

From the Allergy Clinic, the Wright-Fleming Institute and Pharmacology Department, St. Mary's Hospital Medical School, London W. 2, Great Britain.

SKIN TESTS IN THE INVESTIGATION OF HOUSE DUST ALLERGY

By

L. M. McEWEN

INTRODUCTION

The existence of the house dust allergen has been denied by *Freeman* (10) and *Bruce* (3), who felt that extracts of carpet dust were merely irritant, while other workers, such as *Rowe* (15) *Walzer* (20) and *Davidson* (7), believed that house dust allergy is due to the many specific allergens which are found in the domestic environment.

Evidence has been put forward that the house dust allergen is the product of a Maillard reaction occurring in vegetable matter (*Berrens et al.* (1)), and that it is formed in sterile cotton aged in sealed containers (*Cohen et al.* (5), *Cazort* (4)), while *Wagner & Rackemann* (19) could obtain house dust allergen regularly by the action of moulds on cotton or kapok. Recently, *Voorhorst et al.* (18) have proposed the mite *Dermatophagoides pteronyssinus* as the source of an allergen which behaves like the one found in house dust.

The chemical purification of the house dust allergen has been attempted by a number of workers, but there is disagreement even on the colour of the purified substance (*Leitze & Reed* (11), *Elias et al.* (9), *Versie et al.* (17)).

A large part of this conflicting work is based on the results of skin testing with aqueous extracts of suspected sources of the house dust allergen, or with chemically purified substances which might be the house dust allergen. It seems likely that such widely differing conclusions could not have been reached

TABLE 1
Dusts used for Making Extracts.

Type of Dust	Source	Description
Old Flock	Mattress and upholstery	Mixed rags. Minimum wool about 50 per cent. (Subject to British Standard Specification)
Old Feathers	Pillows	Domestic fowl feathers
Old Hair	Mattress	Hog, Cow and Horsehair
Carpet	Carpet beating machine	Mixed dust from many London carpets
Old Cotton (3 samples)	Cushions	Raw Cotton
Old Kapok (2 samples)	Cushions	Kapok
Old Coconut	Gymnasium matting	Coconut fibre
Old Fibre (2 samples)	Upholstery	Asparto grass fibre
Hair Clippings	Human (LMM)	Dry shaver clippings
New Flock	Bale	As above, fresh from factory
New Feathers	Freshly plucked	Domestic fowl feathers

unless the skin testing method, or its interpretation, were subject to important sources of error which have not been recognised.

The present work was undertaken with two objects. Firstly, to try to decide if a characteristic allergen is present in house dust, and if it is, from what substrate it is formed, and secondly, to try to eliminate the errors in skin testing which have misled workers in the past.

METHOD

Dust samples were obtained as described in Table 1. Aged materials were as free as possible from extraneous contamination.

MEAN WHEEL AREAS PRODUCED BY PRICK TESTING
ON 40 FLOCK SENSITIVE SUBJECTS

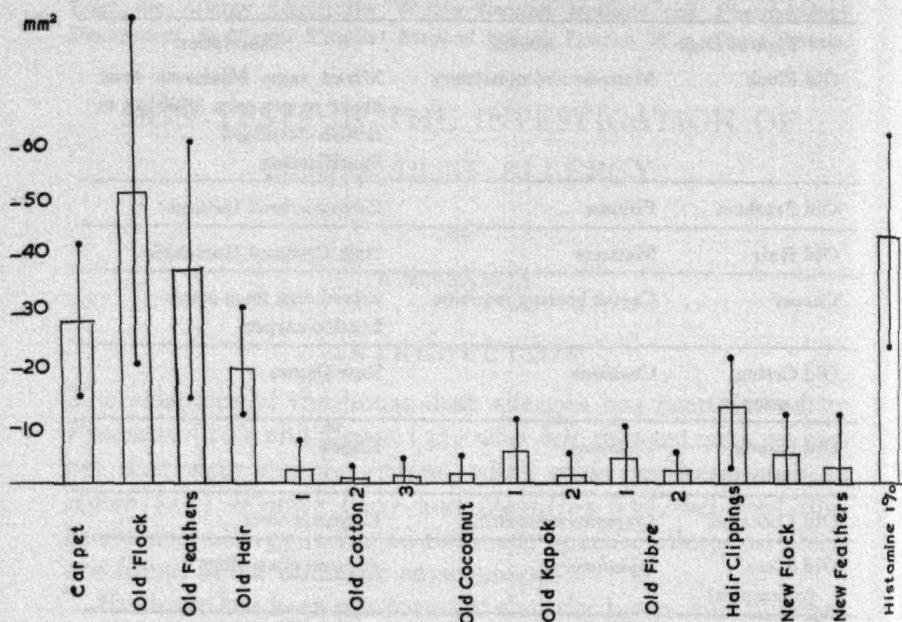


Fig. 1.

The samples of cotton and kapok had been almost entirely reduced to dust by age, but dust was extracted from most of the other materials using a specially cleaned carding machine. Old Feather dust was extracted by agitating the contents of pillows in front of a clean new vacuum cleaner. The old gymnasium mat was beaten over a clean polished floor to obtain coconut fibre dust.

Extracts were made by soaking 10 gm of dust for 7 days at 4° C in 100 ml of fluid of the following composition: Per litre of distilled water: NaCl 5 gm, Na HCO₃ 2.5 gm, NaH₂ PO₄ 0.36 gm, Na₂HPO₄ 1.43 gm, Phenol 4 gm, Propyleneglycol 20 ml, Sodium thioglycollate 0.9 gm, "NIPA mixture" 1.0 gm (Methylhydroxy benzoate 2 parts, Propylhydroxy benzoate 1 part).

In this fluid allergens will not support bacterial growth even when exposed on the bench for long periods, although they should be sterilised by Seitz filtration if intended for injection.

Forty subjects for skin testing were selected because they were known to give wheal and flare skin responses of the immediate type to prick tests with Old Flock dust extract.

Prick tests were made on the arms using needle points inserted into "poly-

there" rods, so that all pricks were of identical depth (*Brown* (2)). The characteristics of the mounted needles were checked using 1 per cent histamine on a forearm, and those used in the trial all made reproducible wheals of comparable size.

Results were recorded by the method of *Macaulay* (12): Wheals were outlined on the skin with a ball-point pen. Transparent adhesive tape was then applied, so that the ink printed on to the adhesive surface. Subsequently the tape could be stuck to paper, giving a permanent record. Wheal areas were determined by using the best fit of standard circles.

RESULTS

Mean wheal areas produced by the various dust extracts, and their standard deviations, are shown in Fig. 1.

All the materials which contained old animal keratin gave rise to dust extracts which produced large skin wheals, while materials of purely vegetable origin did not do so. Sources of keratin dust which had not aged gave extracts with only slight activity. Human shavings also appeared to have some allergic activity, but the mean wheal size was not large enough to justify further conclusions.

TABLE 2
Correlation Coefficient Between Wheals Produced by Extracts of Dust which Contained Old Keratin.

	Feather	Hair	Carpet	Histamine
Flock	0.64	0.40	0.21	0.62
Carpet	0.23	0.59	—	0.16

No. of subjects = 40.

Values of 0.45 or more are significant at the 1 per cent level.

Table 2 shows the coefficients of correlation between the responses to the more active extracts, determined by the formula:

$$r = \frac{\sum xy - \frac{\sum x \sum y}{n}}{\sqrt{\left(\sum x^2 - \frac{(\sum x)^2}{n}\right) \left(\sum y^2 - \frac{(\sum y)^2}{n}\right)}}$$

where x represents the individual wheal areas due to one allergen extract and y the corresponding areas due to another. n is the number of cases.

$$\text{The standard error of } r = \frac{1}{n-1}$$

Values of r are significant at the 5 per cent and 1 per cent levels if they are respectively twice or three times the standard error.

It can be seen that the responses to dust extracts from old flock, feathers and hair are significantly correlated, but the responses to carpet dust extract do not significantly correlate except with those to old hair dust. There was a highly significant correlation between the prick responses to old flock extract and 1 per cent histamine, but this did not apply to the responses produced by carpet dust extract.

DISCUSSION

The first object of the present work was to establish if there is a characteristic allergen in the house, and from what substrate it arises. The results are consistent with the hypothesis that a characteristic allergen develops in old keratin in the domestic environment, whatever the original source of the keratin. Sensitivity to this old keratin allergen seems likely to be the predominant feature of house dust allergy.

The failure of the wheals produced by carpet dust extract to correlate with the responses to extracts of old flock and feather suggests that the former behaves as a weak source of the old keratin allergen mixed with the many other allergens which it is known to contain; human and animal danders, insect debris, food particles, mould spores, and bacteria. The old hair extract occupies an intermediate position, and responses to it correlate significantly with the series of wheals produced by all the old keratin dust extracts. It seems possible that the close relationship between old hair dust and carpet

dust may be due to the animal danders which they both contain.

The second object of the experiment was to investigate potential sources of error in the use of skin tests for the comparison of the identities of allergens. It is clear that if extracts of potential allergens are compared with a "reference" extract on the basis of mean wheal size alone, then the conclusions may be totally misleading, since series of wheals which do not correlate may have equally large mean values.

It should also be pointed out that initial inspection of a series of numerical values for the wheal areas, and of lists of plus signs or histograms to express the results of each case, did not suggest to the author that there would be significant differences between the correlation coefficients of the various series of results. All previous work on the identities of the dust allergens is based on these unsatisfactory methods, and it is easy to understand how the present confused literature has arisen.

The coefficient of correlation gives a numerical value, with a known significance level, to the similarity between the responses to different allergens, and it is clearly essential to use this coefficient in all future comparative work of this kind. The "t" test, which compares mean wheal sizes and their standard deviations, is of doubtful value, although it was used in a recent paper to compare the effects of dust extracts from different countries (*Milner et al.* (13)).

It should be noted that so long as the series of skin wheals are large enough to be within the range of approximate proportionality to the histamine release which produced them (*Squire* (16)), it is unnecessary for the allergens to produce similar mean wheal sizes.

The present work illustrates another pitfall which was not taken into account in the experimental design, hence the results presented are not ideal, but they serve as an excellent example of a major source of error. The subjects used for skin tests were selected because they were known to give a positive skin response to flock extract, and it was found that

there is a highly significant correlation between the effect of this extract and that of histamine. Hence one must rely on an additional series of tests, using subjects not known to be allergic to house dust, for evidence that the extracts were not acting in a non-specific way.

It is natural to choose non-allergic subjects for this purpose, but as *Musso and his co-workers* (14) have shown, an alcoholic extract of dust will produce skin responses in any patient who has an allergic disease, so it is clearly not enough to demonstrate that the extract is non-irritant; it is also necessary to exclude a quasi-allergic response which might occur in all allergic subjects, but not in normal people. Hence for future work it is essential to select a panel of subjects with a variety of "reference" allergies, so that the effects of the allergens which are being studied do not correlate closely with the effect of histamine. Then any substance which has been modified, so that its effect is non-specific, will behave more like histamine than the allergens with which it is being compared.

The results of the present experiment must therefore be defended: none of the extracts used elicited skin wheals in 10 normal subjects, and the extraction procedure involved the minimum of interference with the allergens, so that non-specific properties were unlikely to have been introduced. Clinical experience with the old flock dust extract over 4 years has shown that unexpected skin wheals to it are encountered with about the same frequency as skin wheals to grass pollen extract when there is no supporting history of hay fever.

The ideal criteria have been illustrated by the inclusion of carpet dust in the series of allergens tested. The extract of this dust did not behave like the old flock dust extract, but neither was it similar to histamine. Hence the results contain intrinsic evidence that carpet dust probably behaves as a source of allergen not closely related to old keratin dust.

The close correlation of the wheals produced by old flock dust extract and histamine, in spite of the extremely large standard deviations of the mean wheal size, suggests that if a series of subjects are selected because they are known to give

a positive skin response to a certain allergen, then the variation in the size of the skin wheals from one subject to another will reflect the size of the wheals elicited by histamine in the same individuals. In this situation, the size of the skin responses is clearly not a good guide to the degree of "allergic sensitivity" of the subjects.

The variability of the skin responses confirms the view of *Efron et al.* (8) that little value can be attached to precise measurement of small numbers of skin wheals. Since the present results have been shown to be capable of close correlation, it is felt that it was correct to discard more accurate methods of determining wheal area, and to rely on the statistical treatment of the results from 40 subjects to reduce the importance of random errors of technique and measurement.

The use of special needles (*Brown* (2)) reduced an important source of error in the form of inconsistency of the prick testing technique. This method gives more consistent results than intradermal testing in the hands of the author, although individual skill is very important for the latter technique.

The difference in potency between the extracts used in the present work and those used by most other authors is striking. Intradermal tests have generally been used in the past, and these deliver between 300 and 1,000 times the volume of fluid which is introduced by a prick test (8, 16). It would seem that this was usually necessary because the samples of dust which were employed would not give extracts potent enough for prick testing. In some cases, chemical purification of the allergen has been undertaken in order to provide an extract which was strong enough even for intradermal tests.

The cotton dust extract used in the present experiments elicited skin wheals on intradermal injection, but the old keratin dust extracts would have caused anaphylaxis unless diluted at least 100 fold. Cooke's original dust extracts were of similar potency (6), and since European homes, and those in most temperate regions, contain plenty of old animal keratin, it seems hard to understand why workers should have

attached significance to sources of dust which had very much weaker effects.

SUMMARY

1. Prick tests have been carried out with a variety of dust extracts on 40 patients who were known to give wheal and flare skin responses to old flock dust extract.

2. Extracts of dusts derived from old animal keratin elicited skin wheals with mean sizes from 20 to 50 mm². Extracts of dusts from vegetable sources had little activity.

3. The results of skin tests with old flock, old feather and old hair dust extracts are significantly correlated. It is suggested that sensitivity to an allergen derived from old animal keratin is characteristic of house dust allergy.

4. There was no significant correlation between the responses to carpet dust extract and the responses to old flock and feather dust extracts. It seems possible that carpet dust behaves as a weak source of old keratin allergen mixed with many other specific allergens.

5. The experimental design to be adopted when comparing allergen extracts by skin testing is discussed.

6. The coefficient of correlation between two series of skin responses due to different allergens provides an indication of the similarity between the allergens. Other methods of analysis of the results, which have been used in the past, are inadequate.

ACKNOWLEDGEMENT

I wish to thank Dr. G. A. Rose for his guidance on the statistical treatment of these results

Most of the samples of dust were provided by Nu-Life Upholstery Ltd. and I am deeply indebted to all concerned for their painstaking help and advice.

The vacuum cleaner was kindly loaned by Electrolux Ltd.

REFERENCES

1. *Berrens, L., Morris, J. H. & Versie, R.*: The Complexity of House Dust, with Special Reference to the Presence of Human Dandruff Allergen. *Int. Arch. Allergy* 27, 129-144.
2. *Brown, H. M.*: Personal Communication, 1962.

3. *Bruce, R. A.*: Bronchial and Skin Sensitivity in Asthma. *Int. Arch. Allergy* 22, 294-305, 1963.
4. *Cazort, A. G.*: The House Dust Antigen in Allergy. *South M. J.* 29, 1022-1026, 1936.
5. *Cohen, M. B., Nelson, T. & Reinarz, B. H.*: Observations on the Nature of the House Dust Allergen. *J. Allergy* 6, 517-524, 1935.
6. *Cooke, R. A.*: Studies in Specific Hypersensitiveness 4. New Etiologic Factors in Bronchial Asthma. *J. Immunol.* 7, 147-162, 1922.
7. *Davidson, M. T.*: The Source of the Allergic Activity of House Dust. *J. Allergy* 14, 244-255, 1942.
8. *Efron, B. G., Boatner, C. H. & Pabst, M. R.*: Studies with Antigen 7. The Significance of Reactions to Intracutaneous Tests Performed with Extracts of Purified House Dust. *J. Invest. Dermatol.* 4, 95-101, 1941.
9. *Elias, R. W., Voorhorst R. & Van Krieken, H.*: On the Purification of House Dust Allergen. *Acta Allergol.* 19, 369-386, 1964.
10. *Freeman, J.*: Hay Fever, a key to the Allergic Disorders. William Heinmann, London 1950.
11. *Lietze, A. & Reed, C. E.*: Studies on House Dust Allergens: The Separation of Closely Related Allergens of Different Specificity and their Relationship to Allergenically Inactive Antigens. *Int. Arch. Allergy* 20, 344-354, 1962.
12. *Macaulay, D. B.*: Personal Communication, 1962.
13. *Milner, F. H., Tees, E. C., Dybas, B. & Dean, P. M.*: Preliminary Investigation into the Evaluation of the Extracts of House Dusts Obtained from Different Geographical Locations. *Acta Allergol.* 20, 379-386, 1965.
14. *Musso, E., Brun, R. & Hunziker, N.*: Un Allergene pour Deceler L'Atopie. *Acta Allergol.* 13, 174-179, 1959.
15. *Rowe, A. H.*: House Dust in the Etiology of Bronchial Asthma and of Hay Fever. *Arch. Int. Med.* 39, 498-507, 1927.
16. *Squire, J. R.*: The Relationship Between Horse Dandruff and Horse Serum Antigens in Asthma. *Clin. Sci.* 9, 127-150, 1950.
17. *Versie, R., Monard, Y. & Geubelle, F.*: Comparative Study of Various Extraction and Purification Procedures used for the Preparation of House Dust Allergen. *Int. Arch. Allergy* 30, 368-381, 1966.
18. *Voorhorst, R., Spijksma-Boezeman, M. I. A. & Spijksma, F. Th. M.*: Is a mite (*Dermatophagoides* sp.) the producer of the house-dust allergen? *Allergie und Asthma* 10, 329-334, 1964.
19. *Wagner, H. C. & Rackemann, F. M.*: Kapok and Moulds: An Important Combination. *Ann. Int. Med.* 11, 505-513, 1937.
20. *Walzer, M.*: A Critical Review of the Recent Literature on the Dust Atopen and on Vitamin C in Relation to Hypersensitiveness. *J. Allergy* 10, 72-94, 1938.

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.