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Hookworm Infections

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Pathology

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1. Introduction

Human hookworms are endemic to parts of the world where malnutrition is widespread and where infections with other species of parasites and micro-

organisms are extremely prevalent (Crompton, 1984). Consequently, the pathological sequelae of hookworm infection in field studies are not easily distinguishable from those attributable to concurrent malnutrition and/or heterologous infection (Banwell et al., 1967; Mayoral et al., 1967; Chuttani et al., 1968). To a certain extent histopathological and functional analyses on experimentally infected volunteers (Maxwell et al., 1987) and laboratory animals, notably with the canine species *A. caninum* and *A. ceylanicum* (Verma et al., 1968; Carroll et al., 1984b) have overcome this problem. However, canine and human studies have often differed markedly in their findings and the relevance of canine studies to human hookworm disease has been disputed (Migasena et al., 1972b).

A number of reports have been published since the classic review by Roche and Layrisse (1966), the major conclusions of which are still valid to this day. Subsequent papers by Miller (1968, 1979), Banwell and Schad (1978) and Gilman (1982) should also be consulted for an overview, but the subject as a whole has been relatively neglected in recent years and since it is readily apparent that experimental and clinical techniques have made major advances in the last decade, a concerted effort to apply the tools of modern experimental medicine is urgently required in order to re-evaluate and update our knowledge of the pathological consequences of hookworm infection.

2. The skin penetrating stages

Hookworms, depending on species, invade the host either percutaneously or orally. All three species affecting man and most other species probably have the capacity to penetrate skin, and with the exception of *N. americanus* (Yoshida et al., 1958; Nagahana et al., 1962; Komiya and Yasuraoka, 1966; Behnke et al., 1986), all readily establish after oral ingestion (Foster and Cross, 1934; Kendrick, 1934; Banwell and Schad, 1978). Some hookworm species, e.g., *A. tubaeforme*, penetrate the skin by entirely mechanical means (Matthews, 1972, 1975), but *N. americanus* and *A. caninum* secrete proteolytic enzymes which presumably aid in penetration (Matthews, 1982; Hotez et al., 1985; Lewert and Lee, 1954). The position of *A. duodenale*, *A. braziliense* and *A. ceylanicum* remains equivocal in this context (Matthews, 1977), although all species are likely to have proteolytic enzymes in some measure, because exsheathment is dependent on enzyme mediated changes to the sheath (Gamble et al., 1989).

The infective larvae of *A. ceylanicum*, *A. caninum* and *N. americanus* exsheath before penetrating host skin, but a proportion of *A. tubaeforme*

larvae do not lose their sheaths until they are situated below the epidermis (Matthews, 1975, 1977). Penetration commences by the location of a potential entry site and orientation parallel to the skin surface, followed by entry into the *stratum corneum* (Matthews, 1977), or by entry into hair follicles and passage through the associated glands into the deeper layers of the skin. However, what follows is dependent on the species of hookworm and the host being subjected to invasion (Norris, 1971).

2.1. Ancylostoma caninum

Mouse skin is penetrated rapidly by *A. caninum*. Larvae were observed in the murine epidermis after 5 min of exposure, with the majority completing passage through the skin within 2–3 hours (Banerjee et al., 1970). Dog skin is penetrated mainly via the hair follicle system, with a longer period, 2–6 hours, required for complete passage (Vetter and Leegwater-v.d. Linden, 1977c), but larvae may be recovered from the lungs, trachea and even small intestine within 24 hours of infection (Schwartz and Alicata, 1934b). Rat skin poses more of a problem, as up to 35% of the inoculum may still reside in the skin 12 hours after infection with some larvae persisting for up to 3 days (Norris, 1971; Matsusaki, 1951). Mice and rats being abnormal hosts, the worms subsequently migrate to muscle sites throughout the body and do not follow the usual migratory pathway via the lungs to the intestine (Soh, 1958; Nichols, 1956; Matsusaki, 1951). The passage of *A. caninum* through mouse skin does not appear to be accompanied by inflammation and the sites of entry heal rapidly, leaving no evidence of breached continuity to the skin surface (Banerjee et al., 1970). *A. caninum* larvae secrete proteolytic enzymes during invasion and these may be responsible for the disappearance of the basement membrane at the site of penetration (Lewert and Lee, 1954).

Whilst infection of dogs and rodents with *A. caninum* appears to create little disturbance to the skin, infections in man may result in quite profound changes. Within 15 min of exposure, a prickling sensation is felt and is followed by the appearance of red circular macules indicating points of entry and a widespread erythematous reaction with increasing itching at the site of penetration (White and Dove, 1929; Hunter and Worth, 1945). Urticaria-like swellings develop into papular lesions, but these symptoms generally subside after 48 hours. Although creeping eruptions are not usually attributed to *A. caninum*, Hunter and White (1945) reported a case of extensive cutaneous larva migrans in a volunteer who responded severely to experimental infection. The subject was suspected of sustaining bacterial cellulitis presumably from bacteria contaminating the inoculum, and his exposed limb became swollen and inflamed from wrist to elbow. The site of penetration

was marked by urticarial lesions and papules which persisted for several months. Discontinuous burrows were evident in the skin, possibly as a consequence of the larvae penetrating deeper into the tissues and returning to the surface at a point distant from the entry site. Such creeping eruptions reappeared up to 200 days following infection, reflecting the inability of *A. caninum* larvae to follow the normal migration route in man (see also review by Beaver, 1956b).

2.2. Ancylostoma tubaeforme

The infective larvae of *A. tubaeforme* reside in the skin of rodents for a longer time, most worms leaving the site 12–24 hours after infection, but some persisting for 3 days in rats and 10 days in mice (Norris, 1971). In comparison to orally administered larvae, few successfully entered rodent skin, possibly because of the absence of penetration assisting enzymes in this species. Matthews (1975) concluded that *A. tubaeforme* penetrated cat skin (the normal host) by entirely mechanical means and relatively quickly. Hence, differences in structural qualities between cat and rodent skins and the absence of proteolytic enzymes to assist in penetration, may combine to present particular difficulties to larvae when attempting to enter rodents. On exposure to cat skin, larvae were found in the *stratum corneum* after 5 min and entry into the dermis was observed within 1 h. In this system *A. tubaeforme* did not cause extensive damage to the skin, there being no evidence of penetration tunnels or epidermal cellular destruction during passage through to the dermis.

2.3. Ancylostoma braziliense and A. ceylanicum

These two species are considered together because until the morphological criteria for separate identity were clarified by Biocca (1951) and Yoshida (1971) they were regarded as synonymous. In consequence, the precise identity of the organisms used in earlier studies is uncertain (Beaver, 1956a). It is currently recognized that *A. ceylanicum* may develop to maturity in man, but that *A. braziliense* does not have the capacity to do so (Haydon and Bearup, 1963; Arrekul et al., 1970; Yoshida et al., 1971). Instead the latter species is mainly responsible for human cutaneous larva migrans or creeping eruptions (Beaver, 1964), wandering extensively through the cutaneous layers and surviving for over 3 months after entry (Kirby-Smith et al., 1926).

In rodents and monkeys *A. braziliense* does not produce creeping eruptions reminiscent of those in humans, but the site of penetration may be marked by a severe cutaneous response resulting in chronic lesions (Dove,

1932; Norris, 1971). Larvae enter rat and guinea-pig skin within 2 hours of application, but many seem unable to complete the penetration process and persist for a considerable time. In the first 3 days of infection parasites may be detected in all levels of the skin, but concentrate in the epidermis and in the follicles and glands. Hamster and guinea-pig skin is usually clear by day 6, but large numbers of larvae persist in rat skin for over 2 weeks (Norris, 1971). These observations suggest either that *A. braziliense* does not have penetration enzymes or, if enzymes are present, that these are inappropriate for passing through the basement membrane below the *stratum germinativum* into the rodent dermis.

Penetration and persistence of *A. braziliense* in rodent skin is accompanied by marked inflammatory changes. Norris (1971) found inflamed follicles in erythematous infection sites with neutrophilic and lymphocytic infiltration of the dermal tissues. An acute response followed in rats and guinea-pigs leading to necrosis of follicles, granulomata and abscess formation. Dead and degenerating larvae were reported from these locations. An intense erythema developed at the inoculation site, and by day 14 the follicles were destroyed and replaced by areas of fibrosis. By week 4 fibroplasia was extensive in hamsters and guinea-pigs, but less so in rats and nude areas appeared where the skin had been entirely replaced. Such intense reactions to hookworm infection have only been observed in this species and may be a reflection of persistent antigenic stimulation and/or a response to damage brought about by the parasites.

In contrast, the skin of dogs is penetrated within 0–30 min of larval application, most organisms completing passage by 6 h. This is faster when compared with both *A. ceylanicum* and *A. caninum* (Vetter and Leegwater-v.d. Linden, 1977c). Entry into the dermis is probably mainly through the hair follicles which provide a route to the dermis via sweat glands and to the hypodermis via the apocrine glands. Furthermore direct penetration of the dermis from the epidermis is indicated by tunnels evident on histological sections, linking the two layers (Vetter and Leegwater-v.d. Linden, 1977a,c), a feature not observed when *A. caninum* or *A. ceylanicum* were placed on canine skin. Both of the latter species were seen to congregate in hair follicles and probably exploit the routes provided for passage into the deeper skin layers. Interestingly, when the hairless metacarpal foot pads of dogs were exposed to infection with *A. braziliense*, larvae failed to pass below the epidermis and made no attempts to enter eccrine glands. Adjacent areas which had hair were successfully invaded as described above (Vetter and Leegwater-v.d. Linden, 1977b).

Human skin proves to be a particular problem for *A. braziliense,* the larvae seemingly unable to gain access to the dermis as in dogs. Hence, exposure to human skin is generally, but not invariably, followed by the

classic symptoms of creeping eruptions, which were most vividly described by Sandground (1939) in a case of self infection. A few days after infection the migration began

> "Disappearing occasionally for a few days at a time, but always re-appearing, a single elevated, slightly reddened and oedematous burrow extended itself along a tortuous line at a rate of rather less than 1 centimetre a day."

Eventually the parasite was recovered 55 days after infection and was located within the mucous layer of the epidermis just beneath the *stratum granulosum*, surrounded by eosinophils. The evidence for *A. braziliense* as the principal causative organism of creeping eruptions has been critically reviewed by Beaver (1956b), and the reader is also referred to earlier papers by Dove (1932) and Shelmire (1928) in this context.

The symptoms associated with the penetration of human skin by *A. ceylanicum* are very similar to those described for *A. caninum* and usually equally short lived. Dove (1928) infected volunteers with *A. braziliense*, but since the larvae used were obtained from the stools of a child with a patent infection, it is more likely that the parasite was *A. ceylanicum*. The forearm of the exposed individuals became swollen and edematous. The infection site was marked by urticaria-like lesions and pruritus, but the symptoms receded within 3 days. Areekul et al. (1970) observed nodules up to 2–4 mm in diameter, followed by urticarial lesions which conglomerated into larger blebs and ruptured 4–5 days after infection. Some patients, however, have more persistent skin lesions and these may last for a month (Wijers and Smit, 1966). Dove (1928) observed short (2 cm) tracks marked by rows of pinhead-sized vesicles leading away from the point of exposure before disappearing, presumably as the larvae entered deeper tissues. Comparable signs were reported by Wijers and Smit (1966) and Haydon and Bearup (1963), but neither Areekul et al. (1970) nor Yoshida et al. (1971) found evidence of tracks in the skin which could be assigned to migrating larvae. Carroll and Grove (1986), who used a clean preparation of infective larvae, laced with antibiotics, did not experience any skin reactions in response to penetration by 1200 larvae applied to the inner surface of the forearm. The tortuous tunnels characteristically seen after exposure to *A. braziliense* are therefore not a feature of *A. ceylanicum* infection.

2.4. Ancylostoma duodenale and Necator americanus

'Ground itch' or 'Koi Kabure' in Japan (Matsusaki, 1966), a dermatitis commonly encountered among agricultural workers in the tropics, has long been recognised as a sign of exposure to the penetrating larval stages of parasites, such as the two anthropophilic hookworm species and where contact with

water has been involved, schistosome cercariae. Loos (1911) first demonstrated that the larvae pass through human skin to cause patent infections, but to date the histological consequences of skin penetration in man have not been thoroughly investigated. Bentley (1902) reported that exposure to *A. duodenale* was followed by a dermatitis, characterized by itching and papulovesicular eruptions. Mild local dermatitis, involving local pruritus, erythema and general discomfort, has been reported by several authors both in relation to experimental infections (both species, Yoshida et al., 1958; Maplestone, 1933; *N. americanus*, Cline et al., 1984; Maxwell et al., 1987) and following treatment of polycythemia vera (*A. duodenale*, Nagaty and Zanaty, 1949; Myhre and Wallace, 1956). These symptoms are usually short lived, although punctate hemorrhagic penetration sites may persist for a few days (Cline et al., 1984). On occasion both species may cause limited 'creeping eruptions', with shorter tracks than *A. braziliense*, symptoms disappearing within 2 weeks of infection (Maplestone, 1933; Beaver, 1945, 1956b). However, longer-lasting effects have also been described as for example by Nawalinski and Schad (1974) who found that within hours of exposure to *A. duodenale* macules developed at the infection site. These subsequently became pigmented and lasted for several months. Few of these symptoms have been examined at the histological level but, based on skin biopsies taken 5 days after experimental exposure to *N. americanus*, a moderate perivascular chronic inflammatory infiltrate, consisting predominantly of lymphocytes, but with occasional eosinophils, has been described (Maxwell et al., 1987).

The larvae of *N. americanus* are known to contain and secrete enzymes during penetration, but no comparable information is available for *A. duodenale*. *N. americanus* leaves behind penetration tunnels and causes the destruction of epidermal cells. The basal lamella underlying the *stratum germinativum*, however, may present a particular barrier to penetrating larvae. Once passage into the dermis is achieved, either by by-passing the basal lamella via hair follicle systems or by direct penetration as in *A. braziliense*, movement is considerably easier in the relatively soft gel-like matrix (Matthews, 1977). This obstacle may account for the persistence of the larvae of *N. americanus* at the skin site for up to 1–2 days post exposure. Third stage larvae have been observed in and recovered from skin during the 48 hour period following percutaneous infection in hamsters (Behnke et al., 1986; Nagahama et al., 1963), guinea-pigs (Nagahama et al., 1963), mice (Wells and Behnke, 1988) and pups (Tanake, 1962; Ishikawa, 1966), but again there is no comparable information on *A. duodenale*. The significance of the two day residence by *N. americanus* at the site of penetration is not understood, but it is conceivable that the worms replenish essential enzymes which may have been exhausted during entry of the skin and/or undergo

other changes (perhaps in surface structure) in preparation for movement into the blood and lungs. In support, a temporary reduction in activity has been reported in larvae isolated from dog skin 24 hours post infection (Ishikawa, 1966).

2.5. General observations

The intensity and variability of the cutaneous response to larval penetration may be influenced by the extent to which the larvae are contaminated by bacteria and other microorganisms, as well as prior exposure to hookworms. The eggs and free-living larval stages of hookworms are derived from fecal material on which they feed. Undoubtedly some of these may be responsible for the host reactions in human volunteers (Hunter and Wade, 1945) and variation in bacterial contamination from one study to another may account for some of the differences reported. Larvae which are carefully cleaned by treatment with antibiotics, at least those of *A. ceylanicum*, do not cause symptoms during penetration (Carroll and Grove, 1986).

Repeated exposure to infection leads to a progressively increasing likelihood that larvae (*N. americanus*) may cause creeping eruptions rather than migrate directly to the lungs (Ball and Bartlett, 1969; Beaver, 1945). Cutaneous reactions to hookworm invasion in man probably have little if any host-protective value, at most slowing down larval migration, but not preventing establishment and subsequent development of either adult *N. americanus* (Ball and Bartlett, 1969; Ogilvie et al., 1978) or *A. duodenale* (Nagaty and Zanaty, 1949) in the host intestine.

In contrast there is evidence that infections in rodents are resisted during skin penetration. Mice which have experienced a primary infection with *N. americanus* react to challenge with an intense erythematous response at the site of larval application and fewer worms reach the lungs (Wells and Behnke, 1988b). This is not entirely surprising, because assuming that hookworms utilize evasive strategies for escaping from entrapment by local cellular responses, the anthropophilic species would be expected to show evasive mechanisms highly tuned to their human hosts, and in abnormal hosts such as mice, the balance between entrapment and evasion may be biased in favour of the host. Indeed differences between murine and human immunoregulatory pathways have been recognized and caution against wholesale application of murine studies to humans has been emphasized (Calland and Turner, 1990). Nevertheless, murine studies will help to identify the spectrum of host-protective responses against the invasive larvae of hookworms and with increasing awareness of the intricate immunoregulatory pathways in man, it may be possible in future years to manipulate the human immune system to resist infection despite the parasite's evasive defences.

Cutaneous retention of *A. caninum* has been described in laboratory and cotton rats, in which some larvae become encapsulated in the skin (Lindquist, 1952), but these observations contrast with the report by Banerjee et al. (1970) and Stumberg (1932) and therefore a re-examination of the factors involved is required to resolve the controversy. As was pointed out earlier, *A. braziliense* larvae in rodents initiate a vigorous cutaneous response and may be killed (Norris, 1971). However, it must also be borne in mind that the demonstration of dead or disintegrating larvae, surrounded by host cells in the skin, need not necessarily indicate that an effective skin response has occurred, unless the observations are based on a quantitative comparison with controls (immunosuppressed or naive hosts). A proportion of the larvae would be expected to exhaust energy reserves during penetration and it may be that cellular foci develop around such worms. The host-protective role of skin resistance can only be effectively evaluated by a quantitative assessment of parasite numbers succeeding in migrating to the next organ site or via a quantitative measure of parasites left behind during skin invasion. It is difficult to envisage how this could be achieved other than through radiolabelling experiments such as those carried out to study the migration kinetics of *S. mansoni* in naive and vaccinated hosts (Crabtree and Wilson, 1986a; Dean et al., 1984; Chandiwana, 1988).

The relationships between the pathology and damage created directly by hookworms, their contaminating microorganisms and possible host responses are not understood and much remains to be accomplished in this area. There is a growing interest in the mechanisms of host resistance operating in the skin, especially in the capacity of the skin to mount specific immune responses (Bos and Kapsenberg, 1986). The use of hookworm larvae with their characteristically distinct patterns of behaviour during invasion and penetration, may prove to be useful in unravelling some of its components.

3. The pulmonary stages

After penetrating the skin, hookworm larvae migrate to the lungs and the accompanying respiratory symptoms have been long recognized as indicative of larval migration, although to date they remain poorly investigated. Wakana disease in Japan is associated with the ingestion of fresh vegetables and presumably their accompanying larval contaminants (Harada, 1962; Matsusaki, 1966). Gross respiratory distress is most likely to ensue when a person has been exposed to a large dose of larvae simultaneously (Muhleisen, 1953) or when heavy hookworm infection is compounded by concurrent respiratory disease of viral/bacterial origin (Zimmerman, 1946). In the

more normal course of events, involving contact with small to moderate numbers of larvae per day, respiratory symptoms are mild and probably indistinguishable from those of common viral infections and complications are rare.

3.1. Larval development and gross pathology

Experiments in pups, guinea-pigs and neonatal hamsters infected with *N. americanus* have shown that worms first appear in the lungs 36–48 hours after infection (Tanabe, 1962; Nagahama et al., 1963; Behnke et al., 1986a) in parallel with their disappearance from the skin (Fig. 1). The sudden arrival of larvae in the lungs provokes extensive edema which in mice is seen to fill up to 50% of the alveolar volume (Wilkinson et al., 1991) (Fig. 2). The parasites grow in the lungs, developing into advanced third stage larvae by days 6–7 and, although the L3/L4 moult does not occur in the lungs, by day 7 most larvae have clearly identifiable L4 features within the L3 cuticular sheath. During this period, most of the edema resolves, but extensive damage is caused in the lungs by an inflammatory infiltrate which begins as a peribronchiolar cuffing and amplifies to encompass 25% of the parenchymal tissue (Fig. 2). In experimentally infected mice, hamsters and guinea-pigs, they cause petechial and ecchymotic areas on the lung surface (Schwartz and Alicata; Wilkinson et al., 1991). Human infections are characterized by bronchitis and transient bronchopneumonia (Kalmon, 1954; Muhleisen, 1953) which on intense exposure may be sufficiently severe to cause death, but this has not been proven and is often difficult to dissociate from other compounding respiratory infections, especially under field conditions (Zimmerman, 1946). However, the lungs are resilient organs, at least in rodents, and normally, within a week of the larvae migrating to the intestine, there is a marked improvement in the general appearance. The histopathology of the pulmonary stages of human hookworm infection still remains one of the most poorly documented aspects of hookworm disease.

3.2. Pulmonary cellular responses

Lung lavage in mice infected with *N. americanus* (Brain and Frank, 1973; Egwang et al., 1984) has revealed that the response to infection includes infiltration by all the major types of leukocytic cell. The recovery of eosinophils and neutrophils increased by 1000- and 250-fold, respectively, in NIH mice by day 13 (see Chapter 5), in agreement with the reported increase in eosinophils in human lung lavage fluid (identified in 1 out of 5 patients) following a low intensity experimental exposure to *N. americanus* (Maxwell et al., 1987). Some worms were killed in the mouse lung, particularly during

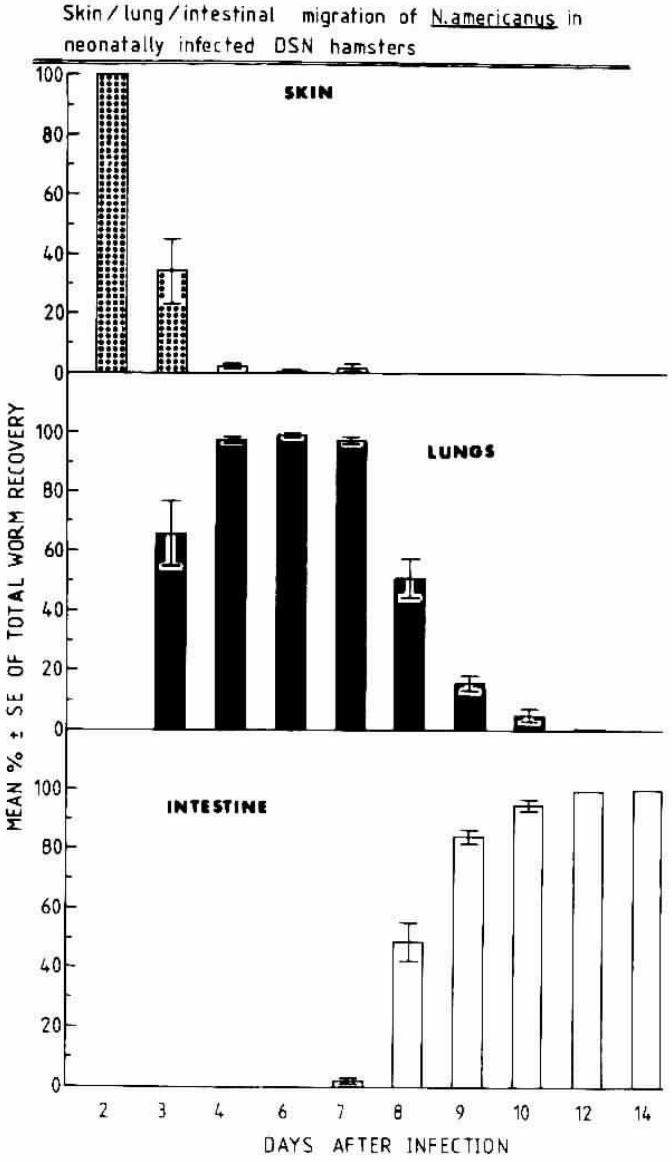


Fig. 1. Migration of *Necator americanus* through the skin and lungs to the intestine of hamsters. The figure shows the mean percentage of larvae recovered from the three tissue sites on various days after exposure of neonatal hamsters to infection with 120 L3 (reproduced from Behnke et al., 1986).

secondary exposure and disintegrating parasites were observed on squashed lung tissue preparations examined by light microscopy as well as on tissue sections (Fig. 3). Comparable human studies are rare, but D'Abrera (1958) reported disintegrating larvae of *N. americanus* surrounded by macrophages and giant cells in pulmonary granulomata from post mortem examinations of patients with tropical eosinophilia, a finding consistent with the known capacity of alveolar macrophages to immobilize and kill parasitic nematode

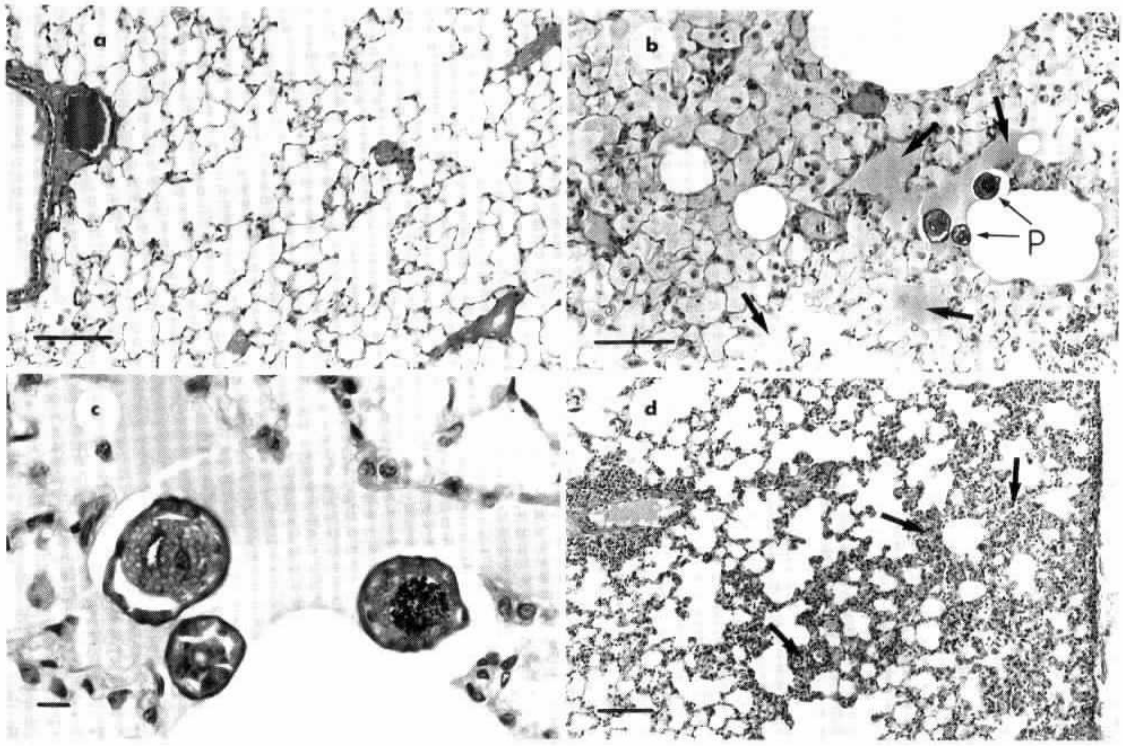


Fig. 2. Histopathological changes in the lungs of mice 5 and 9 days after primary infection with *N. americanus*. (Reproduced from Wilkinson et al., 1990). (a) Normal lung parenchyma, bar =100 μ. (b) Lung parenchyma in mice on day 5 after exposure to 350 larvae. Extensive edema is evident (bold arrows) and sections of parasites can be seen in the small airways (fine arrows and P) with relatively moderate cellular infiltration, bar = 100 μ. (c) Higher magnification of part of 2b, showing more detail of larvae and surrounding tissues. There is no indication of a local response to the parasites, bar = 10 μ. (d) Day 9 after primary infection showing the more intense parenchymal infiltrate (arrows) observed in the later stages of infection and the absence of edema, bar = 100 μ.

larvae (Egwang et al., 1984b). Thus the mouse model may well prove to be relevant for investigating further lung pathology associated with *N. americanus*.

3.3. Migration to the intestine

The mouse intestine does not provide an environment entirely suitable for the establishment of *N. americanus* larvae migrating from the lungs. Only a few poorly developed L4 managed to survive until 2–3 weeks post infection (Wells and Behnke, 1988a). It is conceivable that in the mouse, a proportion of worms is coughed out during passage through the upper respiratory tract and mouth (compare with mice infected with *S. mansoni*, Crabtree and Wilson, 1986b), rather than following the normal migration route. Others may fail to establish in the intestine because of damage incurred during

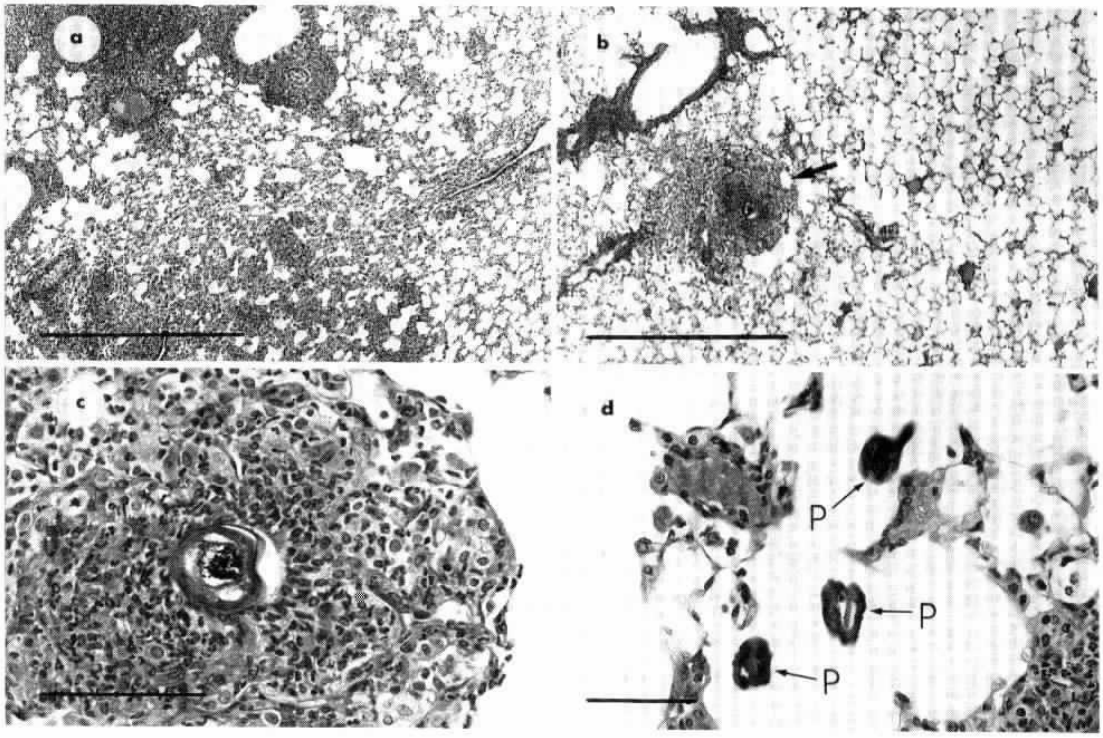


Fig. 3. Histopathological changes in the lungs of mice 5 and 9 days after secondary infection with *N. americanus* (Reproduced from Wilkinson et al., 1990.) (a) Extensive inflammation in the lung parenchyma 5 days after challenge infection, bar = 1000 μ. (b) Inflamed lung parenchyma, peri-bronchiolar infiltration of leukocytes and a granulomatous reaction surrounding a larvae 9 days after secondary infection (bold arrow), bar = 1000 μ. (c) Higher magnification of a section from 3b showing details of the cellular reaction around the parasites, bar = 100 μ. (d) Section of lungs on day 9 after secondary exposure showing parasites free in the bronchioles (fine arrows and P), bar = 100 μ.

residence in the lungs or simply because the physico-chemical environment is inappropriate for their subsequent development. In the former context larvae were recovered in the sputum of a patient who was probably infected with *A. caninum* or *A. braziliense* and in whom the infection, as might be expected with these canine species, never became patent (Muhleisen, 1953).

In contrast to *Necator*, the *Ancylostoma* spp. are not considered to require a period of development in the lungs and on oral ingestion the parasites stay in the intestine. Following skin penetration *Ancylostoma* spp. probably only use the lungs as a convenient staging post on the route to the gut but nevertheless, evoke temporary pulmonary distress during this passage (Yoshida et al., 1958). However, debris suggestive of larval nematode sheaths and hence moulting, or more likely entrapment by pulmonary reactions, was observed in the alveoli and capillaries of the alveolar walls on post-mortem examination of children who had died of heavy hookworm (*A. duodenale*) infection on Guam (Zimmerman, 1946).

Both genera cause irritation during their passage up the trachea and through the throat; tracheitis being a commonly reported symptom often evident in the week following experimental exposure to infective larvae (Nagaty and Zanaty, 1949; Areekul et al., 1970; Matsusaki, 1966) and occasionally persisting until week 3 (Cline et al., 1984).

3.4. General observations

Recent studies have shown that the regulation of protective responses in the lungs is extremely complex, in some way paralleling the intestinal immune system, with equally complex immunoregulatory circuits, reflecting the need to prevent the host from responding unnecessarily to inhaled non-pathogenic materials (Sedgewick and Holt, 1985; Galvin et al., 1986; Danielle, 1988). The regulation of immune responses to hookworm larvae in the lungs including the specificity of elicited reactions, the associated histopathology and the relationship of these processes to subsequent intestinal infections are subjects about which virtually nothing is known at present. However, the development of rodent models provides an excellent opportunity for exciting research in this area.

4. The intestinal stages

4.1. Location within the intestine

The distribution of *A. duodenale* was originally studied by Leichtenstern (1887) whose data was derived from human postmortem examinations and subsequently confirmed by Rep (1975) and Setasuban and Dangsupa, (1981). Much of the anterior section of the small intestine is ground for both species, but *Necator* generally occupies a more anterior section of the gut, principally in the duodenum whereas *A. duodenale* locates slightly further down the intestine with a peak intensity just below the average position for *Necator* and a tail which stretches well into the jejunum (Fig. 4).

There is ample evidence for the jejunum as the preferred site for *A. caninum* in dogs (Nishi, 1936; Yazima and Machida, 1958; Krup, 1961) although in heavy infections worms are distributed throughout the small intestine and even attach in the colon (Migasena et al., 1972). A similar pattern is found in *A. ceylanicum* with the majority of worms in light infections attaching in the anterior half of the gut (Carroll and Grove, 1984). In heavy infections significant numbers may be found in the ileum (Rep, 1966) and in the colon (Carroll and Grove, 1984). As the infection progresses, the more anteriorly attached worms persist and there is some evidence of a general movement

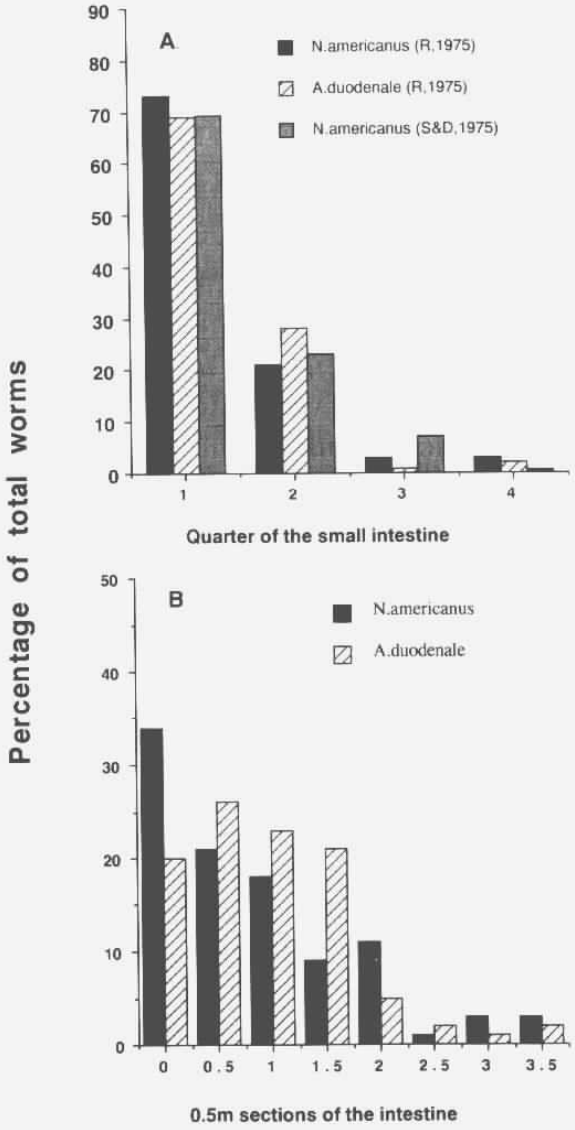


Fig. 4. Distribution of *N. americanus* and *A. duodenale* along the human intestine, as seen following post mortem examination. (A) Data from Rep (1975) and Setasuban and Dangsupa (1981) expressed in terms of the percentage of each species in successive quarters of the small intestine. (B) Data from Rep (1975) giving more precise detail of the distribution in the anterior sections of the small intestine.

towards the duodenum (Rep, 1966). In contrast, *Uncinaria stenocephala* preferentially establishes in the second half of the canine small intestine with a peak density of worms at the junction of the third and fourth quarters (Walker and Jacobs, 1985). *A. ceylanicum* and *N. americanus* both reside throughout the length of the hamster intestine, but the majority locate in the jejunum between 3 and 22 cm from the stomach (Rajasekariah et al., 1985).

4.2. Feeding by adult worms

There has been considerable debate about the method by which hookworms feed, how much blood is taken by individual parasites, and the consequences to the host (Rep, 1980). Hookworms bite into the mucosa, sometimes penetrating deeply into the submucosa (Bonne, 1942; Verma et al., 1968), but there may be important differences in the extent of penetration and the resulting pathology. Bonne (1942) reported that *A. duodenale* often penetrated deeper into the mucosa than *N. americanus*, a finding supported subsequently by Zimmerman (1946) who found the former penetrating well into the submucosa and consistent with the observation that *A. duodenale* is responsible for greater blood loss. Histological sections of dog intestines infected with *A. ceylanicum* show plugs of mucosal tissue enclosed within the parasite's buccal capsule (Carroll et al., 1984b) and many comparable pictures have been published over the years for the human parasites (Zimmerman, 1946). Similarly *A. caninum* can be frequently encountered with the head deep in the canine submucosa. Despite some evidence of feeding tracks extending into the submucosa (Dow et al., 1959), *U. stenocephala* is considered to browse on villus tips and down to the crypt openings, but rarely any deeper (Gibbs, 1958). These differences reflect niche segregation, presumably as a consequence of selection pressure to minimize competition between sympatric species and, given the overall similarities between the organisms, would be expected on ecological and evolutionary considerations (Schad, 1963; Kennedy, 1984; Simberloff, 1990).

Hookworms suck powerfully (Wells, 1931; Roche and Layrisse, 1966), so that mucosal tissue becomes detached from the intestinal layers and underlying capillaries are ruptured. Blood and mucosal cells are drawn into the parasite's gut, but opinions have varied over whether either of these are essential to the worm's metabolism. Some studies have reported apparently intact erythrocytes passing through worms unharmed (*A. caninum*, Wang et al., 1966; Wells, 1931; Zimmerman, 1946), but hookworms are known to secrete proteolytic enzymes and hemolysins and in a recent study of *A. ceylanicum* in dogs Carroll et al. (1984b) reported erythrocytes and mucosal cells in varying degrees of lysis and degradation within the parasite's intestine. Hookworms will draw into their buccal cavity materials other than blood when presented with these in vitro (Roche and Layrisse, 1966) and the evidence on balance indicates that access to the capillaries is achieved incidentally through the internalization of mucosal tissues. Blood is lost both through the continued sucking by the worms, through subsequent leakage from the lesions left behind when the worms move to a fresh site and through changes in intestinal permeability (Walker and Jacobs, 1985). The exact amount lost through each route will depend on the species of worm, the

depth of penetration into the mucosa during feeding, the intensity and duration of infection, concurrent heterologous infection, diet and the state of health of the host. Mucosal tissues, however, cannot be the only food resource for some species because in vitro, serum components appear to be essential for glucose metabolism of *A. caninum* (Fernando and Wong, 1964) and for longterm survival of *A. caninum* and *A. duodenale* (Komiya et al., 1956; Yasuraoka et al., 1960).

4.3. The consequences to the host of hookworm feeding activities

4.3.1. Blood loss and anemia

Various workers have attempted to quantify blood loss by hookworms. The data were reviewed by Roche and Layrisse (1966) who suggested that a realistic figure for *N. americanus* was 0.03 ml, *A. caninum*, 0.05 ml and *A. duodenale* 0.15 ml/worm per day (See also Rep, 1980). In heavily infected hookworm patients, therefore, total blood loss may be as high as 100 ml/day. It is not intended here to add to the debate on the exact quantity of blood loss caused by hookworms. Clearly a very complex set of interactions is involved with species of hookworm being of primary consideration, but influenced by a range of variables as outlined above. Since egg counts are the only readily measurable parameter of the intensity of infection, a rough guide was calculated relating blood loss to EPG (eggs per gram of feces). Venezuelans infected purely with *N. americanus* had an average daily blood loss of 2.1 ml/1000 EPG, whereas in Egyptians with *A. duodenale* the figure was 4.47 ± 1.16 ml/1000 EPG.

Opinions have varied over the relative importance of routes through which blood may be lost. At autopsy or operation, punctate or occasionally coalescent areas of hemorrhage are found around sites of attachment (Edington and Gilles, 1969), and hookworms (*A. caninum*) are believed to change feeding sites regularly (Kalkofen, 1970), leaving behind each time ruptured capillaries from which blood would escape to the intestinal lumen. Although Roche and Layrisse (1966) concluded that blood lost in this way was unlikely to represent a significant proportion of total blood loss, the controversy has been reawakened by recent studies of *A. ceylanicum* in dogs. Carroll et al. (1984b) concluded that a considerable proportion of blood lost from the mucosa originated from tissues around the parasites, from areas of ulceration of mucosa adjacent to the worms. Blood vessels prone to rupture were exposed in the bases of the ulcers and erythrocytes were present between the host tissues and the parasites (See also Fig. 8g). Although the authors did not quantify blood lost through the two routes, their observations suggest that there may be species-specific differences between hookworms in this respect. Taking the canine species as an exam-

ple, it is clear that *A. caninum* (Miller, 1966b) causes the most severe blood loss, *A. ceylanicum* moderate (Arrekul et al., 1975; Rep et al., 1971) and that by comparison *A. braziliense* (Miller, 1966a) and *Uncinaria stenocephala* cause almost negligible loss (Miller, 1968). These differences among canine species are most likely accounted for by niche diversification as explained earlier and reflect distinct patterns of feeding on mucosal tissues. Likewise, the contrasting blood loss caused by the two human hookworm species is attributable to dissimilar feeding strategies.

Anemia, has long been recognized as a possible indication of hookworm infection. There is strong evidence for a direct correlation between the intensity of infection and the severity of anemia (Fig. 5), although field studies of individuals subject to a range of additional variables, including diet, have not always successfully demonstrated this relationship. In light infections the detrimental effects are partially offset by reabsorption and reutilization of iron after passage through the parasite. Roche and Perez-Gimenez (1959) concluded that on average 44.1% of the hemoglobin iron lost during *N. americanus* infection was subsequently reabsorbed (range 13.1–76.4%). On the basis of their work with hookworm-infected Venezuelans, Roche and Layrisse calculated that in an adult carrying 700 *N.*

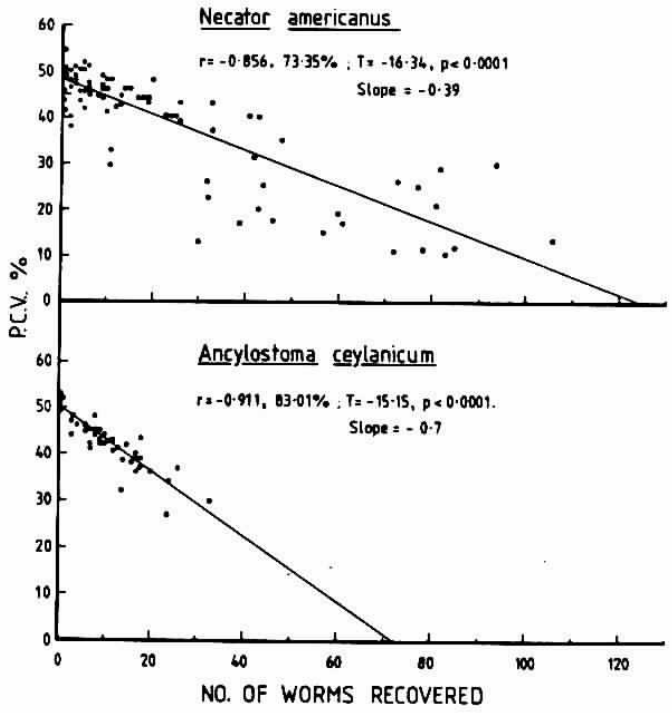


Fig. 5. Comparison of the effect of worm density on PCV of hamsters infected with *N. americanus* and *A. ceylanicum*. Animals with *N. americanus* were killed 35–36 days after infection. Those given *A. ceylanicum* were killed 4–6 weeks after infection. (Reproduced from Behnke, 1990 by kind permission of Taylor and Francis).

americanus and losing about 45 ml of blood per day, hemoglobin levels would fall only after 220 days of infection when the pool of stored reserve iron would become depleted. A new plateau in circulating hemoglobin levels would be achieved some 800–1000 days after infection as the iron balance approached zero. Inevitably this course of events would be accelerated in communities with an iron-deficient diet, and anemia would be precipitated sooner.

4.3.2. Loss of blood protein and albumin

Besides erythrocytes, serum is also withdrawn from the patient by hookworms and lost from the feeding site. Protein losing enteropathy and hypoalbuminemia are well-documented in the tropics and are partially linked to hookworm infection (Roche and Layrisse, 1966; Variyam and Banwell, 1982; Gilles et al., 1964). In patients with moderate to heavy infections with *N. americanus*, fecal albumin corresponded to a value of 0.1 g/100 worms and this was equivalent to loss of 3 ml of plasma/100 worms, a figure in agreement with blood loss estimates using other techniques (Blackman et al., 1965). Patients with mild infections compensated by reabsorption and overall protein loss in such cases may be minimal (Vieira, 1975b). Gupta et al. (1974) using the ^{51}Cr-albumin technique found only one subject, among 11 studied, with protein loss above the upper limits of normal.

There is good experimental evidence for protein losing enteropathy in canine infections. Studies on *A. caninum* (Vieira, 1975a,b; Miller, 1971) and *U. stenocephala* (Walker, 1980; Walker and Jacobs, 1985) have established that protein loss from the intestine is enhanced during hookworm infection but in the latter species, which does not cause marked blood loss, may be confined to the first two weeks after infection (Miller, 1971; Walker, 1980). This temporal relationship, marked differences between species of hookworms and the need for moderate/heavy worm burdens to elicit significant protein loss in animal models explain the inter-study variation on naturally infected humans.

4.4. The effect of hookworms on intestinal structure and function

The small intestine is critically important to the host as the principal site for the uptake of the breakdown products of digestion. However, intestinal tissue has a relatively fast rate of turnover, lesions are rapidly repaired and the organ has enormous reserves in compensating for damage and loss of functional integrity (Castro et al., 1990). Not surprisingly, hookworms have been suspected as causing intestinal disorders, abdominal pain and discomfort being regularly reported by people with hookworms (Sheehy et al., 1962; Chaudhuri and Daha, 1964) and diarrhea a common occurrence, par-

ticularly in experimental human infections (Myhre and Wallace, 1956; Nagaty and Zanaty, 1949; Yoshida, Okamoto and Chui, 1971; Carroll and Grove, 1986). However, controlled studies seeking to define changes in the intestinal structure and function, attributable to hookworms or to the host response have had mixed fortunes and, as with other aspects of hookworm biology, have generated controversy. The subjects in these studies have mostly originated from indigenous populations often affected by malnourishment (Aziz et al., 1968) and by concurrent infection with other parasites (e.g., *Ascaris lumbricoides*, Gupta et al., 1974) or infectious diseases (e.g., tropical sprue, Sheehy et al., 1962; Gardiner, 1958; Banwell et al., 1967), all of which may have effects on the intestine in their own right.

4.4.1. Diarrhea

Diarrhea is widespread in the tropics and considered to be a major cause of mortality especially among children. Naturally acquired hookworm infection has been implicated as a cause of diarrhea (Pimparkar et al., 1970) and transient bouts of diarrhea, usually confined to a period 3–4 weeks after infection with improvement thereafter, have been commonly encountered after experimental exposure of volunteers to hookworm larvae. Occasionally more persistent and severe cases have been reported (Ogilvie et al., 1978) with symptoms lasting for 7 weeks. However, repeated exposure results in less severe symptoms and on tertiary infection diarrhea may not be evident at all (Ogilvie et al., 1978). All three hookworm species affecting man have been found to induce diarrhea in experimentally exposed subjects (*N. americanus*, Maxwell et al., 1987; Ogilvie et al., 1978, Cline et al., 1984; *A. duodenale*, Myhre and Wallace, 1956; Nagaty and Zanaty, 1949; *A. ceylanicum*, Yoshida et al., 1971; Carroll and Grove, 1986).

Although there is no information on the mechanism of hookworm induced diarrhea, studies on other intestinal nematodes suggest that a combination of exudative and secretory diarrhea is likely to be involved. The former is linked to protein losing enteropathy resulting from changes in intestinal permeability, mediated through local inflammation. The latter may be caused by enterotoxins and other biologically active molecules released by endoparasites resulting in perturbations of chloride ion secretion in the gut. The factors involved have been reviewed recently (Castro, 1990).

4.4.2. Intestinal histopathology

When the contributions of heterologous concurrent infections and malnourishment are taken into account, the damage created by hookworms appears to be minimal and extremely local, concentrated at the feeding sites where the worms bite into the gut tissues (Verma et al., 1968). Regeneration and repair of damage are rapid and there is little evidence of persistent scars,

atrophy, progressive necrosis or extensive general pathology (Roche and Layrisse, 1966).

Canine hookworms. The mucosa of dogs naturally infected with *A. caninum* appeared to be normal under stereomicroscopical examination, only minor inflammatory changes being apparent, confined to the attachment sites of the worms in the mucosa and submucosa (Verma et al., 1968). The height, configuration and villus height/crypt length ratio of neighbouring villi were all normal and there was no evidence of significant vascular or tissue hemorrhage. These observations contrast with the more dramatic changes described following experimental administration of larvae. Dogs with moderate to heavy infections showed significantly abnormal villous patterns manifested by villous atrophy (blunted, fused villi), crypt hyperplasia, increased goblet cell activity and local ulceration (Migasena et al., 1972). In the less pathogenic dog hookworm, *U. stenocephala*, damage to the gut was extremely focal, essentially confined to feeding sites (Gibbs, 1958).

Marked changes have also been reported in dogs experimentally infected with *A. ceylanicum* (Carroll et al., 1984b, 1985). Scanning electron microscopy revealed that there was severe atrophy and ulceration of the villi around the sites of worm attachment and, histologically, an intense infiltration by neutrophils and eosinophils was recognised, particularly surrounding the head region of the parasites in the *lamina propria*. Carroll et al. (1984b, 1985) used a single dog killed 6 weeks following primary infection with 5000 larvae for histopathological examination, i.e. just before the onset of the long drawn out period of worm expulsion (Carroll and Grove, 1984a). The time-course of subsequent changes, especially during the period of worm rejection, would make an interesting project for the future. Such studies, focusing on the cellular changes during the course of primary, repeated or secondary infections, have not been reported and for obvious reasons are unlikely to be tackled readily in the dog system. However, we have initiated a systematic analysis of changes in the small intestine of hamsters with hookworms and from preliminary observations it is already evident that *A. ceylanicum* elicits a marked mastocytosis and goblet cell response during the course of a single pulse primary infection (Fig. 6). This is accompanied by villous atrophy, change from long and slender to club-shaped morphology and crypt hyperplasia, all consistent with the involvement of a cellular mucosal response (Figs. 7 and 8). Nevertheless, despite the intensity of these reactions, the worms survive for many weeks after their onset, seemingly unaffected adversely by the mucosal changes.

Human hookworms. The human intestine shows little evidence of more than minor histopathological changes directly attributable to the presence

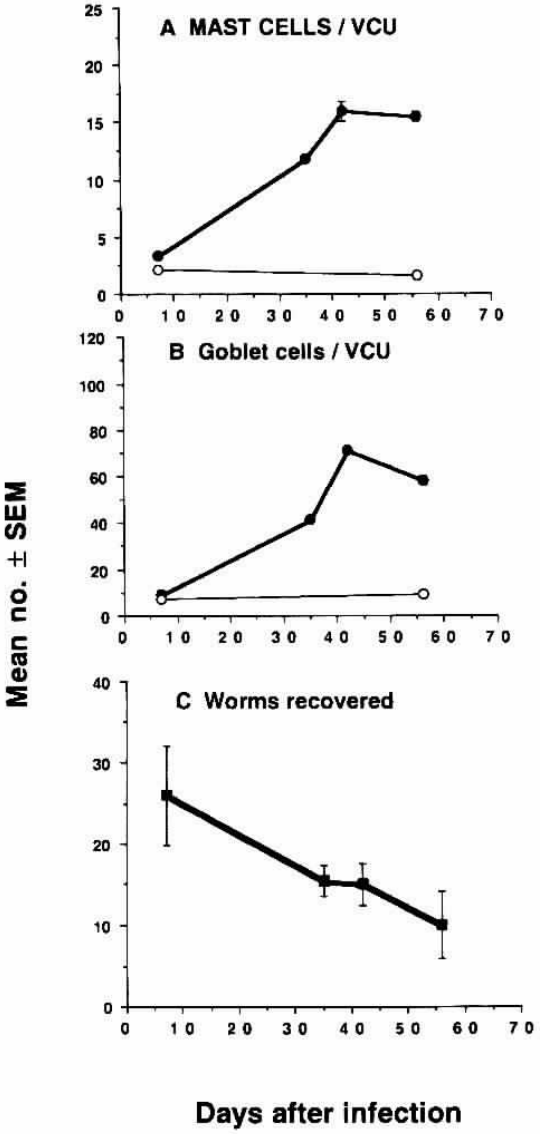
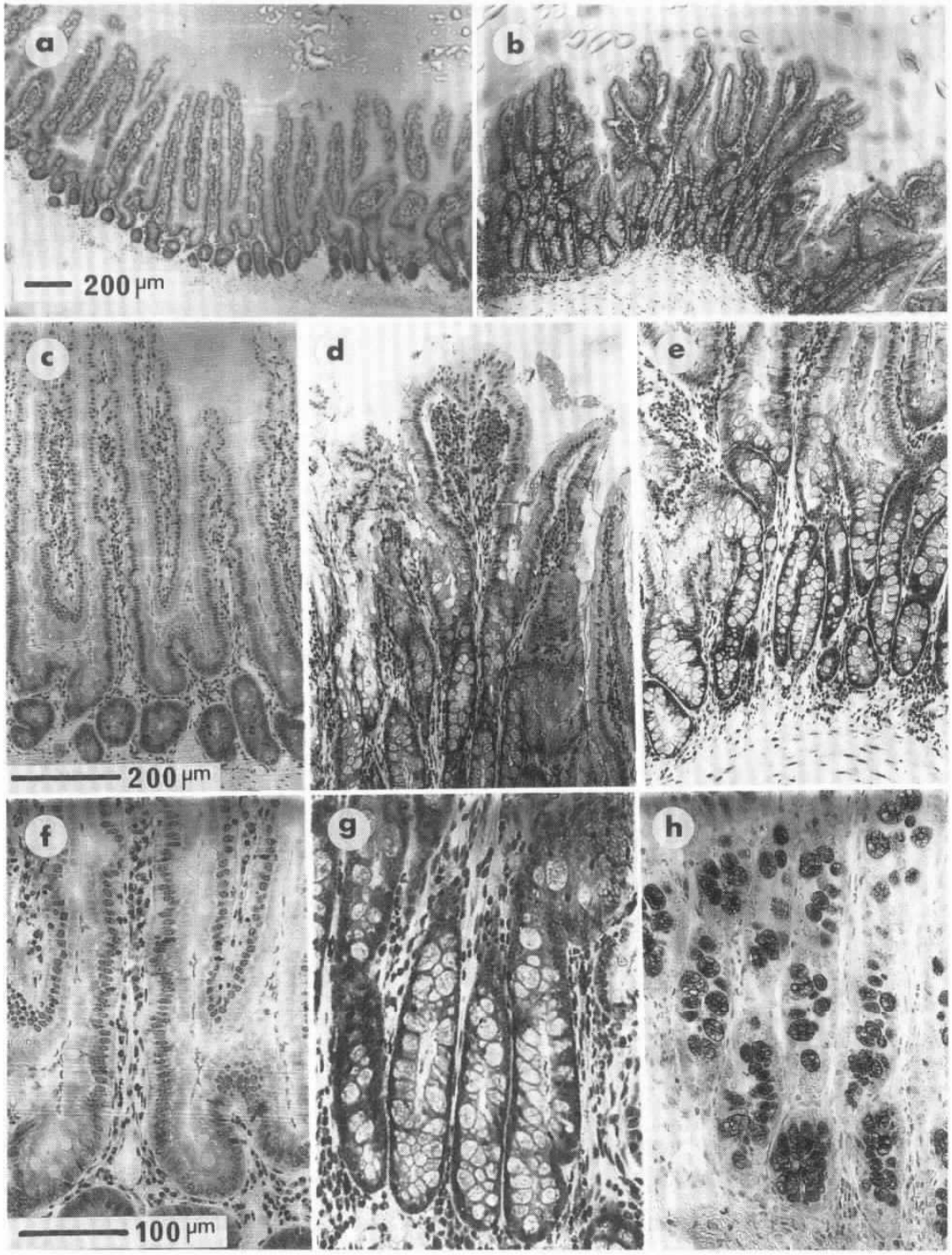


Fig. 6. The intestinal cellular responses of hamsters to infection with *A. ceylanicum*. (A) Mean number of mast cells per villus crypt unit (VCU). (B) Mean number of goblet cells per villous crypt unit. (C) Mean number of worms recovered. The higher mean worm burden on day 7 after infection was attributable to two hamsters with exceptionally high worm counts, but there was no significant loss of worms by day 35, despite the intense mast and goblet cell responses. Overall, including the two high counts on day 7, there was a significant negative correlation of worm burdens with time (P=0.01). Data from Garside (1989).

Fig. 7. Light microscope observations of changes in villus structure, including increased goblet cell number, of hamsters 21 days after infection with 100L3 of *N. americanus*. Samples from control animals were age- and sex-matched. All the photographs, with the exception of h, were taken after staining of tissues with haematoxylin and eosin. h was stained by the periodic acid-schiff reaction to show mucus activity in goblet cells. Data from Brailsford, Rose and Behnke (unpublished observations). (a, c and f) Normal villi in the intestine of control hamsters at progressively higher magnification. (b) Abnormal mucosa of infected



animal (magnification as for a). (d) Abnormal, club-shaped villus tip in mucosa of infected hamster (magnification as for c). (e) Crypt hyperplasia and massive increase in goblet cells in the mucosa of infected hamster (magnification as for c). (g) Details of goblet cells in crypts of the mucosa of infected hamster (magnification as for f). (h) Crypts showing PAS positive cells in the mucosa of an infected hamster (magnifications as for f).

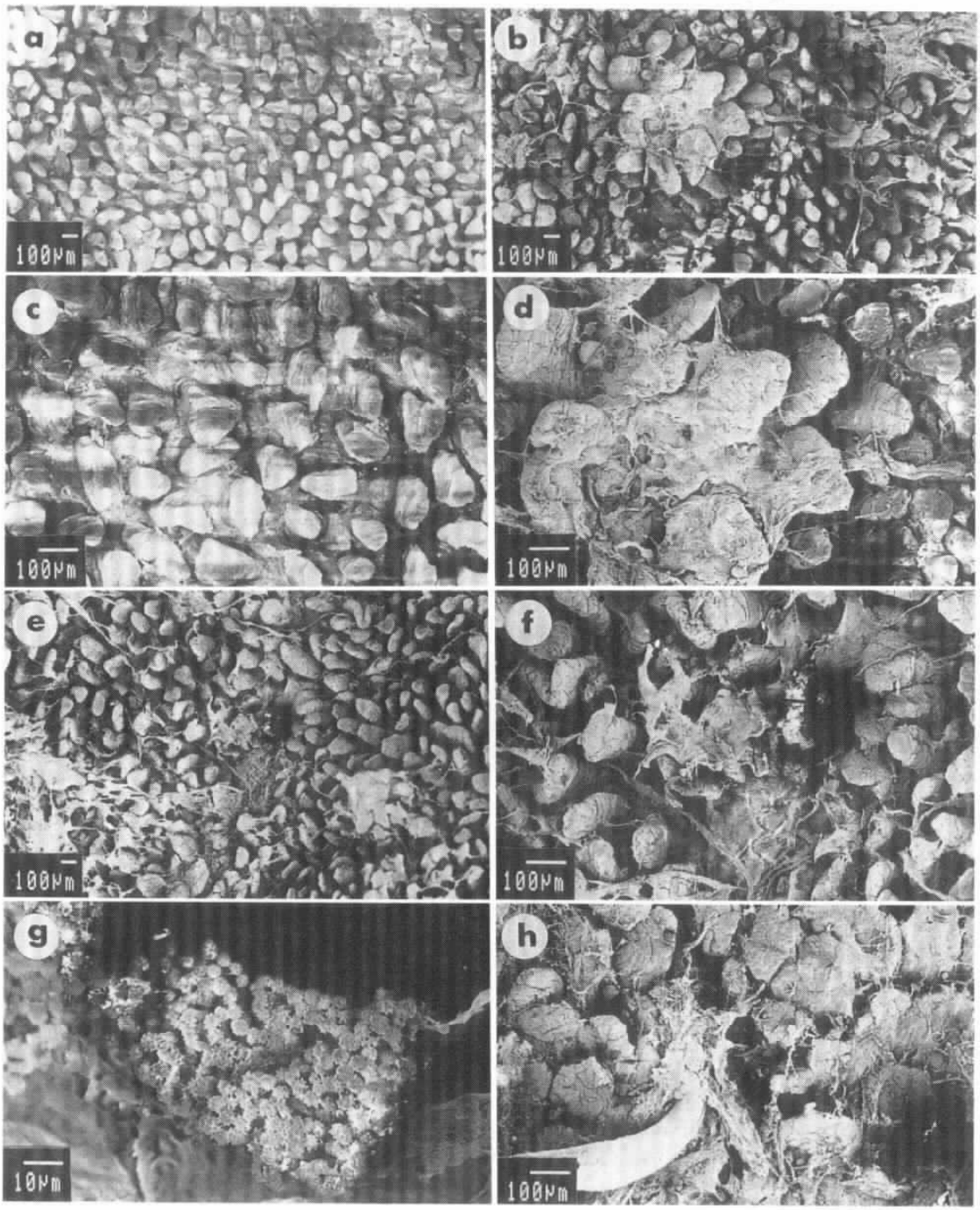


Fig. 8. Scanning electron microscope observations of changes in intestinal structure during infection with *N. americanus* in hamsters. All the samples from infected animals were taken 21 days after exposure to 100L3 and each animal carried approximately 50–60 worms at autopsy. Samples from control animals were age- and sex-matched. Data from Brailsford, Rose and Behnke (unpublished observations). (a) Normal villi in the intestine of a control hamster. (b) Abnormal, enlarged and club-shaped villi in the small intestine of an infected hamster. (c) Higher magnification of a. (d) Higher magnification of b. (e) Part of the small

of hookworms (Pimparkar et al., 1970). Biopsy specimens, usually from hospitalized patients, reveal the most intense infiltration concentrated at the parasite's feeding sites. Chaudhuri and Saha (1964) found no general mucosal damage, but an increase in goblet cells and focal eosinophilic infiltration were observed. In the single case of marked eosinophilic accumulation in the submucosa, the biopsy specimen probably corresponded to a recent feeding site. Burman et al. (1970) found moderate changes in the jejunal mucosa in 4/30 patients with *A. duodenale*, whereas Nath et al. (1971) studying a group of 60 patients from Kanpur, detected moderate changes in 11 with 2 additional cases of extreme abnormality. The intestinal epithelium was flattened, villi were absent and a dense cellular infiltration in the *lamina propria* was observed. Furthermore, a direct relationship between the severity of mucosal changes in the jejunum and the intensity of infection was suggested (see also Rai et al., 1969), but this conclusion contrasts with most other studies. Aziz and Siddiqui (1968) examined 30 patients with *A. duodenale* from West Pakistan, concluding that morphological abnormalities of the intestinal mucosa bear little relationship to the intensity of hookworm infection. One individual passing 4200 EPG had moderately severe changes whilst another with an EPG of 30200 had only minor changes. Moderate structural abnormalities of the jejunal mucosa were reported in 10/27 hospitalized patients in India (Gupta et al., 1973) and in 21/37 *A. duodenale* infected subjects in New Delhi (Chuttani et al., 1967), the latter study recording only 2 individuals with extreme changes. There was little improvement after anthelmintic treatment and it was concluded that hookworms were not responsible for major intestinal injury. Among 11 patients with *N. americanus* studied by Banwell et al. (1967), 6 were found to have minor histological abnormalities and 5 more severe, but no extreme cases were detected and again hookworms were deemed not to be responsible.

In severe *A. duodenale* infection in adults (Salem and Truelove, 1964) and in children (Zimmerman, 1946; the latter based on post mortem of lethal cases), marked intestinal changes were widely observed. These included club-shaped villi and infiltration by neutrophils and eosinophils in both the mucosa and submucosa and ulceration with edema. Zimmerman's observations were complicated by secondary intestinal infection (the 6 cases of peritonitis also had the most extensive lesions in the small intestine), particularly around ulcers, presumably originating from worm feeding sites and also by the absence of controls. It is unlikely that the exact consequences

intestine showing a recently vacated feeding site. (f) Higher magnification of e. (g) Mostly crenulated shapes of erythrocytes emerging from a lacerated blood vessel at a recently vacated feeding site. (h) An adult worm attached in the small intestine with its head buried deep in between villi.

of hookworm infection on intestinal structure will be understood from observations based solely on hospitalized patients or post mortem examinations in tropical areas. There are simply too many variables to take into account and too few 'realistic' controls. Furthermore, from animal studies it is quite evident that the intensity and duration of hookworm infection both exert a major influence on the onset and course of intestinal changes. Under field conditions, these would be difficult to standardize, given that only a few patients at a time could be subjected to endoscopic retrieval of mucosal samples.

4.4.3. Absorption of materials from the gut lumen

The effect of hookworms on the functional integrity of the intestine has been studied by tests assessing the uptake of experimentally administered substances from the gut lumen in both human hookworm cases and in animal models, with contrasting results. Standard techniques have generally been employed in human studies, but criteria have often varied and direct comparisons are not legitimate because of unique variables affecting the subjects in each group studied. Hospitalized patients, initially presenting for treatment for a variety of reasons besides hookworm disease, have been exploited and this has created problems in the choice of appropriate control groups, few of which have been totally satisfactory in all respects. Undoubtedly a further complication affecting the accurate measurement of uptake has been variation in the extent of concurrent loss of material from the circulation into the gut lumen, a variable which has not usually been compensated for.

Uptake of D-*xylose.* The most widely employed assay for monitoring carbohydrate uptake is the D-xylose test in which a 5 g oral dose of D-xylose is given in 500 ml of water and the total excretion in urine is recorded during the following 5 h period (Santini et al., 1961; Roe and Rice, 1948). Excretion of less than 1–1.5 g is considered to represent depressed intestinal absorption. Table 1 summarizes the results from relevant studies, emphasizing the geographical location and where available, the identity of the hookworm species.

It is readily apparent that results have varied widely from one study to another and overall there appears to be little support for a clear, consistent effect. The absorption of D-xylose is not affected by the intensity of infection (two subjects studied by Aziz and Siddiqui (1968), passing 28 600 and 30 200 EPG, had normal absorption) nor by the species of hookworm involved (Tandon et al., 1969). Furthermore, re-examination of individuals, 10–68 weeks after the initial inspection revealed no fresh cases of malabsorption and 4 cases initially detected as depressed returned to normal despite the continuing presence of the parasites.

D-Xylose absorption was monitored in dogs infected with *A. caninum*, through regular blood samples over a course of 5 hours after intragastric administration of 10 grams of the sugar. Peak plasma levels were significantly reduced in dogs with chronic as well as acute infections (Migasena et al., 1972b).

The glucose tolerance test has also been applied to hookworm patients. Fourty-two subjects had essentially normal fasting serum glucose levels, but significantly depressed peaks after glucose loading. The lactose tolerance test did not distinguish between hookworm patients and controls, although 2/16 of the former had values below normal (Pimparkar et al., 1970).

In conclusion, the D-xylose test does not support the concept that hookworms disturb the uptake and excretion of pentose sugars in naturally infected persons and therefore, provided that digestion is unimpaired, dietary carbohydrate should be normally available to the host during hookworm infection. However, animal studies have established that under controlled laboratory conditions, employing age-matched uninfected animals for comparison, impairment of sugar uptake can be detected, by the more sensitive technique of monitoring plasma levels after a sugar meal.

Uptake of dietary fats. A popular method for measuring absorption of dietary fat is that described by Van de Kammer et al. (1949) in which the fat content in a 5 g sample of stools is hydrolysed and the total quantity of fatty acids is subsequently determined by calorimetric titration. Patients are placed on a diet containing known fat content (usually 100 g daily) for 2 days prior to the test and stools are collected for up to 6 days subsequently (Sheehy et al., 1962). Control values vary, but are normally below 5 g daily, e.g., 3.62 ± 2.06 in Puerto Rico (Sheehy et al., 1962), 2.53 ± 1.51 in Uganda (Banwell et al, 1967), 2.81 ± 1.1 Venezuela (Layrisse et al., 1964), 3.7 ± 1.15 and 2.65 ± 0.97 in India (Chuttani et al., 1967 and Burman et al., 1970, respectively), and therefore most workers have considered values above 5–6 g/day to represent malabsorption of fat. An alternative test involves oral administration of ^{131}I-labelled oleic acid or triolein and measurement of total radioactivity in stools or serum collected for 72 h post treatment, but these assays have been used infrequently (Layrisse et al., 1964).

The results of published studies are summarized in Table 1, from which it is apparent that excepting Sheehy et al. (1962), Tandon et al. (1969) and Nath et al. (1971), steatorrhea has been comparatively rarely encountered among hookworm patients. A weak association between increased fecal fat and heavy infection has been reported (Tandon et al., 1969; Nath et al., 1971), but these appear to be exceptional cases. On balance there is little support for the regular occurrence of steatorrhea among hookworm-infected persons. As was pointed out by Banwell et al. (1967) tropical sprue is

TABLE 1
Absorption of dietary D–xylose and fat by subjects infected with *A. duodenale* and/or *N. americanus*

Location	Parasites	D-xylose		Fat		Authors
		positive/ total	percentage +ve	positive/ total	percentage +ve	
Pakistan	*A. duodenale*	8/30	26.7		–	Aziz et al., 1968
India	Both	3/22	13.6	9/22	40.9	Tandon et al., 1966
India	*A. duodenale*	1/25	4.0	3/40	7.5	Chuttani et al., 1967
India	Both	39/66	59.1	21/67	31.3	Tandon et al., 1969
India	*A. duodenale*	14/60	23.3	17/60	28.3	Nath et al., 1971
India	Both	2/27	7.4	0/27	0	Gupta et al., 1973
India	Both	3/7	42.9	0/11	0	Gupta et al., 1974
India	Both	5/30	16.7	3/30	10.0	Burman et al., 1970
India	*A. duodenale*	–	–	10/50	20.0	Rai et al., 1968
India	*A. duodenale*	8/37	21.6	7/24	29.2	Pimparkar et al., 1970
Costa Rica	*N. americanus*	0/11	0	1/44	2.3	Kotcher et al., 1966
Puerto Rico	*N. americanus*	10/14	71.4	14/14	100.0	Sheehy et al., 1962
Venezuela	*N. americanus*	1/18	5.6	3/10	30.0	Layrisse et al., 1964
Uganda	*N. americanus*	1/8	12.5	0/12	0	Banwell et al., 1967
Egypt	*A. duodenale*	0/10	0	0/10	0	Abdalla et al., 1963
Nigeria	*N. americanus*	–	–	1/11	9.1	Gilles et al., 1964

endemic throughout Northern India and in Puerto Rico and the high prevalence of steatorrhea among populations located in these regions may be partially accounted for by concurrent infection.

Again experiments in dogs infected with *A. caninum* have yielded contrasting results. Peak plasma levels (1–5 hours after feeding) of ^{131}I-labelled triolein were significantly depressed in dogs with acute (less than 3 month) infections. Thus hookworm infection can exert a significant effect on fat absorption, at least during the initial stages of infection when inflammatory reactions in the gut might be expected to be at their most intense. Chronic infections were not associated with impairment of fat absorption (Migasena et al., 1972b).

Uptake of other dietary components. Several other dietary constituents have been considered in studies evaluating the effect of hookworms on intestinal function. Sheehy et al. (1962) found that vitamin A absorption was impaired in 8/13 patients of whom 6 improved significantly after anthelmintic treatment. In Uganda it was found that 5/15 patients showed vitamin A malabsorption (Banwell et al., 1967), but this proportion was not considered to be significant (Leonard and Banwell, 1964).

Vitamin B-12 absorption has been monitored by the Schilling test, using ^{58}Co-labelled B-12. Values below 7% of total radioactivity within the first 24 h urine sample are considered to represent malabsorption (Schilling, 1953; Tandon et al., 1969). Studies by Tandon et al. (1969), Banwell et al. (1967), Layrisse et al. (1964), Pimparkar et al. (1970) and Gupta et al. (1974) have only detected the occasional case of impaired absorption and often this has been attributed to other concurrent infections (Pimparkar et al., 1970).

Layrisse et al. (1959, 1964) measured the uptake of folic acid from the intestine and detected significant malfunction in hookworm patients. However, low levels of serum folate activity were frequently encountered among rural communities in Venezuela, unaffected by hookworms and it was concluded that other etiological factors were responsible for the diminution of folate activity in the serum and malabsorption.

Absorption of dietary nitrogen and energy may also be impaired, but generally only in heavily infected persons (Darke, 1959). In dogs with *A. caninum*, however, a significantly slower uptake of the ^{14}C-labelled synthetic amino acid alpha-amino-isobutyric acid, was detected in both chronic and acute infections (Migasena et al., 1972b).

Finally the absorption of iron after an oral dose of ferrous sulphate was essentially normal in individuals infected with hookworms (Gilles et al., 1964; Aziz et al., 1968).

4.5. Concluding remarks

Most papers reporting on the histopathology and functional integrity of the intestine in hookworm patients have been concerned with groups of hospitalized individuals, only some of whom were admitted for the treatment of hookworm disease. The subjects have included extremely malnourished individuals (Aziz and Siddiqui, 1968) sometimes with concurrent heterologous infection Gupta et al., 1974; Kotcher, et al., 1966) and most authors have recognized these as complicating factors, but in all of these studies a central issue has been what to regard as normal and how to select appropriate control groups. Patients frequently come into hospital from distant rural areas and hospitalization would entail improvement in the diet and a major change in life style. Generally, there has been little choice in selecting controls and hence the most accessible groups have been used with volunteers from among other hospital patients or laboratory/medical staff.

A number of papers are based on quite small sample sizes (<20; Table 1). In countries where intestinal disturbances are frequently encountered in the background population (e.g., D-xylose malabsorption and histopathological intestinal changes may occur in up to 50% of apparently healthy Pakistani males (Russell et al., 1964, 1966; Aziz and Siddiqui, 1968) and similar figures have been reported elsewhere (England and O'Brien, 1966; Sprinz et al., 1962)), only large study groups could hope to uncover a significant trend in relating particular disease symptoms to the prevalence of hookworms or other etiological factors, in the face of all the components which disturb intestinal function.

Intestinal disturbances from non hookworm causes may be more frequent among the rural agricultural workers in endemic areas than among better nourished and less exposed urban populations (Layrisse et al., 1964). Furthermore, malnutrition is known to cause intestinal damage including histopathological changes, reduced D-xylose and lactose absorption, diarrhea and steatorrhea (Chuttani et al., 1968; Mayoral et al., 1967; Bowie et al., 1965; Deo and Ramalingaswami, 1964). Mayoral et al. (1967) found that malabsorption in protein malnourished patients was not exacerbated by concomitant hookworm infection and further, the parasites did not retard recovery once the patients were placed on a normal diet. In the absence of protein malnutrition, neither hookworms nor iron-deficiency anemia caused malabsorption (Mayoral et al., 1967; Rawson and Rosenthal, 1960).

Extreme changes in intestinal function and histology, including flattened and fused villi have been recognized in hookworm infection (Layrisse et al., 1964; Sheehy et al., 1962), but appear to be the exception rather than the rule and have not been convincingly shown as directly attributable to the parasites or to the homologous host response in man. On the basis of labora-

tory studies with *A. caninum* and *A. ceylanicum* such changes would be predicted (Miller, 1984; Behnke, Rose, Garside and Brailsford, unpublished observations). It may be difficult to believe that worms which are such voracious feeders with demanding nutritional requirements, do not severely affect the host tissues in which they reside. Indeed hookworms do cause local damage, but this is repaired rapidly in the majority of patients (Rai et al., 1968) with significant mucosal abnormalities being precipitated in some patients only (Chuttani et al., 1967). Malnourished individuals may be the most frequent victims of hookworm disease because they are least capable of compensating for parasite-induced damage. Links between malnutrition and immunosuppression and between the latter and gastrointestinal nematode infections are well established (Bundy and Golden, 1987; Faulk et al., 1975; McGee and McMurray, 1988; WHO, 1972). It may be that one consequence is increased susceptibility to other infectious organisms resulting in a further drain on host reserves and exacerbation of disease symptoms. In other words a vicious circle may ensue with hookworms in turn contributing to the malnourishment of their host (Darke, 1960; Sheehy, 1962; Rubini et al., 1961).

It is also likely that as in other diseases there is a spectrum of capacity to recognize and respond to hookworms within the host population. Some individuals may show extreme histopathological changes in the gut because of their genetically determined sensitivity to parasite antigens (Wakelin, 1985) and if this proves to be a small proportion of the population, as current studies suggest, then convincing data in support will only come from large scale studies involving carefully selected control groups.

Finally it would seem that on balance, hookworms create little overall disturbance and do not affect the absorption of dietary constituents despite biting deep into the intestinal mucosa and causing extensive blood loss. There is therefore still no reason for amending the conclusions arrived at by Roche and Layrisse (1966) that factors other than hookworms are the principal causes of intestinal abnormalities in infected persons. Most subjects have predominantly normal jejunal mucosa, with minor local changes attributable to the feeding of adult worms, these patches being rapidly repaired when the worms change feeding sites. Nevertheless, within the context of this conclusion it must be emphasized that there are likely to be exceptions and that in particular cases, hookworms may indeed be responsible for significant damage to the intestine, as reflected in studies on animal models under controlled laboratory conditions. From these, it is quite clear that the potential to elicit marked intestinal pathology in relation to structure as well as function, does exist.

The pathology of human hookworm infections is still an area of debate which warrants further investigation. Studies on animal models are required

to confirm and extend the observations which have been made so far and to clarify the reasons for the discrepancies between observations on dogs and humans. Histological and functional studies on intestinal changes associated with hookworm infection in dogs have been restricted so far to monitoring the consequences of single-pulse infections, which seldom occur under natural conditions in the field. Perhaps one way forward would be to monitor pathological consequences under trickle infection regimes mimicking low level continuous exposure to hookworm larvae.

Comparable studies could also be contemplated in hamsters infected with *N. americanus* and *A. ceylanicum*, the only rodent models which support long-term survival of adult worms. Experiments along these lines have already been initiated by Indian workers, Kaul et al. (1982) reporting marked disturbances to carbohydrate metabolism in animals which were injected intra-peritoneally with glucose. Blood sugar levels rose higher than in controls and persisted for longer. Although these workers did not measure absorption of carbohydrates, the experiments serve to illustrate that the hamster model does have great potential for future use. Parallel studies in our laboratory have indicated that under experimental conditions, in response to a single pulse administration of infective larvae or following challenge, there are marked changes in intestinal histopathology (Figs. 6, 7 and 8). How these relate to worm survival and/or resistance against hookworms is not yet clear, although it is already apparent from our experiments that adult *A. ceylanicum* do not seem to be severely hampered by mast cell and goblet cell responses capable of making life untenable in the mucosa for other nematode parasites, such as *T. spiralis*.

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