

THE ALLERGEN(S) OF HOUSE DUST.

DIAGNOSIS AND DESENSITIZATION OF DUST-SENSITIVE PATIENTS WITH "CRUDE DUST ANTIGEN."

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RIMINGTON, Stillwell and Maunsell (1947) have reported the purification of the dust antigen and a study of its chemical properties and biological activity. In a later paper (Stillwell, Rimington and Maunsell, 1947) consideration was given to the chemical and biological relationships discernible between the dust antigen and moulds or mould products. With a view to future clinical employment of this antigen, we felt it desirable to obtain some evidence relative to its use in the diagnosis of allergy, and preliminary information concerning its therapeutic value.

Skin titrations with crude dust antigen in unselected persons.

Skin titrations with crude dust antigen were carried out on 59 persons in an unselected population, their age group varying between 20 and 60 years. Notes were taken as to whether or not there occurred in their past or present history such allergic symptoms as hay fever, perennial rhinorrhoea, bronchial spasm with or without bronchitis, urticaria, contact dermatitis or neurodermatitis.

Method.—For source of material, purification and sterilization, and for technique of methods of testing, of skin titrations and reading of reactions see Rimington, Stillwell and Maunsell (1947).

"Crude dust antigen" (10 mg.) was dissolved in 10 ml. of normal saline containing 0.5 per cent of phenol. Starting with this solution three serial dilutions of 1 in 10 were prepared with carbolsaline. The solutions of dust antigen of concentrations (w/v) 10^{-4} , 10^{-5} and 10^{-6} , and carbolsaline as control were then used for testing. The strength of the weakest solution of test material which gave a positive reaction was noted as the "threshold-reaction."

Results.—These are set out in Table I. Out of the 59 persons, 2 who gave positive tests with carbolsaline were excluded from this investigation. The remaining series of 57 persons was divided into a "normal group" of 49 subjects without any allergic history, and an "allergic group" of 10 persons who in some phases of their life had suffered from allergic symptoms. The skin reactions to dust antigen differed considerably in the two groups. In the normal group no individual gave threshold reactions to dust antigen in a dilution 10^{-6} , 3 reacted to 10^{-5} , and 17 to 10^{-4} . In the allergic group 4 persons gave a threshold reaction to a dilution 10^{-6} , 3 to 10^{-5} and 2 to 10^{-4} . As for negative tests, 29 from the normal group (58 per cent) were negative to 10^{-4} , whilst out of the allergic group only 1 person (10 per cent) failed to react.

TABLE I.—*Skin Titrations with Crude Dust Antigen in an Unselected Population. 57 Persons. Age Group 20–60 Years.*

	Number of persons.	Threshold reactions.			
		Positive.			Negative.
		10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	
Carbolsaline	57		0		57
Dust dilutions		10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻⁴
Normal group	49	0	3	17	29
Allergic group	10	4	3	2	1

Since in the normal group about 34 per cent reacted to dust in dilutions of 10⁻⁴, but only about 6 per cent to dust dilutions of 10⁻⁵, it was decided for diagnostic purposes to use the antigen no stronger than in a dilution of 10⁻⁵. We may in so doing, however, have missed a few weak dust-reactors.

Skin tests in patients suffering from allergic syndromes.

Eighty patients were subjected to skin tests because an allergic factor was suspected in relation to their clinical syndrome. 58 persons were suffering from perennial rhinorrhoea (*Group I*), 12 persons were complaining of rhinorrhoea in conjunction with bronchial asthma and (or) bronchitis, neurodermatitis, urticaria (*Group II*), and 10 were suffering from the latter symptoms without rhinorrhoea (*Group III*). The groups are set out in Table II, which records the results of the tests.

TABLE II.—*Skin Reactions in Patients Suffering from Allergic Syndromes.*

	Number of patients.	Positive reactions to dust antigen in dilution 10 ⁻⁵ .
Total tested	80	55
Group I: Perennial rhinorrhoea	58	41
Group II: Perennial rhinorrhoea with bronchial asthma and/or neurodermatitis and/or urticaria	12	7
Group III: Bronchial asthma or neurodermatitis or urticaria	10	7

Fifty-five out of the total 80 patients gave reactions to dust antigen in a dilution of 10⁻⁵ and, so far as one could see from the small numbers, there was the same incidence of positive reactions in all three groups.

In an attempt to correlate the clinical symptoms and the dust reaction, skin titrations were carried out with dust in serial dilutions ranging from 10⁻⁴ to 10⁻⁷ (36 cases of perennial rhinorrhoea with or without asthma), the subjects with a low threshold-reaction (10⁻⁵ and 10⁻⁶) did not seem to be aware of any effect of dust on their symptoms, while 4 persons with a threshold reaction not less than 10⁻⁷ volunteered the statement that unusual amounts of dust, e.g. spring-cleaning, invariably brought on severe attacks.

The relation between reactions to dust and to other allergens was checked in 45 out of the 80 cases. Tests were carried out with dust (10⁻⁵), cat hair, feather (0.1 per cent extracts in carbolsaline) and moulds (for types and preparations see Stillwell, Rimington and Maunsell, 1947). 35 persons reacted to dust,

while 22 out of these 35 were also sensitive to one or more of the other allergens. No case has yet been found which reacts to one of the other allergens but not to dust (see Table III).

TABLE III.—*Skin Tests with Dust Antigen (1×10^{-5}), Extracts of Cat Hair, Feather (0.1 per cent Extracts), and Mould Extracts in Patients Complaining of Allergic Symptoms. Number of Individuals in Brackets.*

Subjects (45)	{	+ to dust (35)	{	+ to other allergens (22)
				— to other allergens (13)
	{	— to dust (10)	{	+ to other allergens (0)
				— to other allergens (10)

Desensitization.

Eighteen allergic patients were treated by desensitization. All of them reacted to dust antigen (10^{-5} or in higher dilutions), and 11 reacted also to other inhalants. 14 patients suffered from perennial rhinorrhoea with or without asthma, and the remaining 4 complained of asthma, urticaria and neurodermatitis without rhinorrhoea. 10 females and 8 males were among this series, their ages ranging from 11 to 52 years. The onset of their symptoms was given as 1 to 20 years previously. In the 14 cases of rhinorrhoea the nasal symptoms consisted of sneezing attacks, especially in the early hours of the morning, obstruction and watery discharge. The colour of the mucosa showed the typical allergic paleness, and was sometimes oedematous. In some cases the mucosa appeared mauve and boggy. Hyperplasia and crenated appearance of the mucosa were recorded twice, and polypi three times. The absence of infective and purulent bronchitis was noted.

During the period of desensitization no other treatment was carried out apart from occasional administration of benadryl and ephedrine when symptomatic relief was necessary. All the patients were advised to avoid contact with excessive amounts of dust, but no special precautions were taken and no change of job or residence was attempted.

Method.—The patients were given a course of intramuscular injections of "crude dust antigen" into the thighs starting with the weaker solution, 1×10^{-4} in carbolsaline, in doses of 0.1, 0.4, 0.8 ml., and this course was followed immediately by injections of the stronger solution, 1×10^{-3} in doses of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 ml., and 4 further injections of 1 ml. were then given. If the patient complained of severe local reactions, or reported marked increase of bronchial or nasal symptoms, the dose was not increased for the next injection. It was either repeated or, in some instances, even decreased. In this way the total amount given during one course of injections was approximately 10 mg. In the majority of cases the injections were made at weekly intervals, but in some patients they were given every second day.

Reactions during desensitization.—Local reactions occurred frequently, the area of injection becoming red and tender after a few hours and remaining painful for a number of days. Attacks of rhinorrhoea and asthma were reported, but there was uncertainty about their nature as constitutional reactions. There was no generalized urticaria brought on by any of the injections. No elevation of temperature was recorded.

Clinical results.—Improvement after treatment, not only of the local complaints but also of the general condition, was stated by 16 out of 18 patients. Sneezing attacks, obstruction and watery discharge were absent or diminished. Attacks of bronchial asthma became rarer and milder. Urticarial rashes (one patient only) disappeared. These statements were remarkable in those subjects who previously had undergone, without favourable results, other forms of treatment, e.g. zinc ionization, removal of polypi, submucous resection and desensitization with mixed inhalants. However, no support for these subjective statements was found by examinations of the nasal mucosa.

The two patients who failed to show subjective improvement were a boy, aged 12, suffering from perennial rhinitis, with dust and mould sensitivity, and a young man, aged 20, who had had for 16 years a severe form of neurodermatitis with multiple sensitiveness. It may be noted that desensitization was attempted using only the dust antigen.

Threshold titre before and after treatment.—Serial dilutions of dust antigen, ranging from 10^{-4} to 10^{-10} were made up in carbolsaline. An attempt was made to do skin titrations before and after treatment in all 18 dust-sensitive cases, but for practical reasons this quantitative assessment could be carried out only in 13 patients of the series. Before and after treatment duplicate injections of 0.04 ml. of each serial dilution were given intradermally into the patient's back, starting with the weakest dilution. The reading of the tests has been described by (Rimington, Stillwell and Maunsell (1947). The threshold reaction obtained before treatment is referred to as threshold A, and after treatment as threshold P. Titration A was in some cases repeated after a few days in order to assess whether or not significant spontaneous variations occurred. No significant variations were encountered in this particular instance.

All 13 patients showed a marked decrease of the threshold reaction after treatment (see Tables IV and V).

TABLE V.—*Typical Examples of Decrease of Titre of Skin Reaction Before and After Treatment with "Crude Dust Antigen."*

Patient . . .	W. A.— Rhinitis. 10 mg.		B. A.— Rhinitis. 15 mg.	
Diagnosis . . .	Feb. 12, 1947.	Feb. 26, 1947.*	Nov. 10, 1946.	Dec. 28, 1946.†
Total dose . . .				
Dilutions of dust antigen.				
1 in :				
10,240,000 . . .	—	—	—	—
5,120,000 . . .	—	—	+	—
2,560,000 . . .	—	—	+	—
1,280,000 . . .	—	—	+	—
640,000 . . .	+	—	+	—
320,000 . . .	++	—	++	—
160,000 . . .	++	—	++	—
80,000 . . .	..	+	..	—
40,000 . . .	..	++	..	+
20,000 . . .	..	++	..	+
10,000 . . .	..	++	..	+

* Tested Feb. 28, 1947.

† Tested Jan. 24, 1947.

TABLE IV.—*Threshold Reactions and Blocking Antibody Before and After Desensitization.*

Number.	Age.	Diagnosis.	Duration (years).	Ante-treatment.			Post-treatment.					
				Date.	Threshold reaction. A.	Blood titre. A.	Date.	Threshold reaction. P.	Blood titre. P.	Total dose, (mg.)	Interval.	Clinical result.
1	20	R.	1	10.xi.46	5,120,000	4	28.xii.46	40,000	8	15	Alternate days	Good
2	38	R, As	20	17.xi.46	10,000,000	..	28.xii.46	1,000,000	..	10	"	"
3	27	R, As	20	1.xi.46	100,000,000	4	17.i.46	1,000,000	8	10	Weekly	Very good
4	45	R	10	14.xii.46	160,000	..	14.ii.47	10,000	..	10	Weekly and fortnightly	"
5	37	R, As	7	5.vii.46	1,280,000	..	1.ii.47	10,000	..	5	Fortnightly	Good
6	19	As, R	12	23.xi.46	2,560,000	4	30.xii.46	640,000	4	15	Alternate days	"
7	35	R, As	6	18.xii.46	320,000	..	17.i.46	10,000	..	10	Weekly	Fair
8	30	R	3	12.ii.47	640,000	16	26.ii.47	80,000	8	10	Daily and alternate days	Good
9	20	N	16	31.xii.46	160,000	..	20.ii.47	20,000	..	10	Twice weekly	Not improved
10	44	B, As	1	14.i.47	1,280,000	4	1.iii.47	160,000	8	5	"	Good
11	40	U	2	15.ix.46	80,000	..	1.iii.47	10,000	..	20	Weekly	Very good
12	14	As	8	16.i.47	100,000,000	..	27.iv.47	80,000	..	10	"	"
13	33	As, R	28	16.i.47	2,560,000	8	23.ii.47	320,000	8	4	Twice weekly	Good

R = Rhinorrhoea; As. = asthma; N = Neurodermatitis; B = bronchitis; U = urticaria.

Direct test for detection of blocking antibody.—Serial dilutions of the crude dust antigen were made in a series of tubes. To each tube was added an equal volume of the patient's serum taken before treatment (dust-serum mixture). A corresponding row was set up in which saline instead of patient's serum was added to each tube (dust-saline mixture). Before desensitization, skin-titrations with these corresponding sets were carried out in the patient from whom the blood was taken. After desensitization, blood was taken again from the patient and the whole experiment was repeated, using the post-treatment serum. The ratio of the threshold titre of the dust-serum mixture to the dust-saline mixture was termed the "blocking titre."

The patients' serum inhibited slightly the skin reaction caused by the crude dust antigen. There was no significant variation between the blocking titre of the anti-treatment and the post-treatment serum (Table IV).

DISCUSSION.

It was observed that skin reactions were provoked by our dust antigen, in a dilution of 10^{-6} in persons with an allergic history but not in others, while reactions to stronger concentrations occurred also in some persons without an allergic history. Efron, Boatner and Pabst (1940, 1941), studying the reactions of their purified house dust extracts, also noted positive reactions in persons with no past or present history of allergy. By using the scratch test they found 7.5 per cent of positive reactions in the normal group and 78.1 per cent in the allergic group. When using the intradermal method and injecting 0.01 ml. of the material, they found that dilutions stronger than 2×10^{-5} reacted not only in the allergic group, but also in the normal group.

The reactions to dust in persons without allergic symptoms are susceptible to explanation in two ways: Firstly, weak antibodies directed against dust may be present in the majority of persons, but reach a high titre only in those individuals who have been more than usually exposed to dust or who naturally produce antibodies of high titre. Secondly, the reactions may be due to the presence of histamine or histamine-like substances in the dust antigen or to irritants which cause the liberation of these substances in normal skin. Similar observations on the reaction to dust have been made in animal experiments. Coulson and Stevens (1939) reported not only anaphylactic reactions in guinea-pigs sensitized to dust, but also anaphylactoid reactions to intravenous injections of dust solutions in unsensitized guinea-pigs. Friedmann (1939) demonstrated the narrow margin between the minimal anaphylactic dose of dust antigen in the sensitized guinea-pig and the anaphylactoid dose in the unsensitized animal.

The observation that dust in stronger concentrations may simulate allergic reactions in normals does not contradict the theory of a dust antigen-antibody reaction in allergic persons. In fact, the general irritating properties of dust may simulate the production of antibodies in non-sensitive persons, and there seems to be little doubt that the mucous membrane of the respiratory tract, when sensitized, can respond to contact with dust by rhinorrhoea and bronchial asthma. Multiple reactions of allergic patients to dust and to other inhalants were noted also by Wagner and Rackemann (1937), and the theory that the dust antigen may consist of a variety of antigens cannot be rejected. However, since in some cases

there occurs an apparently specific dust sensitivity, there may exist a specific dust antigen, not identical, but closely related to the other inhalants.

Desensitization with dust extracts in dust-sensitive cases has been carried out by Sutherland (1942), and by Efron and his co-workers (1941). Both authors reported good results. In our series of 18 cases there also appeared to be improvement, which was supported by the raising of the threshold reactions. We are, however, hesitant to assess the clinical results of treatment in a comparatively small number of allergic cases observed only for a short time after termination of desensitization. Allergic patients are known to pass through phases of attack and of spontaneous remission. These fluctuations may be due either to variations in extrinsic factors, such as the seasonal appearance of pollen and spores of moulds in the air, or spontaneous remissions may be caused by intrinsic factors of unknown origin. Great caution is therefore necessary in assessing improvement of allergic patients after desensitization. The regular decrease of the threshold reaction after treatment to dust is, however, an encouraging objective sign, denoting immunological changes induced by the injection of the antigen. More serological work is needed to reveal the mechanism leading to this decrease in the dust threshold reaction. The titre of the dust reagins, before and after treatment, has not yet been determined. The failure to detect a blocking antibody should not discourage further investigation.

The clinical and biological results make us feel that more work on this subject should and must be done. It should be carried out on a large number of cases, with follow-up of varying length of time and with an equal number of controls given courses of carbolsaline only. In such a way it might be shown whether improvement occurs in the majority of cases, whether it is lasting or of short duration, and whether a maintenance dose given at various intervals will prevent relapse. Furthermore, it should be ascertained whether the disappearance of crenated and hyperplastic mucosa and of polypi can be achieved.

SUMMARY.

1. "Crude dust antigen" dilutions of 10^{-5} produced skin reactions by intradermal tests in about 6 per cent of normal subjects and in 70 per cent of persons with allergic symptoms.

2. Allergic symptoms of the respiratory tract seemed to be provoked by a dusty atmosphere in subjects with a threshold reaction to dust in dilutions not less than 10^{-7} .

3. Sensitivity to dust and to other inhalants occurred often together in the same subjects. No case was found, among 80 allergic patients tested, which was sensitive to feather, cat hair or moulds and not to dust. Many patients exhibited sensitivity to the dust antigen but not to moulds or any other material tested.

4. Eighteen allergic patients suffering from perennial rhinitis and allied conditions who received one course of injections of dust antigen claimed improvement during treatment. The changes in the nasal mucosa did not return to normal.

5. A decrease of the threshold reaction to dust was obtained during treatment.

6. A blocking antibody was not found.

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SEROLOGICAL TYPING OF *C. DIPHTHERIAE*.

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THE object of this communication is the development of the serological typing of *C. diphtheriae* as a tool in studying the epidemiology of diphtheria and in the elucidation of certain more theoretical problems.

Earlier workers (Nicolas, 1896; Eagleton and Baxter, 1921) were handicapped because the organisms had not been classified into the gravis, intermedius and mitis groups (Anderson, Happold, McLeod and Thomson, 1931). There has been progress since this classification was introduced (Ewing, 1933; Robinson and Peeney, 1936; Sia and Huang, 1939; Tarnowski, 1942), but serological typing is still confined mainly to gravis strains, and has not become a routine procedure with diphtheria as with other organisms despite its potential value. In part this lack of development is due to difficulties in technique, and the typing of intermedius and mitis strains has received little attention.

Confusion is caused by using the word types to express the gravis, intermedius and mitis groups of strains, although a bacterial type is usually regarded as a serological entity. Since the classifications gravis, intermedius and mitis are not based on serological differentiation, and since each group consists of a number of different serological types, it is suggested that they should be described as *groups* and that *types* should be confined to serologically distinct strains. In this communication therefore grouping is the separation into the gravis, intermedius and mitis forms, and typing is the further differentiation of strains by serological methods, in this case by agglutination.