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STUDIES ON COTTON DUST IN RELATION TO BYSSINOSIS

PART II: SKIN TESTS FOR ALLERGY WITH EXTRACTS OF COTTON DUST

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The clinical features of byssinosis which have suggested an allergic factor in its aetiology have been discussed in an earlier paper (Furness and Maitland, 1952). Prausnitz (1936) concluded, on the basis of a wheal and flare type of skin reaction elicited by injecting extracts of cotton dust, that he had demonstrated hypersensitivity specific to byssinosis and supported this view with collateral evidence. He noted also a delayed type of skin reaction which he thought was due to a non-specific toxic substance in the extract and he suggested that a direct irritant action of dust on the lungs was of primary importance in causing byssinosis.

Bramwell and Ellis (1932) and Brown (1932) obtained an early type of skin reaction with extracts of cotton dust but their extracts contained histamine (Maitland, Heap, and Macdonald, 1932) and their results were of doubtful significance. That histamine or a similar substance can be extracted from cotton dust has been shown by Macdonald and Maitland (1934) and by Macdonald and Prausnitz (1936); its possible role in causing byssinosis has been discussed by Haworth and Macdonald (1937).

Van Leeuwen (1932) made extracts of cotton dust which did not contain histamine. They produced an early reaction of wheal and erythema in nearly all asthmatics and a late reaction in asthmatics and in healthy people.

If hypersensitivity is a major factor in byssinosis it might be possible to detect persons who had become hypersensitive before they were clinically affected and to take preventive measures. The possibility of desensitizing those with symptomatic byssinosis would also arise. The work reported here was undertaken to investigate these possibilities, but as it progressed the results of the skin tests raised doubts as to the validity of the assumption that the skin reactions indicated an allergy specific to byssinotic subjects. It is necessary, therefore, to describe the samples of dust examined, the methods used for extracting them, the properties of the extracts, and to assess critically the results of skin-testing.

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Methods of Extracting Cotton Dust

During preparation all extracts if they had to stand for a time were kept at 4° C. unless otherwise stated.

All extracts conformed to the standards laid down for sterility by the Statutory Rules and Orders (1931).

The samples of dust extracted and the method of extracting them are shown in Table 1. (For further particulars regarding the source and physical character of these samples as well as the bacteria and fungi which they contained Furness and Maitland (1952) should be consulted.)

The undiluted extracts contained about 0·1% of dried residue (Table 1); the Rimington extract was made up in this strength. Thus the dilutions referred to during this work were further dilutions of a solution of about 1/1000 weight/volume.

Method Based on Prausnitz' Method.—Shake 300 g. dust with 1,200 ml. petroleum ether continuously for six hours; after settling overnight filter the supernatant fluid through paper, discard the filtrate, and return the residue in the filter to the bulk of material. Do this four times. Filter through paper in a Buchner funnel, wash with petroleum ether, and dry the "fat-free" dust by sucking air through the mass.

To the dried residue add 1 litre of the solution (*p*H 7·0) described by Grove and Coca (1925) and shake for six hours. Leave overnight, add a further litre of the extracting solution, squeeze the suspension through four thicknesses of muslin, and filter the extract through paper pulp and a Seitz clarifying K5 disc. Retain the residue for further extraction. On saturation of the partially clarified extract with ammonium sulphate a light, buff-coloured precipitate appears which is filtered off with glass-wool and redissolved in distilled water. Dialyse for 48 hours in running water. Reduce the

TABLE 1

THE SOURCE OF COTTON DUST AND PREPARATION OF EXTRACTS

Designation of Extract	Sample of Dust*	Source of Dust	Viable Counts of Dust† (m. per g.)		Method of Extraction	Dried Weight of Extract (g. per 100 ml.)
			Aerobic Bacteria	Fungi		
I	G	Shirley cage*	620	12	Prausnitz (simplified)	0·12
II					Prausnitz‡	0·11
III					New York	0·22
IV					Rimington	0·1
V	F	Shirley cage	700	18	New York	—
VI					Phosphate buffer	—
VII	H	Air cleaning plant	4,000	8·5	Prausnitz§	—
VIII					Rimington	0·1
IX	E	Flue dust	108	60	Morgan	0·10
X					New York (simplified)	0·09

* Full details given in Furness and Maitland (1952) Table 1.
† Taken from Tables 3 and 6 in Furness and Maitland (1952), which give further details.
‡ Based on the method described by Prausnitz.
§ Filtered through kaolin instead of paper pulp.

volume to 50 ml. by evaporation under reduced pressure and not above 40° C. and freeze-dry the extract.

To the residue from the first extraction add 1 litre of Grove and Coca's solution, leave overnight, squeeze through muslin, filter, precipitate, and dialyse as before. Add the freeze-dried extract, dissolved in a minimum of distilled water, and make up to 700 ml.

To this somewhat cloudy extract, *p*H 5·0, add N/10 NaOH to bring the *p*H to 7·0–7·2, and remove the aggregated particles by centrifuging. Dilute two parts extract with three parts distilled water. Filter in 100 ml. amounts through a 6 cm. Seitz EK disc at 200 mm. Hg reduced pressure, taking about 30 minutes. Store at 4° C.

This technique departed somewhat from the procedure described by Prausnitz (1936). He sterilized the extract before precipitation by filtering it through paper pulp, a Seitz clarifying disc, and a Seitz EK disc, and carried out precipitation and dialysis aseptically. Filtration was relatively slow. The final dried extract, which he termed " protein ", dissolved incompletely and formed a thick viscous layer on the filter so that the possibility of filtration at this stage was abandoned. We also found filtration before precipitation to be slow and for that reason avoided it in order to reduce the possibility of adsorption of biologically active material on the filter. We, too, have noted that the final dried extract dissolved with difficulty and made a viscous suspension which could not be filtered. We have, however, resolved this difficulty by adjusting the *p*H which aggregated small particles, and by diluting the supernatant fluid after centrifuging. The solution then filtered easily and quickly. Prausnitz did not record *p*H values.

Simplified Prausnitz' Method.—To lessen the possibility of losing active substances manipulation was reduced by the following modifications.

Treat 100 g. dust with 400 ml. petroleum ether, shaking occasionally for 24 hours, and repeat the procedure. Extract the residue with 500 ml. Grove and Coca's solution, squeeze through muslin ; also repeat this procedure. Pool the extracts, and adjust with N/10 NaOH to *p*H 7·3. Filter through paper pulp in a Buchner funnel. Saturate the filtrate with ammonium sulphate, stand for three days, and then filter through glass wool. Dissolve the precipitate in 200–300 ml. distilled water, dialyse in running water for two days, and adjust the *p*H to 7·1. Dilute two parts of extract with three parts water. Pass through a Seitz EK 6 cm. disc with a positive-pressure filter, using a pressure of 600 mm. Hg. (about 300 ml. goes through fairly rapidly). Sterilize by filtering 100 ml. amounts through 6 cm. Seitz EK discs with 200 mm. Hg suction. Store at 4° C.

The New York Method.—This was described by Cooke (1947) and used by the allergy laboratory of the New York Postgraduate Medical School and Hospital for extracting allergens from dust. There were no difficulties in this procedure.

Treat 100 g. dust with 400 ml. petroleum ether, shaking occasionally for 24 hours and repeat. (Petroleum ether was substituted for the neutral solvent Sovasol No. 5 which was not available.) Extract with 500 ml. Coca's (1922) solution, *p*H 8·2, at room temperature for two days, shaking occasionally. Squeeze through four thicknesses of muslin and filter through paper pulp in a Buchner funnel. Dialyse the filtrate, about *p*H 8·0, against Evans' (1922) saline, *p*H 7·0, changing the saline daily until the *p*H value of the filtrate is the same as that of the saline. Filter off through paper the deposit that forms during dialysis. Remove the cloudiness by centrifuging the filtrate (*p*H 7·1), throwing down an appreciable deposit. Dilute two parts of extract with three parts of distilled water, filter in 100 ml. amounts through 6 cm. Seitz EK discs. Store at 4° C.

Simplified New York Method.—The following modifications were made to limit the possible loss of active substances. After extraction with Coca's solution partially clarify by centrifuging. Dialyse for four days changing the saline daily. Clear the extract by

centrifuging before sterilizing it by filtration through a gradacol membrane with a pore diameter of 0·99μ.

Rimington's Method.—Rimington, Stillwell, and Maunsell (1947) in extracting allergens from house dust based their technique on that of Sutherland (1942).

Mix 100 g. dust with 350 ml. N/100 ammonia and leave for 24 hours shaking occasionally. Squeeze through muslin and re-extract with 175 ml. N/100 ammonia. Pool the extracts, centrifuge, and filter through kaolin. To the supernatant fluid (260 ml.) add 5 g. sodium benzoate and leave for 24 hours; add HCl (1 part concentrated HCl to 5 parts water) while stirring until just acid to congo red. Filter off the precipitate of benzoic acid and redissolve it in acetone. Deposit the insoluble material by centrifuging, wash it twice with acetone, once with alcohol and once with ether, and dry *in vacuo*. Mix the powder (0·105 g.) with 5–10 ml. distilled water using a syringe and wide-bore needle, leave for 48 hours, centrifuge, remove the supernatant fluid, re-extract the deposit similarly, pool the extracts, and discard the solid residue. Freeze-dry the extract. Dissolve the dried extract (0·033 g.) in saline making a 0·1% weight/volume solution; sterilize by heating for 30 minutes in the steamer on three successive days. Store at 4° C.

Rimington and others (1947) found that their extracts of house dust were fully active at this stage of preparation, but they purified them further (Rimington and Maunsell, 1950).

Morgan's Method.—In view of the large numbers of bacteria and fungi in the dust (Furness and Maitland, 1952) and the possibility that the biologically active substances in dust might come from them, a further extract was made using the method devised by Morgan (1937) for extracting antigen from *Shigella shigae*.

To 100 g. dust add 200 ml. diethylene glycol; stand at 4° C. for seven days shaking occasionally. Squeeze through muslin and centrifuge to remove as much suspended material as possible. Dialyse for 24 hours in running water at room temperature and centrifuge. Dialyse for a further 24 hours and centrifuge. To 100 ml. of extract (Table 1) containing 0·103 g. of solid (*p*H 6·8) add 0·85% NaCl. Sterilize with a Seitz filter and store at 4° C.

Extraction with Citric Acid and Phosphate Buffer.—As the extracting fluids mentioned so far have been either alkaline or neutral, one extract was made using a slightly acid solution of the following composition, 0·2 M Na_2HPO_4 126·3 ml.; 0·1 M citric acid 73·7 ml. to which was added 1% NaCl and 0·4% phenol. Its reaction was *p*H 6·0.

Remove the wax from 85 g. dust with petroleum ether. Extract with 200 ml. buffer at 4° C. for two days, shaking occasionally. Squeeze through muslin and re-extract similarly with 100 ml. buffer solution. Pool the extracts and centrifuge, clearing them completely. Dialyse for four days against Evans' saline, renewing the saline daily. Sterilize with a Seitz filter. (The extract filters readily.) Store at 4° C.

The Chemical Examination of Extracts of Cotton Dust

Three extracts, obtained by different methods from one sample of dust, were examined qualitatively by a number of chemical tests. The results were very similar to those obtained by Rimington and others (1947) with extracts of house dust, although neither the source of the dust nor the method of extraction was the same. Further chemical studies of cotton dust will be reported in a later paper.

Several of the more potent extracts were found not to contain histamine or histamine-like substances.

Skin Tests with Extracts of Cotton Dust

The total number of persons tested was 291. All were observed for an early type of reaction and 143 of them for a late reaction.

The skin of the forearm was cleaned with alcohol and allowed to dry. Between 0·02 and 0·03 ml. of extract was injected into the superficial layers of the skin with a 26 gauge needle having a short bevel. Not more than four tests, with at least 5 cm. between them, were made on one arm and in addition one injection of physiological saline was always included as a control.

Reactions were read after 10 to 15 minutes, at intervals during the first hour, thereafter every two or three hours up to 12 hours, and finally at 24 hours. The size of the reaction was recorded as the mean of the two diameters at right angles, measured in millimetres.

Types of Reaction.—Two types of skin reaction, designated early and late, have occurred. Marked differences in the inherent reactivity of the skin of different individuals has been noted, resulting in differences in the size and intensity of each type of reaction, in response to the same concentration of extract. The reaction of different persons to the control injection of saline also varied.

Early Reactions.—A reaction was considered " positive " if there was a wheal (usually 5 mm. or more across) and erythema, with an overall diameter of 10 mm. or more. Within five to 10 minutes, occasionally even sooner, the pale papule raised by the injection enlarged, first as a flat pale wheal. The degree of pallor differed in different persons; it was seldom white and tense or pseudopodial at the edges. It soon became pink, and was usually surrounded by erythema of varying extent and intensity (referred to as the " flare "). The wheal was often 5–15 mm. and the whole reaction up to 20 to 30 mm. or even more in diameter. The

edges of the wheal diffused and merged with the erythema. The reaction then had the appearance of a slightly thickened red central area merging into a paler erythematous periphery with no very clear demarcation between the two zones. The whole reaction gradually became fainter and smaller ; the periphery receded but the centre remained thickened and red. If no late reaction supervened the central part of the reaction also faded in a few hours.

Variations in the intensity of reaction were noticeable in different persons with the same dilution of an extract or in the same person with different dilutions. The reactions varied in the area and degree of redness and the amount of thickening. Individual differences in reactivity were also apparent in the threshold dilution of extract below which there was no reaction. In some, but not all, elderly people whose skin seemed somewhat atrophic there was a comparatively faint redness with little or no thickening, yet clearly a reaction 10 mm. or more across.

Late Reactions.—The development of the late reaction could be followed most clearly when there was no early reaction preceding it. After the injection nothing was to be seen for three or four hours, the period varying somewhat in different persons. Then a small area of redness developed, gradually increasing in size, with a concurrent thickening of the central part, which, especially with stronger dilutions, was more intensely red than the outer zone, though with weaker dilutions there was no real demarcation bwtween them. The peak of the reaction as judged by size, thickening, and intensity of colour, was reached usually in 10 to 12 hours ; from then onwards it gradually faded. After 24 hours the reaction could often be detected by a faint redness which was sometimes nearly as large as at 12 hours. Thereafter, as a rule, it soon disappeared ; only in a few instances did it fade slowly and persist through the second day. A reaction of 5 mm. or more was regarded as positive.

The time elapsing between the injection and the beginning of the reaction, as well as the size and intensity of the reaction produced, were affected by the strength of the solution injected. The end-point of dilution of extract beyond which no reaction occurred varied tenfold in different persons. Some elderly people with an atrophic skin had little if any thickening. No one reacted with unusual severity to moderate doses. Severe reactions were caused in two members of the staff by injecting 100 times the strength of solution that would elicit a moderate reaction. Marked swelling and livid redness of the whole forearm occurred within three hours together with red streaks over lymph vessels, swollen and painful lymph nodes, and a rise in temperature. Prausnitz (1936) described a similar reaction in one person which he attributed to hypersensitivity brought on by a previous injection, but in the instances noted here there had been no previous injection, and moderate reactions were obtained with the usual doses. Sometimes a red streak in the skin over a lymphatic vessel of the upper forearm developed in test subjects showing three or four moderate reactions.

In many persons the late reaction was superimposed on an early reaction. Its character was not altered when this occurred but sometimes its beginning was not clearly marked because it was merged with the fading early reaction.

The grounds for considering the early and late reactions as separate entities are (1) the difference in time of beginning ; (2) the different appearance of the reactions ; (3) the fact that the late reaction often occurred without a preceding early reaction.

Control Reactions.—Physiological saline was included each time a person was tested. The very small pale papule raised by the injection sometimes disappeared in a few minutes without any reaction. More often the papule enlarged very slightly, became pink, occasionally surrounded with a very narrow ring of slight redness, and then faded. All these were minimal reactions, presumably due to the trauma of the injection, often less and rarely more than 3–5 mm. across ; they usually disappeared within 20 minutes and were regarded as negative.

A control reaction of the early type was positive when there was a wheal (usually 5 mm. or more across) and flare with a total diameter of 10 mm. or more. It developed within five to 10 minutes, usually had a flat white spreading wheal, occasionally with pseudopodial borders, surrounded by a flare of erythema several millimetres wide with an irregular margin. The wheal became pink, the edges less well defined, and then the whole reaction began to fade until after 30 minutes it had almost disappeared, but might persist for an hour or two. Persons with a positive control were obviously more reactive than the usual person and they were excluded from the series although their reactions to dust extracts were often much larger than their control reactions. The control was positive in 14 of 291 persons tested. Of these, 43 were tested on more than one occasion and their control reactions were consistent, with two exceptions which were negative the first time and positive three to four months later.

A late reaction sometimes developed in the control as a small red, slightly thickened area; anything over 4 mm. as an arbitrary limit was regarded as positive and the accompanying reactions were not included in the results. This control reaction coincided in time of appearance, rate of development, and onset of fading with the late reaction after the injection of extract. Sometimes it occurred when there had been no early reaction; in other cases it supervened after an early control reaction had faded and left a trace of thickening which then began to enlarge at about the same time as the late test reaction appeared. The control was positive in 15 of 143 persons tested. Of these 27 were tested more than once. Two who had negative controls on the first occasion had reactions of 4 and 7 mm. three to four months later. The remainder were alike each time.

A marked control reaction, 5 mm. or more in diameter, seemed to indicate a greater degree of reactivity of the skin, because in such persons the reactions to injections of extract were larger than usual and dilutions which were normally inactive elicited reactions. The cause of the late control reaction presumably differs from that of the early one, not merely on the basis of time but also because of the absence of flare.

Categories Tested for Reactions to Cotton Dust Extracts

Individuals tested for their reactions to intradermal injections of cotton dust extracts have been placed in the following categories according to their medical histories and occupations.

Normal Persons.—These were persons with no history of allergy, who had never been employed in cotton mills. Some were patients in hospital.

Allergic Persons.—Hypersensitive individuals, suffering from asthma, hay fever, or urticaria, were placed in this category.

Advanced Byssinotics.—These were men having a long history of the complaint and in many cases receiving disability pensions.

Early Byssinotics.—Workers having asthmatic symptoms on Monday and presumably in the early stages of the disease were thus classified.

Unaffected Card-room (CR) and Blowing-room (BR) Workers.—These were operatives working in the dust but without symptoms of byssinosis.

Other Mill-workers.—Persons who were or had been employed in cotton mills but who had not been in contact with the dust or only for a short period were included in this category.

The numbers in each category, including those who had positive control reactions, were as follows.

Category	Number Tested and Observed for Early Reaction	Number Also Observed for Late Reaction
Normal persons	146	84
Allergic persons	61	nil
Advanced byssinotics ..	31	11
Early byssinotics	16	12
CR and BR workers ..	21	20
Other mill workers	16	16
Total	291	143

Results with Early Reactions

The results of testing 10 extracts are summarized in Table 2.

Three groups of people, normal, allergic, and advanced byssinotic, were tested with all the extracts, sometimes with two dilutions of an extract. On the whole, the incidence of reactors among advanced byssinotics, the normal, and allergic groups was similar. The χ^2 test showed there was no statistically significant difference between the proportions of reactors in the various groups.

Extracts III, IV, and V, which, judging by the number of reactors, seemed to be the most active, were used for testing three further groups, early byssinotics, unaffected card-room and blowing-room workers, and other workers in cotton mills not exposed to the dust. Each of these groups had a similar incidence of reactors, which was of the same order as the first three groups.

Another approach in comparing byssinotics with others was to determine the threshold at which falling dilutions of extract ceased to cause reactions. Information on this point was obtained with extract III (Table 2). There was no indication that byssinotics were more reactive to higher dilutions. The one normal reactor to high dilutions is discussed in a later section.

Thus the general conclusion can be made that the incidence of an early type of skin reaction following the intradermal injection of cotton dust extracts was similar in workers exposed to cotton dust, whether they were suffering from byssinosis or not, and in other groups of the adult urban population. There was no evidence of specific hypersensitivity to something in cotton dust peculiar to byssinosis. Specific generalized hypersensitivity is unlikely to be the cause of byssinosis. Whether the early type of skin reaction produced by these extracts was to be regarded as an indication of hypersensitivity in a percentage of the whole

TABLE 2

INCIDENCE OF EARLY SKIN REACTIONS IN GROUPS INCLUDING NORMAL PERSONS AND BYSSINOTICS

Extract	Dilution	Persons Tested	Number Tested	Number Positive	Percentage Positive
I	1/10	Normal	4	0	—
		Allergic	27	3	11·1
		Advanced byssinotic	8	0	—
II	Undiluted	Normal	23	4	17·4
		Allergic	5	4	—
		Advanced byssinotic	11	1	9·1
	1/10	Normal	4	2	—
		Allergic	31	3	9·7
		Advanced byssinotic	17	2	11·8
III	1/10	Normal	68	36	52·9
		Allergic	11	7	63·6
		Advanced byssinotic	30	13	43·3
		Early byssinotic	15	5	33·3
		C.R. and B.R. workers*	19	9	47·4
		Other mill workers	13	6	46·2
	1/100	Normal	24	10	41·7
		Allergic	48	21	43·7
		Advanced byssinotic	19	2	10·5
		Early byssinotic	11	2	18·2
		C.R. and B.R. workers	11	3	27·3
		Other mill workers	6	1	—
	1/1,000	Normal	24	3	12·5
	1/10,000	Normal	15	1	6·7
		Advanced byssinotic	5	0	—
		Early byssinotic	7	0	—
		C.R. and B.R. workers	9	0	—
		Other mill workers	6	0	—
	1/100,000	Normal	15	1	6·7
IV	1/10	Normal	58	26	44·8
		Allergic	13	6	46·2
		Advanced byssinotic	28	10	35·7
		Early byssinotic	15	5	33·3
		C.R. and B.R. workers	18	7	38·9
		Other mill workers	12	5	41·7
	1/100	Normal	6	2	—
		Allergic	37	12	32·4
		Advanced byssinotic	17	1	5·9
V	1/10	Normal	59	25	42·4
		Allergic	15	9	60·0
		Advanced byssinotic	30	8	26·7
		Early byssinotic	15	6	40·0
		C. R. and B.R. workers	19	6	31·6
		Other mill workers	12	5	41·7
VI	1/10	Normal	—	—	—
		Allergic	6	5	—
		Advanced byssinotic	5	2	—
VII	1/10	Normal	17	9	52·9
		Allergic	6	5	—
		Advanced byssinotic	5	4	—
VIII	1/10	Normal	23	4	17·4
		Allergic	14	7	50·0
		Advanced byssinotic	11	3	27·3
IX	1/10	Normal	18	5	27·8
		Allergic	12	3	25·0
		Advanced byssinotic	5	2	—
X	1/1,000	Normal	81	20	25·1
		Advanced byssinotic	15	4	26·7
		Early byssinotic	15	3	20·0
		C.R. and B.R. workers	19	1	5·3
		Other mill workers	12	3	25·0

*C.R. and B.R. workers = unaffected card-room and blowing-room workers.

population or was due to some toxic action of the extract at the site of injection will be considered later.

All extracts produced reactions (Table 2) but some extracts were stronger than others, judged by the percentage of reactors in relation to the dilution

of the extract injected. Comparing extracts obtained from one sample of dust by four methods of extraction it is seen that extracts I and II obtained by the method of Prausnitz and its simplification respectively, were weaker than those, III and IV, obtained by the New York and Rimington methods. However with another sample of dust the Prausnitz method gave a strong extract, VII, about equal in potency to the Rimington extract, VIII. It is probably unwarranted to draw firm conclusions about the relative efficiency of the different methods used for extraction. All of them appear to be able to extract the substance responsible for eliciting the early reaction. The fact that extract IX, obtained by Morgan's method for extracting bacterial antigens, was effective, is of interest.

It was possible also to compare extracts II–IX by noting the incidence of reactors when several extracts were tested on each person of a series (Table 3). All the extracts caused reactions, but they differed in potency. The data suggested that the differences were quantitative rather than qualitative but there was no direct evidence on this point. The extracts were, of course, far from pure. Extracts III, IV, V, and VII were shown again to cause more reactions than extract II (cf. Table 2).

On the basis of wheal size alone, an analysis of variance applied to the results given in Table 3 (omitting extracts II and VI) showed no significant difference between extracts or between byssinotics and normals ; the interaction between those groups and the extracts was not significant.

Extract X (Table 2) was tested when freshly prepared for comparison with extracts I–IX which had been stored at 4° C. for periods up to 18 months. There was nothing in the results to suggest that storage had affected the extracts as regards their property of causing early reactions.

Results with Late Reactions

Five groups of people were tested, including normal controls, byssinotics, and unaffected workers in cotton mills (Table 4). An allergic group was omitted because it was not feasible to follow the reactions in out-patients for 12 to 24 hours. All groups reacted similarly. With a 1/10 dilution of extracts III, IV, and V over 90% of people had a reaction of 5 mm. or larger, mostly over 10 mm. These extracts were similar in potency although two samples of dust and two methods of extraction were used in making them (Table 1).

Tenfold dilutions of extract III were tested to determine its threshold in eliciting a late reaction in byssinotics and others (Table 5). With falling dilutions the size of the reactions and the incidence of reactors decreased but the limiting dilution was not sharp owing to differences in individual reactivity. The threshold was about the same in byssinotics, unaffected cotton mill workers, and normal persons.

Extract III which had been stored for some months was compared with freshly prepared extract X, both diluted 1/100, in eight normal

TABLE 3
COMPARISON OF ABILITY OF EXTRACTS TO ELICIT EARLY REACTIONS ON THE SAME PERSON

Person Tested	Extract and Dilution									Control
	II		III	IV	V	VI	VII	VIII	IX	
	Undiluted	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	
A	*5 . 30**	—	*5 . 26*	*5 . 28*	0 . 24	0 . 5	0 . 25	0 . 3	5 . 5	0 . 3
B	0 . 5	0 . 0	*7 . 13*	tr.	8 . 9	0 . 7	*8 . 22*	*7 . 15*	7 . 7	0 . 0
C	7 . 9	—	*11 . 28*	*9 . 31*	*10 . 30*	*7 . 24*	*8 . 18*	*11 . 28*	*5 . 14*	0 . 0
D	tr. . 11	—	*8 . 23*	*9 . 21*	0 . 8	0 . 6	*6 . 11*	9 . 9	6 . 6	0 . 6
E	0 . 0	0 . 0	*7 . 22*	0 . 0	*8 . 19*	—	—	0 . 25	—	0 . 0
F	—	*4 . 13*	*10 . 24*	*10 . 25*	*8 . 25*	*14 . 35*	*11 . 33*	*9 . 22*	*8 . 22*	0 . 6
G	0 . 0	—	6 . 6	*6 . 20*	6 . 6	—	*7 . 28*	5 . 5	6 . 6	0 . 0
H	0 . 6	—	*12 . 32*	5 . 5	5 . 5	—	*9 . 26*	0 . 0	*7 . 25*	0 . 0
	0 . 0	—	*5 . 35*	*6 . 35*	*6 . 22*	—	*7 . 30*	6 . 6	*6 . 28*	0 . 0
K	0 . 0	—	0 . 0	0 . 0	0 . 0	—	*5 . 19*	*6 . 14*	0 . 0	0 . 0
L	0 . 0	—	*7 . 13*	*7 . 25*	0 . 0	—	*5 . 16*	0 . 0	0 . 0	0 . 0

A – F were advanced byssinotics, G–L were normal persons.
* 5 . 30 = wheal of 5 mm. whole reaction 30 mm. The difference represents the width of the flare. 0 . 0 = no reaction. — = not tested.
Positive reactions shown in italics.

TABLE 4
INCIDENCE OF LATE REACTIONS IN NORMAL PERSONS AND COTTON OPERATIVES

Extract	Dilution	Group	Number Tested	Diameter of Reaction							
				>10 mm.		9–5 mm.		<5 mm.		No reaction	
				No.	%	No.	%	No.	%	No.	%
III	1/10	Normal	31	22	71	7	22·6	2	6·4	—	—
		Advanced byssinotic	10	9	90	1	10	—	—	—	—
		Early byssinotic	9	8	88·9	1	11·1	—	—	—	—
		C.R. and B.R. workers*	19	19	100	—	—	—	—	—	—
		Other mill workers	12	9	75	3	25	—	—	—	—
IV	1/10	Normal	25	15	65·2	5	21·7	2	8·7	1	4·3
		Advanced byssinotic	10	10	100	—	—	—	—	—	—
		Early byssinotic	9	9	100	—	—	—	—	—	—
		C.R. and B.R. workers	18	15	83·3	3	16·6	—	—	—	—
		Other mill workers	12	8	66·6	4	33·3	—	—	—	—
V	1/10	Normal	23	16	69·6	4	17·4	3	13	—	—
		Advanced byssinotic	10	9	90	1	10	—	—	—	—
		Early byssinotic	9	9	100	—	—	—	—	—	—
		C.R. and B.R. workers	19	18	94·7	1	5·3	—	—	—	—
		Other mill workers	12	11	91·6	1	8·3	—	—	—	—
X	1/1,000	Normal	23	8	34·8	4	17·4	3	13	8	34·8
		Advanced byssinotic	10	4	40	3	30	—	—	3	30
		Early byssinotic	9	1	11·1	4	44·4	—	—	4	44·4
		C.R. and B.R. workers	19	6	31·6	11	57·9	1	5·2	1	5·2
		Other mill workers	12	1	8·3	10	83·3	1	8·3	—	—

*C.R. and B.R. workers = unaffected workers in card-room and blowing-room.

persons. They elicited similar reactions. Storage had not therefore affected the ability of the extract to cause the late type of reaction.

There was nothing in the character of the late reaction to indicate the mechanism of its production, but the fact that 90% or more of normal persons reacted strongly supported the view that it was not due to hypersensitivity ; it seemed to be, therefore, an indication of the presence of some toxic substance in the extracts. The grounds for believing that the substances causing the early and late reactions may be different have already been stated.

Significance of the Results of Skin Tests

The main point to try to decide is whether the results recorded here do or do not indicate an element of sensitization in byssinosis.

One point, at least, is clear : there is no evidence of a sensitization which is peculiar to byssinosis. All groups of people reacted similarly, either with reference to the early or the late type of reaction, and in this respect byssinotics did not differ from other groups in the population.

Reasons have been given for considering that the late type of reaction is not due to sensitization.

TABLE 5
RESULTS OF SKIN TESTS WITH TENFOLD DILUTIONS OF EXTRACT III COMPARING THRESHOLDS IN ELICITING LATE REACTIONS IN BYSSINOTICS AND OTHERS

Dilution	Group	Number Tested	Number and Diameter of Reactions			Number with No Reaction
			>10 mm.	9–5 mm.	<5 mm.	
1/10	Normal	31	22	7	2	—
1/100	Normal	23	20	3	—	—
1/1,000	Normal	23	12	7	2	2
1/10,000	Normal	15	2	3	5	5
1/100,000	Normal	15	—	—	3	12
1/100	Advanced byssinotic	2	2	—	—	—
	Early byssinotic	6	5	1	—	—
	C.R. and B.R. workers*	7	7	—	—	—
1/10,000	Advanced byssinotic	2	—	—	—	2
	Early byssinotic	6	—	2	1	3
	C.R. and B.R. workers	7	1	2	—	4

*C.R. and B.R. workers = unaffected workers in card-room and blowing-room.

The significance that should be attached to the early reaction is much more difficult to decide. The reaction could be due to hypersensitivity, and if it is, then as determined by the technique described, about 30% of the adult urban population are hypersensitive to something in cotton dust extracts. On the other hand the reaction could be due to a direct toxic action on the tissues, and whether a person reacted or not would depend on the level of reactivity inherent in his tissues apart from any factor of specific sensitization or question of allergy. Again, since the extracts are far from pure, both an allergic factor and a toxic factor could be involved in the reaction. To differentiate between these possibilities is the problem.

The results reported here differ from those of Prausnitz (1936) in the incidence of early reactors found in the byssinotic and control groups. He tested smaller numbers of people but concluded that he had demonstrated hypersensitivity specific to byssinosis because nearly all byssinotics reacted and only one-third of control groups. The significance of this number of reactors in non-byssinotics was not discussed. He also transferred hypersensitivity passively to the skin of non-reactors. The detailed appearances and time of reaction were said to be peculiar to reactions obtained with cotton dust extract. It is impossible to determine whether the extracts used by us were the same as the one he used. They were, however, made from several samples of dust, by several recognized methods for extracting allergens, including one based on that of Prausnitz, and all the products were similar in causing skin reactions. The probability is that not all these extracts would have missed an allergen had it been present.

Prausnitz did not mention any reactions caused by control injections although his dose was 0·1 ml. of 0·5% phenol-saline. Controls were said to be negative. Undoubtedly control reactions do occur, as discussed in an earlier section, and should be fully considered if results of tests are to be assessed critically.

Results with Prick Tests

In testing for allergy a prick test is often preferred to intradermal injection in order to reduce trauma to a minimum. Comparisons were made, therefore, in the same persons of the reactions produced by a prick test with extract III undiluted and by intradermal injection of 0·02 ml. of a 1/100 dilution. For the prick test a needle protruding from a rubber bung was pressed into the skin through a drop of extract. The amount of extract given intradermally was about 70 times the amount estimated by Squire (1950) to be introduced into the skin by a prick test. Only highly reactive persons would be expected to respond to a prick test, and any who did react might be specially considered as possibly allergic subjects.

Early Reactions in Normal Persons.—The saline control was negative in all cases with the prick test (a wheal of not more than 1 mm. was regarded as negative) but in one subject the intradermal control was positive and this test was excluded. Only 8·5% of the prick tests were positive compared with 41% of intradermal tests (Table 6). The question arises, Does the difference in results with these two tests indicate a qualitative or only a quantitative difference in the reactivity of an individual? Similarly in the case of the normal person who reacted to an intradermal injection of 1/100,000 extract III (Table 2), Was he qualitatively or only quantitatively different from the other 14 who were tested and did not react? In other words, Were the few who reacted to the prick test and to high dilutions by the intradermal test to be regarded as hypersensitive and all the others who reacted to the intradermal test as not hypersensitive, or were all the reactors hypersensitive and some much more so than others, assuming that the early reaction does in fact indicate hypersensitivity?

Early Reactions in Cotton Workers.—A number of workers exposed to cotton dust, some of them with symptoms of byssinosis, were tested by the prick test with 0·1% histamine and extract X

TABLE 6

COMPARISON OF THE ACTIVITY OF EXTRACT X IN NORMALS WITH PRICK AND INTRADERMAL TESTS

Method	Dilution	Early Reactions			Late Reactions					
					No. Tested	Size of Reaction (mm.)				No Reaction
		No. Tested	No. Positive	Percentage Positive		19–15 (no.)	14–10 (no.)	9–5 (no.)	<5 (no.)	(no.)
Intradermal ..	1/100	34	14	41·1	30	5	14	9	1	1
Prick*	Undiluted	35	3	8·57	16	—	—	5	5	6

*Any early reaction having a wheal of 1 mm. or less was considered negative for the prick test.

undiluted. The object was to determine whether those who reacted to extract were more sensitive than normal to histamine. In 51 persons tested all the saline controls were negative. Two failed to react either to extract or histamine. The remaining 49 all reacted to histamine of whom three reacted to extract; the details of their reactions follow.

Case	Histamine (0·1%)	Prick Test with Extract X		Earlier Intradermal Test with Extract X	
		Dilution	Reaction	Dilution	Reaction
1	W3·5 E14*	Undiluted 1/10	W1·75 E9 W1·0 E3	1/1000	Nil
2	W4 E16	Undiluted 1/100	W4 E19 Nil	—	—
3	W3·5 E14	Undiluted	W2 E3	1/1000	W6·5 E27

* W3·5 E14 = wheal 3·5 mm., erythema 14 mm. in diameter.

In all those who reacted to histamine the mean diameter of the wheal was 3·5 mm. and of the erythema 18 mm. In another group of 20 students the figures were 4·5 and 20 mm. This agrees well with the sizes of the histamine wheal recorded by Squire (1950) in his Table III for normal persons and in his Table V for two asthmatics. In both series there was little individual variation. It may be concluded therefore that the three persons who were much more reactive to extract than the majority were not more sensitive to histamine. All three had symptoms of byssinosis but there were 17 byssinotics who did not react. There was no correlation between reaction to extract by the prick test and symptoms of byssinosis. Normal persons reacted equally with symptomless workers and byssinotics.

Late Reactions.—The prick test with undiluted extract X caused some late reactions, but fewer and smaller than after the intradermal injection of 0·02 ml. of 1/100 dilution (Table 6). The prick test merely stressed that the incidence and size of the late reaction is related to the amount of extract introduced into the skin.

Comment

The conclusion to be drawn from all the results is that the skin testing of byssinotic persons with cotton dust extracts fails to distinguish them from their symptomless fellow workers, and similarly card-room workers as a group are not differentiated from the rest of the population. Reactors are distributed equally between the affected and the unaffected groups so that even if further investigations should demonstrate that the wheal and flare type of reaction to cotton dust extracts is a true allergic response it would appear to have little importance in the genesis of byssinosis.

Chemical tests made on cotton dust extracts suggest a relationship between them and house dust extracts and it is relevant that house dust extracts cause a wheal and flare type of skin reaction which is considered to denote a specific allergy (Rimington and Maunsell, 1950). It may be that there is a similar allergen in cotton dust and house dust.

The fact that a few persons are highly reactive to cotton dust extracts, either by the prick test or following intradermal injection of high dilutions, although they react normally to histamine, might be regarded as an indication of hypersensitivity to an allergen in the cotton dust extract. These reactors occur, however, in normal persons who have no allergic manifestations and who have never been exposed to cotton dust. Supplementary evidence is necessary to settle the question as to the essential nature of these reactions, such as might be obtained from attempts to demonstrate antibody in the serum of reactors by passive transfer of reactivity to human or animal tissue, by serological tests for the demonstration of antibody, by sensitizing animals with extracts, or by tests for toxicity. It is necessary to determine also whether the few who react to the prick test differ essentially in the mechanism of their reaction from the larger number who react to an intradermal injection and who likewise have no allergic symptoms and have never been exposed to cotton dust. Investigations of these and related problems are proceeding.

The late reaction produced in more than 90% of persons by the intradermal inoculation of 0·002 mg. of dried extract, and in some by the small amount introduced by pricking the skin, is almost certainly a direct toxic effect on the tissues. The incidence and intensity of the reaction is related to the amount and the strength of the extract injected. It is clearly desirable to separate this toxic substance and study its direct effect on tissue as a factor in the

causation of byssinosis. The source of the substances in dust which are responsible for both the early and the late reaction also requires elucidation.

Summary

Ten extracts have been made from four samples of cotton dust using seven extractive methods including standard methods for the extraction of allergens and bacterial antigens.

All the extracts were used for skin tests and were similar in causing two types of reaction, an early reaction of wheal and flare and a late reaction, maximal in about 12 hours, of redness and induration. Extracts varied somewhat in potency. Several groups of persons, 291 in all, were compared, including early and advanced byssinotics, unaffected workers in the card-room and blowing-room, other mill-workers not exposed to cotton dust, allergic persons not associated with cotton mills, and persons with no history of allergy likewise never exposed to cotton dust. The incidence of reactors in all these groups was similar.

About 90% had a late reaction, which is considered to be due to the direct action on the tissues of a toxic substance extracted from the dust.

About 30 to 40% had an early reaction following intradermal injection of a suitable dilution of extract. A small percentage reacted to intradermal injection of high dilutions or to the prick test. This reaction may indicate hypersensitivity to something in cotton dust, but that is not certain and further work is required to establish its significance. It is clear however that the reaction does not indicate hypersensitivity specific to byssinosis since reactors occurred equally in all groups of the adult urban population. Specific generalized hypersensitivity is unlikely to be the cause of byssinosis. A possible relationship between cotton dust and house dust is suggested by the chemical properties of the respective extracts and the skin reactions they produce.

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