

Effects of one-year hyposensitization in allergic rhinitis

Comparison of two house dust mite extracts

A. PÉCOUD, L. NICOD, M. BADAN, B. AGRELL,¹ S. DREBORG² & M. KOLLY

Division of Clinical Immunology and Allergy, Lausanne, Switzerland, ¹Pharmacia AB, Uppsala, Sweden, and ²Department of Pediatrics, University of Linköping, Sweden

In an open study, 21 patients suffering from chronic non-seasonal rhinitis and allergic to house mites (HDM) have been treated for 1 year with either a new extract (Pharmalgen; $n = 10$) or an allergoid, pyridine denatured, extract (Alavac; $n = 11$), both precipitated with $\text{Al}(\text{OH})_3$ (depot). The following investigations were performed before and after therapy: clinical scoring (for 4 weeks), quantified skin prick tests (SPT) and nasal provocation tests (NPT) with HDM, and determination in serum of HDM-specific IgE and IgG. Both groups were compared with six patients who remained untreated and underwent the same investigations. Hyposensitization with either extract induced an improvement in clinical scores ($P < 0.05$), a decrease in SPT reactivity (Pharmalgen: $P < 0.001$; Alavac: $P < 0.01$), a marked increase in the nasal tolerance to HDM ($P < 0.001$) and in HDM-specific IgG ($P < 0.001$). In the group of untreated patients, all these parameters remained unchanged. Compared with the Alavac extract, the Pharmalgen extract was more active in decreasing SPT reactions ($P < 0.05$) and inducing a HDM-specific IgG rise ($P < 0.05$). Although both extracts induced some untoward allergic reactions, no adrenaline was used at any time during the study. These data suggest that hyposensitization with depot extracts of HDM can be considered a safe and active adjunct to the treatment of allergic rhinitis.

Key words: allergic rhinitis; house dust mites; hyposensitization.

Allergy to house dust mites (HDM) is a frequent cause of chronic, non-seasonal rhinitis or asthma (29). Hyposensitization therapy (specific immunotherapy) with HDM extracts is often used in these patients, although positive (1, 2, 4, 5, 15, 22-25, 30) and negative (8, 16, 17, 26) results have been obtained in several controlled studies. The majority of the studies have been performed in asthma and fewer in rhinitis (1, 4, 5, 24). Allergen extracts now available are characterized by a better standardisation of their allergen content due to the use of newer techniques, such as crossed immunoelectrophoresis and RAST inhibition. Moreover extract activity is preserved by freeze drying and reconstitution with diluents containing stabilizers.

Recently, hyposensitization with such extracts has been shown effective in allergic rhinitis, as assessed after 3 months of therapy (5). In the present study, we compared the effects of two different extracts after 1-year desensitization of patients suffering from perennial rhinitis and allergic to HDM.

MATERIAL AND METHODS

Patients

Twenty-nine patients were studied (15 females and 14 males): 23 adults (mean age: 29 years, range: 16-45) and six children (range: 10-14).

They had all had chronic, non-seasonal rhinitis for at least 2 years and their disease had not been sufficiently relieved by symptomatic therapy. In all patients, the diagnosis of allergy to HDM was based on the following three criteria: 1) a positive skin prick test (3 + or more) to HDM extract; 2) a RAST class 2 or more for HDM (Phadezym IgE RAST®); 3) a nasal provocation test (NPT) positive for HDM; patients allergic (positive SPT and/or RAST) and regularly exposed to pets were excluded. No patient had been hyposensitized in the 10 years prior to the study.

Allergen preparations

Two HDM extracts (*Dermatophagoides pteronyssinus*) were obtained from the manufacturers. Pharmalgen® (Pharmacia, Uppsala/ALK, Copenhagen) is a freeze-dried biologically standardized allergen preparation available in four 10-fold potencies: 100, 1 000, 10 000 and 100 000 Biological Units/ml (BU/ml). 100 000 BU/ml contains 60 µg of the major allergen of HDM (Der pI) and the potency corresponds to 660 000 International Units/ml. The same batch was used throughout the study. For diagnosis a new vial was reconstituted every second day; Albumin diluent® (0.9 % phenol, 0.03 % albumin, 0.9 % NaCl) was used for NPT, and Glycerol diluent® (50 % glycerol) for SPT. The same extract was reconstituted with Depot diluent® (0.13 % aluminium hydroxide in saline) for treatment. It was used within 6 months (documented stability).

Alavac® (Bencard, London, UK) is obtained by extraction in pyridine and adsorption of AlOH. It was delivered in three concentrations: 10, 100 and 1 000 PNU/ml. The potency of this extract can not be determined by common methods because of the extraction and adsorption procedures.

Methods

Clinical score. All patients were asked to fill in a symptom diary during a 4-week period; they

estimated the intensity of the three nasal symptoms (obstruction, rhinorrhea and sneezings) twice daily on a scale 0 (no symptom) to 4 (intolerable). The H₁ antagonist terfenadine (Teldane® Merrell-Dow) and nasal vasoconstrictor drops were used as the only allowed rescue medication. A drug score was made: one point was given to each administration of any drug (nasal drops or 1 antihistamine tablet). The clinical score consisted of symptom score plus drug score during the 4-week period.

Skin prick test (SPT) was performed in duplicate on the volar side of one forearm by means of a disposable prick tester (Morrow-Brown needle) with the following solutions: a) the glycerol diluent, b) the allergen solution containing 100 000 BU/ml of the *D. pteronyssinus* extract (Pharmalgen), and c) histamine dihydrochloride 1 mg/ml (5.43 mmol/l). After exactly 15 min the size of the wheal was traced on a transparent plastic sheet. The mean diameter of the wheal was measured according to the following method: the longest (d1) and the midpoint orthogonal (d2) diameters of the wheals were measured and the mean calculated (d1 + d2/2). The size of the allergen wheal was expressed as the mean diameter of the allergen wheal in % of the diameter of the wheal obtained with histamine.

Nasal provocation test (NPT) was performed at the end of the 4 weeks of clinical scoring according to a technique described in detail elsewhere (12). Briefly, 0.4 ml of the diluent followed by 10-fold increasing concentrations of the HDM extract (Pharmalgen) were nebulised in both nostrils, every 15 min, by means of a De Vilbiss vaporisator. The test was stopped and considered positive when two of the following three criteria were met: a) nasal obstruction as defined by at least a 40 % drop in nasal inspiratory peak flow (NIPF, Wright peak-flowmeter); b) at least 0.5 g rhinorrhea as determined by the weight difference of paper handkerchiefs used during the 10-min interval, and c) at least 4 sneezes. The criteria: obstruction, rhinorrhea and sneezes were met in 87 %, 100 % and 30 % of the NPTs respectively. The allergen concen-

tration (in BU/ml) reached when the NPT was positive was considered as the "nasal allergic reaction threshold".

HDM-specific IgG and IgE. Serum levels of HDM-specific IgE and IgG antibodies were determined before and after 3 and 12 months of treatment by using the commercially available Phadebas IgE RAST® (Pharmacia) by strictly following the instructions of the manufacturer. Briefly, HDM-specific serum IgE (respectively IgG) is bound to an allergen coated cellulose disc and detected by a rabbit-I¹²⁵ labelled anti-human IgE (IgG respectively). Results were compared with values obtained on a standard curve and expressed in PRU/ml (Phadebas® RAST Units) for HDM-specific IgE and in % of bound radio-activity for HDM-specific IgG.

Study design

The whole initial investigation (symptom scoring followed by SPT/NPT) was performed in winter, between December and February. The first 23 patients were randomly allocated to one of two treatment groups, in a randomization based on the "nasal allergic threshold": 11 patients were allocated to the Pharmalgen HDM preparation and 12 to the Bencard preparation. Six additional patients seen in succession remained untreated and served as open controls. Two children were included in each of the three groups. The same investigations were repeated after 12 months of hyposensitization.

Hyposensitization treatment

Patients received one injection/week subcutaneously for 3 to 4 months, until the maintenance dose was reached: the interval between injections was then increased to 1 month.

After each injection, the patients were asked to remain under the doctor's supervision for 30 min. The following day, they filled in a questionnaire indicating any abnormal reaction occurring during the 24 h after the injection.

All patients gave written informed consent and the study was approved by the local Ethics Committee.

RESULTS

Of the 23 patients, 21 completed the study and were investigated after 12 months. One child dropped out in the Pharmalgen group at 5 000 BU because of the development of asthma and one adult in the Bencard group because of lack of compliance. The maintenance dose reached was 1 000 PNU in all patients with the Bencard and between 40 000 and 80 000 BU with Pharmalgen.

The mean clinical score of the treated and untreated patients is shown in Table 1: a significant decrease in the clinical score was noted in both groups of patients treated with immunotherapy but not in the untreated group. No significant difference was observed between the two treatment groups.

The outcome of the skin reactivity to 100 000 BU/ml of HDM in the three groups of patients is shown in Fig. 1. There was a significant decrease in wheal diameter in both treated groups, slightly more pronounced in the Pharmalgen group ($P < 0.05$). No consistent change was observed in the six controls.

Fig. 2 shows the "nasal allergic reaction thresholds" of the 27 patients individually before and after 1 year. In both treated groups there was a marked increase in the thresholds, whereas they remained essentially unchanged in the six untreated patients. There was a trend for the Pharmalgen treated patients to have a higher threshold after therapy than those treated with the Alavac preparation.

The evolution of HDM-specific IgE and IgG is given in Fig. 3. Large individual variations were noted with both isotypes. Regarding HDM-specific IgE, the only significant difference observed at any time was a rise after 3 months of therapy with the Pharmalgen preparation. For HDM-specific IgG, a significant rise, compared with that in non-treated patients, was obtained at 3 months ($P < 0.01$) and 12 months ($P < 0.001$) with both extracts. At 12 months, the IgG rise was slightly but signifi-

Table 1

Clinical scores before and after 1-year immunotherapy (IT) (mean±SEM)

	Treatment with		No treatment
	Pharmalgen	Bencard	
Before IT	220±25	163±16	183±45
After 1 year IT	110±32	82±20	174±53
Difference in changes within groups	$P < 0.05$	$P < 0.05$	NS

Difference between groups was not statistically significant either before or after IT.

cantly more pronounced in the patients treated with the Pharmalgen extract than in those treated with the Bencard ($P < 0.05$).

The incidence of side effects during 1 year of therapy is summarized in Table 2: large local reactions (diameter > to 10 cm) were more frequently reported by patients treated with Pharmalgen than by those treated with Alavac. Mild systemic reactions (mostly in the form of an exacerbation of rhinitis during the 24 h after injection) were reported with equal frequency by patients in both treatment groups. No serious side effect was reported. No injection of adrenaline was administered during the whole year of treatment.

DISCUSSION

The value of hyposensitization with allergens in the treatment of allergic rhinitis has been the

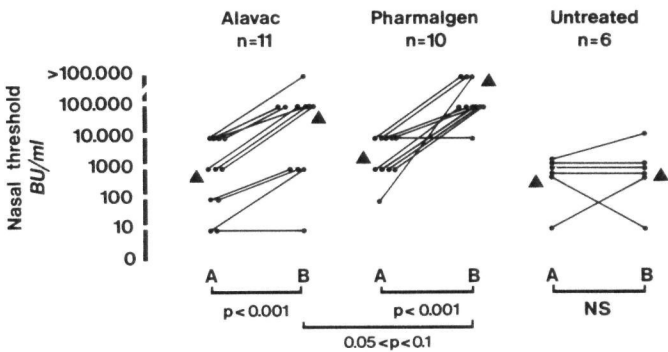


Fig.2. Nasal reaction threshold before and after 1 year. ▲ Indicates median. *P* value determined by the Wilcoxon rank test.

subject of several studies. The efficacy of therapy with pollen extracts in patients suffering from hay fever has been shown (6, 14, 18, 20); in this disease however, the effect of immunotherapy on pollen-induced asthma has been questioned (10). In non-seasonal allergic rhinitis and asthma, results of hyposensitization with HDM have been more conflicting: at least 10 studies have shown the benefit of such treatments (1, 2, 4, 5, 15, 22–25, 30), whereas at least three had not (8, 16, 17). The heterogeneity of the results might be due to differences in

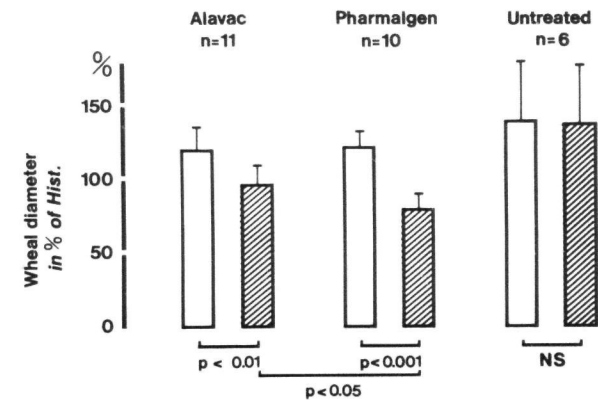


Fig.1. Mean diameter (± SEM) of the wheal induced by 100 000 BU/ml before and after 1-year therapy. *P* value determined by Student's *t* test on paired or unpaired data when appropriate.

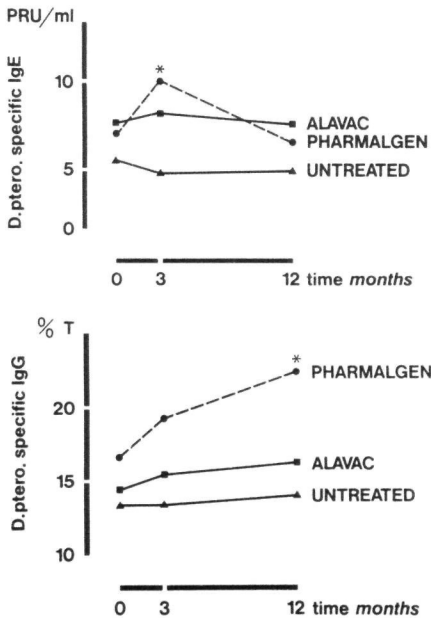


Fig.3. Evolution of HDM-specific IgE and IgG. See text for statistical evaluation of the results. * Difference between Pharmalgen and Alavac: $P < 0.05$ (Student's *t* test).

the diseases treated (rhinitis and asthma, or asthma alone), the methods to establish the diagnosis of hypersensitivity (SPT, RAST, provocation test), the quantitative and qualitative differences among the allergen extracts, and the methods used to test the efficacy of the treatment (clinical judgement, symptom scoring or results of provocation test with allergens).

Manufacturers have now provided clinicians with better standardized extracts which should help to limit the heterogeneity of the results. Since they are freeze-dried, they retain their allergenic potency for a longer period and thus permit comparison of skin or provocation tests performed many months apart. These extracts should also contribute to a more reproducible and accurate diagnosis: all the patients studied in the present trial suffered from chronic non-seasonal rhinitis and had positive SPT, RAST and NPT for a well-characterized allergen. Their disease could not have been influenced by other known allergens since hypersensitivity to pets was excluded and both investigations, before and after treatment, took place in winter, i.e. a period free from pollen and outdoor molds in our area. Finally, to assess treatment efficacy we determined both clinical scores and objective criteria, such as quantified skin prick tests, nasal provocation tests and serum levels of specific IgE or IgG.

Unfortunately, a double blind controlled study was difficult to perform, due to the ethical difficulty of administering a placebo for 1 year. The group of untreated patients, which was

similar by all criteria to the treated patients, provides some information on the natural course of the disease.

Our data indicate that the symptoms nasal tolerance and skin reactivity to HDM were clearly decreased by immunotherapy with both HDM extracts when comparing treated with untreated patients.

The most clear-cut result obtained in this study was a strong increase in the nasal tolerance to HDM, as determined by NPT. This was observed in 19 of 21 treated patients, and the mean threshold after therapy with either extracts increased significantly, whereas no definite change was noticed in the untreated group. Similar results were obtained when nasal challenges were performed after immunotherapy with pollen extracts (3, 7, 20). An increased nasal tolerance to HDM had also been found in the studies of D'Souza et al. (4) and Ewan et al. (5) but not in the two other trials with positive clinical results (1, 24). As also Ewan et al., the NPT used in our study is based on the assessment of the three main symptoms of rhinitis, instead of only nasal obstruction. Moreover we have used freeze-dried extracts of the same allergen batch for therapy as well for SPT/NPT performed before and after 1 year. All these technical differences might explain the discrepancies between studies.

Although moderate, there was a definite decrease in the skin reactivity to HDM, which was slightly more pronounced in the Pharmalgen patients. This finding is very similar to that reported by several studies in which potent and well-characterized allergen extracts have been used, such as hymenoptera venoms (27), pollens (11, 20), cat (19), or HDM (2, 8). That immunotherapy might decrease skin reactivity had been suggested in earlier studies but, at that time, the lack of well-standardized and freeze-dried extracts had raised questions about the value of comparing SPT performed many months apart.

Numerous studies have shown a rise of allergen-specific IgG during immunotherapy, and a rise of specific IgE after 3 months has been shown in studies using potent extracts (9, 13). The main difference we have found between our

Table 2
Incidence of untoward allergic reactions during 1-year treatment

	Alavac 11 patients 220 injections		Pharmalgen 10 patients 250 injections	
	n	% inj.	n	% inj.
Large local	3	1.5	7	1.8
Systemic mild*	6	1.8	7	2.2
Systemic severe**	6	0	0	0
Subcutaneous nodule	0	-	2	-
Fever	1	(0.4 %)	3	(1.6 %)

*Not requiring adrenaline; **requiring adrenaline.

two extracts concerns the changes in serum HDM-specific IgG or IgE. The IgE rise at 3 months and the IgG rise at 3 and 12 months were more pronounced in patients treated with the Pharmalgen extract than in those who received the Alavac. This probably indicates that Pharmalgen had a higher HDM content than Alavac. Unfortunately, it is difficult to compare exactly the allergen concentration of the two extracts since in Alavac, pyridine extraction and early adsorption to ALOH makes it impossible to determine the allergen content.

The hypothesis that treatment with the Pharmalgen extract resulted in the administration of higher quantities of HDM-allergens is also supported by the higher incidence of local side effects, since the incidence of large local reactions has been related to the allergen dose (21). Although no serious reaction occurred during our study, the incidence of systemic minor reactions was relatively high compared with other studies; however, it was no different from that reported when using highly potent allergenic extracts (28). Ewan et al. reported that with an aqueous extract of Pharmalgen 15% of the injections produced generalized allergic reactions, many of them quite severe and all treated with adrenaline (5). This is in contrast to our findings: adrenaline was not used at any time during the 1-year treatment with either extract. This improved tolerance might be related to the use of extracts precipitated on ALOH (depot) rather than in the aqueous form.

In conclusion, in patients allergic to house dust mites, 1-year immunotherapy with two alun-precipitated HDM extracts, one of them pyridine denatured, improved the clinical outcome of patients with chronic rhinitis and showed a low incidence of side effects.

REFERENCES

1. Blainey, A. D., Phillips, M. J., Ollier, S. & Davies, R. J.: Hyposensitization with a tyrosine absorbed extract of *Dermatophagoides pteronyssinus* in adults with perennial rhinitis. *Allergy* 39, 521-528, 1984.
2. Bousquet, J., Calvayrac, P., Guérin, B., et al.: Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. *J. Allergy Clin. Immunol.* 76, 734-744, 1985.
3. Creticos, P. S., Adkinson, N. F., Jr., Kagey-Sobotka, A., et al.: Nasal challenge with ragweed pollen in hay fever patients: effect of immunotherapy. *J. Clin. Invest.* 76, 2247-2253, 1985.
4. D'Souza, M. F., Pepys, J., Wells, I. D., et al.: Hyposensitization with *Dermatophagoides pteronyssinus* in housedust allergy; a controlled study of clinical and immunological effects. *Clin. Allergy* 3, 177-193, 1973.
5. Ewan, P. W., Alexander, M. M., Snape, C., Ind, P. W., Agrell, B. & Dreborg, S.: Effective hyposensitization in allergic rhinitis using a potent partially purified extract of house dust mite. *Clin. Allergy* 18, 501-508, 1988.
6. Frankland, A. W. & Augustin, R.: Prophylaxis of summer hay fever and asthma. *Lancet* i, 1055-1057, 1054.
7. Frostad, A. B., Grimmer, O., Sandvik, L., Moxnes, A. & Has, K.: Clinical effects of hyposensitization using a purified allergen preparation from timothy pollen as compared to crude aqueous extracts from timothy pollen and a four-grass mixture respectively. *Clin. Allergy* 13, 337-357, 1983.
8. Gaddie, J., Skinner, C. & Palmer, K. N. V.: Hyposensitization with house dust mite vaccine in bronchial asthma. *Br. Med. J.* 561-562, 1976.
9. Graft, D. F. & Valentine, M. D.: Immunotherapy. In Kaplan, A. P. (ed.): *Allergy*, pp 679-692. Churchill Livingstone, New-York, 1985.
10. Hill, D. J., Hosking, C. S., Shelton, J. J. & Turner, M. W.: Failure of hyposensitization in treatment of children with grass-pollen asthma. *Br. Med. J.* 284, 306-309, 1982.
11. Kjellman, N. I. M. & Lanner, A.: Hyposensitization in childhood hay fever. A comparison of refined and whole timothy extracts. *Allergy* 35, 323-334, 1980.
11. Kolly, M. & Pécoud, A.: Comparison of levocabastine, a new selective H1 receptor antagonist, and disodium cromoglycate in a nasal provocation test with allergens. *Br. J. Pharmacol.* 22, 389-394, 1986.
13. Lichtenstein, L. M., Ishizaka, K., Norman, P. S., Sobotka, A. K. & Hill, B. M.: IgE antibody measurements in ragweed hay fever: relationship to clinical severity and the results of immunotherapy. *J. Clin. Invest.* 53, 472-482, 1973.
14. Lowel, F. C., & Franklin, W.: A double-blind study of the effectiveness and specificity of injections in ragweed hay fever. *N. Engl. J. Med.* 273, 675-679, 1965.
15. Maunsel, K., Wraith, D. G. & Hughes, A. M.: Hyposensitization in mite asthma. *Lancet* i, 967-968, 1971.
16. Mite Allergy Subcommittee of the Research Committee of the British Thoracic Association. A trial of house dust mite extract in bronchial asthma. *Br. J. Dis. Chest* 73, 260-270, 1979.
17. Newton, D. A. G., Maberley, D. J. & Wilson, R.: House dust mite hyposensitization. *Br. J. Dis. Chest* 72, 21-28, 1978.
18. Norman, P. S.: Allergic rhinitis. *J. Allergy Clin. Immunol.* 75, 531-545, 1985.
19. Ohman, J. L., Jr., 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, 230-239, 1984.
20. Østerballe, O.: Nasal and skin sensitivity during immunotherapy with two major allergens 19, 25 and partially purified extract of timothy grass pollen. *Allergy* 37, 169-177, 1982.
 21. Østerballe, O.: Side effects during immunotherapy with purified grass pollen extracts. *Allergy* 37, 553-562, 1982.
 22. Pauli, G., Bessot, J. C., Bigot, H., et al.: Clinical and immunologic evaluation of tyrosine-adsorbed *Dermatophagoides pteronyssinus* extract: a double-blind placebo-controlled trial. *J. Allergy Clin. Immunol.* 74, 524-535, 1984.
 23. Price, J. F., Warner, J. F., Ney, E. N., Turner, M. W. & Soothill, J. F.: A controlled trial of hyposensitization with adsorbed tyrosine *Dermatophagoides pteronyssinus* antigen in childhood asthma: in vivo aspects. *Clin. Allergy* 14, 109-216, 1984.
 24. Sister Mary Gabriel, H. K., Allan, W. G. L., Hill, L. E. & Nunn, A. J.: Study of prolonged hyposensitization with *Dermatophagoides pteronyssinus* extract in allergic rhinitis. *Clin. Allergy* 7, 325-336, 1977.
 25. Smith, A. P.: Hyposensitization with *Dermatophagoides pteronyssinus* antigen: trial in asthma induced by house-dust. *Br. Med. J.* 4, 204-206, 1971.
 26. Taylor, B., Sanders, S. S. & Norman, A. P.: A double blind trial of house dust mite fortified house dust vaccine in childhood asthma. *Clin. Allergy* 4, 35-40, 1974.
 27. Valentine, M. D.: Insect venom allergy; diagnosis and treatment. *J. Allergy Clin. Immunol.* 73, 299-304, 1984.
 28. Vervloet, D., Khariallah, E., Arnoud, A. & Charpin, J.: A prospective national study of the safety of immunotherapy. *Clin Allergy* 10, 59-64, 1980.
 29. Voorhorst, R., Spiekma, F. T. M., Varekamp, H., Leupen, M. J. & Lyklema, A. W.: The house dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces. Identity with the house dust allergen. *J. Allergy* 39, 325-335, 1969.
 30. Warner, J. O., Price J.-F., Soothill, J. F. & Hey, E. N.: Controlled trial of hyposensitization to *Dermatophagoides pteronyssinus* in children with asthma. *Lancet* *ii*, 912-915, 1978.

Address:

Dr A. Péroud, M.D.

Division of Clinical Immunology and Allergy
 Centre Hospitalier Universitaire Vaudois
 CH - 1011 Lausanne
 Switzerland

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.