THE UNIVERSITY OF MANITOBA

Experimental concurrent infections with *Trichinella spiralis* (Owen, 1835) Railliet, 1895 (Nematoda) and *Hymenolepis diminuta* (Rudolphi, 1819) Weinland, 1859 (Cestoda) in laboratory rats.

by

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"EXPERIMENTAL CONCURRENT INFECTIONS WITH *TRICHINELLA SPIRALIS* (OWEN, 1835) RAILLIET, 1895 (NEMATODA) AND *HYMENOLEPIS DIMINUTA* (RUDOLPHI, 1819) WEINLAND, 1858 (CESTODA) IN LABORATORY RATS"

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MASTER OF SCIENCE

ABSTRACT

Distributional patterns and recovery rates with Trichinella spiralis at 10, 20, and 30 L/g[1] in male and female rats in single-species infections were similar. Most nematodes were situated in the anterior 25% of the small intestine, numbers progressively decrease in the posterior 75% of the small intestine, and 28.5% of the nematodes were recovered after 5 days infection. In concurrent infections the duration of the intestinal phase was not affected, nematodes were more anterior than in single-species infections, and 44% of the nematodes were recovered after 5 days infection.

Distributional patterns and recovery rates of Hymenolepis diminuta 20 and 30 days after infection in male and female rats in single-species infections were similar.

Scolices occupied the first 60% of the small intestine and strobila extended along the entire small intestine but biomass was located primarily in the mid regions. The recovery of tapeworms was 80%.

The position of tapeworms was more posterior, and dry weight per tapeworm and egg production decreased as the number of nematodes infected increased. Tapeworms

[1] larvae per gram body weight of host.

reached their most posterior position 8 days after infections with 1000 T. spiralis and five and eight days after infections with 4000 T. spiralis. Dry weight per tapeworm was not affected during the intestinal phase of 1000 nematode infections but the number of eggs produced decreased compared to single-species infections. Destrobilated tapeworms were present in the mid regions of the small intestine 8 to 18 days after infection of 4000 T. spiralis larvae. Recovery rates of H. diminuta were not affected during concurrent infections.

A loss of normal villi pattern, aberrant epithelial cells, aberrant glycocalyx, inflammation of the lamina propria, and haemorrhaging was observed to be dependent on the number and location of the nematodes in the small intestine. Chronologically, these alterations were more apparent between the 5th and 8th days after nematode infections. There appears to be a relationship between the degree of pathological damage along the small intestine and the position of the tapeworms. Immunological, pathological and nutritional factors which may affect these two parasites in concurrent infections are discussed.

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INTRODUCTION

Localization of parasites at specific sites in the alimentary tract of vertebrates has been known for a long time (Crompton, 1973). Localizations may be more restricted in some host-parasite systems than in others. Distribution of Hymenolepis diminuta (Rudolphi, 1819) Weinland, 1858 (Cestoda) and factors governing such localization were extensively studied by Hopkins (1970) and Mettrick and Podesta (1974). Distribution of Trichinella spiralis (Owen, 1835) Railliet, 1895 (Nematoda) in the intestine of the rat was examined by Tyzzer and Honeji (1916), and Gursh (1949). Some factors governing the nematode localization in mice were examined by Larsh and Hendricks (1949).

Strong evidence suggests that the behaviour of a parasite in a host is influenced by the presence of another species of parasite. Interspecific interaction in the intestine of the vertebrate host was reviewed by Ulmer (1971), Crompton (1973) and Holmes (1973). These interspecific interactions may be mediated through several mechanisms; directly through mechanical or chemical contact, and space or nutrient competition, and indirectly through the response of the host to parasite infection.

This study was undertaken to examine the interspecific interaction of different nematode population levels, and at different times during the intestinal phase of T. spiralis with established H. diminuta. The objectives of this study were:

1. To examine distribution, numbers and rate of recovery of single-species T. spiralis infections in the rat intestine.
2. To examine distribution, recovery, dry weight, and fecundity of H. diminuta in the rat intestine.
3. To determine if T. spiralis and H. diminuta can co-exist in the rat intestine.
4. To determine the effect of these parasites on each other at five nematode population levels and at selected times during the intestinal phase of both a high and a low nematode population level.
5. To study the effect of host sex on each parasite in both single and concurrent infections.
6. To study and relate intestinal pathology to parasite distribution.

REVIEW OF LITERATURE

Trichinella spiralis Infections

Tyzzer and Honeji (1916) reported *T. spiralis* in the mid and posterior regions of the intestine of rats. Gursh (1949) showed for low and high population levels that most of the nematodes occupied the anterior quarter of the intestine during the intestinal phase of their life cycle. Larsh and Hendricks (1949) reported more nematodes in anterior intestinal regions of 6-month-old rats and more nematodes in the posterior intestinal regions of 1-month-old rats.

Larsh and Hendricks (1949) found that young mice, 28-32 days old, had significantly greater number of nematodes in the posterior half of the small intestine while the reverse was true in old mice, 130-140 days old. Larsh, *et al.* (1952) and Gouldson (1958) reported a shift in worm distribution in mice from an anterior position during the early stages of infection to a posterior one after Day 8 of infection. Campbell (1967) found *T. spiralis* distributed anteriorly in young mice, 21 and 35 days old, and Denham (1965, 1968) reported a posterior position of nematodes and no migration in 56 day old mice. Dick and Silver (unpublished data) found *T. spiralis* in the mid and posterior regions of the small intestine in hamsters; in

anterior regions in white mice, 44 days old, 5 and 12 days after infection and in 30 days old white mice, 5 days after infection; in more posterior regions of Sec J mice than white mice; and in the anterior regions of guinea pigs, 5 days after infection.

Discrepancies in the reported distribution of T. spiralis in different hosts and of different ages makes it difficult to determine which factors affect distribution. Larsh and Hendricks (1949) decreased the intestinal emptying rate of young mice with intraperitoneal injections of morphine sulphate and reversed the distribution of Trichinella in the small intestine. Morphine sulphate also closes the common bile duct sphincter therefore altering the intestinal environment through lack of bile and pancreatic juices. Distribution may also be affected by such factors as size (as distinct from age), diet, intestinal flora, stress, and concurrent infections (Campbell, 1967).

The duration of the intestinal phase of T. spiralis increased as the initial dose level increased (McCoy, 1932; Gursh, 1949; Castro, et al., 1967) and most nematodes were expelled 15 to 21 days after infection.

Hymenolepis diminuta Infections

Distribution: Distribution of H. diminuta in the rat intestine is determined by ontogenetic and diurnal migrations of the tapeworm. Ontogenetic migration is the anterior movement of the tapeworm during its development, and diurnal migration is the daily anterior and posterior shifting of the tapeworm.

Chandler (1939) first recorded that the scolex of H. diminuta moved forward in the intestine during tapeworm growth. This observation was confirmed and amplified by Holmes (1962a), Braten and Hopkins (1969), Turton (1971, 1972), and Cannon and Mettrick (1970). These authors generally agree that newly excysted 5-day-old tapeworms were attached in a region 30-40% along the intestine and during cestode growth their scolices and biomass moved anteriorly to the 10-20% region along the intestine. Anterior migration was influenced by the number of tapeworms in the intestine; migration was faster, the larger the number of worms. They attained their most anterior position in 2 days with a 100 worm infection (Goodchild and Harrison, 1961), 7 days with 10 worms (Cannon and Mettrick, 1970), 14 days with 5 worms (Holmes, 1962a), and 16 to 21 days with one worm (Braten and Hopkins, 1969; Turton, 1971). After

attaining the most anterior position there was a posterior spreading of the scolices distribution (Holmes, 1962a; Mettrick and Dunkley, 1969; Cannon and Mettrick, 1970).

Read and Kilejian (1969) demonstrated that a shift occurred during the day in the amount of worm tissue in different regions of the intestine and this was subsequently confirmed by Hopkins (1969) and Mettrick (1971b, 1972). Tapeworms were more anterior when food was available to the host than when food was not available. Withholding food for long periods of time changed this basic pattern and worms migrated anteriorly after 15 h starvation. Reversal of feeding regime caused a reversal in migratory behaviour which brought it into accord with feeding time (Read and Kilejian, 1969). Changes in the position of the tapeworms may be due either to movement of the scolices (Hopkins, 1969, 1970) or, to shortening and to extension of the proglottids (Bailey, 1971), or to folding and to coiling of the strobilae (Turton, 1971).

Chappell, et al. (1970) found three distinct circadian migration patterns in 30-worm infections when tapeworm age was constant throughout the day. Tapeworms 5- to 7-days old moved anteriorly during host fasting, worms 7- to 8-days old did not migrate, and worms 8- to 14-days old moved

anteriorly during host feeding. Tanaka and MacInnis (1975) showed that the apparent reversal of migration reported by Chappell, et al. (1970) in young worms did not occur when worms progressively older through the day were used. These authors suggested that the reversal of migration reported by Chappell, et al. (1970) could be due to variation in the rate of worm growth between hosts infected at different times of the day. H. diminuta growth is exponential during early development (Roberts, 1961) and growth may vary depending upon the physiological state of the host, therefore, a small change in growth rate because worms were infected at different times of the day can cause significant changes in size.

Migrational stimuli: Goodchild (1958a) reported that surgically transplanted tapeworms migrated in recipient rats to the same region of the intestine which they had occupied in donor rats. Braten and Hopkins (1969) confirmed these results and showed that migration was completed in 24 h. Tapeworms when placed in the ileum were smaller than tapeworms placed in the duodenum or jejunum. This suggests that tapeworms actively select a specific region of the intestine and that the posterior intestine provides poor conditions for growth. Crompton and Whitfield (1968) suggested that tapeworms monitor their environment through

stimuli input into the scolex and neck region, and into the strobila region of the tapeworm. Migration is an attempt to keep the greater part of their surface areas within the optimum region.

Goodchild (1958b, 1960) observed that worms were more posteriorly situated and smaller in bileless rats than those in normal rats. He suggested that a change in intestinal position was due to changes of the stimuli input into the shorter worms. However, Braten and Hopkins (1969) found that transplanted worms were not influenced by lack of bile. Suppression of peristalsis by intraperitoneal injection of opium "(anhydrous morphine)" hindered worm migration, but the effect of opium on the intestine and the tapeworm is unknown.

Cannon and Mettrick (1970) showed that the tapeworm biomass moved anteriorly during the early developmental stages and posteriorly during the later stages. These authors suggested that tapeworm selection of different intestinal regions reflected changes in intestinal nutrient or physiochemical gradients or reflected changing metabolic requirements of the tapeworms. They also suggested that long term migration during prepatent development is related, but distinct, from the daily-migrational movements.

Circadian migration may be related to the host feeding pattern (Read and Kilejian, 1969; Hopkins, 1969) or changes in the physiological state of the intestine due to food intake. Migration may result from tapeworms either

actively selecting an optimal site during their absorptive stage, then drifting passively with peristalsis during the post-absorptive stage (Hopkins, 1970), or actively selecting an optimal site which shifts with the host feeding cycle (Chappell, et al., 1970).

Anterior movement of tapeworms during starvation (Read and Kilejian, 1969) and suppression of the posteriad phase of diurnal migration in rats fed suboptimal diets (Chappell, et al., 1970) both support an active selection of anterior or posterior sites by the tapeworm. If the posteriad movement of worms was passive then at times of low energy availability it would be expected that the anteriad movement would be suppressed, and as this is not the case, an active selection of sites is more probable (Holmes, 1973).

Extensive studies (reviewed by Mettrick and Podesta, 1974) have examined the rat - H. diminuta parasite system with respect to host diet and subsequent glucose, lipid, protein, pH, 0_2, and $C0_2$ gradients; microbial flora and 5-hydroxytryptamine levels, as well as the movement and chemical composition of the tapeworms. Mettrick (1971a, b, c; 1972) showed that the quality of the food was not an important factor and that subsequent gradient changes were neither the cause of, nor the result of the migrational movements of the tapeworms. He suggested that the general physiochemical conditions of the intestinal environment

following food intake may produce or result in the circadian rhythm exhibited by the tapeworms, rather than particular gradients along the intestine.

Concurrent Infections

Concurrent infections can result in competitive exclusion, interactive site segregation and selective site segregation (Holmes, 1973).

Competitive exclusion reduces the numbers of parasites. The interaction in some cases may be mediated through the response of the host to one or both of the parasites. Frequently a tissue response, such as inflammation, is involved.

Cross (1934) showed an inverse relationship between the numbers of the cestode Proteocephalus exiguus (La Rue) and the numbers of the acanthocephalan species "Neoicanthorinchus" in fishes. Since the cestodes attached in the gastric caeca and the acanthocephalans attached in the middle of the intestine, Cross (1934) suggested the inverse numbers relationship was the result of a non-specific immunity and not the result of crowding. Both Larsh and Donaldson (1944) and Larsh and Campbell (1952) showed that infections involving H. nana (Siebold, 1853) and either Nippostrongylus brasiliensis (Travassos, 1914), or T. spiralis resulted in decreased numbers of the cestode, and Louch (1962) showed increased resistance to T. spiralis with prior N. muris (=N. brasiliensis) infections. Heyneman (1962, 1963) reported that cross-immunity may be implicated in the reduced reproduction potential of H. diminuta in rats with previous or concurrent infections of H. nana. Previous infections of H. diminuta did not affect H. nana while previous infections with H. citelli (McLeod, 1933) and H. microstoma (Daj., 1845) resulted in increased resistance (Weinman, 1964). Prior infections of T. spiralis in mice (Cox, 1952; Gouldson, 1958) and in

rats (Weinman, 1964) were detrimental to H. nana infections but they showed a dependence on the length of time that the host was infected with the nematode. The dependence on length of infections was also shown in other studies. Cox (1952) showed that prior 2-day oral infections or 10- and 20-day subcutaneous infections with Ancylostoma caninum (Ercolani, 1859) decreased the numbers of T. spiralis recovered. It was also noted that the effect on the recovery rate decreased if prior oral infection was longer than 2 days. Similarly, Gouldson (1958) reported that increased resistance to T. spiralis occurred 24 to 48 h after an A. caninum infection, but no increase in resistance if the previous infection was less than or greater than 24-48 h. Prior infections of 5 or 20 days with T. spiralis had little or no effect on the depression of Aspiculuris tetraptera (Nitzsch, 1821) while a 12 day prior infection showed a decrease in aspiculurid burden (Stahl, 1966).

The sequence of infection is important in determining which parasite is successful in establishment in the host. Keeling (1961) showed that the first species of either Trichuris muris (Shrank, 1788), A. tetraptera or Syphacia obvelata (Reed, 1802) given to a host were successful and susceptibility to T. muris increased after removal of the other parasites. Stahl (1966) reported that A. tetraptera numbers decreased by 50% when infected after S. obvelata but numbers decreased by only 10% when infected before S. obvelata. Hendrix, et al, (1975)

showed that prior or simultaneous infections of N. brasiliensis caused stunting and a more posterior position of H. diminuta and the effect on the tapeworm was more severe at higher nematode population levels. If H. diminuta was allowed to establish before infection with the nematode, then there was no effect on position and weight of the tapeworms. Similar findings for H. diminuta and T. spiralis concurrent infections were found in hamsters (Dick and Silver, unpublished data).

Increased resistance to secondarily infected species when the first species was capable of producing a host immune response and the length of time after first species infection or the sequence of infection are salient features implicating a host mediated, interspecific competitive exclusion. Cross-immunity is a probable interspecific reaction but with unrelated parasites a non-specific immune response is more probable (Schad, 1966). The importance of timing of secondary infections suggests the need to understand parasite life cycles. For instance,the decline in A. tetraptera recovery only if infected 12 days after T. spiralis infection coincides with intense changes to the intestinal environment (Castro and Olson, 1967) and chronic tissue cellular reaction (Larsh and Race, 1975). The production of larvae at this time and the longer adult association with the mice may have provided the necessary stimulation to account for the increased resistance.

Competitive exclusion was found in naturally infected populations. John (1926) showed that cestode species in small intestines of 358 rabbits had little spatial overlap but no concurrent infections occurred. Similar findings were reported in trematode species infections in 593 moles (Frankland, 1959). However, a complex helminth community composed of 10 parasite species in the small intestine of lesser scaup (Aytha affinis) was reported by Hare and Holmes (1973). These authors suggested helminth distributions and populations were determined by specific microhabitat selection and interspecific competition.

The host immune response may suppress superior competitors, prevent them from monopolizing the available space, and increase the parasite diversity of that habitat. Carp which have recovered from an infection with Dactylogyrus vastator Nybelin, 1924 regenerate normal gill tissue and allow invasion by D. extensus (Mueller and Van Cleave, 1932) and D. anchoratus. The specific immune response prevented reinfections by D. vastator the adaptively superior competitor (Paperna, 1964). It seems then that immunity against parasites can regulate their populations (Ractliffe, et al., 1969).

In concurrent infections parasites occur in a more confined area than they would normally occupy and the change in localization segregates them from other parasites. Riley and Owen (1975) studied the distribution of two closely related cestodes in the bird Tulmaris glacialis (L.). Tetrabothrius micro (Lonnberg, 1893) and T. procercus (Spatlich, 1909) favoured different but overlapping areas of the intestine. In concurrent infections T. procercus is posteriorly displaced. Chappell (1969) found that

both the cestode Proteocephalus fillicollis (Reid, 1819) and an immature acanthocephalan Neoechinorhynchus rutili (Muller, 1780) showed restricted distribution in concurrent infections, and this resulted in partial spatial segregation. A similar interaction exists between H. diminuta and Moniliformis dubius (Meyer, 1933) in rats. In concurrent infections the distribution of both species was reduced to part of their normal range in the small intestine. Both worms were reduced in size, and concurrent infections interfered with the tapeworm ontogenic migration (Holmes, 1961, 1962a). Concurrent infections in hamsters reveal no adverse effects on either parasite (Holmes, 1962b) suggesting that the host as well as the parasite was important in parasite interactions.

When interacting parasites commonly co-exist in the same host, one would expect interactive segregation to be replaced by genetically based selective segregation (Holmes, 1973). The distribution of two blood flukes Aporocotyle macfarlani (Smith, 1967) and Psextarium sebastodorum (Goto and Ozahi, 1930) are basically different in single-species infections though they occupy the same general areas of the heart valve (Holmes, 1971). Wertheim (1970) showed that Strongyloides ratti (Sandground, 1925) and S. venezuelensis (Brumpt, 1934) differed only slightly in their distribution along the intestine of rats in concurrent infections, but the former was limited to the crypt regions and the latter

to the villi, close to the surface of the mucosa. The two co-exist without interfering with each other. Similarly, Schad (1963) reported radial and longitudinal distributional differences in ten species of Tachygonetria (Oxyuroidea) in the colon of the European tortoise.

In some concurrent infections, the infectivity and fecundity of one of the species may be enhanced. Liu and Ivery (1961) demonstrated prior infections of A.caninum resulted in decreased take but increased egg production per female of Nematospiroides dubius Baylis, 1926. Colwell and Wescott (1973) reported N. brasiliensis increased in numbers and fecundity when concurrently infected with N. dubius. Weinman (1964) reported increased H. nana viability occurred in mice previously infected with Ascaris lumbricoides Linnaeus, 1758. Courtney and Forrester (1973) showed that greater weight yields of H. microstoma were recovered from mice concurrently infected with Heligmosomoides polygyrum (Duj., 1845) than from mice infected with the tapeworm only.

Immunity and Pathology of Infections

Nematode infections: Considerable information is available on the intestinal pathology caused by the mucosal dwelling nematodes, T. spiralis and N. brasiliensis. Infected animals do not gain weight at a normal rate

which may be a result of reduced food and water intake (Castro and Gentner, 1972) and intestinal malabsorption (Castro, et al., 1967).

The small intestines of mice infected with T. spiralis had shorter villi and deeper crypt regions as early as the first day after infection (Day 1) (Richardson and Olson, 1974). On Day 4 shorter villi had fused bases in low nematode populations and changes were more evident in high population levels in guinea pigs (Castro, et al., 1967) and in mice (Richardson and Olson, 1974). The severest changes in intestinal morphology occurred between Day 7 and Day 14. Villi were short, not extending above the crypt regions, blood and fluid accumulated in the lamina propria, and cuboidal epithelial cells had reduced cytoplasm (Castro and Olson, 1967). The severity of damage depends on the nematode population level. Richardson and Olson (1974) demonstrated severe shortening and fused bases of the villi in infections of 42 and 100 L/g and villi were shortened and blunted at doses of 150 L/g. By Day 21 the villi were shorter and wider than normal but not to the extent of those on previous days and by Day 28 the villi appeared normal (Castro, et al., 1967).

During the first 6 days after N. brasiliensis infection cell production and villi length increased (Symons, 1965).

Symons and Fairbairn (1963) reported villi with normal configuration (protruded into lumen with aligned columnar epithelium) and with normal brush borders. There was blood and fluid accumulation in the lamina propria, enlarged jejunum (due to formation and growth of new cells) and enlarged muscularis externa (due to cell enlargement).

On Day 9 and Day 10 in moderate infections the villi were short, wide, irregularly shaped and apparently fused. An increase in the number of cells in the lamina propria occurred and the brush border appeared normal. In severe infections the villi disappear, crypts were abnormally deep, intervillus ridges hypertrophied, cuboidal epithelial cells in evidence and the brush border thin (Symons and Fairbairn, 1963; Loehry and Creamer, 1969). The flattened appearance of the mucosa may be due to increased cell loss, increased cell production and hypertrophied intervillus ridges (Loehry and Creamer, 1969). Symons (1976) reported that short villi had rough and irregular margins. The ends of the microvilli were of a greater diameter and of uneven height when compared to normal microvilli. On Day 21 villi had a normal configuration, intact brush border, enlarged jejunum, edema and hyperplasia (increased volume due to formation and growth of new cells), and muscularis externa remained grossly hypertrophied.

Hymenolepis diminuta infections: Immunity to H. diminuta has only recently been studied. Pathology due to this tapeworm has not been shown to be related to the host immune response, as in nematode infections, but is related to the release of metabolic end products such as lactic acid and changes in the intestinal environment (Mettrick 1971a, b, c, 1972; Podesta and Mettrick, 1974).

Hopkins, et al. (1972a) showed that H. diminuta can establish in mice. Destrobilation and lack of growth occurred after Day 9 and secondary infections resulted in smaller recovery rates and severely stunted worms. Mice given immunosuppressants allowed worms to mature by Days 16 to 18, as they would in a rat. Cortisone injections not only prevented destrobilation, but also enhanced growth. They suggested that an immune response was elicited by antigens which may be absorbed from the lumen in the form of organism antigens and dietary proteins. Befus and Featherston (1974) reported that old mice, 5-7 weeks old rejected H. diminuta faster than young mice, 2-4 weeks old. They attributed delayed rejection of the tapeworm in younger mice to the immature state of gut-associated lymphoid tissue and differences in the physiological state of the intestinal environments of young mice as compared to older mice. Andreassen, et al. (1974) showed that in infections with 100 cysticercoides in "specific pathogen-free" rats there was destrobilation and expulsion of primary worms,

suppression of this rejection by cortisone, and increased host resistance in secondary infections. Befus (1975) reported an immunoglobulin covering on H. diminuta in mice and suggested that this resulted in depression of the digestive-absorptive functions of the tegument. Befus (1975) showed that mice with six worm infections rejected primary infections faster than one worm infections and suggested the larger worm population stimulated the host immune response to a greater degree. An increase in host immune response to larger infections was shown by Befus and Threadgold (1975) in mice and rats. Changes in the tegument of the tapeworm represented as opaque or darkened areas (DA) are thought to be sites of worm pathology induced by the host immunity. In the more immunogenic infections with six cysticercoids there were more DA per worm than in infections with one cysticercoid.

If this is the case then multiple infections would potentiate stunting of tapeworms via the host immune response. One must be careful in comparing these results from mice (an abnormal host) and SPF-rats to non-SPF rats. Roberts and Mong (1968) showed that after 60 days of initial infection with 50 cysticercoids in a non-SPF host (Holtzman rats) approximately 95% of the tapeworms were recovered while Hesselberg and Andreassen (1975) reported that after 56 days of initial infection with 50 cysticercoid in a SPF-host

24% of the tapeworms were recovered. Apparently there may be a different host-parasite relationship between these two groups of hosts.

Bland (1976) examined the immune response mechanism. He showed that congenitally athymic nude mice retained H. diminuta infections longer than normal thymic mice. This showed a thymus-dependent host immune response but it was not established if the mechanism of response is humoral or cellular.

MATERIALS AND METHODS

Experimental Animals

Sprague-Dawley strain rats (Bio-breeding Co., Ottawa), with males weighing initially 159 ± 24* g and females 143 ± 17 g were used throughout the experiments. Males and females were housed separately, five to a cage and maintained at 22°C on a 15h light, 9h dark photoperiod. in animal holding facilities. Purina Laboratory Chow was fed ad libitum and water was always available. Rats were allowed at least three rest days after arrival. Ears were notched to identify individual animals in each cage.

Collection and Inoculation of Parasites

Trichinella spiralis collection: Maintenance rats were infected by stomach tube with 4500 larvae each, 4-8 weeks prior to infection of experimental animals. Rats were killed by a sharp blow on the head followed by cervical dislocation (per: Appendix 2 - Canadian Council on Animal Care) and the tongue or diaphragm examined for cysts. After positive identification of infection, rats were skinned, eviscerated and ground in a meat grinder. Larvae were excysted from the muscle by

* ± one standard error.

incubation of 16 g of meat per 25 ml of 1% pepsin (1:10,000 Sigma Chemical Co.) and 1% concentrated HCl at 37°C for 45 to 60 minutes. The digest was filtered through four layers of cheesecloth in a Baerman funnel with lights placed over the funnels to maintain warmth. Larvae were collected after 20 to 40 minutes in test tubes by taking 15 to 25 ml from the bottom of the funnels.

Hymenolepis diminuta collection: Eggs were collected from the feces of two maintenance rats infected 10 months previous to the experiments. Flour beetles, Tenebrio molitor (Linnaeus, 1758), were infected by feeding one or two proglottids at least 28 days prior to infection of experimental animals. Infected beetles were dissected and cysticercoids placed in tap water. Whenever possible larvae from one beetle were used, otherwise the larvae obtained from several beetles were mixed and used.

Inoculation with T. spiralis: Nematodes were suspended in a 0.16% agar solution and diluted to the correct concentration after collection from the funnels. Larvae were counted by taking two - 0.1 ml samples and averaging the counts if they were within 10% of each other. If the counts were not similar, the

nematode suspension was reshaken and the procedure repeated. A standard suspension volume of 0.5 or 0.6 ml was given via stomach tube to weighed, ether (Fisher Scientific Co.) anaesthetized rats. Care was taken not to force the suspension into the lungs.

Inoculation with H. diminuta: Weighed, ether anaesthetized rats were inoculated with five (5) cysticercoids via stomach tube. To ensure rats received all the larvae, the stomach tube was washed with 0.4 ml of tap water while it was still in the oesophagus and a new stomach tube used for each animal.

Necropsy Procedure

Experimental animals were killed and weighed to the nearest gram. An incision from the anus to the diaphragm was made and a 1:10,000 solution of warm adrenalin (L-Epinephrine) (Sigma Chemical Co.) poured over the intestinal area to stop peristaltic movement. The small intestine was cut at the pyloric sphincter and at the ileo-caecal junction and laid out on a waxed board marked into 20 sections by a biased grid (Brambell, 1965). The grid allowed cutting of the intestine into 20 equal parts so that equivalent regions of the intestine could be compared.

Counts and Weights of Parasites

Trichinella spiralis counts: Individual regions of the intestine were placed in 3 dram vials containing 10 ml of 0.85% saline and kept refrigerated until examined. Intestinal sections were examined within 24 h after necropsy. Each section was cut open in a petri dish, which was divided into eight triangular areas, and the mucosal layer was scraped off. The contents of the dish were mixed and the nematodes counted under a dissecting microscope. Preliminary experiments showed that counting of one quarter of the area gave an accurate count for calculating the total numbers. This method was used throughout the experiment.

Hymenolepis diminuta counts and weights: Prior to examination, the intestinal sections were handled in the same way as in the nematode counts. Contents of each intestinal section were squeezed out onto a petri dish and the number of scolices counted and recorded. Portions of the worms found in each region were placed on preweighed glass slides and dried for 35 hrs at 70°C. Slides were removed from the incubator and allowed to cool before weighing on a Sartorius 2400 balance.

Counts and Weights of Parasites from Concurrent Infections: Intestinal regions were handled as described. Sections were cut open and if tapeworms were not present the contents were examined for nematodes. If portions of tapeworms were present, they were removed from the counting dish, placed on slides, and then the contents of the dish examined for nematodes.

Fecal Collection and Egg Counts

Feces were collected from each group of five rats over the 24 h period prior to necropsy, placed in plastic bags and frozen until examination. The method for counting eggs was adapted from Beck (1951) and Goodchild(1960) and was performed as follows: Feces were removed from the freezer and weighed. A five gram sample was placed in a waring blender for two minutes and 350 mls water added. Five gram samples were used because the total fecal matter collected over a 24 h period was too large to allow accurate counting. The blended mixture was transferred to a 500 ml Erlenmeyer flask and 150 ml of water added. While the mixture was stirring, one 0.4 ml sample was removed and the number of eggs counted.

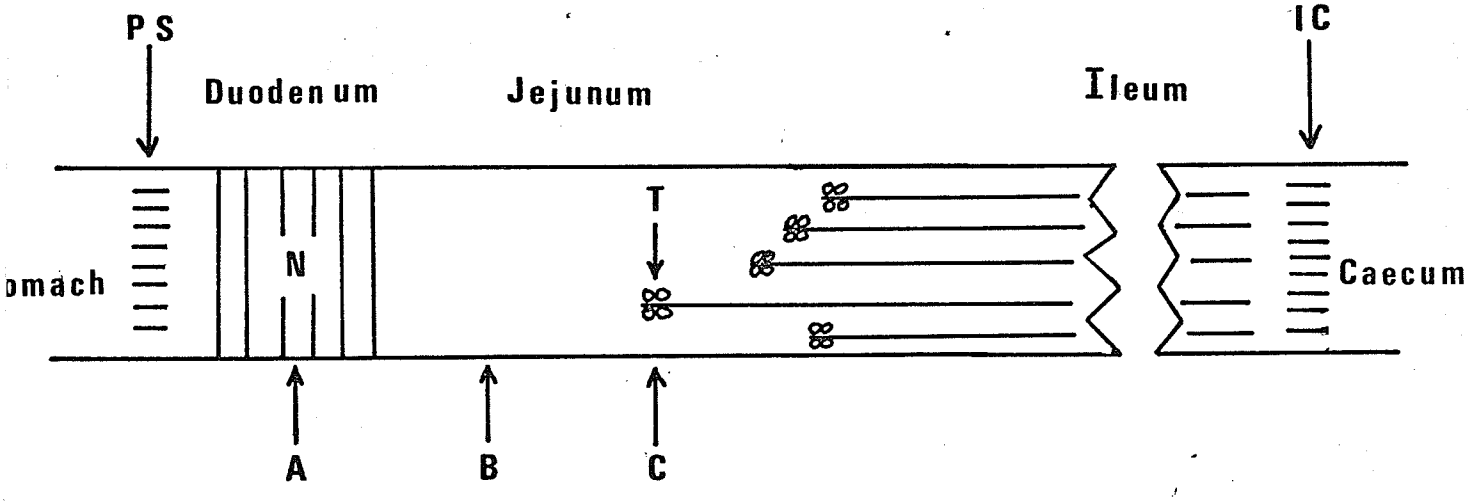
Histology

Intestinal tissue samples were taken from three regions of the intestine (Fig. 1). The sections were taken to examine the intestinal conditions in the regions occupied by the nematodes and the tapeworm scolices.

Samples taken from the intestine were immediately fixed in cold 5% gluteraldehyde (pH 7.2-7.4) for 2.5 to 4 h and washed overnight in 1.5% Sorensen's buffer (pH 7.2-7.4). Following a 70-100% ethanol dehydration series the tissue blocks were embedded in methacrylate (Polyscience Ltd.). Methacrylate solution A and 1% organic peroxidase was made fresh for each embedding experiment. Infiltration of tissues for 30 minutes in a 50:50 mixture of solution A and 100% ethanol was followed by five hours in solution A. The tissue samples were placed in embedding capsules (Micron) with a 20:1 mixture of solution A and solution B and capped with paraffin to prevent oxidation and aid polymerization.

Tissue sections of 1.5 µm thickness were cut on a Sorvall J.B.4 ultramicrotome with glass knives made on a LKB knife breaker, and then placed in water and ammonium hydroxide (two drops of ammonium hydroxide:100 ml of water) and dried onto a glass slide.

Figure 1. Regions of the intestine from which tissue samples were taken. Abbreviations: A - anterior tissue sample; B - middle tissue sample; C - posterior tissue sample; IC - ileo-caecal junction; N - nematode location; PS - pyloric sphincter; T - most anterior tapeworm scolex.


P S
Duodenum
Jejunum
Ileum
I C
omach
N
T
Caecum
A
B
C

Tissue sections were stained with laboratory prepared Giemsa (Gurr, lot number 13900), for 5-10 minutes at 55°C (Humason, 1972) or placed in periodic acid (G. Fredrick Smith Chemical Co.) and 90% ethyl alcohol for two hours and then in Schiff's Reagent (Fisher Scientific Co., lot number 78077) for one hour (Humason, 1972). Slides stained with periodic acid-Schiff (PAS) were washed overnight in 1% saline and 2% acetic acid. This was required because fixation was carried out using gluteraldehyde. The procedure oxidizes the Schiff-positive groups of the aldehydes to carboxyl groups, thus rendering them inactive to the stain.

Photomicrographs of Giemsa and PAS stained tissue sections were taken with a Zeiss Photomicroscope II using bright field illumination and Nomarski differential interference contrast (DIC). Kodak Panatomic X film was developed in Microdol X (Kodak).

Experimental Procedure

Each experiment consisted of one or more groups of five male and of five female rats in each group. The ages within each group were equivalent but varied between groups due to the length of the experiment. Inoculation and necropsies were done between 1130 h and 1330 h to

prevent changes in tapeworm position due to either ontogenetic or circadian migrations (see Review of Literature, pp. 5-7). Changes in position of the tapeworms would, therefore, be related to causes other than their natural movements.

Unless otherwise stated, T. spiralis dose levels were based on host weight (i.e., L/g) as it was not known if host weight affected nematode distribution. Comparison of T. spiralis distribution for dose level (10 L/g, 20 L/g, 30 L/g) was investigated 5 days after infection at the time of constant nematode number (Gursh, 1949). By 5 days after infection the nematodes are well established and have reached sexual maturity in the small intestine (Gursh, 1949).

Cannon and Mettrick (1970) showed that in infections of 10 tapeworms, scolices were bimodally distributed and Roberts (1961) showed that maximum weight of tapeworm populations was obtained in infections of five or more tapeworms. In order to ensure changes were related to causes other than apparent intraspecific competition and also to maximize the use of the rat intestinal environment by tapeworm populations, five tapeworm infections have been used in the experiments.

The prepatent period in H. diminuta infections was given by Hager (in Roberts, 1961) as 17 to 24 days, Beck (1951) as 18 to 20 days, Roberts (1961) as 16 to 17 days,

and Braten and Hopkins (1969) as 16 to 17 days. According to Braten and Hopkins (1969) for one worm infections and to Holmes (1962a) for five worm infections, maximum weight was reached 18 to 21 days after infection. Since H. diminuta weight varied little after the prepatent period it was decided to examine tapeworm distribution after 20 days of infection.

Statistical analysis

Analysis of variance was used to compare sample midpoints. The midpoint of a distribution is that point in the intestine at which the same amount or number of parasites are anterior and posterior to it. The midpoint was found by the formula $\left[\left(\sum_{i=1}^{20} (i)(X_i) \Big/ \sum_{i=1}^{20} X_i\right)\right] - 0.5 \quad X\ 5$ where X_i is the quantity found in the i^{th} region. Raw data were stored in the APL/360 computer system and analyses were done with the aid of the computer. Data were considered significantly different if the means were different at the 5% level of significance.

RESULTS

Experiment 1

Examination of T. spiralis in Single-Species Infections

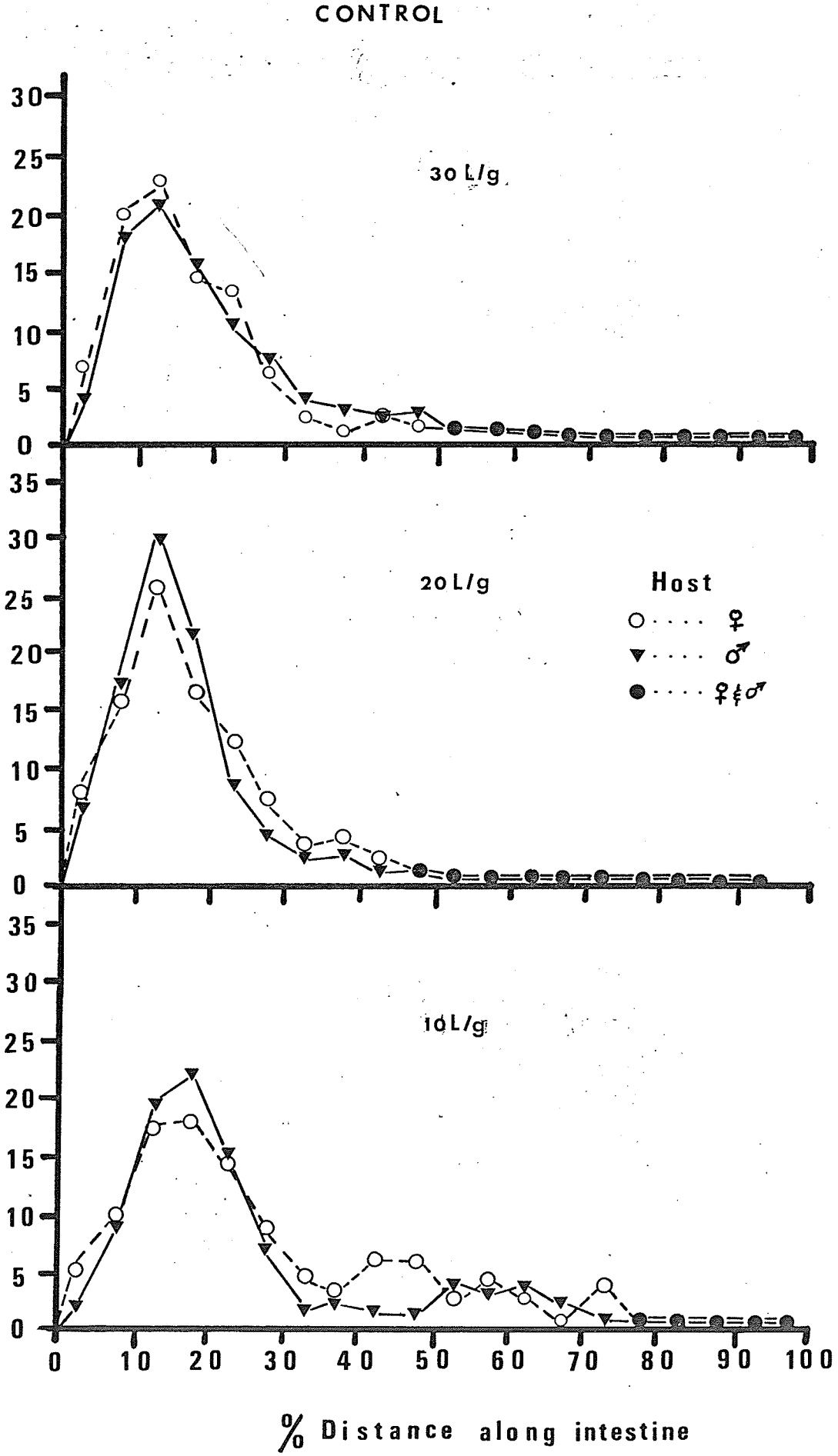
Distribution and recovery rate in male and female rats were determined for three different dose levels of T. spiralis. Discrepancies in the literature concerning single-species distributions of this worm necessitated this experiment.

Three groups of five male and five female rats were given 10, 20 and 30 L/g and examined 5 days after infection. Results are shown in Fig. 2 and Table I, and statistical analysis is shown in Appendix 1, Table I.

Nematodes were situated anteriorly. Most nematodes were located in the first quarter of the intestine, with an average location of the midpoint of the nematode numbers (nematode midpoint) of 21.4 $\pm$ 1.6% along the intestine. The nematode midpoints were not significantly different between male and female rats but a significant difference was noted between dose levels. These differences did not show any trends. The average recovery rate of nematodes 5 days after infection was 28.5%.

The weights of the rats at the different dose levels are shown in Appendix 2, Fig. 1. As the number of nematodes increased, the weight of the rats after 5 days infection decreased.

Figure 2. Distribution of T. spiralis in single-species infections.


CONTROL
30 L/g
20 L/g
10 L/g
Host
♀
♂
♀♂
0
5
10
15
20
25
30
35
0 10 20 30 40 50 60 70 80 90 100
% Distance along intestine

ralis

Table I. Number of T. spiralis inoculated, number recovered, % recovery and nematode midpoints of T. spiralis in single-species infections at dose levels of 10, 20 and 30 L/g.

	Dose Level					
	10 L/g		20 L/g		30 L/g	
Host Sex	♂	♀	♂	♀	♂	♀
# of rats	4	5	5	5	5	5
# T. spiralis inoculated	1500	1450	3000	3000	4800	4500
Average # recovered	410 ±87*	300 ±58	1260 ±88	880 ±92	1180 ±52	1191 ±87
Recovery (%)	27.0	20.7	42.0	27.3	24.6	27.0
Nematode Midpt. (% distance)	26.5 ±3.5*	24.1 ±1.5	17.9 ±1.0	17.4 ±1.1	20.9 ±1.5	22.7 ±1.1

* one standard error.

Experiment 2

Examination of *H. diminuta* in Single-Species Infections

This experiment was designed to reveal single-species distribution, recovery rates and fecundity of *H. diminuta* in male and female rats at standard inoculum of five cysticercoids, 20 and 30 days after infection. Twenty day old tapeworm infections were studied because in Experiment 4 the effect of nematode dose on similarly aged tapeworms is examined. Thirty day old tapeworm infections were studied because in Experiments 5 and 6 the effect of concurrent infection during the intestinal phase of *T. spiralis* is examined and in these experiments worm ages would be between 20 and 35 days old. It was shown in the literature that tapeworm weight increased until the 20th day and then declined slightly (Holmes, 1962a). It was important to evaluate the above parameters with respect to age of tapeworm to ensure that any changes would be related to experimental design rather than aging of the tapeworm.

Twenty day infections: Five groups of five male and five female rats were examined 20 days after infection and results are shown in Fig. 3 and in Table II.

Figure 3. Distribution of H. diminuta in single-species infections.
Histograms represent distribution of scolices, and line graphs represent distribution of biomass.

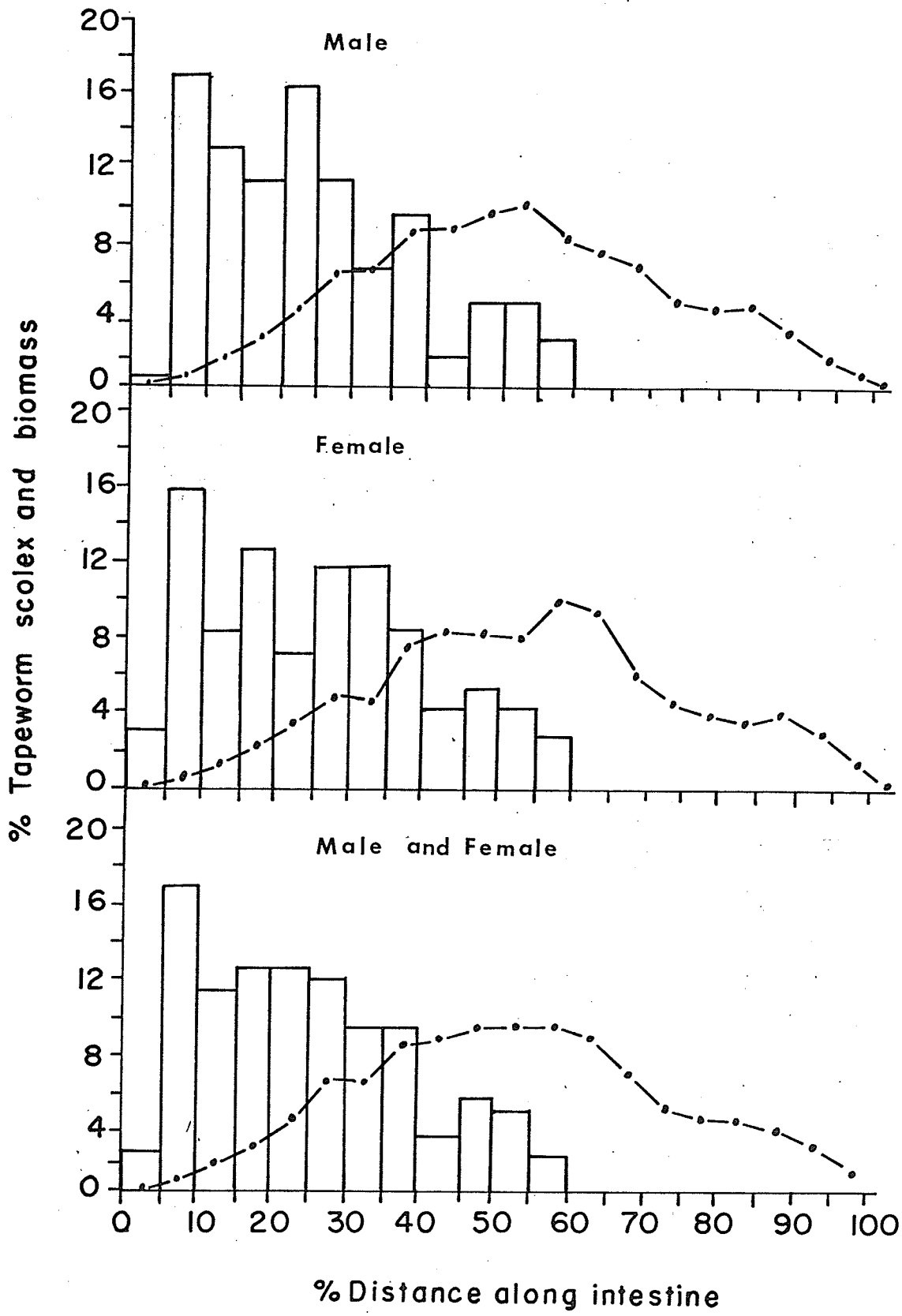

Male
Female
Male and Female
% Tapeworm scolex and biomass
% Distance along intestine
0
4
8
12
16
20
0
10
20
30
40
50
60
70
80
90
100

Table II. Percent recovery, scolex and biomass midpoint location, dry weight per tapeworm and fecundity of H. diminuta in 20 and 30 day infections.

	20 day old worms										30 day old worms	
	1		2		3		4		5			
Host Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
# of rats	5	4	5	5	5	5	5	3	5	5	3	5
Recovery (%)	100	80	80	80	96	96	65	47	72	72	80	84
Scolex midpt. (% distance)	31.8 ±2.4*	27.4 ±5.7	21.8 ±3.9	29.4 ±3.1	20.7 ±2.3	25.5 ±2.8	24.5 ±3.4	26.9 ±3.1	30.4 ±0.8	31.5 ±3.2	17.6 ±5.1	29.1 ±4.2
Biomass midpt. (% distance)	64.0 ±3.4*	59.2 ±1.9	55.5 ±2.3	58.5 ±2.5	52.4 ±1.0	57.9 ±3.9	48.8 ±1.8	47.8 ±5.6	52.6 ±2.6	56.6 ±2.8	52.7 ±9.6	58.0 ±5.7
Dry weight (mg/worm)	60.4 ±3.4*	173.5 ±4.4	242.0 ±17.8	198.2 ±12.7	216.4 ±10.3	188.3 ±3.8	204.3 ±35.1	105.0 ±60.6	175.8 ±13.3	216.6 ±21.9	120.2 ±14.4	140.5 ±4.4
Fecundity (10^3 eggs/24h)	-	-	24	33	60	74	78	78	-	-	54	90

* one standard error.

Tapeworm scolices occurred in the first 60% of the intestine. The average location of the scolex midpoints of tapeworms from male and female rats was 22.1 ± 1.5% and 25.7 ± 2.9%, respectively along the intestine. The strobilae extended along the entire length of the intestine, but the main mass of the strobilae occurred in the central regions of the small intestine. The average location of the biomass midpoints of tapeworms from male and female rats was 52.6 ± 1.1% and 53.6 ± 2.0% along the intestine respectively. The average dry weight per tapeworm was 177 ± 21 mg from male rats and 190 ± 19 mg from female rats. Recovery rates from male and female rats were 83% and 75% respectively. Fecal samples from three male and female groups showed an average of 53,700 eggs per tapeworm (range 33,000 - 78,000).

The analysis of variance of the scolex and biomass midpoints and the dry weight per worm is shown in Appendix 1, Table II. There were no statistically significant differences between the distribution of tapeworms or the dry weight per tapeworm from male and female rats. There were significant differences shown between the biomass midpoints and the dry weight per worm of tapeworms from the various groups. The 95% confidence interval for the biomass midpoint was 47.2% - 58.8% and for the dry weight per worm was 102 mg - 264 mg. The tapeworms which were not included in these limits were found only in group 1, therefore showing that the other 4 groups did not significantly differ from each other.

The weight of the rats after infection with the tapeworms is shown in Appendix 2, Fig. 2. The rate of growth was slower and the maximum weight was less in infected rats than in non-infected rats.

Thirty day infections: Tapeworms were located in the same areas, and though the average weight per worm was less than the 20 day old tapeworms, there was no significant difference (Table II). The number of eggs produced per worm per day was greater than 20 day old worms.

Experiment 3

Examination of Concurrent Infections with 4000 T. spiralis larvae:

Infections of T. spiralis used in this experiment and in the following experiments were changed from dosage based on host weight (L/g) to dosage based on total number of nematodes. This was done because experiment one revealed that host body weight did not affect the distribution of T. spiralis, providing experimental animals are reasonably homogeneous with respect to weight (110 - 130 gms).

Five groups of five male and five female rats were infected with 4000 T. spiralis larvae 15 days after infection

with H. diminuta cysticercoids. Rats were necropsied 5 days after nematode infection.

Trichinella spiralis: Results of this experiment are shown in Table III and analyses of variance are presented in Appendix 1, Table III. The anterior distribution of T. spiralis in this experiment (Fig. 4) was similar to the distribution of T. spiralis in single-species infections. Most nematodes were located in the first quarter of the intestine, with an average nematode midpoint of 19.5 ± 1.5% along the intestine. This average nematode midpoint was not significantly different from the nematode midpoints of single-species infections, though the number of nematodes in the present experiment was greater. Average recovery of T. spiralis was 49.7% and was significantly greater ($P < .05$) than recovery of T. spiralis in single-species infections.

Hymenolepis diminuta: The distribution in male and female rats is shown in Fig. 5 and the location of the scolex and biomass midpoints, dry weight per worm and percentage recovery in each group of rats are shown in Table IV. Tapeworm scolices occurred in the 30% to 85% region of the intestine. The average location of the scolex midpoints of tapeworms from male and female rats was 57.2 ± 2.0% and 60.0 ± 1.6% respectively along the intestine.

Table III. Average number recovered, % recovery, and nematode midpoints of T. spiralis in concurrent infections 5 days after infection of 4000 T. spiralis larvae.

	Group									
	1		2		3		4		5	
Host Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
# of rats	2	4	3	5	4	5	5	5	5	5
Average # recovered	2373 ±183*	1752 ±120	2797 ±280	2257 ±184	1943 ±186	2069 ±243	1280 ±240	2017 ±84	1698 ±142	1715 ±155
Recovery (%)	59.3	43.8	69.9	56.4	48.6	51.7	32.0	50.4	42.5	42.8
Nematode midpt (% distance)	20.9 ±0.8*	29.4 ±2.0	23.2 ±1.8	21.0 ±1.4	20.8 ±1.3	17.7 ±1.6	16.9 ±0.6	17.4 ±0.9	13.1 ±0.6	14.7 ±1.4

* one standard error.

Figure 4. Distribution of T. spiralis in concurrent infections 5 days after infection of 4000 T. spiralis larvae.

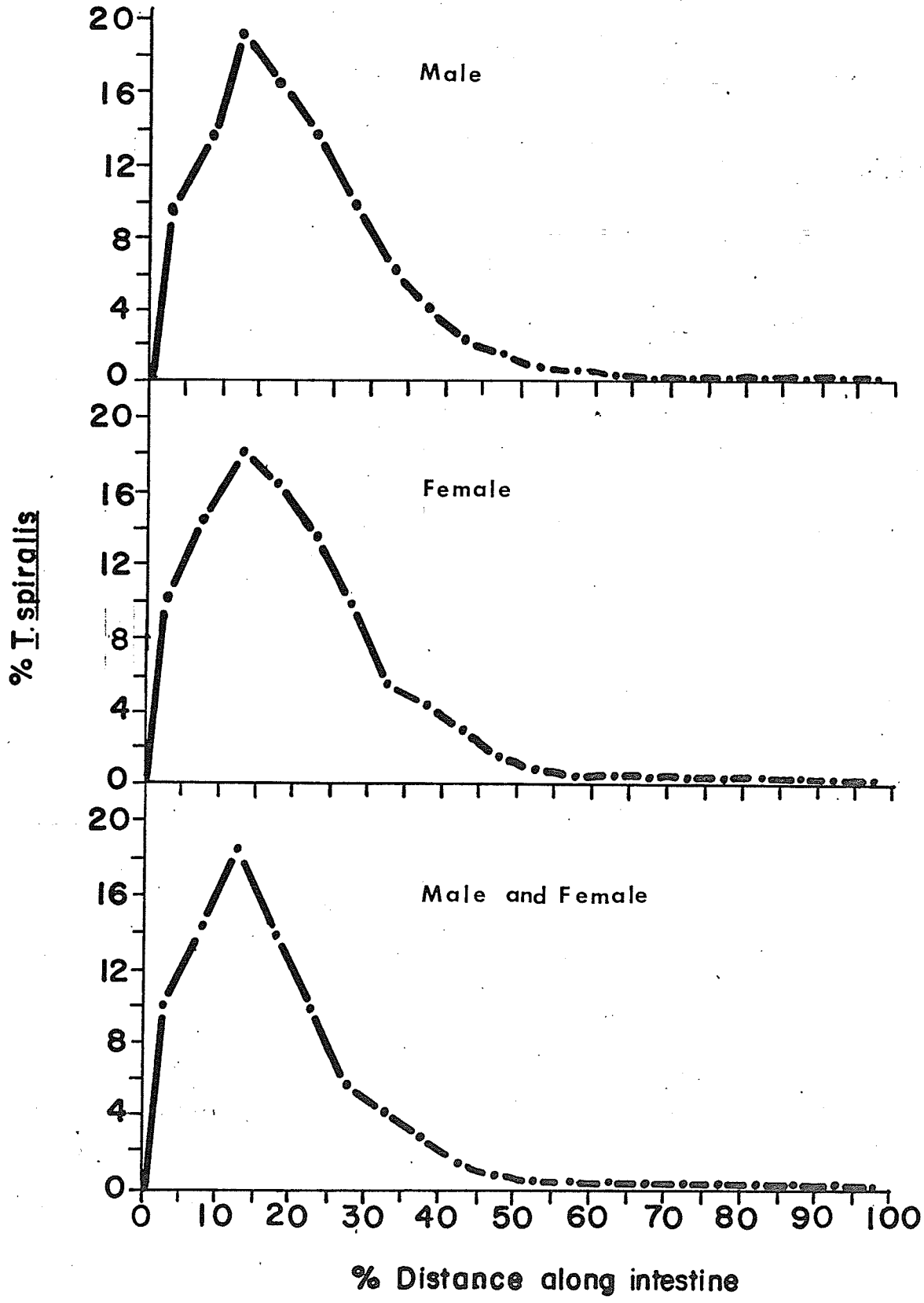

Male
Female
Male and Female
% T. spiralis
0
4
8
12
16
20
0 10 20 30 40 50 60 70 80 90 100
% Distance along intestine

Figure 5. Distribution of H. diminuta in concurrent infections 5 days after infection of 4000 T. spiralis larvae.
Histograms represent distribution of scolices and line graphs represent distribution of biomass.

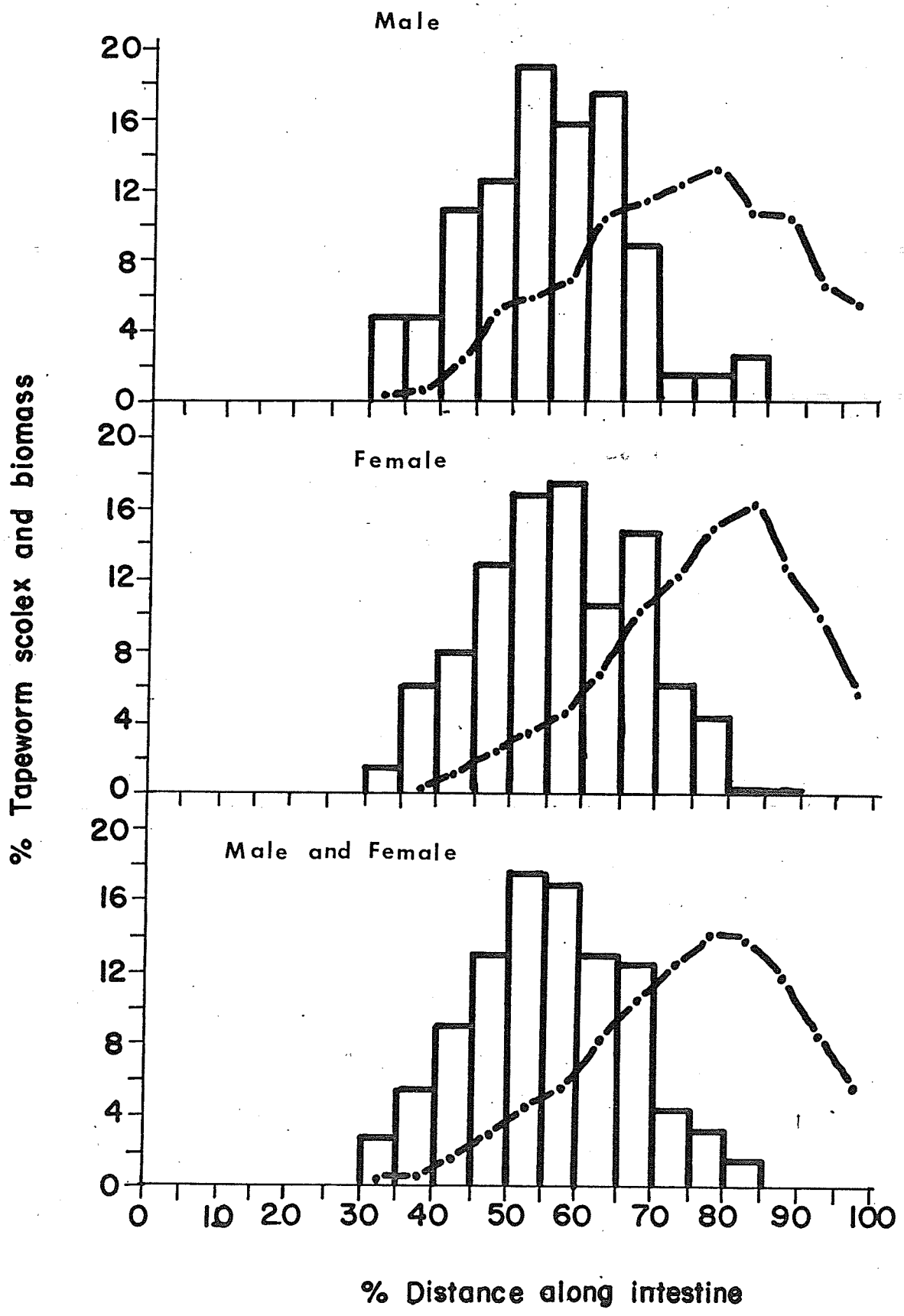

Male
Female
Male and Female
% Tapeworm scolex and biomass
% Distance along intestine
0
4
8
12
16
20
0
10
20
30
40
50
60
70
80
90
100

Table IV. Percent recovery, scolex and biomass midpoint location, dry weight per tapeworm and fecundity of H. diminuta in concurrent infections 5 days after 4000 T. spiralis larvae infection.

	Group 1		2		3		4		5	
Host Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
# of rats	2	4	3	5	4	5	5	5	5	5
Recovery (%)	60	80	100	84	80	88	64	78	100	100
Scolex midpt. (% distance)	54.4 ±3.5*	55.3 ±4.5	53.6 ±6.5	63.4 ±3.0	63.3 ±9.2	65.1 ±5.1	55.9 ±3.4	56.4 ±2.0	56.0 ±1.9	61.8 ±2.6
Biomass midpt. (% distance)	83.6 ±6.9*	83.5 ±4.1	70.6 ±4.7	75.2 ±3.0	72.0 ±3.5	72.0 ±3.7	78.5 ±3.8	75.1 ±4.3	78.0 ±1.3	80.6 ±2.7
Dry weight (per worm)	190.0 ±4.0*	69.5 ±3.9	56.3 ±4.3	69.4 ±13.3	71.7 ±3.8	83.1 ±3.4	91.5 ±5.6	136.7 ±19.4	104.3 ±15.3	132.9 ±13.7
Fecundity (10^3 eggs/24h)	-	-	17	6	29	45	50	44	-	-

* one standard error.

The strobilae were restricted to the posterior 70% of the small intestine, the average location of the biomass midpoints from male and female rats were 75.2 ± 1.9% and 77.4 ± 1.7% along the intestine respectively. The average dry weight per worm in male rats was 95.5 ± 9.77 mg and in female rats was 101.6 ± 7.55 mg. Recovery rates from male and female rats were 81% and 86% respectively. Fecal samples from three male and female groups showed an average of 31,600 eggs per tapeworm with a range from 6,000 to 50,000 eggs.

The analysis of variance of the scolex and biomass midpoints and the dry weight per worm is shown in Appendix 1, Table IV. There was no statistical difference in the distribution or the dry weight per tapeworm for male and female rats. Only the dry weight per tapeworm showed significant differences between groups.

Comparison between *H. diminuta* in single-species infections and concurrent infections: The analysis of variance of *H. diminuta* scolex and biomass midpoints and dry weight per worm is shown in Appendix 1, Table V. Tapeworms recovered from single-species infections and concurrent infections were significantly different in distribution but no significant differences occurred between

the groups. Tapeworms recovered from concurrent infections were more posterior and weigh less than tapeworms in single-species infections. Variation between rat groups were similar in the concurrent and single-species infections.

Experiment 4

Examination of Concurrent Infections at Different *T. spiralis* Dose Levels

This experiment was designed to study the effect of increasing dose levels of *T. spiralis* on its recovery and distribution and on the distribution, weight, recovery and fecundity of *H. diminuta*. Figure 9 shows the number of nematodes recovered rather than the initial infection dose of *T. spiralis*. This was done because nematode recovery is a more valid parameter to examine than initial dose level as it reflects the actual number of nematodes in the concurrent infections at time of examination.

Two groups of five male and five female rats were infected with 500 *T. spiralis* larvae and three groups of five male and five female rats were infected with either 1000, 2000 or 3000 larvae. Rats were necropsied 5 days after nematode infections. Information from Experiment 3 was utilized with these data.

Trichinella spiralis: At all population levels most nematodes are situated in the anterior quarter of the small intestine and their numbers progressively decrease in the posterior 75% of the intestine (Fig. 6). Results are shown in Table V (for analysis of variance see Appendix 1, Table VI). As the number of nematodes increase, the nematode midpoints moved significantly posterior, indicating a posterior spreading of their distribution (Fig. 7). The average nematode midpoint was 15.1 $\pm$ 1.6% along the intestine. The average recovery of nematodes from male and female rats from the five population levels was 45.4%.

Weights of the rats at the different population levels is shown in Appendix 2, Fig. 1. There was an inverse relationship between weight of rats and an increasing nematode population 5 days after infection.

Comparison between T. spiralis in single-species and in concurrent infections: Comparison between groups was based on similar number of nematodes recovered (Appendix 1, Table VII). Variation in nematode midpoints can be attributed to host sex, dose level and type of infection. The largest variation was due to whether the infections were single-species or concurrent. Nematode midpoints in concurrent infections were significantly anterior to nematode midpoints in single-species infections.

Figure 6. Distribution of T. spiralis in concurrent infections 5 days after infection of 500, 1000, 2000 and 3000 T. spiralis larvae.

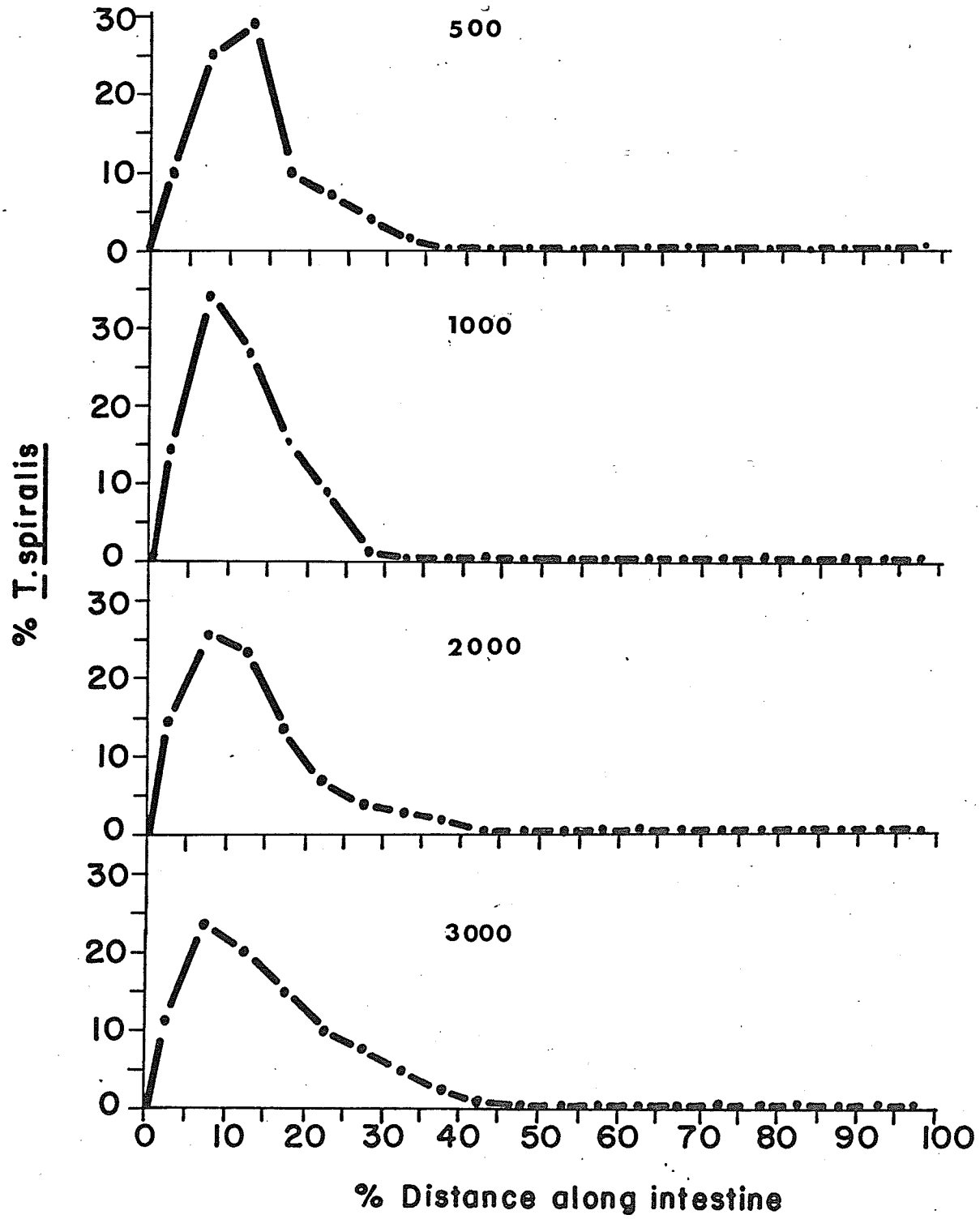
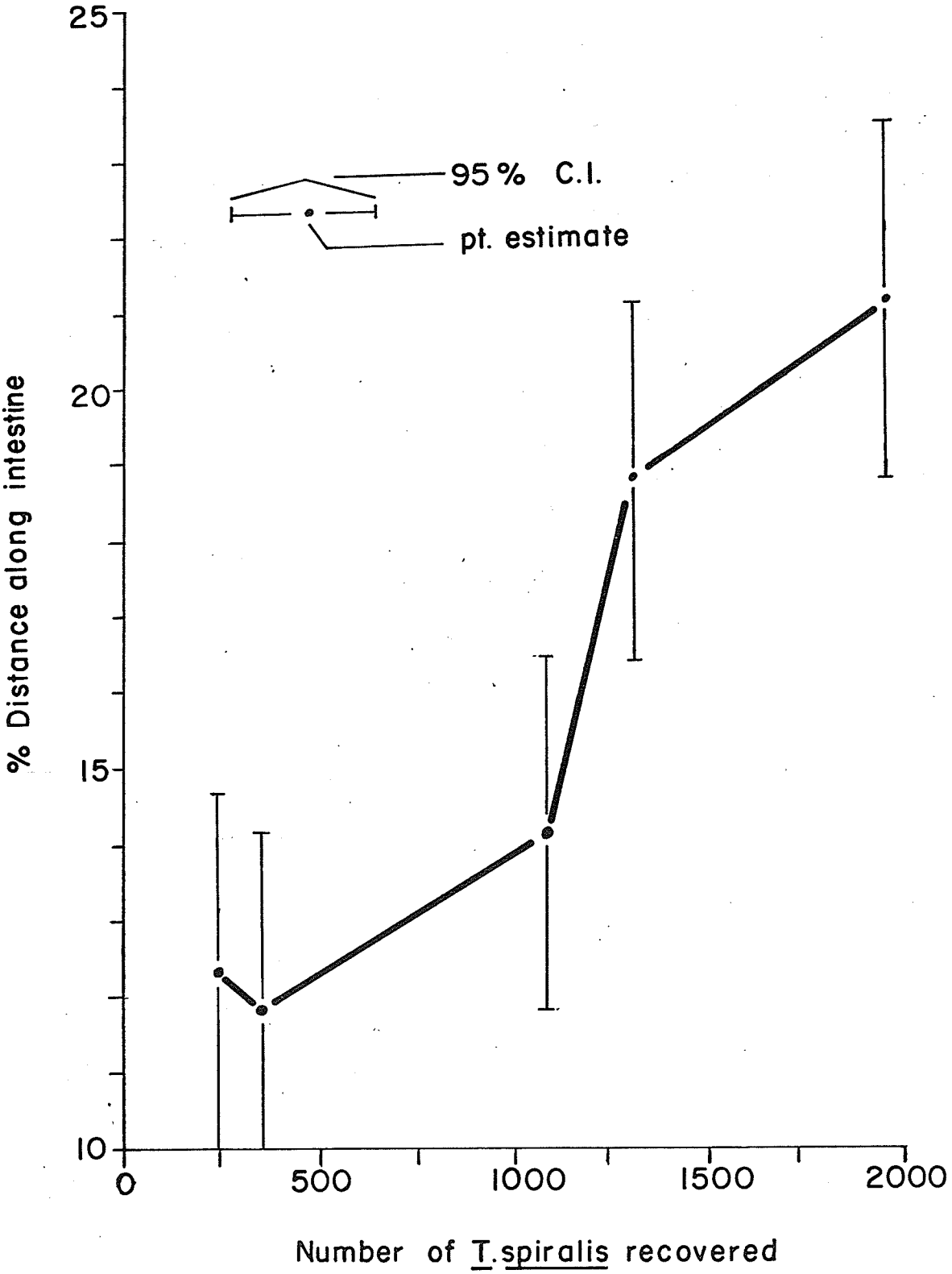

500
1000
2000
3000
30
20
10
0
0 10 20 30 40 50 60 70 80 90 100
% T. spiralis
% Distance along intestine

Table V. Average number recovered, % recovery and nematode midpoints of T. spiralis in concurrent infection 5 days after infection of 500, 1000, 2000, 3000 and 4000 larvae.

	DOSE LEVELS									
	500		1000		2000		3000		4000 (Expt. 3)	
Host Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
# of Rats	10	10	5	5	4	5	3	3	19	24
Average # Recovered	302 ±72*	201 ±19	300 ±35	295 ±27	1326 51	830 ±66	1250 ±138	1400 ±137	2019 ±263	1903 ±98
% Recovered	60.4	40.2	30	29.5	66.3	41.5	41.6	46.7	50.5	47.6
Nematode Midpt. (% Distance)	12.3 ±1.3*	12.2 ±0.8	14.7 ±0.9	8.8 ±0.6	15.9 ±1.3	12.3 ±1.7	20.6 ±0.9	11.1 ±0.6	20.4 ±1.3	21.4 ±2.8

* ± one standard error.

Figure 7. Location of nematode midpoints in concurrent infections 5 days after infection of 500, 1000, 2000, 3000 and 4000 T. spiralis larvae.


95% C.I.
pt. estimate
% Distance along intestine
25
20
15
10
0
500
1000
1500
2000
Number of T. spiralis recovered

Analysis of variance of recovery rate of T. spiralis in single-species infections and in concurrent infections revealed a significantly higher recovery rate of worms during concurrent infections than during single-species infections.

Hymenolepis diminuta: Tapeworm distribution, dry weight per tapeworm and fecundity were affected by the number of nematodes present in the rat small intestine but were not affected by host sex, see Table VI and Appendix 1, Table VIII for analyses of variance. There was a reduction in the anterior limit of tapeworm scolex and biomass distribution and an increase in the posterior limit of the scolex distribution with an increase in nematode numbers (Fig. 5 and 8). The shift in tapeworm populations is more readily seen in Fig. 9. This figure also reveals that with an increase in nematode numbers there is not a direct effect on the position of the tapeworms. When the nematode population exceeded 310 (1000 dose level) there was a decreased rate of posterior movement of the tapeworms. The extent of this posterior movement of the scolices and of the biomass midpoints was different. The change in scolex position over the various dose levels was 23% while the change in biomass position was 16% showing that the scolex position was affected to a greater degree than the biomass position. The dry weight per

Table VI. Percent recovery, scolex and biomass midpoint location, dry weight per tapeworm and fecundity of H. diminuta in concurrent infections 5 days after 500, 1000, 2000, 3000 and 4000 larvae infection.

	DOSE LEVELS									
	500		1000		2000		3000		4000 (Expt.3)	
Host Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
# of Rats	10	10	5	5	4	5	3	3	19	24
Recovery (%)	80	100	80	88	95	92	67	75	80	86
Scolex Midpt. (% distance)	34.1 ±1.9*	41.1 ±2.1	38.1 ±1.6	44.3 ±1.6	50.9 ±0.5	47.4 ±2.4	53.6 ±1.9	48.7 ±1.7	54.2 ±1.0	57.9 ±1.1
Biomass Midpt. (% distance)	54.9 ±1.8*	59.9 ±1.5	60.2 ±1.6	66.5 ±1.2	72.1 ±0.63	69.35 ±1.8	70.7 ±0.99	75.6 ±2.1	72.6 ±0.7	74.8 ±0.9
Dry Weight per worm (mg)	173.8 ±43.2*	155.8 ±19.9	165.4 ±41.9	187.2 ±17.6	163.3 ±47.1	112.6 ±60.7	76.7 ±28.9	150.0 ±14.7	95.5 ±9.8	101.6 ±7.5
Fecundity (10^3 eggs/24h)	73	-	72	-	30	-	22	-	26	32

* ± one standard error.

Figure 8. Distribution of H. diminuta in concurrent infections 5 days after infection of 500, 1000, 2000 and 3000 T. spiralis larvae. Histograms represent distribution of scolices and line graphs represent distribution of biomass.

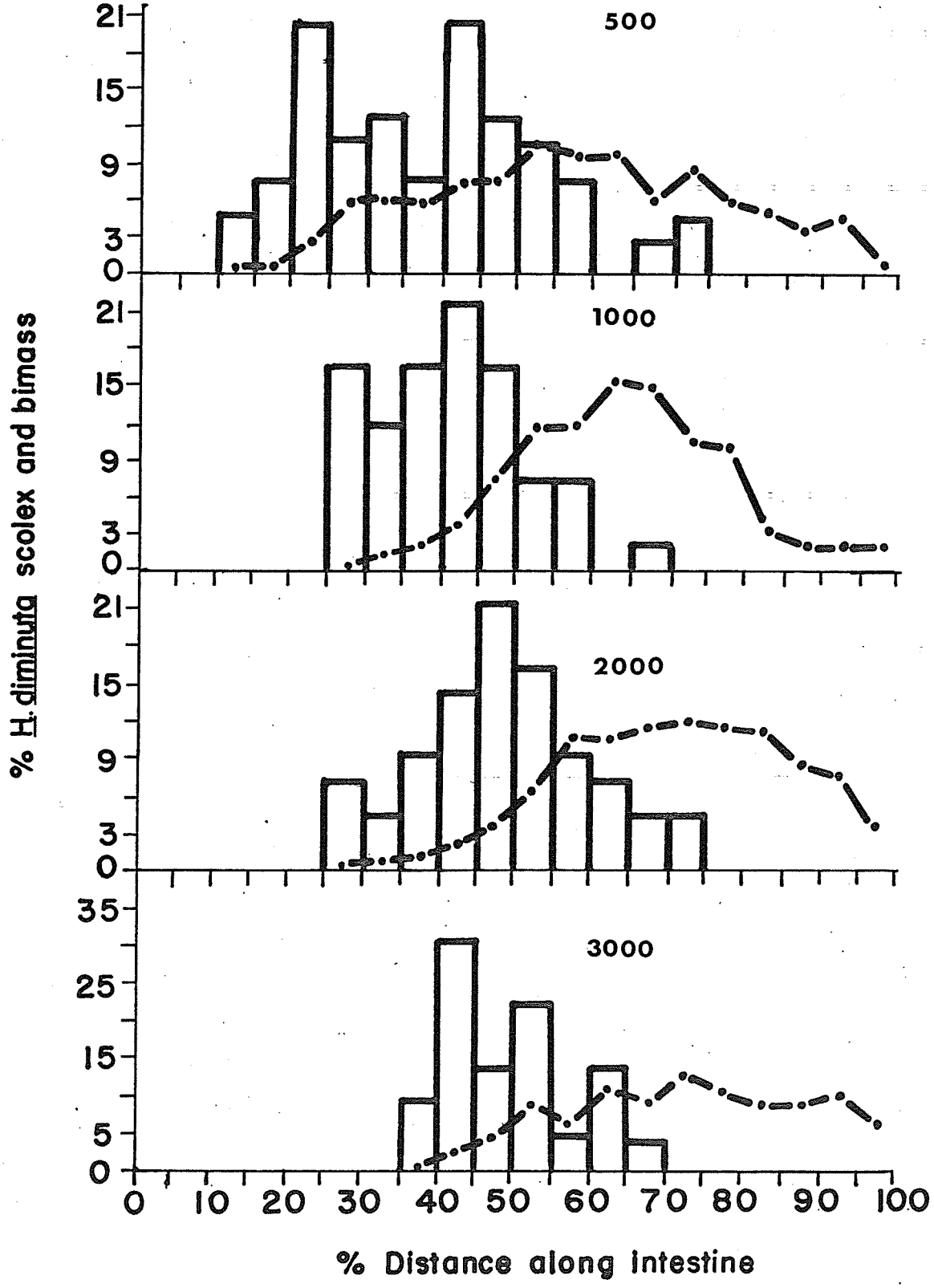
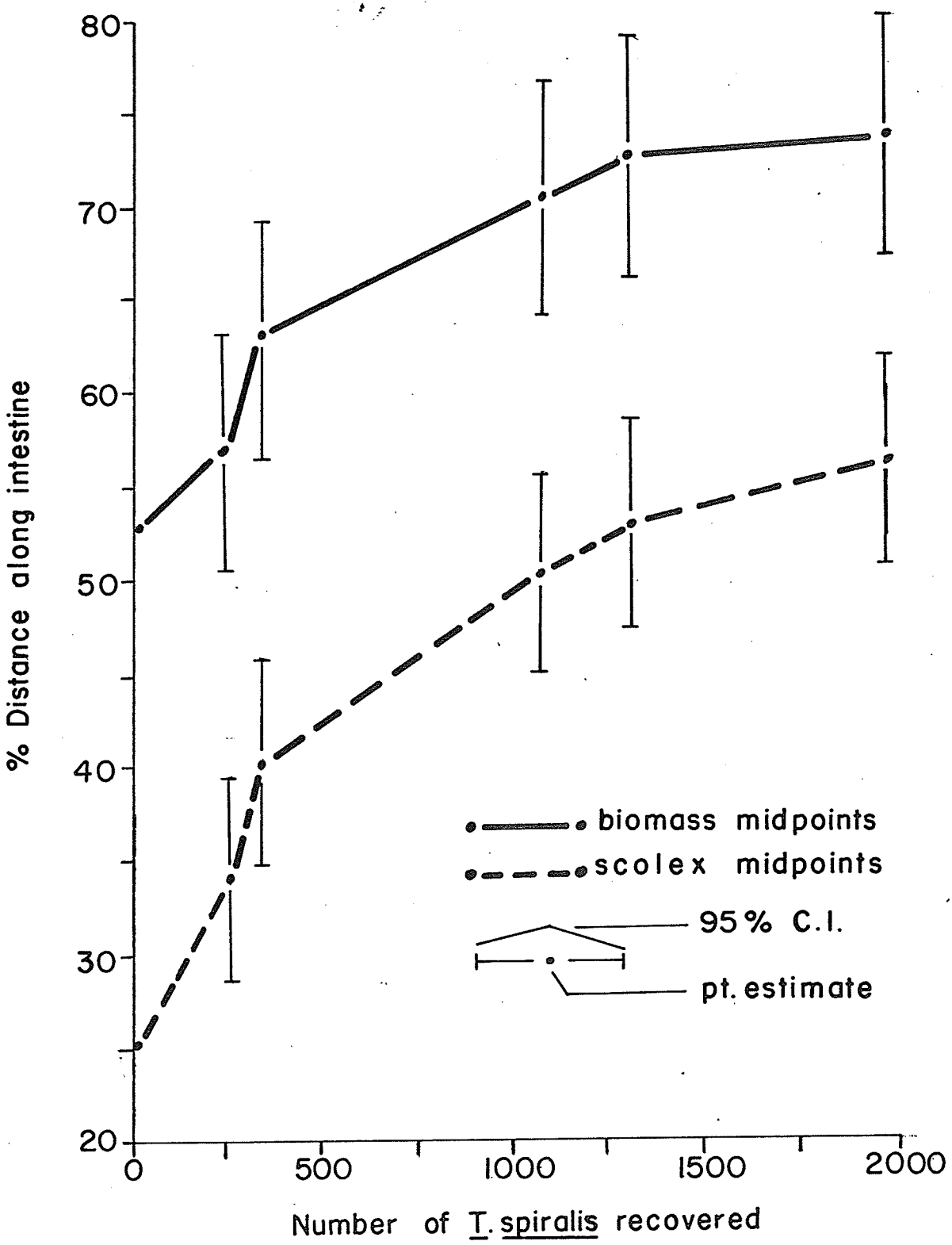

500
1000
2000
3000
21
15
9
3
0
35
25
5
0 10 20 30 40 50 60 70 80 90 100
% H. diminuta scolex and bimass
% Distance along intestine

Figure 9. Location of H. diminuta scolex and biomass midpoints in concurrent infections 5 days after infection of 500, 1000, 2000, 3000 and 4000 T. spiralis larvae.


80
70
60
50
40
30
20
% Distance along intestine
0
500
1000
1500
2000
Number of T. spiralis recovered
biomass midpoints
scolex midpoints
95% C.I.
pt. estimate

tapeworm decreased significantly as the number of nematodes increased. There was a loss of weight when the nematode numbers exceed 310 (1000 dose level) (Appendix 2, Fig. 3), and weight loss became significant when nematode numbers were greater than 1100 (2000 dose level). The recovery of tapeworms was 84.3% and therefore was not affected in concurrent infections.

Number of eggs produced by the tapeworms was less when the nematode dose was 2000, 3000 and 4000 larvae than when the nematode dose was 500 or 1000 larvae.

In summary, these data showed that H. diminuta responded similarly to dose levels of 500 and 1000 nematode larvae, and similarly to dose levels of 2000, 3000 and 4000 nematode larvae 5 days after infection of T. spiralis.

Experiments were carried out to study the effect of concurrent infections throughout the duration of the T. spiralis intestinal phase. Since Experiment 4 revealed the importance of nematode numbers, Experiment 5 was designed to study the effect of a low initial dose of T. spiralis, and Experiment 6, the effect of a high initial dose of T. spiralis.

Experiment 5

Examination of Concurrent Infections Throughout the Duration of a 1000 *T. spiralis* Dose Infection

Six groups of five male rats were infected with *H. diminuta* and 15 days later were infected with 1000 *T. spiralis* larvae. Rats were examined 1, 2, 8, 12, 16 and 20 days after nematode infection. Data from male rats from Day 5, 1000 dose level in Experiment 4, were included in all analyses.

Trichinella spiralis: Most nematodes were situated in the first quarter of the small intestine (Fig. 10). The nematode midpoints, number recovered and recovery rates are shown in Table VII (for analyses of variance see Appendix 1, Table IX).

The majority of nematodes on all days were found in the anterior quarter of the small intestine and the number progressively decreased in the posterior 75% of the intestine. The average nematode midpoint was located 15.2 $\pm$ 3.8% along the intestine. There was no statistically significant difference between the nematode midpoints in this experiment and the nematode midpoints in Experiment 4 ($P < .05$).

There was an increase in the number of nematodes recovered between the 1st and 2nd day after infection and then a decrease until no nematodes were found after Day 12. There was no significant difference between the number of nematodes recovered on each of the days.

Figure 10. Distribution of T. spiralis in concurrent infections after infection of 1000 T. spiralis larvae.

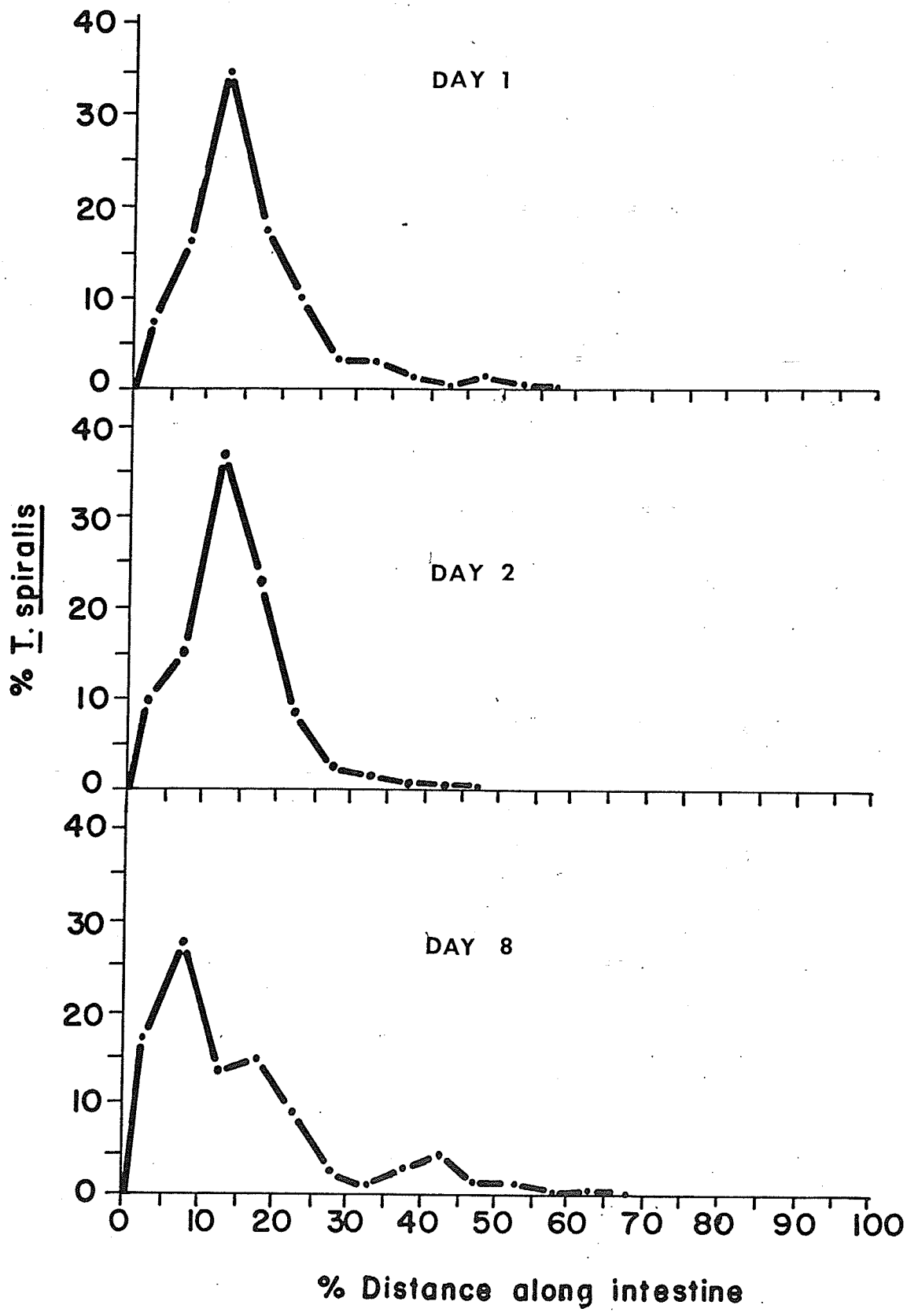

DAY 1
DAY 2
DAY 8
% T. spiralis
% Distance along intestine
0
10
20
30
40
50
60
70
80
90
100

Table VII. Average number recovered, % recovery and nematode midpoint location of T. spiralis after infection of 1000 T. spiralis larvae.

	Days after nematode infection					
	1	2	8	12	16	20
# of Rats	5	5	5	5	5	5
Average # Recovered	308.1 ±42.5*	433.6 ±61.3	264.8 ±84.3	205.1 ±53.8	0	0
Recovery (%)	30.8	43.4	26.5	20.5	0	0
Nematode Midpt. (% Distance)	16.3 ±1.7*	13.5 ±0.7	16.1 ±4.2	14.2 ±3.6	-	-

* ± one standard error.

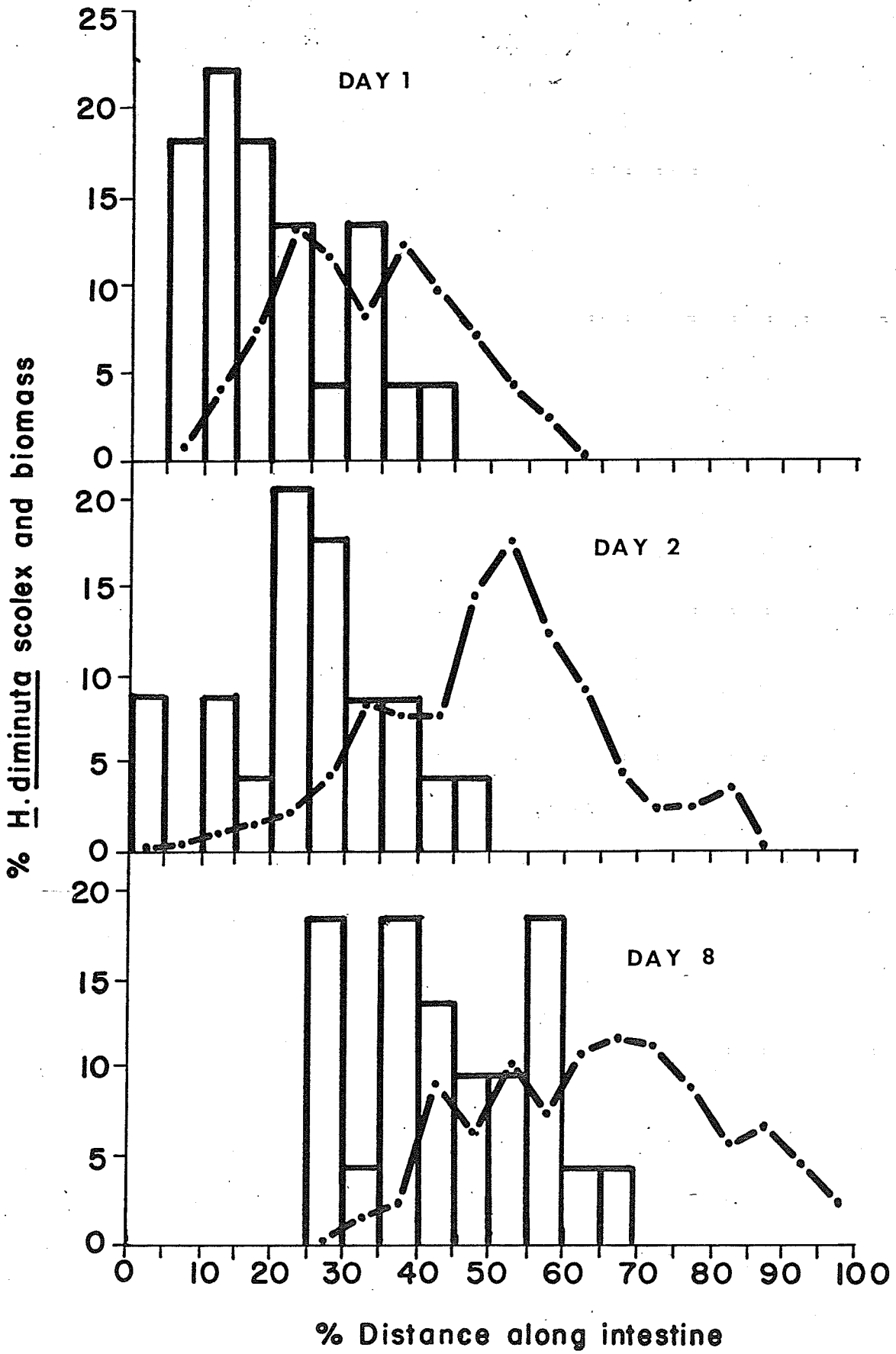
The rat weight changes on the different days after nematode infection is shown in Appendix 2, Fig. 1B. Rats increased in weight throughout the experiment but at a much reduced rate when compared to normal growth.

There was no significant difference between the nematode midpoints in this experiment and the nematode midpoints in Experiment 4 ($P > .05$). The nematode midpoints were significantly more anterior in this experiment than the nematode midpoints in Experiment 1 ($P < .05$).

Hymenolepis diminuta: Tapeworm distribution for the different days is shown in Fig. 11 and 11A, and the location of the scolices and biomass midpoints, dry weight per worm, recovery rate and fecundity are shown in Table VIII, (for analyses of variance see Appendix 1, Table X). Tapeworms moved posteriorly until the 8th day of the concurrent infection, and then moved steadily anterior until the end of the experiment (Fig. 12).

The average dry weight per worm significantly increased until Day 12 (Appendix 2, Fig. 4). There was a decrease in the weight per worm after Day 12 and the weight of worms recovered 20 days after nematode infections

Figure 11, 11A. Distribution of H. diminuta scolices and biomass after infections of 1000 T. spiralis larvae.
Histograms represent distribution of scolices and line graphs represent distribution of biomass.


DAY 1
DAY 2
DAY 8
% H. diminuta scolex and biomass
% Distance along intestine
0
5
10
15
20
25
0
10
20
30
40
50
60
70
80
90
100

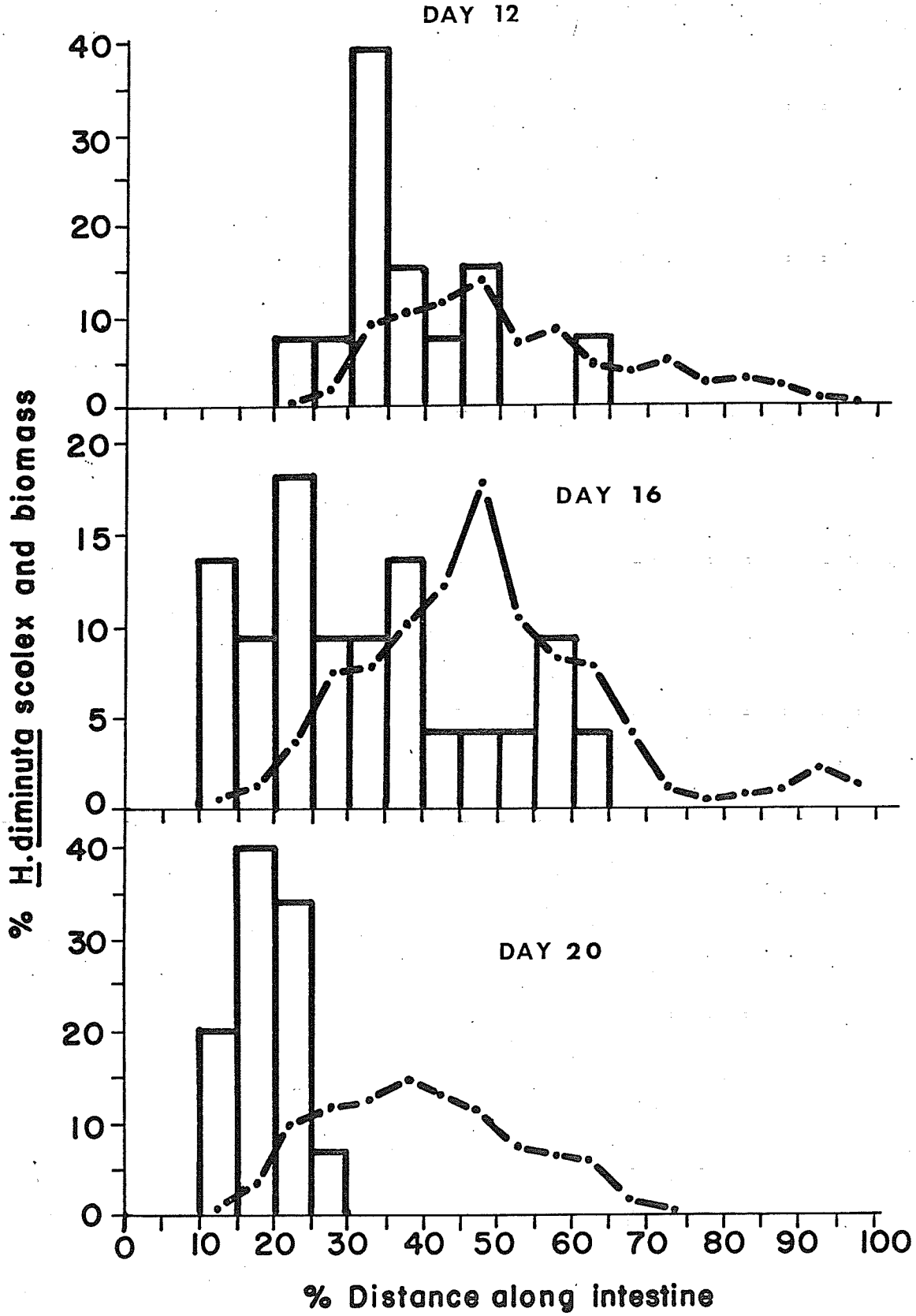
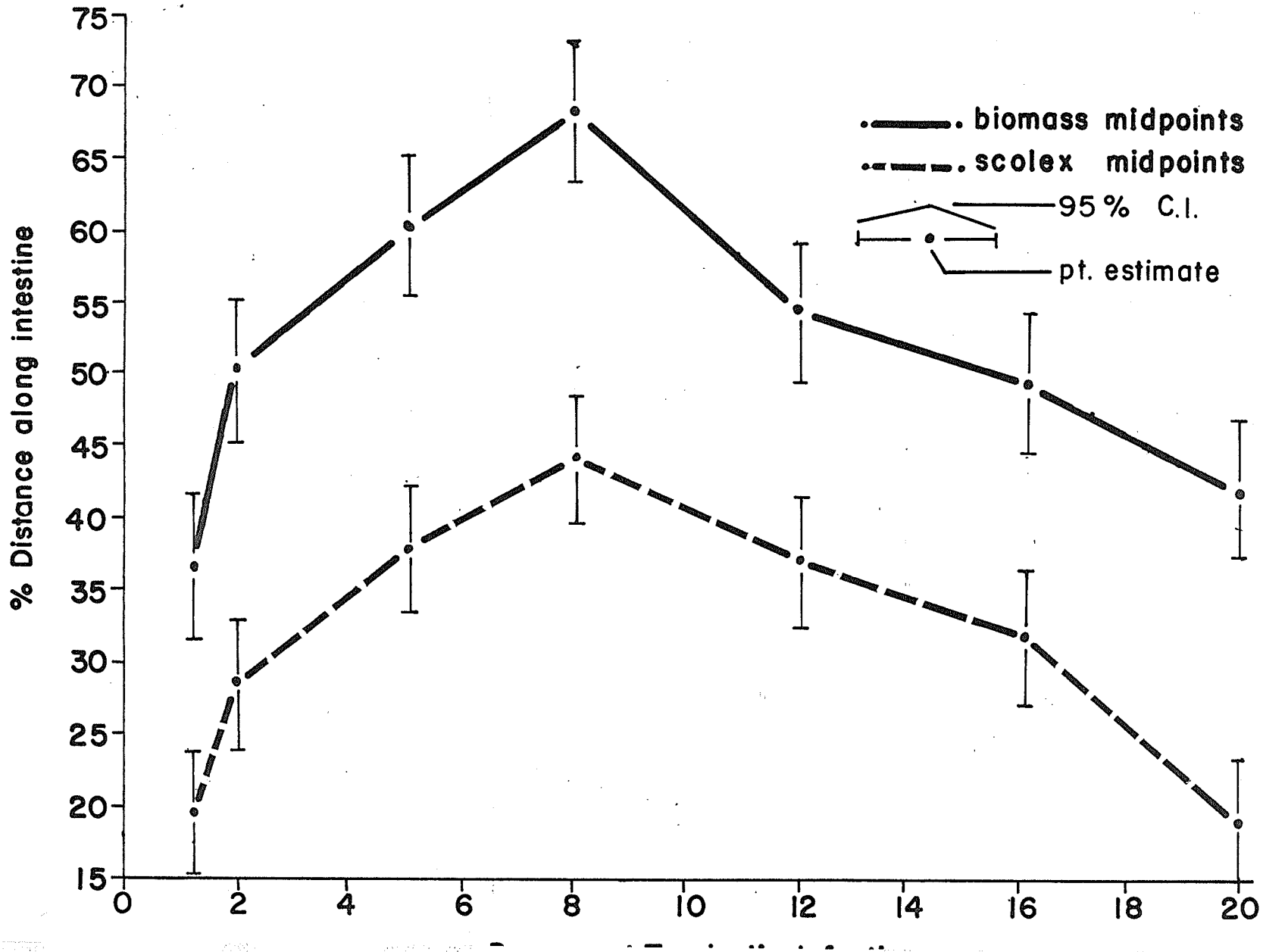

DAY 12
DAY 16
DAY 20
% H.diminuta scolex and biomass
% Distance along intestine
0
10
20
30
40
50
60
70
80
90
100
5
15

Table VIII. Percent recovery, scolex and biomass midpoint location, dry weight per tapeworm and fecundity of H. diminuta after 1000 T. spiralis infection.

	Days after nematode infection					
	1	2	8	12	16	20
# of Rats	5	5	5	5	5	5
Recovery (%)	92	92	88	60	88	60
Scolex Midpt. (% Distance)	19.8 ±1.9*	28.7 ±4.2	44.3 ±5.3	37.3 ±2.4	31.6 ±6.2	21.3 ±1.4
Biomass Midpt. (% Distance)	36.4 ±2.8*	51.25 ±3.0	68.04 ±5.2	54.8 ±3.6	49.7 ±6.5	41.6 ±2.7
Dry Weight per worm(mg)	79.0 ±9.2*	116.2 ±12.3	166.4 ±25.2	191.2 ±24.4	177.4 ±20.1	123.6 ±12.3
Fecundity (10^3 eggs/24h)	0	0	31	71	47	41

* ± one standard error.

Figure 12. Location of H. diminuta scolex and biomass midpoints after infection of 1000 T. spiralis larvae.


% Distance along intestine
75
70
65
60
55
50
45
40
35
30
25
20
15
0
2
4
6
8
10
12
14
16
18
20
biomass midpoints
scolex midpoints
95 % C.I.
pt. estimate

were significantly lighter than Day 12 tapeworms. Analysis of variance between the tapeworm weights of this experiment and Experiment 2 (single-species H. diminuta) revealed no significant difference (Appendix 1, Table XI).

The number of eggs produced decreased between Day 5 and 8, increased between Day 8 and 12 and decreased on Day 16 and 20. The average number of eggs produced per tapeworm per day between the 5th and 20th days after nematode infections was 53,000 (range 31,000 - 70,600).

Experiment 6

Examination of Concurrent Infections Throughout the Duration of a 4000 T. spiralis Dose Infection

Two trials were performed. The first trial used seven groups of five male and five female rats and the second trial used seven groups of five male rats. Analyses of variance of the scolices and biomass midpoints and dry weights per worm indicated similar trends in both groups and the combined information is presented below. Rats infected with H. diminuta were infected with 4000 T. spiralis larvae 15 days later and were examined on Days 8, 10, 12, 14, 16, 18 and 20 after nematode inoculation. Data from Group 4 of the 5 day concurrent infections with 4000 T. spiralis larvae were (Experiment 3) included in all analyses.

Trichinella spiralis: Nematode midpoints and number and recovery rate are shown in Table IX (for analyses of variance see Appendix 1, Table XII). Most of the nematodes were situated in the first quarter of the small intestine and numbers progressively decreased in the posterior three-quarters (Fig.13). There was no significant difference between nematode midpoints during the intestinal phase of T. spiralis infection. The average nematode midpoint was 16.2 ± 1.7% along the intestine and was not significantly different from previous nematode midpoints during concurrent infections ($P < .05$). The nematode midpoints in this experiment were significantly anterior to the nematode midpoints of worms in single-species infections. The number of T. spiralis recovered decreased between Day 5 and 10 after infection, no substantial loss between Day 10 and 14, and after Day 14 the loss of nematodes continued until Day 20 (Appendix 2, Fig. 5). No relationship exists between the loss of nematodes and the position and dry weight per tapeworm (Table X).

Changes in rat weight on the different days after nematode infection are shown in Appendix 2, Fig. 1. Weight decreased until Day 10 then remained constant until Day 14, after which weight increased.

Table IX. Average number recovered, % recovery and nematode midpoint location of T. spiralis after 4000 T. spiralis infection.

	Days after T. spiralis infection						
	8	10	12	14	16	18	20
# of Rats	15	15	15	14	15	15	15
Average # Recovered	1048 ±104*	548 ±65	521 ±58	463 ±137	255 ±88	129 ±68	-
Recovery (%)	26.2	13.7	13.0	11.6	6.4	3.3	-
Nematode Midpt (% Distance)	15.9 ±1.2*	19.2 ±2.4	18.4 ±1.5	16.4 ±1.4	19.3 ±2.3	16.4 ±1.7	-

* ± one standard error.

Figure 13. Distribution of T. spiralis in concurrent infections during the intestinal phase after infection of 4000 T. spiralis larvae.

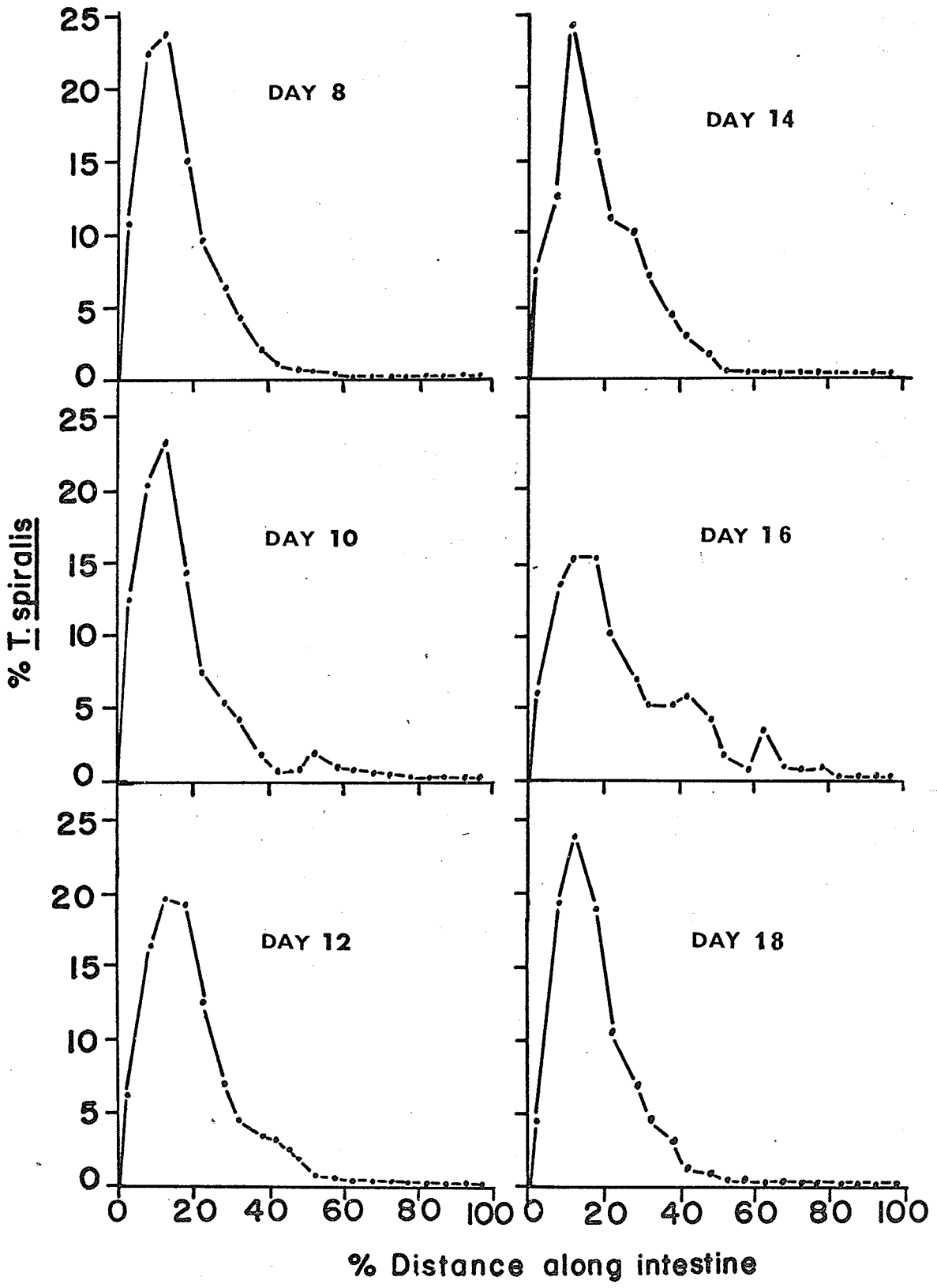

DAY 8
DAY 14
DAY 10
DAY 16
DAY 12
DAY 18
% T. spiralis
% Distance along intestine
0
5
10
15
20
25
0
20
40
60
80
100

Table X. Change of T. spiralis numbers, weight per tapeworm, scolex and biomass midpoint location after 4000 T. spiralis infection.

Time (Days)	Change of T. spiralis Numbers	Change in wt/worm (mg)	Change in scolex midpt. position (% distance)	Change in biomass midpt. position (% distance)
5-8	-912	-33	*+0.75	*-1.05
8-10	-500	-20	+5.80	+10.35
10-12	-27	+4	-1.10	-1.85
12-14	-58	-20	+6.95	+13.65
14-16	-208	-20	-2.85	-1.85
16-18	-126	+49	+9.15	+6.10
18-20	-129	+30	+4.75	-1.35

* + indicates anterior movement

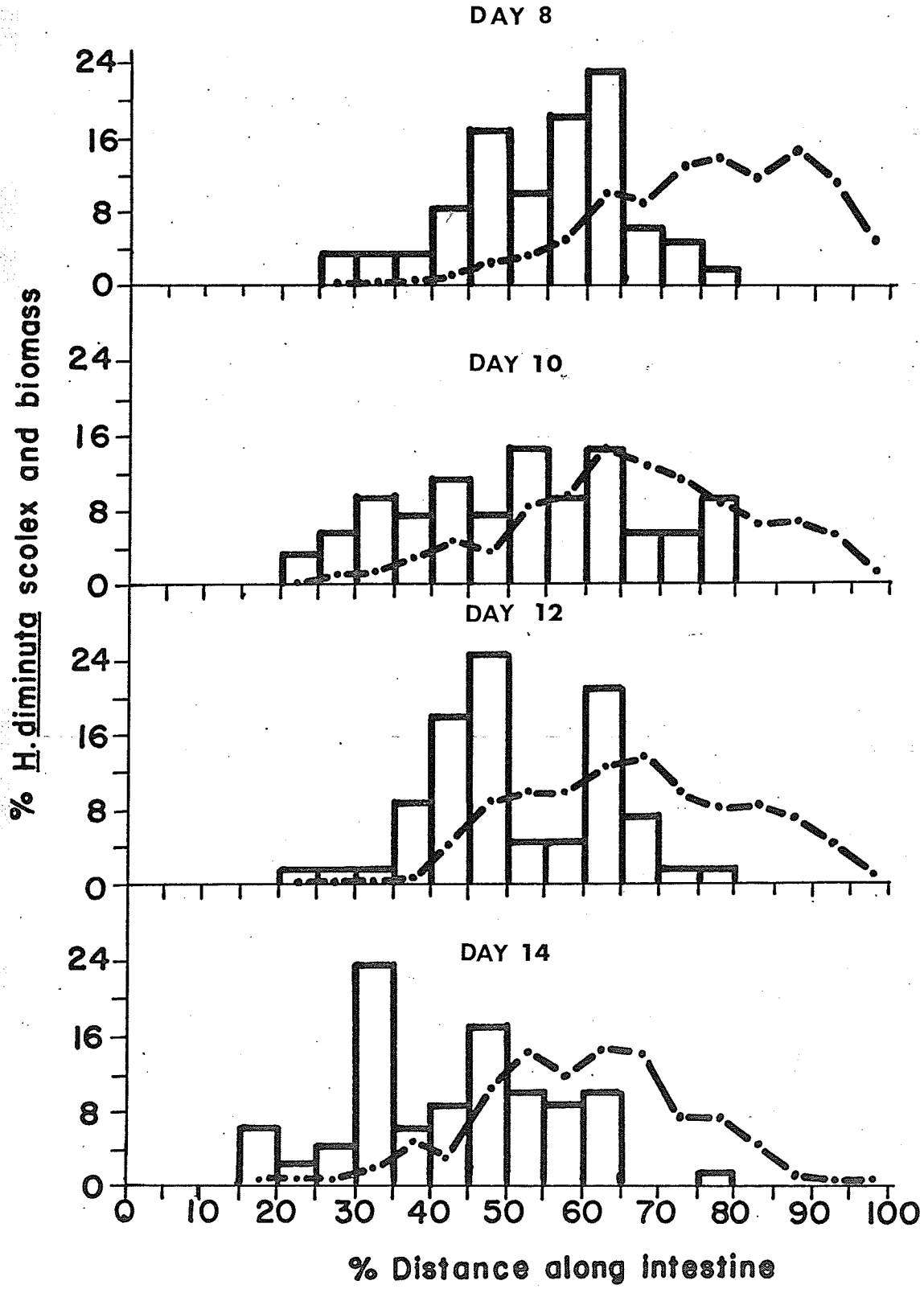
* - indicates posterior movement.

Hymenolepis diminuta: Tapeworm distribution for the different days is shown in Fig. 14 and 14A, and the location of the scolex and biomass midpoints, dry weight per worm, percent recovery and destrobilated worms, and fecundity is seen in Table XI (for analysis of variance see Appendix 1, Table XIII). Tapeworms were located posteriorly on Days 5 and 8 after the nematode infections and a stepwise anterior migration occurred between Days 8 and 18 (Fig. 15). Destrobilated tapeworms were first found on Day 8 after nematode infection, increasing numbers of destrobilated worms were found until Day 16, but destrobilated worms were infrequent on Day 18 and none were found on Day 20. Destrobilated tapeworms were anterior to strobilated worms and occurred in the mid regions of the intestine.

The dry weight of tapeworms showed significant differences between the days after nematode infections. Average weight of tapeworms in the rats declined until Day 16 after nematode infections after which tapeworms regrew. Dry weight of strobilated worms was consistent except for that of Day 16 worms. This value may have been enhanced by the presence of strobila from newly destrobilated worms.

The decrease in recovery of tapeworms during Days 14 and 16 did not appear to be real because the recovery of tapeworms on Days 18 and 20 were similar to

Figure 14, 14A. Distribution of H. diminuta scolices and biomass after infections of 4000 T. spiralis larvae.
Histograms represent distribution of scolices and line graphs represent distribution of biomass.


DAY 8
DAY 10
DAY 12
DAY 14
24
16
8
0
0 10 20 30 40 50 60 70 80 90 100
% H. diminuta scolex and biomass
% Distance along intestine

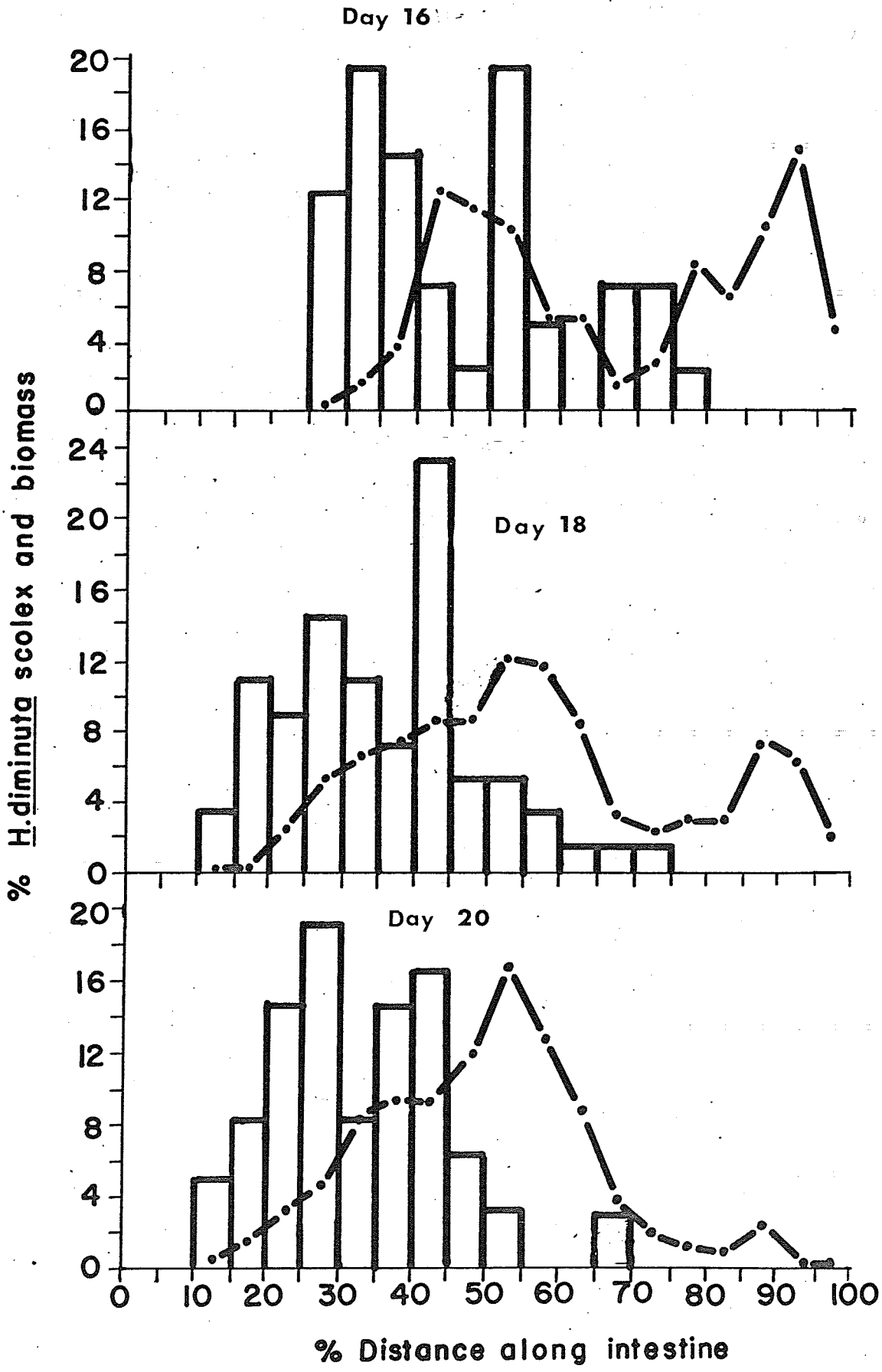
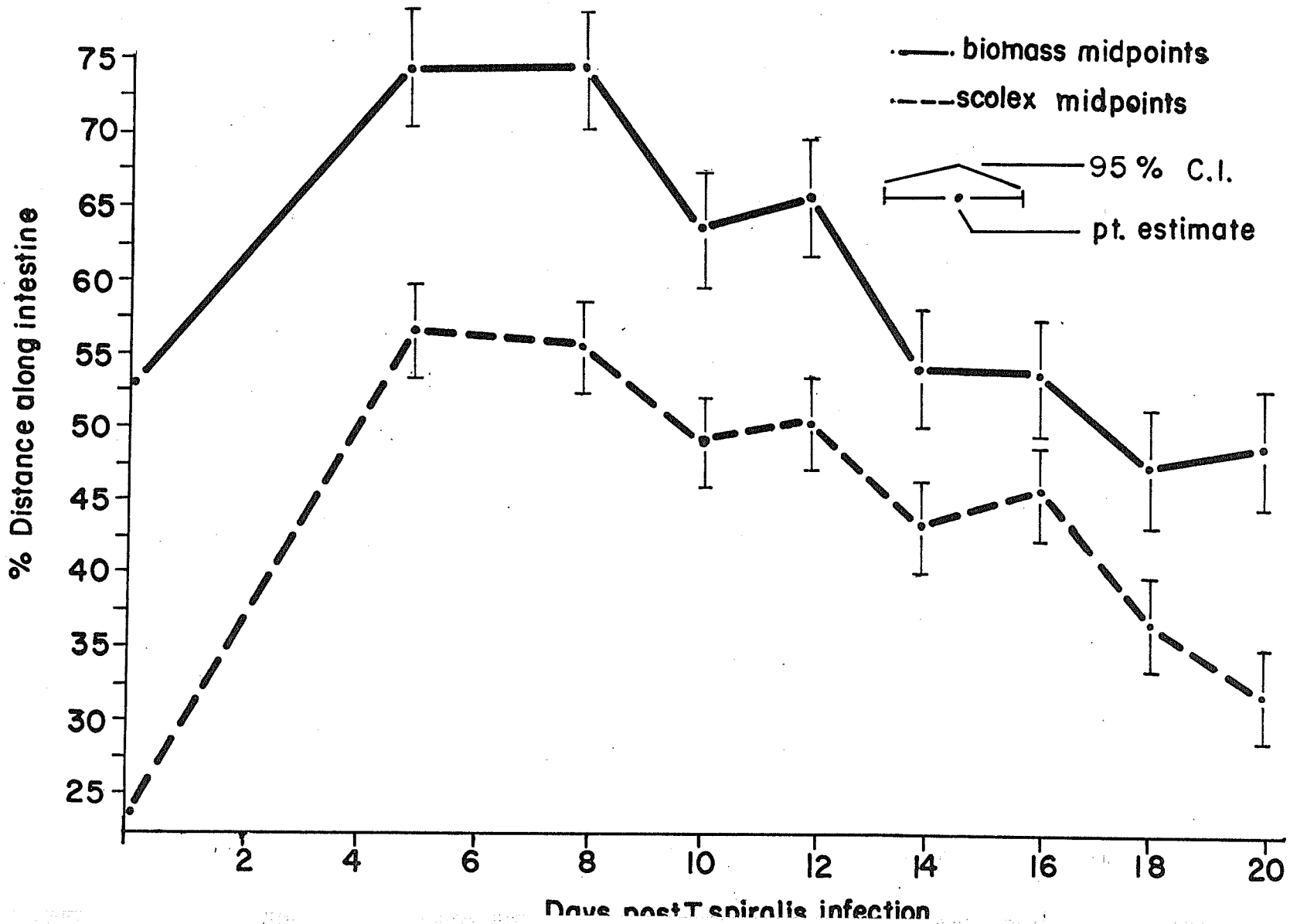

Day 16
Day 18
Day 20
% H.diminuta scolex and biomass
% Distance along intestine
0
4
8
12
16
20
24
0
10
20
30
40
50
60
70
80
90
100

Table XI. Percent recovery, scolex and biomass midpoints, dry weight worm, % worm destrobilated, dry weight per strobilated worm, and fecundity of H. diminuta after 4000 T. spiralis infection.

	Days after nematode infection						
	8	10	12	14	16	18	20
# of Rats	15	15	15	14	15	15	15
% Recovered	80	80	78	68	56	76	81.6
Scolex Midpt. (% Distance)	54.9 ±2.2*	49.5 ±3.5	50.5 ±2.3	43.6 ±3.2	46.3 ±3.3	37.3 ±3.1	32.5 ±1.9
Biomass Midpt. (% Distance)	74.8 ±2.2*	64.6 ±3.1	66.3 ±1.9	52.7 ±4.4	54.5 ±4.0	48.4 ±2.8	49.8 ±2.15
Dry weight per worm (mg)	78.0 ±11.3*	54.6 ±13.5	63.1 ±8.5	40.1 ±10.4	21.7 ±8.5	68.0 ±9.0	98.0 ±13.0
% Worms Destrobilated	11	31	25	53	90	18	0
Dry weight per strobilated worm (mg)	87.6	79.1	84.1	85.3	217.3	82.9	98.0
Fecundity (10^3 eggs/24h)	44	44	14	11	9	55	28

± one standard error.

Figure 15. Location of H. diminuta scolex and biomass midpoints after infection of 4000 T. spiralis larvae.


biomass midpoints
scolex midpoints
95 % C.I.
pt. estimate
% Distance along intestine
75
70
65
60
55
50
45
40
35
30
25
2
4
6
8
10
12
14
16
18
20
Days post T spiralis infection

recovery rates of single-species tapeworm infections. The decreased recovery may be attributed to failure to find all the scolices from the destrobilated tapeworms.

The number of eggs produced increased between Days 5 and 8, was constant between Days 8 and 10, decreased until Day 16 and then rose so that the numbers of eggs produced on Days 18 and 20 were greater than on Day 16. Eggs taken from the Day 18 and Day 20 infections, fed to Tenebrio molitor and the cysticercoids used to infect rats, produced normal egg producing adult tapeworms.

Histological Results

Severity of damage: Trichinella spiralis induced changes to the villi and glycocalyx of the intestinal mucosa were ranked as normal, slight, moderate, or severe.

The intestinal mucosa of uninfected rats revealed well formed villi with narrow lamina propria (Fig. 16), aligned columnar epithelium (Fig.17) and evenly stained glycocalyx (Fig. 18). Villi were observed to be approximately twice as long as the intestinal crypts were deep, and protruded straight into the lumen.

Stained sections of slightly damaged mucosa showed folded villi with larger than normal lamina propria (Fig. 19), both aligned and non-aligned columnar epithelial cells near folds in the villi (Fig. 20) and as well an evenly stained glycocalyx (Fig. 21) that appeared slightly thinner than normal. Villi were observed to be shorter than normal. Stained sections of moderate damaged mucosa showed flattened villi with a greatly expanded lamina propria (Fig. 22), aligned cuboidal epithelial cells with some loss of cell to cell junction (Fig. 23) and glycocalyx thin with a beaded appearance (Fig. 24). Haemorrhaging was apparent in these sections of moderately damaged tissue (Fig . 22) by blood cells outside the villi.

Stained sections of severe damaged mucosa showed villi not protruding above the crypt region, a larger than normal lamina propria (Fig. 25), non-aligned epithelial

cells of indefinite shape with loss of cell to cell junctions or loss of cells (Fig. 26) and a thin glycocalyx with a definite beaded appearance (Fig. 27). Haemorrhaging was apparent in these sections (Fig. 26).

A summary of pathological damage in the experiments and the number of nematodes recovered is presented in Tables XII, XIII,and XIV. Tissue samples were examined from rats harbouring the number of nematodes approximately equal to the average number recovered at a particular dose level or on a particular day.

Anterior tissue samples were taken from 10% along the intestine. Middle samples were taken from the 20 to 35% regions along the intestine. Posterior samples were taken from the 20 to 50% regions of the small intestine.

Experiment 4: A definite relationship existed between dosage level of T. spiralis and damage to the small intestine 5 days after nematode infection. In the anterior

Table XII. Pathological damage* to villi (v) and glycocalyx (g) of the rat small intestine at five dose levels of T. spiralis after 5 days infections.

dose level		Dose Levels 500	1000	2000	3000	4000
# of T. spiralis recovered		212	328	692	1156	1646
anterior	(v)	+	++	++	+++	+++
	(g)	0	+	++	+++	+++
middle	(v)	+	++	++	++	++
	(g)	0	0	+	+	+
posterior	(v)	+	+	+	+	+
	(g)	0	0	0	0	0

0 normal
\+ slight
++ moderate
+++ severe

Table XIII. Pathological damage* to villi (v) and glycocalyx (g) of the rat small intestine during the intestinal phase after infection of 1000 T. spiralis larvae.

		Days						
		1	2	5	8	12	16	20
# of T. spiralis recovered		460	460	328	224	4	0	0
anterior	(v)	+	+	++	++	+	+	+
	(g)	0	0	+	++	0	0	0
middle	(v)	0	0	++	++	+	+	0
	(g)	0	0	0	++	0	0	0
posterior	(v)	0	0	+	0	0	0	0
	(g)	0	0	0	0	0	0	0

0 normal
\+ slight
++ moderate
+++ severe

Table XIV. Pathological damage* to villi (v) and glycocalyx (g) of the rat small intestine during the intestinal phase after infection of 4000 T. spiralis larvae.

		Days							
		5	8	10	12	14	16	18	20
# of T. spiralis recovered		1646	1476	668	544	460	20	4	0
anterior	(v)	+++	+++	++	++	++	++	+	+
	(g)	+++	+++	++	++	+	+	0	0
middle	(v)	++	++	++	+	+	+	+	+
	(g)	+	++	0	0	0	0	0	0
posterior	(v)	+	+	+	+	+	+	+	+
	(g)	0	0	0	0	0	0	0	0

0 normal
\+ slight
++ moderate
+++ severe

regions of the small intestine villi damage was slight at the 500 dose level (Figs. 19, 20), moderate at the 1000 and 2000 dose levels (Figs. 22, 23), and severe at the 3000 and 4000 dose levels (Figs. 25, 26). All the middle regions with the exception of the 500 dose level showed moderate damage with some haemorrhaging (Figs. 22, 23). The middle 500 dose level region and the posterior regions from the 500, 1000, 2000, 3000 and 4000 dose levels showed slight damage (Figs. 16, 17). The beaded appearance of the glycocalyx was present at all dose levels higher than the 500 dose level in the anterior regions. The glycocalyx appeared normal at the 500 dose level (Fig. 18). Slight damage was apparent at the 1000 dose level (Fig. 21), moderate damage was apparent at the 2000 dose level (Fig. 24) and severe damage was apparent at the 3000 and 4000 dose level (Fig. 27).

Generally, as the dose level increased the villi shortened in relation to the crypt depth, lamina propria increased in width, epithelial cells became more unaligned, lose their columnar shape to become cuboidal and then have indefinite shape and there was an increase in loss of cell to cell junction. Fluid and blood accumulation was observed to increase and beaded appearance became more pronounced as the dose level increased (Table XII).

Experiment 5: Morphological changes in the anterior regions were apparent by the 1st day after an initial infection of 1000 T. spiralis larvae and were ranked as slight (Figs. 16, 17). The anterior regions of Days 5 and 8 revealed moderate changes (Fig. 22) while the remainder of the days showed slight changes in the anterior regions. Cuboidal cells were in evidence on Day 5 only (Fig. 23). Beading of the glycocalyx was seen only on Days 5 and 8 and was moderate in appearance (Fig. 24).

The middle regions of the small intestine 1 and 2 days after infection appeared normal (Figs. 16, 17), moderate damage was apparent on Days 5 and 8 (Figs. 22, 23) and slight damage was apparent on Days 12 and 16 (Figs. 19, 20). Villi on Day 20 appeared normal but were longer. The beaded appearance of the glycocalyx was observed to be present on Days 5 and 8 and was moderate in appearance (Fig. 24).

All the posterior regions showed a normal villi and glycocalyx pattern (Figs. 16, 17, 18).

Generally, the greatest damage to the small intestine during the intestinal phase of an initial dose of 1000 T. spiralis larvae was confined to the area occupied by the nematodes and was most evident on Days 5 and 8 (Table XIII).

Experiment 6: Morphological changes in the anterior regions were severe on Days 5 and 8 (Figs. 25, 26), moderate on Days 10, 12, 14, and 16 (Figs. 22, 23) and slight on Days 18 and 20 (Figs. 19, 20) after initial infections of 4000 T. spiralis larvae. Cuboidal cells were evident until Day 10, blood and fluid accumulation, and wider than normal lamina propria were evident until Day 12. The epithelial lining failed to remain intact and haemorrhaging was apparent until Day 14, and unaligned epithelial cells and the beaded appearance of the glycocalyx were evident until Day 16. A pronounced beaded appearance was apparent on Days 5 and 8 (Fig. 27), moderate on Days 10 and 12, slight on Days 14 and 16 (Fig. 21) and normal on Days 18 and 20.

The middle regions of Days 5 through 10 showed moderate damage (Figs. 22, 23) and Days 12 through 20 showed slight damage (Figs. 20, 21). A moderate beaded appearance was apparent only on Days 5 and 8 (Fig. 24) and in the remaining days the glycocalyx appeared normal (Fig. 18).

The posterior regions showed slight damage on Days 5 through 20 (Figs. 19, 20) and a normal glycocalyx (Fig. 18).

Generally, the damage to the glycocalyx of the small intestine was more intense after an initial infection of 4000 nematode larvae than after an initial infection of 1000 nematode larvae, but followed the same pattern during the nematode intestinal phase (Table XIV).

Figure 16. Morphology of a normal villus taken from the small intestine of an uninfected rat (region A, Fig. 1). x 1250.
Giemsa stained 1.5 um methacrylate section. Bright-field microscopy. Abbreviations: bv - blood vessel; ep - epithelial cell; lp - lamina propria.

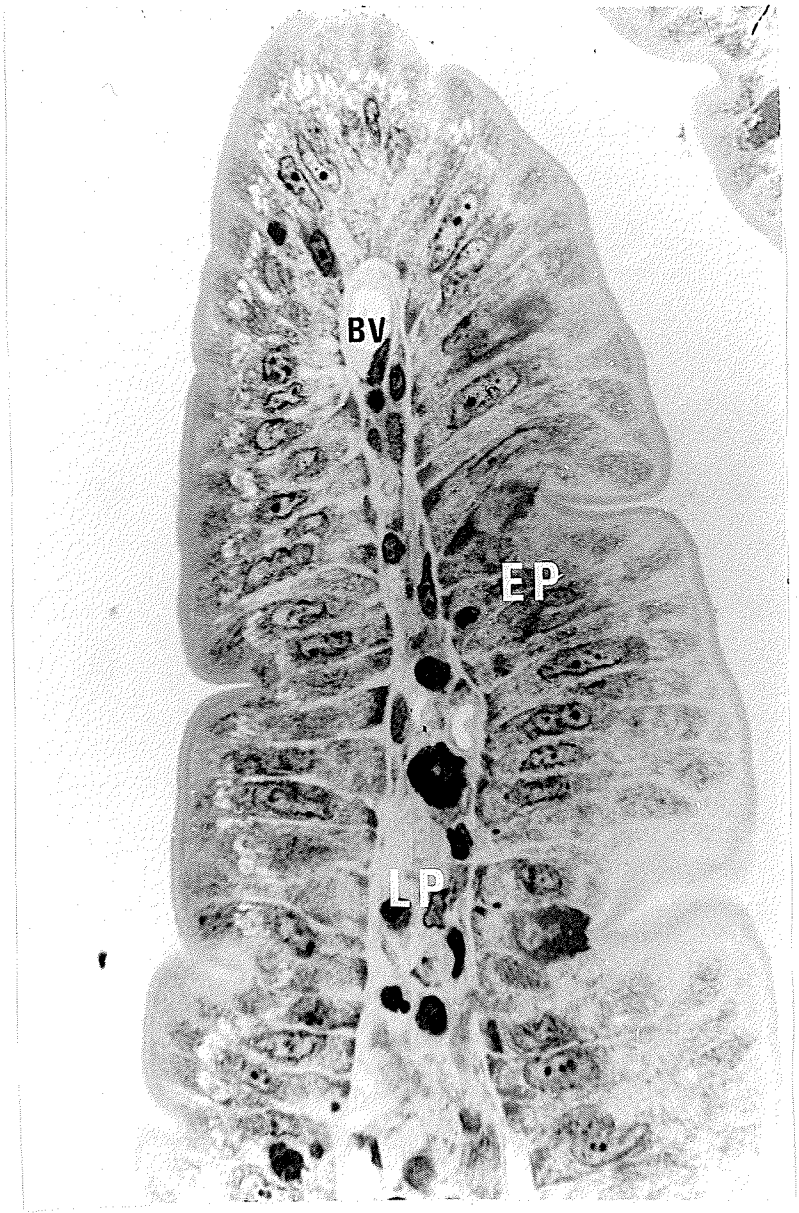

BV
EP
LP

Figure 17. Morphology of epithelial cells of a normal villus taken from the small intestine of an uninfected rat (region A, Fig. 1). x 1875. Giemsa stained 1.5 um methacrylate section. Bright-field microscopy. Abbreviations: bv - blood vessel; ep - epithelial cell; icj - intercell junction; lp - lamina propria.

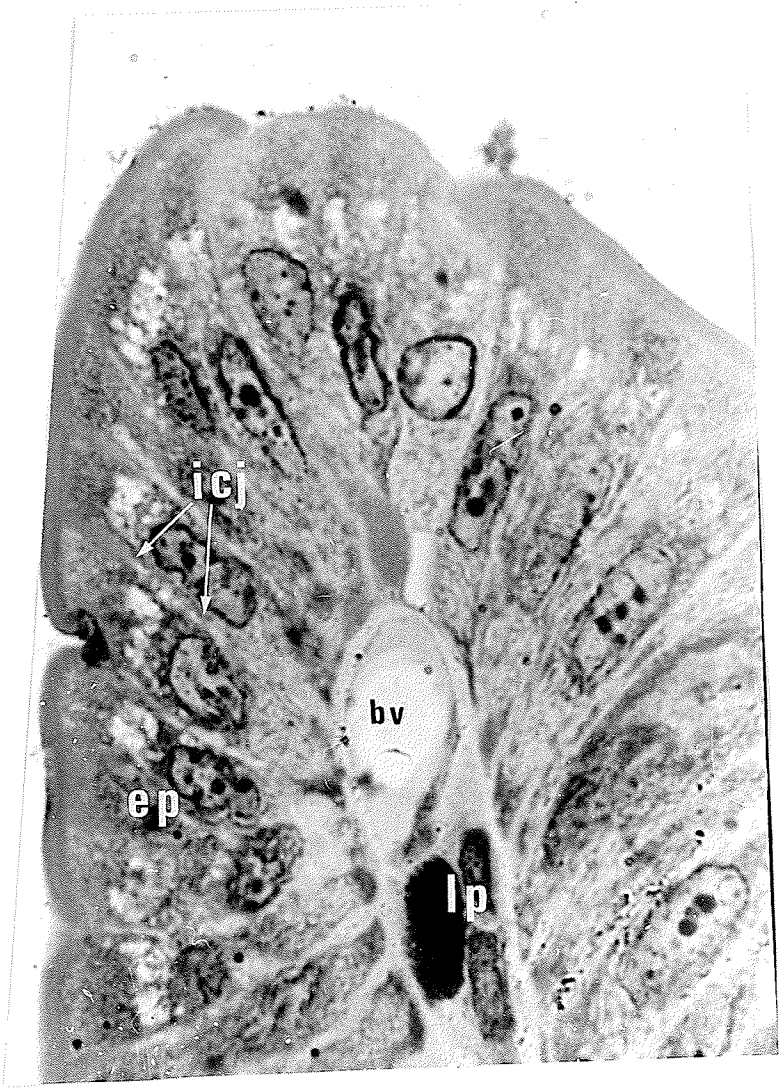

icj
bv
ep
lp

Figure 18. Morphology of the glycocalyx of a normal villus taken from the small intestine of an uninfected rat (region A, Fig. 1). X 3125.
PAS stained 1.5 um methacrylate section.
Interference microscopy. Arrows indicate extent of brush border.
Abbreviations: g - glycocalyx; icj - intercell junction.
Note: smooth appearance of glycocalyx.

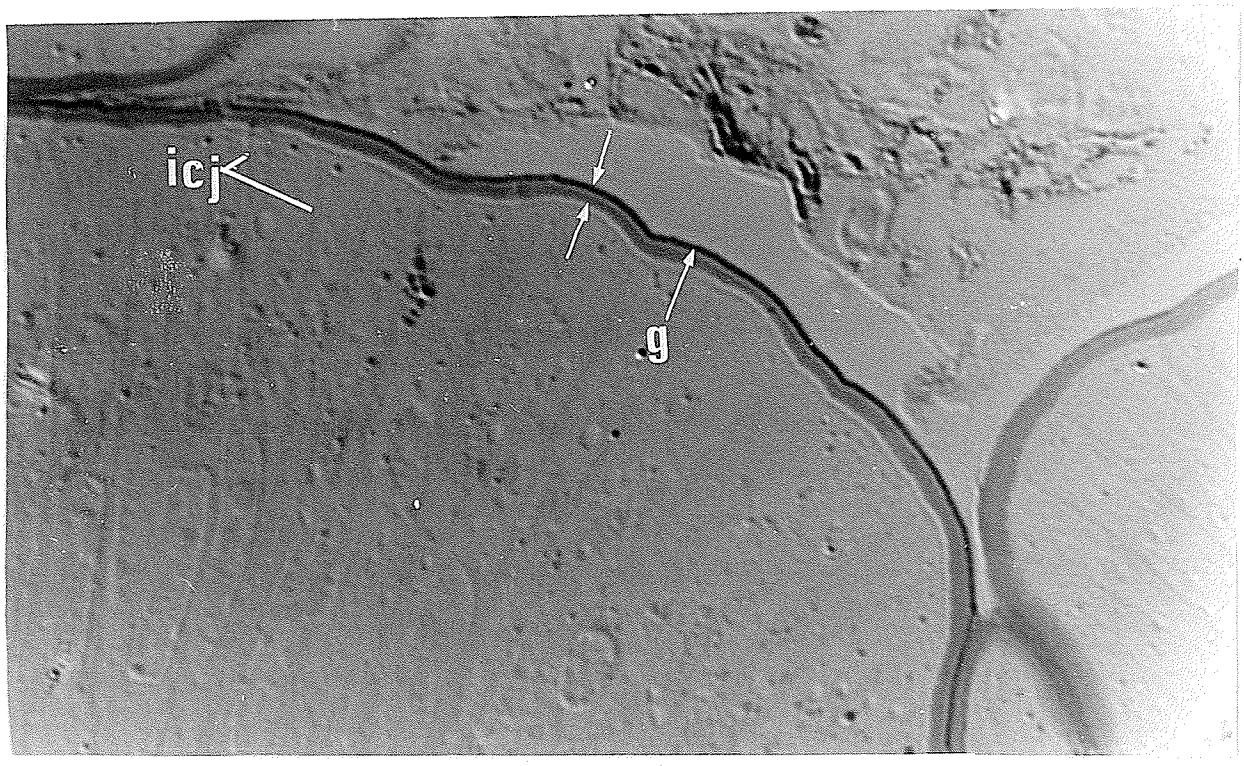

icj
g

Figure 19. Morphology of a slightly damaged villus taken from the small intestine of a rat (region C, Fig. 1) infected 12 days previously with 4000 T. spiralis. X 1800.
Giemsa stained, 1.5 um methacrylate section. Bright-field microscopy. Abbreviations: cr - crypt regions; ep - epithelial cells; gc - goblet cell; lp - lamina propria.

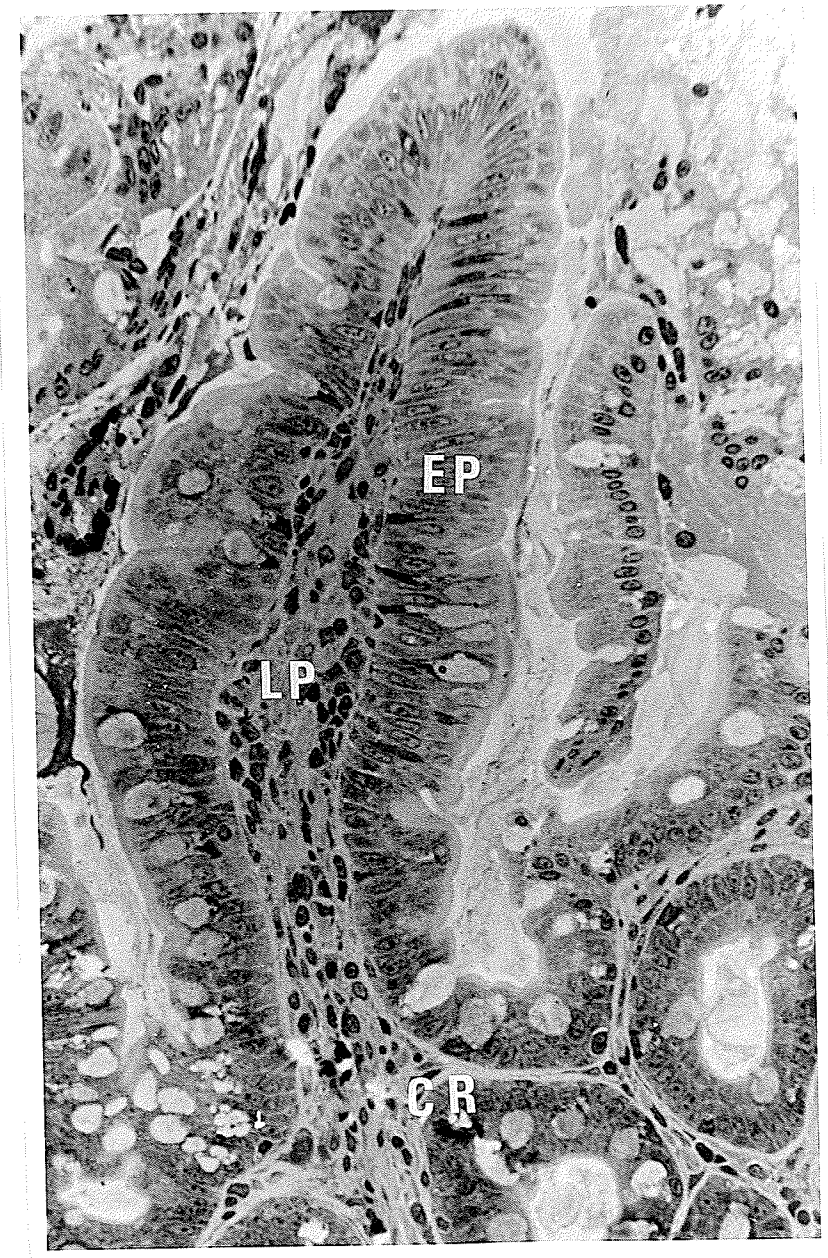

EP
LP
CR

Figure 20. Morphology of epithelial cells of a slightly damaged villus taken from the small intestine of a rat (region C, Fig. 1) infected 12 days previously with 4000 T. spiralis larvae. X 2000. Giemsa stained, 1.5 um methacrylate section. Bright-field microscopy. Abbreviations: ep - epithelial cells; icj - intercell junction; lp - lamina propria; vf - villous fold.

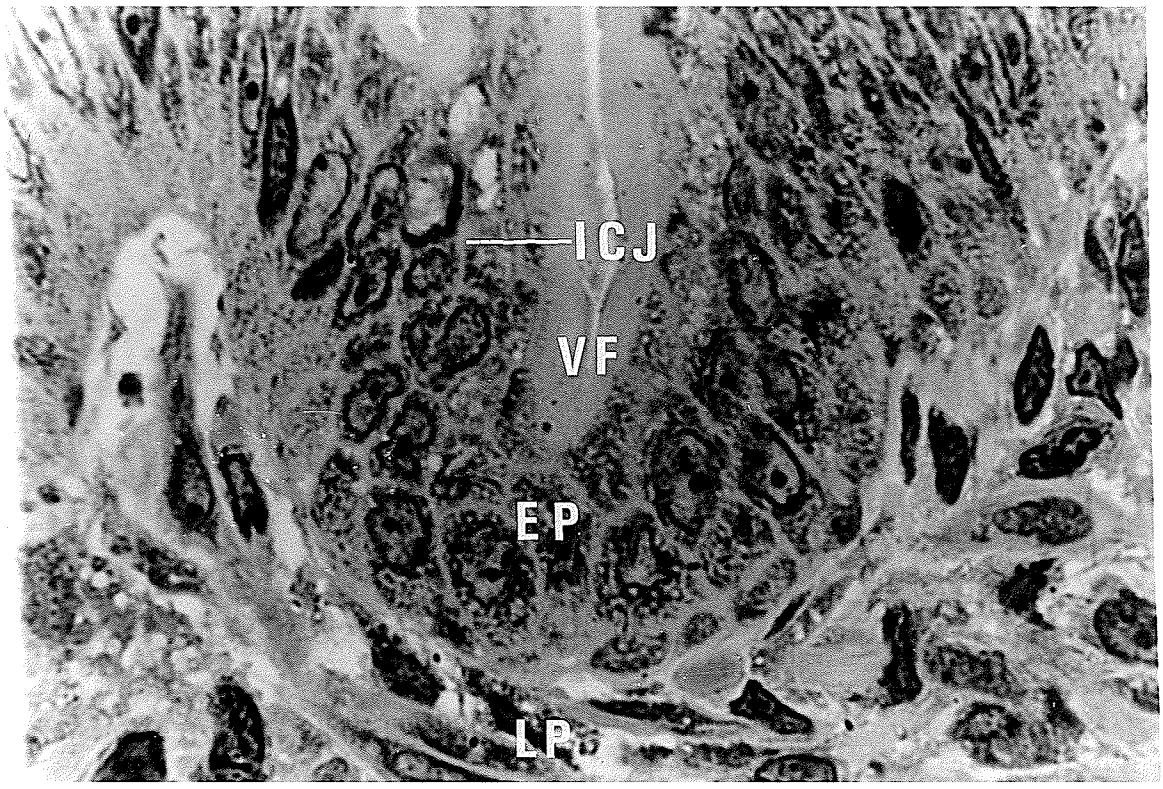

ICJ
VF
EP
LP

Figure 21. Morphology of the glycocalyx of a slightly damaged villus taken from the small intestine of a rat (region C, Fig. 1) infected 12 days previously with 4000 T. spiralis. X 3750. PAS stained, 1.5 um methacrylate section. Interference microscopy. Arrows indicate extent of brush border. Abbreviations: ec - epithelial cells; g - glycocalyx; gc - goblet cell.
Note: slight beading of glycocalyx.

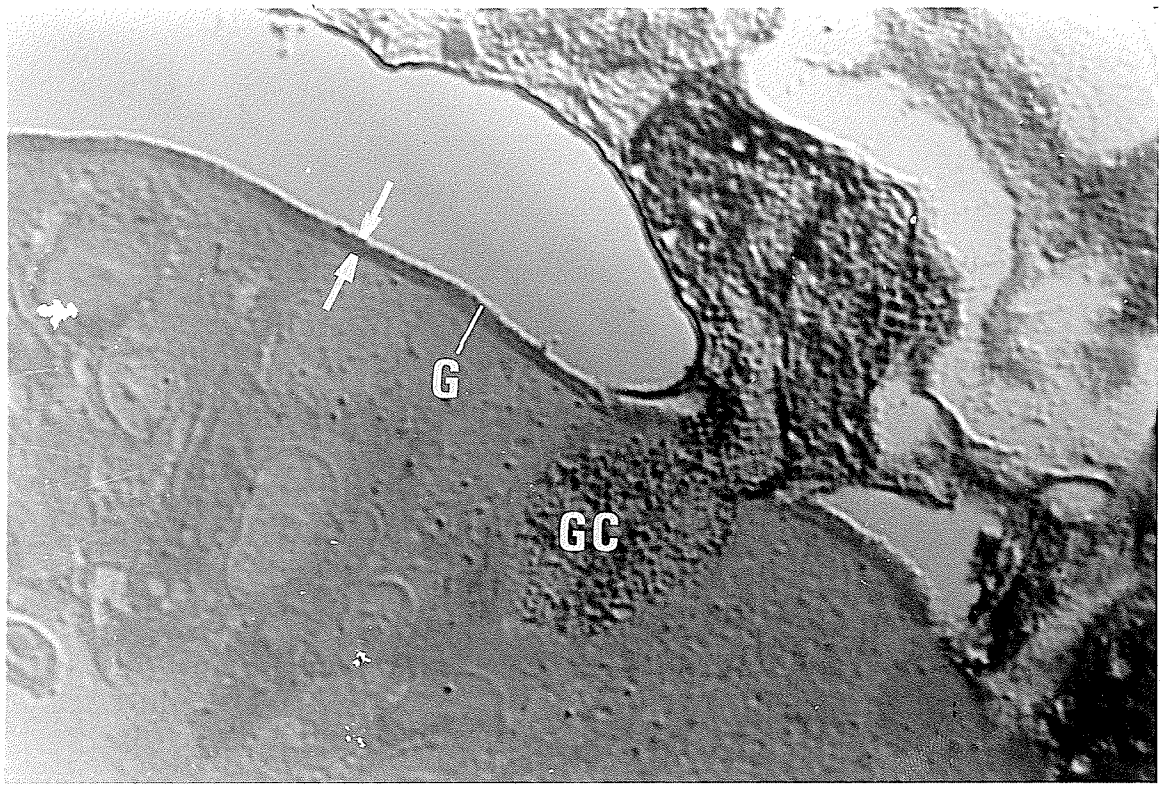

G
GC

Figure 22. Morphology of a moderately damaged villus taken from the small intestine of a rat (region B, Fig. 1) infected 8 days previously with 4000 T. spiralis. X 512.
Giemsa stained, 1.5 um methacrylate section. Bright-field microscopy. Abbreviations: bv - blood vessel; cr - crypt region; ep - epithelial cells; lp -lamina propria, rbc - red blood cells.

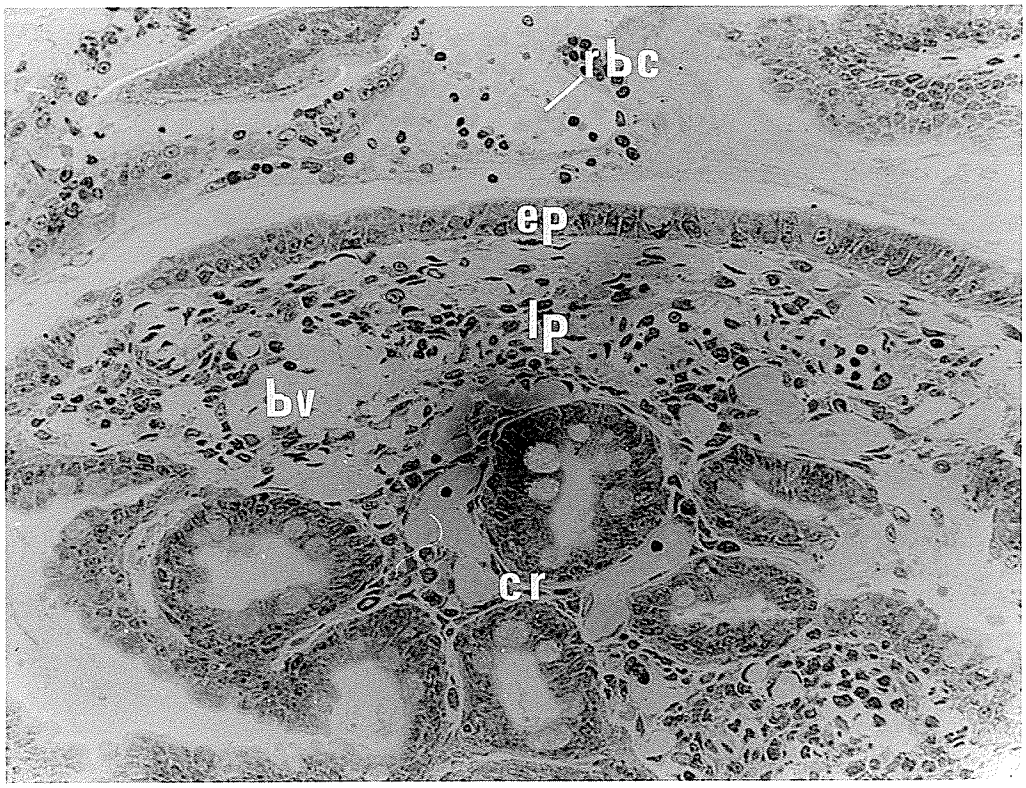

rbc
ep
lp
bv
cr

Figure 23. Morphology of epithelial cells of a moderately damaged villus taken from the small intestine of a rat (region B, Fig. 1) infected 8 days previously with 4000 T. spiralis larvae. X 1575. Giemsa stained, 1.5 um methacrylate section. Bright-field microscopy. Abbreviations: bv - blood vessel; ep - epithelial cells; ics - intercell space; lp - lamina propria.

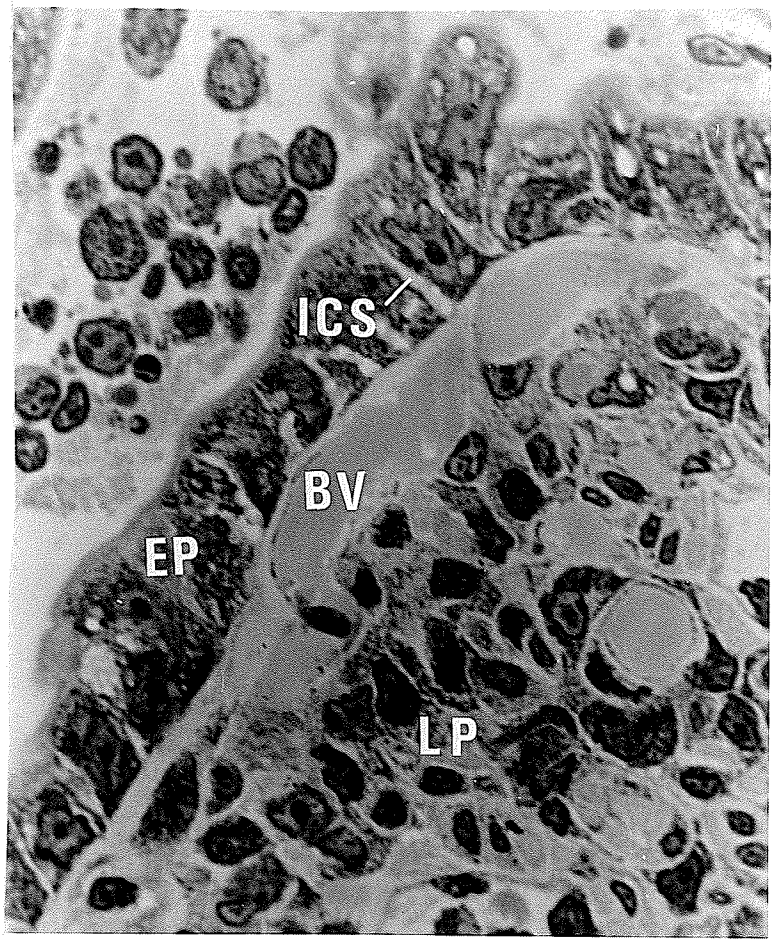

ICS
BV
EP
LP

Figure 24. Morphology of the glycocalyx of a moderately damaged villus taken from the small intestine of a rat (region B, Fig. 1) infected 8 days previously with 4000 T. spiralis larvae. X 3780. PAS stained, 1.5 um methacrylate section. Interference microscopy. Arrows indicate extent of brush border. Abbreviations: g - glycocalyx; gc - goblet cell; ics - intercell space.

Note: moderate beading of glycocalyx.

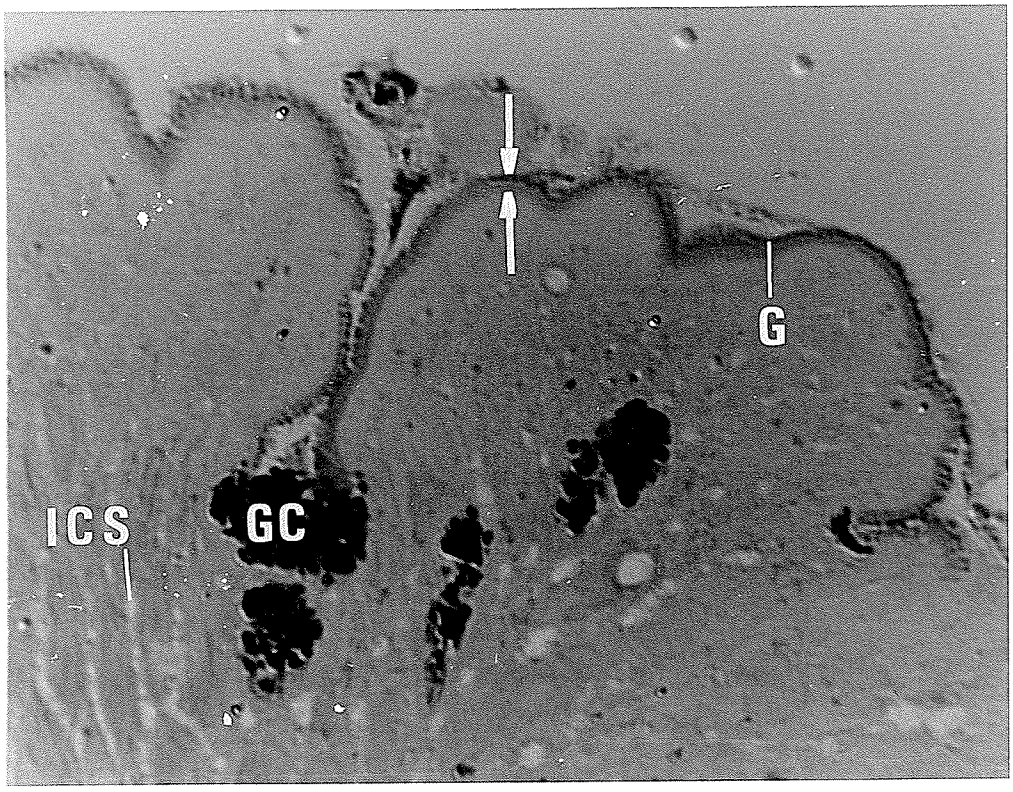

G
GC
ICS

Figure 25. Morphology of a severely damaged villus taken from the small intestine of a rat (region A, Fig. 1) infected 5 days previousl with 4000 T. spiralis larvae. X 100.
Giemsa stained, 1.5 um methacrylate section. Bright-field microscopy. Abbreviations: cr - crypt region; ep - epithelial cells; lp - lamina propria.

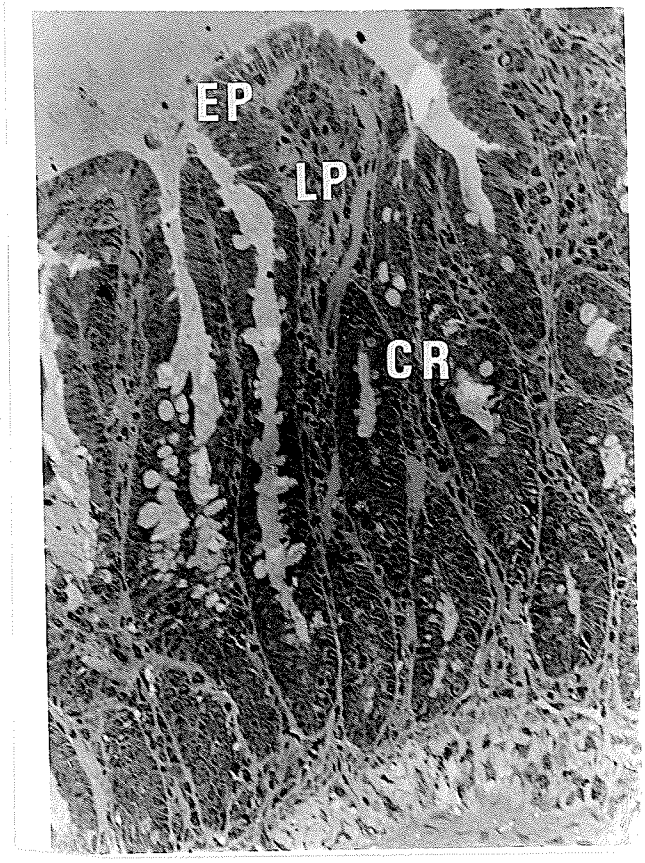

EP
LP
CR

Figure 26. Morphology of epithelial cells of severely damaged villus taken from the small intestine of a rat (region A, Fig.1) infected 5 days previously with 4000 T. spiralis larvae. X 2500. Giemsa stained, 1.5 um methacrylate section. Bright-field microscopy. Abbreviations: bv - blood vessle; ep - epithelial cells; ics - intercell space; lp - lamina propria; rbc - red blood cells.

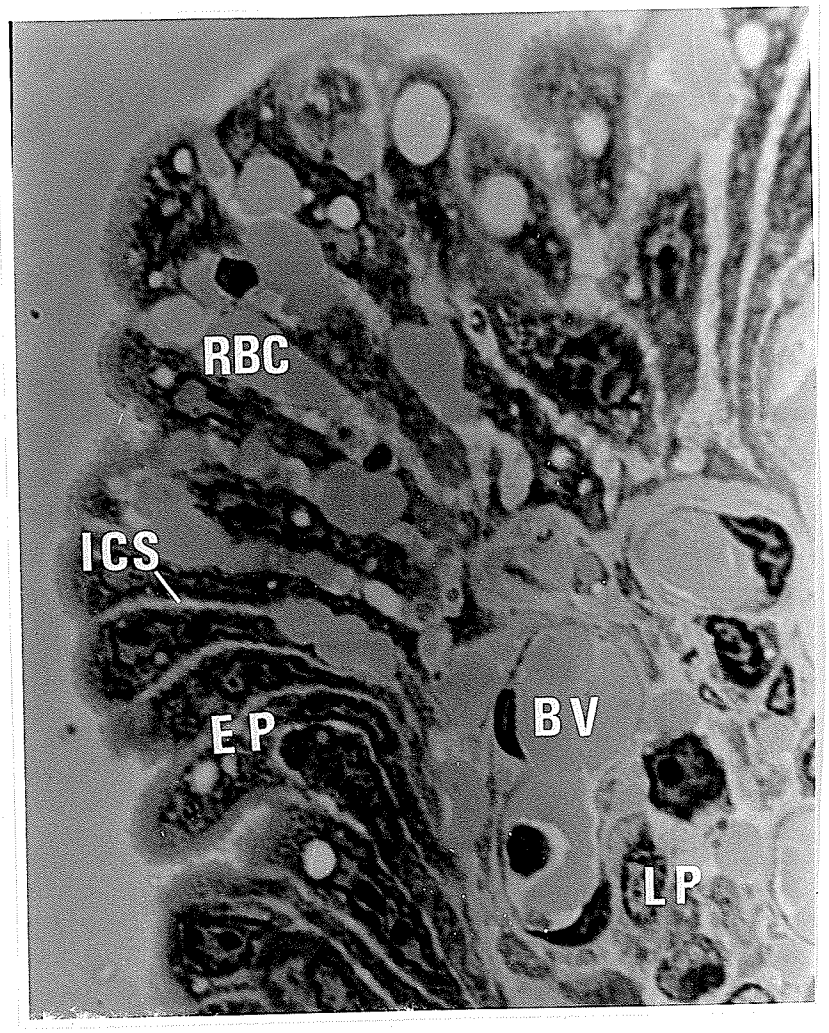
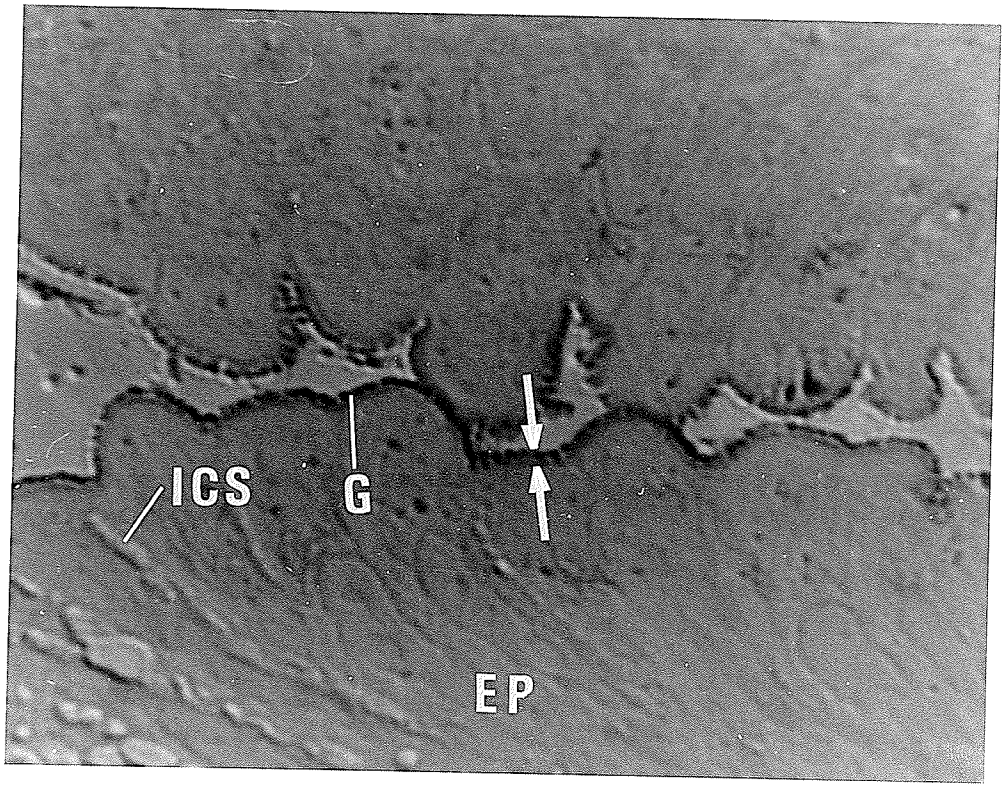

RBC
ICS
EP
BV
LP

Figure 27. Morphology of the glycocalyx of severely damaged villus taken from the small intestine of a rat (region A, Fig. 1) infected 5 days previously with 4000 T. spiralis larvae. X 2500. PAS stained, 1.5 um methacrylate section. Interference microscopy. Arrows indicate extent of brush border. Abbreviations: ep - epithelial cells; g - glycocalyx; ics - intercell space.
Note: severe beading of glycocalyx.


ICS
G
EP

DISCUSSION

Single-Species T. spiralis Infections

T. spiralis in single-species infections is distributed anteriorly in the small intestine of rats and this distribution is similar at the three dose levels examined. This agrees with Gursh (1949). Examination of every five percent region has shown the distribution more specifically than that previously shown for examination of quarter intestinal regions (Gursh, 1949; Larsh and Hendricks, 1949; Smith and Castro, 1975). This is of considerable importance when one studies possible shifts in populations in concurrent infections as normal distribution of T. spiralis is so restricted.

Location of T. spiralis in the small intestine varies in different hosts and in hosts of different ages. Posterior location of nematodes was reported in rats of unknown age (Tyzzer and Honeji, 1916), one month old rats and mice (Larsh and Hendricks, 1949) and 56 day old mice (Denham, 1968). Anterior location of nematodes was reported in 130-140 day old mice (Larsh and Hendricks, 1949), 21-35 day old mice (Campbell, 1967), young and old mice and guinea pigs (Dick and Silver, unpublished data) and a mid to posterior location in hamsters (Dick and Silver, unpublished data). An anterior to posterior shift in T. spiralis distribution during its intestinal phase occurs in mice (Larsh, et al.,

1952 and Gouldson, 1958) and in guinea pigs (Castro, et al., 1967) but was not found in rats (Dick and Silver, unpublished data). Location of the nematodes depends on host and host age and may indicate a reliance of nematodes on the physiology of the small intestine of the host rather than specific site-finding mechanisms of the parasite. Emptying time of the stomach and intestinal motility may influence the nematode distribution (Larsh and Hendricks, 1949). Morphine sulphate intraperitoneal injections slowed stomach emptying time and intestinal motility and reversed the nematode distribution in young mice. This compound influences the flow of bile and pancreatic juices by closing the sphincter of the common bile duct during the first hour after administration (Menguy, In Mallenbeck, 1967) therefore the effect of morphine sulphate on the small intestine is not restricted only to intestinal motility. The bile duct of rats is located 9-12% along the intestine (Cannon and Mettrick, 1970) and the bile and pancreatic juice secretions or associated changes in pH may influence the location of T. spiralis in this region.

Recovery rates of T. spiralis in single-species infections is similar at the three dose levels and the average recovery rate of nematodes was 28.5%. This is low compared to other values reported in the literature. Gursh (1949)

reported 50% recovery, McCoy (1932) 62% recovery, and Nolf and Zaiman (1941) 73% recovery of nematodes in rats 5 days after infection. Various T. spiralis strains have been identified by the variation in the number of muscle larvae recovered (Kozar and Kozar, 1965; Arakawa and Todd, 1971). It was also shown that strain differences of the host may affect the recovery of T. spiralis during the intestinal phase (Rivera-Ortiz and Nussenzweig, 1976). Therefore, the low recovery of T. spiralis in this experiment may be due to the effect of the strain of the nematode or the strain of host.

Single-Species H. diminuta Infections

The distribution and dry weight per tapeworm of H. diminuta reported in this study for single-species five tapeworm infections (Table II) agrees with the information in the literature (Holmes, 1961; Hesselberg and Andreassen, 1975). Position did not depend on host sex as Mettrick and Dunkley (1969) reported. The average scolex midpoints of 23.9% along the intestine agrees with the 19 and 20% location of scolex midpoints reported by Holmes (1961) and Hesselberg and Andreassen (1975) for five tapeworm infections. The literature contains information dealing with biomass position of one and ten worm infections therefore allowing

comparison of the relative positioning of the biomass to the scolex position. The scolex midpoints of one worm infections (Goodchild, 1960; Braten and Hopkins, 1969; Turton, 1971) are anterior to the scolex midpoints of five worm infections and the scolex midpoints of ten worm infections (Mettrick and Dunkley, 1969; Cannon and Mettrick, 1970) are posterior to the scolex midpoints of five worm infections. This pattern was shown by Hesselberg and Andreassen (1975). However, the biomass midpoints of five worm infections is posterior to both one and ten worm infections. That the biomass of five worm infections is posterior to ten worm infections was not expected as Braten and Hopkins (1969) suggested that the scolex position was determined by the position of the strobila in single worm infections.

Examination of the scolex distribution of ten worm infections (Mettrick and Dunkley, 1969) reveals a bimodal distribution which is not present in five worm infections (Fig. 3). The different relative scolex and biomass positioning of worms from different population levels may be attributed to an intraspecific competition occurring in ten worm infections. Intraspecific competition results in decreased weight per tapeworm (Read, 1951; Roberts, 1961) and the weight loss may be associated with the tapeworm distribution.

The dry weight per tapeworm of 20 and 30 day old worms is statistically similar though 30 day old worms weigh less than 20 day old worms (Table II). This supports the observations of Braten and Hopkins (1969) with one worm infections and Holmes (1961) with five worm infections where tapeworm weight was found to increase until the 3rd week after infection and then leveled off before decreasing slightly by the 4th week.

The average dry weight per tapeworm found in this study was 183 ± 20 mg for 20 day old worms and 130 ± 8 mg for 30 day old worms. The dry weight per tapeworm in five worm infections in rats after the 16 to 20 day prepatent period (Roberts, 1961) was 200 mg for 21 day old worms; 160 mg for 28 day old worms (Holmes, 1961); 174.1 mg for 17 day old worms (Roberts, 1961); 142.75 mg for 19 day old worms (Rigby and Chobator, 1966); and 62 mg for 20 day old worms (Hesselberg and Andreassen, 1975). The low weight values of Hesselberg and Andreassen (1975) may be explained by their use of "specific pathogen-free" (SPF) rats, which, because of their lack of intestinal microflora, may influence the growth of the tapeworms. Intestinal microflora influences bile acid metabolism and fat absorption (Rosenberg, In Mettrick, 1971c) and their absence would alter the intestinal environment of SPF rats when compared to normal rats. The dry weight is not affected by host sex and this corroborates the findings of Mettrick and Dunkley (1969) on tapeworm weight and of Addis (1946) on tapeworm length.

Although there is ample information on the development of H. diminuta to the egg producing stage (Chandler, 1939; Holmes, 1962a; Hopkins, 1969; Cannon and Mettrick, 1970) there is only limited information on egg production (Beck, 1951; Hesselberg and Andreassen, 1975). Little work has been done on egg production at various population levels over extended periods of time and consequently extrapolation between this work and other studies is difficult. Egg production in this study averaged 53,000 eggs by 20 day old worms and 76,000 eggs by 30 day old worms. The increase in egg production by older worms agrees with that found by Beck (1951) who showed that in single-species infections egg production by 20 day old worms increased from 50,000 to an egg production by 30 day old worms of 150,000. Similar findings to this study were found by M. Boddington (personal communication)[1] with five worm infections.

Average recovery in five worm infections is 80%, which agrees with the 81% of Roberts (1961) and 91% of Holmes (1961) both of whom worked with 5 worm infections.

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[1] (Canadian Society of Zoology meetings, June 1976, from M. Boddington, Department of Zoology, University of Toronto).

Trichinella spiralis in Concurrent Infections

The average nematode midpoints of *T. spiralis* in concurrent infections are 15.1% (Experiment 4), 15.2% (Experiment 5) and 16.2% (Experiment 6) which are significantly anterior to the average nematode midpoint of 21.4% in single-species infections. This change in nematode midpoint would be detected only by examination of five percent regions of the intestine. The recovery rate of nematodes after 5 days of concurrent infection (45.4%) is 63% greater than the recovery rate of nematodes in single-species infections (28.5%). Under the experimental conditions of this study, concurrent infections with *H. diminuta* enhance nematode infections. Anterior changes of distribution were shown with *H. polygyrus* when infected into mice harbouring *H. microstoma* (Courtney and Forrester, 1973). They reported a decreased recovery of the nematodes in concurrent infections and suggested that *H. microstoma* unfavourably altered the physiochemical character of the paramucosal lumen of the intestine. It is well known that *H. diminuta* alters the intestinal physiochemical nature of the rat small intestine by decreasing pH and pCO_2 levels (Podesta and Mettrick, 1974), by decreasing the amount of TCA-insoluble carbohydrates, TCA-soluable nitrogen (non-protein nitrogen) and lipid levels and by adversely affecting the microbial flora (Mettrick, 1971c).

In concurrent infections with H. diminuta, Read and Phifer (1959) showed reduced weight of H. citelli and Holmes (1961) showed reduced weight, length, weight:length ratio and a slight anterior shift in the distribution of M. dubius in rats. These authors suggested that nutrient competition with H. diminuta adversely affected the other parasites. As T. spiralis feeds on host tissue and H. diminuta absorbs nutrients which are present in the intestinal lumen, nutritional competition would not be expected between these two parasites.

The nematode midpoints in concurrent infections showed a significant posterior trend with increasing nematode dose levels reflecting a posterior spreading of the distribution. Examination of Fig. 6 shows that the distributional mode always occurred in the 5% to 10% region of the intestine though total numbers increased with higher nematode doses. This suggests that the nematodes prefer a specific region of the intestine and with increasing numbers of nematodes, competition for space forces nematodes into more posterior regions.

This is the first study to show the effects of concurrent infections at different times during the nematode intestinal phase in a nematode-cestode system. The length of the intestinal phase of T. spiralis after

1000 larvae infections in concurrent infections is between 12 and 15 days (Table VII) and after 4000 larvae infections is between 18 and 20 days (Table IX). The larger the inoculum the longer the intestinal phase. This supports McCoy (1932), Gursh (1949), and Castro, et al. (1967) who reported increased duration of the intestinal phase of larger T. spiralis inocula and they showed the length of the intestinal phase to vary between 15 and 21 days. The duration of the intestinal phase of a 2000 larvae single-species infection using the same strain of rat and parasite is 18 days (Dick and Silver, unpublished data). Therefore, the duration of the nematode intestinal phase is not affected by concurrent infections with H. diminuta.

McCoy (1932) suggested that heavy infections break down a resistance in the rats which ordinarily limit the duration of adult trichinae in the intestine of the rats and hence in heavier infections T. spiralis remains in the intestine longer.

Hymenolepis diminuta in Concurrent Infections

Hymenolepis diminuta responds to concurrent infections of T. spiralis by locating more posteriorly in the intestine. The higher the dose level of T. spiralis the more posteriorly situated are the tapeworms and the greater the loss of

parasite weight (Table VI). During the intestinal phase of T. spiralis, the tapeworms are at their most posterior position on Day 8 of a 1000 larvae infection and Day 5 and 8 for a 4000 larvae infection (Figs. 12, 15). The response of H. diminuta to T. spiralis does not seem to be due to direct competition because (1) there is a more drastic posterior shift in the tapeworms than would be expected from a relatively non-shifting nematode distribution, (2) tapeworm distribution was affected when no nematodes were present on Day 16 in Experiment 5, and (3) tapeworm distribution changes were independent of changes in the number of nematodes present in the small intestine (Table X).

It is possible that in the present study tapeworms are responding to a non-specific inflammatory reaction of the intestine caused by the nematode infection rather than the nematodes themselves. No tapeworms were present in the highly inflammed regions of the anterior small intestine. Tissue samples taken from areas near the peak numbers of nematodes showed the greatest damage. Tissue samples taken from the middle region (Fig. 1-B) between the nematodes and the tapeworms showed less pathology than sections more anterior, but greater pathology than sections more posterior. In Experiments 3 and 4 it was shown that tapeworms were more posterior as the nematode dose level increased and it was shown that increasing the nematode population increased pathological damage (Table XII).
Damage progressively decreased posteriorly along the intestine. The gradation of damage along the intestine

supports the findings of Castro, et al. (1967) in guinea pigs and Richardson and Olson (1974) in mice.

The most posterior position occupied by the tapeworms during the nematode intestinal phase occurred on days of the greatest intestinal damage (Day 5 in 1000 larvae infections, Days 5 and 8 in 4000 larvae infections) (See Tables XIII, XIV). Also tapeworms recovered from rats initially infected with 4000 nematode larvae were more posterior on similar days (Days 5, 8, 12, 16, and 20) than tapeworms recovered from rats infected with 1000 nematode larvae (Figs. 11,14). The more posterior position of tapeworms and their relative positioning is related to the pathology observed on each similar day after the nematode infection.

Interspecific interaction possibly mediated through a non-specific host response was shown by Cross (1934) in natural infections between P. exiguus and acanthocephalan species of "Neoicanthorinchus" in ciscoes. It has also been shown by Larsh and Donaldson (1944) in experimental infections of H. nana and T. spiralis in mice, and by Louch (1962) using T. spiralis and N. brasiliensis in rats.

Further evidence to support the idea that distribution of H. diminuta is influenced by the host response to T. spiralis is found in the following observations.

Tapeworms in cortisone treated T. spiralis infected rats are more anterior and their weights are similar to the weights of tapeworms in single-species infections (Dick and Silver, unpublished data), but greater than the weights of tapeworms in untreated T. spiralis infected rats. H. diminuta is adversely affected by a host immune response (Hopkins, et al., 1972a, b) and it is thought that the immunoglobulin covering (Befus, 1975) results in decreased digestive absorption function of the tegument. In multiple tapeworm infections immune damage is greater (Befus and Threadgood, 1975) and rejection is faster in mice (Befus, 1975) than in single tapeworm infections. It is possible that concurrent infections with T. spiralis produces a greater effect toward the tapeworm by an enhancement of the immune response, therefore increasing the amount of immunoglobulins covering H. diminuta. It has also been shown that T. spiralis shares antigens with cestodes (Oliver-Gonzalez, In Weinmann, 1964), and it is possible that a cross-immunity may occur in this present study. Interspecific interactions possibly due to cross-immunity were shown by Heyneman (1953, 1962) in experimental infection of H. diminuta and H. nana in rats and by Weinmann (1964) in experimental infections of T. spiralis and H. nana in rats.

A note of caution on the interpretation of the present data: Nippostrongylus brasiliensis does not affect the distribution and weight of established H. diminuta

(Hendrix, et al., 1975). This nematode produces pathological conditions similar to T. spiralis in the rat small intestine, but the distribution and hence the areas of the small intestine affected are more variable. Brambell (1965) reported a similar distribution of N. brasiliensis to T. spiralis whereas Symons and Fairbairn (1963), Loehry and Creamer (1969) and Symons (1976) found N. brasiliensis distributed primarily in the jejunum of the small intestine. This discrepancy in N. brasiliensis distribution, and the lack of response by H. diminuta to N. brasiliensis makes it difficult to generalize as to the importance of the immune response on this tapeworm in concurrent infections. Similarly, there is a lack of response by H. diminuta to T. spiralis infections in hamsters (Dick and Silver, unpublished data), and in hamsters, T. spiralis is distributed in the mid and posterior regions of the small intestine (Dick and Silver, unpublished data). Therefore, location of the parasite eliciting an immune response may be another factor important in determining the effect of concurrent infections on H. diminuta. It is interesting to speculate that since the germinative region of the tapeworm is sensitive to adverse conditions induced in the gut by crowding (Roberts, 1961), the location of a nematode population near or anterior to this region of the tapeworm may be an important factor in determining the response of H. diminuta.

Other conditions associated with T. spiralis infections may affect H. diminuta. The beaded appearance of the glycocalyx (Figs. 24, 27) may affect the spatial arrangement of enzymes resulting in glucose malabsorption found in T. spiralis infected animals (Castro, et al., 1967). Malabsorption of glucose might lead to a higher concentration of glucose in the intestine which may be detrimental to the tapeworms (Hopkins, 1970). It has not been determined if the level of glucose is increased sufficiently to affect the tapeworms. Also it is known that animals with T. spiralis infections have a loss of appetite during the early stages of infection (Castro and Olson, 1967). The beaded appearance of the glycocalyx found in this study has not been shown before in T. spiralis or N. brasiliensis infections. More detailed histochemical and ultrastructural studies are required to evaluate it properly. Symons (1976) reported shorter and less dense microvilli in rats infected with N. brasiliensis and reported clumping of the microvilli. Whether the beaded appearance of the glycocalyx results form changes in the underlying microvilli or a modification of the glycocalyx results in clumping of the microvilli is now known at this time.

Tapeworms recovered after infections of 1000 T. spiralis (Experiment 5) showed positioning posterior to single-species infections, normal weight and reduced egg

production (Table VIII). Holmes (1961, 1962a, b) examined the position, length, and weights of H. diminuta concurrently infected with M. dubius but did not examine fecundity. He concluded that no correlation existed between position and the detrimental effects on the tapeworm and suggested that tapeworms could attach anywhere along the anterior 75% of the intestine and get along very well. The results of this study do not support this conclusion as tapeworms, though able to reach maximum weight, were not able to reach maximum egg production. This suggests that the detrimental affects to the tapeworms were due to position and/or lack of nutrients. This is supported by Braten and Hopkins' (1969) findings where tapeworms transplanted in the posterior regions of the small intestine weighed less than tapeworms transplanted in the anterior regions of the intestine.

In concurrent infections it is unlikely that H. diminuta is able to maintain a normal diurnal migration because of the intestinal pathology caused by T. spiralis. Holmes (1961, 1962a) showed that concurrent infections with M. dubius affected the ontogenetic migration of H. diminuta and a similar situation is possible in the present study with respect to the diurnal migration.

Although it was not the intent of this study to evaluate the affects of T. spiralis infections on the diurnal migration of H. diminuta, preliminary observations at 0800h and 1730h showed that circadian rhythm did not occur.

Reid (1942) and Mettrick (1972) showed changes in the tapeworm glucose levels of Raillietina cesticillus (Molin, 1933) and H. diminuta respectively, depending on the feeding time of the hosts and correlated this with the more anterior position of the tapeworms. It appears, therefore, that if the tapeworms are restricted to a posterior location decreased growth may occur because of lack of nutrients.

Tapeworms recovered following a 4000 T. spiralis larvae infection (Experiment 6) showed a different response to adverse intestinal conditions than tapeworms recovered following a 1000 T. spiralis infection (Experiment 5). Destrobilation and the subsequent anterior migration can account for the decrease in weight, egg production and stepwise anterior movement of the scolex and biomass midpoints between the 8th and 18th days after infection with T. spiralis (Table IX). In the intestine of its definitive host a tapeworm strobilates and matures into an egg producing adult and lives in equilibrium between loss and production of proglottids. This

equilibrium if disrupted sufficiently, results in partial or complete loss of strobila from the scolex and neck regions, by unfavourable conditions such as anthelminthic treatment (Hopkins, et al., 1973); host starvation of fowl infected with R. cesticillus (Reid, 1942); hibernation (Ford, 1972); immunity (Hopkins, et al., 1972a, b; Andreassen, et al., 1974), intravenous feeding (Castro, et al., 1975) or crowding in SPF (Hesselberg and Andreassen, 1975), and, when conditions are improved the strobila regrows.

The regrowth of H. diminuta in this present study did not coincide with the growth of the rats. Growth of the tapeworm started after Day 16 (Table IX) at the time when rats did not show any more weight loss (showing return of normal function of the intestine) on the 10th day after nematode infection (Appendix 2, Fig. 1B). Perhaps there is competition for nutrients between the host and the tapeworm (the host may be more successful in the early stages of recovery), tapeworms may respond more slowly to improved gut conditions than the rat, the intestine while appearing normal in function for the rat is not so for the tapeworms, or amine levels may be abnormal having an adverse affect on the tapeworm (Mettrick and Podesta, 1974). Lack of nutrients to the tapeworms, via intravenous feeding of the host, caused

destrobilation of H. diminuta (Castro, et al., 1975) and then worms regrew, even though rats were still being fed intravenously. This suggests that tapeworms could acquire the necessary factors for growth from the exocrino-enteric circulation. If this is the case then competition for nutrients may not take place in the intestine itself but may depend on the level of nutrients present in the host exocrino-enteric circulatory system. Regrowth of the tapeworms after destrobilation is faster than normal in the present experiment (compare Appendix 2, Fig. 2 and 6) and this may have resulted from increased glycogenesis which has been known to occur in tapeworms from starved rats (Goodchild, 1960).

The lag time between growth of the rat and growth of the tapeworm may also be associated with the host immune response. The lower numbers of nematodes present in concurrent infections between Days 10 and 14 may allow for growth of the rat and yet be in sufficiently high numbers to elicit a strong enough immune response to adversely affect the tapeworms.

The more anterior position of destrobilated worms than strobilated worms shows that the scolex and neck regions respond differently from strobilated tapeworms. The anterior migration of the destrobilated worms was shown for transplanted tapeworms (Goodchild, 1958a; Braten and Hopkins, 1969) and this suggests that the posterior positioning of the tapeworms may be due to adverse stimuli on the strobila. Tapeworm movement from the mid regions of the

intestine occur because of the growth pattern of the tapeworm (Chandler, 1939; Braten and Hopkins, 1969; Mettrick and Dunkley, 1969), and supports the pattern in the present study that movement from the mid region of the small intestine occurs when growth occurs after Day 16.

Hymenolepis diminuta in concurrent infections with T. spiralis can survive because of the adaptive ability of the tapeworm. Concurrent infections in this study resulted in competitive exclusion of H. diminuta which adversely affected the tapeworm but did not affect its population levels. T. spiralis is also affected by concurrent infections but to a lesser extent than H. diminuta. Adverse affects to the tapeworm can be due to either the host immune system, or the lack of available nutrients or the position of the tapeworm. All these factors may be of importance to some degree during the concurrent infections but it seems that the immune response and nutrient availability are the main factors affecting H. diminuta.

CONCLUSION

1. The distribution, numbers and rate of recovery of single-species infections of T. spiralis agree with the information in the literature.

2. The distribution, recovery, dry weight and fecundity of single-species infections of H. diminuta agree with the information in the literature.

3. Trichinella spiralis and H. diminuta can co-exist in rat intestine.

4. Trichinella spiralis in situated more anteriorly and recovery rates increase in concurrent infections with H. diminuta. Competitive exclusion of H. diminuta in concurrent infections is dependent on nematode dose levels and results in drastic changes in position, weight and fecundity.

5. Host sex does not affect T. spiralis and H. diminuta in single-species or in concurrent infections.

6. Trichinella spiralis appears to affect H. diminuta indirectly through modification of the intestinal wall and through availability of nutrients for the tapeworm.

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APPENDIX 1

Table I. Analysis of variance of T. spiralis distributional midpoints and number of nematodes recovered from male and female rats at 10 L/g, 20 L/g, and 30 L/g single-species infections.

Source	DF	Sum of Squares	Mean Square	F Statistic
T. spiralis midpoint				
sex	1	2.60	2.60	.188*
dose	2	322.55	161.25	11.72**
error	23	317.50	13.80	
number recovered				
sex	1	1.82×10^5	1.82×10^5	5.99*
dose	2	3.88×10^6	1.94×10^6	63.82**
error	23	6.99×10^5	3.04×10^4	

* not significant
** significant at 5% level

Table II. Analysis of variance of sex and groupings of the scolex and biomass midpoints and dry weight per *H. diminuta* in single-species infections.

Source	DF	Sum of Squares	Mean Square	F Statistic
Mean scolex midpt				
sex	1	63.61	63.61	1.286*
group	4	394.45	98.62	1.994*
error	38	1879.18	49.45	
Mean biomass midpt				
sex	1	10.59	10.59	.2796*
group	4	886.37	221.59	5.85**
error	36	1363.47	37.87	
Mean weight/worm				
sex	1	1284.56	1284.56	.1403*
group	4	320388.90	80097.20	8.749**
error	36	329591.37	9155.31	

* no significance

** significant at 5% level

Table III. Analysis of variance of sex and groups of the number of T. spiralis recovered and T. spiralis midpoints in concurrent infections 5 days post infection with 4000 T. spiralis.

Source	DF	Sum of Squares	Mean Square	F Statistic
Number of T. spiralis recovered				
sex	1	1.82×10^5	1.82×10^5	0.213*
group	4	2.2948×10^7	5.737×10^6	6.718**
error	36	2.224×10^7	8.54×10^5	
T. spiralis midpoints				
sex	1	0.358	0.358	1.0347*
group	4	28.101	7.025	20.314**
error	36	12.450	0.346	

* not significant

** significant at 5% level

Table IV. Analysis of variance of sex and grouping of the scolex and biomass midpoints and dry weight per *H. diminuta* in concurrent infections with 4000 *T. spiralis* larvae.

Source	DF	Sum of Squares	Mean Squares	F Statistic
Mean scolex midpt				
sex	1	152.97	152.97	2.039*
group	4	483.17	120.79	1.610*
error	37	2775.00	75.0	
Mean biomass midpt				
sex	1	44.08	44.08	0.674*
group	4	420.42	105.11	1.608*
error	33	2157.03	65.36	
Mean wt/worm				
sex	1	183.78	183.78	0.928*
group	4	4214.80	1053.70	5.322**
error	33	6533.98	197.99	

* not significant

** significant at 5% level

Table V. Analysis of variance comparing single-species H.diminuta scolex and biomass midpoints and dry weight per worm to H. diminuta from concurrent infections with 4000 T. spiralis larvae.

Source	DF	Sum of Squares	Mean Square	F Statistic
scolex midpoint				
treatment	1	905.83	905.83	380.80**
group	4	16.91	4.23	1.78*
error	83	197.44	2.38	
biomass midpoint				
treatment	1	370.10	370.10	155.90**
group	4	10.80	2.70	1.14*
error	79	187.54	2.37	
weight per worm				
treatment	1	1.6×10^5	1.6×10^5	54.27**
group	4	1.4×10^4	3.6×10^3	1.20*
error	79	2.4×10^5	3.0×10^3	

* not significant

** significant at the 5% level

Table VI. Analysis of variance of T. spiralis midpoints and numbers recovered in male and female rats with infections of 500, 1000, 2000, 3000 and 4000 nematodes in concurrent infections. (One group only of 5 male and 5 female rats from dose levels of 500 and 4000 are included in analyses).

Source	DF	Sum of Squares	Mean Square	F Statistic
T. spiralis midpoints				
sex	1	207.68	207.68	27.48**
dose	4	488.42	122.11	16.15**
error	36	272.2	7.56	
T. spiralis recovery				
sex	1	2.77×10^5	2.77×10^5	3.49*
dose	4	1.69×10^7	4.24×10^6	63.37**
error	36	3.02×10^6	1.47×10^5	

* not significant

** significant at the 5% level

Table VII. Analysis of variance comparing nematode midpoints of and recovery rates of 10 L/g, 20 L/g, and 30 L/g in single-species infections of T. spiralis and 1000, 2000, and 3000 dose levels of concurrent infections in male and female rats and analysis of variance of recovery rates.

Source	DF	Sum of Squares	Mean Square	F Statistic
Nematode midpoints				
host sex	1	105.47	105.47	8.85**
dose levels	2	110.07	55.03	4.61**
error	21	250.38	11.92	
treatment	1	909.30	909.30	107.26**
error	21	178.03	8.43	
Recovery rates				
treatment	1	2874.94	2874.94	19.731**
dose levels	2	226.35	113.17	.777*
error	53	7722.65	145.71	

* not significant

** significant at the 5% level.

Table VIII. Analysis of variance of H. diminuta scolex and biomass midpoints and weight per tapeworm from male and female rats at dose levels of 500, 1000, 2000, 3000 and 4000 T. spiralis larvae 5 days post infection (one group only of 5 male and 5 female rats from dose levels of 500 and 4000 are included in analyses).

Source	DF	Sum of Squares	Mean Square	F Statistic
scolex midpoints				
sex	1	31.94	31.94	0.749*
dose	4	1950.43	487.60	11.437**
error	35	1492.18	42.63	
biomass midpoints				
sex	1	108.63	108.63	2.153*
dose	4	1712.47	428.12	8.486**
error	35	1765.77	50.45	
Weight per worm				
sex	1	1027.25	1027.25	0.658*
dose	4	36874.50	9218.6	5.913**
error	35	54571.01	1559.17	

* not significant

** significant at 5% level.

Table IX. Analysis of variance of nematode midpoint and numbers recovered of *T. spiralis* after initial infection of 1000 larvae.

Source	DF	Sum of Squares	Mean Square	F Statistic
Nematode midpoint				
days	3	25.93	8.64	.3222*
error	16	429.28	26.83	
Number recovered				
days	3	8.14×10^4	2.7×10^4	1.567*
error	16	2.8×10^5	1.7×10^4	

* not significant at 5% level

Table X. Analysis of variance of scolex and biomass midpoints and dry weight per *H. diminuta* after an initial infection of 1000 *T. spiralis* larvae.

Source	DF	Sum of Squares	Mean Square	F Statistic
scolex midpoints				
days	6	2740.00	456.67	6.227**
error	28	2053.00	73.34	
biomass midpoints				
days	6	3454.00	575.67	6.7559**
error	28	2460.85	87.88	
weight per worm				
days	6	54849.54	9141.50	6.09**
error	28	42030.00	1501.10	

** significant at the 5% level

Table XI. Analysis of variance of the dry weight per H. diminuta in single-species infections and dry weight per H. diminuta recovered after an initial dose of 1000 T. spiralis.

Source	DF	Sum of Squares	Mean Square	F Statistic
treatment	1	2339.30	2339.30	1.047*
group	4	61073.00	15268.25	6.834**
error	39	87131.5	2234.13	

* not significant

** significant at the 5% level

Table XII. Analysis of variance for nematode midpoints and numbers recovered during the intestinal phase of 4000 T. spiralis larvae in concurrent infections with H. diminuta. (Analyses include data from Group 4, Experiment 3).

Source	DF	Sum of Squares	Mean Square	F Statistic
nematode midpoints				
treatment	6	38.58	6.430	.74545*
error	79	655.576	8.626	
numbers recovered				
treatment	6	8596469.16	1432744.86	11.7105**
error	79	9665427.22	122347.18	

* not significant

** significant at the 5% level.

Table XIII. Comparison of groups and days of H. diminuta scolex and biomass midpoints and weight per worm after initial infection of 4000 T. spiralis larvae. (Analyses include data from Group 4, Experiment 3).

Source	DF	Sum of Squares	Mean Square	F Statistic
Scolex midpoints				
group	2	2865.75	1432.87	16.33**
days	7	14067.69	2009.67	22.91**
error	82	7191.25	87.70	
biomass midpoints				
group	2	462.00	231.00	1.95*
days	7	10179.68	1454.24	12.32**
error	82	9790.50	119.40	
Weight per worm				
days	6	828857.94	138142.04	4.606**
error	12	2459338.26	29991.93	

* not significant

** significant at the 5% level.

APPENDIX 2

Fig. 1A Change in rat weight 5 days after infection of various dose levels of T. spiralis in single-species and concurrent infections.

Fig. 1B Change in rat weight during the intestinal phase of 1000 and 4000 T. spiralis larvae infections.

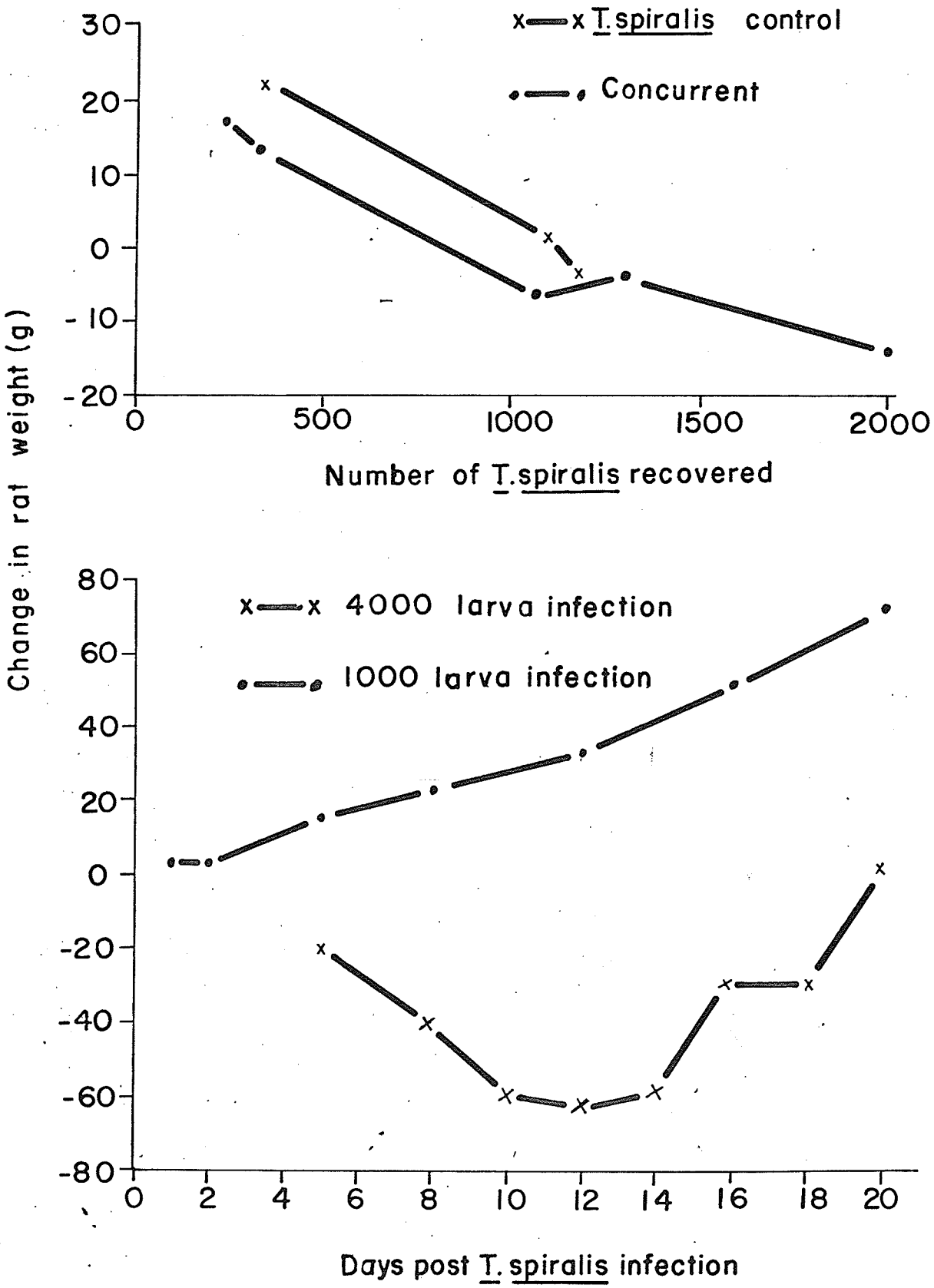

x—x T. spiralis control
Concurrent
30
20
10
0
-10
-20
0
500
1000
1500
2000
Number of T. spiralis recovered
Change in rat weight (g)
x—x 4000 larva infection
1000 larva infection
80
60
40
20
0
-20
-40
-60
-80
0
2
4
6
8
10
12
14
16
18
20
Days post T. spiralis infection

Figure 2. Change in weight of uninfected and H. diminuta infected rats.

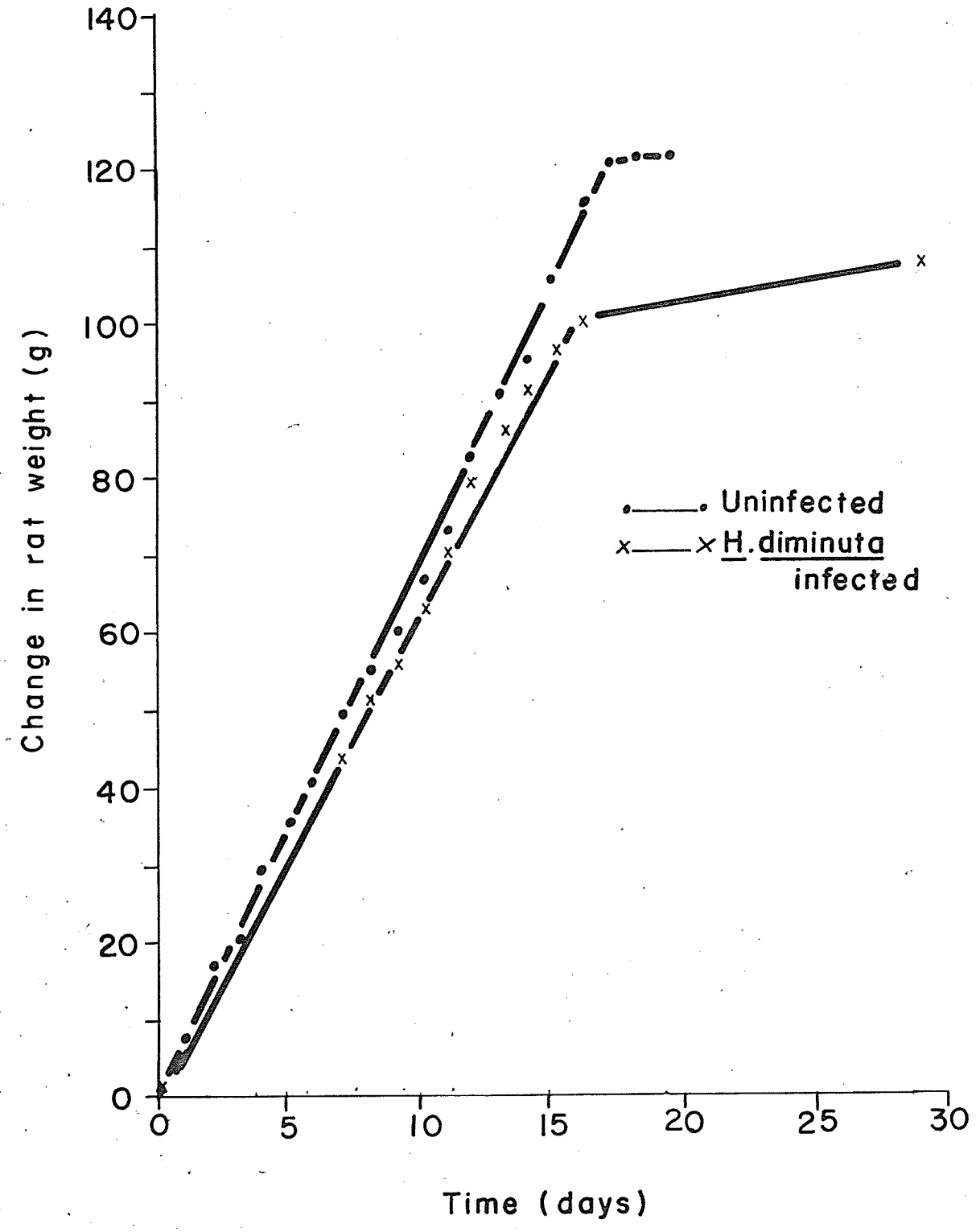

140
120
100
80
60
40
20
0
0
5
10
15
20
25
30
Change in rat weight (g)
Time (days)
Uninfected
H. diminuta infected

Figure 3. Dry weight of H. diminuta 5 days after infection of 500, 1000, 2000, 3000 and 4000 T. spiralis larvae.

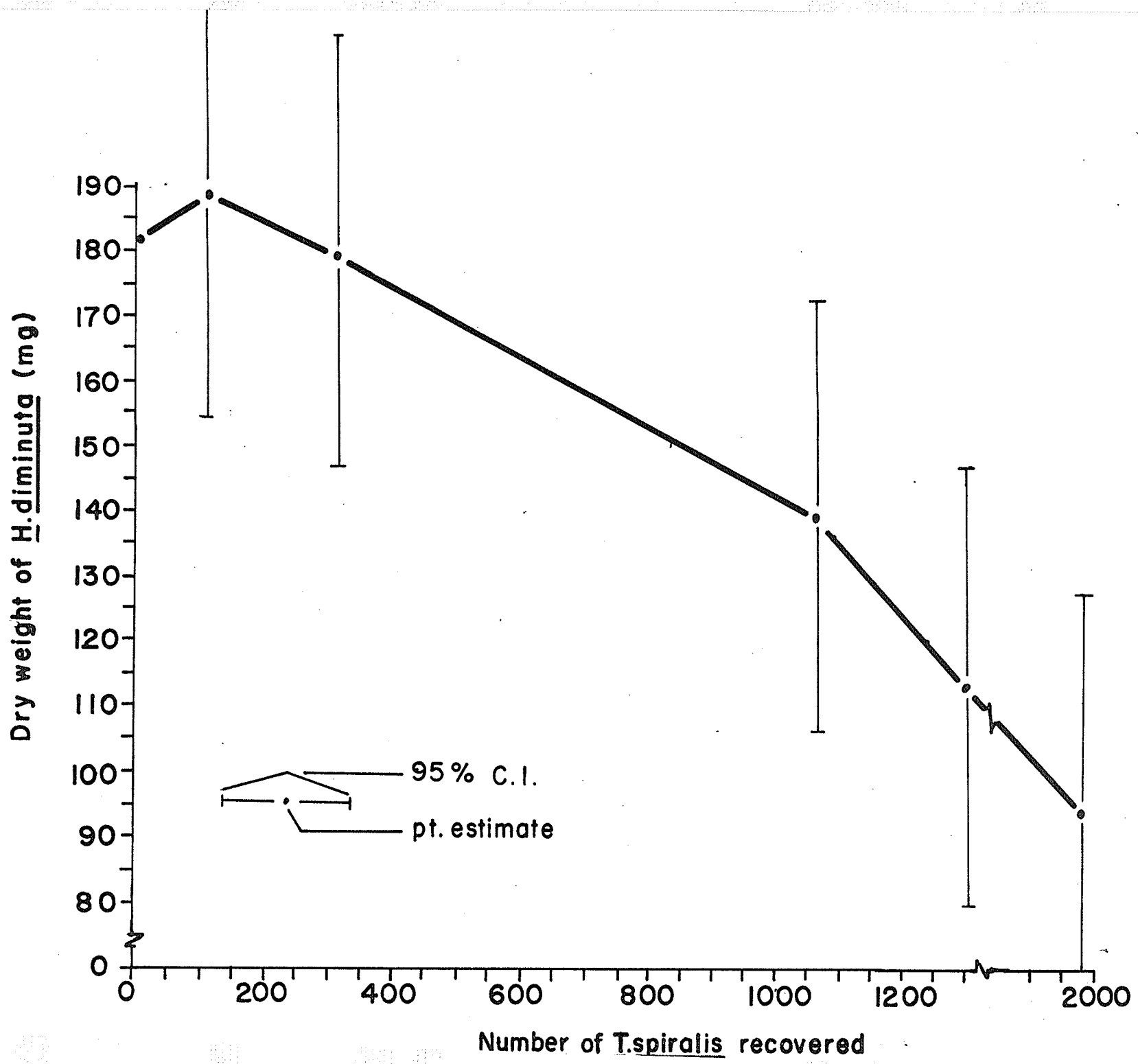

Dry weight of H.diminuta (mg)
190
180
170
160
150
140
130
120
110
100
90
80
0
0
200
400
600
800
1000
1200
2000
95% C.I.
pt. estimate
Number of T.spiralis recovered

Figure 4. Dry weight of H. diminuta during the intestinal phase of 1000 T. spiralis larvae infections.

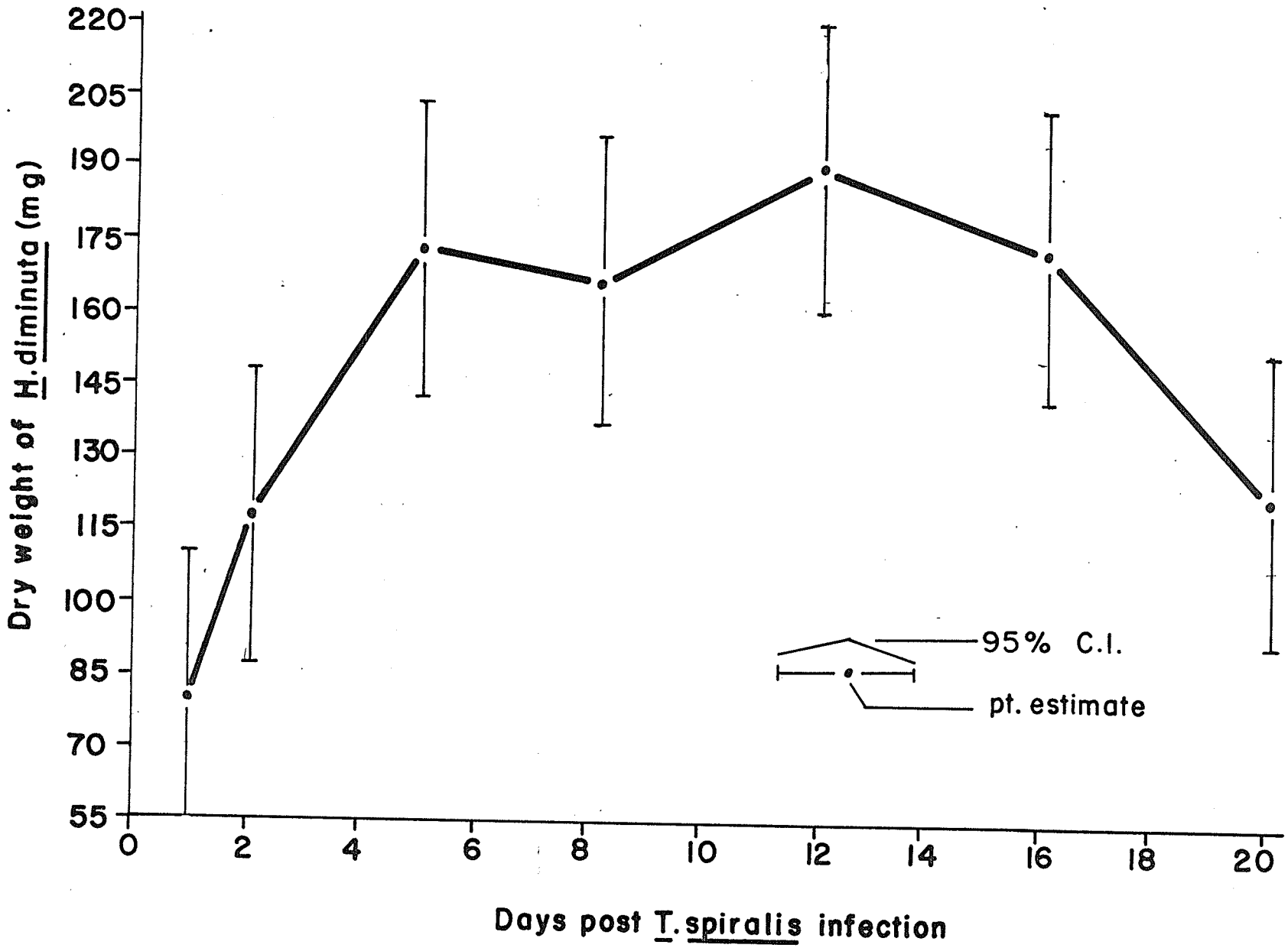

220
205
190
175
160
145
130
115
100
85
70
55
0
2
4
6
8
10
12
14
16
18
20
Dry weight of H.diminuta (mg)
Days post T.spiralis infection
95% C.I.
pt. estimate

Figure 5. Number of T. spiralis recovered during the intestinal phase of 4000 T. spiralis infections.

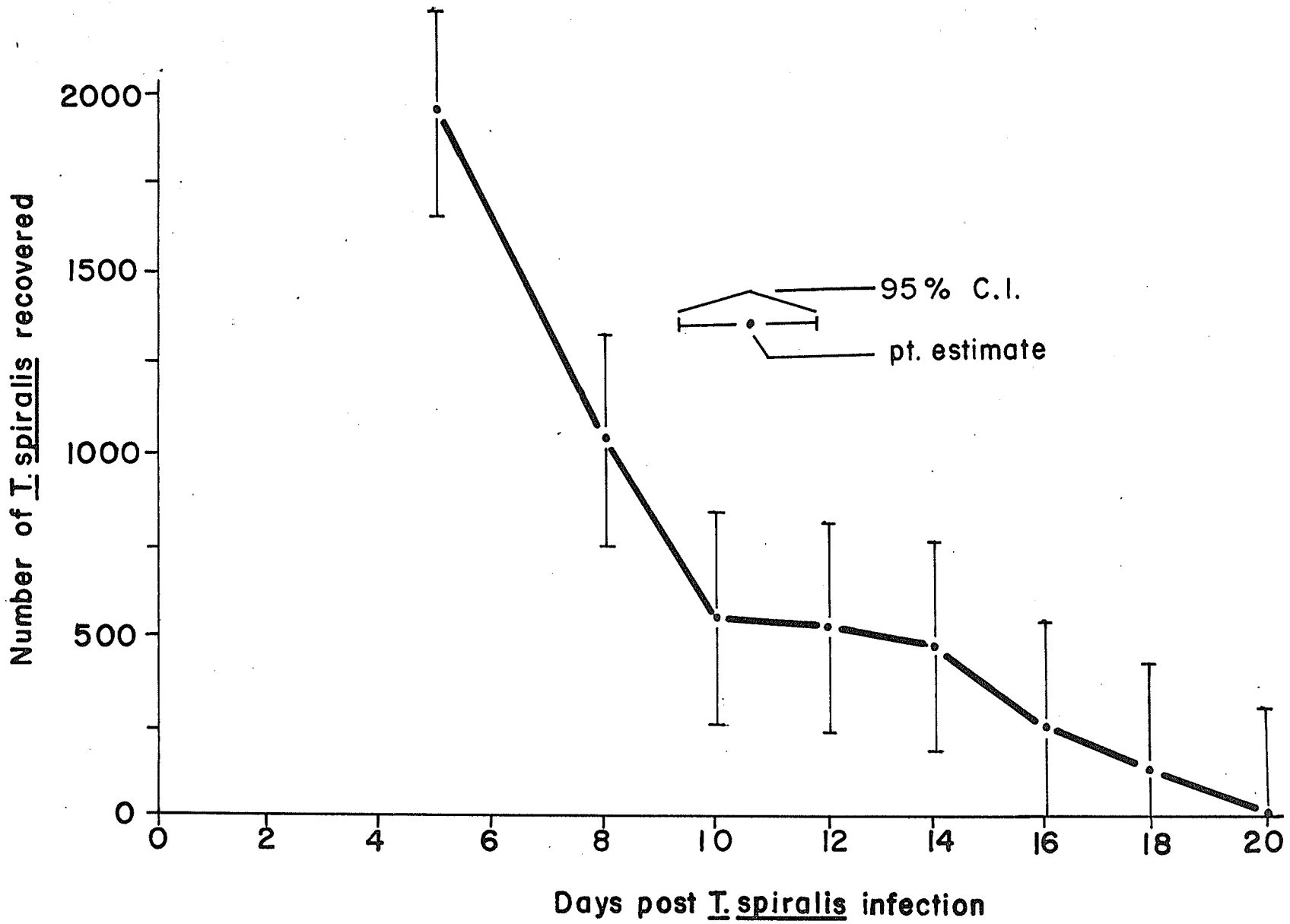
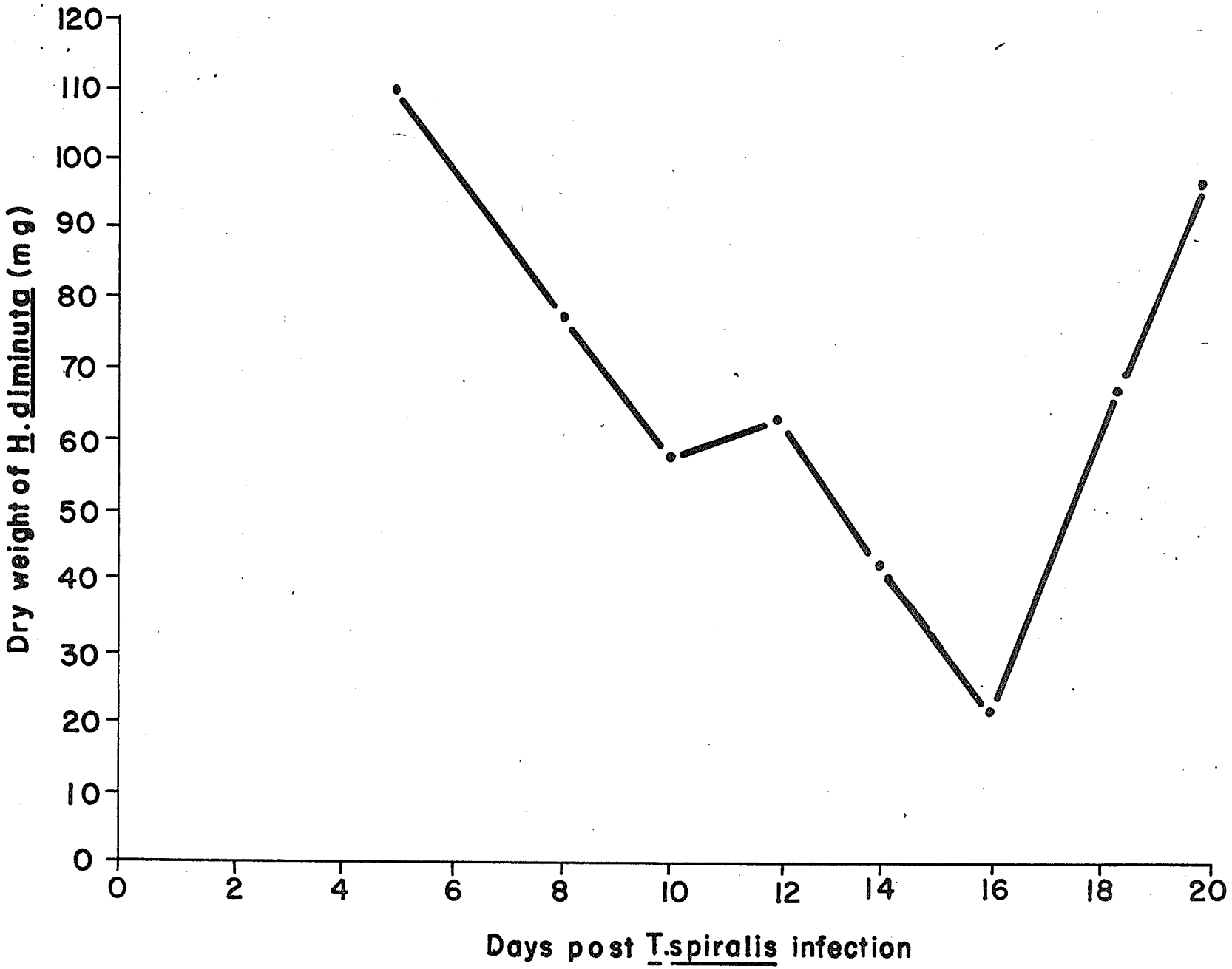

2000
1500
1000
500
0
Number of T. spiralis recovered
95% C.I.
pt. estimate
0
2
4
6
8
10
12
14
16
18
20
Days post T. spiralis infection

Figure 6. Dry weight of H. diminuta during the intestinal phase of 4000 T. spiralis infections.


120
110
100
90
80
70
60
50
40
30
20
10
0
Dry weight of H. diminuta (mg)
0
2
4
6
8
10
12
14
16
18
20
Days post T. spiralis infection

APPENDIX 3

Classification of Parasites

Phylum:	Platyhelminthes
Class:	Cestoda
Subclass:	Eucestoda
Order:	Cyclophyllidea
Family:	Hymenolepidae
Genus:	*Hymenolepis*
Species:	*diminuta*

Phylum:	Nematoda
Class:	Adenophorea
Order:	Trichinellida
Family:	Trichinellidae
Genus:	*Trichinella*
Species:	*spiralis*