

SHORT TITLE

CELLULAR RESPONSE IN NIPPOSTRONGYLOSIS

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CELLULAR RESPONSE IN NIPPOSTRONGYLOSIS

by

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ABSTRACT

Ph.D.

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Parasitology

CELLULAR RESPONSE IN NIPPOSTRONGYLOSIS

A study has been made of the basophil, eosinophil and mast cell changes in rats infected with Nippostrongylus brasiliensis. Self-cure occurs while mast cell numbers are low in the small intestine; mast cell numbers increase after worm loss. Self-cure and mast cell numbers are dose dependent, as is the intestinal eosinophilia found during infection. Eosinophilia and basophilia occur early during the infection in the bone marrow and in the peripheral circulation; they continue until well after worm loss. It is proposed that self-cure in nippostrongylosis is due, not to an immunological response, but to a "non-specific" alteration of the intestine which makes it an unsuitable habitat for the parasite.

ABREGE

Ph.D

John Rogerson

Parasitologie

REPONSE CELLULAIRE CHEZ LE RAT INFECTE AVEC NIPPOSTRONGYLUS BRASILIENSIS

Les changements au niveau des cellules basophiles, éosinophiles et mastocytes ont été étudiés chez le rat infecté avec Nippostrongylus brasiliensis. Le phénomène "self-cure" se produit lorsque le nombre de mastocytes est bas au niveau du petit intestin; le nombre de mastocytes augmente après la disparition du ver. Le phénomène "self-cure", le nombre de mastocytes ainsi que l'éosinophilie rencontrée au niveau de l'intestin durant l'infection sont dépendantes de la dose. L'éosinophilie et la basophilie apparaissent au début de l'infection au niveau de la moëlle osseuse et dans la circulation sanguine et demeure ainsi même après la disparition du ver. Il est proposé que le phénomène "self-cure" dans les infections à N. brasiliensis chez le rat est dû à un changement "non-spécifique" au niveau de l'intestin qui rendrait cet habitat défavorable au parasite. La réponse immunologique qui découle de cette stimulation antigénique est exclue comme explication possible du phénomène "self-cure".

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CLAIM OF ORIGINALITY

1. This is the first study of the eosinophil and basophil changes in the bone marrow of rats infected with N. brasiliensis; these changes have been compared at different levels of infection.
2. This is the first integrated study of the eosinophil changes in the bone marrow, peripheral circulation and small intestine of N. brasiliensis infected rats. 4
3. This is the first comparative study of the mast cell and eosinophil changes in different regions of the rat small intestine at different levels of infection.
4. A new hypothesis is proposed to explain the loss of N. brasiliensis from the rat small intestine.
5. It is proposed that the mast cell to globule leucocyte conversion is a process by which the host eliminates mast cells.

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CHAPTER I

INTRODUCTION

Nippostrongylus brasiliensis infections in rats have been studied extensively in recent years both as a model for obtaining a better understanding of host/parasite relationships in general and, more specifically, for determining the reasons for the loss of this parasite from the small intestine of the rat. If adult rats are given an inoculation of more than 500 N. brasiliensis infective larvae, 9-10 days after infection there is a drop in egg production, followed by the rapid loss of worms from the small intestine over the next few days; by about day 16 of the infection, few worms are left in the intestine. The drop in egg production and loss of worms have been called the self-cure reaction (Mulligan et al., 1965). From the review by Ogilvie and Jones (1971) of the cellular and humoral responses occurring during infection with this parasite, it is obvious that many points concerning the mechanism of expulsion remain to be clarified.

Wells (1962) and Rogerson (1967) studied the mast cell and eosinophil changes in the small intestine of infected rats and, in view of suggestions that eosinophils act as antagonists of histamine and/or serotonin, it was thought that the N. brasiliensis/rat system might be a good model for the further elucidation of the relationship between these two cell types. Preliminary experiments using compounds with an antihistaminic and/or an antiserotonin function failed to produce any effect on the course of infection; in view of this and the fact that

the results obtained varied enormously and that treatments had adverse effects on the health of the rats, this line of investigation was discontinued.

At the time the decision was made to discontinue the above study, Jarrett et al., (1968a) reported a rise in mast cell numbers, in the small intestine, which started just before the commencement of worm loss; they proposed a hypothesis to explain the loss of worms in which this rise played an integral part. Wells (1962) and Rogerson (1967), however, had found this rise in mast cell numbers taking place after worm loss and it was decided to investigate the mast cell response at different levels of infection and in different regions of the gut to see if the mast cell changes differed between levels of infection and between the regions of the gut. As there were discrepancies in the reports of Wells (1962) and Rogerson (1967) concerning the eosinophil changes in the small intestine during infection, they were reinvestigated in the present study. As, at that time, there had been no studies of the eosinophil response in the bone marrow and peripheral circulation of rats infected with N. brasiliensis these were investigated since the bone marrow is the source of leucocytes and, to reach the small intestine they must, presumably, travel via the blood stream. During the counting of bone marrow eosinophils it was obvious that changes in the numbers of basophils also occurred during infection and, in view of their close similarity to mast cells, these cells were also counted in the bone marrow and a separate experiment performed to determine the level of these in the circulation during infection.

-CHAPTER II

HISTORICAL REVIEW

A. Nippostrongylus brasiliensis

Nippostrongylus brasiliensis is a common and widely distributed parasite of the Norway rat, Rattus norvegicus, although natural infections have been observed in Rattus rattus and Mus musculus. A detailed account of the systematics, hosts (both natural and experimental) and geographical distribution of this nematode has been given by Haley (1961).

Rats can become infected with N. brasiliensis following ingestion of the filariform larvae, but the normal route of infection is via the host's skin (Yokogawa, 1922), ingested larvae developing to maturity only after they migrate to the lungs and return to the small intestine (Schwartz and Alicata, 1934). The precise route taken by the larvae in their journey from the skin to the lungs is not known, but it seems likely that both the bloodstream (Yokogawa, 1922) and lymphatics are utilised (Gharib, 1953, 1955; Clarke, 1967). A logarithmic growth occurs in the lungs from about the 19th to the 32nd hour after inoculation when the third moult begins; there is no further growth until the larvae reach the intestine (Twohy, 1955; 1956). The 4th stage larvae migrate up the trachea by the 35th hour after inoculation (Wells, 1971); they then pass down the oesophagus and through the stomach to reach the small intestine between 37 (Wells, 1971) and 40.5 (Twohy, 1955, 1956) hours. By the third day most of the larvae have left the lungs (Twohy, 1956; Jarrett et al., 1968b).

4

The fourth stage larvae grow rapidly after reaching the intestine. The final moult begins from 48 (Ogilvie, 1971) to 90 (Yokogawa, 1922) hours after inoculation and lasts for 12-15 hours (Yokogawa, 1922). After this moult the worms rapidly become sexually mature and grow to their final size. The worms copulate for the first time approximately at the 120th hour (Weinstein and Jones 1956; Phillipson, 1969) and by the 137th hour most females are inseminated (Weinstein and Jones, 1956). Phillipson (1969) found that females usually contain infertile eggs before mating and that each worm can produce approximately 1000 fertile eggs every 24 hours after a single full insemination; since females can store only 4 - 5000 sperm, they must copulate at least once every other day to maintain this egg output (Phillipson, 1969). Male worms are capable of fertilizing one female every two hours during the first 15-20 hours after reaching sexual maturity, this potential being greatly in excess of the minimum requirements for maintaining the maximum fertile egg output of females in normal infections (Phillipson, 1970). Egg production continues at a high level for about a week after eggs first appear in the faeces; most of the adult worms are then lost from the rat intestine over a period of several days.

Eggs are passed out in the faeces in the early stages of segmentation, starting most frequently on the 6th day of infection, although Porter (1935) found them as early as the 4th day. Development of the first stage rhabditiform larvae is rapid and a few eggs may hatch within 12-15 hours of leaving the host, but most of them hatch only after 20-24 hours (Yokogawa, 1922). Second stage larvae, and larvae undergoing the first moult, can be isolated from the charcoal/faecal cultures 48-72

hours after the eggs have been laid (Lucker, 1936; Haley, 1962).

The exact time relationships of the second moult have not been determined, but many investigators have noted that filariform larvae are abundant on the surface of 4 day old charcoal cultures kept at room temperature; the normal food of these larvae is living bacteria (Boardman, 1933). Yokogawa (1922) stated that the filariform larva of N. brasiliensis differs from that of other known "Strongylidae" in not having a sheath. Chandler (1932) however, pointed out that this was only partly true since the anterior end of the sheath is split open and the larvae alternately extend their bodies from the broken end or withdraw entirely within the sheath.

N. brasiliensis adult worms live in the duodenum, jejunum and, occasionally, in the upper ileum, where they are found among the villi and the crypts of the mucosa; these are the same sites occupied by the pre-adult 4th stage larvae (Brambell, 1965; Alphey, 1970). Habitat location is highly developed in this parasite since 4th stage larvae and adult worms surgically transferred to points at varying distances from the normally "inhabited" region of the intestine are able to quickly migrate to the "normal" site (Alphey, 1970).

The adult worms are characterised by the red colour of an endogenous haemoglobin which is distinct from that of the host (Rogers, 1949; Davenport, 1949), although these worms feed on traumatised tissue cells and blood (Porter, 1935; Taliaferro and Sarles, 1939).

Weinstein and Jones (1956) regarded the fact that intestinal flagellates of rats are frequently seen in the gut of adult N. brasiliensis as evidence that adult worms also ingest intestinal contents. Using radioisotopes Rogers and Lazarus (1949), Mulligan et al., (1965) and Neilson (1969) have shown that adult worms do not actively suck blood and that their feeding activities cause little blood loss from the damaged intestine.

The morphology, physiology and biochemistry of the infected rat small intestine has been investigated extensively by L.E.A. Symons. He (1957) found that, 10 days after a moderate or severe inoculation with infective larvae, the jejunum was thick and flabby, the villi irregularly shaped and that the muscularis externa was twice as thick as in normal rats. There was also a two-fold increase in the water content of the small intestine, a 50% increase in the dry weight of the tissue, increased concentrations of Na^+ and Cl^- , a two-fold increase in the volume of whole blood and a decreased fat content of the jejunal tissue. The changes in fat and electrolytes were regarded as a possible reflexion of undernourishment, since a loss of appetite can accompany heavy N. brasiliensis infections (Symons, 1960a). Absorption of Na^+ and Cl^- and H_2O from the intestine is reduced during infection, leading to the accumulation of fluid in the lumen; this is, probably, the main cause of the diarrhoea characteristic of heavily infected rats (Symons, 1960b).

Symons (1960c) found that, although the rate of absorption of d-glucose and l-histidine from the entire small intestine was not affected by N. brasiliensis, d-glucose absorption was greatly reduced in the infected jejunum. The balance was maintained by an increase in the absorption of glucose and maltose in the non-parasitised distal ileum of the infected animal (Symons, 1961). Using ¹³¹I-labelled egg albumin, Symons (1960d) found that, two hours after feeding, the digestion of this protein is depressed by approximately 20% in infected rats; absorption of maltose was also significantly lower in these animals than in the uninfected controls. Later work, however, (Symons, 1966; Symons and Jones, 1970) showed that the differences between test and control groups had disappeared 3-4 hours after feeding the protein and that, by 20 hours almost total digestion had occurred in both infected and uninfected rats.

Symons and Fairbairn (1962, 1963) concluded that the pathological changes which occur in nippostrongylosis are not necessarily the result of a direct action by the parasite on the host and that reductions in enzyme activities are not sufficient to explain the impairment of digestion in intestinal nematode infections. In fact, the pathological changes in the jejunum of rats infected with N. brasiliensis appear to be non-specific because similar changes occur, not only in other helminth infections, but also in unrelated intestinal damage due to non-tropical sprue, niacin deficiency or after total body irradiation (Symons and Fairbairn, 1962).

Because of the ease with which the parasite can be maintained in the laboratory and because of its short life cycle, the

Nippostrongylus brasiliensis/rat system has been used extensively in recent years as a model for the investigation of various aspects of the host-parasite relationship.

Of particular interest has been what is known as the "self-cure" reaction (Africa, 1931; Mulligan *et al.*, 1965) Nippostrongylus brasiliensis starts producing eggs approximately five days after inoculation of larvae into previously uninfected rats. Egg production rises to a peak by about the 10th day after which there is a self-cure characterised by a rapid decline in both the number of eggs produced and in the number of adult worms in the small intestine. By about the 16th day, egg production has virtually ceased and few worms are present in the small intestine. Providing that the rats are mature and providing that they receive an inoculation of at least 500 larvae, the timing of the self-cure reaction is constant within a few days for all strains of rats used.

Studies of the changes which occur during the course of the infection were concentrated for many years on the host rat, but studies have recently been made on the alterations which the parasite undergoes in this animal host. Knowledge of the effect of N. brasiliensis on the rat has to a large extent been based on histological studies of the cellular changes occurring in the small intestine and on studies of antibody production and the relation of these two phenomena to self-cure.

The classical study of the histology of the infected rat small intestine is the work of Taliaferro and Sarles (1939). They found little evidence of mechanical damage or cellular infiltration early in the infection, $3\frac{1}{2}$ - $5\frac{1}{2}$ days after inoculation. At the peak of egg production (10 days), lymphocytes, polyblasts, macrophages and a few eosinophils and heterophils had infiltrated the intestinal tissue. Sixteen days after inoculation the lamina propria was packed with eosinophils, connective tissue basophils (mast cells), globule leucocytes and a few agranulocytes, a histological picture similar to that described in such pathological conditions as idiopathic steatorrhea (non-tropical sprue) or ulcerative colitis (Truelove and Wright, 1968). Recent studies on the cellular changes which occur in the small intestine during nippostrongylosis have, to a large extent, concentrated on mast cells and globule leucocytes.

Weill (1919, 1920) was the first worker to call attention to cells containing characteristic acidophilic granules in the epithelium and, occasionally, in the lamina propria of the intestine of the cat, dog, rabbit and mouse. In consideration of their granular inclusions and presumed mesenchymal origin, he (Weill, 1919) called them Schollenleukocyten, a name that has passed into English as globule, or globular, leucocyte. It has been suggested that this cell type is derived from lymphocytes (Weill, 1920; Dawson, 1943; Kent, 1966; Toner, 1965),

from plasma cells (Dobson, 1966; Carr, 1967; Whur and Johnston, 1967), from eosinophils (Casley-Smith, 1968) or from mast cells (Murray et al., 1968). Globule leucocytes appear in large numbers at sites of parasitic infection in the gastrointestinal tract (Taliaferro and Sarles, 1939; Sommerville, 1956) and are found on the epithelial side of the basement membrane of the villi in the small intestine of rats infected with N. Brasiliensis (Taliaferro and Sarles, 1939; Jarrett et al., 1968a).

The ultrastructural and cytochemical studies of Murray et al., (1968) in cattle infected with Ostertagia ostertagi, sheep infected with Haemonchus contortus or Fasciola hepatica and rats infected with N. brasiliensis have provided convincing evidence that the globule leucocyte is a modified mast cell and that the changes in the granules which occur in the formation of this cell are caused by an alteration in the relationship between acid mucopolysaccharide and basic protein. The globule leucocyte is a mast cell which has migrated from the lamina propria to an intraepithelial position, losing some of its contents in the process.

The presence or absence of mast cells in the rat intestinal lamina propria has been the subject of conflicting reports. Some workers (Mota et al., 1956; Kent, 1966) have found few or no mast cells in this region while others (Lindholm, 1960; Wells, 1962; Rogerson, 1967) have

shown a high density. Enerback (1966a,b) resolved this conflict when he demonstrated not only that the identification of mast cells in the rat small intestine depends on the fixative used, but also that rat gastrointestinal mast cells have different staining properties compared to those found in the skin.

The first quantitative studies on the changes in mast cell numbers in the wall of the small intestine of rats infected with N. brasiliensis were made by Wells (1962) who examined sections from three carefully defined regions of the proximal small intestine of three month old male rats inoculated with 5000 larvae. She found a significant rise in cell numbers 23 days after inoculation, at a time when few worms were present in the small intestine. On days 5, 9, 12 and 16 after inoculation, there were indications of a decrease in mast cells, although this was not statistically significant; the changes occurred to the same extent in the three regions examined (Wells, personal communication).

Rogerson (1967), using female rats 6-7 weeks old at the time of inoculation with 500 N. brasiliensis larvae, found a significant reduction in mast cell numbers on the 5th and 9th days after infection. The peak worm burden occurred on day 9 and, although the number of worms in the small intestine was greatly reduced on day 12, the number of mast cells was still below normal. On the 16th and 23rd days after inoculation,

when few worms are present in the intestine, mast cell numbers rose to values well above normal levels. These results have subsequently been confirmed by Keller (1971), who found large numbers of mast cells only during the later stages of the infection when most of the worms had left the intestine. In contrast to these results, Jarrett et al., (1968a) and Murray et al., (1971a,b), reported an increase in mast cell numbers at the time of worm loss from the intestine and suggested that this rise plays an integral part in worm elimination.

Wells (1962) found raised eosinophil levels in the wall of the small intestine from the 12th day of a primary infection, with no increase before this time, while Rogerson (1967) found an increase from the 12th day and also an increase five days after infection with numbers dropping back to normal on the 9th day.

Mast cell numbers in the lungs are below normal levels while larvae are present and increase after most of larvae have left; there is a slight eosinophilia while larvae are present and a pronounced eosinophilia after they have left (Wells, 1971). In the peripheral circulation basophil and eosinophil levels were raised from the 13th day of a primary infection (Hogarth-Scott and Bingley, 1971).

A great stimulus to the study of antibody production in nippostrongylosis was given when Ogilvie (1964, 1967) found that antibodies identical to human reagins (Bloch, 1967; Ogilvie and Jones, 1969) are formed during the infection. Similar antibodies are also found in rabbits infected with Trichinella spiralis (Ivey and Slanga, 1965), in sheep infected with Trichostrongylus colubriformis (Ogilvie, 1964), in monkeys infected with Schistosoma mansoni (Smithers and Ogilvie, 1965; Ogilvie *et al.*, 1966), in guinea pigs (Ivey, 1965) and rats (Dobson *et al.*, 1967) infected with Toxocara canis, in sheep infected with Oesophogostomum columbianum (Dobson, 1967) and in rats infected with Fasciola hepatica (Jarrett, 1972).

The idea that homocytotropic antibodies might be responsible for protective immunity to helminths was put forward by Ogilvie (1964). This hypothesis was supported by the work of Jones and Ogilvie (1967a,b), who found that protection to N. brasiliensis could be passively transferred with sera of high reaginic titre and that this effect of serum could be neutralised by worm extracts. The protective function of this type of antibody was tentatively associated with its known role in anaphylactic hypersensitivity processes (Wilson and Bloch, 1968). Results from later studies, however, have cast doubts on a role for homocytotropic antibodies in helminth immunity. Not only is serum reagin extremely variable in titre in similarly infected and comparably resistant rats (Ogilvie and Jones, 1967; Wilson *et al.*, 1967), but also repeated vaccinations with worm extracts

results in reagin production but no protection (Ogilvie, 1967). Reagins are only detected in the circulation after worms have left the intestine following an initial infection (Ogilvie, 1967; Keller, 1970a) and, although superimposed infections elicit an anamnestic rise in reagin titre by the 6th day of challenge, the adult worm population is eliminated by the 4th day.

Ogilvie (1967) and Keller (1970a) found no correlation between the titre of circulating reagins in nippostrongylosis and the sensitivity of the infected rats to systemic anaphylaxis; reagin titres continue to rise following repeated infections, but rats can be shocked only after a first or fourth, but not after a 2nd or 3rd infection (Keller, 1970a). Although peritoneal mast cells moderately sensitive to reagents are found 9 days after infection (Wilson and Bloch, 1968), Keller (1970) found only a few mast cells in the peritoneal cavity before days 14-20; it was not until day 14, when worm loss was almost complete, that he was able to detect allergen-induced histamine release from peritoneal mast cells.

Numerous workers have investigated the mechanisms of protection in the N. brasiliensis/rat system by attempting to passively transfer immunity with the serum of infected rats (Graham, 1934; Porter, 1935; Barles and Taliaferro, 1936;

Chandler, 1937; Sarles, 1939; Thorson, 1953, 1954; Mulligan et al., 1965; Ogilvie and Jones, 1969; Jones, 1969). In most of these experiments, however, the protection induced in normal rats required large amounts of serum from multiply infected rats; the amount of protection conferred was extremely variable and the level of protection induced was seldom comparable to that produced by an active infection.

Ogilvie and Jones (1968) found protective antibodies in the circulation 17 days after a primary infection. These authors reported, however, that only about one third of antiserum pools would protect rats passively, and that serum from rats given one infection protected as strongly, and as frequently, as antiserum taken from multiply infected rats. In other words, protective antibodies can be detected only sporadically in the circulation, and an anamnestic rise in these antibodies is not induced by reinfection.

Antibodies are produced during infection against the three isoenzymes of N. brasiliensis acetylcholinesterase, but there is no correlation between the occurrence of these antibodies in an antiserum pool and the ability of such a serum to protect against this parasite

(Jones and Ogilvie, 1972). These workers found antibodies against all three isoenzymes 27, 30 and 84 days after a primary infection of N. brasiliensis when few worms are present in the intestine but could not find any 12 days after infection, when worms are leaving the host.

Attempts to demonstrate protection by the transfer of immune cells have met with the same variable results as have attempts to transfer immunity with serum. Hunter and Leigh (1961) failed to protect N. brasiliensis infected rats by the transfer of spleen and lymph node cells from highly immunised donors; Ogilvie and Jones (1968) achieved some degree of protection with cells from the mesenteric lymph nodes, peritoneal cavity and spleen in only three of eleven experiments. Using either spleen or lymph node cells, Kassai and Szepes (1970) failed to transfer protection and concluded that worm elimination involves one or more factors which cannot be transferred by sensitized cells. Greater success was achieved by Kelly and Dineen (1972) who used mesenteric lymph node cells taken either 7 days after rats had been given a third infection of N. brasiliensis or 15 days after a primary infection (at 15 days few worms are present in the small intestine). Using this procedure they suppressed egg production and obtained 75% expulsion of the worms by the 10th day of infection. When some measure of success has been achieved in these protection experiments, it has been with serum or cells taken from rats which had been multiply infected with N. brasiliensis or from rats with a primary infection at a time when worm loss has either finished or almost finished; an attempt to find protective antibodies

at the time of worm loss during a primary infection was unsuccessful (Jones and Ogilvie, 1972).

When the majority of adult worms have left rats after a primary infection, the residual, mainly male population, is found in the duodenum near the pyloric sphincter and not in the regions of the small intestine which are preferentially inhabited before the onset of self-cure (Brambell, 1965). Chandler (1936) showed that the residual population in rats which had become immune to reinfection was able to reestablish and renew egg production when transplanted into the small intestine of normal rats; a successful transfer of adults could be made at 18 days but not four weeks after infection (Chandler, 1938). These results were, in general, confirmed by Ogilvie and Hockley (1968), who made a detailed study of the effect of the host on the parasite at the end of a primary infection by transferring adult worms to the intestine of normal rats. The behaviour of three populations of worms were compared after transplantation: "normal" worms (found in the intestine 6-9 days after a primary infection) "damaged" worms (found in the intestine after the 10th day of a primary infection) and "adapted" worms (these are able to "adapt" to the response of the host during their development and are found 6-9 days after reinoculation).

The latter two populations produced fewer eggs in recipient rats than "normal" worms, but more than they would have in their original

host. Compared with "normal" worms, the latter two populations also established less efficiently because fewer worms were recovered 5-6 days after transplantation into the new host. Therefore, by the criteria of reproductive capacity and ability to reestablish in normal rats, the damaging effects on the worms at the end of a primary infection are irreversible. Furthermore, the residual population after self-cure is neither more nor less affected than worms just before expulsion, as judged by their ability to recover when transplanted into normal rats. Thus, the residual worms are not a selected population which is more capable of withstanding the effects of the host than worms which are expelled, as has been suggested by Jarrett *et al.*, (1968b,c). It was also shown by the former authors that adult worms from neonatally infected rats, which are not expelled, are also irreversibly damaged during infection because they do not reestablish when transplanted into normal rats, confirming the conclusion that the residual worm population is not a selected population (Ogilvie and Hockley, 1968). These same authors showed that the damage to the worms is caused by a host reaction and not by senescence since "normal" worms transferred every fourth day until the worms were 20 days old did not differ in infectivity and reproductive capacity.

Changes in the biological behaviour of worms from immune rats are associated with changes in their morphology. Taliaferro and Sarles (1939) noted that the worms, in rats which had become immune, were

vacuolated and their guts dilated. Detailed examination of the structural changes in adult worms from immune rats were made by Ogilvie and Hockley (1968) and Lee (1969). These authors found that at the end of a primary infection, the drop in egg production and the onset of loss of the adult worms were preceded by signs of damage which appeared firstly, and were most severe, in the cells of the gut of the worms where the ribosomes are replaced by vacuoles, some of which contain lipid (Lee, 1969). Toward the end of the infection, lipid droplets also appear in the hypodermis, muscle and excretory glands; resorption of spermatozoa occurs in the male and egg production ceases although there is no alteration in the cuticle of damaged worms (Lee, 1969). This latter author suggested that the accumulation of lipid droplets may result from the movement of the parasite from the aerobic environment close to the mucosa where the worms live and feed (Rogers, 1949) to the anaerobic environment of the intestinal lumen where the adults are found in passively immunised rats (Taliaferro and Sarles, 1942). Ogilvie and Hockley (1968) showed that, even 5 days after transplantation into normal rats, this structural damage was not repaired and concluded that these worms were permanently damaged by the host's immunity. Quantities of material thought to be immune precipitates were frequently seen in the lumen of the gut of parasites taken from actively or passively immunised rats (Taliaferro and Sarles, 1939, 1942) but neither Ogilvie and Hockley (1968) nor Lee (1969) were able to confirm this, although worms from immune rats which had been stained for non-specific esterase sometimes had plugs showing

esterase activity at the excretory pore (Ogilvie and Hockley, 1968), plugs which were not seen at the oral or anal openings (Edwards et al., 1971).

Recent work has shown that changes in a number of N. brasiliensis enzymes occur during infection (Edwards et al., 1971). Sanderson (1969) showed that adults contain and release large quantities of acetylcholinesterase into culture fluids and Sanderson and Ogilvie (1971) found that the level of this enzyme increases rapidly in the worms as the host rats become immune. Of the three isoenzymes of acetylcholinesterase, one is reduced in worms damaged by the reaction of the host but is greatly increased in "adapted" worms; the other two increase in both "damaged" and "adapted" worms. In addition, plugs staining for non-specific esterase activity are found in the excretory pore of both "damaged" and "adapted" worms but are not seen in the oral or anal openings nor in the gut lumen (Edwards et al., 1971). Several other non-specific esterases were found in extracts of adult worms and in culture fluids, but the levels of these did not change in worms taken at various times during infection (Edwards et al., 1971).

Acid phosphatase is located in the gut of the parasite (Edwards et al., 1971). There are several isoenzymes of this enzyme in "normal" worm extracts and the pattern of these was changed in "damaged" worms, but the pattern in "adapted" was similar to that in "normal" worms. Edwards and Gutteridge (1969) found that adults of N. brasiliensis are rich in malic

dehydrogenase, but the level of this enzyme does not change markedly during infection and they were not able to show that immunised rats produced any antibodies to it. Leucine-amino-peptidase was found in the "excretory gland" (Lee, 1969, 1970) but it appeared to be present in similar quantities in "normal", "damaged" and "adapted" worms (Edwards et al., 1971).

Sarles and Taliaferro (1936) found that adult worms in rats 7 days after reinfection are stunted, but that, on transfer to a previously uninfected rat, they complete their development and produce large numbers of eggs. Ogilvie and Hockley (1968) confirmed this and found that stunted adults found 6-9 days after reinfection ("adapted" worms) are as capable of reestablishing in clean rats as "normal" worms and rapidly renew their egg production, maintaining it at a high level for more than six days. "Damaged" worms, taken 14 days after infection, do not recover their egg production on transfer. Structurally, some disorganisation of the cytoplasm of the gut cells occurs, but this is probably not as severe as in "damaged" worms (Ogilvie and Hockley, 1968) and, although they remain stunted, the internal structure of the worms is regenerated on transfer to previously uninfected rats.

In this review of N. brasiliensis we have seen that many changes occur in both the parasite and the host during the course of infection. The possible causes and significance of these changes will be mentioned in greater detail in the discussion.

B. The Eosinophil

It is the view of several authors (Sundell, 1958; Rytomaa, 1960; Archer, 1963) that "the coarse granular corpuscles" described by Wharton-Jones (1846) were the cells now known as eosinophil leucocytes. However, it was not until the classical work of Ehrlich (1879) that eosinophils were so named when he showed that the cytoplasmic granules of these cells had an intense affinity for eosin. At the same time he suggested that these cells were produced in the bone marrow and discharged into the circulation in the same way as other blood cells.

The intramedullary origin of eosinophils was confirmed by Opie (1904a) who concluded that they arose by the mitotic division of large mononuclear cells. There has been, however, considerable controversy in the subsequent literature concerning the origin and function of these cells. Ringeon (1921) suggested that the mononuclear eosinophils seen in the peritoneal cavity of the guinea pig arise from the omentum, since cells of myeloid origin are characterised by polymorphic nuclei. Maximow (1927) proposed that eosinopoiesis occurs physiologically in the digestive tract and, in certain pathological states, in inflammatory tissue as well. Cooke (1932) reported that, not only did the eosinophil leucocyte count in the blood and in the tissues vary greatly, but also that the eosinophils in blood and tissue exudates

were not all morphologically similar. These observations were not confirmed by Biggart (1932): he found no morphological difference between blood and tissue eosinophils and reported that both gave identical oxidase and peroxidase reactions. Duran-Jorda (1943) suggested that eosinophils develop from lymphocytes through the stage of Paneth cells in the bowel mucosa. According to this author, the granules in the eosinophils are preformed erythrocytes; when the erythrocytes are finally extruded, the parent cell once more becomes a lymphocyte. His hypothesis, that eosinophils are carriers of preformed red corpuscles from the gastric and intestinal mucosa to the haemopoietic organs has, however, never been confirmed.

Godlowski (1952) proposed that concurrent eosinopoiesis occurs in the bone marrow and in other tissues, such as the mucosa of the gut; he suggested that some circulating blood eosinophils are originally produced in the epithelial lining of the intestinal crypts and that this process is intensified both by the ingestion of antigenic proteins and by various traumata inflicted on the intestinal canal. According to this worker, the most immature forms have a columnar shape similar to the neighbouring epithelial cells; with maturity, the cells become smaller, their granules condense and their nuclei become more compact, thus resembling circulating eosinophils. These observations were not, however, confirmed by Sundell (1958) who made an extensive study of the variation in the level of eosinophils in the wall of the small intestine of the rat after

various stimuli.

Dzury and Cohen (1958) observed that the cutaneous eosinophil infiltration produced in rabbits in response to injections of crude beef extract was not influenced by the level of circulating eosinophils; tissue eosinophilia according to these authors, can occur when blood eosinophil levels are high, or low, or when it is reversed with nitrogen mustard. This observation prompted them to suggest that cutaneous eosinophils may have an origin independent of those in the peripheral circulation. Foote (1965) confirmed the earlier work of Kindred (1942) and Bro-Rasmussen and Henriksen (1964) that, although most of the eosinophils are formed in the bone marrow, some eosinopoiesis can occur elsewhere, since 63% of the spleen eosinophils were formed there. Despite these theories it is generally accepted that most, if not all, eosinophils develop from myeloblasts in the bone marrow; (Undritz, 1952; Wegelius, 1957; Sundell, 1958; Rytomä, 1960; Archer, 1963).

An extensive study of the distribution of eosinophils in various tissues and organs of rats led Rytomä (1960) to conclude that the bone marrow is the tissue containing the largest numbers of these cells; almost three quarters of the eosinophils in this tissue are of the adult, or at least, the "staff" form. This author reported that the gastrointestinal tract was also a rich source of eosinophils; it contains 35, whereas the

bone marrow contains 300 times more eosinophils than the peripheral blood. Even excluding those cells in the erythropoietic organs, most of the body's leucocytes are probably located extra-vascularly (Osgood et al., 1954).

The life span of eosinophils is relatively brief. Using bone marrow cultures, Osgood (1937) showed that the total life span of the human leucocyte is only 10-12 days. This was confirmed in vivo by Kline and Cliffton (1952) in studies using labelled leucocytes. From data available in the literature and from that of his own studies, Osgood (1954) calculated the generation time of human eosinophils in the bone marrow to be about 60 hours. Foote (1965) reported a similar situation in rats; he found that the time from DNA synthesis by the myelocyte in the bone marrow to the appearance of cells in the blood is 60-73 hours. Eosinophils spend a very short time in the peripheral circulation; estimates of their "blood stream phase" range from a short 15-90 minutes (Rytomaa, 1960), to 3-4 hours (Dustin and De Harven, 1954) and to 8-12 hours (Foote, 1965). Foote (1965) estimates that 97% of the cells spend less than 6.2 days in the tissues and that the half-life of eosinophils in the gut is 22 hours.

The ultimate fate of the eosinophil has still not been established. Padawer and Gordon (1952, 1953) have described degenerate eosinophils in the blood, suggesting a form of intravascular lysis.

Archer (1963) believes that intact or broken eosinophils may be engulfed by macrophages, or that they may be able to pass through the intestinal and respiratory mucosae and be excreted. Teir et al., (1963) showed that many eosinophils are eliminated into the lumen of the bowel.

The eosinophil, then, is a short lived white cell, formed primarily in the bone marrow, and found in many parts of the body. The kinetics of this cell are characterised by a dynamic turnover: production and maintenance of a reserve pool of cells in the bone marrow and transport via the blood stream to the tissues.

The distribution of eosinophils in normal tissues and under pathological conditions has led to considerable speculation on the control and function of these cells. Eosinophilia has been classically associated with two broad groups of conditions; allergic states (Knott and Pearson, 1934; Samter, 1949; Andersson, 1958; Eidingen et al., 1962) and parasitic infections; (Craig and Faust, 1957; Beaver, 1962; Wells, 1962; Ismail and Tanner, 1972a). The occurrence of eosinophils in these two groups of conditions has led to the view of some investigators that these leucocytes are, in some manner, connected with the host immune response.

More specifically, four main functions have been ascribed to eosinophils; these are:

1. that they act as non-specific phagocytes, similar in

function to neutrophils (Archer and Hirsch, 1963);

2. that they ingest and destroy antigen-antibody complexes to which they are attracted (Litt, 1964a);
3. that they process the immunological information contained in antigens into a form which stimulates lymphocytes to enter an immunological response (Speirs, 1958a,b);
4. that they act as antagonists to inactivate excess histamine, serotonin and bradykinin which have been released from mast cells during an inflammatory response (Archer, 1963).

Phagocytosis of bacteria by eosinophils was first reported by Mesnil in 1895. Since that time there have been numerous reports of the ability, or of the inability, of eosinophils to phagocytose a wide variety of substances. As early as 1904, Opie found that bacteria exert a chemotactic influence upon eosinophils, attracting them from the circulation to the site of inoculation of the bacteria. These cells, however, rarely acted as phagocytes; instead they underwent degenerative changes and later were ingested by large macrophages (Opie, 1904b). Weinberg and Seguin (1915) found that guinea pig eosinophils ingested inert particles, a variety of microorganisms and red cells and also could absorb hydatid fluid in vitro and in vivo. These results were

confirmed by Parish (1970).

The phagocytic activity of horse eosinophils has been the subject of contradictory reports. G.T. Archer and Hirsch (1963) found that horse eosinophils did not ingest bacteria in vitro although they phagocytosed zymosan, erythrocytes and antigen-antibody complexes. This report could not be confirmed by R.K. Archer (1968) who suggested that the eosinophils used by Archer and Hirsch had, perhaps, been modified by the method used to separate them from the other blood leucocytes. Phagocytosis of mycoplasma by human eosinophils has been reported by Zucker-Franklin et al., (1966).

Eosinophils have been reported capable of ingesting soluble antigen-antibody complexes, as indicated in the phase contrast observations of Archer and Hirsch (1963), by electron microscopy (Sabesin, 1963) and in the immunofluorescence studies of Litt, (1964a,b). These cells are capable of ingesting tritiated soluble antigen in immunised and non-immunised mice (Speirs and Speirs, 1963), even in the absence of antibody (Roberts, 1966a,b). Ferritin can also be absorbed by these cells in the absence of natural antibody in unsensitised rats (Bro-Rasmussen and Egeberg, 1966) although, according to Litt (1964b), soluble antigen is not ingested in the absence of antibody. Archer (1969), working with antigen consisting of a saline extract of the nematode Amplificum robertsi and antisera obtained from rats infected with the parasite,

observed that eosinophils were attracted to insoluble antigen-antibody precipitates in vitro; phagocytosis of the complexes was followed by lysis of those eosinophil granules nearest the phagocytic vacuole.

The possible homeostatic role of eosinophils in the ingestion and degradation of antigen-antibody complexes has been studied by Litt (1961, 1964a). He found that peritoneal eosinophilia resulted within 24 hours when the peritoneal lining, blood or serum from an animal previously sensitised to a foreign protein was transferred, along with antigen, into normal animals. No eosinophilia resulted when tissue extracts of similarly immune animals and the homologous antigen were placed in a cellophane bag and the bag introduced into the peritoneal cavity of normal animals, suggesting that the mediator was not histamine as this latter is a dialyzable substance.

Litt (1961) also reported that one injection of bovine serum, albumin, γ -globulin, whole serum or haemocyanin into guinea pigs increased, 24 hours later, the number of eosinophils in the draining lymph nodes but not at the site of injection; the eosinophilia in the draining lymph nodes reached massive proportions if the injections were repeated weekly. Litt considered this as evidence that eosinophils were concerned in the initiation of the immune response. Furthermore, Roberts (1966a) showed that mouse eosinophils rapidly took tritiated antigen and that eosinophilia resulted in the draining lymph nodes within 4 hours of a primary stimulation. Besides labelling of the

eosinophils, there was a heavy concentration of tritium in the cytoplasm of macrophages, mast cells and in the large mononuclear leucocytes; the latter showed the labelling not only in the cytoplasm, but also in the nucleus.

The eosinophilotactic effect of antigen-antibody complexes is not entirely settled. In Arthus reactions, where tissue damage is due almost entirely to antigen-antibody complexes in the vessel walls, only neutrophils are attracted to ingest the complexes and to liberate proteolytic enzymes; local tissue eosinophilia occurs two days later (Humphrey, 1955). Similarly, Parish and Rhodes (1967) in studies on nodular vasculitis, found neutrophil infiltration but only an occasional eosinophil in baboon skin injected with complexes containing rabbit antibody.

Eosinophils accumulate in tissues in which mast cells shed their granules, as in anaphylaxis, or in tissues in which mast cells are unusually numerous or active, as in mastocytomas or granulation tissue.. Many eosinophils are found in tissues rich in mast cells after the mast cell histamine has been released by compounds such as 48/80 (Riley, 1956, 1959). Electron micrographs of such tissues show that eosinophils ingest mast cell granules (Welsh and Greer, 1959; Mann, 1969). If, instead of the sudden changes caused by chemical histamine releasers, the mast cell damage is prolonged, as occurs when repeatedly washing out the peritoneal cavity of rats with saline (Archer and Hirsch, 1963; Parish, 1970) or as a result of magnesium deficiency in rats (Hungerford and Karson, 1960),

the number of eosinophils in the peritoneal fluid rises as the number of mast cells falls. This phenomenon is possibly due to a constant slow release of substances from the mast cells which attract eosinophils.

The third suggested function for eosinophils, that they are directly concerned in the synthesis of antibody, has been studied extensively by Speirs (1958; 1960; 1963a; 1963b) and Speirs and Osada (1962). Speirs (1960) observed that an eosinophil response always occurs prior to serum antibody following intraperitoneal injections of tetanus toxoid and that an increase in these cells parallels in some respects a rise in antibody titre. Marked accumulation of eosinophils only occurs in actively immunised mice. This result was taken to indicate that eosinophilia is not the result of the reaction of antigen with circulating antibody. Furthermore, the fact that both cortisone and whole body irradiation depress eosinophils and antibodies in a parallel fashion (Speirs, 1956), convinced the author that eosinophils are concerned in the initiation of the antibody response. The results of further work (Speirs and Speirs, 1963) indicated that eosinophils are initially associated primarily with mononuclear cells which contain radioactively labelled antigen, rather than with free antigen-antibody complexes. Labelled eosinophils were later found within viable macrophages of the reticulo-endothelial system. This data suggested to these authors that the role of the eosinophil may be to transfer material from cells injured by antigen to viable cells capable of taking part in the immune response.

The fourth suggested function for eosinophils is that they inactivate excess histamine, serotonin and bradykinin in the tissues (Archer, 1963). Kline et al. (1932) were the first to report that a local tissue eosinophilia occurred when histamine was injected into the skin, an observation which has been subsequently confirmed by various investigators (Knott and Pearson, 1935; Vaughn, 1952, 1953; Archer, R.K., 1959; 1960; 1963). The capacity of histamine to attract eosinophils into the tissues has, however, been the subject of conflicting reports for many years. Vaughn (1952) noted that the eosinophilia-inducing effects of extracts of Ascaris could be completely prevented by the administration of anti-histamine. This led him to support the view that histamine, or some closely related substance, was being set free in abnormal quantities. Similar results were reported by Ismail and Tanner (1972b) who inhibited the eosinophilia induced by Trichinella spiralis in rats by treatment with antihistamines. Samter (1949), however, stated that antihistamines are incapable of blocking the eosinophil response in immunologically hypersensitised guinea pigs and that the severity of an anaphylactic shock has no influence on the eosinophil response.

Archer (1956; 1959; 1960) has done considerable work on the eosinophil-attracting effect of histamine in the skin and in the bone marrow of the horse. He found that the degree of eosinophil infiltration in the skin is related to the dose of histamine injected; a marked inhibition of eosinophilia occurs when histamine is immediately followed

by an injection of the anti-histamine, Mepyramine. He also found an increase in eosinophils - both metamyelocytes and mature cells - in the bone marrow after an intramedullary injection of histamine; this response appeared specific, since there was no "material change in the cellularity of the aspirates". This author was also of the opinion that one of the main functions of eosinophils was, possibly, to counteract the toxic effects of histamine.

There have, on the other hand, been many reports of the failure of histamine to cause eosinophilia. Certain authors claim that eosinophilia is not induced by local histamine injections in rats (Halpern and Benos, 1951; Hungerford, 1964) or consistently in guinea pigs (Campbell, 1943; Samter, 1949; Litt, 1962; 1964a; Parish and Coombs, 1968) or mice (Speirs, 1955). In man, intradermal injections or topical application of histamine to the abraded skin of allergic persons are reported to induce a local eosinophilia (Knott and Pearson, 1934; Eidinger et al., 1964; Wolf-Jürgensen and Zachariae, 1965; Fernex and Fernex, 1966; Feinberg et al., 1967), although not in all investigations (Fowler and Lowell, 1966). Intramuscular histamine in quantities sufficient to induce systemic signs has been found to cause, on the other hand, a peripheral blood eosinopenia (Halpern and Benos, 1951).

Parish (1970) has suggested that some of these discrepancies

are attributable to the time of sampling after injection of the amine and to the allergic state of the individual. Up to three hours after intravenous, intramuscular or intraperitoneal injection of histamine, the number of eosinophils in the blood is decreased by 50% or more in man and rats (Halpern and Benos, 1951), mice (Speirs, 1955), horses (Archer, 1963) and guinea pigs (Parish and Coombs, 1968) although with physiological doses of 2 or 20 g histamine base, the eosinopenia is mild or absent (Parish and Coombs, 1968). However, 24 hours after the injection, there is a mild eosinophilia in the peritoneal fluid and blood of mice (Speirs, 1955) and sometimes in the peripheral blood of guinea pigs (Litt, 1962; Parish and Coombs, 1968). The response to histamine may be modified by the allergic state of the individual. Samter (1949) found that intracardiac histamine causes blood eosinophilia only in sensitised guinea pigs, not in unsensitised animals and the work of Andersson (1953) indicates that histamine causes a decrease in eosinophils in normal humans while the same dose stimulates an eosinophilia in patients suffering from bronchial asthma.

Archer (1963) objected to conclusions based upon changes in the cell composition of peritoneal fluid after histamine infusions, not only because little is known concerning the precise relationship between peritoneal fluid and the blood, but also because there have not as yet been any reports upon histamine levels in this fluid. Histamine is a highly active, soluble and diffusible substance and there is no

evidence that it remains localised in the peritoneum after injection there. Furthermore, it is known that the bowel wall exhibits potent diamine oxidase activity and that there is frequently an extensive eosinophilia of this organ in the absence of any obvious stimulating agent; evidence as to the eosinophil leucocyte content of the intestinal wall following intraperitoneal histamine has not yet been published.

There is a further consideration: it has been shown that an increase in blood histamine concentration is difficult to achieve and that injection, in fact, leads to a decrease in peripheral histamine with extraordinary rapidity. Rose and Brown (1938) demonstrated this phenomenon in rats when they found that blood histamine levels are subnormal about 10 minutes after an intravenous injection of the amine. Using ^{14}C -labelled histamine, Beall and van Ardsel (1960), furthermore, showed that the clearance of radioactive histamine from plasma after intravenous injection was extremely rapid both in normal and asthmatic patients. No comparable studies have been made on peritoneal fluid, but it would seem likely that some comparably rapid and efficient system for the removal of histamine also exists there. There is also the question of the resemblance of an injection of histamine into an animal and the subsequent changes in the animal to the release and effect of histamine in normal animals and in animals under pathological conditions.

The antihistaminic effect of eosinophils was first reported by Kovacs (1950). Using an extract of the buffy coat layer of rabbit

blood, he found that it protected guinea pigs to a lethal histamine aerosol. It was later reported (Kovacs and Juhasz, 1951) that there was a linear relationship between this protective effect and the number of eosinophils in the leucocyte suspensions extracted. Feldberg and Kovacs (1960) showed that extracts of the buffy coat also exerted antihistaminic effects on bronchial and on ileal smooth muscle. Studies on the effect of extracts of the cytoplasmic granules of horse eosinophils (Vercauteren and Peeters, 1952; Vercauteren, 1953) showed that they have an antihistaminic effect on the isolated guinea pig ileum. Further evidence for an antihistaminic effect of eosinophils was provided by Archer and his co-workers when they showed that, in addition to the protection against bronchioconstriction in guinea pigs, the oedema and eosinophil infiltration produced in tissue by histamine could be reduced by saline suspensions of eosinophils (Archer *et al.*, 1962; Archer, 1963); isolated eosinophil granules or aqueous extracts of these cells were also effective. Archer (1968) reported a reduction in the oedema produced in ponies by the intradermal injection of impure bovine fibrinogen when an extract of horse eosinophils was injected; the infiltration of eosinophils was reduced if Mepyramine, a histamine antagonist, was injected.

Aschkenaszy (1962) reported that eosinophils take up and inactivate both histamine and heparin from mast cells during allergic and anaphylactic reactions. He suggested that, while the nature of the substance attracting the eosinophils was probably histamine, heparin

should also be taken into account. Evidence in the literature on the influence of heparin on eosinophils is, however, inconclusive.

Godlowski (1951) working with dogs, and Barr et al., (1960) with humans reported an increase on peripheral eosinophils after the administration of heparin, but Archer (1963) found no such increase in horses.

There is some evidence that another biologically active amine, 5-hydroxytryptamine (serotonin), may also play a part in the pathogenesis of allergic states in some animals, especially mice and rats (Humphrey and Jaques, 1955; Fink, 1956; Munoz, 1957). Rosa et al. (1958) observed that both intramuscular and intravenous injections of serotonin produced a constant decrease in the number of blood eosinophils and that this decrease was proportional to the dose of the amine. Archer (1959) was unable to confirm this report, concluding that serotonin is neither chemotactic for, nor destructive to, eosinophils; he stated, however, that eosinophil leucocytes in tissue could reduce the oedema producing effect of this amine. Archer (1959) also noted that pretreatment with bromylsergic acid diethylamine (BOL 148), a specific antagonist of serotonin, did not reduce either oedema or tissue infiltration induced by an injection of histamine.

There is also some evidence that another vaso-active amine found in mast cells, bradykinin, has some relationship with eosinophils. Archer (1963) found that oedema produced in horse skin by the injection

of crude bradykinin, or pure synthetic bradykinin, was markedly reduced by the subcutaneous injection of an aqueous extract of horse eosinophils; antihistamine (Mepyramine maleate) has no effect on this reaction. Wolf-Jürgensen et al., (1969), using the "skin window" technique in humans, found that intradermal injections of bradykinin produce an initial dose-dependent eosinophilia and suggested that eosinophils may inactivate bradykinin; they pointed out, however, that the eosinophilia might be an indicator of a bradykinin-induced histamine release.

Of the four functions proposed for the eosinophil, three infer that this cell is part of the animal's homeostatic mechanism and the fourth is the suggestion that eosinophils are involved in antibody production. Of the homeostatic functions, phagocytosis is the only process on which investigators generally agree, but there is a reluctance to accept this as the primary function of the eosinophil since this would duplicate the function of neutrophils and macrophages. The emphasis has been placed, to a certain degree, in recent years on the role of eosinophils as antagonists of histamine, serotonin and, to a lesser extent, bradykinin; all of these are vasoactive substances, found in mast cells, which are responsible in large measure for changes in permeability associated with the inflammatory response.

The Mast Cell

Ehrlich (1877, 1879) found cells in tissue containing granules which stained red with the basic blue-violet triphenylmethane dye "Dahlia", a reaction which he was the first to call metachromatic. He called these cells "Mastzellen" because he believed that the granules, of which these cells were full, were the products of overfeeding.

Selye (1965) has defined the mast cell as "a connective tissue element which possesses cytoplasmic granules that stain metachromatically under ordinary conditions". This definition separates the mast cell from epithelial elements which contain metachromatically-staining granules of mucous and from the blood borne, myelogenous basophils. The definition includes immature, neoplastic and discharged true mast cells (whose metachromasia has been lost) and excludes other cells which merely phagocytose metachromatic foreign materials, or which become metachromatic as a result of abnormal histochemical manipulations. The size, shape and degree of granulation of mast cells varies considerably from species to species and in different tissues from individual animals. They range in diameter from $5-25\mu$ or more; they tend to be round or oval in loose connective tissue and elongated along the walls of blood vessels. The granules are $0.5-0.75\mu$ in diameter and may be sparsely or closely packed within the cell.

The origin of the mast cell constitutes something of a problem.

There was a good deal of confusion among early investigators with regard to its classification and it is therefore not surprising to find suggestions in the literature that mast cells originate from almost every type of connective tissue element including fixed cells, lymphocytes, plasma cells, adventitial cells and leucocytes (Michels, 1938). Light microscope studies in the rat by Combs et al., (1965) showed that embryonic mast cells arise from primitive, undifferentiated mesenchymal elements; the characteristic granules are formed in mitotically active cells. After a certain level of differentiation is reached, mitotic activity ceases; mast cells then mature by continued elaboration of the granules. Maximow, as early as 1904, suggested that mature mast cells increase in number primarily by mitosis, but Michels (1938) was the first to show a definitive series of mitotic stages in these cells.

From the discovery of the mast cell until about 1937, interest was confined largely to its morphology, distribution and staining properties, matters comprehensively reviewed by Michels (1938). In 1937 Jorpes et al., advanced the hypothesis that mast cells contain the anticoagulant heparin, thereby stimulating research into the functional role of this cell. In more recent years a great impetus to the investigation of the function of this cell was given by the introduction of the hypothesis that it contained histamine, as well as heparin (Riley and West, 1953). The implications of this possibility led to a massive exploration in which practically all the tools of modern cytology have been applied to the determination of the origin, control, chemical composition and function of the mast cell (Selye, 1965).

Although it had been suggested as early as 1942 by Cazal that the mast cell might be the source of tissue histamine, it was not until 1953 that actual evidence for such a possibility was produced. In that year Riley and West (1953) and Riley (1953) found a strong positive correlation between the histamine and mast cell content of various organs of the rat, cat, dog, sheep, pig, cow and ox. They also observed that both the mast cell and histamine levels in tissues from foetal and very young animals were low, whereas both values were very much higher in the adult; a similar correlation between histamine and mast cells was also found in pathological states. These investigators also found that treatment of tissue with histamine liberators, which reduce the numbers of mast cells, also diminishes the histamine content of the tissue.

More direct evidence that mast cells are the principal source of tissue histamine comes from the work of Fawcett (1954a,b) who found that the injection of distilled water into the peritoneum of rats causes the peritoneal mast cells to discharge their granules and increases the histamine levels in the surrounding fluid. This author also found that the intraperitoneal injection of the histamine liberator, compound 48/80, causes disruption of mast cells and the release of histamine into the peritoneal fluid. Administration of compound 48/80 several days after the degranulation of mast cells by distilled water; however, produces little or no further increase in histamine levels. Direct measurements of the histamine content of normal mast cells have been made on purified

cells from peritoneal washings of rats by bioassay (Benditt et al., 1955) and by chromatography (Keller, 1957).

The fact that histamine is located within the granules of mast cells was shown by Mota et al., (1954), who released the amine from a relatively pure fraction of granules by heating them in an acid medium or by the use of compound 48/80. Schayer (1956) found that cell suspensions from rat peritoneal fluid have a marked ability to decarboxylate ^{14}C -L-histidine and to bind the resulting histamine in a stable form. He believed that this activity is due almost entirely to the mast cells; the disruption of these cells resulted in a considerable loss of histamine-forming ability.

The presence of serotonin (5-hydroxytryptamine) in mast cells was first shown by Benditt et al., (1955) who demonstrated by direct analysis that isolated rat peritoneal mast cells contain substantial amounts of this amine; these results were subsequently confirmed by Keller (1957). Parrat and West (1957), in a comparative study of different species, found that serotonin is a common component of rat and mouse mast cells, but that it is not present in significant amounts in guinea pig, dog, man, rabbit, cow, hamster or cat mast cells. By the use of fractionation-separation procedures, serotonin has been shown to reside in the mast cell granules together with histamine and heparin (Hagen et al., 1959). It has been suggested that both histamine and serotonin may be synthesised in the intergranular cytoplasm and then

stored in the granules in an inactive form (Hagen, 1961). Benditt et al., (1955), Riley (1959), Lagunoff and Benditt (1959) and Hagen (1961) have shown that rat and mouse mast cells are capable of producing serotonin by decarboxylation of 5-hydroxytryptophane. It has also been shown that these cells are able to take up serotonin both in vivo and in vitro (Jansson, 1970) and that, if all the granule binding sites are filled, additional serotonin can be found in the cytoplasm (Furano and Green, 1964).

Mast cell granules have also been shown to contain proteolytic enzymes (Darzynkiewicz and Barnard, 1967, Budd et al., 1967) and Uvnas (1963) showed that slow reacting substance (SRS) is released simultaneously with histamine from isolated rat mast cells under the influence of compound 48/80.

Ever since Ehrlich discovered the mast cell, it has been examined in a variety of physiological and pathological states and under experimental conditions with a view to establishing possible functions for it. Selye (1965) discussed more than 30 hypotheses that were then attracting interest. In the discussion of the eosinophil leucocyte it was suggested that this cell could function as an antagonist of histamine and serotonin, which are found in mast cells. Since mast cells, eosinophils and the biogenic amines are so closely related, a brief discussion of the role of these amines in the body follows.

There is not a single human or animal tissue that does not contain histamine; most of this histamine is found in the mast cells located near the small vessels of the microcirculatory network. When injury occurs the loosely bound histamine of the mast cell is released upon degranulation and vasodilatation is provoked (Riley and West, 1966; Mota, 1966). Such a response follows almost instantaneously the vasoconstrictive effect of epinephrine released from the nerve endings at the moment of injury (Gözszy and Kato, 1970). The histamine which is present in other leucocytes is more strongly bound and is liberated later than the histamine from mast cells (Inderbitzin, 1955).

The most characteristic property of mast cells is that they react to stimuli by discharging their granules (Higginbotham, 1966). Most of the granules undergo rapid lysis upon degranulation with consequent liberation of the active components, histamine and serotonin. It was postulated by Uvnäs (1964), Uvnäs and Thon (1966) and Thon and Uvnäs (1967) that histamine release is a two-step process involving the initial energy-requiring extrusion of granules followed by a passive exchange of histamine in the extruded granules for cations in the surrounding medium. Carlsson and Ritzen (1969) however, have presented evidence from microspectrofluorometric and interference microscope studies that serotonin release from mast cells can occur without a concomitant granule release in response to compound 48/80 and to antigen-antibody reactions. On the basis of ultrastructural studies which showed granule alterations

not only at the periphery of mast cells but also deep within the central parts of the cytoplasm, Bloom and Chakravarty (1970) concluded that it was likely that histamine release in response to antigen or to compound 48/80 can take place both intra and extracellularly. Light microscope studies on rat mast cells exposed to toluidine blue (Smith, 1958a,b) or ATP (Diamant and Krüger, 1967, 1968) had previously shown that intracellular morphological changes and histamine release might occur without degranulation. It has been proposed (Krüger et al., 1970) that degranulation induced by antigen and toluidine blue represents a response which occurs when too much of the cell membrane is stimulated, whereas intracellular morphological changes without degranulation is consistent with a more discrete stimulation.

Following injury, the first histological changes are always the same, regardless of the nature of the injury: alterations in the staining properties of the mast cells and lysis of their granules into the tissues (Mota, 1966). Whenever alterations occur in the morphology of mast cells, histamine is liberated and appears at the capillary bed; there is no injury without mast cell disruption and no mast cell disruption without liberation of histamine (Gözy and Kato, 1970). Mast cell disruption and the release of its contents is, then, a rapid response to injury (Benditt, 1954, Riley, 1961), followed within seconds by vasodilatation and increased capillary permeability. This latter

phenomenon is considered as the principal defense feature in the early inflammatory response (Spector, 1958), since it sets the stage for the migration of leucocytes into the injured area. In the skin of rats, the acute inflammatory reaction is clearly mediated by histamine and serotonin (Rowley and Benditt, 1956; Gözsy and Kato, 1966).

It must always be remembered that, although there are several biological, fluorometric and quantitative methods available for the estimation of histamine in tissue, none of them differentiates between the free physiologically active histamine and bound histamine in the mast cell. The quantitative measurement of histamine in a given tissue gives relatively meaningless data because any functional alterations in tissues can be attributed only to free active histamine and no technique is available to provide such information. This point will be made again later in the discussion in reference to the interpretation of data from work on N. brasiliensis.

D. The Basophil

Basophilic leucocyte is the term used to designate a cell, normally occurring in the blood, which has granules staining metachromatically with basic dyes. The cell was first described by Ehrlich (1879); it originates in the bone marrow where it develops from promyelocytes by gradual accumulation of metachromatic granules and segmentation of the nucleus; (Dantchev, 1932). Like neutrophils and eosinophils it is a circulating polymorphonuclear granulocyte normally present in the blood, but in varying frequency depending on the species. It is characterised in the light microscope by prominent metachromatically staining granules which resemble those seen in tissue mast cells. Basophils and mast cells may be readily distinguished on the basis of nuclear morphology and granule ultra-structure; none the less these cells would seem to have important properties in common since the granules of both contain histamine and heparin, (Selye, 1965). In the rat, basophils form approximately 0.5% of the cells present in the blood (Boseila, 1959). Tissue mast cells and blood basophils show a striking similarity in most of their structural and chemical characteristics and they tend to respond to various stimuli in a similar manner, although this is not always the case. In view of these facts Selye (1965) concluded that it seems reasonable to assume that the blood basophil represents the circulating form of the mast cell system

CHAPTER III

THE EXPERIMENTS

A. 1. The experimental animal

White rats of the Wistar strain were used from a colony maintained by the author in this Institute. Rats were from 12-14 weeks old and weighed approximately 300 gms at the start of each experiment. The rats were housed singly in cages with wire bottoms and food and water were available at all times. Only male rats were used in the experimental infections to eliminate any variations due to sex.

2. Maintenance of *Nippostrongylus brasiliensis*

The parasite was obtained from the University of Salford, England, in 1968 and was maintained in female Wistar rats.

The technique used to obtain infective larvae was based on that of Keeling (1960): rats which had received an inoculation of 1000 third stage larvae in the loose skin at the back of the neck were used as a source of material for strain maintenance and for experimental purposes. Faeces were collected from rats carrying a seven day infection of *N. brasiliensis* and thoroughly comminuted with tap water to form an emulsion which was then mixed with an approximately equal quantity of granular animal charcoal. A part of this mixture was spread in the middle of a 7cm diameter No. 1 Whatman filter paper to leave a 1cm clear

margin. The filter paper holding the faeces/charcoal mixture was placed on top of a layer of fully water saturated Micafil in a 9 cm Petri dish. The dishes were covered and incubated in the dark at 27°C for seven days; during this time the parasite eggs in the faeces hatched and the second stage larvae migrated to the edge of the filter paper. The second moult took place in this location and the third stage larvae could be seen at the edge of the filter paper protruding from the larval cuticle cast during this moult.

Larvae were collected from the cultures using the Baermann technique: two pieces of cotton gauze with a layer of absorbent cotton wool placed between them were placed on top of a circle of wire gauze in a 20 cm diameter funnel. A 10 cm length of rubber tubing was attached to the stem of the funnel and closed with a clip. The funnel was then filled with 0.85% saline at 35°C. The edge of the filter paper, with the larvae attached, was cut off and placed in the funnel. Within two hours most of the larvae had passed through the layers of cheesecloth and cotton wool and had accumulated in the tubing at the bottom of the funnel. They were collected in a measuring cylinder by opening the clip.

3. Infection of the host

The saline from the Baermann funnel which contained the infective third stage larvae was shaken to ensure an even distribution and the number of larvae collected was enumerated by dilution counts.

Three 0.1 ml samples were taken in a 1 ml tuberculin syringe and each was diluted to 10 ml with saline. Three 0.1 ml sub-samples were counted from each sample in small Petri dishes using a x20 binocular dissection microscope. If the counts within each group of three sub-samples varied by more than 10%, extra sub-samples were counted until three were obtained which fell within this range. When the average count for a sample varied by more than 10% from the other two, it was discarded and a new sample was diluted.

In a normal count, the average of nine sub-samples, multiplied by 1000, gave the number of larvae in 1 ml of the suspension. The total volume of the suspension was then adjusted to give the required number of larvae per 0.1 ml. The rats were infected by injecting the appropriate number of larvae, in 0.1 ml of saline, subcutaneously at the back of the neck.

4. Egg counts

Faecal egg counts were made using a modification of the McMaster technique (Gordon and Whitlock, 1939). Two grams of faeces from each rat were ground through a 256 mesh wire gauze into 29 ml of water using a pestle and mortar. This suspension was then made up to 60 ml with saturated sucrose solution (453 gms in 350 ml of water) and thoroughly shaken. A small quantity of the suspension was withdrawn

with a wide mouthed Pasteur pipette and run into a two-chambered McMaster slide. The eggs on both sides of the slide were counted at a magnification of 100x. The count multiplied by 100, gave the number of eggs/gm of faeces. Daily egg counts were made from all infected rats from the 5th day after infection until, and including the day of sacrifice.

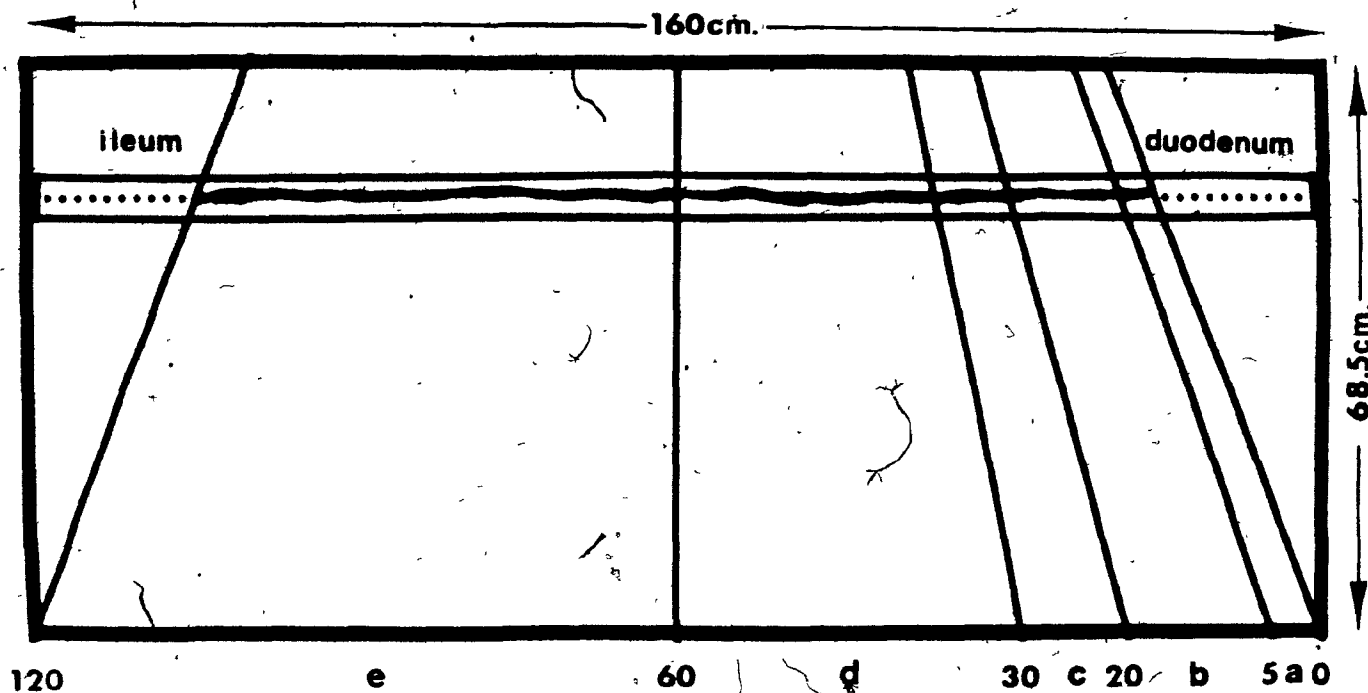
5. Selection of tissue from, and counting of cells in, the small intestine

The intestine from the pylorus to the terminal ileum was removed immediately after death. As intestines from different animals vary in length, equivalent regions were removed using the marking board described by Wells (1962)* shown in Fig. 1, p.52. The intestine was placed on a separate piece of Plexiglass, 160 cm x 2.5 cm, which had closely spaced holes drilled along the middle and pinned through the holes to reduce errors due to contraction. Pieces of small intestine approximately 1cm long were removed from the regions on the board marked 5, 20, 30 and 60, fixed in aqueous Bouins and embedded in paraffin wax. The tissues were sectioned at 7 microns and stained in Giemsa in deionised water. Forty microscope fields (800x) on each of four non-adjacent sections were examined from each region, 20 fields in the region of Brunner's glands and 20 in the lamina propria of the villi to give 160 counts per region and a total of 640 counts per animal. Mast

* The marking board described by Wells (1962) is drawn incorrectly (Wells, personal communication) and should be as shown here in Fig. 1).

Fig.1

**Marking board for removing equivalent regions from
intestines of different lengths.**



**5,20,30 and 60 are tissue selection sites
a,b,c,d and e are regions for worm counts**

cells, globule leucocytes and eosinophils were counted in the same field.

6. Counting of worms in the small intestine

After removal of the four pieces of tissue for histological examination, the five adjacent regions of the small intestine were each placed in separate jars containing warm saline. The intestine was cut down the middle with fine scissors and the contents were scraped into saline in a Petri dish using the back of a scalpel blade. The scraped gut was closely examined for any worms which might have been missed. Worms which had escaped into the saline in the jars were pooled with those obtained in the scrapings and then males and females were counted using a x20 binocular dissection microscope. Any worms which escaped during removal of tissue for histological examination were recorded and added to the worms counted above.

Worms were counted in regions a, b, c, d and e on the marking board Fig. 1, p.52; a is the distance between regions 0 and 5; b between 5 and 20; c between 20 and 30; d between 30 and 60 and e is the distance between 60 and 120. As the lengths of the regions examined for worms varied because of the sampling method and as the sampling method resulted in the intestine being divided into one hundred and twenty

parts and as most workers express results on the basis of the intestine being divided into 100 parts, the counts were converted into the number of worms per 1% of the length of the small intestine.

7. Counting of cells in the peripheral circulation

(a) Eosinophils

Total leucocyte and eosinophil counts were made. Since diurnal variations in cell counts have been reported (Panzenhagen and Speirs, 1953; Halberg et al., 1954), all blood samples were taken at approximately the same time of the day - between 12.30 and 14.30 hours. The rat was immobilised in a plastic restraining cage, the tip of its tail cut off with a sharp scalpel and the first drop of blood wiped off. Subsequent drops were drawn into Exax white blood cell pipettes and diluted 20-fold with Randolph's fluid (Randolph, 1949), details of which are given in the Appendix p. XXX. The pipettes were then shaken well and allowed to stand for at least half an hour before counting. The cells were counted in a Spencer brightline haemocytometer and the number of eosinophils expressed as a percentage of the total leucocyte count.

(b) Basophils

In the experiment in which basophils were counted the procedure was as for eosinophils except that Randolph's fluid was replaced with a toluidine blue stain (Hunt and Hunt, 1959) details of which are given in the Appendix, p. XXI.

8. Counting of cells in the bone marrow

Bone marrow smears were made at the time of sacrifice according to the method of Burke *et al.*, (1955). The musculature was removed from the femur and the bone cut at the junction of the upper third and lower two thirds. A "No. 0" camel hair brush was dipped "ink well" fashion into the exposed marrow cavity and then gently drawn across a clean microscope slide. Brushes were cleaned and dried in alcohol each time before use. The smears were fixed immediately in methanol and subsequently stained in weak Giemsa (20 drops in 100ml of deionised water). 1000 nucleated cells were counted from smears made from each rat and the number of eosinophils and basophils expressed as cells per 1000 nucleated cells.

9. Analysis of Data

Data from the experiments using compound 48/80, reserpine and polymyxin B were not analysed as major differences between treated and control groups were necessary for this line of investigation to be continued and these did not occur. In the main experiments analyses of variance were carried out on faecal egg counts; intestinal worm numbers; worm distribution along the length of the small intestine; intestinal mast cell, globule leucocyte and eosinophil counts and on the eosinophil and basophil counts in the peripheral circulation and bone marrow; means were compared with Duncan's (1955) New Multiple Range Test.

Because of the wide range of the counts, faecal egg counts were transformed, replacing the actual count(x) by $\log x$, and the analysis of variance performed on the transformed data for days 6-12 after infection; before the 6th day no eggs were found and few were present after the 12th day.

Worm counts in the small intestine were transformed into the log of the number and an analysis of variance carried out on the transformed data for days 5, 9 and 12 after infection. On the remaining experimental days few worms were present. After the worm counts in different regions of the intestine had been converted into the number of

worms per 1% of the length of the small intestine the counts(x) were transformed to $\log(x + 1)$, (Snedecor and Cochran, 1967), and an analysis of variance performed on the transformed data for days 5, 9 and 12 after infection to determine the gross distribution of the worms. To do this, the assumption was made that within any given region of the intestine the distribution of the worms was uniform. This assumption is not entirely correct since Alpey (1970) showed that, although there may be more worms present in one region than another, within any given region the worms generally occur in clumps. The transformation, $\log(x + 1)$, was performed in order that the scale be appropriate for the statistical methods applied. The major effect of this transformation was to stabilise the variance which changed directly with the mean in the data being compared.

Mast cell and eosinophil counts in the small intestine were analysed on the basis of the number of cells per region of intestine (the number per 160 fields at 800x); the counts were transformed to the log of the number and the analysis of variance carried out on the transformed counts. Globule leucocyte counts were analysed in the same manner except that, as no globule leucocytes were found in control animals or in infected animals on days 5 and 9 after infection and very few were found in infected animals on the 12th day, the analysis covers only the infected groups on the 16th, 23rd and 30th days after infection.

In the peripheral circulation the total leucocyte count in the

infected animals was the same as in controls and the numbers of eosinophils and basophils are expressed as a percentage of the total leucocyte count. The percentages were transformed by the arcsine/percentage transformation (Snedecor, 1956) and an analysis of variance performed on the transformed data. This transformation weights more heavily the small percentages which have small variance.

Bone marrow eosinophil and basophil data were analysed on the actual counts (the number of eosinophils or basophils per 1000 nucleated cells).

B. Experiments using antihistamine and/or antiserotonin compounds

1. Introduction.

As has already been discussed there is much experimental evidence which suggests that there is some relation between mast cells and eosinophils. The pattern of N. brasiliensis infection in rats has been well established and it has been shown that eosinophil and mast cell changes occur in the small intestine during the course of the infection. Bearing these facts in mind it was thought that the N. brasiliensis/rat system might prove a good system for the further elucidation of the mast cell/eosinophil relationship and of the function of these two cells in parasite infections.

Campbell et al., (1963) using antihistamine or antiserotonin compounds showed that it was possible to prolong the intestinal phase of Trichinella spiralis in mice; Ismail (1966) found that treatment of rats with the antiserotonin compound, BAS, or the antihistamine, promethazine, both resulted in higher levels of muscle parasitism in T. spiralis infected rats and that promethazine treatment also induced a fall in the numbers of eosinophils in the blood, small intestine and bone marrow of infected rats. Working with N. brasiliensis, Urquhart et al., (1965) found that neither the antihistaminic, mepyramine maleate, nor the antiserotonin compound, lysergic acid, had any effect on the self-cure reaction, although subcutaneous injection of the antihistaminic, promethazine hydrochloride,

modified the self-cure reaction in that more worms were found in treated rats than in controls, but no difference in egg counts between the two groups was found. Sharp and Jarrett (1968) showed that treatment of rats with the antiserotonin compound, reserpine, suppressed the self-cure reaction in N. brasiliensis infected rats but at the same time had adverse effect on the rat's health.

Treating N. brasiliensis infected rats with the cortisone derivatives, prednisolone or betamethasone, Ogilvie (1965) found that it was possible to suppress the self-cure reaction and also to suppress all manifestations of acquired resistance. Urquhart et al., (1965) confirmed that betamethasone suppresses self-cure and suggested that because of the speed with which it acted it was more likely that it acted at the cellular level than in suppressing antibody action. Gözay and Kato (1966), studying the inflammatory reaction in rat skin using histamine, serotonin or histamine and serotonin depleting compounds found that both histamine and serotonin were necessary for a maximum inflammatory response.

A series of preliminary experiments were performed using a number of compounds known to antagonise histamine, serotonin or histamine and serotonin to determine what effect, if any, they had on the course of a primary N. brasiliensis infection; faecal egg counts and worm counts on the day of autopsy were used as criteria. If any clear cut effect was observed, the intention was then to study in detail the intestinal mast cell and eosinophil changes using the active compound(s).

Unfortunately, none of the three compounds investigated, compound 48/80, reserpine and polymyxin B had any major effect on the course of the infection. Although by changing treatment levels, times of inoculation or routes of inoculation or by using other compounds it might have been possible to alter the course of the infection, because of the variability of the results obtained and, more especially, because of the severe effects of some of the compounds on the rats, it was decided to discontinue this line of investigation. Details and results of these experiments follow; a detailed examination of the use of such compounds will be found in the main discussion.

2. The experiments

Male rats of the Wistar strain were used; they weighed approximately 200 gms at the time of infection and were obtained from a commercial animal dealer. All animals were given an infection of 1000 *N. brasiliensis* 3rd stage larvae. Faecal egg counts were made daily from the 5th day after infection until, and including, the day of autopsy. On days when drugs were administered this was always done after the counts for that day had been made. All animals were housed singly, unless otherwise stated, in wire bottomed cages. Food and water were available at all times. Maintenance of the parasite and method of infection were as described previously.

(a) Experiment 1: Compound 48/80

Compound 48/80, which results in almost complete mast cell disruption and histamine and serotonin depletion (Görsz and Kato, 1964) was administered intraperitoneally while the animals were under light ether anaesthesia at the dose of 120 µg/0.5 ml saline/100 gm body

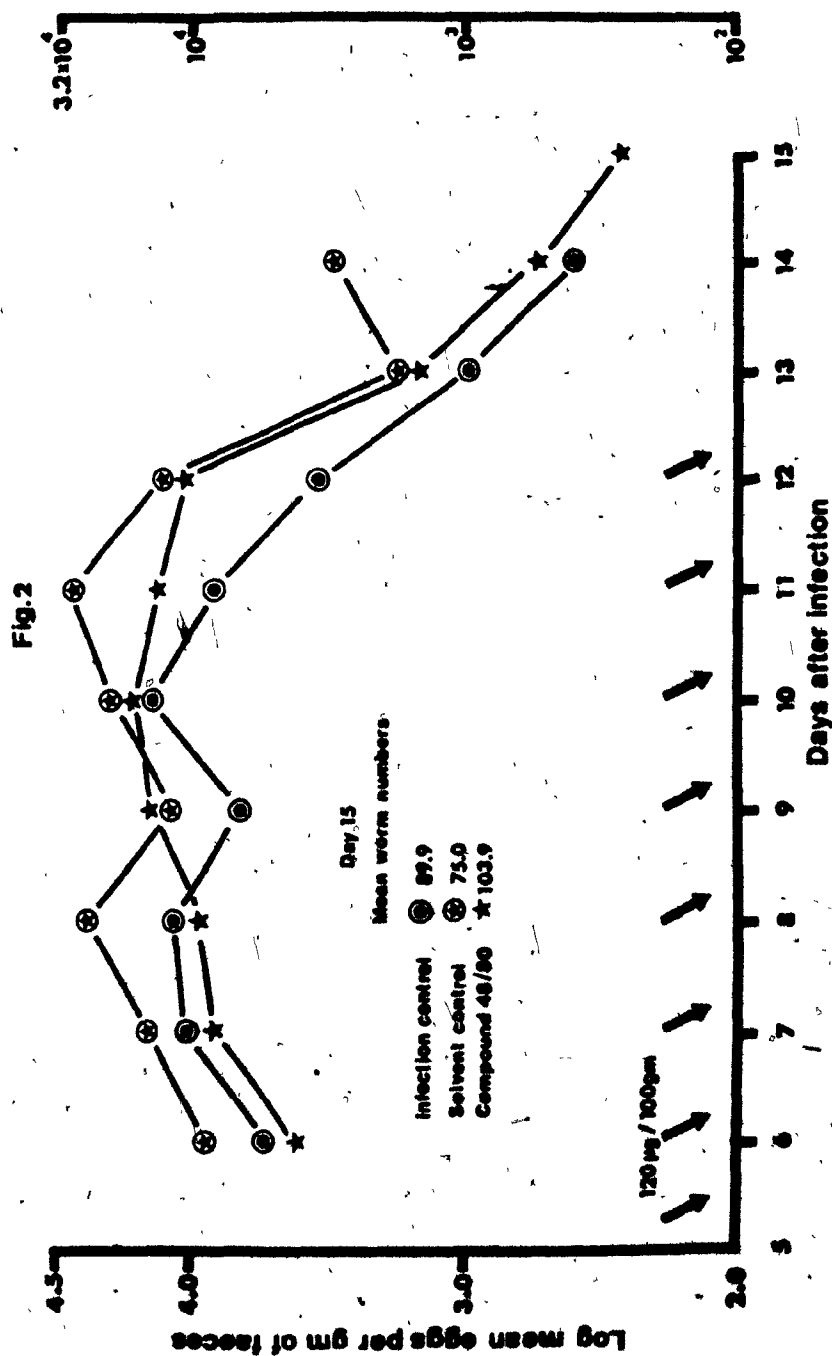
weight on the 5th, 6th, 7th, 8th, 9th, 10th, 11th and 12th days after infection; the animals were sacrificed on the 15th day. Thirty-four rats were used: 10 served as infection controls, 10 as solvent controls and 14 were given compound 48/80. Rats were housed two to a cage. Results are shown in Fig. 2 and the individual counts are given in the Appendix, Table 1.

(b) Experiment 2: Reserpine

Reserpine, which depletes catecholamines and serotonin from mast cells without mast cell disruption (Bhattacharya and Lewis, 1956; West, 1957), was administered intraperitoneally while the animals were under light ether anaesthesia at a dose of 0.05 mg/0.5 ml ethyl acetate/100 gm body weight. Injections were given on the 6th, 7th, 8th and 9th days after infection and discontinued thereafter due to the adverse effects on the rats. The animals were killed on the 15th day. Twenty-four rats were used: 6 served as infection controls, 6 as solvent controls and 9 were given reserpine; the remaining three were lightly anaesthetised on each day of dosing to determine any effect of the anaesthetic. Results are given in Fig. 3, and individual counts in the Appendix, Table 2.

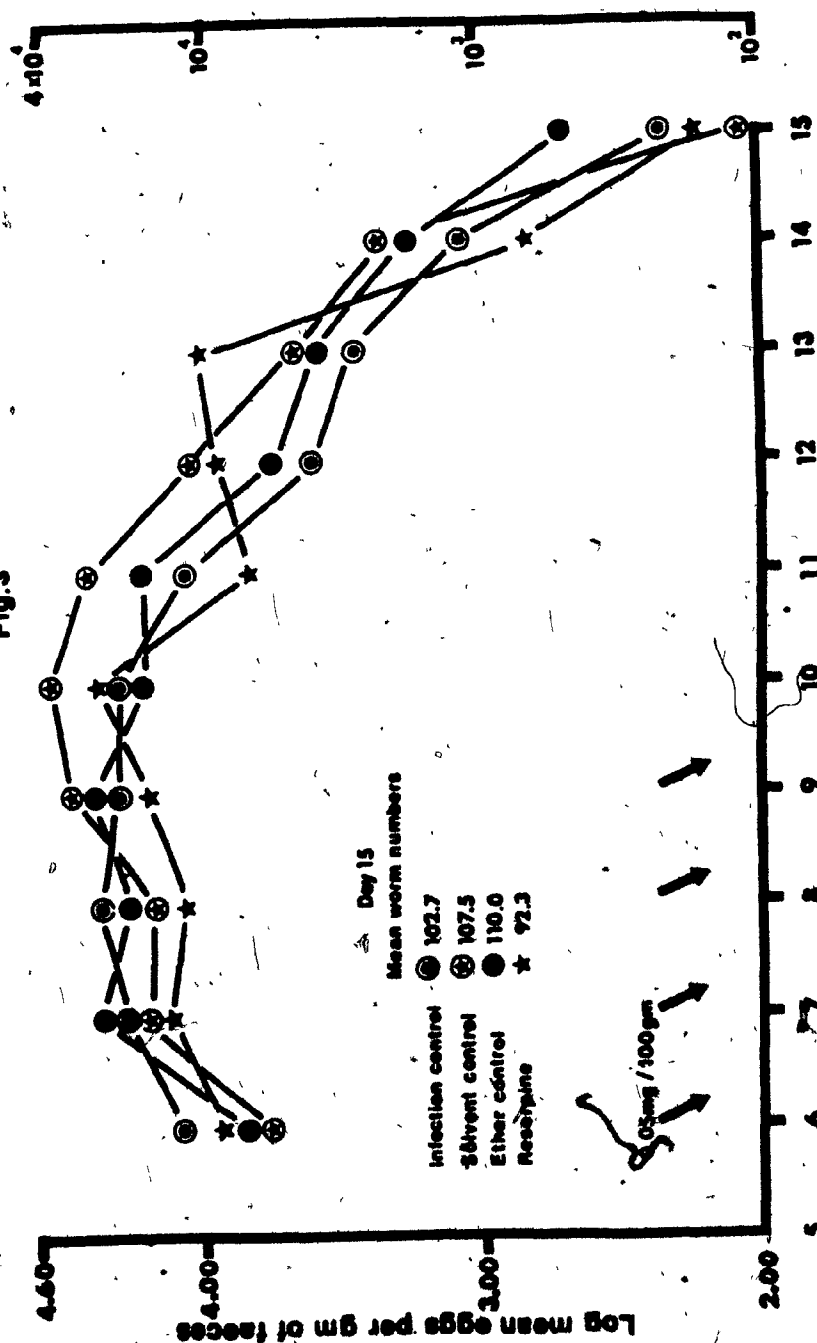
(c) Polymyxin B

Polymyxin B, which causes depletion of histamine, but not of serotonin (Parrat and West, 1956), was administered intramuscularly at a dose of 600 µg/0.5 ml saline/100 gm body weight, on the 6th, 7th, 8th, 9th, 10th and 11th days following infection; the animals were sacrificed on the 12th day. Twenty-seven animals were used: 9 served as infection controls, 9 as solvent controls and 9 were given polymyxin B. Results are given in Fig. 4, and individual counts in the Appendix, Table 3.

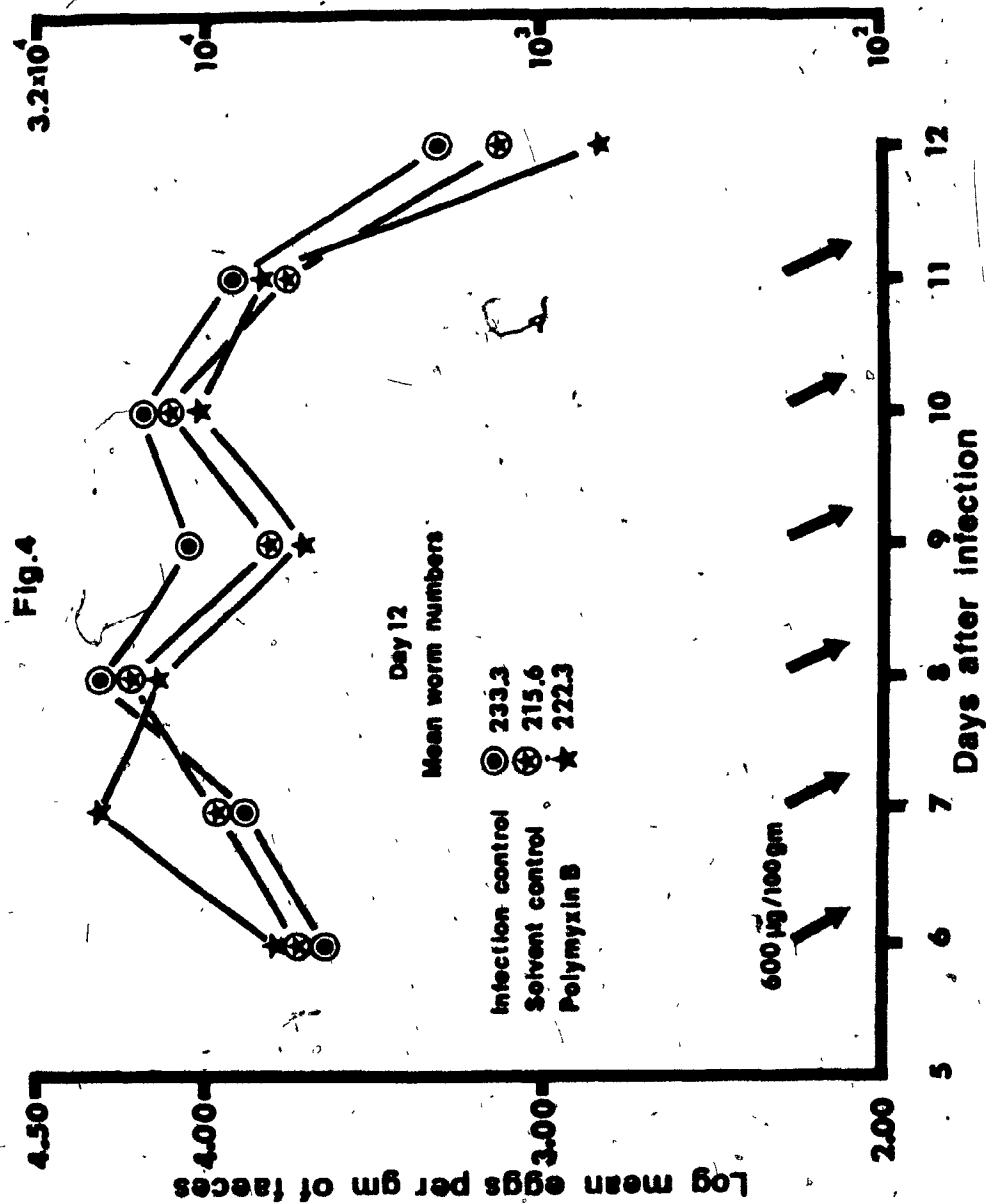


Eggs per gram of faeces after infection with 1000 *M. brailiensis* third stage larvae and treatment with compound 48/80, as indicated by the arrows, and the mean worm recovery on day 15. Due to the limitations of the counting techniques less than 100 eggs per gram of faeces cannot be counted.

Fig. 3



Eggs per gram of faeces after infection with 1000 *N. brasiliensis* third stage larvae and treatment with reserpine, as indicated by the arrows, and the mean worm recovery on day 15. Due to the limitations of the counting techniques less than 100 eggs per gram of faeces cannot be counted.



Eggs per gram of faeces after infection with 1000 *N. brasiliensis* third stage larvae and treatment with polymyxin B, as indicated by the arrows, and the mean worm recovery on day 12. Due to the limitations of the counting technique less than 100 eggs per gram of faeces cannot be counted.

C. CELLULAR RESPONSE DURING NIPFOSTRONGYLOSIS

1. INTRODUCTION

At the time when the decision was made to discontinue the drug study Jarrett et al., (1968a) reported a rise in mast cell numbers in the small intestine of N. brasiliensis infected rats starting just before the commencement of worm loss and reaching a peak at the time when the worms were being lost at the fastest rate; they suggested that this rise in mast cells was involved in the mechanism of the self-cure reaction. These results were contrary to those of Wells (1962) and Rogerson (1967) who found worm loss took place while mast cell levels in the small intestine were below normal and that a rise in numbers occurred only after most of the worms had been lost from the intestine. Obviously, a rise in mast cell numbers could not be involved in the self-cure reaction if it occurred after the loss of worms. In view of these conflicting reports it was decided to investigate the intestinal mast cell changes during primary infections to determine not only when, but also where, this increase occurred and to determine whether any relationship existed between the numbers of worms present and the cellular changes. As Wells (1962) and Rogerson (1967) had reported different eosinophil changes in the small intestine during the course of infection the eosinophil response was also investigated. At this time no studies had been made of the bone marrow and circulating eosinophil responses during infection and it was decided to investigate these to determine if the eosinophilia in the small intestine (Wells, 1962; Rogerson, 1967) was merely a local event caused by influx of cells from the surrounding

tissues or if it was a generalised event stemming from the bone marrow. It became evident, while counting bone marrow eosinophils that there was also a basophil response in the bone marrow during infection; these were also counted in view of their close similarity to mast cells (Selye, 1965), and a separate experiment performed to determine the basophil changes in the peripheral circulation during primary infections.

The cell changes were studied at two levels of infection and the course of infection was followed by daily faecal egg counts and by worm counts at specified times during the experimental period; the gross distribution of worms in the small intestine was measured to determine if there were significant differences between levels of infection. In order to maintain a certain conformity in techniques these, where possible, were those used by Wells (1962) and Rogerson (1967). The same regions of the intestine investigated by these workers were taken using the marking board shown in Fig. 1; these are from regions marked 5, 20 and 30 corresponding, respectively, to areas where few worms are ever found, the beginning of significant worm distribution and the site of maximum worm concentration. In addition, a fourth piece of tissue was taken at the point marked 60 which is at the posterior limit of the range of worm distribution. The experimental days were the same as those of these authors, animals being killed on the 5th, 9th, 12th, 16th and 23rd days after infection; an additional day, the 30th, was added to determine if, by that time, cell numbers were returning to normal. Cells in the intestine were counted using the same technique described by them and intestinal tissue was fixed in Bouin's solution which both workers had found adequate for the preservation of intestinal mast cells.

2. PLAN OF INVESTIGATION

Eighteen rats were divided into three groups of six and caged individually; rats in two of the groups received a subcutaneous injection of either 1000 or 5000 third stage N. brasiliensis larvae in 0.1 ml of saline; rats in the third group served as controls and received 0.1 ml of saline. One rat from each group was sacrificed on days 5, 9, 12, 16, 23 and 30 after infection.

Circulating eosinophil and total leucocyte counts were made on the day of infection (day 0), before the rats were infected, and on all rats on the day of sacrifice. After killing, pieces of tissue from the intestine were placed in Bouin's fixative for histological examination; the remaining regions of the small intestine were placed in jars of warm saline for worm counts. The femur was then exposed and smears made and fixed in methanol for bone marrow counts. Faecal egg counts were made daily from all infected rats starting on the 5th day after infection.

The experiment was replicated six times to give a total of 108 rats, 36 per treatment. Because of the staggered nature of the experiment the results are based on different numbers of animals. Bone marrow counts, intestinal cell counts and worm numbers are from six rats per treatment on each sampling day. Cell counts in the peripheral circulation are from 36 rats per treatment on days 0 and 5; from 30 on day 9; 24 on day 12; 18 on day 16; 12 on day 23 and from 6 rats in each group on day 30. Faecal egg counts are from 36 rats per infection level on day 5; from 30 rats on days 6 - 9; from 24 on days 10 - 12; 18 on days 13 - 16; 12 on

days 17 - 23 and from 6 rats per infection level on days 24 - 30.

A separate experiment was carried out to determine circulating basophil levels. Eighteen rats were divided into three groups of 6 animals, caged individually and infected as above. Counts were made from all rats on the same sampling days as above. As the course of infection had by now been firmly established, no faecal egg counts or worm counts were made in this experiment. After the cell counts on day 30 all animals were killed. This gave six counts per treatment per sampling day.

3. RESULTS

a) Worm numbers and distribution in the small intestine.

Worm counts per region of the intestine and analyses of variance for worm counts and worm distribution are shown in the Appendix, Tables 4, 5, 20 and 21.

Worm numbers in the small intestine following infection with 1000 larvae were constant on days 5, 9 and 12; there was an increase in the number of worms from days 5 - 9 and a decrease between days 9 - 12 after infection with 5000 larvae (Fig. 5); the appearance of worms in the faeces of infected rats indicated that this decrease began on the 10th day. Although there was no statistically significant loss of worms between days 9 and 12 in the rats infected with 1000 *H. brasiliensis* a few worms were found in the faeces on days 10 and 11 and, on day 12, many worms had left the surface of the mucosa and were free in the lumen of the gut. By the 16th day, most of the worms had left the intestine and on the 23rd and 30th days few parasites were present, most of those remaining being males (Figs. 6 and 7).

At the 1000 level of infection, there was no difference in the distribution of worms along the length of the small intestine on days 5, 9 and 12. (Fig. 8). Most of them occupied region c and the first half of region d; there was no difference in the number of worms

per unit length of intestine between these two regions. Few worms were found in regions a and b which are closest to the stomach or in region e, which consists of the entire second half of the small intestine. By the 16th day, however, when most of the worms had left the intestine, a change in the distribution of the parasites had occurred: most of those present inhabited regions a and b. Apparently, while most of the worms leave the small intestine by the 16th day a small number migrate toward the stomach to inhabit areas not normally populated by the parasite. Coinciding with the loss of worms, there is a change in the sex ratio of the worms present. On days 5, 9 and 12 there are relatively more females than males, but, after worm loss has begun, most of the worms present are males.

The distribution of worms in the 5000 infection is different on each of days 5, 9 and 12 because the maximum worm population is not reached until after the 5th day and because by the 12th day worm loss has started. Assuming that the population on day 9 is the more stable of the three populations, the worms are more widely distributed at this time than after infection with 1000 larvae. The majority of them live in regions c and d, there being no difference in the number of worms per unit length between the two regions. Whereas in the 1000 infection most of the worms occur in region c and the first half of region d, in the 5000 infection they occupy almost all of region d and also spread up the intestine to inhabit region b where there are, however, fewer worms per unit length than in regions c and d. As in the 1000 infection few worms live in

regions a and e. By the 12th day after infection approximately one third of the worm population on day 9 has been lost although without any change in the sex ratio. The movement of worms toward the stomach to inhabit region a, seen on day 16 in the 1000 infection, occurs by the 12th day in the 5000 infection. After the 12th day, the pattern of infection is the same as that found in the 1000 infection: most of the worms have left the small intestine by the 16th day and the majority of the parasites remaining are males occupying regions a and b; on days 23 and 30 few, if any, worms are present.

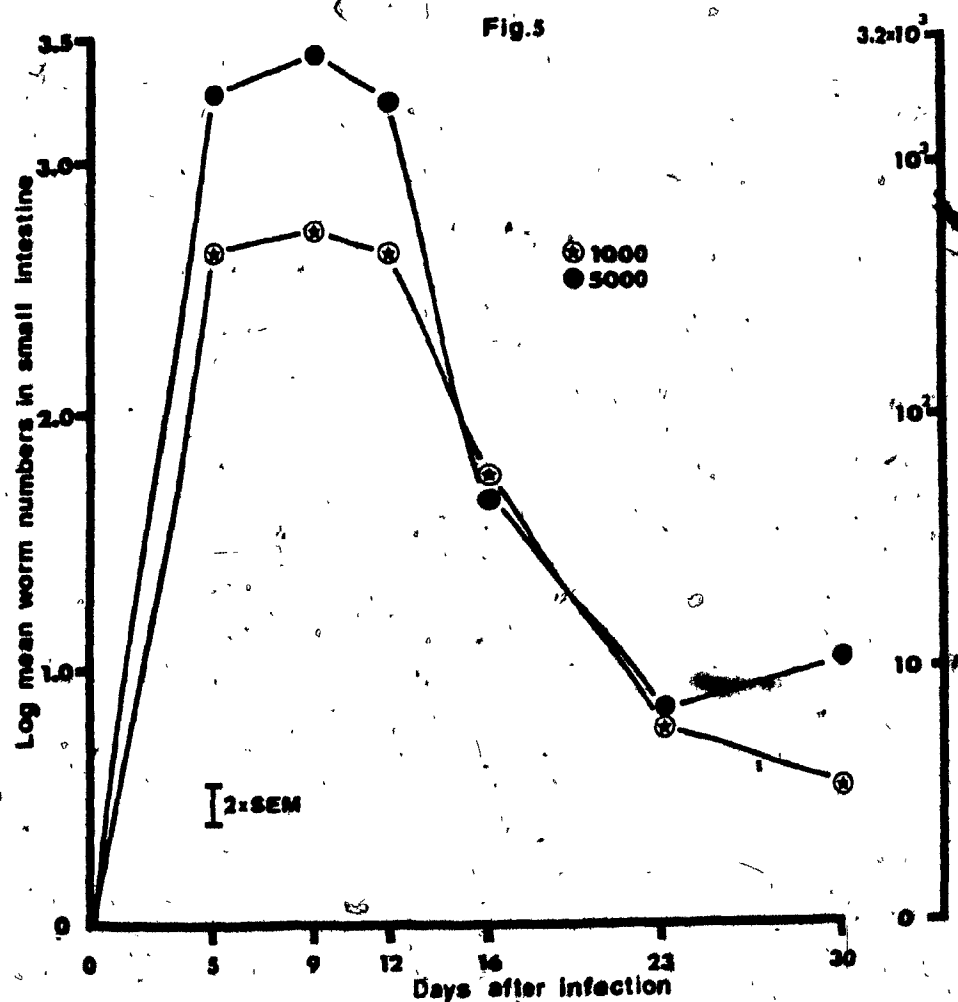
Brambell (1965) and Jarrett *et al.*, (1968b) found that most of the larvae which will reach the small intestine do so by the 4th or 5th day of infection as was the case here after inoculation with 1000 larvae. In the 5000 infection it appears that some larvae take longer to reach the intestine, perhaps delayed by a stronger host reaction in the lungs, since the peak worm burden is not reached until after the 5th day.

The total number of worms reaching maturity in the intestine was approximately the same proportion of the infecting dose at both levels of infection: 58.26% and 60.0% at the 1000 and 5000 levels respectively on the 9th day (these are, however, not absolute values since the worms in the pieces of intestine taken for histological examination were not counted). This observation is in agreement with the work of Jarrett *et al.*, (1968b) and Haley (1961) who found that the number of worms reaching

maturity is a relatively fixed proportion of the infecting dose. The movement of a small number of worms anteriorly to region a, once worm loss has started, agrees with the results of others (eg. Brambell, 1965; Alpey, 1970). Alpey (1970) suggested that this migration might be due to the possibility that the intestinal wall in this region presented a more favourable site at the time of worm loss. As will be discussed later, this suggestion is attractive, since the inflammatory reaction in this part of the intestine is not as pronounced as in regions of high worm density.

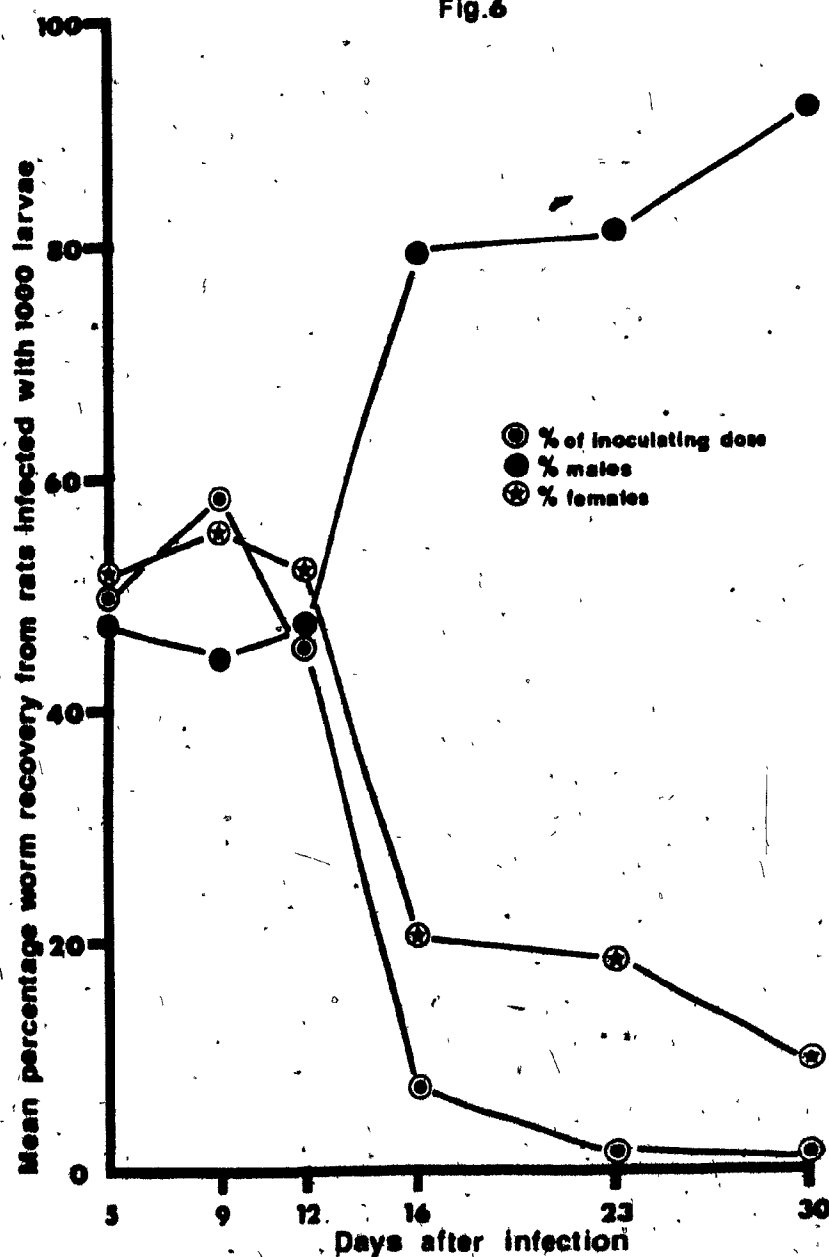
The change in the ratio of male to female worms from a slight preponderance of females in the early stages of the infection to a preponderance of males once most of the worms have been lost is in agreement with the findings of Jarrett et al., (1968b). These authors suggested that the 40 worms which remained in the intestine at the end of their experiments might be a threshold population below which the effect of immune expulsion cannot be manifest. This suggestion is not supported by the results obtained in these experiments. The numbers of worms present on the 30th day varied considerably from rat to rat: 0, 0, 3, 6, 8 and 9 worms were present in the six animals given 1000 larvae and 0, 4, 17, 19, 42 and 46 in the six given 5000. The mean number of worms recovered, expressed as a percentage of the infective dose was, however the same at both levels: 0.43% and 0.42% at the 1000 and 5000 levels of infection respectively. The number of worms in the residual population may depend on the strain, age and sex of the rat and/or the strain of the parasite.

and/or the size of the infecting dose; it is also possible that this population is entirely independent of any of these factors. As might be expected in any large population, a few worms could be genetically better able to withstand the changes which occur in the intestine at the time of self-cure and, instead of leaving the intestinal habitat move towards the stomach where conditions are more favourable to their existence. There is also the possibility that the worms found in region a in the late stages of the infection are not worms which have migrated from the main worm population but are worms which have just entered the small intestine. In these experiments about 40% of the infecting dose did not reach the intestine. Jarrett et al., (1968b) have suggested that most of these larvae are lost either at the site of infection or on their journey to the lungs; Weinstein (1955) suggested that these larvae might be swept into some other organ, perhaps the liver. As the ultimate fate of these larvae is not known, it is possible that some of them may not be killed and eventually reach the intestine to occupy sites in region a as it is in a more favourable condition for their existence than those regions formerly colonized by the previous population.

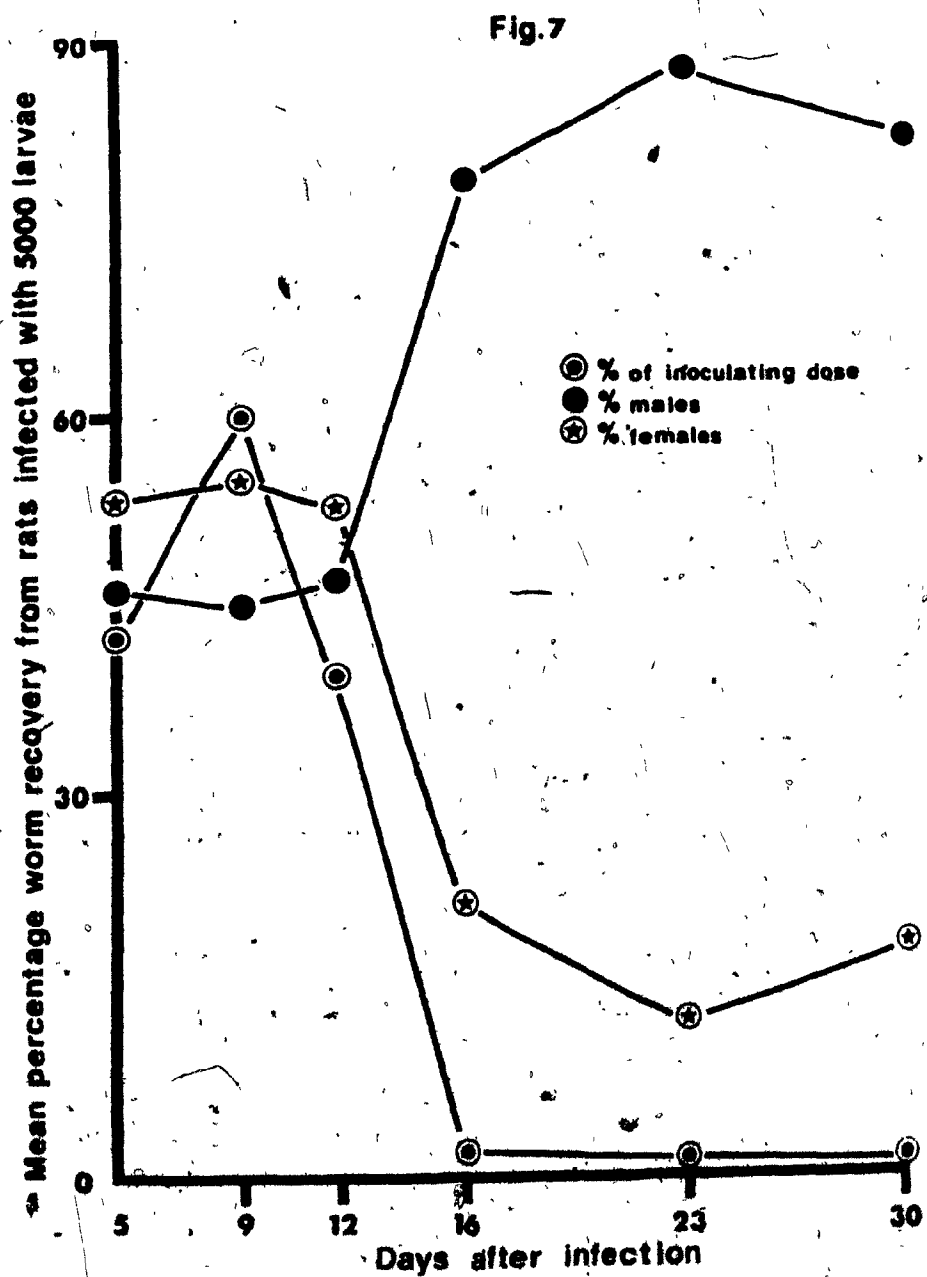


Intestinal worm recovery after infection with 1000 or 5000 *N. brasiliensis* third stage larvae. Standard error of the mean (SEM) for six rats per treatment per experimental day.

Fig. 6

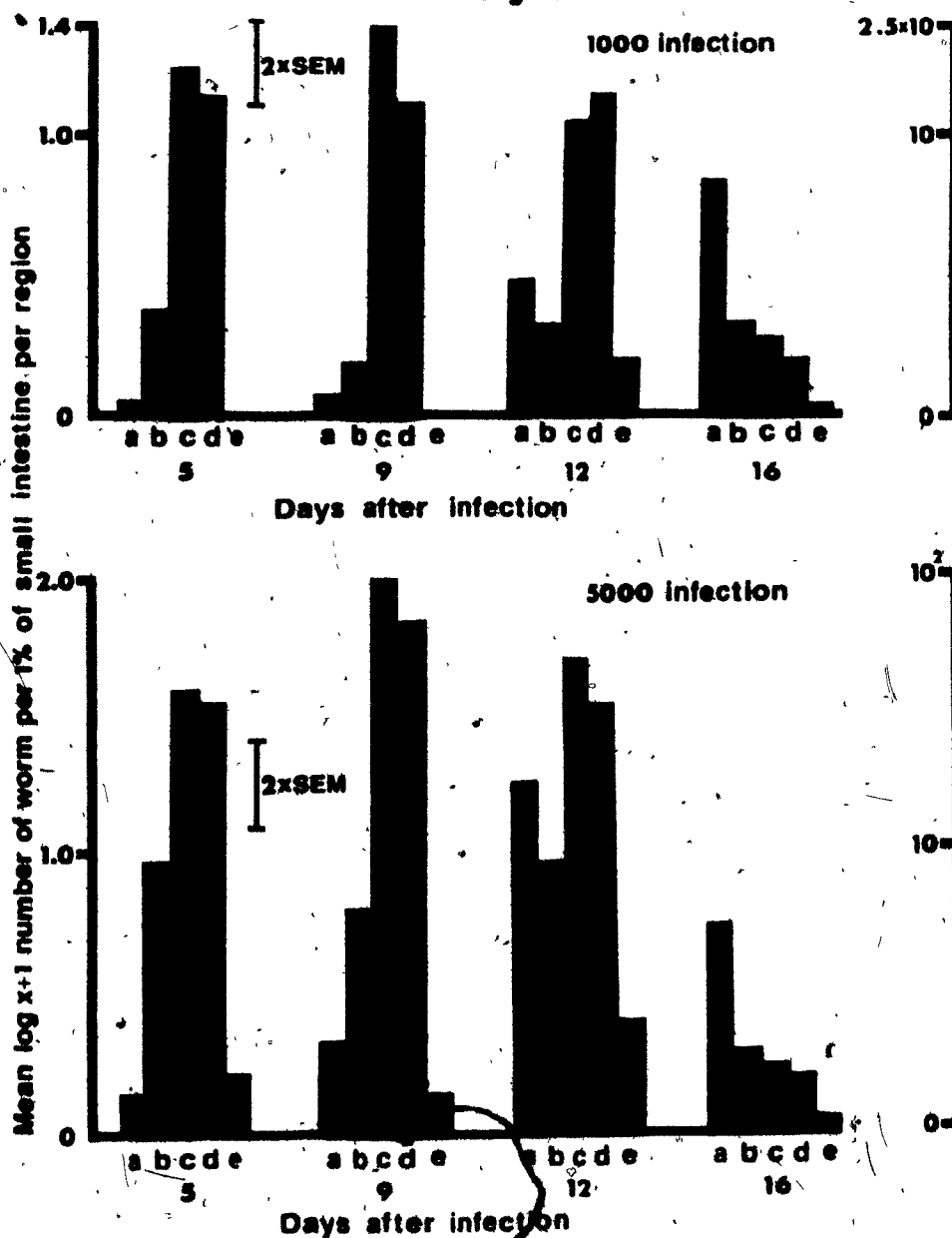


Worm recovery, after infection with 1000 *N. brasiliensis* third stage larvae, expressed as a percentage of the inoculating dose and the percentage, of the population remaining, by sex.



Worm recovery, after infection with 5000 *N. brasiliensis* third stage larvae, expressed as a percentage of the inoculating dose and the percentage, of the population remaining, by sex.

Fig. 8

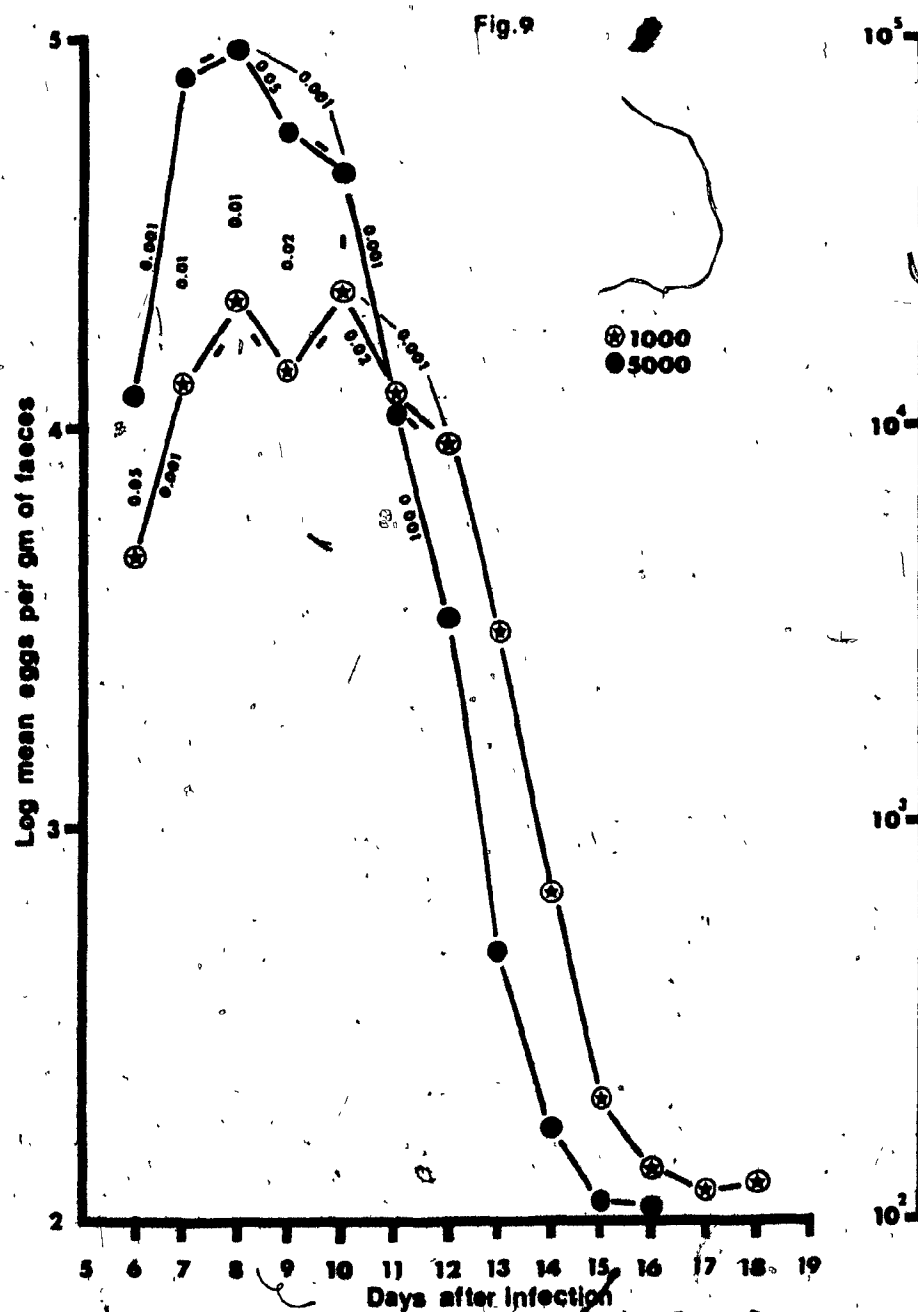


Distribution of worms along the length of the small intestine after infection with 1000 or 5000 *N. brasiliensis* third stage larvae. Standard error of the mean (SEM) for six rats per treatment for days 5, 9 and 12.

b) Egg Production

Egg counts and an analysis of variance are shown in the Appendix, Tables 6, 7 and 22.

Eggs were found in the faeces for the first time on the 6th day at both levels of infection (Fig. 9). Maximum egg production was reached on day 7 at both levels of infection and was maintained until the 10th day in the 1000 infection and until the 8th day in the 5000 infection; it fell slowly for the next two days and rapidly thereafter. No eggs were found in the faeces after the 18th day in rats given 1000 larvae and after the 16th day in those given 5000 larvae. The pattern of egg production shown by the 1000 infection is identical to that reported by Jarrett *et al.*, (1966). The egg production following infection with 5000 larvae differs in that maximum egg production is maintained for only two days and in that the decline in egg production starts two days earlier. At both levels of infection, a decline in egg production occurred before worm loss began. Egg production started to drop between days 10-11 in the 1000 infection and between days 8-9 in the 5000 infection while worm loss occurred between days 12-16 at the lower level of infection and started between days 10-12 at the higher level of infection. Maximum egg production was five times higher at the 5000 level than at the 1000 level but only lasted two days compared to four days at the 1000 level.



Faecal egg counts after infection with 1000 or 5000 *N. brasiliensis* third stage larvae. Statistical analysis performed on data for days 6-12 inclusive. Counts are from 30 rats per infection level for days 6-9 and from 24 for days 10-12. Due to the limitations of the counting technique less than 100 eggs per gram of faeces cannot be counted.

c) Mast cells and globule leucocytes in the small intestine

Mast cell counts per region of intestine (160 fields at 800x) and an analysis of variance are shown in the Appendix, Tables 8, 9, 10 and 23. Globule leucocyte counts per region of intestine (160 fields at 800x) and an analysis of variance are shown in the Appendix, Tables 11 and 24.

The regions of the small intestine where cell counts were made are shown in Fig. 1. Briefly these regions are:

Region 5: proximal to the stomach (where worms do not normally live)

20: one-sixth of the length down the intestine (at the beginning of the range of worm distribution)

30: one-quarter of the length down the intestine (region of greatest worm density)

60: half way down the small intestine (at the terminal end of the range of worm distribution.)

As the mast cell changes in the four regions were basically similar (Fig. 11), the description below is of the mean changes for the four regions together (Fig. 10). The infections are characterised by a decrease in the number of mast cells in the wall of the small intestine,

apparent five days after infection with both 1000 or 5000 larvae.

By the 9th day a further drop in numbers occurred at the higher level of infection: there were fewer mast cells present at this time in the intestine of rats given 5000 larvae than in the rats given 1000 larvae; in the latter group, numbers remained at the level found on the 5th day. A new population of mast cells appeared by the 12th day of infection in the rats given 1000 larvae, the numbers rising to those in the uninfected controls where they remained until the 16th day when few worms were present in the intestine; a few globule leucocytes were present at this time (Figs. 12 and 13.). Mast cell numbers following infection with 1000 larvae were above control levels on the 23rd and 30th days after infection; large numbers of globule leucocytes were present at these times. At the higher level of infection, approximately 30% of the worm burden found on the 9th day had been lost by the 12th day but mast cell numbers remained at the level found on the 9th day; only a few globule leucocytes were present on day 12. By the 16th day a new population of mast cells, which first appeared on the 12th day in the rats infected with 1000 larvae, had appeared and numbers rose above control levels and remained there until the termination of the experiment on the 30th day; large numbers of globule leucocytes were present in the rats infected with 5000 larvae from the 16th day onwards.

The important points which this study has clarified concerning changes in mast cell numbers in the wall of the small intestine during experimental nipprostrongylosis in rats are, as follows:

- 1) throughout the time of worm loss, between days 12 - 16 after inoculation with 1000 larvae, mast cell numbers are at normal levels in regions 5, 20 and 60 and below normal in region 30, which is that of the greatest worm density; mast cell numbers, however, rise above normal levels on days 23 and 30 when worm loss has finished.
- 2) At the 5000 level of infection, where worm loss starts on the 10th day, mast cell numbers are below normal levels in all four regions on day 12 when a third of the worm burden has already been lost from the small intestine; the decrease in numbers, as in the 1000 infection is most marked in region 30, where worm density is greatest; numbers are above control levels from the 16th day onwards.
- 3) Globule leucocytes were found for the first time at the 1000 level of infection on the 16th day, when worm loss was almost complete (six globule leucocytes were found in the 3840 microscope fields examined); large numbers were found on the 23rd and 30th days.
- 4) At the 5000 level, three globule leucocytes were found on the 12th day (in 3840 microscope fields), when a third of the worms had already been lost; large numbers were found from the 16th day onwards.

The changes in mast cell numbers, then, are basically similar at both levels of infection throughout the experimental period in the four regions examined. They are below, or at, control levels when worm loss starts and rise above normal after most of the worms have left the small intestine; the decrease in numbers is more pronounced in the 5000 infection and the changes in numbers at both levels of infection are most marked in region 30, where worms are present in the highest density.

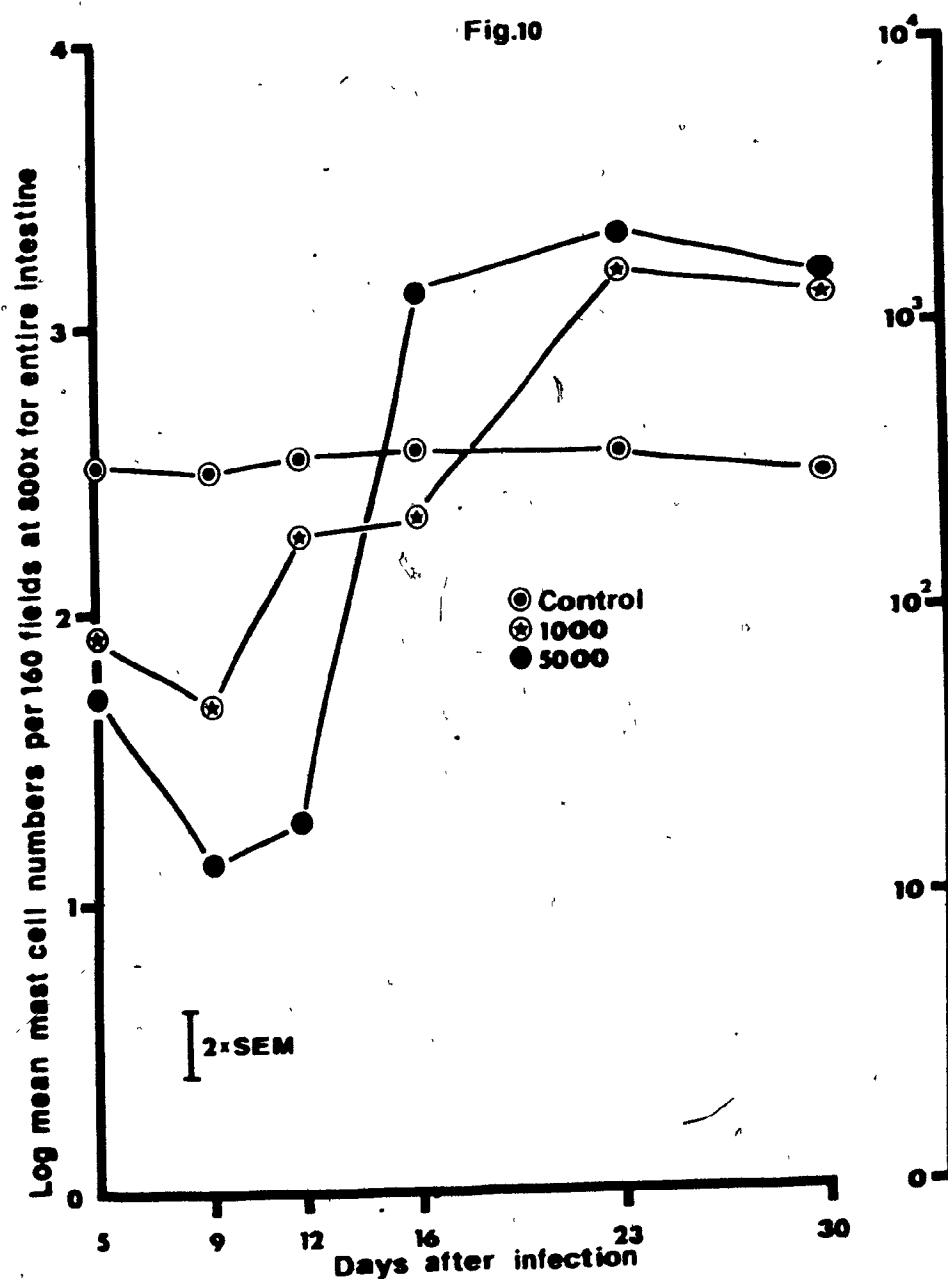
The fluctuations in mast cell numbers in the wall of the small intestine obtained in this study are in agreement with the results of other workers (Wells, 1962; Rogerson, 1967; Jarrett et al., 1968a; Miller, 1970; Miller and Jarrett, 1971; Murray et al., 1971a,b; Keller, 1971), in that, at the peak of infection, there is a considerable decrease in the numbers of these cells; this decrease is especially pronounced in the areas of the intestine where the adult worms are present in the highest concentration. In the present work, however, worm loss occurred while mast cell numbers were below or at, normal levels and only rose above normal levels after many of the worms had been lost, in agreement with the work of Wells (1962), Rogerson (1967) and Keller (1971) and contrary to the results of Jarrett et al., (1968a); Miller (1971); Miller and Jarrett (1971); Murray et al., (1971a,b). These latter investigators reported a rise in mast cell numbers shortly before the commencement of worm loss; this rise occurred slightly earlier in female than in male rats, the females losing their worm burdens slightly faster than males.

Globule leucocytes appeared in the present work on the 12th day in the heavier infection, after the start of worm loss; large numbers of these cells were found from the 16th day when few worms were present in the intestine. In the lower level of infection a few globule leucocytes were found on day 16 and large numbers were not found until the 23rd day. The appearance of globule leucocytes well after worm loss has begun has also been reported by Keller (1971), contrary to the observations of Miller and Jarrett (1971) who found that the mast cell to globule leucocyte conversion started just before the start of worm loss and reached a maximum at a time when the worms were being lost at the fastest rate. This finding led these authors to propose that the conversion of mast cells to globule leucocytes is an integral part of the mechanism which is responsible for the worms leaving the small intestine. If, in fact, these two events are an integral part of the self-cure reaction, one would expect self-cure and conversion to occur at the same time in all strains of rats since, providing that the rats are mature and the infective dose of larvae is more than 500, the timing of worm loss occurs within a day or two in all strains. From the results already discussed, it is obvious that the timing of the increase in mast cell numbers and the mast cell to globule leucocyte conversion are both variable, sometimes beginning before the onset of worm loss and sometimes after. Wells (1962), using three month old male rats infected with 5000 larvae, found that mast cell numbers were above normal 23 days after infection while worm loss began after 9 - 12 days. Female Wistar rats

6 - 7 weeks old when infected with 5000 N. brasiliensis larvae had increased numbers of mast cells after 16 days while worm loss began between the 9th and 12th days (Rogerson, 1967). Using Osborne-Mendel rats approximately 200gm in weight infected with 4000 larvae, Keller (1971) found that worm loss began 7 - 9 days after infection, but that the number of mast cells did not increase until the 12th day. Mast cell numbers were above normal in the present work 16 days after infection with 5000 larvae and 23 days after infection with 1000 larvae; the mast cell globule leucocyte conversion started 12 - 16 days after the heavier infection and 16 - 23 days after the lighter infection; worm loss started after 10 and 12 days following the 5000 and 1000 infections respectively. Whur (1966), counting only globule leucocytes, found that they first appeared 12 days after 170gm hooded Lister female rats were infected with 3,500 larvae, while worm loss began between days 8 - 10.

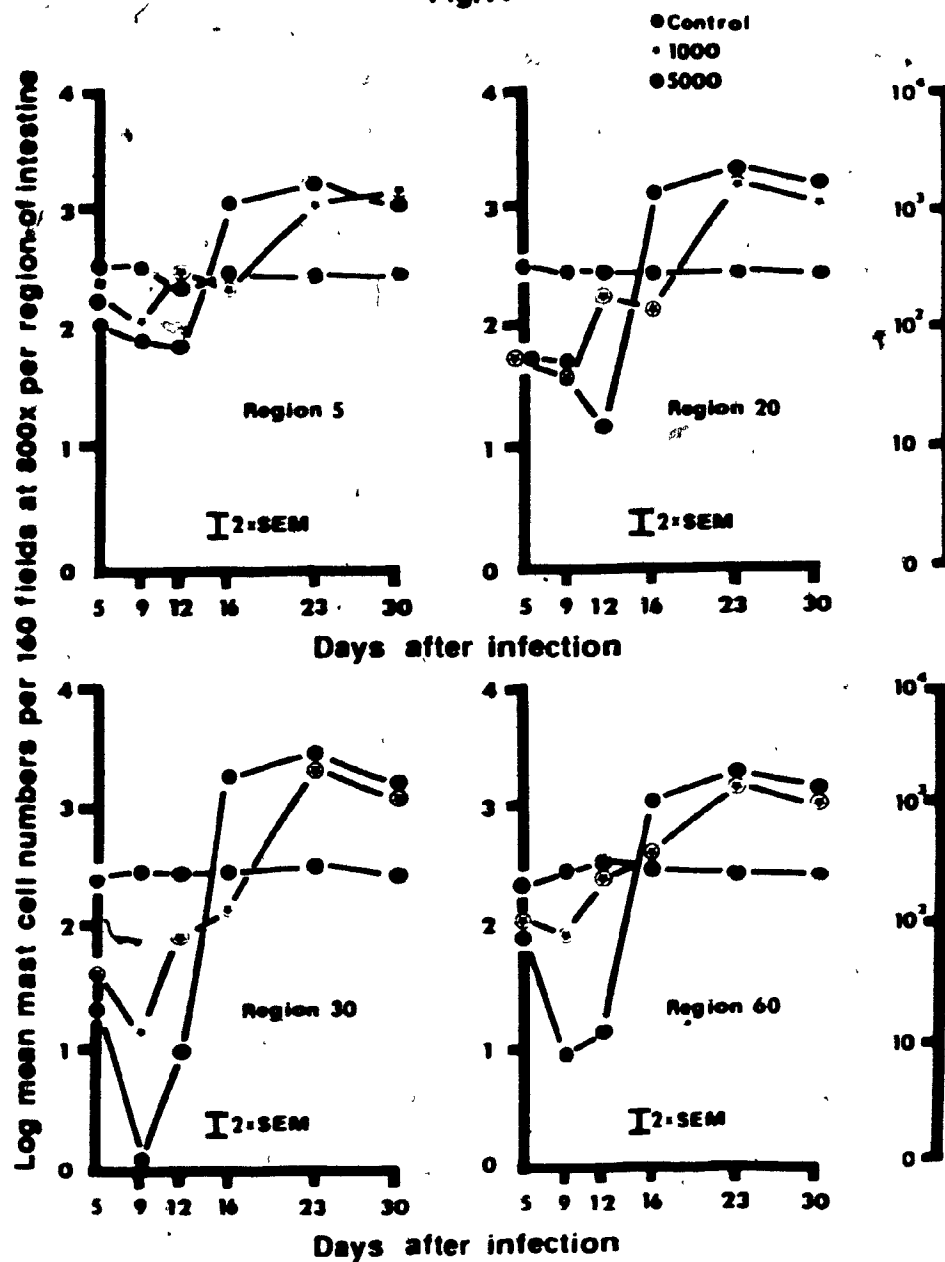
On the other hand, Miller and Jarrett (1971), using female hooded Lister rats weighing 170 - 220gm and infected with 3000 N. brasiliensis larvae, found that worm loss started by the 12th day and that globule leucocytes first appeared on the 10th day. Murray et al., (1971a,b) found that the drop in egg production occurred earlier in 150gm female hooded Lister rats infected with 3000 larvae and that the exponential worm expulsion started earlier and occurred at a faster rate in females than in males. They also found that intestinal mast cells, which disappeared completely from the intestine at the site of maximum worm concentration, reappeared after the 11th day in females and a day after in males.

From the results of workers using different strains of rats, different infective doses and both sexes, it is apparent that the time of the onset of worm loss in a primary N. brasiliensis infection in rats is constant within a few days. At the same time, it is also apparent that there is a wide variation in the time when globule leucocytes first appear and in the time when mast cells increase in number. It is unlikely that events which vary so greatly in time play an important role in the mechanism responsible for worm loss; as in many cases the rise in numbers of mast cells occurs well after worm loss, it is possible that this event has no part in the phenomenon of self-cure.



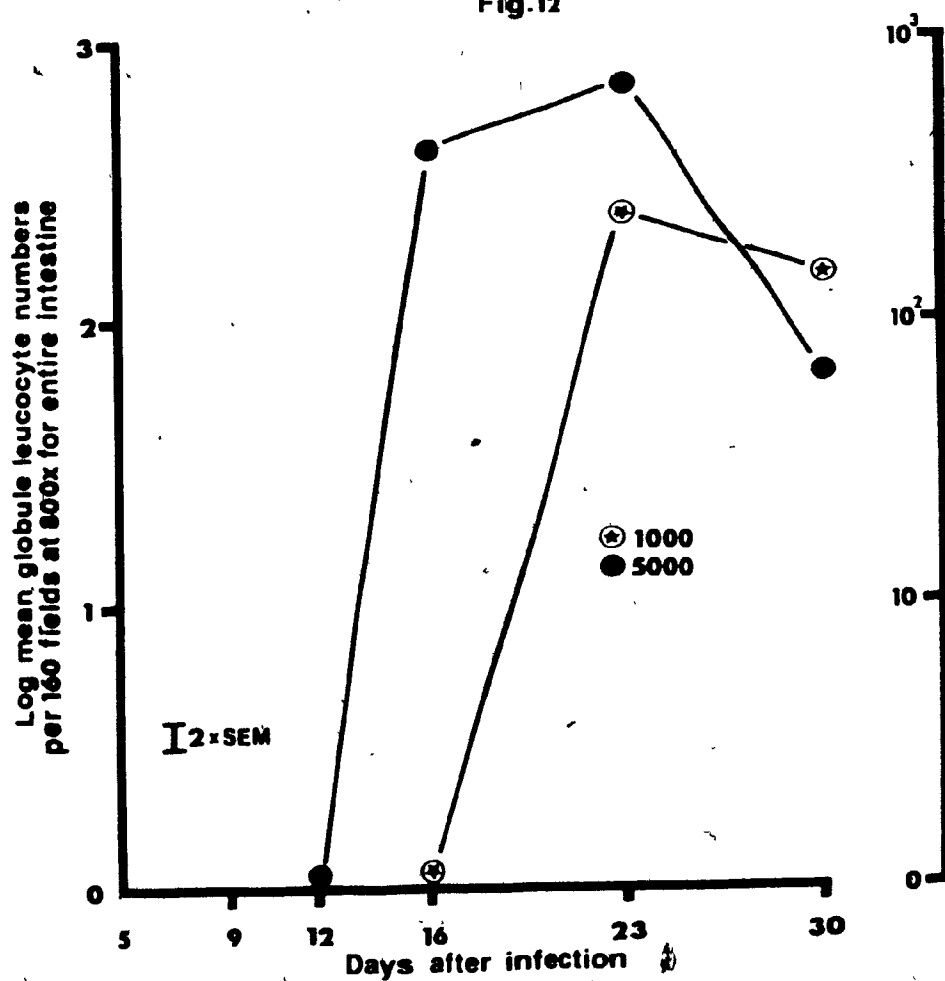
Mast cell response in the small intestine after infection with 1000 or 5000 *N. brasiliensis* third stage larvae. Standard error of the mean (SEM) for six rats per treatment per experimental day.

Fig. 11



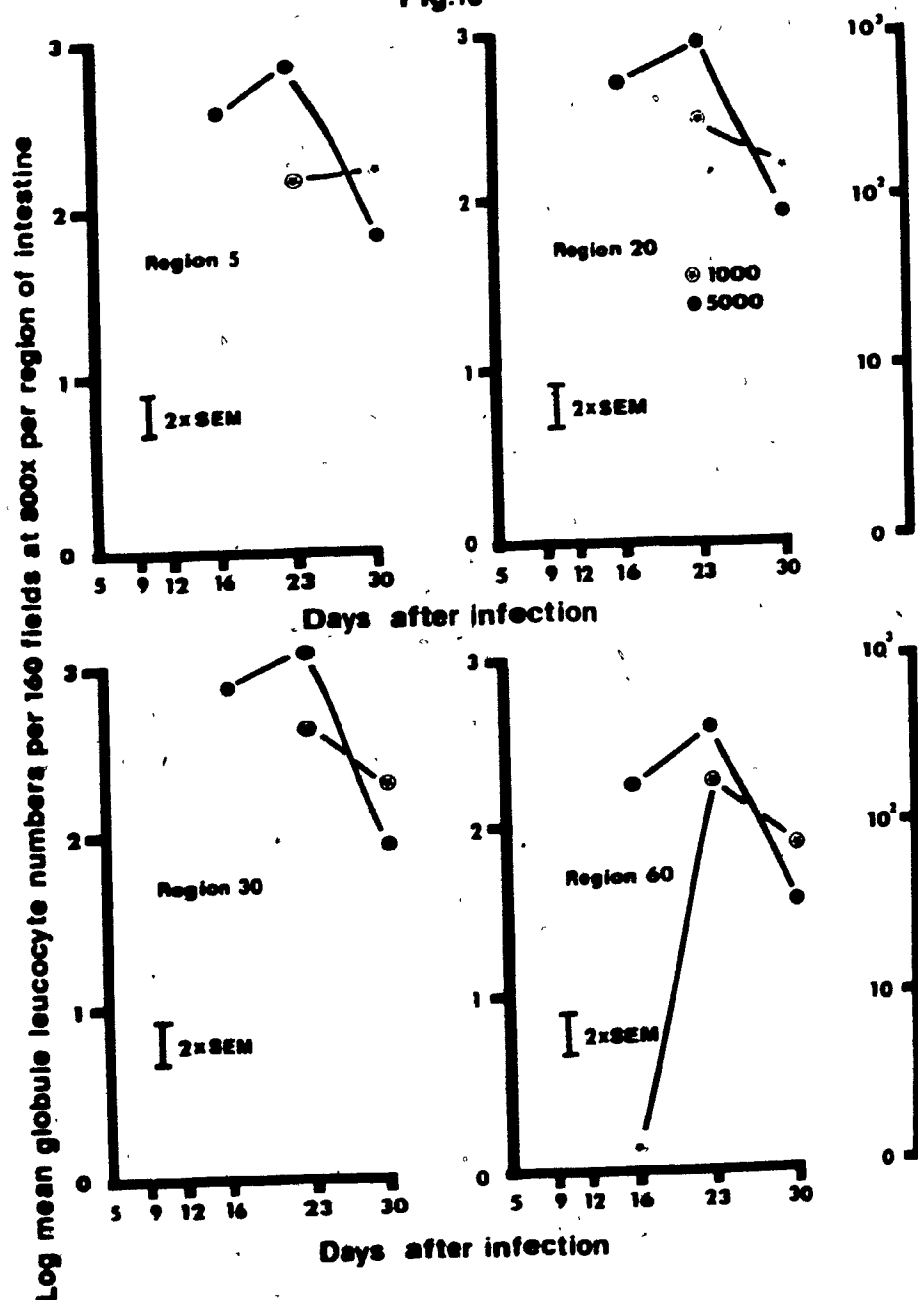
Mast cell response for regions 5, 20, 30 and 60 of the small intestine after infection with 1000 or 5000 *N. brasiliensis* third stage larvae. Standard error of the mean (SEM) for 6 rats per treatment per experimental day.

Fig. 12



Globule leucocyte response in the small intestine after infection with 1000 or 5000 *N. brasiliensis* third stage larvae. Standard error of the mean (SEM) for 6 rats per treatment per experimental day.

Fig.13



Globule leucocyte response for regions 5, 20, 30 and 60 of the small intestine after infection with 1000 or 5000 *H. brasiliensis* third stage larvae. Standard error of the mean (SEM) for 6 rats per treatment per experimental day. On day 12, at the higher level of infection one globule leucocyte was found in each of regions 5, 20 and 60.

d) Eosinophils in the small intestine

Eosinophils per region of intestine (160 fields at 800x) and an analysis of variance are shown in the Appendix, Tables 12, 13, 14 and 25.

Changes in eosinophil numbers at both levels of infection followed basically the same pattern in the four regions of the intestine examined; these changes were most pronounced in region 30 where the worms were present in the greatest density and where the mast cell response was most pronounced. The following is a description of the mean changes for the four regions together (Fig. 14). The changes in the individual regions are shown in Fig. 15. At both levels of infection eosinophil numbers were above control levels throughout the experimental period; there was no difference in the numbers present between the two levels of infection on any given experimental day.

Rogerson (1967), working with young female rats infected with 500 N. brasiliensis larvae, found raised eosinophil numbers on the 5th, 16th and 23rd days after infection; in that study numbers were at normal levels on days 9 and 12. The results for the 1000 infection in the present work are similar since there is a drop in numbers in all four regions between the 5th and 9th days, although numbers remain well above control levels. At the 5000 level of infection there is a fall in numbers to normal levels,

in only region 30. Wells (1962), using adult male rats infected with 5000 N. brasiliensis larvae, found increased eosinophil levels on the 12th, 16th and 23rd days after infection with no change in numbers on days 5 and 9; Kelly and Ogilvie (1972), working with adult female rats infected with 3000 N. brasiliensis, found a depression in eosinophil numbers on the 6th, 9th and 11th days after infection, with numbers rising above control levels after the 13th day.

From the results of Wells (1962), Rogerson (1967) and Kelly and Ogilvie (1972), it is clear that eosinophil numbers in the wall of the small intestine are raised in the later stages of a primary infection when worm loss has started. In the earlier stages of the infection, when worms are present in the intestine, there is some conflict in the results of these authors. From the results reported here, it is apparent that there are differences in the eosinophil response not only at different levels of infection but also between the different regions of the gut which were studied. Although numbers remain above control levels, between days 5 - 9 there is a drop in eosinophil numbers in all four regions at the lower level of infection while at the higher level of infection there is a drop to normal levels in region 30, the site of maximum worm concentration. Kelly and Ogilvie (1972) examined only one region of the small intestine, the site of maximum worm concentration and found no change in eosinophil numbers three days after infection with 3000 larvae and below normal levels on days 6, 9 and 11.

Rogerson (1967), and in the work reported here, found raised eosinophil levels 5 days after infection and Wells (1962) reported an apparent, but not a statistically significant rise in eosinophil numbers on the 5th and 9th days after a primary infection with 5000 larvae. This latter author used only two rats per treatment per day and an apparent drop in mast cell numbers at these times was also not statistically significant.

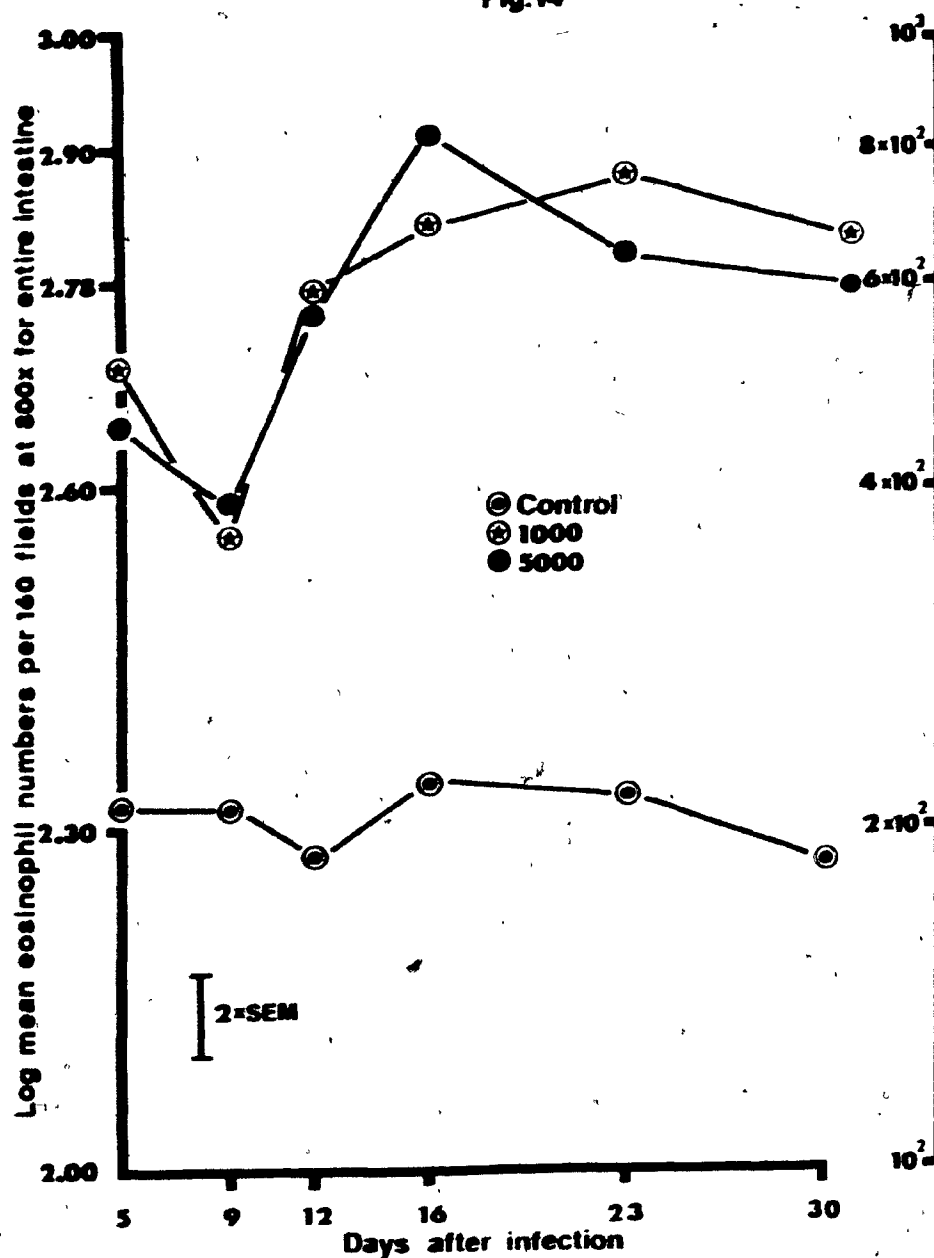
In response to a wide range of stimuli mast cells release the vasoactive amines serotonin and histamine which are mediators of the inflammatory response (Gözy and Kato, 1970). The inflammatory reaction, although part of the host's defence mechanism, is in itself a pathological process (Gözy and Kato, 1970) and, consequently, there must be a means of limiting its effects. It is suggested here that the eosinophil is at least partly responsible for limiting the effects of the inflammatory reaction in the wall of the small intestine of rats infected with N. brasiliensis by antagonising the effects of histamine and/or serotonin. It is suggested furthermore that the release of histamine and/or serotonin from degranulated mast cells during the early stages of N. brasiliensis infection stimulates the bone marrow to increase its production of eosinophils. These cells enter the tissue of the small intestine via the blood stream to antagonize the effects of the two amines; this migration accounts for the increase in eosinophil numbers found in the present work at both levels of infection on the 5th day after infection.

The drop in eosinophil numbers in the intestine between days 5 - 9 at the 1000 level of infection could be explained on the grounds that the eosinophils already present are capable of successfully dealing with the amines that have been released at this time. Consequently there is no migration of eosinophils from the blood stream to the intestine; consequently with the natural loss of eosinophils, their number in the intestine falls. At the higher level of infection, however, more worms are present on day 9 than on day 5 and, consequently, mast cell numbers are considerably lower on day 9 than on day 5; because of this increased mast cell degranulation eosinophils are stimulated to enter the intestine from the blood stream, so that with the exception of region 30, numbers do not decline. It is possible that the normal eosinophil levels in region 30 are due to the fact that mast cells are not abundant in this region because of degranulation earlier in the infection. The consequent lack of amine release here means that there is no stimulation for eosinophils to enter this part of the intestine and, as before, with the natural loss of eosinophils from the intestine, numbers return to normal levels. By the 12th day of the infection eosinophil numbers have risen to their normal high levels at both levels of infection; this increase may be due to amine release from the new population of mast cells which has appeared in the animals infected with 1000 larvae and, at the higher level of infection, to a further release of amines from the few remaining mast cells.

On day 16 there has been a further rise in eosinophil numbers

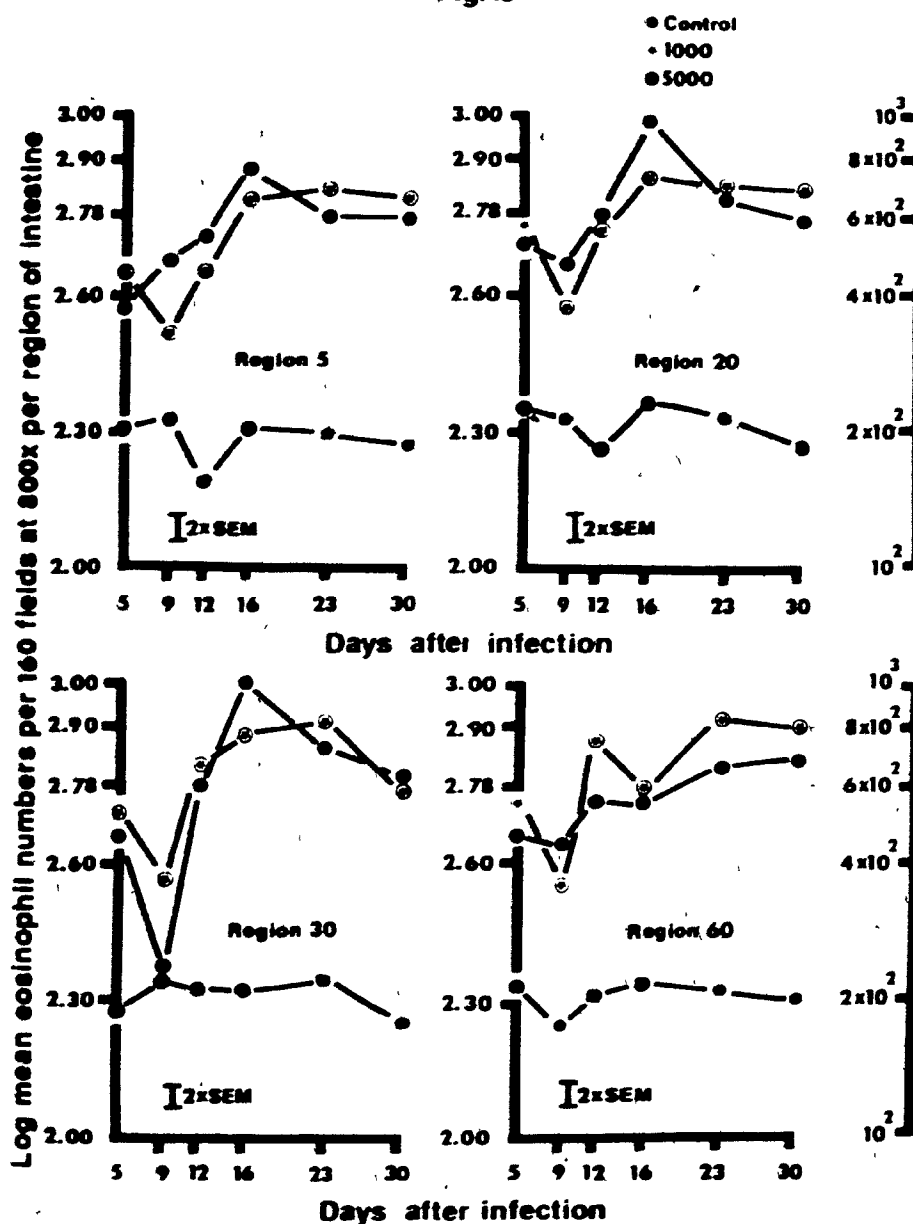
in animals infected with 5000 larvae; it is suggested that this is a response to amine release from the new population of mast cells which is now present in the intestine of these rats. No increase in eosinophil numbers occurs after day 16 at the higher level of infection, nor after day 12 at the lower level of infection. It is suggested that an equilibrium is reached where the eosinophils that are present are capable of dealing with any amines released; the only stimulus for eosinophils to enter the small intestine is probably to replace those eosinophils lost naturally.

Fig. 14



Eosinophil response in the small intestine following infection with 1000 or 5000 *H. brasiliensis* third stage larvae. Standard error of the mean (SEM) for six rats per treatment per experimental day.

Fig. 15



Eosinophil response for regions 5, 20, 30 and 60 of the small intestine after infection with 1000 or 5000 *N. brasiliensis* third stage larvae. Standard error of the mean (SEM) for six rats per treatment per experimental day.

e) Eosinophils in the peripheral circulation and bone marrow

Eosinophil counts in the peripheral circulation, expressed as a percentage of the total leucocyte count, and analyses of variance are shown in the Appendix, Tables 15, 16, 17 and 26. Eosinophil counts in the bone marrow (cells/1000 nucleated cells) and an analysis of variance are shown in the Appendix, Tables 19 and 28.

Eosinophil levels were above normal at both levels of infection throughout the experimental period in both the blood and the bone marrow (Figs. 16 and 17). In the blood the peak response was on day 9 at both levels of infection. The peak was maintained until the 12th day in rats given 5000 larvae and fell thereafter until the 23rd day to a level maintained until day 30. At the lower level of infection numbers fell between days 9 - 12 to the level reached on day 23 at the higher level of infection. More eosinophils were present at the higher than the lower level of infection on days 9, 12 and 16.

In the bone marrow, whether the infection was with 1000 or 5000 larvae, eosinophils rose from levels above normal on day 5 to reach a maximum on day 9; this level was maintained until the 16th day in the rats infected with 5000 larvae. At the lower level of infection medullary eosinophils fell between days 9 - 16 to levels also reached on the 23rd day by animals infected with 5000 larvae; the numbers of eosinophils then

remained constant in both groups until the 30th day. On only two days, the 12th and 16th, were there more eosinophils present in the bone marrow at the higher than at the lower level of infection.

The bone marrow is the production centre for eosinophils from where they travel via the blood stream to the tissue where they are required.

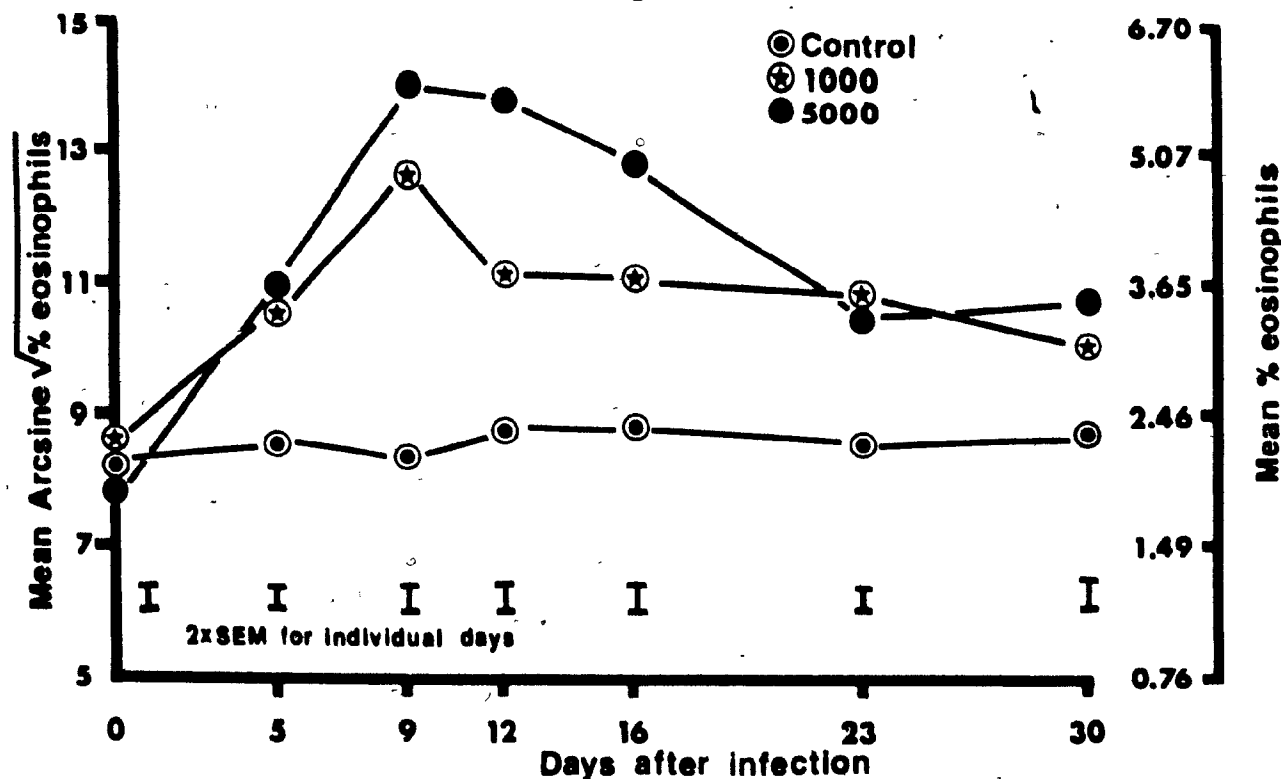
Since reactions involving eosinophils are occurring in other parts of the body of rats infected with N. brasiliensis it is difficult to correlate changes in the blood and bone marrow with those occurring in the small intestine. Wells (1971) found alterations in mast cell numbers in the lungs due to the passage of larvae; these changes were similar to those caused by the adult worms in the small intestine: she noted a moderate eosinophilia while larvae were present in the lungs and a marked eosinophilia after the larvae had left there. Many larvae are lost during migration to the lungs (Jarrett et al., (1968b); Love et al., (1974) and it is quite likely that changes in eosinophil levels also occur in the tissues where these larvae are trapped in their migration, perhaps in the liver (Weinstein, 1955).

In the present study eosinophil levels were found to be above normal in the bone marrow and in the peripheral circulation throughout the experimental period, reaching a peak on day 9; at this time there is eosinophilia in the small intestine at both levels of infection and there.

is a marked eosinophilia in the lungs (Wells, 1971). The decline in eosinophils in the bone marrow and blood after day 9 at the lower level of infection is probably due to a combination of a reduced demand for these cells by both the lungs and the intestine. Peak production is maintained until the 16th day in the bone marrow of rats infected with 5000 larvae probably because the greater number of larvae migrating through the tissues and the larger number of worms in the small intestine cause considerably more physical damage and mast cell degranulation than at the lower level of infection and thus there is a greater demand for the anti-biogenic amine action of eosinophils.

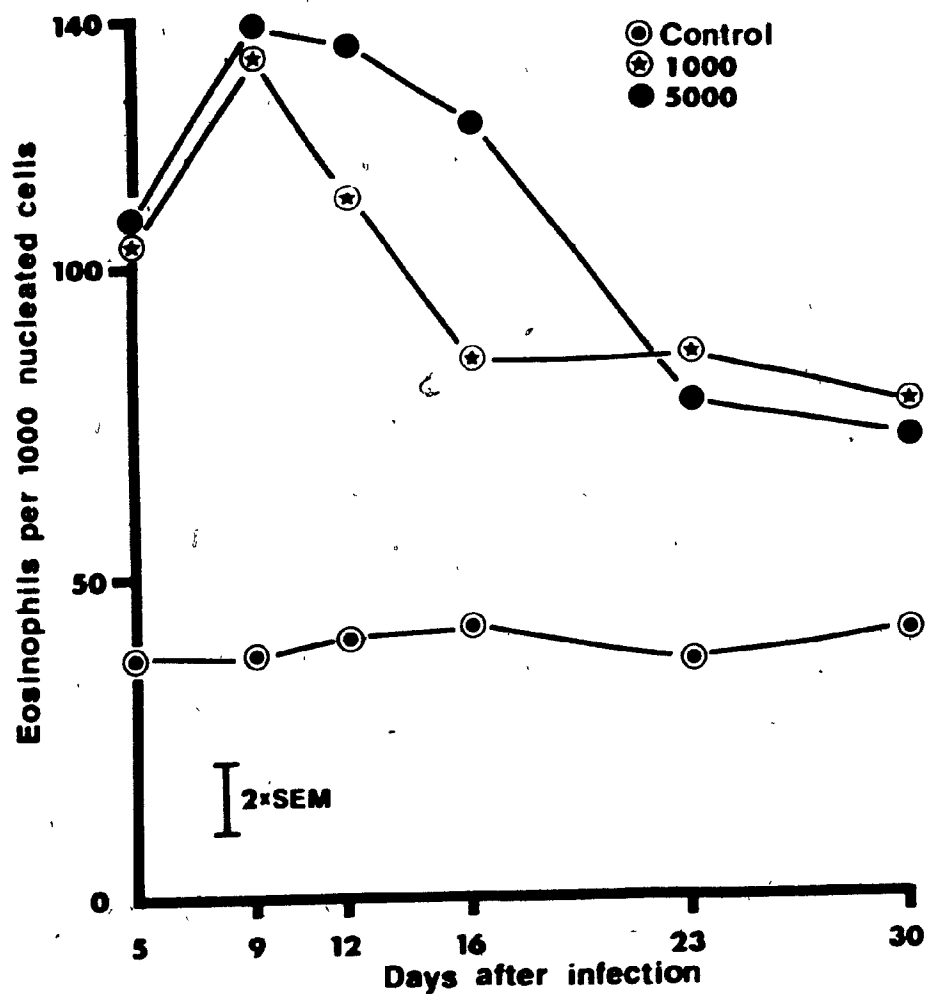
In the only other report dealing with eosinophils in the blood, Hogarth-Scott and Bingley (1971) found eosinophilia from the 13th day of the infection in 200gm female rats infected with 1400 N. brasiliensis larvae but no changes in eosinophil numbers before this time. These authors report only few eggs passed by their infected animals and it is, therefore, likely that their infections were low-grade which might account for their finding no eosinophil changes in the early stages of the infection.

Fig.16



Eosinophil response, expressed as a percentage of the total leucocyte count, in the peripheral circulation after infection with 1000 or 5000 *N. brasiliensis* third stage larvae. Standard error of the mean (SEM) for 36 rats per treatment on days 0 and 5; for 30 on day 9; for 24 on day 12; for 18 on day 16; for 12 on day 23 and for 6 rats per treatment on day 30.

Fig.17



Eosinophil response in the bone marrow after infection with 1000 or 5000 *N. brasiliensis* third stage larvae. Standard error of the mean for six rats per treatment per experimental day.

f) Basophils in the peripheral circulation and bone marrow

Basophil counts in the peripheral circulation, expressed as a percentage of the total leucocyte count, and an analysis of variance are shown in the Appendix, Tables 18 and 27. Basophil counts in the bone marrow (cells/1000 nucleated cells) and an analysis of variance are shown in the Appendix, Tables 19 and 29.

In the peripheral circulation basophil levels at both levels of infection are above normal from the 9th day and no difference exists on any given day between the two levels of infection (Fig. 18). At both infection levels basophil numbers reach a peak on the 12th day, increasing in number between days 5 - 9 and again between days 9 - 12. Between days 12 - 16, at both levels of infection, there is a drop in numbers followed by an increase between days 16 - 23 to the level found on the 9th day; this level is maintained until the 30th day.

Basophil numbers in the bone marrow of rats infected with 1000 or 5000 *N. brasiliensis* larvae were at control levels 5 days after infection (Fig. 19); numbers then rose at both levels of infection to reach a peak on day 9 which was maintained until the 12th day. Between days 12 - 16 there was a decrease in numbers at both levels of infection with numbers returning to control levels at the higher level of infection. After the 16th day numbers rose to the levels of the 9th and 12th days at

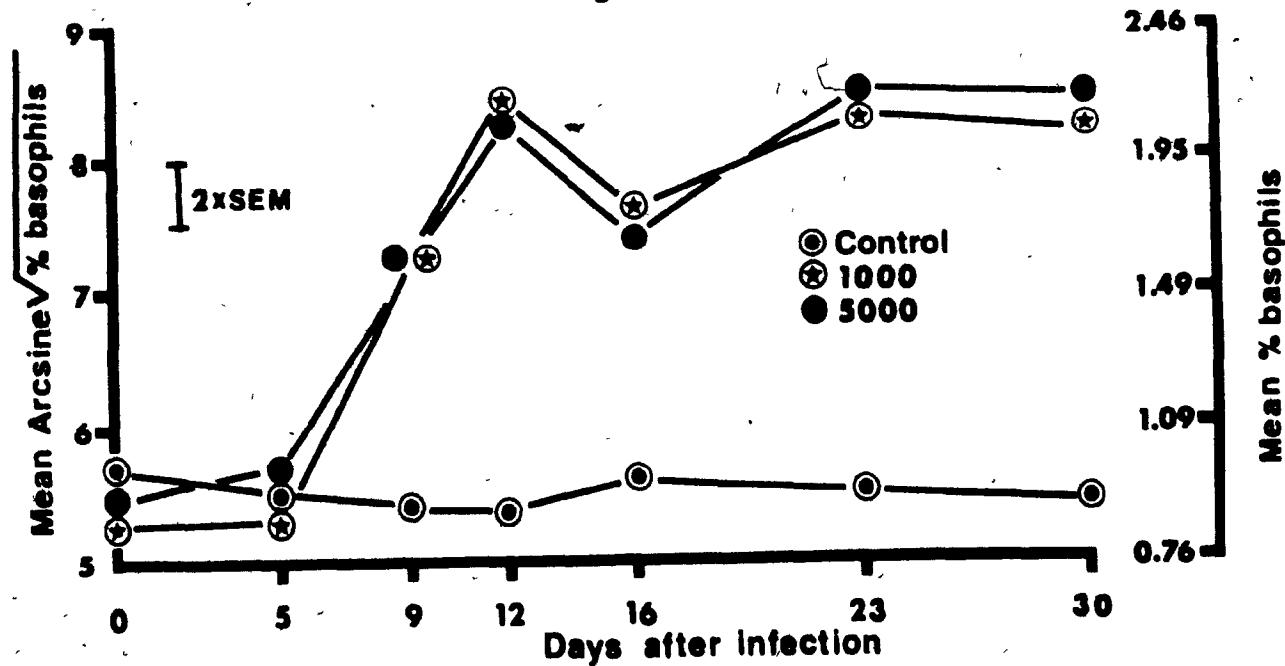
both levels of infection and remained so until the end of the experiment.

In the only other report dealing with basophils in the blood of N. brasiliensis infected rats, Hogarth-Scott and Bingley (1971) found basophilia from the 13th day of the infection in female rats infected with 1400 N. brasiliensis. As was mentioned in the discussion of blood eosinophils the fact that these authors did not find increased levels of basophils earlier in the infection might be because their infections were low grade.

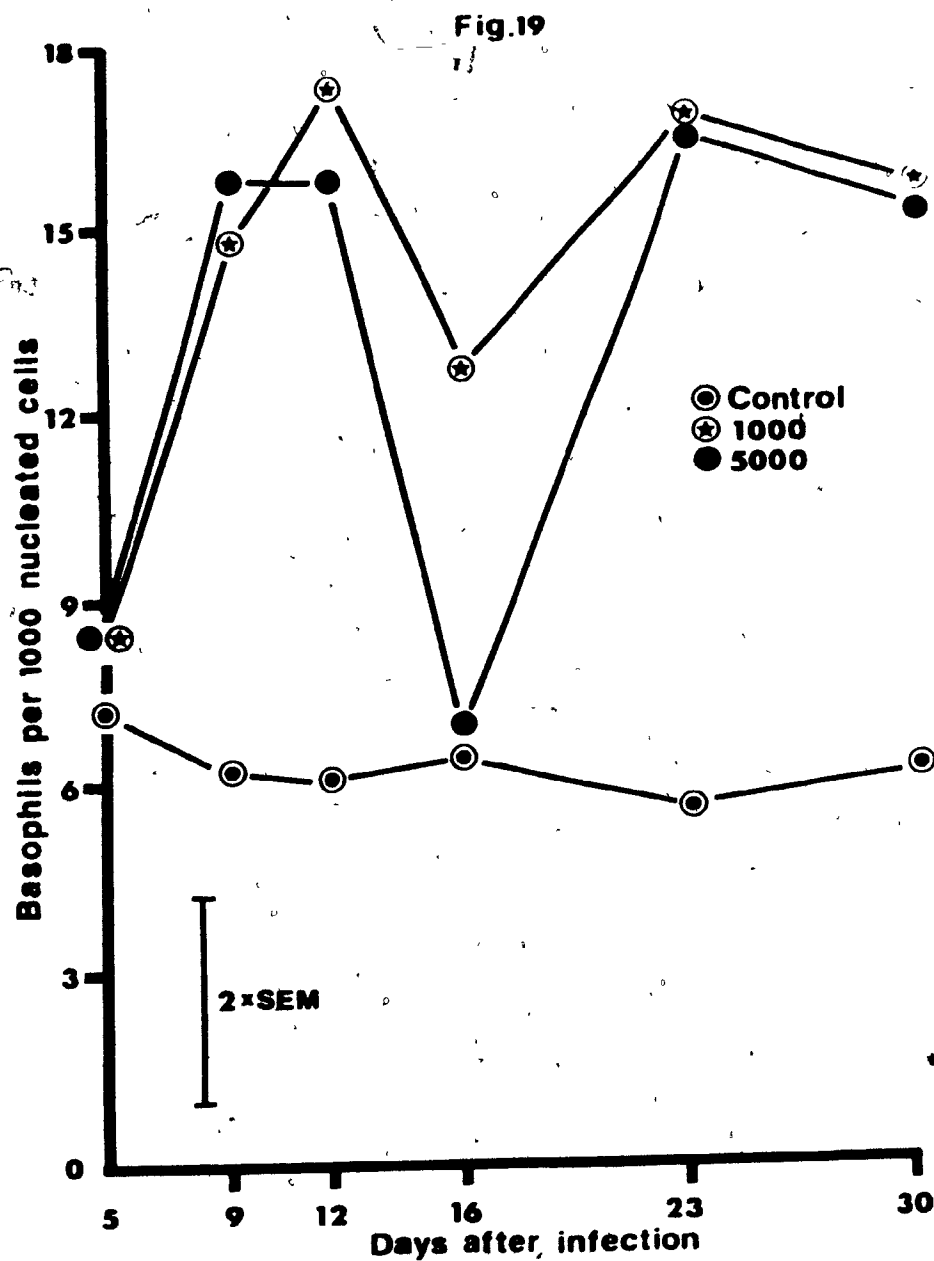
Trichostrongylus colubriformis infections in guinea-pigs are characterised by basophilia in the bone marrow and in the circulation (Rothwell and Dineen, 1972) but the function of the cell remains a mystery. It is tentatively suggested here that the basophil acts as a reserve supply of amines. After the loss of amines due to mast cell degranulation in the lungs and intestine during N. brasiliensis infection the animal is stimulated to produce new mast cells and the bone marrow is stimulated to increase production and release of basophils, thus accounting for the increase in numbers found in this study 9 and 12 days after infection. Once the animals start producing new mast cells the stimulus for basophil production decreases with a consequent drop in numbers in both the bone marrow and blood which occurs between days 12 - 16. Once the mast cells in the new population start releasing amines by degranulation and during the mast cell to globule leucocyte

conversion there is a further stimulus for the bone marrow to increase production and release of basophils leading to the increase in numbers between days 16 - 23.

Fig.18



Basophil response, expressed as a percentage of the total leucocyte count, in the peripheral circulation after infection with 1000 or 5000 *N. brasiliensis* third stage larvae. Standard error of the mean (SEM) for six rats per treatment per experimental day.



Basophil response in the bone marrow after infection with 1000 or 5000 *N. brasiliensis* third stage larvae. Standard error of the mean for six rats per treatment per experimental day.

CHAPTER IV

DISCUSSION

Mulligan et al., (1965) suggested three possible hypotheses to explain the mechanism by which an antibody reaction could induce the self-cure reaction: 1) that the action of immune serum is directed solely against the worms, i.e. an antiworm-antibody effect;

2) that the importance of antibody lies not in direct action against the parasite but in the production of a state of hypersensitivity in the intestine (reaction between tissue-fixed antibody and antigenic material from the worms may give rise to a local anaphylactic lesion which might render the environment unsuitable for the worms); and

3) a combination of the two previous hypotheses where the increased permeability associated with local anaphylaxis might allow a significant extravascular leak of plasma into the sub-epithelial spaces of the villi or into the intestinal lumen; the antibody might then act directly on the worms.

Work since then has led to two current hypotheses to account for the self-cure phenomenon; these will be discussed shortly in detail. Objections are, however, raised here to these hypotheses and, in the following discussion, it will be suggested that neither is correct because they involve the action of specific antibodies. A new hypothesis to explain

the loss of worms from the intestine will be proposed which does not involve antibodies, but in which the mast cell plays a prominent role. The mast cells which are important in the self-cure reaction are those present in the wall of the small intestine when the worms arrive in that habitat, those mast cells which degranulate and decline in number until the time when worm expulsion occurs, not those which appear after worm loss has started.

Of all the changes occurring in the rat during an N. brasiliensis primary infection, the non-specific inflammatory reactions in the small intestine, while the adult worms are present there, are the only changes about which there are no arguments as to whether, or when, they occur.

These changes have been noted, at times in considerable detail, by other workers, but have been generally ignored when reasons have been sought to explain the mechanism by which N. brasiliensis is induced to leave the small intestine. It will be suggested here that it is the inflammatory reaction and associated morphological changes in the wall of the small intestine which are the root cause of the expulsion of the parasite; it is proposed that these changes alter conditions at the surface of the mucosa to such an extent that the environment is no longer suitable for the existence of the worms in that habitat.

Adult N. brasiliensis live in the small intestine coiled between the villi. Mast cells are found in the lamina propria of the mucosa and contain the vasoactive amines histamine and serotonin, which are mediators of the inflammatory response (Gözy and Kato, 1970). These mast cells degranulate, releasing their amines, in response to a wide range of stimuli. During the early stages of N. brasiliensis infection when worms are present in the intestine, there is a decrease in the number of mast cells in the wall of the small intestine which is most pronounced in areas where the worms are most abundant. This drop in cell numbers starts at least as soon as worms appear in the intestine, if not before. Wells (1971) found a decrease in mast cells in the lungs before the migrating larvae reached there. This decrease could have, possibly, been caused by the stress of the infection and/or by substances released by the larvae during their migration to that organ; it is possible that a similar phenomenon occurs in the small intestine. The degranulation of mast cells in the intestine during the early stages of the infection, before self-cure, could be caused by any one, or by any combination, of the following factors: 1) general stress caused by the presence of the worms;

2) mechanical damage caused by the cuticular ridges of the parasite or by its feeding activities;

3) damage caused by the action of substances secreted by the parasite, perhaps digestive enzymes (a low molecular weight polypeptide which causes mast cell degranulation has been isolated from Ascaris suum (Uvnäs and Wold, 1967; Thompson, 1972)

and Jarrett et al., (1969) and Murray (1972) have shown that N. brasiliensis secretes a mast cell degranulator); or

4) by the action of

intestinal bacteria penetrating the damaged epithelium to produce an inflammatory reaction. Although it is known that antigen/antibody reactions can cause the degranulation of mast cells, this mechanism is not seriously considered here since, assuming the host to be immunologically naive before infection, the relatively short life cycle of the parasite in the host could not have induced an antibody response of such a magnitude capable of producing degranulation. Mast cell degranulation in the lungs has been found as early as one hour after infection (Wells, 1971); sensitised cells have not, however, been detected before the eighth day (Wilson and Bloch, 1968).

It is important to distinguish between the mast cell population present in the small intestine at the time of arrival of the worms and the new population which arises either after worm loss or as the worms are being lost. This distinction is necessary because the degranulation of mast cells while worms are present in the intestine results in a decline in the number of these cells and leads to inflammation and the accompanying morphological changes in the small intestine. After worm loss, the morphology of the intestine quickly returns more or less to normal so that the new population of mast cells arriving at the sites once infected does not lead to inflammation. It is unlikely that the initial mast cell degranulation is caused by antigen/antibody reaction

as reagins are not found during the early stages of nippostrongylosis. Antigen/antibody reactions may, however, be responsible for some amine release after self-cure, as reagins can be found at this time (Ogilvie, 1967).

During the early stages of infection, when there is a reduction in mast cell numbers, reduced levels of histamine are found in the wall of the small intestine (Wells, 1962; Keller, 1971). Keller (1971) interpreted low mast cell numbers and reduced histamine levels at the time of the start of worm loss as indicating that mast cells and histamine were unlikely to play a part in the mechanism of self-cure. This, surely, is a misunderstanding of the action of histamine in the body. Histamine, when it is present in the mast cell, is biologically inactive and performs its physiological function to cause increased capillary permeability only when released from the cell; it then rapidly disappears. Techniques for assaying histamine in tissues or organs measure only the amount of histamine which is present within the mast cell (Gözy and Kato, 1970). Hence, the finding of reduced levels of histamine in the small intestine during the early stages of N. brasiliensis infection, far from indicating that it is not involved in the mechanism of worm loss, is a strong indication that it has probably already performed its physiological function, a function which could be directly involved in the loss of worms. The release of histamine from the mast cell is an early characteristic of the host's inflammatory response, largely contributing to the increased capillary permeability which results in the flooding of the tissue with a protein-rich oedema fluid (Barth et al., 1966).

Extensive changes occur in the mucosa at the peak of infection with N. brasiliensis and include: 1) an increase in diameter of the intestine;

2) irregularities in, and partial fusion of, the villi;

3) extensive hyperplasia of the mucosa and hypertrophy of the muscularis externa (Symons and Fairbairn, 1962) and;

4) a doubling of the water and whole blood contents of the small intestine (Symons, 1960a).

In the infected small intestine the changes in villar structure depend on the number of worms present. When there are few worms, some blunting and fusion and a moderate increase in cells in the lamina propria occurs. In areas where many worms are present, many villi are replaced by a more or less continuous sheet of epithelial cells to give a flat mucosal surface; the crypts are deeper and contain many cells (Symons and Fairbairn, 1962). Similar changes were observed in the present work and were most pronounced in the areas of greatest worm density (Plates 1,2 and 3); these were also the areas of greatest mast cell disruption.

Sprinz (1962) has stated that the morphological structure of the intestinal mucosa depends on function and that the structure changes with the alteration of functional demands. The normal stimuli of food and intestinal flora have an effect on the morphology of the intestinal mucosa and help to determine the structure that is generally considered normal. The intestinal mucosa forms part of a biological unit in which the epithelium has primacy in initiating mucosal responses to stimuli;

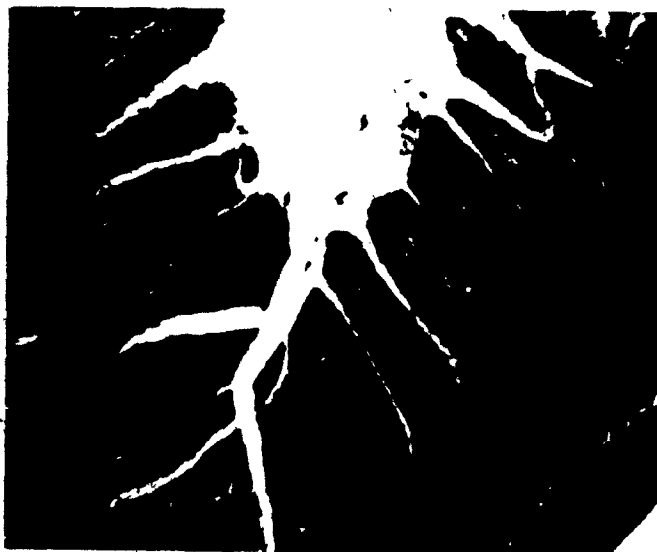
Plate 1.

Changes in the rat small intestine during infection

with N. brasiliensis.

- a) Uninfected control; 135x. Note the regular structure of the villi, the compactness of the tissue and the narrow intestinal lumen.
- b) Five days after infection with 1000 N. brasiliensis; 135x.
Note the infiltration of cells and separation of the capillaries from the epithelial lining of the villi.
- c) Five days after infection with 5000 N. brasiliensis; 135x.
Note changes in villar structure, erosion of the epithelial lining of the villi and influx of cells.

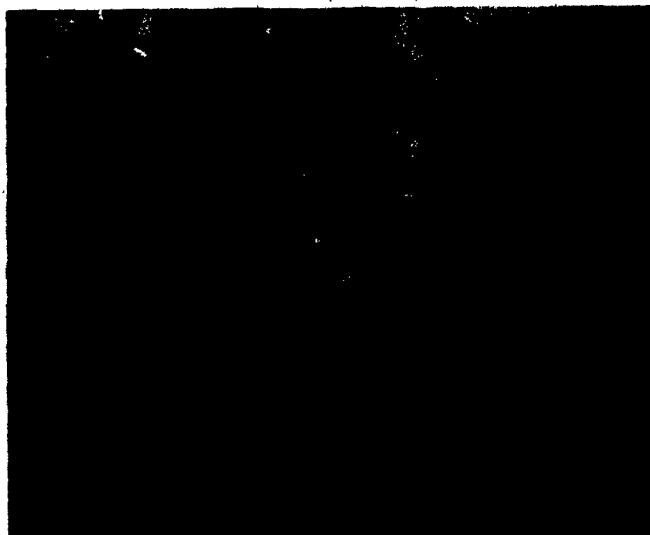
Plate 1



a



b



c

Plate 2.

Changes in the rat small intestine during infection
with N. brasiliensis.

- d) Nine days after infection with 5000 N. brasiliensis; 135x.

Note the hyperplasia of Brunner's glands, loss of villi and the increase in diameter of the intestine.

- e) Nine days after infection with 5000 N. brasiliensis; 135x.

Note the complete loss of villi and increase in diameter of the intestine.

- f) Twenty-three days after infection with 5000 N. brasiliensis;

135x. Note that the structure of the intestine has returned to normal although many mast cells and globule leucocytes are present.

Plate 2

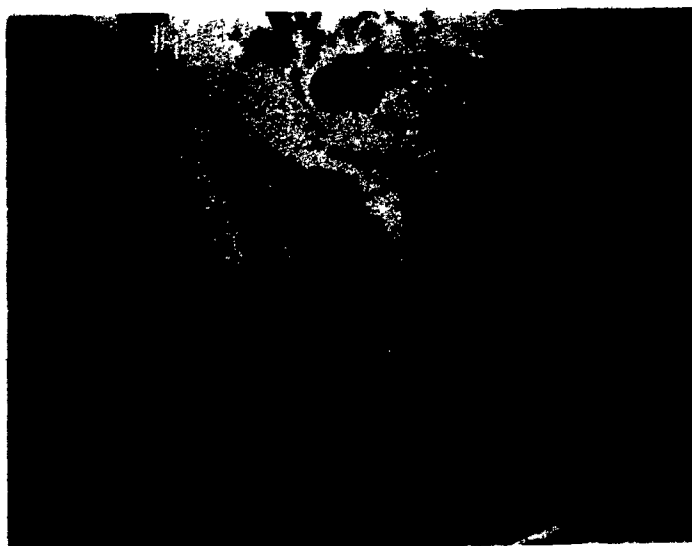
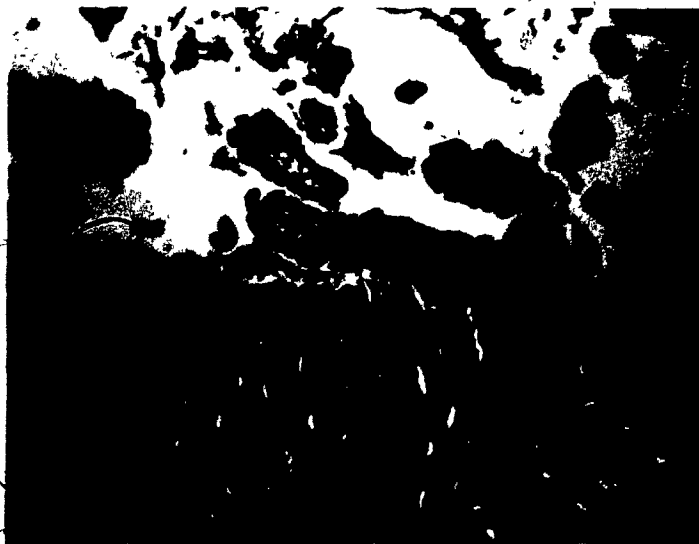
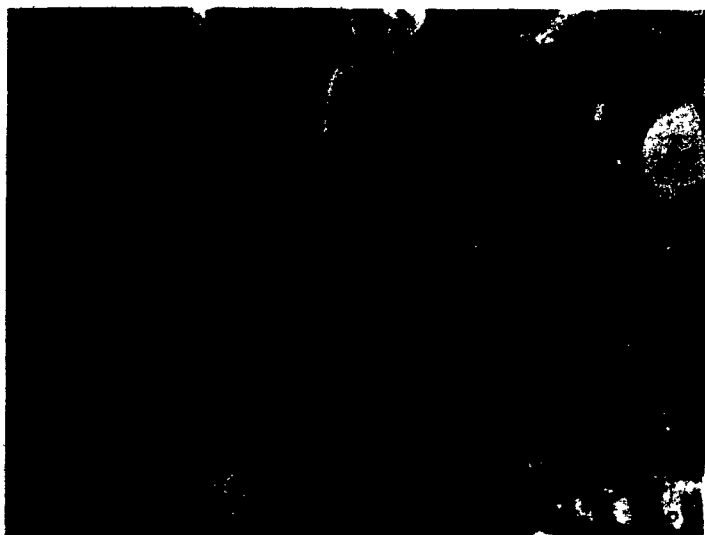


Plate 3.

Changes in the rat small intestine during infection
with N. brasiliensis.

- g) Mast cell (m) and eosinophil (e) in mesentery attached to the intestine; 860x. Note the intense affinity the mesenteric mast cell has for the stain compared to the intestinal mast cell in (i).
- h) Sixteen days after infection with 5000 N. brasiliensis; 860x. Note the globule leucocytes (g) within the epithelial lining of the villus and mast cells (m) in the lamina propria.
- i) Twenty-three days after infection with 5000 N. brasiliensis; 860x. Note the globule leucocytes (g) within the epithelial lining of Brunner's glands and the mast cells (m) outside the glands.

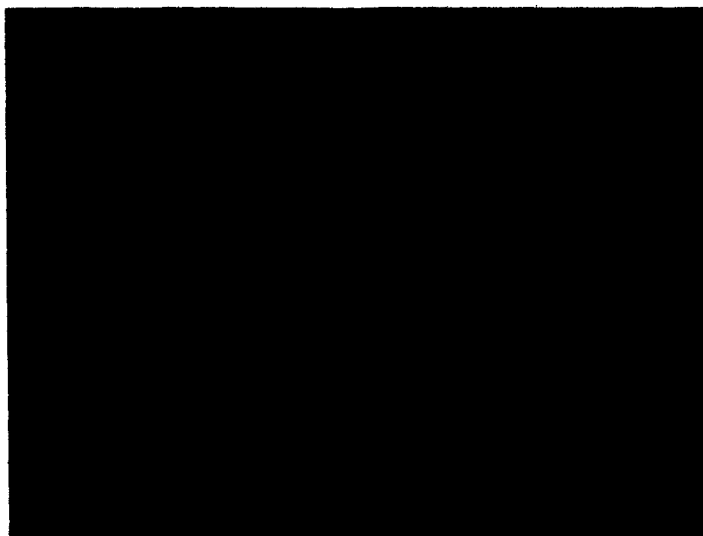
Plate 3



g



h



i

these responses are mediated through the neuro-vascular and lympho-reticular apparatuses of the lamina propria. Sprinz found that germ free guinea pigs infected with Shigella flexneri developed blunting and fusion of the villi and attributed this to hyperaemia and exudation of fluid and cells into the lamina propria. The inflamed villi were less mobile and tended to adhere to each other. Oedema has been suggested as the cause of the morphological changes in the mucosa in coeliac disease (Doniach and Shiner, 1957) and in N. brasiliensis infection (Barth et al., 1966). Sprinz stated that the changes occurring in sprue caused by sensitivity to gluten, folic acid deficiency or by enteric infection were histologically indistinguishable and were in fact a non-specific morphological manifestation of a functional adaptation by the intestinal mucosa to resorption, digestion and defence of the host. An indication of the non-specific nature of the response is found in the work of Cameron and Khanna (1959) who found similar changes in rats following the mechanical stimulus of clamping the mesenteric artery.

The mucosa of the small intestine maintains a constant appearance because of a continuous state of proliferation within the crypts of Lieberkühn, from which immature cells stream up the villi in regular fashion to be lost at the tip (Leblond and Stevens, 1948; Creamer et al., 1961). The epithelium of the whole small intestine is replaced about every two days and, if the production of new cells is abruptly stopped, the normal villus structure collapses (Philips and Thiersch, 1949; Wiernik et al., 1962). This suggests that the

structure of the mucosa of the small intestine is plastic and that variations in epithelial cell turnover may well determine the shape and size of the villi (Creamer, 1964a,b). Symons (1965) showed that there was a more rapid proliferation and a faster movement of epithelial cells towards the apices of the villi in N. brasiliensis infected rats than in normal animals. In infected rats, therefore, we have both effects, of hyperemia and influx of fluid and cells and changes in the rate of epithelial cell turnover, which can cause changes in villus structure. Perhaps the two effects are complementary. The inflammatory response produces marked changes in the structure of the small intestine at the peak of N. brasiliensis infection and it is suggested that it is these changes which are primarily responsible for the loss of the parasite from the small intestine.

Many parasite are highly site specific. N. brasiliensis occupies the first half of the small intestine and within this region it lodges in certain areas in preference to others. Presumably, when they first enter the small intestine, the worms occupy sites between the villi at the surface of the mucosa where the requirements for their existence can best be met. Lee (1969) suggested that the effect of the host's response to the parasite may be to alter the worm's normal behavioural patterns. Whilst in the lumen of the gut the worms move about actively but, when they change to a position between the villi where their body surface is touched on all sides, they exhibit thigmokinesis

(Alphey, 1971), slow down and are thus able to maintain their position. When the host reacts to the presence of the parasite there are drastic changes in villar structure and, in heavily populated regions, there may be a total elimination of villi; their natural environment no longer exists and, as they are no longer touched on all sides, there is, quite possibly, an increase in body movements. This increase in movement could, possibly, be enhanced by the direct action on the parasite of amines released from mast cells in the vicinity. In vitro work by Mansour (1957) showed that serotonin has a stimulatory effect on the rhythmical movements of Fasciola hepatica. It has been suggested that amines regulate the production of the intracellular regulator cyclic 3', 5' - adenosine monophosphate in mammals (Goodman, 1968) and invertebrates (Mansour, 1967); Rothwell and Dineen (1972) suggested that amine release in the parasite's microenvironment may inhibit crucial metabolic pathways and lead to the elimination of T. colubriformis in guinea pigs. Possibly a similar situation could occur in rats infected with N. brasiliensis. One has, however, to be extremely careful in comparing the two parasites as T. colubriformis is expelled when large numbers of mast cells are present and degranulating in the intestine, whereas N. brasiliensis, on the other hand, is usually eliminated when few mast cells are present. However, a relatively slow release of amines over a period of several days while the worms are present may effect this latter parasite.

Following the appearance of a new population of mast cells in

the intestine in nippostrongylosis they increase in an exponential fashion (Miller and Jarrett, 1971); Murray *et al.*, (1971b) suggested that the parasite produces a substance, or substances, which stimulate this increase. During the exponential rise in cell numbers there is a corresponding increase in the level of serotonin either at the site of actual maximum worm concentration or, if the worms had been removed by an anthelmintic, amine levels were highest in the areas previously occupied by the parasite (Murray *et al.*, 1971b). Amine release can be demonstrated when worms are present by histochemical methods and mast cell discharge has been seen by electron microscopy (Miller *et al.*, 1967); Murray *et al.*, 1968; W.F.H. Jarrett *et al.*, 1970; Miller, 1971a). Murray *et al.*, (1971a) found that between days 11-16 of the infection the permeability of the bowel wall to macromolecules increases; there is a very sharp rise in permeability on the 14th day coinciding with the onset of the rapid phase of self-cure. At this time epithelial cells, blood vessels and cells in the lamina propria are extensively damaged (Murray, 1972) and intercellular pathways open up between epithelial cells through which macromolecules can pass (Murray, 1972); it was proposed that these pathways present a route for the rapid translocation across the mucous membrane of circulating or locally produced antibodies and that the formation of these passages was mediated by substances released from the mast cells. Majno *et al.*, (1967) showed that histamine and serotonin cause endothelial cell contraction and the formation of intercellular gaps and Murray (1972) suggested that these amines might have a similar effect on epithelial cells.

However, as has been mentioned previously, there is one major objection to involving the rise in mast cell numbers with the loss of the parasite: the rise in mast cells invariably occurs either after many of the worms have been lost or after worm loss has finished. The theory of the relationship of the increase in mast cells to the self-cure of N. brasiliensis infection has been evolved by other investigators from results obtained with one particular strain of rats; this is the only strain in which a rise in mast cell numbers has been found to coincide with loss of worms. As the increase in mast cell numbers and the mast cell to globule leucocyte transformation usually take place in most strains of rats after worm loss, they, therefore, cannot form part of the mechanism which results in self-cure.

In the early stages of nippostrongylosis, up to the commencement of worm loss, there is an inflammatory reaction in the wall of the small intestine which is marked by the degranulation of many of the rat's intestinal mast cells. Acute inflammatory reactions are known to be followed by a reactive phase in which there is a large increase in the number of these cells (Keller, 1971). During a primary N. brasiliensis infection, the rat, having lost many of its intestinal mast cells by degranulation in the generalised inflammatory reaction induced by the parasite, responds by overproducing a new generation of these cells to replace those which were lost; it is suggested that this is the reason for the large increase in mast cell numbers which characterises the infection. The main extrinsic factor which determines the speed with which the rise occurs is probably the size of the infecting dose: the more worms present in the small intestine, the greater the loss of

mast cells due to degranulation; the greater the loss of mast cells, the greater the stimulus to produce new mast cells. In the present work, a more pronounced loss of mast cells was obtained in rats infected with 5000 larvae than in those given 1000; the rise in mast cell numbers also occurred faster at the higher level of infection. Intrinsic factors appear to include the sex of rats: Murray *et al.*, (1971a,b) found that female rats effected the rapid phase of worm loss at least one day earlier and 1.5 times faster than male rats given the same infecting dose; there was also a corresponding difference between the sexes in the timing of both the mast cell rise and the peak of the macromolecular leak. From the results of various other workers it is also apparent that the strain of rats infected has an influence on the time at which the rise in mast cells occurs and it is interesting to note that, even with the same strain of rats, the time of the rise varies even when the same infecting dose is used. Mature, male, Wistar rats infected with 5000 larvae were used by Wells (1962), and also here, and the rise in numbers occurred on the 23rd and 16th days respectively. The N. brasiliensis used here was from the colony used by Wells (1962).

Globule leucocytes are mast cells which have discharged or are discharging some or all of their contents and have migrated into an intraepithelial position (Murray *et al.*, 1968). It is suggested here that this migration and transformation of the mast cell into a globule leucocyte is a step by which excess mast cells are excreted. Globule

leucocytes are found within the epithelial lining of the villi from where they can easily pass into the lumen of the gut; they are also found within the epithelial lining of the acini of Brunner's glands from where they can easily pass into the ducts and from there into the lumen of the intestine. It is known that eosinophils may be eliminated from the body via the intestinal lumen (Teir et al., 1963). Although it is possible that some, if not all, the globule leucocytes which are excreted are eliminated because more mast cells were produced than required, it is possible, as the mast cells lose some or all of their contents in their change to globule leucocytes, that the substances released provide material to help in the repair of damaged tissues. Kelsall and Crabb (1954) suggested that mast cells may provide mucopolysaccharides for the synthesis of mucin and it is known that 16 days after a primary infection of 5000 N. brasiliensis there is an increase in both the mucin secreting cells lining Brunner's glands and in the goblet cells lining the villi of the small intestine (Wells, 1963).

Murray et al., (1971b) found that, if N. brasiliensis worms were removed with an anthelmintic from the intestine by the 7th day of a primary infection, the rise in mast cells still occurred, but that the mast cell to globule leucocyte conversion did not take place; they concluded from this that the continuing presence of the parasite is not necessary for the rise to take place and that the worms produce a

substance, or substances, which induce the rat to produce new mast cells. There is, however, no reason to postulate the production by the worms of substances specifically stimulating mast cell formation: the rat will normally produce new mast cells to replace those lost by degranulation during the inflammatory reaction. If the worms are not removed by anthelmintic, a continued breakdown of mast cells occurs with a consequent continuing stimulation to produce new mast cells. There is, however, a limit beyond which the rat will excrete mast cells as globule leucocytes. When worms are removed before the 7th day this limit may not be reached and as the cause of mast cell degranulation is removed no conversion of mast cells to globule leucocytes occurs. Furthermore, if degranulation supplies material to repair damaged tissue, the damage occurring during the 4 or 5 days before worms are removed by anthelmintic might not be sufficient to induce the mast cells to convert into globule leucocytes.

Having proposed a hypothesis, which does not involve antigens or antibodies to explain the loss of N. brasiliensis from the rat small intestine during a primary infection, what can be said about the work on which theories involving antigens and antibodies are based? The basic argument which will be used here is that the worms are expelled by an inflammatory reaction mounted by the host in response to the parasite during the early stages of the infection; this response is accompanied by morphological and biochemical changes in the small intestine

which render the intestinal environment unsuitable for the worms' continued existence. It will be shown that the experimental results which other workers have used to support the involvement of antigen-antibody reactions in the self-cure reaction can be reinterpreted to support the mechanism suggested here. Before discussing these experimental results in detail, a number of general comments should be made.

When N. brasiliensis first enters the small intestine, conditions exist which are conducive to the parasite's well being. As the host responds to the presence of the worms, conditions in the small intestine change and, by the 9th or 10th day of the infection, the environment is no longer suitable for the parasite and the worms are lost from the intestine. By the 16th day when, apart from a few individuals, worm loss is complete, yet another condition exists in the small intestine which arises as a consequence of the changes which occurred during the earlier stages of the infection. It must be stressed that the conditions at the time of self-cure are the ones responsible for worm loss, not those when the worms first enter the small intestine nor those after completion of worm loss.

It is not argued that the rat infected with N. brasiliensis does not produce antibodies in response to the parasite. Just as any animal produces antibodies to foreign proteins so too does the rat in response to secretions of the parasite, e.g. to the isoenzymes of

acetylcholinesterase (Edwards et al., 1971). It is suggested, however, that the fact that antibodies are produced does not automatically mean that they are involved in the self-cure reaction, although this is the conclusion that most workers have drawn.

The arbitrary division of the infection into three time periods (before, during and after worm loss) has resulted, to a large extent, from work which has involved the transfer of cells or sera from infected to normal rats under various experimental conditions. The conclusion from these experiments has been that transferred cells or sera play the same role in passive immunity as they do in a primary infection. This conclusion might be valid if the cells or sera were taken from donor rats at, or before, the time of self-cure, but this has never been the case. If they have been taken from donors with a primary infection, this has been either, after self-cure has been completed, when conditions in the rat are different from those existing at the time of self-cure, or several days after donor rats have received the last of a series of infections, when conditions are different from those existing at any time during a primary infection. The reason for this has been presumably, that only at these times are sufficient quantities of immune cells or sera present to produce an effect in the recipient. However, what if the mechanism of the self-cure reaction itself does not involve an immunological reaction? If this is true, the transfer of large

quantities of cells or serum from rats which have undergone self-cure would superimpose in the recipients additional factors on a normal rejection mechanism.

Let us now look at the two hypotheses which implicate antigen-antibody reactions in the self-cure reaction. Jones and Ogilvie (1971) suggested a two-step process in which the initial and essential first step is the action of protective antibodies which damage the parasite: changes occur in the worms' acetylcholinesterases and the structure of their gut cells deteriorates. Once these antibody-induced irreversible changes have occurred, the damaged worms are susceptible to a second step which acts independently of protective antibodies, but which cannot act unless the first step has occurred. These authors gave no evidence as to the nature of the second step, but stated that it is prevented by irradiation and slightly affected by treatment with anti-lymphocyte serum; they suggested that, if the release of pharmacologically active amines is important in self-cure, it would be involved in this step. Later, work by Keller and Keist (1972), Kelly and Dineen (1972a,b, 1973), Dineen and Kelly (1972, 1973), Dineen et al., (1973) suggested the involvement of lymphocytes in the second step and work by Keller (1971) and Keller and Ogilvie (1972) suggested that vasoactive amines were unlikely to play any part in worm expulsion. In addition to the two steps suggested above, namely antibody-mediated damage and lymphocyte-mediated action, Kelly et al., (1973) suggested the presence of a third step which might be of myeloid origin.

The second hypothesis suggests that worm antigens stimulate the production of specific protective antiworm-antibodies and that among these antigens there are also allergens which induce the formation of reagins. The reagins attach to mast cells in the lamina propria and, on contact with the allergen, cause the discharge of vasoactive amines from mast cells resulting in increased mucosal permeability. This causes the passage of antiworm-antibody through the intestinal wall at the sites of attachment of the parasite and results in their elimination (Jarrett et al., 1968a, 1970). This theory was expanded by Murray et al., (1971b) to include a third stage in which there is a stimulus, or stimuli, from the parasite which causes the synchronous development of new populations of mast cells, IgE-producing plasma cells and plasma cells producing other classes of antibodies capable of an anti-worm effect.

Jones and Ogilvie (1971) based their hypothesis on work in which uninfected rats were each given either 6ml of normal serum or 6ml of serum known to contain antibodies; one day later, approximately 1000 "normal" worms taken from donors 7 days after infection were transplanted into the small intestine of each rat; similar numbers of worms were found 3 days later in both groups, indicating that worm expulsion had not occurred. However, histological examination and enzyme studies on worms recovered from rats given antiserum showed structural degeneration of the gut cells similar to that seen in worms damaged by the phenomena which lead to worm loss in self-cure (Ogilvie and Hockley, 1968): the acetylcholinesterase patterns of these worms had begun to change in a manner similar to that reported in "damaged" worms from an

active infection (Edwards et al., 1971). Similar changes were not found in the worms obtained from rats given normal serum. Three hundred worms from serum treated rats were re-implanted into the small intestine of other uninfected rats and recovered six days later: worms from rats given antiserum were expelled, whereas the worms from rats given normal serum were not. It was concluded that protective antibodies cause worm damage in both active and passive immunity (Jones and Ogilvie, 1971). These authors also found that irradiated rats failed to expel "damaged" worms which had been transplanted into them and that protective antiserum did not induce irradiated recipients to expel "normal" or "damaged" worms; they concluded that irradiation destroys a factor in the host essential for expulsion which is not transferred by antiserum and that worm expulsion is not a passive process which occurs after damage but involves an active process. They also found that ALS treatment had a slight, but not a statistically significant, effect on the expulsion of transplanted worms damaged during the self-cure phenomenon and that, in ALS treated rats, damage to "normal" worms occurred later than in rats given normal rabbit serum and concluded that self-cure was unlikely to be a specific immunological event as ALS treatment did not prevent, but only suppressed the expulsion of "damaged" worms. The antisera used by Jones and Ogilvie (1971) were either taken after self-cure (17-21 days after a primary infection) or 5-9 days after rats had received the last of a series of infections. It is true that transplanted worms were damaged when their hosts were treated with these antisera, but this is not sufficient evidence that the damage worms sustain during a primary infection is caused by antibody action. Their experiments only show that antisera administered in quantities

approximating the total serum volume of the recipients (6ml), can produce changes in the worms similar to those found during self-cure.

The hypothesis presented here suggests that changes in the parasite during self-cure are degenerative changes which result from worm-induced damage to its environment which prevent the parasite from feeding. Since the wall of the small intestine shows increased permeability as early as four days after a primary infection with N. brasiliensis (Barth et al., 1966), the treatment of rats with large quantities of antiserum could result in the antibodies passing into the host's intestine changing the parasites' environment, perhaps blocking its digestive system and, thus, inducing the same degenerative changes found during self-cure. The expulsion of "damaged" worms within six days of transfer into normal rats can result from an inflammatory reaction mounted by the host to the transplanted parasite; since the worms are already damaged, they are rapidly expelled because they are less able than "normal" worms to withstand the antagonistic effect of the inflammatory reaction occurring in the intestine as the host responds to their presence.

Jarrett et al., (1970) have suggested that the failure of irradiated rats to expel "damaged" worms may well be due to the possibility that irradiation alters the permeability of the intestinal wall to serum and that the damaged worms are able to exploit this plasma-

enriched environment. Furthermore, it is well known that irradiation suppresses the inflammatory reaction (Taliaferro *et al.* 1963) and, therefore, such treatment would inhibit an inflammation-mediated reaction against the parasite and favour the establishment even of "damaged" worms. These authors suggested that the inability of antisera to induce worm expulsion in irradiated rats could be due to the elution of protective antibody into enlarged extra-vascular pools created by a generalised vascular leak caused by total body irradiation; antibody would, therefore, not reach the parasites in the intestine in sufficient quantities to be effective. There is yet another factor which could also be involved in reducing the amount of antibody reaching the worms in the small intestine of irradiated rats: in an active infection, the permeability of the wall of the small intestine is increased in areas where worms are present. When large quantities of antisera are given to non-irradiated rats, most of the antibody entering the small intestine would do so in regions where worms are present; in irradiated rats, however, the permeability of the entire small intestine would be increased and any effect of the antibodies would be limited because of the diluting effect of their penetrating into the intestine throughout its entire length.

A number of reports have implicated lymphocytes in the second step of worm expulsion. Keller and Keist (1972) found that the expulsion of "damaged" worms could be induced in irradiated rats by the

transfer of lymph node cells from uninfected, syngeneic adult donors; worm expulsion did not occur in these rats after the transfer of in vitro irradiated lymph node cells. They also found that self-cure occurred more or less normally in neonatally-thymectomised rats, but was delayed when thymectomised rats were treated with anti-thymus serum intraperitoneally; these authors concluded that the lymphocyte plays a decisive role in the elimination of worms previously damaged by antibodies. They found that "damaged" worms were not expelled when transplanted into young rats but that, when lymph node cells from uninfected adults were transferred to them, self-cure was greatly enhanced; they suggested that the inability of young rats to expel worms was due to the immaturity of their cell-mediated immune response.

Kelly and Dineen (1972a) found that the transfer of lymph node cells from infected rats to animals with a primary infection of N. brasiliensis suppressed egg production, caused a reduction in the number of eggs/uterus in gravid females and stimulated the rejection of a substantial proportion of the intestinal worm population in the recipients 6 and 10 days after infection. Lymph node cells from normal rats had no effect on the course of infection in the recipients. Dineen and Kelly (1972) found that lactating rats showed significant increases in worm fecundity and total worm burdens compared with infected nulliparous controls; the transfer of lymph node cells from infected, nulliparous female donors to infected lactating recipients caused a suppression of worm fecundity and the rejection of a substantial proportion

of the worms by the 10th day of the infection. Lymph node cells from lactating donors, when transferred syngeneically to nulliparous recipients, also suppressed worm fecundity and caused rejection of the parasite; they had, however, a far less pronounced effect when transferred to lactating recipients; these authors suggested that, in lactating rats, the inductive processes of the immune response occur normally but that differentiation to effector cells is impaired.

Urquhart et al., (1965) found that treatment of rats with promethazine hydrochloride suppressed rejection of N. brasiliensis, a finding which was confirmed by Kelly and Dineen (1972b). These latter authors extended the work to show that the functional activity of lymph node cells from infected rats, as assessed by their ability to produce self-cure, was reduced by treating recipient rats with promethazine; lymphocytes from donors treated with promethazine were markedly less effective in syngeneic recipients in stimulating self-cure than cells from untreated donors. These authors suggested that the in vivo effect of promethazine may be mediated at the specific lymphoid level, as well as at the myeloid-amine level. They also found that serum from promethazine-treated rats seriously impaired the activity of immune lymphocytes in the cellular transfer of resistance. Kelly and Dineen (1973) reported that, when castrated male rats were treated parenterally with prolactin during a primary N. brasiliensis infection, survival of the worms in the small intestine was prolonged. They also found that the functional activity of lymph node cells from infected

rats was reduced when recipients were treated with prolactin and also that lymphocytes from donors treated with prolactin were less efficient in inducing self-cure in syngeneic recipients than cells from untreated donors. In addition, they reported that lymphocytes from prolactin-treated immune donors suppressed rejection to the same extent in prolactin-treated and untreated syngeneic recipients; they concluded that prolactin treatment affected a lymphoid-specific component of the rejection mechanism rather than a non-specific component.

Dineen et al., (1973) found that lymph node cells from infected rats, when transferred into recipients into which "normal", "damaged", or "adapted" worms had been transplanted, eliminated all the "damaged" worms and 50% of the normal worms within eight days, but had little effect on "adapted" worms. (These cells also caused that premature rejection of a larval infection; they concluded that this was further evidence that sensitised lymphocytes expel worms but that they do not affect the worms until antibodies have acted on them. Dineen and Kelly (1973) found that lymph node cells from infected adult rats induced self-cure when transferred into syngeneic neonatal rats, whereas lymphocytes from infected neonatal rats had no effect in infected adult rats; this led them to suggest that, in neonatal rats, the lymphocytes are not competent to initiate worm expulsion, either because they are impotent as a result of tolerance or because of a deficiency in the immune response as a result of delayed ontogeny.

It has been reported by a number of workers that lymphoid

cells are able to develop into mast cells. Ginsburg (1963) and Ginsburg and Sachs (1963) described the differentiation to mast cells of normal mouse thymic lymphoid cells grown on embryonic fibroblast monolayers. Csaba and Barath (1970) also reported the transformation in vitro of thymic lymphoid cells and small peritoneal lymphocytes, but not lymph node cells, into mast cells. Burnett (1965) found large numbers of mast cells in the thymuses of NZB mice and proposed that they, like plasma cells, were specialised "executive derivatives" of the lymphoid series and that transformation into one or the other depends on the nature of the stimulus; Miller and Coles (1968) reported that antigenic stimulation can produce an increase in the mast cell population of rat lymph nodes. The in vitro studies by Ginsburg and Lagunoff (1967) showed that, when lymph node or thoracic duct cells from hyperimmunised mice are cultured on embryonic fibroblast monolayers in the presence of antigen, numerous clones of mast cells appeared in the vicinity of large lymphoid cells; however, in cultures from unimmunised mice or in cultures without antigen, phagocytic histiocytes were evident.

If it is possible for lymphocytes to develop into mast cells after transplantation into recipients, these mast cells could either augment an existing inflammatory reaction or, if prior treatment has eliminated a capacity to mount such a reaction, initiate inflammation in the small intestine of the recipient; it is also possible that any treatment of recipients or donors which interferes with the ability of lymphocytes to effect a self-cure reaction in recipients could be due

to an inhibition of the ability of the lymphocytes to transform into mast cells.

The finding of Keller and Keist (1972) that "damaged" worms are expelled from irradiated rats several days after the transfer of normal lymphocytes suggested to them that functional lymphocytes were essential for the second step of worm expulsion; the lag period of 2-3 days between transfer and expulsion was required according to these authors, for the transferred lymphocytes to be sensitised by antigen and to react in the self-cure reaction. They offer no suggestion concerning the mechanism by which sensitised lymphocytes induce worm expulsion; they felt, however, that the release of vasoactive amines from mast cells was not likely to be involved as the development of mast cells from possible lymphocyte precursors takes 5 days (Ginsburg and Lagunoff, 1967) and the irradiation dose they used destroys mast cells. The argument that transferred lymphocytes cannot develop into mast cells because of the time difference between this process and the time taken for transferred cells to bring about worm expulsion is, at best, a tenuous one. The period of 5 days for this differentiation found by Ginsburg and Lagunoff (1967) was the result of in vitro work with mouse lymphocytes. Wlodarski et al., (1973) found that the numbers of mast cells in the lymph nodes of mice increase within 5 hours following antigenic and non-antigenic stimulation. In contrast to the report of Keller and Keist (1972), Dineen et al., (1973) showed that the lag of 2-3 days between lymphocyte transfer and worm expulsion was not required for the sensitisation of lymphocytes

as they found a similar lag when lymphocytes were taken from infected donor rats.

Since irradiation destroys mast cells, the ability of irradiated rats to mount an intestinal inflammatory reaction to the parasite is destroyed or, at least, severely reduced. It is suggested here that, when normal or "immune" lymphocytes are transferred to irradiated rats, they develop into mast cells which initiate an inflammatory reaction in the small intestine of the recipients which results in self-cure; the 2-3 day period between cell transfer and worm elimination is required for the lymphocytes to migrate to the gut, transform into mast cells and initiate the inflammatory reaction. Parrott and Ferguson (1974) found that mesenteric lymph node cells from uninfected mice, or mice with a six day primary infection of N. brasiliensis, will home on the lamina propria of the gut of recipient mice within 24-72 hours of inoculation.

Kelly et al., (1973) found that immune lymphocytes initiated an expulsion of "damaged" worms in both non-irradiated recipients and in recipients irradiated with 400 rad, but not in recipients irradiated with 750 rad, and concluded that both non-irradiated and irradiated (400 rad) recipients actively participated in worm expulsion; therefore, the factor(s) actively contributing to self-cure in the two groups where this occurred was abolished at the higher radiation dose. There must, therefore, be a factor(s) additional to antibody-mediated damage

and lymphocyte action which is (are) required for worm expulsion. In the hypothesis presented here, "damaged" worms are eliminated from non-irradiated rats as a consequence of the inflammatory reaction mounted in the intestine of the recipient. In the lower dose irradiated rats, mast cells are destroyed and consequently the recipients' capacity to mount an inflammatory response is eliminated, or at least severely reduced. Immune lymphocytes transferred to these animals transformed into mast cells and restored this capacity. There may also be some participation by residual mast cells in irradiated recipients.

Kelly et al., (1973) found that there was substantial regeneration of immunological reactivity during the 4-6 day time period of their studies in rats irradiated at 400 rad, but not in those irradiated at 750 rad. If the ability of rats irradiated at the lower dose to produce new mast cells to replace those lost by irradiation is not completely lost, they then may do this and the new mast cells could work with the transferred cells to produce an inflammatory reaction. At the higher irradiation dose the damage may be so great that the rats cannot produce any new mast cells of their own and the transferred cells alone may not be capable of mounting an inflammatory reaction of sufficient intensity to cause worm elimination.

Kelly and Dineen (1972a) found that lymph node cells from infected rats accelerate the self-cure reaction in recipients whereas

normal lymph node cells had no effect. The lymph node cells from infected rats were taken on the 15th day of a primary infection, when mast cell numbers are rising in the small intestine of the donor rats; it is suggested here that the transplanted lymph node cells were stimulated in the donor rats to differentiate into mast cells and do this on transfer to the recipients, so augmenting the recipient's inflammatory response to the parasite.

Connan (1973) found that the rise in intestinal mast cell numbers and the appearance of globule leucocytes occurred at the same time and was of the same magnitude in lactating and nulliparous rats. Similar results were also obtained by Kelly and Ogilvie (1972) who also found that the fall in mast cell numbers in the early stages of the infection was similar in both lactating and nulliparous rats infected with N. brasiliensis. Neither group of workers provided any information on the inflammatory and morphological changes in the intestine or on the distribution of worms in the different groups. Cells were counted from only one region of the intestine, a section chosen because it was the site of maximum worm concentration in nulliparous rats. A direct comparison of counts in one region in nulliparous and lactating rats is meaningful only if the worm distribution in both groups is the same; as has been shown in this study, the mast cell responses in any given region are dependent on the density of worms present in that region and in lactating rats worm counts 10 days after infection showed more worms

present than in nulliparous rats (Kelly and Ogilvie, 1972) while Connan (1973) found fewer. It is suggested that, although apparently there is a similar mast cell response in lactating and nulliparous rats, in the lactating rats the hormonal changes associated with lactation affect the ability of mast cell constituents to initiate inflammation and consequently the changes necessary for self-cure do not occur.

The reduced ability of lactating rats to eliminate adult worms could be due to a reduced ability on the part of these animals to mount an inflammatory reaction in the small intestine as a result of the hormonal changes during lactation since it is well known that corticosteroids can exert an anti-inflammatory effect. The ability of lymph node cells from infected nulliparous donors to bring about self-cure in lactating recipients could be due to the differentiation of the lymphocytes into mast cells which then augment the intestinal inflammatory response in the recipients; lymph node cells from lactating rats could possibly do the same thing on transfer to nulliparous recipients.

Work with promethazine hydrochloride led Kelly and Dineen (1972b) to suggest that this compound might act on specific lymphoid cells as well as at the more normally accepted myeloid-amine level; they also suggested that prolactin works at the specific lymphoid level rather than at the non-specific myeloid-amine level. However, if one accepts the evidence that lymphoid cells can differentiate into mast cells,

the morphological and functional distinction of these two cell types is meaningless, since differentiation is a function only of the need for a specific cellular response. It is suggested that promethazine and prolactin reduce the capacity to eliminate N. brasiliensis by virtue of their ability to exert an anti-inflammatory effect.

Prolactin is known to synergise steroid hormones (Nicoll and Bern, 1972) and to stimulate the pituitary-adrenal system (Grosvenor et al., 1970) and, as corticosteroids are known to be anti-inflammatory, prolactin may work in this way; the reduced ability of lymph node cells from promethazine or prolactin treated rats to accelerate self-cure in recipients may be due to these two compounds directly or indirectly reducing the ability of transferred lymph node cells to differentiate into mast cells.

Having considered the first of the two hypotheses which are currently proposed to explain the loss of N. brasiliensis from the rat small intestine, let us now look at some of the earlier experimental work upon which the Jarretts and their associates have based their ideas. Much of their later work, concerning the reappearance of mast cells in the small intestine, the mast cell to globule leucocyte conversion and the relevance of these two events to self-cure, has already been discussed.

Mulligan et al., (1965) found that the passive transfer of serum from infected rats caused the premature rejection of adult worms which had been transplanted into the intestines of normal rats. This led them to conclude that antibody action must be involved in the self-cure of

N. brasiliensis infections. The sera used were taken either 20 days after a primary infection or from rats which had been reinfected twice and the sera were given in large quantities (0.03 ml/gm) body weight. The fact that worm expulsion is enhanced under these conditions is not necessarily compelling evidence that antibodies play a part in self-cure; the superimposition of specific antibodies in large quantities on a non-specific inflammatory mechanism would enhance the departure of the worms since these antibodies would easily pass out through the intestinal mucosa through the opened intercellular spaces and specifically react with the parasites.

Urquhart et al., (1965), studying the possible role of anaphylaxis in self-cure, demonstrated areas of increased capillary permeability which appeared to be roughly proportional to the concentration of worms 5, 10 and 14 but not 19 days after a primary infection; they suggested that this was not necessarily evidence of anaphylaxis but could possibly be caused by irritation produced by the worms. Intravenous injection of an Evans blue/N. brasiliensis antigen mixture into hyperimmune rats and into rats with 7, 21 and 26 day primary infections resulted in extensive blueing of the mucosa, a macroscopically apparent hyperemia and an increased amount, and fluidity of, deeply stained mucus; the intensity of the reaction increased in the order: control, 7, 21 and 26 day infections, with the hyperimmune rats being similar to those with the 26 day infection. These authors

also injected Evans blue/antigen mixture intravenously into rats passively immunised with hyperimmune serum and found that the intestinal changes described above also occurred in these rats. These results suggested to them that the effects observed upon injection of antigen might occur in a more localised form during the self-cure reaction at points where the antigenic stimulus was being supplied by the worms themselves; the associated changes (fluid and mucus passing into the gut) might then be responsible for self-cure or, if antibody had any role in eliminating the parasite, this might be the means by which antibody escaped into the lumen of the gut to react with the worms.

Working with compounds which might abolish the self-cure reaction (the anti-serotonin compound, lysergic acid, and the antihistaminics, mepyramine maleate and promethazine hydrochloride) Urquhart *et al.*, (1965) indicated that they had no effect as judged by parasite egg counts although the latter antihistaminic slowed the rate at which the worms were expelled. Ogilvie (1965) showed that cortisone inhibited the self-cure reaction to a primary infection, and Urquhart *et al.*, (1965) found that, when rats with a three week old primary infection of *N. brasiliensis* were treated with the cortisone derivative betamethasone prior to a shocking dose with antigen, the clinical signs of shock were lessened; due to the timing of the reaction these authors concluded that the effect of cortisone was other than by suppressing antibody production.

Barth et al., (1966) found that ovalbumin-induced anaphylaxis in rats passively immunised with serum to N. brasiliensis produced a significant reduction in their populations of transplanted adult worms compared either to rats which were passively immunised alone or to rats subjected to ovalbumin anaphylaxis alone. They described lesions in the villi of the small intestine which were evident four days after infection with N. brasiliensis larvae and which persisted until the time of self-cure. In these lesions the epithelium becomes separated from the underlying capillaries and the space so formed fills with a protein containing fluid. The lesion was most marked at the tips of the villi, where it gave rise to instability or actual shedding of the overlying epithelium; it also extended down the lateral faces of the villi but was absent from the crypt area. The extent of the lesion was proportional to the dose of larvae and the large volume of induced fluid caused stretching of the epithelial sheet. A similar, but transient, lesion was found in ovalbumin-shocked rats. A severe lesion was produced by the intravenous injection of N. brasiliensis antigen into rats hyperimmune to the parasite and they concluded that the production of the lesion was necessary in self-cure since it would allow antibody to come into contact with the worms. They did, however, suggest two other possibilities: that hypersensitivity might not primarily be concerned with leak production but have some other obscure role or that anaphylaxis might be merely coincidental and not play a beneficial role in the self-cure response.

That anaphylaxis in itself will not bring about worm expulsion (Barth *et al.*, 1966) is hardly surprising. In a primary infection the host is stimulated continuously from the time the worms enter the small intestine until the time of expulsion - the host's inflammatory response mounts and the accompanying morphological changes become more severe until the environment has changed to such an extent that the worms can no longer survive. Anaphylaxis is a transitory phenomenon: it occurs and then it is over; mast cell degranulation occurs when the shocking dose is given and there is a temporary inflammation but, for sufficient changes to occur which will lead to the elimination of the parasite it is suggested that there must be a continuing stimulation over a period of time which is not supplied by an anaphylactic shock. The lesions described by Barth *et al.*, (1966) occurring during a primary infection and two days after adult worm transplant are but part of the changes arising as a result of the host's inflammatory response and are, in themselves, not responsible for worm elimination. However, when anaphylaxis is induced in the presence of large quantities of antiserum from multiply infected rats as was done by Urquhart *et al.*, (1965), specific antibodies can then utilise the lesion, pass into the intestine, and cause worm elimination by directly acting on the worms; it is suggested, however, that this is an artificial situation which does not occur during a primary infection.

Since the preliminary experiments reported in this study on

the use of antihistaminic or antiserotonin compounds to affect the course of N. brasiliensis infections, there have been a number of reports dealing with the action of compounds affecting mast cell constituents. Murray et al., (1971c) found that the antihistaminic Phenergan and the anti-serotonin 501C67 prevented the onset of the rapid phase of worm expulsion when administered separately; this effect was considerably more pronounced when they were administered together. Jarrett et al., (1968a) found that cortisone suppresses the mast cell response and inhibits self-cure and Murray et al., (1971b) showed that increased mucosal permeability did not occur in cortisone-treated rats.

Rothwell et al., (1971), working with Trichostrongylus colubriformis in guinea pigs, found that promethazine, mepyramine, the α -hydrazino analogue of histidine and reserpine substantially reduced or inhibited the rejection of the parasite while methysergide and α -methyl dopa had no effect; oral administration of histidine and 5-hydroxytryptamine during the 4th larval stage gave significantly lower egg and worm counts than infections in untreated controls. They concluded that biogenic amines play a role in worm expulsion.

Keller and Ogilvie (1972), studying the effects of a wide range of compounds on the expulsion of N. brasiliensis, found that the chronic administration of histamine (which increases vascular permeability),

compound 48/80 (which degranulates mast cells), prednisolone (which has multiples effects), phenylbutazone (an anti-inflammatory compound), isoprenaline or theophylline (which increase cellular 3', 5',c-AMP) inhibit the expulsion of transferred "damaged" adult worms; the expulsion of these worms was accelerated by treatment with the histidine decarboxylase inhibitor, p-toluene sulphonyl hydrazine. Mepyramine (a histamine antagonist), UML 419 (a serotonin antagonist), oxyphenonium bromide (which decreases motility of the gut) and saline had no effect. They also found that treatment with histamine, compound 48/80 and prednisolone delayed worm expulsion in rats infected with larvae while saline and cyproheptadine (a serotonin and histamine antagonist) had no effect. The four main objections raised by these workers to the experimental approach used by them and by others investigating amine function in worm expulsion are not in themselves sufficient to conclude that amines are not involved in worm expulsion. These objections will be discussed below but it should be pointed out that they apply equally as well to drawing any conclusions that amines are involved in worm expulsion.

Their first objection was that the drugs used may exert their action by interfering with mechanisms other than those being investigated. Compound 48/80 reduces circulating reagin titres in N. brasiliensis infected rats; Promethazine inhibits lymphocyte action as well as worm expulsion (Kelly and Dineen 1972b) although, as was mentioned earlier, it may not be possible to separate this effect from an effect on mast cells:

Glucocorticosteroids which have consistently been shown to inhibit worm expulsion have a wide range of effects (Zurier and Weissman, 1973). The second objection raised was that drugs which inhibit amines do not consistently affect worm expulsion. They suggested that this inconsistency might be explained by faults in administration of drugs which resulted in the failure of drug action at the parasitised site; but it was equally likely that the inhibitory drugs also affect mechanisms not involving amines. Keller and Ogilvie (1972) found that compound 48/80 inhibited the expulsion of N. brasiliensis; they had expected it to accelerate the expulsion if amines played a role in self-cure. On the other hand, Karmanska et al., (1973) found that this compound accelerated the expulsion of Trichinella spiralis from the rat intestine, but that the dosage and timing of administration were critical to obtain this effect. Other objections raised were that effective drugs are all given over a prolonged period of time and at high levels and also that, with the exception of their report (Keller and Ogilvie, 1972) drugs known to affect release mechanisms in general (Stormorken, 1969) have not been investigated (i.e. experiments have been biased to obtaining an effect on amines; effects on the release of other pharmacologically active substances have not been considered). Their final objection was that it is possible that the release mechanisms being affected are those of the parasite as well as those of the host.

These objections are all valid but there is, perhaps, a more basic objection to the administration of pharmacologically active compounds to study a possible role for biogenic amine compounds in

the self-cure reaction. The degranulation of mast cells and release of their constituents up to the time of self-cure is not an isolated event from two points of view: 1) degranulation is a continuing process which starts as soon as the worms attach to the intestinal mucosa, if not before; this process increases in intensity until around the time of self-cure when few, if any, mast cells are present in the intestinal wall in areas where worms are found, and

2) from a physiological point of view, mast cell degranulation is only one of a continuous series of events, albeit an important one since this reaction initiates the inflammatory response; furthermore, it must be remembered that, while inflammation is part of a basic defence mechanism whose purpose is to isolate and eliminate damage and/or the causative agent, it is in itself a pathological process whose effects must be limited by control mechanisms in the animal (Götsz and Kato, 1970).

To what extent do the effects of administering pharmacological compounds once or twice a day resemble the changes occurring in response to the parasite as the worm presumably provides stimulation continuously? Apart from a few isolated observations that oedema is reduced after treatment with several of the compounds mentioned above (eg. Murray *et al.*, 1971c), we have no idea of the condition of the intestine of the rat under treatment in comparison to the condition of that organ during infection with *M. brasiliensis*. Until we have a far greater knowledge of these changes, this author is in agreement with Keller and Ogilvie (1972) that drug treatment is an unsatisfactory technique from which to

draw any conclusions concerning the role of biogenic amines.

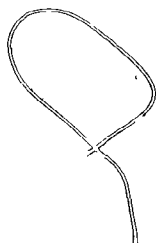
It is interesting to note, however, that prednisolone, which delays worm expulsion in N. brasiliensis infected rats (Keller and Ogilvie, 1972), brings about an improvement in the jejunal histology of humans suffering from coeliac sprue (Wall et al., 1970).

In a series of experiments, E.E.E. Jarrett and associates (1966, 1968, 1969; Jarrett and Urquhart, 1969; Jarrett, 1971) found a degree of immunological unresponsiveness in young rats up to 6 or 7 weeks of age. Worm expulsion commenced later and proceeded more slowly in the young animals than in adults and a stable population of around 200 worms was left as compared with 40 in adults, providing infective doses of less than 500 N. brasiliensis larvae were given. With infective doses greater than 500 larvae, however, worm expulsion occurs, albeit at a slower rate and commencing a day or so later than in adult rats (Murray, 1972). It is suggested that the inability of young rats to eliminate their worm burdens when infective doses less than 500 larvae are used is due to a combination of fewer mast cells in the wall of the small intestine (Murray, 1972), a wider spread of the worm population in the young rats (Murray, 1972) and the small number of worms actually present. At any given point in the intestine of these animals there are not sufficient worms present to cause the host to mount an inflammatory reaction of sufficient intensity to change the mucosa to the extent required for worm expulsion or, if such a response can be

mounted, with so few worms being present they can move to a previously uninhabited region of the intestine. When doses of more than 500 larvae are given, the host can mount an inflammatory response capable of causing parasite elimination and, as more worms are present, the worms in inflamed regions cannot reestablish in uninfected areas. That expulsion takes longer to occur than in adult rats and takes place more slowly could be due to the young rats possessing fewer mast cells and consequently they would take longer to mount an inflammatory response sufficient to eliminate the worms.

When rats are exposed to a number of small daily infections of N. brasiliensis larvae over a period of weeks, what are known as 'trickle' infections, the infection is cumulative and does not elicit an acute immune response from the host. (Jenkins and Phillipson, 1971). As with low infections in young rats it is suggested that this lack of expulsion is due either to there being too few worms in any given part of the intestine (Jenkins, 1973) to stimulate an inflammatory reaction of sufficient intensity to change the environment to bring about expulsion or, if such a reaction does occur, there are sufficient alternate suitable sites for the worms to reestablish and continue to live because the density of the worm population is so low.

Finally, let us take a look at challenge infections. In challenge infections fewer larvae reach the small intestine (Africa, 1931; Love et al., 1974) and the adult worms are expelled earlier



and at a faster rate (Jarrett et al., 1968b) than in primary infections. It is proposed that the mechanism of expulsion of challenge infections is basically that suggested for a primary infection. Challenge infections are normally given shortly after a primary infection when mast cell levels in the wall of the small intestine are well above normal and it is suggested that this enables the host to mount a faster and more intense inflammatory reaction which causes an earlier and faster expulsion of the worms. As reagins are present in the circulation up to seven months after a primary infection (Ogilvie, 1967) it may be that these also participate in the release of amines from mast cells. It is suggested that "protective" antibodies probably do not play a part as they are not present in high enough concentration to have any effect on the parasite.

CONCLUSION

It is suggested that the self-cure of N. brasiliensis infections is the result of the inflammatory response in the host's intestine and that the associated morphological changes in the mucosa render the environment unsuitable for the continued existence there of the parasite. These changes are probably not antibody mediated and the alteration in the structure of the worms are degenerative changes consequent to the changed environment. It is not suggested that this mechanism is responsible for the elimination of all other intestinal parasites although, as similar changes occur in other intestinal parasitic infections, this may well be so. It is suggested that N. brasiliensis is particularly sensitive to modifications in the environment; other parasites may be better able to adapt to environmental changes and, thus, some other mechanism would be necessary. Although it would be convenient to have one mechanism for the elimination of all intestinal parasites, considering the complexities of individual host-parasite relationships and comparing these to other host-parasite relationships with differences in hosts, life cycles, behaviour and feeding habits etc., it is, perhaps, too much to expect that there be but one mechanism.

The advantage of the hypothesis proposed here, as compared, to others, is that it utilises events which occur before and at the time of self-cure and which are attributable to the host's basic defence response-inflammation; this response is mediated by the amines released

from the mast cells which degranulate while the worms are in the intestine. The two other hypotheses which are currently propounded involve antibody action. The supporters of these theories base their evidence for antibody involvement on the action of serum or cells taken either from multiply infected animals or animals after they have undergone self-cure; it is proposed that treatment with such material superimposes on the inflammatory response additional factors which can enhance self-cure; these tactics do not, however, reproduce conditions as they exist during an active infection.

It would seem that the rise in mast cell numbers is a consequence of the acute inflammatory reaction. The animal loses its mast cells by degranulation and, in trying to return to normal, overproduces mast cells: a 'rebound effect'. What better way of eliminating the excess of mast cells than by excreting them into the intestinal lumen as globule leucocytes? During the rise in mast cell numbers and the mast cell to globule leucocyte conversion, the inflammation in the intestine subsides and the structure of the mucosa returns to normal; the presence of large numbers of eosinophils in the intestine at this time may help by antagonising the effects of the amines released.

Studies of the morphological changes occurring in rats where worm elimination does not occur, e.g. in lactating rats, would help clarify the involvement of mast cells in self-cure. However, if similar morphological changes also occur in these animals in the absence of self-cure we are faced with other questions. What amount of change is necessary

for elimination? In severe infections there is total elimination of the villi, but this amount of change is probably not necessary for worm elimination. In animals, such as lactating rats, which do not expel their worms there may be additional factors favouring the retention of worms which are not present in nulliparous rats.

SUMMARY

- 1) Preliminary experiments using compounds with an antihistaminic and/or an anti-serotonin effect were discontinued because no effect on the course of infection was observed and because of the wide variation in results and the adverse effects the compounds had on the health of the rats.
- 2) The cellular changes occurring in the bone marrow, peripheral circulation and in four regions of the small intestine were studied in rats infected with either 1000 or 5000 N. brasiliensis third stage larvae with respect to eosinophils, basophils and mast cells. The course of the infection was followed by daily faecal egg counts and worm counts on the 5th, 9th, 12th, 16th, 23rd and 30th days after infection.
- 3) Egg counts reach a peak 7 days after infection with 1000 larvae; this peak is maintained until the 10th day. On days 10 and 11 there is a slight drop in egg numbers, followed by a rapid drop thereafter. At the higher level of infection, peak egg production is again reached on the 7th day but this level is maintained only until the 8th day. On days 9 and 10 there is a slight drop in egg production followed by a rapid drop. At both levels of infection egg production has virtually ceased by the 16th day.
- 4) Worm numbers in the small intestine reach a peak 5 days after infection with 1000 larvae and remain constant until the 12th day.

Between days 12-16 most of the worms are lost from the intestine.

Worm numbers at the 5000 level of infection reach a maximum after day 5.

Between days 9-12 approximately 30% of the worms present on day 9 are lost from the intestine with most of the remainder being lost between days 12-16. At both levels of infection the small residual population after day 16 occupies a region close to the stomach which is not inhabited by the worms while the main worm population is present. The residual population consists largely of male worms.

- 5) Worm distribution at the peak of infection on day 9 is essentially the same at both levels of infection, with the greatest concentration of worms being in regions c and d; the population at the 5000 level of infection occupies a greater length of the small intestine.
- 6) Eosinophil numbers in the bone marrow and peripheral circulation are above control levels throughout the experimental period at both levels of infection. They reach a peak after 9 days at both levels of infection; this level is maintained until the 16th day in rats infected with 5000 larvae. At the lower level of infection there is a fall in numbers between days 9-16 to levels also reached on the 23rd day by animals infected with 5000 larvae; the numbers then remain constant until the 30th day.
- 7) Basophil numbers are above control levels from the 9th day after infection at both infection levels in the bone marrow and peripheral circulation apart from the 16th day at the higher level of infection where they return to normal in the bone marrow. The magnitude of the response is the same at both levels of infection.
- 8) In the small intestine mast cell numbers are below normal at the 5000 level of infection when worm expulsion begins in the four regions of

intestine examined; at the 1000 level of infection they are below normal in region 30 and at normal levels in the other three regions; worm expulsion commences when mast cell levels are below, or at, control levels. Mast cell numbers rise above normal levels, and globule leucocytes appear in large numbers on the 16th and 23rd days after infection at the 5000 and 1000 levels of infection respectively. The extent of the mast cell changes depends on the number of worms present being more pronounced at the higher level of infection; at both levels of infection these changes are most pronounced in region 30, which is the region of maximum worm density.

- 9) Eosinophil numbers are above control levels in all regions of the intestine at both levels of infection apart from region three where, at the higher level of infection, these cells return to normal on the 9th day. Maximum numbers of eosinophils were found at both levels of infection 16 days after inoculation and were maintained until the 30th day. Changes at both levels were most pronounced at the site of maximum worm concentration.
- 10) A new hypothesis is proposed to explain the loss of worms in which the inflammatory and associated morphological changes in the mucosa of the infected small intestine render the environment unsuitable for the worms' continued existence. The non-antibody mediated degranulation of mast cells while worms are present in the intestine releases the vasoactive amines histamine and serotonin which are mediators of the inflammatory response.

- 11) It is suggested that the rise in mast cells is a consequence of the loss of mast cells by degranulation during the acute inflammatory response while worms are present in the intestine and that this rise plays no part in worm expulsion.
- 12) It is suggested that the mast cell to globule leucocyte conversion which occurs during the rise in mast cells numbers is a process by which the host eliminates from the body mast cells which have been produced in excess of the animals requirements.
- 13) It is suggested that eosinophils act as antagonists of histamine and/or serotonin and so limit the effects of the inflammation.

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ADDENDUM

Since the work of Kelly and Dineen (1972a), who reported the enhancement of self-cure to N. brasiliensis in recipient rats by the transfer of lymph node cells from donors infected with N. brasiliensis, there have been a number of reports implicating lymphocytes in worm expulsion. Keller and Keist (1972) found that "damaged" worms were expelled earlier from irradiated rats following the transfer of lymph node cells from uninfected donor rats; no expulsion occurred, however, if the donor's lymph node cells were irradiated in vitro before transfer. They also reported that lymph node cells from uninfected adult rats stimulated the expulsion of the parasite from young rats which normally keep their parasite burden longer than adult animals. Kelly and Dineen (1972a), however, had previously found that lymph node cells from uninfected rats had no effect when transferred to adult rats. Kelly et al. (1973) reported that immune lymphocytes initiated expulsion of "damaged" worms in both non-irradiated recipients and in recipients irradiated with 400 rad but not in recipients irradiated with 750 rad. Kelly and Dineen (1972b) found that the functional activity of lymph node cells in inducing self-cure on transfer was reduced by treating recipients or donors with promethazine, and later Kelly and Dineen (1973) obtained a similar effect by treating donors or recipients with prolactin. Dineen and Kelly (1972) showed that lymph node cells from nulliparous females induced expulsion of the parasite when transferred to lactating rats.

Dineen et al. (1973) found that lymph node cells from infected rats, on transfer to recipients given "normal", "damaged" or "adapted" worms, induced the expulsion of "normal" and "damaged" worms but had

little effect on the "adapted" worms and Dineen and Kelly (1973) found that lymph node cells from adults induced expulsion of the parasite on transfer to neonatal recipients.

Jones and Ogilvie (1971) suggested a two-step process in the self-cure of nippostrongylosis, involving an initial step in which antibodies damage the parasite followed by a second step which expels the parasite. Results obtained by these authors (Dineen and Kelly, 1972; Keller and Keist, 1972; Kelly and Dineen, 1972a, b; Dineen et al., 1973; Kelly and Dineen, 1973) following the transfer of lymph node cells, implicated lymphocytes in the second step and Kelly et al. (1973) suggested the presence of a third step which might be of myeloid origin. Smith et al. (1974) have recently indicated the possible involvement of prostaglandins in expulsion and Kelly (1974) has suggested that the trigger to a prostaglandin response at the site of infection may be the interaction of immune lymphocytes with a suggested bone marrow derived component.

A second hypothesis to explain the self-cure of nippostrongylosis suggests that worm antigens stimulate the production of specific antibodies and that among the antigens which stimulate the immune mechanism there are also allergens which induce the formation of reagins; these reagins attach to mast cells in the lamina propria and, on contact with allergen, cause the discharge of vasoactive amines resulting in increased mucosal permeability. Increased permeability would permit the passage of antiworm-antibody through the intestinal wall at the sites of attachment of the parasite and result in their

elimination (Jarrett et al., 1970; Miller, 1971a, b; Miller and Jarrett, 1971; Murray et al., 1971a). This hypothesis was expanded by Murray et al. (1971b) to include a third stage in which there is a stimulus, or stimuli, from the parasite which causes the synchronous development of new populations of mast cells, IgE-producing plasma cells and plasma cells producing other classes of antibodies capable of an antiworm effect. Keller (1971), studying the intestinal mast cell response, and Keller and Ogilvie (1972), studying the effect of a wide range of compounds on the self-cure reaction, have suggested that the hypothesis of Jarrett's group is not correct since vasoactive amines are unlikely to be involved in the self-cure reaction. Kelly and Ogilvie (1972) had found that the fall in mast cell numbers which occurs during the early stages of the infection was similar in both lactating and nulliparous rats infected with N. brasiliensis and Connan (1973) reported that the rise in intestinal mast cell numbers and the appearance of globule leucocytes occurred at the same time and was of the same order of magnitude in infected lactating and nulliparous rats. Connan (1974) subsequently found that in lactating rats, which do not expel their worm burdens, the worm population migrates to a position close to the stomach, this migration being inhibited by daily treatment with betamethasone. Jenkins (1974) found a similar migration in neonatal rats which also do not expel N. brasiliensis.

Although it has been shown that lymphocytes might be involved in the self-cure reaction there is no direct evidence as to their exact role in this phenomenon. In the present study it is suggested that lymphocytes might transform into mast cells which then initiate or augment the normal anti-parasite inflammatory reaction in the small

intestine and, thus, enhance expulsion; treatment with such compounds as prolactin or promethazine may act by preventing or hindering this transformation and, thus, prolong the survival of the parasites in their habitat. Results from the present work indicate that the mast cell response in any given region of the infected small intestine is dependent on worm density; it is suggested that, when studying the response in lactating or neonatal rats, the distribution of worms in the small intestine, their density in the region being studied and the mast cell concentration in that region must be ascertained before a meaningful comparison can be made between the cellular response in these animals and in normal rats.

A characteristic of experimental animals is that they do not respond uniformly to stimuli which elicit an eosinophil response. Litt (1973) found that, in guinea-pigs, histamine intraperitoneally injected elicited systemic eosinophilia and haemocyanin injected into footpads induced eosinophilia of draining lymph nodes but no systemic eosinophilia. He found that the strain, sex and age of the guinea-pigs and the dose level were contributing factors in determining whether histamine elicits systemic eosinophilia. An individual animal which responded to one agent was not necessarily more prone to respond to the other and neither agent provoked eosinophilia in all animals. In the case of histamine, the incidence of response in various groups used ranged from 0-88% and he also found that it was important to take into account, in calculations of relative cell changes, the marked fluctuation in counts which occur in normal untreated control groups.

Early studies of the eosinophil response to antigen and/or antibodies were made using whole rabbit or guinea-pig sera which are mixtures of antibodies with differing biological activities. Litt (1967), Kay (1970) and Parish (1970) have shown that, when purified guinea-pig globulins are used, the ability to prepare tissues for eosinophilia is found to be a property of anaphylactic sensitising IgG₁ (γ 1) antibodies and not of the non-anaphylactic sensitising IgG₂ (γ 2) antibodies. IgG₁, alone or complexed with antigens, mediates generalised and localised eosinophilia in passive anaphylaxis in vivo whereas IgG₂-antigen complexes, which are completely ineffective in vivo, are much more effective in attracting eosinophils in vitro than those containing IgG₂ (Parish, 1970). Parish (1972) found that eosinophils accumulate in the anaphylactic skin around changed mast cells and that the number of eosinophils in anaphylactic skin reflects the numbers present in the blood at the time of challenge; the number of blood eosinophils did not increase between the time of challenge and the time of sampling. He suggested that eosinophils are not selectively attracted to sites of anaphylaxis but enter them with neutrophils in a relation equivalent to their relative proportions in the blood; eosinophils are, however, selectively retained in anaphylactic or anaphylactoid tissue, while neutrophils continue to emigrate.

In the intestinal phase of trichinosis as many as 30% of the lymphocytes in the thoracic duct are large pyroninophilic cells which, when injected into syngeneic donors, induce an increase in

blood eosinophils 4-8 days later (Basten and Beeson, 1970). The selective destruction of large lymphocytes abolished this effect and serum and lymphocyte-free lymph from rats with trichinosis did not induce eosinophilia in rats (Basten and Beeson, 1970). Kinetic studies on the eosinophil response of rats infected with Trichinella spiralis have shown that the peripheral eosinophilia of trichinosis reflects increased production in the bone marrow (Spry, 1971a, b). Spry (1972) has suggested that eosinophilia can be increased by the local interaction between lymphocytes and eosinophil precursors.

Specific thymus-dependent lymphocytes have been implicated in the direct induction of eosinophilia (Basten et al., 1970; Basten and Beeson, 1970; Walls et al., 1971) and in the production of a factor generated by the interaction of antigen and specific lymphocytes. The factor in turn reacts with homologous antigen-antibody complexes and becomes chemotactic for eosinophils (Cohen and Ward, 1971). Colley (1973) found that eosinophil migration is stimulated by the exposure of eosinophil-rich peritoneal cell populations from Schistosoma mansoni-infected mice to soluble schistosomal egg antigens. Supernatant fluids from serum-free lymph node cell cultures stimulated with antigens to which the donor mice had been specifically sensitised, or stimulation with phytohaemagglutinin, also induced eosinophil migration. The active principle in such culture supernatant fluids is soluble, non-dialysable and heat stable (56°C/30 minutes) and Colley (1973) has tentatively classed it as a lymphokine and designated it 'eosinophil stimulation promoter' (ESP).

Two chemotactic factors have been described which selectively attract eosinophil leucocytes from a mixed cell population. The first, (ECF-C), requires the presence of an intact complement system and is generated by incubating fresh guinea-pig serum with antigen-antibody complexes prepared from homologous IgG₁ or IgG₂ (Kay, 1970). The second factor, an eosinophil chemotactic factor of anaphylaxis (ECF-A) is released following antigen challenge of actively-sensitised lung cells or from lung cells passively sensitised with IgG₁ (Kay et al., 1971). The release of ECF-A does not require complement and it is further distinguished from ECF-C by its different molecular weight. Kay et al. (1973) found that ECF-A and C5a were chemotactic for both eosinophils and neutrophils. When eosinophils comprise more than about 10% of a mixed leucocyte population they are preferentially attracted by both ECF-A and C5a; a marked synergism was observed between the two in their ability to attract leucocytes.

Kay et al. (1971) and Wasserman et al. (1974a) have demonstrated that ECF-A exists preformed in human and guinea-pig lung cells and also in isolated rat mast cells; in the latter it resides in association with the granules. Wasserman et al. (1974b) have shown that the cells responsible for the immune release of ECF-A contain adrenergic and cholinergic receptors and are modulated by cyclic nucleotides in the same manner as the immunerelease of histamine and SRS-A.

The effect of X-irradiation on tissue mast cells has been the subject of several conflicting reports. Smith and Lewis (1953) reported that a lethal dose of irradiation induced a series of

striking changes in hamster and mouse tissue mast cells which led, finally, to their disruption. Hamaoka et al. (1973) found that the injection of IgE antibody-forming spleen cells into irradiated syngeneic mice induced a higher titre of IgE antibody in the recipient than was present in the serum of the donor of these cells. These authors proposed that depletion of mast cells in irradiated recipient mice might be one of the reasons for the high IgE titre. However, Kojima and Ovary (1974) found that lethal doses of irradiation in mice do not demonstrably change either the mast cell membrane receptors for antibody or the amount of vasoactive components contained in the cell nor do they inhibit the release of these substances during anaphylactic reactions.

Ackerman (1963) and Sampson and Archer (1967) showed that the histamine in basophils is located in the granules of these cells and that these granules are morphologically identical in both mast cells and basophils. The granules are produced in the Golgi complex and mature by the reorganisation and condensation of the granule contents (Chan, 1969). This latter author (1972) demonstrated that the ultrastructural changes which occur in guinea-pig bone marrow basophils during anaphylaxis are related to the severity of the reaction; degranulation is the result of the reaction of antigen with cytophilic reaginic antibodies which have a selective affinity for the basophil cell membrane. Guinea-pig bone marrow basophils from sensitised animals degranulate when challenged with specific antigen regardless of whether or not the animals have received an injection of the histamine antagonist, mepyramine maleate (Chan, 1974). The degranulation is most pronounced in the first five

minutes following challenge; the discharged granules are taken up by neutrophils, macrophages and mononuclear leucocytes in the bone marrow, with the neutrophils responding most actively and promptly. Phagocytosis is most active 10-15 minutes after challenge and the discharged granules have disappeared completely from the bone marrow one hour after challenge; this removal is accomplished by digestion in the bone marrow as well as by the dispersal of the granules through the sinusoids into the circulation.

Ishizaka et al. (1970, 1972) detected IgE molecules on the surface of basophils by autoradiography, a finding which has been confirmed by Sullivan et al. (1971) with the electron microscope. Ishizaka et al. (1973) estimated that the average number of IgE molecules per basophil is between 10-40,000. A rough inverse relationship was observed between the number of cell-bound IgE molecules and the optimum concentration of anti-IgE required for maximum histamine release from the cells. Their results suggested to them that the number and affinity of cell-bound IgE molecules probably determine the sensitivity of basophils to degranulation by allergens.

Studies have revealed that cellular hypersensitivity is not a homogeneous entity and that several forms of delayed onset lymphocyte-mediated hypersensitivity reactions in animals and man are characterised by substantial infiltrations of basophils. Such reactions have been designated "cutaneous basophil hypersensitivity" (CBH) by Inderbitzin (1961) to distinguish them from the true delayed type hypersensitivity (DTH), epitomised by the tuberculin reaction, in which basophils are

characteristically relatively infrequent. CBH-type reactions (the so-called "Jones-Mote" reactions) can be induced to a variety of protein antigens; basophilia also characterises the lesions of contact allergy, hypersensitivity to vaccines and viruses and skin allograft rejection in guinea-pigs. While true delayed reactions may also contain some basophils, these cells constitute only a small fraction of the total infiltrate (Dvorak et al., 1970). Goldman et al. (1973) have shown that there is a direct correlation between histamine concentration and basophil frequency in CBH. Dvorak and Dvorak (1972) have suggested that the action and function of mast cells and basophils may be modified by sensitised lymphocytes or their products, possibly without the need for homocytotropic antibody. Evidence that sensitised lymphocytes are involved in CBH comes from Richerson et al. (1970) who found that the reaction could be passively transferred with sensitised lymph node cells but not with immune serum and that the reaction could be eliminated by treating animals with anti-lymphocyte serum before skin testing. Stadecker and Leskowitz (1974) induced basophil-rich skin reactions in guinea-pigs with local injections of several mitogens and suggested that T-cells may mediate such reactions by releasing a factor chemotactic for basophils.

Colvin et al. (1973) showed that CBH is probably elicited only soon after sensitisation and that it is superseded by another form of cutaneous reaction with a more rapid time course, termed the "late reaction". This latter reaction is composed of three sequential,

but overlapping, phases which include cutaneous anaphylaxis, Arthus reactivity and a residual CBH component. In "late reactions" in guinea-pigs ^{125}I -HSA-antibody complex is concentrated by basophils and, less commonly, by eosinophils. Whereas the anaphylactic and, to a lesser extent, the Arthus component of the "late reaction" can be passively transferred with small quantities of hyperimmune serum, the CBH component can not be transferred; hyperimmune serum given before immunisation does not interfere with the induction of CBH reactivity, as measured by basophil infiltration; this reaction is not immunologically specific.

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APPENDIX

I

Table 1. Compound 48/80; eggs/gm/faeces ($\times 10^{-2}$) and worm counts
(2 rats/cage)

Day	6	7	8	9	10	11	12	13	14	15	Worms	
<u>Infection Control</u>												
	31	65	37	13	136	141	24	28	2	0	137	62
	118	140	247	101	268	135	102	9	4	0	173	56
	33	147	138	88	139	61	13	5	0	0	23	80
	64	37	75	100	98	25	2	0	0	0	57	72
	30	73	62	31	113	55	39	8	15	0	138	101
<u>Solvent Control</u>												
	69	171	198	75	60	161	72	16	10	0	211	81
	72	252	322	348	267	169	177	22	3	0	72	112
	72	0	**									
	122	152	143	44	98	493	122	15	9	*	32	92
	65	116	315	36	343	*	*	*	73	0	51	40
<u>Compound 48/80</u>												
	21	74	42	35	172	113	34	9	10	1	139	237
	33	85	100	69	100	111	82	5	11	0	111	61
	119	202	313	724	471	258	217	39	1	1	38	93
	17	92	57	72	82	95	155	14	9	11	141	102
	46	63	47	64	128	88	58	16	6	1	47	29
	49	106	44	49	66	239	116	3	12	3	132	117
	8	22	88	4	119	88	172	26	0	**		

* faeces available; ** both animals died

Table 2. Reserpine: eggs/gm/faeces (x10) and worm counts

Day	6	7	8	9	10	11	12	13	14	15	Worms
<u>Infection control</u>											
	213	273	294	311	239	20	13	3	1	0	189
	160	342	514	285	409	375	117	88	19	3	76
	68	124	203	134	155	20	9	2	1	0	117
	44	188	185	194	234	91	15	1	1	0	39
	77	116	120	256	188	98	37	9	1	0	64
	66	70	120	179	126	105	62	50	50	10	131
<u>Ether control</u>											
	95	231	121	153	151	55	15	2	2	0	139
	102	201	136	384	311	345	99	76	21	1	90
	23	146	256	156	179	110	68	52	36	15	101
<u>Solvent control</u>											
	39	209	268	227	395	335	230	47	40	0	80
	149	172	206	262	461	157	180	124	33	0	105
	28	283	169	133	56	28	26	10	2	0	36
	48	140	219	722	697	287	**				
	42	47	61	123	265	*	128	43	22	4	209
	61	177	57	321	325	459	14	1	**		
<u>Reserpine</u>											
	157	377	124	347	195	28	6	*	0	0	119
	100	158	219	147	302	249	204	70	19	1	76
	156	126	157	147	228	31	39	*	1	**	
	150	241	203	173	327	54	*	8	6	2	161
	75	186	166	163	210	152	46	13	5	0	113
	33	195	59	197	211	81	26	7	1	1	53
	63	101	185	209	566	28	304	508	3	0	32
	41	83	70	46	132	1	**				
	37	162	10	103	110	119	**				

*no faeces available;

** animal died

Table 3. Polymyxin B: eggs/gm/feces ($\times 10^{-2}$) and worm counts

Day	6	7	8	9	10	11	12	Worms
-----	---	---	---	---	----	----	----	-------

Infection Control

44	135	216	161	156	144	80	189
7	99	372	122	280	145	20	312
31	68	78	88	114	109	28	163
13	33	67	48	55	37	8	133
27	37	65	56	127	29	29	231
54	155	241	185	231	22	0	297
64	68	75	100	95	11	7	157
130	95	284	220	223	67	26	372
63	150	224	122	233	76	5	246

Solvent Control

12	41	86	33	73	38	7	181
29	48	69	31	74	59	13	150
32	159	**					
46	41	145	42	120	24	14	162
74	81	128	55	91	124	14	211
103	160	406	189	294	150	8	279
59	160	334	74	225	11	37	294
86	124	140	40	125	29	9	232
17	42	151	**				

Polymyxin B

13	10	60	39	63	38	4	170
21	108	37	48	69	63	2	321
34	268	227	145	242	32	22	284
49	330	368	64	217	46	7	169
87	2	156	44	8	98	10	247
104	899	202	7	204	160	5	143
65	59	75	16	7	**		
84	**						
17	37	**					

** animal died

Table 4. Worm counts by sex per region of gut in rats infected with 1000 *N. brasiliensis*

		Day 5		Day 9		Day 12		Day 16		Day 23		Day 30	
		M	F	M	F	M	F	M	F	M	F	M	F
I	a	0	0	0	0	0	0	14	5	0	0	0	0
	b	5	5	0	1	0	3	2	0	0	0	0	0
	c	59	53	35	49	112	28	0	1	0	0	0	0
	d	60	36	29	170	125	138	0	0	0	0	0	0
	e	0	0	0	0	0	1	0	0	0	0	0	0
II	a	0	0	0	0	2	8	10	1	0	0	0	0
	b	0	0	2	3	23	8	18	1	1	0	0	0
	c	4	2	135	151	45	34	9	0	0	0	0	0
	d	108	132	67	56	144	147	2	0	0	0	0	0
	e	0	0	0	0	1	4	2	1	0	0	0	0
III	a	0	0	0	0	3	6	17	10	4	0	0	0
	b	3	10	7	11	2	2	7	0	0	0	1	0
	c	56	118	80	118	47	65	2	0	0	0	2	0
	d	102	159	106	125	148	191	3	4	0	0	0	0
	e	7	2	0	1	0	0	2	2	0	0	0	0
IV	a	0	0	0	0	7	11	9	0	3	0	0	0
	b	34	32	3	1	9	6	20	0	5	2	4	0
	c	182	100	110	157	32	41	6	1	3	0	2	0
	d	89	47	207	215	186	240	28	0	0	0	0	0
	e	0	0	0	1	0	3	2	0	0	0	0	0
V	a	0	1	0	0	23	9	51	0	8	0	3	0
	b	2	6	0	0	33	20	6	1	14	0	1	0
	c	80	101	68	82	65	44	6	4	0	0	2	0
	d	283	251	295	309	219	207	1	0	2	0	1	1
	e	0	0	2	0	12	47	0	0	0	0	0	0
VI	a	0	0	1	4	1	0	56	2	15	0	1	0
	b	20	22	9	11	2	3	36	1	18	10	7	0
	c	306	322	132	165	64	70	25	4	1	4	0	0
	d	97	114	282	293	154	157	22	52	0	1	1	0
	e	0	0	0	3	0	2	0	4	0	0	0	0

I, II, III, IV, V and VI: Replicates; a,b,c,d and e: Regions of intestine; M: male; F: female

Table 5. Worm counts by sex per region of gut in rats infected with 5000 *N. brasiliensis*

		Day 5		Day 9		Day 12		Day 16		Day 23		Day 30	
		M	F	M	F	M	F	M	F	M	F	M	F
I	a	0	0	0	0	131	110	3	0	0	0	0	0
	b	78	85	5	5	196	92	0	0	0	0	0	0
	c	17	30	284	336	62	30	2	0	0	0	0	0
	d	339	284	406	340	51	58	0	0	0	0	0	0
	e	42	11	3	1	3	4	0	0	0	0	0	0
II	a	0	0	68	53	40	22	3	5	1	0	1	0
	b	0	10	130	78	66	61	16	8	0	0	1	0
	c	36	40	99	112	329	326	1	0	0	0	2	0
	d	888	744	876	1407	515	362	0	0	0	0	0	0
	e	5	3	1	0	28	102	0	0	0	0	0	0
III	a	12	5	0	1	25	7	4	4	3	1	14	0
	b	283	274	9	4	50	15	0	0	0	0	0	0
	c	720	695	231	241	207	283	5	1	0	0	3	0
	d	2	0	1058	1230	485	859	34	22	0	0	0	0
	e	119	100	26	25	28	85	14	9	0	0	0	0
IV	a	0	0	0	0	34	6	47	46	4	2	7	0
	b	27	18	1	3	23	10	0	0	1	1	11	0
	c	449	647	315	401	206	323	0	0	0	0	1	0
	d	757	1006	1012	1327	775	610	0	0	0	0	0	0
	e	2	1	10	14	53	75	0	0	0	0	0	0
V	a	0	0	2	2	41	4	43	1	8	7	4	0
	b	73	72	90	79	216	172	67	1	4	3	12	17
	c	140	158	546	732	364	462	66	3	0	0	0	0
	d	991	1515	1011	1369	408	428	89	5	0	0	0	0
	e	0	0	2	6	32	57	2	1	0	0	0	0
VI	a	0	1	2	0	84	60	13	7	78	6	28	3
	b	36	90	274	234	16	16	14	3	64	3	13	1
	c	250	279	1002	1080	168	223	1	0	24	1	0	0
	d	563	684	632	807	893	1352	0	0	22	5	1	0
	e	13	7	8	13	17	29	0	0	0	0	0	0

I, II, III, IV, V and VI: Replicates; a,b,c,d and e: Regions of gut; M: male; F: female

Table 6. Eggs/gm/faeces ($\times 10^{-2}$) in rats infected with
1000 N. brasiliensis

Day	6	7	8	9	10	11	12	13	14	15	16	17	18
I	34	233	105	51									
	58	62	219	208	227	74	80						
	42	77	167	173	128	48	208	67	104	13	8		
	24	97	154	28	93	53	104	66	49	14	4	0	0
	11	66	108	54	73	46	101	28	6	1	0	0	0
II	33	91	116	99									
	37	64	93	51	85	99	66						
	16	66	267	236	155	134	32	20	3	0	0		
	28	229	315	296	315	177	169	125	18	2	0	0	0
	18	225	245	201	219	155	24	10	0	0	0	0	0
III	33	135	76	89									
	89	154	292	277	495	239	102						
	68	139	313	281	266	216	195	33	5	1	0		
	33	31	195	170	167	162	98	132	12	3	0	7	6
	62	196	326	151	307	171	222	80	52	0	2	2	5
IV	129	246	261	318									
	73	245	211	274	330	93	43						
	97	180	260	192	228	201	193	146	4	6	0		
	125	287	287	249	255	143	96	36	0	0	0	0	0
	48	149	204	282	338	55	14	1	0	0	0	0	0
V	57	128	154	72									
	15	60	105	74	150	146	95						
	42	130	187	89	205	96	42	13	13	1	0		
	72	145	132	21	123	98	64	26	52	0	0	0	0
	48	214	352	66	166	157	42	56	10	0	0	0	0
VI	52	227	111	176									
	76	228	243	172	311	153	119						
	92	217	193	182	179	196	48	17	4	1	0		
	66	185	171	203	193	122	63	9	0	0	0	0	0
	44	219	272	237	201	93	116	5	1	0	0	0	0

I, II, III, IV, V and VI: Replicates

No eggs were found on day 5 or after day 18.

Table 7. Eggs/gm/faeces ($\times 10^{-2}$) in rats infected with
5000 *N. brasiliensis*

Day	6	7	8	9	10	11	12	13	14	15	16
I	54	638	1079	664							
	84	918	1392	1246	448	465	651				
	74	404	489	411	467	27	151	14	2	1	0
	80	340	1065	358	178	59	16	2	1	0	0
	60	695	940	703	328	92	814	333	95	5	1
II	91	1002	2297	977							
	12	402	385	73	38	6	0				
	137	1163	1996	841	1230	666	115	23	2	0	0
	80	554	801	457	337	41	1	0	0	1	0
	115	293	1154	397	751	440	105	24	0	0	0
III	15	860	445	698							
	188	719	722	363	53	17	3				
	111	584	1077	953	1370	670	212	7	2	0	4
	146	792	1382	1132	1057	833	658	5	0	0	0
	73	1199	1069	663	1053	680	89	5	3	0	0
IV	83	194	1554	981							
	139	1431	1333	1072	970	75	17				
	145	1233	1412	398	170	377	75	5	0	0	0
	308	1701	1454	1121	1113	26	1	0	0	0	0
	334	1201	837	1065	862	51	45	1	0	0	0
V	210	301	460	222							
	480	544	597	350	852	278	70				
	422	791	362	288	584	721	394	293	1	0	0
	164	858	1048	321	635	213	2	2	0	0	0
	239	642	358	179	301	19	3	3	13	1	1
VI	835	1713	1540	1402							
	176	806	1231	932	1225	320	121				
	220	681	725	985	463	115	57	2	0	0	0
	500	602	740	740	260	75	8	0	0	0	0
	310	593	684	684	94	12	5	2	0	0	0

I, II, III, IV, V and VI: Replicates
No eggs were found on day 5 or after day 16

Table 8. **Mast cell counts per region of intestine (160 fields at 800x) from uninfected control rats**

Day		5	9	12	16	23	30
I	5	289	296	275	252	241	357
	20	277	269	262	248	225	267
	30	218	240	262	373	262	272
	60	213	283	317	430	333	249
II	5	266	381	300	243	293	333
	20	259	316	290	282	232	244
	30	255	357	313	301	296	283
	60	212	217	319	355	343	303
III	5	259	404	317	314	314	307
	20	221	426	288	308	256	258
	30	302	501	320	242	375	283
	60	239	216	412	321	294	320
IV	5	447	316	290	367	328	266
	20	446	271	239	229	395	238
	30	293	237	308	273	309	224
	60	291	233	362	383	317	233
V	5	481	295	240	280	310	301
	20	459	279	322	302	346	243
	30	250	288	300	344	344	274
	60	245	260	317	312	253	250
VI	5	453	250	184	286	297	295
	20	409	284	330	360	336	296
	30	306	325	302	303	408	314
	60	238	263	311	291	259	279

I, II, III, IV, V and VI: Replicates

5, 20, 30 and 60: Regions of intestine

Table 9. Mast cell counts per region of intestine (160 fields at 800 x) from rats infected with 1000 *H. brasiliensis*

Day	5	9	12	16	23	30
I	5	111	97	457	1265	2065
	20	5	215	171	1949	934
	30	1	152	59	1921	1447
	60	33	342	102	2020	1496
II	5	237	89	136	1424	1500
	20	56	2	24	1645	944
	30	181	179	9	2211	1215
	60	92	289	158	2015	1261
III	5	138	95	323	781	587
	20	64	94	19	1488	619
	30	18	6	9	1917	953
	60	212	7	88	2074	854
IV	5	222	26	161	857	2668
	20	163	59	385	6	1954
	30	64	41	448	28	1904
	60	152	100	242	69	1924
V	5	265	132	41	985	2533
	20	209	3	246	1733	1581
	30	173	1	87	2223	1246
	60	159	114	286	1786	1204
VI	5	217	85	330	1037	1204
	20	51	57	317	1925	917
	30	174	2	260	2349	1050
	60	186	59	343	1666	856

I, II, III, IV, V and VI: Replicates

5, 20, 30 and 60: Regions of intestine

Table 10. Mast cell counts per region of intestine (160 fields at 800x) from rats infected with 5000 *N. brasiliensis*

Day	5	9	12	16	23	30
I	5	172	127	406	1579	2353
	20	5	16	35	3267	2644
	30	25	0	0	4170	3466
	60	115	141	93	2329	1535
II	5	65	46	135	666	1875
	20	162	66	0	676	2151
	30	28	0	245	787	2912
	60	88	0	1	430	1973
III	5	93	77	204	1779	1602
	20	126	86	0	1681	1929
	30	10	0	0	2134	2758
	60	55	28	233	1367	1776
IV	5	105	34	9	1174	1274
	20	65	73	69	1291	1999
	30	9	0	23	1797	2733
	60	84	0	3	858	1699
V	5	94	58	65	1318	1877
	20	102	17	44	1272	2002
	30	73	1	7	2309	2746
	60	116	2	2	1087	1763
VI	5	132	46	31	1012	1515
	20	29	8	128	848	1803
	30	14	2	16	1249	2706
	60	67	44	31	1042	1743

I, II, III, IV, V and VI: Replicates

5, 20, 30 and 60 : Regions of intestine

**Table 11. Globule leucocyte counts per region of intestine
(160 fields at 800x) from rats infected
with 1000 or 5000 *N. brasiliensis***

	Day	1000 Infection				5000 Infection			
		12	16	23	30	12	16	23	30
I	5	0	0	35	816	1	321	539	3
	20	0	0	152	233	0	1225	650	104
	30	0	0	94	185	0	1989	1120	1
	60	0	0	28	107	0	651	36	76
II	5	0	0	236	277	0	207	746	708
	20	0	0	381	207	0	178	812	422
	30	0	0	781	227	0	226	1231	333
	60	0	0	136	22	0	1	461	23
III	5	0	0	134	1	0	625	713	3
	20	0	0	277	12	1	636	849	1
	30	0	0	497	91	0	848	1309	53
	60	0	0	374	44	1	687	638	15
IV	5	0	0	110	622	0	343	538	488
	20	0	0	279	434	0	646	955	1390
	30	0	0	597	417	0	728	1395	1242
	60	0	0	185	209	0	437	709	172
V	5	0	0	236	456	0	596	747	43
	20	0	0	324	386	0	554	880	36
	30	0	0	512	141	0	1017	1289	48
	60	0	0	504	115	0	512	741	35
VI	5	0	0	427	420	0	324	694	625
	20	0	0	535	247	0	329	744	124
	30	0	0	692	283	0	585	1287	534
	60	0	6	407	101	0	378	900	21

No globule leucocytes were found in the uninfected control rats or in the infected groups on days 5 and 9.

I, II, III, IV, V and VI: Replicates
5, 20, 30 and 60: Regions of intestine

Table 12. Eosinophil counts per region of intestine (160 fields at 800x) from uninfected control rats

Day	5	9	12	16	23	30
I	5	195	184	159	193	213
	20	169	176	213	214	225
	30	201	207	253	213	267
	60	242	172	251	208	251
II	5	169	195	148	191	194
	20	285	212	171	207	207
	30	188	207	267	218	267
	60	203	182	212	215	226
III	5	172	172	153	202	183
	20	344	262	158	197	222
	30	158	234	243	222	231
	60	160	178	196	194	211
IV	5	263	192	167	243	211
	20	193	184	173	295	206
	30	246	176	172	246	197
	60	292	183	188	310	206
V	5	237	259	167	208	200
	20	194	226	197	296	211
	30	213	271	202	221	211
	60	240	179	211	213	211
VI	5	184	296	144	199	195
	20	213	259	199	239	239
	30	194	333	203	190	199
	60	197	196	185	197	196

I, II, III, IV, V and VI: Replicates

5, 20, 30 and 60 : Regions of intestine

Table 13. Eosinophil counts per region of intestine (160 fields at 800x) from rats infected with 1000 N. brasiliensis

Day	5	9	12	16	23	30	
I	5	332	388	389	756	718	454
	20	372	581	739	918	599	949
	30	304	405	604	959	788	600
	60	408	296	839	645	1148	446
II	5	591	263	621	750	631	543
	20	829	296	704	493	833	699
	30	792	453	1158	483	898	463
	60	1159	352	824	657	820	734
III	5	704	510	385	432	773	424
	20	734	463	644	702	729	517
	30	721	369	592	770	838	472
	60	723	643	664	355	860	799
IV	5	361	370	349	864	568	901
	20	239	400	449	787	685	639
	30	307	414	489	773	771	827
	60	410	368	907	580	736	1825
V	5	366	187	558	614	640	998
	20	682	302	399	1417	684	805
	30	694	274	534	1056	788	648
	60	635	329	757	671	680	783
VI	5	359	316	412	538	826	760
	20	589	331	427	464	721	638
	30	445	330	762	745	703	534
	60	354	279	668	592	738	679

I, II, III, IV, V and VI: Replicates
 5, 20, 30 and 60 : Regions of intestine

Table 14. Eosinophil counts per region of intestine (160 fields at 800x) from rats infected with 5000 *N. brasiliensis*

Day	5	9	12	16	23	30	
I	5	458	471	388	884	639	494
	20	446	749	617	1134	694	284
	30	390	261	825	1338	623	380
	60	572	848	724	494	679	630
II	5	414	632	541	480	495	459
	20	955	357	579	937	517	524
	30	767	599	598	604	620	596
	60	681	555	522	250	486	992
III	5	484	617	448	1033	418	831
	20	406	599	636	1087	511	930
	30	366	178	546	1121	804	1146
	60	507	495	647	696	524	1017
IV	5	279	379	592	702	670	645
	20	555	330	461	738	656	669
	30	511	147	359	948	654	722
	60	351	670	533	635	682	502
V	5	428	351	446	866	698	446
	20	424	359	488	1171	751	649
	30	428	121	520	1199	829	514
	60	422	159	385	750	731	512
VI	5	262	372	899	771	632	624
	20	396	501	961	913	768	551
	30	404	334	896	1100	806	458
	60	341	359	599	791	781	576

I, II, III, IV, V and VI: Replicates
 5, 20, 30 and 60: Regions of intestine

Table 15. Eosinophils, as a percentage of the total leucocyte count, in the peripheral circulation of uninfected control rats

Day	0	5	9	12	16	23	30
I	3.00	2.64					
	2.02	2.28	2.68				
	1.76	2.21	2.24	2.33			
	2.38	2.69	2.26	2.07	2.04		
	3.04	2.52	2.33	1.87	2.41	2.30	
	2.34	2.57	2.43	2.25	1.88	2.26	2.65
II	1.72	2.49					
	1.99	2.47	2.07				
	1.27	2.56	1.99	1.84			
	2.00	2.53	1.69	1.90	2.04		
	2.24	1.99	2.17	2.04	2.14	1.90	
	2.15	1.69	2.13	2.14	1.82	1.86	1.82
III	2.43	1.91					
	2.61	2.70	2.13				
	1.79	2.16	1.95	2.11			
	2.77	2.42	1.90	2.53	2.84		
	3.02	1.61	2.18	2.90	2.43	2.00	
	2.23	2.19	1.97	2.51	3.59	1.95	1.86
IV	1.71	3.33					
	1.56	2.11	2.25				
	2.40	1.82	2.11	2.35			
	1.51	1.46	2.37	3.49	2.86		
	2.03	2.08	2.01	3.19	1.83	1.71	
	2.96	2.20	2.70	2.78	2.58	2.23	1.79
V	1.94	2.27					
	2.30	1.49	2.12				
	2.16	1.90	2.07	1.79			
	1.93	1.51	1.94	1.88	2.48		
	2.20	1.86	2.05	3.05	3.11	2.33	
	2.14	2.70	1.89	3.72	2.61	4.10	2.82
VI	2.35	2.25					
	3.14	2.67	2.53				
	1.61	2.63	2.43	2.07			
	1.27	2.70	2.58	1.81	2.20		
	3.05	2.48	3.42	1.46	2.25	2.32	
	3.34	2.22	2.05	2.36	2.10	2.01	2.90

I, II, III, IV, V and VI: Replicates

Table 16. Eosinophils, as a percentage of the total leucocyte count, in the peripheral circulation of rats infected with 1000 N. brasiliensis

Day	0	5	9	12	16	23	30
I	2.45	3.47					
	2.80	3.97	8.27				
	3.19	4.18	6.38	3.47			
	2.18	3.25	6.75	3.59	4.31		
	2.55	3.90	6.70	5.03	4.17	3.34	
	1.86	2.74	4.81	3.76	3.48	3.75	2.99
II	2.24	3.34					
	1.57	2.89	4.21				
	2.12	3.64	4.45	3.76			
	2.45	3.95	4.14	3.73	3.33		
	2.07	2.97	3.91	3.39	3.55	3.87	
	2.00	3.67	3.91	3.64	3.57	3.10	3.18
III	1.40	2.50					
	1.50	2.75	6.09				
	2.00	2.99	5.68	3.71			
	2.13	3.54	4.97	3.76	4.49		
	2.30	3.87	5.18	4.08	3.53	2.95	
	2.00	3.15	4.09	3.33	4.31	3.55	2.71
IV	2.42	3.66					
	2.37	3.52	5.66				
	2.78	3.94	5.61	4.90			
	2.00	2.80	3.51	2.81	3.38		
	1.67	2.44	2.46	3.94	4.06	3.66	
	1.72	2.55	3.44	3.55	3.47	3.06	2.80
V	3.30	3.68					
	1.71	3.39	5.09				
	2.10	3.26	3.58	3.76			
	2.18	4.24	4.66	4.50	3.72		
	2.47	3.62	5.43	4.59	3.05	3.80	
	2.75	4.55	5.93	3.64	3.96	4.22	3.68
VI	2.10	4.45					
	1.90	3.13	4.14				
	2.24	3.49	3.76	3.66			
	1.82	3.61	4.58	2.92	3.33		
	3.72	4.02	4.59	3.04	3.76	3.34	
	2.77	4.50	5.93	3.70	3.64	3.80	3.33

I, II, III, IV, V and VI : Replicates

Table 17. Eosinophils, as a percentage of the total leucocyte count, in the peripheral circulation of rats infected with 5000 *N. brasiliensis*

Day	0	5	9	12	16	23	30
I	1.53	3.47					
	1.52	2.65	7.01				
	1.70	3.77	5.68	5.31			
	1.87	3.12	4.80	5.48	6.07		
	1.74	3.34	6.76	5.81	5.08	3.23	
	1.89	4.13	5.01	5.12	4.05	3.40	3.70
II	1.53	2.65					
	1.81	3.79	5.41				
	2.19	3.68	6.96	5.87			
	1.54	3.47	8.03	8.06	7.25		
	1.81	4.23	7.47	8.65	6.38	3.94	
	2.11	3.29	4.73	4.46	3.91	2.86	3.19
III	1.95	3.31					
	2.58	2.77	7.87				
	2.05	3.81	7.17	8.17			
	1.84	3.88	5.45	4.91	4.39		
	1.76	3.55	5.63	6.06	5.08	2.97	
	2.23	3.96	6.39	6.84	5.77	3.40	3.55
IV	2.11	4.17					
	1.86	3.68	5.70				
	2.24	3.53	5.43	6.87			
	1.66	2.89	7.79	6.73	4.14		
	1.78	3.72	7.47	7.14	5.99	3.20	
	2.34	3.34	6.20	5.40	6.26	3.53	3.72
V	2.77	4.49					
	2.12	3.82	4.02				
	1.83	3.19	5.88	4.89			
	1.71	3.07	6.55	5.58	4.56		
	1.93	4.67	5.24	4.62	4.97	3.45	
	1.90	3.82	5.77	4.64	3.67	3.76	3.42
VI	1.86	3.54					
	2.12	3.42	5.01				
	1.85	4.72	5.10	5.67			
	2.01	4.58	6.52	6.27	6.44		
	1.63	2.91	3.68	3.33	3.71	3.49	
	2.26	3.39	6.12	5.28	4.04	3.39	3.48

I, II, III, IV, V and VI: Replicates

Table 18. Basophils, as a percentage of the total leucocyte count, in the peripheral circulation of uninfected control rats and rats infected with 1000 or 5000 *H. brasiliensis*

Uninfected controls

Day	0	5	9	12	16	23	30
I	0.78	0.89	1.23	0.76	1.20	0.77	0.61
II	1.07	1.20	0.94	1.22	1.09	1.56	1.34
III	1.16	0.94	0.70	0.69	0.66	0.85	0.87
IV	0.61	0.85	0.60	0.96	1.11	0.79	0.91
V	1.12	1.03	1.15	0.62	0.78	0.63	1.22
VI	0.96	0.80	0.83	1.20	0.98	1.07	0.62

1000 Infection

Day	0	5	9	12	16	23	30
I	0.65	0.80	1.49	2.49	2.63	2.10	2.00
II	1.11	1.22	1.96	2.00	1.99	2.49	2.30
III	0.85	0.67	2.12	2.24	1.86	1.74	2.00
IV	1.22	1.13	1.75	2.23	1.46	2.05	1.78
V	0.53	1.01	1.21	2.42	1.40	1.79	1.80
VI	0.87	0.75	1.44	1.76	1.70	2.40	2.50

5000 Infection

Day	0	5	9	12	16	23	30
I	0.67	0.82	2.17	2.58	1.48	2.55	2.45
II	1.13	0.94	1.49	2.07	1.60	2.31	2.50
III	1.06	0.78	1.40	2.46	1.69	1.75	2.60
IV	0.96	1.18	2.21	2.10	1.55	2.31	1.73
V	0.61	1.16	1.25	1.92	1.57	2.04	2.14
VI	0.96	1.00	1.35	1.85	2.22	1.96	1.92

I, II, III, IV, V and VI: Replicates

Table 19. Eosinophils and Basophils/1000 nucleated cells in the bone marrow of uninfected control rats and rats infected with 1000 or 5000 M. brasiliensis

<u>EOSINOPHILS</u>																		
<u>Day</u>	5	9	<u>Control</u>				5	<u>1000 Infection</u>					5	<u>5000 Infection</u>				
			12	16	23	30		9	12	16	23	30		9	12	16	23	30
I	47	31	43	45	39	47	108	131	103	90	64	97	142	135	115	130	51	61
II	43	40	37	45	38	45	123	136	99	79	87	72	98	147	116	117	87	69
III	30	34	42	47	34	47	107	140	121	104	81	97	109	136	161	114	77	83
IV	37	37	43	42	34	37	76	125	118	81	86	68	98	141	168	124	67	74
V	34	44	41	41	41	33	104	133	120	76	101	77	98	150	121	114	68	80
VI	37	43	40	40	42	43	104	142	108	82	98	60	89	129	136	140	122	67

<u>BASOPHILS</u>																		
<u>Day</u>	5	9	<u>Control</u>				5	<u>1000 Infection</u>					5	<u>5000 Infection</u>				
			12	16	23	30		9	12	16	23	30		9	12	16	23	30
I	5	8	5	7	8	6	5	13	12	15	21	10	13	15	14	3	11	20
II	8	4	6	7	6	9	8	11	16	16	18	13	4	17	20	9	21	15
III	8	7	6	3	6	7	7	23	16	10	15	16	14	23	17	7	27	11
IV	8	8	7	5	2	4	8	10	20	6	20	11	3	19	14	6	13	15
V	6	6	4	9	9	5	14	15	23	24	17	16	5	9	17	9	10	17
VI	9	5	9	8	3	7	10	17	17	5	10	29	12	12	13	8	18	14

I, II, III, IV, V and VI: Replicates

Table 20. Intestinal worm numbers: statistical analysis for days 5, 9 and 12 following infection with 1000 or 5000 *N. brasiliensis* after transformation of the worm counts (x) to log x.

Source of variance	d.f.	var. est.	F
Replicates	5	0.1762	23.1842***
Infections	1	3.8481	506.3289***
Time	2	0.0634	16.6711***
Infections x Time	2	0.0120	1.5789
Residual error	25	0.0076	

***P < 0.001.

Table 21. Intestinal worm distribution; statistical analysis
for days 5, 9 and 12 following infection with 1000
or 5000 *N. brasiliensis* after transformation of the
number of worms per 1% length of the intestine(x) to $\log(x + 1)$.

Source of variance	d.f.	var. est.	F
<u>Main Plots</u>			
Replicates	5	0.2634	3.1621*
Infections (I)	1	8.8922	106.7491***
Time (T)	2	0.5107	6.1309**
I x T	2	0.1609	1.9316
error (a)	25	0.0833	
<u>Sub-Plots</u>			
Length (L)	4	13.1986	102.1563***
T x L	8	0.5738	4.4412***
L x I	4	0.2433	1.8831
L x I x T	8	0.1033	
error (b)	121	0.1292	

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table 22. Faecal egg counts: statistical analysis for days 6 - 12 following infection with 1000 or 5000 N. brasiliensis after transformation of the actual count(x) to log x.

Source of variance	d.f.	var. est.	F
<u>Main Plots</u>			
Blocks	9	0.3333	
Infections	6	2.8167	3.0329*
error (a)	45	0.9287	
<u>Sub-Plots</u>			
Time	9	4.4944	191.1414***
Infections x Time	54	0.3677	15.6468***
error (b)	264	0.0235	

* $P < 0.05$

*** $P < 0.001$

The actual count(x) when multiplied by 100 gives the number of eggs/gm faeces.

Table 23. Intestinal mast cell numbers: statistical analysis
after transformation of the number of cells(x) per
160 fields at 800x to log x.

Source of variance	d.f.	var. est.	F
<u>Main Plots</u>			
Replicates	5	0.1262	
Infections (I)	2	1.262	4.4343*
Time (T)	5	19.1851	67.4108***
Infections x Time	10	7.0437	24.7495***
error (a)	85	0.2846	
<u>Sub-Plots</u>			
Parts of gut (G)	3	1.4395	9.2632***
T x G	15	0.5725	3.684 ***
G x I	6	0.625	4.0219***
G x I x T	30	0.2667	1.7162**
error ^a (b)	271	0.1554	

*P<0.05

**P<0.01

***P<0.001

Table 24. Intestinal globule leucocyte numbers: statistical analysis for days 16, 23 and 30 following infection with 1000 or 5000 *N. brasiliensis* after transformation of the number of cells (x) per 160 fields at 800x to log x.

Source of variance	d.f.	var. est.	F
<u>Main Plots</u>			
Replicates	5	1.056	1.453
Infections (I)	1	28.765	39.567***
Time (T)	2	20.664	28.424***
Infections x time	2	27.264	26.537***
error (a)	25	0.727	
<u>Sub-Plots</u>			
Parts of gut (G)	3	0.803	4.511**
T x G	6	0.064	
G x I	3	0.128	
G x I x T	6	0.113	
error (b)	91	0.178	

**P<0.01

***P<0.001

Table 25. Intestinal eosinophil numbers: statistical analysis
after transformation of the number of cells (x) per
160 fields at 800x to log x.

Source of variance	d.f.	var. est.	F
<u>Main Plots</u>			
Replicates	5	0.0084	
Infections	2	9.4155	339.9098***
Time	5	0.4109	14.8339***
Infections x time	10	0.1247	4.5*
error (a)	85	0.0277	
<u>Sub-Plots</u>			
Parts of gut (G)	3	0.0428	4.7033***
T x G	15	0.0375	4.1209***
G x I	6	0.0212	2.3296*
G.x.I x T	30	0.0165	1.8132***
error (b)	271	0.0091	

*P < 0.05

***P < 0.001

Table 26. Eosinophils in the peripheral circulation: statistical analysis of the percentage of eosinophils (x) after transformation of (x) by the arcsine $\sqrt{\text{percentage}}$ transformation

Day 0

Source of variance	d.f.	var. est.	F
Replicates	35	0.795 ^a	1.01
Infections	7	4.173	4.96*
Residual error	70	0.787	

Day 5

Source of variance	d.f.	var. est.	F
Replicates	35	0.794	
Infections	2	57.353	51.60***
Residual error	70	1.111	

Day 9

Source of variance	d.f.	var. est.	F
Replicates	29	1.346	
Infections	2	262.707	171.68***
Residual error	58	1.53	

Table 26 (continued)

Day 12

Source of variance	d.f.	var. est	F
Replicates	23	1.460	1.07
Infections	2	161.684	118.73***
Residual error	46	1.362	

Day 16

Source of variance	d.f.	var. est	F
Replicates	17	9.993	
Infections	2	77.178	68.80***
Residual error	34	1.222	

Day 23

Source of variance	d.f.	var. est	F
Replicates	11	1.035	3.16*
Infections	2	18.78	57.32***
Residual error	22	0.328	

Day 30

Source of variance	d.f.	var. est	F
Replicates	5	0.710	2.03
Infections	2	7.156	20.46***
Residual error	10	0.349	

*P < 0.05

**P < 0.01

***P < 0.001

Table 27. Basophils in the peripheral circulation: statistical analysis of the percentage of eosinophils (x) after transformation of (x) by the arcsine/percentage transformation

Source of variance	d.f.	var. est	F
Replicates	5	1.199	3.13*
Infections (I)	2	44.997	117.66***
Time (T)	6	13.515	35.34***
I x T	12	3.844	10.05***
Residual error	120	0.382	

* $P < 0.05$

*** $P < 0.001$

Table 28. Eosinophils in the bone marrow: statistical analysis of the actual counts (number of eosinophils per 1000 nucleated cells)

Source of variance	d.f.	var. est.	F
Replicates	5	127.6	
Infection	2	50,746.0	268.00***
Time	5	4,352.4	22.99***
Infection x Time	10	1,656.5	8.75***
Residual error	85	189.3	

***P < 0.001

Table 29. Basophils in the bone marrow: statistical analysis of the actual counts (number of eosinophils per 1000 nucleated cells)

Source of variance	d.f.	var. est.	F
Replicates	5	14.5	
Infection	2	667.5	39.90***
Time	5	91.2	5.45***
Infection x Time	10	41.4	2.48*
Residual error	85	16.7	

*P < 0.05

***P < 0.001

Randolph's fluid (Randolph, 1949) for the staining of eosinophilsStock solution A

Methylene blue	0.1 gm
Propylene glycol	50 ml
Distilled water	50 ml

Stock solution B

Phloxine	0.1 gm
Propylene glycol	50 ml
Distilled water	50 ml

Both stock solutions were prepared by first mixing the propylene glycol and distilled water and then adding the dye. The working solution consisted of equal volumes of the two stock solutions; after the two stock solutions were combined they were filtered. The working solution was unstable and was always used within two hours of preparation. By this method nuclei were stained blue and eosinophil granules a bright red.

Staining of Basophils^a (Hunt and Hunt, 1959)

Stock Solution

M/15 Na_2HPO_4	9.29 ml
m/15 KH_2PO_4	0.71 ml
Isotonic saline	5.00 ml
0.1% toluidine blue	5.00 ml
Absolute ethanol	5.00 ml

The stock solution must be kept well stoppered and refrigerated; to 5 ml of the stock solution are added 0.4 ml of freshly prepared saponin solution made by dissolving 0.3 gm in 1 ml of distilled water. The mixture of stain and saponin must be filtered and used at temperatures below 80°F. Nuclei are stained blue and basophils reddish-purple.

END

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