hypersensitivity (Type IV allergy) in patients with allergic rhinitis. They have drawn their conclusions from immunological tests *in vitro*[4–13] or from allergy skin tests.[4,5,10,13] Clinical experience in addition to immunological tests indicates that delayed hypersensitivity in the "delayed nasal response" to allergen challenge cannot be excluded. In fact, the "delayed nasal mucosa response" may be responsible for many of the nasal symptoms of some allergic rhinitis patients, and may be the reason why the usual treatment fails. This type of response is often overlooked in practice.

This study is a continuation of our previous studies dealing with the effects of disodium cromoglycate (DSCG) and beclomethasone dipropionate (BDA) on the immediate and late nasal mucosa responses.[3,14–16] The purpose of this study was to investigate the presence of protective effects of DSCG and BDA on the "delayed nasal response" to allergen challenge, to compare them and to determine their therapeutic possibilities. Thus we hoped to define the indications of both these drugs for the treatment of allergic rhinitis where the "delayed nasal response" is present. In addition, using both the

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drugs with their known pharmacological qualities and effects, we hoped to further clarify the pathophysiological mechanism of the "delayed nasal mucosa response."

Materials and Methods

*Allergens.** The dialyzed and lyophilyzed water extracts were diluted in Coca's Solution (dry weight of allergen in mg per ml of Coca's Solution) and were used in the following concentrations.

For nasal challenges. (a) House dust — 5 mg/ml. (b) Hairs and feather mixture,** dog danders, cat danders: each 2.5 mg/ml. (c) Moulds mixture,*** Aspergillus fumigatus: each 2.0 mg/ml. (d) Grass pollen mixture,† spring pollen mixture,‡ weed pollen mixture:†† each 10,000 Noon Units (NU)/ml.⊗

For intracutaneous tests. Allergens identical to those used for the nasal challenges, except that they were diluted in a 1:10 ratio.

Evaluation of the skin tests. The skin response was evaluated at 20 minutes, eight, 24, 36, 48 and 72 hours after intracutaneous injection of 0.05 ml of an allergen. If necessary, the skin response was evaluated at 24 hour intervals until the reaction totally disappeared.

The skin response was interpreted as follows. Immediate skin response was a positive reaction within 20 minutes and delayed skin response was a positive reaction of more than 24 hours after the test (usually between 36 and 72 hours).

The evaluation parameters are presented in the legends of Table I.

Drugs. (a) Disodium cromoglycate (DSCG)* in powder form placed in gelatine capsules were applied topically to the nasal mucosa by means of a special nasal applicator as follows: One capsule, containing 10 mg of DSCG powder, was placed into the applicator and then perforated and blown into the nose by an air-flow. A single dose consisted of one capsule, the contents of which (10 mg DSCG) were divided equally into both nostrils. (b) Beclomethasone dipropionate aerosol (BDA)** was administered topically to the nasal mucosa by means of a special nasal adaptor. Two puffs were always given in each nostril. (One puff = 50 μg; one dose = two puffs in each nostril = 2 × 2 puffs = 4 × 50 μg = 200 μg). A single dose always consisted of four puffs.

Apparatus and Equipment. This consisted of a one-channel recorder, an electrical differential transducer, a water manometer, a small rubber balloon of 5 mm diameter, and 25 mm length and a polyethylene tubes connecting system.

Principle of the Method. The nasal mucosa of the patient with allergic rhinitis, when challenged topically by an allergen to which he is sensitive, reacts with swelling, hypersecretion, sneezing and itching. This interferes with the passage of air through the nose, resulting in a pressure differences between the nasopharyngeal cavity and the outside air while the air-flow is constant. These pressure differences, the so-called NPG (nasopharynx-nostril-pressure gradients), expressed in cm H_2O, were recorded and considered as a parameter for the assessment of the nasal mucosa reactivity. The mean values of the NPG were calculated during regular breathing over 90 to 120 seconds. This "balloon technique," being a combination of rhinomamometry anterior and posterior, has been described in detail in our previous papers.[2,3,14–21]

Patients. Of the 268 allergic rhinitis patients, examined by a routine diagnostic procedure including short term nasal challenges, 39 complained of acute nasal symptoms within 26–50 hours after allergen challenge. These nasal symptoms were then confirmed by long term nasal provocation tests in 37 of the patients and interpreted as a "delayed nasal response" to allergen challenge. These 37 patients were selected for the present study. They all gave a history of (1) nasal complaints, (2) a positive history of one or more allergens, (3) positive immediate skin responses to one or more "inhalant" allergens and (4) a delayed nasal response to one or more allergens.

These patients were investigated during a symptom-free period. None had used DSCG, corticosteroids or had received immunotherapy in the past. No antihistaminics or topical decongestants were prescribed during the three day interval prior to the study. The clinical characteristics of the patients studied are presented in Table I.

Procedure. In each of the patients, three experimental procedures (a provocation test, protection test with DSCG and protection test with BDA), and one control test with Coca's Solution were performed. There was an interval of three to five days between the procedures.

Provocation Test with Allergen

The "initial test" (base line). The NPG values, the so-called "initial values," were recorded at 0,5 and 10 minutes.

The "Coca's Solution Test." The NPG values, the so-called "Coca's Solution values," were recorded at 0.5 and 10 minutes after a three minute application of Coca's Solution to the nasal mucosa under the medial turbinate of the non-intubated nasal cavity. This was done by means of a saturated wad of cotton wool on a nasal probe. If no changes of the mean "Coca's Solution NPG" value with respect to the "initial" NPG values occurred, the test was continued.

The allergen challenge. The nasal mucosa was challenged by the allergen for three minutes in the same way and on the same site as by the Coca's Solution. The NPG values were recorded at 0, 5, 10, 20, 30, 45 and 60 minutes, and then every hour up to the 11th hour; after this every second hour during the 24th–36th and 47–56th hour intervals.

The provocation tests (nasal mucosa response) were considered to be positive when the NPG values after allergen challenge increased by at least 2.0 cmH_2O with respect to the "initial" and 'Coca's Solution" values. (The value of 2SD is 1.6 cmH_2O for this technique).

Protection Test with Disodium Cromoglycate (DSCG)

Protection Test with Beclomethasone Dipropionate (BDA)

Both these test were performed in the same manner and according to the same schedule. The patients were pretreated with the appropriate drug in a daily dose of 4 × 1, during a four day interval, starting two days before allergen challenge, and continuing through the "challenge day" up to two days after the "challenge day." The drug was always administered at 7a.m.,

* All allergen extracts were prepared by Pharmacy "Diephuis," Groningen, The Netherlands.

** Cat, dog, cattle, goat, hog, horse, rabbit, rat, mouse, hamster, guinea pig, canary, goose, duck, turkey, hen, pigeon, parrot, in equal proportions by weight.

*** Cladosporium cladosporioides-elatum-herbarum, Penicillium brevi-compactum-expansum-notatum-frequentans-commune, Aspergillus versicolor-niger-fumigatus, Mucor spinosus-mucedo-racemosus, Pullularia pullulans, Botryris cinerea, Merculius domesticus, Epicoccum purpurascens, Alternaris tenuis, Stemphylium botryosum, Rhizopus nigricans, Fusarium culmorum and Trichoderma viride, in equal proportions by weight.

† Dry weight percentage: Secale cereale 15%, Dactylis glomerata 15%, Lolium perenne 10%, Anthoxanthum odoratum 10%, Agrostis alba 10%, Holcus lanatus 10%, Phleum pratense 10%, Cynosurus cristatus 5%, Alopecurus pratensis 15%.

‡ Dry weight percentage: Corylus avellana 20%, Alnus species 30%, Salis species 20%, Betula species 20%, Myrica species 10%.

†† Dry weight percentage: Artemisia vulgaris 33%, Rumex acetosa 33%, Plantago lanceolata 33%.

⊗ 1 Noon Unit (NU) = 0.001 mg of dry pollen (powder) = 0.5 PNU = 1.3 TNU.

* Henceforth abbreviated as DSCG.

** Henceforth abbreviated as BDA.

Table I. Clinical Characteristics of the Patients Investigated.

Patient	Nasal complaints: Obstruction	Nasal complaints: Hypersecretion	Nasal complaints: Sneezing	Lividity of nasal mucosa	Blood eosinophilic cells per mm3	Skin tests (i.c.)—immediate response: Allergens: Basic: HD	Basic: HF	Basic: GP	Basic: SP	Complementary	Allergen investigated and type of nasal response observed: I + IV	O + IV
1	+	+	+	−	451	+	+	+±	±	+ D	GP	
2	+++	+++	+++	−	440	+	+	−	−	+ C	HD	
3	++	++	++	−	551	++	++	+++	++	++ Mm	GP	
4	−	−	+++	−	649	++	−	+++	++	+± TP	SP	
5	+	+	+	+++	770	+	±	+	+	+ Mm	GP	
6	++	++	++	+++	241	+	±	−	−	− C	HD	
7	+++	+++	+++	+++	781	+	+±	+±	−	+± FP	GP	
8	++	++	−	++	583	++	+±	+±	++	+++± WP	WP	
9	+++	+++	+++	+++	737	+±	+±	+	−	+++ D	GP	
10	+++	+++	+++	+	682	+	−	−	−	+ Mm	HD	
11	+++	+	+++	−	682	+	+	++	++	+± D	GP	
12	+++	−	−	+	495	+±	+	−	−	+ Mm	HD	
13	+	+	+	+	363	+±	+	+++	+	+ TP	GP	
14	+	+	+	+	1540	+±	+++	+++	+	++ C	GP	
15	++	++	++	−	297	+	−	−	−	− Mm	GP	
16	+	+	+	−	649	+±	+±	++	+	+ FP	GP	
17	++	++	++	+++	671	+±	+±	+	+	+± C	C	
18	+	++	+	−	495	+++	++	+++	−	+++ C	C	
19	++	+	+	+	1485	++	++	+	+	± Mm	Mm	
20	+++	+++	+	+	1155	+	−	−	−	++ AF	AF	
21	+++	+++	+++	+	1144	±	−	±	−	+ Mm	HD	
22	++	++	++	+++	1100	+±	++	+	±	+ PF	GP	
23	++	++	++	−	616	++	++	+±	++	++ C	HD, GP	
24	+++	+++	+++	++	308	+	−	−	−	± WP	HD	GP, SP
25	+	+	+	+++	1462	±	+	−	−	+ C	HD	HF
26	−	+	−	++	715	+	±	−	−	− Mm	GP	Mm
27	+++	+++	+++	−	440	−	−	−	−	− WP		GP
28	+	+	+	+	451	+	−	+±	−	+ Mm		GP
29	+++	+++	+++	−	1958	±	−	−	−	− WP		GP
30	+++	++	+	−	440	++	+	+	−	− D		HD, SP
31	+++	+++	+++	+++	363	+±	+	±	±	± C		HD
32	+++	+++	+++	+++	341	−	−	−	−	− D		HD
33	+++	+++	+++	+++	275	+	±	−	−	+± HeF		HF
34	+	−	+	−	776	+±	−	+	+	+FP		GP
35	++	++	−	+	682	+	+	+++	++	± D		D
36	+	−	−	+++	264	++	+±	+	−	+± D		HF
37	+++	+++	+	+++	550	+	−	−	−	− Mm		Mm

Legends: HD = house dust; HF = hairs and feathers mixture; GP = grass pollen mixture; SP = spring pollen mixture; C = cat danders; D = dog danders; PF = pigeon feathers; HeF = hen feathers; TP = tree pollen mixture; WP = weed pollen mixture; FP = flower pollen mixture; Mm = moulds mixture; AF = Aspergillus fumigatus.
Nasal mucosa responses: O = immediate response negative; I = immediate response positive; IV = delayed response positive.
The evaluation of the intracutaneous tests:
−= normal skin appearance
±= wheal not greater than original injected papule.
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5	+	+	+	+++	770	+	±	+	+	+ Mm	GP	
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12	+++	–	–	+	495	+±	+	–	–	+ Mm	HD	
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15	++	++	++	–	297	+	–	–	–	– Mm	GP	
16	+	+	+	–	649	+±	+±	++	+	+ FP	GP	
17	++	++	++	+++	671	+±	+±	+	+	+± C	C	
18	+	++	+	–	495	+++	++	+++	–	+++ C	C	
19	++	+	+	+	1485	++	++	+	+	± Mm	Mm	
20	+++	+++	+	+	1155	+	–	–	–	++ AF	AF	
21	+++	+++	+++	+	1144	±	–	±	–	+ Mm	HD	
22	++	++	++	+++	1100	+±	++	+	±	+ PF	GP	
23	++	++	++	–	616	++	++	+±	++	++ C	HD, GP	
24	+++	+++	+++	++	308	+	–	–	–	± WP	HD	GP, SP
25	+	+	+	+++	1462	±	+	–	–	+ C	HD	HF
26	–	+	–	++	715	+	±	–	–	– Mm	GP	Mm
27	+++	+++	+++	–	440	–	–	–	–	– WP		GP
28	+	+	+	+	451	+	–	+±	–	+ Mm		GP
29	+++	+++	+++	–	1958	±	–	–	–	– WP		GP
30	+++	++	+	–	440	++	+	+	–	– D		HD, SP
31	+++	+++	+++	+++	363	+±	+	±	±	± C		HD
32	+++	+++	+++	+++	341	–	–	–	–	– D		HD
33	+++	+++	+++	+++	275	+	±	–	–	+± HeF		HF
34	+	–	+	–	776	+±	–	+	+	+FP		GP
35	++	++	–	+	682	+	+	+++	++	± D		D
36	+	–	–	+++	264	++	+±	+	–	+± D		HF
37	+++	+++	+	+++	550	+	–	–	–	– Mm		Mm

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++± = wheal increase up to 15.0 mm in diameter.
+++ = wheal increase greater than 15 mm, with surrounding erythema and sometimes with "pseudopodia".

12a.m., 6p.m. and 11p.m. On the "challenge day" an extra drug dose was administered after the "Coca's Solution" values and 10 minutes prior to the allergen challenge.

The schedule of the protection tests was similar to that of the provocation tests, the only exception being the administration of the drugs. The protection tests were performed as "single-blind" with respect to the patient.

The protective effects of the drug were considered to be significant when the NPG values recorded after the pretreated allergen challenge decreased by at least 50% with respect to the NPG values recorded after the non-pretreated allergen challenge.

Control Test with Coca's Solution

After recording of the "initial values," Coca's Solution was applied for three minutes to the nasal mucosa in the same way as described above and the NPG values were recorded up to 56 hours.

*Statistical Analysis**

The results were analyzed and statistically evaluated by means of polynomials which were fitted, and the hypotheses were tested by Potthoff and Roy's generalized multivariate analysis of variance model (generalized Manova Model)[22] presented by Timm.[23]

Results

Provocation Tests

The 37 patients developed a total of 43 nasal mucosa responses within 26–52 hours after allergen challenge. This response began within 24–30 hours, reached its maximum within 30–40 hours and disappeared in most cases within 56 hours after allergen challenge. This type was considered to be the "delayed nasal mucosa response" to allergen challenge.

Two modifications of the "delayed mucosa response"* were observed. In 16 patients there was as an "isolated delayed response"** only. In 27 patients a "dual delayed response"*** occurred in which there was first an immediate response† and then, after a symptom-free interval of more than 24 hours, the delayed response†† appeared.

A review of the NPG values recorded during the nasal provocation tests in individual cases is presented in abbreviated form in Table II.

Control Test with Coca's Solution

The NPG values recorded after Coca's Solution application did not differ among them significantly during the whole 56 hour period; the changes varied within 1.2 cm H_2O.

Protection Test with BDA

The NPG values recorded during the protection tests with BDA are given in abbreviated form in Table IV. Comparing the results presented in Table IV with those in Table II, the following conclusions could be drawn.

BDA, when applied topically in a sufficient dose before and after allergen challenge, significantly decreased the "delayed nasal response" to allergen challenge in both its modifications. However, the "isolated delayed response" was decreased by BDA to a higher degree than the "delayed nasal response," being a part of the "dual delayed response."

The "immediate response" being a part of the "dual delayed response" was not decreased significantly by BDA.

Protection Tests with DSCG

The NPG values recorded during the protection tests with DSCG in individual cases are shown in abbreviated form in Table III. Comparing the results given in Table III with those in Table II, the following can be concluded.

DSCG when applied topically in a sufficient dose before and after allergen challenge was not able to prevent or significantly decrease the development of the "delayed nasal response" to allergen challenge in any of the patients studied.

The "delayed nasal response" being a part of the "dual delayed response" was decreased to a very slight degree. This, however, was not significant. In the case of the "isolated delayed response" DSCG was completely ineffective. The "immediate nasal response" recorded during the "dual delayed response" was decreased significantly by DSCG.

The NPG values recorded during all three tests, with respect to the "Coca's Solution" NPG values in the case of the IDNR are summarized in Figure 1 and those in the case of the DDNR in Figure 2.

Statistical Analysis

A. Isolated delayed nasal response

Hypothesis Number 1: All three curves (ALL, DSCG, BDA) coincide. This hypothesis is rejected ($p < 0.01$). Hypothesis Number 2: the ALL and DSCG curves coincide. This hypothesis is not rejected ($p = 0.40$). Hypothesis Number 3: the BDA curve runs horizontally. This hypothesis is not rejected ($p > 0.05$).

B. Dual delayed nasal response

(a) *Immediate response.* Hypothesis Number 1: all three curves (ALL, DSCG, BDA) coincide. This hypothesis is rejected ($p < 0.05$). Hypothesis Number 2: the ALL and BDA curves coincide. This hypothesis is not rejected ($p > 0.05$). Hypothesis Number 3: the DSCG curve runs horizontally. This hypothesis is not rejected ($p > 0.05$).

(*continued on page 118*)

* The calculations were performed by Drs. M.P.F. Berger (Dept. Psychology, University of Tilburg, Tilburg, the Netherlands), using PDP 11/45 computer.
* Delayed Nasal Mucosa Response = henceforth abbreviated as DNR.
** Isolated Delayed Nasal Mucosa Response = henceforth abbreviated as IDNR.
*** Dual Delayed Nasal Mucosa Response = henceforth abbreviated as DDNR.
† Immediate Nasal Mucosa Respone = henceforth abbreviated as INR.
†† Delayed Nasal Mucosa Response being a part of the dual response = henceforth abbreviated as DLDNR.

Table II. Abbreviated Survey of the Mean Nasopharynx-Nostril-Pressure Gradient (NPG) Values in cm H_2O Recorded During the Nasal Provocation Tests with Allergen in Individual Patients.

Patient	Coca value	Allergen	Time in minutes after challenge 0′	10′	20′	30′	45′	60′	10h	24h	26h	Time in hours after challenge 28h	30h	32h	34h	36h	48	54h
1	2.2	GP	3.0	8.8	11.6	13.6	9.6	5.6	2.9	2.9	4.0	7.2	8.7	14.1	4.4	5.0	2.5	2.4
2	3.2	HD	3.5	7.5	10.6	11.0	10.1	6.2	2.9	2.8	3.2	11.3	15.4	12.8	8.4	4.7	2.8	3.7
3	3.0	GP	7.2	8.2	24.0	24.7	21.0	7.3	4.2	4.0	9.4	10.1	26.6	20.9	14.7	—	9.9	2.1
4	2.1	SP	2.3	2.7	6.4	9.0	9.9	10.2	2.9	1.7	1.5	4.2	9.7	15.8	11.2	10.7	8.4	—
5	4.7	GP	13.7	24.8	26.0	21.7	20.1	20.0	5.0	5.1	18.0	20.0	15.4	11.2	10.1	7.9	4.6	—
6	6.4	HD	6.9	8.3	8.8	10.9	10.9	11.8	5.7	3.1	5.7	5.1	7.6	9.5	8.1	—	4.3	—
7	4.8	GP	30.6	30.7	30.7	30.7	30.7	30.7	4.8	12.1	28.1	19.9	15.7	17.3	18.1	—	3.7	—
8	3.3	WP	7.7	10.2	13.7	23.3	19.0	15.9	3.5	6.2	11.3	12.7	20.9	20.0	25.1	24.9	15.5	10.5
9	2.6	GP	5.5	11.9	10.3	9.4	3.4	3.4	3.9	9.2	7.5	9.8	10.2	7.5	6.6	—	2.9	—
10	3.2	HD	3.5	5.7	5.5	7.7	6.6	7.6	4.1	3.4	3.2	7.0	8.0	6.6	5.6	5.1	3.4	—
11	2.7	GP	10.9	24.8	26.1	18.2	8.1	9.8	2.5	3.5	5.0	4.9	4.6	3.5	4.0	3.9	2.2	—
12	1.5	HD	3.6	4.7	5.7	5.1	9.4	8.3	2.4	2.2	2.5	4.1	6.5	5.1	6.1	6.3	7.0	—
13	3.6	GP	6.4	12.1	9.9	8.2	8.0	7.6	4.6	8.4	12.9	12.5	13.2	10.8	10.8	—	4.7	—
14	2.2	GP	2.5	5.5	5.3	5.6	4.8	4.5	2.5	3.8	7.7	9.7	16.8	16.3	7.2	7.1	3.0	3.3
15	1.4	GP	1.3	1.7	15.2	15.8	6.8	5.2	1.6	2.4	4.7	4.5	4.9	8.2	12.1	6.2	2.7	1.6
16	4.5	GP	4.2	6.2	6.8	15.8	8.5	11.5	4.6	5.5	7.5	27.3	24.7	22.4	27.6	27.8	27.8	12.8
17	2.1	Cat	5.2	10.3	14.2	16.9	11.2	8.7	1.9	8.3	16.0	17.1	10.9	11.0	8.1	6.7	2.0	—
18	1.3	Cat	7.7	21.4	21.7	23.8	13.8	7.4	—	2.2	1.7	1.8	10.8	8.5	8.2	7.5	7.0	—
19	2.0	Mm,	3.6	6.5	8.1	7.8	7.1	7.3	3.5	2.9	7.3	6.7	8.1	9.9	6.0	6.3	2.9	—
20	2.5	AF	2.5	4.8	12.4	19.5	15.4	12.9	2.7	2.4	2.5	2.5	2.7	15.4	17.0	14.3	3.2	—
21	3.6	HD	4.9	6.0	7.8	11.7	15.8	13.9	4.0	4.0	9.8	13.3	8.7	8.2	10.9	8.6	2.3	—
22	1.1	GP	2.4	3.2	4.3	5.2	6.2	2.6	2.4	2.2	6.3	6.0	10.5	16.3	5.0	6.2	2.2	—
23	1.4	GP	3.0	4.6	7.1	8.1	9.4	7.0	3.1	2.9	2.7	2.5	5.1	6.4	10.1	8.8	1.6	—
	3.2	HD	6.2	24.4	23.4	26.7	26.7	26.8	2.6	7.1	6.4	10.1	7.4	8.5	5.5	4.3	1.9	—
24	2.4	HD	4.1	6.8	11.0	9.4	4.6	3.8	2.6	2.7	3.4	7.6	10.8	9.7	11.1	3.4	2.7	2.6
25	4.0	HD	4.8	6.3	7.3	11.1	8.8	7.3	3.9	2.9	4.9	9.2	11.9	8.0	3.8	2.6	—	—
26	3.7	GP	5.2	5.3	12.3	10.6	12.0	10.1	4.3	6.6	9.5	11.4	4.6	12.1	11.6	8.0	7.0	—
27	0.9	GP	0.4	0.7	0.5	0.4	0.5	0.5	0.9	1.2	2.1	2.8	3.4	11.6	11.7	6.7	7.1	1.0
28	1.3	SP	3.0	3.5	3.5	3.9	3.1	3.0	2.5	3.2	3.6	3.4	13.1	7.9	9.4	21.6	3.4	—
29	2.2	GP	2.0	2.3	2.2	2.5	1.9	1.9	2.0	1.7	2.5	4.5	5.9	3.7	5.8	—	4.5	—
25	1.5	SP	1.8	1.8	1.7	1.7	1.9	2.4	2.9	1.9	3.2	2.0	6.7	5.5	5.9	8.6	7.8	2.8
26	1.2	Mm	1.4	1.1	2.2	2.1	2.4	1.1	1.5	3.7	8.2	10.4	15.6	14.0	10.3	15.5	11.4	3.4
27	0.9	GP	0.8	0.9	0.8	0.8	0.9	1.0	0.9	2.5	3.6	6.3	9.2	6.4	5.5	4.4	8.0	—
28	1.3	GP	1.2	1.0	1.4	1.5	2.9	2.1	1.6	1.6	4.3	7.0	11.6	8.9	14.5	—	5.6	—
29	2.2	GP	2.0	2.3	2.2	2.5	1.9	1.9	2.0	1.7	2.5	4.5	5.9	3.7	5.8	—	4.5	—
30	2.7	HD	2.4	2.4	2.9	3.1	2.6	2.7	2.4	2.8	2.9	10.5	10.5	6.7	4.4	—	2.4	—
31	2.1	SP	2.3	2.4	2.7	2.7	3.0	2.7	2.5	2.0	4.6	10.3	10.8	9.7	16.5	10.5	12.2	—
32	2.5	HD	3.2	3.5	2.4	3.0	2.6	2.7	2.4	3.2	7.1	6.4	6.7	10.4	9.8	10.3	5.6	—
33	1.2	HD	1.2	1.1	1.1	1.3	1.3	1.5	1.7	1.6	3.3	4.9	5.3	5.2	7.1	4.9	1.3	—
34	2.7	HF	3.2	2.6	2.9	2.6	2.0	2.0	2.7	2.4	6.5	6.1	5.7	3.4	1.9	2.1	2.5	—
35	4.1	GP	4.3	3.8	4.4	4.6	3.9	2.8	4.4	4.7	4.8	8.6	8.4	9.9	9.6	12.1	6.9	—
36	1.7	Dog	1.6	1.3	1.3	1.2	1.4	1.4	1.3	1.9	7.9	5.6	8.3	13.1	11.1	8.4	8.0	2.4
37	3.4	HF	2.6	3.2	2.8	2.8	2.8	3.4	3.1	13.2	12.6	15.8	13.8	15.2	16.4	17.7	20.0	—
	1.7	Mm	1.6	1.3	1.5	2.0	2.0	2.0	1.4	2.5	3.0	4.5	6.2	7.0	7.3	—	2.4	—

Legends: GP = grass pollen mixture; HD = house dust; SP = spring pollen 18 mixture; WP = weed pollen mixture; Cat = cat danders; Mn = moulds mixture; AF = Aspergillus fumigatus; HF = hairs and feathers mixture; Dog = dog danders; — = not recorded.
The "Coca value" is the mean NPG value, calculated always from three values, as recorded at 0, 5 and 10 minutes after the application of Coca's Solution.

Table III. Abbreviated Survey of the Mean Nasopharynx-Nostril-Pressure Gradient (NPG) in cm H_2O Recorded During the Nasal Protection Tests with Disodium Cromoglycate (DSCG) in Individual Patients.

Patient	Coca value	DSCG value	Allergen	Time in minutes after challenge						Time in hours after challenge									
				0′	10′	20′	30′	45′	60′	10h	24h	26h	28h	30h	32h	34h	36h	48h	50h
1	2.7	3.0	GP	3.8	4.1	2.8	2.8	2.7	3.7	2.6	2.8	2.2	3.0	2.6	2.4	3.4	3.4	2.2	2.2
2	3.5	3.5	HD	3.8	4.8	5.3	5.8	4.6	5.1	3.2	3.4	3.7	3.0	2.6	10.9	8.2	10.0	3.1	—
3	4.4	4.8	GP	3.5	3.9	4.4	6.2	6.1	3.8	3.8	3.1	3.4	3.0	15.1	15.0	9.2	3.1	3.5	3.2
4	1.9	1.9	SP	1.6	1.8	1.6	1.5	2.1	2.1	1.9	1.5	2.0	4.5	3.5	5.8	4.6	3.4	3.8	3.3
5	2.6	2.6	GP	3.4	4.1	3.4	3.5	3.8	3.9	2.7	6.8	4.1	5.6	8.0	6.4	4.6	—	5.0	4.3
6	6.5	6.0	HD	5.8	5.1	5.8	4.0	5.1	4.8	6.8	4.5	5.0	4.3	6.7	3.3	4.4	—	5.1	—
7	1.2	1.0	GP	4.3	3.3	2.9	3.8	1.1	1.1	1.4	1.8	1.5	8.2	5.0	6.0	8.1	—	1.9	—
8	2.3	2.2	WP	2.2	2.0	2.0	1.9	2.0	2.2	2.8	2.9	6.6	17.6	26.8	16.5	21.9	23.8	11.1	10.4
9	3.1	2.6	GP	4.9	5.1	4.9	3.1	3.9	3.0	3.0	2.2	3.1	2.4	2.7	3.4	2.6	2.7	1.9	2.8
10	4.3	3.5	HD	4.7	4.9	4.7	5.0	4.3	4.8	3.6	4.9	6.6	4.3	5.4	6.6	3.3	5.5	4.8	—
11	2.5	2.1	GP	2.4	3.8	3.2	2.8	2.3	2.9	2.2	3.0	2.5	6.4	5.2	3.9	4.2	2.0	2.2	2.5
12	1.9	2.8	HD	2.7	2.4	2.7	2.4	2.3	2.3	1.8	1.7	5.9	3.2	6.2	4.6	2.8	2.2	1.8	1.8
13	3.9	3.5	GP	4.0	4.2	4.0	4.9	5.1	4.4	4.2	9.3	17.9	13.8	18.8	19.1	20.6	—	8.4	5.6
14	2.2	—	GP	2.3	2.2	4.0	3.9	2.1	2.1	2.4	1.9	3.0	4.2	7.3	2.3	3.8	3.8	1.8	2.4
15	2.7	2.5	GP	1.9	3.1	3.7	4.0	3.6	2.3	3.7	3.5	3.3	2.5	2.6	2.4	2.4	2.3	3.4	3.6
16	4.7	—	GP	5.3	6.9	4.4	4.5	5.3	4.4	5.1	6.5	10.1	9.2	7.6	14.6	8.6	—	5.7	—
17	4.1	4.3	Cat	3.6	4.5	4.5	4.3	3.7	3.9	3.1	3.3	3.7	3.0	3.0	2.4	2.5	2.7	3.6	2.7
18	1.6	1.6	Cat	1.5	1.5	1.4	1.6	1.8	1.9	1.5	1.3	1.6	5.3	3.8	3.4	3.4	2.9	1.7	2.3
19	1.2	1.3	Mm	1.3	1.7	1.7	1.5	1.7	1.7	1.8	1.5	2.1	2.4	3.2	3.6	4.4	3.8	4.4	1.4
20	2.6	2.7	AF	3.4	3.3	4.8	4.4	4.3	4.2	3.1	2.6	2.2	2.5	2.7	3.6	2.7	3.5	2.4	3.3
21	1.1	1.4	HD	1.5	1.4	1.3	1.7	1.6	1.3	1.7	2.1	4.5	2.7	2.7	4.0	3.2	4.6	1.5	1.4
22	3.1	2.8	GP	2.4	3.9	3.5	2.6	3.0	2.6	2.1	3.4	3.7	3.9	5.7	4.8	3.3	—	2.5	2.6
23	1.0	1.0	GP	1.5	2.6	1.7	1.1	1.1	1.2	1.1	0.9	1.0	1.1	1.2	1.2	1.5	1.1	1.5	1.7
	0.9	0.9	HD	1.8	2.0	2.0	1.4	2.6	1.9	1.4	1.8	3.1	6.2	8.1	7.0	1.6	—	—	—
24	3.2	3.2	HD	—	3.1	3.5	3.9	3.1	3.0	2.6	3.0	3.8	6.8	9.8	10.7	21.6	—	3.4	3.4
25	3.2	2.9	HD	3.1	2.8	2.4	2.7	2.3	2.6	—	2.7	3.4	2.3	3.5	3.2	2.5	3.2	2.6	2.2
26	0.9	1.3	GP	1.3	1.1	1.1	0.9	0.9	1.3	0.9	2.0	1.7	3.9	2.6	3.1	3.4	2.4	0.7	0.8
24	2.1	2.2	GP	2.4	2.3	2.1	2.1	2.0	2.0	2.2	1.4	1.4	5.9	6.5	7.2	5.5	—	1.3	1.4
	2.8	3.0	SP	2.9	2.1	3.1	3.1	3.2	3.0	2.5	1.9	2.9	6.5	6.8	6.4	6.3	—	2.9	—
25	2.6	2.9	HF	2.4	2.9	3.7	3.1	3.2	3.3	2.2	2.7	4.3	5.9	6.9	6.3	9.5	6.0	8.8	8.0
26	0.4	0.5	Mm	0.4	0.6	0.6	0.7	0.7	0.6	1.6	0.7	1.3	3.1	8.5	18.2	4.5	4.5	6.4	4.4
27	2.6	2.6	GP	2.3	2.6	2.6	2.7	3.2	2.8	1.7	2.1	2.6	7.6	7.1	9.5	8.0	—	6.2	6.2
28	2.2	5.2	GP	2.3	2.5	2.4	2.4	2.3	2.8	2.6	3.4	4.6	16.5	11.3	11.0	19.5	—	6.9	—
29	2.7	2.9	GP	2.2	2.8	2.7	2.9	2.7	—	2.4	3.8	3.0	6.6	9.5	8.7	7.7	9.5	2.7	2.9
30	3.8	4.0	HD	3.8	4.5	3.8	3.2	3.4	3.7	3.9	11.7	10.7	15.2	20.8	14.9	16.5	—	—	6.0
	1.6	1.9	SP	1.8	2.4	2.8	2.3	2.8	1.9	1.9	2.5	2.9	5.6	6.7	5.9	5.3	4.7	—	1.8
31	1.1	—	HD	1.0	1.1	1.2	1.1	2.0	1.8	1.1	1.5	2.6	5.8	9.9	9.7	8.1	10.2	3.5	4.9
32	1.9	—	HD	1.6	1.5	1.5	2.7	2.7	2.4	1.7	1.7	1.9	8.1	12.5	11.1	10.6	9.2	—	—
33	5.0	—	HF	4.4	4.5	4.2	4.3	4.3	3.6	3.1	3.3	2.8	3.6	3.3	3.8	3.8	5.0	4.9	3.9
34	4.3	3.8	GP	3.0	2.9	3.6	3.8	4.9	4.9	2.8	7.2	7.5	8.1	9.9	8.1	8.1	—	2.9	3.4
35	2.4	2.3	Dog	2.2	2.3	2.6	2.4	2.8	2.5	2.6	2.8	2.8	2.3	2.4	2.7	2.6	—	2.3	2.2
36	2.5	2.3	HF	2.4	1.6	2.9	2.4	2.3	2.9	2.4	2.9	2.9	3.6	14.0	14.8	14.6	—	8.5	2.3
37	1.4	1.2	Mm	2.1	1.4	1.4	1.7	1.9	1.5	1.6	2.3	1.6	1.5	8.5	9.9	9.0	—	2.6	1.7

Legends: GP = grass pollen mixture; HD = house dust; SP = spring pollen mixture; WP = weed pollen mixture; Cat = cat danders; Mm = moulds mixture; AF = Aspergillus fumigatus; HF = hairs and feathers mixture; Dog = dog danders; — = not recorded.
The "Coca value" as well as the "DSCG value" are the mean NPG values, calculated always from three values, as recorded at 0, 5 and 10 minutes after the Coca's Solution, respectively DSCG administration.

Table IV. Abbreviated Survey of the Mean Nasopharyx-Nostril-Pressure Gradient (NPG) Values in cm H_2O Recorded During the Nasal Protection Tests with Beclomethasone Dipropionate (BDA) in Individual Patients.

Patient	Coca value	BDA value	Allergen	Time in minutes after challenge						Time in hours after challenge									
				0′	10′	20′	30′	45′	60′	10h	24h	26h	28h	30h	32h	34h	36h	48h	50h
1	1.9	1.7	GP	3.3	4.9	8.3	12.0	10.4	5.3	2.3	2.4	2.2	2.0	1.8	2.2	2.1	—	2.7	2.2
2	2.8	2.6	HD	6.6	12.3	14.4	18.3	11.0	0.9	2.8	2.3	2.7	2.3	2.3	2.7	2.1	—	3.2	—
3	3.7	3.7	GP	13.1	28.0	28.0	15.4	15.5	16.2	3.4	3.8	3.2	3.4	3.0	4.4	3.0	—	3.0	3.0
4	2.5	2.6	SP	5.8	7.9	9.1	8.7	10.0	7.2	2.6	1.4	2.3	1.9	1.8	4.8	2.8	—	3.1	3.4
5	1.0	1.0	GP	13.7	15.0	13.7	13.7	14.7	17.8	1.0	1.0	1.1	1.6	1.7	2.1	3.0	3.5	—	—
6	5.1	4.9	HD	10.7	12.8	13.9	15.9	7.8	7.1	3.8	4.5	4.1	4.4	5.8	4.1	4.2	—	4.5	—
7	1.1	1.1	GP	4.3	20.8	28.1	30.1	30.1	27.2	2.0	8.9	1.4	4.2	10.9	1.2	7.2	2.1	0.9	—
8	2.1	1.9	WP	8.9	9.4	12.0	10.5	16.0	14.8	2.1	1.4	1.7	1.8	1.6	2.1	1.9	2.1	3.9	1.8
9	3.9	4.2	GP	7.5	11.6	13.8	14.0	10.4	10.5	4.2	2.6	1.9	2.6	2.7	4.7	2.8	2.4	2.7	—
10	3.2	—	HD	7.6	9.0	9.4	11.4	9.5	7.9	4.0	4.6	5.7	4.2	6.0	4.0	3.6	—	3.8	—
11	1.4	1.5	GP	10.0	15.0	13.6	23.1	24.5	8.9	1.5	2.8	2.5	2.8	4.2	2.9	2.8	1.7	1.8	—
12	2.5	2.4	HD	4.5	8.6	7.0	8.1	10.3	10.6	2.8	2.0	2.8	2.9	4.4	2.5	1.4	1.9	2.4	2.4
13	4.1	4.3	GP	7.8	9.0	10.6	9.2	11.6	11.0	5.3	3.0	4.0	4.6	5.6	3.7	4.5	4.2	4.9	4.3
14	1.8	—	GP	10.3	17.3	13.1	11.1	11.1	3.9	2.8	1.6	1.6	3.0	2.1	1.7	2.3	2.5	1.7	2.2
15	2.1	2.1	GP	18.4	17.5	18.6	24.6	12.6	14.7	2.0	2.1	2.3	2.1	2.5	2.0	2.3	2.2	2.0	2.1
16	4.7	—	GP	9.7	14.0	19.1	15.2	10.1	7.3	6.0	5.9	5.5	4.7	5.7	5.5	5	4.2	—	—
17	1.0	1.0	Cat	5.3	5.9	5.1	7.9	11.8	7.7	1.8	0.7	0.7	0.8	0.7	0.9	0.8	0.9	0.7	1.0
18	2.6	2.6	Cat	7.5	9.9	22.9	19.6	18.3	16.0	2.0	2.0	2.2	2.6	2.2	2.2	3.2	—	2.2	—
19	2.7	3.1	Mm	9.0	6.8	11.2	7.9	6.8	6.5	2.6	1.6	1.6	2.9	3.4	4.5	3.3	3.5	1.8	2.3
20	3.8	3.6	AF	5.2	9.9	13.1	17.8	17.6	14.5	4.3	3.8	3.7	4.1	3.7	3.7	4.9	3.9	3.7	3.9
21	0.8	0.7	HD	3.0	8.8	7.8	7.2	8.3	8.4	0.8	1.2	1.4	1.1	1.7	1.4	1.4	1.2	1.7	1.5
22	1.7	1.6	GP	5.7	8.9	8.0	9.1	10.7	11.9	2.3	2.1	1.9	1.5	1.8	1.6	2.1	—	2.3	2.4
23	1.3	0.8	GP	1.9	8.8	7.0	3.7	1.9	1.9	1.0	1.2	1.2	1.2	2.3	1.5	1.0	—	—	1.2
	0.8	0.7	HD	12.4	19.4	25.2	21.5	16.0	11.7	1.0	1.4	2.2	3.9	3.0	1.4	1.0	—	—	—
24	2.7	2.8	HD	3.0	14.3	10.2	10.6	10.4	10.6	3.4	2.3	3.2	7.6	3.4	4.2	4.9	—	3.1	3.8
25	2.5	2.6	HD	4.8	6.0	9.4	8.8	7.4	3.0	2.6	1.9	2.5	2.5	2.4	3.6	3.6	2.2	1.7	2.4
26	0.8	0.9	GP	4.6	4.9	6.0	5.0	4.6	4.8	—	1.0	0.7	0.8	0.8	1.0	0.8	1.1	0.8	0.6
24	2.5	2.5	GP	2.2	2.5	2.8	2.6	2.8	2.6	1.9	2.1	3.7	3.5	3.0	3.0	2.7	—	2.5	—
	1.3	1.4	SP	1.2	1.5	1.2	1.3	1.4	1.4	2.0	1.8	2.1	1.8	2.0	2.1	2.5	—	2.7	—
25	1.6	1.5	HF	1.4	1.7	1.8	1.8	1.8	1.8	1.8	2.5	2.3	2.5	2.3	3.3	3.1	3.7	2.5	2.8
26	1.1	1.1	Mm	1.0	1.1	1.3	1.2	1.1	1.1	1.4	1.2	2.0	1.5	2.8	2.6	2.3	—	1.0	1.1
27	3.3	2.9	GP	3.7	3.6	3.1	3.3	3.7	4.1	3.9	3.6	3.1	3.0	3.1	2.8	3.3	—	3.2	5.3
28	1.5	1.8	GP	1.8	1.6	1.6	1.7	1.7	1.3	2.6	2.6	2.6	2.4	2.9	2.1	2.6	—	1.5	1.9
29	2.9	3.0	GP	3.0	2.6	2.7	2.8	2.5	2.9	3.4	2.9	3.5	3.1	2.9	2.5	2.8	2.8	—	—
30	3.7	3.3	HD	4.8	4.7	4.5	4.1	3.8	3.0	3.5	3.3	4.9	6.5	7.1	6.6	5.8	—	3.7	2.8
	3.8	3.5	SP	3.8	4.9	4.8	3.8	3.8	4.0	3.6	2.9	3.9	4.8	4.9	3.1	3.6	3.4	3.1	3.8
31	1.8	—	HD	2.0	2.1	2.0	2.0	2.4	2.6	3.0	3.3	4.3	2.8	4.2	4.3	3.9	—	—	—
32	2.5	2.6	HD	3.1	2.6	3.0	2.7	2.7	2.5	2.0	1.8	2.2	2.3	2.4	2.5	2.7	2.7	—	—
33	2.3	—	HF	2.2	2.4	2.4	2.4	2.3	2.4	2.1	2.8	3.1	2.3	2.6	4.6	4.3	3.2	2.7	2.0
34	1.4	1.8	GP	1.6	1.8	1.3	1.3	1.3	1.4	2.2	2.1	2.2	1.5	1.6	2.1	2.1	—	2.9	3.2
35	1.6	1.7	Dog	1.6	2.0	2.0	2.3	1.7	1.8	2.3	2.0	2.9	3.0	3.6	3.0	2.5	—	1.9	1.7
36	2.4	2.5	HF	2.7	2.8	1.8	2.3	2.5	2.5	2.4	2.4	2.5	2.0	2.2	2.7	2.5	—	1.4	2.5
37	1.2	1.2	Mm	1.1	1.2	1.7	1.3	1.5	1.3	1.2	2.0	1.7	1.7	2.2	1.6	2.0	—	1.0	1.1

Legends: GP = grass pollen mixture; HD = house dust; SP = spring pollen mixture; WP = weed pollen mixture; Cat = cat danders; Mm = moulds mixture; AF = Aspergillus fumigatus; HF = hairs and feathers mixture; Dog = dog danders; — = not recorded.
The "Coca value" as well as the "BDA value" are the mean NPG values, calculated always from three values, as recorded at 0, 5 and 10 minutes after the Coca's Solution, respectively BDA administration.

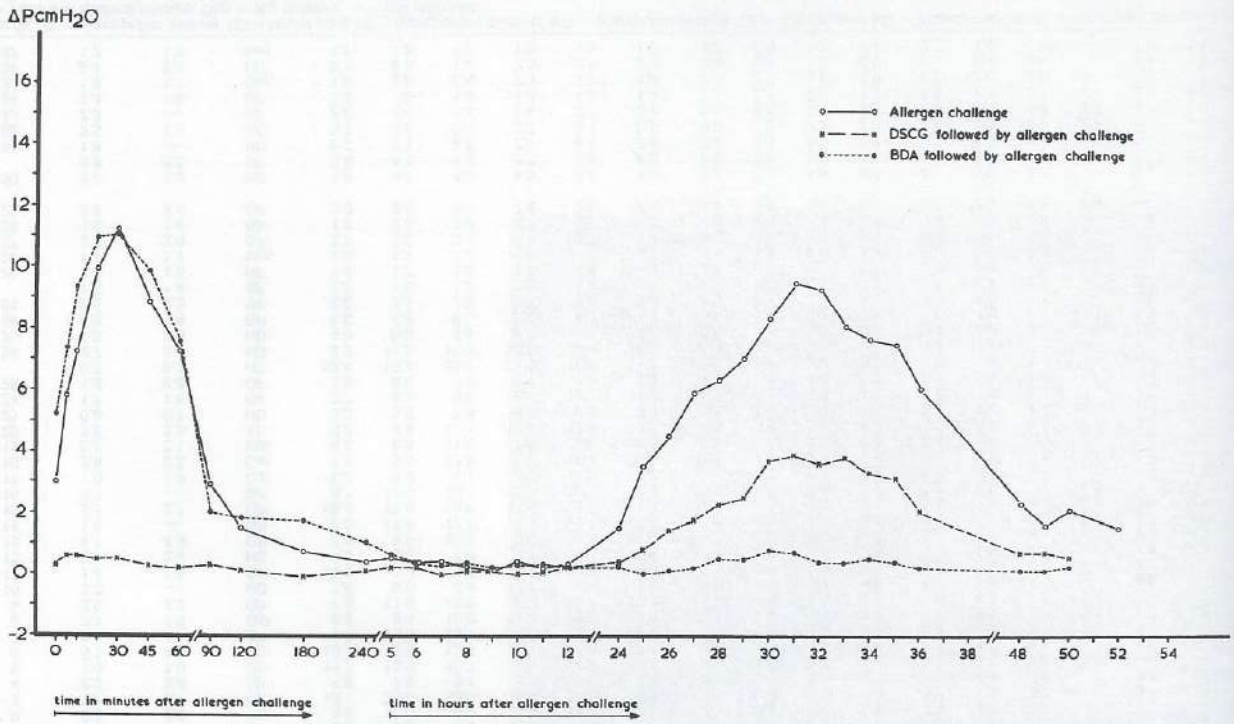

Figure 1. Group I and IV (positive immediate and delayed responses). Provocation tests and protection tests with DSCG and BDA. The mean NPG values recorded from all patients demonstrating "dual nasal mucosa response" (due to the Type I and to the Type IV hypersensitivity) during these tests, with respect to the appropriate Coca's solution mean NPG values. DSCG = disodium cromoglycate. BDA = beclomethasone dipropionate.

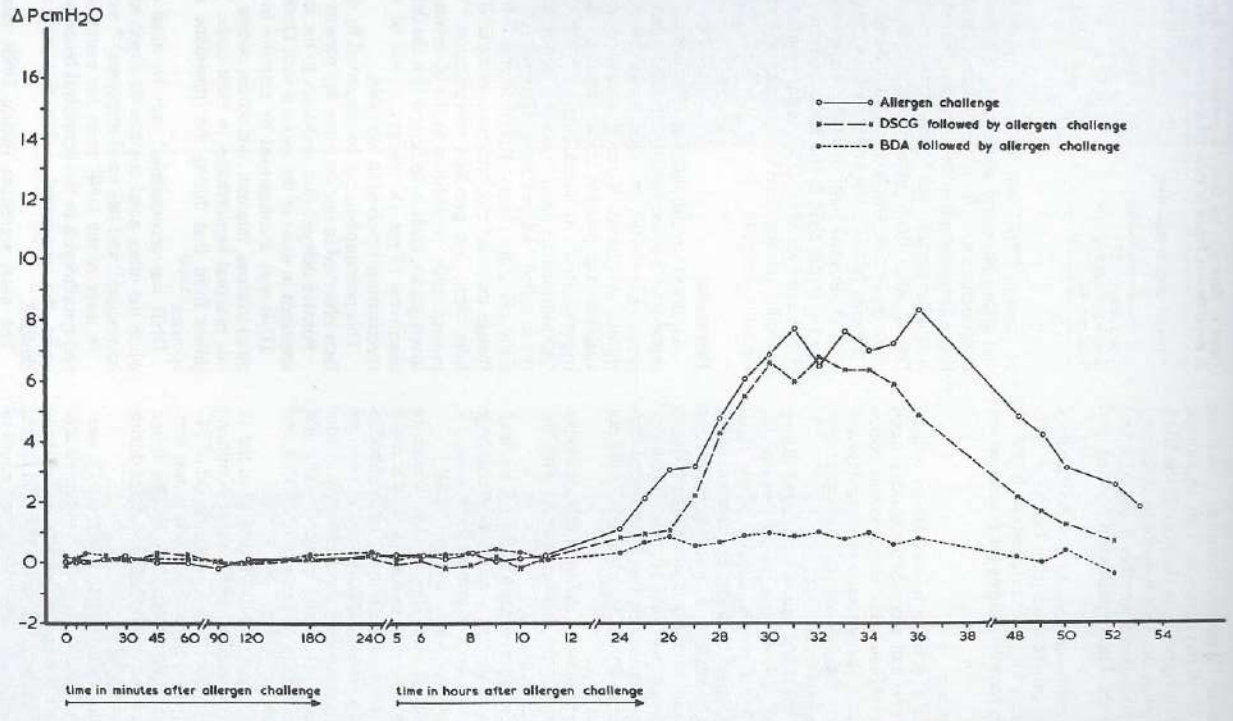

Figure 2. Group O and IV (positive isolated delayed response). Provocation tests and protection tests with DSCG and BDA. The mean NPG values recorded from all patients demonstrating "isolated delayed nasal mucosa response" (due to the Type IV hypersensitivity) during these tests, with respect to the appropriate Coca's solution mean NPG values. DSCG = disodium cromoglycate. BDA = beclomethasone dipropionate.

(b) *Delayed response:* Hypothesis Number 1: all three curves (ALL, DSCG, BDA) coincide. This hypothesis is rejected ($p < 0.01$). Hypothesis Number 2: the BDA and DSCG curves coincide. This hypothesis is rejected ($0.05 > p > 0.01$). Hypothesis Number 3: the ALL and DSCG curves coincide. This hypothesis is rejected ($p < 0.05$).

[Explanation of abbreviations: ALL = NPG values recorded during the provocation tests; DSCG = NPG values recorded during the protection tests with DSCG; BDA = NPG values recorded during the protection test with BDA.]

Accompanying Findings

All patients with a positive immediate as well as delayed nasal response had accompanying acute nasal complaints. The immediate nasal response was associated with obstruction, hypersecretion, sneezing and itching, while during the delayed nasal response only distinct obstruction was noted.

Mild lividity of the nasal mucosa occurred during the cases of immediate response. In all cases of the "delayed response" pronounced lividity of the nasal mucosa was accompanied by distinct induration of the nasal mucosa, by a decrease in the nasal secretions and in most cases by small multiple mucosal hemorrhages localized on the septum nasi and all three turbinates. These changes were significantly less pronounced during the protection tests with BDA, while DSCG did not influence them at all.

The serum concentration of the total IgE antibodies (PRIST) was elevated only in two patients with the DDNR (numbers seven and 18).

The serum concentration of the specific IgE antibodies (RAST) to the same allergen as used for the challenge was elevated in five patients (numbers three, nine, 15, 18, 23), all of whom developed only the DDNR.

The concentration of antibodies of other classes in the patient's serum were determined by double radial immuno-diffusion (Mancini technique). IgG antibodies were elevated in two cases of DDNR and in one case of IDNR. The IgA antibodies were elevated in four cases of DDNR and in five cases of IDNR. The IgM antibodies were elevated in three cases of DDNR and in three cases of IDNR.

Fourteen of the 27 cases of DDNR and 13 of the 16 cases of IDNR were associated with a slight peripheral leucocytosis and lymphocytosis during a period of 24–48 hours after challenge. Slight leucocytosis and lymphocytosis in the peripheral blood were also found during the protection tests with DSCG, but not during the protection tests with BDA.

The nasal secretions during 21 cases of INR were characterized by an elevated count of eosinophils and mast cells, which both disappeared within the following two to four hours. A similar picture was found during INR pretreated by BDA. A clearly decreased count of eosinophils and mast cells was observed during INR pretreated by DSCG. During 19 cases of DLDNR and 14 cases of IDNR, an elevated count of lymphocytes and neutrophils and sometimes of plasma cells and macrophages were found in the nasal secretions, while the eosinophils and mast cells disappeared completely. A similar picture was found after pretreatment with DSCG. After BDA pretreatment the number of lymphocytes and neutrophils definitely decreased.

Table V. Survey of the Skin Responses (i.c. Tests) with Respect to the Different Types of Nasal Mucosa Responses.

Nasal mucosa response	Skin response	
	immediate	delayed
Dual delayed (immediate + delayed) n = 27	22 (= 81%)	16 (= 59%)
Isolated delayed n = 16	2 (= 12%)	10 (= 63%)
Total n = 43		26 (= 60%)

Results of the intracutaneous tests are presented in Table V. Positive immediate skin responses were found in 81% of the INR to the same allergen. The 63% of IDNR and 59% of DLDNR being a part of the DDNR, were also accompanied by "delayed skin responses" to the same allergens.

Discussion

As already mentioned, in the classical conception, immediate hypersensitivity (Type I allergy) has been presumed to be the only mechanism causing the allergic rhinitis symptoms. Recently some investigators have suggested the possible involvement of other types of hypersensitivity in allergic rhinitis.[1,2,4–10,21,24] One of the "non-immediate nasal responses" to allergen challenge, the so-called "delayed nasal response" (DNR), was initially clinically observed and described by us.[2,21] Although the exact mechanisms causing DNR are not yet fully clear, our previous investigations[2,3,21] and this present study, in addition to the observations of other investigators, leads us to believe that the delayed hypersensitivity (Type IV allergy) is one of the possible mechanisms involved in the DNR.

This presumption can be supported by the following facts observed in our previous and present studies.[2,3,21]

Positive delayed skin responses to the same allergen appeared in most of the patients with DNR.

DNR was accompanied by extreme lividity of the nasal mucosa, mucosal induration, edema and numerous mucosal hemorrhages in most cases. This picture differs from that during the immediate and late responses.[15,16,21]

DNR was accompanied only by nasal obstruction, while the other nasal complaints observed during the immediate[15] and late nasal responses[2,16] were absent.

In most of the DNR cases the count of leucocytes and lymphocytes in the peripheral blood was elevated, while the eosinophil count was not significantly changed.

The nasal secretions during DNR showed an increased number of neutrophils, lymphocytes and epithelial cells. Other cells were found sporadically. Sometimes erythrocytes from disrupted capillaries appeared. This finding differs from that observed during the immediate response[21] and the late response.[21]

No significant changes in the serum concentrations of the immunoglobulins were found in most of the patients with DNR. The serum concentration of total IgE was not elevated in any of these patients. The serum concentration of specific IgE was elevated only in a minority of these patients. An increased serum concentration of IgG, IgM and IgA was found only sporadically. These findings do not support the possible role of these immunoglobulins in the DNR.

However, this conclusion has two controversial aspects.[25] First, the complete role of the immunoglobulins of individual classes in detail, in various hypersensitivity reactions in the nasal mucosa is not yet fully known. Secondly, the participation of the central parts (in the serum) and of the topical parts (in the nasal mucosa) in the nasal response and their feedback mechanisms are not yet fully explained.

In a few cases of DNR, total IgE, specific IgE, IgG, IgA, IgM immunoglobulins in the nasal secretions were quantitatively determined. No elevated concentration of any of these was found.

Our hypothesis that the delayed hypersensitivity (Type IV allergy) could possibly be involved in the "delayed nasal response" is supported by other investigators' observations.

Slavin et al[5,13] suggested "that symptoms of rhinitis might also result from delayed hypersensitivity." Other investigators[4,6,7–10,12] found evidence for a cellular immune response to some antigens in patients with hay fever from various immunological tests *in vitro*. Alfosso et al[26] demonstrated the mitogenic activity of pollen, which can lead to the stimulation of lymphocytes. Ashida et al[27] observed that endothelial cells can support the induction of mitogen-induced T-cell activation, and they suggested that cells lining blood vessels may play an active role in the initiation of immune responses *in vitro*. This fact seems to us to be important because hemorrhages were observed during DNR. Connel[28] found lymphocytes and plasma cell infiltrates in the nasal biopsies of patients with allergic rhinitis. He interpreted these findings as "being compatible with Type IV immune mechanism." Other investigators have suggested a possible role of delayed hypersensitivity in patients with allergic rhinitis from the results of allergy skin tests.[4,10,12] Michell and Platts-Mills[31] found a delayed cutaneous response caused by "inhalation allergens" involving T-cells. Their observation, as well as the frequently described cutaneous basophil hypersensitivity being considered as attributed to the delayed hypersensitivity mechanism,[29,30] could be analogical reactions to the "delayed nasal response" observed by us.

With respect to the kind of allergens, some investigators[4,9–12,26,31–34] suggested that "inhalation allergens" can also be involved in the cell-mediate reactions (delayed hypersensitivity).

Rocklin et al[35–37] observed possible suppression of delayed hypersensitivity (lymphocyte functions) by histamine *in vitro*. Their findings could explain why some patients develop only the immediate response. In these cases the histamine is probably the most important mediator or is released in greater amounts than the other mediators, and besides its mediating activity in immediate hypersensitivity it also prevents the development of delayed hypersensitivity. Other patients develop the dual nasal response. In them, the mediators different from histamine probably play a more important role in mediating of the immediate hypersensitivity, or the histamine is released in a lower concentration which cannot prevent the development of the delayed nasal response.

In delayed hypersensitivity (cell-mediated immune reaction) the antigen reacts with sensitized lymphocytes (T-cells). They release a variety of factors (primary and secondary mediators of delayed hypersensitivity) which then act through several pathways on the further systems and organs resulting in immunological tissue injury.[32,38,39]

The role of the basophils in cutaneous basophil hypersensitivity has been confirmed.[29,30] The possible role of mast cells and/or basophils in other kinds of delayed hypersensitivity has also been suggested.[29–31,40,41,42]

The varied presence of basophils led Dvorak et al to formulate a theory of two different types of delayed hypersensitivity reactions.[40,42]

The suggested dual role of basophils and/or mast cells in the immediate as well as the delayed hypersensitivity could probably explain the appearance of the dual delayed nasal response, and could also be interpreted as the linkage between these two very different types of hypersensitivity.

In this study, DSCG demonstrated significant protective effects on the immediate response, which can be explained by the ability of DSCG to prevent degranulation of mast cells and basophils and the subsequent release of the mediators.[14,15,17,43] On the other hand, the full ineffectiveness of DSCG on the "delayed nasal response" cannot support the suggested role of the mast cells and/or basophils in the "delayed nasal response".[4,10,21] BDA showed opposite effects. The immediate nasal response was not influenced significantly by BDA,[14–16,21] while the "delayed nasal response" was decreased significantly by BDA.

These results indicate that the mechanisms involved in DNR might differ from those participating in the immediate nasal response. Moreover, the excellent protective effects of BDA on DNR support the hypothesis of the possible involvement of delayed hypersensitivity in DNR, since the suppressive effects of corticosteroids on delayed hypersensitivity have been demonstrated.[21,44–52]

Glucocorticosteroids, including BDA, possess a high anti-inflammatory activity[44,46,47,51,52] sometimes combined with decongestant effects. However, their direct

protective effects on the mast cells and basophils have not yet been confirmed.[44,50,51] They decrease and inhibit the action of cGMP, and increase and potentiate cAMP[44,46,52,53,55] leading to inhibition of the lymphocytes' proliferation and the production of lymphokines.[52] They also inhibit the synthesis of some prostaglandins by preventing the release of the arachidonic acid precursors.[44,46,47,49,52,53,56] By increasing the resistance of the capillary wall, corticosteroids inhibit the extravasation of fluid and reduce the accumulation of leucocytes and lymphocytes at the site of injury.[44] Glucocorticosteroids also influence the various functions of leucocytes and their redistribution. Neutrophils marginating the capillary endothelium in tissue re-enter the circulation, while eosinophils, monocytes and lymphocytes disappear from it.[44] They also antagonize the effects of various chemotactic factors.[54] Nevertheless, the effects of glucocorticosteroids in detail on the delayed hypersensitivity are still uncertain.[44]

The results of our present study lead us to believe that a possible involvement of the delayed hypersensitivity in DNR can be presumed. Our hypothesis might be analogical to the suggestion of some authors of the possible existence of delayed hypersensitivity in certain pulmonary diseases in man.[57–59] We are aware that more concurrent immunological investigation will be necessary to clarify the mechanisms underlying the "delayed nasal mucosa response."

As a result of this study, the following procedure for the practical use of disodium cromoglyconate (DSCG) and beclomethasone dipropionate (BDA) is suggested.

In patients with allergic rhinitis with the "delayed nasal response," BDA (800 μg daily intranasally) should be the primary therapeutic choice.

When immediate responses to the same allergen (DDNR) or also to other allergens are present, then in addition to prolonged BDA therapy (800 μg daily intranasally), DSCG (4 × 1 capsule daily intranasally) should be added temporarily for a period of six to ten months at the beginning of the treatment.

With such a schedule, we achieved excellent therapeutic improvement in most of our patients with the delayed nasal response.

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THE DIAGNOSTIC APPROACH TO IMMEDIATE HYPERSENSITIVITY IN PATIENTS WITH ALLERGIC RHINITIS; A COMPARISON OF NASAL CHALLENGES AND SERUM RAST

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a communication from The Netherlands

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Z. PELIKAN, M.D., F.A.C.A

Nasal challenges performed in 182 patients with allergic rhinitis revealed 273 positive and 125 negative responses to house dust, grass pollen, cat and dog danders and aspergillus fumigatus, which all correlated with disease history and intracutaneous tests. In these randomly selected patients the specific IgE antibodies in the serum to the same allergens as those used for nasal challenges were determined quantitatively by serum RAST.

Additionally, blood and nasal eosinophilia were recorded during the nasal provocation tests. In all 273 positive challenges protection tests with disodium cromoglycate (DSCG) were also performed.

In 24.2% of the positive nasal challenges serum RAST was found positive and in 18.7% of them serum RAST was doubtful. In 52% of the negative nasal challenges negative serum RAST also occurred.

No significant correlation was found between serum RAST and nasal challenges ($X^2 = 0.92$; d.f. = 1; $0.20 < p < 0.40$). No significant correlation was found between serum RAST and nasal challenges for individual allergens tested.

Since the nasal provocation test is an in vivo technique, it is likely to be of value for the diagnostic confirmation of the allergic component (due to immediate hypersensitivity) in patients with allergic rhinitis than serum RAST alone. Although serum RAST cannot substitute the nasal provocation tests, it may be a useful supplementary diagnostic parameter.

Introduction

THE MAGNITUDE OF ALLERGIC RHINITIS is often underestimated in practice, despite the fact that this condition can be a long-term problem and a source of considerable discomfort to the patient, affecting several somatic, psychosocial and occupational aspects of his life. In predisposed individuals, allergic rhinitis may be associated with bronchial asthma, asthmatic bronchitis or other complications (e.g. sinusitis, otitis, conjunctivitis).

The diagnostic approach to the patient with allergic rhinitis varies in practice and usually consists of a brief disease history, skin tests and serum RAST. Sometimes, serum RAST, as an *in vitro* technique, is preferred to the whole *in vivo* tests, e.g. nasal provocation tests.

The aim of this present study was to compare serum RAST and the nasal provocation tests with allergens in patients with allergic rhinitis, using common allergens (house dust, grass pollen, cat danders, dog danders, aspergillus fumigatus). One of the anticipated outcomes of this study was to evaluate the values and limitations of serum RAST and nasal challenges for the diagnosis of allergic rhinitis.

Materials and Methods

*Allergens**. Aqueous extracts were dialyzed, lyophilyzed and then diluted in Coca's Solution (dry weight of lyophilyzed allergen in mg per 1 ml of Coca's Solution) and used in the following concentrations:

	Scratch + intracutaneous tests	Nasal challenges
House dust	0.50 mg/ml	5.0 mg/ml
Cat danders	0.25 mg/ml	2.5 mg/ml

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* Prepared by Allergen Laboratory "Diephuis," Groningen, The Netherlands.

	Scratch + intracutaneous tests	Nasal challenges
Dog danders	0.25 mg/ml	2.5 mg/ml
Aspergillus fumigatus	0.25 mg/ml	2.5 mg/ml
Grass pollen**	1 000 NU/ml	10 000 NU/ml

Skin tests. Scratch tests were performed initially and evaluated at 20 minutes. If they were negative, intracutaneous tests (i.c.) were carried out and evaluated 20 minutes later.*

*Nasal provocation tests***. These were performed by means of a modified rhinomanometry technique (balloon technique) measuring the nasal resistance, which methods have been described previously.[1-4] Every test consisted of (a) "initial value" (base line)—parameters were recorded at 0, 5 and 10 minutes, (b) "Coca's Solution value"—parameters were recorded at 0, 5 and 10 minutes after 3 minutes' application of Coca's Solution to the nasal mucosa, and (c) allergen challenge values—parameters were recorded at 0, 5, 10, 20, 30, 45, 60, 90 and 120 minutes after the 3 minutes' challenge of allergen to the nasal mucosa.

RAST (radio-allergo-sorbent test). Specific serum IgE antibodies were determined quantitatively by means of the standard Phadebas RAST technique. Grass pollens were used for RAST as individual antigens and not as a mixture. The RAST was considered to be positive for grass pollen if at least one of the nine basic grass antigens was positive. The RAST was considered to be negative for grass pollen if all the nine grass pollen antigens were negative.

Total blood eosinophil count. This was performed three times during each nasal provocation test: before and at 30 and 60 minutes after the allergen challenge. The samples were stained with Eosin Y technique.[5] An eosinophil count higher than 300 eos per mm^3 ($300 \times 10^6/l$) was considered to be elevated.

Nasal eosinophil count. Nasal secretions' samples obtained before allergen challenge and at 30 and 60 minutes after allergen challenge were processed and stained by Hansel's technique,[6,7] modified by the author.[8] The eosinophil cells were enumerated microscopically and expressed as a percentage of 100 leucocytes.[8]

Protection tests with intranasal disodium cromoglycate (DSCG). A 2% solution of DSCG in distilled water was applied to the nasal mucosa for three minutes by means of wad of cotton wool on a nasal probe introduced under the middle concha. Seven minutes after the end of the DSCG administration, challenge with allergen was performed on the same site and the parameters were recorded up to 120 minutes.[1]

Patients. In patients with seasonal or perennial rhinitis the following baseline diagnostic studies were performed. A. *Basic general examination*: extensive disease history, physical examination, basic laboratory tests, X-ray examination of sinuses (if necessary also chest X-ray) and basic pulmonary functions. B. *Basic allergic and immunologic examination*: (a) Skin tests (first scratch tests; if these were negative then intracutaneous tests), (b) Nasal Histamine Threshold, (c) nasal provocation tests with various allergens as determined by history and skin tests, (d) nasal secretions' cytograms and eosinophil count, and (e) blood leucocyte and eosinophil count.

In 182 patients with allergic rhinitis, a total of 398 nasal provocation tests with one or more of the above-mentioned allergens which correlated with the intracutaneous tests and disease history were randomly selected. Of these 398 nasal provocation tests, there were 273 positive NPT ("positive correlating NPT group")* and 125 negative NPT ("negative correlating NPT group").**

In all patients studied the specific serum IgE antibodies to house dust, cat danders, dog danders, Aspergillus fumigatus and the nine individual grass pollens were also determined quantitatively by serum RAST.

In addition, the protection tests with intranasal disodium cromoglycate were performed in the case of all 273 positive nasal challenges from the "positive correlating NPT group" as described above.

Statistical analysis. The Chi-square test was employed for statistical evaluation of the results. A p value of < 0.05 was considered to be statistically significant.[9,10]

Results

Of the group of 273 positive nasal challenges, serum RAST was found positive (score 4,3) in 66 cases (24.2%) and doubtful (score 2) in 51 cases (18.7%). In the remaining 156 positive nasal challenges (57.1%), the serum RAST was negative (score 1,0).

Of the group of 125 negative nasal challenges, negative serum RAST (score 1,0) was found in 65 cases (52.0%). In the remaining 60 cases, serum RAST was positive in 21 cases (16.8%) and questionable in 39 cases (31.2%). It can then be concluded that in 127 of the 273 positive nasal challenges (42.9%) serum RAST was also found positive. In 65 of the 125 negative nasal challenges (52.0%) serum RAST was also found negative. These results are summarized in Table I.

The distribution of the serum RAST results with respect to the individual allergens in both groups of nasal challenges is presented in Tables II and III. The correlation between the serum RAST results and the nasal

** Dry weight percentage: Secale cereale 15%, Dactylis glomerata 15%, Lolium perenne 10%, Anthoxanthum odoratum 10%, Agrostis alba 10%, Holcus lanatus 10%, Phleum pratense 10%, Cyosurus cristatus 5%, Alopecurus pratensis 15%.

* The evaluation of the intracutaneous tests: − = normal skin appearance; ± = wheal diameter up to 5.0 mm (original injected papule); + = wheal diameter up to 7.5 mm; ++ = wheal diameter up to 10 mm; ++ = wheal diameter up to 12.5 mm; +++ = wheal diameter up to 15.0 mm; +++ = wheal diameter greater than 15 mm, with surrounding erythema and sometimes with pseudopodia.

** The nasal provocation tests are henceforth abbreviated as NPT.

* "Positive correlating NPT group" = positive NPT, positive skin tests and positive disease history to the same allergen.

** "Negative correlating NPT group" = negative NPT, negative skin tests and negative disease history to the same allergen.

Table I. The Survey of Serum RAST Results in the "Positive Correlating NPT Group" and in the "Negative Correlating NPT Group."

Disease history + intracutaneous tests	Nasal provocation tests	RAST (specific IgE antibodies in the serum) Score	Interpretation
Positive = 273	Positive = 273	4 = 35 (12.8%) 3 = 31 (11.4%)	positive = 66 (24.2%)
		2 = 51 (18.7%)	doubtful = 51 (18.7%)
		1 = 60 (22.0%) 0 = 96 (35.1%)	negative = 156 (57.1%)
Negative = 125	Negative = 125	4 = 5 (4.0%) 3 = 16 (12.8%)	positive = 21 (16.8%)
		2 = 39 (31.2%)	doubtful = 39 (31.2%)
		1 = 22 (17.6%) 0 = 43 (34.4%)	negative = 65 (52.0%)

Results of RAST evaluated by the standard Pharmacia RAST Score; the interpretation of which has been modified by us:

Score 0 = concentration less than 0.35 U/ml — negative
1 = 0.35 − 0.70 U/ml — doubtful
2 = 0.70 − 3.50 U/ml — doubtful
3 = 3.50 − 17.50 U/ml — positive
4 = concentration higher than 17.5 U/ml — positive

Table II. The Distribution of the RAST Results with Respect to the Individual Allergens in the "Positive Correlating NPT Group."

Allergen	RAST Positive	RAST Doubtful	RAST Negative	Number of Nasal provocation tests
House dust	18 (20.2%)	25 (28.1%)	46 (51.7%)	89
Grass pollen	27 (31.1%)	19 (21.8%)	41 (47.1%)	87
Cat danders	11 (22.0%)	5 (6.0%)	34 (72.0%)	50
Dog danders	9 (22.5%)	1 (2.5%)	30 (75.0%)	40
Aspergillus fumigatus	1 (14.3%)	1 (14.3%)	5 (71.4%)	7
Total	66 (24.2%)	51 (18.7%)	156 (57.1%)	273

Table III. The Distribution of the RAST Results with Respect to the Individual Allergens in the "Negative Correlating NPT Group."

Allergen	RAST Positive	RAST Doubtful	RAST Negative	Number of Nasal provocation tests
House dust	3 (12.0%)	11 (44.0%)	11 (44.0%)	25
Grass pollen	5 (12.8%)	12 (30.8%)	22 (56.4%)	39
Cat danders	4 (16.7%)	7 (29.1%)	13 (54.2%)	24
Dog danders	7 (22.6%)	6 (19.4%)	18 (58.0%)	31
Aspergillus fumigatus	2 (33.3%)	3 (50.0%)	1 (16.7%)	6
Total	21 (16.8%)	39 (31.2%)	65 (52.0%)	125

enges for individual allergens was found to be nonficant and was as follows: house dust ($X^2 = 2.5$; d.f. $0.15 < p < 0.30$); grass pollen ($X^2 = 4.72$; d.f. = 2; $< p < 0.10$); cat danders ($X^2 = 4.29$; d.f. = 2; $0.10 < 0.20$); dog danders ($X^2 = 5.6$; d.f. = 2; $0.05 < p$ 10); aspergillus fumigatus ($X^2 = 3.95$; d.f. = 2; $0.10 < 0.20$).

o significant correlation was found between the score e serum RAST and the degree of nasal response as as intracutaneous tests to the same allergens in nts from both the groups.

a elevated blood eosinophilia was found during 131 ive nasal challenges (=48%) and only in 8 negative l challenges (=6%). Changes in the count of the l secretions' eosinophils between before and after gen challenge were recorded in 223 positive chales (=82%) and in 11 negative challenges (=9%). No ionship was found between the number of eosinos in the nasal secretions and the score of serum ST in both the groups.

DSCG demonstrated distinct protective effects on the positive nasal mucosa response. From the 273 positive responses, DSCG protected fully 161 responses (58.9%), decreased significantly (improvement higher than 50%) 108 challenges (39.6%) and was ineffective in 4 cases (1.5%).

Discussion

Numerous papers have dealt with the diagnostic value of serum RAST for the allergic component due to the immediate hypersensitivity (Type I allergy) in patients with allergic rhinitis. The majority of these papers report a correlation between serum RAST and various *in vivo* diagnostic tests, especially skin tests.[11,12] However, in most of these studies the allergic component in the nose has not been confirmed by nasal provocation tests.

The nasal challenges with allergens are accepted as an important diagnostic parameter and as a definitive confirmation of the allergic component due to the immediate hypersensitivity in allergic rhinitis patients.[3,4,13-17]

A very important step in the development of clinical allergy during the last decade has been the introduction of the quantitative determination of specific serum IgE antibodies, i.e, RAST in the routine diagnosis of allergic diseases due to the immediate hypersensitivity.[11,12,18–20] However, due to inappropriate use the RAST has come under criticism.

Studies dealing with the correlation between serum RAST and the nasal challenges are not numerous and, moreover, their results vary greatly, from a significant to a non-significant correlation.[17,21–29]

Our results, which indicate a less than optimal correlation between serum RAST and NPT are comparable with the findings of other authors[21,22,24,25,27,29] as well as with our previous studies.[23]

Wüthrich et al[25] found a correlation between serum RAST and nasal challenges of 42–47%. Santrach et al[24] observed 62% "incorrectly" negative RAST in positive nasal challenges and a total discorrelation of 50%. Nagaya et al[29] concluded that many allergic rhinitis patients had a negative RAST and low total serum IgE.

Other investigators found a significant correlation between NPT and serum RAST, being approximately 60–80%.[11,18–20,26,28]

The RAST results were evaluated by means of the RAST Score, divided into five classes (0–4), the interpretation of which, however, has already been modified by us.[23] With respect to the irregular statistical distribution, to our clinical experience and to the results of therapy, we concluded that only classes 3 and 4 of the RAST Score can be accepted as real positive. Class 2 seems to us to be doubtful and classes 1 and 0 as negative. Our difficulties with the interpretation of the RAST Score and our suggestion to modify it were later supported by other investigators.[24,30–34] These authors concluded that the expectations of the RAST as a conclusive test were exaggerated. They suggested some technical and interpretative improvements of the RAST technique and a re-evaluation of the position of serum RAST in the diagnosis of allergic diseases.

Although this study deals with the relationships between nasal challenges and serum RAST, some additional tests, i.e., blood and nasal eosinophilia, and protection tests with DSCG, were performed. The results of these procedures should be considered as supplementary parameters supporting the diagnostic certainty of the allergic component in the nasal mucosa.[14]

The positive NPT were accompanied in a high percentage (82%) by changes in the count of the nasal secretions' eosinophils (= local eosinophils) and, in a rather low percentage (48%), by an elevated blood eosinophilia. This fact could indicate that the nasal mucosa response to allergen challenge is a more topical event, which does not necessarily need to invoke a further central response (e.g., elevation of blood eosinophilia or elevation of the immunoglobulin concentration in the serum, in this case specific IgE).[14,21–23,35,37–39]

DSCG is a very important drug in the treatment of allergic disorders due to immediate hypersensitivity, with respect to its protective action on the mast cells, which play a pivotal role in immediate hypersensitivity (Type I allergy). Good protective effects of DSCG observed in 98.5% of the 273 cases of positive nasal challenges could therefore be accepted as supplementary confirmation that the positive nasal responses selected for this study were indeed due to the immediate hypersensitivity (Type I allergy).[1,14]

The suboptimal correlation between nasal challenges and serum RAST observed in this study could be caused and explained by the following factors: (1) The largest part of the specific IgE antibodies playing a role in the allergic reaction in the nose is probably synthetized in the nasal mucosa, which is directly stimulated by the allergen. This topical process cannot influence and increase significantly the level of specific IgE antibodies in the serum.[14,21,23,39–41] (2) The role of antibodies of other classes, different from the IgE class, e.g., IgG and/or IgA in immediate hypersensitivity (Type I allergy) in the nasal mucosa cannot be excluded.[14,25,29,32,34,38,40,42–46] (3) Nasal challenges with allergens are carried out directly on the site of the antigen-antibody interaction (allergic reaction). They are, therefore, a more sensitive and adequate technique for the detection of the topical allergic reaction than a general determination of specific IgE antibodies circulating in the serum. Their concentration can namely be influenced by various factors as well as by other antigen-antibody interactions occurring in other parts of the body.[4,14,16,46] (4) With respect to the variation in the specificity and antigenicity of the allergens, the origin of the allergens could also be of importance for this lack of correlation. The nasal challenges and skin tests are usually carried out with allergens originating from the same country (sometimes from the same area as where the patient lives. In contrast, most of the standard allergens used for the commercial RAST kits are collected in certain countries (e.g., in the Scandinavian countries) and are then used for tests in patients in other countries. If this fact is confirmed as one of the causes of this discorrelation, it seems to us to be necessary to perform also the serum RAST with allergens originating from the patient's own country or from the patient's own area.[24,46] (5) Finally, some technical and/or interpretation disadvantages of RAST as possible source of the above-mentioned lack of correlation cannot be excluded.[23,30–32,34]

The following practical conclusions may be drawn from this study. (A) The correlation between serum RAST and the nasal provocation tests was found to be non-significant. (B) The nasal provocation tests with allergens seem to be of greater value for the diagnosis of allergic rhinitis than serum RAST only. RAST cannot substitute the nasal provocation tests and other *in vivo* diagnostic tests generally. This would only be possible in such cases where the nasal challenges cannot be performed or are contra-indicated.[8,16,23,25] (C) The diagnosis of allergic rhinitis, based only on disease history and RAST, or on serum RAST alone can lead to the risk of an incorrect diagnostic conclusion, at least part of the patients.

In patients with suspected allergic rhinitis who demonstrated negative RAST, supplementary skin tests and provocation tests should be performed.

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A New Disease: a Nasal Form of Pigeon Breeder's Disease

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In 47 allergic rhinitis patients regularly exposed to pigeons, nasal provocation tests with pigeon dropping extracts, in combination with other parameters, were performed. Twenty-five of the 47 patients developed a late nasal response (LNR) to pigeon droppings (53%), six of them an isolated response and 19 a dual response (immediate + late). The precipitating antibodies to pigeon droppings were positive in 20 (80%) and to pigeon serum in nine LNR patients (36%). Late skin responses to pigeon droppings occurred in 15 (60%) and to pigeon serum in seven LNR patients (28%). In 13 patients (52%) general malaise, in seven (28%) increased blood eosinophilia, in nine (36%) increased body temperature and in 11 (44%) haemorrhages in the nasal mucosa were observed during the LNR. The results of this study provide evidence for the existence of a new disease, a nasal form of pigeon breeder's disease.

Key words: late nasal response; pigeon breeder's disease, nasal form; pigeon droppings.

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CLINICAL ASPECTS

Late nasal response to pigeon droppings challenge was recorded in 53% of patients with nasal complaints. The patients were, directly or indirectly, regularly exposed to pigeons. Late nasal response was observed in two forms, either isolated or dual (immediate + late response). Late nasal response to pigeon droppings associated in different degrees with various in vitro and in vivo parameters (late skin response, precipitating IgG antibodies to pigeon droppings in the patient's serum, general malaise, increased body temperature, blood eosinophilia), provides diagnostic confirmation of a nasal form of pigeon breeder's disease.

Pigeon breeder's disease (PBD), in its pulmonary form belonging to the group of hypersensitivity pneumonitis or extrinsic alveolitis, is observed regularly in a number of exposed breeders (1, 2, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 28).

Pigeon breeder's disease is characterized clinically by a late broncho-obstructive response appearing several hours (4–8 h) after exposure to pigeon droppings (24), accompanied by formation of precipitins in the lung tissues (sometimes visible on chest X-rays), an increase in body temperature and general malaise (1, 2, 6, 9, 10, 25, 29, 30).

Immunologically, this disease, being a non-IgE mediated hypersensitivity state, is characterized by the occurrence of precipitating antibodies to the proteins of pigeon droppings in the serum of exposed individuals (6, 8, 9, 14, 18, 25, 28, 31).

Type III (late) hypersensitivity (Arthus phenomenon) has been suggested as the mechanism involved in PBD (3, 5, 9, 11, 13, 14, 24, 25, 27, 31).

A nasal form of PBD, or a nasal equivalent to this pulmonary disorder, has, to our knowledge, not yet been described in the literature.

Among the patients referred to our department, we see, regularly, pigeon breeders with the pulmonary form of PBD, and also patients with nasal complaints only, which can sometimes be related to pigeon excretions. This observa-

tion caused us to carry out the present investigation, which is a continuation of our preliminary report (23). The purpose of this study was to investigate the existence of a nasal form of pigeon breeder's disease and its clinical features and frequency.

MATERIAL AND METHODS

Allergens

Dialyzed and lyophilized extracts were diluted in Coca's solution (dry weight of allergen in mg/1 ml Coca's solution) and used in the following concentrations: a) for intracutaneous tests: pigeon faeces, feathers and serum – 0.25 mg/ml each; b) for nasal challenges: pigeon faeces 2.5 mg/ml.

Skin tests

Scratch tests were performed first. If these were negative, then intracutaneous tests (i.c.) were carried out and evaluated at 20 min, 8, 24 and 48 h after allergen injection. Skin tests were interpreted as follows: an immediate skin response – positivity within 20 min; a late skin response – positivity within 8–24 h (sometimes persisting up to 48 h). The evaluation parameters are presented in Table 2.

Nasal provocation tests (NPT)

Apparatus and equipment consisted of a one-channel recorder, an electric differential transducer, a water manometer, a small rubber balloon 5 mm in diameter and 25 mm in length and a connecting system of polyethylene tubes (19, 21, 22, 23).

Principle of method. The nasal mucosa of a patient with allergic rhinitis, when challenged topically by an allergen to which he is sensitive, reacts with swelling, hypersecretion, sneezing and itching. The changes influence the passage of air through the nose, resulting in pressure differences between the nasopharyngeal cavity and the outside air, while the air-flow is constant. These pressure differences, the so-called NPG (nasopharynx-nostril-pressure gradients) expressed in cm H_2O, were recorded and considered as a parameter for assessment of the nasal mucosa response. The mean NPG values were calculated during regular breathing for 90 to 120 sec. This "balloon technique", being a combination of anterior and posterior rhinomanometry, has been described in detail in our previous papers (19, 21, 22, 23).

Procedure. After equipment calibration, a rubber balloon was inserted into the nasopharynx through one of the nasal cavities, filled with 2 ml air and connected to the pressure transducer. During the recording of NPG, the patient breathed only through the non-intubated cavity, the mouth being closed and the intubated cavity being closed by the patient's finger placed on the alae nasi. (The finger closing the nostril did not influence the air pressure inside the tubing because the tubing material was of PVC with a thick wall and low elasticity). Within 15 min of balloon insertion, when the patient was breathing regularly, the test was begun according to the following schedule:

a) *Initial (basic) values.* The NPG values were recorded at 0, 5 and 10 min to obtain the so-called "initial (basic) values"; b) *Coca's solution values.* Coca's solution was applied for 3 min to the nasal mucosa of the non-intubated cavity by means of a saturated wad of cotton wool on a nasal probe. The NPG values were then recorded at 0, 5 and 10 min. If no significant changes in mean NPG "Coca's values" compared with mean "initial NPG values" were measured, the investigation was continued; c) *Allergen challenge.* The nasal mucosa was challenged with the allergen for 3 min in the same way and at the same site as with Coca's solution. The NPG values were recorded at 0, 5, 10, 20, 30, 45, 60, (90), 120 min and then every hour up to 30 h, with the exception of an interval between the 11th and 24th hour during which the patient slept.

The nasal response was considered positive when the mean NPG values after allergen challenge increased by at least 2.0 cm H_2O with respect to the "Initial" and "Coca's solution" values. (The 2SD value with this techique is 1.6 cm H_2O). A significant increase in NPG values within 60 min of allergen challenge was considered as an immediate nasal response and within 4–24 h as a late response.

The control test with Coca's solution was performed 4 days later in all patients with positive nasal responses. After the "initial NPG values", Coca's solution was applied to the nasal mucosa as previously and the mean NPG values were recorded up to 30 h.

Body temperature was recorded four times daily, starting 1 day before allergen challenge and continuing up to 48 h after challenge. *Leucocyte and eosinophil count* in the peripheral blood was recorded three times on the day prior to challenge; on the "challenge day" once before and then at 1,4 and 8 h after challenge; and three times on the day after challenge. *Subjective nasal and general complaints* were recorded continually during an interval from 24 h before challenge until 30 h after. *Precipitating antibodies (IgG)* in the patient's serum to pigeon faeces and pigeon serum were determined by double immuno-diffusion in gel according to Ouchterlony & Nilsson (17) and Hudson & Hay (12).

Histology of nasal secretions. Samples of nasal secretions were obtained before allergen challenge and after challenge at 0,30 and 60 min and then every 2nd h. After processing, the samples were stained by Hansel's method, modified by the author, and evaluated microscopically.

Total IgE antibodies in serum (PRIST) was determined once in the Medical Chemical Laboratory in Assen, The Netherlands. *X-ray of sinusses* was performed 24 h after allergen challenge in four patients.

Patients

From a total of 273 patients with rhinitis complaints only, referred to our department during a 16 month period, 47 patients, in whom a perennial allergic component was suspected, also reported regular exposure to pigeons and/or their excretions either through their hobby as a pigeon breeder, or as a member of a breeder's family or a close neighbour.

In these 47 patients, besides the routine allergologic diagnostic procedure (including short-term nasal challenges with basic "inhalation" allergens), long-term nasal provocation tests with pigeon faeces extracts supplemented by other diagnostic parameters were performed.

The patients were investigated during a period without acute complaints. None of them had previously received immunotherapy or corticosteroids, and a few had used disodium cromoglycate. They received no antihistaminics or topical decongestants during the 3 days prior to the study. The clinical characteristics of the patients studied are presented in Table 1.

RESULTS

From a total of 273 patients suffering from rhinitis complaints, with possible involvement of an allergic component of the perennial type, 47 patients (17.2%) reported regular contact with pigeons or regular exposure to pigeon excretions.

Long-term nasal provocation tests with pigeon faeces extracts were performed in these 47 patients. Twenty-five developed a "late nasal response" to pigeon faeces antigen (53%) and are referred to as the "positive late response group" (PLRG). The remaining 22 patients (47%) developed no nasal responses and are referred to as the "negative late response group" (NLRG).

Of the 25 patients demonstrating a late nasal response (LNR), six developed only an *isolated late nasal response* (ILNR), while the other 19 showed a *dual nasal response* (DNR), which is a combination of the "immediate nasal response" (INR) and the "late nasal response" (DLNR).

In four patients with LNR (2 with ILNR and 2 with DNR) the long-term provocation test with pigeon droppings extract was repeated 8–10 days after the first challenge, to check the reproducibility of the test. The nasal response after the repeated challenge did not differ significantly from the first response in any of the four patients investigated.

The "isolated late response" began within 4–6 h, reached its maximum within 6–12 h and resolved within 26 h of allergen challenge. In

Table 1

Clinical characteristics of the patients with late nasal response to pigeon dropping extract

Patient	Age	Sex	Nasal complaints			Disease history related to pigeons	Approx. No. pigeons	Duration of exposure (years)	Frequency of exposure	Direct or indirect exposure	Distance of pigeon lofts from patient's house (meters)
			Obstruction	Hypersecretion	Sneezing						
1	13	F	+++	+	−	−	40	13	daily	neighbours	8
2	9	F	++	++	+	−	40	9	daily	neighbours	8
3	50	F	+++	+	++	+	80	15	daily	neighbours	5
4	27	F	+++	+++	+	+	200	4	daily	neighbours	8
5	27	F	++	++	++	+	20	5	daily	neighbours	7
6	26	F	+++	−	++	+	200	10	daily	neighbours	4
7	44	M	++	+++	+	±	20	20	daily	breeder	5
8	33	M	+++	++	++	+	60	10	daily	breeder	10
9	25	F	+++	−	−	−	100	20	daily	husband	3
10	64	M	+++	+	+	+	60	40	daily	breeder	5
11	34	M	+++	−	−	+	140	8	daily	breeder	8
12	20	F	++	+	++	−	25	20	daily	father	4
13	40	M	+++	+++	−	+	40	33	daily	breeder	3
14	37	F	++	−	−	−	50	13	daily	neighbours	8
15	10	M	++	+	+	−	100	10	daily	father	6
16	24	M	+++	−	−	+	50	24	daily	father	10
17	26	M	+++	++	+	+	80	20	weekend	father	10
18	15	M	++	−	+	−	25	15	daily	father	10
19	24	F	+++	+	+++	+	50	24	daily	father	6
20	25	F	++	+	−	−	20	3	daily	neighbours	15
21	42	M	++	−	−	+	120	10	daily	neighbours	8
22	36	M	+	+	+	−	800	36	daily	father	4
23	18	F	+++	−	−	+	30	18	daily	neighbours	8
24	37	F	++	+	+	−	10	8	daily	neighbours	10
25	15	M	++	−	+	+	200	15	weekly	grandfather	7

the case of the "dual response", an "immediate response" appeared first (onset within 10 min, maximum within 30 min, resolving within 120 min of allergen challenge) and then after a free interval of 2–4 h the late response occurred. The late nasal responses are summarized in Fig. 1.

Three of the 22 patients in the NLRG developed an "immediate nasal response" to pigeon faeces to a slight degree.

The results of other diagnostic parameters are presented in Tables 2 and 3.

The association of LNR, in both its modifications, with *other diagnostic parameters* was as follows:

A late skin response to pigeon faeces was found in 15 patients with LNR (60%) (4 with ILNR (16%) and 11 with DLNR (44%)). A late skin response to pigeon serum was observed in seven patients with LNR (28%) (2 with ILNR (8%) and 5 with DLNR (20%)).

An immediate skin response to pigeon faeces was found in 10 of the 19 DNR and in two of the six ILNR patients. An immediate skin response to pigeon serum was found in four of the 19 DNR patients and in none of the six ILNR patients.

Positive precipitating antibodies (IgG) in serum were found in 20 LNR patients (80%) to pigeon faeces antigen (6 with ILNR and 14 with DLNR) and to pigeon serum antigen in nine

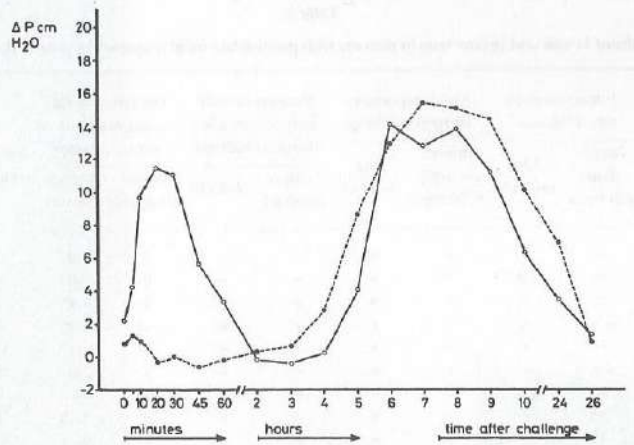

Fig. 1. Dual (immediate + late) and isolated nasal mucosa response to nasal challenge with pigeon dropping extract. The mean NPG values after allergen challenge with respect to the appropriate "Coca's solution NPG values" were calculated from the positively reacting patients of both types of response.
○ = Dual nasal response (immediate + late); ● = Isolated late nasal response.

LNR patients (36%) (3 with ILNR and 6 with DLNR).

In seven patients, an increased *blood eosinophil count* (28%) and in six patients an *increased blood leucocyte count* (24%) were recorded during the LNR.

A slight increase in *body temperature* during the positive LNR was recorded in nine patients (36%) (4 with ILNR and 5 with DLNR).

Thirteen patients (52%) (5 with ILNR and 8 with DLNR) complained of general malaise (headache, tiredness, malaise) during the LNR.

Nasal complaints, obstruction, hypersecretion, sneezing and itching appeared equally during the INR, while nasal obstruction was the most pronounced complaint during all cases of LNR.

In four of the six ILNR patients and seven of the 19 DNR patients, *small mucosal haemorrhages* appeared during the late response (6–12 h) and lasted up to approximately 30 h after allergen challenge.

An increased number of eosinophils, mast cells and goblet cells were found in the *nasal secretions* during most cases of INR. During the LNR, the nasal secretions showed a mixture of cells (a moderate number of eosinophils and goblet cells, sporadic mast cells) with a slight prevalence of neutrophilic leucocytes. No differences were observed in the nasal secretions between both modifications of LNR.

Serum concentration of the total IgE antibodies (serum PRIST) was slightly elevated in eight patients with DLNR and in one patient with ILNR.

In four of the ILNR positive patients, *X-rays of the sinusses* were performed 24 h after allergen challenge. Three of them developed slight oedema of the mucosal membrane in the maxillar sinusses.

The disease history parameters related to pigeons are summarized in Table 1. The pigeon-related disease history (nasal complaints) was suggestive in 14 patients with LNR. In most of the patients, exposure to pigeons and/or their excretions was longer than 4–5 years, the frequency was daily and the number of pigeons kept was more than 10, usually 50 or more.

The results of the other diagnostic parameters found in 22 patients with negative LNR (NLRG) are presented in Table 3.

Clinical improvement after 1 year

Eleven of the 25 LNR patients avoided all contact with pigeons. They all reported a significant improvement in their nasal complaints.

Table 2

Survey of results of *in vivo* and *in vitro* tests in patients with positive late nasal responses to pigeon dropping extract

Patient	Allergen	Intracutaneous tests response[1]		Nasal response to allergen challenge		Increase in body temperature after allergen challenge		Precipitating IgG antibodies in serum against[3]		Blood eosinophilic cells/mm 3[2]	General malaise symptoms 4–24 h after allergen challenge
		Immediate (20 min)	Late (4–24 h)	Immediate (within 60 min)	Late (4–24 h)	(within 60 min)	(4–24 h)	Pigeon droppings	Pigeon serum		
1	PD	−	+	+	+	−	−	4	0	638	+
2	PD	−	++	+	+	−	−	2	0	550	−
3	PD	±	−	+	+	−	−	2	2	308	−
4	PD	++	−	−	+	+	+	1–2	2	330	+
5	PD	−	+	−	+	−	−	3	1	1364	−
6	PD	++	+	+	+	−	−	2	0	352	−
7	PD	+	++	+	+	−	−	0	2	495	−
8	PD	++±	+	+	+	−	−	3	2–3	605	+
9	PD	−	+	+	+	−	−	2	2–3	407	−
10	PD	++	++	−	+	−	+	2–3	1–2	550	+
11	PD	−	+±	−	+	−	+	3–4	0	407	+
12	PD	−	+	−	+	−	+	2	2–3	1331	+
13	PD	−	−	+	+	+	−	1	0	603	−
14	PD	−	+	+	+	−	−	1	1	275	−
15	PD	++	−	+	+	−	−	0	1	1749	−
16	PD	−	−	+	+	−	+	3	0	660	+
17	PD	++	−	+	+	−	−	1	1	275	−
18	PD	+±	+	+	+	−	−	2	1–2	1397	+
19	PD	+++	+++	+	+	−	+	2	0	385	+
20	PD	+	+	+	+	−	+	1–2	1	1199	+
21	PD	−	−	+	+	−	+	2	1	803	+
22	PD	−	−	−	+	−	−	3	0	770	+
23	PD	−	++	+	+	−	−	4	0	1353	−
24	PD	−	−	+	+	−	+	2	0	814	+
25	PD	±	−	+	+	−	−	2	1–2	1496	−

PD = Pigeon dropping extract.

[1] Evaluation of intracutaneous tests:
− = normal skin appearance
± = wheal not greater than original injected papule
+ = wheal increase up to 7.5 mm diam.
+± = wheal increase up to 10.0 mm diam.
++ = wheal increase up to 12.5 mm diam.
++± = wheal increase up to 15.0 mm diam.
+++ = wheal increase >15 mm diam. with surrounding erythema and sometimes with "pseudopodia".

[2] Blood eosinophil count evalution: <300 eos/mm^3 = normal; >300 eos/mm^3 = increased.

[3] Number of precipitating lines.

Six others reduced their contact with pigeons and their nasal complaints improved only partially. The eight patients who did not alter their contact with pigeons reported persisting nasal complaints despite therapy.

DISCUSSION

Pigeon breeder's disease has been detected not only in pigeon breeders (1, 2, 3, 4, 5, 6, 7), but also in their wives (29), children (11, 30, 31),

Table 3

Summarized results of diagnostic parameters in patients with positive late nasal responses and with negative late nasal responses

			Nasal response		
			Positive late (n = 25)		Negative late (n = 22)
			Dual (n = 19)	Isolated late (n = 6)	
Positive skin response	immediate	PD	10	2	6
		PS	4	0	2
	late	PD	11	4	3
		PS	5	2	1
Positive PA in patient's serum		PD	14	6	2
		PS	6	3	0
Increase of blood eos/leucos			4/4	3/2	0/1
Increase in body temperature			5	4	0
General malaise			8	5	0
Late nasal complaints			18	6	0
Appearance of mucosal haemorrhages			7	4	0

PD = Pigeon dropping extract.
PS = Pigeon serum extract.
PA = Precipitating antibodies.

and neighbours (24), all of whom having been directly or indirectly exposed to pigeon droppings. The frequency of PBD varies from country to country and has been found in 6–12% of breeders (3, 4). In all individuals exposed, the frequency of PBD found by us was 31% (24).

It has been demonstrated that PBD is caused by inhalation of dried pigeon droppings (6). Edwards et al. (5) demonstrated that antigens present in the pigeon alimentary tract cross-react with pigeon serum proteins, probably gamma globulin. Pigeon 7S globulin has been suggested as the main antigen responsible for this disorder (8).

The immunological feature of PBD is the occurrence of precipitating antibodies in the serum of persons exposed to pigeons and/or their droppings over a longer period (6, 8, 9, 15, 18, 25, 28, 31). Precipitating antibodies of the IgG class (5, 6, 14, 25), but also from other classes, e.g. IgM (14), or P1-antibodies (a variant of IgM) have been regularly detected in the patient's serum (16).

Clinically, PBD has been observed in acute and sub-acute forms (9). Some investigators have suggested two types of PBD, a symptomatic and an asymptomatic (6, 9, 14). In both types, precipitating antibodies in the patient's serum to pigeon dropping antigens have been detected; however, after exposure to pigeon droppings, the patient with the symptomatic type develops a typical clinical picture, while the patient with the asymptomatic type does not. The acute pulmonary form of PBD is characterized by the so-called bronchial response occurring several hours after exposure to pigeon droppings (4–8 h), where the obstructive and restrictive components in various ratios, are regularly accompanied by other symptoms (general malaise, increased body temperature, cough, chills, myalgia). Changes on the chest X-ray also occur, e.g. pronounced broncho-vascular markings combined with fine sharp nodulations and retriculation, or so-called honeycombing (9, 13, 27). The late broncho-obstructive response has been observed either as an isolated late response or as a dual response, where the late response was preceded by an immediate response (24).

We found the late broncho-obstructive response to bronchial challenge with pigeon dropping antigen in 31% of exposed individuals (breeders, their family members, neighbours) (24). The study showed that the positive precipitating antibodies in the serum of individuals exposed to pigeon droppings are an important diagnostic parameter for PBD. However, the definitive diagnostic confirmation of PBD should be provided by bronchial challenge with pigeon dropping extract, which with its lower antigen concentration is safer than challenge by natural exposure (1, 2, 16). The existence of the so-called asymptomatic PBD form could not be confirmed by our study.

PBD as well as its clinical manifestation, the late bronchial response, are presumed to be caused by Type III (late) hypersensitivit

(Arthus reaction, immune-complex mechanism) (3, 6, 9, 10, 14, 15, 25, 26, 28, 31). This hypothesis is based on the existence of precipitating antibodies (IgG, IgM) in the patient's serum which are involved in the forming of immune complexes (11, 27), and on the histological changes in lung biopsies corresponding to the changes typical for the Arthus reaction (10, 24, 26).

Other authors, however, have suggested the possible involvement of Type IV (delayed) hypersensitivity (cell-mediated hypersensitivity) in PBD. They have detected lymphocyte transformation and the macrophage migration-inhibition factors in some of the PBD patients (7).

Classically, it has been presumed that PBD is a typical hypersensitivity state localized only in the lung tissue and in no other organ. In the literature available we have not yet found any report of possible extrapulmonary localization of PBD. Our preliminary study (23) probably provided the first evidence for the possible existence of a nasal form of PBD.

Late nasal response to challenge with pigeon dropping antigens, described in our previous (23), and this present study seems to be clinically similar to the late broncho-obstructive response observed by us after bronchial challenge with the same antigen (24). The presence of precipitating antibodies in the serum of patients with LNR to pigeon droppings and the results of other diagnostic parameters support this hypothesis.

The LNR to pigeon droppings has also many similarities with the LNR observed by us after challenge with "inhalation" allergens (19, 21, 22). However, there are some differences between them (e.g. general malaise, blood leucocytes and eosinophils, body temperature, precipitating antibodies, etc.).

LNR to pigeon droppings was associated in different degrees with various *in vitro* and *in vivo* parameters; late nasal complaints being highest (100%), followed by precipitating IgG antibodies in the patient's serum to pigeon droppings (80%), late skin responses to the same antigen (60%) and general malaise (52%).

Interpretation of the double immuno-diffusion results is not without difficulties. These results are characterized by the appearance of precipitating lines, which can vary both in number and thickness. According to our previous experience, two or more lines are considered significantly positive. Only one line could be a non-specific result (impurity of material) or possibly "cross-allergy".

A similar problem arises with the interpretation of skin tests. Ten of the 25 patients with positive LNR had negative late skin responses, while three patients with negative LNR showed positive late skin responses. This could perhaps be explained by a false positive skin response or by the presence of mechanisms in the skin, which differ from those in the nasal mucosa, or by the presence of precipitating IgG antibodies in the skin and/or serum which are absent in the nasal mucosa.

This study shows that intracutaneous tests are a useful *in vivo* supplementary parameter, but not the most important. The dilemma with skin tests is that they are performed on one organ and conclusions are drawn for other organs, in this case the nasal mucosa. Such an application also has its limitations. It is very important to follow the skin tests for at least 48 h after injection with respect to late skin response.

The nasal secretions during the LNR showed a mixture of various cells, with a slight prevalence of neutrophils in contrast to the picture found during the INR, where the eosinophils and the mast cells dominated. These findings are similar to those observed by us during the LNR to inhalation allergens (22). The presence of neutrophils in the nasal secretions could be seen as a supplementary argument for the possible involvement of Type III hypersensitivity in the LNR.

Another important finding was the appearance of mucosal haemorrhages in 52% of the patients during the LNR. Under certain circumstances, these mucosal haemorrhages can be considered comparable to histopathological changes typical of the Arthus reaction (Type III hypersensitivity) having been found in the lung tissue of hypersensitivity pneumonitis patients. For technical reasons, biopsies of the nasal mucosa have not been performed as routine examination. The results of incidental biopsies were therefore not reported. However, a microscopic deposition of precipitating antibodies in the nasal mucosa around the capillaries (with respect to haemorrhages) cannot be excluded. Therefore, in the future, nasal mucosa biopsies, X-rays of the sinusses and immunological tests of the nasal secretions in patients with the nasal form of PBD will be a useful supplement to the routine diagnostic procedure.

Another interesting fact was the appearance of a dual nasal response, as well as a dual skin response. This observation could indicate the presence of two different mechanisms, one due to the IgE and the other to the IgG (and IgM, IgA) antibodies, or a possible dual role of the IgG antibodies in the immediate and late responses (19, 21, 22, 23, 25, 26, 27, 28).

Conclusion

The late nasal response to pigeon dropping challenge accompanied by late skin responses, positive precipitating serum IgG antibodies, and other diagnostic parameters, suggests the existence of a new disease, a nasal form of pigeon breeder's disease.

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THE CHANGES IN THE NASAL SECRETIONS OF EOSINOPHILS DURING THE IMMEDIATE NASAL RESPONSE TO ALLERGEN CHALLENGE

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The changes in the nasal secretions of eosinophils during the immediate nasal response to allergen challenge

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In 162 patients with allergic rhinitis due to immediate hypersensitivity, nasal provocation tests (NPT) were supplemented by recording of the eosinophils in the nasal secretions (NS). Nasal secretion specimens were obtained before and repeatedly after allergen challenge and stained by a modified Hansel's method. The 188 positive immediate nasal responses (INR) that correlated with positive skin tests and history and 92 negative INR that correlated with negative skin tests and history were studied. Several different types of eosinophil response to allergen challenge were observed. Eosinophilia was found in the NS in 84% of patients with positive INR but in only 49% of patients with negative INR. The eosinophil count before allergen challenge was low in 79.5% of positive INR and in 76.5% of negative INR, whereas it was high in 20.5% of positive INR and in 23.5% of negative INR. The positive INR were accompanied by significant changes ($p < 0.01$) in the eosinophil count between before and after allergen challenge in 74% and the negative INR in only 19% of the cases. These changes appeared within 30 min after allergen challenge. This study shows that only a single count of eosinophils in the NS is not a suitable indicator of nasal allergy. The recording of eosinophils in the NS can be considered as a useful supplementary diagnostic parameter for the possible involvement of immediate hypersensitivity in the nasal mucosa if (1) the eosinophil count is related to a certain allergen and (2) the eosinophils are recorded before and repeatedly up to 60 min after allergen challenge. (J ALLERGY CLIN IMMUNOL 72:657-662, 1983.)

The eosinophil leukocyte, besides having a variety of other functions, plays one of the pivotal roles in the mechanism of the allergic reaction.[1-14] Its role in immediate hypersensitivity has been studied extensively.[2, 4, 6-10, 14, 15] However, some studies suggest that its role also in other types of hypersensitivity cannot be excluded.[6, 7, 10] The involvement of eosinophils in the allergic reaction is principally of two kinds: as a target cell, which is influenced and affected by previously released compounds from other cells (e.g., mast cells or basophil leukocytes), and as a source of a number of substances that, after release, participate in the modulating of other steps.[2, 5-8]

Several, sometimes controversial, papers discuss the possible role and significance of the eosinophil in allergic rhinitis.[8, 10-14, 16-39] The eosinophils in the blood,[19, 23, 27, 30, 31, 38] the nasal mucosa tissue,[4, 18, 21-23, 38, 39] and NS[10, 11, 13, 18, 20-38] have been studied in patients with allergic rhinitis. Nevertheless, the exact role of the eosinophil in allergic reactions in the nasal mucosa is not yet known in detail.

In practice, the appearance of eosinophils in the NS is presumed to be one of the clinical indicators of the involvement of an allergic reaction in rhinitis patients. This conclusion is usually drawn from only a single sample of the patient's nasal smear and in most cases is not directly related to other factors.[13, 22, 26, 31, 33, 35]

We regularly see patients suffering from allergic

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Abbreviations used

NPT: Nasal provocation test
INR: Immediate nasal response
NPG: Nasopharynx-nostril pressure gradient
MGG: May-Grünwald-Giemsa staining technique
NS: Nasal secretions
i.c.: Intracutaneous

rhinitis with an allergic component in which several diagnostic parameters are positive and the NS do not demonstrate any significant eosinophilia, or patients where the high number of NS eosinophils is one of the few clinical findings, or patients with some positive clinical parameters who demonstrate a high variation in the number of eosinophils in the NS.

The purpose of this study, being a continuation of our preliminary papers,[10-12] was to investigate the changes in the number of eosinophils in the NS in relation to challenge with a certain allergen. We also tried to describe these changes, to interpret them, and to determine the criteria upon which the occurrence of eosinophils in the NS could be accepted as a diagnostic parameter for nasal allergy.

MATERIALS AND METHODS

Patients

In 162 patients suffering from allergic rhinitis with a perennial or seasonal allergic component, or a combination of both, a routine diagnostic procedure was performed, including short-term NPTs with allergens. All these patients showed (1) nasal complaints (obstruction, hypersecretion, sneezing, itching) to various degrees; (2) positive disease history to one or more allergens; (3) positive immediate skin responses to more than one allergen; (4) positive results of short-term NPTs with various allergens. The patients were investigated during a symptom-free period. None of them had received previous immunotherapy, disodium cromoglycate or corticosteroids, within at least 1 yr before this study.

No antihistaminics or topical decongestants were given to them during the 3-day interval prior to this study.

Allergens

Allergens in the form of dialyzed and lyophilized extracts were diluted in Coca's solution (dry weight of allergen reported in milligrams per 1 milliliter of Coca's solution) and used for nasal challenges and skin tests in the concentrations summarized in Table I.

Skin testing

The scratch tests were performed first. If they were negative, then i.c. tests were carried out and evaluated after 20 min.

Nasal provocation tests

NPTs were carried out by the rhinomanometry technique, which has been described in detail in our previous papers.[10-12, 40, 41] The NPG values (expressed in cm H_2O) recorded by this technique were considered as basic parameters of the nasal mucosa response (= nasal obstruction). The schedule of the NPT was as follows: (1) recording of the "initial values" at 0, 5, and 10 min; (2) recording of the "Coca's solution values" at 0, 5, and 10 min after 3 min application of Coca's solution to the nasal mucosa of the nonintubated nasal cavity by means of a saturated wad of cotton wool on a nasal probe; (3) challenge of allergen for 3 min in the same way and manner as Coca's solution and recording of NPG values at 0, 5, 10, 20, 30, 45, 60, 90, and 120 min. The NPT was considered to be positive when the mean NPG values after allergen challenge increased by at least 2.0 cm H_2O with respect to the initial and Coca's solution values.

Nasal secretions

During the NPTs, samples of NS were obtained from the nonintubated nasal cavity by having the patient blow his nose onto a polyethylene sheet. Two specimens of NS were always taken and that twice before the allergen challenge (after the initial values and after the Coca's solution values) and six times after the allergen challenge at 0, 10, 20, 30, 45, and 60 min. In some of the patients, specimens of the NS were collected every hour up to 10 hr after the allergen challenge, and in sporadic cases the NS specimens collecting was continued up to 3 days after NPT.

One series of air-dried specimens was processed by polyethylene glycol and stained by Hansel's method,[13, 28, 29] modified by the author,[10] and the other series was stained by a modified MGG[10, 14] as a control technique. According to our previous studies, in the case of Hansel's method, we used double fixation, air-drying, and polyethylene glycol (Carbowax; Union Carbide Corp., New York, N. Y.), allowing a better adhesion of the material to the glass slide.

The specimens were dehydrated by methyl alcohol, mounted in Canada balsam, and then scanned microscopically as "double-blind" for the technician. The whole slide, usually 50 microscopic fields (magnification 250×), was always examined and the leukocytes including eosinophils were recorded by means of a differentiation count. Doubtful cells were re-examined under oil immersion (magnification 1000×). The number of eosinophils was expressed as a percentage of 100 leukocytes, calculated from the whole slide.

The eosinophil percentage was graded as follows: 0 to 5% = −; 5% to 10% = ±; 10% to 25% = +; 25% to 40% = +±; 40% to 60% = ++; 60% to 80% = ++±; 80% to 100% = +++. Changes in the eosinophil count less than 10% were regarded as nonsignificant ($p > 0.05$).

Control test with Coca's solution

In 56 patients a control test with Coca's solution was also performed 5 days after the NPT. The Coca's solution was applied to the nasal mucosa in the same way as previously and the NPG were then recorded up to 120 min by the same schedule as that during the NPT. Samples of NS were obtained, processed, and stained in the same way as those during the NPT with allergen.

TABLE I. Survey of the allergens used for skin tests and nasal challenges

	Concentration (per 1 ml of Coca's solution)	
	Nasal challenge	Scratch and intracutaneous tests
House dust	5.0 mg	0.5 mg
Dog danders	2.5 mg	0.25 mg
Cat danders	2.5 mg	0.25 mg
Hamster danders	2.5 mg	0.25 mg
Horse danders	2.5 mg	0.25 mg
Moulds mixture	2.0 mg	0.2 mg
Mites *(D. pteronyssinus)*	100 NU	10 NU
Grass pollen mixture	10,000 NU	1,000 NU
Spring pollen mixture	10,000 NU	1,000 NU
Weed pollen mixture	10,000 NU	1,000 NU

Allergens: dry weight of dialyzed and lyophilized allergen extracts in mg; 1 Noon unit (NU) = 0.001 mg of dry pollen (powder) = 0.5 PNU = 1.3 TNU.

Statistical evaluation

The nasal response to allergen challenge was statistically evaluated by Wilcoxon matched paired test. The changes between the eosinophil count before and after allergen challenge within the same group (positive NPT group and negative NPT group) were evaluated by Wilcoxon paired test. A p value <0.05 was considered to be significant.

RESULTS

In 162 patients, a total of 373 NFTs with allergen were performed. From the 373, 304 NPTs (81%) correlated with disease history and i.c. tests. The 205 positive INRs ($p < 0.05$) correlated with positive disease history and positive skin tests, and 99 negative INRs ($p < 0.01$) correlated with negative disease history and negative skin tests. The 69 noncorrelating nasal responses (41 positive and 28 negative) were rejected from this study and will later be the subject of another investigation. Twenty-four correlating INRs (17 positive and 7 negative) were withdrawn from this study because NS could not be obtained.

Several different types of eosinophil response to allergen challenge were observed during the 188 positive and 92 negative INRs. They can be summarized as follows: (1) no changes in the eosinophil number between before and after allergen challenge; (2) an increase in the number after challenge, which persisted throughout the period of observation; (3) an initial increase in number, followed by a decrease; (4) a decrease; (5) a decrease initially, followed by an increase in the number of eosinophils. The survey of the changes in the eosinophil count between before and after allergen challenge in the positive and negative INR groups is presented in Table II.

TABLE II. Survey of the changes in the eosinophil count in NS during the positive and negative nasal challenges

	Eosinophil count in NS (% of total leukocytes)	
INR	Before challenge	30-60 min after challenge
Positive (n = 188)	Low = 79.5%*	High = 57.5% Low = 22.0%
	High = 20.5%	High = 4.0% Low = 16.5%
Negative (n = 92)	Low = 76.5%†	High = 8.5% Low = 68.0%
	High = 23.5%	High = 13.0% Low = 10.5%

*Including 16% of total absence of eosinophils.
†Including 51% of total absence of eosinophils.

No significant differences in the eosinophil count were found between the initial value and the Coca's solution value ($p > 0.1$). The presence of eosinophils in the NS was found in 84% of patients with positive INR but in only 49% of those with negative INR. The eosinophil count before allergen challenge was low (up to +) in 79.5% of patients with positive INR and in 76.5% of those with negative INR and it was high (up to +++) in 20.5% of positive INRs and in 23.5% of negative INRs. The positive INRs were accompanied by significant changes ($p < 0.01$) in the eosinophil count between before and after allergen challenge in 74% of cases, which appeared within 30 min after allergen challenge and lasted several hours. In 26% of the INR positive, this count was not significantly changed ($p > 0.05$). During the negative INR, the eosinophil count between before and after allergen challenge changed significantly ($p < 0.05$) in 19% of cases, but in 81% of cases this count did not show any changes ($p > 0.1$). No relationships were observed between the individual allergens tested and the individual types of eosinophil response. The changes of the NPG values recorded during the con-

trol tests with Coca's solution in all 56 patients varied only within 1.4 cm H_2O and were not significant with respect to the initial NPG values ($p < 0.1$). No significant changes in the eosinophil count were observed between before and after application of Coca's solution during the control test with Coca's solution ($p > 0.05$) in all 56 patients. These changes varied within 10%. The reproducibility of the technique was satisfactory. The results of the control series of specimens, stained by MGG did not differ significantly from results with the specimens stained by Hansel's staining, either with respect to the differential count of leukocytes or with respect to the percentage of eosinophils. However, the specimens stained by the modified Hansel's technique were of slightly better quality and easier to evaluate than those stained by MGG.

DISCUSSION

Various papers deal with the presence of eosinophils in the nasal mucosa and in the NS[8, 13, 14, 17, 18, 20–38, 42] and with their significance for nasal allergy, especially of the immediate type. In most of these papers, only a single specimen of nasal smear has been studied.

We were unable to find any report in the available literature that investigated extensively the changes in the eosinophil count in the NS and their types in relation to allergen challenge in a large group of patients with allergic rhinitis.

The allergic reaction in the nasal mucosa is a process that is always caused by a certain allergen. Therefore, if the eosinophils are involved in this reaction, then their count should be related to the certain allergen. The involvement of the eosinophil in the allergic reaction, being a dynamic process, can be characterized only by comparing of the changes before and after the certain allergen. The eosinophil count and its changes should be regarded as a confirmation of the role of a certain allergen in causing a certain response in the nasal mucosa and not as a general diagnostic parameter only.

The examination of the NS for eosinophils seems to be a single technique, at first sight. However, with respect to some technical aspects concerning the collecting and processing of the NS, as well as to the interpretation of the results, this technique is not so easy and requires a certain degree of experience. The NS can be collected by wiping from the nasal cavity with a cotton-tipped wiper or swab,[13, 14, 22, 25, 26, 31, 34–36] by aspiration with a suction tip or syringe,[13, 30] or by blowing the nose onto a waxpaper or polyethylene sheet.[13, 23, 24, 36] Each of these techniques has its advantages and disadvantages. We used the blowing technique because it seems to us to be the most similar to the natural clearance of the nose.

Various methods of recording, evaluating, and interpreting the cytologic findings in the NS have been described.[13, 14, 19, 22–26, 28, 29, 31, 34, 35] Hansel's method, which records the eosinophils in relationship to the neutrophils by means of plus scores,[13, 26, 28, 29, 31] is used frequently. Some authors recorded the eosinophils only by means of an approximated grade system,[23, 24, 34, 35] or counted the eosinophils per microscopic field (e.g., high-power field),[25] or recorded the eosinophils as a percentage of the total leukocyte count.[14, 19, 21, 22, 43] However, in most of these studies, the specimens were examined by scanning of a limited number of microscopic fields. The distribution of the cells in the NS, as well as the density of the secretions, is not always regular and cannot satisfactorily be standardized. Therefore we consider this technique as only semiquantitative. With respect to this disadvantage, we always examined the whole slide, and all leukocytes were recorded. The eosinophils were then expressed as a percentage of 100 leukocytes calculated from the whole slide. In this very laborious manner, we tried to minimize the technical disadvantage. Our method is similar to that used by O'Connell et al.[44] for the counting of sputum eosinophils.

Although the differential count of leukocytes is more suitable for the evaluation of blood smears, which are a homogeneous material,[7] we found from our preliminary studies that this method is also valuable for the counting of eosinophils in the NS. The exfoliative process in the nasal mucosa is not limited only to eosinophils. Therefore the differential count supplies information about the whole spectrum of leukocytes in the specimen and their proportions, especially that between eosinophils and neutrophils.

In most of the papers where the presence of eosinophils in the NS was used as one of the indicators of nasal allergy,[13, 20, 23–29] the eosinophil count was performed as a single determination at an arbitrarily chosen time. The presence of a high number of eosinophils in the NS was interpreted as an additional confirmation of nasal allergy. A low number of eosinophils or their absence in the NS was explained as a less probable involvement of an allergic reaction.[13, 22–35]

The results of our present study demonstrate that in the majority of patients the number of eosinophils before allergen challenge was low (79.5% of positive INRs and 76.5% of negative INRs) and that no differences were found between the positive INR group and the negative INR group. Therefore only a single count of eosinophils in the NS seems to us to be a doubtful test.

Our findings of a high-level eosinophil count without changes in 13.0% of patients with negative INR can be similar to the NARES syndrome described by Jacobs et al.,[19] Mullarkey et al.,[17] and Connell,[18] who found nasal eosinophilia in patients with nonallergic rhinitis. Our results of a total absence of eosinophils in 16.0% of positive INRs can be similar to the observation of Connell,[18] who did not find any eosinophils in the nasal mucosa and NS in a number of allergic rhinitis patients.

Our results demonstrating that only the changes in the eosinophil count in the NS related to challenge with a certain allergen can be an indicative parameter for nasal allergy are supported by Mygind's[14] similar findings. On the other hand, Mygind's and our study are not fully comparable because he studied only five patients and recorded the eosinophils up to 3 days after allergen challenge. In our opinion, the recording of the eosinophils before and up to 1 hr after allergen challenge is a more adequate schedule that can be better related to a certain allergen. During a longer interval, an exposure to other allergens or nonspecific agents influencing the eosinophil kinetics cannot be excluded.

The most important finding in this present study was the observation of the significant changes in the number of eosinophils before and after allergen challenge in 74% of patients having positive INR and only in 19% of cases of patients with negative INR. This finding emphasizes that only the changes in the number of eosinophils related to a certain allergen (recorded before and after allergen challenge) can be accepted as a decisive supplementary parameter for the possible involvement of the immediate hypersensitivity in the nasal mucosa.

The role and involvement of the eosinophil in the immediate hypersensitivity reactions are manifold.[1–9, 13–15, 38] However, they are not yet known in detail. One of the most important questions concerns the mode of involvement. Is the eosinophil a part of this mechanism or a consequence of it? Or is its involvement a combination of both of these (e.g., a dual role)? Our results can imply the following hypothesis. The fact that the number of eosinophils in the NS increased within 30 min after allergen challenge in 57.5% of positive INRs but in only 8.5% of negative INRs can indicate that eosinophils have been attracted to the nasal mucosa and subsequently into the NS during the later stage of the allergic reaction mechanism. After antigen-antibody interaction, the mediators and eosinophil chemotactic factors released from the mast cells (basophils) acted on the effector organs, resulting in the appearance of various symptoms, one of these being an increase in the eosinophil number. The topical eosinophil accumulation can therefore be interpreted as a consequence of the positive allergic reaction of the immediate type.

On the other hand, the decrease in the number of eosinophils after allergen challenge in 16.5% of patients with positive INR can be considered as a consumption of directly involved eosinophils. This can indicate the possible involvement of eosinophils in the early stage of the allergic reaction mechanism, probably as an integrated part of this reaction.

From another point of view, 74% of "positive INRs with changes in the eosinophil count," characterized by the relationships "increase-decrease" and "decrease-increase," can be seen as a possible dual involvement of the eosinophil in the allergic reaction. In this way, the eosinophil can be regarded as an integrated part of some of the steps and also as a consequence of other steps of the immediate hypersensitivity mechanism.

Implications for clinical practice

1. Only a single recording of the eosinophils in the NS is not a suitable indicator of immediate hypersensitivity in the nasal mucosa.
2. The recording of the eosinophils in the NS can be considered as a useful diagnostic supplement if (a) the eosinophil count is related to a certain allergen and (b) the eosinophils are recorded before and repeatedly after the allergen challenge.
3. Only the changes in the eosinophil count after allergen challenge compared to that before challenge should be accepted as a supplementary diagnostic parameter.

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CYTOLOGIC CHANGES IN THE NASAL SECRETIONS DURING THE IMMEDIATE NASAL RESPONSE

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Cytologic changes in the nasal secretions during the immediate nasal response

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In 102 randomly selected patients with allergic rhinitis caused by immediate hypersensitivity, nasal provocation tests (NPTs) with allergens were evaluated by means of rhinomanometry, and cytologic examination of the nasal secretions (NSs) was performed. The cells in NS of 117 positive immediate nasal responses (INRs) and in 68 negative INRs, correlating with history and skin tests, and in 102 control challenges with phosphate-buffered saline were stained by modified May-Grünwald-Giemsa, toluidine blue, and Hansel's method. The positive INR was accompanied by significant changes in the count of eosinophils (increase followed by decrease) in 67% of neutrophils (decrease followed by increase) in 40%, goblet cells (increase followed by decrease) in 41%, and basophils (decrease) in 13% of the NSs. No significant changes in the count of other types of cells in the NSs were recorded during most of the cases of INR. No significant changes in the count of individual cell types in NSs were found during most cases of negative INR. During the phosphate-buffered saline control challenges, the individual cell types appeared irregularly, and no significant changes in their count were recorded in any patient. The cytologic examination of NS, evoked by allergen, appears therefore to be a valuable supplementary diagnostic parameter for nasal allergy. The repeated counting of eosinophils in NS, before and after allergen challenge, appears to be the best way to discriminate between positive and negative nasal responses, since the eosinophils demonstrated significant changes in their count during 67% of the positive and only 11% of the negative INRs. (J ALLERGY CLIN IMMUNOL 1988;82:1103-12.)

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The appearance of various cells in NS, especially of eosinophils and mast cells/basophils, and their significance for the nasal allergy has been studied for several years.[1-29]

Studies concerning the appearance of other cell kinds in NS are not numerous.[16, 22] We did not find any article in the available literature describing extensively the appearance of all types of cells and the

Abbreviations used
NPT: Nasal provocation test
INR: Immediate nasal response
NS: Nasal secretions
PBS: Phosphate-buffered saline
IH: Immediate hypersensitivity
NPG: Nasopharynx-nostril pressure gradient expressed in cmH_2O

changes in their count during INR in a large group of patients.

In practice, the appearance of eosinophils in NS is presumed to be an indicator for the nasal allergy. This presumption is usually made from a single sample of the patient's NS and is, in most cases, not directly related to a particular allergen.[9, 14, 16, 18, 27] Other authors suggested the appearance of mast cells/basophils in NS to be a diagnostic parameter for the nasal allergen.[1, 12-14, 17, 19, 21, 23]

Nevertheless, the exact role and involvement of eosinophils, as well as of mast cells/basophils in hypersensitivity reactions in the nasal mucosa and in various types of nasal response, is not yet known in detail. There is a dearth of information concerning the role and significance also of other cell kinds in NS, for example, neutrophils, goblet cells, lymphocytes, etc., for the particular types of hypersensitivity in the nose and the types of nasal response.

The purpose of this study, since it is a continuation of our previous studies,[2-5] was (1) to investigate the changes in the count of individual cell types in NS during the INR to allergen challenge caused by IH (type I) mechanism and (2) to evaluate the possible significance of such changes for the IH in the nose.

MATERIAL AND METHODS

Patients

The 102 patients, 18 to 40 years of age, suffering only from allergic rhinitis, with perennial or seasonal allergic component or a combination of both, were randomly selected for this study. All these patients demonstrated (1) nasal symptoms (obstruction, hypersecretion, sneezing, and itching) in various degrees, (2) positive disease history to one or more allergens, (3) positive skin tests (immediate skin response) to one or more allergens, and (4) no increased responsiveness to histamine in the nose. Patients were investigated in a period of minimal complaints and without nasal infections, most of them during hospitalization. None were suffering from other disorders, other forms of allergy, adverse reactions to foods, had polyps, or had had surgery of the nose. None had had previous immunotherapy or had received oral corticosteroids. No disodium cromoglycate or topical corticosteroids were used for 8 weeks and no antihistamines or topical decongestants for 24 hours before this study. With respect to disease history, skin tests, and RAST, all being either positive or negative, allergens were chosen for the nasal challenges. The 128 positive INRs ($p < 0.05$), correlating with positive history and positive skin tests, and the 80 negative INRs ($p < 0.01$), correlating with negative history and negative skin tests, were repeated 3 to 5 days later and supplemented by collection of the NS. In each of the 102 patients, a control challenge with PBS was also carried out and supplemented by histologic examination of the NS. During 23 responses (11 positive and 12 negative), no NS could be obtained, and these patients were therefore excluded from the study.

Control group

In a group of 78 healthy volunteers, without any rhinitis complaints, NS samples were collected before and four times after the challenge with PBS, at 0, 30, 60, and 120 minutes, and histologically examined by the same technique as will be described.

Allergens

Allergens (Diephuis Laboratory, Groningen, The Netherlands), in the form of dialyzed and lyophilyzed extracts, were diluted in PBS (dry weight of allergen in milligrams per 1 ml of PBS) and used for skin tests in the concentrations as follows: house dust, 0.5 mg/ml; animal danders, 0.25 mg/ml; molds, 0.2 mg/ml; *Dermatophagoides pieronyssinus*, 10 nU/ml; and pollen kinds, 1000 Noon units per milliliter. The concentrations of allergens used for the nasal challenges were always 10 times higher.

Skin tests

Scratch tests were first performed. If these tests were negative, intracutaneous tests were carried out and evaluated after 20 minutes and 6 hours and were then followed every 24 hours, up to 96 hours, according to a standard routine schedule. Skin reaction (wheal), appearing 20 minutes after the intradermal allergen injection, was considered a positive immediate skin response.

NPTs

NPTs were performed by means of the rhinomanometry technique, described in detail in our previous articles.[30, 31] The NPG values recorded by this technique were considered to be basic parameters of the nasal mucosa response (nasal obstruction). The schedule of the NPT was as follows: (1) initial values recorded at 0, 5, and 10 minutes, (2) PBS values recorded at 0, 5, and 10 minutes after a 3-minute application of PBS to the nasal mucosa of the nonintubated nasal cavity by means of a saturated wad of cotton wool on a nasal probe, and (3) challenge with allergen for 3 minutes by the same technique as for the PBS and recording of NPG values at 0, 5, 10, 20, 30, 45, 60, 90, and 120 minutes. The INR was considered to be positive when the mean NPG values after allergen challenge increased by at least 2.0 cmH_2O (1.2 ± 0.3 = mean ± SE) with respect to the PBS values and at three consecutive time intervals within 60 minutes.

TABLE I. Survey of the presence of the individual cell types in NS and the significant changes in their count during the positive and negative INR

	Presence of cells		Significant changes in the cell count before and after allergen	
	INRs		INRs	
	Positive (n = 117) (%)	Negative (n = 68) (%)	Positive (n = 117) (%)	Negative (n = 68) (%)
Eosinophils	85	56	67	11
Basophils plus mast cells	16	11	13	2
Neutrophils	91	66	40	4
Goblet cells	87	34	41	4
Lymphocytes	11	2	2	0
Epithelial cells	68	63	0	0
Plasma cells	7	5	0	0
Monocytes	3	4	0	0

NSs

The NS specimens were obtained from the nonintubated nasal cavity by having the patient blow onto a polyethylene sheet immediately after every NPG recording, twice before (initial and PBS values), and seven times after allergen challenge at 0, 10, 20, 30, 45, 60, and 120 minutes. The NS specimens were divided into three portions, transferred to microscopic slides, and spread out on the slide surface by means of a glass probe.

The first series of air-dried specimens was additionally fixed by polyethlene glycol and stained according to the method of Hansel,[1, 13] modified by the authors,[2, 4, 5] the second air-dried series was stained by a modified May-Grünwald-Giemsa,[2, 4] and the third series was fixed by methanol and stained by the toluidine blue method.[32] Specimens were dehydrated by methyl alcohol, mounted in Canada balsam, and scanned microscopically.

Two techniques of cell counting were used: (1) the absolute number of individual cell types was counted per microscopic field (magnification ×250), and means were calculated from 25 fields spread over the whole slide, and (2) the leukocytes were recorded by means of a differential count from 25 fields spread over the whole slide (magnification ×250), and the number of their individual subtypes was expressed as a percentage of 100 leukocytes. The differential count was used as a control technique. Doubtful cells were reexamined under oil immersion (magnification ×1200).

The appearance of the individual cell types was evaluated as follows: −, no appearance at all; ±, very slight; +, slight; + ±, moderate; + +, distinct; + + ±, large; and + + +, very large.

The statistically significant magnitude of changes in the count of individual cell types in NS in patients with allergic rhinitis between two consecutive degrees was as follows (mean ± SE): eosinophis, 7 (7.17 ± 0.91); basophils and mast cells, 2 (2.26 ± 0.71); neutrophils, 8(8.33 ± 0.56); lymphocytes, 2 (2.0 ± 0.25); monocytes, 1 (1.26 ± 0.21); plasma cells, 1 (1.27 ± 0.21); epithelial cells, 5 (5.00 ± 0.63); and goblet cells, 4 (4.15 ± 0.65). The lowest difference in the count of the particular cell types, which was found to be statistically significant (significant magnitude), is ± (very slight), as is expressed in the figures.

Control tests with PBS

This test was performed in each patient, 3 days before the first NPT, and the NS samples were processed in the same way as during the NPT with allergen.

Statistical analysis

The INR was statistically evaluated by Wilcoxon matched-pair signed-rank test, comparing the NPG values recorded after the allergen challenge with the mean NPG value of PBS. A p value of <0.05 was considered to be statistically significant.

The positive and negative INRs were compared and statistically evaluated by means of the Mann-Whitney U test. (A $p < 0.05$ was considered to be statistically significant.)

The changes in the count of individual cell types were statistically analyzed by the Wilcoxon paired test. A p value <0.05 was considered to be statistically significant. Each of the cell types recorded during the individual NPT, positive INR, negative INR, or control PBS, was analyzed separately. The numbers of cells recorded after allergen challenge at each of the times were compared with that recorded after PBS.

If significant differences in the cell count were found at least three times after allergen challenge, compared with that after PBS, it was concluded that this cell type demonstrated significant changes during that nasal response.

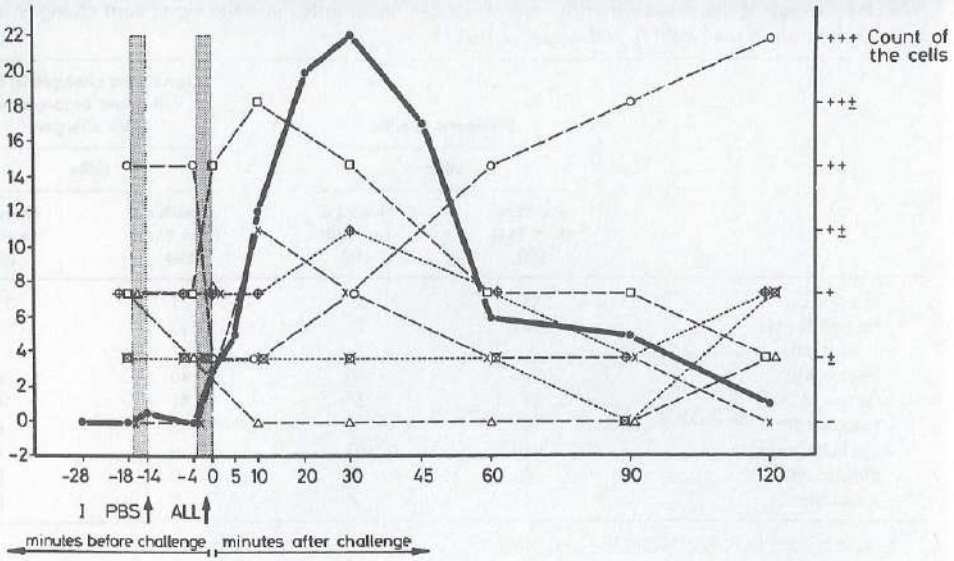

FIG. 1. The mean NPG values recorded after the allergen challenge with respect to the appropriate PBS NPG values were calculated from all 117 positive INRs. The mean changes in the count of the individual types of cells in the NSs were calculated from all positive INRs; positive immediate nasal response (●——●); eosinophils (□ - - - □); basophils (△ - - - △); neutrophils (○ - - - ○); goblet cells (x - - - x); lymphocytes (⊠ · · · · · ⊠); epithelial cells (⊕ · · · · · ⊕); I, initial value; PBS, PBS value; ALL, allergen challenge.

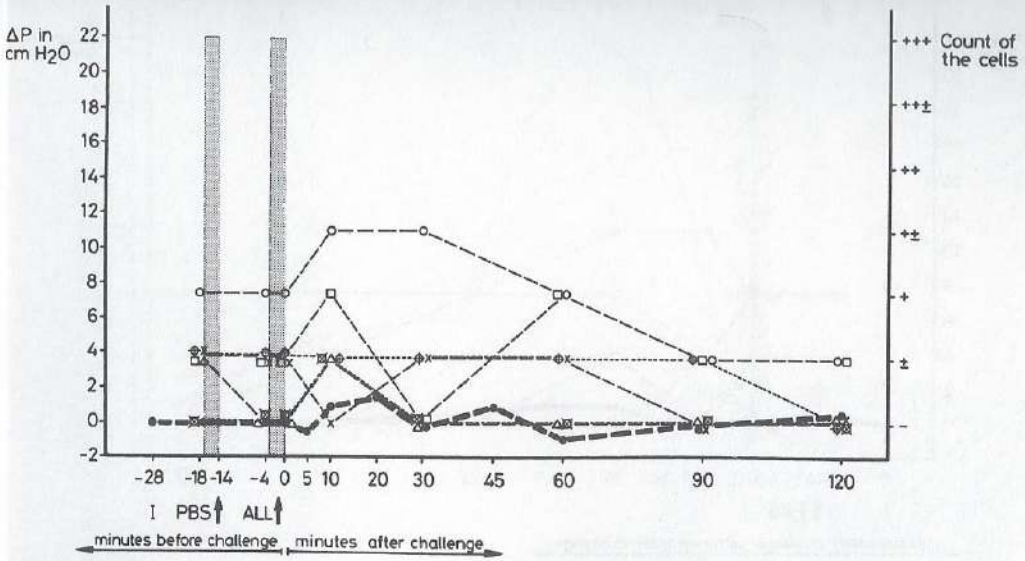

FIG. 2. The mean NPG values recorded after the allergen challenge with respect to the appropriate PBS NPG values were calculated from all 68 negative INRs. The mean changes in the count of the individual types of cells in the NSs were calculated from all negative INRs; negative immediate nasal response (●——●); eosinophils (□ - - - □); basophils (△ - - - △); neutrophils (○ - - - ○); goblet cells (x - - - x); lymphocytes (⊠ · · · · · ⊠); epithelial cells (⊕ · · · · · ⊕); I, initial value; PBS, PBS value; ALL, allergen challenge.

RESULTS

The 117 positive INRs ($p < 0.05$), 68 negative INRs ($p < 0.01$), and 102 control PBS tests were the subject of this study. The differences between the positive and negative INRs, evaluated by the Mann-Whitney U test, were statistically highly significant ($p < 0.0001$) at all time intervals.

The 102 control challenges with PBS did not demonstrate any significant changes of NPG ($p > 0.1$).

The presence of the individual types of cells in the NS and the changes in their count before and after the allergen challenge are summarized in Table I.

The cell influx into NS in the appropriate groups was as follows:

Positive immediate nasal response (n = 117)

The changes in the count of the particular cell types in NS during the positive INR are summarized in Fig. 1.

No significant changes in the count of any type of cell were found between the initial and PBS values ($p > 0.05$).

Eosinophils were present in NS in 85% of the positive INRs. Before the allergen challenge, their count was low (up to +) in 65% and high (up to + + +) in 20%. The positive INRs were accompanied by significant changes ($p < 0.05$) in the eosinophil count after allergen challenge, compared with that before the challenge in 67% of the cases. In 54% of the INR cases, the eosinophil count increased after allergen challenge and then decreased, whereas in 13% of the cases, their count decreased. The changes appeared mostly within 30 minutes after the allergen challenge and lasted up to 2 hours at least.

Neutrophils were present in NS in 91% of the positive INRs. Before allergen challenge, their count was low (up to +) in 23% and high (up to + + +) in 68% of the INR cases. Their count demonstrated significant changes after the allergen challenge, compared with that before the challenge in 40% of the INRs ($p < 0.05$). In most of the INR cases (37%), the neutrophil count decreased after allergen challenge and then increased.

Basophils and mast cells were found in NS in 16% of the positive INRs. Their count demonstrated significant decrease after allergen challenge during 13% of the INRs ($p < 0.01$).

Epithelial cells were present in NS in 68% of the positive INRs. The changes in their count, recorded during 11% of the positive INRs, were nonsignificant ($p > 0.1$) and irregular.

Goblet cells were present in NS in 87% of the positive INRs. Their count increased significantly after the allergen challenge in 41% of the INR cases ($p < 0.05$).

Lymphocytes appeared in NS in 11% of the positive INRs. Their count changed significantly in only 2% of the INRs ($p < 0.05$), demonstrating a slight decrease, followed by a slight increase during the nasal response.

Plasma cells and monocytes appeared in NS in 7%, respectively, in 3% of the INRs without any significant changes in their count ($p > 0.1$).

Statistically significant correlation of the NPG changes during the positive INR and changes in the count of the particular cell types were found as follows: (1) for eosinophils, 0, 10, 30, and 120 minutes ($p = 0.0001$); (2) for neutrophils, 0, 10, 30, and 120 minutes ($p = 0.0001$); (3) for goblet cells, 0, 10, 30, 60, and 90 minutes ($p = 0.0001$); (4) for basophils/mast cells, 10 and 30 minutes ($p = 0.0001$); and (5) for epithelial cells, 30 minutes ($p = 0.0001$).

TABLE II. Survey of the count (mean value ± SE) of individual cell types in NS in control subjects before PBS challenge

Eosinophils	4 (3.90 ± 0.40)
Basophils plus mast cells	0 (0.48 ± 0.12)
Neutrophils	5 (5.00 ± 0.63)
Epithelial cells	6 (6.20 ± 0.92)
Goblet cells	1 (1.20 ± 0.28)
Lymphocytes	1 (1.20 ± 0.26)
Plasma cells	0 (0.40 ± 0.13)
Monocytes	1 (1.10 ± 0.30)

The statistically nonsignificant changes in the count of lymphocytes, plasma cells, and monocytes did not correlate with INR at all ($p > 0.1614$).

Negative INR (n = 68)

The changes in the count of individual cell types in NS during the negative INR are presented in Fig. 2.

No significant changes in the count of any type of cell were found between initial and PBS values ($p > 0.1$).

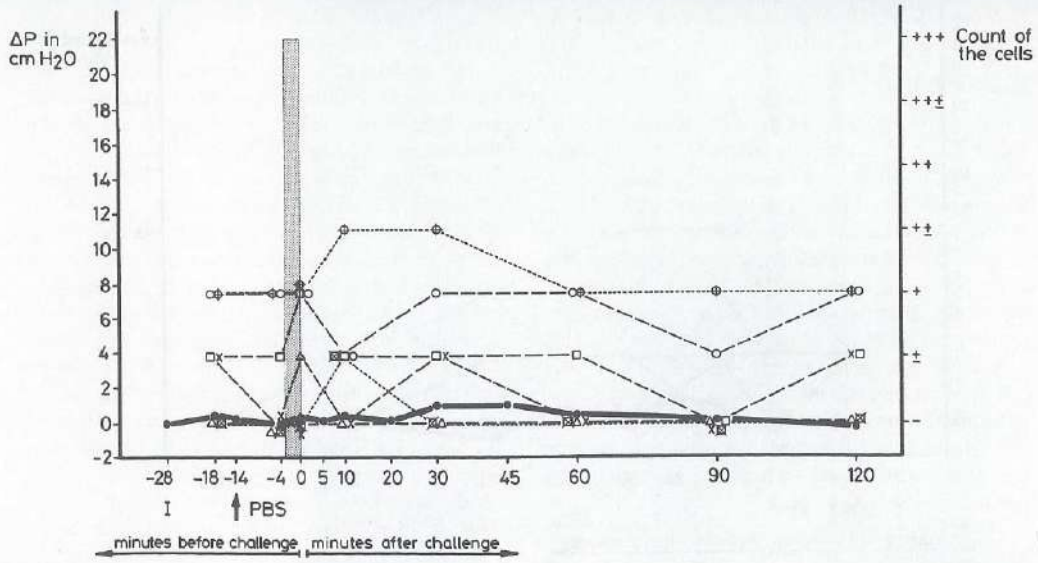

FIG. 3. The mean NPG values recorded after PBS challenge (control) with respect to the appropriate initial NPG values were calculated from all 102 control challenges; control challenge with PBS (●——●) (n = 102 = 100%); eosinophils (□ - - - □); basophils (△ - - - △); neutrophils (O—O); goblet cells (x - - - x); lymphocytes (⊠ · · · · · ⊠); epithelial cells (⊕ · · · · · ⊕); I, initial value; PBS, PBS value.

Eosinophils were present in NS in 56% of negative INRs. Their count before allergen challenge was low (up to +) in 35% and high (up to + + +) in 21% of the INRs. Significant changes in their count ($p < 0.05$), an increase in eosinophils within 30 minutes after the challenge lasting up to 2 hours, were recorded in only 11% of the INR cases.

Neutrophils were present in the NS in 66% of the negative INRs. Significant changes in their count, a slight increase in their number after allergen challenge, followed by a slow decrease, were recorded in 4% of the INR cases ($p < 0.05$).

Basophils and mast cells were found in NS in 11% of the negative INRs. Significant changes in their count, a slight increase after allergen challenge, were recorded during 2% of the responses ($p < 0.05$).

Epithelial cells were present in NS in 63% of the negative INRs, however without any significant changes in their count after the allergen challenge ($p > 0.1$).

Goblet cells were present in NS in 34% of the INRs. Significant changes in their count, a slight decrease after allergen challenge, were found in 4% of the negative INRs ($p < 0.05$).

Lymphocytes, plasma cells, and monocytes appeared in the NS only sporadically: lymphocytes in 2%, plasma cells in 5%, and monocytes in 4%, without any significant changes in their count between before and after allergen challenge ($p > 0.2$).

PBS control challenge (n = 102)

Changes in the count of individual cell types and their course during this challenge are illustrated in Fig. 3. The individual types of cells appeared in NS before the PBS challenge to a low degree and irregularly.

No significant changes in the count of any of the individual cell types were recorded after the PBS challenge, compared with that before the challenge. The statistical significance for the individual cell types was as follows: eosinophils, $p > 0.05$; neutrophils, $p > 0.1$; basophils and mast cells, $p > 0.05$; epithelial cells, $p > 0.05$; goblet cells, $p > 0.05$; lymphocytes, $p > 0.1$; and plasma cells and monocytes, $p > 0.2$.

Control group of healthy subjects

The count of individual kinds of cells in NS of the control subjects (n = 78) before PBS challenge (baseline) is presented in Table II. No significant changes in the count of particular types of cells were found between the baseline and any of the four time intervals (0, 30, 60, and 120 minutes) after the PBS challenge ($0.1 > p > 0.05$).

DISCUSSION

The allergy reaction in the nasal mucosa is a dynamic process caused by a certain allergen in which various types of cells are involved in various steps. This is also an exfoliative process leading to release of various cells into the NS. The involvement of individual cell types in the allergic reaction can be characterized only by comparing the changes before and, repeatedly, after the specific allergen.[5] The cytologic examination of NS is a relatively easy and valuable technique for evaluating the changes in the particular cell types appearing in NS during the allergic responses. By contrast, this method is limited only to the NS and does not evaluate the cellular changes in the nasal mucosa tissue, which could, however, be derived from a biopsy specimen only.

The NS cytogram, collecting and processing of NS specimens and interpretation of the results, requires a certain experience. The NS can be collected by wiping with a cotton-tipped wiper or swab,[9, 11, 14, 17-19, 29] by aspiration with a suction tip or syringe,[33] or by blowing the nose onto a wax paper or polyethylene sheet.[5, 10, 13, 19] We used the blowing technique because it is most similar to the natural clearance of the nose and not traumatizing to the nasal mucosa.

Various methods of recording and evaluating of cytologic findings in the NS have already been described.[6-11, 21-23, 29]

According to the method of Hansel,[1] recording eosinophils in relationship to the neutrophils by means of plus scores is used frequently.[1, 13, 14] Other authors recorded eosinophils in NS by means of an approximated grade system only,[10, 17, 18] counted the cells per microscopic field,[11] or recorded the cells as a percentage of the total leukocyte count.[6, 8, 9, 29] In most of these studies, only a limited number of microscopic fields was scanned. The distribution of the cells in NS, as well as the density of the secretions, is not always regular and cannot be satisfactorily standardized. Therefore, the counting techniques of the cells in NS should only be regarded a semiquantitative method.

With respect to this disadvantage, we always scanned the whole slide, at least 25 fields, recorded all cells, and calculated their mean values per microscopic field. The counting of absolute numbers of all cell types was used by us as the basic assessment of cytograms, while the leukocyte differential count was used as an additional control only. In this laborious manner, we tried to minimize the technical disadvantage. The differential count of leukocytes is more suitable for blood smears, being homogenous material,[34] than for the nasal smears.

The basic results were obtained from specimens stained by May-Grünwald-Giemsa, whereas staining according to the method of Hansel[1, 13] and toluidine blue staining were an additional control only.

In most studies of the cells in NS, the appearance of eosinophils and/or basophils/mast cells was investigated. The high number of eosinophils in a single sample of NS was interpreted as an indicator for nasal allergy, whereas their low number or absence to the contrary.[1, 7, 9-20, 33] The results of our previous study, as well as of this study, demonstrated that in most subjects the number of eosinophils before allergen challenge was low (in 76% of positive INRs and 79% of negative INRs) without any differences between the positive and the negative INR group. Therefore, only a single count of eosinophils in NS does not appear to us to be a suitable test. Although involvement of the eosinophil in the IH mechanism and its various steps are manifold, its role is not yet fully clarified in all detail.[5, 20, 29, 34-45]

Our findings of a high eosinophil count in 21% of the negative INRs may be similar to other investigators' studies of nasal eosinophilia in patients with nonallergic rhinitis.[6, 27] Our results of a total absence of eosinophils in 15% of the positive INRs would be similar to Connell's[28] observation.

The most important result of the present study was the recording of significant changes in the eosinophil count between before and after allergen challenge in 67% of the positive INRs and in only 11% of the negative INRs. This finding suggests that only the changes in the eosinophil count related to a certain allergen can be accepted as a supplementary parameter for the possible involvement of IH in the INR. This conclusion might be supported by Mygind's[29] similar findings.

An important question concerns the mode of involvement of eosinophils in INR. Is the eosinophil part of the IH mechanism, a consequence, or is its involvement a combination of both? Our results of increased number of eosinophils in the NS within 30 minutes after allergen challenge in 54% of the positive INRs, but in only 11% of the negative INRs, might indicate that eosinophils were attracted to the nasal mucosa and, subsequently, into the NS during the later stage of the IH mechanism. The topical eosinophil accumulation might therefore be interpreted as a consequence of the positive allergic reaction of the immediate type.[4, 5] Otherwise, the decreased number of eosinophils after allergen challenge in 13% of the positive INRs might be interpreted as a direct involvement of eosinophils in the early stages of the allergic

reaction, probably as its integrated part.[4, 5] Finally, 67% of the positive INRs were accompanied by changes in the eosinophil count, characterized by "increase-decrease" and "decrease-increase," which can be considered a "dual involvement" of the eosinophil in the allergic reaction. In this way, to us, the most probable way, the eosinophil can be regarded as an integrated part of some of the early steps and also a consequence or a target cell of another, mostly later, steps of the IH mechanism.[4, 5]

The pivotal role of basophils and mast cells in the classically understood IH mechanism has been confirmed frequently in the literature.[42-48]

The results concerning the appearance of mast cells and/or basophils in the NS vary highly. Some investigators, finding an increased number of mast cells in the NS, interpreted this as an indicator for the nasal allergy.[8, 14, 17-19, 21, 23, 26] Other investigators found no significant number of mast cells in the NS of patients with allergic rhinitis at all.[25, 29]

Other authors described only basophils in the NS.[24, 25] Still other investigators concluded that mast cells migrate into the epithelium and therefore occur predominantly in the nasal mucosa, whereas basophils migrate through the epithelium into the NS.[49, 50]

We were unable to find any study in the available literature of the appearance of basophils and mast cells, their relationship, differentiation, and kinetics in NS during the INR. With respect to some anatomic and functional differences between the mast cells and basophils,[43, 44, 46, 47] their exact differentiation, especially in the NS, is not easy and needs complicated immunoenzymatic methods.[49] With the high-power magnification (12 × 100) with oil immersion, we were convinced to identify basophils and also mast cells in the NS, according to the criteria described by Schleimer et al.[43, 48] and Jalowayski et al.[49] However, from a practical point of view, both these cell kinds are described together as basophils/mast cells. Our finding of basophils in the NS, which are contrary to the conclusions of other authors,[14, 17, 19, 23] could be explained by increased vascular permeability and microdamage of the mucosal capillaries in the nasal mucosa during the allergic reaction and a possible migration of some cells from the capillaries. This suggestion may be supported by some investigators' findings of the so-called fenestrated type of blood capillaries in the nasal mucosa.[29, 51]

The migration of mast cells from the nasal mucosa into the NS can probably be explained by functional and anatomic changes in the nasal mucosa during the INR, for example, microdamage of epithelium, appearance of edema, etc.,[29] leading to a temporary decrease in the compactness and integrity of the epithelial layer and also partly of the whole mucosal membrane, allowing the migration of mast cells. Our findings of mast cells in the NS is in agreement with similar results of Mygind and Thomsen.[8]

Our results, demonstrating the presence of basophils and/or mast cells in only 16% of positive and 11% of negative INRs, would not support the significance of their appearance in NS as a diagnostic parameter for nasal allergy and are in agreement with conclusions of Sasaki et al.,[17] Connell,[26] Shioda et al.,[21] Bryan and Bryan,[23] and Okuda and Ohtsuka.[24]

The articles dealing with the appearance of neutrophils in NS are not numerous.[1, 8, 13, 29, 52] The neutrophils in NS are usually assumed to be related predominantly to a possible infectious agent.[1, 19, 52] Despite the fact that various studies concerning the role of neutrophils in hypersensitivity mechanisms were published,[44, 53-56] there is a dearth of information concerning their involvement in the nasal allergy and in the INR.

Our results demonstrate the presence of neutrophils in NS in 91% of the positive, in only 66% of the negative INRs, and significant changes in their count during 40% of the positive INRs, and only during 4% of the negative INRs. These findings may suggest a dynamic involvement of neutrophils in the INR. This assumption is in agreement with some investigators,[55, 56] whereas it partly disagrees with other authors.[8]

The goblet cells producing mucous secretions[8, 18, 19, 29] are located unevenly in the nasal epithelium. There is a dearth of knowledge of their exact function and biochemical feature in the nose.[29] Bryan and Bryan[19] observed a marked increase in their count in the NS of patients with nasal allergy, whereas Murray and Anderson[16] did not. Our results demonstrated appearance of goblet cells in the NS in 87% of the positive and in only 34% of the negative INRs. In most of the positive INRs, their count increased after the allergen challenge and then decreased parallel to the resolving of the nasal response, but during the negative INRs, their low count remained unchanged. These results may indicate the involement of goblet cells in the later stage of the INR and are in agreement with the conclusion of Bryan and Bryan.[19] Their migration into the NS might probably be explained by decrease of epithelial compactness during the INR caused by topical edema.

The epithelial cells were found in NS in 68% of the positive and in 63% of the negative INRs, however without any significant changes in their count.

The lymphocytes, plasma cells, and monocytes appeared in the NS sporadically and without any significance for the positive as well as the negative INRs. These results are in agreement with findings of Mygind and Thomsen.[8]

In conclusion, the NS should be examined with 10-minute intervals until 30 minutes after the allergen challenge in the individual patient to detect the significant changes in the count of eosinophils and neutrophils, in particular.

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Cytologic changes in the nasal secretions during the late nasal response

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One hundred sixty-four patients with allergic rhinitis (104 patients with positive late nasal responses (LNRs) and 60 patients with negative nasal responses to allergen challenge), correlating with history and skin tests, were randomly selected for this study. Nasal challenges were repeated and supplemented by cytologic examination of the nasal secretions (NSs). The cytologic examination of NS was also performed during 164 control challenges with phosphate-buffered saline. The positive LNR was accompanied by significant changes in the count of neutrophils in 84% of the cases (increase immediately before and decrease during appearance of LNR, with increase again during resolving of late nasal response), of eosinophils in 58% (increase immediately before and decrease during appearance of LNR), of epithelial cells in 73% (increase followed by decrease, running parallel with LNR), of goblet cells in 63% (increase followed by decrease), of basophils in 8%, and of lymphocytes in 6% (both of these cells demonstrated a slight increase during LNR) in the NS. No significant changes in the count of other types of cells in the NS during most of the LNR cases were recorded. Most cases of negative response were not accompanied by significant changes in the count of individual cell types. No significant changes in the count of any cell type in the NS were recorded in any patient during the phosphate-buffered saline–control challenges. The cytologic examination of NS, accompanying the nasal challenge with allergen, appears therefore to be a valuable supplementary diagnostic parameter for the LNR and a promising model for further immunologic and clinical studies of the LNR. (J ALLERGY CLIN IMMUNOL 1989;83:1068-79.)

Patients with nasal allergy may develop different types of nasal response to the challenge with allergen, including the LNR. The LNR has been studied and described extensively by the authors of this article[1-4] and is becoming recognized as an important clinical phenomenon in patients with allergic rhinitis.[5-7] This response can regularly play an important role in patients with chronic allergic rhinitis and may be responsible for failure of current treatment in some of these patients.

There are little data available to illustrate the appearance of particular kinds of cells and the changes in their count in NSs during the LNR.

The purpose of this study was (1) to investigate the appearance of individual types of cells in NS and the changes in their count during the LNR and (2) to determine the significance of individual kinds of cells in the NS and differences noted during LNR.

Abbreviations used

NPT:	Nasal provocation test
NS:	Nasal secretion
LNR:	Late nasal response
NNR:	Negative late nasal response (no nasal response)
MGG:	May-Grünwald-Giemsa
HS:	Hansels's stain
TB:	Toluidine blue staining
PBS:	Phosphate-buffered saline
LSR:	Late skin response
NPG:	Nasopharynx-nostril pressure gradient

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MATERIAL AND METHODS

Patients

Two hundred thirty-five patients with a history of allergic rhinitis participated in this study. In all patients, routine diagnostic procedures, including skin tests, short-term NPTs, and long-term NPTs with various inhalant allergens

TABLE I. Frequency of the LSR and its relationship to the LNR for the same allergen

LNR	LSR	
	Positive	Negative
Positive (n = 147)	111 (76%)	36 (24%)
Negative (n = 171)	53 (31%)	118 (69%)

Positive LSR: induration >7.5 mm in diameter appearing 6 to 10 hours after injection.

with rhinomanometry were performed. All patients demonstrated (1) nasal symptoms, including obstruction, hypersecretion, sneezing, and itching, (2) positive history to one or more allergen, (3) positive immediate skin response to one or more allergens, (4) positive nasal response of the immediate type to one or more allergens during the short-term NPT, and (5) nonincreased nasal responsiveness to histamine.

In addition, 318 long-term nasal challenges were performed. Allergens for the long-term NPT were chosen according to the following criteria: (1) history suspected from a late onset of the nasal complaints (in some patients, nasal complaints already appeared after the short-term challenge) and/or positive LSR and (2) negative history and negative skin response of any type. An allergen presumed to be negative was randomly selected (e.g., mites, animal danders in patients with seasonal rhinitis, or one of the pollens in patients with perennial rhinitis).

The 235 patients developed a total of 147 positive LNRs and 171 NNRs (Table I). The 104 positive LNRs ($p < 0.05$), correlating with positive history and positive LSRs, and 60 NNRs ($p > 0.25$), correlating with negative history and negative LSRs, recorded in 164 patients from whom a sufficient quantity of NS could be obtained, were randomly selected for this study. Tests were repeated 3 to 5 days later and supplemented by collection of NSs. In each of the 164 patients, a control challenge with PBS was also performed and supplemented by histologic examination of the NSs.

While patients were undergoing tests, they were hospitalized under standard conditions and were free from nasal infections and suffered from minimal nasal complaints only. None of the patients had received previous immunotherapy or corticosteroids. No disodium cromoglycate was used by the patients for at least 8 weeks before this study. No antihistamines or topical decongestants were taken by the patients during a 48-hour period before this study.

Control group

In a group of 15 healthy volunteers without any rhinitis complaints, NS samples were collected before and seven times after the challenge with PBS (every 2 hours from 8 AM until 8 PM) and histologically examined by the same procedure as described below.

TABLE II. Review of the allergens used for skin tests and nasal challenges

	Concentration (per 1 ml of PBS)	
	Nasal challenge	Scratch and intracutaneous tests
House dust	5.0 mg	0.5 mg
Animal danders (each)	2.5 mg	0.25 mg
Mold mixture	2.0 mg	0.2 mg
Aspergillus fumigatus	2.0 mg	0.2 mg
Mites (*D. pteronyssinus*)	100 NU	10 NU
Grass-, spring-, weed-pollen mixture (each)	10.000 NU	1.000 NU

NU, Noon unit; *PNU*, protein nitrogen unit.
Allergens: dry weight of dialyzed and lyophilized allergen extracts in milligrams; 1 NU equals 0.001 mg of dry pollen (powder) equals 0.5 PNU.

Allergens

Allergens (Diephuis Laboratory, Groningen, The Netherlands), in the form of dialyzed and lyophilized extracts, were diluted in PBS (dry weight of allergens in milligrams per 1 ml of PBS) and used for nasal challenges and skin tests in the concentrations reviewed in Table II.

Skin tests

Scratch tests were performed and evaluated after 20 minutes. If tests were negative, intracutaneous tests were carried out and evaluated after 20 minutes, 2, 4, 6, 8, 10, and 12 hours, and were then followed every 12 hours up to 96 hours, according to a standard routine schedule. The appearance of wheal (or induration) of >7.5 mm in diameter was interpreted to be a positive skin response. The positive skin response appearing within 20 minutes after allergen injection (wheal) was considered to be a positive immediate skin response, whereas that appearing within 6 to 10 hours (induration) was considered to be a positive LSR.

NPTs

NPTs were performed by means of the rhinomanometry technique, as described previously.[1,2] The NPG values (expressed in cmH_2O) recorded by this technique were considered to be basic parameters of the nasal obstruction (nasal response). The NPT was performed according to the following schedule: (1) recording of the base values (initial values) at 0, 5, and 10 minutes, (2) recording of the PBS values (control values) at 0, 5, and 10 minutes after a 3-minute application of PBS to the nasal mucosa of the nonintubated nasal cavity by means of a saturated wad of cotton

wool on a nasal probe, (3) challenge of allergen for 3 minutes in the same way and at the same site as the PBS and recording of the NPG values at 0, 5, 10, 20, 30, 45, 60, 90, and 120 minutes, and then every hour up to 12 hours. The nasal response was considered positive when the mean NPG values after the allergen challenge increased by at least 2.0 cmH_2O (1.2 ± 0.3, mean ± SE) with respect to the PBS values, recorded during at least three consecutive time intervals. The NPG changes within 60 minutes after the allergen challenge were considered to be positive immediate nasal response, whereas those appearing within 4 to 12 hours were considered a positive LNR.

NSs

Three series of the NS specimens were collected from the nonintubated nasal cavity by having the patient blow onto a polyethylene sheet twice before the allergen challenge (initial value at 10 minutes, and PBS value at 10 minutes) and 15 times after the allergen challenge. The first series of air-dried specimens was fixed additionally by polyethylene glycol and stained by Hansel's method (modified by the authors).[8-11] The second air-dried series was stained by the MGG method (modified by the authors).[6, 8] The third series was fixed by methanol and stained by the TB method.[12] After dehydration with methyl alcohol, the specimens were mounted in Canada balsam and scanned microscopically with the technician blinded to the conditions. Two counting techniques were used. (1) The counting of absolute numbers of individual kinds of cells per microscopic field was at 250 times magnification, and means per microscopic field were always calculated from 25 fields spread over the whole slide. (2) The differential count of leukocytes was expressed as a percentage of 100. The cells were counted in 25 microscopic fields (250 times magnification) spread over the whole slide. The differential count was used as a control technique. Doubtful cells were reexamined under oil immersion (magnification 1200 times). The results of the MGG were considered by the authors to be basic results, whereas the Hansel's method and TB staining were an additional control.

The particular cell types were identified by the authors according to the following morphologic criteria:

1. *Eosinophils:* large cells (size 10 to 14 μm) with blue-stained bilobed nucleus; in MGG, pink cytoplasm containing large, bright yellowish-red (orange) granules; in HS, red cytoplasm containing large deep-red granules
2. *Neutrophils:* large cells (size 10 to 12 μm) with blue-stained segmented or stab nucleus; in MGG, pink cytoplasm containing slightly visible, fine, pinkish-purple-lilac–stained granules; in HS, pale-pink cytoplasm containing pink-purple, fine, slightly visible granules
3. *Basophils:* metachromatically stained cells (size 10 to 14 μm), round form, sometimes dissected surface, with a large lobulated nucleus and numerous large granules in cytoplasm; in MGG, blue nucleus, round blue to pink cytoplasm, dark-bluish granules; in HS, blue nucleus, light-blue cytoplasm, reddish-purple, large granules; in TB, dark-blue nucleus, light-blue cytoplasm, red-violet, large granules
4. *Mast cells:* large cells (size 15 to 30 μm), oval or elongated form, irregular shape, sometimes with pseudopod-like structure, stained with metachromatic dyes to a higher degree (more intensively) than basophils, blue oval nucleus, usually located centrally, but often obscured by numerous granules; in MGG, blue nucleus, light-blue cytoplasm, numerous dark-blue cytoplasmatic granules; in HS, blue nucleus, light-blue cytoplasm, sometimes visible only in a peripheral ring, sometimes with vacuoles, and contains a large number of dark-blue granules (smaller size but darker stained than those in basophils); in TB, dark-blue cytoplasm, violet-blue, small-size granules in a large number
5. *Epithelial cells:* large cells (size varies from 8 to 40 μm), may be of various forms, dependent on their original location in the nose, may be cylindric (columnar) or stellate (squamous), ciliated or nonciliated, stratified or pseudostratified, with or without microvilli, mostly oval nucleus with nucleoli, finely granular chromatin structure and homogenous cytoplasm, sometimes vacuolized; in MGG, dark-violet, irregularly stained nucleus, light-violet cytoplasm; in HS, blue nucleus, irregularly stained, light-blue cytoplasm with vacuoles to a varied degree
6. *Goblet cells:* elongated cells (size 20 to 25 μm), very typical form, characterized by a narrow base and a wide ciliated top; nucleus usually located in the basal part and contains visible nucleoli, regularly bizarrely defleated and bent; sometimes filled in by mucinous droplets located in the top part; in MGG, dark violet-blue nucleus, light violet-blue cytoplasm; in HS, dark-bluish irregularly stained nucleus with nucleoli, light-blue cytoplasm, sometimes with irregularly blue-stained mucinous droplets.
7. *Plasma cells:* large egg-shaped cells (size 8 to 18 μm) narrower at one end; ovoid nucleus often located excentrically with a purplish and coarse chromatin of a wheel-spokes structure, without nucleoli, a perinuclear halo usually visible; abundant cytoplasm regularly contains a number of vacuoles present near the cell border; ratio nucleus: cytoplasm approximately 1:2; in MGG, round dark-blue nucleus with purplish, somewhat coarser chromatin, light-blue cytoplasm with nonstained vacuoles; HS method does not stain plasma cells sufficiently.
8. *Monocytes:* oval cells (sized 14 to 19 μm), irregular surface, excentrically located nucleus, which is lobulated, kidney bean– or horseshoe-shaped form, contains fine lightly purple-pink–stained chromatin; abundant cytoplasm contains very fine (punctuate) azurophilic granules; ratio nucleus: cytoplasm approximately 2.5 to 3.0:1.0; in MGG, dark-red to pale-violet nucleus, sometimes deformed, marine-blue cytoplasm containing very fine azurophilic granules; HS method does not stain monocytes sufficiently.
9. *Lymphocytes:* size varies from small (7 to 9 μm) to large (8 to 16 μm); nucleus usually round shaped, located excentrically, contains dark, coarse, and clumped chromatin; nucleoli usually absent, nuclear membrane usually sharply defined; cytoplasm varies in quantity from only a rim around the nucleus to relative abundance; perinuclear clear zone occasionally contains azurophilic granules, especially in larger lymphocytes; ratio nucleus: cytoplasm approximately 1.5 to 2.5:1.0; in MGG, blue-violet, large nucleus, blue (robin's egg blue) cytoplasm; HS is not a suitable stain for lymphocytes.

In approximately 1% to 2% (one, sporadically two cells per 100 cells), the cells could not be definitely identified and established.

The statistically significant magnitude of changes in the count of individual cell types in NSs in patients with allergic rhinitis between two consecutive degrees was as follows (mean ± SE): eosinophils, 7 (7.17 ± 0.91); basophils and mast cells, 2 (2.26 ± 0.71); neutrophils, 8 (8.33 ± 0.56); lymphocytes, 2 (2.0 ± 0.25); monocytes, 1 (1.26 ± 0.21); plasma cells, 1 (1.27 ± 0.21); epithelial cells, 5 (5.00 ± 0.63); and goblet cells, 4 (4.15 ± 0.65). The lowest difference in the count of the individual cell type, which was found to be still statistically significant (significant magnitude), is expressed in the figures as ± (very slight).

The appearance of the individual cell type in NS was evaluated in a summarized form in the figures as follows: −, no appearance at all; ±, very slight; +, slight; + ±, moderate; + +, distinct; + + ±, large; and + + +, very large appearance. The evaluation criteria used for appearance of particular cell types in NS are described in detail in Table III.

Control test with PBS

This test was performed in each of the patients studied, in the same way, by the same schedule, and with the same processing of NS samples as that used during the experimental allergen challenge.

Statistical analysis

The nasal response to allergen challenge was statistically evaluated by Wilcoxon matched-paired signed-rank test, comparing the NPG values recorded after the allergen challenge with the mean NPG value of PBS. A p value of <0.05 was considered to be statistically significant.

The positive LNRs and NNRs were compared and statistically evaluated by means of the Mann-Whitney U test ($p < 0.05$ was considered to be statistically significant).

The changes in the count of individual cell types were statistically analyzed by Wilcoxon paired test. A p value <0.05 was considered to be statistically significant. Each of the cell types recorded during the individual NPT, positive LNR, NNR, or control PBS test was analyzed separately. The numbers of cells recorded after allergen challenge on each occasion were compared with numbers recorded after PBS.

If significant differences in the cell count were found at least three times after allergen challenge compared with the cell count after PBS, it was concluded that this cell type demonstrated significant changes in its count during that nasal response.

TABLE III. Evaluation criteria of appearance of individual cell types in NS (average number of particular cell type per moderate power field (250 times magnification)

	−	±	+	+ ±	+ +	+ + ±	+ + +
Eosinophils	0-6	7-13	14-20	21-27	28-34	35-41	> 42
Neutrophils	0-7	8-15	16-23	24-31	32-39	40-47	> 48
Basophils and mast cells	0-1	2-3	4-5	6-7	8-9	10-11	> 12
Epithelial cells	0-4	5-9	10-14	15-19	20-24	25-29	> 30
Goblet cells	0-3	4-7	8-11	12-15	16-19	20-23	> 24
Plasma cells	0	1	2	3	4	5	> 6
Monocytes	0	1	2	3	4	5	> 6
Lymphocytes	0-1	2-3	4-5	6-7	8-9	10-11	> 12

RESULTS

The 104 positive LNRs ($p < 0.05$), 60 NNRs ($p > 0.25$), and 104 control PBS tests were subject to this study. The difference between the positive LNR and NNR, evaluated by the Mann-Whitney U test, was statistically highly significant ($p < 0.0001$) at all time intervals.

The 164 PBS control tests did not demonstrate any significant changes of NPG ($p > 0.05$).

Positive LNR (n = 104)

The changes in the count of the particular types of cells in NS and their course during the positive LNR are reviewed in Figs. 1A and 1B.

No significant changes in the count of any type of cells were found between initial and PBS values ($p > 0.05$).

Neutrophils. Neutrophils were present in NS in 96% of positive LNRs. Before the allergen challenge, their count was low (up to +) in 89% and high (up to + + +) in 7% of the LNR cases. The significant changes in their count were recorded during 84% of

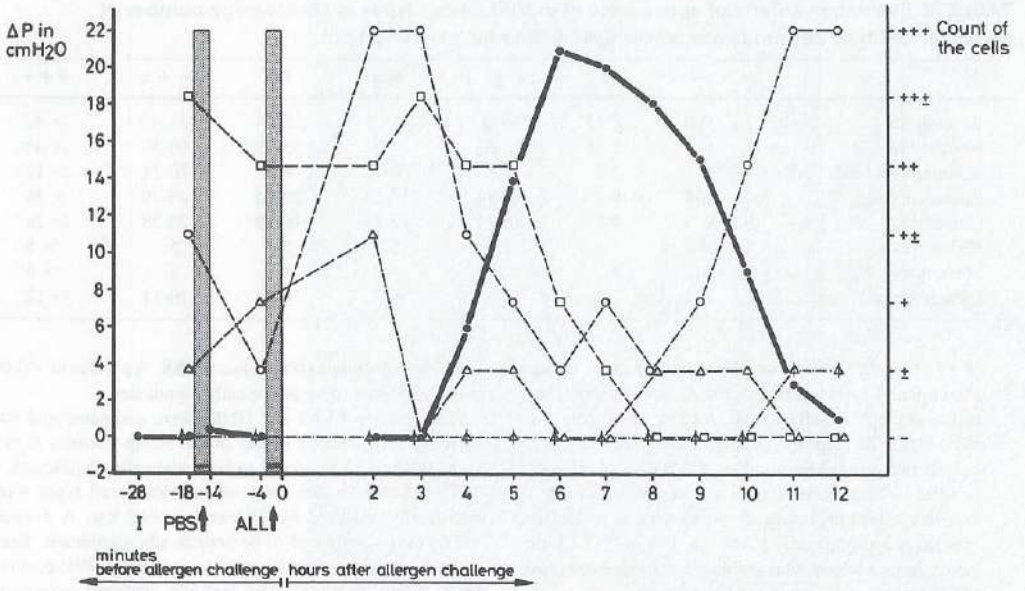

FIG. 1A. The mean NPG values recorded after the allergen challenge with respect to the control NPG values (PBS), calculated from 104 positive LNRs. The mean changes in the count of the individual cell types in the NSs calculated from 104 positive LNR; *I*, initial value; *PBS*, control challenge; *ALL*, allergen challenge; positive LNR (●—●); neutrophils (○ - - - ○); eosinophils (□ - - - □); basophils (△ - - - △); mast cells (⅄ — • — ⅄).

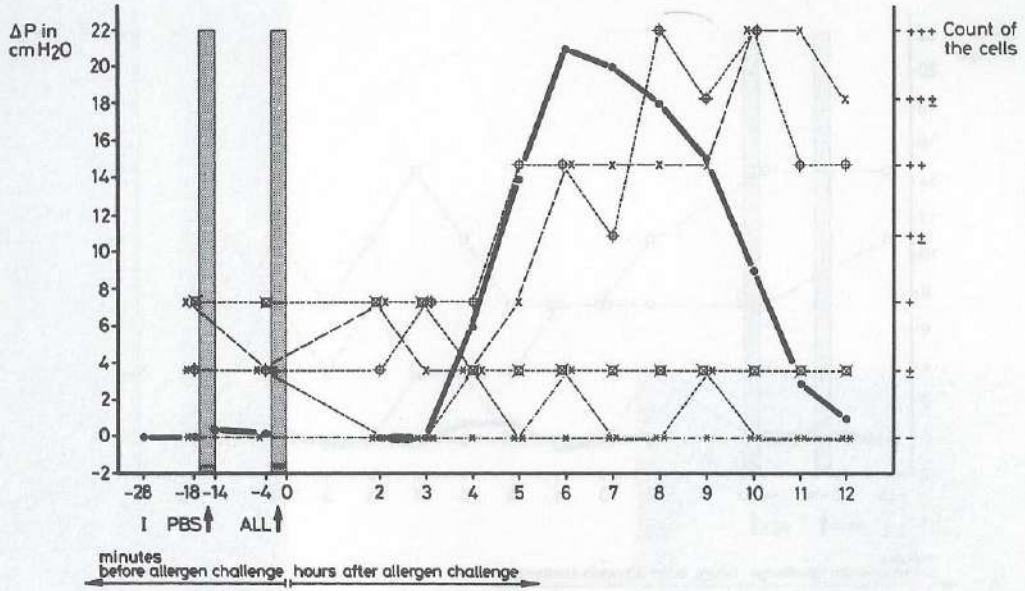

FIG. 1B. The mean NPG values recorded after the allergen challenge with respect to the control NPG values (PBS), calculated from 104 positive LNRs. The mean changes in the count of the individual cell types in the NSs calculated from 104 positive LNRs; *I*, initial value; *PBS*, control challenge; *ALL*, allergen challenge; positive LNR (●—●); epithelial cells (○ · · · ○); goblet cells (X - - - - X); lymphocytes (□ · · · · □); plasma cells (* - - - - *); monocytes (* – • – *).

the LNR cases ($p < 0.05$). In most cases of positive LNR (75%), the neutrophil count was low before, and up to 5 to 6 hours after the allergen challenge, increased rapidly immediately before the appearance of the LNR (within 1 hour), decreased gradually during the response, and increased again after resolving of the response.

Eosinophils. Eosinophils were present in NS in 61% of the positive cases. The significant changes in their count were recorded during 58% of the LNRs ($p < 0.05$). In most cases of positive LNR (51%), the eosinophil count increased immediately before the appearance of the LNR (within 1 hour), then decreased so rapidly, that at the time of the peak of the response, the eosinophils had disappeared from the NS. The absence of eosinophils in the NS lasted for more than 24 hours after the positive LNR.

Basophils. Basophils were found in NS in 15% of positive LNRs. In 8% of the LNR cases, their count changed significantly ($p < 0.05$). It was low before the allergen challenge and increased slightly during the response.

Mast cells. Most cells appeared in NS in 3% of the LNR cases. Their count was low and without significant changes ($p > 0.1$).

Epithelial cells. Epithelial cells were present in 100% of the positive LNRs. Significant changes in cases ($p < 0.05$). In most cases their count was low before the allergen challenge, increased during appearance of LNR, and decreased during resolving of the response.

Goblet cells. Goblet cells were found in NS in 82% of the LNRs. Their count changed significantly during 63% of the LNRs ($p < 0.05$). In most cases their count increased gradually during the LNR, reached its maximum during resolving of the LNR, and then decreased within 12 hours after resolving of the LNR.

Lymphocytes. Lymphocytes appeared in NS in 18% of the LNRs. Their count increased significantly during the LNR in 6% of the cases ($p < 0.05$).

Plasma cells. Plasma cells were present in NS in 4% of the LNR cases, however, without any significant changes in their count ($p > 0.1$).

Monocytes. Monocytes appeared in NS during LNR sporadically and irregularly.

NNR (n = 60)

The course of changes in the count of individual cell types in NS during the NNR is presented in Figs. 2A and 2B.

No significant changes in the count of any type of cells were found between initial and PBS values ($p > 0.1$).

Neutrophils. Neutrophils were present in NS in 17% was low in most cases (15%). Significant changes in their count, a slight increase between 6 to 8 hours after the challenge, followed by decrease, were recorded in only 3% of the NNR cases ($p < 0.05$).

Eosinophils. Eosinophils were found in NS in 19% of the NNRs. Significant changes in their count ($p < 0.05$), a slight increase between 6 to 8 hours after the challenge, and then stability up to 12 hours were recorded in 5% of the NNRs.

Basophils. Basophils appeared in NS in 9% of the negative cases. Their count did not reveal any significant changes ($p > 0.1$).

Mast cells. Mast cells were present in NS in 2% of these cases. Their count was low and without any significant changes ($p = 0.25$).

Epithelial cells. Epithelial cells were present in NS in 23% of the NNRs. Significant changes in their count ($p < 0.05$), a slight increase between 7 to 9 hours after the challenge, were recorded in 3% of these cases.

Goblet cells. Goblet cells appeared in NS in 13% of the NNRs. Their count changed significantly ($p < 0.05$) in 3% of the NNRs, characterized by a slight increase, followed by decrease between 6 to 9 hours after the challenge.

Monocytes. Monocytes were present in NS in 3% of this response. Significant changes in their count, a lenge, were observed in 2% of the cases ($p < 0.05$).

Plasma cells. Plasma cells were found in NS in 2% of the NNRs without any significant changes in their count ($p > 0.1$).

Monocytes. Monocytes were completely absent from the NS in NNRs.

PBS control challenge (n = 164)

The changes in the count of the individual cell types and their course during the PBS challenge, performed in all patients studied, are illustrated in Figs. 3A and 3B. The individual types of cells appeared in NS before the PBS challenge sporadically or to a very low degree (±).

No significant changes in the count of any of the particular types of cells were recorded after the PBS challenge up to 12 hours. The statistical significance for the particular cell types was found as follows: neutrophils, $p > 0.01$; eosinophils, $p > 0.05$; basophils, $p > 0.1$; mast cells, $p = 0.1$; epithelial cells, $p > 0.05$; goblet cells, $p > 0.1$; lymphocytes, $p > 0.1$; plasma cells, $p > 0.1$; and monocytes, calculation of p value was impossible.

Control group of healthy subjects

The count of individual kinds of cells in NS of the control subjects (n = 15) before PBS challenge

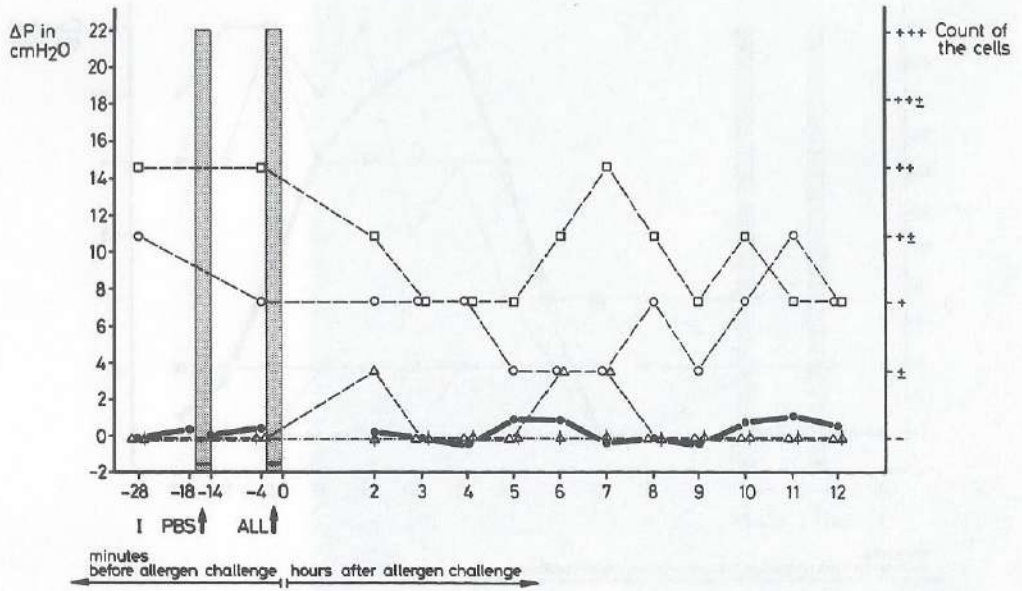

FIG. 2A. The mean NPG values recorded after the allergen challenge with respect to the control NPG values (PBS), calculated from 60 negative LNRs. The mean changes in the count of the individual cell types in the NSs calculated from 60 LNRs; *I*, Initial values; *PBS*, control challenge; *ALL*, allergen challenge; negative LNR (●—●); neutrophils (○ - - - ○); eosinophils (□ - - - □); basophils (△ - - - △); mast cells (⏃ –●– ⏃).

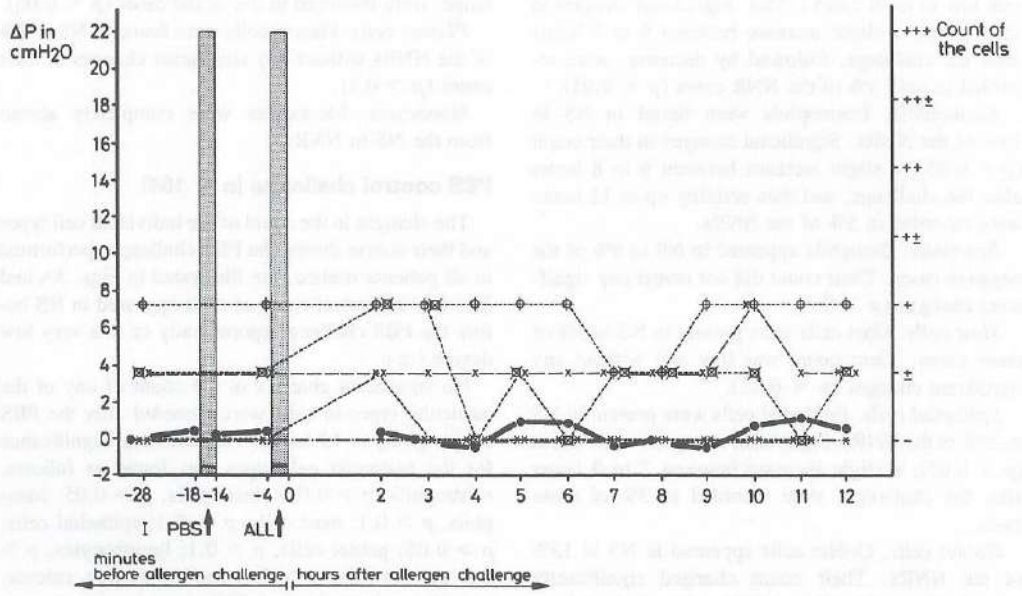

FIG. 2B. The mean NPG values recorded after the allergen challenge with respect to the control NPG values (PBS), calculated from 60 negative LNRs. The mean changes in the count of the individual cell types in the NSs calculated from 60 negative LNRs; *I*, initial value; *PBS*, control challenge; *ALL*, allergen challenge; negative LNR (●—●); epithelial cells (⊕ · · · · ⊕); goblet cells (X - - - - X); lymphocytes (⊞ · · · · ⊞); plasma cells (* - - - - *); monocytes (* –●– *).

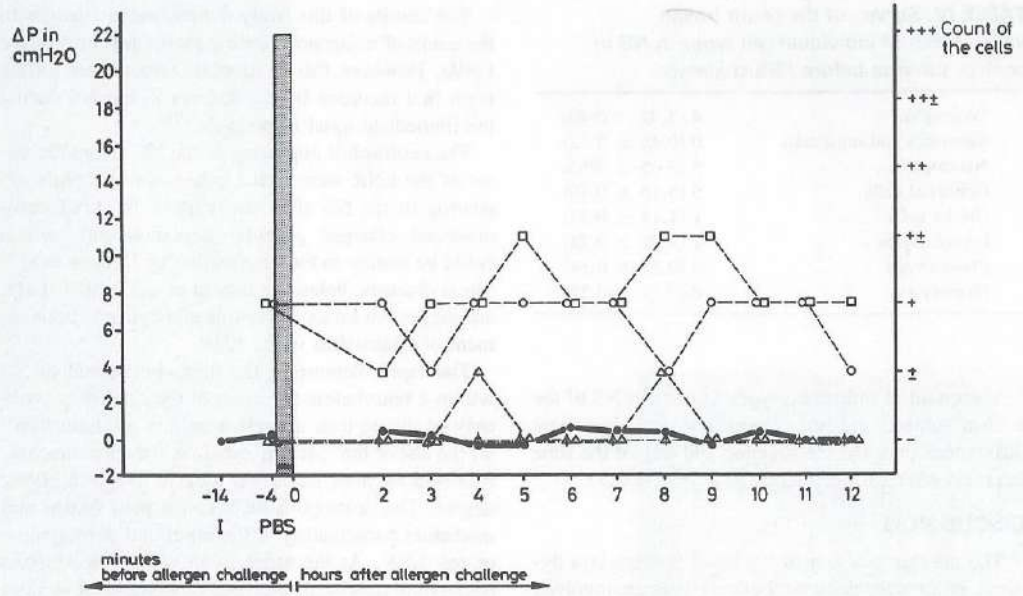

FIG. 3A. The mean NPG values recorded after PBS control challenge with respect to the initial NPG values, calculated from all 164 control challenges. The mean changes in the count of individual cell types in the NSs were calculated from 164 PBS control challenges. *I*, initial value; *PBS*, control challenge; PBS control challenge (●—●); neutrophils (○ - - - ○); eosinophils (□ - - - □); basophils (△ - - - △); mast cells (⏃ –●– ⏃).

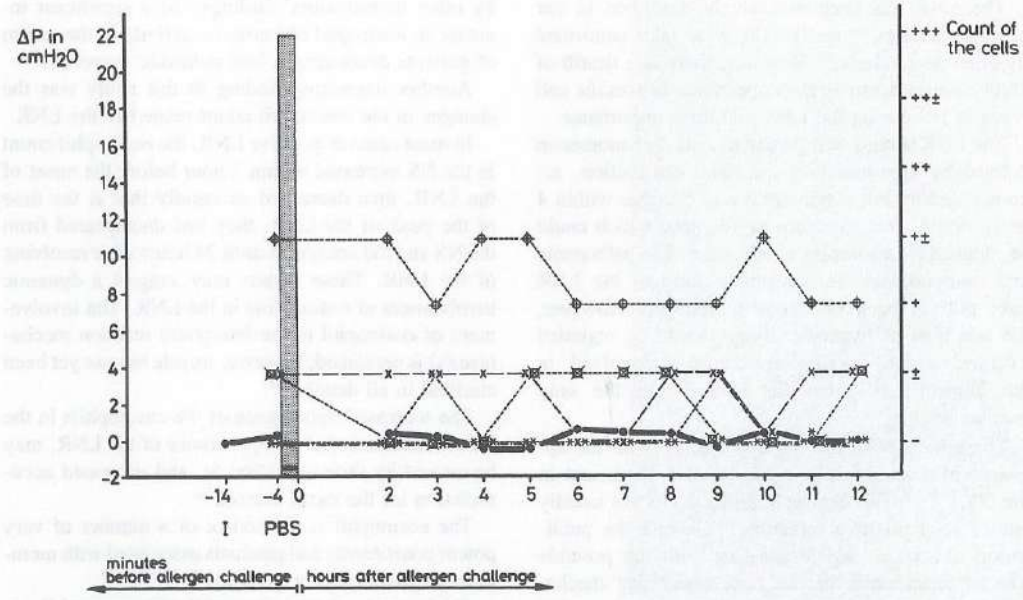

FIG. 3B. The mean NPG values recorded after PBS control challenge with respect to the initial NPG values, calculated from all 164 control challenges. The mean changes in the count of individual cell types in the NSs were calculated from 164 PBS control challenges; *I*, initial value; *PBS*, control challenge; PBS control challenge (●—●); epithelial cells (⊕ · · · · ⊕); goblet cells (X - - - - X); lymphocytes (⊞ · · · · ⊞); plasma cells (* - - - - *); monocytes (* –●– *).

TABLE IV. Survey of the count (mean value ± SE) of individual cell types in NS in control subjects before PBS challenge

Eosinophils	4 (3.92 ± 0.40)
Basophils and mast cells	0 (0.48 ± 0.12)
Neutrophils	5 (5.05 ± 0.63)
Epithelial cells	5 (5.10 ± 0.79)
Goblet cells	1 (1.15 ± 0.31)
Lymphocytes	1 (1.20 ± 0.24)
Plasma cells	0 (0.38 ± 0.14)
Monocytes	0 (0.31 ± 0.25)

The count of individual types of cells in NS of the control subjects did not demonstrate any significant differences between the baseline and any of the time intervals after PBS challenge ($0.2 > p > 0.1$).

DISCUSSION

The allergic reaction in the nasal mucosa is a dynamic process in which various cell types are involved at different times.[13-19] This reaction is associated with migration of some kinds of cells in the nasal mucosa and into the NS.[20-27] The involvement of individual cell types can only be characterized by comparison of the changes in their count before and, repeatedly, after the challenge with a particular allergen.

The LNR has been extensively described in our previous articles,[1-4] and results were later confirmed by other researchers.[5-7] However, there is a dearth of knowledge concerning the appearance of specific cell types in NS during the LNR and their importance.

The LNR should be regarded a clinical phenomenon defined by appearance of the nasal obstruction, accompanied by other symptoms and changes within 4 to 12 hours after exposure to allergen, which could be induced by a complex mechanism. The pathogenic and immunologic mechanisms leading to the LNR have not yet been satisfactorily clarified. However, the late type of hypersensitivity should be regarded as one of the possible mechanisms involved in the clinical LNR, but far from being the only mechanism.[1-3, 28]

There have been few studies dealing with the appearance of neutrophils in the nasal mucosa and in the NS.[18-20, 29] The neutrophils in the NS are usually related to a possible infection.[15] Despite the publication of various articles dealing with the possible role of neutrophils in the hypersensitivity mechanism,[18, 30-35] there is a dearth of information concerning their involvement in the nasal allergy, and especially their possible role in LNR. Neutrophils, besides platelets, may play a pivotal role in the case of the late hypersensitivity.[31-33, 35-37]

The results of this study demonstrated changes in the count of neutrophils during most cases of positive LNRs. However, this neutrophil count pattern differs from that recorded by the authors in the NS during the immediate nasal response.[3, 9, 11]

The neutrophils appearing in the NS before the onset of the LNR were intact, whereas neutrophils appearing in the NS after resolving of the LNR demonstrated changed granule (degranulation), which could be similar to the observation by Henson et al.[36] These changes, being the subject of our further study, are suggestive for an active role and dynamic involvement of neutrophils in the LNR.

The rapid increase in the neutrophil count in NS within 1 hour before the onset of the LNR may probably be due to their attraction to, and accumulation[38] at, the site of the reaction, which is the nasal mucosa, followed by their migration into the NS to a higher degree. The neutrophils then release their factors and mediators participating in the onset and development of the LNR. At the same moment, the neutrophils become immobilized, they gather in the nasal mucosa tissue and do not migrate further into the NS. During the resolving of the LNR, the accumulated neutrophils, which had already released their factors, were expelled into the NS, and in this way their count in the NS increased and reached its prechallenge level within 24 hours. This hypothesis may be supported by other investigators' findings[30] of a significant increase in neutrophil chemotactic activity in the serum of patients developing a late asthmatic response.

Another interesting finding in this study was the changes in the eosinophil count related to the LNR.

In most cases of positive LNR, the eosinophil count in the NS increased within 1 hour before the onset of the LNR, then decreased so rapidly that at the time of the peak of the LNR, they had disappeared from the NS and did not return until 24 hours after resolving of the LNR. These results may suggest a dynamic involvement of eosinophils in the LNR. The involvement of eosinophil in the late-phase reaction mechanism(s) is presumed; however, its role has not yet been clarified in all detail.[38-41]

The increased appearance of the eosinophils in the NS, immediately before appearance of the LNR, may be caused by their attraction to, and increased accumulation in, the nasal mucosa.[41]

The eosinophil is the source of a number of very potent constituents and products associated with membrane, cytoplasma, or granules.[31, 32, 38, 40-43]

At the peak of the eosinophil and neutrophil accumulation in the nasal mucosa, they become activated and release their factors contributing to the development of the LNR. At the same time, the eosinophils also gather in the nasal mucosa and do not migrate into the NS. During the resolving of the LNR, the eosinophils are retained in the tissue and may participate in the later stages of the hypersensitivity mechanism, for example, inactivating of some mediators.[40, 41, 43] Such involvement could also explain their absence from the NS during a period of more than 24 hours after the LNR.

Our results demonstrating increased migration of neutrophils and eosinophils into the NS, preceding the appearance of LNR, are supported by findings of other investigators of an increased neutrophil and eosinophil infiltration before and during the late-phase allergic reaction.[42]

The important role of basophils and mast cells in the classic immediate hypersensitivity mechanism has repeatedly been stressed in the literature.[31, 44-46] The basophils and mast cells with IgE and IgG receptors on their membrane and with membrane-bound IgE antibodies can generate and release various potent constituents associated with their granules, cytoplasm, or membrane, the so-called primary and secondary mediators, having manifold effects on various effector organs, cells, and steps of the hypersensitivity mechanism.[31, 40, 41, 44, 45, 47, 48]

The views on the possible involvement of basophils and/or mast cells in the late hypersensitivity mechanism, as well as the late nasal and bronchial response, vary greatly. Some investigators presume that mast cells and/or basophils may play the main role in these responses,[39, 47] whereas other investigators do not.[28] We have identified both the basophils and the mast cells in NS during the LNR by means of naphthol AS-D chloracetate esterase method.[49, 50]

The presence of basophils and mast cells in the NS before and during the LNR, recorded by the authors in a very low number of cases, did not allow for a clear interpretation. In contrast, it can be concluded that the count of basophils and mast cells in the NS is not a suitable indicator for the LNR.

Another interesting result of this study concerns the epithelial cells and goblet cells. The epithelial cells appeared in 100% of the positive LNRs and in only 23% of the NNRs. In most of the positive LNRs, the count of epithelial cells demonstrated significant changes running parallel to the course of the LNR. This finding may suggest that the appearance of the epithelial cells should be regarded as a consequence of the LNR, during which reversible changes of the nasal mucosa, for example, edema of the mucosa diminishing the compactness of the epithelial layer, lead to the expulsion of epithelial cells into the NS.[17]

The goblet cells, being monocellular secretory elements producing mucous secretions,[20] may probably also be influenced by some mediators released during the hypersensitivity mechanism, although the goblet cells themselves do not participate in this mechanism.[13, 15] Their appearance and the changes in their count in NS during the LNR may probably be explained in the same way as appearance and changes of the epithelial cells.

The appearance of lymphocytes in the NS in 18% of the positive LNRs and in only 3% of the NNRs, demonstrating a slight increase in their count between 9 to 12 hours after the challenge, could also be regarded as an interesting finding. The lymphocytes are not presumed to be directly involved in the LNR.[39] In contrast, this finding could be indicative for a large dilatation of the mucosal capillary network during the LNR, leading to reversible damage of the capillary wall (opening of the fenestrae, breaking of the intercellular junction between endothelial cells, etc.)[18, 51] and then allowing the lymphocytes to migrate from the capillary stream into the tissue and then into the NS.

The incidental appearance of plasma cells and monocytes in the NS during the LNR did not allow the authors to draw any conclusion.

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Nasal Response to Food Ingestion Challenge

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• **A routine diagnostic procedure, the food-focused history, intracutaneous tests with food extracts, and ingestion challenge with selected foods in combination with rhinomanometry were performed in 142 patients suffering from perennial allergic rhinitis. Forty-one of the 47 patients with a "positive food allergy history" developed 65 (90%) positive nasal responses during 72 food ingestion challenges. Of the 95 patients with an "unknown food allergy history," 54 developed 68 (50%) positive nasal responses during 132 food ingestion challenges. The following responses were recorded: 29 isolated immediate (within three hours), 38 isolated late (six to 24 hours), 42 dual late (immediate + late), 11 isolated delayed (28 to 52 hours), and 13 dual delayed (immediate + delayed). It can be concluded that the involvement of foods in allergic rhinitis is more frequent than is usually expected. The definite confirmation of the role of a certain food in these patients should be provided by the food ingestion challenge demonstrating one of the clinical types of nasal response. The mechanisms underlying the nasal response to foods are not yet fully clarified. The involvement of different types of hypersensitivity in the individual types of nasal response cannot be excluded.**

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Allergic rhinitis has classically been attributed to the immediate hypersensitivity mechanism (type 1 allergy) caused by inhalant allergens. The possible role of food allergy in patients with nasal complaints is still underestimated. The involvement of foods in patients with allergic disorders is complex and has various forms, of which the hypersensitivity mechanism is only one.

The participation of foods in patients with allergic rhinitis and the involvement of the hypersensitivity mechanism as one of the possible mechanisms has already been discussed in the literature.[1-9] We were unable to find any investigation of the nasal response and its types due to food ingestion in a sufficiently large number of patients. The purpose of this study was to investigate the possible role of foods in patients with allergic rhinitis and its frequency, as well as the types of nasal response to food ingestion, their clinical features, and correlation with other in vivo and in vitro diagnostic results.

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PATIENTS, MATERIALS, AND METHODS

We studied 142 patients, aged 16 to 65 years, suffering from a perennial allergic rhinitis (nasal obstruction accompanied by hypersecretion, sneezing, and itching) for longer than five years. A routine diagnostic procedure, including nasal challenges with inhalant allergens, food-focused history, intracutaneous tests with standard series of food extracts, and ingestion challenge with selected foods in combination with rhinomanometry were performed in these patients.

None of the patients had a history of anaphylaxis from food. None had received previous immunotherapy, and none had used cromolyn sodium (disodium cromoglycate), oral corticosteroids, or long-acting antihistamines. Treatment with short-acting antihistamines and topical decongestants was stopped at least 48 hours before the food challenge. The food selected for ingestion challenge and related foods were avoided for at least seven days before the challenge.

The patients were divided into two groups. The first group consisted of 47 patients with highly suggestive food histories related to their nasal complaints. Eleven of these patients gave a history suggestive of only food hypersensitivities. The histories of the remaining 36 patients were also suggestive of inhalant allergens. The second group consisted of 95 patients with unknown food history with respect to their nasal complaints, but with positive intracutaneous tests for one or more foods.

Skin Tests

Dialyzed and lyophilized food extracts, diluted in phosphate-buffered saline (dry weight of food extract in milligrams per 1 mL of phosphate-buffered saline) (Pharmacy "Diephuis"), were used in the following concentrations: cheese and eggs, each in 1 mg/mL; nuts, chocolate, cocoa powder, Dutch sweets, nonalcoholic beverages, beers, and wines, each in 0.5 mg/mL; meat, spices, and beans, each in 0.2 mg/mL; all other foods, each in 0.1 mg/mL. The extracts were standardized according to the European standard quality criteria (histamine-releasing tests, radioallergosorbent test [RAST], crossed immunoelectrophoresis, crossed radioimmunoelectrophoresis, and skin titration).

Scratch tests were performed. If they were negative, then intracutaneous tests were carried out and evaluated after 20 minutes, 4, 8, 12, 24, 36, 48, 60, 72, 84, and 96 hours. The positive skin response, appearing within 20 minutes, was considered to be an immediate skin response, that appearing between six and 24 hours was considered to be a late skin response, and that appearing after 48 hours was considered to be a delayed skin response.

Rhinomanometry

A modification of the posterior technique, described previously by us in detail,[10-13] was used for the assessment of the nasal mucosa response. The nasal resistance parameters (nasopharynx-nostril-pressure gradient values [NPG]) were recorded. The nasal response was considered to be positive when the mean NPG values after food ingestion challenge increased by at least 2.7 ± 0.6 cm H_2O (mean $\pm$ 2 SDs) with respect to the "initial" values, recorded at least at three consecutive time intervals.

Food Ingestion Challenge

The open food ingestion challenge was performed according to the following schedule: (1) the parameters were recorded at 0, 5, and 10 minutes, the so-called initial or baseline values; (2) the food was ingested within five minutes and then a one-hour waiting interval always followed to allow the food to be digested; during this interval the nasal parameters were measured every 15 minutes to exclude an unexpected or early nasal response; (3) after the one-hour waiting interval the actual post-challenge nasal factors were recorded at 0, 10, 20, 30, 45, 60, 90, and 120 minutes and then every hour up to the 11th hour and

every second hour during the 24th- to 36th-hour intervals and the 47th- to 56th-hour intervals.

The foods used for ingestion challenge and their quantities, presented in Table 1, were identical to those used by the patients; this allowed us to obtain the highest degree of reproducibility.

The Control Test

The control ingestion challenge with either cooked rice, cooked potatoes, or glucose solution, the choice of which depended on the patient's problem, was performed in the same way as the experimental food challenge in all patients.

Double-blind Food Challenge

In 26 randomly selected patients, besides the open food challenge, a double-blind ingestion challenge with the same food was performed eight days later to compare these techniques. The double-blind, placebo-matched, crossover technique was performed according to the method described in the literature.[19,20]

Control Group

In 12 healthy subjects, 22 ingestion challenges with selected foods (cheese, chocolate) were performed so there would be a control for the method of ingestion challenge as well as for the technique of rhinomanometry.

RAST

The specific IgE antibodies to the foods in the serum were determined quantitatively in the Central Laboratory of the Dutch Red Cross Blood Transfusion Service, Amsterdam.

Single Radial Immunodiffusion (Mancini Technique)

The determination of IgG, IgM, and IgA antibodies in the serum was performed by means of standard plates (Kallestad Co, and De Beer Immunological Supplies).

Blood Leukocyte and Eosinophil Count and Eosinophils in the Nasal Secretions

The blood leukocyte and eosinophil counts were performed four times daily, starting on the day before and continuing up to day 2 after the challenge. At the same time intervals, samples of the nasal secretions were also taken and then stained by a modified[17] Hansel's method.

Rhinoscopy, Nasal, and Other Complaints

The appearance of the nasal mucosa, nasal complaints, and other complaints were recorded before and then every two hours during the 0 to 12-, 24- to 36-, and 47- to 56-hour periods after the food ingestion challenge.

Table 1.—Quantities of the Food Used for Ingestion Challenge*

Food Type	Total Quantity
Basic foods with a "solid consistency;" more than 50 g consumed at one time of: cheese, chocolate, vegetables and fruits, nuts, meat, Dutch sweets, etc	100 g each
Basic foods with a "fluid consistency," more than 50 mL consumed at one time of: milk, yogurt, nonalcoholic beverages	200 mL each
Foods, parts of foods or foodstuffs, with a well-defined flavor, taste, or consistency, only added to the basic foods in very small quantities: spices, aromatic vegetables (garlic, onion, etc), varia (casein, vinegar, dressings, etc)	5 g each on brown bread with butter or in 100 mL of water
Soft alcoholic beverages: beer, wine, sherry, port, etc	100 mL each

*The frequency of the individual foods used for the ingestion challenge was as follows: (1) high frequency: cheese, peanuts, milk, Dutch sweets, chocolate; (2) moderate frequency: sherry, shrimps, tomato, walnuts, wine, beer, almonds, mustard, apple, egg, onion, hazelnuts; (3) low frequency: individual spices, fruits, vegetables, meat, fish.

Statistical Analysis

The Student t test was used for the statistical evaluation of the results. A P value <.05 was considered to be statistically significant.

RESULTS

A total of 207 food ingestion challenges were performed in 142 patients. In 47 patients with highly suggestive food allergy histories, 72 ingestion challenges were carried out; and in 95 patients with unknown food allergy histories, 135 ingestion challenges were carried out.

Three basic types of nasal response were recorded: (1) the immediate nasal response (INR): onset within 70 minutes, maximum within 105 minutes, and resolving within 180 minutes; (2) the late nasal response (LNR): onset within six hours, maximum within ten hours, and resolving within 24 hours; (3) the delayed nasal response (DNR): onset within 24 to 28 hours, maximum within 32 to 36 hours, resolving within 48 to 52 hours after the food ingestion challenge. Beside the three basic types of nasal response, two other modifications were also recorded, the so-called dual late nasal response (DLNR)—a combination of an INR and an LNR—and the dual delayed nasal response (DDNR)—a combination of an INR and a DNR (Figs 1 and 2).

The Positive or Highly Suggestive Food Allergy History Group

Of the 72 food ingestion challenges performed in this group of 47 patients, 41 patients developed 65 (90%) significantly positive nasal responses ($P < .05$): 16 (22%) isolated INR (IINR); 23 (32%) DLNR; 11 (15%) isolated LNR (ILNR); ten (14%) DDNR; and five (7%) isolated DNR (IDNR). In the remaining six patients seven (10%) negative nasal responses (NNR) were recorded during the seven food ingestion challenges ($.05 < P < .10$).

The Unknown Food Allergy History Group

A total of 135 ingestion challenges with food were performed in the 95 patients in this group. Fifty-four patients developed 68 (50%) significantly positive nasal responses ($.01 < P < .05$): 13 (10%) IINR; 19 (14%) DLNR; 27 (20%) ILNR; three (2%) DDNR; and six (4%) IDNR. In the remaining 41 patients, 67 (50%) NNR were recorded during the 67 food ingestion challenges ($P > .1$).

The Control Ingestion Challenges

No significant changes were recorded during the 142 control tests ($.05 < P < .1$).

Reproducibility of the Food Ingestion Challenge

None of the 18 patients with repeated food ingestion challenge (six IINR, four ILNR, four IDNR, four NNR) showed any statistically significant differences between the first and the second challenge ($P > .05$). The technique of food ingestion challenge used therefore demonstrated a good reproducibility.

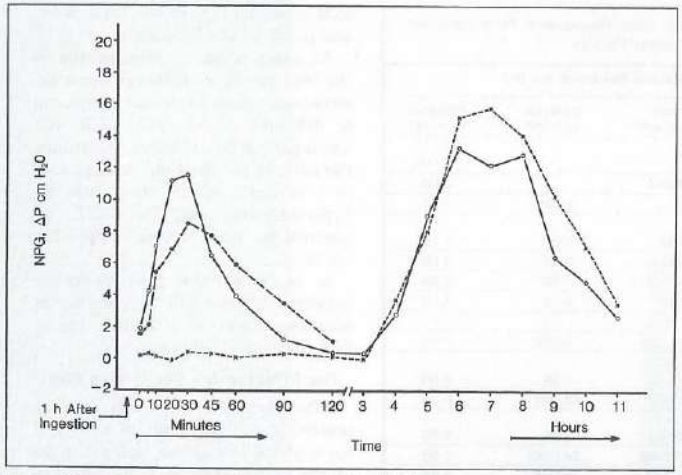

Fig 1.—Mean nasopharynx-nostril-pressure gradient (NPG) values recorded after food ingestion challenge, with respect to appropriate "control" NPG values, were calculated from all patients developing same type of nasal response. Closed circles indicate isolated immediate response (n = 29); open circles, dual late response (n = 42); x's, isolated late response (n = 38).

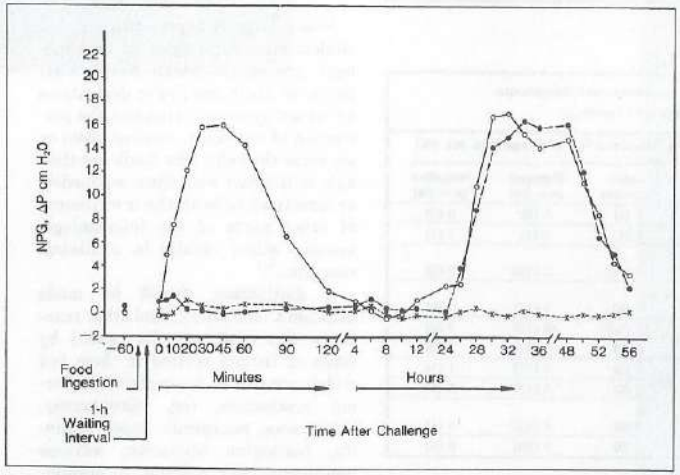

Fig 2.—Mean nasopharynx-nostril-pressure gradient (NPG) values recorded after food ingestion challenge, with respect to appropriate "control" NPG values, were calculated from all patients developing same type of nasal response. Closed circles indicate isolated delayed response (n = 11); open circles, dual delayed response (n = 13); x's, control ingestion challenge with indifferent food (n = 142).

Double-blind Food Challenge

In the 26 randomly selected patients developing eight IINR, six DLNR, two ILNR, four DDNR, two IDNR, and four NNR due to the food ingestion challenge by open schedule, a food ingestion challenge by double-blind schedule was performed eight days later. One patient developed a DLNR to the open challenge but an ILNR to the double-blind challenge. No statistically significant differences were found between the nasal responses recorded after the open and double-blind challenges in any of the remaining 25 patients ($P > .05$).

Control Group

None of the 12 control subjects developed any significant nasal response to the food ingestion challenge ($P > .1$).

Other Diagnostic Factors

The association of the individual types of nasal mucosa response to food ingestion challenge with other diagnostic factors is reviewed in Table 2. In the positive food history group, 65 foods (90%), suspected from the allergy symptom history, caused a nasal response of some type. In the unknown food history group, 68 foods (50%) caused a nasal response of some type. The overall correlation between the allergy symptom history to foods and the nasal response to oral challenge was not significant ($P > .05$).

A positive immediate skin response was found in 47 INR cases (56%), a positive late skin response in 32 cases of LNR (40%), and a positive delayed response in nine DNR cases (38%). The overall correlation found between the skin and the appropriate nasal response in any of the three types was not significant ($P > .05$). In 18 cases (24%) of negative nasal response, the skin tests were positive.

Specific IgE antibodies in the serum for the appropriate foods were positive in 17 INR cases (20%) and in two LNR cases (3%). This correlation was not significant ($P > .1$). The increased serum concentration of IgG, IgM, and IgA antibodies during the nasal response showed no statistically significant correlation with any type of nasal response ($P > .05$). However, the increase of IgG (>18 g/L [>1800 mg/dL]) in 19 cases (24%) and of IgM (>3.8 g/L [>380 mg/dL]) in eight cases (10%) of LNR may be an interesting finding.

Table 2.—The Association of the Various Types of Nasal Response to Food Ingestion Challenge With Other Diagnostic Factors

	Nasal Mucosa Response, No. (%)			
	Immediate (n = 84)	Late (n = 80)	Delayed (n = 24)	Negative (n = 74)
Positive skin response				
Immediate	47 (56)	...	...	14 (19)
Late	...	32 (40)	...	3 (4)
Delayed	...	...	9 (38)	1 (1)
Positive specific IgE in the serum (RAST)*	17 (20)	2 (3)	0 (0)	2 (3)
Increased serum IgG	6 (7)	19 (24)	0 (0)	3 (4)
Increased serum IgM	1 (1)	8 (10)	1 (4)	0 (0)
Increased serum IgA	0 (0)	1 (1)	0 (0)	1 (1)
Increase in blood	7/4	9/15	1/2	1/1
Eosinophils/leukocytes	(8/5)	(11/19)	(4/8)	(1/1)
Nasal mucosa appearance				
Hyperemic	33 (39)	12 (15)	1 (4)	3 (4)
Violaceous	51 (61)	68 (85)	22 (92)	0 (0)
Nasal mucosa hemorrhages	0 (0)	13 (16)	1 (4)	0 (0)
Nasal symptoms	84 (100)	80 (100)	24 (100)	7 (9)
Obstruction	81 (96)	77 (96)	24 (100)	0 (0)
Hypersecretion	83 (99)	11 (14)	0 (0)	3 (4)
Sneezing	49 (58)	0 (0)	0 (0)	2 (3)
Itching	12 (14)	41 (51)	16 (67)	2 (3)
Changes in the nasal secretions eosinophil count	39 (46)	20 (25)	1 (4)	5 (7)

*RAST indicates radioallergosorbent test.

Table 3.—Review of Other Organs' Responses and Complaints Recorded After the Food Ingestion Challenge

	Nasal Mucosa Response to Food Ingested, No. (%)			
	Immediate (n = 84)	Late (n = 80)	Delayed (n = 24)	Negative (n = 74)
Conjunctival response	9 (11)	6 (8)	1 (8)	0 (0)
Bronchial obstruction	2 (2)	3 (4)	1 (4)	1 (1)
Middle-ear response (otalgia, changes in middle-ear pressure)	7 (8)	8 (10)	3 (13)	2 (3)
Responses of sinuses (acute edema of sinus mucosa)	3 (4)	7 (9)	4 (17)	0 (0)
Cephalalgia	14 (17)	15 (19)	18 (75)	3 (4)
Urticaria	1 (1)	5 (6)	5 (21)	2 (3)
Angioneurotic edema	3 (4)	2 (3)	4 (17)	1 (1)
Body temperature increase	0 (0)	0 (0)	3 (13)	0 (0)
Gastrointestinal complaints (nausea, vomiting, diarrhea)	2 (2)	7 (9)	5 (21)	1 (1)
General malaise complaints	4 (5)	4 (5)	8 (33)	0 (0)

Increased blood eosinophilia (>300 $\times 10^6$/L [>300 $\times 10^3$/mm^3]) was found in seven cases of INR (8%), in nine cases of LNR (11%), and in one case of DNR (4%), while increased blood leukocytosis (>10 $\times 10^9$/L [10 $\times 10^3$/mm^3]) was found in four cases of INR (5%), in 15 cases of LNR (19%), and in only two cases of DNR (8%).

The nasal mucosa was hyperemic in 39% and it was violaceous in 61% of the INR cases. The violaceous nasal mucosa was found in 85% of the LNR cases and in 92% of the DNR cases. Small hemorrhages on the nasal mucosa were found in 13 cases of LNR (16%). The changes in the eosinophil count of the nasal secretions, compared before and after food ingestion challenge, were found in 46% of the INR cases, in 25% of the LNR cases, and in 4% of the DNR cases.

All cases of nasal response due to the food ingestion challenge, however, were accompanied by nasal symptoms to different degrees. The INR was accompanied by all nasal symptoms; the LNR by obstruction, itching, and, in a minority of the cases, also by hypersecretion; and the DNR by obstruction and itching only (Table 1).

In some of the patients other organs' responses or other complaints also were observed after food ingestion challenge (Table 3).

The Effect of the Elimination Diet

After avoiding the foods causing positive nasal responses of any type for a period of eight to 12 months, 38 of the 41 patients from the positive food history group and 49 of the 54 patients from the unknown food history group reported a distinct decrease in their nasal symptoms.

COMMENT

Food allergy or hypersensitivity is a clinical manifestation of an immunologic process in which foods, their parts, or their metabolic derivatives act as antigens and stimulate the production of antibodies against them or sensitize the cells. The foods are then able to interact with these antibodies or sensitized cells on the involvement of other parts of the immunologic system, which results in a clinical reaction.[1,5,21]

A distinction should be made between a food allergy and other reactions that could also be caused by foods or factors related to them but which are due to a completely different mechanism (eg, idiosyncrasy, intolerance, nonspecific hyperreactivity, histamine liberation; adverse nonimmunologic reaction to noncontrolled chemical compounds, microorganisms and their products, controlled chemical compounds, and additives).[1,22,23]

The phrase "adverse reactions to foods" would seem most appropriate when immunologic mechanisms cannot be demonstrated. Food allergy would then be one of the suspected mechanisms.[1,19]

Additives, being controlled chemical compounds in foods, form a special problem. They are commonly used and their mode of action in patients with adverse reactions to them is not yet known in detail.[24,25]

The participation of foods in patients with allergic rhinitis has regularly been discussed in the literature, sometimes from controversial points of view.[1-5,8,9,16,20,26,27] The involvement of foods through hypersensitivity mechanisms has been suggested as one of the possible causes.[4-5,8,22,28-31]

The three types of nasal response due to the food ingestion challenge recorded in this study may be comparable to the basic types of nasal response to nasal challenge with inhalant allergens.[10,11,13] A positive allergy symptom history for a certain food was confirmed by food ingestion challenge in 90% of the patients. However, 50% of the patients with rhinitis with an unknown history for foods allergy developed a nasal response after food ingestion. It can be concluded that a positive history for certain foods in patients with rhinitis is a valuable diagnostic factor, while the unknown history cannot exclude such a role. Skin tests with food extracts were found to be useful diagnostic factors by some authors[20,28,29] while not by others.[4,8,32]

Our results demonstrated the positive immediate skin response in 56% of the INR cases, the late skin response in 40% of the LNR cases, and the delayed skin response in 38% of the DNR cases. On the other hand, in 24% of the negative nasal responses, a positive skin response was observed. The correlation between the positive immediate skin response and the positive INR to food of 56% differs from other investigators' data, which find either a lower (30%)[3] or a higher correlation (90%).[28,29] This difference might be caused by the clinical feature of the patients studied. Our patients suffered from rhinitis only, while other investigators' patients formed a less comparable group.[28,29] Our results of 24% positive skin response in patients with negative nasal response to foods is similar to the results of Atkins et al,[28,29] who found 21% false-positive skin tests. The skin tests with food extracts therefore should be regarded as a screening procedure only.

Specific IgE in the serum (RAST) for the particular foods was found only in 20% of the INR cases. The correlation between the RAST for foods and the INR was not significant. These results are similar to other investigators' findings.[27-29,33,34] The RAST with foods is frequently used, but it is also one of the controversial methods.[5,16,33-36]

Increased serum concentration of IgG antibodies was found in 24% and that of IgM in 10% of the LNR cases. Although this correlation was not significant, it might be suggestive of involvement of IgG and IgM antibodies in some cases of LNR to the foods ingested.

An interesting finding was the appearance of the nasal mucosa during the individual types of nasal response. The violaceous nasal mucosa was found during the INR in 61%, during the LNR in 85%, and during the DNR in 92% of the cases, while hyperemic nasal mucosa was observed in 39% of the INR, in 15% of the LNR, and in 4% of the DNR cases. These results, together with the findings of small mucosal hemorrhages in 16% of the LNR cases might suggest involvement of different mechanisms in the individual types of nasal response.

The changes in the eosinophil count in nasal secretions in 46% of the INR cases can be indicative of a possible involvement of the immediate hypersensitivity mechanism in the INR. This percentage, however, was lower than during the INR to inhalant allergens (74%).[11] Ingestion challenge with foods should be considered one of the important diagnostic tools for the assessment of the role of a certain food in a patient's complaints.[27-31,33,34,37]

Despite various reports dealing with provocative testing with foods, studies concerning the nasal response to food ingestion by recording of the objective factors in patients with allergic rhinitis are not numerous.[5,16]

The following requirements should be met for the ingestion challenge with foods[5,16]: (1) It should be safe, reproducible, sensitive, independent of the influence of the patient and investigator. (2) The use of technical equipment, the methods, materials, and factors recorded should be monitored. (3) The evaluation of indications and contraindications for the individual patient should be documented. Detailed history, physical examination, and basic laboratory tests should precede the oral challenge: an absolute contraindication is the presumption of an anaphylactic reaction to the specific food,[9,19,20,28,29] pregnancy, and any disorder that could lead to irreversible damage in the patient or to an emergency situation; the relative contraindication is any state influencing the results of the oral challenge or leading to any undesirable complication in the patient. (4) Oral challenges concerning a response, in which the nonvital factors are recorded (eg, nasal resistance) and in which no serious reaction is to be expected, can be performed in outpatients. The challenges in which the vital factors are recorded (eg, lung function, distinct diarrhea, hypothermia, or hyperthermia) or in which a late onset of the organ response is expected should be performed during hospitalization, under surveillance. Oral challenge should be carried out in a well-equipped department with resuscitation and intensive care facilities and well-trained staff. (5) The allergens (foods or extracts), their processing, and quality should be checked continuously. (6) The particular foods, their parts, and the related foods should be excluded from the diet for a sufficiently long period before the oral challenge. Some authors recommend one to two weeks,[8,20] while our clinical experience indicates a four- to six-day exclusion to be sufficient.[5,16]

There are two basic techniques for food ingestion challenges, the double-blind challenge[19,20] or the open challenge with natural foods.[5,9,16,33,36] Both these techniques have advantages and disadvantages.[5,21] The open challenge, in our experience, was sufficient in patients in whom objective factors, independent of the patient's influence, can be recorded, eg, the nasal resistance.[16] This fact is supported by the results of this study, demonstrating nonsignificant differences between

nasal responses recorded after both the schedules of food ingestion challenge in 25 of 26 patients. The double-blind crossover schedule seems to us to be important in patients in whom the objective factors cannot be recorded (eg, migraine, gastrointestinal complaints, skin itching), or in whom the organ response may be influenced by the patient, or in whom drug effects are investigated.[18]

Despite the important advantage of the double-blind technique, that its results are less influenceable, there are some disadvantages: (1) The capsules to be swallowed are maximally of 500-mg content. The dose of the food may then be distinctly lower, or the number of capsules would have to be increased distinctly to equal the natural consumption (eg, for 100 g of meat 200 capsules would be necessary).[2,3,10] (2) The foods must be colorless, odorless, and tasteless. Such preparation is either not always possible or can lead to changes in physical or chemical properties. (3) Providing a suitable placebo regarding quantity, consistency, color, and taste is not always possible.[2] (4) The administering of food in capsules excludes the buccal mucosa, tongue, and esophagus. These organs may be the site of the response in some patients (edema of the tongue or epiglottis, vesicular eruption of the buccal mucosa, gingivitis, dysfunction of the esophagus, etc).[16,21,35]

It can be concluded that a definite confirmation of the role of a certain food in nasal complaints of patients should be provided by food ingestion challenge combined with rhinomanometry.[2,3,15] The results of our study, according to our previous investigations,[5,16] demonstrated three basic types of nasal response due to the food ingestion. The immunologic mechanisms underlying the individual types of nasal response to foods could not be fully clarified; therefore, further concurrent immunologic studies will be necessary.

R. Aalberse, PhD, and C. Aalj, MD, of the Central Laboratory of the Dutch Red Cross Transfusion Service performed the RAST.

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Effects of Oral Cromolyn on the Nasal Response due to Foods

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• Thirty-eight patients with a perennial allergic rhinitis, who developed a nasal response to ingestion challenge with certain foods, were randomly selected for protection tests with oral cromolyn sodium (Nalcrom). The food challenges were performed in combination with rhinomanometry. The patients were pretreated with cromolyn or placebo by double-blind crossover schedule, in a daily oral dose of 200 mg (four times), starting 3 days before and continuing up to 3 days after the food ingestion challenge. The 38 patients previously developed 25 immediate, 24 late, and 6 delayed nasal responses to food ingestion challenge. Cromolyn fully prevented 15, significantly decreased 9, and was ineffective in 1 case of immediate nasal response. Of the 24 cases of late response, cromolyn fully prevented 10, significantly decreased 12, and was ineffective in 2. Of the 6 cases of delayed response, 2 cases were decreased significantly by cromolyn, while the other 4 cases were not. The protection effects of oral cromolyn were highly significant for the immediate and late nasal responses and nonsignificant for delayed responses. It can be concluded that cromolyn in a daily oral dose of 200 mg four times prevented the immediate and late nasal responses to ingested food.

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Allergic rhinitis due to adverse reactions to foods and the involvement of hypersensitivity as one of the suspected mechanisms has already been reported in the literature.[1-14] However, the possible role of food allergy and food in general in subjects suffering from nasal complaints remains underestimated by clinicians. In the literature there is a dearth of information concerning not only the involvement of foods and food allergy in the allergic disorders of the airway, but also the pharmacologic control and treatment of such disorders.[1,4,5,7-24]

Oral cromolyn sodium and its possible protective effects on various clinical manifestations of food hypersensitivity have regularly been discussed in the literature, though sometimes from controversial points of view.* However, we were unable to find any report in the available literature concerning the investigation of the possible protective effects of oral cromolyn on the nasal response to food ingestion challenge, especially on the particular types of nasal response, in a sufficiently large and well-diagnosed group of patients.

The purpose of this study, being a continuation of our preliminary articles,[7,9,11,13] was to investigate the possible existence of protective effects of oral cromolyn sodium (Nalcrom) on the three basic types of nasal response to food ingestion challenge in patients with allergic rhinitis[14] and to define the indications for the practical use of this drug in patients suffering from nasal complaints due to the food ingested.

*References 2, 8, 9, 11, 13, 15, 17, 18, 21, 22, 24-37.

PATIENTS AND METHODS

Patients

Thirty-eight patients who developed a nasal response to ingestion challenge with certain foods, correlating with positive history and/or positive intracutaneous tests, were included in this study. These 38 patients were randomly selected from a large group of 142 patients who had been studied and extensively described in our previous study.[14] Patients were aged 14 to 60 years and had perennial allergic rhinitis (nasal obstruction accompanied by hypersecretion, sneezing, and itching to various degrees) for longer than 5 years.

A routine diagnostic procedure was done in all patients, which included skin tests and nasal challenges with inhalant allergens, food-focused history, intracutaneous tests with standard and supplementary series of food extracts, and ingestion challenge with selected foods in combination with recording of nasal resistance by means of rhinomanometry.

None of the patients studied had had an anaphylactic or other serious life-threatening reaction to foods in the past. None had received previous immunotherapy or used oral corticosteroids or long-acting antihistamines. Treatment with topical corticosteroids and intranasal cromolyn was stopped at least 4 weeks before the study. No short-acting antihistamines were given during 46 hours and no topical decongestants during 12 hours before each of the food ingestion challenges. The food selected for ingestion challenge and related foods were always avoided for at least 7 days before each of the ingestion challenges.

The 38 patients developed the following types of nasal response to food ingestion challenge: 8 isolated immediate, 10 isolated late, 14 dual late (a combination of an immediate and a late), 3 isolated delayed, and 3 dual delayed responses (a combination of an immediate and a delayed). In 21 of these patients, the nasal response to the food ingestion challenge was verified by the double-blind placebo-matched crossover technique, as was reported in our previous study.[14]

Table 1.—Survey of Foods Used and Particular Types of Nasal Responses*

	Total (N = 38)	IINR (n = 8)	ILNR (n = 10)	DLNR (n = 14)	IDNR (n = 3)	DDNR (n = 3)
Cheese	7	1	2	2	1	1
Chocolate	6	2	2	1	1	0
Peanuts	4	1	1	0	1	1
Milk	4	1	1	1	0	1
Dutch sweets	3	1	1	1	0	0
Shrimp	2	0	1	1	0	0
Apple	2	0	1	1	0	0
Tomato	2	1	0	1	0	0
Egg	1	0	0	1	0	0
Onion	1	0	1	0	0	0
Hazelnuts	1	0	0	1	0	0
Codfish	1	1	0	0	0	0
Banana	1	0	0	1	0	0
Garlic	1	0	0	1	0	0
Pork	1	0	0	1	0	0
Sherry	1	0	0	1	0	0

*IINR indicates isolated immediate nasal response; ILNR, isolated late; DLNR, dual late; IDNR, isolated delayed; and DDNR, dual delayed.

Skin Tests

Dialyzed and lyophilized food extracts, diluted in phosphate-buffered saline (dry weight of food extract in milligrams per 1 mL of phosphate-buffered saline; Laboratory "Diephuis," Groningen, the Netherlands), were used in the following concentrations: cheese and eggs, each in 1 mg/mL; nuts, chocolate, cocoa powder, Dutch sweets, nonalcoholic beverages, beers, and wines, each in 0.5 mg/mL; meat, spices, and beans, each in 0.2 mg/mL; all other foods, each in 0.1 mg/mL.

Scratch tests were performed and evaluated after 20 minutes. If they were negative, then intracutaneous tests were carried out and the results evaluated after 20 minutes and 4, 8, 12, 36, 48, 60, 72, 84, and 96 hours. A positive skin response, appearing within 20 minutes, was considered to be an immediate skin response, that appearing between 6 and 24 hours was considered to be a late skin response, and that appearing after 48 hours was considered to be a delayed skin response.[14]

Rhinomanometry

A modification of the posterior technique, described previously by us in detail,[7,38-42] was used for assessing the nasal mucosa response. The nasal resistance values (nasopharynx-nostril-pressure gradient [NPG] values) were recorded. The nasal response was considered to be positive when the mean NPG values after food ingestion challenge increased by at least 2.7 ± 0.6 cm H_2O (mean ± 2 SDs) with respect to the "initial" values, recorded at least at three consecutive intervals.[14]

Food Ingestion Challenge

The open food ingestion challenge was performed according to the following schedule: (1) baseline values were recorded at 0.5 and 10 minutes; (2) the food was ingested within 5 minutes, and then a 1-hour waiting period followed to allow the food to be digested; during this hour interval the nasal values were measured every 15 minutes to exclude an unexpected or too-early nasal response; (3) after the 1-hour waiting interval, the actual postchallenge nasal values were recorded at 0, 10, 20, 30, 45, 60, 90, and 120 minutes and then every hour up to the 12th hour and every second hour during the 24- to 36-hour intervals and the 47- to 56-hour intervals.[14]

A survey of foods used for ingestion challenge, protection tests with cromolyn, and the particular types of nasal response is given in Table 1.

Control Test

The control ingestion challenge conducted with cooked rice, cooked potatoes, or glucose solution, depending on the patient's problem, was performed in the same way as the experimental food challenge.[14]

Rhinoscopy Complaints

The appearance of the nasal mucosa, nasal complaints, and other complaints were recorded before and then every 2 hours during the 0- to 12-, 24- to 35-, and 47- to 56-hour periods after non-pretreated as well as pretreated ingestion challenges.[14]

Other Diagnostic Factors

Radioallergosorbent test, single radial immunodiffusion (Mancini technique), blood leukocyte and eosinophil count, and eosinophil count in nasal secretions were performed as previously described.[14]

Drugs

Cromolyn sodium (Nalcrom) was used in powder form administered orally in capsules (one capsule being 100 mg). Placebo was the tablet material in powder form administered orally in capsules (one capsule being 100 mg of tablet material).

Protection Tests and Study Design

In the patients selected, the food ingestion challenge as well as the control ingestion challenge with an indifferent food were repeated, and then two protection tests, one with oral cromolyn and another with placebo, were performed. The design of the study was double-blind crossover placebo-matched. The basic schedule of the protection tests (pretreated challenge) was similar to that of the non-pretreated challenge.

The patients were pretreated with cromolyn sodium and placebo in a daily oral dose of 4 × 2 capsules (4 × 200 mg) taken 30 minutes before meals (at 8 AM and at 1, 7, and 11 PM) starting 3 days before the challenge and continuing throughout the challenge day up to 3 days after the challenge. The tests were separated by an interval of 7 days.

The protection effects with the oral cromolyn were considered to be clinically significant when the NPG values recorded after the pretreated food ingestion challenge decreased by at least 50% with respect to the NPG values recorded after the non-pretreated food ingestion challenge.

Statistical Analysis

The particular types of the nasal response to food ingestion challenge were statistically evaluated by the Wilcoxon Matched Paired-Signed-Ranks Test, comparing the NPG values recorded after food challenge with mean NPG values of baseline (before the challenge).

The positive nasal responses of a particular type were compared with appropriate control challenges and statistically evaluated by the Mann-Whitney *U* Test. The results of the repeated food challenges were compared with those observed in the previous study[14] (reproducibility) and statistically evaluated by the Mann-Whitney *U* Test.

The results of both protection tests (cro-

Table 2.—Time Course of Basic Types of Nasal Response to Food Ingestion Challenge

	Onset	Maximum	Resolving
Immediate, min	60-70	90-105	150-180
Late, h	4-6	8-10	12-24
Delayed, h	26-30	32-38	48-56

molyn and placebo) and their differences were statistically analyzed and evaluated by the Wilcoxon Paired-Signed-Ranks Test. A P value below .05 was considered to be statistically significant in all analyses.

RESULTS

Types of Nasal Response

The 38 patients developed 25 immediate (INR; $P < .02$), 24 late (LNR; $P < .01$), and 6 delayed (DNR; $P < .05$) nasal responses to food ingestion challenge. Eight of them were isolated immediate, 10 isolated late, 3 isolated delayed, 14 dual late, and 3 dual delayed nasal responses. The time course of the basic types of nasal response to food ingestion challenge is described in Table 2.

Control Ingestion Challenge

No significant NPG changes were recorded during the 38 control food ingestion challenges ($P > .1$).

Comparison Between Previous and Actual Food Ingestion Challenges

None of the 38 patients studied showed any statistically significant differences in NPG values between the first (previous) and the second (actual) challenges. The P values for the particular types of nasal response were as follows: INR, $P > .1$; LNR, $P > .05$; and DNR, $P > .05$.

Protection Tests With Oral Cromolyn

The oral cromolyn demonstrated the following protective effects on the particular types of nasal response to food ingestion challenge. (1) For INR, the cromolyn fully prevented 15 (60%), significantly decreased 9 (36%), and was ineffective in 1 case (4%). (2) For LNR, the cromolyn fully protected 10 (42%), significantly decreased 12 (50%), and was ineffective in 2 cases (8%). (3) For DNR, the cromolyn significantly decreased 2 (33%) and nonsignificantly decreased 4 cases (67%). The protective effects of oral cromolyn as compared with placebo were statistically highly significant for INR ($P < .001$), distinctly significant for LNR ($P < .01$), and nonsignificant for DNR ($P \leq .05$). The non-pretreated nasal responses to food ingestion challenge as well as those pretreated with

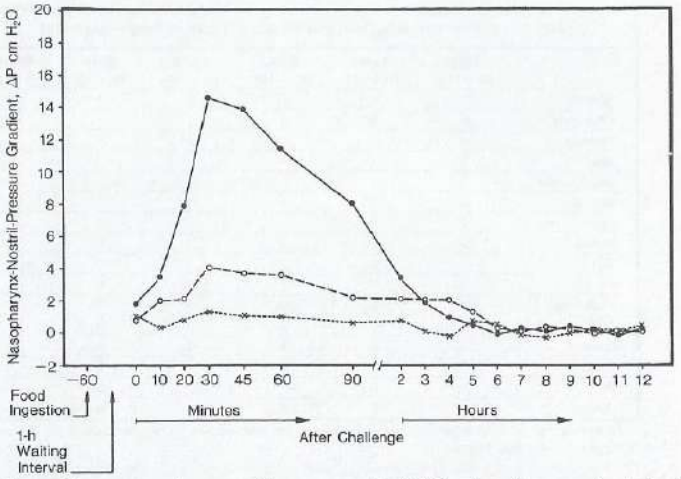

Fig 1.—The mean nasopharynx-nostril-pressure gradient (NPG) values after non-pretreated and pretreated responses due to the food ingestion challenge, with respect to the appropriate control NPG values calculated from all patients developing the same type of nasal response, immediate nasal response (n = 23); closed circles indicate non-pretreated response; open circles, response pretreated with cromolyn sodium (Nalcrom); and crosses, control test (n = 23).

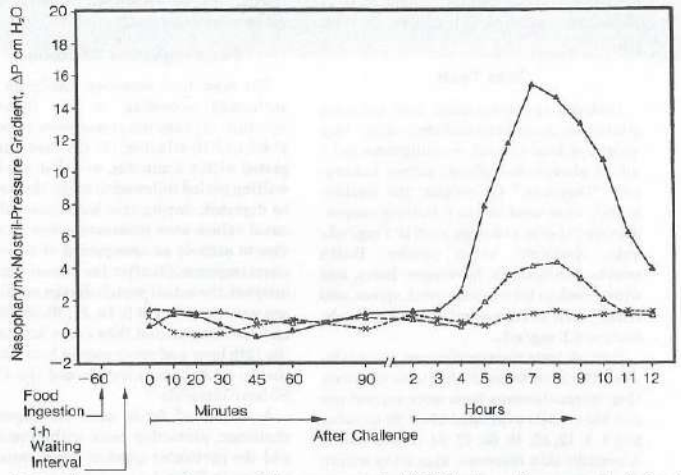

Fig 2.—The mean nasopharynx-nostril-pressure gradient (NPG) values after non-pretreated and pretreated response due to the food ingestion challenge, with respect to the appropriate control NPG values calculated from all patients developing the same type of nasal response, late nasal response (n = 24); closed triangles indicate non-pretreated response; open triangles, response pretreated with cromolyn sodium (Nalcrom); and crosses, control test (n = 24).

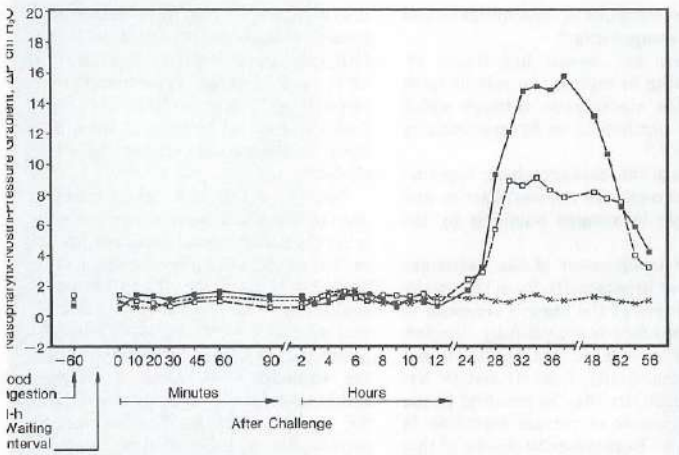

Fig 3.—The mean nasopharynx-nostril-pressure gradient (NPG) values after non-pretreated and retreated responses due to the food ingestion challenge, with respect to the appropriate control IPG values calculated from all patients developing the same type of nasal response, delayed nasal response (n = 6); closed squares indicate non-pretreated response; open squares, response retreated with cromolyn sodium (Nalcrom); and crosses, control test (n = 6).

ral cromolyn are summarized in Figs through 3.

COMMENT

The participation of food in allergic hinitis symptoms has already been iscussed in the literature, though ometimes from controversial points f view.[1-14] There is a dearth of infornation concerning the well-documented data confirming the causative ole of foods in nasal complaints in a ufficiently large group of patients vith rhinitis.[14]

The three types of nasal response o food ingestion challenge described n our previous studies[7-9,11,13,14] may be omparable with the basic types of naal response to inhalant allergens reorted previously by us.[38-43] A hypernsitivity mechanism has been uggested as one of the possible pathvays.[8,14] However, the exact mechnisms underlying the particular ypes of nasal response to foods and hose to inhalant allergens must not ecessarily be similar and comarable.[8,14] Unfortunately, the exact echanisms leading to the particular ypes of nasal response to food ingested are not yet fully clarified and eed more concurrent immunologic tudies.[14]

The food ingestion challenge should be considered one of the important diagnostic tools for the detection and confirmation of the role of a certain food in patients' complaints.[9,14] Its technical and interpretation aspects as well as basic methods employed (open vs double-blind challenge) have already been exhaustively discussed in our previous studies.[9,14]

The ingestion challenge with selected foods is utilized at our department as a standard part of the routine diagnostic procedure in patients suffering from various disorders to confirm the participation of a certain food in the patient's complaints and to detect the particular type of organ response, in this case nasal response.[7-18,44-47]

We also routinely use another modification, the so-called protection tests, where the challenge is pretreated with a certain drug.[9,11,13,44-47] The "protective effects" of the drug investigated refer then to its ability to prevent or significantly decrease the development of the organ response due to the allergen challenge, in this case food ingestion challenge.

The protective effects of oral cromolyn on various clinical manifestations due to the adverse reactions to foods have regularly been studied and published in the literature. In most of these studies, however, the possible protective effects of oral cromolyn on the skin disorders (atopic eczema, urticaria),[27,33-36] colon disorders (colitis ulcerosa, Crohn's disease),[31,37] or multiple symptoms[21,23,25,26] due to adverse reactions to foods, have been investigated.

Moreover, in a majority of these studies, the protective effects of oral cromolyn were evaluated only by the recording of symptom scores or clinical improvement and they were not related to a qualitatively and quantitatively well-defined stimulus such as ingestion challenge with a certain food. There are few data available to illustrate the protective effects of oral cromolyn on nasal responses to the food ingested.[8,9,11-23]

The results of this study demonstrated significant protective effects of oral cromolyn on the INR ($P < .001$) and on the LNR ($P < .01$) but not on the DNR ($P \leq .05$), as compared with placebo. On the other hand, the low number of DNR cases did not allow statistical evaluation sufficient to demonstrate real clinical utility.

The positive protective effects of cromolyn on the INR as well as on the LNR due to the food ingested can suggest the following hypothesis: either the mechanisms underlying both types of nasal response may include some similar parts and steps, or these mechanisms are different and cromolyn can affect all of them.

Cromolyn possesses manifold pharmacologic effects. It protects the mast cell and basophil from degranulation and release or selective secretion of mediators, possibly by stabilizing cell membranes, by blocking calcium transport, and by inhibiting calcium gate opening induced by antigen. There is also evidence suggesting that cromolyn elevates membrane-associated cyclic adenosine monophosphate, either directly or indirectly through the inhibition of phosphodiesterase. This increase may inhibit release of mediators.[48-53]

Cromolyn also seems to decrease the neutrophil chemotactic activity, to increase cyclic adenosine monophosphate, and to decrease cyclic guanosine

monophosphate in cells such as neutrophils and platelets, and tissue cells like lung tissue cells.[32,49] The exact mechanism(s) underlying the pharmacologic actions of the oral cromolyn in the gut, resulting in its protective effects on the various organs' response to food ingested, is not yet fully clarified.[14]

On the other side there is a lack of exact knowledge of the process and mechanisms in the gut through which food and/or its parts are able to act as an antigen and initiate the hypersensitivity reactions leading to the response of a certain organ.[14] The crucial questions in this poorly understood area of allergology include the role of the intestine in controlling uptake of ingested antigens, the mechanisms directly involved during the resorption of the potential antigens, and lastly the presentation of food antigens and their components.[14]

There are several hypotheses attempting to explain the role of foods and the mechanisms through which foods participate in hypersensitivity states.[54-57]

One of the most promising hypotheses concerns the mucosal barrier and its role in antigen handling by the gut.[58]

The involvement of the particular types of hypersensitivity in the particular types of the organ's response to ingested food is not yet fully clarified. There is evidence that besides type I hypersensitivity, type III and IV hypersensitivity may be involved in the pathogenesis of certain reactions to foods.[55-57] Regarding the results of this study, as well as our previous investigations[9,11,13,18] and those of other investigators,[55-57] the involvement of immedate hypersensitivity (type I) in INR, late hypersensitivity (type III) in LNR, and delayed hypersensitivity (type IV; eg, T cells) in the DNR to the food ingested, or at least of some of their modifications, cannot be excluded.

Despite a dearth of sufficient knowledge of the exact mechanisms underlying the basic types of nasal response to food as well as of pharmacologic action of oral cromolyn, it can be concluded that cromolyn sodium in a daily oral dose of 4 × 200 mg significantly prevented the INR and the LNR due to the ingested food. Oral cromolyn seems therefore to be a suitable drug for the prophylaxis of nasal complaints due to ingested food, acting probably through food allergy mechanism(s).

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Role of nasal allergy in chronic maxillary sinusitis—Diagnostic value of nasal challenge with allergen

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The role of nasal allergy in chronic maxillary sinusitis without an air-fluid level was studied in 37 patients. Seventy-three nasal provocation tests with various inhalant allergens were performed in 37 patients by means of rhinomanometry, and maxillary sinus radiographs were performed before and repeatedly after the allergen challenge. Forty-one positive nasal responses (NRs) occurred in 29 patients; 13 were immediate only, 18 were late only, and 10 NRs were dual responses. Of these responses, 32 demonstrated radiographic changes, primarily an increase in mucosal edema and/or opacification. These responses were accompanied by increased pressure in the maxillary sinuses, acute headache, and sometimes otalgia. Eight patients did not develop any NRs; however, increased thickening of the mucosal membrane of the maxillary sinuses, accompanied by subjective symptoms, was recorded in three of these nonresponders. These results demonstrate the role of nasal allergy in some patients with chronic maxillary sinusitis, which may affect the diagnostic and therapeutic approaches to this disorder. (J ALLERGY CLIN IMMUNOL 1990;86:484-91.)

Chronic sinusitis, especially CMS, is a common disorder in adults[1-4] and sometimes in children.[5-7] The structure and function of paranasal sinuses have been well established[1, 3, 8-17] as well as the diagnosis and treatment of sinusitis.[1-4, 13, 18-21] However, most of these studies reflect the role of bacterial and viral infections in chronic sinusitis.[2-4, 6, 7, 11, 22, 23] The association of CMS with rhinitis has been confirmed.[1, 2, 7, 10, 12, 13, 17, 21-24] The etiologic role of nasal allergy in CMS, and the involvement of hypersensitivity as a possible mechanism leading to the CMS, has already been discussed in the literature.[1, 5, 10, 13, 18, 24]

However, little data are available to illustrate the direct causal relationship of hypersensitivity mechanisms appearing primarily in the nasal mucosa tha lead to the secondary response in the mucosal mem brane of the maxillary sinuses. We were unable t find any data in the available literature that studie the changes in the maxillary sinuses after allerge challenge with relation to particular types of NRs.

The purpose of this study as a continuation of ou previous work[25-27] was to investigate (1) the possibl role of the nasal mucosa and nasal allergy in CMS (2) the clinical features and types of the sinus max types of the NR to allergen challenge, and (3) the diagnostic value of nasal challenge with allergen and other supplementary diagnostic parameters for the assessment of the allergic component in patients with CMS.

Abbreviations used

CMS:	Chronic maxillary sinusitis
NPT:	Nasal provocation test with allergen
NR:	Nasal response
PBS:	Phosphate-buffered saline
INR:	Immediate nasal response
IINR:	Isolated immediate nasal response
LNR:	Late nasal response
ILNR:	Isolated late nasal response
DLNR:	Dual late nasal response (immediate plus late response)
NNR:	Negative nasal response (no nasal response)
NPG:	Nasopharynx-nostril pressure gradient expressed in centimeters of water
MSR:	Maxillary sinus response (radiographic changes)
NS:	Nasal secretion

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MATERIAL AND METHODS

Patients

Thirty-seven patients, 18 to 50 years of age, with a history of resistant CMS without an air-fluid level on the radiograph, were referred to our department.

All patients had been treated during the last 3 to 5 years with antibiotics and decongestants. Some of these patients received various kinds of surgical intervention, such as repeated sinus puncture, nasal septoplasty, conchotomy, etc., however, without sufficient improvement. No patient had significant health or allergic disorders, food allergies, or nasal polyps. None of the patients received immunotherapy, oral or topical corticosteroids, disodium cromoglycate, or long-acting antihistamines in the past.

All patients underwent the routine diagnostic procedures consisting of complete disease history, skin tests, blood eosinophil and leukocyte count, NS cytogram, PRIST, and RAST determination in serum. In addition, nasal histamine thresholds were determined, and 73 NPTs with various inhalant allergens with rhinomanometry were also performed and supplemented by radiographs of the maxillary sinuses. Patients were studied during a period of minimal complaints and without symptoms of nasal infections, most of them during hospitalization. No antihistamines or topical decongestants were taken within 24 hours before this study. In each patient, a control nasal challenge with PBS was also performed according to the same schedule as that used for the NPT with allergen. When changes on the radiographs were observed during NPT with allergen, then the PBS control challenge was also supplemented with radiographs of maxillary sinuses, performed at the same time intervals as during NPT with allergen.

Allergens

Dialyzed and lyophilized allergen extracts (Diephuis Laboratory, Groningen, The Netherlands) were diluted in PBS (dry weight of allergen in milligrams per 1 ml of PBS) and used for skin tests in the following concentrations: house dust, 0.25 mg/ml; animal danders and feathers, 0.125 mg/ml; various kinds of molds, 0.1 mg/ml; mites *(Dermatophagoides pteronyssinus)*, 5 NU/ml; and various pollens, 100 NU/ml. The concentration of allergen extracts used for the nasal challenges was tenfold greater.

Skin tests

Scratch tests were initially performed. If these tests were negative, intracutaneous tests were performed and evaluated 20 minutes, 6, 12, 24, 48, 72, and 96 hours after injection. A skin wheal reaction (>7.5 mm) appearing 20 minutes after the intradermal allergen injection was considered a positive immediate skin response, whereas significant induration occurring 6 to 12 hours later was considered a positive late skin response.

NPTs

NPTs were performed by means of a rhinomanometry method, described in our previous articles.[25-36] The NPG values recorded by this method were considered to be the basic parameters of the mucosa NR, principally nasal obstruction. In short, these tests were performed according to the following schedule: (1) initial values (baseline) were recorded at 0, 5, and 10 minutes, (2) PBS control values were recorded at 0, 5, and 10 minutes after a 3-minute application of PBS to the nasal mucosa of the nonintubated nasal cavity by means of a saturated wad of cotton wool on a nasal probe, and (3) test values were recorded similarly after a 3-minute challenge with allergen at 0, 5, 10, 20, 30, 45, 60, 90, and 120 minutes and then every hour up to the twelfth hour and at the twenty-fourth hour.

An NR was considered positive when mean NPG values after allergen challenge increased by a minimum of 2.0 cm H_2O (1.2 ± 0.3 equals mean ± SE) over the PBS control values for at least three consecutive time intervals. The NPG changes occurring within 60 minutes after allergen challenge were considered to be a positive INR, whereas changes appearing 4 to 12 hours after challenge were considered to be a positive LNR.

Allergens for the NPTs were chosen with respect to patient history, skin tests, or RAST.

Cytograms of NSs

NS specimens were collected from the nonintubated nasal cavity by having the patient blow onto a polyethylene sheet immediately after each NPG recording. Specimens were processed and stained according to a modified method of Hansel, described in our previous articles.[28-30, 33]

Radiographs

Maxillary sinus radiographs by Water's projection[19] (other parts having been shielded) were performed at baseline, at 2 hours, and again at either 6 or 12 hours after the allergen challenge. In the cases of isolated INR or NNR, an additional radiograph was performed 24 hours after the allergen challenge and again at 48 hours in the case of LNR.

The radiographs were evaluated in their totality; however, the changes in the mucosal thickening (especially its increase) as an indicator of mucosal edema or infiltration, and the changes in the aeration or opacification of the sinuses were recorded predominantly.

In patients demonstrating changes on radiographs after allergen challenge, the PBS control challenge has also been supplemented with radiographs performed at the same time intervals as radiographs after the allergen challenge, for comparison.

Control group

In eight volunteers suffering from atopic eczema only without any sinusitis complaints, NPT with house dust mites was performed and supplemented by radiographs of the maxillary sinuses. Four of these patients had radiographs before and 2 hours after allergen challenge, whereas another four patients had radiographs before and 24 hours after allergen challenge.

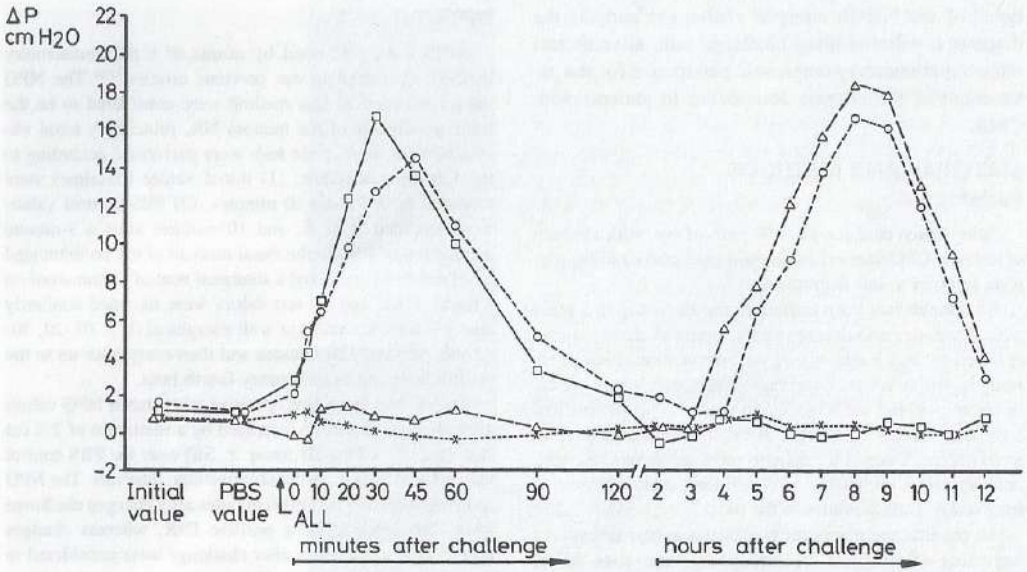

FIG. 1. The mean NPG values recorded after the allergen challenge with respect to the appropriate PBS NPG values were calculated from all patients reacting positively with the same type of response; IINR (□—□) (n = 13), ILNR (△— —△) (n = 18), DLNR (○- - -○) (n = 10), control challenge with PBS (X · · · X) (n = 37); and *ALL*, allergen challenge.

Statistical analysis

The NR to allergen challenge was statistically evaluated by the Wilcoxon matched-pair, signed-rank test, comparing the NPG values recorded after the allergen challenge with the mean NPG value of PBS. A p value <0.05 was considered to be statistically significant.

The positive and negative nasal responses of the same type (INR or LNR) were compared and statistically evaluated by means of the Mann-Whitney U test. A p value <0.05 was considered to be statistically significant.

The correlation between NRs and radiographic changes was evaluated by means of a Fischer's exact test, which is a specialized chi-square test. (A $p < 0.05$ was considered to be statistically significant.)

RESULTS

Of the 37 patients in whom 73 NPTs were performed, 29 patients developed 41 positive NRs, 13 IINRs, 18 ILNRs, 10 DLNRs, and 11 NNRs (Fig. 1). The remaining eight patients demonstrated 21 NNRs (Table I). The differences between the positive and negative NRs were statistically highly significant ($p < 0.001$) at all time intervals.

The 37 control challenges with PBS did not demonstrate any significant changes of NPG when they were compared to baseline values ($p > 0.1$).

Characteristics of the particular types of NR were as follows: (1) INR: onset within 10, maximum within 20 to 45, and resolving within 120 minutes after allergen challenge, (2) LNR: onset within 4 to 6, maximum within 6 to 10, and resolving within 12 to 24 hours, and (3) DLNR is a combination of INR and LNR.

Radiographs of the maxillary sinuses

In 34 of the 37 patients studied (92%), slight baseline thickening of the mucosal membranes in the maxillary sinuses was noted on the radiographs (<3 mm) before the control and allergen challenges were performed.

Thirty-two of the 41 positive NRs (11 IINRs, 15 ILNRs, and six DLNRs) and two of the 11 NNRs, recorded in 29 patients, were associated with changes on the radiographs of the maxillary sinuses, mostly a distinct increase in thickening (edema) of the mucosal membrane (>3.0 mm, usually 5 to 8 mm) and/or increase in opacification or a decrease in aeration (Figs. 2 and 3). The agreement between the positive NRs and positive MSRs on one side and the negative NR and negative MSR on the other side was highly significant ($p < 0.001$). NRs and MSRs are presented in Table I, while particular maxillary sinus changes are reviewed in Table II. In nine of the 11 positive IINRs and both NNR cases, mucosal thickening in the maxillary sinuses and other accompanying changes resolved fully within 24 hours after the allergen challenge, whereas in 12 of 15 ILNRs and all six DLNR cases within 48 hours. The radiographic changes were accompanied by clinical symptoms in all patients, such as pressure in the maxillary sinuses, acute headache, sometimes also otalgia, appearing parallel to the course of the NR (INR or LNR), with a slight delay.

All positive NRs were accompanied by acute nasal symptoms. Cases of INR were characterized by nasal blockage, hypersecretion, sneezing, and itching, whereas LNR cases were characterized predominantly by blockage and hypersecretion.

Three of eight patients with NNRs demonstrated an increased thickening of the maxillary sinus mucosal membrane and symptoms of sinus pressure during three NNRs (Table I). These changes and symptoms resolved within 24 hours after the allergen challenge.

No significant radiographic changes of the maxillary sinuses nor nasal symptoms were recorded in 37 control challenges with PBS. The spontaneous variations in the thickening of the mucosal membrane in maxillary sinuses during the PBS control challenges were always <2 mm and sometimes not even measurable.

Other diagnostic parameters

The results of other diagnostic parameters are summarized in Tables III and IV.

Control group

None of the eight control patients (four in each group) developed any NRs to allergen challenge or demonstrated any changes in the maxillary sinuses or measurable thickening of the mucosal membrane at either 2 or 24 hours after allergen challenge, respectively.

DISCUSSION

The relationship between the nose and sinuses, especially maxillary sinuses, has been well established.[1-7, 10-13, 17, 21-24] Most of these studies, however, reflect the role of bacterial and viral infections in the pathogenesis of CMS. In some patients, the CMS has been referred to as a complication of perennial allergic rhinitis. This conclusion has been drawn either from clinical observation or from epidemiologic follow-up studies.[7, 13, 18, 20] We were unable to find any data correlating the direct role of hypersensitivity reactions affecting the nasal mucosa in the development of maxillary sinus pathology.

There are many similarities in anatomic, physiologic, and pathologic characteristics of the nose and paranasal sinuses, especially the maxillary sinuses.[1, 3, 7, 10, 12, 13, 15-17, 22] Some authors, therefore, report about one type of the mucosal membrane with little distinction.[10, 17]

The maxillary sinus communicates with the nasal cavity through the ostium, which plays a pivotal role for the maxillary sinus and its mucosal membrane

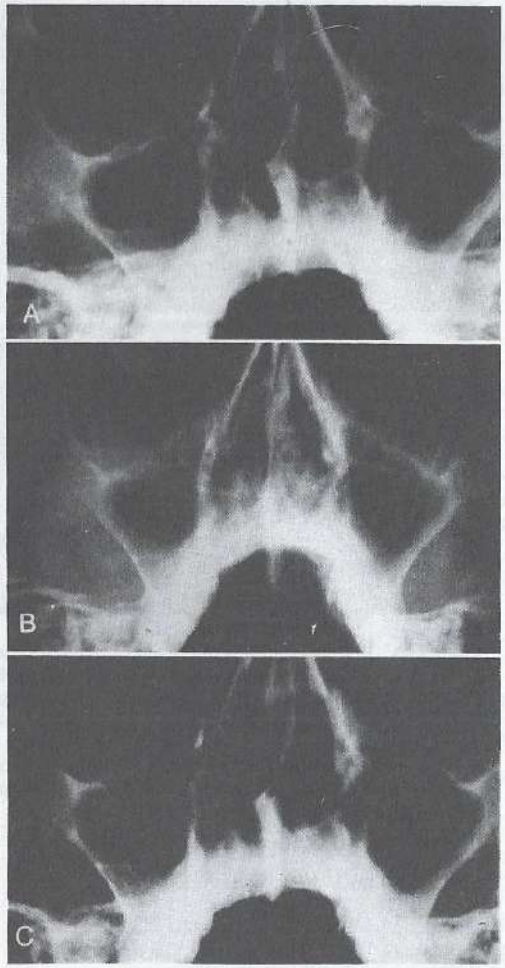

FIG. 2. Radiographs of maxillary sinuses in patient developing an IINR to cat danders in a concentration of 1.25 mg/ml. **A**, Before allergen challenge; thickness of maxillary sinuses mucosa, "baseline"; *right*, 2 mm; *left*, 2 mm. **B**, One hundred twenty minutes after allergen challenge; the thickening of the mucosal membrane in the maxillary sinuses increased with respect to the "baseline"; *right*, 10 mm; *left*, 7 mm. **C**, Twenty-four hours after allergen challenge, the mucosal thickening decreased; *right*, 2 mm; *left*, 2 mm.

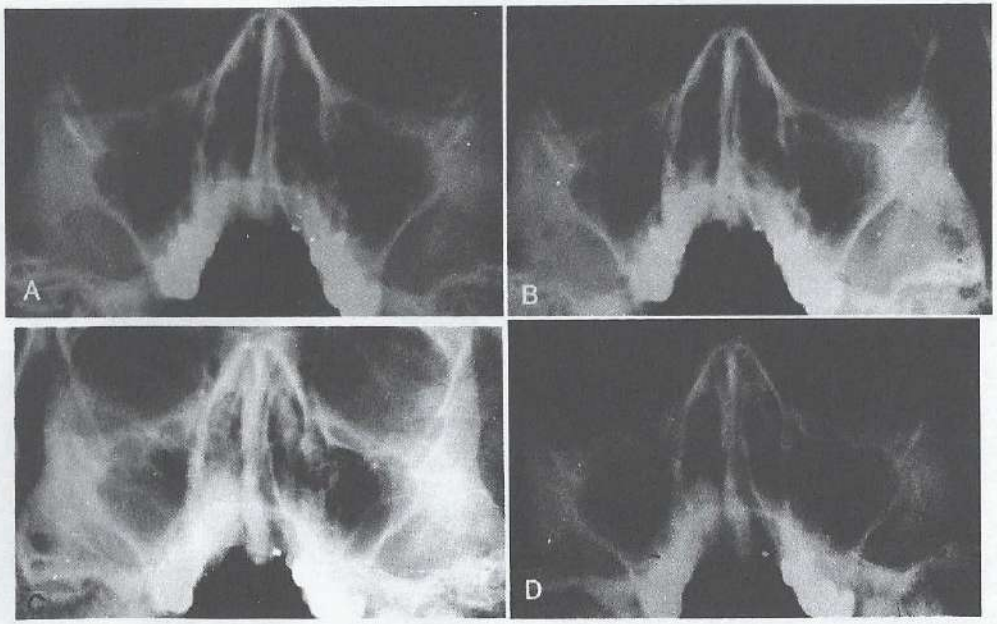

FIG. 3. Radiographs of maxillary sinuses in patient developing an ILNR to mites *(Dermatophagoides pteronyssinus)* in a concentration of 50 NU/ml. **A,** Before allergen challenge; mucosa thickness in maxillary sinuses, "baseline": *right,* 7 mm; *left,* 5 mm. **B,** Six hours after allergen challenge; mucosal thickening in maxillary sinuses increased with respect to the "baseline"; *right,* 14 mm; *left,* 12 mm. **C,** Twelve hours after allergen challenge, mucosal thickening increased; *right,* 17 mm; *left,* 14 mm. **D,** Forty-eight hours after allergen challenge; mucosa thickening decreased; *right,* 12 mm; *left,* 7 mm.

TABLE I. NR and MSRs after nasal challenge with allergen

Patients (n = 37)	MSR	
	Positive	Negative
29 patients		
41 positive NR	32	9
11 negative NR	2	9
8 patients		
21 negative NR	3	18
37 PBS controls	0	37

The agreement between positive NR and positive MSR as well as between negative NR and negative MSR was highly significant ($p < 0.001$).

with the drainage of secretions being the primary function.[1, 3, 10-13, 17, 22]

Several factors may disturb the draining function of the ostia, resulting in retention of secretions in the maxillary sinuses: (1) swelling of the nasal mucosa, leading to reduced patency of ostia, (2) reduced transport capacity related to abnormalities of the cilia, including quantitative reduction, retardation of movement, and insufficient coordination of movement, and (3) overproduction of secretions in the maxillary sinuses.[1, 3, 10, 13, 17] The cross-sectional area of the ostium is also the primary determinant of gas exchange in the human sinuses.[15, 16] Another factor in determinating gas exchange is the nasal airflow.[15, 16] Sinus cavity air exchanges are twice as fast during nasal breathing than during oral breathing.[16]

The hypersensitivity mechanism, that is, antigen-antibody interaction with subsequent steps, occurs in the nasal mucosa with various symptoms. Nasal obstruction caused by swelling of the nasal mucosa is one of these symptoms.[1, 34-36] The nasal mucosa edema leads to an edematic obstruction of the nasal ostia, decreased paranasal sinus ciliary action, and increased mucus production. The whole process then results in the accumulation of mucus and gas in the sinuses, with the subsequent thickening of the mucosal membrane in the sinuses (edema and/or infiltration), a

TABLE II. Changes in the maxillary sinuses mucosal membrane during particular types of NR

Changes in maxillary sinuses	NR			
	IINR (n = 11)	ILRN (n = 15)	DLNR (n = 6)	NNR (n = 5)
Increase in mucosal edema	10*	15	6	5
Increase in opacification	8	11	6	1
Decrease in aeration	7	10	2	3

*One patient demonstrated no increase in mucosal edema, but a distinct increase in opacification and decrease in aeration was noted.

TABLE III. Other diagnostic parameters related to NR with allergen

Positive	NR			
	IINR (n = 13)	ILNR (n = 18)	DLNR (n = 10)	NNR (n = 32)
Disease history	6	7	5	15
Skin test	9	12	6	18
RAST	6	1	4	4
Increased eosinophil count				
Nasal secretions	10	12	7	4
Peripheral blood	8	9	6	1

TABLE IV. Other patient characteristics

Patients (n = 37)	Increased	Nonincreased
Total IgE in serum (PRIST)*	6 (2)	31 (6)
Blood eosinophil count†	13 (1)	24 (7)
Blood leukocyte count‡	1 (0)	36 (8)
Nasal responsiveness to histamine§	12 (4)	25 (4)

*More than 500 IU/ml.
†More than 300×10^6/L.
‡More than 10×10^9/L.
§Less than 12 mg/ml (36 mmol/ml); numbers in parentheses refer to the eight patients with NNRs.

decrease in aeration, and an increase in opacification, and sometimes the formation of an increased fluid level and soft tissue mass.[1, 9, 10, 13, 25-27]

Another mechanism that can be involved in the development of sinusitis has been suggested by Slavin[1] and Slavin et al.[24] These authors postulated that foreign particles that escape the filtering apparatus of the nose can be trapped in the mucus of the sinuses. In such a case, it might be possible that an antigen would also pass the nasal barrier, would be trapped in the maxillary sinuses, and cause an antigen-antibody interaction with mediator release topically in the mucosal membrane of maxillary sinuses.

The existence of these two mechanisms, both of them resulting in the changes of the mucosal membrane in the sinuses, could probably explain our results and, likewise, our results could support the existence of such mechanisms.

Thirty-two of 41 (78%) positive NRs to allergen challenge were accompanied by radiographic changes of the maxillary sinuses (Table I). In this case, designated by us as an "associated form" of maxillary sinusitis, the hypersensitivity reaction is located primarily in the nasal mucosa and leads to a secondary response of the maxillary sinuses. This mechanism is identical to that leading to the obstruction of the ostia through the swelling of the nasal mucosa as noted by other investigators.[1, 9, 10, 13]

In contrast, during five of 32 (16%) negative NRs to allergen challenge, the MSR was documented (Table I). In such a case, called by us a "nonassociated form" of maxillary sinusitis, the hypersensitivity re-

action (antigen-antibody interaction with subsequent steps) takes place locally in the mucosal membrane of maxillary sinuses, and the same organ develops the clinical response. This mechanism may be similar to that described by Slavin[1] and Slavin et al.[24] as "trapping of foreign particles in the mucus of the sinuses."

The relationship between allergic rhinitis and sinusitis is not only academically interesting but also has important diagnostic and therapeutic use, and yet the relationship has not been well studied.[1, 3, 25] Allergic rhinitis and sinusitis are frequently regarded as two different disorders because of different etiologics, since sinusitis is assumed to be caused mainly by infection with microorganisms.[3-6, 11]

The use of the term "sinusitis" for a disorder caused by an immunologic process does not appear to be fully suitable. It would probably be better to speak of the "sinus response" or "allergic sinusopathy."[27]

From the practical point of view, some investigators have tried to replace the radiographs of sinuses by echography to reduce radiation exposure.[21] Unfortunately, the echographic results have not yet been found to be fully comparable with the radiography.[21] Perhaps a new technique, SPECT (single photon emission computerized tomography), will offer a suitable alternative in examining the maxillary sinuses without unnecessary radiation.[24]

In conclusion, the nasal mucosa and nasal allergy may regularly play a role in changes of the mucosal membrane in maxillary sinuses. The nasal challenge with allergen, combined with radiographs of maxillary sinuses, can be a useful diagnostic approach leading to improved therapeutic opportunities.

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IMMEDIATE NASAL RESPONSE TO ALLERGEN CHALLENGE CYTOLOGIC CHANGES IN THE NASAL SECRETIONS AND HISTOLOGIC CHANGES IN THE NASAL MUCOSA

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INTRODUCTION

Patients with nasal allergy may develop different types of nasal response to challenge with an allergen, e.g. immediate (INR), late (LNR) or delayed (DYNR),[1] due to different hypersensitivity mechanisms.[1,2] The immediate nasal response having been classically attributed to the immediate hypersensitivity mechanism (Type I allergy) has been studied most frequently.[2] However, some evidence recently has been provided for possible involvement of other mechanisms in INR.[1-3]

The INR should be regarded as a clinical phenomenon defined by appearance of nasal obstruction accompanied by other nasal complaints and changes within 2 h after exposure to allergen, induced by complex mechanisms. The antigen-antibody interaction leads to various changes and further steps on various levels and to involvement of various cell types, mediators and compounds, resulting in appearance of the INR.[1] The INR may therefore be associated with various cellular, immunological, biochemical and biophysical changes, e. g. in the nasal secretions (NS) and nasal mucosa.[1-3,5]

The purpose of this study, as a continuation of our previous work,[1-5] was to investigate: 1) cytologic changes in the nasal secretions (NS) simultaneously with the histologic changes in the nasal mucosa during the INR; (2) the kinetics and traffic of particular cell types and their involvement in the INR; (3) the significance of such changes for the INR, its mechanism and diagnostic approach.

Patients

Two hundred patients suffering from allergic rhinitis, 117 of them developing INR to challenge with one of the "inhalant" allergens (house dust, animal danders, pollen species, *D. pteronyssinus*) correlating with positive history and intracutaneous (i.c.) tests and 83 patients developing negative nasal responses (NNR) were randomly selected for this study. Nasal challenges were repeated with the same allergens as well as control challenges with phosphate buffered saline (PBS). Cytological examination of the nasal secretions (NS) was also performed in some subjects. In 6 patients with NNR and in 12 patients with positive INR, 6 of whom also received a PBS control challenge, the repeated challenge was supplemented by biopsy of the nasal mucosa. The control tests with PBS were performed 3 days before the first NPT with allergen according to the same schedule, and the NS

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samples were processed in the same way as those obtained during the NPT with allergen (see below).

Nasal Provocation Tests (NPT)

The NPTs with allergens as well as the control challenges with PBS were performed by means of rhinomanometry (the so-called "Balloon method", which is a modification of the posterior technique), described in detail in our previous papers.[2-4]

Nasal Secretions (NS)

Three series of NS specimens were obtained from the non-intubated nasal cavity by having the patient blow onto a polyethylene sheet after every NPG recording, twice at baseline and after PBS challenge and 7 times after the allergen challenge. The NS specimens were processed, stained and evaluated according to the same techniques described in detail in our previous studies.[3,4]

Biopsy Technique

Ten minutes after local anesthesia (1% xylocaine spray), the biopsy was performed by means of cup forceps from the anterior part of the middle turbinate of the challenged nasal cavity. The second biopsy was taken from the central part of the same turbinate. The 1st biopsy was performed between 24 to 2 hours before the allergen challenge, while the 2nd biopsy was taken 30 minutes after the allergen challenge (at the maximum of the INR). The specimens were fixed in 10% buffered formaldehyde, dehydrated, embedded in wax, sectioned and slides were stained with May-Grunwald-Giemsa.

RESULTS

The 117 positive INRs ($p<0.01$), 83 NNRs ($p>0.25$) and 200 PBS control challenges ($p>0.1$) were studied. The differences between INR and NNR as well as between INR and PBS controls were statistically significant ($p<0.001$) at all time intervals. No significant differences were found between the first and the second INR ($p>0.1$), NNR ($p>0.05$) or PBS controls ($p>0.05$).

Cytologic Examination of the Nasal Secretions (NS)

Positive Immediate Nasal Response (INR; n = 117). The presence of the particular cell types in the NS and the changes in their counts are summarized in Table 1, while the course of the changes is shown in Fig. 1. No significant changes in the count of any cell type were found between the baseline and PBS values ($p>0.05$). (a) Eosinophils (ES) were present in NS in 85% of the positive INRs. Before challenge, the ES count was low in 60%, moderate in 15% and high in 10% of the INRs. The positive INR was accompanied by significant changes ($p<0.05$) in the ES count in 68% of cases. In most INR cases, the count increased after allergen challenge and decreased during development of the INR. (b) Neutrophils (NE) were present in NS in 71% of the positive INRs. Before challenge, the NE count was low in 10%, moderate in 15% and high in 46% of the INR cases. Their count demonstrated significant changes during 37% of the INRs ($p<0.05$). The NE count decreased after the allergen challenge and increased slowly during the development of the INR. (c) Basophils (BS) were recorded in NS in 16%, while mast cells (MC) were observed in 4% of the positive INRs. The BS count decreased significantly after the challenge during 13% of the INRs ($p<0.05$), while the count of mast cells did not change. (d) Epithelial cells (EC) were present in NS in 68% of the INRs. The changes in their count (slight increase followed by decrease), recorded in 9% of the INRs, were not significant ($p>0.05$). (e) Goblet cells (GC) appeared in NS in 57% of INR cases. Their

Table 1. Presence of individual cell types in the nasal secretions and changes in their count during the nasal response (in %).

	Presence of cells			Changes in the cell counts between before and after the challenge		
	INR	NNR	PBS	INR	NNR	PBS
Eosinophils	85	19	48	68*	5	3
Neutrophils	71	17	40	37*	3	0
Basophils	16	91	31	3*	0	0
Epithelial cells	68	23	25	9	7	4
Goblet cells	57	13	11	16*	4	2
Lymphocytes	11	4	7	2	3	0
Mast cells	4	2	3	0	0	0
Plasma cells	7	2	3	0	0	0
Monocytes	1	0	0	0	0	0

INR+immediate nasal response; NNR = negative nasal response; PBS = phosphate buffered saline (=control); *=statistically significant

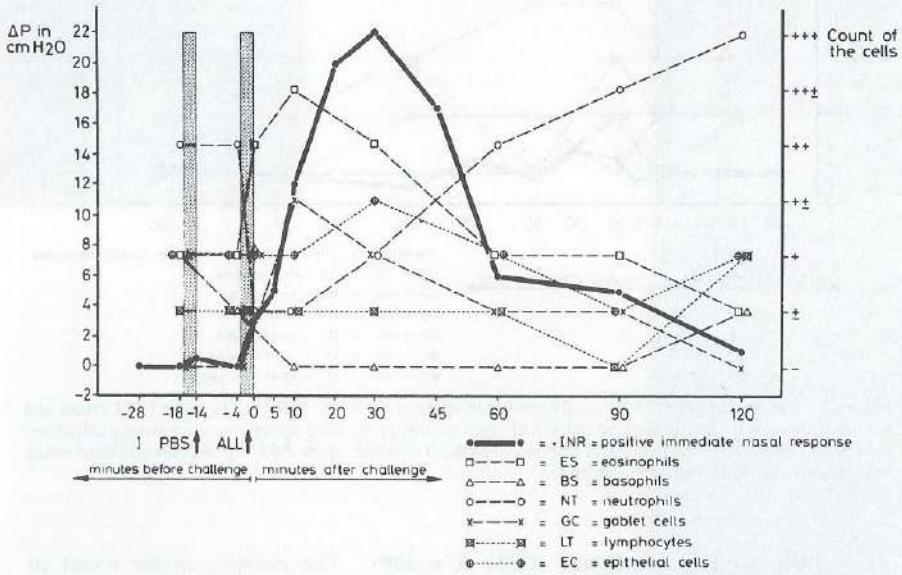

Figure 1. The positive immediate nasal response to allergen challenge (INR). The mean NPG values and the mean changes in the count of the individual types of cells in the nasal secretions, were always calculated from all 117 positive immediate nasal response (+INR). I = initial value; PBS = phosphate buffered saline value (=control); ALL = allergen challenge.

count increased significantly during 16% of the INRs ($p<0.05$). (f) Lymphocytes (LT) were present in NS in 11%, plasma cells (PC) in 7% and monocytes (MT) in 3% of the INR, without any significant changes in their counts ($p>0.1$).

The ES, NE, BS and MC found in NS before the allergen challenge were mostly intact and without any intracellular changes. In most of the ES (76%), NE (70%), MC

(4%) and BSs (16%), appearing in NS during the INR, degranulation and other cellular changes (vacuolization, diminished intake of stain, wrinkling of cellular membrane, sometimes cellular disruption) were recorded.

Negative Nasal Response (NNR; n = 83). The appearance of the individual cell types in the NS and the changes in their count are summarized in Table 1. The course of these changes is presented in Fig 2. The NNR was accompanied by presence of individual cell types in NS to a low degree and only in a minority of cases. The small changes in the count of the particular cell types in NS during the NNRs were statistically not significant ($p>0.05$). The cells recorded in NS during the NNR were intact and without any intracellular changes.

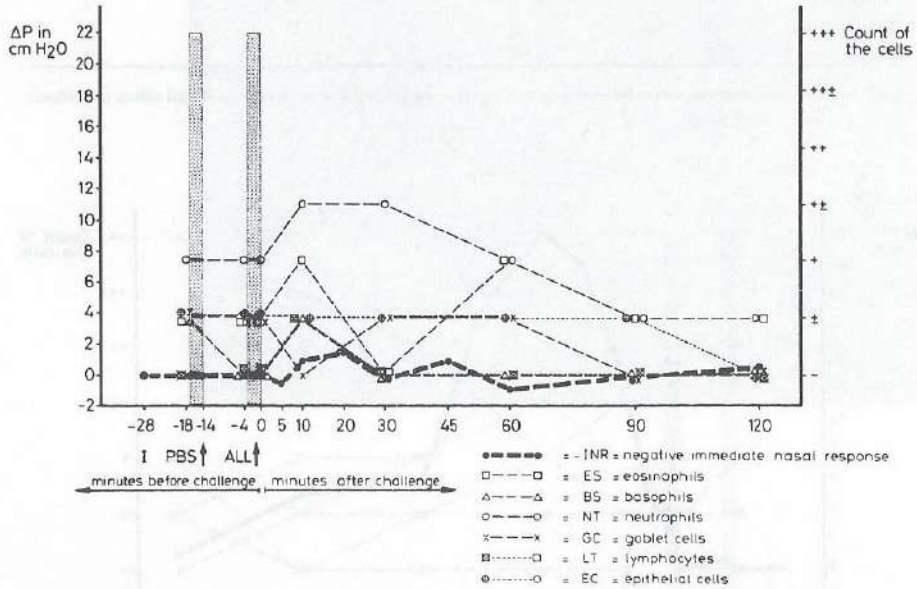

Figure 2. The negative immediate nasal response to allergen challenge (-INR). The mean NPG values and the mean changes in the count of the individual types of cells in the nasal secretions, were always calculated from all 83 negative immediate nasal response (-INR). I = initial value; PBS = phosphate buffered saline value (=control); ALL = allergen challenge.

PBS Control Challenge (PBS; n = 200). The changes in the count of particular cell types, shown in Fig. 3, were not statistically significant ($p>0.1$).

Histologic Examination Of The Nasal Mucosa

Positive Immediate Nasal Response (INR; n = 12). Before the allergen challenge (=baseline), the nasal mucosa was compact and did not demonstrate any histologic or functional changes. In the upper layer of the lamina propria intact eosinophils, tissue mast cells, neutrophils and lymphocytes were recorded to a very slight degree (±). During the positive INR, the following changes were recorded in the nasal mucosa (Figs. 4, 5): 1) increased serous secretions; 2) enlarged ducti of mucosal glands; 3) enlarged intercellular spaces in the epithelium; 4) the basement membrane was intact; 5) eosinophil and tissue mast cell accumulation but not infiltrates in upper layer of lamina propria (30% of

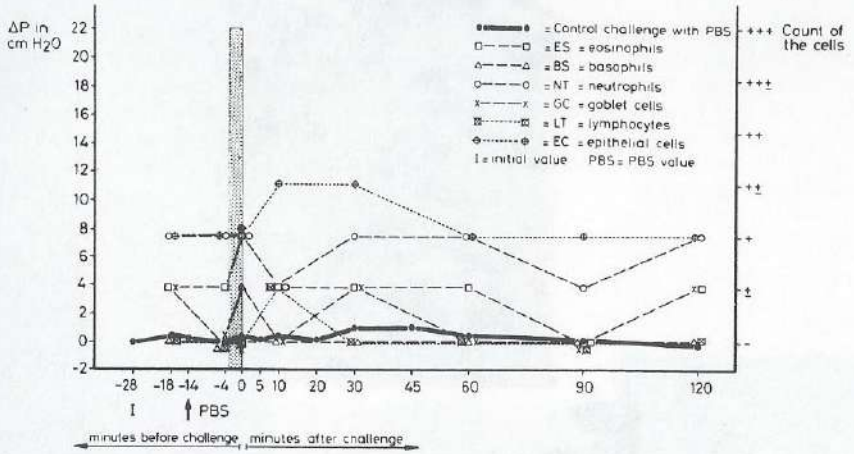

Figure 3. Control challenge with phosphate buffered saline (PBS). The mean NPG values and the mean changes in the count of the individual cells types in the nasal secretions were calculated from all 200 control challenges.

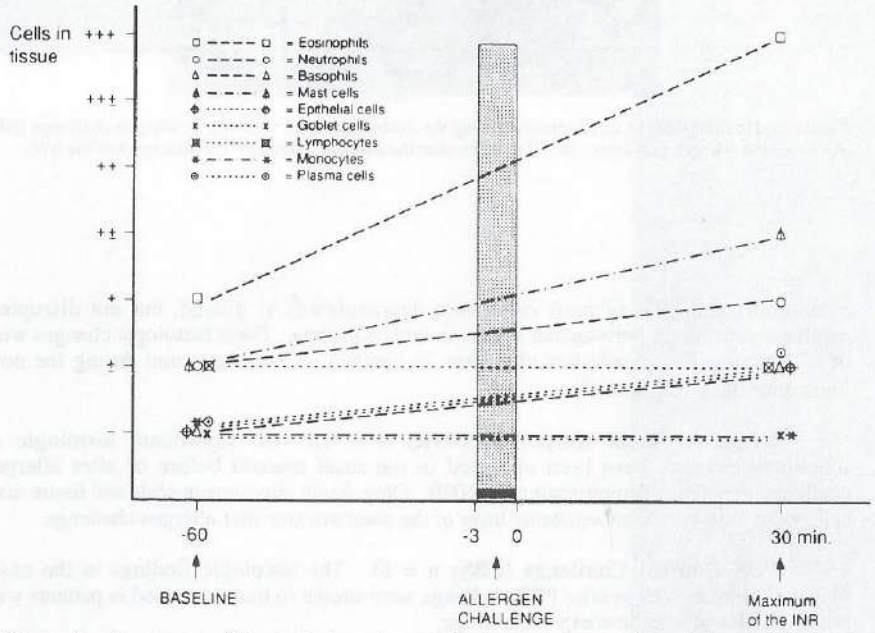

Figure 4. Appearance of the particular cell types in the nasal mucosa tissue before and during the immediate nasal response to allergen challenge (n=12).

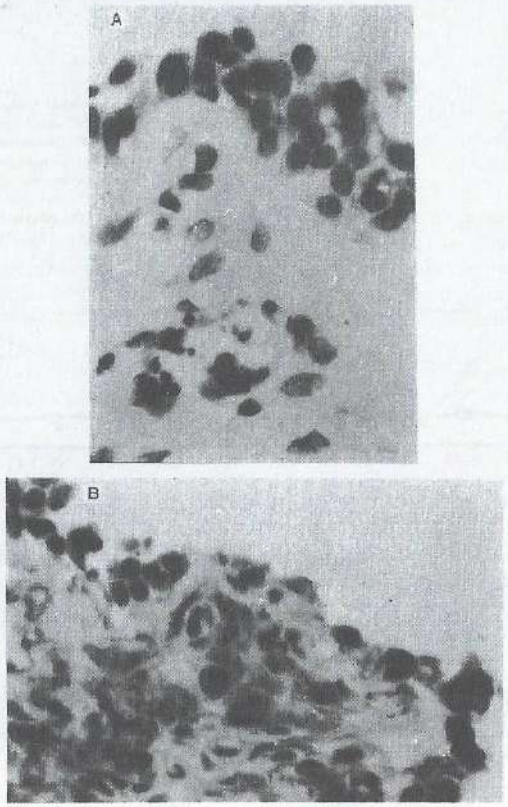

Figure 5. Histology of the nasal mucosa during the immediate nasal response to allergen challenge (NR). (A) before the allergen challenge, (B) 30 minutes after the allergen challenge = at maximum of the INR.

eosinophils and 80% of mast cells were degranulated) 6) dilated, but not disrupted, capillaries and slight perivascular edema in lamina propria. These histologic changes were of a "functional" and transient character, in contrast to those recorded during the non-immediate nasal responses.[5]

Negative Nasal Response (NNR; n = 6). No significant histologic or functional changes have been observed in the nasal mucosa before or after allergen challenge in patients demonstrating the NNR. Only single intact eosinophils and tissue mast cells were found in the subepithelial layer of the nasal mucosa after allergen challenge.

PBS Control Challenge (PBS; n = 6). The histologic findings in the nasal mucosa before as well as after PBS challenge were similar to those recorded in patients with positive INRs before the allergen challenge.

DISCUSSION

Cytologic examination of (NS) is a relatively easy and valuable technique for studying kinetics of particular cell types and the changes in their count related to a certain type of nasal response.[1-4] However, this method is limited only to NS and does not provide data concerning the histologic changes in the nasal mucosa tissue, preceding the appearance of the nasal response. Such information can only be derived from biopsy of nasal mucosa. On the other hand, nasal mucosa biopsy has also some limitations, technical disadvantages and interpretation problems; e. g. the number of biopsies is very limited, trauma and bleeding occur, choice of local anesthetics may influence the results, etc.

The histologic findings in the nasal mucosa tissue and those in the NS during the INR are only partly in agreement. The accumulation of eosinophils in the tissue was preceded by their influx and increased count in the NS. The slight accumulation of mast cells in the tissue was not preceded or followed by increased influx into the NS. Surprisingly, the neutrophils were found in the tissue only sporadically, while they appeared in NS in the majority of the INR cases and their counts showed significant changes.

These results allow us to formulate the following hypothesis of the involvement of particular cell types in the mechanism underlying the INR. The antigen-antibody interaction activates the tissue mast cells (possibly also basophils), which then attract the eosinophils into the tissue and stimulate their influx into the NS through their factors (ECF-A, lipid chemotactic factor). During the influx into NS, the activated eosinophils release their constituents that participate in the development of the nasal mucosa edema (major basic protein, prostaglandins, leukotrienes) and in inhibition of mast cells, neutrophils and histamine. Reaching the NS, the eosinophils are already degranulated and they become immobilized. The maximum eosinophil accumulation in the NS is followed by the maximum nasal mucosa edema and nasal obstruction (= INR). At this time the role of eosinophils as well as of mast cells is finished, they gather in the tissue and do not migrate further into NS. At the same time, regarding the decreasing influence of eosinophil constituents, the inhibition of neutrophils also decreases, they become activated, and their influx into the NS increases. During this process the neutrophils release a number of factors participating in the inhibition of the previous effects of mast cell and eosinophil factors and in diminishing of the nasal mucosa edema. However, additional investigations will be necessary to clarify the cellular kinetics accompanying the INR.

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LATE NASAL RESPONSE TO ALLERGEN CHALLENGE CYTOLOGIC CHANGES IN THE NASAL SECRETIONS AND HISTOLOGIC CHANGES IN THE NASAL MUCOSA

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INTRODUCTION

The purpose of this study was: 1) to investigate the cellular traffic in the nasal secretions (NS) and in the nasal mucosa during the late nasal response to allergen challenge (LNR); 2) to compare the changes in the count of particular cell types in NS with those in the nasal mucosa and in this way to study their kinetics, role and involvement in the LNR mechanism(s); 3) to determine the significance of such changes for the LNR, its mechanism and diagnostic approach.

MATERIALS AND METHODS

In 104 patients with allergic rhinitis developing positive LNR to challenge with "inhalant" allergens (onset within 4-6 h, maximum within 6-12 h, resolving within 24 h)[1,2], correlating with positive history and intradermal tests and in 83 patients developing negative nasal responses (NNR), the nasal challenges with allergens as well as 187 phosphate buffered saline (PBS) control challenges were repeated and supplemented by cytologic examination of NS. In 6 patients with NNR, in 12 subjects with positive LNR, 6 of which also received a PBS control challenge, the repeated challenge was supplemented by biopsy of the nasal mucosa 24 to 2 h before and at 6 h after the challenge. The nasal provocation tests (NPT) with allergens were performed by means of rhinomanometry (the "balloon method", being a modification of the posterior technique), described in detail in our previous work.[1-4] The collection of the NS specimens, their processing, staining and evaluation were similar to those described in detail in our previous papers.[3] The biopsy of the nasal mucosa was performed 10 minutes after the application of 1% xylocaine spray by means of cup forceps from the anterior part of the concha media of the challenged nasal cavity, while the second biopsy (at 6 h after the challenge) was taken from the central part of the same turbinate. The specimens were fixed in 10% buffered formaldehyde, dehydrated, embedded in wax, sectioned and slides were stained with May-Grünwald-Giemsa technique.[4]

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RESULTS

The 104 positive LNRs ($p<0.01$), 83 negative responses ($p>0.25$) and 187 PBS control challenges ($p>0.05$) were studied. The statistical differences between LNR and PBS as well as between LNR and NNR were significant at all time intervals ($p<0.01$). No significant differences were found between the first and second LNR ($p>0.2$), NNR ($p> 0.1$) or PBS ($p>0.1$). The presence of particular cell types in the NS and the changes in their counts during the late nasal response (LNR; n=104) are presented in Table 1, while the course of the changes is shown in Fig. 1. The LNR was accompanied by significant changes in the count of neutrophils in 84% (increase before, decrease during the appearance and increase during the resolving of the LNR), eosinophils in 58% (increase before and decrease during the LNR), epithelial cells in 73% (increase followed by decrease), goblet cells in 63% (increase followed by decrease), basophils (and/or mast cells) in 8% and lymphocytes in 6% (both of them increased slightly), in NS. In most of the neutrophils, eosinophils, basophils and mast cells appearing in NS during the LNR, intracellular changes (degranulation, vacuolization, diminished intake of stain, wrinkling of cellular membrane) were recorded.

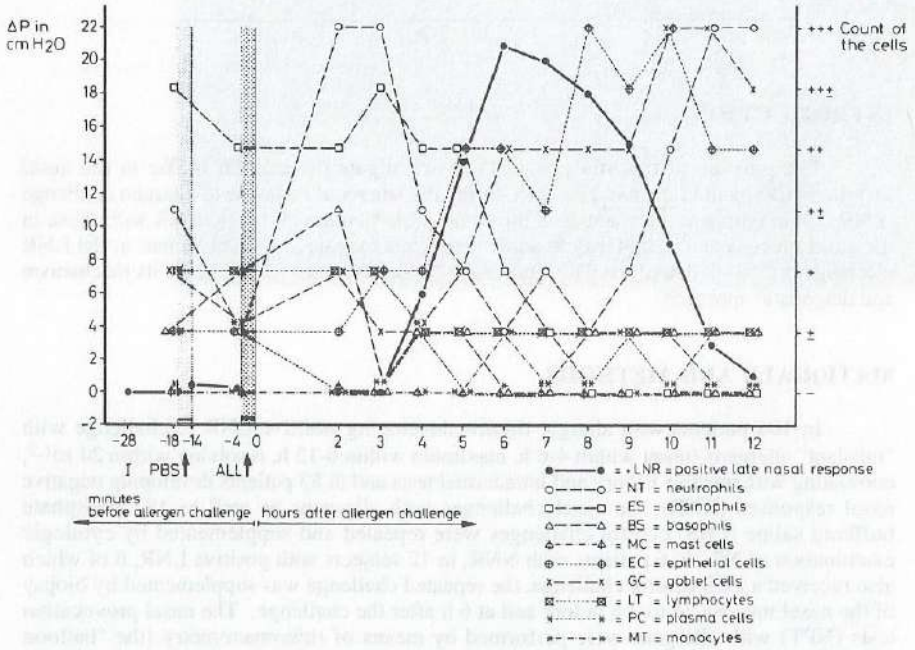

Figure 1. The positive late nasal response to allergen challenge (+LNR). The mean NPG values and the mean changes in the count of the individual types of cells in the nasal secretions, were always calculated from all 104 positive late nasal responses (+LNR). I = initial value; PBS = phosphate buffered saline value (= control); ALL = allergen challenge.

The appearance of individual cell types in the NS and the changes in their counts during the NNR (n=83) are summarized in Table 1, while the course of the changes is presented in Fig. 2. The NNR as well as the PBS control challenge were accompanied by presence of particular cell types in NS to a low degree and without significant changes in their count ($p>0.1$) (Fig.3). The cells appearing in NS during the NNR and PBS control were mostly intact and without any intracellular changes.

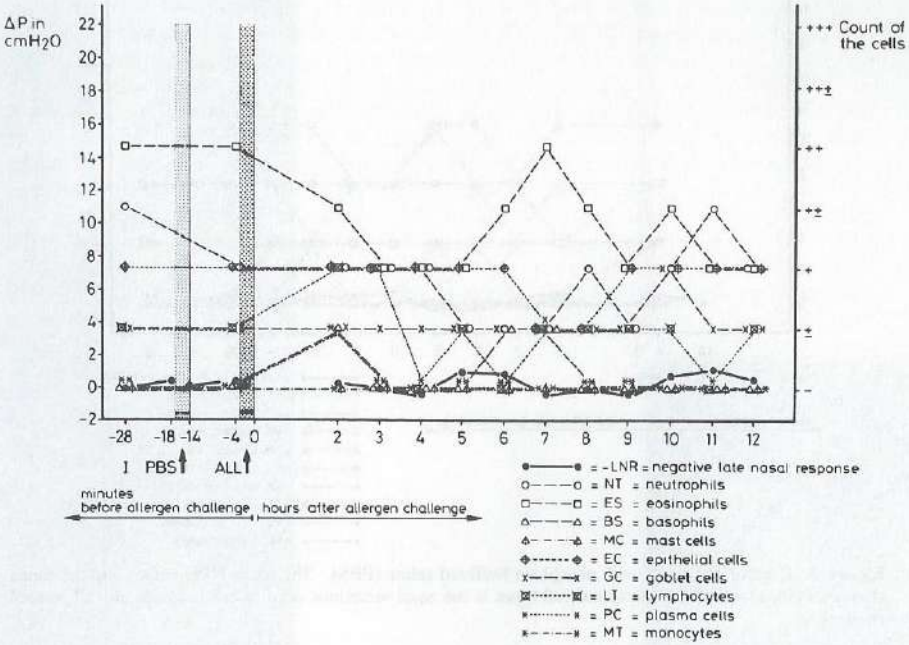

Figure 2. The negative late nasal response to allergen challenge (-NNR). The mean NPG values and the mean changes in the count of the individual types of cells in the nasal secretions were always calculated from all 83 negative nasal responses (NNR). I = initial value; PBS = phosphate buffered saline value (= control); ALL = allergen challenge.

Table 1. Presence of particular cell types in the nasal secretions and changes in their counts during the particular nasal response (in %).

	Presence of the cells			Changes in the cell counts between before and after the challenge		
	INR	NNR	PBS	INR	NNR	PBS
Eosinophils	61	19	49	58*	5	1
Neutrophils	96	17	45	84*	3	2
Basophils	15	9	10	8*	0	0
Epithelial cells	100	23	41	73	4	1
Goblet cells	82	13	35	63*	3	0
Lymphocytes	18	4	9	6	0	0
Mast cells	3	2	1	0	0	0
Plasma cells	4	2	1	0	0	0
Monocytes	1	0	0	0	0	0

LNR = nasal response; NNR = negative nasal response; PBS = phosphage buffered saline (=control); *=statistically significant

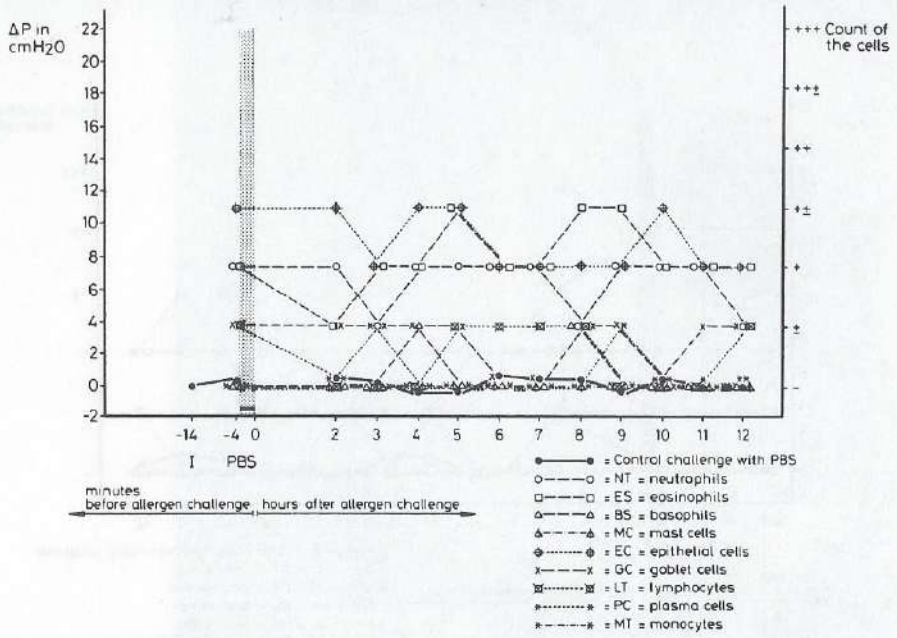

Figure 3. Control challenge with phosphate buffered saline (PBS). The mean NPG values and the mean changes in the count of the individual cell types in the nasal secretions were calculated from all 187 control challenges.

The histologic changes in the nasal mucosa during the LNR, as compared with the "pre-challenge baseline", represented slight tissue damage with inflammatory components (Figs. 4, 5): 1) edematous and damaged epithelium; 2) enlarged intercellular spaces and breaches in the epithelial layer; 3) some epithelial and goblet cells were expelled; 4) single breaches in the basement membrane; 5) edematous sub-epithelial layer containing mixed eosinophil-neutrophil infiltrates and single mast cells, basophils and monocytes; 6) dilated, sometimes disrupted capillaries and perivascular edema in lamina propria. No significant histologic changes were recorded in the nasal mucosa during the NNR or PBS controls.

CONCLUSIONS

The positive LNR is associated with changes in the count of neutrophils, eosinophils, epithelial and goblet cells in the nasal secretions, and with histologic changes in the nasal mucosa tissue representing a slight damage of the tissue, which may be reversible.[1-4] These findings indicating the involvement of inflammatory component in the mechanism(s) underlying the LNR, differ from those recorded during the immediate nasal response (INR) and represent "functional" changes only, without any tissue damage.[5] These findings suggest different involvement of the particular cell types as well as hypersensitivity mechanisms in the LNR, compared with those participating in the INR.[2,5]

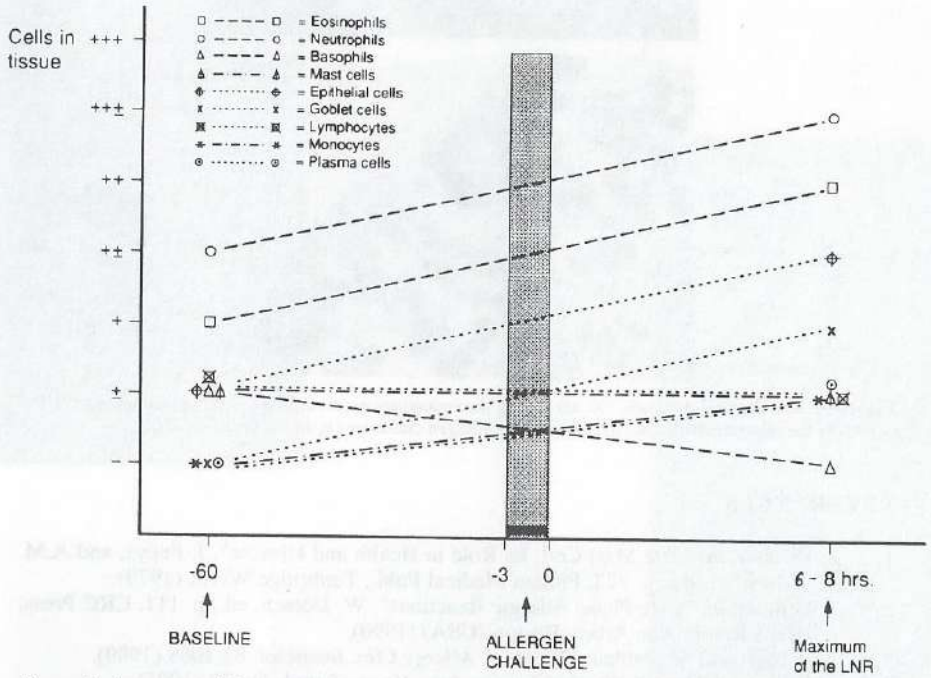

Figure 4. Apperance of the particular cell types in the nasal mucosa tissue before and during the late nasal response to allergen challenge (n=12).

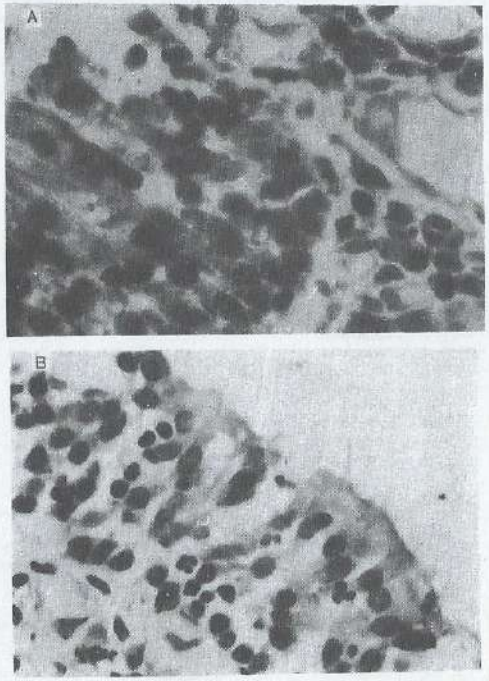

Figure 5. Histology of the nasal mucosa during the immediate nasal response to allergen challenge (NR). (A) before the allergen challenge, (B) 6 h after the allergen challenge (at maximum of the INR).

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THE LATE ASTHMATIC RESPONSE TO ALLERGEN CHALLENGE—PART I

Z. PELIKAN, MD, FACA* and M. PELIKAN-FILIPEK, MD

Of the 251 patients with bronchial asthma, 61 (24%) developed 83 late asthmatic (bronchus-obstructive) responses (LAR) to the bronchial challenge with allergen (BP). The LAR began within 6 to 12 hours, reached its maximum within 4 to 8 hours, and resolved within 24 to 26 hours after the allergen challenge. All LARs were highly significant in comparison with the control test ($P < .01$). The LAR was observed either as an isolated late response (ILAR) in 35 cases (10%) or as a dual late response (DAR) in 48 cases (14%), being a combination of an immediate (IDAR) and a late response (LDAR). The LAR demonstrated six basic patterns. The association of LAR with other diagnostic parameters was as follows: positive disease history in 49 LAR cases (59%); positive late skin response (LSR) in 51 LAR cases (61%); increased total serum IgE in 17 LAR (20%); positive specific IgE in the serum in 24 LAR cases (29%); increased serum concentration of IgG in 55 LAR cases (66%), of IgM in 41 LAR cases (49%), of IgA in one LAR case (1%); increased serum concentration of IgG1 in seven LARs (8%), of IgG3 in 21 LARs (25%), of IgG4 in 43 LARs (52%), the serum concentration of IgG2 increased in two LARs, while it decreased in 45 LARs (54%); increased blood eosinophilia in 42 LAR cases (51%) and blood leukocytosis in 29 LAR cases (35%); increased body temperature in 15 LAR cases (18%); appearance of late bronchial complaints during 83 LAR cases (100%), and of general malaise complaints during 67 LAR cases (81%). Increased bronchial reactivity to histamine was found in 40 of the 61 patients with LAR (65%). The LAR demonstrated a very good reproducibility and a significant dose-response relationship ($P < .01$). The LAR occurring regularly in patients with allergic bronchial asthma can play an important role in the bronchial complaints of these patients. We consider it important to focus the routine diagnostic procedure in bronchial asthma patients also on the LAR. The definite confirmation of the LAR should be provided by bronchial challenge with allergen.

Abbreviations

Asthma, bronchial asthma
BP, bronchial provocation test (challenge)
DAR, dual late asthmatic response
FEV_1, forced expiratory 1-second volume
IAR, immediate asthmatic response
IDAR, immediate asthmatic response being a part of the dual response
ILAR, isolated late asthmatic response
LAR, late asthmatic (bronchus-obstructive) response
LDAR, late asthmatic response being a part of the dual response
NAR, negative asthmatic response
VC, forced vital capacity

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Introduction

THE ALLERGIC COMPONENT involved in patients with bronchial asthma and chronic asthmatic bronchitis has classically been attributed to the immediate hypersensitivity (Type I allergy) mechanism.[1–3] Patients with bronchial allergy, having been challenged by allergen using BP, may develop different types of asthmatic (bronchus-obstructive) responses.[1–2,4–21] The IAR has been described by several authors and analyzed extensively in our previous papers.[4,7–9] The other types, so-called "non-immediate bronchial responses" were also recorded by us, the LAR (bronchus-obstructive) being one of them.[6–8]

Despite the fact that some investigators reported the existence of LAR,[1,2,6–8,10–13,16,17,20] there is a dearth of information concerning the clinical features of LAR and its association with other *in vivo* and *in vitro* diagnostic parameters. The pathogenetic and immunologic mechanisms underlying LAR are not yet sufficiently known. The involvement of the late hypersensitivity (type III allergy) in LAR cannot, however, be excluded.[1,6–8,20,22–24]

Late asthmatic response can play an important role in the bronchial complaints of some patients with bronchial allergy and may be responsible for the failure of the usual treatment in these patients. This type of response is often overlooked in practice.

The purpose of this study, being a continuation of our previous studies,[4–9] was to investigate the frequency and clinical characteristics of LAR, its association with other *in vivo* and *in vitro* diagnostic parameters and to contribute to the knowledge concerning the mechanisms underlying the LAR.

Materials and methods

Patients

The 251 patients suffering from bronchial asthma where an allergy component was suspected, were preselected according to the following criteria. They were all between 18 to 55 years of age and showed reversible bronchial constriction alternating with symptom-free periods. Their pulmonary functions did not demonstrate any restrictive changes and they did not suffer from chronic infections of the airways. These patients had never used oral corticosteroids and had not received any immunotherapy in the past. They were examined by a diagnostic procedure consisting of: (1) general part (performed once during the first visit to obtain not only general information on the patient but also to exclude other disorders that might lead to bronchial complaints and to exclude basic contraindications for BP with allergens): disease history, physical examination, basic laboratory tests, x-rays of the chest and sinuses, electrocardiogram, basic pulmonary functions, blood gases estimation, bacteriologic examination of the sputum. (2) Allergologic and immunologic part consisting of:
—skin tests,
—bronchial histamine threshold (PD_{20}),[25,26]
—determination of the total IgE antibodies in the serum (PRIST) and the specific IgE in the serum (RAST) to the same allergens as those used for the BP,
—determination of the IgM, IgA, IgG antibodies and IgG sub-classes (1, 2, 3, 4) in the serum by single radial immunodiffusion (Mancini technique), and
—BPs with allergens recorded up to 56 hours after the allergen challenge and supplemented by recording of the leukocyte and eosinophil count, body temperature, bronchial and general complaints before and up to 56 hours after the allergen challenge.

The allergens for bronchial challenges were chosen mostly with respect to the disease history and/or the skin tests and, in some exceptional cases, with respect to the frequency of the patient's exposure to the particular allergen (Table 2).

The 61 patients who had developed LAR to the challenge with one or more allergens are included in this study. The dose-response relationship was investigated in 36 cases of LAR (25 ILBR and 11 LDBR cases) all of them demonstrating either distinctly positive skin response and/or a disease history that was suspect for a very strong reaction to the particular allergen. The pollen allergens and mites (*D. pteronyssinus*) were used in dilution 1:1000, 1:100, 1:10 and, if necessary, undiluted. The other allergens were used in dilutions 1:10, 1:2 and, if necessary, undiluted, with respect to the standard concentrations for the bronchial challenge given in Table 1. The sequence of the BPs was from the lowest to the highest concentration, always with a free interval of at least three days between consecutive challenges. If the FEV_1 or both FEV_1 and VC decreased by 50% or more during the BP, no challenge with the higher concentration followed. In another 20 patients developing a positive LAR (ten ILBR and ten LDBR cases), the 20 bronchial challenges

Table 1. Survey of the Allergens Used for Skin Tests and Bronchial Challenges

	Concentration (per 1 mL of Coca's Solution)	
	Scratch and Intracutaneous Tests	Bronchial Challenges
House dust	0.5 mg	5.0 mg
Hairs and feathers mix*	0.25 mg	2.5 mg
Dog, cat, horse, cow danders and hen, parrot, canary feathers, each of them	0.25 mg	2.5 mg
Grasspollen mix†, spring pollen mix,‡ weed pollen mix,§ each of them	1000 NU‖	10,000 NU‖
Mites (*Dermatophagoides pteronyssinus*)	10 NU	100 NU

* Cat, dog, cattle, goat, hog, horse, rabbit, rat, mouse, hamster, guinea pig, canary, goose, duck, turkey, hen, pigeon, parrot, in equal portions by weight.
† Dry weight percentage: *Secale cereale* 15%, *Dactylis glomerata* 15%, *Lolium perenne* 10%, *Anthoxanthum odoratum* 10%, *Agrostis alba* 10%, *Holcus lanatus* 10%, *Phleum pratense* 10%, *Cynosurus cristatus* 5%, and *Alopecurus pratensis* 15%.
‡ Dry weight percentage: *Corylus avellana* 20%, Alnus species 30%, Salis species 20%, Betula species 20%, and Myrica species 10%.
§ Dry weight percentage: *Artemisia vulgaris* 33%, *Rumex acetosa* 33%, *Plantago lanceolata* 33%.
‖ 1 Noon Unit (NU) = 0.001 mg of dry pollen (powder) = 0.5 PNU = 1.3 TNU.

were repeated with the same allergen 3 to 4 weeks later to evaluate the reproducibility of the LAR.

All BPs were performed during hospitalization of the patients under standard conditions during which period the patients were free from infections and manifest bronchial complaints. No disodium cromoglycate, aerosolized corticosteroids, or anticholinergics were used by them during at least 4 weeks prior to the study. No bronchodilators or other therapy was given during the 48 hours prior to this study. In the case of the FEV_1, or both the FEV_1 and VC decreasing by 50% or more during the BP, a single inhalation dose of 100 μg salbutamol was given to stop a further drop of FEV_1. If the bronchial response required more extensive treatment, the patient was excluded from the study.

Allergens

The dialyzed and lyophilyzed extracts (Allergen Laboratory Diephuis, Groningen, the Netherlands) were diluted in Coca's solution (dry weight of allergen, in milligrams of NU per 1 mL of Coca's solution) and used for the skin tests and bronchial challenges in concentrations presented in Table 1.

Skin Tests

The scratch tests were performed and evaluated after 20 minutes. If they were negative, the intracutaneous tests were carried out and evaluated after 20 minutes, 8, 24, 36, 48, 72, and 96 hours. The results of the intracutaneous tests were interpreted as follows: (1) immediate skin response = onset within 20 minutes and disappearance within 120 minutes after allergen injection and (2) late skin response = onset within six to ten hours and disappearance within 24 to 36 hours after the intradermal injection of allergen.

Bronchial Provocation Tests

The BPs were performed by means of spirography, recording the forced VC and the FEV_1. The FEV_1 was considered to be the indicative parameter for the assessment of the bronchial obstruction (bronchospasm).

The Coca's solution (control test) as well as the allergen extracts were inhaled in the form of an aerosol administered to the patient by means of the Wiesbadener Doppel-Inhalator at an air flow of 10 L/min. (The aerosol particles had a mass median diameter of 2.8 to 3.6 μ.)

The BP consisted of the following steps: (1) recording of base values ("initial values") at zero, five, and ten minutes; (2) inhalation of the Coca's solution for ten minutes and then recording of the "Coca's Solution" values at zero, five, and ten minutes; (3) inhalation of allergen aerosol for ten minutes (two × five minutes) followed by recording of the parameters at 0, 5, 10, 20, 30, 45, 60, 90, and 120 minutes and then every hour up to the 12th hour and every second hour during the 24th to 38th-hour interval and the 47th to 56th-hour interval after the end of the challenge. The BPs were evaluated according to the following criteria: (1) the decrease of FEV_1 or both FEV_1 and VC of less than 10% with respect to the control values (initial and Coca's values) as negative, from 10% to 20% as doubtful and of 20% (PD_{20}) or more as positive; (2) the decrease of FEV_1 or both FEV_1 and VC within 60 minutes after the allergen challenge, recorded at least at three consecutive time-intervals, was considered to be a positive IAR (bronchus-obstructive). The FEV_1 decrease within an interval of four to 24 hours after the allergen challenge, recorded at least at three consecutive time-intervals, was considered to be a positive LAR (bronchus-obstructive).

The Control Test with Coca's Solution

This test was carried out in each of the patients investigated, at least three days before BP with allergen. The VC and FEV_1 were recorded up to 56 hours after the inhalation of Coca's solution. The control test was considered to be negative when the FEV_1 changes did not vary more than 5% ± 2% (mean ± SE) with respect to the "initial values."

The PRIST and RAST

The tests were carried out by means of the standard Pharmacia Phadebas RAST and PRIST kits. The grass pollens were used for RAST as individual grass pollens and not as a mixture. Even if only one of the nine basic grass types was positive, the RAST was considered to be positive for grass pollen. The RAST was considered to be negative for grass pollen if all nine grass pollens were negative. The same criterion was used for the spring pollen, weed pollen, flower pollen, and tree pollen.

Single Radial Immunodiffusion (Mancini Technique)

The determination of the total serum IgG, IgA, and IgM was performed by means of the standard plates (Kallestad Co, USA) provided by "De Beer Immunologic Supplies," the Netherlands. The IgG subclasses were determined by means of the plates prepared at the author's department, using antisera provided by the Central Laboratory of the Dutch National Red Cross Blood Transfusion Service (CBL, Amsterdam).

Bronchial Histamine Threshold

Bronchial histamine threshold was performed by a serial inhalation of histamine diphosphate diluted in physiologic solution (saline), starting with 0.25 mg/mL (0.75 mmol/mL). The concentration of histamine was progressively doubled until a decrease in FEV_1 of at least 20% of the "initial value" had appeared or the maximum concentration of 32 mg/mL (96 mmol/mL) had been reached. The lowest concentration of histamine, which produced a decrease in FEV_1 of 20% or more, was called the "bronchial histamine threshold." The method used by the authors was basically similar to the standard technique used by other investigators.[25,26] But one modification was introduced by the authors, ie, a 1-hour interval was always inserted between the two subsequent concentrations of histamine in order to exclude the cumulation effect.

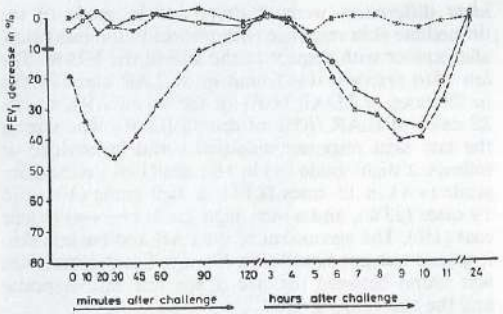

Figure 1. The mean percent changes in FEV_1 with respect to the "initial FEV_1 values," calculated from 48 dual late asthmatic responses (Δ- - -Δ), 35 isolated late asthmatic responses (O——O), and 116 control challenges with Coca's solution (×- - -×).

Statistical Analysis

The results were statistically evaluated by means of fitting polynominals to the mean curves over time (six time points within 120 minutes and eight time points between 6 to 12 and 22 to 28 hours after the allergen challenge). The appropriate hypotheses were tested by means of the generalized MANOVA model (generalized multivariate analysis of variance model), proposed by Potthoff and Roy.[27] The statistical analysis was performed by means of the computer program described by Berger,[28] using a VAX 11/780 computer. A $P < .01$ was considered to be highly significant, $P < .05$ to be statistically significant and $P > .05$ to be statistically non-significant.

Results

Of the 356 BPs performed in 251 patients, the 83 positive LAR (24%) in 61 patients, and the 98 NAR (28%) in 55 patients were recorded. The 83 positive LARs, in comparison with the 98 NARs, are subject of this study. The other types of bronchial responses, the immediate asthmatic response (37%) as well as the so-called delayed asthmatic response (12%), are the subject of other studies.

The LAR began within four to eight hours, reached its maximum within six to twelve hours, and resolved within 24 to 26 hours after the allergen challenge in most cases (Fig 1). The LAR was observed in two forms, either as an ILAR in 35 cases (10%) or as a DAR in 48 cases (14%), where at first an immediate response (IDAR) appeared and then, after a symptom-free interval of three to five hours, the late response (LDAR) occurred.

Statistical Analysis of the Results

Hypothesis No. 1. The ILAR recorded in individual patients (decrease in FEV_1 values) showed no mean trend (no significant changes). This hypothesis is rejected with $P < .01$.

Hypothesis No. 2. The IDAR, being a part of the DAR, showed no mean trend (no significant changes in FEV_1 values). This hypothesis is rejected with $P < .01$.

Hypothesis No. 3. The LDAR, being a part of DAR, showed no mean trend. This hypothesis is rejected with $P < .01$.

Hypothesis No. 4. The Coca's solution curves (FEV_1 values) has no mean trend. This hypothesis cannot be rejected ($P > .05$).

It can be concluded that all LARs (ILAR and LDAR) as well as all IDARs were highly significantly positive, while all control curves (Coca's solution) were significantly negative.

The LAR has been recorded in six patterns as shown in Figure 2. (1) *The "flat form."* The FEV_1 decreased quickly, remained stable for a longer time, and then increased again quickly (29% of LAR cases). (2) *The "peak form."* This is similar to the "flat form," while an additional peak (an extra decrease of FEV_1) appeared during the flat interval, usually at the beginning, in the center or at the end of this interval (45% of the LAR cases). (3) *The "zig-zag form."* After its quick decrease, the FEV_1 demonstrated small changes (a so-called "zig-zag" course) during the middle part, which lasted several hours and was followed by a quick increase (13% of LAR cases). (4) The *"prolonged form."* The FEV_1 decreased gradually, remained stable for a longer time and then increased very slowly, sometimes exceeding 24 to 26 hours (5% of the cases). (5) The *"step form."* This was characterized by the step-wise course of the FEV_1 changes during the decrease as well as the increase phase (4% of the LAR cases). (6) The *"reverse prolonged form."* In this form, the FEV_1 decreased very

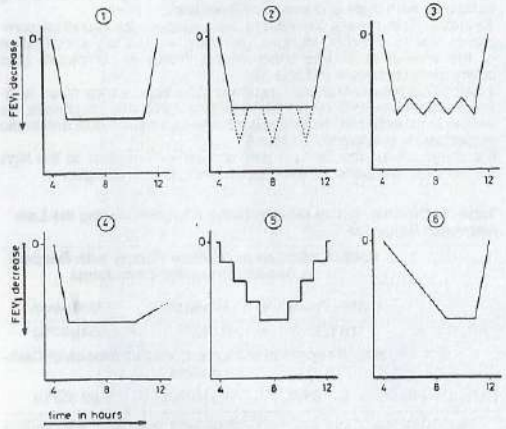

Figure 2. The survey of the six subtypes of LAR recorded.

slowly and after reaching its maximum, increased very quickly (4% of the LAR cases).

The Association of the LAR with Other Diagnostic Parameters

This association is summarized in Tables 2, 3, 4, and 5. The following discussion is presented.

(*1*) *The positive disease history* to the same allergen was found in 49 LAR cases (59%) (Tables 2 and 3) of which in 14 cases, all belonging to the ILAR, the disease history was indicative for the late onset of the bronchial complaints (17% of the 83 LAR in total or 40% of the 35 ILAR). This correlation was found to be unsatisfactory. No significant differences were found with respect to the individual allergens.

(*2*) *The skin tests. The immediate skin response* to the same allergen as that causing the LAR was found in 33 DARs (69% of the 48 DAR cases) and only in seven ILARs (20% of the 35 ILAR cases). These results indicate that the immediate skin response is related predominantly to the immediate asthmatic response, being a part of the DAR. The size of the skin response recorded in the group of the 33 DAR cases varied as follows: a slight degree (+) in 13 cases (27%), a medium degree (++) in 16 cases (34%), and a high degree (+++) in four cases (8%). The correlation of the immediate skin response and IDAR was not significant. No significant differences were observed in the grade of the immediate skin response with respect to the individual allergens or with respect to the size of the IDAR. *The late skin response* was found in 51 LAR cases (61%), in 29 cases of LDAR (60% of the 48 LDAR), and in 22 cases of ILAR (63% of the 35 ILAR). The size of the late skin response recorded could be divided as follows: a slight grade (+) in 16 cases (19%), a medium grade (++) in 15 cases (18%), a high grade (+++) in 19 cases (23%), and a very high grade (++++) in one case (1%). The agreement of the LAR and the late skin response was not significant. No significant correlation was found between the size of the late skin response and the size of the LAR.

(*3*) *The concentration of the total IgE antibodies in the serum (PRIST)* was increased significantly (>500 IU/mL) in only 17 cases of LAR (20%), 16 of them belonging to the DAR and one case to the ILAR. This result suggests a possible relationship of the PRIST to the IDAR, although this relationship was not statistically significant. No significant differences were found in the PRIST results with respect to the individual allergens used.

(4) *The specific IgE antibodies in the serum (RAST)* to the same allergen as that causing the clinical asthmatic response was significantly positive (Pharmacia score grade 3 or 4) in 24 LAR cases (29%), 22 of them were DARs (IDAR + LDAR) and only two were ILARs. No significant differences in the RAST results were found with respect to the individual allergens, although the RAST for grass pollen and cat danders were slightly more frequently positive than for the other allergens.

(*5*) *The serum concentration of the total IgG antibodies* was increased in 55 LAR cases (66%) of which 25 were ILARs (71% of the 35 ILAR) and 30 were LDAR (63% of the 48 LDAR). *The total IgM antibodies* were increased in 41 cases of LAR (49%), in 19 ILAR cases (54% of the total ILARs), and in 22 cases of LDAR (45% of the total LDARs). The *total IgA antibodies* were increased in one ILAR case (1%). No significant differences were observed in the increase of the serum concentration of individual antibodies with respect to the individual allergens causing the LAR.

(*6*) *The serum concentration of the individual IgG subclasses* was found as follows: IgG1 was elevated in seven LAR (8%), in four ILAR and three LDAR cases. IgG3 increased in 21 LAR (25%), in 13 LDAR, and in eight ILAR cases; IgG4 was elevated in 43 cases of LAR (52%), 25 of which were ILAR and 18 were LDAR. The IgG2 antibodies were elevated in only two cases of LDAR, while they decreased significantly in 45 LAR cases (54%), in 30 ILBR cases and in 15 LDAR cases.

(*7*) *The blood eosinophil count increased* (more than 300×10^6/L) in 30 (36%) and decreased in 23 (28%) LAR cases and *the blood leukocyte count increased* (more than 10×10^9/L) in 16 LAR cases (19%). No significant differences were found in blood eosinophilia or leukocytosis with respect to the individual allergens or with respect to the type of LAR (ILAR or LDAR).

(*8*) *The body temperature increased* (more than 37 °C = 98.6 °F axillary) during 15 cases of LAR (18%), belonging mostly to the ILAR.

(*9*) All cases of LAR (100%) were accompanied by the late appearance of *bronchial complaints* (Tables 4 and 5) in which dyspnea and wheezing dominated. *General malaise complaints* were observed during 67 cases of LAR (81%), of which 31 were ILARs and 36 were LDARs (Tables 4 and 5). No differences were observed in the appearance of bronchial or general malaise complaints with respect to the individual allergens.

(*10*) *Thorax x-ray* was performed in 11 cases of LAR before and repeatedly after allergen challenge. In seven of the 11 LAR cases the bronchovascular markings increased significantly during the period of six to 24 hours after the allergen challenge.

The increased bronchial reactivity to histamine (PD_{20}) or so-called decreased bronchial histamine threshold was found in 40 of the 61 patients with LAR (65%) (as shown in Table 4). The normal value of the bronchial histamine threshold is higher than 32 mg/mL (96 mmol/mL). The increased bronchial reactivity to histamine was found to a low degree (up to 48 mmol/mL) in 13 patients (21%), to a moderate degree (up to 24 mmol/mL) in 22 patients (36%), and to a high degree (less than 24 mmol/mL) in five patients with LAR (8%). In 21 patients with LAR (35%), no increased bronchial reactivity to histamine was recorded although the histamine challenge was repeated twice, with an interval of 4 weeks between both challenges. No significant differences were found in the frequency or in the degree of the increased bronchial reactivity to histamine with respect to the individual allergens causing the LAR, or with respect to the appearance or size of both the types of LAR, namely ILAR or LDAR.

Table 2. Survey of the History, Skin Tests, and Exposure Frequency Related to Particular Allergens*

	†History + ‡Skin −	History − Skin +	History + Skin +	§History − §Skin −
Late asthmatic responses n = 83	21	23	28	11
Negative asthmatic responses n = 98	55	18	9	16

* Allergens were chosen for the bronchial challenges for the 83 late asthmatic responses and 98 negative asthmatic responses according to history, skin tests and exposure frequency.
† History. This means the history was suspect for bronchial complaints due to a certain allergen, generally without any specification of the immediate or late onset of the bronchial complaints (this specification is shown in Table 3).
‡ Skin. This means late skin response. The appearance of an additional immediate skin response in all late asthmatic responses, as well as in its individual types (isolated late responses, dual asthmatic responses) is presented in Table 4.
§ In these cases, the allergen was chosen with respect to the high frequency of the patient's exposure to this specific allergen.

Table 3. Disease History Related to the Allergen Causing the Late Asthmatic Response

	Role of Allergen in Disease History with Respect to General Bronchial Complaints		
	Highly Probable	Probable	Unknown
LAR, n = 83	18 (22%)	31 (37%)	34 (41%)
	With Respect to the Late Onset of Bronchial Complaints		
LAR, n = 83	5 (6%)*	9 (11%)*	69 (83%)

* These 14 cases of late asthmatic responses were all of the isolated late type.

Table 4. The Association of the Late Asthmatic Response (LAR) and the Negative Asthmatic Response (NAR) with Other Diagnostic Parameters

Allergen and Bronchial Response-Related Parameters	LAR n = 83	Isolated Late Response n = 35	Dual Response n = 48	NAR n = 98
Positive skin response				
Immediate	40	7	33	28
Late	51	22	29	4
Increased total IgE in the serum (PRIST)	17	1	16	9
Positive specific IgE in the serum (RAST)	24	2	22	17
Increased concentration in serum				
Total IgG	55	25	30	2
IgG1	7	4	3	1
IgG2*	2	0	2	0
IgG3	21	8	13	1
IgG4	43	25	18	0
Total IgM	41	19	22	0
Total IgA	1	1	0	0
Increase in blood eosinophils/leukocytes	30/16	13/9	17/7	10/3
Increase in body temperature (more than 37 °C = 98.6 °F axillary)	15	11	4	0
Appearance of bronchial complaints	83	35	48	1
General malaise complaints	67	31	36	0
Thorax x-ray†	11/7	9/6	2/1	4/0
Auscultation				
Crepitation	44	28	16	0
Bronchioli effect	29	21	8	0
Patient-related Parameters	**LAR patients n = 61**		**NAR Patients n = 55**	
Increased bronchial reactivity to histamine	40		52	

* IgG2 in the serum decreased in 45 LAR cases (54%) and that in 38 isolated late responses and in 15 late, being part of dual responses.
† Performed/appearance or increase of pronounced bronchovascular markings.

Table 5. Survey of Bronchial and General Malaise Complaints Accompanying Late Asthmatic Response (LAR) and Negative Asthmatic Response (NAR)

	LAR n = 83	NAR n = 98
Dyspnea	72 (86.7%)	0
Wheezing	80 (96.4%)	0
Cough	3 (3.6%)	13 (13%)
Productive	0 (0%)	0
Non-productive	3 (3.6%)	13 (13%)
Expectoration	0 (0%)	0
Thick sputum	0 (0%)	0
Thin sputum	0 (0%)	0
Pressure on the chest	67 (81%)	2 (2%)
Chills	35 (42%)	0
Tiredness	67 (81%)	8 (8%)
Weakness	61 (73%)	5 (5%)
Headache	49 (59%)	9 (9%)
Acral cyanosis	2 (2.4%)	0
Nasal obstruction	1 (1.2%)	3 (3%)
Eye irritation (conjunctivitis)	1 (1.2%)	1 (1%)
Angio-neurotic edema	8 (9.6%)	0
Pressure in the sinuses maxill. and front.	6 (7.2%)	3 (3%)
Acute skin eruption (exanthema, urticaria, rush)	9 (10.8%)	0
Gastrointestinal complaints	2 (2.4%)	0
Tachycardia	5 (6.0%)	0
Bradycardia	1 (1.2%)	0
Blood pressure		
Increase	4 (4.8%)	0
Decrease	1 (1.2%)	0

The reproducibility of LAR measured by spirography was found to be very good. In 19 of the 20 patients with LAR in whom the BPs were repeated, the variations in FEV_1 values between the first and second challenges, being 5% or less, were statistically non-significant ($P > .05$). In one case of LDAR, the FEV_1 values varied significantly ($P < .05$).

The correlation between the allergen dose and the size of the LAR the dose-response relationship, was statistically significant in 32 of the 36 LAR cases ($P < .01$). In four cases (three ILAR and one LDAR), two of them due to house dust and two to grass pollen, the dose-response was not significant ($P > .05$); the FEV_1 value variations were 23% or more.

The 55 patients who did not demonstrate any kind of bronchial response during the 98 BPs, therefore called NAR, were considered a control group. The review of the other diagnostic parameters found in these patients is presented in Table 4, in comparison with those found in patients with positive LAR. The finding of the positive immediate skin response in 28%, of the positive late skin response in 4%, increased total serum IgE antibodies in 9%, and positive specific IgE antibodies in the serum in 17% of these patients did not correspond with the fully negative bronchial response. These results could therefore be interpreted either to be possibly related to other allergic reactions, occurring in other organs of the body besides the bronchial mucosa and tree, or possibly be false positive results. The NAR was not accompanied by bronchial complaints at all. In some cases, however, general malaise complaints and response of other organs were observed (Table 4). In the majority of these patients (52 of 55 = 95%), the distinctly increased bronchial reactivity to histamine (decreased bronchial histamine threshold) was found.

THE LATE ASTHMATIC RESPONSE TO ALLERGEN CHALLENGE—Part II

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The views on the pathogenetic and immunologic mechanisms presumably involved in the late asthmatic response (LAR) vary. Some investigators suggested the role of late hypersensitivity and IgG antibodies. Other authors assumed the role of the immediate hypersensitivity, mediated by IgE antibodies, with involvement of mast cells (and/or basophils). Another group of investigators assume that non-specific hyperreactivity could contribute to the development of the LAR. Finally, other investigators assumed that various combinations and modifications of established types of hypersensitivity mechanisms could be involved in the LAR.

In the literature, there is a large quantity of various information, results, and hypotheses concerning the late asthmatic response which are, however, often controversial. The authors attempted to summarize the hypotheses and facts concerning LAR and to compare them with the results of their study in order to better understand LAR.

Abbreviations

BP, bronchial challenge
IAR, immediate asthmatic response
ILAR, isolated late asthmatic response
LAR, late asthmatic response
LDAR, late asthmatic response being a part of the dual response.

Introduction

BRONCHIAL PROVOCATION TESTS WITH ALLERGEN are becoming recognized as one of the most important *in vivo* diagnostic parameters for the detection and confirmation of the role of the allergic component in patients with bronchial asthma and chronic asthmatic bronchitis.[1–9,12,13,16–18,20,22,25,26,29–33] The BP is performed directly at the site of the antigen-antibody interaction leading to the bronchial response, which is quantitatively recorded. The BP is therefore a more sensitive and reliable technique for the detection of the allergic reaction and its clinical consequences than some single *in vitro* or *in vivo* tests performed on the other organs (eg, skin tests). Further, BP is a more dynamic technique, recording the clinical event during its development, than an only once-performed "static measurement *in vitro*." BPs, despite their high diagnostic value, require certain special conditions and have some limitations, relative disadvantages, and contraindications that have been discussed already in our previous paper concerning the IAR.[9]

The bronchial response to the allergen challenge can be of various types. Besides the IAR, the non-immediate bronchial responses were also observed, the LAR being one of them. The results of the present study describing the LAR, its clinical features and relationship with other *in vivo* and *in vitro* diagnostic parameters are in accordance with the results of our preliminary studies,[7,8] as well as with other investigators.[1,12,17,19,21,32] Although a number of papers concerning the LAR have already been presented, the exact pathogenetic and immunologic mechanisms underlying LAR have not yet been satisfactorily clarified.

Late Asthmatic Response vs Late Type Hypersensitivity

A difference should be made between LAR and the late type of hypersensitivity (type III allergy, Arthus reaction, immune complex state). The late hypersensitivity is a well-defined immunologic mechanism, characterized by involvement of IgG and possibly also IgM antibodies, forming immune complexes and resulting in the complex inflammatory reactions leading to tissue damage.[35,37,38] The LAR should be regarded as a clinical phenomenon, defined by the appearance of the bron-

choconstriction, accompanied by other symptoms and changes, within four to 12 hours after the allergen challenge (antigen-antibody interaction), which could be induced by a complex mechanism.[33,34,36] Although the pathogenetic and immunologic mechanisms leading to LAR could probably be different, the late type of hypersensitivity should be regarded as one of the possible mechanisms involved in the clinical LAR, but far from the only one.

Late Asthmatic Response

There is no doubt of the existence of LAR, which has been repeatedly demonstrated and confirmed by several investigators.[1,6–8,10,12,13,16–17,20,31–33] The views on the pathogenetic and immunologic mechanisms, presumably underlying clinical LAR, however, vary highly. Some investigators suggested the involvement of the late hypersensitivity and IgG antibodies in the LAR.[1,6–8,19,22–24,30,34,40,48,49,51] Other investigators suggested that the immediate hypersensitivity, mediated by IgE antibodies with involvement of mast cells (and/or basophils), may play the main role in the LAR.[10,12,15,35,39,43] Another group of authors presented a hypothesis that the non-specific bronchial hyperreactivity plays an important role in the development of LAR.[16,32,45] Other investigators presumed that various combinations and modifications of the above-mentioned mechanisms could be involved in LAR.[13,14,17,20,31,36,37,41,42,44,46,47,50,52]

Literature Review vs Our Results

There is a variety of controversial information, results, and hypotheses in the literature trying to explain the clinical phenomenon of LAR. We decided to summarize the known facts and hypotheses concerning the possible mechanisms that may be involved in LAR and to combine them with the results of Part I of this paper. The summary is presented in schematic form in Figure 1. The main reason for such a schedule was to outline the main possible relationships and interactions between the individual parts of the immunologic system and cells involved. The effects of the individual mediators, intermediators, precursors, and derivates are not included in detail in this diagram.

Generally, in systemic late hypersensitivity (Type III allergy, Arthus reaction, immune complex state), circulating antibodies of the IgG and possibly also of the IgM class (presumed to play a major role) interact with the circulating antigens in the blood stream or in the vascular wall, sometimes also in the tissue, and in this way form immune complexes.[1,23,24,35,37,48,51,53,54] Immune complexes then activate the complement cascade[35,55] (especially C3a, C5a, C5b, C6, C7) with subsequent activation of the blood clotting and kinin systems,[36] release of lysosomal enzymes, vascular permeability factors, LTB_4,[33,56] and other factors[35,36,57] (eg, PGE_2) from the polymorphonuclear neutrophil leukocytes[35–37,41,58–63] plus release of vasoactive amines, lysosomal enzymes, and other proteins and factors from the platelets[35,36,38,60,63–69] and activation of the eosinophils.[55,57,70–75] These factors, as well as platelets and neutrophils themselves[20,76] may be involved directly or indirectly in the development of a complex of various inflammatory reactions typical for the type III hypersensitivity[35–38,53,58,60,65] and leading to the tissue injury, sometimes also tissue damage[35,38,55,68] (Fig 1, pathway No. 2).

Some investigators reported the findings of immune complexes in the patients with allergic bronchial asthma.[19,23,24,48] We were, however, unable to find any satisfactory investigation concerning the immune complex in patients demonstrating the LAR, in the literature. The changes in the concentration of the IgG and IgM antibodies and the IgG subclasses in the serum, sputum, and in the bronchial mucosal membrane; the changes of the chemical mediators, their precursors, derivates and metabolites; the changes of the complement system parts and of the individual types of cells possibly participating in the systemic type of late hypersensitivity have, in our opinion, not yet been sufficiently investigated in patients with LAR and require further study.

The IgG and possibly also IgM antibodies are presumed to play a major role in the forming of immune complexes after their interaction with the antigen,[1,35–38,46,51,53,54] occurring in the blood stream or in the vascular wall and sometimes also in the tissue. The IgG antibodies can also directly activate the eosinophils through their membrane IgG receptors,[40,42,54,55,57,70,75] stimulate macrophages,[77,80] neutrophils,[42,54,57,75,77–79] and platelets.[54,68,77,81] IgG and possibly also IgM antibodies are suspected to be responsible for the late hypersensitivity mechanism.[1,7,54] Such a presumption could be supported by our results, demonstrating an increase in the serum concentration of IgG during 66% and of IgM during 49% of the LAR cases.

With respect to the IgG subclasses, we found a significant increase in the serum concentration of IgG4 during 52% of IgG3 during 25%, and of IgG1 during 8% of LARs, while IgG2 increased in only 2% but decreased during 54% of LAR cases. These results are partly in agreement with the findings of other authors who performed bronchial challenges and concluded that IgG4 antibodies could be involved in late onset bronchial reactions in patients with bronchial asthma with negative prick test and without specific serum IgE antibodies.[22,82] Homburger et al[49] found a significantly higher concentration of IgG4 antibodies in the serum of patients with bronchial asthma or with "inflammation of the airways" than in normal control subjects. Stanworth,[82] Nakagawa et al,[23,83] Goodwin,[84] and Perelmutter[85] suggested the possible involvement of IgG4 antibodies in the hypersensitivity states. The exact mode of this involvement is, however, not yet fully clarified. The question of the role of the individual IgG subclasses in bronchial asthma and especially in the LAR would become more complicated if some investigators' suggestion of the existence of the allergen-specific IgG subclasses were to be confirmed.[22,82,86]

The decrease of IgG2 antibodies during 54% of LARs was another interesting finding of this study. We do not have a clear explanation for the exact role of the IgG2 antibodies in the LAR as well as for their decrease during the LAR. On the other hand, a possible involvement of the IgG2 and their changes during LAR cannot be excluded.[87] The exact involvement and significance of the individual IgG subclasses for the hypersensitivity mechanism, not only of the immediate type, but also of other types, is still uncertain and controversial.[82] The changes in the IgG subclasses during LAR that we observed could implicate the possibility of differentiated involvement of individual IgG subclasses in the interaction with antigen and in LAR. Our results do not confirm the involvement of IgG as a uniform complex antibody.

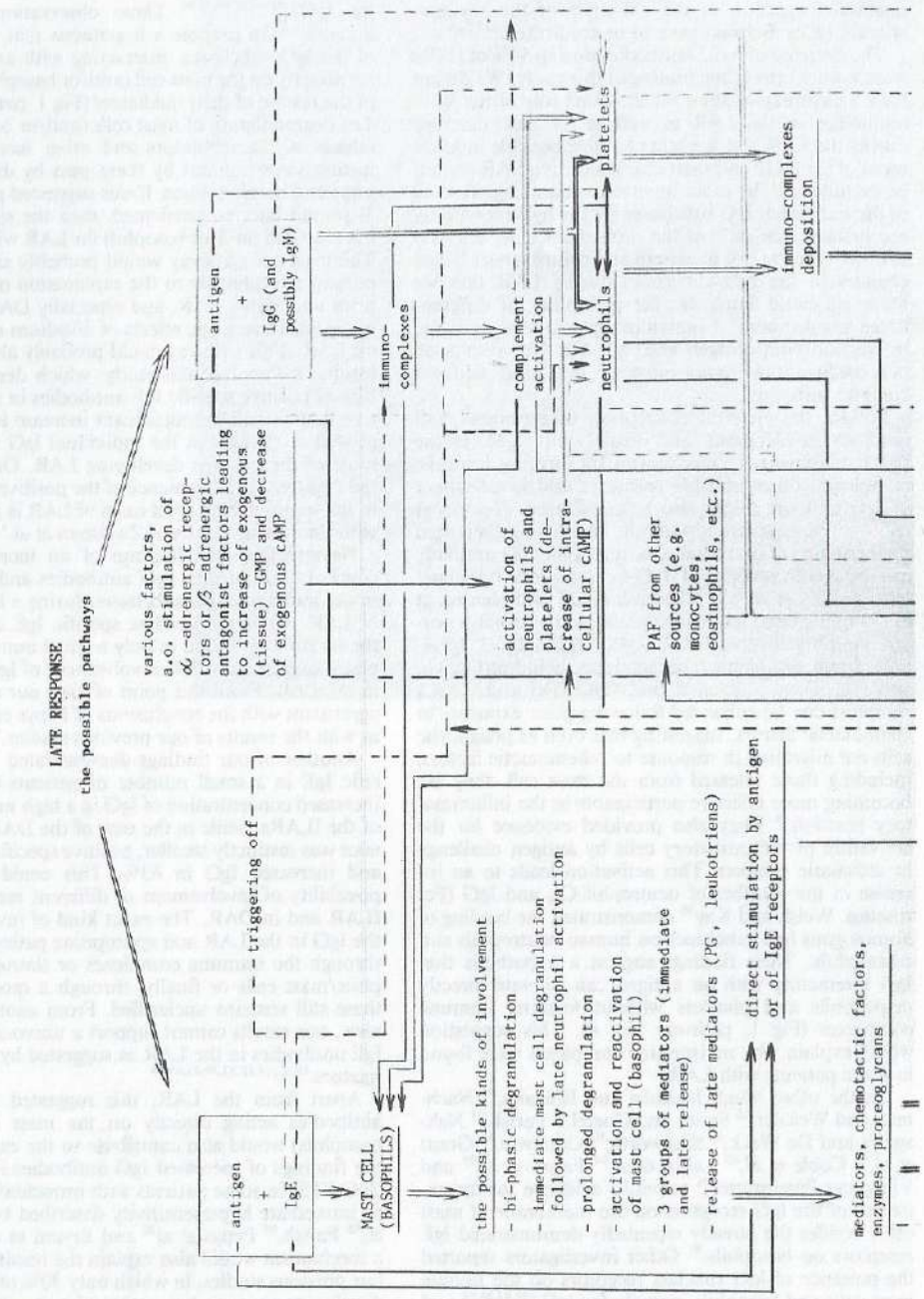
Besides the classical conception of presumed IgG antibody involvement (and possibly also IgM) in the late hypersensitivity mechanism by forming immune complexes, other possible pathways and involvement of IgG in LAR might also be considered. Henson et al[54,78,79,81] Rabellino and Metcalf,[88] and Hong[77] reported the existence of membrane receptors for IgG antibody on the neutrophils, and IgG-Fc receptors on platelets.[77,81] Kay et al,[42,57,75,89] Walsh et al[40] and Henson et al[81] demonstrated that eosinophils and neutrophils possess membrane receptors for IgG1, IgG2, IgG3, IgG4, and certain complement components including C_{3b}, C_4, and C_{5a}. They concluded that "IgG (Fc) and C_{3b}/C_4 receptors can be enhanced following prior exposure to chemotactic factors, suggesting that even as phagocytic cells are migrating in response to "chemotactic factors, including those released from the mast cell, they are becoming more effective participants in the inflammatory reaction." They also provided evidence for the activation of inflammatory cells by antigen challenge in asthmatic subjects. This activation leads to an increase in the number of neutrophil C_{3b} and IgG (Fc) rosettes. Welsh and Kay[40] demonstrated the binding of homologous IgG subclasses on human neutrophils and eosinophils. Their findings suggest a hypothesis that IgG interacting with an antigen can activate directly neutrophils and platelets without forming immune complexes (Fig 1, pathway No. 4). This suggestion would explain why no immune complexes were found in some patients with LAR.

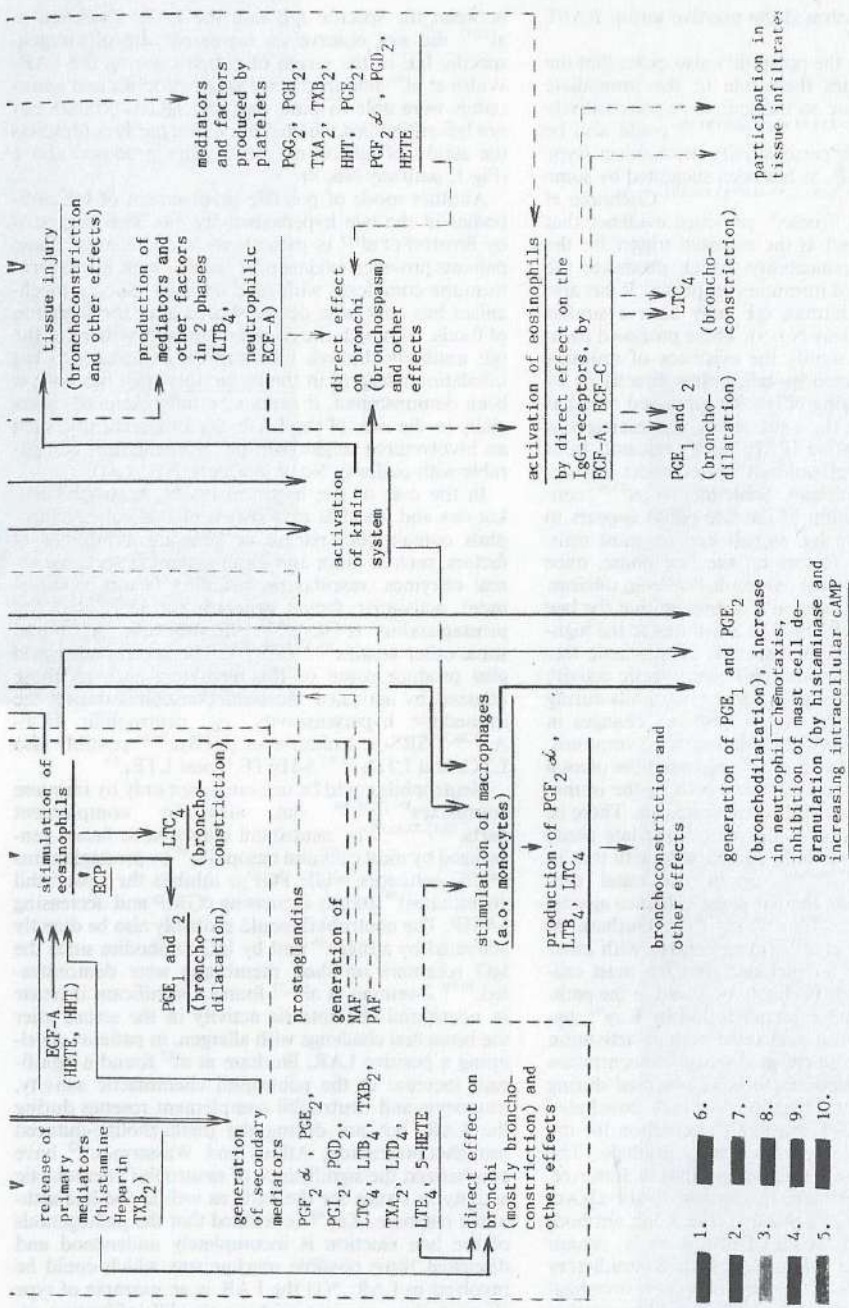
On the other hand, Ishizaka and Ishizaka,[90] Nachman and Weksler,[68] Smith and Goetzl,[55] Parish,[91] Nakagawa and De Weck,[83] Stanworth,[82] Goodwin,[84] Grant et al,[92] Coble et al,[93] Lakin et al,[94] Fagan et al,[95] and Vijay and Perelmutter[96] provided evidence for the existence of the IgG receptors on the membrane of mast cells, besides the already repeatedly demonstrated IgE receptors on basophils.[97] Other investigators reported the presence of IgG subclass receptors on the human mast cells and basophils, namely for IgG4[68,83,84,96] and for IgG2.[68,83,87,94,96,98] These observations led other authors[55,83] to propose a hypothesis that IgG or some of the IgG subclasses, interacting with antigen, might act directly on the mast cell (and/or basophil) and result in the release of their mediators (Fig 1, pathway No. 3). The degranulation of mast cells (and/or basophils), the release of the mediators and other factors, and the further steps induced by them pass by different pathways and modifications. If this suggested pathway (No. 3) should later be confirmed, then the significance of the mast cell (and/or basophil) for LAR will be evident. The mast-cell pathway would probably signify an important contribution to the explanation of the mechanism underlying LAR, and especially DAR, as well as of the pharmacologic effects of disodium cromoglycate on LAR. This pathway could probably also clarify the results of Part I of this study, which demonstrated a lack of positive specific IgE antibodies in serum (negative RAST) and the significant increase in serum IgG as well as changes in the individual IgG subclasses in most of the patients developing LAR. Our finding of the negative RAST (absence of the positive specific IgE) in the serum during most cases of LAR is in agreement with the similar results of Zweiman et al.[13,31]

Nevertheless, our findings of an increased serum concentration of total IgG antibodies and the changes in the individual IgG subclasses during a large number of LAR (while the positive specific IgE antibodies in the serum were found in only a small number of LAR) might suggest a possible involvement of IgG antibodies in the LAR. From this point of view our results are in agreement with the conclusions of Pepys et al[1,30] as well as with the results of our previous studies.[6,8]

Another of our findings demonstrated positive specific IgE in a small number of patients (6%) but an increased concentration of IgG in a high number (71%) of the ILARs, while in the case of the DAR this difference was distinctly smaller, positive specific IgE in 46% and increased IgG in 63%. This could suggest the possibility of involvement of different mechanisms in ILAR and in DAR. The exact kind of involvement of the IgG in the LAR and appropriate pathway(s), either through the immune complexes or through the basophils/mast cells or finally, through a modification of these still remains unclarified. From another point of view, our results cannot support a univocal role of the IgE antibodies in the LAR as suggested by other investigators.[10,12,14,15,32,39,43,45,99]

Apart from the LAR, this suggested role of IgG antibodies acting directly on the mast cell (and/or basophils) would also contribute to the explanation of the findings of increased IgG antibodies in the serum (IgG-STS) in some patients with bronchial asthma due to immediate hypersensitivity described by Etievan et al,[86] Parish,[91] Pepys et al[30] and Bryant et al.[34,100] Such a mechanism would also explain the results of some of our previous studies, in which only 30% of the patients developing an immediate asthmatic response to allergen


LATE RESPONSE
the possible pathways
- antigen + IgE
triggering off
various factors, a.o. stimulation of α-adrenergic receptors or β-adrenergic antagonist factors leading to increase of exogenous (tissue) cGMP and decrease of exogenous cAMP
antigen + IgG (and possibly IgM)
MAST CELL (BASOPHILS)
immuno-complexes
complement activation
neutrophils
platelets
immuno-complexes deposition
the possible kinds of involvement
- bi-phasic degranulation
- immediate mast cell degranulation followed by late neutrophil activation
- prolonged degranulation
- activation and reactivation of mast cell (basophil)
- 3 groups of mediators (immediate and late release)
- release of late mediators (PG, leukotriens)
activation of neutrophils and platelets (decrease of intracellular cAMP)
PAF from other sources (e.g. monocytes, eosinophils, etc.)
direct stimulation by antigen or of IgE receptors
mediators, chemotactic factors, enzymes, proteoglycans


release of primary mediators (histamine Heparin TXB2)
generation of secondary mediators
PGF2α, PGE2, PGI2, PGD2, LTC4, LTB4, TXB2, TXA2, LTD4, LTE4, 5-HETE
direct effect on bronchi (mostly bronchoconstriction) and other effects
ECF-A (HETE, HMT)
stimulation of eosinophils
ECP
LTC4 (bronchoconstriction)
PGE1 and 2 (bronchodilatation)
prostaglandins
generation of NAF PAF
stimulation of macrophages (a.o. monocytes)
production of PGF2α, LTB4, LTC4
bronchoconstriction and other effects
tissue injury (bronchoconstriction and other effects)
production of mediators and other factors in 2 phases (LTB4, neutrophilic ECF-A)
activation of kinin system
direct effect on bronchi (bronchospasm) and other effects
activation of eosinophils by direct effect on the IgG-receptors, by ECF-A, ECF-C
mediators and factors produced by platelets
PGG2, PGH2, TXA2, TXB2, HHT, PGE2, PGF2α, PGD2, HETE
PGE1 and 2 (bronchodilatation)
LTC4 (bronchoconstriction)
generation of PGE1 and PGE2 (bronchodilatation), increase in neutrophil chemotaxis, inhibition of mast cell degranulation (by histaminase and increasing intracellular cAMP
participation in tissue infiltrates
1. 2. 3. 4. 5. 6. 7. 8. 9. 10.

challenge also demonstrated the positive serum RAST to the same allergen.[4]

On the other hand, the possibility also exists that the IgE antibodies, besides their role in the immediate asthmatic response due to immediate hypersensitivity (type I allergy),[36,39,41,43,74,98,99,101,103,104,106] could also be involved in the late hypersensitivity mechanism (type III allergy) and in LAR, as has been suggested by some investigators.[1,10,12,14,15,20,30,32,38,44,47,85,102,105] Cochrane et al,[37,38] Henson,[79] and Froese,[97] provided evidence that IgE in animals may act as the essential trigger for the increased vascular permeability, which promotes the vascular localization of immune complexes. It has also been suggested that human IgE may have a similar role[35,37,38] (Fig 1, pathway No. 9). These proposed pathways, however, may signify the existence of immune complexes being affected by IgE, either directly or by means of their triggering effect for increased vascular permeability through the neutrophils and platelets as well as by the mediators (PAF, NAF) released from basophils after the IgE-antibody interaction has occurred on their membrane. Schleimer et al[43,99] concluded that the induction of the late phase appears to be dependent both on IgE signals and on mast cells. The actual causative factors on the late phase, once cells have migrated into the tissue are, however, unclear. Wasserman[61] and Atkins et al[102] suggested that the late phase bronchial response may be attributed to the high-molecular weight neutrophil-specific chemotactic factors (HMW-NCF) and neutrophil chemotactic activity being released from mast cells and/or basophils during the early phase. Metzger et al[10] observed changes in specific IgE during the LAR correlating with symptoms. Solley et al[107] and Dolovich et al[20] suggested the pivotal role of the IgE antibodies and mast cells in the immediate and the late phase of the skin reactions. There is, however, no unequivocal evidence that the late phase of reaction in the skin is fully comparable with that in the bronchial tree[7,13,14,44,101] or in the nasal mucosa.[108,110] Furthermore, there is some evidence against such a comparison.[1,2,6–8,13,22,30,31,47,108,111] Durham et al,[12] Kay,[46] and Nagy et al[112] (in agreement with other authors[14,15,20,41,43,44,61,99]) concluded that the mast cell-associated mediators are probably involved in the pathogenesis of LAR. Other evidence found by Kay[46] suggests that LAR might be associated with re-activation of the mast cells, since an elevated serum concentration of neutrophil chemotactic factor was observed during the late phase asthmatic reaction. Atkins[47] concluded that responsibility of IgE-mast cell interaction for the late phase bronchial responses seems unlikely. The proposed biphasic mast cell activation should, however, be taken into consideration. In contrast to the above-mentioned suggestion of a possible role of IgE antibodies in LAR, the results of Part I of this study, cannot confirm this suggested role of IgE. Our contradictory results are supported by results of other investigators.[13,31,32,41] Lam et al[32] did not find any correlation between the specific IgE and the LAR. Zweiman et al[13,31] did not observe an increased titre of antigen-specific IgE in the serum of patients during the LAR. Walsh et al[40] demonstrated that neutrophils and eosinophils were able to bind only the IgG subclasses but not IgE antibodies, which observation partly contradicts the action of IgE on the neutrophils proposed above (Fig 1, pathway No. 8).

Another mode of possible involvement of IgE antibodies in the late hypersensitivity has been suggested by Brostoff et al[113] in patients with food allergy. These patients provided evidence for the ability of IgE to form immune complexes with food antigens. Such a mechanism has only been demonstrated after the ingestion of foods. Although the comparable involvement of the IgE antibody through the immune complexes to the inhalation allergens in the respiratory tract has not yet been demonstrated, it cannot be fully excluded, especially in the case of the LAR. Seen theoretically, such an involvement might then be approximately comparable with pathway No. 9 in Figure 1.

In the case of late hypersensitivity, neutrophil leukocytes and platelets may play a pivotal role. Neutrophils contain and release or generate a number of factors, such as kinin and kinin system factors, lysosomal enzymes, vascular permeability factors, complement activating factor generating C5a,[35–37,53,58–60,64] prostaglandins (PGE_2),[36,63] thromboxane A_2,[63] and some other factors.[35–37,53,58–60,64] The neutrophils could also produce some of the mediators such as those released by activated mast cells/basophils during the immediate hypersensitivity, eg, neutrophilic ECF-A,[35,36,58] SRS-A, leukotrienes (LTB_4),[56,67] possibly also LTC_4 and LTD_4,[61,63] 5-HETE,[63] and LTE_4.[61]

Neutrophils could be activated, not only by immune complexes[35–57,58–60] but also by complement parts,[35,42,75,81,89] by neutrophil chemotactic factor generalized by mast cells and basophils,[36] by prostaglandins (PGE_1 enhances, while $PGF_2\alpha$ inhibits the neutrophil chemotaxis)[39] and by increasing cGMP and decreasing cAMP. The neutrophils could probably also be directly activated by antigen[56] and by IgG antibodies since the IgG receptors on their membrane were demonstrated.[78,79] Zweiman et al[13,31] found a significant increase in neutrophil chemotactic activity in the serum after the bronchial challenge with allergen, in patients developing a positive LAR. Durham et al[12] found a significant increase in the neutrophil chemotactic activity, monocyte and neutrophil complement rosettes during the LAR, but not during the methacholine-induced bronchoconstriction. Atkins and Wasserman[102] have emphasized the significance of neutrophil chemotactic activity in serum for the early as well as the late asthmatic response. Kay[46] concluded that the pathogenesis of the late reaction is incompletely understood and discussed three possible mechanisms which could be involved in LAR: "(1) the LAR is an example of type III or Arthus response with neutrophil infiltration resulting from the generation of chemotactic factors, following activation of complement by immune complexes; (2) LAR might be associated with re-activation of mast cells, since elevated serum concentration of neutrophil chemotactic factor was observed during LAR; and (3) the leukotrienes, prostaglandins and thromboxanes play a role in LAR as these mediators tend to have sustained biologic effects and might, for instance, cause prolonged contraction of the bronchial smooth muscles together with edema of the submucosa as a result of their effects on the microvasculature." A similar role of the neutrophils has been suggested by Casale et al[44] and Atkins et al,[47,102] who postulated the involvement of neutrophils in the LAR by forming cellular infiltrates, after their stimulation by previously released granule constituents from the mast cell.

The role of platelets and neutrophils in mediating the hypersensitivity reaction seems to us to still be underestimated and not fully understood.[68] The platelets and neutrophils can be involved during the late hypersensitivity in various ways and manners and they stimulate and induce other steps through various pathways (Fig 1). The platelets contain intracellular granules, similar to classical lysosomes of polymorphonuclear leukocytes, which can degranulate during the formation of the platelet plug.[68] Platelets accumulate in vessels adjacent to inflammatory foci and interact with antigen-antibody complexes.[68]

The platelet response to surface stimulation by release and secretion of various factors is also accompanied by formation of prostaglandins and thromboxanes.[68] Platelets contribute to the inflammatory process accompanying tissue injury by releasing potent intracellular constituents that increase vascular permeability in two phases: the acute phase, appearing within 15 minutes and the secondary phase, reaching its maximum in three hours. The secondary phase is usually accompanied by polymorphonuclear infiltration.[68] Treatment with anti-histamine blocked the acute phase but did not block the secondary phase in rabbit.[68] The platelets contain, and can also release, a number of factors including vasoactive amines, lysosomal enzymes, vascular permeability factors,[35–37,53,64,65] prostaglandins[36] (PGG_2, PGH_2, PGE_2, $PGF_2\alpha$, PGD_2),[61,68] and other metabolites of arachidonic acids.[61,63,68,69] Some authors suggested that prostaglandins present in blood are almost entirely produced by platelets during aggregation and blood clotting.[66,68]

The activation and aggregation of platelets is stimulated by a variety of factors,[36] among others by platelet activating factors generated and/or released directly from the activated mast cells and basophils,[10,35,37–39,59,65,103] by decreasing of exogenous cAMP and/or increasing of cGMP with subsequent decreasing of intracellular cAMP in the platelets, by complement parts (C3a, C5a)[54] and probably also by other pathways.[68] The platelets may be stimulated directly by IgG antibodies and/or immune complexes, since Fc receptors for all four subclasses of IgG and complement parts (C3a, C5a) have been demonstrated on their membrane.[79,81]

The platelet activating factors could be generated and/or released from activated mast cells and basophils[61] and also from other cells, eg, polymorphonuclear leukocytes such as eosinophils and neutrophils[39,66,102] or monocytes.[39] Lysosomal enzymes are involved in the further stages of the immune complex-mediated tissue injury.[35,36,38,64] Neutrophils are also involved in immune complex-mediated tissue injury by their influx into the site of the immune complexes as well as platelets, which interact with damaged epithelium and adhere to immune complexes.[35,38,56,65] The neutrophils are then additionally activated by immune complexes through the complement system.[35,36,53]

Besides the possible involvement of the late hypersensitivity mechanism (Arthus reaction, type III allergy) and of IgG antibody in LAR discussed above, there are investigators who suggest the involvement of the IgE antibodies, basophils, and/or mast cells in LAR.[10,12,15,35,39,43,47] This involvement may be realized either by means of interaction and interference with some parts and components of the classical late hypersensitivity mechanism or through the classical immediate hypersensitivity mechanism (type I allergy) or through some pathways and steps belonging principally to the classical immediate hypersensitivity, but which have been re-evaluated in light of recent data. With respect to the late hypersensitivity mechanism and its parts, human IgE may play a role in triggering increased vascular permeability and deposition of immune complexes.[35–37] Basophils and mast cells with membrane-bound IgE can generate and release a soluble platelet activating factor,[35,37–39,65] factors chemotactic for neutrophils (NAF, HMW-NCF),[36] and arachidonic acid metabolites having manifold effects, eg, stimulating neutrophils (chemokinesis, granule release), platelet aggregation, enhance the inflammation mechanism (induction of fever, increase of vascular permeability, and edema), besides their direct effects on the bronchial smooth muscle and receptors.[63]

Involvement of IgE antibodies, basophils, and mast cells in LAR through the classically understood immediate hypersensitivity has been assumed by various authors.[10,12,14,15,20,35,39,43,47,62,74] This assumption is partly based on the finds of Solley et al[107] and Dolovich et al[20] concerning the skin during the late skin response. Similar changes in the bronchial mucosal membrane during LAR have, however, not been demonstrated. Furthermore, despite some relationship between skin tests and bronchial allergy, the results obtained on the skin cannot be applied to the bronchial tree without any limitations because of the distinct anatomic, immunologic, and pathophysiologic differences between these organs, as well as of the difference in their response patterns.

The evidence against the presumed main role of the classical immediate hypersensitivity mechanism in LAR is, nevertheless, growing. These facts led a number of investigators to suggest various modifications of the established classical immediate hypersensitivity mechanism or even alternative pathways which might be involved in LAR, as is discussed further on.[13–15,17,20,31,36,37,41,42,44,46,47,50]

The results of Part I of our study, especially the findings of positive specific IgE antibodies in only 29%, the increased serum concentration of IgG in 66%, and of IgM in 49% cases of LAR; the increase in blood leukocytes during 19%, eosinophils during 36%, and neutrophils during 40% of the cases of LAR; as well as the increase in body temperature during some LAR cases and appearance of general malaise complaints during most LAR cases (81%) support the evidence against the main role of the classical immediate hypersensitivity mechanism in the LAR. These results may indicate the involvement either of the late hypersensitivity mechanism, or at least some of its parts or finally, of an intermediary mechanism, being a modification or combination of some parts, previously ascribed to one or another classical hypersensitivity mechanism, immediate or late. Regarding some differences in our results observed between the ILAR and LDAR, an involvement of different mechanisms or of different modifications of one mechanism, in both these subtypes of LAR cannot be excluded.

Another possible role of the mast cells and basophils in the LAR, especially in the LDAR, could be related to some authors' suggestions of the modified functions of these cells.[11,39,61,101,107] Wasserman,[39] Lewis et al,[101] and Terral et al[11] suggested the bi-phasic degranulation of mast cells and basophils. The bi-phasic response of airways to inhaled allergen may then be comparable to the bi-phasic cutaneous response to the IgE-dependent activation of mast cells.[107] This could be the process by which cells' mediators might provoke a bi-phasic inflammatory response. Casale and Kaliner[44] described three groups of mast cell-derived mediators: (1) preformed or primary mediators (eg, histamine); (2) secondary or newly generated mediators (eg, prostaglandins); (3) granule matrix mediators (eg, peroxidases, heparin). They presumed that the preformed and partly newly generated mediators, appearing rapidly, cause "the immediate allergic reactions," while the granule-matrix mediators and some of the newly generated mediators might lead to the polymorphonuclear leukocyte infiltration, followed by mononuclear/macrophage infiltration and resulting in the late phase allergic reactions. Kay,[46] Nagy et al[112] and Durham et al[12] formulated the existence of three phases of airway obstruction in bronchial asthma: the rapid (spasmogenic), the late (sustained), and the subacute inflammatory phase. All of these phases are caused by the mast cell-derived mediators. The rapid phase (within 10 to 15 minutes) may be mediated by histamine; the late (sustained) phase (four to eight hours) may be associated with, what they call "re-activation of the mast cells," and with an increase of circulating serum neutrophil chemotactic factor, and in which leukotrienes, prostaglandins and thromboxanes presumably play a role; and finally, the subacute inflammatory phase, which is characterized by infiltration of eosinophils, neutrophils, and mononuclear cells and is probably mediated by chemotactic factors from mast cells (NCF, LTB_4, ECF-A). Schleimer et al[43] suggested the release of two groups of mediators during the IgE-dependent hypersensitivity mechanism, the primary mediators from basophils and the secondary mediators from lung tissue. The release of two mediator groups seems to them to correspond with the two phases of the IgE-dependent hypersensitivity, namely, the early and the late phase. There is evidence that "after activation of mast cells and other cells that may be triggered during the first phase of the immediate hypersensitivity response, a late phase response occurs that involves migration of cells such as eosinophils, basophils, neutrophils, and some mononuclear cells into the tissue site." Zweiman et al,[31] Atkins and Wasserman,[102] and Atkins[47] observed that blood neutrophils increased two hours after the allergen, but not methacholine inhalation. They suggested that immediate phase bronchial response may be caused by the mediators released from the degranulated mast cell, followed by the late neutrophils activation, which may be involved in the late phase bronchial response. Talbot et al[114] appointed the possibility of a "prolonged release" of mediators from the mast cells. The findings of PGD_2 in the nasal secretions during early nasal response but not during the late nasal response led Naclerio et al[115] to conclude that the mast cell is probably the main source of mediators during the early response and the basophil during the late response.

As mentioned above, with respect to the results of Part I of our study, the participation of different mechanisms in both modifications of the LAR, namely the ILAR and the LDAR, cannot be excluded. In the case of LDAR, the mast cells and/or basophils could play the predominant role. This could, however, be realized in different ways: either directly (the suggested bi-phasic degranulation,[11,39,102] reactivation,[46,112] degranulation of three mediator groups[44]) or indirectly (the generating of platelet activating factor,[41,43,61,65,99,102] to triggering increased vascular permeability or to generating chemotactic factor for neutrophils[35,37,38,41,65] and that for eosinophils,[41,70,89,103] prostaglandins,[41,46,63,112] leukotrienes and other arachidonic acid metabolites.[46,61,63,112] In the case of the ILAR, the other mechanisms and pathways (such as activation of neutrophils,[35–37,58–60] activation of complement,[35] activation of platelets by platelet activating factor from sources other than the mast cells and basophils,[39,101] decrease of exogenous cAMP and/or increase of cGMP,[35,59] leading to the decrease in the intracellular cAMP and increase in the intracellular cGMP) may probably play the predominant role, while the mast cells and/or basophils are involved to a lesser degree.

Another cell being presumed to play an important role in the hypersensitivity mechanism is the eosinophil, although its role is not yet fully clarified in all detail. The involvement of the eosinophil in the immediate hypersensitivity mechanism has been repeatedly demonstrated.[36,43,54,61,70,89,116] Its involvement in the classical late hypersensitivity mechanism (Arthus reaction) has historically been limited to participation in the eosinophil-neutrophil infiltrates.[14,15,35,38,43,44,46,52,54,55,73,89] The evidence, however, for its manifold function and involvement in various steps, also of the late hypersensitivity mechanism or its modifications increases.[70,73] The eosinophil can be activated directly by antigen-antibody complexes,[51,71,73] by IgE antibodies through the surface IgE receptors,[55,70,117,118] by IgG antibodies through the surface IgG receptors[40,55,75] and by sub-classes IgG1–4 and complement parts (C3b, C4b, C5a), the receptors of which are also present on its membrane.[42,57,75,119] The chemotaxis of eosinophils and their attraction to the site of the hypersensitivity reaction is modulated by various factors: (1) basophils and mast cells release and generate the eosinophil chemotactic factors of anaphylaxis[14,36,44,55,61,70,103] lipid chemotactic factor,[120] (2) metabolites of arachidonic acid,[14,44,61,63] (3) neutrophils release eosinophil chemotactic factors,[55,70] (4) activation of platelets contribute to generation of HETE and HHT, which are both chemotactic for eosinophils,[67,103] (5) by eosinophil chemotactic factors derived from complement,[55,70] and (6) by histamine.[61]

Histamine, by acting on the H_2 receptors, can inhibit migration and chemotaxis of human eosinophils. By acting on the H_1 receptors, histamine can augment eosinophil chemokinesis and migration.[61] The eosinophil chemotactic factors of anaphylaxis and histamine, after attracting eosinophils to the site of the reaction, are able to inactivate them.[61,102] Smith and Goetzl[55] concluded that the initial event in the stimulation of eosinophil chemotaxis involves the activation of pathways that generate specific chemotactic factors. Activation by immune complexes of the classical complement pathway elaborates chemotactic fragments with broad leukocyte specificity, most notably the minor fragment of the C5a or complexes such as C567, while the alternative pathway alone contributes to the chemotactic factors C3Bb and Ba. Histamine and eosinophil chemotactic factors of anaphylaxis increase the density of C3b receptors on eosinophils.[75] The eosinophils, after having been attracted to the site of the antigen-antibody interaction, become immobilized and they then retain other cellular and metabolic functions. The eosinophils also retain a full complement of lysosomal and other enzymes which permit them to modulate the ongoing mast cell and/or basophils reaction by inhibiting the release of chemical mediators and inactivating some of the already released mediators.[106] "The interaction of an antigen with eosinophil-bound IgE also results in the synthesis of prostaglandins E_1 and E_2.[118] The mixtures of PGE_1 and PGE_2 inhibit the release of histamine from human basophils and/or mast cells by virtue of inducing elevations in their intracellular levels of cAMP.[55,70] The major basic protein inactivates the heparin,[121] eosinophil histaminase deaminates histamine,[55,122,123] and eosinophil peroxidase and/or aryl sulfatase might be involved in inactivation of leukotrienes (LTB_4) which has previously been described as SRS-A.[70,76,124]

The eosinophil is also a source of a number of very potent constituents and products, associated with membrane, cytoplasm or granules, such as major basic protein (neutralization of heparin, stimulation of histamine release from basophils, damage of human bronchial epithelium),[121] peroxidase, aryl-sulfatase B (intracellular inactivation of leukotrienes), phospholipase D (inactivation of mast cell-derived platelet lytic factor), lysophospholipase (inactivation of lysophospholipids), PGE_1 and PGE_2 (stimulation of adenylate cyclase, inhibition of histamine release from mast cells and basophils),[55] LTC_4 (bronchoconstriction and vasoactive compound) and a small amount of LTB_4,[33,35] eosinophil-derived neurotoxin, and cationic peptides.[41] Eosinophil has also been suggested to generate platelet activating factor and lipoxygenese products.[55,61]

Henson[54] postulated "that since that time we have gained even more understanding of the hypersensitivity mechanism but have also found that the mediators we thought were exclusive to such immediate hypersensitivity reactions (type I allergy) are in fact ubiquitous mediators of inflammatory processes and are derived from many cell types, not only from the mast cells and basophils." Henson's statement may also be principally applicable to eosinophils, regarding their manifold role and involvement. From this point of view, the eosinophil seems to us to perhaps play an important role in both the classical types of immediate and late hypersensitivity. In this way, the eosinophils may probably act as a linking factor between both the hypersensitivity mechanisms, resulting in the occurrence of some modified pathways and intermechanisms which may, however, differ from the classically understood hypersensitivity mechanisms.

Our results of the changes in the count of the eosinophils in the blood during LAR, may indicate the possible involvement of the eosinophils in the mechanism underlying LAR. The changes in the eosinophil count during LAR were characterized by two patterns: (Part I, Table 4) in 30 (36%) LAR cases the initial increase was followed by a slow decrease of this count, while in 23 (28%) LAR cases the eosinophil count first decreased and then, after the maximum of the LAR, increased. The explanation and interpretation of the different patterns of the eosinophil count changes during LAR seems to us a difficult problem. A different

mode of involvement of the eosinophils in the same type of bronchial response (LAR) cannot be excluded. Unfortunately, we were unable to find any study concerning the investigation of the blood eosinophil count and its changes during the LAR, in a sufficient number of patients, available in the literature. Dahl et al[105] described an initial decrease in the eosinophil count, accompanied by a decrease in the serum eosinophil cationic protein, followed by an increase of both parameters during the LAR; however, this occurred in only 12 patients.

Dolovich et al[20] found perivascular infiltration of eosinophils and neutrophils in skin biopsies during the late skin reaction. Other authors[14,44] postulated that the late phase reactions occur in response to the granule-derived mediators inducing an influx of polymorphonuclear leukocytes (eosinophils and neutrophils) at the site of mast cell degranulation at two to eight hours, followed by mononuclear infiltration and tissue damage. In our opinion and according to the above mentioned facts and results, the eosinophil may play one of the pivotal roles in the LAR and therefore further research, focused on the eosinophil and its role, seems to us to be of high importance.

Another very interesting result in Part I of this study concerns the bronchial reactivity to histamine in patients with LAR. The bronchial reactivity to inhaled histamine (and/or methacholine) is also called a non-specific hyperreactivity of the bronchial tree, whose counter-value is the bronchial histamine (methacholine) threshold. The decrease of the bronchial histamine threshold indicates the increased bronchial reactivity to histamine or increased non-specific hyperreactivity. In our study, the increased bronchial reactivity to histamine was found in 40 (66%) patients demonstrating LAR, while the other 21 (34%) patients with LAR showed no increased bronchial histamine reactivity. On the other hand, most of the patients with negative bronchial response to allergen challenge (52 of the 55 = 95%) demonstrated a distinctly increased bronchial reactivity to histamine. Our findings in Part I of an increased bronchial reactivity to histamine in only some of the patients with positive LAR, are in agreement with results of some other investigators.[21] On the other hand, our findings disagree with some authors, who reported a much higher percentage of increased bronchial reactivity to histamine or methacholine in patients with positive LAR to allergen.[16,32,45] Cartier et al[16] concluded that LAR to allergen can be associated with increased bronchial responsiveness to histamine. They also suggested that allergen-induced LAR virtually always increases responsiveness to histamine. Furthermore, Lam et al[32] postulated that non-specific bronchial hyperreactivity is an important factor in determining the type and the severity of the asthmatic reaction induced by inhalation challenge with allergen. Unfortunately, our results cannot support their conclusions. The discrepancy between their findings and ours can be explained by the following facts. (1) The criteria for the patients' selection differ. Some of the above mentioned investigators characterized their patients as asthmatics only. This definition cannot, however, exclude a present or just-passed viral or bacterial infection. In all our patients, the sputum was examined bacteriologically just before the bronchial challenge. Our patients with bronchial asthma, having completely symptom-free intervals between the bronchoconstriction attacks, were always challenged only during a symptom-free period. (2) The conditions under which all challenges were carried out seem to us to be very important. All our patients were challenged during hospitalization under standard conditions, such as allergen-poor environment and surroundings (the air in the testing rooms were continuously controlled and standardized) and they followed a standard diet and regimen. On the other hand, some of the above mentioned investigators performed the challenge on out-patients, where the conditions preceding the challenges cannot be standardized and guaranteed. (3) The differences in the method employed could also be a source of the discrepancy in results. The other authors mostly used a schedule where the individual concentrations of histamine or methacholine, in our opinion, followed one another too quickly (five minutes) and therefore the cumulative effect can be expected. In our method, which corresponds principally with the standard technique described by several investigators,[25,26] we always inserted a 1-hour interval between two subsequent concentrations of histamine in order to exclude the cumulative effects. This modification was introduced by us as a result of one of our other studies, which will be published, demonstrating that bronchial response to inhaled histamine resolves within 30 to 45 minutes after the challenge, independent of the degree of FEV_1 decrease. Another point in the method used by some investigators,[16,45] namely the performance of the histamine challenge on the same day as the challenge with allergen, seems to us to be questionable. A part of LAR does not disappear within eight hours, but lasts for a longer time. In such cases, when the LAR is not completely resolved, the challenge with histamine may inevitably lead to a cumulative effect which does not, however, represent the real bronchial reactivity to histamine. It is therefore not surprising to us that the bronchial responsiveness to histamine increased when the histamine challenge was performed at this stage. In our opinion, the increased bronchial reactivity to histamine, recorded after the LAR, does not allow the conclusion that the non-specific hyperreactivity is an important factor in determining LAR. A positive LAR can lead to a temporary increase of non-specific hyperreactivity that remains for hours. This is caused by an increase of the general susceptibility of the bronchial tree and can be observed also after the just-passed viral or other kind of infection. All bronchial response to histamine challenge resolved within 30 to 45 minutes and we never

recorded any late response to the histamine in our patients. With respect to this fact, we are not convinced of such an important role of the non-specific hyperreactivity in the pathogenesis of the LAR to allergen, as is believed by these investigators.[16,32,45] The recent findings of Ramsdale et al[125] of a normal responsiveness to methacholine in some patients with asthma who demonstrate LAR, are in agreement with the results of our study.

The interpretation of the increased bronchial reactivity to histamine (or methacholine) for the diagnosis of bronchial asthma, varies highly. In our opinion, the positive LAR to allergen challenge is an indicator for an allergic component, while the decreased bronchial histamine threshold is an indicator for the increased non-specific hyperreactivity component in the bronchial tree. Both these components can participate in the patients with asthma, to various degrees. We consider the allergic and the non-specific hyperreactivity components two different mechanisms, which result in the same spectrum of bronchial complaints (bronchial response). They can both exist beside one another in the same patients, but neither can be regarded a necessary condition for the other. In a majority of the patients with asthma (70%), we usually found both these components to various degrees. On the other hand, in approximately 30% to 40% of the asthma patients we found only one of these components. In this study, in 40 of the 61 patients with LAR (66%) the bronchial histamine threshold was decreased, while in 21 patients (34%) the bronchial histamine threshold was unchanged and remained unchanged, even after two repetitions (1 and 4 weeks later). On the other hand, in 52 of the 55 patients (95%) who did not develop any kind of bronchial response to allergen challenge, the bronchial histamine threshold was distinctly decreased. This fact indicates that the bronchial complaints of these patients were solely due to the non-specific hyperreactivity, while the allergy component was absent.

The positive late skin response was found by us in 51 LAR cases (61%). This unsatisfactory correlation observed in our patients implicates the conclusion that skin tests cannot replace the bronchial challenge and they may be regarded as a screening and supplementary diagnostic parameter to the bronchial challenge. The unsatisfactory correlation between the late skin response and LAR could probably be explained by the following factors. (1) The possible involvement of different antibodies in the LSR and LAR.[6-8,12,14,15,20,46,61] (2) The antigen-antibody interaction and its subsequent stages could be a process limited to the bronchial tree only, at least in some of the patients with LAR. Such a topical process, involving the topical immunoglobulins only, does not sufficiently influence the concentration of the immunoglobulins in the serum and in the skin. (3) The possible existence of the different sensitivity degrees between the skin and the bronchial tree to the same allergen. (4) The possible existence of the different mechanisms involved in the skin response and in the LAR.

The increase in body temperature during 15 LAR cases (18%), the appearance of general malaise complaints during 67 LAR cases (81%), and slight changes in the roentgenogram (increase of pronounced bronchovascular markings) during some cases of LAR could suggest a possible involvement of an inflammatory mechanism, such as late hypersensitivity (type III reaction) and probably IgG antibodies, rather than an immediate hypersensitivity with IgE, at least in some of these patients.

The very good reproducibility of LAR and a significant dose-response relationship in most cases of LAR confirm the suitability of the method employed and the concentrations of allergen extracts used by us for BP. It also exclude the false positivity of LAR.

The appearance of LAR in Part I of our study as an isolated form (ILAR) and another time in combination with an immediate response (DAR = IDAR + LDAR) might also be the subject of a large discussion. In the case of the dual late asthmatic response, with its two-step course (immediate and late response), the bi-phasic mast cell degranulation suggested by Wasserman,[41] the activation and reactivation process of mast cells proposed by Kay et al,[46,112] the release of three groups of mediators in three subsequent time-periods postulated by Casale and Kaliner,[44] or lastly, the combined mechanism described by Zweiman et al[13,31] and Kaliner[14] (where the degranulation of mast cells leads to an immediate response but, at the same time, also to a neutrophil activation then causing the late response) could be considered. In the case of the isolated late asthmatic response, the involvement of the classical inflammatory mechanism (including the neutrophils, eosinophils, platelets, complement parts, immune complexes, and IgG) which might be similar to the late hypersensitivity, cannot be excluded. This hypothesis can be supported by our observation that total IgE did not increase and by the lack of specific IgE antibodies in the serum, but increases in the total IgG and the IgG4 and decrease in the IgG2 in the serum, slight leukocytosis, increase in the body temperature, appearance of general malaise complaints and lastly, slight changes on the thorax x-ray accompanying the isolated late asthmatic response to a higher degree than the LDAR (see "Abbreviations"). The LAR recorded in this study was very similar to that observed by us after the bronchial challenge with pigeon feces.[6] The existence of the isolated late asthmatic response does not support some authors' suggestion[15,20] that the late response only appears after the preceding immediate response; moreover, the isolated late asthmatic responses excludes this suggestion. The six patterns of the LAR we recorded, seem to us to be an interesting finding. Although we do not have any clear explanation for it yet, the involvement of the different parts of the

immunologic system, in the individual LAR sub-forms cannot be fully excluded (Figure 1).

Another very important problem seems to us to be the question of why a certain allergen causes an isolated immediate response in one patient, a dual response in another, and, an isolated late response in a third patient. A similar question is, why the same patient develops various types of asthmatic responses to different allergens. Unfortunately, we do not yet have a clear answer to these questions. We are aware that more concurrent investigations will be necessary to clarify the development of the LAR as well as the mechanism(s) underlying this clinical phenomenon.

Implications for the Clinical Practice

(1) The LAR occurs in approximately 24% of the patients with bronchial asthma.

(2) The LAR can play an important role in the bronchial complaints of some patients with allergic bronchial asthma.

(3) This type of response can regularly be overlooked in practice.

(4) The mechanism underlying the LAR is not yet satisfactorily clarified. The involvement of the late hypersensitivity (type III allergy) or at least some of its parts in the LAR, however, cannot be excluded. The role of the immediate hypersensitivity mechanism (type I allergy) in the LAR can also not be excluded. The existence of the two modifications of the LAR, namely ILAR and DAR, might support the hypothesis of the involvement of different mechanisms, or at least of different modifications.

(5) The correlation of the LAR with other *in vivo* and *in vitro* diagnostic parameters, found to various degrees, was not statistically significant. These tests should therefore be regarded as useful supplementary diagnostic parameters only.

(6) The definite confirmation of the LAR in patients with bronchial allergy should be provided by BP with allergens, followed by recording of the parameters for at least 24 hours.

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Effects of disodium cromoglycate and beclomethasone dipropionate on the asthmatic response to allergen challenge I. Immediate response (IAR)

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This study deals with comparative investigation of the protective effects of disodium cromoglycate (DSCG, Lomudal®, Intal®) and beclomethasone dipropionate aerosol (BDA, Aldecin®, Becotide®, Beclovent®) on 103 immediate asthmatic responses (IARs) to allergen challenge recorded in 103 patients with an allergic bronchial asthma. Disodium cromoglycate demonstrated highly significant protective effects on the IAR in patients investigated ($P < .01$). The protective effects of BDA on the IAR were found to be non-significant ($P > .01$). It is suggested that DSCG should be the first choice in controlling allergic bronchial asthma when the immediate asthmatic response to allergen plays the predominant role.

ABBREVIATIONS

BDA = beclomethasone dipropionate aerosol
BHT = bronchial histamine threshold
BP = bronchial provocation test
DSCG = disodium cromoglycate
FEV_1 = forced expiratory volume in one second
IAR = immediate asthmatic response
IH = immediate hypersensitivity
PBS = phosphate buffered saline
VC = vital capacity

Some of the results reported in this paper were presented as a preliminary communication at the XIth International Congress of Allergology & Clinical Immunology in London 1982,[11] and some of these results were included in the paper presented at the XIIth International Congress of Allergology & Clinical Immunology in Washington, DC in 1985.[12]

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INTRODUCTION

The effects of disodium cromoglycate (DSCG) as well as beclomethasone dipropionate aerosol (BDA) have been investigated extensively in patients with allergic bronchial asthma.[1-3] In most of these studies the patients were treated with these drugs for a certain period of time and the effectiveness of the drugs was recorded by means of clinical scores. Papers comparing the effects of both these drugs in the same group of patients[4,5] or with the effects of both drugs on the asthmatic response to allergen challenge are limited.[6-13]

In the literature there is a dearth of information concerning a comparison of the effects of DSCG and BDA on the immediate asthmatic response to allergen challenge during bronchial provocation tests with allergen (BP) in a sufficiently large group of well-defined and diagnosed patients with allergic bronchial asthma.

A comparative study would be of interest.

Despite frequent use of these drugs in practice, there is a lack of well-defined indications for their use and both these drugs are often considered in practice to be equivalent alternatives, although they have completely different pharmacologic effects and actions.

This study is a continuation of our preliminary studies[11-19] and is part of a large clinical investigation dealing with a comparison of the effects of DSCG and BDA on the various types of bronchial response to allergen challenge.

MATERIALS AND METHODS

Patients

A total of 103 patients suffering from bronchial asthma with an allergic component and developing only an isolated IAR (bronchus-obstructive) to allergen challenge were included in this study. Of these 103 patients, 133 positive IARs developed. Only 103 IARs (is one IAR per patient) were, however, the subject of this investigation. These patients, as well as the IARs and their clinical feature, were extensively described in our previous paper.[17]

The patients investigated were between 18 and 55 years of age, showed reversible bronchoconstriction alternating with symptom-free periods. Their pulmonary functions did not demonstrate restrictive changes, they did not suffer from chronic infections of the airways, and none had received immunotherapy or used oral corticosteroids. All these patients were previously examined by a diagnostic procedure consisting of (1) a general part (disease history, physical examination, basic laboratory tests including bacteriologic examination of the sputum, x-rays of the chest and sinuses, electrocardiogram, pulmonary functions, and blood gases estimation) and (2) an allergologic and immunologic part consisting of skin tests; bronchial histamine threshold (PD20); blood leukocyte and eosinophils counts; determination of total serum IgE by PRIST, specific IgE by RAST, IgG, IgM, and IgA by single radial immunodiffusion (Mancini technique); and BPs with allergens recorded up to 56 hours and supplemented by recording of the body temperature and bronchial and general complaints.[17]

All BPs, non-pretreated as well as pretreated, and the control challenges were performed during hospitalization of the patients under standard conditions. At this time the patients were free from manifest bronchial complaints and infection. Anti-allergic therapy had been stopped at least 4 weeks and bronchodilators 48 hours prior to this study.

In the event that FEV_1, or both FEV_1 and VC decreased by 50% or more during the BPs, a single inhalation of 100 μg of salbutamol aerosol was given to prevent a further drop of FEV_1. If the bronchial response needed more extensive treatment, the patient was excluded from the study.

Allergens

The dialyzed and lyophilyzed extracts (Allergen Laboratory Diephuis, Groningen, The Netherlands) were diluted in PBS (dry weight of allergen in milligrams per 1 milliliter of PBS) and used for skin tests and bronchial challenges in concentrations as shown in Table 1.

Skin Tests

The scratch tests were performed and evaluated after 20 minutes. If they were negative, intracutaneous tests were carried out and evaluated after 20 minutes and then up to 96 hours and, if necessary, for longer, until their disappearance. The results of the intracutaneous tests were interpreted as follows: (a) immediate skin response—onset within 20 minutes and disappearance within 120 minutes; (b) late skin response—onset within six to 10 hours and disappearance within 36 hours after the intradermal skin injection.

Bronchial Provocation Tests (BP)

The VC and FEV_1 were recorded by means of a spirograph (LODE, Model D-75, The Netherlands). The FEV_1 was considered to be the basic parameter for assessment of the bronchial obstruction. The PBS as well as the allergen extracts were inhaled in the form of an aerosol administered by means of the Wiesbadener Doppel-Inhalator at an air flow of 10 L/min (the aerosol particles had a mass median diameter of 2.8 to 3.6 μ).

The schedule of the BP was as follows: (1) recording of the base values ("initial values") at 0.5 and 10 minutes; (2) inhalation of PBS for 10 minutes and then recording of the "PBS values" (= control) at 0, 5, and 10 minutes; (3) inhalation of the allergen aerosol for 10 minutes (2 × 5 minutes) followed by recording of the VC and FEV_1 values at 0, 5, 10, 20, 30, 45, 60, 90, and 120 minutes and then every hour up to the 12th hour and every second hour during the 24th and 38th and 47th to 56th hour intervals after the end of the challenge. The BPs were evaluated as follows: (1) the decrease of FEV_1 or both FEV_1 and VC of less than 10% with respect to the control values as negative, from 10% to 20% as doubtful and of 20% (PD20) or more as positive; (2) the decrease of FEV_1 or both FEV_1 and VC within 60 minutes after allergen challenge, recorded at least at three consecutive time-intervals, was considered to be a positive IAR (bronchus-obstructive). The decrease of FEV_1 within a period of 4 to 24 hours after allergen challenge, recorded at least at three consecutive time-intervals, was considered to be a positive late asthmatic (bronchus-obstructive) response.

Table 1. Survey of the Allergens Used for Skin Tests* and Bronchial Challenges

	Concentration, per 1 mL of PBS	
	Scratch and Intracutaneous Tests	Bronchial Challenges
House dust	0.5 mg	5.0 mg
Hairs and feathers mix**	0.25 mg	2.5 mg
Dog, cat, horse, cow danders and hen, parrot, canary feathers—each of them	0.25 mg	2.5 mg
Grass pollen mix,† spring pollen mix,‡ weed pollen mix‖—each of them	1000 NU¶	10,000 NU¶
Mites (*Dermatophagoides pteronyssinus*)	10 NU	100 NU

* The criteria for evaluation of the intracutaneous tests: −, normal skin appearance; ±, wheal not larger than original (5.0 mm) injected papule; +, wheal increase up to 7.5 mm in diameter; ++, wheal increase up to 10.0 mm in diameter; ++ wheal increase up to 12.5 mm in diameter; +++± wheal increase up to 15.0 mm in diameter; and +++, wheal increase larger than 15.0 mm, sometimes with "pseudopodia."

** Cat, dog, cattle, goat, hog, horse, rabbit, rat, mouse, hamster, guinea pig, canary, goose, duck, turkey, hen, pigeon, and parrot in equal portions by weight.

† Dry weight percentage: *Secale cereale* 15%, *Dactylis glomerata* 15%, *Lolium perenne* 10%, *Anthoxanthum odoratum* 10%, *Agrostis alba* 10%, *Holcus lanatus* 10%, *Phleum pratense* 10%, *Cynosurus cristatus* 5%, and *Alopecurus pratensis* 15%.

‡ Dry weight percentage: *Corylus avellana* 20%, Alnus species 30%, Salix species 20%, Betula species 20%, Myrica species 10%.

‖ Dry weight percentage: *Artemisia vulgaris* 33%, *Rumex acetosa* 33%, *Plantago lanceolata* 33%.

¶ 1 Noon Unit (NU) = 0.001 mg of dry pollen (powder) = 0.5 PNU = 1.3 TNU.

The Control Test with PBS

This test was performed in each patient three days before the BP with allergen, in the same way as during the BP, and the VC and FEV_1 were recorded up to 56 hours. The control test was considered negative when the changes of the FEV_1 values varied no more than 5% ± 2% (mean ± SE) with respect to the "initial values."

Drugs

(1) Disodium cromoglycate (Lomudal®, Intal®), in powder form contained in capsules, was inhaled by means of a special applicator (spinhaler). One dose of DSCG was always one capsule containing 20 mg of DSCG powder. The capsule was put into the spinhaler, perforated and then the DSCG powder was inhaled by an air flow produced by the patient's active inspiration.

(b) Beclomethasone dipropionate (Becotide®, Aldecin®, Beclovent®) was used in the form of a metered aerosol. One dose of BDA was always four inhalations = 200 μg (1 inhalation = 50 μg).

Protection Tests with DSCG and BDA

The protection tests were carried out by means of spirography, which technique was identical to that used for the BPs.

Both the protection tests were performed in the same manner and according to the same schedule.

The patients were always pretreated with the appropriate drug in a daily dose of 4 × 1, starting two days before the allergen challenge and continuing throughout the "challenge day." The drug was always administered at 7 AM, 12 AM, 6 PM, and 11 PM. On the "challenge day" one additional dose of the drug was given 10 minutes before the allergen challenge. With the exception of this extra drug dose, the basic schedule of both the protection tests was similar to that of the provocation tests (BPs). The protection tests were performed as "single blind," where the patient did not know which drug was being used* and "crossover" randomized.

An interval of three to six days was always allowed between the individual tests, bronchial provocation test, and individual protection tests.

The protective effects of the drug were considered clinically significant when the FEV_1 or both FEV_1 and VC values, recorded after the pretreated challenge, improved by at least 50% with respect to the values recorded after the non-pretreated challenge (BP).

In ten patients ten protection tests with DSCG and in another ten patients ten protection tests with BDA were repeated 2 to 4 weeks later to evaluate reproducibility. In two other groups of patients, each of them consisting of 12 subjects, 12 protection tests with DSCG and 12 with BDA were repeated after pretreatment of 28 days with these drugs (daily dose of 4 × 1) in order to evaluate the sufficiency of the length of the pretreatment period.

Statistical Analysis

The results were statistically evaluated by means of fitting polynominals to the mean curves over time (five time points within 120 minutes after the allergen challenge). The appropriate hypotheses were tested by means of the generalized MANOVA model (generalized multivariate analysis of variance model), proposed by Potthoff and Roy[20] and reviewed by Timm.[21] The statistical analysis was performed by means of the computer program described by Berger[22] using a VAX 11/780 computer. A $P < .01$ was considered to be significant, and $P > .01$ to be statistically non-significant.

* The "single blinding" of the drugs for the patient was possible since both the DSCG and the BDA in the powder as well as in the metered aerosol form are available in The Netherlands. The patient was always informed about all four forms of these drugs and could therefore not identify exactly which of these drugs was being used.

RESULTS

The 103 patients developed 103 positive IARs. The IAR began within 10 minutes, reached its maximum within 45 minutes and disappeared within 90 to 120 minutes after the end of the allergen challenge in most cases[17] (Fig 1).

The IAR was associated with other diagnostic parameters as follows: (1) positive disease history in 62%; (2) positive immediate skin response in 68.4%; (3) increased total IgE in the serum in 41%; (4) positive specific IgE in the serum in 44.4%; (5) increased serum IgG in 27%, IgM in 12.8%, IgA in 2.3%; (6) increased blood eosinophils in 27% and leukocytes in 14.3%; (7) increased body temperature in 7%; (8) appearance of bronchial complaints in 98.5%; (9) occurrence of general malaise complaints in 46.6%.[17] The positive IAR was accompanied by various bronchial and general complaints as is presented in Table 2.

Protection Tests with DSCG and BDA

The bronchial provocation tests, the protection tests, and control challenges are summarized in Figure 1.

Protection Tests with DSCG

Disodium cromoglycate prevented fully 62 IAR cases (60%), decreased significantly 39 (38%), and was ineffective in 2 IAR cases (2%) (Fig 2). The DSCG effects lasted for at least four hours.

The reproducibility of the protection tests with DSCG was found to be very good. No significant differences were found between the first and the second DSCG protection test, either in the time course or in the changes of the appropriate FEV_1 values in any of the ten patients examined ($P > .15$).

No significant differences in the FEV_1 values were found between the short-pretreated and the long-pretreated DSCG protection tests in

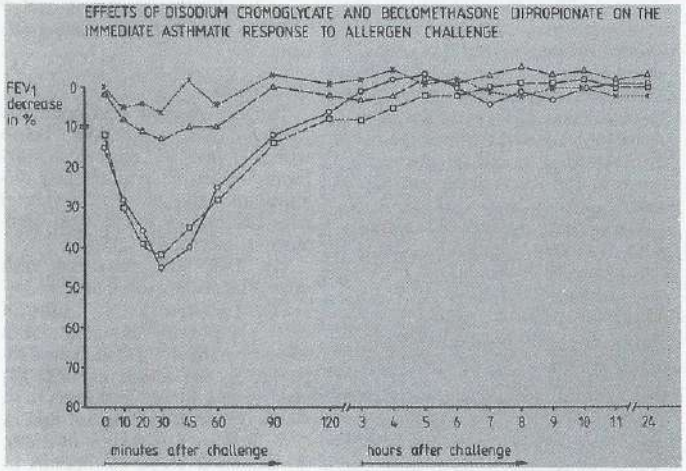

Figure 1. Mean percentage changes in FEV_1 with respect to the "initial FEV_1 values" calculated from all non-pretreated and pretreated immediate asthmatic responses (IAR) (n = 103); O——O = non-pretreated IARs (= bronchial provocation tests); Δ- - - -Δ = IARs pretreated with DSCG (= DSCG protection tests); □- - - -□ = IARs pretreated with BDA (= BDA protection tests); ×- - - -× = control challenges with PBS.

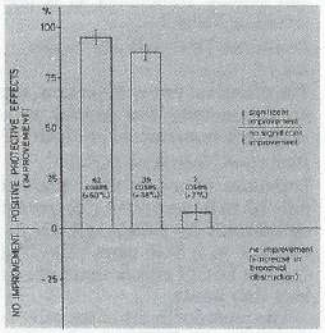

Figure 2. Distribution of the protective effects of DSCG on the immediate asthmatic response (n = 103).

any of the 12 patients studied ($P > .05$).

The bronchial challenge pretreated with DSCG was accompanied by the bronchial and general complaints to a distinctly lower degree than the non-pretreated challenge, as is shown in Table 2.

Protection Tests with BDA

Beclomethasone dipropionate aerosol decreased significantly 3 IAR cases (3%), decreased non-significantly 18 (18%), was fully ineffective in 73 (71%), and increased 9 IAR cases (8%) (Fig 3).

The reproducibility of the BDA protection tests was found to be very good. No significant differences were found between the first and the second BDA protection tests in any of the ten patients investigated ($P > .1$).

No significant differences in the effects of BDA on the IAR were found between the pretreatment of 2.5 days and that of 28 days in any of the 12 patients studied ($P > .05$). It could therefore be concluded that the pretreatment with BDA for a period of 28 days, as compared with that for 2.5 days, did not significantly increase the protective effects of this drug on the IAR.

The results summarized in Table 2 show that the BDA was not able to decrease bronchial and general complaints accompanying the IAR.

Statistical Analysis

I. Non-pretreated asthmatic responses.

Hypothesis No. 1: The immediate asthmatic response (decrease in FEV_1 values), recorded in individual patients, showed no mean trend (= no significant changes). This hypothesis is rejected ($P < .01$). It can be concluded that all IARs recorded were significantly positive. *Hypothesis No. 2:* The PBS curves recorded in individual patients showed no mean trend (= no significant changes). This hypothesis cannot be rejected ($P > .05$) but must be accepted. It can therefore be concluded that PBS curves did not show any significant changes ($P > .05$) and they were therefore significantly negative.

II. Pretreated asthmatic responses.

Hypothesis No. 3: All three curves (ALL, DSCG, BDA) coincide; this hypothesis is rejected ($P < .01$).

Hypothesis No. 4: The ALL and BDA curves coincide; this hypothesis is not rejected but accepted ($P > .01$).

Explanation of the abbreviations: ALL = non-pretreated IAR (provocation test); DSCG = IAR pretreated with DSCG (= DSCG protection test); and BDA = IAR pretreated with BDA (= BDA protection test).

With respect to the results of hypotheses 3 and 4, it could be concluded that the DSCG demonstrated highly significant protective effects on the IAR, while BDA did not show any significant protective effects on the IAR to allergen challenge at all.

DISCUSSION

The BPs with allergen have become accepted as a very important *in vivo* part of the diagnostic procedure for the detection and confirmation of the role of the allergy component

Table 2. Bronchial and General Complaints Accompanying the Non-pretreated Immediate Asthmatic Response (IAR) and the IAR Pretreated with Disodium cromoglycate (DSCG) and Beclomethasone Dipropionate Aerosol (BDA)

n = 103	IAR Non-pretreated	IAR Pretreated with	
		DSCG	BDA
Dyspnea	101 (98%)	5 (5%)	92 (90%)
Wheezing	85 (83%)	6 (6%)	81 (79%)
Cough	73 (71%)	11 (11%)	68 (66%)
Productive	19 (18%)	0	19 (18%)
Non-productive	54 (53%)	11 (11%)	49 (48%)
Expectoration	19 (18%)	0	19 (18%)
Thick sputum	3 (2%)	0	3 (3%)
Thin sputum	16 (16%)	0	16 (15%)
Pressure on the chest	47 (46%)	3 (3%)	31 (30%)
Chills	9 (9%)	1 (1%)	5 (5%)
Tiredness	55 (53%)	4 (4%)	47 (46%)
Weakness	38 (37%)	1 (1%)	25 (24%)
Headache	27 (26%)	1 (1%)	27 (26%)
Acral cyanosis	12 (12%)	0	8 (8%)
Nasal obstruction	9 (9%)	1 (1%)	9 (9%)
Conjunctival irritation	3 (2%)	0	3 (3%)
Pressure in the sinuses maxill and front.	6 (6%)	1 (1%)	6 (6%)
Angioedema	2 (2%)	0	1 (1%)
Acute skin eruption (rash, urticaria, exanthema)	4 (4%)	0	4 (4%)
Tachycardia	8 (8%)	1 (1%)	8 (8%)
Bradycardia	1 (1%)	0	0
Blood pressure	10 (10%)	0	10 (10%)
Increase	9 (9%)	0	9 (9%)
Decrease	1 (1%)	0	0
Gastrointestinal complaints	1 (1%)	0	1 (1%)

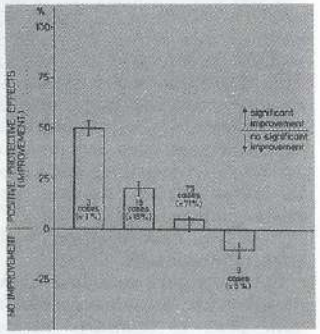

Figure 3. Distribution of the protective effects of BDA on the immediate asthmatic response (n = 103).

and of the role of certain allergens in patients with bronchial asthma and chronic asthmatic bronchitis.[8,11-19,23-29] During these tests, the bronchial tree is challenged directly by a certain allergen and the bronchial response, due to the antigen-antibody interaction with its subsequent steps, is recorded quantitatively. Some investigators, however, believe that the IAR can be predicted by knowledge of non-specific bronchial reactivity and level of allergen sensitization, as is extensively discussed in our previous papers.[17-19]

We use the BPs as a standard part of the routine diagnostic procedure in patients with bronchial asthma and chronic asthmatic bronchitis for detection of the bronchial response to allergen challenge of the immediate as well as the non-immediate types (late, delayed).[12,13,15-19]

We also use these tests in another modification, the so-called protection tests, where the pretreatment with the drug investigated during a certain period of time precedes the allergen challenge. The "protective effects" of the drug investigated refer to its ability to prevent or significantly decrease the development of the bronchus-obstructive response caused by the antigen-antibody interaction.[11-14]

The methods employed for BP used in this study were described in our previous papers.[11-14,17-19]

Numerous papers concerning the IAR, as well as the role of IH have already been published. The exact pathologic and immunologic mechanisms underlying the IAR have, however, not yet been fully clarified. In our opinion a difference should be made between the IAR and IH in patients with bronchial asthma. Immediate hypersensitivity is a well-defined immunologic mechanism, mediated by IgE antibodies, in which the mast cells (and/or basophils) and most of the known primary mediators are involved. The IAR should be regarded as a clinical phenomenon, defined by the appearance within 60 minutes after the allergen challenge, which could be induced by complex mechanisms. The pathologic and immunologic mechanisms leading to the IAR could probably be different.[17-19,23-25,29-34]

In a large part of the IAR the pivotal role of the IH mechanism, thus IgE mediated, could be presumed. On the other hand it is not a matter of course that every IAR is caused by the IH mechanism, as was believed for years. There is evidence that in some IAR cases the antibodies of other classes, eg, IgG and mechanisms different from IH could be involved.[17,23,24,29,34]

The results of this study demonstrated the highly significant protective effects of DSCG on the IAR ($P < .01$), while the protective effects of BDA on the IAR were non-significant ($P > .01$).

These results are in agreement with findings of some other investigators.[6,7,10]

On the other hand, our study disagrees with Burge's findings[35] reporting the protective effects of BDA on the IAR to allergen challenge, after a week's pretreatment of a daily dose of 800 μg. His number of patients was, however, low and no reliable statistical analysis of the data was employed.

Our findings of the lack of protective effects of BDA on the IAR disagree with some authors describing the beneficial therapeutic effects of BDA in patients with bronchial asthma.[3,9] These so-called beneficial effects of BDA can perhaps be explained by the following facts: (1)

the bronchial complaints in their patients were not due to the immediate hypersensitivity but to nonspecific hyperreactivity or to nonimmediate type of hypersensitivity; (2) the simple recording of the bronchial complaints score during the BDA administration cannot demonstrate that the BDA really modulated the antigen-antibody interaction with its subsequent steps; (3) the relief of the bronchial complaints in these patients could also have been caused by diminishing of the inflammatory component by the BDA; and (4) in some of these studies the diagnostic procedure and the selection of patients were not fully standardized.

Both the drugs were used in this study in doses corresponding with those reported in the literature,[2-4] DSCG 4 × 20 mg and BDA 4 × 200 μg. The lack of protective effects of BDA observed in our study could therefore not have been caused by an under-dosage.

No significant differences in the effects of BDA on the IAR were found between the pretreatment of 2.5 days and that of 28 days, in any of the 12 patients investigated ($P > .05$). These results do not confirm the findings of some investigators, reporting the appearance of the protective effects of BDA on the IAR after pretreatment of longer than 1 week (1 to 3 weeks).[8,35]

The explanation for the different protective effects of DSCG and those of BDA on the IAR and also on the accompanying bronchial and general complaints could be provided by the pharmacologic properties of these drugs, which are highly different.

The majority of IARs may be due to the IH mechanism, both in the classically understood IH and in its various modifications,[17-19,23-25,31-33] mediated by IgE antibodies, where the mast cells and/or basophils play a pivotal role. There is, however, some evidence for a possible involvement of antibodies of other classes, eg, IgG and IgM in, at least, a part of the IARs.[17-19,23-25,29,30,34]

Disodium cromoglycate protects the mast cell and basophil from degranulation and release of mediators by stabilizing their cell membrane, due to the blocking of calcium transport and inhibition of calcium gate opening induced by antigen. There is also evidence for possible increase of the membrane-associated cAMP by DSCG directly or indirectly through the inhibition of phosphodiesterase, the increase of which inhibits the mediator release.[36-41]

Disodium cromoglycate is therefore the indicated compound for the treatment of such bronchial asthma in which the IAR due to the immediate hypersensitivity mechanism plays the main role.

Beclomethasone dipropionate aerosol, being a glucocorticosteroid, has a high topical anti-inflammatory activity.[42,43] The corticosteroids, including BDA, increase and potentiate the action of cAMP, decrease and inhibit the action of cGMP,[42-44] inhibit synthesis of some prostaglandins,[43-45] influence the calcium transport in some types of cells, decrease neutrophil chemotaxis,[44] decrease the release of lysosomal enzymes from neutrophils, decrease vascular permeability and increase the resistance of the capillary wall, inhibit the synthesis and release vasoactive kinins and prostaglandins and have inhibitory effects on some complement parts.[42-44] With respect to the suggested involvement of cAMP and cGMP in modulation of release of certain mediators (decrease of cAMP and increase of cGMP stimulates mediator release), corticosteroids such as BDA could also indirectly influence the release of some mediators.[44] The corticosteroids might also prevent re-accumulation of histamine in tissue, decrease the histamine tissue level and decrease the synthesis and metabolism of histamine, leukotrienes, and other products or arachidonic acid.[42,46] On the other hand, various other important mediators and their metabolism are not affected by corticosteroids at all.

Despite some reports suggesting the possible effects of glucocorticosteroids on the human basophils and mouse mast cells, by *in vitro* inhibition of histamine release,[47,48] other studies demonstrated that incubation of human lung fragments or human lung mast cells with glucocorticosteroids *in vitro* did not inhibit IgE-mediated release of histamine or the mast cell cyclooxygenase metabolites, PGD_2 and thromboxanes.[49]

Nevertheless, the direct protective effects of corticosteroids, including BDA, on the human mast cells and basophils, by preventing release of their mediators, have not yet been unequivocally demonstrated.[42]

These pharmacologic properties of glucocorticosteroids could explain the lack of protective effects of BDA on the IARs observed in this study. On the other hand, glucocorticosteroids distinctly inhibit other types of hypersensitivity reactions, different from the immediate (Type I allergy), eg, late (Type III allergy, Arthus reaction, immune-complex state),[7,9,10,12,13,15,16,23-25,42,49,51] or delayed (Type IV allergy, cell-mediated).[42,43,50,52]

Corticosteroids, including BDA, may therefore be more successful in such cases of bronchial asthma where: (1) non-immediate bronchial responses are involved, (2) an inflammatory component and a pronounced edema of the bronchial mucosa appears, (3) as adjunct therapy to DSCG in the case of the combination of different types of hypersensitivity, (4) emergency state appears, either in an acute form or in a chronic form (ineffectiveness of DSCG, severe decrease of lung functions, distinct decrease in tissue elasticity, etc).

See reprint address and reference list in Part II.

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Effects of disodium cromoglycate and beclomethasone dipropionate on the asthmatic response to allergen challenge II. Late response (LAR)

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The protective effects of disodium cromoglycate (DSCG; Lomudal®, Intal®) and beclomethasone dipropionate (BDA; Aldecin®, Becotide®, Beclovent®) on the late asthmatic response to allergen challenge (LAR) were investigated in 61 patients with allergic bronchial asthma. The 61 patients developed a total of 83 late asthmatic responses, 35 isolated late responses (ILAR), and 48 dual late responses (DLAR), which is a combination of an immediate response (IDLAR) and a late response (LDLAR). Disodium cromoglycate demonstrated significant protective effects on the LAR ($P < .01$), however, the LDLAR as part of the DLAR was decreased by DSCG to a slightly higher degree than the ILAR. The BDA also showed significant protective effects on the LAR ($P < .01$), but the ILAR was protected by BDA to a slightly higher degree than the LDLAR as part of the DLAR. The immediate asthmatic response as part of the DLAR was prevented by DSCG significantly ($P < .01$) while the BDA was ineffective ($P > .05$). It can be concluded that both DSCG and BDA demonstrated significant effects on the LAR. It is suggested that DSCG should be used as a drug of the first choice to control bronchial asthma with an allergy component where the LAR plays a role. The BDA should be added temporarily at the beginning of the treatment of patients in whom the isolated late asthmatic response plays the predominant role, or of patients in whom the DSCG does not provide full control of the LAR during a certain period, eg, during the peak of the pollen season. In those patients with LAR, in whom DSCG is ineffective, the bronchial asthma should be controlled solely by BDA.

ABBREVIATIONS

BDA = beclomethasone dipropionate aerosol
BP = bronchial provocation test
DLAR = dual asthmatic response
DSCG = disodium cromoglycate
FEV_1 = forced expiratory volume in 1 second
IDLAR = immediate response as part of the dual
ILAR = isolated late asthmatic response
LAR = late asthmatic response
LDLAR = late response as part of the dual
PBS = phosphate buffered saline
VC = forced vital capacity

Part of the results reported in this paper have previously been presented as a preliminary communication at the XIIth International Congress of Allergology & Clinical Immunology in Washington (DC), October 20–25, 1985[12] and at the 42nd Annual Meeting of the American Academy of Allergy and Immunology, New Orleans, March 24–26, 1986.[13]

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INTRODUCTION

Patients with bronchial allergy, when challenged by allergen, may develop different types of bronchial (bronchus-obstructive) response, the LAR being one of them.[11-13,15-19,23-25] The LAR has been extensively investigated and described in our previous papers.[12,13,15-19]

The LAR plays an important role in bronchial complaints in some patients with bronchial allergy and it may be responsible for failure of the usual treatment in these patients. The LAR is also overlooked in practice because only in a few departments, where the diagnostic procedure of bronchial allergy takes place, BPs with allergen are routinely performed.[18,19].

We were unable to find any paper in the available literature concerning the comparative investigation of the protective effects of DSCG and BDA on the LAR to allergen challenge in a sufficiently large group of well-defined and diagnosed patients with allergic bronchial asthma.

This study is a continuation of our preliminary and previous papers concerning the protective effects of DSCG and BDA on the IAR and LAR.[12,13,15,16]

MATERIALS AND METHODS

Patients

The 61 patients, selected from a group of 251 patients, suffering from bronchial asthma with an allergic component and developing 83 late asthmatic (bronchus-obstruc-

tive) responses to allergen challenge (LAR) were included in this study. These patients, as well as the LARs and their clinical characteristics were extensively described in our previous papers.[18,19]

The criteria for selection of these patients as well as the diagnostic procedure performed in them were identical to those in the patients with IAR, as described in part I.

Allergens, skin tests, BP, control test with PBS, drug protection tests with DSCG and BDA were identical to those which are already described in part I.

Of ten patients, ten protection tests with DSCG and in another ten patients, ten protection tests with BDA were selected. These tests were repeated 3 to 4 weeks later in order to evaluate their reproducibility. In another two groups of patients, each group consisting of eight subjects, eight protection tests with DSCG and eight with BDA were repeated after 28 days of pretreatment with these drugs (daily dose 4 × 1) to evaluate the possible influence of the length of pretreatment on the effectiveness of the drug.

Statistical Analysis

The results were statistically evaluated by means of fitting polynomials to the mean curves over time (five time points within 120 minutes and 6 time points between 4 to 11 hours after the allergen challenge). The appropriate hypotheses were tested by means of the same method as described in part I (MANOVA model). A $P < .01$ was considered to be highly significant, $.01 < P < .05$ to be moderately significant and $P > .05$ to be statistically non-significant.

RESULTS

The 61 patients developed 83 positive LAR responses. The LAR began within 4 to 8, reached maximum within 6 to 12, and resolved within 24 to 26 hours after allergen challenge in most cases. The LAR was modified in two ways: either as an ILAR (35 cases) or as a DLAR (48 cases), where at first an IDLAR appeared and then, after a symptom-free interval of 3 to 5 hours, the LDLAR occurred.[18,19]

The LAR was associated with other diagnostic parameters as follows: (1) positive disease history in 59%; (2) positive late skin response in 61%; (3) increased total IgE in the serum in 20%; (4) positive specific IgE in the serum in 29%; (5) increase in serum IgG in 66%, IgM in 49% and IgA in 1%; (6) increase in serum IgG1 in 8%, IgG3 in 25%, IgG4 in 52% and decrease in serum IgG2 in 54%; (7) increased blood leukocyte count in 19% and eosinophil count in 36%; (8) increased body temperature in 18%; (9) general malaise complaints in 81%; (10) bronchial complaints in 100%; and (11) changes on the thorax x-ray in 6%.[18,19]

The positive LAR was accompanied by various bronchial and general complaints as is presented in Table 1.[18,19]

Protection Tests with DSCG and BDA

The mean percentage changes in FEV_1 (calculated from all patients investigated during all the challenges), the bronchial provocation tests (BPs = non-pretreated challenge), and the bronchial protection tests with DSCG and BDA (= pretreated challenges) and control challenge with PBS, are summarized in Figures 1 and 2.

Protection Tests with DSCG

Disodium cromoglycate prevented fully 49 LAR cases (59%), decreased significantly 33 LAR cases (40%), and was ineffective in 1 LAR case (1%) (Fig 3).

Slight, however non-significant, differences in the protective effects of DSCG with respect to both the LAR modifications were observed. (1) LDLAR: thirty cases (36%) were prevented fully and 18 cases (22%) were decreased significantly. (2)

Table 1. Bronchial and General Complaints Accompanying the Non-pretreated Late Asthmatic Response (LAR) and LAR Pretreated with Disodium Cromoglycate (DSCG) and Beclomethasone Dipropionate Aerosol (BDA)

n = 83	LAR Non-pretreated	LAR Pretreated with DSCG	LAR Pretreated with BDA
Dyspnea	72 (87%)	0 (0%)	0 (0%)
Wheezing	80 (96%)	2 (2%)	1 (1%)
Cough	3 (4%)	1 (1%)	0 (0%)
Productive	0 (0%)	0 (0%)	0 (0%)
Non-productive	3 (4%)	1 (1%)	0 (0%)
Expectoration	0 (0%)	0 (0%)	0 (0%)
Thick sputum	0 (0%)	0 (0%)	0 (0%)
Thin sputum	0 (0%)	0 (0%)	0 (0%)
Pressure on the chest	67 (81%)	1 (1%)	3 (4%)
Chills	35 (42%)	1 (1%)	1 (1%)
Tiredness	67 (81%)	3 (4%)	5 (6%)
Weakness	61 (73%)	2 (2%)	4 (5%)
Headache	49 (59%)	0 (0%)	5 (6%)
Acral cyanosis	2 (2%)	0 (0%)	0 (0%)
Nasal obstruction	1 (1%)	0 (0%)	0 (0%)
Eye irritation (= conjunctivitis)	1 (1%)	0 (0%)	1 (1%)
Angioneurotic edema	8 (10%)	1 (1%)	2 (2%)
Pressure in the sinuses maxill and front.	6 (7%)	0 (0%)	2 (2%)
Acute skin eruption (exanthema, rash, urticaria)	9 (11%)	1 (1%)	1 (1%)
Gastrointestinal complaints	2 (2%)	0 (0%)	2 (2%)
Tachycardia	5 (6%)	0 (0%)	1 (1%)
Bradycardia	1 (1%)	0 (0%)	0 (0%)
Blood pressure increase	4 (5%)	0 (0%)	2 (2%)
Decrease	1 (1%)	1 (1%)	0 (0%)

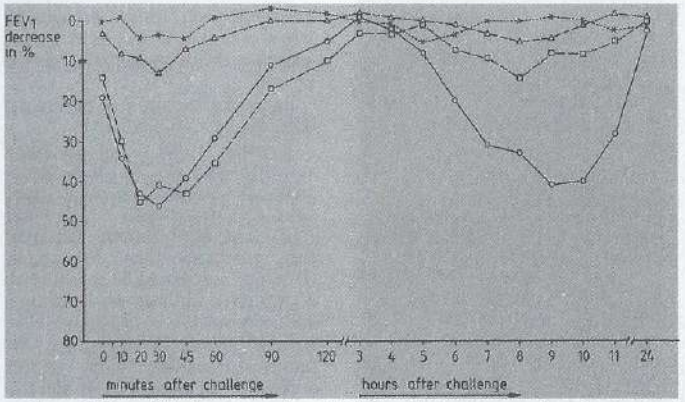

Figure 1. Effects of disodium cromoglycate (DSCG) and beclomethasone dipropionate aerosol (BDA) on the dual late asthmatic response to allergen challenge (DLAR). Mean percentage changes in FEV_1 with respect to the "initial FEV_1 values" calculated from all non-pretreated and pretreated dual late asthmatic responses (DLAR = IDLAR + LDLAR) (n = 48). O——O = Non-pretreated DLARs (= bronchial provocation tests), Δ---Δ = DLARs pretreated with DSCG (= DSCG protection tests), □---□ = DLARs pretreated with BDA (= BDA protection tests), and ×·····× = control challenges with PBS.

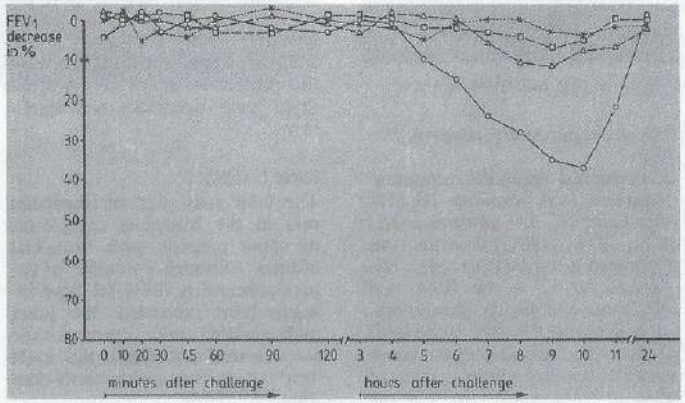

Figure 2. Effects of DSCG and BDA on the isolated late asthmatic response to allergen challenge (ILAR). Mean percentage changes in FEV_1 with respect to the "initial FEV_1 values" calculated from all non-pretreated and pretreated isolated late asthmatic responses (ILAR) (n = 35). O——O = Non-pretreated ILARs (= bronchial provocation tests), Δ---Δ = ILARs pretreated with DSCG (= DSCG protection tests), □---□ = ILARs pretreated with BDA (= BDA protection tests), and ×·····× = control challenges with PBS.

ILAR: Nineteen cases (23%) were prevented fully, 15 cases (18%) were decreased significantly, and 1 case (1%) was not influenced.

The reproducibility of the protection tests with DSCG was found to be very good. No significant differences ($P > .1$) were found between the first and the second DSCG protection test, either in the time course or in the changes of the corresponding FEV_1 values, in any of the ten patients investigated.

No significant differences in the FEV_1 values were found between the short-time and long-time pretreatment with DSCG in any of the eight patients studied ($P > .1$).

The bronchial and general malaise complaints accompanying the LAR were decreased distinctly by DSCG (Table 1).

Protection Tests with BDA

Beclomethasone diproprionate aerosol prevented fully 46 LAR cases (56%), decreased significantly 36 LAR cases (43%), and was ineffective in 1 LAR case (1%) (Fig 3). With respect to both the LAR modifications the following BDA effects were observed. (1) LDLAR: Twenty-five cases (30%) were prevented fully, 22 cases (27%) were decreased significantly, and 1 case (1%) was decreased non-significantly. (2) ILAR: Twenty-one, cases (25%) were prevented fully and 14 cases (17%) were decreased significantly.

No significant differences were found between the first and second BDA protection tests in any of the ten patients investigated ($P > .05$). It could be concluded therefore that the reproducibility of the BDA protection tests was good.

No significant differences were observed between the appropriate FEV_1 values recorded after the short and the long pre-treatment with BDA in any of the eight patients ($P > .05$).

Bronchial and general malaise complaints accompanying the LAR were decreased distinctly by BDA as is shown in Table 1.

Statistical Analysis

I. Non-pretreated asthmatic responses.

Hypothesis No. 1 = the isolated late response (ILAR) recorded in individual patients (= decrease in FEV_1 values) showed no mean trend (= no significant changes). This hypothesis is rejected ($P < .01$).

Hypothesis No. 2 = the immedi-

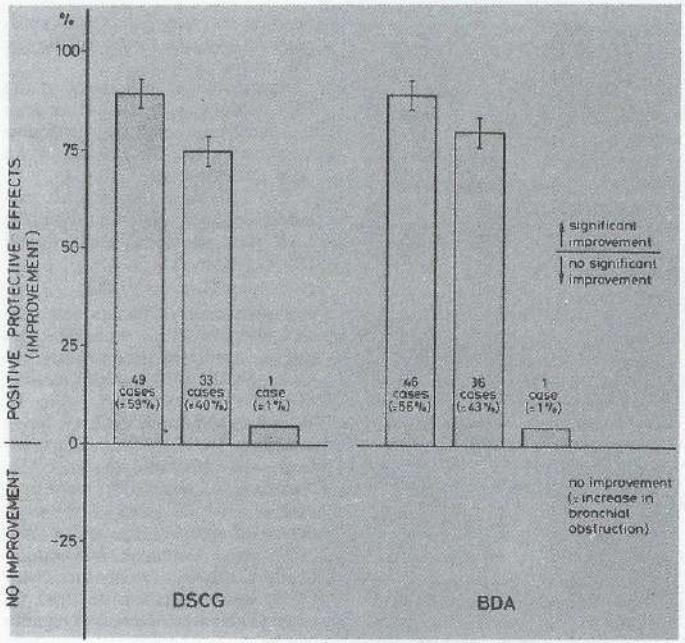

Figure 3. Distribution of the protective effects of DSCG and BDA on the LAR (n = 83).

ate response (IDLAR), as part of the dual response (DLAR) recorded in individual patients, showed no mean trend (= no significant changes in FEV_1 values). This hypothesis is rejected ($P < .01$).

Hypothesis No. 3 = the late response (LDLAR), as part of the dual response (DLAR) recorded in individual patients, showed no mean trend. This hypothesis is rejected ($P < .01$).

Hypothesis No. 4 = the PBS curves, recorded in individual patients, had no mean trend (= no significant changes in FEV_1 values). This hypothesis cannot be rejected ($P > .05$) but must be accepted.

It can be concluded therefore that the late asthmatic response, in both of its modifications (ILAR, LDLAR) as well as the immediate asthmatic response (IDLAR) were significantly positive ($P < .01$), while the control challenge with PBS was significantly negative ($P > .05$).

II. Pretreated asthmatic responses.

Isolated late response (ILAR):

Hypothesis No. 1 = all three curves (ALL, BDA, DSCG) coincide; this hypothesis is rejected ($P < .01$). *Hypothesis No. 2* = the BDA and DSCG curves coincide; this hypothesis is rejected ($P = .01$). *Hypothesis No. 3* = the ALL and BDA curves coincide; this hypothesis is rejected ($P < .01$). *Hypothesis No. 4* = the ALL and DSCG coincide; this hypothesis is rejected ($P < .01$).

Late response as part of the dual response (LDLAR):

Hypothesis No. 1 = all three curves (ALL, DSCG, BDA) coincide; this hypothesis is rejected ($P < .01$). *Hypothesis No. 2* = the BDA and DSCG curves coincide; this hypothesis is rejected ($P = .04$). *Hypothesis No. 3* = the ALL and DSCG curves coincide; this hypothesis is rejected ($P < .01$). *Hypothesis No. 4* = the ALL and BDA curves coincide; this hypothesis is rejected ($P < .01$).

Immediate response as part of the dual response (IDLAR):

Hypothesis No. 1 = all three curves (ALL, DSCG, BDA) coincide; this hypothesis is rejected ($P < .01$). *Hypothesis No. 2* = the ALL and BDA curves coincide; this hypothesis cannot be rejected ($P > .05$) but should be accepted.

Explanation of the abbreviations: ALL = non-pretreated asthmatic response; DSCG = asthmatic response pretreated with DSCG (= DSCG protection test); BDA = asthmatic response pretreated with BDA (= BDA protection test).

It can therefore be concluded that both DSCG and BDA demonstrated significant protective effects on the LAR. The LDLAR as part of the DLAR was decreased by DSCG to a slightly higher degree than the ILAR, while BDA was slightly more effective in the ILAR than in the LDLAR. These small differences in the protective effects of both the drugs were statistically non-significant.

DISCUSSION

The LAR may play an important role in the bronchial complaints in some patients with bronchial asthma. Although a number of papers concerning the LAR have already been presented, the exact pathogenetic and immunologic mechanisms underlying the LAR have not yet been satisfactorily clarified.[7,18,19,23,32,53-61]

A difference should be made between the LAR and the late type of hypersensitivity (type III allergy, Arthus reaction, immune-complex state). The late hypersensitivity is a well-defined immunologic mechanism, characterized by involvement of IgG and possibly also IgM antibodies forming immune-complexes and resulting in the complex inflammatory reactions.[63-65]

The LAR should be regarded a clinical phenomenon defined by the appearance of broncho-constriction, accompanied by other symptoms within 4 to 12 hours after exposure to allergen, which could be induced by a complex mechanism.[18,19,23,32,34,53-60] Although the pathogenetic and immunologic mechanisms leading to the LAR could be different, the late type of hypersensitivity should be regarded as one of the possible mechanisms involved in the clinical LAR, but far from the only one.[18,19]

The views on the pathogenetic and immunologic mechanisms presumably underlying the clinical LAR, however, vary highly. Some investigators suggested the involvement of the late hypersensitivity and IgG antibodies in the LAR.[18,19,23,25,29,32,34,66-69] Other investigators presumed that the immediate hypersensitivity mediated by IgE antibodies with involvement of mast cells (and/or basophils) may play the main role in the LAR.[53,57,58,70,71] Another group of authors suggested that varius combinations and modifications of the above-mentioned mechanisms could be involved in the LAR.[32,33,54-56,64,65,70,72,73]

In our previous papers we attempted to summarize the known facts and hypotheses concerning the possible mechanisms that may be involved in the LAR and to combine them with the results of our studies.[18,19]

The papers dealing with the protective effects of DSCG or BDA on the LAR are not numerous,[38,62] while the papers dealing with comparative investigation of the effects of both these drugs on the LAR to allergen challenge, are only very few.[6,7,12,13,15,16]

Pepys et al[6] and Cockcroft et al[7] observed that the IAR as well as the LAR to allergen challenge were inhibited significantly by pretreatment with inhaled DSCG. They also found that inhaled BDA prevented the LAR but did not decrease the IAR. Similar conclusions, comparing the effects of DSCG and oral corticosteroids, were reported by Booij-Noord et al.[62]

The results of our present study are principally in agreement with findings of the above mentioned investigators. Small differences, however, from their results were observed. Although DSCG significantly prevented the LAR, the LDLAR was inhibited to a slightly higher degree than the ILAR. Beclomethasone dipropionate aerosol showed an adverse effect by inhibiting the ILAR to a slightly higher degree than the LDLAR.

This observation could support the suggestion of some investigators that IAR can play an introductory role for LAR in some patients demonstrating the "dual late asthmatic response."[56,58]

The other organ related and general malaise complaints accompanying the LAR were decreased distinctly by DSCG and BDA to a similar degree (Table 1).

The reproducibility of the protection tests with DSCG and BDA was found to be very good regarding the non-significant differences ($P > .1$; $P > .05$) between the first and the second protection tests performed with each of these drugs in the appropriate group of ten patients.

The length of the pretreatment of 2.5 days was found to be sufficient for DSCG as well as for BDA to achieve their significant protective effects regarding the non-significant differences between the short-term pretreatment and the long-term pretreatment ($P > .1$; $P > .05$).

The results of this study, demonstrating the positive protective effects of both the DSCG and BDA on the LAR to allergen challenge, could probably be explained by the presumption of the involvement of the different mechanisms or, at least, different modifications, as mentioned above.

The protective effects of DSCG on the immediate as well as the late asthmatic response to allergen challenge could be explained by the ability of DSCG to protect the mast cells and basophils and in this way prevent their degranulation with subsequent release of the mediators.[38,39,41] Two possible actions of DSCG have been suggested: blocking of calcium transport and inhibition of calcium gate opening induced by antigen. There is also evidence for possible increase of the membrane-associated cAMP by DSCG, either directly or indirectly through inhibition of phosphodiesterase, the increase of which inhibits the mediator release.[37,41] On the other hand, DSCG also seems to possess other pharmacologic effects such as decreasing the neutrophil chemotactic activity, increasing cAMP and/or decreasing cGMP, either exogenous or those associated with other cells, eg, neutrophils, platelets, lung tissue.[38,72]

Our results demonstrated significant protective effects of DSCG on the LAR, however, the effects on the LDLAR were slightly higher than those on the ILAR. These differences could probably depend on the participation of some of the above mentioned mechanisms in both these modifications of the LAR.

In the case of LDLAR the mast cells and/or basophils could play the predominant role. This could, however, be realized in different ways: either directly (the suggested biphasic degranulation,[31,39,70] reactivation,[32,33,56,60,71] release of three mediator groups[33,56]) or indirectly (the generating of PAFs,[31,70,71] to triggering increased vascular permeability or to generating chemotactic factors for neutrophils,[31,63-65,70] eosinophils,[31,61,70,74,75] prostaglandins,[31,32,60,70,76] leukotrienes, and other arachidonic acid metabolites[31,32,60,76]). Disodium cromoglycate, protecting the mast cells and basophils, therefore demonstrated highly significant protective effects on this type of LAR.

In the case of the ILAR, other mechanisms and pathways (such as neutrophil activation,[44,63,65,77] com-

plement activation,[63] activation of platelets by PAFs from sources other than the mast cells and basophils,[70,78,79] decrease of cAMP and/or increase of cGMP[63,77]) probably play the predominant role, while the mast cells and/or basophils are involved to a lesser degree. Therefore DSCG, by protecting the mast cells and/or basophils to a higher degree than the other cells and components, demonstrated significant protective effects on the ILAR, but to a slightly lower degree.

The glucocorticosteroids, including BDA, possess a high topical anti-inflammatory activity.[42–45] They increase and potentiate the action and level of cAMP and decrease and inhibit action of cGMP,[42,44] inhibit synthesis of some prostaglandins,[43,45] influence the calcium transport in some types of cells,[44] decrease neutrophil chemotaxis, decrease release of lysosomal enzymes from neutrophil, decrease vascular permeability, increase the resistance of the capillary wall, inhibit the synthesis and release of vasoactive kinins and prostaglandins, and have inhibitory effects on some complement parts.[42–44] The increase of exogenous cAMP and decrease of exogenous cGMP levels by corticosteroids lead to the increase of intracellular cAMP and the decrease of intracellular cGMP, resulting in inhibition of platelet activation and accumulation[42,43] and could, indirectly, influence the release of some mediators.[44] The direct protective effects of corticosteroids, including BDA, on the human mast cells and basophils, by directly preventing the release of their mediators, have not yet been unequivocally demonstrated,[42] as has already been discussed in part I.

Beclomethasone dipropionate aerosol demonstrated significant protective effects on the LAR, but to a slightly higher degree on the ILAR than on the LDAR. These small differences could probably be explained in a similar manner as in the case of DSCG. In the ILAR, where the other mechanisms probably play a more important role than mast cells and/or basophils, the BDA demonstrated protective effects to a slightly higher degree than in the case of LDLAR, where mast cells and/or basophils are probably more involved than the other mechanisms.

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Inhibition of the late asthmatic response by nedocromil sodium administered more than two hours after allergen challenge

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Fourteen adult patients with bronchial asthma who were known late responders to bronchial allergen challenge were entered into a double-blind crossover study to compare the protective effects of nedocromil sodium (3 × 4 mg) and placebo aerosols on the late asthmatic response (LAR). After screening for development of an LAR (fall in forced expiratory volume in 1 second [FEV_1] ≥20% at at least 3 consecutive time points, 4 to 10 hours after challenge), patients were randomized to test treatment on 2 study days, with an interval of at least 3 days. Nine of the patients had a dual late response, which is a combination of an immediate and a late response, and five other patients had an isolated late response only. On each study day the concentration of allergen that previously elicited a response was inhaled for 10 minutes. FEV_1 was recorded every 10 or 15 minutes for up to 1 hour after challenge, and then at hourly intervals for 12 hours and every second hour on the next 2 days. Test treatments were administered in three doses at 30-minute intervals, with the first dose given 90 minutes before the expected onset of the LAR for each patient. Compared with placebo, nedocromil sodium significantly inhibited the LAR ($p < 0.05$) at each time point measured from 6 to 10 hours after challenge and reduced the maximum fall in FEV_1 by 21.0% overall ($p = 0.003$). (J ALLERGY CLIN IMMUNOL 1993;92:19-28.)

Key words: *Late asthmatic response, effects of nedocromil sodium*

Patients with bronchial allergy, having been challenged by allergen, may develop different types of asthmatic (bronchus-obstructive) response.[1-8] The immediate asthmatic response (IAR) has been repeatedly described.[1, 4]

Other, so-called "nonimmediate asthmatic responses," the late asthmatic response (LAR) being one of them, were also described in our previous articles.[5-7, 9-13] The LAR is a well recognized clinical phenomenon in patients with bronchial asthma.[1-3, 5-7, 10-15]

Abbreviations used

BPT:	Bronchial provocation test
BDP:	Beclomethasone dipropionate
DSCG:	Disodium cromoglycate
FEV_1:	Forced expiratory volume in 1 second
IAR:	Immediate asthmatic response
LAR:	Late asthmatic response
NS:	Nedocromil sodium
PBS:	Phosphate-buffered saline
VC:	Vital capacity

Its incidence has been variously quoted as about 50% in adults[2] and up to 73% in some selected groups of children,[3] after allergen challenge. The LAR may occur in two clinical forms, as an isolated form or as a part of a dual response, in which an early response precedes the late one.[6, 11]

In our previous studies, which included large groups of patients with bronchial asthma ($n = 251$), 24% of the patients developed an LAR to allergen challenge, 10% demonstrated an

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TABLE I. Patient characteristics

Patient No.	Age (yr)	Sex	Histamine PD_{20} (mg/ml)	Bronchial challenge: Allergen	Bronchial challenge: Dose/ml	Nonpretreated response [FEV_1]: Baseline (L)	IAR: Max.fall (%)	LAR: Onset (hr)	LAR: Max.fall (%)	Treatment order
Dual response										
2	46	M	16	HD	5.0 mg	4.7	31	6	34	b
4	20	F	8	HD	0.5 mg	2.5	32	5	36	b
5	42	M	>32	GP	10,000 NU	3.9	25	4	26	a
6	28	M	32	GP	1000 NU	5.3	43	6	40	a
7	22	F	8	HD	1.0 mg	2.7	26	7	44	a
8	19	M	>32	GP	1000 NU	3.6	34	5	39	a
9	27	F	>32	HD	0.5 mg	3.3	33	7	39	a
10	26	F	16	GP	10,000 NU	1.6	38	6	38	b
13	25	F	32	HD	1.0 mg	3.4	42	5	38	b
Isolated LAR										
1	25	F	>32	GP	10,000 NU	3.0	–	6	37	b
3	25	F	16	GP	10,000 NU	2.1	–	5	38	a
11	32	F	>32	HD	0.5 mg	3.0	–	6	50	b
12	28	M	32	HD	5.0 mg	4.4	–	5	30	b
14	25	F	>32	HD	1.0 mg	3.6	–	4	44	a

Treatment order: a = nedocromil sodium/placebo; b = placebo/nedocromil sodium.
PD_{20}, Provocative dose causing a 20% fall in FEV_1; *HD*, house dust; *GP*, grass pollen; *NU*, Noon units.

isolated form of LAR, and 14% showed a dual form of LAR.[6] The exact pathophysiologic and immunologic mechanisms presumably underlying the LAR and its different subforms have not yet been satisfactorily clarified.[12] However, in contrast to the IAR, there is strong evidence for involvement of various inflammatory mechanisms in the LAR.[6, 7, 12-14, 16]

Nedocromil sodium (NS), the disodium salt of a novel pyranoquinoline dicarboxylic acid, demonstrated antiallergic and antiinflammatory effects both in animal and human studies, especially in the respiratory tract.[17-22]

The protective effects of NS on the LAR in patients with bronchial asthma have already been reported.[22, 23] However, only the LAR, being a part of dual response, has been studied, and NS was administered before the allergen challenge.[22]

Few data are available to illustrate the possible protective effects of NS on the isolated form of LAR. There is also a dearth of information concerning the effects of NS administered in a single dose after the allergen challenge, before the predicted onset of the LAR.

The purpose of this study was to investigate: (1) the effects of NS as a single prophylaxis if administered after the allergen challenge, just before the predicted onset of LAR; (2) the existence of some differences in the effects of NS on the isolated form of LAR and that being a part of the dual response; (3) the indication for the use of NS in cases of LAR.

METHODS

Patients

Fourteen patients who had allergic bronchial asthma that required regular therapy (13 inhaled bronchodilators, 5 inhaled corticosteroids and 6 inhaled cromolyn) and who developed an LAR to bronchial provocation test (BPT) with an inhalant allergen were randomly selected for this study. The patients ranged in age from 19 to 46 years (Table I). They all showed reversible bronchoconstriction alternating with symptom-free periods. Their pulmonary function did not demonstrate any restrictive changes, and they did not have chronic infections of the airways; none had received immunotherapy or orally administered corticosteroids in the past. None of the female participants were pregnant. All patients were previously examined by the following diagnostic procedure: (1) a general part including disease history, physical examination, basic laboratory tests, chest x-ray, electrocardiogram, pulmonary functions and blood gases; (2) an allergologic and immunologic part including skin tests, bronchial histamine threshold (provocative dose causing a 20% decrease in forced expiratory volume in 1 second [FEV_1]), blood leukocyte and eosinophil count, total serum IgE (paper

TABLE II. Bronchial and general complaints accompanying the nonpretreated and pretreated late asthmatic response (LAR)

	LAR: Nonpretreated	LAR: Pretreated with NS	LAR: Pretreated with Placebo
Dyspnea	+++	±	+++
Wheezing	+	–	+
Cough	+	–	+
Expectoration – thick sputum	+	–	++
Pressure on the chest	++	–	+
Chills	+	–	+
Tiredness	+	–	+
Headache	++	+	++
Weakness	+++	+	+++
General malaise	++	–	++
Increase in body temperature*	+	–	+

The appearance of the symptoms: –, No appearance; +, slight degree; ++, moderate degree; +++, distinct degree.
*Higher than 37° C = 98.6° F axillary.

radioimmunosorbent test), specific serum IgE (RAST), total serum IgG, IgM, and IgA (single radial immunodiffusion), and bronchial challenges with inhalant allergens, recorded up to 56 hours.

All bronchial challenges were performed during hospitalization of the patients under standard conditions.[4, 6] At the start of the study all patients were free of symptoms of asthma and infections and had a baseline FEV_1 of 80% or more of predicted value. Antiallergic therapy (cromolyn, inhaled corticosteroids, long-term–acting oral antihistamines) had been withdrawn for at least 6 weeks, short-term–acting antihistamines for at least 12 hours, and inhaled short-term–acting bronchodilators (β_2-agonists) for at least 8 hours before the study. In cases in which FEV_1 or both FEV_1 and vital capacity (VC) decreased by 50% or more during the BPTs, a single inhalation of 100 μg of salbutamol aerosol was given to prevent a further decrease in FEV_1. If more extensive treatment was necessary, the patient was excluded from the study. The study was approved by the Hospital Ethical Committee, and informed consent was obtained from each participant.

Allergens

The dialyzed and lyophilyzed extracts (Allergen Laboratory Diephuis, Groningen, The Netherlands) were diluted in phosphate-buffered saline (PBS). The basic (undiluted) concentrations of allergens used for bronchial challenges were as follows: house dust, 5 mg/ml; and grass pollen, 10,000 NU/ml. The allergen extracts used for skin tests were diluted 10 times.

BPTs

The VC and FEV_1 were recorded by means of a spirograph (model D-75, LODE, The Netherlands). FEV_1 was considered to be the basic parameter for assessment of the bronchial obstruction. Both PBS and allergen extracts were inhaled in the form of an aerosol administered by means of the Wiesbadener Doppel-Inhalator at an air flow of 10 L/min. The aerosol particles had a mass median diameter of 2.8 to 3.6 μm.

The bronchial challenge was performed according to the following schedule:[4, 6, 9] (1) recording of the base values ("initial values") at 0, 5, and 10 minutes; (2) inhalation of PBS for 10 minutes followed by recording of the "PBS values" (control) at 0, 5, and 10 minutes; (3) inhalation of the allergen aerosol for 10 minutes (2 × 5 minutes) followed by recording of the VC and FEV_1 values at 0, 5, 10, 20, 30, 45, 60, 90, and 120 minutes and then every hour up to the twelfth hour and every second hour between the twenty-fourth and thirty-eighth hours and between the forty-seventh and fifty-sixth hours after the challenge. The BPTs were evaluated as follows: (1) the decrease of FEV_1 or both FEV_1 and VC of less than 10% with respect to the control values as negative, from 10% to 20% as doubtful, and of 20% (provocative dose causing a 20% fall in FEV_1) or more as positive; (2) the decrease in FEV_1 within 60 minutes after allergen challenge, recorded at a minimum of three consecutive time intervals, was considered to be a positive IAR; the decrease in FEV_1 within a period of 4 to 24 hours after allergen challenge, recorded at a minimum of three consecutive time intervals was considered to be a positive LAR. All bronchial challenges were performed with allergen extracts in a standard dilution sequence (pollen allergens 1:100, 1:10, and undiluted; and perennial allergens 1:10, 1:5, 1:2, and undiluted), with respect to the basic concentrations described above. A time interval of 6 days was always inserted betw

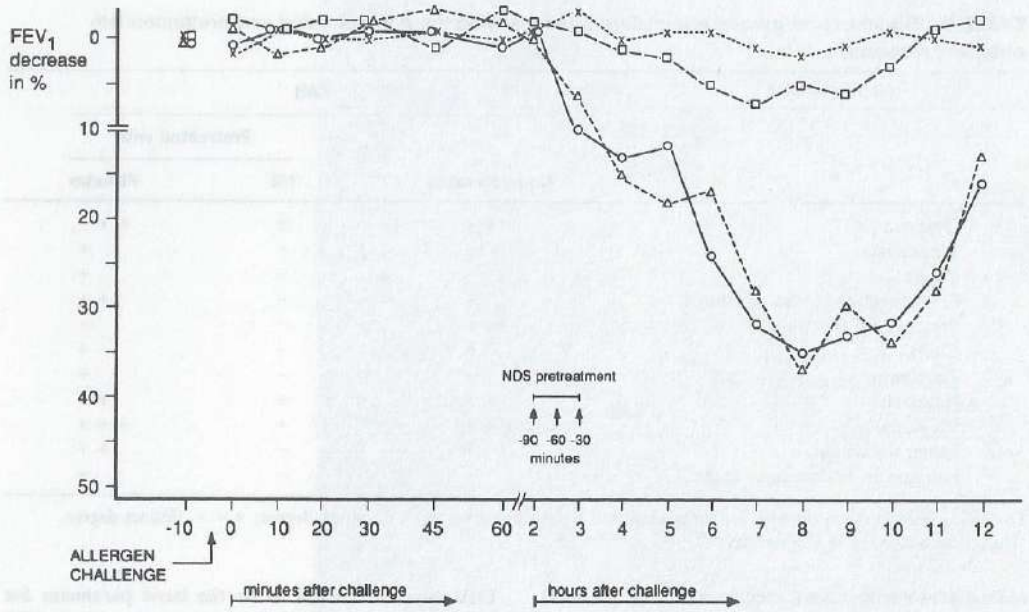

FIG. 1. Isolated late asthmatic response (ILAR) to allergen challenge and the protective effects of NS in five patients. The mean percentage change in FEV_1 values calculated from the nonpretreated ILARs (○), the ILARs pretreated with NS (□) and with placebo (△), and from PBS control challenges (x).

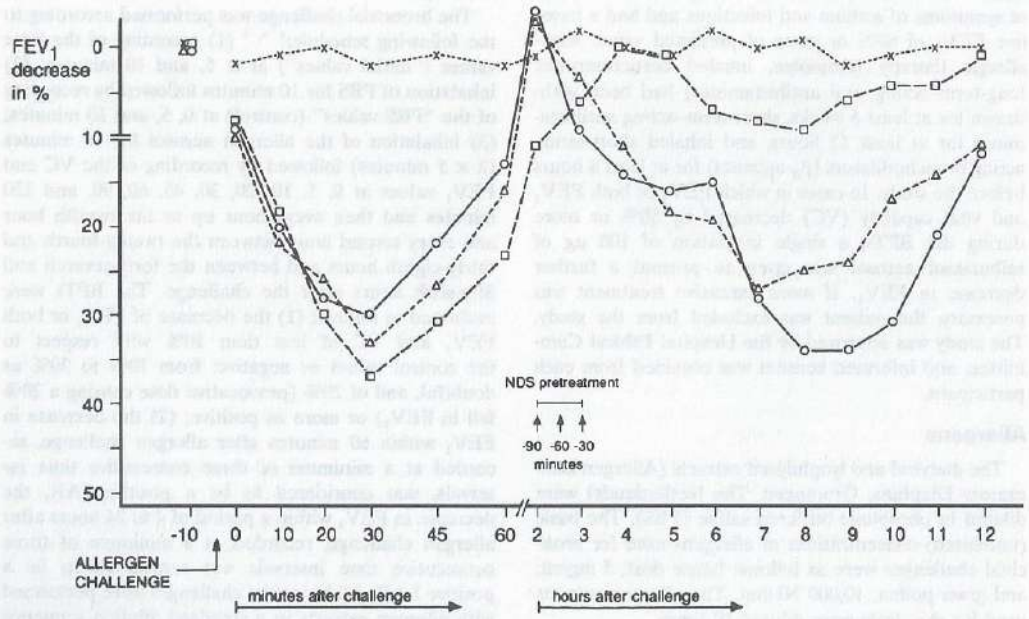

FIG. 2. Dual late asthmatic response (DLAR) to allergen challenge (DLAR, immediate + late) and the protective effects of NS in nine patients. The mean percentage change in FEV_1 values calculated from the nonpretreated DLARs (○), the DLARs pretreated with NS (□) and with placebo (△), and from PBS control challenges (x).

TABLE III. Mean maximum percentage falls from prechallenge baseline FEV_1 during LAR due to allergen inhalation

Type of response	Study treatment	Baseline FEV_1 (L)	Max % fall in FEV_1
Dual LAR (n = 9)	No treatment	3.44 ± 1.12	37.1 ± 5.15
	Placebo	3.43 ± 1.10	28.5 ± 19.05
	NS	3.42 ± 1.08	11.4 ± 15.00
Isolated LAR (n = 5)	No treatment	3.22 ± 0.85	39.7 ± 7.81
	Placebo	3.20 ± 0.86	42.1 ± 16.48
	NS	3.20 ± 0.88	14.3 ± 11.29
All LARs (n = 14)	No treatment	3.36 ± 1.01	38.0 ± 6.07
	Placebo	3.35 ± 0.99	33.4 ± 18.77
	NS	3.34 ± 0.98	12.4 ± 13.40
Treatment difference (all):			
Standard deviation (df)		0.067	14.93
p Value			
Treatment order		0.78	0.18
Treatment effect		0.78	0.003*
(Treatment × responder type)		(0.84)	(0.40)

*$p < 0.01$.

the consecutive bronchial challenges. The concentration of allergen producing a significant asthmatic response (FEV_1 fall ≥20%) of any type was considered to be a final dose and was also used for the protection tests. All bronchial challenges, both nonpretreated and pretreated, were supplemented by recording of various bronchial and general complaints and symptoms (Table II).

Control test with PBS

A control test with PBS was performed in all patients 3 days before the bronchial challenge with allergen, and the spirometric values were recorded for up to 56 hours. Results of the control test were considered to be negative when the changes in FEV_1 values varied within 8% with respect to baseline values.

Drug administration and schedule of protection tests

Nedocromil sodium (Tilade) (1 puff = 2 mg NS) and placebo were used in the form of a metered pressurized aerosol. Each treatment consisted of 2 puffs administered at 3 time intervals (90, 60, and 30 minutes) before the predicted onset of the LAR.

The protection tests with NS and with placebo were performed with a time interval of 5 days, in the same way and according to the same schedule as that used for the nonpretreated allergen challenge. The design of the study was randomized, double-blind, crossover, placebo-matched.

Statistical analysis

The FEV_1 before allergen challenge (baseline value) and the percentage change in FEV_1 after challenge were compared by analysis of variance with patient, treatment, and study day as factors. The maximum percentage fall in FEV_1 during the LAR was taken as the primary variable, although the fall in FEV_1 at each time point was also analyzed. The nonparametric method of Koch[24] was used to confirm the primary analysis. The possible difference in treatment effects on the LAR between isolated and dual responders was examined by means of analysis of variance with treatment and responder type as factors. A two-tailed significance level of 5% was used throughout. A p value of less than 0.05 was considered to be statistically significant, a p value of less than 0.01 to be distinctly significant, and a p value of less than 0.001 to be highly significant.

RESULTS

The 14 patients developed 14 LARs to allergen challenge. Each LAR began between 4 and 6 hours, reached its maximum between 8 and 10 hours, and resolved within 12 to 24 hours after the allergen challenge. Five patients demonstrated an isolated late response (Fig. 1), and nine showed a dual late response, which is a combination of an immediate and a late response (Fig. 2). All LARs were highly significantly positive as compared with PBS control challenge ($p < 0.001$). During the nonpretreated LAR, the mean maximum percentage fall in FEV_1 was 38.0% (range, 25.6% to 50.0%) from a mean baseline value of 3.36 L (range, 1.60 to 5.30 L) (Table III). There were no withdrawals from the study. Some patients needed rescue therapy, a single inhalation of 100 μg salbutamol, when their FEV_1 fell below 50% during the nonpretreated LAR or during admin-

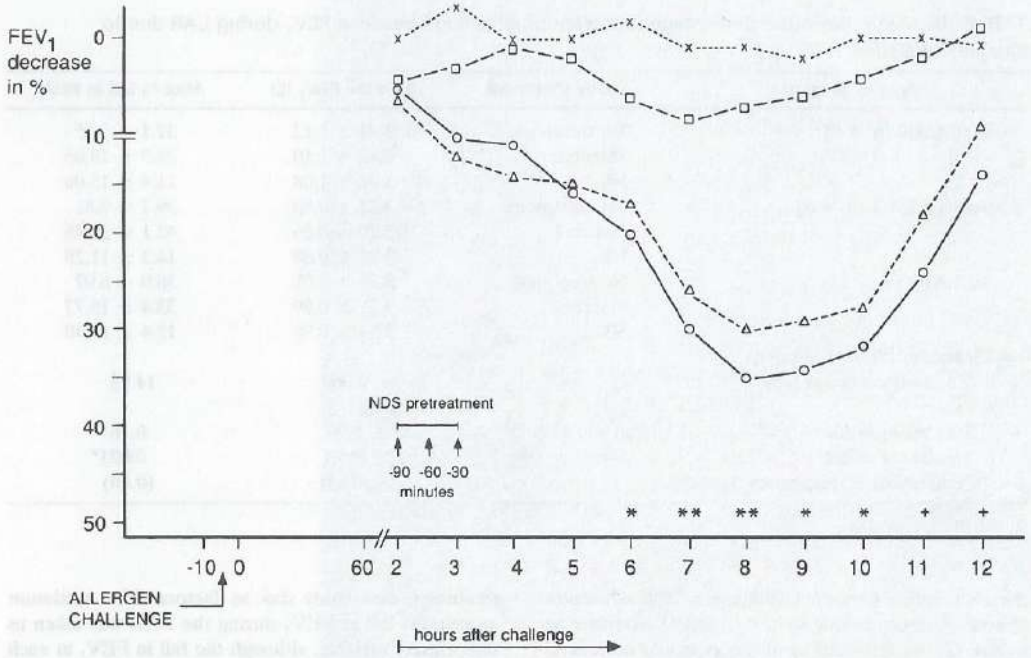

FIG. 3. LAR to allergen challenge and the overall protective effects of NS in all 14 patients. The mean percentage change in FEV_1 values calculated from the nonpretreated LARs (○), the LARs pretreated with NS (□) and with placebo (△), and from PBS control challenges (x). Significance of the protective effects of NS on the LAR as compared with those of placebo: $*p < 0.05$; $**p < 0.01$; $^{+}0.05 < p < 0.1$.

istration of placebo. The values obtained after salbutamol administration were excluded from the analysis. None of the patients required rescue therapy after administration of NS. All PBS control challenges were significantly negative ($p > 0.2$) (Figs. 1 and 2).

NS prevented 12 cases of LAR (86%) significantly ($p < 0.01$) and was ineffective in two LAR cases (14%) ($p \geq 0.05$). The mean maximum percentage fall in FEV_1 during the LAR pretreated with NS was significantly reduced ($p = 0.003$) and showed an improvement of 21.0% as compared with placebo (95% confidence interval = 8.6% to 33.2%). Only three of the 14 patients demonstrated a fall in FEV_1 greater than 20% after NS pretreatment. This protection lasted over the entire time course of the LAR, showing significance ($p < 0.05$) whether viewed from 6 to 10 hours after allergen challenge or from 2.5 to 6.5 hours after the first treatment dose, allowing for individual variation in the time of onset.

Placebo demonstrated slight protective effects in two cases of LAR (14%) ($p \leq 0.05$) and was fully ineffective in 12 cases of LAR (86%) ($p > 0.05$). Eleven of the 14 patients showed a maximum fall in FEV_1 of more than 20% after placebo treatment.

The overall protective effects of NS on the LAR as compared with those of placebo were found to be highly statistically significant ($0.002 < p < 0.01$) (Fig. 3). The treatment differences were slightly greater in patients with isolated LAR. However, analysis of variance indicated no significant differences between the two LAR types in comparing test treatments (Table III).

In all patients studied, the bronchial, as well as general, complaints accompanying the nonpretreated LAR were distinctly prevented by NS but not by placebo (Table II).

DISCUSSION

The LAR may play an important role in bronchial asthma and may be responsible for failure of the usual treatment in these patients.[6, 7, 12]

The views on the pathophysiologic and immunologic mechanisms leading to the LAR, however, vary. Some investigators have suggested involvement of the late hypersensitivity and IgG antibodies in the LAR.[1, 6, 7, 12, 25, 26] Other investigators have presumed that immediate hypersensitivity, mediated by IgE antibodies, on involvement of mast cells (and/or basophils), may play the main role in the LAR.[13, 14] Others have assumed that various combinations and modifications of the above-mentioned mechanisms may be involved.[12, 27-29]

In our opinion, the LAR should be regarded as a clinical phenomenon, defined by the appearance of bronchoconstriction, accompanied by other symptoms and changes, within 4 to 12 hours after allergen exposure.[6, 7, 10-12] Although the pathogenetic and immunologic mechanisms leading to the LAR could be different, the late type of hypersensitivity should be regarded as one of the possibly involved mechanisms but certainly not the only one.[6, 7, 11, 12] Nevertheless, evidence has been provided for involvement of various inflammatory mechanisms including various cell types, chemical mediators, and chemotactic factors in clinical LAR.* This very complex topic is extensively discussed elsewhere.[6, 7, 12, 34, 35]

Some investigators have presumed the role of IAR in the development of the LAR and postulated that the LAR is preceded by an IAR.[3, 14] Our results, which demonstrate unequivocally the existence of an isolated form of LAR, do not agree with this presumption.[5-7, 10-12, 17]

Because the LAR has been recognized as an important clinical phenomenon, its pharmacologic modulation has also become a current topic.[6, 7, 10-12, 31] The effects of various drugs have been studied with varying results.† The antihistamines, anticholinergics, and theophylline derivatives did not demonstrate any significant effects; β-agonists were slightly effective; while disodium cromoglycate (DSCG), corticosteroids, and NS showed significant protective effects on the LAR.‡

The comparative studies of the effects of DSCG and topical corticosteroids (beclomethasone dipropionate [BDP] and budesonide) on the LAR are not numerous.[1, 10, 11, 31] In our previous studies, both DSCG and BDP/budesonide significantly prevented the LAR ($p < 0.01$), with small differences.[10, 11] In patients who had the dual response, however, the IAR was prevented significantly by DSCG ($p < 0.01$), although not by BDP ($p > 0.05$).[9-11] Similar results were also reported by Pepys and Hutchcroft[1] and Cockcroft and Murdock.[31]

In our preliminary studies the patients with dual asthmatic response were pretreated with DSCG or BDP/budesonide for 48 hours before allergen challenge. DSCG, the administration of which was finished 10 minutes before the allergen challenge, only prevented significantly the IAR ($p < 0.05$), but not the LAR ($p > 0.05$). Although if administration was continued up to 12 hours after the allergen challenge, it prevented significantly both the IAR ($p < 0.02$) and the LAR ($p < 0.05$).[37, 41] In contrast, BDP/budesonide demonstrated significant protective effects on the LAR ($p < 0.01$) without any differences between the two treatment schedules, but they did not affect the IAR at all ($p > 0.05$).[41, 42]

We also compared the effects of a single dose of DSCG or budesonide on the LAR, administered either before or after allergen challenge (before the onset of LAR), in patients with dual asthmatic response. (Some of these data have not yet been published). DSCG administered 30 minutes before allergen challenge prevented the IAR significantly ($p < 0.05$) but did not affect the LAR ($p > 0.05$). The DSCG given 1, 2, 3, or 4 hours after the challenge did not prevent the LAR ($p > 0.05$). The budesonide administered 30 minutes before the allergen challenge prevented the LAR significantly ($p < 0.01$) but did not affect the IAR ($p > 0.05$). Budesonide administered 1, 2, or 3 hours after the allergen challenge prevented the LAR significantly ($p < 0.05$).[41, 42]

The differences in the pharmacologic modulation of the early (IAR), as well as the late (LAR) phase, of asthmatic response by DSCG and BDP/budesonide, administered at various time intervals with respect to the allergen challenge, would suggest involvement of different mechanisms in the early and in the late phase.[4-7, 12] The allergen exposure may activate the mechanisms that cause the IAR, or those that lead to the LAR, or both the groups simultaneously, resulting in parallel appearance of IAR and LAR. With respect to these preliminary results, the so-called "dual asthmatic response" may perhaps be considered as an appearance of two independent responses.

The differences in the protective effects of DSCG and topical corticosteroids (BDP/budesonide) on the particular types of asthmatic response may be explained by their different pharmacologic effects.[12, 43-45] The effects of DSCG on

*References 6, 7, 10-13, 16, 26, 27, 30-36.
†References 10, 11, 15, 17, 21-23, 36-42.
‡References 10, 11, 15, 17, 21-23, 31, 36, 37, 39-42.

the IAR, as well as on the LAR, could be explained by the ability of DSCG to protect mast cells and basophils from degranulation and subsequent release of mediators.[43] The glucocorticosteroids possess a manifold antiinflammatory activity including effects on the regulation of cyclic adenosine monophosphate, cyclic guanosine monophosphate, prostaglandins, various chemotactic factors, complement parts, and vasoactive kinins, as well as effects on various cell types (e.g., eosinophils, neutrophils and platelets).[44, 45]

NS has a number of unique pharmacologic properties, including antiinflammatory effects.[18-20, 22, 46] In animal in vitro studies, NS protected mast cells from degranulation[20] and inhibited the secretory response of neutrophils.[19] In animal in vivo studies, NS inhibited the release of histamine, leukotriene C_4 and prostaglandin D_2 from mast cells in bronchoalveolar lavage and was more potent in preventing antigen-induced bronchoconstriction in primates than DSCG.[18] In guinea pigs NS that was inhaled 15 minutes before the antigen challenge inhibited both IAR and LAR, whereas salbutamol inhibited the IAR only. When inhaled 6 hours after the challenge, NS prevented LAR significantly, but salbutamol prevented LAR only partially. NS also reduced accumulation of eosinophils, but not of neutrophils, in bronchoalveolar lavage from these animals.[20]

In the human in vitro studies NS inhibited histamine release from human "chopped lung" and inhibited activation of eosinophils and neutrophils.[19] NS also prevented the chemotactic factor–induced activation of eosinophils and neutrophils (formyl-methionyl-leucyl-phenylalanine) by means of inhibition of expression of their C3b and IgG (Fc) membrane receptors,[46] the eosinophil activation induced by platelet activating factor, and the increase in IgG-dependent release of leukotriene C_4 from human eosinophils.[46] NS, like DSCG, was able to inhibit the sepharose-C3b-induced protein release from eosinophil granules.[47] NS also prevented the IgE-dependent activation of human alveolar macrophages, blood monocytes, and platelets and the release of their mediators.[48] NS showed a higher inhibitory activity on the human mast cells from bronchoalveolar lavage and from dispersed lung fragments than DSCG.[49, 50]

In human in vivo studies, when administered 30 minutes before the challenge, NS inhibited the bronchoconstriction caused by inhaled sulfur dioxide, fog,[51, 52] and adenosine, but not by methacholine.[53] In bronchial challenge studies with antigen, NS demonstrated significant protection on both IAR and LAR, when administered in a single dose of 4 mg before the challenge.[21] NS inhibited the IAR to allergen challenge in patients with asthma to a higher degree than DSCG, when administered in a single dose of 2 or 4 mg, 30 minutes, 2 hours, or 3 hours before the challenge.[54, 55] The safety of NS and a high measure of tolerability have also been confirmed.[38, 51] In long-term clinical trials, NS demonstrated a significant reduction in the requirement for steroids in patients with bronchial asthma.[38]

It can be concluded that NS shares some pharmacologic effects with DSCG, especially those concerning activities expressed on mast cells and eosinophils. NS also demonstrates antiinflammatory effects, which are similar to those of the glucocorticosteroids. This unique position of NS should be stressed, following the recognition of the key role of inflammatory events in the pathogenesis of asthma and the clinical importance of the LAR.[5, 7, 10-13, 16, 33]

According to our preliminary data and other investigators' findings, DSCG administered before the predicted onset of LAR was not able to inhibit this response.[40, 43] Results of this study show that NS, given 30 to 90 minutes before the expected onset, inhibited the LAR significantly. These data may indicate the differences in the effects and properties between DSCG and NS, and they also implicate the involvement of different mechanisms in IAR and those in LAR. From a practical point of view, these results demonstrate an effective prophylaxis of LAR by a single dose of NS given even after allergen exposure. NS might therefore be considered a very promising drug for the control and preventive treatment of allergic bronchial asthma.

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Bronchial response to the food ingestion challenge

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A total of 143 food ingestion challenges were carried out in 107 patients suffering from bronchial asthma of a perennial type. In the group of 21 patients with "positive or highly suggestive food history" related to their bronchial complaints, 21 food challenges were performed. In the group of 86 patients with "unknown food history," 122 ingestion challenges with foods causing a positive skin response were performed.

Fifteen of the 21 patients with "positive food history" developed 15 (71%) bronchus-obstructive responses to foods ingested, while 45 of the 86 patients with "unknown food history" developed 68 (56%) bronchial responses to the food ingestion challenge. Twenty-three isolated immediate (within 2 hours), 11 isolated late (4 to 24 hours), 34 dual late (a combination of an immediate and a late), 6 isolated delayed (28 to 56 hours), and 9 dual delayed (a combination of an immediate and delayed) bronchus-obstructive responses were recorded. No significant correlation of the individual types of bronchial response to food ingested with other in vivo *and* in vitro *diagnostic parameters were found.*

Although the exact pathogenetic and immunologic mechanisms underlying the particular types of bronchial response to foods are not yet fully clarified, the involvement of different types of hypersensitivity cannot be excluded. It could be concluded that the involvement of foods in bronchial asthma is more frequent than is usually expected. The diagnostic value of the food ingestion challenge seems to be superior to that of other diagnostic parameters. The definite confirmation of the role of a certain food, in patients with bronchial asthma, should therefore be provided by the food ingestion challenge demonstrating one of the clinical types of bronchus-obstructive response.

ABBREVIATIONS

BA = bronchial asthma
BPT = bronchial provocation tests
BR = bronchus-obstructive response
DDR = dual delayed bronchial response
DLR = dual late bronchial response
DR = delayed bronchial response
DSR = delayed skin response
FEV_1 = forced expiratory volume in one second
IC = intracutaneous tests
IDR = isolated delayed bronchial response
IIR = isolated immediate bronchial response
ILR = isolated late bronchial response
IR = immediate bronchial response
ISR = immediate skin response
LR = late bronchial response
LSR = late skin response
NR = negative bronchial response
VC = vital capacity

Some of the results reported in this paper, were presented as preliminary communications at the Fifth Charles Blackley Symposium in Nottingham (UK), July 8–13th, 1984[7] and at the XIIth International Congress of Allergology and Clinical Immunology, Washington, October 20–25, 1985.[8]

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INTRODUCTION

Bronchial asthma has classically been attributed to the immediate hypersensitivity mechanism (Type I allergy), caused predominantly by "inhalation" allergens.[1] Later, evidence has been generated that other types of immunologic mechanisms might also be involved in bronchial asthma.[1–4] This evidence was supported by demonstration of the so-called "non-immediate asthmatic responses" to allergen challenge, the late and delayed response.[1–9]

The role of food allergy in patients with bronchial complaints is still underestimated by clinicians because (1) of the dearth of information in this area. (2) The involvement of foods in patients with allergic disorders is very complex and has various forms, of which the hypersensitivity mechanism due to the foods is only one. (3) The diagnostic procedure and confirmation of the involvement of adverse reactions to foods in the patient's complaints is a difficult problem.

We have presented some of our observations concerning involvement of foods in patients with bronchial asthma.[8,9]

The purpose of the present study was to investigate the possible role of foods in patients with BA, the frequency of such a role as well as the bronchial response to the food ingestion challenge, its clinical features, types and correlation with other *in vivo* and *in vitro* diagnostic parameters. The value of food ingestion challenge and the other diagnostic parameters for the diagnostic assessment of the role of foods in patients with bronchial asthma is also evaluated.

MATERIALS AND METHODS

Patients

One hundred and seven patients between 20 to 50 years of age were studied. These patients, suffering from BA with an allergic component of a perennial type for longer than 3 years, were characterized by reversible bronchoconstriction alternating with symptom-free periods.

In these patients, a routine diagnostic procedure including 241 BPT with "inhalation" allergens was carried out. The food-focused history, IC with basic and, if necessary, with supplementary series of food extracts, ingestion challenge with selected foods, in combination with spirography, were also performed.

Their pulmonary functions did not demonstrate any restrictive changes. They did not suffer from any chronic or acute infections of the airways and they had not had immunotherapy or used oral corticosteroids. None of the patients studied had suffered an anaphylactic or other serious life-threatening reaction to foods in the past. The patients were investigated during a period without manifest bronchial complaints. All BPTs were performed during hospitalization under standard conditions. Use of aerosolized corticosteroids and disodium cromoglycate was stopped at least 4 weeks before the study. No other anti-allergic therapy was given during 48 hours, and no bronchodilators during 24 hours prior to this study. The foods selected for the ingestion challenge, as well as the related foods were always avoided by the patients for at least seven days before the ingestion challenge.

The patients were divided into two groups: (a) 21 patients with "positive or highly suggestive food history" concerning one or more foods related to their bronchial complaints, of which in four patients the history was suggestive only to foods, while in the other 17 patients the history was positive or highly suggestive, not only to foods but also to various "inhalation allergens."[6] Group two consisted of 86 patients with an "unknown food history" with respect to their bronchial complaints. In these patients the foods for ingestion challenge were chosen with respect to the positive skin tests, RAST, or to the frequency of their consumption.

Allergens for Skin Tests

The dialyzed and lyophilized food extracts were diluted in Coca's solution (dry weight of food extract in mg per 1 mL of Coca's solution) (Diephuis Laboratory, Groningen, The Netherlands) and used in the following concentrations: cheese (eg, young or old Gouda, Frisian cheese, Leerdammer cheese, etc), egg white and yolk—each in 1 mg/mL; nuts, chocolate, cocoa, dutch sweets, wines, beers—each in 0.5 mg/mL; meats (eg, pork, beef, lamb, chicken, etc), spices, beans (eg, green beans, brown beans, broad beans, etc)—each in 0.2 mg/mL; all other foods such as individual vegetables, fruits, fish, crustacea, honey, etc—each in 0.1 mg/mL.

Skin Tests

The scratch tests were performed first and evaluated after 20 minutes. If these were negative, the IC were carried out (injection of 0.05 mL extract intracutaneously) and evaluated after 20 minutes, 4, 8, 12, 24, 36, 48, 60, 72, 84, and 96 hours and, if necessary, for longer, up to complete disappearance of the reaction.

The skin response appearing after the scratch as well as the IC were evaluated according to the following criteria: − = normal skin appearance; ± = wheal up to 5.0 mm in diameter; + = wheal up to 7.5 mm; +± = wheal up to 10 mm; ++ = wheal up to 12.5 mm; ++± = wheal up to 15.0 mm; +++ = wheal larger than 15.0 mm, sometimes with pseudopodia; and ++++ = extremely large wheal of more than 25 mm. The responses − and ± were interpreted as negative, and those of + or more as positive.

The positive skin response, appearing within 20 minutes, was considered to be an ISR, response appearing between 6 to 24 hours to be a LSR, and response appearing after 48 hours to be a DSR.

Spirography

The VC and FEV_1 were recorded by means of a spirograph (Lode Co, Groningen, The Netherlands, Model D-75). The Coca's solution was inhaled in the form of an aerosol, administered by means of the Wiesbadener-Doppel-Inhalator at an air flow of 10 L/min. The FEV_1 was considered to be the basic parameter for the assessment of the bronchial obstruction.

Food Ingestion Challenge

The open food ingestion challenge was performed by the following schedule (Fig 1): (1) recording base values ("initial values") at zero, five, and ten minutes; (2) inhalation of Coca's solution for ten minutes and then recording of the Coca's solution values (control) at zero, five, and ten minutes. If no significant changes of the mean "Coca's solution values" with respect to the mean "initial values" were measured (<5 ± 2%), the investigation was continued. (3) The appropriate food was ingested within five minutes and then a 1-hour waiting interval always followed in order to allow the food to be digested. During the 1-hour waiting interval, the VC and FEV_1 were measured four times to exclude an unexpected or too early bronchial response. (4) "Food challenge values." After the 1-hour waiting interval, the actual post-challenge spirographic parameters were recorded at 0, 5, 10, 20, 30, 45, 60, 90, and 120 minutes and then every hour up to the 12th hour and every second hour during the 24 to 36th and the 47 to 56th hour intervals, after the end of the challenge. The spirographic parameters were evaluated as follows: (a) decrease of FEV_1 or both FEV_1 and VC of less than 10% with respect to the control values were considered negative, between 10% to 20% as doubtful and of 20% (PD_{20}) or more as positive. (b) The decrease of FEV_1 or both FEV_1 and VC, recorded at least at three consecutive time intervals, was considered to be a positive bronchial response. (c) A bronchial response appearing within two hours after the food ingestion, followed by the 1-hour waiting interval, was considered to be an IR; that occurring between 4 to 24 hours to be a LR; and that appearing after more than 24 hours a DR.

In the event of the patient developing a decrease in FEV_1 of 40% or more, a single inhalation of salbu-

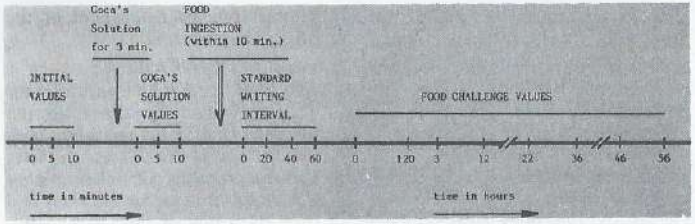

Figure 1 Schedule of food ingestion challenge combined with spirography.

Table 1. Review of Individual Foods Used for the Ingestion Challenge (n = 143).

I. Basic foods of "solid consistency"—ingestion of 100 g: cheese 18 (12), peanuts 15 (9), chocolate 15 (11), shrimps 5 (5), Dutch sweets 5 (3), beans 4 (1), honey 3 (2), pork 3 (2), egg 2 (2), cucumber 2 (1), hazelnuts 2 (1), almonds 2 (1), tomato 1 (1), pear 1 (1), spinach 1 (1), lettuce 1 (1), strawberries 2 (1), blueberries 3 (0), raspberries 1 (0), banana 2 (0), herring 2 (1), mushrooms 1 (1), cabbage 1 (1).
II. Basic foods of "fluid consistency"—ingestion of 200 mL each of milk 18 (9), coca-cola 4 (1).
III. Foods with a well-defined flavour, taste or consistency, only added to the basic foods in very small quantities—ingestion of 5 g on brown bread with butter or in 100 mL of water: spices = nutmeg 1 (0), ginger 1 (0), vanilla 1 (1); vegetables = onion 3 (3), garlic 2 (2); and vana: butter 1 (0).
IV. Alcoholic beverages: (a) soft—ingestion of 100 mL each of beer 7 (3), wine 10 (4), sherry 2 (1); (b) liquors—ingestion of 5 mL: cognac 1 (1).

* The numbers in parentheses indicate positive bronchial response of some type to the particular food (n = 83).

tamol aerosol (100 μg) was given in order to stop a further drop. If the bronchial response required more extensive treatment, the patient was excluded from this study.

Foods Used for Ingestion Challenge

The foods used for ingestion challenge were identical to those usually used by the patients in order to obtain the highest degree of simulation and reproducibility. The quantities of the individual foods for the ingestion challenge, corresponding roughly to the real consumption by the patients and the frequency of the ingestion challenge with the particular foods, are presented in Table 1.

The Control Test

The control ingestion challenge with either cooked rice, glucose solution or cooked potatoes, the choice of which depended on the patient's problem, was performed in the same way and according to the same schedule as the experimental food ingestion challenge in all patients with a positive bronchial response to food (n = 83).

Double-blind Food Challenge

In 22 patients, a double-blind ingestion challenge with the same foods was also performed six days later in order to compare both of the techniques. The technique used was identical to that described by other investigators.[13,17,25,30] The schedule of the double-blind challenge was, however, identical to that used by us for the open challenge.

Reproducibility of the Food Ingestion Challenge

In 14 patients, the open ingestion challenge with the same foods was repeated 2 weeks later to evaluate the reproducibility of this test.

Control group of patients

In a control group of 12 patients suffering from atopic eczema, who had never had any nasal or bronchial complaints, 12 ingestion challenges with selected foods, corresponding with the positive skin tests, were carried out. These patients were considered by us to be control patients with respect to the method of food ingestion challenge as well as to the technique of spirography used.

RAST

Specific IgE antibodies to the foods in the serum were determined quantitatively in the Central Laboratory of the Dutch Red Cross Blood Transfusion Service (CBL) in Amsterdam by Dr. R. Aalberse and Dr. C. Aaij.

Single Radial Immuno-diffusion (Mancini technique)

The determination of the IgG, IgM, and IgA antibodies in the serum was performed by means of the standard plates, a product of Kallestad Co, USA and provided by "De Beer Immunological Supplies," The Netherlands.

Blood Leukocyte and Eosinophil Count

Blood leukocytes and eosinophils were counted four times on the day before the ingestion challenge, five times on the day of the challenge (60 minutes before and 1, 4, 8, and 11 hours after the challenge), and four times on each of the two days following the challenge day. The counting was performed using flow cytometry (4-chloro-1-naphthol and peroxidase staining).

Bronchial and Other Complaints

These were recorded during the whole test up to 56 hours after the ingestion challenge at least and, if necessary, up to complete disappearance.

Statistical Analysis

The Student's *t* test was used for the statistical evaluation of the results. A $P < .05$ was considered to be statistically significant.

RESULTS

A total of 143 food ingestion challenges were performed in 107 patients. In the group of 21 patients with "positive or highly suggestive food history" 21 food challenges, and in the group of 86 patients with "unknown food history" 122 ingestion challenges were performed.

Three basic types of BR to food ingested were recorded: (1) the IR = onset within 10 to 20 minutes, maximum within 30 to 45, and resolving within 120 minutes; (2) the LR = onset within 4 to 6 hours, maximum within 5 to 12, and resolving within 24 hours; (3) the DR = onset within 28 to 32 hours, maximum within 32 to 36, resolving within 48 to 56 hours after the food ingestion challenge, followed by a 1-hour waiting interval. Beside the three basic types of bronchial response recorded in an isolated form, two other modifications were also observed, the so-called "dual late bronchial response," being a combination of an immediate and a late response, and the "dual delayed bronchial response," being a combination of an immediate and a delayed bronchial response (Fig 2).

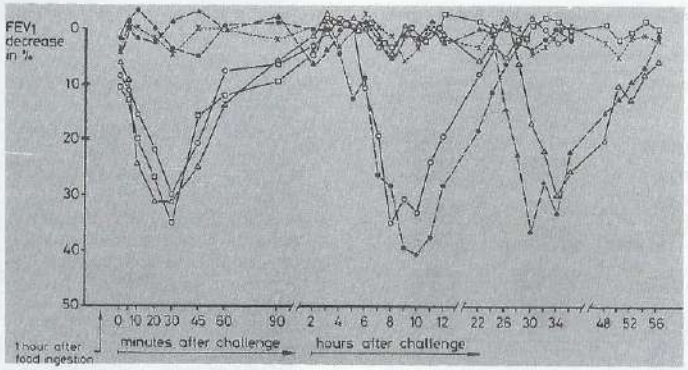

Figure 2 The mean percentage changes in the FEV_1 calculated from all patients with the same type of bronchial response to the food ingestion challenge. □——□ = IIR = isolated immediate bronchial response (n = 23); ○——○ = DLR = dual late bronchial response (n = 34); ●- - - -● = ILR = isolated late bronchial response (n = 11); △——△ = DDR = dual delayed bronchial response (n = 9); ▲- - - -▲ = IDR = isolated delayed bronchial response (n = 6); and ×- - - -× = control ingestion challenge (n = 83).

Table 2. Review of Bronchial Response Types Recorded in the "Positive Food History" Group and in the "Unknown Food History" Group

	Food Ingestion Challenges	Bronchial Response*					
		IIR	ILR	DLR	IDR	DDR	NNR
"Positive or highly suggestive food history" group (patients n = 21)	21	4	3	6	1	1	6
"Unknown food history" group (patients n = 86)	122	19	8	28	5	8	54

* For definitions of IIR, ILR, DLR, etc, see "Abbreviations."

The remaining six patients did not develop any significantly positive bronchial response during six food ingestion challenges, which are therefore called six negative bronchial responses (NR) (29%) (Table 2).

The "Positive or Highly Suggestive Food History" Group

Of the 21 patients belonging to this group, in whom 21 food challenges were performed, 15 patients developed 15 (71%) bronchial responses to foods as follows: four IIR (19%), six DLR (28%), three ILR (14%), one DDR (5%), and one IDR (5%).

The "Unknown Food History" Group

Of the 86 patients belonging to this group, in whom 122 food ingestion challenges were performed, 45 patients developed 68 (56%) bronchial responses to foods as follows: 19 IIR (16%), 28 DLR (22%), 8 ILR (7%), eight DDR (7%), and 5 IDR (4%). The remaining 41 patients did not develop any significantly positive bronchial response to foods during the 54 food ingestion challenges. They are therefore called 54 NR (44%) (Table 2).

Statistical Evaluation

The individual types of bronchial response were statistically significantly positive when compared with the control ingestion challenge: IR ($P < .01$), LR ($P < .05$), DR ($P < .05$).

The Control Test

The 60 control ingestion challenges performed in all patients demonstrating a positive bronchial response of any type were negative. The changes in FEV_1, measured during the control challenges, were statistically non-significant ($P = .1$).

Double-blind Food Challenge

In the 22 patients developing four IIR, five DLR, three ILR, four DDR, three IDR, and three NR due to the food ingestion challenge by open schedule, the food ingestion challenge by a double-blind schedule was carried out six days later. One patient, developing the IIR by the open challenge, demonstrated DLR by double-blind challenge. One patient showing the DLR to the open challenge developed an IIR by the double-blind schedule. The remaining 20 patients developed similar bronchial response after both the challenge techniques. The bronchial responses recorded after the open challenge and those after the double-blind challenges did not demonstrate any statistically significant differences ($P > .05$).

Reproducibility of the Food Ingestion Challenge

None of the 14 patients with repeated food ingestion challenge (five IIR, five DLR, two ILR, one DDR, one NR) demonstrated any statistically significant differences between the first and the second challenge ($.05 < P < .1$).

Control Group of Patients

None of the 12 control patients developed any significant bronchial or nasal response to the food ingestion challenge ($P > .1$). Five of these patients, however, demonstrated an acute activation of eczematic eruptions; one, a whole-body itching; and one, a severe headache.

Table 3. Review of Other Diagnostic Parameters and Their Association with Individual Types of Bronchial Response to Food Ingestion Challenge

Response Related Parameters	Bronchus-obstructive Response			
	Immediate (IR) n = 66	Late (LR) n = 45	Delayed (DR) n = 15	Negative (NR) n = 60
Positive skin response				
immediate	37 (56%)			11 (18%)
late		22 (49%)		7 (12%)
delayed			6 (40%)	1 (2%)
Positive specific IgE in the serum (RAST)	11 (17%)	4 (9%)	0 (0%)	4 (6%)
Increased IgG in the serum	10 (15%)	17 (38%)	0 (0%)	2 (3%)
Increased IgM in the serum	0 (0%)	8 (18%)	0 (0%)	0 (0%)
Increased IgA in the serum	1 (2%)	0 (0%)	0 (0%)	1 (2%)
Increase in blood eosinophils/leukocytes	12/2 (35%/3%)	22/21 (49%/47%)	0/3 (0%/20%)	2/2 (3%/3%)
Bronchial complaints	66 (100%)	45 (100%)	15 (100%)	5 (8%)
Dyspnea	63 (95%)	44 (98%)	15 (100%)	0 (0%)
Wheezing	64 (96%)	45 (100%)	3 (20%)	0 (0%)
Cough (non-productive)	3 (5%)	23 (51%)	0 (0%)	3 (5%)
Pressure on the chest	2 (3)	18 (40%)	1 (7%)	2 (3%)

Table 4. Survey of the Skin Response Degrees in Individual Types of Bronchial Response to Food Ingestion Challenge

Intracutaneous Tests	Bronchial Response			
	Immediate (IR) n = 66	Late (LR) n = 45	Delayed (DR) n = 15	Negative (NR) n = 60
Negative				
−	21	13	9	29
±	8	10	0	12
Positive				
+	7	5	1	9
+±	10	1	0	5
++	11	8	0	4
++±	5	6	4	0
+++	3	2	1	0
++++	1	0	0	0

The Other Diagnostic Parameters

The association of the particular types of bronchial response to food ingestion challenge with other diagnostic parameters is reviewed in Table 3.

Disease history. In the "positive food history" group, 15 of the 21 foods presumed from the history, caused a bronchial response of some type (71%). In the "unknown food history" group, 68 of the 122 foods caused a bronchial response of some type (56%). The overall correlation between the disease history to foods and the bronchial response of some type, due to the ingestion of the appropriate foods, was found to be non-significant ($P > .1$).

Skin response. A positive ISR was found in 37 of the 66 cases of IR (56%), a positive LSR in 22 of the 45 LR cases (49%), and a positive DSR in 6 of the 15 cases of DR (40%). The correlation between the skin and bronchial response was found to be non-significant ($P > .05$). In 19 of the 60 cases of NB (32%) the skin tests were, however, positive (Table 4).

Specific IgE antibodies in the serum. Pharmacia score—grade 3 or 4 were positive in 11 of the 66 cases of IR (17%), in 4 of the 45 cases of LR (9%), and in no case of DR (0%). This correlation was not satisfactory ($P > .1$).

Concentration of IgG, IgM, and IgA antibodies in the serum. The IgG increased (>18 g/L) during ten cases of IR (15%) and during 17 cases of LR (38%). The IgM increased (>3.8 g/L) in eight cases of LR (18%). The IgA increased (>4.0 g/L) during one IR (2%) only. No statistically significant correlation was, however, found between any type of bronchial response and these antibodies ($P > .05$).

Increased blood leukocytosis (>10 × 10^9/L) was found during 2 IRs (3%), 21 LRs (47%), and 3 DRs (20%), while the *increased blood eosinophilia* (>300 × 10^6/L) appeared during 23 IR cases (35%), 22 LR cases (49%), and no DR case (0%).

Body temperature increased slightly (between 37 to 38 °C = 98.6 to 100.4 °F) during three LR cases (7%) and during six DR cases (40%).

Bronchial complaints. All cases of bronchial response to food ingestion were accompanied by bronchial complaints, however, to various degrees. The IR was accompanied by dyspnea and wheezing; the LR by dyspnea, wheezing and dry, non-productive cough; and the DR by dyspnea only.

General malaise complaints appeared during 27 bronchial responses (33%) and that during 8 IRs (10%), 15 LRs (33%), and 4 DRs (27%).

Other organ response and other complaints recorded after the food ingestion challenge are summarized in Table 5.

The Effect of the Elimination Diet

According to the patients' reports, avoidance for 8 to 12 months of the individual foods causing the positive bronchial response of any type led to a distinct decrease in the bronchial complaints in 93%.

DISCUSSION

The "food allergy" or "food hypersensitivity" is a clinical manifestation of an immunologic process, in which foods, their parts or their metabolic derivates are able to act either as antigens or haptens to stimulate the production of antibodies against them or to sensitize the cells. The foods are then also able to interact with these antibodies or sensitized cells upon the involvement of other parts of the immunologic system, which results in hypersensitivity.[8,9,15]

Food allergy should be differentiated from other disorders which could also be caused by foods, but which are, however, due to a completely different mechanism (eg, intolerance, non-specific hyperreactivity, histamine liberation, idiosyncrasy, non-immunologic reactions to non-controlled or controlled (additives) chemical compounds, microorganisms and their products, etc).[15,24,31]

With respect to the lack of exact data in many patients developing reactions to foods (eg, regularly no antibody of any class could be detected, no part of the food responsible for the patient's response is known, the exact pathogenetic and immunologic mechanism involved in the patient's response remains unknown, etc), the term "food allergy" seems to us to be slightly uncertain. It would therefore be more appropriate to speak of "adverse reactions to foods," while the real food hypersensitivity remains one of the suspect mechanisms.[15,25]

The additives, which are controlled chemical compounds in foods, form a special problem and not only with respect to their large number, heterogeneity, variations in their use, but also the lack of sufficient knowledge concerning their mode of action, involvement and pathogenetic mechanisms.[15,31-33]

The participation of foods in the patients with bronchial asthma and the role of the food allergy in producing bronchial complaints has been regularly discussed in the literature.[8,9,11,13-15,17,20,22,27,30-32,34-37,39] Although this role has become recognized, the involvement of foods through the hypersensitivity mechanism has been suggested as one of the possible mechanisms.[8-11,14,15-17,19-23,26-30,34-37]

There is a dearth of information concerning the clinical features of the bronchial response and its various types due to food ingestion challenge as well as their correlation with other *in vivo* and *in vitro* diagnostic parameters in a large number of sufficiently defined and selected patients. The three types of bronchus-obstructive response to the food ingestion challenge recorded in this study correspond with the basic types of asthmatic response to the bronchial challenge with inhalation allergens previously reported by us.[4-7] The individual types of bronchus-obstructive response to food ingestion challenge can be associated with other diagnostic parameters, to varying degrees, as reviewed in Table 3.

The positive disease history for a certain food with respect to the bronchial response of any type was confirmed in 71% of patients in this study. Contrary to this, patients with unknown history for foods developed a bronchial response of some type after a food ingestion in 56% of patients. These results indicate that a positive history for certain foods in bronchial asthma patients is a valuable diagnostic parameter, while the unknown history for certain foods cannot exclude such a role. These results confirm the diagnostic value of the food ingestion challenge.

The diagnostic value of skin tests with food extracts has frequently been discussed in the literature from controversial points of view. Some investigators found the skin test a useful diagnostic parameter for the food allergy.[15-17,21,25,28-30]

We found the positive ISR in 56% of IR to food ingestion, the positive LSR in 49% of LR, and the positive DSR in 40% of the DR cases. On the other hand, in 32% of the negative bronchial responses a positive skin response was also found. The correlation between the positive ISR and the positive IR to food of 56% is not in full agreement with other investigators' data, reporting either a lower (30%) or higher correlation (90%).[17,28,29] This discrepancy can probably be attributed to differences in the patients investigated. Our patients formed a "homogenic group," all of them suffering from bronchial asthma, while the other investigators' patients suffered from various allergic disorders.[28,29] Our result of 32% positive skin responses in patients with negative bronchial responses to foods, corresponds with Atkins et al[28] finding of 21% false positive skin tests. It could be concluded that skin tests with food extracts should be considered a screening diagnostic parameter only, while the definite confirmation of the role of a particular food in bronchial asthma should be provided by the food ingestion challenge.[8,9,16,17]

The specific IgE antibody in the serum (RAST) for the appropriate foods was found in only 17% of the IRs, in 9% of the LRs, and in 0% of the DRs. In 6% of the negative bronchial responses, the RAST was positive for the particular food. This unsatisfactory correlation between the RAST and IR to foods is in agreement with our previous studies[4,6-9] as well as with other investigators' results.[28,39,42]

The increased serum concentration of IgG antibodies was found in 15% of IR, in 38% of LR, and in 0% of DR, while that of IgM was found in only 18% of the LR cases. These results, despite their being statistically non-significant ($P > .05$), could suggest some involvement of IgG and IgM antibodies in some

Table 5. Review of Other Organs' Response and Complaints Appearing After the Food Ingestion Challenge

	Bronchial Response to Food Ingestion			
	Immediate n = 66	Late n = 45	Delayed n = 15	Negative n = 60
Conjunctival injection and itching	2 (3%)	3 (7%)	1 (7%)	0 (0%)
Nasal obstruction	4 (6%)	2 (4%)	3 (20%)	1 (2%)
Increase in middle ear pressure	0 (0%)	1 (2%)	1 (7%)	0 (0%)
Response of sinuses (pressure, acute edema of sinus-mucosa)	1 (2%)	3 (7%)	0 (0%)	1 (2%)
Cephalgia	13 (20%)	17 (38%)	8 (53%)	2 (3%)
Urticaria	0 (0%)	2 (4%)	4 (27%)	1 (2%)
Angio-neurotic edema	1 (2%)	1 (2%)	4 (27%)	0 (0%)
Increase in body temperature (>37 °C axillary)	0 (0%)	3 (7%)	6 (40%)	0 (0%)
Gastrointestinal complaints (nausea, vomitting, diarrhea)	3 (5%)	4 (9%)	3 (20%)	1 (2%)
General malaise complaints	8 (12%)	15 (33%)	4 (27%)	1 (2%)

cases of bronchial response to foods, especially of the LR type.[1,6–9,11,14,18–20,22,26,27,30,38–41]

The increase in blood eosinophil count in 35% of IRs and especially in 49% of LRs may suggest the involvement of eosinophils in both the clinical types of bronchial response to foods; however, these results were not statistically significant. The blood leukocyte count increased during 47% of the LR cases. Both these results can probably be explained by Durham et al[2] suggestion of the role of eosinophils and neutrophils in the late-phase asthmatic reactions. They could also be suggestive for a possible involvement of different mechanisms in the individual types of bronchial response to foods.

The IR was accompanied by dyspnea and wheezing only, DR by dyspnea, while during the LR, besides the dyspnea and wheezing, a non-productive cough and pressure on the chest were also observed. These differences in bronchial complaints, appearing during the individual clinical types of bronchial response to foods, may also be suggestive for involvement of different mechanisms.

In some patients the food ingested also led to other organ responses, occurring simultaneously with the bronchial response as is shown in Table 3. This finding is similar to the observation of Atkins et al.[28,29]

The importance of the ingestion challenge with foods has frequently been confirmed in the literature.[16,17,19–21,23,28–30] Papers concerning the role of foods and the food allergy in patients with bronchial asthma, especially those dealing with the bronchial response to food ingestion challenge by quantitative recording of the objective parameters are, however, not numerous.[8,9,16,17,19,23,38]

On the other hand, the ingestion challenge with foods is not completely free from some risks for the patient and therefore this technique should be performed under certain conditions. (1) The tests should be safe, reproducible, sensitive, free from artifacts, and independent of the patient's and investigator's influence. (2) The tests should be performed by experienced personnel, using suitable techniques and equipment. (3) A resuscitation and intensive care unit should be directly available.

The indications and contraindications for these tests must be carefully considered in every patient. A detailed disease history, general physical examination, and basic laboratory screening must precede the oral challenge. (1) The absolute contraindication is the presumption of an anaphylactic reaction to the particular food,[16,20,25,28,29] pregnancy, and any disorder which could lead either to a severe emergency situation requiring surgery, intensive care of resuscitation, the so-called "life-threatening reaction." (2) The relative contraindication for the oral challenge may be any state or disorder leading to any undesirable complication in the patient (eg, post-infectious stage, metabolic disorders, etc), or which can distinctly influence the results of the oral challenge, (eg, medication). (3) The oral challenges, where the so-called vital organ responses are recorded (eg, lung function, severe diarrhea, hypo- or hyperthermia, changes in the blood pressure, etc) or where a late onset of the organ response is expected, should be performed during hospitalization for a sufficiently long period, 56 hours at least. Those oral challenges where the "non-vital parameters" are measured (eg, nasal resistance) can be performed in the out-patients, however, during a long enough period of time to exclude a non-immediate response. (4) The allergen extracts as well as foods for oral challenge, their processing and quality, should be continuously checked; (5) The foods, their parts, and related foods that are suspected of causing the adverse reaction in the patients should be excluded from the diet for a sufficiently long period of time before the challenge. The recommendation concerning the exclusion period varies.[30] Some authors suggest 1 to 2 weeks. According to our clinical experience, a four to six day exclusion of the particular foods before the oral challenge is, however, sufficient.[8]

The ingestion challenge with foods is usually performed according to two basic schedules, either as a double-blind challenge[13,17,25,30] or as an open challenge with natural foods.[8,9,15,16,20,27–29] Both these techniques have their advantages and disadvantages. The open challenge with foods was found by us to be sufficient in such cases where the objective parameters can be recorded (eg, nasal resistance, spirographic parameters, temperature, etc). Such results were not found by us to be inferior to those of the double-blind technique.[8] This statement is also supported by the results of this study demonstrating non-significant differences between bronchial response recorded after the open and those after the double-blind schedule in 20 of the 22 patients. The double-blind schedule seems to us to be very important in those cases of adverse reactions to foods where the objective parameters cannot be recorded or where the organ response may be influenced by the patient (eg, migraine, headache, gastrointestinal or joint complaints, skin itching, general malaise, etc), or where the effects of drugs should be investigated. The double-blind schedule, despite its very important advantage of not being influenced by the patient, also has some disadvantages. (1) The content of the capsules to be swallowed is maximally 500 mg. The particular food may then either be taken in a lower dose or the number of capsules would increase very drastically (eg, 100 g of cheese would be processed in 200 capsules.[16,26,29] (2) One of the requirements is that the food must not be identified, therefore colourless, tasteless, odourless, etc. Such a preparation of foods is either not always possible or can lead to changes in physical or chemical properties.[16] (3) Providing a suitable placebo that matches the offending food in quantity, consistence, colour, and taste is not always possible.[16] (4) The administering of the food in capsules excludes the buccal mucosa, tongue, esophagus, and partly the stomach. These organs, however, could play an important role or are themselves the site of the response in a number of patients with adverse reactions to foods (eg, dysfunction of esophagus, gingivitis, vesicular eruption on the buccal mucosa, edema of the tongue, lips, or epiglottis, etc).[8,9,43] (5) By administering the food in capsules the digestive process, already starting in the mouth, may be clearly longer than that under natural conditions.

The results of our study demonstrated the appearance of three types of bronchus-obstructive response—immediate, late, and delayed—due to the food ingestion. Unfortunately, the pathogenetic and immunologic mechanisms underlying the individual clinical types of bronchial response to foods could not yet be fully clarified by us.

According to the results of this study, as well as the evidence provided by other investigators,[8–11,13–19,21,22,26,30,34–41] the involvement of the immediate hypersensitivity in the IR, the late hypersensitivity in the LR, and the involvement of the delayed hypersensitivity mechanism (eg, T-cells) in the DR, or at least of some of their modifications, cannot be excluded. Much further concurrent immunologic and clinical study will have to be done to approach clarification of the mechanisms underlying the particular types of bronchial response to foods.

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