

Hyposensitization with *Dermatophagoides pteronyssinus* in house dust allergy: a controlled study of clinical and immunological effects

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Summary

A double-blind statistically controlled study was carried out on ninety-six patients with Type I allergy to *D. pteronyssinus* and a history of perennial asthma and/or rhinitis. Forty-eight received injections of an extract of *Dermatophagoides pteronyssinus* and forty-eight controls received injections of carbol saline. Both groups were closely comparable in all respects. Ninety-one were available for assessment, forty-six controls, and forty-five treated.

The *D. pteronyssinus* treated group showed a statistically significant decrease in nasal sensitivity to the mite extract after treatment and recorded less asthma, less use of drugs and an increased clinical tolerance of household dust.

A significant rise in specific IgE to *D. pteronyssinus* was found in twenty-three out of forty (58%) of the treated compared with eight out of forty-one (20%) of the controls and a rise in total IgE in twenty out of forty (50%) of the treated compared with nine out of thirty-nine (23%) of the control group.

Specific IgG antibodies to *D. pteronyssinus* were found prior to treatment in twelve out of thirty-nine (30%) of controls and twelve out of forty (31%) of the treated group. There was a significant increase in the number of patients with specific IgG antibody after injections of *D. pteronyssinus* extract as compared with the controls. This increase was associated with clinical improvement which occurred mainly in subjects who showed a decrease, little change or an increase of less than 30% in specific IgE antibody. In those with an increase of more than 30% in specific IgE antibody there was no evidence of clinical improvement even if specific IgG was produced.

Diminished release of histamine from leucocytes was found after treatment in six out of ten treated compared with three out of eleven controls. There were, however, no differences between treatment and control groups in lymphocyte transformation and the liberation of leucocyte inhibitory agents.

Clinical improvement was significantly better in those treated patients who gave strong nasal and skin reactions before treatment than in those with weak nasal and skin reactivity, who were no different from the controls.

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Introduction

The effectiveness of hyposensitization in relieving symptoms due to inhalant allergens has been shown by a number of controlled clinical trials (Frankland & Augustin, 1954; Lowell & Franklin, 1965; Aas 1971; Lichtenstein, Norman & Winkenwerder, 1971). There would appear to be great variability of response depending on the nature and total dose of the allergen used, the duration of the treatment and the selection of patients. The assessment of clinical improvement in naturally remitting conditions such as asthma and rhinitis is difficult. A number of trials have already appeared in the literature reporting favourable results after hyposensitization with extracts of house dust and the house dust mite *Dermatophagoides* spp. (Aas, 1971; Maunsell, Wraith & Hughes, 1971; Smith, 1971). The majority of patients with clinical allergy to household dust in the United Kingdom and Europe are allergic to *Dermatophagoides pteronyssinus* (Voorhorst, Spieksma-Boezeman & Spieksma, 1964; Maunsell, Hughes & Wraith, 1970; Holford-Strevens *et al.*, 1970; van Bronswijk & Sinha, 1971).

In undertaking the present study a number of clinical and laboratory methods of investigation were used to assess the effects of hyposensitization, in the hope of providing a basis for further larger co-operative studies.

Patients and methods

Selection of patients

Ninety-six patients with a history of asthma and/or rhinitis attending the Outpatient Clinic at the Brompton Hospital were chosen so that after random allocation of

Table 1. Age groups of subjects

Age group	Controls			Treated		
	Male	Female	Total	Male	Female	Total
10-20	6	5	11	9	3	12
21-30	9	6	15	4	11	15
31-40	7	3	10	7	2	9
41 and over	8	2	10	4	5	9
Totals	30	16	46	24	21	45

Table 2. Clinical history of subjects

	Controls	Treated
Totals	46	45
Asthma	40	43
Rhinitis	43	41
Eczema	13	6
Family history of allergy	20	27
Previous treatment with steroids	12	11
Previous hyposensitization with house dust	10	10
Intal treated	21	16

Table 3. Atopic status

Allergen	No. of patients reacting to allergen	
	Controls (46)	Treated (45)
Dermatophagoides and house dust only	11	9
Pollens*	25	30
Animals†	16	18
Moulds‡	10	9
Foods§	9	2
No. positive to: 2-5 allergens	31	27
6 or more allergens	4	9

* Grass pollens (B₂); tree pollens (B₃); shrub pollens (B₁).

† Cat; dog; horse; feathers.

‡ *Alternaria*; *Aspergillus fumigatus*; *Aspergillus terreus*; *Cladosporium*; dry rot; *Sporobolomyces*; yeast; *Candida albicans*.

§ Milk; egg; wheat; fish; nuts.

treatment there were equal numbers receiving *D. pteronyssinus* or carbol saline injections in each age group and analysis was also possible within the age groups. Ninety-one were available for assessment, forty-six controls and forty-five treated. As can be seen from Tables 1-3, they were comparable in all respects. Also, as can be seen from Fig. 1, the pollen sensitive subjects in the control and treatment groups began their injections at similar times with respect to the pollen season which might influence the clinical assessment.

Criteria for inclusion in the study were taken as: (1) A positive skin prick test reaction to *D. pteronyssinus* 1% mass/vol. In all patients the weal diameter was 3 mm or more. (2) Either (a) an unequivocal history of symptoms being provoked by contact with household dust, or (b) a positive reaction to nasal challenge with *D. pteronyssinus* 0.33% mass/vol.

No patient was included who had received steroid therapy within the previous 3 months or house dust hyposensitization within the previous 3 years.

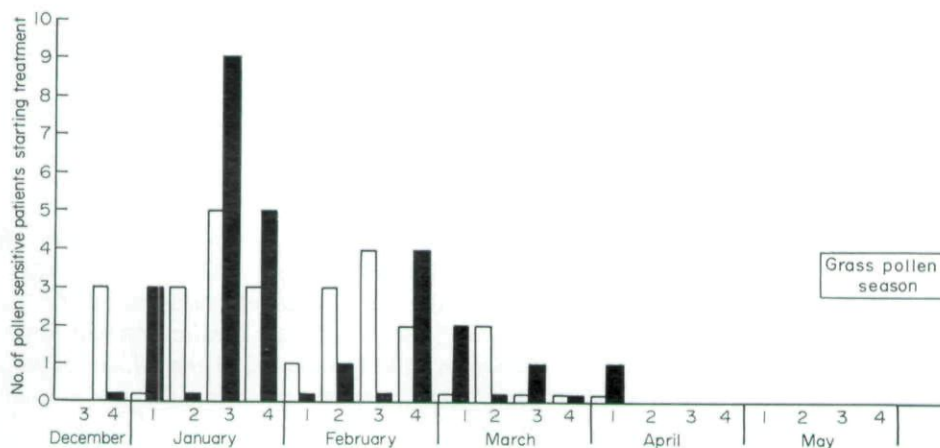


Fig. 1. Time of entry into trial of pollen sensitive patients related to start of grass pollen season. Open columns, controls; hatched columns, treated. Final accumulated totals: 24 controls, 25 treated.

Test and treatment materials

Treatment material. The placebo treatment consisted of 0.5% carbol saline solution. The *D. pteronyssinus* extract was prepared by Beecham Research Laboratories. A 1 g sample of mites and mite excreta separated from the culture medium was extracted in 100 ml of glycerinated extraction fluid to make a 1% extract. This was dialysed and sterilized by membrane filtration. Treatment sets were made up in three 10 ml vials, so that the top concentration, the No. 3 vial, was 0.33%. This was diluted 1/8 for vial No. 2 and 1/64 for vial No. 1. The control injections were made up in identical bottles. Both treatment and control preparations were indistinguishable clear solutions.

Test materials. Prick test solutions were made up in 50% glycerol and 1% carbol saline solution. The nasal test solutions had no glycerol. Concentrations of 0.005%, 0.04%, 0.33%, 1% were used for the skin tests and the lower three for the nasal tests. The same batch of mite growth was used to produce all test materials both for clinical and laboratory purposes and for the treatment sets.

Trial procedure

All patients had the nature of the trial explained to them and their co-operation was obtained in the full knowledge that they might receive placebo injections.

A standard panel of palliative drugs was agreed upon for the trial period, consisting of any of the following: Franol, Orciprenaline, Phenergan or any other specified antihistamine, Mogadon, Isoprenaline inhaler, Ventolin inhaler and Intal. Antihistamines were omitted 48 hr prior to each clinic attendance to permit skin and nasal testing.

House dust control

All patients who had not previously been instructed on house dust control were advised to vacuum clean their mattresses and pillows, damp dust their bedrooms and wash their blankets every 6–8 weeks and to exclude feathers from their beds.

Diary records

Diary charts were issued with instructions on how to score each symptom on a scale of 0–3, a separate recording being made for day and night time and for the quantities of medication used. Reactions to the injections were also recorded.

An assessment was made for 1 month prior to the start of treatment and the diaries were collected at the end of each month.

Skin and nasal tests

At the commencement, all the patients were prick tested with a routine series of twenty-four common allergens (Table 3). A further series of skin tests were then performed consisting of saline control, four dilutions of *D. pteronyssinus*, 0.005%, 0.04%, 0.33%, 1%; two dilutions, 10% and 1%, of the culture medium on which the mite had been grown and three concentrations of histamine HCl 1 mg/ml, 10 mg/ml, 100 mg/ml. Results were recorded after 10–15 min by measuring the mean weal diameters.

All the patients who at the time were free of rhinitis had nasal tests with *D. pteronyssinus* extract commencing at 0.005% and rising to a maximum of 0.33%.

Table 4. Progress of patients who had generalized reactions to *Dermatophagoides* treatment

Vials	Injection no.	Patient D.R.	Patient J.L.	Patient A.A.	Patient J.G.
Low dose	1	No reaction	Local reaction	No reaction	Local reaction
	2	No reaction	Local reaction	No reaction	Local reaction
	3	No reaction	Local reaction	No reaction	Local reaction
	4	No reaction	Local reaction	No reaction	Local reaction
Medium dose	5	No reaction	Local reaction	No reaction	Local reaction
	6	Minor local reaction	Local reaction	No reaction	Local reaction
	7	Minor local reaction	Local reaction	Nasal reaction	Local reaction
	8	Minor local reaction	Local reaction	Urticaria	Local reaction
High dose	9	Minor local reaction	Bigger local reaction	Injections continued	Local reaction
	10	Local reaction	Bigger local reaction	but doses reduced	Local reaction
	11	Urticaria and syncope	Nasal reaction		Urticaria
	12	Injections stopped	Urticaria and asthma		Injections stopped

Test extract was instilled into one nostril and control test carbol saline in the other. A positive result was taken as sneezing, blockage, rhinorrhoea or irritation in the challenged nostril in the absence of any reaction in the control nostril. Reactions were read at 10 min after each dilution and recorded as negative, mild, moderate or severe. The same nostril was challenged with each successive higher concentration of the test extract. The interval between each test was 15 min.

Serological methods

Total IgE measurements. Total IgE was measured using a radioactive radial immunodiffusion technique (Rowe, 1969; Ishizaka & Newcombe, 1970). Comparative readings before and after treatment were available in forty treated and thirty-nine controls. A change of greater than 10% of the pre-treatment value was considered significant as this was shown to be greater than the variability of the test itself.

Specific IgE to *D. pteronyssinus*. This was measured by the RAST technique (Wide, 1969). 20 mg of *D. pteronyssinus* extract was coupled to 100 mg of cyanogen bromide activated cellulose. 0.5 mg of this conjugate was reacted with 0.1 ml of the patient's serum and the presence of specific IgE antibody was tested by a ^{125}I labelled rabbit anti-human IgE serum. A change in the radioactive count of 10% or more over the pre-treatment reading was considered significant as this was over twice the experimental error.

Specific IgG antibodies to *D. pteronyssinus*. Radioimmuno-electrophoretic tests were used to detect the presence of specific IgG to *D. pteronyssinus* (Pepys *et al.*, 1973).

Lymphocyte transformation on allergen challenge

Lymphocyte transformation after challenge with *D. pteronyssinus* was measured by the

uptake of radioactive thymidine in twenty-three patients at the Kennedy Institute (Maini et al., 1971). Further studies on leucocyte inhibitory factors were also done.

Histamine release from leucocytes

Histamine release from leucocytes after challenge with *D. pteronyssinus* was measured in twenty-five patients, by a bioassay using guinea-pig ileum by Beecham Research Laboratories. The technique used was essentially that of Starr & Weinstock (1970).

The treatment

As a precaution the first injection was given in the clinic. Thereafter seventy-one patients were treated by their family doctors returning each month for clinical appraisal and exchange of diaries while the remaining twenty-five received their injections at weekly intervals in the clinic.

Injections of 0.1, 0.2, 0.4 and 0.8 ml were given weekly from each vial starting with the No. 1 low dose vial, proceeding to the medium and then to the high dose vial. The treatment was modified according to individual patient's reactions.

It was advised that where local reactions of redness or swelling of over 24 hr duration were elicited the same dose should be repeated, and where focal reactions of asthma or rhinitis were provoked the dose should be reduced to a lower dose that had not occasioned any reaction. The course was stopped after twelve injections irrespective of whether the top dose had been reached. The top (0.8 ml) dose of each of the lower dilutions corresponded approximately in allergen content to the bottom 0.1 ml dose of the next stronger vial in an attempt to obviate undue reactions on changing vials.

Results

Out of a total of the ninety-six patients given the first injection five are not included in the analysis. Two of these completed the twelve injections, but failed to return for clinical assessment. Two dropped out while under therapy, no reasons being given. The fifth patient had a mild asthmatic reaction to the second injection and was excluded from the trial for this and other reasons. Of these five, two were in the control and three in the treated group.

Reactions

Reactions to the injections were mainly local redness and swelling at the site of treatment in thirty-four patients. Where this lasted for over 24 hr most of the patients had the causative dose repeated. In general when this precaution was observed, no further problems were encountered. However, a total of eighteen patients on treatment had mild asthma and/or rhinitis within 48 hr of an injection, but on eleven occasions this was when previous warning local reactions had been ignored. In four patients there was short-term generalized urticaria and in three fainting and dizziness (Table 4). In all of these patients there were mainly local warning reactions prior to other reactions.

Twenty-three treated patients reached the top dose and all but five finished both lower strength vials. Most reactions were observed on changing to higher dose vials. In four patients the *D. pteronyssinus* injections were stopped due to reactions whilst receiving the high dose vial. In one, treatment was stopped because of purulent bronchitis.

In the control group two patients stopped treatment because of increasing asthma.

Table 5. Clinical results after treatment (self-assessment questionnaire)

	1 month			3 months		
	Controls (46)	Treated (45)	P	Controls (43)	Treated (40)	P
Asthma improved	35% (16/46)	58% (26/45)	NS	33% (14/43)	62.5% (25/40)	0.02
Rhinitis improved	22% (10/46)	29% (13/45)	NS	14% (6/43)	35% (14/40)	NS
Exercise tolerance better	13% (6/46)	27% (12/45)	NS	19% (8/43)	35% (14/40)	NS
Dust tolerance better	Not assessed			30% (13/43)	55% (22/40)	0.05
Time off work less	9% (4/46)	2% (1/45)	NS	14% (6/43)	25% (10/40)	NS
Drug use less	Diary			19% (8/43)	43% (17/40)	0.05
Overall self-assessment						
'very much better'	13% (6/46)	31% (14/45)	NS	19% (8/43)	35% (14/40)	NS

Questionnaire assessment

At 1 and 3 months after treatment all patients were interviewed and asked to assess their overall progress as a result of treatment, as 'Improved' (very much, moderately or slightly), 'No change' or 'Worse' (slightly, moderately or very much). They were also asked to specify in what way they were changed, e.g. less asthma, less time off work, etc. In all ninety-one patients were assessed, forty-five treated and forty-six controls (Table 5).

Significant improvement in asthma, drug use and dust tolerance was recorded in the treated group as compared with controls at 3 months after treatment, but not at 1 month after treatment. The overall self-assessment at 1 and 3 months after treatment also showed differences in those recording themselves as 'Very much improved'. 31% (14/45) of the treated as opposed to 13% (6/46) of the controls recording themselves as very much improved at 1 month and 35% (14/40) compared with 19% (8/43) at 3 months after the end of the treatment. The treatment group showed better results at 3 months, in all seven criteria assessed (Table 5).

Nasal challenge

Serial nasal tests were done on fifty-two patients who were free from rhinitis at the time. Nasal sensitivity was considered to have decreased only when a higher dosage of test solution was required to provoke a reaction. A significant improvement was seen in the treated group 70% (19/27) as compared with the controls 36% (9/25) ($P < 0.025$) (Table 6).

Table 6. Changes in nasal provocation after immunotherapy with *Dermatophagoides pteronyssinus*

Compared with pre-treatment reaction	Controls (25)	Treated (27)
Nasal sensitivity decreased	36% (9/25)	70% (19/27)*
No change	36% (9/25)	19% (5/27)
Increased sensitivity	28% (7/25)	11% (3/27)

* $P < 0.025$.

Prick tests

The results of prick tests before and after treatment showed little change. Nine out of thirty-six treated patients became skin negative to the most dilute *D. pteronyssinus* extract after treatment as compared to four out of twenty-eight controls. Histamine reactivity was unchanged and no patient became skin sensitive to the culture medium on which the mite was grown.

Diary scores (patient assessment of presence and severity of symptoms)

Records were adequately completed and available for comparison in seventy patients, thirty-three treated and thirty-seven controls. The pre-treatment severity of all symptoms as measured by diary scores was similar in both groups (e.g. mean scores for asthma were 1.13 per day for controls and 1.04 per day for treated).

Table 7. Changes in diary scores after treatment

Diary scores	Controls	Treated
Wheeze		
Improved	64% (18/28)	75% (21/28)
No change or worse	36% (10/28)	25% (7/28)
Rhinitis		
Improved	66% (23/35)	64% (21/33)
No change or worse	34% (12/35)	36% (12/33)
Inhaler use		
Less	50% (9/18)	50% (8/16)
Unchanged or more	50% (9/18)	50% (8/16)
Night asthma		
Improved	33% (9/27)	23% (5/22)
Unchanged or worse	66% (18/27)	77% (17/22)

Symptomatic improvement was thought to have occurred if the total of the scores recorded for the month after treatment was less than that for the month prior to treatment. As can be seen from Table 7, both treated and control groups recorded improvement with no difference between the two groups except for less asthma in the treated group. The mean improvement in asthma scores for the treated group was 36% with a range of 14–100%, whereas for the controls it was 25% with a range of 5–100%. It should be noted that not all patients gave scores for all the items shown in the table and that the comparisons were made between a winter month pre-treatment and a summer month post-treatment.

Overall assessment (in terms of a combination of questionnaires, nasal challenges and diary scores)

In contrast to the usual procedure of assessing clinical improvement in terms of single individual test results, for the purposes of statistical analysis a combination of the three main clinical criteria was used. Questionnaires, nasal challenges and diaries were looked at, and individuals were considered to have improved only if they were better by all three measures after treatment. In thirty-six patients, seventeen treated and nineteen controls, full records were available for diary scores, nasal challenges and

questionnaires. The reason for failure to complete diaries was lack of patient co-operation, while nasal challenges were often impossible because of rhinitis. However, those available for this combined assessment were distributed comparably within the age groups (Table 8).

There was a significant improvement ($P = 0.02$) in the treated group (9/17) as compared with the control (2/19) when improvement was measured in this way.

Table 8. Sub-group who recorded all three clinical criteria: (a) nasal challenge; (b) diary scores; (c) questionnaire

Age group	No. improved in		No. improved in	
	Controls	a, b, c	Treated	a, b, c
10 to 20	4	0	4	2
21 to 30	6	0	7	4
31 to 40	4	1	4	3
41 and over	5	1	2	0
Totals	19	2	17	9
Clinically improved*	2/19 (10%)		9/17 (53%)	

$P = 0.02$ (Fisher's exact test).

* Clinically improved patients showed: (1) Increased nasal tolerance to *D. pteronyssinus* on challenge; (2) improved diary scores for asthma (or rhinitis if not asthmatic); (3) registered improvement on questionnaire for asthma (or rhinitis if not asthmatic).

Relationship of prick and nasal test sensitivity to treatment response

Table 9 shows that the more highly skin sensitive subjects who gave positive prick test reactions to the lowest concentration tested of *D. pteronyssinus* were better in most respects after treatment than those with lower degrees of skin sensitivity who gave negative prick tests to this but positive reactions to higher concentrations. The subjects who gave positive nasal test reactions to the lower two concentrations of *D. pteronyssinus*, i.e. those with the highest degree of nasal sensitivity, fared significantly better than those who gave negative nasal reactions to them.

Serological results

Total IgE. A rise in total IgE was found in 50% (20/40) of the treated patients compared with 23% (9/39) controls. A fall in total IgE levels was found in 25% (10/40) of the treated patients compared with 52% (20/39) of the controls (Table 10).

IgE specific to *D. pteronyssinus*. Results showed a highly significant rise ($P = 0.001$); greater than 10% in specific IgE after therapy, in 58% the *D. pteronyssinus* treated group (23/40), compared with 20% (8/41) in the control group (Table 11). It was noted that 58% (18/31) of the patients who showed a rise in specific IgE also had a rise in total IgE. A significant difference in clinical outcome was observed when individual patients with changes in specific IgE greater or less than 30% were compared. Those with higher post treatment specific IgE changes were found to have fared worse as a group by all clinical measurements (Table 11).

Table 9. Clinical results of treated patients in respect of pre-treatment skin and nasal sensitivity

Clinical response to treatment	Skin reactivity to <i>D. pteronyssinus</i>				Nasal reactivity to <i>D. pteronyssinus</i>					
	Weak reactors		Strong reactors		Weak reactors		Strong reactors			
	Prick test negative to 0.005%	Prick test positive to 0.005%	<i>P</i>		No reaction to 0.04%	Nasal reaction to 0.04% or less	<i>P</i>			
(1) Increased nasal tolerance on challenge	75%	6/8	66%	12/18	NS	29%	2/7	94%	17/18	0.01
(2) Improved diary score										
(a) For asthma	50%	3/6	85%	11/13	NS	43%	3/7	91%	10/11	0.01
(b) For exercise asthma	0%	0/3	82%	9/11	NS	40%	2/5	78%	7/9	NS
(c) For rhinitis	66%	6/9	53%	8/15	NS	60%	6/10	54%	7/13	NS
(d) For drug use	0%	0/7	57%	8/14	0.05	13%	1/8	58%	7/12	NS
(3) Questionnaire										
(a) 'Very much better overall' 1 month after	17%	2/12	43%	10/23	NS	27%	4/15	40%	8/20	NS
(b) 'Very much better overall' 3 months after	25%	3/12	30%	6/20	NS	8%	1/12	40%	8/20	NS
(c) House dust tolerance improved	42%	5/12	45%	9/20	NS	17%	2/12	65%	13/20	0.02
(d) Assessed as improved by an independent physician	13%	1/8	67%	8/12	0.05	27%	3/11	67%	6/9	NS
(4) Improved by all three criteria 1 + 2(a)(or 2c) + 3(a)	20%	1/5	54%	7/13	NS	0%	0/5	69%	9/13	0.05
(5) Improved in any two of these criteria	20%	2/10	65%	13/20	0.05	21%	3/14	75%	12/16	0.02
(6) Improved in any one of these criteria	83%	10/12	78%	18/23	NS	60%	9/15	95%	19/20	<0.05

IgG specific to D. pteronyssinus. From forty treated and thirty-nine control patients specific IgG antibodies to *D. pteronyssinus* were detected before treatment in twenty-four patients and a further two controls developed them during the 3 months of observation (Table 12). Of these twenty-six patients, nine had had previous hyposensitization with a house dust extract 3 or more years ago. Specific IgG antibodies were found in 28% (17/61) who had never received any house dust immunotherapy.

Table 10. Total IgE levels after immunotherapy with *Dermatophagoides pteronyssinus*

	Controls (39)	Treated (40)
Mean total IgE before treatment (ng/ml)	729	646
Mean total IgE after treatment (ng/ml)	425	674
Number of patients with a rise* in total IgE after treatment	23% (9/39)	50% (20/40)
No change	25% (10/39)	25% (10/40)
Fall*	52% (20/39)	25% (10/40)

* A change of 10% or more over the pre-treatment value.

Table 11. Changes in specific IgE (RAST) after hyposensitization with *Dermatophagoides pteronyssinus*

	Controls (41)	Treated (40)	Significance
Rise after treatment*	20% (8/41)	58% (23/40)	$P = 0.001$
No change	54% (22/41)	20% (8/40)	
Fall*	27% (11/41)	23% (9/40)	

* A change of 10% or more over the pre-treatment value.

Table 12. Specific IgG to *D. pteronyssinus*

	Controls (39)	Treated (40)
Specific IgG before treatment	12 (31%)	12 (30%)
IgG -ve before and +ve after treatment	2/27 (7%)	13/28 (46%)*
IgG +ve before and -ve after treatment	7/12	3/12

* $\chi^2 = 8.68$, $P = 0.003$.

After treatment there was a very significant increase in the numbers of treated patients showing specific IgG compared with controls. As can be seen from the table, three of the twelve treated patients who had IgG antibodies before treatment failed to show them afterwards (during the summer months) as compared with seven of the twelve controls.

Specific IgG and IgE antibodies

Table 13 shows that in the treated group a general trend for improvement in nasal tolerance, improvement in the asthma scores and subjective improvement was present in the patients who developed IgG antibody, and for those in whom specific

Table 13. Clinical results after hyposensitization correlated with changes in specific IgG and changes in specific IgE

Post-treatment clinical measure	Changes in specific IgG				Changes in specific IgE		
	O → O	No specific IgG detected before or after treatment	O → O	O → G	P*	Low specific IgE (IgE rise not greater than 30% after treatment)	High specific IgE (IgE rise greater than 30%)
(1) Increased nasal tolerance on challenge	73%	8/11	75%	6/8	NS	83%	38%
(2) Improved diary scores							3/8
(a) For asthma	63%	5/7	89%	8/9	NS	83%	60%
(b) For exercise asthma	60%	3/5	86%	6/7	NS	77%	60%
(c) For rhinitis	50%	5/10	82%	9/11	NS	67%	43%
(d) For drug use	38%	3/8	67%	6/9	NS	63%	33%
(3) Questionnaire							2/6
(a) 'Very much better overall' 1 month after	7%	1/15	46%	6/13	0.03	38%	21%
(b) 'Very much better overall' 3 months after	21%	3/14	42%	5/12	NS	43%	23%
(c) Tolerance of house dust improved	36%	5/14	75%	9/12	NS	61%	31%
(d) Assessed as improved by an independent physician	22%	2/9	64%	7/11	0.09	53%	33%
(4) Improved by all three criteria combined (1 + 2a(or 2c) + 3a)	0%	0/8	57%	4/7	0.03	36%	0%
(5) Improved in any two of these criteria	43%	6/14	58%	7/12	NS	58%	25%
(6) Improved in any one of these criteria	73%	11/15	85%	11/13	NS	92%	50%
							3/14
							3/13
							4/13
							3/9
							0/3
							3/12
							7/14
							0.008

* Fisher's exact test.

IgE did not rise more than 30%. Furthermore, the thirteen out of twenty-eight treated patients (Table 12) who developed IgG antibodies showed as a group no significant increase in the specific IgE (RAST mean counts for the group pre-treatment 5449 and post-treatment 6039) and they fared better as a group in all respects than the patients who did not produce IgG. The improvement in this group was in fact attributable to ten of the thirteen who showed no increase above 30% in specific IgE, in contrast to the remaining three whose specific IgE increased more than 30% and who showed no improvement. In the fifteen treated patients who did not develop IgG antibodies the specific IgE was significantly increased (RAST mean counts pre-treatment 3852 and post-treatment 5358). In the control group only two developed IgG antibodies and there was no change in specific IgE (RAST mean counts pre-treatment 5223 and post-treatment 5114).

Table 14. Lymphocyte transformation and leucocyte inhibition changes after hypsensitization with *D. pteronyssinus*

	Treated (12)		Controls (11)	
	Before	After	Before	After
(A) % Leucocyte inhibition				
15-20 (doubtful)	1	2	3	1
≥20 (significant)	3	3	3	5
None	7	7	5	5
(B) Lymphocyte transformation (<i>T/C</i> ratios)				
1.5-2.0 (doubtful)	2	2	1	3
≥2 (significant)	0	2	0	1
None	7	5	10	4
(C) Leucocyte inhibition factor				
15-20	1	2	0	1
≥20	1	2	1	2
None	3	5	3	4

Lymphocyte transformation and leucocyte inhibition studies

The results are shown in Table 14. As can be seen, very little difference was observed between the control and treatment groups, the most significant finding being that the majority of patients did not show any very strong cellular reactions to this antigen.

Histamine release

The results before and after treatment in twenty-one patients, ten treated and eleven controls (Table 15) show a greater number of patients in the treated group with a decrease in histamine release after treatment as compared with the control. 'Blocking antibody' measurements using this technique were attempted. Results were obtained in only twelve patients; of these four controls and three treated showed increased blocking activity after treatment.

Further analysis

Analysis of the clinical data by sex, age group and atopic status did not reveal any

differences. The patients who reacted on skin testing only to house dust and *Dermatophagoides* fared no better or worse than other patients.

Table 15. Maximum histamine release from leucocytes

	Controls (11)	Treated (10)
Decreased histamine released after treatment*	27% (3/11)	60% (6/10)
No change or increase	73% (8/11)	40% (4/10)

* Maximum histamine release was expressed as a percentage of total releasable histamine. 'Decreased' implied a difference of more than 10% between pre- and post-treatment readings.

Discussion

The clinical evaluation of patients undergoing hyposensitization has been made in many different ways. When the first report of this form of therapy appeared in the literature, Noon & Freeman (1911) recorded diminished mucosal reactivity in the conjunctiva with anecdotal evidence of improvement in their uncontrolled group of patients after grass pollen injections. In the first controlled study in 1954, Frankland & Augustin used evidence from a questionnaire to measure clinical improvement. Since then diary scores and physiological measurements have also been used to assess clinical change.

Controlled studies of hyposensitization using house dust and mites have so far shown differing clinical results. The B.T.A. trial (1968) using a house dust extract showed no benefit after a 3 month course of injections when compared with a saline control, measuring change by questionnaires; whereas Aas (1971) showed marked improvement using diary scores and bronchial challenges as measurements of change after 3 years treatment comparing two different house dust preparations with saline. More recently Maunsell *et al.* (1971) using *D. farinae* and Smith (1971) using *D. pteronyssinus* have both recorded moderate levels of improvement over controls in small groups of patients using diary scores and questionnaires. Morrison-Smith & Pizarro (1972) using very dilute solutions of *D. pteronyssinus*, about one-tenth weaker than those used in this present study, failed to demonstrate any difference between this preparation and house dust.

The degree of improvement in trials of hyposensitization can be difficult to assess. Large numbers are needed if comparisons are to be made of certain of the test criteria, because not all of the subjects are clinically suitable for assessment of these and because the numbers showing particular changes may be small.

In this study we have utilized most of the previous methods of clinical assessment, namely diary scores, questionnaires and mucosal challenges and have found that when looked at individually there were statistically significant differences between control and treatment groups in both questionnaire and nasal challenges. In the case of the diaries, however, there was considerable improvement for both treatment and control groups, but no significant difference between them. One possible explanation for this was that improvement in terms of diary scores was taken as a fall in score between a pre-treatment month of assessment made during the winter time and a post-treatment

month in the summer time. Evidence from Voorhorst *et al.* (1964) and van Bronswijk & Sinha (1971) suggests that symptoms in mite sensitive subjects vary according to the seasons, being worse in the winter months when mite populations are higher. Therefore in assessing whether improvement had in fact taken place after these injections, the combination of diary, questionnaire and nasal challenge results was considered to be the most sensitive indicator and in this assessment a significant improvement was observed. Its duration as measured by questionnaire showed that seven out of nine treated patients and one out of two controls assessed as improved by all three clinical criteria were still registering improvement 6 months after treatment.

In an attempt to define clinically a group of patients who might benefit most from these injections, comparisons were made within the treatment group between the clinical outcome of patients with differing degrees of skin and nasal reactivity to *D. pteronyssinus* as measured before treatment. Using the same comparative means of clinical assessment on different groups of treated patients it became apparent that those with a high degree of pre-treatment skin reactivity to *D. pteronyssinus* did significantly better than those with a lower degree of reactivity. This difference was even more marked with initial high degrees of nasal reactivity. These patients as a group were better statistically after hyposensitization in terms of nasal tolerance, diary score for asthma, house dust tolerance and the combined assessment when compared with those who initially were less nasally reactive. The severity of asthma in both these groups as measured by pre-treatment diary scores was similar (the mean score for the strong nasal reactors was 1.4 per day and for the weak reactors 1.2 per day).

Whereas the patients with high degrees of nasal sensitivity to *D. pteronyssinus* fared better than those with low degrees in terms of asthma, this did not apply to rhinitis.

There were thirteen patients who showed both marked nasal and skin sensitivity according to the same criteria outlined above. It is of interest that in these patients the best clinical response of all was observed. All ten of those with completed diary scores showed a big improvement for asthma with a mean fall of 63.2% over pre-treatment scores and eleven out of twelve who completed nasal challenges showed improvement. Also of interest was the finding that those with either weak nasal or weak skin reactivity showed no difference in clinical results compared with controls.

After hyposensitization in grass pollenosis (Starr & Weinstock, 1968) and in ragweed allergy (Lichtenstein *et al.*, 1971) evidence of clinical improvement correlating with the production of 'blocking antibodies' has been found using the *in vitro* technique of inhibition of histamine release from leucocytes. In the present study specific IgG antibodies to *D. pteronyssinus* were found in nearly one-third of previously untreated patients. Their presence before treatment was not related to clinical outcome as measured. However, those patients who showed no specific IgG before treatment, but produced it after treatment were improved as a group when compared with those who failed to show specific IgG before or after treatment. For the questionnaire and combined assessments this improvement was statistically significant. The group who failed to show IgG after treatment was in addition found to be similar to the control group. The group who produced no specific IgG after treatment showed a significant increase in specific IgE, whereas in the group who produced specific IgG the increase in specific IgE was not significant.

Specific IgE to *D. pteronyssinus* rose significantly in the treated group as a whole and also seemed to be related to the clinical outcome. The treated patients who showed a rise in their specific IgE to levels greater than 30% following treatment were clinically

unimproved when compared with patients who failed to show such an increase. Patients who showed an increase of less than 30%, however, were clinically just as improved as those with a decrease or no change. Three patients who produced both specific IgG and a greater than 30% increase in specific IgE showed no clinical improvement. There were ten patients with less than 30% increase in specific IgE who also produced specific IgG and these were clinically significantly very much better than all the others; all those with full records (8/9) were improved on diary score for asthma, five of the six tested showed decreased sensitivity on nasal challenge and eight out of nine registered improvement in clinical tolerance to house dust.

The total IgE levels showed a fall in the control group that was not matched by the treated patients. There is some evidence that natural fluctuations in total IgE coincide with seasonal variations in allergenic exposure (Berg & Johansson, 1971), and the fact that in the treated patients total IgE levels did not fall might be in part due to the increase in specific IgE resulting from the injections. It was also noted that in the control group in addition to the seasonal fall in total and specific IgE levels there was also a decrease in the numbers showing specific IgG in summer after the trial as compared with the winter.

The histamine release experiments showed decreases in histamine release from leucocytes in six treated patients and three controls. These findings, however, did not correlate with other changes. Lichtenstein *et al.* (1971) have suggested that decreased leucocyte sensitivity is a phenomenon in its own right and is unrelated to changes in immunoglobulin levels or the measured 'blocking activity' of post-treatment serum.

Lymphocyte transformation and leucocyte migration inhibition studies showed little difference between treated and control patients.

Our conclusions from these clinical and immunological results are that injections of *D. pteronyssinus* extracts are clinically acceptable and that this form of therapy is active and also safe if given with due precautions. There is evidence that the use of a range of concentrations of this allergen for skin and nasal testing may be of value for assessing degrees of sensitivity and identifying patients most likely to benefit from the therapy.

The periods of treatment and follow-up in this present study were short and there is a need for further more prolonged studies before any full assessment can be made.

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