Significance of late reactions after bronchial challenge with house dust mite

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Warner, J. O. (1976). *Archives of Disease in Childhood*, 51, 905. **Significance of late reactions after bronchial challenge with house dust mite.** Sixty-nine children with moderately severe perennial asthma were submitted to bronchial provocation tests using aqueous extracts of *Dermatophagoides pteronyssinus* (house dust mite). 60 (87%) had immediate positive reactions. 73% also had late reactions occurring 4 to 8 hours after the challenge. A significant correlation was found between frequent attacks of asthma in the year before bronchial challenge and the occurrence of late reactions. The late reaction was more prolonged and severe than the immediate reaction. The changes in lung mechanics during a late reaction were similar to those found in severe naturally occurring asthma. The possible relevance of the late reaction in antigen-induced asthma is discussed.

BPTs are rarely considered to be an essential part of the investigation of bronchial asthma in childhood. There are misgivings about the safety of such procedures (Bristow, 1974). The specificity of the reactions is doubted in a disease where nonspecific airways lability is a frequent problem. It is also possible that hypersensitivity or primary sensitization might be produced by the test. However, in a situation where major family upheaval is often necessary to achieve adequate antigen avoidance, or where hyposensitization is contemplated, clinical histories and prick skin testing are inadequate methods of allergen identification (Aas, 1970; Spector and Farr, 1974).

Unfortunately BPTs neither mimic the conditions which occur during natural allergen exposure

(Kim, 1965; Bronsky and Ellis, 1969), nor produce a reaction which could be equated with severe asthmatic attacks (Engström, 1964). However, pulmonary allergy may be associated with immediate and late responses to the inhalation of allergens (Pepys, 1973) and the relevance of these two reactions to symptoms in different diseases is not clear.

Bronchial responses to direct *Dermatophagoides pteronyssinus* (house dust mite) challenge were studied to investigate the relevance of late reactions in childhood asthma, and to answer some of the criticisms of BPTs.

Methods

D. pteronyssinus provocation test solutions.

The test solutions of *D. pteronyssinus* were kindly supplied by Bencard Ltd. 1.2 g of mite raw material (mite bodies and excreta) were extracted into 100 ml glycerinated extraction medium, dialysed, concentrated under vacuum, reconstituted as a glycerinated extract, and sterilized by filtration. Quality control tests included measurement of protein nitrogen and polysaccharide, skin test activity compared with freeze-dried standards in sensitive human volunteers, and tests for acute toxicity by intraperitoneal injection into mice and for irritancy by intradermal injection in guinea pig skin. Serial dilutions of the extract were prepared aseptically diluting in sterile 0.5% phenol saline. Three strengths, 0.001%, 0.01%, and 0.1% were dispensed in 10 ml amber vials and stored at 4°C. A single batch was used throughout.

Abbreviations

BPT:	bronchial provocation tests
PEF:	peak expiratory flow
FEV _{0.75} :	forced expiratory volume at 0.75 s
FVC:	forced vital capacity
TGV:	thoracic gas volume
RV:	residual volume
TLC:	total lung capacity
SGaw:	specific conductance

Bronchial provocation test. The allergen extracts were inhaled through a mouth piece after nebulization by a Wright nebulizer, containing 2 ml of fluid, with an airflow of 8 l/min from an air cylinder. Expired air, containing allergen, was removed from the room by a closed circuit to an extractor fan system, so that the room atmosphere was not contaminated by the allergen.

Children were admitted to hospital 24 hours before the procedure so that bronchodilators, antihistamines, disodium cromoglycate, and occasionally inhaled steroids, could be discontinued in a stable environment where there was no further exposure to the house mite (Sarsfield, 1974). PEF measurements, using a Wright Peak Flow Meter, were recorded hourly during waking hours for comparison with similar readings after provocation (to establish a stable baseline record). The BPT only proceeded if the baseline PEF was greater than 50% of the normal value.

Clinical histories were obtained from the parents at the time of admission using a standard questionnaire. Patients were selected for study because they had a history suggestive of house mite allergy. The children were prick skin tested with the different dilutions of the *D. pteronyssinus* extract and the highest concentration which failed to elicit a skin response was used as the starting dose for inhalation. Each patient had two 5-minute control inhalations, one with 0.5% phenol saline and the second with an extract of the mite culture medium. Provided there was no response to these inhalations, the allergen extracts were then inhaled first for 1 minute and, if no response after 20 minutes, for 5 minutes, in increasing concentrations until the top dose was reached or a positive response obtained. Patients with a negative bronchial response to *D. pteronyssinus* were challenged with a further allergen selected on the basis of the skin test results.

Each inhalation was followed by a 20-minute interval to allow detection of a response, a fall in PEF of 20% or greater at both 10 and 20 minutes after inhalation being considered positive. After a positive reaction various respiratory function parameters were measured at frequent intervals for the next 24 hours before the patient was discharged. FEV_{0.75} and VC were measured using the Vitalograph, and TGV, RV, TLC, and SGaw were measured by total body plethysmograph (DuBois *et al.*, 1956). The results are presented as changes from baseline expressed as a percentage of the normal predicted values for height using our own published normal data (Cogswell *et al.*, 1975).

Patients

Sixty-nine children (51 males, 18 females) between the ages of 5 and 14 years completed the tests. All had been selected from medical clinics in the hospital as having asthma of sufficient severity to warrant further investigation and treatment. All had at least 6 attacks of wheezing per year and none was satisfactorily controlled on bronchodilators alone; 38 were on prophylactic disodium cromoglycate and 17 on inhaled steroid preparations. All had perennial asthma with a predominance of nocturnal attacks. 34 had coexistent eczema and 42 had symptoms suggestive of allergic rhinitis (11 seasonal, 31 perennial). 10 additional children did not complete the tests because they had positive reactions to the control solution (0.5% phenosaline) and were excluded; none reacted to the mite culture medium.

Results

Sixty (87%) of the 69 patients completing the tests had an immediate bronchial sensitivity to *D. pteronyssinus*. None of the 69 children reacted to the control solutions. 9 had no bronchial response to *D. pteronyssinus*, but 5 had positive responses to other allergens (1 to cat fur, 2 to Timothy grass pollen, 1 to *Alternaria* mould, and 1 to *Cladosporium* mould). 4 of the negative *D. pteronyssinus* BPT patients had positive prick skin tests to the standard 1.2% skin testing solution and only 3 of these reacted to the 0.1% dilution. Only 5 positive BPT patients had negative skin responses to the 0.1% dilution (Table I). The correlation between skin test and BPT result was 94%.

Many children had a late skin response to 1.2% *D. pteronyssinus* solution and occasionally to 0.1% but not to the more dilute solutions, with induration and erythema developing at the site of the immediate response 3 to 4 hours after it had disappeared. 24 of 39 (62%) patients had late skin reactions and all these had positive BPTs to *D. pteronyssinus*. 36 (73%) of the 49 children with positive BPTs to *D. pteronyssinus* also had late bronchial reactions beginning 4 to 8 hours after inhalation challenge and lasting for at least 12 hours.

TABLE I

D. pteronyssinus prick skin test threshold in bronchial provocation positive and negative children

	Skin test threshold dilution giving a positive reaction (%)				Skin test negative at all dilutions
	0.001	0.01	0.1	1.2	
Bronchial provocation positive	7	31	17	5	0
Bronchial provocation negative	0	0	3	1	5

In addition, 4 of 9 patients who continued inhaled steroids on the day they were tested had late reactions. There was a significant correlation ($P < 0.005$) of patients having both a late skin reaction and late bronchial reactions excluding the steroid-treated patients (Table II). The mean fall in PEF

TABLE II

Late skin reactions and late reactions after bronchial challenge

	Late skin reaction	No late skin reaction
Late reaction on bronchial challenge	22	2
No late reaction on bronchial challenge	4	11

Fisher's exact test $P < 0.005$.

in the immediate reactions was 39% (range 20–80%) from baseline, but all falls in PEF in the late reactions were greater than 50%.

The late reaction was characteristically more prolonged and more severe than the immediate one (Fig.). 41 of the immediate reactions resolved spontaneously, causing no distress to the patient nor necessitating bronchodilator therapy, whereas all late reactions required some treatment. Where bronchodilators were necessary during an immediate reaction, complete correlation of the PEF always ensued. Though it tended to be less effective in controlling late reactions (Fig.), nebulized salbutamol was always sufficient and prevented distress in the patients.

Severity of asthma did not correlate with the degree of bronchial sensitivity as judged by the magnitude of the immediate reaction or the concentration of allergen necessary to produce a positive response. However, the presence of a late reaction on BPT was significantly associated with frequent attacks of asthma ($P < 0.001$) (Table III). There

D. pteronyssinus
bronchial challenge

FIG.—Change in various respiratory function parameters in one patient with an immediate and late reaction to *D. pteronyssinus* bronchial challenge.

was no significant difference in the antiasthma therapies that the two groups were receiving (Table IV).

With regard to pulmonary function changes, late reactions were more severe and showed large and early rises in TGV and RV:TLC (Fig.), whereas in the immediate reactions PEF, FEV_{0.75}, and SGaw fell rapidly, with variable and often negligible changes in TGV and RV:TLC. The percentage change in each respiratory function parameter at 20 minutes (during an immediate reaction) and at 5 hours (during a late reaction) after inhalation was calculated in 19 patients and the result of the immediate reaction subtracted from the late to

TABLE III

Frequency of attacks of asthma in the year before bronchial challenge in patients with and without late reactions

	<1 per month	1 per month	2–4 per month	> 1 per week
Late reaction on bronchial challenge	0	6	17	13
No late reaction on bronchial challenge	6	4	3	0

2 × 2 Fisher's exact test $P < 0.01$.

TABLE IV

Therapy taken by patients, with and without late reactions, during the one year before bronchial challenge

	Inhaled steroids	Disodium cromoglycate	Regular bronchodilators	Bronchodilators as required
Late reaction on bronchial challenge	3	24	6	3
No late reaction on bronchial challenge	1	7	1	4

indicate which response produced the greater change. 20 minutes and 5 hours were chosen because the fall in PEF was then roughly similar in most patients. TGV, FEV_{0.75}, and the RV:TLC ratio changed proportionally more than PEF and TGV proportionally more than SGaw during the late reaction using the paired t-test (Table V).

Discussion

BPTs, using antigen extracts, have been used in the study of bronchial asthma for many years. They provide the only method of making a precise allergy diagnosis (Aas, 1975; Bronsky and Ellis, 1969; Colldahl, 1952; Hill, 1974; Spector and Farr, 1974), and can be used to assess the efficacy of

TABLE V

Difference between various respiratory function parameters 20 minutes and 5 hours after bronchial challenge (expressed as a percentage)

Case no.	PEF	FEV _{0.75}	FEV _{0.75} :FVC	TGV	RV:TLC	SGaw
1	+12	+11	+5	+21	+10	+6
2	-20	-14	+2	-19	-4	-7
3	-14	-2	+9	-8	+1	-5
4	-3	-8	-5	+18	-2	-3
5	+10	+4	-8	+18	+4	+11
6	-5	0	0	0	-4	-1
7	+12	+8	+4	+25	+8	
8	+16	+11	-4	+13	+13	+1
9	+6	+11	+3	+13	+2	+12
10	-9	0	-1	+23	+18	+11
11	-8	-7	+4	-9	+2	+6
12	-24	-9	-3	0	0	-22
13	+2	+9	+1	-8	+5	-17
14	-10	+7	-8	+7	+11	+9
15	+7	+3	+5	-2	+2	+1
16	-12	-15	-11	+17	-5	-7
17	+16	+31	-1	+4	+5	-7
18	-14	+4	+3	+20	+6	+17
19	-2	+13	+6	+13	+11	+7
Mean paired difference between change in PEF and change in test		+5.1	+2.2	+9.8	+6.5	+3.6
P value for paired t-test (no. of pairs)		<0.02 (19)	NS (19)	<0.01 (19)	0.025 (19)	NS (18)
Mean paired difference between change in SGaw and change in test	-3.6	+2.1	-0.8	+6.1	+3.5	
P value for paired t-test (no. of pairs)	NS (18)	NS (18)	NS (18)	<0.05 (18)	NS (18)	

Note: Positive values occur when change was greater at 5 hours (in late reactions) than at 20 minutes (in immediate reactions). NS, not significant, P > 0.05.

various antiasthma drugs (Altounyan, 1964) and of hyposensitization (Aas, 1971; McAllen, 1961). They have even been used as a means of administering antigen for hyposensitization programmes (Herxheimer, 1951; McAllen, 1961). While most of the above studies have been on small groups of patients in experimental situations, Aas (1970) has experience of this procedure in routine diagnostic work on over 1000 children, involving nearly 10 000 inhalation tests.

None of the above authors has encountered serious reactions to the procedure, and concluded that the test is safe in experienced hands. In the present study no major reactions occurred but all the patients having late reactions became symptomatic. Bronchodilator therapy has so far been effective in controlling these reactions, but perhaps without prompt treatment status asthmaticus would develop. Therefore careful observation is necessary for at least 24 hours after a BPT.

The specificity of the *D. pteronyssinus* reaction has been confirmed by the lack of reaction to control solutions (including mite culture medium) and the lack of response of some asthmatics who reacted to other allergens. Specificity of reactions has been examined in detail by other authors with similar conclusions (Aas, 1970; Hill, 1974; Townley, Dennis and Itkin, 1965).

The present study has not attempted to show the reproducibility of BPTs or excluded the possibility of hypersensitization. However, Bronsky and Ellis (1969) showed no change in BPT threshold in 2 patients having 6 challenges over a 2-month period, and in 22 patients tested on two occasions with *D. pteronyssinus* no significant differences in threshold were noted by Bryant, Burns and Lazarus, (1975).

Correlation with skin tests. Correlations between clinical histories of allergen exposure, reactions on allergen skin testing and on BPTs have varied widely, depending on the allergen, the techniques used, and the workers involved. However, generally the correlations are poor (Aas, 1970; Spector and Farr, 1974).

Despite the fact that all the patients selected had perennial asthma occurring particularly nocturnally, suggesting a house mite allergy, only 87% had a positive BPT to *D. pteronyssinus*. Allergy to the house mite is undoubtedly common in bronchial asthma (Hendrick *et al.*, 1975) and the percentage with positive reactions would necessarily be high even in randomly selected asthmatics. Thus clinical histories are not particularly reliable for identifying house mite-sensitive asthmatics.

The correlation between positive BPT and positive skin test to *D. pteronyssinus* (1.2%) was 94%. The 3 BPT-negative patients who had positive skin reactions to the 0.1% dilution had wheal sizes on these reactions of less than 3 mm diameter. Taking 3 mm as the minimum wheal size for skin test positivity and using *D. pteronyssinus*, 0.1% will give a 100% correlation with positive BPTs. Furthermore, all those patients with late skin reactions had positive BPTs. Thus the combination of clinical history with skin testing to *D. pteronyssinus* 1.2% and 0.1% and observation for late skin reactions provides a reliable indication of a bronchial sensitivity to that allergen.

Late skin reactions were mostly observed with the 1.2% dilution and rarely with 0.1%, suggesting that the larger dose of allergen, and perhaps the more sensitive the patient to that allergen, the more likely a late reaction will occur. Similarly, in ragweed pollen skin tests late reactions could be elicited in virtually all sensitive subjects when a large concentration of allergen was used (Robertson *et al.*, 1974). Of Robertson's 15 cases, 9 had late reactions on BPT with ragweed pollen and these occurred in the more sensitive subjects. The late reactions on skin tests correlated well with late reactions to *D. pteronyssinus* inhalation in the present study. These reactions are frequently attributed to the Gell and Coombs (1968) type III Arthus reaction (Pepys, 1973), but in this study they were always preceded by an immediate reaction. Furthermore, no fever, leucocytosis, crepitations, or pulmonary infiltrates have been shown during late reactions to house dust extracts (Booij-Noord *et al.*, 1972). Thus it is possible that the late reaction is merely an IgE-mediated Gell and Coombs (1968) type I hypersensitivity phenomenon which is dose related, requiring the release of large quantities of mediator amines which have a delayed effect.

Late reactions to the BPT. Late reactions have been observed with increasing frequency on BPTs (Herxheimer, 1952; Van Lookeren Campagne, Knol, and de Vries, 1969; Booij-Noord *et al.*, 1972), particularly with *Aspergillus fumigatus* (Pepys, 1973) and house dust allergy. Van Lookeren Campagne *et al.* (1969) had a 54%, and Booij-Noord *et al.* a 47% incidence of late reactions in house dust-sensitive asthmatics. In this study the incidence was even higher at 73%, perhaps due to the difference of allergen and method of administration. The occurrence of late reactions correlated very well with the presence of frequent asthma attacks. In order for BPTs to have any meaning in relation to clinical manifestations of the disease, such an

association must be present. This is the first time that a correlation between bronchial sensitivity at BPT and clinical aspects of asthma has been made.

The respiratory function changes that occur during an immediate reaction after BPT are unlike those which normally occur during severe exacerbations of asthma. In this study these reactions were of short duration. Almost all resolved spontaneously; the few that did not resolved completely after bronchodilator administration. The most consistent respiratory function changes were in PEF, $FEV_{0.75}$, and SGaw. The major component contributing to changes in these parameters is large airways resistance (Macklem and Mead, 1967). When overinflation did occur during the reaction it resolved as completely and rapidly as the other measurements, spontaneously or with bronchodilators. Therefore one must assume that the immediate reaction is predominantly due to bronchospasm.

The pattern of abnormality in exacerbations of asthma is very variable, but in its more severe forms is similar to that found in late reactions after BPT. Status asthmaticus almost by definition is relatively bronchodilator unresponsive, and Palmer and Diamant (1969) showed that airways obstruction was not reduced significantly by bronchodilators in their group of patients with severe exacerbations of asthma. Gross hyperinflation in severe asthma which often persists long after measurements of large airways obstruction have returned to normal has been noted (Engström, 1964; Palmer and Diamant, 1969; Weng and Levison, 1969; Woolcock, and Read, 1966). In this study late reaction was characterized by poor responsiveness to bronchodilators, by a duration of several hours, and by significantly greater changes in measurements of overinflation (TGV and RV: TLC) for the same degree of airways resistance (measured by PEF and SGaw) than in immediate reactions. This suggests that there is a greater involvement of small airways, perhaps due to mucosal oedema and viscous secretions resulting in early airways closure during expiration and thus gas trapping. The increased small airways obstruction would not necessarily be shown by changes in PEF or SGaw but would result in overinflation. Mansell *et al.* (1974) and Olive and Hyatt (1972) described two varieties of change of lung mechanics in antigen-induced asthma which could have been due to the two different reactions, immediate and late, as observed in this study.

The conditions at BPT are very dissimilar to those which occur during natural allergen exposure,

with soluble rather than particulate allergen and the duration of exposure to the allergen being limited rather than prolonged or repeated. Despite this, a significant correlation between severe asthma and the occurrence of late reactions on BPT has been shown. The similarity of lung function changes in late reactions and severe naturally occurring asthma has also been described. The chemopathological mechanisms occurring in severe asthma or status asthmaticus may well be similar to those occurring in late reactions. They therefore form the ideal clinical model, under controlled conditions, for studying the pathophysiology of asthma and its response to different therapies.

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