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ON CONCENTRATED PURIFIED DUST EXTRACT

By

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Previous investigations at the Department of Medicine, Lund, have shown that hypersensitivity to dust is one of the most common demonstrable causal factors of bronchial asthma (*Arner, Fürst & Truedsson*, 1958). It has also been shown that the use of a variety of different potent standard extracts and of specially prepared "auto" dust extracts is of diagnostic value (*Arner, Ekwall & Fürst*, 1959). This is in agreement with the assumption that extracts from different sorts of house dust contain a common major allergen as well as one or more minor allergens specific for the environments and of the individual (*Wodehouse* 1954).

Boatner & Efron (1942) devised methods for purifying and concentrating allergen in water extracts of house dust by fractionation with dioxane and ammonium sulphate. Similar methods have since been described by *Sutherland* (1942), *Rimington* (1947) *Vannier & Campbell* (1959) and others.

Purified and concentrated dust extracts are widely used abroad for diagnostic and therapeutic purposes. *Winge-Flensburg* (1950) reported an investigation of such extracts and claimed that they are diagnostically superior to ordinary crude "auto" house-dust extracts. *Winge-Flensburg's* conclusions are based on skin tests with the scratch and intracutaneous technique. Since it is now possible to assess the clinical importance of positive skin reactions by provocation tests, it was considered worth while investigating whether concentrated, purified extracts prepared by the method of *Boatner & Efron* could re-

place crude extracts in diagnostics when used in the way described previously (*Arner, Ekwall & Fürst, 1959*). If so, it would imply a considerable simplification of the diagnostic procedure.

PERSONAL INVESTIGATIONS

In the evaluation of an allergic extract it is necessary first to ascertain in what concentration the extract can be used without the content of non-specific substances giving a false positive test. In addition the capacity of the extract to give positive skin reactions and provocation tests in persons with known allergy should be checked.

MATERIAL AND METHODS

1) *Allergen*.—A Danish, purified concentrated dust allergen, called C.D., was used. According to the manufacturers, this allergen is prepared in accordance with the method of *Boatner & Efron*. It is commercially available in a maximum concentration of 1 per cent. It is prepared from dust collected from the homes of patients with asthma. The manufacturers recommend a concentration of 0.1 per cent for intracutaneous tests.

Two crude dust extracts, L₂ and L₃, were also used. These extracts were prepared at our laboratory and proved to be strong allergens. Crude "auto" dust extracts, called A.D., were prepared for a number of patients with dust asthma. The strength of the crude extracts is given in terms of weight per volume, according to which 1:100, for example, means that the extract contains the water soluble components in 1 g of raw material dissolved in 100 parts by volume of solvent (Coca's solution with 0.5 per cent phenol). The crude extracts were prepared in a maximum strength of 1:10.

2) *Skin test technique*.—The intracutaneous skin tests were always made on the skin of the back with a dose of 0.05–0.1 ml. The reactions were read 15 minutes later and judged by measurement of the largest perpendicular diameters of the wheals. The results were classified as follows:

Sum of diameters of wheal	Reaction
< 18	—
Ⅷ 18	±
Ⅷ 20	+
Ⅷ 30	++
Ⅷ 40	+++
Ⅷ 50	++++

Control tests with Coca's solution were made in each subject.

3) *Provocation tests.*—The subjects inhaled allergen extract nebulized in a de Vilbiss spray No. 40, driven by compressed air. All the patients were symptomfree and free of wheezing before the test and after a control test in which they inhaled 1.0 ml of Coca's solution. Subsequent inhalation of allergen was discontinued as soon as any signs of asthma appeared. If no such signs developed, the inhalation was continued until 2.0 ml of allergen had been nebulized. The amount of allergen used in each test was estimated and noted. The test was said to be positive if clear cut signs of asthma occurred during or immediately after the inhalation of the allergen. If no such signs appeared after inhalation of 2.0 ml of allergen extract the test was said to be negative.

4) *Patients.*—Intracutaneous skin tests were performed on 20 healthy persons (undergraduates), aged 20 to 25. None of them or their closest relatives had allergy in their history.

Intracutaneous and provocation tests were performed on a group of 20 patients (10 males and 10 females) aged 6–40. All of these patients had asthma, with a typical history of dust allergy and, by means of inhalation tests, we checked that they all had manifest dust allergy.

I. *Content of non-specific irritants in the concentrated purified dust extract.*

Purification of allergen extracts aims at securing extracts as free as possible from non-specific irritants, so that they might be used in high concentration in intracutaneous skin tests.

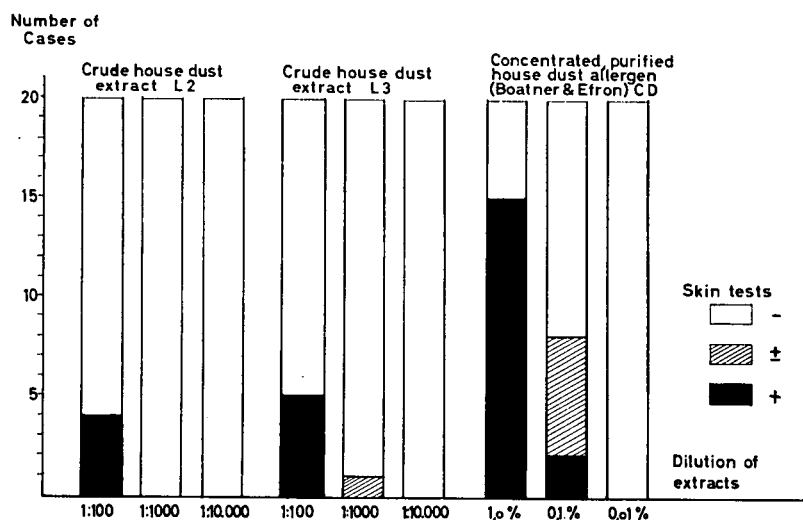


Fig. 1.

Results of intracutaneous skin tests with different house dust extracts in 20 normals.

The degree of purity of the extracts was therefore tested on the controls, who received simultaneous intracutaneous injections of various dilutions of the concentrated purified dust extract and of the two crude extracts, L2 and L3. The results are summarized in Fig. 1.

The investigation shows that the crude extracts can be used in intracutaneous tests in a concentration of 1:1000 without producing false positive tests. When used in a concentration of 0.01 per cent the concentrated dust extract regularly gave a negative reaction. In a concentration of 0.1 per cent it produced wheals ≥ 20 mm (+) in 2 subjects and with a total diameter of 18–19 mm (±) in 6. It is true that a concentration of 0.1 per cent fills the conventional requirements of the *Nordisk Förening för Allergiforskning* (The Northern Society of Allergology), but in view of the inaccuracy of the method and the relatively high number of ± reactions to a concentration of 0.1 per cent, we consider it advisable to use a concentration of 0.01 per cent if non-specific reactions are to be avoided.

It thus appears that the concentrated dust extract in a strength of 0.01 per cent and the crude extract 1:1000 contained an approximately equal amount of non-specific irritants. The investigation suggests that the purification procedure could not have been so very effective.

II. *Dust allergen in the concentrated purified dust extract.*

The concentration of dust allergen in a given extract can be assessed by ascertaining which concentration gives a positive skin test in patients with dust allergy. Simultaneous skin tests were performed on the 20 patients with asthma with the two crude extract and of the concentrated purified dust extract (18 patients) and with concentrated purified dust extract. The skin tests were carried out with a series of dilutions of the extracts, so-called skin titration. The highest concentrations of the crude extracts and of the concentrated purified dust extract used were 1:1000 and 0.1 per cent respectively. The results are given in Table 1.

In 19 of the 20 patients the concentrated dust extract in a concentration of 0.01 per cent or lower gave a positive reaction. The crude extracts, L2 and L3, regularly gave a positive reaction in a concentration of 1:1000 or lower.

Comparison of the titer limits with the various extracts in the individual patients showed that the allergen content of the crude extracts in a concentration of 1:10,000 is comparable to the allergen content of the extract 0.01 per cent regarding the capacity to produce specific skin reactions.

In 3 cases out of 18 the specially prepared "auto" dust extracts gave negative reactions. This might have been due to faulty technique used in the collection of the dust from which the extract has been prepared. Since these "auto" dust extracts have not been tried systematically on other patients with known dust allergy, their potency cannot be judged from the skin tests performed. On the average, however, the skin titer for the "auto" dust extracts appears to be high and comparable to the other crude extracts and the concentrated dust extract.

TABLE 1
Results of Intracutaneous Skin Tests and Provocation Tests with Different House
Dust Extracts in 20 Cases of Asthma.

Case		Skin tests				Provocation tests							
		Lowest extract conc. just giving a pos. skin reaction				Inhaled amount of extracts employed							
		Extracts employed				L 2, 10 ⁻¹		L 3, 10 ⁻¹		AD, 10 ⁻¹		CD, 10 ⁻²	
Nr.	Age	L 2	L 3	AD	CD	pos.	neg.	pos.	neg.	pos.	neg.	pos.	neg.
1	21	10 ⁻⁵	10 ⁻⁴		10 ⁻⁵	5 breaths						8 breaths	
2	40	10 ⁻⁴	10 ⁻⁴	10 ⁻⁴	10 ⁻⁴					1.0 ml			2.0 ml
3	15	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻⁶					2.0 ml		0.5 ml	
4	11	10 ⁻⁴	10 ⁻³	10 ⁻⁴	10 ⁻⁴					30 breaths		1.0 ml	
5	14	10 ⁻⁶	10 ⁻⁵	10 ⁻⁶	10 ⁻⁵					3 breaths		1.5 ml	
6	38	10 ⁻⁵	10 ⁻⁴	10 ⁻⁵	10 ⁻⁵					6 breaths		30 breaths	
7	27	10 ⁻⁵	10 ⁻⁴	>10 ⁻³	10 ⁻⁴	30 breaths		1.5 ml				45 breaths	
8	14	10 ⁻³	10 ⁻³	10 ⁻⁴	10 ⁻⁴			1.0 ml		0.5 ml		30 breaths	
9	9	10 ⁻³	>10 ⁻³	10 ⁻³	>10 ⁻⁴					1.5 ml		1.5 ml	
10	23	10 ⁻⁶	10 ⁻⁴		10 ⁻⁶	5 breaths						0.5 ml	
11	12	10 ⁻³	10 ⁻³	10 ⁻⁵	10 ⁻⁴					4 breaths			2.0 ml
12	10	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻⁵					0.3 ml			2.0 ml
13	20	10 ⁻⁴	10 ⁻⁴	10 ⁻⁶	10 ⁻⁴					2.0 ml		2.0 ml	
14	14	10 ⁻⁵	10 ⁻⁵	10 ⁻³	10 ⁻⁵	2.0 ml							2.0 ml
15	23	10 ⁻⁴	10 ⁻³	10 ⁻⁴	10 ⁻⁴					2.0 ml			2.0 ml
16	19	10 ⁻⁴	10 ⁻³	>10 ⁻³	10 ⁻⁴	6 breaths			2.0 ml				2.0 ml
17	27	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁵	2.0 ml							2.0 ml
18	11	10 ⁻⁵	10 ⁻⁵	>10 ⁻³	10 ⁻⁵	0.5 ml							2.0 ml
19	6	10 ⁻⁵	10 ⁻⁵	10 ⁻⁵	10 ⁻⁴					1.0 ml		0.7 ml	
20	9	10 ⁻⁴	10 ⁻⁴	10 ⁻³	10 ⁻⁵					0.5 ml		2.0 ml	
										1.0 ml		1.0 ml	

The investigation thus showed that the concentrated purified dust extract may be used instead of potent crude extract from house dust. On the other hand, the investigation did not reveal any advantages of purified dust extract over such crude extracts for skin tests. It does not appear probable that concentrated purified dust extract prepared in accordance with the method of *Boatner & Efron* will offer better possibilities of detecting cases of slight hypersensitiveness to house dust.

III. *Provocation tests by inhalation of concentrated purified dust extract.*

It is well known that in asthma a positive skin reaction to dust extract does not always imply that dust sensitivity is a causal factor of the disease. In all cases of allergy where the skin is not the shock organ, the skin test is only a supplementary aid. Hypersensitivity can only be demonstrated with certainty if the actual shock organ is directly exposed to the allergen in question and then responds with a typical allergic reaction. Therefore, it must be ascertained to what extent concentrated purified dust extract will produce symptoms on inhalation by patients with manifest dust allergy. Small amounts of allergen should be sufficient to produce typical symptoms if the extract is strong. We therefore carried out provocation tests with concentrated purified dust extract on the previous mentioned group of patients with asthma. The results are given in Table 1, which also shows the approximate amounts of allergen that gave a positive provocation test. Each of the patients reacted positively to at least one of the 3 crude extracts. Extract L2 gave a positive reaction in 7 out of 7 and L3 in 2 out of 3 patients. The specially prepared crude "auto" dust extracts, tried on every single patient, gave a positive reaction in 13 out of 15 patients studied. The concentrated dust extract gave a positive provocation test in 13 out of 20 studied. The investigation also showed that it was necessary to use at least equally large volumes of the concentrated dust extract in 1.0 per cent concentration as of the crude extract 1:10.

Though the number of patients studied is relatively small, the investigation suggest that, as far as the content of dust allergen is concerned, the extract L2 was better than the concentrated purified dust extract. It is also clear from the table that of the 7 patients in whom the concentrated purified dust allergen gave a negative provocation test, 4 showed a high titer limit for the skin test, namely 10^{-5} (0.0001 per cent). It is also apparent that in those cases in which concentrated, purified dust extract 1 per cent gave a positive provocation test it was often necessary to use relatively large doses of the extract.

The investigation showed that the method used for concentrating the extract did not produce the desired result.

SUMMARY

Several authors have described methods for concentrating and purifying water extracts of house dust, and on the basis of serologic reactions and skin tests it has been claimed that the extracts are potent allergens. Concentrated dust extracts contain several different allergen factors but are believed to contain one major allergen. Concentrated, purified dust extracts would be clinically valuable if they filled the following requirements:

1. The allergen should be free of toxic substances so that it can be used in high concentration in skin tests without any risk of false positive reactions.
2. Low concentrations of the extract should give clear-cut positive reactions in skin tests in patients with known dust allergy.
3. The concentrated dust extract should give positive provocation tests in persons with known allergy, *e.g.*, on inhalation of the extract by patients with asthma.

A concentrated, purified dust extract, prepared according to *Boatner & Efron*, was compared with 3 different crude extracts (2 stock extracts, L2 and L3, which were proved to be potent allergens, and so-called "auto" dust extracts, prepared specially for each patient).

It was found necessary to use the concentrated purified dust extract in a dilution of 0.01 per cent in skin tests, if non-specific reactions were to be avoided.

In 20 patients with known dust allergy the concentrated dust extract gave a positive skin reaction in 19 with titer limits of 0.01–0.0001 per cent.

As to the capacity to elicit specific skin reactions, 0.01 per cent of the concentrated dust extract was found to be equal to the crude extracts in 1 : 10,000 dilution. The investigation thus produced no evidence that the type of concentrated purified dust extract tried was superior to our crude extracts in diagnostic skin tests.

In 7 of 20 patients with clinically manifest dust allergy and in whom skin tests with the concentrated dust extract had given a positive reaction, inhalation of this extract failed to provoke any allergic symptoms. It thus appears that the extract was not rich in allergens of causal significance in these cases. The dust extract used was thus not a universal dust allergen.

To summarize, we would stress that intracutaneous and provocation tests by various different potent crude dust extracts and with "auto" dust extracts is of indispensable diagnostic aid in allergologic practice.

LITERATURE

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