THE IMPACT OF INTESTINAL HELMINTHS

ON MAMMALIAN NUTRITIONAL PHYSIOLOGY

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DECLARATION:

I declare that this thesis describes research carried out by myself unless otherwise cited or acknowledged. It is from my own composition and has not, in whole or part, been presented for any other degree.

Lucy J. Robertson

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CONTENTS

SUMMARY

A critical review of the literature (Section 1) interprets evidence to show how various mammalian intestinal helminth infections have been associated with alterations in the nutritional physiology of the mammalian host. The effects have been observed during all stages of metabolism, from initial ingestion through all the intra-luminal events, including digestion and nutrient absorption, and finally to the post-absorptive events of intermediary metabolism, and the mechanisms appear to be as diverse as structural histopathologic changes and alterations in the concentrations of circulating hormones.

An experimental investigation of interactions between rat host metabolism and infection with *Nippostrongylus brasiliensis* (Nematoda), (Section 2), was conducted. Plasma glucose concentrations were observed to fall between days 6 - 14 p.i. of a primary infection of rats with *N.brasiliensis*, and although food intake was also observed to fall during this intake, with marked troughs on days 3 and 9 p.i., this is considered to be no more than contributory to the reduced plasma glucose concentrations.

Measurements of the activity of the hepatic gluconeogenic enzyme alanine-amino-transferase (ALT), by *in vitro* assay, during *N.brasiliensis* infections revealed striking fluctuations. An elevation followed by a reduction in the activity of the enzyme was observed, and these alterations were found to be dose-dependent. In secondary infections similar fluctuations were observed, but appeared to be attenuated. A reduction in the activity of the enzyme was detected when the infection was initiated by oral transfer of adult *N.brasiliensis* worms, but when an infection initiated by subcutaneous inoculation of *N.brasiliensis* larvae was terminated on day 4 p.i. by anthelmintic treatment, ALT activity measured on day 10 p.i. was not found to be different from that measured in control rats.

Addition of parasitic homogenate to the liver assay caused no change in the

measurements, but addition of serum was associated with a decrease in the ALT activity measured, and this was particularly marked with serum from immune rats. These results suggested that an immune response of the host to the *N.brasiliensis* infection may be influencing ALT activity. Measurements of hepatic ALT activity in immunosuppressed rats infected with *N.brasiliensis* supported this theory. Accordingly measurements were made in rats during *N.brasiliensis* infections of parameters known to be indicators of an immune response involving cytokines, known to influence a number of aspects of metabolism (Klasing, 1988). Although body temperature, leukocyte numbers and plasma zinc concentrations provided no evidence of an immune response involving cytokines, a marked elevation in the concentration of plasma α_2-macroglobulin was observed, suggesting that cytokines may be produced.

Measurements of plasma corticosterone concentrations during both primary and secondary *N.brasiliensis* infections in rats revealed fluctuations in concentration that provided a tempting suggestion that alterations in concentrations of this immunosuppressant hormone may be contributing to the fluctuations in ALT activity described. The possibility that metabolic fluctuations may be a "trade off" for an effective immune response is discussed in terms of these results.

A collaborative study of a human community in Coclé Province, Panama (Section 3) was undertaken to investigate associations between nutritional variables, in particular vitamin A status, and soil-transmitted helminth infections in primary school children, with measurements being made of nutritional parameters both before and after anthelmintic intervention.

The epidemiological data collected revealed that *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* were the most common intestinal helminth infections observed, with prevalences of 18.2, 12.0 and 27.5% respectively. This data could be compared not only to results of intestinal helminth surveys conducted in the same area over the past 60 years, but also to more contemporary data from various

regions of Central America. The efficacy of levamisole as a control measure was assessed, and considered to have an effective role.

Plasma retinol concentrations were not found to be indicative of vitamin A deficiency, and no association between lower concentrations and intestinal helminths was detected. Over 20% of the children, however, were found to have blood haemoglobin concentrations indicative of anaemia, and concomitant infections of *T.trichiura* and hookworm were considered to be contributory. Heavier infections of *T.trichiura* were also found to be associated with lower anthropometric variables.

These results suggest that even relatively light infections of soil-transmitted helminths may exert an insidious effect on the nutritional status of growing children, despite the availability of adequate nutritional resources.

SECTION 1

INFLUENCES OF INTESTINAL HELMINTH INFECTION ON MAMMALIAN NUTRITIONAL PHYSIOLOGY: A REVIEW

1.1. INTRODUCTION

The effects of parasitic infection on mammalian host nutrition have long been a subject of research and discussion. General theories have been proposed, but there are cases of contrasting views and conflicting evidence. Even the now classic work of Scrimshaw, Taylor & Gordon (1968), that initially promoted the concepts of synergism and antagonism in this context, has received criticism (Beisel, 1982; Bundy & Golden, 1987). Criticism of theory and practical research is essential in the development of an accurate assessment of the interactions between host nutrition and parasitic infection, but in practical research, especially with human community studies, the ideal research situation is probably impossible.

In this section, I have outlined facets of host nutritional physiology that have been shown to be affected by infection with intestinal helminths. I have included influences on food intake, intestinal propulsion, digestion, absorption of nutrients, and events of intermediary metabolism. There is, however, no discussion of nutrient loss, especially salts and minerals, through diarrhoea and/or vomiting, both of which have been associated with several infections with intestinal helminths.

1.2. FOOD INTAKE AND ANOREXIA

A commonly observed feature of intestinal helminth infections in mammalian hosts is a reduction in voluntary food intake (Table 1.2.1). It is often regarded as an important factor in the pathology of such infections and is frequently accompanied by loss of body weight, or a reduction in the rate of body weight gain (Symons & Jones, 1975). Although the words appetite, appetence and anorexia are frequently used in this context, this can cause semantic confusion. Appetite and appetence are highly subjective terms which describe the complex of sensations in which there is an awareness of the desire for food, and anorexia is the pathological lack of appetite (Mayer, 1973). In animal studies, it is not possible to measure the extent of appetite or lack of it. Instead, the variable measured is food intake, and it is assumed that a voluntary reduction in food intake is indicative of a decreased appetite and an

anorexic state. I have continued to exploit this assumption in this review, and have used the words appetence, appetite, and anorexia freely, although I am aware that in animal studies at least, it is food intake, rather than appetite, which is being measured.

The degree of inappetence that has been associated with intestinal helminth infections is variable, and in some studies has been shown to be related to the intensity of infection (Crompton, Walters & Arnold, 1981; Forsum, Nesheim & Crompton, 1981; Keymer, Crompton & Walters, 1983; Ovington, 1985a). It is a common observation that the degree of inappetence in sheep harbouring gastrointestinal helminths is related to disease severity. In some infections, inappetence is transitory and normal appetite is regained. During primary experimental infections of rats with *Nippostrongylus brasiliensis* a periodic inappetence, apparently related to parasitic development, has been described; periods of severe anorexia seem to be associated with particular stages of the parasitic life cycle (Ovington, 1985a).

Although loss of appetite is commonly reported by clinicians (Stephenson, 1987), reduction in food intake is a difficult parameter to measure in human communities for both ethical and operational reasons. Thus, most of the literature concerned with food intake and infection relates to animal studies.

The reasons for inappetence during intestinal helminth infection have not yet been explained, although various speculative theories have been proposed (Crompton, 1984; Rosenberg & Bowman, 1984; Symons, 1985). Food intake is influenced by events both in the C.N.S. and the gastrointestinal tract, with central regulation of appetite apparently controlled by two interacting centres in the hypothalamus (Morley, 1980). Food intake may also be affected by systemic changes that accompany parasitic infection; fever, toxin production, metabolic and hormonal alterations. These could influence both the central control of appetite, and local functions, such as gastric emptying.

Four basic theories have been proposed to describe the control of food intake

during intestinal helminth infection. These are:-

1) Reduced appetite during helminth infections could be a consequence of alterations in concentrations of plasma hormones, particularly cholecystokinin (CCK). Symons and Hennessy (1981) postulate an association between elevated CCK concentration and anorexia in sheep infected with *Trichostrongylus colubriformis*. Ovington (1985b) measured an increase in plasma CCK concentration during primary *N.brasiliensis* infections in rats between days 5 and 9 p.i., but as this did not coincide with the periods of greatest inappetence, she was unable to correlate plasma CCK concentrations with reduced food intake.

2) Alterations in the rate of digestive flow and the motility in the digestive tract might contribute to alterations in the voluntary intake of food (Rosenberg & Bowman, 1984; see Section 1.3.1).

3) Alterations in the pH of the digestive tract during infection with gastrointestinal helminths have been recorded by some researchers (Bueno, Dakkak & Fioramonti, 1982; McLeay, Anderson, Bingley & Titchen, 1973). This might affect appetite directly or indirectly by altering protein digestion and thus the availability of amino acids, some of which have been demonstrated to stimulate appetite (Leng, 1981). Another factor suggested by Leng (1981), that may reduce the availability of amino acids for absorption and thereby depress appetite in ruminants, is quantitative alteration in the populations of fermentive bacteria of the digestive tract. However, research on this is scanty, and available data contradictory.

4) Anorexia might also be precipitated by pain. Gibson (1955) suggested that it is the pain felt by sheep during infections with *Trichostrongylus axei* that causes a reduction in their appetite. However, the assessment of a subjective phenomenon is difficult, especially in animal studies. Miller (1979) could be using more objective evidence in stating that hookworm infection in humans is associated with abdominal pain accompanied by anorexia.

These four approaches to explain reduced host food intake during intestinal helminth infections are not exclusive and other ideas have been explored. For

example, Keymer, Crompton & Sahakian (1983) presented data to indicate that rats may develop a learned taste aversion by associating a particular flavour of food with the trauma of the initial phase of a primary infection with *N.brasiliensis*, originating from a large inoculum of larvae.

In concluding a comprehensive review concerned with anorexia during parasitic infections, Symons (1985) terms the present state of knowledge regarding this interaction as "rudimentary". Further research in this area is clearly indicated.

TABLE 1.2.1

OBSERVATIONS ON REDUCED FOOD INTAKE IN MAMMALS DURING INFECTION WITH INTESTINAL HELMINTHS*1

PARASITE	HOST	REFERENCES*2
Digenea		
Fasciola hepatica	Sheep	Sykes, Coop & Rushton, (1980)
Fasciola hepatica	Cattle	Cawdery, Strickland, Conway & Crowe, (1977)
Paramphistomum microbothrium	Sheep	Horak, (1971)
Nematoda		
Hyostrongylus rubidus	Pigs	Castelino, Herbert & Lean, (1970)
Ostertagia circumcinta	Sheep	Sykes & Coop, (1977)
Haemonchus contortus	Sheep	Abbott, (1982)
Trichostrongylus colubriformis	Sheep	Steel, Symons & Jones, (1980)
Nematodirus battus	Sheep	Rowlands & Probert, (1972)
Cooperia pectinata	Calves	Keith, (1967)
Ascaris suum	Pigs	Forsum *et al*, (1981)
Ancylostoma duodenale) *Ancylostoma ceylanicum*) *Necator americanus*)	Humans	Miller, (1979)
Capillaria philippinensis	Humans	Whalen, Strickland, Cross, Rosenberg, Gutman, Watten, Uylangco & Dizon, (1969)
Trichuris suis	Pigs	Hale & Stewart, (1979)
Oesophagostomum radiatum	Cattle	Bremner, (1961)
Chabertina ovina	Sheep	Herd, (1971)
Nippostrongylus brasiliensis	Rats	Ovington, (1985a)
Haemonchus longistipes	Camels	Arzoun, Hussein & Hussein, (1984)

*1 Adapted from Symons (1985) and Crompton (1984).

*2 Only one reference is given, although in several cases more could be cited

1.3. INTESTINAL PROPULSION AND MOTILITY

In view of their location in the gastrointestinal (GI) tract it is probable that gut helminths will influence and interact with gastrointestinal functions. During digestive function in the absence of helminthiases, chyme is released from the stomach into the duodenum by a controlled relaxation of the pyloric sphincter. The intestinal contents then remain in the tract long enough for further digestion and absorption. If gut motility increases, as well as influencing food intake (see Section 1.2), the time for the digesta mixing with biliary and pancreatic secretions will be reduced, as will the time for digestion and nutrient absorption. Controlled gut motility is also an important factor in the maintenance of a balanced microbial gut flora population. Alterations in the population structure and dynamics of cellulose-digesting and nitrogen fixing microbes in ruminants have important nutritional consequences.

The data on intestinal propulsion and gut motility during experimental intestinal helminth infections is not extensive, and is particularly limited for infections in humans. Food transit time from mouth to caecum was compared between uninfected children and children infected with *Ascaris lumbricoides* in Panama and not found to be significantly different (Taren, Nesheim, Crompton, Holland, Barbeau, Rivera, Sanjur, Tiffany & Tucker, 1987). One infected child had an exceptionally long transit time of over 140 min, and when this child was excluded from the data set, a significant negative correlation between the intensity of infection (log e.p.g. for *A.lumbricoides*) and transit time was produced. Also in humans, the contractile movements of the small intestine have been reported to increase during hookworm infections (Roche & Layrisse, 1966).

Castro, Badial-Aceves, Smith, Dudrick & Weisbrodt (1976) measured gut motility in mice during the intestinal phase of the infection with *Trichinella spiralis*. They linked an increase in propulsive activity with inflammatory changes which were also observed by Sukhdeo and Croll (1981) during *T.spiralis* infections

in mice. Sukhdeo and Croll (1981) measured a significant increase in gut propulsion rate on day 5 p.i., but by day 9 p.i. this had returned to control levels. Intestinal motility has also been observed to increase during the enteric phase of *T.spiralis* infections in dogs (Schanbacher, Nations, Weisbrodt & Castro, 1978). Symons (1966) recorded an increase in the rate of propulsion of intestinal contents during *Nippostrongylus brasiliensis* infection in rats, but only in certain regions of the small intestine, and in some regions flow rate was reduced.

Contrastingly, during intestinal helminth infections in ruminants, the rate of digesta flow through the gut appears to be generally reduced rather than increased (Holmes, 1986). For example, in infections of sheep with *Trichostrongylus colubriformis* there appears to be an inhibition in digesta flow in the abomasum and proximal small intestine (Gregory, Wenham, Poppi, Coop, MacRae & Miller, 1985). However, in sheep infected with *Haemonchus contortus* gastrointestinal motility and the duodenal flow rate of digesta have been observed to increase, mostly between days 2 and 12 p.i. in association with the activities of immature worms in the abomasal mucosa (Bueno *et al*, 1982). Despite the apparently conflicting sets of data, it is evident that digesta propulsion and intestinal motility are affected by intestinal helminth infections.

1.4. INTRALUMINAL EVENTS

1.4.1. GASTRIC FUNCTION

The stomach acts as a reservoir for ingested food, and its secretory activity provides both enzymes and hydrochloric acid required for various functions (Keele, Neil & Joels, 1982). Peptic cells contain granular pepsinogen, which in an acidic environment (optimally pH 2) is converted to pepsin, a proteolytic enzyme that hydrolyses peptide bonds between phenyl alanine and other amino acids. Gastric hydrochloric acid is secreted by the parietal cells, and is important for various reasons. As well as providing the optimum pH for the initiation of enzymic digestion, the acid limits bacterial growth in the gut and facilitates the breakdown

of sucrose to glucose and fructose. The secreted gastric juice also includes mucus, gastrin, intrinsic factor and rennin (in young mammals other than man) which curdles milk. Intrinsic factor is a heat-labile muco-protein that combines with dietary vitamin B-12; failure to absorb vitamin B-12 can result from gastric atrophy (Keele *et al*, 1982).

McLeay *et al* (1973) measured a rise in pH in the abomasum of sheep harbouring *Ostertagia circumcinta*. It was suggested that this might be either due to a failure of secretion, or to a lesion in the reabsorptive permeability. Although the parietal cells appeared to respond to secretory stimuli, the cells of the gastric mucosa seemed to be suppressed. Calves infected with *Ostertagia ostertagi* also exhibited a rise in gastric pH (Jarrett, 1966). This was concluded to be a consequence of the loss of functional HCl-secreting parietal cells. A similar pH rise was also measured in lambs infected with *Haemonchus contortus* during the histotrophic phase of larval development (Bueno *et al*, 1982), the gastric pH rising from 1.9 $\pm$ 0.1 to 3.0 $\pm$ 0.2, with a maximal measurement of 4.0 $\pm$ 0.3.

Decreased gastric secretion has also been reported in human infections with intestinal helminths, but clinical studies do not always take into account other concomitant infections or malnutrition. Gianella, Broitman & Zamcheck (1973) described decreased gastric acid secretion in symptomatic strongyloidiasis, and Gupta, Pitamber, Gupta & Dube (1980) reported similarly for human hookworm infections. During infections with *Diphyllobothrium latum*, evidence has been presented that suggests gastric juice production is impaired (Siurala, Palva & Nyberg, 1964), and it is possible that this may contribute to the vitamin B-12 deficiency anaemia with which this infection is implicated.

Some infections, however, have been associated with increased stomach acidity. In contrast to the results of McLeay *et al* (1973), hypersecretion of hydrochloric acid was observed in surgically-prepared and parasite-free fundic pouches of sheep with abomasal *O.circumcinta* infections (Anderson, Blake & Titchen, 1976). This

they associated with an augmentation in the secretion of gastrin, and in a subsequent study of the same parasite in sheep, hypergastrinemia was observed (Anderson, Hansky & Titchen, 1981). Furthermore, direct transfer of adult *O.circumcinta* into the abomasum of a previously uninfected sheep has been followed by an elevation in gastrin concentrations within 24 h (Titchen, 1982), and a similar effect has been detected in calves given abomasal transplants of adult *O.ostertagi* (McKellar, 1984). Gastrin is a hormone that affects gastric acid secretion, gut motility and epithelial growth. Elevated gastrin concentrations have also been measured in rats during experimental infections with *Trichinella spiralis* (Castro, Copeland, Dudrick & Johnson, 1976), and *Taenia taeniaeformis* (Cooke, Williams & Lichtenberger, 1981), but in experimental infections of rats with *Hymenolepis diminuta* (Castro *et al*, 1976) and *Nippostrongylus brasiliensis* (Ovington, 1985b) gastrin concentrations were not significantly different from those measured in uninfected controls. Experimental infections of sheep with *Trichostrongylus colubriformis* have revealed depression in plasma gastrin concentrations, and this may have affected the gastric acid secretion, which was also found to be reduced (Titchen, 1982).

Thus, it has been demonstrated that infection with intestinal helminths influences host gastric secretion, and this may be mediated through an alteration in gastrin production.

1.4.2. INTESTINAL DIGESTION

Before nutrient absorption can occur in the GI tract, complex carbohydrates must be broken down to simple sugars, fats must be emulsified and reduced to glycerol and monoglycerides, and proteins hydrolysed to small peptides and amino acids. The physical location of intestinal helminths facilitates the possibility of their interference with these processes.

Symons and Fairbairn (1962, 1963) measured the activity of pancreatic amylase and epithelial brush border maltase activity in rats infected with *Nippostrongylus brasiliensis*. Although the pancreatic secretion of amylase was found to be within normal limits, the enzyme concentration in the jejunal fluid was abnormally low,

apparently due to the increased volume of liquid in the intestine of infected animals. The maltase activity of the brush border, even after adjustment for dry weights, was found to be reduced by approximately 50% in infected animals. The mechanism of this reduction is not clear, but inhibitor production is unlikely to be responsible, as *N.brasiliensis* has not been observed to produce measurable amounts of antienzymes. It is possible that the loss of enzymic activity correlates with damage to the macrostructure of the intestinal surface, which is known to occur during *N.brasiliensis* infection in rats (Martin, 1980; Cheema & Scofield, 1984). Whether losses of enzyme activity in the enteric mucosa will necessarily have a critical effect on digestion is questionable. Symons (1966) suggests that, apart from during the very heaviest infections, intestinal enzyme reserves will allow for normal digestion. However, in the very heavy infections appreciable amounts of maltose and glucose were measured to pass into the large intestine. Sucrase is another disaccharidase that has been implicated in being affected by nippostrongylosis. The activity of this enzyme has been demonstrated to be significantly reduced on day 11 p.i. of primary experimental infections of *N.brasiliensis* in the rat (Mayberry, Bristol, Cajas & Tellez, 1986), but no attempt was made to correlate this with alterations in intestinal structure, and the data was inadequate to decide whether the enzymic depression was detrimental to the health of the host. Reduction in the activity of maltase and lactase has also been measured in lambs harbouring infections of *Nematodirus battus* between days 12 and 16 p.i. (Coop, Mapes & Angus, 1972).

Lactase activity has been demonstrated to be reduced during ascariasis. Forsum *et al* (1981) observed a decrease related to infection intensity in the lactase activity of the mucosa of pigs infected with *Ascaris suum.* When given an oral dose of lactose, the blood glucose levels rose significantly less than in uninfected control pigs, suggesting an associated lactose intolerance. The activity of mucosal sucrase and maltase however, remained at control levels suggesting that this was an enzyme

specific effect. It could be that as lactase is the most superficially located disaccharidase of the mucosa, this reduction in activity is a consequence of either physical damage to the brush border by the worms, or an increase in the rate of turn-over of mucosal cells to such an extent that full lactase activity is never developed before the cell is lost. There is indirect evidence that lactase activity is likely to be reduced in *Ascaris lumbricoides* infections in man. Lactose digestion was quantified in Panamanian pre-school children by following the fate of an oral lactose load by monitoring breath hydrogen. A significant difference was detected between uninfected children and those harbouring *A.lumbricoides*, and following anthelminthic treatment, lactose digestion increased (Carrera, Nesheim & Crompton, 1984, Taren *et al*, 1987).

There is less evidence to show that intestinal helminths affect protein and fat digestion. Inhibitors of pancreatic proteases have been isolated from *A.suum* (Homandberg & Peanasky, 1976), and *Hymenolepis diminuta* has been reported to inactivate trypsin (Schroeder, Pappas & Means, 1981), but there is no direct evidence of impaired protein digestion. In rats, the pancreatic secretion of trypsin was not impaired during infection with *N.brasiliensis* (Symons & Fairbairn, 1962), but in sheep infected with *Trichostrongylus vitrinus*, a significant decrease has been detected in the activity of both trypsin and chymotrypsin, particularly during the first 9 weeks of infection (Jones, 1982). Mechanical obstruction of the biliary tree sometimes occurs during ascariasis in humans (Pawlowski & Davis, 1989) and accumulations of flukes in the bile duct of sheep with *Fasciola hepatica* infections can cause biliary congestion (Sykes, 1983). A consequence of this could be a deficiency in luminal bile salt concentration, and hence decreased emulsification of fat and absorption of its digestion products.

1.4.3. COMPETITION FOR LUMINAL NUTRIENTS

It is generally considered that intestinal helminths are unlikely to deprive their hosts of appreciable quantities of macronutrients (Von Brand, 1979). However, some investigations have indicated that appropriation of nutrients by some parasites do

have significant effects on the host. For example, Mettrick (1973) demonstrated that although rats infected with *Hymenolepis diminuta* utilised the same percentage of ingested nutrients as their uninfected controls feeding *ad lib.*, the parasitised rats also exhibited a 20% decrease in growth rate, and an 18% increase in energy intake/g increase in body weight. Crompton, Singhvi, Nesheim, & Walters (1981) observed that rats infected with *Moniliformis moniliformis* and feeding on a 4% fructose diet had significantly lower blood sugar concentrations than their uninfected controls feeding on the same diet. They also showed that both male and female *M.moniliformis* were lighter if host rats were fed on a 2% fructose diet, rather than a 4% fructose diet. They suggested these results are indicative of a competition between the *M.moniliformis* and the rat for dietary fructose.

Despite these examples, micronutrients are more likely to be potential candidates for competition between intestinal helminth and host resulting in pathological damage to the host (Rosenberg & Bowman, 1984). The best documented case of this appears to be the competition between *Diphyllobothrium latum* and its mammalian host for vitamin B-12 (Nyberg, 1958). Although host vitamin B-12 deficiency is related to infection intensity, and only observed in a relatively small proportion of those infected, it can result in a severe megaloblastic anaemia.

1.4.4. EPITHELIAL STRUCTURE: ABSORPTION AND PERMEABILITY

The development of structural lesions in the GI tract, particularly the mucosa, is one of the most striking features of intestinal helminth infections. Consequences of these lesions may include leakage of blood into the GI tract due to the disruption in intestinal integrity, and malabsorption of nutrients from the intestine due to a loss of absorptive surface area and a disruption of intestinal macrostructure.

Nutrient loss through leakage has been observed in infections in both man and experimental animals. Protein is the major macronutrient lost, in plasma, red cells, exfoliated epithelial cells and mucus (Holmes, 1985). Lunn, Northrop & Wainwright (1988) measured loss of endogenous protein through parasitic lesions in the gut

during nippostrongylosis in rats, by measuring faecal excretion of ^{51}Cr-labeled albumin which had been administered by intraperitoneal injection. They correlated this protein-losing enteropathy with alterations in plasma albumin concentrations. In concomitantly undernourished animals these effects were observed to be much greater. Similar observations were made by Nawa (1979), using the appearance of intravenously injected Evans' blue dye in the GI tract as a measurement of plasma protein translocation across the intestine during nippostrongylosis in rats. Increased mucosal permeability was associated with the apparent breakdown of the tight junctions between epithelial cells, and shown to be related to the intensity of infection. Leakage of plasma proteins has also been observed in sheep with abomasal infections of *Ostertagia circumcinta* (Symons, Steel & Jones, 1981) and infections of the small intestine with *Trichostrongylus colubriformis* (Steel, Symons & Jones, 1980). Protein-losing enteropathy is also severe during capillariasis in man (Whalen *et al*, 1969).

It is possible that in studies where nitrogen excretion is used as a measure of protein malabsorption, what is really being measured is loss of endogenous protein. Nevertheless, some of the protein lost into the GI tract may be reabsorbed further down the small intestine. Studies of *T.colubriformis* in lambs suggested to Poppi, MacRae & Corrigall (1981) that 60% of plasma protein lost into the GI tract was later reabsorbed, and Rowe, Abbott, Dargie & Holmes (1982) estimated a total reabsorption of all nitrogen lost into the GI tract by the end of the small intestine, during *Haemonchus contortus* infections in sheep.

Blood leakage into the GI tract during intestinal helminthiases must result in loss of nutrients other than protein. Iron deficiency may result from extensive loss of red blood cells. With each new attachment of hookworms within the gut, the microhaemorrhagic lesion which occurs may contribute to host iron deficiency (Miller, 1979). Massive loss of red cells is also an apparent consequence of infections with haematophagic parasites such as *H.contortus* (Abbott, 1982). Again, some of the iron lost will probably be reabsorbed further down the GI tract.

Whether the apparent malabsorption syndrome that has been reported in association with many cases of infections with intestinal helminths is as important as initially thought, has been a matter of controversy. Different researchers have used different criteria in the assessment of malabsorption, and infection intensity may be a critical factor as to whether absorption efficiency remains within limits considered to be normal. It is probably unjustified to conclude that malabsorption must occur merely because mucosal lesions appear in the duodenum and/or jejunum where nutrient absorption occurs to the greatest extent (Symons, 1976). A retrospective assessment of many experiments has revealed flawed methodology, in that only one region of the intestine has been investigated, or the exploratory period has been short. The intestinal functional reserve is large, and disturbance of absorption in one section, may be compensated for in the organ as a whole. Despite these misgivings, much research suggests that malabsorption occurs during intestinal helminth infections, that this may be exacerbated by poor nutritional status, and that it probably originates from major histopathologic alterations in gut morphology.

During infections of *Trichinella spiralis* in guinea pigs, inflammation of the lamnia propria and a flattening of the mucosa characterised by villi being shorter, blunter and fewer in number were associated with malabsorption of glucose in particular sections of the intestine (Castro, Olson & Baker, 1967). An alternative theory proposed for the reduced glucose absorption was an alteration in the carrier protein in the active transport of glucose that is located in the epithelial cell brush border.

Detailed studies of the gut morphology of rats infected with *N.brasiliensis* have been performed (Symons & Fairbairn, 1962; Symons, 1976; Cheema & Scofield, 1982). In general, by day 10 p.i. some areas of the jejunum have become dilated and fluid filled and the walls thickened and flabby. There is partial villous fusion, accumulation of inflammatory cells in the lamnia propria, hypertrophy of the muscularis externa and an increase in the rate of crypt cell production (crypt

hyperplasia). These events result in the usual regular surface topography of the uninfected rat gut, being altered to an abnormal flattened form. In hosts feeding on sub-optimal diets, the lesions appear to be more extensive, and there is evidence that haemorrhage is more probable (Martin, 1980).

Whether a malabsorption syndrome could be a consequence of these alterations has been investigated at length. Symons and Fairbairn (1962) observed a reduction in the absorption of glucose in the jejunum during nippostrongylosis, but this was subsequently qualified when Symons (1966) demonstrated that the absorption of glucose within the entire intestine showed no significant reduction. More recent investigations have shown a depressed absorption of glucose along the entire length of the small intestine during experimental nippostrongylosis in rats (Nolla, Bristol & Mayberry, 1985). These studies involved the effect of single large infections on intestinal pathology. Cheema & Scofield (1984) exposed rats to low level daily doses of *N.brasiliensis* larvae (i.e. 'trickle' infections), but were unable to demonstrate any definite trends in the absorptive capacity of the small intestine with respect to glucose. They associated this difference in their results with an immune adaptation; in thymus deprived rats, crypt hyperplasia and villous atrophy are not observed during *N.brasiliensis* infection (Ferguson & Jarrett, 1975). That glucose malabsorption occurs, and is rectified, more rapidly in secondary than primary infections with *N.brasiliensis* in rats has also been related to host immunological adaptation (Scofield, 1980).

Subtotal villous atrophy, flat mucosa, low surface epithelium and protruding crypt openings have also been observed during infections with *T.colubriformis* in sheep (Barker, 1975). In man, Tripathy, Duque, Bolanos, Lotero & Mayoral (1972) have associated altered gut structure with impaired absorption of fat and d-xylose in children harbouring heavy burdens of *Ascaris lumbricoides.* Particularly, jejunal mucosa biopsies revealed broadening and shortening of villi, elongation of crypts and cellular infiltration of the lamnia propria. Brown, Gilman, Khatun & Ahmed (1980) observed an apparent improvement in fat and nitrogen absorption in children

infected with *A.lumbricoides* after anthelmintic therapy, but no attempt was made to correlate nutrient absorption with gut structure. Some studies of *A.lumbricoides* have revealed no significant association between malabsorption of nutrients and infection with *A.lumbricoides*, but this may be related to study design and intensity of infection. For example, Freij, Meeuwisse, Berg, Wall & Gebre-Medhin (1979) found no significant differences in nitrogen, fat and xylose absorption between lightly infected and uninfected children, but mean worm burden was low, chemotherapy was not successful, and dietary intake was not measured or considered. A reduction in fat absorption was associated with infections of pigs with *Ascaris suum*, but the reduction was not found to be significant (Stephenson, Pond, Nesheim, Krook & Crompton, 1980).

A number of studies have linked ascariasis with impaired absorption of fat soluble vitamin A. Sivakumar & Reddy (1975) demonstrated that infected children absorbed significantly less of a test dose of radioactively labelled vitamin A than uninfected children. A comparable study by Mahalanabis, Jalan, Maitra & Agarwal (1976) obtained similar results.

Abnormal xylose absorption has been associated with hookworm infections (Sheehy, Meroney, Cox & Soler, 1962), but amongst human intestinal helminth infections, strongyloidiasis is most commonly associated with a malabsorption syndrome. This conclusion seems to be based upon relatively small scale studies. For example, Milner, Irvine, Barton, Bras & Richards (1965) connected a malabsorption syndrome and strongyloidiasis on the basis of only four patients, and O'Brien (1975) directly related steattorhoea and malabsorption of D-xylose and vitamin B-12 to strongyloidiasis after a study involving only seven subjects. Also, not all studies have detected this association. Kotcher, Miranda, Esquivel, Pena-Chavarria, Donohugh, Baldizon, Acosta & Apuy (1966) were unable to find evidence of malabsorption during chronic strongyloidiasis, and similarly malabsorption did not seem to be a consequence of *Strongyloides stercoralis* infection in the study by

Garcia, Sessions, Strum, Schweistris, Tripathy, Bolanos, Lotero, Duque, Ramelli & Mayoral (1977), unless malnutrition was already observed.

It appears probable that malabsorption and plasma leakage into the G.I. tract occurs to a certain extent during several intestinal helminth infections. This can be correlated with pathological alterations in gut morphology, and these may be associated with the immune response of the host to the infection. However, the relative importance of these events in influencing the nutritional status of the host has yet to be ascertained.

1.5. POST-ABSORPTIVE EVENTS

1.5.1. ENZYMES OF INTERMEDIARY METABOLISM

The association of intestinal helminths with alterations in the activity of intermediary metabolism is less well researched than other areas of nutritional physiology, presumably because of technical difficulties. Nevertheless, what research has been done indicates that this field is worthy of further exploration.

Symons & Fairbairn (1963) found the activity of the cytoplasmic enzyme, succinate dehydrogenase, was strikingly reduced in the jejunal villi of rats during infections with *Nippostrongylus brasiliensis*, as measured by a histochemical technique. This enzyme was selected for investigation because of its essential respiratory role and because Padykula, Strauss, Ladman & Gardner (1961) had measured a reduction in its activity during non-tropical sprue. Ovington (1985b) measured a reduction in activity of a number of enzymes, by *in vitro* assay, during infection of rats with *N.brasiliensis*, for example liver fructose-1,6-bisphosphatase and liver glucose-6-phosphatase. The activity of liver phosphoenolpyruvate carboxykinase, however, was shown to be elevated during nippostrongylosis (Ovington, 1985b), whereas the activity of liver alanine-amino-transferase has been seen to fluctuate in rats during the course of infection with *N.brasiliensis* (see Section 2, chapter 4; Robertson, 1989). The reason for these alterations in the activity of the enzymes of intermediary metabolism is unclear and awaits further

work. It is possible that they are associated with a phase of the host immune response. As more evidence accumulates that cytokines may have a role in the in the immune response to intestinal helminths, it is probable that further aspects of host intermediary metabolism will be observed to be affected by infection with parasites (Klasing, 1988).

1.5.2. ENDOCRINE RESPONSES

The fluctuating and steady concentrations of metabolites circulating in the blood and body fluids are influenced by the actions of various hormones. During parasitic diseases profound alterations in the host endocrinological status have been measured (Titchen, 1982).

Crompton, Arnold, Coward & Lunn (1978) measured an increased plasma insulin concentration in rats on a 2% protein diet infected with *Nippostrongylus brasiliensis*. However, Ash, Crompton & Lunn (1985) found that plasma insulin concentrations were highly variable during *N.brasiliensis* infection, but in general presented a reduction. Ash *et al* (1985) also measured a reduction in the concentration of plasma glucocorticoid, adrenocorticotrophic and thyroid hormones. Ovington (1985b) also measured concentrations of metabolically important hormones during nippostrongylosis in rats. A reduction in plasma insulin was observed, whereas the concentrations of entero- and pancreatic glucagon increased significantly.

During the terminal stages of trichostrongylosis in sheep increases in the concentration of plasma corticosteroids as great as fifteenfold have been identified, and also significant reductions in the concentrations of plasma insulin and thyroxine (Prichard, Hennessy & Griffiths, 1974). Similarly in pigs infected with *Strongyloides ransomi* an increase in the concentration of plasma corticosteroids, and a decrease in plasma insulin concentrations have been detected (Enigk & Dey-Hazra, 1978). Pair-feeding experiments involving sheep harbouring *Trichostrongylus colubriformis* have suggested that the reductions in plasma insulin concentrations may be related to food intake, but plasma corticosteroid levels are influenced by

other factors associated with the infection.

Bailenger & Carcenac (1974) measured a temporary elevation in plasma corticosterone concentrations in rats during the initial stages of infections with *Strongyloides ratti*, but a reduction in plasma corticosterone concentrations shortly before the nematode was eliminated by a spontaneous "self-cure". I observed a similar pattern in *N.brasiliensis* infections in rats (see Section 2, chapter 6).

The aetiology and effects of these alterations in hormone concentrations is debatable. Some changes may occur initially as a physiological response to maintain homeostasis, but by developing into considerable perturbations, the host's health and chances of survival may be compromised (Titchen, 1982). It has been suggested that the body's response to the stress of frequent infections such as hookworm disease and ascariasis may cause an increase in plasma cortisol concentrations, which may in turn, cause wasting in malnourished children (Whitehead, Coward, Lunn & Rutishauser, 1977). It is also possible that high levels of circulating corticosteroids may compromise immunological protective mechanisms, perhaps exacerbating other deleterious effects of intestinal helminth infection.

These data suggest that although a hormonal effect is apparent in a number of infections with intestinal helminths, further research is indicated to elucidate what occurs and why, to what extent, and the metabolic and pathological importance of these fluctuations.

1.6. OVERVIEW

Although this review has demonstrated that intestinal helminths are associated with a plethora of alterations in the nutritional physiology of the mammalian hosts, it has also clearly indicated the requirement for further research within this field. The published literature on this wide topic is sometimes unclear and contradictory. There are some cases of conclusions being drawn from poor experimental or inadequate clinical studies, and occasionally these studies have been used as a basis for further investigation by other researchers. The requirement for further careful

observation and experimentation is highlighted.

SECTION 2

INFLUENCES OF *NIPPOSTRONGYLUS BRASILIENSIS* INFECTION ON RAT CARBOHYDRATE METABOLISM: AN EXPERIMENTAL STUDY

SECTION 2

CHAPTER 1: *NIPPOSTRONGYLUS BRASILIENSIS* INFECTION OF RATS: A MODEL SYSTEM FOR STUDYING THE INTERACTION OF INFECTION AND HOST NUTRITION

Nippostrongylus brasiliensis (Nematoda: Strongylida) has a direct life cycle and a cosmopolitan distribution involving various species of rodent as host. The rat, being the most typical and natural host, is used preferentially in laboratory studies. The life cycle can be divided into a number of phases: a free-living stage; a parasitic systemic stage; and a parasitic intestinal stage. Eggs, passed in the faeces of the parasitised host, hatch within 24 h at room temperature releasing first-stage rhabditiform juveniles, commonly referred to as larvae (L1). These free living larvae moult twice within 6 days, resulting in the formation of exsheathed filariform infective third-stage larvae (L3). Host rodents are believed to be naturally infected by active penetration of L3 through the intact skin, but in the laboratory situation the hosts are usually infected by subcutaneous injection of L3 in order to monitor more carefully the number of larvae administered. Hosts can also be infected orally (Schwartz & Alicata, 1934). During an experimentally established primary infection the larvae migrate via the blood stream to the lungs, the usual site for the third moult. The resultant L4 larvae then migrate via the trachea, oesophagus and stomach to the small intestine where the final moult occurs. The immature adult worms, grow and attain sexual maturity. By the end of the day 5 p.i. inseminated females can be found in the gut, and by day 6 p.i. eggs are being passed in the faeces (Kassai, 1982).

For a number of reasons *N.brasiliensis* is a widely studied parasite. First, the life cycle of the parasite is similar to that of a number of gastrointestinal nematodes of medical and veterinary importance. Indeed, it is occasionally referred to as the 'rat hookworm'. However, although its development does resemble that of

hookworms closely, it is not closely related to the *Ancylostomatidae*. Secondly, the life cycle of the parasite is relatively rapid, and there is no involvement of an intermediate host. Furthermore, its maintenance requires minimum labour and time. Thirdly, handling of *N.brasiliensis* is relatively uncomplicated and safe. It is easy to count, and its host is commonly utilised in experimental biology and is comparatively inexpensive. Fourthly, abundant research has already been performed on this host/parasite model. The extensive background literature that this has generated (Kassai, 1982), forms a basis for further work and provides the context in which ones own observations can be placed.

Investigations into how *N.brasiliensis* may interact with the nutrition of its host first concentrated on pathological damage to the intestine, and how this may affect both the rate of propulsion of intestinal contents, and absorption of nutrients. Largely initiated by Symons (1966 & 1976), this work also included investigating the possibility of interference with digestive enzyme activity (Symons & Fairbairn, 1962 & 1963; Symons, Gibbins & Jones, 1971), and expanded to study morphological damage to the epithelial structure and function by scanning electron microscopy (Ferguson & Jarrett, 1975, Martin, 1980). Further research has diversified from centralising on localised intestinal damage, and now includes studies concerned with altered appetence (Crompton, Walters & Arnold, 1981; Keymer, Crompton & Walters, 1983; Ovington, 1985a) including learned taste aversion (Keymer, Crompton & Sahakian, 1983), alteration in the activity of enzymes of intermediary carbohydrate metabolism (Symons & Fairbairn, 1962; Ovington, 1985b), and altered endocrine response (Crompton *et al*, 1978; Ovington, 1985b; Ash *et al*, 1985). Fluctuations in the concentrations of plasma constituents, such as total protein (Crompton *et al*, 1978; Ovington, 1985b), albumin (Crompton *et al*, 1978; Ovington, 1985b; Lunn *et al*, 1988), and glucose, triglyceride and non-esterified-fatty-acid (Ovington, 1985b) have also been measured.

The experimental approach employed in the work described below focuses on a particular aspect of hepatic intermediary carbohydrate metabolism, namely the

activity of the gluconeogenic enzyme, alanine-amino-transferase, during nippostrongylosis. A significant alteration in the activity of this enzyme in rats on day 9 of a primary infection with *N.brasiliensis* was reported by Ovington (1985b). My research has developed from her observation, not by investigating other similar parameters, but by exploring the mechanism of this fluctuation in enzyme activity and its physiological basis.

SECTION 2

CHAPTER 2: MATERIALS AND METHODS.

1. Introduction.

The description of materials and methods in this chapter provides a synopsis of the procedures employed during the experimental investigations. Further details concerning, for example, host sex and experimental chronometry, are specified in the relevant chapters. All procedures employed were covered by both a Home Office Project Licence (PPL 60/00370), and a Home Office Personal Licence (PIL 60/01789).

2. Parasite. *Nippostrongylus brasiliensis.*

1. Source.

The isolate of *N.brasiliensis* used in the research described in this dissertation was initially obtained from the Moredun Institute, Edinburgh, where it has been maintained since at least 1976. From October 1986, I maintained this line in the Department of Zoology at the University of Glasgow.

2. Maintenance.

Rats of known age, sex and strain were infected with approximately 4000 third-stage larvae to establish primary infections. Faecal pellets were collected on days 6,7 and 8 p.i. The pellets were softened by soaking in tap water at room temperature, then gently comminuted to a paste by pestle and mortar. Activated charcoal granules were mixed with the faecal paste, and spoonfuls of this mixture were placed onto 70mm discs of hardened filter paper, resting on small pads of damp cotton wool within plastic Petri dishes. The lids of the Petri dishes were raised, to avoid contact with the faeces, by strips of plastic. The faeces were incubated for 6 days in the dark, in an incubator set at a temperature of 25^{o}C, and checked on alternate days to ensure that the cultures remained moist. Larvae were collected from the edge of the filter paper by a modified Baermann procedure (Markell & Voge, 1976),

concentrated by centrifugation, rinsed, and stored in tap water in glass Petri dishes at room temperature in the dark.

3. Infection of rats.

Subcutaneous inoculation. In all experiments, except where stated otherwise, infections of *N.brasiliensis* were initiated by subcutaneous inoculation of an estimated 4000 third-stage larvae. The larvae used were aged between 7 - 30 days; larval infectivity shows little variation within this age range (Keymer, Martin & Wainwright, 1983). Doses were estimated by serialised dilution and aliquot counts. When a range of doses was required for a particular experiment (number of larvae per individual rat), the suspension involved in the infection of the highest dose was diluted as appropriate to obtain the lower doses. Calculation of larval dose on a host body weight basis is not advised by Kassai (1982) and consequently was not employed. To check all estimated doses, replicates were inoculated into tubes of formalin so that subsequent counts of known aliquots could provide a closer assessment of the number of larvae actually administered to the rats.

Light anaesthesia was induced in rats with diethyl ether to reduce stress, and the larvae were administered by subcutaneous inoculation into the skin of the lower abdomen in a volume of tap water not exceeding 0.4 ml, using a 21G or 23G needle. Uninfected (=control) rats were similarly injected, not with larvae, but with the same volume of the water in which the larvae had been stored. For primary infections, the day of infection was termed day 0. In some experiments, secondary infections were required. Secondary infections were always commenced between days 18 - 20 p.i. of a primary infection, and the day that the secondary infection commenced termed day 0(2).

Oral transfer of adult worms. In some experiments *N.brasiliensis* infections were set up which by-passed the migratory larval phase. Rather than use surgical techniques of transplant as have been employed by some researchers (Ogilvie, 1965; Ovington, 1985b), I considered that it would be less traumatic for the rat to attempt to modify

for *N.brasiliensis*, the method outlined by Stoddart & Crompton (1988) for oral transfer of *Moniliformis moniliformis* between rats. Adult *N.brasiliensis* were collected from the gut of infected rats on day 7 p.i. by the Baermann technique. It was calculated that if the collected worms were allowed to stand undisturbed for an hour then 250 µl of settled worms of this age should number approximately 1000. Although not of great accuracy, this method of approximating worm numbers is rapid, avoids much interference with the worms and minimises the possibility of causing damage to them.

Recipient rats were dosed with Cimetidine, in the form of Tagamet (Smith, Kline and French, Welwyn Garden City), a specific, competitive, H_2-receptor antagonist that inhibits gastric acid secretion. The concentration of Cimetidine used was 480mg/ml water, and the dosage 0.5 ml/250g rat body weight. An hour after dosing with Cimetidine, light anaesthesia was induced in the rats with diethyl ether, and the 250 µl of worms administered orally to each rat using a Pasteur pipette. Control rats received 250 µl of the 0.6% saline in which the worms had been stored. Replicate aliquots of 250 µl of settled worms were fixed in 10% formalin for individual counting. These replicate doses were always found to exceed 1000 worms, but the excess was generally compensated for by loss of worms through adherence to the walls of the Pasteur pipette. Eggs could be found in the faeces of the infected rats within 3 days p.i. and if rats were killed 4 days p.i. establishment of approximately 75% of the inoculum was observed.

3. Host rats.

In all experiments, the rats used were outbred Wistars aged between 6- and 12-weeks old, and obtained from Bantin and Kingman, Hull. Both female and male rats were utilised. Throughout the experiments, the diet was a commercially obtained open formula pellet (Labsure) and rats had free access to water at all times.

Experimental groups always consisted of seven rats per group. The rats were housed either together in their groups of seven, or individually, according to the

nature of the experiment. Uninfected rats being fed *ad lib* were always housed in their experimental groups; although this was often not experimentally ideal, cage space restrictions made this necessary. The rats were held in a well serviced animal suite with a 12h regime of light and darkness. In order to reduce the risk of stress at sampling, rats were regularly handled throughout the course of the experiments.

1. Diet, food intake and pair-feeding.

Experimental work was limited to well-nourished rats. Both infected and uninfected control rats were allowed to feed *ad libitum* from the pelleted standard diet. When food intake was being measured, 100.0g of food was offered to individually caged rats between 10.00 - 11.00h. Twenty-four hours later the remaining food weighed to the nearest 0.5g and then made up to 100.0g again. No rat feeding *ad lib.* ever ate all of the available food.

In certain experiments, each infected rat had an uninfected, pair-fed partner of similar age, weight and appetence (as determined by measurement of daily food intake for several days prior to infection). The inclusion of pair-fed controls was made to assess whether reduction in food intake due to the infection contributed to any of the variables under investigation. Each day after day 0, between 10.00 - 11.00h, pair-fed rats were given the weight of diet that their infected partners had consumed in the previous 24 h. However, pair-feeding experiments are not entirely satisfactory as enforced reduction in food-intake not only is a stress factor, but also alters feeding behaviour. Pair-fed control rats consistently ate all of their food, and it was generally consumed rapidly, and as soon as it was offered.

In all experiments food was withheld overnight before sampling, for no longer than 15h.

2. Anthelmintic treatment.

In some experiments, elimination of the parasite population before the natural termination of the infection (approximately 14 days p.i.) was required. Ovington (1985b) cites Valbazen, a commercially prepared drug (Smith, Kline and French, Welwyn Garden City) containing 10% albendazole as effective in eliminating

substantial *N.brasiliensis* infections. Accordingly, Valbazen provided by the Department of Pathology, Glasgow Veterinary School, was utilised in some experiments by oral dose. Each rat received 5mg albendazole/100g rat body weight. At *post-mortem* examination efficient elimination of the parasite at this dosage was apparent.

3. Body temperature.

Rectal temperatures of rats were measured daily for 2 days before an infection with *N.brasiliensis* and thereafter daily for 15 days p.i. Temperatures were taken between 08.30 - 10.00h with a standard rectal thermometer inserted approximately 15mm into the rectum for between 60 to 120 sec.

4. Blood collection.

Blood samples (see below) were obtained from rats for experiments requiring measurements of metabolites, minerals, hormones, enzymes, cell counts and the preparation of plasma or serum for other purposes. For all experiments other than the measurement of plasma glucose and α_2-macroglobulin, in which blood was collected by superficial venesection, blood was collected by cardiac puncture. Blood was always collected in the morning between 09.30 - 12.00 h.

Plasma preparation for glucose assays. A longitudinal study was conducted to monitor the plasma glucose concentrations of the same group of rats on alternate days over the course of an infection with *N.brasiliensis*. Blood collecting procedures were designed to cause minimal stress to the rats. Each rat was encouraged to run into a restraining tube so that blood could be obtained quickly by superficial venesection from the tails. Blood flow to the tail was increased by immersing the tail in warm water, the tail was swabbed with 70% ethanol and then the extreme tip snipped off cleanly. Blood was collected into paediatric-size plastic bottles coated with fluoride oxalate (Sterilin) and swirled gently to ensure thorough mixing. Fluoride oxalate prevents clotting and halts glycolysis. Plasma was prepared by spinning at 11600g for 4 min. The plasma was held on ice until the glucose assays

were performed, no more than 6 h after the collection of blood.

Preparation of plasma/serum for other assays and measurements. Anaesthesia was induced in rats with diethyl ether and blood was collected by cardiac puncture into a 10 ml syringe. In the preparation of plasma, the blood was promptly transferred to tubes containing sodium heparin (Sigma), and swirled gently to ensure mixing. For preparation of serum the blood was transferred to Eppendorf tubes and allowed to clot. The tubes were spun at 11600g for 4 min for the preparation of both serum and plasma. Corticosterone, α_2-macroglobulin and zinc concentrations were measured in plasma. All other experiments utilised serum. In most experiments involving the measurement of factors within the serum or plasma, these were performed on the same day as blood collection, with the serum/plasma held on ice until required. However, for the determination of zinc and α_2-macroglobulin, plasma aliquots were frozen in order that measurements could be performed on the same day. It has been shown that freezing causes no alteration to serum zinc concentrations, (Pekarek, Beisel, Bartelloni & Bostian, 1972).

Leukocyte counts. When blood was required for leukocyte counts, the blood was collected by cardiac puncture into a 5 ml syringe treated with Hanks's-heparin solution. A sample of 0.05 ml blood was added to 0.95 ml of white cell diluting fluid (2% acetic acid with a few grains of gentian violet) which lyses the red blood cells. Each blood sample was diluted 5 times, and eight counts were made on each dilution using a Neubauer haemocytometer.

5. Immunosuppression.

In some experiments, both infected and uninfected rats were immunosuppressed by the administration of betamethasone (Betsolan, Glaxovet). The betamethasone, which was provided by Glasgow Veterinary School, was injected subcutaneously into the rats at a dosage of 0.8mg/kg body weight. In rats with *N.brasiliensis* infections, the betamethasone administration commenced on day 7 p.i. and continued at this dosage on alternate days until the experiment was terminated on day 14 p.i. An antibiotic (Terramycin, Pfizer) was given in the drinking water to all rats regardless

of immunosuppression at a concentration of 200mg oxytetracycline hydrochloride/l tap water. Fresh antibiotic solution was prepared every 24h.

4. Biochemical assays.

1. Plasma glucose.

Plasma glucose concentrations were measured with a glucose oxidase assay kit (Sigma Diagnostics no. 315) in which any glucose in the sample is initially oxidised to gluconic acid and hydrogen peroxide in a glucose oxidase catalysed reaction. The hydrogen peroxide reacts with 4-aminoantipyrine and p-hydroxybenzene sulphonate in the presence of peroxidase to form a quinoneimine dye which has an absorbance maximum at 505 nm. The concentration of glucose is directly proportional to the colour formed. The absorbance was measured using a Phillips PU-8610 UV/VIS kinetics spectrophotometer.

2. Alanine-amino-transferase

Alanine-amino-transferase (ALT) activity was measured by an *in vitro* assay in rat serum and preparations of rat liver. Preparation of liver samples was based upon the method described by Ovington (1985b). The liver was removed whole from the rat and weighed to one decimal place. A small piece of between 1.5 - 2.5 g was taken, weighed, rinsed in homogenising medium (0.15M KCl, 5mM $MgCl_2$, 5mM EDTA.2Na; pH 5.5) and blotted dry. From this piece of liver 10 ml of liver homogenate were prepared. The homogenate was centrifuged at 5000**g** for 5 min at $4^{o}C$ and the supernatant fluid retained for assay. All samples, homogenates and supernatant fluids were held on ice, for no longer than an hour *post mortem.*

The assay (Sigma procedure no. 59-UV) involves the enzyme's catalysing the transfer of the amino group from alanine to 2-oxoglutarate to form glutamate and pyruvate. The pyruvate formed is then reduced to lactate in the presence of lactate dehydrogenase, with the simultaneous oxidation of reduced nicotinamide adenine dinucleotide (NADH). The rate of decrease in the concentration of NADH, which can be monitored by following the rate of decrease in absorbance at 340 nm using

cuvettes of suitable optical activity, is directly proportional to ALT activity. Duplicate assays were performed on each sample. A Phillips PU-8610 UV/VIS kinetics spectrophotometer was used in all the absorbance measurements.

In some of the liver assays, the effect of adding particular serum samples or homogenate supernatants of either larval or adult *N.brasiliensis* was investigated. Samples of 0.02 ml of the serum or parasite homogenate was added to the reagents immediately before the addition of the liver homogenate supernatant fluid.

A number of tests were run to eliminate procedural errors, identify artifacts, and standardise the methods. These included checks on enzymic delay with time, effects of temperature fluctuations and interference by glutamate dehydrogenase in the liver assays, (see Appendix 2.2.1). One unit of enzymic activity (U) is defined as the amount of enzyme which produces 1 μmol of NAD/min under the conditions used.

3. Plasma corticosterone.

Corticosterone measurements were made on rat plasma and employed a method adapted from Ovington (1985b). This method utilises the steroid binding properties of the plasma protein transcortin (Lunn, Whitehead, Baker & Austin, 1976). Plasma aliquots of 10 μl were taken and protein was precipitated by addition of 100 μl of absolute ethanol. The sample tubes were shaken and dried overnight by evaporation in an incubator set at 37°C. The unlabelled hormone in the sample was allowed to compete with a known amount of tritiated hormone for the binding sites of transcortin. One ml of protein binding reagent (1l of phosphosaline buffer at pH 7.4 containing 3ml rat plasma and 75μCi ^{3}H-corticosterone (Amersham International Ltd, Amersham, UK) was added to each sample before incubation at 45°C for 10 min to allow the steroid to redissolve. The samples were cooled in ice and remained at this temperature until completion of the assay.

After an incubation period of between 30 - 40 min, the bound and free hormone were separated by the absorption of the free corticosterone onto dextran-coated charcoal. A suspension had been prepared containing 0.65 g dextran T-70

(Pharmacia Ltd) and 6.25 g Norit A charcoal (Sigma) in 1l of phosphosaline buffer. Within 30 sec, 400µl of this suspension were added to each sample. Exactly 12 min after the first addition the tubes were centrifuged for 7.5 min at 2000g, 4.5°C to pellet both charcoal and unbound hormone. Immediately after centrifugation 0.75 ml of the supernatant fluid was removed and added to 8 ml of scintillant (Ecoscint A, National Diagnostics) in a plastic scintillation vial and counted with quench correction. Samples were processed continuously in batches of 4, as the centrifuge rotor could only accommodate 4 tubes at a time. A standard curve over the range 0 - 52 µg/100ml (Hydrocortisone, Sigma Chemical Co. Poole, UK) was constructed and used to estimate corticosterone concentrations in the samples.

4. Plasma zinc.

To avoid contamination of plasma samples with exogenous zinc, all glassware, which was to come into contact with the blood or plasma, was acid-cleaned. Additionally, care was taken to ensure that white or colourless, rather than tinted plastics were used, for example, in the pipette tips. Plasma was frozen immediately after preparation, and held at -20°C for up to 3 months, by which time all the samples for zinc determination had been collected. Individual 0.6ml aliquots of plasma were diluted 1:1 with deionised water. Zinc concentrations were measured with an automated PU9200 atomic absorption spectrophotometer with an aspiration of 2 sec per sample of approximately 1 ml. Working standards of 0.1, 0.25, 0.5 and 1.0 ppm provided a calibration curve, (see Appendix 2.2.2), and deionised water was aspirated between samples to ensure that the line remained clean.

5. Plasma α_2-macroglobulin.

Frozen plasma samples were sent packed in dry ice to the M.R.C. Dunn Nutrition Laboratories, Cambridge for the measurement of α_2-macroglobulin concentrations. All the measurements were performed by Dr. G. Jennings. Measurements were made on 5 µl samples of plasma using a single radial immunodiffusion technique (Mancini, Carbonara & Heremans, 1965). The samples were left to diffuse into the 1.5% agar gells for 72h, washed in buffer and water, and then dried overnight.

Coomasie blue was used to stain the precipitin rings. The area within the precipitin ring, measured as ring diameter squared, is proportional to the antigen (α_2-macroglobulin) concentration which could be derived from interpolation from a standard curve

5.Data analysis.

The Mann-Whitney U-test and Kruskall-Wallis test were routinely used to compare values of parameters measured between the rat groups. In the text, a difference is referred to as significant when $P \leq 0.05$.

SECTION 2

CHAPTER 3: PLASMA GLUCOSE CONCENTRATIONS AND FOOD INTAKE.

1. Introduction.

Most of the monosaccharides absorbed from the gut are transported to the liver, but, in general, their metabolic usefulness requires that they be converted to glucose. Carbohydrate metabolism can thus be described as synonymous with glucose metabolism. Circulating blood glucose is an energy source for all of the body's cells, and a major substrate for the synthesis of other compounds. An elegant matrix of several mechanisms regulate its dynamic equilibrium, and therefore any disruption in carbohydrate metabolism can be detected simply by marked or prolonged fluctuation in blood glucose concentrations.

In many host-parasite relationships, disturbances in host carbohydrate metabolism have been detected, in both vertebrate and invertebrate hosts with both protozoan and helminth infections. In vertebrate hosts, these disturbances are often reflected in alterations in plasma glucose concentrations. For example, fluctuations in host plasma glucose have been associated with infections with African trypanosomes, *Fasciola hepatica*, various species of *Schistosoma*, and malarial infections (Von Brand, 1979). The physiological and biochemical backgrounds to these fluctuations are diverse, but a reduction in food intake is one of the better understood mechanisms that could result in a temporarily hypoglycaemic state. In this chapter, observations on the effect of *Nippostrongylus brasiliensis* infection, on food intake and on the concentration of plasma glucose are described.

That plasma glucose concentrations are subject to fluctuation under various stresses, including those related to intestinal helminth infections, is well known. Ovington (1985b) provided a detailed study of these alterations during *N.brasiliensis* infections in rats in a cross-sectional study that found a significant, dose-dependent, decrease in plasma glucose concentrations between days 8 - 9 p.i. For my research

such a complete analysis would be both wasteful and unnecessary. Instead, and to complement her results, a longitudinal study involving the same group of rats throughout the course of the infection was accomplished. The effect of albendazole (Valbazen; Smith, Kline & French, Welwyn Garden City) treatment on day 8 p.i. was also studied. The rationale for this, was to investigate whether elimination of the established adult parasite influenced plasma glucose concentrations.

Food intake was primarily measured as a by-product of experiments that necessitated pair-feeding. Extensive research has been carried out relatively recently on the alterations in food intake in association with *N.brasiliensis* infections (Ovington, 1985a; Crompton, Walters & Arnold, 1981). My results demonstrate supporting evidence, and enable my work to be placed in context with other research on this host-parasite relationship.

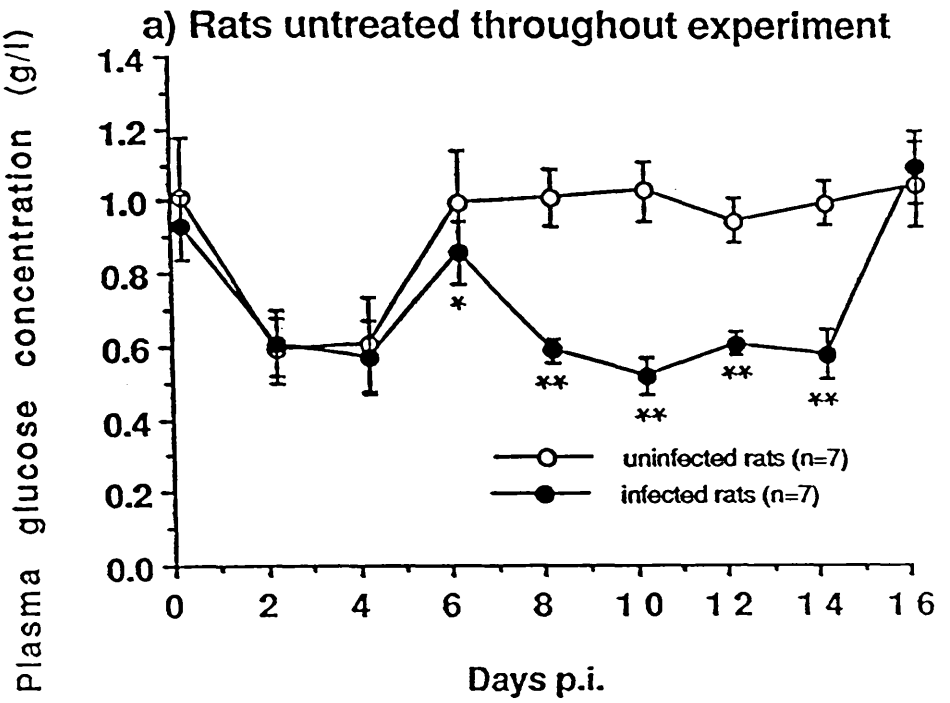
2.Results.

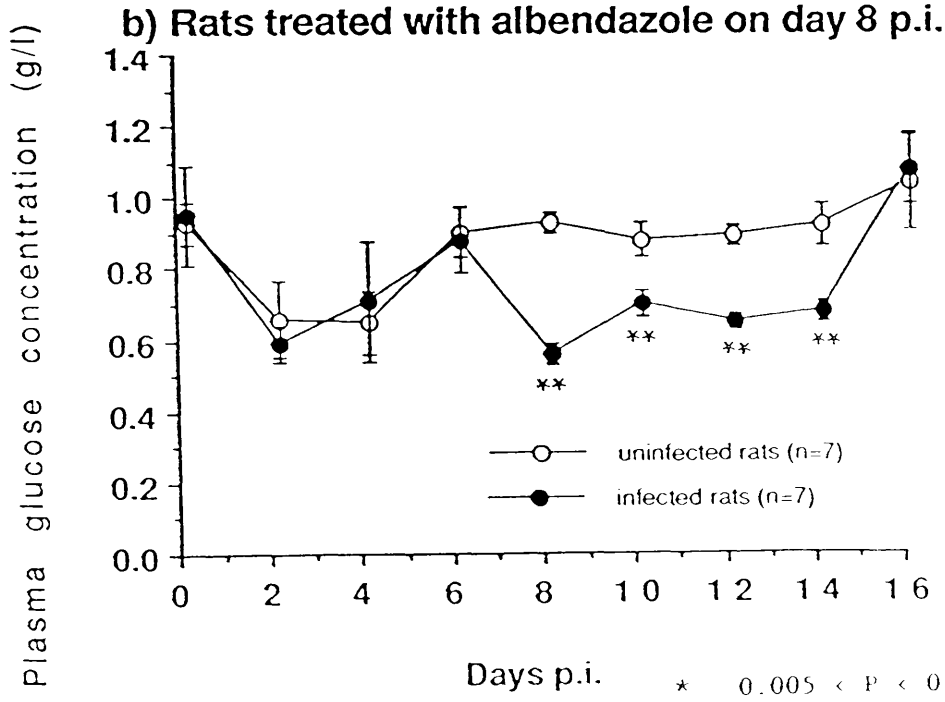
1. Plasma glucose concentrations.

Significantly different mean concentrations of plasma glucose were observed between uninfected and infected rats on days 6, 8, 10, 12, and 14 p.i. (Fig. 2.3.1.a and b). On day 10 p.i. the lowest mean concentration of plasma glucose in infected rats was measured (0.49 ± 0.05 g/l plasma, as compared with 1.00 ± 0.08 g/l plasma, $P = 0.0006$). On day 10 p.i. infected rats that had received albendazole treatment had significantly elevated plasma glucose concentrations, as compared to their untreated infected partners (0.67 ± 0.04 g/l plasma as compared to 0.49 ± 0.05 g/l plasma, $P < 0.05$). Until day 16 p.i. the plasma glucose concentrations of these rats remained significantly higher ($P = 0.0006$), although still very significantly below that of rats that had remained uninfected throughout the experiment ($P = 0.0006$). Although *N.brasiliensis* infections are effectively eliminated by day 14 p.i., plasma glucose levels were still low in both infected rats that had remained untreated and in those that had received albendazole treatment on day 14 p.i.

Intriguingly, a significant decrease in plasma glucose concentration was observed

FIGURE 2.3.1. RAT PLASMA GLUCOSE CONCENTRATIONS DURING PRIMARY NIPPOSTRONGYLOSIS





* $0.005 < P < 0.05$

** $0.0005 < P < 0.005$

in uninfected control rats 2 days after they had been dosed with albendazole ($P = 0.0023$). However, the reduction is not large, especially if compared to an inter-control group base line, and the values lie within the range considered to be normal (Horton; Smith, Kline and French, Welwyn Garden City, pers.comm.).

Another intriguing occurrence was the substantial decrease in plasma glucose concentration measured in all four groups of rats on days 2 - 4 p.i. Perhaps an external factor, for example, handling stress, might have contributed to this reduction.

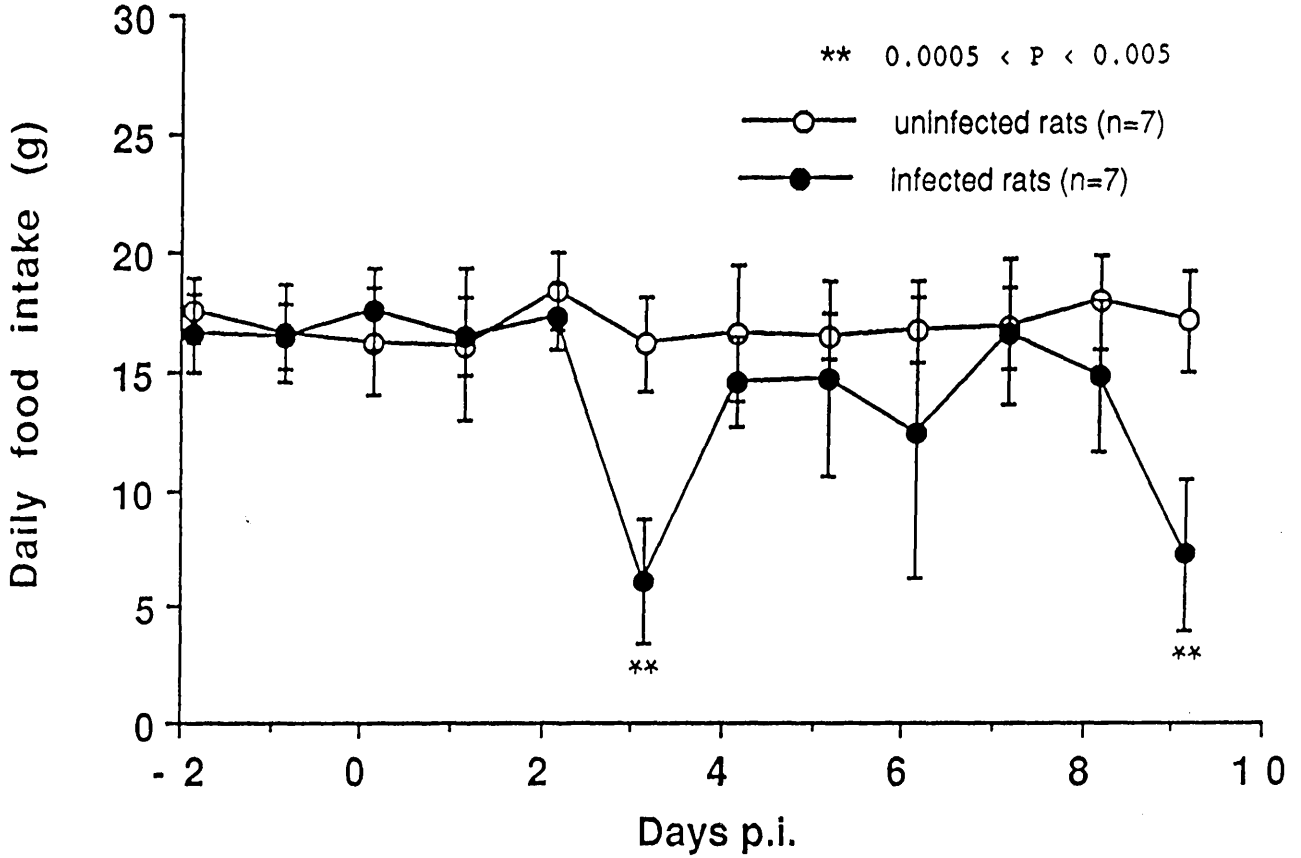
2. Food intake.

Food intake measurements (Fig. 2.3.2) show a striking and significant reduction in food intake on days 3 and 9 p.i. ($P = 0.0006$). A recovery to pre-infection levels subsequently would be expected, but measurements were not made beyond this time. Between days 4 - 8 p.i. food intake is reduced, but not significantly ($0.07 < P < 0.90$).

3.Discussion.

Ovington's (1985b) conclusion that *N.brasiliensis* infection in rats is associated with a reduction in plasma glucose concentration towards the end of the infection is largely borne out by the results I obtained. However, the decrease observed by Ovington is of considerably smaller magnitude than that which I found (on day 8 p.i. she measured a mean plasma glucose concentration of 0.86 g/l compared with 0.56 g/l). Ovington also measured a return to 'normal' or control levels by day 10 p.i., which I did not observe until day 16 p.i. by which time the infection would have been effectively eliminated. These differences might be explained by strain differences in the parasite and/or rats. Indeed, even in rats whose parasite populations were expelled on day 8 p.i. by albendazole treatment, it is not until day 16 p.i. that plasma glucose concentrations fall within the range considered normal. By day 8 p.i. the adult worm population is well established in the intestine and is at a stage of maximum egg production. By this time a large part of the parasites' effect

FIGURE 2.3.2. FOOD INTAKE DURING PRIMARY NIPPOSTRONGYLOSIS IN RATS



on the host will be in progress, including pathological events and the initiation of the immune response. It is not unduly surprising that albendazole treatment of infected rats on day 8 p.i. is only associated with a slight, but significant, attenuation of the observed decrease in plasma glucose concentration.

Mammalian plasma glucose concentrations are sustained in a state of variable homeostasis by a vast array of regulatory interactions (Newsholme & Start, 1981). It is important that glucose levels are maintained above a minimum concentration, for the functioning of tissues such as the nervous system, kidney medulla, and testis (Newsholme & Start, 1981). Glucose is an obligatory requirement, and when glucose concentrations are decreased due to, for example, reduced exogenous input, endogenous production and a reduced rate of utilisation will compensate. In certain situations the compensatory mechanisms may be compromised, resulting in hypoglycaemia. In the primary *N.brasiliensis* infection studied, between days 8 - 14 p.i. the rats are temporarily hypoglycaemic, indicating that rates of glucose production and intake are being exceeded by the rate of glucose utilisation. Either utilisation has increased greatly, or production and intake reduced greatly, or, more probably, both of these may have occurred to a lesser extent.

Ovington (1985a) demonstrated that primary infections with *N.brasiliensis* in adult rats are associated with alterations in food intake that are precise, reproducible and dose dependent. There are two minimal intake troughs on days 3 and 9 p.i. which are separated by 6 days of food intake that is reduced, but to a lesser extent. Within certain limits, the values that I obtained correspond closely to those described by Ovington. This decrease in food intake cannot solely be responsible for the decrease in plasma glucose concentrations. Endogenous gluconeogenic mechanisms and reduced glucose utilisation compensate for the loss of glucose due to reduced food intake in animals that are temporarily starved.

4. Summary

1. Plasma glucose concentrations and food intake were measured during the course of primary *N.brasiliensis* infections in outbred rats considered to be immunocompetent. The infection was initiated by a subcutaneous inoculation of an estimated 4000 3rd-stage larvae.

2. Plasma glucose concentration was observed to be reduced in association with the infection between days 6 - 14 p.i.

3. If the infection was eliminated on day 8 p.i. by anthelmintic treatment, this reduction was attenuated, but plasma glucose concentrations were still significantly depressed until day 16 p.i.

4. Food intake was observed to be reduced during the infection with troughs of minimal intake on days 3 and 9 p.i., separated by a plateau of higher, but still reduced, food intake.

5. These results are discussed in the context of the observations of other researchers.

SECTION 2

CHAPTER 4: ALANINE-AMINO-TRANSFERASE: A FOCUS FOR ATTENTION IN HEPATIC INTERMEDIARY METABOLISM

1. Introduction

In 1959 Gallagher and Symons hypothesised that should *N.brasiliensis* infection in rats cause specific nutrient deficiencies or precipitate the absorption of toxins by the host, then the organ most likely to demonstrate a defective metabolism would be the liver. However, they were unable to detect any disturbance in anaerobic glycolysis, the tricarboxylic acid cycle, or mitochondrial oxidative phosphorylation. They therefore concluded that their results provided no evidence that infection with *N.brasiliensis* disturbed the host's intermediary metabolism.

In contrast to the conclusion suggested by Gallagher & Symons (1959), Ovington (1985b) presented evidence to show that *N.brasiliensis* infection exerted a considerable influence on the intermediary metabolism of the rat host. In her work, alterations in the activity of four important hepatic gluconeogenic enzymes, namely glucose-6-phosphatase (EC 3.1.3.9), fructose-1,6-bisphosphatase (EC 4.1.2.13), phophoenolpyruvate carboxykinase (EC 4.1.1.32), and alanine-amino-transferase (EC 2.6.1.2, glutamate-pyruvate transaminase) on day 9 p.i. were observed.

The physiological basis and roles of many of the metabolic alterations during parasitic infection described by various researchers have been but sketchily suggested. In general, a diversion of nutrients away from the "normal" biosynthetic pathways to other pathways seem indicated. This lack of clearly defined interpretations of the alterations, including uncertainty as to whether they are facets of physiological adaptation or of pathological lesion, is not to be criticised. It merely reflects the complexity of the interactions between parasitic infections and their hosts, the paucity of studies concerned with host intermediary metabolism during helminth infection, and the apparently conflicting evidence arising from the studies

conducted.

In my research, in an effort to uncover more of the basis for alterations in hepatic intermediary metabolism during *N.brasiliensis* infections, I investigated fluctuations in activity of one particular gluconeogenic enzyme, alanine-amino-transferase, (ALT).

In metabolic processes involving a number of sequential steps, particular stages involve key reactions which are rate limiting. Key reactions in gluconeogenesis involve the enzymes fructose-1,6-bisphosphatase, glucose-6-phosphatase, and phosphoenolpyruvate carboxykinase. The reaction which initiates the metabolic sequence is also optimally placed to determine the reaction rate (Krebs, 1964). Alanine is the major precursor in gluconeogenesis from amino acids (Ross, Hems & Krebs, 1967; Felig, Pozefsky, Marliss & Cahill, 1970), and during its conversion to glucose, the initial stage is catalysed by ALT (Fig. 2.4.1). During my studies, I followed the activity of this enzyme in the rat host liver during the course of primary and secondary *N.brasiliensis* infections, and I measured its activity in the host serum. Furthermore, the effect of non-immune or immune serum or *N.brasiliensis* extracts on the *in vitro* assay of liver ALT activity was measured. It is important to remember that it is an assumption that the measurements made by an *in vitro* assay are of significance *in vivo.*

2. Results.

1. Liver ALT activity

Primary infections. Between days 0 - 14 p.i. of a primary infection, the ALT activities of liver, as measured by *in vitro* assay, follow a precise, reproducible pattern (Fig. 2.4.2). Initially the activity increases from control levels peaking on day 4 p.i. (9.67±0.79 U/g liver as compared with 3.24±0.54 U/g liver), followed by a decrease in activity between days 9 - 13 p.i., with the trough of activity measured on day 10 p.i. (O.44±0.18 U/g liver). On day 14 p.i. ALT activity is observed to have returned to control levels. Pair-feeding experiments on days 4 and 10 p.i.,

FIGURE 2.4.1
GLUCONEOGENESIS: THE ENTRY OF PRECURSORS INTO THE PATHWAY.
(Adapted from Newsholme and Start, 1981)

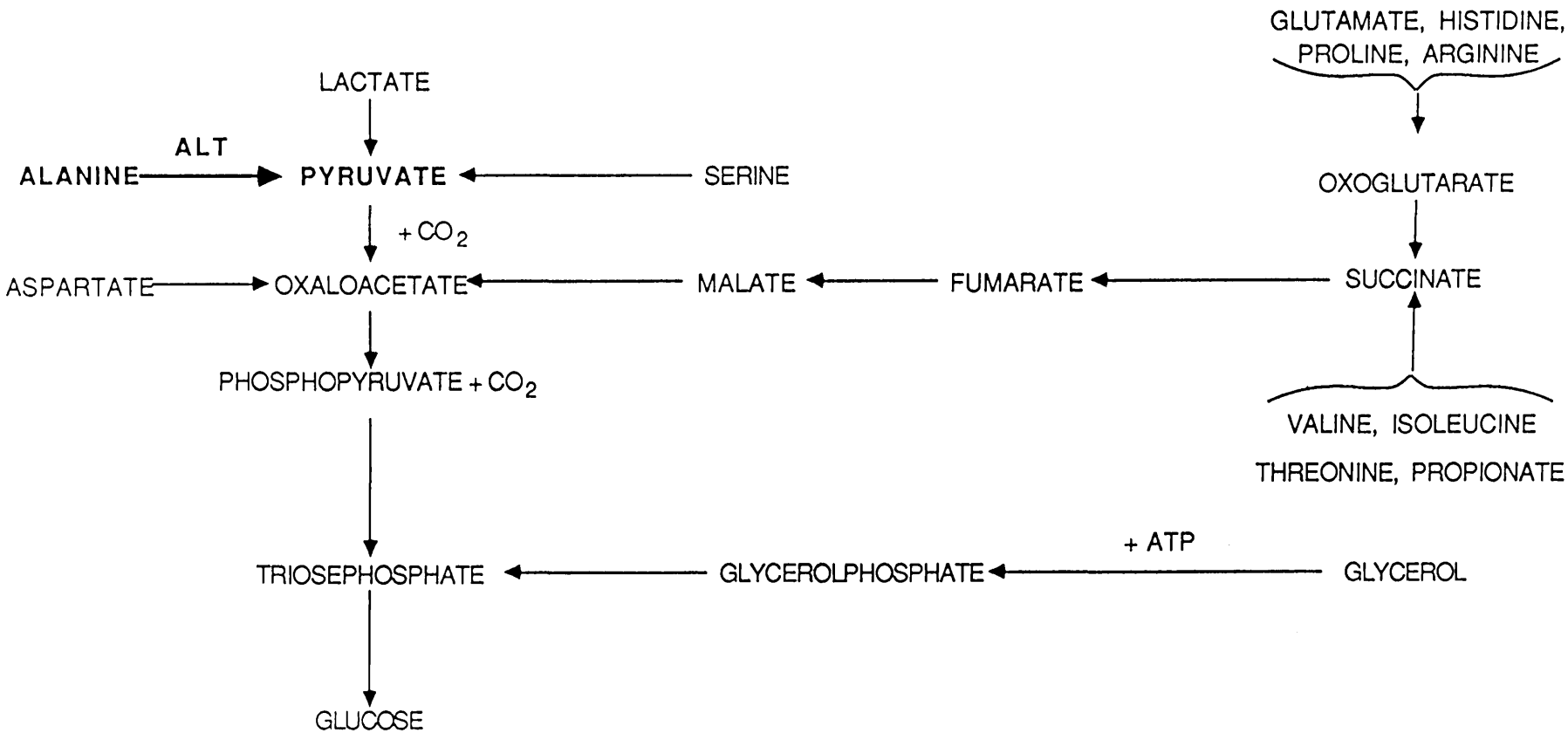
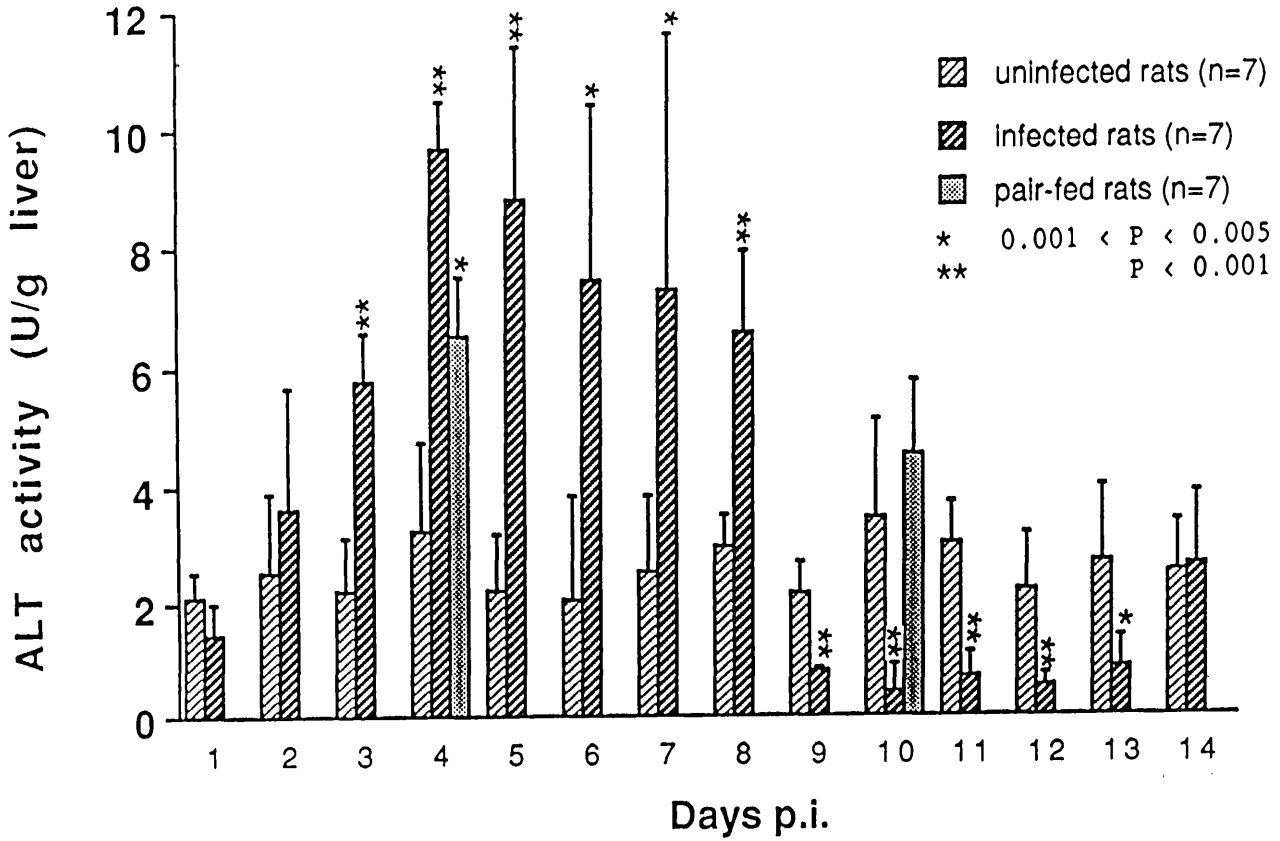


FIGURE 2.4.2. RAT LIVER ALT ACTIVITY DURING A PRIMARY INFECTION OF NIPPOSTRONGYLOSIS



demonstrated that uninfected pair-fed controls also had somewhat elevated ALT activities. On day 4 p.i. this elevation was observed to be significantly different to that measured in uninfected rats feeding *ad libitum* (P = 0.0012).

In one experiment the infection was terminated on day 4 p.i. by treatment with albendazole. The liver ALT activity was measured on day 10 p.i. and shown to be no different from that measured in uninfected controls, but was significantly different from that in rats infected at the same time that had not received albendazole treatment (P = 0.0006) (Table 2.4.1).

When the infection was initiated by oral transfer of adult worms, liver ALT measurements performed on day 4 p.i. revealed a significant reduction in activity as compared with uninfected controls (Table 2.4.2).

Dose dependency On days 4 and 10 p.i. of a primary infection the alterations in liver ALT activity were found to be dose dependent. (Fig.s 2.4.3 a and b). It is of interest to note that on day 10 p.i. doses as low as 500 larvae per rat were associated with a significant difference. On day 4 p.i. differences were not found to be significant until the dose was greater than 1000 larvae per rat.

Effect of the addition of serum The effect of the addition of serum from either immune or non-immune rats to the assay of ALT activity in liver from either uninfected rats or rats on day 4 p.i. of a primary infection was measured. The reason for this measurement was to investigate the possibility that some component of the host blood might be directly influencing the activity of the enzyme. Significant decreases were measured, in particular upon the addition of the immune serum (Fig. 2.4.4).

Effect of the addition of *N.brasiliensis* extracts. The effect of the addition of the homogenate supernatant fluid from either adult or larval *N.brasiliensis* to the *in vitro* assay of livers from uninfected rats was measured. The rationale for this measurement was to explore the possibility that a biochemical factor elaborated by the parasite may be directly influencing the liver ALT activity of the host. No significant differences in the ALT activities were detected. (Table 2.4.3).

TABLE 2.4.1.

LIVER ALT ACTIVITY ON DAY 10 P.I. OF A PRIMARY INFECTION WITH *N.BRASILIENSIS*. EFFECT OF ALBENDAZOLE TREATMENT ON DAY 4 P.I.

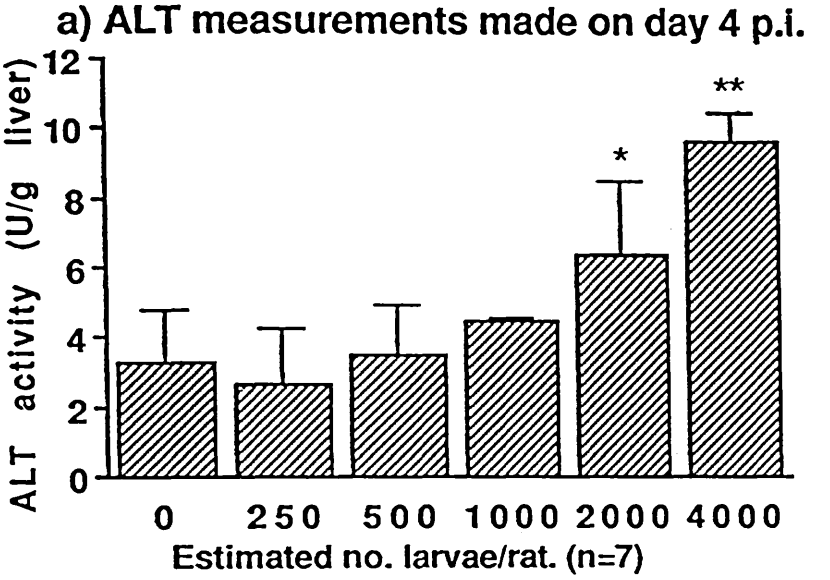
	Mean liver ALT activity ± SD (U/g liver) Uninfected rats (n=7)	Infected rats (n=7)	P
No albendazole treatment	2.28 ± 0.37	0.39 ± 0.35	0.0006
Albendazole (day 4 p.i.)	2.20 ± 0.24	2.39 ± 0.93	0.8048
P	0.9015	0.0006	

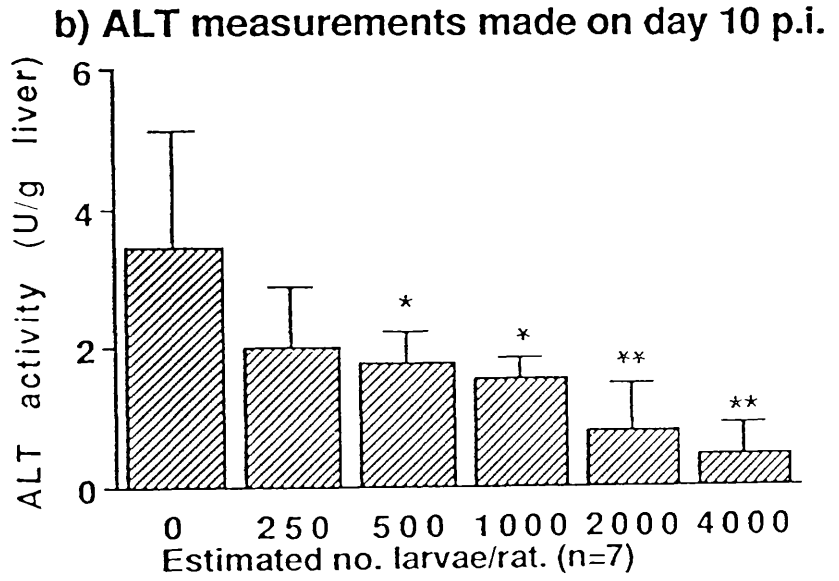
TABLE 2.4.2.

LIVER ALT ACTIVITY ON DAY 4 P.I. OF AN INFECTION INITIATED BY ORAL TRANSFER OF ADULT *N.BRASILIENSIS*.

	Mean liver ALT activity ± SD (U/g liver)
Infected rats (n=7)	0.30 ± 0.22
Uninfected rats (n=7)	2.60 ± 0.79
P	0.0022

FIGURE 2.4.3. DOSE DEPENDENCY OF LIVER ALT ACTIVITY DURING PRIMARY INFECTIONS OF NIPPOSTRONGYLOSIS IN RATS





* 0.005 < P < 0.05
** 0.0005 < P < 0.005

FIGURE 2.4.4. LIVER ALT ACTIVITY IN UNINFECTED OR INFECTED RATS (DAY 4 P.I.): EFFECT OF ADDITION OF IMMUNE OR NON-IMMUNE SERUM

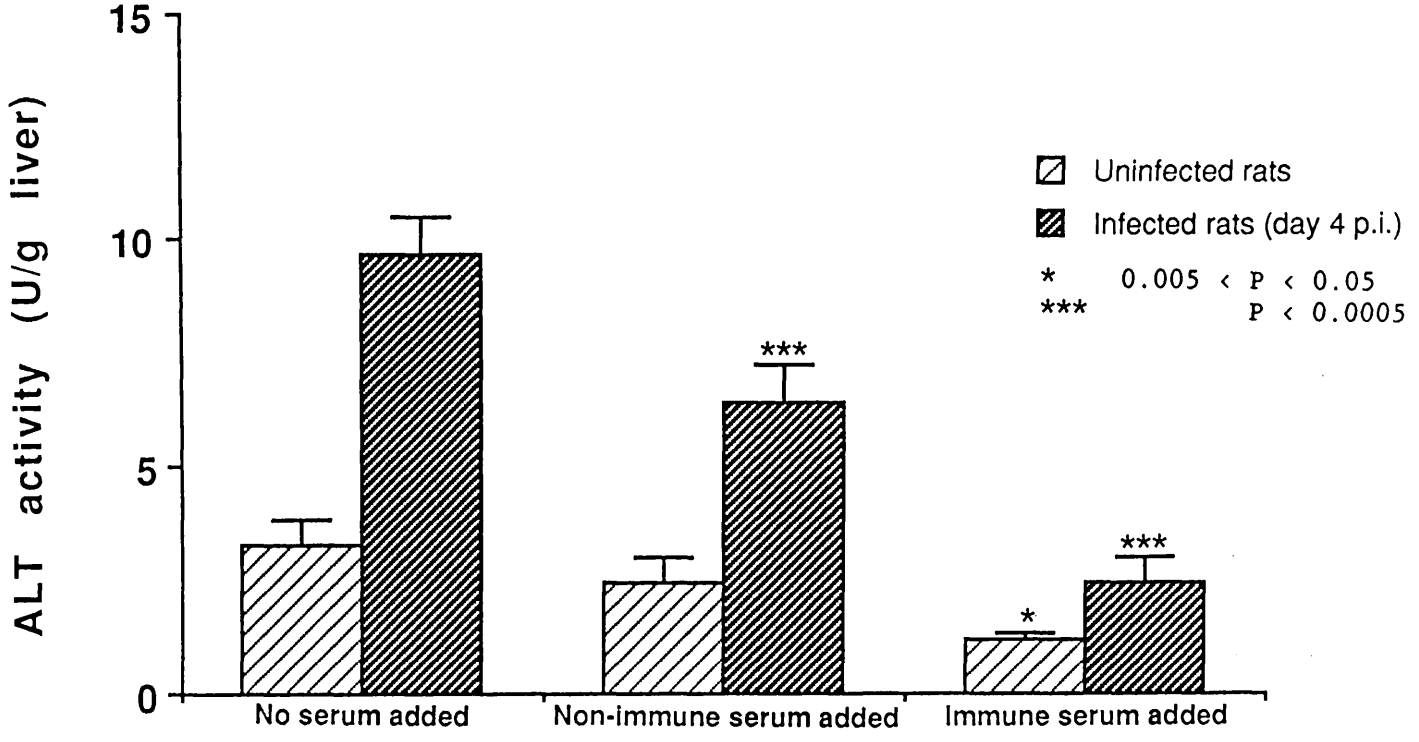


TABLE 2.4.3.

LIVER ALT ACTIVITY IN UNINFECTED RATS:
EFFECT OF ADDITION OF *N.BRASILIENSIS* HOMOGENATES.

	Mean liver ALT activity ± SD (U/g liver)
Control group (n=7)	2.19 ± 0.64
Larval homogenate *N.brasiliensis* added (n=7)	2.12 ± 0.52
Adult homogenate *N.brasiliensis* added (n=7)	2.11 ± 0.48
Kruskall-Wallis Associated probability	0.9816

Secondary infections. In rats given a secondary challenge with *N.brasiliensis* larvae 18 days after the primary challenge, a similar pattern in measured ALT activity to that seen in primary infections was observed. (Fig. 2.4.5). However, in the secondary infection, the timescale was compressed, with elevated ALT activities measured on day 2 p.i. (2), and reduced ALT activities on day 4 p.i. (2). By day 7 p.i., ALT activities were not significantly different from those measured in controls. *Post mortem* examination on day 7 p.i., revealed that the secondary infection had been effectively eliminated.

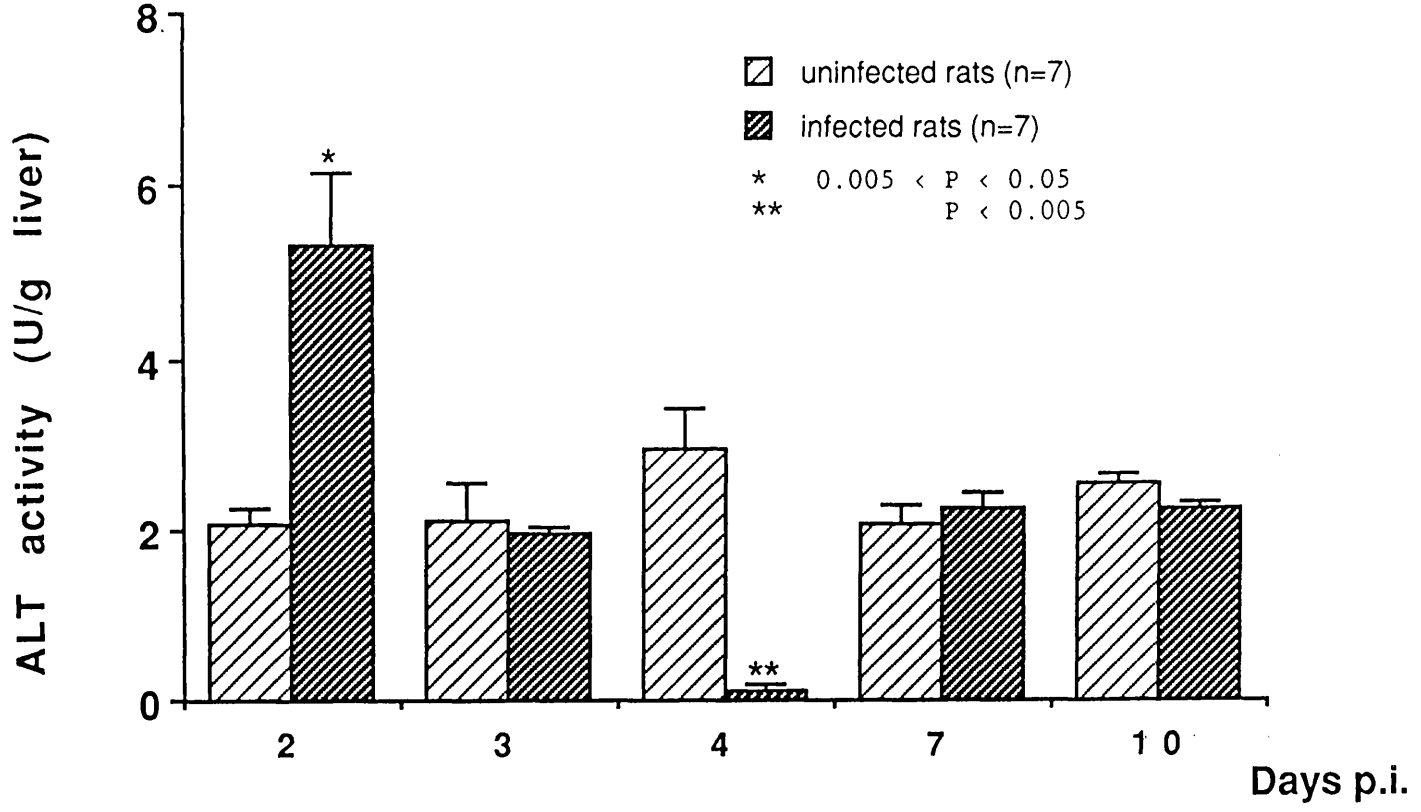
2. Serum ALT activity. Serum ALT activity was measured in rats on days 4 and 10 p.i. of primary infection, and in uninfected control rats. Mean results (U/l serum) of 23.74 (uninfected control rats), 14.15 (infected rats day 4p.i.) and 18.61 (infected rats day 10 p.i.) were obtained, and found to be not significantly different ($P > 0.3$).

3.Discussion.

The marked pattern of elevation followed by reduction in the activity of liver ALT, as measured by *in vitro* assay throughout the course of a primary *N.brasiliensis* infection, suggests that the infection is mediating a metabolic response within the rat. Importantly, the extent of this response is dependent upon the dose of infective larvae administered.

Initially the metabolic effects seem to be a reflection of an alteration of balance in the glucose-alanine cycle (Felig, 1973). The concentration of plasma alanine will be determined by the rate of its release from peripheral tissues, and the rate of its utilisation, including the proportion taken by the liver during gluconeogenesis. Wannemacher (1977) observed that in man, the pattern of alteration in individual free amino acids during infections differs from that found during starvation. Alanine is released in increased amounts by skeletal muscle, but is rapidly used as a gluconeogenic substrate by the liver to compensate for the increased rate of glucose oxidation in the infected host. Wannemacher, Neufeld & Canonico (1976) found that in rats with pneumococcal infections, during the earlier stages of infection the rate

FIGURE 2.4.5. LIVER ALT ACTIVITY DURING A SECONDARY INFECTION OF NIPPOSTRONGYLOSIS



of gluconeogenesis from alanine was elevated, but during the agonal stages of the infection the liver exhibited a decreased gluconeogenic capacity. Although it has been suggested that these observations could be associated with an enhanced and deranged activity of liver gluconeogenic enzymes (Beisel, 1982), measurements of enzymic activity were not made. Similarly Wolfe (1981) described how during gram-negative bacterial infections in the acute phase of the infection the ability to use alanine as a gluconeogenic precursor increases, with a resultant decrease in plasma alanine concentrations, but in the agonal phase the utilisation of alanine in gluconeogenesis significantly decreases.

The increase, followed by a decrease, in ALT activity that I observed repeatedly in primary *N.brasiliensis* infections seems initially to parallel with the results described by Wannemacher *et al* (1976) from rats with pneumococcal infections, and those described by Wolfe (1981) with reference to gram-negative bacterial infections. However, the latter stage of the infection with *N.brasiliensis* cannot be described as agonal; rats recover from this magnitude of infection and develop a protective immunity (Kassai, 1982). Beisel (1972) suggested that a latterly observed reduction in gluconeogenesis could be dependent upon the earlier elevation, which results in increased plasma glucose concentrations, causing a negative feedback response. Investigations of plasma glucose concentrations throughout *N.brasiliensis* infections (see Section 2, chapter 2) are not commensurate with this explanation. Furthermore, two sets of experimental data reported in this chapter indicate that the two phases in the fluctuations in liver ALT activity, as measured by the *in vitro* assay, are independent of each other. First, that the drop in liver ALT activity on day 10 p.i. is not an obligatory, subsequent effect of the earlier rise in activity, is demonstrated by the experiment in which the infection is eliminated on day 4 p.i. by anthelmintic treatment. The ALT activity in these infected, treated rats on what would be described as day 10 p.i. is comparable to that measured in control rats. Secondly, that the drop in ALT activity measured on day 10 p.i. does not have to be preceded by the rise in ALT activity, and is dependent on the presence of the adult

worms in the gut rather than the larval migration, is demonstrated by the experiment in which the infection has been established by oral transfer of adult worms. Liver ALT activity measured on day 4 p.i. is significantly decreased.

The initial elevation in ALT activity during a primary infection of *N.brasiliensis* may partially be a response to reduced food intake, as indicated by the pair-feeding experiment to day 4 p.i. As body carbohydrate stores are small, reduced food intake almost inevitably results in increased gluconeogenesis. Also liver ALT activity has been shown to involve a feeding-cued circadian rhythm (Sitren & Stevenson, 1978).

Glucose concentrations would also be negatively affected by malabsorption. Malabsorption has been demonstrated to occur throughout the rat intestine from day 6 p.i. of a primary *N.brasiliensis* infection (Nolla *et al*, 1985), and could result in elevated gluconeogenic activity in the liver.

The decrease in liver ALT activity from day 9 p.i. seems not to be due to elevated plasma glucose levels (see Section 2, chapter 3). However, it may be a response to an alteration in host protein status. Caldwell & McHenry (1953) have demonstrated that protein depletion will reduce liver ALT activity. Nawa (1979) and Lunn *et al* (1988) have reported that hypoalbuminaemia occurs by day 6 p.i. in primary *N.brasiliensis* infections, due to protein leakage into the gut mediated by a complex of factors associated with parasite-induced lesions. Intestinal protein loss may have a significant influence on the activity of ALT.

In secondary *N.brasiliensis* infections, the reduction in liver ALT activity, as measured by the *in vitro* assay, occurs much earlier than in primary infections in terms of days p.i., but at an equivalent stage in terms of the parasites' expulsion. This finding suggests that the drop in liver ALT activity is linked either to the *N.brasiliensis* development, or in some way to the development of the host immune response to the infection. Results of the experiment involving measurements of liver ALT activity when either adult or larval *N.brasiliensis* homogenate is added to the

assay, suggests a direct interaction is improbable. The worms themselves show little ALT activity (Barrett, 1981), and I did not detect that they affected the liver assay. However addition of either immune or non-immune serum to the assay demonstrates that a serological factor does disturb the assay, and this factor is enhanced in immune serum. However, as host serum ALT activity remains at control levels on both days 4 and 10 p.i., it seems probable that the factor that is perturbing ALT activities obtained when serum is added to the liver assays is acting on the *in vitro* assay technique rather than on the ALT itself. Nevertheless, the identification of this perturbant serological factor remains of interest, and reinforces the proposal that an immunological factor may be contributing to the measured decrease in liver ALT activity during the primary and secondary infection.

One possible non-specific factor of immunological importance that may be affecting liver ALT activities during *N.brasiliensis*, is a fluctuation in plasma corticosterone concentration. This possibility is examined more closely in Section 2, chapter 6, and other immunological factors in Section 2, chapter 5.

4.Summary

1. The activity of ALT was measured by an *in vitro* assay:

a. In rat livers during primary and secondary infections of *N.brasiliensis* in rats initiated by estimated inocula of 4000 third-stage larvae.

b. In rat livers on days 4 and 10 p.i. of primary infections of *N.brasiliensis* initiated by a range of doses of larvae.

c. In rat livers of both uninfected rats and rats infected with *N.brasiliensis* on day 4 p.i. of a primary infection. Sera from non-immune and immune rats were added to the assay.

d. In rat serum on days 4 and 10 p.i. of a primary infection with *N.brasiliensis* initiated by estimated inocula of 4000 larvae.

2. During primary infections of *N.brasiliensis* the hepatic ALT activity, as measured by *in vitro* assay, followed a precise reproducible pattern, peaking on day 4 p.i

(9.67±0.79 U/g liver) and with a trough of activity on day 10 p.i. (0.44±0.18 U/g liver), returning to control levels by day 14 p.i. On both these days these fluctuations in activity were demonstrated to be dependent upon the initial dose of larvae inoculated.

3. When the infection was terminated by anthelmintic treatment on day 4 p.i., by day 10 p.i. hepatic ALT activity was measured to be comparable to initially uninfected controls.

4. When the infection was initiated by oral transfer of adult worms, measurement of liver ALT activity on day 4 p.i. demonstrated a reduction in level.

5. Addition of parasitic homogenates to the liver assay caused no effect to the measurements. The addition of either immune or non-immune serum, however, caused a reduction in the measurements made.

6. During secondary infections with *N.brasiliensis*, hepatic ALT activity followed a similar pattern of an increase followed by a reduction, but this was in a shorter time interval than that observed during primary infections, and control levels were regained by day 7 p.i.

7. Serum ALT was not detected to fluctuate in activity during primary infections with *N.brasiliensis*.

8. These results are discussed in relation to the glucose-alanine cycle and carbohydrate and protein constraints upon the host during this infection. The possibility of a contributory immunological factor is proposed.

SECTION 2

CHAPTER 5: IMMUNOLOGICAL FACTORS: A POSSIBLE INTERACTION OF CYTOKINES WITH CARBOHYDRATE METABOLISM

1. Introduction

Nippostrongylus brasiliensis infection in rats generates a strong acquired immunity that results in the termination of the initial infection after about two weeks. In my investigations, *post-mortem* examination on day 14 p.i. of a primary infection revealed that the infection had been largely eliminated by this time, the number of remaining worms found never numbering more than 25, and often no more than 4. The host also develops a resistance to further infections of the same parasite.

As in most host-parasite systems, the host immune response is complex, and a cause of controversy amongst researchers. However, it is accepted that the adult *N.brasiliensis* is the most important stage in eliciting the immune response in the rat, and the contribution of larval stages is less significant (Kassai, 1982). Despite this, it is known that many of the larvae injected fail to establish as adults in the gut. Jarrett, Jarrett & Urquhart (1968) found that over 60% of their initial inoculum of 3000 larvae failed to reach the intestine, and I found that from an initial inoculum of an estimated 4000 third-stage larvae only approximately 25% established as adults in the gut. Larval loss will occur as a consequence of non-specific host defence mechanisms during larval migration.

That the spontaneous self-cure of rats infected with *N.brasiliensis* has an immunological basis and is thymus dependent has been often demonstrated. Confirmatory evidence includes: a) the absence of cure in T-deficient or T-deprived hosts, for example in congenitally nude (athymic) mice (Jacobson & Reed, 1974); b) the suppression of cure by host irradiation, and the reconstitution of this capacity by transplanting lymphocyte cell populations (Ogilvie, Love, Jarra & Brown, 1977);

c) the acceleration of cure in secondary infections (Jarrett *et al*, 1968); and d) the acceleration of cure in hosts that have received both serum and lymphocytes from immune hosts (Love, 1975).

However, there are indications that humoral and lymphocytic responses are not the only components of the mechanisms that result in spontaneous self-cure, and other events, for example an inflammatory response, play a significant role. The components for a hypersensitivity reaction are a feature of infections with *N.brasiliensis*. Mast cells proliferate, and IgE production is stimulated. Indeed, of the humoral response, antibody IgE is of primary dominance. Parasite specific IgE enhances IgE response to unrelated antigens, and this potentiated response causes a massive elevation of total serum IgE (Jarrett, 1978). Other antibodies are also found in the serum, intestinal secretions and milk. They are present in the serum from approximately day 7 p.i., peaking with worm expulsion, and then declining, although positive titres are in evidence for several weeks at least.

In an attempt to examine the importance of IgE-mast cell-mediated hypersensitivity in the spontaneous cure of *N.brasiliensis* three hypotheses have been proposed. These involve either a) direct worm damage by amines, b) an alteration of the intestinal environment by amines, so that it becomes unsuitable for worm survival, or c) amine induced permeability of epithelium, allowing translocation of anti-worm antibodies into the gut lumen (Wakelin, 1984). However, none of these ideas is entirely satisfactory, and the most that can be said is that while the reagin-mast cell interaction may be involved in the self cure, it is not essential. This view is reinforced by experiments in which complete abrogation of antibody production appears to cause little impairment to the worm expulsion capacity of the host (Jacobson, Reed & Manning, 1977).

It remains uncertain whether myeloid cells are an essential requirement for a successful self-cure mechanism (Wakelin, 1984). During infections with *N.brasiliensis*, alterations in blood granulocytes, as well as in tissue granulocytes (mast cells) occur; their levels in circulation increase in defined and reproducible

patterns (Ogilvie, Hesketh & Rose, 1978), that indicate the possibility of an immunological role. Basophils especially increase greatly in number, but the significance of this remains unclear. Eosinophils, attracted by the release of an eosinophil chemotactic factor (Czarnetzki, 1978) associated with a humoral interaction, are more probably involved in reducing the effects of the local anaphylactic reaction, in their role as histamine antagonists, than in the elimination of the infection (Ogilvie, Mackenzie & Love, 1977). However, a lethal effect of eosinophil enriched peritoneal cells on adult worms has been demonstrated *in vitro* (Mackenzie, Preston & Ogilvie, 1978).

As a reduction in liver ALT activity was measured between days 9 - 13 p.i., concurrent with the activation of the immune response, possible synergistic interactions between these events were investigated. It seems unlikely that humoral responses could affect liver ALT activity *in vivo*, although it is possible that they may influence an *in vitro* assay. The reduction in enzymic activity when adult worms are transferred to naive hosts occurs too rapidly to suggest the involvement of antibodies. Also, by day 14 p.i. the majority of worms would be expelled, but the antibody titre would still be high. ALT measurements at this time show a return to control levels.

Hypersensitivity reactions are unlikely to have a direct effect on the activity of liver enzymes. However, they would aggravate parasite induced lesions in the gut, causing increased mucosal permeability and protein leakage into the gastrointestinal tract. It has been suggested, that hypoalbuminaemia would result from this leakage (Nawa, 1979; Lunn *et al*, 1988), and protein depletion has been linked to reduced liver ALT activity (Caldwell & McHenry, 1953). Again, however, mucosal permeability and resultant protein leakage, is not rectified by day 14 p.i., but by this time liver ALT measurements correlate with activities measured in control rats. Mast cells also release a number of aspecific proteases which may disrupt the enzyme assay system *in vitro*.

Leukocytes, primarily macrophages, have been shown to secrete a particular type of proteinaceous mediators in a number of viral, bacterial and parasitic infections (Wannemacher, Pekarek, Klainer, Bartelloni, Dupont, Hornick & Beisel, 1975). These mediators, which originally were known collectively as leukocytic-endogenous-mediator (LEM), are now referred to as cytokines. Cytokines have been classified by the employment of advanced techniques into a number of component proteins including interleukins, cachectin or tumour necrosis factor, and interferons (Dinarello, Cannon & Wolff, 1988).

The ability of the liver to increase dramatically the synthesis of certain proteins in response to infections or inflammatory challenges is well recognised (Roitt, 1984). These proteins are frequently referred to as acute phase reactants, although they are elevated in both short-lived and chronic or inflammatory disease. Dinarello (1984), divided acute phase proteins into two categories; the first includes those proteins that are normally present in the blood, but their concentration increases considerably, e.g. fibrinogen, haptoglobin, cerruloplasmin and serum amyloid. The second embraces those proteins that are normally either not present in blood at all, or are found in very low concentrations, and their concentration increases several hundred fold, e.g. c-reactive protein and 2-macroferroprotein. In general, the increase in the synthesis of acute phase proteins is associated with decreases in albumin and transferrin concentrations. Interleukin-1 (IL-1) has been the focus of attention among the cytokines as the mediator of production of acute phase reactants, particularly as relatively impure preparations of IL-1 have been demonstrated to induce many of the metabolic alterations which characterise an acute phase response (Powanda & Beisel, 1982). More recently, however, the importance of other cytokines and conventional hormones in regulating the metabolic alterations which accompany an acute phase immunological response has been realised (Klasing, 1988).

The liver is stimulated by IL-1 in the uptake of amino acids from the plasma for the production of acute phase reactants. As this uptake has been observed in rats

that have been adrenalectomised, hypophysectomised, thyroidectomised or rendered diabetic, then hormonal mediation is not essential (Wannemacher, Pekarek, Thompson, Curnow, Beall, Zenser, DeRubertis & Beisel, 1975). Physiological quantities of hormones e.g. adrenal corticoids, however, may be necessary to stimulate RNA and acute phase globulin synthesis. The specific functions of acute phase reactants in host defence or protection after release from the liver is unclear, although it has been suggested that they are immunoregulatory (Dinarello, 1984). Should IL-1 be produced by the rat host during infection with *N.brasiliensis*, then the increase in the synthesis of acute phase proteins, as mediated by IL-1, could contribute to a reduction in the activity of hepatic ALT, which is extremely sensitive to the direction of protein metabolism (Beaton, Curry & Veen, 1957).

IL-1 and other cytokines have been demonstrated to be mediators of a number of other metabolic alterations. these include decreases in the concentration of plasma iron and zinc, and increases in plasma copper concentrations. Barber and Cousins (1988) suggest that the alteration in zinc levels is due to IL-1 acting as an inducer that enhances the expression of the metallotheionein gene.

Another demonstrable and major alteration that cytokines are associated with is elevation in body temperature. Fever has been demonstrated to be associated with at least three cytokines (Blatteis, Shibata & Dinarello, 1987), and is believed to be achieved by a prostaglandin-induced alteration in the relatives rates at which the heat- and cold-sensitive neurons are fired within the hypothalamus (Klasing, 1988).

Cachectin, another hormone secreted by macrophages, and a component of the cytokine system has been demonstrated to suppress systemically lipoprotein lipase, a membrane bound enzyme that normally mediates the clearance of circulating lipid (Tracey, Lowry & Cerami, 1988). There are high affinity cachectin receptors on a variety of normally functioning tissues, including liver. L6 myoblasts incubated with cachectin mobilise energy stores, causing a rapid depletion in intracellular glycogen, increased expression of hexose transporters and enhancement of lactate efflux. Cells

that produce cachectin must be induced. Such cells (in particular monocyte/macrophage) have been demonstrated to be induced by endotoxin/lipopolysaccharide and cell lysate products of various parasites, viral particles and endotoxins. Beutler, Milsark & Cerami (1985) suggest that the suppression of the biosynthesis of specific cellular proteins by cachectin is enacted at the level of transcription. The descriptions of the direct suppression of various cellular proteins synthesised in the liver by different components of the cytokine system suggest that it is possible that liver ALT could be directly suppressed *in vivo* either by direct suppression of its synthesis at a molecular level, or by another mechanism.

Yang, Moldawer, Sakamoto, Keenan, Matthews, Young, Wannnemacher, Blackburn & Bistrian (1983) suggest that amino acid oxidation should increase in the liver upon the administration of cytokines, as the availability of amino acids will exceed hepatic protein synthetic capacity and require clearance. This would involve elevation in the activity of ALT. In support of this theory, Roh, Moldawer, Ekman, Dinarello, Bistrian, Jeevanandam & Brennan (1986) describe how in their experiments they found that intra-peritoneal injections of crude IL-1 preparations increased the rate of alanine transport into rat hepatocytes, and also increased oxygen consumption, and gluconeogenesis from alanine.

In an attempt to detect any clues that might suggest an involvement of cytokines in the immune response of rats to *N.brasiliensis*, and to speculate upon an association of cytokines with either the initial increase in liver ALT activity or the later decrease, a number of measurements were made of factors associated with cytokine production. Leukocyte counts were performed on days 4 and 10 p.i. of primary infections, rat body temperature was measured from day 1 until day 14 p.i. of a primary infection, and plasma zinc concentrations were measured on days 4 and 10 p.i. of a primary infection, and day 4 p .i. of a secondary infection. However, the measurement most directly associated with cytokines, was the quantification of the acute phase protein in plasma, α_2-macroglobulin (α_2M), in a longitudinal study

on days 3, 7, 10, 14 and 21 p.i. of a primary infection.

2.Results

1. Leukocyte counts on days 4 and 10 p.i. The heterogeneity of the results obtained from these counts, reflect both individual variation and the inaccuracies of the methodology (Table 2.5.1). No significant difference was detected between counts in infected and uninfected rats on day 4 p.i., although it is of interest to note the considerable increase in standard error in infected rats as compared to uninfected rats. On day 10 p.i. the leukocytic count in infected rats was found to be significantly higher in infected rats than uninfected control rats (7980 as compared to 5330 cells/µl blood). Both of these values were considerably higher than the values obtained on day 4 p.i. These values for days 4 and 10 p.i. were not obtained as a longitudinal study, ie measurements made on day 10 p.i. were from a different group of rats than had cell counts made on day 4 p.i. Therefore, the rise in blood leukocyte count on day 10 p.i. I have interpreted as a real perturbation rather than an artifact. Crompton *et al* (1978) have also measured a rise in leukocyte count on day 10 p.i. in infections of rats with *N.brasiliensis*.

TABLE 2.5.1.

RAT LEUKOCYTE COUNTS ON DAYS 4 AND 10 P.I.
OF A PRIMARY INFECTION WITH *N.BRASILIENSIS*.

	Mean leukocyte counts ± SD (Cells/µl blood)		
	Uninfected rats	Infected rats	P
Day 4 p.i.	3225 ± 430 (n=7)	3393 ± 1113 (n=7)	0.8048
Day 10 p.i.	5330 ± 1534 (n=6)	7980 ± 1846 (n=7)	0.0140

2. Rat body temperature. Great fluctuation in rat body temperature as measured by a standard rectal thermometer was apparent over the 17 day period of measurement both in infected and uninfected rats (Fig. 2.5.1). On days 5 and 12 p.i. infected and uninfected rat temperatures were found to be significantly different. Examination of the actual values, however, indicates that this difference may be a result of a decrease in the temperature of the uninfected rats rather than an increase in body temperature in the infected rats. As rats in both uninfected and infected groups were subjected to as similar environmental conditions as possible, then it is possible that these results do indicate a real effect of the infection. Kampschmidt, Upchurch, Eddington & Pulliam (1973) record a maximum temperature elevation of 1.16^{o}C after intravenous injection with cytokines, and Tocco-Bradley, Georgieff, Jones, Moldawer, Dinarello, Blackburn & Bistrian (1987) an elevation of 1.3^{o}C 4 - 8 h post-infusion with a crude IL-1 preparation. In my study individual temperature fluctuations were as great as 1.8^{o}C, even in uninfected rats, despite great care being taken about the timing and consistency of the method of measurement. Thus, despite the statistically significant differences between infected and uninfected rats on two of the days p.i., I feel that more accurate measurements need to be made before the hypothesis that *N.brasiliensis* infection is associated with an increase in body temperature can be justified.

3. Plasma zinc concentrations. Some results for the infected rats on day 10 p.i. and most of the results for their uninfected pair-fed controls were unobtainable, due to blockages in the aspirator tube of the atomic absorption spectrophotometer. Nevertheless, enough measurements were made to conclude that no apparent significant difference could be detected in plasma zinc concentration between any of the seven groups of rats investigated (Table 2.5.2).

Unlike with the estimation of blood leukocytes and the measurement of body temperature throughout the infection, the method utilised for the measurement of plasma zinc concentration was precise, and intra-group variation was not large.

FIGURE 2.5.1. RAT BODY TEMPERATURE DURING A PRIMARY INFECTION OF NIPPOSTRONGYLOSIS

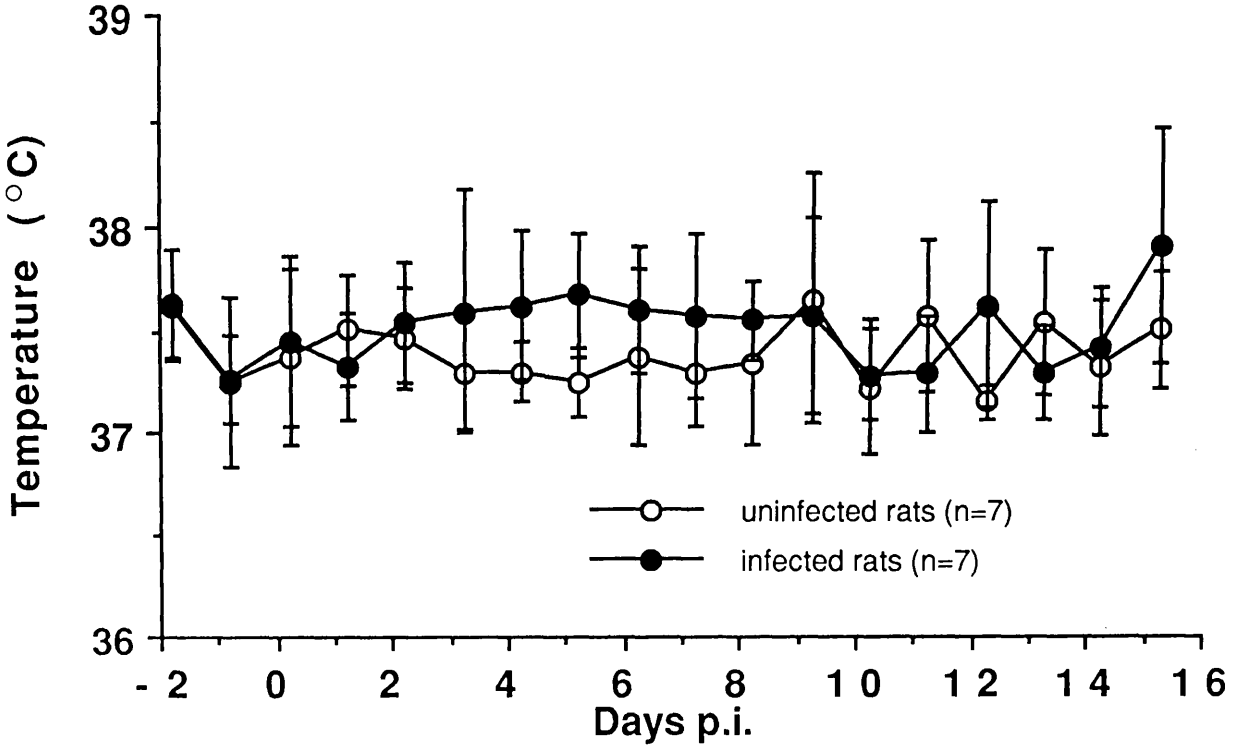


TABLE 2.5.2.

RAT PLASMA ZINC CONCENTRATIONS ON DAYS 4 AND 10 P.I. OF A PRIMARY INFECTION, AND DAY 4 PI. OF A SECONDARY INFECTION WITH *N.BRASILIENSIS*.

	Mean plasma zinc concentrations ± SD (ppm)		
	Uninfected rats	Infected rats	Pair-fed rats
Day 4 p.i. (primary)	2.72 ± 0.11 (n=7)	2.98 ± 0.27 (n=7)	2.73 ± 0.40 (n=7)
Day 10 p.i. (primary)	2.57 ± 0.40 (n=7)	2.59 ± 0.13 (n=5)	2.56 (n=1)
Day 4 p.i. (secondary)	2.65 ± 0.32 (n=6)		

4. Plasma α_2-macroglobulin measurements

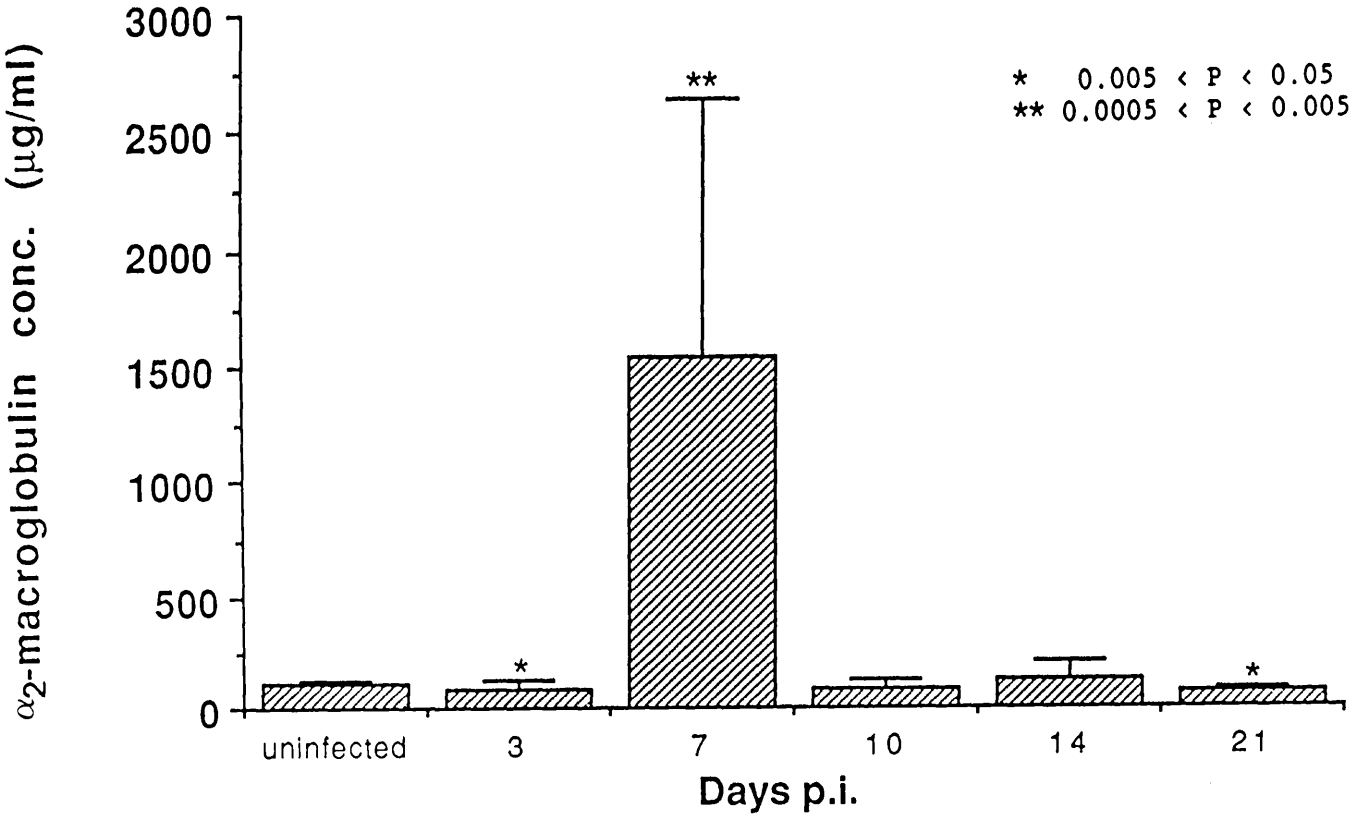
Although the values measured in this longitudinal study reveal a large amount of intra-group variation, as seen in the large standard deviations, the results reveal that during primary infections with *N.brasiliensis* there are very significant fluctuations in the concentration of plasma α_2M (Fig. 2.5.2). Initially there is a significant reduction in concentration, but by day 7 p.i. the concentration has increased by approximately ten-fold. By day 10 p.i. the concentration has returned to levels that are not significantly different from that measured in controls, and this concentration is also observed on day 14 p.i., by which time the infection will have largely been eliminated. It is, therefore, curious that on day 21 p.i. a further significant dip in the plasma concentration of this protein should have been measured.

3. Discussion

In this study a number of parameters have been measured, and particular alterations in them could have been interpreted as indicators of the production of cytokines. The quantitative alteration in leukocytes measured on day 10 p.i., although demonstrating a leukocytic proliferation, provides no evidence of cytokine production. Eosinophilia is often used as circumstantial diagnosis of helminth infections. The body temperature measurements are too variable to enable any conclusions to be made from them. The two most precise and useful measurements made were those of plasma zinc and plasma α_2M concentrations, but intriguingly they provided contrasting results. The plasma zinc measurements provided no evidence for the production of cytokines during nippostrongylosis, but measurements of the acute phase protein suggested that cytokines are involved in the immune response to primary *N.brasiliensis* infections, and this was particularly marked on day 7 p.i. when a very significant increase in the concentration of α_2M was measured.

Indirect or direct evidence by other workers of the involvement of cytokines in

FIGURE 2.5.2. RAT PLASMA α_2-MACROGLOBULIN CONCENTRATION DURING PRIMARY NIPPOSTRONGYLOSIS



the immune response to *N.brasiliensis* infections is scanty, and to a certain extent, contradictory. Experiments by Selivanova, Molodykh & Pavlova (1975), revealed decreases in rat plasma copper concentrations during infections with *N.brasiliensis*, which does not suggest the involvement of cytokines, as they would be expected to be associated with a rise in the concentration of plasma copper.

Work by Lamontagne, Gauldie, Befus, McAdam, Baltz & Pepys (1984), however, indicates that during *N.brasiliensis* infection in mice, an acute phase response occurs. Serum measurements of four acute phase reactants were made, namely 1-proteinase inhibitor, complement C_3, serum amyloid A protein and serum amyloid P component. During the migratory larval stage, serum changes in the latter 2 of these reactants was measured. Alterations in the synthesis of all 4, however, were observed in primary hepatocyte cultures. Subsequently, during the immune response in the gut, which eliminated the parasite by day 14 p.i., both serum and hepatocyte cultures demonstrated an acute inflammatory response in all four reactants. Lamontagne *et al* (1984) concluded that two acute inflammatory responses occurred. They suggest that the initial response is a result of the traumatic non-immune episode of larval migration through the lungs. The latter phase they describe as originating as a non-specific immune response. Lamontagne, Gauldie, Stadnyk, Richards & Jenkins (1985), have also detected apparent active secretion by alveolar macrophages of significant amounts IL-1, or a similar molecule, during *N.brasiliensis* infections in mice.

The studies by Lamontagne and colleagues (1984; 1985) with mice, indicative of a two-stage acute phase response, does not correlate closely with the results obtained in our study in rats, in which only one peak is indicated. Although, from the work by Lamontagne *et al* (1984) it is apparent that quantification of blood concentrations of some acute phase reactants may not be a satisfactory indicator of an acute phase response; the synthesis or secretion of reactants should also be studied. Recent work by Ramirez, Mayberry & Bristol (1989) has, nevertheless, indicated that *N.brasiliensis* infection suppresses the production of interleukins.

Despite the contradictory nature of the available data, it appears that the possibility of the immune response to *N.brasiliensis* infection involving cytokines is not remote. This may have far reaching implications for intestinal nematode infections of medical and veterinary importance. However, to attempt to correlate the involvement of cytokines with fluctuations in hepatic ALT activity measured in rats during nippostrongylosis, would be only speculative. Further research is clearly indicated before any associations can be investigated. Other work on cytokines, and metabolic alterations measured by myself and other researchers during *N.brasiliensis* infection in rats, are evaluated in the final discussion.

4.Summary

1. Factors of the rat immune response to *N.brasiliensis* infection that may affect host intermediary metabolism are considered, with particular reference to cytokines of leukocytic origin, that are well known to mediate an effect on some aspects of mammalian metabolism.

2. Experiments involving measurements of blood leukocyte number, body temperature and plasma zinc concentrations provide no evidence for the involvement of cytokines in the rat immune response to *N.brasiliensis* infection, but measurements of plasma α_2-macroglobulin indicated otherwise. On day 7 p.i. a very significant elevation in the concentration of α_2M was measured.

3. These observations are discussed in relation to measurements made by other workers, but the relative paucity of work in this field means that it is difficult to draw firm conclusions, and it is not yet possible to relate these results to the observed fluctuations in hepatic ALT activity.

SECTION 2

CHAPTER 6: CORTICOSTERONE: A POSSIBLE IMMUNO-HORMONAL INFLUENCE ON CARBOHYDRATE METABOLISM

1.Introduction

Corticosterone is a glucocorticoid hormone secreted by the adrenal cortex. Glucocorticoids have a role in regulating the distribution of body water and electrolytes, and an important influence on carbohydrate metabolism (Schmidt-Nielsen, 1983). The influence of corticosterone on carbohydrate metabolism in the rat is expressed as acceleration of gluconeogenic processes in the liver by increasing the activity of the appropriate enzymes, and by inhibiting glucose uptake by the tissues (Beato & Doencke, 1980). Glucocorticoids are also effective immunosuppressants (Keele *et al*, 1982), decreasing hyperaemia, reducing exudation, and diminishing the migration and infiltration of leukocytes at the sites of injuries. They have a stabilising influence on lysosomal membranes, and thus diminish the possibility of proteases and hydrolytic enzymes being released into the tissue fluid. They also inhibit the intracellular synthesis of histamine, an important anaphylactic compound.

During primary *N.brasiliensis* infections in rats, plasma corticosterone concentration has been demonstrated to fall on day 9 p.i. (Crompton *et al*, 1978; Ash *et al*, 1985). This decrease in plasma corticosterone concentration has been observed during other nematode infections, and often occurs shortly before the nematode is eliminated by a host "self-cure" mechanism (Bailenger & Carcenac, 1974). In their own experiments, Bailenger and Carcenac (1974) found, as well as a decrease in rat host plasma corticosterone concentration towards the end of an infection with *Strongyloides ratti*, a temporary elevation in corticosterone concentration in the initial stages of the infection. The immunosuppressant qualities of corticosteroids have been utilised to modify both natural resistance and acquired immunity to a

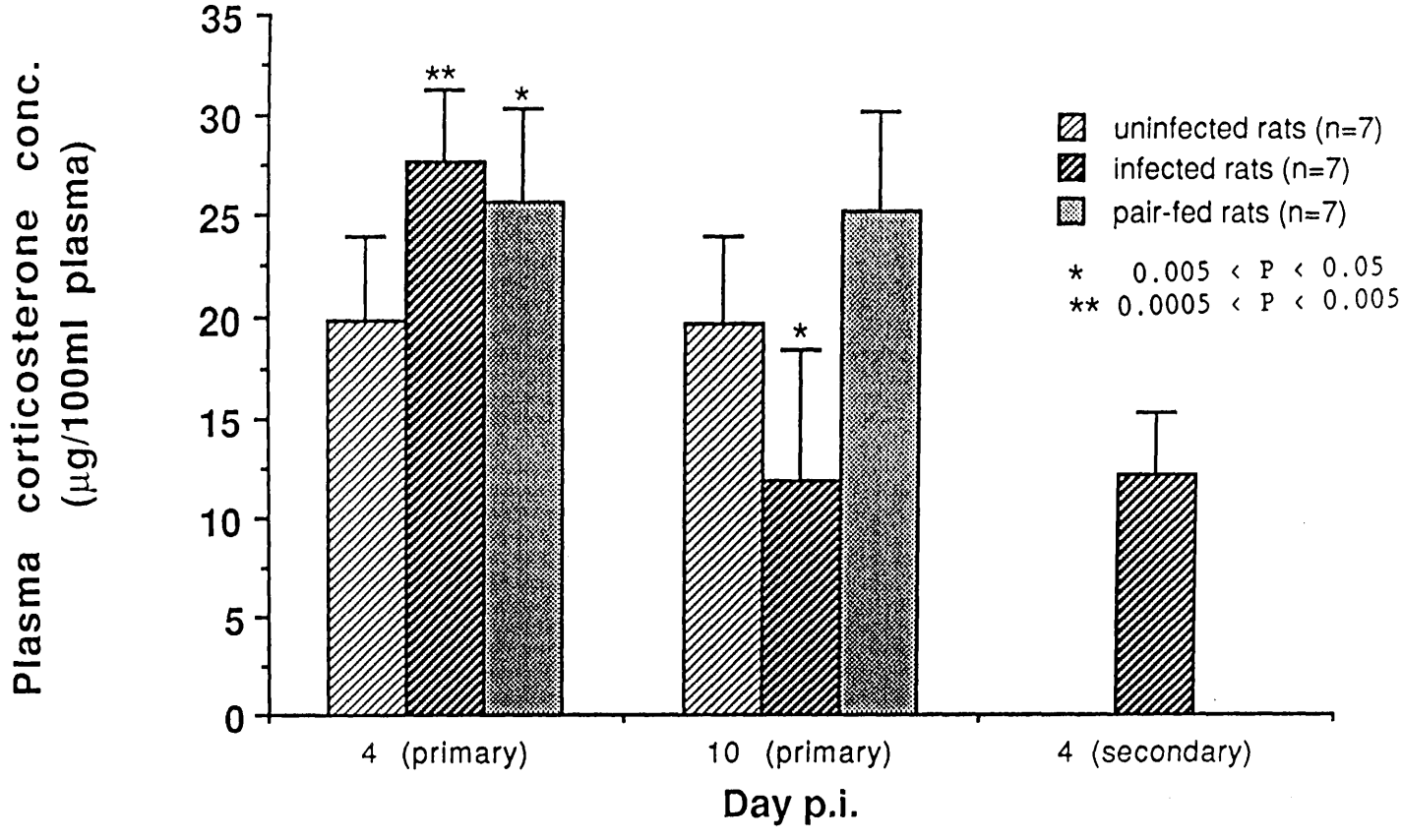
number of parasitic nematodes, including *N.brasiliensis*, by the administration of corticosteroid analogue drugs (Parker, 1961; Luffau, Forgereau & Paraf, 1969; Harley & Gallichio, 1970; Kennedy, 1980).

Administration of corticosteroids and related compounds to uninfected animals has been long known to promote an increase in the activity of ALT (Gavosto, Pileri & Brusca, 1957; Rosen, Roberts & Nichol, 1958; Segal, Beattie & Hopper, 1962; Segal, Rosso, Hopper & Weber, 1962). ALT activity has also been induced in cultured hepatostoma cells by adrenal steroids (Lee & Kenney, 1970). I therefore suspected that alterations in plasma corticosterone concentrations might be associated with the fluctuations in liver ALT activity described in Section 2, chapter 4. This possibility was studied by measurement of plasma corticosterone concentrations on days 4 and 10 p.i. of a primary infection, and day 4 p.i. of a secondary infection. All the rats used in this series of experiments were male so that interference by female hormonal cycles could be avoided.

2.Results

The results (Fig. 2.6.1) show large intra-group variation, as reflected in the large values of standard deviation. This is probably a reflection of the technique, the complexity of which permits the introduction of various sources of error at a number of stages. However, it can be seen that on day 4 p.i. of a primary infection of *N.brasiliensis* substantially increased plasma corticosterone concentrations were measured in infected rats as compared with uninfected controls feeding *ad libitum*, (27.6 ± 3.6 µg/100ml plasma as compared with 19.8 ± 4.1 µg/100ml plasma). The results are not significantly different from those measured in pair-fed uninfected controls ($P = 0.3176$). On day 10 p.i. of a primary infection, the infected rats were found to have significantly decreased plasma corticosterone concentrations compared with both *ad lib* and pair-fed uninfected controls (Fig. 2.6.1). The pair-fed rats tended to show elevated plasma corticosterone concentrations (25.2 ± 4.9 µg/100ml plasma), but not at a significantly higher value than in those feeding *ad lib* ($P = 0.0530$). On day

FIGURE 2.6.1. RAT PLASMA CORTICOSTERONE CONCENTRATIONS DURING NIPPOSTRONGYLOSIS



4 p.i. of the secondary infection with *N.brasiliensis*, plasma corticosterone concentrations were found to be similar to those obtained from infected rats on day 10 p.i. of a primary infection (P = 0.8048).

3. Discussion

The results obtained from this experiment are in general agreement with those obtained by other researchers in this field (Crompton *et al*, 1978; Ash *et al*, 1985; Bailenger & Carcenac, 1974). The initial elevation in plasma corticosterone concentration probably represents a non-specific stress response. As no significant difference in corticosterone concentration could be detected on day 4 p.i. between uninfected pair-fed rats and those that were infected, this is probably associated mainly with reduced food intake. It is also of interest to note that on day 4 p.i. a significant difference was observed between uninfected rats feeding *ad lib* and their pair-fed partners with reference to ALT activity.

The later decrease in plasma corticosterone concentration has been observed in other studies, although how the reduction occurs remains unclear. Crompton *et al* (1978) suggest that the parasite itself might have produced, or caused the production of, a substance with glucocorticoid activity that interferes with endocrine control mechanisms. This was at least partially refuted by Ash *et al* (1985) who argued, in the light of evidence from Barrett (1981), that helminths inhabiting the gastro-intestinal tract are unlikely to synthesise steroids. The reduction in plasma corticosteroid concentration towards the termination of an infection with *S.ratti* in rats (Bailenger & Carcenac, 1974) was subsequently considered to be related to the hypothalamic secretion of cortico-releasing factor and associated with an inhibition of the nervous mechanism which regulates this neurosecretion (Bailenger & Faraggi, 1975 a and b).

Another possible reason for the decrease in plasma corticosterone could be a reduction in the synthesis of transcortin. In the blood stream, corticosterone is reversibly bound to a specific glycoprotein called transcortin or corticosteroid-

binding globulin (CBG), and it is upon this that the assay is based. Transcortin is synthesised in the liver, and in cirrhosis of the liver or nephrosis the initial decrease in CBG will result in a greater percentage of free or unbound corticosterone. This is the biologically active fraction which will depress further synthesis of corticosterone, thus reducing the overall total corticosterone concentration. However, there is as yet no obvious explanation as to why the infection should cause a reduction in transcortin synthesis, as *N.brasiliensis* causes no known physical disruption of the liver. Since reduced transcortin synthesis would not affect the concentration of biologically active corticosterone, any influence on the activity of hepatic ALT would be unlikely.

Interesting observations have also been made on plasma corticosterone concentrations during nematode infections in lactating mammals. In lactating mammals, or mammals in the late stages of pregnancy, plasma corticosteroid concentrations rise due to an increase in transcortin production (Keele *et al*, 1982). While protection against *N.brasiliensis* larvae is unimpaired in lactating rats, immunity to the adult stages appears to be abrogated (Connan, 1972). The ability of the rats to reject the worms is restored by the transfer of mesenteric lymph node cells from nulliparous donor rats (Dineen & Kelly, 1972). However, when Bailenger & Cabannes (1976) investigated the effect of lactation in rats on infections of *S.ratti*, they observed that the intensity of the infection was reduced. Lactation was thought to act as a buffer on the alterations in corticosterone production normally associated with the presence of *S.ratti* (Bailenger & Cabannes, 1976). The initial 'hypercorticosteronomy' is suppressed, allowing non-specific defence mechanisms to diminish the intensity of the infection, and latterly the 'hypocorticosteronomy' is attenuated, prolonging the duration of the infection. Bailenger & Cabannes (1976) extended their observations to explain the phenomenon of 'spring-rise' in lactating sheep harbouring trichostrongyle nematodes. Kassai (1982) mentions that immunosuppression during lactation seems to be particularly associated with helminth infections. In infections with viruses or coccidia, lactation does not

suppress immunity, although exogenous treatment with corticosteroids may lead to immunosuppression regardless of infectious agent. For example, administration of hydrocortisone to rabbits infected with *Trypanosoma gambiense* suppresses the formation of skin lesions (Seed, Marcus & Risby, 1972), and its administration to mice infected with *Trypanosoma brucei* attenuates the anaemia (Balber, 1974). There appears to be no information about the activity of hepatic gluconeogenic enzymes during lactation in either infected or uninfected animals, and further work on this topic would be of great interest.

It remains tempting to suggest that the decrease in rat host plasma corticosterone concentration towards the end of the infection with *N.brasiliensis*, is an intrinsic and important component of the rat host response that contributes to the eventual elimination of the parasite. Also, despite the lack of a satisfactory explanation as to how the reduction in plasma corticosterone is effected towards the end of both primary and secondary *N.brasiliensis* infections in rats, it is tempting to associate both the initial increase and the later decrease with the increase and decrease in hepatic ALT activity that are occurring simultaneously.

4. Summary

1. Plasma corticosterone concentrations were measured on days 4 and 10 of a primary infection with *N.brasiliensis* in rats, and day 4 p.i. of a secondary infection. Measurements of the plasma corticosterone concentrations of pair-fed controls were included for the primary infection.

2. Although intra-group variation was large, a significant increase in concentration was detected on day 4 p.i. of the primary infection in both infected rats and their pair-fed controls, and a significant decrease was observed in infected rats on day 10 p.i., which did not occur in their pair-fed controls. On day 4 p.i. of a secondary infection plasma corticosterone concentrations were found not to be significantly different to those measured in infected rats on day 10 p.i. of a primary infection. This apparent elevation followed by a reduction in plasma corticosterone

concentrations during primary *N.brasiliensis* infections is discussed with respect to the similar pattern observed in the activity of liver ALT during primary *N.brasiliensis* infections.

4. Measurements of plasma corticosterone concentrations made by other researchers are discussed, notably during infections of rats with *S.ratti*. The influence of lactation on plasma concentrations of this hormone is also mentioned. It is proposed that measurements of liver ALT activity during lactation would make an interesting continuation of this line of study.

SECTION 2

CHAPTER 7: *NIPPOSTRONGYLUS BRASILIENSIS* INFECTION IN IMMUNOSUPPRESSED RATS

1.Introduction

The development of an immune response to an infection is dependent upon the active proliferation of a relatively small number of antigen-sensitive lymphocytes (Roitt, 1984). Immunosuppressive agents can interfere with this process in a number of ways (Roitt, 1984). Cyclophosphamides tend to be antimitotic, inhibiting the synthesis of nucleic acids during mitosis. Cyclosporin, an insoluble metabolite of fungi, selectively inhibits the type II RNA polymerase of antigen-sensitive T-cells during a particular phase. Steroids intervene at several points in the immune response, affecting, for example, the recirculation of lymphocytes and the proliferation of cytotoxic effector cells, as well as having a powerful anti-anaphylactic effect.

The administration of steroids has been shown to be immunosuppressive in various parasitic infections. Although many published results appear to be contradictory, administration of cortisone and related compounds often reduces the resistance of animals to helminth infections (Von Brand, 1979). Some infections which are usually not fatal, have proved to be lethal in hosts that have received cortisone treatment, e.g. *Strongyloides stercoralis* infection in humans (Civantos & Robinson, 1969).

Corticosteroid treatment has been shown to suppress the immune response of the host to *N.brasiliensis* infection, resulting in the protraction of the infection (Parker, 1961; Luffau *et al*, 1969; Harley & Gallichio, 1970; Kennedy, 1980). I decided to investigate liver ALT activity in rats experiencing a *N.brasiliensis* infection prolonged by immunosuppression with betamethasone. As well as measuring the liver ALT activity in these rats and their uninfected controls, the concentration of

endogenous plasma corticosterone was measured and the number of circulating leukocytes was estimated.

2.Results

The rats, all of which were male, were randomly arranged in 4 groups: group A, infected immunosuppressed rats; group B, uninfected, immunosuppressed, pair-fed controls; group C, uninfected, pair-fed controls that were not immunosuppressed; group D, uninfected immunosuppressed controls fed *ad lib.*

All rats in group A had a substantial worm burden at *post-mortem* examination on day 14 p.i. (Table 2.7.1) indicating that administration of betamethasone had disrupted the usual course of infection, probably through immunosuppression.

TABLE 2.7.1.

WORM BURDEN OF IMMUNOSUPPRESSED RATS (GROUP A) ON DAY 14 P.I.

Rats	No. worms counted at post mortem
a	567
b	1015
c	660
d	735
e	719
f	942
g	737
Mean ± SD	767 ± 157

The number of leukocytes/µl blood was significantly higher in infected rats (group A), as compared with rats from the other three groups ($P \leq 0.005$). There was no significant difference ($1.00 \geq P > 0.3$) in leukocyte number between any of the other three groups, (Table 2.7.2).

TABLE 2.7.2.

MEAN LEUKOCYTE COUNT FOR EACH GROUP OF RATS.

Rat groups*	Mean ± SD leukocyte count (no. leukocytes/µl blood)
Infected immunosuppresed rats (A)	10539 ± 3289
Pair-fed uninfected immunosuppressed rats (B)	4296 ± 1262
Pair-fed uninfected non-immunosuppressed rats (C)	5161 ± 1428
Uninfected immunosuppressed rats (D)	5157 ± 1145

* In subsequent tables rat groups are identified by letter

Plasma corticosterone concentration was significantly higher in rats of group C (uninfected, non-immunosuppressed, pair-fed) than in any of the other three groups ($P = 0.0006$). No significant difference ($1.00 > P > 0.600$) in plasma corticosteroid concentration was measured between any of the other three groups (Table 2.7.3).

TABLE 2.7.3.

MEAN PLASMA CORTICOSTERONE CONCENTRATION FOR EACH RAT GROUP.

Rat group	Mean plasma corticosterone concentration ± SD (μg/100 ml plasma)
A (n=7)	7.8 ± 2.8
B (n=7)	8.2 ± 2.6
C (n=7)	25.7 ± 4.4
D (n=7)	6.1 ± 1.1

There was no significant difference (0.9>P>0.4) in liver ALT activity, based on the *in vitro* assay, between any of the four groups of rats (Table 2.7.4). Due to unforeseen circumstances some liver samples were not assayed within the hour *post mortem*, and the activities of these samples are not included.

TABLE 2.7.4.

MEAN LIVER ALT ACTIVITY
FOR EACH RAT GROUP.

Rat group	Mean liver ALT activity ± SD (U/g liver)
A (n=6)	7.48 ± 1.35
B (n=7)	4.86 ± 2.57
C (n=7)	6.56 ± 2.03
D (n=5)	6.89 ± 1.77

3.Discussion

The betamethasone administered in this experiment prolonged the parasite's occupation of the host intestine. From an estimated dose of 4000 larvae, I have usually found approximately 1000 worms at *post-mortem* examination between days 5 - 8 p.i. On day 14 p.i. the number of worms counted at *post-mortem* examination has always been no more than 25, and frequently no more than 4 have been found. In this experiment, approximately 75% of the expected population at day 7 p.i. continued to be occupying the host intestine on day 14 p.i. Although the *N.brasiliensis* continues to survive in the rat gut on day 14 p.i., part of the immune response has clearly been mounted against the parasite, as indicated by the elevated leukocyte numbers. Despite this evidence of some immunoresponsiveness by the host, betamethasone treatment modified the immune response to impair its effectiveness in eliminating the infection.

Betamethasone treatment appeared to have a significant effect on the concentration of circulating corticosterone, (c.f. Section 2, chapter 6). Those rats in group C, that were pair-fed to the infected rats, but did not receive betamethasone treatment demonstrated elevated plasma corticosterone concentrations. This is to be expected in rats stressed by a reduced food intake. The method utilised for the measurement of corticosterone has been described in detail in Section 2, chapter 2, and the results here appear to indicate that betamethasone neither binds to, nor competes with endogenous corticosterone for, transcortin.

Although the liver ALT activity was slightly elevated in rats from all four groups, the fact that no significant difference in ALT activity was demonstrated suggests that both the endogenous increase in corticosteroid concentrations, and the exogenous administration of betamethasone, increased the activity of liver ALT to the same extent.

In non-immunosuppressed rats that had the same intensity of adult *N.brasiliensis* infection as was established in the infected rats in this experiment, ALT activity has been demonstrated to be significantly reduced, (Section 2, chapter

4, Table 2.4.2). This has two implications; firstly, that it is unlikely to be the parasite in itself that is responsible for the depression in liver ALT activity. The parasite is present in the immunosuppressed host, but the activity of ALT is not observed to be reduced in the *in vitro* assay. Secondly, that as the factor that is responsible for the reduction in ALT activity is ineffective or inactive in immunosuppressed rats, then this factor itself, is likely to be a component of the immune response.

4. Summary

1. Rats were arranged in 4 groups: group A, immunosuppressed rats infected with *N.brasiliensis*; group B, uninfected, immunosuppressed pair-fed controls; group C, uninfected, pair-fed controls that were not immunosuppressed; group D, uninfected, immunosuppressed controls fed *ad lib.* Infections were initiated by subcutaneous inoculation with an estimated dose of 4000 3rd-stage *N.brasiliensis* larvae.

2. *Post-mortem* examination revealed a substantial worm burden in group A on day 14 p.i.

3. Leukocyte counts, measurement of plasma corticosterone concentration and liver ALT activity were also conducted at this time in all 4 groups. Group A had a significantly higher leukocyte count ($P<0.005$), and group C a significantly higher plasma corticosterone concentration ($P=0.0006$). No difference in ALT activity between the groups was detected.

4. These results are discussed in relation to whether the parasite itself, or the host immune response to the parasite, is more probably influencing the activity of hepatic ALT.

SECTION 2

CHAPTER 8: ALTERED HOST METABOLISM IN NIPPOSTRONGYLOSIS: THE DEBT OF IMMUNITY ?

The experimental results described in the previous chapters, and the plethora of papers published on the pathology of *N.brasiliensis* infections in rats (Kassai, 1982), indicate that a variety of aspects of host nutrition and metabolism alter in response to this infection. Some experimental results also suggest that, despite the opinion of Gallagher and Symons (1959), certain features of the host's intermediary metabolism are affected by the infection. A tabulated summary of some of these disturbances has been prepared (Table 2.8.1).

In an attempt to improve the basis for understanding the physiological roles of altered metabolism in this host-parasite relationship, research has been directed at investigating mechanisms by which *N.brasilensis* infection may influence a selected feature of host metabolism. The metabolic component studied was the activity of the gluconeogenic enzyme, ALT. This was selected not only because it is an important factor of intermediary hepatic metabolism, but also because comparatively recent research (Ovington, 1985b) has shown that this enzyme alters significantly in activity during infection with *N.brasiliensis*.

The initial increase in hepatic ALT activity seems to correlate strongly with the reduction in food intake and increase in the concentration of circulating corticosterone, but the later decrease in the activity of this enzyme is less easily explained. This coincides with the establishment of an effective immune response, resulting in expulsion of the parasite, whether during a primary or secondary infection, and is of considerable interest.

In Section 2, chapter 7, I put forward the hypothesis that the factor that causes the reduction of hepatic ALT activity, assuming that the results obtained from the *in vitro* assay can be interpreted as a reflection of the *in vivo* situation, is likely to

TABLE 2.8.1.

PHYSIOLOGICAL RESPONSES OF RATS DURING A PRIMARY EXPERIMENTAL INFECTION WITH *N.BRASILIENSIS*.

Parameter	Response
Food intake[a,b]	▼
Body weight[b]	▼
Liver wet weight[a]	◇
Liver dry weight[a]	◇
Gastrocnemius muscle weight[b]	▼
Weight of gut and contents[b]	▲
Body temperature[a,b]	◇
Plasma glucose conc.[a,b]	▼
Plasma triglyceride conc.[b]	▲
Plasma total protein conc.[b,d]	▲▼
Plasma albumin conc.[b,d,e]	▼
Plama globulin conc.[b]	▲▼
Plasma non-esterified fatty acid conc.[b]	▲
Plasma corticosterone conc.[a,c,d]	▲▼
Plasma secretin conc.[b]	▲
Plasma enteroglucagon conc.[b]	▲
Plasma insulin conc.[b]	▼
Plasma gastrin conc.[b]	◇
Plasma pancreatic polypeptide conc.[b]	◇
Plasma cholecystokinin conc.[b]	▲
Plamsa ACTH conc.[c]	▼
Plasma triodothyronine conc.[c]	▼
Plasma thyroxine conc.[c]	▼
Plasma zinc conc.[a]	◇
Copper conc. (blood, muscle & liver)[f]	▼
Leukocyte number[a]	▲
Serum IgE conc.[g]	▲
Plasma a_2-macroglobulin conc.[a]	▲
Liver total protein conc.[c]	▲
Liver glycogen conc.[b]	▼
Liver ALT activity[a,b]	▲▼
Liver PEPCK activity[b]	▲
Liver glucose-6-phosphatase activity[b]	▼
Brush border sucrase activity[h]	▼
Brush border maltase activity[i]	▼

Key

▲ Elevation in measured parameter
▼ Reduction in measured parameter
◇ No alteration in parameter detected

a Robertson, (1986-1989): own observations
b Ovington, 1985b
c Ash *et al*, 1985
d Crompton *et al*, 1978
e Lunn *et al*, 1988
f Selivanova *et al*, 1975
g Jarrett & Bazin, 1977
h Mayberry *et al* 1986
i Symons & Fairbairn, 1963

be a consequence of the immune response to the infection. The basis for this hypothesis is twofold. First, in immunosuppressed rats with a particular adult *N.brasiliensis* burden, the alteration in liver ALT activity measured in rats that have not been immunosuppressed and have a similar worm burden, is not observed. Secondly, while addition of non-immune serum to the *in vitro* assay is accompanied by an apparent decrease in activity measured, the addition of immune serum, leads to a significantly greater reduction.

The identification of this proposed factor is of immense interest. In Section 2, chapter 5, components of the immune response that are possible candidates were explored, with particular emphasis on the possible involvement of cytokines. Although not all the results indicated an involvement in the immune response, various cytokines are known to influence several aspects of metabolism, and this requires that further investigation into this be undertaken. In a recent review by Klasing (1988), a number of ways in which cytokines can affect metabolic processes were considered and these are summarised in Table 2.8.2. It is interesting to compare this table with Table 2.8.1, which lists various metabolic fluctuations that occur during *N.brasiliensis* infections. Although several factors listed alter in apparently opposite directions, notably gluconeogenesis and corticosteroid release, an overall similarity between the two tables is apparent. This reinforces the need for further research into the involvement of cytokines in the immunological response of rats to *N.brasiliensis* infection.

The view that corticosterone plays an important role in both the immune response of rats to *N.brasiliensis* infection, and in the regulation of hepatic ALT activity was discussed in section 2, chapter 6. The possibility that this hormone has a major role in orchestrating the immune response of rats to this infection, and could have consequences for the regulation of ALT activity, suggests a model for the interaction between the immuno-hormonal response of the rats and their metabolism. Observations made on immunosuppressed rats (Section 2, chapter 7) do

TABLE 2.8.2.

ROLES OF LEUKOCYTIC CYTOKINES ON METABOLISM REGULATION*.

Parameter	Response
Food intake	▼
Resting energy expenditure	▲
Body temperature	▲
Glucose oxidation	▲
Gluconeogenesis	▲
Lipoprotein lipase activity	▼
Adipocyte fatty acid synthesis	▼
Adipocyte lipolysis	▲
Hepatic triglyceride synthesis	▲
Hepatic cholesterol synthesis	▲
Hepatic acute phase protein sythesis	▲
Skeletal muscle protein degradation	▲
Hepatic metallothionein synthesis	▲
Hepatic cerruloplasmin synthesis	▲
Corticosteroid release	▲
Thyroxin release	▼
Glucagon release	▲
Insulin release	▲

Key

▲ Elevation in measured parameter

▼ Reduction in measured parameter

◇ No alteration in parameter detected

* Adapted from Klasing, 1988

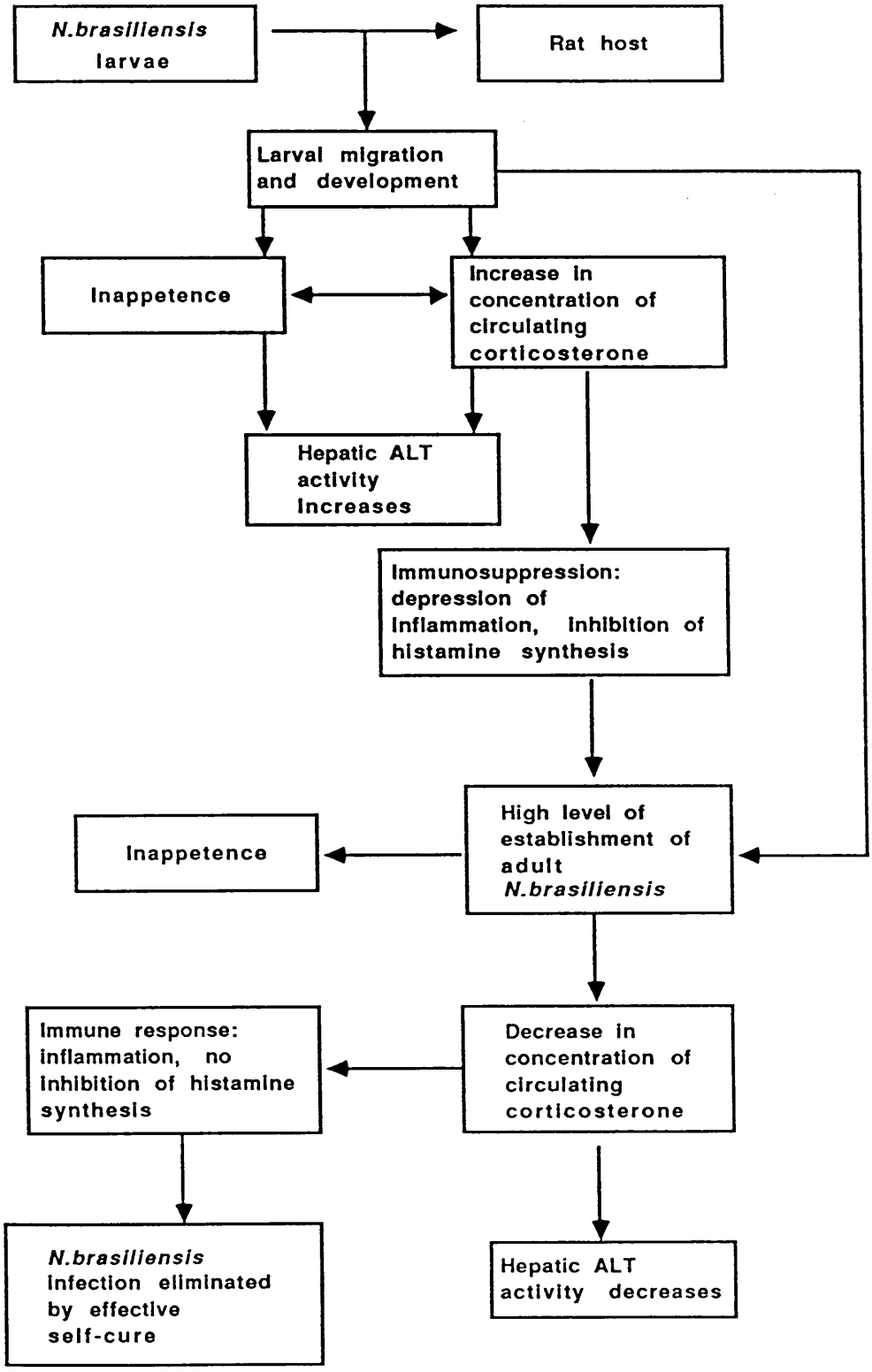
not oppose this idea, but such a theory does not explain how addition of immune serum to the *in vitro* assay for ALT activity should be observed to be more inhibitory than addition of non-immune serum. Some interactions between rats and *N.brasiliensis* suggested by the results of this research are depicted in Figure 2.8.1.

The physiological implications of the metabolic alterations that occur in the host during *N.brasiliensis* infection raise other questions. Within the literature concerned with host responses to parasitic infections, the expressions 'pathological lesion' and 'physiological adaptation' are frequently employed. Which of these most appropriately describes the metabolic alterations observed in rats during nippostrongylosis is questionable, although these expressions may prove to be synonymous. A possibly more interesting and useful way of addressing the interaction between pathology and physiology, is to consider whether or not some of the alterations in host metabolism are inevitable consequences of the successful immunological reaction against the parasite, and serve no real purpose in themselves. In other words, are some of these temporary fluctuations in host metabolism the necessary debt of eliminating the parasite, analogous to the concept of "trade off" employed by behavioural ecologists,

For a host-parasite relationship to succeed, in biological terms, the parasite must be able to reproduce at a rate that maintains itself without eliminating the host population. It could be argued that the laboratory situation is sufficiently far removed from the 'natural' system, as to be irrelevant. The three main arguments for this being first, that the levels of infection used in the laboratory may be much greater than those experienced in the natural situation, secondly, that concurrent infections, which occur commonly in the natural situation, are avoided, and thirdly, that the availability of the host species for infection in the laboratory situation is determined entirely by the researcher. The first argument can be counteracted by knowledge that overdispersion of helminths amongst a natural host population is almost ubiquitous (Keymer, 1982). Although most hosts will be only lightly infected, a minority will have very substantial parasite burdens. It is also possible that

FIGURE 2.8.1.

PRIMARY NIPPOSTRONGYLOSIS IN RATS: SOME POSSIBLE IMMUNOLOGICAL, HORMONAL AND METABOLIC INTERACTIONS



amongst a natural host population, some individuals will only be harbouring single infections. This suggests that, if it is intended to draw implications from the laboratory system to the natural situation, then only a small sub-population of the natural population is being examined. This section being those individuals harbouring single heavy infections. However, the third argument, that the continued existence of a host population is not affected by the morbidity of the parasitic infection in the laboratory situation, but may be in the natural situation cannot be refuted. I believe it is essential to remember that this may have a substantial influence on the evolutionary pressures exerted on the host-parasite relationship.

The popular concept of the relative rates of evolution of parasites and their hosts, considers that endoparasites evolve more slowly than their hosts (Noble & Noble, 1982). The basis of this theory is that the immediate external environment of the parasite, (the internal environment provided by the host), will alter as a response to alterations in the external environment of the host. Therefore pressures for parasite adaptation will occur chronologically later than pressures for host adaptation, thus resulting in a slower rate of evolution. Of course, there are many parasites, including *N.brasiliensis*, which have stages in their life cycles that are directly in contact the environment experienced by their hosts. It is possible that during laboratory culture of *N.brasiliensis* larvae unintentional selection pressures may be imposed on the parasite, particularly selecting for ease of passage and maintenance.

In laboratory situations similar to those described in the preceding chapters, it is believed that host responses to the infection will be unlikely to have altered (Ovington, 1985b). However, there is evidence that during the last 50 - 60 years the rat-*N.brasiliensis* interaction in the laboratory has changed significantly. This is supportive of Holmes's (1983) view that helminths 'track local conditions' and are capable of rapid evolutionary change. The pathogenicity of *N.brasiliensis* has clearly attenuated throughout its many generations of culture in the laboratory. In the

experiments of Africa (1931), a dose of 500 larvae per rat made the rats 'ill'. Faeces were mucoid and the rats became 'apathetic' and emaciated. Lindquist (1950) reported that within 3 days p.i. of an infection with 2500 larvae, many rats died. Symons (1969) infected rats on a body weight basis. His infections per rat would have been between approximately 3000 - 3750 larvae, at which level his rats experienced quite a considerable pathology, including diarrhoea. Although the rats described in the preceding chapters appeared to suffer physical discomfort as a consequence of infection with *N.brasiliensis*, diarrhoea was only observed on one occasion at the maximum dose utilised of 4000 larvae per rat. Ovington (1985a) reported that diarrhoea was not detectable unless a dose as high as 9,400 larvae per rat was given.

The pressure for the pathology associated with a parasite to become attenuated is obvious. If *N.brasiliensis* kills its host, it will simultaneously be destroying itself and, as Lindquist (1950) discovered, this may occur before the parasite has matured and reproduced. However, less easily explained, is the decrease in the time that *N.brasiliensis* can survive within the rat. In 1932 Chandler observed that eggs could be found in the faeces of rats several months after they had been infected with *N.brasiliensis*. Haley (1962) gives examples of research that have demonstrated patent infections lasting for between 30 - 60 days. More recently, egg production has been demonstrated to be minimal by day 10 p.i. (Ogilvie and Jones, 1971), and in my maintenance of this infection, it was not worthwhile to collect faeces for the propagation of larvae after day 8 p.i. Woodbury, Miller, Huntley, Newlands, Palliser & Wakelin (1984) found that between days 9 - 10 p.i. intestinal worm populations were significantly reduced, and in rats that were not immunosuppressed, I never found more than 25 worms on day 14 p.i., from an initial inoculum estimated at 4000 larvae. The pressure for the reduction in duration of infection could be that worms that put all their energy into reproduction as soon as they are mature, rather than immune evasion, are positively selected for. In contrast, worms that would remain longer in the host producing eggs at a slower rate may be

negatively selected for, as the rat may be killed, before the parasite has maximised egg production; the researcher controls the destiny of both the individual rat and if it remains there, the *N.brasiliensis*.

In my opinion, discussion of these evolutionary trends is of importance when addressing the original question as to whether the metabolic fluctuations observed in the host represent the 'debt' of the successful immune response to the *N.brasiliensis* infection, as it facilitates observation of the presence or absence of evolutionary pressure for the rat to experience an altered metabolism during a *N.brasiliensis* infection. In the laboratory situation, the progression towards an attenuated pathology and a relatively rapid susceptibility to the immune response could have evolved for the infective agent, and a similar situation could be envisaged in a 'natural' system. By contrast to a 'natural' system, it is improbable that the host rats in the laboratory situation will evolve a more attenuated response to *N.brasiliensis* infection. The experimental rats are bred from rats that will probably have had no contact with *N.brasiliensis* infection. It is equally improbable, for the same reason, that rats will have evolved a more efficient immune response to *N.brasiliensis* infections. The most probable explanation for some of the observed alterations in host metabolism during *N.brasiliensis* infections, is that they are an inevitable consequence and cost of the immune response that the rat is mounting against the parasitic infection.

SECTION 3

INFLUENCES OF INTESTINAL HELMINTHS ON HUMAN NUTRITIONAL STATUS: A COMMUNITY STUDY

SECTION 3

CHAPTER 1: INTESTINAL HELMINTHIASES IN CENTRAL AMERICA: A POSSIBLE IMPACT ON HUMAN NUTRITION.

Intestinal helminth infections are amongst the most prevalent and persistent health problems in developing countries. It is widely acknowledged that they may have a synergistic effect on nutritional status, especially in children, which is frequently already compromised by dietary inadequacy combined with socio-economic and educational problems. In 1989, the World Health Organisation promoted the setting up of a database concerned with *A.lumbricoides*, one of the most important intestinal helminthiases. In assisting with this, I had the opportunity to study over 60 papers, abstracts and original data sets from Central America, dating from 1975 onwards. The distribution of information available by country is described in Table 3.1.1. Not surprisingly, data from Belize, Honduras and Nicaragua was not accessible, and only one paper with data from El Salvador was found. Many of the papers (40%) described epidemiological surveys, and tended to reiterate the well known association between intestinal helminth infection and poor socio-economic status, and almost as many of the reports (37%) were concerned solely with children. In 18% of the publications, cases of ascariasis proving fatal or requiring surgery were described. Another 18% of the papers were primarily involved with drug trials, and evaluated reinfection rates after particular treatment regimes. Less than 8% of the data was concerned with the possible impact of helminthiases on the nutritional status of the populations, although the occurrence of malnutrition was noted in a number of papers. Those papers which did attempt to investigate associations between malnutrition and intestinal helminthiases often provided convincing clues that these infections were influencing the nutritional status of some communities within Central America (e.g. Taren *et al*, 1987).

Between November 1987 and November 1988, I became a member of a collaborative team (Table 3.2.1.) involved in studying associations between intestinal

helminth infections and malnutrition in the Republic of Panama. Although the project was primarily designed to investigate whether *A.lumbricoides* infection influenced the vitamin A status of the study community, it was possible to incorporate subsidiary lines of investigation into the programme. This study is described in the following chapters.

TABLE 3.1.1.

SOURCES OF DATA CONCERNED WITH INTESTINAL HELMINTHIASES IN CENTRAL AMERICA FROM 1975 TO DATE: DISTRIBUTION BY COUNTRY.

CENTRAL AMERICAN COUNTRY	NO. PAPERS STUDIED
Belize	0
Costa Rica	12
El Salvador	1
Guatemala	8
Honduras	0
Mexico	32
Nicaragua	0
Panama	11
TOTAL	64

SECTION 3

CHAPTER 2: MATERIALS AND METHODS.

1. Investigative team

In a community study of this type and scale, it is generally considered that a collaborative team, optimally involving local workers, is essential. The structure of the team involved in our study in Coclé, Panama is described in Table 3.2.1.

TABLE 3.2.1.

STUDY TEAM STRUCTURE.

TEAM MEMBER	MAJOR ROLE
DWT Crompton; Glasgow, UK MC Nesheim; Cornell, USA D Sanjur; Cornell, USA	Organisation
LJ Robertson; Glasgow, UK	Parasitology Hb measurements Plasma preparation
A Garcia; Cornell, USA R Furumoto; Cornell, USA	Dietary and Socio-economic surveys
P Harvey; Cornell, USA B Parker; Cornell, USA	Plasma retinol measurements
V Caillouet; S.I.S., Panama	Health worker coordination
M De Proll; S.I.S., Panama	Nutrition & anthropometry
C Quiros; S.I.S., Panama	Paediatrician
E De Melamed; S.I.S., Panama R Caceres & colleagues; S.I.S., Panama	Blood collection & technical support
A Batista; ICI, Panama	Anthelmintic supply & ground support

2. Study design protocol.

The field work protocol designed for this study is outlined in Table 3.2.2. Initially it was anticipated to include three periods of data collection over one year, between November 1987 and November 1988. Unfortunately, due to political unrest in the Republic of Panama, it was impossible to undertake the second data collection in June 1988. Through considerable effort, however, Panamanian health workers and Dr. Sanjur were able to distribute anthelmintic to the majority of children (almost 80%) intended to receive it at this time.

TABLE 3.2.2.

PROPOSED STUDY PROTOCOL.
INTENDED FIELD WORK IN COCLÉ PROVINCE

	2 PRIMARY SCHOOLS	2 PRIMARY SCHOOLS
NOVEMBER 1987 (1)	Collection of parasitological, dietary, anthropometric, socio-economic and blood biochemistry data.	
	Anthelmintic* treatment.	No anthelmintic treatment.
JUNE 1988 (2)	Collection of parasitological, dietary, anthropometric, socio-economic and blood biochemistry data.	
	Anthelmintic treatment.	No anthelmintic treatment.
NOVEMBER 1988 (3)	Collection of parasitological, dietary, anthropometric, socio-economic and blood biochemistry data.	
	Anthelmintic treatment for all children.	

* Levamisole, supplied as Ketrax syrup; ICI Pharmaceuticals, UK.

3. Study sites and subjects.

Four primary schools located in the towns of Penonomé, Rio Hato, Anton and Nata in the province of Coclé comprised the study sites. All four communities, the environments of which are generally rural, are located on the Pan-American Highway near the Pacific coast and are of similar size apart from Penonomé which is the provincial capital and considerably larger (Fig. 3.2.1). Children from grades I and II of the schools were initially enrolled in the study which had been described to their families by local health workers. Signed letters of consent were provided by the school on behalf of the children (see Appendix 3.1.1). Each of the children was given a clinical examination by a paediatrician, as detailed in the protocol for the proposed study that had already been reviewed by the Human Subjects Committee of Cornell University. Details of the 661 children initially enrolled in the survey are described in Table 3.2.2. Sub-sets of this group participated in different sections of this project (Section 3, chapters 4, 5, 6, and 7).

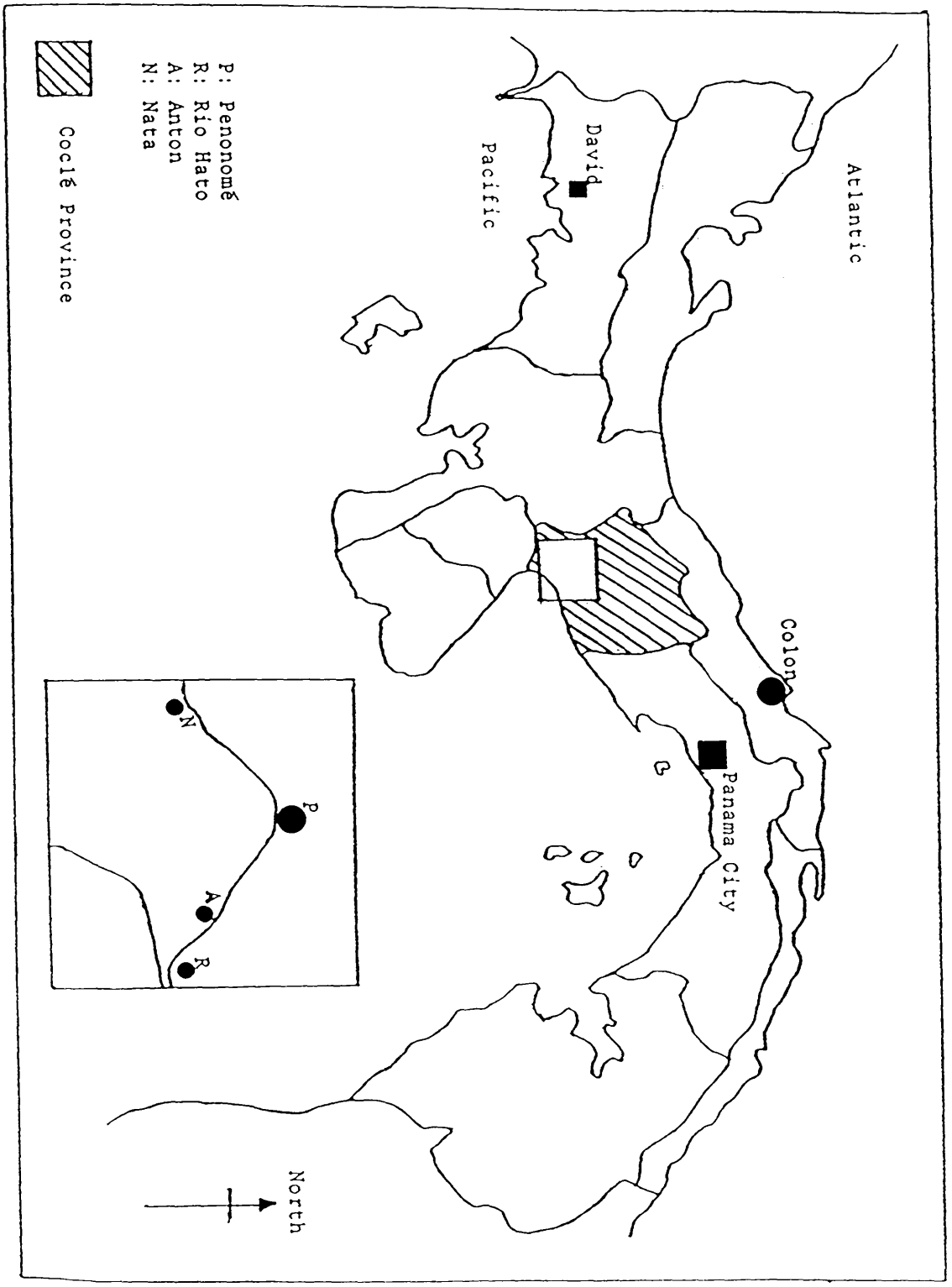
TABLE 3.2.3.

DETAILS OF THE STUDY POPULATION OF PRIMARY SCHOOL CHILDREN

SCHOOL LOCATION	NO. OF BOYS	NO. OF GIRLS	TOTAL	AGE (YEARS) MEAN ± SD	NO. OF SIBLINGS
Penonomé (A)*	62	65	127	7.6 ± 1.0	24
Rio Hato (B)	106	102	208	7.8 ± 1.1	34
Anton (C)	76	77	153	7.3 ± 0.9	30
Nata (D)	90	83	173	7.6 ± 1.5	29
TOTAL	334	327	661	7.6 ± 1.1	117

* In subsequent tables the schools are identified by these letters.

FIGURE 3.2.1. MAP OF PANAMA INDICATING LOCATION OF STUDY SITES.



4. Parasitological techniques.

Faecal samples were collected from the participating children in November 1987 and November 1988. Plastic containers with screw-top lids were distributed to each child at school for collection of a fresh stool specimen to be retrieved on the following day. Each stool was thoroughly mixed, a portion was fixed in 10% aqueous formalin solution and samples were examined in the laboratory using a modified Kato-Katz method (WHO, 1985). Before examination, each stool sample was centrifuged for 5 min at 2000 rpm and material from the pellet was passed through a 100 mesh sieve into a stainless steel template to provide an aliquot of approximately 50 mg. This material was spread on a slide, thoroughly mixed with 2-3 drops of 3% malachite green in 50% glycerol (Martin & Beaver, 1968). The preparation was allowed to stand for at least 30 min, but not longer than 48 h, before examination at x100 magnification. Although some workers feel that malachite green/glycerol preparations do not aid in the examination of stools that have been fixed in formalin, it was found, after a series of tests, that parasite eggs were easily observed.

The anthelmintic levamisole (Ketrax, I.C.I. Pharmaceuticals, Macclesfield) was distributed to project participants attending schools in Rio Hato and Anton in November 1987, after stool collection, and in June 1988. Distribution of the anthelmintic at the latter date was accomplished by local health workers visiting the children at their homes, political unrest having led to the temporary closure of the schools. In November 1988, it was distributed to all children attending the four schools, including those not participating in the project. The anthelmintic was given as a flavoured syrup, which was well received by the children; adverse clinical reactions were not reported. The dosage for the age group being treated was 10ml (27 mg equivalent) which was distributed in disposable paper cups. In November 1987, an attempt was made to collect all worms passed in the faeces following anthelmintic treatment in Rio Hato and Anton. Opaque plastic bags and securely fastened labels were distributed to the children as they received the anthelmintic

dose, and health-workers were asked to explain to the children that all faeces passed in the following 48 h were to be retained. Unfortunately, an apparent misunderstanding resulted in only a minority of faeces being collected, and few of these were more than a small sample. It is improbable that this was a reflection of unwillingness on the part of the study community, as compliance with all other aspects of the project was good.

In subsequent chapters, children in whom evidence of helminth infection was detected are referred to as having a helminthiasis (e.g. ascariasis, trichuriasis). Although this term should strictly be applied only to those children who present with disease symptoms due to the infection, for brevity this rule has not been adhered to. This imprecise terminology is often used in publications by WHO and by Public Health workers.

5.Anthropometry and collection of socio-economic and dietary data

The heights and weights of children were measured in November 1987 and 1988 using an upright beam scale. Weight for age, height for age and weight for height percentages were calculated from standard NCHS charts and tables (Stephenson, Latham & Jansen, 1983). Socio-economic and dietary data were collected between November 1987-March 1988, and November 1988-December 1988 by interviews of care-givers at their homes using food frequency and 24-h recall methods and completion of questionnaires.

6. Blood biochemistry

Blood was collected from children in November 1987 and November 1988. Trained health workers, authorised by the local Director of Health, collected approximately 3 ml of venous blood from the upper arm of each child into a numbered heparinised tube. Blood collection was conducted in a room with subdued lighting and immediately after collection each tube was gently swirled and wrapped in foil to protect from light. The tubes were kept in a cooler on ice-packs until they

could be transferred to a refrigerator, in no longer than 8 h. Results of experiments on the stability of retinol in blood (Mejia & Arroyave, 1983) suggest that blood samples collected in the field can be stored in an ice box for at least 24 h without detriment to the stability of the retinol.

Haemoglobin concentration was measured in a small portion of each blood sample, by the cyanmethaemoglobin method (Crosby, Munn & Furth, 1954) within 3 to 10 h after blood collection. The remainder of each sample was centrifuged at 1750 rpm for 15 min in a darkened laboratory. The plasma obtained from centrifugation was divided into two aliquots in two foil wrapped microtubes, one of which contained sodium ascorbate (approximately 5mg/ml plasma). The plasma aliquots were frozen to -40^{o}C, and kept frozen until vitamin A measurements had been performed within 10 months.

Retinol measurements were performed immediately after thawing the plasma. Retinol was extracted into 1.5 ml hexane, evaporated under nitrogen and redissolved in 100 µl ethanol. Retinol concentrations were determined by reverse-phase high perfomance liquid chromatography (HPLC) on a 15 cm C18 Resolve column (Water Associates, Milford, MA). The HPLC system (Water Associates, Milford, MA) consisted of a Model 510 pump, U6K injector and a Model 450 variable wavelength detector set at 326 nm, connected to a 3390 A integrator (Hewlett-Packard, Paramus, NJ). Methanol:water (90:10) was used as an elutant, and all-*trans*-retinyl-acetate (Sigma, St. Louis, MO) was used as an internal standard.

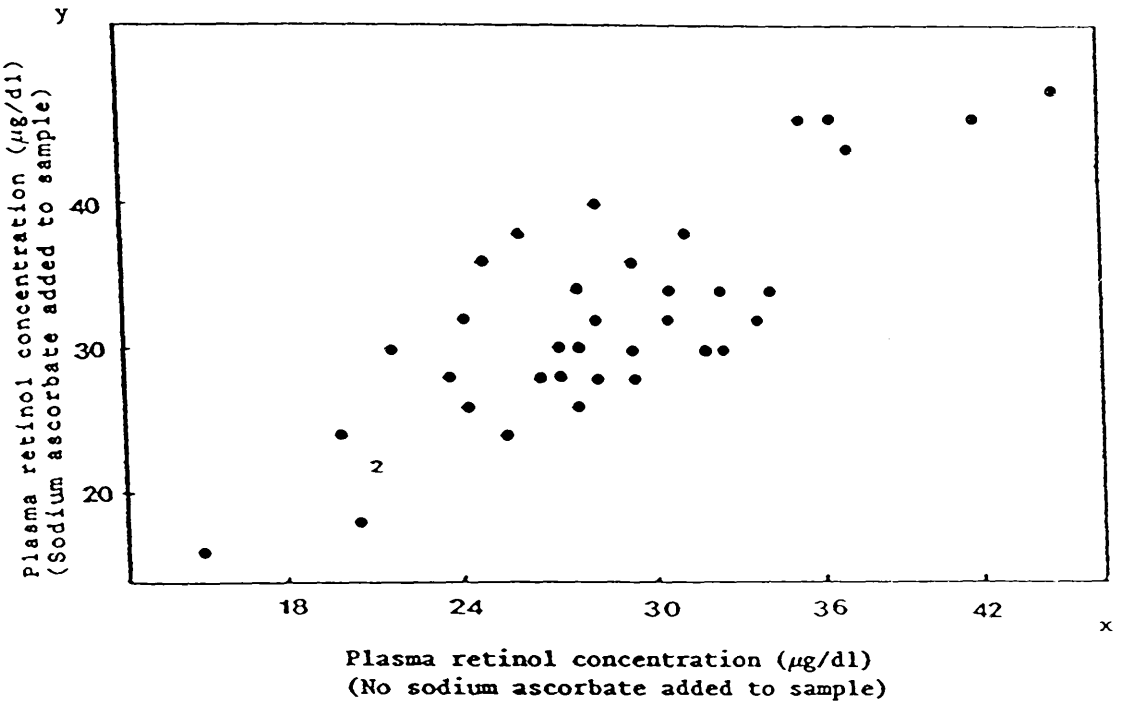
Although all the plasma retinol measurements were conducted at Cornell University, Ithaca, NY, unfortunately it was not possible for the same individual to measure both the 1987 and 1988 samples, thus, inter-analyst error was unavoidable. A further problem arose as the retinol analyses of the 1987 samples were performed on plasma to which sodium ascorbate had not been added. The retinol analyses of the 1988 samples, however, were performed on plasma samples to which sodium ascorbate had been added. Retinol measurements were also conducted on samples from 37 children, both with and without the addition of sodium ascorbate. This not

only demonstrated that sodium ascorbate significantly reduced retinol degradation, but also demonstrated a linear correlation between measurements of plasma retinol concentrations with and without sodium ascorbate addition (Fig. 3.2.2). Accordingly all retinol concentration values obtained by measurements on samples collected in 1987 were transformed by the regression equation given in Figure 3.2.2. before further analysis.

7. Statistical investigation

The quantity of data that this project generated required that a variety of statistical methods be employed. The tests used are mentioned in the relevant chapters, but mostly involved the formation of contingency tables, analyses of variance, paired T-tests and correlations. Where parametric tests were employed, the Kolmogorov-Smirnov test was used to check the normality of the data. GENSTAT, AMSTAT and MINITAB statistical packages were used to handle the data.

FIGURE 3.2.2. THE EFFECT OF THE ADDITION OF SODIUM ASCORBATE TO PLASMA SAMPLES ON THE CONCENTRATION OF RETINOL MEASURED



Correlation of x and y = 0.839

The regression equation is:-
y = 2.37 + 1.03x

Predictor	Coeff	S.D.	t-ratio	P
Constant	2.367	3.304	0.72	0.478
x	1.0339	0.1133	9.13	0.000

s = 4.173 R^2 = 70.4% R^2(adjusted) = 69.6%

Analysis of variance

Source	D.F.	S.S.	M.S.	F	P
Regression	1	1451.1	1451.1	83.35	0.000
Error	35	609.4	17.4		
Total	36	2060.4			

SECTION 3

CHAPTER 3: EPIDEMIOLOGY OF SOIL-TRANSMITTED HELMINTHS IN THE STUDY COMMUNITIES

1. Introduction.

Since the study was investigating relationships between helminthiases and other variables, it was important that an estimate of the prevalence and intensity of soil-transmitted helminths in the study community was undertaken. This assessment was performed from stool samples collected in November 1987 and involved all the 661 children enrolled. Its results enabled comparisons to be made with epidemiological surveys of soil-transmitted helminthiases conducted in Coclé Province by Cort, Stoll, Sweet, Riley & Shapiro (1929) and Ramos (1975), and could also be added to the body of information on the prevalence of intestinal helminth infections within Central America.

2. Results.

1. Infections identified. The common intestinal helminths detected, on the basis of stool examinations, were *Ascaris lumbricoides*, hookworm and *Trichuris trichiura*, but 3 cases of *Strongyloides stercoralis* and 4 cases of *Enterobius vermicularis* were also observed. The species of hookworm was not identified, but geographical evidence suggests it is most probably *Necator americanus* (Migasena & Gilles, 1987).

2. Infection prevalences. The overall prevalences of *A.lumbricoides*, hookworm and *T.trichiura* in the children were observed to be 18.2%, 12.0% and 27.5% respectively. No evidence of a sex effect was detected in the prevalence of the 3 species of helminth. Significant differences were, however, detected between the prevalences in the children attending the 4 different primary schools (Table 3.3.1) by constructing 95% confidence limits on the observed difference for various categories (Walters, 1986).

TABLE 3.3.1.

COMPARISONS OF THE PREVALENCE VALUES OF *A.LUMBRICOIDES*, HOOKWORM AND *T.TRICHIURA* BETWEEN CHILDREN ATTENDING THE 4 SCHOOLS

HELMINTH	PRIMARY SCHOOL[a]	DIFFERENCE	95% LIMITS	P
ASCARIS LUMBRICOIDES	B v. A	0.187	0.103 & 0.260	< 0.001
	D v. A	0.116	0.035 & 0.190	< 0.010
	B v. C	0.163	0.096 & 0.215	< 0.001
	D v. C	0.091	0.009 & 0.168	< 0.050
HOOKWORM	B v. A	0.087	0.003 & 0.159	< 0.050
	B v. C	0.099	0.023 & 0.168	< 0.050
	B v. D	0.145	0.080 & 0.207	< 0.001
TRICHURIS TRICHIURA	B v. A	0.411	0.323 & 0.499	< 0.001
	B v. C	0.268	0.171 & 0.365	< 0.001
	B v. D	0.431	0.350 & 0.512	< 0.001
	C v. A	0.143	0.051 & 0.230	< 0.010
	C v. D	0.163	0.080 & 0.245	< 0.001

a School identity (see Table 3.2.3.)

TABLE 3.3.2.

ASSOCIATIONS BETWEEN THE PREVALENCES OF *A.LUMBRICOIDES*, HOOKWORM AND *T.TRICHIURA* IN THE CHILDREN ATTENDING THE 4 SCHOOLS.

HELMINTH ASSOCIATION	PRIMARY SCHOOL[a] A	B	C	D
ASCARIS/HOOKWORM	1.7[b]	6.0	6.0	0.1
ASCARIS/TRICHURIS	4.6	14.8	5.6	0.5
HOOKWORM/*TRICHURIS*	11.4	23.3	11.9	9.0

a School identity (see Table 3.2.3.)
b Chi-squared values; those above 3.84 are statistically significant at the 5% level.

3. Association between infections. The prevalence data were analysed for possible associations between different helminth infections. As in a study by Annan, Crompton, Walters & Arnold (1986), the data was investigated to see if some infections are more likely to occur together, or apparently avoid each other, or whether such relationships may be generated randomly. Chi-squared tests on data revealed (Table 3.3.2) that there was often significant positive association between infection, and that the most pronounced association was between hookworm and *T.trichiura* in all 4 schools. It is important, however, to remember that trichuriasis was the most prevalent intestinal helminth infection in the study population.

4. Infection in siblings. The prevalence data were investigated with regard to the occurrence of the helminth infections amongst siblings (see Table 3.2.2). The prevalence values were compared for each primary school, between single children, and children known to have a brother or sister in the study population by Fisher's Exact Test (Table 3.3.3). Evidence of a positive association between siblings at Penonomé was detected for each helminth infection, but not in siblings attending the other 3 schools. The observed proportion of infected children in the sibling group at Penonomé was significantly higher than the corresponding proportion in the group of single children

5. Intensity of infections. Large variation in the egg counts was observed for all 3 infections. For example, for *A.lumbricoides* the egg counts ranged from 20 e.p.g. to 126180 e.p.g. Egg counts provide an indirect measure of infection intensity; because it was not possible to obtain worm counts following expulsion chemotherapy, density-dependent effects could not be allowed for. In the combined study population the egg counts were highly over-dispersed for each helminth infection. The frequency distribution in each case was best described by the negative binomial, fitted by using the GENSTAT statistical package. The average values of k for the frequency distributions of egg counts for *A.lumbricoides*, hookworm and *T.trichiura* were 0.015, 0.012 and 0.017 respectively (Table 3.3.4).

The mean egg counts (e.p.g.) for the three helminths in the infected children

TABLE 3.3.3.

A COMPARISON OF THE PREVALENCES OF *A.LUMBRICOIDES*, HOOKWORM AND *T.TRICHIURA* INFECTIONS IN SINGLE CHILDREN AND SIBLING CHILDREN.

HELMINTH	PRIMARY SCHOOL[a]	SINGLE CHILD	SIBLING CHILD	TEST RESULT
ASCARIS LUMBRICOIDES	A	0.058	0.208	$P < 0.05$
	B	0.274	0.273	N.S.
	C	0.106	0.133	N.S.
	D	0.204	0.192	N.S.
HOOKWORM	A	0.068	0.292	$P < 0.01$
	B	0.188	0.273	N.S.
	C	0.089	0.133	N.S.
	D	0.054	0.038	N.S.
TRICHURIS TRICHIURA	A	0.068	0.292	$P < 0.01$
	B	0.511	0.682	N.S.
	C	0.260	0.267	N.S.
	D	0.095	0.115	N.S.

a School identity (see Table 3.2.3)

TABLE 3.3.4.

ESTIMATES OF THE *k* PARAMETER OBTAINED BY FITTING THE NEGATIVE BINOMIAL DISTRIBUTION TO THE EGG COUNT DATA.

PRIMARY SCHOOL[a]	VALUE OF THE *k* PARAMETER		
	ASCARIS LUMBRICOIDES	HOOKWORM	*TRICHURIS TRICHIURA*
A	0.0084	0.0032	-[b]
B	0.0313	0.0074	0.0332
C	0.0066	0.0760	0.0199
D	0.0672	-	0.0092
OVERALL	0.0153 ± 0.0040	0.0124 ± 0.0071	0.0171 ± 0.0046
CHI-SQUARE (6 df)	5.60	6.11	6.01

a School identity (see Table 3.2.3.)
b Failure of convergence with the maximum likelihood process.

attending the 4 primary schools are compared in Table 3.3.5. Analysis of variance on this data revealed significant differences between the mean intensities of *A.lumbricoides* infection by primary school, but not for the 2 other infections. The mean intensity of *A.lumbricoides* infections at Nata was observed to be significantly lower than for children attending the other 3 schools ($P<0.01$). This is particularly interesting because the prevalence of *A.lumbricoides* infection at Nata was found to be significantly higher than those found at Penonomé and Anton, although not significantly different from that at Rio Hato. Analysis of variance revealed no evidence of a relationship between child sex or age, and the intensities of any of the infections.

The transformed intensity data was also examined for any associations between infections. For example, would children with high egg counts for *A.lumbricoides* be expected to have high counts for hookworm or *T.trichiura*? The most convincing finding was the detection of a strong positive association between the intensities of *A.lumbricoides* and *T.trichiura* ($P<0.001$, as assessed by Kendall's coefficient of rank correlation). This appears to be contradictory to the results based solely on prevalence data (Table 3.3.2), but here it applies to infected children only, rather than all those involved in the survey. The striking association between the intensities of *A.lumbricoides* and *T.trichiura* infections as measured by egg counts, is depicted graphically in Figure 3.3.1. The data fall within a broad band indicative of a positive association in which children heavily infected with *A.lumbricoides* are likely to be heavily infected with *T.trichiura*.

3. Discussion

Five major points are evident as a consequence of this epidemiological survey. First, it appears that the prevalences of *A.lumbricoides*, hookworm and *T.trichiura* are steadily declining in Coclé Province. Cort *et al* (1929) studied more than 2000 subjects and found prevalence values of 51.0, 83.1 and 49.9% for *A.lumbricoides*, hookworm and *T.trichiura* respectively in Coclé Province, with research centred, as

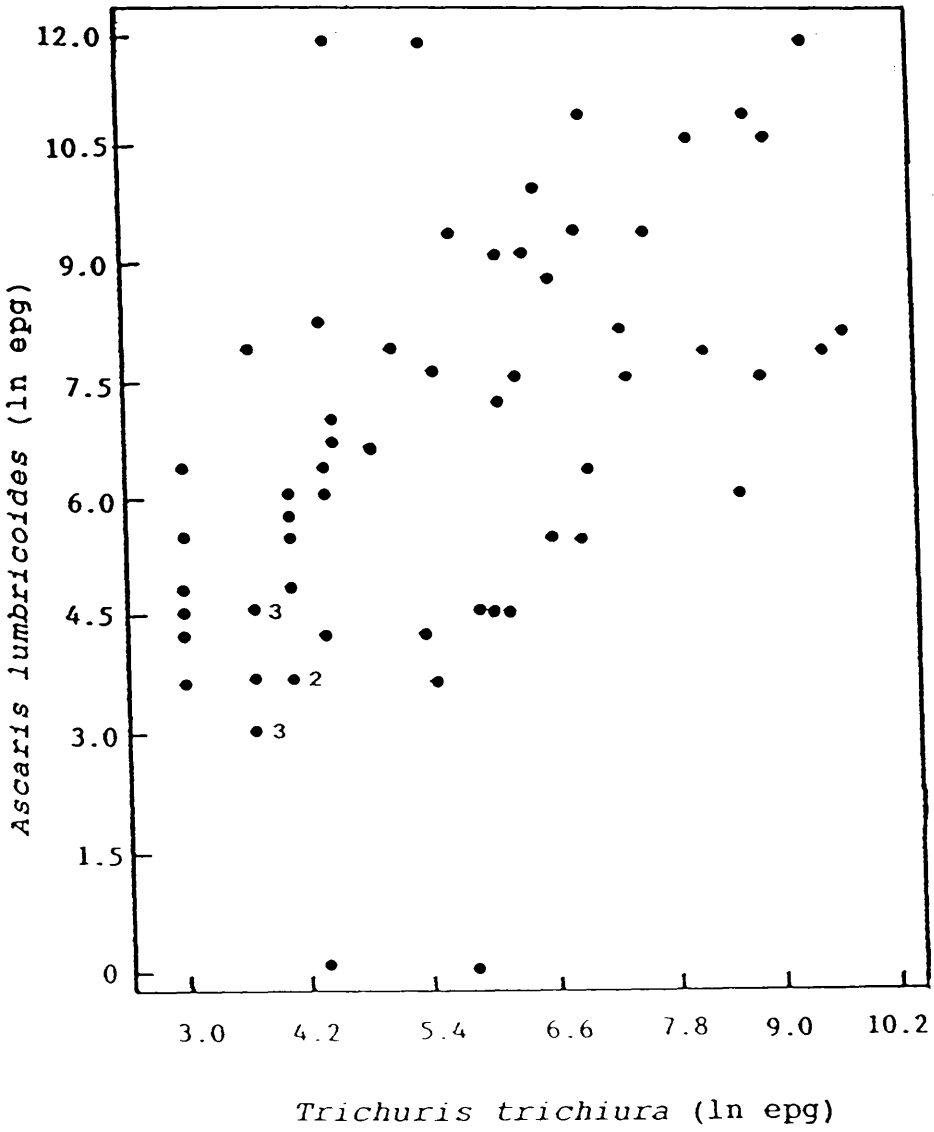
TABLE 3.3.5.

MEAN INTENSITIES (E.P.G.) OF *A.LUMBRICOIDES*, HOOKWORM AND *T.TRICHIURA* INFECTIONS IN CHILDREN ATTENDING THE 4 SCHOOLS.

HELMINTH	SCHOOL[a] A	B	C	D
ASCARIS LUMBRICOIDES	395 (5.980)[b]	438 (6.082)	392 (5.970)	53 (3.967)
HOOKWORM	148 (4.999)	108 (4.684)	159 (5.070)	76 (4.331)
TRICHURIS TRICHIURA	114 (4.737)	250 (5.520)	185 (5.227)	104 (4.648)

a School identity (see Table 3.2.3)
b Logarithmic value

FIGURE 3.3.1. THE RELATIONSHIP BETWEEN THE INTENSITIES (epg) OF A.LUMBRICOIDES AND T.TRICHIURA IN INDIVIDUAL CHILDREN



Where numbers are shown beside solid circles, these represent the number of observations at that point.

in our study, on the provincial capital of Penonomé. Ramos (1975) conducted an epidemiological study among communities in mountainous regions of Coclé, and obtained prevalence values of 22.9, 21.3 and 27.8%. The values from the present study of 18.2, 12.0 and 27.5 for the three helminth infections, although limited by involving only primary school children, demonstrate a decline in prevalence. Most striking is the reduction in the prevalence of hookworm infections. This is probably related to the health care provided by the Sistema Integrada de Salud, the extension of education, improved hygiene, and the availability and use of shoes.

The second point of interest was that for a given infection, the highest or lowest values for prevalence and intensity did not necessarily coincide. For example, the overall prevalence of *A.lumbricoides* in children attending school in Penonomé was 8.7%. However, the mean intensity there was much higher than among infected children attending school at Nata which had a prevalence of 20.2%.

The third point was the observation that the three infections were only significantly associated among siblings in the children at the school in Penonomé, and not at the other schools. *A.lumbricoides* is recognised as being associated with family members (WHO, 1967; Williams, Burke & Owen-Hendley, 1974), and more recently a clustering for both *A.lumbricoides* and *T.trichiura* has been described by Forrester, Scott, Bundy & Golden (1988) from a community in Mexico. The restriction of the association of all three infections with siblings to one location in this survey indicates a possible source of focal transmission which merits further investigation.

The fourth point of particular interest and potential importance, was the finding that individual children tended to harbour similar intensities of infection of *A.lumbricoides* and *T.trichiura*, as measured indirectly by egg counts. A number of studies in various regions have demonstrated that individuals may be predisposed to either heavy or light intestinal nematode infections (Haswell-Elkins, Elkins & Anderson, 1987; Holland, Asaolu, Crompton, Stoddart, MacDonald & Torimiro, 1989). Proposed explanations for the observed predispositions include genetic,

behavioural and environmental factors (Keymer & Pagel, 1989). The possibility of predisposition to given intensity levels for more than one helminth infection simultaneously in the same individual deserves to be investigated more fully.

Finally the data was compared with contemporary prevalence data from other regions of Central America, obtained from epidemiological reports published in or after 1977 (10 years prior to the start of our study in Panama). This data (Table 3.3.6) indicates that within Central America intestinal helminths are a ubiquitous problem, with *T.trichiura* frequently the most prevalent.

4. Summary

1. The prevalence and intensity of soil-transmitted helminths in the study community was investigated by faecal egg counts.

2. The three most common helminth infections observed were *A.lumbricoides*, hookworm and *T.trichiura*. The overall prevalences were, respectively, 18.2, 12.0 and 27.5%.

3. Significant differences were observed between prevalences of infection at the three schools. No evidence of a sex effect was detected.

4. Association between infections was often noticed, and was most pronounced between hookworm and *T.trichiura*.

5. In the children attending school at Penonomé, but at none of the 3 other schools, a positive association between siblings was found for prevalence of each of the 3 infections.

6. Intensity of infection, as measured by faecal egg counts, had large variation. The frequency distributions were best described by the negative binomial. Mean egg counts were significantly lower for *A.lumbricoides* infection at Nata, than at the other 3 schools, despite the prevalence of ascariasis being considerably higher at this school than at Penonomé or Anton.

7. Transformed intensity data also revealed a strong positive correlation between infection intensities of *T.trichiura* and *A.lumbricoides*.

8. The results are discussed in relation to associations between prevalence and intensity of infection, associations of infections with siblings, and associations of high levels of intensity between infections. The data is also compared to results from previous studies conducted in the same area over a period of 60 years, and to more contemporary data from all over Central America.

9. Comparison of this prevalence data with more contemporary data (published in the last 10 years) from other parts of Central America, revealed great regional variation, but that *T.trichiura* was often the most prevalent intestinal helminth infection.

TABLE 3.3.6.

CONTEMPORARY DATA ON THE PREVALENCE OF INTESTINAL HELMINTH INFECTIONS IN CENTRAL AMERICA.

COUNTRY	REGION	SAMPLE SIZE	SAMPLE AGE-GROUP	INFECTION PREVALENCES* AL	HW	TT	OTHER INTESTINAL HELMINTHS DETECTED	DATA SOURCE
Costa Rica	Northern Region	162	Children	18.5	-	69.8	*S.stercoralis, H.nana*	Bullock (1980)
Costa Rica	San José	271	Children	32.1	25.1	100.0	*S.stercoralis, H.nana E.vermicularis*	Cortes, Madrigal, Chavarria, Piedra & Castro (1977)
Costa Rica	Cartago	156	Children	16.0	1.3	61.5	*S.stercoralis, H.nana*	Porras, Vindas & Solano (1978)
Costa Rica	Nicoya Peninsula	138	Children	4.3	-	28.0	-	Serrato, Garcia, Gomez, Chavez, Ramirez & Quiros (1978)
Costa Rica	Puriscal Area	1901	-	10.0	8.5	35.0	-	Zamora, Mora, Soto & Beauregard (1978)
El Salvador	-	210	Children	18.0	-	31.0	*H.nana*	Reinthaler, Linck, Klem, Mascher & Sixl (1988)
Guatemala	-	15383	-	72.6	18.6	2.7	*S.stercoralis*	Aguilar, (1981)
Guatemala	Department of Sacatepequez	159	Children	60.0	-	-	-	Gupta & Urrutia (1982)
Guatemala	Department of Sacatepequez	45	Children	93.0	-	49.0	-	Mata, Kromal, Urrutia & Garcia (1977)
Guatemala	San Pedro Ayumpac	102	60+ years	41.0	-	49.0	-	Santizo, Zepeda, King, Castaneda, Mazariegos & Solomons (1989)
Guatemala	- Blind	102	Children	14.7	12.7	43.1	*H.nana*	Scott, Santizo, Zepeda, Ramirez & Forrester (1989)
	Deaf	218	Children	20.6	4.6	39.3	*H.nana*	
Mexico	Jalisco State	144	Children	36.8	-	27.8	-	Arcinga, Amaral, Sandoval, Orendain, Hunz & Saavedra (1982)
Mexico	Chiapas: Village 1	150	-	68.0	66.7	86.7	-	Carrillo & Hernandez (1977)
	Village 2	108	-	31.0	25.3	27.2	-	
	Village 3	104	-	11.5	58.7	14.4	-	
Mexico	Tabasco: Site 1	74	Children	35.1	4.1	51.4	*S.stercoralis, H.nana H.diminuta*	Dewey (1983)
	Site 2	37	Children	16.2	-	10.8	*S.stercoralis*	
Mexico	Merida City	9071	-	6.16	0.12	4.33	*S.stercoralis, H.nana*	Duarte-Zapata, Escalante-Triay & Novelo de Ceballos (1984)
Mexico	Chiapas: Indigenous	204	-	45.5	37.0	36.4	*S.stercoralis, E.vermicularis*	Garcia, Gallardo, Munoz, De Lara, Altamirano, Huerta Galnares, Carrasco & Hernandez (1987)
	Refugees	318	-	36.3	44.1	37.7	*S.stercoralis, E.vermicularis*	
Mexico	Mexico City	833	13 - 15 years	3.7	0.001	1.2	*H.diminuta, H.nana, E.vermicularis*	Guerrero, (1983)
Mexico	Mexico City	100	7 - 10 years	19.0	11.0	31.0	*S.stercoralis, E.vermicularis*	Ortega, (1982)
Mexico	Tulpetlac	380	Children	35.5	-	19.5	*E.vermicularis, H.nana*	Ponce, Alvarez-Chacon & Perez-Amador (1978)
Mexico	Southern Region, Federal District	538	-	14.1	-	5.2	*H.nana*	Salazar-Schettino, Garcia-Yanez, Ruiz-Hernandez, Alonso-Guerrero, Quintero-Garcia, De Auajare-Cinta, & Rodriguez-Ramos, (1981)
Mexico	Yucatan Peninsula	50	-	44.0	4.0	80.0	*H.diminuta, H.nana S.stercoralis*	Urquiaga & Pavia (1982)
Mexico	Lagunera Region	80	-	1.3	-	-	*E.vermicularis, H.nana*	Valdez, Albores, Cebrian & Tellez (1982)
Panama	East Chiriqui	56	Children	42.8	62.5	42.8	*S.stercoralis*	Crompton, (1984). Unpublished.
Panama	Chiriqui	140	-	27.0	14.0	34.0	*S.stercoralis*	Holland, Crompton, Taren, Nesheim, Sanjur, Barbeau & Tucker (1987)
Panama	Coclé	661	Children	18.2	12.0	27.5	*S.stercoralis E.vermicularis*	Robertson, Crompton, Walters, Nesheim, Sanjur & Walsh (1989)

* AL: *Ascaris lumbricoides*
HW: Hookworm
TT: *Trichuris trichiura*

SECTION 3

CHAPTER 4: HELMINTHIASES IN 1987 AND 1988: EFFICACY OF LEVAMISOLE AS A CONTROL MEASURE.

1.Introduction

A comparison of the prevalence and intensity of the three common intestinal helminthiases observed in Coclé, Panama between November 1987 and November 1988 was made in a sub-group of 177 children selected from the 661 children originally enrolled. The children in the sub-group were selected on the basis of 4 criteria. First, several children had left the study community in the year, and so were unavailable to contribute further to the study. Secondly, children were only included if they had both had helminth egg counts conducted on faecal samples collected in 1987, and had had successful retinol measurements made on plasma collected in 1987. It was not considered ethical to collect more samples than necessary from the children; this considerably reduced the number of children participating in this stage of the study. Thirdly, samples were only collected from children attending the schools in Rio Hato and Nata if they had received anthelmintic treatment from the project in both November 1987 and June 1988. Fourthly, in order to increase the independence of the observations, only one child out of sibling sets was included.

Data analysis allowed comparisons be made of both prevalence and intensity of the three helminthiases between November 1987 and June 1988 between the four schools, in two of which children had received anthelminthic treatment twice at six month intervals.

2.Results

1. Infection prevalences. The prevalence of the three intestinal helminths *A.lumbricoides*, hookworm and *T.trichiura*, in the sub-group of 177 children in both 1987 and 1988 is described by school in Table 3.4.1. Application of Chi-squared and

McNemar tests revealed that in these children in 1987 the prevalence of ascariasis was not significantly different between the 4 schools, that the prevalence of hookworm only revealed a significant difference when data from Anton and Nata were compared ($0.025<P<0.05$), and that the prevalence of trichuriasis was significantly lower in Nata than in both Rio Hato ($P<0.005$) and Anton ($0.01<P<0.025$).

In 1988, the prevalence of ascariasis was significantly higher in Anton than in both Penonomé ($0.025<P<0.05$) and Nata ($P<0.005$), and also the prevalence of ascariasis in Rio Hato was significantly higher than in Nata ($0.025<P<0.05$). As in 1987 the only significant difference detected in hookworm prevalence was between Anton and Nata ($0.025<P<0.05$). In Rio Hato the prevalence of trichuriasis was significantly higher than in both Penonomé ($0.01<P<0.025$) and Nata ($P<0.005$).

With the exceptions of *T.trichiura* infection in Nata, the prevalence of all 3 infections dropped between 1987 and 1988 in all 4 schools. In order to try and determine whether children who received levamisole (Ketrax) in November 1987 and June 1988 showed a significant improvement in helminth infection prevalence as compared to those children to whom levamisole was not distributed until the end of sample collection in November 1989, data from Penonomé and Anton was pooled, as was data from Rio Hato and Nata. Improvements in percentage prevalence of the three infections in the two treatment groups are compared in Figures 3.4.1. a, b and c. Chi-squared and Fisher's Exact Probability tests demonstrated a significant reduction in the prevalence of hookworm and *A.lumbricoides* infections in the group that had received the levamisole treatment; this effect was particularly marked in *A.lumbricoides* infections. No significant reduction was detected in the prevalence of trichuriasis. In the group of children that had not received anthelmintic treatment from the project at this stage, a significant reduction in prevalence was only observed in ascariasis.

2. Infection intensities The mean intensities of the 3 intestinal helminths infections, as measured by egg per gramme of sieved faecal pellet in the sub-group of 177

TABLE 3.4.1

PREVALENCES OF *A.LUMBRICOIDES*, HOOKWORM & *T.TRICHIURA* INFECTIONS IN THE SUB-GROUP OF 177 CHILDREN IN 1987 AND 1988. A COMPARISON BY SCHOOL.

SCHOOL	A[a]		B		C		D	
HELMINTH INFECTION	NOV. 1987	NOV. 1988	NOV. 1987	NOV. 1988	NOV. 1987	NOV. 1988	NOV. 1987	NOV. 1988
Ascaris lumbricoides	8[b]/18[c] 0.444[d]	1/18 0.056	30/80 0.375	14/80 0.175	14/33 0.424	10/33 0.305	20/46 0.435	2/46 0.043
Hookworm	3/18 0.167	1/18 0.056	12/80 0.150	3/80 0.038	6/33 0.182	3/33 0.091	2/46 0.043	0/46 0.000
Trichuris trichiura	3/18 0.167	1/18 0.056	28/80 0.350	27/80 0.338	11/33 0.333	9/33 0.273	5/46 0.109	5/46 0.109

a School identity (see Table 3.2.3)
b Number of children infected
c Number of children from school participating
d Proportion of children infected

TABLE 3.4.2.

MEAN INTENSITIES (E.P.G.) OF *A.LUMBRICOIDES*, HOOKWORM AND *T.TRICHIURA* INFECTIONS IN THE INFECTED CHILDREN IN THE SUB-GROUP OF 177, IN NOVEMBER 1987 AND 1988. A COMPARISON BY SCHOOL.

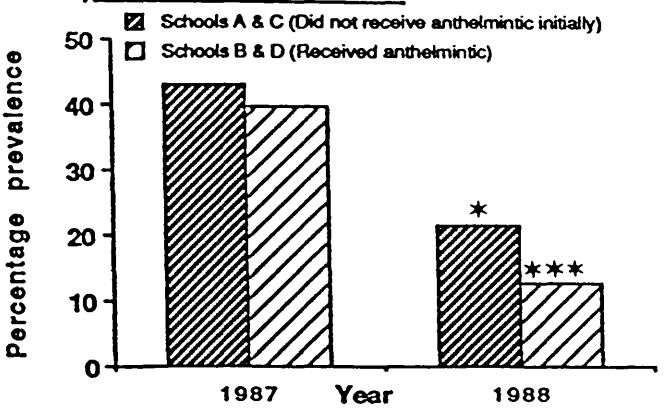
SCHOOL	A[a]		B		C		D	
HELMINTH INFECTION	NOV. 1987	NOV. 1988	NOV. 1987	NOV. 1988	NOV. 1987	NOV. 1988	NOV. 1987	NOV. 1988
Ascaris lumbricoides	16667[b] 9.72[c] (8)[d]	5780 8.66 (1)	10647 9.27 (30)	3041 8.01 (14)	6834 8.83 (14)	21974 10.00 (10)	244 5.50 (20)	20 3.00 (2)
Hookworm	2727 7.91 (3)	1240 7.12 (1)	285 5.65 (12)	40 3.69 (3)	520 6.25 (6)	293 5.68 (3)	1330 7.19 (2)	-- -- (0)
Trichuris trichiura	93 4.53 (3)	20 3.00 (1)	2434 7.80 (28)	1762 7.47 (27)	1384 7.23 (11)	4578 8.43 (9)	2868 7.96 (5)	5352 8.59 (5)

a School identity (see Table 3.2.3.)
b Mean intensity (e.p.g.)
c Logarithmic value
d n

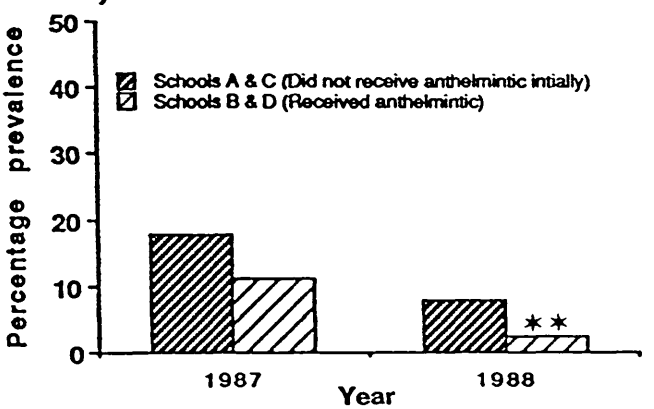
FIGURE 3.4.1. REDUCTION IN PREVALENCES OF HELMINTHIASES BETWEEN NOVEMBER 1987 AND NOVEMBER 1988. COMPARISON BETWEEN TREATMENT GROUPS.

0.01<P<0.025 *
0.005<P<0.01 **
P<0.005 ***

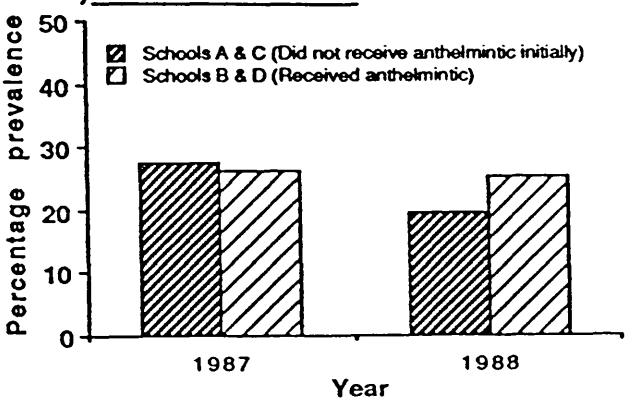
a) Ascaris lumbricoides



b) Hookworm



c) Trichuris trichiura



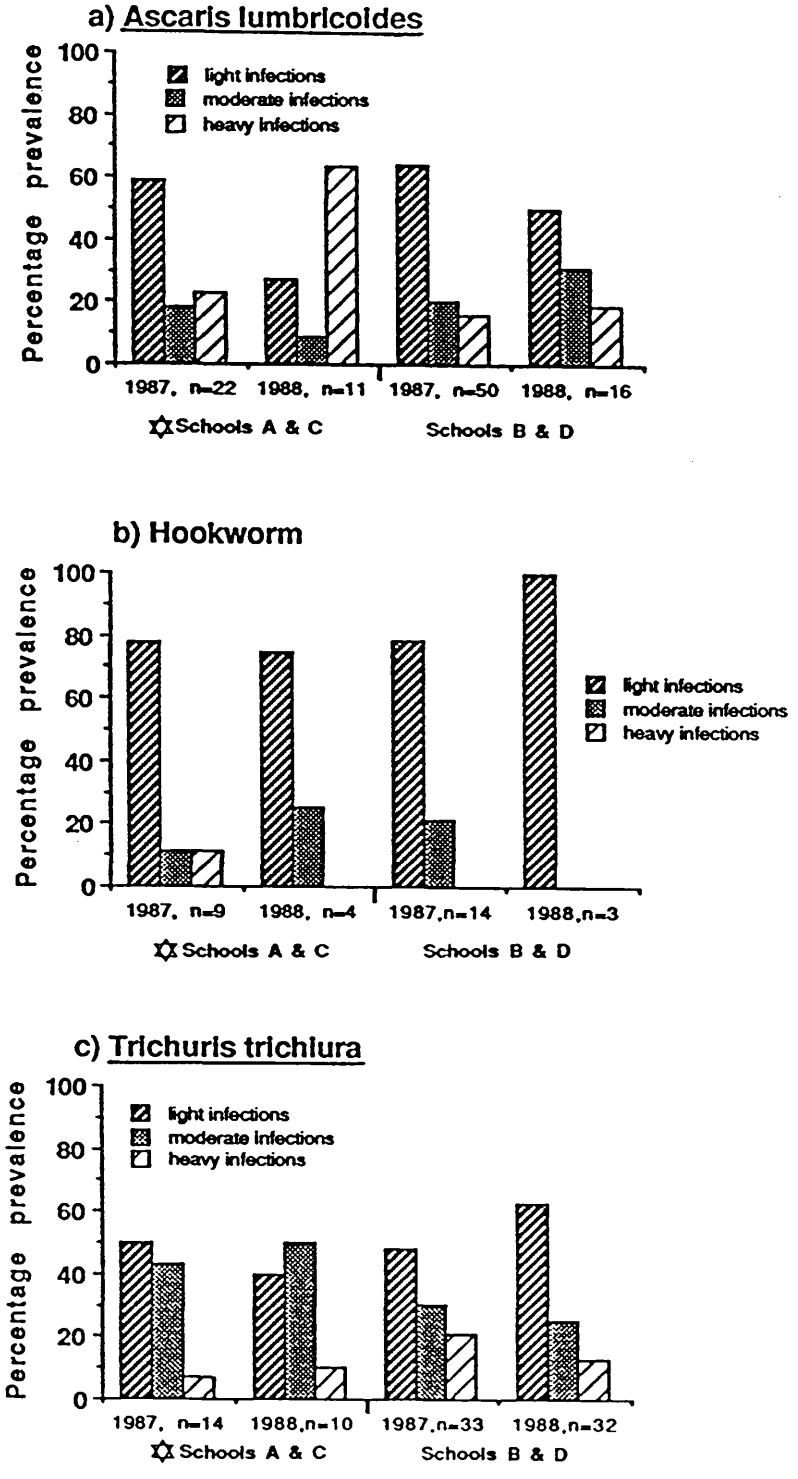
children, is described by school for both November 1987 and 1988 in Table 3.4.2. Uninfected children are not included in the table.

Analysis of variance on the transformed faecal egg count data did not reveal any significant difference in infection intensity between schools in either 1987 or 1988, apart from in the intensity of ascariasis in 1987 ($P<0.001$). This difference was due to the mean intensity of ascariasis being considerably lower in Nata than in the other four schools in November 1987.

Intensity data from children attending schools in Rio Hato and Nata, and from Penonomé and Anton, were pooled to compare the two treatment groups. Again, analysis of variance did not reveal any significant alteration in infection intensity between 1987 and 1988 for any of the three infections in either group. In further investigations on pooled treatment group intensity data, infection intensities were classified as either light (20-499 e.p.g.), moderate (500-4999 e.p.g.) or high ($\geq$ 5000 e.p.g.) for each helminth infection. The intensity ranges for each category have been selected arbitrarily. A large proportion of the infections are light (20-499 e.p.g.), and the definitions of infection intensity sometimes used in field studies (for example an infection of *A.lumbricoides* is only classified as heavy when e.p.g are greater than 50000 (WHO 1987)) were not considered appropriate.

Alterations in the percentage prevalence between 1987 and 1988 of each infection intensity category among the infected children of the two treatment groups are compared in Figures 3.4.2. a, b and c. Although in Penonomé and Anton, Chi-squared values suggest an increase in the proportion of more heavily infected children with ascariasis, the size of the category classes are too small for any other statistically significant changes to be detected. It is apparent that, in general, children infected with hookworm or *T.trichuria* attending school in either Rio Hato or Nata were more likely to belong to a lower intensity class in November 1988 than November 1987. The same generality cannot be applied to similarly infected children attending school in either Penonomé or Anton.

FIGURE 3.4.2. PERCENTAGE PREVALENCES OF EACH INFECTION INTENSITY CATEGORY IN NOVEMBER 1987 AND NOVEMBER 1988. A COMPARISON BETWEEN TREATMENT GROUPS. INFECTED CHILDREN ONLY.



✡School identity (see Table 3.2.3)

3.Discussion

Over the course of a year between November 1987 and November 1988 there has been an overall reduction in the prevalence of all 3 infections. This observation is significant for the reduction in the prevalence of ascariasis in both the treatment group that received anthelmintic in November 1987 and June 1988, and the treatment group to which levamisole was not initially distributed. Efficient anthelmintics are readily available commercially in Panama, and interviews with care-givers revealed that such drugs are often administered to their children. This is especially evident in Penonomé, the regional capital of Coclé, where the school is situated close to the hospital. It is also possible that the implementation of this research project prompted some care-givers to seek anthelmintic treatment for their children. Consequently, the fact that children did not receive anthelmintic treatment from this research project until November 1988, does not preclude their having received similar treatment from another source before this date. In this sense the group to which levamisole was not initially distributed cannot be regarded as a true control in this data set.

Despite the lack of a true control group, it is evident that bi-annual treatment with levamisole does have a significant impact on the prevalence of both ascariasis and hookworm within the community. Unfortunately the sample size was not great enough to demonstrate any alteration in infection intensity, but the general trend suggests that this treatment regime reduces the intensity of infection, especially with *T.trichiura*. Chemotherapeutic trials with levamisole have demonstrated a significant impact on the prevalence of ascariasis and hookworm, that is not also observed with trichuriasis (Theinpont, Brugmans, Abadi & Tanamal 1969; Seo 1980). It is generally agreed that the control of soil-transmitted helminthiases would be most effectively enforced by an combined approach involving not only chemotherapy, but also improvements in environmental sanitation and basic hygiene measures (Kan 1986). It is possible that in areas where the initial prevalence of *A.lumbricoides* infections is

higher and standards of environmental sanitation are, perhaps lower, bi-annual treatment with levamisole or other anthelmintic will have little or no impact on the prevalence of intestinal helminthiases. For example, in a village in rural Burma, Thein Hlaing, Than Saw & Myint Lwin (1987) found that 6-monthly treatment with levamisole (Ketrax) did not result in a reduction in the prevalence of ascariasis one year after the initial treatment.

Anderson (1989) has argued that prevalence is not a useful score when considering parameters, such as nutritional status or immunological response, associated with infectious disease agents such as soil-transmitted helminthiases. If, as in animal studies, pathological features associated with intestinal helminth infections are intensity related, then control programmes which reduce intensity of these infections may be as important as those which reduce prevalence. In the community study conducted by Thein Hlaing *et al* (1987), although prevalence of ascariasis was not reduced by two 6-monthly treatments with levamisole (Ketrax), the intensity was significantly reduced in both children and adults, and a single levamisole treatment was sufficient to have caused a definite reduction in the intensity of *A.lumbricoides* infections in adults. In our study in Panama, the sizes of the intensity classes became too small to allow the detection of statistical significance, but the trend suggested that the 6-monthly treatment with levamisole resulted in a reduction in the intensity of hookworm and *T.trichiura* infections.

These findings reveal that bi-annual treatment with levamisole could play an important role in a programme to control intestinal helminth infections within this community.

4.Summary

1. The intensity and prevalence of *A.lumbricoides*, hookworm and *T.trichiura* infections were compared between November 1987 and November 1988 in a sub-group of 177 children.

2. Intensities and prevalences were compared by school at both these occasions.

3. Alterations in prevalence and intensity between November 1987 and November 1988 were compared within treatment group; data from children attending schools in Rio Hato and Nata where levamisole (Ketrax) was distributed in November 1987 and June 1988 were pooled. Likewise data were pooled from children attending schools in Penonomé and Anton where anthelmintic treatment was not offered until November 1988.

4. Significant decreases in the prevalence of ascariasis and hookworm infection were detected between 1987 and 1988 in the group that received the anthelmintic, but in the second treatment group only a decrease in the prevalence of *A.lumbricoides* infection was detected.

5. Sample sizes were too small to observe any statistically significant changes in the intensities of the three helminthiases, but the general trend revealed a decrease in the proportion of heavier intensities of infection in children infected with hookworm and *T.trichiura* in the treated group.

6. These results are discussed in relation to the validity of including anthelmintic treatment in a programme to control intestinal helminthiases in the study community.

SECTION 3

CHAPTER 5: ANTHROPOMETRIC VARIABLES: A POSSIBLE IMPACT OF INTESTINAL HELMINTHIASES

1. Introduction.

The combined variables of height, weight and age provide indications of malnourishment in children. By calculating percentage weight for age, height for age, and weight for height, children can be classified into 4 nutritional categories (Latham, Latham & Basta, 1977). These 4 categories are:-

a. Essentially normal: Although the weight for age of children belonging to this category may be slightly below 90%, weight for height and height for age will be close to 100%.

b. Past chronic malnutrition: Children in this category will be of low weight and height for their age, but of normal weight for height. This is indicative of an adequate diet at present, but the effects of earlier energy or protein deficiencies are apparent.

c. Current acute malnutrition (short term): Children falling within this category will be of normal height for age, but a low weight for both age and height will indicate a recent deficiency of energy and/or protein, but no evidence of a long term deficiency.

d. Current acute malnutrition (long term): Children classified as belonging to this category will have low values for all 3 variables; evidence of both a present and past deficiency of protein and/or energy.

Some degree of retardation in child growth has long been associated with helminth infections, including schistosomiasis, ascariasis, trichuriasis and hookworm infections (Stephenson, 1987; Holland, 1987). In our Panamanian study we had the opportunity to use two approaches to investigate the impact or otherwise of intestinal helminthiases on anthropometric measurements. First, a cross-sectional study of 625 children in November 1987 allowed comparisons to be made between

uninfected and infected children. Secondly, in a smaller group of 169 children, a longitudinal study between November 1987 and November 1988 enabled a comparison to be made between alterations in anthropometric measurements and changes in helminthological status.

2. Results.

1. Base line anthropometric measurements. The overall means ± standard deviations of percentage weight for height, height for age and weight for age are presented in Table 3.5.1. Using the 4 nutrition categories described above, over 98% of the children were classified as being of normal nutritional status. Less than 2% were classified as belonging either to past chronic or chronic acute (short duration) malnutrition categories.

Boys tended to be of a lower weight for height, height for age and weight for age than the girls, but only in the latter of these three variables was the difference between girls and boys significant (Table 3.5.1). The breakdown of percentage weight for age, height for age and weight for height followed the classic pattern of the values becoming lower for each variable with increasing age (Fig.s 3.5.1 a, b and c).

Comparison of the three anthropometric variables by school (Table 3.5.2) revealed that children attending school in Rio Hato tended to be of lower weight and height for age, and children attending school in Penonomé tended to be of a greater weight for height than children attending the other three schools.

2. Anthropometric variables and intestinal helminthiases. Of the 625 children participating in this stage of the study, in 386 no evidence of intestinal helminth infection was detected. In the other 239 children, infections of *A.lumbricoides*, *T.trichiura* and hookworm were observed. In some children the infections were detected singly; other children were considered to harbour 2 of these infections. Eggs from all 3 parasites were observed in faecal samples from 22 of the children.

TABLE 3.5.1.

MEAN ANTHROPOMETRIC MEASUREMENTS.
COMPARISON BY SEX.

	TOTAL	BOYS	GIRLS	P (BOYS c.f. GIRLS)
N	625	314	311	
% WEIGHT FOR AGE (MEAN ± SD)	98.25 ± 17.11	96.61 ± 14.02	99.90 ± 19.63	0.016
% HEIGHT FOR AGE (MEAN ± SD)	98.16 ± 4.77	97.85 ± 4.41	98.46 ± 5.09	0.108
% WEIGHT FOR HEIGHT (MEAN ± SD)	101.85 ± 11.25	101.35 ± 9.68	102.36 ± 12.64	0.264

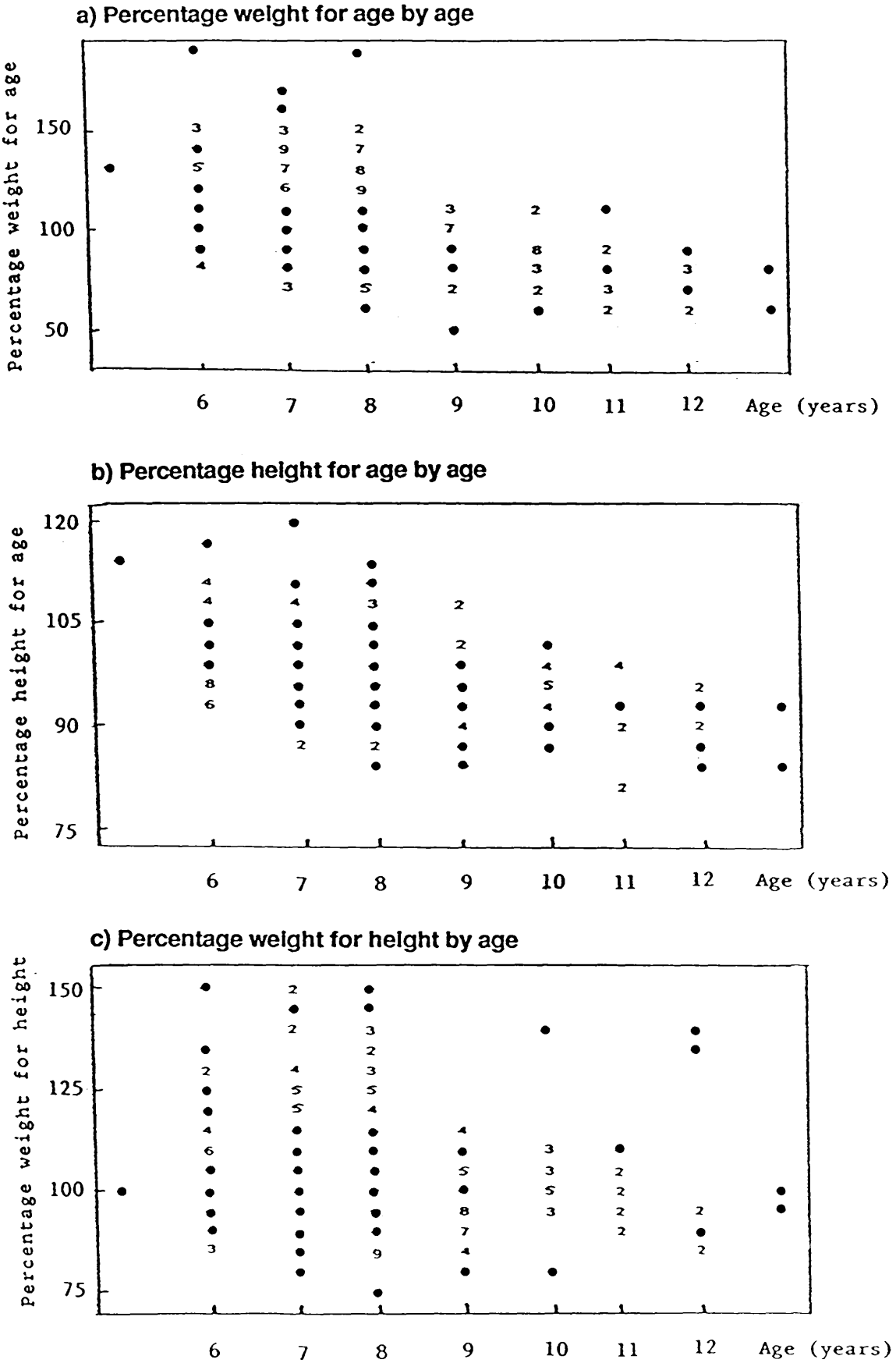
TABLE 3.5.2.

MEAN ANTHROPOMETRIC MEASUREMENTS.
COMPARISON BY SCHOOL.

	A[a]	B	C	D
N	123	193	148	161
% WEIGHT FOR AGE (MEAN ± SD)	102.13 ± 20.01	94.07 ± 13.43	100.33 ± 18.50	98.38 ± 16.41
% HEIGHT FOR AGE (MEAN ± SD)	98.07 ± 4.69	97.22 ± 4.61	99.04 ± 4.41	98.54 ± 5.15
% WEIGHT FOR HEIGHT (MEAN ± SD)	105.56 ± 13.36	100.05 ± 8.12	101.58 ± 12.64	101.43 ± 10.81

a School identity (see Table 3.2.3)

FIGURE 3.5.1. ANTHROPOMETRIC VARIABLES BY AGE



Where numbers are shown in place of solid circles, these represent the number of observations at that point.

Analysis of variance for each of the three anthropometric variables by infection, revealed a significant association between *T.trichiura* infections and lower values for each parameter (Table 3.5.3; P=0.003). Initial analysis of the data for weight and height by age suggested that children with hookworm infections were likely to be lighter and smaller for their age than children not harbouring hookworm infections (P=0.02). Closer analysis, however, revealed that only children with concomitant *T.trichiura* infections were associated with lower mean percentage heights and weights for age; children with single hookworm infections did not demonstrate this association (P>0.05; Table 3.5.3).

In order to investigate whether the association between infection with *T.trichiura* and lower anthropometric variables is density dependent, the infection was classified into intensity categories by egg count. As in Section 3, chapter 4, the *T.trichiura* infections were arbitrarily classified as light (20 - 499 e.p.g.), moderate (500 - 4999 e.p.g.) or heavy ($\geq$ 5000 e.p.g.). Analysis of variance was used to compare mean anthropometric variable for each intensity category. Despite a trend in the data suggesting that children with heavier *T.trichiura* infections were of lower mean anthropometric status, particularly weight for age, no significant differences were detected (Fig.s 3.5.2 a, b and c). Multiple regression analysis on each anthropometric variable for boys and girls revealed that age was a more important predictor than *T.trichiura* egg counts. Low R^2 values indicated that other important predictors (e.g. socio-economic status) had been omitted from the analysis.

3. Longitudinal observations on anthropometric status and intestinal helminth infections. Comparison between November 1987 and 1988 of the anthropometric status of the sub-group of 169 children, drawn from the original group of 625, revealed significant alterations. Mean percentage weight for age was reduced from 96.33$\pm$15.44 to 95.09$\pm$15.99 (P=0.0006), mean percentage height for age was reduced from 97.81$\pm$4.95 to 95.44$\pm$4.89 (P=0.019), but percentage weight for height increased, although not significantly, from 100.74$\pm$9.09 to 101.17$\pm$9.82. This trend of a

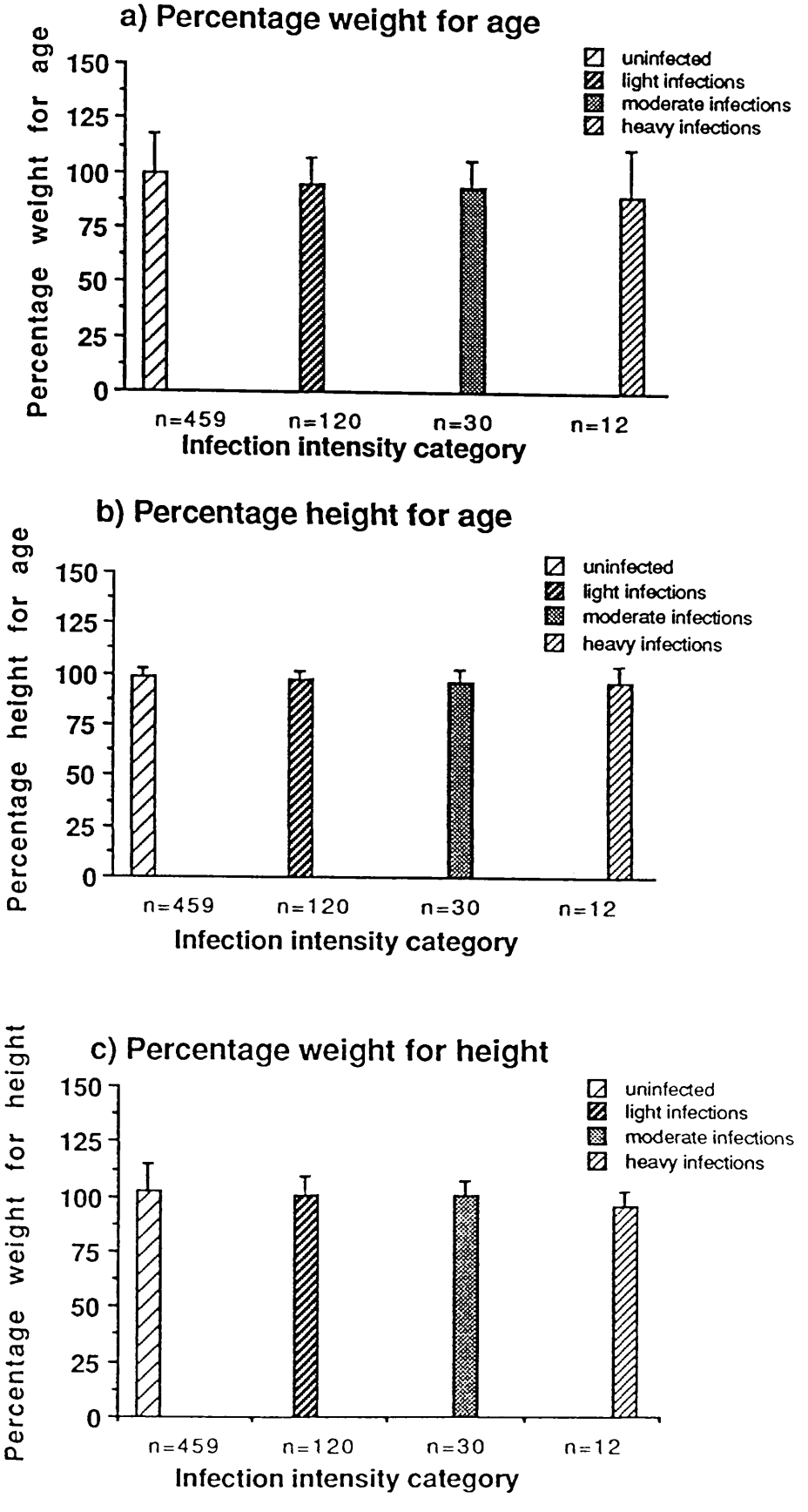
TABLE 3.5.3.

MEAN ANTHROPOMETRIC MEASUREMENTS.
COMPARISON BY INFECTION.

	INFECTION GROUP[a]				
	1	2	3	4	5
N	386	112	18	81	50
% WEIGHT FOR AGE (MEAN ± SD)	99.91 ± 18.46	97.32 ± 15.00	98.20 ± 18.77	93.86 ± 12.77	92.43 ± 12.45
% HEIGHT FOR AGE (MEAN ± SD)	98.52 ± 4.59	98.12 ± 4.73	98.52 ± 6.93	97.20 ± 4.74	96.01 ± 4.10
% WEIGHT FOR HEIGHT (MEAN ± SD)	102.53 ± 12.27	101.31 ± 10.29	101.55 ± 10.02	99.65 ± 8.47	101.51 ± 8.78

a Infection groups:
1. Children who were not considered to be harbouring infections of intestinal helminths.
2. Children considered to be harbouring single infections of *A.lumbricoides*.
3. Children considered to be harbouring single infections of hookworm.
4. Children considered to be harbouring single infections of *T.trichiura*.
5. Children considered to be harbouring dual infections of *T.trichiura* and hookworm.

FIGURE 3.5.2. ANTHROPOMETRIC VARIABLES: COMPARISON BY INTENSITY OF INFECTION WITH T.TRICHIURA



reduction in mean anthropometric variables is consistent with the positive increment in mean age of the group. It is not, however, consistent with the reduction in the prevalence and intensity of helminthiases, including trichuriasis, within the group.

Rather than compare changes in anthropometric data by treatment group, it was considered to be more appropriate to classify children by alterations in helminth infection status, and compare alterations in helminth status these groups. Although children whose *T.trichiura* infections had improved, (as defined by a reduction in faecal egg count; n=26), tended to have a more positive alteration in percentage weight and height for age than children whose *T.trichiura* infection status had deteriorated, (as defined by an increase in faecal egg counts; n=25), this was not statistically significant. Mean alteration in percentage weight for height, however, was negative in children whose *T.trichiura* infection status had improved (-0.70 ± 3.93), and positive in children whose infection status had deteriorated (1.13 ± 10.56). No association between alterations in infection status of *A.lumbricoides* or hookworm with anthropometric changes were detected, nor were any trends observed.

3. Discussion

Three interesting observations have emerged from this section of the study in Coclé. First, despite over 98% of the children being classified of essentially normal nutritional status by measurement of height and weight, the classic pattern of percentage weight and height for age, and weight for height decreasing with increasing age is evident. This suggests that even in a community that is adequately nourished, the compounded effects of those problems which result in a growth deficit, will have an impact on the growth potential of children.

Secondly, the difference between the anthropometric measurements in boys and girls is intriguing. Other studies have usually either found no differences between sexes, or have associated boys with a better nutritional status, especially in surveys conducted in Latin America and Asia where there is often a cultural bias in favour

of males (Stephenson *et al*, 1983). The third point of interest is the association between *T.trichiura* infections and lower anthropometric values. Previous studies that have investigated associations of *T.trichiura* infections with reductions in growth and weight gain, have tended to concentrate on heavily infected children, frequently with symptomatic rectal prolapse, chronic dysentery and finger clubbing. These studies have been both cross-sectional (Cooper & Bundy, 1986) and longitudinal (Gilman, Chong, Davis, Greenberg, Virik & Dixon, 1983). Both have produced provocative results suggesting that trichuriasis has an important impact on child growth and nutrition. In contrast, Bowie, Morison, Ireland & Duys (1978) concluded that one of the most important reasons for improved weight gain following treatment of children with moderate *T.trichuriasis* infections, was the nutritious diet available in hospital. In our study in Panama, an association between *T.trichiura* infections and reduced anthropometric measurements was detected, and although a trend in the data suggests that heavier infections are associated with lower values for anthropometric variables, none of the infections would be considered heavy by the standards employed by Cooper & Bundy (1986) and by Gilman *et al* (1983). Surprisingly, the findings of nutritional surveys revealed a significantly lower intake of all 3 macronutrients in 75 children with *A.lumbricoides* infections than in 75 children considered not to be infected with *A.lumbricoides*, but no difference between dietary macronutrient intakes of children with *T.trichiura* infections compared with children not considered to be harbouring this infection was reported (Garcia, pers. comm.).

Although the longitudinal study did not reveal any significant association between an improvement in *T.trichiura* infection status and anthropometric variables, a trend was observed. It must also be remembered that the increased age of the study group would be a confounding problem. This data implies that it may be important not to exclude lighter infections of *T.trichiura* when considering the possible impact of this infection on the growth of children.

4. Summary.

1. The weights and heights of a group of 625 children were measured. Knowledge of their ages allowed the calculation of percentage weight for age, height for age and weight for height. These three anthropometric parameters are frequently employed as indicators of nutritional status in children. Over 98% of the children were considered to be of normal nutritional status by these indicators.

2. Analysis of variance revealed that boys tended to be of lower nutritional status than girls. Significant differences between schools were also revealed. The classic pattern of lower anthropometric values being measured with increasing age was also observed.

3. Children considered to be harbouring *T.trichiura* infections were found to be of lower mean anthropometric status than children not infected with this parasite. *A.lumbricoides* and hookworm infections were not found to be associated with lower anthropometric measurements.

4. Although a trend in the data suggested that heavier *T.trichiura* infections as determined by faecal egg count were associated with a lower anthropometric values, this was not observed to be significant. Multiple regression analysis suggested that other factors, not considered here, are important in predicting the nutritional status of this group of children

5. A longitudinal study on a sub-group of 169 children revealed a significant reduction in mean weight and height for age between November 1987 and November 1988. This was considered to be related to the increasing age of the group. Although improvement in *T.trichiura* status was not significantly associated with increased anthropometric status, trends in the data did indicate this as a possibility.

6. The results are discussed in relation to other anthropometric studies, particularly those concerned with *T.trichiura* infections. It is suggested that lighter infections of this parasite than previously considered, may have an influence on the growth of children.

SECTION 3

CHAPTER 6: BLOOD HAEMOGLOBIN CONCENTRATIONS: IMPACT OF HOOKWORM AND *TRICHURIS TRICHIURA* INFECTIONS.

1. Introduction

Synthesis of haemoglobin requires the provision of nutrients such as proteins, vitamins and minerals (Keele *et al.*, 1982), and occurs in erythrocytic precursors. A low blood haemoglobin concentration may indicate abnormal haemoglobin synthesis for reasons which may include genetic factors, acquired enzyme disorders, or dietary deficiencies, especially of iron and protein. Alternatively, low blood haemoglobin concentrations may indicate blood loss. It is with this latter origin of reduced blood haemoglobin values, that hookworm and *T.trichiura* infections have been particularly associated.

Intestinal blood loss is one of the most important nutritional impacts that hookworm has upon its host (Schad & Banwell, 1984), and clinical symptoms will be those of iron deficiency anaemia. In a person passing approximately 2000 *N.americanus* e.p.g. faeces, an estimated 1.3 mg of iron are likely to be lost every day (Stephenson, 1987). Whether the loss results in clinical disease or not, will depend on a number of other factors including host iron reserves, pregnancy, menstruation, the adequacy of iron and protein intake and their bioavailability, and other constraints upon the health of the host. Good nutrition may not necessarily compensate for blood loss (Roche & Layrisse, 1966). The clinical severity of hookworm anaemia tends also to be related to the intensity of infection, and highly significant relationships between hookworm egg counts and haemoglobin levels have been found (Layrisse & Roche, 1964; Stephenson, Latham, Kurz, Kinoti, Oduori & Crompton, 1985).

The association between trichuriasis and reduced haemoglobin concentrations has been the focus of less attention. Some evidence suggests that heavy *T.trichiura* infections may cause blood loss extensive enough to result in anaemia, although

whether this is due to lesions in the colon, or haematophagous activity by the worms is still unclear (Holland, 1987). According to Greenberg & Cline (1979) blood loss in less heavy *T.trichiura* infections may have no pathological association with iron deficiency, but their data indicates a considerable inverse relationship between egg counts and haemoglobin concentration, and this could not be accounted for by dietary iron intake.

In our Panamanian study, two approaches were employed to detect associations between haemoglobin concentration and hookworm and/or *T.trichiura* infections. First, a cross-sectional study of 658 children in November 1987 allowed comparison of haemoglobin concentrations between infected and uninfected children. Secondly, in a smaller group of 171 children, a longitudinal study from November 1987 to November 1988 enabled a comparison to be made between alterations in haemoglobin concentration and changes in helminthological status.

2. Results

1. Blood haemoglobin concentrations in the study population. The overall mean ± standard deviation of blood haemoglobin concentration in the children was 12.19 ± 1.19 g/dl (n=658). Children of this age group living at this altitude will probably be anaemic if their haemoglobin concentrations are less than 11.5 g/dl (INACG, 1979). The proportion of children with haemoglobin concentrations of less than 11.5 g/dl was 22.3%. No evidence of a sex effect was detected, and in this age group, none would be expected. Significant differences were detected, however, between children by school using one way analysis of variance (P=0.000), with the mean haemoglobin concentration being considerably lower in Anton, than in Nata (Table 3.6.1).

2. Blood haemoglobin concentrations in children with intestinal helminth infections. Of the 658 children participating in this study, 78 were harbouring hookworm infections, 179 were harbouring *T.trichiura* infections, and no evidence of infection with either of these nematodes was found in 456 children. Of those children that

TABLE 3.6.1.

MEAN HAEMOGLOBIN CONCENTRATION: COMPARISON BY SCHOOL.

	SCHOOLS[a]				
	A	B	C	D	TOTAL
MEAN HAEMOGLOBIN CONC. (g/dl) ± SD	12.27 ± 0.90	12.07 ± 1.18	11.90 ± 1.01	12.59 ± 1.39	12.19 ± 1.19
N	127	204	153	174	658

a School identity (see Table 3.2.3.)

TABLE 3.6.2.

MEAN HAEMOGLOBIN CONCENTRATION: COMPARISON BY INFECTION

	INFECTION GROUP[a]				
	1	2	3	4	TOTAL
MEAN HAEMOGLOBIN CONC. (g/dl) ± SD	12.27 ± 1.13	12.25 ± 1.34	12.12 ± 1.16	11.68 ± 1.52	12.19 ± 1.19
N	456	23	124	55	658

a Infection groups:
1. Children who were not considered to be harbouring infections of hookworm or *T.trichiura*.
2. Children considered to be harbouring infections of hookworm, but not of *T.trichiura*.
3. Children considered to be harbouring infections of *T.trichiura*, but not hookworm.
4. Children considered to be harbouring infections of both *T.trichiura* and hookworm.

were infected, 55 were harbouring both infections.

Analysis of variance revealed that children infected with hookworm had significantly lower blood haemoglobin concentrations than those that were judged not to be infected by this parasite (P=0.006). Similarly, those children harbouring *T.trichiura* had significantly lower blood haemoglobin concentrations (P=0.006), than those which were not. Closer analysis of the data revealed that children with single infections of either hookworm or *T.trichiura*, but not both, had mean haemoglobin concentrations that were not significantly different from those measured in children with neither infection, but those children harbouring both infections were observed to have significantly lower blood haemoglobin concentrations (Table 3.6.2; P=0.005). No evidence of ascariasis being associated with reduced haemoglobin concentration was detected.

International guidelines for the association of haemoglobin concentrations with the likelihood of anaemia have been prepared by WHO (1972) and INACG (1979). Using the more conservative estimate for the study children of this age group, anaemia is more likely to be present in individuals with haemoglobin concentrations of less than 11.5 g/dl. Contingency tables were constructed to compare the percentages of children with haemoglobin concentrations less than 11.5 g/dl that were either uninfected, infected with either hookworm or *T.trichiura*, or infected with both of these nematodes. Chi-squared tests revealed that children with both infections were significantly more likely to be associated with haemoglobin concentrations indicative of anaemia than uninfected children ($P<0.005$), but children with single infections with either of these helminths in this population were no more likely to be associated with haemoglobin concentrations of less than 11.5 g/dl than uninfected children.

In order to investigate whether reduced haemoglobin concentration in children with hookworm and *T.trichiura* infections is related to infection intensity, the infections were classified into intensity categories. As in section 3, chapter 4, the *T.trichiura* infections were classified as either light (20 - 499 e.p.g.), moderate (500

- 4999 e.p.g.) or heavy (≥ 5000 e.p.g.), but for hookworm, as only one child had an infection greater than 5000 e.p.g., the infections were classified as either light (20 - 499 e.p.g.) or moderate (≥ 500 e.p.g.). The mean haemoglobin concentration for each intensity category were compared by analysis of variance. Although a trend in the data suggests that children with heavier hookworm or *T.trichiura* infections will have lower blood haemoglobin concentrations (Fig.s 3.6.1.a & b), a significantly lower measurement was detected only in the group with the heaviest *T.trichiura* infections (P=0.014).

3. Longitudinal observations on haemoglobin concentration and infections with hookworm and *T.trichiura*. Comparison between November 1987 and 1988 of the haemoglobin status of the sub-group of 171 children drawn from the original group of 658, revealed no alteration in mean haemoglobin concentration, which remained at 12.4 g/dl, although the standard deviation was reduced from 1.2 to 0.7 g/dl.

Although children attending two of the schools had received anthelmintic from the project in both November 1987 and June 1988, it was decided not to compare changes in haemoglobin concentration by treatment group, as trichuriasis was not reduced significantly in either group. Instead, for studying both hookworm and *T.trichiura* infections, children were allocated into groups which reflected any alteration in status concerned with the particular helminth (Table 3.6.3). Mean alterations in blood haemoglobin concentration for each group were calculated, and compared either by analysis of variance or Kruskall-Wallis tests, depending of the normality of the data. For trichuriasis, although improvement in infection status tended to be associated with an increase in haemoglobin concentration, this was not found to be statistically significant (Table 3.6.3). Likewise, for hookworm infections there was no statistically significant increase in haemoglobin status in children whose infections had improved.

In the cross-sectional study, only children with both hookworm and *T.trichiura* infections were observed to be associated with lower haemoglobin concentrations.

FIGURE 3.6.1. BLOOD HAEMOGLOBIN CONCENTRATIONS. COMPARISON BY INFECTION INTENSITY FOR T.TRICHIURA AND HOOKWORM INFECTIONS

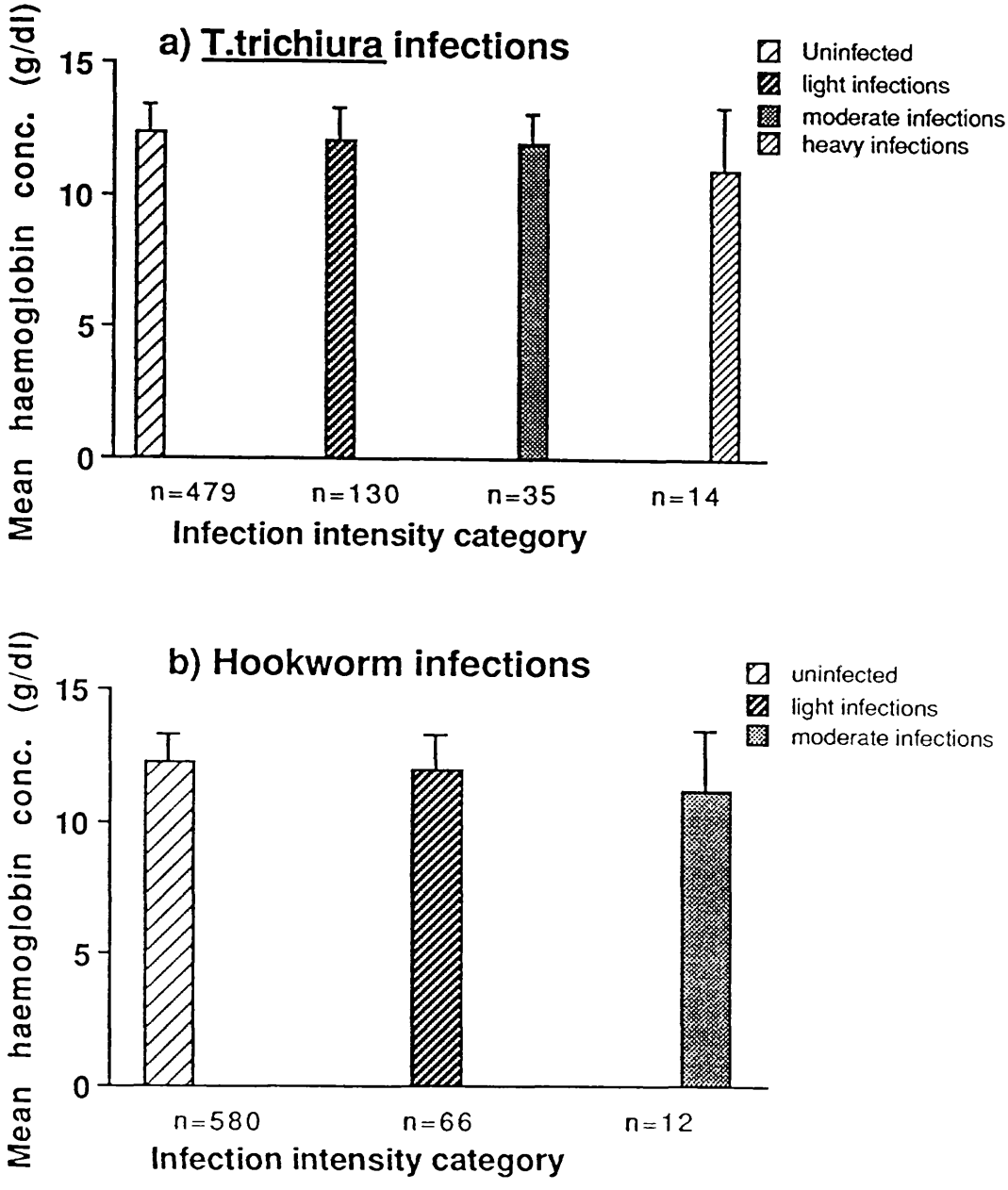


TABLE 3.6.3.

MEAN ALTERATION IN BLOOD HAEMOGLOBIN CONCENTRATION BETWEEN NOVEMBER 1987 AND NOVEMBER 1988. COMPARISON BY ALTERATION IN HOOKWORM AND *T.TRICHIURA* INFECTION STATUS.

		TRICHURIS TRICHIURA			HOOKWORM		
	GROUP[a]	A	B	C	A	B	C
CHANGE IN MEAN HAEMOGLOBIN CONC. ± SD (g/dl).		-0.10 ± 1.3	+0.40 ± 1.4	-0.10 ± 1.5	-0.10 ± 1.3	+0.10 ± 1.8	+0.10
N		119	26	26	149	21	1

a Group:

A. No evidence of the particular infection was detected in either November 1987 or November 1988.

B. Children were considered to be infected by the parasite in November 1987, but in November 1988 either the infection was no longer detected, or there had been a reduction in the infection intensity, as measured by a decrease in the faecal egg count.

C. Children were either considered to be uninfected by the parasite in November 1987, but were found to be infected in November 1988, or the children were detected to be infected in November 1987, and in November 1988 the infection was considered to have escalated, as measured by an increase in faecal egg count.

TABLE 3.6.4.

MEAN ALTERATION IN BLOOD HAEMOGLOBIN CONCENTRATION BETWEEN NOVEMBER 1987 AND NOVEMBER 1988. COMPARISON BETWEEN CHILDREN REMAINING UNINFECTED AND CHILDREN IN WHOM AN IMPROVEMENT IN BOTH HOOKWORM AND *T.TRICHIURA* INFECTIONS WAS DETECTED.

	GROUPS[a]	
	A	B
CHANGE IN MEAN HAEMOGLOBIN CONCENTRATION ± SD (g/dl).	-0.1±1.3	+1.0±1.6
N	116	10

a Group:

A. Children in whom no evidence of hookworm or *T.trichiura* infection was detected in either November 1987 or November 1988.

B. Children in whom an improvement in both hookworm and *T.trichiura* infections was detected in November 1988 from their status in November 1987.

Therefore, in the longitudinal study a comparison was made of mean alteration in haemoglobin concentration between children in whom no evidence of infection with either parasite was found in both November 1987 and November 1988, and children in which an improvement had been detected for both infections, as indicated by reductions in faecal egg counts (Table 3.6.4). Analysis of variance of this data revealed that in children in whom a decrease in the magnitude of both these infections was detected, the alteration in haemoglobin concentration over the year was considerably greater than in children in whom no evidence of either of these infections was detected on either occasion. No evidence was found of change in haemoglobin concentration being affected by altered levels of ascariasis.

3. Discussion.

The prevalence of anaemia in the Central American population has long been a matter of concern. From data reported by Viteri & Guzman (1972), it can be calculated that of 1065 Central American children in their study in several countries, aged between 5 - 12 years and living at a similar altitude to that of Coclé, Panama, 11.6% had haemoglobin concentrations of less than 11.5 g/dl. They suggest that marginal iron and folate intake, hookworm infection and sweat iron losses may be of contributory importance.

In the group of 658 children participating in the cross-sectional study in Coclé, 22.3% had haemoglobin concentrations of less than 11.5 g/dl. Although comparison of multi-national and localised data may be of limited value, this does suggest that anaemia in this age group is becoming a problem of increasing importance in Central America, despite an apparent reduction in the prevalence of hookworm (Section 3, chapter 3). Marginal iron and protein intake in some individuals may be contributory, but the mean iron intake of 150 children selected from the 658 participating in this section of the study was judged by dietary survey to be adequate (>10mg iron daily) (Garcia, pers.comm.). Among these children, those infected with *A.lumbricoides* rather than hookworm or *T.trichiura* appeared to be

associated with lower dietary iron intake (Garcia, pers. comm.), but no association between ascariasis and lower haemoglobin concentration was detected in this study. It is possible that hookworm infections may interfere with absorption of iron, but this complex and controversial hypothesis was not tested.

An important observation revealed by both the cross-sectional and longitudinal parts of this study, was the association of lower haemoglobin with dual infections with hookworm and *T.trichiura*, and improvement in both these infections being associated with significant improvements in haemoglobin status. Initially it was suspected that the reason for this finding, would be that those children with heavy *T.trichiura* infections would be predisposed to also having heavy hookworm infections, and *vice versa*. Investigation of this possibility did not indicate this to be an adequate explanation. Another explanation involves the relative positioning in the gut of the two helminth populations. Adult hookworms tend to inhabit the upper small intestine, whereas mature *T.trichiura* inhabit the caecum and colon. At the relatively low infection intensities observed in this study population, where infection intensity, as measured by faecal egg counts, was only detected to be associated with a significantly lower haemoglobin concentrations in the heaviest *T.trichiura* infections, and not at all in hookworm infections, it is unlikely that a single infection with either parasite would cause extensive blood loss. Bleeding due to hookworm infections occurs in the upper small intestine, and some of the iron lost will be reabsorbed further down the G.I. tract. Estimates by different researchers of the amount reabsorbed have ranged from between 20 to 80% (Stephenson, 1987). It is possible that if there is a concomitant infection of trichuriasis, then the reabsorption of iron is impaired, either by ingestion of the iron by the *T.trichiura* worms or by the absorptive surface of the gut being affected. A greater loss of iron may result than would be anticipated from the intensity of the hookworm infection, and have a nutritional impact on the host, evident in a reduced blood haemoglobin concentration. Although there may be alternative explanations, the results of this study do emphasise the importance of taking into consideration concomitant

infections, an aspect which often appears to be omitted in research exploring associations between a given infection and nutritional deficiencies.

4. Summary

1. The blood haemoglobin concentrations of a group of 658 children were measured. The mean value was found to be 12.19 g/dl. The percentage of children with blood haemoglobin concentrations of less than 11.5 g/dl was 22.3%. Analysis of variance revealed significant differences in mean haemoglobin concentration between schools, but not between ages or sexes.

2. Children with hookworm or *T.trichiura* infections were found to have lower mean blood haemoglobin concentration than children judged to be uninfected. Closer analysis revealed that only children with dual hookworm and *T.trichiura* infections showed this association, and children with single infections were not significantly more likely to have lower haemoglobin concentrations than uninfected children. No association was detected between lower blood haemoglobin concentration and ascariasis.

3. Infection intensities for both hookworm and *T.trichiura* infections tended to be low, but children with *T.trichiura* infections of greater than 5000 e.p.g. faeces had significantly lower mean blood haemoglobin concentration than those children with lighter *T.trichiura* infections.

4. A longitudinal study on a group of 171 children revealed no significant increase in blood haemoglobin concentrations over the course of the year in children whose hookworm or *T.trichiura* infections had improved as compared to children in whom no evidence of either infection had been detected on both occasions. However, a significant increase in blood haemoglobin concentrations was detected in children in whom an improvement in both hookworm and *T.trichiura* infections was found, as compared to children in whom no evidence of either infection had been detected on both occasions. No evidence of haemoglobin concentration being influenced by alteration in status of *A.lumbricoides* infection was detected.

5. These results are discussed in relation to a previous survey on blood haemoglobin concentrations in Central America, dietary iron intake and hookworm and *T.trichiura* infections. The importance of considering concomitant infections when investigating nutritional deficiencies is emphasised.

SECTION 3

CHAPTER 7: PLASMA VITAMIN A STATUS: POSSIBLE ASSOCIATION WITH INTESTINAL HELMINTHIASES.

1. Introduction

Vitamin A* is an essential nutrient. If inadequate intake of vitamin A, or its carotenoid precursors, occurs pathological symptoms may be expected (WHO, 1982). Amongst the symptoms of vitamin A deficiency are appetite loss, weight loss, keratinisation of epithelial tissues, a reduction in mucus secretion, and a negative nitrogen balance. As the severity of the deficiency progresses, further symptoms appear. Stomach emptying becomes disturbed, reproduction is disrupted, balance and motor skills become defective, and night blindness, eye lesions and blindness occur. The resistance to infection is considerably diminished, with both humoral and cell-mediated responses being compromised, and the eventual outcome of the disease will be death (WHO 1976).

The extent of human vitamin A deficiency in the world is extensive, but an accurate assessment is difficult to make. Estimates in promotional literature (e.g. 10 million new childhood xeropthalmia cases per year, 500,000 of which will result in blindness; PATH, 1985) are derived by extrapolation from community data and may give only a limited insight into the real situation. Nevertheless, it is obvious that the identification of factors that precipitate or exacerbate vitamin A deficiency is an important concern.

Although the primary cause of vitamin A deficiency is inadequate dietary resources (Feachem, 1987), evidence is accumulating to suggest that vitamin A status may be affected by various infections. Nearly all serious illnesses, acute or

* *Vitamin A is used here as a general term for all forms of vitamin A1 and A2.*

chronic, depress food intake, increase the requirement for vitamin A and have other general metabolic effects which may reduce vitamin A status. However, association of an infection with reduced blood vitamin A concentrations does not provide evidence that the infection is the aetiological agent. Vitamin A deficiency causes metaplasia at various sites, and will increase the susceptibility to pathogens. Thus, infections will be more likely to establish in hosts whose vitamin A status is compromised. Longitudinal studies, on the effect of eliminating an infection on nutritional status, provide more helpful clues, as do studies which focus on a particular aspect of vitamin A nutriture, for example dietary absorption.

Although chronic nutrient depletion will undoubtedly be harmful, Vitale (1977) suggested that in acute infections, a reduction in blood concentration of a vitamin may represent a redistribution by the host, indicative of an adaptive and ultimately beneficial response. Consequently, the significance of a measured reduction in blood vitamin A concentration is difficult to interpret, and the possible beneficial effect of vitamin administration in infection is not easy to evaluate.

The diseases which have been primarily associated with vitamin A deficiency tend to be those of populations of developing countries, where vitamin A deficiency due to inadequate dietary intake occurs most frequently. For example, the relationship of measles to ocular lesions associated with vitamin A deficiency is of major importance (Male, 1989). This is not only because measles is, to some extent, an eye disease, but also because of the marked impairment of the cell-mediated immune response, as indicated by the prolonged excretion of giant cells (Scheifele & Forbes, 1972). Low serum retinol concentrations have also been associated with malaria (Nesheim, pers.comm.; Sturchler, Tanner, Hanck, Betschart, Gautschi, Weiss, Burnier, Del Guidice & Degremont, 1987), but Sturchler *et al* (1987) were unable to exclude age as a confounding factor. Low serum retinol concentrations have also been linked to intestinal schistosomiasis (Mikhail & Mansour, 1982; Sturchler, Holzer, Hanck, & Degremont, 1983), but malabsorption of the vitamin during

schistosomiasis may be more directly due to concomitant chronic salmonella septicemia, which frequently occurs with *Schistosoma mansoni* infections (Mansour, Mikhail, Farid & Bassily, 1979). Diarrhoea of any aetiology has been long associated with steatorrhoea, and hence malabsorption of fat soluble vitamins. Feachem (1987), in a detailed review of interrelationships of vitamin A deficiency and diarrhoea, suggests that diarrhoea is a risk factor for the development of xerophthalmia. Giardiasis has likewise been implicated in impairing vitamin A absorption through steatorrhoea (Ament & Rubin, 1972). However, a particular focus of attention in more recent years has been the contribution of intestinal helminth infections, in particular ascariasis, to a reduction in vitamin A status.

Although there is little convincing evidence to link *A.lumbricoides* infection with a low vitamin A status, an association of ascariasis with vitamin A deficiency is not a new idea; for example, McLaren, Oomen & Escapini (1964) reported that in Djakarta, if a mother discovered Bitot's spots in the eyes of her child, she concluded that "he has worms". Vitamin A deficiency and ascariasis are likely to be correlated with similar socio-economic conditions, and it is important to be able to distinguish between a simple association between the two, and a causal relationship. Taren *et al* (1987) found that a significant relationship remained between ascariasis and reduced plasma vitamin A and carotenoid concentrations when a range of socio-economic variables had been controlled for. Close clinical associations between xerophthalmia and ascariasis have also been noted in a number of studies (Bhattacharyya, 1988), where the people from the uninfected group were from a socio-economic background very similar to that of the infected group.

The mechanism by which vitamin A status would be affected by infection with *A.lumbricoides* is uncertain. A number of studies have reported that vitamin A absorption is affected by ascariasis (Sikavumar & Reddy, 1975; Mahalanabis *et al.*, 1976; Krause, Moraleda, Leon, Franjola, Puga, Munoz & Dolz, 1986), and this would be consistent with the reduced fat absorption that is a regular observation during ascariasis. Further research has suggested that children infected with both

A.lumbricoides and *Giardia intestinalis* suffer the most severe impairment of vitamin A absorption (Mahalanabis, Simpson, Chakraborty, Ganguli, Bhattacharjee & Mukherjee, 1979). Alternative theories have also been put forward including reduced dietary intake of vitamin A, sequestration or consumption of the vitamin by the worms themselves (Singh, Vijayavargava, Dubke & Pohowala, 1968), although this is considered improbable, a decrease in the concentration of plasma retinol binding protein, and reduced activity of the enzymes of the intestinal mucosa that convert carotene to vitamin A. This latter suggestion is of considerable interest, as most populations living where ascariasis is endemic will consume the majority of their vitamin A as carotenoid precursors.

In a number of literature reviews of ascariasis and nutrition, it has been noted that no study has yet been undertaken to monitor plasma vitamin A levels both before and after anthelmintic treatment. In Panama, we employed two approaches to investigate the influence of intestinal helminths on vitamin A status. First, we were able to conduct a cross-sectional study of 211 children in November 1987, to allow comparison of plasma retinol concentrations between infected and uninfected children. Secondly, in a smaller group of 152 children, a longitudinal study from November 1987 and November 1988, enabled comparisons to be made between alterations in plasma retinol concentrations and changes in helminthological status following intervention with levamisole.

2. Results.

1. Plasma retinol concentrations in the study population. The overall mean ± standard deviation of plasma retinol concentrations in the children was 31.82 ± 5.68 µg/dl (n=211). Plasma vitamin A values in excess of 20 µg/dl are not associated with the deficiency state (WHO 1982). In this study only 2 children had retinol concentrations of less than 20 µg/dl; less than 1% of the study group. No child was found to have a retinol concentration of less than 10 µg/dl. No evidence of a sex or age effect was detected. Significant differences were detected, however, between

children by school using one way analysis of variance (P=0.037), with mean retinol concentrations being considerably lower in children attending school in Nata, and considerably higher in children attending school in Rio Hato (Table 3.7.1). Both children with plasma retinol concentrations of less than 20 μg/dl attended school in Nata.

2. Plasma retinol concentrations in children with intestinal helminth infections. Of the 211 children participating in this study, 105 were harbouring *A.lumbricoides* infections and 106 children were considered not to be infected with intestinal helminths. Of those 105 children found to be infected with *A.lumbricoides*, 23 were found to be infected with both hookworm and *T.trichiura*. Concomitant infection of hookworm alone was detected in 3 of the children, and of *T.trichiura* in 32 of the children.

Although the mean plasma retinol concentration was found to be higher in uninfected children than children harbouring *A.lumbricoides* infections (Table 3.7.2), analysis of variance revealed no significant difference between infected and uninfected children. No association was detected either between lower plasma retinol concentrations and concomitant infections. No evidence of intestinal helminth infection was detected in either child with plasma retinol concentrations of less than 20 μg/dl.

In order to investigate whether heavier infections with *A.lumbricoides* might be associated with reduced plasma retinol concentrations, the infection was classified into intensity categories by egg count. As in section 3, chapter 4, the *A.lumbricoides* infections were arbitrarily classified as light (20 - 499 e.p.g.), moderate (500 - 4999 e.p.g.) or heavy ($\geq$ 5000 e.p.g.). The mean plasma retinol concentrations for each intensity category were compared by analysis of variance and the mean plasma retinol concentrations of children belonging to the heavier infection categories were compared with the mean plasma retinol concentrations of children in whom no evidence of an infection was detected. Despite a trend in the

TABLE 3.7.1.

MEAN PLASMA RETINOL CONCENTRATIONS. COMPARISON BY SCHOOL.

	SCHOOLS[a]				
	A	B	C	D	TOTAL
MEAN PLASMA RETINOL CONC.S ± SD (μg/dl)	31.90 ±4.93	32.83 ±5.36	31.91 ±6.79	30.11 ±5.48	31.82 ±5.68
N	22	96	34	59	211

a School identity (see Table 3.2.3.)

TABLE 3.7.2.

MEAN PLASMA RETINOL CONCENTRATIONS.
COMPARISON BETWEEN CHILDREN HARBOURING
A.LUMBRICOIDES INFECTIONS AND THOSE CONSIDERED
NOT TO HARBOUR THIS INFECTION.

	CHILDREN WITH *A.LUMBRICOIDES* INFECTIONS	CHILDREN WITHOUT *A.LUMBRICOIDES* INFECTIONS
MEAN PLASMA RETINOL CONC. ± SD (μg/dl)	31.52±5.50	32.12±5.87
N	105	106

data suggesting that children with infections heavier than 500 e.p.g. were likely to have a lower vitamin A status (Fig. 3.7.1), no significant differences were detected. A surprising observation was that children with the heaviest infections tended to have plasma retinol concentrations that were higher than children with moderate infections.

3. Longitudinal observations on plasma retinol concentrations and infections with *Ascaris lumbricoides*. Comparison of values measured in November 1987 and 1988 of the vitamin A status of the sub-group of 152 children drawn from the original group of 211, revealed a non-significant rise in mean plasma retinol concentration from 32.1 ± 5.7 to 33.2 ± 7.7 μg/dl.

Children at two of the schools had received anthelmintic drug in both November 1987 and June 1988, but it was decided not to compare changes in plasma retinol concentration by treatment group, as the prevalence of ascariasis had been significantly reduced in both groups. Instead, for all three infections, children were allocated into groups which reflected any alteration in status concerned with the particular helminth infection (Table 3.7.3). Mean alterations in plasma retinol concentration were calculated for each group and compared by analysis of variance. For trichuriasis, although improvement in infection status, as detected by a reduction in faecal egg counts, tended to be associated with an increase in plasma retinol concentrations, this was not found to be statistically significant (Table 3.7.3). Likewise, for hookworm infections, an improvement in infection status appeared to be associated with an increase in retinol concentrations, and a deterioration in infection status appeared to be associated with a reduction in plasma retinol concentration (Table 3.7.3), but again these differences were not found to be statistically significant. For ascariasis, a similar pattern was detected, although, surprisingly, children in whom the infection status had deteriorated had a greater mean elevation in plasma retinol concentration, than children who had remained uninfected or whose infection status had improved (Table 3.7.3).

FIGURE 3.7.1. MEAN PLASMA RETINOL CONCENTRATIONS. COMPARISON BY A.LUMBRICOIDES INFECTION INTENSITY.

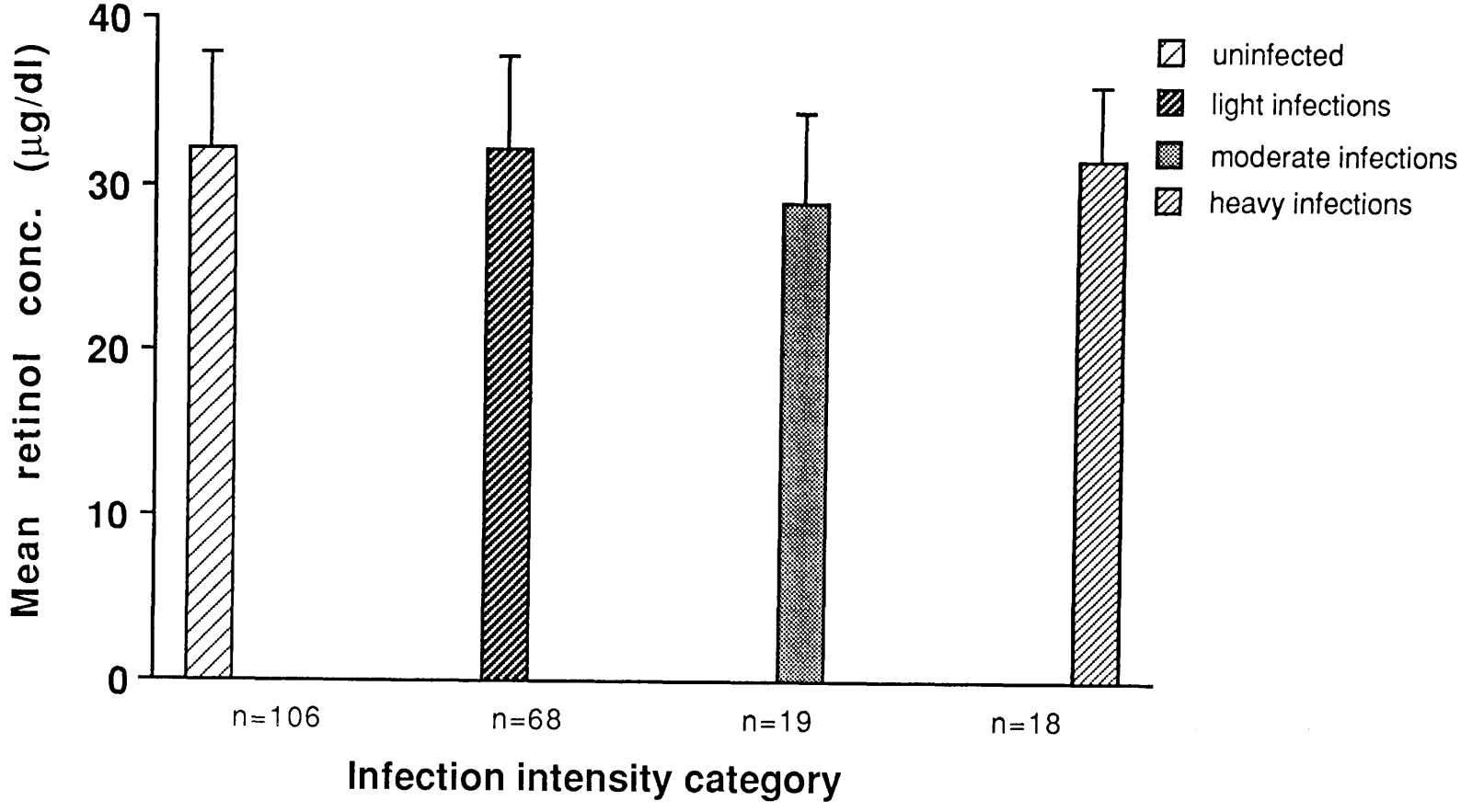


TABLE 3.7.3.

MEAN ALTERATION IN PLASMA RETINOL CONCENTRATION BETWEEN NOVEMBER 1987 AND NOVEMBER 1988. COMPARISON BY ALTERATION IN INTESTINAL HELMINTH INFECTION STATUS.

GROUPS[a]	*A. LUMBRICOIDES*			*T. TRICHIURA*			HOOKWORM		
	A	B	C	A	B	C	A	B	C
CHANGE IN MEAN PLASMA RETINOL CONC. ±SD (μg/dl)	+0.74 ± 6.41	+1.49 ± 6.12	+1.68 ± 5.87	+1.07 ± 5.94	+2.63 ± 6.53	-0.65 ± 7.48	+0.94 ± 6.17	+2.41 ± 6.84	---
N	82	60	10	112	22	18	136	16	0

a Groups:

A. Children in whom no evidence of the particular infection was detected in either November 1987 or November 1988.

B. Children considered to be infected with the particular helminth in November 1987, but in November 1988 either the infection was no longer detected, or there was a reduction in intensity, as measured by a decrease in faecal egg count.

C. Children who were either, a) considered to be uninfected with a particular helminth in November 1987, but the infection was detected in November 1988, or b) the infection was detected in both November 1987 and November 1988, but the intensity of the infection was considered to have escalated, as measured by an increase in faecal egg count.

3. Discussion

Since the 1960s the occurrence of vitamin A deficiency in Central America has been a subject of concern. In a nutrition survey by Guzman, Arroyave & Scrimshaw (1961), the serum concentrations of vitamin A were measured in 849 children attending school in Costa Rica, El Salvador, Guatemala, Honduras and Panama. Analysis of the data revealed that rural children had lower serum vitamin A concentrations than urban children, and that these concentrations fluctuated seasonally. Despite the mean concentration being within limits considered to be healthy, and no evidence of xerophthalmia or ocular lesions were found by clinical examination, approximately 15% of the children had serum vitamin A concentrations below 20 µg/dl. The reason for these low values was considered to be dietary insufficiency. Later research conducted by the Organizacion Panamericana de la Salud (1970), indicated that of 5879 Central Americans residing in either Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua or Panama, 12.8% had blood vitamin A concentrations of between 10 - 19 µg/dl, and 1.5% had vitamin A concentrations of below 10 µg/dl. In children aged under 15 years, these percentages were almost doubled. As a consequence of these surveys, the Institute of Nutrition of Central America and Panama (INCAP) embarked on a dietary vitamin A fortification programme, in which white sugar was selected as the dietary vehicle. This scheme initially received massive support, to the extent that the governments of Costa Rica, Guatemala, Honduras and Panama passed laws to the effect that all sugar for home consumption should be fortified with vitamin A (WHO, 1982). A longitudinal evaluation of the programme in Guatemala in 1975 and 1976, indicated that it had been successful in increasing serum retinol concentrations (Arroyave, Mejia & Aguilar, 1981). Nevertheless, in 1979 economic constraints brought resistance to the sugar fortification programme, and in Panama it has been halted.

In view of this background, it is particularly interesting to observe that in the primary school children participating in our project in Coclé, vitamin A deficiency did not appear to be an extensive problem. Less than 1 % of the children had

vitamin A concentrations of under 20 μg/dl. This is most probably a reflection of an improvement in nutrition, education and other socio-economic variables over the last 25 years, but it is also noteworthy that in the survey by Guzman *et al* (1961), ascorbate was not added to their serum samples to retard degradation of the retinol. In our project we found that the addition of sodium ascorbate to the plasma samples had a significant effect (see Section 3, chapter 2). It is possible, therefore, that earlier measurements could have been an under estimate of the actual vitamin A status in the study communities.

Our results also revealed that, in this community where base-line vitamin A status is normal, an impact of intestinal helminthiases on plasma retinol concentrations could not be detected. This result is confirmatory of those reported by Ostwald, Fitch, Arnhold, Shield, Louie, Kilner & Kimber (1984), from a study concerned with vitamin A status and intestinal helminthiases in Papua New Guinea. Ostwald *et al* (1984) suggested in their conclusion that, when intestinal helminth infection is not severe and base-line nutritional status adequate, an adverse nutritional state precipitated by the infections is unlikely to be detected. In other studies, where nutrition is poor, there is conflicting evidence as to whether ascariasis precipitates vitamin A deficiency. The study of Taren *et al* (1987) in Chiriqui Province, Panama, associated reduced plasma vitamin A concentrations with infection with *A.lumbricoides*. In contrast, in another study in Central America where malnutrition was evident, Dewey (1983) was unable to detect any negative relationship between vitamin A status and ascariasis.

Vitamin A is stored in relatively high quantities in the liver, and if acquisition is reduced, for example, as a result of malabsorption during ascariasis, reduced plasma retinol concentrations may be masked by mobilisation of liver stores. Nevertheless, a prolonged reduction in vitamin A intake will result in a detectable decrease in plasma retinol concentrations. These biochemical results suggest that in our study, dietary intake of vitamin A or carotenoid precursors will be normal in

both uninfected children and children harbouring intestinal helminth infections. In contrast, the findings of a dietary survey, based on interviews, involving 75 of the children with *A.lumbricoides* infections, and 75 children in whom no evidence of *A.lumbricoides* infection was detected, did not support this conclusion (Garcia, pers. comm.). Although the mean daily dietary intake of uninfected children was normal (401.9 Retinol Equivalents (RE)), the mean daily dietary intake of infected children was apparently significantly lower (296.6 RE), and below the recommended dietary intake for their age group (WHO, 1967). Why this inadequate dietary intake was not reflected in the biochemical analysis is not clear. It is possible that the marginal intake was recent, and liver stores were still adequate. It must also be remembered that the gathering of dietary data can be subjective and requires considerable experience. Analysis of dietary data is an area of some controversy, with methodological inaccuracies in the chemical determination of carotenoids, and discrepancies between the vitamin A and carotenoids in foods actually ingested and values given in the food composition tables (WHO, 1982).

Although the results of this study contribute to the debate surrounding the possible impact of helminthiases on nutritional status, conclusions are difficult to draw. It would be interesting and worthwhile to conduct a similar study in an area where marked vitamin A deficiency occurs. Animal studies could also provide useful results. There appears to have been little research concerned with host vitamin A status during *Ascaris suum* infections in pigs, although the literature which is available has revealed some interesting observations. Boroskova, Benkova, Berekzo, Rosival & Toth (1985) found that oral administration of vitamin A was accompanied by reduced numbers of migrating *A.suum* larvae in the lungs by 50%, possibly through immunostimulation. Chroustova, Raszyk, Herzig, Toulova, Dvorak & Urbanova (1986) observed that during experimental infections of *A.suum* in pigs, serum vitamin A levels were reduced between days 5 - 8 p.i., when the larvae would be migrating, but the vitamin A content of the liver was comparable to that measured in the control group. There is clearly an incentive for further research in

this field.

4. Summary.

1. The plasma retinol concentrations of a group of 211 children were measured. The mean value was found to be 31.82 ± 5.68 µg/dl. The proportion of children with plasma retinol concentrations of less than 20 µg/dl was less than 1 %. Analysis of variance revealed significant differences in mean plasma retinol concentrations by school, but not by age or sex.

2. No significant association between lower plasma retinol concentrations and *A.lumbricoides* infection was detected. Similarly infections with hookworm and *T.trichiura* appeared not to be associated with low plasma retinol concentrations.

3. Children with higher infection intensities of *A.lumbricoides*, as judged by faecal egg counts, did not have significantly lower plasma retinol concentrations than children with lower infection intensities, or than uninfected children.

4. A longitudinal study on a group of 152 children revealed no significant alterations in plasma retinol concentrations over the course of the year, regardless of alterations in intestinal helminth status.

5. These results are discussed in relation to a previous research on vitamin A status in Central America. The possibility that negative nutritional impacts from helminth infections will not be observed in populations with adequate nutrition rather than marginal nutrition, is discussed in relation to studies by other researchers concerned with vitamin A status and intestinal helminth infection. Despite the inconclusiveness of these results, the requirement for further diverse study in this field is highlighted.

SECTION 3

CHAPTER 8: CONCLUSIONS FROM THE COMMUNITY STUDY IN PANAMA

The observations on the relationships between soil-transmitted helminth infections and the nutritional status of their host children, provided some insight into the conditions experienced by many children in developing countries.

Intestinal helminth infections can, perhaps, all be described as "neglected infections". Although they mostly enjoy a cosmopolitan distribution, the majority are most prevalent in developing countries, where economic resources are already stretched. Medical research tends to be directed to those diseases which are a problem of financially solvent communities. Efficient chemotherapeutic cures for most helminth infections have been formulated, but mass elimination of these parasites within a population remains complicated, an impossibility for many nations, and often not even a high priority.

T.trichiura is probably one of the helminth infections that has attracted the least attention. Cooper, Bundy & Henry (1986) suggest that previous calculations of global morbidity rate from trichuriasis have been gross underestimates, falsely reducing its importance in the world public health. The wearing of shoes has reduced the prevalence of hookworm infections, and the steady decline in the prevalence of hookworm infections in Coclé Province, Panama over the past 60 years is noteworthy. The relatively large size of *A.lumbricoides* has probably been a factor that has encouraged a response from infected communities; unfortunately drugs that are efficacious against *A.lumbricoides* are seldom as effective against *T.trichiura*. In our study in Panama, *T.trichiura* was the most prevalent intestinal helminth and the decline in the prevalence of this infection since 1975 was negligible. Nevertheless, the anthelmintics most obviously available commercially were not the best for the treatment of this infection.

Most research concerned with intestinal helminthiases is, rightly, directed to

those individuals that are affected most seriously by the infection (e.g. children), and to those individuals that harbour the heaviest infections. Symptoms nearly always show a strong, positive correlation with infection intensity, and this is true of *T.trichiura* infections (Holland, 1987). Nevertheless, results from our study in Panama suggest that even infections which are relatively light may negatively affect the growth of children. Despite the debate about what constitutes optimal growth for children, in general it is agreed that although it is not desirable to achieve full potential for weight, children should have the ability to reach their full potential for stature (Stephenson *et al*, 1983). That more severe cases of trichuriasis cause dysentery, which is itself associated with stunting, has been described (Cooper *et al*, 1986), but our data indicates that lighter infections may too be implicated in precluding children from reaching their full potential for height.

Another important point that this research has indicated is that intestinal helminths should not be considered separately. Very often children that are infected with one intestinal helminth will also be harbouring an infection of another intestinal helminth. Dual or multiple infections may have an impact on the health or nutrition of a child which would not be seen in a single infection of a similar intensity. Haemoglobin concentrations were significantly lower in children with both hookworm and *T.trichiura* infections, than children considered to be infected with one of these parasites. The data obtained from the longitudinal study also supported these observations. As over 20% of the children had haemoglobin concentrations considered to be likely to be associated with anaemia, this observation may be of particular importance.

Although no associations between vitamin A status and intestinal helminth infections were detected, it was noted that despite adequate nutritional resources being generally available to the children, they were significantly negatively affected in some aspects of their nutritional status by relatively light *T.trichiura* and hookworm infections. Such findings should increase the pitch of concern about the impact that intestinal helminth infections are having upon the nutritional welfare of

children in more impoverished communities.

SIGNIFICANCE OF STUDIES

Three approaches have been adopted in my investigation of the proposition that intestinal helminth infections have an impact on the nutritional physiology of their mammalian hosts.

Reviewing the results from a range of previous studies revealed the extent of practical and technical difficulties such research entails and the intricacy of the interactions between intestinal helminth infections and mammalian nutrition.

The experimental study of rats experiencing infections with *N.brasiliensisis* concentrated on fluctuations in the activity of hepatic alanine-amino-transferase during primary and secondary infections. One interpretation of these results is that a metabolic biochemical lesion might be an inevitable consequence of a successful immunological response of the host to this infection.

The community study in Coclé, Panama, not only demonstrated the problems involved in human studies, and the necessity, benefits and difficulties of collaborative research, but also emphasised how the interactions between intestinal helminth infections and host nutrition are complicated by factors such as socio-economic status, political climate and traditional community attitudes.

Within this framework, the impact that intestinal helminth infections have on host nutritional physiology is shown to be of greater complexity than previously indicated by established concepts of synergism and antagonism.

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APPENDIX 2.2.1.

ALT ASSAY: CONTROL OF ASSAY PARAMETERS

The assay used for the measurement of ALT activity involved the use of a kit (Sigma Procedure no. 59-UV), laboratory tested to work optimally within certain limits. These limits were described in the protocol issued with each kit. For experimental accuracy I performed my own tests on particular aspects.

Temperature. The Sigma protocol recommends that the assay, which is temperature dependent, be performed at 30°C. Temperature correction factors allow the assay to be performed at other temperatures. By making use of a temperature controllable cuvette compartment, operated by water being pumped from a thermostatically controlled water bath, I conducted all my assays at 30°C.

Linearity. The linearity of the reaction was checked by measuring ALT activity in liver samples from an uninfected rat every 15 sec for up to 240 sec. following commencement of assay. After between 45-60 sec the reaction became linear, and in my experimental assays I chose to follow Sigma's recommended protocol involving a 90 sec incubation period followed by a 60 sec period of measurement, with a reading also taken after 30 sec to ensure linearity.

Enzymic decay. It was suggested that enzymic decay with time might be incorrectly interpreted as experimental results. In order to investigate this ALT assays were conducted on liver homogenate samples from uninfected rats every 20 min for 3 h post-sacrifice. From the 5th reading (1 h 20 min post-sacrifice) values obtained were no longer constant. Consequently, I ensured that all ALT assays were performed within 1 h post-sacrifice.

Interference by glutamate dehydrogenase. The ALT assay kit has been designed to measure ALT activity in plasma or serum, in which glutamate dehydrogenase activity is negligible. However, this enzyme is rich in some tissues,notably liver. In order to assess whether the perceived fluctuations in liver ALT activity during

nippostrongylosis were in fact reflections of fluctuations in glutamate dehydrogenase activity, the activity of hepatic glutamate dehydrogenase was assayed on various days p.i. during infections with *N.brasiliensis*. The assay procedure is that described by Schmidt (1974). In this assay, glutamate is formed from oxoglutarate and ammonium ion substrates. The oxidation of the co-enzyme NADH is monitored by following changes in absorbance at 340 nm. The pyruvate content of the sample is removed by a 5 min pre-incubation with lactate dehydrogenase. Subtraction of the preliminary non-specific reaction rate before the addition of 2-oxoglutarate eliminates other sources of error. Constant activities throughout the infection support the view of Ovington (1985b) that glutamate dehydrogenase is not involved in the metabolic fluctuations observed in the ALT assay.

APPENDIX 2.2.2.

ZINC CONCENTRATIONS: CALIBRATION CURVE