

A controlled trial of hyposensitization with adsorbed tyrosine *Dermatophagoides pteronyssinus* antigen in childhood asthma: *in vivo* aspects

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Summary

Continuing study for a second year and further analysis of a double-blind placebo controlled trial, already briefly reported, of injections of tyrosine-adsorbed, glutaraldehyde-modified *Dermatophagoides pteronyssinus* antigen in fifty-one children with perennial asthma and positive bronchial challenge to the antigen, confirms that the patients receiving the treatment reduced their symptomatic medication more than controls, without deterioration of symptoms. Some became symptom-free, when off all treatment.

A double-blind placebo controlled trial of continuing treatment for a second year gave evidence of deterioration when the treatment was stopped.

Within the treatment group, the improvement was associated with loss of late (6 hr) reaction to bronchial provocation with the antigen, but was not associated with change of immediate (20 min) reaction in lungs or skin. Those who improved in the placebo group did not lose their late reaction.

There was a trend for similar benefit from active treatment in the control group, during the second year, though less than in the original active group, and only one lost his late reaction.

Only one of the six children with very severe early onset asthma improved.

Local reactions to either active or placebo (tyrosine) were seen in half the patients; these were mild and did not influence the treatment. Systemic symptoms occurred shortly after four active injections and after two placebo injections; only one patient stopped the treatment.

Introduction

There have been many enthusiastic claims for symptomatic relief of allergic disease by various forms of hyposensitization, first introduced by Noon (1911) based on unsound

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theory. Controlled trials have confirmed such effects for pollen allergy (Frankland & Augustin, 1954; Lichtenstein, Norman & Winkerwerder, 1968), including children who may do even better (Sadan *et al.*, 1969) though the evidence is not so good, and it is not very strong for other allergic diseases. Some benefit has been reported in adults with asthma or rhinitis due to *Dermatophagoides pteronyssinus* (D'Souza *et al.*, 1973; Gabriel *et al.*, 1977) and preparations are available and widely used. The mechanism of this effect is unknown and failure to detect effects on responses associated with allergy, such as IgE antibody, has contributed to the limited acceptance of this line of treatment; so have the rapid advances in drug therapy, and, for children, the awareness of the probability of spontaneous improvement. The recognition of the predominant role of *D. pteronyssinus* in children with perennial asthma in Britain, reports of symptomatic improvement with injections of such crude material as housedust extract (Aas, 1971; Taylor, Sanders & Norman, 1974) and the development of preparations of gluteraldehyde-modified antigens adsorbed onto tyrosine crystals, which act as adjuvants (Wheeler *et al.*, 1982) and which have been used successfully in hyposensitization to pollens without the need to increase doses to potentially toxic levels (Miller, 1976), led to a need for further study. We have already briefly reported the first phase of a double-blind controlled trial of such treatment in children with moderate to severe perennial childhood asthma, shown to be due to *D. pteronyssinus* sensitivity by bronchial provocation (Warner *et al.*, 1978) which showed an impressive benefit, which was associated with loss of their late reaction on bronchial provocation testing. We now report *in vivo* aspects of the completed study, including reanalysis of the first year and a report of the second year, during which the treated patients undertook a trial of continuation therapy, and the original placebo group were treated. *In vitro* findings are presented in the accompanying paper (Turner *et al.*, 1984).

Methods

Patients aged between 5 and 15 years attending a paediatric respiratory disease clinic, who had perennial asthma inadequately controlled by conventional treatment, were considered for the study. Those who had had previous hyposensitization treatment, or who were receiving long-term oral steroid treatment were rejected. Skin prick tests to ten common allergens (Bencard) were done, and those reacting (> 3 -mm wheal at 15 min) to *Dermatophagoides pteronyssinus* had bronchial provocation tests to this antigen (Warner, 1976). Peak expiratory flow rate was measured at intervals for at least 24 hr after each test and immediate and late reactions noted. An immediate bronchial reaction was defined as a fall in peak flow of at least 20% from baseline within 20 min of challenge, and a late bronchial reaction as a fall in peak flow of at least 50% from baseline 4–8 hr after challenge.

The sixty-three children who gave a positive immediate reaction were enrolled in the study. All were receiving intermittent bronchodilator treatment, usually inhaled salbutamol, some were receiving regular inhaled or oral salbutamol, and some inhaled sodium cromoglycate and/or beclamethasone. During a 3-month pre-treatment period, housedust-mite avoidance measures were introduced and this drug therapy was progressively reduced, in the reverse of this sequence to the lowest dose which controlled the symptoms. That is, first the frequency of dose of inhaled steroid was reduced from 4 (rare) to 3 to 2 to 0 daily, introducing sodium cromoglycate if necessary. Then sodium cromoglycate was similarly reduced, and then the oral and then the inhaled bronchodilators. At the end of the pre-treatment period, seven

patients were symptom-free after all drugs had been withdrawn, so they were excluded from the trial. The remaining fifty-six children entered it; twelve were taking inhaled steroid, twenty-nine cromoglycate, and fourteen oral or inhaled bronchodilators continuously; nine were taking intermittent bronchodilators only. The attempt to reduce the drug therapy was maintained, though, in those requiring more treatment, the agents were introduced usually in the reverse of the order of withdrawal. When a severe asthma attack occurred, appropriate treatment was given, including oral and injected bronchodilators and systemic steroids. Records were kept of the drug treatment, and treatment scores were derived from these, as described below.

The fifty-six children were randomly allocated on a double-blind basis either to active treatment (tyrosine glutaraldehyde modified *D. pteronyssinus* antigen, 'Migen', Bencard) or to placebo (tyrosine suspension) by injections given by their family doctor, weekly for the first 6 weeks, followed by injections every 8 weeks. The active group received injections from prefilled syringes, containing 4, 10, 25, 80, 150 and 400 Noon units in the first 6 weeks, and 400 Noon units for each subsequent injection. Each injection was preceded by an oral dose of 4 mg chlorpheniramine, and the doctor and the patient recorded any reactions which occurred on a diary card. Five patients were withdrawn from the trial, one on active treatment who objected to the injections and four on placebo (one each because of similar objections, emigration, a severe attack of asthma requiring a long course of oral steroids, and a generalized utricularial rash). Thus twenty-seven completed the active treatment, and twenty-four completed the placebo course; drug score and other data (initial or at 1 year) were incomplete in seven subjects, so the numbers in the groups vary slightly.

At the end of the first year, the code was broken. The twenty-seven in the active group were then randomly allocated on a double-blind basis to six more injections of *D. pteronyssinus* at 2-monthly intervals, or to a control group who received six injections of isotonic saline (the tyrosine control suspension was not used because of the local reactions—see below); this constitutes a trial of treatment withdrawal at the end of the first year. Thirteen children completed a second year of active therapy and twelve completed the placebo injections. One patient declined further injections after 1 year because he had become symptom-free, and one was lost to follow-up for 4 months, after which this regimen was contraindicated.

Twenty-four children (eighteen boys and six girls) had received placebo injections for the first year. Two patients were then lost to follow-up; the other twenty-two were given active therapy in the second year. One of these patients was withdrawn after a possible systemic reaction to the fifth injection. Twenty-one children completed the treatment. The patients and their parents did not know in which of the 2 years they had received active therapy until the second year assessment was completed.

As reported previously (Warner *et al.*, 1978) the patients kept a daily account on diary cards of symptoms and drugs taken, and also recorded morning and evening peak expiratory flow rates. Every 2 months they attended outpatients, when records were checked, the drug therapy was adjusted, the patients and parents were asked whether the asthma was better, unchanged or worse, and forced expiratory volume after 0.75 sec (reverse plethysmography) and thoracic gas volume (whole-body plethysmography) were measured. The patients (and parents) kept daily diary cards, grading (0–3) night cough, night wheeze, and day activity, and recording all drugs taken.

Drug scores were derived from the record of doses taken during each 2 month

period, assigning 1 for each dose of bronchodilator (regular or intermittent) 2 for each dose of sodium chromoglycate, 3 for inhaled steroid, 4 for oral steroid or injected bronchodilator, and 5 for injected steroid. Skin and bronchial provocation tests were repeated after the first and second year.

Results were analysed by Fishers exact test (2-tail) and by Mann-Whitney *U* and Wilcoxon matched-pair tests.

Results

At onset, the two groups did not differ significantly for skin-test results, associated symptoms (eczema, or perennial allergic rhinitis), frequency of attack, diary-card score of wheeze, day or night (rather higher in treatment group), peak flow, thoracic gas volume, or drug score, as reported by Warner *et al.* (1978). The median drug score was less after 1 year of treatment than in the placebo group ($P=0.007$, Mann-Whitney *U* test) (Warner *et al.*, 1978), despite the large scatter of initial scores (Fig. 1). Some patients were taking so few drugs at the start of the trial that further reduction was not attainable. Excluding the five subjects with an initial drug score < 30 (i.e. taking less than one dose of Salbutamol/day), the prevalence of fall of drug score is significantly greater in the treatment group (Table 1). In only one of the four patients in the

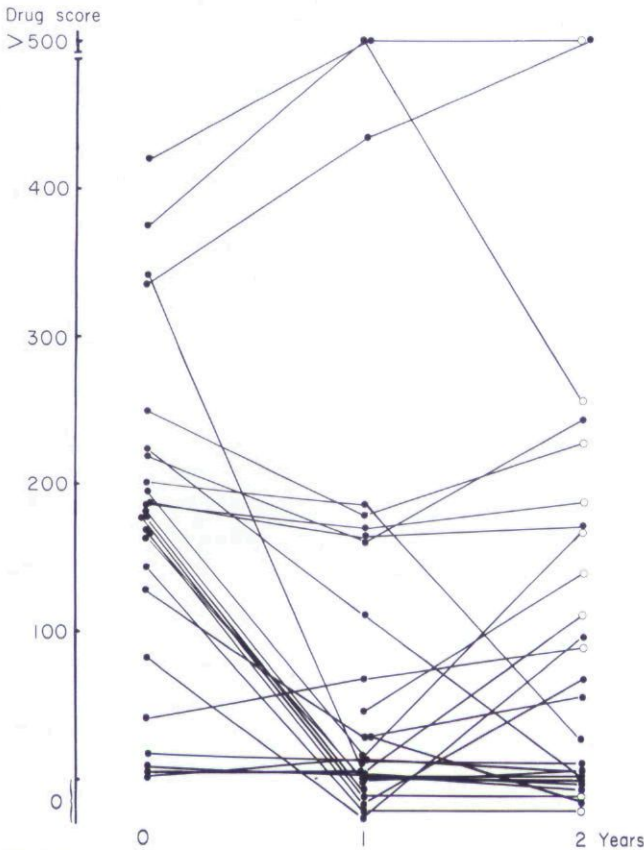


Fig. 1. Drug score for each patient in the initial active group who received *D. pteronyssinus* hypsensitization in the first year. Those who received it in the second year too are shown with closed symbols (●) and those who received placebo in the second year are shown with open symbols (○).

Table 1. Association of fall of drug score at 1 year with treatment (first year)

	(a) Treatment group*		(b) Treatment group†	
	Active	Placebo	Active	Placebo
Fall	17	7	16	6
No fall	4	10	1	8
	$P=0.0278$		$P=0.0052$	

* Omitting all subjects with initial drug score < 30 , and † also omitting those with initial drug score > 300 .

treatment group with an initial drug score > 300 did it fall (Fig. 1), but the drug score fell in all but one of the seventeen in the active group with initial drug scores between 30 and 300, significantly more frequent than in the control group (Table 1). The fall of drug scores in the treatment group (Fig. 2) occurred despite no deterioration of symptoms. Indeed, the diary-card record of night wheeze fell in the first year in the treatment group ($P=0.008$ Wilcoxon rank sum matched pair test) as did the thoracic gas volume ($P=0.01$, Fig. 3).

The median drug score of the placebo group remained constant for the first year (Fig. 4) but fell in the second year when they received the active treatment, on a single-blind basis. However, neither of the two of these children who completed the treatment who had initial drug scores > 300 improved during the second year. If these and the two patients with drug score at one year < 30 are omitted, all but two of the eleven patients for whom data is available showed a fall in drug score. This fall was not as evident as that of the first-year treatment group, and only one lost his late reaction on bronchial provocation tests. The association of fall of drug score to $< 50\%$ and loss of late reaction was highly significant, not only in the first year but in both groups together (Table 2). After 1 year's treatment in the two groups (omitting those with drug score < 30 initially and with incomplete data—total thirty-eight) eight were off all drugs and another eight had drug scores < 30 .

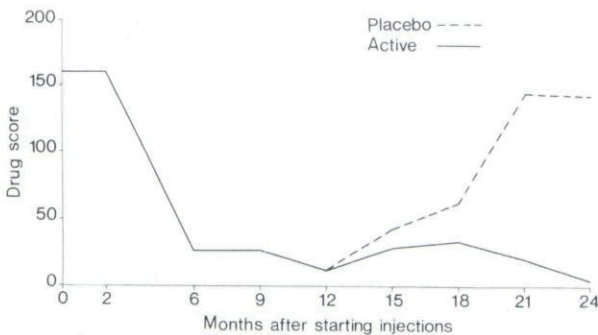


Fig. 2. Median drug score in the patients receiving active treatment in the first year. In the second year the median values are shown for those continuing the treatment (continuous line) and for those receiving placebo (dotted line).

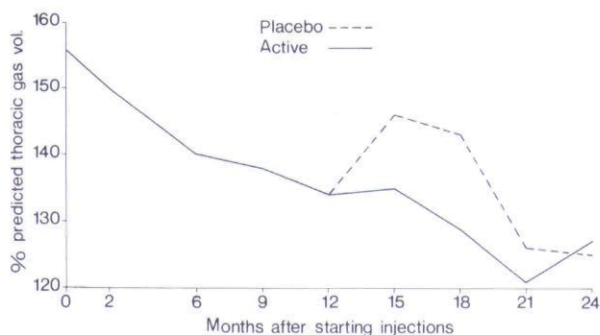


Fig. 3. Median values for thoracic gas volume, expressed as % of expected value, in the patients who received active treatment in the first year, plotted as in Fig. 2.

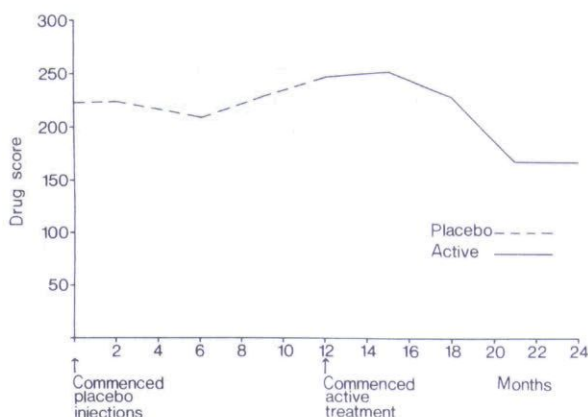


Fig. 4. Median drug score in the patients receiving placebo in the first year, and active treatment in the second year, plotted as in Fig. 2.

In the second year, the initial treatment group was randomly allocated either to continued treatment or to placebo injections. First the lung function deteriorated in those receiving placebo (e.g. median thoracic gas volume (TGV) rose by 15 and 18 months, Fig. 3), and then the drug score rose, effectively to control the asthma again (21–24 months; Figs 2 and 3). The data on individual patients are shown in Fig. 1. A fall in drug score in the second year was significantly more frequent in those who continued with the treatment (Table 3) than in those who received the placebo. Of the patients treated in the first year, with an initial drug score > 30 , relevant data from the outcome of the second year is complete in nineteen; late reactions disappeared in another two in the second year of active treatment and their drug scores fell. Late reactions recurred during the second year in three of the four who had lost them after a year of treatment, but who then received no further treatment in the second year.

As has already been published (Warner *et al.*, 1978) patients' and parents' diary scores also improved significantly more frequently in the first year in the treated group than in the placebo group for asthma, rhinitis and eczema (Table 4); Warner *et al.* (1978) omitted the probability value for eczema. Similarly, the asthma and eczema of most of the initial placebo group improved in the second year when they received treatment, and the diary cards showed continued improvement in asthma and rhinitis

Table 2. Association of drug-score improvement (to < 50%) with loss of late reaction in the treated patients

	Drug score			
	First year		Both groups	
	Better	Not better	Better	Not better
Late reaction:				
Lost	8	0	9	0
Not lost	4	8	4	16
	$P=0.008$		$P<0.001$	

Table 3. Association of fall of drug score during the second year of the study in the initial treated group, with continued treatment or withdrawal

Drug score	Treatment	
	Continued	Withdrawn
Fell	9	1
Rose	4	9
	$P=0.012$	

Patients whose drug scores (0 in two children and 3 in another) did not change, all in the withdrawal group, were omitted. Similarly low values were retained if they changed.

in more of those of the initial active group who continued treatment than in those who received placebo in the second year, but these differences are not significant, and such an effect was not seen in the eczema of the small number who had it.

Seven patients had systemic symptoms following injections, which may have been reactions. Of those receiving active treatment, one developed wheezing and vomiting and fainted the day after receiving 150 Noon units of *D. pteronyssinus* and was withdrawn from the trial. Two became wheezy and one developed general weakness and headaches for 24 hr after injections; treatment was maintained. Of the patients on the tyrosine placebo, one developed urticaria and one severe asthma; they were withdrawn from the study. One patient became jaundiced briefly, after the last injection of tyrosine suspension.

Local discomfort, redness and swelling lasting from 24 to 48 hr was seen in twenty of the forty-nine patients who received active therapy, in ten of the twenty-four who received the tyrosine suspension, and in none of the twelve given saline injections.

Discussion

The further study of this series, the first year of which has already been reported (Warner *et al.*, 1978), provides evidence to support our previous conclusions that the injections of tyrosine-adsorbed *D. pteronyssinus* antigen reduced the severity of

Table 4. Parents' and patients' assessment of asthma, perennial rhinitis and eczema in the first-year trial (already published Warner *et al.*, 1978) and in the studies in the second year, a randomized controlled trial of continuing in the group who had received the active treatment (results not significant) and in the initial placebo controlled group, who received active treatment in the second year.

Year of study	Treatment		Number	Asthma		Perennial rhinitis		Eczema	
	First year	Second year		Improved	No change or worse	Improved	No change or worse	Improved	No change or worse
1	{ Active	—	27	23*	4*	14†	9†	7‡	2‡
	{ Placebo	—	24	12*	12*	5†	17†	3	8
2	Placebo	Active	21	16	5	10	10	7	4
2	{ Active	Active	13	10	3	7	3	2	2
	{ Active	Placebo	12	8	4	6	6	3	1

* $P = 0.015$; † $P = 0.02$; ‡ $P = 0.038$.

asthma. Confirmation came from the significantly greater improvement which occurred in those patients in the active group who maintained treatment in the second year, in the randomized controlled trial of treatment withdrawal. Similar improvement was seen in the initial placebo group who received treatment in the second year, but it was less evident. The difficulty of the quantitative assessment of long-term treatment effects in childhood asthma result from the wide range of severity and intermittency, its tendency for spontaneous recovery, and the effectiveness of symptom-controlling drugs. The validity of our primary parameter—the drug score during continuous attempts to lower the dose of symptom-controlling drugs—is clearly shown in the second year trial of withdrawal, in which those receiving placebo, after a year of active treatment first showed a rise in TGV and then a rise in drug score with a fall in TGV, but we found evidence of benefit from other systems of assessment too. That recorded by parent and patient assessment presumably is the basis for the continuous use of hyposensitization despite lack of convincing associated changes in objective tests or knowledge of the mechanism of the effect. The additional data provide some support for the benefit for rhinitis and eczema too; Warner *et al.* (1978) did not comment on the latter, but it is interesting in view of the increasing evidence of a role for inhalant allergens in eczema, as well as foods.

This therapy did not help everyone. Some had such mild symptoms and such low drug scores that, retrospectively, we feel that we should have had a higher cut-off for selection to the trial. However, we could not assess benefit effectively in these. Of the six children with most severe asthma only one showed considerable improvement but of the twenty-eight with drug scores between these extremes (the considerable majority) all improved except three. This certainly did not happen in the placebo group in the first year and the trial data strongly suggests that this improvement was at least partly the effect of the treatment. Since nearly all the children with moderately severe asthma improved, it is not surprising that *in vitro* tests do not predict who will benefit, apart from the greater benefit in those with relative immunoglobulin deficiency (Turner *et al.*, 1984).

This improvement was achieved with very few adverse effects. The local reactions, never bad enough to preclude continuation, were as frequent in the placebo group as in the active treatment group, and were therefore presumably an effect of the tyrosine.

There is no evidence that the few systemic episodes were related to either treatment. However, a report of a fatal illness (presumed reaction) following injection of this preparation (Rands, 1980) suggests that there may be some risk. Careful supervision of dose and facilities for resuscitation are critical, and the disease must be severe enough to justify this risk.

The benefit is consistent with reports of other trials of hyposensitization to *D. pteronyssinus* in asthma, only a few controlled and none studied in such detail, in adult and child asthmatics, with a range of extracts in various media and adjuvants (Price, 1981). However, we know of no other controlled trial of hyposensitization of asthmatic children with tyrosine-adsorbed glutaraldehyde-modified *D. pteronyssinus* antigen. Standard regimens for aqueous extracts entail increasing the dose until reactions become common. Achieving an effect, without such reactions is a useful advance. As with this study (Turner *et al.*, 1984), no consistent change in *in vitro* test results from any of these studies provides even a suggestion of possible mechanism for this effect.

But was the effect big enough to warrant the demanding regimen? Clearly minor or seasonal asthma can be adequately controlled by drugs, until it recovers spon-

taneously, and our evidence suggests that the treatment we gave has little to offer for children with very severe asthma who are most likely to have symptoms persisting into adult life. But the consistency of the effect in the intermediate group, who were greatly relieved from distressing symptoms, may well have a place, when symptoms persist, despite optimal drug treatment supervised in an experienced clinic. We believe that bronchial provocation tests, preferably with observation of the late reaction, to confirm the allergy to *D. pteronyssinus*, is very desirable before undertaking this treatment, though a strong skin-test reaction does give some evidence for this (Price *et al.*, 1984). So decisions about such treatment in asthmatic children should be taken in special centres.

This regimen of injection was selected as a result of limited experience in adults, and of experience from other preparations which is not necessarily relevant. Perhaps the children with very severe asthma would have benefited if more had been given or if it had been given more often. Perhaps those who benefited would have done as well with less, though the positive trial of withdrawal suggests that continued injections for at least 2 years are needed by many. It will be interesting to know whether such treatment reduces the bronchial responsiveness to such non-specific stimuli as exercise and cold. If, as we believe, such non-specific reactivity is an effect of allergy, and may also improve with the treatment, such an effect might well be worth having.

Satisfactory appraisal of dose is difficult while we are dependent on slow and varied clinical evidence for assessing an effect. Theories of mechanisms include Noon's antitoxin hypothesis, IgG blocking antibodies, and suppression of IgE antibody production by stimulation of antigen- and isotype-specific suppressor cells. We have no evidence to support any of these. We made the study in view of the possibility that the effect might be related to the normal process of spontaneous recovery of childhood allergy, which we have linked to recovery from transient IgA deficiency (Taylor *et al.*, 1973). If this is so, children might particularly benefit from hyposensitization as suggested by Sadan *et al.* (1969). The greater improvement in those with lower serum IgA provides some support for this view, though we think that the IgA deficiency itself may well be only a way of identifying an immunodeficient subject, and not necessarily the relevant deficient mechanism. We did not detect an association of improvement with the development of IgA antibodies.

Our previous report (Warner *et al.*, 1978) of the association of loss of the late reaction on bronchial provocation with successful hyposensitization, in this series suggested that new theories should be considered. Though the significance of this association was even stronger in the group as a whole than in the first years' data, the association was less strong in the second year. However, such an association has since been observed in skin tests by Dieges, Aalberse and Schut-Mer (1982). Unfortunately we did not observe the effect of the treatment on late skin and nose reactions. However, since the mechanism of the late reaction is not known (Price *et al.*, 1982), this does not help much in identifying mechanisms for hyposensitization, but it does provide an explanation for improvement without change in immediate reactions, or IgE-related tests. Neither the lack of effect on *in vitro* tests, nor that of an established mechanism of effect, need deter the cautious continued clinical development of such treatment. The use of antigens associated with tyrosine is based on the non-toxic effectiveness of tyrosine as an adjuvant in other animals (Wheeler *et al.*, 1982). Its application to human atopy remains sensible if this non-toxic effectiveness is maintained, but, without clearer ideas of mechanism, it is very difficult to optimize the system.

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