Intestinal nematode infections in children: the pathophysiological price paid

E. S. COOPER$^1$, C. A. M. WHYTE-ALLENG$^2$, J. S. FINZI-SMITH$^1$ and T. T. MacDONALD$^4$

1 Tropical Metabolism Research Unit, University of the West Indies, Mona, Kingston 7, Jamaica
2 Parasite Epidemiology Research Group, Department of Biology, Imperial College, London SW7 2AZ, UK
3 Department of Zoology, University of the West Indies (Jamaica)
4 Department of Paediatric Gastroenterology, St Bartholomew’s Hospital Medical College, London EC1A 7BE, UK

SUMMARY

The mechanism by which small animals such as rodents resist or eliminate nematode parasites requires mucosal inflammation as the final effector of the immune response. The resulting freedom from chronic infection may be worth the price of short-term illness. Putative vaccines which attempt to enhance the natural effect will have to take into account the inflammatory cost to the host. Human helminthiases involve a more stable equilibrium between host and parasite. The medical literature on hookworm disease and clinical ascariasis describes, for the former, some chronic inflammatory effects correlated with worm burden, but for the latter a less quantified or predictable set of detrimental effects. We describe a current, systematic study of the inflammatory response to whipworm infection, in which anaemia, growth retardation and intestinal leakiness are viewed as predictable consequences related to infection intensity. There is evidence for the absence of cell-mediated immunopathology. However, a specific, IgE-mediated local anaphylaxis may, at least partly, mediate the deleterious effects. Increased numbers of mucosal macrophages may also contribute to the chronic, systemic effects through their output of cytokines. Similar attempts to show the mechanisms of pathogenesis and quantify the effects of hookworm disease should be undertaken.

Key words: mucosal inflammation, local anaphylaxis, helminthiases, human growth.

INTRODUCTION

The similarities in basic organization among the parasitic nematodes, so striking to the zoologist, may make the differences in the way in which they produce disease appear to be relatively small details. But to the physician it is these details that are in the foreground. So, while the three most prevalent intestinal nematode infections of man, hookworm, roundworm and whipworm, make an obvious group—roundworm and whipworm occupy the small and large bowels respectively; hookworm and roundworm are quite different in their mode of attachment to the small-intestinal mucosa; and whipworm has no phase of extra-intestinal migration, unlike roundworm and hookworm. In the latter pair both the larvae and the adults are pathogenic, although each stage causes a different syndrome.

All three parasites can be the principal agents of well-defined diseases, susceptible to accurate diagnosis and completely effective, specific treatment. However, such defined diseases occur in a small minority of infected people, those with the most intense infections. In spite of the clarity of the diseases, little is known of the quantity of the parasites’ detrimental effects in the great majority of their hosts, most of whom will be relatively lightly infected, and will also have intercurrent bacterial and viral infections as well as multiple environmental deprivations. Even in the specific diseases, in the most intensely infected hosts, not much is known of the mechanisms of pathogenesis.

It is a tenet of this paper that the second of these unknowns, the mechanism of pathogenesis, should be the one to be tackled first. From an understanding of the mechanisms we can generate expectations of community-wide effects, and then check on their real existence in community studies.

We shall first look briefly at some evidence from animal studies on the mechanisms by which a price is paid in inflammation—but the goods are duly delivered, worm infection is resisted. Next, we review medical literature on hookworm disease and clinical ascariasis, but find it difficult to build the reports of specific clinical associations of infection towards a coherent total view of the pathophysiological costs to the hosts. Finally, we give an overall picture of our own current work on trichuriasis, including unpublished work in progress, in order to show an attempt at constructing such a view. We shall not try to hide the difficulties in quantifying clinical observations in the field.

ANIMAL HOSTS: THE PRICE PAID FOR WORM EXPULSION

Several mammalian species, much studied in the laboratory, have highly effective immune responses against intestinal nematodes. The effector arm of this response is concentrated in the mucosa, where
induction of immunity. This can be seen in the history of vaccines against *Mycobacterium tuberculosis* (BCG), *Bordetella pertussis* and *Salmonella typhi*.

**HUMAN DISEASE**

**Larval migration phase**

**Hookworm** (*Ancylostoma duodenale* and *Necator americanus*). In naive adult hosts entering an endemic area for the first time, the severe itching of the area of skin penetrated by the filariform (L3) larvae, known as 'ground itch', is more obvious than it is in children native to the region. The greatest opportunity to observe it has been provided to the medical officers of troops on the move (Rogers & Damm, 1946). The condition appears to be self-limiting, the course being only two weeks. Although it is difficult to say whether equivalent symptoms occur in children, it is likely that they do at least to some extent: the important consequence is then probably secondary infection and a generalized pyoderma. The area then provides a source for the spread of the secondary pathogens to other parts of the skin and to that of other children. There is little prospect of measuring the proportion of pyoderma in hookworm-endemic areas that can be ascribed to larval skin invasion, as opposed to insect bites, scabies and direct trauma; but it remains likely that hookworm contributes to the general load of skin infection affecting most children in the tropics.

Hookworm larval migration through the lungs is said to cause less wheezing and coughing than the migration of ascarid larvae. As usual, this view is based on the experience of exceptional infections in naive adult hosts. There is no evidence for or against the proposition in children.

**Roundworm** (*Ascaris lumbricoides*). Granulomata surrounding larvae have been described in the liver (Beaver & Danaraj, 1958). It is not known whether this reaction is associated with any adverse functional consequences.

Cough is said to be the principal symptom as the larvae cross the capillary bed and parenchyma of the lungs and emerge through the bronchial epithelium into the lumen of the bronchial tree. Wheezing and breathlessness, the symptomatic manifestations of muscular broncho-constriction and mucous plugging of the airways, may also occur; but views on these points are no more than extrapolations from unusual and extreme situations in adults. An immediate hypersensitivity type of immune reaction may occur in the lungs, known as Loeffler's pneumonia. Clinical experience suggests that this type of reaction is more severe, and therefore more easily discerned against the general background of children's coughs, colds and mild dyspnoea, when the...
parasitic larva is a zoonotic agent, e.g. *Toxocara*. Respiratory symptoms in children with larval human ascariasis, therefore, should at present be regarded as of unknown extent and severity in populations where these infections are endemic.

**Adult helminths in intestine**

**Hookworm.** The morphological and biological differences between the two species, *A. duodenale* and *N. americanus*, and the different geographical regions in which each predominates, are of relatively little clinical importance: this is why it is usual to speak of the parasite simply as hookworm. Severe iron deficiency occurs in prolonged and intense hookworm infection, and this is the principal, and sometimes the only, component widely recognized as 'hookworm disease.' However, hypo-albuminaemia is also recognized as part of the syndrome, frequently associated with oedema of the lower limbs.

Recently, there has been renewed interest in growth impairment as a consequence of hookworm infection in children. This has come about because of the availability of safe and effective drugs (the carbimazole carbamate family). These have allowed Stephenson and her colleagues (1989) to show, in double-blind clinical trials, improvements in the growth of Kenyan children infected with hookworm and other intestinal parasites, following treatment with a broad-spectrum anthelmintic (albendazole). Moreover, Stephenson et al. (1989) have drawn attention to literature describing studies (Smillie & Augustine, 1926; Keller et al. 1935) dating back to 1903 which associated hookworm infection with stunted growth and delayed puberty. The clinical descriptions of this neglected aspect of 'hookworm disease' include loss of appetite, although this symptom is difficult to validate and quantify. Reduced food intake is certainly an expected effect of infection in children. This has come about because of the mere loss of the plasma accompanying the limited exudation. This can also be expressed as a protein clearance of 35 ml of plasma (since the plasma itself is replaced from the extracellular fluid). However, using albumen labelled with $^{125}$I or $^{51}$Cr clearances of over 100 ml/day have often been shown. This exudation is likely to represent inflamation, ultimately expressed as loosening of the tight junctions between the epithelial cells. A direct relationship between the extent of protein-losing enteropathy and the intensity of infection has been shown by several authors (Blackman et al. 1965; Aveekul et al. 1971). In *Nippostrongylus* infection in the rat, the quantity of protein lost into the stool is associated not only with the intensity of infection (positively), but also with the pre-existing protein nutrition of the animal (negatively) (Lunn et al. 1986).

Although protein-losing enteropathy occurs, it may not be the complete explanation of hypo-albuminaemia in hookworm infection. Hypo-albuminaemia due to depressed hepatic synthesis is expected as part of the systemic response to inflamation. That there is such a systemic change has been reported in ancylostomiasis (Blom, Prag & Norredam, 1979). In the course of such a response, the presence of increased circulating tumour necrosis factor (TNF) has been particularly associated with depressed albumen synthesis (Grimble, 1989). The systemic cytokine profile has not been reported in hookworm infection, but we have noted high plasma concentrations of TNF in severe trichuriasis (Cooper et al. 1990a), and so it seems a study well worth undertaking.

The morbidity of *A. brasiliensis* infection in dogs has been studied (Kalkofen, 1974) more thoroughly than that of hookworm in man, although several studies of human intestinal biopsies have been reported (Tandon et al. 1966; Burman et al. 1970). Several villi are drawn into the worm's buccal cavity. The epithelial cells are detached from the lamina propria, in which the capillary loops burst. There is therefore direct contact between antigenic substances from the worm, probably including proteolytic enzymes, and the macrophages and lymphocytes of the lamina propria. Nevertheless, the striking finding is a negative one: namely, that the villi not ingested by the worm usually appear normal. Immuno-pathology mediated by T-cell activation would have been expected to lead to villous atrophy (MacDonald & Spencer, 1988). The histological effects of hookworm infection in man appear to consist only of local tissue destruction, haemorrhage within the ingested tissue and transepithelial migration of erythrocytes in adjacent tissue, as well as oedema, increased mitotic activity in the neighboring crypts with corresponding immaturity of the cells migrating up the sides, and neutrophil infiltration of the lamina propria on the edge of the lesion. All of these effects are consistent with a local
Table 1. Hookworm disease

<table>
<thead>
<tr>
<th>Larval stage</th>
<th>Adult in the intestine</th>
<th>Systemic secondary effects</th>
</tr>
</thead>
</table>

Table 2. Roundworm disease

<table>
<thead>
<tr>
<th>Larval stage</th>
<th>Adult in the intestine</th>
<th>Adult in ectopic site</th>
<th>Systemic secondary effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bile-duct</td>
<td>Obstructive jaundice.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pancreatic duct</td>
<td>Acute pancreatitis.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Larynx</td>
<td>Asphyxia.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Many other ectopic sites and unusual presentations.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vitamin A deficiency.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Malnutrition? (Not established).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

response to mechanical and chemical trauma and no T cell-mediated immune response needs to be invoked as an explanation. So far, there are no reports of immunohistochemical studies to confirm this inactivity. Swelling of the retroperitoneal lymph-nodes has been noted (Miller, 1979), but its immunological basis has not been investigated.

In Table 1, a summary of the relatively clear and established manifestations of hookworm disease is set out. It is very probable that Type I hypersensitivity underlies the manifestations of larval migration, but it is not established that there is any immunological mechanism behind the intestinal manifestations of mucosal attachment of adult worms. However, there is clearly an inflammatory response, both local and systemic, in the broader sense of the word.

Roundworm (ascariasis). During the intestinal phase of ascariasis there is no symptomatology that can clearly be ascribed to the operation of immune or inflammatory mechanisms. All the well-founded symptoms and signs of roundworms in the gut are explicable on a mechanical basis. In the most intense infections a knot of worms may obstruct the bowel. The adult worms, maintaining their position in the lumen by muscular bracing, are capable of moving back up through the alimentary canal and appearing in the mouth or upper respiratory tract. They are also capable of blocking the common bile-duct or the pancreatic duct, and of obstructing the larynx. These various accidents will have some mathematical expectation of occurrence in a large population where the parasitosis is endemic, but they do not amount to a predictable price paid by the host in its own defence. The ectopic migration seems to be exacerbated by fever in the child or by the initial metabolic attack of the carbimazole drugs (Chanco & Vidad, 1978).

It has been suggested that lactose intolerance is induced by ascariasis (Taren et al. 1987), and that villous atrophy and malabsorption may occur (Tripathy et al. 1972), but the evidence for these as pure effects of the helminthiasis is not strong. It is
Table 3. Whipworm disease

<table>
<thead>
<tr>
<th>Adult in intestine</th>
<th>Systemic secondary effects</th>
</tr>
</thead>
</table>

possible that the parasite has a profound influence on the intestinal microflora, mediating some of these claimed effects, but very little work has been done in this difficult area.

Table 2 is a summary of the commoner and better established symptoms of ascariasis.

**Whipworm** (trichuriasis). The principal consequences of intense infection by *Trichuris trichiura* (Table 3) are chronic diarrhoea, anaemia and stunting of growth (Gilman et al. 1983; Cooper & Bundy, 1987). All of these are likely to be the detrimental consequences of some form of inflammatory response to the worm.

(1) Anaemia – relationship to worm burden. Anaemia in the *Trichuris* dysentery syndrome can be very severe. Only 3 out of 45 Jamaican cases of this syndrome (aged 1–12 years) had blood haemoglobin concentrations above 110 g/l, the standard accepted as the cut-off between normal and anaemia (WHO, 1975). Our patients, aged 3–6, had a mean haemoglobin concentration of 80 g/l (standard at that age 128, s.d. $\approx 18$ g). We have recently seen an 8-year-old girl whose haemoglobin was 19 g/l, approaching the limit of dilution compatible with life, who responded completely to simple oral iron therapy and cure of the syndrome with mebendazole.

However, anaemia is a clear example of the non-linearity of the relationship of morbidity to the prevalence of infection. Two community-wide studies (Otto, 1935; Greenberg & Cline, 1979) showed a complete lack of association between trichuriasis and anaemia, but then low-intensity infections greatly predominated in their surveys. In our own study of a village with hyperendemic infection rates (Cooper & Bundy, 1986), although the difference in haemoglobin levels between intensely infected and uninfected children was highly significant statistically, it was not large, and not on the same scale as the difference between hospital cases of *Trichuris* dysentery syndrome and their neighbourhood controls (unpublished data of J. E. M. Callender).

The mechanism of blood loss in severe trichuriasis is likely to be both by gross loss from the inflamed rectum and by passage of red cells across the entire bowel epithelium, rendered permeable by the anaaphylactic inflammation (see below). Leakage of red cells across the colonic epithelium was likewise considered by Beer, Sansom & Taylor (1974) to be the likely mechanism of blood loss in trichuriasis in the pig.

(2) Growth retardation – relationship to worm burden. Growth retardation is unlikely to represent the effects of direct loss of nutrients and host substances to the worm on the outmoded model that the host’s loss is the worm’s gain. An adult *T. trichiura* weighs, at most, 10 mg. Therefore, a burden of 1000 whipworms, which would be associated with all the clinical features of the *Trichuris* dysentery syndrome, represents less than 10 g of nematode tissue. This should not pose significant metabolic competition to the 10 or 20 kg host.

The outcome of somatic growth is attained mass and length (height). If the rate of growth is depressed proportionately by the extant worm burden, then the deficit in attained size should correlate with the instantaneous worm burden integrated over time: not a quantity that can be measured or even estimated in practice. To show this, we have attempted, in a recent data-set, correlations among current worm burden, duration of bowel symptoms by mother’s account, and height deficit; but the deficit did not correlate with the current infection intensity, the alleged duration of symptoms, or the product of the two. In another, published data-set (Cooper et al. 1990b), however, the correlation between duration of symptoms and height deficit was highly significant (Kendall’s tau 0.55, $n = 19$, $P < 0.001$). We must conclude that no reliable retrospective estimation is possible for the clinician, which seems likely enough to be the case. In fact, our evidence for the retardation of growth in the *Trichuris* dysentery syndrome is strong but indirect, and depends on the reversal of growth retardation upon treatment of the helminthiasis (Cooper et al. 1990b). Then, exceptionally high growth velocities are achieved in a recovery or ‘catch-up’ phase, even though the child’s environment and available diet remain unchanged.

We have the opportunity from time to time to make more direct observations. This is when we
Fig. 1. Symptomatic periods, height velocity and anthelmintic treatment over 5 years in a young, multiply deprived, urban Jamaican boy. TDS, Trichuris Dysentery Syndrome. Circles represent the points of estimated height velocity; horizontal lines between inward arrowheads represent the intervals between the height measurements leading to this estimate. (O) Height velocities disaggregated from the mean velocities (•) within which they are nested. Where the measurement interval is much less than 0.5 years accuracy is increasingly sacrificed for time-precision, a general feature of growth velocity estimations derived over short periods. The height velocities are here not shown as cm/year, but in terms of standard deviation for age. The Tanner–Whitehouse (1976) median and ±4σ boundaries are thus shown as horizontal lines to provide a vertical scale. Downward arrows represent full, supervised courses of benzimidazole carbamate (mebendazole or albendazole). [ ] indicates unsupervised treatment at home, which we have independent reasons to believe is unlikely to have been completed. On both occasions when the boy was admitted and treated under supervision, acceleration in height-growth followed. On the first occasion, height velocity fell precipitously some 3 months afterwards, and symptoms of dysentery later appeared and worsened. On the second occasion, 3-monthly follow-up in the community was sustained, with supervised treatments. Height velocity was then maintained at a catch-up rate, and symptoms did not recur.

observe the effect of re-infection to the point of a recurrence of the dysentery syndrome in our patients, whom we then have an ethical obligation to re-treat. Height velocity does diminish on heavy re-infection, and increases again on re-treatment (Fig. 1).

The mechanism of growth retardation is unknown, but may be related to the increased concentration of circulating cytokines alluded to below. Possible mechanisms by which TNFα (below) might mediate growth retardation are: anorexia (Moldawer et al. 1988), inhibition of proteoglycan synthesis and promotion of its resorption (Saklatvala, 1986), increased production of collagenase by fibroblasts (Bremner et al. 1989), cachexia and local inflammation (Tracey et al. 1988), and inhibition of growth hormone release by pituitary cells (Walton & Cronin, 1989). However, much of the growth retardation may be a consequence of lack of available substrate through nutrient loss as part of the protein-losing enteropathy described below.

(3) Inflammation. There is macroscopical evidence of colonic inflammation in the Trichuris dysentery syndrome, seen through the colonoscope, but it is confined to hyperaemia and oedema. Ulceration of the mucosa does not occur.

Microscopically, the lamina propria is richly cellular, mostly with mononuclear cells, but also with an infiltration of eosinophils in some cases.
However, these changes in the lamina propria are not specific to *T. trichiura* infection, for they are also seen in children with mucoid diarrhoea of other origin from the same environment. These other children are not seriously ill. Microbiological and parasitological investigations on them have been negative. They recover from their mucoid diarrhoea spontaneously, typically within a month or two.

What is more striking in trichuriasis is the many absences of the markers of cell-mediated immunopathology, despite the enormous antigenic load inside the epithelial barrier of the mucosa (MacDonald et al. 1991). Intra-epithelial lymphocytes are reduced in number, as is the proportion bearing gamma-delta T cell receptors, so markedly increased in the immunopathological condition of coeliac disease (Spencer et al. 1991). In the lamina propria, the proportion of cells bearing the CD3 marker is the same in trichuriasis as in local controls, implying that there is no increase in T cell number. Lamina propria CD25+ cell numbers are also highly variable within each group of children but with no difference between the groups. Since this implies that there is no general increase in the number of cells bearing receptors for IL2, it also implies that there is no general activation of T cells or macrophages. The epithelium also fails to show the consequences of a local T cell activation: not only is the cellular architecture undisturbed, but there is a lack of consistent expression of HLA-DR or VLA-1, both of which are characteristic of mucosal inflammation (Selby et al. 1983; MacDonald et al. 1990). Despite the apparent lack of T cell activation, there are two indications of an inflammatory response to trichuriasis within the lamina propria: (i) increased numbers of macrophages, with evidence of their production of cytokines, and (ii) an IgE-mediated mucosal mast cell response. Both of these have consequences which are costly to the host.

In an immunohistochemical study on formalin-fixed caecal biopsies, control children (i.e. those requiring colonoscopy for other conditions) showed staining of 5–17% of the lamina propria cells with the pan-macrophage monoclonal antibody MAC 387. In contrast, in children with trichuriasis, MAC 387 stained 23–45% of lamina propria cells. This difference is clear-cut, especially when one takes account of the clinical heterogeneity of the controls.

There is an increase in the monocyte/macrophage population of the colonic lamina propria in *Trichuris* colitis. Perhaps this finding alone would not constitute evidence of inflammation: the macrophages may be degrading the copious soluble antigen produced by the *T. trichiura* stichocytes without deleterious effects on the host. However, there is evidence of the production of TNFα by these mononuclear cells (Cooper et al. 1990a). This work may be considered as 'in progress'. We have cultured mononuclear cells from the caecal mucosa in trichuriasis, and shown an increased concentration of TNF in the supernatant fraction when compared with that from mononuclear cells isolated from the caecal mucosa of control children. Immunostaining of cryopreserved mucosa using a specific monoclonal antibody against TNF has also shown intense staining in tissue from children with the *Trichuris* dysentery syndrome (S. H. Murch, unpublished data).

If TNF is produced in the colonic mucosa it will be drained by the portal venous system and so pass directly to the liver, where it is degraded (Tracey et al. 1988). However, we have shown (Cooper et al. 1990a) increased plasma concentrations of TNF in the systemic circulation in children with the *Trichuris* dysentery syndrome. If the origin of this is the gut mucosa, then this is the fraction that has survived a first pass through the liver. The assay we used, a commercially available ELISA, has been shown to be relatively insensitive (Butcher et al. 1990). Despite these two points, the trichuriasis children were clearly different from two sets of controls, surgical patients and stunted but non-parasitized children from similar communities, who had normal levels. Nine of thirteen children at the time of admission with *Trichuris* dysentery syndrome had raised concentrations of TNF.

In Crohn's disease, a severe form of inflammatory bowel disease, similarly increased concentrations of serum TNF have been recorded (Murch et al. 1991), especially during relapses and associated with periods of reduced growth-rates in children. However, *Trichuris* dysentery syndrome is associated with much less severe inflammatory responses than full-blown Crohn's disease, both in the bowel and systemically.

There is other evidence of a systemic response to trichuriasis: low plasma albumen (Gilman et al. 1983), reversed albumen/globulin ratio (unpublished data), raised alpha1-antitrypsin (similar to that reported with hookworm infections by Blom et al. 1979), moderately increased C-reactive protein and high plasma fibronectin levels (unpublished data). There has so far been little description of these changes in circulating proteins in children with helminthiasis, despite interest in the non-specific 'acute-phase response' to infection (Stadnyk & Gauldie, 1991).

We have found it interesting to observe that finger-clubbing - not itself, perhaps, a cost of infection, since the change in the nailbed does not appear actually to harm the patient - correlates more closely with intensity of infection than any other clinical effect of which we are aware (Table 4). Even rectal prolapse, often thought of as the nearest thing to a pathognomonic sign of trichuriasis in endemic areas (Cooper & Bundy, 1987), does not correlate so perfectly. Despite the harmfulness of clubbing, it is nevertheless significant, for it is inextricable...
Table 4. Frequencies of rectal prolapse and finger clubbing at different cut-offs of infection intensity in a set of 45 cases of *Trichuris* dysentery syndrome

<table>
<thead>
<tr>
<th>Intensity (Adult <em>T. trichiura</em>)</th>
<th>Prolapse n/N(%)</th>
<th>Clubbing n/N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 1</td>
<td>15/45(33)</td>
<td>15/45(33)</td>
</tr>
<tr>
<td>&gt; 200</td>
<td>14/37(38)</td>
<td>14/32(44)</td>
</tr>
<tr>
<td>&gt; 400</td>
<td>12/31(45)</td>
<td>14/31(45)</td>
</tr>
<tr>
<td>&gt; 600</td>
<td>11/27(41)</td>
<td>14/27(52)</td>
</tr>
<tr>
<td>&gt; 800</td>
<td>9/19(58)</td>
<td>11/19(58)</td>
</tr>
<tr>
<td>&gt; 1000</td>
<td>2/6(33)</td>
<td>4/6(67)</td>
</tr>
</tbody>
</table>

Evidence that a signal arising in the bowel mucosa has distant corporal effects. It has been suggested (Braegger, Corrigan & MacDonald, 1990) that TNF may be the mediator of clubbing.

(4) Anaphylaxis. Immunohistochemical examination of formalin-fixed caecal mucosal biopsies showed a 10-fold increase in the proportion of lamina propria cells with surface IgE in those children with intense trichuriasis, when compared with uninfected children with non-specific mucoid diarrhoea (Cooper *et al.* 1991). About 10% of the lamina propria cells were positive to the peroxidase-conjugated anti-human IgE monoclonal antibody; the expectation is that many of these cells will be mucosal mast cells. Direct staining for mast cells with Alcian blue on tissue fixed in Carnoy’s fluid (Strobel, Miller & Ferguson, 1981) has confirmed an increase in their numbers in trichuriasis. The cells in trichuriasis stained more lightly than in controls, however, and granules were also seen in the intercellular spaces. The implication is that these more abundant mast cells were also degranulating. The degranulation was corroborated by electron microscopy on rectal biopsies in trichuriasis.

On a series of 11 children with the *Trichuris* dysentery syndrome we took rectal biopsies and placed the tissue directly into ice-cold Tyrode’s buffer. This was a serial experiment, for we repeated the biopsies both 3 days after worm expulsion and upon complete recovery from the syndrome, which is for us the moment when we have established an estimate of height velocity accelerated above the normal value for age (‘catch-up growth’ – Prader, Tanner & von Harnack (1963)). Within an hour of the biopsy the tissue was weighed and then suspended in buffer at 37 °C. Spontaneous release of histamine, release after the addition of rabbit anti-human epsilon chain, release after the addition of *T. trichiura* excretory–secretory (ES) protein (final concentration 10 μg/ml) and, representing the total histamine in the sample, release after disrupting the cells by boiling the tissue in perchloric acid could all be assayed. Histamine was assayed by the double-radioisotope method (Cromwell, 1986).

As shown in Fig. 2, many of the biopsies taken at the time of infection and disease released much of their histamine spontaneously. The effect could also be induced by adding the ES protein, especially in the biopsies taken after recovery, when there was increased total histamine in the samples of mucosa and an apparent stabilization of the cells towards non-release of their granule-associated mediator. The evidence for considering much of the inflammation in *Trichuris* colitis as an instance of a local, tissue anaphylactic response is presented in Table 5. This form of inflammation, although it is mediated by specific antibody (IgE) (Lillywhite *et al.* 1991), represents a considerable pathophysiological cost to the host with no apparent benefit in compensation.
The adult nematodes are firmly attached and appear vigorous. These children had long histories of symptoms almost certainly due to their *Trichuris trichiura* infection. It may be that in the absence of T cell activation the effects of IgE and mediator release from mast cells are, as a defence mechanism, very much a second-best buy.

(5) Plasma protein loss and abnormal intestinal permeability. Recently, the leakage of protein across the mucosal surface of the gut in severe trichuriasis has been investigated. This was assessed as the clearance of plasma into stool of alpha,-antitrypsin — an abundant 54 kDa globulin which is resistant to degradation by trypsin in the bowel lumen. The total mass of alpha,-antitrypsin in stool passed over 24 h, and the concentration in the child’s plasma at the same time, are assayed in order to allow this calculation, ultimately expressed as the notional volume of plasma completely cleared of the protein by the gut over 24 h. The higher the clearance (ml plasma/24 h) the greater the leakage of the marker protein into the gut. It is assumed that all the other plasma proteins are leaking at a similar rate. This is a well-founded supposition: the gut mucosa is not an effective molecular sieve, and proteins of diverse molecular size tend to be lost together in protein-losing enteropathy. As Fig. 3 shows, it is typical for some 120 ml of plasma/day to be cleared by the gut. This represents about 7 g of protein. A typical daily dietary intake for a 10 kg, stunted Kingston child is 25–30 g protein (Walker, Powell & Grantham-McGregor, 1990), so the enteric loss is around one quarter of the intake.

The leakage of protein across the intestinal mucosa is not the only manifestation of the loss of mucosal integrity. We have also studied the permeability to the disaccharide lactulose, in the lactulose–rhamnose differential sugar absorption test (Sanderson et al. 1987). Normally, not more than 1 % of lactulose, a sugar that does not occur in the human gut naturally, is absorbed: it is excluded by the tight junctions of the enterocytes of the small intestine. Rhamnose, a monosaccharide, is by contrast absorbed rapidly through abundant aqueous pores in the cell membranes of the enterocytes, but it is not metabolized and, like lactulose, is excreted unchanged in the urine. The test involves the ingestion by the child of a hyperosmolar solution of these two sugars and their subsequent recovery in the urine. By deriving the lactulose–rhamnose ratio, rather than the absolute values, a number of factors common to the intestinal fate of both sugars are controlled.

The lactulose–rhamnose ratios of all 20 children with *Trichuris* dysentery syndrome studied were found to be abnormally high, and higher than Jamaican controls. Eighteen of these children were re-tested 6 weeks after treatment, when estimations of height velocity indicated that they were in a recovery growth-spurt. In every case, the lactulose–rhamnose ratio had diminished. They did not all reach the value of 0.05 ±0.02 suggested as the standard for West European/North Americans

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**Table 5. Evidence that inflammation in *Trichuris* colitis is a local, tissue anaphylactic response**

| 1 | Specific anti-*Trichuris trichiura* IgE in sera of infected people (immunoblot). |
| 2 | 10-fold increase in cells with membrane IgE in caecal lamina propria. |
| 3 | Increased numbers of large, granular cells staining with Alcan blue in subepithelial mucosa of caecum and rectum. |
| 4 | Paler staining cells; intercellular granules seen. |
| 5 | Electron microscopy shows degranulation in most mast cells. |
| 6 | Spontaneous histamine release from rectal biopsies diminishes and total histamine in tissue increases upon recovery from *Trichuris* dysentery syndrome. |
| 7 | Addition of small amounts of *T. trichiura* excretory-secretory protein provokes histamine release from rectal biopsies, in inverse proportion to the spontaneous release, and more so after recovery. |
| 8 | Distended, mucus-secreting goblet cells in normal numbers are seen histologically. |
| 9 | Intestinal anaphylaxis is a sufficient (although not a necessary) explanation for the mucus production, watery stool, plasma protein loss and increased intestinal permeability of the *Trichuris* dysentery syndrome. |
(Menzies et al. 1979); but they did become similar to those of the asymptomatic Jamaican children whom we had chosen as controls.

Those control children with abnormal ratios by European standards did not have increased lactulose absorption, but reduced rhamnose absorption. A similar finding has been reported from apparently healthy children in The Gambia (Behrens et al. 1987; Lunn, Northrop-Clewes & Downes, 1991). In our children with trichuriasis, it appeared to be the increased lactulose absorption that diminished on treatment, rather than the monosaccharide absorption that increased. This result is consistent with the findings of protein leakage from plasma, and suggests that the tight junctions between enterocytes in the small intestine are involved. The protein-losing enteropathy described above could be of small or large bowel origin, but the disaccharide permeability effect is unlikely to be in the large intestine (Lobley et al. 1990). If this work is confirmed, then an explanation for the effect of a large bowel parasite on small intestinal function must be sought. A circulatory factor may be involved. Mast cell or macrophage products could be implicated. Local anaesthesia in the small intestine of the rat produces watery diarrhoea (Forbes et al. 1988). TNF affects tight junctions (Mullin & Snock, 1990). In our patients, both protein loss and intestinal permeability are significantly correlated with worm burden.

The complete nutritional cost to the child associated with the loss of 5–10 g protein/day has not yet been assessed. The level of the gut at which it is occurring is relevant to this. If the loss is in the proximal small intestine, the nitrogen is likely to be salvaged, since the plasma proteins (other than those resistant to proteolysis such as our marker alpha,-antitrypsin) will be digested and the amino acids reabsorbed. The principal cost will then be one of energy, used in the resynthesis of the peptides. If the loss is more from the distal bowel, nitrogen will be irretrievably lost in the stool. This requires investigation in the future. Nitrogen may not be a limiting nutrient: micronutrient deficiency is generally associated with protein deficiency, especially that of zinc. Zinc deficiency is in turn associated with stunted growth (Prasad, 1983; Golden, 1988). Bundy (1986) reported an inverse correlation between intensity of T. trichiura infection and plasma zinc concentration in a community study; there are several possible interpretations of this simple association. It now requires more detailed investigation in the more intensely infected children.

(6) Cognitive function. It would be wrong to leave even the most preliminary look at the pathophysiological price of a chronic parasitosis without mentioning that important human organ, the brain. Chronic infection may depress cognitive function, with cumulative effects on the development of children's intellectual accomplishments. This is hypothetical at present, with several investigators now looking for impaired cognitive function, and the effects of anthelmintic treatment upon it, in the field. We await their findings. The subject holds the greatest potential of all for complementary work between field and laboratory, in view of the emerging knowledge of the effect of cytokines on the nervous system (Clark, Chaudri & Cowden, 1989).

CONCLUSIONS

The interaction between small mammals and the parasitic nematodes within their mucosal tissue, as exemplified by the rat and N. brasiliensis, and that between children and theirs, as exemplified by T. trichiura, differ in one fundamental respect: rats expel their worms and develop effective anamnestic responses to reinfestation. To do this, they pay a short-term price in acute illness, since the effector mechanisms for the immune response involve mucosal inflammation. Children mount an inflammatory response that is less effective but longer-lasting. There is good evidence of a correlation between the intensity of whipworm infection and the ill effects on the human host of the inflammatory response; but so far no evidence of a correlation between the extent of the inflammatory response and a tendency to self-cure. We may speculate that the human strategy is appropriate to a large and extremely ‘r-selected’ animal, if a more vigorous inflammatory response would increase the risk of death to the child; however, this is no more than speculation with current evidence.

What is clear is that there are a number of adverse consequences' (Gilman et al. 1983) to heavy whipworm infection, and that they are highly predictable at a given intensity of infection.

We suggest that a similarly systematic investigation of the pathology of hookworm infection in children, which somewhat lost its impetus around the 1970s, is now due for a revival.

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REFERENCES


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and its influence on albumin homeostasis in rats fed two levels of dietary protein. Clinical Science 70, 469–75.


Walker, S. P., Powell, C. A. & Grantham-McGregor,
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