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STUDIES IN HYPOSENSITISATION WITH ALLPYRAL

Objective Evaluation of the Results

By

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The conventional therapy in allergic rhinitis and asthma—when the causative allergen has been confirmed—is specific hyposensitisation—except for the use of symptomatic drugs and/or elimination of the offending allergen. Results of specific hyposensitisation have been considered to be favourable with several allergens, e. g. 75–80 per cent in pollen allergies. However, there are certain disadvantages in using aqueous extracts—a very large number of injections has to be given at frequent intervals in order to reach the optimal individual maintenance dose. Side-effects are not uncommon. These may be local with large swellings, itching and erythema, or general with urticaria, running nose, asthma and allergic shock. They may occur any time during treatment but especially when switching to a higher concentration of the extract or to a new vial of a freshly prepared extract. The risks of severe reactions are particularly great when intervals between injections are too long—more than 1 week in the initial stage and more than 4 weeks in maintenance treatment.

In trying to exclude these disadvantages, new allergen extracts have been developed, where the allergen is slowly

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released. One of these products is Allpyral, which is a pyridine extracted alum precipitated allergenic extract suspended in 0.9 per cent saline. The strength is given in protein-nitrogen-units per ml (PNU/ml). 10,000 PNU is equivalent to 0.1 mg protein nitrogen. The advantages with Allpyral compared to the traditional aqueous extracts are said to be: The allergen is released slowly after the injection and therefore the risk of severe reactions is reduced. The dose need not be changed when switching to a new vial of the same strength. The number of injections is reduced and intervals are longer between injections.

Since the first results of treatment with Allpyral were published in 1959 by *Fuchs & Strauss* (16), a large number of clinical trials from various countries have been published and they have reported good results (4, 10, 12, 15, 17, 18, 19, 20, 23, 29, 33, 35). However, most of the authors seem to have based their diagnosis on case histories and skin tests only. The results have been the patient's opinion as to relief of symptoms. In our trial we also wanted to include objective evaluations of the results of treatment with Allpyral extracts.

MATERIAL AND METHODS

First, a comparison was made between aqueous extracts manufactured according to the weight/volume principle (Vitrum) and aqueous extracts, for which the strength is given in PNU/ml (Dome). In 75 patients with known sensitivities for certain allergens, titrated intracutaneous skin tests and provocation tests were carried out with both types of extracts. When testing intracutaneously, 0.1 ml was injected, starting with the dilution 10^{-6} of the Vitrum extract and 10 PNU/ml of the Dome extract. Reactions were read after 10-15 minutes and were considered positive if the sum of the largest diameter of the wheal and the diameter at right angles to that was 20 mm or more. If the reaction was not positive, testing was continued with successively higher concentrations until a positive reaction occurred or the final strength 10^{-2} (Vitrum) and 1,000 PNU/ml (Dome) had been reached.

Provocation tests were performed in patients with allergic rhinitis by dropping the allergen solution into the nostril and in asthma patients by inhalation from a nebulizer. In order to avoid non-specific reactions the patient was not told in advance which allergens would be used and, furthermore, each test was started with the diluent—0.9 per cent saline with 0.5 per cent phenol—

as a control. The provocation tests were then continued with dilutions of the allergen extracts—in the nose with 0.1 ml and in the bronchi by inhalation of 1.0 ml. They were started with the lowest concentration that had given a positive intracutaneous reaction. Then the patient was under observation—after the nasal test for 15–20 minutes and after the bronchial test for 30–45 minutes. If no reaction occurred the test continued with successively increasing concentrations until symptoms occurred or until the highest strength of the extract had been reached. The provocation test was considered positive in the nasal application if the patient had a running nose, sneezing and/or a stuffed nose, and in the bronchial test if the patient got ronchi, reduced values in dynamic spirometry by at least 15 per cent and subjective symptoms such as coughing and a feeling of tightness in the chest.

After the allergologic evaluation described above, specific hyposensitisation with Allpyral was started in some patients. The injections were given subcutaneously at weekly intervals up to the optimal tolerable dose, usually 0.4–1.0 ml of the strength 10,000 PNU/ml. After this dose had been reached, provocation tests were repeated according to the same method as used before treatment. In all, 36 patients were examined in this manner. All were men, 34 in the age group of 16–30 and 2 in the group 40–50 years. 24 patients were treated with one single allergen, 10 with two and 2 with three allergens. The patients treated with 2 and 3 allergens were not treated with a mixture of the allergens but with separate injections of the different allergen extracts. Thus 50 treatment courses were given and in every course provocation tests were carried out before and after treatment.

In order to study circulating reagins and blocking antibodies, blood was taken from 15 of the patients in a closed system in sterile glass bottles. The bottles were centrifuged within 12 hours and serum was transferred to low-pressure glass vials of 20 ml which were then stored at -20°C . Blood samples were taken before the start of the Allpyral treatment (serum A) and after the maintenance dose had been reached (serum P). With serum A and serum P, the following experiments were carried out:

1. Dilution Tests

Serum A and serum P respectively were diluted 1:2, 1:4, 1:8, etc. with the above-mentioned diluent (0.9 per cent saline with 0.5 per cent phenol). The dilutions were kept at $+2$ to $+6^{\circ}\text{C}$ for 12 hours. Then transfer tests according to Prausnitz-Küstner (P-K) (30) were carried out, i. e. 0.1 ml of each dilution was injected intracutaneously in vertical lines on the back of a non-allergic recipient. Each wheal was marked with ink. 24 hours later challenge was performed with aqueous allergen extracts in the strength of 1,000 PNU/ml, and thus 0.1 ml (i. e. 100 PNU of the respective allergen) was injected in each wheal (Table 1). This reaction was read after 15–20 minutes. The same criteria for positive reactions were used as in the intracutaneous test described above.

TABLE 1

Dilution test. 0.1 ml of serum dilutions 1:2, 1:4 etc. injected intracutaneously as table below. 24 hrs later challenge with aqueous allergen-extract 1,000 PNU/ml—0.1 ml extract in each site.

Serum A dil.	Result 15 min. after challenge	Serum P dil.	Result 15 min. after challenge
1 : 2	POS	1 : 2	POS
1 : 4	POS	1 : 4	POS
1 : 8	POS	1 : 8	POS
1 : 16	POS	1 : 16	POS
1 : 32	POS	1 : 32	POS
1 : 64	NEG	1 : 64	NEG

2. Neutralisation test

Neutralisation tests were carried out according to *Cooke* (9) and are illustrated in Fig. 1. To serum A and serum P was added in equal parts the aqueous allergen extract in increasing strengths, e.g. 0.3, 0.6 and 1.0 ml of serum A from a patient sensitive to horse epithelia was mixed with 0.3, 0.6 and 1.0 ml respectively of horse epithelia extract Dome in the strength of 1,000 PNU/ml. The mixtures thus contained 300, 600 and 1,000 PNU respectively. In the same way all other mixtures of sera A and P were prepared with allergen extracts according to Fig. 1. For the controls, 0.5 ml of the serum was mixed with 0.5 ml diluent (0.9 per cent saline with 0.5 per cent phenol). After storing for 12 hours at +2 to +6°C, passive homologous sensitisation was performed with these mixtures in accordance with the above-mentioned dilution test. 0.1 ml of each serum allergen extract mixture was injected on the back of a non-allergic recipient. The injections were made in vertical lines after increasing allergen strengths and marked with an ink ring. After 24 hours, challenge was made with 0.1 ml of aqueous allergen extract 1,000 PNU/ml, i.e. 100 PNU of the respective allergen. The results were read as usual after 15–20 minutes.

3. With serum from a dog epithelia sensitive patient (M.L.) the following experiment was carried out, illustrated in Fig. 2, according to *Walzer & Bowman* (36):

1 ml of serum was mixed with 1 ml of Dome aqueous dog epithelia extract of the strength 10,000 PNU/ml and the mixture was stored for 12 hours at +2 to +6°C. Transfer tests were then carried out in a non-allergic recipient. In each of four sites 0.1 ml of the mixture was injected. 24 hours later challenge was performed with 0.1 ml of aqueous dog epithelia extract. In one of the wheals the Dome extract of 1,000 PNU/ml was used. In the other injection sites Vitrum's extracts in the strength of 10^{-3} were given of Boxer, "Drever"

Neutralisation test.

0.1 ml. OF SERUM MIXT INJECT. I.C.	CHALL. 24 HRS WITH	RES. 15' AFT. CHALL.	0.1 ml. OF SERUM MIXT INJECT. I.C.	CHALL. 24 HRS WITH	RES. 15' AFT. CHALL.
SERUM A + CONTROL } ○	HORSE 100 PNU	POS	SERUM P + CONTROL } ○	HORSE 100 PNU	POS
SERUM A + HORSE 300 PNU } ○	" "	" "	SERUM P + HORSE 300 PNU } ○	" "	" "
SERUM A + HORSE 600 PNU } ○	" "	" "	SERUM P + HORSE 600 PNU } ○	" "	" "
SERUM A + HORSE 1000 PNU } ○	" "	NEG	SERUM P + HORSE 1000 PNU } ○	" "	" "
SERUM A + HORSE 3000 PNU } ○	" "	" "	SERUM P + HORSE 3000 PNU } ○	" "	" "
SERUM A + HORSE 6000 PNU } ○	" "	" "	SERUM P + HORSE 6000 PNU } ○	" "	" "
SERUM A + HORSE 10,000 PNU } ○	" "	" "	SERUM P + HORSE 10,000 PNU } ○	" "	NEG

Fig. 1.

Neutralisation test according to Cooke. Serum A and serum P mixed with increasing doses of allergen extract are injected intracutaneously in non allergic recipient. After 24 hrs challenge with 100 PNU of the same allergen extract in the wheal; read after 15 minutes.

MIXT OF	CHALL. AFT.	RES.
SERUM A 1.0 ml + DOG 10^4 PNU } ○	24 HRS WITH 0.1 ml OF DOG- EXTR. 10^3 PNU/ml	AFT. 15' NEG
" " } ○	0.1 ml OF "BOXER" 10^{-3}	POS
" " } ○	0.1 ml OF "DREVER" 10^{-3}	" "
" " } ○	0.1 ml OF ALSATIAN 10^{-3}	NEG

Fig. 2.

Neutralisation test according to Walzer & Bowman. From a mixture of serum 1.0 ml and allergen extract 10,000 PNU lots of 0.1 ml are injected intracutaneously in 4 sites on non allergic recipient. After 24 hrs challenge with 0.1 ml of different allergen extracts in shown strengths.

and Alsatian epithelia respectively—one in each wheal. Reading as usual after 15–20 minutes.

RESULTS

The results of the comparative experiments with intracutaneous skin tests and provocation tests are presented in Table 2. From this table it can be seen that the Dome extracts showed positive intracutaneous reactions in all cases where the Vitrum extracts showed positive reactions. Also the provocation tests correlated well. In one case the provocation test with the Vitrum dog epithelia extract was negative but positive with the corresponding Dome extract. The allergens tested for were birch pollen, timothy pollen, dog epithelia, cat epithelia, horse epithelia, cow epithelia and perennial rye pollen. It can also be seen from Table 2 that positive intracutaneous reactions occurred with low concentrations of the allergen solutions in the majority of patients (56/75 patients in the dilution 10^{-5} or 10^{-4} with the Vitrum extracts and 72/75 in the dilution 10 or 100 PNU/ml with the Dome extracts).

The results of the provocation tests before and after treatment in the 50 treatment courses can be seen from Fig. 3, where the abscissa shows the lowest concentration of the allergen extract giving symptoms before treatment and the ordinate the corresponding results after treatment. From the figure it can be seen that 7 provocation tests gave positive reactions with the same strength of extract before and after treatment. The remaining 43 tests showed a reduced sensitivity in the shock tissues after hyposensitisation. This reduction was 100 fold in 5 treatment courses, 10 fold in 32 and bifold in 5. Six of the 7 patients who showed unchanged results in provocation testing, however, had subjectively improved during the pollen season or when exposed to the offending allergen. The remaining patient was subjectively unimproved during the first season but during the third season after three years of treatment with Allpyral he had improved both subjectively and objectively. When asking the 29 patients who had improved according to objective criteria, 28 said that

TABLE 2

Allergens:	Intracutaneous tests				Provocation tests							
	Vitrum (W/v)		Dome (PNU/ml)		Vitrum (W/v)				Dome (PNU/ml)			
	pos.	neg.	pos.	neg.	pos.	neg.	pos.	neg.	pos.	neg.	pos.	neg.
Birch pollen	10 ⁻⁵ 4	10 ⁻⁴ 3	10 16	1,000 2	10 ⁻⁴ 5	10 ⁻³ 12	10 ⁻³ 3	10 ⁻¹ 8	1,000 10	10,000 2		
Timothy pollen	11	2	19	5	8	12*	2		4	14*	5	
Dog epithelia	1	10	11	9		10	10	1	3	9	9	
Cat epithelia	2	4	5	1			6		1	3	2	
Horse epithelia	1		1		1			1				
Cow epithelia	1	1	1	1		2			1	1	1	
Perennial rye pollen	1		1			1		1				

Intracutaneous skin tests and provocation tests with extracts from Vitrum (Weight/Volume) and Dome Inc. (PNU/ml) in 75 patients with rhinitis allergica and/or asthma bronchiale. The figures represent the number of positive and negative reactions respectively on titration.

* Provocation tests were not done in 2 patients with Vitrum's extract and in 1 patient with Dome's extract.

** Two intracutaneous tests were doubtfully positive.

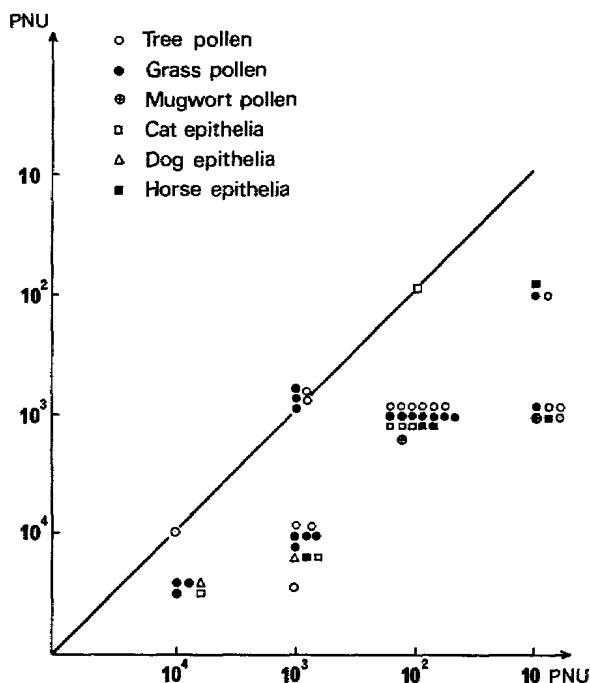


Fig. 3.

Sensitivity to allergen extracts in provocation tests before (Absciss) and after (Ordinate) specific hyposensitization.

they had been considerably better. A dog epithelia sensitive patient (M.L.) said, however, that he still had reactions when he came into contact with a certain breed, "drever", but he no more got symptoms when exposed to other dogs. With the serum from this patient the passive transfer test was performed according to the description Fig. 2, with mixtures of the patient's serum and dog epithelia extract 10,000 PNU/ml. The reaction was negative with the Dome extract and with the Alsatian extract but positive with both Boxer and "Drever" epithelia. This shows that the patient's serum

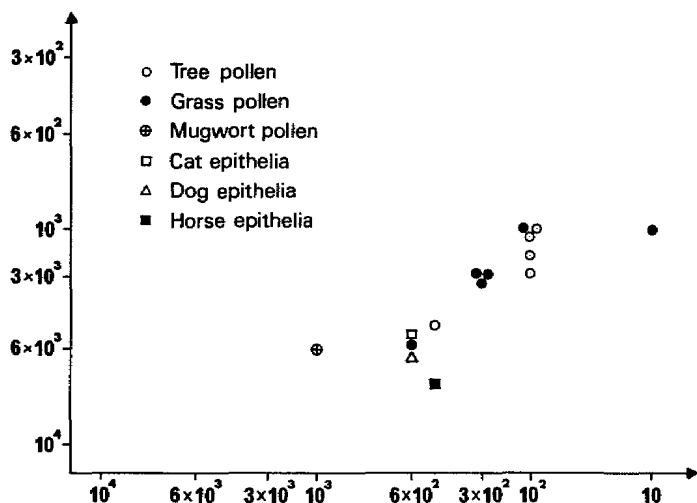


Fig. 4.

Serum A (Absciss) and serum P (Ordinate) mixed with increasing doses of allergen extract giving positive reaction on challenge after 24 hrs with 100 PNU of the same allergen extract.

contained reagins also against "Drever" epithelia and these reagins were not neutralised by the Dome dog epithelia extract. It also indicates that the latter extract did not contain "Drever" epithelia. The experiment also seems to demonstrate that there are probably breed specific allergenic determinants as well as allergenic structures common to several dog breeds. These observations are in agreement with previous experiences (14).

The neutralisation tests with mixtures of sera A and P with the allergen extracts in increasing strengths gave the following results, which are illustrated in Figs. 1 and 4. After challenge with 100 PNU of the respective allergen extract a positive reaction occurred—as an indication that there had been a reaction between non-neutralised reagins and the added allergen—in the wheals with mixtures of serum A and aller-

gen extract up to a certain strength. In the corresponding challenge in the wheals with serum P the reaction was positive in more wheals, i. e. there were still reagins available for the reaction with the added allergen extract.

On comparison between the endpoints for positive challenge in P serum and A serum, i.e. in the example of Fig. 1 $\frac{6,000}{600} = 10$, the following blocking indexes were found in these 15 sera: 100 in one, 30 in two, 20 in one, 10 in ten and 6 in one serum.

DISCUSSION

It has been shown in Sweden, among other countries, and especially by *Salén & Juhlin-Dannfelt* (31) that the correlation between results of intracutaneous tests and clinical hypersensitivity is poor. In order to establish the final diagnosis, provocation tests were therefore carried out in this trial by applying the allergen direct to the shock tissues according to *Juhlin-Dannfelt* (22) and *Arner* (1). As a basis for the diagnosis it was essential that there was a complete correlation between history, intracutaneous tests and provocation tests. All patients in this trial fulfilled these claims. The Dome aqueous extracts showed positive intracutaneous and provocative tests in all cases where the Vitrum extracts had been positive.

The majority of cases showed positive intracutaneous skin tests with allergen solutions which were more diluted than the standard solutions, which are routinely used for intracutaneous tests, i. e. 10^{-3} with the Vitrum extracts and 1,000 PNU/ml with the Dome extracts. Put together with the results of the provocation testing, our findings confirm previous observations by e. g. *Colldahl* (6) and *Engström & Kraepelien* (11), that there is a positive correlation between results of intracutaneous tests and clinical allergy. If a very low concentration of an allergen gives a positive reaction, it is more probable that such an allergen is of importance for

the clinical symptoms of the patient, than if the intracutaneous test is not positive until a higher concentration is used. It must, however, be pointed out that this is not always true in the individual case. There have been reports of clinical hypersensitivity to specific allergens which have given negative intracutaneous skin tests, e. g. *Fagerberg* (13), just as there are cases demonstrating positive reactions with weak dilutions of an allergen extract which has no significance as far as the patient's symptoms are concerned. Every allergic patient must, therefore, be subjected to a detailed allergy diagnosis according to the criteria above. If a patient has not improved after adequate specific hyposensitisation he should be examined again thoroughly to make a detailed allergic diagnosis.

As was mentioned on page 3 comparisons were made between the results of titrated provocation tests before and after treatment of 36 patients. In Fig. 3, however, the results are presented after 50 treatment courses, and this number is explained by the fact that 10 patients were treated with two allergen extracts and 2 with three extracts. The patients who were treated with more than one extract, however, had a clinical, established hypersensitivity to allergens of different types, e. g. animal epithelia and pollen. It could be questioned whether it is correct to look upon the results of different treatment courses in the same patient as separate treatments. However, such a method is backed up by the results of previous investigations (38) where titrated provocation tests have been carried out in patients with an established clinical hypersensitivity to different allergens, but where it has not been considered necessary to treat with more than one extract. The provocation tests were repeated after treatment also with the extracts, with which the patient had not been treated and showed an unchanged titre. Provocation tests with the extract used for treatment, however, showed a reduced sensitivity in the shock tissues. In three of those patients in the present investigation, who were treated with two extracts, the provocation tests also showed a different degree of reduced sensitivity to the different allergens. Provocation tests carried

out after the patients had reached their maintenance dose demonstrated objective improvement in 43/50 cases. In the 7 cases with unchanged values in provocation testing after treatment there could, however, have been indicated an objective improvement. In the provocation tests, namely only 10, 100, 1,000 and 10,000 PNU/ml strengths were used, and if we had used a greater range of dilutions, small variations in sensitivity might have been detectable and accordingly sharper limits between the results of provocations could have been obtained. The results of this trial suggest that titrated provocation tests are valuable aids in the evaluation of the effect of specific hyposensitisation. This is also in accordance with earlier investigations by *Baldwin & Glaser 1936 (2)*, *Loveless 1940 (25)* and *Wiholm 1962 (37)*.

The mechanism behind the effect of specific hyposensitisation has been discussed in the literature. Cooke et al. already in 1935 launched the theory that the active factor could be the "property" which had appeared in serum after hyposensitisation and which they called blocking antibody. Further support for this theory according to Cooke was that after transfusing blood from hyposensitised patients to untreated patients with the same type of hypersensitivity he could see a reduced sensitivity in the untreated patients. Later on other authors have demonstrated that the blocking antibody could be the active factor, e. g. *Loveless 1940 (24)*, *Maunsell 1946 (28)*, *Wiholm 1962 (37)*, *Starr & Weinstock 1970 (34)*, *Connell & Klein 1970 (8)*.

On the other hand, this has been questioned by some authors, e. g. *Scully & Rackemann 1941 (32)* who could not find any correlation between the subjective clinical improvement of the patient and the concentration of blocking antibodies. A survey of this problem was published by *Burdon 1967 (5)*. In our material there is a correlation considering that blocking antibodies were demonstrated in the sera of all the above-mentioned 15 patients. All 15 were both subjectively and objectively improved.

Another theory is that the effect could be explained by a

reduction or total neutralisation of the reagins. *Connell & Sherman* (7) have shown that the concentration of reagins in serum may be reduced after several years of treatment. In our trial there was, however, no difference in the concentration of reagins from the same patient before and after treatment at the time, when objective improvement could already be demonstrated. No placebo control has been carried out in this trial. Such trials have already been published by e.g. *Bruun* 1949 (3), *Johnstone et al.* 1961 (21), *Wiholm* 1962 (37), *Lowell & Franklin* 1963 (26) and *McAllen* 1969 (27). We have considered these trials sufficient to prove the difference between hyposensitisation and placebo treatment.

SUMMARY

Comparisons were made between Dome aqueous extracts—strength in PNU/ml—and Vitrum aqueous extracts—strength in weight/volume—in 75 patients, in which clinical sensitivity to some common allergens had been established. The two types of extracts corresponded well. 36 patients received a controlled specific hyposensitisation with Allpyral allergen extracts. Provocation tests carried out before and after treatment showed a reduced sensitivity in 43/50 treatment courses. The possible causes for the “failures” in the remaining 7 are discussed. All the patients but two were subjectively improved. Serum was taken from 15 patients and in all 15 blocking antibodies were demonstrated. Passive transfer tests using the Prausnitz-Küstner test with dilutions of sera showed an unchanged reagin titre in all patients at the time, when blocking antibodies and reduced sensitivity in the shock tissues could be demonstrated. The results are interpreted as a confirmation that treatment with Allpyral extracts is effective and that this effect cannot be explained by a reduction in reagin titres in serum in the initial stage of treatment.

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