

The value of inhalation tests in perennial bronchial asthma

Valentin Popa, M.D., Dan Teculescu, M.D., Dan Stănescu, M.D., and Nicolae Gavrilescu, M.D., Bucharest, Rumania

The authors report the results obtained by routine utilization of inhalation tests with allergens in perennial asthma. One hundred seventy-five subjects from 230 consecutive asthmatic adults were submitted to inhalation tests. Both clinical observation and a simple spirographic recording ($FEV_{1.0}$) were used to assess the bronchial responses. The results are discussed from the point of view of clinical practice. It was found that a very suggestive anamnesis, supported by positive skin tests, was always associated with positive inhalation tests. In contrast, a somewhat suggestive anamnesis, with positive skin test to the incriminated allergen, was accompanied in only 23 per cent of the patients by a positive inhalation test to this allergen. Negative skin tests lowered considerably the incidence of positive inhalation tests. Allergens not indicated by anamnesis never determined a positive inhalation test, irrespective of the skin reaction. The authors support the view that inhalation tests are the only method available for an accurate etiologic diagnosis in perennial asthma.

Inhalation tests with allergens have been employed for research purposes by many authors, usually in selected cases of bronchial asthma, to demonstrate sensitivity to a certain allergen in an asthmatic population,¹⁻⁸ to single out the offending allergen from a group of potential allergens,⁹⁻¹¹ or to study the functional pattern of an asthma attack.¹²⁻¹⁵

In contrast to the widespread use of inhalation tests in research, they enjoy a limited popularity in allergy practice.¹⁶ Many allergists perform inhalation tests only sporadically, rather than as a routine diagnostic procedure.^{17, 18} The two most significant reasons for limiting the routine application of them in allergy practice are: the technical difficulties and the surprisingly little information yet available concerning the inhalation test importance as a systematic means of diagnosis of bronchial asthma.¹⁹⁻²³

The aim of the present investigation was to assess, for clinical practice, the value of inhalation tests in the etiologic diagnosis of perennial asthma. In our

From the Allergy Unit, Department of Occupational Diseases, Colentina Hospital, Bucharest.

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opinion, in the diagnosis of bronchial asthma, the tests should be assessed separately for perennial and pollen asthma. In cases of sensitivity to pollen, anamnesis is characteristic, skin tests are practically always positive, and inhalation tests are positive too.¹⁻³ In perennial asthma, anamnesis is less informative, skin tests are less contributory, and inhalation tests are not always positive. Thus, in any large group of asthmatic patients submitted to the inhalation tests the incidence of pollen allergy may significantly alter the per cent of positive tests and, accordingly, their importance in the etiological diagnosis of bronchial asthma.

For an accurate evaluation of the inhalation tests in allergy practice, the following 3 conditions are necessary:

1. The study should include all consecutive subjects with bronchial asthma examined in an Allergy Division during a certain period. If some subjects could not perform the inhalation tests, the reasons should be clearly outlined.

2. Every subject should submit to all of the inhalation tests deemed necessary, not only to part of them.

3. Since we are concerned with the allergy practice, a simple and reliable spiropgraphic criterion should be chosen for the estimation of the bronchial response to the inhalation tests; from our experience, FEV_{1.0} seems to be most suitable.

MATERIAL AND METHODS

Patients

Two hundred thirty consecutive adult patients with bronchial asthma of the Allergy Unit of the Department of Occupational Diseases, Colentina Hospital, Bucharest, participated in this study.

Methods

All the subjects had: (1) A detailed allergologic anamnesis. Anamnesis was considered as "highly suggestive" for an allergen if asthma attacks occurred unmistakably after contact with this allergen. Anamnesis was "suggestive" (positive) if it pointed to a probable allergen; and anamnesis was labeled as "not suggestive" (negative) if no relation could be found between asthma attacks and a certain allergen. Thus, the same subject might have a suggestive or highly suggestive anamnesis for a certain allergen, as well as negative anamnesis for others. (2) Intradermal tests were then given with the allergens pointed out by anamnesis, as well as with other allergens most commonly involved in the etiology of bronchial asthma (house dust, feathers, animal hair, bacteria and fungi). The allergens used were supplied by the Bencard Allergy Laboratory; 5 other allergens were prepared in our Institute (cotton, hemp, flax, jute, sericine) and used simultaneously with those of Bencard. If castor bean allergy was suspected, scratch tests with crude castor bean dust were carried out.

- (3) One hundred seventy-five out of 230 patients were submitted to inhalation tests. Everyone tested was subjected to the tests with all the allergens revealed by anamnesis (usually 1 to 3 allergens). Some patients also performed inhalation tests with allergens not indicated by anamnesis. This was for various reasons, such as a very strong intradermal reaction or occupational allergy

claimed by the patient. A total of 570 inhalation tests were performed, not including tests which were repeated. If a patient was submitted to many bacterial inhalation tests, these tests were not considered as one inhalation test; the same applies to these tests with fungi.

The inhalation tests were conducted as follows: the allergen was nebulized 5 minutes in an open-circuit system with a Dräger Aerolette apparatus, having a flow rate of 0.2 ml. per minute and delivering an aerosol with particle size 0.5 to 5 μ . Vital capacity and FEV_{1.0} were performed on the subjects before and after nebulization, at 30 minutes, 60 minutes and, if an asthmatic attack occurred, immediately after its onset. At least 3 similar records of vital capacity and FEV_{1.0} were obtained. In measuring the latter, the convention recommended by the American College of Chest Physicians²⁴ was applied: the highest of 3 values was retained both for vital capacity and FEV_{1.0}. Auscultation of the chest was carried out before every spiropgraphic recording.^{6, 9, 14, 25-27} Inhalation tests were considered positive if there was a FEV_{1.0} decrease of at least 10 per cent, and if the subject had clinical signs of acute bronchial obstruction (dyspnea, wheezing, and, eventually, rales) at the same time. In 3 subjects, inhalation tests were considered positive when there were clinical signs of bronchial spasm, but only minor spiropgraphic changes (reduction of FEV_{1.0} by less than 10 per cent). Doubtful inhalation tests (dissociation of spiropgraphic and clinical response) and most of the positive inhalation tests were repeated. It must be stressed that if inhalation of an allergen produced only a 10 per cent diminution of FEV_{1.0} without subjective and objective signs of bronchospasm, the test proved to be clearly clinically and spiropgraphically negative, upon subsequent retesting. In 4 subjects with asthmatic attacks occurring during vital capacity and FEV_{1.0} determination, inhalations tests were twice repeated and proved to be negative. In 8 subjects, clinical and spiropgraphic criteria could not establish

Table I. *Concentration of intradermal and inhalation Bencard allergens*

<i>Allergen</i>	<i>Concentration</i>			
	<i>Intradermal test</i>		<i>Inhalation test</i>	
	<i>% w/v*</i>	<i>Units/ml.†</i>	<i>% w/v*</i>	<i>Units/ml.†</i>
House dust	12.5	125,000	250	250,000
Horse hair	0.2	2,000	8	80,000
Other inhalant allergens	2	20,000	50	500,000
Bacteria	500 million per ml.		2,500 million per ml.	
Molds	1	10,000	10	100,000
Ingestants‡	2	20,000	10	100,000

*Proportions per cent indicate weight of original water-free allergenic material used per 100 ml. of extracting fluid.

†One unit represents the extract from 1 μ g of original water-free allergenic material; it has the same connotation as the Noon unit for pollens.

‡Foods.

whether the tests were positive or not. There were minor spirographic changes, accompanied by minor clinical signs, which sometimes were difficult to evaluate (e.g., accentuation of a pre-existing wheezing or more numerous bronchial rales). In these cases, pulmonary compliance was used as functional criterion.²⁸

The allergens used were procured from the Bencard Allergy Laboratory specifically for the inhalation tests. Table I presents the per cent weight to volume and units content of the Bencard allergens. Inhalation extracts, in concentration 1:1, contain 50 per cent glycerine and 0.5 per cent phenol, which is the extracting fluid. The control tests (concentration 1:1) had exactly the same composition, except that they contained no allergenic material. The inhalation test solution was diluted 1:10 with physiologic-buffered saline in the case of house dust, horse hair, and various other inhalants and 1:5 in the case of bacteria, fungi, and ingestants. A stronger concentration (1:1) was used if these dilutions produced a negative or dubious respiratory response in subjects with a suggestive anamnesis. The stronger concentration was also used if the corresponding allergen either elicited a large skin reaction (> 1.5 cm.) or was the only one to give a positive skin test. This procedure was applied for 35 subjects (40 inhalation tests).

Two types of control inhalation tests were conducted with Bencard allergens. (I) Control test showed that the diluent used was not irritant. In a preliminary study, 20 subjects with bronchial asthma were submitted to inhalation tests with a control inhalation test solution in 1:1 concentration; none of these asthmatic subjects presented any evidence of immediate or delayed (12 or 24 hours) bronchospasm. As all subjects performing inhalation tests with Bencard allergens had at least 1 negative test to an allergen from the same source in an identical dilution, those negative inhalation tests served also as control inhalation tests. (II) Control tests indicated that the allergens used were not irritating. The following allergens were used in 1:1 concentration for 33 asthmatic subjects not suspected of being sensitive to them: house dust (7 subjects), bacteria (10 subjects), cotton (5 subjects), hemp (4 subjects), jute (2 subjects), molds (5 subjects). These allergens were the ones which it was necessary to employ in a 1:1 concentration during the present investigation (they were chosen for control testing just for this reason). From the 8 subjects mentioned previously, in whom pulmonary compliance determinations were also made, 7 subjects submitted to inhalation tests with Bencard allergens: One had a positive (1:10 dilution) and 6 had a negative inhalation test. Among these 6 nonreactors, 4 subjects took tests with 1:10 allergen dilution, and 2 subjects with 1:1 concentration. All control inhalation tests (with diluent or allergens) were not included in the inhalation test statistics.

The allergens prepared in our Institute were employed for inhalation tests in a concentration of 1,000 PNU per milliliter. Control inhalation tests were made with phosphate-buffered physiologic saline, pH 7.2, containing 1:100,000 Merthiolate, which was the diluent of these allergens.

The inhalation tests of 15 subjects were performed with nonirritant dilutions of potentially irritant substances (sulfathiazole, ethylenediamine, paraphenylenediamine, chloramine, bichromate, urelit-formaldehydeurea resin). A detailed

report will be published elsewhere. Briefly, the nonirritant threshold dilution was established for each substance by selecting a group of 6 asthmatic individuals at random. In these subjects, nebulization of the threshold-nonirritating concentration did not produce positive inhalation tests, and pulmonary compliance was not affected by these dilutions (2 control asthmatic subjects for each substance).

For inhalation tests, 1:5 and 1:10 dilutions of the threshold-nonirritative concentrations were used. The diluent, phosphate-buffered physiologic saline, pH 7.2, containing 1:100,000 Merthiolate was used as a control test for each subject.

Positive diagnosis of bronchial asthma was supported by visualization of the asthma attack and/or by provocation tests with acetylcholine (AcCh). Patients with long-term asthmatic bronchitis were not included in the present study. The AcCh inhalation test was a qualitative one,²⁹ since it attempted only to demonstrate the characteristic cholinergic hyperreactivity in bronchial asthma. A solution of 1 per cent AcCh was nebulized 3 minutes in the same open-circuit system, approximately 2,000 μ g being delivered per minute; vital capacity, FEV_{1.0}, and physical examination were performed as in the inhalation tests.

RESULTS

Subjects performing inhalation tests

Of 230 patients with bronchial asthma, 175 (62 per cent) were submitted to 597 inhalation tests, an average of 3.2 tests per patient. From the 175 subjects, 98 (5 per cent) had 112 positive inhalation tests (20 per cent of all inhalation

Table II. *Allergens detected by inhalation tests*

<i>Allergens</i>	<i>Number of patients</i>
House dust	19 (6)*
Bacteria	19 (2)
Fungi	3 (1)
Food (milk)	1 (2)
Insects (bee)	1
Rabbit fur, cat fur, horse hair, wool	7 (3)
Sericine	2
Cotton	8
Hemp	8
Flax	1
Jute	1
Woods	1
Wheat flour	7
ACTH	3
Penicillin	1
Sulfathiazole (0.01%)	1
Ethylenediamine (0.1%)	3
Paraphenylenediamine (0.01%)	5
Chloramine (0.05%)	1
Bichromate (0.0001%)	4
Urelit (fume)	1
Total	98 (14)

*Numbers in parentheses equal polysensitization.

tests). All but 2 of the subjects with inhalation tests positive to Bencard allergens reacted to the 1:10 or 1:5 dilutions. Of the subjects responding only to the 1:1 concentration, one was sensitive to house dust and the other to bacteria. The allergens responsible for positive tests are shown in Table II.

For 77 patients (44 per cent), inhalation tests remained negative. The correlation of anamnesis, skin tests, and inhalation tests is presented in Table III.

Subjects not performing inhalation tests

Fifty-five subjects (34 per cent) did not submit to the inhalation tests. Table IV shows the reasons for not performing these tests.

DISCUSSION

Subjects performing inhalation tests

Subjects with positive tests. As indicated by Table II, an allergic factor is involved in the etiology of 56 per cent of the subjects with perennial bronchial asthma. This is a much higher incidence than is currently assumed (20 to 30 per cent by many authors employing the inhalation tests in the diagnosis of bron-

Table III. *Anamnesis and skin tests versus inhalation tests*

<i>Anamnesis and skin tests</i>	<i>Inhalation tests</i>		
	<i>Total number</i>	<i>Positive</i>	<i>Negative</i>
Anamnesis ++,* skin tests +†	33	33 (100)‡	0
Anamnesis +, skin tests +	339	78 (23)‡	261
Anamnesis +, skin tests -§	52	1 (2)‡	51
Anamnesis -, skin tests +	162	0 (0)	162
Anamnesis -, skin tests -	11	0 (0)	11
Total	597	112	485

*Highly suggestive.

†Positive.

‡Figures in parentheses are percentages.

§Negative.

Table IV. *Subjects not performing inhalation tests*

<i>Reasons for not performing inhalation tests</i>	<i>Number of patients</i>
Focal and/or general reaction (to ACTH, penicillin, ypeca)	6
Potent allergens with limited distribution (castor bean and penicillin)	17
Severe ventilatory impairment*	22
Failure to perform FEV _{1.0}	10
Total	55

*FEV_{1.0} less than 1,000 ml.

chial asthma.^{4-6, 10, 11, 19, 20, 22} Of course, as long as the reported series (with the exception of Colldahl's²²) are still relatively small, it is difficult to exclude a sampling effect. We should, however, stress the fact that the prevalence of allergic asthma in any one of the series may be affected by 2 other factors: the number of subjects with pollen asthma and the criteria utilized for the positive diagnosis of bronchial asthma. We restricted our investigation to perennial asthma; thus, an important number of subjects with allergic asthma were excluded. Yet, the incidence of allergic asthma in our series was higher than in the previous large studies which included also pollen asthma.^{19, 20, 22} Perhaps the criteria of diagnosis might have been different in our series. We deliberately chose for study only subjects with typical bronchial asthma, whose attacks were observed by a physician of our Department or induced by AcCh inhalation. Patients with long-term asthmatic bronchitis were excluded.

Polysensitization was relatively rare in our series: 14 of 98 subjects (14 per cent) had positive inhalation test reaction to 2 or 3 allergens. This is in contrast to the data of Aamodt,²⁰ who found polysensitization in 50 per cent of his patients. However, our series is too small to permit firm conclusions about the incidence of polysensitization. The same holds true in regard to the incidence of sensitivity to a certain allergen in the asthmatic population.

Correlation of anamnesis, skin tests, and inhalation tests

Highly suggestive anamnesis, positive skin tests. Highly suggestive anamnesis was found in 33 subjects, 34 per cent of all subjects having positive inhalation tests. Bronchial asthma with highly suggestive anamnesis has some particular features:

Allergens accounting for highly suggestive anamnesis were seldom the ubiquitous ones: 2 out of 19 patients with house dust asthma (13 per cent) and 3 out of 19 patients with bacterial allergic asthma (14 per cent) had a highly suggestive anamnesis (Table III). Highly suggestive anamnesis was due especially to wool, sericine, fungi, food, penicillin, ACTH, and micromolecular irritant substances.

All patients with highly suggestive anamnesis had positive skin tests. All patients with highly suggestive anamnesis had positive inhalation tests; in this respect, these patients resemble bronchial asthmatic patients sensitive to pollen, since pollen asthma has a seasonal, highly suggestive anamnesis. For practical purposes, if the anamnesis is highly suggestive, the inhalation tests would appear to be unnecessary. However, even for these patients, we believe that inhalation tests should be performed if an occupational etiology has to be substantiated (these tests are objective and reproducible) and if micromolecular irritant substances are the offending agents.^{27, 30}

Suggestive anamnesis and positive skin tests. In this group, there were 78 positive (23 per cent) and 261 negative inhalation tests. Fuchs and Gronemeyer³¹ reported that suggestive anamnesis and positive skin tests are supported in 80 per cent of the cases by positive inhalation tests. The unexpected high incidence of negative inhalation tests in our patients could be ascribed to many factors:

Occupational contact with irritant dusts or substances may have caused such

negative results because, in our series, 95 per cent of the subjects were factory workers or farmers. When these substances are also well-known allergens, confusion between a simple irritative action and an antigen-antibody reaction is unavoidable. The most illustrative situation arises in the textile industry, in which textile dust is both allergenic and irritant.³² We studied 35 textile workers with bronchial asthma.³³ All had suggestive anamnesis for allergy to textile dust and positive skin tests to those allergens. Inhalation tests revealed that only in 19 of them was bronchial asthma due to textile dust allergy.

If many allergens are present in the circumstances when asthmatic attacks occur, anamnesis and skin tests are not able to point out the offending allergen. Two examples may be given: (a) In some textile factories, for technical reasons, cat hair, rabbit fur, horse hair, and so forth are included in the shuttle.³⁴ For many weavers with a suggestive anamnesis of occupational asthma and positive skin tests to textile fibers and animal hairs, inhalation tests are negative to the textile fibers they handle, but positive to the animal hair or fur from the shuttle. (b) Perennial nocturnal asthmatic attacks are frequently related to domestic aeroallergens, to bacterial allergens, or to fungi. In a certain subject, skin tests to these allergens are positive more often than not; while inhalation tests are positive to 1 or 2 of them.

As respiratory infections tend to precipitate attacks in all asthmatic subjects, anamnesis could not clearly distinguish bacterial-allergic asthma, in which positive inhalation tests to bacteria would be expected, from infectious (non-allergic) asthma and mixed asthma (allergic asthma with superimposed bronchial infection). Positive skin test to bacteria were frequently encountered in all adult asthmatic subjects. Thus, many cases of suggestive anamnesis with positive skin tests, but negative inhalation tests, are seen in patients with asthma with concomitant bronchial infection. A detailed account of our inhalation test studies with bacteria will be published elsewhere.

Suggestive anamnesis with negative skin tests. In our series, inhalation tests were positive only once. Many authors have reported that in 6 to 15 per cent of their patients inhalation tests were positive, despite negative skin tests.^{1, 2, 5, 6, 9, 10, 19, 20, 31, 35} At first, the number of patients with negative skin tests associated with positive inhalation tests appeared to be greater (5 cases). We retested these patients many times at various intervals (10 to 30 days) and the intradermal tests became slightly positive. The only subject who could not be retested was the exception mentioned previously. It is not assumed that fluctuation of the skin reactivity might explain all similar cases reported in the literature. The authors want only to point out that intradermal testing in a negative phase of skin reactivity should not be overlooked as a possibility in patients with positive inhalation tests and negative intradermal tests.

As indicated by Table III, suggestive anamnesis and negative skin tests were associated with negative inhalation tests on 51 of 52 occasions; while negative skin tests were associated with positive inhalation tests only once in 112 trials. All the remaining positive inhalation test subjects (111) had positive skin tests. This difference is statistically significant at a $p < 0.001$. In clinical practice, we are reluctant to recommend inhalation tests with allergens giving repeated

negative intradermal responses. However, if no other allergen can be found, such tests are carried out.

Negative anamnesis with positive skin tests. Inhalation tests were always negative when skin tests were positive (162 occasions). There was no surprising bronchial allergy, i.e., not indicated by anamnesis, as some authors have reported.³¹ Positive skin tests alone have no importance in etiological diagnosis of bronchial asthma. This is a long-accepted fact, stressed again recently by several authors, Bruce,¹⁰ Colldahl,²² and Ripe.⁶ Aamodt²⁰ reported that 50 per cent of his asthmatic subjects had positive skin tests to a certain allergen, and only half of these showed positive inhalation tests. In our series (Table III), 534 positive skin tests were confirmed by positive inhalation tests, 111 times (20 per cent).

Negative anamnesis with negative skin tests. In this situation inhalation tests were always negative.

Subjects not performing inhalation tests

We attempted to use inhalation tests for all our subjects, but in some instances this was impossible. We deliberately refrained from bronchial testing of the 55 subjects belonging to 1 of the 4 categories listed in Table IV. One of these groups merits comment: with a highly suggestive anamnesis for a potent allergen with limited distribution, intradermal or scratch tests are positive. The most representative allergens for this group are penicillin (3 cases) and castor bean (14 cases). Though used sporadically by some authors, even in Rumania,³⁶ inhalation tests with castor bean extracts may be dangerous. The most dramatic bronchial reaction we observed was due to inhalation of highly diluted castor bean extract. For similar reasons, inhalation tests with penicillin are not recommended.³⁷

In conclusion, inhalation tests contributed significantly to a correct etiological diagnosis of bronchial asthma. The method that was used requires no expensive devices, but it is time-consuming to both physician and patient. Unfortunately, there is no alternative, since direct bronchial testing is still the only proof of bronchial hypersensitivity. Our findings are in full agreement with those reported by many who recommend inhalation tests for an accurate etiological diagnosis of asthmatic patients.

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