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GUT MOTILITY IN THE EXPULSION OF PARASITES

A thesis submitted for the degree of

Doctor of Philosophy

by

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CONTENTS

SUMMARY

Summary

The aim of this project was to investigate the intestinal motility during infection with Nippostrongylus brasiliensis in the rat, and the possible role of motility in the expulsion of this parasite from the gut.

1. Intestinal propulsive motility was measured in rats infected with 4,000 N. brasiliensis larvae by following the transit of radioactive chromium (^{51}Cr), a non-absorbable marker, through the gut. On days 6, 8, 10, 12 and 14 post-infection, ^{51}Cr was injected through an indwelling catheter into the duodenum. 15 minutes later the animals were killed and the distribution of radioactivity in the small intestine measured. A group of uninfected, catheterised rats served as controls. Intestinal propulsive activity was increased only on day 8 of infection. On day 6, although the overall motility appeared unchanged, propulsion in the upper small intestine may have been reduced. The possible causes of these observations, and their role during infection and/or expulsion is discussed.

2. To examine the effect of artificially stimulating intestinal motility upon an established worm population, a dose of carbachol which caused diarrhoea, but no other marked physical symptoms, was employed. Rats were injected regularly with carbachol on days 5 and 6 post-infection, killed on day 7 and their worm burdens determined. Similarly, another group was treated on days 7 and 8 and killed on day 9. However, increased gut motility, as influenced by carbachol, had no apparent effect upon the worm population size, either before or after the worms had probably sustained some immunological damage.

3. The responses and sensitivity of isolated rat intestinal segments to field stimulation and a number of agonists were examined on days 6 - 20 post-infection with 5,000 N. brasiliensis larvae. In addition, the spontaneous contractile activity of the preparations was recorded. Infection with Nippostrongylus was associated with

a dramatic increase in the maximum contractile responses of intestinal smooth muscle to both electrical stimulation and drugs, which reached a peak around day 14 of infection. The amplitude of spontaneous contractions also increased markedly on days 10, 12 and 14, but this activity was either very erratic or non-existent on days 6 and 8. These changes were not associated with any shift of the dose-% response curves for acetylcholine or carbachol. The dose-% response curve for 5-hydroxytryptamine (5-HT) however, was displaced to the right. This was probably due to desensitization of the preparations, due to the high intestinal levels of 5-HT known to occur during infection. It is not known how these changes in gut sensitivity were produced, and at present it is not possible to assess their relevance to worm expulsion. Their possible causes and significance are discussed.

4. Because of its inhibitory effect upon worm expulsion, one wondered whether the drug betamethasone would also prevent the onset of hypersensitivity of isolated gut preparations from infected rats. This was indeed the case, though betamethasone did not completely abolish the effect. Neither did pretreatment with the drug have any effect upon the sensitivity of the gut smooth muscle from uninfected control rats. It is suggested therefore, that the increased reactivity of the intestine was due to some aspect of the immune response, rather than to a direct action of the worms themselves. However, it is still not known whether supersensitivity plays a role in worm expulsion, or whether it is simply an ineffectual consequence of infection.

5. Supersensitivity similar to that occurring in the rat small intestine during nippostrongylosis has been reported in other tissues following various treatments, and alterations in the blood levels of various hormones have been implicated as a possible cause. It was therfore decided to measure the serum levels of thyroxine and corticosterone during infection in order to determine if any changes occurred. However, no change in the blood level of corticosterone was apparent, and although the thyroxine levels appeared to be lower

during infection, difficulty in the interpretation of this result was encountered since the serum thyroxine levels were also reduced in uninfected, control rats which had received a single saline injection.

6. Alterations in the circulating levels of hormones (or indeed some other chemical mediator) during N. brasiliensis infection render possible the development of supersensitivity of the type described in other smooth muscle tissues not directly concerned with either the parasites or their expulsion. The pressor responses of the vasculature of the rat hind limb preparation were therefore measured during infection, using the α-adrenoceptor agonist, phenylephrine. The maximum response to this drug was significantly increased on day 14, and on days 6, 10 and 20, although apparently greater than control, the maximum responses were not significantly so. As in the gut, this increase in maximum response was not associated with any shift of the dose-% response curve.

GENERAL INTRODUCTION

1. *Nippostrongylus brasiliensis* infection in the rat.

2. Intestinal smooth muscle: function and control.

3. The role of gut motility in parasite infections.

GENERAL INTRODUCTION

1. Nippostrongylus brasiliensis infection in the rat

The gastrointestinal parasite, Nippostrongylus brasiliensis has been extensively used as a model system for studying the immunity to helminths (see reviews by Ogilvie and Jones, 1971; Murray, 1972). The life-cycle of this parasite was first described by Yokagawa (1922), and Haley (1961, 1962) gave a detailed description of the systematics, hosts, geographical distribution and life-cycle of the nematode. The natural hosts of N. brasiliensis are Rattus rattus, Rattus norvegicus, and the mouse, Mus muscularis, but the parasite does not develop so successfully in the latter (Solomon and Haley, 1966).

N. brasiliensis provides a very useful laboratory system in that the experimental techniques are relatively simple, the hosts are cheap and the life-cycle of the parasite is short. As a model in the study of immunity to gastro-intestinal helminths, Nippostrongylus is excellent in that the host's immune response can be measured by worm egg output and the time of onset and rate of worm expulsion, and the constant initial localisation of the majority of the adult worm population in a 4 - 5 cm area of the jejenum facilitates the detailed study of the nature of the changes associated with worm loss. Immunity to N. brasiliensis has been studied perhaps more than immunity to any other nematode. Its popularity is because the life-cycle and the host response resemble those of similar nematodes which are of medical and veterinary importance.

The life-cycle of N. brasiliensis is as follows. The parasite enters the rat percutaneously as a third larval stage (L3) having developed from the egg through L1 and L2 stages outside the body of the rat. (Experimental infections are conveniently produced by subcutaneous injection of a suspension of L3 larvae.) After spending several hours in the hypodermis,

the infective larvae migrate, probably via the blood and lymph, to the lungs (12 - 15 hours post-infection). The exact route of migration is still a matter for speculation (Ogilvie and Jones, 1971).

The larvae grow in the lungs (18 - 32 hours) then moult to give L4 larvae, and sex-differentiation takes place (32 - 46 hours). The L4 larvae migrate up the bronchi and trachea, then via the oesophagus and the stomach, to the small intestine (50 - 60 hours post-infection) where rapid growth occurs. In the small intestine the final moult to the adult worm begins on the third day post-infection, and egg production by the mature worms starts at the end of the fifth day. Like most helminths, N. brasiliensis does not multiply within the host and eggs pass out in the faeces of the rat (950 eggs/female worm/day approximately).

Nippostrongylus is a parasite of the upper part of the small intestine, localized predominantly in the proximal jejenum, in a 4 - 5 cm area, 20 cm distal to the pylorus (Murray, 1972). How the parasites feed is a matter of some controversy, although it has been conclusively shown that they neither suck blood nor do their feeding activities cause much blood-loss into the intestine (Mulligan et al., 1965; Neilson, 1969). It is unlikely that absorption of nutrients occurs via the cuticle (Roberts and Fairbairn, 1965) and therefore the worms probably feed solely by absorption through their gut. Lee (1969a) suggested that the parasite braces itself against the villi, with its cuticular ridges pressed into the villi, and thereby damages the microvilli and outer edge of the epithelial cells of the rat small intestine. In common with this mechanical damage, parasite enzymes are probably poured into the host cells so that the worms can then suck the cellular debris and contents into their intestine via the oesophagus. The digestive enzymes of the parasite appear to be located in the dorsal gland and ampulla of the eosophagus, which have strong non-specific esterase activity, and in the subventral glands of the excretory

system which have strong non-specific esterase, aminopeptidase and cholinesterase activity (Lee, 1969a, 1970).

From the sixth to the tenth days post-infection with N. brasiliensis, the egg production rises rapidly, and from the tenth to the thirteenth day a rapid fall sets in to give very low egg counts by day 13. In parallel with this the worm population found in the proximal jejenum falls gradually between days 10 and 12, and rapidly from day 12 almost to zero by day 20 (Murray et al., 1971a). The timing of the various stages in the life-cycle and expulsion of the worms can vary by one or two days, depending on the sex and strain of the host rats.

The expulsion of N. brasiliensis is termed the 'self-cure' reaction. The term was first used by Stoll (1929) to describe the sudden dramatic fall in faecal egg counts in lambs infected with the ovine parasite Haemonchus contortus. He assumed this to be due to the expulsion of the worm burden. In N. brasiliensis infection the self-cure reaction was first described by Africa (1931) and Taliaferro and Sarles (1937) demonstrated that the loss of parasite burden was not the result of ageing of the worms, but of an immune reaction in the host. These authors showed that acquired immunity to Nippostrongylus muris, as the parasite was then called, was to some extent antibody-based since it was passively transferable. In the intestine there was also evidence of immune pressure in that the worms were stunted. Taliaferro and Sarles also noticed that during the development of immunity, there was a mobilization of host basophils and eosinophils, but the exact function of this was obscure.

The immune expulsion, or 'self-cure' is a phenomenon which occurs in many host/helminth systems but it is probably seen at its most dramatic in rats infected with N. brasiliensis. The self-cure reaction in rats infected with this parasite is characterized by a sudden termination of primary infection which is not associated with a fresh uptake of larvae. After primary infection rats have a strong resistance to further infection.

The mechanisms involved in the immune expulsion of N. brasiliensis from the rat have been widely investigated (see reviews by Ogilvie and Jones, 1971; Ogilvie and Love, 1974; Murray, 1972). The preoccupation of most researchers has been concerned largely with purely immunological explanations of the self-cure phenomenon. Studies have centred around the role of antibodies of different classes, immune cells such as lymphocytes and mast-cells, and local anaphylaxis in the gut, but exactly how the parasite is expelled from the gut is, as yet, obscure.

The role of humoral antibody in the sequence of events has been investigated, and it was demonstrated that rats could be passively immunized by the transfer of immune serum (Chandler, 1938; Neilson, 1965; Sarles and Taliaferro, 1936). It was further shown that when adult parasites were transferred to rats which had been passively immunized with hyperimmune serum, the expulsion was accelerated (Mulligan et al., 1965; Ogilvie and Jones, 1968). However, in these experiments large quantities of hyperimmune serum were needed to produce significant effects. It has also been shown that hyperimmune serum has little effect or none, on adult worms in vitro (Weinstein, 1967). Similarly, the ability of pools of antisera to cause worm expulsion from recipient rats varied enormously. For example, in a series involving 48 large pools of antisera, only one in three pools caused worm rejection. It was also found that pools of antisera prepared from animals which had had a single immunizing infection were, paradoxically, as potent in causing worm expulsion as pools prepared from rats which had been hyperimmunized with several infections (Ogilvie and Jones, 1968).

Thus, the accumulating evidence tended to suggest that the presence of circulating antibody alone was insufficient to cause expulsion and that some other mechanism was required to produce it.

Because of the varying and weak effects of antiserum on the worms, it was suggested that some mechanism might be required to cause an increase in the concentration of anti-worm antibodies round the parasites because their environment is in the lumen of the rat small intestine (Mulligan et al., 1965; Urquhart et al., 1965). As was discussed previously, the parasite does not ingest host blood, and no significant amount of blood is found in the lumen of the rat gut during infection. It was suggested therefore, by Mulligan et al. (1965) that some other mechanism caused an elevated antibody concentration around the worms. Because of the sudden termination of the worm population, these workers proposed that worm expulsion may involve an anaphylactic reaction in the gut. Indeed, Urquhart et al., (1965) showed that rats immunized against N. brasiliensis were very susceptible to systemic anaphylaxis when injected intravenously with a worm extract, and that severe intestinal damage occurred. However, when an anaphylactic reaction was produced in the intestine of infected, sensitized rats using ovalbumin as the antigen, the resulting shock alone did not expel the worms (Barth et al., 1966; Ogilvie, 1967). It was also shown that if hyperimmune serum was administered before the ovalbumin shock, expulsion of the worms occurred. Thus, even although anaphylactic shock had no effect on the worms, and hyperimmune serum caused weak and varying results, both together brought about expulsion.

The 'leak-lesion' hypothesis was proposed (Mulligan et al., 1965; Urquhart et al., 1965; Barth et al., 1966), which stated that the immune expulsion of N. brasiliensis was a two-stage process. It was proposed that the first stage involved hypersensitivity in the immune rat induced by reaginic type antibody, followed by the release of vasoactive amines such as histamine and 5-hydroxytryptamine (5-HT). The amines released by the anaphylactic reaction would cause an increased capillary permeability which in turn would allow

significant amounts of anti-worm antibody to leak into the gut lumen where they would exert a direct action on the parasites. It has in fact been shown that during primary infection with N. brasiliensis, the macromolecular leak into the intestine does increase markedly, reaching a maximum between days 10 and 12 post-infection, corresponding to the time of worm expulsion (Maclean, 1974).

In N. brasiliensis infected rats the development of immunity is closely associated with the appearance of IgE antibodies in the serum (Ogilvie, 1964). These were detected in the serum by passive cutaneous anaphylaxis and by mast-cell degranulation in the presence of worm antigen slightly after the onset of worm expulsion (Ogilvie, 1967; Wilson and Bloch, 1968).

However, it became obvious that the direct or indirect action of IgE on worms could not be the only mechanism concerned with worm rejection since there are certain situations in which these antibodies occur, and yet the worms are not expelled. Neonatal or lactating, infected rats do not expel their worm burdens, and yet the serum IgE levels are similar to those in infected, mature, non-parous rats (Jarrett et al., 1966; Connan, 1973). It has also been demonstrated that rats can be passively immunized by antiserum free from any detectable IgE (Jones, et al., 1970). Further, it has been shown that the antibodies cause structural and metabolic damage to the worms by day 10 of the infection (Ogilvie and Hockley, 1968; Lee, 1969b); i.e. before the macromolecular leak is maximal. For example, Henney et al. (1971) demonstrated metabolic damage to the parasite as early as day 8, reflected as a reduced capacity of worms to take up ^{32}P-labelled phosphate from the host. This metabolic damage increased so that by day 10, the uptake of ^{32}P by the worms was 10% of that on days 6 or 7 post-infection. Maclean (1977) showed that the uptake by the worms of ^{75}Se-methionine and ^{14}C-glucose were similarly reduced, and he proposed that measurement of the uptake of

metabolites by adult worms from the host's tissue fluids might provide a useful index of immunological damage sustained by the parasites.

It was then suggested significant immunological damage might precede the macromolecular leak (Ogilvie and Jones, 1971), the termination of infection involving the expulsion of worms which have been irreversibly damaged by immunological action. However, the mechanisms involved in the actual expulsion of damaged worms from the gut are yet to be elucidated.

One factor which may be of importance is the fact that N. brasiliensis worms secrete acetylcholinesterase (AChE). This enzyme is present in large amounts in the secretory glands of several nematodes including N. brasiliensis(Sanderson, 1969), existing as at least three isoenzymes (Edwards et al., 1971), and there are several possible roles which AChE might play in the host/parasite relationship. However, there is no convincing evidence to support any of them. Firstly, the function of worm-secreted AChE has been suggested to be that of a 'biochemical holdfast', (Ogilvie and Jones, 1971), in that the injection of AChE into the host gut could affect nervous transmission to the intestinal smooth muscle, and thereby prevent local peristalsis which would be of advantage to the parasite in maintaining its position in the villi of the intestinal mucosa. It has also been shown that acetylcholine and its analogues enhance several processes known to be concerned in the immune response such as secretion of chemical mediators from mast-cells and basophils, destruction of target cells by lymphocytes and release of lysosomal enzymes from polymorphs (Kaliner and Austen, 1974). If these latter effects are important in the gut, then it may be that parasite-secreted AChE, by virtue of its action on acetylcholine, reduces the immune response of the host to the parasites.

Changes in the properties of worm AChE occur as infection progresses (Lee, 1970) and it was suggested by Edwards and his co-workers (1971) that the worms are attacked by circulating antibodies, which not only produce structural changes in the worms

themselves, but also in AChE. Anti-AChE antibodies have been found in the sera from immune rats (Jones and Ogilvie, 1972). Thus, antibodies attacking worm AChE may interfere with the 'biochemical holdfast' action and force the worms into the lumen of the gut where the environment is less favourable Ogilvie and Love, 1974). The occurrence of lipid droplets and an accumulation of neutral lipid in antibody-damaged worms (Lee, 1971) supports the hypothesis that antibodies make the worms move into the gut lumen where the oxygen tension is unfavourable to them.

Similarly, if AChE reduces the immune response to the parasite as described above, then antibodies damaging the enzyme may prevent this and allow the immune response of mast-cells, basophils and lymphocytes to proceed. Immunological damage is certainly incurred by the worms before expulsion begins, reflected by a drop in egg production (Mulligan et al., 1965), decrease in metabolite uptake (Henney et al., 1971; Maclean, 1977), damage to the worm gut-cells (Ogilvie and Hockley, 1968; Lee, 1970).

However, if antibody-damage of AChE is important it is unlikely to be sufficient to cause worm expulsion, and a further step is necessary. Recent evidence has been produced which indicates that the sequence of worm expulsion involves damage of the worms by circulating antibodies followed by the action of cells, this cellular action being necessary to produce expulsion. The cooperation between antibodies and cells in immunity to N. brasiliensis was the subject of a recent review (Ogilvie and Love, 1974).

Jones and Ogilvie (1971) criticized the leak-lesion hypothesis of worm rejection because they found that if damaged worms were transplanted into the intestine of rats which had been subjected to total body irradiation, no expulsion took place, even if the irradiated recipients were also treated with immune serum. According to the leak-lesion hypothesis, a local anaphylactic reaction causes a greatly increased permeability of the gut mucosa, thus allowing protective antibodies to leak

rapidly into the gut lumen and act on the worms to cause expulsion. Jones and Ogilvie (1971) claimed that if this were the case, then irradiation, which destroys the integrity of the gut, should also have caused an increased leakage of antibodies into the gut lumen leading to worm expulsion. However, irradiation prevented the expulsion of damaged worms and these authors proposed that a second step in the expulsion sequence, the step which expels the parasite, was the radiosensitive one. They also felt that if anaphylaxis plays a part in expulsion, its action must follow antibody damage. Indeed, it has been shown that when worms were damaged by antibodies (step 1), expulsion (step 2) could be accelerated by anaphylactic shock (Barth et al., 1966).

However, Murray (1972) concluded that it was not necessary to postulate a second step for the above reasons. He suggested that it may be that damaged worms, with the loss of isoenzymes and possibly feeding ability, die rapidly when transferred to normal rats, but when transferred into the slightly 'leaking' intestine of irradiated rats, are able to survive for a longer period in this plasma enriched environment. Murray also found that total body irradiation produced little increased leakage of plasma into the intestinal lumen, whereas in rats undergoing anaphylactic shock, there was a significant increase. Thus, 'total body irradiation mimics neither the macromolecular leak produced by anaphylactic shock nor the massive leak lesion which occurs in naturally infected rats' (Murray, 1972). Similarly, he suggested that a possible reason for Jones' and Ogilvie's failure to accomplish expulsion by immune serum in irradiated rats was that the antibody given may have been eluted into the enlarged extravascular pool created by the generalized leak caused by irradiation, and thus diluted. As was mentioned previously, large quantities of immune serum are required to bring about expulsion.

Nevertheless, further evidence for a second step was produced by Keller and Keist (1972), who showed that injection of normal mesenteric lymph node cells, obtained from

syngeneic donors, into irradiated rats restored the ability to expel damaged worms. If however, the mesenteric lymph node cells were irradiated prior to injection, then they failed to restore the expulsion mechanism. Keller and Keist also found that the effect of irradiation on expulsion could be mimicked in rats which had been thymectomized at birth and treated with antilymphocytic serum (ALS). Similarly, Ogilvie and Jones (1971) showed that ALS suppressed the induction of active immunity as reflected by a prolongation of worm egg output.

As was stated previously, neonatal rats do not expel N. brasiliensis unless a large infection is given, and even then expulsion is very slow (Jarrett et al., 1966; Jarrett, 1971). However, it has been demonstrated that the transfer of normal mesenteric lymph node cells, from syngeneic donors, to neonates restores fully the ability to cause worm rejection (Keller and Keist, 1972). Thus, it was proposed that during infection with N. brasiliensis, antibodies develop first and damage the worms, making them susceptible to the expulsive effect of lymphocytes (Ogilvie and Love, 1974). Further evidence in support of this came when it was found that worms transferred into recipients before they were damaged by antibodies were affected much less rapidly by the expulsive action of lymphoid cells given to the recipient rats, than worms transferred after they had been damaged by antibodies (Dineen et al., 1973). The delay in expulsion of undamaged worms was explained by the proposal that transferred immune cells must first produce antibodies, which caused damage to the worms which are then expelled by the lymphocytes.

The mechanism whereby lymphocytes cause worm expulsion is unknown, and no differences between worms damaged by antibodies and those which are being attacked by lymphocytes have been observed. Worms are not killed by cell action, but simply leave the intestine, and if transferred immediately into the intestine of a non-immune rat will re-establish and behave like antibody-damaged worms (Love et al., 1975).

It has been suggested that lymphocytes act in conjuction with bone marrow-derived cells to cause expulsion of damaged worms, since lymphocytes restore to rats irradiated with 400 rads, the ability to expel worms, but not to rats given 750 R. These latter animals expelled their worm burdens only when given both lymphocytes and bone marrow-derived cells (Dineen et al., 1974a; Dineen and Kelly, 1974). However, Ogilvie and her co-workers (1977) found that the expulsion of antibody-damaged worms from rats irradiated with 750 R could be induced either by thoracic duct lymph cells, or by mesenteric lymph node cells, apparently without the help of bone marrow-derived cells.

It would seem therefore that lymphocytes are certainly important in the expulsion of antibody-damaged worms though, as has been stressed, it is unknown how these cells bring about expulsion. Another cell-type which has been implicated in the mechanisms concerned with self-cure is the mast-cell, and a great deal of work has been carried out concerning the role of mast-cells and amine release in expulsion.

The occurrence of large numbers of mast-cells in the intestinal wall of rats which had become immune to N. brasiliensis was first described by Taliaferro and Sarles (1939). They found an accumulation of connective tissue basophils, now known to be mast-cells, in the gut mucosa, but not the lungs of immune rats.

Many studies have been carried out concerned with the temporal relationship between the sudden onset of mast-cell proliferation in the lamina propria and worm expulsion, since Wells (1962) found that following loss of N. brasiliensis, intestinal mast-cell numbers and histamine levels in the gut increased. A role for histamine in the self-cure phenomenon was first proposed by Stewart (1953), who found that during the expulsion of H. contortus from the sheep intestine, there was a rapid rise in blood histamine, that the wall of the gut became oedematous and that in some cases, expulsion could be inhibited by the administration of antihistamine drugs. He suggested that tha local reaction produced an environment unfavourable to the survival of the parasites.

Several studies have demonstrated that mast-cell numbers in the intestinal wall increase during expulsion of N. brasiliensis and that there is a corresponding increase in the concentration of 5-HT at the site of maximum worm localisation (Murray et al., 1971b). Rat subepithelial mast-cells contain 5-HT (Enerback, 1966; Miller, 1970), and several workers have demonstrated that amines are released from intestinal mast-cells during expulsion (Miller et al., 1968; Murray et al., 1968, 1971b; Jarrett et al., 1970).

For example, 5-HT levels were measured spectrofluorimetrically in the intestine of Nippostrongylus-infected rats, and were shown to be related to intestinal mast-cell numbers and worm-burden kinetics (Murray et al., 1971b). These workers demonstrated that on days 8 - 9, when the worms were resident in the small intestine, very few subepithelial mast-cells were present, and the gut was largely depleted of 5-HT. It has been shown that N. brasiliensis worms secrete a mast-cell degranulating agent (Jarrett et al., 1969; Miller, 1970) and that the subsequent amine discharge from mast-cells creates a local vascular leak in the rat bowel (Jarrett et al., 1968), which may be part of the worms' feeding mechanism (Murray et al., 1971b). Associated with worm expulsion however, at days 10 - 14, there is a marked increase in intestinal subepithelial mast-cell numbers and a corresponding rise in 5- HT levels in the gut wall (Murray et al., 1971b). Using an anthelminthic (thiabendazole), these authors also showed that the mast-cell numbers increased whether the worms were removed or not. However, if the worms were removed, mast-cell discharge of 5-HT did not occur.

In another experiment Murray and his co-workers (1971a) found that female rats expel their worm burdens sooner, and at a faster rate, than males did, and that there is a corresponding difference in the mast-cell response and permeability of the rat gut wall.

It seems therefore, that the onset of the immune expulsion of N. brasiliensis is related to the rise in intestinal mast-cell

numbers and to the discharge of their 5-HT content (Murray, 1972). Ogilvie and Jones (1971) stated that 'the release of amines from mast-cells can be triggered either by the interaction of reaginic antibodies and worm allergen, or by the action of a degranulator produced by the worms which does not require the cooperation of antibodies for its action'. If important in worm rejection, then 'the latter method of amine release explains why the presence of reaginic antibodies is not essential for worm expulsion to occur in passive immunity. In active immunity, the former method of amine release from mast-cells is probably the more effective trigger'.

The role of the vasoactive amines histamine and 5-HT in the self-cure reaction is a matter of much controversy, though it is generally agreed that they play at least a minor role. In order to elucidate the function of histamine and 5-HT in immune expulsion, studies have centred around treating infected rats with the amines themselves, either enterally or parenterally, with drugs such as reserpine or compound 48/80, known to release 5-HT and histamine respectively from mast-cells (Lewis, 1958), and with antagonists of histamine, 5-HT or both such as mepyramine, methysergide or cyproheptadine respectively. It is worth mentioning that even if these amines play a part in the rejection of N. brasiliensis, it may be that they have some role other than in the anaphylactic reaction, since it has been shown that shock will still occur in rats when both histamine and 5-HT levels have been depleted or specifically inhibited by antagonists (Sanyal and West, 1958). These workers concluded that, in the rat, histamine and 5-HT play little, if any part in anaphylaxis, and that anaphylaxis in rats is mediated by other substances, or that antigen-antibody reactions directly damage the susceptible cells.

The role of histamine in N. brasiliensis expulsion in the rat was indicated by using the antihistamine promethazine, which also exhibits some anti-5-HT activity (Goodman and Gilman, 1975). Promethazine partially inhibited worm loss (Urquhart et al., 1965).

In another study on the role of vasoactive amines, Murray et al. (1971c) confirmed the above findings. These workers showed that the daily administration of an antihistamine (promethazine) or an anti-5-HT (501C67) did not prevent the initial slow phase of worm expulsion, or the fall in egg production which always precedes expulsion. However, treatment of infected rats did prevent the onset of the rapid phase of worm loss. Further, if both antagonists were given together, their effects appeared to be additive. If amine-mediated antibody translocation into the gut lumen is necessary to cause worm damage (reflected as a drop in worm egg production) these results were interpreted as showing that amine antagonists do not inhibit small amounts of anti-worm antibody initiating worm damage, or the first gradual phase of worm loss, but they prevent the rapid phase of worm loss. It was suggested therefore, that the rapid phase of expulsion must be mediated, at least partly, by histamine and 5-HT. Murray et al. (1971c) also found that these drugs did not prevent the increase in the number of mast-cells or their discharge, but did inhibit the effects of the amines which the mast-cells released. The intestinal oedema normally seen in untreated, infected rats was not observed in treated rats. Similarly, Sharp and Jarrett (1968) demonstrated that reserpine, a drug which depletes mast-cells of 5-HT, inhibited the expulsion of N. brasiliensis, again suggesting a role for amines in the rejection mechanism.

A further study on the action of promethazine in N. brasiliensis expulsion attempted to elucidate the mechanism whereby the drug inhibited worm loss (Kelly and Dineen, 1972). It was found that the functional activity of immune mesenteric lymph node cells, as assessed by their capacity to cause worm rejection, was reduced by treatment of cell recipients with promethazine. In addition, lymphocytes from promethazine-treated donors were markedly less effective than cells from

untreated donors, when transferred to recipients. Kelly and Dineen (1972) suggested that promethazine may inhibit resistance to N. brasiliensis by an immunosuppressive action at the lymphoid level either in addition to, or instead of, antagonism of amines. This hypothesis was confirmed by the finding that when immune lymphocytes were incubated with serum from promethazine-treated rats, their ability to transfer immunity was seriously impaired. Thus, in addition to its antagonism of histamine, promethazine may have an immunosuppressive activity at the lymphoid level.

Nevertheless, conflicting evidence as to the roles of histamine and 5-HT in worm expulsion has been obtained. For example, Kelly and his co-workers (1974) found that either histamine or 5-HT administered intraduodenally to Nippostrongylus-infected rats were without effect upon worm expulsion. It should however, be borne in mind that histamine, even in large amounts, is converted by intestinal bacteria to inactive N-acetylhistamine, and any free histamine absorbed is mostly inactivated as it traverses the intestinal wall or passes through the liver. Similarly, 5-HT is also rapidly degraded in the gut (Goodman and Gilman, 1975).

Keller (1970) found that the potent mast-cell depletor, compound 48/80 administered daily from days 9 - 19 post-infection reduced worm loss. This author concluded that if histamine and 5-HT were important in worm expulsion, then treatment with 48/80 should have accelerated worm loss. However, as pointed out by Murray (1972), the likely explanation of this result was that 'by continued daily treatment with 48/80, the intestinal mucosa was depleted of biogenic amines and hence the effector mechanism for antibody translocation was not fully functional'. Nevertheless, Keller and Ogilvie (1972) found that chronic treatment with histamine (intraperitoneally) or compound 48/80 prevented worm expulsion, and they showed that gut histamine levels were also increased during the latter treatment. Conversely, these workers

demonstrated that administration of p-toluene sulphonyl-hydrazine, a histidine decarboxylase inhibitor (Reilly and Schayer, 1968), accelerated worm expulsion. These results were interpreted as showing that high levels of gut histamine enable worms to resist the action of immunity, and that reduction of histidine decarboxylase activity reduces histamine levels and makes the parasite more susceptible to expulsion. Thus, Keller and Ogilvie (1972) proposed that it was most unlikely that the release of histamine from mast-cells is responsible for expulsion. They also found that treatment of infected rats with either methysergide, an anti-5-HT, or with cyproheptadine, a potent antihistamine and anti-5-HT (Stone et al.,1961) were both without effect upon worm expulsion and egg production.

Keller and Ogilvie (1972) also demonstrated that worm expulsion was delayed by drugs which increased intracellular levels of cyclic 3', 5'-adenosine monophosphate (cAMP), such as isoprenaline and theophylline. Drugs which increase cellular cAMP levels have been shown to prevent antigen-induced release of histamine from human lung (Assem and Schild, 1969; Kaliner and Austen, 1974), from basophils (Ishizaka et al., 1971) and from rat mast-cells (Keller and Ogilvie, 1972). Thus, although it appears that cAMP is in some way involved in worm expulsion, Keller and Ogilvie suggested that its role is probably not only in amine release. On the other hand, if the theory of Murray and his associates, discussed previously, that amines are involved in worm expulsion, then one would expect that drugs which increase intracellular cAMP, and thus inhibit histamine release, could as a consequence, delay rejection of the parasite.

It can be seen, therefore, that the evidence for a role of histamine and 5-HT in the immune rejection of N. brasiliensis is indeed conflicting. The reasons for the diversity of results obtained are not known, but as pointed out by Keller and Ogilvie (1972), caution is necessary in

interpreting results as the drugs used may have their action by interfering with mechanisms other than their 'classical' effects. Also, inconsistencies may result because of differences in drug administration route, dosage and treatment schedules. Another very important factor which should be considered is, that it is not unlikely that the use of drugs in the study of host/parasite relationships can be complicated by the possibility that the drugs may act by affecting the host, the parasites themselves, or both. Again, this may depend upon the route of administration and dosage.

Further, evidence has recently been obtained which implicates mast-cells and amine release in worm expulsion. MacDonald et al., (1980) showed that there is an increase in mast-cell numbers in sterile, worm-free grafts of small intestine heterotopically transplanted under the kidney capsule, as well as in the intestine of infected rats. Beginning at day 12 of infection there was a rapid increase in the number of globule leukocyctes in the infected intestine, but not in the isografts. The globule leukocyte is thought to be derived from the lamina propria mast-cell, and increases greatly in numbers during worm expulsion, at the time of extensive mast-cell degranulation (Murray et al., 1968). No degranulation of mast-cells occurred in the isografts during worm rejection. It was suggested therefore, that the mast-cell increase at the site of worm infection is not antigen specific, nor is it related to the presence of worms in the gut lumen, but local factors related to the worms or the immune response against them seem necessary before degranulation can occur. It was further proposed that since mast-cell proliferation was found not only at the site of infection, but at other sites in the gut and in the intestinal isografts under the kidney, that this represented an increased homing of mast-cells or their precursors to intestinal tissue, regardless of the presence of worm antigen.

Recently, Befus and Bienenstock (1980) have obtained results which suggest a relationship between the effects of

transferred immune mesenteric lymph node (IMLN) cells upon worm expulsion, and intestinal mast-cell numbers. These workers found that adoptive transfer of IMLN cells from immune donors to infected recipient rats hastened both worm rejection and the proliferation of intestinal mast-cells by up to 5 days. The IMLN cells exhibited this mastopoietic activity in the presence, but not the absence, of concurrent infection with N. brasiliensis. Lymph node cells from normal, non-immune, donors were without effect. It was also found that injection of rats at day 14 post-infection with immune serum hastened mastocytosis. It was suggested that the IMLN cells may be a source of intestinal mast-cell precursors, and it is possible therefore, that the action of lymphocytes in hastening expulsion as described previously, may be partly due to an inducement of mast-cell proliferation.

It is unknown how lymphocytes, mast-cells and amine discharge bring about worm expulsion, and it is worth mentioning that pharmacological mediators other than histamine and 5-HT may be involved. For example, it has been shown that allergen-IgE-mediated mast-cell discharge releases slow reacting substance of anaphylaxis (SRS-A) as well as histamine and 5-HT (Orange et al., 1970). Similarly if these substances are involved in worm expulsion, it is not known whether they produce an environment unfavourable to the parasites in the gut, or whether they directly cause metabolic damage to the worms.

Another family of pharmacological mediators have recently been implicated in the expulsion of N. brasiliensis from the rat: these are the ubiquitous prostaglandins (PG). Much evidence has been forthcoming which suggests a role for PG's in worm rejection (reviewed by Kelly and Dineen, 1976), and this has stemmed from studies of intestinal PG levels during expulsion, the effect of PG administration to infected rats upon worm expulsion, and the effect of inhibitors of PG synthesis upon rejection.

Dineen et al. (1974b) demonstrated that the introduodenal injection of chloroform extracts of acidified rat semen (containing PG-like factors) caused accelerated expulsion of N. brasiliensis worms from the gut of rats. It was also shown that these active chloroform extracts did not contain physiologically significant levels of 5-HT or histamine and, using thin layer chromatography it was found that most of the active fraction contained bands with Rf values corresponding to those of synthetic PG's (Smith et al., 1974). In a further study it was found that intraduodenal injections of PGE_1 and E_2 were highly effective in causing worm rejection. PGA and PGB were less effective, and PGF was without effect (Kelly et al., 1974). As mentioned earlier, these authors also showed that intraduodenal administration of either histamine or 5-HT were without effect in causing expulsion. However, as was also discussed previously, both amines are rapidly metabolized in the gut, and it may be that they do not exert their actions by a direct effect on the worms.

Confirmation that PG's are involved in the immune expulsion of N. brasiliensis was obtained in experiments using inhibitors of PG synthesis and activity. It has been widely documented that the mechanisms of aspirin's action are produced by its capacity to inhibit PG synthesis and release (Vane, 1971; Ferreira and Vane, 1974), and it has also been shown that this drug antagonizes the contractile responses of the uterus both in vivo and in vitro, to $PGF_{2\alpha}$ (Smith and Temple, 1973). Dextropropoxyphene has properties of both narcotic and non-narcotic analgesic drugs (Goodman and Gilman, 1975) and Smith and Temple (1973) also demonstrated that this drug, in physiological concentrations, inhibits $PGF_{2\alpha}$- induced contractions of uterus.

Dineen et al. (1974b) showed that the daily administration of aspirin or dextropropoxyphene prevented both, the drop in faecal worm egg counts which normally precedes expulsion, and the expulsion of N. brasiliensis worms from the rat. Unlike

promethazine (see Kelly and Dineen, 1972), the analgesics did not affect production of immune lymphocytes, and it was suggested that they prevented worm expulsion by interfering with the synthesis and activity of PG.

The view that PG's are involved in worm expulsion demands that intestinal levels of these compounds are elevated at the time of expulsion, and it has been demonstrated the PGE levels increase 10-fold in the gut all during primary infection with N. brasilinsis (Dineen and Kelly, 1976). Peak levels of PGE at the site of infection were measured on day 7. Posterior to this site in the gut, the level of PGE increased even further by day 10. Intestinal levels of PGF also increased, but not until after expulsion. Thus, worm expulsion was preceded by increased intestinal levels of PGE, and it is worth noting that, as mentioned earlier, PGE was the most effective class of PG in causing worm rejection following intraduodenal injection (Kelly et al., 1974).

Therefore, it would seem that PG's especially PGE, are important in the expulsion of N. brasiliensis. Their mechanism of action in expulsion however can only be a matter for conjecture. The increased intestinal levels of PGE at day 7 post-injection described by Dineen and Kelly (1976) preceded the onset of expulsion by 3 or 4 days. However, expulsion of N. brasiliensis is preceded by metabolic and structural damage to the worms (Edwards et al., 1971; Henney et al., 1971; Maclean, 1977; Ogilvie and Hockley, 1968). Henney and his co-authors (1971) described how the uptake of labelled metabolites by adult worms drops from day 7 of infection, and this coincides with the rise in PGE which led Dineen and Kelly (1976) to suggest that the damage sustained by the worms is due to a direct effect of PGE, and not to antibodies as proposed by Jones and Ogilvie (1971). It was suggested that antibodies may provide the specific immunological trigger for the release of PG's, and this is supported by the fact that PGE, at physiological concentrations, causes extensive

metabolic damage to adult Nippostrongylus worms cultured in vitro (Richards et al., 1975). As was mentioned previously, attempts to demonstrate a direct effect of antibodies on the parasite in vitro have been unsuccessful (Weinstein, 1967; Ogilvie and Jones, 1971, Zander et al., 1974). Recently the nature of the lesion produced in worms by PGE_1 has been described (Richards et al., 1977). These workers found that the aerobic incubation of adult N. brasiliensis worms in vitro in the presence of PGE_1 produced a depression in the adenylate energy charge and ATP/ADP ratio, and reduced glucose uptake by the parasites as well as causing alterations in the concentrations of various metabolic intermediates. In addition, the action of PGE_1 on worms in vitro adversely affected their ability to re-establish when transferred to rats , and caused marked structural damage to the parasite alimentary and reproductive tracts similar to that which occurs in vivo during primary infection as described by Ogilvie and Hockley (1968).

However, the effect of PG's on worm expulsion may not simply be a direct effect on the parasites themselves. Dineen and Kelly (1976) suggested that, (i) PGE directly affects the morphological and metabolic aspects of the worms and (ii), that elevated levels of PGE act indirectly by affecting gastro-intestinal function which alters the microenvironment at the site of infection. They showed that the concentration of PGE in the small intestine anterior to the main site of infection was elevated, reaching a peak level at day 14, at least 4 days after significantly increased levels were recorded either at the infection site (20 - 25 cm from the pylorus), or further down the small intestine. Dineen and Kelly suggested that this finding may be related to the anterior migration of parasites known to occur before the onset of expulsion (Brambell, 1965; Hindsbro, 1974; Love and Ogilvie, 1974). Love and Ogilvie proposed that antibodies cause damage by forcing worms to migrate from their normal site in the gut, to a less favourable one anterior to this. Dineen and Kelly(1976), however, suggested

that an unfavourable microenvironment, due to increased PGE levels at the site of infection, may be temporarily avoided by the worms, by anterior migration.

Nevertheless, like histamine and 5-HT, the role played by PG's in worm expulsion is obscure. As mentioned previously, it has been shown that catecholamines and xanthines, drugs which increase intracellular levels of cAMP, cause a delay in the rejection of N. brasiliensis from rats. It appears that PG's can regulate virtually every class of immunological responsiveness including immediate hypersensitivity (mediated by IgE), cell-mediated immunity, production or release of humoral antibody, proliferation of lymphocytes, degranulation of mast-cells, and that these effects of PG are also mediated by cAMP (Robinson et al., 1971; Tauber et al., 1973; Bourne, 1974). However, in most of the systems tested, prostaglandins (chiefly PGE) were inhibitory in their effects. Thus, the action of catecholamines and xanthine derivatives in delaying expulsion may be due to a general depression of the immune response, mediated by cAMP. PG's on the other hand, which also increase intracellular cAMP and depress immunity (Bourne, 1974), accelerated worm rejection as has been discussed. Therefore, it seems unlikely that the effect of PG on expulsion is due to their action on immunological responsiveness, although the suppression of PG action by anti-inflammatory drugs indicates a role for PG's in inflammation (Willoughby et al., 1973). This provides further evidence that an inflammatory response, possibly mediated by PG's and cAMP, plays a role in worm expulsion.

PG's have numerous effects on gastrointestinal function, and they could substantially alter the gastrointestinal environment, possibly producing an unfavourable habitat for parasite survival, as well as their direct action on the worms. For example, PG's of the E and A classes inhibit gastric acid and pepsin secretion in several species (Bennett et al., 1973) and it has been shown that in vivo infusion of PGE_1, PGA_1 or $PGF_{2\alpha}$ cause prompt secretion of water and electrolytes into the small intestine

of dogs (Pierce et al., 1971). It is worth noting that both PGE_1 and cholera endotoxin produce similar effects on secretion, apparently mediated by cAMP, and it has been proposed that the profuse diarrhoea of cholera is due to the production and release of PG's (Bennett, 1971). These effects on secretion, fluid and electrolyte balance and pH may produce an unsuitable micro-environment for N. brasiliensis worms at the site of infection. PGE produces increased vascular permeability in the skin (Velo et al., 1973) and if this effect also occurs in the gut, PG's may facilitate leakage of antibodies into the gut lumen in a way similar to that previously described for histamine and 5-HT.

Thus, it can be seen that the sequence of events which expels N. brasiliensis from the small intestine is indeed complex and controversial, involving antibodies, immune lymphocytes, mast-cells and amine release, and prostaglandins. However, to what extent these factors are important and the exact sequence of events which damages the parasite and eventually expels it are, as yet, unknown. It is even possible that one or more of these factors may simply be a consequence of some other mechanism involved in the expulsion sequence.

A factor which may be of major significance in the expulsion of gastrointestinal parasites, but which has not been investigated to any extent, especially in reference to Nippostrongylus brasiliensis infections, is the role of gut motility. For example, 5-HT and certain of the prostaglandins, as well as their involvement in immune phenomena and secretions, cause increased motility of intestinal smooth muscle in the rat. As has been described, intestinal levels of these substances are elevated during the expulsion of N. brasiliensis, and their effects on intestinal motility may be significant. Similarly, the parasites themselves may irritate the gut mucosa and stimulate intestinal contractions. It is possible that if the first stage in expulsion is immunological damage, the second may be physiological, a change in motility bringing about the removal of the already damaged worms.

2. Intestinal smooth muscle: function and control

Before discussing the possible role of gut motility in the expulsion of parasites however, it is perhaps of value to discuss the smooth muscle of the small intestine, and some aspects of its control and function. For a more detailed description the reader is referred to Bulbring *et al.* (1970), Bulbring and Bolton (1979) and Code and Heidal (1968).

The muscle lining vertebrate viscera such as the stomach and intestine is described as 'smooth' or 'unstriated', because of the absence of the characteristic cross-striations seen in skeletal and cardiac muscles. It is also called 'involuntary' muscle because it is brought into action either spontaneously or through activity of the autonomic nervous system.

As in the rest of the gastrointestinal tract, the small intestine consists basically of four layers surrounding the lumen. The two innermost layers are the mucosa and submucosa, which are separated by a thin muscular layer, the muscularis mucosa. The two outermost layers consist of smooth muscle whose function is to mix and propel the contents of the gut lumen (Chyme). This muscle can be divided into groups of fibres running longitudinally and circularly, and are referred to as the 'longitudinal' and 'circular' muscle layers respectively. The circular smooth muscle layer is the greater in mass and is the innermost layer of the two. The relationship between these two muscle layers is very close, and the activity in one can often be transmitted to the other. However, much of the coordinated activity of the two intestinal muscle layers may be controlled independently.

It is worth mentioning at this point, that by far the most widely used preparations in studying the physiology and pharmacology of the gut smooth muscle is the isolated guinea-pig ileum (GPI) and many generalizations concerning smooth muscle of other parts of the gut, and indeed of other species, have been drawn from observations of this preparation.

As in the rest of the alimentary canal, the innervation of the small intestine is both intrinsic and extrinsic. There are many intrinsic neurones lying in several plexuses, the most prominent being the myenteric, or Auerbach's plexus, which lies between the circular and longitudinal muscle layers. Nerves of this plexus probably constitute the major intrinsic neural influence on the gut. Most of the nerve cell bodies react positively to cholinesterase staining and are considered to be cholinergic. Other cells exist, however, which do not take cholinesterase stains (Schofield, 1968) and the chemical transmitter is unknown. Axons from the myenteric plexus innervate both smooth muscle layers. The nerve cell bodies of the plexus receive input from many different receptors in the mucosa and within the muscle wall itself, constituting local reflex areas within the wall of the intestine, the most important being that which controls peristalsis (see later). Another nerve plexus, Meissner's plexus, lies in the submucosa. It is not as extensive as the myenteric nerve plexus however, and is thought to be important in the control of secretion and in the reception of sensory stimulation such as distension or irritation of the mucosa and submucosa.

In addition to this intrinsic control of smooth muscle function, axons from cells of the parasympathetic and sympathetic nervous systems enter the nerve plexuses. Parasympathetic input comes from the vagus nerve which is generally cholinergic and excitatory. Sympathetic input originates from cell bodies located in the coeliac and superior mesenteric ganglia. The axons synapse mainly with cells of the nerve plexuses and are inhibitory.

Small intestinal contractions are regulated by at least four mechanisms:- inherent, spontaneous smooth muscle cell activity, activity of the intrinsic nerves, activity of the extrinsic nerves, and circulating or locally released chemical mediators such as adrenaline, 5-HT, substance P and prostaglandins.

Smooth muscle cells of both the circular and longitudinal layers have an unstable resting membrane potential of around -50 mV. Periodic depolarisations (often called the basal electric rhythm or BER) occur at regular intervals. This activity is always in evidence whether or not contractions occur. Its frequency depends upon the species and on the area of the intestine. Occurence of action potentials is accompanied by contraction, and the greater the frequency of spikes in a given burst, the more powerful the contraction. Spike potentials occur primarily during the depolarisation phase of the rhythmic slow waves and appear to be superimposed on the slow wave recording. They do not occur with every slow wave, but when they do, they are followed by contraction of the muscle which generated them. Muscle cells from both the longitudinal and circular layers generate spike activity and the basal tone of the tissues is directly related to the frequency of spikes. Spontaneous contractions of intestinal smooth muscle probably arise from the simultaneous depolarisation of many cells (Creed, 1979) and are myogenic in origin, i.e. this activity originates within the muscle itself as it is not affected by application of tetrodotoxin, a drug which blocks nervous transmission.

Although the BER can be recorded from either muscle layer of the intact intestine, it has been found that if the circular and longitudinal layers are separated, then the slow waves of depolarisation are only generated by cells of the latter (Bortoff,1965). It was later demonstrated by Bortoff and Sachs (1970) that slow waves are generated by cells of longitudinal muscle and spread electrotonically into the circular muscle layer. Thus, if in the intestine, the longitudinal muscle layer is removed, the circular layer no longer exhibits spontaneous rhythmic activity. Kobayashi et al. (1966), in studies involving the cat intestine, reported that although the circular layer appears to be unnecessary for the generation of slow waves, it may be necessary for the coordination of the waves at certain sites. These authors

suggested that the pathways for coordination of slow wave activity between the two layers are via smooth muscle cells penetrating the myenteric plexus and directly connecting the two layers. Controversy has arisen over this however, since Hirst et al. (1975) could not demonstrate electrical coupling between the two muscle layers of the guinea-pig ileum (GPI). This may be attributed to species difference.

Intestinal slow waves are propagated down the bowel, and it was originally thought that propagation was via regions of fusion of the membranes of adjacent smooth muscle cells (or nexal contacts). However, Daniel and his co-workers (1972) found that there are no nexal contacts in the smooth muscle of the longitudinal layer. Close contacts of another type exist and these may be involved in the electrical interactions between cells of the longitudinal layer.

Although the slow waves, and accompanying spike potentials and contractions are generated by the muscle cells themselves, they can be modified by nervous and humoral factors. The innervation of the small intestine is diffuse. Few nerve fibres are within 100 nm of the smooth muscle cells and the ratio of nerves to muscle cells is low (Burnstock, 1970). Transmitters released by these nerves generally act on the intramural plexuses as opposed to acting directly on the muscle cells, and modify the basic activity by increasing or decreasing spike activity. However, complex patterns of activity such as peristalsis and segmentation are coordinated by the intrinsic nerve plexuses (Hirst,1979).

The most familiar intrinsic neural reflex of the intestine is the peristaltic reflex. Peristaltic movements were first described by Bayliss and Starling (1899) who found that when a linseed oil-soaked cotton ball was introduced into a denervated segment of small intestine, it was propelled aborally. Peristalsis is the main form of propulsion in

the small intestine, and the mechanisms controlling it are very complex. (For a detailed description see Hirst, 1979 and Kottegoda, 1970.)

The fundamental feature of peristalsis is a ring of contraction of the circular smooth muscle passing down the alimentary canal in an aboral direction, the contraction being preceded by a greater or less degree of relaxation of the muscle directly in front. Trendelenburg (1917) introduced the now classical preparation in which an isolated segment of GPI was subjected to increased intraluminal pressure. The reflex so initiated was considered to consist of two phases (Trendelenburg, 1917; Kottegoda, 1970). The longitudinal muscle contracted first, causing a shortening of the intestinal segment. This was followed by the contraction of the circular muscle with simultaneous relaxation of the longitudinal muscle. The circular muscular contraction spread as a wave in an aboral direction, propelling the intestinal contents. Trendelenburg described the longitudinal muscle contraction as the 'filling' or 'preparatory' phase, and the circular contraction as the 'emptying' phase.

However, it has been shown that the peristaltic wave of contraction of the circular muscle layer can take place in the absence of contractions of longitudinal muscle (Kosterlitz et al., 1956). Therefore, although contractions of both muscle layers follow a definite temporal pattern during peristalsis, the contraction of the circular muscle would appear not to be a consequence of the longitudinal muscle contraction, but requires its own stimulus of gut distension. When the circular muscle contracts it causes inhibition of the longitudinal muscle (Kosterlitz et al., 1956). Nevertheless, the fact that the wave of peristalsis can occur independently of the longitudinal muscle, does not mean that the latter, or 'filling phase', is without physiological significance. It is possible that the longitudinal muscle contraction opens

the gut lumen and favours mixing of the chyme in preparation for the propulsive phase of peristalsis (Raiford and Mulinos, 1936; Kosterlitz et al., 1956).

The stimulus for peristalsis is radial stretch of the intestine, and the nervous pathways responsible lie in the intramural nerve plexuses (Kosterlitz, 1968). The importance of the submucosal and myenteric plexuses is indicated by the ability of local anaesthetics such as cocaine (Bulbring et al., 1958), ganglion blockers like hexamethonium (Paton and Zaimis, 1949) and acetylcholine blockers such as atropine (Kosterlitz, 1968; Kottegoda, 1970) to disrupt the peristaltic reflex.

Thus, peristalsis is provoked by the stretch of the gut produced by a bolus in the lumen, and as formulated by Bayliss' and Starling's 'Law of the Intestine' (1899), this stimulation produces excitation above the stimulated point - ascending contraction - and relaxation below the stimulated point - descending inhibition. The peristaltic reflex is not dependent upon the extrinsic innervation of the gut, but is a coordinated process which is mediated by the neurones of the intrinsic nerve plexuses, initiated by sensory endings in the mucosa that responsive to stretch.

Apart from peristalsis, other motility patterns occur in the small intestine and these can be divided into pendular movements, segmentation contractions, tonus changes and movements of the villi (Ganong, 1969; Hightower, 1968). Their function is to churn the chyme, breaking it up and mixing it with the digestive enzymes, thus aiding digestion.

Segmentation contractions may be defined as localised circumferential contractions of the circular muscle which section the intraluminal contents into a series of approximately equal segments, with the segments being further divided and joined with great rapidity. The chyme is thus mixed, circulated and pushed to and fro, but no net propulsion occurs. W.B. Cannon (1902) first used the term 'rhythmic segmentation' for

this, the most common type of intestinal motility, and his description is worth quoting:- 'A string-like mass of food is seen lying quietly in one of the intestinal loops. Suddenly an undefined activity appears in the mass, and a moment later, constrictions at regular intervals along its length cut it into small ovoid pieces. The solid string is thus quickly transformed by a simultaneous sectioning, into a series of uniform segments. A moment later, each of these segments is divided into two particles, and immediately after the division, neighbouring particles rush together, often with the rapidity of flying shuttles, and merge to form new segments. The next moment these new segments are divided and neighbouring particles unite to form a third series, and so on'. This eloquent description adequately describes the rhythmic division of a length of the (cat) small intestine into segments by regularly recurring segmental contractions.

Pendular movements were described in the denervated small intestine of the dog by Bayliss and Starling, as a gentle swaying motion of the gut. These spontaneous contractions of the longitudinal muscle result in rhythmic shortenings and lengthenings of the gut. Tonus changes are local changes in the rate or degree of smooth muscle contraction occurring slowly in all parts of the tract independently of the volume or nature of the contents. Movements of the intestinal villi have also been observed, which are caused by contraction of the muscularis mucosa.

Thus, the motility patterns of the small intestine which mix and propel the chyme are complex in their nature and control, the control being mediated by the smooth muscle cells themselves, and the intrinsic nerve plexuses.

The role of the extrinsic innervation of gastrointestinal smooth muscle is a modulatory one, and as stated previously, it comes from both the sympathetic and parasympathetic divisions of the autonomic nervous system.

The sympathetic postganglionic neurones are adrenergic and modern techniques of identifying adrenergic neurones through their fluorescence (Falck et al., 1962) have shown that most adrenergic nerves pass to the intramural nerve plexuses and not to the muscle layers (C.Y. Lee, 1970; Gabella, 1976). Within the myenteric plexus they terminate around the intramural cholinergic ganglion cells, where release of their transmitter, noradrenaline, is inhibitory in its action. Except at sphincter regions, where increased tone may result, the effect of sympathetic activity in the gut is inhibitory, and blockage of sympathetic nerve action often leads to increased gastrointestinal motility. It is worth mentioning that some disagreement does exist concerning the sympathetic innervation of the gut, since Silva and his fellow workers (1971) found that, whereas the longitudinal muscle layer of the ileum has little or no adrenergic innervation, the circular layer is densely innervated, as well as the neurones of the myenteric plexus. Thus, in circular muscle, inhibition may be brought about directly in the smooth muscle, and indirectly through the intramural plexus.

The vagus nerve provides the parasympathetic innervation of the small intestine and it is generally excitatory, activity producing increased motility. A great deal of the parasympathetic activity is exerted indirectly via the interneurones of the intramural plexuses, the transmitter being acetylcholine. Thus, generally speaking, the parasympathetic innervation of the gut is excitatory and the sympathetic innervation is inhibitory, except at the sphincter where contraction results.

In addition to the above, the existence of what are known as 'non-cholinergic, non-adrenergic inhibitory nerves' has been described, and a great deal of evidence has been produced which suggests that the inhibitory neurotransmitter released from these nerves is ATP (Burnstock, 1972, 1979). Thus, stimulation of the parasympathetic supply to the intestine in the presence of cholinergic and adrenergic antagonists produces relaxation of the gut muscle. The physiological function of purinergic nerves in the gut is unknown. They

may be implicated in inhibitory reflexes during peristalsis which facilitate passage of material through the gut by opening sphincters and relaxing the intestine in front of the advancing bolus (Burnstock, 1979). In contrast, the inhibitory action of adrenergic nerves is concerned with modulating reflex activity.

Apart from adrenergic, cholinergic and purinergic nerves, several other putative transmitters have been proposed for autonomic nerves and these include 5-HT, dopamine, γ-amino-butyric acid (GABA) and various polypeptides including substance P and the enkephalins.

The role of nerves containing these different substances in unresolved. It may be that in the gut, substance P exists in sensory nerves, and GABA, 5-HT and enkephalin appear to be contained in interneurones. However, unlike ATP, none of them mimic the response of intestinal muscle to stimulation of non-adrenergic, non-cholinergic inhibitory nerves (Cocks and Burnstock, 1979).

As described previously, the two smooth muscle layers of the intestine can act together in a definite temporal and coordinated fashion, though they can also act independently. It is therefore worth briefly discussing the action of substances upon intestinal smooth muscle and the differences in sensitivity which exist between the circular and longitudinal muscle layers to certain chemical mediators.

The circular smooth muscle of the GPI receives both excitatory and inhibitory innervation, whereas the longitudinal layer receives only excitatory innervation (Hirst et al., 1975), and while there is little doubt that the excitatory transmitter to longitudinal muscle is acetylcholine (ACh) (Paton, 1955; Paton and Zar, 1968), the excitatory transmission to the circular muscle may not be cholinergic. For example, it has been demonstrated that when the circular smooth muscle of GPI is separated from the longitudinal muscle layer and the myenteric plexus, it is relatively or completely insensitive to ACh (Harry, 1963). This is unlike the longitudinal muscle which

is very sensitive to ACh. It has been found that there is a higher concentration of AChE associated with the circular muscle of the rabbit ileum than with the longitudinal muscle, and this may account for the relative insensitivity of the former to ACh (Koelle et al., 1950). After inhibition of AChE Harry (1963) found that circular muscle, although still relatively insensitive, responded to ACh. Evidence that the circular muscle of the gut receives non-cholinergic excitatory innervation came when Kottegoda (1968, 1969) found that contractions in response to repetitive electrical stimulation were not blocked by antagonists of cholinergic transmission.

Generally speaking, the longitudinal muscle of the intestine tends to be more sensitive than the circular muscle. Paton and Zar (1968) showed that the longitudinal muscle of GPI when innervated and, to a lesser extent, in the absence of nerves, contracted readily in response to minute doses of ACh, and also to low concentrations of numerous other drugs such as muscarine, histamine, oxytocin and substance P. These substances all acted directly on the smooth muscle, and angiotensin, potassium and 5-HT produced, in the order listed, a progressively increasing effect on the nerve plexus. On the other hand, the circular muscle is particularly insensitive to these compounds, and Brownlee and Harry (1963) showed that the circular muscle is completely insensitive to substance P and angiotensin. Histamine which acts directly on the longitudinal muscle of GPI, causes contraction of the circular muscle, probably via the intrinsic nerve plexus since the action is blocked by the ACh antagonist, atropine, or by morphine, which blocks the release of ACh from autonomic nerves in the gut (Schaumann, 1957; Cowie et al., 1968).

It is worth mentioning at this stage, that although the smooth muscle of GPI is very sensitive to the contractile action of histamine, that of rat intestine is completely insensitive, and histamine is almost without effect on this tissue (Livingstone, 1968; Sakai et al., 1979).

Prostaglandins and 5-HT generally cause contraction of intestinal smooth muscle, though their roles in the control of gut motility are obscure. Most experimental work on the effect of 5-HT has been carried out on GPI, and in this preparation 5-HT causes contraction of the longitudinal muscle by both a direct action on the muscle, and an indirect action by stimulating the intrinsic nerve plexus. Bulbring and Lin (1958) found that when 5-HT, which is a potent stimulant of sensory receptors in general, was introduced into the lumen of a section of small intestine, there was a stimulation of peristalsis. They attributed this effect to stimulation and sensitization of the sensory receptors in the mucosa which normally trigger the peristaltic reflex. Small amounts of 5-HT are continuously released into the lumen of the gut, and the amount released is directly proportional to the filling pressure and is greatly increased during peristalsis (Bulbring and Crema, 1959a, b). Further evidence that 5-HT is involved in the peristaltic reflex arises from the observation that antagonists of 5-HT such as lysergic acid diethylamide (LSD) and the specific inhibitor of 5-HT synthesis, parachlorophenylalanine, inhibit peristalsis (Bulbring and Lin, 1958; Ginzel, 1957; Kottegoda, 1970). This could explain why most of the body's 5-HT is found in the gut, stored in the mucosa (Feldberg and Toh, 1953) and in the myenteric plexus (Gershon and Ross, 1966).

The prostaglandins (PG) owe their discovery to their contractile activity upon smooth muscle (Goldblatt, 1935; von Euler, 1936), and this group of unsaturated hydroxy fatty acids are distributed widely in animal and human tissues. The occurrence of PG's and their effects, have been widely documented (Bergstrom et al., 1968; Horrobin, 1978; Horton, 1969; Kadowitz et al., 1975), and the actions of PG's on smooth muscle and the gut have been reviewed by Bennett and Fleshler (1970), Bennett (1976) and Horton (1979).

Generally, PGE_2 relaxes circular but contracts longitudinal intestinal smooth muscle, $PGF_{2\alpha}$ contracts both types and PGA and B have relatively little effect (Bennett,

1976; Bennett and Fleshler, 1970). PGE and F occur naturally in the gut wall, and the ability of PG's of these classes to affect smooth muscle in very low concentrations would suggest that these compounds may be involved in gut motility.

Evidence has been produced which suggests that the resting tone of the intestine, generally classified as myogenic in origin, may be maintained by the continuous generation of PG. Several isolated smooth muscle preparations release PG into their bathing medium (Vogt and Distelkotter, 1967; Posner, 1970; Ferreira et al., 1972), and it has been found that PG synthesis is related to the basal tone. Indomethacin, which blocks PG synthesis reduced or abolished both the resting tone of rabbit intestine and the release of PG into the bathing fluid (Ferreira et al., 1972), and Bennett and Posner (1971) found that PG-antagonists reduced the tone of several preparations of human, guinea-pig and rat gastrointestinal muscle. However, it should be borne in mind that the production of PG's by isolated smooth muscle preparations may be associated with the trauma of removal and preparation of the tissues, since it has been demonstrated that rabbit jejenum releases PG in proportion to the amount of handling of, or damage to the tissue (Ferreira et al., 1976).

The mechanisms of action of PG's on intestinal smooth muscle have been examined in order to determine whether the effects observed are due to stimulation of the intrinsic nerve supply or to a direct action on the muscle. One action of PG's which has received a lot of interest in recent years, is their ability to prevent the release of noradrenaline (NA) from sympathetic nerves (Hedqvist, 1973, 1977; Horten, 1973). From his work on spleen, Hedqvist proposed that PG released from splenic smooth muscle acts as a negative feed-back mechanism which attenuates the effects of nerve stimulation. This hypothesis has been extended to other sympathetically innervated tissues. PGE_1 and E_2 counteract or abolish the inhibition of gut motility resulting from stimulation of the sympathetic nerves, without affecting the responses to NA, and this provides indirect evidence that PG's inhibit NA

release in intestinal smooth muscle (Hedqvist, 1977). This could partly account for the excitatory action on gut muscle generally exerted by PG's. PGE_1 and E_2 have also been found to enhance the contractile response of intestinal muscle elicited by cholinergic nerve stimulation (Harry, 1968; Kadlec et al., 1974; Hall et al., 1975). Similarly, contractions of gut muscle induced by cholinergic nerve stimulation are markedly reduced by indomethacin, and partially or completely restored by subsequent administration of small, sub-contractile doses of PGE_1 and E_2 (Hedqvist, 1977). Indomethacin has little effect on the contractile response to exogenous ACh (Kadlec et al., 1974), and these observations provide evidence that PGE stimulates cholinergic transmission in the gut, and imply that part of their effect is prejunctional stimulation of cholinergic nerves.

PG's have also been shown to exert a direct effect on the gastrointestinal tract. The inhibitory effect of PGE on circular muscle appears to be mediated by a direct action on the muscle, as are the contractile responses of the longitudinal muscle of rat intestine, and of some preparations of human gut (Bennett and Fleshler, 1970). It is likely therefore, that the contractile responses of gut muscle to PG's are due to a combined direct action on the muscle, and an indirect action on nerves. It is of interest to mention here that the stimulant actions of bradykinin, angiotensin and 5-HT on gut motility may be partly mediated by a stimulation of PG synthesis and release (Chong and Downing, 1973; Kadlec et al., 1974; Crocker and Willavoys, 1976; Famaey et al., 1977).

To conclude this discussion of the control of intestinal smooth muscle, it is perhaps relevant to discuss briefly the actions of some of the gastrointestinal hormones, whose more pronounced activity is on secretion rather than motility. Gastrin, which normally stimulates the secretion of hydrochloric acid and pepsin in the stomach, has been shown to increase contractile activity in both the stomach and the intestine

(Bennett, 1965; Davson and Segal, 1976). This effect is blocked by tetrodotoxin, atropine, hyoscine and morphine. It was suggested therefore, that gastrin acted by stimulating parasympathetic nerves to release ACh. Secretin normally causes the secretion of bicarbonate from the pancreas and gall bladder. This hormone also decreases intestinal motility (Katz, 1973; Waterfall et al., 1973). Choleocystokinin(CCK) and caerulin (a decapeptide whose C-terminal pentapeptide amino acids are identical to those of CCK), which increases intestinal, pancreatic and biliary secretions, also contracts human intestinal smooth muscle by an action which is antagonized by secretin (Gutierrez et al., 1974). The actions of these and other gastrointestinal hormones are discussed in the review by Burks (1976).

From this somewhat abridged description, it becomes obvious that the control of small intestinal motility is indeed complex, involving a multitude of myogenic, nervous and chemical mechanisms. How these factors all cooperate in the coordinated control of intestinal smooth muscle activity is not, as yet, fully understood, and a good deal of work into this fascinating field of study is still necessary.

3. The role of gut motility in parasitic infections

A possible role for gut motility in the expulsion of intestinal parasites was first proposed by Stewart (1953, 1955), who showed that in sheep infected with, and suitably sensitized by H. contortus, a challenge dose of infective larvae would cause a sudden and dramatic loss of adult worms from the gut. Blood histamine levels rose at the same time as the complement-fixing antibody titres increased, and he explained this as the effect of an antibody-cell complex and the subsequent discharge of mast-cell granules by the interaction with antigen. It was suggested that the pharmacologically active substances released (histamine and possibly 5-HT) probably caused an increased gut motility and marked oedema, which in turn resulted in expulsion of the worms.

Symptoms of several intestinal helminthiases include nausea, vomiting, abdominal pain and diarrhoea or constipation which are due, presumably, to inflammation and abnormal intestinal motility, and it is conceivable that alterations in motility could affect the establishment or course of a parasitic infection. Recently, interest has arisen in the possible role of gut motility associated with the intestinal phase of Trichinella spiralis infection in rats. By following the transit through the small bowel of a non-absorbable radioactive marker ($Na^{51}CrO_4$), Castro and his fellow workers (1976) demonstrated that intestinal transit was significantly increased during the intestinal phase of T. spiralis infection. The increased activity was associated with mucosal inflammation, which paralleled the site of worm localization, and some of the experimental animals were diarrhoeic. It was presumed that this increase in intestinal propulsion was due to changes in smooth muscle activity, since it had previously been shown that trichinosis in the dog was accompanied by abnormal electrical activity of the intestinal muscle of infected animals (Schanbacher et al., 1975, 1978). These authors described an increased frequency of spike potentials recorded from the intestine of

trichinosed dogs. Castro et al. (1976) however, did not make any suggestions as to whether increased intestinal propulsion was due to stimuli provided by the inflammatory response to the parasites, or was due to a direct response to products secreted by the worms. As described previously, the stimulus for peristalsis in the small intestine is radial stretch, and Castro and his associates suggested the possibility that increased intestinal volume due to hypersecretion or malabsorption of water and solute may have provided the stimulus for increased propulsion. It has in fact been shown that, whereas uninfected rats show net absorption of H_2O from the intestine, net secretion occurs in animals at the fifth day of a primary infection with T. spiralis (Castro et al., 1979). These workers also showed that this response was more marked and occurred almost immediately following a secondary infection. However, a challenge infection with T. spiralis in previously immunized rats does not induce an increase in intestinal transit (Castro et al., 1977). Similarly, Schnabacher et al. (1975) did not observe any change in gut motility in immune dogs given a challenge infection with T. spiralis. Thus, it appears that primary infection, but not secondary infection with T. spiralis is associated with increased gut propulsive activity, even though intestinal fluid accumulation occurs in both cases. Therefore, excess fluid in the gut would seem not to be the stimulus for increased motility. The number of worms which establish in the gut following secondary infection is less than that following primary infection (McCoy, 1940; Love et al., 1976) and it may be that the smaller number of established adult worms does not provide adequate stimulus to induce intestinal motility changes.

Marked fluid accumulation and thickening of the small intestine of rats infected with N. brasiliensis also occurs (Symons, 1960). This author found that when segments of the intestine of infected rats were perfused in vivo with sodium chlroide solutions, there was a net movement of Na^+

and Cl^- ions into the intestine. The use of ^{24}Na showed that the fluid accumulation was an osmotic effect due to impaired absorption of Na^+ ions during infection, and not due to hypersecretion by the intestine. Symons (1966) subsequently obtained evidence of altered intestinal propulsion during N. brasiliensis infection. Using a ^{14}C-labelled polyethylene glycol marker introduced into the stomach of infected rats, gastric emptying was found to be unaltered, but the propulsion of intestinal contents appeared to be reduced in the proximal two-thirds of the small intestine, and increased in the distal ileum, as compared to uninfected controls.

In addition to fluid accumulation in the gut, the intestinal levels of prostaglandins and 5-HT are greatly elevated during infection with N. brasiliensis, as previously discussed. It is plausible therefore, that these compounds, which have marked stimulant effect on gut smooth muscle could cause motility changes during infection. Also, Symons' finding that intestinal propulsion appeared to be reduced in the upper part of the small intestine may have been a manifestation of the 'biochemical-holdfast' action of worm-secreted AChE, suggested to occur by Ogilvie and Jones (1971).

The primary purpose of the work presented here was to investigate whether changes in gut motility occur during N. brasiliensis infection, and if so, their nature and possible role in worm rejection. Experiments are described in which the propulsive activity of the small intestine at various times post-infection with N. brasiliensis were measured in vivo. The effect of artificially stimulating gut motility with drugs, upon an established worm population was also investigated. In addition, possible alterations in spontaneous contractions and responses to electrical stimulation and drugs of isolated preparations of rat gut during infection were examined. As will be seen, whereas no marked changes in propulsive motility in vivo were evident, the in vitro

experiments with isolated preparations of intestinal smooth yielded unexpected, and unusual results which were worthy of further investigation.

GENERAL METHODS AND MATERIALS

1. Experimental animals

All rats used were male hooded Listers (Animal Suppliers, London, Ltd.), weighing approximately 150 - 250 g. The animals were housed in polypropylene or metal cages (50 x 32 x 25 cm) with wire mesh floors suspended above sawdust trays, normally no more than ten rats per cage. Animals which had undergone intraduodenal catheterisation were housed individually because they tended to chew each others' catheters and wounds. Rats were maintained on a pellet type diet (Labsure, C.R.M.) and tap water ad libitum in a constant temperature of approximately 22°C.

2. Parasitological Techniques

(i) Culture of Infective Larvae

The culture of N. brasiliensis larvae was as described by Jennings et al. (1963). Faeces were collected from rats with a patent infection over the period of days 7 - 10 post-infection. The faecal pellets were placed in a mortar with a little warm water, and allowed to soak for 15 - 30 minutes before being broken up and mixed to a paste. Using a spatula, a portion of the faecal paste was spread on to the centre of a piece of Whatman's No. 1 filter paper, diameter 7 cm, the faecal smear being approximately 3 cm in diameter, such that the periphery of the paper was kept clear. The paper was then immersed in water and placed on a foam rubber pad, which had been soaked in water, in a plastic Petri dish. After replacing the lids, the Petri dishes were stored in a humid incubator at 27°C.

Normally, after 5 days, the larvae could be seen collecting in a fringe about the periphery of the filter paper. The larvae were always harvested 5 to 7 days after setting up the culture and were used to infect rats on the same day.

Harvesting was accomplished by flooding the Petri dishes with water at 35°C, thus allowing the larvae to swim off into the water. The water containing the larvae was collected and filtered under suction in a large Buchner funnel, through strong filter paper (Green's Hyduro 904, 18.5 cm diameter). This paper was then inverted and placed on an Endecott sieve (53 µm aperture) in a

Baermann apparatus filled with water at 35°C. The larvae swam through the sieve to the bottom of the funnel, leaving the faecal debris trapped on the sieve, and were run off into a measuring cylinder.

Larval counts were made by a dilution technique. A 1.0 ml sample was diluted to 100 mls with water and 0.1 ml samples of this suspension (which had been mixed well) placed on a slide and the larvae counted with the aid of a dissecting microscope. Ten counts were normally made. The number of larvae in the original suspension was then calculated.

(ii) Infection of Rats with Larvae

The original larval suspension was diluted so that the required number of larvae was contained in a 1.0 ml volume. Rats were lightly anaesthetized with halothane and injected subcutaneously in the groin region using a 1.0 ml disposable syringe and a No. 19 hypodermic needle. Due to errors in the counting technique, only the approximate number of larvae administered can be stated, but in any one experiment each rat should receive the same larval burden. The level of infection in the *in vivo* motility experiments (see experiment 1) was 4,000 larvae, and in all others, 5,000 larvae approximately.

(iii) Recovery and Preparation of Worms for Analysis

Infected animals, which had been starved overnight, were killed by stunning and cervical dislocation. The skin and abdominal wall were incised along the midventral line and the whole of the small intestine removed. The intestine was slit longitudinally with blunt scissors, cut into 4 inch lengths and placed in a muslin bag suspended in a 250 ml beaker filled with isotonic saline at 37°C. This was then placed in a water bath at 37°C for 30 minutes, during which time the worms had swum out of the bag and collected at the bottom ot the beaker. Worms were then collected using a Pasteur pipette and placed on Whatman's No. 50 filter paper in a micro-Buchner funnel. The sample thus surface dried was transferred to a previously weighed coverslip, and the weights of the worm sample found by weight difference.

3. Radioisotopes

The radionuclide used, $Na^{51}CrO_4$, was obtained from the Radiochemical Centre, Ltd., Amersham, England. $Na^{51}CrO_4$ (referred to as '^{51}Cr') was dissolved in sterile isotonic saline. Dilutions to the required activity and volume were carried out prior to the start of an experiment in isotonic saline.

Intraduodenal injections of radioisotope were administered via an indwelling intraduodenal catheter, and flushed in with 0.2 ml saline, which was sufficient to wash in any isotope remaining in the catheter. The volume and activity of ^{51}Cr administered is discussed in experiment 1.

Radioactivity was measured in a Packard Automatic Scintillation 'well-type' gamma counter, each vial being counted for 5 - 10 minutes to reduce statistical errors to an acceptable level. The natural 'background' radioactivity was measured and subtracted from all observed count-rates. No quenching corrections were necessary as ^{51}Cr is a gamma-emitting radionuclide.

The Thyopac - 4 kit used to determine serum thyroxine levels in experiment 5 was also obtained from the Radiochemical Centre, Ltd. ^{125}I is also a gamma- emitter, and radioactivity was therefore determined in the gamma counter.

4 Drugs

All drugs employed were dissolved in 0.9% saline, except for sulphanilamide, which was dissolved in 70% ethanol, and betamethasone, which was suspended in 0.9% sterile saline.

Ficoll, an artificial colloid, is a synthetic polymer of sucrose and epichloro - F hydrin, and has a molecular weight of approximately 70,000.

The suppliers of the drugs used were as follows:-

Acetylcholine chloride	Sigma
Betamethasone (Betsolan injection)	Glaxovet
Carbachol chloride	Sigma
Ficoll	Sigma
Halothane (Fluothane)	ICI

Heparin (Pularin injection)	Evans
Histamine acid phosphate	BDH
5-Hydroxytryptamine creatinine sulphate	Sigma
Oxytetracycline HCl (Terramycin)	Pfizer
L-Phenylephrine HCl	Sigma
Sulphanilamide	ICI

THE ROLE OF GUT MOTILITY IN THE EXPULSION OF *NIPPOSTRONGYLUS BRASILIENSIS*

1. Propulsive motility of the gastrointestinal tract in rats infected with *N. brasiliensis*.

2. The effect of carbachol on the worm population of infected rats.

3. Investigation of the sensitivity of isolated segments of small intestine from infected rats.

4. Is the intestinal supersensitivity observed during infection abolished by betamethasone?

5. Corticosterone and thyroxine blood levels in infected rats.

6. Pressor responses of the isolated hind limb preparation from infected rats.

1. PROPULSIVE ACTIVITY OF THE GASTROINTESTINAL TRACT IN RATS INFECTED WITH N. brasiliensis

1. Propulsive Activity of the Gastrointestinal Tract in Rats Infected with N. brasiliensis

Introduction

As discussed in the general introduction, Nippostrongylus brasiliensis infection in the rat has been extensively used as an experimental model in studying the immune expulsion of gastrointestinal parasites (see reviews by Murray, 1972 and Ogilvie and Love, 1974). By far the greatest proportion of work carried out has concentrated upon purely immunological aspects of the expulsion, and very little has been done concerning the possible role of changes in gut motility in the expulsion mechanism.

A number of workers have recently become interested in the role of abnormal gut motility in the expulsion of parasites already suffering some immunological damage (Castro et al., 1976, 1977; Schànbacher et al., 1975, 1978). Castro and his associates carried out a quantitative study which demonstrated a significant increase in intestinal propulsive activity in rats during the intestinal phase of a primary infection with T. spiralis. Their method depended upon following the progress of radioactive chromate through the small intestine of the rat. This altered intestinal propulsion was associated with mucosal inflammation, disaccharidase deficiency and fluid accumulation in the gut, which coincided with the intestinal phase of T. spiralis infection.

The purpose of the present experiment was to measure intestinal propulsive motility during a primary infection with N. brasiliensis. ^{51}Cr-labelled chromate is ideally suited for the measurement of gut propulsion since it does not produce changes in motility itself, is easily and accurately measured even when mixed with chyme, and is better suited to give accurate information about distribution than non-radioactive materials such as dyes (Derblom et al., 1966; Summers et al., 1970.

Methods

(i) Implantation of Intraduodenal Catheters

Intraduodenal catheters were implanted in rats weighing 150 - 200 g. The technique employed was essentially the same as that described by Castro et al. (1976, 1977), but some modification was found to be necessary since difficulties were experienced initially. These included the fact that the animals were very adept at removing their implanted catheters, even when individually caged. This was overcome by closing the cutaneous wound in the abdomen with clips instead of stitches, as the animals tended to chew the latter. Also, the stitches used to hold the catheter in place at the back of the neck had to be slack. If they were too tight the surrounding tissue died (presumably due to loss of blood supply) and the catheter had no anchor in the back of the neck to hold it in place. Following surgery it was found necessary to flush the catheters at least once a day, as they were prone to becoming blocked with intestinal contents. This also meant that the rats were handled frequently and consequently became relaxed and accustomed to handling.

Each PVC catheter (1.0 mm, id; 2.0 mm, od) of approximately 15 cm in length had a blunted hypodermic needle (No. 19 swg) inserted into one end and glued in place. The injection site in the needle hub was sealed with a silicone rubber bung.

Rats were anaesthetized with 4% halothane in a 2:1 O_2/N_2O mixture, and the abdomen and midscapular regions shaved. A midline incision in the abdominal skin of approximately 3.0 cm was made, and the catheter passed through a small puncture in the skin at the midscapular region from left to right to the abdominal incision. It was found necessary to make a subcutaneous 'skin tunnel' to facilitate passage of the catheter. A small hole was made at the midscapular region, and a thin, sharp metal rod inside a Teflon tube (2.5 mm, id) was pushed through the hole between the skin and musculature until it protruded at the abdominal skin incision.

Care had to be taken here to ensure that the thoracic cavity was not punctured. The metal rod was withdrawn leaving the Teflon 'skin-tunnel' in place. The catheter was then inserted through the tunnel and the latter removed, leaving the catheter in place under the skin from the mid-abdomen to the back of the neck, where it was stitched in place using 4-0 surgical silk. The needle hub was left projecting outside the skin where it would serve for injection purposes.

The peritoneum was then incised and the free end of the catheter passed through the musculature of the abdominal wall into the abdominal cavity. The stomach was exposed and a small 6-0 silk purse-string suture made in the middle of the greater curvature at the junction of the pyloric and cardiac regions. A small incision was then made in the stomach wall in the centre of the purse-string suture, and the catheter inserted and advanced until it was approximately 2.5 cm into the duodenum, care being observed not to damage the pyloric sphincter. The purse-string was used to close the gastric incision around the catheter.

The abdominal incision was then closed in two layers, the musculature being stitched with 4-0 silk. The catheter was also stitched to the abdominal muscle at the site of its entry. The skin incision was closed with 9.0 mm stainless-steel autoclips. To prevent infection, the wounds were sprayed with an oxytetracycline aerosol and each animal given an intraperitoneal injection of 100 mg/kg sulphanilamide.

Post-operative rats were given 5% glucose solution overnight before receiving normal stock diet and water ad libitum. They were checked daily and the catheters were flushed each time with 0.2 ml of 0.9% saline.

(ii) Experimental and Autopsy Procedures

Rats were infected with 4,000 N. brasiliensis larvae at least one week after implantation of the catheter. The intestinal propulsive activity was studied in groups of animals on days 6 (n = 7), 8 (n = 11), 10 (n = 9), 12 (n = 10) and 14 (n = 10) post-infection, and in a group of 14 catheterized, non-infected control rats.

On the day of the experiment, the catheter in each rat was perfused with 0.1 ml normal saline, followed by subsequent injection of 0.1 ml saline containing approximately 10 μCi of $Na_2{}^{51}CrO_4$ (referred to as ^{51}Cr). Following the intraduodenal injection of radio-isotope 0.15 ml saline was immediately injected to wash in any ^{51}Cr remaining in the catheter.

Fifteen minutes later, the animals were killed by a blow to the head and cervical dislocation. Two ml of blood were drawn by cardiac puncture for the assay of radioactivity.

The abdomen was opened and silk was used to tie the small intestine at the tip of the catheter, and at the ileo-caecal junction. The gastrointestinal tract was then cut at the point of these ties and removed from the animal. The intestine was divided by small artery clips into 10 equal segments of approximately 8 cm in length, beginning at the catheter tip and working distally. It was then cut at the clips and each segment, as well as the caecum and a blood sample from each rat was placed in a numbered vial.

The amount of ^{51}Cr in each segment, caecum and blood sample was assayed by measuring the activity in each vial in a gamma counter (Packard Automatic Scintillation Counter).

Since no radioactivity was detected in any of the blood samples, it was presumed that all of the radioactivity had remained in the gut and the activity in the segments from each animal was calculated as a percentage of that injected. Activities were expressed as percentage distribution present in, or passing through, a given section of small intestine during the 15 minute test period.

(iii) Statistics

Data for the percent radioactivity present in, or passing through a given segment of intestine were graphed and analysed by regression analysis. Regression equations represent the percentage of radioactivity (Y) as a function of gut length (X). Significance of the slopes of the lines was determined by $t = b/S_b$, and probabilities of less than 0.05 were considered significant (Snedecor, 1956).

Differences between the graphs for each group obtained were measured by analysis of the variance (anovar) between regression coefficients. Probability values of less than 0.05 for group comparisons were considered significant.

Results

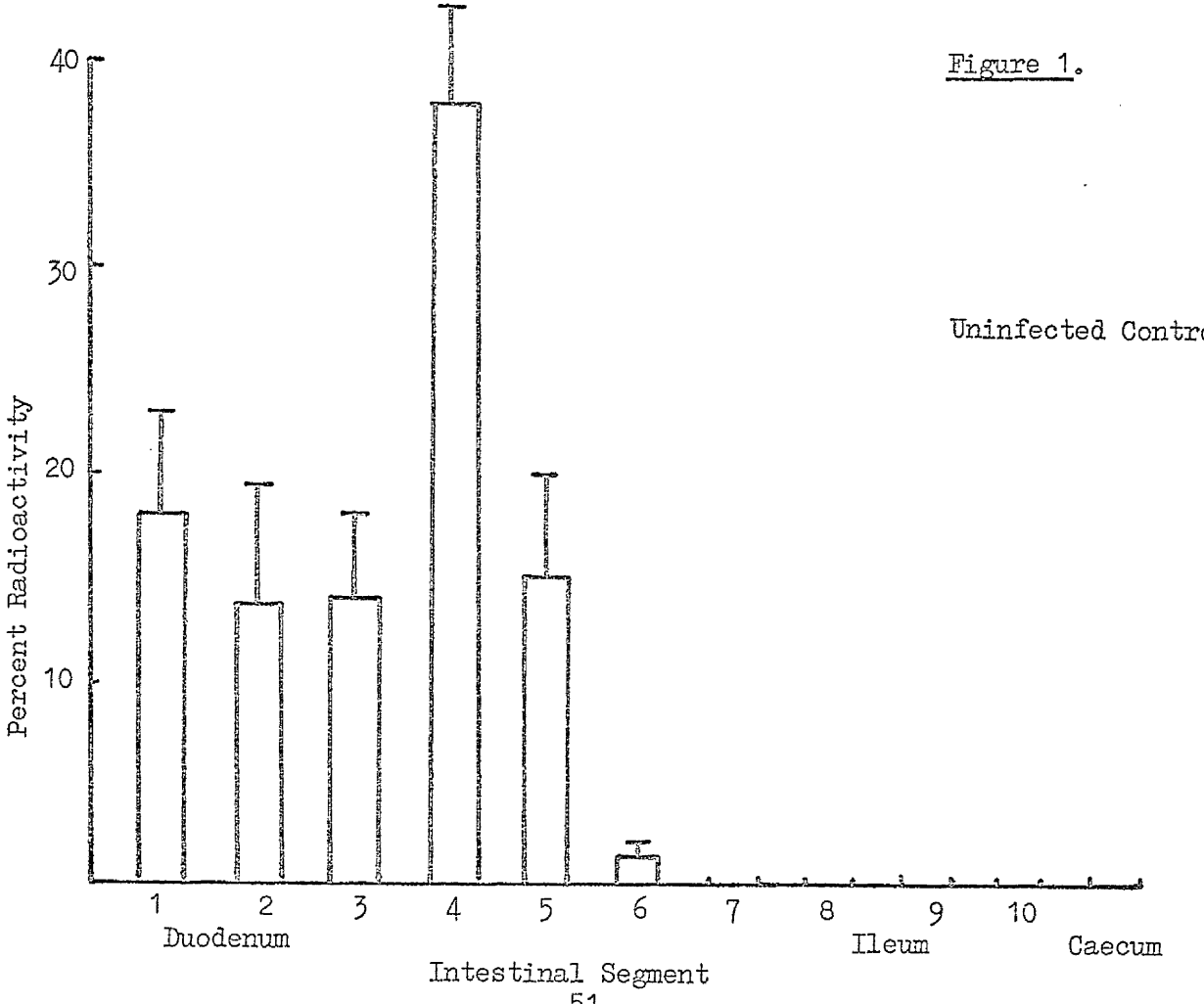
It was observed that the physical appearance of the small intestine of infected animals, especially on days 6 and 8 post-infection, was markedly different than that of controls. Infected guts were very swollen and contained evident amounts of yellow fluid. Great care had to be exercised when removing the intestines from infected animals as they were easily torn, allowing leakage of the gut contents and thus causing error in determination of the radioactivity present.

No significant changes occurred in animal weights following surgery or infection. Because they were handled so often, the rats became very tame and manageable. This was convenient from a practical point of view since injection of the radioisotope was without fuss, and fewer bites were sustained by the author.

The distributions of radioactivity in the small intestines of control rats and rats at different days post-infection are shown in Figures 1 - 6. Some variation in the pattern occurred between different groups, but the important measurement here was the extent to which the ^{51}Cr marker had traversed the small intestine. It is clear from Figs. 1 - 6 that only in the day 8 group had the marker traversed any further through the small intestine than in controls. The ^{51}Cr marker reached segment 6, or 60% of the small intestine in controls (Fig. 1) as compared with segments 8, or 80%, on day 8 post-infection (Fig. 3). On days 6, 10, 12 and 14, very little or none of the marker traversed the small intestine further than in controls (Figs. 2, 4, 5 and 6).

The data in Figs 1 - 6 were transformed to yield the percentage of radioactivity which had passed through, or was

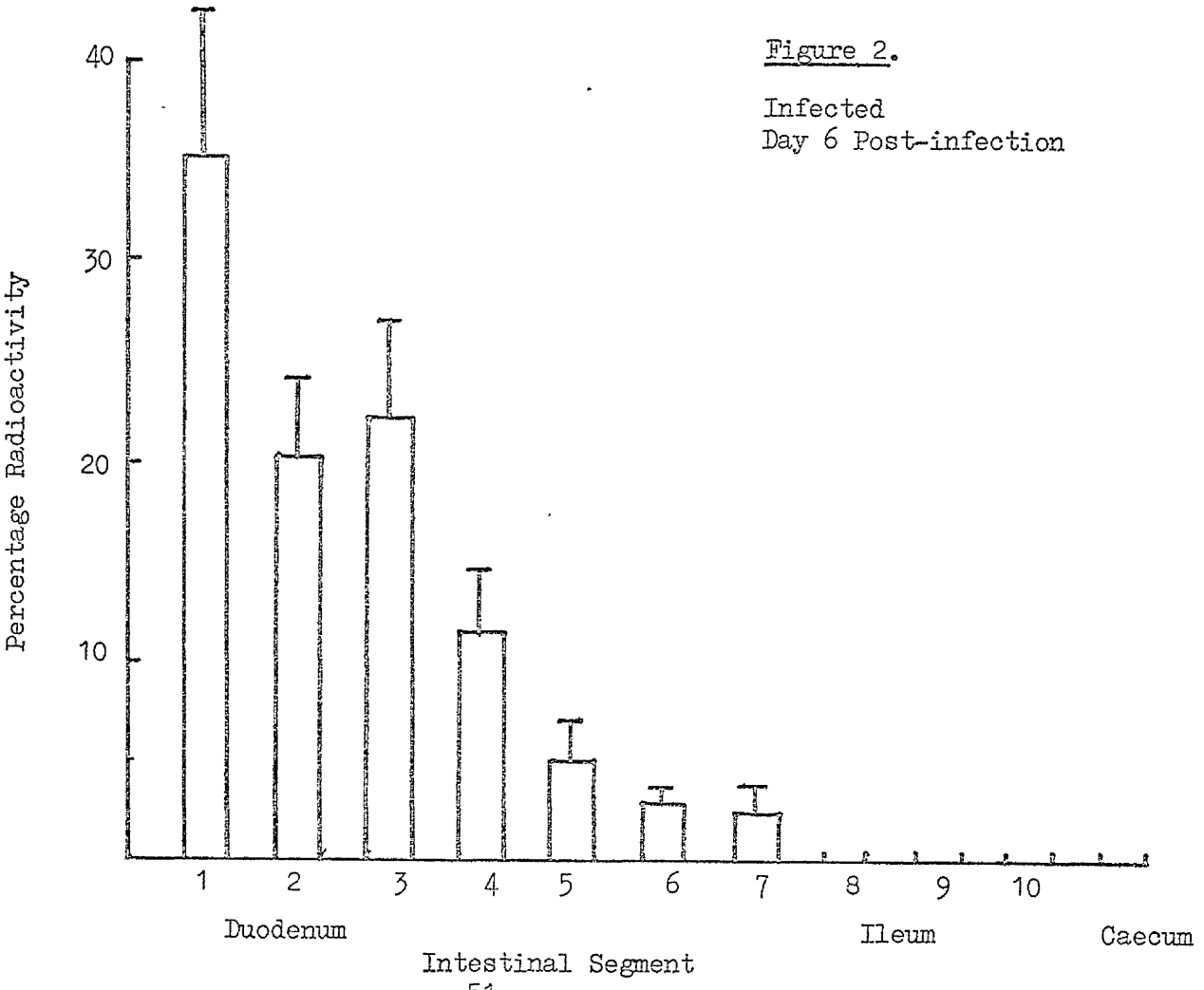
Figure 1.



Distribution of radioactivity (^{51}Cr) along the length of intestine of uninfected rats (n = 14) at the end of the 15 minute test-period. Each column represents the mean ± standard error of the mean (SEM)

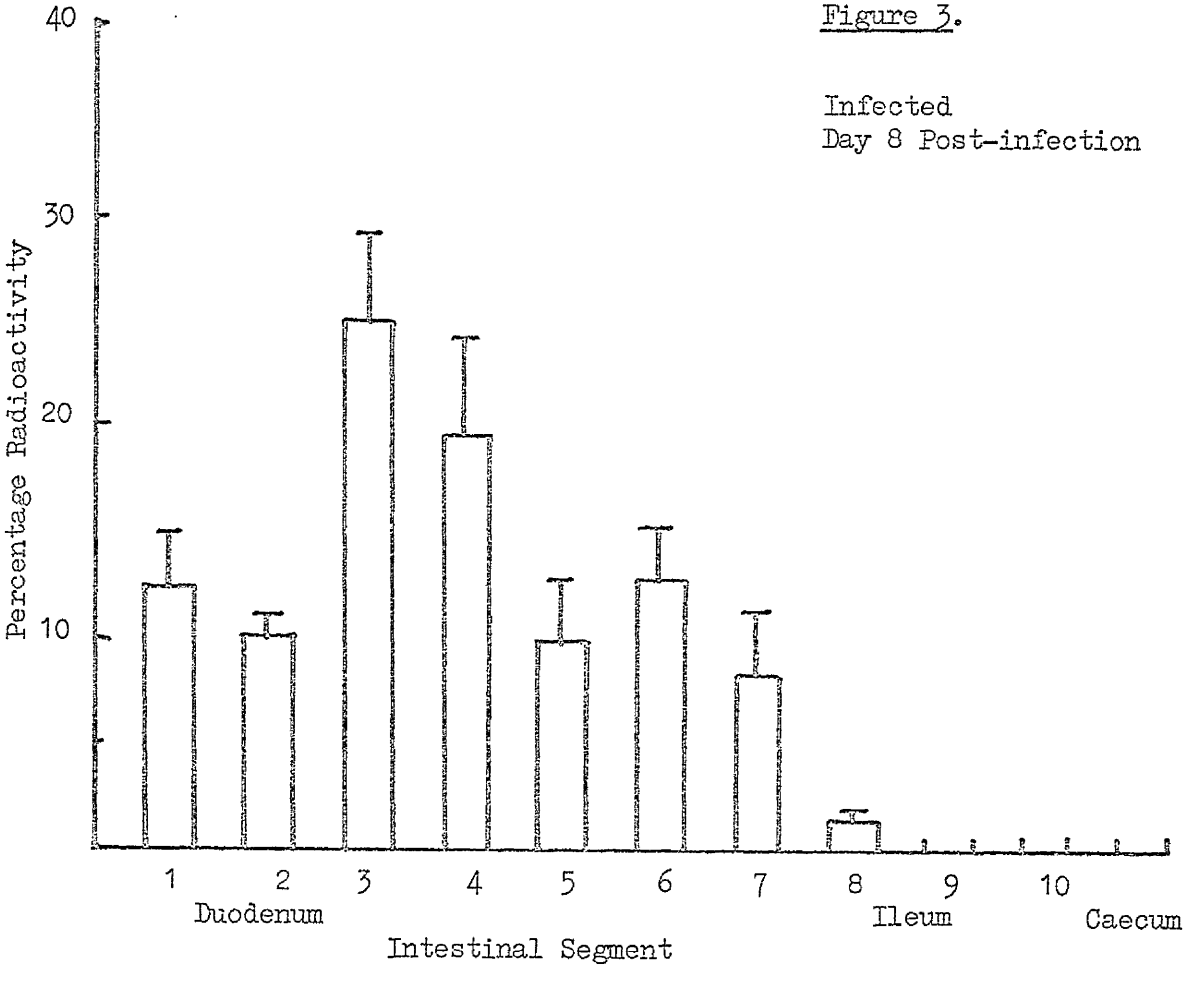
Figure 2.

Infected
Day 6 Post-infection



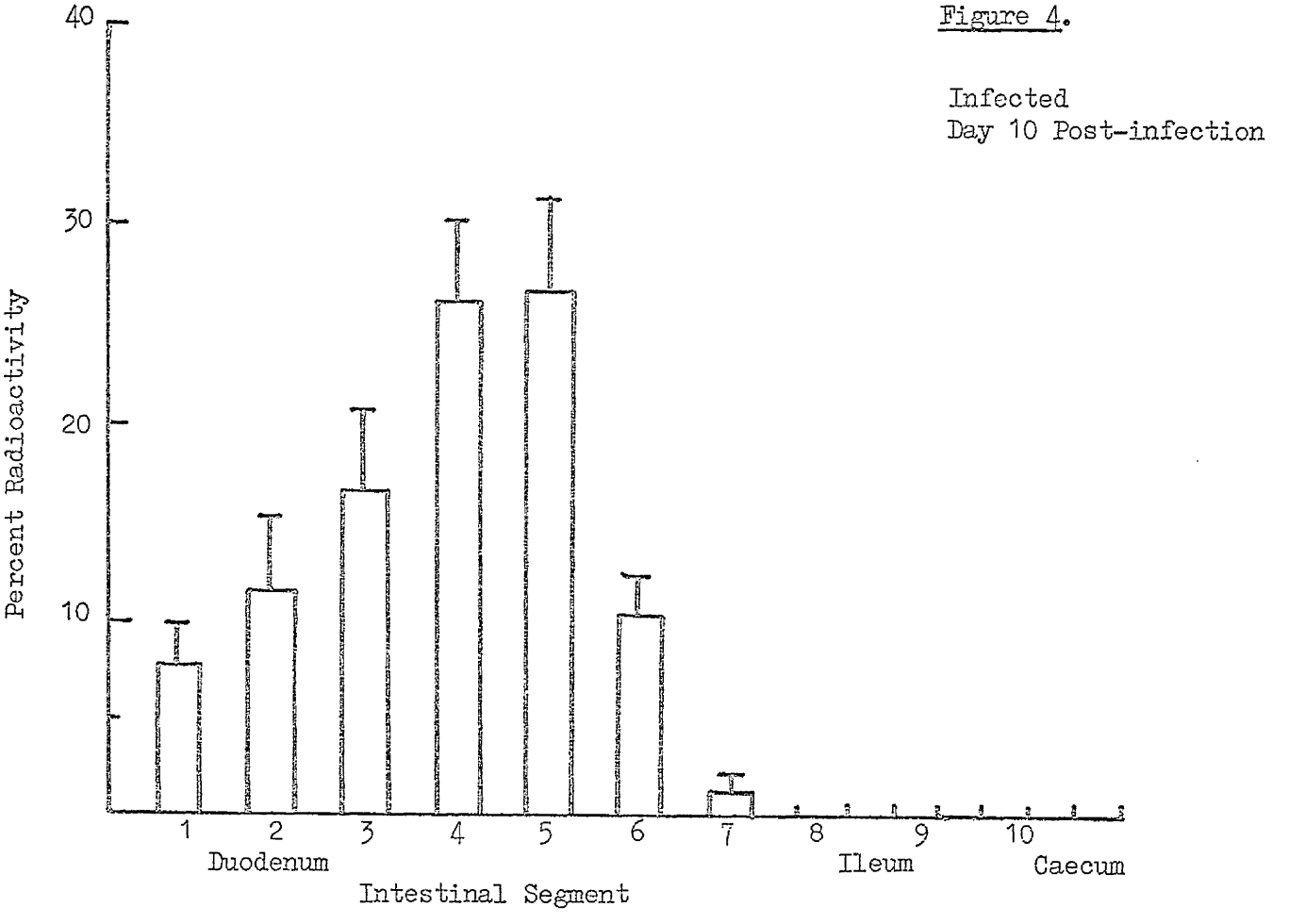
Distribution of radioactivity (^{51}Cr) along the length of intestine of rats on day 6 post-infection with N. brasiliensis (n = 7). Each column represents the mean ± SEM.

Figure 3.



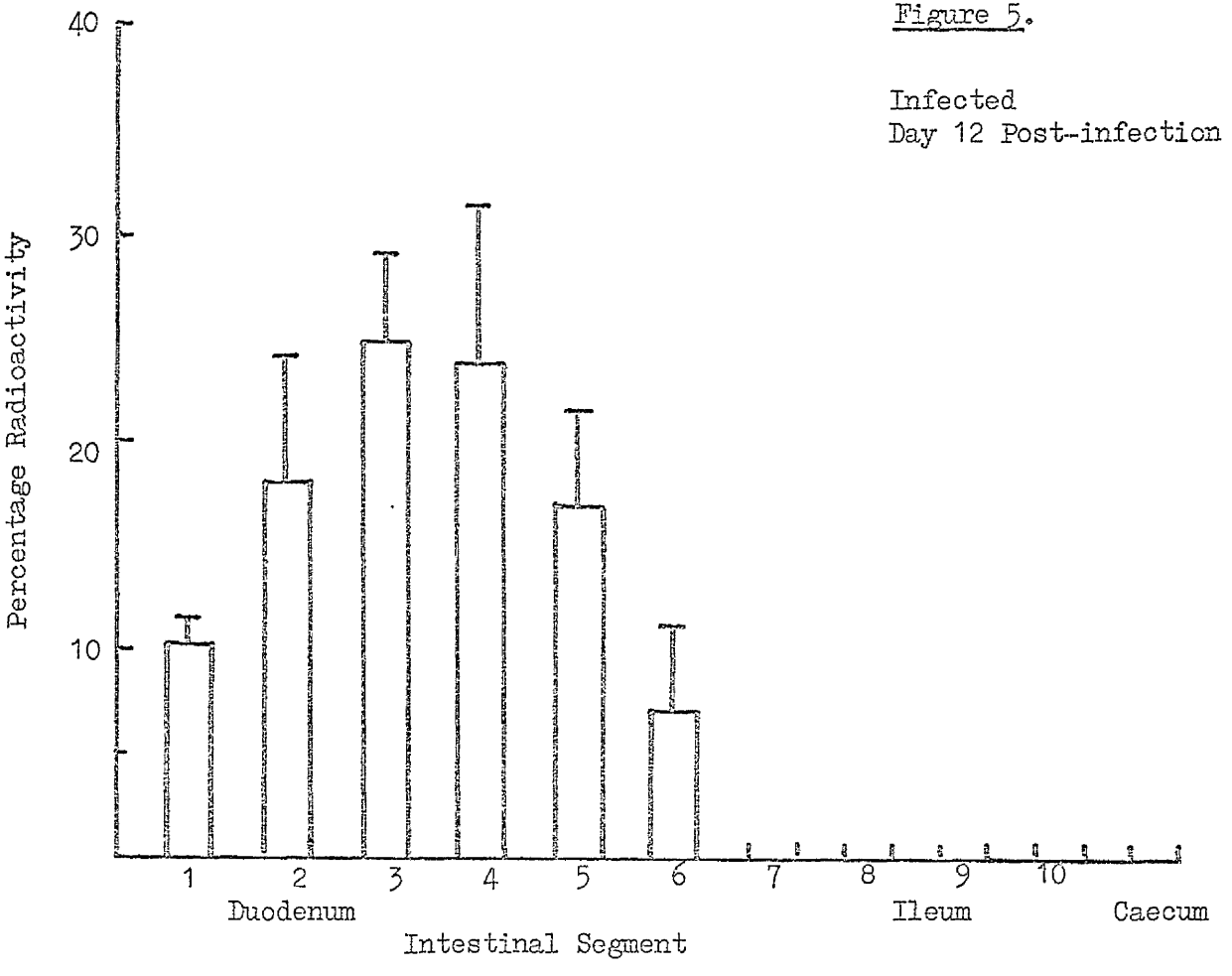
Distribution of radioactivity (^{51}Cr) along the length of intestine of rats on day 8 post-infection with N. brasiliensis (n = 11). Each column represents the mean ± SEM.

Figure 4.



Distribution of radioactivity (^{51}Cr) along the length of intestine of rats on day 10 post-infection with N. brasiliensis (n = 9). Each column represents the mean ± SEM.

Figure 5.



Distribution of radioactivity (^{51}Cr) along the length of intestine of rats on day 12 post-infection with N. brasiliensis (n = 10). Each column represents the mean ± SEM.

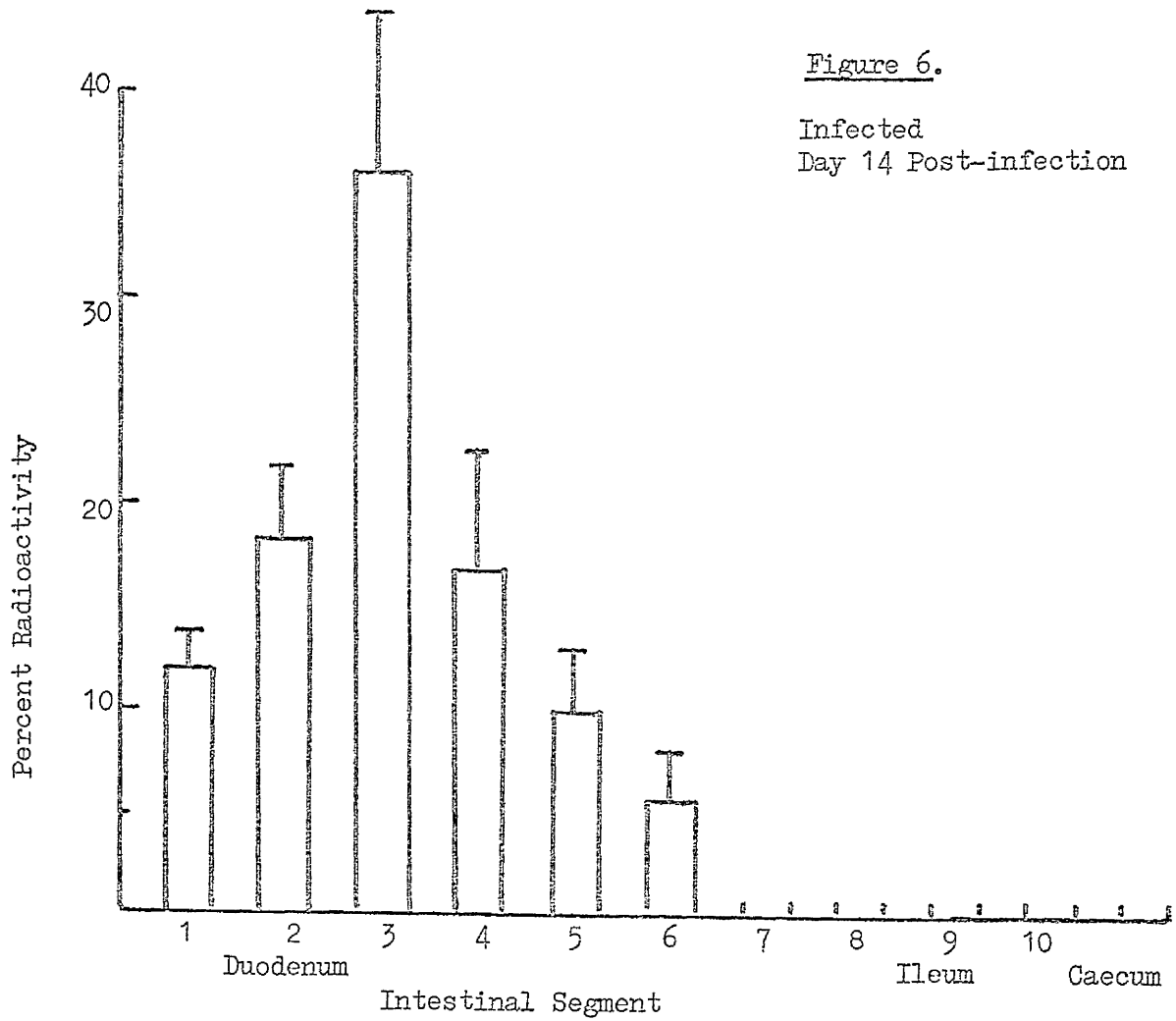


Figure 6.

Infected
Day 14 Post-infection

Distribution of radioactivity (^{51}Cr) along the length of intestine of rats on day 14 post-infection with N. brasiliensis (n = 10). Each column represents the mean ± SEM.

present in a given segment of the small intestine during the 15 minute test-period. It is obvious therefore, that 100% of the marker would pass through segment 1, and that the percent activity passing through the other segments would decrease with intestinal length traversed. Thus, for each group a line with a negative slope resulted which could be analysed by linear regression. These graphs are plotted in Figs. 7 - 12.

Upon analysis of the variance between the regression coefficients obtained for each group (Table 1), that for day 8 (Fig. 9) was found to be significantly different from control (Fig. 7). No significance was found between control and days 6, 10, 12 or 14. By using the regression equations, the amount of radioactivity traversing the midpoint of the gut (segment 5) was estimated as 24% in uninfected rats, and 40% in rats on day 8 post-infection (Table 2). These observed changes in the distribution of radioactivity on day 8 could be explained in terms of a change in intestinal propulsion.

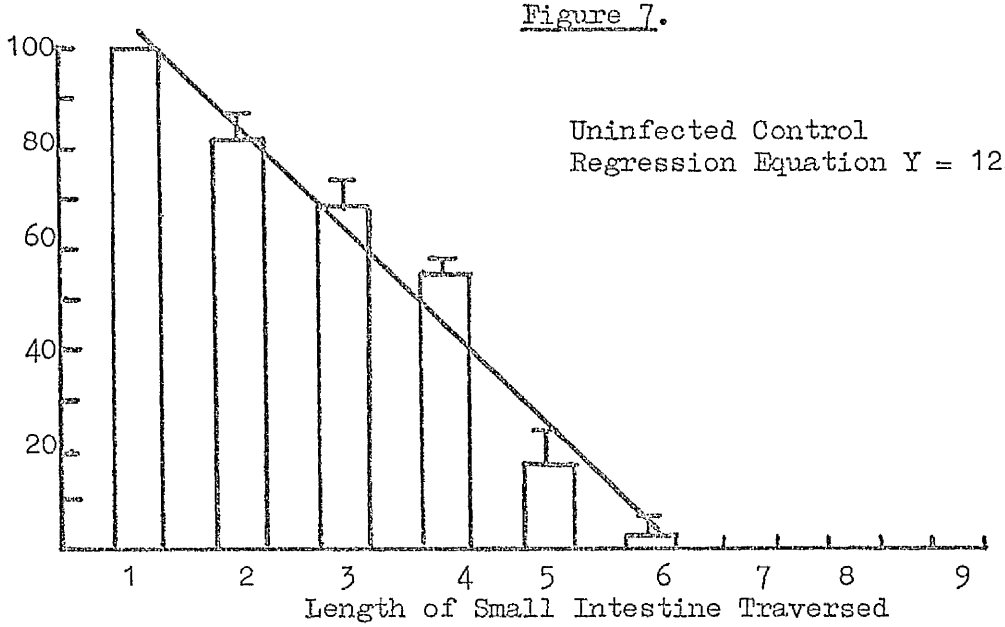
By comparing the control graphs (Figs. 1 and 7) with those for day 6 (Figs. 2 and 8), it would appear that the propulsion in the upper small intestine (segments 1 and 2) is reduced on day 6, even though no significant difference was obtained in the overall propulsion.

Summary and Conclusions

In the experiment described, intestinal propulsion was measured by monitoring the transit of a radioactive marker (^{51}Cr) through the small intestine of rats infected with 4,000 *N. brasiliensis* larvae at various days post-infection. Intestinal propulsion was slightly increased on day 8 post-infection, the leading edge of radioactivity traversing 80% of the small intestine, as opposed to 60% in control (uninfected) rats. However, no alterations in the propulsive motility of the small bowel occurred on days 6, 10, 12 and 14. Propulsion in the upper small intestine appeared to be reduced on day 6 as compared with control.

In view of the greatly increased levels of intestinal prostaglandins (PG) and 5-hydroxytryptamine (5-HT) known to occur during infection and expulsion, it is perhaps surprising that

Figure 7.

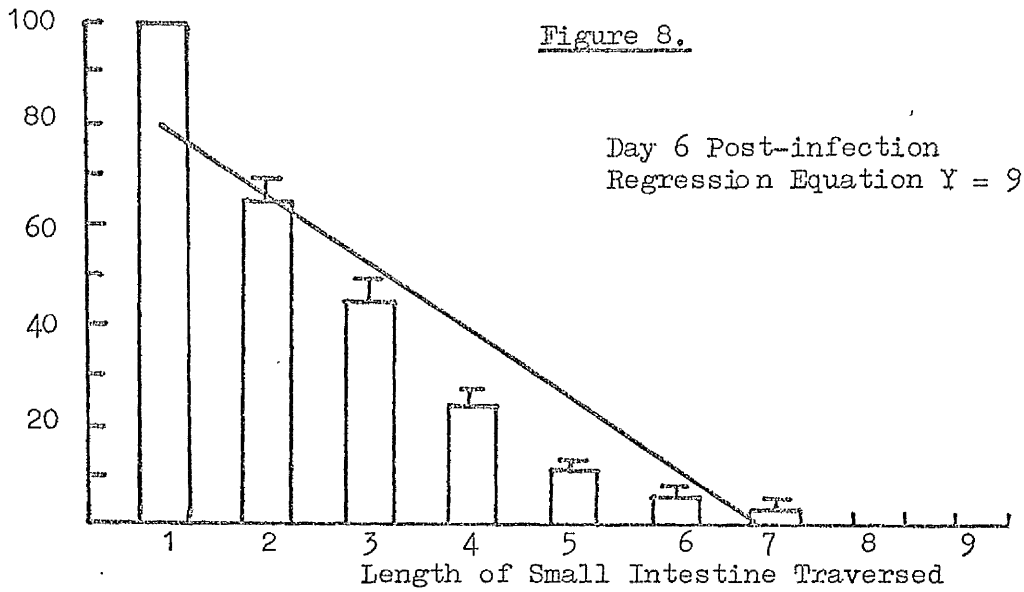


Uninfected Control
Regression Equation Y = 124-20.05X

The percentage of radioactivity (Y) passing through a given segment of intestine in 15 minutes were added and plotted as a function of gut length (X). Means and standard errors are for 14 uninfected rats.

Percent Radioactivity

Figure 8.



Day 6 Post-infection
Regression Equation Y = 92-13.32X

The percentage of radioactivity (Y) passing through a given segment of intestine in 15 minutes were added and plotted as a function of gut length (X). Means and standard errors are for 7 rats on day 6 post-infection with N. brasiliensis.

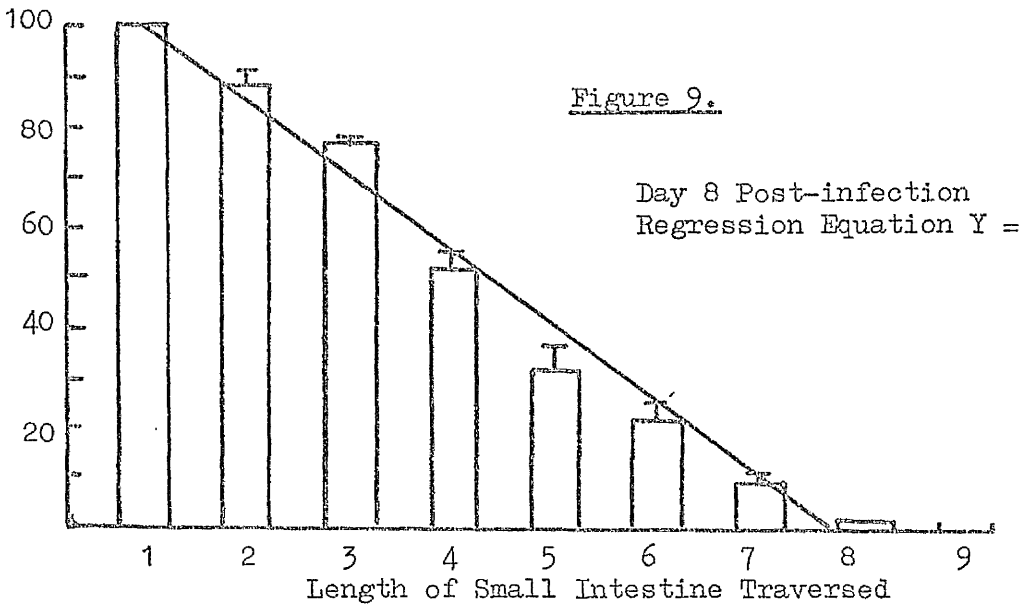


Figure 9.

Day 8 Post-infection
Regression Equation Y = 116-15.11

The percentage of radioactivity (Y) passing through a given segment of intestine in 15 minutes were added and plotted as a function of gut length (X). Means and standard errors are for 11 rats on day 8 post-infection with N. brasiliensis.

Percent Radioactivity

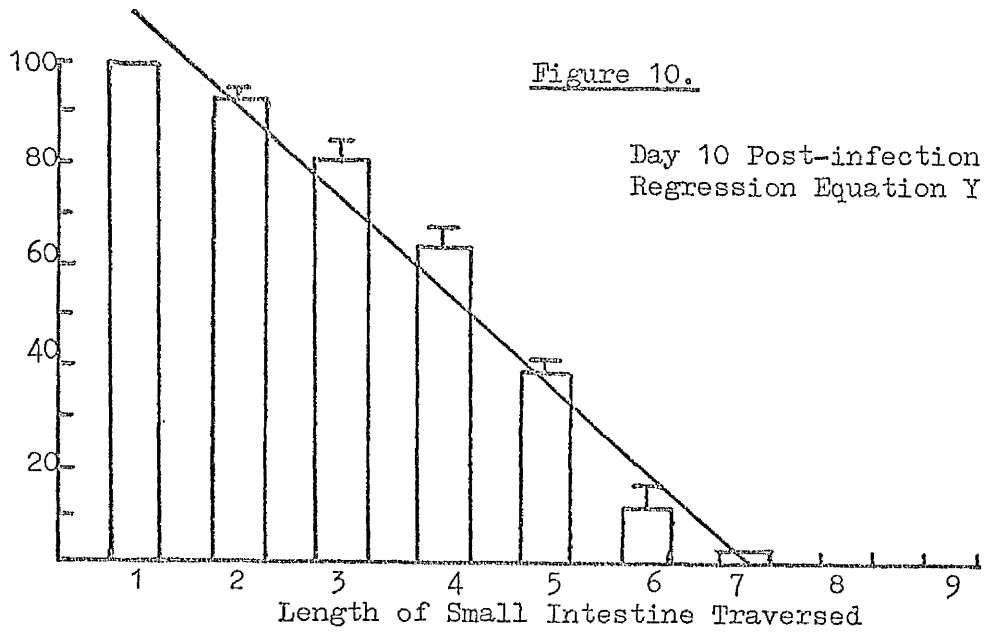


Figure 10.

Day 10 Post-infection
Regression Equation Y = 127-17.86

The percentage of radioactivity (Y) passing through a given segment of intestine in 15 minutes were added and plotted as a function of gut length (X). Means and standard errors are for 9 rats on day 10 post-infection with N. brasiliensis.

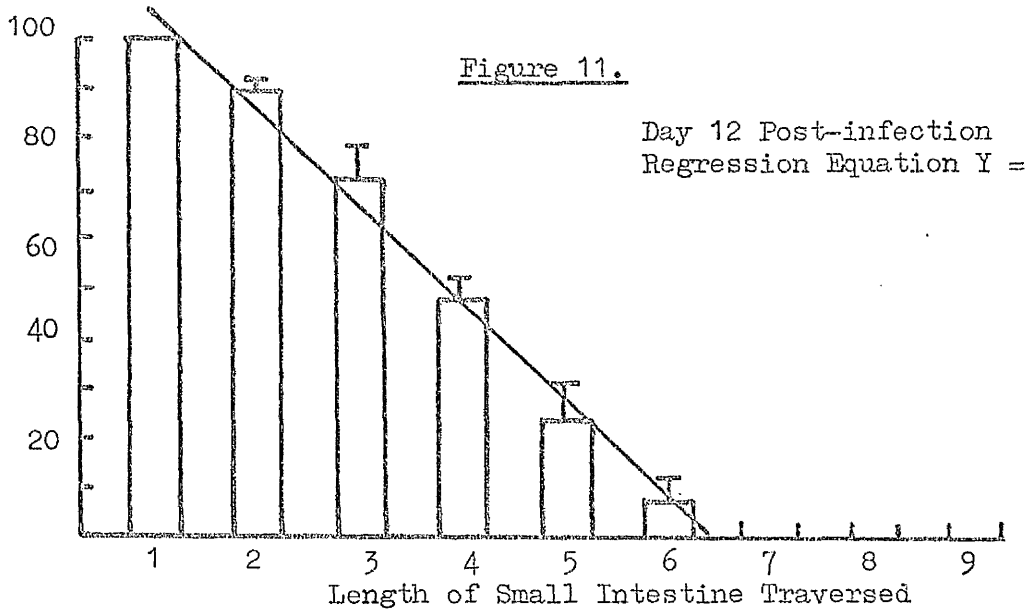


Figure 11.

Day 12 Post-infection
Regression Equation Y = 125-19.55

The percentage of radioactivity (Y) passing through a given segment of intestine in 15 minutes were added and plotted as a function of gut length (X). Means and standard errors are for 10 rats on day 12 post-infection with N. brasiliensis.

Percent Radioactivity

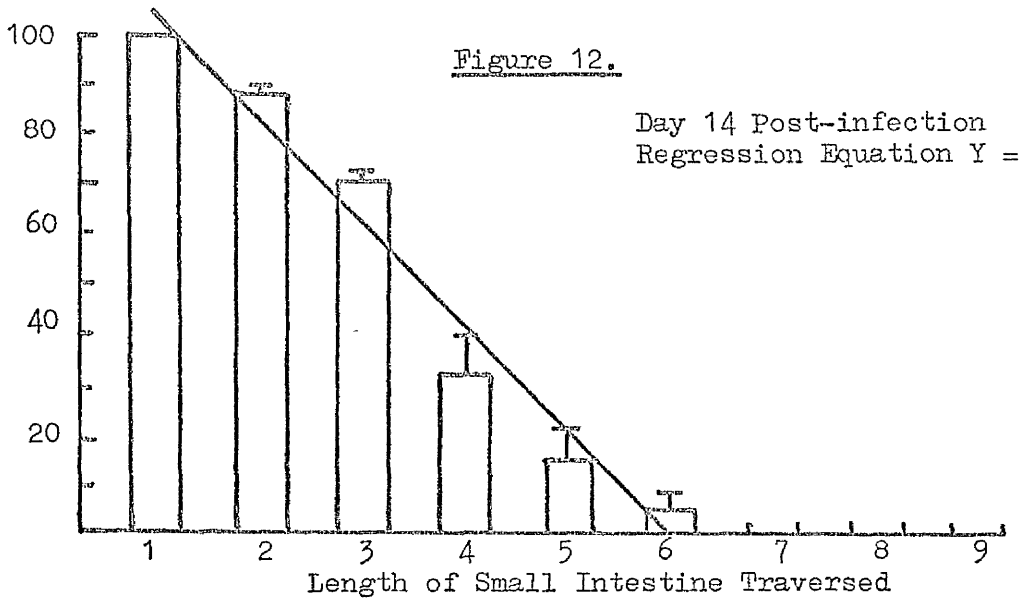


Figure 12.

Day 14 Post-infection
Regression Equation Y = 124-20.62

The percentage of radioactivity (Y) passing through a given segment of intestine in 15 minutes were added and plotted as a function of gut length (X). Means and standard errors are for 10 rats on day 14 post-infection with N. brasiliensis.

Table 1: Statistics for Regression of Percentage of Radioactivity (Y) or Length of Intestine Traversed (X) in Rats Infected with 4,000 Nippostrongylus brasiliensis Larvae. Regression Equations Represent Y as a Function of X.

Group	No.	Regression Coefficient	Regression Equation	Anovar[b] on Regression Equation		
				Groups Compared	F	P
1. Control	14	- 20.05[a]	Y = 124 - 20.05X			
				1 vs 2	3.900	ns[c]
2. Day 6	7	- 13.32[a]	Y = 92 - 13.32X			
				1 vs 3	7.985	< 0.025
3. Day 8	11	- 15.11[a]	Y = 116 - 15.11X			
				1 vs 4	0.966	ns[c]
4. Day 10	9	- 17.86[a]	Y = 127 - 17.86X			
				1 vs 5	0.060	ns[c]
5. Day 12	10	- 19.55[a]	Y = 125 - 19.55X			
				1 vs 6	0.053	ns[c]
6. Day 14	10	- 20.62[a]	Y = 124 - 20.62X			

a Indicates that the slope of the line was significant as determined by $t = b/S_b$ (Snedecor, 1956). In all cases $P < 0.001$.

b Statistics for all regressions are plotted in Figures 7 - 12. Regression coefficients were compared for significant differences by analysis of variance (anovar).

c ns indicates no significance at the 5% level between groups compared.

Table 2: The Amount of Injected Radioactivity Traversing the Midpoint (50% of the length) of the Small Intestine at Various Times Post-infection with 4,000 N.brasiliensis Larvae.

Days Post-Infection	% Activity Traversing Mid-point of the Gut
Control	23.75
Day 6	25.40
Day 8	40.20
Day 10	37.5
Day 12	25.25
Day 14	20.90

the observed changes in intestinal propulsion were so slight and transient. Murray et al. (1971b) described a marked episode of mast-cell activity associated with worm expulsion, and a corresponding rise in 5-HT levels in the intestinal wall. They proposed that 5-HT plays a major role in creating a hyperpermeable intestinal wall which facilitates antibody translocation into the gut lumen. Rat intestinal smooth muscle is very sensitive to 5-HT, and it has been demonstrated that small amounts of this compound are continuously released into the gut lumen, the amount released being directly proportional to the filling pressure and increased during peristalsis (Bulbring and Crema, 1959a, b; Bulbring and Lin, 1958). Similarly Bulbring and Lin (1958) showed that 5-HT introduced into the gut lumen stimulated peristalsis. It is tempting therefore, to attribute the increased intestinal propulsion observed at day 8 post-infection with N. brasiliensis in the present experiment to the increased levels of 5-HT in the gut mucosa. However, if this is the case, it is strange that no changes in propulsive activity were observed on days 10 - 14 post-infection, since Murray and his associated (1971b) found that 5-HT levels increased in the gut by 300% during this time.

Similarly, the marked oedema and fluid accumulation which occurs in the rat small intestine at the site of worm infection may cause gut distension. As was described previously, the stimulus for peristalsis is radial stretch of the small intestine, and gut distension due to fluid accumulation may, at least in part, have been the cause of increased intestinal propulsion on day 8 post-infection. However, it should be remembered that Castro and his co-workers (1976, 1977) found that, although intestinal propulsion increased during a primary infection with T. spiralis, no changes in propulsion occurred during secondary infection, even though marked intestinal fluid accumulation accompanied both.

Some evidence has accumulated which suggests that PG's, especially of the E class are involved in the expulsion of N. brasiliensis (see general introduction and review by Kelly

and Dineen, 1976). It has, for example, been shown that intraduodenal administration of PGE_1 and E_2 cause rapid worm rejection (Kelly et al., 1974), and Dineen and Kelly (1976) found that gut levels of PGE increased during N. brasiliensis infection. Peak levels at the site of worm location were attained on day 7. As has been said, this precedes expulsion by 3 or 4 days, but it has been shown that metabolic and structural damage to the worms occurs prior to expulsion (Ogilvie and Hockley, 1968; Edwards et al., 1971; Henney et al., 1971). Richards and his co-workers (1975, 1977) demonstrated that PGE_1 causes metabolic and structural damage to worms in vitro, and Dineen and Kelly (1976) proposed that the increased intestinal levels of PGE cause expulsion by a direct effect on N. brasiliensis worms. It should be mentioned here that Kassai and his associates (1980) reported recently that they could find no substantial untoward effect either in vitro or in vivo of PG's on adult worms.

However, the effects of PG's on intestinal smooth muscle may also be of some relevance in worm damage or expulsion. It must be admitted that the increased intestinal propulsion on day 8 observed in the present investigation was rather slight and transient. Therefore, it is difficult to suggest that it plays a major factor in expulsion of the worms which does not begin until around day 10 post-infection. PG's of the E class generally cause relaxation of the circular smooth muscle of the small intestine (Bennett, 1976; Bennett and Fleshler, 1970) and it is therefore unlikely that the elevated levels of PGE found by Dineen and Kelly (1976) would cause increased intestinal propulsion. However, PG's of the E class cause contraction of the gut longitudinal muscle layer through several possible mechanisms (see general introduction). It is possible therefore, that the weak increase in intestinal propulsion observed at day 8 may have been a reflection of some other motility change of the longitudinal muscle (such as pendular contractions), which was stimulated by increased intestinal levels of PGE on days 7 - 9. This could merely be an ineffectual

consequence of PG's or, it is possible that increased local contractions of the small intestinal longitudinal muscle may help dislodge damaged worms.

It has been found that the passage of food through the upper intestine is decreased in rats infected with N. brasiliensis (Symons, 1966). As discussed earlier, this parasite secretes large amounts of acetylcholinesterase (AChE), and the decreased propulsion observed by Symons tends to support the hypothesis that worm-secreted AChE may act to inhibit local peristalsis by hydrolysing neurally released ACh (Ogilvie and Jones, 1971). Supporting evidence for this was obtained in the present study, in that intestinal transit appeared to be reduced in the upper part of the small intestine on day 6 post-infection (see Figs 1, 2, 7 and 8). N. brasiliensis worms have just matured at this time, and if worm-AChE is responsible for decreased local propulsion, it may be a mechanism to aid the establishment of adult worms. It is of interest to note that most of the worms are located in the upper small intestine.

In conclusion, it would appear that increased intestinal propulsive motility does not play a significant part in the expulsion of N. brasiliensis, though this does not preclude the possibility that other non-propulsive alterations in motility, such as segmentation or pendular contractions, may do so.

2. THE EFFECT OF CARBACHOL ON THE WORM POPULATION OF INFECTED RATS

2. The Effect of Carbachol on the Worm Population of Infected Rats

Introduction

In the previous experiment, it was shown that during primary infection with N. brasiliensis, no alteration in the propulsive motility of the rat small intestine occurred except at day 8, when a slight increase was observed. It was thought that this slight increase in propulsion perhaps reflected some other motility change which may dislodge damaged worms from the intestinal wall. If gut motility is important in expulsion, it is conceivable that a drug such as carbachol which stimulates motility, when given at the proper time, might bring forward the onset of expulsion, or increase the rate of expulsion once it has started.

Keller and Ogilvie (1972) investigated the effect of oxyphenonium, which decreases gut motility by antagonizing ACh, upon worm expulsion in N. brasiliensis infected rats, and found that the drug had no effect. However, these authors had no way of determining the extent of effect which the dose of oxyphenonium used (1 mg/kg) had on gut motility.

It has been found that medroxyprogesterone acetate (Depo-provera) delays the expulsion of N. brasiliensis from the intestine of infected rats (Lloyd, Allonby and Mulligan, personal communication). This long-acting, synthetic progestegin delayed the actual expulsion of the parasites, but seemed to have no effect upon the damage which the parasite suffers due to the development of host immunity. It was suggested that this action of Depo-provera is related to its properties as a smooth muscle-relaxant. Progesterone itself is known to produce a general sluggishness of the alimentary tract in humans, manifested as delayed gastric emptying and constipation in early pregnancy (Rhodes, 1969).

The purpose of the present experiment was to determine what effect increased gut motility has upon an established worm population. As discussed previously, N. brasiliensis worms undergo metabolic damage 3 or 4 days prior to expulsion, reflected as a decrease in uptake of labelled metabolites from the host beginning at around

day 7 post-infection (Henney et al., 1971; Maclean, 1977). This study therefore set out to find whether increasing gut motility with carbachol would bring forward the expulsion of adult worms both before and after they had been damaged. Carbachol is a parasympathomimetic drug which stimulates intestinal smooth muscles.

Methods

The LD_{50} for carbachol in rats is quoted as 4 mg/kg (Molitor, 1936). However, it was found in this laboratory that a subcutaneous injection of as little as 1 mg/kg was lethal in 6 rats treated and therefore a much smaller dose was required. Nine rats were divided into 3 groups of 3, and 0.3, 0.5 and 0.7 mg/kg carbachol were administered to each group respectively in order to ascertain a dose which was sufficient to produce increased gut motility (manifested as diarrhoea), but which did not cause any other marked physical effects.

Rats which were given 0.3 mg/kg carbachol showed physical symptoms within 10 minutes, of profuse salivation, chromodacryorrhesis and slight diarrhoea. These effects had worn off after 25 minutes. 0.5 mg/kg caused more profuse diarrhoea than 0.3 mg/kg, but the animals tremored and were unusually quiescent. This was presumably a central effect of the drug. 0.7 mg/kg produced severe physical symptoms. Thus, it was thought that a dose of 0.4 mg/kg would be sufficient to stimulate the gut without markedly producing other effects.

Twenty-four rats were infected with 5,000 N. brasiliensis larvae each, and divided into 4 groups of 6. The rats of group 1 were not given any drug, and those of group 2 were treated with 0.4 mg/kg carbachol every two hours on days 5 and 6 post-infection, i.e. before metabolic damage to the worms occurred. These groups, termed 'pre-damage, pre-expulsion', were killed by cervical dislocation on day 7 and their worm burdens determined. The rats of group 3 were also untreated, and those of group 4 were treated with 0.4 mg/kg carbachol on days 7 and 8 post-infection. Groups 3 and 4, termed 'pre-expulsion', were killed on day 9, and their

worm burdens determined. Control saline injections were not given to the untreated groups since a previous study showed that subcutaneous injection of saline has no effect upon worm burdens.

The individual worm burdens for each group were meaned and the means compared by Student's 't' test. For the method of worm burden recovery and determination, the reader is referred to general methods and materials, part 2(iii).

Results

The individual worm burdens from each rat are shown in Table 3. It is clear that although some variation does exist, no apparent difference between different groups occurred. The worm burdens were meaned and the means for each group compared by student's t-test. No significant difference was found between any groups.

Thus, it appears that increased gut motility, as influenced by carbachol, did not bring forward worm expulsion, even when the parasites would have sustained at least some metabolic and structural damage.

Summary and Conclusions

Expulsion of N. brasiliensis normally begins around day 10 post-infection. However, prior to this metabolic and structural damage to worms occurs. Metabolic damage to the parasite has been demonstrated as early as day 8, reflected as a reduced capacity of the worms to take up labelled metabolites from the host (Henney et al., 1971; Maclean, 1977). By day 10, when expulsion normally begins, the uptake by worms of ^{32}P-phosphate was 10% of that on days 6 or 7 post-infection. In the present experiment, treatment of rats with carbachol on days 5 and 6 post-infection, before the worms had been damaged, did not bring about expulsion, since their worm burdens on day 7 were no different from those of untreated, infected rats. Similarly, expulsion was not brought forward by treatment of infected rats on days 7 and 8, after some damage had been incurred by the worms.

Table 3: Individual Worm Burdens (mg) in Control Rats and in Rats Treated with 0.4 mg/kg Carbachol. Rats in Groups 1 and 2 were Killed on Day 7 Post-infection and are Termed 'Pre-damage, Pre-expulsion'. Rats in Groups 3 and 4 were Killed on Day 9 and are Termed 'Pre-expulsion'.

	Rat	Treatment	Worm wts. (mg)		Rat	Treatment	Worm wts. (mg)
Group 1	1	-	74.6	Group 3	1	-	56.9
	2	-	77.2		2	-	31.4
	3	-	49.9		3	-	73.5
	4	-	64.2		4	-	41.5
	5	-	47.6		5	-	60.4
	6	-	56.6		6	-	36.7
Group 2	1	Carbachol	23.2	Group 4	1	Carbachol	43.9
	2	"	49.6		2	"	62.9
	3	"	66.0		3	"	76.3
	4	"	22.6		4	"	65.3
	5	"	73.1		5	"	78.0
	6	"	74.3		6	"	62.2

Carbachol treated rats were diarrhoeic, presumably due to stimulation of cholinergic muscarinic receptors in the gut causing increased motility. The infected rats of group 2 were treated with this drug on days 5 - 6. Since the parasites probably maintain a fairly firm hold intertwined among the rat intestinal villi (Lee, 1969a), it is perhaps not surprising that increased gut motility did not expel them at this time. However, the rats of group 4 were treated with carbachol on days 7 - 8 post-infection, and it is probable that during this time their parasite populations had sustained some immunological damage. Therefore, it is surprising that increased gut motility did not expel at least part of the worm population.

It is of relevance to mention here that most anthelminthic drugs do not kill intestinal parasites, but damage them such that their hold on the gut is loosened and they are swept out by normal peristalsis (Bowman et al, 1968; Crossland, 1970). For example, piperazine which is probably the most widely used anthelminthic in man, blocks the action of ACh in the worm myoneural junctions. This produces flaccid paralysis, which in turn facilitates the elimination of the living worms by intestinal peristalsis (Botero, 1978). Indeed, many anthelminthic drug treatments are followed by administration of a purgative which stimulates increased peristalsis and expulsion of the drug-impaired parasites.

Nevertheless, it is possible that the N. brasiliensis worms in the present experiment were not sufficiently damaged on days 7 - 8 to be susceptible to expulsion by the increased gut motility produced by carbachol. It would be of interest to discover if increased gut motility, induced by carbachol, would expel the parasites at a greater 'rate' than normal between days 10 and 14, i.e. without altering the time of onset of expulsion.

3. INVESTIGATION OF THE SENSITIVITY OF ISOLATED SEGMENTS OF THE SMALL INTESTINE FROM INFECTED RATS

3. Investigation of the Sensitivity of Isolated Segments of Small Intestine from Infected Rats

Introduction

In the study of smooth muscle function, its responses to various stimuli and pathological conditions, and its possible role in worm expulsion, it is of advantage to study the tissue *in vivo*. For obvious reasons, this will give the most accurate information as the muscle is in its normal anatomical and physiological environment. This is particularly true in the case of the parasitized gut, in that parasites such as *Nippostrongylus brasiliensis* are very sensitive to their microenvironment and factors which may alter it. One should therefore be cautious in extrapolating results obtained from *in vitro* experiments involving parasitized gut to the situation *in vivo*.

However, by far the easiest and most convenient way to examine the responses and sensitivity of intestinal smooth muscle is to do so *in vitro*. It has been found that isolated pieces of small intestine from rats infected with *N. brasiliensis* appeared to exhibit a greater amplitude of spontaneous contractions than small intestine from uninfected animals (H.R.P. Miller, Personal Communication). It was therefore decided to investigate further not only spontaneous activity, but responses to electrical and chemical stimuli, of isolated segments of small intestine from rats at different stages of infection with *N. brasiliensis*.

As described previously, propulsion in the intestine (such as that measured in experiment 1) is mediated in the main by co-ordinated contractions of the circular smooth muscle layer. However, intestinal motility need not be propulsive in nature, and indeed the most common intestinal movements are concerned with mixing the chyme, with no net propulsion occurring. Mixing movements such as segmentation and pendular contractions are due to contractions of the circular and longitudinal muscle layers respectively. These two types of muscle are known to differ in their sensitivity to several substances such as ACh and 5-HT (Harry, 1963; Paton and Zar, 1968), the circular layer generally being less sensitive.

In the experiments described below, contractions of the longitudinal layer were measured. Contractions of this muscle layer *in vivo* could have a profound effect on the worm micro-environment which would not necessarily be manifested as increased propulsion.

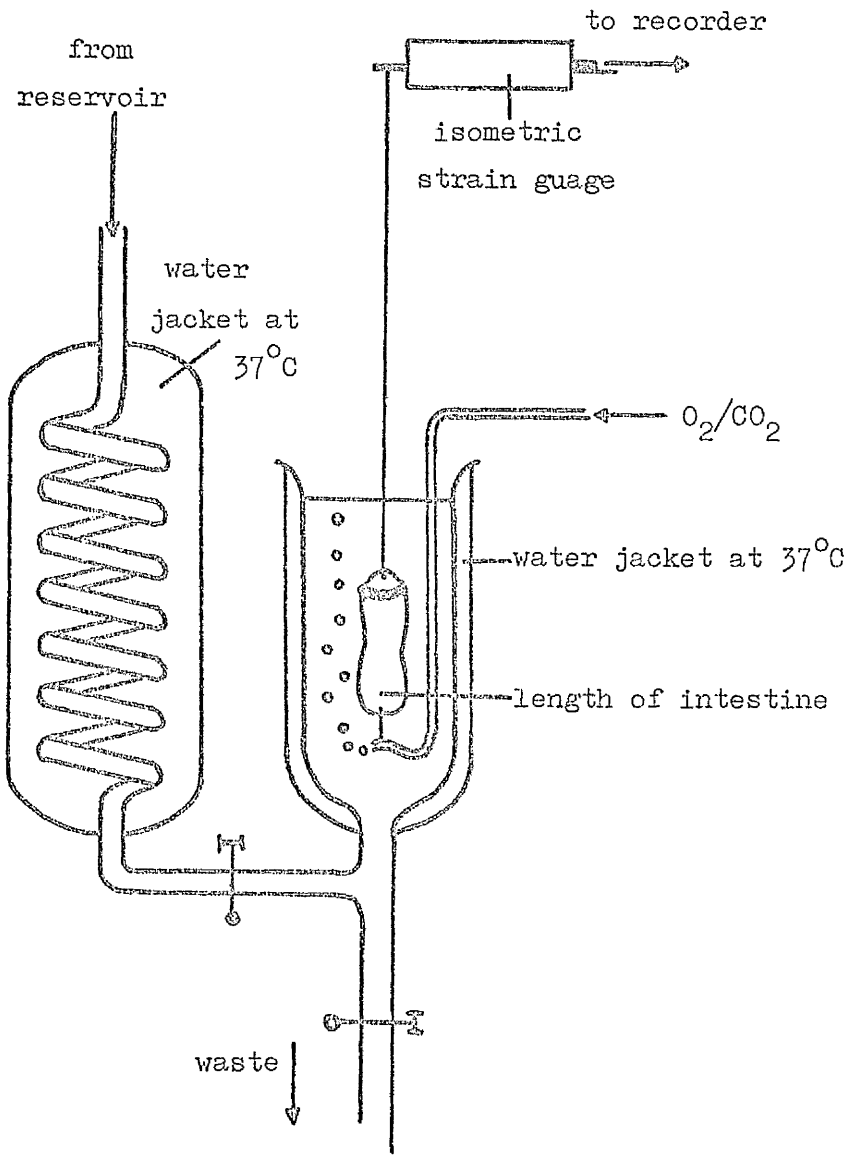
Methods

(i) Preparation of Intestinal Muscle

Rats were killed by a blow to the head and cervical dislocation, the small intestine excised and a segment of about 2 cm in length removed from approximately 20 cm distal to the pylorus. It is in this region of the small intestine that the worms localize. Attached mesentery was carefully dissected from the piece of gut and the tissue suspended in a 40 ml organ bath containing Krebs' solution, pH 7.4, of the following composition (mM): NaCl, 118.5; KCl, 4.8; KH_2PO_4, 1.2; $MgSO_4$, 1.0; $NaHCO_3$, 25.0; $CaCl_2$, 2.5; Glucose, 11.1. This was gassed with 95% O_2 and 5% CO_2 and maintained at 37°C. Using thread the piece of gut was suspended between a small hook at the bottom of the organ bath and an isometric strain guage, which was connected to a Servoscribe pen recorder via a pre-amplifier (see Fig. 13). The initial resting tension was adjusted to 10 g and the tissue allowed to equilibrate for at least 30 minutes. Field stimulation of the intramural nerves in the tissue was provided by a Palmer Electronic Square Wave Stimulator via ring type platinum electrodes (Burn and Rand, 1960). Stimuli were delivered at a supramaximal voltage of 70V and a pulse-width of 0.5m s. Using this short pulse width ensured that only nervous tissue, and not muscle, was being stimulated.

The spontaneous activity of the tissue was observed for not less than 30 minutes before stimuli or drugs were applied, and the rate and mean amplitude of contractions measured. In addition, frequency-responses at supramaximal voltage were carried out within a range of 0.05 - 10Hz. At 1Hz or more stimulus duration did not exceed 25 seconds.

Figure 13.



Arrangements for recording the contractions of a segment of small intestine. The water jackets were maintained at 37°C by means of a thermostatically controlled circulation pump.

Dose-responses were carried out to 5-HT, to ACh and to the synthetic carbamyl choline ester, carbachol. The chloride salts of ACh and carbachol, and the creatinine sulphate salt of 5-HT were used. ACh and carbachol contract gut muscle by acting on muscarinic receptors, the latter having a more prolonged effect since it is resistant to hydrolysis by cholinesterase. 5-HT also causes contraction of intestinal muscle by direct and indirect actions. For a detailed description of the actions of ACh and 5-HT, see the general introduction. Drugs were made up freshly on the day of each experiment, dissolved in 0.9% saline, and volumes not exceeding 0.5 ml were added to the organ bath.

Responses were measured as the peak rise in tension produced by field stimulation or agonists, and subsequent doses were not added until the tissue had returned to its resting tension following washout of the drug.

(ii) Calculation of Results

The spontaneous activity, and the responses to field stimulation and drugs were examined in preparations taken from uninfected, control animals and in those from animals on days 6, 8, 10, 12, 14 and 20 post-infection with 5,000 *N. brasiliensis* larvae. The maximum amplitudes of spontaneous contractions (g) and responses to field stimulation were recorded at the days mentioned, the individual results for each group meaned and differences between groups compared by Student's t-test.

Two methods of plotting dose-responses are commonly used, the doses being plotted on the abscissa in a logarithmic scale in both cases. The response of the tissue is developed tension, in g, under conditions of constant length (isometric tension), and the maximum response is the greatest tension developed by the tissue to different concentrations of drug. Thus, in this experiment, log dose - responses to agonists were plotted, each response being in absolute units, and the maximum responses recorded and expressed as a mean for each group. Differences in the mean maximum response between groups were analysed by t-testing.

Another method of plotting data, called 'normalization', is to express each drug response as a percentage of the maximum response. The dose of agonist which produces 50% of the maximum

response of the tissue is termed 'D_{50}', and the negative logarithm of D_{50} has been termed the 'pD_2' value (Ariëns and van Rossum, 1957). Thus, log dose - percent response curves for each agonist were plotted, and the pD_2 value for each curve calculated from regression line-analysis of the straight line part of the curve. The mean pD_2 values for each experimental group were determined, and those of different groups compared by t-testing.

Consequently, for each tissue on the days post-infection concerned, there are two useful criteria of drug effect. Firstly, the pD_2 value gives an indication of the sensitivity or susceptibility of the tissue to the agonist in question. In other words the size of drug dose required to produce a particular, sub-maximal response (D_{50}). Secondly, the maximum response developed due to drug action (or indeed some other form of stimulation) gives an indication of the reactivity of the preparation. In other words, the responses the tissue is capable of eliciting. Changes in the maximum response are generally recognized to be unusual and not as frequently encountered as alterations in pD_2 (Fleming et al., 1973).

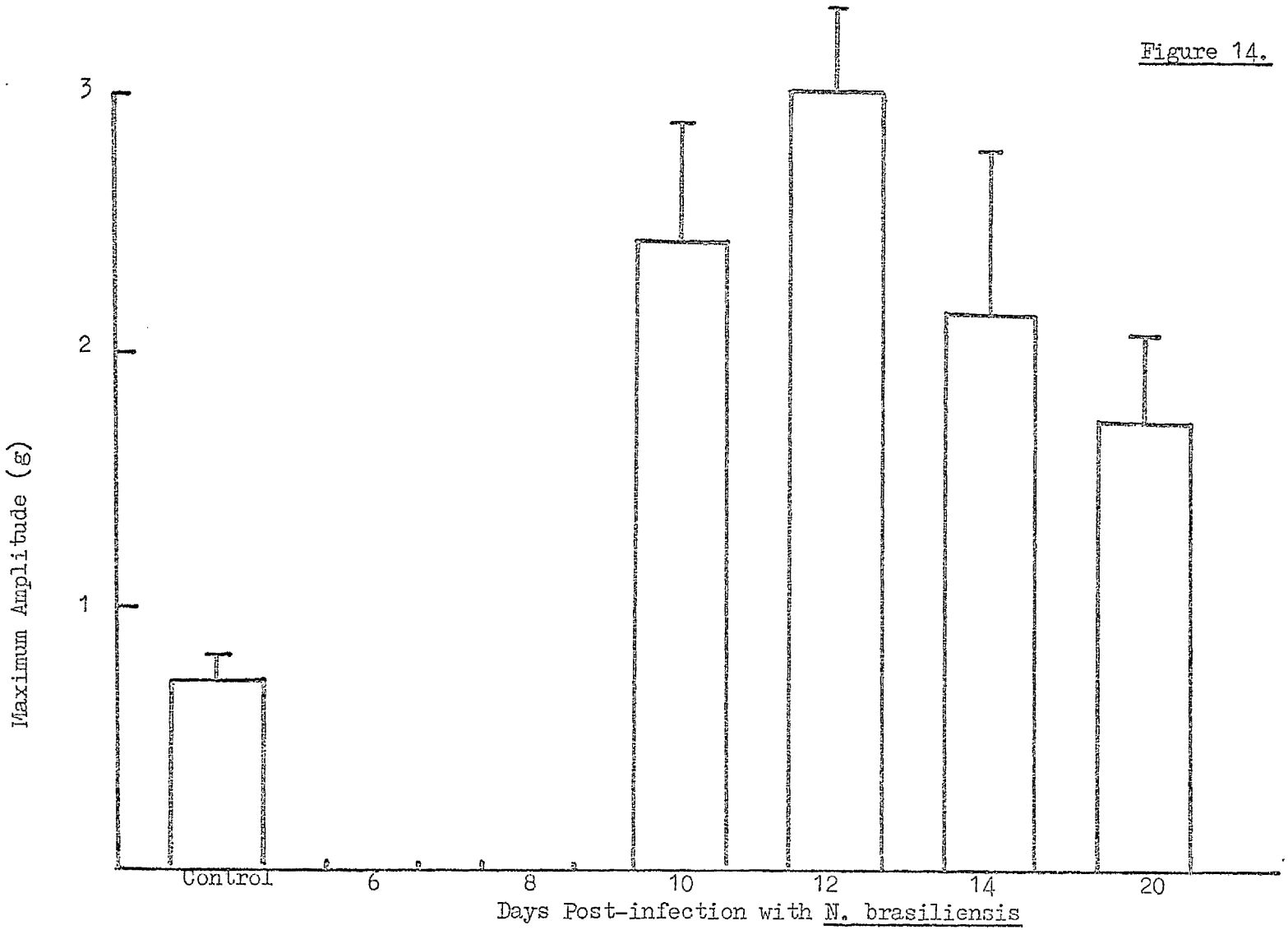
Results

(i) Spontaneous Activity

Intestinal preparations from control rats generally exhibited rhythmic contractions of 29 - 32 per minute. The mean amplitude of spontaneous contractions of control tissues was 0.73 g.

Infection with 5,000 *N. brasiliensis* larvae had a marked effect on the amplitude of spontaneous contractions of infected intestinal muscle. On days 6 and 8 post-infection activity was noticeably reduced in amplitude and tended to be extremely erratic, exhibiting long periods of inactivity, interrupted by bursts of spontaneous movements. Tissues taken from rats at this stage of infection were very sensitive to changing of the bathing medium, which caused cessation of any spontaneous activity completely. This was in contrast to control preparations which stopped rhythmic contractions for only a few seconds, if at all, following washout. Because of the erratic nature of spontaneous activity on days 6 and 8, it was impossible to measure for plotting on figure 14, which shows the average amplitudes of spontaneous contractions as infection progressed.

Figure 14.



Mean amplitudes of spontaneous contractions ± SEM of gut from infected rats.

The spontaneous activity of intestinal smooth muscle taken on day 10 of infection again showed regular rhythmic contractions, and from Table 4 and Fig. 14 it can be seen that the mean amplitude of contractions had increased from 0.73 g in control tissues, to 2.56 g. The spontaneous tension developed by the preparations had increased even further by day 12, reaching a mean value of 3.02 g. The amplitude had fallen by day 14, but even by day 20 it was still more than double the value for control preparations.

The difference in mean amplitudes between control and days 10 and 12 were compared by Student's t-test and found to be very significant ($P < 0.001$). Similarly the fall in amplitude between days 12 and 20 was also significant ($P < 0.05$). The frequency of spontaneous contractions did not change during infection, remaining at around 30/min.

(ii) Frequency-Responses to Field Stimulation

Field stimulation of the rat small intestinal preparations produced complex responses, which varied according to the frequency of stimulation. Low frequencies (0.05 - 0.5Hz) produced either a marked bi-phasic response which consisted of an inhibition, followed by a 're-bound' contraction in the case of control tissues, or only an inhibitory response in tissues from animals at day 6 post-infection. In some day 6 tissues, no response at all was elicited by low frequencies of stimulation. Similarly, in tissues from animals at day 8, low frequencies produced either inhibition or no response at all. Intestinal smooth muscle from rats at days 10, 12, 14 and 20 of infection contracted to stimuli at 0.05 - 0.5Hz, but rarely was this response preceded by an inhibition.

Higher frequencies of stimulation (1 - 10Hz) rarely produced a contractile response in tissues from rats at day 6 post-infection. If a response occurred at all it was very feeble. Control preparations, and those on days 8 - 20 gave graded contractile responses to frequencies of 1, 2, 5 and 10Hz, a maximum response usually occurring at 5 or 10Hz. The rebound contractions which occurred at low frequencies, sometimes also occurred at the higher frequencies in that there was a further brief contraction of the tissue on cessation of stimulation. Typical frequency-responses from a control preparation are shown in Figure 15. Note the spontaneous rhythmic pendular movements and the rebound contractions.

Table 4: Mean Amplitudes of Spontaneous Rhythmic Contractions of Pieces of Small Intestine from Rats at Various Times After Infection with *N. brasiliensis*. Means for Infected Tissues were Compared with the Control Value by Student's t-test.
***, $P < 0.001$;
**, $P < 0.01$.

Days Post-Infection	Mean Amplitude (g)	Standard Deviation (SD)	n
Control	0.73	0.38	12
6	-	-	6
8	-	-	6
10	2.56***	1.12	6
12	3.02 ***	0.79	6
14	2.16 **	1.60	6
20	1.72 **	0.84	6

Fig. 15

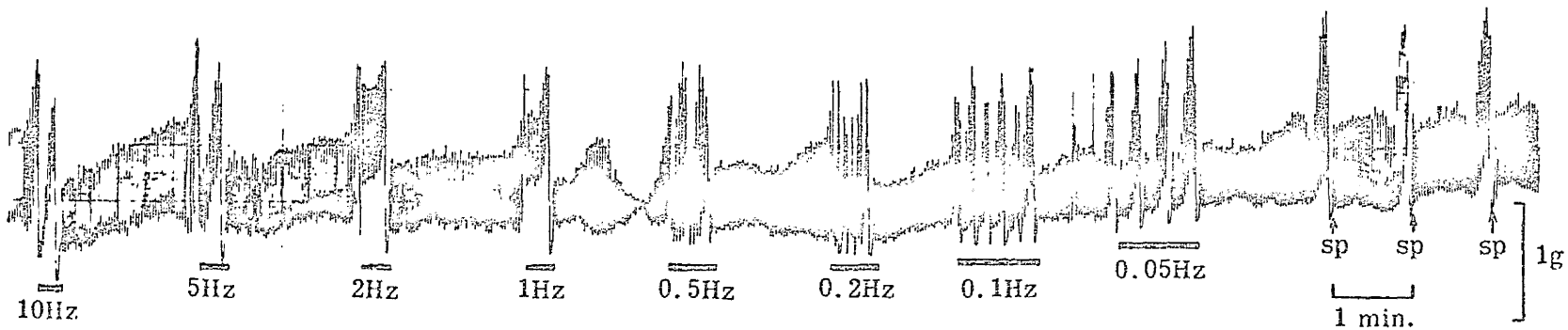


Fig. 15 A typical frequency-response curve from a control (uninfected) preparation. sp = single pulse. bars donate period of stimulation

The maximum tension developed by tissues due to field stimulation increased markedly as infection progressed as compared with controls (table 5), reaching a peak on day 12 post-infection. The mean maximum response to field stimulation in controls was 1.26 g as compared with 7.05 g on day 12 ($P< 0.001$). The mean maximum response remained high on day 14, but fell to 4.19 g on day 20. Maximum responses to field stimulation are plotted on Fig. 16.

(iii) Responses of Infected Tissues to Acetylcholine

The individual log dose - % maximum response curves for ACh were analysed by regression analysis and the pD_2 values calculated for control and infected tissues. The mean pD_2 values for each group were compared by Student's t-test, and no significant differences were found between the control and infected groups, i.e. there was no displacement of the log dose - % response curve to ACh during infection. Mean pD_2 values for control and infected tissues are given in Table 6 and typical dose-responses to ACh are shown in Figure 17.

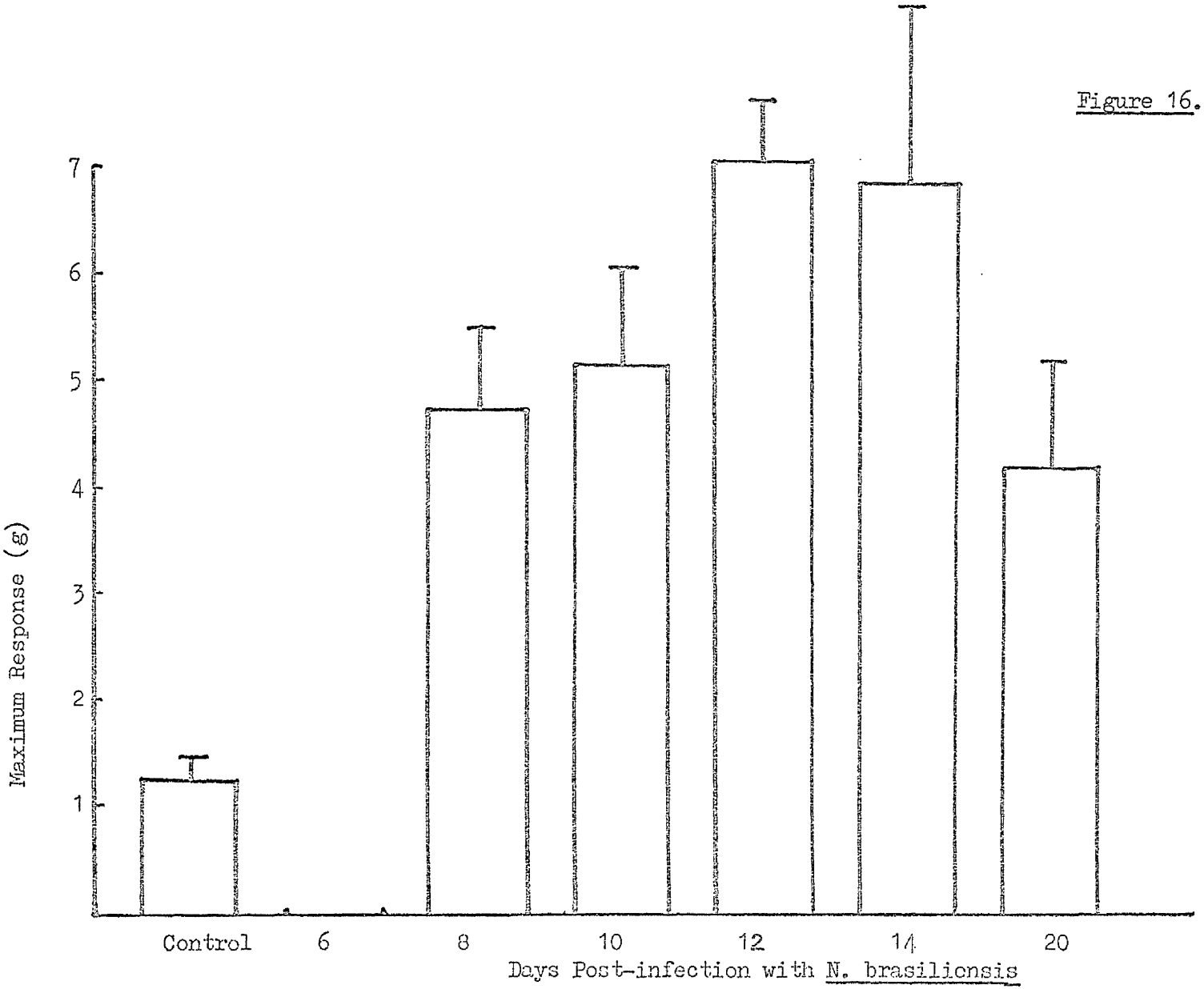
One factor which was observed was that, even though no changes in pD_2 value for ACh were found, the threshold for a drug-response tended to be lower during infection, in that infected preparations gave a contractile response to doses which elicited no response in controls. This was particularly true on day 14 post-infection, where the preparations responded to a dose of ACh as low as 7×10^{-8}M. The dose - % response curves to ACh during infection are given in Figures 18 - 23.

Although no change in the pD_2 to ACh occurred during infection, a striking increase in the maximum response of the preparations to this drug was observed. As infection progressed, the maximum response elicited by ACh in isolated gut increased markedly, reaching a peak on day 14, after which it decreased. However, on day 20 it was still significantly greater than in controls ($P< 0.001$). Figure 24 shows the dose-response curves for ACh in controls and in parasitized gut as infection progressed. The increases in maximum response to ACh as infection progressed can be clearly

Table 5: The Maximum Responses Elicited by Field Stimulation in Small Intestinal Preparations from Rats at Various Stages Post-infection. Means for Infected Tissues were Compared with the Control Value by Student's t-test.
***, $P < 0.001$;
**, $P < 0.01$.

Days Post-infection	Mean Maximum Response (g)	SD	n
Control	1.26	0.56	10
6	-	-	-
8	4.69***	1.55	4
10	5.14***	2.12	6
12	7.05***	1.34	6
14	6.82***	3.98	6
20	4.19**	2.51	6

Figure 16.

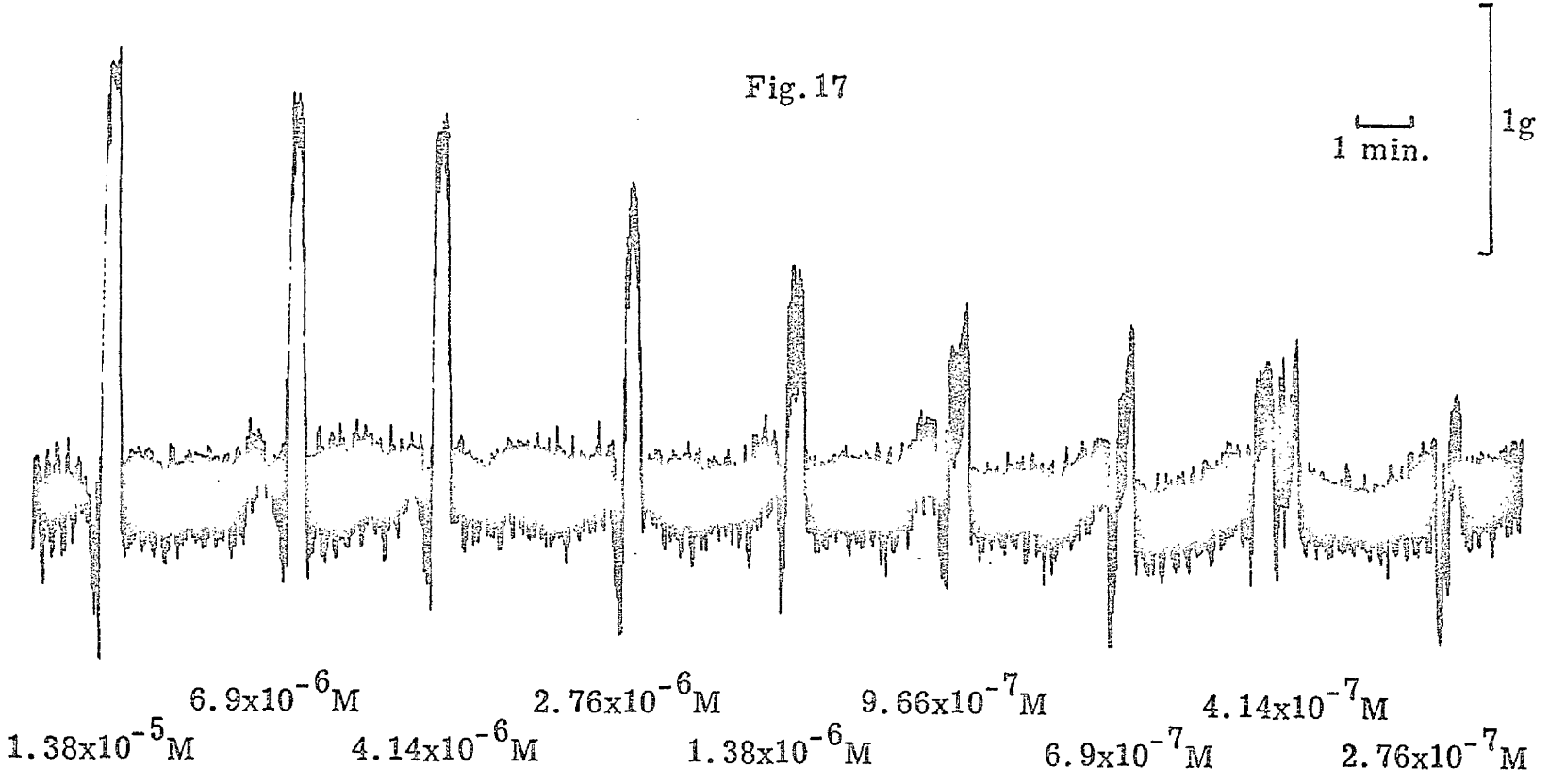


Mean maximum responses to field-stimulation ± SEM of gut from infected rats.

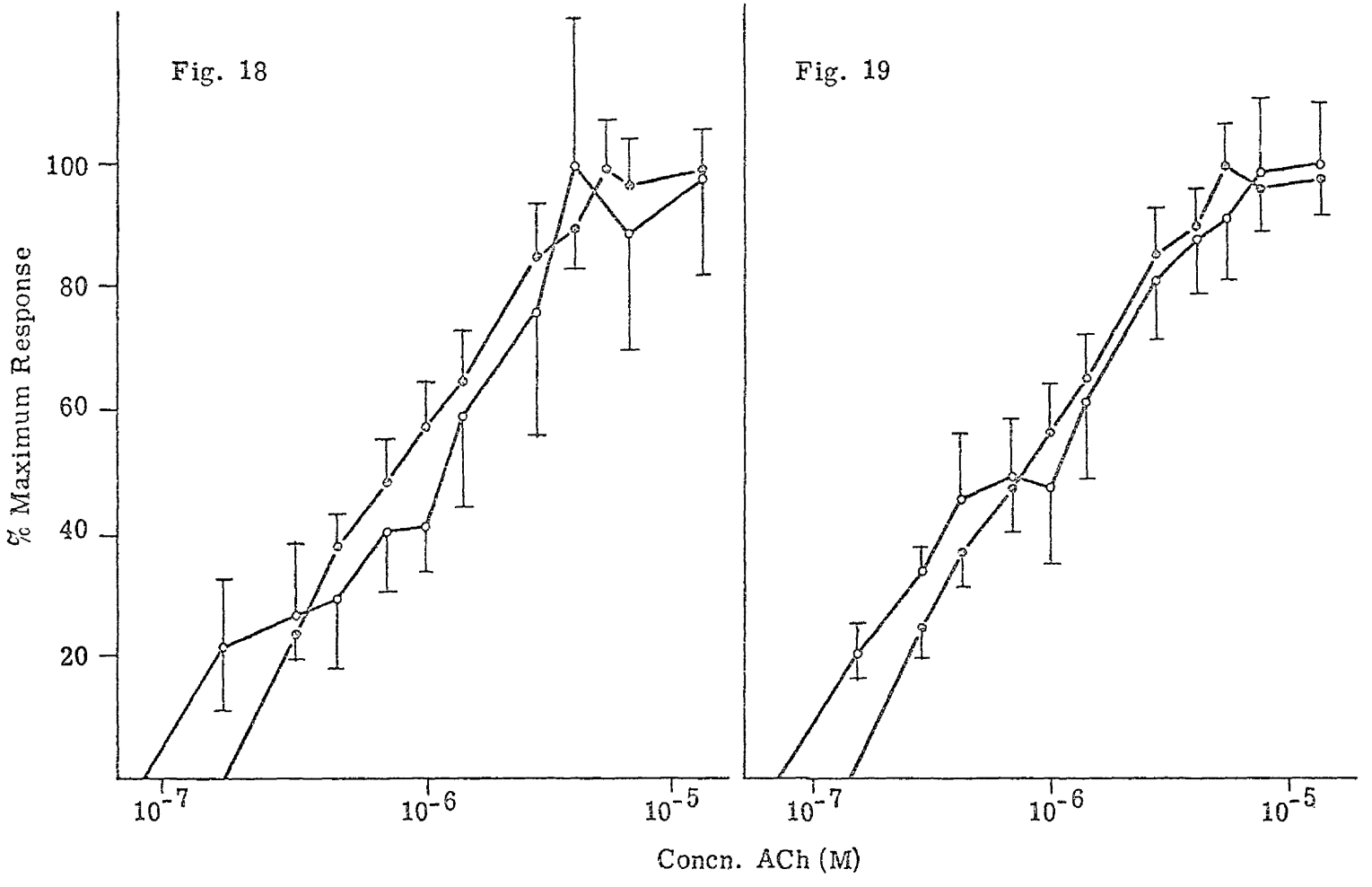
Table 6: The Mean pD_2 Values for ACh in *in vitro* Intestinal Smooth Muscle from Animals at Various Times Post-infection with *N. brasiliensis*. Each pD_2 Mean was Compared to the Control Value by Student's t-test. No Significant Difference was found.

Days Post-infection	Mean pD_2	SD	n
Control	6.03	0.17	11
6	6.05	0.34	5
8	6.14	0.36	5
10	6.11	0.42	6
12	6.16	0.10	6
14	6.14	0.11	6
20	6.09	0.15	6

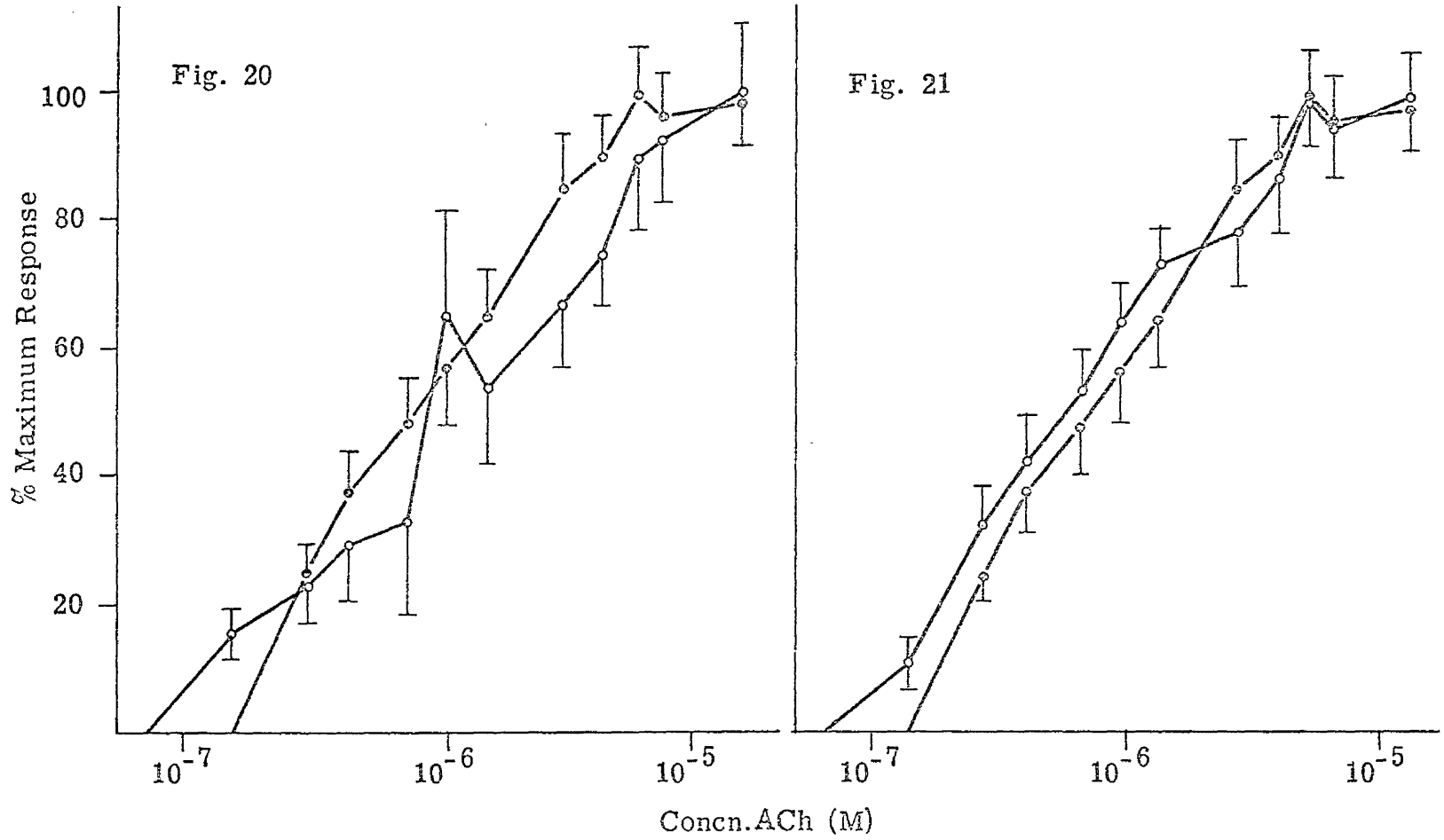
Fig. 17



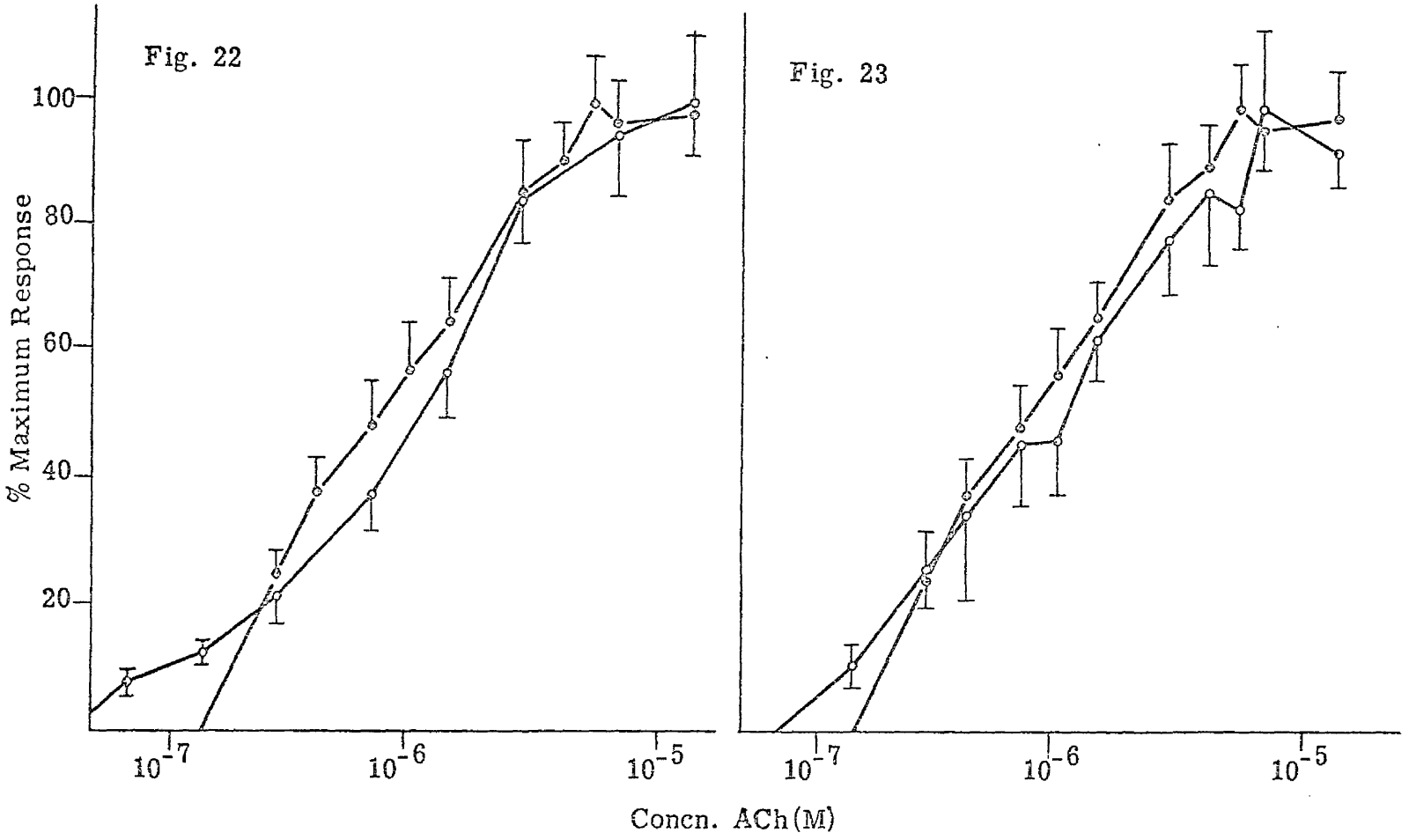
A typical dose-response curve to ACh in control (uninfected) tissues. Doses were left in the bath for 20 seconds before washout



Log dose - % response curves to ACh in small intestine from control rats (∘), and from rats at days 6 and 8 post-infection with N.brasiliensis (Figs. 18 and 19 respectively, o). I-bars are standard errors.



Log dose - % response curves to ACh in small intestine from control rats (o), and from rats at days 10 and 12 post-infection with N. brasiliensis (Figs. 20 and 21 respectively, o). I-bars are standard errors.



Log dose - % response curves to ACh in small intestine from control rats (o), and from rats at days 14 and 20 post-infection with N. brasiliensis (Figs. 22 and 23 respectively, o). I-bars are standard errors.

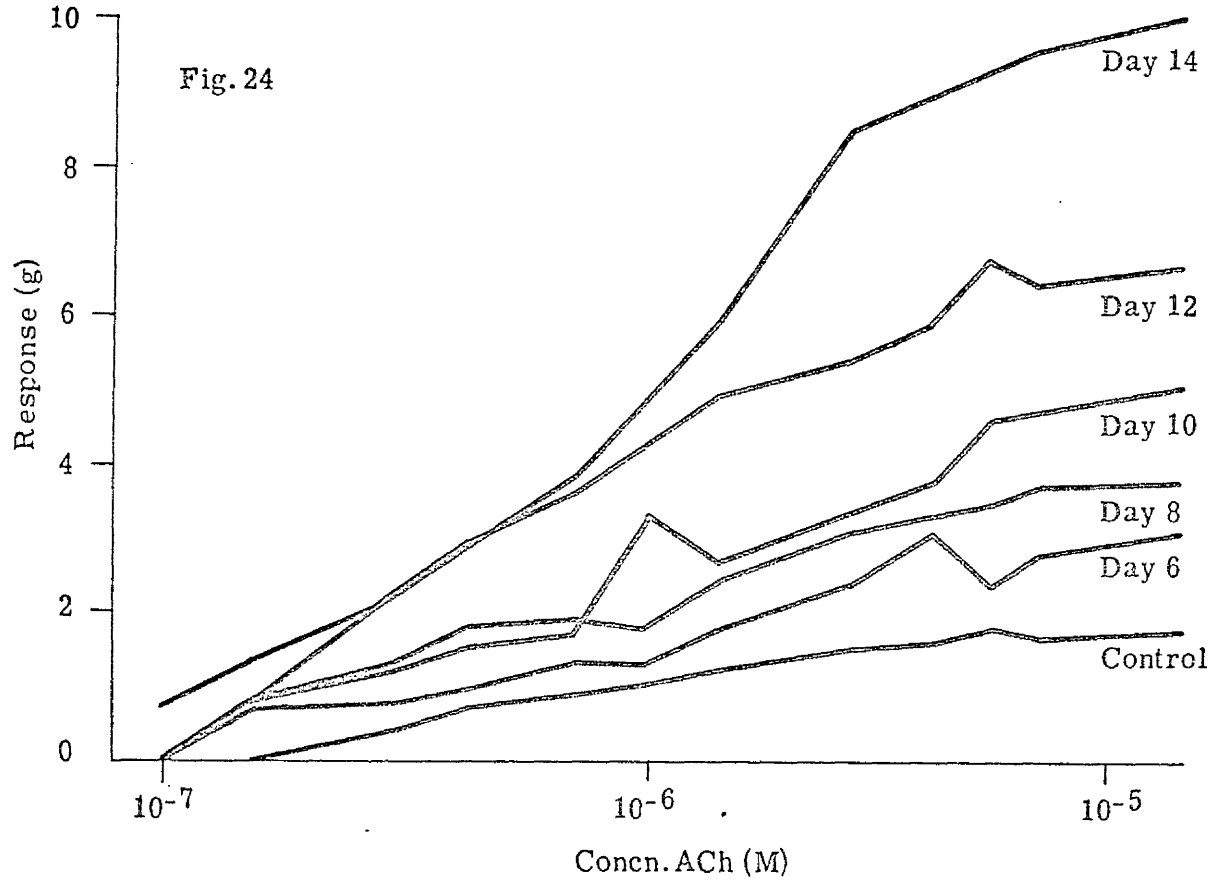


Fig. 24. Log dose-response curves to acetylcholine (ACh) in isolated rat small intestinal preparations at various times post-infection with 5,000 N. brasiliensis larvae. Each point is the mean of at least 6 observations, and in the control curve each point is the mean of at least 10 observations. For the sake of clarity, error bars have been omitted.

seen, even as early as day 6. Error-bars have been omitted from the graph for the sake of clarity.

The mean maximum responses to ACh were calculated for each day post-infection, and compared with that for controls by Student's t-test, as shown in Figure 25. Again, the increase in maximum response as infection progressed is obvious. Upon statistical analysis it was found that the mean maximum response to ACh on day 6 (3.1 g) was significantly greater than in preparations from control rats (1.6 g), P being less than 0.05. Upon comparison with controls, the maximum responses of the infected tissues from animals at days 8 - 20 post-infection were also significantly increased, $P < 0.001$ in all cases. Similarly, the difference in maximum response to ACh between day 6 and day 14 was significant ($P < 0.001$).

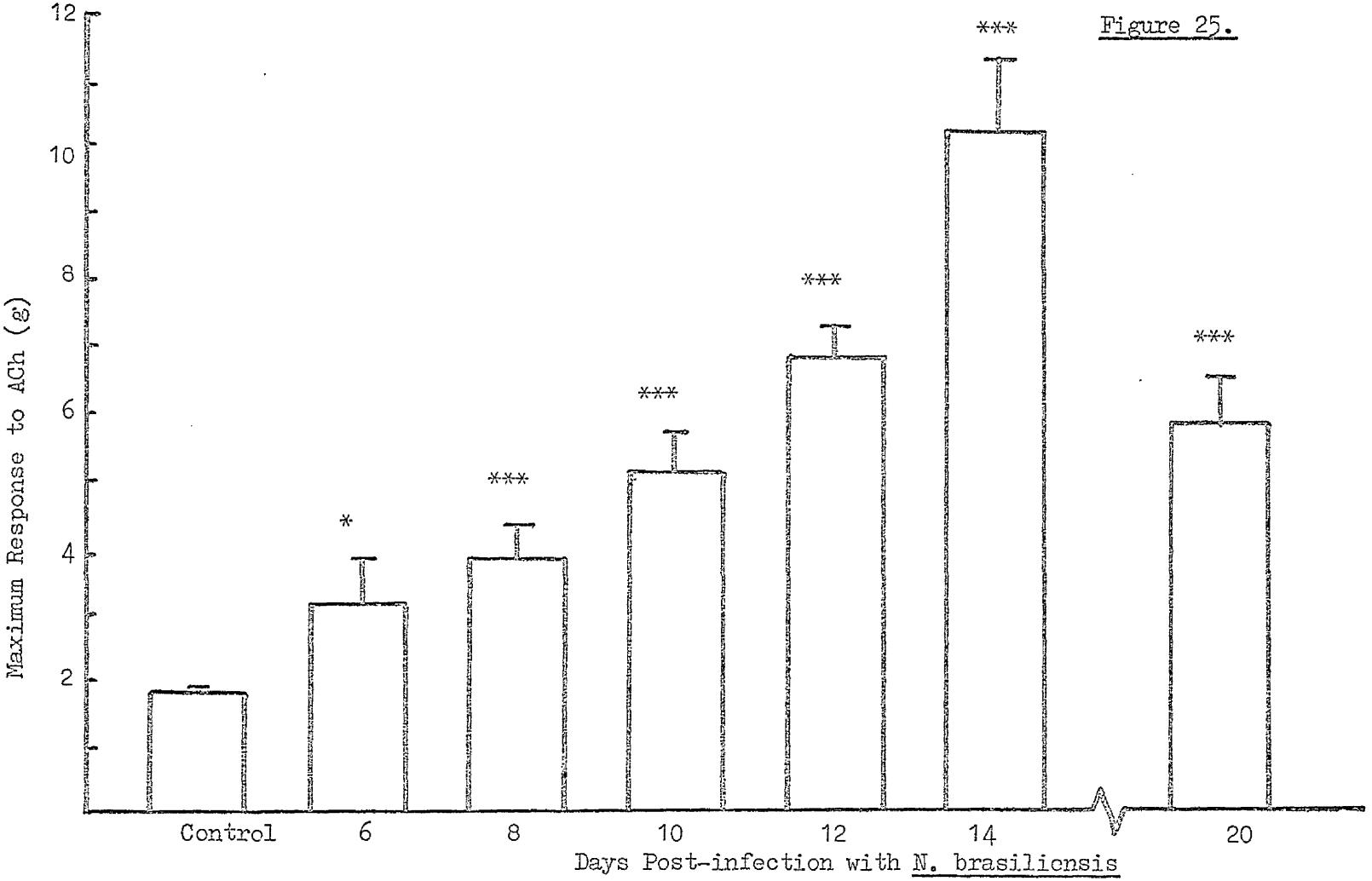
From figure 25 it can be seen that the maximum response to ACh increased in a graded fashion, reaching a peak of 10.2 g on day 14. This was a striking increase by a factor of almost 6 from controls where the mean maximum response was 1.8 g.

(iv) Responses of Infected Tissue to Carbachol

No shift of the dose-response curve to carbachol occurred during infection since the mean pD_2 values at each day post-infection were not significantly different from that for control preparations (Table 7). However, as with ACh, infected tissues were responsive to lower doses of carbachol than those from uninfected rats. Again, it seems as though the threshold for drug-response was lowered in infected tissues.

In much the same way as with ACh, the mean maximum response to carbachol increased markedly as infection progressed, reaching a peak of 9.7 g on day 14, as compared with 2.4 g in the control intestinal muscle preparations. These two means were compared by t-testing and found to be significantly different ($P < 0.001$). Figure 26 shows the dose-response curves to carbachol as infection progressed and it can be seen that the maximum response produced by the drug increased with time post-infection. The maximum response on day 6 was significantly higher than in controls ($P < 0.001$), and

Figure 25.



Mean maximum responses to acetylcholine (ACh) ± SEM of gut from infected rats. Each mean value was compared to that for uninfected controls by Student's t-test.
***, $P < 0.001$; *, $P < 0.05$.

Table 7: The Mean pD_2 Values for Carbachol in *in vitro* Intestinal Smooth Muscle from Animals at Various Times Post-infection with *N. brasiliensis*. Each Mean pD_2 was Compared to the Control Value by Student's t-test and no Significance was Found.

Days Post-infection	Mean pD_2	SD	n
Control	5.94	0.21	11
6	6.04	0.61	6
8	5.83	0.14	5
10	5.85	0.11	6
12	5.93	0.17	5
14	5.91	0.08	5
20	5.82	0.06	6

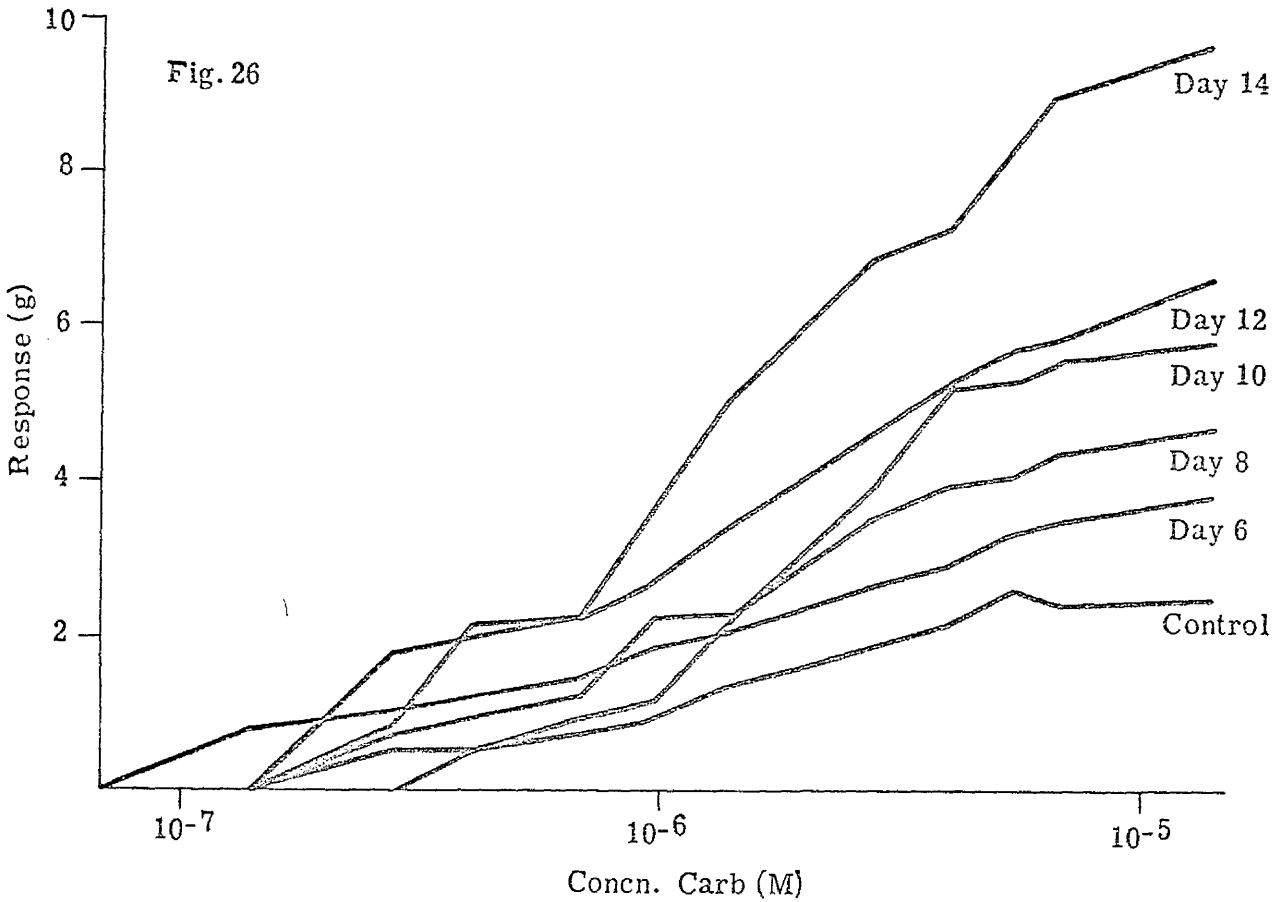


Fig. 26. Log dose-response curves to carbachol (carb) in isolated rat small intestine at various times post-infection with N. brasiliensis. Each point is the mean of at least six observations, and in the control curve, each point is the mean of at least ten observations. Error bars have been omitted for clarity.

the increase between day 6 and 14 was also significant ($P < 0.001$). By day 20 the maximum response to carbachol had fallen to a mean of 5.8 g though it was still significantly higher than the control value of 2.4 g ($P < 0.001$). The mean maximum responses to carbachol were plotted as a histogram, shown in Figure 27.

(v) Responses of Infected Tissues to 5-Hydroxytryptamine

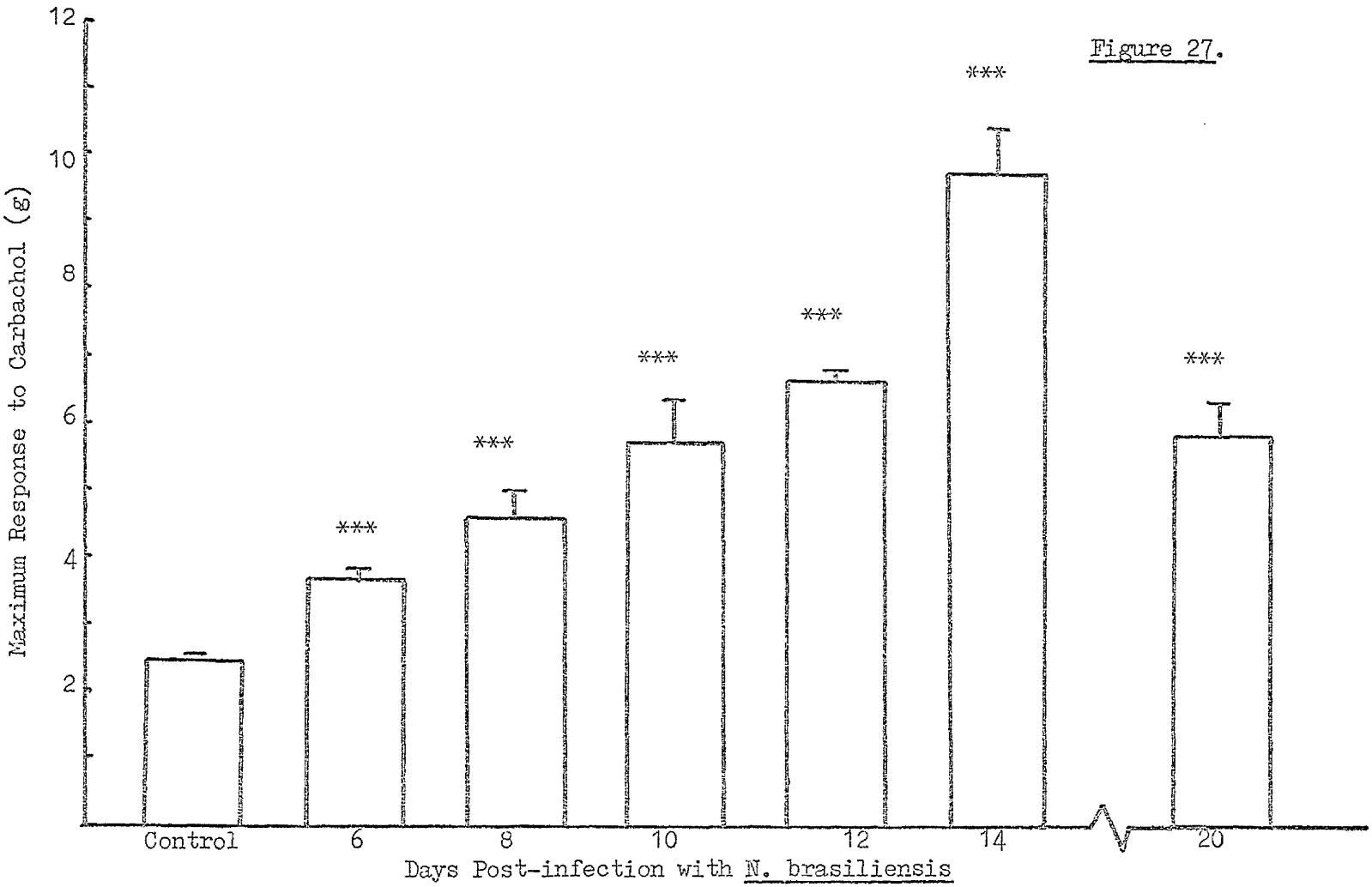
The mean pD_2 values for 5-hydroxytryptamine (5-HT) for infected tissues were compared with that for controls by Student's t-test, and no significant difference occurred on days 6, 10 and 12. The pD_2 value for 5-HT on day 8 was significantly reduced, though only marginally ($P < 0.05$). On days 14 and 20 however, a reduction in pD_2 occurred which was markedly significant ($P < 0.001$, $P < 0.02$ respectively). The mean pD_2 values for control and infected tissues are given in Table 8, and the log dose - % response curves to 5-HT during infection, in Figures 28 - 33.

The mean maximum response to 5-HT increased as infection advanced, again reaching a peak value on day 14, of 5.3g. This was in contrast to the maximum response of 1.8 g in controls. Upon statistical analysis, the increase in maximum response at day 14 was found to be significant ($P < 0.01$). As early as day 6, the maximum response elicited by 5-HT of 3.3 g was significantly greater than controls ($P < 0.001$). By day 20 post-infection, the mean maximum response to 5-HT of 4.4 g was less than that on day 14, but it was still significantly greater than in control preparations ($P < 0.01$). The dose-response curves to 5-HT during infection are given in Figure 34, and the trend of increasing responsiveness can clearly be seen. The mean maximum responses to 5-HT were plotted as a histogram as shown in Figure 35.

Summary and Conclusions

The spontaneous contractile activity of rat isolated intestinal segments, and their responses to electrical and chemical stimulation were studied during infection with N. brasiliensis. Intestinal preparations from uninfected, control animals exhibited rhythmic, spontaneous contractions of around 30/minute, with a

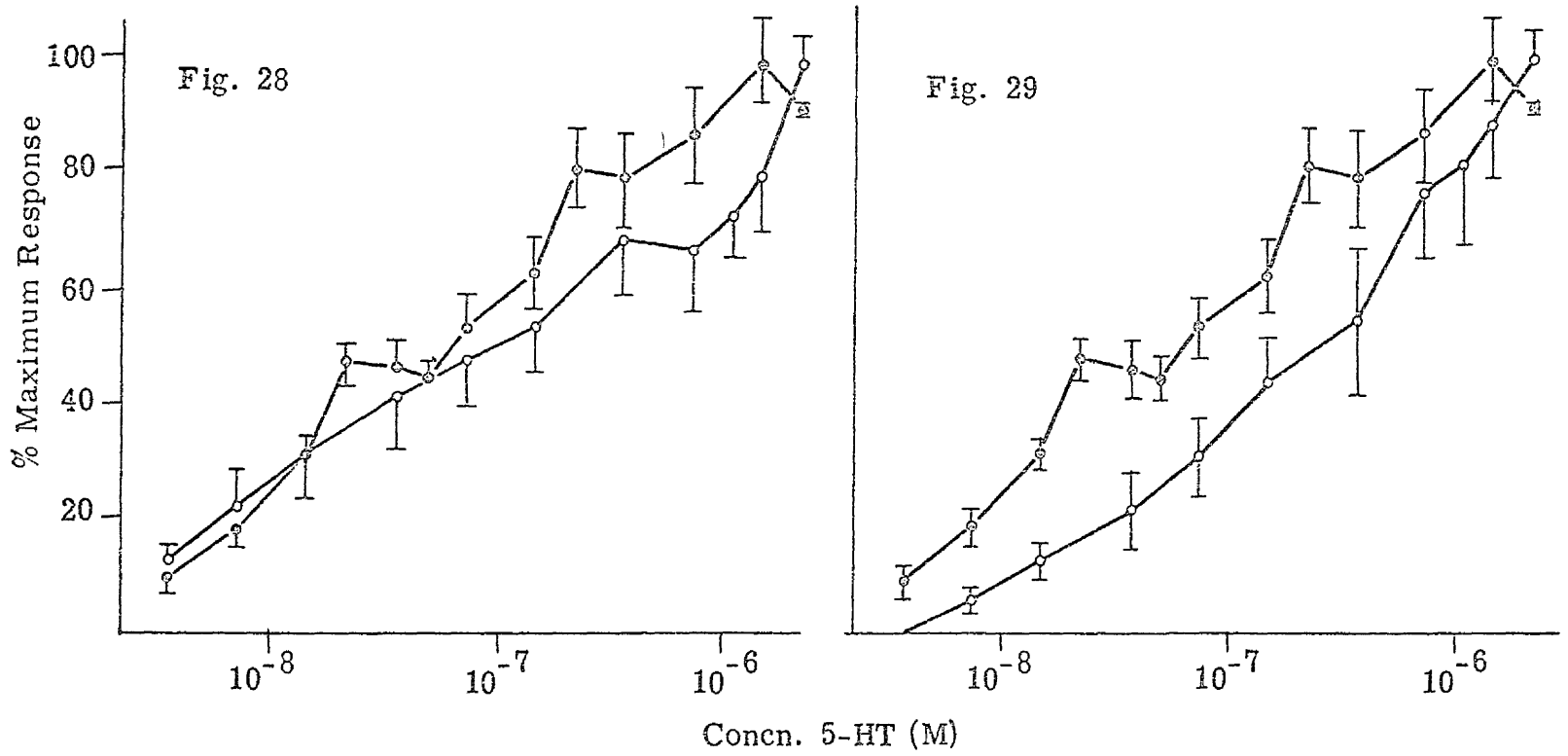
Figure 27.



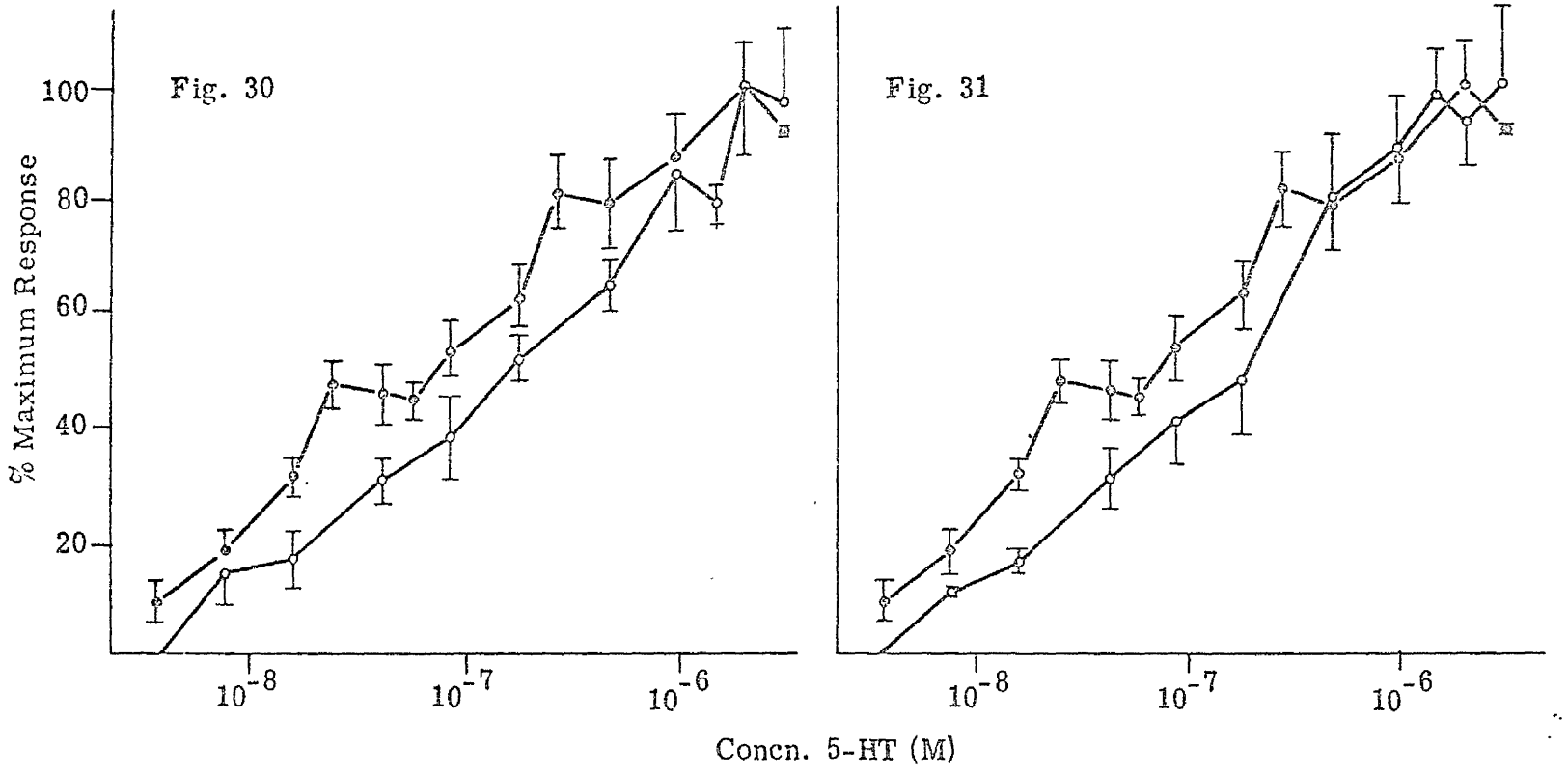
Mean maximum responses to carbachol ± SEM of gut from infected rats. Each mean value was compared to that for uninfected controls by Student's t-test.
***, $P < 0.001$.

Table 8: The Mean pD_2 Values for 5-HT in *in vitro* Intestinal Smooth Muscle from Animals at Various Times Post-infection with *N. brasiliensis*. Values for Infected Tissues were Compared to the Control Value by t-test.
*, $P < 0.05$; **, $P < 0.02$; ***, $P < 0.001$.
NS, no significance.

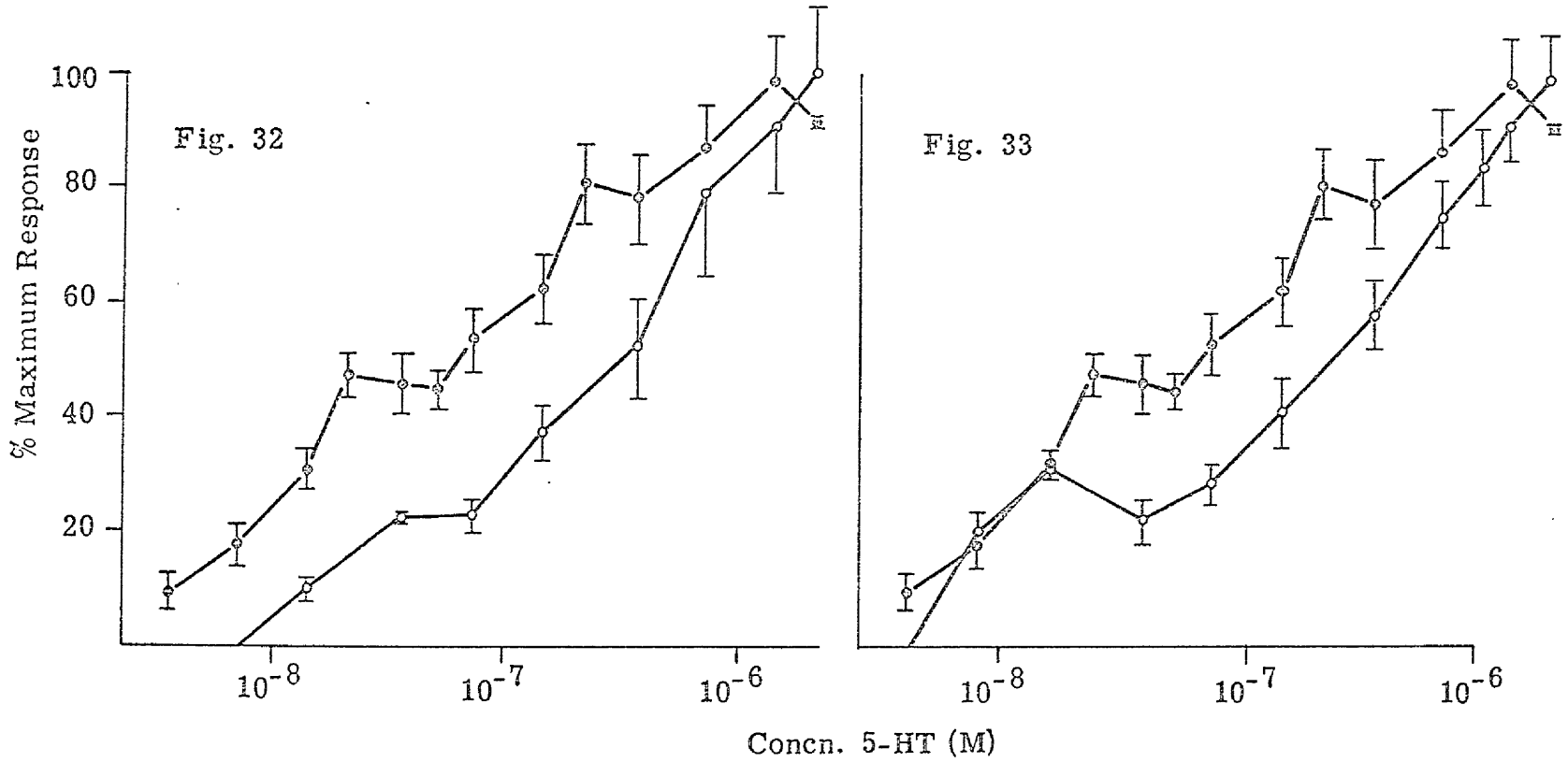
Days Post-infection	Mean pD_2	SD	n
Control	7.28	0.29	9
6	7.29 NS	0.32	6
8	6.83*	0.28	4
10	7.08 NS	0.15	6
12	7.00 NS	0.10	4
14	6.81***	0.11	5
20	6.89**	0.23	6



Log dose - % response curves to 5-HT in small intestine from control rats (o), and from rats at days 6 and 8 post-infection with N.brasiliensis (Figs. 28 and 29 respectively, o). I-bars are standard errors.



Log dose - % response curves to 5-HT in small intestine from control rats (•), and from rats at days 10 and 12 post-infection with N. brasiliensis (Figs. 30 and 31 respectively, ○). I-bars are standard errors.



Log dose - % response curves to 5-HT in small intestine from control rat (o), and from rats at days 14 and 20 post-infection with N. brasiliensis (Figs. 32 and 33 respectively, o). I-bars are standard errors.

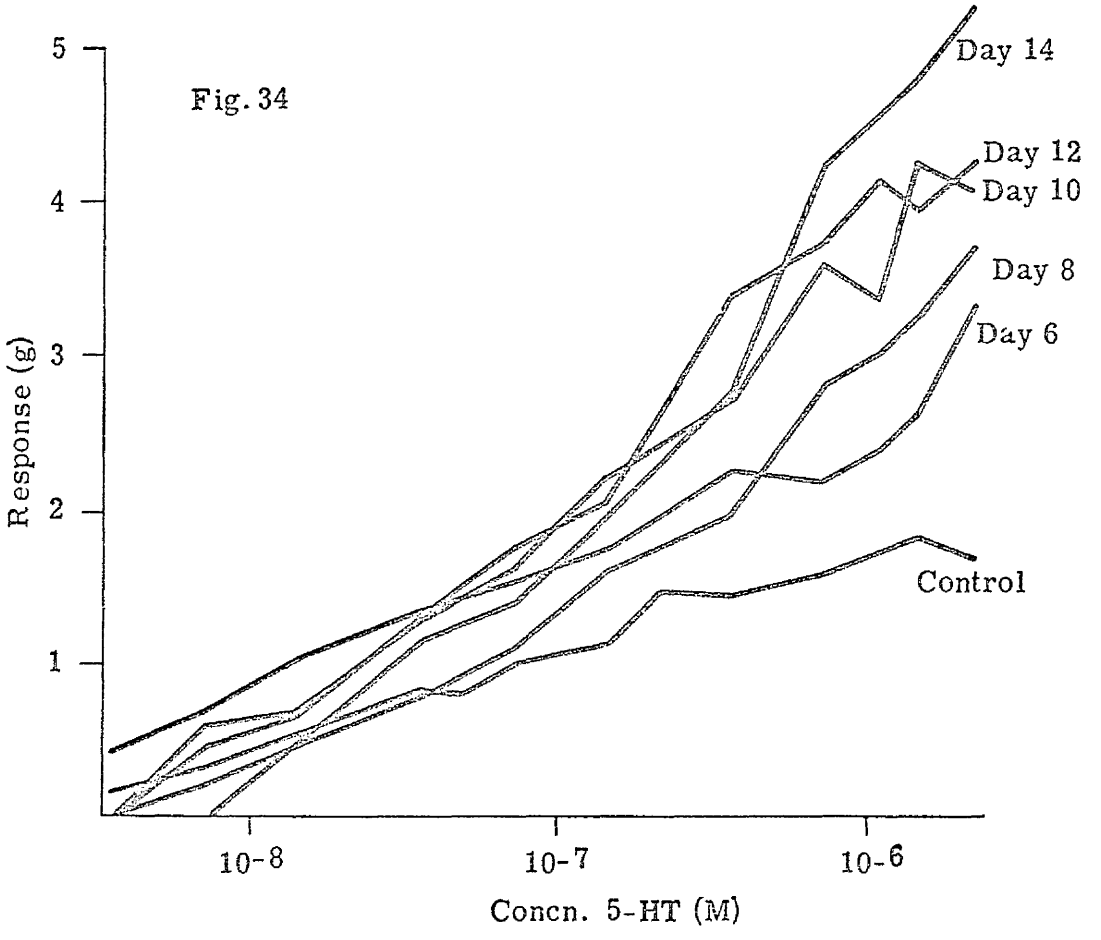
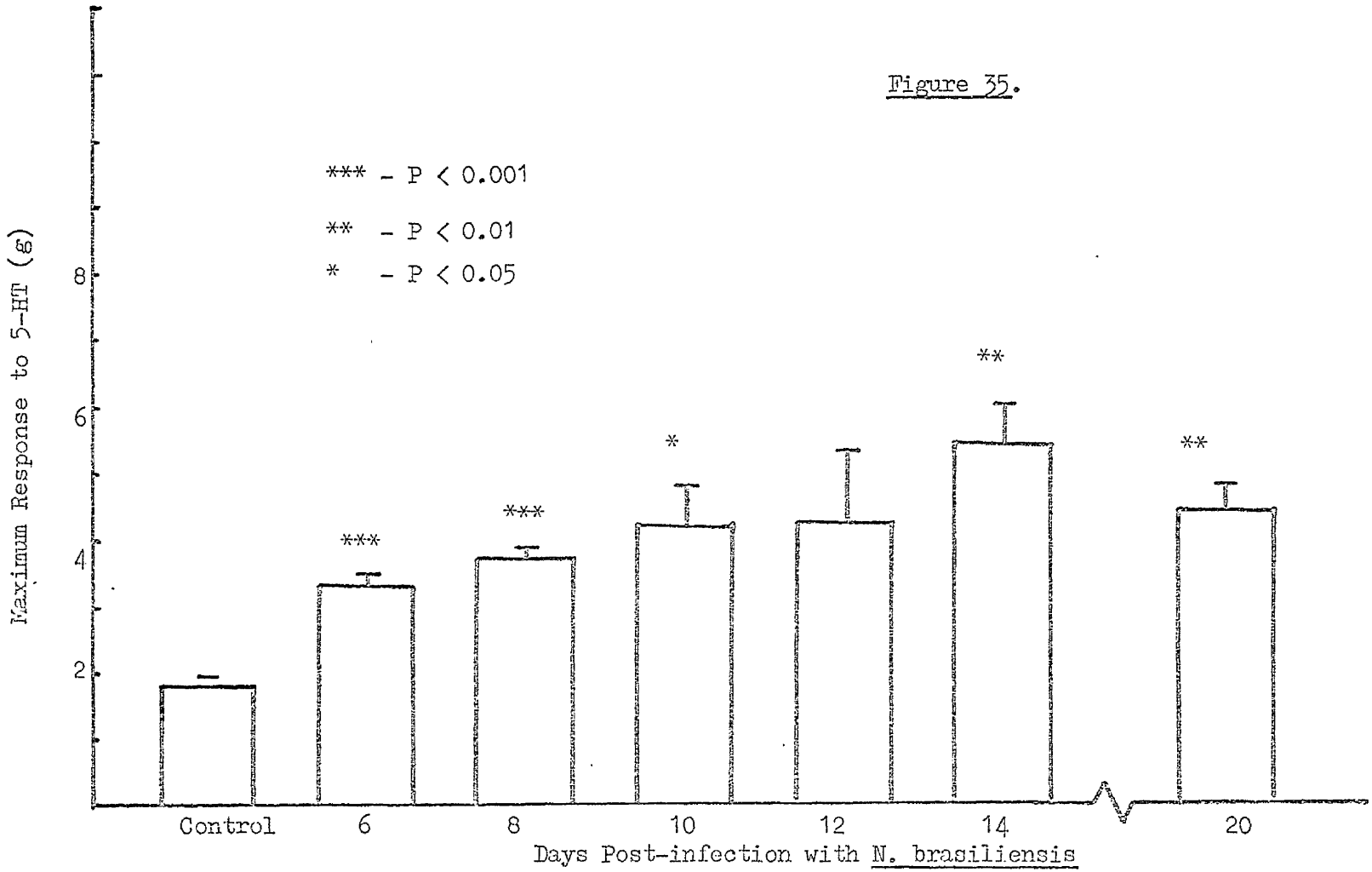


Fig. 34. Log dose-response curves to 5-hydroxytryptamine (5-HT) in isolated rat small intestine at various times post-infection with N.brasiliensis. Each point is the mean of at least 6 observations. For clarity, error bars have been omitted.

Figure 35.



Mean maximum responses to 5-hydroxytryptamine (5-HT) ± SEM of gut from infected rats. Each mean value was compared to that for uninfected controls by Student's t-test.

mean amplitude of 0.7 g. In infected rats on days 6 and 8 post-infection this activity was very erratic and noticeably reduced in amplitude, tissues being quiescent for most of the time. However, on days 10 - 20 spontaneous activity was once again in evidence. The mean amplitudes of contraction during this period were significantly greater than in control preparations, the greatest mean amplitude occurring on day 12, when it was more than 4 times that in control preparations. After day 12, the mean amplitude fell, but by day 20 it was still significantly greater than controls. No change occurred in the frequency of spontaneous contractions after infection with N. brasiliensis.

The responses produced by field stimulation in intestinal preparations were complex and varied with the frequency of stimulation and the stage of infection (see results section). However, by far the most striking observation was the increase in maximum response to stimulation at higher frequencies (5 - 10Hz) which occurred during infection. Except at day 6, when stimulation produced at best feeble responses, the mean maximum responses increased in a graded fashion between days 8 and 14 as compared with control preparations. The greatest maximum response to field stimulation was seen at day 12 when it was more than 5 times greater than the mean maximum response of control tissues. The maximum response to field stimulation was still elevated on day 14 and it had fallen by day 20, although at this time it was still significantly greater than in controls.

The maximum responses produced by ACh, carbachol, and 5-HT also increased as infection progressed, reaching a peak on day 14. The lowest maximum responses to all three agonists was seen at day 6 and even at this stage of infection they were significantly greater than in controls. As with field stimulation, the increased maximum responses to agonists had decreased by day 20 though they were still greater than control. However, the elevation in maximum responses to ACh and carbachol were much more marked than those produced by field stimulation or 5-HT, particularly at days 12 and 14 post-infection where they peaked. The mean maximum responses of control preparations to ACh and carbachol were 1.8 g and 2.46 g respectively.

On day 14 the mean maximum response to ACh was 10.2 g and to carbachol was 9.68 g.

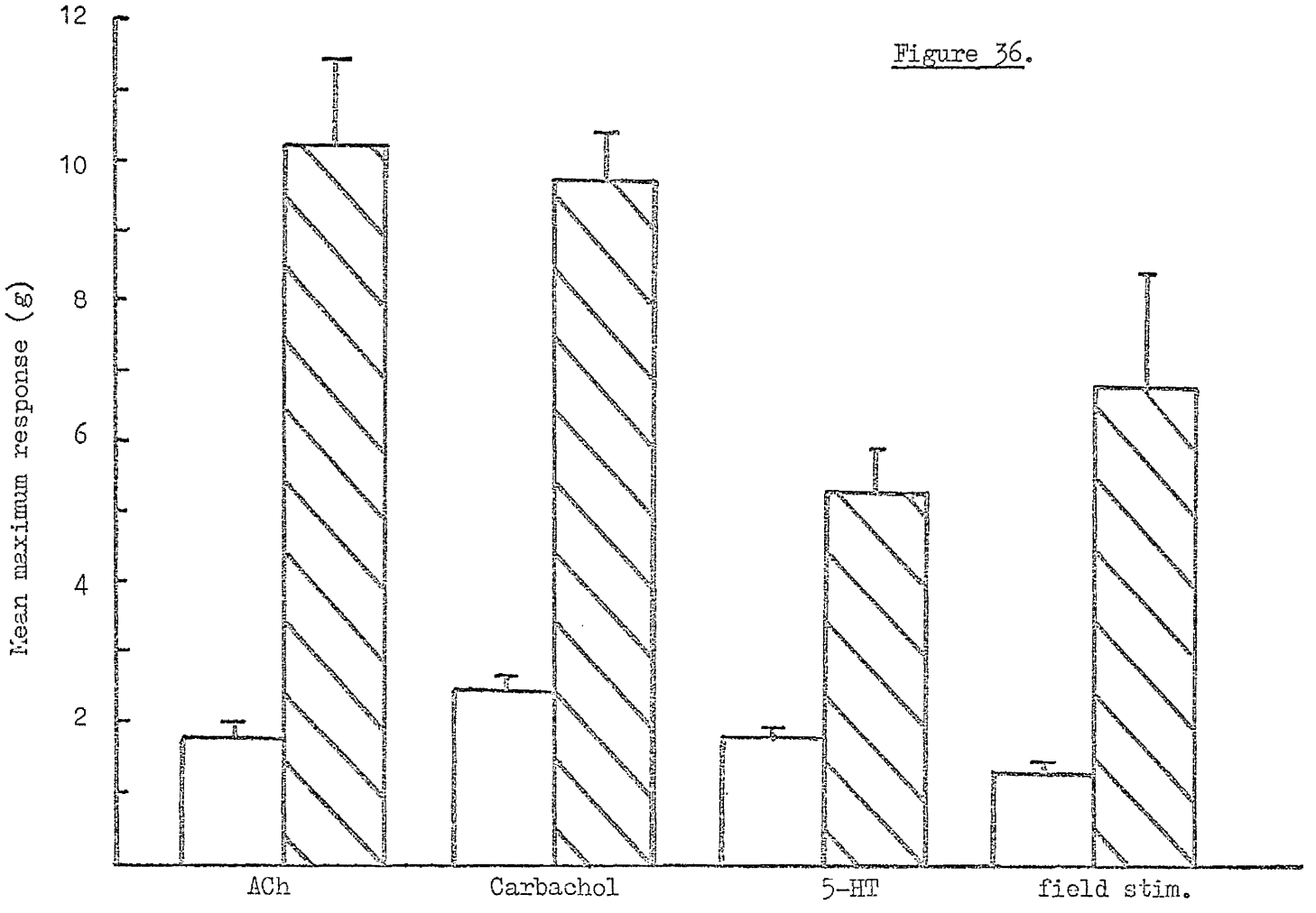
Although the maximum response of intestinal preparations to 5-HT increased as infection progressed, the effect was not as marked as with the choline esters. There tended to be more variation in the responses to 5-HT, leading to greater standard deviations. This probably accounts for the lack of significance between the mean maximum response of control preparations and that of day 12 preparations, even though the latter was greater by a factor of more than 2 (see Figure 35).

Thus, during infection with N. brasiliensis the maximum responses to field stimulation, ACh, carbachol and 5-HT of rat gut preparations all increased markedly. Maximum responses increased in a graded fashion as infection advanced, reaching a peak at around day 14 of the infection - see Figure 36.

The explanation of these changes in intestinal smooth muscle activity can, at this stage, only be a matter for speculation. It is strange that spontaneous activity was drastically reduced and very erratic on days 6 and 8 post-infection, but became rhythmic once more from day 10 onwards with a significantly greater force of contraction. Similarly, any spontaneous activity which was present on days 6 and 8 was abolished merely by changing the organ bath fluid. Between the organ bath and the heating coil which maintains solution from the reservoir at 37°C (see Fig. 13) there is a section of tubing where the fluid is not heated and it subsequently cools slightly between washouts. It is this cooler solution which initially replaces the bathing medium following washout. Since smooth muscle is sensitive to drops in temperature, this probably accounts for the brief reduction of spontaneous activity which occurred in control preparations after washout. It may be therefore, that gut muscle from rats on days 6 and 8 is particularly sensitive to lower temperatures.

On days 6 and 8 post-infection, the spontaneous activity of intestinal preparations was impaired in that any activity occurring was erratic and appeared uncoordinated. It may be that the marked oedema which is prevalent at this stage of infection, interferes with

Figure 36.



Mean maximum responses to ACh, carbachol, 5-HT and field stimulation of isolated gut from uninfected rats (empty columns) and from rats on day 14 post-infection with N. brasiliensis (hatched columns). I-bars are standard errors.

the integrity and synchronisation of electrical impulse conduction between smooth muscle cells. Thus, even though individual cells are capable of contraction, the electrical connections between them may have been altered in such a way as to impair the synchronized activity which leads to coordinated contractions of the muscle preparation as a whole. This could be resolved by histological and electron microscopical study of the muscle cells and the junctions between them.

On the other hand, the reduced spontaneous contractions may have been due to abnormal nervous activity within the gut wall. Although spontaneous contractions are myogenic in origin, they are subject to control by neurotransmitters released by the intrinsic and extrinsic nervous systems in the gut. As stated earlier, that as well as the classical cholinergic and adrenergic nerves in the intestine, there are non-cholinergic, non-adrenergic inhibitory (NCNAI) nerves whose putative transmitter is ATP (Burnstock et al., 1970; Burnstock, 1972, 1975). It is possible that some factor causes increased activity of these nerves on days 6 and 8 of infection with N. brasiliensis, thereby decreasing spontaneous activity. Unlike the small intestine, rat colon does not normally exhibit spontaneous activity in vitro. However, it has been demonstrated recently that addition of morphine or enkephalins to the bathing medium has an excitatory action, causing regular waves of contraction of rat colon (Gillan and Pollock, 1980). These authors obtained results which suggested that this action of opiates was mediated via an inhibitory effect uponNCNAI transmission. Thus, in the colon, morphine may unmask the inherent myogenic activity which is normally inhibited by NCNAI nerves. In the present experiment, it is possible that the activity of these nerves is not normally sufficient to block spontaneous myogenic contractions, but some factor due to the presence of N. brasiliensis worms interferes with either muscle cell metabolism, or with inhibitory nerves, to block spontaneous activity. However, if this is the case, it is surprising that on day 10, parasitized gut exhibited rhythmic spontaneous activity with an amplitude significantly greater than in uninfected gut. By day 10

worms have undergone substantial immunological damage and expulsion is just beginning. If the above hypothesis is correct, then it is possible that before sufficient damage has been sustained by the parasite, it in some way provokes activity of NCNAI nerves. It would be of interest to see whether morphine restores spontaneous activity on days 6 and 8. Similarly, would ATP or stimulation of the inhibitory nerves block the spontaneous contractions of control gut preparations, or those from rats at day 10 or later? If the worms do exert this inhibitory effect on gut motility it may be an aid to their establishment in the gut, and could act in conjunction with the co-called 'biochemical holdfast' action of parasite-secreted AChE. Nevertheless, even if these proposals are true, they would be of more relevance in vivo and it is surprising that spontaneous activity is inhibited in vitro, since worms do not remain in the isolated gut preparation and are removed from the organ bath by washout.

Intestinal preparations from rats at days 6 and 8 were also relatively insensitive to low frequencies of field stimulation of the intramural nerves, which either had no effect, or produced an inhibitory response. Control tissues on the other hand, exhibited a biphasic response to field stimulation. This type of response is characteristic of several intestinal preparations. For example, stimulation of the purinergic nerves of guinea pig taenia coli produced inhibition, and upon cessation of stimulation the preparation underwent a 'rebound contraction' (Bennett, 1966). This type of contraction, which is not blocked by atropine, usually occurs at the end of stimulation, especially at low frequencies. However, rebound contractions may also 'break through' during a period of repetitive stimulation at higher frequencies (Burnstock, 1979). Rebound excitation is blocked by indomethacin (Burnstock et al., 1975; Gillan and Pollock, 1980), and it has been suggested that it is due to the action of PG's whose synthesis is induced by ATP released from purinergic nerves (Burnstock et al., 1975).

Why rebound contractions following low frequency stimulation disappeared on days 6 and 8 can only be surmised at this stage.

As stated previously, Dineen and Kelly (1976) found that intestinal PGE levels increased 10 fold during N. brasiliensis infection, reaching a peak on day 7 at the site of worm localization. If rebound contractions are caused by PG's, it is possible that the gut smooth muscle had become less sensitive to their action due to the increased levels of PGE at this time. However, this is unlikely since levels remained high on days 10 - 14 and as described, field stimulation caused contraction of the preparations at this time. Conversely, contractions on days 10 - 14 were not rebound contractions and may have been due to some mediator other than PG, such as ACh released from nerves by field stimulation. This could be resolved by the use of atropine. The fact that stimulation-induced contractions were not preceded by inhibition from day 10 onwards provides indirect evidence for the proposal earlier that, before the worms are damaged, they somehow stimulate activity of inhibitory nerves.

Higher frequencies of stimulation (1 - 10Hz) caused contractions of control preparations and of those from rats at days 8 - 20 post-infection. Tissues from animals on day 6 rarely gave more than a feeble response. Again this may have been due to either a predominance of inhibitory nerve activity or to a decreased sensitivity to PG's. The contractions of control and days 8 - 20 preparations were maintained during the period of stimulation and increased in amplitude as the frequency increased reaching a maximum at 5 or 10 Hz. As mentioned previously, the most striking observation was the increase in the maximum response to field stimulation as infection progressed. This will be discussed later.

Supersensitivity has been defined as the phenomenon in which the amount of a drug required to elicit a given biological response from a tissue is less than 'normal'. This increase in susceptibility of the tissue is characterised by a shift to the left of the dose-response curve and a subsequent increase in the pD_2 for the particular drug (Fleming et al., 1973). Similarly, a shift to the right of the dose-response curve and a decrease in the pD_2 value indicates that the tissue is less sensitive to a drug than 'normal'.

In the present experiment, no changes in the pD_2 values for ACh or carbachol occurred during infection with N. brasiliensis. Nevertheless, although no change in absolute sensitivity as defined above was evident, infection did have a slight effect on the susceptibility of gut muscle to drugs. The threshold for drug-response appeared to have decreased, in that preparations from rats at day 14 responded to doses of as low as 7×10^{-8}M ACh or carbachol. This dose elicited no response from control tissues. Thus, even though no shift in the dose-response curve occurred overall, the foot of the curve was displaced leftward during infection. The cause of this decrease in threshold however, is obscure. Chronic treatment with the ganglion blocking drug chlorisondamine for 5 - 7 days produces a similar effect in the guinea-pig ileum (Fleming, 1968). This treatment produced an increase in sensitivity to ACh, 5-HT, histamine and potassium, the increased sensitivity to ACh being the greater. Fleming found however, that the dose-response curve was not shifted to the left in a parallel fashion. The lower part of the curve was shifted much more than the upper portion. Generally, supersensitivity is brought about by decreasing the amount of neuro-transmitter to which a tissue is exposed (reviewed by Cannon and Rosenbleuth, 1949; Fleming et al., 1973). This is achieved by denervation, either physical or chemical, or with the use of drugs which block either release of transmitter or its combination with its receptors on the effector organ. As discussed earlier, a suggested function of worm-secreted AChE is to hydrolyse neurally released ACh, thus preventing its excitatory action on the gut muscle. This 'biochemical holdfast' action may aid the establishment of N. brasiliensis in the gut (Ogilvie and Jones, 1971). This being the case, the gut smooth muscle cells may to an extent have a reduced exposure to neurally released ACh during infection, thus bringing about an increase in the tissue sensitivity to choline esters such as ACh and carbachol. However, if this is the case, it is strange that only the foot, and not the whole dose-response curves for ACh and carbachol were shifted.

In contrast to ACh and carbachol, infection with N. brasiliensis brought about a marked decrease in the sensitivity of intestinal preparations to 5-HT, reflected as a rightward shift of the dose-response curves and a decrease in pD_2 on days 14 and 20. The pD_2 for 5-HT was also slightly reduced on day 8, as were the values obtained on days 10 and 12, though the latter two were not significantly reduced. The relatively large amount of variation in individual results at these times possibly accounts for the lack of significance.

It has been shown that intestinal levels of 5-HT increase markedly during infection with N. brasiliensis (Murray et al., 1971b). These authors found that levels in the bowel wall rose from day 8 of infection, reached a peak around day 12 and remained high as late as day 20. It was proposed that 5-HT plays a major role in creating a hyperpermeable intestinal wall which facilitates antibody translocation into the gut lumen.

If 5-HT is repeatedly added to, and washed out from an isolated preparation of guinea-pig ileum, successive responses are progressively reduced(Crossland, 1970). This rapidly developing tolerance is the well known pharmacological phenomenon of tachyphylaxis. Similarly, prolonged exposure of a piece of rat colon to 5-HT renders the tissue unresponsive to this compound (Gillan and Pollock, 1980). In many isolated preparations, the response to a given concentration of drug is reduced (or abolished) after prior exposure to the same or to a different drug. For example, if a large dose of ACh is added to an organ bath in which is suspended a piece of guinea-pig ileum, the tissue will be temporarily insensitive to a subsequent dose of either ACh or histamine (Cantoni and Eastman, 1946). In a preparation of fowl gut, the response to histamine was abolished by prior prolonged exposure to histamine, but the responses to other drugs such as ACh or adrenaline were only slightly diminished (Barsoum and Gaddum, 1935). This diminution in drug response, whether specific or non-specific, is termed 'desensitization'.

Therefore, in view of the continuous, abnormally high intestinal levels of 5-HT as from day 8 post-infection with N. brasiliensis, it is hardly surprising that the gut muscle became less sensitive to the action of 5-HT. In Experiment 1, described earlier, it was suggested that the slight increase in intestinal propulsive motility on day 8 post-infection may have been due to increased 5-HT levels in the gut wall. Some doubt was cast on this however, since no changes in propulsion occurred on days 10 - 14, when 5-HT levels are even higher. In view of the results of the present experiment however, it is possible that, after day 8, no further change in propulsive motility occurred because the gut smooth muscle had become desensitized to 5-HT.

By far the most dramatic results obtained from isolated rat gut in the present series of experiments, were the increases in maximum response to field stimulation and drugs which occurred during nippostrongylosis. This is of great interest not only for its possible relevance in worm expulsion, but also because it provides an excellent system for the study of supersensitivity in smooth muscle physiology.

In smooth muscle the most commonly observed supersensitivity is that in which a displacement to the left of the dose-response curve for a particular agonist or agonists occurs (Fleming et al., 1973). In other words, the response of the tissue to a submaximal stimulus is increased, but the maximum response remains unchanged. However, a less common type of supersensitivity, in which the maximum response of the tissue increases, also occurs which may or may not be associated with a shift of the dose-percent response curve. According to Fleming and his co-authors (1973), this type of supersensitivity is 'not a regular occurrence', and no treatment consistently produces it.

Nevertheless, an increased maximum response of the rat vas deferens and colon is seen during morphine withdrawal, and in the rat anococcygeus muscle during morphine withdrawal, following thyroidectomy, treatment with reserpine, or chronic treatment with

corticosterone (Pollock et al., 1972; Gibson and Pollock, 1975a). Similarly, the ability of certain treatments to produce this type of supersensitivity have on occasion been overlooked. For example, it was reported that pretreatment of rabbits with reserpine for 3 days produced an increase in the maximum response of aortic strips to ACh, but not to the α-adrenoceptor agonist, phenylephrine (Taylor and Green, 1971). These workers concluded that since phenylephrine produced a greater contraction than ACh in control preparations, then reserpine pretreatment simply allowed the maximum response to ACh to more nearly attain that to the full agonist, phenylephrine. However, these results were recalculated by Gibson (1975) who demonstrated that the maximum response to phenylephrine was also significantly increased by reserpine treatment.

Considering the relevance to worm expulsion, from the present experiment, it is obvious that the supersensitivity characterized by an increase in maximum response occurs in rat intestine during N. brasiliensis infection. This was not associated with any shift in the dose-percent response curves for ACh or carbachol. However, a rightward shift of the dose-response curve for 5-HT, with a subsequent decrease in the pD_2 value did occur. In the analysis of drug effects many people follow the convention of plotting only percent responses, thus risking any change in the maximum response going unnoticed. From the present responses to 5-HT however, a curious observation arises in that, by definition, infected tissues were subsensitive to 5-HT as indicated by a reduction in pD_2. Conversely, infected tissues also exhibited increased maximum responses to 5-HT, and were therefore displaying supersensitivity of the increased reactivity type. Thus, infected tissues were, in a way, simultaneously subsensitive and supersensitive to 5-HT. It is possible that the maximum responses to 5-HT did not increase during infection to the same extent as those for ACh and carbachol due to the fact that tissues were subsensitive to 5-HT.

The mechanisms involved in this type of supersensitivity are unknown. Similarly, there are problems in the interpretation of its relationship with worm expulsion. For example, it is

conceivable that if these increased contractile responses also occur in vivo in the rat, they may help to dislodge damaged worms from the gut mucosa, or otherwise embarrass them. It is not clear whether gut supersensitivity is actually connected with the expulsion process or whether it is simply coincidental in time. Several questions arise. For example, is this supersensitivity due to some mediator secreted by the worms, or is it a consequence of the host's immune response to the parasites? It is possible that the gut supersensitivity plays no part in the rejection of N. brasiliensis.

However, it is tempting to think that the gut supersensitivity observed may be related to the expulsion process in that its development roughly parallels other events known to occur during the self-cure phenomenon. Self-cure begins at around day 10, reflected as a drop in worm egg output and a gradual loss of worms from the gut between days 10 and 13. Similarly the macromolecular leak into the gut is maximal between days 10 and 14 (Maclean, 1974). It was at this time when gut supersensitivity was also at its maximum. Rapid worm loss begins at around day 14, when intestinal maximum responses were also at a peak level. Intestinal mast-cell numbers and 5-HT levels increase dramatically between days 8 and 14 (Murray et al., 1971b), and it would be of interest to find whether this was in any way related to gut supersensitivity. Murray and his associates also reported that administration of the anthelminthic drug thiabendazole on day 6 of infection eliminated the worm burden by day 8. Intestinal mast-cell numbers increased as they do in untreated rats, but no degranulation and subsequent 5-HT release occurred. It would be of value to investigate whether the gut sensitivity to drugs still increased in rats whose worm burdens had similarly been removed. If it did not, it would be reasonable to suggest that the gut supersensitivity was related to the presence of the worms as opposed to the local inflammatory reaction. On the other hand, if gut sensitivity increased even though the worms had been removed, it would be due to some stimulus other than the worms themselves or some mediator secreted by them.

Several factors are known to interfere with the rejection of N. brasiliensis worms from the rat intestine. For example, expulsion is delayed or inhibited in neonatal rats (Jarrett et al., 1966) and in lactating females (Connan, 1973). It would be of interest to see if the maximum responses of intestinal preparations from these animals were altered during infection. Similarly, irradiated rats do not expel their worm burdens. Would these rats also exhibit no changes in gut sensitivity?

Evidence for a connection between intestinal supersensitivity and worm expulsion may be obtained by investigating the effect on gut sensitivity of factors known to interfere with expulsion. It has been known for some time that treatment of infected animals with cortisone derivatives delays the immune rejection of N. brasiliensis (Ogilvie, 1965; Henney et al., 1971). The following experiments were designed to look into this aspect.

4. IS THE INTESTINAL SUPERSENSITIVITY OBSERVED DURING INFECTION ABOLISHED BY BETAMETHASONE?

4. Is the Intestinal Supersensitivity Observed During Infection Abolished by Betamethasone?

Introduction

Several factors can delay or prevent the immune expulsion of adult Nippostrongylus brasiliensis from the intestine of infected rats. As discussed previously, irradiated rats do not expel their worm burdens (Jones and Ogilvie, 1971), and it has been shown that in neonatal rats, the expulsion process begins much later than in adults, and proceeds at a slower rate (Jarrett et al., 1966, 1968). Similarly, in lactating rats the duration of infection is greatly prolonged, so that the worms are expelled very much more gradually than from non-lactating rats (Connan, 1970, 1973). It has been suggested that the hormones concerned with the initiation and maintenance of lactation either directly or indirectly exert an inhibitory effect on lymphoid cell differentiation, and it was shown that administration of prolactin to infected male rats suppressed the expulsion of N. brasiliensis (Kelly and Dineen, 1973).

Treatment of infected rats with cortisone derivatives also delays the immune expulsion of N. brasiliensis, presumably by virtue of the immunosuppressive and anti-inflammatory capacity of these drugs (Ogilvie, 1965; Henney et al., 1971). It is possible however, that compounds of this type may also have some influence on gut motility.

The purpose of the experiment described here was to determine the effect of treating infected rats with betamethasone upon the sensitivity of the isolated small intestine to ACh and 5-HT. Betamethasone is a synthetic derivative of cortisone which has a much more powerful anti-inflammatory potency (about 40:1) than cortisone itself, but has negligible sodium retaining activity (Goodman and Gilman, 1975).

Because of its inhibitory effect upon worm expulsion, the question arises, does betamethasone also prevent the increased contractility of the isolated gut preparations from infected animals already described? It is also of relevance to consider the effect of betamethasone itself on the sensitivity of gut muscle from normal, uninfected rats which have been treated with the drug.

Methods

Thirty rats were each infected with 5,000 N. brasiliensis larvae, and on the mornings of days 6, 8, 10, 12, 14 and 17 post-infection were injected subcutaneously with 1.5 mg/kg betamethasone. This is a schedule which has been shown to inhibit worm expulsion. A further 14 uninfected rats were also treated with the same dose on corresponding days.

On days 8, 10, 12, 14 and 20 groups of both infected and uninfected, betamethasone-treated rats were killed and isolated intestinal preparations set up as described in the previous experiment. The spontaneous activity of each preparation was recorded for 30 minutes, and dose-responses to ACh and 5-HT carried out.

Animals were divided into four groups as follows:

Group I (control) - uninfected, untreated

Group II - infected, untreated

Group III - uninfected, betamethasone treated

Group IV - infected, betamethasone treated.

As before, individual maximum contractile responses to agonists were recorded, the mean maximum responses calculated for each group, and means compared by Student's t-test. Probability values of less than 0.05 were taken as significant. Similarly, individual dose-percent response curves were drawn and the pD_2 values for each curve calculated. Mean pD_2 values for each group were compared by t-testing.

The following comparisons were made between groups:

I and II - Analysis of the effect which infection alone had upon the activity and responses of the small intestine. This was described in the previous experiment.

I and III - Analysis of the effect of betamethasone treatment alone upon the activity and responses of intestinal preparations. This comparison was made since it was possible that betamethasone treatment itself may produce changes in gut sensitivity or motility.

The most important comparison was between groups II and IV to discover if betamethasone treatment abolished the supersensitivity associated with infection, which was described earlier.

Results

Uninfected rats withstood betamethasone treatment well considering the relatively high dosage and duration of the experiment, although slight weight loss did occur in a few. Infected rats on the other hand, did not tolerate treatment very well. Weight loss and diarrhoea were very marked, and by day 14 of the experiment, the animals were all severely ill, and several died. As a consequence, results from only 2 animals were obtained for day 20.

It was observed that the marked inflammation and fluid accumulation which usually occurs in the small intestine of infected rats, especially around days 8 - 10, was not in evidence in infected rats treated with betamethasone, even as late as day 14. Also, worm expulsion was obviously delayed, since the parasites were still much in evidence at this time.

Comparison between groups I (control) and III (uninfected, betamethasone treated) showed that treatment of uninfected rats had no effect upon the amplitude and rate of the spontaneous, rhythmic contractions of the small intestine. Similarly, betamethasone treatment neither produced any shift in the dose-response curves to ACh or 5-HT, as indicated by the pD_2 values (see Table 9), nor did it cause any changes in the maximum responses of gut preparations to either agonist (see Table 10).

It was reported in the previous experiment that the spontaneous activity normally elicited by isolated gut from control rats was either non-existent, or very erratic on day 8 post-infection with N. brasiliensis. In the present experiment, gut preparations from betamethasone-treated rats at day 8 post-infection exhibited regular rhythmic contractions at a frequency of about 30/min, and with a mean amplitude of 1.01 ± 0.56 g ($n = 6$). On days 10 and 14 post-infection, gut preparations from betamethasone-treated rats (Group IV) also exhibited spontaneous rhythmic activity, but the mean amplitudes of contraction were not significantly different from those measured in gut from untreated, infected rats (Group II) at the same days of infection. (They were however, significantly greater than the mean amplitude of control (Group I) spontaneous contractions. This is discussed later.)

Table 9: The Mean pD_2 Values to ACh (top) and 5-HT(bottom) in *in vitro* Intestinal Preparations from Control Rats and from Uninfected Rats Treated with Betamethasone. 1.5 mg/kg was Administered on the Sixth Day of the Experiment, and on alternate Days up to Day 17. Figures are Mean ± SD, and those in Parentheses are the Number of Observations. No Significant Difference was Found Between Control and any of the Experimental Groups.

	Control (Group I)	Betamethasone Treated (Group III) Day 8	Day 10	Day 14
ACh	6.03 ± 0.17 (11)	6.11 ± 0.08 (4)	6.19 ± 0.02 (4)	6.14 ± 0.19 (6)
5-HT	7.25 ± 0.29 (9)	7.44 ± 0.23 (4)	7.16 ± 0.20 (4)	7.09 ± 0.22 (6)

Table 10: Mean Maximum Responses to ACh (Top) and 5-HT (Bottom) of Preparations of Isolated Small Intestine from Control Rats and from Uninfected Rats Treated with Betamethasone. 1.5 mg/kg Betamethasone was administered on Day 6 of the Experiment and on Alternate Days up to Day 17. Figures are Mean ± SD and those in Parentheses are the Number of Observations. No Significant Difference was found between Control and any of the Betamethasone Treated Groups.

	Control (Group I)	Betamethasone Treated (Group III) Day 8	Day 10	Day 14
ACh	1.80 ± 0.45 (7)	1.61 ± 0.34 (4)	1.50 ± 0.16 (4)	1.52 ± 0.61 (6)
5HT	1.81 ± 0.42 (9)	1.52 ± 0.10 (4)	1.55 ± 0.26 (4)	1.66 ± 0.63 (6)

As discussed in the previous experiment, the maximum responses elicited by ACh and 5-HT from isolated gut increased significantly as infection with Nippostrongylus progressed (see Figs. 25, 35 and 36), reaching a peak on day 14. Treatment of infected rats with betamethasone significantly reduced this effect. From Table 11 it can be seen that gut from betamethasone-treated rats at days 8, 10 and 14 post-infection with N. brasiliensis (Group IV) showed maximum responses to ACh significantly lower than gut from untreated, infected animals (Group II) at the corresponding days of infection. This was most marked at day 14, where the mean maximum response to ACh of betamethasone-treated, infected gut was 2.98 g, as compared with 10.23 g in untreated, infected gut - a reduction of over 70%.

Similarly, mean maximum responses to 5-HT were all reduced in infected preparations from betamethasone-treated rats. Significant reductions occurred on days 8 and 14, but not on day 10 (see Table 11).

The mean maximum responses to both agonists for controls (Group I), day 14 post-infection (Group II), and betamethasone-treatment, day 14 post-infection (Group IV) were plotted as a histogram shown in Figure 37. From this diagram it is clear that betamethasone-treatment dramatically reduced the intestinal supersensitivity associated with nippostrongylosis. However, it is also evident that the supersensitivity was not completely abolished, and it was therefore thought to be of interest to compare the results obtained for controls (Group I) with those for betamethasone-treated, infected preparations (Group IV).

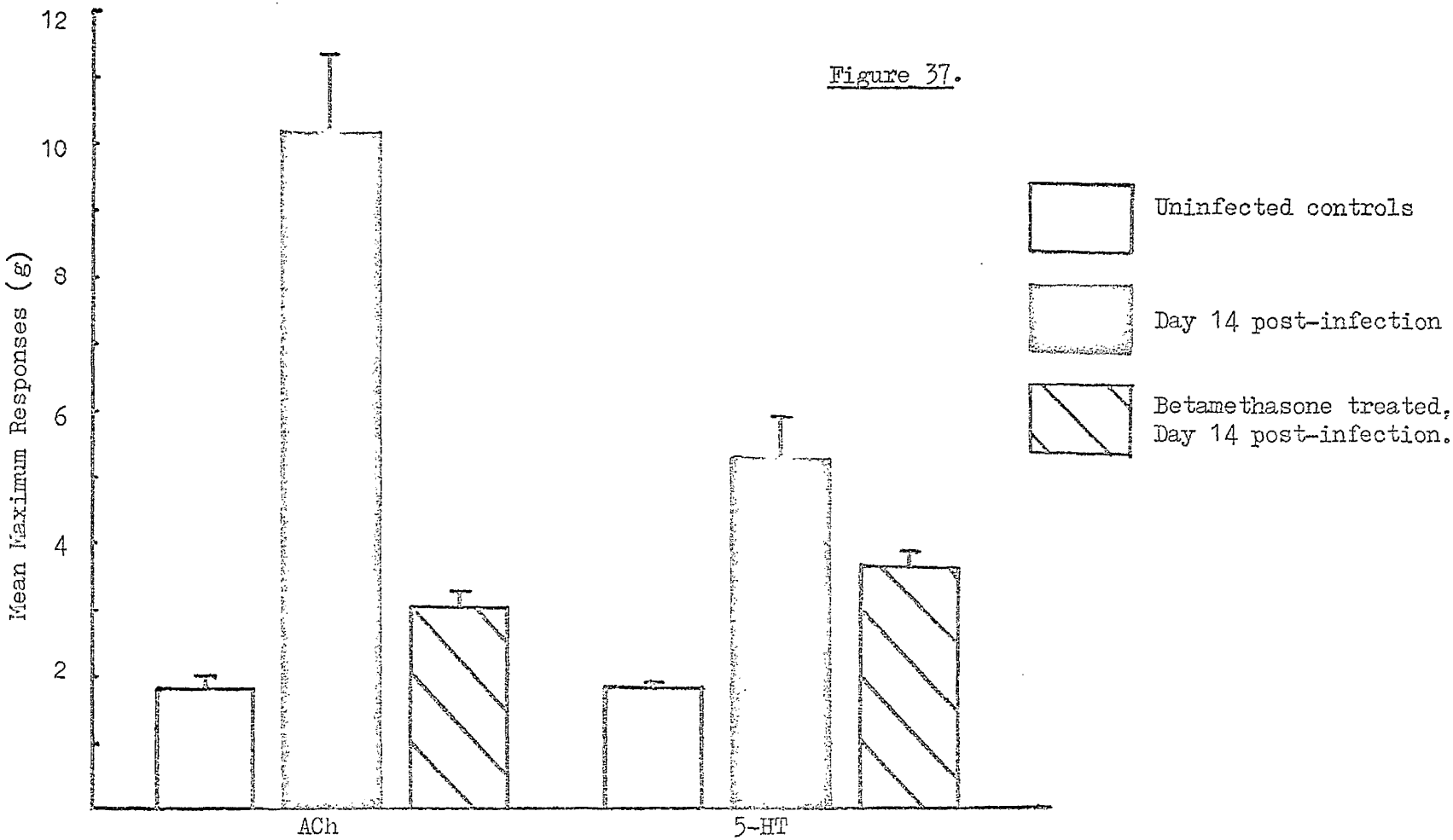
As mentioned earlier, although no difference in the amplitude of spontaneous contractions occurred between infected (Group II) and betamethasone-treated, infected (Group IV), the mean amplitude of contractions of Group IV were significantly greater than in controls (Group I). From Table 12 it can be seen that spontaneous contractions elicited by preparations from betamethasone-treated rats at days 10 and 14 post-infection, were significantly greater than those of controls.

The mean maximum response to ACh and 5-HT elicited by betamethasone-treated preparations from rats at days 10 or 14 post-

Table 11: Mean Maximum Responses to ACh and 5-HT of Isolated Gut Preparations from Rats at Days 8, 10 and 14 Post-infection with N. brasiliensis (Group II), and from Betamethasone-treated Rats at the Same Days of Infection (Group IV).
Student's t-test were carried out for each agonist between the means for Groups II and IV at the same days of infection.
***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; NS, not significant.

Group	Day of Infection	Mean max. Response to ACh (g)	SD	n	Mean max. response to 5-HT (g)	SD	n
II	8	3.78	0.99	4	3.65	0.40	4
IV		1.67*	0.63	4	1.95**	0.70	4
II	10	5.11	1.32	6	4.23	1.39	5
IV		3.79*	0.55	7	3.98 NS	0.96	7
II	14	10.23	2.6	5	5.26	1.56	6
IV		2.98***	0.95	8	3.56*	0.95	8

Figure 37.



Mean maximum responses to ACh (left) and 5-HT (right) of isolated rat gut for uninfected rats, rats on day 14 post-infection and betamethasone-treated rats on day 14 post-infection. I-bars are standard errors.

Table 12: Mean Amplitudes of Spontaneous, Rhythmic Contractions of Isolated Intestinal Preparations from Control Rats (Group I) and from Betamethasone-treated Rats at Various Days Post-infection (PI) with 5,000 *N. brasiliensis* Larvae (Group IV). Each mean from Group IV was compared to the Control Mean by Student's t-test.
***, $P < 0.001$; N.S., Not Significant.

Group	Mean Amplitude (g)	SD	n
I (Control)	0.73	0.38	12
IV (day 8 P.I.)	1.01 NS	0.56	6
IV (day 10 P.I.)	1.55***	0.48	7
IV (day 14 P.I.)	1.85***	0.44	8

infection were also significantly greater than control responses (Table 13). Thus, although betamethasone treatment of infected rats markedly reduced the intestinal supersensitivity associated with infection, it did not completely abolish it, since on days 10 and 14 the amplitudes of spontaneous contractions and the maximum responses to ACh and 5-HT of treated, infected preparations were greater than those of controls.

It was found in the previous experiment that during infection, a rightward shift of the dose-% response curve to 5-HT, with a subsequent decrease in the pD_2 value occurred. As was discussed, this observation produced a paradox, in that infected preparations were both subsensitive to 5-HT, as indicated by the reduction in pD_2, and supersensitive, since the maximum response was increased. It was therefore thought relevant to investigate the possibility that betamethasone treatment, which greatly reduced the abnormally high maximum response to 5-HT associated with nippostrongylosis, might also inhibit the rightward shift of the dose-% response curve which occurred during infection.

The mean pD_2 values for 5-HT were calculated for gut preparations from infected, betamethasone-treated rats on days 8, 10 and 14 post-infection (Group IV), and each compared to that for controls (Group I). As can be seen from table 14, the pD_2 values for 5-HT of gut preparations from betamethasone-treated rats at days 10 and 14 post-infection were significantly less than in controls. Thus, betamethasone did not prevent the rightward shift of the dose-%response curve which occurs during N. brasiliensis infection.

Although infection with N. brasiliensis produced no significant changes in the pD_2 for ACh, as infection progressed the mean pD_2 for ACh did increase slightly (see Table 6 in the previous experiment). Similarly, treatment of uninfected rats, in the present experiment, with betamethasone produced no significant changes in the pD_2 for ACh, but the pD_2 values were marginally greater than control during treatment (Table 9). One wondered whether both infection and betamethasone treatment of rats would have an additive effect, and thereby produce an increase in the pD_2 for ACh of gut preparations. From Table 15 it is seen that the pD_2 values for ACh in tissues from

Table 13: The Mean Maximum Responses to ACh and 5-HT of Isolated Gut Preparations from Betamethasone-treated Rats at Various Times Post-infection (P.I.) with N. brasiliensis (Group IV) were Compared with Those of Control Preparations (Group I) by Student's t-test.
***, $P < 0.001$; * $P < 0.05$; NS, not significant

Group	Agonist	Mean Max. Response (g)	SD	n
I (Control)	ACh	1.80	0.45	7
IV (day 8 PI)	"	1.67 NS	0.63	4
IV (day 10 PI)	"	3.79***	0.55	7
IV (day 14 PI)	"	2.98*	0.95	8
IV (day 20 PI)	"	2.5 NS	0.05	2
I (Control)	5-HT	1.81	0.42	9
IV (day 8 PI)	"	1.95 NS	0.70	4
IV (day 10 PI)	"	3.98 ***	0.96	7
IV (day 14 PI)	"	3.56 ***	0.95	8
IV (day 20 PI)	"	2.34 NS	0.49	2

Table 14: Mean pD_2 Values for 5-HT in Isolated Gut Preparations from Control Rats (Group I), and Betamethasone-treated Rats at Various Times Post-infection (PI) with *N. brasiliensis* (GROUP IV). Each Mean Value for Group IV was compared by Student's t-test with that for Group I.
***, $P < 0.001$; NS, not significant.

Group	Mean pD_2	SD	n
I (Control)	7.28	0.29	9
IV (Day 8 PI)	6.98 NS	0.13	4
IV (Day 10 PI)	6.77 ***	0.16	7
IV (Day 14 PI)	6.84 ***	0.11	7

Table 15: Mean pD_2 Values for ACh in Isolated Gut Preparations from Control Rats (Group I), and Betamethasone-treated Rats at Various Times Post-infection (PI) with N. brasiliensis (Group IV). Each Mean Value for Group IV was Compared by Student's t-test with that for Group I.
***, $P < 0.001$; *$P < 0.05$; NS, not significant.

Group	Mean pD_2	SD	n
I (Control)	6.03	0.17	11
IV (Day 8 PI)	6.25*	0.09	4
IV (Day 10 PI)	6.18 NS	0.16	7
IV (Day 14 PI)	6.50***	0.21	8

betamethasone-treated animals at days 8 and 14 were significantly greater than control. This was especially so on day 14 ($P < 0.001$). The pD_2 value for ACh on day 10, although apparently greater than control, was not significantly increased. Therefore, a leftward displacement of the dose-% response curves for ACh occurred on days 8 and 14 post-infection in preparations from betamethasone-treated rats.

Summary and Conclusions

The aim of the present investigation was to find whether betamethasone treatment, which inhibits the expulsion of N. brasiliensis, also prevented the onset of intestinal supersensitivity observed during infection. This was indeed the case, and further interesting results were obtained.

Treatment of rats with betamethasone had no effect on the spontaneous activity of isolated segments of small intestine. Similarly, neither the maximum contractile responses to ACh or 5-HT nor the pD_2 for each agonist were affected. Betamethasone treatment did however, markedly reduce the increased maximum responses to ACh and 5-HT which were elicited by isolated gut preparations from rats infected with N. brasiliensis. The drug did not completely abolish infection-associated supersensitivity since the maximum responses of betamethasone-treated, infected tissues (on days 10 and 14) were still greater than control (see Fig. 37).

Unlike gut from untreated rats at day 8 post-infection, preparations from betamethasone-treated rats at day 8 exhibited spontaneous, rhythmic contractile activity. However, the increased amplitude of spontaneous contractions during infection was not reduced by pretreatment with betamethasone.

The pD_2 values for 5-HT on days 10 and 14 were significantly less in preparations from betamethasone-treated, infected rats than controls. Therefore, betamethasone did not prevent the rightward shift of the dose-response curve to 5-HT which occurred during N. brasiliensis infection.

Conversely, betamethasone treatment of infected rats produced a leftward displacement of the dose-% response curve for ACh, reflected as an increase in the pD_2. This increase was significant on days 8 and 14 of infection. On day 10, although the pD_2 for ACh was apparently increased, there was no significant difference from control.

The immunosuppressive and anti-inflammatory actions of betamethasone and other corticosteroids have been widely documented (see reviews by Asboe-Hansen, 1958; Melby, 1977 and Parrillo and Fauchi, 1979). It is highly probable that betamethasone inhibits the rejection of N. brasiliensis from the rat intestine by virtue of these actions. As betamethasone treatment also inhibited the development of the intestinal supersensitivity associated with nippostrongylosis, it is conceivable that this supersensitivity is a consequence of the immune response to the parasites, rather than being a direct effect of the worms themselves. It would be interesting to discover whether this type of gut supersensitivity is also inhibited in other situations where the immune response to N. brasiliensis is impaired. For example, would the increase in intestinal maximum responses also be inhibited in lactating, neonatal or irradiated, infected rats? Similarly, would other types of immunosuppressives such as antimetabolites or cytotoxic agents also inhibit the onset of supersensitivity of gut from infected animals? Keller and Ogilvie (1972) reported that the powerful anti-inflammatory drug, phenylbutazone (but not aspirin) inhibited the expulsion of Nippostrongylus. It would be of value to see if this drug also blocks the occurrence of gut supersensitivity.

Nevertheless, the most important question in this study - the involvement of gut supersensitivity in worm expulsion - remains unanswered. Since betamethasone inhibited both worm expulsion and the development of supersensitivity, it is tempting to think that both phenomena may be related. However, there is no direct evidence for supersensitivity being involved in expulsion.

As mentioned earlier, several factors are known to produce increases in the maximum responses of other tissues. Morphine withdrawal for example, produces this type of supersensitivity in

the rat colon, vas deferens and anoccygeus muscle (Pollock et al., 1972; Gibson and Pollock, 1975a). A single dose of reserpine or thyroidectomy also causes increased maximum responses of the anococcygeus muscle. Although these treatments may appear to be unrelated, there is a common factor in all of them. Gibson and Pollock (1975a) proposed that the increased maximum responses of rat anococcygeus muscle seen during morphine withdrawal, or following thyroidectomy or a single dose of reserpine are mediated by abnormally high blood levels of corticosterone, and they obtained sound evidence for this. These authors demonstrated that, (i) chronic administration of corticosterone to rats produced supersensitivity of the anococcygeus similar to that seen during morphine withdrawal or thyroidectomy; (ii) Metyrapone, a corticosteroid synthesis inhibitor, prevented the development of morphine withdrawal supersensitivity; (iii) A single dose of reserpine (1 mg/kg) which produced this type of supersensitivity, also raised plasma corticosterone levels; (iv) The supersensitivity produced by reserpine could be prevented by adrenalectomy; (v) Plasma corticosterone levels were raised in thyroidectomized rats. It therefore seems likely that an increased blood-corticosterone level is the common factor involved in the ability of these treatments to increase the maximum responses of the rat anococcygeus. It is possible that the supersensitivity of rat colon and vas deferens observed during morphine withdrawal is also due in some way to increased blood corticosterone. It is also feasible that the above treatments may produce an increase in the maximum response of the rat small intestine. This being the case, it would be very interesting to see if the expulsion of N. brasiliensis could be brought forward, or accelerated by a single dose of reserpine (which is less traumatic than morphine withdrawal) or by chronic treatment with corticosterone. Several questions need to be answered before a possible interrelationship between expulsion and gut supersensitivity can be postulated. For example, are the corticosterone blood levels of rats increased during infection? (This is discussed

in the next section). Would adrenalectomy prevent both worm rejection and the development of intestinal supersensitivity? Neonatal rats do not expel N. brasiliensis nearly as effectively as do adults, presumably because their immune systems are not fully developed (Jarrett et al., 1966, 1968). Similarly, in lactating female rats the ability to reject N. brasiliensis is impaired (Connan, 1970, 1973) and it has been suggested that the hormones involved with lactation have an immunosuppressive effect (Kelly and Dineen, 1973). It would be of great interest to discover if the production of gut supersensitivity, in neonatal or lactating rats, with corticosterone would restore the ability of these animals to expel N. brasiliensis. Conversely, does this type of supersensitivity also exist during infection of neonates or lactating rats? If some aspect of the immune response produces increased maximum responses, this would be unlikely.

It should perhaps be pointed out that, although corticosterone and betamethasone are related compounds, they differ greatly in their pharmacological actions. Corticosterone is a powerful mineralocorticoid with very little anti-inflammatory activity (Goodman and Gilman, 1975), and it is probably that its ability to induce hypercontractility of the anococcygeus may be related to its effects on ion distribution. Indeed, it has been found that intramuscular redistribution of Na^+ ions seems to be involved in the supersensitivity produced by corticosterone administration (Gibson and Pollock, 1976). Betamethasone on the other hand, is a glucocorticoid, with very potent anti-inflammatory effects and negligible mineralocorticoid activity. This provides further, indirect evidence that corticosterone-induced supersensitivity is due to its mineralocorticoid activity, since betamethasone (with negligible mineralocorticoid activity) did not produce any changes in the sensitivity of isolated gut preparations from uninfected rats.

In the previous section it was described how the spontaneous activity of isolated gut was either very erratic or non-existent on days 6 and 8 post-infection with N. brasiliensis. In the present experiment betamethasone treatment, which commenced on

day 6, restored the spontaneous, rhythmic contractile activity of gut from animals at day 8 of infection. However, the increased amplitudes of spontaneous contraction which occurred on days 10 - 14 post-infection were not reduced by betamethasone. It was suggested that spontaneous activity was impaired on days 6 and 8 due, either to intestinal oedema interfering with the coordinated electrical conduction between smooth muscle cells, or to a possible stimulatory effect of the parasites on inhibitory nerves in the gut, which disappears when the worms are immunologically damaged. If the latter is the case, it seems strange that during betamethasone treatment, which presumably prevents the immune response to the parasites, spontaneous activity was in evidence throughout the course of infection. On the other hand, betamethasone treatment prevented the marked intestinal inflammation and oedema which normally occurs around days 6 - 8 of Nippostrongylus infection, thus providing indirect evidence that oedema and inflammation may indeed impair smooth muscle activity.

During N. brasiliensis infection, the pD_2 for 5-HT of isolated gut decreased, and it was suggested that tissues became subsensitive to 5-HT due to the abnormally high levels of this compound in the gut wall during infection (see previous section). Because of its numerous anti-imflammatory effects, including inhibition of mast-cell function (Melby, 1977), it was thought that betamethasone might inhibit the release and action of 5-HT, and thus prevent the development of subsensitivity, as well as decreasing the maximum responses of infected gut. However, this was not the case, even though no visible inflammatory changes were evident in betamethasone-treated, infected gut preparations. Thus, it would appear that, in the rat, betamethasone does not affect 5-HT release from mast-cells. It is possible that the intestinal oedema seen during nippostrongylosis is not due to 5-HT, but may be caused by some other products of immunity, such as histamine, kradykinin or slow reacting substance, which may be inhibited by betamethasone. On the other hand, it is known that corticosteroids block the increase in permeability of capillary endothelium induced by acute inflammation (Melby, 1977).

There is a reduction both in the leakage of oedematous fluid and in the transport of proteins into areas of injury. Thus, tissue swelling is minimized, if not prevented. Glucocorticoids such as betamethasone cause vasoconstriction in localised areas of the capillary bed (Parrillo and Fauchi, 1979) and Schayer (1963) suggested that this may physiologically antagonize histamine-induced vasodilation. If this is true, then betamethasone would prevent oedema without altering 5-HT release in the gut, and thus have no effect upon the development of subsensitivity to 5-HT of the intestinal smooth muscle.

Although infection or betamethasone treatment alone had no significant effect on the intestinal pD_2 for ACh, both produced an increase. The reasons for this are obscure, but there are two possible explanations. However, there is no evidence in support of either:

(i) As well as the non-specific increase in the maximum response of the rat anococcygeus to ACh and noradrenaline which Gibson and Pollock (1975a) demonstrated could be produced by chronic corticosterone treatment, morphine withdrawal or reserpine, these authors also reported a specific supersensitivity to ACh, in that the pD_2 for this compound was also increased. In a further study, Gibson and Pollock (1975b) showed that corticosterone administration also caused a 47% reduction in the cholinesterase (ChE) activity of homogenates of the anococcygeus muscle. Further, a reduction in ChE activity could also be produced both by morphine withdrawal (which was prevented by metyrapone), and a single dose of reserpine (prevented by adrenalectomy). It was therefore suggested that the specific leftward displacement of the dose-% response curve to ACh during corticosterone treatment could be explained by the reduction in ChE activity. In other words, because ChE activity is less than 'normal' during corticosterone treatment, a smaller dose of ACh is required to produce a given effect and the dose-% response curve is consequently shifted to the left. However, Gibson and Pollock stressed that the ability of the above treatments to increase the maximum response of the anococcygeus to agonists could not be explained by inhibition

of ChE activity. The mechanism whereby corticosterone inhibits ChE activity is unknown, but Gibson and Pollock (1975b) suggested that corticosteroid-induced reduction of enzyme activity *in vivo* is probably related to the effect of these hormones on protein synthesis and degradation (White *et al.*, 1961; Parvez and Parvez, 1972).

If betamethasone treatment of rats infected with *N. brasiliensis* has a similar effect on the activity of AChE in the small intestine, then the increase in the pD_2 for ACh in betamethasone-treated, infected gut observed in the present experiment might also be explained by reduced degradation of exogenously applied ACh. However, this seems unlikely since betamethasone treatment of uninfected animals had no effect on the sensitivity of the gut to ACh.

(ii) Another possible explanation for the increase in pD_2 for ACh produced by betamethasone administration to infected animals involves the 'biochemical-holdfast' action of worm-secreted AChE. Briefly, it has been proposed that the function of worm-AChE is to hydrolyse neurally released ACh, preventing local intestinal motility and thus aiding the establishment of *Nippostrongylus* worms in the gut (see General Introduction). It has been shown that, as immunity to the parasites develops, changes in the properties of worm-AChE occur (Lee, 1970), and Jones and Ogilvie (1972) have found anti-AChE antibodies in the sera from immune rats. Thus, immunity may interfere with the biochemical holdfast action of worm-AChE and, thereby contribute to the expulsion of the parasite. However, treatment of infected rats with betamethasone will prevent immunological damage being incurred not only by the worms themselves, but also by their AChE. If the function of this AChE is indeed to prevent the action of the host's neurally released ACh on gut motility, then this action will be prolonged in rats whose immune responses have been suppressed by betamethasone, and the gut smooth muscle may be deprived of the action of neural ACh.

Generally, supersensitivity of the type characterised by an increase in the pD_2 for one or more agonists, is produced

by a reduction of the amount of neurotransmitter to which a tissue is exposed (Fleming *et al.*, 1973). Therefore, the increased pD_2 for exogenous ACh observed in the present experiment may have been caused by a reduced exposure of the gut to ACh, which was being degraded to a greater degree than normal, by the prolonged production of worm-AChE due to the immunosuppressive effects of betamethasone. Nevertheless, as has been said, no direct evidence for either of these hypotheses exists.

From the present experiment then, it is obvious that the causes and function of the increase in maximum response of the gut during *N. brasiliensis* infection are still unresolved. The responses of isolated gut from betamethasone-treated, infected rats are very complex and a great deal more work is necessary before the relationship, if any, of this supersensitivity to infection and/or expulsion can be found. Similarly, it is unknown whether the effects of infection upon the gut smooth muscle are of relevance *in vivo*.

5. CORTICOSTEROID AND THYROXINE SERUM LEVELS IN INFECTED RATS

5. Corticosteroid and Thyroxine Serum Levels in Infected Rats

Introduction

Hypercontractility similar to that previously described in the intestinal smooth muscle of N. brasiliensis-infected rats is an atypical and little understood phenomenon (Fleming et al., 1973), but it also occurs in other smooth muscles under different circumstances. For example, both the colon and vas deferens of rats undergoing morphine withdrawal exhibit increases in maximum contractile responses (Pollock et al., 1972). A closely related supersensitivity also occurs in the rat anococcygeus muscle during morphine withdrawal or following thyroidectomy (Gardiner et al., 1974). Serum thyroxine levels are reduced by both of these treatments, although the level is even lower during morphine administration than withdrawal (no increased maximum responses of the anococcygeus were observed during treatment, but only during the withdrawal syndrome).

It was proposed that this hypercontractility of the anococcygeus may not be due to a direct effect of reduced serum thyroxine, but to an adaption which occurs following thyroidectomy and which appears only transiently during morphine withdrawal (Muir and Pollock, 1973; Gardiner et al., 1974). The metabolism of the corticosteroids is associated with thyroxine (Pitot and Yatvin, 1973), and corticosteroid blood levels are elevated during morphine withdrawal (Eisenmann et al., 1961; Paroli and Melchiorri, 1961), and following thyroidectomy (Gibson and Pollock, 1975a).

As was described in the last section, a good deal of evidence has been accumulated which implicates a role for corticosterone in the hypercontractility of the anococcygeus (Gibson and Pollock, 1975a). A single dose of reserpine, which increases ACTH secretion (Wells et al., 1956) and as a result raises plasma corticosteroid levels, produces increased maximum responses of this muscle. In contrast, repeated injections of reserpine, which after 5 days loses its ability to release ACTH (Wells et al., 1956) presumably due to depletion of brain monoamines, have no effect on the maximum response

(Gibson and Pollock, 1973). Chronically administered corticosterone produces increased maximum responses of the anococcygeus, and metyrapone, which inhibits corticosteroid synthesis, prevents the hypercontractility which normally occurs during morphine withdrawal (Gibson and Pollock, 1975a). Similarly, a single dose of reserpine has no effect upon anococcygeal maximum responses in adrenalectomized rats.

It was therefore of interest to investigate the possibility that the gut supersensitivity which occurs during nippostrongylosis, may also be associated with altered serum levels of these hormones. Thus, in the present experiment serum levels of corticosterone were measured at various stages of a primary infection with N. brasiliensis. In addition, the serum levels of thyroxine were assayed.

Methods

Twenty two rats, while lightly anaesthetized with halothane, were infected subcutaneously with 5,000 N. brasiliensis larvae, and a further 20 were anaesthetized and injected subcutaneously with the same volume (1 ml) of 0.9% saline. On days 1, 6, 10 and 14 post-infection groups of both infected and saline-control rats were anaesthetized in halothane, and a blood sample (normally 5 - 6 ml) collected from the abdominal aorta. Following blood sampling, animals which were not already dead, were killed. In addition, blood samples were obtained from 10 rats which had received no previous injections (controls). Blood samples were allowed to clot at room temperature for about an hour, after which time the serum was obtained by centrifugation at 5,000 rpm for 10 minutes. Each serum sample was then stored under refrigeration until hormone assays were carried out.

Measurement of Serum Corticosterone Levels

Serum was assayed fluorimetrically for corticosterone, essentially using the procedure described by Zenker and Bernstein (1958). A 1 ml aliquot of serum from each sample was transferred to 2 ml distilled water, and the steroids extracted in 50 ml chloroform by shaking for 60 seconds. Steroids other than corticosterone were destroyed by mixing the separated chloroform layer for 15 seconds

with 4.5 ml 0.01N NaOH. The corticosterone in the chloroform was converted to a fluorescent product by mixing for 15 seconds, 10 ml of the chloroform solution with 3 ml of an acid-ethanol mixture, which contained concentrated sulphuric acid (2.4 vols) and 50% ethanol (1.0 vols).

The tubes were then shaken for 15 seconds and left to stand at room temperature for 2 hours. Standards and blanks were also carried through the procedure. After 2 hours, the fluorescence of the acid layer was determined using an Aminco-Bowman spectrofluorimeter, with the excitation wavelength set at 470 mμ and the fluorescence wavelength at 520 mμ.

Plasma corticosterone levels were calculated by reference to a standard curve prepared each day the assay was carried out. Results were expressed as μg corticosterone/100 ml plasma.

Measurement of Serum Thyroxine Levels

Serum thyroxine (T_4) was assayed using a Thyopac-4 kit, obtained from the Radiochemical Centre, Ltd., Amersham. A 0.5 ml aliquot of serum was added to 1 ml absolute ethanol and mixed for 3 minutes. The precipitated proteins were separated by centrifugation and 0.5 ml supernatant was transferred to a vial containing adsorbent granules suspended in a buffer solution containing ^{125}I-labelled T_4, which was bound to thyroxine binding globulin (TBG). The contents of the vial were mixed for 40 minutes. During mixing, the unlabelled T_4 displaced labelled hormone from TBG, and the displaced label was bound by the adsorbent granules. The amount of T_4 in the sample was then inversely proportional to the radioactivity remaining in the supernatant buffer solution, and could therefore be calculated by reference to the radioactivity of supernatant of vials to which had been added standard serum. The radioactivity of a 1 ml aliquot of supernatant was determined in a gamma counter.

Because of the large number of serum samples, the corticosterone assays were carried out in two batches on different days. The first batch consisted of the samples from the untreated, control group (n = 10), from the saline-control (SC) one day following injection with saline (day 1 SC, n = 5), and from the infected group one day post-infection

(day 1 PI, n = 5). The second batch consisted of the remainder of the saline control samples (days 6, 10 and 14 SC) and infected samples (days 6, 10 and 14 PI). The thyroxine assay in all samples was carried out on a separate day also.

Results

Some difficulty was experienced in obtaining a sufficient blood sample from some animals, and in these cases, the amount of serum obtained was not enough to use in both assays.

No results in the corticosterone assay were obtained for samples from control or the day 1 saline-control and infected samples. This was probably because the amounts of corticosterone used to draw the standard curve for these groups were too large. Thus, the standard curve was not 'sensitive' enough for the amount of corticosterone in the controls and day 1 saline-control (SC) and post-infection (PI) groups. However, the results may also have been affected to a small extent by some quenching which subsequent internal standards indicated was occurring.

Consequently, the standards used in the assay of the days 6, 10 and 14 SC and PI samples were smaller. They were reduced from 5, 10, 20 and 50 μg/ml to 0.5, 1, 2 and 5 μg/ml and the concentrations of corticosterone in these samples are given in Table 16. As can be seen in each experimental group there was considerable variation in individual results, as indicated by the large standard deviations (SD). For days 6, 10 and 14 of the experiment, t-tests were carried out between the mean corticosterone serum levels in saline-controls and infected rats. No significant differences occurred. It would appear therefore, that infection with N. brasiliensis had no effect upon blood corticosterone levels.

On the other hand, the serum levels of thyroxine (T_4) were significantly lower than controls, not only in infected rats, but also in rats which had been injected with saline (see Table 17). The serum level of T_4 decreased with time following subcutaneous injection with 1.0 ml of normal saline, until 14 days later, it was 5.9 μg/100 ml as compared with 21.5 μg/100 ml in rats which had had no previous treatment.

Table 16: Concentrations of Corticosterone (µg/100 ml) in the Serum of Saline-injected Control (S.C.) Rats and Rats on Days 6, 10 and 14 Post-Infection (P.I.) with *N. brasiliensis*.

Group	µg Corticosterone per 100 ml ± SD	n
Control	Not determined	10
Day 1 SC	" "	5
Day 1 PI	" "	5
Day 6 SC	19.9 ± 11.7	5
Day 6 PI	11.4 ± 7.4	6
Day 10 SC	18.5 ± 16.4	5
Day 10 PI	20.6 ± 7.5	5
Day 14 SC	11.0 ± 10.0	5
Day 14 PI	14.8 ± 8.1	6

Table 17: Concentrations of Thyroxine (µg/100 ml) in the Serum of Untreated, Control Rats, Saline-injected Control (SC) Rats and Rats on Days 1, 6, 10 and 14 Post-infection (PI) with N. brasiliensis. Differences between all Values and Control were Analysed by Student's t-test. ***, $P < 0.001$; **, $P < 0.02$; *, $P < 0.05$.

Group	µg Thyroxine per 100 ml ± SD	n
Control	21.5 ± 4.2	10
Day 1 SC	16.5 ± 1.6 *	5
Day 1 PI	9.8 ± 2.0 ***	5
Day 6 SC	15.4 ± 2.3 **	4
Day 6 PI	11.0 ± 1.2 ***	5
Day 10 SC	8.4 ± 1.4 ***	3
Day 10 PI	13.9 ± 4.7 *	2
Day 14 SC	5.9 ± 2.6 ***	5
Day 14 PI	6.4 ± 2.4 ***	6

The serum levels of T_4 in rats on days 1, 6, 10 and 14 post-infection with N. brasiliensis were all significantly lower than control. However, unlike the saline-controls, where T_4 levels decreased with time after injection, levels of T_4 in infected animals did not show any trend, though there was some variation between different days.

Differences between values obtained for injected and saline-injected groups were also analysed by t-testing. Significant differences were found on days 1 ($P < 0.001$) and 6 ($P < 0.01$), but not on days 10 and 14.

Summary and Conclusions

In the present experiment the serum levels of corticosterone and thyroxine (T_4) were assayed during infection with N. brasiliensis. Although infection had no apparent effect upon corticosterone levels, both infection and a single control injection with isotonic saline had a marked effect on T_4 levels, which decreased significantly as compared with non-injected controls. Infection caused a greater decrease in serum T_4 on days 1 and 6 of infection than did saline 1 and 6 days following its injection.

Therefore, it seems likely that increased levels of circulating corticosterone are not involved in the production of infection-associated supersensitivity. This is perhaps surprising since Gibson and Pollock (1975a) found the corticosterone levels were elevated in rats whose anococcygeus muscles exhibited a similar type of supersensitivity.

In view of the effect which a control saline injection had on serum T_4 levels it is not possible to say whether infection with Nippostrongylus caused a decrease in T_4. Similarly, it is not clear how a saline injection brought about this marked reduction in serum T_4 levels. It is possible that the stress to the animals due to anaesthesia may have caused a transient decrease in serum T_4, since it has been reported that thyroid stimulating hormone (TSH) secretion is inhibited by stress in several species, including the rat (Martin, 1974). Kooh and his co-workers (1972) found that ether administration to rats

caused a decrease in plasma TSH levels within 30 minutes, and presumably a consequent reduction in plasma T_4.

Nevertheless, the results obtained in the present experiment do not preclude the possibility that some other circulating substance is involved in the production of intestinal hypercontractility during nippostrongylosis. The fact that suppression of worm rejection with betamethasone also prevented, to a great extent, the development of gut supersensitivity (see Experiment 4) suggests that supersensitivity may be a consequence of some mediator produced by the inflammatory and immune responses. If this mediator is indeed in the circulation, it is possible that other types of smooth muscle, not directly concerned with N. brasiliensis or its expulsion, may also be induced to exhibit increased maximum responses. The following experiment was designed to look into this possibility.

6. PRESSOR RESPONSES OF THE ISOLATED HIND-LIMB PREPARATION FROM INFECTED RATS

6. Pressor Responses of the Isolated Hind Limb Preparation from Infected Rats

Introduction

If alterations in the levels of circulating hormones (or indeed, some other chemical mediator) are involved with the increased maximum responses previously described in the rat intestine during nippostrongylosis, it is conceivable that the phenomenon may occur in smooth muscle tissues other than the small intestine. It was therefore thought of interest to investigate the possibility that supersensitivity of this type may also occur in other sites in infected rats, which are not directly concerned with the parasite or its expulsion, such as the vascular smooth muscle.

The isolated, perfused rat hind limbs preparation has been widely used in the study of vascular smooth muscle reactivity (Folkow et al., 1970; Schomig et al., 1976), and the purpose of the present experiment was to examine the pressor-responses of this preparation to phenylephine, an α-adrenoceptor agonist, at various times post-infection with N. brasiliensis.

Methods

The pressor responses of hind limb preparations to phenylephrine were carried out on controls (n = 5) and on preparations from rats at day 6 post-infection (n = 6), day 10 (n = 8), day 14 (n = 12) and day 20 (n = 6). Animals were infected with 5,000 larvae.

On the day of experiment, rats were heparinised (500 i.u./100 g), killed by halothane anaesthesia and eviscerated. The abdominal aorta was located and a polythene cannula inserted, distal to the renal arteries, as far as the aortic bufurcation and tied in place. The hind quarters were separated from the rest of the animal and perfused via the cannula with a constant flow (5 ml per minute) of oxygenated Krebs' solution containing 3% Ficoll, an artificial colloid, to reduce oedema formation. The perfusate was of the same composition as that used to maintain the isolated intestinal preparations except that it contained 22.2 mM glucose and was maintained at a temperature of 35°C.

After equilibrating for 15 - 20 minutes, cumulative dose-responses were obtained for phenylephrine. Perfusion pressure was measured, via a T-tube, by a Stratham P23AC pressure transducer connected to a Grass Model 7 Polygraph.

The maximum pressor responses of each preparation were determined, and the individual values in each group converted to a mean. Differences between group means were compared by Student's t-test. In addition, individual log dose-% maximum response curves were constructed, and the pD_2 for phenylephrine found from regression analysis of the straight line part of each curve. The individual pD_2 values in each group were converted to a mean, and differences between group means compared by Student's t-test.

Results

As can be seen from Table 18, no significant changes in the pD_2 for phenylephrine occurred during infection with N. brasiliensis. However, changes in the maximum responses to phenylephrine were evident, as shown in Table 19. In each group there was a fair amount of variation in individual results and this was reflected in the relatively large standard deviations (SD). On days 6 and 10 post-infection, although apparently greater than control, the mean maximum pressor responses to phenylephrine were not significantly so. However, on day 14 the maximum response was 222.7 mmHg. This was a significant increase ($P < 0.01$). By day 20, the maximum response to phenylephrine had fallen to a level not significantly different from control. The increase in maximum response to phenylephrine can be clearly seen in Figure 38.

Summary and Conclusions

In the present experiment the responses of the isolated, perfused rat hind quarters to the sympathominetic amine, phenylephrine were examined during infection with N. brasiliensis. No change occurred in the pD_2 value for this drug, but the maximum pressor response it elicited was increased significantly on day 14 of infection.

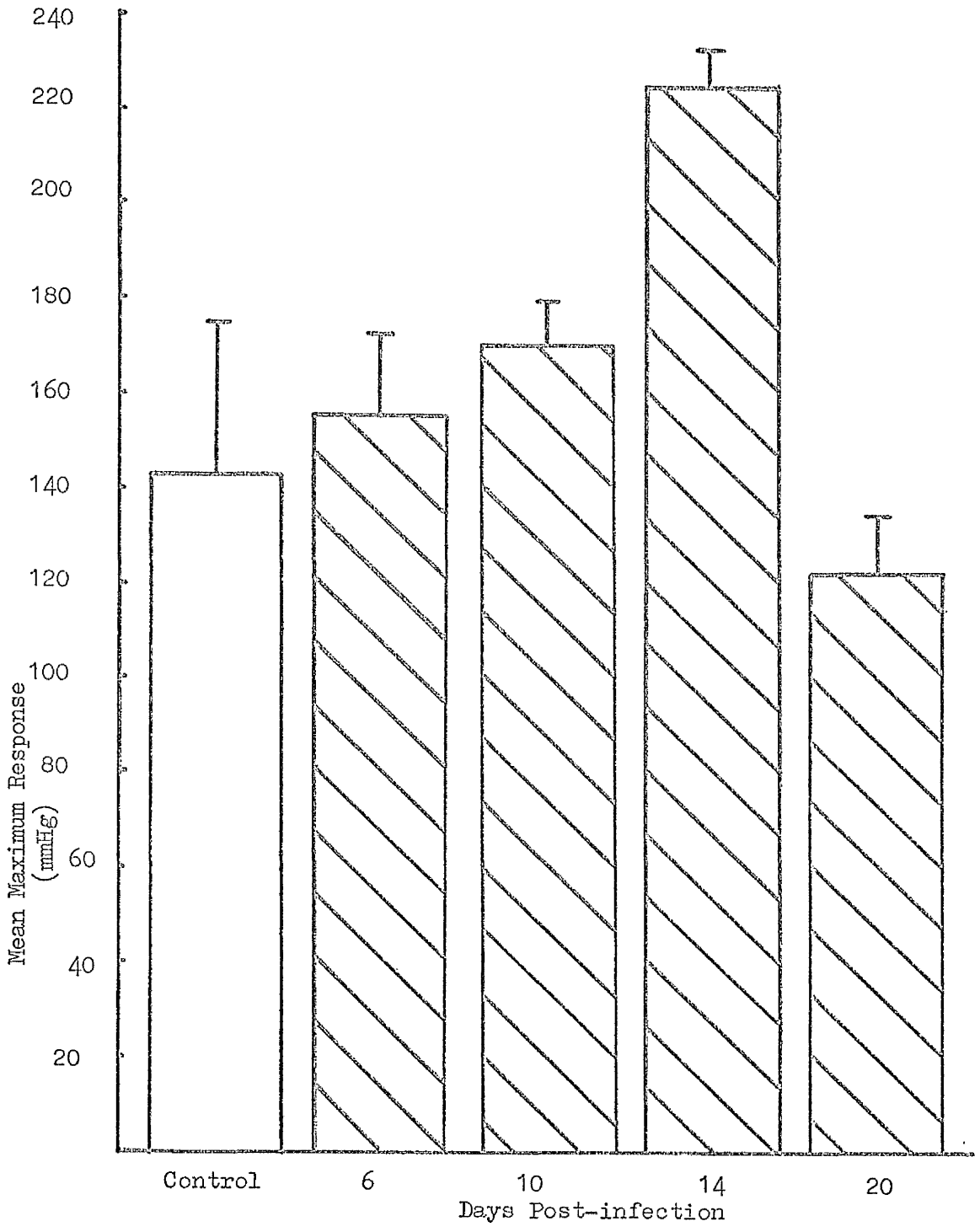
Table 18: Mean pD_2 Values for Phenylephrine of the Pressor Responses of the Perfused Rat Hind Quarters at Various Times Post-infection with *N. brasiliensis*

Days Post-Infection	Mean pD_2	SD	n
Control	4.50	0.11	4
Day 6	4.54	0.07	6
Day 10	4.63	0.12	7
Day 14	4.47	0.07	12
Day 20	4.47	0.13	4

Table 19: The Mean Maximum Pressor Responses of the Perfused Rat Hind Quarters to Phenylephrine. Groups from Infected Rats were compared to Control by Student's t-test.
**, $P < 0.01$; NS, not significant.

Days post-Infection	Mean Maximum Pressor Response (mmHg)	SD	n
Control	143.0	72.1	5
6	154.2 NS	40.8	6
10	169.8 NS	26.4	8
14	222.7 **	27.3	12
20	120.8 NS	28.7	6

Figure 38



Mean maximum pressor responses of the rat hind limbs preparation at various times post-infection with N. brasiliensis. I-bars represent standard errors of the mean.

Thus, the supersensitivity which occurs during Nippostrongylus infection is not confined to the small intestine, but is also observed in the smooth muscle of blood vessels in the perfused hind quarters, and presumably other vasculature in the rat.

This provides further evidence that the increased smooth muscle reactivity observed in the gut is not directly due to the parasites, but to some factor occurring generally in the host which is an indirect consequence of infection. The supersensitivities in both vasculature and gut smooth muscle were similar, in that they were characterised by an increase in the maximum response to agonists, which was not associated with any increase in the pD_2 for those agonists. In both preparations, this increased maximum response reached a peak on day 14 post-infection, around which time the rapid phase of worm expulsion begins. By day 20, when very few worms remain in the gut, the maximum responses of both gut and vascular smooth muscle had decreased towards control levels. This also would seem to suggest that the supersensitivity is indeed associated with some factor stimulated, or inhibited, by the rat's immune response, as opposed to the worms themselves. Similarly, it is improbable that supersensitivity is induced by some secretory product of the worms, since suppression of worm expulsion with betamethasone considerably reduced the maximum responses associated with infection (though it did not cause complete abolition; see Expt. 4).

Again, the question of a possible involvement of alterations in circulating hormones, or some other substance, with the production of smooth muscle hypercontractility arises. Because supersensitivity of this type also occurred in the vascular smooth muscle in the hind quarters during nippostrongylosis, it is conceivable that some aspect of the immune response produces an alteration in the blood levels of some chemical mediator, which in turn induces the development of muscle hypercontractility.

As discussed earlier, both thyroxine and corticosterone have been implicated in the development of a similar type of supersensitivity of the rat anococcygeus muscle (Gardiner et al., 1974; Gibson and Pollock, 1975a). In Experiment 5, inconclusive

evidence for a possible role of thyroxine in infection-associated supersensitivity was obtained, though it seemed unlikely. Similarly, no change in serum corticosterone levels occurred during nippostrongylosis. It is therefore not clear how increased maximum responses are induced in vascular and intestinal smooth muscle during primary infection with N. brasiliensis. A great deal of work is still necessary before the mechanisms responsible for this type of supersensitivity, and its possible relation to worm expulsion are elucidated.

GENERAL DISCUSSION

GENERAL DISCUSSION

A detailed discussion of the results of the individual experiments has already been given in the relevant sections. What is presented here is more in the way of general observations.

A great deal of work has been carried out concerning the rejection of Nippostrongylus brasiliensis from the rat small intestine. While there can be no doubt that the parasite is damaged by a specific immunological reaction on the part of the host, the evidence suggests that a non-specific component is also involved.

Although the immune response which protects the host animal is thought to be highly specific for the parasite species which induces immunity, it is clear that so called 'immune-expulsion' can include other parasites occupying the same environment. For example, Stewart (1955) found that the intake of Haemonchus contortus larvae by sheep will cause 'self-cure' of infestations of other abomasal nematodes such as Ostertagia circumcincta and Trichostrongylus axei. He also reported that the intake of larvae of these two species will bring about self-cure of infestations with H. contortus or with T. colubriformis. Similarly, Louch (1962) found that infection with N. brasiliensis increased the rat's resistance to T. spiralis.

Recently, the mechanisms involved in cross-immunity to nematode parasites have been investigated in the mouse infected with both T. spiralis and Trichuris muris (Bruce and Wakelin, 1977). These workers reported that the expulsion of T. spiralis from the small intestine was accompanied by simultaneous, premature expulsion of T. muris from the caecum and large intestine. This, and the ability of indomethacin administration to prevent the expulsion of both parasite species, led these authors to suggest that the expulsion of T. muris was non-specific, in that it arose from the inflammatory response to T. spiralis infection. This inflammation is thymus-dependent (Walls et al., 1973; Bruce and Wakelin, 1977), and the reduction

of the expulsive effect of T. spiralis upon T. muris in thymectomized mice further suggests that the non-specific expulsion is a cell-mediated inflammatory response (Bruce and Wakelin, 1977). These authors also showed that mice immunized against one or the other parasite species did not show cross-immunity upon heterologous challenge infection. Thus, the interaction did not involve similar antigens in the two species. Indeed, it was earlier concluded by Larsh and Race (1975) that the expulsion of T. spiralis is due purely to cell-mediated inflammation in the gut wall, and that antibodies play no part in the process.

Thus, Bruce and Wakelin (1977) proposed that there are two ways in which immunological interactions between nematodes can occur. There may be a specific cross-immunity, in which antibodies and/or sensitized lymphoid cells produced in response to one species' antigens, have a direct action against the antigens of the other species - direct cross-immunity. On the other hand, cross-immunity may be the indirect result of a specific response to one species in that, an immunologically mediated inflammation acts non-specifically to expel the other species concerned without antigenic similarities being involved. It was further suggested by Ogilvie and Rose (1977), that although the immune response to nematodes must be induced specifically, its action once triggered may be non-specific, and the actual expulsion of the parasites involves the non-specific components of the immune response produced by macrophages, eosinophils or mast-cells, which are triggered subsequent to antigen recognition.

However, as stated several times earlier, the mechanism whereby cells and inflammation bring about the expulsion of antibody-damaged N. brasiliensis worms is unknown. (Indeed, from the experiments described above, where immunity to one species brings about the simultaneous expulsion of another, it appears that antibody damage to the worms, in certain circumstances, may not be necessary for expulsion to occur.) Cell action does not kill the parasites which simply leave the intestine, and if

transferred immediately into the intestine of a non-immune rat, will re-establish and behave like antibody-damaged worms (Love et al., 1975).

Although little doubt exists as to the basis for self-cure in N. brasiliensis infected rats being immunological, it is possible that the non-specific stage of the actual expulsion is more physiological in nature. Stewart (1955) found that when two sheep, hypersensitive to H. contortus, were given a challenge infection, the abomasum showed increased peristalsis and segmentation within 10 minutes of injection of the parasites. After an hour, the abomasum was oedematous and had contracted in diameter. The reaction began to decrease in 1½ - 2 hours and both animals self-cured in under a week. Similarly, it was demonstrated by Castro and his associates, (1976) that intestinal propulsive motility increased significantly during the intestinal phase of T. spiralis infection. As discussed, the specific expulsion of T. spiralis also brought about the non-specific, premature expulsion of T. muris from a different part of the intestine (Bruce and Wakelin, 1977).

This non-specific component was inhibited by treating infected rats with the anti-inflammatory drug, indomethacin. It is possible therefore, that the non-specific inflammatory mechanism which brought about rejection of both parasite species involved altered intestinal motility such as that described by Castro and his fellow workers (1976).

The purpose of the present series of experiments therefore, was to investigate the possibility that changes in gut motility may occur during infection with N. brasiliensis and be involved to some extent in worm expulsion. In Experiment 1, the propulsive motility in vivo of the rat small intestine during nippostrongylosis was measured, and a slight and transient increase occurred only on day 8 of infection. It is difficult to suggest that this slight increase in intestinal propulsion plays a major factor in expulsion, which does not normally commence until about day 10, and it was proposed that it may have been a manifestation of some other motility change which was not propulsive in nature. For

example, increased contractions of the gut longitudinal smooth muscle, which would not be propulsive, may have been stimulated by the high intestinal levels of PGE known to occur on days 7 - 9 of infection (Dineen and Kelly, 1976). Coordinated contractions of the circular muscle layer cause propulsion of intestinal contents. This muscle is relaxed by PGE (Bennett and Fleshler, 1970; Bennett, 1976), and increased levels would consequently, be unlikely to stimulate intestinal propulsion.

The role of increased intestinal PGE levels during N. brasiliensis infection is a matter of much controversy (Kelly and Dineen, 1976; Kassai et al., 1980). As discussed earlier, PG's inhibit several aspects of the immune response (Bourne, 1974), and it is therefore unlikely that their action in accelerating worm loss is due to their effects on immunological responsiveness. Conversely, the suppression of their synthesis and action by anti-inflammatory drugs indicates a role for PG's in inflammation (Willoughby et al., 1973). It has been demonstrated that N. brasiliensis worms in vitro are severely impaired metabolically and structurally by PGE_1 (Richards et al., 1975, 1977). PGE_1 also has many effects on gastro-intestinal function including stimulation of secretion of water and electrolytes into the small intestine (Pierce et al., 1971), and stimulation, in low concentrations, of intestinal longitudinal smooth muscle (Bennett and Fleshler, 1970; Bennett, 1976).

Again the question of the non-specific mechanism which brings about worm expulsion arises, and there are several possible ways in which increased PGE levels in the gut, accompanying inflammation, may cause rejection of one or more species of parasite. As mentioned, N. brasiliensis worms are damaged directly by PGE_1, and it is conceivable that the effects on intestinal secretions may produce an unfavourable environment for the parasites. It therefore seems feasible that these effects, in conjunction with increased contractions of the

longitudinal muscle which could dislodge the worms from the mucosa, could bring about their removal from the gut.

The most striking results in the present experiments were those obtained from the isolated intestinal segments from parasitized rats (see Expts 3 and 4). The amplitude of spontaneous contractions, and the maximum contractile responses of gut to field stimulation and drugs (ACh, 5-HT) increased dramatically during infection, reaching a peak at around Day 14, after which time they decreased towards control levels.

The mechanisms involved in this type of intestinal supersensitivity, and their possible connection with worm expulsion, are unknown. The fact that betamethasone, which inhibits immunity to N. brasiliensis, also markedly reduced the increase in maximum responses, would suggest that the intestinal supersensitivity observed was due to some aspect of the host's immune response, rather than a direct effect of the worms. This is supported by the fact that in Experiment 6, a similar supersensitivity occurred in the vascular smooth muscle of the hind quarters, which is not directly concerned with the parasite or its expulsion. This would also imply that the effect is systemic, rather than a local effect upon the gut. The smooth muscle supersensitivities were similar in gut and vasculature in that both were characterised by an increased maximum response to agonists (ACh and phenylephrine respectively), which was not associated with any shift in the dose-% maximum response curve. (The pD_2 value for 5-HT in the gut was decreased, but this was probably due to desensitization caused by elevated levels of this substance during infection. See Expt. 3.)

In both types of preparation, the increase in muscle reactivity reached a peak on day 14 of infection, after which it decreased. This suggests that the phenomenon may be associated with worm expulsion which is also at its peak at around day 14. However, the factors which produced supersensitivity are unknown. In Experiment 5 it was shown that corticosterone serum levels

are unaltered during nippostrongylosis. It will be remembered that this steroid has been implicated in a similar type of supersensitivity of the rat anococcygeus muscle (Gibson and Pollock, 1975a). Similarly, inconclusive evidence for a possible role of thyroxine in infection-associated supersensitivity was obtained.

Thus, it can be seen that more work will have to be carried out before the relationship of intestinal supersensitivity to the expulsion of N. brasiliensis will be elucidated. It is possible that the increase in gut reactivity may play no part in the expulsion mechanism, but is simply an indirect consequence of infection. Nevertheless, it will be of great value to discover not only the role of supersensitivity in parasitic expulsion, but also to find the mechanism whereby nippostrongylosis produces it. Although generally believed to be unusual (Fleming et al., 1973), this type of supersensitivity has been reported in several tissue preparations following various pretreatments (Lee, 1942; Pollock et al., 1972; Gibson and Pollock, 1975a), as well as in the intestinal and vascular smooth muscle of N. brasiliensis infected rats and it may be that a common mechanism exists.

There are several possible changes which could take place in the gut smooth muscle to cause an increase in the maximum force of contraction. Perhaps the most obvious change is an increase in muscle size. Symons (1975), in a study of pathological changes in the gut during a Nippostrongylus infection, reported the occurrence of hypertrophy and hyperplasia of smooth muscle cells in the small intestine. This could account for the increase in force of spontaneous contractions and increased maximum responses of isolated gut from infected rats. It would be of interest to examine the vascular smooth muscle of infected rats, to see if this tissue had also undergone hypertrophy or hyperplasia. It will be remembered that increased maximum responses to phenylephrine, of the hind quarters, were encountered during infection.

It is strange that betamethasone had no effect on the increased amplitude of spontaneous contractions which occurred during infection. Similarly, this drug did not completely abolish the increased maximum responses to ACh and 5-HT (see Fig. 37). If both were due to some aspect of the immune response, it is possible that betmaethasone did not cause complete immunosuppression.

As well as an increase in muscle size, some other mechanism may have been involved in producing increased maximum responses of intestinal muscle. It is possible for example, that some alteration occurred in the utilization of ions such as Na^+ or Ca^{2+}, which are very important in smooth muscle contraction (Godfraind, 1973). Indeed, Gibson and Pollock (1976) reported a possible involvement of Na^+ ions in the supersensitivity produced in the anococcygeus muscle by chronic treatment with corticosterone. It will be of value to determine whether increased maximum responses of the gut during infection depend on changes in cell membrane permeability to Na^+ or Ca^{2+} and/or the intracellular metabolism of these ions.

The findings which have emerged from the present study have demonstrated that infection with N. brasiliensis brings about dramatic changes in the reactivity not only of intestinal smooth muscle of the host, but also of vascular smooth muscle in other parts of the body. The particular type of change, which is referred to as 'supersensitivity' is of great physiological interest, but it has to be admitted that its occurrence does little to clarify an increasingly complex picture of the mechanisms involved in self-cure of the parasites. In addition, if this type of supersensitivity in vitro of the longitudinal smooth muscle, peaking at around day 14, also occurs in vivo, one can speculate that it could embarrass the worms. For example, greater forces of spontaneous or chemically-induced contractions of the longitudinal smooth muscle could help to dislodge immunologically damaged worms.

However, as with most of the other parameters that have been studied in Nippostrongylus infection - mast-cell numbers, 5-HT and histamine levels, macromolecular leak etc., one is merely obtaining a temporal relationship between a particular measurement and expulsion of the parasites. To establish causal relationships is much more difficult. What does seem clear at present, is that earlier expectations of a relatively simple mechanism for worm rejection were optimistic. It seems likely that a number of non-specific features such as local oedema or alterations in intestinal motility affecting the worms or their position in the gut, may be superimposed on, and arise from a specific immunological reaction involving antibodies and immune cells. The relative importance of specific and non-specific mechanisms may vary greatly between host/parasite systems, and within systems under different conditions.

Thus, in conclusion it can be said that the role of gut motility in the expulsion of N. brasiliensis is as yet not clear and a great deal more work is necessary. As well as its wide use as a model in studying immunity to intestinal nematodes, Nippostrongylus also provides a useful system for the study of an unusual and poorly understood type of smooth muscle supersensitivity.

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