Bronchial Provocation Tests in Asthma

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Aas, K. (1970). Archives of Disease in Childhood, 45, 221. Bronchial provocation tests in asthma. Bronchial provocation tests are necessary for precise aetiological diagnosis in bronchial asthma. This is illustrated by the results in 9,364 bronchial provocation tests compared with skin tests and nasal tests.

The technique of bronchial provocation testing and its interpretation are described in detail.

In allergic asthma a precise aetiological diagnosis is a sine qua non for specific treatment, while the diagnosis of non-allergic or 'intrinsic asthma' is one of exclusion to be made only when no allergic cause can be shown. In some cases a precise allergy diagnosis may be made from history and skin test reactions, but these diagnostic aids are often unsatisfactory, leaving doubt as to whether the allergy diagnosis is correct for the asthmatic disease, relates to other organs only, or is quite irrelevant (Holt, 1967). Allergy diagnosis may therefore be completed by applying provocative tests to the diseased organ (Aas, 1966a, 1969; Bronsky and Ellis, 1969; Colldahl, 1952a, b, c; Fagerberg, 1960; Fuchs and Gronemeyer, 1967; Lowell and Schiller, 1947; Mélon et al., 1964; Popa et al., 1969; Tuft et al., 1962). The results of bronchial provocative tests in small groups of patients have been published by Aas (1966a), Bronsky and Ellis (1969), Citron (1967), Colldahl (1952b, c), McAllen (1961), Popa et al. (1969), and Ryssing (1959). Most of these represent experimental studies. It is the purpose of this paper to present our experiences in routine diagnostic work which has included 9,364 inhalation tests performed on a total of 1,035 asthmatic children over a 5-year period. Some of the data have been presented previously (Aas, 1966a, b, c; 1969; Aas et al., 1963).

Methods

The allergen extract or control solution was nebulized by compressed air using an electric pump, and a plastic nebulizer (Pari-Optimal, Pari-Werk, Starnberg am See, Germany). The patient inhaled the nebulized fluid through a loosely fitting plastic face mask (Pari-Optimal). Initially the bronchial provocation tests (BPT) were performed in an ordinary hospital room. A powerful electric fan connected to a wide tube collected the contaminated air from in front of the patient and carried it outside the building. The room air was renewed through a fresh-air intake at the opposite side of the room. However, when more than about six BPTs were performed during the day, the room air became so contaminated by the nebulized allergens that sensitive patients reacted to them soon after entering the room. There was also some risk of sensitization of medical staff, and the system had to be abandoned.

Two small neighbouring test chambers separated by glass walls and doors from a main test laboratory were then used. This enabled the staff in charge to keep the patients undergoing BPT under close observation while they themselves continued their duties in the main laboratory. Two patients with nurses or parents attending them could be tested simultaneously in each test box, provided they were tested with the same allergen.

To secure satisfactory ventilation of the test boxes without contaminating the air of neighbouring rooms, a modified air-conditioning system was used. Prewarmed (winter) and filtered air entered the main laboratory and passed through vent openings into the BPT boxes. A slight positive pressure was maintained in the main laboratory to counteract any backflow from the test boxes. A flexible tube close to the patient collected the contaminated air with the aid of an electrical fan and led it out of the building.

Infants and children unable to use a face mask could be tested by allowing the patient to play or sleep in the test box. The vents were closed and the air in the box slowly charged with the nebulized allergen.

Allergen extracts. The extracts used for intracutaneous testing* were used for BPT. A few extracts

*Allergologisk Laboratorium, Copenhagen (Denmark); Bencard Ltd., Brentford, Middlesex (Great Britain); Center Lab., Port Wash, New York, N.Y. (U.S.A.); Nyegaard et Comp., Oslo (Norway); Sahlgrenska Hospital, Allergy Division, Gothenburg (Sweden); and Vitrum AB, Stockholm (Sweden) (Aas, 1966c).
were made in the laboratory of the Allergy Unit by methods described elsewhere (Aas, 1966b). Stock solutions were diluted with 0.9% saline containing 0.5% phenol. The latter solution was used as a control solution in addition to the control solutions supplied commercially with the allergen extracts.

**Skin tests.** Scratch, prick, and intracutaneous tests were carried out by accepted methods, as described elsewhere (Aas, 1966a). In the present paper only the results of intracutaneous tests are reported when comparing skin tests with bronchial tests. Skin tests were read and registered after 20 minutes, only urticarial wheal reactions being considered. Histamine solutions of 0.1 mg./ml. were used as positive controls for intracutaneous tests to allow for the individual skin reactivity of each patient. The reaction to the histamine test was recorded as three plus (+++). Test reactions were recorded against the histamine control. Reactions larger than that of the histamine control received additional plus marks, such that each new plus mark indicated a twofold increase in the wheal area. Four plus (++++) reactions thus indicated wheals twice the size of a three plus (+++) histamine reaction.

**Bronchial provocation test (BPT).** The child was first allowed to become thoroughly acquainted with the equipment and the test was not begun until he was relaxed and confident. Small children sometimes needed up to 4 play sessions before this was achieved, but usually the test could be started after only a few minutes. The child first inhaled nebulized saline, and then control solution inhalation of allergen extract followed. To avoid bias the tests were performed without the patient, parents, or nurse knowing the type of allergen, the allergen extract vials being coded. Only one allergen was used per day.

In addition to allergens used in BPT because they were suspected as causes of the patient’s disease, allergens were included which obviously should be tolerated by the particular patient according to the history (for instance pollen extract in winter asthma, house dust extract in summer asthma). This use of ‘control allergens’ served a double purpose. First, the specificity of positive reactions with other allergens was confirmed by the fact that a control allergen did not produce obstruction in that patient, though it was known to be active in other patients allergic to that allergen. Secondly, negative reactions to the control allergen extracts in patients reacting to other allergen extracts confirmed that these did not contain irritants producing non-specific bronchial reactions. BPTs were started with allergen concentrations of $10^{-4}$ to $10^{-6}$. Pollen and animal dander extracts were always initiated at very low concentrations. The more sensitive the patient as judged from history and skin test reactivity, the lower the initial concentration.

The patient was allowed to inhale the aerosol from approximately 1 ml. of each dilution, the concentration being progressively increased until reactions occurred, or until 1 ml. of the highest concentration used had been nebulized. Each dilution step was completed in 3–5 minutes, and the time needed for one complete BPT was approximately 20–40 minutes, allowing for auscultation, lung function tests, and refilling of nebulizers. After completion of a test, the patient was observed for another 20–40 minutes in a waiting room, after which time auscultation and lung function tests were repeated.

**Safety precautions.** As in all allergy testing, safety measures must be employed to minimize the risk of severe reactions and if necessary to treat them. BPT was not carried out on patients with infections, pulmonary infiltrations, or signs of bronchial obstruction, though ronchi heard on auscultation were ignored if lung function tests could be shown to be satisfactory. Patients who had corticosteroid medication recently, or antihistamines within 24 hours were not tested. Tests were started with dilute extracts even when it was expected that the patient would not react to that particular allergen. The starting concentration selected was such that the patient was unlikely to react to the first two dilution steps used, two to three steps representing a safety margin for the tests. Patients were under close observation during the test and for the following 30 minutes, or longer if necessary.

**Registation of bronchial reactions.** Before BPT the patient was examined by chest auscultation and with anterior rhinoscopy. In children old enough to do so, lung function tests were recorded: peak expiratory flow (PEF) by means of a Wright peak flow meter, vital capacity (VC), and forced expiratory volume in one and/or a half second (FEV$_{1.5}$ or FEV$_{1.0}$) on a Bernstein spirometer. During the test the patient was observed through the glass windows of the test box. If cough or signs of nasal reactions, bronchial reactions, or changed respiration occurred, nebulization was immediately stopped and the child was examined more closely by auscultation and PEF measurements. If there was any sign of bronchial reaction spirometry was also performed. The test was allowed to proceed as before if no convincing obstruction was found. The lowest concentration and dose at which bronchial obstruction became evident was registered as the bronchial sensitivity threshold dose.

**Treatment of positive reactions.** As soon as positive reactions were observed and registered, treatment was started with inhalation of nebulized isoprenaline 0.5% and with antihistamines, ephedrine, and occasionally a theophylline preparation in that order. If sudden or severe reactions occurred, an injection of adrenaline was given in place of the isoprenaline inhalation, but this was rarely needed. Medication was continued for at least 12 hours or as long as any bronchial obstruction could be registered.

**Results**

**Types of reaction.** Anaphylactic or other severe reactions have not been encountered.
Some patients reacted to very dilute extracts, especially extracts of pollen or animal danders. With few exceptions, such early reactions were found in patients in whom a high degree of sensitivity was suspected from the history. More than 70% of positive reactions occurred only at the highest allergen extract concentration used.

In most patients a positive bronchial reaction was signalled by early symptoms and signs of typical asthma such as cough, characteristic expiratory dyspnoea, and wheezing. This usually occurred as an immediate reaction so typical that it left no doubt. PEF was much reduced and spirometry showed reduction of VC, and a flattened and prolonged expiratory curve with reduced FEV₁ (Fig. 1). An initial 25% reduction of PEF was regularly followed by increasing bronchial obstruction if not immediately treated. In practice, the BPT was immediately stopped when PEF was clearly reduced; the patient was then observed without further provocation, bronchial obstruction being allowed to become evident by auscultation and spirometry before treatment was given. The use of the peak flow meter as a screening test for this purpose made it possible to detect reactions early when they were easily reversed by treatment.

Immediate reactions of the type described were regularly relieved within a few minutes by isoprenaline inhalation, indicating that the obstruction mainly was caused by spasm of the bronchial smooth muscle at this stage, and that histamine release probably played an important part. In some patients the positive bronchial reaction developed more slowly, becoming evident only 1–6 hours after completion of the test. This type of reaction was often accompanied by auscultatory rhonchi, râles, and coarse crepitations, findings of obstructive bronchitis. It was usually less easily relieved by isoprenaline, but responded much better to adrenaline. The type of reaction could be indicative of mediator mechanisms other than those of histamine, such as SRS-A (slow reacting substance) or bradykinin (Aas, 1965, 1969). Late reactions of this kind could also be due to immune mechanisms of an Arthus-like type in combination with a reagin-dependent one, as suggested for inhalant allergies to moulds (Pepys et al., 1968). In several children a combination of these reactions occurred.

In some children lung function tests showed slight bronchial obstruction without other symptoms or signs of asthma, but the obstruction usually then became slowly more pronounced and eventually clinically evident in the course of a few hours. In young children in whom lung function tests could not be used, such slow reactions were particularly common and these children had to be examined by auscultation at regular intervals for several hours after completion of the test, while the test often had to be repeated to confirm the diagnosis. In retesting, if the top concentration of the extract was given for a prolonged period, the reaction was accelerated.

In some other children a positive reaction was signalled by slight symptoms of impaired ventilation, and on auscultation by crepitations of a kind that would usually arouse a suspicion of pneumonitis or bronchopneumonia. After a few minutes, however, crepitations were replaced by more typically obstructive sounds, and would also disappear if adrenaline was given.

Allergic reactions were also induced in other
organs as a result of BPT with or without simultaneous reactions in the bronchi. Nasal reactions were the most common, but allergic conjunctivitis, urticaria, and delayed eczematous eruptions occurred as well. Some children complained of general malaise, itching, or headache.

In a small number of children BPT resulted in prolonged bronchial obstruction which was resistant to symptomatic treatment. This sometimes appeared to be caused by simultaneous exposure to naturally occurring allergens, and sometimes by respiratory infection. In 8 such cases transient atelectasis of a pulmonary segment was present radiologically after the BPT.

Allergens provoking bronchial obstruction in BPT. A total of 9,364 BPTs was performed in 1,035 patients, the number of BPT's per individual ranging from 2 to 24. Positive bronchial reactions were elicited in 3,832 instances. House dust was the most common offender, with 1,061 positive reactions in 3,161 tests (Table I). Animal danders reactions were found when the inhalation test was performed with separate mould extracts. Cladosporium (Hormodendron) was the most active of the separate moulds included in this study, but was also the one most frequently used in BPT. Feather extracts were not used so regularly as they should have been according to the patient's history, as it was considered necessary to eliminate feathers from the environment in many cases without proving the existence of feather allergy.

Pollens allergies were also registered less frequently than expected, mainly due to the selection of patients, since mild cases, such as pollen allergies, often are, are not usually admitted. Furthermore, in 98 cases the history and skin tests were considered adequate to diagnose pollen allergy, sometimes confirmed by nasal tests with pollen.

Three patients showed bronchial obstructive reactions to the control solution containing 0.5% phenol. This was considered a result of excessive hyperreactivity of the bronchi, and such patients were not submitted to further BPT.

Bacterial vaccines were used in 424 BPTs, as will be outlined. In 12 instances positive bronchial reactions were provoked, but some of these reactions were probably non-specific as regards the microorganisms in question.

The distribution of the allergic causes of asthma in 809 consecutive cases using BPT has been reported elsewhere (Aas, 1969). One or more allergens causing asthma could be defined in 688 of 715 children tested, all but 94 being inhalant allergies.

Non-specific reactions. In three patients bronchial obstruction was provoked by the control solution containing 0.5% phenol. No attempt was made to exclude this being due to phenol allergy, as the reactions were accepted as non-specific ones. Much effort was concentrated on avoiding false positive reactions to BPT. The use of 'control allergens' was useful in recognizing the existence of non-specific irritants. In very few instances the control allergens elicited unexpected bronchial reactions in contradiction to the history. This happened, however, occasionally with one particular house dust extract, several feather extracts, a few animal dander extracts, and with sheep wool extracts. It occurred regularly with bacterial vaccines used in BPT (see below).

Extracts suspected of eliciting reactions of a non-specific nature were studied in patients known to be allergic to the allergen in question, and in other patients known to tolerate it. The highest concentration of the extract was diluted 2 and 4.
times and given in BPT in doses 2 to 4 times the usual, so that the total amount of allergen used equalled one dose of the undiluted allergen extract. Patients truly allergic to the particular allergen regularly reacted to prolonged BPT with the diluted extract, whereas no reactions were elicited in the patients who on clinical grounds were thought to be tolerant to that allergen.

Subsequent to this trial, BPT was performed with 2 ml. twice diluted bacterial vaccines, sheep wool extract, feather extracts, and the particular house dust extract containing irritants, without provoking non-specific reactions.

Reactions to bacterial vaccines. To investigate the specificity of response to bacterial vaccines used in BPT in the concentrations reported by others (Hajos, 1960; Hampton, Johnson, and Galakatos, 1963; Popa et al., 1969) 8 patients were selected who suffered from isolated pollen allergy asthma; none of these had any bronchial obstruction except in the pollen season, nor did they have asthmatic or bronchitic episodes in connexion with common colds and other respiratory infections during winter. BPT was carried out with Bencard F 2 bacterial extract (Hajos, 1960) and with a stock bacterial vaccine containing approximately 1800 million bacteria per ml. (Aas et al., 1963). Five of the pollen-allergic subjects reacted with bronchial obstruction to the undiluted top concentrations of both extracts, showing them to contain non-specific irritants. This was confirmed by the fact that none of the 8 patients showed bronchial reactions after receiving subcutaneous injections of 0·5 ml. bacterial vaccines though they experienced both local and systemic effects including fever and headache.

BPT with bacterial extracts was performed in a total of 424 patients with a history indicating that asthma was triggered by upper respiratory infections. In these patients 2 ml. twofold diluted bacterial vaccine was used as the maximum dose. Positive reactions were elicited in 12 of the patients (2·8%). In one of these patients a positive bronchial reaction was provoked by an extract of Neisseria catarrhalis diluted 1 : 100 (Aas, 1966a), but when the same bacteria were cultivated on another medium (potato starch) and used in a repeated BPT four months later, no reaction occurred (Aas, 1963).

In 70 consecutive cases not reacting to BPT with the mixed bacterial vaccine, despite reporting the asthma to be triggered by respiratory infections, an injection 0·5 of a 1 : 10 dilution of the bacterial vaccine was given as a subcutaneous test injection. In no instances were bronchial reactions or any other allergic reactions elicited by such injections, though slight fever and local inflammation occurred in more than half the patients. Bronchial reactions to other known allergens were more easily elicited than previously within 72 hours of the bacterial vaccine injection, as shown by a lowered bronchial sensitivity threshold in a number of patients so studied.

Comparison of skin test and bronchial test reaction. The reactions to skin tests and to bronchial tests were compared in patients with a suggestive but not completely convincing history of bronchial allergy to various allergens. Intracutaneous tests and BPT's were performed with the same extract of the allergen in question. Discrepancies were common, comparing skin test reactivity and bronchial allergy in patients with house dust or mould allergy history (Aas, 1969). Reliance on skin test reactions would have resulted in a 25–40% diagnostic error in these patients, and similar observations were made with animal danders, sheep wool, and pollen (Tables II, III,

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**TABLE II**

Intradermal and Bronchial Tests with Animal Dander (Horse, Cow, Dog, Cat) Extract in 637 Asthmatic Children with History Suggestive of Animal Dander Allergy*

<table>
<thead>
<tr>
<th>Skin Test Reaction</th>
<th>No. of Cases</th>
<th>Bronchial Test Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive Cases</td>
</tr>
<tr>
<td>—</td>
<td>133</td>
<td>43</td>
</tr>
<tr>
<td>+/++</td>
<td>300</td>
<td>109</td>
</tr>
<tr>
<td>+/++/+//++</td>
<td>204</td>
<td>159</td>
</tr>
<tr>
<td>Totals</td>
<td>637</td>
<td>311</td>
</tr>
</tbody>
</table>

*In 177 additional cases history and skin test reactions were together considered diagnostic of animal dander allergy as cause of asthma.

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**TABLE III**

Intradermal and Bronchial Tests with Different Pollen Extracts in 387 Asthmatic Children with History Suggestive of Pollen Allergy*

<table>
<thead>
<tr>
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<th>No. of Cases</th>
<th>Bronchial Test Reaction</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td>Positive Cases</td>
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<tr>
<td>—</td>
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<td>8</td>
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<td>174</td>
</tr>
<tr>
<td>Totals</td>
<td>387</td>
<td>267</td>
</tr>
</tbody>
</table>

*In 96 additional cases history and skin test reactions were together considered conclusively diagnostic of pollen allergy as cause of asthma.
and IV). However, a 3+ skin test usually was
found to be significant provided the history was
also strongly suggestive, a fact that is partly con-
cealed in the Tables shown. The largest dis-
crepancies were found in the cases in which the
history was only slightly suggestive, which was
often the case for house dust, moulds, and sheep
wool allergies.

**TABLE IV**
Intradermal and Bronchial Tests with Sheep Wool
Extract in 354 Asthmatic Children with a History
Suggestive of Sheep Wool Allergy*

<table>
<thead>
<tr>
<th>Skin Test Reaction</th>
<th>No. of Cases</th>
<th>Bronchial Test Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive Cases</td>
</tr>
<tr>
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<tr>
<td>Totals</td>
<td></td>
<td>354</td>
</tr>
<tr>
<td></td>
<td></td>
<td>301</td>
</tr>
</tbody>
</table>

*In 1 additional case history and skin test reactions were together
considered diagnostic of sheep wool allergy as cause of asthma.

Comparison of nasal test and bronchial test
reactions. In a large proportion of children the
BPT resulted in nasal allergic reactions, some with
and some without simultaneous bronchial reactions
(Table V). The nasal reactions were characterized
by sneezing, nasal discharge, and swelling of the
mucosa. They were regularly followed by in-
creased eosinophilia in the nasal mucus within a
few hours. Simultaneous nasal and bronchial
reactions were most often found in BPT with pollen
and animal dander extracts. For all allergens
used in BPT, discrepancies were observed between
nasal and bronchial reactivity. The correlation
between nasal and bronchial allergy was studied in
more detail in (a) 20 patients with both nasal and
bronchial allergy to the same allergen, and (b) 18
patients with bronchial allergy but no nasal reaction
to the BPT. In these patients a repeated allergen
challenge was performed by instilling the appropri-
ate allergen into the nasal cavity. The natural dry
pollen or a dried protein concentrate of the allergen
was used. In the group (a) patients, a positive
nasal reaction occurred in all, but a bronchial
reaction resulting from the nasal test was detected
in only 6 patients. In the group (b) patients, the
nasal test resulted in nasal reactions in 2 instances,
one of whom also developed slight bronchial
obstruction after the nasal test. Two other patients
reacted with bronchial obstruction but no nasal
symptoms. The bronchial reaction to nasal test
developed rather slowly and sometimes not for 4
hours.

**Bronchial provocation test in assessment of
effect of treatment.** BPT was used for the
assessment of bronchial reactivity after treatment
with an allergen extract for 2 to 4 years, in order to
decide whether the hyposensitization ought to be
continued or not (Pegelow et al., 1967). BPT was
also used when there was doubt as to whether
clinical reactions in hyposensitized patients were due
to exposure to the known allergens included in the
hyposensitization programme or were caused by
new or unknown allergens. The bronchial sensi-
tivity threshold after treatment was compared to
that found before treatment. Care was taken to
perform the second BPT under the same conditions.
Such assessment often but not always showed that
the bronchial reactivity had been reduced following
hyposensitization (Fig. 2). In several patients
who had obtained bronchial tolerance to the
maximum dose and concentration of the allergen
used, the BPT still resulted in positive allergic
reactions in the nose.

BPT is currently being used in a double-blind
study of the efficacy of house dust hyposensitization
but the study is not yet concluded. Some patients
have reduced bronchial reactivity to the house
dust extract after 2–4 years of treatment, others
have not, but we still do not know which individu-
ally received house dust extract, and which placebo.
There are, however, important discrepancies be-
tween the clinical effect as subjectively reported by
the patients and/or parents, and the actual effect as
assessed by BPT. This may be due to other
allergens not treated but affecting the patient's
condition in addition to the house dust.
Bronchial Provocation Tests in Asthma

There is now ample evidence that bronchial provocation tests are valuable and offer indispensable aid for precise allergy diagnosis in asthma. The reproducibility of the method has been studied by Colldahl (1952a). BPT is certainly more reliable than the other diagnostic methods used in allergy; indeed there are few diseases in which the aetiological diagnosis can now be made so precisely as bronchial asthma. By means of BPT, the disease under study can be reproduced at will within a few minutes. When the allergen exposure is discontinued and symptomatic medication given, the patient is rapidly restored to normal function. Furthermore, this procedure may be quantitated to a certain degree (Itkin et al., 1963), and the functional changes recorded.

The present material largely confirms the careful studies of bronchial provocation tests in adult patients performed by Colldahl (1952a, b, c), except that a much higher incidence of bronchial allergy is found in childhood asthma than is the case for adult patients. As reported both by Colldahl (1952, a, b, c) and others (Bronsky and Ellis, 1969; Debelić and Virchow, 1968; Fagerberg, 1957; 1960; Holt, 1967; Kaude, 1960; Melon et al., 1964; Popa et al., 1969; Ripe, 1967), it was found that skin tests were not to be trusted diagnostically even when the history of the patient was suggestive for the allergens giving positive skin reactions. Clinical or experimental studies based on skin test diagnosis are, therefore, of restricted value. This applies, for instance, to attempts to evaluate the efficacy of hyposensitization treatment.

Bronchial allergy may be present when the skin test is negative (Fagerberg, 1960; Fastman and Glaser, 1963), while positive skin tests are found in many normal non-allergic individuals (Curran and Goldman, 1961). The reliability of skin tests in allergy diagnosis varies from allergen to allergen. Thus Colldahl (1952a, b, c) found BPT to be positive in 43% of patients with positive skin tests to the same allergen, varying from 72% for house dust to 20% for animal dander extracts. He found a positive BPT reaction in 16% of patients with a negative skin test but with a history suggesting a particular allergen.

These findings do not mean that the skin test is of no value, but rather that skin testing should be regarded as a screening procedure, which ought to be followed by more specific diagnostic tests such as BPT. BPT was indicated when discrepancies were found between history and skin test reactions, for allergies in which the history was diffuse or non-informative, and in patients with poor as well as excessive skin test reactivity.

Bronchial provocation tests are time consuming. They are mandatory, however, for clinical research concerned with diagnosis of bronchial asthma. They are superfluous when the patient's history and skin test reactions together form conclusive evidence.

By using nasal tests in asthma, the target organ (bronchi) is approached; though more reliable than the skin test (Popa and Al-George, 1969), it is not an adequate substitute for bronchial tests.

The time should come when practical in vitro tests may be used as screening tests for allergy diagnosis in asthma. In vitro tests demonstrating specific reagins in serum will not, however, replace the provocation test, for it is the immunological state of the various organs that determines the allergic disease, and circulating reagins may play varying roles in different organs.

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