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COMPARISON OF SKIN REACTIONS TO EXTRACTS OF HOUSE DUST, MITES, AND HUMAN SKIN SCALES

By

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INTRODUCTION

During the last four years many reports have been published on the significance of mites for allergic diseases, especially on the role of the "house-dust mite" *Dermatophagoides pteronyssinus*, in relation to house-dust atopy (1, 6-13, 15-17, 20, 23, 25, 26, 28, 29). It has been shown in a number of these reports that there is a relation between the numbers of mites occurring in house dust (5-7, 9, 14, 19) and the allergen content of house dust (12, 17, 20, 25, 26). Another, more direct proof of the causal relation between the occurrence of *D. pteronyssinus* and the allergenic potency of house dust is given by the comparison of skin reactions to extracts of house dust and this species of mite (1, 10, 12, 13, 16, 17, 23, 25, 26, 28, 29), and also by the comparison of the reactions in inhalation tests (1, 10, 13, 16).

Although there seems to be a growing acceptance of the idea that the "house-dust mite" *Dermatophagoides pteronyssinus* is responsible for the presence of a specific allergen in dust, no solution has yet been found for the problem of the extent to which other species of mites occurring less frequently in house dust contribute to the allergenic potency of house dust. This aspect has been studied in an investigation in which skin reactions to extracts of house dust and a number of species of mites were compared. The species used in this study

were *D. pteronyssinus*, *D. farinae*, *Glycyphagus destructor*, *Tyrophagus putrescentiae*, and *Acarus siro*. These are the species of mites occurring most frequently in house dust (5-7, 12-17, 19, 20). The only species missing from this list is *Euroglyphus maynei*, a species which is, however, difficult to rear in the laboratory. Experiments with this species are now in progress.

In the present study attention was also given to the suggestions that the allergen in human skin scales, which are present in house dust, and are used in the culture medium for *D. pteronyssinus* and *D. farinae*, affect the potency of house dust and of our mite cultures (2-4, 13, 16, 27).

MATERIALS AND METHODS

Cultivation of Mites

Cultures of *D. pteronyssinus* and *D. farinae* were reared on a medium consisting of human skin scales and powdered yeast, at 25° C and 80 per cent relative humidity of the air. Under these conditions these species show relatively rapid growth (17). *G. destructor* was cultured on whole meal flour, *T. putrescentiae* on powdered milk, and *A. siro* on wheat germ flakes; powdered yeast was added to all media. The cultures were kept at about 20° C and 75 per cent relative humidity, in flat-bottomed round glass containers ("crystallization cups").

Preparation and Testing of Extracts

The mites were killed by heating the culture for 6 hours at 60° C.

For intradermal testing of *D. pteronyssinus* and *D. farinae*, extracts prepared from the whole full-grown culture were used, since for these species it has been shown that much more allergen is present in the culture than in the mites themselves (17). The cultures were weighed and placed in a solution of 0.5 per cent phenol in saline solution (21) to give a 10 per cent suspension, which was extracted for 24 hours at room temperature and then filtered through sintered glass bacterial filters. For testing, the extracts were freshly diluted every week to the desired dilutions. The same procedure of extraction and dilution was followed for all the other extracts. The extracts of these mite cultures were tested in three tenfold dilutions of 0.001, 0.0001, and 0.00001 per cent (1:100,000, 1:1,000,000, and 1:10,000,000 by weight).

The other species of mites were removed from the culture medium by sieving and washing in acetone, and then extracted in suspensions of 1 or 0.1 per cent, depending on the amount of material available. The culture medium of *G. de-*

structor contained very small amounts or no allergen (18), which is in sharp contrast with the situation in the cultures of *D. pteronyssinus* (17). For this reason, extracts of *G. destructor*, and also of the two other stored-food mites, were prepared from pure mites. These extracts were tested in only one dilution of 0.001 per cent.

Extract of house dust was prepared from a pool of many house dust samples collected in Leiden and environment. After mixing the samples, the dust was suspended to give a 40 per cent suspension. The extract was then concentrated by evaporation under vacuum at 50° C to 100 per cent. This stock extract served as our "standard house-dust extract". The extract was tested in three tenfold dilutions of 1, 0.1, and 0.01 per cent.

Human skin scales were washed in acetone from hairs collected in hair-dressers' shops, and then extracted in a 10 per cent suspension. Tests were done in three tenfold dilutions of 1, 0.1, and 0.01 per cent.

About 0.1 ml of the diluted extracts was injected into the skin of the back. The reactions of the skin were noted after 20 minutes. The strengths are indicated by plus signs (21, 22).

The tested subjects included "new" patients, who visited the out-patient clinic of the Department of Allergology, University Hospital, Leiden, for the first time, with diverse complaints, and "selected" patients with pronounced skin reactions to house dust extract.

RESULTS

Introduction

Before discussing the results of the comparison of skin reactions after intradermal testing of several allergen extracts, we must consider some of the factors that influence the reliability of this method.

1. *Multiple sensitizations.* The aim of testing two or more extracts in a number of subjects is to determine the extent to which these extracts contain related or identical allergens. The closer the allergens are related, the greater the correlation will be in the frequencies and the strengths of the reactions. However, properties of the group of tested subjects also influence the correlation. Several allergological studies have demonstrated that patients who have become sensitized to one atopic allergen are very often atopic to other allergens as well (21). In most cases these additional sensitizations are of no clinical importance and cause very few or no complaints.

The more similar the intensity and frequency of exposure to the different allergens, the greater the chance that these atopic multiple sensitizations will be of equal strength. This multiple sensitization explains why it is to be expected that in a group of atopic subjects a certain degree of correlation will almost always be found between skin reactions to different allergens. These "false" correlations will be high if there is great similarity in the occurrence of the allergens. For instance, a high correlation due to multiple sensitization between the reactions to the allergens of grass pollen and the mite *Acarus siro* is very improbable. On the other hand, the correlation between the reactions to the allergens of house dust and human skin scales can be expected to be high, since their intensity and frequency of occurrence is rather similar.

2. *Errors and variations.* Unlike the above-mentioned effect of multiple sensitization, which falsely strengthens correlations or even causes false correlations, the errors and variations related to the method and the material will have a diminishing effect on existing correlations. The testing of allergen extracts on human skin is a bio-assay, and will therefore show considerable variation. Such variations can be caused by biological variability in the population of the tested subjects, but also, and perhaps to a greater extent, by variations in the skin of the back of the subject, *e.g.* the thickness of the skin, the presence or absence of nearby blood capillaries, deformation of the reaction in the direction of the costae, etc. Other sources of errors or variations are diurnal rythm in the reactivity of the skin, dermographism, use of antihistamines by the patient, etc. Furthermore, the technique of testing contributes to a number of inaccuracies, *e.g.* the amount of extract that is injected into the skin *de facto*, the depth to which the extract is injected into the skin, and the estimation of the reaction and its indication by plus signs. These are a number of the most important factors that can cause variations and errors in skin testing (21, 22).

To summarize, it can be stated that variations in the tested

subjects and inaccuracies in the technique will have a diminishing effect on the correlations between skin reactions to related or identical allergens. Multiple sensitization to various atopic allergens, however, will strengthen the correlations between reactions to different allergens, especially when there is similarity in the occurrence of the allergens.

3. *Equivalence of test fluids.* For a correct comparison of the skin reactions to several allergen extracts the dilutions used must be identical in their allergen content (21, 22). This is achieved by comparing the strengths of the largest skin reactions to the various allergen extracts in a group of tested subjects, which must be done before the final skin-test series is performed.

In this method of making the test fluids equally strong, it is assumed that persons with the largest skin reactions have the maximal reagin production against the corresponding allergen. And it is also assumed that the levels of the maximal reagin production in different persons against the different corresponding allergens is about equal.

On the basis of these assumptions, the amount of allergen in the test fluid can be made equal by comparing the skin reactions to a given allergen, of persons whose skin cells are maximally saturated with reagins, *i.e.* of persons with the largest skin reactions, with the skin reactions of persons with the largest skin reactions to another allergen.

The chance of finding a high number of these persons with maximal reagin production is small in the case of a rare allergen, for example in the case of the allergens of stored-food mites. Therefore, the test series carried out to make the test fluids equally strong must not be too small.

Once the dilutions of the extracts giving roughly equally strong skin reactions in the persons with maximal reagin production, are established, a check can be made by comparing the skin reactions of these persons in the final test series, as shown below.

In our opinion, this essential comparison of skin reactions

to equivalent test fluids is too often overlooked. Although other authors often come to the same conclusions as we do in this and earlier reports, some are comparing reactions to unequivalent extract dilutions. For instance, one of these authors (16) gives as the average wheal diameters of reactions to extracts of house dust, *D. culinae*, *A. siro*, and *G. domesticus* respectively, 1.2, 5.8, 2.8, and 1.6 mm in 27 persons. These differences in average diameters make it almost certain that the largest reactions in this group of tested subjects also showed considerable differences. The results of that study would have been better if a more dilute extract of *D. culinae* had been used.

We realize very well that, especially for diagnostic purposes, it must never be the aim of the investigator to perform as many, or as strong, or as equal skin reactions as possible, certainly not in individual patients. But in studying relations between various allergen extracts, the equivalence of the test fluids, which must be established before the final test series is done, is a *conditio sine qua non*.

Qualitative Distributions of Skin Reactions of "new" Patients

During the period October 1967–March 1968, 336 patients who visited the out-patient clinic for the first time, were intradermally tested with extracts of house dust (1, 0.1, 0.01 per cent); culture of *Dermatophagoides pteronyssinus* (0.001, 0.0001, 0.00001 per cent); pure *Glycyphagus destructor* (0.001 per cent); *Tyrophagus putrescentiae* (0.001 per cent); *Acarus siro* (0.001 per cent); and human skin scales (1, 0.1, 0.01 per cent). Of the three extracts, which were tested in three dilutions, the reactions to the middle dilution were used for this examination, to permit their comparison with the reactions to the extracts of the stored-food mites, which could only be tested in one dilution.

Of the 336 tested patients, 200 showed a skin reaction (at least one +) to at least one of the 6 extracts. These 200 patients are considered atopic; 121 had a reaction to house-dust

extract (average reaction of 5 largest: 3.0; average reaction of all 121: 1.6); 123 had a reaction to *D. pteronyssinus*-culture extract (3.0; 1.8); 83 to *G. destructor* (3.2; 1.6); 68 to *T. putrescentiae* (3.0; 1.6); 68 to *A. siro* (3.0; 1.5); and 184 to human skin-scale extract (3.0; 1.7).

The average skin reactions of the 5 patients with largest reactions to the 6 allergen extracts provide the confirmation that equivalent dilutions of the extracts were used.

The most important pairs of extracts are now considered with respect to the distribution of the skin reactions over the 200 patients.

1. House dust; *D. pteronyssinus* culture; human skin scales (Table 1). First we shall examine a group of three pairs, viz. skin reactions to extracts of house dust, *D. pteronyssinus* culture, and human skin scales. We have distributed the 200 atopic patients according to the qualitative equalness and unequalness of their reactions. In other words, how many patients have equal reactions, either + (or more), or — (or $\pm$) to the two extracts under examination, and how many have unequal skin reactions, + to the one extract and — to the other, and vice versa? These distributions are shown in Table 1.

In part *a* of Table 1 the distribution of the patients is given according to their skin reactions to extracts of house dust and *D. pteronyssinus* culture. The average skin reactions are also given in the tables. The 28 patients with unequal skin reactions are symmetrically distributed (13 to 15) over the two possible combinations. This suggests that this relatively small number of cases with unequal reactions is due to the variations in the tested patients and in the method as pointed out above. Obviously, these variations have their greatest influence on the distribution of the cases with a weak atopy: i.e. with small skin reactions. The skin reactions of these patients will lie closely around the one + limit, which separates the cases with "positive" reactions from those with "negative" reactions. The average "positive" reactions (one + or more) to one of the two extracts of the 28 unequal cases are indeed smaller

TABLE 1

Distribution of 200 Atopic "New" Patients According to Their Skin Reactions to Extracts of House Dust (0.1 Per Cent), *D. pteronyssinus* culture (0.0001 Per Cent), and Human Skin Scales (0.1 Per Cent).

Equal			Unequal					
	Average skin reactions	Number and percentage of patients		Average skin reactions	Number and percentage of patients			
a	House dust	+	1.7	108 (54)	House dust	+	1.2	13 (6.5)
	<i>D. pter.</i> cult.	+	1.9		<i>D. pter.</i> cult.	—	0.1	
	House dust	—	0.0	64 (32)	House dust	—	0.3	15 (7.5)
	<i>D. pter.</i> cult.	—	0.0	172 (86)	<i>D. pter.</i> cult.	+	1.2	28 (14)
b	House dust	+	1.7	117 (58.5)	House dust	+	1.0	4 (2)
	Human skin sc.	+	1.9		Human skin sc.	—	0.5	
	House dust	—	0.2	12 (6)	House dust	—	0.1	67 (33.5)
	Human skin sc.	—	0.1	129 (64.5)	Human skin sc.	+	1.4	71 (35.5)
c	<i>D. pter.</i> cult.	+	1.8	121 (60.5)	<i>D. pter.</i> cult.	+	1.5	2 (1)
	Human skin sc.	+	1.9		Human skin sc.	—	0.5	
	<i>D. pter.</i> cult.	—	0.2	14 (7)	<i>D. pter.</i> cult.	—	0.0	63 (31.5)
	Human skin sc.	—	0.1	135 (67.5)	Human skin sc.	+	1.5	65 (32.5)

(1.2) than those of the 108 equals with skin reactions to both extracts (1.7 and 1.9). And the "negative" reactions ($\pm$ or —) of the 28 unequals are larger (0.1 and 0.3) than those of the 64 equals without reactions to both extracts (0.0).

It is evident that on the basis of this qualitative distribution, skin reactions to extracts of house dust and culture of the "house dust mite" *D. pteronyssinus* occur in the same frequency and in almost the same patients. This distribution provides a strong argument in favour of the idea that the specific house-dust allergen originates from this species of mite.

If we make the same distribution of the 200 atopic patients according to their skin reactions to extracts of house dust and of human skin scales, which some authors believe to be a constant and essential constituent for the allergenic potency of house dust, we get the results shown in part *b* of Table 1. Here, the 71 unequal cases form a much larger number than the 28 in Table 1*a*. It is also very clear that the distribution of these 71 unequals is strongly asymmetrical (4 to 67; $p < 0.01$). The variations in the patients and in the method will have the same influence on the distribution here as on that of Table 1*a*, and will be largest again in the cases with small reactions. However, although in the 67 unequals without skin reactions to house-dust extract, the reactions to human skin-scale extract are smaller (1.4) than those in the 117 equals with reactions to both extracts (1.9), this difference is smaller in Table 1*b* than in 1*a*. Moreover, the "negative" reactions to house dust extract in these 67 unequals are not greater, but, to the contrary, smaller (0.1) than those in the 12 equals without reactions (0.2). Although the differences of these average skin reactions are small, they nevertheless suggest that at least in the group of the 67 unequals the influence of the variations is of minor importance; and that the majority of cases in this group has become sensitized against the human skin-scale allergen, and not to the house-dust allergen.

The conclusions to be drawn from the distribution in Table 1*b* confirm the findings of many earlier investigations that human skin scales and house dust contain different aller-

gens. It must be kept in mind, however, that house dust contains human skin scales as well, albeit in small amounts. If the skin reactions of the 117 "positive" equals were caused by the human skin-scale allergen, house dust would have to be composed exclusively of skin scales. But this quantity is probably not more than 1 per cent, which means that a 1 per cent dilution of house-dust extract is comparable, as to the content of human skin-scale allergen, with a 0.01 per cent dilution of human skin-scale extract. Extracts of human skin scales of this dilution cause skin reactions of this strength relatively seldom.

Almost exactly the same distribution is found in the reactions to extracts of *D. pteronyssinus* culture and human skin scales (Table 1c). Here too, we see a strongly asymmetrical distribution of the 65 unequals (2 to 63; $p < 0.01$), and a small influence of the variations in the material and method in the group of 63 unequals without skin reactions to *D. pteronyssinus*-culture extract.

From Table 1c the same conclusions can be drawn as from Table 1b. The 121 "positive" equals cannot be due to the human skin scales in the nutrient medium of *D. pteronyssinus* (about 40 per cent), because a 0.001 per cent dilution of mite-culture extract is comparable with a less than 0.0005 per cent dilution of human skin-scale extract, according to the content of human skin-scale allergen.

2. House dust; *G. destructor*; *T. putrescentiae*; *A. siro* (Tables 2 and 3). Next, we may compare the distributions of the 200 atopic patients according to their skin reactions to extracts of house dust and three other species of mites, viz. *G. destructor*, *T. putrescentiae*, and *A. siro*. The result is shown in Table 2. The numbers of cases with unequal skin reactions are high (74, 81, and 79, respectively), and the distributions of these cases over the two possible combinations is asymmetrical (56 to 18, 67 to 14, and 66 to 13; $p < 0.01$). From these distributions it becomes clear that these mites do not contribute to the specific allergenic potency of house dust. This is not surprising, because their occurrence in house dust

TABLE 2
Distribution of 200 Atopic "New" Patients According to Their Skin Reactions to Extracts of House Dust (0.1 Per Cent), and *G. destructor* (0.001 per cent), *T. putrescentiae* (0.001 Per Cent), and *A. siro* (0.001 Per Cent).

	Equal		Unequal	
	Average skin reactions	Number and percentage of patients	Average skin reactions	Number and percentage of patients
a	House dust	+	1.6	56(28)
	<i>G. destr.</i>	+	1.7	
	House dust	—	0.1	18(9)
	<i>G. destr.</i>	—	0.1	74(37)
b	House dust	+	1.6	67(33.5)
	<i>T. putr.</i>	+	0.1	
	House dust	—	0.1	14(7)
	<i>T. putr.</i>	—	1.5	81(40.5)
c	House dust	+	1.6	66(33)
	<i>A. siro</i>	+	0.1	
	House dust	—	0.2	13(6.5)
	<i>A. siro</i>	—	1.4	79(39.5)

TABLE 3

Distribution of 200 Atopic "New" Patients According to Their Skin Reactions to Extracts of *G. destructor* (0.001 Per Cent), *T. putrescentiae* (0.001 Per Cent), and *A. siro* (0.001 Per Cent).

Equal			Unequal		
	Average skin reactions	Number and percentage of patients		Average skin reactions	Number and percentage of patients
a	<i>G. destr.</i>	+	1.7	+	1.6
	<i>T. putr.</i>	+	1.7	—	0.1
	<i>G. destr.</i>	—	0.1	—	0.1
	<i>T. putr.</i>	—	0.0	+	1.2
		51 (25.5)			32 (16)
		100 (50)			17 (8.5)
		151 (75.5)			49 (24.5)
b	<i>G. destr.</i>	+	1.7	+	1.6
	<i>A. siro</i>	+	1.6	—	0.1
	<i>G. destr.</i>	—	0.1	—	0.2
	<i>A. siro</i>	—	0.0	+	1.2
		48 (24)			35 (17.5)
		97 (48.5)			20 (10)
		145 (72.5)			55 (27.5)
c	<i>T. putr.</i>	+	1.6	+	1.2
	<i>A. siro</i>	+	1.6	—	0.2
	<i>T. putr.</i>	—	0.0	—	0.2
	<i>A. siro</i>	—	0.0	+	1.2
		53 (26.5)			15 (7.5)
		117 (58.5)			15 (7.5)
		170 (85)			30 (15)

is unusual (5-7, 12-17, 19, 20). It must be regarded as exceptional that complaints of atopic patients are caused by a sensitization to one of these three species of mites. Only in a few cases of people living on a farm, or working in a flour mill, etc., where these mites occur more regularly and in greater numbers are sensitizations of clinical importance possible (24).

Comparing the distributions of the skin reactions of the 200 patients atopic to extracts of the three last mentioned species of mites (Table 3), we see that the numbers of unequal cases is relatively low (49, 55, and 30). The distribution of the unequals over the two possible combinations is only slightly asymmetrical (32 to 17, 35 to 20; $p = 0.1$) for the skin reactions to extracts of *G. destructor* and *T. putrescentiae*, and of *G. destructor* and *A. siro*. This distribution for the skin reactions to extracts of *T. putrescentiae* and *A. siro* is completely symmetrical, suggesting a relationship of the allergens produced by these two species of mites. This suggestion is also supported by the fact that the "positive" skin reactions in the 30 unequals (1.2) are smaller than those of the 53 "positive" equals (1.6) and by the fact that the "negative" reactions in the 30 unequals are larger (0.2) than those of the 117 "negative" equals (0.0).

The relationship between the allergens of these two species of mites is not very surprising, because biologically they are relatively closely related species of one sub-family, Tyroglyphinae.

Correlations in the Strengths of the Skin Reactions of 200 Atopic "new" Patients

A more quantitative way of comparing skin reactions to two extracts is to compute the correlation coefficient (r), which, in this case, indicates the correlation between the strengths of the reactions (30). This correlation can also be illustrated by a diagram in which the reactions of each patient to the two extracts is shown. For these quantitative comparisons of the strengths of the skin reactions, the equal cases

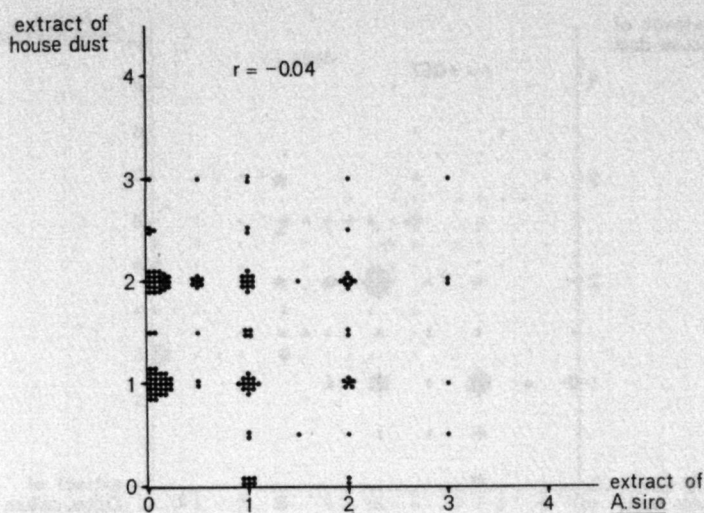


Fig. 1.

Comparison of the strengths of skin reactions to extracts of house dust (0.1 per cent) and *Acarus siro* (0.001 per cent), in 134 patients.

without reactions to both extracts under examination are left out of consideration. In the report of the Japanese workers (13) persons without skin reactions to the two compared extracts were also included in their correlation study. They find higher coefficients than we do, but these cases contribute to the correlation unproportionally. For that reason a different number of cases is used for the comparison of the strengths of their reactions, *i.e.* only those patients who have skin reactions of one + or more to at least one of the two extracts.

1. *One dilution* (Table 4; Figs. 1 and 2). For this part of the study of the extracts tested in three dilutions, only the reactions to the middle dilution are taken into consideration so that they can be compared with the reactions to the extracts tested in one dilution. In Table 4 the correlation coefficients of the most important pairs of extracts are shown. Diagrams of the pairs with the lowest and the highest correlation coefficient are given in Figs. 1 and 2.

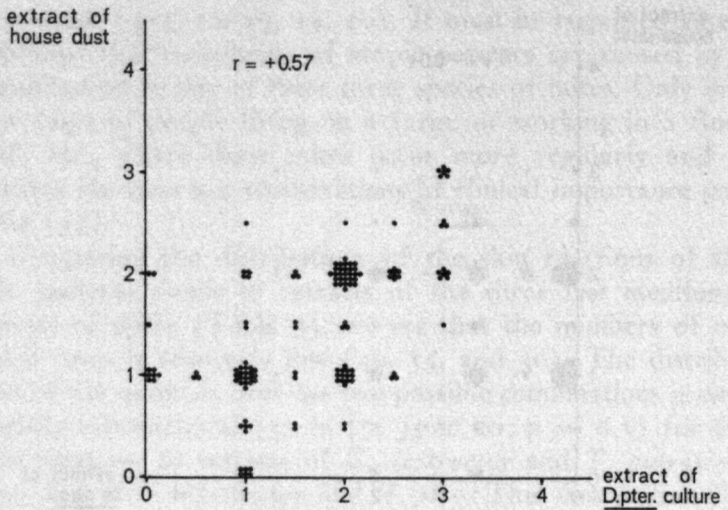


Fig. 2.

Comparison of the strengths of skin reactions to extracts of house dust (0.1 per cent) and *Dermatophagoides pteronyssinus* culture (0.0001 per cent), in 136 patients.

TABLE 4

Correlation Coefficients of Skin Reactions to Nine Pairs of Extracts Consisting of House Dust (0.1 Per Cent), *D. pteronyssinus* culture (0.001 Per Cent), *G. destructor* (0.001 Per Cent), *T. putrescentiae* (0.001 Per Cent), *A. siro* (0.001 Per Cent), and Human Skin Scales (0.1 Per Cent), in 200 Atopic "New" Patients.

Paired extracts	Number of patients with reactions (n)	Correlation coefficient (r)	Level of significance (p)
House dust - <i>D. pter.</i> cult.	136	+0.57	<<0.001
House dust - Human skin sc.	188	+0.52	<<0.001
<i>D. pter.</i> cult. - Human skin sc.	186	+0.47	<<0.001
House dust - <i>G. destr.</i>	139	+0.04	>0.5
House dust - <i>T. putr.</i>	135	+0.01	>0.5
House dust - <i>A. siro</i>	134	-0.04	>0.5
<i>G. destr.</i> - <i>T. putr.</i>	100	+0.10	0.5
<i>G. destr.</i> - <i>A. siro</i>	103	+0.09	0.5
<i>T. putr.</i> - <i>A. siro</i>	83	+0.44	<0.001

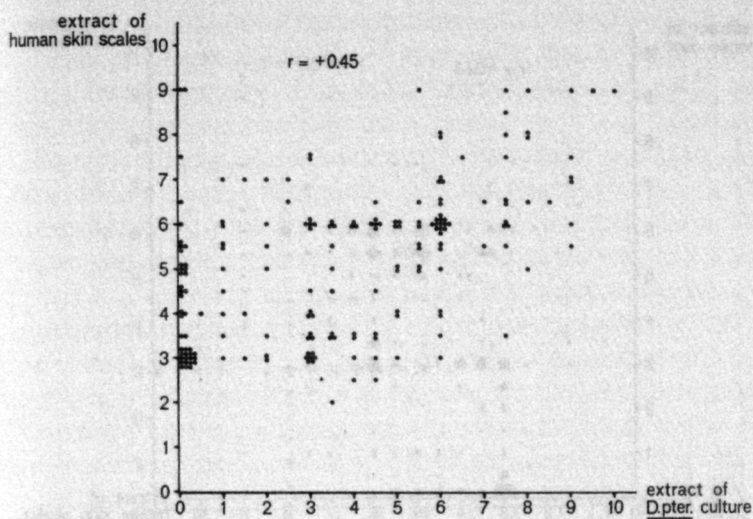


Fig. 3.

Comparison of the strengths of skin reactions to extracts of human skin scales (1, 0.1, 0.01 per cent) and *Dermatophagoides pteronyssinus* culture (0.001, 0.0001, 0.00001 per cent), in 186 patients.

Table 4 clearly shows that there is no correlation between the strengths of the skin reactions to extracts of house dust and of the three species of mites viz. *G. destructor* ($r = +0.04$), *T. putrescentiae* ($r = +0.01$), and *A. siro* ($r = -0.04$) (Fig. 1). Also no correlations are found between reactions to extracts of *G. destructor* and *T. putrescentiae* ($r = +0.10$), and of *G. destructor* and *A. siro* ($r = +0.09$). A weak, although highly significant, correlation is found between the reactions to extracts of *T. putrescentiae* and *A. siro* ($r = +0.44$; $p < 0.001$). These findings confirm the results of the qualitative distribution, as has been discussed in the preceding section.

2. *Three dilutions* (Table 5; Figs. 3-5). The correlation coefficients of the three first pairs in the list of Table 4 lie so close together that it is impossible to decide whether there are relationships between the allergens in these extracts or not. A more differentiated result is found concerning these three

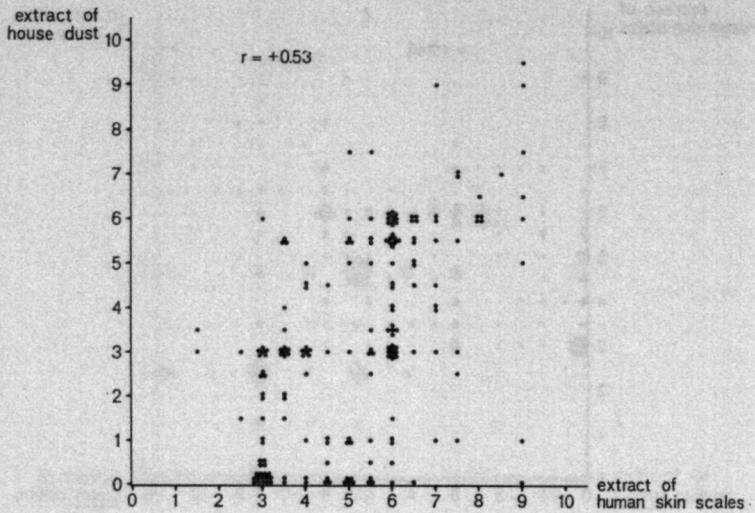


Fig. 4.

Comparison of the strengths of skin reactions to extracts of house dust (1, 0.1, 0.01 per cent) and human skin scales (1, 0.1, 0.01 per cent), in 188 patients.

TABLE 5

Correlation Coefficients of Skin Reactions to Extracts of House Dust (1, 0.1, 0.01 Per Cent), *D. pteronyssinus* Culture (0.001, 0.0001, 0.00001 Per Cent), and Human Skin Scales (1, 0.1, 0.01 Per Cent), in 200 Atopic "New" Patients.

Paired extracts	Number of patients with reactions (n)	Correlation coefficient (r)	Level of significance (p)
House dust - <i>D. pter. cult.</i>	136	+0.70	<<<0.001
House dust - Human skin sc.	188	+0.53	<<0.001
<i>D. pter. cult.</i> - Human skin sc.	186	+0.45	<<0.001

extracts when not only the middle dilutions of the three tested is taken, but all three dilutions together (Table 5; Figs. 3-5).

Just as was the case in the qualitative distributions of the skin reactions (preceding section), here too, influences of multiple sensitization and variations in the tested patients and in

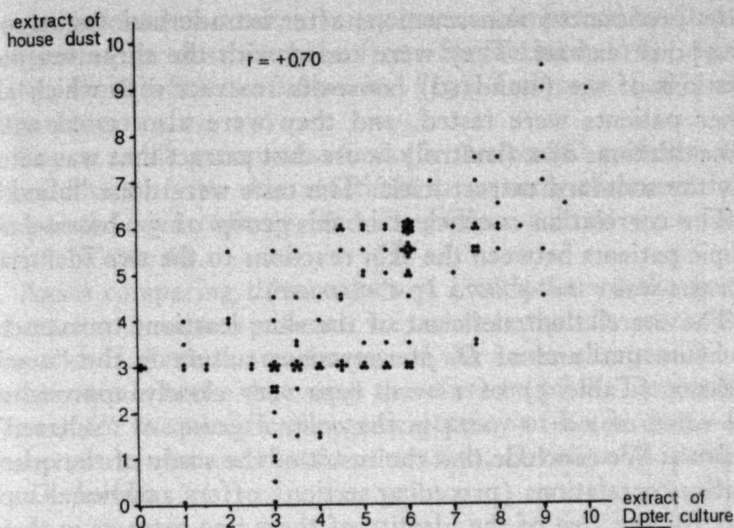


Fig. 5.

Comparison of the strengths of skin reactions to extracts of house dust (1, 0.1, 0.01 per cent) and *Dermatophagoides pteronyssinus* culture (0.001, 0.0001, 0.00001 per cent), in 136 patients.

the technique have a strengthening or diminishing effect, respectively, on the correlations found. In the following section, where the results of the testing of "selected" patients are reported, the determination is described of the maximal correlation that can be found, *i.e.* the correlation between skin reactions after intradermal testing with two completely identical extracts. There, this maximal correlation will be compared with the correlations listed in Table 5.

Correlations in the Strengths of the Skin Reactions of Atopic "Selected" Patients

1. *Control group of patients.* The influence of the variations and errors on the results of the study of correlations in skin reactions can be estimated in a control group of atopic patients. For this purpose we selected 34 patients known to

show pronounced skin reactions after intradermal testing of house-dust extract. They were tested with the three tenfold dilutions of the (standard) house-dust extract with which all other patients were tested, and they were also tested with three dilutions of a (control) house-dust extract that was actually the standard extract itself. The tests were done "blind".

The correlation coefficient in this group of 34 house-dust atopic patients between the skin reactions to the two identical extracts was $r = +0.74$ ($p < 0.001$).

The correlation coefficient of the skin reactions to extracts of house dust and of *D. pteronyssinus* culture in the "new" patients (Table 5) of $r = +0.70$ very closely approaches the value of $r = +0.74$ in the control group of "selected" patients. We conclude that the result of the study of the quantitative correlations (preceding section) offers additional support for the idea of the identity of these two extracts in their effect on the skin of atopic patients.

The other correlation coefficients are considerably lower.

TABLE 6

Correlation Coefficients of Skin Reactions to Extracts of House Dust (1, 0.1, 0.01 Per Cent), D. pteronyssinus Culture (0.001, 0.0001, 0.00001 Per Cent), and D. farinae Culture (0.001, 0.0001, 0.00001 Per Cent) in 16 Atopic "Selected" Patients.

Paired extracts	Number of patients with reactions (n)	Correlation coefficient (r)	Level of significance (p)
House dust - <i>D. pter.</i> cult.	16	+0.71	0.002
House dust - <i>D. far.</i> cult.	16	+0.69	0.01
<i>D. pter.</i> cult. - <i>D. far.</i> cult.	16	+0.72	0.002

2. *D. farinae* (Table 6). Another question concerning mites in house dust as sources of the specific house-dust allergen is, do the other species of the genus *Dermatophagoides*, e.g. *D. farinae*, play the same role as *D. pteronyssinus*? *D. farinae*

has been found to occur, in many cases accompanied by *D. pteronyssinus*, in dust samples from Switzerland (18), Spain (18), United States of America (17), Indonesia (18), and Japan (10, 13, 14, 15).

For a small group of 16 atopic "selected" patients, the correlations of the skin reactions to extracts of house dust, *D. pteronyssinus* culture, and *D. farinae* culture were computed (Table 6).

Again comparing these values of r with that of the group of 34 atopic control patients, we conclude that *D. pteronyssinus* and *D. farinae* are sources of the same allergen. Their occurrence in house dust must therefore be of the same significance for the specific allergenic potency of house dust.

In our opinion and that of others (9), *D. culinae* is identical to *D. farinae*.

CONCLUSIONS AND SUMMARY

To summarize the foregoing, the following conclusions can be drawn from the distributions of the skin reactions of 200 atopic "new" patients and from the correlations of the skin reactions of those same patients and of atopic "selected" patients:

a. Skin reactions to extracts of house dust and *D. pteronyssinus* culture are seen in the same frequency in the same patients. The small number of cases with qualitatively unequal reactions is symmetrically distributed over the two possible combinations. Quantitatively the skin reactions show a high correlation ($r = +0.70$), which very closely approaches the maximal obtainable correlation between the reactions to two completely identical extracts ($r = +0.74$). The extracts of house dust and *D. pteronyssinus* culture contain an allergen that, with the method of skin testing, cannot be distinguished from each other, and are therefore considered to be biologically identical.

b. There is a large group of patients showing skin reactions to extracts of human skin scales and not to extracts of house dust and *D. pteronyssinus* culture. The correlations of the strengths of the skin reactions to these two pairs of extracts

are relatively low ($r = + 0.53$; $r = + 0.45$, respectively). It is concluded that human skin scales, on the one hand, and house dust and *D. pteronyssinus* culture on the other, contain allergens that differ in their biological effect on the skin of atopic patients.

c. The number of atopic patients who have become sensitized to the allergens found in extracts of three other species of mites occasionally occurring in house dust, viz. *G. destructor*, *T. putrescentiae*, and *A. siro*, is relatively small. From the distributions and the correlations of the strengths of the skin reactions to extracts of house dust and each of the three species, it appears that they do not share allergens with the same effect in skin tests. These species do not contribute to the specific allergenic potency of house dust, and sensitizations of clinical importance are considered exceptional.

d. The distribution of the skin reactions to extracts of *T. putrescentiae* and *A. siro* suggests that there is a relationship between the allergens of these mites. These two allergens are probably not completely identical, because the correlation of the strengths of the reactions (to one dilution only) is weak ($r = + 0.44$).

e. Extracts of *D. farinae* (= *D. culinae*) culture contain the same allergen as those of *D. pteronyssinus*.

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