SKIN REACTIONS TO NECATOR AMERICANUS IN PERSONS INFECTED WITH THE COMMON INTESTINAL PARASITES

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During recent years intradermal testing with parasitic extracts has received considerable attention. Some investigators claim promising diagnostic possibilities for the skin test as an aid in detecting the presence of helminths, while others have found little or no correlation between positive reactions and the presence or absence of the parasites in the skin-tested host. Considerable work has been done with regard to the hypersensitivity to the various species of ascarids while only a few articles have appeared dealing with the sensitivity to hookworm (*Necator americanus*). The statistical study of Stumberg and Rodríguez Molina(1) on intradermal injections with various extracts of *Necator americanus,* the works of Coventry and Taliaferro(2) and of Pirie, Retief and Ferguson(3) are of leading importance. Stumberg and Rodríguez-Molina noted that "all people gave an immediate type of reaction" but found no correlation between the presence or absence of the parasite and the skin test. They, therefore, confirmed the findings of Coventry and Taliaferro's work in Honduras.

The present study of cutaneous hypersensitivity to hookworm extract (*Necator americanus*) was carried out on the Island of Porto Rico among three widely separated classes of individuals: A group of farmers living on a large estate where they were employed as farm-hands in the cultivation of fruit and sugar-cane; a group of boys and girls from all parts of the Island, living as orphans in the "asilos" of Santurce; and a group of children living in the mountains of the northwestern part of the Island.

In this investigation attempts were made to determine the degree of hypersensitiveness to helminthic protein, before and after treatment, in individuals positive and negative for intestinal worms; to study the specificity of the reactions, and to observe atopic sensitiveness in children under 24 months of age; to measure the reactions

at time intervals and study the importance of erythema and the formation of pseudopods to extracts of *Necator americanus*. Furthermore, to test out the efficacy of cutaneous hypersensitiveness expresed by the skin reactions, as an aid in the diagnosis of hookworm infection.

DESCRIPTION OF AREAS AND GROUPS

Three general groups of people, widely separated geographically and living under varied conditions, were studied. The first general group was a small population of farmers inhabiting a coastal plain in the northern part of the Island, in the district of Dorado. This group was divided into two sections, A and B.* Section A, 168 people, were occupied in the cultivation of grapefruit. They were rather energetic as a class and were provided by the landowner with good houses, each house having a latrine. Section B, 206 people, worked for a neighboring Central, and spent their time in the growth, cultivation and harvesting of sugar-cane. The living conditions in this section were not good and little provision was made for sanitation. Only fourteen of the houses in this section had latrines.

In sections A and B the soil was sandy or sandy loam. In Section A, coconut palms, grapefruit trees and numerous shade trees furnished the principal vegetation; while on Area B there were sugar-cane fields and a few scattered trees. The houses in both sections, A and B, were widely separated. In each section ideal conditions were found favorable for the development of *Necator americanus* and *Ascaris lumbricoides*. The minimum temperature was never below 57° F. with a maximum temperature of 99° F. The minimum monthly temperature was between 70° and 80° F. Rainfall varied with the season. The minimum precipitation occurred in the spring, with an annual rainfall of about 70 inches.

The people of both sections, A and B, were examined one or several times for intestinal parasites before treatment and again post

* Throughout the greater part of the experiment on Sections A and B a hookworm-prevention campaign was conducted with the assistance of Miss Blanca Canales, a resident social worker who made house-to-house visits explaining the rules of good health and the importance of the use and care of latrines. Through her vigilance the percentage of reinfected cases was kept comparatively low. Miss Canales' assistance was made possible through the courtesy of the Board of Education.

treatment.* In determining the degree of infestation with intestinal helminths the Stoll(4) dilution egg counting method as employed by Cort, Riley and Payne(5) in connection with their epidemiological studies of hookworm disease in Porto Rico, was used.

Of 168 farmers and their children examined in Section A, 80 per cent were found infected with *N. americanus,* 65 per cent with *A. lumbricoides,* and 86 per cent with *Trichuris trichiura.* Fourteen days after treatment, 11 per cent, 4 per cent and 32 per cent remained infected with Necator, Ascaris and Trichuris, respectively. During the second month post treatment, 14 per cent, 23 per cent and 74 per cent were infected, while in the third and fifth months there was no appreciable change in the degree of infection. By the ninth month little change was observed, the per cent of infection being 11, 34 and 57 for Necator, Ascaris and Trichuris, respectively. The positive cases had egg counts ranging from 50 to 417,000 eggs per gram. In section B, 87 per cent of the cases were positive for Necator, 51 per cent for Ascaris and 81 per cent for Trichuris. Egg counts in this section varied from 100 to 81,400 eggs per gram.

The second general group included 189 country children from 7 to 20 years of age, living in the mountainous district of Aguadilla, where little hookworm-prevention work had been done. Within this group 98 per cent were positive for *Necator americanus,* 28 per cent positive for *Ascaris lumbricoides* and 96.4 per cent positive for *Trichuris trichiura.* Within two months after treatment 164 of these

* All the infected cases in Section A were treated in a temporary hospital arranged with laboratory, kitchen and bedroom facilities. Patients were admitted in the evening, and on the following morning, before breakfast were given a mixture of carbon tetrachloride (2.2 cc.) and oil of chenopodium (0.8 cc.). Adults over fifteen years of age were given 3 cc. of the mixture in cold black coffee. The strong extract of coffee camouflaged the taste of the tetrachloride and oil of chenopodium, thereby making it less objectionable to the patient. To children under fifteen years of age the following doses were given from the mixture:

For 3 years old child	0.6 cc.
For 4 years old child	0.8 cc.
For 5 years old child	1.0 cc.
For 6 years old child	1.2 cc.
For 7 years old child	1.4 cc.
For 8 years old child	1.6 cc.
For 9 years old child	1.8 cc.
For 10 years old child	2.0 cc.
For 11 years old child	2.2 cc.
For 12 years old child	2.4 cc.
For 13 years old child	2.6 cc.
For 14 years old child	2.8 cc.
After 15 years	3.0 cc.

One to two hours after administering the mixture a saturated solution of sodium sulphate was given. In most cases, on the morning of the second day a second purge was given with excellent results as determined by worm counts. This method of treatment was followed in all cases. Hospitalization was only done, however, in Dorado and the Asilo groups.

children were reexamined and showed 45 per cent, 9 per cent and 77 per cent positive for Necator, Ascaris and Trichuris, respectively.

The third general group consisted of 263 boys and 301 girls from various parts of the Island, living in the two Asilos of Santurce. This group was examined for intestinal helminths and was skin-tested. In the boys' group, 23.9 per cent were positive for Necator, 10.2 per cent positive for Ascaris, and 44.1 per cent positive for Trichuris. Within this same group 1.7 per cent of the boys were found infected with *Schistosoma mansoni* and 0.7 per cent with *Strongyloides stercoralis*. Protozoan parasites appeared as follows: *Giardia* 1.9 per cent; *E. histolytica* 1.5 per cent; and *Entamoeba coli* 11.4 per cent. Within the girls' group 12 per cent were positive for Necator, 9 per cent positive for Ascaris, and 29 per cent positive for Trichuris. Besides, 0.3 per cent were positive for *Taenia saginata* and 7 per cent positive for microfilaria. The protozoan infections were: 2 per cent positive for *E. histolytica,* 5 per cent positive for *Entamoeba coli,* and 0.3 per cent positive for *Giardia*. Only two examinations for intestinal parasites were made in the boys' and girls' groups, since there was little chance for hookworm reinfestation to occur during the time of the experiment.

In the compilation of our data no attempt has been made to separate into groups people infected with *Trichuris trichiura*. The fact that a very large percentage of cases positive for Trichuris was also infected with either Necator or Ascaris made it somewhat difficult to consider Trichuris in a group by itself. The other helminths as *Filaria (Wuchereria) bancrofti, Schistosoma mansoni, Strongyloides stercoralis* and *Taenia saginata,* and the protozoan infections will not be considered in this study.

MATERIALS AND METHODS

The adult *Necator americanus* and *Ascaris lumbricoides* used in the preparation of the extracts (test-antigens)* were obtained from human patients on treatment. The infective hookworm larvae were cultivated in charcoal and obtained by the Baermann isolation method. The *Fasciola hepatica,* commonly found here in the liver of cattle and goats, was secured fresh from the abattoir, immediately after killing the cattle. The bacterial test-antigen was obtained by washing off with sterile distilled water a 48-hour culture of pure *B. coli* grown on nutrient agar.

* The term "test-antigen" is used to signify solutions used in the test tube experiments, as differentiated from the term "antigen" signifying substances which stimulate the production of antibodies within the animal body.

In the preparation of the above test-antigens the material was thoroughly dried *in vacuo* over H_2SO_4 or in a Freas hot-air electric oven, ground by hand and then pulverized in an agate mortar with a mechanical grinder. All test-antigens were made up in proportion of 0.5 gram of dried powder to 100 cu. cm. of 0.85 per cent saline and Coca's alkaline solution. Extraction was continued for twelve hours, then filtered through a Jenken's porcelain filter, stored in sterile ampoules of about 2 cc. capacity and kept in the ice-box until ready for use. In the preparation of several test-antigens the extract was filtered through hard Whatman's filter paper No. 2, collected into sterile ampoules, sealed and autoclaved for 30 minutes at 120 pounds pressure.

In an endeavor to find a suitable test-antigen, the following preparations were made and tried out on individuals positive or negative for either Necator or Ascaris (Table 1).

(*a*) Adult *Necator americanus* in Coca's alkaline solution.
(*b*) Adult *Necator americanus* in Coca's alkaline solution autoclaved and not filtered.
(*c*) Adult *Necator americanus* in Coca's alkaline solution autoclaved and filtered.
(*d*) Adult *Necator americanus* in Coca's solution and not filtered.
(*e*) Adult *Necator americanus* in 0.85 per cent saline solution.
(*f*) Infective larvae of *Necator americanus* in 0.85 per cent saline solution, and in Coca's alkaline solution.
(*g*) Adult *Ascaris lumbricoides* (from man) in Coca's alkaline solution.
(*h*) Liver fluke, *Fasciola hepatica*, in Coca's alkaline solution.
(*i*) B. (*Escherichia*) *coli* in Coca's alkaline solution.

In the compilation of data obtained from the use of the various test-antigens it may be readily seen that three or four of these test-antigens were potent (Table 1). However, for convenience of preparation and keeping qualities, Coca's alkaline solution was preferred as the diluent. Coca's autoclaved, unfiltered, gave a surprisingly high percentage of positive reactions. The autoclaved filtered extract showed less potency than any of the other test-antigens used.

In making the intradermal tests the inner surface of the arm was thoroughly cleaned with alcohol and allowed to dry. With a 27½-inch Gage needle 0.025 cc. of the test-antigen was injected intradermally. In every case a control injection of the diluent was made on the same arm, a short distance above the site where the hookworm test-antigen was injected. In cases where multiple injections were made both arms were used.

Just what constitutes a true positive immediate type of skin reaction is at present disputable. Cooke([6]) in his treatise on broncho-asthma, considers a marked cutaneous reaction in which the urticarial wheal is 2 or 3 cu. mm. in diameter with the formation of pseudopods. Peshkin and Fineman([7]) in their work on asthma in children computed the size by measuring the diameter of the wheal. A 3 plus reaction was a wheal of more than 10 mm. in diameter with a zone of erythema and the formation of pseudopods. Fülleborn([8]) considers all wheals over 20 mm. as positive. Fairley, Fairley, and Williams([9]) studied the immediate type of reaction following the intradermal injection of hydatid test-antigen and attempted to evaluate the reaction by the size of the wheal and the presence of pseudopods. They regarded edema and erythema as too variable for measuring characteristics. In their measurements, they included within the greatest diameter the length of one pseudopod and the measurement of the second diameter at right angles to the first. They considered a reaction positive when the wheal measured 24 mm. in one diameter and at least an average of 22 mm. in both.

Throughout this study the authors have continued to use the method as adopted by Taliaferro and Hoffman([10]) and followed by Stumberg and Rodríguez-Molina([1]). Their method and the one here followed is essentially the measurement of an average of two diameters, not including the pseudopods measured at right angles to one another. The pseudopods were measured from the edge of the wheal. All measurements were taken to the nearest millimeter. All wheals made by the injection of the test-antigen and the diluent were measured at intervals of 10, 30 and 60 minutes. Pseudopods which generally disappeared by the end of 30 minutes were measured at intervals of 10 and 30 minutes. All intradermal reactions with an average diameter of 5 mm. or less were considered negative. This limit was followed consistently throughout the entire study since it was found that 0.025 cu. cm. of the diluent when injected intradermally, produced a small bleb ranging from 2 to 4 mm. in diameter.

Upon the injection of the test-antigen an immediate type of reaction generally occurred. This reaction was characterized by a wheal formation, an erythema and the presence of pseudopods. Pruritus frequently accompanied the reaction. All of these characteristics were not manifested in every test, nor did they bear any consistent relation to one another, either in their order of appearance or in the intensity of the reaction. In only a few cases was a delayed reaction observed. In infants under 24 months of age erythema was observed in a few cases without the formation of a definite wheal.

In all of the intradermal tests the authors noted only two cases which showed a marked systemic reaction. In one case, following the injection of *Necator americanus,* severe edema of the eyes, fever, tachychardia, arrhythmia and headache were well pronounced. The edema of the face and eyes persisted for several days. In the second case an extensive lymphangitis occurred on the arm and extended to the axillary glands; intensive pruritus and tenderness of the axillary glands were also observed. There was no shortness of breath nor rapid pulse. In all respects the patient seemed perfectly normal except for tenderness in the axillary region.

EXPERIMENTAL RESULTS

For convenience of description, the general groups as outlined above (Dorado, Aguadilla and the Asilos) were considered under eleven major groups. Each major group was divided into 4 minor groups according to the type of worms harboured:

(1) *Necator americanus* and *Ascaris lumbricoides* negative group.
(2) *Necator americanus* and *Ascaris lumbricoides* positive group.
(3) *Necator americanus* positive and *Ascaris lumbricoides* negative group and
(4) *Necator americanus* negative and *Ascaris lumbricoides* positive group.

Each of the eleven major divisions ~~are~~ described as follows:

1. Intradermal test before and after treatment, on individuals harboring *Necator americanus* and *Ascaris lumbricoides* (Tables 2, 2A, and 2B).
2. Control 1. Two intradermal tests at various intervals, on cases positive for intestinal nematodes. Cases were not treated. (Table 3).
3. Control 2. Intradermal tests with various test-antigens on cases negative for Necator and Ascaris but living under the same environmental conditions as 1 and 2 (Table 4).
4. Observations on the variability of wheal size at intervals of 3 and 5 months (Table 5).
5. Control 3. Intradermal test on 251 children from 7 to 20 years of age, living in the State of Michigan, where intestinal parasites are rare (Table 6).
6. Study of specificity to various test antigens as *A. lumbricoides, F. hepatica,* and *B. coli* (Table 7).
7. Comparison of intradermal test in relation to sex (Table 8).
8. Intradermal test on various age groups from 0 to 70 years (Table 9).

9. Atopic sensitiveness in mothers, and their infants under 24 months of age (Tables 10 and 11).
10. Intradermal test on two miscellaneous groups: (*a*) continentals inhabiting the Island from one to thirty-two years, and (*b*) group of native professionals such as teachers and physicians.
11. A study of the significance of the wheal size, erythema and pseudopod formation. (Table of Erythema 12). (Summary Table 13).

In the first major group (see Tables 2, 2A and 2B), 512 intradermal tests before treatment and 517 tests after treatment were made.* The object of making such a large number of tests was to determine an average per cent of reactivity in a population harboring an infection of intestinal nematodes and in order to determine the average difference in hypersensitiveness before and at various intervals of time after the administration of an anthelmintic. Post-treatment intradermal tests were performed on the same individuals on which pre-treatment tests were made. The tests were made at various intervals, from 1 to 2 months, 3 to 5 months, and as long as 10 months after treatment.

In table 2, 315 children of three of the minor groups gave 76 per cent, 91 per cent and 84 per cent positive skin reactions in 10 minutes, before treatment, while 278 of the same individuals, free from intestinal parasites, gave 93 per cent positive reactions in 10 minutes, one to two months post-treatment. It will be noted that slight differences occurred in the percentage of positive reactions before and after treatment in the 30 and 60-minute periods. There was also little difference between the formation of pseudopods before and after treatment. The average percentage of pseudopods of the three minor groups before treatment was 25.6 per cent and 18 per cent after treatment, a decrease of only 7.6 per cent in the treated group. There was no marked difference in the average size of the wheals before and after treatment.

In the minor group, Necator and Ascaris positive, in 10 minutes, 84 per cent of the total 32 positive reactions gave wheals within 5.5 to 10 mm. in diameter, and only 16 per cent of the positive reactions were 10.5–15 mm. in diameter. In the second minor group, Necator positive and Ascaris negative, 61 per cent of 186 positive reactions gave wheals of an average diameter of 5.5 to 10 mm. and 37 per cent fell within the 10.5 to 15 mm. diameter. The third minor group, Necator negative and Ascaris positive, showed 81 per cent

* Five additional cases from another group are here included.

and 19 per cent of the 59 positive reactions with wheals within the first- and second-size groups, respectively.

One to two months after treatment, 74 and 23 per cent of 259 positive reactions were 5.5 to 10 mm. and 10.5 to 15 mm. in diameter, respectively. Before and after treatment there was a difference of about 10 per cent, which is of no significance. Table 2A shows a considerable difference in the size of wheals before and after treatment. In the two minor groups, before treatment, 60 and 62 per cent of the total 46 and 97 positive reactions, respectively, gave wheals with an average diameter of 5.5 to 10 mm. Three months post-treatment the average wheal size in the two groups decreased, since the average per cent of wheals increased within the smallest wheal size-group to 83 and 82 per cent. In the two other groups the size of the wheals remained about the same.

There is also a difference in the percentage of wheals with pseudopods before and three months post-treatment (Table 2A). The indefinite reactions remain about the same for the pre- and post-treatment tests.

In table 2B, five and ten months after treament, there is an increase in the number of smaller wheals. In the minor group, positive for Necator and Ascaris, 12 per cent of the positive reactions were 5.5 to 10 mm. in diameter before treatment. In the group negative for Necator and Ascaris, 30 and 57 per cent, respectively, gave smaller wheals. The other minor groups also gave a corresponding decrease in the wheal size with time after treatment. The percentages of indefinite reactions increased in 60 minutes. The formation of wheals with pseudopods decreased with time after treatment.

Stumberg and Rodríguez-Molina[1] in their statistical study of 60 individuals of the same group, found a significant difference between the size of the 10-minute wheal before and after treatment, in favor of the former (P.E. Diff. is 12.18).

In the first major groups (Tables (2, 2A and 2B) the percentage of positive reactions within the 10-minute period did not show any marked change before and after treatment. However, there was a considerable difference in the rate at which the wheals became indefinite in 30- and 60-minute periods, five months post-treatment. Stumberg and Rodríguez-Molina also found a difference in the rate at which the wheals became indefinite post-treatment, the X^2s for 30- and 60-minute readings being 38.9944 and 76.5938, respectively. Furthermore, the formation of pseudopods, before and after treatment, shows considerable variation. Table 2, one to two months after treatment, gives an average of 25.6 per cent of wheals with

pseudopods in the three groups before treatment, and 18 per cent post-treatment. In Table 2A pre-treatment, an average of 72 per cent of wheals contained pseudopods, against 27.8 per cent, three months after treatment, in the four groups. Likewise, in the five and ten months post-treatment periods (Table 2B) the average for the two groups is 79 per cent before treatment, as compared to 48.6 per cent and 35.3 per cent, five and ten months after treatment, respectively.

The question arises, in a series of intradermal tests as described above, what might have been the results of the intradermal test if the treatment had not been administered? Is the slight reduction in the wheal size and the decrease in the percentage of wheals with pseudopods after treatment due to elimination of the parasite, or is it nothing more than a biological variant? The writers are quite aware of the fact that the intervals between the intradermal tests were not long, and, furthermore, that reinfection in the treated cases was difficult to control, especially among farmers. The latter, we feel, has been well controlled in the boys and girls from the Asilos, where there was no opportunity for reinfection, particularly with *N. americanus*. To determine the extent of variation in the wheal size and other characteristic changes, the following control groups were studied:

1. Group positive for intestinal parasites and skin-tested several times before treatment (Table 3).
2. Group of individuals negative for intestinal parasites and skin-tested several times with various test-antigens (Table 4).
3. Multiple injections on the same cases at intervals of three and five months (Table 5).

In the two minor groups of cases, positive for Necator and Ascaris, and positive for Necator, (Table 3) when the tests were made twice before treatment (cases were not treated until close of experiment) the size of the wheals varied somewhat between the first and second skin tests. The average wheal size for the first group is larger than for the second, but there is no change in 10 minutes between the per cent of positive reactions, in general, in the two tests of the two groups; i. e., the average per cent of positives in 10 minutes was 98 per cent and, likewise, 98 per cent for the first two tests, and 98 and 100 per cent for the second tests. The rate of appearance of indefinite reactions in the 60-minute periods remained about the same for the first and second tests. Also, there was no significant change in the formation of pseudopods.

The group of individuals negative for intestinal nematodes, were skin-tested several times with various test-antigens. The table is divided into five antigen groups, each group showing the result of a test-antigen (Table 4). With each antigen group are two minor groups showing the results of the first and second intradermal tests on the same individuals, performed at intervals of 1 to 4 months apart with a homologous test-antigen. In the first and second tests with the same antigens the per cent of positive reactions in the 10-minute period showed a close similarity while on the other hand, the size of the wheals varied between the first and second tests of the same antigen groups. For example, the ten minute reactions of the first and second skin tests for the five different antigen groups are as follows: 98 per cent (first test) and 93 per cent (second test); 80 per cent and 82 per cent; 75 and 72 per cent; 81 and 81 per cent; and 100 per cent and 98 per cent. This similarity does not hold for the 30 and 60-minute readings. In the saline larvae group the percentage of wheals with pseudopods remains fairly constant throughout the two tests. For the Coca's adult antigen and Coca's adult unfiltered tests there is a variation of 16 to 8.7 per cent and 26 to 14.9 per cent, respectively.

Of 528 skin tests made, using Coca's adult of *Necator americanus*, 289 positive reactions on the first test gave 37 per cent of the wheals with pseudopods, while on the second test only 3 per cent of the 137 positive reactions contained pseudopods. There is a significant change in the formation of pseudopods between the first and second intradermal tests with the same antigen. These facts show a considerable variation in the formation of pseudopods.

In the 60-minute period the indefinite reactions were fairly constant in all antigen groups, except in two. The difference between the two intradermal tests was: 0 first test, 70 per cent second test; 52 per cent and 59 per cent; 63 per cent and 65 per cent; 80 per cent and 84 per cent; 0 and 40 per cent. For a complete summary of figures see Table 4. The writers believe that the high percentage of postive skin tests within these groups of cases negative for *Necator americanus* is due to the fact that most, if not all, of these people have had at some time or other an infection of Necator and that the positive skin reactions may be nothing more than remainders of past infections.

Schloss[11] has found that the size of a positive reaction varied, depending on its location in the arm. This observation was later confirmed by Larsen, Paddock and Alexander[12] by making tests with ragweed pollen on sensitive patients. Recently, Alexander[13]

has shown that the position where injections are made on the body influences to a great extent a negative or a positive reaction. In making multiple injections for comparison of wheal size it seemed advisable that, in skin testing, the site of operation should be made as constant a factor as possible. To this end, we used the inner surface of the arms a short distance above the wrists and below the elbow. Care was taken not to use the same site twice.

In carrying out this study a group of 37 individuals was divided into three test-antigen groups, each group being tested three times; second and third tests were made 3 and 8 months after the first. All of these cases were positive for Necator; a few were also infected with Ascaris. None received treatment for intestinal helminths. The averages for the first, second and third tests at the 10- and 30-minute periods were very much alike. On comparison, the general averages of the three tests for the three antigen groups were similar. A fairly constant result was obtained in the average diameter of the individual wheals. For example, in case 23, the 10- and 30-minute readings for the three tests varied little. On the other hand, in case 22 there was no constancy. The formation of pseudopods was likewise variable. (Table 5)

The intradermal tests as above described have been performed on individuals who have spent their entire life on the Island, and without doubt have had, or in some way have come in contact, with intestinal helminths. This fact alone may account for an average of 80 per cent positive skin reactions for 10-minute periods in individuals of all the groups who were negative for Necator and Ascaris (Table 4).

In order to determine the degree of hypersensitiveness caused by the presence of Necator and Ascaris, it was essential to have as control tests individuals who were not only free from Necator but who had lived all their life in an area free from hookworm. For this study, a group of 251 Michigan children was selected. The clinical histories of these 251 cases showed no record of worms. On a large number of these children fecal examinations for parasites were previously made from time to time, their stools being negative. In carrying out the intradermal tests the same technique was used as in the Porto Rican groups. The test-antigen, Coca's adult of *Necator americanus*, and the diluent, were from the same preparations as those of the former tests. Also all wheals over an average diameter of 5 mm. were considered positive. Table 6 shows the following results: Within the 10-minute period 20 per cent of the wheals were positive and within 30 and 60 minutes, 17 per cent and 3 per cent gave positive readings, respectively. The average diam-

eter of the wheals was small: 5.5–10 mm. Erythema was present in 3 per cent, 7 per cent and 5 per cent, within the 10-, 30- and 60-minute periods, respectively. In the control-diluent test, 2 per cent of the total number of tests gave wheals, and 18 per cent gave erythema. Thirteen per cent of the wheals contained pseudopods in 10 minutes and all pseudopods had completely disappeared by the 30-minute period. It will be of general interest to note here that Schick tests were made on the opposite arm at the same time that the injections of *Necator americanus* antigen were given. Over 93 per cent of the reactions in 10 and 30 minutes due to diphtheria toxin compared in intensity of reaction to the wheals produced by the injection of the Necator extract. By eliminating those reactions due to the diluent, which were 2 per cent of the total number, and the reactions similar in the 10- and 30-minute periods to the pseudo-Schicks, it can be safely stated that only 7 of the 251 cases or 2.7 per cent gave a positive reaction. It therefore seems reasonable to assume from the present experimental results that marked cutaneous hypersensitiveness to extracts of *Necator americanus* is demonstrated among groups infected or who have had contact with *Necator americanus,* while those individuals free from infection and who had lived outside of the infected area, were not sensitive to a similar amount of the test-antigen.

Rackemann and Stevens[14] as well as many others, specially those working with *Ascaris lumbricoides*[15], have demonstrated that sensitiveness can be acquired through contact. On a miscellaneous group of 13 continentals who have inhabited the Island from 5 to 32 years, attempts were made to demonstrate the possibility of an acquired hypersensitiveness through contact. The individuals of this group, as fas as they knew, had never been infected with *Necator americanus* or *Ascaris lumbricoides.* Fecal examinations made at the time of the tests gave negative stools for worms. Of six continentals with 8 to 32 years' residence in the Island, five gave marked positive cutaneous reactions and one gave a slight reaction. Of seven with 1 to 4 years' residence, four gave negative results and three gave slight reactions. While the continental group of 13 cases is too small to make any generalized statement, yet from the data obtained it has been found that sensitiveness seems to increase with the years of residence.

In connection with the studies on acquired sensitiveness, a group of 18 teachers and physicians (all of whom were born and for the most part, educated in the Island and negative for intestinal nematodes for ten years or more) gave positive reactions in 50 per cent,

39 per cent, and 11 per cent, in 10, 30 and 60 minutes, respectively. Fifty per cent of the wheals had an average size of 5.5–10 mm. in diameter. In the negative group as described above (Table 4) the percentages of positive reactions were much higher. The data from such a small group may not be of significance; yet it does support Fülleborn's([8]) view that sensitivity persists for a period of years following the cessation of intestinal worms.

In the analysis of the above data nothing has been said as to specificity of the reactions. Brünner ([16]) postulates a group reaction embracing the various members of the nematode family as Ascaris, Trichuris, Oxyuris, and Necator, because individuals show a marked reaction when infected with other nematodes and not necessarily infected with Ascaris. Fülleborn ([8]) reports evidence of a specific reaction between Ascaris and Strongyloides, while, on the other hand, he obtained immediate reactions with hydatid fluid in two patients injected with *Strongyloides stercoralis,* but showed no evidence of hydatid disease.

The purpose of making specificity tests with various test-antigens of *Ascaris lumbricoides, F. hepatica** and a common bacterium of the intestine, *B. coli,* was to see if the reactions were specific in nature in individuals whom we knew were infected with *Necator americanus* and *Ascaris lumbricoides.* With the test-antigen of *B. coli* and *F. hepatica* we tested 121 cases, using the right and left arms, respectively. Another 60 individuals were tested with extracts of *Ascaris lumbricoides.* Boys from 12–17 years of age were chosen for the above tests. The average size of the wheals due to intracutaneous injections with *B. Coli* and *F. hepatica* was the smallest obtained in any of the previous infected groups (Table 7). Ninety and 93 per cent of the wheals had an average diameter of 5.5–10 mm. Of those cases injected with Ascaris extracts, 78 per cent gave a wheal of an average diameter of 5.5–10 mm.

A comparison of the average diameter of the wheals in the three control test-antigens and Necator test-antigen in 10, 30 and 60 minutes, is as follows:

	10 Min.	30 Min.	60 Min.	Control
B. coli	8.05 mm.	9.83 mm.	10.66 mm.	7.04 mm.
F. hepatica	7.44 mm.	9.03 mm.	13.50 mm.	7.04 mm.
A. lumbricoides	9.32 mm.	10.82 mm.	11.45 mm.	7.45 mm.
N. americanus	10.98 mm.	13.39 mm.	7.94 mm.	7.24 mm.

A significant fact is that individuals infected with Necator and

F. hepatica is not a common infection in man on the Island, since only six cases have been reported so far.

Ascaris gave the largest wheals to a homologous test-antigen. The size of the wheals increased as to time in all the groups except in the group tested with *Necator americanus*, where the average for the 60–min. period was the smallest. Its maximum size was reached in about 30 minutes.

The rate at which the indefinite reactions appeared is of no importance. There is, however a considerable difference in the formation of pseudopods. In the cases positive for Necator and Ascaris, and not treated (Table 3), an average of 72 per cent of the total wheals showed pseudopods while only 15 and 12 per cent of the wheals for *B. coli* and *F. hepatica*, respectively, (Table 7) showed pseudopods. The figures are comparable to the Michigan group (Table 6) where only 13 per cent of the wheals had pseudopods and 100 per cent of the wheals had an average of 5.5 to 10 mm. in diameter. The percentages of wheals containing pseudopods in the tests for *B. coli*, *F. hepatica* and *A. lumbricoides* were 15, 12 and 40 per cent, respectively. Ascaris test-antigen gave the highest per cent, which was expected, since most of the cases were infected with ascaris.

From the data shown in Table 7 there is some difference between the reactions to the various test-antigens. The most significant one occurred in the formation of pseudopods. The difference in the average wheal size is not large enough to warrant any specificity of reaction. This seems to support the group-reaction theory as postulated by Brünner. However, it must be considered that a large per cent of the above cases were infected with two or more nematodes and therefore, before the question of specificity of group reaction can be established, the authors feel it would be necessary to use a larger number of individuals living in an area infected with only one of the common intestinal parasites.

There is the opinion that hypersensitiveness may not bear any relation to the degree of infestation, since many individuals with a low degree of infection have often given the most marked intracutaneous reactions, and, again, those with a heavy infestation responded only slightly to worm extracts.

SEX

Stumberg and Rodríguez-Molina's findings have demonstrated that the degree of infestation is not correlated to the mean wheal size. Hill([17]) and Daengsvang([18]) have both found a higher rate of infestation among men than women. They give as an explanation to this the result of frequent exposures, especially in the coastal

plains where sugar-cane is the main crop. If men are more exposed to the infective larvae than women, it seems reasonable to suppose that there should be a significant difference between the hypersensitiveness of men and women. In this study a well-controlled group of 315 cases (179 boys and 136 girls, from 7 to 20 years of age) was tested. All the 315 individuals were examined for intestinal parasites and arranged in groups, according to the type of infection.

Three groups were used: (1) A group infected with Necator and Ascaris. (2) A group positive for Necator and negative for Ascaris. (3) A group positive for Ascaris and negative for Necator (Table 8). Within the three groups the girls gave a much higher percentage of wheals of an average size of 5.5 to 10 mm. in diameter than the boys; that is, the wheals were smaller among girls than among boys. For instance, the per cent of reactions with an average wheal size of 5.5 to 10 mm. in the 10-minute period Necator and Ascaris positive group was 75 per cent for boys while among girls the percentage was 100.

In the Necator positive and Ascaris negative group, the same held, true; for boys 45 per cent of the wheals were of the smallest average size, against 97 per cent for the girls.

The per cent of positive cases in the Necator negative and Ascaris positive group within the 10-min. period was 69 per cent for the boys and 89 per cent for the girls.

The positive reactions of the total number of boys was from 95 per cent to 100 per cent and from 57 to 79 per cent for girls. In other words, the percentage of positive reactions was higher among the boys than among the girls.

A difference was also noted in the formation of pseudopods in relation to sex. The percentage of wheals with pseudopods was higher among boys than among girls. For instance, for boys and girls the results were 25 and 8 per cent; 35 and 26 per cent and 43 and 14 per cent in the three groups, respectively.

The degree of hypersensitiviness to *Necator americanus,* in regard to sex is well established in the above results (Table 8). However, we feel that this difference is not entirely due to a physiological sex factor but rather to the number of exposures to infection.

AGE

The question of age in connection with hypersensitiveness is a much disputed one. Brünner[16] has found in his study of intracutaneous tests for Ascaris in adults and children from 11 months to 17 years of age, that only 3.5 per cent of 181 cases gave significant

intracutaneous reactions. About 40 per cent of these reactions occurred in adults infected with intestinal nematodes. All of his children tested, under 4 years of age, gave negative stools and negative skin reactions. He therefore came to the conclusion that positive intracutaneous reaction to Ascaris extract is a result of infection with one or more members of the nematode group. Stumberg and Rodríguez-Molina([1]), however, did not find any relation between age and wheal size among their cases.

Kobak and Pilot([19]), on the other hand, seem to think that the negative results obtained in infants with various protein and worm extracts were due to a lack of reacting power of the skin rather than to circulating antibodies. Ratner and Greenburgh([20]) present still another point of view. They feel that the mechanism of congenital hypersensitiveness depends on placental permeability and is a result of the passage of the antigen from an unsensitive mother to her susceptible fetus, or of sensitizing antibodies from an allergic mother to her susceptible offspring.

In view of our own conceptions, we arranged 276 persons, not including 111 mothers, into various age-groups from 1 week to 40 years and over. (Table 9). Age groups were from 1 week to 2 years, 3–9 years, 10–14 years, 15–25 years, 26–39 years, and 40 years and over. From the 10-year old to the oldest age group no appreciable difference was observed in the percentage of positive reactions, nor was there any difference in the rate of appearance of indefinite reactions. The pseudopod formation remained about the same. However, in the age groups below 9 years of age there is a considerable difference as compared with the older groups. In the group 3–9 years the percentage of positive reactions was 81 per cent as compared with 100 per cent in the older age groups and pseudopods were 40 per cent, as against 69–74 per cent in the older age groups. A significant change was noticed in the group 1 week–24 months old, where only 5 per cent positive reactions occurred in the 10-minute period. All infants under 12 months of age gave negative reactions though 2 cases from 7–9 months old gave positive erythemas but no wheals. In another series of 106 infants who did not give any reaction to Coca's extract of *Necator americanus* (Table 10), 78 per cent were negative for parasites, 11.3 per cent positive for Ascaris, 5.6 per cent positive for Necator and 6.5 per cent positive for Trichuris. The mothers of these infants gave 100 per cent marked intracutaneous reactions. (Table 11).

In addition to the above figure (106 infants), nine from 7–24 months old gave positive reactions. These are included in a separate

table (Table 11) on account of marked erythema without any measurable wheal being obtained, while in some cases a measurable wheal was present without erythema. The average wheal sizes in children were small, being only 6.7 and 7.7 mm. in the 10-and 30-minute readings, respectively. Six of the 9 infants showed erythema, and four of the 9 cases were infected with Ascaris and Trichuris.

From the above data it seems reasonable to assume that age (as a physiological factor) does not influence the degree of intensity in the reaction, but rather, that hypersensitiveness bears a relation to exposure. A marked difference occurred in the age group 1 week–24 months of age, as compared to the other age groups. This difference, we believe, can be attributed to exposure to infection. Furthermore, the study of mothers and their infants tends to show that the immediate type of reaction is not truly atopic in character but develops with exposure.

Negative reactions in cases of positive stools seem to indicate lack of time in the development of worm hypersensitiveness. In only 3 cases (children of one year of age) did we obtain negative cutaneous reactions with positive stools.

ERYTHEMA

Erythema is generally the most common and constant component of the intradermal reaction although at times it may be entirely lacking. In the present study it occurred in about 72 per cent of the cases tested, before treatment. Erythema may occur, as in the case of children (Table 11), without the presence of wheals or pruritus.

It was observed that the minimum and maximum size of erythema in the 10- 30- and 60-minute periods varied little between the 225 tests performed before treatment and the 223 tests made after treatment. The average sizes of erythema for the pre-treatment tests were 13.3, 22 and 14 mm.; and post-treatment, 22.5, 22.5 and 11.3 mm. for 10-, 30- and 60-minute periods, respectively. No difference was noticed in the percentage of erythema to the various extracts used throughout the study.

LOCAL POSITIVE TRANSFER OF SKIN SENSITIVITY TO *Necator americanus*

The experiment of Prausnitz and Küstner([21]) was performed on five individuals who had previously given negative reactions to *Nec. americanus*. The serum from a case that had given a marked intra-

cutaneous reaction was diluted with an equal amount of 0.85 per cent saline solution filtered through a Jenken's porcelain filter and tested for sterility. The inner surface of the arms of non-reacting recipients was cleaned with alcohol and then 0.2 cc of the sterile filtrate was injected intradermally at two sites. Twenty-four hours later 0.025 cc of the Necator extract and Coca's diluent was injected at the prepared sensitized sites. Two controls, one Coca's and another Necator test-antigen, were injected on normal sites. In the case of successful local passive transfer a positive reaction should be obtained on the first and not on the control sites. Out of five passive transfers made we obtained four positive and one negative. Three were well marked reactions and one only slight. All three controls were uniformly negative.

CONCLUSIONS

Experimental results of these studies demonstrate that in certain persons there is a marked intracutaneous hypersensitiveness to extracts of *Necator americanus*. In a total of 2,901 intradermal tests this hypersensitiveness was detected in 81 to 98 per cent of all Porto Ricans tested, while infants under 12 months of age were uniformly negative. Of those children tested in the State of Michigan, 20 per cent gave positive reactions and only 7 of the 251 gave reactions of any significance.

In general, five months after treatment, compared to its size before treatment, the wheal size decreased greatly. There was also an appreciable reduction in the number of wheals with pseudopods after treatment.

In cases with positive stools, untreated, the percentage of wheals with pseudopods remained about the same throughout two or three intracutaneous tests. Cases with negative stools gave the lowest percentage of positive reactions in 10 minutes. Eighty-three and three-tenths (83.3) per cent of the wheals had an average diameter of 5.5 to 10 mm. Only 20.5 per cent of the wheals contained pseudopods. These positive cutaneous reactions undoubtedly represent remainders of previous infections and demonstrate that sensitiveness may persist indefinitely after cessation of the parasite.

The controls for specificity tests with *B. coli*, *F. hepatica* and *A. lumbricoides* are significant. The lowest percentage of positive reactions and of wheals with pseudopods was obtained in cases with positive stools for Necator and Ascaris, with extracts of *B. coli* and *F. hepatica*.

Ascaris test-antigen gave results comparable to those of *Necator americanus*. These, of course, may be due to the fact that a large percentage of the cases was infected with Ascaris. The results are compatible with the assumption that sensitivity to *Necator americanus* in man is a group phenomenon arising as a result of sufficient contact with the excitants or due to the presence of intestinal nematodes. If we consider only those cases infected with Ascaris and Trichuris, the correlation between infection and sensitivity to extracts of *Necator americanus* is high. However, it should be noted that a large per cent of the cases tested have had in all probability a previous infection of *Necator americanus*.

There seems to be no relation between the wheal size and the presence or absence of erythema to the incidence of infection. Within the various age groups a marked difference was noted. This seems to bear out the contention that positive intracutaneous reactions to extracts of worm powder, immediate in reaction, are not of a true atopic character but rather due to exposure and infestation. Negative findings among the Michigan school children and the continental group seems to support this statement.

The authors feel that for practical application of the skin test in detecting the presence of *Necator americanus* in individuals infected with two or more intestinal nematodes, the measurable characteristics are too variable, and the reactions are not significantly specific.

SUMMARY

(1) Hypersensitiveness to *Necator americanus* test-antigen was demonstrated in 90 to 98 per cent of 1,081 Porto Ricans positive for intestinal nematodes in the ten-minute period.

(2) This sensitiveness was found to diminish in intensity and there was a decrease in the formation of pseudopods five to ten months post-treatment.

(3) Hypersensitiveness was also shown in 81 per cent of 1,042 tests on patients negative for intestinal parasites with 20 per cent of the wheals containing pseudopods.

(4) Wheal formation appeared in 98 per cent of 189 tests, in ten minutes, in patients positive for intestinal worms, with 72 per cent of the wheals containing pseudopods.

(5) Skin sensitivity to *Necator americanus* was tested in 251 Michigan children. Twenty per cent reacted in ten minutes, 13 per cent of the wheals contained pseudopods and all of the wheals were under an average of 10 mm in diameter.

(6) Hypersensitiveness to *Necator americanus* was found in only 5 per cent of fifteen children under twenty-four months of age. The mothers of these children gave 100 per cent positive reactions.

(7) Extracts of *B. coli* and *F. hepatica* on one hundred and twenty-one patients were positive in 73 and 55 per cent of these cases, respectively. Only 15 and 12 per cent of the wheals contained pseudopods; 90 and 93 per cent of the wheals were under an average diameter of 10 mm.

(8) Intradermal tests to *A. lumbricoides* were positive in 92 per cent of sixty cases, 40 per cent of which showed pseudopods.

(9) The wheal size and formation of pseudopods were found to vary in the same individual.

(10) Continentals living on the Island for various lengths of time seem to acquire hypersensitiveness to *Necator americanus*.

(11) Hypersensitiveness was more marked in boys than girls. This difference is probably due to exposure rather than to the degree of infection.

(12) The average per cent of positive reactions in people over 10 years of age was fairly constant in the 10 and 30-minute periods. In age group 3–9 years old there is a considerable difference in the intensity of the reaction compared with the other groups. A significant change was noted in the group 1 week–24 months old, where only 5 per cent of 115 children gave positive reactions in the 10-minute period.

(13) Erythema is not a constant factor, but when present the average size of the erythemic area varied but little.

(14) No relation was found between the positive skin reaction and the presence of Necator or other intestinal nematodes.

(15) Table 13 shows a summary of 2,901 skin tests and the per cent of positive, negative and indefinite reactions and pseudopods to extracts of *Nec. americanus* in 10-, 30- and 60-minute periods.

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TABLE 1

SHOWING THE AVERAGE PER CENT OF POSITIVE, NEGATIVE AND INDEFINITE REACTIONS IN TWO GROUPS (NECATOR POSITIVE—ASCARIS NEGATIVE AND NECATOR NEGATIVE—ASCARIS POSITIVE) WITH VARIOUS EXTRACTS OF *NECATOR AMERICANUS*

Antigen	Necator Positive—Ascaris Negative												Necator Negative — Ascaris Positive											
	Per cent of Positive Reactions				Per cent of Indefinite Reactions			Per cent of Psds.	Per cent of Negative Reactions				Per cent of Positive Reactions				Per cent of Indefinite Reactions			Per cent of Psds.	Per cent of Negative Reactions			
	C*	*10 min.*	*30 min.*	*60 min.*	*10 min.*	*30 min.*	*60 min.*		C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*	*10 min.*	*30 min.*	*60 min.*		C*	*10 min.*	*30 min.*	*60 min.*
Saline Larvae	28	100	28	—	—	—	—	50	72	—	72	—	28	100	28	—	—	—	—	50.7	72	—	72	—
Coca's Larvae	15	100	60	—	—	—	—	35	85	—	40	—	50	100	50	—	—	—	—	50	50	—	50	—
Coca's Adult Autoclaved Unfiltered	—	100	86	39	—	14	60	11	100	—	—	—	—	100	50	—	—	50	100	—	100	—	—	—
Coca's Adult	5	95	84	30	—	8	64	48	95	5	8	6	—	100	40	—	—	20	100	40	100	—	4	—
Coca's Adult Unfiltered	—	85	50	7	—	3	85	30	100	15	7	7	—	81	63	9	—	18	72	—	100	19	18	18
Saline Adult	—	80	80	26	—	3	60	26	100	20	16	13	—	86	81	19	—	—	66	19	100	14	19	14
Coca's Adult Autoclaved Filtered	—	75	58	12	—	25	70	8	100	25	16	16	—	60	47	—	6	30	79	7	100	30	20	20

C*—Diluent control.

TABLE 2

SHOWING RESULTS OF SKIN TESTS WITH COCA'S EXTRACT OF ADULT *NECATOR AMERICANUS* ON SCHOOL CHILDREN FROM 7 TO 20 YEARS OF AGE, BEFORE, AND ONE TO TWO MONTHS AFTER TREATMENT

Infection	Before Treatment												1 – 2 Months after Treatment			
	Positive Necator and Ascaris				Positive Necator Negative Ascaris				Positive Ascaris Negative Necator				Negative Necator and Ascaris			
Size of wheal in mm.	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*
5.5 — 10	2	27(84%)	17	2	8	112(61%)	67	20	5	48(81%)	29	2	26	193(74%)	103	4
10.5 — 15	—	5(16%)	6	3	—	70(37%)	70	14	—	11(19%)	8	3	—	50(23%)	69	31
15.5 — 20	—	—	1	1	—	4(2%)	11	5	—	—	2	—	—	4(2%)	9	2
20.5 — 25	—	—	—	—	—	—	2	—	—	—	—	—	—	2(1%)	2	—
Total Positive	2	32	24	6	8	186	150	39	5	59	39	5	26	259	183	37
Per cent Positive	4%	76%	58%	20%	3%	91%	73%	29%	7%	84%	55%	9%	9%	93%	65%	15%
Indefinite	—	—	9	20	—	—	23	93	—	1	9	38	—	—	78	178
Per cent	—	—	21%	69%	—	—	11%	65%	—	2%	19%	74%	—	—	28%	74%
Total	2	32	33	26	8	186	173	132	5	60	48	43	26	259	261	215
Negative	40	10	9	3	195	17	30	11	65	10	22	8	252	19	17	24
Per cent	96%	24%	21%	11%	97%	9%	15%	6%	93%	14%	31%	17%	91%	7%	7%	11%
Total	42	42	42	29	203	203	203	143	70	70	70	51	278	278	278	239
Total Number of Cases	315												278			
Pseudopods																
1 — 3 mm	3				30				8				19			
3 — 9 mm	2				28				5				23			
9 mm. up	1				4				2				6			
Total Pseudopods	6(19%)				62(33%)				15(25%)				48(18%)			

*C — Diluent control.

TABLE 3

SHOWING INTRADERMAL TESTS TO ADULT *NECATOR AMERICANUS* ON TWO GROUPS, POSITIVE FOR NECATOR AND ASCARIS AND POSITIVE FOR NECATOR
THESE CASES WERE NOT TREATED UNTIL CLOSE OF THE EXPERIMENT

Size of wheal in mm.	First Test								Second Test							
	Positive for Necator and Ascaris				Positive for Necator				Positive for Necator and Ascaris				Positive for Necator			
	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60*
5.5 — 10	11	22(50%)	9	9	20	28(58%)	19	16	11	19(43%)	9	7	15	17(34%)	8	6
10.5 — 15	3	20(44%)	16	13	1	18(38%)	19	14	—	23(52%)	21	13	2	30(61%)	27	19
15.5 — 20	—	2(4%)	7	7	—	2(4%)	7	5	—	2(4%)	11	8	—	2(4%)	11	8
20.5 — 25	—	1(2%)	3	3	—	—	—	—	—	—	1	1	—	—	1	3
25.5 — 30	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1
Total Positive	14	45	35	32	21	48	45	35	11	44	42	30	17	49	47	37
Per cent	30%	98%	76%	69%	42%	98%	92%	71%	24%	98%	93%	66%	34%	100%	95%	74%
Indefinite	—	—	10	13	—	—	3	11	—	—	3	11	—	—	1	9
Per cent	—	—	22%	28%	—	—	6%	20%	—	—	6%	24%	—	—	2%	24%
Total	14	45	45	45	21	48	48	46	11	44	45	41	17	49	48	46
Negative	32	1	1	1	28	1	1	3	34	1	—	4	32	—	1	3
Per cent	70%	2%	2%	2%	58%	2%	2%	6%	76%	2%	—	9%	66%	—	2%	8%
Total	46	46	46	46	49	49	49	49	45	45	45	45	49	49	49	49
Total No. of Cases	95								94							
Length of Pseudopods in mm.																
1 — 3 mm	21				17				19				18			
4 — 9	13				13				13				12			
9 mm. up	2				1				1				5			
Total Pseudopods	36				31				33				35			
Per cent Pseudopods	80%				64%				75%				71%			

*C—Diluent control

TABLE 2—A

SUMMARY OF SKIN TESTS WITH COCA'S EXTRACT OF DRIED PULVERIZED LARVAE OF *NECATOR AMERICANUS* BEFORE, AND THREE MONTHS AFTER TREATMENT

Size of wheal in mm.	Before Treatment						Three Months After Treatment											
	Positive for Necator and Ascaris			Necator positive Ascaris negative			Negative for Necator and Ascaris			Necator positive Ascaris negative			Necator negative Ascaris positive			Positive for Necator and Ascaris		
	C*	10 min.	30 min.	C*	10 min.	30 min.	C*	10 min.	30 min.	C*	10 min.	30 min.	C*	10 min.	30 min.	C*	10 min.	30 min.
5.5 — 10	20	28(60%)	13	29	61(62%)	15	8	54(83%)	35	11	46(82%)	39	1	4(66%)	3	2	5(63%)	6
10.5 — 15	—	16(34%)	20	1	35(36%)	50	—	9(14%)	20	—	8(14%)	17	—	2(34%)	2	—	3(37%)	2
15.5 — 20	—	2 (6%)	5	—	1 (2%)	8	—	2(3%)	—	—	2(4%)	—	—	—	—	—	—	—
Total Positive	20	46	38	30	97	73	8	65	55	11	56	56	1	6	5	2	8	8
Per cent Positive	43%	97%	80%	30%	97%	73%	11%	90%	76%	19%	94%	94%	12%	75%	62%	22%	88%	88%
Indefinite	—	—	7	—	—	23	—	—	15	—	—	2	—	—	1	—	—	1
Per cent	—	—	15%	—	—	23%	—	—	21%	—	—	3%	—	—	13%	—	—	12%
Total	20	46	45	30	97	96	8	65	70	11	56	58	1	6	6	2	8	9
Negative	27	1	2	70	3	4	64	7	2	48	3	1	7	2	2	7	1	—
Per cent	56%	3%	4%	70%	3%	4%	89%	10%	2%	81%	5%	1.7%	88%	25%	25%	78%	12%	—
Total	47	47	47	100	100	100	72	72	72	59	59	59	8	8	8	9	9	9
Total No. of cases	147						148											
Length of Pseudopods																		
1 — 3 mm		19			48			10			11			1			1	
4 — 9 mm		12			15			6			8			1			—	
9 mm up		3			5			1			2			—				
Total Psds		34			68			17			21			2			1	
Per cent		74%			70%			26%			38%			33%			12.5%	

C*—Diluent control.

TABLE 2—B

SKIN TESTS ON COUNTRY PEOPLE WITH COCA'S EXTRACT OF DRIED PULVERIZED ADULT *NECATOR AMERICANUS* BEFORE, FIVE, AND TEN MONTHS AFTER TREATMENT

Size of wheal in mm.	Before Treatment								Five Months after Treatment												Ten Months after Treatment											
	Positive Necator & Ascaris				Positive Necator Negative Ascaris				Negative Ascaris and Necator				Positive Necator Negative Ascaris				Positive Ascaris Negative Necator				Negative Ascaris and Necator				Positive Necator Negative Ascaris				Positive Ascaris Negative Necator			
	C*	10 min.	30 min.	60 min.	C*	10 min.	30 min.	60 min.	C*	10 min.	30 min.	60 min.	C*	10 min.	30 min.	60 min.	C*	10 min.	30 min.	60 min.	C*	10 min.	30 min.	60 min.	C*	10 min.	30 min.	60 min.	C*	10 min.	30 min.	60 min.
5.5 — 10	—	4(12%)	7	7	—	3(18%)	2	4	5	8(30%)	2	—	—	—	2	1	4	2(22%)	—	—	2	21(57%)	19	4	—	1(33%)	1	—	—	6(50%)	3	1
10.5 — 15	—	22(66%)	16	10	—	5(29%)	4	1	1	14(55%)	3	—	—	4(100%)	—	—	—	5(56%)	1	—	—	12(32%)	10	3	—	2(67%)	1	—	—	6(50%)	6	—
15.5 — 20	1	4(12%)	6	7	—	8(47%)	9	4	—	4(15%)	9	1	—	—	1	—	—	2(22%)	—	—	—	4(11%)	7	2	—	—	—	—	—	—	—	—
20.5 — 25	—	2(6%)	3	1	—	1(6%)	1	6	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
25.5 — 30	—	1(3%)	—	1	—	—	1	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
30.5 — 35	—	—	1	2	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total Positive	1	33	33	28	—	17	17	16	6	26	16	2	—	4	3	1	4	9	1	—	2	37	36	9	—	3	2	—	—	12	9	1
Per cent Positive	3%	100%	100%	84%	—	100%	100%	94%	22%	100%	61%	8%	—	100%	75%	—	44%	100%	11%	—	5%	100%	97%	24%	—	100%	66%	—	—	100%	75%	8%
Indefinites	—	—	—	—	—	—	—	—	—	—	2	3	—	—	1	—	—	—	2	4	—	—	1	22	—	—	1	1	—	—	3	8
Per cent	—	—	—	—	—	—	—	—	—	—	8%	11%	—	—	25%	—	—	—	22%	44%	—	—	3%	69%	—	—	33%	33%	—	—	25%	67%
Total	1	33	33	28	—	17	17	16	6	26	18	5	—	4	4	1	4	9	3	4	2	37	37	31	—	3	3	1	—	12	12	9
Negatives	32	—	—	5	17	—	—	1	20	—	8	21	4	—	—	3	5	—	6	5	35	—	—	6	3	—	—	2	12	—	—	3
Per cent	97%	—	—	15%	100%	—	—	5%	77%	—	30%	80%	100%	—	—	75%	56%	—	66%	55%	95%	—	—	16%	100%	—	—	66%	100%	—	—	25%
Total	33	33	33	33	17	17	17	17	26	26	26	26	4	4	4	4	9	9	9	9	37	37	37	37	3	3	3	3	12	12	12	12
Total Number of Cases	50								39												52											
Length of Pseudopods in mm.																																
1 — 3 mm		27				13				2				—				—				4				—				—		
4 — 9 mm		—				—				10				2				4				8				1				3		
9 mm. up		—				—				2				—				—				3				—				1		
Total Pseudopods		27				13				14				2				4				15				1				4		
Per cent		81%				77%				52%				50%				44%				40%				33%				33%		

C*—Diluent control.

TABLE 5

SHOWING VARIABILITY IN SIZE OF WHEAL TO EXTRACT OF *NECATOR AMERICANUS* AT INTERVALS OF THREE AND FIVE MONTHS IN CASES POSITIVE FOR NECATOR AND ASCARIS

Case No.		First Skin Test				Second Skin Test 3 months later					Third Skin Test 5 months later				
		C*	*10 min.*	*30 min.*	Psd.	C*	*10 min.*	*30 min.*	*60 min.*	Psd.	C*	*10 min.*	*30 min.*	*60 min.*	Psd.
1	Adult Coca's filtered antigen	7	10	15	—	—	8.5	10	Not taken	—	—	10	Ind.	Ind..	—
2		—	13.5	20	5	—	13	22.5		3	—	10	12.5	12	—
3		7	10	—	—	—	11.5	11		5	—	7	9	Ind..	—
4		—	11	10	—	—	12.5	13		5	—	7	15	Ind..	—
5		—	10	—	2	—	8.5	7.5		—	—	8	7	Ind..	2
6		—	12	—	1	—	10	10		6	—	13.5	12.5	10	—
7		—	13	17.5	8	—	8	11.5		6	—	10	10	11	—
8		—	8	10	—	—	8.5	11		3	—	10	12	8	—
9		—	10	10	—	—	5	6.5		—	—	10	11	13	—
10		—	7	8	—	—	7	10		3	—	14.5	15	18	—
11		—	12	13	—	—	6	6.5		4	—	11	13	13	—
12		—	13	15	5	—	11	11		12	—	13	15	15	—
13		—	11	15	1	—	11	11.5		—	Left School				
Average Size of Wheals..		—	10.8	13.3	3.6	—	9.2	12.9	—	5.2	—	10.3	12	—	2
14	Larvae Coca's	—	13	—	1	—	7	10	8	—	—	9	10	12	—
15		—	11	11	—	—	—	7	6	—	—	7	11	12	4
16		7	10.5	—	2	—	6	7	6	—	—	17	17	17	—
17		—	11	12	—	—	8	7.5	7.5	—	—	8	10	12	—
18		—	16	—	1	—	8	10	Ind..	—	—	10	10	12	—
19		—	13	—	—	—	6	6	Ind..	—	—	10	10	10	—
20		—	10	10	—	—	7.5	8.5	7	—	—	7.5	8	8	5
21		—	9	14	—	—	—	—	—	—	—	10	12.5	13	—
22		6	12	—	1	—	11	10.5	Ind..	—	—	12.5	15	13.5	—
Average Size of Wheals..		—	11.7	11.8	1.2	—	9.6	8.6	—	—	—	10.1	11.5	—	4.5
23	Larvae Saline	10	12	13.5	1	—	9	13.5	10	7	—	9	12	12	—
24		—	8	10	—	—	8	8.5	7	—	—	11	10	10	—
25		—	8.5	10.1	—	—	10	10.5	11.5	—	—	8.5	8.5	10	—
26		—	14	—	1	—	10	11.5	12.5	—	—	11	12.5	12.5	—
27		—	11.5	—	—	—	9.5	12.5	Ind..	—	—	9	10	12	3
28		—	12	19	—	—	10	11	12	5	—	10	10	10	—
29		—	11	—	—	—	10.5	13.5	12	4	—	15	20	20	3
30		—	8.5	—	—	—	8	10.5	Ind..	—	—	7	8	Ind..	—
31		—	8	9	—	—	9	11	11	6	—	20	20	11	—
32		—	8	—	—	—	7	8.5	7	—	—	7	7	8	—
33		11	14	13	1	—	19.5	17.5	Ind..	—	—	12.5	12.5	11.5	—
34		—	9	12	—	—	10	12	11	—	—	10	13	12	—
35		—	10	12	—	—	11	11	8	3	—	8	9	8	—
36		7	13	—	—	—	7.5	13	12.5	5	—	8.5	10	11	—
37		—	5	—	—	—	8	11	13	—	—	7	8	8	—
Average Size of Wheals...		—	10.1	12.3	1	—	9.2	11.7	—	5	—	10.2	10.7	—	3
General Average.....		—	10.8	12.4	1.9	—	9.3	11.0	—	5.1	—	10.2	11.4	—	3.1

C*—Diluent control.

TABLE 6

SHOWING RESULTS OF INTRADERMAL TEST ON 251 CHILDREN LIVING IN THE STATE OF MICHIGAN

Size of wheal in mm.	C*	*10 Min.*	*30 Min.*	*60 Min.*
5.5 — 10	6	52 (100%)	39	7
10.5 — 15	—	—	5	1
15.5 — 20	—	—	—	—
20.5 — 25	—	—	—	—
Positive	6	52	44	8
Per cent	2%	20%	17%	3%
Indefinite	—	—	17	38
Per cent	—	—	7%	16%
Negative	245	199	190	205
Per cent	98%	80%	76%	81%
Total	251	251	251	251
Pseudopods				
1 — 3 mm	—	5	—	—
4 — 9 mm	—	1	—	—
9 mm up	—	1	—	—
Pseudopods				
Total	—	7	—	—
Per cent	—	13%	—	—
Erythema				
5.5 — 15	1	—	—	1
15.5 — 25	1	—	2	—
25.5 — 45	—	7	18	11
Total erythema	2	7	20	12
Per cent of erythema	18%	3%	7%	5%

C*—Diluent control.

TABLE 7

SHOWING TOTALS AND PER CENTS OF POSITIVE, INDEFINITE, AND NEGATIVE REACTIONS; TOTALS AND PER CENTS OF PSEUDOPODS AND ERYTHEMA, WITH COCA'S EXTRACT OF *B. COLI*, *F. HEPATICA*, AND *A. LUMBRICOIDES*, IN BOYS FROM 12 TO 17 YEARS OF AGE

Size of wheal in mm.	B. coli				F. hepatica			A. lumbricoides			
	10 min.	*30 min.*	*60 min.*	Control*	*10 min.*	*30 min.*	*60 min.*	*10 min.*	*30 min.*	*60 min.*	Control*
5.5 — 10	80(90%)	58	25	10	62(93%)	56	22	43(78%)	35	24	12
10.5 — 15	8(90%)	27	15	1	5(7%)	10	11	10(18%)	13	13	—
15.5 — 20	1(1%)	—	2	—	—	—	2	1(2%)	3	2	—
20.5 — 30	—	—	—	—	—	—	—	1(2%)	1	1	—
Total	89	86	42	11	67	66	35	55	52	40	12
Per cent	73%	71%	34%	18%	55%	54%	28%	92%	87%	66%	20%
Indefinite	—	12	54	—	1	14	47	1	5	17	—
Per cent	—	9%	45%	—	2%	11%	40%	2%	8%	28%	—
Total	89	98	96	—	68	80	82	56	57	57	12
Negative	32	24	25	49	53	41	39	4	3	3	48
Per cent	26%	20%	21%	82%	43%	34%	32%	6%	5%	5%	80%
Total	121	121	121	61	121	121	121	60	60	60	60
Average size of wheal	8.05	9.83	10.66	7.04	7.44	9.03	13.5	9.32	10.82	11.45	7.45
Psds. in mm.											
1 — 3	—	4	—	—	—	2	—	—	6	—	—
4 — 9	—	9	—	—	—	5	—	—	11	—	—
9+	—	1	—	—	—	1	—	—	5	—	—
Total	—	14	—	—	—	8	—	—	23	—	—
Per cent	—	15%	—	—	—	12%	—	—	40%	—	—
Erythema											
5.5 — 15	25	10	4	—	10	13	4	15	25	18	8
15.5 — 25	3	17	8	2	6	10	4	6	3	3	3
25.5 — 30	3	4	0	—	0	3	0	5	5	2	1
30.5 — 35	1	1	0	—	—	1	1	3	1	1	1
Total	32	32	12	2	16	27	9	29	34	24	13

*C—Diluent control.

TABLE 8

COMPARISON OF INTRADERMAL TESTS TO COCA'S ADULT *NECATOR AMERICANUS* ON BOYS AND GIRLS

Size of wheal in mm.	Boys Age 7 — 20												Girls Age 7 — 20											
	Positive Necator and Ascaris				Necator Positive Ascaris Negative				Ascaris Positive Necator Negative				Positive Necator and Ascaris				Necator Positive Ascaris Negative				Ascaris Positive Necator Negative			
	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*
5.5 — 10	2	15(75%)	9	2	8	60(45%)	31	10	5	16(69%)	5	—	—	12(100%)	8	—	—	52(97%)	36	9	—	32(89%)	24	2
10.5 — 15	—	5(25%)	6	3	—	68(52%)	61	11	-	7(31%)	3	—	—	—	—	—	—	2(3%)	9	3	—	4(11%)	6	3
15.5 — 20	—	—	1	1	—	4(3%)	11	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—
20.5 — 25	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total Positive	2	20	16	6	8	132	105	26	5	23	8	—	—	12	8	—	—	54	45	12	—	36	31	5
Per cent	9%	95%	76%	75%	5%	98%	77%	35%	21%	100%	35%	—	—	57%	38%	—	—	79%	66%	17%	—	76%	66%	10%
Indefinite	—	—	1	2	—	—	10	46	—	—	2	4	—	—	8	18	—	—	13	47	—	1	7	34
Per cent	—	—	4%	25%	—	—	7%	62%	—	9%	10%	100%	—	—	38%	85%	—	—	19%	70%	—	27%	15%	73%
Total	2	20	17	8	8	132	115	72	5	23	10	4	—	12	16	18	—	54	58	59	—	37	38	39
Negative	19	1	4	—	127	3	20	2	18	—	13	—	21	9	5	3	68	14	10	9	47	10	9	8
Per cent	91%	4%	19%	—	95%	2%	16%	2%	79%	—	55%	—	100%	43%	23%	15%	100%	20%	15%	13%	100%	21%	19%	17%
Total	21	21	21	8	135	135	135	74	23	23	23	4	21	21	21	21	68	68	68	68	47	47	47	47
Total No. of Cases	179												136											
Pseudopods																								
1 — 3 mm		3				28				7				—				—				1		
4 — 9 mm		1				16				2				1				2				3		
9 mm. +		1				3				1				—				12				1		
Total Psds		5				47				10				1				14				5		
Per cent		25%				35%				43%				8%				26%				14%		

*C—Diluent control.

TABLE 9

SHOWING INTRACUTANEOUS REACTION IN VARIOUS AGE GROUPS, WITH COCA'S EXTRACT OF ADULT *NECATOR AMERICANUS*

Age groups	1 week — 2 years				3 — 9 years				10 — 14 years				15 —25 years				26 — 39 years				40 years up			
Size of wheal in mm.	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*
5.5 — 10	—	6(100%)	4	0	5	25(71%)	17	10	7	12(39%)	12	8	8	16(38%)	10	12	7	11(55%)	5	5	6	9(37%)	2	4
10.5 — 15	—	—	—	—	—	9(26%)	6	5	2	17(55%)	10	6	1	20(47%)	20	12	1	7(35%)	10	7	1	11(46%)	11	6
15.5 — 20	—	—	—	—	—	1(3%)	4	2	—	2(6%)	5	3	—	4(10%)	7	8	—	2(10%)	2	3	—	4(17%)	7	3
20.5 — 25	—	—	—	—	—	—	—	2	—	—	1	—	—	1(2.5%)	2	4	—	—	1	1	—	—	—	1
25.5 — 30	—	—	—	—	—	—	—	—	—	—	—	—	—	1(2.5%)	—	—	—	—	1	1	—	—	—	—
30.5 — 35	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—	—
Total Positive	—	6	4	0	5	35	27	19	9	31	28	17	9	42	40	37	8	20	19	17	7	24	20	14
Per cent	—	5%	3%	—	11%	81%	63%	44%	29%	100%	90%	59%	21	100%	95%	88%	38%	96%	92%	81%	29%	100%	83%	58%
Indefinite	—	0	2	3	—	—	6	10	—	—	3	11	—	—	2	4	—	—	1	3	—	—	1	3
Per cent	—	—	2%	2%	—	—	15%	22%	—	—	9%	32%	—	—	4%	9%	—	—	4%	14%	—	—	4%	13%
Total	—	6	6	3	5	35	33	29	9	31	31	28	9	42	42	41	8	20	20	20	7	24	21	17
Negative	—	109	109	112	38	8	10	14	22	—	—	3	33	—	—	1	13	1	1	1	17	—	3	7
Per cent	—	94%	94%	97%	88%	18%	22%	33%	71%	—	—	9%	78%	—	—	2%	61%	4%	4%	4%	71%	—	12%	29%
Total	—	115	115	115	43	43	43	43	31	31	31	31	42	42	42	42	21	21	21	21	24	24	24	24
Total Number of cases	276																							
Size of Pseudopods																								
1 — 3 mm		0				11				19				22				7				5		
4 — 9 mm		0				3				2				7				7				11		
9 mm. up		0				—				—				2				—				1		
Total Pseudopods		0				14				21				31				14				17		
Per cent		0				40%				69%				74%				70%				71%		

* C—Diluent control.

TABLE 10

SKIN SENSITIVITY IN MOTHERS AND THEIR CHILDREN UNDER 2 YEARS OF AGE, WITH COCA'S EXTRACT OF *N. AMERICANUS*

Mothers: 20–50 years								Children: 0–24 months										
No. of Cases	Controls*	Average size of wheal in mm.			No. of reactions with pseudopods	No. of Indefinite Reactions in		No. of Cases	Age in months	Controls*	Average size of wheal in mm.			No. of reactions with pseudopods	No. of Cases Positive for Intestinal Parasites			No. of cases neg. for parasites
		10 Min.	*30 Min.*	*60 Min.*		*30 Min.*	*60 Min.*				*10 Min.*	*30 Min.*	*60 Min.*		Asc.	Nec.	Trich.	
1	—	7	7	—	—	—	1	1	1	—	—	—	—	—	—	—	—	1
6	—	7.6	9.4	8.5	1	—	—	7	2	—	—	—	—	—	—	—	—	7
2	—	9	10	—	—	—	1	2	3	—	—	—	—	—	1	—	—	1
5	—	9	12.1	12.7	2	—	2	5	4	—	—	—	—	—	—	—	—	5
7	—	7.3	8.3	9.3	4	1	3	7	5	—	—	—	—	—	1	1	—	6
13	—	7.6	8.8	8.3	1	—	4	14	6	—	—	—	—	—	—	—	—	14
5	—	8.4	11.5	11	2	—	2	5	7	—	—	—	—	—	—	—	—	5
10	—	9	12.3	15	4	2	5	11	8	—	—	—	—	—	—	1	—	10
8	—	7.3	11.7	11.6	4	—	4	8	9	—	—	—	—	—	—	—	—	8
6	—	6.6	—	—	—	3	4	6	10	—	—	—	—	—	2	—	—	4
5	—	8	12.3	11.5	1	—	1	5	12	—	—	—	—	—	—	—	—	5
2	—	7	9	10	1	—	1	2	13	—	—	—	—	—	—	—	—	2
4	—	8.3	10.8	15	2	—	2	4	14	—	—	—	—	—	—	—	—	4
1	—	9	12	9	—	—	—	1	15	—	—	—	—	—	—	—	—	1
2	—	9.7	10	9	1	1	1	2	17	—	—	—	—	—	No samples			
8	—	10	12.1	12	3	—	2	8	18	—	—	—	—	—	1	—	3	4
Mother Dead						—	—	1	20	—	—	—	—	—	—	—	—	1
3	—	7.8	9.1	7.1	2	—	—	3	21	—	—	—	—	—	1	—	—	2
1	—	7	7	—	—	—	1	1	22	—	—	—	—	—	—	—	—	1
2	—	13	15.5	17.5	1	—	—	2	23	—	—	—	—	—	1	1	—	0
11	—	8	9.5	13.3	1	—	2	11	24	—	—	—	—	—	5	3	4	1
102	—	166.6	198.4	188.8	30	7	36	106	Total...	—	—	—	—	—	12	6	7	82
Average size of wheal....	—	8.33	10.4	11.8					Per cent of Infection.....						11.3	5.6	6.5	78%

*Diluent control.

TABLE 11

SKIN SENSITIVITY IN MOTHERS AND THEIR CHILDREN UNDER 2 YEARS OF AGE WHO REACTED TO COCA'S ADULT OF *NECATOR AMERICANUS*

Mothers						Children											
Case No.	Controls	Size of wheal in mm.				Age in months	Controls	Size of wheal in mm.				Erythema			Intestinal Parasites		
		10 Min.	*30 Min.*	*60 Min.*	Psd.			*10 Min.*	*30 Min.*	*60 Min.*	Psd.	*10 Min.*	*30 Min.*	*60 Min.*	Asc.	Nec.	Tric.
103	—	11.5	12	13	14	7	—	—	—	—	—	—	12	12.5	—	—	—
104	—	10	13	Ind....	—	9	—	—	—	—	—	12	12	17.5	+	—	+
105	7	14.5	14	17.5	10	12	—	6	Ind...	—	—	—	—	—	—	—	—
106	—	12	20	Ind...	10	13	—	6	6	—	—	—	—	—	—	—	—
107	—	15	15	Ind...	5	18	—	7.5	9	Ind...	—	25	16	13	Not Exam.		
108	—	12	12	17.5	4	20	—	7	8	Ind...	—	—	20	Ind...	Not Exam.		
109	No mother			—	—	24	—	8	8	—	—	—	—	—	+	—	—
110	—	14.5	14	Ind...	7	24	—	—	—	—	—	—	25	—	+	—	+
111	—	11.5	12.5	12	14	24	—	6	Ind...	Ind...	—	15	—	—	—	—	+
Average Size of wheal.	—	12.6	14.0	15	—	—	—	6.7	7.7	—	—	17	17	16	3 42%	0 —	3 42%

TABLE 13

SUMMARY OF 2931 SKIN TESTS AND PER CENT OF POSITIVE, NEGATIVE AND INDEFINITE REACTIONS AND PSEUDOPODS TO EXTRACTS OF *NECATOR AMERICANUS* IN 10, 30, AND 60 MINUTES

Groups of cases positive and negative for necator and ascaris	Total number of tests made	Tests giving positive reaction	Per cent of reactivity at different periods of time			Per cent of Indefinite Reactions			Per cent of wheals with pseudopods	Per cent of reactions with an average wheal of		
			10 min.	*30 min.*	*60 min.*	*10 min.*	*30 min.*	*60 min.*		5.5 – 10 *mm.*	10.5 – 15 *mm.*	15.5+ *mm.*
Positive Cases (Before Treatment)	516	474	90	71	54	1	13	53	41	62	31	7
Negative Cases (After Treatment)	376	350	93	67	25	0	25	60	22	67	27	6
Positive Cases (Not Treated)	189	186	98	89	70	0	8	23	72	46	49	5
Negative Cases (Not Treated)	1,042	868	81	74	28	2	11	60	20	83	16	.8
Mothers and their Children												
Mothers	110	110	100	94	87	0	6	36	34	92	8	0
Children	115	6	5	3	0	0	1	2	0	100	0	0
Michigan Children Negative for Parasites	251	52	20	17	3	0	7	16	13	100	0	0
Control Tests with Coca's Extracts of:												
B. coli	121	89	73	71	34	0	9	45	15	90	9	1
F. hepatica	121	67	55	54	28	2	11	40	12	93	7	0
A. lumbricoides	60	55	92	87	66	2	8	28	40	78	18	4

TABLE 12

SHOWING THE PERCENTAGE AND AVERAGE SIZE IN MILLIMETERS OF ERYTHEMA INCASES POSITIVE FOR *NECATOR AMERICANUS* AND *ASCARIS LUMBRICOIDES* AT 10, 30, AND 60-MINUTE INTERVALS WITH EXTRACTS OF *NECATOR AMERICANUS*

Size of Erythema	Before Treatment — Positive for *N. americanus* and *A. lumbricoides*												After Treatment — Negative for *N. americanus* and *A. lumbricoides*											
	Adult Coca's				Adult Coca's Autoclaved				Adult Coca's Not Filtered				Adult Coca's				Adult Coca's Autoclaved				Adult Coca's Not Filtered			
	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*	C*	*10 min.*	*30 min.*	*60 min.*
10 –15	7	1(2.5%)	3	7	—	4(8%)	3	1	2	—	—	2	4	4(5%)	10	13	—	3(5%)	6	6	1	1(1%)	3	11
15.5 — 20	4	15(27%)	24	16	—	11(20%)	21	24	1	12(21%)	1	18	9	25(34%)	20	16	—	20(29%)	15	17	2	30(44%)	18	25
20.5 — 30	3	32(58%)	35	20	—	26(48%)	31	13	2	29(22%)	18	26	5	34(47%)	33	15	—	37(54%)	37	13	—	34(50%)	40	6
30.5 — 40	1	6(10%)	3	2	—	11(20%)	6	4	—	11(50%)	6	2	1	9(14%)	8	2	—	7(11%)	8	2	—	3(5%)	7	2
40.5 — 50	—	1(2.5%)	2	—	—	2(4%)	—	—	—	3(7%)	1	—	—	—	—	1	—	1(1%)	—	—	—	—	1	—
Total positive	15	55	68	45	—	54	61	42	5	55	68	48	19	72	71	47	—	68	66	38	3	68	69	44
Per cent	20%	73%	90%	60%	—	72%	81%	56%	6%	73%	125	64%	25%	97%	95%	63%	—	90%	88%	50.5%	4%	91%	93%	59%
Negative	60	20	7	30	75	21	14	33	70	20	6	27	55	2	3	27	75	7	9	37.	71	6	5	30
Per cent	80%	26%	10%	40%	100%	28%	18%	44%	93%	26%	8%	36%	74%	2%	4%	36%	100%	10%	22%	49.5%	95%	8%	6%	40%
Total	75	75	75	75	75	75	75	75	75	75	75	75	74	74	74	74	75	75	75	75	74	74	74	74
Total No. of Cases	225												223											
Minimum size	8	19.5	12.5	10	—	12.5	10	10	10	19.5	15	15	10	10	10	15	—	10	10	10	10	15	15	10
Maximum size	32.5	45	52.5	40	—	42	40	27.5	30	50	50	35.5	32.5	40	40	45	—	45	40	35	20	37.5	42.5	35
Average size	3.9	18	21.3	13.2	—	20.3	20	13	1.3	19.5	24	15.5	5.9	23.1	23	14.2	—	22.4	21.5	10.3	0.8	22	23	9.5

Average size Erythema of the group	C*	10 *min.*	30 *min.*	60 *min.*
	2	19.3	22	14
	2.2	22.5	22.5	11.3

*C—Diluent control.