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(Continued)

FACTORS INFLUENCING REAGIN FORMATION IN EXPERIMENTAL HUMAN SENSITIZATION TO *ASCARIS LUMBRICOIDES* ANTIGEN

I. INFLUENCE OF CHRONIC INFECTION (TUBERCULOSIS) ON RATE OF SENSITIZATION*†

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THIS series of investigations was started in 1935 in an attempt to study some of the factors which influence the production of skin-sensitizing antibodies, the atopic reagins. Fülleborn and Kikuth,¹ Coca and associates,² Brunner,³ and others found that sensitization of the skin to *Ascaris lumbricoides* could be easily induced in humans, sometimes by a single injection of *Ascaris* extract. By means of the Prausnitz-Küstner technique Rackemann and Stevens demonstrated that the antibodies responsible for the immediate positive skin reaction in *Ascaris* hypersensitiveness were the atopic reagins. Since *Ascaris* sensitization is so readily induced and produces no clinical hazard in the experimental subjects, *Ascaris* extract seemed particularly suitable as an antigen for the study of reagin formation.

The extract used in these studies was prepared in the following manner: *Ascaris lumbricoides* (pig) worms were first washed in water, then in toluol. They were then covered with a slight excess of buffered saline and with a layer of toluol. This was allowed to stand at room temperature until most of the worms had disintegrated. The material was paper-filtered and sterilized by Seitz filtration on June 2, 1933. The extract was bottled, covered with a layer of toluol, and stored in the ice box. On Sept. 3, 1935, it was again passed through a Seitz filter. At this time the total nitrogen content determined by the Macro-Kjeldahl method was 5.3 mg. N per ml. From this stock material, proper dilutions for use in this study were made.

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For the selection of experimental subjects, an intracutaneous test was performed with 0.025 ml. of an *Ascaris* extract containing 0.1 mg. N per ml. Readings of the reactions to these tests were made at the end of fifteen minutes. Only patients with completely negative reactions were used in the subsequent experiments.

Sensitizing doses of 0.1 ml. of the 0.1 mg. N *Ascaris* extract were, with only an occasional exception, given at weekly intervals until definite positive skin reactions developed or until the investigation was terminated. The successive sensitizing doses are noted in the graphs and tables as "S₁," "S₂," "S₃," etc., and the test dose as "T."

In the first nine patients of Group 1, only the test dose of 0.025 ml. was given on the first day. These subjects received the first 0.1 ml. dose the following week. In the remaining patients of Group 1 (Nos. 10 to 35) and in subsequent groups, the first 0.1 ml. dose was given on the same day as the test dose of 0.025 ml. That this variation in technique produced no significant difference in the final results was demonstrated in a subsequent study (Group 3).

The skin reactions were read and recorded as follows: 0, wheal and erythema negative; ±, erythema slight and wheal doubtful; +, wheal and erythema slightly positive; ++ and +++, more pronounced degrees of whealing and erythema.

The occurrence of erythema alone was not considered definite evidence of sensitization, since some subjects with ± reactions showed negative reactions upon subsequent test. However, a + reading almost invariably remained positive on subsequent test. Positive reactions were routinely confirmed before sensitization was considered to be effected.

The positive reactions in tuberculous patients, who were the subjects in many of these studies, usually developed less vigorously than did those in non-tuberculous subjects. The impairment in skin reactivity was particularly pronounced in the acutely ill and in the cachectic patients. These reactions, nevertheless, were sufficiently definite to be read.

In addition to the immediate whealing response, delayed reactions frequently occurred at the injection site, usually after an interval of two to four days. Such reactions were most often associated with early doses in the sensitizing series. They were characterized by itching, redness, and deep-seated edema. Their sites frequently persisted as discolored and sometimes scaly areas for several weeks. The onset of the delayed type of reaction frequently preceded that of the immediate type, but it occasionally occurred in individuals who did not exhibit immediate wheals throughout the course of the experiment.

From the actively sensitized patients in the first group, bloods were taken in four cases chosen at random and passive transfer tests were performed according to the technique employed by Brunner⁵ in his studies on hypersensitiveness to *Ascaris lumbricoides*. All four cases (Nos. 1, 3, 15, and 30, Chart I) gave pronounced positive transfer reactions. Because of the regularity with which reagins were demonstrated in the bloods of patients with immediate positive skin reactions to *Ascaris* in these experiments, as well as in those of Brunner,⁵ Prausnitz-Küstner tests were not performed in the later studies.

STUDIES ON GROUP 1

The purpose of the first study was to determine the influence of chronic infection on reagin production. The subjects for this investigation were selected from male patients with active pulmonary tuberculosis at Sea View Hospital in New York City. The tests on this group were begun on November 5, 1935. Sensitizing doses were repeated at weekly intervals on each subject until he either became sensitized or had received twelve injections.

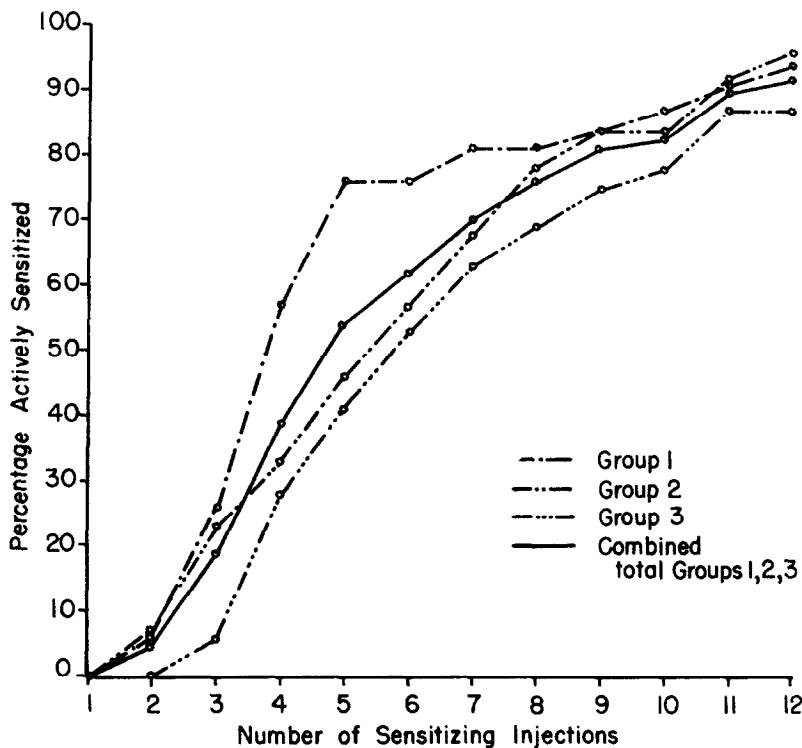


Fig. 1.—Sensitization rates, groups 1, 2, and 3.

Sixty-two patients were tested with *Ascaris* extract on the first day. Of these, twenty-two showed positive or doubtful reactions. Of the remaining forty cases, five were discharged before they had received at least four sensitizing doses and were eliminated from the study. The remaining thirty-five cases constituted Group 1. (See Chart 1 and Table I.)

TABLE I. SUMMARY OF RESULTS OF SENSITIZATION IN MALE TUBERCULOUS PATIENTS (GROUP 1)

WEEKLY SENSITIZING DOSE	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀	S ₁₁	S ₁₂
No. of patients	35	35	35	35	33	33	32	32	32	32	32	31
No. of positive reactors(+)	0	2	9	20	25	25	26	26	27	28	29	29
Per cent positive reactors	0	6	26	57	76	76	81	81	84	87	91	94

CHART I. GROUP 1. MALE TUBERCULOUS PATIENTS

CASE NO.		T	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀	S ₁₁	S ₁₂
1	Wheal	0		0	0	++	+++	+++						
	Eryth.	0		0	0	+++	+++	+++						
2	W	0		0	0	++	++	++						
	E	0		0	0	++	++	++						
3	W	0		±	+	+	+							
	E	0		++	++	++	++							
4	W	0		0	±	+++	+++							
	E	0		0	++	+++	+++							
5	W	0		0	0	0	0	0	±	±	+	+	+	+
	E	0		0	+	0	0	0	±	+	++	+	+	+
6*	W	0		0	0	0	++	++	++	++				
	E	0		0	0	0	?	?	?	++?				
7	W	0		0	++	++								
	E	0		0	++	+++								
8*	W	0		0	±	+++	+++							
	E	0		0	?	?	?							
9	W	0		0	0	±	(discharged)							
	E	0		0	0	0								
10	W	0	0	±	++	+++								
	E	0	0	++	+++	+++								
11	W	0	0	+	++	+++								
	E	0	0	++	++	+++								
12	W	0	0	0	0	0	0	0	0±	0±	0±	0±	0±	0
	E	0	0	0	0	0	0	0	+	+	+	+	+	+
13	W	0	0	0	+	++	+++							
	E	0	0	0	++	++	+++							
14	W	0	0	0	0	0	0	0	0	0	0	0	0	0
	E	0	0	0	0	0	0	0	0	±	+	+	+	+
15	W	0	0	0	0	0±	0	0	±	±	±	±	++	++
	E	0	0	0	0	0	0	0	+	+	+	+	+++	++
16	W	0	0	0	0	0	(discharged)							
	E	0	0	0	0	0								
17	W	0	0	0	0	±	++	++	++					
	E	0	0	0	0	+	++	++	++					
18	W	0	0	0	0	+	++	+++	+++					
	E	0	0	0	+	++	++	+++	++					
19	W	0	0	0	0	±	+	++	++					
	E	0	0	0	0	+	++	++	++					
20	W	0	0	0	±	+	+++	+++						
	E	0	0	0	++	++	++	+++						
21	W	0	0	0	0	+	++	+++						
	E	0	0	0	+	+	++	+++						
22	W	0	0	0	0	+	++	+++						
	E	0	0	0	+	++	++	+++						
23	W	0	0	0	±	+	+	+	+	++				
	E	0	0	0	+	++	++	++	+	+++				
24	W	0	0	±	+	++	+++							
	E	0	++	+	++	++	+++							
25	W	0	0	0	0	0	0	0	0	0	0	+	+++	+++
	E	0	0	0	0	+	±	±	0	0	0	++	+++	+++
26	W	0	0	0	0	0	0	0	+	++				
	E	0	0	0	0	0	0	0	+	++				
27	W	0	0	0	+	+++								
	E	0	0	0	+++	+++								

CHART I. GROUP 1. MALE TUBERCULOUS PATIENTS (CONT'D)

CASE NO.		T	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀	S ₁₁	S ₁₂
28	Wheal	0	0	±	±	++	+++	+++						
	Eryth.	0	0	+	+	++	+++	+++						
29*	W	0	0	0	0	±	+	+	++					
	E	0	0	0	0	?	?	?	?					
30*	W	0	0	0	++	+++	++							
	E	0	0	0	?	+	?							
31	W	0	0	0	0	0	0	0						
	E	0	0	+	0	0	+	+	(discharged)					
32	W	0	0	0	0	0	0	0	0	0	0	0	0	0
	E	0	0	0	0	0	0	0	0	0	0	0	0	0
33	W	0	0	0	0	±	++	+++						
	E	0	0	0	0	+	++	+++						
34	W	0	0	+	+	+	++	+++						
	E	0	+	++	+	++	++	+++						
35	W	0	0	0	0	+	++							
	E	0	0	0	0	+	++							

T = Initial test dose of 0.025 ml. Ascaris antigen.

S₁ = Additional sensitizing dose of 0.1 ml. given on first day.

S₂, S₃, etc. = Reactions to repeated sensitizing doses of 0.1 ml. given at weekly intervals (with occasional exceptions).

*Negro patients.

? = Erythema difficult to assess.

The ages of the patients in this group varied from 17 to 66 years. The average age was 35.8 years. Eight patients were over 50 years of age. There were four Negroes among the thirty-five males in this group.

The individual reactions to the successive sensitizing doses of the antigen for all patients in this group are tabulated in detail in Chart 1. Cases 9, 16, and 31 received from four to six doses of antigen without becoming sensitized but were eliminated from further study when they were discharged from the hospital. In Negro patients 6, 8, 29 and 30, it was difficult to grade the erythema because of the skin pigmentation.

It may be noted in Chart 1 that sensitivity sometimes appeared suddenly; in such individuals a strong positive reaction was obtained only one week after a negative test. In most individuals, however, the development of sensitivity was more gradual, and the progression from the first indication of a reaction to a definite positive usually took from two to three weeks.

The final results obtained in this study are summarized in Table I and in Fig. 1. In these and in subsequent tables and graphs, only confirmed positive reactions were included. Sensitization was present in 6 per cent of thirty-five cases at S₂, and rapidly increased to 76 per cent of the group at S₅ (four weeks after the first sensitizing dose). Thereafter, the rate of increase was much slower, reaching 84 per cent positive at S₉ and 94 per cent at S₁₂, when the study was terminated. The average time for sensitization was 3.3 weeks.

STUDIES ON GROUP 2

The study of sensitization to Ascaris in a nontuberculous group of patients was carried out in the medical Out-Patient Department of the Jewish Hospital

of Brooklyn. It was begun on Dec. 18, 1935, while the study on Group 1 was still in progress.

Twelve sensitizing doses were given in this series. The attendance of some of these subjects at the clinic was somewhat irregular, so that the sensitizing injections were occasionally given at intervals greater than one week.

The same *Ascaris* extract was employed for sensitization as for Group 1. All patients were tested intracutaneously with 0.025 ml. of a 0.1 mg. N dilution. If the reaction was totally negative at the end of fifteen minutes, a sensitizing intracutaneous dose of 0.1 ml. of the 0.1 mg. N dilution was administered on the same day.

There were forty-three negative reactors in the group at the start of the investigation. They included eight males and thirty-five females, all white, ranging in age from 13 to 65 years. The average age was 42.1 years. Twelve exceeded 50 years of age. Only twenty-four of the forty-three patients became sensitized or received their full quota of twelve sensitizing injections. The results of sensitization in these nontuberculous individuals are summarized in Tables II A, II B, II C, and II D.

In this group, 7 per cent of forty-three subjects showed definite evidence of sensitization at the second injection, S_2 (Table II A), 46 per cent of 39 sub-

TABLE II A. SUMMARY OF RESULTS OF SENSITIZATION IN NONTUBERCULOUS SUBJECTS (GROUP 2)

WEEKLY SENSITIZING DOSE	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}
No. of patients	43	43	43	43	39	35	31	27	25	25	24	24
No. of positive reactors (+)	0	3	10	14	18	20	21	21	21	21	22	23
Per cent positive reactors	0	7	23	33	46	57	68	78	84	84	92	96

TABLE II B. RESULTS OF SENSITIZATION IN MALE NONTUBERCULOUS SUBJECTS (GROUP 2)

WEEKLY SENSITIZING DOSE	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}
No. of patients	8	8	8	8	8	8	7	7	7	7	7	7
No. of positive reactors (+)	0	0	1	4	5	6	6	6	6	6	6	7
Per cent positive reactors	0	0	12	50	63	75	86	86	86	86	86	100

TABLE II C. RESULTS OF SENSITIZATION IN FEMALE NONTUBERCULOUS SUBJECTS (GROUP 2)

WEEKLY SENSITIZING DOSE	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}
No. of patients	35	35	35	35	31	27	24	20	18	18	17	17
No. of positive reactors (+)	0	3	9	10	13	14	15	15	15	15	16	16
Per cent positive reactors	0	9	26	29	42	52	62	75	83	83	94	94

TABLE II D. COMBINED RESULTS OF NONTUBERCULOUS MALES AND FEMALES CORRECTED FOR THE NUMERICAL INEQUALITY OF THE SEXES

WEEKLY SENSITIZING DOSE	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}
Per cent females sensitized	0	9	26	29	42	52	62	75	83	83	94	94
Per cent males sensitized	0	0	12	50	63	75	86	86	86	86	86	100
Unweighted average per cent sensitized	0	4.5	19	39.5	52.5	63.5	74	80.5	84.5	84.5	90	97

jects were positive at S₅, 84 per cent of 25 patients at S₉, and 96 per cent of 24 patients at S₁₂.

The rate of sensitization for Group 2 was not so rapid as that for Group 1. This is clearly illustrated in Fig. 1. The differences between the two groups were most pronounced at S₄, S₅, S₆, and S₇. There was virtually no difference between the two groups at S₉ and thereafter.

In attempting to account for the differences in the rates of sensitization between Groups 1 and 2, several factors had to be considered: (1) Group 1 was comprised of tuberculous individuals, whereas those in Group 2 were non-tuberculous. (2) The sensitizing extract had aged several months between the studies on the two groups. (3) There was a difference in the sex distribution of the two groups, Group 1 consisting entirely of males, whereas Group 2 was preponderantly female. (4) The irregularity in attendance of subjects in Group 2 may have distorted the results obtained in this series. (5) Group 1 remained fairly constant throughout the study, whereas there was a progressive loss of subjects in Group 2 as the investigation continued.

An evaluation of these factors was impossible at the time because of the limited amount of data available.

The possibility that the difference in the results obtained in Group 1, consisting entirely of males, and in Group 2 which was preponderantly female, might depend on a sex factor seemed to find some support in an analysis of the results obtained in Group 2 (Tables II B and II C, and Fig. 3). Unfortunately, at the beginning of this study, there were only eight males, as compared to 35 females in the group, an inequality which minimized the value of any comparative analysis. It was therefore decided to obtain sensitization data on a larger group of female tuberculous patients in order to make a more valid comparison with the results obtained in the male tuberculous patients of Group 1.

STUDY ON GROUP 3

This study was begun on Sept. 30, 1936, and was completed in twelve weeks. The antigen was the same one employed in the first two studies. The subjects consisted of thirty-two female patients under treatment for tuberculosis at Sea View Hospital. All gave negative immediate reactions to intracutaneous tests with *Ascaris lumbricoides* extract. Adequate data were obtained on thirty-one of these patients before the termination of the study after the twelfth sensitizing dose. The ages of the patients varied from 19 to 65 years, the average age being 33.6 years. Three patients were over 50 years of age. Two in the series were Negroes.

In order to evaluate the importance of a variation in sensitizing technique which had occurred in Group 1, eighteen patients received only 0.025 ml. of an 0.1 mg. N extract intracutaneously on the first day, while the remaining fourteen received, in addition, an intracutaneous injection of 0.1 ml. at this time. Thereafter, all patients received the same dosage. The variation in the first sensitizing dose produced no significant difference in the results obtained. The data for the entire group are presented in Table III and Fig. 1.

TABLE III. SUMMARY OF RESULTS OF SENSITIZATION IN FEMALE TUBERCULOUS PATIENTS (GROUP 3)

WEEKLY SENSITIZING DOSE	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀	S ₁₁	S ₁₂
No. of patients	32	32	32	32	32	32	32	32	32	32	31	31
No. of positive reactors (+)	0	0	2	9	13	17	20	22	24	25	27	27
Per cent positive reactors	0	0	6	28	41	53	63	69	75	78	87	87

In Group 3, sensitization was present in 6 per cent of cases at S₃. The percentages of positives rose to 41 per cent at S₅, 75 per cent at S₉, and 87 per cent at S₁₁.

Comparison of the rates of sensitization in the female tuberculous patients in Group 3 and the male tuberculous patients in Group 1 again showed a more rapid rate of sensitization in the males, a finding similar to that noted in the previous study with Group 2 (Figs. 1 and 3, and Tables I, II, and III).

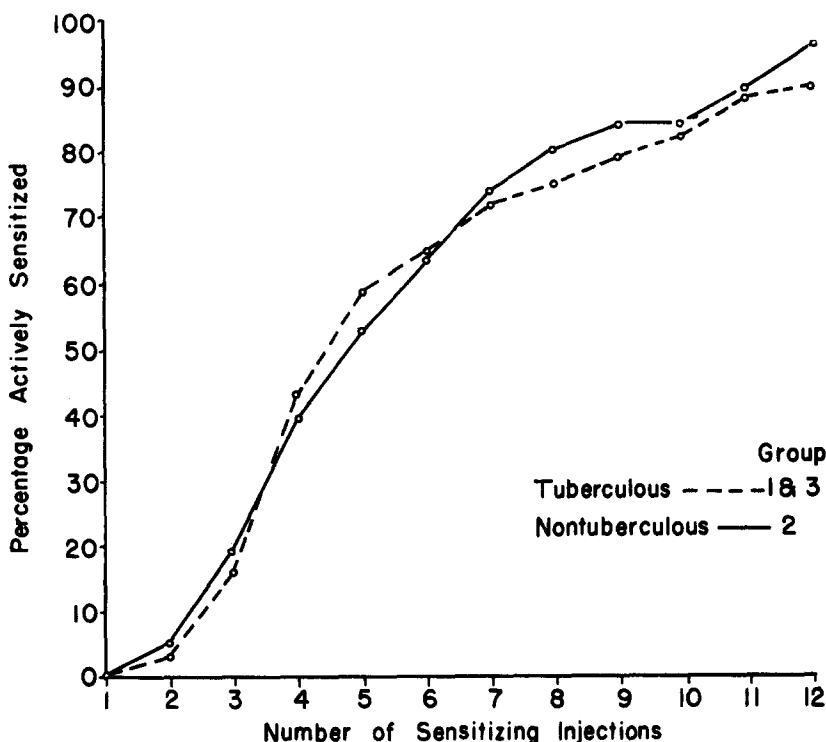


Fig. 2.—Sensitization rates in tuberculous and nontuberculous groups.

ANALYSIS OF THE COMBINED RESULTS OBTAINED IN GROUPS 1, 2, AND 3

An analysis of the data obtained in the three group studies provided answers to some of the questions prompting this series of investigations. A summary of the combined totals of the three groups, tabulated in Table IV and charted in Fig. 1, includes data on 110 originally negative reactors who were subjected to the sensitization procedures.

TABLE IV. COMBINED RESULTS OF SENSITIZATION IN GROUPS 1, 2, AND 3
(TOTALS OF ALL INDIVIDUALS)

WEEKLY SENSITIZING DOSE	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀	S ₁₁	S ₁₂
No. of patients	110	110	110	110	104	100	95	91	89	89	87	86
No. of positive reactors (+)	0	5	21	43	56	62	67	69	72	74	78	79
Per cent positive reactors	0	5	19	39	54	62	70	76	81	83	90	92

In four of the five instances of sensitization found to be present at the time of S₂, there was a fourteen-day interval instead of the customary seven days between S₁ and S₂. Hence, in only one doubtful instance in the 110 cases studied did sensitization appear by the end of the first week.

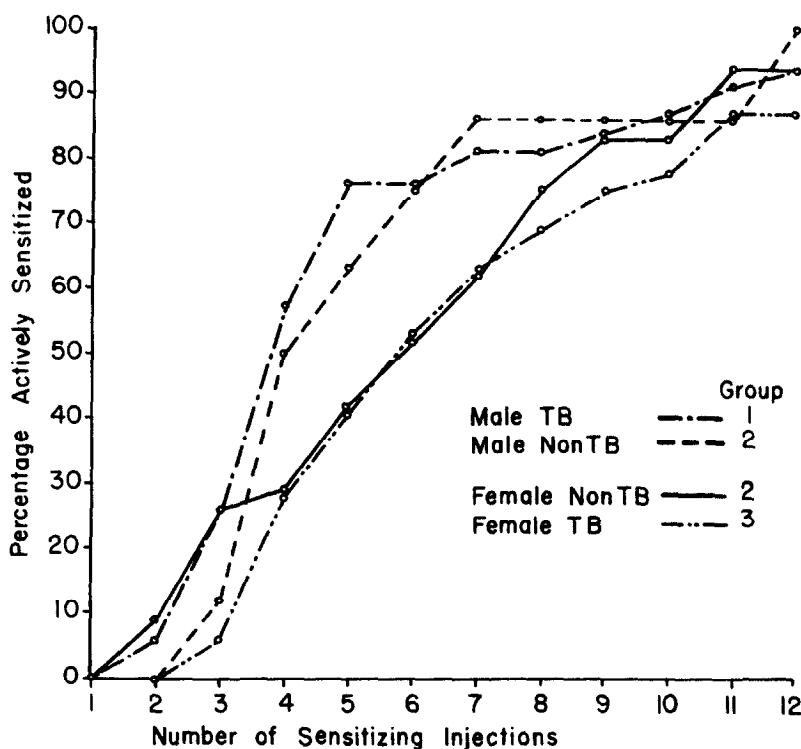


Fig. 3.—Influence of infection and sex on sensitization rates.

Nineteen per cent of the 110 subjects showed positive reactions at S₃. Fifty-four per cent of 104 were positive at S₅, 81 per cent of 89 at S₉, and 92 per cent of 86 at S₁₂. As in previous studies by Brunner,³ a few individuals were found who, for unknown reasons, remained resistant to sensitization.

The effect of a chronic infection, such as tuberculosis, on reaginic sensitization was determined by comparing the results obtained in the tuberculous patients (Groups 1 and 2) with those in the nontuberculous subjects (Group 2, Table V and Fig. 2). The sensitization rates for the two groups were found to be virtually identical. In these studies, chronic infection, in the absence of

cachexia, appeared to exert no influence on the rate of sensitization to *Ascaris lumbricoides*.

TABLE V. INFLUENCE OF TUBERCULOUS INFECTION ON RATE OF SENSITIZATION
TUBERCULOUS: GROUPS 1 AND 3.* NONTUBERCULOUS: GROUP 2*

WEEKLY SENSITIZING DOSE	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀	S ₁₁	S ₁₂
Per cent tuberculous patients positive	0	3	16	42.5	58.5	64.5	72	75	79.5	82.5	89	90.5
Per cent nontuberculous patients positive	0	4.5	19	39.5	52.5	63.5	74	80.5	84.5	84.5	90	97

*Corrected for numerical inequality of the sexes.

Fig. 3 illustrates the influence of the sex factor on the sensitization rate noted in the various studies. There is a striking similarity in the character of the curves for each sex regardless of the presence or absence of tuberculosis infection. The difference in the rates of sensitization for the two sexes appears most pronounced between the fourth and seventh sensitizing injections.

There remained one uncontrolled factor to be evaluated. This was the influence of age of the antigen extract upon the results obtained. Approximately fourteen months had elapsed between the beginning of these studies on Group 1 and their completion on Group 3. This factor became the subject of a subsequent investigation.

SUMMARY

1. Active sensitization to *Ascaris lumbricoides* antigen was experimentally induced, by repeated intracutaneous injections, in over 90 per cent of negative reactors, most of whom were not atopic.

2. Sensitization to *Ascaris lumbricoides* antigen was demonstrable in 19 per cent of 110 subjects at the time of S₃ (fourteenth day in most cases), in 54 per cent of 104 cases at S₅, in 81 per cent of 89 patients at S₆, and in 92 per cent of 86 cases at S₁₂.

3. The antibody responsible for the immediate skin reaction was the atopic reagin. The presence of this antibody in the sera of sensitized subjects was demonstrated by the Prausnitz-Küstner technique.

4. Chronic tuberculous infection did not appear to be a factor affecting the rate of sensitization to *Ascaris* antigen.

5. Evidence was uncovered which suggested that the rate of active sensitization to *Ascaris lumbricoides* might be influenced by a sex factor.

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