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A STUDY OF PROVOCATION TESTS ON PATIENTS WITH BRONCHIAL ASTHMA

II. *The outcome of provocation tests with different antigens.*

By

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Different authors who have performed provocation tests, have obtained very different results. This will be evident from the following survey of investigations on the subject.

Peipers (1931) was one of the first who made inhalation tests with house dust. He emphasised that one often finds patients sensitive to house dust showing only a positive intradermal reaction to dust from their own house. He states that inhalation of dust extract that has given a positive intradermal test is able to give asthmatic symptoms. Nothing is mentioned, however, as to how often this was obtained and how many patients were examined. He mentions that Storm von Leeuwen has shown that those extracts of house dust which are prepared from places where asthma is common are the strongest ones.

Stevens (1934) used provocation tests for some time in his routine work but did not continue these tests as they took too much time, and because the asthma provoked could be severe and of long duration. According to Stevens, however, provocation tests, when compared with the intradermal test, give important information as to the value of the latter as an indicator of the hypersensitivity of the bronchial mucosa. 45 patients with asthma, aged 10-55, were studied. The extracts used in intradermal testing, but 25 times stronger, were

employed for the inhalation tests. A spray was used for the inhalation. Only one strength of the inhaled extract was used for the inhalations, the inhalation time being varied. Sometimes the patients got severe asthma after only a few inhalation breaths of the extract. Only sensitizations to inhaled allergens were examined. Among 45 patients studied, 11 were not cutaneously sensitive to any of 90 common allergens. All the inhalation tests in this group were negative. 34 patients reacted to one or more of the 90 common allergens used. On these patients 17 inhalent allergens were tested intradermally and by inhalation. For each of the 578 tests an inhalation was made. In all there were 25 positive inhalations on 15 patients. 39 of the dermal tests were strongly positive, 53 moderately positive, 76 were weak, and 410 negative. Symptoms of asthma occurred 7 times when the corresponding dermal test was strongly positive, 6 times with a moderate test, twice with a weak test, and 10 times where the skin failed to react. The approximate ratios of pulmonary sensibility to skin reactivity as derived from the figures were 1:5,5-1:9-1:38-1:41 respectively in the above mentioned groups. According to this material only half the clinical sensitizations in asthma uncomplicated by allergic rhinitis and conjunctivites are indicated by positive or strongly positive tests. Dust was not included among the 17 allergens tested both intradermally and by inhalation.

Harris (1941) made an investigation in an attempt to evaluate skin reactions to alternaria by the experimental reproduction of respiratory allergy in a group of patients reacting to this mould by dermal testing. The patients were first tested in a room with a fixed amount of mould spores in the air, and subsequently nasal tests were performed with the same mould. Four normal controls and 3 allergic control patients developed no symptoms after 1 hour in the mould-room. In an effort to evaluate clinical mould sensitivity 22 patients reacting intradermally to alternaria were subjected to the mould-room test. 10 of 12 patients whose histories suggested mould allergy gave a positive test, but only one

out of 10 doubtful mould cases developed a positive mould-room test. Nasal tests (powdered alternaria were blown or inhaled by deep breaths) gave about the same result.

Lowell and Schiller (1948) believed that valuable information may be obtained by studying provoked asthma by measurements of changes in vital capacity as a means of detecting pulmonary reaction to inhaled aerosolized allergenic extracts in asthmatic subjects both as a diagnostic procedure and as a means of studying the asthmatic attack under controlled conditions. Every subject was tested one or more times with the extracting fluid alone. In no instance did this cause a significant reduction in vital capacity or any allergic symptoms. There is, however, no information as to whether the patients had normal auscultatory findings on the lungs or not before the tests started. The authors could find no evidence that indicated, as was stated by Urbach, that the phenol in the extracts administered in this manner is likely to cause symptoms due to irritation. In no instance did the skin fail to react to the endermal injection of an extract which gave a reduction in vital capacity by this method of testing. However, there were many instances in which substances which reacted by skin testing failed to produce any change in the vital capacity when inhaled as an aerosol. The correlation was good between allergenic substances causing reductions in vital capacity on the one hand, and clinical sensitivity as judged by statement made by the subject on the other. In the investigation mentioned pollen and dust were studied in 10 cases of asthma.

Christensen and Sonne (1948) were of the opinion that the reason why the result of provocatory inhalation tests have never been published to any particular extent can presumably be found mainly in the following three circumstances: 1) Risk of a severe attack of asthma, 2) Inhalatory tests were considered unnecessary when skin tests were available, 3) When provocation tests were performed no reaction usually appeared. The authors described 7 patients in whom the skin reaction was strongest to dust from their own homes. These

patients were exposed in two different ways. First they had to stir dust in a dish with a stick for half an hour. Only one patient had an attack and not before 6 hours after the exposure. Further the patients had to inhale extracts of the dust. The inhalation experiments were commenced with weak extracts (10^{-4}), the strongest extract inhaled were 10^{-2} . This procedure, even with the 1 % extract, never resulted in an asthmatic attack. The authors conclude that their experiments have only contributed to increasing the scepticism felt by many about the justification for desensitizing therapy in bronchial asthma merely on the basis of the skin reaction.

In an investigation of mould hypersensitivity in Sweden, Nilsby (1949) examined asthma patients sensitive to different antigens with inhalation experiments. Out of 100 provocation tests he got positive results only in 3 cases (2 with pollen allergy and 1 patient sensitive to horse epithelium). No positive results were obtained with mould inhalations, although in 12 cases it was probable from the history that mould had been of etiological importance.

In studying asthma patients Juhlin-Dannfelt (1950) made a provocation test for each of 165 strongly positive intradermal reactions. 99 of the provocation tests were positive and 66 negative. In most cases the tests for hypersensitivity to animals and for alimentary allergy were carried out as exposure tests, whereas the tests for other inhalation allergens were performed as provocation tests, mostly by sniffing the substance in question. In the material of Juhlin-Dannfelt the latent form of allergy was no less than $\frac{2}{5}$ of the cases of hypersensitivity with pronounced skin reactions. The patients belonging to this material had in the main suffered from asthma throughout the year, and Juhlin-Dannfelt stresses that it has usually not been practicable to decide in any other way than by means of exposure or provocation tests whether the demonstrable skin reactions were due to manifest or latent allergy. The positive reactions which were examined by provocation tests in the material of Juhlin-Dannfelt were to a

large extent caused by animal epithelium and pollen (124 reactions). House dust was only examined in one case.

According to the above investigations different opinions exist, especially concerning the following questions:

1. How often does the inhalation test give a positive result?
2. What is the result of provocation tests with auto-dust extracts? This question is of special interest, since according to Salén (1948) house dust is the most common cause of asthma in Sweden and since provocation tests with auto-dust have not been published to any very large extent.
3. Can one get a positive inhalation test even if the intradermal test is negative to the antigen in question?
4. How great are the risks with provocation tests?

The different results of the above investigations may depend to a large extent upon the different methods used and the different concentrations of the inhaled antigen solutions. Juhlin-Dannfelt emphasises (1950) that a very important point concerning provocation tests is that the strength of the solution to which the patient is exposed should be sufficiently great, especially in the case of weak allergens, as for instance house dust, otherwise the provocation test may turn out negatively in manifest allergy.

It is shown in previous papers (Colldahl and Lundin 1952, Colldahl 1952) that it is possible to perform provocation tests with the same reliability on patients with rough breathing sound as in cases of normal breathing sound provided that the patients have no breathing difficulties. In this investigation the results from both groups of patients are therefore taken together. The method and the extracts used are described in a previous paper (Colldahl, 1952).

RESULTS

44 asthma patients were examined both by intradermal titration of especially those antigens that had given a positive

reaction in the intradermal routine skin test and by provocation tests. The outcome and number of these tests are found in table 1. In all 169 provocation tests were performed. On some patients only one provocation test was made, but on others up to nine provocation tests with different antigens were performed. Of the 169 provocation tests performed 57 were positive and 90 negative. It was possible by the method used, therefore, to get a clear result in about 87 % of the examined cases. In the remaining 13 % minor symptoms occurred after inhalation of the strongest antigen solution (10^{-1}), but they were not sufficiently characteristic to indicate their being positive. If the method of Colldahl and Lundin mentioned above of registering the washing-out time after each inhalation had been used in all cases, it is possible that the number of questionable results from the provocation tests could have been reduced.

A very high incidence of positive results from the provocation tests has been obtained in cases of auto-dust allergy, no less than 64 % of the provocation tests being positive. Of the patients showing a positive skin reaction for auto-dust (by skin titration at least + for a concentration 10^{-3}) the provocation tests turned out positively in 72 % for this antigen. The incidence of positive provocation tests of the whole material is about 34 %. Of the patients showing a positive skin reaction the provocation tests turned out positively for the whole material in 43 %. And, as is seen also from table 1, the highest incidence of skin allergy among the patients examined was due to house dust. This material therefore confirms the earlier observations by Salén regarding the great importance of house dust as a cause of asthma in Sweden. In cases of a positive skin reaction for auto-dust only 14 % indicates a latent form of allergy.

As the provocation tests by inhalation of auto-dust extracts have turned out positively to such a large extent it was considered necessary to let asthma patients with a negative history for dust and with negative skin reactions to different kinds of auto-dust from other patients on whom provocation

tests with their respective auto-dust extract have given positive results, inhale one of these auto-dust extracts. This was done on 6 patients. The results of these provocation tests were negative. (Only 2 of these patients are included in the material in this investigation, table 1). Therefore one may conclude that the auto-dust extracts themselves are not irritating when inhaled and do not therefore cause unspecific reactions.

As it is the opinion of several investigators that mould spores are the active agent in house dust, provocation tests with an extract of mixed mould spores have been performed on dust-sensitive patients. As a rule a provocation test with mould spores has been performed on such patients before the provocation test with auto-dust. As is seen from table 1, the tests with mould spores in these cases where the skin reaction was negative as a rule turned out negatively. On the other hand it has been clearly shown that mould has great importance in giving potency to different sorts of dust (Rimington).

Compared with the high percentage of positive provocation tests in cases with positive skin reactions to auto-dust it is interesting to find that of 41 positive skin reactions to animal dander only 8 provocation tests by inhalation of animal dander were positive. 9 were questionable and 24 negative. Therefore only about 20 % of the provocation tests in cases of positive skin reactions to animal dander turned out positively. 59 % of the positive skin reactions to animal dander indicate a latent form of allergy. Of the positive skin reactions for the whole material of asthma patients 42 % indicate with certainty a latent form of allergy.

The patients in this investigation showed 17 positive skin reactions for different kinds of pollen. The provocation tests turned out positively in 10 tests, questionably in 1 test, and negatively in 6 tests, i.e. about 59 %, 6 % and 35 % respectively. Therefore, in cases of positive skin reactions on asthma patients for different kinds of pollen, about 35 % of them indicate latent allergy.

From table 1 it is evident that the higher the skin sensitivity, the larger is the percentage of positive results from the provocation tests. When an antigen concentration of 10^{-5} by skin titration just gives a positive reaction, 62 % of the provocation tests turn out positively; by a skin titer of 10^{-4} 45 % of the provocation tests are positive; by a skin titer of 10^{-3} 32 % are positive; and in those cases where still higher antigen concentrations are necessary in order to give a positive skin reaction, the provocation tests turn out positively in 16 %. These figures refer to the whole material.

As is seen from table 1, nine out of 58 provocation tests turned out positively even though the skin test was negative, i.e. a one plus reaction at least does not occur after an intradermal injection of an antigen concentration = 10^{-3} . This means that about 16 % of the provocation tests turn out positively in cases of a negative skin test in asthmatic patients under the conditions for the material in this investigation. Therefore, not a few patients with a clinically significant allergy may be missed, if a negative skin test is considered as showing that a manifest (or semilient) form of allergy does not exist.

About 56 % of the positive provocation tests do not become positive until the patients have inhaled the strongest antigen solution used, i.e. 1:10 (one part of the antigen material to 9 parts of Coca's solution). This is evident from table 1. Therefore one can with certainty conclude that a provocation test ought never to be considered as negative until this antigen concentration has been tested.

Table 1 shows that, in order to get a positive outcome from the provocation test, it is in most cases necessary to inhale a solution with a higher antigen concentration than that which has just given a positive reaction by intradermal titration. In most cases, with the method used, the skin sensitivity was about 100 times stronger than the bronchial sensitivity, when the previous given definitions of skin sensitivity and bronchial sensitivity are used (see under Method, Paper 1 of this series). It was rather rare for the bronchial sensitivity

TABLE I
Total provocation tests performed.

Extract	Result of provocation tests (+ ? -) in different degrees of skin sensitivity determined by titration																		The concentration of extract giving by inhalation just a positive provocation test										Skin sensitivity / Bronchial sensitivity										Number of provocation tests																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																															
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to be higher than the skin sensitivity. In this material this has been the case only in some provocation tests with pollen and animal dander. Can this be the reason why these antigens belong to the strong ones?

In this investigation, where the patients inhaled the antigen in gradually increasing amounts and finished the inhalations as soon as they felt any difficulties, severe asthma occurred only in one patient. After oxygen inhalation and injections of adrenaline and theophyllamine the patient very soon recovered. About 20 patients received injections of adrenaline after the inhalations in order more quickly to make them symptom-free.

DISCUSSION

In this paper it is shown that in case of positive skin reactions to different antigens, these reactions indicate a latent form of allergy to a very different extent for different antigens. For auto-dust 14 %, for pollen 35 % and for animal dander 59 % indicate a latent form of allergy. If by skin testing, therefore, one finds a positive reaction for animal dander, there is a much greater possibility that this skin allergy has no importance for the disease, as it would be if one got a positive skin reaction for auto-dust. These results agree well with earlier investigations (Colmes, Guild, Rackemann, Salén and Juhlin-Dannfelt).

The fact that Juhlin-Dannfelt (1950) got positive provocation tests in 60 % of cases and that the percentage of positive results of the provocation tests, in cases of positive skin reactions, in this investigation are only 43 %, although Juhlin-Dannfelt had in his material only 1 case of allergy to house dust, which antigen has in this investigation given the highest percentage of positive results of the provocation tests, may to a large extent depend on the cases examined. Juhlin-Dannfelt only examined cases with pronounced skin reactions, and therefore the percentage of positive results of the provocation tests ought to be greater.

According to Juhlin-Dannfelt (1950) it is exceedingly seldom that a manifest allergy gives only a weak positive skin reaction. This result cannot be confirmed by the present investigation. The provocation tests turned out positively in 32 % of cases when the skin titer was just positive (a positive skin reaction was just obtained by an antigen conc. $= 10^{-3}$) for the whole material, and in the case of dust allergy in a still higher percentage.

SUMMARY

On 44 asthma patients 169 provocation tests were performed. A clear positive or negative result was obtained in 87 % of cases. In 43 % the provocation tests turned out positively, if the skin test with the antigen examined was positive.

Auto-dust was the most common cause of skin sensitivity among the patients examined. Auto-dust was the antigen that gave the highest percentage of positive provocation tests. Of the patients showing a positive skin reaction for auto-dust, the provocation tests were positive in 72 % of cases.

Patients on whom the provocation test became positive by inhalation of auto-dust did not as a rule react by inhalation of an extract of mixed moulds spores.

In case of positive skin reactions to animal dander the provocation tests turned out positively in only 20 % of cases.

In 59 % the provocation tests gave a positive result if the patients had a positive intradermal test for pollen.

The higher the skin sensitivity, the larger was the percentage of positive outcomes in the provocation tests.

In 16 % of cases the provocation tests turned out positively, though the intradermal test was negative.

With the method used most of the positive provocation tests did not become positive until inhalation of an antigen concentration $= 10^{-1}$.

With few exceptions (some cases of sensitivity to pollen and animal dander) the skin sensitivity was higher than the

bronchial sensitivity with the method used in this investigation.

The risks with provocation tests with the method used are small, but of course careful observation of the patient is necessary.

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