

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

IV. THE INFLUENCE OF A PREVIOUS SENSITIZATION ON RATE OF SENSITIZATION*

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IN PREVIOUS studies by the writers, 243 adult human beings were subjected to experimental sensitization with *Ascaris lumbricoides* antigen.^{1, 2, 3} The results obtained suggested that skin-sensitizing antibodies to *Ascaris* tended to appear more rapidly in Negroes than in whites, and more rapidly in males than in females. In seeking to account for these findings, the present investigation was undertaken to determine the influence of previous exposure to *Ascaris* antigen upon the rate of antibody response in a second experimental sensitization.

SUBJECTS

Three groups of adult subjects, patients in an institution for mental illnesses, who had been actively sensitized to *Ascaris* antigen in a previous study in 1946 were available for restudy two years later. Of 14 Negro females, 11 (78.6 per cent) were found to have lost their former reactivity to intracutaneous tests with 0.1 ml. of an *Ascaris lumbricoides* extract containing 0.1 mg. nitrogen per ml. Similarly, 9 of 11 white males (81.8 per cent) and 6 of 8 white females (75 per cent) had lost their cutaneous sensitivity within the same two-year period. These individuals, who now showed no evidence of their former sensitivity to *Ascaris*, were subjected to a second series of sensitizing injections.

Controls consisted of groups of nonsensitive patients in the same institution who, for the most part, corresponded in age, race, and sex to those previously sensitized. Unfortunately, the only Negro males available for the investigation differed from those employed in earlier studies in that they were older, had been hospitalized longer, suffered from mental illnesses of a more chronic type, and showed a higher rate of pre-existent reactivity to *Ascaris* antigen. Nevertheless, a number of these subjects were studied, but data pertaining to this group were not included in the comparative analysis of the 1946 and 1948 series presented in Table II and Figs. 1 and 2.

In selecting the control groups for this study, preliminary skin tests to screen subjects already sensitive resulted in the exclusion of 9 out of 29 Negro females (31 per cent), 9 out of 33 white females (27 per cent), 12 out of 32 white males (37.5 per cent), and 27 out of 48 Negro males (56 per cent). The corresponding rejection rates for the groups studied in 1946 were 42.5 per cent, 32 per cent, 42 per cent, and 31 per cent, respectively.

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METHOD

The technique employed in the present study reduplicated that used in previous studies.³ Antigen was freshly prepared in late 1947 from the same powdered worm material used in the previous experiments and was diluted to contain 0.1 mg. nitrogen per ml. A preliminary intracutaneous test was performed on each subject with 0.01 ml. of an extract containing 0.01 N. per ml. in order to detect extremely sensitive subjects in whom stronger tests might have produced constitutional symptoms. If, at the end of fifteen minutes, this test was completely negative, 0.1 ml. of 0.1 mg. N. extract was injected intracutaneously. The cutaneous reaction to this test was read in twenty minutes. The presence of either a wheal or erythema at the test site disqualified the subject from the study.

TABLE I. A COMPARISON OF THE RAPIDITY OF SENSITIZATION AND OF RESENSITIZATION IN THE SAME SUBJECTS

PATIENTS	NUMBER OF INJECTIONS REQUIRED TO SENSITIZE THE FIRST TIME IN 1946	NUMBER OF INJECTIONS REQUIRED TO SENSITIZE IN 1948
Negro Females		
L. H.	6	6
M. D.	7	4
B. J.	4	4
P. M.	3	3
E. W.	5	4
E. E.	7	3
L. McK.	7	4
E. L.	6	3
A. C.	4	4
P. T.	6	3
Average number of injections re- quired for sensitization	5.5	3.8
White Females		
H. A.	8	3
G. D.	3	10
R. J.	8	4
M. K.	5	3
E. B.*	10	3
C. J.	9	4
Average number of injections re- quired for sensitization	7.2	4.5
White Males		
P. B.	6	3
A. F.*	8	2
J. H.	9	4
R. W.	10	4
C. K.	10	5
P. R.	6	3
C. C.*	5	3
J. McK.	10	2
R. G.	10	4
Average number of injections re- quired for sensitization	8.2	3.3

*Tested (therefore restimulated) six months after initial sensitization, but negative eighteen months later.

Following these initial screening tests, which took the place of the first sensitizing injection (S_1), 0.1 ml. of 0.1 mg. N. extract was administered intracutaneously every week until a definite immediate whealing reaction, in-

dicative of the onset of sensitivity, developed at the site of injection. Simultaneously with each sensitizing dose, the subject was skin tested with 0.01 ml. of the same extract. The reaction to this small test served as a means of checking the specificity of the reaction to the larger test dose. Whenever a positive reaction appeared for the first time the test was repeated the following week for the purpose of confirmation. With few exceptions, positives were always elicited on retest.

RESULTS

Of the 25 individuals previously sensitized, 20 showed a more rapid rate of reagin formation on restimulation than on initial sensitization (Table I). In 4 subjects, all Negro females, the rates of sensitization remained the same. Only one subject, a white female, showed a slower rate of reaginic response to the second series of injections than that shown to the first series.

TABLE II. A COMPARISON OF THE RAPIDITY OF SENSITIZATION IN A RESENSITIZED GROUP AND IN A CONTROL GROUP

	WEEKLY SENSITIZING DOSE									
	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀	
<i>Resensitized Groups*</i>										
Number of subjects studied	25	25	25	25	25	25	25	25	25	
Number of positive reactors	2	12	22	24	24	24	24	24	25	
Per cent of positive reactors	8	48	88	96	96	96	96	96	100	
<i>Control Group*</i>										
Number of subjects studied	58	56	55	54	54	53	53	53		
Number of positive reactors	1	6	11	17	23	29	32	36		
Per cent of positive reactors	1.7	11	20	31	43	55	60	68		

*Negro males are not included.

The average sensitizing dose at which cutaneous sensitivity appeared on resensitization was 3.8, representing an interval of 2.8 weeks between the first sensitizing dose and the appearance of the positive reaction. In the original sensitization of the same subjects two years previously, the average sensitizing dose was 6.9, representing an interval of 5.9 weeks. A more rapid rate of resensitization occurred in each of the three groups of experimental subjects sorted according to sex and race.

The pronounced difference between the sensitization rates of the resensitized groups and their controls is brought out in Table II and is illustrated in Fig. 1. Ninety-six per cent of the 25 restimulated subjects were sensitized by the end of the fourth week (S₅) as compared with 31 per cent of 54 subjects of similar sex and race who were being injected for the first time. At the end of the eighth week (S₉), when sensitization was discontinued, only 68 per cent of the latter group had developed positive reactions.

Analysis of the results obtained in the control groups failed to confirm the influence of race and sex factors on rate of sensitization which the writers observed in their former studies. This finding will be discussed later.

DISCUSSION

In a previous communication,³ the writers reported that of 5 white males tested six months after sensitization 3 had lost their cutaneous sensitivity, one

showed a doubtful reaction, and one was still positive. Three Negro males were found to be as strongly positive at six months as they had been when first sensitized. In the present study conducted two years after sensitization, cutaneous sensitivity was no longer demonstrable in 26 of 33 persons (78.8 per cent) in a group containing no Negro males.

It has been demonstrated in this study that subjects who have lost their former sensitivity to *Ascaris* antigen tend to become resensitized more readily than do individuals undergoing an initial sensitization. In most of the former subjects, the rate of sensitization the second time was much more rapid than it had been the first time.

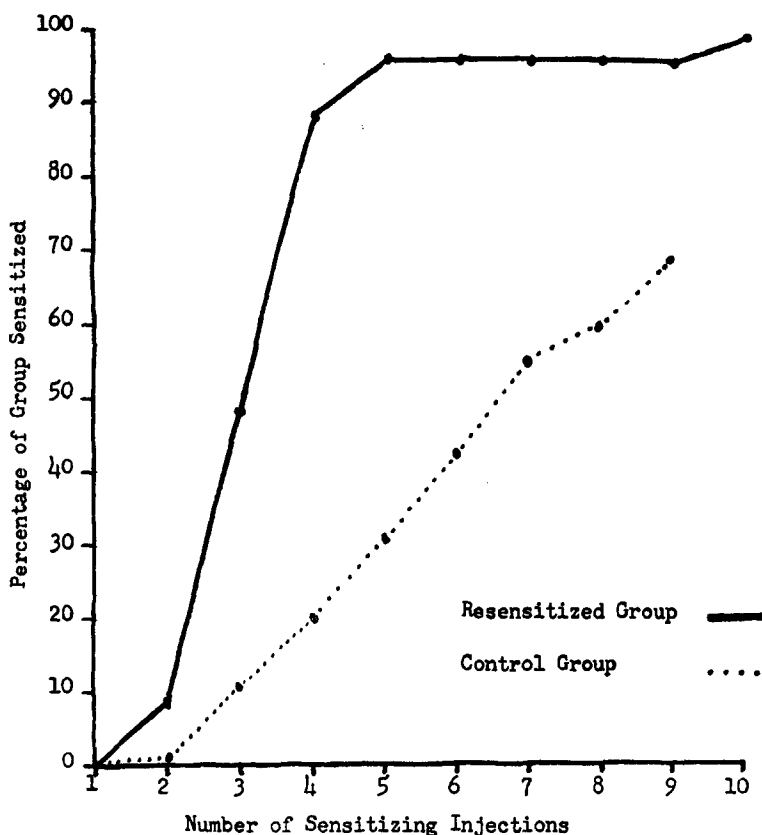


Fig. 1.—Rates of sensitization in resensitized and control groups.

Since the immediate positive cutaneous reaction to *Ascaris lumbricoides* is mediated by the skin-sensitizing antibody, the accelerated response obtained upon resensitization with this antigen may be considered an example of an anamnestic reaction involving antibodies of the reaginic type. This is noteworthy in that the human, passively transferable, skin-sensitizing antibody has not readily lent itself, in the past, to experimental demonstration of the anamnestic reaction.

Among those previously sensitive individuals who were subjected to re-sensitization, only 5 failed to respond more rapidly the second time (Table I). Of these, 4 were Negro females who showed the same rate of sensitization in both series. This observation suggests the possibility that, in these 4 subjects, the first experimental sensitization in 1946 may already have been in the nature of an anamnestic response. If such were the case, these 4 subjects should be eliminated from the protocols of the first sensitization series, a circumstance which would materially influence the interpretation of the results of that experiment.

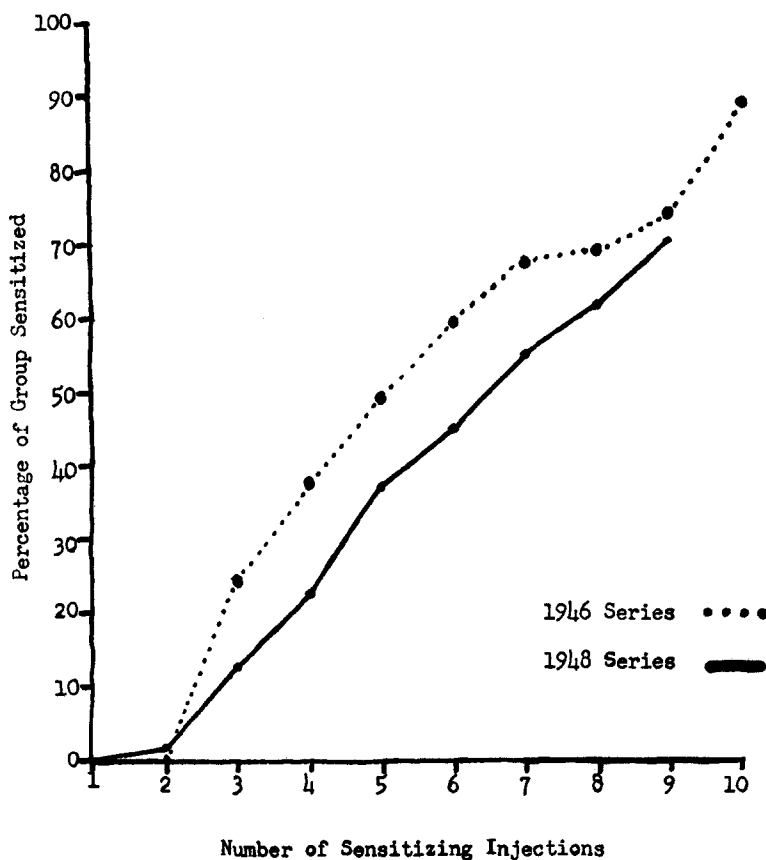


Fig. 2.—Sensitization rates in subjects experimentally sensitized for the first time.

In the present study, one of 79 subjects in the control group (1.3 per cent) developed a positive reaction by the end of the first week. Among the 25 resensitized persons, 2 subjects (8 per cent) showed definite positive reactions at that time. On the basis of the accelerated response in previously sensitized subjects, it seems likely that the single control subjects who exhibited a positive reaction at seven days had also been sensitized by a roundworm infestation at some time in the past.

There is no ready explanation to account for the single instance in which the sensitization rate in a subject was slower the second time than the first.

The patient, G. D., was a white female (Table I) who was hospitalized for the entire two years between sensitizations and had experienced no important acute illness during this period.

The sensitizing extracts used in the 1946 and 1948 experiments were not significantly different in antigenic potency. One may arrive at this conclusion by comparing the sensitization rates of subjects injected for the first time in the 1946 and 1948 groups. The slight difference between the two rates seems to indicate that the 1948 extract was, if anything, slightly weaker than the one used in 1946, and that antigenic potency was therefore not a factor in causing the accelerated responses obtained on restimulation of previously sensitive cases in 1948 (Fig. 2).

The findings in the present study may have a direct bearing upon the race and sex factors reported by the writers in their previous communications. The more rapid sensitization rate which they had observed in Negroes may, in part, have been based upon a higher rate of previous roundworm infestation in the particular Negro subjects employed in the investigation. Under these circumstances, the sensitization artificially induced in these subjects by injection of *Ascaris* antigen would have been in the nature of a resensitization in which a more rapid antibody formation would be expected to occur.

A definite indication of a greater rate of previous exposure to roundworms in Negroes is not reflected in the incidence of pre-existing positive skin reactions to *Ascaris* in the adult groups from which the subjects were chosen for these studies. Positive reactions to *Ascaris* were found by preliminary testing in 31 per cent and 43 per cent, respectively, of the Negro females in the present and former studies, in 27 per cent and 32 per cent of the white females, in 56 per cent and 31 per cent of the Negro males, and in 37 per cent and 42 per cent of the white males. However, the transient nature of the cutaneous sensitization produced by the *Ascaris* antigen limits the value of these figures as accurate indices of the incidence of previous infestation among adult populations. Hence, the writers are not in a position to exclude a greater incidence of previous infestation among the original Negro groups as one of the factors responsible for the more rapid sensitization rates manifested by these groups in the former studies.

The evidence uncovered in the previous studies which suggested a more rapid sensitization rate among males than females was less definite and convincing than that in support of a racial factor. Whether this sex factor may also be dependent upon the incidence of previous infestation in the groups studied is not apparent at the present time.

The whole problem is further complicated by the fact that, in the present study, the control groups, divided on the basis of race and sex, did not manifest the pronounced differences in rates of sensitization which were noted repeatedly in the writers' earlier studies. The causes of these discrepancies are not discernible at present. Their explanation must await the outcome of additional studies on larger and more varied populations.

SUMMARY

Artificially induced sensitivity to *Ascaris lumbricoides* antigen was found to have disappeared in 79 per cent of 33 subjects tested two years after sensitization. Among 25 of these negative reactors who were subjected to a second series of sensitizing injections, the average time for the reappearance of immediate positive skin reactions was 2.8 weeks as compared to 5.9 weeks in their former sensitization. This more rapid response is interpreted as an anamnestic response involving the skin-sensitizing antibody. The possible significance of these findings in interpreting the results of previous studies dealing with race and sex factors in sensitization to *Ascaris* antigen is discussed.

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