Role of parasites in the pathogenesis of intestinal malabsorption in hookworm disease

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Functional and structural changes in the small bowel in hookworm disease have been convincingly demonstrated by several investigators (Sheehy, Meroney, Cox, and Soler, 1962; Salem and Truelove, 1964; Chaudhuri and Saha, 1964; Tandon, Das, Saraya, and Deo, 1966; Burman, 1966; Chuttani, Puri, and Misra, 1967). The pathogenesis of these changes is not clear. Patients with hookworm infection have varying degrees of anaemia and malnutrition associated with protein deficiency. Anaemia in adult patients does not contribute to the intestinal abnormalities (Rawson and Rosenthal, 1960; Layrisse, Blumenfeld, Carbonell, Desenne, and Roche, 1964; Halsted, Prasad, and Nadimi, 1965; Magotra, 1966). Hypoalbuminaemia, often due to associated malnutrition with protein deficiency, bears a close relationship to the abnormal absorption tests in hookworm disease, and may be a significant aetiological factor (Mayoral, Tripathy, García, Klahr, Bolaños, and Ghitis, 1967; Tandon, Magotra, Saraya, and Ramalingaswami, 1968). Some investigators have suggested that hookworm infestation can cause functional and structural changes in the small bowel (Sheehy *et al*, 1962; Sheehy, 1962; Vélez and Orrego, 1963); others have reported that hookworm is not the aetiological agent and the associated malnutrition (Mayoral *et al*, 1967; Tandon *et al*, 1968) or chance coexistence of tropical sprue (Layrisse *et al*, 1964; Banwell, Marsden, Blackman, Leonard, and Hutt, 1967) may be responsible for the malabsorption in patients with hookworm disease. The object of this paper is to present the results of a study of the influence of parasites in the pathogenesis of the intestinal changes.

METHODS AND MATERIAL

Adult patients with stools positive for hookworm ova were selected for the study. None had any associated systemic disease. Three aspects of parasitic infestation were studied.

LOAD OF HOOKWORM INFECTION The load of worm infestation was assessed by doing an ova count in 67 cases (Stoll, 1963) on three consecutive daily samples and calculating the mean count per gram of faeces per day.

SPECIES OF WORM *Ankylostome duodenale* and *Necator americanus* were recognized by studying the larval cultures in 65 cases (Hsieh, 1963).

DURATION OF INFESTATION A prospective follow-up study of intestinal function and structure was done in 17 patients. Worm infestation was left untreated, anaemia was corrected by oral iron therapy, and the patients ate their normal home diet during the period of follow up. No additional vitamin or protein supplement was provided.

TESTS Haemoglobin and packed cell volume were estimated (Dacie, 1956), and total serum proteins, albumin and globulin (King and Wootton, 1956). D-xylose absorption with a 5-g dose (Roe and Rice, 1948; Santini, Sheehy, and Martinez de Jesus, 1961), a fat balance test (King and Wootton, 1956), a Schilling test (Schilling, 1953), and intestinal biopsy studies were carried out (Brandborg, Rubin, and Quinton, 1959). Biopsy was obtained from the jejunum within 10 to 12 cm from the ligament of Treitz under fluoroscopic control. Tissue was properly orientated and serial sections were cut and stained according to the standard technique.

Five-hour urinary excretion of less than 1 g of D-xylose on a 5-g dose, faecal fat of more than 5 g per 24 hr, and less than 7% of the labelled B₁₂ in urine by Schilling test were considered as indicative of malabsorption in our laboratory (Magotra, 1966). Intestinal biopsy was considered abnormal only if the villus:crypt ratio had shown distinct change to 2:1 or less, epithelial cells were atypical, and the lamina propria was infiltrated with inflammatory cells. Non-specific change, such as branching and blunting of the villi with a slight to moderate degree of infiltration of the lamina propria with inflammatory cells, was not considered a definite abnormality. These investigations were repeated again during the follow-up period of 10 to 16 weeks in 12 cases, and at the longer interval of 17 weeks to 68 weeks in five cases. The maximal follow-up period was 68 weeks in one case.

RESULTS

HOOKWORM LOAD AND ITS ASSOCIATION WITH INTES-TINAL FUNCTIONAL AND STRUCTURAL ABNORMALITIES The load of ova was less than 1,000 per gram of faeces in 25 cases, 1,000 to 5,000 in 23 cases, 5,000 to 15,000 in six cases, and above 15,000 in 13 cases. The maximum load was 93,000/g of faeces. The relationship of the ova load to the d-xylose excretion in urine is presented in Table I. Normal d-xylose excretion is recorded in 83% of the cases with an ova load of 1,000 or less compared with 70%, 50%, and 54% respectively of the patients with a load of 1,000 to 5,000, 5,000 to 15,000, and 15,000 or more. Since there is no apparent difference between the last two groups, for statistical analysis they have been considered together as one group of heavy infestation. The association of ova load with d-xylose absorption is statistically not significant ($\chi^2=4.73$, $P=0.10$). Table II presents the association of faecal fat on the balance test with the ova load. Eighty-four per cent of the patients with a load of less than 1,000, 70% between 1,000 to 5,000, and 47% with ova load of more than 5,000 had a normal fat balance test. Statistical analysis suggests a weak association between increased faecal fat with heavy worm infestation ($\chi^2=6.74$, $P=0.04$). A statistically significant association between heavy hookworm infestation and hypoalbuminaemia had already

been observed (Tandon *et al*, 1968) ($\chi^2=19.17$, $P=0.01$). Therefore, a cross analysis of ova load and serum albumin level with the fat balance test was done and the results are presented in Table III. Normal faecal fat is recorded in 79% of the cases with an ova load of less than 5,000 compared with 80% with an ova load of more than 5,000 at the serum albumin level of 3.26 g or more. However, at the serum albumin level of less than 2.75 g and 2.75 to 3.25 g %, normal fat absorption is noted in 67% and 70% cases, with an ova load of less than 5,000 compared with 29% and 43% cases with an ova load of more than 5,000 per gram of faeces. The intestinal biopsy (Table IV) does not reveal a statistically significant association of histological abnormality with a heavy load of ova. The intestinal biopsy was noted as normal in 83% of the cases with an ova load of less than 1,000, and in 96% and 88% of the cases with an ova load 1,000 to 5,000 and more than 5,000 respectively. Twelve per cent of cases with an ova load less than 5,000 and only 6.2% of cases with heavy infestation (ova load more than 5,000) had an abnormal intestinal biopsy.

EFFECT OF HOOKWORM SPECIES ON THE INTESTINAL CHANGES *Ankylostome duodenale* was detected in 49 cases and 16 had *Necator americanus* infestation. Species relationship to the intestinal absorption and morphological studies is presented in Table V.

TABLE I

D-XYLOSE ABSORPTION TEST IN RELATION TO THE OVA LOAD IN 66 CASES OF HOOKWORM DISEASE

Ova Load per g Faeces	No. of Patients	Five-hour D-xylose Excretion in Urine (g) ¹						Mean Five-hr D-xylose Excretion in Urine (g)
		Less than 0.50	0.50-0.99	1.00-1.49	1.50-1.99	2.00 or More	1.00 or More	
Less than 1,000	24	1	3	9	4	7	20 (83%) ²	1.73
1,000-4,999	23	1	6	6	5	5	16 (70%)	1.48
5,000-14,999	6	2	1	1	2	0	3 (50%)	1.06
15,000 and more	13	0	6	3	2	2	7 (54%)	1.21

$$\chi^2_{(2)}=4.73; P=0.10$$

¹5 g dose was used for xylose absorption test.

²Percentages based on fewer than 25 observations.

TABLE II

FAT BALANCE TEST IN RELATION TO THE OVA LOAD IN 67 CASES OF HOOKWORM DISEASE

Ova Load per g Faeces	No. of Patients	24-Hour Faecal Fat (g) on 7-Day Fat Balance Test					Mean Faecal Fat Excretion (g/24 hr)
		Less than 1.00	1.00-4.99	5.00-8.99	9.00 and More	Less than 5.00	
Less than 1,000	25	3	18	4	0	21 (84%)	2.97
1,000-4,999	23	2	14	6	1	16 (70%) ¹	3.99
5,000-14,999	6	0	3	3	0	3 (50%) ¹	4.71
15,000 and more	13	1	5	4	3	6 (46%) ¹	6.12

$$\chi^2_{(2)}=6.74; P=0.04$$

¹Percentages based on fewer than 25 observations.

TABLE III

CROSS ANALYSIS OF THE ASSOCIATION OF FAT BALANCE TEST WITH THE OVA LOAD AND SERUM ALBUMIN LEVELS

<i>Ova load per g Faeces</i>	<i>Serum Albumin Level (g per 100 ml)</i>					
	<i>Less than 2.75</i>		<i>2.75-3.24</i>		<i>3.25 or More</i>	
	<i>Faecal Fat (g/24 hr)</i>					
	<i>Less than 5.00</i>	<i>5.00 or More</i>	<i>Less than 5.00</i>	<i>5.00 or More</i>	<i>Less than 5.00</i>	<i>5.00 or More</i>
Less than 5,000	2 (67%)	1	7 (70%)	3	27 (79%)	7
5,000 or more	2 (29%)	5	3 (43%)	4	4 (80%)	1

TABLE IV

RELATIONSHIP OF INTESTINAL BIOPSY FINDINGS WITH THE OVA LOAD IN 64 CASES OF HOOKWORM DISEASE

Ova Load per g Faeces	No. of Cases	Normal Biopsy	Abnormal Biopsy
Less than 1,000	23	19 (83%) ¹	4
1,000-4,999	24	23 (96%) ¹	1
5,000 and more	17	15 (88%) ¹	2

¹Percentage calculated on fewer than 25 observations.

Normal d-xylose, fat absorption, and intestinal biopsy are noted respectively in 69.39%, 66.62%, and 89.13% of the patients with *Ankylostome duodenale* infection compared with 73.33%, 75.00%, and 87.50% for *Necator americanus* infection. Intestinal function and structure changes do not show statistically significant differences in the two types of worm infection. The mean ova load for *Ankylostome duodenale* was 16,115 with a range of 200 to 91,000 and corresponding figures for *Necator americanus* were mean ova load 1,575 with a range of 200 to 7,500/g faeces.

EFFECT OF DURATION OF HOOKWORM INFESTATION ON INTESTINAL FUNCTION AND STRUCTURE Table VI presents the results of the initial and the follow-up studies of d-xylose absorption, fat balance test, Schilling test, and intestinal biopsy. It is noted that

during the period of follow-up, the percentage of abnormal tests became less than at the time of the initial study. No fresh case developed any structural or functional abnormality during this period of persistent hookworm infestation which extended from a minimum of 10 weeks to a maximum of 68 weeks. D-xylose absorption, which was initially abnormal in four cases, became normal in all during the period of 10, 12, 14, and 18 weeks respectively.

TABLE VI

INITIAL AND FOLLOW-UP OBSERVATIONS OF D-XYLOSE ABSORPTION, FAT BALANCE TEST, SCHILLING TEST, AND INTESTINAL BIOPSY IN HOOKWORM DISEASE

Test	No. of Cases	Percentage Abnormal at First Study	Percentage Abnormal at Follow-up Study ¹
D-xylose absorption	17	25.00	0.0
Fat balance	17	43.75	17.64
Schilling test	8	18.1	12.50
Intestinal biopsy	15	11.76	6.60

¹Percentages calculated on fewer than 25 observations.

The fat balance test was abnormal in seven of the 16 cases at the initial study. During the follow-up period it became normal in all but one case. This particular patient of the present series had the heaviest ova load of 93,000/g, a serum albumin level of 2.6 g %, and a haemoglobin of 3.1 g %. The Schilling test was done during the follow-up study (10 weeks to 13 weeks) in eight cases. Only one case showed an abnormality of 3.4% excretion in urine. An initial study, done 10 weeks earlier in this case, had shown 1.9% excretion. The remaining seven cases had normal results.

Intestinal biopsy showed distinct villous atrophy (Fig. 1) in two cases at the initial study. Repeat observations at 14 weeks showed abnormality in one of them. The rest of the 16 cases showed normal morphology at the follow-up study. The mean haemoglobin of the 17 cases at the initial study was 6.81 g % with a range of 1.9 g % to 13.0 g %. All, except one woman with haemoglobin of 12.7 g %, during the period of follow-up, the percentage of abnormal tests became less than at the time of the initial study.

TABLE V

D-XYLOSE ABSORPTION, FAT BALANCE TEST, AND INTESTINAL BIOPSY STUDY IN RELATION TO SPECIES OF THE HOOKWORM INFESTATION

Species of Hookworm	No. of Cases	Percentage 5-hr Urinary D-xylose (g)		Percentage 24-hr Faecal Fat (g)		Intestinal Biopsy	
		1.0 or More	Less than 1.0	Less than 5.0	5.0 or More	Normal (%)	Abnormal (%)
<i>Ankylostome duodenale</i>	49	69.39	30.61	66.6	33.33	89.13	10.87
<i>Necator americanus</i>	16	73.33 ¹	26.66 ¹	75.0 ¹	25.0 ¹	87.5 ¹	12.5 ¹

¹Percentage calculated on fewer than 25 observations.

FIG. 1. Jejunal biopsy from a patient with hookworm disease. There is broadening and atrophy of the villi. The villus:crypt ratio shows a significant reduction. Lining epithelial cells are atypical with loss of polarity and the lamina propria contains an increased number of chronic inflammatory cells. Haematoxylin and eosin $\times 135$.

were anaemic. The mean haemoglobin at the time of follow-up of intestinal function and structure studies became 11.46 g % with a range of 5.6 to 14.8%. In all haemoglobin had risen significantly. Serum albumin levels ranged from 2.1 to 4.1 g %, with a mean of 3.21 g % at the initial study; this showed no change when the intestinal studies were repeated.

DISCUSSION

Hookworm infestation is widely prevalent in the world (World Health Organization, 1963). Recent reports (Sheehy *et al*, 1962; Sheehy, 1962; Vélez and Orrego, 1963) suggesting hookworm as a cause of the malabsorption syndrome have been of concern to investigators in tropical countries. The present study, which was planned to investigate the role of parasitic factors in the pathogenesis of structural and functional changes in the small bowel, indicates that hookworm infestation as such is not a cause of

the malabsorption syndrome. The load of worm infestation had been reported to correlate with the anaemia (Hill and Andrews, 1942; Layrisse, Paz, Blumenfeld, and Roche, 1961; Foy and Kondi, 1961; Layrisse and Roche, 1964), with plasma and albumin loss from the gut (Blackman, Marsden, Banwell, and Craggs, 1965), and with nitrogen absorption (Darke, 1959). Our investigations, however, fail to reveal a statistically significant association of defective d-xylose and fat absorption and intestinal biopsy abnormality with a heavy load of ova in faeces. This confirms the observations of Layrisse and Roche (1964), who in a study of d-xylose absorption, fat balance test, Schilling test, and intestinal biopsy in 18 patients, with heavy infestation (ova load 5,000/g faeces) did not find evidence of malabsorption in any of these parameters, except that d-xylose absorption was abnormal in one case and intestinal biopsy showed only non-specific changes in 14 cases. A weak association between heavy infestation with increased excretion of fat in the stools in moderately and severely hypoalbuminaemic patients, however, was recorded in the present study (Table II). The mechanism of defective fat absorption in these patients had not been studied. Darke's (1959) hypothesis that dietary deficiency of protein, leading to a deficiency of digestive enzymes and possibly to the secretion of antienzymes by parasites, in heavy infestation, needs to be investigated. Indeed, deficiency of lipase has been demonstrated in hypoproteinaemia associated with protein malnutrition (George, 1968).

The prevalence of *Necator americanus* and *Ankylostome duodenale* is variable in different parts of the world (World Health Organization, 1963). *Ankylostome duodenale*, with its larger size, toothed mouth parts, and migratory habits has been considered more injurious than the *Necator americanus* (Bonne, 1942). Therefore the intestinal changes in relation to the species were studied. *Ankylostome duodenale* infestation was three times more common than that of *Necator americanus*. Chhuttani and Chugh (1959) reported an almost exclusive incidence of *Ankylostome duodenale* in the adjoining state of Punjab. The present study does not reveal any difference in the incidence of intestinal abnormalities in relation to the species of the worm (Table V), but this finding is less specific when applied to a smaller number of cases with fewer ova per gram faeces in which *Necator americanus* was the species of worm implicated compared with *Ankylostome duodenale*. However, all the cases of hookworm disease with malabsorption syndrome reported by Sheehy *et al* (1962) were infested with *Necator americanus*. The species of worm responsible for the infestation cannot be correlated with intestinal abnormalities.

Reports of the variable incidence of malabsorption in hookworm disease are not related to the prevalence of any particular species of the hookworm in different parts of the world.

Hookworm infestation is a chronic disease persisting for several years. The duration of infection as judged by the day of onset of symptoms is unreliable, hence a prospective follow-up study was undertaken to find out if intestinal abnormalities were related to a prolonged and persistent infestation. Since hypoalbuminaemia had been reported to be closely associated with intestinal changes (Tandon *et al*, 1968; Mayoral *et al*, 1967) this factor was not corrected during the follow-up study and the mean value for serum albumin at the initial and the follow-up period of study were the same. Neither absorption tests nor intestinal biopsy studies revealed any deleterious effects of persistent hookworm infestation for a period of 10 weeks to 68 weeks. The small intestinal mucosa is one of the most dynamic structures in the body (Creamer, 1967). If hookworm infestation had any noxious effect on the structure and functions of the small bowel this would have been seen during the several weeks of observation recorded in the present report, and it is unlikely that a longer follow-up would yield any different results. Mayoral *et al* (1967) failed to find any adverse effect due to persistent hookworm infestation in four cases followed from 52 to 247 days. These patients had intestinal function and structure abnormalities which reverted to normal despite persistent hookworm infestation as the associated protein caloric malnutrition was treated.

A critical study of various parasitic factors leads to the conclusion that neither the heavy load nor the duration of infection nor the species of worm are of any significance in the pathogenesis of intestinal abnormalities in hookworm disease. The infested patients very often come from a low socio-economic class with a poor dietary history, and investigations suggesting the causative role of hookworm (Sheehy *et al*, 1962; Vélez and Orrego, 1963) may have overlooked the significance of associated protein malnutrition in the pathogenesis of intestinal abnormalities. A statistically significant association of hypoalbuminaemia with defective d-xylose and fat absorption ($P < 0.01$) and a weak association with an abnormal intestinal biopsy have been observed in hookworm infestation (Tandon *et al*, 1968). The severity of the anaemia had no relationship to the intestinal changes in these cases (Tandon *et al*, 1968). A study of 12 cases with hookworm infestation (four patients with severe protein malnutrition and eight without) and three cases of protein malnutrition with anaemia but without hookworm infestation led Mayoral *et al* (1967) to the similar conclusion that

protein malnutrition was the important pathogenic factor in intestinal abnormalities but anaemia and a heavy hookworm infestation did not have a causative role. Several other authors have suspected a relationship between hookworm disease and protein malnutrition (Darke, 1959; Vélez and Orrego, 1963; Fülleborn, 1929). Indeed, the role of protein depletion in the pathogenesis of malabsorption due to small bowel damage has been reported in several experimental and clinical studies (Zubirán, 1961; Deo and Ramalingaswami, 1964; Platt, Heard, and Stewart, 1964; Takano, 1964; Stanfield, Hutt, and Tunnicliffe, 1965; Mayoral *et al*, 1967; Tandon *et al*, 1968). The 'malabsorption syndrome' observed in hookworm disease is not due to any of the parasitic factors but is primarily related to the associated malnutrition and protein deficiency.

SUMMARY AND CONCLUSIONS

The role of parasites in the pathogenesis of malabsorption was investigated in 67 patients with hookworm disease. The relationship of d-xylose absorption, fat balance test, and intestinal biopsy abnormalities with the ova load and species of the parasite was studied. A follow-up study was carried out in 17 cases to find out the effect of 10 to 68 weeks' persistent hookworm infestation on absorption parameters and intestinal morphology. Functional and structural abnormalities had statistically no significant association with the ova load or the species of the hookworm, nor any deleterious effect on the small bowel. It is concluded that neither the load or infection, nor the species, nor the duration of infestation has any direct causative relationship with the malabsorption syndrome in hookworm disease.

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