

Immunochemical evaluation of antigenicity of house dust extract

Specificity of dermal wheal reactions and responses to injections for immunotherapy

Charles D. May, M.D.,* Margaret Lyman, M.D., Rosalina Alberto, M.D., and Jane Cheng, M.D., New York, N. Y.

Immunochemical procedures were applied to an evaluation of antigenicity of house dust extract. A dermal wheal reaction to house dust extract was invariably accompanied by release of histamine from the subject's leukocytes exposed to the extract in vitro; when no dermal reaction occurred, histamine was not released. Serum of every subject with a dermal wheal reaction to house dust extract was able to neutralize the antigenic activity of the extract to some degree, while serum of nonreactive persons did not. Serial injections of house dust extract produced up to 56-fold increases in specific antigen neutralization capacity of the serum in a small portion of hypersensitive subjects who received unusually large amounts, but the leukocytes were not desensitized to antigenic release of histamine. The findings support the contention that house dust contains immunologically specific antigenic material capable of causing specific hypersensitivity and provoking specific immune response upon injection in atopic persons. The clinical implications are considered.

The idea that something in house dust may be a cause of allergic rhinitis gained prominence in clinical practice about 50 years ago^{1, 2} through support from an immunologic study by Cooke.^{1†} In the ensuing years extracts of house dust were found to evoke wheal reactions in the skin of the majority of atopic individuals.³ The opinion has often been expressed that extracts of house dust contain irritative materials that may produce confusing dermal reactions in "normal" persons which are not indicative of specific immunologic response.⁴

From the Department of Pediatrics, New York University School of Medicine.

Presented in part at the Annual Meeting of the American Academy of Allergy, March 18, 1969.

Received for publication Sept. 10, 1969.

*Career Scientist of the Health Research Council of the City of New York. Supported by Grant HD-01892, Public Health Service.

†The suggestion that house dust may play an etiologic role in asthma first appeared in the literature at least 100 years ago in Henry Salter's book, *On asthma* (London, 1860), pp. 126-127.

Nevertheless the practice of giving injections of house dust extract for "desensitization" became widespread without ever being subjected to objective evaluation of the clinical or immunologic effects.

The purpose of the present study was to apply immunochemical procedures to the question of the immunologic specificity of wheal reactions to intradermal administration of a house dust extract and to the determination of immunologic responses to a series of subcutaneous injections of the house dust extract.

PLAN OF STUDY AND SUBJECTS

The subjects were children of ages 6 to 16 years from the patient population of the Pediatric Allergy Clinic of Bellevue Hospital. They were predominantly of Puerto Rican extraction, mostly born and reared in New York City. They were from the poor economically, but the mothers generally struggled to keep the crowded, vermin-invaded homes clean, though bedding and furnishings were well worn. The subjects enrolled in the study proved to be remarkably dependable for regular examination. Studies could not be completed on only 3 of the originally selected group, because they moved to Puerto Rico.

Subjects selected for study of immunotherapy had been shown to be hypersensitive to a house dust extract by a 2 to 4+ dermal wheal reaction and substantial release of histamine from leukocytes with the house dust extract. They also had the following qualifications: (1) definite clinical manifestations of allergic rhinitis or bronchial asthma or both; (2) dermal wheal reactions to other allergens like ragweed and others as further evidence of an atopic constitution; and (3) a reasonable prospect of faithful attendance throughout a long-term study.

The subjects were unsystematically divided into two groups, one group to

Table 1. *Customary dosage schedule for injection therapy with the house dust extract used in the present study*

<i>Weekly dose No.</i>	<i>HDE* (μg PN†)</i>
1	0.1
2	0.2
3	0.4
4	0.7
5	1
6	2
7	4
8	7
9	10
10	15
11	20
12	30
13	40
14	50

50 μ g of PN every 2 weeks thereafter as a maintenance dose. This program provides 1,084 μ g of PN the first year and a total of 1,980 μ g of PN after 20 months.

Excerpt based on suggested schedule provided by the manufacturer, Center Laboratories, Port Washington, N. Y., in instructions accompanying each vial. Current textbooks of clinical allergy recommend essentially the same dosage schedule.

*HDE: house dust extract.

†To convert to PNU multiply by 100.

receive serial injections of the house dust extract and the other to receive no injections. Both groups were allowed drugs commonly employed for relief of symptoms. Initially and at 3 to 4 month intervals blood was obtained from the subjects for determination of leukocyte sensitivity and antigen neutralization by serum with the house dust extract by procedures to be described. All subjects were examined and appropriate drug therapy prescribed at 2 to 4 week intervals in the Pediatric Allergy Clinic at Bellevue Hospital by the same group of 4 pediatricians occupied full-time in the Pediatric Allergy Service of New York University Medical Center.

Treatment of those subjects receiving subcutaneous injections of the house dust extract was instituted about as suggested in the customary dosage schedule in the instructions provided with each vial of house dust extract by the manufacturer (Center Laboratories) (Table I). By graduated weekly doses the amount injected was increased according to tolerance until the full recommended dosage was achieved. Then the interval between injections and the dosage was further increased until the majority ultimately were receiving 1 ml. of house dust extract containing 200 μ g of protein nitrogen (PN) (20,000 protein nitrogen units) every 3 to 4 weeks. Total dosage received will be tabulated in the Results section.

In addition to these groups of subjects involved in determination of the immunochemical effects of a customary dosage schedule of injections of house dust extract, 3 subjects with the same qualifications were studied while receiving a more intensive schedule of injections to ascertain the effect of greater dosage in shorter time.

While careful clinical records were kept, precise clinical evaluation by a scoring system was not undertaken. The purpose of this study was to ascertain the immunologic response to injections of house dust extract preparatory to selection of a suitable dosage regimen for clinical evaluation, in case encouraging immunologic response was observed.

MATERIALS

To have an antigen free of preservative and which could reasonably be expected to be stable, the house dust extract chosen as antigen in this study was a sterile freeze-dried product manufactured by Center Laboratories, Port Washington, New York, from old mattresses and carpet sweepings. It was purchased through regular marketing channels in sufficient quantity so that use of only 2 lots was required. These lots were found to give identical quantitative release of histamine from leukocytes of a sensitive subject in simultaneous *in vitro* analyses with the procedures employed in the study.

Solutions of the dried house dust extract were prepared aseptically with sterile tris-CM buffer, pH 7.6, of the composition used for suspensions of leukocytes in the histamine release procedures.⁵ A stock dilution containing 200 μ g of PN per milliliter* was prepared freshly every 2 months, and lower concentra-

*Throughout this report amounts of allergen extracts are expressed in terms of micrograms of protein nitrogen (μ g of PN) and concentrations as μ g of PN per milliliter; 1 μ g PN = 100 protein nitrogen units in the familiar terminology of clinical allergy.

tions were not used longer than 2 weeks. No preservatives were added, and great care was taken to preserve sterility. If contamination was remotely suspected, the solution was discarded.

A fresh supply of normal serum was obtained about every 3 months from a single donor, blood group AB. The donor was free of allergic or other disease, gave no dermal reaction to the house dust extract (10 μ g of PN per milliliter) or three other common allergens, and had ingested no drugs during the week prior to withdrawal of blood. This donor's serum always enhanced histamine release compared with release in the presence of serum from a dust-sensitive subject or in the presence of crystallized human albumin and may be presumed to have been without any capacity to neutralize the antigenic activity of the house dust extract.

PROCEDURES

Our methods for collection of blood and separation of leukocytes, measurement of antigenic release of histamine from leukocytes, and determination of antigen neutralization by serum have been described in detail.⁵

Detection of hypersensitivity to the house dust extract and other allergens was by means of intradermal injection of about 0.01 ml. of extract containing 1 μ g of PN per milliliter, as a first test, and 10 μ g of PN per milliliter if a higher concentration was required to demonstrate a definite reaction or a lack of reaction. Wheals with diameters greater than that of a buffer control by about 5 mm., 10 mm., 15 mm., or 15 mm. with pseudopodia were recorded as 1, 2, 3, and 4+, respectively. Disposable needles and syringes were used.

EXPRESSION OF RESULTS

The nature of the data derived from determinations of leukocyte sensitivity and antigen neutralization by serum is depicted graphically in Figs. 1 and 2.

Leukocyte sensitivity

The curves for concentration of antigen versus per cent of histamine released from leukocytes in the presence of normal serum (Fig. 1) reveal changes in leukocyte sensitivity during and after a period of injections of house dust extract. Note that 100 per cent of the histamine was not released, the maxima being 62 to 75 per cent. Maximal release in many subjects at some time in a series of observations was observed to be less than 50 per cent. Also the point of maximal release cannot always be located when economy of blood sample limits the range of concentrations of antigen tested. Consequently, to compare leukocyte sensitivity by calculation of the familiar "EC-50" (effective concentration for 50 per cent release) would lack precision when release is less than 100 per cent and cannot be done at all in case of less than 50 per cent release. We did not wish to arbitrarily exclude cases of the latter type from the study.

Because of these considerations, the leukocyte sensitivity in a series of observations is expressed in terms of the per cent of histamine released by a given or

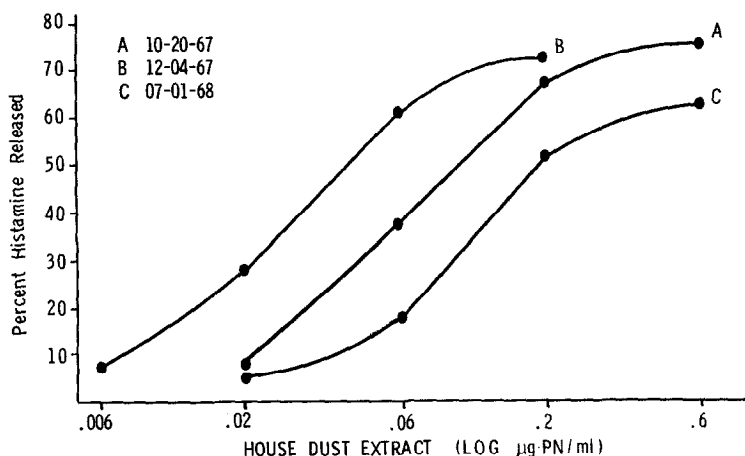


Fig. 1

Series of concentration-response curves to illustrate procedure described in Expression of results for calculation of leukocyte sensitivity. Serial determinations of release of histamine from leukocytes by house dust extract. Patient No. 253; sensitive to house dust; injections of house dust extract Nov. 3, 1967 to April 29, 1968.

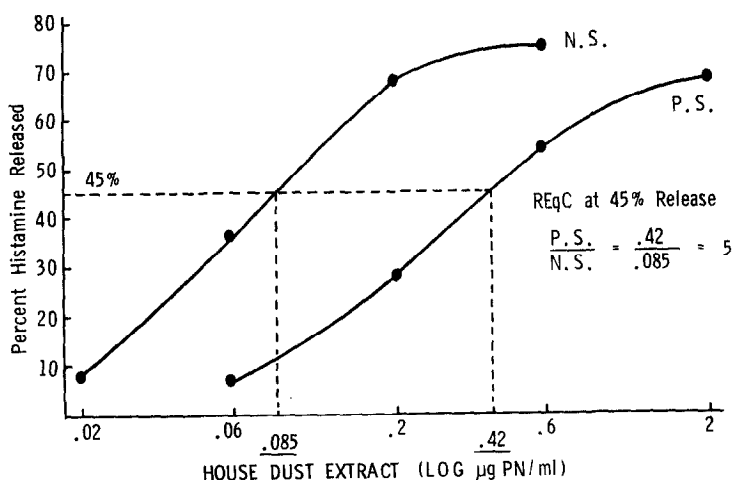


Fig. 2

Example to illustrate procedure described in Expression of results for calculation of REqC or antigen neutralization capacity of serum. Histamine released from leukocytes by antigen incubated with normal serum (N.S.) versus patient's serum (P.S.). Patient No. 253A; sensitive to house dust; antigen, house dust extract.

fixed concentration of antigen. This concentration is selected by inspection of graphic plots of the concentration-response curve of each determination, whereby a concentration of antigen is chosen which will give a value for per cent of release lying on the most linear portions of the curves from a series of determinations. Thus, in the example in Fig. 1, per cent of release by 0.06 μg of PN per milliliter for each curve is: $A = 37$, $B = 61$, and $C = 18$. The ratios of the latter values to the initial value provide an expression of the magnitude of the change in sensitivity— B/A or $61/37 = 1.64$; C/A or $18/37 = .43$. A ratio above 1.0 represents an increase in leukocyte sensitivity, and below 1.0 a decrease.

Antigen neutralization by serum

Antigen neutralization by a subject's serum is determined by comparing the release of histamine from leukocytes by antigen incubated with a normal serum to release with the subject's serum by procedures described elsewhere.⁵ Fig. 2 shows the manner of plotting typical data and how the calculation is made. A percentage release of histamine is selected which will give a point within the most nearly linear portions of both concentration-response curves. From these points the concentrations of antigen previously incubated with normal or subject's serum that were required to release the same per cent of histamine are read off the scale. These are designated "equivalent concentrations" of antigen (EqC), and the ratio between them is the REqC. Because sera from normal, nonallergic individuals always enhance release and sera of allergic subjects inhibit release by neutralization of antigen (or more antigen is needed for equivalent release), the ratio of the equivalent concentrations is arranged with EqC for subject's serum/EqC for normal serum to give a value greater than 1. The REqC is an expression of the antigen neutralization capacity of the subject's serum. Referring to Fig. 2, the procedure for calculating the REqC can be easily traced: at 45 per cent release the EqC's for subject's and normal sera are 0.42 and 0.085 μg of PN per milliliter, and the REqC at 45 per cent is $0.42/0.085$ or 5.

Change in the REqC or antigen neutralization capacity over a period of time can be stated simply by the ratio of the final REqC over the initial REqC, as will be seen in tabulations of data in the Results section. A ratio greater than 1.0 means an increase in antigen neutralization capacity, and less than 1.0 a decrease.

RESULTS

Specificity of dermal wheal reactions to house dust extract

Concentrations of house dust extract between 6×10^{-5} and 2×10^2 μg of PN per milliliter were used for tests of histamine release from leukocytes. In no case was histamine released by less than 2×10^{-3} μg of PN per milliliter. As may be seen in Table II, a dermal wheal reaction to house dust extract was invariably associated with in vitro release of histamine from leukocytes, and, conversely, when there was no dermal reaction, histamine was never released from the leukocytes in vitro. Every individual found to have a wheal reaction to intradermal injection

Table II. *Dermal wheal reaction versus in vitro release of histamine from leukocytes with house dust extract*

	<i>Histamine released</i>	
	<i>Yes</i>	<i>No</i>
<i>No dermal reaction*</i> 50 subjects	0	50
<i>Dermal wheal reaction*</i> 115 subjects	115†	0

*Approximately 0.01 ml. of extract given intradermally, first 1 μ g of PN per milliliter and, if no reaction, 10 μ g of PN per milliliter. Wheals larger than bleb from injected fluid were considered positive, usually 10 to 15 mm. greater in diameter and often 15 mm. even with the initial weaker dose of 1 μ g of PN per milliliter.

†Maximal release of histamine ranged from 25 to 100 per cent of total available in leukocytes, usually 60 to 80 per cent. To obtain this a concentration of extract of from 0.06 to 20 μ g of PN per milliliter, mostly between 0.2 and 6 μ g of PN per milliliter, was required.

tion of the house dust extract and who invariably exhibited in vitro release of histamine from the leukocytes with the extract also gave a history of definite clinical manifestations of allergic disorders and had wheal reactions to intradermal injection of other allergens. Careful questioning was sometimes required to identify allergic disorders in the history of subjects; even some physicians in residency training were unaware of the significance of their symptoms in the past or present until their memories or powers of observation were rendered more acute by the demonstration of hypersensitivity to several allergens by dermal reactions and in vitro release of histamine from leukocytes.

In other words, every individual found to give a wheal reaction to intradermal injection of the amounts of the house dust extract used in this study was shown to possess the characteristics of an allergic or atopic person. In our population fully 90 per cent of atopic persons have a wheal reaction to intradermal injection of this house dust extract, and no nonallergic persons have been discovered to have such a reaction.

Not only did the house dust extract release histamine exclusively from the leukocytes of dermal reactors, but the sera of reactive persons invariably had the capacity to neutralize the antigenic property of house dust extract. This sort of antigen neutralization capacity is ascribed to presence of specific antibody in the serum.⁶ The magnitude of antigen neutralization by serum of house dust-sensitive subjects is shown in Tables IV and V as the initial REqC in the untreated and before injections in the treated subjects (REqC-I). The REqC-I values indicate that the sera of subjects sensitive to house dust extract were able to neutralize the histamine-releasing antigenic property of the extract in every case and sometimes strikingly—an average value of 8 and up to 33. Sera of normal, nonallergic subjects having no dermal reaction to house dust extract do not neutralize the antigenic property of this material and in contrast actually enhance the antigenic release of histamine from leukocytes.

The antigen neutralization capacity of the serum in hypersensitive subjects can be specifically increased by subcutaneous injections of the antigen. Table III gives an example of specific increase in REqC of the serum for house dust

Table III. *Specificity of response in antigen neutralization capacity of serum to injections of antigenic extracts*

<i>Subject W. L. No. 102 (sensitive to HDE and ragweed)</i>		
	<i>REqC</i>	
	<i>House dust extract</i>	<i>Ragweed extract</i>
2-19-68 Before injections	5	3
2-20 to 4-8-68 4,250 µg PN HDE injected		
4-8-68 After injections	178	5
<i>Subject N. J. No. 56 (sensitive to HDE and Alternaria)</i>		
	<i>REqC</i>	
	<i>House dust extract</i>	<i>Alternaria extract</i>
5-15-68 Before injections	10	66
6-28 to 11-15-68 1,820 µg PN Alternaria extract injected		
11-15-68 After injections	2.7	274

extract in a subject also hypersensitive to ragweed. The REqC for ragweed was essentially unchanged during injections of house dust extract, for which the REqC was increased 36-fold. Also given is an example of the negligible effect of injection of Alternaria extract on the REqC for house dust extract, while increasing the REqC for Alternaria fourfold.

A few individuals found to give no dermal reaction or histamine release from leukocytes with house dust extract were retested on several occasions, and these results were repeatedly confirmed. A dermal wheal reaction and substantial release of histamine from leukocytes with house dust extract has been observed as early as 1½ years of age.

Thus, evidence for the immunologic specificity of antigenic material in the house dust extract studied includes: (1) Wheal reactions to intradermal injection were elicited only in atopic individuals with allergic manifestations. (2) Histamine could be released substantially in vitro by house dust extract from leukocytes of all subjects with wheal reactions to dermal injection of the extract. Histamine release never occurred with leukocytes of subjects unreactive to intradermal injection of house dust extract, as might be expected if it were a nonspecific irritant. (3) Sensitivity of skin and leukocytes to house dust extract is invariably associated with specific neutralizing antibody in the serum, which can be specifically increased by injection of house dust extract.

Immunologic responses to injections of house dust extract

Customary dosage schedule. The data pertaining to leukocyte sensitivity and antigen neutralization by serum at the beginning and at the end of the relatively long periods of observation of the treated and untreated groups are provided in Tables IV and V. Representative findings in serial determinations are shown in Figs. 3 and 4.

The two groups turned out to be quite similar in the initial leukocyte sensitivities and antigen neutralization capacities of the sera. Neither group had a significant change in mean leukocyte sensitivity ($F/I = 1.32 \pm 1.12$ and

Table IV. Initial and final leukocyte sensitivity and antigen neutralization capacity of serum of 12 subjects hypersensitive to house dust extract observed for prolonged period without injection therapy

No.	Age (yrs.)	Period (mo.)	Leukocyte sensitivity				REqC‡ (Antigen neutralization)		
			Anti- gen* (μ g PN/ ml.)	I†	F†	F/I‡	I	F	F/I
37	16½	28	0.6	61	32	0.52	2.3	2.2	0.96
167	11	19	2.0	44	60	1.36	10.0	10.0	1.00
163	11	18	0.06	29	68	2.34	20.0	60.0	3.00
174	14½	13	6.0	97	34	0.35	5.0	1.0	0.20
165	9¼	23	2.0	17	72	4.22	10.0	11.3	1.13
181	15	19	0.6	50	23	0.46	3.3	3.3	1.00
56	13¾	23	0.2	61	49	0.80	2.5	2.7	1.17
107	10½	21	6.0	26	25	1.04	10.0	10.0	1.00
205	8¼	19	2.0	67	65	0.97	11.1	2.0	0.18
171	13¾	22	0.6	27	62	2.30	3.0	7.0	2.33
82	9¼	19	20.0	73	53	0.72	8.0	10.0	1.25
168	12½	20	2.0	65	50	0.77	10.0	7.7	0.77
Mean		20				1.32	7.93	10.6	1.16
S.D.						1.12	4.89	15.3	0.77
Range		(13-28)				(0.35-4.22)	(2.3-20.0)	(1.0-60.0)	(0.18-3.0)

I = initial; F = final; REqC = ratio equivalent concentrations.

*House dust extract.

†Per cent of available histamine released from leukocytes by the concentration of antigen shown, initial and final.

‡For meaning of F/I ratios and REqC see section on Expression of results and discussion of data from this table in Results.

1.19 $\pm$.45), and repetition of intradermal injections of house dust extract still gave 3 to 4+ wheal reactions in all subjects. There were modest net increases in leukocyte sensitivity (F/I) in 6 of 12 untreated and 5 of 21 treated subjects.

The mean value of 31 for final antigen neutralization by serum (REqC-F) in the treated group was clearly greater than the mean of 10.6 in the untreated group. But inspection of the data reveals that the difference was largely dependent upon considerable increases in REqC in 3 subjects (Nos. 52, 79 and 136) with final values (REqC-F) of 185, 100, and 150. Calculations of the mean values of REqC-F and REqC-I and F/I for the treated group excluding the 3 exceptional subjects reveals that the great majority of the remaining 18 treated subjects had insignificant increases in antigen neutralization capacity of the serum, compared with that of the untreated, as a result of injections of customary dosage of house dust extract (mean F/I for REqC: 1.92 $\pm$ 2.3 and 1.16 $\pm$.77). The treated subjects received a mean total dose of 1,460 μ g of PN of house dust extract in the mean of 21 months, compared with 1,980 μ g of PN that would be provided in 20 months by the manufacturer's suggested schedule (Table I). The difference is probably due to slowness in reaching the maintenance dose in some subjects in this study; ultimately a larger maintenance dose was being given to nearly all treated subjects, though less frequently than that suggested in the manufacturer's schedule (200 versus 50 μ g PN and every 4 versus every 2 weeks).

Table V. Initial and final leukocyte sensitivity and antigen neutralization capacity of period of injection therapy with customary dosage of house dust extract

No.	Age (yrs.)	Period (mo.)	Total HDE (μ g PN/ml.)*	Leukocyte	
				Antigen (μ g PN/ml.)	I†
30	11	24	1,820	0.6	67
105	9	26	2,310	2.0	40
95	10	17	990	0.6	74
35	9	22	1,210	0.6	55
52	5½	21	1,920	0.06	48
50	8	23	1,290	2.0	50
84	7	17	660	0.6	44
162	8½	23	610	2.0	66
97	9	12	530	2.0	44
40	12	22	1,430	2.0	62
146	7	22	1,900	20.0	38
178	7	24	1,940	0.6	86
65	9½	21	1,000	0.6	32
143	12	23	2,270	0.2	83
79	15	18	2,190	0.02	51
49	10	20	1,340	0.6	63
136	12	18	1,380	0.06	45
41	10	19	1,290	0.2	50
86	9	21	1,300	0.06	85
73	8	24	1,560	2.0	34
57	11½	21	1,650	6.0	45
Mean		21	1,460		
S.D.					
Range		(12-26)	(530-2,310)		

Excluding Nos. 52, 79, 136

Mean
S.D.
Range

I = initial; F = final; REqC = ratio equivalent concentrations.

*1 μ g PN = 100 PNU.

†Per cent of available histamine released from leukocytes by the concentration of antigen

‡For meaning of F/I ratios and REqC see section on Expression of results and discussion of

The 3 exceptional treated subjects did indicate a substantial immunologic response could be evoked in some hypersensitive subjects by house dust extract. As they had been the recipients of some of the larger total doses of house dust extract and treated for the longer periods, it was decided to test the effect of a more intensive dosage regimen—higher dose in shorter time.

Intensive schedule. Three more subjects were hypersensitive to house dust extract, possessed the same qualifications as the groups already described, and had never received injection therapy with house dust extract. They were given injections of house dust extract by relatively intensive regimens: 2,300 μ g of PN in 6½ months; 3,000 μ g of PN in 6½ months; and 6,250 μ g of PN in 3 months (compared with the mean of 1,460 μ g of PN in 21 months by the customary schedule). The effects on leukocyte sensitivity and antigen neutralization by serum are shown in Figs. 5, 6, and 7.

The responses in the least intensively treated subject (Fig. 5) were within the range of responses in the group treated by a customary schedule. Immunologic responses evoked in the subject receiving injections of house dust extract

serum of 21 subjects hypersensitive to house dust extract observed for prolonged

<i>sensitivity</i>		<i>REqC†</i> (<i>Antigen neutralization</i>)		
<i>F†</i>	<i>F/I†</i>	<i>I</i>	<i>F</i>	<i>F/I</i>
22	0.33	10.0	10.0	1.00
55	1.37	3.0	15.0	5.00
74	1.00	10.0	10.0	1.00
80	1.45	1.3	5.6	4.30
47	1.00	3.3	185.0	56.00
67	1.14	1.4	3.7	2.60
85	1.93	10.0	10.0	1.00
50	0.76	5.0	5.3	1.06
70	1.60	8.0	33.3	4.20
62	1.00	7.3	20.0	2.73
42	1.10	4.5	6.0	1.30
81	0.94	10.0	10.0	1.0
70	2.20	10.0	10.0	1.00
78	0.83	10.0	35.0	3.50
65	1.27	10.0	100.0	10.00
49	0.77	10.0	10.0	1.00
70	1.55	10.0	150.0	15.00
94	1.87	10.0	14.0	1.40
57	0.67	10.0	6.5	.65
38	1.11	10.0	10.0	1.00
48	1.07	3.0	3.0	1.00
1.19		7.47	31.0	5.50
0.45		3.3	48.9	11.9
(0.33-2.20)		(1.3-10.0)	(3.0-185.0)	(0.7-56.0)
		7.52	12.0	1.93
		3.3	8.9	2.3
		(1.3-10.0)	(3.0-35.0)	(0.7-5.0)

shown, initial and final.
data from this table in Results.

by a dosage regimen of intermediate intensity were more impressive (Fig. 6). REqC rose to as high as 50 (8-fold increase), promptly declined on cessation of injections in mid-April, 1958, and was back to the initial level by October, 1968. It is interesting to note that leukocyte sensitivity fluctuated considerably, but the increases during injections of house dust extract were no greater than those in the latter months of the following year when no injections were given. The same holds for times of lowest leukocyte sensitivity.

With the most intensive dosage regimen for injections of house dust extract, the immunologic responses were striking (Fig. 7). The REqC rose from 5 to 178 (36-fold) within a few weeks of the start of injections and declined gradually in the 7 months after discontinuance. Leukocyte sensitivity increased steeply in the early weeks of injections, remained at a high level during the injection period, and gradually declined thereafter. The program of injections that this subject received is too rigorous for general use but was judged necessary to complete immunologic evaluation of the efficacy of injections of a commonly employed house dust extract. The subject was housed in a clinical

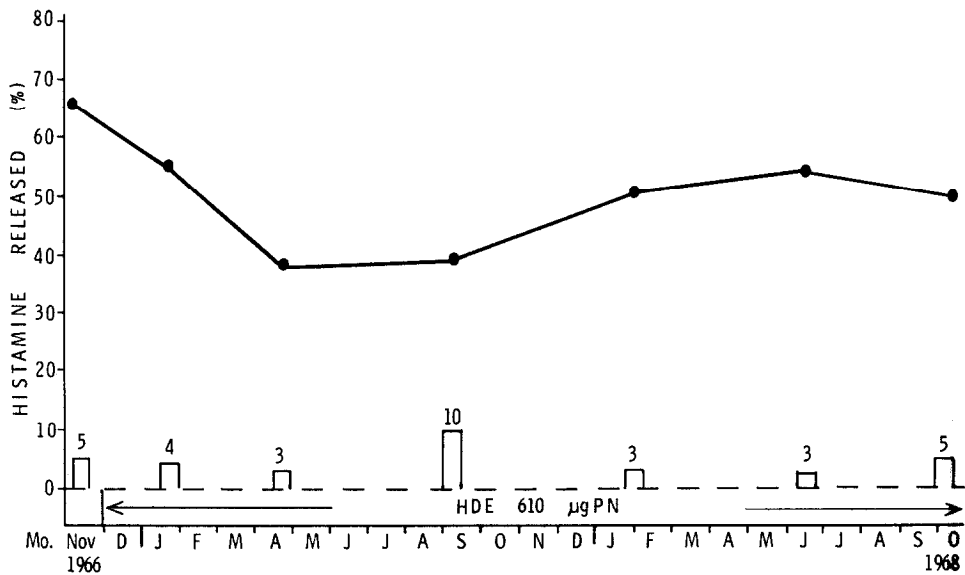


Fig. 3

Patient L. P., No. 162; Nov. 2, 1966 to Oct. 11, 1968; antigen, house dust extract. Subject hypersensitive to house dust extract (*HDE*). Continuous line traces leukocyte sensitivity to *HDE* at concentration of 2 µg of PN per milliliter. Bars with values at top represent REqC. Changes during administration of injections of *HDE* by customary injection therapy but low total dosage are within ranges observed in untreated subjects.

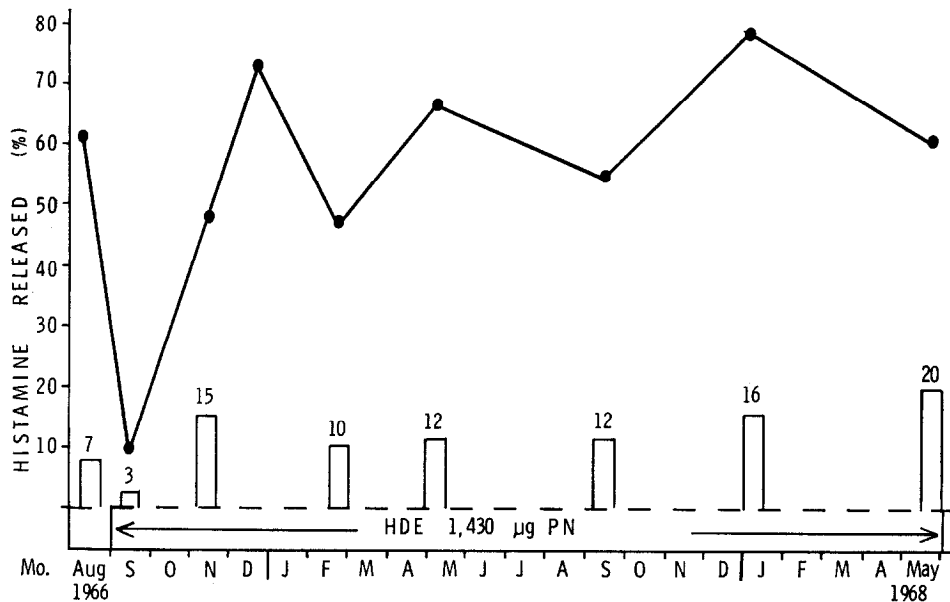


Fig. 4

Patient B. R., No. 40; Aug. 4, 1966, to May 17, 1968; antigen, house dust extract. Subject hypersensitive to house dust extract (*HDE*). Continuous line traces leukocyte sensitivity to *HDE* at concentration of 2 µg of PN per milliliter. Bars with values at top represent REqC. After injection of 1,430 µg of PN of *HDE* during 22 months by customary injection therapy, REqC had increased threefold, and leukocyte sensitivity was same as that before injections.

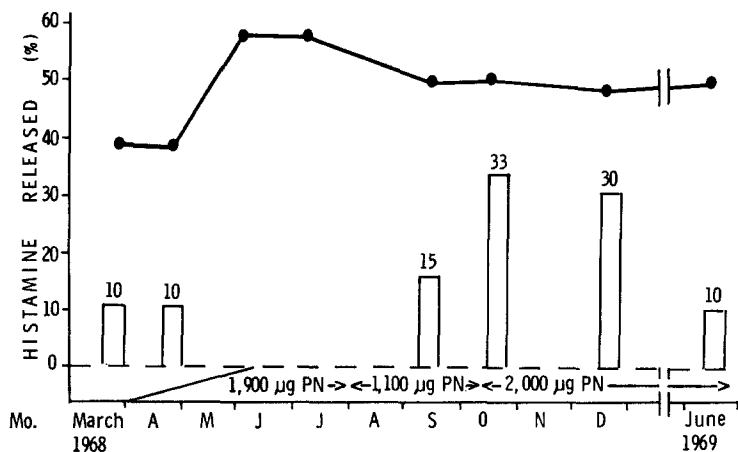


Fig. 5

Patient A. R., No. 120; March 27, 1968, to June 20, 1969; antigen, house dust extract. Subject hypersensitive to house dust extract. Moderately intensive house dust extract injection therapy in 6 month period, then continued at reduced dosage. Continuous line traces leukocyte sensitivity to house dust extract at concentration of 2 µg of PN per milliliter. Bars with values at top show REqC before and during injection therapy. Modest increase in REqC during moderately intensive dosage not sustained by reduced dosage. Leukocyte sensitivity remained essentially unchanged throughout, except for slight increase at start of injections.

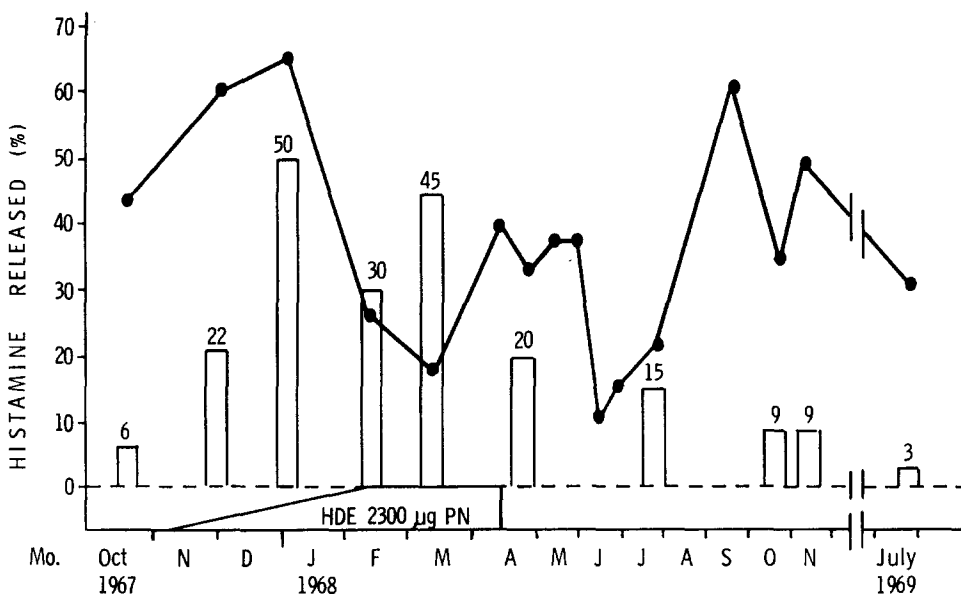
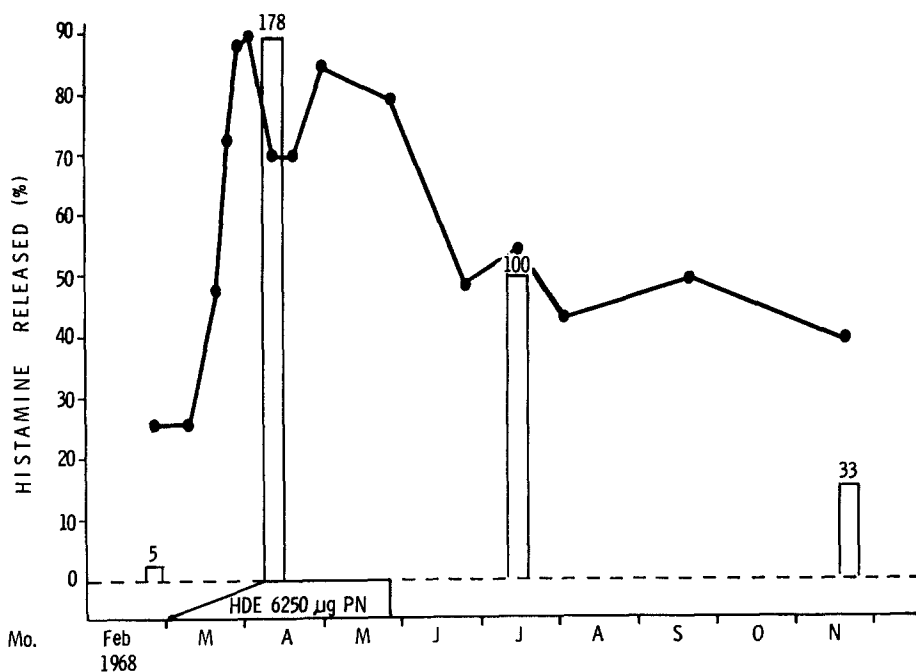


Fig. 6

Patient J. P., No. 253; Oct. 20, 1967, to July 18, 1969; antigen, house dust extract (HDE). Subject hypersensitive to HDE. Moderately intensive HDE injection therapy in 6 month period. For discussion of therapy and dosage, refer to text. Continuous line traces leukocyte sensitivity to HDE at concentration of 0.06 µg of PN per milliliter. Bars with values at top show REqC before, during, and after injection therapy. An impressive increase in REqC occurred during treatment and gradually returned toward original level after discontinuance of injections. Changes in leukocyte sensitivity correlated only partly and were as great after as during period of injections.

**Fig. 7**

Patient W. L., No. 102; Feb. 19, 1968, to Nov. 18, 1969; antigen, house dust extract (*HDE*). Subject hypersensitive to *HDE*. Intensive *HDE* injection therapy in 3 month period. For discussion of therapy and dosage, refer to text. Continuous line traces leukocyte sensitivity to *HDE* at concentration of 0.06 µg of PN per milliliter. Bars with values at top show REqC. A sharp increase in leukocyte sensitivity and in REqC occurred soon after onset of treatment. Both gradually declined toward pretreatment values when injections of *HDE* were discontinued.

research unit under constant surveillance. During the first 14 days of inauguration of the regimen, he received injections 3 to 4 times a day up to tolerance, then daily for a month and weekly thereafter, in order to safely give a large amount of house dust extract in a short period. In the last 2 months of injections he received more than 25 ml. of a dilution of the extract containing 200 µg of PN (20,000 PNU) per milliliter—a dark-brown crude material. No untoward reactions or side effects were apparent in this patient or any of the others in this entire study.

From the foregoing findings, the possibility arises that the magnitude of immunologic response to injections of house dust extract may depend more on total dose administered than on duration of treatment.

DISCUSSION

Substantial evidence has been presented that the house dust extract studied contains specific antigenic material. No evidence was found for nonspecific irritative qualities of the house dust extract in the concentrations employed for intradermal tests. In our experience a wheal reaction to intradermal injection of 0.01 to 0.02 ml. of a solution of this house dust extract containing

1 to 10 μg of PN (100 to 1,000 PNU) per milliliter occurs so regularly and exclusively in the atopic-type person as to serve as an indicator of this state as well as of hypersensitivity to the antigenic material in house dust. Our findings are in keeping with the evidence for a close association between presence of specific skin-sensitizing antibody to house dust extract in the serum and a dermal wheal reaction to the extract which had been accumulated through the passive transfer (Prausnitz-Küstner) procedure by investigators years ago^{3, 7} as well as recently.⁸ Mention was made that even with physician patients a thorough history for manifestations of allergic disorders, past and present, and appropriate diagnostic procedures to detect hypersensitivities may be required to discover that an individual who has considered himself "normal" is in fact atopic. If this were not carefully done by us or others,⁴ specific dermal wheal reactions to house dust extract could easily have been mistaken as nonspecific irritation. Considering the inevitability of continuous exposure to house dust from birth and the evidence for antigenic properties of dust extract, it is not surprising that hypersensitivity to house dust extract is found to be so prevalent in atopic persons.

To what extent clinical manifestations of allergic disorders can be ascribed to hypersensitivity to house dust has not been well delineated. The present study was not designed to examine this question. Unless one is satisfied that the amount of extract used to identify hypersensitivity to house dust produces only specific immunologic reactions and can have some assurance that a dosage schedule selected for injections of the extract will stimulate a promising immune response, appraisal of the clinical importance of hypersensitivity to house dust and clinical evaluation of injection therapy are apt to prove futile.

The data obtained indicate that the immune response elicited by injections of a popular commercial house dust extract, given approximately according to a customary schedule and dosage for nearly 2 years, was erratic and generally meager. Only 3 of 21 subjects showed an impressive increase in capacity of the serum to neutralize the house dust antigen, and none was "desensitized" as judged by persistence of dermal wheal reaction and leukocyte sensitivity to histamine release with house dust extract. Another subject exhibited a great increase in antigen neutralization capacity in the serum during a much more intensive regimen of injections providing a large dose of house dust extract in 3 months.

The present studies were done with a particular dust extract, but the potency of several available extracts has been examined. The extract used herein was the most potent (by 3- to 10-fold on a dry weight basis) as judged by simultaneous quantitative determinations of histamine release from leukocytes of a dust-sensitive individual.

Thus we found an impressive specific immune response could be provoked occasionally by injections of house dust extract, but more vigorous treatment than has been customary may be required for dependable results. It is conceivable that observations of immune responses after continuation of a customary dosage schedule for an even longer period than the nearly 2 years of this study will disclose greater efficacy. This would still seem unsatisfactorily

slow and inefficient. Unfortunately administration of large doses of presently available crude extracts entails the giving of considerable extraneous material and probably frequent injections indefinitely to sustain any immune response. While no overt adverse effects were noted in subjects given the larger doses of crude extract, an admonition is in order regarding the possibility of producing potentially injurious soluble antigen-antibody complexes. Furthermore it is noteworthy that "desensitization" was not accomplished with any dosage given in this study.

Considerable fluctuations in leukocyte sensitivity were observed during serial determinations with both treated and untreated dust-sensitive children. This differs from the finding in earlier studies by Lichtenstein and associates⁹ of ragweed-sensitive adults selected for high sensitivity of their leukocytes to antigenic release of histamine, where no change in leukocyte sensitivity was demonstrated. Subsequent studies from that clinic concerning adults¹⁰ and especially children¹¹ report marked fluctuations in leukocyte sensitivity in treated and untreated ragweed-sensitive subjects.

Dr. Voorhorst and co-workers have proposed a mite (*Dermatophagoides pteronyssinus*) as a major source of the antigenic activity in house dust,¹² and others have confirmed their findings and suggested that more than one species of mite may be implicated.¹³ Identity or cross-reactivity of antigens derived from various species has been found.^{14, 15} Voorhorst considers house dust to contain a single allergen derived from mites.¹² Contrary to this view, Berrens and Versie¹⁶ contend their chemical and immunologic studies show that the allergenic property of house dust is due to a mixture of closely related glycopeptide constituents of various substances found in dust.

Although house dust clearly contains material to which hypersensitivity is widespread, clinical applications must take into account: (1) the uncertainty as to the nature and homogeneity of the antigenic material in house dust; (2) the failure of injection therapy with customary dosage of house dust extract to evoke a significant immune response dependably; (3) misgivings regarding consequences of injection of larger amounts of crude dust extracts over long periods; and (4) the lack of a controlled clinical evaluation of injection therapy with house dust extract. Even the extent to which hypersensitivity to material in house dust may be responsible for allergic disorders has not been ascertained objectively. The foregoing considerations lend no support to the practice of injection therapy with house dust extract in the hope of "desensitization" or "immunization."

The technical assistance of Bonnie Boilini and Janet Strollo made this study possible. The children who served as subjects, their parents, and clinic personnel earned our sincere appreciation. Use of the Clinical Research Unit in the Department of Pediatrics, sponsored by the Public Health Service, was most helpful in obtaining data during intensive immunotherapy in one subject.

REFERENCES

1. Cooke, R. A.: New etiologic factors in bronchial asthma, *J. Immun.* 7: 147, 1922.
2. Kerr, A.: Dust sensitization in bronchial asthma, *Med. Clin. N. Amer.* 5: 751, 1921.
3. Pratt, H. N.: House dust hypersensitivity in perennial asthma of childhood, *J. ALLERG.* 8: 60, 1936.

4. Fontana, V. J., Wittig, H., and Holt, L. E., Jr.: Observations on the specificity of the skin test, *J. ALLERG.* **34**: 348, 1963.
5. May, C. D., Lyman, M. S., Alberto, R., and Cheng, J.: Procedures for immunochemical study of histamine release from leukocytes with small volume of blood, *J. ALLERG.* **46**: 12, 1970.
6. Lichtenstein, L. M., Holtzman, N. A., and Burnett, L. S.: Quantitative in vitro study of chromatographic distribution and immunoglobulin characteristics of human blocking antibody, *J. Immun.* **101**: 317, 1968.
7. Spivack, C. A., and Grove, E. F.: A study of the house dust atopen in asthma, *J. Immun.* **10**: 465, 1925.
8. Lindbald, J. H., and Farr, R. S.: The incidence of positive intradermal reactions and the demonstration of skin-sensitizing antibody to extracts of ragweed and dust in humans without history of rhinitis or asthma, *J. ALLERG.* **32**: 392, 1961.
9. Lichtenstein, L. M., Norman, P. S., and Winkenwerder, W. L.: Clinical and in vitro studies on the role of immunotherapy in ragweed hayfever, *Amer. J. Med.* **44**: 514, 1968.
10. Norman, P. S.: A rational approach to desensitization, *J. ALLERG.* **44**: 129, 1969.
11. Sadan, N. et al.: Immunotherapy of pollinosis in children, *New Eng. J. Med.* **280**: 623, 1969.
12. Voorhorst, R., Spieksma, F. Th. M., Varekamp, H., Leupen, M. J., and Lyklema, A. W.: The house-dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces. Identity with the house-dust allergen, *J. ALLERG.* **39**: 325, 1967.
13. Leading article: Mites and asthma, *Lancet* **1**: 1295, 1968.
14. Voorhorst, R., and Spieksma, F. Th. M.: Recent progress in the house dust mite problem, *Acta Allerg.* **24**: 115, 1969.
15. Spieksma, F. Th. M., and Voorhorst, R.: Comparison of skin reactions to extracts of house dust, mites, and human skin scales, *Acta Allerg.* **24**: 124, 1969.
16. Berrens, L., and Versie, R.: Cross reactions of anti-house dust sera with inhalant glycoprotein allergens from different sources, *Acta Allerg.* **22**: 347, 1967.