STUDIES ON SITE SELECTION IN SOME GASTROINTESTINAL NEMATODES OF MAMMALS

BY

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ABSTRACT

This study has investigated the early establishment of Trichinella spiralis and Nematospiroides dubius in the gastrointestinal tracts of their hosts. By using different vehicles of infection, different pre-treatments of the infective stages, by changing intestinal motility and by implantation of the infective stages directly into the intestine, significant changes in the longitudinal distribution were obtained. Surgical procedures, including gastrectomy, duodenal by-pass, ligation of the pancreatic duct and ligation or relocation of the bile duct, performed on rats prior to infection, have elucidated the central roles of bile, chloride and enterokinase. In vitro studies have corroborated in vivo studies. New categories of movement were observed in T. spiralis and new activity patterns were found to be stimulated in N. dubius exposed to bile.

These observations are discussed in the context of site selection by intestinal nematodes with emphasis on critical factors which activate infective larval stages during their passive transport down the intestinal tract.

ABREGE

L'établissement précoce de Trichinella spiralis et Nematospiroides dubius dans l'intestin de leurs hôtes a été étudié. Des changements significatifs ont été obtenus dans la distribution longitudinale sous les conditions suivantes: differents modes d'infection, dans les cas ou les larves infectieuses ont été traités au préalable, lors du changement de la motilité de l'intestin et aussi par implantation de larves directement au niveau de l'intestin. Chez le rat de laboratoire, la gastrectomie, l'évitement des voies duodénales, le ligation des conduits pancréatiques, la ligature ou le déplacement des canaux biliaires avant infection ont permis de déterminer le rôle de la bile, les ions de chlore ainsi que l'entérokinase. D'autre part, les études in vitro confirment les observations faites in vivo. Des nouvelles catégories de mouvement ont été observés chez T. spiralis tandis que des activités nouvelles furent notées chez N. dubius lorsqu'ils étaient incubés dans la bile.

Les résultats de cette étude sont discutés en tenant compte du site de sélection choisi par les nématodes intestinaux et des facteurs critiques pouvant activer les larves infectieuses durant leur transport qui est assuré par les mouvements de l'intestin.

Suggested Short Title: -

SITE SELECTION IN GASTROINTESTINAL NEMATODES

TO MY WIFE

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TABLE OF CONTENTS

TABLE OF CONTENTS (cont'd)

TABLE OF CONTENTS (cont'd)

TABLE OF CONTENTS (cont'd)

TABLE OF CONTENTS (cont'd)

TABLE OF CONTENTS (cont d)

LIST OF TABLES

LIST OF TABLES (cont'd)

LIST OF TABLES (cont'd)

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ERRATUM

All p's used in indications of statistical significance should be capitalized.

All references to log dilutions mean $\log_{10}$ dilutions.

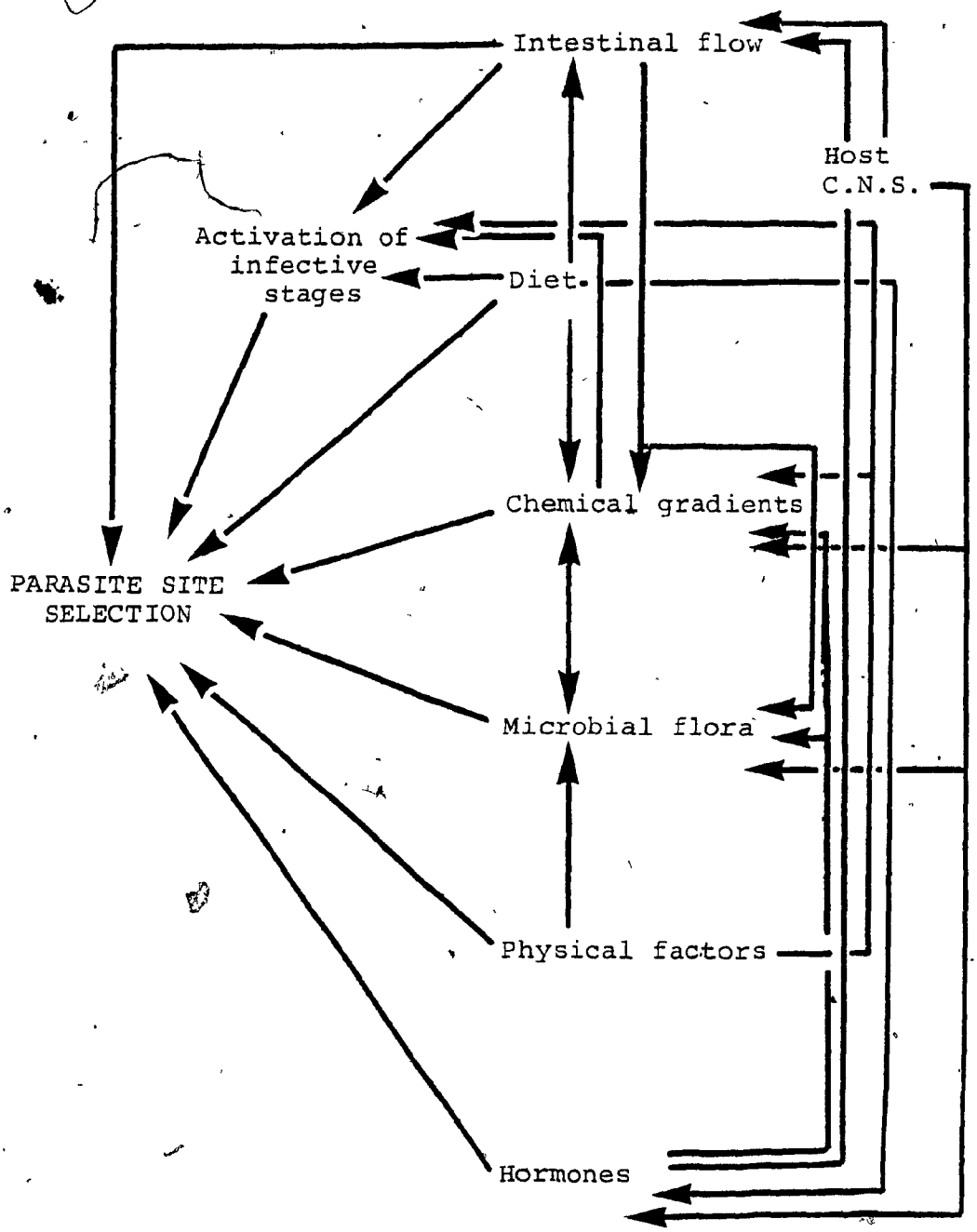
INTRODUCTION

The gastrointestinal tract is frequently parasitized by a wide range of protozoa and helminths. Several investigators have shown that gut parasites are restricted within regions of the gut and that they may return to their "normal" sites after implantation in abnormal sites. Several hypotheses have been proposed to explain the mechanisms controlling these phenomena but experimental verification is rare and the basis of site selection by parasites of the gut remains largely obscure. Definitive conclusions remain elusive because the alimentary tract is a complex series of tissues and organs which differ physiologically, biochemically and morphologically and little is known of the specific requirements of parasites.

The purpose of this study was to investigate the relationship between the alimentary tract and two gastrointestinal nematodes, Trichinella spiralis and Nematospiroides dubius in order to understand more of the controlling mechanisms in site selection. Following a review of the literature, it was decided that there were six significant groups of factors that may influence the site selection of gastrointestinal nematodes. These were: 1), intestinal flow; 2), diet; 3), chemical gradients; 4), microbial flora; 5), physical factors and 6), hormones. Clearly, these labile components of the parasite's environment are superimposed on the more constant architectural substrate of cells, tissues and organs. These

groups of variable factors are known to be interdependent and to interact in numerous ways (Figure 0.1). Because of these complexities and the relative paucity of specific information, a variety of approaches to these problems was used. Thus, it was hoped, a comparison of two parasites and the use of several types of experiments would generate a more general understanding of the mechanisms that are important in site selection.

FIGURE 0.1 Schematic model of the significant factors that influence the selection of sites by parasitic nematodes of the gastrointestinal tract. Lines and arrows indicate the main ways in which the six factors interact, there are many more subtle interactions between all the factors.


Intestinal flow
Host
C.N.S.
Activation of
infective
stages
Diet
Chemical gradients
PARASITE SITE
SELECTION
Microbial flora
Physical factors
Hormones

CHAPTER 1. LITERATURE REVIEW

1A. THE LIFE CYCLES OF TRICHINELLA SPIRALIS AND NEMATOSPIROIDES DUBIUS

1) Trichinella spiralis

T. spiralis (Owen, 1845) is an unusual nematode because all of the stages in the life cycle occur within the same host. It is also remarkable for its lack of host specificity since it can live in almost any mammal and under certain conditions, in some birds.

The infective larvae penetrate into the host mucosa soon after infection (Despommier, et al., 1978) where they remain for the duration of their larval and adult lives in the epithelial cells of the small intestine (Gardiner, 1976; Despommier, et al., 1978; Wright, 1979). The adult female is ovoviviparous (Christensen, 1950) and the newborn larvae are passed from the vulva directly into the mucosa. The newborn larvae then enter the host's striated musculature via the mesenteric venules and lymphatic circulatory system into the circulatory system (Berntzen, 1965; Harley & Gallichio, 1971) or via the peritoneal cavity and connective tissue (Shanta & Meerovitch, 1967a, b). The larvae penetrate the muscle fibers eliciting a response that changes the morphology and physiology of the cells which accomodate the larvae (Pukerson & Despommier, 1974; Despommier, 1975, 1976). The muscle cell contributes to the development of a collagenous cyst while the larvae matures (Ritterson, 1966; Bruce, 1970; Gould, 1970; Stewart & Read, 1972; Teppema et al., 1973; Despommier, 1975, 1976).

The larvae remain in the muscles and when eaten, survive passage through the stomach and establish in the intestine of another host.

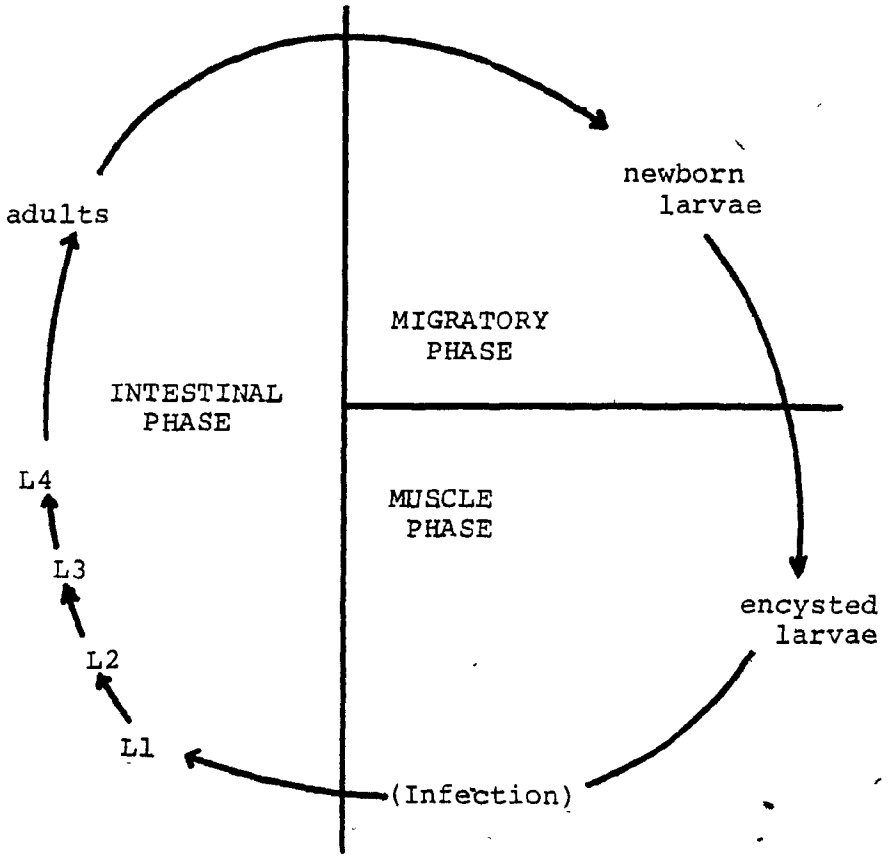
Transmission of the parasite occurs through the ingestion of muscle tissue containing viable encapsulated larvae. The ability of the encapsulated larvae to survive extremes of cold and putrefaction has led to the suggestion that this may be considered a free-living stage (Madsen, 1974). Upon ingestion, the larvae are freed after the digestion of the flesh and the capsule surrounding them and they penetrate into the mucosa. Development in the intestine has been frequently studied and there are conflicting reports regarding the number of larval moults prior to adulthood (Wiu & Kingscote, 1957; Villela, 1958; Podhajecky, 1964; Berntzen, 1965; Ali Khan, 1966; Shanta & Meerovitch, 1967a, b). An extensive study by Kozek (1971a, b) using light and electron microscopy seems to have resolved this problem. Kozek (1970a, b) found that the larvae undergo 4 moults in quick succession and that the adult stage is reached within 48 hours of infection. A diagram of the life cycle is presented in Figure 1.1.

Under the present taxonomic classification, there is only one species recognized in the genus Trichinella (Yorke & Maplestone, 1926; Chitwood & Chitwood, 1941).

Superfamily - Trichuroidea
Family - Trichinellidae
Genus - Trichinella

Recently, evidence has suggested that Trichinella may not be a monospecific genus and that there may be several

FIGURE 1.1 The life cycle of T. spiralis.

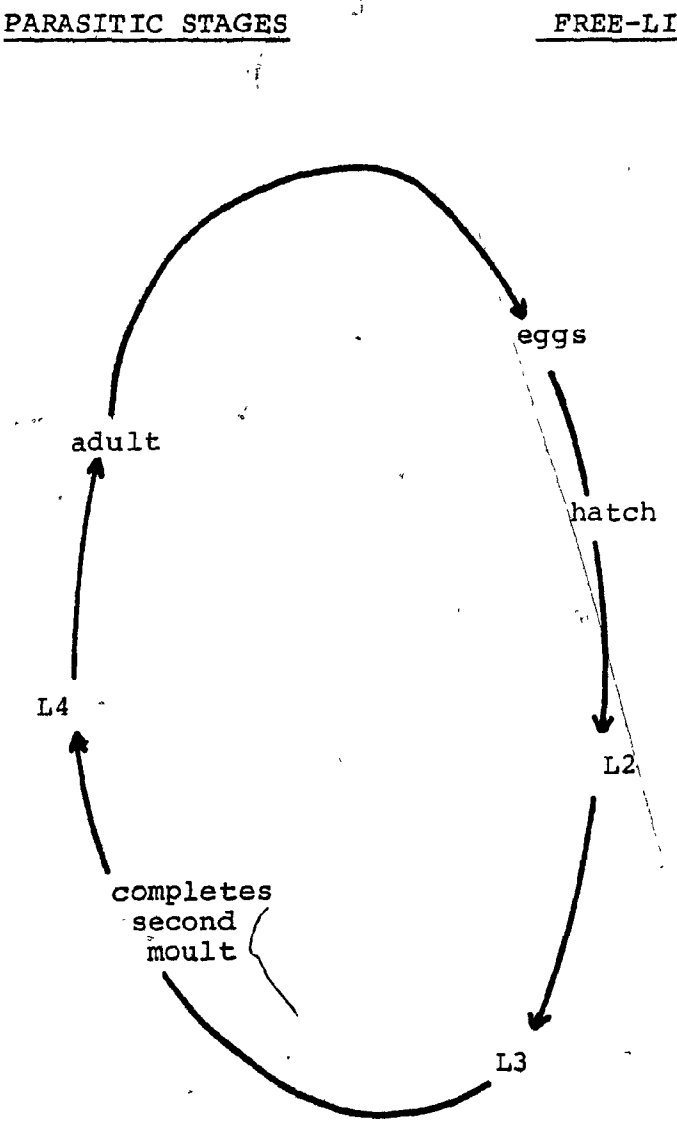

adults
newborn
larvae
MIGRATORY
PHASE
INTESTINAL
PHASE
MUSCLE
PHASE
L4
L3
L2
L1
encysted
larvae
(Infection)

species, "strains" or "biological species" (Forrester et al., 1961; Nelson et al., 1961, 1966; Kruger, 1966; Gretillat & Vassiliades, 1967; Pereverseva, 1966; Read & Schiller, 1969; Ozeretskovskaya et al., 1969; Britov, 1969). In a series of reports, Britov and his colleagues (Britov, 1971a, b, c, 1974; Britov et al., 1971; Britov & Boev, 1972) have provided evidence to justify the existence of three species of Trichinella: T. spiralis, T. nativa and T. nelsoni and a fourth species that differs considerably from the other three, T. pseudospiralis (Garkavi, 1972a, b, 1974; Britov, 1974, 1975a, b; Pereverseva et al., 1974, Britov & Garkavi, 1975). Although geographical, biological, ecological, serological and morphological differences have been found, recent evidence was thought not to justify as many as 4 separate species (Sukhdeo & Meerovitch, 1977, 1979, 1980; Belosevic & Dick, 1980a, b, c). The problems in the taxonomy of this genus have not yet been adequately resolved.

2) Nematospiroides dubius

N. dubius (Baylis, 1926) is a parasite of rodents with a direct life cycle involving both free-living and parasitic stages (Figure 1.2). The eggs are passed in the faeces, hatch and develop to the ensheathed infective L_3 larvae. Ehrenford (1954) and Dobson (1960) believed that there was 1 free-living moult and 3 parasitic moults, Spurlock (1943), Fahmy (1954) and Bryant (1973) reported that there were 2 free-living moults and 2 parasitic moults. Two free-living moults are frequent in trichostrongylids and the results of the latter authors are now widely accepted.

FIGURE 1.2 The life cycle of N. dubius.


PARASITIC STAGES
FREE-LIVING STAGES
eggs
hatch
L2
L3
completes second moult
L4
adult

Following ingestion, infective larvae have been found in the lumen of the gut for several hours before exsheathment and penetration (Ehrenford, 1954; Bryant, 1973). However, Ehrenford (1943) believed that exsheathment occurred upon ingestion and Sommerville and Bailey (1973) have reported that 89% of the larvae had exsheathed after 5 minutes in the stomach.

The larvae penetrate the intestinal mucosa where they encyst in or close to the muscles of the intestinal wall (Spurlock, 1943; Ehrenford, 1956). Liu (1965a, b) reported that the larvae may penetrate the gastric mucosa and remain there for up to 24 hours before leaving to penetrate the intestinal mucosa. The larvae develop to adults in the tissue of the small intestine and then move to the lumen where they occupy their adult sites in the duodenum. After copulation, the eggs are passed in the host's faeces.

The taxonomy of N. dubius (Baylis, 1926) has recently been subject to revision and it is now thought to be the same as Heligmosomoides polygrus (Dusjardin, 1845) following the studies of Durrette-Desset (1968a, b). Several investigators now working with this parasite synonomize the names (Forrester, 1971; Forrester & Neilson, 1973; Crandall et al., 1975; Cypess & Zidian, 1975; Cypess et al., 1977a; Molinari, et al., 1978).

Superfamily - Trichostrongyloidea
Family - Trichostrongylidae
Subfamily - Heligmosominae
Genus - Nematospiroides
(Yamaguti, 1961)

Strongylus polygyrus was first described by Dusjardin (1845) as a parasite from the intestine of rodents and it was subsequently put into the genus Heligmosomum (Raillet & Henry, 1909). It was later transferred to the genus Viannaia (Hall, 1916) on the authority of Travassos (1914) although Hall did not feel that there was sufficient evidence to justify the change. Hall (1916) erected a new genus, Heligmosomoides to accomodate H. linstowi, a parasite that was similar to V. polygyrus. Boulenger (1922) reported that H. linstowi and V. polygyrus were identical species and he proposed that V. polygyrus should be transferred from Viannaia to Heligmosomoides where it remains (Baylis & Daubney, 1926; Yorke & Maplestone, 1926).

N. dubius was first described by Baylis (1926) as an intestinal parasite of the woodmouse and it was placed in the subfamily Heligmosominae (Travassos, 1937, Yamaguti, 1961). Durette-Desset (1968a, b) reported that N. dubius and H. polygyrus were identical species.

I have retained the use of Nematospiroides dubius to describe the parasite used in this study pending taxonomic confirmation of the status of my species by the Commonwealth Institute of Helminthology, St. Albans, England.

1B. SITE SELECTION BY GASTROINTESTINAL NEMATODES

1) Introduction

Acanthocephalans and cestodes are usually restricted to the small intestine, a region where there is absorption of nutrients by the host (Ulmer, 1971; Crompton, 1973). The

trematodes and nematodes that inhabit the gut are much more mobile and varied in their linear distribution in the gut than the cestodes and acanthocephalans. Trematodes are believed to have a high degree of site specificity and this is demonstrated by some of the unusual locations of the infective stages e.g. the attraction of Diplostomum flexicaudatum cercariae for the lens of fish (Ferguson, 1943), the predisposition of echinostome cercariae for renal organs (Heyneman, 1966) or the specific migration of Brachylecithum musquensis to the supraoesophageal ganglia of ants (Carney, 1967, 1969). Among nematodes, microhabitat specificity also appears to be highly developed, adult gastrointestinal nematodes can be found in all regions of the gut. The sites occupied by nematodes may differ both linearly and radially along the gut (Schad 1963a; Weirtheim, 1970). Table 1.1 summarizes the results of a literature search and lists the sites selected by some nematodes that inhabit the gastrointestinal tract. The site specificity of these nematodes probably depend on a large number of factors of both host and parasite origin, however, most of these factors have not been elucidated.

2) Activation of the infective stages of gastrointestinal nematodes

The system that controls activation in some nematodes is believed to consist of a mechanism which regulates (the timing of) the production of "internal secretions", the internal secretions themselves and the structures and tissues on which the secretions act (Rogers & Sommerville, 1963). The host produces either (a), a stimulus that acts directly

TABLE 1.1 THE SITES OCCUPIED BY ADULT NEMATODES IN THE GASTROINTESTINAL TRACT

(a) Nematodes inhabiting the mouth and oesophagus

Parasite	Host	Comments	Reference
Amplicaecum robertsi	python	capsules in eosophagus	Sprent, 1963
Anatrichosoma buccalis	opposum	lesions in mucosa of palate	Pence & Little, 1972 Kinsella & Winegarner, 1975
Capillaria blarinae	shrew	tunnels of epithelial cell of eosophagus	Ogren, 1953
Kalicephalus inermis coronellae	boa constrictor	- - - -	Schad, 1962
Spirocerca lupi	dog	in nodules in eosophagus	Nazarova, 1964 Bailey, 1972 Chhabra & Singh, 1972
Gongylonema macroguberna aculum	monkey	in the mucosa of the eosophagus	Lubimov, 1931

(b) Nematodes inhabiting the stomach

Anatrichosoma gerbillis	gerbil	in the mucosa	Bernard, 1964
Ancylostoma pluridendatum	ocelot	- - - -	Canavan, 1931
Capillaria contorta	crow	in the mucosa	Canavan, 1931

(b) Nematodes inhabiting the stomach (cont'd)

C. gastrica	rat	epithelium of cardiac portion	Baylis, 1926b Lopez-Neyra, 1947
Cloacina spp.	kangaroo	anterior stomach	Mykytowycz, 1964
Contracaecum microcephalus	fish eating birds	attach to mucosa by labia	Semenova, 1974
Coronostrongylus sp.	kangaroo	mid to posterior intestine	Mykytowycz, 1964
Filarinema spp	kangaroo	pyloric glandular portion	Mykytowycz, 1964
Gongylonema neoplasticum	rat	forestomach	Hitchcock & Bell, 1952
Haemonchus contortus	sheep	abomasal mucosa	Stoll, 1929 Canavan, 1931 Turner et al., 1962 Sommerville, 1963
H. placei	cattle	abomasal mucosa	Roberts, 1957
Habronema muscae	horse	buried in mucosa	Foster, 1930
H. megastoma	horse	abscesses in cardiac region	Foster, 1930
H. microstoma	horse	associated with eroded mucosa	Foster, 1930
Labiostrongylus longispicularis	kangaroo	midstomach, cardia	Dudzinski & Mykytowza, 1965
L. eugenii	wallaby	- - - -	Smales, 1977 a,b

(b) Nematodes inhabiting the stomach (cont'd)

Macropostrongylus sp.	kangaroo	mid to posterior stomach	Mykytowycz, 1964
Ophidascaris moreliae	pythons	- - - -	Sprent, 1970
Ostertagia circumcinta	cattle	- - - -	Turner, et al., 1962
O. ostertagi	cattle	- - - -	Michel, 1963
Rugopharnynx austrialis	kangaroo	mid to posterior stomach	Mykytowycz, 1964
Spinitectus ranae	frog	- - - -	Hasegawa & Otsuru, 1977
Trichostrongylus axei	sheep and cattle	mucosa	Leland & Drudge, 1957 Sommerville, 1963 Purcell et al., 1971
T. colubriformis	sheep and cattle	mucosa	Barker, 1973, 1974, 1975a
T. retortaeformis	sheep and cattle	mucosa	Drudge et al., 1955 Bailey, 1968 Barker & Ford, 1975

(c) Nematodes inhabiting the small intestine

Ascaridia columbae	pigeons	- - - -	Melendez & Lindquist, 1979
A. galli	chicken	duodenum	Todd & Crowdus, 1952 Khouri & Pande, 1970
Ascaris lumbricoides	man	jejenum	Makidono, 1956

(α) Nematodes inhabiting the small intestine (cont'd)

A. suum	pig	ant. small intestine	Stephenson et al., 1980
Baylisascaris tasmaniensis	tasmanian devil	- - - -	Sprent, et al., 1973
Bunostomum phlebotomum	cattle	- - - -	Sprent, 1946
Capillaria caudinflata	chicken	duodenum	Morehouse, 1944 Madsen, 1952
C. columbae	chicken	ileum	Levine, 1938
C. obsignata	chicken	- - - -	Wakelin, 1965
Cooperia oncophora	cattle	ant. s. i.	Isenstein, 1963
C. punctata	cattle	ant. s. i.	Stewart, 1954
Cucullanus cirratus	fish	ant. s. i.	Valovaya, 1979
Dochomoides stenocephala	dog	- - - -	Gibbs, 1961
Hedrurus ijimai	frog	- - - -	Hasegawa & Otsuru, 1979
Kalicephalus parvus	boa constrictor	ant. s. i.	Schad, 1962
Nematodirus battus	sheep	ant. s. i.	Mapes & Coop, 1972
N. fillicolis	sheep	- - - -	Thomas, 1959

(c) Nematodes inhabiting the small intestine (cont'd)

N. helviatanus	cattle	- - - -	Herlich, 1954
Nematospiroides dubius	mouse	ant. s. i.	Bryant, 1973 Lewis & Bryant, 1976 Sukhdeo & Croll, in press
Nippostrongylus brasiliensis	rat	- - - -	Alphey, 1970
Oesophagostomum vigintimembrum	camel	- - - -	Canavan, 1931
Oswaldocruzia pipiens	frog	ant. s. i.	Barker, 1978a
Parascaris equorum	horse	ant. s. i.	Foster, 1931 Clayton & Duncan, 1979
Polydelphis quadrangularis	rattlesnake	ant. s. i.	Araujo, 1972
Porracaecum angusticalle	buzzard	duodenum	Canavan, 1931
P. depressum	blackfinch	- - - -	Canavan, 1931
P. ventriculatum	heron	ileum	Canavan, 1931
P. serpentulus	crane	duodenum	Canavan, 1931
Placoconus lotoris	raccoon	post s. i.	Balsingham, 1964
Rhabdocona ergensi	fish	- - - -	Moravec, 1972
Streptopharagus pigmentatus	monkey	- - - -	Machida *et al.*, 1978

(c) Nematodes inhabiting the small intestine (cont'd)

Strongyloides agoutii	guinea pig	duodenum	Griffiths, 1940
S. ratti	rat	ant. s. i., mucosa at base of glands	Abadie, 1963 Wertheim, 1970
S. venezuelensis	rat	ant. s. i., mucosa of villi	Wertheim, 1970
Strongylus equinus	zebra	- - - -	Canavan, 1931
Toxocara canis	dog	- - - -	Warren, 1969
T. vitulorum	cattle	- - - -	Warren, 1971
Trichinella spiralis	mouse	ant. s. i.	Larsh & Hendricks, 1949 Sukhdeo & Meerovitch, 1980
Trichostrongylus colubriformis	guinea pig	ant. s. i.	Connan, 1966
Truttaedacritis stelmoides	lamprey	- - - -	Pybus *et al.*, 1978

(d) Nematodes inhabiting the large intestine

Aspiculuris tetraptera	mouse	proximal colon	Chan, 1955
Castorstrongylus castoris	beaver	caecum	Canavan, 1931
Chabertia ovina	sheep	- - - -	Herd, 1971

(d) Nematodes inhabiting the large intestine (cont'd)

Cosmocerca kashmiriensis	toad	- - - -	Fotedar & Tihoo, 1968
Cosmocercoides dukae	toad	rectum	Baker, 1978b
Craterstomum micronatum	horse	dorsal colon	Foster, 1936
Cyathostomum coronatum	horse	caecum	Foster, 1936
C. labiatum	horse	ventral colon	Foster, 1936
Cyclobractus brericapulatis	horse	dorsal colon	Foster, 1936
Cyclostephanus calicatus	horse	caecum	Foster, 1936
C. poculatus	horse	caecum	Foster, 1936
C. minutus	horse	ventral colon	Foster, 1936
C. longibursatus	horse	ventral colon	Foster, 1936
C. hybridus	horse	ventral colon	Foster, 1936
Cylicocercus catinatus	horse	ventral colon	Foster, 1936
C. goldi	horse	dorsal colon	Foster, 1936
C. pateratus	horse	ventral colon	Foster, 1936
Cylicocylus radiatus	horse	ventral colon	Foster, 1936
C. elongatus	horse	dorsal colon	Foster, 1936

(d) Nematodes inhabiting the large intestine (cont'd)

C. nossatus	horse	ventral colon	Foster, 1936
C. insigne	horse	dorsal colon	Foster, 1936
C. leptostomus	horse	ventral colon	Foster, 1936
Cylicondontophorus euproctus	horse	dorsal colon	Foster, 1936
C. bicoronatus	horse	ventral colon	Foster, 1936
C. ultrajectinus	horse	dorsal colon	Foster, 1936
Cylicotetrapedon asymetricum	horse	dorsal colon	Foster, 1936
Gyalocephalus capitatis	horse	ventral colon	Foster, 1936
Heterakis gallinae	chicken	distal caecum and colon	Clapham, 1933 Kazam & Barya, 1973
H. papillosa	chicken	caecum	Uribe, 1922
Hypodontus macropi	kangaroo	caecum	Mykytowycz, 1964
Kalicephalus rectophilus	boa constrictor	rectum	Schad, 1962
Oxyuris equii	horse	dorsal colon	Foster, 1936
Oesophagostomum columbianum	sheep	- - - -	Dash, 1973
O. venulosum	sheep	caecum	Goldberg, 1951
Posteriostomum imparidentatum	horse	dorsal colon	Foster, 1936

(d) Nematodes inhabiting the large intestine (cont'd)

P. ratzii	horse	dorsal colon	Foster, 1936
Strongylus edentatus	horse	ventral colon	Foster, 1936
S. equinus	horse	caecum	Foster, 1936
S. vulgaris	horse	caecum and colon	Drudge et al., 1966
Tachygonetria spp	Tortoise	colon	Schad, 1963a
Ternidens deminutus	baboons and man	- - - -	Goldsmid, 1971
Thelandros magnavulvaris	salamander	- - - -	Schad, 1936b
Trichostrongylus ovis	sheep	- - - -	Canavan, 1931
T. sigmodontis	cotton rats	caecum	Thatcher & Scott, 1962
T. tenuis	grouse and partridge	caecum	Cram & Cuvillier, 1934
Trichuris muris	mouse	caecum	Wakelin, 1969 Panesar & Croll, in press
T. leporis	rabbit	caecum	Shlikas, 1978
T. vulpis	dog	caecum	Miller, 1947
Tridontophorus serratus	horse	caecum	Foster, 1936
T. minor	horse	ventral colon	Foster, 1936

(d) Nematodes inhabiting the large intestine (cont'd)

T. tenuicollis	horse	ventral colon	Foster, 1936
T. brevicauda	horse	ventral colon	Foster, 1936

on the infective stage to start the activation process and production of the internal secretions (believed to be enzymic in nature) or (b), an indirect stimulus which initiates the same process (Rogers, 1962; Rogers & Sommerville, 1963, 1968; Lackie, 1975).

Many of the stimuli believed to be involved in the activation process (hatching, excystment or exsheathment) have been elucidated by in vitro experimentation where simulation of 'natural' conditions are believed to enhance success (Silverman & Hansen, 1971) and are believed to represent the factors normally present in the alimentary tract (Fairbairn, 1960, 1961; Rogers, 1958, 1962; Lackie, 1975). The activation and hatching of Ascaris lumbricoides eggs are dependent upon the temperature, pCO_2, pH and reducing factors that can be found in the homeothermic host (Fairbairn, 1960, 1961). The exsheathment of infective strongylid L_3 larvae require similar stimuli (Rogers, 1962).

The evidence suggests that under the ideal conditions of its host, the larvae are stimulated to secrete substances that aid in hatching or exsheathment. The exsheathing fluid produced by the stimulated larvae contains an aminopeptidase that is specific for the species (Rogers, 1965) but these enzymes have not always been found in other nematodes (Ozerol & Silverman, 1969; Slocombe & Whitlock, 1969; Slocombe, 1974).

In addition, it has been suggested that the activation of some nematodes may be aided by exogenous enzymes provided by the host. Dictyocaulus viviparus has an absolute requirement for pepsin, Trichostrongylus colubriformis has a relative requirement for pepsin and Haemonchus contortus larvae are indifferent to pepsin (Silverman, 1963; Silverman & Podger, 1964). This has been challenged by Parker and Croll (1975) who found that the larvae of D. viviparus do not require pepsin but that sheath degradation in vivo is a result of intestinal enzyme activity.

Bile salts may also be involved in the activation of nematode larvae. The rate of exsheathment of the larvae of T. colubriformis and Nematodirus battus is increased and the hatching of eggs of Trichosomoides crassicauda is enhanced, in the presence of bile salts (Chapman & Undeen, 1968; Mapes, 1972).

The specificity of the nematode larva's response to the activating stimuli has led to the suggestion that sensory receptors are involved in the activation process (Rogers & Sommerville, 1960) but experimental evidence is not convincing.

3) The nematode sensory system

All organisms that actively select their sites require a sensory capacity. Nematodes possess several receptor structures and respond to different input modalities (Ward, 1973, Ward et al., 1975, Ware et al., 1975, Dusenbery, 1980). While these have not been proven to be functional in site selection, the body of circumstantial evidence favours this assumption.

The nervous system of a nematode consists of a "brain" or nerve ring (circumoesophageal ganglia and nerve cell bodies) to which are connected the main dorsal and ventral nerve chords and the lateral accessory nerve chords which travel to the posterior of the worm (del Castillo & Morales, 1969; White et al., 1976, 1978; Stretton et al., 1978). Sensory nerves also extend from the nerve ring and travel to the anterior (Ward et al., 1975; Ware et al., 1975). The various cephalic, cervical and caudal sense organs or "sensillae" (Lee & Atkinson, 1976) are all in continuity with the nervous system. The sense organs of nematodes and their neural connections have been the subject of several reviews (Ward et al., 1975, Ware et al., 1975, McLaren, 1976; Croll, 1977; and others).

The nematode sensory system shows discriminatory ability with a wide range of organic and inorganic chemicals but for the most part the biological function of these responses are unknown (Ward, 1978; Dusenbery, 1980; Croll & Sukhdeo, in press). In gastrointestinal nematodes, the sensory mechanisms have not been fully quantified but the sensory response of some nematodes to sexual attractants has been demonstrated (Roche, 1966; Bonner & Etges, 1967; Bone & Shorey, 1977, 1978). In the activation of the infective stage of some nematodes, the larvae are capable of responding to specific stimuli and this suggests that one or more receptors are involved (Rogers & Sommerville, 1960; Rogers, 1966a, b). A discrete chemorecptor which initiates a complex hormonal and neurosecretory response when stimulated has been postulated (Rogers 1966a, b). Indirect evidence for such a receptor may be the reversible inhibition of exsheathment of the infective stage when treated

with an oxidizing agent (Rogers, 1966a). Unfortunately, little else is known at the present time and before the mechanisms of stimulus reception and activation are understood, more information is needed on the role of hormonal and neurosecretory responses in nematodes.

4) Categories of site selection behaviour of nematodes in the gastrointestinal tract

In a review of the sites occupied by parasites in the alimentary tract, Crompton (1973) distinguished three categories of parasite behaviour based on the events leading to the establishment of gastrointestinal parasites in their sites: (a), direct arrival of the immature stages in the sites of the adults; (b), emigration of the adults in, or (c), against the direction of gastrointestinal flow subsequent to establishment of the immature stages. In his and other reviews on the sites selected by gut parasites, the site selection behaviour of the adults is usually emphasized. I have modified and changed Crompton's (1973) categories, to apply specifically to the site selection behaviour of gastrointestinal nematodes, thus: (a), nematodes with the site in the gastrointestinal tract the same at different stages; (b), nematodes with the sites in the gastrointestinal tract and extra-gastrointestinal locations different at diffent stages and (c), nematodes with the sites in the gastrointestinal tract different at different stages.

a) Nematodes with the site selection in the gastrointestinal tract the same at different stages.

In this group, site selection may be active in all stages or just in the establishing stage. It is also suggested

that the nematodes with passive mechanisms of site selection would be found in this group although the evidence is circumstantial. Among the helminths in which the sites are selected by the infective stages are the cestodes and acanthocephalans (Ulmer, 1971; Crompton, 1973; Holmes, 1973). The cystacanths of Polymorphus minutus passively select their sites and their site selection can be correlated with the physico-chemical conditions and the rate of propulsion of the gut (Lingard & Crompton, 1971; Crompton, 1973). The adults are also found at the site of larval establishment.

Amongst the gastrointestinal nematodes there are several that seem to fit into this category although the site selection by the larvae has not been demonstrated to be active or passive.

The distribution of T. spiralis adults is known to be aggregated in the small intestine (Tyzzer & Honeij, 1916; Roth, 1938, 1939; Gursch, 1949; Larsh & Hendricks, 1949; Sukhdeo & Meerovitch, 1980). The infective larvae of this nematode localize in the epithelium of the small intestine as early as ten minutes post-inoculation (Despommier et al., 1978). The larvae select their sites between the epithelial cells and basement membrane (Despommier et al., 1978) or within a syncytium of the epithelial cells (Wright, 1979) and the adult worms are recovered from the same site (Gardiner, 1976). The restriction to the mucosal epithelium may be due to the lamina propria; in vitro studies have demonstrated that lamina propria cells or their products are toxic to T. spiralis (Castro et al., 1975).

A number of other nematodes including Trichostrongylus axei (Purcell et al., 1971), T. colubriformis (Barker, 1973, 1974, 1975a, b), T. retortaeformis (Barker & Ford, 1975), T. muris (Panesar & Croll, in press) and Trichuris suis (Jenkins, 1970) occupy similar sites in the epithelial cells of the gut. This site selection suggests independence of the luminal contents of the gut which is consistent with the findings that the parenteral feeding of rats does not affect the development of T. spiralis (Castro et al., 1974, 1976b).

In T. spiralis infections, the adults are recovered from the site of larval establishment (Crompton, 1973). However, several investigators found that transplanted worms from all stages of the life cycle can establish successfully in the recipient host (Katz, 1960; Matoff, 1963; Denham, 1966). These studies suggest that although the adults remain in the same site as the larvae, the adults are also capable of site selection and establishment i.e. selection may be active in all stages. This is not so in T. muris infections.

Members of the genus Trichuris show considerable site specificity with intimate tissue contact. The adults are usually found with their anterior ends buried in the mucosa (Jenkins, 1970; Panesar & Croll, in press). Unhatched eggs of T. vulpis in the dog and T. muris in the laboratory mouse were both in the small intestine 30 minutes after infection (Miller, 1947; Fahmy, 1954; Panesar & Croll, in prep.). Histological studies with T. vulpis showed that there was a histotrophic phase in the small intestine and that the larvae

did not migrate to the large intestine until ten days after infection (Miller, 1947). Fahmy (1954) was able to recover larvae of T. vulpis from the caecum within 24 hours after infection and suggested that there was no histrotrophic phase in the small intestine. These latter results are consistent with those of Panesar & Croll (in prep.) using T. muris who found that in normal mice, the infective eggs were rapidly carried down the gut to the caecum. The larvae were quickly stimulated to hatch by factors present in the caecum (Panesar & Croll, in prep.). The hatched larvae penetrated the caecal mucosa where they developed in a "syncytium" of epithelial cells (Lee & Wright, 1978; Panesar & Croll, in press). The larvae retained their ability to penetrate and establish an infection for up to 10 days after hatching (Panesar & Croll, in press). Adults of T. muris were recovered from the same site as the larvae but the adults failed to establish when transplanted. The evidence suggests that site selection in this nematode does not occur in all stages, just in the establishing stage.

b) Nematodes with the sites in the gastrointestinal tract and extra-gastrointestinal locations different at different stages.

Several gastrointestinal nematodes have a migration phase through the host prior to establishment in the gut. In these nematodes, the critical factors for site selection may be very different for each stage. The complex migratory behaviour by the larvae of Ascaris lumbricoides and Spirocerca spp. before they establish in the gut strongly suggests a complex site selection behaviour in the larvae with various

cues at different stages (Kelley et al., 1957; Galvin, 1968; Douvres & Tromba, 1971; Bailey, 1972). Implantation of the larvae of Spirocerca lupi in various abnormal locations of the host all resulted in the larval recovery from the same site (Bailey, 1972). Visceral larva migrans, cutaneous larva migrans and Anisakis infections in paratenic hosts result from the less specific site selection of infective ascaroid larvae (Beaver, 1969; Croll et al., in press) and may be explained by the lack of specific sensory cues to guide them.

After the migration through the body, the worms enter the gut where the site selected is dependent on both the late larvae stages and the adults. Nippostrongylus brasiliensis has been described as a parasite of the upper intestinal tract (Yokogawa, 1920; Chandler, 1935; Haley, 1961; Brambell, 1965; Alphey, 1970). An interesting feature of the distribution pattern of N. brasiliensis is the presence of clumps of worms in the small intestine. It was suggested that the selection of the anterior small intestine by the larvae and adults of this nematode may be due to the availability of nutrients, concentration of bile and the concentration of pancreatic juices or other physiochemical factors (Alphey, 1970, 1971, 1972) and that the clumping behaviour was due to intrinsic activity patterns such as sexual attraction and thigmokinesis (Alphey, 1971). N. brasiliensis has an inflated balloon of cuticle which is associated with presumptive mechanoreceptor sense organs (Wright, 1976). This arrangement may considerably increase mechanoreception and may be relevant in thigmokinetic function.

It was postulated that site specificity of this nematode was due to the worm's requirement for oxygen (Alphey, 1972) in which they responded to the pO_2 gradient between the stomach and the caecum (Rogers, 1949) and from the mucosa to the lumen (Crompton et al., 1965). Once the nematode had migrated to its optimal site, thigmokinetic responses operated to keep the worms near to the mucosa (Alphey, 1972).

Recent findings challenged this hypothesis. There was a significant difference in the distribution of N. brasiliense in the intestine of fasted rats (Croll, 1976). The worms assumed a less clumped and much more dispersed distribution along the small intestine and even entered the caecum in rats fasted for 2 days. When the rats were returned to food (the nature of the food was not a dependent factor) the worms migrated to the food and assumed their normal distribution, confirming an earlier report by Alphey (1970) that the worms could migrate over long distances. Croll and Smith (1977) also disagreed with previous reports that N. brasiliensis lives near the mucosa; the worms were predominantly found within the food bolus and only in starved rats in which there is no lumen, were the worms found in the mucosa. The evidence suggests that these parasites simply orientate with respect to food using an orthokinetic response; the worms are active in the absence of food and having found food, they become relatively inactive and feed (Croll & Smith, 1977). This interpretation was partly confirmed in a recent study of Trichostrongylus vitrinus in sheep (Taylor & Kilpatrick, 1980).

In N. brasiliensis infections, the site is determined by labile factors resulting from the temporary fluctuation of environmental factors e.g. food in the gut. The possible importance of labile versus constant factors in site selection of nematodes will be discussed in a later section.

c) Nematodes with the sites in the gastrointestinal tract different for different stages.

Site selection by the nematodes in this group must be a property of more than one stage. The adults occupy sites that are different from the sites occupied by the larvae and this implies an active site selection where the worms are able to respond to specific cues and respond in a directed manner. It is generally accepted that there are two mechanisms by which the nematodes arrive at their sites: (a), a passive selection dominated by host influences or (b), an active selection dependent on the behavioural response of the nematode when stimulated by host-derived cues. Passive site selection dominated by the host would be largely determined by the rate of movement of luminal contents and the location of activation of the infective stages (Rogers, 1958, 1960; Sommerville, 1957; Lingard & Crompton, 1971; Crompton, 1973) and although active site selection may also be affected by these factors, the directional behaviour of the parasite would be a major part in site selection (Ulmer, 1971; Crompton, 1973; Holmes, 1973). Active site selection by nematodes is not found only in nematodes of this category but also in categories (a) and (b), however, the evidence for active site selection is mostly indirect and it is in this category that the evidence is most

convincing i.e. the adults leave the site of larval selection and migrate with or against the flow of ingesta and maintain themselves in specific locations.

The infective larvae of Placoconus lotoris encyst in the duodenum and the adults migrate along the gut to the posterior small intestine as they mature (Balasingham, 1964). The infective larvae of Kalicephalus parvus encyst in the stomach mucosa and the adults live in the small intestine (Schad, 1956) while the infective larvae of Dochomoides stenocephala encyst in the gastric and duodenal mucosa and the adults live in the small intestine (Gibbs, 1961). Finally, the infective larvae of Chabertia ovina and Oesophagostomum venulosum encyst in the small intestine and the adults live in the large intestine (Goldberg, 1951; Herd, 1971). In these examples, the emigration was posteriad and may be passive; and it is reported that the adults of O. venulosum may be swept out with the faeces after the histotrophic phase (Goldberg, 1951).

In mice infected with N. dubius the larvae encyst in the wall of the small intestine, at maturity, the worms enter the lumen and migrate anteriad, against the gastrointestinal flow, to their site in the duodenum (Lewis & Bryant, 1976). Panter (1969) argued that these shifts were due to changes within the preferred sites that made them unfavourable and the probable cause was the host immune response. The more recent literature has suggested for many nematode infections that immunological reactions or tissue pathology can cause the chosen site to become "uninhabitable", while

this lacks experimental verification, it is a popular notion.

Bawden (1969) believed that the anteriad migration of N. dubius was due either to pH or pCO_2 gradients or dietary factors. Dobson (1961) suggested that the stimulus for emigration may have been an adverse reaction to bile or the effects of bile, since the adults migrated to a site anteriad of the bile duct. Two possible mechanisms have been postulated in the most recent study of N. dubius by Lewis & Bryant (1976). Firstly, a response to an unknown factor(s) that is secreted by the anterior small intestine, or, secondly, orientation to a pO_2 gradient along the small intestine. Although the first suggestion should not be discarded, there is some experimental support for the second suggestion. Aerobic metabolism is of functional importance to this species and oxygen was found to be necessary in all parasitic stages for survival and motility (Bryant, 1974). A pO_2 gradient exists in the gut with the highest concentrations at the pylorus (Rogers, 1949). An orientation response to higher concentrations of oxygen could explain the adult distribution.

Pinworms inhabit the large intestine and because of the relative ease of maintainance and facility of infection, they have been studied by several workers (Philpot, 1924; Schnuffner & Swellengrebel, 1949; Prince, 1950; Chan, 1952, 1955; Brown et al., 1954a, b; Mathies, 1954; Schad, 1963a).

Pinworm eggs travel to the large intestine before they hatch (Philpot, 1924; Chan, 1955), the larvae establish in the caecum and colon and the adults are recovered from the

same site (Prince, 1950). However, the adults of the mouse pinworm, Aspiculuris tetraptera are usually found in a more restricted habitat than the larvae. Early in the infection, the majority of larvae are found in the distal colon but as the infection progresses, the adults are found to be concentrated in the proximal colon (Chan, 1955). Site specificity within the large intestine has also been demonstrated in other pinworms. Large numbers of Tachygonetria spp. infected the colon of their tortoise host (Testudo graeca), but despite these high infections, there was little overlap in the distributions among the more than 10 species studied (Schad, 1963a). Among the species with similar linear distributions, the radial distributions differed. The niche specialization that occurs between these closely related species in the colon illustrates the complexity of factors that must be involved in the selection of sites.

5) Factors affecting site selection of gastrointestinal parasites

There is little information available on the cues that are used in site selection of gastrointestinal nematodes. Most investigators suggest that sites are determined by labile factors resulting from the temporary fluctuation of environmental factors e.g. food on the site selection of N. brasiliensis (Croll, 1976) or various physico-chemical and nutritive gradients on the site selection of N. dubius (Bawden, 1969; Lewis & Bryant, 1976). Very few workers have considered that site selection may also be determined by critical architectural factors but there are several examples of host and site selection

of cestodes based on the architecture of the gut (Williams, 1960; Williams et al, 1970; Carvajal & Dailey, 1976; McVicar, 1977, 1979). The evidence for site selection of nematodes based on gut architecture is scanty but it can be argued that the epithelial cells are essential for the site selection of T. spiralis and T. muris (Despommier et al., 1978; Lee & Wright, 1978, Wright, 1979; Panesar & Croll, in press) and that the size and topography of the duodenal villi are important in the attachment and therefore site selection of N. dubius (Kleinschuster et al., 1978). In studies of site selection, the notion of labile and constant factors, the interaction between the labile and constant factors and the critical thresholds that may be involved in these interactions has not been well developed. It is an area that requires much more work.

The following is a summary of the factors that have been reported to affect the site selection and distribution of parasites of the gastrointestinal tract.

a) Diet

Several investigators have reported a relationship between diet and intestinal helminths (Chandler, 1943, 1953; Hunter, 1953; Gordon, 1960; Gibson, 1963, Hopkins, 1969, 1970a, b; Bawden, 1969; Dunkely & Mettrick, 1969; Read & Kilejian, 1969; Mettrick, 1971a, b; Croll 1976). The cestode, H. dimunita displays a migration response that is related to the ingestion of food by the rat host (Hopkins, 1969; Mettrick, 1971a, b, 1972). The migration was related to the nutritional requirements of the host and that there was a

positive correlation between host feeding and increases in the worm's biomass (Mettrick, 1970; Mettrick & Cannon, 1970). However, although many investigators have found that the normal development of this parasite is greatly affected by the quantity and quality of the diet (Chandler, 1943; Chandler et al., 1950; Read & Rothman, 1957a, b; Roberts, 1966; Roberts & Platzer, 1967; Dunkely & Mettrick, 1969), it has been reported that H. dimunita could develop normally in the gut of rats that were parenterally fed (Castro et al., 1976b). This evidence suggests that the host may be secreting nutrients into the intestinal lumen which can be utilized by the cestode; and it strongly supports the concept of luminal homeostasis proposed by Read (1950, 1971) (discussed in the next section).

Among the nematodes that inhabit the gut, the effects of diet may be important depending on their mode of feeding. The longitudinal distribution of N. dubius and N. brasiliensis are affected by the diet of their hosts (Bawden, 1969; Croll, 1976). Although a relationship has been described between the diet and distribution of N. dubius (the adults of which live close to the mucosa) it was complicated by several factors including the sex of the host and intraspecific sexual attraction (Bawden, 1969). The distribution of N. brasiliensis (a lumen dweller) was definitely affected by host diet (Croll, 1976) and the location of T. spiralis (a tissue dweller) was not found to be affected by the presence or absence of food (Castro et al., 1974, 1976b).

b) Gradients

In several reviews on the selection of sites by parasites it has been implied that the sites found in the alimentary tract are homeostatic (Read, 1971; Ulmer, 1971; Crompton, 1973; Holmes, 1973). The concept of a site as a constant topographic region with chemical homeostasis has been questioned by some investigators (Mettrick & Podesta, 1974; Croll, 1976). The concepts of sites and habitats in the gastrointestinal tract should be considered more as dynamic environmental interactions between hosts and their parasites and that all play an important part in site selection (Croll, 1976b).

There are a number of physico-chemical gradients along the gut and these include pO_2, pCO_2, pH, bile salts, amino acids, enzymes, ionic, osmotic and redox potentials (Mettrick & Podesta, 1974). Although many or all of these gradients have been implicated in parasite site selection (Read, 1950, 1971; Dobson, 1961; Bawden, 1969; Mettrick, 1970, 1971; Alphey, 1971, Smyth & Hasslewood, 1973; Mettrick & Podesta, 1974; Podesta & Mettrick, 1974, 1975; Lewis & Bryant, 1976), there has been little experimental substantiation and the assertions of these investigators have not been rigourously tested.

c) Microbial flora

There exists an interrelationship between bacterial flora and parasite infection in the gastrointestinal tract (Mettrick, 1971c; Podesta & Mettrick, 1974). The bacterial flora may alter the pO_2, pH or redox potentials in the gut, which, in turn, affects the parasite distribution (Podesta &

Mettrick, 1974). On the other hand, H. dimunita reduces the pH of the gut which may reduce the density of microflora in their habitat (Mettrick & Podesta, 1974).

The relationship between nematode infections and microbial flora has not been extensively studied. In nematode infections, there is a synergistic and an antagonistic component in the microbial flora (Westcott, 1970; Mettrick & Podesta, 1974; Hall et al., 1976). The normal flora of the small intestine of mice significantly favoured the development of T. spiralis whereas in bacteria-free mice, establishment was decreased (Stefanski & Przyjalkowski, 1965, 1966; Przyjalkowski & Westcott, 1969). Although migrations may be more successful in gnotobiotic animals e.g. with infections of N. brasiliensis and Angiostrongylus cantonensis, the healing of lesions produced by the migrating larvae and the development of adults in the gut is much more successful in animals with the normal flora (Newton et al., 1959; Westcott & Todd, 1964; Westcott, 1968, 1970; Przyjalkowski & Gorecka, 1976). Because of possible avitaminosis, dietary factors and other experimental conditions, the interpretation of these results is very speculative.

The nematodes and the microbial flora may also interact synergistically to increase the pathological effects of the parasite. The pathology of T. suis infection is much more severe in conventional pigs than in germ-free pigs (Rutter & Beer, 1971; Hall et al., 1976).

d) Parenteral factors

The relationship between the host immune response and the distribution of nematodes in the gut has been demonstrated

by several investigators (Larsh et al., 1952; Brambell, 1963; Panther, 1969; Kennedy, 1978). Nematode distributions are also affected by increased host age, possibly due to increased immunity (Dobson, 1962; Benkhe, 1975b; 1976) and by corticosteroid treatment (Coker, 1955, 1956; Markell & Lewis, 1957; Robinson, 1961; Campbell & Collette, 1962; Mathies, 1962; Olivier, 1962; Briggs, 1963; Campbell, 1963; Ogilvie, 1965).

Sex hormones have also been implicated in the alteration of nematode distributions in the gut. Nematode infections may be altered by host sex (Mathies, 1954, 1959b; Dobson, 1961; Stahl, 1962; Benkhe, 1976; Lewis & Bryant, 1976), host pregnancy (Oshima, 1961; Dunn & Brown, 1962), host lactation (Connan, 1970, 1972, 1974; Dineen & Kelly, 1972) and gonadectomy or hormone treatment of the host (Solomon, 1966). The role of hormones in the host-parasite relationship is not fully understood (Solomon, 1966, 1969) but hormones play an important role in intestinal function and both sex and gastrointestinal hormones of the host may affect parasites (Solomon, 1969).

e) Intra-specific and inter-specific interactions between parasites

The hookworm, Ancylostoma caninum, has a preferred location in the jejunum of dogs, but in heavy infections the crowded worms may locate along the entire length of the small intestine (Krupp, 1961). Similarly in heavy infections of T. spiralis (Roth, 1938) and C. ovina (Herd, 1971), there is an increased longitudinal dispersion. The host's immune response is density dependent and results in the rejection of both parasites

before it completes its development (Herd, 1971; Kennedy, 1975).

Interactions with the opposite sex may also affect the distribution of nematodes in the gut. Male and female hookworms (A. caninum and Necator americanus) and T. spiralis can find each other in the gut even if they are inoculated at separate locations (Doerr & Menzi, 1933; Beaver, 1955; Oshima et al., 1962; Roche, 1966; Kozlov, 1971; Sukhdeo & Meerovitch, 1977). Sex attraction and orientation towards the opposite sex has been observed in several nematodes (Green, 1966; Roche, 1966; Bonner & Etges, 1967; Anya, 1976a, b; Bone & Shorey, 1977, 1978; Roberts & Thornson, 1977a, b) and the movements of worms to the opposite sex may result in altered distributions. In hookworm infections, when the sexes are unbalanced with more males than females, there is an increase in the laceration of the mucosa and subsequent blood loss as the males migrate more actively to find the females (Beaver et al., 1964).

CHAPTER 2. GENERAL METHODS AND MATERIALS

2A. THE PARASITES

The isolate of T. spiralis used in this investigation was originally recovered from a naturally infected pig encountered by the Animal Diseases Research Institute, Canada Department of Agriculture, Quebec in 1959. It has been continuously maintained for more than 20 years in rats and mice at the Institute of Parasitology and has been extensively studied.

The isolate of N. dubius used in this investigation was donated by Dr. Jon Wetzel of Ayerst Research Laboratories, New York in 1978 and has been maintained for the past 2 years in Swiss mice at the Institute of Parasitology.

2B. THE HOSTS

The mice used in this investigation were female Swiss outbred mice, weighing 20-25 g, purchased from Canadian Breeding Farms, Quebec. Unless otherwise stated, they were housed in groups of 5 in plastic "shoebox" cages (12" x 6") and given Purina Mouse Chow (Ralston Purina Company of Canada, Quebec) and water ad libitum.

The rats used were male and female Evans, Long-Haired (ELH) hooded rats, weighing 250-350 g, purchased from Canadian Breeding Farms, Quebec. They were housed in groups of up to 6 animals in metal mesh cages (17" x 10") and, unless otherwise stated, they were given Purina Rat Chow (Ralston Purina Company of Canada, Quebec) and water ad libitum.

All animals were housed in an animal room at 21°C ± 1°C, on a 14 hour and 8 hour light/dark photoperiod.

2C. METHODS OF HANDLING TRICHINELLA SPIRALIS

1) Collection of infective larvae

Mice or rats which had been infected with T. spiralis for more than 30 days and therefore carried infective muscle larvae, served as the source of infective larvae. The animals were killed by decapitation, skinned, eviscerated, the bones crushed and the muscles of the carcass cut into small pieces. These pieces were homogenized with the "digestion solution" (below) for 30 seconds in a high speed Waring blender. The "digestion solution" was an artificial gastric juice consisting of a final concentration of 0.06 N HCl made up in tap water with 0.3%(W/V) pepsin (Fischer Scientific Company, Quebec). Approximately 30 ml of this solution were used for each gram of meat to be digested. The homogenate was incubated at 37°C with slow agitation. After 3 hours the homogenate was filtered through 2 layers of cheesecloth and the larvae were sedimented by tilting the collecting flask. The sedimented larvae were collected in a centrifuge tube using a suction device, washed thrice in 0.85% NaCl solution (saline) and stored at 4°C until used. The larvae were used within 12 hours of storage.

2) Counting the larvae

The isolated larvae were re-suspended in fresh saline and the volume was made up to 10 ml; from this, appropriate dilutions were made and counted using a McMaster, nematode, egg-counting chamber. At least 10 chambers were counted and

the mean was calculated.

3) Infecting with T. spiralis

An infecting dose of 1000 larvae for rats and 500 larvae for mice was delivered in 0.1 ml saline (unless otherwise stated) with a 1 ml syringe attached to a long blunt 18 gauge needle. The animals were infected by inserting the needle down the esophagus to the stomach and releasing the larvae. Hosts were given standard doses of larvae regardless of their age and weight and differences between hosts were always kept at a minimum.

4) Recovery of T. spiralis from the gut

Maximum numbers of adults of T. spiralis occur on the fifth day after infection (Sukhdeo & Meerovitch, 1980); adults were, therefore, collected on the fifth day. Hosts were fasted for 12 hours prior to sacrifice, to reduce the gut contents and facilitate fast and accurate counting. Mice were killed by decapitation, the small intestine was immediately removed, was lightly stretched and was divided into 12 equal segments using a modified grid similar to that employed by Brambell (1965). Each segment was slit open and was placed in a petri dish of warm NaCl (0.85%) and was incubated for 1½ hours at 37 °C. The worms which migrated out of the tissue could be easily counted under a dissecting microscope. Rats were treated similarly but the small intestine was divided into 5 cm segments. When worms were recovered from other parts of the gut, the caecum and colon were each considered as segments.

2D. METHODS OF HANDLING NEMATOSPIROIDES DUBIUS

1) Collection of infective larvae

Faeces of mice which had been infected with N. dubius for 2-6 weeks were used to culture infective larvae. The mice were put into cages with wire mesh bottoms and the faeces were collected after 24 hours. These faeces were examined for the presence of eggs of N. dubius and, if positive were cultured. The faeces were made into a slurry with water, strained through cheesecloth and the filtrate was centrifuged at 800 rpm for 10 minutes. The supernatant was discarded and the sediment containing the eggs was spread in the centre of a No. 1 Whatman filter paper with a diameter of 12 cm. A small 5 cm petri dish was inverted in the centre of a 15 cm petri dish containing a small amount of water (to a height of 0.5 cm). The filter paper was placed in the large petri dish so that the centre was raised above water level. Ten days after incubation at 25°C, the larvae had migrated into the water and were collected. The larvae were washed in water and stored at 4°C for up to 2 weeks.

2) Counting the larvae and infecting with N. dubius

The procedures for counting the larvae and infecting the host animals were similar to those for T. spiralis. Mice and rats were given standard doses regardless of their age and weight, rats were inoculated with 300 and mice with 100 infective, third stage larvae.

3) Recovery of N. dubius from the gut

The larvae of N. dubius in the gut were counted 6

days after infection while they were encysted within the tissue of the gut (Lewis & Bryant, 1976) and were easy to see because of their large size. The infected mice were killed by decapitation and the small intestine removed and divided into 12 equal segments (see p. 46). Each segment was then slit open longitudinally, pressed between 2 plates of glass each 8 x 4 cm, and was examined under the dissecting microscope. In rats, infected with N. dubius, the procedures were similar but the small intestine was divided into 5 cm segments.

2E. THE MEASUREMENTS OF LARVAL BEHAVIOUR IN VITRO

Larval behaviour of both T. spiralis and N. dubius was observed in closed watchglasses with even, cold illumination (fluorescent lighting under translucent plexiglass) at temperatures of 22 °C and 37 °C. A manual event recorder was used to record a temporal pattern of behavioural events.

Time-lapse and real speed recordings were made of mobile larvae using a Hitachi video taperecorder, Model SV-512, and a Hitachi closed-circuit television camera, Model HV-165, mounted on a Zeiss microscope over a warmed-stage (Croll, 1975). This permitted detailed measurements of certain behavioural parameters from the display monitor.

2F. THE MEASUREMENT OF GUT PROPULSION

Gut propulsion was measured in mice using Affi Gel Blue affinity chromatography beads, measuring 75-150 μ (Bio Rad Laboratories, Missisauga) as non-absorbable markers.

Each mouse was fasted for 12 hours prior to experimentation. Approximately 50,000 beads in 0.1 ml distilled water were given by temporary intragastric intubation. Fifteen minutes after intubation, the animals were killed and the small intestines were quickly removed and were divided into 12 equal segments (see p 46). The stomach and intestinal segments were each flushed with 10 ml distilled water to remove the beads and aliquants of the suspended beads were counted in a McMaster nematode egg-counting chamber. The distribution of beads was expressed as the percentage of beads in, or passing through, each segment during the 15 minute test period (modified from Castro et al., 1976, 1977). The rate of propulsion was described by the slope of the distribution and permitted fitted lines to be compared by regression analysis (e.g. Figure 3.13).

2G. GENERAL SURGICAL PROCEDURES

All animals were fasted for 12 hours before undergoing gastrointestinal surgery to reduce ingesta. This minimized possible infection and reduced gastrointestinal propulsion. They were then anesthetized with a single intraperitoneal dose of 1 mg/gm sodium pentobarbital (Somnothol , MTC Pharmaceuticals Limited, Hamilton). Using sterile procedures, laparotomy and surgery were performed and the abdomen was closed. The animal was then given an intramuscular injection of 10 mg/kg of a penicillin-dihydrostreptomycin antibiotic (Derapene®, Ayerst Research Laboratories, Montreal) and fed only glucose in their ad libitum water supply for the first

post-operative day. The animals were returned to a normal feeding regime on the second post-operative day.

2H. SOURCES OF BILE FOR EXPERIMENTATION

The standard crude bile used in this investigation came from the pooled gall bladder contents of 50 pigs. The bile was removed from these gall bladders using sterile procedures and was stored in small aliquants at -20° C until used. All of the other bile used were recovered from the gall bladders of experimental animals and in naturally acholecystic animals, the bile was collected through cannulation of the bile duct.

2I. HISTOLOGICAL PROCEDURES

All tissues for histological examination were fixed for a minimum of 24 hours in AFA (glacial acetic acid (5%) formaldehyde (10%), glycerine (10%), 95% ethyl alcohol, (25%) in distilled water (Croll, 1966)). The fixed tissues were dehydrated in a graded series of ethanols to absolute ethyl alcohol, transferred to benzene and then infiltrated and embedded in paraffin wax (M.P. 56° C). The embedded tissues were sectioned at 7 µm thickness and stained using the haematoxylin-eosin-method (Carleton, 1962).

CHAPTER 3. FACTORS AFFECTING THE LONGITUDINAL DISTRIBUTION OF TRICHINELLA SPIRALIS AND NEMATOSPIROIDES DUBIUS IN THEIR HOSTS

3A. INTRODUCTION

There are three groups of factors which may influence the site of intestinal helminths and which can be estimated through an analysis of their longitudinal distribution. Firstly, the activation (hatching, exsheathment or excystment) of the infective stage. Secondly, the morphology and motility of the gastrointestinal tract which impose passive influences on the dispersion of parasites. Finally, those sensory and behavioural phenomena, attributable to the parasite, which lead to active selection of specific sites. For a full discussion of these see pp 13 - 43. The relative contributions of these components is to be examined, through analysis of longitudinal distributions for T. spiralis and N. dubius.

T. spiralis is localized to certain areas of the small intestine although there have been some conflicting reports. Most worms are found in the anterior half of the small intestine of mature rats (Gursch, 1943a), mature mice (Larsh & Hendricks, 1949) and young mice (Campbell, 1967; Sukhdeo & Meerovitch, 1980). However, there are several reports in which T. spiralis inhabited the posterior half of the small intestine of young rats and mice (Larsh & Hendricks, 1949) and in mice, rats and guinea pigs of unknown ages (Tyzzer & Honeij, 1916; Roth, 1938, 1939). Larsh & Hendricks (1949)

suggested that the age of the host determined the longitudinal distribution of the worms because the intestinal motility of young mice was faster than in old mice. When the intestinal motility of young mice was generally suppressed with morphine sulphate, the worms established more anteriorly. These investigators did not quantify the rate of intestinal motility between the two groups. Furthermore, their results were contradicted by the results of Campbell (1967) who reported that the majority of T. spiralis localize in the anterior small intestine of normal young mice.

Infective larvae of N. dubius enter the host per os and exsheath in, and penetrate the stomach (Liu, 1964; Sommerville & Bailey, 1973). They penetrate and encyst in the small intestine and later they emerge as adults in the lumen (Bryant, 1973). The positions of larvae and adults of N. dubius in the small intestine differ as the infection progressed (Lewis & Bryant, 1976) and while several physico-chemical factors have been implicated in the dispersion of adults of N. dubius in the intestine e.g. diet, host sex, host age and intestinal microflora (Dobson, 1961, 1962; Newton et al., 1962; Bawden, 1969; Panther, 1969; Lewis & Bryant, 1976); little is known of the factors affecting early larval establishment (Bawden, 1969).

Because of the contradictions in some of these reports and as part of our program to determine factors that affect the localization of parasites within their hosts, I have examined the effects of several physical factors on the establishment behaviour of both T. spiralis and N. dubius.

3B. MATERIALS AND METHODS

1) Trichinella spiralis

a) The longitudinal distribution of T. spiralis in the small intestine of mice with differing vehicles of infection.

Fifteen mice were divided into 3 equal groups, each group was given one of three types of dose of T. spiralis larvae: (a), 500 excysted larvae (HCl-pepsin digested); (b), 500 encysted larvae (trypsin digested, see below); or, (c), a piece of infected diaphragm containing an estimated 500 larval cysts. To recover encysted larvae of T. spiralis, the homogenized carcass of the infected host was digested in a 5% solution of trypsin in phosphate buffered saline (pH 7.2) while slowly agitated at 37°C for 3 hours. Five days after infection, the animals were killed and the distribution of worms in the small intestine was determined. The influence of the vehicle of infection on the longitudinal dispersion was therefore examined.

b) The effect of a surfactant on the longitudinal distribution of T. spiralis in the small intestine.

Twenty mice were divided into 4 equal groups. Two groups, the controls, were given 0.1 ml and 0.2 ml of 0.85% NaCl and the remaining groups were given 0.1 ml and 0.2 ml of the surfactant, Tween 80 (Polyoxyethylenesorbitan monooleate, Sigma Chemical Company, St. Louis). Thirty minutes after receiving the saline or surfactants, 500 larvae of T. spiralis were given to each animal by intragastric intubation. Five days after infection, the animals were killed and the distribution of worms in the intestine was determined. The influence

of a surfactant on longitudinal dispersion was therefore examined.

c) The effect of continuous antibiotic treatment on the longitudinal distribution of T. spiralis in the small intestine.

Two groups of mice were used in this study. One group was given a general antibiotic, penicillin (base) 10,000 μ/ml - Streptomycin (base) 10,000 mcg/ml (Grand Island Biological Company, Canada) at a 5% concentration in the drinking water ad libitum and the control group had nothing added to their drinking water. After 7 days of this treatment, all animals were infected with 500 larvae of T. spiralis and antibiotic treatment was discontinued in the experimental group. Five days after infection, the animals were killed and the distribution of worms in the small intestine was determined. Establishment and longitudinal dispersion were then examined with an altered or diminished intestinal bacterial flora.

d) The effects of the quantity of food in the gut on the longitudinal distribution of T. spiralis in the small intestine.

Seventy-nine mice were divided into 13 groups, 12 groups of 6 mice each and 1 control group of 7 mice which was used to monitor weight changes. The control group was fed ad libitum throughout the experiment and the remaining groups were given food for a 1-hour period each day, i.e. food was presented at 1000 hours and removed at 1100 hours each day, water was provided ad libitum throughout this regimen. To determine the adaptation of the mice to this feeding regimen, the mice

were weighed every 3 days. Initially, there was some loss of weight, but starting 9 days after the beginning of the experiment, the mice showed weight gains at a similar rate to that in the controls.

After continuing this regime for 3 weeks, 6 groups of the mice were infected with 500 larvae of T. spiralis in 0.1 ml saline at different times of the day: (a), infection at 1000 hours with no food presented then or for a further 12 hours; (b), infection at 1000 hours, at the time of food presentation; (c), infection at 1100 hours, at the time of food withdrawal; (d), infection at 1300 hours, 2 hours after food withdrawal; (e), infection at 1500 hours, 4 hours after food withdrawal and (f), infection at 2130 hours, 12½ hours after food withdrawal. The remaining 6 groups were given 0.1 ml saline at the times described above, then they were killed within 15 minutes and the distribution of food in the stomach and small intestine was determined. The small intestine of each mouse was removed and divided into 12 equal segments. The contents of the stomach and of each segment were put onto pre-weighed tin-foil containers, dried overnight at 60 °C and weighed. Five days after infection, the remaining mice were killed and the distribution of worms in the small intestine was determined. The influence of food distribution in the gut on the longitudinal dispersion of T. spiralis was therefore examined.

e) Implantation of excysted larvae of T. spiralis in different locations of the small intestine without temporary ligatures.

Fifteen mice were divided into 3 equal groups and all

mice were anaesthetized and laparotomized. Five hundred larvae in 0.1 ml saline were implanted with a syringe into the duodenum or ileum. A control group was infected by intragastric inoculation. Five days later, the animals were killed and the distribution of worms in the small intestine was determined. The influence of larval implantation on longitudinal dispersion was examined.

(f) Implantation of excysted larvae of T. spiralis in different locations of the gut using temporary ligatures.

Thirty mice were divided into 6 equal groups, all mice were anaesthetized, laparotomized and regions of the gut were temporarily ligatured into pouches. Five hundred larvae in 0.1 ml saline were implanted into each pouch; the ligatures were released 30 minutes after the larvae had been injected and the abdomens were closed. One control group was given 500 larvae by intragastric intubation. The 5 experimental groups were given the larvae into pouches at the following locations: (a), duodenum; (b), jejunum and upper ileum; (c), distal ileum; (d), caecum and (e), colon. Five days later, the animals were killed and the distribution of worms in the gut was determined. The influence of implantation of larvae into ligatured pouches on the longitudinal dispersion was therefore examined.

g) The effect of altered intestinal propulsion on the longitudinal distribution of T. spiralis in the small intestine.

(i) Twenty mice were divided into 2 equal groups. One group was treated with 50 mg/kg diphenoxylate hydrochloride (Lomotil®, Searle Pharmaceuticals, Oakville) in 0.1 ml

distilled water, 3 doses were given per day from 2 days prior to infection to the time of infection. Lomotil® reduces intestinal motility and peristaltic activity (Council on Drugs, 1964; Kinnear, 1964; van Neuten, 1964). The control group was given 0.1 ml distilled water at corresponding times. Five hundred larvae of T. spiralis were given to each of 5 mice in each of the two groups. At the time of infection, the remaining 5 mice from each group were tested for the rate of intestinal propulsion using Affi Gel Blue affinity chromatography beads (see general materials and methods section).

Five days after infection, the remaining mice were killed and the distribution of worms in the small intestine was determined. The influence of reduced intestinal propulsion in the longitudinal dispersion of T. spiralis was examined.

(ii) Thirty mice were divided into 6 equal groups. Five hundred larvae of T. spiralis in 0.1 ml saline were given to the control group and 500 larvae in 0.5 ml and 1.0 ml saline were given to the mice in two groups. The rate of intestinal propulsion was determined in the remaining 3 groups using Affi Gel Blue affinity chromatography beads delivered in 0.1 ml, 0.5 ml and 1.0 ml saline.

Five days after infection, the animals were killed and the distribution of worms in the small intestine was determined. The influence of increased intestinal propulsion on the longitudinal dispersion of T. spiralis was therefore examined.

h) The effects of different inocula on the longitudinal distribution of T. spiralis in the small intestine.

Twenty mice were divided into 4 equal groups. The mice in each group were given an intragastric inoculation of one of the following larval doses: (a), 100; (b), 500; (c), 1000 and (d), 2000 (all inocula were delivered in 0.1 ml saline). Five days later, the animals were killed and the distribution of worms in the gut was determined. The influence of different larval inocula on the longitudinal dispersion of T. spiralis was therefore examined.

i) The effects of single-sex infections on the longitudinal distribution of T. spiralis in the small intestine.

Fifteen mice were divided into 3 groups. The mice in one group were each given 250 female larvae, the mice in the second group were each given 250 male larvae and the mice in the control group were each given 250 mixed larvae (male: female = 2:1.3). The method used in distinguishing larval sex was given in a previous paper (Sukhdeo & Meerovitch, 1977). Five days after infection, the animals were killed and the effects of the parasite's sex on longitudinal dispersion was therefore examined.

2) Nematospiroides dubius

a) The longitudinal distribution of N. dubius in the small intestine of mice.

The mice were divided into 2 groups. The mice were infected with 100 larvae of N. dubius that were either: (a), ensheathed, or, (b), exsheathed by treatment in 0.3% sodium hypochlorite for 3 minutes (Croll et al., 1967). Six days

after infection, the mice were killed and the distribution of worms in the small intestine was determined. The influence of the sheath during infection on the subsequent longitudinal dispersion of N. dubius was examined.

b) The effects of a surfactant on the longitudinal distribution of N. dubius in the small intestine.

Ten mice were divided into 2 groups. The control group was given 0.2 ml saline and the experimental group was given 0.2 ml Tween 80 (see p 53) by intragastric intubation. Thirty minutes after this treatment, 100 larvae of N. dubius were given to each animal by intragastric intubation. Six days after infection, the animals were killed and the distribution of worms in the small intestine was determined. The influence of a surfactant on the longitudinal dispersion was therefore examined.

c) The effect of continuous antibiotic treatment on the longitudinal distribution of N. dubius in the small intestine.

Ten mice were divided into 2 groups. One group was given a penicillin-streptomycin solution in their drinking water (see p 54) ad libitum. The control group was given only water. Seven days later, all animals were infected with 100 larvae of N. dubius and antibiotic treatment in the experimental group was discontinued. Six days after infection, the animals were killed and the distribution of worms in the small intestine was determined. The influence of an altered or diminished intestinal bacterial flora on longitudinal dispersion was therefore examined.

d) The effects of the quantity of food in the gut on the longitudinal distribution of N. dubius in the small intestine.

Thirty mice were divided into 6 equal groups. The mice were given food from 1000 to 1100 hours daily for 3 weeks (see p 55). The mice in 3 groups were then infected with 100 larvae of N. dubius at different times of the day: (a), infected at 1000 hours without food and with no food presented for a further 12 hours; (b), infected at 1000 hours, at the time of food presentation and (c), infected at 1500 hours, 4 hours after withdrawal of the food. The remaining groups were given 0.1 ml saline at the times described above, were killed within 15 minutes and the distribution of food in the stomach and small intestine was determined (see p 55). Six days after infection, the mice in the remaining groups were killed and the distribution of worms in the small intestine was determined. The influence of food distribution in the gut on the longitudinal dispersion of N. dubius was examined.

e) Implantation of larvae of N. dubius in different locations of the gut using temporary ligatures.

Sixty mice were divided into 12 equal groups; all of the mice were anaesthetized, laparotomized and regions of the gut were temporarily ligatured into pouches. One hundred larvae in 0.1 ml suspensions were implanted into each pouch and the ligatures were released after 30 minutes. The infective larvae were delivered in one of 4 ways: (a), ensheathed larvae in distilled water; (b), ensheathed larvae in a 10% aqueous solution of bile; (c), exsheathed larvae in distilled

water and (d), exsheathed larvae in a 10% aqueous solution of bile. The larvae in groups (c) and (d) were ensheathed by pretreatment for 3 minutes in sodium hypochlorite. These 4 groups of larvae were implanted into each of the following locations: (a), duodenum; (b), ileum and (c), caecum. Six days after infection the animals were killed and the distribution of worms in the gut was determined. Establishment and longitudinal dispersion were examined after implantation into normal and abnormal sites.

f) The effects of altered intestinal propulsion on the longitudinal distribution of N. dubius in the small intestine.

(i) Twenty mice were divided into 2 equal groups. One group was treated with 50 mg/kg Lomotil® (see p 56) in 0.1 ml distilled water; 3 doses were given per day from 2 days prior to infection to the day of infection. The control group was given 0.1 ml distilled water at corresponding times. One hundred larvae of N. dubius were given by intragastric intubation to 5 mice from each group. At the time of infection, the remaining 5 mice from each group were tested for the rate of intestinal propulsion using Affi Gel Blue affinity chromatography beads. Six days later, the infected animals were killed and the distribution of worms in the small intestine was determined. The influence of reduced intestinal propulsion on establishment and longitudinal dispersion was therefore examined.

(ii) Twenty mice were divided into 2 equal groups. Five mice from each group were given 100 larvae of N. dubius by

intragastric inoculation in 0.1 ml saline and 0.5 ml saline respectively. At the time of infection, the rates of intestinal propulsion were tested in the remaining 5 mice of each group using Affi Gel Blue affinity chromatography beads delivered in 0.1 ml and 0.5 ml saline respectively. Six days after infection, the remaining animals were killed and the distribution of worms in the small intestine was determined. The influence of increased intestinal propulsion on the establishment and longitudinal dispersion was therefore examined.

g) The effect of different inocula on the longitudinal distribution of N. dubius in the small intestine.

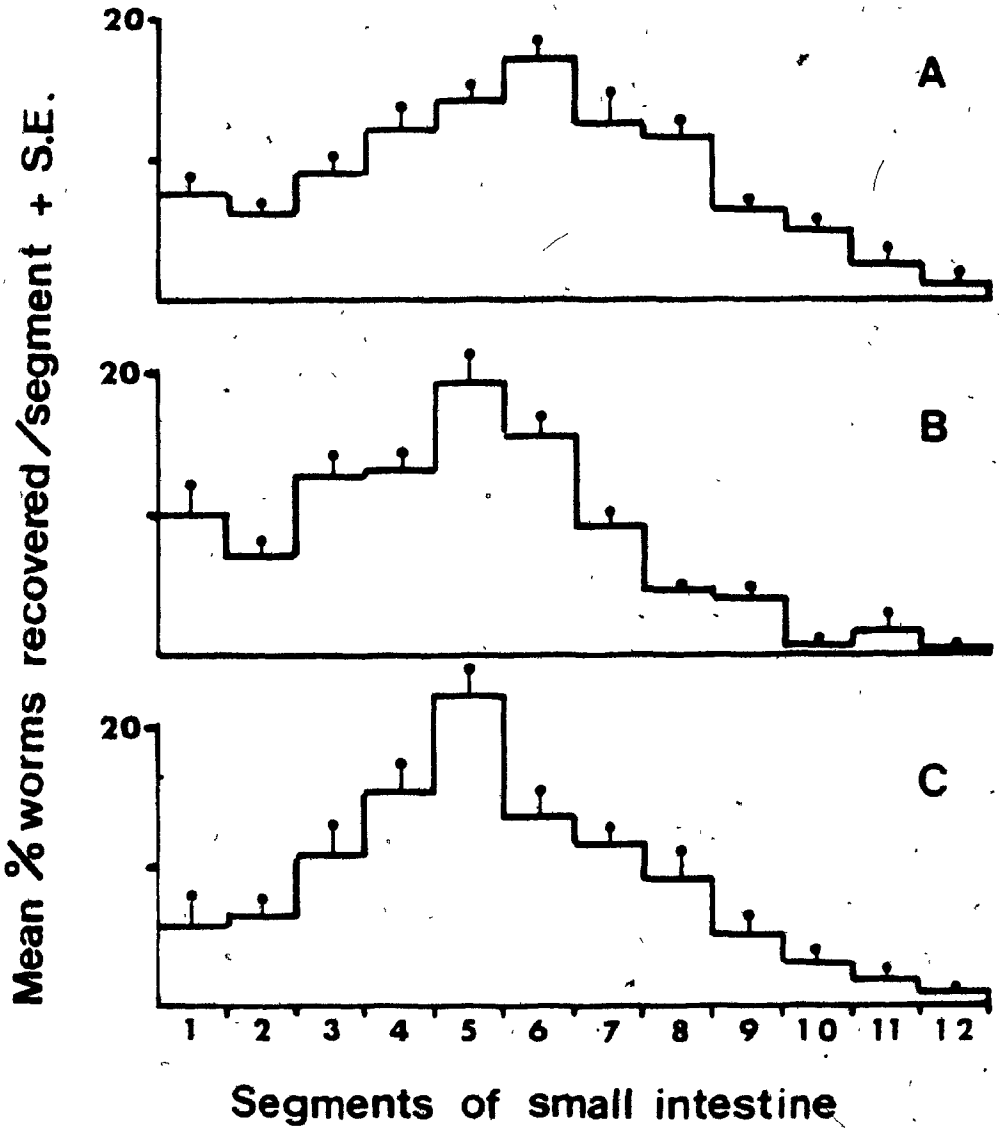
Twenty mice were divided into 4 groups. Each group was given an intragastric inoculation of one of the following larval doses of N. dubius: (a), 100; (b), 200; (c), 500 and (d), 1500, each in 0.1 ml. Six days after infection, the animals were killed and the distribution of worms in the small intestine was determined. The influence of larval inocula on longitudinal dispersion was therefore examined.

3C. RESULTS

1) Trichinella spiralis

The longitudinal distributions of adults of T. spiralis resulting from infections by encysted larvae, excysted larvae and larvae in infected meat were similar (Figure 3.1). About 50% of all adults were in the second quarter of the small intestine with less than 10% in the posterior quarter. In mice pretreated with the surfactant,

FIGURE 3.1 The distribution of adults in the small intestine of mice 5 days after oral infection with 500 larvae of T. spiralis. A, encysted larvae; B, excysted larvae and C, infected meat.


20
A
20
B
20
C
Mean % worms recovered/segment + S.E.
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

Tween 80, there was a more posterior peak in distribution pattern (Figure 3.2) which was greater with 0.2 ml than 0.1 ml treatments. Continuous antibiotic treatment caused a somewhat more anterior longitudinal distribution of the adults than in the controls (Figure 3.3). Table 3.1 lists the recoveries from these three experimental groups.

The distribution of food in the gut had an effect on the distribution pattern of T. spiralis in the small intestine. Figures 3.4, 3.5, 3.6, 3.7, 3.8 and 3.9 show the distribution of worms in the small intestine and the corresponding distribution of food in the gut at the time of infection. In all but one group, the majority of the adult worms were recovered from the second quarter of the small intestine independent of food distribution; in these groups the majority of the ingesta was recovered from the posterior half of the small intestine (Figures 3.6, 3.7, 3.8 and 3.9) or there was little or no food in the gut (Figure 3.4). In group (b) (Figure 3.5), the larvae were administered at the time of food presentation and the majority of adults, about 70%, were recovered from the posterior half of the small intestine (equal numbers in both quarters). The food distribution in these animals parallelled the parasite distribution. The mean numbers of adults recovered from these groups are shown in Table 3.2.

The distribution of adults in the small intestine of mice implanted with the excysted larvae of T. spiralis is shown in Figure 3.10. When the larvae were implanted into the duodenum, the majority of the worms were recovered from

FIGURE 3.2 The distribution of adults in the small intestine of mice 5 days after oral infection with 500 larvae of T. spiralis. A, control, 0.1 ml distilled water; B, control, 0.2 ml distilled water; C, Tween 80, 0.1 ml; D, Tween 80, 0.2 ml.

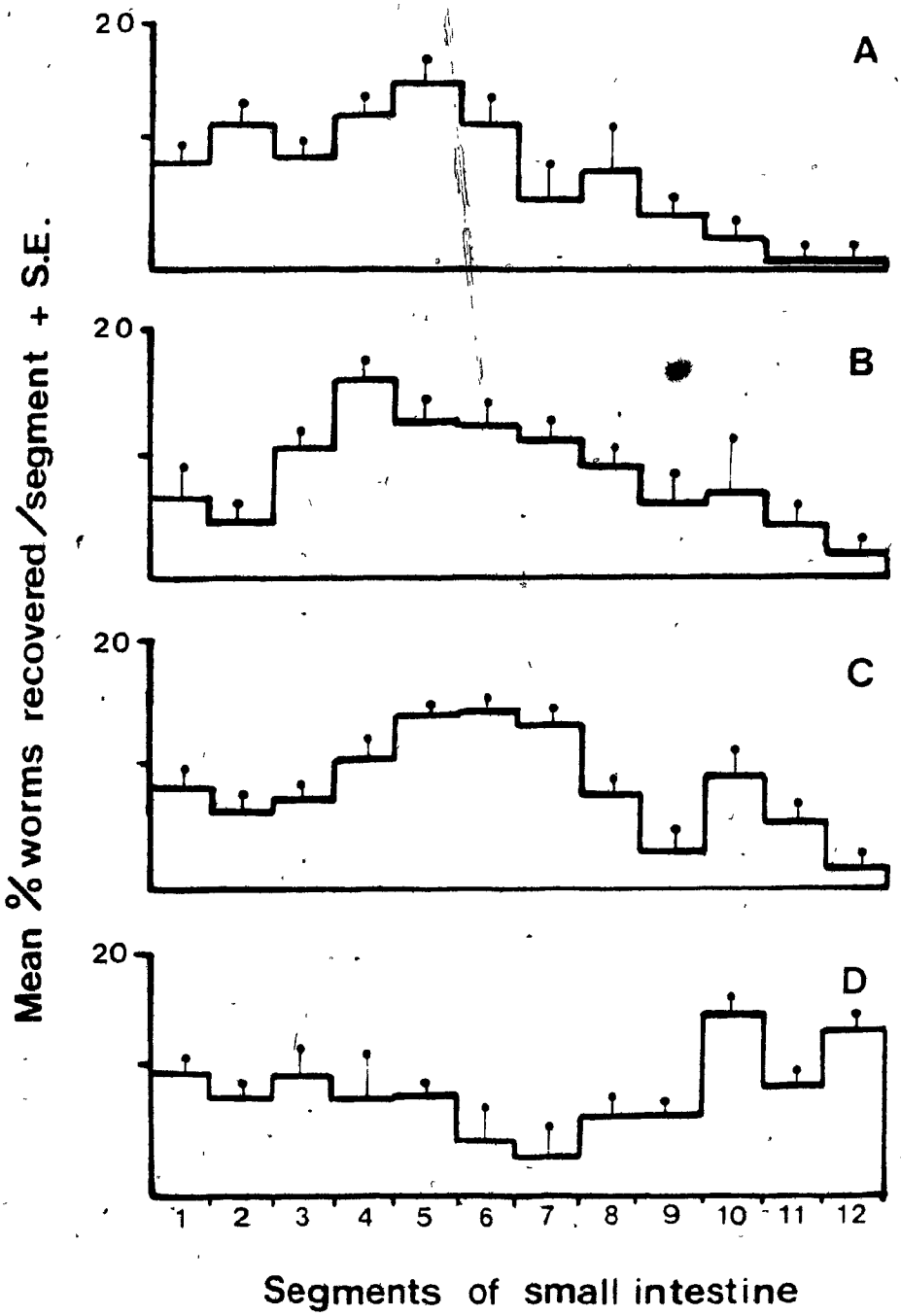

20
A
20
B
20
C
20
D
Mean % worms recovered/segment + S.E.
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

FIGURE 3.3 The distribution of adults in the small intestine of mice 5 days after oral infection with 500 larvae of T. spiralis. A, controls; B, mice received antibiotic treatment.

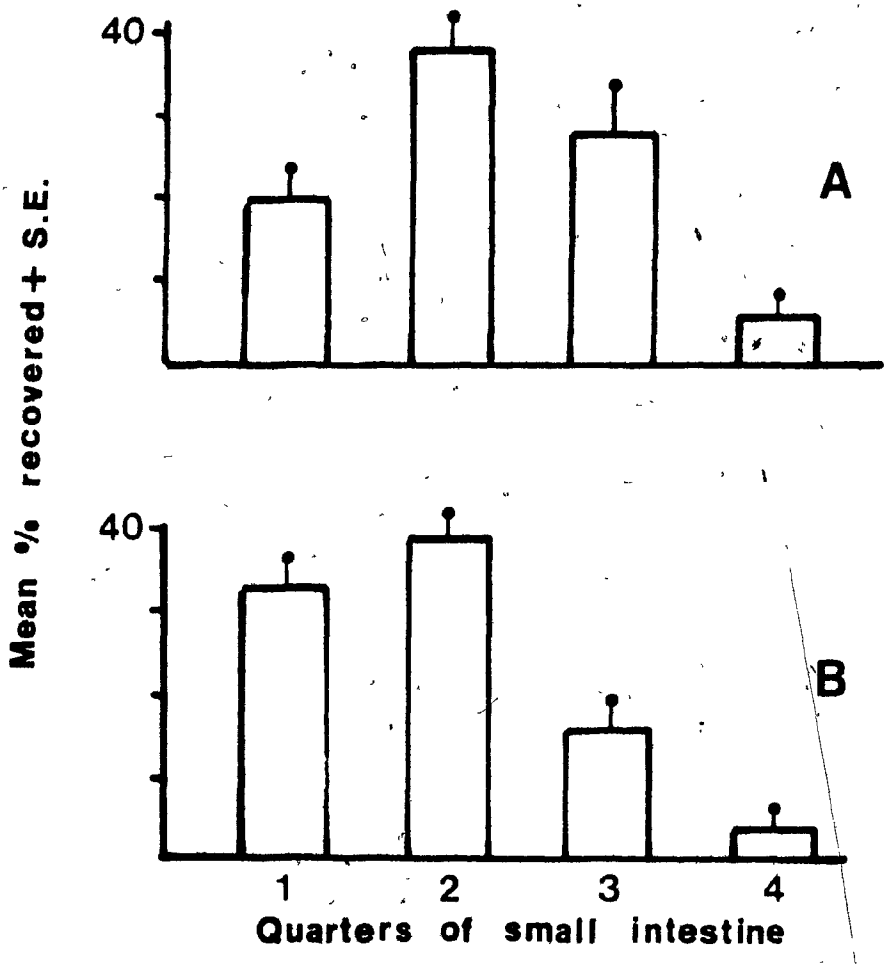

40
A
40
B
Mean % recovered + S.E.
1
2
3
4
Quarters of small intestine

TABLE 3.1 THE MEAN RECOVERY OF ADULTS FROM THE SMALL INTESTINE 5 DAYS AFTER INFECTION WITH 500 LARVAE OF T. SPIRALIS

Group	Treatment	Mean # Adults Recovered ± S. E.		% Recovery
1	encysted	229.3 ± 20.5	(a)	45.8
	excysted	207.0 ± 31.4	(a)	41.4
	infected meat	286.5 ± 29.6	(a)	57.3
2	Control (0.1 ml)	172.2 ± 7.5	(b)	34.4
	Tween 80 (0.1 ml)	161.5 ± 21.3	(b)	32.2
	Control (0.2 ml)	64.0 ± 8.5	(c)	12.8
	Tween 80 (0.2 ml)	43.4 ± 11.1	(c)	8.7
3	Control	223.7 ± 37.7	(d)	44.6
	Antibiotics	186.5 ± 25.4	(d)	37.3

(a) (b) (c) (d) All values with the same superscript do not differ significantly from each other ($p \leq 0.05$)

FIGURE 3.4. The distribution of adults in the small intestine of mice 5 days after infection with 500 larvae of T. spiralis and the distribution of food in the gut at the time of infection. Mice were infected at 1000 hours, and no food was presented then or for a further 12 hours. A, distribution of adults; B, distribution of food.

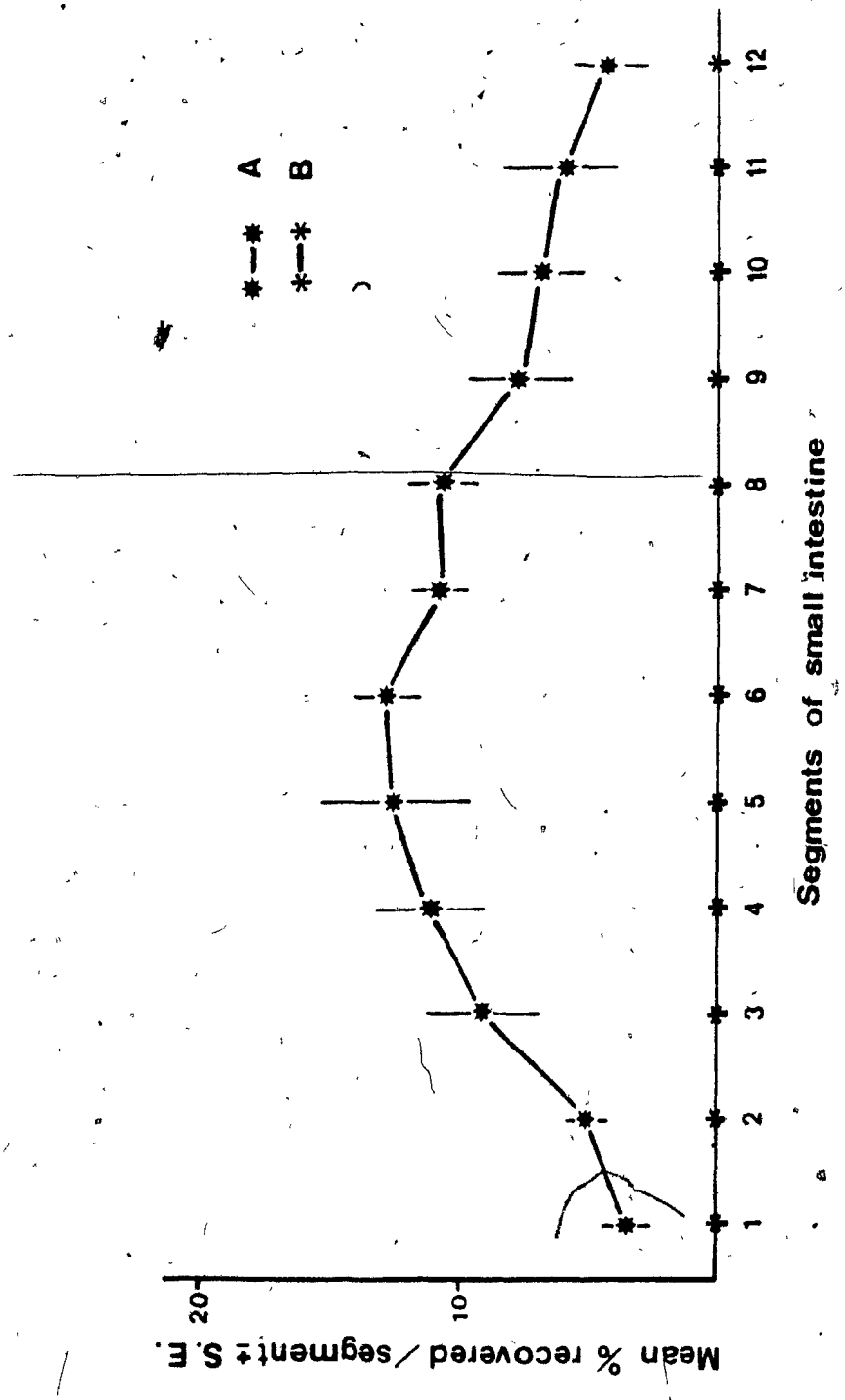

A
B
Mean % recovered / segment ± S.E.
20
10
1
2
3
4
5
6
7
8
9
10
11
12
Segments of small intestine

FIGURE 3.5 The distribution of adults in the small intestine of mice 5 days after infection with 500 larvae of T. spiralis and the distribution of food in the gut at the time of infection. The mice were infected at 1000 hours at the time of food presentation. A, distribution of adults; B, distribution of food.

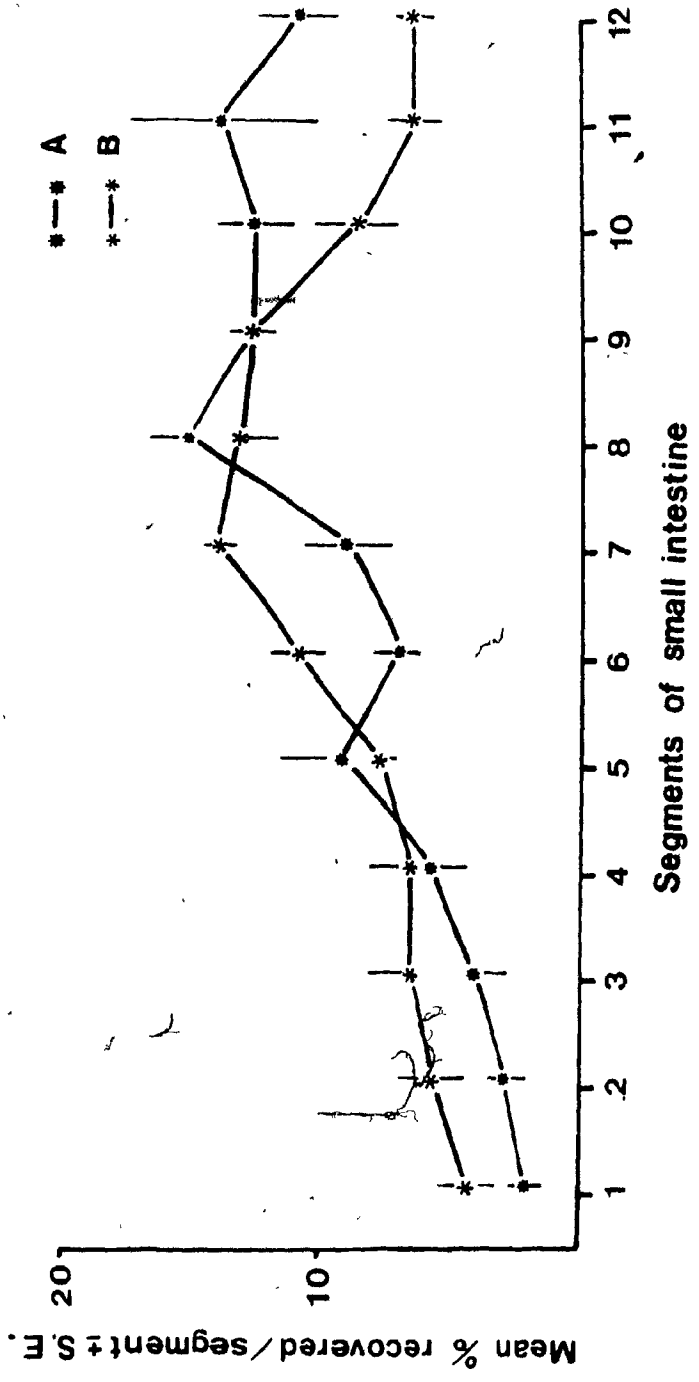

A
B
20
10
Mean % recovered/segment ± S.E.
1
2
3
4
5
6
7
8
9
10
11
12
Segments of small intestine

FIGURE 3.6 The distribution of adults in the small intestine of mice 5 days after infection with 500 larvae of T. spiralis and the distribution of food in the gut at the time of infection. The mice were infected at 1100 hours at the time of food withdrawal. A, distribution of adults; B, distribution of food.

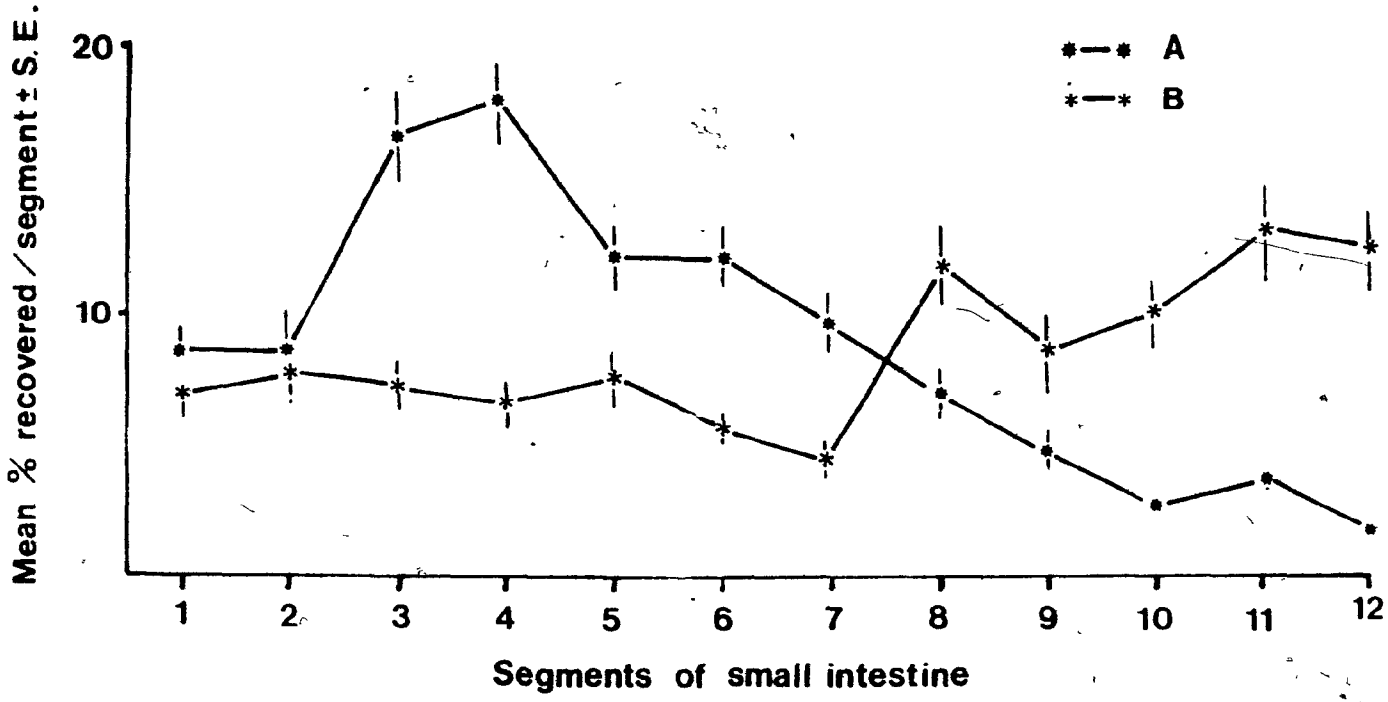

Mean % recovered / segment ± S.E.
20
10
A
B
1
2
3
4
5
6
7
8
9
10
11
12
Segments of small intestine

FIGURE 3.7 The distribution of adults in the small intestine of mice 5 days after infection with 500 larvae of T. spiralis and the distribution of food in the gut at the time of infection. The mice were infected at 1300 hours, 2 hours after food withdrawal. A, distribution of adults; B, distribution of food.

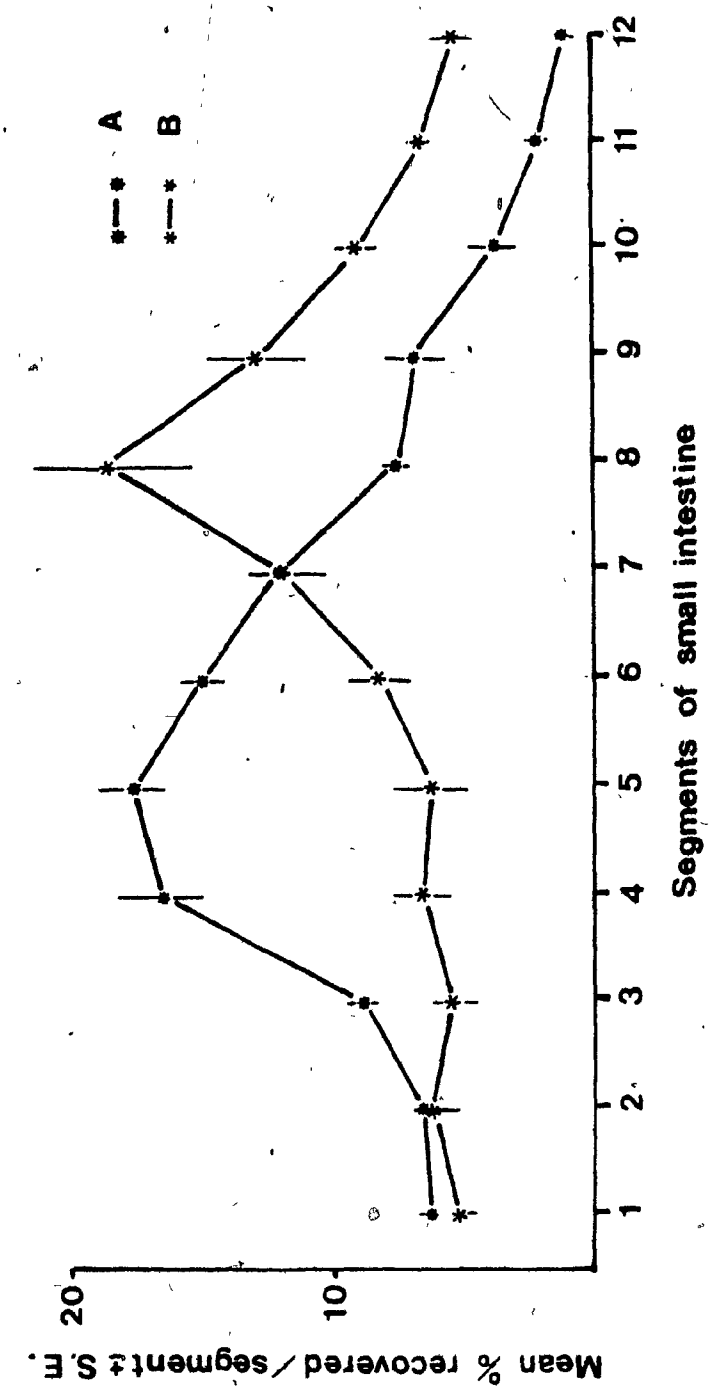

A
B
20
10
Mean % recovered / segment ± S.E.
1
2
3
4
5
6
7
8
9
10
11
12
Segments of small intestine

FIGURE 3.8 The distribution of adults in the small intestine of mice 5 days after infection with 500 larvae of T. spiralis and the distribution of food in the gut at the time of infection. The mice were infected at 1500 hours, 4 hours after food withdrawal. A, distribution of adults; B, distribution of food.

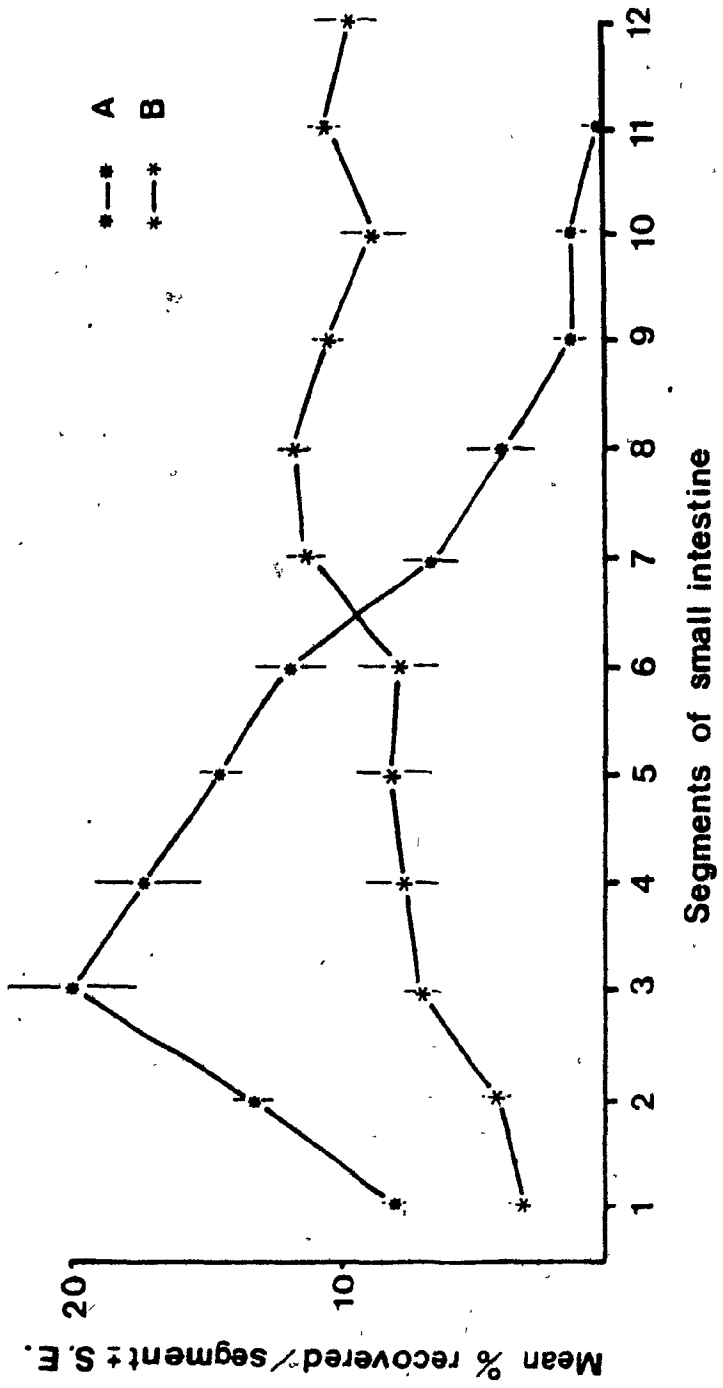

A
B
Mean % recovered/segment ± S. E.
20
10
1
2
3
4
5
6
7
8
9
10
11
12
Segments of small intestine

FIGURE 3.9 The distribution of adults in the small intestine of mice 5 days after infection with 500 larvae of T. spiralis and the distribution of food in the gut at the time of infection. A, distribution of adults; B, distribution of food.

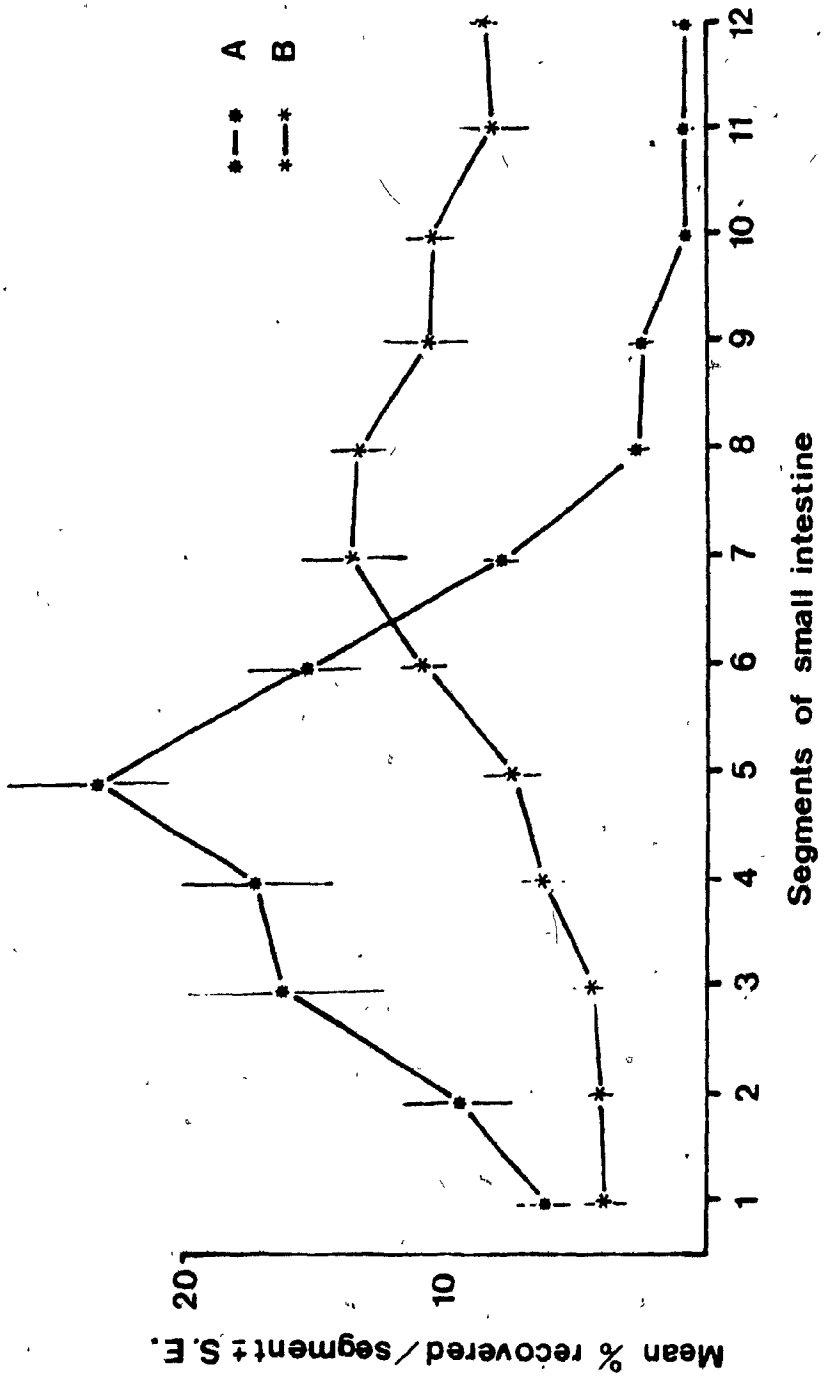

A
B
Mean % recovered / segment ± S.E.
20
10
1
2
3
4
5
6
7
8
9
10
11
12
Segments of small intestine

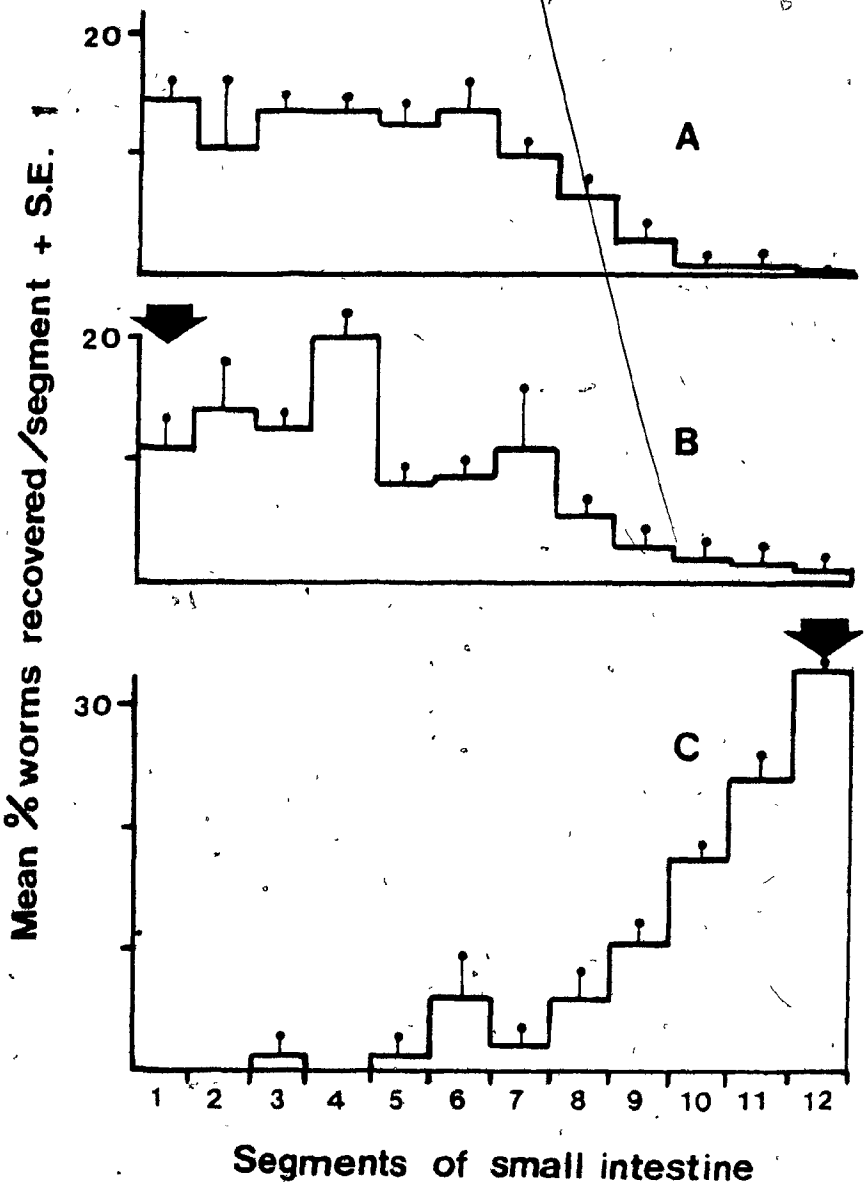
TABLE 3.2 THE MEAN TOTAL RECOVERY OF ADULT WORMS OF *T. SPIRALIS* FROM THE INTESTINE OF MICE, AFTER ADJUSTING TO FEEDING DURING A 1-HOUR PERIOD EACH DAY, INFECTED WITH 500 LARVAE

Time of Infection (hours)	1000	1100	1300	2130	1500	1000 (a)
Mean Total Recovery ± S. E.	88.8 ± 14.0*	122.2 ± 11.7	130.8 ± 11.2	131.0 ± 13.9	147.8 ± 20.0	254.2 ± 23.8

(a) 1000 group infected and fasted

* all values underscored by the same line are not significantly different ($p \leq 0.05$)

FIGURE 3.10 -The distribution of adults in the small intestine of mice 5 days after infection with 500 larvae of T. spiralis. A, controls; B, implantation into the duodenum and C, implantation into the ileum.


20
A
20
B
30
C
Mean % worms recovered/segment + S.E. 1
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

the anterior small intestine with a distribution similar to control animals given an oral infection. When the larvae were implanted into the ileum, the peak recovery was from the site of implantation with more worms anterior to the site of implantation. The numbers of adults recovered from these groups is shown in Table 3.3.

The mean recovery of adults was lowest in the ileum implantations and only slightly better in the duodenum implantations when compared to controls.

In mice implanted with excysted larvae between temporary ligatured pouches, the majority of the adult worms were consistently recovered from the site of implantation (with the possible exception of 3.12B) (Figures 3.11 and 3.12).

There was some longitudinal movement towards the mid-intestine in anterior or posterior implants. Larvae established but the percentage of recoveries varied from 7.1% in the colon to 29.1% in the mid-intestine (Table 3.4).

In mice in which the rate of intestinal propulsion was changed, there were changes in the distribution of worms in the intestine. The intestinal propulsion rate in animals treated with Lomotil® was significantly slower than that in the control animals ($p \leq 0.05$) (Figure 3.13). The rate of propulsion in the gut is given by the slope m. The distribution of adults recovered from the intestines of mice treated with Lomotil® was anterior of controls with the majority of larvae in the first quarter and with peak establishment in segment 2 (Figure 3.14). The adult recoveries are shown in Table 3.5.

TABLE 3.3 THE MEAN RECOVERY OF ADULT WORMS FROM THE SMALL INTESTINE FOLLOWING THE IMPLANTATION OF 500 LARVAE OF T. SPIRALIS INTO DIFFERENT LOCATIONS OF THE GUT

Treatment	Position	N	Mean # Adults Recovered ± S. E.	% Recovery
Control	intragastric	5	209.6 ± 18.8	41.9
Implantation	duodenum	5	39.2 ± 7.0 (a)	7.8
Implantation	ileum	5	33.2 ± 4.5 (a)	6.6

(a) significantly different from control ($p \leq 0.05$)

FIGURE 3.11 The distribution of adults in the gut of mice 5 days after implantation of 500 larvae of T. spiralis into temporarily ligatured pouches of gut. A, controls; B, implantation into the duodenum; C, implantation into the ileum; D, implantation into the caecum and E, implantation into the colon.

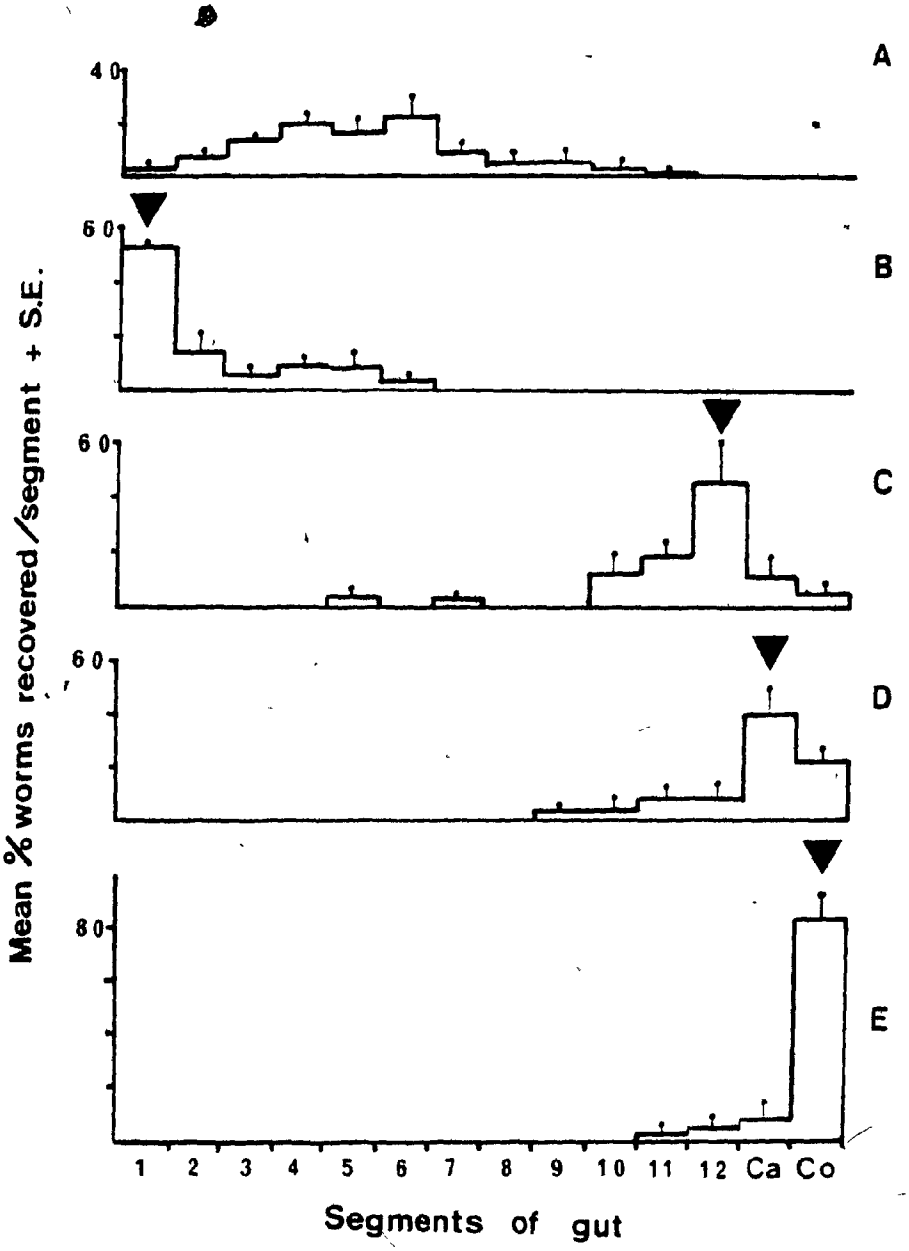

A
B
C
D
E
40
60
60
60
80
Mean % worms recovered / segment + S.E.
1 2 3 4 5 6 7 8 9 10 11 12 Ca Co
Segments of gut

FIGURE 3.12 The distribution of adults in the gut of mice 5 days after implantation of 500 larvae of T. spiralis into temporarily ligatured pouches of the mid small intestine.

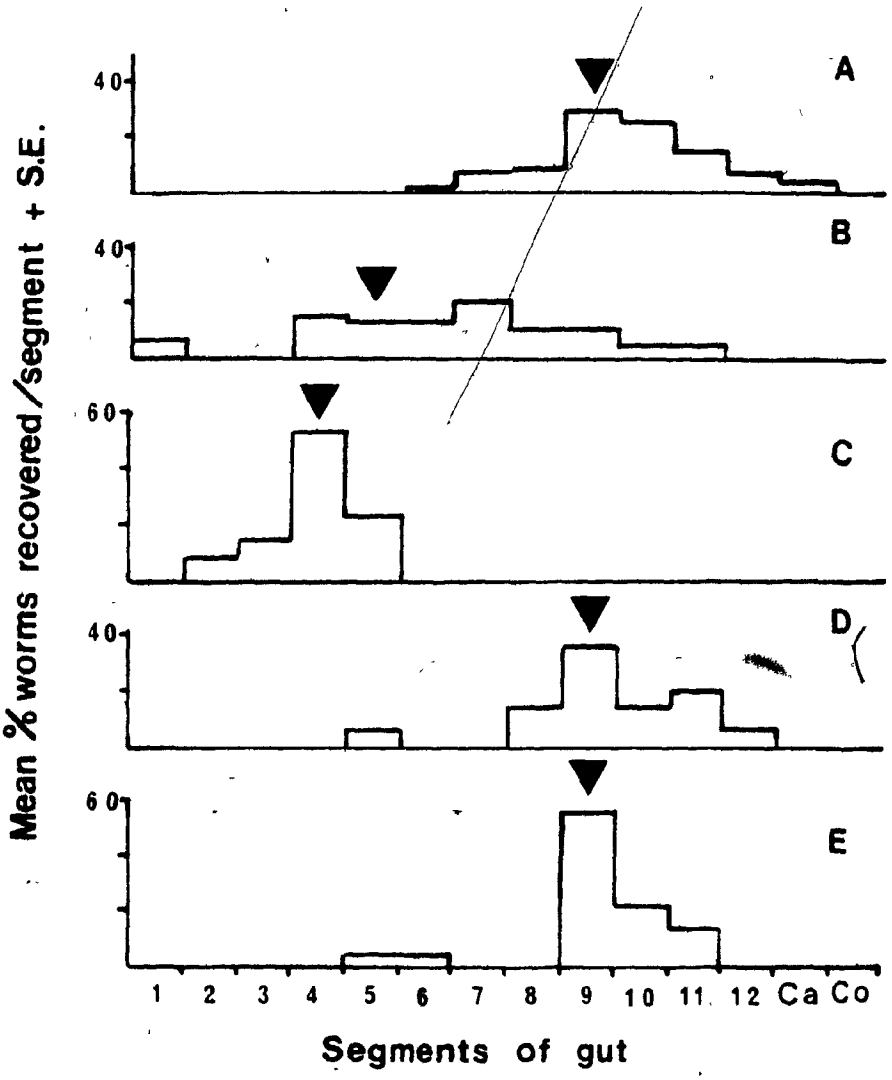

Mean % worms recovered /segment + S.E.
40
A
40
B
60
C
40
D
60
E
1 2 3 4 5 6 7 8 9 10 11 12 Ca Co
Segments of gut

TABLE 3.4 THE MEAN RECOVERY OF ADULTS FROM THE SMALL INTESTINE FOLLOWING THE IMPLANTATION OF 500 LARVAE OF T. SPIRALIS INTO LIGATURED POUCHES IN DIFFERENT LOCATIONS OF THE GUT

Position of Implant	N	Mean # Adults Recovered ± S. E.	% Recovery
Control	5	175.0 ± 12.1 (a)	35.0
Midgut	5	145.5 ± 29.5 (a) (b)	29.1
Duodenum	5	103.4 ± 13.5 (b)	20.6
Ileum	5	81.2 ± 25.5 (b) (c)	16.2
Caecum	5	52.5 ± 23.0 (c)	10.5
Colon	5	35.5 ± 16.0 (c)	7.1

(a) (b) (c) all values with the same superscript do not differ significantly from each other ($p \leq 0.05$)

FIGURE 3.13 The rate of intestinal propulsion in mice. A, controls; B, mice treated with Lomotil®. M = slope, * significantly different from the control ($p \leq 0.05$)

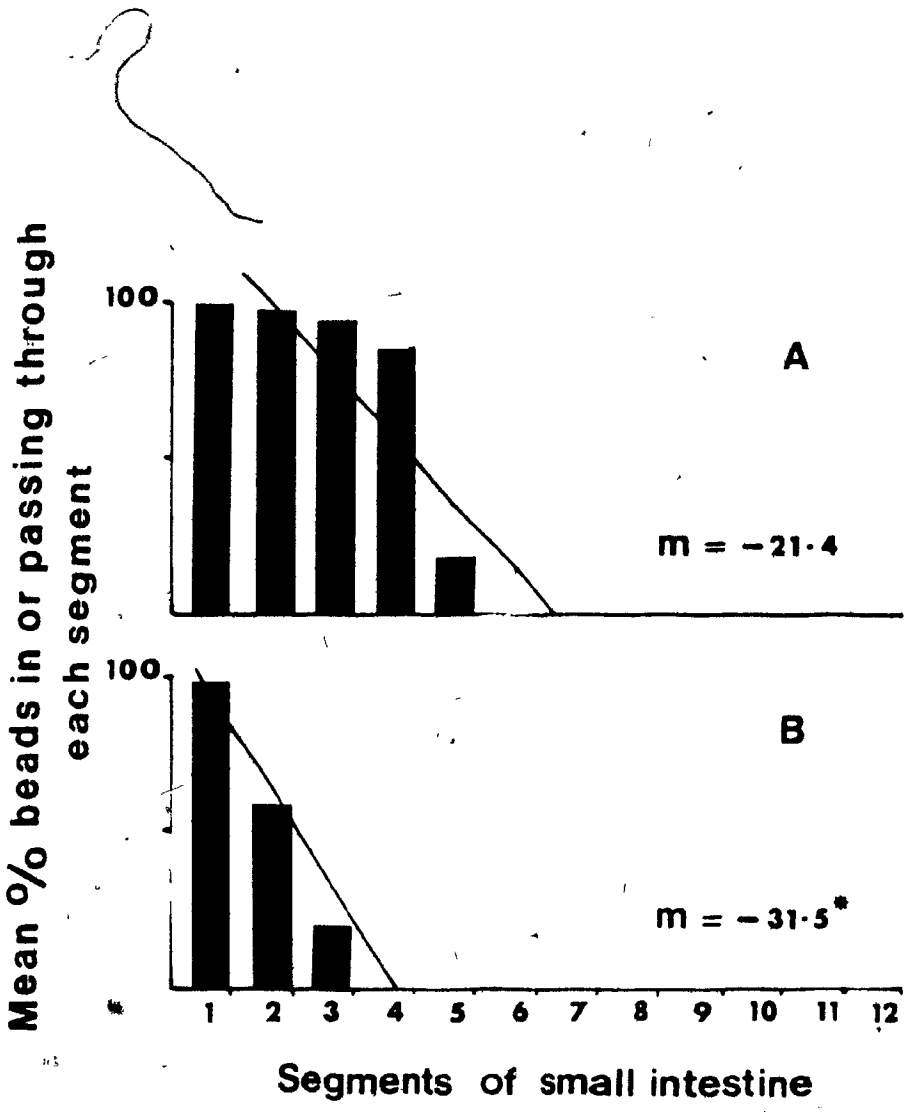

Mean % beads in or passing through each segment
100
A
m = −21·4
100
B
m = −31·5*
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

FIGURE 3.14 The distribution of adults in the small intestine of mice 5 days after infection with 500 larvae of T. spiralis. A, animals treated with Lomotil®; B, controls.

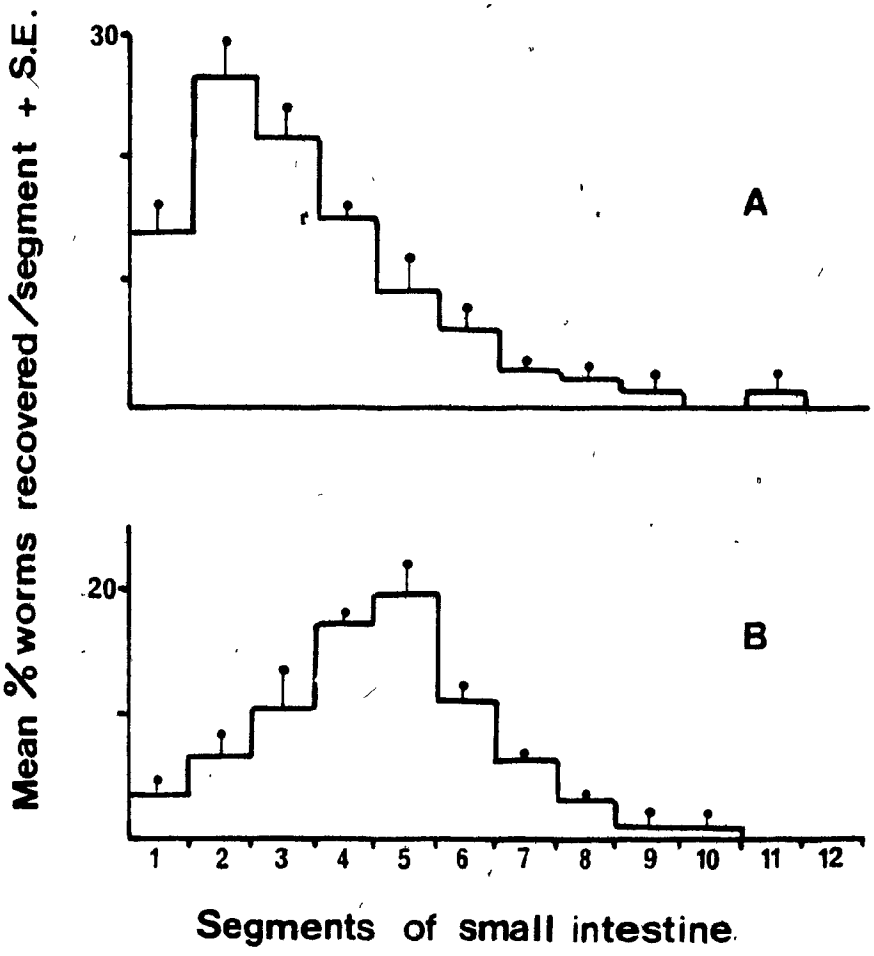

Mean % worms recovered/segment + S.E.
30
20
A
B
1
2
3
4
5
6
7
8
9
10
11
12
Segments of small intestine

TABLE 3.5 THE MEAN RECOVERY OF ADULTS OF *T. SPIRALIS* IN MICE TREATED WITH LOMOTIL® 5 DAYS AFTER INFECTION WITH 500 LARVAE

Treatment	N	Mean # Adults Recovered ± S. E.	% Recovery	$p < 0.05$
Control	5	238.8 ± 40.0	47.6	
Lomotil®	5	253.4 ± 10.5	50.7	N. S.

The intestinal propulsion rates of animals given 0.5 ml and 1.0 ml saline were significantly faster than the controls given 0.1 ml saline ($p \leq 0.05$) (Figure 3.15). In animals with increased propulsion, the adults were recovered from a more posterior position than controls. Peak recovery of adults in the animals given 0.5 ml and 1.0 ml saline occurred in segments 8 and 10 respectively while in control animals given 0.1 ml saline, peak establishment occurred in segment 4 (Figure 3.16). The adult recoveries are shown in Table 3.6.

With larval inocula of 500 to 2000, there was a posteriad shift in the establishment pattern with increasing dosages. The majority of adults recovered from infections with 500 larvae were from the anterior small intestine while the majority of adults of the infection with 2000 larvae were recovered from the posterior intestine (Figure 3.17). Low recoveries from the mice given 100 larvae make interpretation somewhat arbitrary. The adult recoveries from these groups are shown in Table 3.7.

From the results with the single-sex infections of T. spiralis it was discovered that the infections were not purely homosexual and some members of the opposite sex were always recovered. However, in no instance was the contamination greater than 10%. The distribution of adults in the female-only infections closely approximated the

FIGURE 3.15 The rate of intestinal propulsion in mice. A, controls given 0.1 ml saline; B, mice given 0.5 ml saline and C, mice given 1.0 ml saline. M = slope, * significantly different from the control ($p \leq 0.05$).

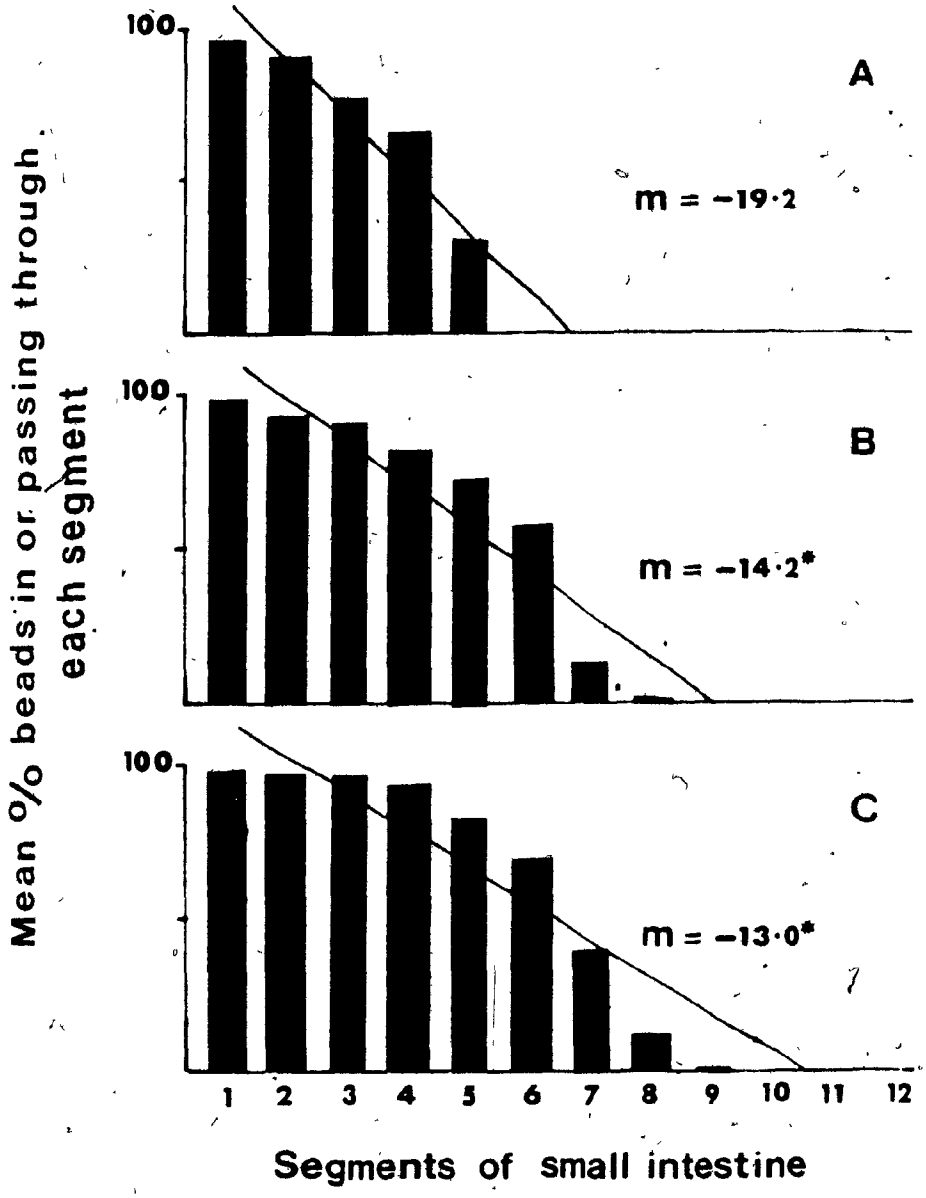

100
A
m = −19·2
100
B
m = −14·2*
100
C
m = −13·0*
Mean % beads in or passing through each segment
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

FIGURE 3.16 The distribution of adults in the small intestine of mice 5 days after infection with 500 larvae of T. spiralis. A, mice given 0.1 ml saline; B, mice given 0.5 ml saline and C, mice given 1.0 ml saline.

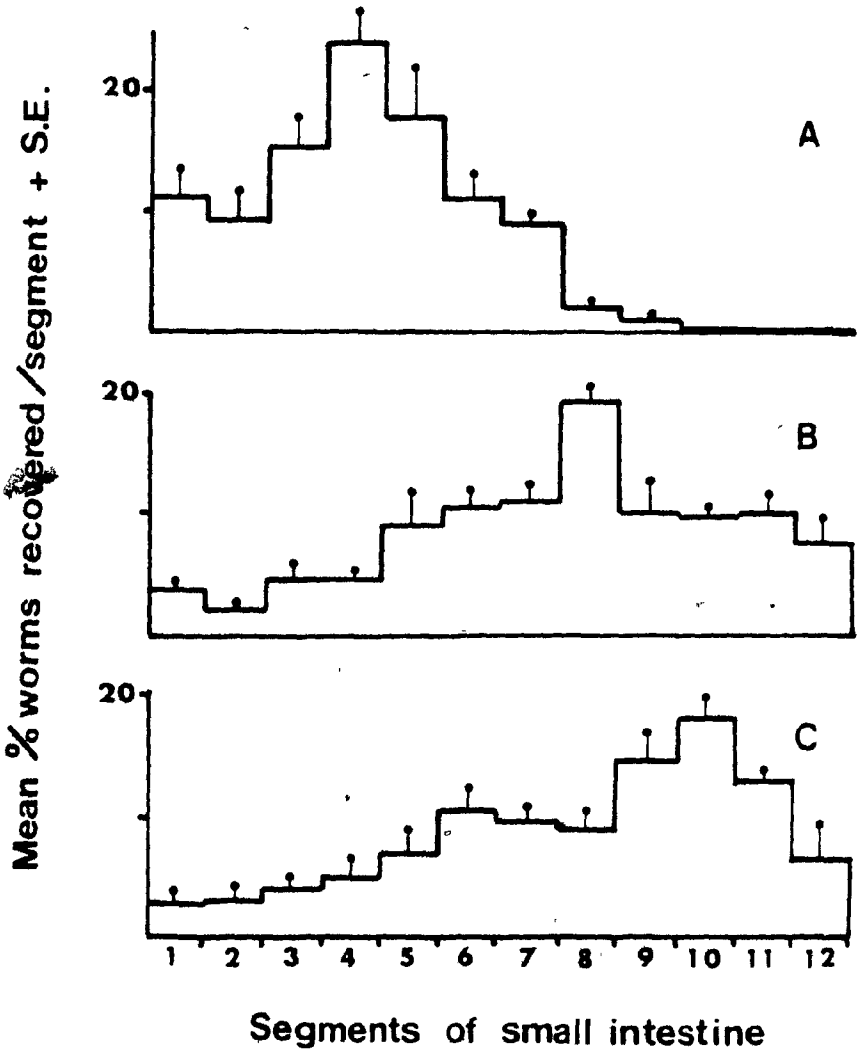

Mean % worms recovered/segment + S.E.
20
A
20
B
20
C
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

TABLE 3.6 THE MEAN RECOVERY OF ADULTS OF *T. SPIRALIS* IN MICE WITH ALTERED INTESTINAL PROPULSION, 5 DAYS AFTER INFECTION WITH 500 LARVAE

Treatment	N	Mean # Adults Recovered ± S. E.	% Recovery	$p \leq 0.05$
Control 0.1 ml	5	186.5 ± 12.6	37.3	
0.5 ml	5	162.3 ± 3.9	32.5	N. S.
1.0 ml	5	162.0 ± 37.8	32.4	N. S.

FIGURE 3.17 The distribution of adults in the small intestine of mice infected 5 days previously with: A, 100; B, 500; C, 1000 and D, 2000 larvae of T. spiralis.

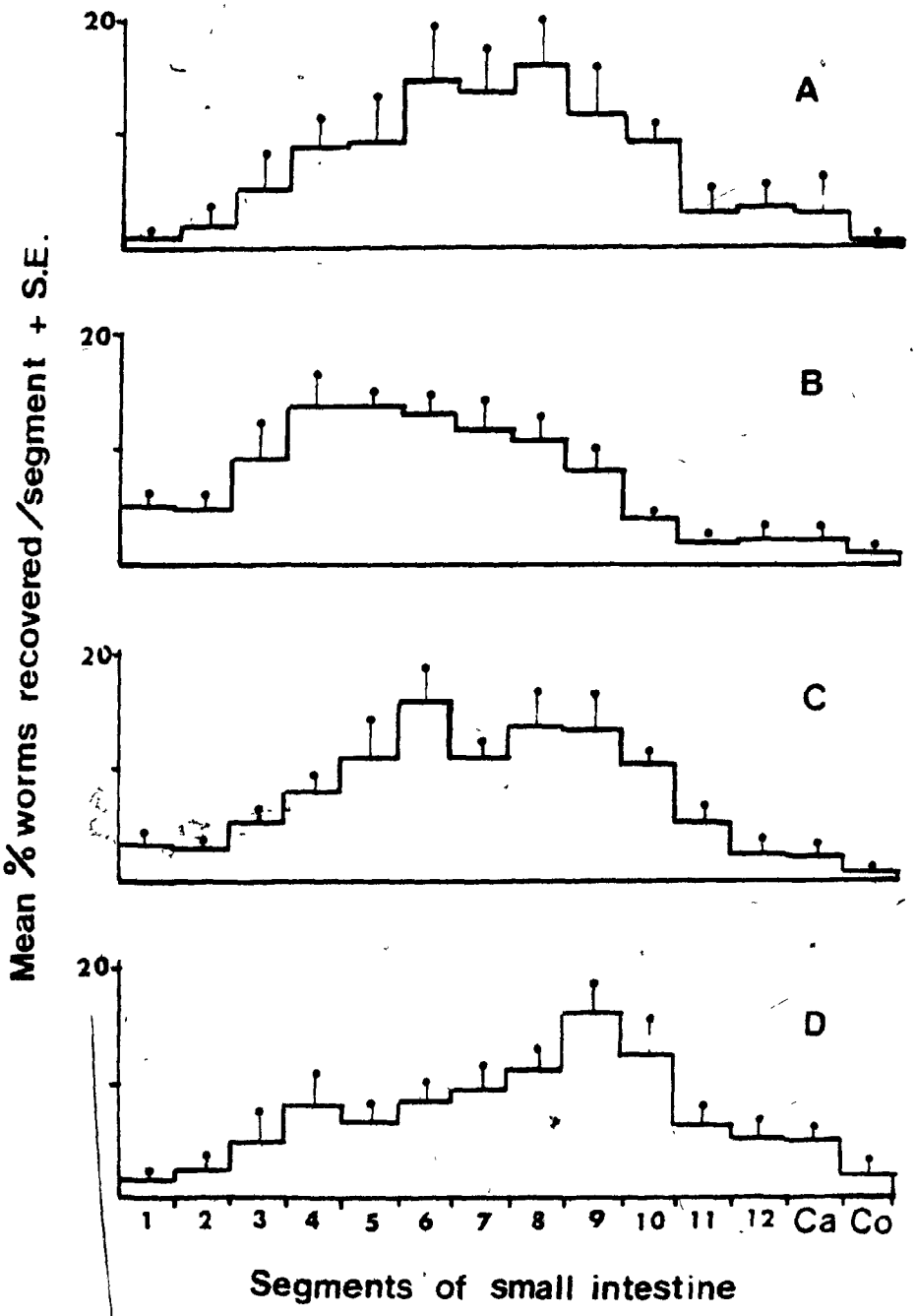

20
A
20
B
20
C
20
D
Mean % worms recovered/segment + S.E.
1 2 3 4 5 6 7 8 9 10 11 12 Ca Co
Segments of small intestine

TABLE 3.7 THE MEAN NUMBER OF ADULTS RECOVERED FROM THE SMALL INTESTINE, 5 DAYS AFTER INFECTION WITH DIFFERENT INOCULA OF T. SPIRALIS

Inocula	N	Mean # Adults Recovered ± S. E.	Mean % Adults Recovered ± S. E.
100	5	32.8 ± 0.6	32.8 ± 0.6 (a)
500	5	201.6 ± 21.5	40.2 ± 4.3 (a)
1000	5	529.6 ± 40.3	52.9 ± 4.3 (b)
2000	5	1216.4 ± 77.4	60.8 ± 3.8 (b)

(a) (b) all values with similar superscript are not significantly different from each other ($p \leq 0.05$)

distribution pattern in the control mixed infections. The aggregation of adults was less consistent and the worms were more irregularly distributed in the male-only infections (Figure 3.18). The adult recoveries are shown in Table 3.8.

2) Nematospiroides dubius

The distribution of the larvae of N. dubius along the small intestine of mice is shown in Figure 3.19 and the pattern of establishment was similar for infections with ensheathed and exsheathed larvae. The majority of the larvae were recovered from the anterior small intestine with peak recoveries in segments 1 and 2. The numbers of larvae establishing per segment decreases distally and larvae rarely establish past the sixth segment. There were significantly fewer larvae establishing in the group given exsheathed larvae (Table 3.9).

There was no change in the distribution pattern when the animals were pre-treated with a surfactant, Tween 80, prior to infection (Figure 3.20). Likewise, in mice treated with antibiotics prior to infection, there was no effect on larval distribution in the small intestine (Figure 3.21). In addition, the distribution of food in the gut at the time of infection did not significantly affect the longitudinal distribution of worms in the small intestine (Figure 3.22). The larval recoveries from these groups are shown in Table 3.10.

The distribution of larvae of N. dubius implanted into the duodenum was similar when the larvae were implanted in the presence or absence of bile (Figure 3.23) with 90% of

FIGURE 3.18 The distribution of adults of T. spiralis in the gut of mice 5 days after infection with: A, 250 female larvae; B, 250 male larvae and C, 250 mixed larvae (female: male = 2.3:1.0).

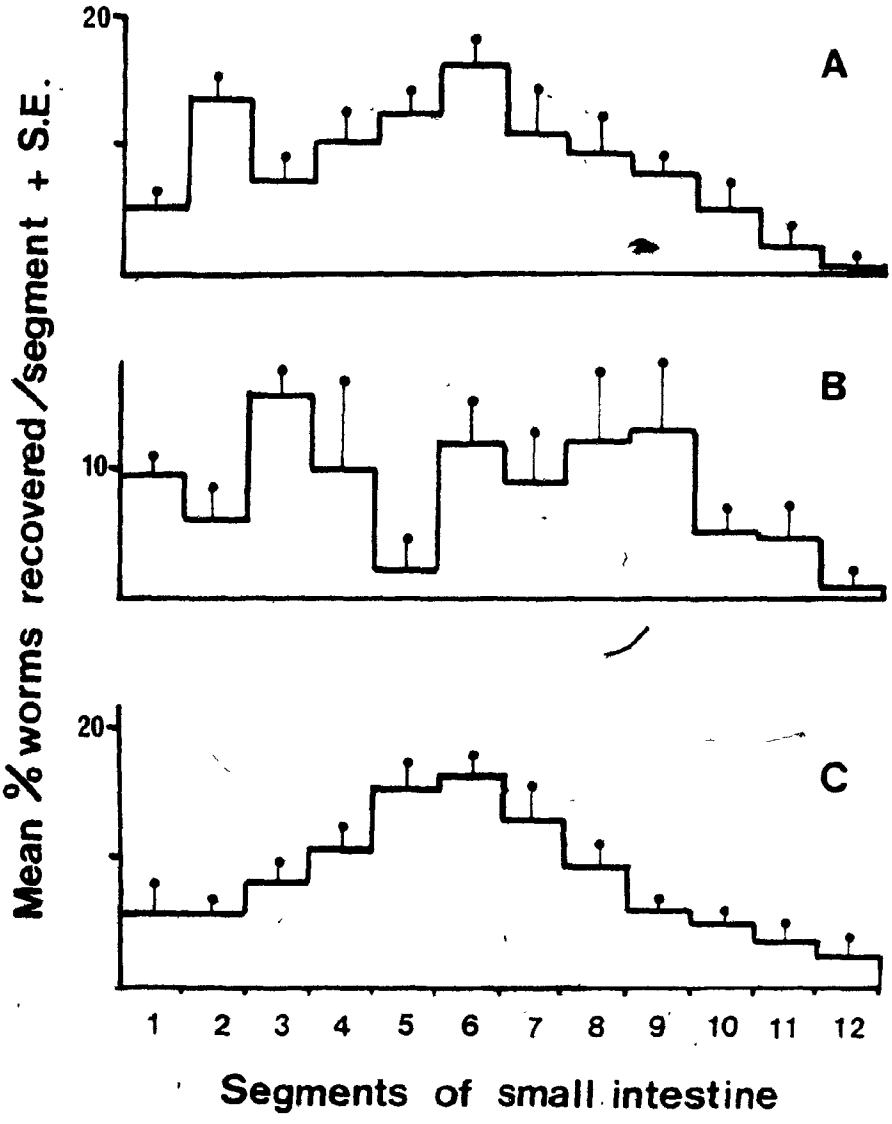

20
A
10
B
20
C
Mean % worms recovered/segment + S.E.
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

TABLE 3.8 THE MEAN NUMBER OF ADULTS RECOVERED FROM THE SMALL INTESTINE 5 DAYS AFTER SINGLE-SEX INFECTIONS WITH 250 LARVAE OF T. SPIRALIS

Treatment	N	Mean # Adults Recovered ± S. E.	% Adults Recovered	$p \leq 0.05$
Control	5	93.3 ± 8.5	37.3	
Female-only	5	74.0 ± 20.1	29.6	N. S.
Male-only	5	68.3 ± 16.4	27.3	N. S.

FIGURE 3.19 The distribution of larvae in the small intestine of mice 6 days after infection with 100 larvae of N. dubius. A, ensheathed larvae and B. exsheathed larvae.

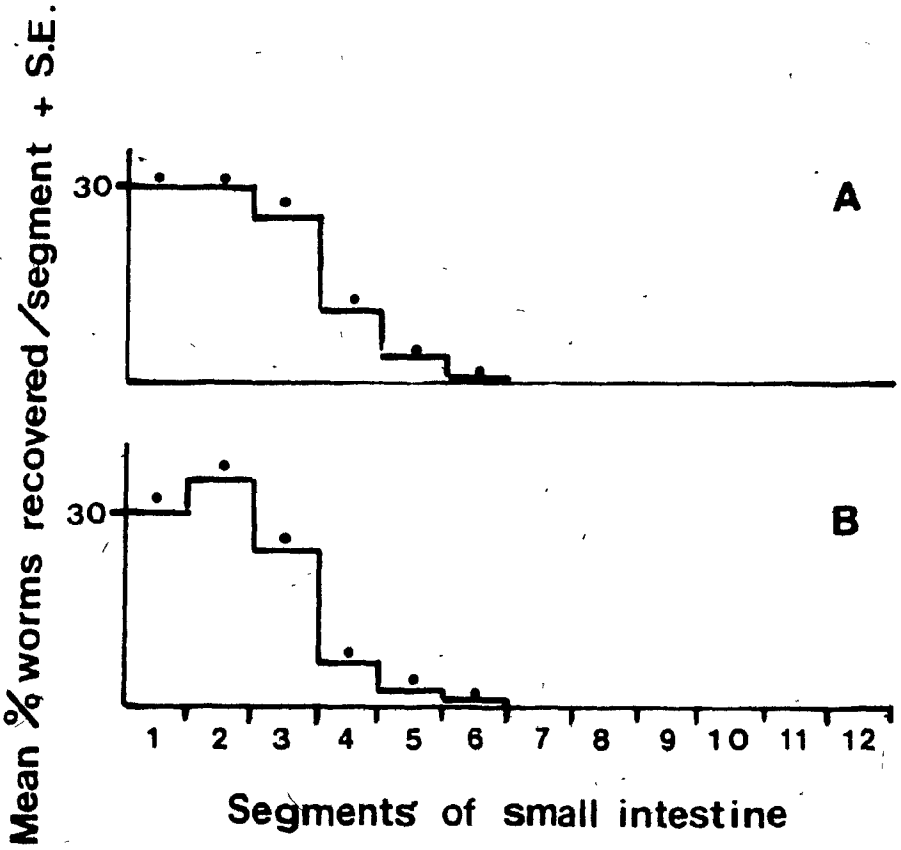

Mean % worms recovered/segment + S.E.
30
A
30
B
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

TABLE 3.9 THE MEAN RECOVERY OF LARVAE OF *N. DUBIUS* FROM THE SMALL INTESTINE 6 DAYS AFTER INFECTION WITH 100 LARVAE

Treatment	N	Mean % Adults Recovered ± S. E.
Ensheathed	5	80.2 ± 3.2
Exsheathed	5	59.8 ± 3.7 (a)

(a) significantly different from controls ($p \leq 0.05$)

FIGURE 3.20 The distribution of larvae in the small intestine of mice 6 days after infection with 100 larvae of N. dubius. A, controls; B, the mice were given 0.2 ml Tween 80 prior to infection.

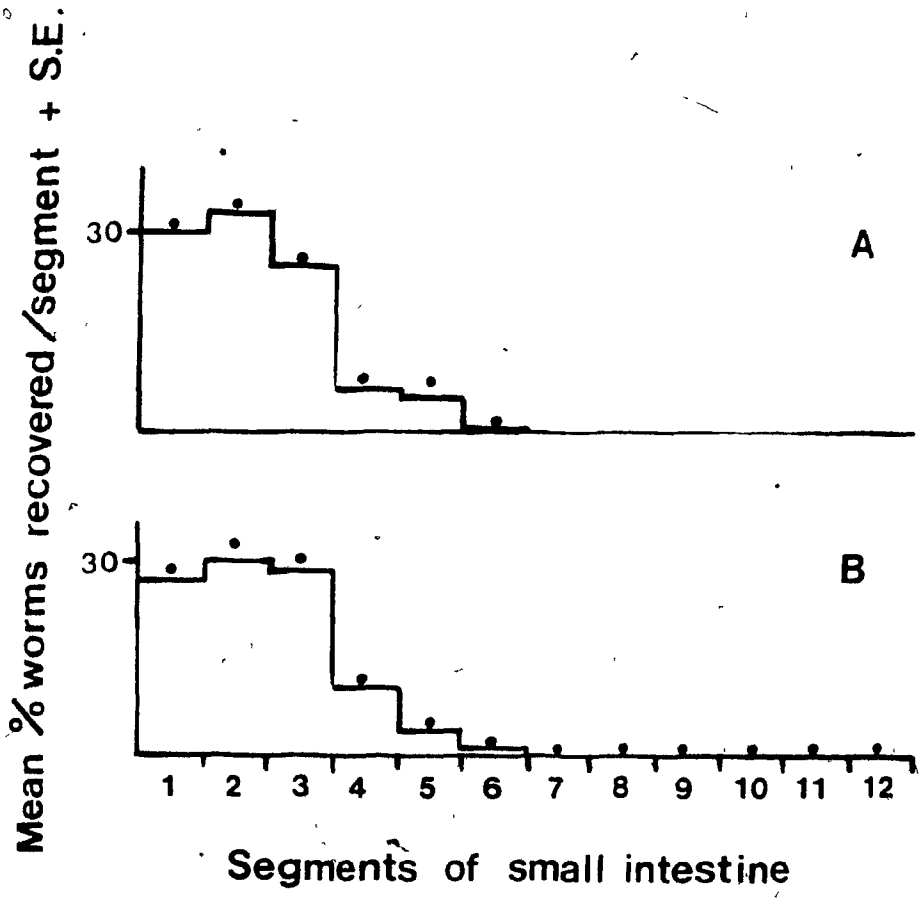

Mean % worms recovered/segment + S.E.
30
A
30
B
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

FIGURE 3.21 The distribution of larvae in the small intestine of mice 6 days after infection with 100 larvae of N. dubius. A, controls; B, the mice were treated with antibiotics prior to infection.

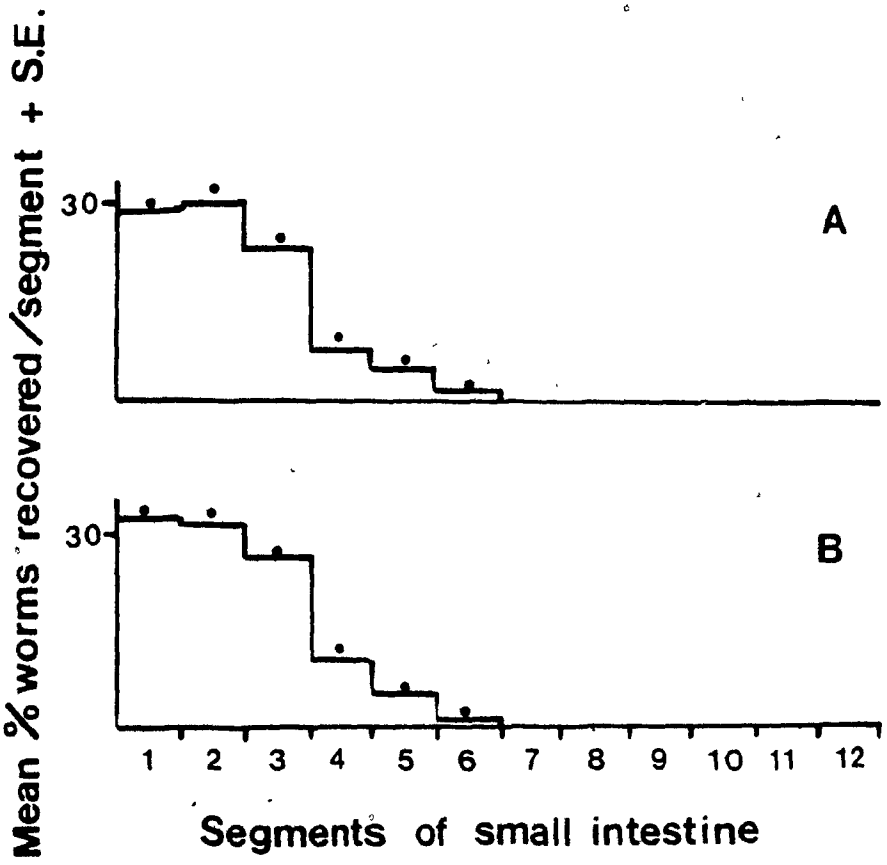

Mean % worms recovered/segment + S.E.
30
A
30
B
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

FIGURE 3.22 The distribution of larvae in the small intestine of mice 6 days after infection with 100 larvae of N. dubius and the distribution of food in the gut at the time of infection. A, the mice were infected at 1000 hours and no food was presented for a further 12 hours; B, the mice were infected at 1000 hours at the time of food presentation and C, the mice were infected at 1500 hours, 4 hours after food withdrawal. The histogram shows the worm distribution and the ogive shows food distribution

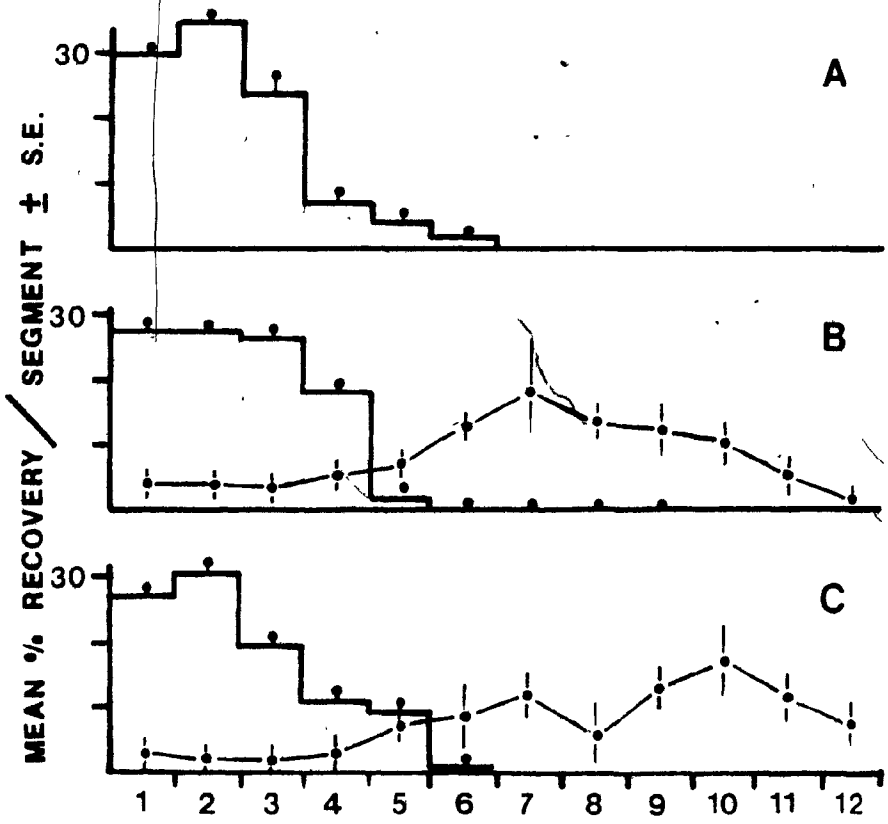

30
A
30
B
30
C
MEAN % RECOVERY / SEGMENT ± S.E.
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

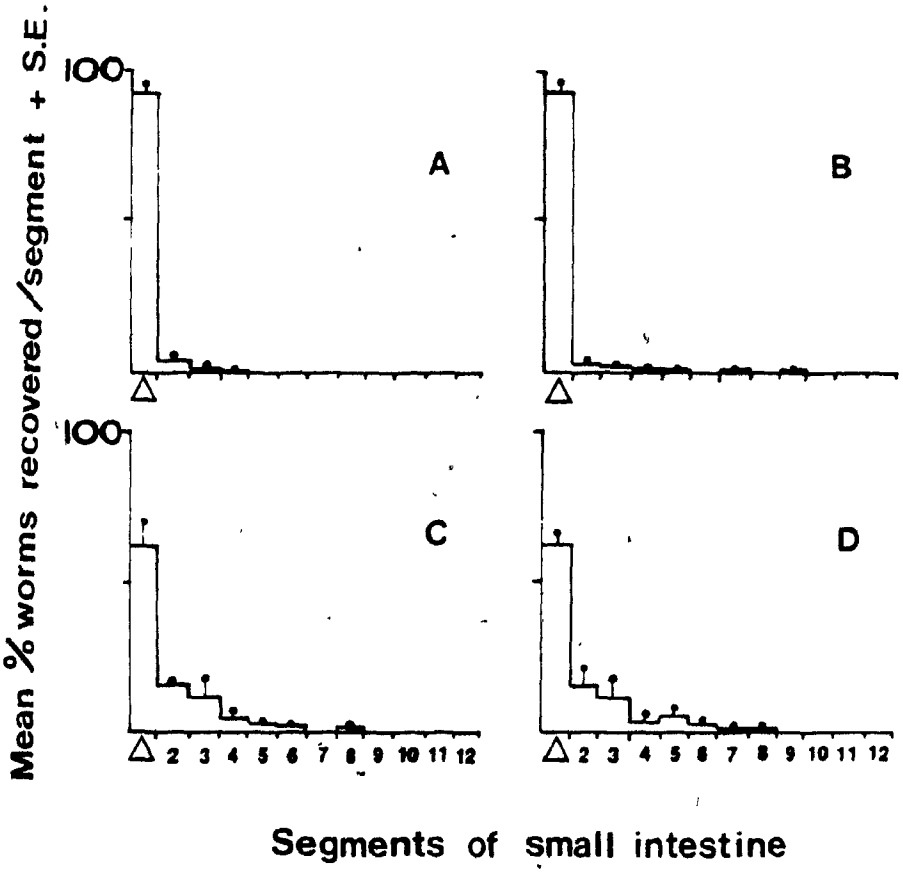
TABLE 3.10 . THE MEAN PERCENT LARVAL RECOVERIES 6 DAYS AFTER INFECTION WITH 100 LARVAE OF N. DUBIUS

Group	Treatment	Mean % Recovery ± S. E.	$p \leq$ *
1	Control	59.6 ± 12.3	
1	Tween 80	52.2 ± 5.1	N. S.
2	Control	81.2 ± 7.4	
2	Antibiotics	78.1 ± 3.6	N. S.
3	1000 hr. fasted	90.3 ± 12.5 (a)	N. S.
3	1000 hr. fed	74.6 ± 6.2 (a)	N. S.
3	1500 hr. fed	82.3 ± 5.9 (a)	N. S.

* Significantly different from controls within the group ($p \leq 0.05$)

(a) All groups with the same superscript do not differ significantly from each other ($p \leq 0.05$)

FIGURE 3.23 The mean recovery of worms from the small intestine of mice 6 days after implantation of 100 larvae of N. dubius into the duodenum. (Arrowhead points to site of implantation). A, implanted with ensheathed larvae; B, implanted with ensheathed larvae with bile; C, implanted with exsheathed larvae and D, implanted with exsheathed larvae with bile.


Mean % worms recovered / segment + S.E.
100
A
B
100
C
D
2 3 4 5 6 7 8 9 10 11 12
2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

the ensheathed larvae and 60% of the exsheathed larvae recovered from the area between the ligatures. Larger numbers of larvae established posterior of the implantation site when exsheathed larvae were used. In addition, the recovery was significantly lower in the animals implanted with exsheathed larvae when compared to ensheathed larvae (Table 3.11). Implantation of the 4 treatment groups into the ileum resulted in very small recoveries (Figure 3.24; Table 3.11) with the majority of larvae being found at the site of implantation. While the recoveries were very small, the numbers of larvae establishing in the ileum in the presence of bile was almost twice as much as the numbers establishing in its absence (Table 3.11). There was no significant increase in establishment with bile in the duodenum or ileum. Implantation into the caecum resulted in no establishment regardless of treatment (Table 3.11).

Alteration of the rate of intestinal propulsion changed the distribution pattern of N. dubius in the small intestine. Lomotil® treatment significantly reduced the propulsion rate when compared to controls ($p \leq 0.05$) (Figure 3.25). The establishment of larvae in animals with this treatment was anteriad of controls with 67% of the worms established in the first segment and 99% in the first 2 segments (Figure 3.26). The larval recoveries are shown in Table 3.12.

Intestinal propulsion was significantly increased ($p \leq 0.05$) when compared to controls in the animals treated with larger doses of saline (Figure 3.27). However, there

TABLE 3.11 PERCENTAGE RECOVERY OF LARVAE OF *N. DUBIUS* FOLLOWING IMPLANTATION OF 100 3RD STAGE LARVAE INTO THE GUT

Larvae	Bile added to inoculum	Position in the alimentary tract	Mean % Recovery ± S. E.	
Ensheathed	-	Duodenum	90.3 ± 6.8	(a)*
Ensheathed		Duodenum	90.8 ± 6.0	(a)
Exsheathed	-	Duodenum	43.0 ± 2.6	(b)
Exsheathed		Duodenum	32.4 ± 9.4	(b)
Ensheathed	-	Ileum	0	
Ensheathed		Ileum	2.0 ± 1.7	(c)
Exsheathed	-	Ileum	0.4 ± 0.2	(c)
Exsheathed		Ileum	0.9 ± 0.6	(c)
Ensheathed	-		0	
Ensheathed			0	
Exsheathed	-		0	
Exsheathed			0	

* all values with the same superscript do not differ significantly from each other ($p \leq 0.05$)

FIGURE 3.24 The mean recovery of worms from the small intestine of mice 6 days after implantation of 100 larvae of N. dubius into the ileum. (Arrowhead points to site of implantation). A, implanted with ensheathed larvae; B, implanted with ensheathed larvae with bile; C, implanted with exsheathed larvae and D, implanted with exsheathed larvae with bile.

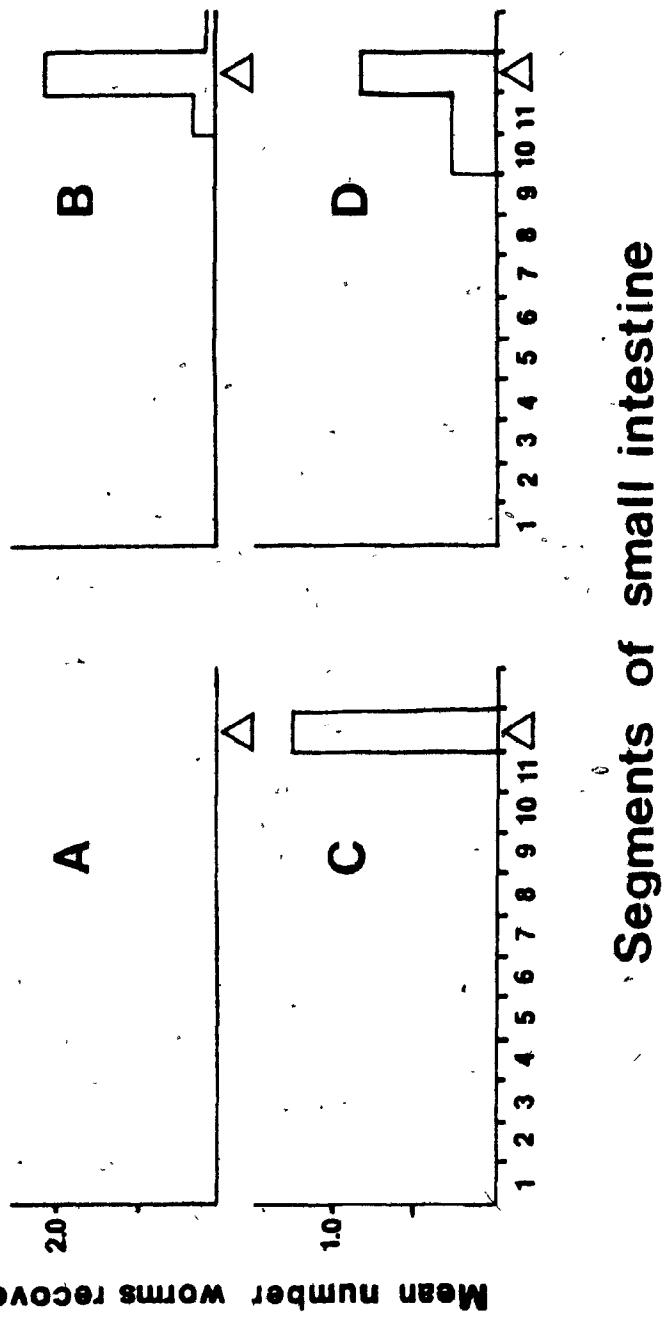

A
B
C
D
2.0
1.0
1 2 3 4 5 6 7 8 9 10 11
Mean number worms recovered
Segments of small intestine

FIGURE 3.25 The rate of intestinal propulsion in mice. A, controls; B, mice treated with Lomotil®, M = slope, * significantly different from the control ($p \leq 0.05$).

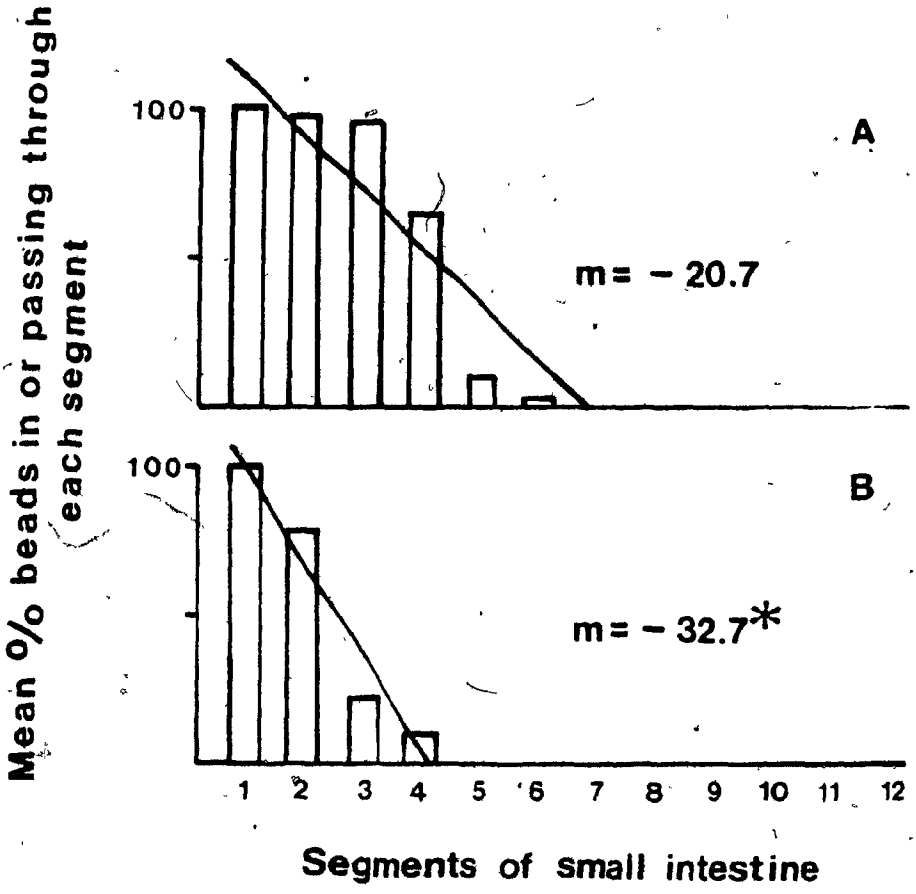

Mean % beads in or passing through each segment
100
A
m = − 20.7
100
B
m = − 32.7*
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

FIGURE 3.26 The distribution of worms in the small intestine of mice 6 days after infection with 100 larvae of N. dubius. A, controls; B, the mice were treated with Lomotil®.

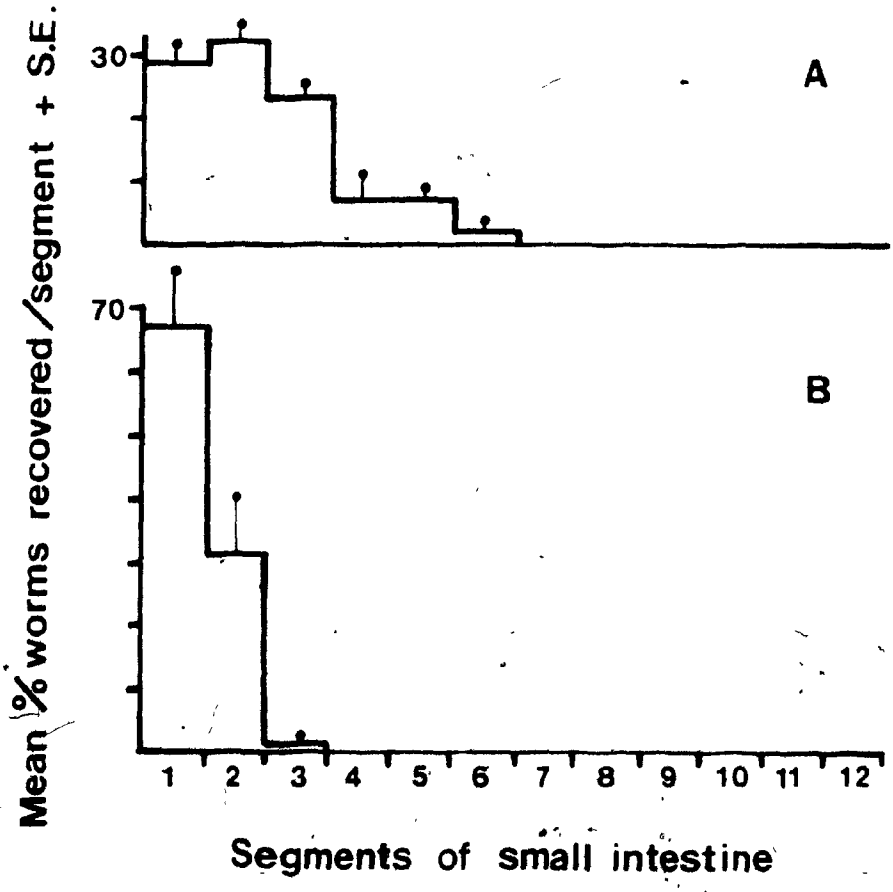
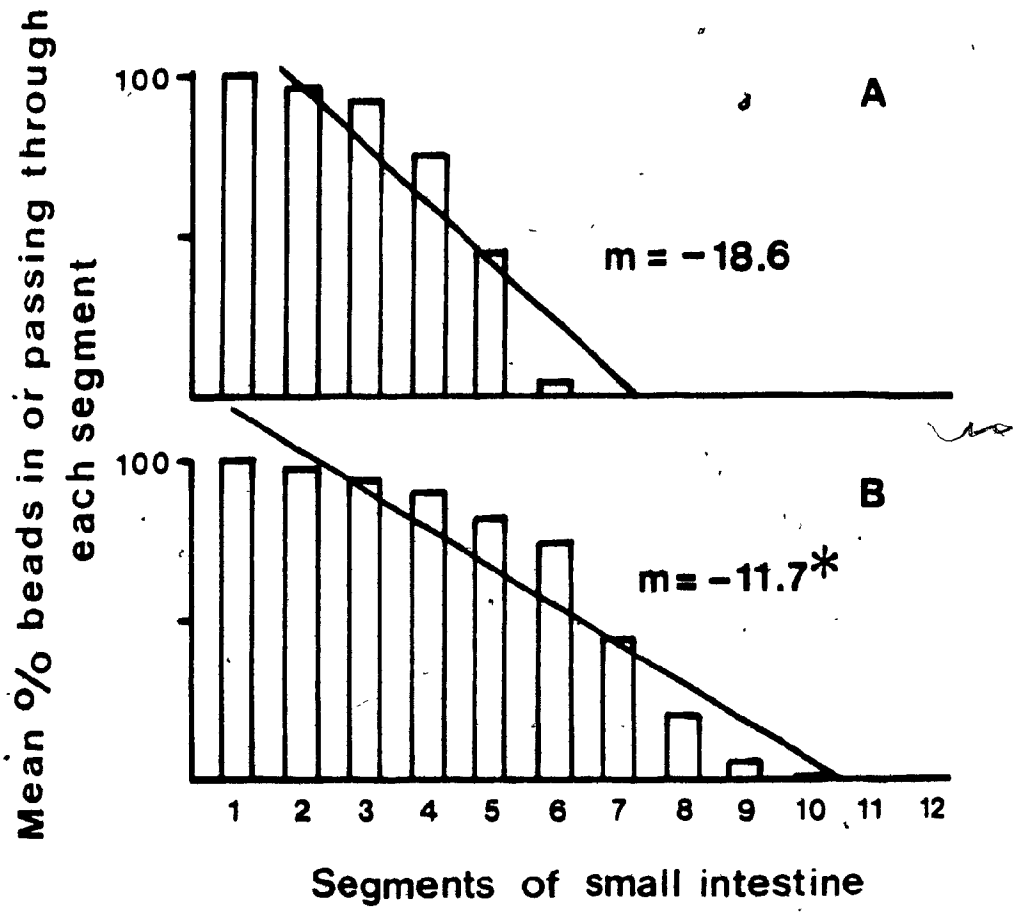

Mean % worms recovered/segment + S.E.
30
A
70
B
1
2
3
4
5
6
7
8
9
10
11
12
Segments of small intestine

TABLE 3.12 THE MEAN LARVAL RECOVERIES FROM THE SMALL INTESTINE OF MICE WITH ALTERED INTESTINAL PROPULSION, 6 DAYS AFTER INFECTION WITH 100 LARVAE OF N. DUBIUS

Group	Treatment	Mean # Adults Recovered ± S. E.	p ≤
1	Control	84.4 ± 7.1	N. S.
1	Lomotil®	87.9 ± 3.5	
2	Saline 0.1 ml	75.3 ± 5.8	0.05
2	Saline 0.5 ml	38.6 ± 9.9	

FIGURE 3.27 The rate of intestinal propulsion in mice. A, mice given 0.1 ml saline; B, mice given 0.5 ml saline. M = slope, * significantly different from the control ($p \leq 0.05$).


Mean % beads in or passing through each segment
100
A
m = −18.6
100
B
m = −11.7*
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

were no measurable changes in the distribution of larvae in the intestine. The peak recoveries in animals with increased propulsion were recorded from segments 2 and 3, while in the controls, the highest recoveries were recorded from segments 1 and 2 (Figure 3.28). In addition, there was a significant reduction ($p \leq 0.05$) in the number of larvae that establish in the animals with increased propulsion when compared to controls (Table 3.12).

The effects of different inocula on the longitudinal distribution of N. dubius is shown in Figure 3.29. With larval dosages up to 500, the distribution pattern remained similar, the peak recoveries were recorded from the first or second segments. With the very high larval dose of 1500, there was an initial high establishment in the first segment followed by a rapid decrease in establishment posteriorly, but larvae were recovered in small numbers as far posterior as the eleventh segment. The larval recoveries for these groups are shown in Table 3.13.

3D. DISCUSSION

1) T. spiralis

These results confirm earlier observations that the worms are aggregated in the small intestine (see p 29) and show the peak populations in the second quarter of the small intestine of the mouse. In addition, the vehicle of infection, infected meat, excysted larvae or encysted larvae, did not

FIGURE 3.28 The distribution of worms in the small intestine of mice 6 days after infection with 100 larvae of N. dubius. A, mice given 0.1 ml saline; B, mice given 0.5 ml saline.

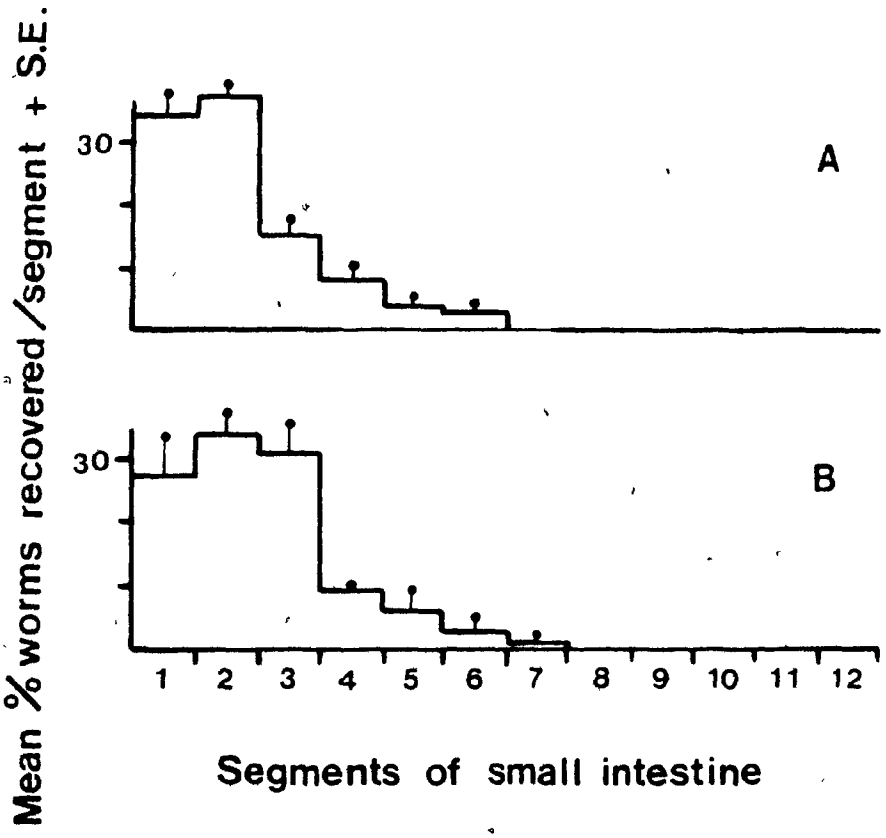

Mean % worms recovered/segment + S.E.
30
A
30
B
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

FIGURE 3.29 The distribution of worms in the small intestine of mice 6 days after infection with: A, 100; B, 200; C, 500 and D, 1500 larvae of N. dubius.

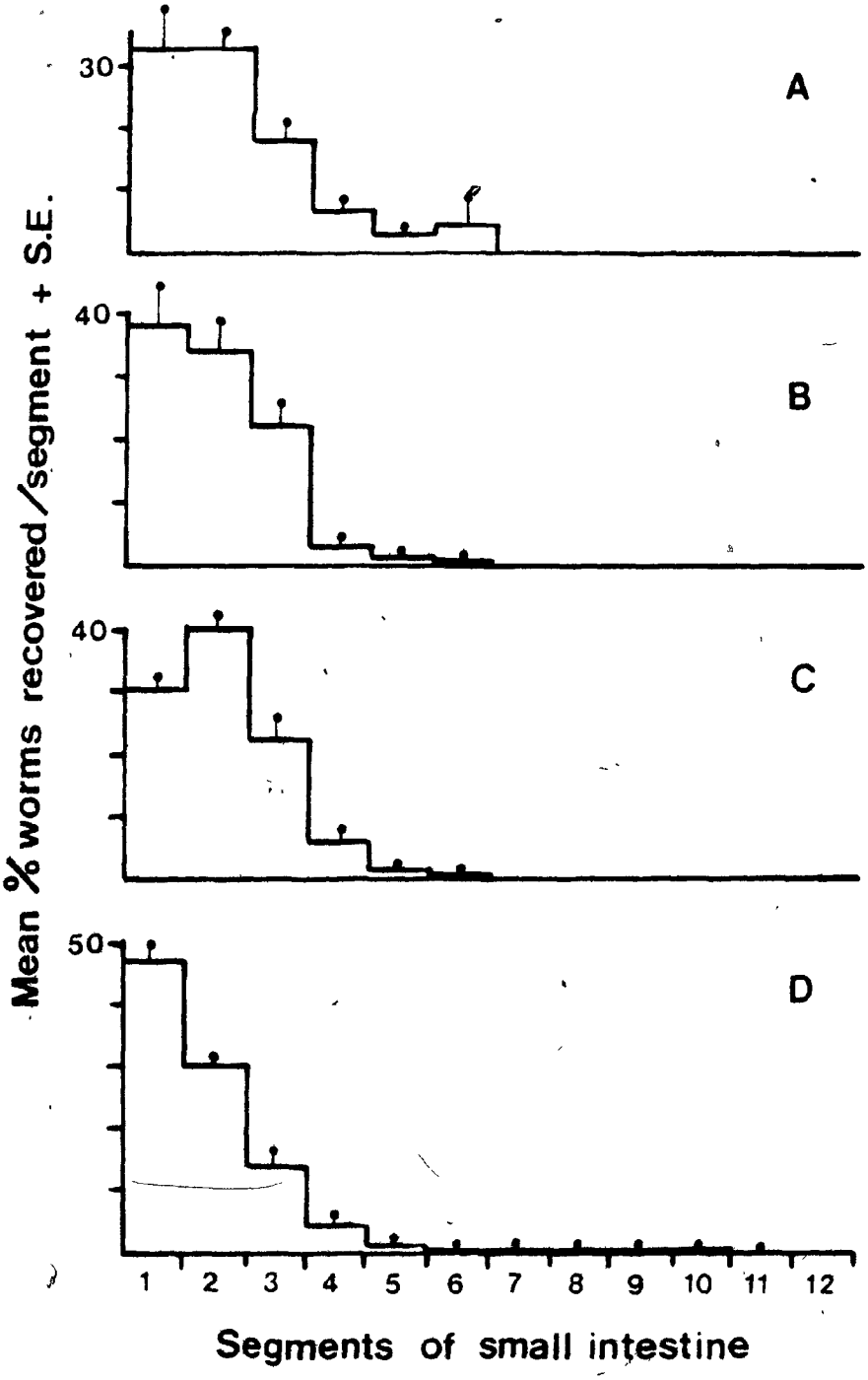

30
A
40
B
40
C
50
D
Mean % worms recovered/segment + S.E.
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

TABLE 3.13 THE MEAN LARVAL RECOVERIES FROM THE SMALL INTESTINE 6 DAYS AFTER INFECTION WITH DIFFERENT INOCULA OF N. DUBIUS

Treatment	Mean # Adults Recovered ± S. E.	Mean % Adults Recovered ± S. E.
100	80.7 ± 5.7	80.7 ± 5.7
200	160.0 ± 14.0	80.0 ± 7.0
500	441.3 ± 80.3	88.2 ± 16.1
1500	1106 ± 40.5	73.7 ± 2.7

change this pattern. My results suggest that the larvae are freed in the stomach, regardless of the vehicle of infection, and that the larvae entering the small intestine established in similar patterns.

Inherent in the above argument and throughout this investigation is the assumption that the sites selected by the larvae are the same as the sites selected by the adults. There are practical and theoretical reasons for this assumption.

Firstly, it is difficult to recover the larvae within the first 2 days and before the fifth day of infection the counts do not accurately represent the numbers of worms in the gut (Sukhdeo & Meerovitch, 1980).

Secondly, there is a lot of indirect evidence to support the assumption. When the larvae were implanted within temporarily ligatured pouches in the gut, the majority of the adult worms were subsequently recovered from the site of implantation. The larvae can enter the mucosa of the small intestine within 10 minutes of oral infection (Despommier et al., 1978) and in the present study it was assumed that the larvae had established during the 30 minutes of temporary containment. Therefore, the adults were recovered from the site of larval establishment. In addition, in several experiments in this section, e.g. altered propulsion rates, antibiotic treatments and surfactant treatments, the distribution of adults in the gut has been altered. However, it was the conditions at the time of larval establishment that were changed. This again suggests that the larval site

selection determines the adult distribution. Therefore, throughout this investigation, larval establishment has been examined by the adult distribution pattern 5 days after infection.

One of the most dramatic and significant changes in the larval establishment occurred subsequent to the alteration of the rate of gut propulsion. When propulsion was decreased with Lomotil® treatment, the larvae established more anteriad of the controls and when propulsion was increased, the larvae established posteriad of the controls. The rate of intestinal propulsion was measured by the rate of movement of the chromatography bead markers through the stomach and small intestine. This method was chosen to eliminate the need for surgery whereby markers could be placed directly into the gut sections. One of the problems with this method is that because gastric emptying and intestinal propulsion are intrinsically related (Smith, 1973), the measured rate of intestinal propulsion may actually be the rate of gastric emptying. In a previous study (Sukhdeo & Croll, submitted for publication) we have found that the rate of gastric emptying did not have any significant effects on the measurement of gut propulsion. These results, therefore, are consistent with the hypothesis that the rate of movement of ingesta along the gut affects the selection of the site. The results of other experiments, e.g. antibiotic or surfactant treatment, in which there was a change in distribution pattern will now be explained within this assumption.

There were differences in the distribution patterns of the worms when implanted into ligatured pouches and implanted

without ligatured pouches. The dispersion seen without ligatured pouches may have been due to the movements of ingesta in the gut that moved the worm before penetration.

Distribution patterns of worms in the mice fed for only one hour per day may also be explained by differences in gut propulsion. (In studies of rats adapted to intermittent starvation, there were several anatomical and physiological changes including hyperphagia, gastric hypertrophy, intestinal hypertrophy and increased enzyme secretion (Holeckova & Fabry, 1959). The hypertrophy was also seen in my animals and the possible effects of increased enzyme secretions in site selection are discussed in a later chapter on the behavioural cues used by the worms in site selection. The larval establishment patterns of T. spiralis were similar in all groups except the group that was fasted, then infected and fed at the same time. Perhaps the larval inoculum was incorporated into the first few boli to leave the stomach. In pigs, fed one large meal after a period of fasting, there was a burst of electrical activity with increased muscular contractions and motility in the stomach and small intestine which lasted several hours (Ruckebusch & Bueno, 1975). In the group fed and infected at the same time, such an increase in motility in mice would cause the posteriad establishment of larvae seen in this group and it could also account for the low recoveries in this group. More larvae would be swept out of the gut before they could establish and there would only be a brief exposure to pepsin-HCl in the stomach. Subsequent to this initial burst of activity, the gut motility returned to normal and propulsion is slower. Again this is consistent

with the hypothesis and in the remaining fed groups the larvae established in the anterior small intestine.

In the animals that were infected but not fed then or for a further 12 hours, the distribution of the worms in the small intestine was normal, however, significantly more larvae established in these mice than in all of the other groups. T. spiralis can develop in parenterally fed rats but the establishment is lower than in animals with food in their guts (Castro et al., 1974, 1976). The latter authors suggested that the larvae required the food as transport mechanisms to carry the larvae along the gut. The present results only partly agree with their hypothesis, although food appears to be used as a transport mechanism. I have found that the establishment of larvae is greater in fasted animals. Fasting results in changes in the pattern of intestinal motility, the rate of propulsion becomes significantly slower than fed animals (Reinke, et al., 1967; Carlson et al., 1967; Szutszewski, 1969; Ruckebusch & Bueno, 1975; Summers et al., 1976). Perhaps in the fasting mice given the infective larvae, the rate of flow along the gut was slower than in the fed animals and more larvae had the time to establish than in the fed animals.

The changes in distribution following surfactant and antibiotic treatment may also be explained by altered propulsion. Animals pre-treated with the surfactant, Tween 80, showed a distinct posteriad shift in the distribution of T. spiralis. A consequence of surfactant pre-treatment may have been an increased propulsion rate. The rate of intestinal propulsion

in these animals was not, however, measured. In mice pretreated with antibiotics, the larvae established anteriad of the controls. Comparison between conventional and germ-free mice show that the rate of propulsion in the conventional mice was significantly faster than the germ-free animals (Abram & Bishop, 1966, 1967). In the present study, a slower propulsion rate caused by the suppression of the normal flora could explain the observed results.

Increased inocula resulted in a 'skewed' distribution posteriad. It may have been that the higher densities anteriorly, resulted in intraspecific competition and therefore more larvae penetrated posteriorly. Alternatively, because the motility of the gut is higher anteriorly (Bennet, 1974; Ritter, 1975), high densities of larvae are carried posteriorly, resulting in some establishment in the posterior segments.

Roth (1938, 1939) used very high larval densities (~ 8000 larvae per guinea pig) and the posterior distributions may have resulted from this. Most investigators who have described anterior distributions have used relatively small doses (Gursh, 1949; Larsh & Hendricks, 1949; Campbell, 1967; Sukhdeo & Meerovitch, 1980).

Homosexual infections of T. spiralis females resulted in normally aggregated distributions while homosexual male infections result in much less aggregated distributions. The males may be more active than the females and are probably in search of the females. Intraspecific sexual interactions are well known among nematodes (Green, 1966;

Croll, 1972; Anya, 1976 a.b; Bone et al., 1977) and the influence of sexual attraction on the distribution of gastrointestinal nematodes is suggested (Roche, 1966; Alphey, 1971). Most males pursue their females and this is consistent with my results; in the absence of females the males are less aggregated. Bonner and Etges (1967) reported that both sexes of T. spiralis are attracted to the other but the females move more. Belosevic and Dick (1980) found that males are more actively attracted to the females and not vice versa which is more consistent with the present data. The site selected by the males would seem to be at least partly determined by the position of the females.

2) N. dubius

Most of the larvae of N. dubius established in the anteriormost segments of the small intestine with about 60% of the total recoveries occurring in segments 1 and 2. These results confirm the results of several investigators who have worked with this parasite (Bawden, 1969; Panther, 1969, Lewis & Bryant, 1976). Histological observations in the sites selected by this parasite (see Chapter 4) indicate that larvae penetrate the intestinal wall between 10 and 30 minutes after inoculation. There is no available evidence for subsequent migrations, so the encystment sites are believed to be adjacent to those of penetration.

The distribution pattern was unaffected by the presence of a sheath. Eighty per cent of the larvae exsheath within 5 minutes of infection (Sommerville & Bailey, 1973) therefore similar distributions would have been expected.

There was a significant reduction of larval establishment in the animals given exsheathed larvae. The method used to exsheath the larvae may have been traumatic and there may have been a subsequent reduction in infectivity. In another experiment, exsheathed larvae were implanted into the duodenum and a decrease in recovery was also observed.

Surfactant, antibiotic treatment and food in the gut had significant effects on the distribution of T. spiralis but their effects were all insignificant on the distribution of N. dubius in the intestine. The results of the implantation studies of N. dubius may partially explain this difference. Establishment in the duodenum following implantation into temporary pouches was very high with sheathed larvae (>90%) and exsheathed larvae (>60%). Further results suggest that the larvae are incapable of effectively establishing elsewhere in the gut. This is strongly supported by numerous studies and observations that the larvae are rarely or never recovered from the lower gut. These results suggest that the conditions essential for the establishment of the infective larvae are probably found only in the upper small intestine. The similarity in distributions after different treatments would therefore be expected.

In the experiments with surfactant pre-treatment, antibiotic pre-treatment and intermittent fasting, there were no differences in the distributions or the numbers of larvae establishing when compared to controls. These results contradict the results of Westcott (1967) and Bawden (1969). It had been reported that the microbial flora and the direct or indirect effects of diet exert a significant influence on

the establishment, distribution and development of N. dubius (Westcott, 1967; Bawden, 1969). Bawden (loc. cit.) suggested that the changes that were found in his experiment may have been due solely to altered propulsion. The effects of altered propulsion on the larvae of N. dubius partly supports this hypothesis.

In several of these experiments with N. dubius, the conditions at the time of infection were altered, and there was little change in the longitudinal distributions when compared to controls. It has been reported that some larvae can enter the mucosa of the stomach and remain there for longer than 24 hours before re-entering the lumen and penetrating the intestinal mucosa (Liu, 1965a). The inability of short term alterations in conditions to affect the larvae in the gastric mucosa may therefore explain the unaltered distribution pattern. However, when intestinal propulsion was suppressed with Lomotil®, more larvae established anteriad of the controls. When intestinal propulsion was increased, there was little change in the distribution but significantly fewer larvae became established ($p < 0.05$) when compared to controls. It is possible that the larvae were swept beyond their potential site in the animals with increased propulsion and could not establish further down and the larvae that established in the normal distribution pattern came from the gastric mucosa. The reason for the anteriad establishment in mice treated with Lomotil® may have been due to the prolonged effects of this drug.

Inocula of 100 to 500 larvae of N. dubius resulted in similar distributions. However, with inocula of 1500 larvae, although the majority of the larvae were recovered from the first 3 segments, encysted larvae were consistently recovered posteriad, as far as the 11th segment. I have found that larvae can establish in the ileum in small numbers and I believe that because of the overwhelming number of larvae given in the large inocula, more larvae were able to penetrate the lower small intestine.

3) Summary

a) The larvae of T. spiralis could establish anywhere along the gut but the larvae of N. dubius would establish best in the anterior small intestine.

b) The vehicle of entry into the host does not affect the distributions of T. spiralis or N. dubius in the small intestine.

c) The larvae of T. spiralis determine the sites selected by the adults.

d) The distributions of T. spiralis and N. dubius are affected by the rate of intestinal propulsion.

e) The direct or indirect effects of a surfactant, antibiotics and food in the gut are important in the distribution of T. spiralis but do not affect the distribution of N. dubius.

f) Increased inocula results in more posteriad establishment in T. spiralis and N. dubius.

g) Intraspecific sexual interactions are important in the distribution of T. spiralis.

CHAPTER 4. THE TRANSVERSE SITE OF THE LARVAE OF NEMATOSPIROIDES DUBIUS IN THE GASTROINTESTINAL TRACT OF THE MOUSE

4A. INTRODUCTION

The life cycle of N. dubius has been studied by several investigators (Spurlock, 1943; Ehrenford, 1954, Fahmy, 1956; Bryant, 1973) who have reported that the infective larva of this nematode penetrates the intestinal mucosa of the anterior small intestine and encysts in the intestinal wall. Liu (1965a) reported that the larvae also penetrate the gastric mucosa where they may remain for up to 36 hours before re-entering the lumen and penetrating the mucosa of the small intestine. The purpose of this study was to determine the transverse site selected by the larvae of N. dubius in the small intestine of the mouse.

4B. MATERIALS AND METHODS

Thirty-two mice were divided into 8 equal groups. The mice in four of the groups were each given 1000 larvae of N. dubius by intragastric inoculation. The mice in these groups were killed at intervals of 10, 30, 60 minutes and 24 hours after inoculation. Segments of the stomach and duodenum of these mice were prepared for histological examination. The mice in the remaining 4 groups were each given 500 larvae of N. dubius by intragastric inoculation and they were killed at intervals of 3, 5, 7 and 9 days after infection and the duodenum of these mice were prepared for histological examination.

All tissue for histological examination were prepared as described previously (Chapter 2. General Materials and Methods). In addition, some sections of intestinal tissue were stained with Milligan Trichrome stain, a muscle specific stain (Humason, 1972) and with Picro-Ponceau with haemotoxylin stain, a collagen stain (Humason, 1972).

4C. RESULTS AND DISCUSSION

The larvae of N. dubius are found in the tissues of the stomach and the duodenum during the first 24 hours of infection. In the stomach, the larvae penetrate the epithelial cells within 10 - 30 minutes of infection and migrate into the mucosa (Figure 4.1). Penetration occurs predominantly in the glandular region of the pyloric area of the stomach, and the larvae are usually found penetrated into the fundic glands (Figure 4.2a). The larvae that had penetrated the epithelium of the duodenum were found in the mucosa, and rarely in the submucosa, during the first 24 hours of infection (Figure 4.3). It was reported that the larvae that penetrated the mucosa of the stomach did not stay in the gastric mucosa for longer than 36 hours when they migrated to the lumen and penetrated the intestinal mucosa. In the first day after infection, the larvae are never found in the final site (described below) in the muscularis externa. My results suggest that early in the infection, the larvae either wander around in the tissue or are very slow in making their way to the final site. However, since the larvae are found in the same region as the

FIGURE 4.1 Histological sections of the stomach of mice A, 30 minutes and B, 60 minutes after infection with the larvae of N. dubius. In both cases, the larvae are found in the fundic glands. Arrows point to the larvae (bar = 100 μm).

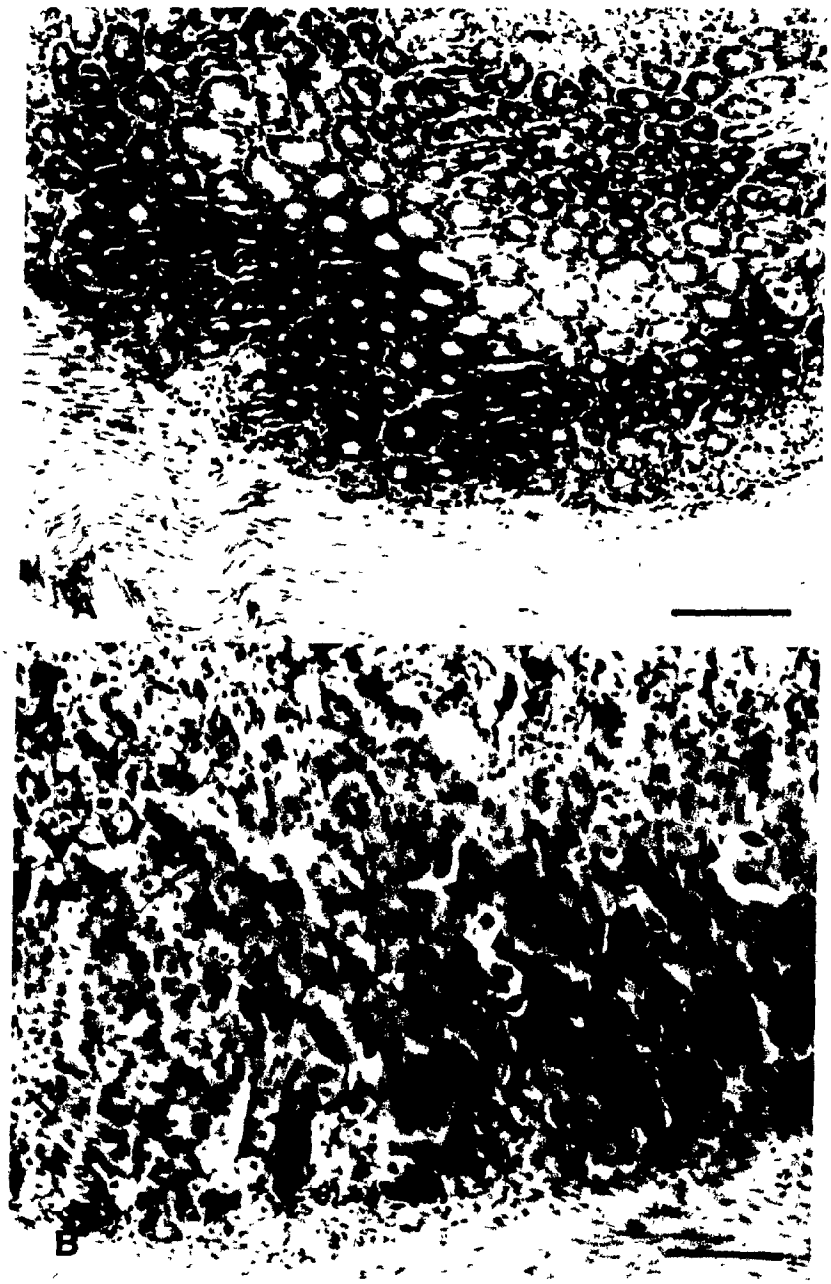
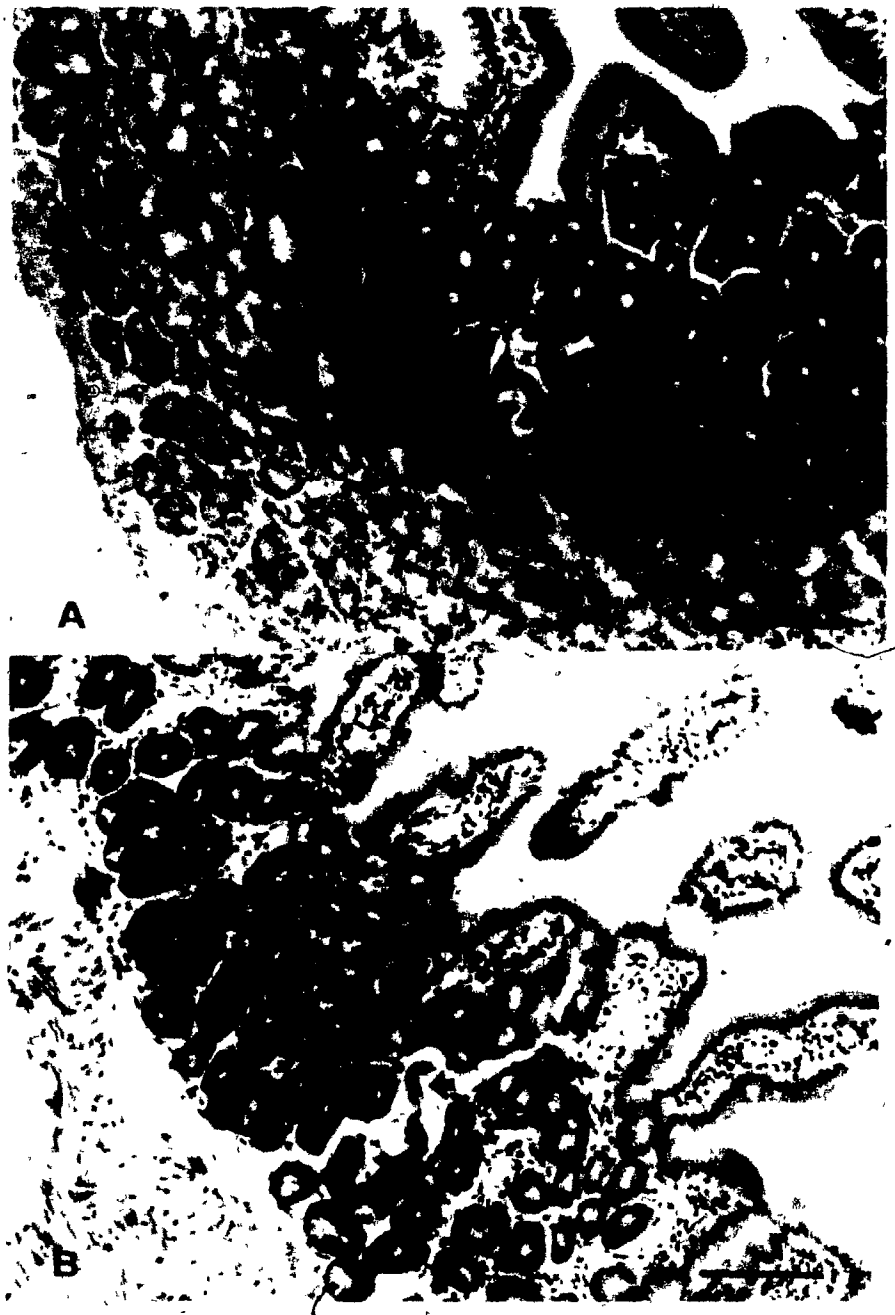

B

FIGURE 4.2 The location of the larvae of N. dubius in the stomach and small intestine 1 day after infection. A, a larva in the stomach, the larvae are still found in the region of the fundic glands. B, a larva in the small intestine, probably in the process of penetrating. The larvae are rarely found in the submucosa during the first 24 hours of infection (bar = 100 μm).


B

FIGURE 4.3 The location of the larvae of N. dubius in the small intestine A, 10 minutes and B, 30 minutes after infection. The larvae are found in the mucosa, arrow points to larva (bar = 100 μm).


A
B

final site, I suggest that the penetration is a slow process.

It has been reported that the larvae cause a local necrosis as they burrow through the mucosa and it was postulated that the larvae secrete a substance(s) which acts as a necrosin to lyse the surrounding tissue (Liu, 1965). In addition, Liu (loc. cit.) has reported that in Webster and C_3H mice, there is a dense infiltration of polymorphonuclear leucocytes around the larvae in the small intestine and in the lamina propria and submucosa of the stomach, as early as 24 hours after infection. I have found that in the first 24 hours of infection, the only damage in the stomach and the small intestine appeared to be caused by the mechanical passage of the larvae through the mucosa and that there was no obvious increase in the infiltration of polymorphonuclear leucocytes in either region during the early stages of infection.

From 3 days after infection, there was a localized eosinophilic response around the worms that increased slightly in severity as the infection progressed (Figures 4.4 - 4.6). This response was similar to the response of Webster and C_3H mice to N. dubius infection (Liu, 1965a) but of a much lesser degree. The differences in severity of response may have been due to strain differences in the host and parasite.

From the 3rd to the 7th day after infection, the larvae were found in the muscularis externa (Figures 4.4; 4.5). When the sections were stained with Milligan Trichrome stain for muscle tissue, it was found that the larvae were completely surrounded by the cells of the circular muscle layer of the muscularis externa (Figure 4.5a) but never in the

FIGURE 4.4 The location of the larvae of N. dubius in the small intestine A, 3 days and B, 7 days after infection. The larvae are found embedded in the muscle layers of the wall surrounded by polymorphonuclear leucocytes. The leucocytic response increases as the infection progresses (bar = 100 μm).


A
B

FIGURE 4.5 The location of the larvae of N. dubius in the small intestine 5 days after infection. A, section stained with Milligan Trichrome stain, showing the circular muscle layers surrounding the worm. B, section stained with Picro-Ponceau, showing a layer of collagen in the submucosa but not around the larva (bar = 100 µm).

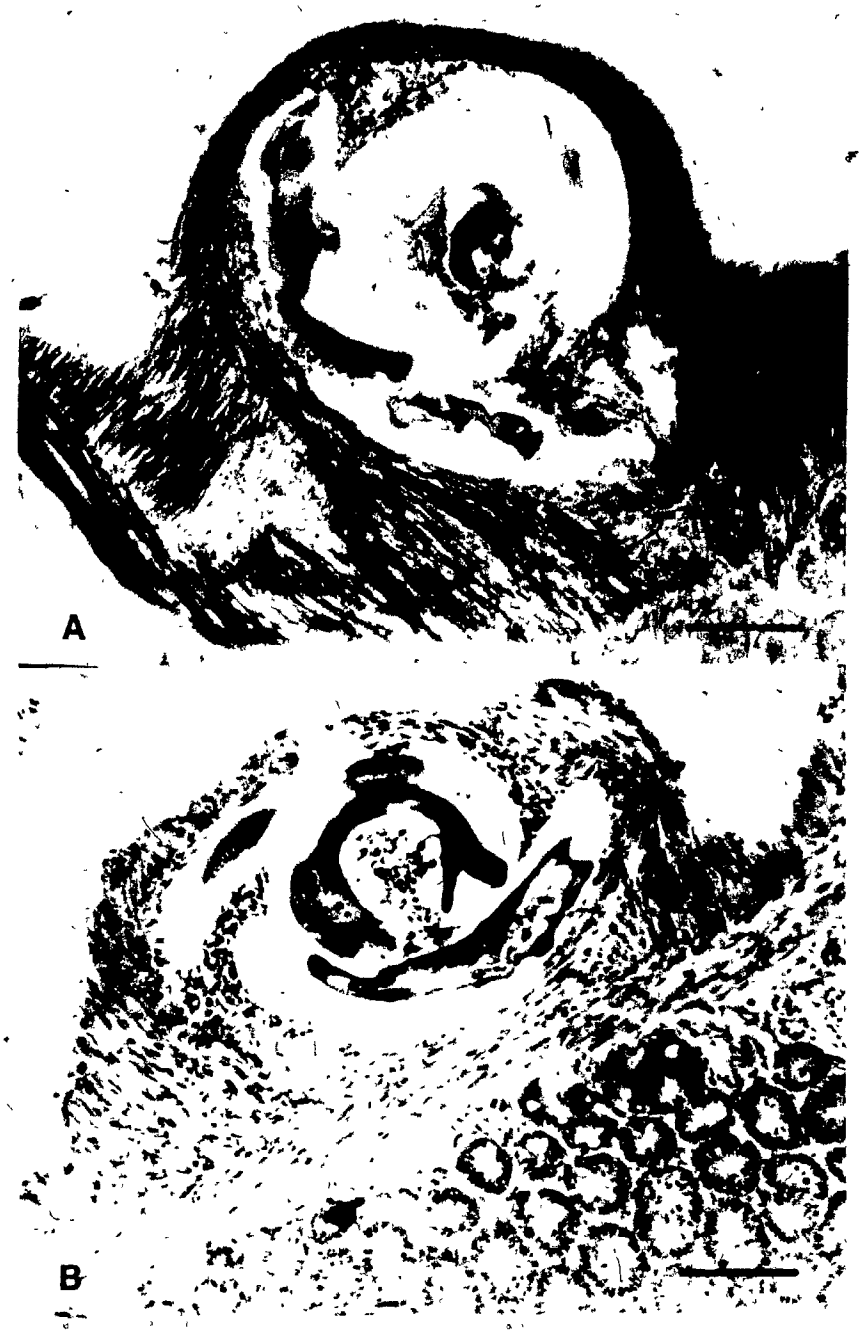

A
B

FIGURE 4.6 The location of the adults of N. dubius in the small intestine 9 days after infection. The adults are found in the lumen of the small intestine, entwined around the villi. The striated cuticle probably has a function in anchoring the worms to the villi and particulate matter is found in the gut (bar = 100 µm).

longitudinal muscles. Although several investigators have suggested that the larvae are found within cysts (Liu, 1965a; Bryant, 1973), I have found no evidence of a cyst. In sections stained for collagen (Picro-Ponceau stain), a layer of collagen was seen in the submucosa and the worms were usually found below this layer. There was no collagen seen around the larvae (Figure 4.5b). My results suggest that the larvae are merely embedded in the circular muscle tissue and sequestered by the localized leucocytic response, this is not a true cyst.

The method by which nematodes feed when apparently immobile and within tissue is not understood. The encysted larvae of T. spiralis have the ability to absorb various metabolites and nutrients (McCoy, et al., 1941; Stoner & Hankes, 1955, 1958; Hankes & Stoner, 1956, 1958; Mills & Kent, 1965; Kozar, et al., 1969). The mechanism of nutrient uptake in the larvae of T. spiralis has not been elucidated. In the entomophilic nematode, Mermis nigrescens, uptake of nutrients occurs across the cuticle (Rutherford & Webster, 1974, 1978; Rutherford et al., 1977) and the mechanism may be similar for the larvae of T. spiralis. The mechanism of nutrient uptake of the larvae of N. dubius during its histotrophic phase has not been previously described.

I have found that the gut of the larvae of N. dubius is filled with particulate matter while it is embedded within the muscle (Figure 4.7a) and occasionally the gut may contain collagenous material similar to that found in the nearby submucosa (Figure 4.5b).

FIGURE 4.7 The location of the larvae of N. dubius in the small intestine A, 5 days and B, 7 days after infection. Particulate matter is found in the gut (A) and the spike of the larva is extruded (B) (bar = 100 μm).

B

A spike-like object, reminiscent of an underdeveloped stylet, has been seen in some but not all L_4 larvae (Figure 4.7b). This appendage has not been previously described from trichostrongylid larvae but it may be used to disrupt the cell membranes.

The presence of particulate matter within the gut of the nematode suggests that it is actively feeding on the host's tissue during development.

4D. SUMMARY

a) The larvae of N. dubius do not immediately go to their final site after penetration but the final site selected in the small intestine is adjacent to the sites of penetration.

b) The final site of the larvae in the small intestine is the circular muscle layer of the muscularis externa.

c) The larvae feed on the surrounding tissue during its development in the small intestine.

CHAPTER 5. EXCYSTMENT BEHAVIOUR IN *TRICHINELLA SPIRALIS*: THE EFFECTS OF GASTRIC SECRETIONS

5A. INTRODUCTION

On its arrival in the host, the infective stage of an intestinal parasite emerges from its protective egg, cyst or sheath and establishes itself. The initial activation occurs anywhere along the gut from the stomach to the large intestine (Rogers, 1960; Christie & Charleston, 1965; Sommerville & Bailey, 1973; Lackie, 1975; Panesar & Croll, in press). The infective larvae of *N. dubius* lose their protective sheaths in the stomach of the host when 80% of the larvae exsheath in less than 5 minutes (Sommerville & Bailey, 1973). The latter authors also found that the major components of the stimulus for exsheathment of *N. dubius in vitro* are the high concentrations of hydrogen ions and temperatures similar to that of their host. There are several other nematodes, including *Trichostongylus colubriformis*, *Trichostrongylus retortaeformis* and *Nematodirus battus* (Rogers, 1960; Christie & Charleston, 1965; Bailey, 1968; Lackie, 1975) which have similar requirements for exsheathment and these nematodes also exsheath in the stomach of their hosts.

T. spiralis is generally believed to excyst following the enzymatic degradation of the capsule by pepsin in the stomach (Gould, 1970). The normal laboratory practice of excysting larvae from infected meat is by digestion in pepsin

and HCl which also supports this belief. This section attempts to quantify more precisely the active and passive components of excystment in T. spiralis.

5B. MATERIALS AND METHODS

1) The method of recovery of encysted larvae of T. spiralis

The encysted larvae of T. spiralis were recovered from infected meat by homogenization. Infected animals were killed by decapitation, skinned, eviscerated and the carcass was cut into small pieces. These pieces were homogenized with saline (unless otherwise stated) in a Servall Omni Mixer (Dupont Instruments, Newton, U.S.A.).

The homogenate was strained through cheesecloth and the filtrate was sedimented in "pilsner" glass. The sediment containing the encysted larvae was washed thrice in saline. The cysts were then individually collected with a Pasteur pipette drawn to a fine point. Cysts containing 1 larva and with a minimum of muscle debris attached were used in these studies.

2) Experiments on the excystation of T. spiralis

a) The effect of the concentration of pepsin on the excystment of T. spiralis larvae.

(i) Infected meat was homogenized in 0.85% saline and aliquants of between 50 and 100 cysts were transferred to each of 15 watchglasses. The watchglasses were covered and incubated at 37° C for 30 minutes prior to the commencement of the experiment to acclimate the worms to this temperature.

Log dilutions of purified pepsin (Fisher Scientific Company Limited, Montreal) from 10% to 10^{-4} % were prepared in a solution of 0.06 N HCl (pH 1.9 - 2.0) that was previously warmed to 37° C. Two ml of each dilution was added to each of 3 watchglasses to a final volume of 2.1 ml and the numbers of larvae excysting were counted at intervals.

(ii) This experiment was identical in all respects with experiment 2(i), except that the infected meat was initially homogenized in distilled water instead of saline.

b) The activity and behaviour of T. spiralis larvae.

The activity and behaviour of the larvae was observed just prior to and subsequent to excystment, after being placed in HCl-pepsin solution at 37° C. The CCTV was used to record the larval movements.

c) The effects of pH and several enzymes on the encystment of T. spiralis larvae.

The infected meat was homogenized in saline and the cysts extracted. The following enzymes were tested: pepsin, trypsin, papain, α-chymotrypsin, α-amylase, lactase and lipase (Fisher Scientific Company Limited, Montreal; Sigma Chemical Company, St. Louis). A 1% solution of each enzyme was prepared in each of a series of 9 buffers ranging between pH 1 to pH 9 (Parker & Croll, 1975) and a 1.0 ml sample of each solution was added to each of 4 tubes containing 50 - 100 cysts to a final volume of 1.1 ml. The tubes were then incubated in a slow-shaking water bath at 37° C for 1 hour, at the end of this incubation the tubes were placed in crushed ice to arrest enzymatic reaction. Controls of 1% pepsin in buffered

HCl solution (pH 2.0) were included to assess the viability of the cysts. The effect of collagenase (Sigma Chemical Company, St. Louis) was also assayed at pH 7.9, the pH optimum of this enzyme.

The influence of these enzymes on the ratio of excysted to encysted larvae was examined.

d) The effects of different concentrations of acids with pepsin on the excystment of T. spiralis larvae.

Infected meat was homogenized in distilled water and the cysts were extracted. One percent solutions of pepsin in log dilutions of HCl, H_2SO_4 and CH_3COOH were prepared. One ml samples of each solution were put into test tubes containing 50 - 100 cysts in 0.1 ml distilled water. The tubes were incubated in a slow-shaking water bath at 37° C, for 1 hour before being chilled to arrest enzymatic reaction. The influence of acid concentration on excystment of the larvae was examined.

e) The effects of chloride ions on the activity of the excysted larvae of T. spiralis.

(i) Infected meat was homogenized in distilled water and 0.1 ml aliquants containing between 50 - 100 cysts were transferred to each of 16 watchglasses. The watchglasses were covered and incubated at 37° C for 30 minutes prior to commencement of the experiment. One ml of the following solutions was added to each group of 4 watchglasses: (a), a 1% solution of pepsin in HCl (pH 2.0); (b), a 1% solution of pepsin in H_2SO_4 (pH 2.0); (c), a 1% solution of pepsin

in H_2SO_4 (pH 2.0) plus the addition of NaCl to a final concentration of 1% and (d), a 1% solution of pepsin in H_2SO_4 (pH 2.0) plus the addition of $NaSO_4$ to a final concentration of 1%. The population activity and the mean activity rate was monitored after 30 minutes incubation at 37° C in these solutions.

(ii) Infected meat was homogenized in distilled water and 0.1 ml aliquants containing 50 - 100 cysts were transferred to each of 16 watchglasses. The watchglasses were covered and incubated at 37° C for 30 minutes before the start of the experiment. One ml of the following solutions was added to each group of 4 watchglasses: (a), a 1% solution of pepsin in HCl (pH 2.0); (b), a 1% solution of pepsin in H_2SO_4 (pH 2.0); (c), a 1% solution of pepsin in H_2SO_4 (pH 2.0) plus NaCl to a final concentration of 0.1% and (d), a 1% solution of pepsin in H_2SO_4 (pH 2.0) plus NaCl to a final concentration of 1.0%. At intervals after incubation at 37° C the percentage motile in each treatment were examined.

f) The effects of intestinal secretions on the excystment of T. spiralis larvae.

In order to examine the possibility of unspecified factor(s) from the intestine affecting excystment of T. spiralis, a crude "succus entericus" was prepared. A 10 cm portion of the mid small intestine was resected in anaesthetized, laparotomized rats. This was washed with sterile saline and closed at both ends to form a fistula having its

own blood supply. The gut was anastomosed without this section and the abdomen was closed. Four days after surgery, the contents of the fistulae were collected, pooled and diluted to a concentration of 10% in buffered saline (pH 7.2). This solution was used as the crude "succus entericus" and small aliquants were stored at -20° C until used.

Log dilutions of (a), trypsin, (b), "succus entericus" and (c), elastase (Sigma Chemical Company, St. Louis) were prepared in (a), buffered saline, and (b), a 10% solution of crude pig bile in buffered saline. Infected meat was homogenized in distilled water and 0.1 ml aliquants containing 50 - 100 cysts were transferred to test tubes. One ml of each dilution was added to groups of 3 tubes. The tubes were incubated in a slow-shaking water bath at 37° C for 1 hour before being chilled to arrest the enzymatic reaction. The percentage excystment in each treatment was examined.

g) The effect of gastric secretions in vivo on activation, excystment and establishment of the larvae of T. spiralis.

Fifteen rats were anaesthetized and laparotomized. Five equal groups of rats were surgically prepared: (a), controls, closed without further surgery and infected with 1000 encysted larvae; (b), total gastrectomy (see below) and infected with 1000 encysted larvae; (c), total gastrectomy and bile duct ligature (see below) and infected with 1000 encysted larvae; (d), no surgery and infected with 1000 excysted larvae and (e), total gastrectomy and infected with 1000 excysted larvae.

The animals in which the stomach was removed were anaesthetized and laparotomized. The avascular peritoneal folds linking the stomach to the neighbouring organs were divided and the stomach was pulled out of the abdomen. In order, the left gastric vein, the caeliac artery, the portal vein, the gastric short vein and several branches of the right gastro-epiploic vein were divided between 2 ligatures. The stomach was removed after division at the eosophagus and duodenum. These were joined by end-to-end anastomosis. The abdomens were then closed (Figure 5.1).

The animals in which the bile duct was ligatured were anaesthetized and laparotomized. The upper bile duct was ligatured and divided above the entrance of the pancreatic secretions into the common bile duct. The abdomens were then closed (Figure 5.2).

In all cases, the larvae were given by intragastric intubation 3 days after surgery. Five days after infection, the animals were killed and the numbers and distribution of worms in the small intestine were determined. The influence of gastric secretions *in vivo* on establishment and longitudinal dispersion was examined.

h) The resistance of the infective stage of *T. spiralis* to gastric secretion.

Thirty animals were divided into 3 equal groups and each animal was given 500 larvae of *T. spiralis* by intragastric inoculation. The mice in the groups were killed at intervals of: (a) 1 hour; (b) 6 hours, and (c) 18 hours, after infection; the small intestine was immediately removed and the mucosa was scraped with a glass slide. The mucosal

FIGURE 5.1 The removal of the stomach in the rat. S, shows the removed stomach; X, shows the suture line between the oesophagus and the duodenum.


S

FIGURE 5.2 Bile duct ligature. Arrow points to the two ligatures of the common bile duct, the duct is divided between the ligatures.

scrapings of 5 mice in each group were fed individually to rats and the scrapings of the remaining 5 mice were implanted individually into the duodenum of anaesthetized laparotomized rats. Five days later, the rats were killed and the number of adults in the gut was determined. The influence of larval maturity on the ability to survive gastric secretions was examined.

5C. RESULTS

1) Pepsin and exsheathment.

The excystment of larvae of T. spiralis occurs in pepsin-HCl solutions and the rate of excystment is dependent on the concentration of pepsin (Figure 5.3). The most rapid excystment occurred in 1% solutions of pepsin and the maximum excystment was attained within 30 minutes of incubation. The time required for encystment increased as the concentration of pepsin decreased and below 0.001% solutions, the encystment was 25% even after 90 minutes. There was no excystment in pepsin concentrations of 0.0001% and less. Excystment was slower in the corresponding dilutions of pepsin when the infected carcass was homogenized in distilled water, and at pepsin concentrations of 0.1% and lower, fewer than 90% of the larvae excysted after 90 minutes (Figure 5.4).

2) Behaviour of larvae.

On immersion in HCl-pepsin solution, the worms began to move while still within their cysts. The worm follows a "figure of 8" movement with the appearance of spinning around within the cyst. As more space is made within the

FIGURE 5.3 The rate of excystment of the larvae of T. spiralis in different concentrations of pepsin after homogenization in saline. The numbers on the curves refer to the concentration of pepsin.

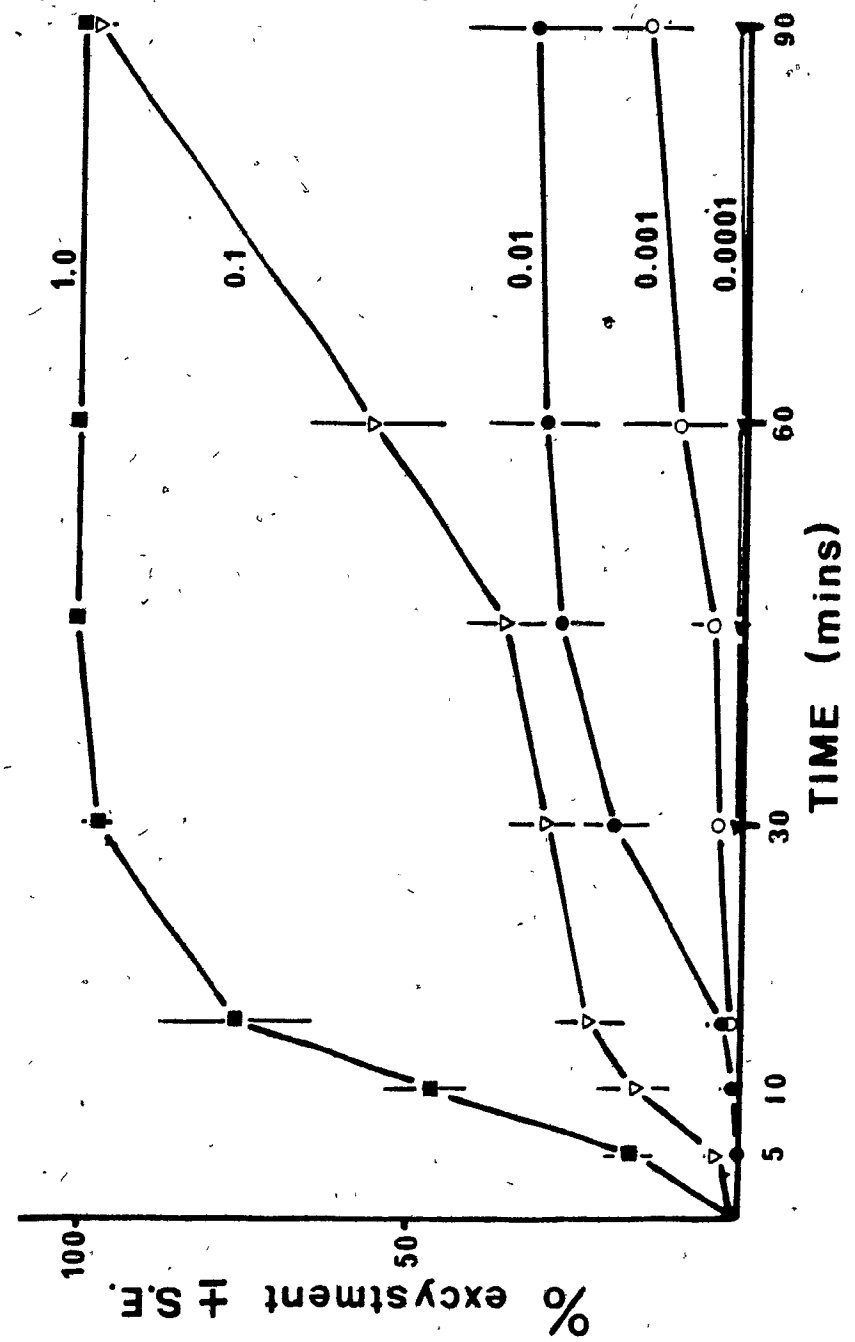
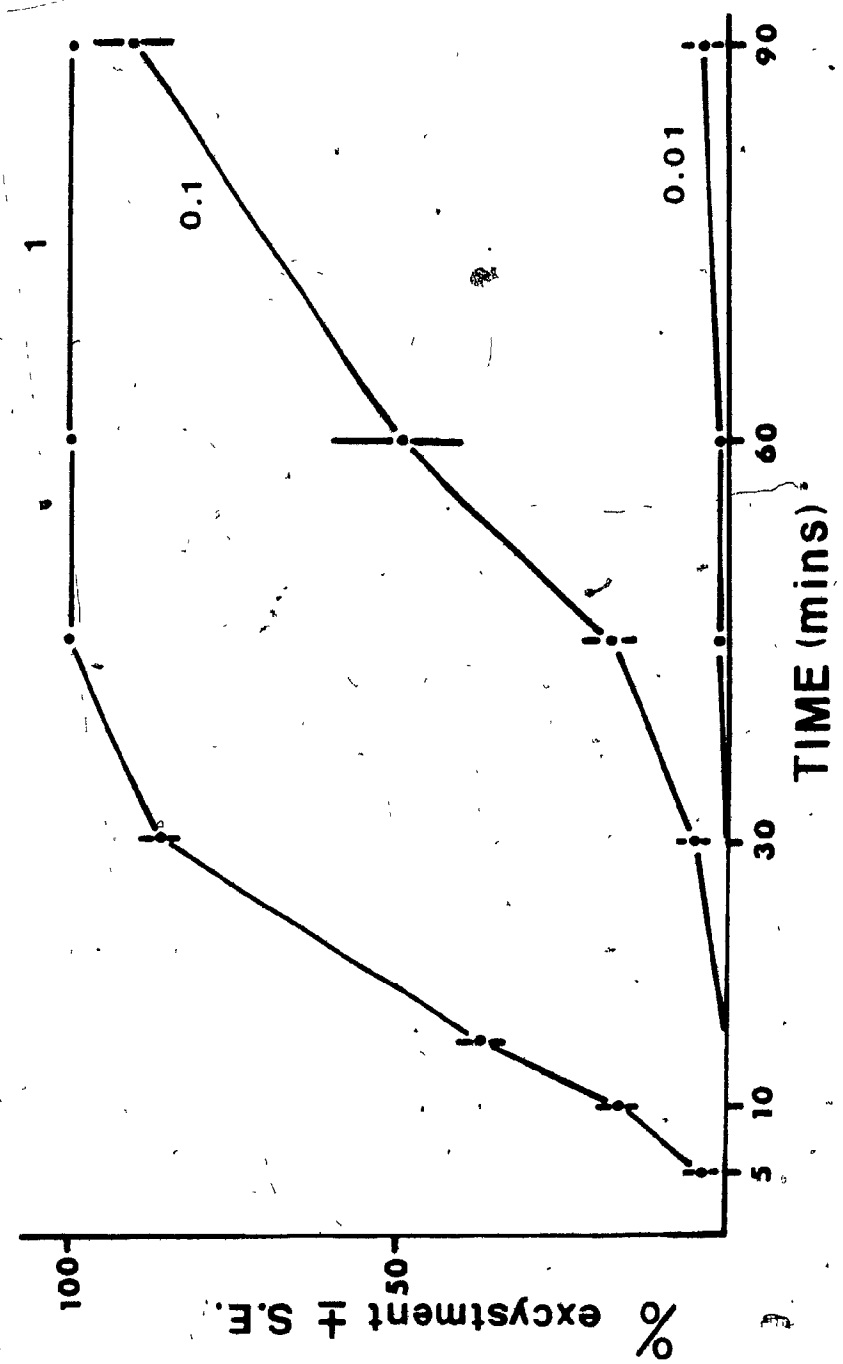

% excystment ± S.E.
100
50
5
10
30
60
90
TIME (mins)
1.0
0.1
0.01
0.001
0.0001

FIGURE 5.4 The rate of excystment of the larvae of T. spiralis in different concentrations of pepsin after homogenization in distilled water. The numbers on the curves refer to the concentration of pepsin.


% excystment ± S.E.
100
50
5
10
30
60
90
TIME (mins)
1
0.1
0.01

cyst, small lashing movements of the tail begin. These tail lashing movements help the larvae in breaking out of the cyst before the cyst is completely digested (Figure 5.5). The tail lashing behaviour continues for several hours after the larvae are free of the cyst. The head end remains coiled and immobile while the posterior half of the worm rythmically lashes out. This behaviour pattern is designated as type 1. A frame-by-frame analysis taken from CCTV recordings of this behaviour pattern is shown in Figure 5.6. Figure 5.7 shows the rate of activity (Type 1) and the population activity of the larvae after encystment and in the absence of any other stimulation.

3) Enzymes and excystment.

The only enzymes that had a dramatic effect on the excystment of the larvae of T. spiralis were pepsin at a low pH and collagenase at its optimum pH (Figure 5.8). The other enzymes tested appeared to have little effect on excystment with the exception of trypsin. In trypsin, there was some excystment at pH 8 to 9 but maximum excystment with this enzyme was not higher than 8%. The cysts used in all of the assays were active and the controls (HCl-pepsin) included in each assay all resulted in 100% excystment.

4) Acid, chloride ion and excystation.

The efficiency of excystment with a 1% pepsin solution increased with increasing concentrations of acid (Figure 5.9). Excystment was higher in the strong acids HCl and H_2SO_4 and extremely low in the weak acid CH_3COOH. The excystment response differed in the strong acids even at the

same pH and the numbers of larvae excysting were consistently higher in the HCl concentrations (Figure 5.10). It is possible that the presence of Cl^- ions influenced the efficiency of excystment.

The process of excystment was much slower and the activity of larvae in a solution of pepsin with no Cl^- ions was significantly lower ($p \leq 0.05$) than the activity of larvae in pepsin solutions with Cl^- ions (Table 5.1).

The numbers of mobile larvae in the population increased and persisted for several hours as the Cl^- ion concentration in the incubation medium was increased (Figure 5.11).

5) Intestinal secretions and excystment.

Bile, trypsin, "succus entericus" and elastase alone or in combination with each other had no effect on the excystment of the larvae. Like the saline controls, there was no excystment of the larvae. (No data are given for these results, as there was zero excystment.)

6) Excystment and Gastrectomy.

In rats with total gastrectomies, encysted larvae were still able to establish. The distribution of worms in the gut differed from the control animals (Figure 5.12) but was similar to the distribution of worms in the gastrectomized rats given excysted larvae (Figure 5.13), with most of the adults recovered in the posterior small intestine. Infection with encysted larvae in rats with total gastrectomies and bile duct ligature resulted in adult distributions that did not differ from the infection in the gastrectomy-only rats (Figure 5.14). The percentage establishment of these

FIGURE 5.5 Larval excystment, the larva of T. spiralis is shown breaking out of the cyst tail-first after pepsin-HCl stimulation.

FIGURE 5.6 Frame-by-frame analysis of the movement pattern of a larva of T. spiralis in type 1 movement. The sequence moves from the top left hand to the bottom right hand. The anterior end of the worm is depicted by the arrow and the elapsed time between each frame is 0.017 seconds.

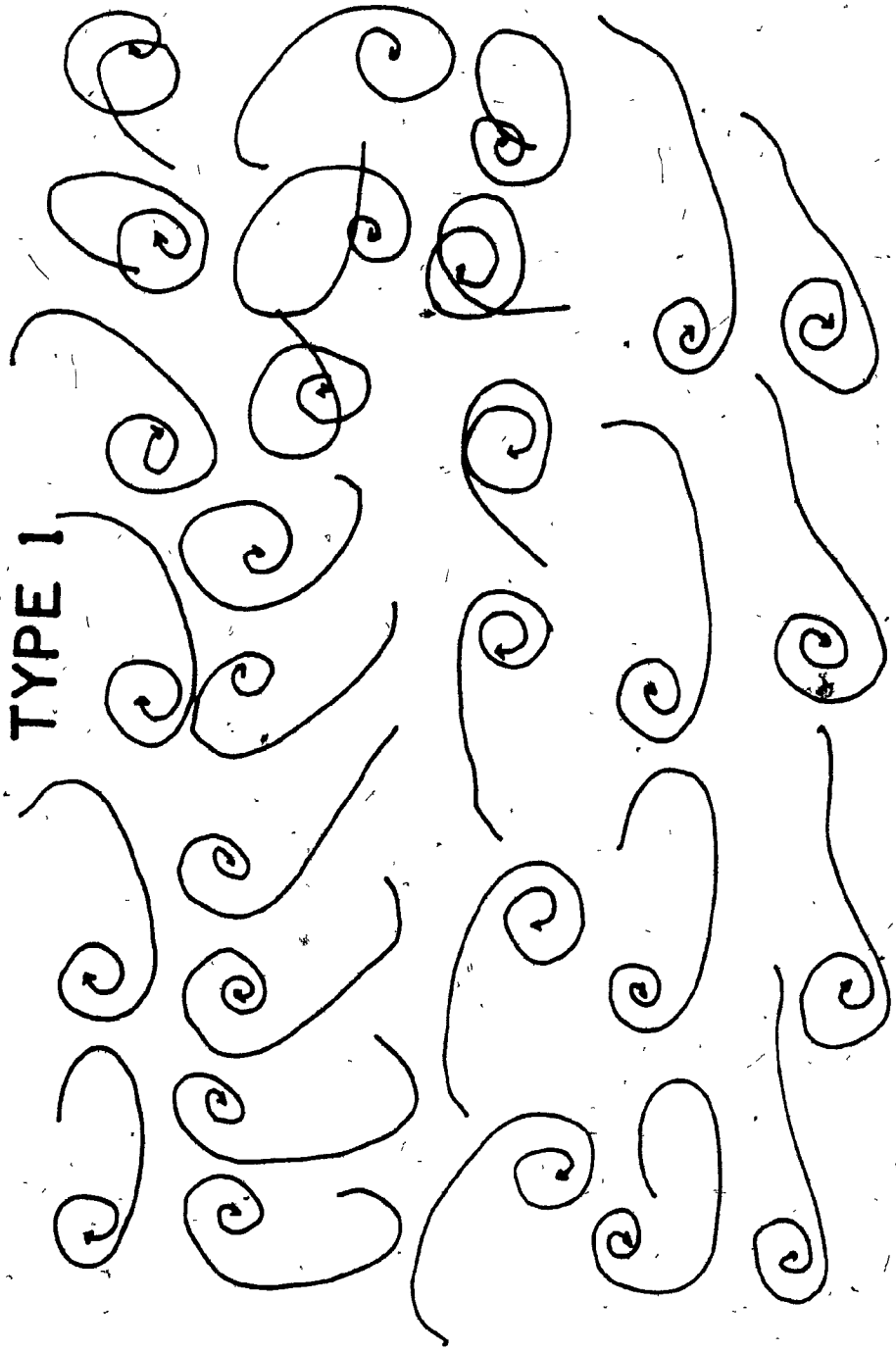

TYPE 1

FIGURE 5.7 The rate of activity (type 1) and the population activity of the larvae after excystment in the HCl-pepsin solution and in the absence of other stimuli.

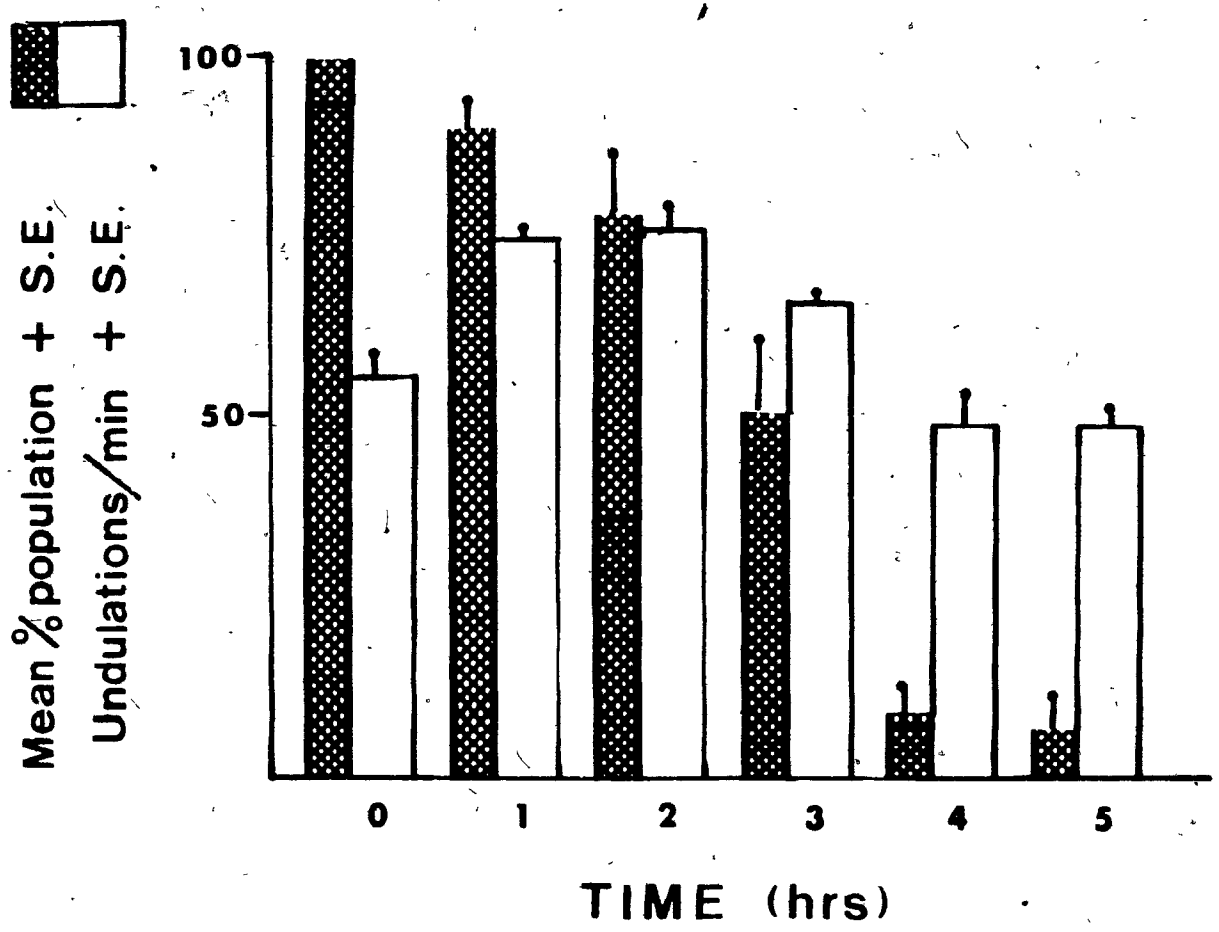

Mean % population + S.E.
Undulations/min + S.E.
100
50
0
1
2
3
4
5
TIME (hrs)

FIGURE 5.8 The percentage excystment of the cysts of T. spiralis after 30 minutes incubation at 37° C in various enzyme solutions in a series of buffers.

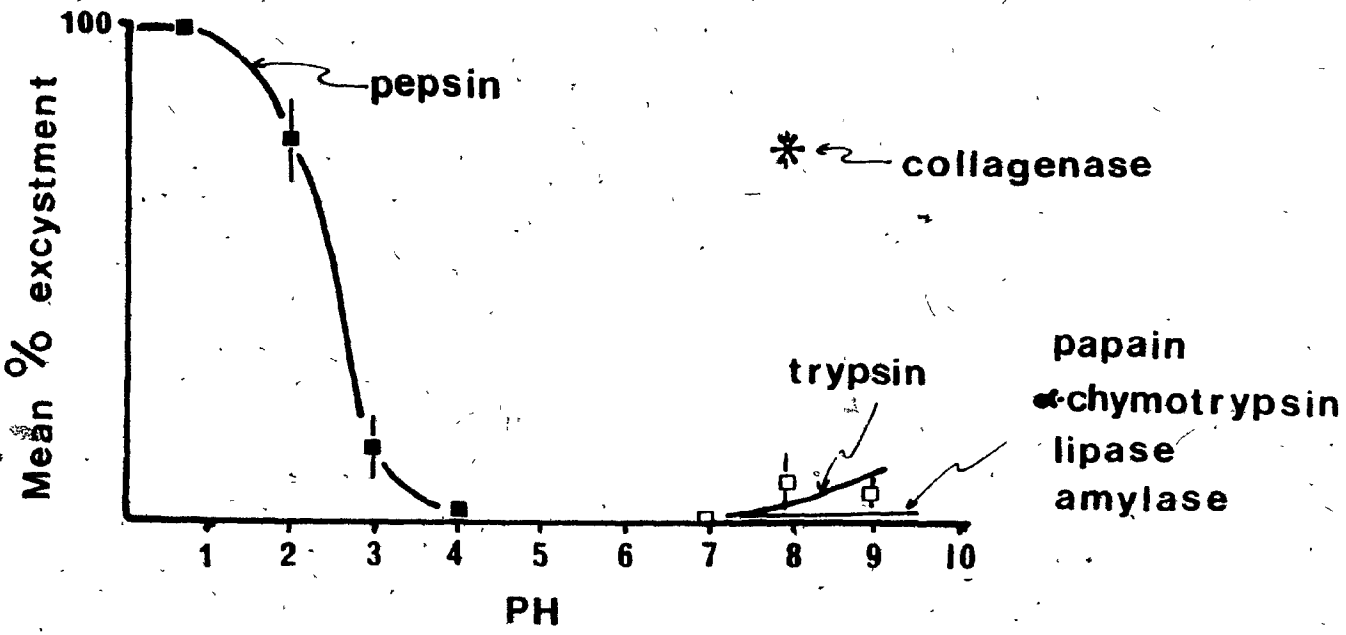

100
Mean % excystment
pepsin
collagenase
trypsin
papain
chymotrypsin
lipase
amylase
1
2
3
4
5
6
7
8
9
10
PH

FIGURE 5.9 The percentage excystment of the cysts of T. spiralis after 30 minutes incubation at 37° C in 1% pepsin solutions of different concentrations of acid. A, HCl; b, H_2SO_4; c, CH_3COOH.

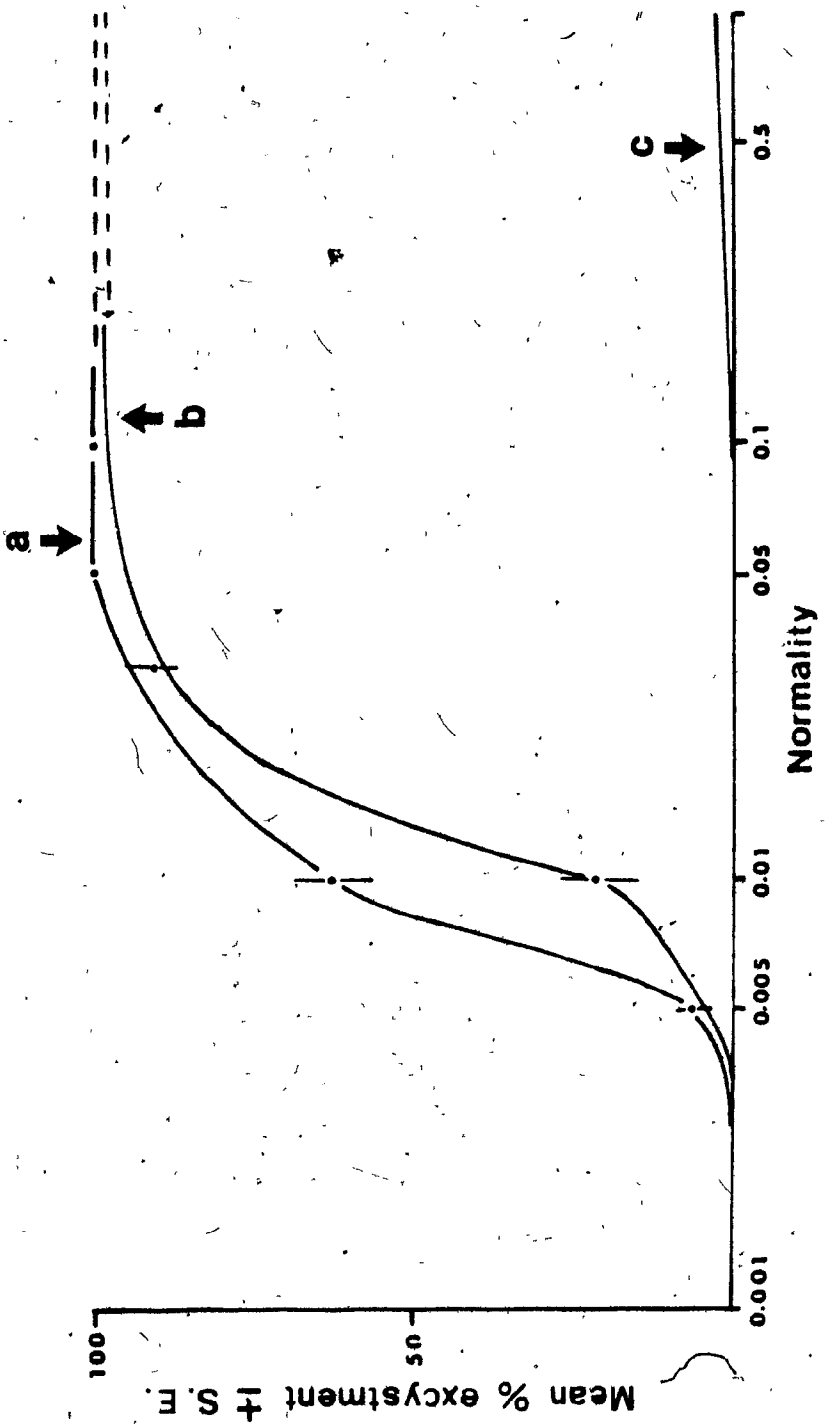

a
b
c
0.5
0.1
0.05
0.01
0.005
0.001
Normality
100
50
Mean % excystment ± S.E.

FIGURE 5.10 The percentage excystment of the cysts of T. spiralis after 30 minutes incubation at 37° C in 1% pepsin solutions at different pH.

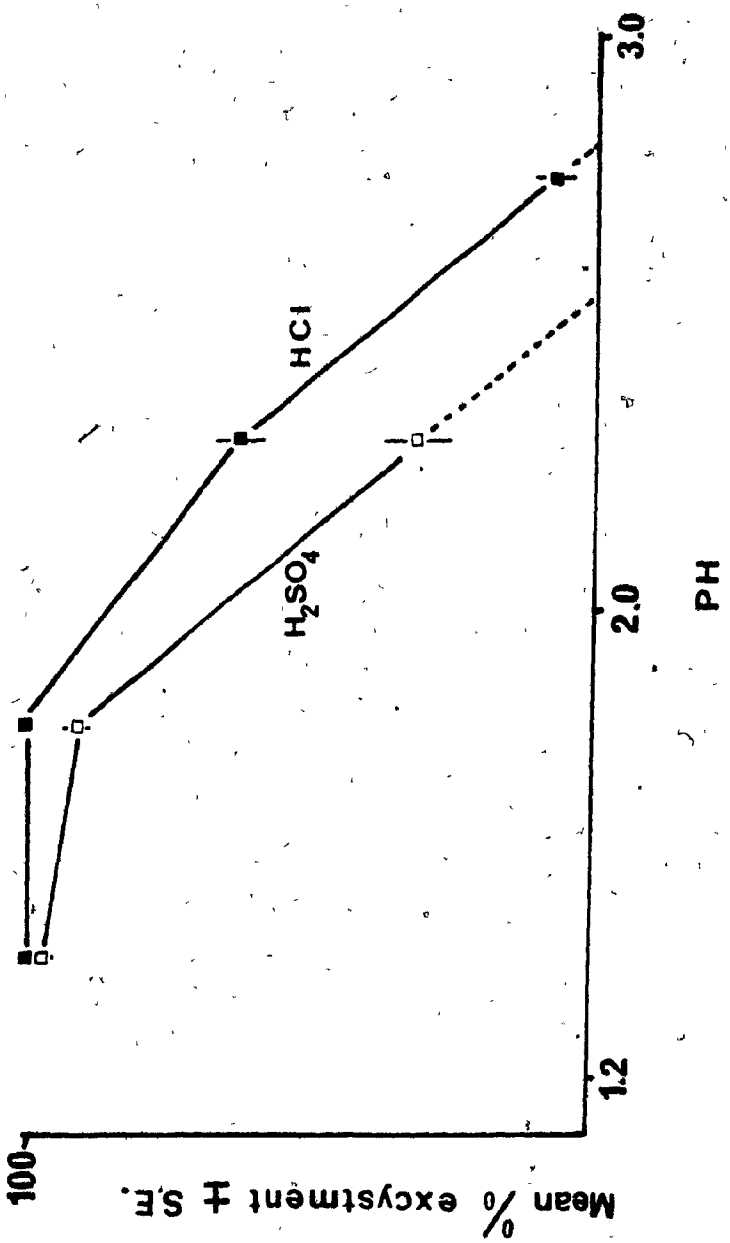

Mean % excystment ± S.E.
100
1.2
2.0
3.0
PH
HCl
H_2SO_4

TABLE 5.1 THE RATE OF ACTIVITY AND THE PERCENT OF THE POPULATION ACTIVE 5 MINUTES AFTER EXCYSTMENT IN THE PRESENCE AND ABSENCE OF Cl^- IONS

Treatment	HCl + pepsin	H_2SO_4 + pepsin	H_2SO_4 + pepsin + NaCl	H_2SO_4 + pepsin + NaSO
% pop. active	97.8 ±1.2	32.2 (a) ±4.5	82.1 ±2.3	43.1 (a) ±7.6
Activity undulations/min	73.8 ±1.5	49.1 (a) ±4.7	68.1 ±3.9	45.4 (a) ±5.4

(a) significantly different from controls at $p \leq 0.05$

FIGURE 5.11 The mean % population active after excystment of the larvae of T. spiralis in various solutions.

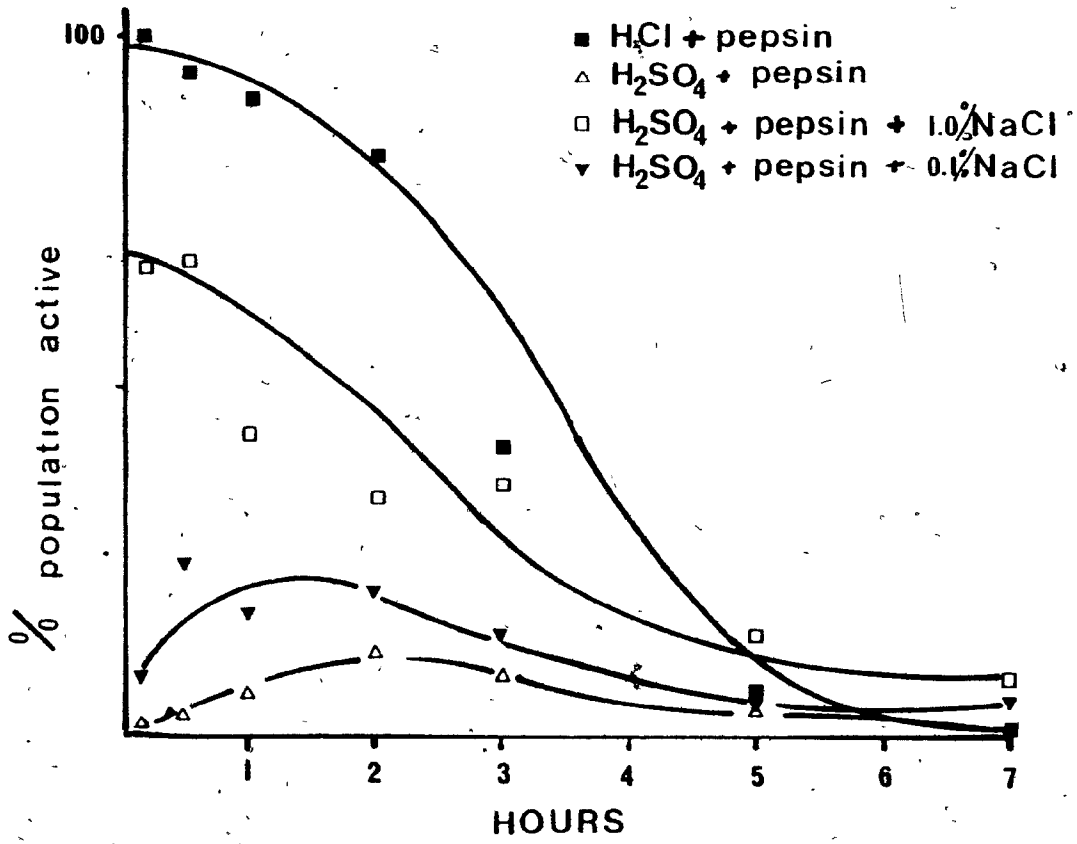

100
% population active
HCl + pepsin
H_2SO_4 + pepsin
H_2SO_4 + pepsin + 1.0% NaCl
H_2SO_4 + pepsin + 0.1% NaCl
1
2
3
4
5
6
7
HOURS

FIGURE 5.12 The distribution of adults in the small intestine of rats 5 days after infection with 1000 larvae of T. spiralis. A-C, sham operated controls given encysted larvae; D-F, gastrectomized rats given encysted larvae.

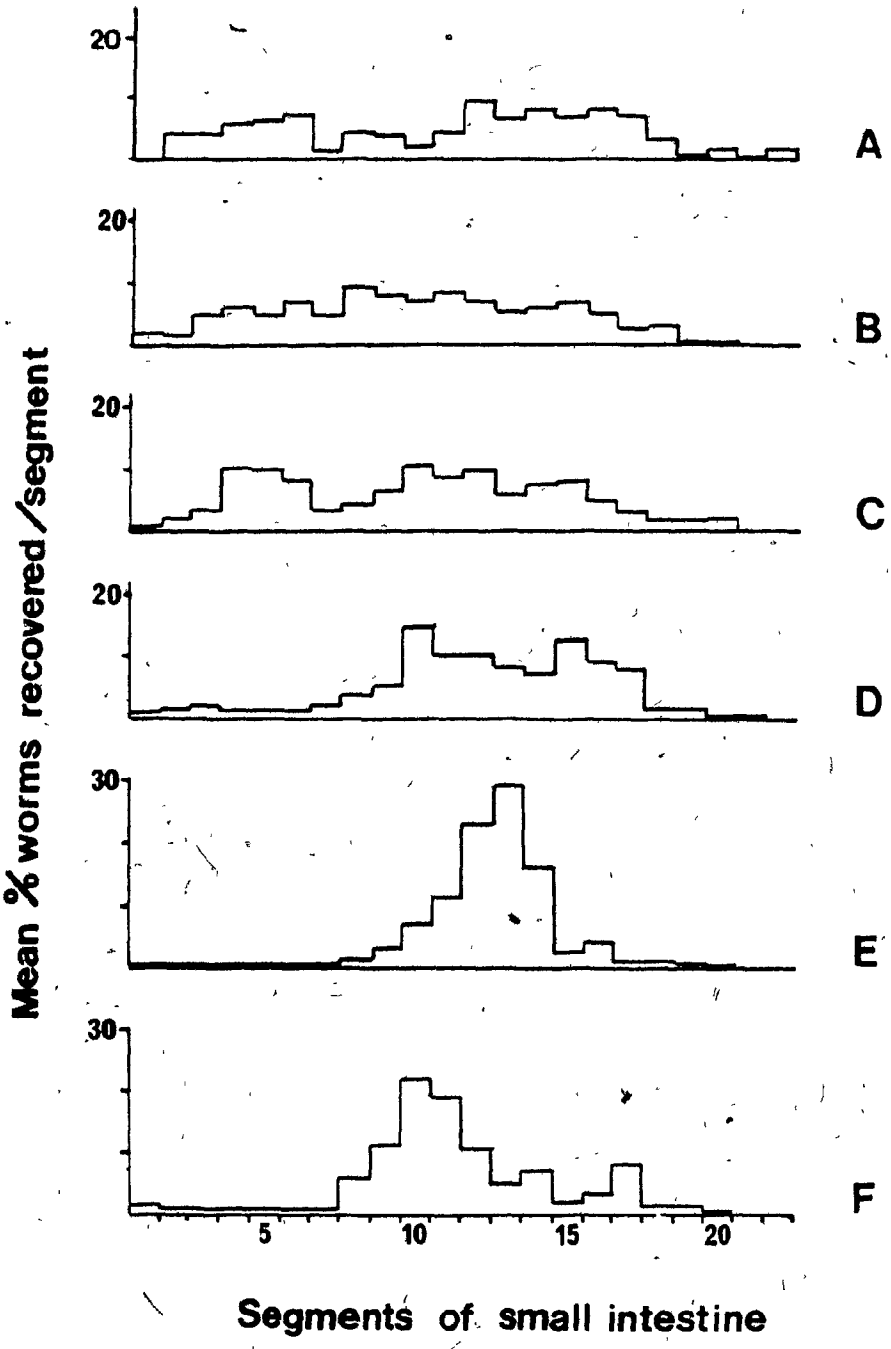

Mean % worms recovered/segment
20
A
20
B
20
C
20
D
30
E
30
F
5
10
15
20
Segments of small intestine

FIGURE 5.13 The distribution of adults in the small intestine of rats 5 days after infection with 1000 larvae of T. spirals. A-C, sham operated controls given excysted larvae; D-F, gastrectomized rats given excysted larvae.

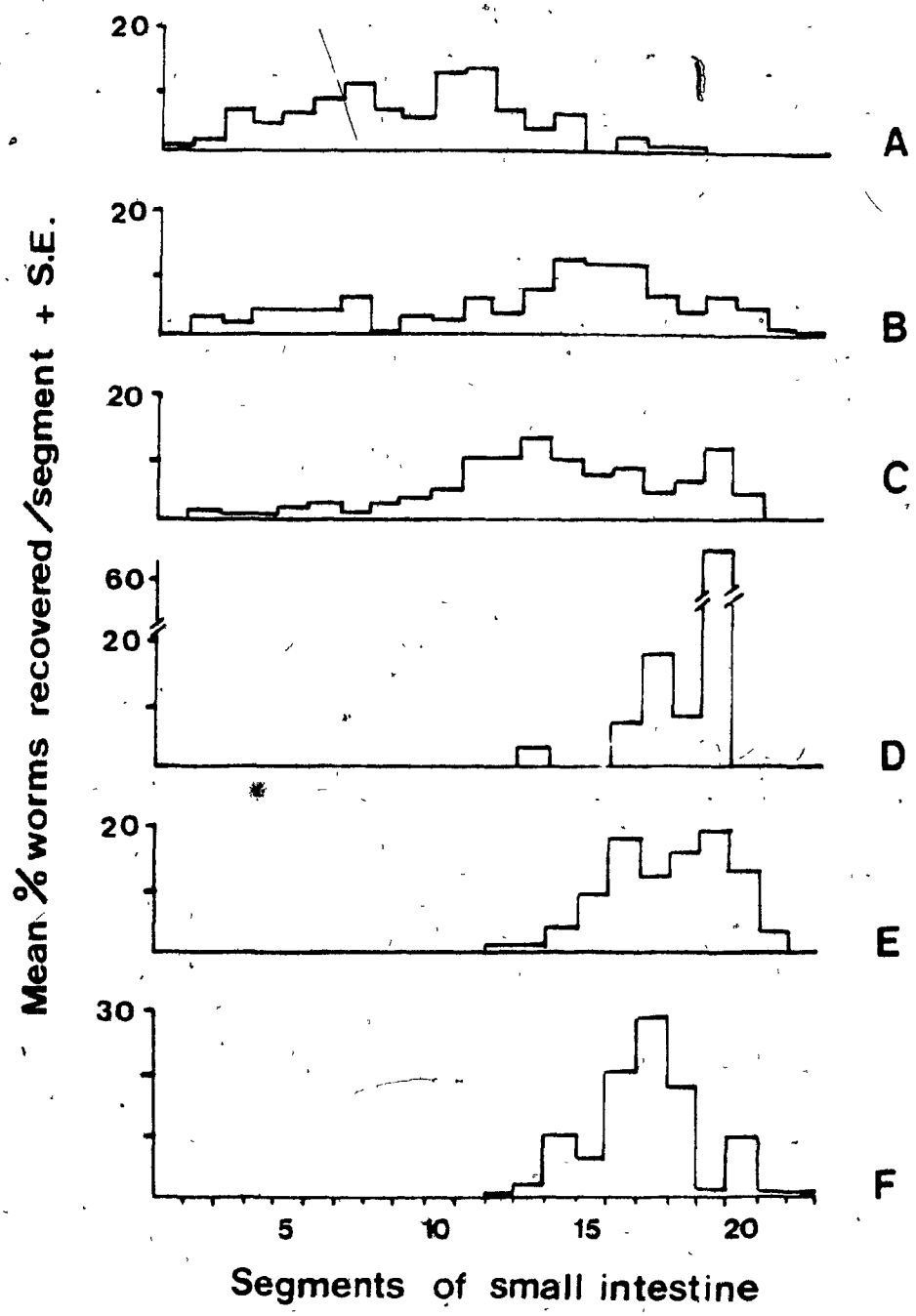
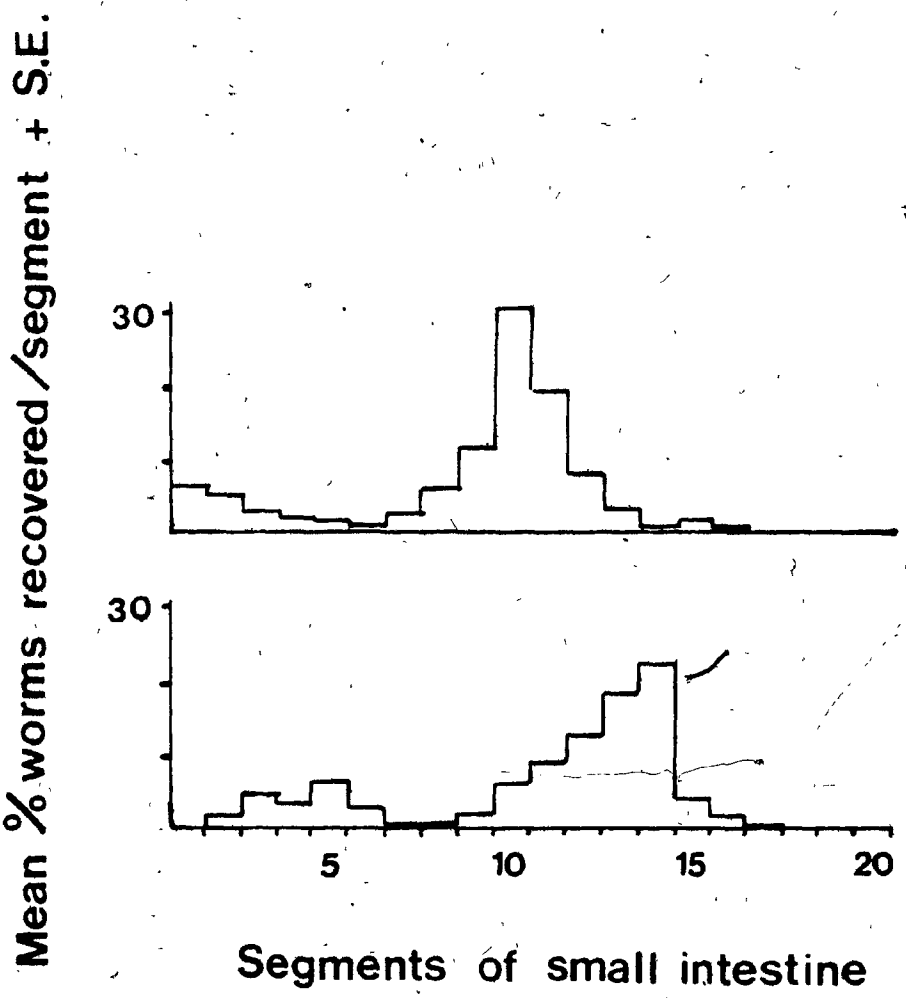

20
A
20
B
20
C
60
20
D
20
E
30
F
5
10
15
20
Mean % worms recovered/segment + S.E.
Segments of small intestine

FIGURE 5.14 The distribution of adults in the small intestine of rats 5 days after infection with 1000 larvae of T. spiralis after gastrectomy and bile duct ligature.


Mean % worms recovered/segment + S.E.
30
30
5
10
15
20
Segments of small intestine

treatments is given in Table 5.2.

7) Resistance of the infective stage.

The larvae of T. spiralis when delivered orally, could successfully establish and infect their hosts a second time at 1 and 6 hours after the initial excystment but not at 18 hours after infection. However, all larvae (1, 6 and 18 hours after infection) were equally infective when implanted into the duodenum (Table 5.3).

5D. DISCUSSION

For several years, the subject of the origin of cyst formation in T. spiralis has been discussed: was it of parasite or host origin? Recently, the role of the muscle fiber in cyst development has been demonstrated (Gould, 1970; Stewart & Read, 1972a; Teppema et al., 1973). After larval penetration, the fiber loses its contractile elements, there is nuclear enlargement, hypertrophy of the sarcoplasmic reticulum and mitochondrial vacuolation (Despommier, 1975). This occurs within 8 days of penetration and the modified myocyte is termed a "nurse cell". The muscle fiber dies but the nurse cell contributes to cyst development and persists for the life of the larva (Pukerson & Despommier, 1974; Despommier, 1976).

On the basis of the reaction of cysts with collagen stains, their sensitivity to dietary ascorbic acid during development, and their resistance to trypsin and collagenase digestion, Ritterson (1966) suggested that the principal structure of the outer cyst wall of T. spiralis was collagen. This

TABLE 5.2 RECOVERY OF ADULTS IN GASTRECTOMIZED RATS 5 DAYS AFTER INFECTION WITH 1000 LARVAE OF *T. SPIRALIS*

Treatment	Larvae	Mean # Adults Recovered ± S. E.	Mean % Recovery
Control	encysted	512.0 ± 62.3 (a)	51
Total gastrectomy	encysted	544.7 ± 47.9 (a)	54
Total gastrectomy & bile duct ligature	encysted	505.3 ± 43.3 (a)	50
Control	excysted	336.7 ± 44.1 (b)	33
Total gastrectomy	excysted	246.7 ± 88.3 (b)	24

(a) (b) all values with similar superscript do not differ significantly ($p \leq 0.05$)

TABLE 5.3 ADULT RECOVERIES OF T. SPIRALIS FROM RATS INFECTED WITH THE MUCOSAL SCRAPING OF INFECTED MICE GIVEN 5000 LARVAE PER OS

Time of larval recovery (hrs)	Route of Infection	Mean # Adults Recovered ± S. E.
1	Oral	1135.5 ± 266.0 (a)
6	Oral	1079.5 ± 87.3 (a)
18	Oral	21.4 ± 10.0 (b)
1	duodenum implant	1223.5 ± 281.5 (a)
6	duodenum implant	963.0 ± 197.4 (a)
18	duodenum implant	621.7 ± 104.1 (a)

(a) all values with the same superscript do not differ significantly from each other ($p \leq 0.05$)

was consistent with previous speculation on the nature of the cyst wall (Gould, 1945). The basic framework of the outer cyst wall is probably the sarcolemma of the muscle fiber with collagen and acid mucopolysaccharides (Bruce, 1970; Harley & Moore, 1974). The inner cyst wall or "matrix" which envelops the larva is believed to be made of collagen fibers interspersed with cytoplasm.

The maximum excystment of encysted larvae in my experiment was between 30 - 45 minutes in 1% pepsin solution at which 80% excysted within 15 minutes. This period is consistent with the time required for nematode activation in the stomachs of their hosts (Bailey, 1969; Fairbairn, 1961; Muller, 1971; Sommerville & Bailey, 1973). Investigations on the movements of ingesta in the mouse gut (Sukhdeo, unpublished) show that the mouse stomach can retain non-absorbable markers for 4 - 6 hours after ingestion with a mean transit time of 4.5 hours. This allows adequate time for excystment.

The pH optimum of pepsin is pH 1.5 to 2.0 (Spector, 1956) and the reduction of excystment coincided with an increase in pH. The mean pH of the contents of the mouse stomach was 4.83 (range, 3.26 - 6.24) (Haiba, 1954) and 5.1 ± 0.25 (Panesar & Croll, in press). Within this pH range, excystment in my systems was less than 10%. I suggest that these recordings of the pH of the mouse stomach are too alkaline. If they are not, then perhaps there are factors other than pepsin digestion which may be responsible for excystment. There is some evidence for this assertion.

The establishment of T. spiralis in gastrectomized rats remains unexplained unless pepsin was present in the small intestine since pepsin and collagenase were the only enzymes to excyst the larvae in vitro. The other intestinal enzymes tested had little effect on encystment at any pH and there were no synergistic effects between bile, trypsin and "succus entericus". In gastrectomized rats given encysted larvae, establishment was comparable to the controls and the distribution of the worms was similar in gastrectomized rats given excysted larvae. This suggested that the altered distribution in the gastrectomized rats given encysted larvae was due to a change in propulsion of the gut rather than being due to excystment in the small intestine. The exclusion of bile in the gastrectomized rats with the ligatured bile duct also had no effect on excystment. This supports the in vitro results in which there were no synergistic effects with bile and other enzymes.

CHAPTER 6. THE ESTABLISHMENT OF *TRICHINELLA SPIRALIS* AND *NEMATOSPIROIDES DUBIUS* IN THE SMALL INTESTINE: THE EFFECTS OF SURGICAL ALTERATION OF INTESTINAL SECRETIONS

6A. INTRODUCTION

Thorson (1969) has summarized some of the stimuli in the host gut that a gastrointestinal parasite encounters: hormonal, cellular, humoral, nutritional, chemical, physiological, thermal, directional cues, interactions with other species and interactions with members of its own species. It is through exploitation of these stimuli that nematode larvae may have evolved mechanisms to establish themselves through their own behavioural efforts.

Bile or its constituents have been implicated in the site selection behaviour of several helminths through their influence on activation. These include trematodes (Dawes & Hughes, 1964; Erasmus & Bennet, 1965; Howell, 1970); acanthocephalans (Lackie, 1974); cestodes (Smyth, 1969; Read, 1970; Caley, 1974) and nematodes (Rogers, 1960; Hwang, 1960; Mapes, 1972; Lackie, 1975). Similarly, the importance of pancreatic secretions have been demonstrated in trematodes (Hoffman, 1958; Kobayashi *et al.*, 1959; Dawes & Hughes, 1964; Yokagawa, 1965; Fried & Roth, 1974); cestodes (Penfold, *et al.*, 1937; Wantland, 1953; Jones *et al.*, 1960; Hoffman & Jones, 1962; Berntzen & Mueller, 1963; Berntzen & Voge, 1965; de Rycke & van Grembergen, 1965; Laws, 1968; Read, 1970; Goodchild & Davis, 1972); acanthocephalans (Lackie, 1974) and nematodes

(Hwang, 1960; Silverman & Podger, 1964; Chapman & Undeen, 1968). These have been mostly in vitro studies and there have been no experimental investigations on parasite microhabitat selection to examine the effects of bile or pancreatic secretions in vivo. This study attempted to determine the importance of bile and pancreatic secretions on T. spiralis and N. dubius in vivo.

6B. MATERIALS AND METHODS

The general methods for anaesthesia, laparotomy and post-surgical care have been given (Chapter 2). Rats and mice were used in this study.

1) Surgery on mice: The effects on the establishment and longitudinal dispersion of T. spiralis and N. dubius.

a) The effects of ligaturing the bile duct.

Two groups of 5 mice were used and all mice were anaesthetized and laparotomized. The mice in the control group were closed without further surgery. The upper bile duct, just below the level where the gall bladder entered the duct, was ligatured and divided in the mice of the second group. The mice were infected 2 days after surgery.

b) The effects of cholestyramine treatment.

Two groups of 5 mice were used. The experimental group was given 50 mg cholestryamine (Questran®, Mead Johnson Company, Montreal) in 0.1 ml distilled water 30 minutes prior to infection to reduce the concentration of conjugated bile acids by an undetermined amount (Hagerman et al., 1971). The

controls were given 0.1 ml distilled water at the same time.

Experiments (a) and (b) were duplicated and the mice were infected with: (a) 500 excysted larvae of T. spiralis or (b) 100 larvae of N. dubius, by intragastric inoculation. Five days after infection with T. spiralis and 6 days after infection with N. dubius the mice were killed and the distribution of worms in the small intestine was determined. The influence of the exclusion of bile on the establishment and longitudinal dispersion of T. spiralis and N. dubius were examined.

2) Surgery on rats: The effects of manipulation of bile and pancreatic secretions on the establishment and longitudinal dispersion of T. spiralis and N. dubius.

Rats were chosen for this part of the study because they were larger than mice and it was easier to handle them in surgery. Ten groups of rats were altered surgically. The numbers of rats in each group varied because of the high post-operative mortality in some groups but an attempt was made to have at least 3 rats per group. The ten groups are as follows:

a) Sham-operated controls.

These animals were laparotomized and the abdomen was then closed.

b) Sham-cannula controls.

A cannula made of polyethylene tubing, I.D. 0.58 mm and O.D. 0.965 mm (Intramedic® PE50, Clay Adams, Becton, Dickinson and Company, Parsippany) with a blind end was inserted at different locations in the small intestine. The end of the cannula to be inserted into the intestine was flared by heating

then inserted into and attached to the small intestine with a purse-string suture.

c) Bile duct ligature.

The bile duct was ligatured and divided above the entry of the pancreatic secretions into the common bile duct. This procedure allowed the continued flow of pancreatic secretions (Figure 6.1).

d) Bile duct cannulation.

The bile duct was ligatured and divided above the entry of the pancreatic secretions into the common bile duct. A catheter of polyethylene tubing (see group (b)) was inserted into the upper bile duct and the bile flow was cannulated to different locations in the small intestine. The end of the cannula to be inserted into the intestine was flared by heating in a flame and inserted and attached (Figure 6.2).

e) Bile flow externalized.

The upper bile duct was cannulated as described above (see group (d)). The cannula was passed through the abdomen, under the skin and passed out at the nape of the rat's neck. The cannula was held firmly in place with dental caulk (Nu Weld®, The L.D. Caulk Company, Delaware), (Figure 6.3).

f) Duodenal bypass.

The small intestine was divided above the entry of the common bile duct. The distal section above the bile duct was closed with invaginating stitches to form a self-emptying blind loop. The proximal section below the pyloric sphincter was joined to the small intestine at different locations using the method of end-to-side anastomosis with 2 guiding threads

FIGURE 6.1 Bile duct ligature in the rat, the arrow points to the distended bile duct, 7 days after surgery.

FIGURE 6.2 Bile duct cannulation in the rat. The arrows point to the points of catheter entrance into the bile duct and the duodenum.

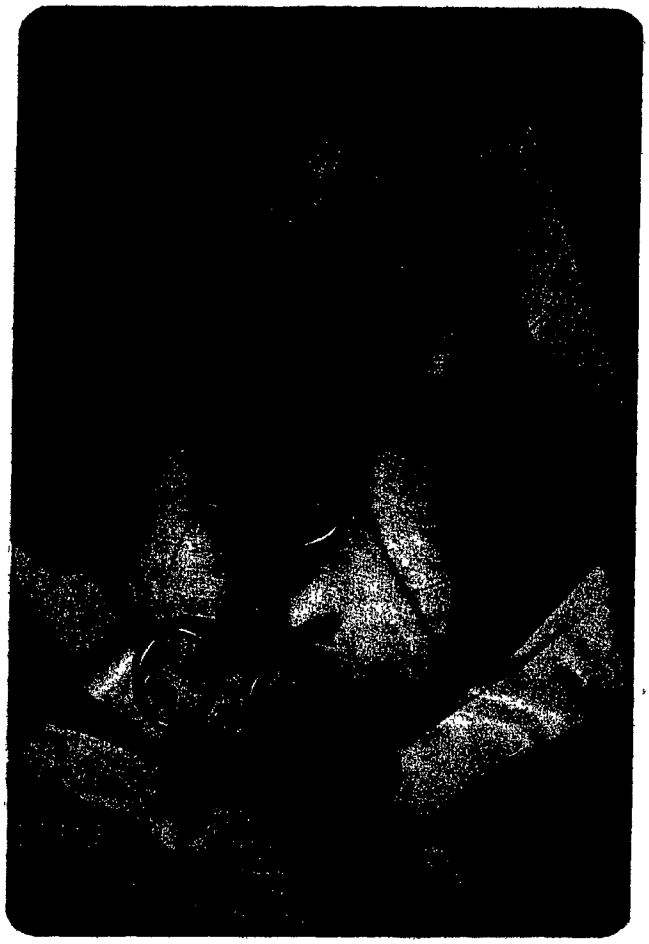


FIGURE 6.3 Bile flow externalized in the rat. The exit of the bile duct cannula on the nape of the neck and the method of securing the cannula is shown.

(Lambert, 1965). Figure 6.4 is a photograph of the gut after recovery from this treatment.

g) Bile and pancreatic duct ligature.

The upper bile duct was ligatured and divided as described above (see group (c)). The common bile duct was ligatured and divided just before entry into the duodenum. The use of both ligatures was necessary to prevent the flow of bile into the pancreas as the pressure due to biliary stasis increased (Figure 6.5).

h) Pancreatic ligature.

The animals were treated in a similar manner as described in the previous group (see group (g)). The upper bile duct was cannulated (see group (d)) and directed to the normal site of bile entry into the duodenum.

i) Bile duct ligature with cholestyramine treatment.

The control rats were laparotomized and closed. The experimental animals were laparotomized and the bile duct ligatured and divided (see group (c)). Thirty minutes prior to infection the rats were given 500 mg cholestyramine per os.

j) Bile duct cannulation with cholestyramine treatment.

The bile duct was ligatured and cannulated to different locations of the gut (see group (d)). Thirty minutes prior to infection the rats were given 500 mg cholestryamine per os.

All animals were infected 3 days after surgery. The groups were duplicated, in one trial each animal was infected with 1000 excysted larvae of T. spiralis and in the

FIGURE 6.4 Duodenal bypass in the rat. The gut was removed from the rat 8 days after surgery. S, stomach; bd, site of entry of bile duct; c, caecum; arrow points to the anastomosis.

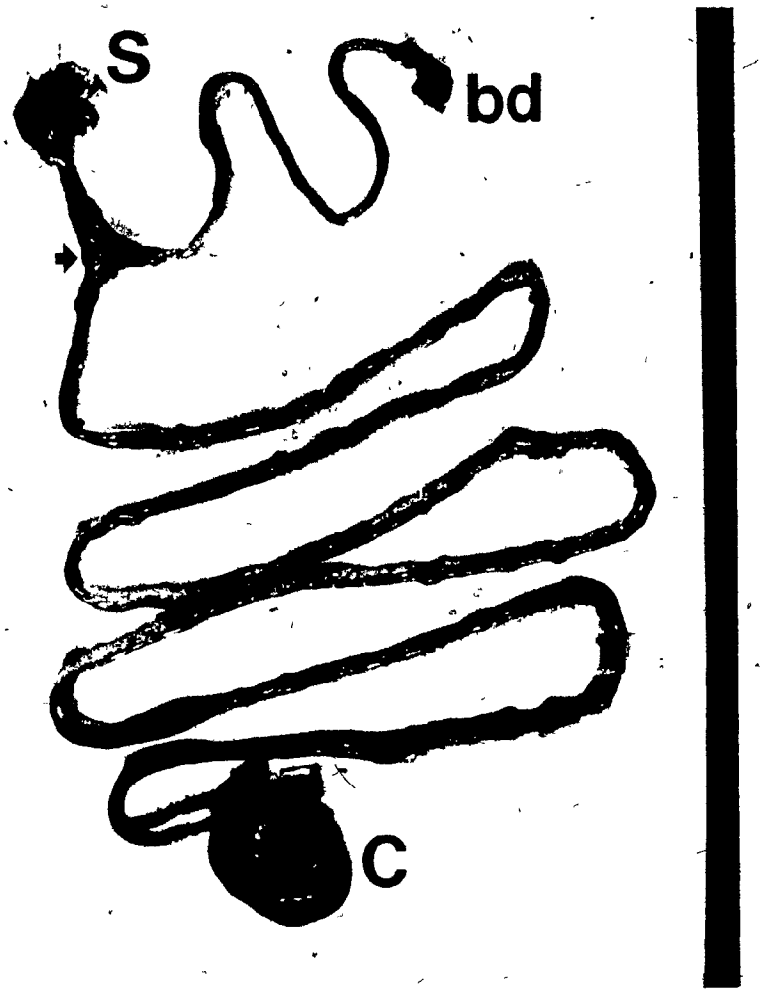

S
bd
C

FIGURE 6.5 Pancreatic duct ligature in the rat. The arrows point to the two points of ligature prior to division of the duct between the ligatures.

second trial (except for groups (h), (i) and (j)) each animal was infected with 300 larvae of N. dubius. The infective doses in both trials were delivered by intragastric intubation. The rats were killed 5 days after infection with T. spiralis and 6 days after infection with N. dubius and the distribution of worms in the small intestine was determined. The influence of altered bile and pancreatic secretions on the establishment and longitudinal dispersions of T. spiralis and N. dubius were examined.

6C. RESULTS

1) Mice: The effects of excluding bile.

a) T. spiralis

Neither ligaturing the bile duct nor treatment with cholestyramine had any significant effect on the longitudinal distribution (Figures 6.6 and 6.7) or the establishment (Table 6.1) of T. spiralis.

b) N. dubius

The distribution of larvae in the small intestines of mice following ligature of the bile duct differed slightly from the controls. In the control mice, all of the larvae established within the first half of the small intestine whereas in animals with the bile duct ligatured the larvae were recovered up to 75% of the way down the small intestine (Figure 6.8). In both groups, the peak recoveries were in the first segments. Significantly, fewer larvae ($p \leq 0.05$) were recovered from the mice in which the bile duct had been ligatured than in control mice (Table 6.2). The results were

FIGURE 6.6 The distribution of adults in the small intestine of mice 5 days after infection with 500 larvae of T. spiralis. A, controls; B, mice with the bile duct ligatured.

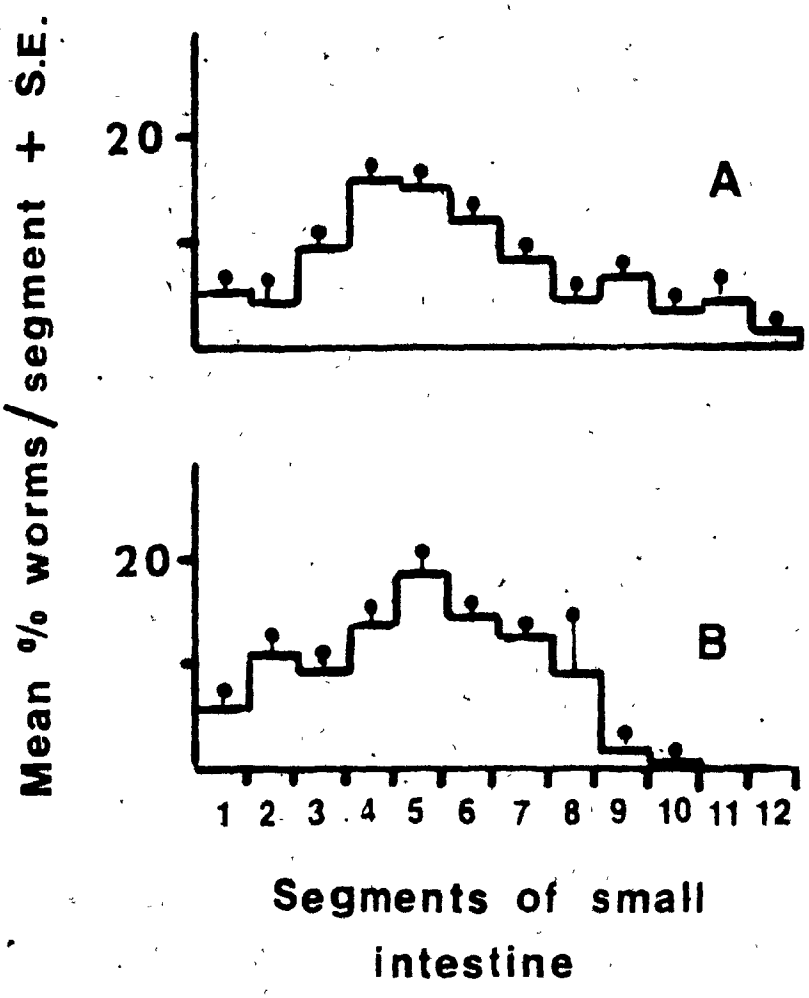

Mean % worms / segment + S.E.
20
A
20
B
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

FIGURE 6.7 The distribution of adults in the small intestine of mice 5 days after infection with 500 larvae of T. sprialis. A, controls; B, the mice were treated with cholestyramine prior to infection.

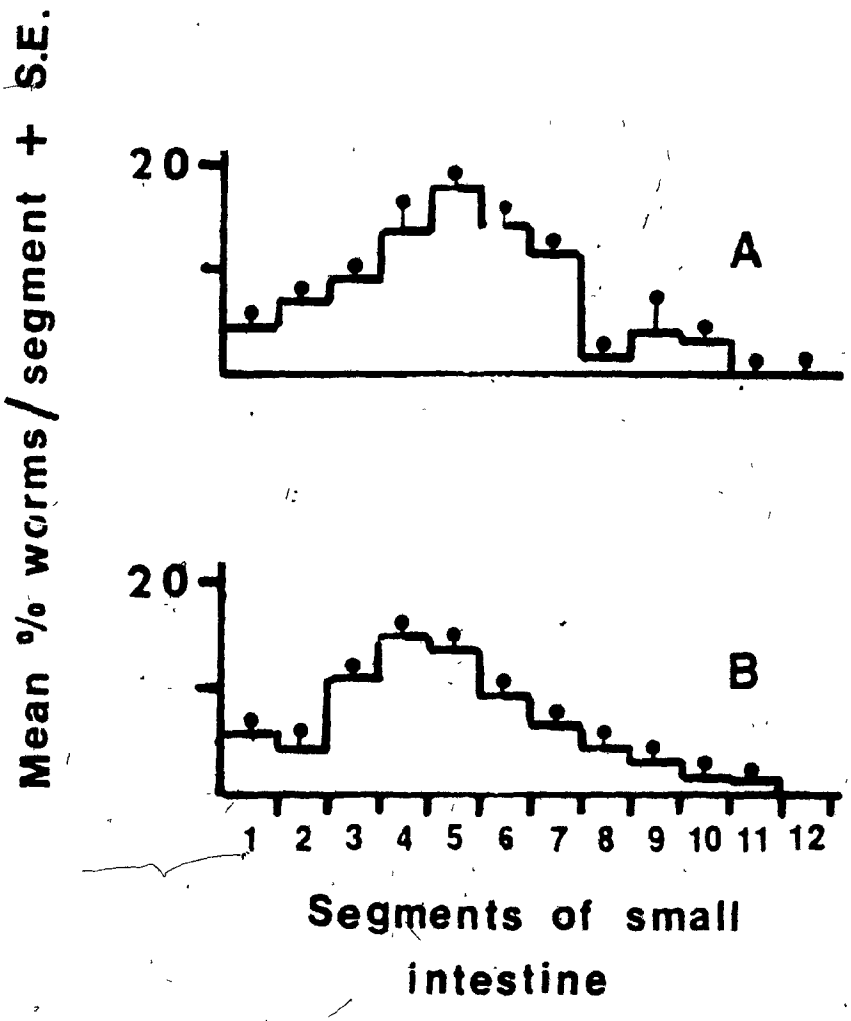

Mean % worms / segment + S.E.
20
A
20
B
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

TABLE 6.1 THE MEAN PERCENTAGE RECOVERY OF *T. SPIRALIS* IN THE SMALL INTESTINE OF MICE 5 DAYS AFTER INFECTION WITH 500 LARVAE EACH

Group	Treatment	Mean % Recovery ± S. E.	$p \leq$
1A	bile duct ligature	27.4 ± 6.1	N. S.
1B	sham controls	41.3 ± 4.8	
2A	cholestyramine treated	34.2 ± 4.3	N. S.
2B	controls	35.8 ± 3.1	

FIGURE 6.8 The distribution of worms in the small intestine of mice 6 days after infection with 100 larvae of N. dubius. A, mice with the bile duct ligatured; B, controls.

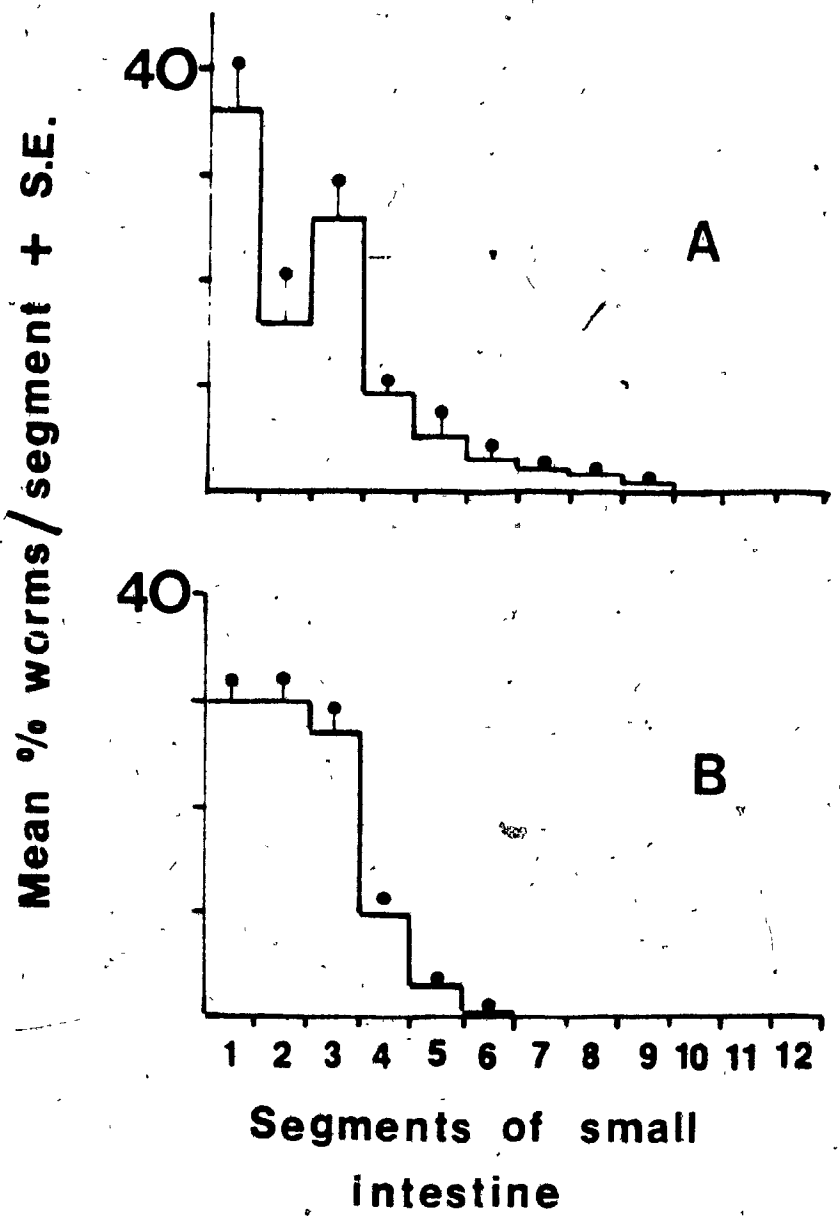

40
A
40
B
Mean % worms / segment + S.E.
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

TABLE 6.2 THE MEAN PERCENTAGE RECOVERY OF LARVAE OF *N. DUBIUS* FROM THE SMALL INTESTINE OF MICE 6 DAYS AFTER INFECTION WITH 100 LARVAE EACH

Group	Treatment	Mean % Recovery ± S. E.	$p \leq$
1A	bile duct ligatured	64.5 ± 5.5	0.05
1B	sham control	93.6 ± 3.9	
2A	cholestyramine treated	71.4 ± 7.8	N. S.
2B	controls	90.6 ± 7.0	

similar in the animals treated with cholestyramine, the larvae were slightly more longitudinally dispersed in the gut than in the controls (Figure 6.9) and there was a reduction in the recovery of larvae (Table 6.2).

2) Rats: The effects of manipulating bile and pancreatic secretions.

a) T. spiralis

The distribution of T. spiralis in rats (Figure 6.10) differed from their distribution in mice (Chapter 3; Figures 6.6 and 6.7). In rats the majority of adults were recovered from the first quarter of the small intestine while in mice the majority of adults were recovered from the second quarter. In rats the distribution also tended to be more longitudinal and did not peak consistently in any one segment. The presence of blind cannulas did not have a significant effect on this distribution (Figure 6.11). The mean total recoveries of these and other groups infected with T. spiralis are given in Table 6.3.

Ligature of the bile ducts of rats resulted in a dramatic change in distribution. A significant number of worms, 40% - 60% of the total recovery, were found in the very first segment with a rapid diminution of numbers establishing more posteriad (Figure 6.12).

Cannulation of the bile duct and rerouting the bile flow also changed the distribution of worms in the small intestine. When the bile duct was routed back to the normal site of bile entry, the peak recovery of adults occurred in segment 1 and the distribution was similar to the distribution

FIGURE 6.9 The distribution of worms in the small intestine of mice 6 days after infection with 100 larvae of N. dubius. A, mice treated with cholestyramine prior to infection; B, controls.

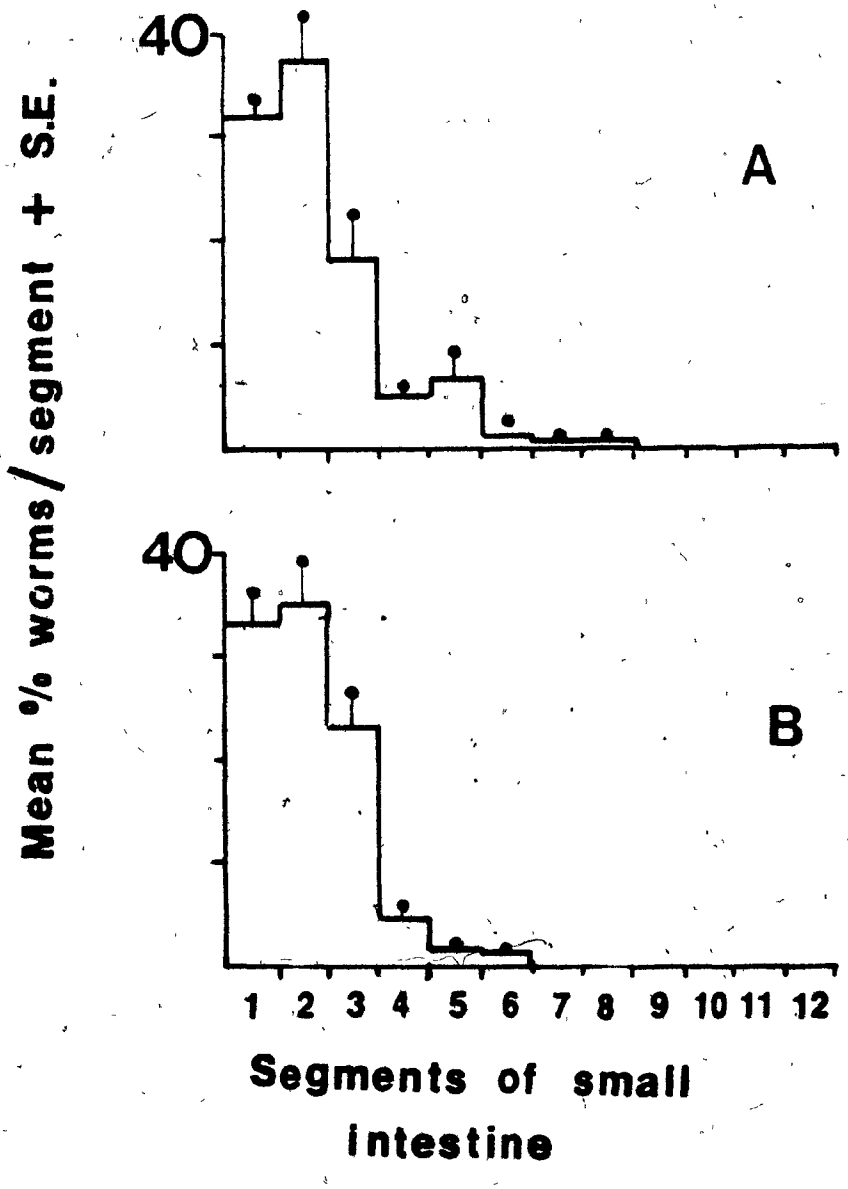

40
A
40
B
Mean % worms / segment + S.E.
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

FIGURE 6.10 The distribution of adults in the small intestine of control rats with sham operations, 5 days after infection with 1000 larvae of T. spiralis.

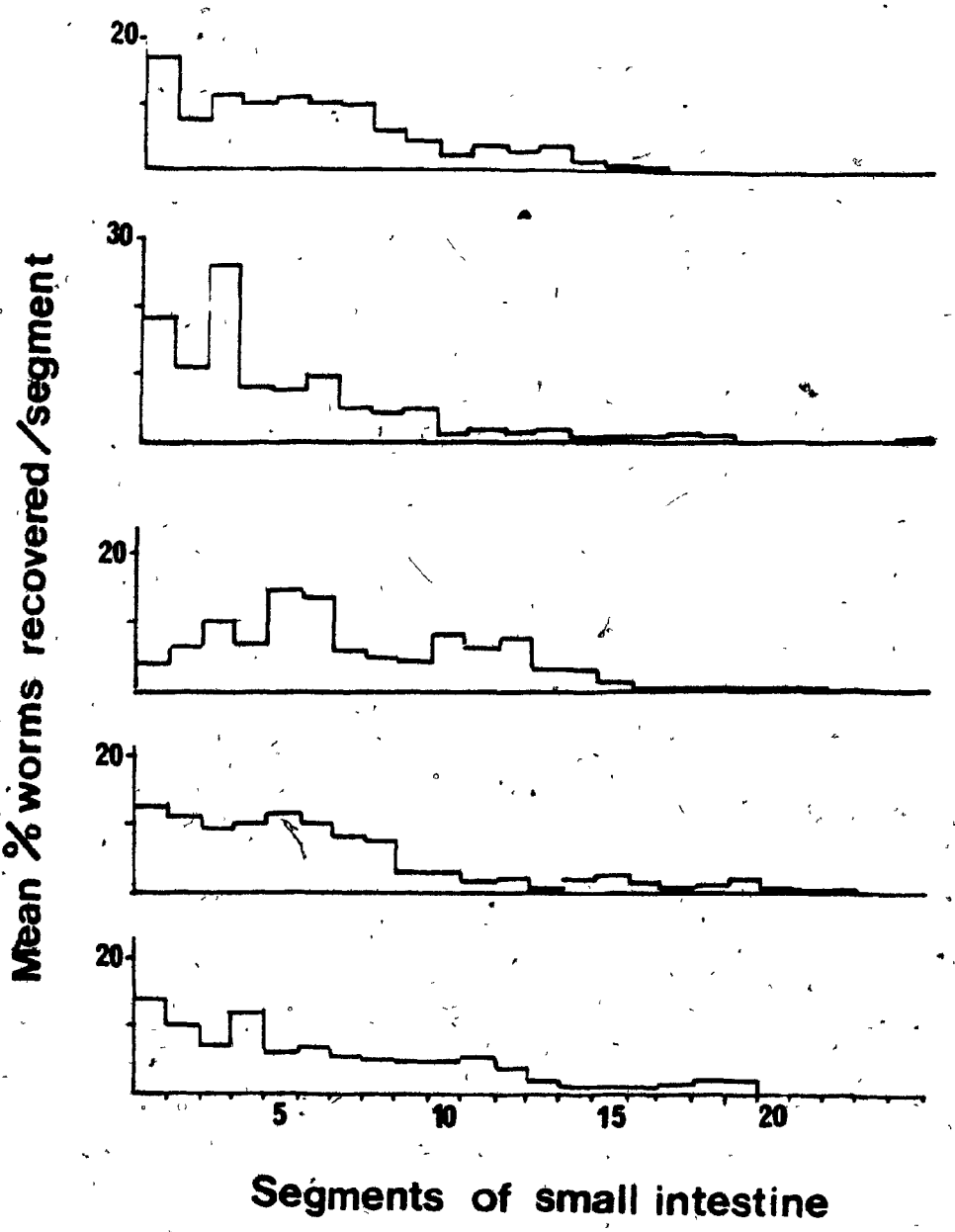

20
30
20
20
20
Mean % worms recovered /segment
5
10
15
20
Segments of small intestine

FIGURE 6.11 The distribution of adults in the small intestine of control rats with blind cannulas, 5 days after infection with 1000 larvae of T. spiralis. The arrowheads point to the site of entry of the blind cannulas.

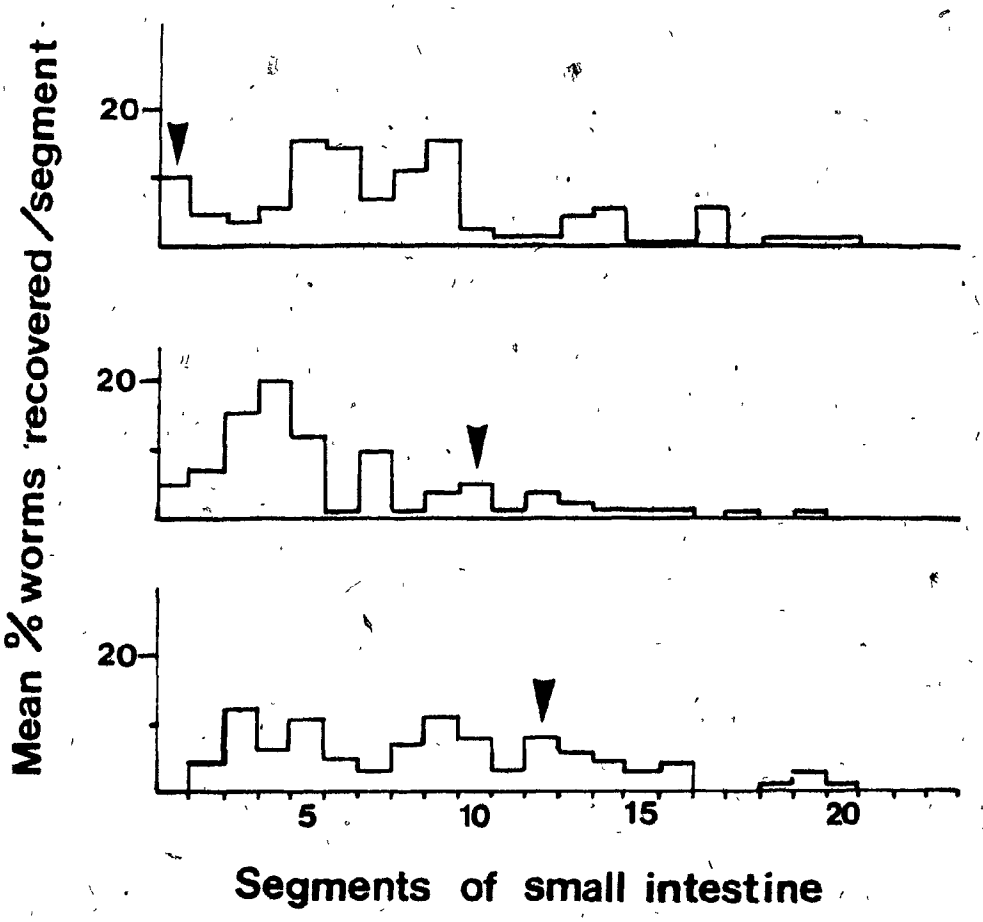
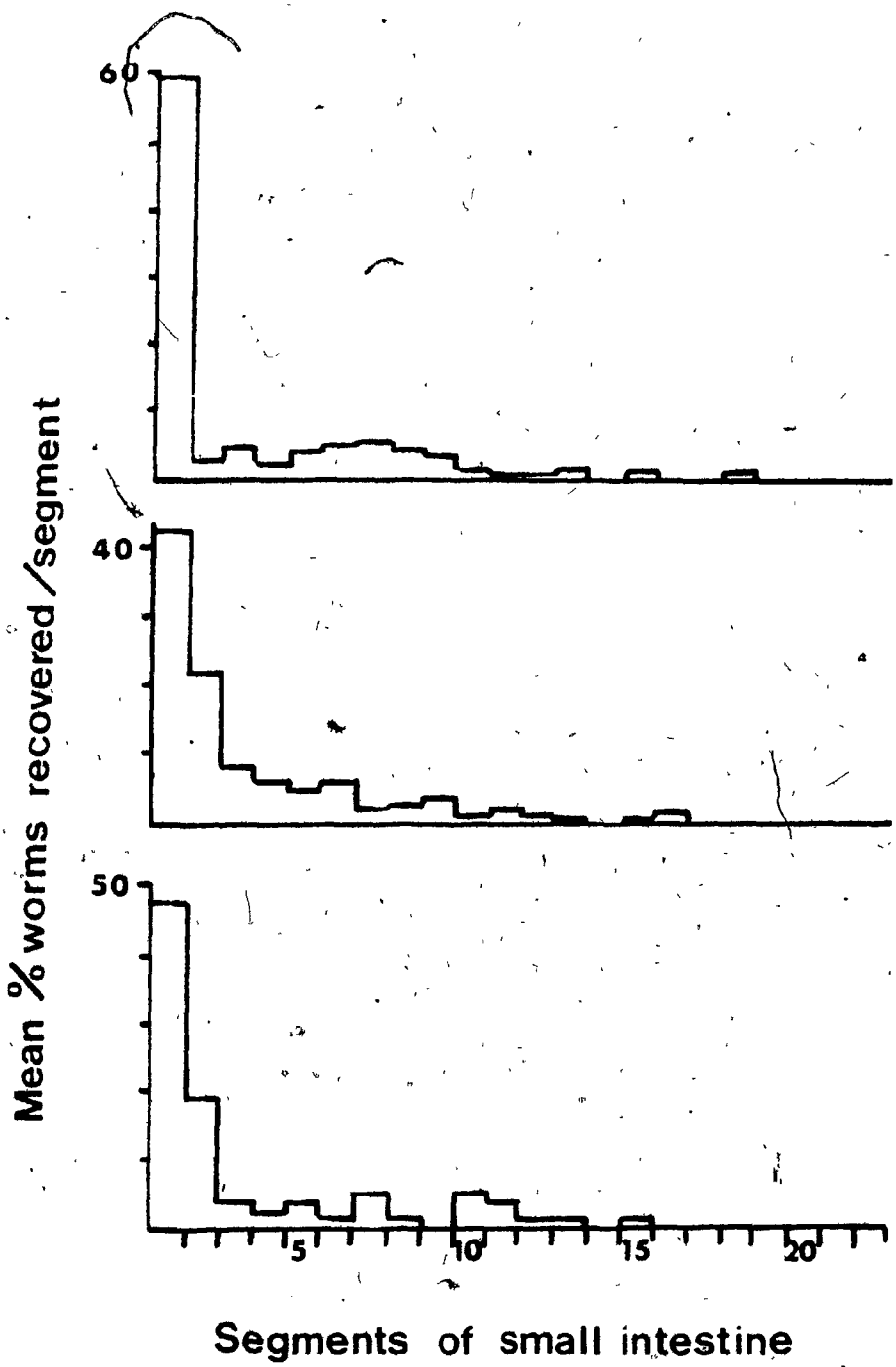

Mean % worms recovered /segment
20
20
20
5
10
15
20
Segments of small intestine

TABLE 6.3 THE MEAN TOTAL RECOVERY OF WORMS FROM THE SMALL INTESTINE OF SURGICALLY ALTERED RATS 5 DAYS AFTER INFECTION WITH 1000 LARVAE OF *T. SPIRALIS*

Treatment	Mean Recovery ± S. E.	$p \leq$ (difference from controls)
Sham Control	412.5 ± 14.5	
Sham Cannula	381.3 ± 28.7	N. S.
Bile Ligature	343.0 ± 29.0	N. S.
Bile Cannula	349.3 ± 46.4	N. S.
Bile Externalized	319.0 ± 21.4	0.05
Duodenal Bypass	366.6 ± 32.1	N. S.
Pancreatic & Bile + Ligature	361.7 ± 63.1	N. S.
Pancreatic Ligature	372.5 ± 34.1	N. S.
Cholestyramine	469.6 ± 108	N. S.
Bile Ligature + Cholestyramine	445.5 ± 5.5	N. S.
Bile Cannulation + Cholestyramine	563.0 ± 4.6	0.05

FIGURE 6.12 The distribution of adults in the small intestine of rats with ligature of the bile duct, 5 days after infection with 1000 larvae of *T. spiralis*.


60
40
50
Mean % worms recovered /segment
5
10
15
20
Segments of small intestine

in animals with ligature of the bile duct (Figure 6.13). When the cannula entered the small intestine above the midpoint, there was an increase in establishment at the site of cannula entry (Figures 6.13 and 6.14) but in animals where the cannula entered the intestine below the midpoint of the small intestine, there was no increase in establishment at the site of cannula entry (Figure 6.15). There was one exception in each case.

When the bile flow was externalized the distribution of worms in the small intestine was similar to the distribution in rats with ligature of the bile duct (Figure 6.16).

T. spiralis did not establish in the bypassed sections in animals in which the duodenum was bypassed. The establishment of larvae began at the site of anastomosis and the distributions from this point were similar to controls (Figure 6.17).

Bile and pancreatic duct ligature (Figure 6.18); pancreatic duct ligature (Figure 6.19); bile ligature with cholestyramine treatment (Figure 6.20); cholestyramine treatment (Figure 6.21) and bile cannulation with cholestyramine treatment (Figure 6.22) all resulted in similar distributions of worms. The majority of the worms established in the first or second segments.

The mean recoveries from the above treatments are shown in Table 6.3. There were no significant differences between the controls and animals in which the bile was not excluded. In rats with the bile flow externalized, there was

FIGURE 6.13 The distribution of adults in the small intestine of rats with the bile duct cannulated to different locations in the small intestine, 5 days after infection with 1000 larvae of T. spiralis. The asterisk shows the site of cannula entry.

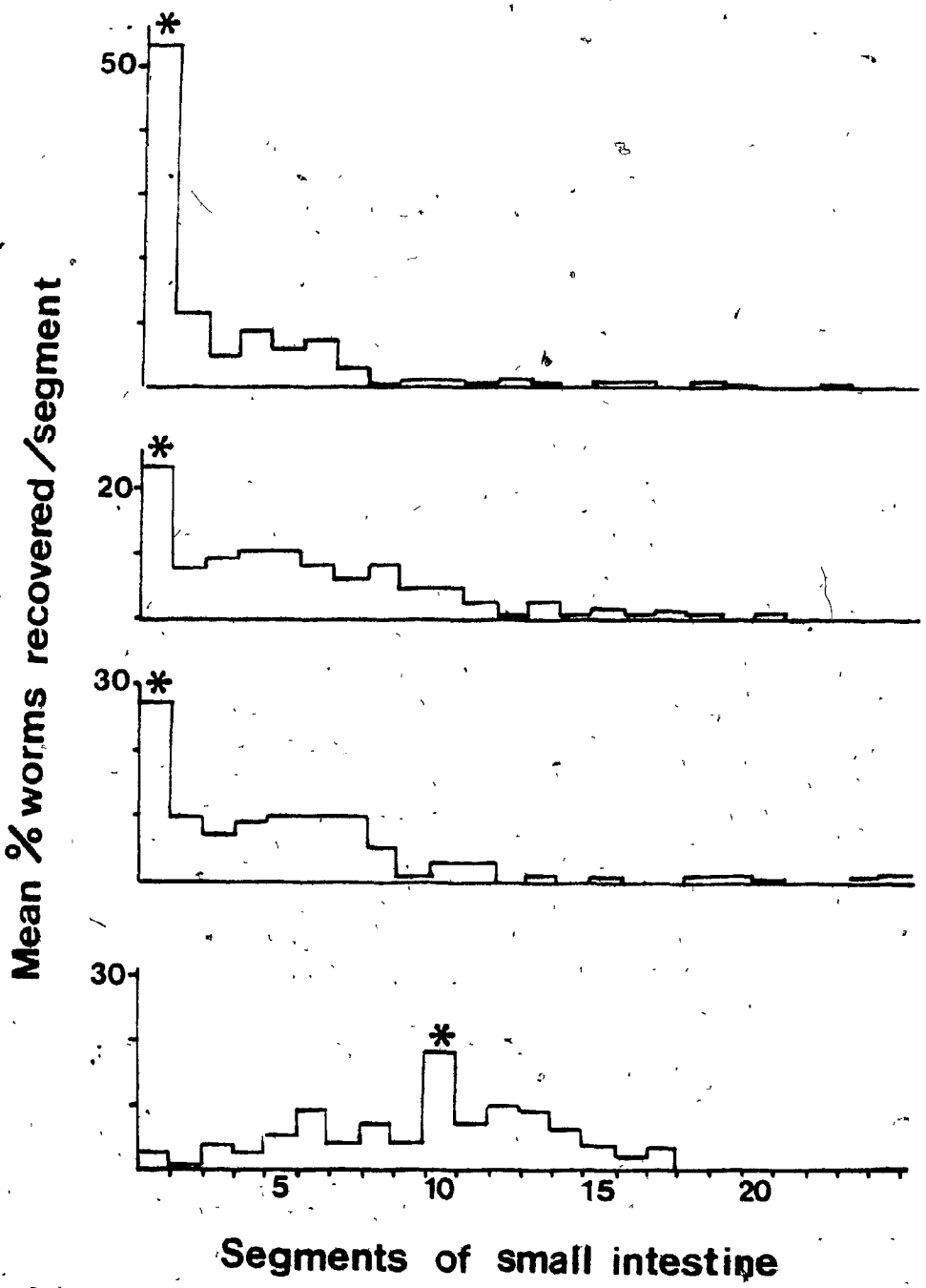

Mean % worms recovered/segment
50
20
30
30
*
5
10
15
20
Segments of small intestine

FIGURE 6.14 The distribution of adults in the small intestine of rats with the bile duct cannulated to different locations in the small intestine, 5 days after infection with 1000 larvae of T. spiralis. The asterisk shows the site of cannula entry.

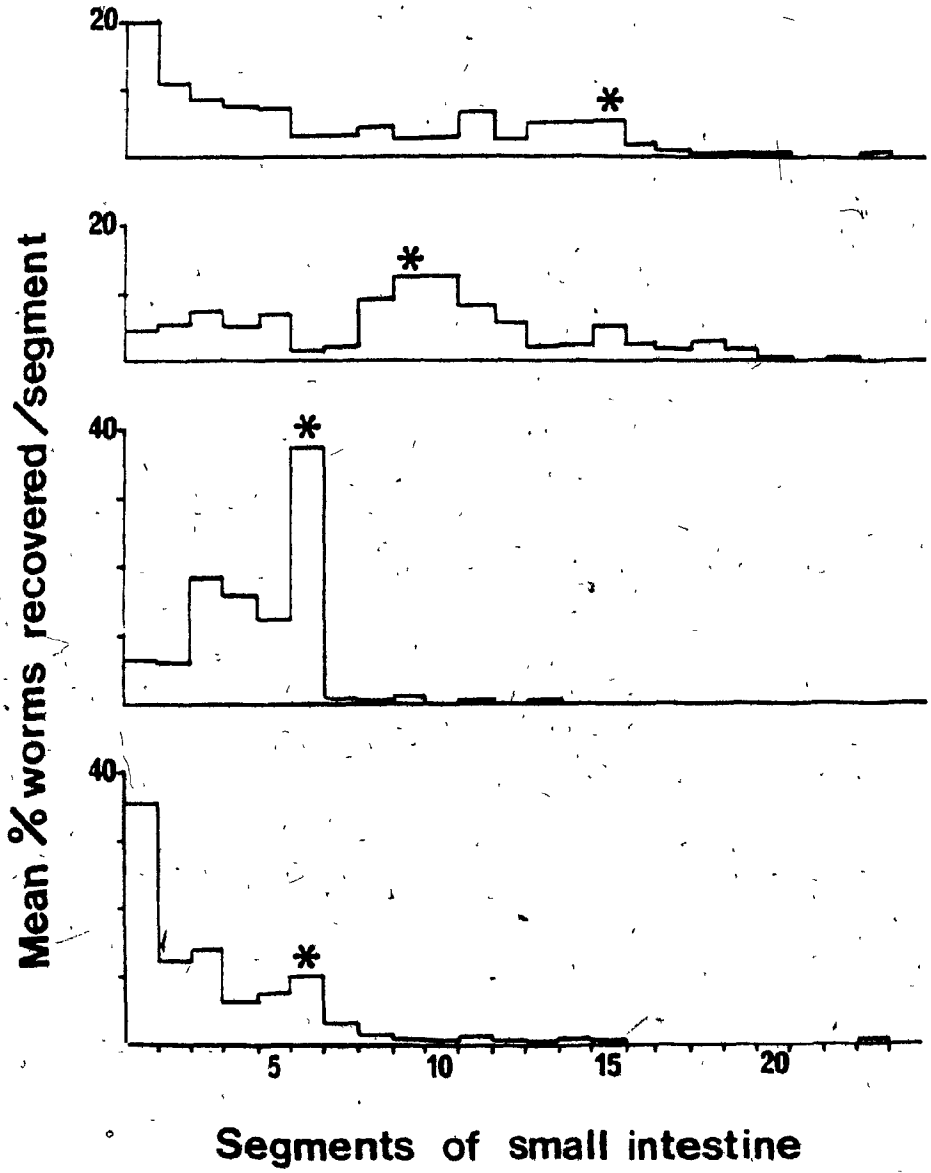

20
20
40
40
*
*
*
*
Mean % worms recovered / segment
5
10
15
20
Segments of small intestine

FIGURE 6.15 The distribution of adults in the small intestine of rats with the bile duct cannulated to different locations in the small intestine, 5 days after infection with 1000 larvae of T. spiralis. The asterisk shows the site of cannula entry.

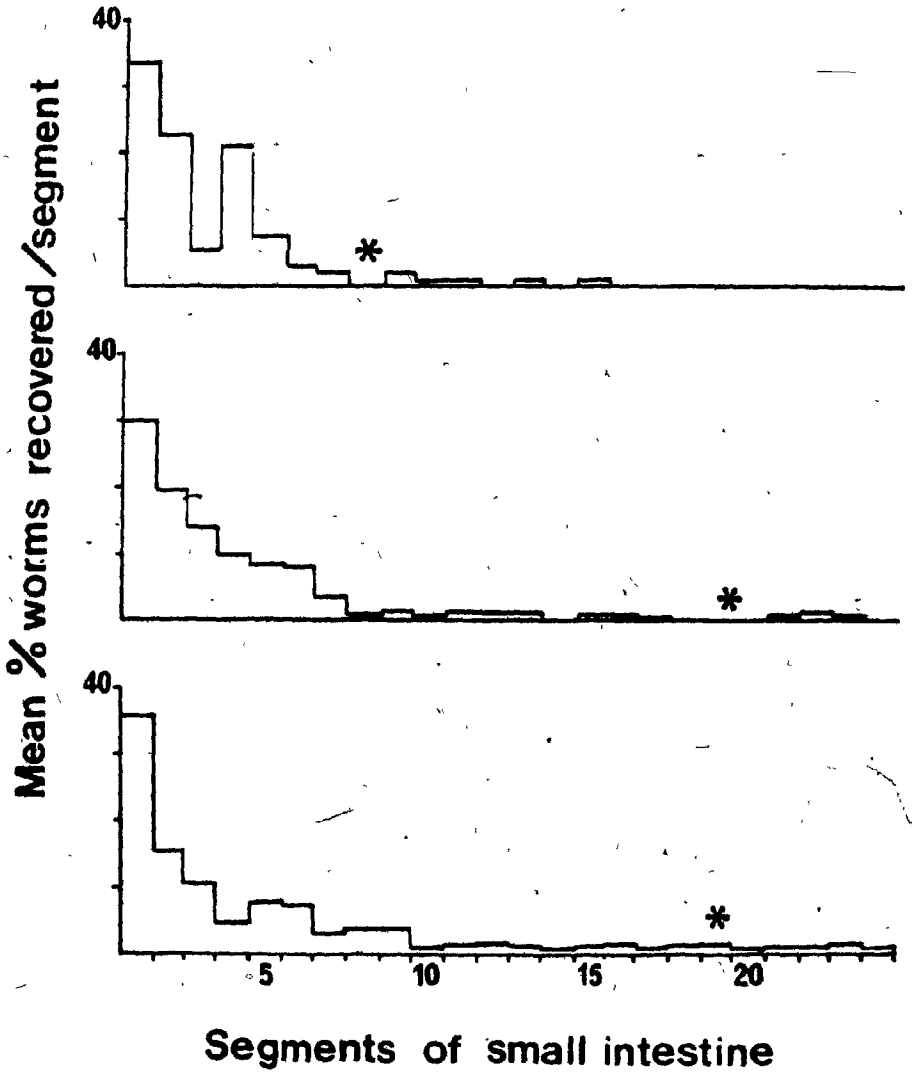

40
40
40
*
*
*
5
10
15
20
Mean % worms recovered/segment
Segments of small intestine

FIGURE 6.16 The distribution of adults in the small intestine of rats with the bile flow externalized, 5 days after infection with 1000 larvae of *T. spiralis*.

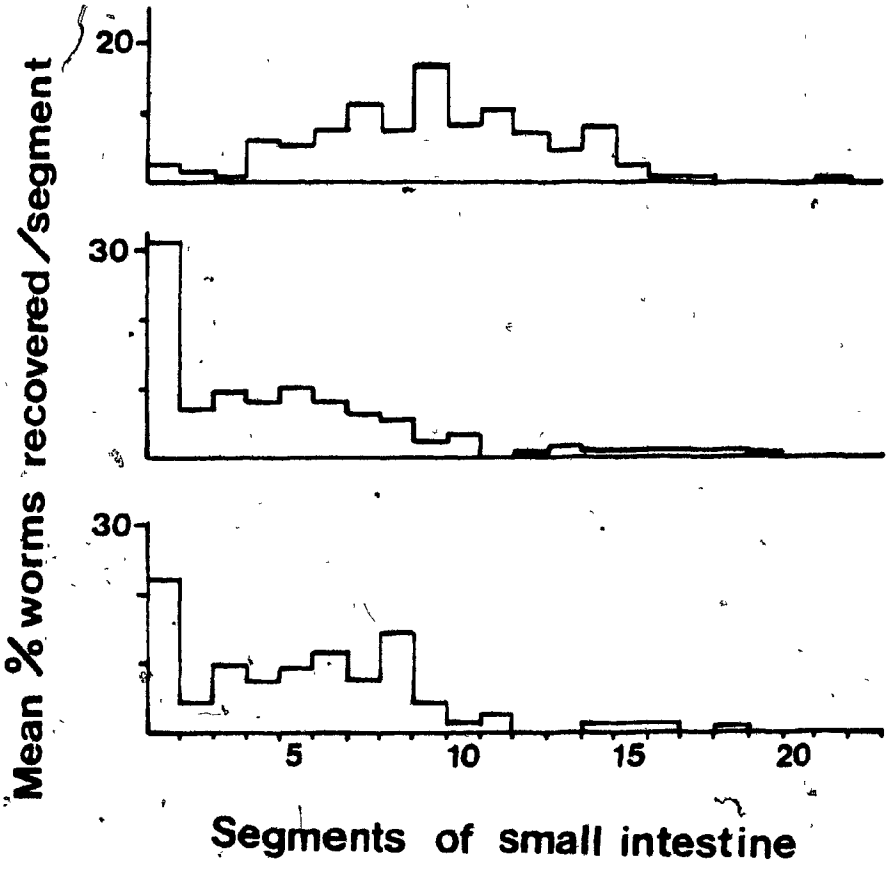

Mean % worms recovered /segment
20
30
30
5
10
15
20
Segments of small intestine

FIGURE 6.17 The distribution of adults in the small intestine of rats with duodenal bypass operations, 5 days after infection with 1000 larvae of T. spiralis. The dotted line is the length of the bypassed segments.

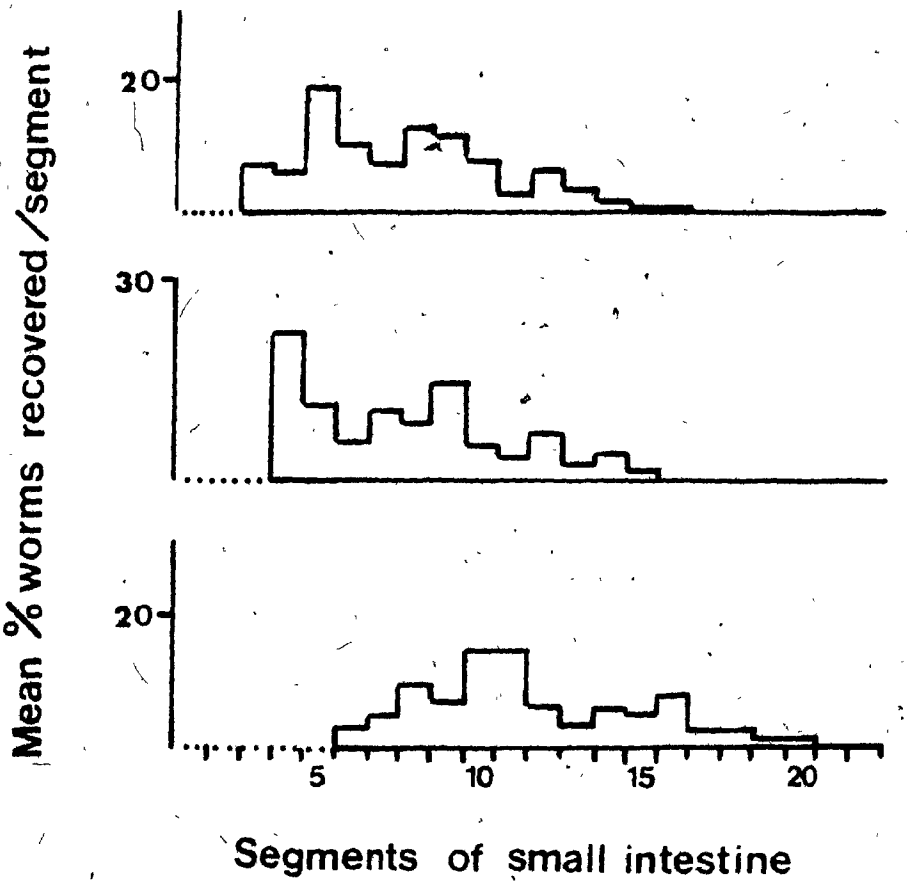

Mean % worms recovered /segment
20
30
20
5
10
15
20
Segments of small intestine

FIGURE 6.18 The distribution of adults in the small intestine of rats with ligature of the bile and pancreatic ducts, 5 days after infection with 1000 larvae of T. spiralis.

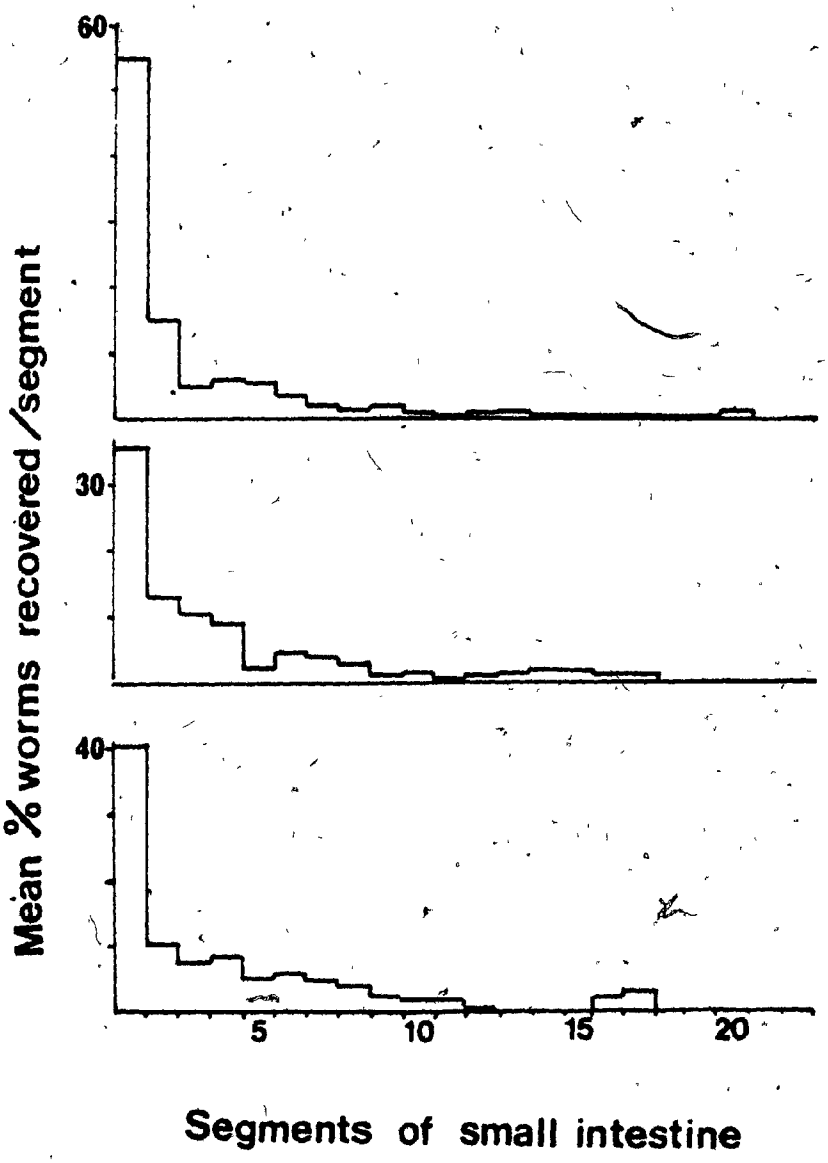
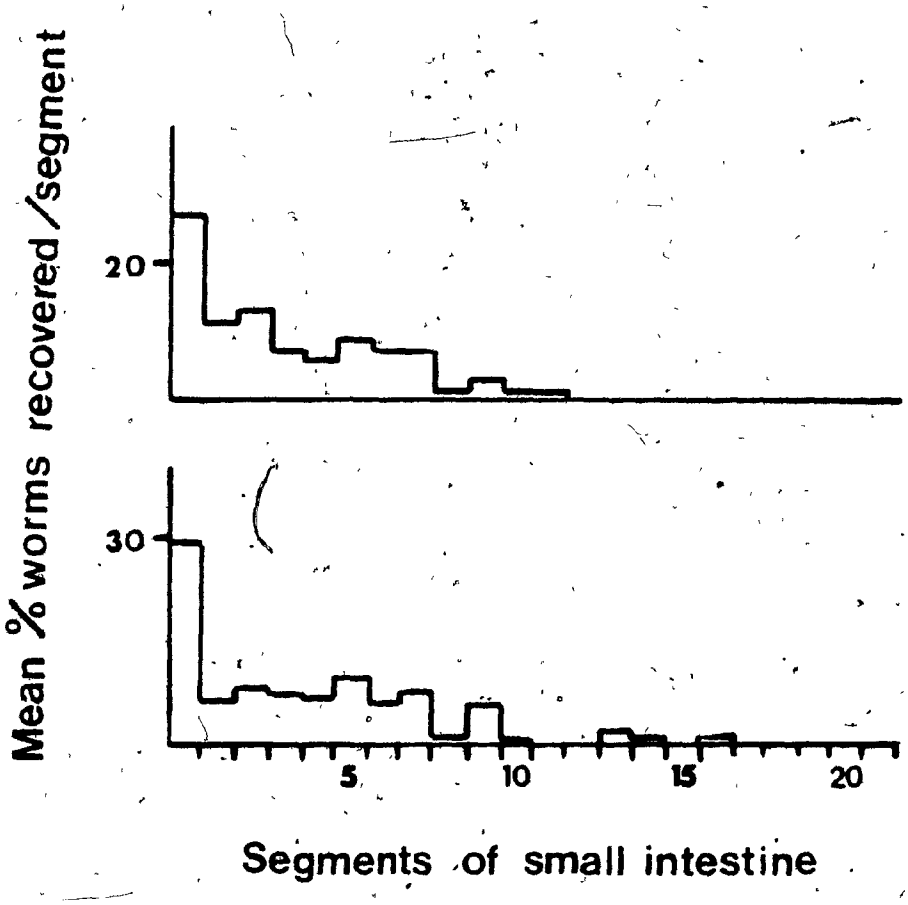

60
30
40
Mean % worms recovered /segment
5
10
15
20
Segments of small intestine

FIGURE 6.19 The distribution of adults in the small intestine of rats with ligature of the pancreatic duct, 5 days after infection with 1000 larvae of *T. spiralis*.


Mean % worms recovered /segment
20
30
5
10
15
20
Segments of small intestine

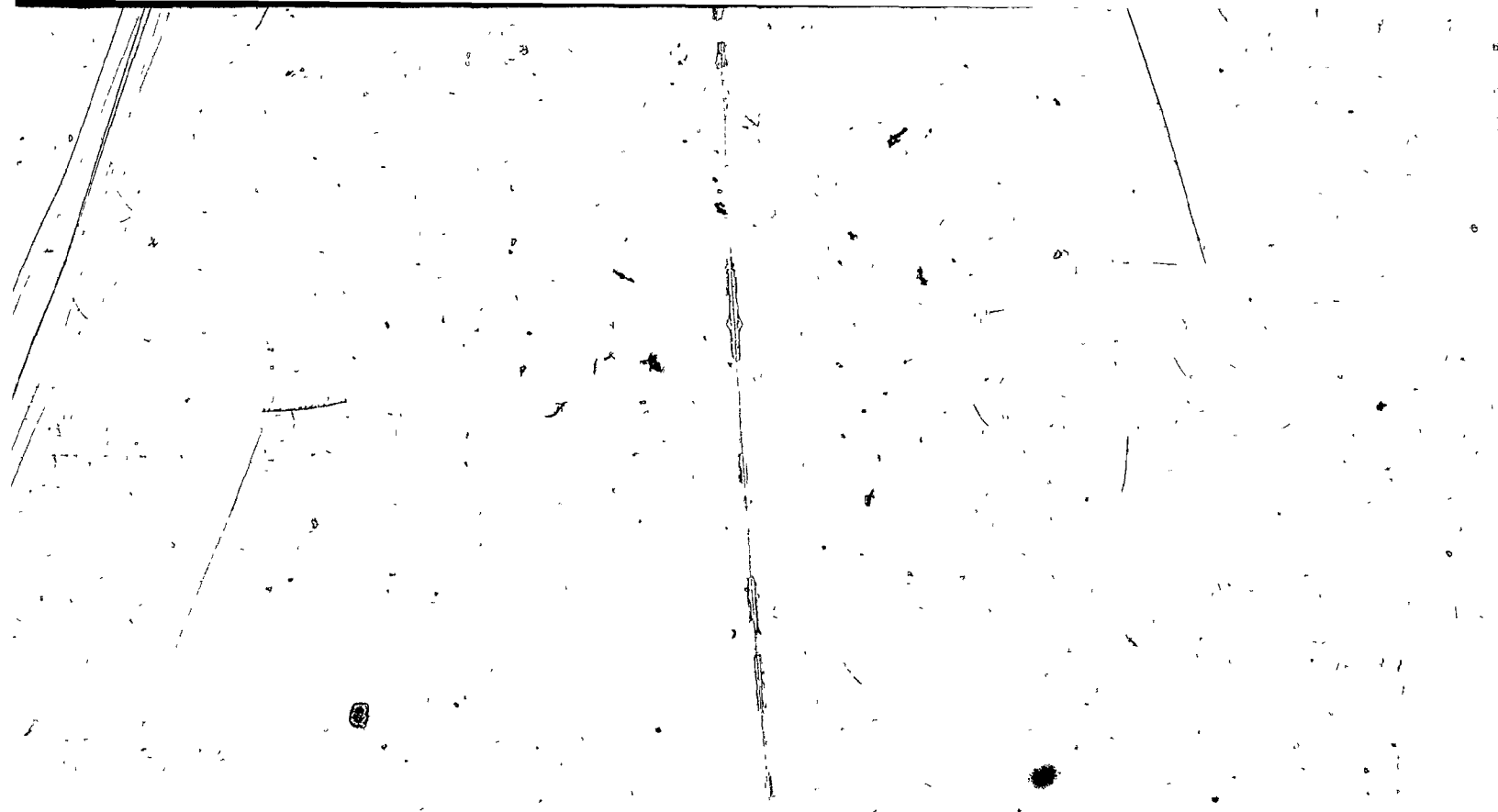


FIGURE 6.20 The distribution of adults in the small intestine of rats with ligature of the bile duct and treated with cholestyramine prior to infection, 5 days after infection with 1000 larvae of T. spiralis.

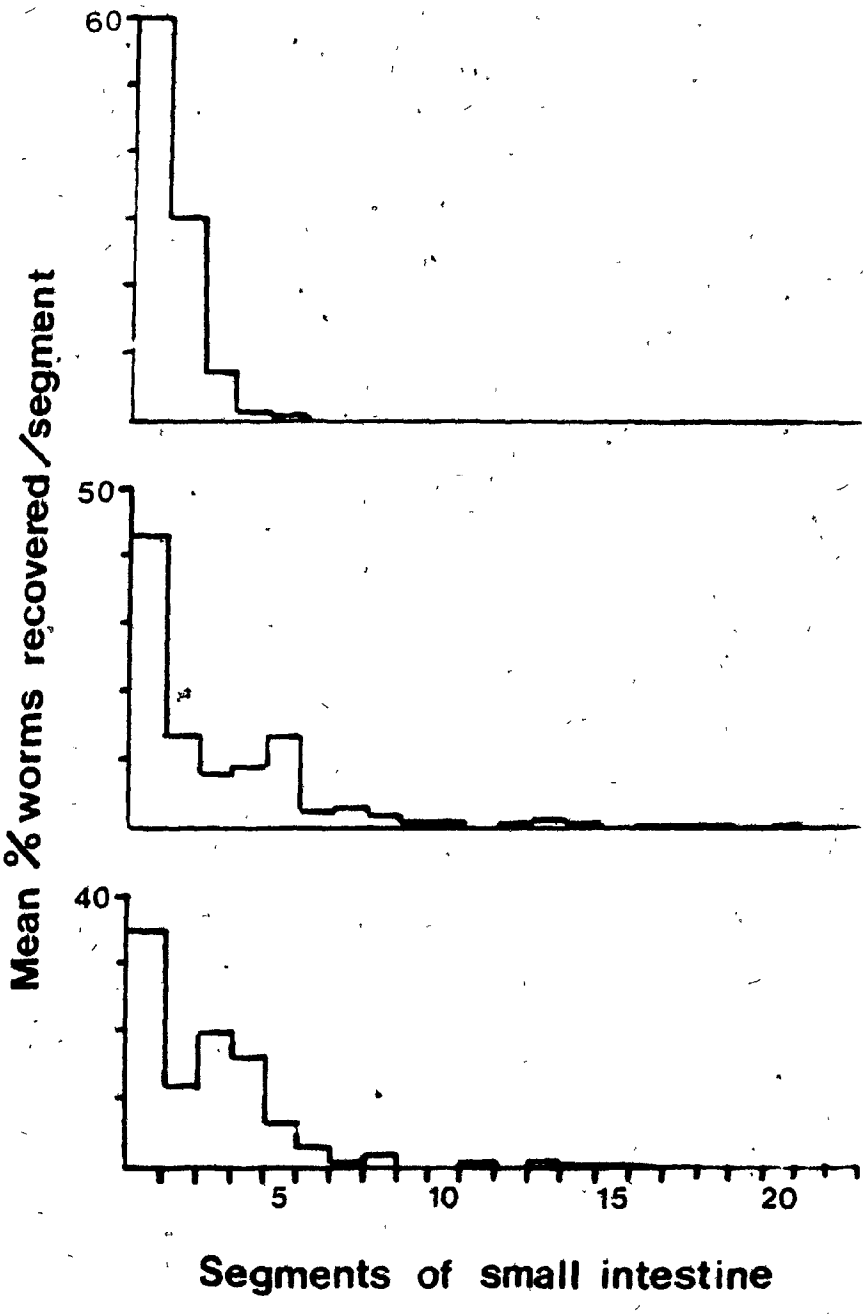

60
50
40
Mean % worms recovered/segment
5
10
15
20
Segments of small intestine

FIGURE 6.21 The distribution of adults in the small intestine of rats treated with cholestyramine prior to infection, 5 days after infection with 1000 larvae of T. spiralis.

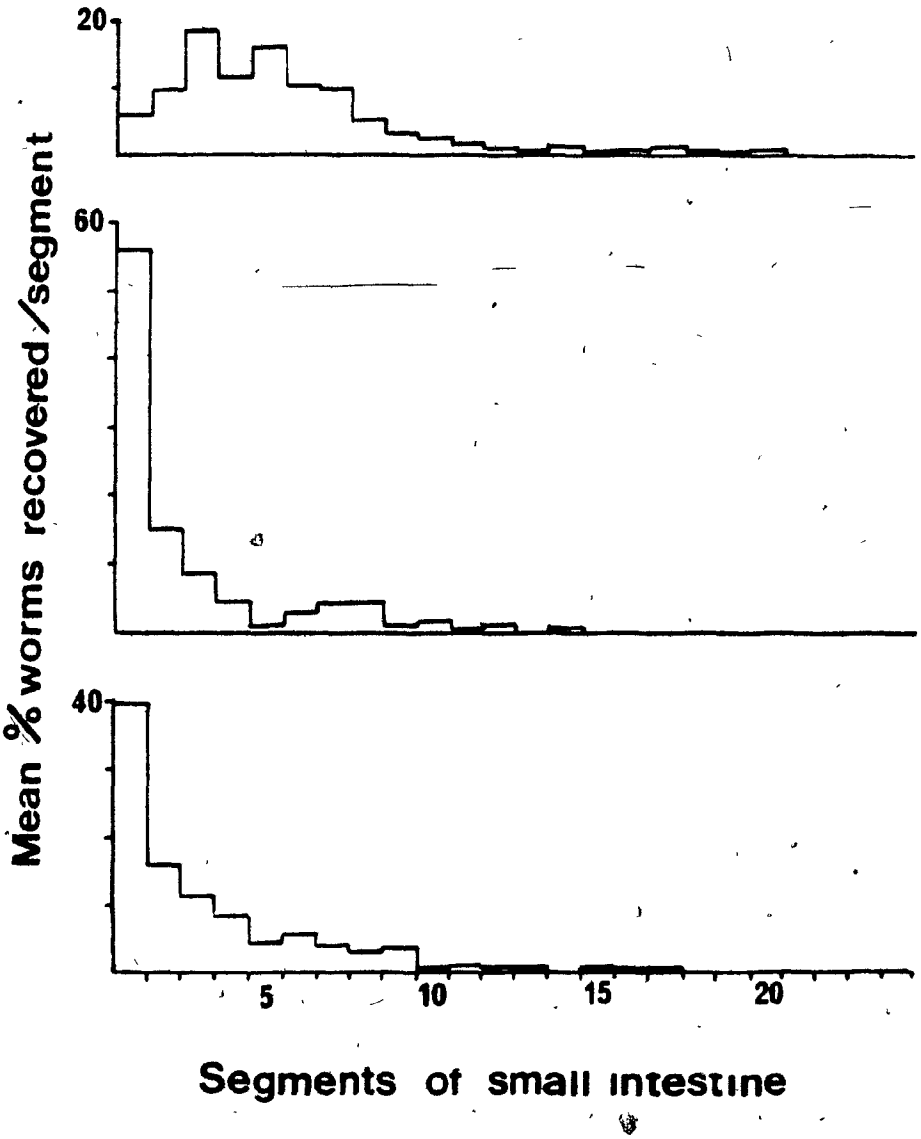
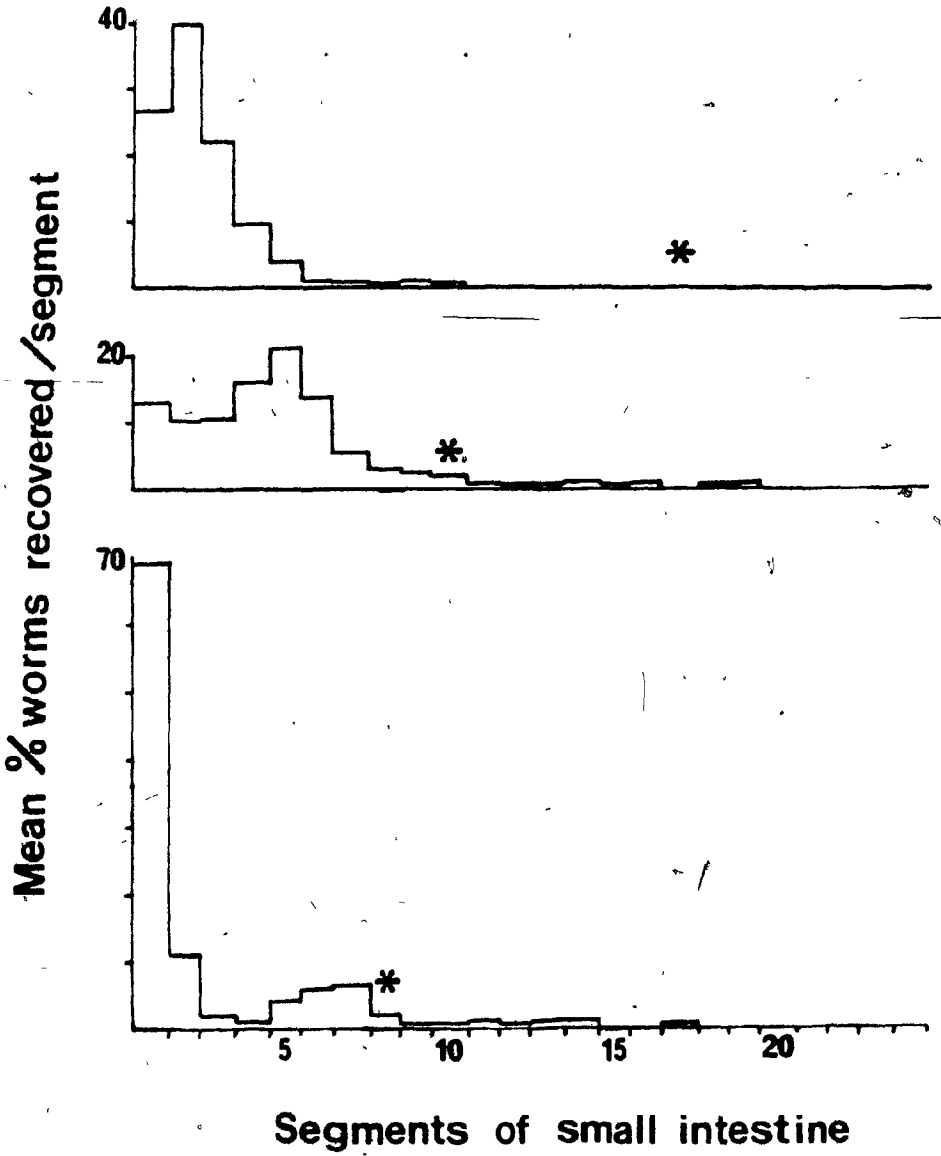

20
60
40
Mean % worms recovered/segment
5
10
15
20
Segments of small intestine

FIGURE 6.22 The distribution of adults in the small intestine of rats with the bile duct cannulated to different locations of the small intestine and treated with cholestyramine prior to infection, 5 days after infection with 1000 larvae of T. spiralis.


40
20
70
*
*
*
Mean % worms recovered /segment
5
10
15
20
Segments of small intestine

a significant reduction ($p \leq 0.05$) in the number of worms that established. The recovery rate of animals treated with cholestyramine was higher than in the controls and in one group in which the animals had been treated with cholestyramine subsequent to bile duct cannulation, the recovery was significantly higher ($p \leq 0.05$) than in the controls.

b) *N. dubius*

The distribution of *N. dubius* in the small intestine of rats (Figure 6.23) was similar to the distribution in mice (Chapter 3; Figures 6.8 and 6.9). The majority of the larvae established in the first segment of the small intestine. Implantation of blind cannulas (Figure 6.23); ligature of the bile duct (Figure 6.27); externalization of the bile duct (Figure 6.28) and ligation of both the pancreatic and bile ducts (Figure 6.29) had no effect on the distribution of larvae in the small intestine. The percentage recoveries of all groups are shown in Table 6.4.

When the bile flow was rerouted, peak larval establishment coincided with the entry point of the bile duct contents (Figures 6.24 and 6.25).

The peak establishment also coincided with bile flow in the rats in which the duodenum had been bypassed. The larvae were not found in the bypassed section (Figure 6.26).

The numbers of larvae establishing did not differ from the controls when the bile was rerouted into the small intestine. In the animals in which the bile flow had been stopped by ligature and division, fewer larvae were recovered. In animals in which the bile flow was externalized, significantly fewer larvae ($p \leq 0.05$) were recovered than controls.

FIGURE 6.23 The distribution of worms in the small intestine of control rats, 6 days after infection with 300 larvae of N. dubius. A and B, sham operated controls; C-E, control rats with blind cannulas (arrowheads point to site of cannula entry).

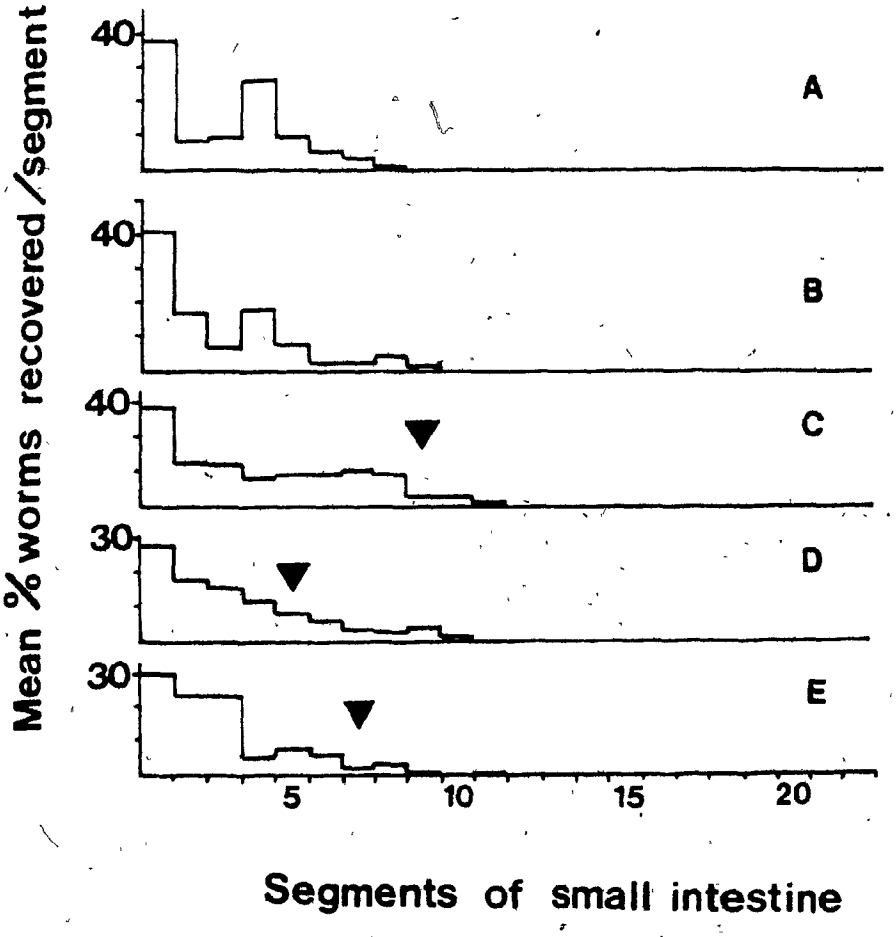

Mean % worms recovered / segment
40
A
40
B
40
C
30
D
30
E
5
10
15
20
Segments of small intestine

FIGURE 6.24 The distribution of worms in the small intestine of rats with the bile duct cannulated to different locations of the small intestine, 6 days after infection with 300 larvae of N. dubius. The arrowheads point to the site of cannula entry.

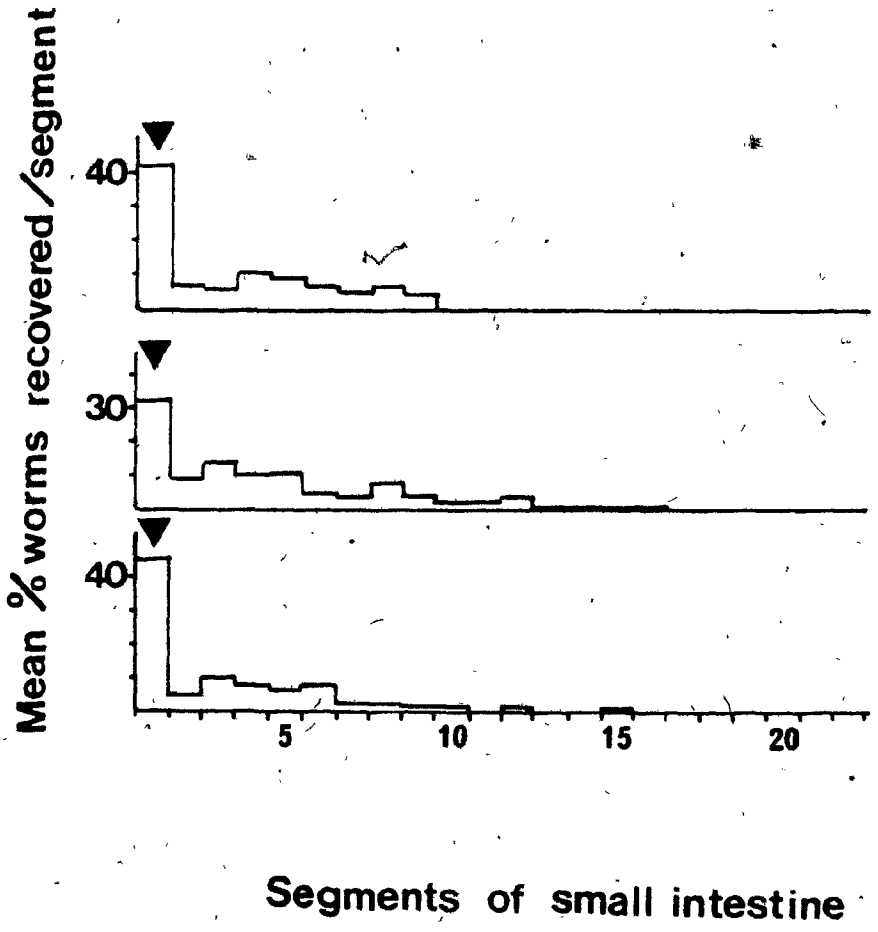

Mean % worms recovered / segment
40
30
40
5
10
15
20
Segments of small intestine

FIGURE 6.25 The distribution of worms in the small intestine of rats with the bile duct cannulated to different locations of the small intestine, 6 days after infection with 300 larvae of N. dubius. The arrowheads point to the site of cannula entry.

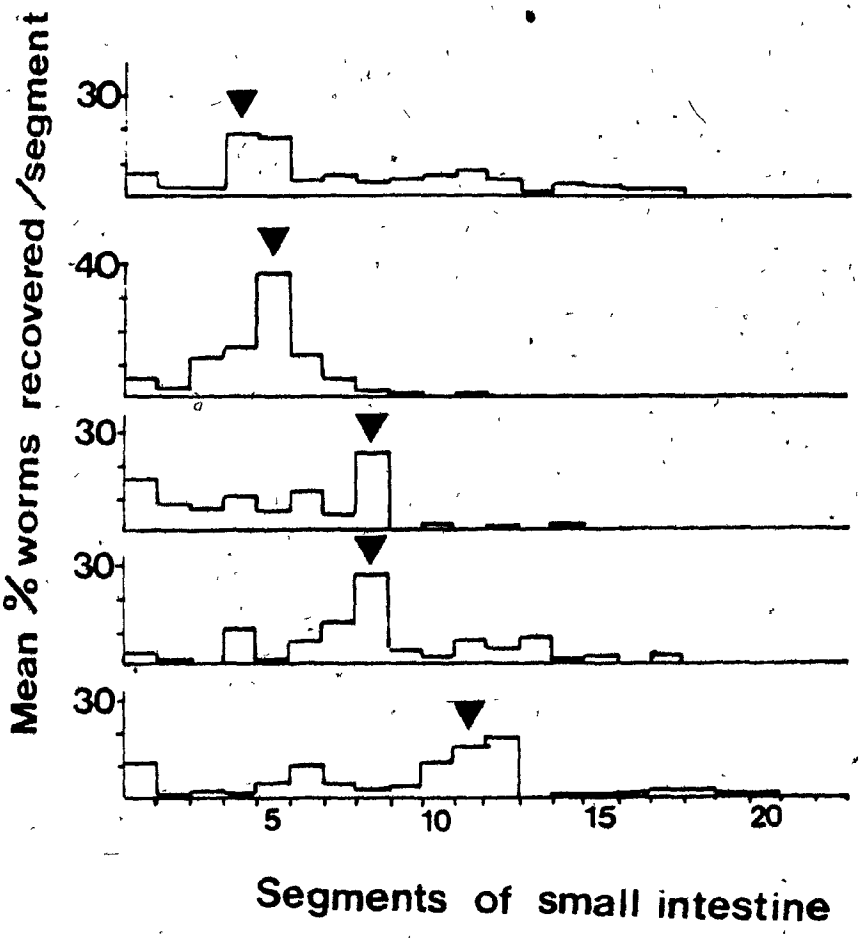

Mean % worms recovered / segment
30
40
30
30
30
5
10
15
20
Segments of small intestine

FIGURE 6.26 The distribution of worms in the small intestine of rats with duodenal bypass operation, 6 days after infection with 300 larvae of N. dubius. The dotted line shows the length of the bypassed segments

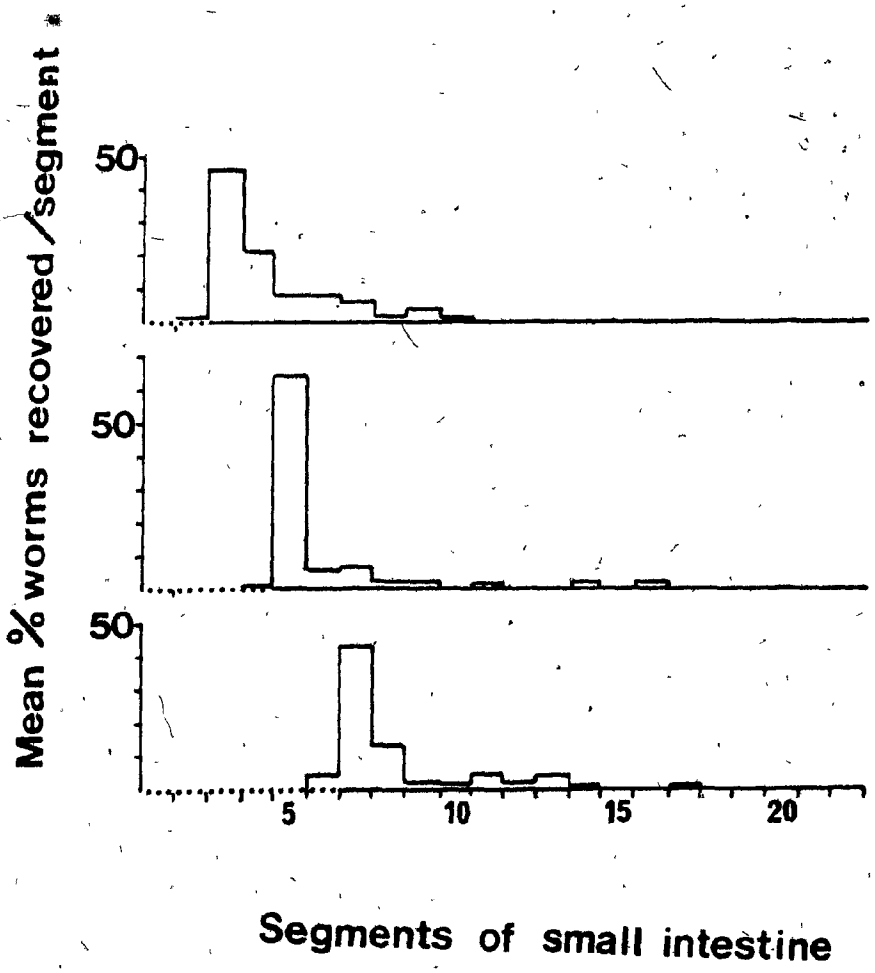

Mean % worms recovered / segment
50
50
50
5
10
15
20
Segments of small intestine

FIGURE 6.27 The distribution of worms in the small intestine of rats with ligature of the bile duct, 6 days after infection with 300 larvae of N. dubius.

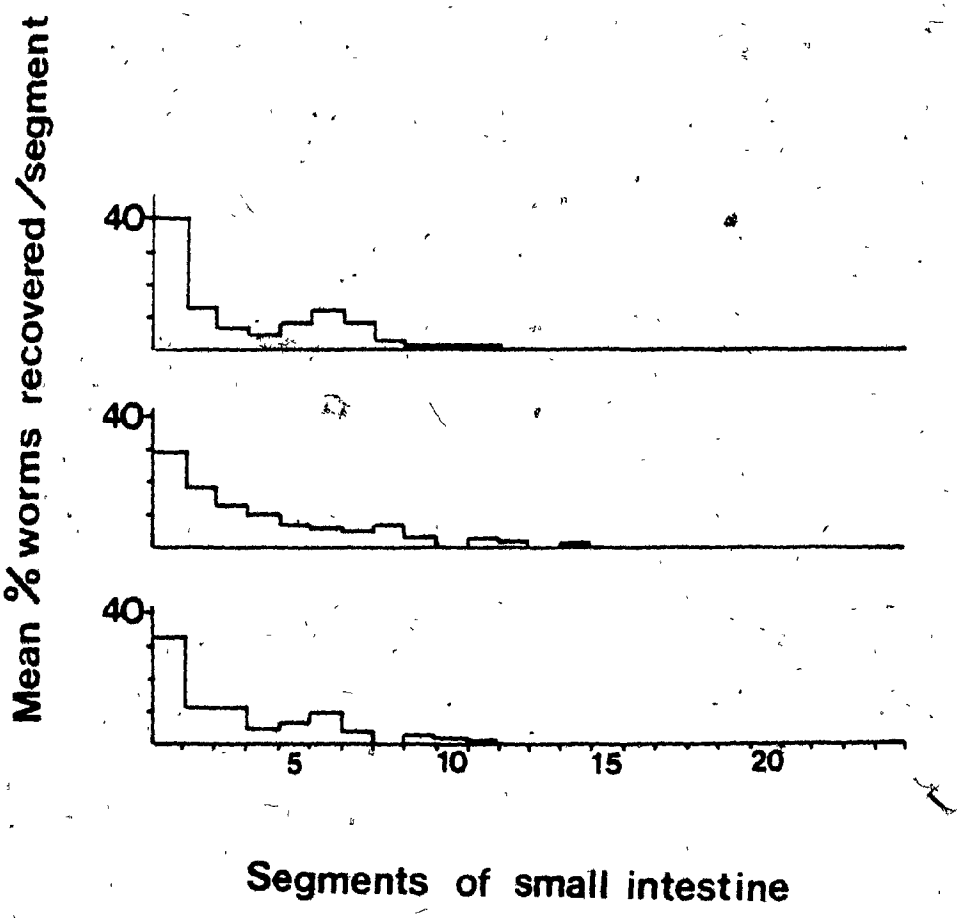

Mean % worms recovered /segment
40
40
40
5
10
15
20
Segments of small intestine

FIGURE 6.28 The distribution of worms in the small intestine of rats with the bile flow externalized, 6 days after infection with 300 larvae of N. dubius.

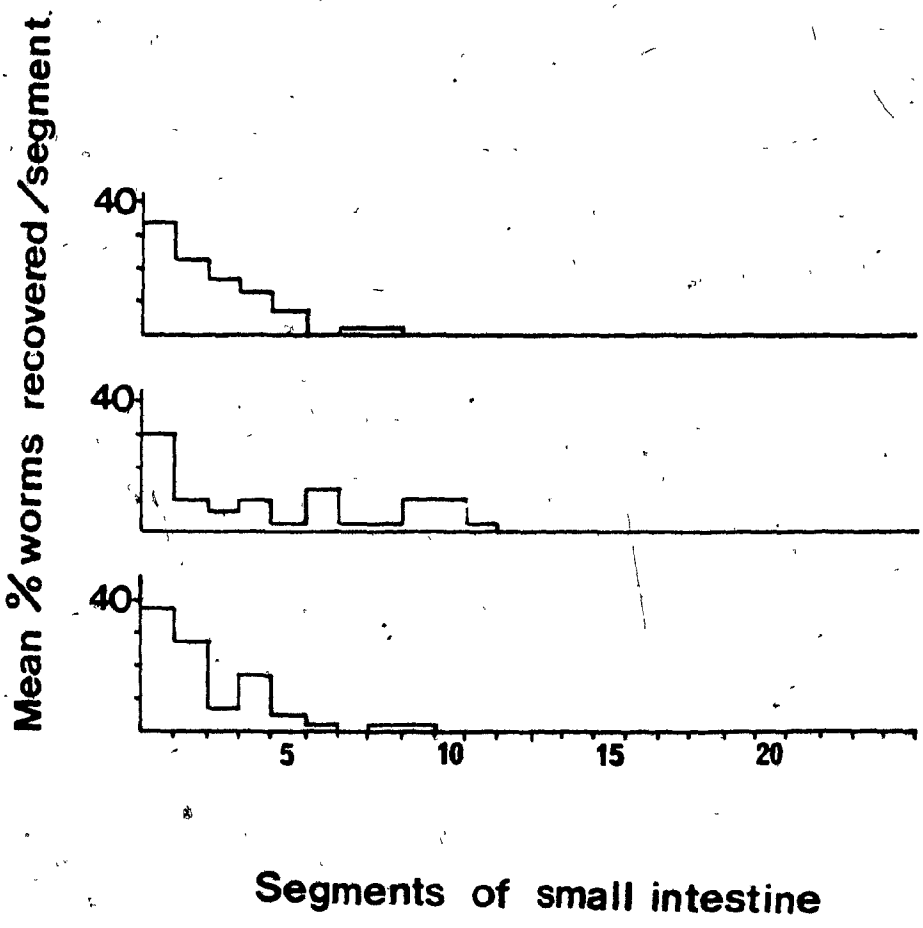

Mean % worms recovered / segment
40
40
40
5
10
15
20
Segments of small intestine

FIGURE 6.29 The distribution of worms in the small intestine of rats with ligature of the bile and pancreatic ducts, 6 days after infection with 300 larvae of N. dubius.

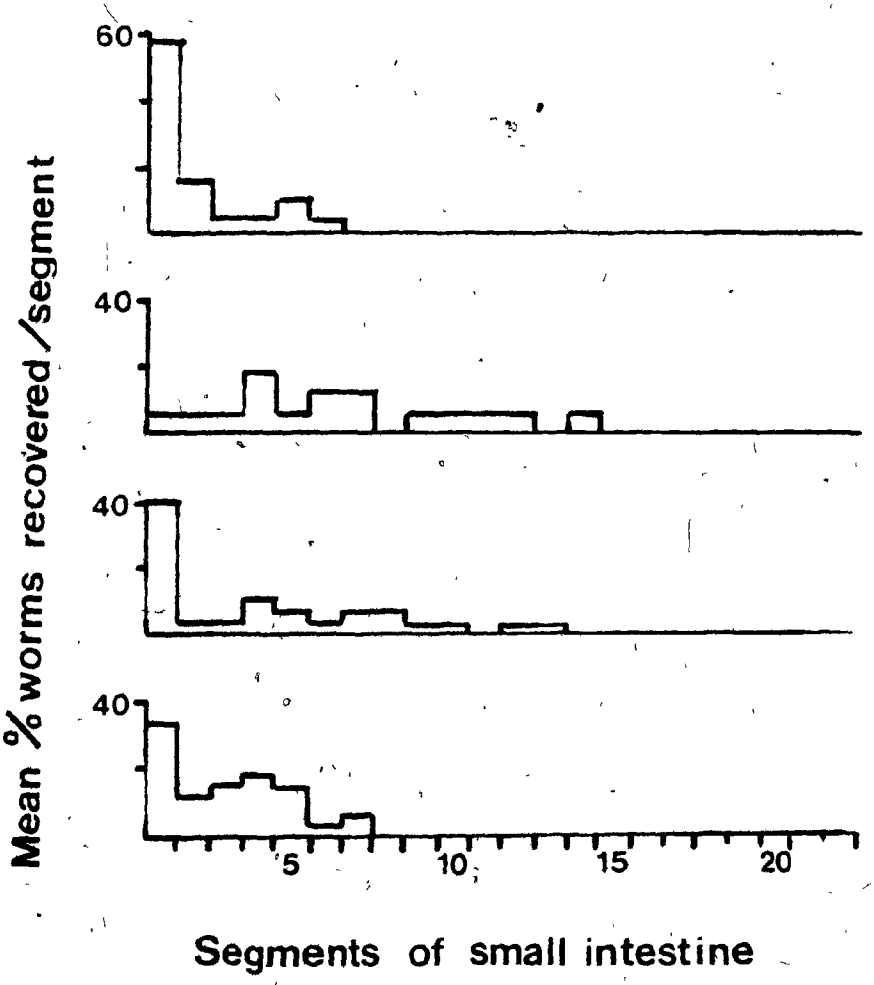

60
40
40
40
Mean % worms recovered/segment
5
10
15
20
Segments of small intestine

TABLE 6.4 THE MEAN TOTAL RECOVERY ± OF WORMS FROM THE SMALL INTESTINE OF SURGICALLY ALTERED RATS 6 DAYS AFTER INFECTION WITH 300 LARVAE OF N. DUBIUS

Treatment	Mean Recovery ± S. E.	$p \leq$ (difference from controls)
Controls	128.2 ± 15.8	
Bile Ligature	91.5 ± 10.5	N. S.
Bile Cannula (Duod)	141.0 ± 37.3	N. S.
Bile Cannula (Ileum)	153.2 ± 12.3	N. S.
Bile Externalized	41.6 ± 6.4	0.005
Duodenal Bypass	98.6 ± 7.8	N. S.
Bile & Pancreatic Ligature	94.83 ± 9.3	N. S.

6D. DISCUSSION

There are a number of factors that have been found to activate the various infective stages of gastrointestinal nematodes *in vitro*, these include: various electrolytes, temperature, pH, appropriate gas phase, redox potentials, pancreatic or intestinal enzymes and bile (see Chapter 1). The results of most of these studies were then extrapolated to *in vivo* conditions in an attempt to interpret the parasite's behaviour. Previous surgical manipulation to study activation and establishment has been only to implant the infective stage, either directly or within dialysis sacs, into different locations of the gut (Ackert, 1931; Hansen, *et al.*, 1956; Sommerville, 1957; Hwang, 1960; Fairbairn, 1961; Bailey, 1968; Chapman & Undeen, 1968; Muller, 1971; Sommerville & Bailey, 1973).

This study examined the manner in which some of the gastrointestinal secretions affected the establishment and longitudinal distribution of *T. spiralis* and *N. dubius* in rats. Although the mechanisms of establishment and site selection are unknown, it is presumed that the activation of the infective larva plays an integral part in the process:

1) *T. spiralis*.

The majority of the larvae established in the anterior-most quarter of the small intestine of rats in normal infections with the larvae of *T. spiralis*. In rats in which the bile duct had been ligatured, there was a decrease in establishment but the majority of the larvae established in the first segment of the small intestine. In mice and rats in which the bile

flow had been externalized, there was a significant reduction ($p \leq 0.05$) in establishment when compared to controls. In the rats in which the bile flow was cannulated to different locations of the small intestine, there was an increased establishment at the site of bile entry into the gut.

The direct effects of bile or its components have been reported in several parasites including: trematodes, cestodes, acanthocephalans and nematodes (Silverman, 1954; Smyth & Hasslewood, 1963; Dawes & Hughes, 1964; Erasmus & Bennet, 1965; Graff & Kitzman, 1965; Howell, 1968, 1970; Read, 1970; Goodchild & Davis, 1972, Jorgensen, 1973; Lackie, 1974 ; Hanna & Jura, 1976). The observation that there are direct effects of bile on these parasites has implied a specific receptor(s) but there is no direct evidence for such a receptor for bile or its components. Bile may also act indirectly via digestive products or through synergistic effects with one or more gastrointestinal enzymes (Lackie, 1975). Although several investigators have implicated bile alone or in combination with other factors in the activation and emergence of some nematodes (Poyntner, 1954; Hwang, 1960; Rogers, 1960; Mapes, 1970; Jorgensen, 1973) no relationship with establishment or penetration has been demonstrated.

Clearly my results indicated that, although bile is important in the establishment of T. spiralis in the gut, it is not essential. In the absence of bile, the larvae are still able to establish but the establishment pattern in these animals differed from controls. Establishment in the absence

of bile suggests that there was another cue (or cues) (designated as factor x) used by the parasite in its site selection. There are two possible explanations for my results: (a), factor x causes penetration and establishment of the larvae of T. spiralis in the small intestine but bile does not and (b), factor x and bile are synergistic in stimulating penetration and establishment of the larvae.

(a) is less likely because there is an increase in larval establishment at the site of bile entry in rats with the bile duct cannulated and there is a significant reduction in establishment in rats with the bile flow externalized. These results suggest that bile also causes penetration and establishment.

(b) is most probably correct. The presence of chyme and fatty acids in the duodenum stimulates increased bile flow via the gastrointestinal hormones, secretin and pancreozymin. Secretin activates the larvae of T. spiralis (see Chapter 7) and if hormone flow is increased in the animals with biliary stasis, the peculiar distribution seen in these animals would be explained. However, gastrointestinal hormones are not secreted into the lumen and increased fatty acids in the lumen do not alter the rate of production of secretin (Guyton, 1964). However, the presence of fatty foods and especially fatty acids in the chyme that enters the duodenum results in the depression of the activity of the pyloric pump. Thus stomach emptying is correspondingly slowed down and so is intestinal propulsion to allow slow digestion of

the fats before they move too far down the gut (Guyton, 1964). Previous experiments (Chapter 3) have demonstrated that the larvae establish anteriad of controls when the propulsion is reduced. In the absence of bile in the small intestine, there is an increase in the fatty acids in the duodenum and a reduction in the propulsion. The site selection behaviour in T. spiralis could then be explained if there was a factor in the lumen to stimulate penetration of the larvae. Potential candidates for this factor may be components of mucus or "succus entericus" secreted into the lumen. This hypothesis is supported by in vitro experiments (Chapter 7). The secretions from the pancreas are discounted because ligature of the pancreatic ducts has no effect on the parasite distribution or establishment.

2) N. dubius

The distribution of N. dubius in normal rats is similar to the distribution in mice, the majority of the larvae were recovered from the first quarter of the small intestine. However, in rats in which the bile duct was cannulated to different locations in the gut and in animals with the duodenum bypassed, peak establishment of the worms occurred at the site of bile entry. This close correlation between bile and N. dubius is further evidenced by the significant reduction in establishment ($p \leq 0.05$) in rats in which the bile flow was externalized and in mice with ligature of the bile duct.

The evidence also suggests that as with infections of T. spiralis, there may be other factors involved in the

establishment of this parasite. These factors are not of pancreatic origin since there were no effects on the distribution of the larvae in rats with the pancreatic flow obstructed. The suggestions made to explain site selection by the larvae of T. spiralis (in previous section) may also apply to site selection by the larvae of N. dubius. The other factors important may include components of the mucus or "succus entericus". In addition, previous experiments (Chapter 3) suggest that there are morphological or anatomical characteristics of the duodenum that encourage penetration since the larvae do not establish in the ileum even in the presence of bile.

These results also suggest that the penetration of the stomach mucosa during the early stages of infection is only a temporary phase. The cues to the selection of a site in the small intestine appear to be dependent on the stimulation of bile and perhaps villus architecture, after the larvae have left the stomach. Ligature of the bile duct in mice significantly reduces establishment, but temporary reduction of the bile with cholestyramine treatment at the time of infection (at which time the larvae can remain in the stomach mucosa) has no significant effect on the establishment. The reason for the phase in the mucosa of the stomach is unknown and the larvae do not remain long enough for developmental changes (Liu, 1965a).

3) Summary

a) Bile is important in the establishment and distribution of T. spiralis and N. dubius in vivo.

b) There are also other factors involved in the site selection by the larvae of T. spiralis and N. dubius in vivo.

c) Pancreatic secretions have no effect on the establishment and distribution of T. spiralis and N. dubius in vivo.

CHAPTER 7. FACTORS AFFECTING THE BEHAVIOUR OF THE INFECTIVE LARVAE OF TRICHINELLA SPIRALIS AND NEMATOSPIROIDES DUBIUS

7A. INTRODUCTION

The previous results suggest that gastrointestinal secretions may be important in the site selection of T. spiralis and N. dubius. Alteration of the extent of bile flow and of its location altered the distribution pattern of establishment. While this measured the effects on the populations it did not provide detailed information on individual behavioural changes. This section attempts to characterize the behavioural changes related to emergence and establishment of T. spiralis and N. dubius.

7B. MATERIALS AND METHODS

1) Trichinella spiralis

The infective excysted larvae of T. spiralis exhibit 2 distinct types of behaviour patterns, designated here as type 1 and type 2. Type 1 behaviour was described in a previous chapter (Chapter 5) and will not be further elaborated in this section. All larvae used in this section were excysted in HCl-pepsin solution.

a) Characterization of type 2 activity.

The behaviour patterns of the larvae of T. spiralis following stimulation with a 10% solution of bile in saline and at 37° C were recorded on the CCTV. Frame-by-frame analysis permitted characterization of the behaviour patterns.

b) The effects of temperature on the activity of the larvae of T. spiralis.

The effects of temperature on the larvae were determined under 4 conditions: (a), in distilled water; (b), in 0.85% NaCl solution; (c), in artificial gastric juice (HCl-pepsin) and (d), in a 10% solution of bile in 0.85% NaCl. 2.0 ml of each solution were added to each of 5 flat-bottomed vials containing approximately 50 larvae in 0.025 ml distilled water. The vials were incubated at various temperatures from 4° C - 43° C (the temperature of the solutions was measured with an electronic thermistor probe). After 3 minutes at each temperature interval, the percentage of the larvae mobile, the numbers of larvae in type 1 and type 2 and the numbers of dead larvae were determined. (Subsequent to this study, all further in vitro studies on T. spiralis were performed at 37° C).

c) The effects of bile on the larvae of T. spiralis.

(i) The effects of pig bile on the population activity of T. spiralis larvae.

Ten percent solutions of different biles in normal saline collected from several laboratory animals were tested for their effects on the larvae at 37° C. One ml of each solution was added to each of 3 watchglasses containing approximately 100 larvae in 0.05 ml normal saline. After 30 minutes incubation, the percentages of the larvae that were mobile in each solution were determined.

(ii) The effects of pig bile on the population activity and the activity rate of T. spiralis larvae.

The percentage of the population that was active in the type 2 behaviour pattern was measured at various intervals after stimulation with a 10% solution of pig bile in buffered saline (pH 7.2) and the mean was calculated for 10 replicates. In addition, rates of activity of 5 representative mobile larvae, chosen at random, were monitored at various intervals after stimulation.

(iii) The effects of dilutions of pig bile on the population activity of T. spiralis larvae.

Log dilutions of bile in buffered saline (pH 7.2) were prepared and tested for their effects on the population activity (type 2) of the larvae of T. spiralis. One ml of each dilution was added to each of 5 watchglasses containing approximately 50 larvae in 0.025 ml buffered saline. After 30 minutes incubation at 37° C the percentage of larvae in type 2 activity was determined.

(iv) The effects of bile salts on the activity of the larvae of T. spiralis.

The effects of various sodium salts of bile on the behaviour of the larvae were monitored. The bile components used were sodium salts of taurocholic acid, deoxycholic acid, cholic acid, taurodeoxycholic acid, glycoholic acid, glycochenodeoxycholic acid, taurochendeoxycholic acid (Sigma Chemical Company, St. Louis); buffered saline (pH 7.2) and 10% pig bile were prepared as controls. These salts were diluted in buffered saline (pH 7.2) see Table 7.2 for the final

concentrations) and 0.5 ml of each salt solution was added to each of 4 flat bottomed vials containing approximately 50 larvae in 0.025 ml buffered saline.

Type 2 activity is preceeded by type 1 and some of these salts stimulated type 1 but not type 2. A simple equation to quantify the effect of stimulation was used:

$$A = \frac{\frac{1}{2}I + II}{0 + I + II}$$

A = Activity

I = The numbers of larvae in type 1

II = The number of larvae in type 2

0 = The number of inactive larvae

After 30 minutes incubation at 37° C, the activity in each treatment was determined.

(v) The effects of sodium taurodeoxycholate on the larvae of T. spiralis.

Log dilutions of sodium taurodeoxycholate (Sigma Chemical Company, St. Louis) were prepared in buffered saline (pH 7.2). 0.5 ml of each dilution was added to each of 4 vials containing approximately 50 larvae in 0.025 ml buffered saline. After 30 minutes incubation, the activity of the larvae in each dilution was determined.

(vi) The response of larvae of T. spiralis to pig bile after intervals of digestion and refrigeration.

Three infected carcasses were separately digested in HCl-pepsin solutions at 37° C. At various intervals; 1, 2, 3, 5, 8, 12, 18 and 24 hours, after the beginning of digestion,

the larvae were tested for their type 2 response. A 10% solution of pig bile in normal saline was added to each of 3 aliquants containing approximately 100 larvae from each of the 3 digesting carcasses, for each interval tested. The percentage of larvae in type 2 activity was determined after 30 minutes of incubation at 37° C. Three aliquants containing approximately 100 larvae taken at corresponding times from each of the 3 digesting carcasses were refrigerated (4° C) for 24 hours. The type 2 response to bile was tested as described above. Three, 8 and 18 hours after digestion had begun, aliquants of larvae were removed from the digesting solutions, pooled and (a), 500 larvae from each interval were given to each of 5 mice by intragastric intubation and (b), the larvae were refrigerated for 24 hours and 500 larvae from each period were given to each of 5 mice by intragastric intubation. Five days after infection, the mice were killed and the worms were recovered from the small intestine. The relationship between the response to bile stimulation and infectivity to mice were examined.

(vii) The longitudinal distribution of larvae stimulated with bile after implantation into the ileum.

Larvae of T. spiralis were treated with a 10% solution of pig bile in normal saline at 37° C for 30 minutes before being implanted into the small intestine. The control larvae were incubated in saline at 37° C for 30 minutes before implantation. Five mice in each group were anaesthetized and laparotomized. Five hundred larvae from each treatment were implanted into the ileum in 0.1 ml solution, without

restriction at the lumen by ligatured pouches. Five days after implantation, the mice were killed and the distributions of worms in the small intestine were determined. The influence of bile pre-treatment on longitudinal dispersion was examined.

(viii) The effects of pig bile on the penetration of intestinal tissue by the larvae of T. spiralis in vitro.

An attempt was made to visualize larval penetration of intestinal tissue using the CCTV. The intestinal tissues were prepared in several ways including: (a) inverted loops; (b), in penetration cells; (c), ileal loops with intact blood supply and (d), saline perfused intestinal segments.

(ix) The effects of prolonged stimulation with bile on the longitudinal distribution and establishment of the larvae of T. spiralis.

Larvae of T. spiralis were incubated in a 10% solution of bile in buffered saline at 37° C for 6 hours. Control larvae were incubated in buffered saline at 37° C for 6 hours. Five hundred larvae of each group were given to each of 5 mice by intragastric intubation. Five days after infection the mice were killed and the establishment and distribution on the small intestine was determined. The influence of prolonged stimulation with bile on the infectivity of the larvae was examined.

d) The effects of crude mucosal extract on the larvae of T. spiralis.

In vivo studies on the site selection behaviour of

T. spiralis (Chapter 6) indicated that although bile had an effect on the site selection behaviour, there were other determining factors involved. In vitro studies of several separate secretions indicated that there were some factors present in the mucosa of the gut that could also stimulate type 2 activity.

The crude mucosal extract was prepared by scraping the mucosa of the gut with a glass slide. The scrapings were diluted in 5 parts of buffered saline (pH 7.2) and homogenized by passing the solution several times through a 10 ml syringe attached to an 18 gauge needle. The mixture was centrifuged at 800 rpm for 10 minutes and the supernatant was used as the crude extract.

(i) The effect of mucosal extracts from different locations of the gut on the larvae of T. spiralis.

Mucosal extracts from the anterior small intestine, posterior small intestine, caecum and colon of 3 mice were prepared and pooled separately. The effects of these extracts as, (a), normal and (b), denatured by immersion in boiling water for 3 minutes, were tested on the activity of the larvae of T. spiralis. One ml of each solution was added to each of 4 watchglasses containing approximately 50 larvae in 0.025 ml buffered saline. Ten percent bile in buffered saline was added to each of 4 watchglasses containing approximately 50 larvae in 0.025 ml buffered saline, these were the controls. After 30 minutes of incubation at 37° C the activity of the larvae in each treatment was determined.

(ii) The effects of mucus components on the larvae of T. spiralis.

To determine whether it was the mucus in the mucosal extract that was responsible for type 2 activity in the larvae, purified mucin and the components of mucus: N-acetyl-D-glucosamine, D (+) galactose, D (+) fucose, N-acetyl-D-galactosamine and N-acetyl-neuramic acid (Sigma Chemical Company, St. Louis) were prepared in solutions of buffered saline. A 10% solution of pig bile in buffered saline (pH 7.2) and buffered saline (pH 7.2) were included as controls. 0.5 ml of each solution was added to each of 4 vials containing approximately 50 larvae in 0.025 ml buffered saline (the final concentrations of the solutions are given in Table 7.3). After 30 minutes of incubation at 37° C, the activity of the larvae in each solution was determined.

e) The effects of "succus entericus" on the activity of the larvae of T. spiralis.

The "succus entericus" from rats was collected as described previously (see p 172). These studies attempted to determine whether the enteric secretions in the crude mucosal extract were responsible for the type 2 activity of the larvae.

(i) The effects of dilutions of "succus entericus" on the activity of the larvae of T. spiralis.

Log dilutions of "succus entericus" were prepared in buffered saline (pH 7.2). 1.0 ml of each dilution was added to each of 3 watchglasses containing approximately 100 larvae in 0.05 ml buffered saline (pH 7.2). After 30 minutes of incubation at 37° C, the activity of the larvae in each vial was determined.

(ii) The effects of "succus entericus" on the rate and duration of activity of larvae of T. spiralis.

The duration of the activity of larvae was monitored in 3 populations after stimulation with a 10% solution of "succus entericus" in buffered saline. In addition, the activity of 5 representative mobile larvae chosen at random, was monitored at various intervals.

(iii) The effects of the components of "succus entericus" on the larvae of T. spiralis.

Dilutions of the components of "succus entericus": peptidase, α-amylase and enterokinase and the hormone, secretin (Sigma Chemical Company, St. Louis) were prepared in buffered saline (pH 7.2). Buffered saline (pH 7.2) was used as a control. 0.5 ml of each solution was added to each of 4 vials containing approximately 100 larvae in 0.05 ml buffered saline (pH 7.2). The final concentration of the treatments is given in Table 7.4. After 30 minutes incubation at 37° C the activity of the larvae was determined.

(iv) The effects of dilutions of enterokinase on the population activity of larvae of T. spiralis.

Log dilutions of enterokinase (80 units/ml) in buffered saline (pH 7.2) were prepared and tested for their effects on the population activity of the larvae of T. spiralis. One ml of each dilution was added to each of 4 watchglasses containing approximately 50 larvae in 0.025 ml buffered saline. After 30 minutes of incubation at 37° C, the activity in each dilution was determined.

(v) The effect of pH on the stimulation of the larvae of T. spiralis by enterokinase.

Dilutions of enterokinase (40 units/ml) were prepared in distilled water, buffered saline (pH 7.2), citrate buffer (pH 5.0) (Parker & Croll, 1975) and Tris buffer (pH 8.0) Parker & Croll, 1975). One ml of each dilution was added to each of 4 watchglasses containing approximately 50 larvae in 0.025 ml buffered saline. After 30 minutes of incubation at 37° C the activity in each solution was determined.

(vi) The longitudinal distribution of T. spiralis in the small intestine following stimulation with "succus entericus" and implantation into the duodenum.

Larvae of T. spiralis were treated with a 10% solution of "succus entericus" at 37° C for 30 minutes. Control larvae were treated with saline at 37° C for 30 minutes. Two groups of 5 mice were anaesthetized, laparotomized and 500 larvae from each treatment were implanted into the duodenum of each mouse without restriction of the lumen by ligatured pouches. Five days after infection, the mice were killed and the distribution of worms in the small intestine was determined. The influence of "succus entericus" on the rate of establishment was examined.

f) The effects of bile and "succus entericus" on the dispersion and penetration of larvae of T. spiralis (in vitro).

(i) The effects of bile and "succus entericus" on dispersion.

Larvae of T. spiralis was added to a warm solution of 1% Noble agar (45° C) to a concentration of 500 larvae/ml.

Ten ml of this solution was poured into 5 cm Petri dishes and allowed to polymerize. Sections of agar, containing approximately 50 larvae, were added to each of 18 petri dishes (5 cm) divided into 3 groups. The petri dishes in each group contained solutions of: (a), 10% bile in buffered saline (pH 7.2) (b), 10% "succus entericus" in buffered saline (pH 7.2) and (c), buffered saline (pH 7.2). After incubation at 37° C for 30 minutes, the ratio of worms within the agar: worms that left the agar and were in the solutions, was determined for each treatment. The influence of these treatments on the dispersion of larvae was examined.

(ii) The effects of bile and "succus entericus" on penetration.

Larvae of T. spiralis were added to a warm solution of 0.5% Noble agar (45° C) to a concentration of 500 larvae/ml. In 15 petri dishes (5 cm) containing 9 ml solidified 1% agar, wells of 1 cm diameter were made and filled with the molten, soft agar containing the larvae. 0.1 ml of (a), 10% bile in buffered saline (pH 7.2) (b), 10% "succus entericus" in buffered saline (pH 7.2) and (c), buffered saline (pH 7.2) was added to each of 5 wells. The plates were immediately incubated for 60 minutes at 37° C and the percentage of worms migrating from the soft agar to the hard agar was counted. The influence of these treatments on the penetration of the hard agar was examined.

g) The effects of CO_2 on the behaviour of the larvae of T. spiralis.

Three mixtures of gases were used: (a), air;

(b), 5% CO_2 - 95% N_2 and (c), 50% CO_2 - 50% N_2. Each mixture was bubbled for 30 seconds in each of 4 vials containing approximately 50 larvae in 0.3 ml buffered saline (pH 7.2). The vials were then sealed and after 30 minutes of incubation at 37° C, the activity in each vial was determined. The influence of various gas phases on the activity of the larvae was examined.

2) Nematospiroides dubius

a) The effects of pig bile on the larvae of N. dubius.

(i) The effects of pig bile on the population activity and rate of activity of N. dubius larvae at 22° C.

Two ml of a 10% aqueous solution of pig bile were added to each of 10 watchglasses containing approximately 100 larvae in 0.05 ml distilled water. In 10 control watchglasses containing 100 larvae in 2.0 ml distilled water, the larvae were mechanically stimulated by drawing them into and expelling them from a 10 ml syringe with blunted 20 gauge needle thrice. The percentage of the population that was mobile was monitored at various intervals in both groups. The rate of activity of the control, mechanically stimulated, larvae was monitored in 4 representative individuals, chosen at random, for the duration of their active periods. The rates of activity on the larvae stimulated with bile were monitored in 5 representative worms, chosen at random, at intervals after stimulation. This experiment was carried out at 22° C under conditions of even, cold, transmitted, illumination (fluorescent lighting below translucent glass).

(ii) The effects of pig bile on the population activity and the rate of activity of N. dubius larvae at 37° C.

The experiment described above a(i) was repeated at 37° C. Subsequent to this study, unless otherwise stated, all further in vitro studies on the larvae of N. dubius were done at 20 - 22° C.

(iii) The effects of log dilutions of pig bile on the population activity of the larvae of N. dubius.

Log dilutions of pig bile were prepared in distilled water. One ml of each dilution was added to each of 5 watch glasses containing approximately 100 larvae in 0.05 ml distilled water. The percentage of the population mobile at each concentration was determined after 30 minutes.

(iv) The response of the larvae of N. dubius to gradients of pig bile.

Gradients of bile were prepared in 5 petri dishes (5 cm) containing 5 ml of 0.05% Noble agar. Using a template to divide the plate into 8 sectors, a well was punched in sector 4. 0.05 ml bile was placed in each well and allowed to diffuse for 24 hours at 25° C; 0.05 ml distilled water was put in the wells of 5 control plates. Approximately 50 larvae were placed in the centre of each plate before the plates were incubated at 25° C in the dark for 30 minutes. The numbers of larvae in each sector were determined and the influence of bile gradients on the dispersion of the larvae was examined.

(vii) The effects of prolonged stimulation with bile on the establishment and longitudinal distribution of the larvae of N. dubius in the small intestine.

Larvae of N. dubius were incubated in a 10% aqueous solution of bile for 6 hours at 22° C, control larvae were incubated in distilled water at 22° C for 6 hours. One hundred larvae from each treatment group were given to each of 5 mice by intragastric intubation. Six days after infection, the mice were killed and the distribution in the small intestine was determined. The influence of prolonged stimulation with bile on the infectivity of the larvae was examined.

b) The effects of gastric sections (in vitro) on the larvae of N. dubius.

Seven solutions were prepared: (a), HCl (pH 2.0); (b), HCl (pH 2.0) plus 1% pepsin; (c), Citrate buffer (pH 4.0) Parker & Croll, 1975) plus 1% pepsin; (d), Tris buffer (pH 8.0) Parker & Croll, 1975) plus 1% pepsin; (e), H_2SO_4 (pH 2.0); (f), H_2SO_4 (pH 2.0) plus 1% pepsin and (g), H_2SO_4 (pH 2.0) plus 1% pepsin plus 1% NaCl. 0.3 ml of each solution was added to each of 16 vials containing approximately 50 larvae of N. dubius in 0.025 ml distilled water. The vials were then equally divided into 4 groups which were treated in the following manner, the vials in each group were: (a), untouched and sealed; (b), gassed with 5% CO_2 - 95% N_2 for 30 seconds before sealing; (c), gassed with 50% CO_2 - 50% N_2 for 30 seconds before sealing and (d), gassed with 100% CO_2 before sealing. Thirty minutes after incubation at 37° C, the percentage of active larvae in each vial was determined. The influence of gastric secretions and gas phase on the larval activity was examined.

c) The effects of crude mucosal extracts on the larvae of N. dubius.

(i) The effects of mucosal extracts from different locations of the gut on the larvae of N. dubius.

Mucosal extracts from the anterior small intestine (duodenum/jejenum), posterior small intestine (ileum), caecum and colon of 3 mice were prepared and pooled separately. Solutions of 10% bile in buffered saline (pH 7.2) and buffered saline (pH 7.2) alone were included as controls. The effects of these solutions (a), normal and (b), denatured by immersion in boiling water for 3 minutes, were tested for their effects on the activity of the larvae of N. dubius. One ml of each solution was added to each of 3 watchglasses containing approximately 50 larvae in 0.025 ml buffered saline. After 30 minutes incubation at 22° C, the percentage of larvae that were mobile were determined.

c) The effects of "succus entericus" on the activity of larvae of N. dubius.

Log dilutions of "succus entericus" were prepared in buffered saline (pH 7.2). 0.5 ml of each dilution was added to each of 3 vials containing approximately 100 larvae in 0.05 ml buffered saline. After 10 minutes incubation at 22° C, the percentage of larvae that were mobile was determined.

d) The effects of bile and "succus entericus" on the dispersion and penetration behaviour of the larvae of N. dubius.

The procedure and methodology used in these experiments were similar to the previous experiments f(i) and f(ii) (Section 1) except that the larvae used were N. dubius.

7C. RESULTS

1) T. spiralis

The activity pattern described as type 2 appears to

be a behavioural progression from type 1, type 1 always preceeds type 2. Type 2 differs from type 1 in that the anterior end of the worm uncoils and waves travel along the entire body resulting in a sinusoidal type movement with a strong dorsal bias. Head waving and strong contractions of the body musculature which resulted in tight coiling movements were frequently seen. Figure 7.1 shows a frame-by-frame analysis from the CCTV screen of a worm in type 2 activity. It is important to note that this description is for worms in liquid media.

The activity of the larvae of T. spiralis is maximal at 37° C under all conditions but the population activity differs in distilled water, saline, gastric juice and 10% bile solutions (Figure 7.2). The response was greatest in gastric juice and 10% bile with 90 - 100% of the larvae becoming mobile at 37° C while in saline and distilled water the maximum activity was 25% and 8% respectively.

Increasing temperatures led to increased death of larvae under some conditions. The numbers of dead larvae in distilled water increased with temperature and approximately 60% of the larvae were dead at 43° C, similarly, approximately 23% of the larvae in the gastric juice were dead at 43° C (Figure 7.3A). Throughout the temperature range tested, there were no larval deaths recorded from the saline or bile treatment. (The criteria used to determine death of the larvae was the irreversible straightening of the larvae into "question mark" shapes).

Except for the larvae in 10% bile, most of the larval behaviour observed was type 1. However, with increasing

FIGURE 7.1 Frame by frame analysis from the CCTV screen of an infective larva of T. spiralis in type 2 activity. The anterior end is depicted by the arrow, the elapsed time between each frame is 0.017 seconds.

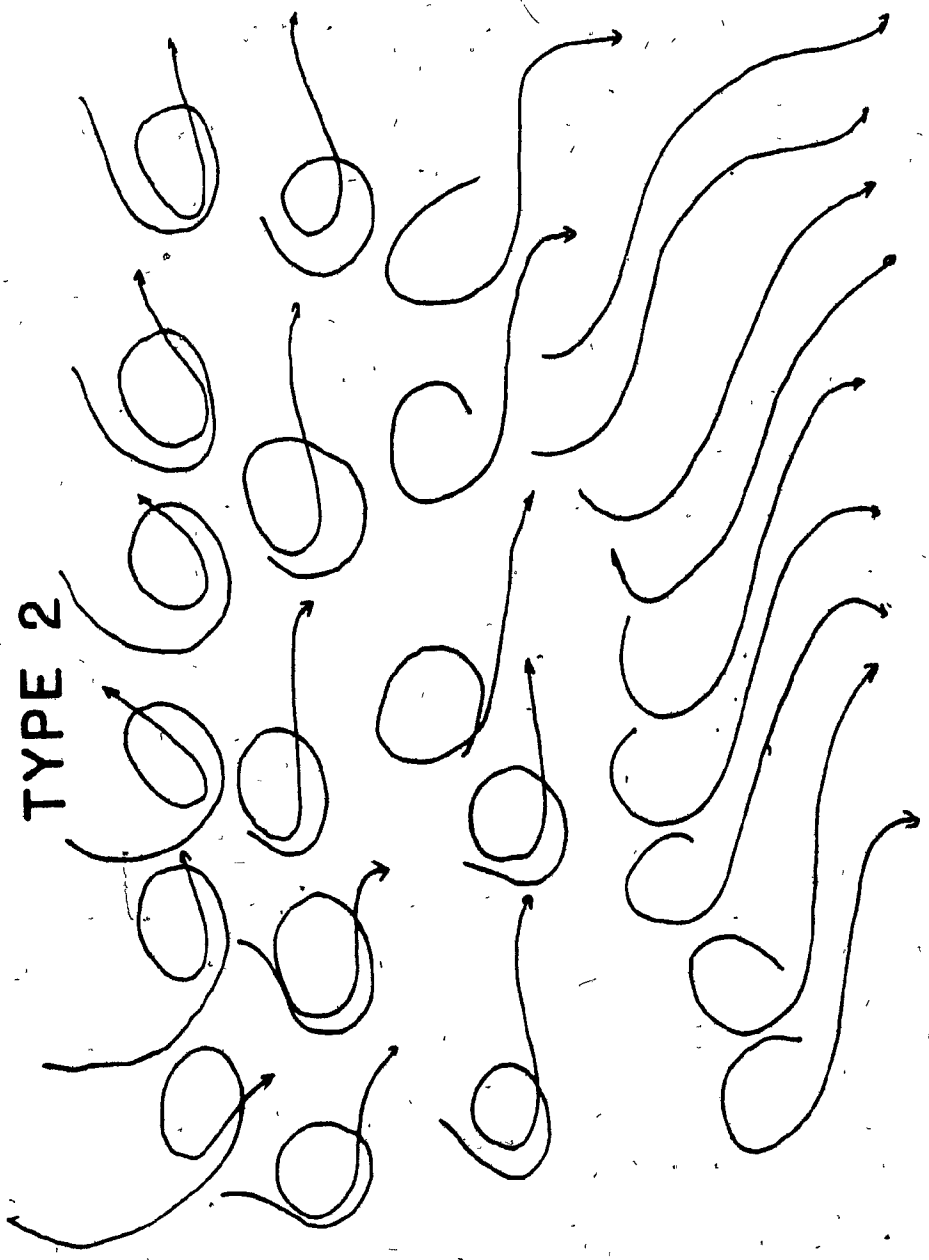

TYPE 2

FIGURE 7.2 The population activity of the excysted larvae of T. spiralis at different temperatures and under different conditions. A, artificial gastric juice; B, 10% pig bile in buffered saline (pH 7.2); C, buffered saline (pH 7.2) and D, distilled water.

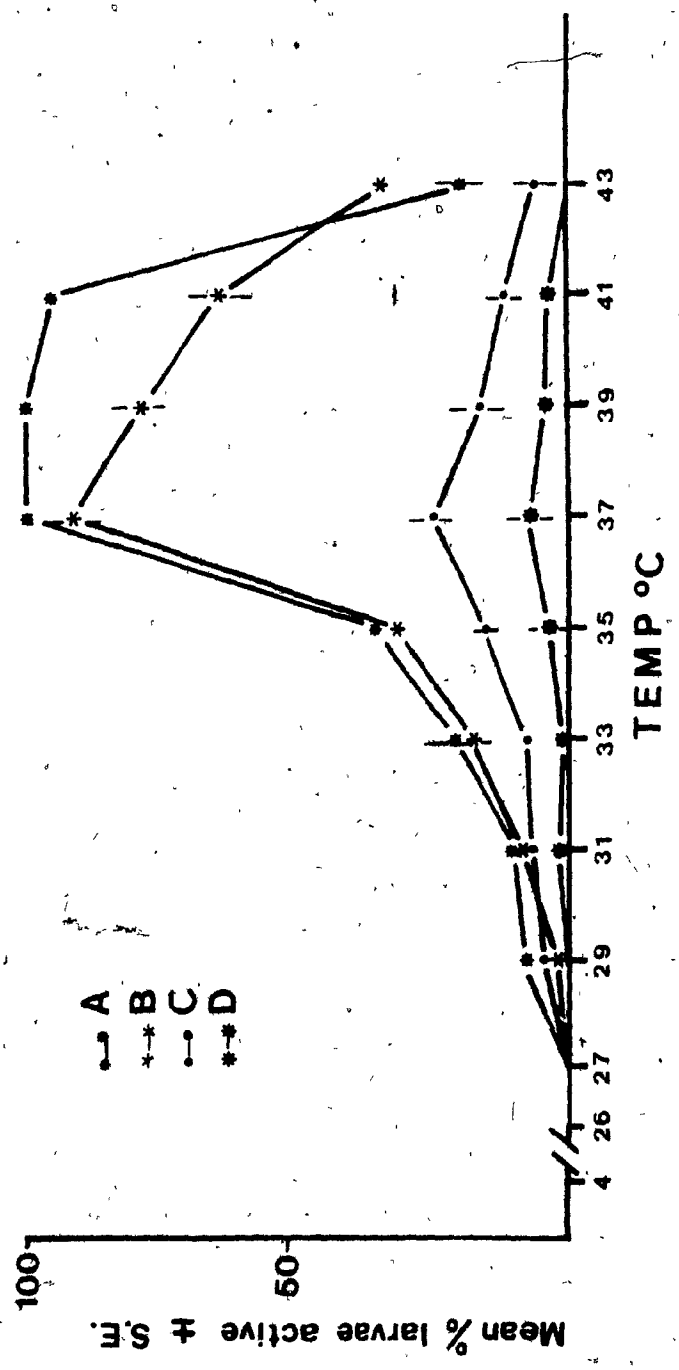
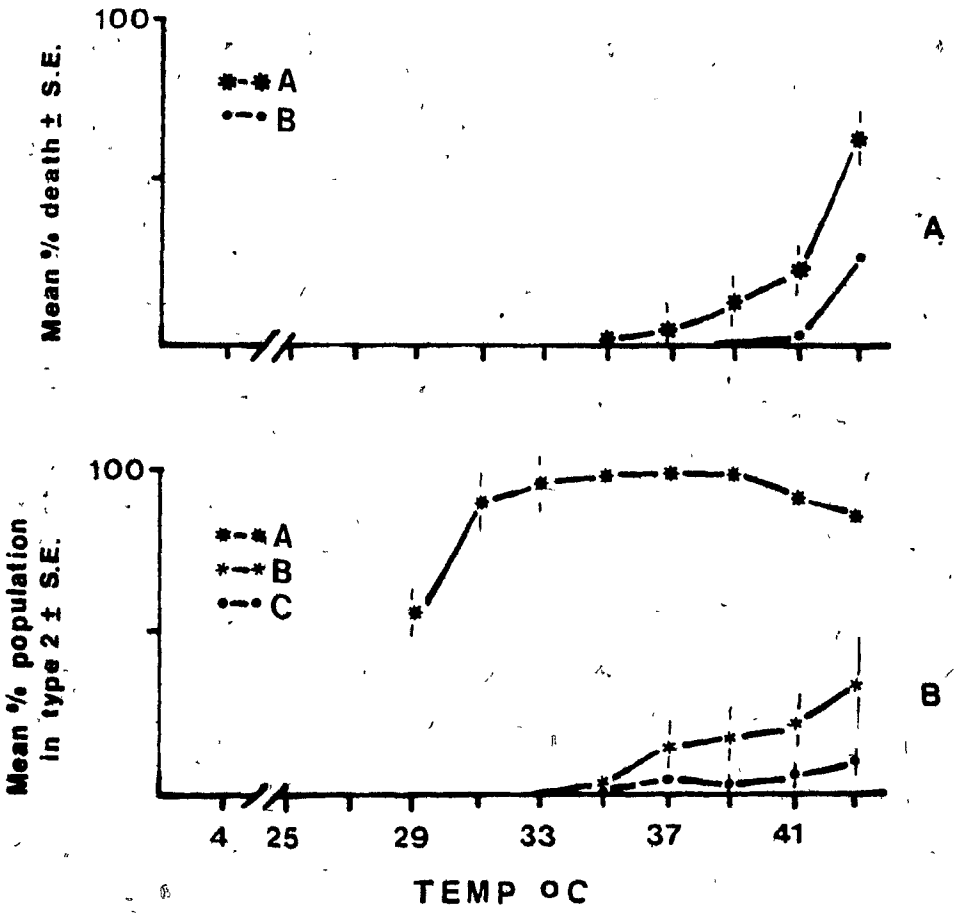

Mean % larvae active ± S.E.
100
50
4
26
27
29
31
33
35
37
39
41
43
TEMP °C
A
B
C
D

FIGURE 7.3 The behaviour of the larvae of T. spiralis at different temperatures and under different conditions. 7.3A: The changes in death rate with increasing temperature; A, distilled water; B, artificial gastric juice. 7.3B: The percent population in type 2 activity with increasing temperature; A, 10% bile in buffered saline (pH 7.2); B, buffered saline (pH 7.2) and C, artificial gastric juice.


100
A
B
Mean % death ± S.E.
A
100
A
B
C
Mean % population in type 2 ± S.E.
B
4
25
29
33
37
41
TEMP °C

temperatures, the numbers of larvae entering type 2 increased in the saline and gastric juice groups. Approximately 30% of the larvae in saline and 10% of the larvae in gastric juice were in type 2 activity at 43° C (Figure 7.3B). Type 2 activity was never observed in the distilled water group.

The biles collected from various experimental animals all caused 100% activation into type 2 when the larvae were incubated in 10% solutions. The exception to this was duck bile (Table 7.1). Larval viability was not a factor in the unresponsiveness to duck bile because the same worms would become active if small amounts of pig bile were added to the medium.

Crude pig bile at a 10% concentration stimulated type 2 activity and this activity was maintained for several hours in the absence of any other stimulus (Figure 7.4). There is a gradual reduction of activity beginning 2 hours after stimulation. The rate of movement after stimulation with bile was initially high with approximately 50 undulations per minute but there was a gradual reduction 3 to 4 hours after stimulation (Figure 7.5). In the log-dose response curve of the larvae to bile, there was a linear increase in population activity from 0.1% to 1.0% above and below which the response plateaus (Figure 7.6).

The components of bile that appear to stimulate the larvae into type 2 activity are the taurine-conjugated bile salts with the sodium salt of taurodeoxycholic acid being the most potent (Table 7.2). The glycine-conjugated bile salts

TABLE 7.1 THE EFFECT OF VARIOUS ANIMAL BILES IN SALINE ON THE POPULATION ACTIVITY OF *T. SPIRALIS* LARVAE IN VITRO AT 37° C

Host	Bile Concentration	% Population Active at 30 minutes
Pig	10%	100
Ox	10%	100
Cat	10%	100
Rabbit	10%	100
Sheep	10%	100
Rat	10%	100
Mouse	10%	100
Raccoon	10%	100
Duck	10%	0

FIGURE 7.4 The activity (see p 292) of the larvae of T. spiralis in response to stimulation with 10% pig bile.

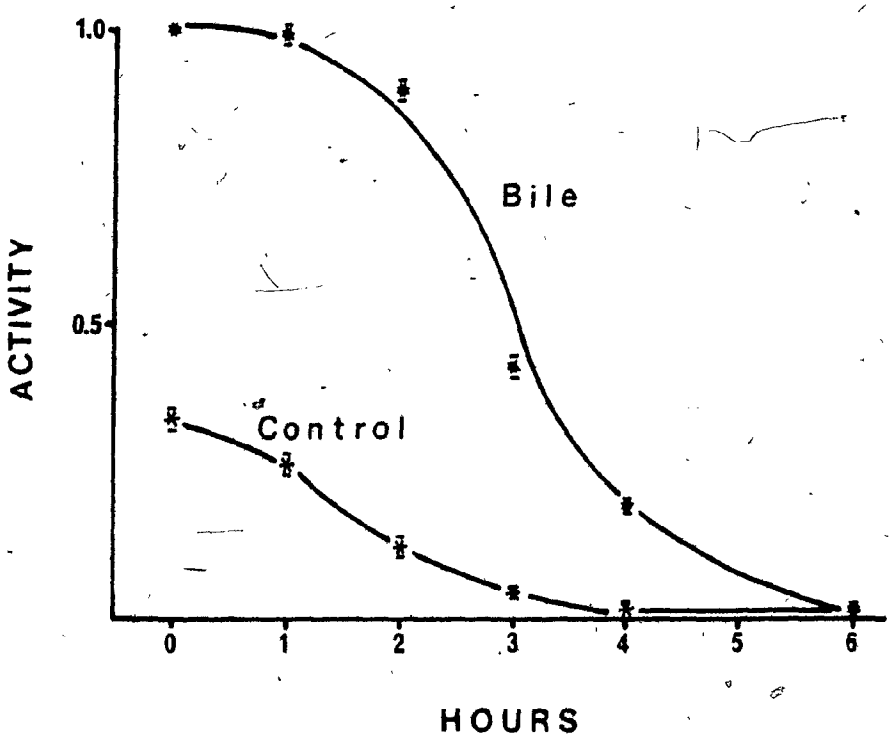

1.0
0.5
ACTIVITY
Bile
Control
0
1
2
3
4
5
6
HOURS

FIGURE 7.5 The rate of movement of the larvae of T. spiralis in response to stimulation with 10% pig bile.

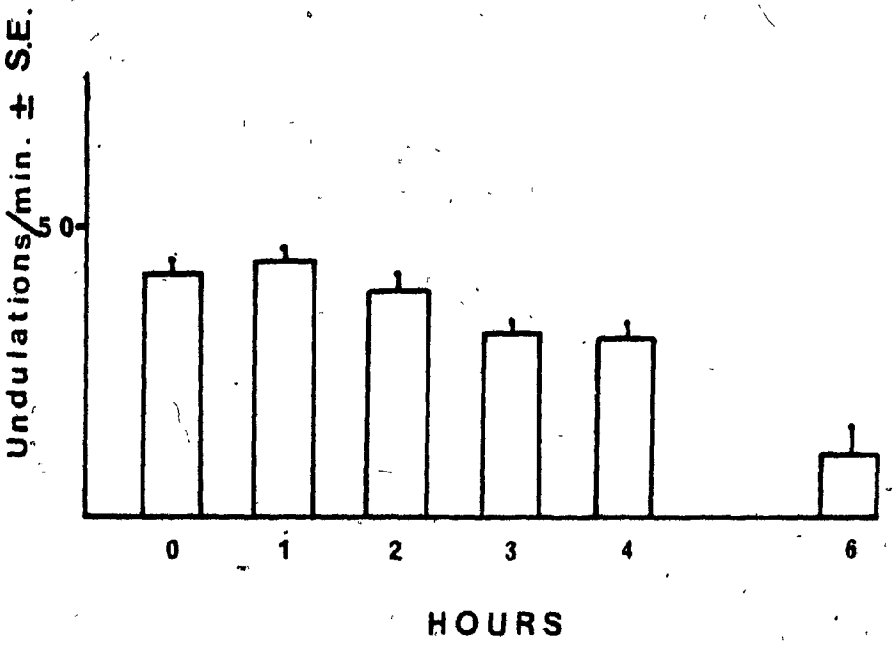

Undulations/min. ± S.E.
50
0
1
2
3
4
6
HOURS

FIGURE 7.6 The population activity of the larvae of T. spiralis in response to log dilutions of bile after 30 minutes incubation at 37° C.

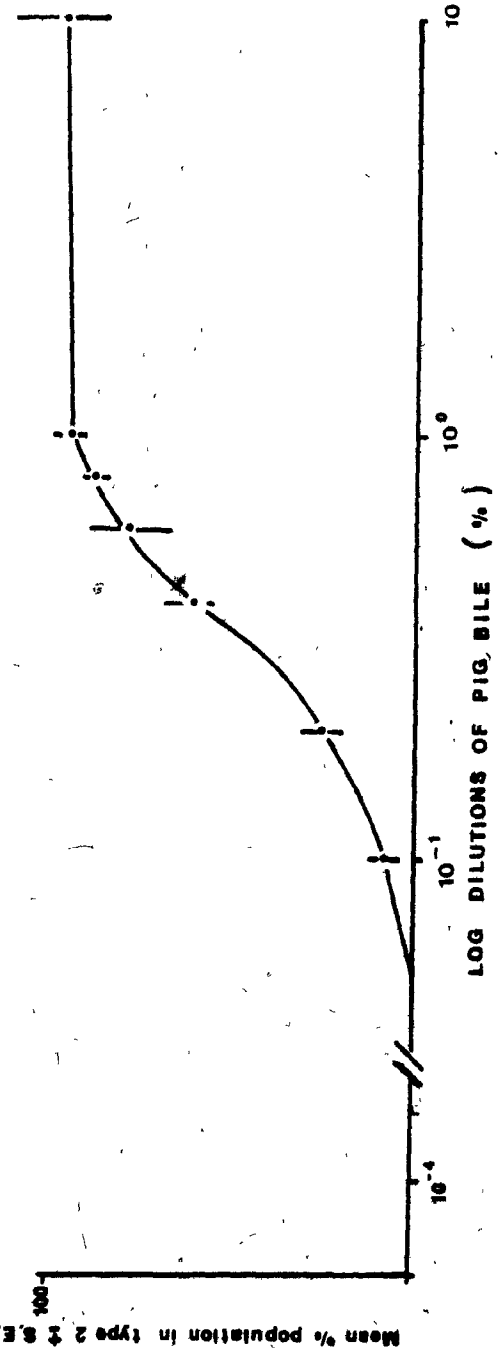

Mean % population in type 2 ± S.E.
100
10
10^{0}
10^{-1}
10^{-4}
LOG DILUTIONS OF PIG BILE (%)

TABLE 7.2 THE EFFECT OF VARIOUS BILE SALTS IN BUFFERED SALINE (pH 7.2) ON THE ACTIVITY OF THE LARVAE OF T. SPIRALIS IN VITRO AT 37° C

Bile salt	Concentration	Activity (b)	$p \leq$ (a)
Sodium taurocholate	0.1	0.65 ± 0.05	0.05
Sodium deoxycholate	0.1	0.18 ± 0.02	0.05
Sodium cholate	0.1	0.02 ± 0.004	N.S.
Sodium taurodeoxycholate	0.1	1.00 ± 0.00	0.05
Sodium glycocholate	0.01	0.01 ± 0.01	N.S.
Sodium glycochenodeoxycholate	0.01	0.03 ± 0.01	N.S.
Sodium taurochenodeoxycholate	0.001	0.23 ± 0.04	0.05
Controls			
Buffered saline (pH 7.2)	0.85%	0.04 ± 0.02	-
Crude bile	10%	0.95 ± 0.02	0.05

(a) significantly different from buffered saline (pH 7.2) control ($p \leq 0.05$)

(b) activity (see p 292)

had no significant effect in stimulating the activity of the larvae (Table 7.2). The log-dose response of the larvae to the sodium salt of taurodeoxycholic acid shows that the larvae were extremely sensitive to this compound, the response is maximal to concentrations as low as 10^{-4}M (Figure 7.7).

The ability of the larvae to respond to pig bile decreased with the duration of digestion. The ability to respond to bile is best in larvae that had been digesting for 3 hours or less. As the duration of digestion increased, the ability to respond to bile decreased (Figure 7.8). Refrigeration for 24 hours after removal of the larvae from the digestion solution further decreased the larva's ability to respond to bile (Figure 7.8). However, the inability to respond to bile did not necessarily indicate reduced ability to infect mice. There were no signficant differences in worm recovery (and hence infectivity) between larvae given to mice immediately after digestion and larvae refrigerated for 24 hours before infection (despite their differences in response to bile) (Figure 7.9, Table 7.3). However, there was a significant decrease in larval infectivity between 8 and 18 hours of digestion (Table 7.3).

Pre-treatment of the larvae with bile for 30 minutes prior to implantation into the ileum did not result in any changes in the distribution of the worms in the small intestine when compared to controls (Figure 7.10, Table 7.4). Prolonged stimulation of the larvae with bile for 6 hours prior to oral infection had no adverse effects on the larvae and the establishment and distribution of worms in the small intestine

FIGURE 7.7 The activity (see p 292) of the larvae of *T. spiralis* in response to log dilutions of tauro-deoxycholic acid after 30 minutes incubation at 37° C.

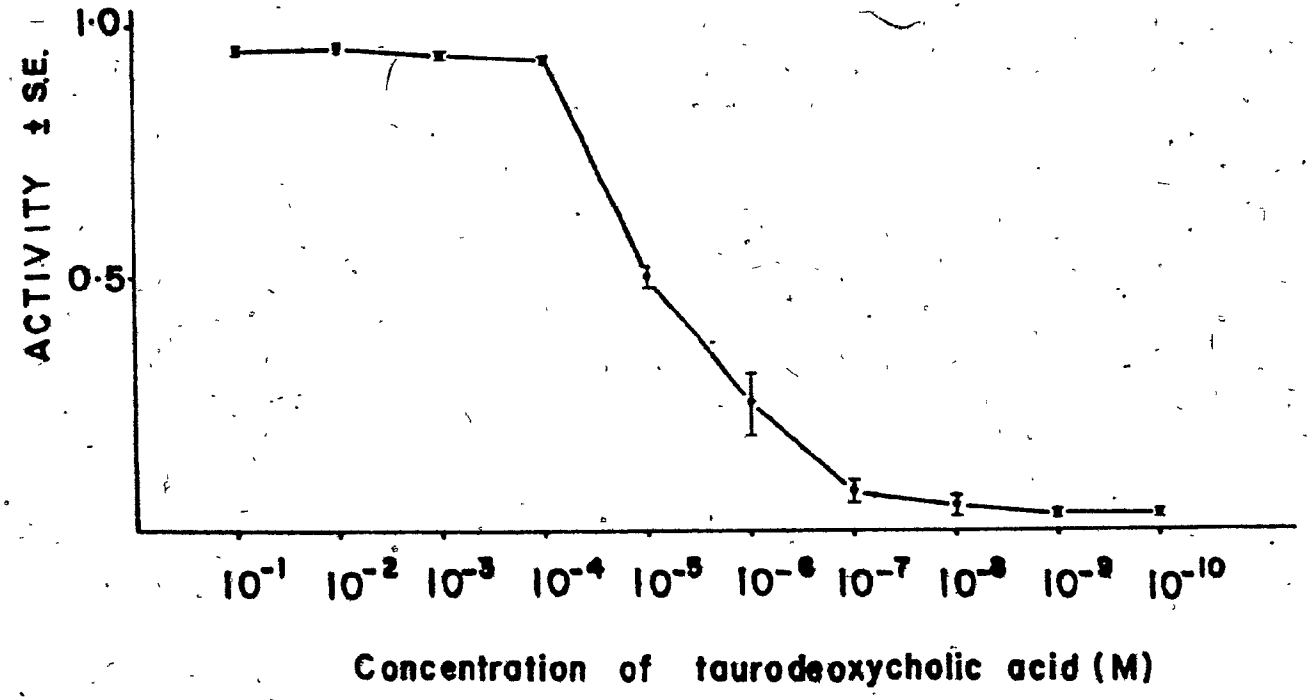

ACTIVITY ± S.E.
1·0
0·5
10⁻¹ 10⁻² 10⁻³ 10⁻⁴ 10⁻⁵ 10⁻⁶ 10⁻⁷ 10⁻⁸ 10⁻⁹ 10⁻¹⁰
Concentration of taurodeoxycholic acid (M)

FIGURE 7.8 The percent population of larvae of T. spiralis in type 2 activity 30 minutes after stimulation by 10% bile solution and after various intervals of digestion and refrigeration. A, the larvae were not refrigerated and B, the larvae were refrigerated at 4° C for 24 hours.

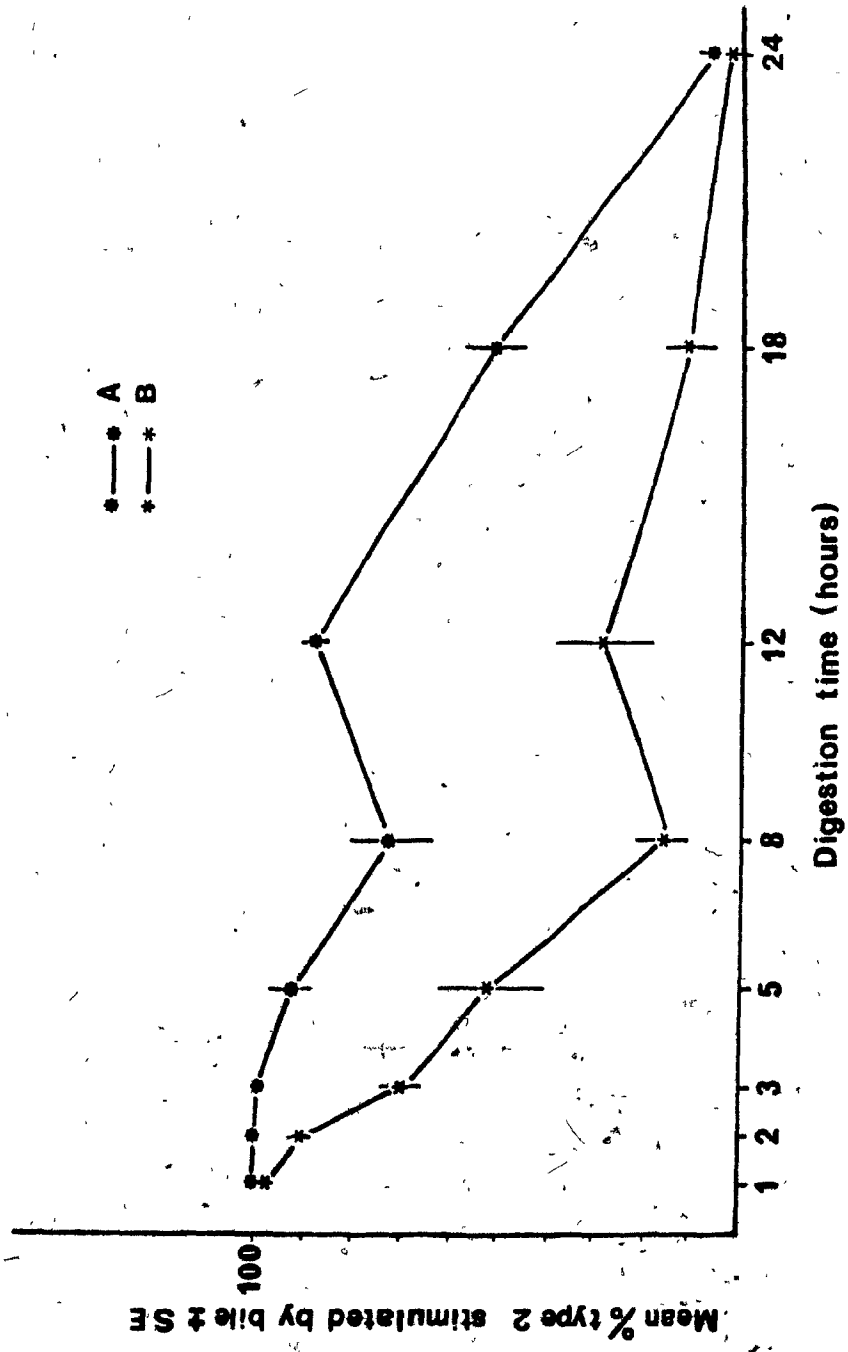

A
B
100
Mean % type 2 stimulated by bile ± SE
1 2 3 5 8 12 18 24
Digestion time (hours)

FIGURE 7.9 The mean % recovery of adults from the small intestine of white mice infected 5 days previously with larvae of T. spiralis that had been treated with various periods of digestion. A, the larvae were not refrigerated; B, the larvae were refrigerated for 24 hours at 4° C.

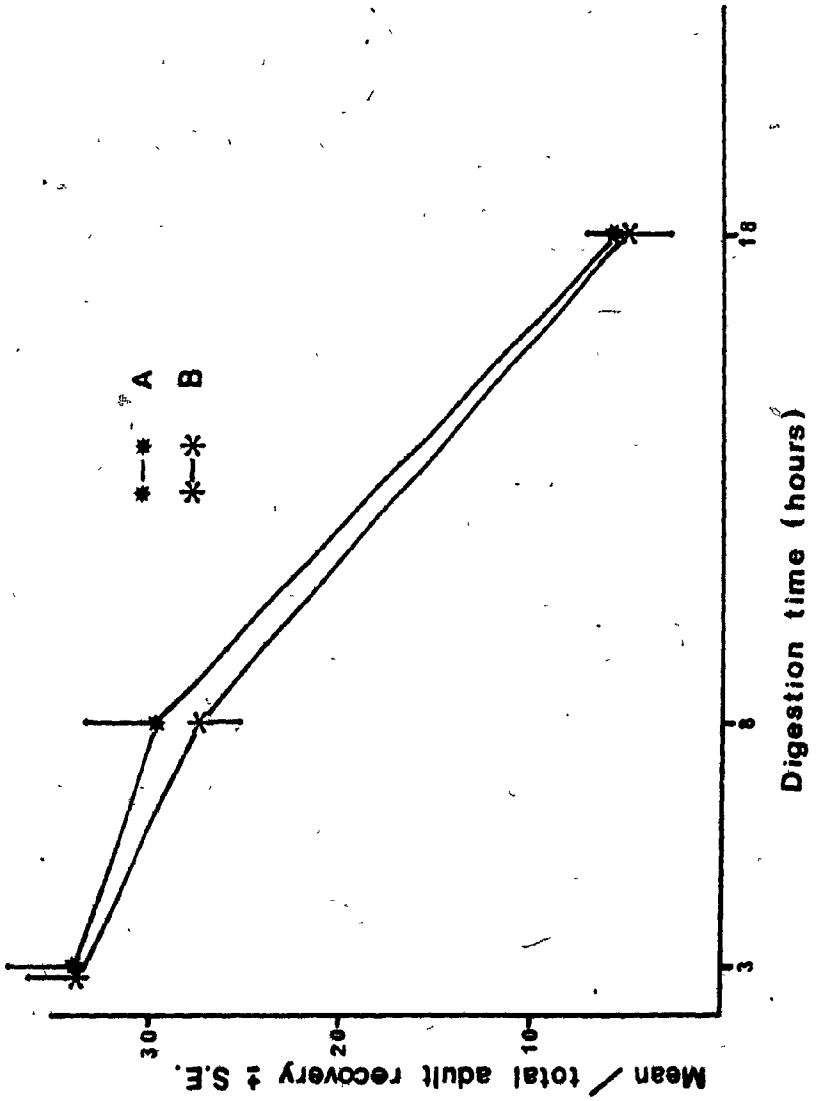

Mean / total adult recovery ± S.E.
30
20
10
3
8
18
Digestion time (hours)
A
B

TABLE 7.3 THE RECOVERY OF ADULTS FROM THE SMALL INTESTINE 5 DAYS AFTER INFECTION WITH 500 LARVAE OF T. SPIRALIS

Digestion time	Refrigeration (4° C)	Mean % Recovery ± S. E.		$p \leq 0.05$
3	0 hrs	34.5 ± 3.3	(a)	
3	24 hrs	33.8 ± 2.8	(a)	N.S.
8	0 hrs	30.3 ± 3.7	(a)	
8	24 hrs	28.2 ± 2.3	(a)	N.S.
18	0 hrs	4.8 ± 1.1	(b)	
18	24 hrs	5.1 ± 2.2	(b)	N.S.

(a)(b) all values with the same superscript do not differ significantly ($p \leq 0.05$)

FIGURE 7.10 The recovery of adults from the small intestine of mice 5 days after infection with 500 larvae of T. spiralis given by implantation into the ileum. A, controls; B, the larvae were treated with 10% bile for 30 minutes at 37° C prior to implantation.

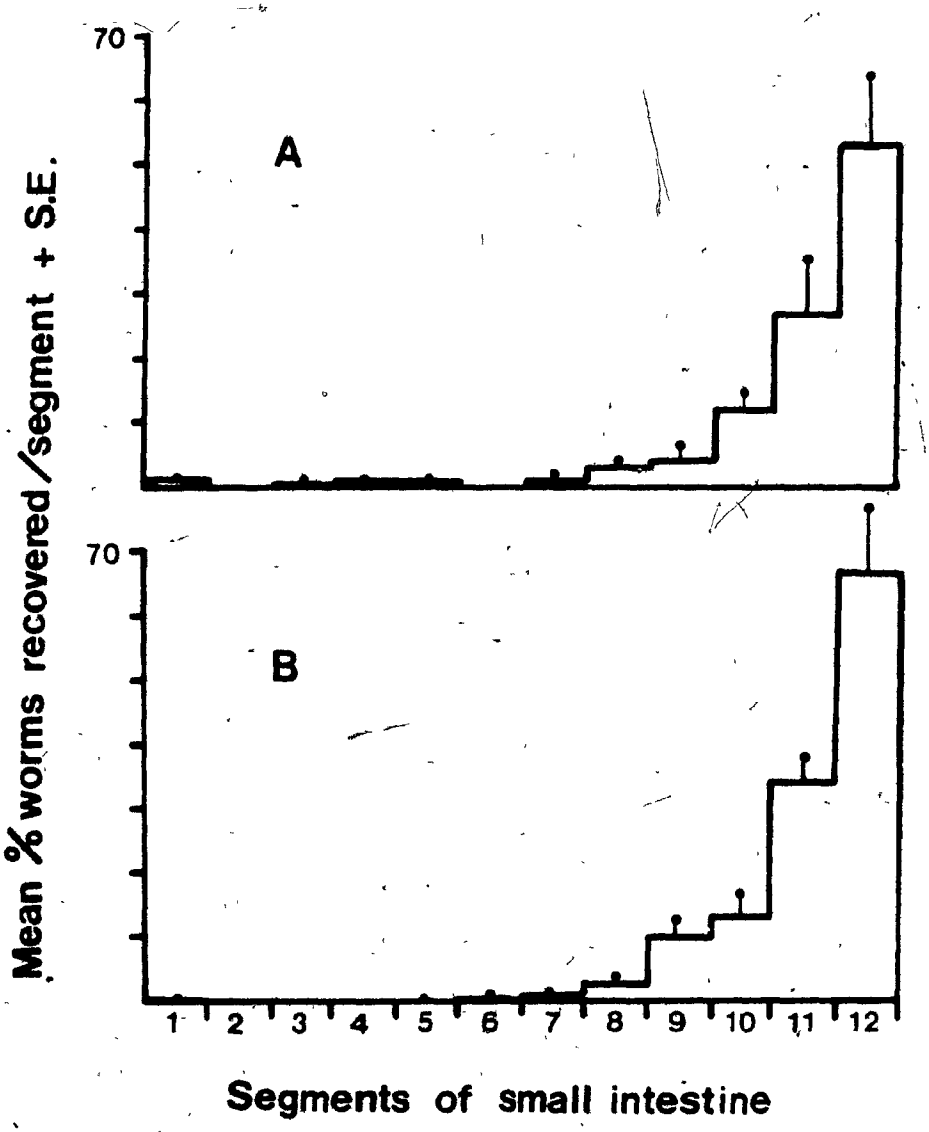

70
A
70
B
Mean % worms recovered/segment + S.E.
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

TABLE 7.4 THE RECOVERY OF ADULTS FROM THE SMALL INTESTINE OF MICE 5 DAYS AFTER INFECTION WITH 500 LARVAE OF T. SPIRALIS

Group	Treatment	Route	Mean # Adults Recovered ± S. E.	$p \leq 0.05$
1	Control	Implanted into ileum	52.5 ± 13.7	N. S.
	Bile Stimulation	"	84.5 ± 10.7	
2	Control	oral	159.0 ± 28.6	N. S.
	Prolonged bile stimulation	"	107.0 ± 32.5	

was normal (Figure 7.11, Table 7.4).

Several attempts were made to stimulate penetration of larvae in vitro using several methods of preparing intestinal tissue. After more than 50 attempts, the act of penetration has never been visualized. This has consistently eluded many previous investigators.

The crude mucosal extract prepared from mouse gut stimulates type 2 activity in the larvae of T. spiralis (Figure 7.12). The factor or factors responsible for this stimulating effect are mainly in the small intestine and its effect is significantly reduced by denaturation.

The crude mucosal extract consists of mucus, "succus entericus" and cell debris. The individual components of mucus did not activate the larvae but crude pig mucin did cause a small, but significant, increase in activity when compared to controls (Table 7.5).

"Succus entericus" activated the larvae (Figure 7.13) and a log-dose response of the larvae shows that the larvae are sensitive to concentrations of 0.001% (Figure 7.14). One component of "succus entericus" that stimulated type 2 activity was enterokinase. α-amylase was not as potent and peptidase did not stimulate any activity (Table 7.6). Included in this assay was the hormone secretin which was also a potent stimulator of type 2 activity. In the log-dose response curve of the larvae to enterokinase there is a linear increase in the activity of the larvae of T. spiralis from 0.008 to 0.08 units enterokinase/ml, above and below these concentrations, the

FIGURE 7.11 The recovery of adults from the small intestine of mice 5 days after infection with 500 larvae of T. spiralis given by oral inoculation. A, controls; B, the larvae were treated with 10% bile for 6 hours at 37° C prior to infection.

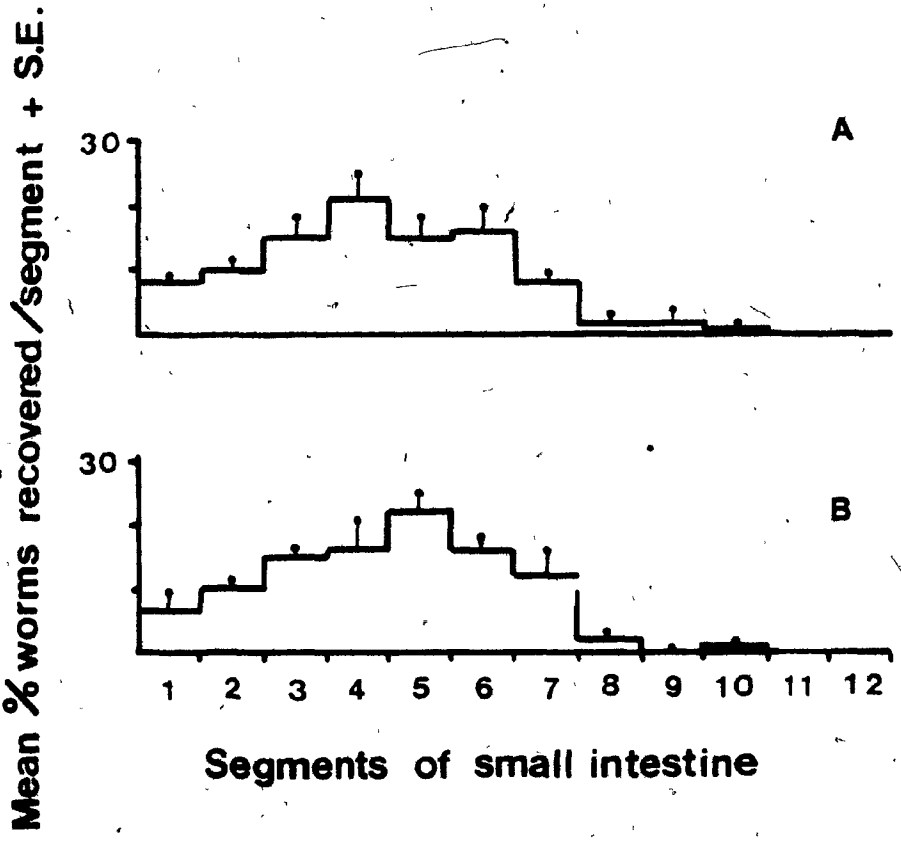

Mean % worms recovered/segment + S.E.
30
A
30
B
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

FIGURE 7.12 The activity (see p 292) of the larva of T. spiralis in response to stimulation with the crude mucosal extract from different regions of the gut. A, normal extracts; B, heat-denatured extract. S, saline control; AI, anterior small intestine; PI, posterior small intestine; CA, caecum; CO, colon; Bile, 10% bile control.

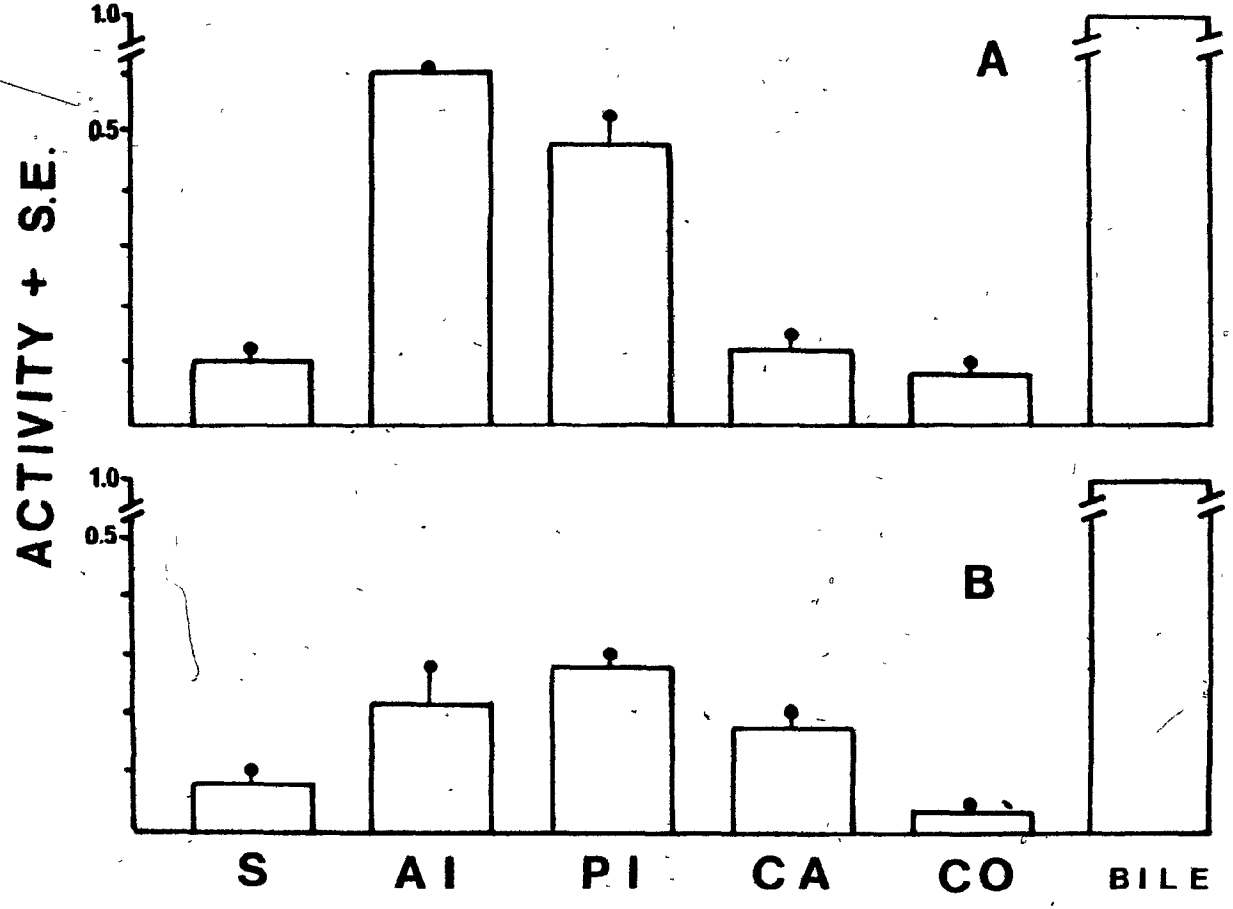

ACTIVITY + S.E.
1.0
0.5
A
1.0
0.5
B
S
AI
PI
CA
CO
BILE

TABLE 7.5 THE EFFECTS OF MUCUS AND COMPONENTS OF MUCUS IN BUFFERED SALINE (pH 7.2) ON THE ACTIVITY OF LARVAE OF T. SPIRALIS IN VITRO AT 37° C

Treatment	Concentration	Activity	$p \leq$
Mucin	1%	0.16 ± 0.01	0.05 (a)
N-acetyl-D-glycosamine	0.1M	0.03 ± 0.01	N.S.
D Galactose	0.1M	0.06 ± 0.01	N.S.
D Fucose	0.1M	0.05 ± 0.01	N.S.
N-acetyl-D-galactosamine	0.1M	0.05 ± 0.01	N.S.
N-acetyl-neuramic acid	0.1M	0.04 ± 0.01	N.S.
Controls			
Buffered Saline (pH 7.2)	0.85%	0.05 ± 0.02	
Bile	10%	0.97 ± 0.02	0.05 (a)

(a) significantly different from buffered saline (pH 7.2) controls ($p \leq 0.05$)

FIGURE 7.13 The behavioural response of the larvae of T. spiralis to stimulation with "succus entericus". A, the rate of movement of the larvae and B, the activity (see p 292) of the larvae.

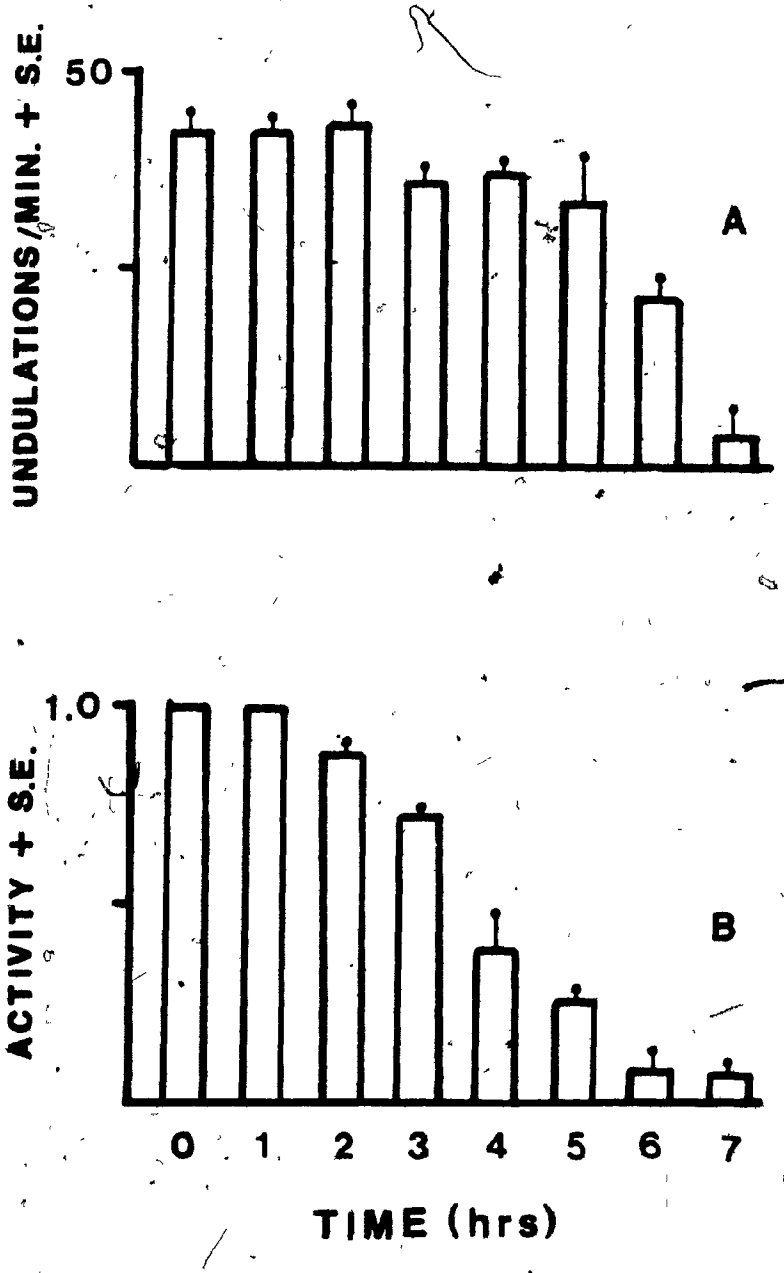

UNDULATIONS/MIN. + S.E.
50
A
ACTIVITY + S.E.
1.0
B
0 1 2 3 4 5 6 7
TIME (hrs)

FIGURE 7.14 The activity (see p 292) of the larvae of T. spiralis in log dilutions of "succus entericus".

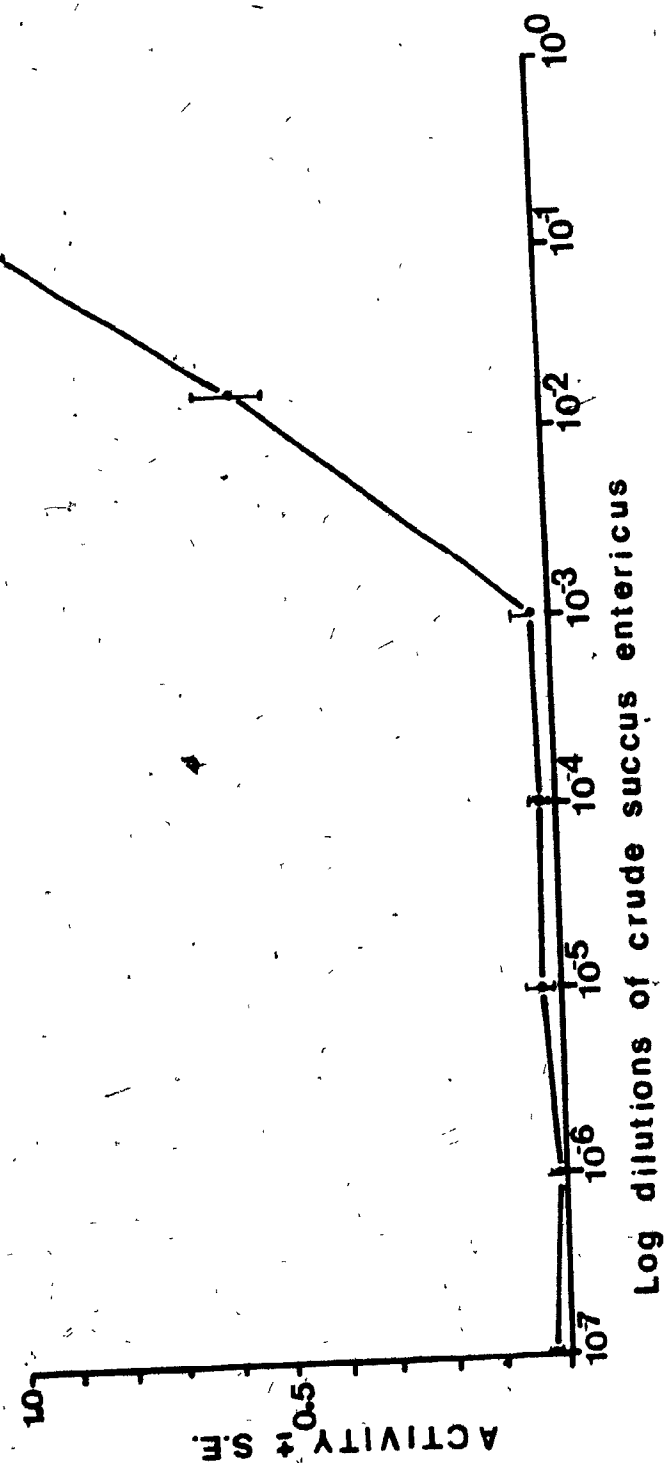

ACTIVITY ± S.E.
1.0
0.5
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
Log dilutions of crude succus entericus

TABLE 7.6 THE EFFECTS OF THE COMPONENTS OF "SUCCUS ENTERICUS" DILUTED IN BUFFERED SALINE (pH 7.2) ON THE ACTIVITY OF LARVAE OF T. SPIRALIS

Treatment	Concentration (a)	Activity	$p \leq$
Peptidase (porcine intestinal mucosa	0.2 units/ml	30.2 6.9	N. S.
α - amylase (porcine pancreas)	2500 units/ml	53.9 ± 8.7	0.05 (b)
Enterokinase (porcine intestine)	80 units/ml	94.5 ± 3.5	0.05
Secretin (porcine	20 units/ml	99.7 ± 0.2	0.05
Saline (control)	0.85%	22.3 ± 3.6	-

(a) - 1 unit peptidase will liberate 1 µmole of B-napthylamine from L-leucine-B-napthylamide per minute at pH 7.1 at 37° C

- 1 unit α-amylase will liberate 1.0 mg maltose from starch in 3 minutes at pH 6.9 at 37° C

- 1 unit enterokinase will activate 0.005 mg crystalline trypsinogen per hour at pH 5.8 at 5° C

- secretin in Crick units

(b) significantly different from saline controls ($p \leq 0.05$)

response plateaus (Figure 7.15). Enterokinase (40 units/ml) in solutions of distilled water and buffered solutions of pH 5.0, 7.2 and 8.0 was equally effective in stimulating 100% type 2 activity in the larvae of T. spiralis (Table 7.7).

Pre-treatment of larvae with "succus entericus" for 30 minutes prior to implantation into the duodenum resulted in a distribution that was anterior to that of the controls (Figure 7.16). There was no significant difference in the numbers of worms establishing between the pre-treated larvae and controls (Table 7.8).

Bile and "succus entericus" both significantly increased the ability of the larvae of T. spiralis to exit from an artificial bolus of agar into which they had been embedded (Table 7.9) and to penetrate into agar (Table 7.10). Prior to dispersion and penetration, the larvae entered type 2 activity. Figure 7.17A shows the sinusoidal tracks of the larvae of T. spiralis moving through agar after stimulation with bile.

There were no significant differences in the activity of the larvae of T. spiralis after the incubation medium was gassed with air, 5% CO_2 - 95% N_2 and 50% CO_2 - 50% N_2 (Table 7.11).

2) N. dubius.

At 22° C, all of the larvae of N. dubius responded rapidly to mechanical stimulation but were inactive within 20 minutes (Figure 7.18A). In bile, the maximum rates of movement were similar when compared to worms that were mechanically stimulated but the activity in the bile-treated worms consistently persisted for 3 hours (Figure 7.18B) with a gradual reduction

FIGURE 7.15 The activity (see p 292) of the larvae of T. spiralis in log dilutions of enterokinase (80 units/ml).

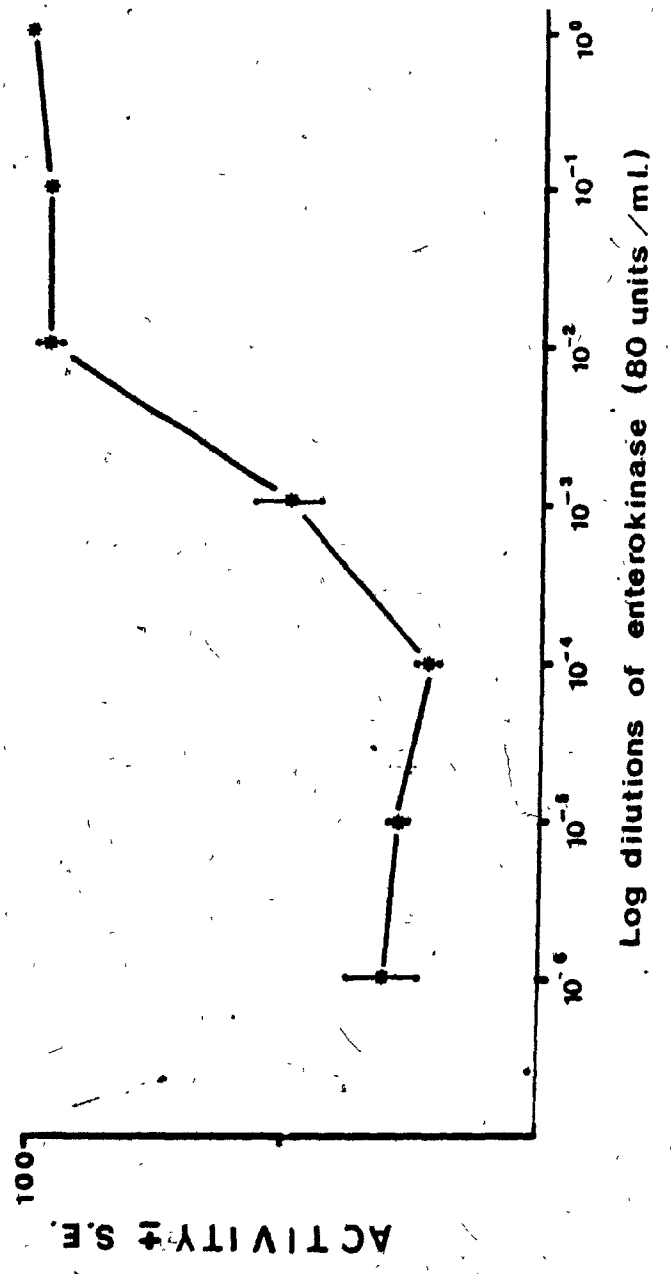

ACTIVITY ± S.E.
100
Log dilutions of enterokinase (80 units/ml.)
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}

TABLE 7.7 THE ACTIVITY OF THE LARVAE OF T. SPIRALIS 30 MINUTES AFTER STIMULATION WITH ENTEROKINASE IN VARIOUS BUFFERED SOLUTIONS AT 37° C

Solution	pH	Activity (a) ± S.E.	p ≤
distilled water	6.8	1.0 ± 0.00	N.S.
citrate buffer	5.0	1.0 ± 0.00	N.S.
buffered saline	7.2	1.0 ± 0.00	N.S.
Tris buffer	8.0	1.0 ± 0.00	N.S.

(a) Activity (see p 292)

FIGURE 7.16 The recovery of adults from the small intestine of mice 5 days after infection with 500 larvae of T. spiralis given by implantation into the duodenum. A, controls and B, the larvae were pre-treated with "succus entericus" for 30 minutes at 37° C prior to implantation.

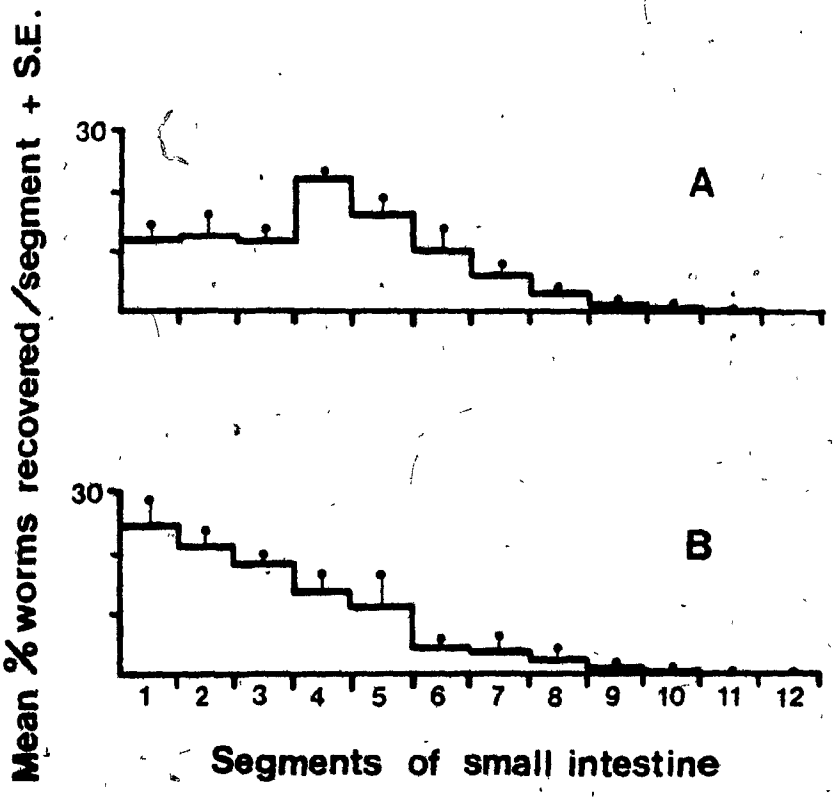

Mean % worms recovered/segment + S.E.
30
A
30
B
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

TABLE 7.8 THE NUMBERS OF ADULTS OF T. SPIRALIS RECOVERED FROM THE SMALL INTESTINE OF MICE 5 DAYS AFTER IMPLANTATION OF 500 LARVAE INTO THE DUODENUM

Treatment	Implanted into	Mean # Adults Recovered ± S. E.	$p \leq 0.05$
Controls	Duodenum	250.3 ± 2.0	
Pre-treatment with "succus entericus"	Duodenum	254.6 ± 23.7	N.S.

TABLE 7.9 THE EFFECT OF BILE AND "SUCCUS ENTERICUS" IN BUFFERED SALINE (pH 7.2) ON THE DISPERSION OF THE LARVAE OF *T. SPIRALIS* FROM AN ARTIFICIAL FOOD BOLUS *IN VITRO* AT 37° C

Treatment	Concentration	Dispersion ratio[b]	$p \leq$
Bile	10%	9.27 ± 1.59	0.05[a]
"Succus entericus"	10%	10.46 ± 2.32	0.05
Saline	0.85%	0.44 ± 0.06	-

(a) significantly different from saline controls ($p \leq 0.05$)

(b) dispersion ratio = $\frac{\text{\# larvae out of bolus}}{\text{\# larvae in bolus}}$

TABLE 7.10 THE EFFECTS OF BILE AND "SUCCUS ENTERICUS" IN BUFFERED SALINE (pH 7.2) ON PENETRATION BEHAVIOUR OF THE LARVAE OF T. SPIRALIS IN VITRO AT 37° C

Treatment	Mean % penetration	$p \leq$
Bile	9.4 ± 2.9	0.05 (a)
"Succus entericus"	14.5 ± 3.4	0.05
Saline	0.9 ± 0.6	-

(a) significantly different from saline controls ($p \leq 0.05$)

FIGURE 7.17 The tracks of worms (visualized through bacterial overgrowth) as they migrate through agar. A, the tracks of the larvae of T. spiralis and B, the tracks of the larvae of N. dubius.

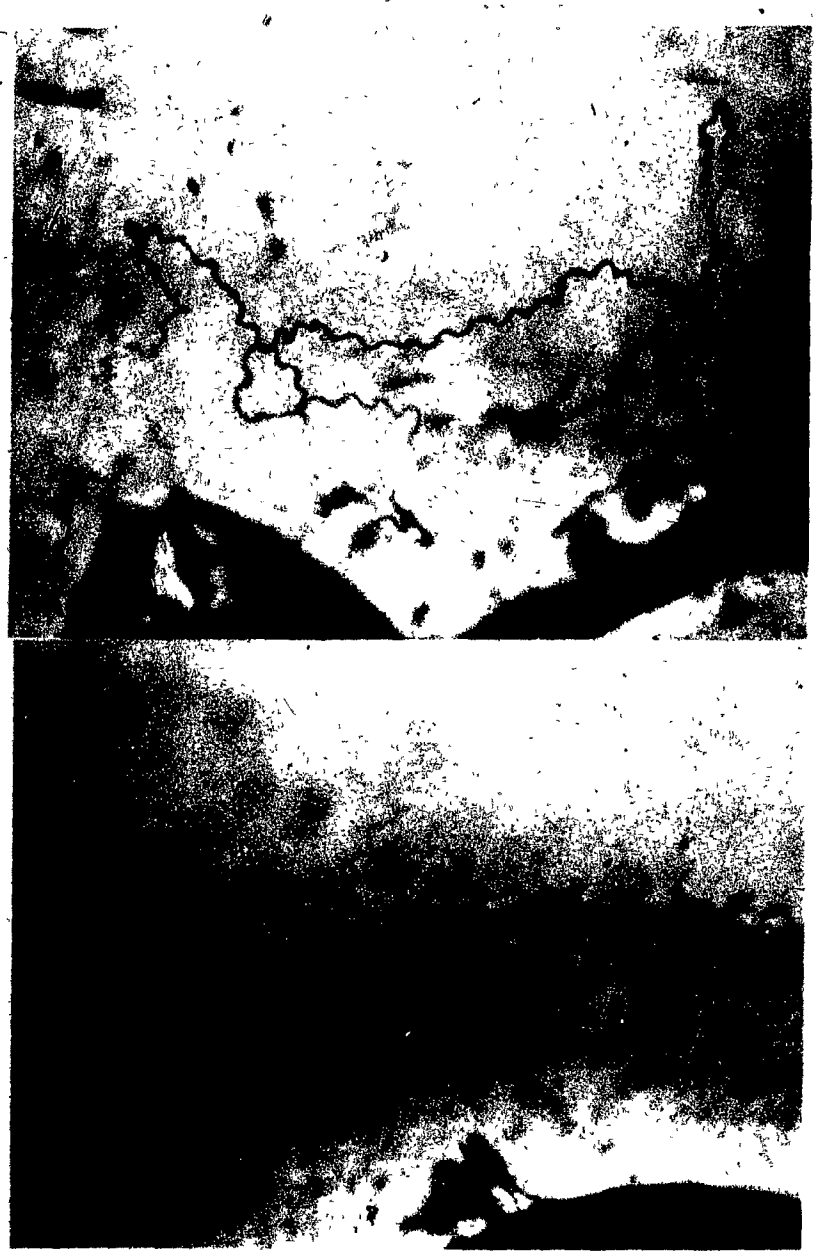
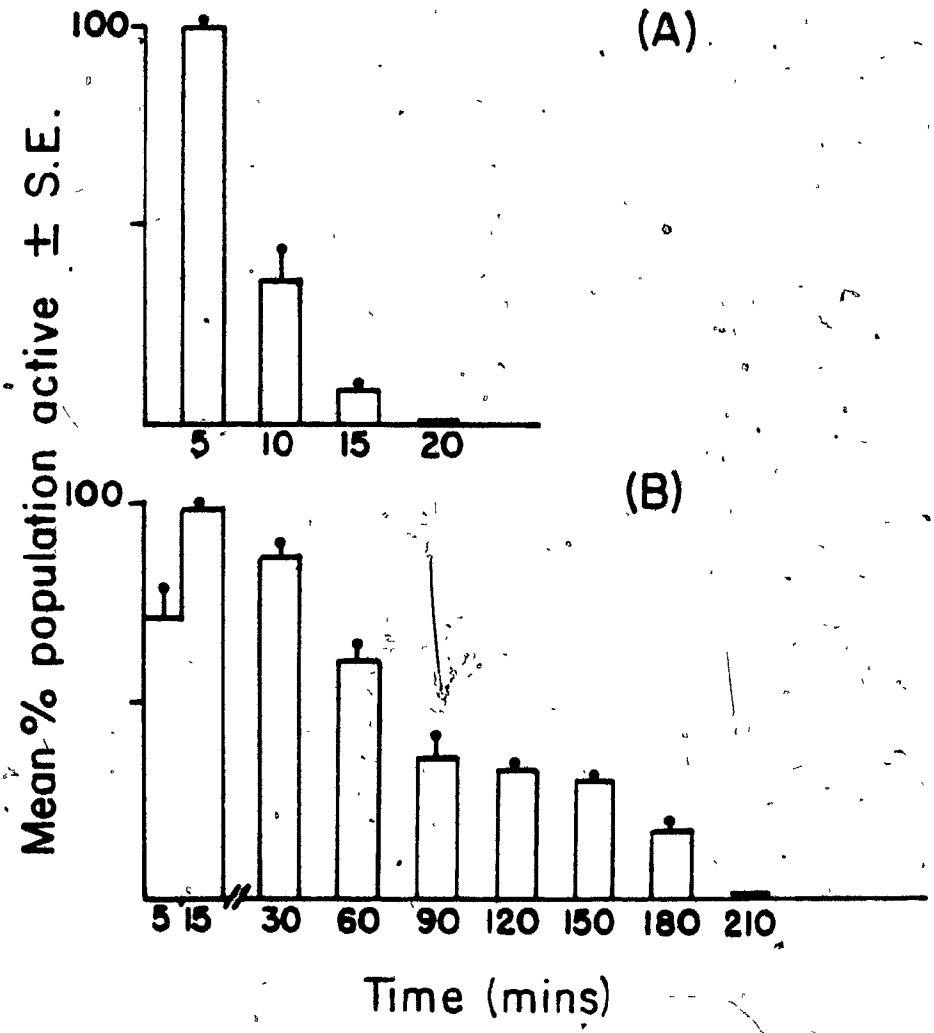


TABLE 7.11 THE EFFECTS OF GAS MIXTURES ON THE ACTIVITY OF THE LARVAE OF T. SPIRALIS INCUBATED IN BUFFERED SALINE (pH 7.2) AT 37° C

Gas Mixture	Activity (a)	$p \leq$
Air	0.12 ± 0.05	-
5% CO_2 - 95% N_2	0.12 ± 0.02	N.S.
50% CO_2 - 50% N_2	0.15 ± 0.05	N.S.

(a) Activity (see p 292).

FIGURE 7.18 The population activity of the larvae of N. dubius in response to stimuli at 22° C. A, mechanical stimuli and B, stimulation with 10% bile.


100
(A)
Mean % population active ± S.E.
5
10
15
20
100
(B)
5
15
30
60
90
120
150
180
210
Time (mins)

in the rate of activity after 90 minutes (Figure 7.20B). This differs from the fairly abrupt cessation of activity after mechanical stimulation; the rates of movement of 4 previously unstimulated worms after mechanical stimulation is shown in Figures 7.19 and 7.20A. A repetition of this experiment at 37° C gave similar results, but, the population activity in larvae stimulated mechanically did not exceed 10% of the population (Figure 7.21A). The responses to bile at 37° C was similar to the response at 22° C and the activity of these larvae persisted for 3 hours (Figure 7.21B). The rates of movement of larvae stimulated mechanically and with bile at 37° C are shown in Figure 7.22; the activity rates of these worms were similar to the activity rates of worms at 22° C.

The log-dose response curve of the activity of the larvae of N. dubius in bile showed a linear increase between 0.2% and 0.5% bile, at concentrations above and below, the response plateaus (Figure 7.23).

In the bile-gradient studies, the larvae placed in the centre of the control plates (template is shown in Figure 7.24) were dispersed randomly. Significantly more larvae ($p \leq 0.05$) were recovered from the sector containing bile than the sector containing distilled water (Figure 7.25).

All the bile salts, with the exception of sodium deoxycholate and sodium taurodeoxycholate, were capable of stimulating some activity in the larvae of N. dubius. The glycine-conjugated bile salts were much more potent than the

FIGURE 7.19 The rate and duration of movement of 4 individual larvae of N. dubius after mechanical stimulation.

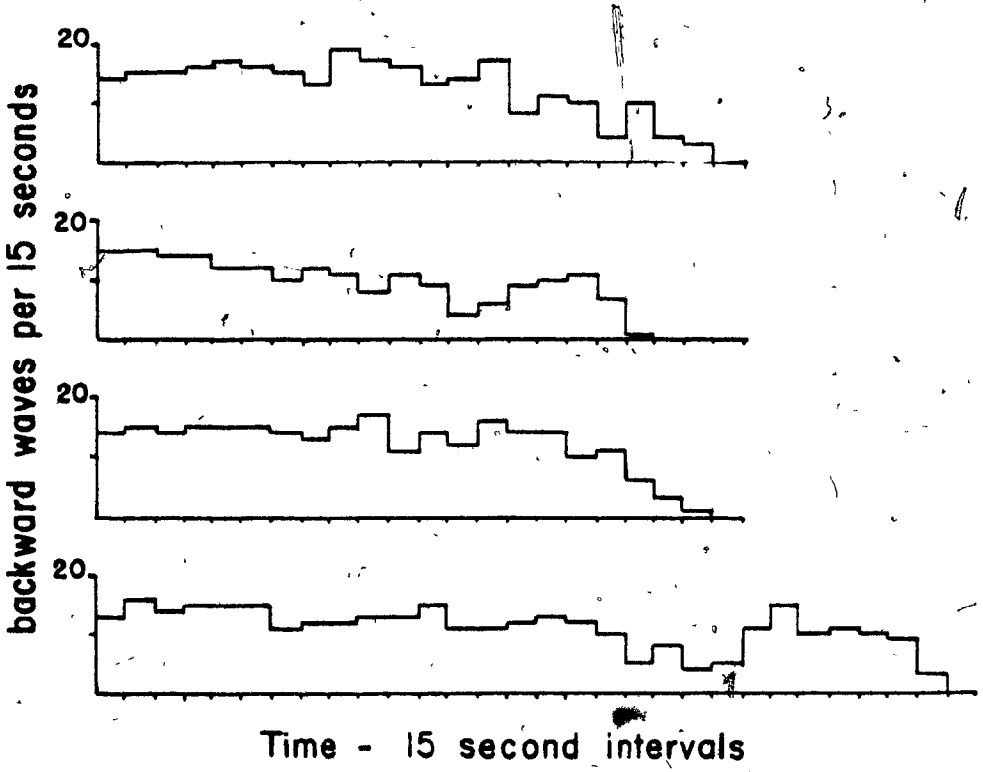

20
20
20
20
backward waves per 15 seconds
Time - 15 second intervals

FIGURE 7.20 The rate of movement of the larvae of N. dubius in response to stimuli at 22° C. A, mechanical stimuli and B, stimulation with 10% bile.

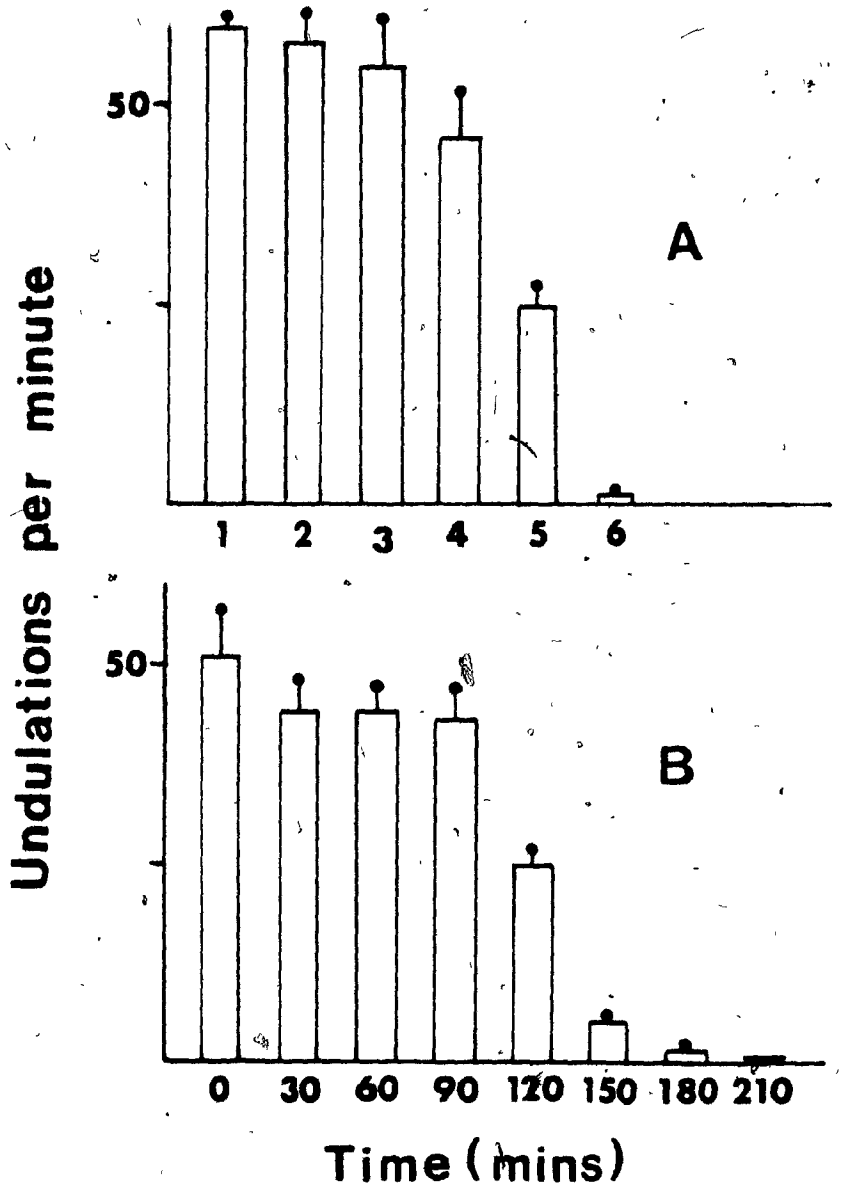

Undulations per minute
50
A
1
2
3
4
5
6
50
B
0
30
60
90
120
150
180
210
Time (mins)

FIGURE 7.21 The population activity of the larvae of N. dubius in response to stimuli at 37° C. A, mechanical stimuli and B, stimulation with 10% bile.

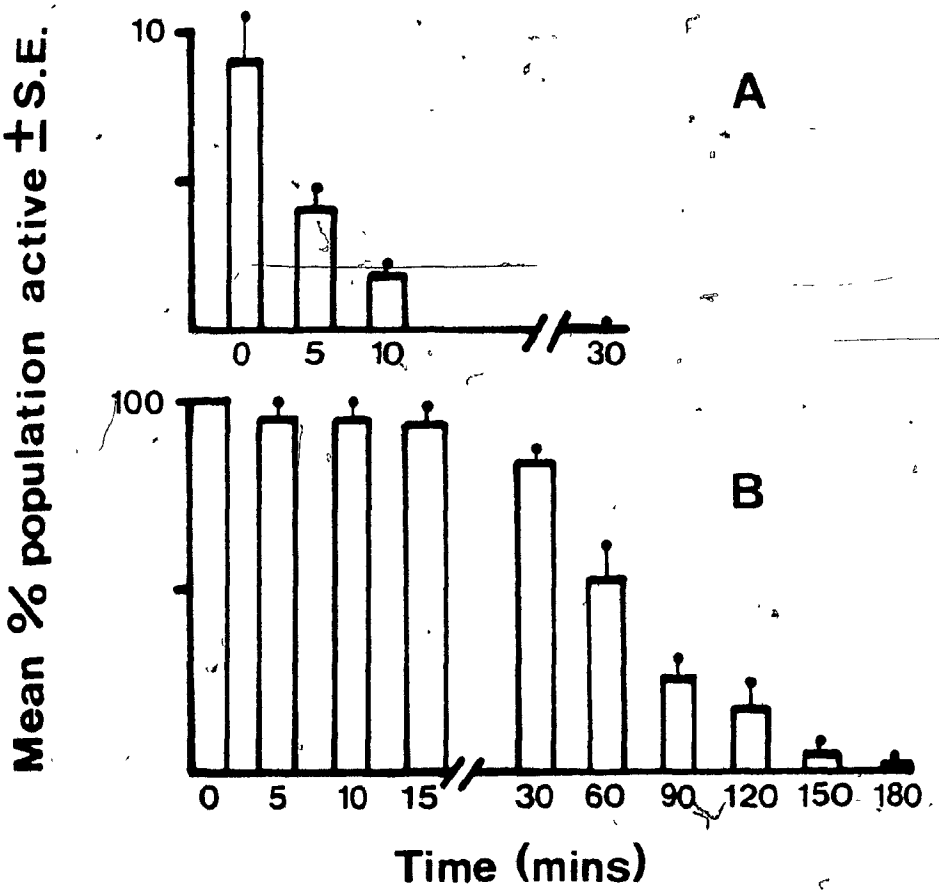

Mean % population active ± S.E.
10
A
0
5
10
30
100
B
0
5
10
15
30
60
90
120
150
180
Time (mins)

FIGURE 7.22 The rate of movement of the larvae of N. dubius in response to stimuli at 37° C. A, mechanical stimuli and B, stimulation with 10% bile.

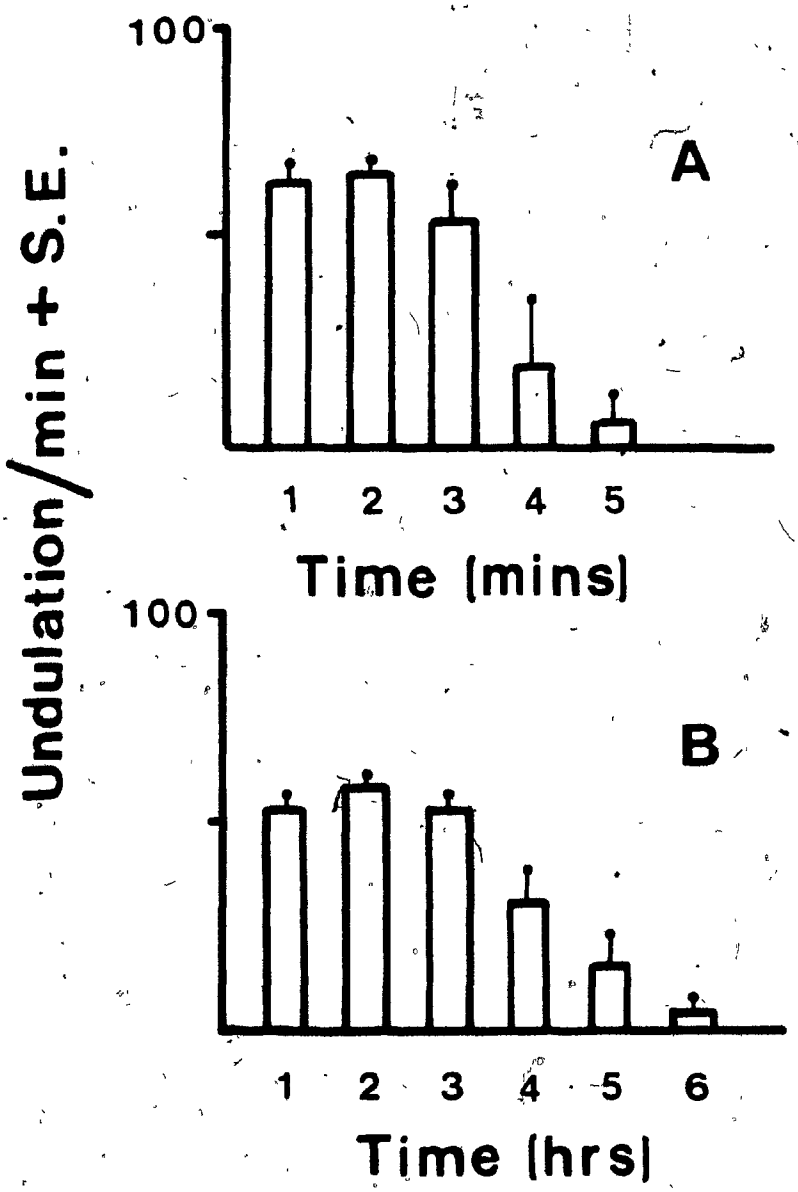
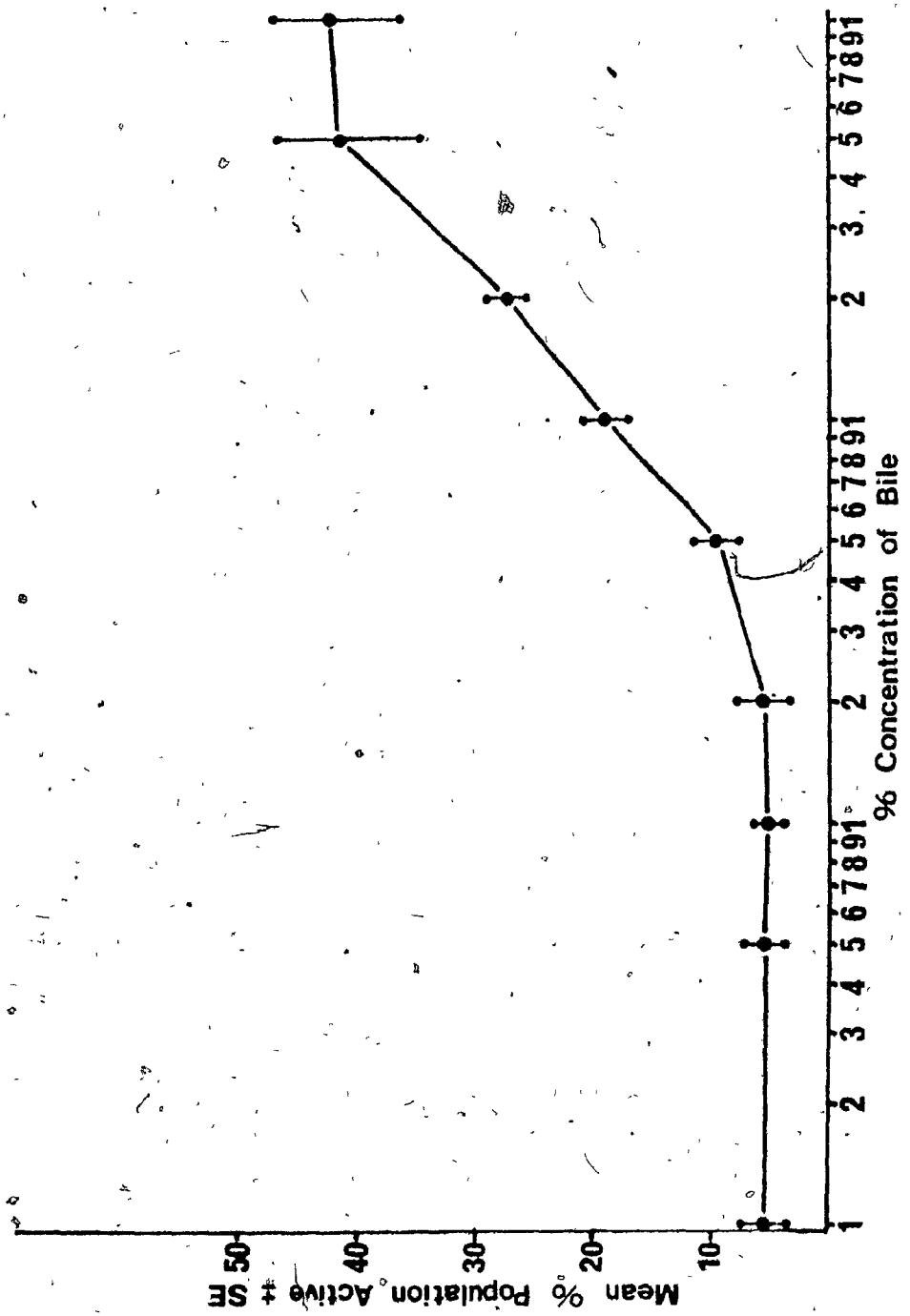

100
A
Undulation/min + S.E.
1 2 3 4 5
Time (mins)
100
B
1 2 3 4 5 6
Time (hrs)

FIGURE 7.23 The population activity of the larvae of N. dubius in response to log dilutions of bile after 30 minutes incubation at 37° C.


Mean % Population Active ± SE
50
40
30
20
10
1 2 3 4 5 6 7 8 9 1 2 3 4 5 6 7 8 9 1 2 3 4 5 6 7 8 9 1
% Concentration of Bile

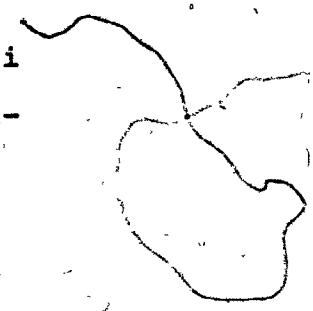


FIGURE 7.24 The template used to divide the petri dishes in the bile gradient studies. Sector 4 contained the well.

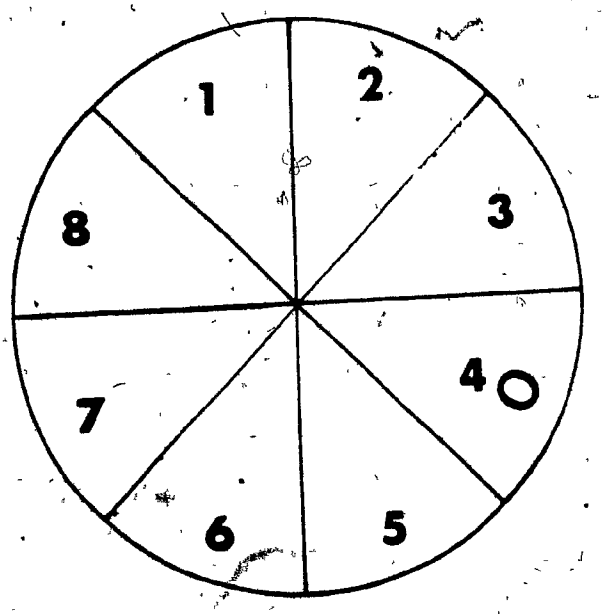
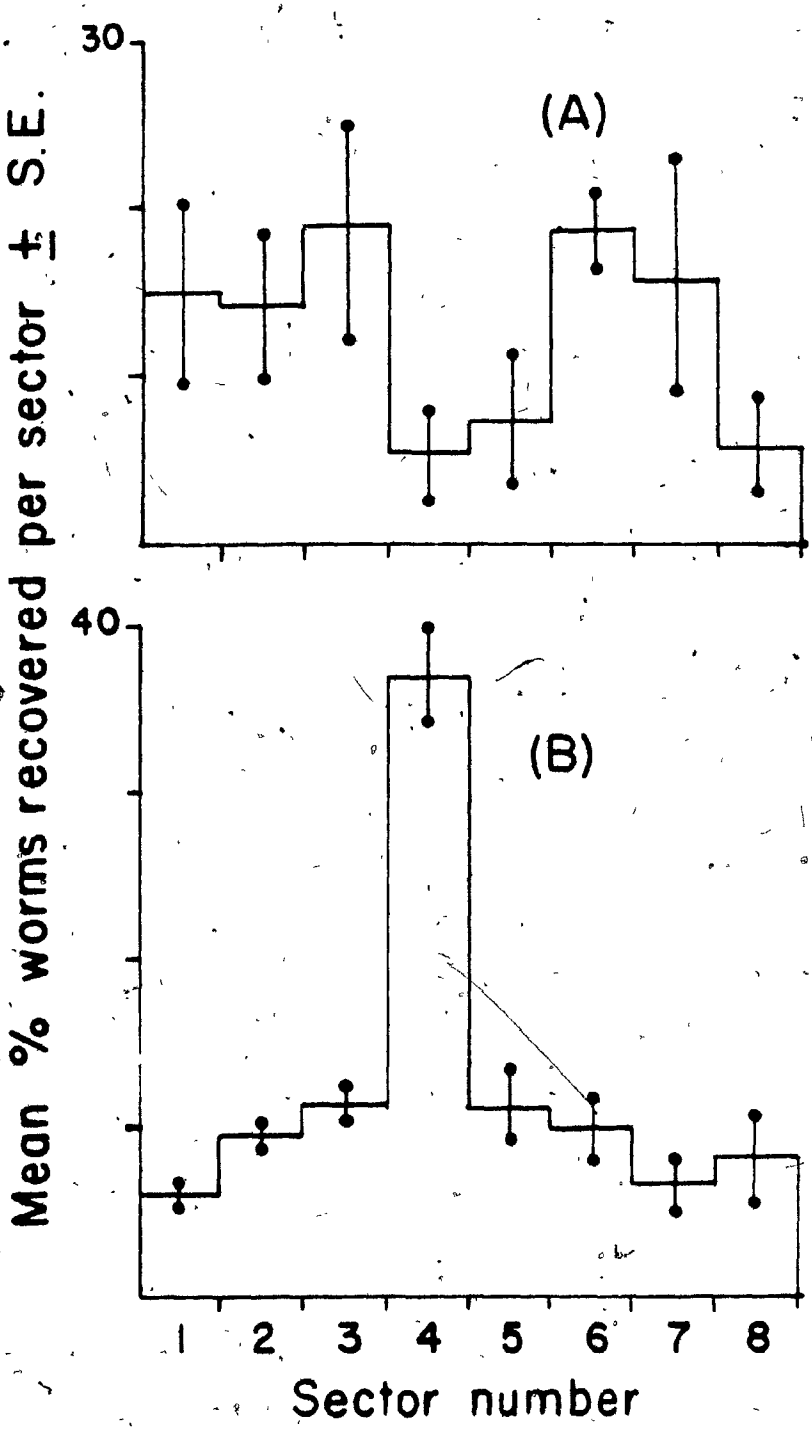

1
2
3
4
5
6
7
8

FIGURE 7.25 The number of larvae of N. dubius recovered from each sector of the petri dish after 30 minutes incubation at 25° C. A, distilled water controls and B, bile was placed in the well.


30
(A)
40
(B)
Mean % worms recovered per sector ± S.E.
1 2 3 4 5 6 7 8
Sector number

other salts; the most potent were sodium glycocholate and sodium glycochenodeoxycholate (Table 7.12). Prolonged pre-treatment with bile did affect the infectivity (Table 7.13) but not the intestinal distribution of the larvae (Figure 7.26).

HCl was the only gastric secretion that caused larval activity of long duration in vitro (Table 7.14). The behaviour stimulated by HCl was similar to the behaviour stimulated by bile. Although a low pH was necessary, H_2SO_4 at the same pH did not stimulate this behaviour. Pepsin and various gas phases had no effect on larval behaviour (Table 7.14).

Crude mucosal extract did not stimulate the larvae (Figure 7.27). "Succus entericus" stimulated activity of the larvae (Figure 7.28) but the activity was transitory (Figure 7.29).

There were no significant differences in the numbers of larvae dispersing from an artificial agar bolus (Table 7.15) or penetrating into agar (Table 7.16) when stimulated by bile or "succus entericus" and compared with controls. Manipulation and handling during this experiment were sufficient stimulus to activate the worms in the controls. Figure 7.17B shows the sinusoidal tracks of a larva of N. dubius moving through agar.

7D. DISCUSSION

1) T. spiralis.

It is now accepted that all stages of T. spiralis are intra-cellular (Gould, 1970; Gardiner, 1977; Despommier

TABLE 7.12 THE EFFECTS OF BILE SALTS IN BUFFERED SALINE (pH 7.2) ON THE ACTIVITY OF THE LARVAE OF N. DUBIUS IN VITRO AT 37° C

Bile salt	Concentration	Activity	p≤ (a)
Sodium cholate	1.0	16.6 ± 2.8	0.05
Sodium deoxycholate	1.0	1.5 ± 0.8	N.S.
Sodium glycocholate	1.0	78.2 ± 5.8	0.05
Sodium taurocholate	1.0	22.1 ± 9.2	0.05
Sodium taurodeoxycholate	1.0	12.3 ± 4.3	N.S.
Sodium glycochenodeoxycholate	0.1	76.8 ± 6.4	0.05
Sodium taurochenodeoxycholate	0.1	33.4 ± 7.6	0.05
Buffered saline (pH 7.2)	0.85%	2.8 ± 1.3	-

(a) significantly different from controls

TABLE 7.13 THE RECOVERY OF WORMS FROM THE SMALL INTESTINE OF MICE 6 DAYS AFTER INFECTION WITH 100 LARVAE OF N. DUBIUS

Treatment	Mean # adults Recovered ± S. E.	$p \leq 0.05$
Control	80.6 ± 1.7	
		0.05
Prolonged bile stimulation	64.3 ± 1.8	

TABLE 7.14 THE EFFECTS OF GASTRIC SECRETIONS AND VARIOUS GAS PHASES ON THE ACTIVITY OF THE LARVAE OF N. DUBIUS IN VITRO AT 37°C

Solution	pH	Mean % population active with time (mins) Time < 5	30	60	120	180
HCl	2.0	100	26.3 ± 7.6	21.4 ± 3.3	23.0 ± 3.4	10.8 ± 0.7
+ 5% CO_2 - 95% N_2	2.0	100	18.7 ± 3.2	0	0	0
+ 50% CO_2 - 50% N_2	2.0	100	11.5 ± 3.1	0	0	0
+ 100% CO_2	2.0	100	16.3 ± 5.1	1.6 ± 1.6	0	0
HCl + 1% pepsin	2.0	100	7.6 ± 1.8	8.3 ± 1.4	3.5 ± 0.5	0
+ 5% CO_2 - 95% N_2	2.0	100	6.1 ± 2.4	0	0	0
+ 50% CO_2 - 50% N_2	2.0	100	6.4 ± 3.5	0	0	0
+ 100% CO_2	2.0	100	10.4 ± 2.7	0	0	0
Citrate buffer 1% pepsin	4.0	100	0	0	0	0
+ 5% CO_2 - 95% N_2	4.0	100	0	0	0	0
+ 50% CO_2 - 50% N_2	4.0	100	0	0	0	0
+ 100% CO_2		100	0	0	0	0

TABLE 7.14 (cont'd)

Solution	pH	Mean % population active with time (mins) Time < 5	30	60	120	180
Tris buffer + 1% pepsin	8.0	100	0	0	0	0
+ 5% CO_2 - 95% N_2	8.0	100	0	0	0	0
+ 50% CO_2 - 95% N_2	8.0	100	0	0	0	0
+ 100% CO_2	8.0	100	0	0	0	0
H_2SO_4	2.0	100	0	0	0	0
+ 5% - 95% N_2	2.0	100	0	0	0	0
+ 50% CO_2 - 50% N_2	2.0	100	0	0	0	0
+ 100% CO_2	2.0	100	0	0	0	0
H_2SO_4 + 1% pepsin	2.0	100	0	0	0	0
+ 5% CO_2 - 95% N_2	2.0	100	0	0	0	0
+ 50% CO_2 - 50% N_2	2.0	100	0	0	0	0
+ 100% CO_2	2.0	100	0	0	0	0
H_2SO_4 + 1% pepsin 1% NaCl	2.0	100	13.4 ± 1.1	0	0	0
+ 5% CO_2 - 95% N_2	2.0	100	0	0	0	0
+ 50% CO_2 - 50% N_2	2.0	100	0	0	0	0
+ 100% CO_2	2.0	100	0	0	0	0

FIGURE 7.26 The recovery of larvae from the small intestine of mice 6 days after infection with 100 larvae of N. dubius given by oral inoculation. A, controls and B, the larvae were treated with 10% bile for 6 hours at 37° C, prior to infection.

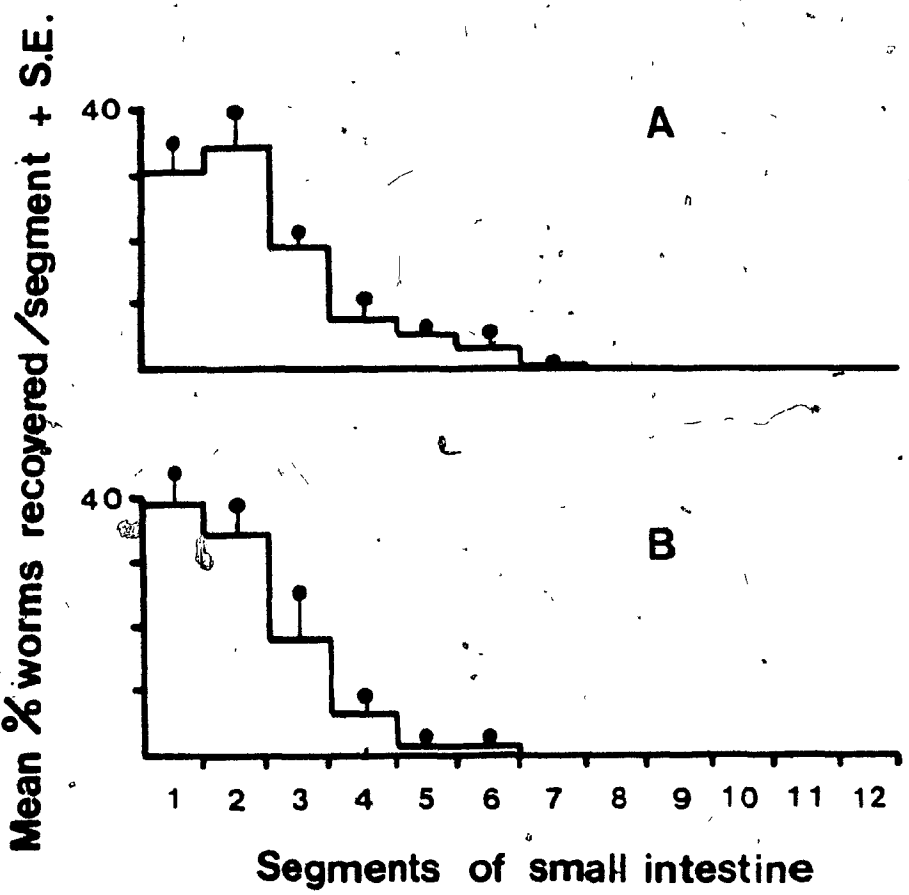

Mean % worms recovered/segment + S.E.
40
A
40
B
1 2 3 4 5 6 7 8 9 10 11 12
Segments of small intestine

FIGURE 7.27 The population activity of the larvae of N. dubius in response to stimulation with the crude mucosal extract from different regions of the gut. A, normal extract and B, heat-denatured extract. S, saline control; AI, anterior small intestine; PI, posterior small intestine; CA, caecum; CO, colon; Bile, 10% bile control.

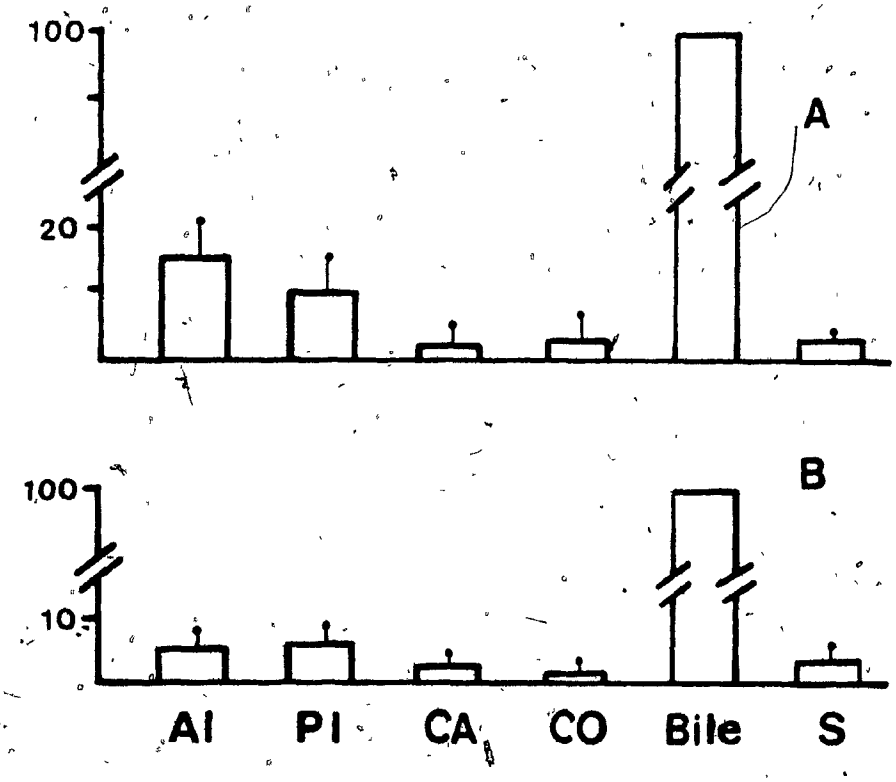

100
20
A
100
10
B
AI
PI
CA
CO
Bile
S

FIGURE 7.28 The population activity of the larvae of N. dubius in response to log dilutions of "succus entericus" after 10 minutes of incubation at 22° C.

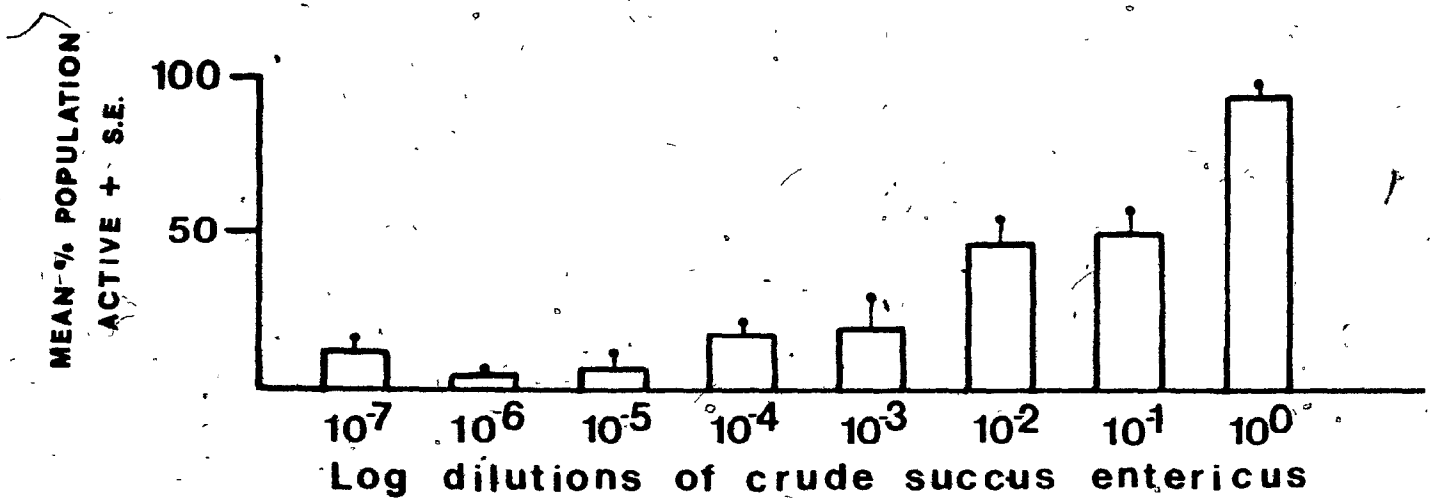

MEAN % POPULATION ACTIVE + S.E.
100
50
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
Log dilutions of crude succus entericus

FIGURE 7.29 The population activity of the larvae of N. dubius in response to stimulation with "succus entericus".

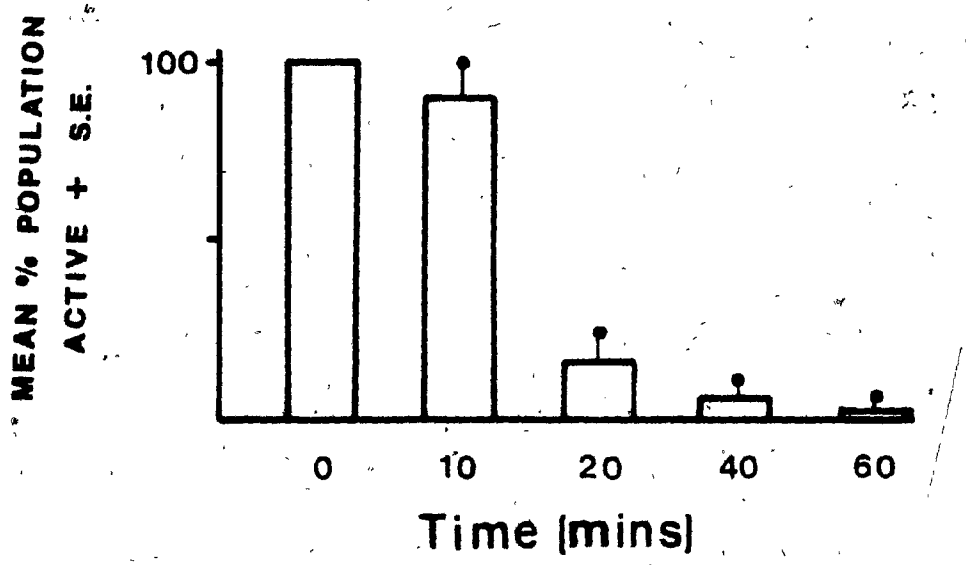

MEAN % POPULATION ACTIVE + S.E.
100
0
10
20
40
60
Time (mins)

TABLE 7.15 THE EFFECT OF BILE AND "SUCCUS ENTERICUS" IN BUFFERED SALINE (pH 7.2) ON THE DISPERSION OF THE LARVAE OF N. DUBIUS FROM AN ARTIFICIAL BOLUS IN VITRO AT 37° C

Treatment	Concentration	Mean % larvae leaving bolus	$p \leq$ (a)
Bile	10%	97.8 ± 2.2	N.S.
"Succus entericus"	10%	100 ± 0.0	N.S.
Buffered saline (pH 7.2)	0.85%	86.9 ± 13.3	-

(a) significantly different from controls ($p \leq 0.05$)

TABLE 7.16 THE EFFECTS OF BILE AND "SUCCUS ENTERICUS" IN BUFFERED SALINE (pH 7.2) ON THE PENETRATION BEHAVIOUR OF THE LARVAE OF N. DUBIUS IN VITRO AT 37° C

Treatment	Concentration	Mean % Penetration	$p \leq$ (a)
Bile	10%	100 ± 0.0	N.S.
"Succus entericus"	10%	98.2 ± 1.1	N.S.
Buffered saline (pH 7.2)	0.85%	95.4 ± 3.4	N.S.

(a) significantly different from controls ($p \leq 0.05$)

et al., 1978, Wright, 1979) and the larvae are constantly at host physiological temperature except for short periods during t ansmission. It is therefore expected that the maximum activity will occur around 37° C.

At 37° C, the activity of the larvae was lowest in distilled water, just slightly higher in 0.85% NaCl and highest in artificial gastric juice (the larvae in bile are not considered in this argument because of the stimulatory effect of bile). In Chapter 5, I have suggested that the Cl^- ion is important in the initiation of activity of the larvae of T. spiralis. These results support the hypothesis since the Cl^- ion content of the artificial gastric juice is high and the activity of the larvae are concomitantly high.

Increasing temperature led to increasing death of the larvae in distilled water and gastric juice. Preliminary studies on the osmotic tolerance of larvae at room temperature indicate that they can withstand both hypo- and hyper-tonic solutions without significant effect on their infectivity to mice (Sukhdeo, unpublished). This resistance may not be operative at higher than physiological temperatures and it may explain why the larvae died in the hypotonic solutions but not in the isotonic solutions at high temperatures.

Type 2 activity was almost immediately stimulated in the larvae in bile while some of the larvae in saline and gastric juice spontaneously switched from type 1 to type 2. Nothing is known of the neural mechanisms that govern these two types of activity and although this phenomenon is interesting it would be speculative to discuss this switchover.

An intestinal nematode *in vivo* is exposed to different amounts of bile depending on the diet of the host and to different forms of the bile salts depending on the ambient pH and the quantity and distribution of the microflora (Mettrick & Podesta, 1974). The different concentrations, forms and types of bile salts may have different effects on the nematode and this leads to some difficulty in the choice of a suitable concentration of bile to use *in vitro*. Five to 10 percent whole bile has been suggested as a concentration that closely approximates the *in vivo* conditions (Crompton & Nesheim, 1970) and for most of our *in vitro* studies we have used a standard concentration of 10% pig, gall bladder, bile.

Type 2 activity results from bile stimulation and the biles from a wide variety of mammals can stimulate the larvae of *T. spiralis*. This does not appear to be the case with bile from the mallard duck (*Anas platyrhynchos*). Bile alcohols, bile acids and the stereochemical configuration differs widely among species although the function remains basically the same (Hasslewood, 1978). There is a tendency for the bile salts of herbivorous mammals to be conjugated with glycine and those of carnivorous animals to be conjugated with taurine (Smyth & Hasslewood, 1963). Rabbit bile is almost exclusively glycine-conjugated, while, bile from cats and dogs are almost exclusively taurine-conjugated (Hasslewood, 1962; Gordon *et al.*, 1963). Bile from rabbits, cats and dogs stimulate type 2 activity yet I have found that it is only the taurine-conjugated bile salts that can stimulate type 2 activity. These results suggest that stimulation is

independent of the functional properties of bile and since the results from the log dose response curves of the larvae to bile and taurodeoxycholate show larval sensitivity at low levels, rabbit bile is probably contaminated with taurine conjugated bile salts. Bile is not generally known for activating nematodes although there is a lot of evidence for its effect on protozoa, cestodes and trematodes (Smyth & Hasslewood, 1963; Mettrick & Podesta, 1974; Lackie, 1975; Hasslewood, 1978). Among nematodes, bile has been shown to activate the larvae of Dictyocaulus viviparus. The levels of bile required for this activation were low and the percentage of active larvae increased with increasing concentrations of bile until a plateau was reached about 4% (Jorgensen, 1973).

In a review of nematode behaviour, Croll (1972) lists 22 activities of nematodes ranging from movements within the egg to oviposition in the adults. The evidence suggests that all nematode activities may be grouped into subsets of 5 basic patterns (Croll & Sukhdeo, in press). In the subset activities of locomotion in nematodes which include: backward waves, forward waves, omega waves, migration and dispersal, swarming and nictating behaviour and leaping motion, the wave patterns generated are sinusoidal. Type 1 and type 2 activities in T. spiralis are not sinusoidal and are atypical in their predominant dorsal bias of movement. Moreover, the existence of 2 distinct patterns of movement in the same larval stage is unusual. The behavioural repertoire of the larvae of T. spiralis has been previously reported by Meerovitch (1965) who found that the coiling-uncoiling movements (type 1) were

predominant after the larvae were freshly digested but that the undulating movements (type 2) were usually a result of developmental changes as they matured to adulthood. My results suggest that type 2 activity is not a result of maturity and larvae have been observed going from type 2 to type 1. I must stress that the behaviour of the larvae of T. spiralis were observed in vitro in liquid media and could be artifacts. However, prior to penetrating agar, the larvae entered type 2 activity and the path through the agar shows a sinusoidal pattern of movement that is not easily distinguishable from the tracks created by N. dubius. N. dubius larvae normally exhibit a more characteristic sinusoidal movement in liquid media.

Bile stimulates activity and maintains the activity for several hours after which the infectivity of the larvae does not appear to be impaired. There are no differences in the infectivity and distribution of the worms in the samll intestine of mice following stimulation by bile for 6 hours at 37° C. There have been reports that bile or bile salts are toxic to the larvae of T. spiralis. Weller (1943) reported adverse effects on the larvae following cultivation with bile salts and Meerovitch (1965) found that incubation for 2 hours in a medium containing bile salts was lethal to the larvae. The latter author suggested that tauroglycocholate and taurocholate sodium salts were detrimental to the larvae. These observations are inconsistent with the results of my studies. Bile is not harmful to the larvae (using infectivity as an assay) and the larvae respond to concentrations of sodium

taurodeoxycholate as low as 10^{-7}M. In addition, the results of several previous experiments show that bile enhances site selection and is important in site selection. There may be an explanation for this disagreement. The larvae used by Meerovitch (1965) were in culture for 24 - 48 hours and were moulting. We have shown in a previous study that the larvae are intracellular at this stage (Despommier et al., 1978) and not in intimate contact with bile. After the first moult, the larvae that were previously resistant to gastric secretions lose their resistance (see p 207). A similar mechanism may be operative with bile, whereby the infective larvae are not affected by bile, it is toxic to the later larval stages.

The ability of the larvae to be stimulated by bile decreases with digestion time. One to 8 hours of digestion and up to 24 hours of refrigeration did not impair the infectivity of the larvae to mice. Similar results were obtained in rats (Gursch, 1948) and this investigator found that longer refrigeration was definitely injurious to the larvae. In my experiments, although there were no differences in the infectivity of larvae between the controls and the refrigerated groups, there was a decrease in the ability of the refrigerated larvae to respond to bile. There is no information available on the mechanisms by which the larvae lose their ability to respond to bile after refrigeration but it is significant that the ability to respond to bile does not affect the ability to infect and establish in the host.

Attempts to view the penetration of the larvae subsequent to bile stimulation were not successful. Although several systems were tried, the worms did not penetrate intestinal tissue in vitro. Investigators have had similar frustrating experiences in visualizing penetration of other intestinal nematodes (Croll, pers. comm.) and there appears to be no explanation.

The site selection behaviour of T. spiralis could not be explained by the actions of bile alone. In previous experiments I have found that larvae established in the absence of bile and that pancreatic secretions were not important in site selection. My results indicate that enterokinase, an enzyme secreted by the intestinal cells can also stimulate type 2 activity. α-amylase, an enzyme of pancreatic origin that is believed to be a contaminant of "succus entericus", also stimulates some type 2 activity in the larvae of T. spiralis. However, the activity stimulated was of a low level, and may have been due to impurities in the preparation. Similarly, "mucin" stimulates type 2 activity but the activity was low when compared to the activity stimulated by the crude mucosal extract. This may have been due to the contamination of the mucin by small amounts of enterokinase. The log-dose response curve shows that the larvae are extremely sensitive to enterokinase and the ability of enterokinase to stimulate larval activity at different pH values suggests that the response is sensory rather than enzymic. In vivo experiments show that the larvae of T. spiralis when pre-treated with "succus entericus",

establish more rapidly than untreated controls. Enterokinase is an excellent choice for a cue to inform the parasite of its arrival in the small intestine of the host since it is secreted by the mucosal cells of several mammals in response to food in the gut (Biol. Handk, 1964).

A second potent stimulator of type 2 activity is secretin, a polypeptide hormone secreted by the mucosa to stimulate bile and pancreatic flow. The sensitivity of the larvae to secretin is puzzling since this hormone is not secreted into the lumen. However, the larvae quickly enter the mucosal cells during infection and the response to secretin may be important to the behaviour of the larvae subsequent to penetration of the epithelial cells. Meerovitch (1965) has found that type 2 activity is present in the developing worms. Secretin may be the cue that maintains the high level of activity during penetration.

The evidence for the importance of intestinal enzymes or "succus entericus" in the site selection behaviour of the larvae is necessarily indirect because of the difficulty of altering mucosal secretions. If the larvae of T. spiralis are pre-treated with "succus entericus" prior to implantation into the duodenum, the larvae establish anteriad of the controls. These results suggest that the unstimulated larvae, upon implantation into the duodenum, are stimulated in type 2 activity by bile or enterokinase and they penetrate the epithelial cells. However, before they establish, they had already been carried along by the intestinal propulsion and the peak establishment occurred in the second quarter. The larvae that were pre-treated

with "succus entericus" penetrated and established immediately upon being implanted into the duodenum and were not as affected by intestinal propulsion. The ability for "succus entericus" to stimulate penetration is supported in vitro. Bile and "succus entericus" increase the ability of the larvae of T. spiralis to penetrate and migrate through agar in vitro.

It is interesting that the larvae were not stimulated by CO_2 since CO_2 plays an important part in the activation and orientation of several plant-parasitic and animal parasitic nematodes (Croll, 1972). The levels of CO_2 are high in both the stomach and duodenum (Mettrick & Podesta, 1974) and it may be that the larvae prefer a more specific cue like bile or enterokinase to identify the small intestine.

2) N. dubius.

Several larval strongyles, trichostrongyles and ancylostomes are activated by sudden changes from the ambient conditions resulting in the release of "activity quanta" or bursts of motion which last for a few seconds or minutes (Croll, 1970; Croll & Al-Hadithi, 1972). The infective larvae of N. dubius respond in this way to mechanical stimuli. In the presence of bile, however, the larvae are activated for significantly longer periods and their activity shows distinctive parameters which suggests that the nature of the stimulus-response may be different from that of other sensory modalities which elicit the "activity quanta". In bile gradients, the larvae accumulate preferentially in bile when compared to controls and the components of bile to which the

larvae respond are the glycine-conjugated bile salts. The pancreatic and enteric secretions of the gut had no measurable effect on the larvae of N. dubius.

The responses of the larvae of N. dubius to bile were similar at 22° C and 37° C but the response to mechanical stimuli differed and the larvae did not respond maximally to mechanical stimuli at 37° C. I speculate that external changes in the environment i.e. photic, thermal or mechanical stimuli, might be cues to the presence of a host and the larvae respond by releasing "activity quanta" that may improve their chances of reaching the host (Croll, 1972). At 37° C the parasite is within the host and does not require this response (see Chapter 8).

The response to bile, i.e. the long lasting activity is also observed when the larvae are stimulated by HCl, and this appears to be a function of the Cl^- ions. Histological studies show that the larvae also penetrate the mucosa of the stomach. The evidence suggests that this persistent behavioural activity may be related to penetration and only HCl and bile in the gut appear to stimulate this response. The penetration of the gastric mucosa appears to be accidental and is not important in the biology of the nematode. This is discussed further in Chapter 8. Selective forces would have worked to maximise the ultimate and persistent behavioural effort of the larvae before it is swept out of its preferred site. The prolonged pattern of activity in response to bile gives these factors a central role in the establishment behaviour of N. dubius.

3) Summary.

a) Bile stimulates prolonged activity in the larvae of T. spiralis and N. dubius.

b) Cl^- stimulates prolonged activity in the larvae of N. dubius.

c) The response of the larvae of T. spiralis to bile is temperature dependent; the response of the larvae of N. dubius to bile is not temperature dependent but the response to mechanical stimuli is.

d) In bile gradients, the larvae of N. dubius accumulated in bile.

e) The larvae of T. spiralis respond most effectively to taurine-conjugated bile salts while the larvae of N. dubius respond to glycine-conjugated bile salts.

f) Crude mucosal extracts stimulate activity in the larvae of T. spiralis but not in the larvae of N. dubius.

g) The component of the mucosal extract that stimulates activity in the larvae of T. spiralis is most probably enterokinase.

h) Bile and "succus entericus" cause an increase in the penetration and migration, through agar, in vitro, of the larvae of T. spiralis.

i) "Succus entericus" causes an increase in the rate of establishment of the larvae of T. spiralis in vivo.

j) CO_2 does not appear to play a critical part in the behaviour of the larvae of T. spiralis and N. dubius.

CHAPTER 8. GENERAL DISCUSSION

This investigation examines the factors operative in the early site selection behaviour of two nematodes, T. spiralis and N. dubius, following their entry into the gastrointestinal tract. The results of in vivo and in vitro experiments suggest several similarities between these nematodes and from these observations and related published studies, I have proposed a simple model (Figure 8.1) for the mechanism of early site selection by T. spiralis, N. dubius and T. muris. After entry into the host, anteriad to or near the preferred sites, the larvae are activated and change or modify their behaviour patterns. Physiological activation of the infective stages of nematodes upon entry is better known than behavioural changes (Lackie, 1975) and while activation usually involves emergence from the cyst and motility, detailed behavioural analyses are rare and specific behavioural changes upon entry are unknown. I suggest that the modifications of behaviour subsequent to stimulation with intestinal secretions observed in this study, may lead to localized dispersion of the nematodes within the lumen and ultimately to penetration and establishment. In this discussion I will present the mechanisms and sensory cues that may be utilized by these nematodes in locating their sites. There are only limited experimental results in the literature to support these hypotheses and most of the specific examples used will draw from the present study and studies on T. muris (Panesar & Croll, in press).

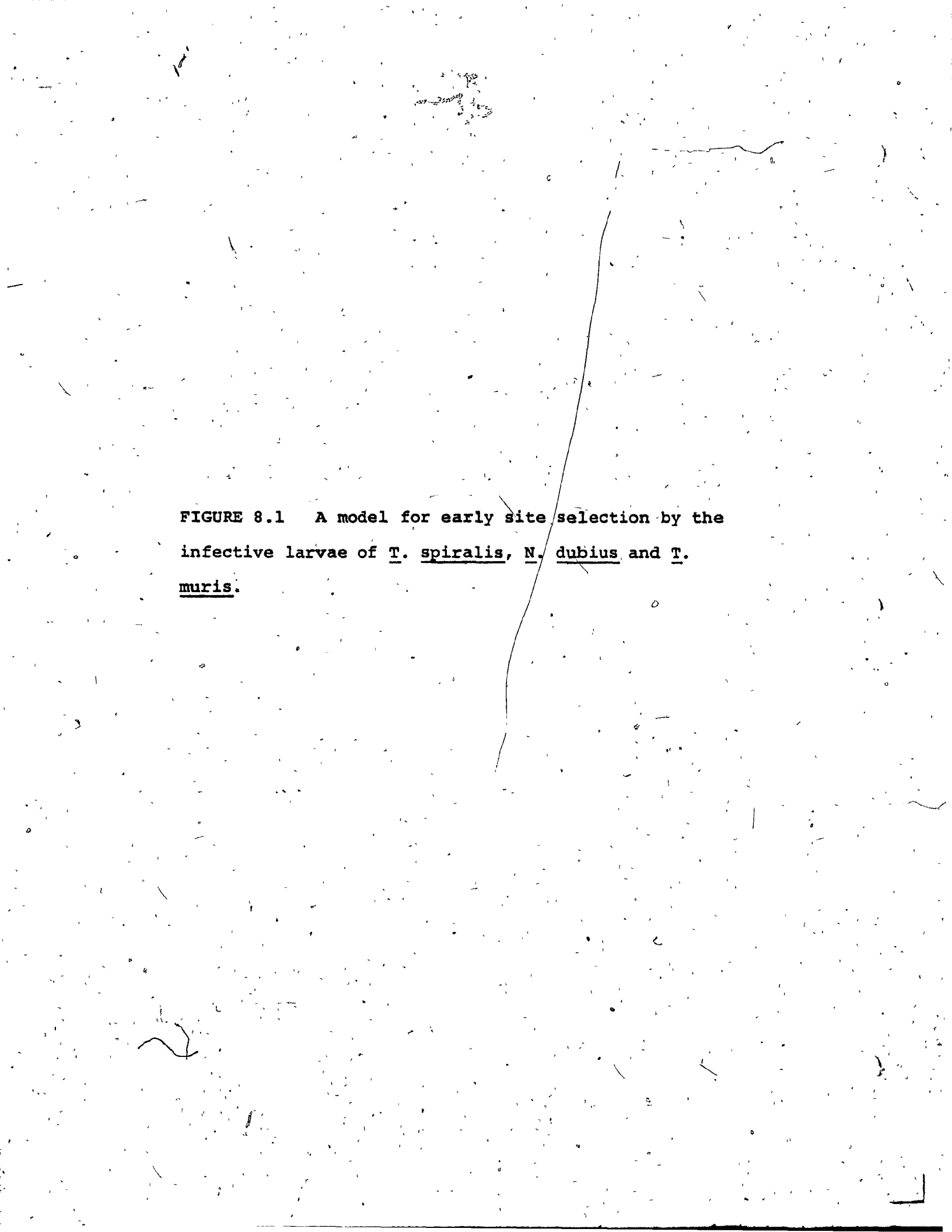
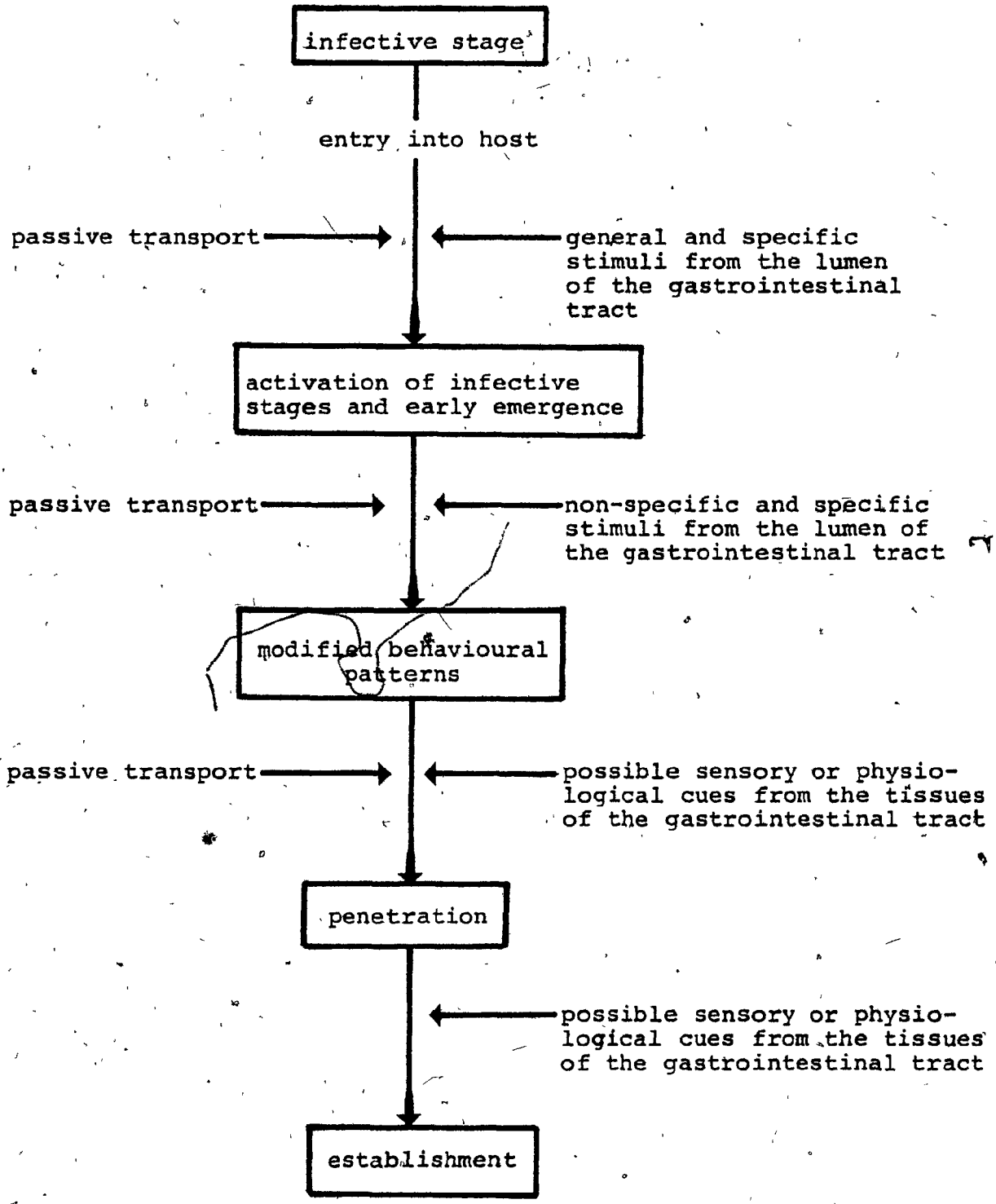


FIGURE 8.1 A model for early site selection by the infective larvae of T. spiralis, N. dubius and T. muris.


infective stage
entry into host
passive transport
general and specific stimuli from the lumen of the gastrointestinal tract
activation of infective stages and early emergence
passive transport
non-specific and specific stimuli from the lumen of the gastrointestinal tract
modified behavioural patterns
passive transport
possible sensory or physiological cues from the tissues of the gastrointestinal tract
penetration
possible sensory or physiological cues from the tissues of the gastrointestinal tract
establishment

1) Entry into the host

The behaviour of the free-living stages of nematode parasites has been reviewed in several places and is beyond the immediate scope of this thesis (Croll, 1970, 1972a, 1975; and others). The behaviour patterns of the infective larvae are generally interpreted as being purposeful in enhancing transmission (Robinson, 1962; Bizzell & Ciordia, 1965; Croll, 1972a; MacInnis, 1976).

Several larval strongyles, trichostrongyles and ancylostomes are activated by sudden changes from ambient conditions, resulting in the release of "activity quanta" or bursts of motion (Croll, 1970; Croll & Al-Hadithi, 1972). These responses may have adaptive significance because energy reserves will be used preferentially in response to rapid environmental changes which may indicate the proximity of their hosts (Croll, 1972a). This behaviour pattern has been observed in my present study in the infective larvae of N. dubius and is consistent with the above hypothesis.

The infective larvae of T. spiralis are generally considered to be immobile within the cyst prior to infection. However, in an indirect manner, they may influence the probability of ingestion by the host. Penetration and excystment within the muscle fibers by the migrating larvae results in the loss of function by the fiber (Ribas-Mujal & Rivera-Pomar, 1968; Despommier, 1975, 1976) and the muscles of infected animals (Ribas-Mujal, 1975; Farris & Harley, 1976). These changes may lead to an increased susceptibility to predation and therefore enhance transmission.

2) General and specific stimuli from the gastrointestinal tract

Subsequent to entry into the appropriate host, a combination of environmental factors and parasite responses must be involved in the emergence of the larvae from its egg, cyst or sheath and the subsequent penetration of the host tissue. I have divided the environmental conditions and factors, encountered by the nematodes in the homeothermic gut into two categories; (a), non-specific stimuli and (b), specific stimuli to the nematodes (Table 8.1).

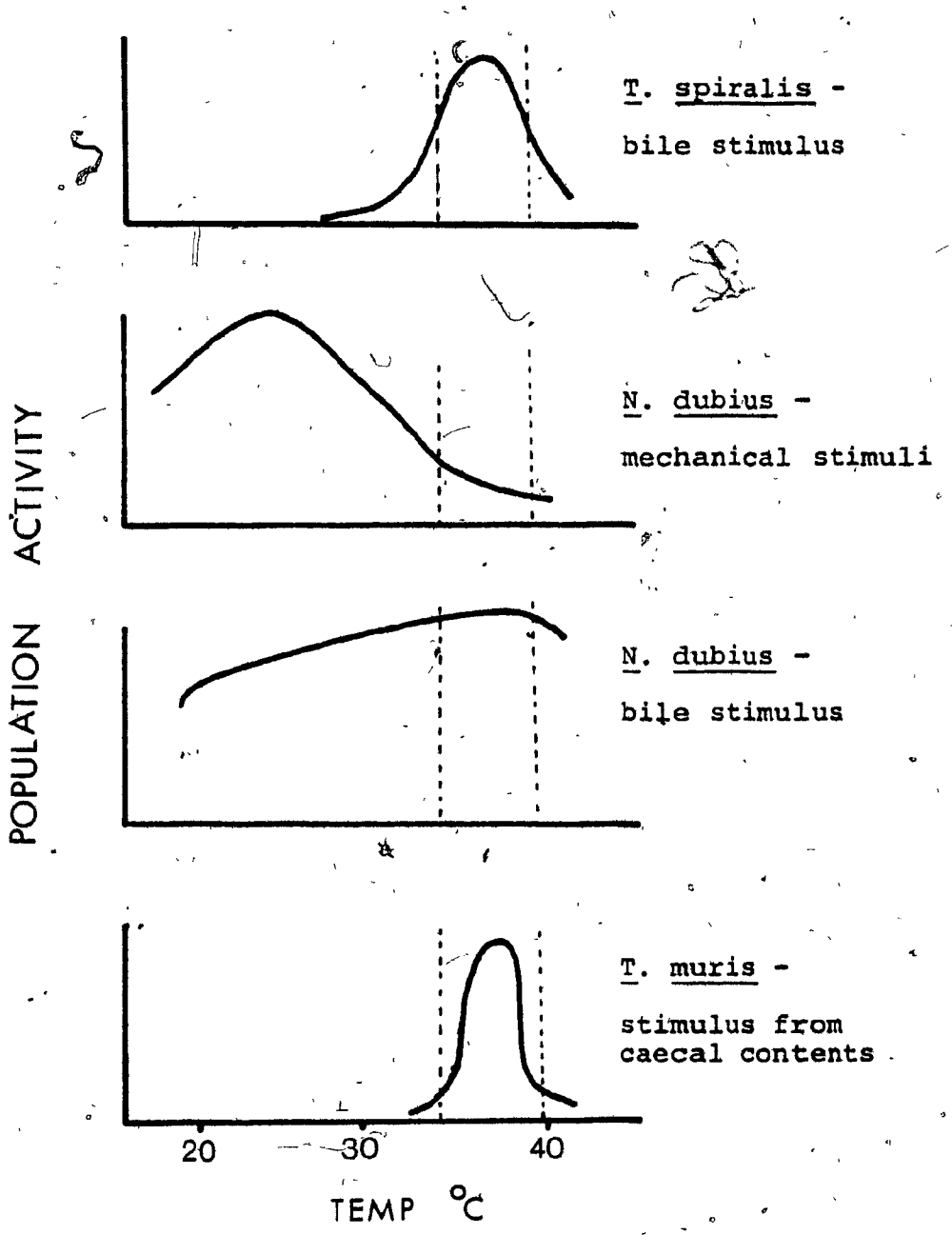
A temperature similar to that of the host appears to be a common requirement of these nematodes. Activity of the larvae of T. spiralis is maximal at 37° C when the larvae have been treated with HCl-pepsin solution or 10.0% bile solutions. However, in distilled water, that is, in the absence of the specific stimuli of Cl^- or bile, activity remains at a low level. This suggests that while a temperature of around 37° C is necessary for activity, temperature by itself is not a specific stimulus of activity. Similarly, the hatched larvae of T. muris are most active at about 37° C in the presence of specific stimuli from the caecum (Panesar & Croll, in prep.). In contrast, the infective larvae of N. dubius are active at the wide range of temperatures encountered during the free-living and parasitic phases of its life cycle (Figure 8.2). However, the response to sudden changes in ambient conditions e.g. the release of "activity quanta" to mechanical stimuli, occurs predominantly at lower temperatures while the larvae are able to respond to specific

TABLE 8.1 SELECTED ENVIRONMENTAL CONDITIONS ENCOUNTERED BY NEMATODES AFTER ENTRY INTO THE GASTROINTESTINAL TRACT OF THE APPROPRIATE HOST

Non-specific	Specific
Temperature	Cl^-
Gut motility	Bile
pH	"Succus entericus"
Some g. i. enzymes	Caecal factors
	Pepsin*

* specific action but not a stimulus

FIGURE 8.2 Diagrammatic representation of the activity of the infective larvae of T. spiralis, N. dubius and T. muris at different temperatures (vertical dotted lines = approximate range of temperatures encountered in the homeothermic host).


T. spiralis -
bile stimulus
N. dubius -
mechanical stimuli
N. dubius -
bile stimulus
T. muris -
stimulus from
caecal contents
POPULATION ACTIVITY
20
30
40
TEMP °C

stimuli e.g. bile, at both low and high temperatures.

In these nematodes, temperature may be a general cue to indicate arrival in the host and may act to "prime" the larvae to respond to the specific cues that determine site selection.

The concept of "priming" by the non-specific host stimuli is also seen in the larval response to pH. The activation and emergence of the encysted larvae of T. spiralis requires a low pH but a low pH in the absence of Cl^- ions does not initiate behaviour of these larvae. Similarly, with the larvae of N. dubius, a low pH is required for exsheathment, but the prolonged activity observed in gastric secretions is not initiated when the larvae are incubated in solutions of low pH without the Cl^- ions. These results suggest that the pH may provide a general cue to indicate arrival in the host stomach but the Cl^- ions are the specific stimuli in initiating behavioural change.

Gastrointestinal motility and some enzymes are included as stimuli because their effects on the larvae entering the gut are inevitable. Intestinal propulsion of the ingesta passively carries the larvae through the gut and some enzymes e.g. pepsin in the case of T. spiralis degrades the cyst. These will be discussed later.

The specific stimuli are gastrointestinal secretions (Table 8.1) that stimulated measurable behavioural changes in the larvae. The stimulus response pattern to these specific stimuli is believed to be closely related to the site selection of these nematodes.

3) Behavioural changes during activation

The encysted larvae of T. spiralis, when stimulated by gastric secretions, initiate a behaviour pattern, designated as type 1. Type 1 activity consists of a prolonged rapid tail lashing behaviour that is always associated with excystment and is presumed to be responsible for the rapid emergence of the larvae from the cyst and surrounding muscles. The specific stimulus for this behaviour is the Cl^- ion and not other gastric factors. Similarly, the ensheathed larvae of N. dubius are also activated by gastric secretions (Sommerville & Bailey, 1973) and I have found that is the Cl^- ions and not other gastric secretions that are responsible for the initiation of a prolonged motility that may aid in exsheathment. In contrast, the embryonated eggs of T. muris are not affected by gastric secretions, but a caecal factor(s) is responsible for initiating a complex pattern of behaviour in the larvae that ultimately leads to hatching (Panesar & Croll, in prep.).

In vitro results, therefore, indicate that the conditions in the stomach are necessary for the activation of the larvae of T. spiralis and N. dubius, while the conditions in the caecum are necessary for the activation of the larvae of T. muris. However, in the absence of these conditions in vivo, these larvae still establish themselves. The encysted larvae of T. spiralis establish in rats that have had total gastrectomy, the ensheathed larvae of N. dubius when implanted directly into the duodenum and the larvae of T. muris establish in caecumectomized mice (Panesar & Croll, in press). Therefore,

available results indicate that although specific conditions for the optimal activation of these larval nematodes can be isolated and tested in vitro, none appear to be absolute requirements.

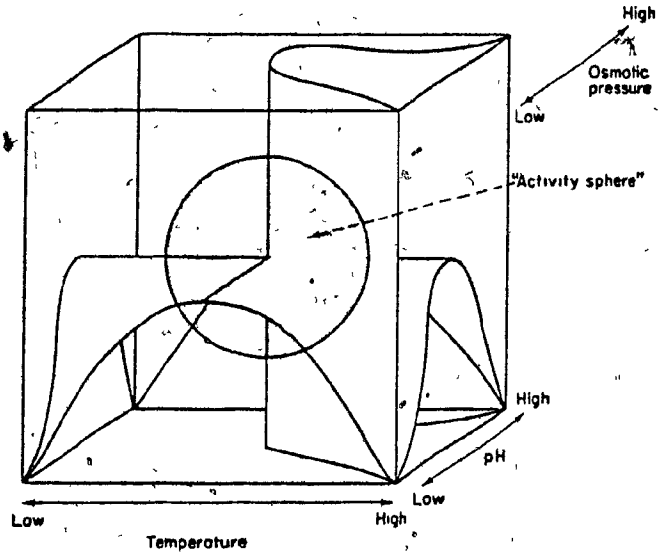
Few absolutes have emerged from the studies of site selection (Croll, 1976; Panesar & Croll, in press) or the studies of cues (Lackie, 1975). The concept of absolute requirements are being replaced by the concept of more relative and flexible responses to the flux of biological conditions encountered within living hosts. Croll (1972b, c) describes an "activity sphere" (Figure 8.3) for the survival and movement of larval nematodes, and this concept may also apply to behaviour of nematodes in the gastrointestinal tract. Activity is only possible under interacting optimal conditions. While the parameters affecting the activation and site selection of gastrointestinal nematodes may differ from those of larval survival, each parameter (temperature, pH, gastrointestinal motility, bile, HCl, "succus entericus", osmosity and so on) has a high, low and optimal value. Trends, optima and synergistic interactions are probably more representative of the true situation in vivo than absolutes.

4) Dispersion

For infective stages entering the gut, the inevitable method of passive longitudinal dispersion is to move with the food. This mode of transportation is clearly used by most gastrointestinal parasites (Crompton, 1973).

Infections of T. spiralis in mice treated with Lomotil® resulted in a more anteriad establishment than in untreated

FIGURE 8.3 Hypothetical survival curve, described by three physiological parameters known to influence infective larval activity and survival (Croll, 1972b).


High
Osmotic pressure
Low
"Activity sphere"
High
pH
Low
Low
High
Temperature

controls. Increasing the propulsion rate of mice at the time of infection resulted in a more posteriad distribution. The changes in the distribution pattern of N. dubius in mice given similar treatments were similar but less marked. There was a more anteriad establishment in mice treated with Lomotil® and there was a significant reduction in the numbers establishing in mice with the propulsion increased at the time of infection, when compared with controls. In the latter group, the larvae may have been carried from potential sites before they could establish. The eggs of T. muris were passively transported along the gut at the same rate as inert markers (Panesar & Croll, in press, Panesar, pers. comm.).

The results of these experiments strongly suggest that the timing of behavioural responses leading to site selection are integrated or coordinated with the rate of passive transport in the gut. That is, the larvae move with the ingesta until they receive the appropriate stimulus(i), whereupon they disperse and establish. It is assumed in these experiments with altered motility in mice infected with T. spiralis and N. dubius, that the altered distributions result from the effects of passive transport and not from the alterations of "homeostatic" physico-chemical gradients that have been implicated (but not proven) in the site selection of a variety of parasites including nematodes (Mettrick & Podesta, 1974). The influence of passive transport is further supported by experiments in surgically modified rats. When the preferred sites in the anterior small intestine were surgically bypassed, the larvae of T. spiralis and N. dubius did not establish in

the bypassed sections through which no food flowed.

5) Penetration and establishment

The infective larvae must leave the ingesta to penetrate the mucosa, while being passively transported along the gut. I suggest that specific stimuli anteriad or adjacent to the preferred sites stimulate increased activity of the larvae, which will cause a general kinetic effect leading to dispersal from the ingesta.

In this study I have attempted to elucidate the cues and behaviour patterns of the larvae of T. spiralis and N. dubius that lead to penetration. Despite many attempts, technical problems have prevented the actual visualization of penetration by these larvae. Histological evidence shows that the larvae of these nematodes can penetrate the gastric and intestinal mucosa within 10 minutes of infection and the sites penetrated in the small intestine are adjacent to the sites of recovery of adults of T. spiralis and larvae of N. dubius several days after infection. Correlating behavioural changes in vitro with gastrointestinal factors simulating "natural" conditions at the site of penetration, gives an indication of the sensory cues that may be important in the penetration behaviour of these nematodes. The major cues are believed to be gastric secretions, bile, "succus entericus" and perhaps the architecture of the small intestine. Stimulation of the larvae of T. spiralis and N. dubius with these gastrointestinal secretions resulted in a prolonged behavioural activity that was not stimulated by other secretions of the gastrointestinal tract. It is clearly seen that the behaviour

elicited by stimulation with these secretions e.g. stimulation of the larvae of T. spiralis with bile, differs from the behaviour observed in the activation and emergence of the larvae.

a) Stimulation of prolonged activity.

The importance of gastric secretions and their possible role in nematode activation and site selection has been recognized by several investigators (Lackie, 1975). Gastric secretions activated the larvae of T. spiralis and N. dubius and probably stimulated the larvae of N. dubius to penetrate the mucosa of the stomach.

The major components of gastric secretions are HCl, pepsin and high pCO_2 (Mettrick & Podesta, 1974). Neither pepsin nor CO_2 appeared to have significant effects on the behaviour of the larvae of N. dubius. However, concentrations of HCl that were similar to in vivo conditions stimulated a prolonged undulating activity in the larvae of N. dubius in vitro. The stimulation was due to the Cl^- ion and not the low pH of the solution. This behavioural response to Cl^- was similar to the response of the larvae to bile and it is believed to result in dispersion from the ingesta and penetration of the mucosa. The penetration of the gastric mucosa by the larvae of N. dubius appears to be accidental and not essential to the biology of the parasite. This hypothesis is supported by the short phase in the gastric mucosa before the worms leave to penetrate the mucosa of the small intestine and the lack of developmental events while the worms are within the gastric mucosa.

In vitro studies on the behaviour of the larvae of T. spiralis and N. dubius showed a prolonged activity (3 - 6 hours' duration) in response to stimulation with a 10% crude bile extract. This pattern is designated as type 2 activity in the larvae of T. spiralis to distinguish it from the prolonged activity (type 1) stimulated by HCl during activation. The response in the larvae of N. dubius is similar to that stimulated by Cl^-. A similar behavioural response has been observed in the infective larvae of D. viviparus when stimulated by ox bile and in the absence of experimental evidence, it is believed that this behaviour is responsible for causing penetration of the gut wall of the host animal (Jorgensen, 1973; Hasslewood, 1978).

In surgically modified rats in which bile was excluded from the gastrointestinal tract, there was a significant reduction in the establishment of both T. spiralis and N. dubius when compared to controls. There was an increased establishment of these nematodes at the site of re-entry of bile following cannulation to different locations in the small intestine. In vitro studies showed that these larvae were sensitive to and responded to concentrations of bile that were below in vivo levels in the lumen. The larvae of N. dubius migrated up gradients of bile and the type 2 activity of the larvae of T. spiralis resulting from stimulation with bile, significantly increased their ability to penetrate and migrate through agar. Bile is therefore likely to be a major component in stimulating penetration of the mucosa by these

larvae or in stimulating behaviour that ultimately leads to penetration. Apart from the preliminary observations on the larvae of D. viviparus (Jorgensen, 1973) this response to bile has not been demonstrated in any other nematodes.

Bile is composed of water, mucin, protein, bile pigments, bile salts (conjugated and unconjugated), phospholipids, neutral fats and various inorganic ions (Hasslewood, 1978). These constituents vary with the diet, pH, presence of parasites, microflora and with the host species (Smyth & Hasslewood, 1963; Mettrick & Podesta, 1974; Hasslewood, 1978). Smyth and Hasslewood (1963) have proposed that the composition of bile is a factor in the determination of host specificity of intestinal parasites. Although these authors support their hypothesis with in vitro studies on E. granulosus, there has been little substantiation from other parasites in the seventeen years since their hypothesis was formulated. I have found that the larvae of T. spiralis are specifically activated by taurine-conjugated bile salts while the larvae of N. dubius are activated by glycine-conjugated bile salts. T. spiralis is naturally transmitted through ingestion by carnivores and there is a tendency for the bile salts of carnivores to be conjugated with taurine (Smyth & Hasslewood, 1963) while the larvae of N. dubius are ingested with herbage and there is a tendency for the bile salts of herbivores to be conjugated with glycine (Smyth & Hasslewood, 1963). Although this may be taken as a broad support for the hypothesis of host specificity due to bile, this specificity is not absolute and there may be several other factors involved. Host specificity

in N. dubius is restricted to mice and some other rodents while T. spiralis has a wide range of carnivorous and omnivorous hosts.

The type 2 activity that is involved in the penetration behaviour of the larvae of T. spiralis is also stimulated by "succus entericus". The behavioural response of "succus entericus" is similar to the response of the larvae to bile. Stimulation of the larvae of T. spiralis with "succus entericus" leads to an increased rate of establishment of the larvae in vivo, and significant increase in penetration of agar in vitro. It was determined that the active component in "succus entericus" was the enzyme enterokinase. The threshold of sensitivity to enterokinase, by the larvae, was extremely low and the kinetics of the response suggest a sensory rather than enzymic stimulation. Enterokinase is a key enzyme in the initial activation of trypsin, and other pancreatic enzymes and is secreted by the mucosa of the anterior small intestine, the preferred site of T. spiralis.

Another stimulus for type 2 activity in the larvae of T. spiralis is secretin. Secretin is a gastrointestinal hormone produced by the epithelial cells of the small intestine (Guyton, 1976) and is not normally found in the lumen. I speculate that this hormone is necessary to maintain the type 2 activity of this nematode while it is penetrating the intestinal cells.

b) Intestinal architecture

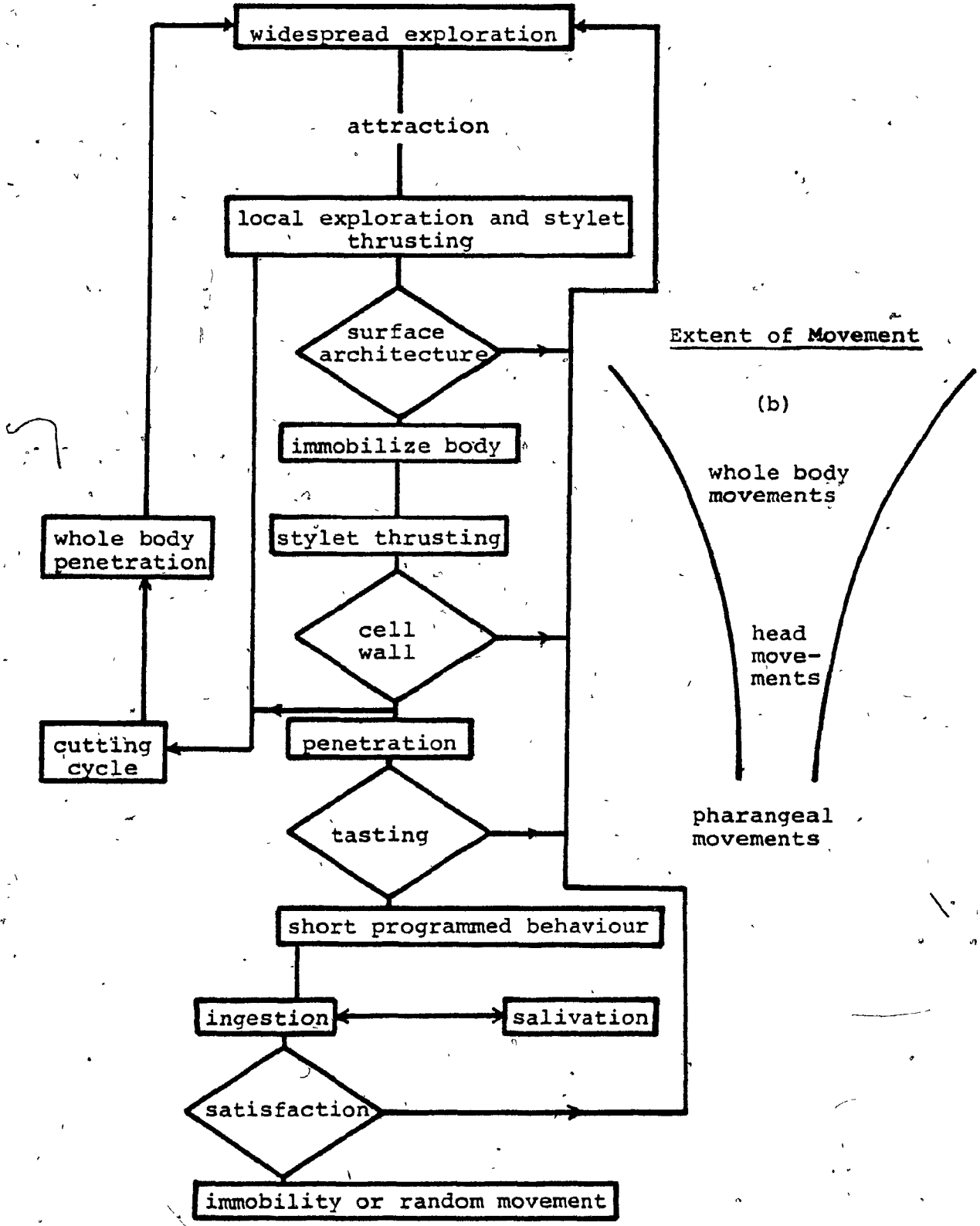
It has been argued above that penetration behaviour results from stimulation by various secretions of the gastrointestinal tract. However, there may be additional factors

involved. Although bile stimulated behaviour that led to penetration of the mucosa of the duodenum by the larvae of N. dubius, the larvae did not penetrate the mucosa of the lower small intestine and large intestine even in the presence of bile. Perhaps there is a tissue specificity or mechanical sense based on the topography or architecture of the intestinal cells. Technical difficulties did not allow in vivo verification of this hypothesis but the following speculations are based on the observations of the behaviour of plant parasitic nematodes as they approach and feed on, or penetrate host roots.

The pattern of feeding behaviour in plant parasitic nematodes is shown diagramatically in Figure 8.4a. This diagram summarizes the observations of several investigators (Doncaster & Seymour, 1973; Robertson, 1975; Trudgill, 1976; Weischer & Wyss, 1976; Wyss, 1977; Jones, 1978). Subsequent to initial activation by host attractants there appears to be an automatic sequence with 4 major decision points which leads to nematode feeding. This is concurrent with a gradual restriction of movement (Figure 8.4b). The whole body movements result in widespread exploration that is random with respect to the host roots prior to moving into the rhizosphere. This leads to more specific orientation which brings them to the roots. At the roots, the nematodes explore the surface of the roots with their cephalic sensillae (Doncaster & Seymour, 1973). If the surface architecture is appropriate, the nematodes penetrate with their stylets to feed or use a combination of head movements and stylet thrusting to enter the cell.

FIGURE 8.4 · A model of the feeding behaviour in plant parasitic nematodes.

(a)


widespread exploration
attraction
local exploration and stylet thrusting
surface architecture
immobilize body
stylet thrusting
cell wall
penetration
tasting
short programmed behaviour
ingestion
salivation
satisfaction
immobility or random movement
whole body penetration
cutting cycle
Extent of Movement
(b)
whole body movements
head move-ments
pharangeal movements

An analogous system is possible in the penetration of the mucosa by the infective stages of gastrointestinal nematodes. A model for this hypothesis is presented diagramatically in Figure 8.5. Dispersal and penetration behaviour is stimulated by the secretions of the gastrointestinal tract. This activity continues until the larvae arrive at the mucosa, the larvae then explore the tissue and if appropriate, they penetrate.

The model is hypothetical but it may explain the phenomena of tissue and host specificity. The tissues of the anterior small intestine of mice and some other rodents may provide the only appropriate cues for penetration by the larvae of N. dubius. There is also some circumstantial evidence to support this hypothesis. The larvae of T. spiralis, when stimulated into type 2 activity with bile, migrate in an apparently random manner through agar, this behaviour is suggestive of the widespread exploratory behaviour of phytonematodes. In the course of their rapid undulating activity after stimulation with bile, the larvae of N. dubius have been frequently observed to explore, with their lips, the sides of the watchglass, cracks and bumps in the glass and even each other, this behaviour is suggestive of the local exploration of the host root by phytonematodes. Exploration of 'non-root structures' have similarly been reported in the feeding behaviour of plant parasitic nematodes (Croll, 1972a). CCTV photomicrography of T. muris in the caecum, shows that a combination of head movements and stylet thrusting are used while penetrating epithelial cells during tunnel formation (Panesar & Croll, pers. comm.).

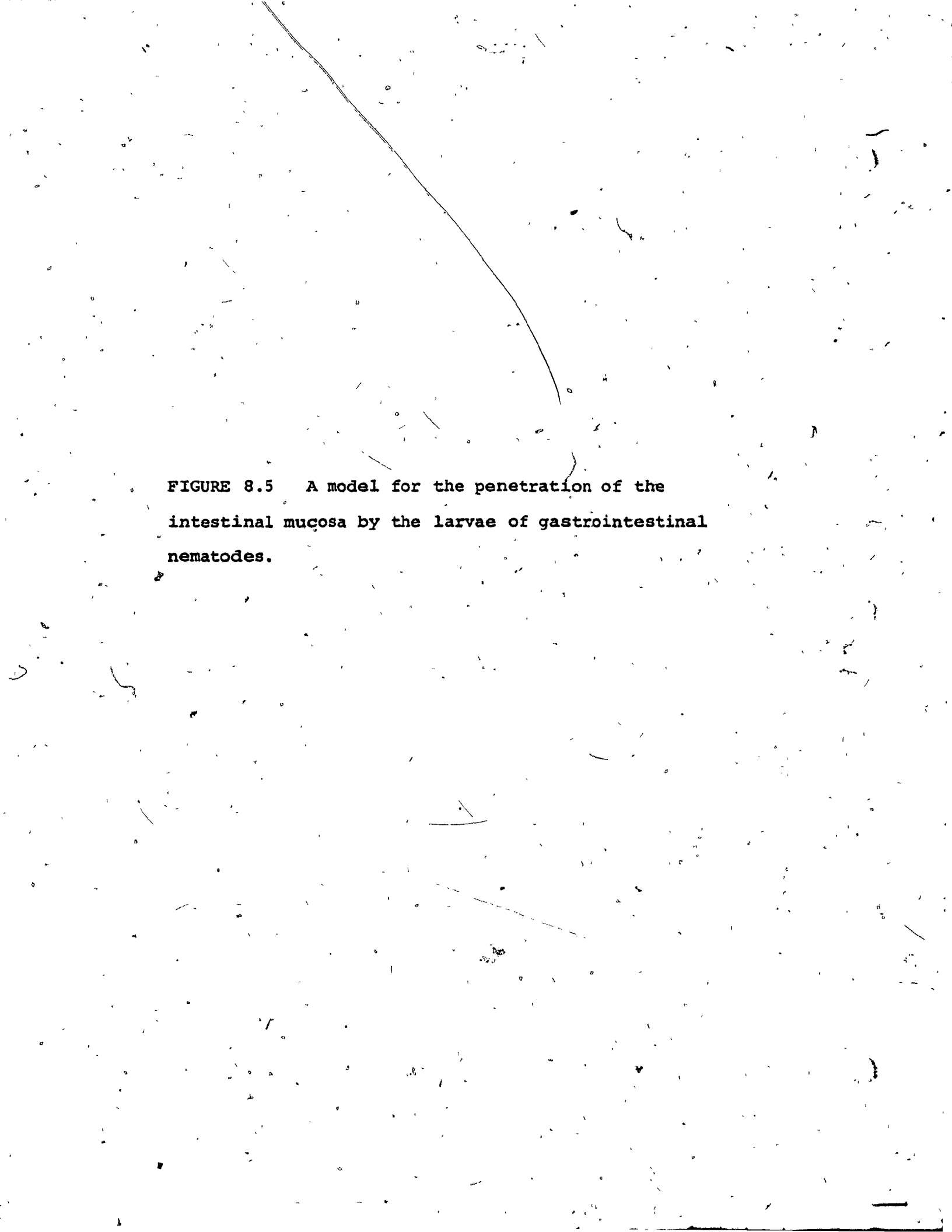
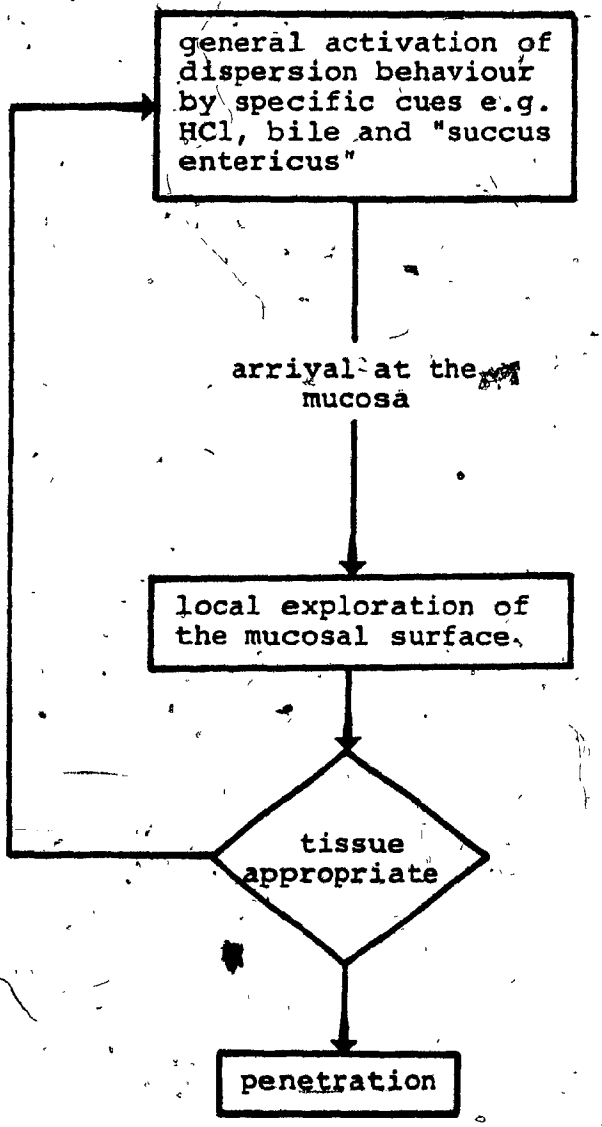


FIGURE 8.5 A model for the penetration of the intestinal mucosa by the larvae of gastrointestinal nematodes.


general activation of dispersion behaviour by specific cues e.g. HCl, bile and "succus entericus"
arrival at the mucosa
local exploration of the mucosal surface
tissue appropriate
penetration

The above observations on the behaviour of these nematodes show several similarities with the feeding behaviour of phytonematodes. The behavioural repertoire of nematodes appears to be finite with approximately 22 behavioural activities in 5 major categories (Croll, 1972a; Croll & Sukhdeo, in press) and the nervous system and the localization of sensory receptors are also remarkably constant among nematodes (Ward et al., 1975; Ware et al., 1975; Wergin & Endo, 1976; Croll, 1977). Several behavioural activities are under endogenous control and appear to be programmed sequences which follow appropriate sensory input (Croll, 1972a, b). I suggest that the behavioural activities of plant parasitic and animal parasitic nematodes may correspond in their penetration of host roots or host mucosa.

6) Summary

After entry into the appropriate host, the larvae of T. spiralis, N. dubius and T. muris are stimulated by various non-specific and specific stimuli while being transported passively along the gastrointestinal tract. The evidence suggests that there is a separation in the stimuli and behavioural responses of activation (and emergence) from dispersal and penetration (Table 8.2). Gastric secretions stimulate activation and emergence of the larvae of T. spiralis and N. dubius while caecal factors stimulate the activation and emergence of T. muris (Panesar & Croll, in prep.). Subsequent to activation these larvae are stimulated by specific cues anteriad or adjacent to their preferred sites. This stimulation produces a prolonged activity in the larvae that

TABLE 8.2 SUMMARY OF THE STIMULI AND BEHAVIOURAL RESPONSES OF T. SPIRALIS, N. DUBIUS AND T. MURIS

Nematode	Trichinella spiralis		Nematospiroides dubius		Trichuris muris	
Behavioural Response	Emergence	Dispersion and Penetration	Emergence	Dispersion and Penetration	Emergence	Dispersion and Penetration
Primary Stimulus	Cl^-	bile enterokinase	Cl^-	bile	caecal* factor	caecal* factor
Relative Requirements	37° C low pH (pepsin)	37° C near neutral pH	37° C low pH	37° C near neutral pH	37° C near neutral pH	37° C near neutral pH
Type of Activity	type 1	type 2	prolonged motility	prolonged motility	crescendo of larval activities in the egg	prolonged motility

* unknown factor from the caecal contents

results in dispersal from the ingesta and penetration of the mucosa. Indirect evidence also suggests that there may be tissue specific factors involved in the penetration of host mucosa.

Lack of experimental evidence makes it difficult to compare these hypotheses with the early site selection behaviour of other gastrointestinal nematodes. It is likely that while the specific cues for activation and penetration may differ, the underlying mechanisms of site selection in other gastrointestinal nematodes are similar.

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CLAIM OF ORIGINALITY

The elements in this thesis that should be considered as contributions to original knowledge are: - (a), the factors that affect the longitudinal distribution of T. spiralis and N. dubius (Chapter 3); (b), the description of the transverse site of N. dubius in the small intestine (Chapter 4); (c), the description of the behavioural changes during, and the factors affecting activation and emergence of T. spiralis (Chapter 5); (d), the effects of surgical modification of the gastrointestinal tract of rats on the longitudinal distribution of T. spiralis and N. dubius (Chapter 6) and (e), the analyses of the behavioural responses of T. spiralis and N. dubius to gastrointestinal secretions in vitro (Chapter 7).