

Report

Provocation tests with allergens

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Introduction

It seems that the first allergen challenge was performed by Blackley in 1873 (1). He applied fresh pollen to the lining membrane of his nares provoking itching, rapidly followed by sneezing and discharge of serum. Moreover, he provoked positive bronchial responses by inhaling particles, including fungal spores. Lowell & Schiller (2) were the first to demonstrate that allergen could be administered by inhalation employing aerosols of allergen extracts, and the responses could be measured quantitatively with a spirometer. Subsequently, the improved techniques for pulmonary function testing, the advent of nebulizers that give reproducible doses, and good penetration and deposition of particles in the lower airways (3) together with standardized allergen extracts have significantly improved the methods of provocation so that results may be considered satisfactory and data from different centers may be compared.

Several articles have appeared regarding standardized bronchoprovocation testing (BPT). A science citation search revealed that the standardized method of BPT proposed by the United States National Institute of Health Committee, later adopted by the American Academy of Allergy and Immunology, is one of the most highly referenced in world literature (4). This technique requires the intermittent administration of graded doses of aerosol, repeated pulmonary function tests, the construction of a dose-response curve and the determination of the PD_{20} FEV_1 : the provocation dose necessary for a 20% decrease in FEV_1 which may be interpolated from the curve. Another technique requires tidal volume breathing of graded concentrations of aerosols for 2-minute periods, with periodic pulmonary function tests and also the construction of a dose-response curve (5). The PC_{20} , or the concentration necessary for a 20% decrease in FEV_1 , is determined from this curve.

Another recently proposed method involves the use of a powder device to deliver a dry powder aerosol to the lung (6). With this innovative technique, incremental doses of dry powder capsules that contain lyophilized antigen are inhaled, and a dose-response curve is constructed after the determination of sequential pulmonary function tests. All three methods have a good reproducibility and there is a good correlation between the two aerosol techniques (7, 8), between provocation with allergen in solution and in powder form (9) and between the dosimeter and powder method (10). Because of inter-laboratory differences, however, each laboratory should be responsible for its own coefficient of reproducibility. Care should be taken not to confuse PD_{20} (breath units) with PC_{20} (a derived concentration). Details of the respective techniques can be found in the source literature for the intermittent method (11), the continuous method (5, 12) and the dry powder method (6).

Although BPT are widely used, there is no international consensus as to how the tests should be performed, nor is there agreement on their standardization, indication and uses. In an attempt to rectify somewhat this situation, an international committee produced an update of guidelines for standardization of BPT with allergens (11). Furthermore, in 1990, the European Academy of Allergy and Clinical Immunology (EAACI) established a Subcommittee on Bronchial Provocation Tests with Allergens. The aims of the Subcommittee were to define indications, to describe the types of allergen extracts, to define the methods for BPT, to define the sensitivity, specificity and reproducibility of the results and to provide cautions and contraindications. Initially, the subcommittee's mandate was limited to bronchial provocation tests; later it was extended to nasal and conjunctival tests. A Steering Committee was appointed to draw up the various chapters, which were circulated to other EAACI members who expressed an interest in the topic.

Provocation tests with allergens

Therefore, the final document represents the opinion of a large number of experts in the field of allergen challenges.

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Indications for bronchial provocation tests

The indications for bronchial provocation tests (BPT) have undergone significant changes over the past few years. Up to 1970, BPT were used mainly for diagnostic purposes. Subsequently, progress in skin test techniques and, chiefly, the introduction

of serological assays for IgE have progressively reduced the diagnostic use of BPT, which are used only in selected cases. In parallel, many groups have reported on indications for specific antigen challenge (1–10). The indications for BPT, at present, are of two types:

A. Clinical

- 1) Actual role of BPT in the etiological diagnosis of asthma
- 2) Bronchial sensitization vs. clinical allergy
- 3) BPT as a criterion for the evaluation of the effects of immunotherapy
- 4) Study of new anti-asthma drugs

B. Research

- 1) Pathophysiology of asthma
- 2) Relationship between specific and non-specific reactivity

BPT continue to be used in the etiological diagnosis of asthma because skin tests and *in vitro* tests may not always reflect the allergic situation at bronchial level (2, 5, 11).

Mosbech et al. (12) studied the sensitivity of some patients with house dust mite asthma by prick test, histamine release and by nasal, conjunctival and bronchial challenge, and showed that the sensitivity of the mucosa in different organs of the same subject was unrelated. A possible explanation for a dissociation of response of different organs may be the local production of IgE. Indeed, Aas (2) found nasal allergy to pollen in patients who had a positive skin response to that pollen and a negative bronchial challenge. Moreover, Huggins & Brostoff (13) showed the presence of specific IgE in nasal secretions after provocation in patients with negative skin test and RAST to the allergen.

It has been demonstrated that there is a correlation between skin testing, RAST and inhalation challenge to a specific allergen, so that BPT are not necessary in most cases of asthma. However, there is general agreement that bronchial challenges are useful when the history and other tests (skin tests and/or RAST) are not conclusive (14). As a general rule, slight reactions of skin tests tend to have minimal significance, whereas strongly positive reactions correlate highly with clinical sensitivity to the corresponding allergen (9).

It appears that the main diagnostic indication of BPT is its use in asthmatics with a low-degree allergy. In these patients with borderline sensitivity, whose skin-test response is classified as “+” or “++” and RAST class “I” or “II”, only bronchial challenge is able to establish the role of the allergen in provoking asthmatic

symptoms. In a study on a group of asthmatics with a low or mild degree allergy it was found that, in some cases, there was no correlation between skin-tests, RAST and BPT, and several subjects with low response to the first two tests experienced either a positive or a negative BPT (Melillo, unpublished data).

BPT may be useful in the identification of new unrecognized allergens (15) and in evaluating the clinical role of different antigenic fractions in a total allergen extract. Fractions P_2 and P_3 of the *Parietaria* pollen extract were found to be more active in inducing bronchial symptoms, while the other fractions induced bronchial reactions only at a very high dosage (17).

Occasionally, BPT may be indicated in polysensitized subjects to establish the role of each allergen in inducing bronchial symptoms.

Many inhalant allergens have been used for BPT. In Europe, house dust mite (HDM), animal danders (cat, dog), pollen and molds are the most widely employed allergens for diagnostic purposes. The indications for BPT in grass pollen asthma are very limited. In fact, in this type of asthma the clinical history, generally well characterized with a definite seasonal pattern, and the very high correlation between history, skin tests and/or RAST render BPT unnecessary. However, if a particular pollen species is suspected of being the causative agent of the patient's asthmatic complaints, BPT with that type of pollen is a useful tool to confirm diagnosis. On the contrary, BPT with pollens is particularly suitable in clinical research. In fact, out of the pollen season, subjects with pollen allergy may appear completely normal when environmental exposure to the offending allergen is absent. Moreover, out of the pollen season, nonspecific hyperresponsiveness is very low or absent, hence, patients can undergo BPT without being exposed to undue risks (16). Several other allergens have been used for BPT: various animal dander extracts (18), molds such as *Alternaria basidio* spore, *Aspergillus fumigatus* (19, 21–23), storage mites (24, 25) and cockroach extracts (26, 27). Of course, other inhalant allergens, if appropriately standardized, could be used for BPT.

Correlation between BPT and clinical allergy

It is well known that positivity to skin tests and/or *in vitro* tests does not always correspond to clinical manifestations of rhinitis or asthma. The belief that allergic asthma is characterized by specific sensitivity of the airways to the offending allergen has led to the use of BPT for diagnostic purposes. This raises the question: is positivity to bronchial challenge always the expression of clinical allergy, or

are there false-positive responses? Data on this aspect are scanty. Bruce et al. (28) failed to show any statistically significant difference between ragweed hay fever and ragweed allergic asthma on the basis of PD_{35} with inhaled ragweed extract. The mean PD_{35} was approximately 2.5 times greater in hay fever patients than in asthmatic patients. Permut et al. (29) showed a 4-fold difference for PD_{35} and a 9-fold difference for PD_{20} in subjects with ragweed hay fever and ragweed asthma and they considered the difference not significant because it was not greater than the variability (10-fold) in a single subject on repeated tests. These results are in contrast with those observed by Aas (2), who reported that BPT with recording of FEV_1 discriminates very well between patients with bronchial and those with nasal allergy. The different results of Permut et al. (29) may be due to the quality and top concentration of inhaled allergen. The experience of Aas (30) is that when the dose of allergen concentration does not exceed 1 histamine equivalent (1 HEP), positive reactions are specific for bronchial allergy in asthma, and bronchial obstruction is never elicited in non asthmatic rhinitis patients. Our findings coincide with those of Aas. In fact, the use of purified, standardized and stable allergen extract has significantly reduced the variability of PD_{20} on repeated tests so that, under controlled conditions, the PD_{20} is reproducible on doubling the dose (31–34).

Another element that might cause false positives is the maximum dose of inhaled allergen. The top dose should be sufficiently high to elicit a reaction in all sensitized patients but not so high as to cause false-positive responses (35). The higher the dose administered, the lower the specificity, i.e. the more patients without bronchial allergy will be diagnosed, e.g., patients suffering from hay fever alone (36). Moreover, in addition to the above-mentioned "true" false-positive response, the possibility exists of some "apparent" false-positive responses. These could be observed in subjects with initial sensitization of bronchial mucosa in whom symptoms of asthma are not yet developed. This type of response implies a preclinical bronchial sensitization and might indicate a high risk of developing asthmatic symptoms.

It seems that there are no reports of false-negative responses to BPT. Consequently, a subject that does not react to the maximum dose, may be considered not sensitized to the inhaled allergen.

BPT in the evaluation of results of immunotherapy

Some investigators have suggested that BPT should be performed before starting immunotherapy in asthmatic patients (37, 38). As no immunologic test

Provocation tests with allergens

or combination of tests is considered able to reflect the effects of immunotherapy, challenge techniques have been devised for the evaluation of these effects (39). A series of studies have shown that immunotherapy can reduce bronchial sensitivity to the allergen. There is some uncertainty regarding the value of BPT as means by which to evaluate immunotherapy with respect to the immediate response. Several authors have shown an increase of PD_{20} of the immediate response after immunotherapy (40–48). Tolerance to the offending allergen has been shown significantly increased after immunotherapy. Moreover, the increase appeared dose-dependent: greater the maintenance dose-extract, higher the specific tolerance at provocation test (49). A significant decrease in bronchial sensitivity was also observed by Melillo during local immunotherapy by inhalation (50). Other studies failed to show any modification in the immediate response after immunotherapy while the late response was decreased or lost (51, 52). In contrast, other investigators have not observed any significant effect of the immunotherapy either on the immediate or on the late asthmatic response (16, 20).

It has been reported that symptoms in patients with allergic disease correlate more closely to the late than to the immediate response to antigen (53–55). These data have prompted studies aimed at assessing the benefits of immunotherapy by examining the late asthmatic response. Inhalation provocation with antigen must be done with great precision since the late asthmatic response may be antigen-dose dependent. Moreover, the late asthmatic response could be affected by nonspecific hyperreactivity. Ideally, a methacholine or histamine challenge should be done prior to antigen challenge in order to demonstrate stability of non-specific responsiveness. Although the data are still scanty, it appears that the clinical efficacy of immunotherapy could be related to changes in the late asthmatic response (51, 52, 56, 57). Moreover, it has been shown that the decrease in severity of the late asthmatic reaction, obtained after the first year of immunotherapy (IT), may be lost, after 1 year, in patients, who stop receiving it while it remains decreased if it is continued (58).

Studies with rabbit models of the late asthmatic response suggest that the way in which immunotherapy in humans may be clinically useful is through modifications of the late reaction associated with an increase in IgG levels (59, 60).

BPT in the evaluation of new anti-asthmatic drugs

Various *in vitro* and *in vivo* models have been employed in the search for tests that have a good

relevance to the clinical disease (16, 20). Often, however, the results obtained with these models were not correlated with the clinical efficiency of drugs. For instance, many compounds were shown to have a biological activity similar to that of cromoglycate in some models but none, except nedocromil sodium, has been accepted in clinical practice (61). Similarly, β -adrenoreceptors have been shown to inhibit *in vitro* the antigen-induced release of mediators from sensitized tissues (62–65). From this observation it was argued that β_2 -adrenergic bronchodilators should exert a prophylactic effect, not only preventing bronchospasm by a direct effect on smooth muscles, but also by inhibiting the production of the mediators causing bronchospasm, inflammation and hyperreactivity. However, long-term treatment with β -adrenoreceptor agonists does not improve airway responsiveness (66, 67). Consequently, these drugs are not used as prophylactic or anti-inflammatory treatment in asthma (68).

In conclusion, *in vitro* and many *in vivo* screening models do not possess a complete clinically predictive value and only BPT are satisfactory in this sense (16, 20).

The functional changes that occur in humans after controlled antigen inhalation are similar to those observed during spontaneous acute attack of asthma (69). It has been observed that asthmatic reactions produced in laboratory are rapidly provoked and easily reversed by an inhaled bronchodilator implying that airway obstruction is mediated by bronchial muscle contraction. However, Hutchcroft (16, 70), performing a local BPT by instilling the antigen through a fiber optic bronchoscope, observed a marked increase in mucosal edema with hyperemia and mucus production, which may contribute to bronchial obstruction. Moreover, the investigations of late phase reactions during BPT, through bronchioloalveolar lavage, have contributed to our knowledge of the underlying mechanisms (i.e. inflammation) of asthma. Many investigators have shown that the response to inhaled allergen correlates with non-specific airway responsiveness whereas others have not found such a relationship (16, 20, 71–73). It is necessary, if BPT are repeated in the evaluation of new drugs, to determine the degree of airway responsiveness before each challenge session (74).

In conclusion, the therapeutical properties of new anti-asthmatic drugs should be investigated directly in humans and under controlled conditions in the laboratory. BPT, performed with standardized allergens and appropriate methods, is considered a reliable predictor of pharmacologic activity and efficacy of anti-asthmatic compounds in clinical asthma (16, 20).

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Provocation tests with allergens

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Allergen extracts for provocation tests

Introduction

Standardization of allergenic extracts has been required by clinical allergists in order to improve the precision of results as well as the safety of the diagnostic procedures.

In recent years, provocation tests have been employed in the investigation of the pathogenesis of allergic asthma and the protective effect of various medications (3). During the last ten years, the procedures have been standardized, and the reproducibility determined (6), just as well-characterized

and standardized allergen extracts have become available.

Allergens causing Type 1 allergy are defined as molecules that specifically bind IgE in at least one serum from a group of humans with clinical symptoms of allergy towards the specific allergen source, and therefore all antigenic components may be regarded as potential allergens.

Criteria for diagnostic allergen preparations

The optimal criteria for allergenic extracts should include: 1) All the potential allergens in 2) the relevant ratios and in 3) native forms, and 4) exclude irrelevant material.

Standardization expressed as the reproduction of a standard, implies that the standard, the in-house reference extract, ought to be well characterized and defined, and proper methods of reproduction must be evaluated and selected, fulfilling the four criteria listed above, to ensure that qualitative and quantitative properties of all successive production batches equal this in-house reference extract. In order to select a clinically relevant potency of the allergen in question, this potency is confirmed in clinical trials. Biological methods are not feasible for routine standardization and depend on the patient population, season of the year of testing, age, and sex, but are useful mainly to establish relevant levels of allergen potency and as a control of *in vitro* investigations. Once established, biological potency of in-house preparations, can be reproduced by supplementary *in vitro* methods better suited for the purpose of reproduction of composition and allergen concentration.

Extract quality

The raw material must be carefully selected, as no method of standardization can compensate for wrongly selected raw material. The raw material must contain all clinically relevant components. Allergens causing Type 1 allergy are defined as molecules that specifically bind IgE in at least one serum from a group of humans with clinical symptoms of allergy towards the specific allergen source, and therefore all antigenic components may be regarded as potential allergens. However, in allergen identification techniques (crossed radio-immunoelectrophoresis, CRIE) some extract components reveal very high IgE binding prevalences when sera from a group of allergic patients are investigated, whereas other components show low binding prevalences. Components binding IgE from more than half of the investigated sera and binding strongly to IgE from more than half of

these sera are defined as major allergens and must be present in relevant ratios in the extracts in question. Furthermore, less important allergic components might have great importance in certain allergic patients and ought therefore also to be present.

International standards

Standardization of allergenic extracts has been increasingly required by allergists and control authorities, since their introduction for diagnosis and immunotherapy. At first this was limited by the lack of biochemical information about allergenic extracts, which only included skin testing for potency measurements and some rather simple physicochemical data, such as weight/volume (w/v) and protein nitrogen unit (PNU) determinations. With the increasing knowledge of the allergenic extracts, and the development of *in vitro* assays applied to the allergens and allergenic extracts, more relevant standardization became possible. A number of different proposals for standardization of allergenic extracts have been suggested. Among these are: overall composition potency determination; concentration determination for some well-defined allergens; or any combination of these principles. Criteria such as specified ratios between characteristic components have also been put forward for consideration (8).

International standards (IS) were established by a group under the International Union of Immunological Societies (IUIS) in 1980–81 (10). Collaboration with the World Health Organization (WHO) attempted to achieve binding agreement at an international level (21). IS extracts or an International Biological Reference Preparation (IBRP) from several allergenic sources have been selected and characterized (*Dermatophagoides pteronyssinus* mite, dog hair/dander, short ragweed pollen, timothy grass pollen, and birch pollen), others are to follow (*Dermatophagoides farinae* mite, *Alternaria alternata* mold, cat hair/dander, bermuda and rye grass pollen). These standards have assigned potency in international units (IU), and one must note that both total allergenic activity and the amount of a specific allergen may be referred to as 100,000 IU, which is an arbitrary unit (15). The IS extracts are only recommended for use as calibrators (standards for measurement of relative potency) and are not recommended for use as prototypes (materials to which an extract is to be matched in all respects) (11).

In 1980 the Nordic Council of Medicine proposed the use of allergen standardization using biological methods and prescribed such standardization in its first guidelines for registration of allergenic preparations (1, 13, 17), later brought

Provocation tests with allergens

into context with the FDA criteria. However, the two systems cannot be directly compared (5, 14, 18). The Nordic guidelines prescribe standardization by skin prick testing, whereas United States FDA guidelines by intracutaneous testing (17) resulting in the allergy unit (AU). Problems concerning which method of skin testing should be the primary method of allergen standardization have been raised: the method of patient selection, skin testing, the response to be measured, the standard use for comparison testing, the method used for statistical analysis of the results, and the unitage in which the results are expressed should be standardized (5). One standardization method is not sufficient to reproduce an in-house reference or standard. Both the composition, the distribution of allergenic components, and the total biologic potency must be reproduced to ensure a reliable tool for bronchial provocation testing which is consistent from batch to batch. Therefore a single method is not sufficient to ensure the reproduction of allergen extracts both qualitatively and quantitatively. However, this can be obtained by combining different supplementary methods (9). IU is an arbitrary, nonclinical unit, an external standard, whereas AU and biological units (BU) are biological units, and a clinical (dose dependent), internal standard.

Allergens characterized by different methods ought to be compared to international reference standards when available, and if not, aliquots of the allergen preparations used in scientific studies should be stored freeze-dried and frozen to permit later comparison with other preparations. However, an in-house standard should be established and the potency of the final product should be labelled after calibration against this standard.

Concerning the labelling of the allergen extracts, only a limited number of IS extracts are available; however, these ought to be used as a yardstick in order to allow labelling with IU of potency in parallel with other units. Highly purified allergens will be designated as follows according to the IS: the first three letters of the genus (*italicized*); space; first letter of the species name (*italicized*); space; a roman numeral, i.e.: "*Der p 1*" refers to *Dermatophagoides pteronyssinus* allergen No. 1. The allergens will usually be numbered in the order in which they were isolated, with the following general exceptions: 1) a major allergen will normally be assigned number 1; 2) structurally homologous components from different species will be assigned the same number.

Clinically relevant potency determination

Allergen standardization using biological methods offers several advantages to the practicing aller-

gists, namely less variability in potency of allergenic extracts, more quantitative and meaningful schedules for diagnostic testing as well as for immunotherapy than i.e. w/v or PNU, and finally a safer comparison from one allergist's clinic to another concerning diagnostic procedures and treatment of patients. The authority-approved units IU, AU, and BU form a basis for legal control of allergenic extracts but do not in themselves guarantee the clinical relevance for clinical purposes such as skin test, provocation test and immunotherapy. IUs are arbitrary units assigned for nonclinical purposes, for *in vitro* comparison of single components only. AU and BU are biological units that reflect biological equilibration of in-house reference preparations, standards, on selected patient material. Any standardization must be followed by clinical documentation in order to establish the optimal dose and regimens for each purpose of the allergenic extract in question (16, 19, 20). Standard quality extracts (SQ) have been evaluated in clinical trials in order to establish a clinical relevant potency.

Defining the biological potency as described above in AU, BU, or HEP, the allergen extract provides a relevant tool for comparison of patients' sensitivity toward different allergenic source materials. Biological equilibration by skin test seems to be relevant for other diagnostic procedures, such as provocation tests in various shock organs (4, 18). The total biologic potency of the in-house reference must be clinically relevant. For bronchial provocation testing, basic dose-response investigations are principally needed; however, undesired adverse reactions when performing such studies are a risk. The sensitivity in the shock organ varies with time, patient status, and other factors, therefore the recommended dosages for provocation testing must be carefully selected. The initial dose should be sufficiently low in order not to lead to unacceptable severe reactions in more sensitive patients. The top dose applied, however, should be sufficiently potent to exclude the risk of false-negative reactions in patients with clinical allergy to the allergen in question concerning provocation tests or sufficiently immunogenic concerning immunotherapy.

The use of biological testing by means of skin prick testing, or intracutaneous testing, gives the possibility for all users in clinical practice to check the potency of the extract they have received (2). As mentioned above, one way to describe the patients for bronchial provocation testing with allergens is to classify them according to the reaction in the skin to the extract in question, combined with the responsiveness to nonspecific bronchoconstrictors, i.e. histamine. This relationship

might be of interest both with the initial and the top concentration of the allergen solution used (4).

Biological equilibration of allergenic extracts

From the above, emphasis has been placed on the clinical relevance of allergen standardization using biological methods (AU, BU) versus other methods such as measuring allergen concentration (ug/ml), PNU, or w/v. The AU and BU establish a guide to the expected potency of extracts. Ideally the same extracts should be used for diagnosis (skin tests, specific IgE determinations, provocation testing) and treatment (immunotherapy).

Stability of extracts

Stability may be maintained by various techniques: preparing and storing lyophilized extract to be reconstituted on the day of challenge, using a proper diluent (such as phosphate-buffered saline = PBS) containing human albumin or without human albumine (7), or as recently proposed by storing the extract as a freeze-dried micronized powder in capsules containing the carrier substance and premeasured sequential doses of allergen extract (12). Negative control, either diluent or freeze-dried carrier substance, should ensure if any preservative influences test results, as irritants.

Conclusions

To improve reproducibility, sensitivity, and specificity of bronchial provocation tests with allergens, standardization of procedures is essential, and as several factors influence the response, and thereby the interpretation, each factor must be described and controlled.

The allergen extracts used for diagnostic tests as well as in treatment, must be compared with qualitatively and quantitatively clinical relevant standards, using a combination of complementary immunochemical and biological methods. Clinical accuracy and reliability depend on the quality of standardization procedures, i.e., the combination and number of tests applied and the quality of every single test performed. Clinical safety and efficacy depend not only on the quality of standardization but also on the applied potencies. Standardization using biological methods is established in allergic patients in order to select a clinically relevant potency of the allergen in question, and this potency is later confirmed in clinical trials. AU and BU are biological units, although not directly comparable, reflecting a biological equilibration of in-house reference preparations on selected patient material. The advantages of extracts

labelled in standardized units are that generally they are very consistent from batch to batch and are of high overall potency. Very few commercial allergen preparations are available that fulfill the above-mentioned criteria.

Standardization is essential for improvement of allergy diagnosis and treatment, but standardization must be followed by clinical documentation in order to establish the optimal dose and regimens for diagnostic purposes.

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Provocation tests with allergens

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Methods of provocation: technical and methodological aspects

Introduction

Inhalation of allergen in allergen-sensitive patients may result in early- and late-phase reactions. The early reaction starts within minutes after allergen challenge, reaching its maximum after 15–20 minutes and usually resolves after 1–2 hours. The severity of the early reaction is, among others, dependent on the level of specific IgE, the degree of non-specific bronchial reactivity and the amount of allergen delivered (1, 2). In 50–80% of the individuals tested the early response is followed by a late asthmatic response, usually starting 3–4 hours after allergen challenge and reaching its maximum after 7–12 hours. In many cases FEV₁ is still depressed 24 hours after challenge.

Patients with a dual response usually show a more severe type of disease than do patients with a single response. While the pathology of the early reaction can mainly be ascribed to the effect of mediators, such as histamine, prostaglandins and leukotrienes, the late phase reaction is characterized by a cellular infiltrate consisting of eosinophils, lymphocytes, basophils and sometimes neutrophils (3–7).

In a laboratory setting, bronchial challenge tests with allergens are often used to induce an asthmatic reaction. Although this setting is different from the situation of natural exposure in many respects (single challenge vs chronic repeated exposure, aerosol inhalation vs inhalation of dry material etc.), the bronchial challenge test has

proved to be a useful tool in research and to certain extent also in the management of patients with allergic asthma.

Patients

The technique of performing bronchial provocation tests is critical in order to obtain reproducible results with minimal discomfort to the patient. Patients should be cooperative and be able to understand some basic facts about their disease. Although bronchial challenge tests with histamine and methacholine have been performed in younger children, bronchial provocation tests with allergens have been performed only in children from 7 years of age (8, 9). Patients must be stable without asthma medication and have FEV₁ values of ≥70% of predicted value at the start of the test. Anti-inflammatory medication should ideally be stopped 4–6 weeks before the test. This, however, cannot be achieved in the majority of patients attending an asthma clinic. In many studies inhaled steroids are stopped 1–2 weeks before the test; a slight carry-over effect of these drugs is to be expected in such cases. Theophylline, oral β_2 -adrenergics and antihistamines should be stopped 48 hours before the test (astemizole 6 weeks); inhaled β_2 -adrenergic drugs 12 hours (short-acting) or 24 hours (long-acting) before the test. Anticholinergic agents should be stopped 12–24 hours before challenge. Sodium cromoglycate and nedocromil sodium should be discontinued 12–24 hours before challenge. Bronchial challenge tests with allergen should not be done in patients who had suffered severe asthmatic attacks in the previous 6 months or respiratory tract infections in the previous 6 weeks.

Studies have been conducted to ascertain whether it is safe to perform bronchial challenge with allergens in patients who have severe bronchial hyperresponsiveness to histamine. Bronchial challenges with house dust mite or grass pollens were performed in 19 allergic patients with asthma who had previously shown late phase reactions. A significant decrease in PC₂₀ histamine (*t*-test, log-transformed data, *P* = 0.02) was found after allergen challenge compared with pre-challenge values. No rescue medication was necessary. There were no significant correlations between the PC₂₀ histamine before the allergen challenge test and the severity of the early and late asthmatic reaction, measured as mean fall in FEV₁ value during early and late response (10). Thus, it seems safe to perform bronchial challenge with allergens in patients with severe histamine-induced responsiveness, when the test is carefully performed and all necessary precautions are adhered to.

The technique of bronchial provocation

Bronchial challenges with allergen are preferably performed in the clinical setting, since it is important that the patient not be exposed to allergic or other stimuli during the test. Also for safety reasons it is preferable that the patient be kept in the hospital. Severe late responses may occur up to 12 hours after challenge. Fig. 1 shows the effect of bronchial challenge with allergens in 20 atopic patients with asthma who were monitored until 11 to 12 hours after challenge. Even 24 hours after challenge, 6 of the 20 patients had a decrease in FEV₁ value $\geq 15\%$ of baseline (10). If patients leave the hospital after the test, they should be treated with inhaled corticosteroids and bronchodilators for at least 24 hours to avoid possible severe reactions occurring at home. In such cases, it is not possible to monitor the entire late response.

Prior to the allergen challenge, a control day is scheduled during which a phosphate buffer aerosol is inhaled 3–4 times with intervals of 15 minutes. Lung function tests are performed at the same intervals as during the day of the allergen challenge. The control day is useful for several reasons. Firstly, it is important to estimate diurnal variation in lung function. A slight increase in lung function during the day is seen in most asthmatic patients, often followed by a gradual decrease in the evening and night. Some patients, however, show an impressive improvement of their lung function even after 1 day in hospital. Arbitrarily, allergen challenge

should not be performed when diurnal variation is $\geq 15\%$ of initial value. Lung function values measured on the allergen challenge day must be corrected for the values measured at the same time as on the control day. Fig. 2 shows the effect of correction for diurnal variations in allergen challenge tests in 1 patient.

Another reason why a control day is necessary is to see whether the inhalation of phosphate buffer saline aerosol results in a fall in FEV₁ value. When there is a fall in FEV₁ $\geq 15\%$ from baseline after inhalation of buffer aerosol, no allergen challenge should be performed. It is also important to check for the presence of spirometry-associated bronchospasm due to deep inspiration to TLC which precedes forced expiration maneuvers. Before the start of the test, a minimum of 3 FEV₁ measurements should be made with a variation of $\leq 5\%$.

Allergen extracts

Test solution. Preferably, a well-standardized allergen preparation is used in order to induce reproducible test results with a minimum of side effects. As allergens may be standardized by different methods, they ought to be compared with international reference standards, when available (11). If preference standards are not available, allergen should be stored freeze-dried at -30°C or freeze-dried at $\pm 4^{\circ}\text{C}$. Testing materials should not contain glycerol. Immediately before the provocation, the relevant dilutions are prepared from the stock solution; diluted material should not be stored after

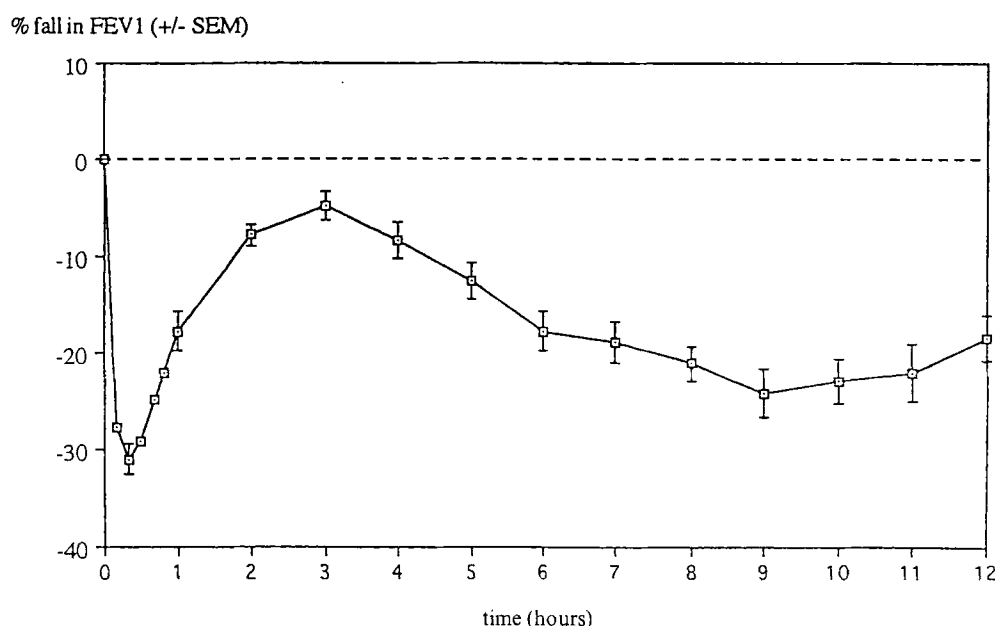


Fig. 1. The time course of the early and late asthmatic reaction following allergen challenge in 20 patients, allergic to house dust mite or grass-pollens. All patients were mild asthmatics and had baseline FEV₁ values $\geq 70\%$ predicted.

Provocation tests with allergens

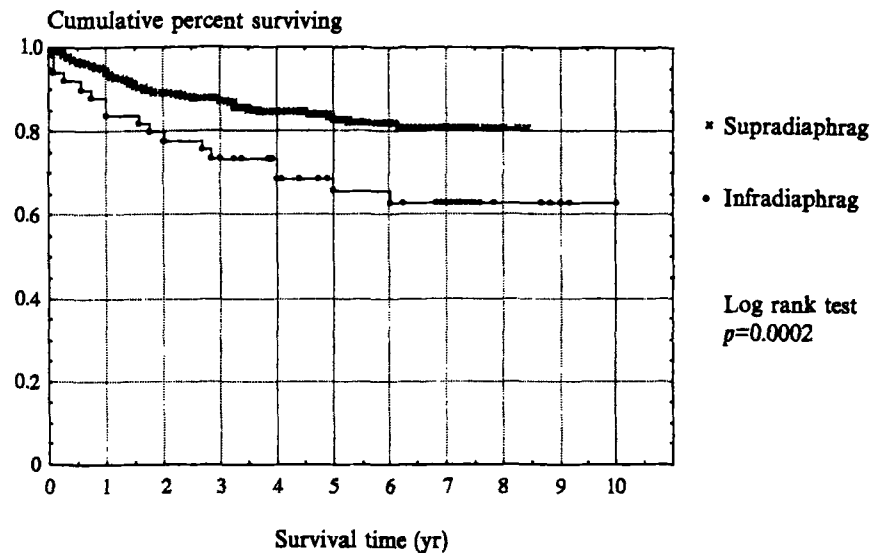


Fig. 2. The effect of correction for diurnal variation on the allergen challenge test in 1 patient who improved during hospitalisation and 1 patient who showed a progressive decrease in lung function during the control day.

the preparation. Recently, an allergen extract in powder has been proposed for bronchial challenge. The extract is prepared as freeze-dried, micronized powder in capsules containing a carrier substance (lactose) and premeasured sequential doses of allergen extract (12).

Methods of provocation. Even if allergen extracts are well standardized and stabilized, the technical procedures for aerosol generation and inhalation must follow definite conditions in order to ensure the reproducibility of the results. In fact, the method of aerosol generation (nebulizer output, particle size, etc.) and inhalation (pattern of breathing) can influence the deposition of inhaled allergens (13).

Aerosol delivery systems and inhalation methods. At present the following techniques for allergen provocation are used: 1) continuous inhalation of allergen extract at tidal volume breathing from a constant output nebulizer; 2) intermittent delivery of the allergen by breath-actuated dosimeter; 3) inhalation of a dry powder allergen in capsules by a powder inhaler; and 4) local allergen challenge.

Jet-nebulizers and dry powder inhalers are the main system of aerosol generation for bronchial provocation tests. Various types of jet-nebulizers are used in different countries (De Vilbiss 646 in the USA, Wright's nebulizer in the UK, Pari

nebulizer in northern Europe, etc.). The "tidal volume" breathing method is used for bronchial provocation tests with jet-nebulizers. According to this method, the aerosol is inhaled breathing continuously at tidal volume for a fixed time (14). Metered-dose devices, such as the Rosenthal-French dosimeter (15) and the Me-Far dosimeter (16), operate with jet-nebulizers in which a fixed volume of aerosol is delivered to airways during inspiration. Results obtained with the different methods are comparable (17–19). A peculiar method of allergen provocation for research purpose is the local allergen challenge using local instillation of allergen solution through the bronchoscope (20–25).

Updosing. Protocols described below concern bronchial provocation tests performed using allergen extracts labeled in biological units (BU).

Continuous inhalation methods. Starting from a fixed concentration and applying four 5-fold increasing doses the starting concentration is usually 80 BU/ml. The solution is inhaled over 60 seconds with continuous nebulization during tidal breathing. The dose of allergen is calculated on the base of a jet nebulizer output of 0.13 ml/min. The nebulizer can be connected to a valve-box with aerosol filter. Lung function is measured immediately after the inhalation and then 15 minutes after inhalation. In a group of 19 patients who under-

went bronchial challenge with allergen, only 6 patients reached their maximal fall in FEV₁ value within 15 minutes; all other patients had a further decrease after 20 and 30 minutes. To avoid severe bronchoconstriction it is important to use an interval of at least 20 minutes between the different allergen concentrations. When the fall of FEV₁ has reached 15% or 20% from baseline value, no further dose is given. If no response is observed, concentrations of 400, 2,000 and 10,000 BU/ml are inhaled for 60 seconds.

Based on variable starting concentrations and increasing with doubling dose steps, the starting concentration is the dose three steps below a calculated response concentration. This calculation is based on the end-point concentration of the prick test and the PC₂₀ histamine value (2-minute method). The formula is:

$$\text{estimated log PC}_{20} \text{ allergen} = 0.69 \times \log \text{PC}_{20} \text{ histamine} \times \text{end point concentration.}$$

All concentrations are inhaled during 2 minutes. The interval between the doubling concentrations is 10 minutes. (In our opinion, however, an interval of 20 minutes is preferable for the above-mentioned reason.) After a 15% or 20% fall in FEV₁ value from baseline, no further dose is given. The highest concentration may consist of 5,000 BU. Also with this method a jet nebulizer with an output of 0.13 mg/ml is advised.

Dosimeter method. The dosimeter method is, on the contrary, a quantitative method, and one may therefore measure the provocation dose (PD) instead of the PC. The dosimeter releases an exact quantity of allergen extract at each delivery. The starting dose is calculated in the same way as for the PC, i.e., three steps below the calculated PD (estimated $\log \text{PD}_{20} \text{ allergen} = 0.69 \times \log \text{PD}_{20} \text{ histamine} \times \text{end-point concentration}$). The dose is doubled at intervals of 20 minutes; no further dose is given after a 15% or 20% fall in FEV₁ value from baseline. The highest dosage inhaled is 8-fold increasing doses starting from the calculated PD.

Powder allergen methods. The allergen extract is prepared in capsules containing premeasured sequential doses of micronized allergen. It is inhaled through a powder inhaler with the same technique as used for powder cromolyn, β_2 -agonists or steroids (12). Doses in the capsules are doubled, each dose is inhaled at intervals of 20 minutes. The provocation dose is recorded after a fall in FEV₁ $\geq 15\%$ or 20% from basal value.

Quantification and follow-up of early and late reaction

Clinical follow-up. A useful parameter for the follow-up of the reaction is the FEV₁ value. To obtain information about the level of bronchoconstriction, other parameters such as vital capacity (VC) and FEV₁/VC can be taken in account. However, these maneuvers require much effort from the patient. At critical points in the test period, for example when the allergen threshold is reached, it is important to evaluate both FEV₁ and the VC, in order to check the effort of the patient. In patients with spirometer-induced asthma or in patients who have technical problems in performing FEV₁/VC maneuvers, it is also possible to use the airway resistance as a follow up parameter (25, 26). The reproducibility of a simplified oscillometer method (Siemens Siregnost FD 5) in comparison to VC and FEV₁ was tested in healthy subjects and in patients with COPD by Gimeno et al. The results of this study indicate that effort-depending tests, such as FEV₁, are more reproducible than effort-independent tests, such as oscillometric way resistance (27).

Several parameters are often used to quantify the EAR and LAR. A simple method is to calculate the maximum fall in FEV₁ during the early and late response as compared with the initial value. As mentioned above, correction must be made for the control day. The disadvantage of this method is that the quantification of the response is based on one single value, which is not always representative of the severity of the reaction. Therefore, additional information comes by calculating the mean fall in FEV₁ during the response or the area above the FEV₁ curve. When comparing allergen challenge tests in which different doses of allergen have been used, the PD₁₅ or PD₂₀ value for allergen can be calculated for both early- and late-phase reactions.

Because the dose-response curve for allergens is not completely linear, the following formula can be used:

$$\text{PD}_{20} = \frac{(0.223 \times \text{dose})}{\text{LN}\left(1 - \frac{\Delta \text{FEV}_1 \text{ max}}{100}\right)}$$

$\Delta \text{FEV}_1 \text{ max}$ is the maximal difference in FEV₁ between control day and after allergen challenge (corrected for initial challenge) (28).

Some authors (28) have studied the time course of the early and late asthmatic reaction to obtain accurate information on both the occurrence and the severity of the late phase reaction. It was observed that the number of late reactions and the mean fall

Provocation tests with allergens

in FEV₁ tended to increase as the interval of hours after the challenge increased (Fig. 3). Therefore, a minimum follow-up of the late allergic reaction of 10 and preferable 12 hours is recommended.

In vitro follow-up. In recent years an increased emphasis has been put on *in vitro* correlates of bronchial challenge tests. These *in vitro* correlates may consist of serum parameters such as histamine and tryptase for the early response and eosinophil numbers, eosinophil cationic protein (ECP), eosinophil chemotactic activity (ECA), and neutrophil chemotactic activity (NCA) for the late response (6, 30–34). Detection of cytokines in peripheral blood after allergen challenge is still cumbersome because of their very low concentrations. Urine samples may show an increased level of urinary methyl histamine following the early reaction and of leukotriene D₄ (LTD₄) during the late-phase reaction (35). Both urinary parameters, however, lack sensitivity. Bronchoalveolar lavage and mucosal biopsies have proved to be an important additional tool with which to investigate the changes in the bronchi after bronchial challenge (4, 36). Detection of cytokines in bronchoalveolar lavage fluid and the use of *in situ* hybridization in bronchial biopsies have made it possible to study immunological mechanisms that are relevant in the expression of the inflammatory response (37–39). Although bronchoscopy and bronchoalveolar lavage can cause side effects, such as alveolar infiltration, wheezing and bronchospasm (40), many studies have been carried out in stable asthmatics before and after bronchoprovocation with allergens or occupational agents without major complications. However, particular attention and

careful monitoring is advised in asthmatics with marked bronchial responsiveness (41).

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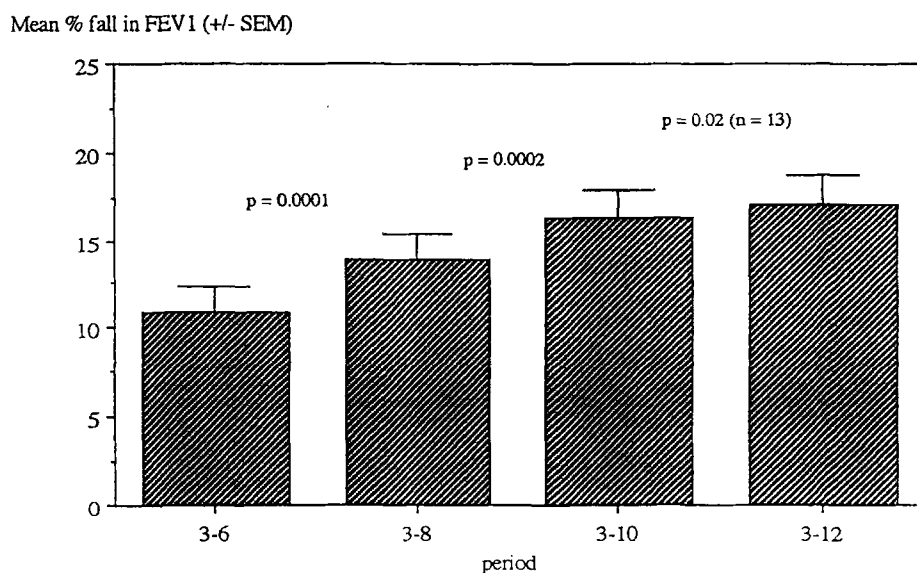


Fig. 3. The mean fall in FEV₁ value measured in different periods after allergen challenge in the same groups of patients as in Figure 1.

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Cautions and contraindications in allergen bronchial provocation testing (BPT)

When safety measures are observed, BPT is as safe as any other *in vivo* allergy test. In a review of 3000 BPT performed in our Centre, only 4 reactions required medical intervention (1).

The SEPCCR "Bronchial Hyperreactivity" Working Group (2) established a set of recommendations and safety procedures to be adopted when performing BPT.

General

Prior to challenge, informed consent must be obtained from the patient. Full cardiopulmonary resuscitation equipment must be available in the laboratory, and the patient must be under supervision by a full-trained doctor for at least 8 h after the last allergen inhalation. β -2 adrenergic stimulants, steroids, vasopressors, intravenous fluids, and oxygen should be readily accessible. The challenge room should be well ventilated, for the rapid removal of aerosolized allergen from environment.

Provocation tests with allergens

Allergen extracts

Allergens must be standardized as described in section entitled Allergen extracts for BPT of this volume.

The concentration or dose of allergen administered first should be the one that produced a 2+ reaction on intracutaneous injection or a 3-mm wheal on skin prick test (3, 4). This "empirical" procedure has been demonstrated to be safe and not to induce degrees of bronchial constriction that could be dangerous. Allergen extract in powder, on the contrary, is administered in pre-established sequential doses (5).

Patient conditions

BPT should not be done at a time when there are active asthmatic symptoms. Many factors can alter basal bronchial tone. Recent upper and lower respiratory tract infections, vaccinations with live attenuated viruses and exposure to occupational sensitizers and to severe atmospheric pollution are known to induce nonspecific bronchial hyperresponsiveness. The recommended time intervals between exposure to these factors and an allergen provocation test vary widely and are indicated in Table 1.

Table 1.

Factor inducing nonspecific bronchial hyperresponsiveness	Recommended interval between exposure and challenge
Upper and lower respiratory tract infections	6 weeks
Vaccinations with live attenuated viruses	6 weeks
Exposure to occupational sensitizers	several months
Exposure to high degree of atmospheric pollution	1 week

Challenge procedures

Herxheimer (6) described hypersensitization to allergen following prior experimental laboratory exposure. Thus, it seems that prior exposure increases the degree of bronchial response to BPT. This resembles the "priming effect" described by Connell (7). Kelly et al. (8) suggested that priming might be triggered also by natural environmental pollen antigen exposure. Moreover, allergen environmental and laboratory exposure dramatically increases nonspecific bronchial responsiveness in sensitized subjects (see section on Correlation between allergen-induced and nonspecific airway responsiveness).

Taken together, the foregoing findings emphasize that BPT should be done at least 1 week after a previous allergen challenge, and, in patients

allergic to pollens, out of the pollen season. The patients' clinical history should be carefully examined for the presence of any of the factors listed in Table 1. If these precautions are not taken, reproducible basal FEV₁ and nonspecific bronchial responsiveness should be measured before performing BPT.

Other general contraindications are: a) diseases of the immune system, or other relevant organic disease; b) conditions that make it difficult to manage adverse reactions, such as coronary artery or hypersensitive disease, or patients on beta-adrenergic blockers; c) patients who developed severe or generalized reactions during previous BPT; and d) pregnancy.

Management of adverse reactions induced by challenge

Most mild bronchospastic reactions require no specific treatment. Severe ones that may last for a few days can be managed by simple measures such as β -2 agonists or steroids.

Asthmatic reactions are treated by conventional means, using nebulized or injected beta-adrenergic agonists, oxygen, and steroids. Although not described to date, stridor due to laryngeal oedema may require parenteral epinephrine, oxygen, intubation, and tracheostomy. Systemic reactions, including hypotension, angioedema and urticaria, are treated by subcutaneous epinephrine and by steroids.

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Reproducibility of bronchial allergen challenges

Introduction

A measurement that is totally unrepeatable clearly has no validity. Repeatability, however, or reproducibility, is an ambiguous concept without precise definition. Comparing populations, a low reproducibility of a test can be compensated for by increasing the number of patients investigated or by investigating each patient many times. When investigating a single patient, reproducibility can, however, only be compensated for by repeating the test. The favorite approach is to calculate the product-moment correlation coefficient, r , between the two methods of measurement, or the slope of the least-squares regression line tested against line of identity, and to express reproducibility as a coefficient of variation (SD/mean). One way proposed to express repeatability is: as the value below in which the difference between two test results may be expected to lie with a specified probability of 95%, in absence of other indications, and reproducibility, as the value below which two single test results, obtained under different conditions, may be expected to lie with a specified probability, 95% (1, 2).

Reproducibility data on bronchial allergen challenges from different centers

Early asthmatic response. There are few data on the reproducibility of response to inhaled allergen, and most published data relate to single dose-response relationships, and the early asthmatic response (EAR). In 1957, Tiffeneau (28, 29) found a significant correlation ($r=0.857$, $P<0.01$) between repeated measurements in 16 asthmatics on 2 days for the "dose liminaire" for antigen. Ruffin (26) found a coefficient of variation equal to 7.2% in pollen challenge tests performed at 4-day intervals. Löwhagen (20) reported a coefficient of variation in $PD_{20}FEV_1$ of 23% for repeated responses to antigen in 20 asthmatics. The same individual allergen doses were inhaled on 3 to 9 occasions at intervals of 1 week or more, during a variable period of up to 24 months. There was no evidence of either priming or desensitisation of the airways to the effects of antigen. Rosenthal (25) demonstrated a ten-fold intra-subject difference in the response to antigen, and agreed that repeated antigen challenge did not systematically alter the response of the patients challenged on 4 successive days. Fish (11) investigated 10 asthmatics, undergoing immunotherapy, receiving a minimum of 5 challenges during a 1-year period. They found a $0.91 \log_{10}$ dose intra-subject variation for

$PD_{35}SG_{aw}$, and $0.70 \log_{10}$ dose intra-subject variation for PD_{20} . Tamura (27) used single-exposure method for inhalation challenge and found good reproducibility, but their technique was very different to others reported, as the essential index studied was the time at which the conductance decreased after the same high allergen dose was administered in all patients. Kopferschmitt-Kubler (17) obtained a satisfactory reproducibility in a 2-week interval analyzing the $PD_{15}FEV_1$, the $PD_{20}FEV_1$, the overall slope and the area under the parabolic curve. PD_{20} was significantly reproducible in a range of 1 doubling dose (2-fold variation). Eiser (10) found the antigen dose-response curve highly repeatable, investigating 9 asthmatics on different days, where the pre-challenge baseline lung function varied less than 20% within an individual, the whole dose-response curves were compared by several types of analyses. Frølund (14) demonstrated a reproducible test evaluated by the Spearman rank correlation coefficient ρ ($\rho=0.90$, $P<0.05$), investigating 15 patients on two different days with a standardized challenge model, using tidal volume breathing. Another study (13) investigated 19 extrinsic, stable asthmatics with a median interval of 15 days, using a tidal volume breathing method, calculating a $PC_{20}FEV_1$ allergen, as well as a $PC_{40}R_1$ allergen, R_1 expressing the total resistance to breathing determined by an opening interruptor method. The reproducibility of $PC_{20}FEV_1$ allergen was high, with a 95% tolerance limit (TL) for a single determination being the observed value ± 0.83 , ten-fold concentration difference, the intraclass correlation (IC) 0.99 and the coefficient of variation 8.46%. Concerning $PC_{40}R_1$ allergen, a 95% TL for a single determination was ± 0.58 , IC was 0.99 and the coefficient of variation was 5.79%. The reproducibility of a provocation test using a dosimeter method (12) expressing results either as a $PC_{20}FEV_1$ or the cumulative dose showed a 95% TL ± 1.80 two-fold concentration difference for PC, and ± 1.76 for the cumulative dose. When bronchial challenge has to be repeated after a long time, reproducibility declines (9).

Late asthmatic response. Concerning the late asthmatic response (LAR), few studies are available giving data on reproducibility. Crimi (9) rechallenged from 1 to 6 months using a dosimeter-method and found the reproducibility of LAR was better in patients with well reproducible EAR (coefficient of variation=8%) than in patients in whom a different dose of allergen was administered on the two occasions (coefficient of variation=22%). Studies (12–15, 18, 19, 21) were able to

Provocation tests with allergens

detect and confirm the dose-dependence on the dose of allergen administered and the occurrence of LAR, using domiciliary peak flow (PF) recordings and symptom score, and Moscato (22) concluded that PF readings are a useful diagnostic tool in assessing the airway response during a specific bronchial challenge and can be used to monitor the LAR in relation to clinical symptoms compared with FEV₁ measurements. Rasmussen (23), however, showed that after one challenge-specific airway reactivity, i.e., reactivity to allergen, at a subsequent rechallenge is increased in the late phase, and that this late-phase hyperreactivity seems to persist for at least 2 weeks after allergen challenge. Furthermore, this study showed a high reproducibility of the two challenges, the mean difference between tests being 0.120 (95% confidence interval: -0.127 to 0.367), $r=0.115$. It has been shown that increased non-specific reactivity can be present up to weeks after allergen exposure (4, 8, 21).

Expressing repeatability and reproducibility statistically

Expressing repeatability and reproducibility as a coefficient of variation is not satisfactory, as the coefficient is influenced by the range of variation between subjects or other units chosen to test the methods (1, 2). As repeatability studies are carried out with just two repeat measurements per subject, the differences between the first and the second measurement for each subject ought to be calculated (2, 5, 6), and the standard deviation of the differences or within subject standard deviation may be reported as a measure of repeatability referred to as the single determination standard deviation and a 95% range given. Dividing the standard deviation by the mean to give the coefficient of variation implies that the value is constant for different mean values and implies that a log transformation should be used before analysis. To achieve a dimensionless index, the ratio of between-subject to total variation, known as the intraclass correlation coefficient, can be calculated. The maximum value of the intraclass correlation coefficient is 1, achieved only when repeatability is perfect. Unfortunately, terminology has been confused concerning 95% ranges, as ranges and standard deviations measure variation in individual measurements, whereas confidence intervals and standard errors relate to the precision of population estimates. Emphasis that a 95% range is not a 95% confidence interval can be made by denoting it a reference range when applicable, or a 95% tolerance range, or tolerance limits.

Sensitivity and specificity of bronchial allergen challenge

There is some logic in the assumption that by testing the organ of involvement, which in the case of the asthmatic patient is the lung, conclusions drawn regarding cause-and-effect relationships with allergens and clinical symptoms might be more meaningful than if another organ, such as the skin, is used to assess this relationship. The sensitivity of a diagnostic test is evaluated by the ability of the test to recognize true disease when it is present, whereas specificity is evaluated by the ability of the test to reject it when the disease is absent. Investigators have found considerable overlap in the allergen provocation response among patients who have extrinsic asthma and patients who suffer from allergic rhinitis (3, 24, 30). A good correlation between skin tests, *in vitro* tests and bronchial provocation has been found; the stronger the skin reaction the greater the chance of a positive bronchial allergen challenge (31), especially correlated to the nonspecific bronchial hyperreactivity (3, 7, 8, 30), whereas this correlation is debatable in others (16). With a few exceptions, a negative allergen challenge to a certain antigen may exclude the possibility that the allergen is responsible for asthma. In contrast, a positive challenge only indicates sensitivity to the allergen and may be clinically unrelated to their asthma. Also there is a difference in allergens, since patients with pollen sensitivity by skin tests and a subsequent positive bronchial challenge numbered more than the patients with house dust reactivity (31), but this may depend on nonstandardized extracts.

Conclusion

It is essential that allergen inhalation challenges are to be performed in a standardized manner, each step of the method described, and reproducibility data presented, however keeping in mind the aforementioned cautions about clinical usefulness, but potential to help in answering other questions. Data on the sensitivity and specificity of the test in the clinical scenario may be a subject of future study.

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Correlation between allergen-induced and nonspecific airway responsiveness

BPT, which utilize aerosols of small liquid droplets or of micronized solid particles, are laboratory tests. They mimic the effects of natural exposure, but with some important differences: the amount of inhaled allergen, inhalation of an allergenic extract instead of allergen in natural form, time course of the inhalation, etc. Therefore, we cannot assume that any laboratory-studied relationship between allergen-induced airway responses and nonspecific responsiveness has an absolute value in clinical practice. Nevertheless, many laboratory phenomena have been confirmed in *in vivo* studies. Therefore, the study of the correlations between allergen-induced and nonspecific bronchial responsiveness can be useful to understand better the pathophysiology of bronchial asthma.

Importance of nonspecific bronchial responsiveness in allergen-induced asthma

Nonspecific bronchial hyperresponsiveness can be responsible for false-positive responses from allergen bronchial provocation tests. In fact, many irritants are contained in commercial allergen extracts. Thus, if their concentration is high, they

Provocation tests with allergens

can induce a nonspecific bronchial response in a hyperresponsive asthmatic. Moreover, due to their molecular weight and osmotic properties, allergenic proteins are irritant *per se*. Therefore, if we administer high doses of allergenic extracts to a hyperresponsive asthmatic, a false-positive response can be induced regardless of irritant particles. Thus, when a BPT is performed, the highest dose that will not induce nonspecific bronchial reactions must be established previous to testing.

Moreover, nonspecific bronchial responsiveness is, at least in part, responsible for the degree of allergen-induced asthma (1–3). Correlations have been observed between airway responsiveness to histamine (4–6) or acetylcholine (7, 8) and allergen. Bryant & Burns (4) and Cockcroft et al. (1) demonstrated that the correlation is higher if the serum level of IgE antibodies and airway responsiveness to histamine are regressed together with airway responsiveness to allergen. Moreover, Cockcroft et al. (1) demonstrated that allergen concentration significantly influences this correlation.

These results suggest that a mild allergic reaction is sufficient to trigger asthma in a patient with severe bronchial hyperresponsiveness. Moreover, significant exposure to a sensitizing allergen could induce a bronchospastic response in an allergic asymptomatic subject (4, 9, 10).

Therefore, bronchial responsiveness to an allergen depends on the degree of nonspecific airway responsiveness and, moreover, on the dose of allergen and on the level of IgE antibodies.

Allergen-induced increase in nonspecific bronchial responsiveness

Natural and laboratory exposure to antigens has been demonstrated to increase nonspecific bronchial responsiveness to a number of nonspecific agonists (11–17) in sensitized subjects and animals. Conversely, removal from environmental exposure often results in decreased bronchial hyperresponsiveness (18–22).

The severity of increase in nonallergic responsiveness may be profound, and it may progress over several days following allergen exposure (23). Moreover, allergen-induced hyperresponsiveness may persist for one or more days up to several weeks (23).

These studies explain why, when allergen provocation doses are compared with the provocation doses of histamine or methacholine in the asthmatic zone, two diametrically opposed conclusions can be reached: good correlation, if the provocation dose of mite or animal dander is studied, or no correlation, if the provocation dose

for pollen is evaluated (24–26). In fact, pollen BPT are generally performed out of the pollen season, when nonspecific bronchial responsiveness decreases naturally, whereas mite and animal dander exposure is continuous, representing a continuous stimulus for bronchial hyperresponsiveness.

The increase in airway responsiveness *in vivo* has been linked with an antigen-induced inflammatory process (27), but the underlying mechanisms remain unknown. Moreover, it cannot be excluded that allergen challenge leads initially to damage of the epithelium, thereby facilitating the passage of the provoking substance: but there is no relationship between increased epithelial permeability and increased nonspecific bronchial responsiveness (28). Therefore, epithelial damage cannot be considered responsible for the allergen-induced increase in nonspecific bronchial responsiveness.

Recently it has been demonstrated that during the late asthmatic reaction following BPT there is a dramatic increase in the number of eosinophils and other inflammatory cells migrating into the airways (29–31). Moreover, many investigators have observed an increase in bronchial responsiveness following the late response induced by allergen provocation in sensitized human subjects (32, 33). Therefore, a relationship was hypothesized between inflammatory cell migration, late asthmatic reactions and allergen-induced bronchial hyperresponsiveness.

Thereafter, it was demonstrated that an increase in airway histamine, methacholine and AMP responsiveness, recorded 3 h after allergen inhalation, precedes the late asthmatic response (34–36), and that there is a significant correlation between the increase in methacholine bronchial hyperresponsiveness at 3 h and the development of late asthmatic reactions (36). Also some hours after the exposure to allergen, mast cells were found to be activated and recruited in bronchial mucosa (37, 38). Moreover, a significant correlation between the percentage of eosinophils in BAL and the late fall in FEV₁ has been demonstrated (30, 39, 40). These findings could be regarded as indirect evidence that the contribution of inflammatory cells plays an important role in allergen-induced increase in nonspecific bronchial responsiveness.

The links between inflammatory cells and bronchial responsiveness are probably complex and are mediated by many different inflammatory mediators. The most likely mechanism involves a mediator-induced alteration of neural and ganglionic function of cholinergic airway nerves rather than an alteration in airway smooth muscle contractility *per se* (17): but the debate continues.

Conclusion

The study of the correlations between allergen-specific and nonspecific bronchial responsiveness is complex, and much more needs to be done before the mechanisms are clarified. Certainly, bronchial responsiveness to an allergen depends at least in part on the degree of nonspecific airway responsiveness. Moreover, there is an antigen-induced increase in bronchial responsiveness, probably due to allergic inflammation and related to the late asthmatic reaction. Therefore, it is necessary to evaluate both antigen-specific and nonspecific bronchial reactivity when performing BPT for clinical or research purposes.

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Standardisation of nasal provocation tests

Introduction

The concept of airway hyperreactivity in allergic disease is well established (1). Particular interest has been directed towards the response of the lower airways of asthmatics to inhaled allergen (2) and to nonspecific pharmacological agents such as histamine and methacholine (3). Large numbers of reports are to be found in the literature that cover all aspects of bronchial reactivity, and the bronchial provocation test has been well standardized (2, 4, 5) and has been shown to be reproducible (6, 7). This is in contrast to the number of studies in the upper respiratory tract, which is surprising considering that 10–15% of the population suffer from hayfever (8) and there is evidence that the incidence is increasing (9). Nasal reactivity to both histamine (10) and methacholine (11) has also been demonstrated in rhinitic subjects, but its usefulness as a diagnostic aid is limited. The diagnostic use of allergen provocation in the nose has not yet been fully explored, and the techniques used vary. Since the mid-1970s there has been an increasing interest in nasal provocation tests, the main reason being that reproduction of allergic disease under controlled conditions allows not only a careful study of its pathophysiology, immunology and pharmacology but also provides definite confirmation of the role of a particular allergen or nonspecific agent in the complaints of the patient (12). The diversity of nasal provocation techniques creates problems with

interpretation, and there is clearly a need for standardization and rationalization of procedures. This section will outline some of those techniques and discuss the advantages and disadvantages associated with each.

Nasal provocation tests

The first documented nasal provocation tests were carried out by Charles Blackley in 1873 (13), who applied pollen grains directly to the nares and mucous membrane. This resulted in the symptoms of rhinorrhea, sneezing, itching and nasal blockage characteristic of allergic rhinitis. It was not until the work of Aschan & Drettner in 1958 (14) that the potential of the nasal provocation test was realised. They used the technique of posterior rhinomanometry to assess the effect of antihistamines on the nasal response to allergen. Since this time a range of substances have been used in nasal provocation, including allergen, anti-IgE, 48/80, histamine, methacholine and NH_3 (15).

Although the importance of nasal provocation as a research tool for the investigation of the pathophysiology and pharmacology of allergic disease is well established, its role as a clinical diagnostic procedure is recognized but not yet fully explored. In general, seasonal allergic rhinitis can be diagnosed by a positive clinical history supported by either positive skin test and/or RAST, and nasal provocation would not always be required. In the case of occupational agents, a clinical history may be far from conclusive and the appropriate nasal provocation should be carried out. Huggins & Brostoff (16) have reported symptoms of dust mite induced rhinitis in patients with negative skin tests and negative RAST, and nasal provocation may help in such cases of diagnostic difficulty. The nasal challenge may confirm the role of a certain allergen or nonspecific agent in the patient's nasal complaints. These tests may also demonstrate the dose-response and time-response curves to a particular allergen or nonspecific agent (12). The nasal provocation test can be used in the demonstration of the early, late and delayed nasal response to allergen (12, 17). Following nasal challenge, repeated recording of NAR can demonstrate such phases of nasal obstruction. Other more specialized indications for nasal provocation include confirmation of occupational allergy (19), and the assessment of different therapies (12, 20–22) and immunotherapy (23). Nasal provocation testing may also cause symptoms and signs in adjacent sites such as conjunctiva, middle ear, sinuses and bronchial tree, which may be of diagnostic value (12, 22, 24).

Allergen application

Unlike the lower airways, the nose provides the ideal site for allergen provocation. Inhalation of allergen into the lower respiratory tract must be carefully controlled to avoid severe bronchospasm. Bronchial challenge with allergen therefore requires strict criteria and protocols, highly qualified personnel and facilities for resuscitation. The nose, however, is readily accessible and allergen can be applied to the mucosa with a high degree of accuracy. If the subjects hold their breath during the application, exposure to the lower airways is minimal. The nose is divided into two parallel orifices by the nasal septum. Beyond the vestibulae and the bony cavum the lateral walls are lined by the inferior, middle and superior turbinates. The turbinates, like the rest of the nasal cavity, are lined by ciliated epithelium and have a rich supply of blood vessels in the submucosa. The blood vessels of the submucosa can be divided into two types. Those closest to the basement membrane are essentially arterioles that supply the surrounding tissue and may be important in the humidification and warming of inspired air. Deeper down in the submucosa are the venous sinusoids, which can rapidly engorge with blood in response to sympathetic stimulation. Rapid engorgement of the sinusoids is a characteristic of the nasal response to allergen and nonspecific agents. The anterior section of the inferior turbinate is clearly visible from the nasal vestibule and provides the ideal site for direct allergen application.

Several techniques have been used to apply allergenic or pharmacological agents into the nose. Dropping allergenic substances onto the nasal mucosa via syringes or bottle droppers is potentially hazardous, since fluid may pass down to the larynx and in some cases may cause laryngeal edema or penetrate into the bronchial tree, causing unexpected bronchospasm. In addition, the deposition and distribution of the allergen applied by this method cannot be predicted. The use of a micropipette to deliver small and exact volumes of allergen onto the turbinate (24) directly improves on the droppers, but the post-application distribution is difficult to control. Allergen extract prepared as freeze-dried micronized powder with lactose as the vehicle stored in ready to use capsules containing 2.5 to 80 allergenic units can be nebulized into the nose by a powder insufflator while the patient holds his or her breath. This method has been shown to be simple, reproducible and not to induce any bronchospastic reactions (25). Impregnated paper discs (26) inserted into the nares as well as administration of allergen via nebulized solutions (8) have also been used. Pelikan et al. (18) intro-

duced the application of allergenic extracts by means of cotton wool on a nasal probe placed under the medial concha. Philips et al. (20) applied allergen extract directly onto the anterior section of the inferior turbinate using a modified airbrush technique. As a safety measure, it is advisable to begin the nasal challenge with allergen concentration lower than that required to produce a 3-mm skin wheal on skin prick testing as postulated by Davies & Corrado (27). Another important factor to consider is a small but existing risk associated with the nasal provocation tests with allergen, and therefore these tests should be performed by well-trained personnel under standard conditions and in a reliable, well-equipped unit. The challenge procedure should be considered to be a model technique for stimulation of natural exposure. The above techniques are all capable of delivering allergen to the nose and initiating a response, but some of them can deliver allergen in higher dose than that occurring 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. However, this method is applicable only for pollen and not for other allergens. Studies by Naclerio (28) and Bousquet (29) have shown that the symptoms produced using this technique correlated with mediators recovered in nasal secretions and with the skin prick test end-point. Unfortunately, they did not relate these findings to clinical measurements of the nasal response.

Other studies with pollen grains by Connell (30) have demonstrated the priming effect in allergic rhinitis. Repeated allergen exposure leads to an increased allergic response to the same dose given subsequently. Nasal challenges with allergens in powder form were also studied by Schiano et al. (31).

Measurement of response

The basic pathophysiologic mechanisms in the nose have been summarized by Mygind (8), and nasal response to allergen provocation should be measured with reference to these mechanisms. In the simplest terms, stimulation of the nasal mucosa by allergen leads to rapid vasodilation, edema and the release of chemical mediators (e.g., histamine, leukotrienes and prostaglandins). These reactions result in the symptoms seen in rhinitis; the nasal mucosa becomes edematous and there is usually marked rhinorrhea and sneezing. A summary of the methods currently available for measuring these responses is given in Table 1. The most obvious and probably the simplest method is to look at the appearance of the nasal mucosa using anterior rhinoscopy (8). This, however, does not

Provocation tests with allergens

Table 1. Methods for assessing nasal response to inhaled allergen

Anterior rhinoscopy (27)	
Sneeze count/sneeze drip score (33)	
Volume/weight of secretion (34)	
Nasal peak inspiratory/expiratory flow rate (36, 37)	
Nasal spirometry (38)	
Tests of NAR	Passive anterior (28) Active anterior (39)
Rhinomanometry	Active posterior (18, 39) Acoustic (46) Balloon method (17, 18)
Oscillometry (52)	
Laser Doppler Velocitometry (48)	

allow any quantitative assessment of the nasal response. Sneeze counts, sneeze/drip scores and volume/weight of secretions have also been used (32, 33). However, these parameters allow only semiquantitative recording. Moreover, not every nasal response is accompanied by significant sneezing. Therefore it is advisable to use these parameters only in conjunction with recording of objective parameters such as nasal patency.

Factors that affect nasal patency have been discussed elsewhere (34), and several methods for its assessment have been developed. Taylor et al. (35) used a modified Wright peak flow meter to measure nasal peak expiratory flow. This method is simple to perform, but following allergen challenge it is impossible to avoid secretions contaminating the apparatus. A modified mini-peak flow meter has been developed to measure peak inspiratory flow (36); again this is simple to perform, but forced maneuvers through the nose can result in reflex changes in nasal patency, and such techniques may not be suitable for provocation procedures.

Modified spirometric procedures have also been investigated (37) with similar limitations to those noted above.

The most widely employed measurements to date are those of nasal airway resistance (NAR) or parameters derived from NAR. Several techniques for the measurement of NAR are available and these are summarized in Table 1.

Rhinomanometry. Rhinomanometry can be used to measure NAR in four different ways. Air pressure and/or air flow or their derivatives (e.g., resistance, conductance, etc.) are recorded. In passive rhinomanometry, pressure and airflow are recorded during breath holding, whereas active rhinomanometry involves their recording during tidal nasal breathing. Active rhinomanometry can be further subdivided into active anterior, active posterior and modified techniques.

Passive anterior rhinomanometry. For this procedure air is passed through one nostril at a fixed flow rate during a breath hold with the mouth open. The air pressure is recorded and the resistance ($\text{kPa}\cdot\text{l}^{-1}\cdot\text{s}$) can be calculated for each nostril independently (27). The main drawback with passive techniques is that they do not truly represent the physiological process, and changes that occur during the period of change over from recording on one side to recording on the other may be missed. On the other hand, this technique is simple, but has a rather high SD and SE and the results can be influenced by anatomical abnormalities in the nose (e.g., distinct deviation of the septum). This is a suitable technique for screening, in children (who may be anxious when using sophisticated equipment), patients with an active gag reflex or those with problematic dental prostheses.

Active anterior rhinomanometry. For the active procedure, air flow and pressure are recorded during tidal breathing. A tube connected to a pressure sensor is placed into one nostril, which is otherwise occluded by tape (38).

Care must be taken to ensure that the sides of the tube do not contact any part of the nasal mucosa. Flow through the other nostril is recorded by a pneumotachograph in a tightly fitting face mask. In this case, care must be taken to ensure a close fit with as little compression of the external tissue as possible. Resistance is calculated by analysis of the pressure/volume or pressure/flow curve (39) for the nostril that has not been occluded. This procedure is easy to perform and requires little instruction of the patients, but well-trained personnel. However, when used for allergen provocation, problems arise if one nostril becomes very occluded, at which point measurement of resistance falls below the limitations of the recording instrument. In addition, the presence of rhinorrhea due to allergen causes the tape to lose its adherence, resulting in leakage, giving a false reading. This technique has a low SD and SE but is not suitable for patients with fragile and bleeding nasal mucosa or with frequent sneezing.

Active posterior rhinomanometry. In this method, resistance is also recorded during tidal breathing. Flow and/or volume rate is recorded by a pneumotachograph placed into a tight-fitting face mask as described above. Pressure is detected via a sensor tube placed into the mouth. The tube is gripped firmly between the lips and is held underneath the palate. The sensor detects the pressure at the back of the mouth, which is usually equal to that behind the nasal passages. Resistance is again calculated from flow/pressure or volume/pressure curves.

However, in this case only total resistance is calculated. The advantage of this system is that it is still possible to take readings of total resistance even when one nostril is markedly occluded or there is a degree of rhinorrhoea.

The major disadvantage is that about 20% of individuals cannot perform the test adequately even with repeated training (34). This technique is laborious, requires well-trained personnel and is not suitable for children (anxiety aspects) or for patients unable to tolerate the close-fitting mask.

The resistance of nasal airways is calculated as:

$$r = \frac{dP}{V}$$

where "r" is the resistance in $\text{kPa} \cdot \text{l}^{-1} \cdot \text{s}$, "dP" is the change in nasal pressure (kPa) and "V" is the flow rate ($\text{l} \cdot \text{s}^{-1}$).

For the two anterior techniques where the resistances of each nostril are calculated separately, the total resistance is calculated from Ohm's law of parallel resistors:

$$\frac{1}{r_l} + \frac{1}{r_r} = \frac{1}{R_t}$$

where r_l = resistance in left side, r_r = resistance in right side, R_t = total resistance. It is important when this technique is used to state that the total resistance has been calculated and not measured.

The two active techniques record pressure and flow (or volume) simultaneously, and in most cases this can be plotted as a flow/pressure or volume/pressure curve. The curve is sigmoid in shape and passes through the origin at the change from inspiration to expiration (Fig. 1). This curve is used to calculate nasal resistance. Use of the relationship between flow/pressure described above oversimplifies the physiological changes occurring in the nose during tidal breathing. It has been shown that nasal airflow can only be considered laminar at pressures below 40 Pa (40) and, as a result, the slope of the curve cannot be used to calculate NAR. Attempts have been made to overcome this problem. Some investigators suggested a technique that uses the angle produced by the curve to record resistance (41). The steeper the curve the greater the resistance. Others (42) proposed sampling the resistance at a range of pressures on the curve. The most widely used interpretation to date was defined by the European Committee on Standardization of Rhinomanometry (39, 43).

This suggested that resistance should be calculated at fixed pressures of 150 Pa for the active anterior method and 75 Pa for the active posterior method. We have found, however, (unpublished data) that

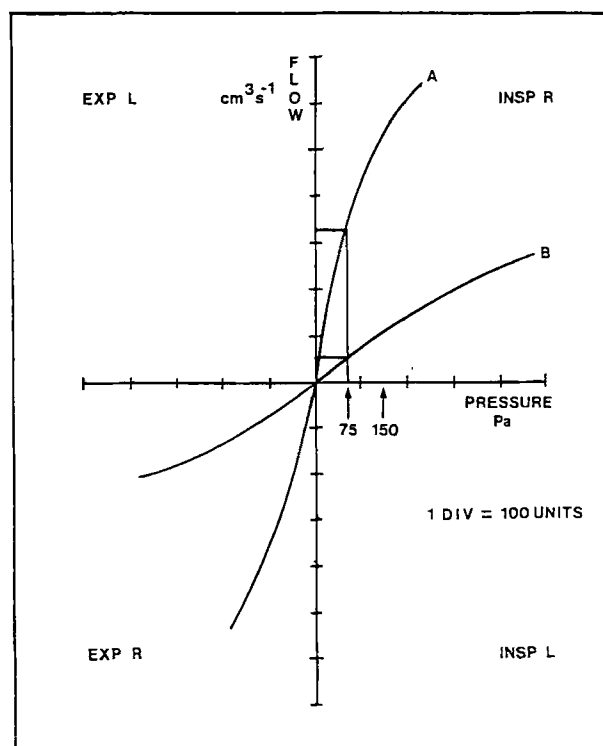


Fig. 1. Examples of pressure/flow curves obtained from a rhinomanometer. Flow is recorded at either 75 (anterior method) or 150 Pa (posterior method) as shown. Curve A represents a relatively low NAR (unblocked) whilst curve B represents a high NAR (blocked).

recording at 150 Pa excludes certain subjects from analysis; patients with relatively unblocked nasal passages and patients of African/Oriental origin with wider nasal passages are often unable to generate such high pressures and NAR cannot be recorded. This factor should be taken into consideration when standardizing the methodology.

Modified techniques of rhinomanometry. Some techniques of rhinomanometry differ from the previous types, either in the parameters recorded or in method of recording of the response.

- The balloon method, used by Pelikan (12, 17, 18), measures the pressure differences (ΔP) between the nasopharynx-nose and outside air by means of a small rubber balloon introduced into the posterior part of one of the nasal cavities. The increase in mucosal edema due to an allergen or nonspecific agent leads to pressure differences between the nasopharyngeal cavity and the outside air, while the airflow is constant. The pressure differences, (the nasopharynx-nostril-pressure gradients or NPG) expressed in cm of H_2O are recorded and considered as a parameter for assessment of the nasal response. This technique is a contralateral method, where

Provocation tests with allergens

one nostril is challenged and the NPG parameters are recorded through the other nostril. This technique is not difficult, has a low SD and SE, and is not influenced by anatomical disproportion. It is not suitable for patients with fragile or bleeding nasal mucosa.

- Body-plethysmography modified for nasal measurements (44).
- Acoustic rhinomanometry, a newer technique for evaluating nasal patency, has been developed recently (45). This technique uses the reflection of sound waves from the nasal cavity to assess the cross-sectional area. Any changes in the cross-sectional area of the nasal cavity result in a change in the acoustic impedance. In acoustic rhinomanometry, sound in the range 2–10,000 Hz is pulsed into the nasal cavity at a fixed interval while the subject breathes in a tidal manner. By accurate recording of the incident and reflected sound waves within a given time lapse, the distance from source to the reflecting body can be determined. This can then be related to cross-sectional area. Recorded pressures in the mouth and flow rates in the nose can be combined with acoustic measurements to give a measurement of NAR. This technique (oscillometry) described by Berdel et al. (46) has been compared to the more common methods of rhinomanometry and has been shown to be highly reproducible (47). The role of such techniques in nasal provocation has yet to be fully assessed.

Non-rhinomanometry techniques. Other techniques based on principles different from those of rhinomanometry, are sometimes used for assessment of the nasal response, mostly for research purposes.

- Laser Doppler velocitometry (48) measuring capillary blood flow in the nasal mucosa, altering after a nasal response to allergen or nonspecific agent.
- ¹³³Xenon washout clearance (49) recording the capillary blood flow in the nasal mucosa, being a result of different components of the nasal vasculature.

Reproducibility of rhinomanometry. Rhinomanometry has been criticized because a number of factors could alter the recorded parameters, for example, compression of the soft exterior nasal tissue by a tight-fitting face mask or air leakage from the tape used to occlude one nostril.

In most cases these problems can be overcome by careful training of the operators and attention to detail. We and others (18, 47, 50) have studied the reproducibility of rhinomanometric tech-

Table 2. Summary of studies of reproducibility of rhinomanometry

Method	Shelton et al. (47)		Ferguson & Thomas (50)
	Coefficient of variation		Coefficient of variation
	Normal	Rhinitics	Rhinitics
Passive anterior	10.0	12.0	27.9
Active anterior	11.0	18.0	30.6
Active posterior	14.0	19.0	23.2

niques. The coefficients of variation were within acceptable limits in each case (Table 2). There is some disagreement as to which technique is the most reproducible. The active anterior technique is the most commonly used (39) and requires relatively little patient instruction. We have found, however, that when using the anterior method with allergen provocation, recording becomes difficult when the nostril tested becomes occluded. In these cases, recording of NAR is not possible as the degree of blockage exceeds the limitations of the recording device.

Similarly, nasal secretions tend to dislodge the tape holding the pressure transducer, and reproducible results are not always possible. We have found that the most acceptable technique to use for challenge is active posterior rhinomanometry. Although it is reported that approximately 20% of individuals cannot perform the technique adequately (34), in those who can be studied, total NAR is nearly always recorded even when one nostril is markedly occluded. It has been reported that the anterior method is useful because one nostril can be challenged and the other serves as a control (27). We have observed (unpublished data) that in some cases the contralateral nostril will occlude. The same effect has been reported by Pelikan et al. using the modified posterior method (18, 51) with contralateral challenge. Their data indicate also a good reproducibility of the balloon technique.

What is the role of nasal provocation? The usefulness of nasal provocation in clinical diagnosis is becoming recognized. However, there is no doubt about the importance of such procedures for research into the pathology of rhinitis. Many workers have used nasal provocation to assess various factors associated with rhinitis.

The basic indications for nasal challenge (10, 12, 17, 19, 20, 22, 27, 51) include:

- to confirm the role of a certain allergen or non-specific agent in provoking nasal symptoms, especially in the case of discordance of skin tests, RAST or history (51, 52);

- to investigate the existence of the particular types of the nasal allergen response: immediate, or late (10–12, 17, 19);
- to establish the dose-response curve (= allergen titration);
- to confirm a possible role of a certain food in the nasal complaints (food allergy) (22); and
- to examine the protective or therapeutic effects of a certain drug on the nasal response to allergens or nonspecific agents (12, 20, 21, 25, 32, 53) or effects of immunotherapy (23).

Other possible applications of nasal provocation tests include:

- the confirmation of the role of an allergic reaction in the nasal mucosa in provocation of symptoms at other sites, e.g., conjunctiva, middle ear, sinuses, etc. (12, 22, 24); and
- to discriminate the role and involvement of allergy (nasal challenge with allergens) and non-specific hyperreactivity (nasal challenge with histamine or methacholine) in rhinitis (51, 52).

Diagnosis. Nasal provocation may have an important role in the diagnosis of rhinitis, as discussed earlier (12, 19, 20, 23, 27).

Late-phase nasal responses. The existence of a late-phase response to allergen in the nose has already been demonstrated; however, studies of the phenomenon are not yet numerous (12, 17, 22, 51, 55). The late-phase nasal response has recently been reviewed elsewhere (51, 52, 56). In this review, the authors highlighted the need for standardizing provocation techniques in order to verify the occurrence of such reactions. Most of the studies have shown that the late-phase reaction occurs in about 30–50% of the individuals tested (12). The intensity of the immediate reaction is not directly related to the magnitude of the late response, nor can it be considered to be a suitable predictor of the late response (12). The mechanisms underlying these reactions have not yet been fully clarified (12).

Cellular response to allergen. Identification of the cellular response to inhaled allergen is essential to our understanding of the pathology of allergic rhinitis. Although some of the techniques used for nasal provocation deliver allergen in amounts greater than those experienced naturally, they have contributed significantly to our understanding of rhinitis. Nasal lavage, biopsy and brushing have all been used to investigate cellular responses to allergen provocation. The techniques used vary widely but have yielded valuable information on the role

of the mast cell (57), the eosinophil (58, 59) and also other cell types and their dynamics in the immediate nasal response to allergen as well as in the late-phase response (12, 51, 55, 59, 60).

Chemical mediators. The appearance of a number of chemical mediators of inflammation has been demonstrated in lavage fluid following allergen provocation (61). These include histamine and kinins, which produce nasal itch, sneezing and rhinorrhea and also prostaglandin D₂ and leukotrienes C₄, D₄, which have been shown to increase NAR (62). A number of cytokines can be detected in nasal lavage (63). Their relevance to the development of allergic rhinitis has yet to be defined.

Pharmacological agents. Nasal provocation can be used to investigate the therapeutic effects of pharmacological agents in rhinitis, the so-called “protection tests”. These have included antihistamines (20), sodium cromoglycate (12, 21) and corticosteroids (12, 21, 64, 65). Efficacy can be assessed by reduction in symptoms, changes in nasal patency, changes in cellular response and reduction in mediator release.

Appendix

Example of the schedule – of nasal provocation tests with (12, 51, 54):

A. Nonspecific hyperreactivity agent (e.g. histamine or methacholine)

Serial dilution in physiological saline
0.125; 0.25; 0.5; 1.0; 2.0; 4.0; 8.0 mg/ml

- Recording of baseline values at 0, 5 and 10 min.
- Challenge with diluent for 30 s and recording of control values at 0, 5, and 10 min.
- Challenge with histamine or methacholine for 30 s and recording of values at 0, 5, 10, (20, 30) min.

B. Allergen

- Recording of baseline values at 0, 5 and 10 min.
- Challenge with diluent and recording of control values at 0, 5 and 10 min.
- Challenge with allergen and recording of post-allergen values at 0, 5, 10, 20, 30, 45, 60 min, followed by every hour up to 10 hours (51) for recording of the LAR.
- In the case of positive nasal response to allergen challenge, the control challenge with the diluent should be performed on another day and the control values should be recorded during the same period of time as post-allergen values (60 min or 10 hours).

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The conjunctival provocation test

In its broadest definition, the conjunctival provocation test (CPT) is a test used to evaluate the ocular effects induced by any substance after topical application on the surface of the eye. In its clinical and experimental application, the conjunc-

tival provocation test is generally associated with ocular allergen challenge.

CPTs have been widely used in the last 50 years to detect the evidence of allergy as a check on both positive and negative skin test reactions to various inhalant allergens (1, 2). The results of these studies suggested that eye tests are extremely helpful both in etiologic diagnosis and in determining subsequent therapeutic management. Despite these findings, for several years provocation eye tests (and other mucosal test as well) were placed as a second line approach in the diagnosis of allergy and, to date, skin tests are the widest routinely used allergy tests in clinical practice.

More recently, however, mucosal provocation tests have attracted the attention of many investigators since, other than their diagnostic purposes, they allow the immunopathologic study of the target organ.

The purpose of this paper is to summarize the indications to CPT, to define its technical aspects, to suggest the criteria in evaluating the ocular response and to propose guidelines for the study of antiallergic treatment.

Indication and possible application of CPT

Four major purposes could be potentially achieved by performing CPT in humans:

- the diagnosis of allergy
- the diagnosis of ocular allergy
- research purposes
- the effectiveness of ocular treatment.

Diagnosis of allergy. Basically, the CPT has been used, in the past: a) to confirm the presence of allergy in patients with a positive skin test but a negative clinical history of allergy; b) to prove when skin tests were negative or an indefinite clinical history of allergy was present.

Several reports indicate that the eye, but not the skin, may be responsive to the allergen when a clinical history of allergy is suspected. These reports, however, lack data on the amount of allergen needed to elicit a conjunctival reaction (1-3).

Is atopic patients conjunctiva more or less sensitive than skin after allergen challenge? In our experience, CPT starting with skin test end-point (that is, the concentration of allergen that induced a wheal more than half the area of histamine reaction after subtracting the response to the diluent control) induced a mild or even absent conjunctival hyperemia (4). Our findings are in agreement with other investigators, which suggest that the dose of allergen needed to elicit a conjunctival allergen reaction is about 10,000 times higher than the dose needed to elicit a positive skin test (5-7).

Provocation tests with allergens

Therefore, it seems likely that conjunctiva is less sensitive than skin after challenge with allergen and that CPTs are of limited value in the diagnosis of allergy.

Diagnosis of ocular allergy. Skin test-positive patients with referred seasonal ocular symptoms of possible allergic origin can be clinically and cytologically evaluated by CPT outside their acute season during the remission of the ocular disease. In this case, the severity of the conjunctival positive response will reflect the intensity of the ocular symptoms in season so that prompt diagnosis and early preventive treatment can be started. Previous reports suggest a high correlation between skin-RAST positive patients and positive CPT responders (8).

On the contrary, some reports claim that skin test-negative patients with suspected ocular allergic symptoms but without any evidence of other associated atopic diseases gave a positive reaction following specific ocular challenge (3, 9, 10). These reports suggest that the conjunctiva may represent the only sensitized tissue and support their findings with the presence of a local production of IgE antibodies in tears (11, 12).

In our experience we did not find positive CPT in skin test negative patients without any other associated atopic disease.

Further studies are needed to prove that the eye is the only target organ of an allergic reaction in the absence of any other systemic sensitization. These studies should exclude any bias such as previous manifestation of other atopic disorders, exclude the toxic effect of the allergen on the eye and define the time of the year and the amount of allergen used for CPT (13).

Therefore, although specific CPT is extremely helpful in evaluating the individual conjunctival reactivity in skin test-positive patients, further studies are required to establish its efficacy when skin tests are negative.

Research purposes. Recently, CPT received major attention because it allows one to follow the pathophysiology of the ocular allergic reaction, of the cellular response and of the release of mediators.

The conjunctiva is so well exposed to the macroscopic clinical observation and so easily accessible to diagnostic and nontraumatic procedures that we suggested the study of the eye as a model for allergic inflammation of other atopic diseases (14).

Basically, with the sensitizing allergen, a clinical, cytologic and humoral response appears within a few minutes, reaches its peak at 20–60 min and then gradually subsides. Clinically it is characterized by redness, chemosis and tearing of the

bulbar conjunctiva. Edema of the lids and occasionally nasal symptoms (sneezing and rhinorrhea) may be associated. Itching and mucous discharge are the most frequent subjective symptoms described. Mediators and cells parallel the clinical early phase reaction (EPR). In fact, neutrophils, eosinophils and the levels of histamine, tryptase, kinins, prostaglandin D₂, albumin and TAME-esterase activity all significantly increase in tears of allergic patients (4, 15–17). The intensity of the immediate hypersensitivity reaction is dose dependent: the higher the dose the more intense the conjunctival reaction (18). High allergen concentrations have more chance of inducing the persistence or reappearance of clinical symptoms and cellular infiltrates. This prolonged or dual inflammatory reaction has been called late-phase reaction (LPR). The ocular LPR is similar to that occurring in the skin, nose or lung following specific allergen challenge (4).

Therefore, major information on the pathophysiology of eye and allergic diseases can be obtained by the study of cells and mediators in tears.

Effectiveness of ocular treatment. The reproducibility and safety of CPTs led investigators to use this test to evaluate the effectiveness of antiallergic treatment.

There are several advantages in testing an antiallergic compound by performing CPT: a) it is a rapid test; b) it gives a clinically reproducible conjunctival response; c) it is practical since it gives an answer in one day instead of one month or more for a clinical trial; d) it can be done outside the acute season without any risk for the patient of treatment failure; e) it does not cause discomfort to the patient; and f) it allows the evaluation of several parameters (clinical signs and symptoms, cells and mediators in tears) of the allergic response.

Technique for performing CPT

Technical problems include:

- preparation and storage of allergen extracts
- dose of allergen.

The allergen extracts most commonly used are quantified by allergy unit (AU) or biological unit (BU): few reports even lacked specific information on allergen preparation. Therefore, results of different reports cannot be compared.

We suggest that allergen quantified by different methods should be compared with international reference standard, when available or with an established in-house standard. Moreover, freeze-dried lyophilized extract should be prepared or

reconstituted at the time of testing using a conventional diluent (19). Finally, the dose of allergen to be topically applied to the conjunctiva should be strictly related to the objective of the study. For diagnostic purposes, the dose of allergen should be equal to the skin prick test concentration. For research purposes, including the study of cells, mediators and the efficacy of antiallergic treatment, high doses of allergen are required. Dose-response curve studies could help in establishing a threshold of conjunctival sensitivity after allergen challenge.

Evaluation of the conjunctival response

The existence of several methods for the evaluation of the conjunctival response (by grading the clinical reaction, measuring the conjunctival temperature, considering the conjunctival cytology or quantifying the level of mediators in tears, etc.) indicates that, at present, none of these is valid, objective and reproducible (4, 5, 14, 16, 17, 19–22).

At present, having a standardized clinical grading of ocular signs and symptoms is the major goal. The other criteria in the evaluation of a positive CPT may be only used to support the clinical findings.

In conclusion, despite the advantages over the other mucosa (easy macroscopic evaluation, non-traumatic histologic techniques, other eye as control), the conjunctiva has two main drawbacks: the absence of standardization for allergen preparation and, the difficulty, at present, in obtaining a reliable, easily available, inexpensive and practice instrument to have standardized, quantifiable and objective measures of the conjunctival allergic reaction.

However, once these problems are resolved by technical evolution, the conjunctival mucosa may be chosen as a model for studying allergy in humans.

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