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House dust mite allergen levels and an anti-mite mattress spray (natamycin) in the treatment of childhood asthma

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Summary

Natamycin, a fungicide marketed as Tymasil, is claimed to reduce house dust mite numbers and would therefore be expected to improve asthma in children with mite sensitivity. We have tested this assertion by a double-blind, placebo-controlled trial. There was no significant effect on levels of *Der p* I in mattress dust between active and placebo groups at the end of the spraying period. Histamine inhalation challenge PC_{20}, clinic visit symptom scores and lung function tests reflecting either large or small airways obstruction were also unchanged. Therefore this product is not a therapeutic option for mite-allergic patients using the manufacturer's recommended dose and method of administration. Other factors influencing the *Der p* I levels were also investigated. Of these, only month of measurement and bedroom wall humidity showed any association.

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Introduction

The house dust mite (HDM), *Dermatophagoides pteronyssinus* (Dpt), is a source of allergens contributing to symptoms in the majority of children with asthma [1]. The main source of nutrition for Dpt are epidermal scales [2], but they also require certain fungi for optimal growth [3,4]. Natamycin is a potent fungicide (marketed as Tymasil) which has been found to decrease growth of Dpt in culture [5]. A previous open, uncontrolled trial in adults with asthma suggested that symptoms were improved when natamycin was regularly sprayed onto mattresses [6]. We have conducted a placebo-controlled, randomized, double-blind trial of natamycin sprayed on mattresses following the manufacturer's instructions to evaluate its effect both on Dpt antigen (*Der p* I) [7,8], content of mattress dust and symptoms of asthma in children sensitive to Dpt.

The HDM has many requirements for optimal growth including temperature and humidity [2]. As these may be influenced by heating and ventilation of houses [9–11], we investigated home environmental conditions in relation to *Der p* I levels in dust.

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Materials and methods

The study was approved by the hospital ethical committee, and informed written consent was obtained from a parent of each child.

Subjects

Fifty-one children aged 5–16 yr (35 males), were selected from the Brompton Hospital asthma clinic and St Mary's Hospital allergy clinic. Five dropped out, three because of non-attendance (one active group, two placebo groups), one because of pruritus following the mattress spraying on two occasions (active group) and one due to bereavement (active group). The diagnosis of asthma was established by a history of recurrent attacks of cough and/or wheeze responsive to bronchodilator therapy; evidence of atopy on history, skin tests and sometimes radioallergosorbent tests (RAST); and evidence of reversible airflow limitation on simple lung function tests (peak expiratory flow or spirometry). The severity varied from mild episodic disease requiring intermittent bronchodilators only, to chronic severe asthma requiring regular inhaled steroids with regular oral bronchodilators as well as intermittent inhaled bronchodilators. Mite sensitivity was suggested by perennial symptoms and confirmed by

Table 1. Trial protocol

Week	0	2	4	6	8	10	12	16	20	24
Spray	+	+	+	+	+	+				
Home	+						+			
Clin	+		+		+		+	+	+	+
LFTs	+		+		+		+	+	+	+
Hist	+						+			+
Diary	----------									→
bd PEFR	----------									→

Home = home visit with collection of mattress dust.
Clin = clinical assessment.
LFTs = pulmonary function tests (flow–volume loop and nitrogen wash out).
Hist = histamine inhalation challenge for bronchial reactivity.
Diary = recording of daily symptom scores and medication use.
bd PEFR = twice daily peak expiratory flow recordings.

Table 2. *Der p* I ELISA assay results (ng/g sieved dust)

	Group A		Group B	
	D1	D2	D1	D2
Geometrical mean	4647	3671	3385	3075
s.d.+	25668	17969	15790	11994
s.d.−	841	751	726	788

D1 = results of 1st sample of pair (baseline), D2 = that of 2nd sample (12 weeks).
Geometrical mean = mean of logged data, s.d.± = ±s.d. antilogged. The geometrical means of the differences between log D1 and log D2 were: group A = 1·2659; group B = 1·1009; showing a non-significant tendency to a greater fall in *Der p* I in group A. Student's *t*-test between logged group A difference and group B difference showed no significant difference.

skin-prick tests (SPT) [12] with Bencard solutions. The weal with Dpt was always larger than the positive control (1% histamine in phosphate buffer) and usually the strongest reaction in a battery of 6–10 allergens.

The children's asthma was considered stable at the time of entry to the trial. No oral steroids had been administered in the last 6 months, and regular medication was continued throughout the trial.

Treatment

The mattresses from the subjects' beds were sprayed once every 2 weeks for 3 months (six applications) with either

Table 3. The provocation concentration of histamine for a fall of 20 (PC_{20}) in lung function (Fev_1) for weeks 0,12 and 24 in groups A (active) and B (placebo)

	Group A			Group B		
	0	12	24	0	12	24
Geometrical mean	4·478	3·027	4·148	4·424	4·972	4·105
s.d.+	18·61	9·014	14·121	19·541	22·832	16·667
s.d.−	1·077	1·016	1·219	1·002	1·083	1·011

Values represent mg (histamine)/ml.
Geometrical mean = mean of logged data, s.d.± = ±s.d. antilogged. The data were log transformed to give a normal distribution.
Using Student's *t*-test there were no significant differences between the groups at baseline or week 12 or within the groups between baseline and week 12.

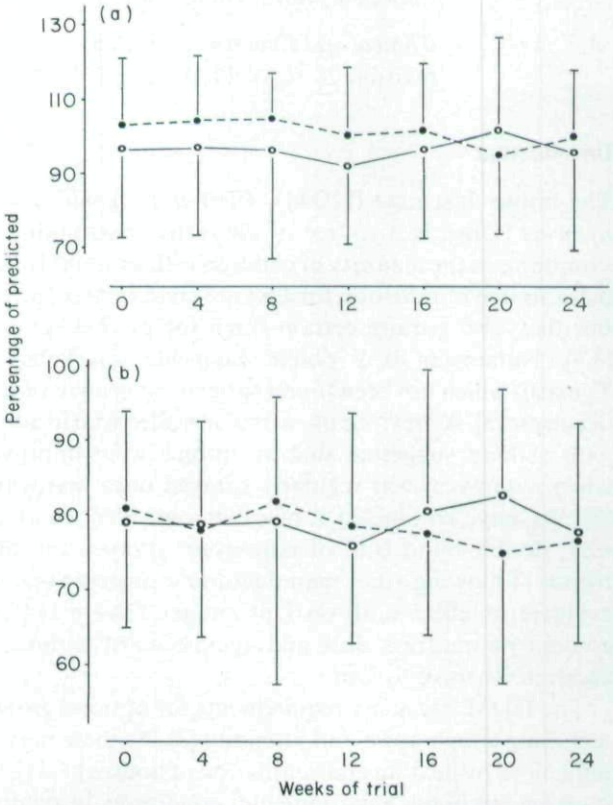

Fig. 1. Peak expiratory flow rates (a) and forced expiratory volumes in 1 sec (b). Means and standard deviations for each clinic visit for groups A (active) (—○—) and B (placebo) (- - - ● - - -).

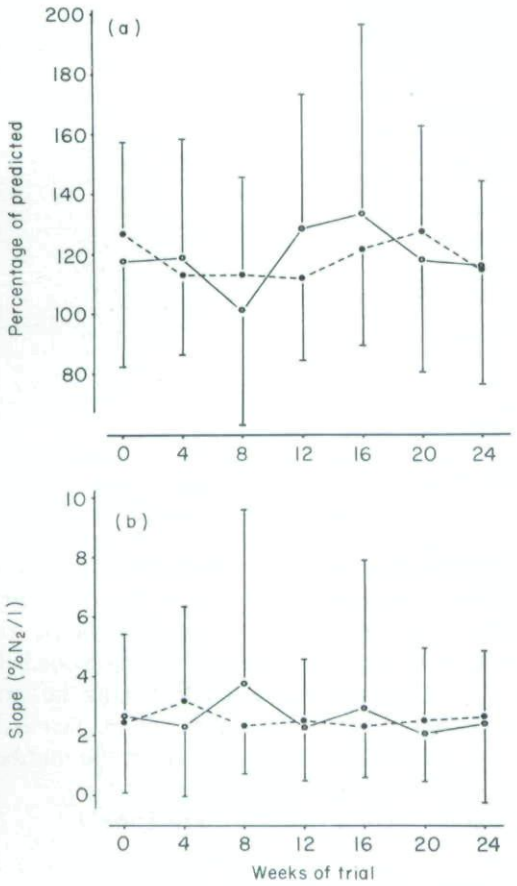

Fig. 2. Residual volumes (a) and slopes of phase 3 (b). Means and standard deviations for each clinic visit for groups A (active) (—○—) and B (placebo) (- - - ● - - -).

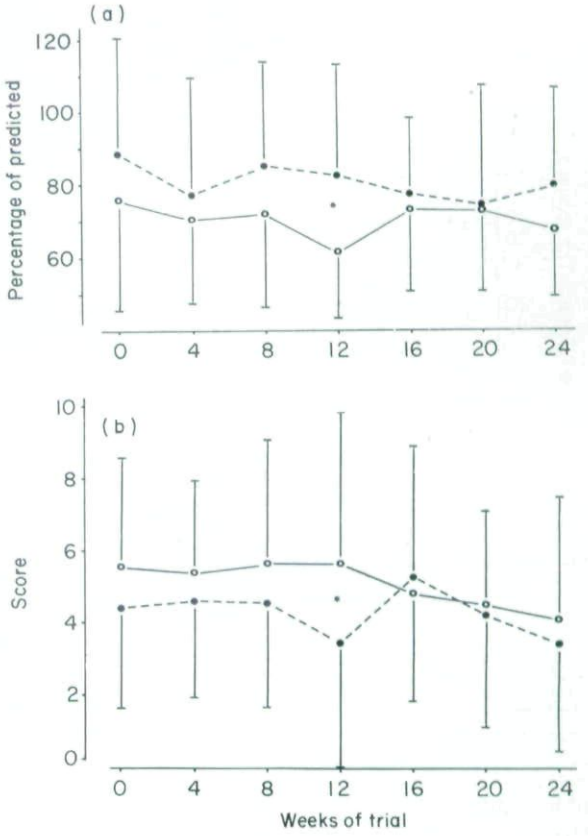

Fig. 3. Maximum expiratory flows at 50% of vital capacity (a) and clinic scores (b). Means and standard deviations for each clinic visit for groups A (active) (—○—) and B (placebo) (- - - ● - - -). * The only differences reaching statistical significance were those between the MEF_{50}s at week 12 ($P=0{\cdot}0098$ in favour of placebo) and the clinical scores at week 12 ($P=0{\cdot}013$ in favour of placebo).

natamycin (500 mg/dose) or placebo. The first spraying was demonstrated by one of us (J.R.) after which the parents used the other aerosol cans provided at appropriate intervals. For consistency the parents were asked to vacuum the mattresses prior to spraying and at weekly intervals. Prior to the trial the anti-dust measures used by the parents varied from none to twice-weekly vacuuming of the mattresses. None were using plastic mattress covers.

Monitoring

(a) The home was visited and mattress dust was collected using a portable suction cleaner. A dust trap was attached to the nozzle and each mattress was thoroughly vacuumed over the top surface. As the concentration of *Der p* I was measured, the area of mattress or time taken was not standardized, but the same technique was employed by the same operator (J.R.) throughout and was, therefore, comparable. The samples of dust were sieved, weighed, extracted in 0·1% Tween 80 borate-buffered saline and aliquots were analysed for *Der p* I allergen using a monoclonal ELISA [13]. The specimens were stored at −20°C until analysis to minimize changes in *Der p* I concentration and all were analysed at one time to avoid interbatch variability.

The relative humidity (RH) was measured with a whirling hygrometer and the humidity of the wall of the bedroom (WH) using a Protimeter (Protimeter Ltd, Marlow, U.K.) at each home visit. The scale on the latter was 1–100, corresponding to humidity values; 0–40≡6–14%, 40–55≡15–19% and >55≡>19%. Absolute humidity (AbH) was derived from the RH and tables [27]. Although both the whirling hygrometer and Protimiter have limitations, the latter being influenced by factors such as salt precipitation in plaster of walls, their portability and ease of use influenced our choice.

The presence of pets was ascertained, as was the type of heating, windows, the presence of carpets in the bedroom

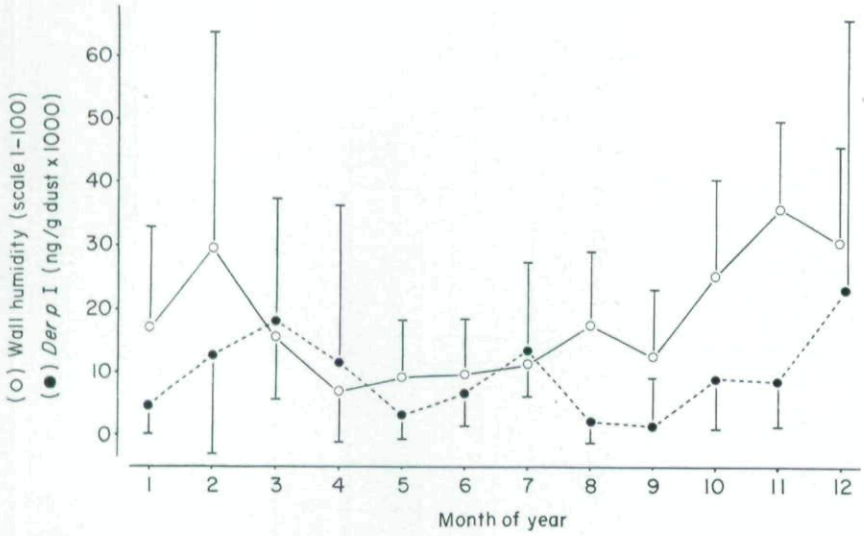

Fig. 4. Levels of wall humidity (—○—) and *Der p* I levels (- - -●- - -) at each month of the year (mean ± s.d.)

Table 4. Influences on wall humidity

	Heating		Glazing		Ownership	
	CH+	CH−	DG	SG	OO	NOO
n	36	10	12	34	40	6
Wall humidity						
Mean	16·9	24·9	20·9	18·1	17·9	24·5
s.d.	16·9	16·3	15·7	17·4	13·8	32·0
P (Mann–Whitney *U*-test)	n.s.		n.s.		n.s.	

CH, central heating; DG, double glazing; SG, single glazing; OO, owner-occupied; NOO, not owner-occupied.

and whether the house was rented, council owned or owner-occupied.

(b) A sample of blood was taken from each child and was later analysed for anti-Dpt IgE by RAST [14].

(c) Clinical progress was monitored using diary card records of symptoms and medication use; twice daily peak expiratory flow rates (PEFR); monthly clinical assessment scores; monthly lung function tests (flow–volume loops, from which were derived PEFR, forced expiratory volume in 1 sec (FEV_1), maximum expiratory flow at 50% and 25% of vital capacity (MEF_{50}, MEF_{25}); and nitrogen wash-outs giving total lung capacity (TLC), residual volume (RV) and slope of phase 3 (S13)) (using a Hewlett-Packard pneumotachograph and nitrogen analyser). Histamine bronchial provocation tests for bronchial reactivity were performed once every 3 months [15].

Statistics

The Mann–Whitney *U*-test was used for comparison of data for lung function tests, clinical scores (there was no significant change in outcome if Student's *t*-test was used) and factors in the homes. The data for *Der p* I levels and PC_{20} values were log transformed and geometric means and standard deviations derived. Correlation coefficients were calculated for suitable paired data using the AMSTAT computer program (© 1986, Coleman JG, Coleman SC). Fischer's exact test was used to compare the number of rises and falls in *Der p* I values.

The trial protocol is represented in Table 1.

Results

There were 46 paired results for SPTs and RASTs for HDM which were significantly correlated ($P \leqslant 0{\cdot}01$). This occurred despite clustering near the top end of the values because of subject selection for mite sensitivity.

The 48 paired results for HDM RASTs and mattress dust *Der p* I did not correlate significantly.

Statistical analysis on the natamycin trial data was done by partial breaking of the code to compare two treatment groups, A and B. Only when all analysis was complete was A revealed to be active natamycin and B placebo.

The *Der p* I ELISA was completed on 46 paired samples. The results are summarized in Table 2 divided into groups A (active) and B (placebo).

The two groups of *Der p* I results were compared for:

(1) the frequency of either increase or decrease in *Der p* I values from dust collection 1 to dust collection 2, showing that group B had more falls (16) and fewer rises (6) in *Der p* I than group A (11 falls and 10 rises), although the difference was not significant ($P = 0{\cdot}14$ Fischer's exact test); and

(2) the magnitudes of the changes, recorded as the increase or decrease in log *Der p* I from the first to the

Table 5. Influences on antigen *Der p* I levels

	Heating		Glazing		Ownership		Pets		Carpets	
	CH+	CH−	DG	SG	OO	NOO	+	−	+	−
n	38	12	13	37	42	8	18	32	41	9
Der p I										
Mean	13678	7139	17947	10057	13335	5670	19873	7741	13411	6178
s.d.	26856	5260	26821	22453	25561	5303	35122	12331	25827	6257
P (Mann–Whitney *U*. test)	n.s.		n.s.		n.s.		n.s.		n.s.	

CH, central heating; DG, double glazing; SG, single glazing; OO, owner-occupied; NOO, not owner-occupied.

second dust sample. There was a small non-significant trend to a fall in *Der p* I antigen in both groups (Table 1).

The results of the histamine provocation PC_{20} for FEV_1 are summarized in Table 3. The value 100 was assigned to tests where there was no reaction at an inhalation concentration of 32 mg/ml of histamine.

The output of the nebulizer used was measured at 0·22 ml/2 min (at 8 l/min flow), allowing conversion of these results to the provocation dose (PD_{20}).

The relationship between change in *Der p* I and change in PC_{20} was also explored and showed no statistically significant association at either 12 or 24 weeks compared to baseline (week 0).

The monthly lung function tests (LFT) representing changes predominantly in central airways (PEFR, FEV_1), predominantly peripheral airways (MEF_{50}, RV and Sl3) and the clinical scores are represented in Fig. 1 (PEFR, FEV_1), Fig. 2 (RV, S13) and Fig. 3 (MEF_{50}, clinical scores). The LFTs are represented as the percentage of predicted value for height [16] to make comparisons between subjects of different ages, sex and sizes possible (except for Sl3 values which are given in absolute numbers as $\%N_2/1$) [17]. No differences were seen for any measurement.

A total of 96 results of log *Der p* I, RH, AbH and WH were correlated. As might be expected, RH, WH and AbH correlated highly significantly ($P \leqslant 0{\cdot}0001$). However, there was no significant correlation between *Der p* I levels and RH, WH or AbH.

Although *Der p* I and WH did not correlate significantly directly, they have been plotted by month of collection, showing that *Der p* I follows the pattern of the wall humidity but about 1 month later (Fig. 4). Unexpectedly, although AbH and RH correlated with WH, they were not related to *Der p* I and month in a similar way.

The association with factors in the house was checked. This showed a trend to lower humidity with central heating, single glazing and home ownership (Table 4), although none of these trends reached statistical significance. There were 18 subjects with either cats or dogs or both in the house with access to the bedroom. There was a trend for double glazing, central heating, home ownership and the presence of pets and carpets to be associated with increased *Der p* I levels. None of these reached statistical significance. The results are presented in Table 5.

Discussion

The correlation between SPT and RAST is to be expected as they both represent specific IgE. The clustering near the top of the ranges confirms that these were subjects with strong sensitivity to HDM, and justifies their selection. The poor correlation of *Der p* I in mattress dust and RAST results suggests either that the range of *Der p* I levels found in these houses was enough to maintain IgE levels in sensitized individuals or, less likely, that the two are not related at all. This contrasts with the findings of Gillies *et al.* [18] whose patients had a wider range of *Der p* I levels and specific IgE responses. We almost certainly influenced the range of mite levels by our subject selection (see below). No home was devoid of mites and there is evidence that in the absence of mites there would be no sensitivity to mites [19,20].

The varying *Der p* I levels over the months of the year are interesting. The peak in wall humidity was between November and February, with peak *Der p* I levels in December and March. The changes in WH (which correlated with *Der p* I levels more closely than RH) were followed about 1 month later by changes in *Der p* I levels. These results are in agreement with *in-vitro* observations indicating the importance of humidity for mites to thrive and with previous observations on fluctuations in mite numbers with seasons [2]. However, a similar relationship was not found with RH or AbH in this study. WH may be influenced less rapidly by outdoor humidity and so

fluctuate less. It may also be more representative of changes in surface microclimate which is more important to mites.

The variations in WH in houses were as might be expected. Central heating and home ownership decreased WH, whilst double glazing increased WH. As none of these associations were statistically significant, they were not analysed independently and may well be related to each other. There has been previous discussion on the influence of pets and carpeting in the house on antigen *Der p* I levels [21,22]. Both factors tended to increase *Der p* I levels, but not statistically significantly in our study. The biological importance of these influences must be viewed in the context of the poor correlation between *Der p* I values and both RAST and histamine PC_{20}. Individually, variations in these factors under normal conditions would be likely to decrease *Der p* I levels below the threshold for response in sensitized individuals. Effective intervention to modify these factors seems unlikely, in standard London homes, as seasonal variation induced large changes in WH but still did not influence *Der p* I levels enough to alter bronchial reactivity or RAST significantly. However, different types of houses, such as some built in Scandinavia, may be more effective in this regard [28], although the outdoor climate may also be crucial.

The variation in month of birth of children with HDM sensitivity which corresponds with months with higher antigen load [1] indicates that fluctuations in *Der p* I levels are more likely to affect primary sensitization than symptoms in already sensitized individuals.

In the natamycin trial, on the criterion of direction of change in *Der p* I (either increase or decrease), there was a trend in favour of group B (placebo); this did not reach statistical significance. When comparing the magnitude of the changes, it was group A (active) which had the favourable trend (again not statistically significant). This indicated that the changes were due to chance alone and not the effect of therapy. The disappointing effect of Tymasil on the level of *Der p* I in the mattress dust may have been because of inadequate penetration of the whole mattress [23]; recolonization from other bedding which was not treated or because the effect of reduction of fungi on Dpt was weak. In the ideal environment, the antifungal effect may have been significant, but in the patients' homes many other factors, such as temperature, humidity and fabric thickness of bedding may have influenced the effect. The spray was, however, used according to the manufacturer's instructions, therefore, as presented, it does not work.

As a previous open study demonstrated some clinical benefit of spraying Tymasil onto mattresses [6], we analysed the results of the clinical response. This also showed no effect on either proximal airways (PEFR, FEV_1), distal airways (RV, Sl3, MEF_{50}), bronchial reactivity (PC_{20}) or clinical scores. The two results showing statistically significant differences at week 12 were the clinical score and the MEF_{50}, both in favour of placebo treatment. It is likely that these results occurred by chance with the number of analyses performed and in any event the active treatment conferred no clinical benefit.

The poor correlation between changes in *Der p* I and changes in PC_{20} is somewhat surprising but indicates that in sensitized subjects small reductions in allergen load will not reduce bronchial hyperresponsiveness. Very strict measures for allergen avoidance or removal of the subject to a low allergen environment are required before improvement in symptoms and bronchial hyperresponsiveness is seen [9–11,19,24–26].

Conclusion

Natamycin, at this dose, sprayed onto mattresses of children with asthma and mite sensitivity had no effect either in reduction of antigen *Der p* I in the mattress dust, in improved bronchial reactivity, improved lung function tests, or clinical symptoms. We have no evidence to justify the use of natamycin spray as part of an allergen avoidance regime. Where *Der p* I levels fell in the mattress dust this did not apparently lead to an improvement in bronchial hyperreactivity. Reductions in *Der p* I must be considerable before reactivity is affected.

The humidity in the walls of bedrooms may influence the level of *Der p* I antigen and is closely related to RH and AbH. This fluctuates with the seasons of the year. Although factors such as heating, glazing, pets, carpets and home ownership are associated with and may influence humidity and perhaps *Der p* I levels, these influences are individually small and unlikely in themselves to change *Der p* I levels significantly in London homes.

The magnitudes of variation in *Der p* I levels seen in mattress dust did not influence either specific IgE levels or bronchial hyperactivity. The possibility of these factors being more important in the primary sensitization of susceptible individuals, during a critical period of immunological maturation in infancy, has not been excluded and requires further study.

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