THE ROLE OF ASCARIS LUMBRICOIDES IN THE NUTRITION OF THE HOST
EFFECT OF ASCARIASIS ON DIGESTION OF PROTEIN

BY

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The wide prevalence of intestinal infestation with Ascaris lumbricoides in human beings, especially the young children in India is well known (Caius and Mhaskar, 1923; Goheen, 1925). The presence of round-worms in the small intestine is often regarded as a common occurrence and the injurious effects if any are lightly dismissed, since in the majority of cases there is an absence of striking clinical manifestations. On the other hand, the rarer complications, mainly surgical in nature, are often unduly emphasized.

The adult worm in the small intestine is known to give rise to varied symptoms, ranging from vague abdominal discomfort to acute colicky pains around the umbilicus. Poor digestion and diarrhoea are often considered to be due to the presence of these parasites. It is reasonable to assume that the presence of the worms in the small intestine, their growth, metabolism and secretions might exert an adverse effect on the nutrition of the host. In general, children with heavy worm infestation are usually of substandard weight and height and show the common symptoms of malnourishment. Ken Ikuri (1926) noted the wide prevalence of ascariasis in children in Osaka, and pointed out that the nutritional status of the infested children was subnormal. Yokogawa and Wakeshima (1932) demonstrated that ascaris-infested children were physically and mentally underdeveloped and that disinfestation improved their nutrition and along with it their mental ability. Villegas (1941) described a syndrome of cachexia in non-syphilitic children with ascariasis, mainly characterised by oedema, diarrhoea and other nutritional deficiency manifestations. Einhorn and Miller (1946), in a clinical survey of helminthic infection among children on the Isthmus of Panama, observed malnutrition, loss of or failure to gain weight and fever in ascaris-infested children. Nearly 55 per cent. of the subjects were underweight and this feature was most prominent in the younger age-groups.

In India, too, infestation with ascariis is fairly common among children. It has been frequently encountered in children patients appearing at the Nutrition Clinic at Coonoor. Of particular interest was the high incidence (85 per cent.) among cases of nutritional oedema syndrome (kwashiorkor) investigated at the Clinic. A similar heavy incidence has been reported by Achar (1950) at Madras in his cases of nutritional dystrophy. The researches of Trowell (1949), Veghelyi (1948) and Altmann (1948) have shown that kwashiorkor is mainly a result of chronic protein deficiency during the post weaning period. This deficiency is primarily caused by inadequate and faulty feeding of the child. Trowell (1949) has also
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drawn attention to the role played by parasitic infection and infestation in the development of the syndrome. In view of these observations, a possible interference by round-worm in the protein nutrition of the child already inadequately supplied with this nutrient was an aspect which it was felt, deserved closer attention than it had hitherto received.

The present communication contains an account of an investigation carried out to determine the effect of moderately heavy or heavy infestation with A. lumbricoides on the digestion and absorption of protein in the host.

MATERIALS AND METHODS

From among the children patients attending the Nutrition Clinic, nine children were selected for this investigation. They did not exhibit specific signs of nutritional deficiency although their physical development appeared to be poor in relation to their age. On examination of faeces, they showed evidence of ascaris infestation; ova of other intestinal parasites were not detected in the faeces of these subjects. Duplicate egg counts by the modified Stoll’s technique (STOLL and HAUSHEER, 1926) were performed and children with an average count of more than 20,000 eggs per g. of faeces were chosen for the study. According to Clayton LANE (1930) the estimation of the number of worms in the gut is not possible from egg counts, howsoever exactly done, since several factors influence the number of eggs passed in the stool, the more important being (1) the consistency and size of the stool, (2) the age and maturity of the worms, (3) the intensity of infestation (it has been found with ancylostomes that as infestation increased, each female worm laid fewer eggs), and (4) the nutrients available; it is stated that oviposition ceases if the worm is deprived of food. In view of the above, no conclusion can be drawn regarding the heaviness of worm load based on egg counts. In fact, however, all except two subjects were found to harbour more than 21 worms.

Diet. The children were kept in hospital and put on a diet consisting of reconstituted skimmed milk and peeled ripe bananas. The nitrogen content of the samples of skim milk powder and peeled bananas was estimated at intervals during the investigation in order to determine the intake of protein. No other article of diet was given throughout the course of the investigation, which lasted for nearly 18 days for each subject.

Collection of stool specimens. The collection of stool specimens for N determination was carried out over 24-hour periods on 2 to 3 successive days after the subjects were on the above regime for 7 to 8 days. Eight minims of oil of chenopodium mixed in one ounce of castor oil were administered on the 8th or 9th day. The subjects passed worms even as late as 24 to 36 hours after the worming treatment. In every one of the cases, the treatment appeared to have been successful since no ascaris ova could be found in the stools examined 72 hours after worming. The collection of stool samples after disinfestations was begun on the 4th day following the treatment after making sure that no more worms were passed.

Estimation of faecal nitrogen.

The 24-hour stool specimens were weighed and the sample was made up to a definite volume with water in a large wide-mouthed flask containing glass beads. The flask was vigorously shaken till the faecal material was uniformly distributed; 25 c.c. of the suspension were taken in duplicate for digestion. The nitrogen content of the samples was estimated by the Kjeldahl’s method, and the total nitrogen in the 24-hour samples was calculated. The average difference in the N values for duplicates of the same samples calculated from all the observations was 4.01 per cent. The above method of determination of faecal N was chosen in preference to determinations on dried samples for the sake of convenience.
It must be admitted, however, that the method adopted was liable to cause some error, the extent of which was, however, such as not to invalidate the conclusions reached. The results are given in Table I.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Egg count per g. of stool</th>
<th>Number of worms passed</th>
<th>Nitrogen intake per day g.</th>
<th>Before disinfection</th>
<th>After disinfection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Number of observations</td>
<td>Average N/24 hours g.</td>
</tr>
<tr>
<td>1</td>
<td>54,000</td>
<td>21</td>
<td>6.29</td>
<td>3</td>
<td>1.803</td>
</tr>
<tr>
<td>2</td>
<td>76,800</td>
<td>17</td>
<td>5.64</td>
<td>2</td>
<td>1.401</td>
</tr>
<tr>
<td>3</td>
<td>36,600</td>
<td>31</td>
<td>8.30</td>
<td>2</td>
<td>1.085</td>
</tr>
<tr>
<td>4</td>
<td>29,400</td>
<td>40</td>
<td>8.30</td>
<td>2</td>
<td>1.272</td>
</tr>
<tr>
<td>5</td>
<td>21,800</td>
<td>26</td>
<td>8.30</td>
<td>2</td>
<td>0.777</td>
</tr>
<tr>
<td>6</td>
<td>28,600</td>
<td>37</td>
<td>8.30</td>
<td>3</td>
<td>1.397</td>
</tr>
<tr>
<td>7</td>
<td>34,800</td>
<td>27</td>
<td>8.30</td>
<td>3</td>
<td>0.875</td>
</tr>
<tr>
<td>8</td>
<td>29,400</td>
<td>13</td>
<td>8.30</td>
<td>2</td>
<td>2.470</td>
</tr>
<tr>
<td>9</td>
<td>31,100</td>
<td>26</td>
<td>8.30</td>
<td>2</td>
<td>0.932</td>
</tr>
</tbody>
</table>

21 Mean 1.315
σ 0.5121

19 Mean 0.755
σ 0.2398

Estimation of the nitrogen content of ascaris eggs.

Dead female adult worms preserved in neutral formalin saline were dissected within 12 hours after being passed out. The uteri were removed, cut into small pieces and shaken gently with 5 per cent. formal saline to release the eggs from the lumen. The free eggs were separated from the floating pieces of uterine tissue by slow centrifuging. The eggs were then transferred to a 100 cc. volumetric flask with a minimum of manipulation to avoid breaking them, and made up to 100 cc. with 1 per cent. NaOH solution. The alkali checked the tendency of the eggs to stick to the walls of the glass container. The flask was gently shaken in a mechanical shaker for half an hour and an egg count was made using 20 c.mm. of the solution on a specially calibrated slide. Four counts were made and the results were found to agree well within the limits of experimental error. The nitrogen content of a known volume of egg solution was estimated in triplicate by Kjeldahl's method.

The results of two such experiments in which eggs from 13 and 15 female worms respectively were used, are set out in Table II.

<table>
<thead>
<tr>
<th>Number of adult female worms used</th>
<th>Total number of eggs (in million)</th>
<th>Total nitrogen in eggs mg.</th>
<th>Nitrogen content per million eggs mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>4.21</td>
<td>18.7</td>
<td>4.44</td>
</tr>
<tr>
<td>15</td>
<td>10.93</td>
<td>44.2</td>
<td>4.04</td>
</tr>
<tr>
<td>Mean — 4.24 mg.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results and Discussion

The average value of faecal nitrogen for all the subjects before disinfection was 1.315 g.
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per 24 hours, the range being 0.711 to 2.655 g. After the worms were removed, the average faecal nitrogen for the same subjects fell to 0.755 g per 24 hours with a range of 0.406 to 1.233 g. The difference was found to be statistically significant ($p = <0.01$). It is to be noted that throughout the experimental period the nitrogen intake for each subject was maintained nearly constant. Figures in Table I show that in every one of the children investigated the faecal nitrogen after disinfection was lower than that excreted prior to worming. That the higher values prior to worming were not due to the contribution made to faecal nitrogen by the ascaris eggs excreted with faeces can be seen by reference to Table III in which relevant data for four subjects are tabulated. It was found that between 19-50 mg of the faecal nitrogen could be due to the N content of eggs, whereas the difference between the mean faecal N before and after worming was 560 mg., i.e. ten times or more of the maximum egg contribution possible. The higher faecal nitrogen before worming must have been due to a greater amount of dietary protein remaining undigested and hence excreted unabsorbed from the gastro-intestinal tract indicating that round-worms did probably interfere in the digestion of proteins.

Table III. Faecal nitrogen contributed by ascaris ova.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Fresh weight of stools—(24 hours) —(Mean value) g.</th>
<th>Egg count per g.</th>
<th>Total number of eggs—(in million)</th>
<th>Nitrogen content of million eggs (From Table II) mg.</th>
<th>Faecal nitrogen contributed by ascaris eggs mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>170</td>
<td>28,600</td>
<td>4.862</td>
<td>4.24</td>
<td>20.6</td>
</tr>
<tr>
<td>7</td>
<td>177</td>
<td>34,800</td>
<td>6.160</td>
<td>4.24</td>
<td>26.1</td>
</tr>
<tr>
<td>8</td>
<td>191</td>
<td>29,400</td>
<td>5.615</td>
<td>4.24</td>
<td>23.8</td>
</tr>
<tr>
<td>9</td>
<td>145</td>
<td>31,100</td>
<td>4.510</td>
<td>4.24</td>
<td>19.2</td>
</tr>
</tbody>
</table>

Before considering the interference in protein digestion caused by round-worms as proved, two factors had to be taken into consideration. It was possible that the administration of anthelminthic and the cathartic might have been partly or wholly responsible for the observed differences in faecal nitrogen. The second consideration was the response of poorly nourished children to a daily intake during the experimental period of 6 to 8 g. of N derived from protein of high biological value. It could be argued that since these children were likely to have been accustomed to low protein diets they would respond to higher levels of dietary protein by gradually adapting themselves to its more efficient digestion and absorption particularly during the latter part of the experimental period. Both these points were put to test as follows.

Influence of worming treatment: Two children found to be free from round-worms on repeated examinations of their faeces, were put through the same regime as described earlier in the case of nine children with worms. One child excreted on an average $0.869$ g. N/24 hours before the worming treatment and $0.808$ g. after. The other excreted $1.323$ g. before and $1.556$ g. after the worming treatment. Both were on a constant N intake of $8.30$ g. N per day. These results clearly show that worming treatment by itself in the absence of worms had no favourable effect on the digestion and absorption of proteins.

Influence of continued feeding of skim milk diet: For this study two children harbouring round-worms were selected. They were kept on a similar diet, as described before, continu-
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Insolently for a period of 19 days, and beginning with the 4th day periodical determinations of faecal N excreted in 24 hours were made. No worming treatment was given. The faecal N in 24-hour stool collections determined on five occasions during this period did not indicate in either of the two subjects any tendency to decrease from the initial values.

In view of these results, it is clear that in the nine children investigated (Table I), neither of the two factors mentioned above could have been responsible for the observed differences between the faecal N values before and after worming.

Round-worm infestation is known to produce vague gastro-intestinal disturbances inducing diarrhoea or constipation, more often the former. It is possible that the intestinal hurry as a result of mechanical irritation caused by the presence of the parasites in the small intestine, moves the semidigested food rapidly down the gut leaving little time for the host's digestive enzymes to act on the food. This would affect both the digestion and absorption of food from the intestine. In the present series all except one subject were found to pass two or three semisolid stools per day before they were disinfested. But in none were the stools watery, nor was the frequency at any time greater than three. After worming, the stools became solid and the frequency was reduced to one. Even in the one subject (Subject No. 8) where the stools were well formed throughout, the difference in faecal nitrogen before and after disinfestation was marked. From these observations it would be reasonable to conclude that the interference with digestion caused by the parasites in the small intestine cannot be solely attributed to gastro-intestinal hurry.

Weinland (1903) was the first to suggest that specific antienzymes are secreted by intestinal helminths. He found that aqueous extracts of *A. lumbricoide* had the power of inactivating trypsin, an observation later confirmed by Hamill (1906) and Harned and Nash (1932). Collier (1941) isolated and purified the antienzyme factor from ascaris and showed that it had both antitryptic and antipeptic properties. These observations would suggest that the major role of the antienzymes is to afford protection for the parasite from dissolution by the host's digestive juices. But it is also probable that in the presence of a large number of worms, the antienzymes may retard effectively, the digestion of dietary protein in the intestine of the host. In fact, Stewart (1932) found that when sheep were heavily infested with nematode parasites, there was only 40 to 60 per cent. utilization of crude protein of the diet. As the degree of infestation became lower under experimental conditions, the utilization of protein improved. The author ascribed the low utilization to antiproteolytic substances produced by the parasites present. In the present investigation no direct correlation between the worm load and the extent of digestive defect was observed. The fact is certain, however, that the presence of round-worms did lower the intestinal digestion and absorption of protein.

The barium X-rays have shown (Fulborn, 1931) that the normal site of *A. lumbricoide* in the small intestine is the jejunum, and in severe cases the worms are found to be stretched side by side throughout the small intestine permitting just enough space for the chyme to pass. Hence, it is quite possible that the antienzymes secreted might mix effectively with the intestinal contents bringing about a slowing of protein digestion.

The intestinal parasites have access to abundant food and it is probable that *A. lumbricoide* obtains its nourishment mainly by direct absorption of materials dissolved in the surrounding semifluid medium. This worm can also swallow particles of solid food. Considerable quantities of barium meal and animal charcoal fed to subjects harboring ascaris have been found in the bodies of the parasites. It has been shown that it is provided with
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digestive enzymes capable of dealing with proteins, fats and carbohydrates (Hobson, 1948). It is unfortunate that little is known of the nitrogen metabolism of ascaris. Brand (1934) estimated that 100 g. of A. lumbricoides eliminated 30 mg. of nitrogen in 24 hours, of which 10 mg. was in the form of eggs and 20 mg. as dissolved material. From Tables II and III, it will be seen that in the present series of experiments the quota of nitrogen contributed by eggs varied between 19 to 50 mg. It has not been possible to estimate the amount of nitrogen excreted by the worms as a result of their metabolic activity although it is not likely to be such as to make appreciable difference in the conclusions reached in the present study.

Shorb and Spindler (1947) have reported that skimmed milk diet fed to young pigs infested with ascaris, resulted in good weight gains since the host eliminated the parasites automatically. It has also been our experience that the administration of high protein diet in the form of skimmed milk to subjects of nutritional oedema syndrome sometimes resulted in the elimination of a few or all of the parasites. Similar effects of a well balanced diet were observed by us in experiments on puppies infested with Toxocara canis (unpublished). It would appear as if the poor diet on which probably the children observed by us subsisted provides a favourable environment for the growth and activity of round-worms which in turn interfere with the digestion and absorption of dietary protein.

The incomplete digestion of protein in the digestive tract of the host would thus deprive him of a part of dietary protein. In the conditions where the diets are such as to meet the nutritional needs of the child, this may not be of serious import. On the other hand, in children who live on diets inadequate in protein, the presence of round-worms in the intestine and the resulting loss of dietary protein through incomplete digestion must accelerate the pathological process of hypoproteinosis, and hence must be considered of great significance in the etiology of nutritional oedema syndrome. It has also been the experience at the Clinic in Coonoor that in children harbouring round-worms, signs and symptoms of other nutritional deficiencies are not uncommon. Whether such developments result in part from the parasitic infestation, is a question deserving further study. In children living on marginal or submarginal diets, intestinal infestation with round-worm must be considered as one of the contributory causes in the development of deficiency syndromes.

Summary

(1) An investigation was undertaken to determine the effect of moderately heavy or heavy infestation with Ascaris lumbricoides on the digestion and absorption of protein in poorly nourished children. Nine children harbouring roundworms were maintained in hospital for 18 to 19 days on a diet permitting a constant nitrogen intake throughout the experimental period. Faecal nitrogen in 24-hour collections of faeces before and after worming was estimated.

(2) In every one of the subjects investigated, the faecal nitrogen per 24 hours was lower after disinfestation than that excreted prior to worming. The average values for faecal N were 1.315 g. and 0.755 g. per 24 hours respectively. The difference was statistically highly significant.

(3) A method has been described for the determination of the N content of ascaris eggs. In four children, the egg N component varied from 19.2 to 26.1 mg. per 24 hours in a total faecal N range of 0.875 g. to 2.470 g.

(4) In two worm-free subjects it was demonstrated that the administration of anthel-
mentric and cathartic did not cause a fall in faecal N as was observed after worming in nine worm-carrying children.

(5) In view of the above, it is suggested that in nine children investigated above, the presence of *Ascaris lumbricoides* in the small intestine interfered with the digestion and absorption of dietary protein. The probable ways in which this interference is caused have been discussed.

(6) It is further suggested that when the protein in the diet is inadequate, incomplete digestion resulting in deficient absorption of protein may be of great significance as one of the aetiological factors in the causation of nutritional oedema syndrome (kwashiorkor) which, in young children, is the result, primarily of subacute protein deficiency.

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