

# A double-blind study of hyposensitization with an alginate conjugated extract of *D. pteronyssinus* (Conjuvac®) in patients with perennial rhinitis

## 1. Clinical aspects

O. J. CORRADO, E. PASTORELLO,<sup>1</sup> S. OLLIER, L. CRESSWELL, C. ZANUSSI,<sup>1</sup>  
C. ORTOLANI,<sup>1</sup> A. INCORVAIA,<sup>1</sup> A. FUGAZZA,<sup>1</sup> J. R. LOVELY,<sup>2</sup> R. I. HARRIS,<sup>2</sup>  
H. K. WATSON<sup>2</sup> & R. J. DAVIES

Academic Department of Respiratory Medicine, St Bartholomew's Hospital, London, U.K., <sup>1</sup>Ospedale S Anna, Como and Policlinico-Universita, Milan, Italy, and <sup>2</sup>Miles Laboratories, Slough, U.K.

The efficacy of hyposensitization with a standardised extract of *Dermatophagoides pteronyssinus* (*D. pteronyssinus*) conjugated to alginate and containing known amounts of antigen P1 (Conjuvac®) was tested in a double blind, placebo controlled, multi-centre study in 66 adult patients with perennial rhinitis. Patients received 11 weekly injections of increasing concentrations of Conjuvac containing from  $56 \times 10^1$  to  $448 \times 10^3$  IU *D. pteronyssinus* or placebo injections of the alginate diluent to some of which 5 µg of histamine has been randomly added. This was followed by 15 monthly injections of Conjuvac or placebo. The severity of nasal blockage, sneezing and rhinorrhoea was recorded twice daily in a diary and visual analogue assessments (VAS) made at each clinic visit. Nasal provocation testing (NPT) was performed with increasing concentrations of the same extract of *D. pteronyssinus* as used in the hyposensitization injections, and changes in nasal airways resistance measured by passive anterior rhinomanometry. VAS was recorded and NPT was performed on entry to the study and after the fifth, ninth and final monthly injection. Conjuvac injections were well tolerated. Large local reactions (> 5 cm) occurred within 30 min in only 1% of patients but later in 23%. No systemic reactions or anaphylaxis occurred within 30 min of injections, but urticaria or worsening of asthma and rhinitis was reported later in 3% of patients. A significant improvement in nasal obstruction occurred in the Conjuvac compared to the placebo treated group ( $P < 0.01$ ) and there was significant increase in the percentage of patients able to tolerate provocation with the highest concentrations of *D. pteronyssinus* extract after nine and 15 maintenance injections of Conjuvac compared to the placebo ( $P < 0.02$ ,  $P < 0.001$ ). Patient use of additional therapy decreased sooner in the Conjuvac treated group but was minimal in both groups after 5 months of the study.

**Key words:** Conjuvac mite alginate; *D. pteronyssinus*; hyposensitization; nasal provocation tests; perennial rhinitis.

Accepted for publication 10 September 1988

The role of the housedust mite in the aetiology of perennial rhinitis is now well established. However, the place of specific hyposensitization with extracts of *Dermatophagoides pteronyssinus* (*D. pteronyssinus*) in the management of the disease is still unclear. There is a small, but significant, inci-

dence of generalised reactions associated with this type of therapy with the attendant risk to the patient. In the view of many respiratory physicians this is a major deterrent to the use of hyposensitization except for patients who have not been helped by allergen avoidance regimes

and symptomatic therapy. In fact, up to 30 % of patients with allergic rhinitis respond only poorly to treatment with topical corticosteroids (4). In such patients an effective form of hyposensitization with minimal unwanted effects would be invaluable.

The efficacy of immunotherapy in adult patients with perennial allergic rhinitis has been reported in controlled studies using both aqueous extracts of *D. pteronyssinus* (6, 7) and a tyrosine adsorbed extract (1), although there is still controversy concerning the underlying mechanisms by which hyposensitization elicits clinical improvement.

One possible mechanism by which immunotherapy may reduce responses to inhaled allergen is by the production of IgG and IgA "blocking" antibodies in respiratory secretions. An indication that this may be the case, at least in patients with allergic rhinitis, has come from studies of mediator release in nasal secretions from treated and untreated subjects (5). If this hypothesis is true it would be unrealistic to expect hyposensitization to be effective unless the allergen extracts used to stimulate specific antibody production are clinically relevant and antigenically "pure". One major advance has been the isolation and characterisation of the P1 antigen derived from *D. pteronyssinus* (3). Since it has been demonstrated that up to 75 % of IgE antibodies to mite are directed against antigen P1 (11) it is possible that hyposensitization with an extract rich in this component might prove more effective than cruder formulations. One such extract is an alginate conjugated preparation, Conjuvac® mite, manufactured by Miles Laboratories Ltd. In this study we have used a protocol including symptomatic assessments and objective assessments by nasal provocation testing to investigate the safety and efficacy of Conjuvac mite therapy in adult patients with perennial rhinitis due to the house-dust mite.

## MATERIAL AND METHODS

### *Study design*

The study was designed in a double-blind, parallel group fashion. Patients entered the study between November 1983 and March 1985. All

participants completed a 4-week assessment period before being randomly allocated to receive either active or placebo treatment. Patients were initially treated with 11 injections at weekly intervals. Those on active therapy received allergen doses increasing from  $56 \times 10^1$  to  $448 \times 10^3$  international units (IU) *D. pteronyssinus*. On completion of the weekly injection course patients went on to receive 15 monthly "maintenance" injections, each containing  $448 \times 10^3$  IU *D. pteronyssinus*. Those who initially received active therapy were maintained on the highest dose achieved during the weekly course, and those who had received placebo therapy were continued on this. The minimum duration of treatment for patients who successfully completed the course was therefore 18 months. The mean total cumulative dose of Conjuvac mite administered to patients on active treatment who completed the study was  $8.16 \times 10^6$  IU (range  $1.19 \times 10^6$  -  $13.7 \times 10^6$  IU).

The clinical progress of the patients was monitored by means of patient symptom diaries, nasal peak flow records, visual analogue scales and nasal provocation testing. All assessments were performed on admission to the study and following 5, 9 and 15 months of maintenance injections. Throughout the study blood samples were obtained for immunological testing. The results of these investigations will be reported elsewhere.

### *Patients*

A total of 66 patients entered the study in three centres; two in Italy and one in the UK. Ages ranged from 17-55 years with a mean age of 29.5 years. Twenty-six were male and 40 female. Thirty-two patients suffered from rhinitis only, with the remainder also suffering from asthma. The mean duration of rhinitis was 10.5 years (range 1-40 years) and of asthma 10.6 years (range 1-40 years). In all cases the housedust mite (*D. pteronyssinus*) was implicated as the main cause of their symptoms on the basis of history and both a positive response to nasal provocation testing and to skin prick testing (a mean weal diameter of  $> 3$  mm being

considered positive). The technique of nasal provocation testing is described below.

Patients who were pregnant or who had nasal polyps or other nasal deformity were excluded from the study as were those having proven sensitivities to animals and who kept pets at home. In addition, patients requiring systemic corticosteroids were also excluded from participation. Patients who became pregnant during the study, were withdrawn.

All participants were required to give written, informed consent to their inclusion in the study which was approved by the local Ethics Committee of the hospitals involved.

#### *Allergen extracts*

Conjuvac mite consists of an extract of *D. pteronyssinus* which is dialysed to remove potentially irritant low molecular weight components of less than 5,000 Daltons. The extract, known as Alpha Fraction contains  $7 \times 10^6$  IU  $\text{mg}^{-1}$  of the critical allergenic component, antigen P1. Following biological standardisation the extract is chemically conjugated to a non-toxic alginate carrier which reduces its allergenicity without reducing its immunogenicity (9).

For the purposes of the study injection material (Conjuvac mite) was in the form of single dose vials of lyophilised material containing, on reconstitution, 5.6, 56 or  $560 \times 10^3$  IU *D. pteronyssinus*  $\text{ml}^{-1}$ . Placebo treatment contained lyophilised sodium alginate diluent (0.2% after reconstitution) used in the manufacture of the active treatment. To preserve the double blind nature of the study some placebo vials also contained 5  $\mu\text{g}$  of histamine dihydrochloride to simulate local skin reactions which are often associated with hyposensitization injections. The order of "alginate only" or "alginate + histamine" vials was randomized within each treatment set.

Alphatest mite for skin prick testing on admission to the study contained  $90 \times 10^3$  IU *D. pteronyssinus*  $\text{ml}^{-1}$ . Stock extract of *D. pteronyssinus* for use in nasal testing was in the form of vials of lyophilised Alphabase containing, on reconstitution in physiological saline,  $440 \times 10^3$  IU *D. pteronyssinus*  $\text{ml}^{-1}$ . This stock extract was

further diluted to provide serial concentrations of  $220 \times 10^3$  (1/2),  $44 \times 10^3$  (1/10),  $22 \times 10^3$  (1/200) and  $8.8 \times 10^3$  (1/50) IU  $\text{ml}^{-1}$ . Alphatest mite extract for skin prick testing, Alphabase mite for nasal provocation testing and Conjuvac mite for hyposensitization injections were all derived from the same parent extract of *D. pteronyssinus*.

#### *Nasal provocation testing*

Nasal provocation testing was performed by the technique of passive anterior rhinomanometry using the PK Morgan nasal airways resistance tester (NART<sup>®</sup>, PK Morgan Ltd, Chatham, UK) as originally described by Britton et al. (2). Resting nasal airways resistance (in  $\text{kPa s}^{-1}$ ) was measured in each nostril prior to provocation. Measurements were repeated 2, 5, 10 and 15 min after instillation of a control solution (isotonic saline) and each of a series of increasing concentrations of allergen into the nostril with the lowest initial resistance. The control solution and each concentration of agonist were nebulised in a volume of 100  $\mu\text{l}$  from a modified airbrush (Humbrol, UK) at an airflow rate of 7  $\text{l min}^{-1}$ . To minimise the exposure of the lower airways to allergen, subjects were instructed to breathhold in inspiration whilst solutions were being instilled into the nostril.

#### *Symptomatic assessments*

Patients were provided with a diary card on which they were asked to record symptoms of nasal blockage, sneezing and rhinorrhoea by scoring severity twice daily on a scale of 0–3. Patients were also supplied with a low range mini-Wright peak expiratory flow meter (Clement Clarke International Ltd, UK) and face mask (Medishield, size 2), and asked to record three readings of nasal expiratory flow rate on the diary cards both on rising and before going to bed. They were also asked to detail any other drugs (beclomethasone dipropionate, xylometazoline, sodium cromoglycate or chlorpheniramine) used for the control of nasal symptoms. Diary cards covered a period of 1 month and were issued prior to treatment

and following, 5, 9 and 15 months of maintenance therapy.

At the same time points patients were asked to record their subjective assessments of nasal blockage, rhinorrhoea and sneezing on visual analogue scales (VAS). Assessments for each symptom individually, obtained whilst the patients were visiting the hospital, were recorded on 10 cm lines scored from "no symptoms" to "could not be worse".

#### *Concomitant medication*

Patients were restricted to three nasal sprays (xylometazoline hydrochloride, beclomethasone dipropionate and sodium cromoglycate) and short-acting antihistamine tablets (chlorpheniramine) as symptomatic medication. All concomitant medication was prescribed and supplied only by the clinicians co-ordinating the study in each centre and no other symptomatic medication was permitted. All patients were encouraged to reduce their medication as much as possible. Consumption was monitored by recordings of tablet counts and cannister weights.

#### *Adverse effects*

Prior to each injection patients were asked a non-leading question about unwanted effects – "Did you notice anything unusual after the last injection?". Reported problems were classified as local or systemic reactions and subdivided into "early" or "late" (where a late reaction is one commencing more than 30 min after the administration of the injection). Systemic reactions included generalised urticaria or angio-oedema, exacerbation of rhinitis or asthma.

To reduce "avoidable" adverse events the dosage given during initial treatment was not increased if a local reaction of > 5 cm in diameter had occurred at injection site within 48 h of the patient's previous injection. In these circumstances the last dose was repeated or the dose reduced to one previously well tolerated. The dose was similarly reduced if the last injection had provoked a mild systemic reaction such as mild itching of the eyes, nose or throat.

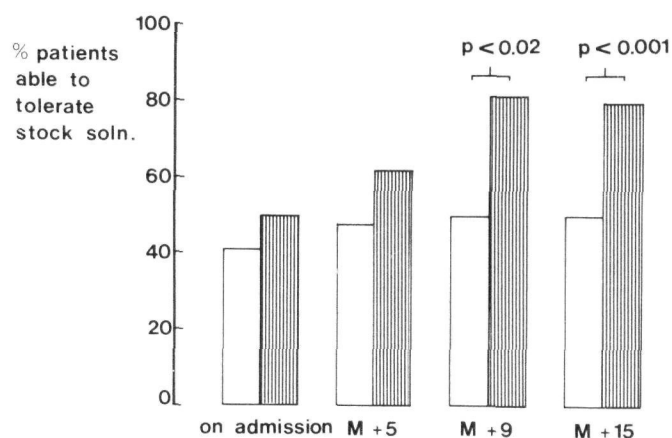


Fig. 1. Percentage of patients treated with Conjuvac Mite (▨) or placebo (□) able to tolerate the most concentrated mite extract available for provocation testing on admission to the study and after 5, 9 and 15 months of maintenance injections.

## RESULTS

Fifty-one patients completed the study; 22 (69%) of those on active therapy and 29 (85%) of those receiving placebo therapy. There were a total of 15 withdrawals from the study, six of which were due to pregnancy. The remaining nine withdrawals consisted of four in whom local reactions were severe or unacceptable (all receiving Conjuvac), two patients who had late or generalised reactions to therapy (one in each treatment group), one patient who felt that the treatment was of no benefit and two patients who defaulted from the study with no reason given.

Because many withdrawals in one treatment group may improve results in that group as treatment failures leave, allowance was made for withdrawals in the analysis of the data. A conservative method was used, substituting a patient's last available observation at all subsequent time points, thus ensuring no bias in favour of the active therapy.

#### *Nasal provocation tests*

The results of nasal provocation tests were variable, mainly due to large differences in the patients' sensitivities to the allergen extract. For this reason results were analysed by considering the proportion of individuals able to tolerate each allergen concentration before reaching the point at which total nasal obstruction occurred

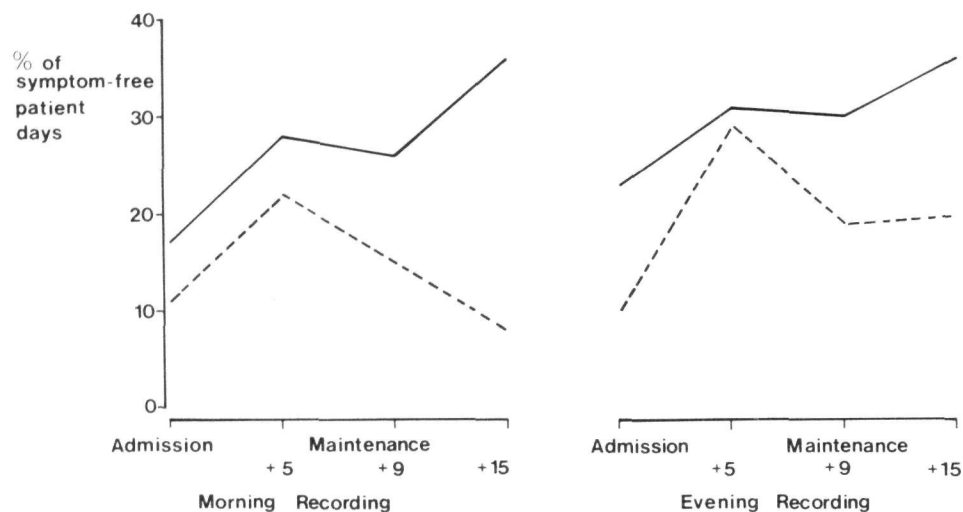


Figure 2. Percentage of symptom-free mornings and evenings recorded in daily diaries by Conjuvac-treated (—) and placebo-treated (----) patients on admission to the study and after 5, 9 and 15 months of maintenance injections.

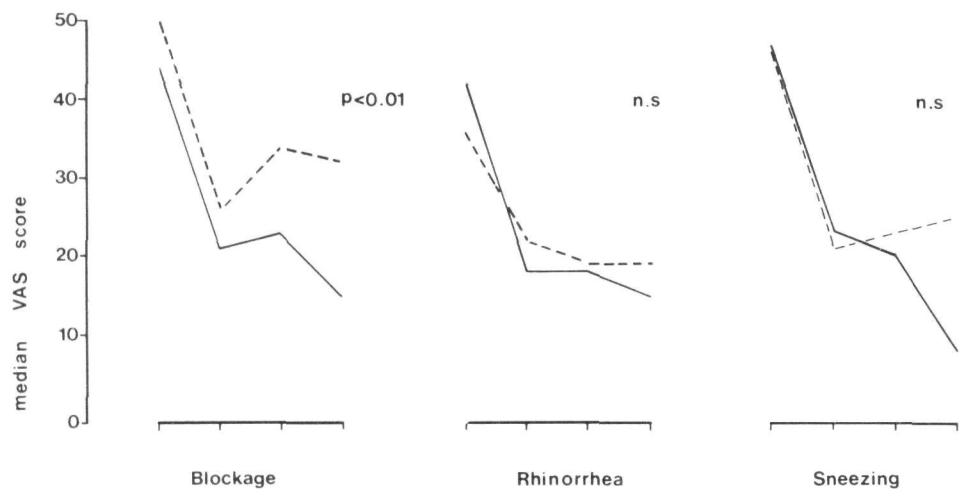


Figure 3. Changes in VAS scores for nasal blockage, rhinorrhoea and sneezing in Conjuvac-treated patients (—) and placebo-treated patients (----) on entry to the study and after 5, 9 and 15 months of maintenance therapy. (0 = no symptoms, 100 = “could not be worse”).

and testing was discontinuing. These results are displayed in Fig. 1. There was no difference in the proportion of patients who could initially reach the highest concentration available, but on completion of the study a significantly greater proportion of Conjuvac-treated patients received this concentration of allergen compared to placebo-treated patients ( $P < 0.001$ ), Chi-square test with Yates’ correction).

Symptoms

The majority of patients returned daily diary cards which were filled out to a high standard,

although some of our otherwise healthy rhinitic patients were poorly motivated to document symptoms on a daily basis over a long time period as other investigators (4) have also reported.

The difference between treatment groups was tested by the Mann-Whitney “U” test on the patients’ mean symptom scores. There was evidence of a difference in the final diary recordings in the morning score ( $P = 0.028$ ), but it was less noticeable in the evening score ( $P = 0.12$ ).

Fig. 2 shows the percentage of symptom-free mornings and evenings. Conjuvac-treated patients recorded more symptom-free mornings during the final month of the study. Nasal peak

Table 1

Incidence of immediate and delayed reactions to injections of Conjuvac Mite or placebo as a percentage of total number of injections of each type administered

|                         | Conjuvac | Placebo |
|-------------------------|----------|---------|
| Immediate reactions (%) |          |         |
| local < 5 cm            | 29       | 3       |
| local > 5 cm            | 1        | Nil     |
| systemic                | Nil      | Nil     |
| Delayed reactions (%)   |          |         |
| local < 5 cm            | 12       | 2       |
| local > 5 cm            | 23       | 1       |
| systemic                | 3        | 1       |

flow meter recordings, both morning and evening, showed relatively little change in either treatment group throughout the study and there was no significant difference between the groups at any time ( $P > 0.2$ , “t” test).

Subjective assessment of symptoms by visual analogue scales showed no difference in severity of nasal obstruction, rhinorrhoea or sneezing between the two treatment groups on entry to the study. During the study there was a general improvement in the patients’ perception of the severity of all symptoms. The only significant difference between the treatment groups was a much greater improvement in the degree of nasal obstruction in the Conjuvac-treated group ( $P < 0.01$ , Mann-Whitney “U”) as shown in Fig. 3.

Medication

An overall daily medication score was calculated for all patients, giving the topical medications equal weights of one unit/g consumed and chlorpheniramine one third of a unit/tablet. Medication consumption in the Conjuvac group fell much sooner than in the placebo-treated group, but after 4 to 5 months most patients used very little medication.

Unwanted effects

A considerable number of Conjuvac injections caused local reactions (see Table 1) but less than 12% were larger than 5 cm in diameter. Late systemic reactions occurred after 3% of Conjuvac injections and 1% of placebo injections.

There were no cases of anaphylaxis or any immediate systemic reactions. Some patients did, however, find the minor unwanted effects sufficiently troublesome to withdraw from the study.

DISCUSSION

A very large placebo effect was apparent in this double blind study, with both active and placebo treatments causing an improvement in all subjective assessments until well into the course of maintenance injections. This finding is in agreement with those of several other investigators. Blainey et al. (1) reported a significant improvement in symptoms of both placebo-treated and patients who received active therapy 4 weeks after an initial course of six weekly injections of tyrosine adsorbed extract of *D. pteronyssinus*. A similar finding was also reported by D’Souza et al. (6) who reported that 65% of patients showed a symptomatic improvement following 12 weekly injections, irrespective of whether they had received active aqueous extract of *D. pteronyssinus* or placebo therapy. Gabriel et al. (7) failed to show any definite benefit to patients actively treated with a tyrosine adsorbed extract of *D. pteronyssinus*, compared to placebo-treated patients, following approximately 20 weekly injections but, again, a trend towards improvement in individual symptoms of rhinitis in both treatment groups was apparent. This was particularly noticeable for nasal obstruction where 54% of patients receiving active therapy and 42% of those on placebo reported improvement on their symptom diary cards. In their study, where weekly treatment was followed by monthly maintenance injections for 1 year, the actively treated group began to show a greater degree of improvement than the placebo group, especially in terms of nasal obstruction and discharge, from 4 months onwards; a difference which reached significance after 1 year’s therapy. Our results are in agreement with these in that symptoms in the placebo-treated patients eventually began to slip back to their original level whilst in Conjuvac-treated patients’ symptomatic improvement was maintained even though

they were no longer taking additional medications.

In all the above studies it is likely that the strong widely recognised "placebo" effect of a course of injections resulted in temporary symptomatic improvements in placebo-treated patients during the early stages of therapy. In most cases this effect was relatively short-lived and, as in the present study, improvement was only maintained in those patients receiving active therapy. The treatment regime reported by D'Souza (6) was probably too short (3 months) to overcome the influence of this placebo effect.

In our study the placebo effect was less apparent in the objective tests of disease severity. In common with other authors (1, 6) we found a decrease in the nasal response to provocation testing with *D. pteronyssinus* in the actively treated group, which occurred early in the treatment schedule and was maintained throughout. D'Souza et al. noted an improvement in nasal tolerance of allergen in 70% of actively treated patients and 36% of placebo-treated patients after 12 weeks' therapy. In our study there was also a slight improvement in nasal allergen tolerance in the placebo group but this was minimal in comparison to that achieved by the Conjuvac-treated group. In contrast, a study in Hong Kong (7) failed to show any consistent changes in nasal provocation tests despite improvement in rhinitis symptoms which occurred after prolonged hyposensitization. This discrepancy is puzzling. However, it is possible that in the latter study sensitivities to other environmental allergens may have contributed to maintaining high levels of non-specific nasal hyperresponsiveness which, in the lung at least, appears to remain unaffected by specific immunotherapy (8, 10). In the present study patients with proven animal allergies who kept pets at home were excluded from participation and all provocation tests were performed outside the local pollen season, in both Italy and the UK. In the Hong Kong study, 85% of the study population had positive skin prick tests to fur and 23% to pollens. If these sensitivities did maintain higher levels of nasal hyperresponsiveness it is

possible that high concentrations of *D. pteronyssinus* applied topically during provocation would continue to evoke a response, whereas lower levels of antigen encountered in the home might be insufficient to cause problems once specific sensitivity to *D. pteronyssinus* had been reduced by hyposensitization.

Even though both treatment groups recorded initial reductions in nasal obstruction on both diary cards and visual analogue scales there was no difference in records of self-administered peak flow. This may have resulted from the use of expiratory peak flow meters (this study having commenced before the introduction of the Youlten inspiratory meter). An expiratory manoeuvre through the nose is unpleasant and more difficult to perform than an inspiratory manoeuvre, especially when there is excess mucus in the nasal cavity, and may therefore have discouraged patient compliance resulting in an inaccurate representation of the patient's condition.

It is clear that the relationship between changes in objective measures such as responses to nasal provocation testing and symptomatic improvement requires further investigation, especially since some patients respond much better to this type of therapy than others, and it has been suggested that an early improvement in nasal response may be a useful method of predicting the eventual outcome of hyposensitization therapy with *D. pteronyssinus* (1). A clearer understanding of the factors which determine which patients will respond well to therapy would be invaluable in helping physicians to decide whether embarking on an expensive and time-consuming procedure is merited.

In conclusion, we have shown that both symptomatic benefit and a significant improvement in the nasal response to allergen can be demonstrated in patients with perennial rhinitis due to the housedust mite following hyposensitization with Conjuvac. Provided the appropriate precautions are observed, Conjuvac mite can be administered with minimal risk: although it is an effective treatment, its superiority to placebo is apparent only after a year of therapy.

## ACKNOWLEDGEMENTS

The authors are grateful to Miles Laboratories for financial support and for provision of allergen extracts. Our thanks also go to Laura Roberts for typing the manuscript.

## REFERENCES

1. Blainey, A. D., Phillips, M. J., Ollier, S. & Davies, R. J.: Hyposensitization with a tyrosine adsorbed extract of *Dermatophagoides pteronyssinus* in adults with perennial rhinitis. *Allergy* 39, 521-528, 1984.
2. Britton, M. G., Empey, D. W., John, G. C., McDonnell, K. A. & Hughes, D. T. D.: Histamine challenge and anterior nasal rhinometry: their use in assessment of pseudoephedrine and triprolidine. *Br. J. Clin. Pharmacol.* 6, 51-58, 1978.
3. Chapman, M. D. & Platts-Mills, T. A. E.: Purification and characterisation of the major allergen from *Dermatophagoides pteronyssinus*, antigen P1. *J. Immunol.* 125, 587-592, 1980.
4. Cockcroft, D. W., Hargreave, F. E. & Dolovich, J.: Intranasal beclomethasone dipropionate in rhinitis. In: Mygind, N. & Clark, T. J. H. (eds.): Topical steroid treatment for asthma and rhinitis. Balliere Tindall, London, 1980.
5. Creticos, P. S., Adkinson, N. F. Jr., Kagey-Sobotka, A., Lichtenstein, L. M., Marsh, D. G. & Norman, P. S.: Characterisation of ragweed sensitive patients by nasal challenge with pollen. *J. Allergy Clin. Immunol.* 71, 90, 1983.
6. D'Souza, M. F., Pepys, J., Wells, I. D., et al.: Hyposensitization with *Dermatophagoides pteronyssinus* in house dust allergy: A controlled study of clinical and immunological effects. *Clin. Allergy* 3, 177-193, 1973.
7. Gabriel, M., Ng, H. K., Allan, W. G. L., Hill, L. E. & Nunn, A.: Study of prolonged sensitization with *D. pteronyssinus* extract in allergic rhinitis. *Clin. Allergy* 7, 325-336, 1977.
8. Ohman, J. L., Findlay, S. R. & Leitermann, K. M.: Immunotherapy in cat induced asthma. Double blind trial with evaluation of *in vivo* and *in vitro* responses. *J. Allergy Clin. Immunol.* 74, 231-239, 1984.
9. Pegelow, K.-O., Belin, L., Broman, P., Heilborn, H., Sundin, B. & Watson, K.: Immunotherapy with alginate-conjugated and alum-precipitated grass pollen extracts in patients with allergic rhinoconjunctivitis. *Allergy* 39, 275-290, 1984.
10. Taylor, W. W., Ohman, J. L. & Lowell, F. C.: Immunotherapy in cat induced asthma. *J. Allergy Clin. Immunol.* 61, 283-287, 1978.
11. Tovey, E. R., Chapman, M. D. & Platts-Mills, T. A. E.: Mite faeces are a major source of housedust allergens. *Nature* 289, 592-593, 1981.

Address:

Dr. Robert J. Davies

Academic Department of Respiratory Medicine

St. Bartholomew's Hospital

London

U.K.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.