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The Haemagglutination Test in Hookworm (*Necator americanus*) Infestation in Ceylon.

by

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THE use of tanned red cells in the haemagglutination test (Boyden, 1951) is familiar to students of immunology, but its use in hookworm infestation has only been reported once (Reports of the Zoological Society of London, 1965). It was Dr. Peter Ball of the Nuffield Institute of Comparative Medicine, London, who stimulated our interest in this test. In a personal communication he stated that an antigen prepared from third stage larvae of hookworm (*A. duodenale*) had given very specific results with African sera, from persons with *A. duodenale* infestation.

It was decided to test sera from hookworm positive persons in Ceylon to detect the presence of antibody to third stage larvae of *Necator americanus*, which is the hookworm of Ceylon.

MATERIAL AND METHODS

The methods used with slight modifications were those recommended by Dr. Peter Ball.

SERUM

Serum was obtained from three groups of persons:—

1. From patients from a ward of the General Hospital, Colombo, who had hookworm ova in their stools. These persons were from a low income group.
2. From the staff of the Medical Research Institute. This group represented different income levels, but being resident in the tropics it is extremely likely that they had been or are exposed to infection with hookworms.
3. As controls from the Nuffield Institute of Comparative Medicine and from the Tropical Laboratories of Messrs Burroughs Wellcome & Co. Ltd., from persons who had been life long residents of England and who were presumably not exposed to hookworm infestation.

Antigen. Stools containing a good concentration of hookworm eggs were cultured and the larvae collected after 7 days. These were filtered and washed in phosphate buffered saline (PBS) and the concentrate of larvae made up to 1/10 in PBS and stored frozen until use. Fresh 1/250 solution of the stock suspension was made up for each days test.

Cells. Citrated sheep cells 2 to 3 days old were used. These were washed in buffer and resuspended to 4%. Tanned cells were prepared by incubating the suspension with an equal volume of freshly prepared 1: 20,000 tannic acid in PBS at 37°C, for exactly 10 minutes. Cells were then washed, coated with antigen and made up to 2% with albumin buffer.

Controls. Tanned uncoated cells were made up to 2% for controls in the same way. The controls used were uncoated cells + serum 1/5 and coated cells with buffer, using albumin buffer as diluent.

Tests were originally done in Wasserman tubes and later repeated in microhaemagglutination plates. Settling was much better defined in the latter.

The results were read after a minimum period of 2 hours at room temperature; plates were then placed in the refrigerator overnight and results reread. Settling was better in the cold.

Stools. Stools were examined by direct saline smear and wherever possible by the merthiolate iodine formaldehyde concentration test (Blagg, Schloegel, Mansour & Khalaf 1955). Egg counts were made by Stoll's Method (1926).

RESULTS

TABLE 1

Results obtained with sera from 78 hookworm ova positive cases from a ward of the General Hospital.

Titre	Number Positive	%	Ova count
1/5	10	12	1—8600
1/10—1/20	6	8	1—400
1/40—1/5120	62	80	1—34,900

A total of 78 were examined from this group. Ten (12 %) were negative for antibodies, 6 showed a low titre and 62 (80 %) showed a diagnostic titre of 1/40 and over. The titres did not have any relation to the egg counts i.e. to the worm lead. Two batches of these sera in groups of 10 were tested twice over to determine consistency of results.

TABLE 2

Results obtained from 70 healthy persons Staff of the Medical Research Institute

Titre	Number examined	%	Number with Hookworm ova and count	Number with Other Ova cysts	Number without parasites
1/5—1/10	0				
1/20	4	5.9	0	1	3
1/40—1/5120	66	93.1	22 (1—2600/gm)	21	23

A total of 70 persons were examined in this group. These sera were tested with a very stable antigen which was used to recheck results of group 1 and was also used in all the tests of group 3. Four of them had titres below diagnostic levels, 3 of whom had no parasites while 1 had cysts of *E. histolytica*. The rest 66 (93.1%) showed diagnostic titres of antibody to the antigen used. (See Plate 1.) Twenty three of this group did not have parasites in their stools.

TABLE 3

Results obtained with sera from 33 healthy persons who had not been exposed to hookworm infestation.

Titre	Number
1/5	31 (93%)
1/10	1
1/160	1

Thirty one of thirty three (93 %) showed no reaction to hookworm. One serum showed a diagnostic titre of 1/160. Nine other sera of this group which were decomposed gave false positive reactions. Fourteen sera of this group were tested twice over and gave repeated negative results. (See Plate 2.)

DISCUSSION

All sera used in this series were obtained from adults. Those in group 1 (Table 1) represent a low income group in whom it could be assumed that the specific antigenic stimulus with hookworm is always present but is probably at low levels throughout the year and over a period of years. Group 2 (Table 2) involves a group all of whom have better home conditions than group 1 but the majority are also probably exposed to low grade hookworm infection in the same manner as group 1.

There are many factors which influence the formation of antibody even with the use of relatively homogeneous antigens in experimental studies. When immunity to metazoa with complex life cycles is considered, the antigenic stimuli may also be assumed to be complex. This is further complicated by the fact that we are not aware of the duration of the antigenic stimulus; but with a knowledge of the home conditions of the persons concerned, we have assumed it to be low grade and continuous. It is known that in the early stages of immunisation antigen antibody union is more specific and less avid while in the later stages antibody becomes less specific and more avid.

It has been reported by other workers (Soulsby, 1960) that in helminth infections the haemagglutination test is more sensitive than the complement fixation test. Soulsby also found that there was no correlation between the level of antibody and resistance. Mandras and Addis (1960) have reported that in hydatid disease too there is no such correlation. In the present series we have not seen any correlation between the worm load and antibody levels.

In view of the complexity of factors influencing both antibody formation and the complexing of antigen and antibody and with the declared sensitivity of the haemagglutination test; our results can be assumed to be an indication of a specific response of the sera tested to *N. americanus* infection. This is further confirmed by the fact that sera from persons not exposed to hookworm infection (with one exception) gave negative results. (Group 3. Table 3).

SUMMARY

Sixty two sera (80%) from 78 hospital patients who had hookworm ova in their stools showed diagnostic titres of antibody (1/40 and over) to the third stage larvae of *N.americanus.*

Sixty six sera (93%) from 70 apparently healthy persons also showed diagnostic titres to the same antigen.

Sera from 33 healthy persons not exposed to infection gave only 1 diagnostically positive result.

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EXPLANATION OF PLATE

Plate I.

FIG. 1. Shows results obtained with sera from group 2. All sera in this qlate show positive titres except the negative control. It is observed that the 2 lower dilutions gave a "prozone" reaction.

FIG. 2. Shows negative results obtained with English and (a few) local sera. (1-10 English; 75 - 77 local.) Positive and negative controls are seen at the top of plate.

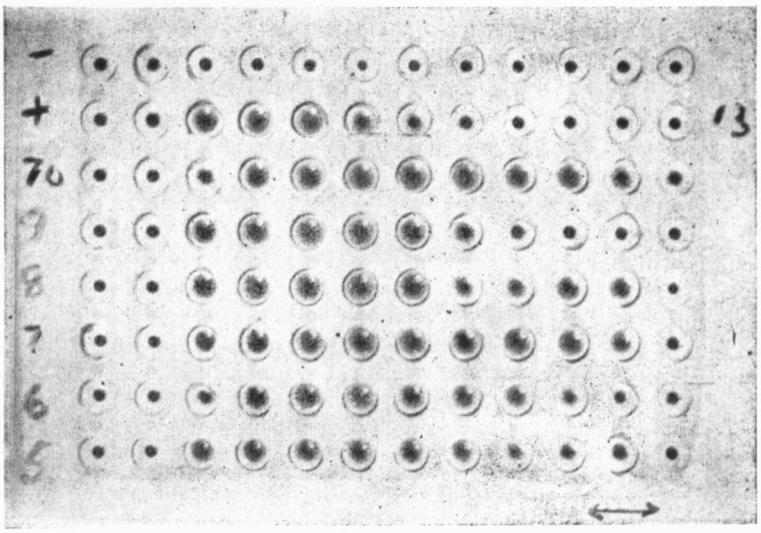

FIG. 1.

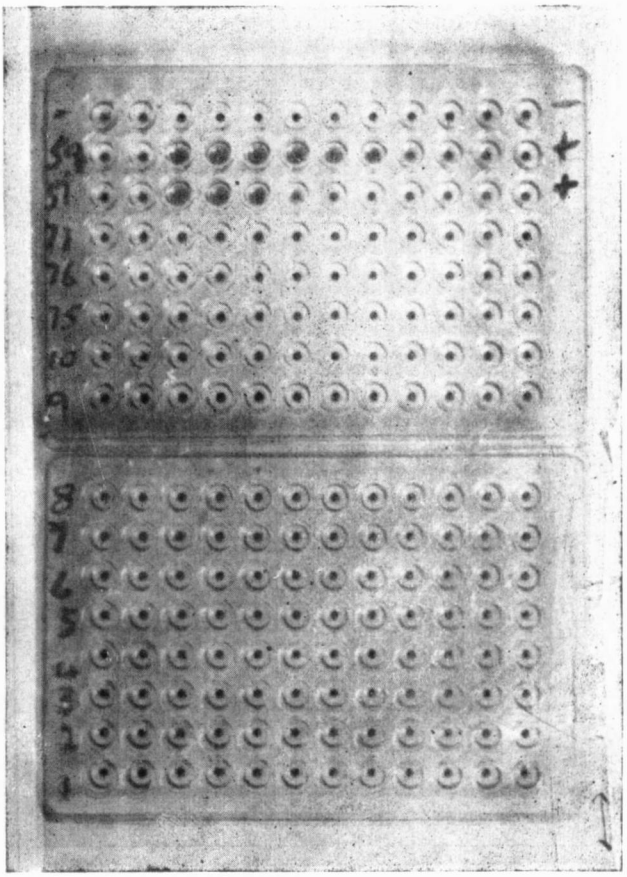

FIG. 2.