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**BIER, JEFFREY WEBSTER  
STUDIES ON ANISAKINE NEMATODES AND  
EXPERIMENTAL ANISAKIASIS.**

**LEHIGH UNIVERSITY, PH.D., 1978**

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STUDIES ON ANISAKINE NEMATODES  
AND EXPERIMENTAL ANISAKIASIS

by

Jeffrey Webster Bier

A Dissertation

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of Lehigh University  
in Candidacy for the Degree of  
Doctor of Philosophy

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## ABSTRACT

The taxonomic systems used to define the primarily aquatic ascarids, i.e., the Anisakinae, are discussed. The subfamily Anasakinae and the subordinate genera (Phocanema, Contracecum, Anisakis, Paranisakiopsis, Sulcascaris, Thynnascaris, Goezia, Raphidascaris and Porrocaecum) are defined on the bases of size, mouth and digestive tract morphology, cuticular ornamentation, structure of the excretory system, structure of the male and female reproductive systems, and definitive hosts. The 30 genera included in the subfamily are distinguished by differences in adult morphology and, whenever possible, of larval morphology.

A survey of 1,010 fresh, fish from Washington, District of Columbia markets revealed 6,547 nematodes. Among fish species of which 25 or more were examined, spotted hake (Urophycis requius) was the most wormy and white perch (Morone americana) the least. Only two of the nematodes were recovered from fish flesh, both were Anisakis sp. larvae. Recovered from fish viscera were 9 Anisakis sp. larvae, 41 Porrocaecum sp. larvae, 3,221 Thynnascaris spp. larvae and adults, 21 Goezia sp. larvae and 1,220 Raphidascaris acus larvae, all of which are considered to be anisakines. In addition, 225 anisakines were too damaged to be identified more precisely. Other nematodes recovered were 71 Spinitectus spp. adults and larvae, 114 Bulbodactinis sp. adults and larvae, 108 Metabronema sp. adults and larvae, 111 spirurinae larvae, 662 Philometra sp. adults and larvae, 1 Capillaria sp. larva, 447 similar small larvae so

undeveloped that they could not be identified, and 294 other nematodes too damaged even for general identification.

Only the Anisakis sp. larvae are considered pathogenic to consumers of raw or semiraw fish. The low incidence of pathogenic anisakines is attributed to the absence of definitive hosts (marine mammals) from the Chesapeake Bay and adjacent waters.

Pigs were force fed Phocanema decipiens and Anisakis sp. larvae from fish. P. decipiens larvae molt once, between 3 and 7 days after infection (usually day 4) and were recovered as long as 18 days after infection. The larvae were usually found attached to the stomach wall, with the anterior 5-8 mm having penetrated into the stomach wall and the remainder free in the gut lumen. Anisakis sp. larvae attach similarly and molt between 4 to 7 days (usually day 5) After 7 days, larvae were found completely embedded in the stomach wall.

Pigs (10) fed P. decipiens and Anisakis sp. (14) were clinically tested (hematology, clinical chemistry). The only parameter tested which varied reliably after infection was an increase in number of eosinophils.

A swollen area surrounded the nematodes at the attachment site. If the nematodes detached or were removed a bloody ulcerous crater remained. Evidence of more stomach lesions than nematodes in the innoculum indicates that the larval nematodes detach and reattach.

Microscopical examination of the lesions revealed a cellular response consisting almost entirely of eosinophils. Surrounding a

parasite-produced attachment cap at the base of the mucosa, some fibroblast-like cells were observed from day 4 onwards.

Fishborne anisakine larvae in vitro, in fish flesh, and in fried fish fingers, do not survive heating to 60°C for 1 min. The recommended time and temperature found in Japanese and European literature for freezing fish to kill anisakine larvae is -20°C for 24 hr; however, some North American species survive for 52 hr at this temperature.

## INTRODUCTION

Anisakine nematodes are of economic, medical, and scientific interest. Larval anisakines of the genus Phocanema have virtually eliminated the smelt and cod fisheries in the northern maritime provinces of Canada because so much of the catch is "wormy". Phocanema also occur in muscles (fillets) of Nova Scotia cod, but with less frequency so that it is practical to candle and remove the worms. McClelland (1976) reports that in fish processing plants the candling personnel often outnumber the filleting personnel by as many as three to one. This additional labor required to detect and remove worms has made the price of fish flesh noncompetitive with other sources of animal protein in the market such as beef, pork, and poultry.

In the northwestern United States, heavy anisakine infestations of certain species of fish have caused them to be rejected as unmarketable. For example, the bocaccio rockfish Sebastes paucispinus was still being sold as recently as during the early spring of 1975. Later, that spring the infestation with larval nematodes had reached such a level that this species of fish became commercially unacceptable. So far, the anisakine problem has not led to the implementation of candling procedures in the United States. Instead, the matter is dealt with by the selective fishing and marketing of only those species with relatively low infestation rates.

The medical importance of anisakine nematodes was generally disregarded until Van Thiel, et al. (1960) reported that a gastrointestinal syndrome in The Netherlands was being caused by a fishborne anisakine larva. Infections were acquired through the consumption of raw fish, specifically a lightly marinated product called "green herring". Subsequently, other cases were newly reported elsewhere in Europe, Japan, and the Americas.

Concurrent with increased interest in the economic and medical ramifications, anisakines have been used as experimental models in both practical and theoretical studies. Determination of the temperature tolerances of these worms has the useful aim of inactivating them as a human health hazard in edible fish. In vitro cultivation of anisakines facilitated the study of molting processes and has theoretical implications for our understanding of nematode development.

The purpose of this dissertation is to review critically the literature on anisakine nematodes and anisakiasis, and to present original work on aspects of these parasite's systematics, distribution and pathogenicity.

## HISTORICAL REVIEW

### Systematics

Members of the nematode subfamily Anisakinae are found as larvae or adults in a wide variety of animals that inhabit several types of environments. For example, larval anisakines have been reported from coelenterates, arthropods, annelids, molluscs, fishes, amphibians, reptiles, birds, and mammals (see Tables 1, 2, 3). Vertebrates are usually the definitive hosts and the larval nematodes encountered within them are third- and fourth-stage larvae ( $L_3$ ,  $L_4$ ) that are in the process of developing into adults. Some fish are second intermediate and or transport hosts for those forms that mature in endothermic hosts. The anisakines and their hosts occur primarily in marine, and freshwater, but in some instances also in terrestrial environments.

The Anisakinae is a large taxonomic group. Yamaguti (1961) considered 28 genera subordinate to this subfamily, and these genera include more than 300 species. In the 17 years since the publication of Yamaguti's monograph, one additional genus and approximately 50 species have been described. Many of the described species are undoubtedly conspecific i.e., with "new species" descriptions often based on a single specimen or only a few, from a host reported to harbor other members of the genus. Furthermore, some of the descriptions are so vague that subsequent identification is impossible.

At the beginning of the 20th Century, the anisakine's superfamily Ascaroidea, included many of the organisms that were included in the genus Ascaris. The first attempt to subdivide this large group into subfamilies was made in 1912. At the same time, the nematodes which are the subject of this study were included by Railliet and Henry (1912) in the two subfamilies: Anisakinae and Heterocheilinae. These groups were established as follows:

"Anisakinae; generes Anisakis Duj., 1845 (Peritrachelius Dies., 1981; Conocephalus Dies., 1851), et peut-etre Crossocephalus Raill., 1909 (Pterocephalus Linst., 1899).

"Heterocheilinae; englobant provisoirement toutes les formes a caecums esophagines ou intestinaux; Heterocheilus Diesing, 1839; Typhlophoros Linst., 1906; Porrocaecum n.g., type P. crassum (Des L.) = Ascaris crassa Des Longchamps, 1824; Crossophorus Hempr. and Ehbr. 1828; Lecanocephalus Dies., 1839; Contraecum n.g., type C. spiculigerum (Rud) = Ascaris spiculigera Rud. 1809."

The groups designated and described by Dujardin (1845) as Ascaris subgenera were thereby raised to the rank of subfamilies by Railliet and Henry (1912). These authors also established several new genera based on the morphology of appendages associated with the digestive tract. Later, Henry and Railliet (1915) suggested raising these subfamilies to family rank.

Baylis (1920) combined the two families of Railliet and Henry's (1915) into the subfamily Anisakinae. He thought that the division

of the esophagus into two histologically distinct portions was a more conservative and uniting character than the occurrence of appendices to the esophageal ventriculus or of caeca (also, blind appendages) to the intestine. Baylis' definition of the subfamily included of six genera: Anisakis; Raphidascaris, Railliet and Henry, 1915; Porrocaecum; Contraecum; Dujardinia Gedoelst, 1916, Angusticaecum Baylis, 1920; Amplificaecum, Baylis, 1920.

Baylis (1920) synonymized Terranova Leiper and Atkinson, 1914 with Porrocaecum, Kathleena Leiper and Atkinson, 1914 with Contraecum, and Hysterothylacium Ward and McGath, 1916 with Raphidascaris. The genus Terranova was resurrected by Johnston and Mawson (1940); its validity is generally accepted today although the diagnosis has been restricted.

Yorke and Maplestone (1926) and Chitwood (1939) accepted the system proposed by Baylis (1920). Mozgovoi (1950) presented a modified taxonomic scheme. This worker, in addition to translating all generic and many specific descriptions into Russian, also established new genera and divided many large genera into subgenera. The reasoning behind some of this work is unclear in Kabata's (1964) translation of the original Russian treatise. Since several obvious errors have been encountered in this translation, discussion of Mozgovoi's actual contribution must be postponed until better translations of the original texts are available

Hartwich (1954, 1957, 1974) presented a new taxonomic system which emphasized the morphology of the excretory system and

digestive glands, in addition to the diagnostic value of variations in the structure of the digestive tract. He also thought that ecological and life cycle information have taxonomic significance.

Osche (1958) presented a system of classification for the Anisakinae which differed in interpretation from Hartwich's but was based on similar criteria, with the exception that major emphasis was given to the morphology of the lips. Osche further refined his system through 1963.

Yamaguti (1961), in his taxonomic monograph of nematodes, did not accept the newer anisakine classifications by Hartwich or Osche, but used Baylis' criteria only changing the group name to Filocapsularinae on the basis of priority. Johnston and Mawson (1945) had performed a similar disservice by resurrecting the name Stomachus and designating the subfamily as Stomachinae. The aforementioned names and Capsularia must be rejected because they are nomen dubia; they refer only to the larval anisakine nematodes found in fish. From their sparse descriptions, these forms cannot even be assigned to a recognized family.

In fairness to Baylis, it should be stated that he placed more emphasis on lip structure after 1920, and that after 1923 he either noted the position of the excretory pore in relation to the nerve ring and lips or stated that he was not able to determine its position in the material available to him.

In this study I have adopted the taxonomic system originally proposed by Baylis (1920). It is the most widely accepted system of classification. The life cycle data, which the other systems consider, are incomplete and often speculative. The morphology of the excretory system in rarely encountered forms is unknown.

For instance, Lichtenfels et al (1976) reported that members of the genus Sulcascaris (= in part, Paranisakopsis) may have an excretory system that differs in the position of the excretory pore and type of gland from the system which Hartwich (1957) ascribes to them. The morphology of the lips, the structure and morphology of the digestive system, the position of the excretory pore and the structure of the excretory gland are selected as criteria of taxonomic importance, in addition to the arrangement of sensory structures and the anatomy of the reproductive system. When more complete ecologic and life cycle data become available, these data might be useful in future taxonomic schemes. Traditionally, however, the International Code of Zoological Nomenclature (Stoll, et al. 1961) has given more weight to morphological criteria. This code is currently undergoing revision and the new version may increase the importance of ecological considerations. A diagnosis of the subfamily and diagnoses of all constituent genera encountered in these studies are presented in the results section.

#### Life History Pattern

Anisakine eggs are passed out of the definitive host in feces. They are either uncleaved or at an early cleavage stage. In a

suitable aquatic environment, embryonic development continues until a first stage larva (L<sub>1</sub>) is formed. These larvae begin their first molt by depositing a second cuticle beneath the old cuticle, which is retained as a sheath. The larvae then hatch as ensheathed L<sub>2</sub> and are free living. They may attach themselves to a substrate by the tail as in the case of Phocanema decipiens (see McClelland, 1975) and Anisakis simplex (Van Banning, 1975), or swim freely as in the case of Contraecaecum osculatum (McClelland, 1976) until they are consumed by an intermediate host. If this does not occur, they die. The L<sub>2</sub> emerges from the sheath in the intermediate host or in vitro.

The life cycles of members of the Anisakinae generally involve one or two invertebrate intermediate hosts, and may involve a vertebrate second or third intermediate host. In addition, paratenic hosts, usually fishes, may be involved. By definition, paratenic hosts are not required by the parasite for its physiological and morphological development to the next stage of its life cycle. However, paratenic hosts may be required because of particular ecological circumstances. For example, the definitive host becomes infected by consuming intermediate or paratenic hosts that contain infective larvae. In the definitive host, the larvae molt and develop into mature male and female nematodes. After copulation, the female produces fertilized eggs.

Information concerning the intermediate hosts of anisakines has been derived mainly from survey data. Table 1 summarizes the information of natural infections in invertebrate hosts. Table 2 summarizes the information on experimental invertebrate intermediate hosts.

Thomas (1937a) presented the first experimental results on the life cycles of Contracaecum spiculigerum and Raphidascaris canadensis. He incubated eggs until they hatched. He then fed hatched larvae to intermediate hosts, and subsequently fed the infected intermediate hosts to definitive hosts. Details of development in either the intermediate or the definitive hosts were not given.

The life cycle patterns of the anisakines may be categorized by the number of intermediate hosts utilized. The life cycle of Porrocaecum ensicaudatum is reviewed at this point as representative of the type involving two hosts, that of Contracaecum spiculigerum is presented as a representative of the three intermediate host type of life cycle, and that of Phocanema decipiens is representative of the four intermediate host life cycle.

A TWO HOST FORM: Porrocaecum ensicaudatum. Levin (1957, 1961) reported that ensheathed infective larvae of P. ensicaudatum are located in the central blood vessels and hearts of the annelids Lumbricus terrestris and Octolasion lacteum. When the larvae in intermediate hosts are ingested by robins, chickens, or starlings, they penetrate the gizzard and come to lie between the horny and the muscle layers. The larvae complete exsheathing within 48 hr, and by 72 hr are found embedded in the intestinal wall. The gonads develop in both sexes and by day 10 preparation for the second molt has commenced. By day 14, this molt is complete and the young adults emerge from the avian hosts intestinal tissues approximately 18 days after infection. These are luminal parasites.

A THREE HOST FORM: Contraecaecum spiculigerum. Huizinga (1966) found that the eggs of C. spiculigerum, a proventricular parasite of marine piscivorous birds, sink in sea water with a salinity of 30%. Newly oviposited eggs include embryos in the initial cleavage stages and development to L<sub>1</sub> occurs in 4-5 days at 21°C. The first molt occurs within the egg capsule (or shell) between the day 5 and 7 after oviposition and an ensheathed L<sub>2</sub> emerges from each egg. Development within the egg capsule is prolonged if maintained at 13°C, but is suspended at 7°C. The L<sub>2</sub> possesses a boring tooth that may aid in its escape from the egg capsule and subsequent penetration of the gut wall of the first intermediate host. The boring tooth is a characteristic common to many anisakine larvae.

The free-living, second-stage larva has a ventricular appendix but its intestinal caecum is not completely developed. It will hatch in sea water, tap water, and distilled water. Although it survives for some time in all three types of water, its longevity is much greater in sea water.

The first intermediate host for C. spiculigerum is a copepod. Huizinga (1965, 1966) reported that both Cyclops vernalis and Tigriopus californicus will actively ingest free-living, L<sub>2</sub>s of C. spiculigerum and serve as compatible hosts. Mozgovi et al. (1965) report that unspiciated Cyclops and Macrocyclus serve as first intermediate hosts for this species of nematodes in Russia. Between 95 and 100% of the exposed copepods became infected with 1 to 9 larvae. The majority of copepods, however, contained 1 or 2 larvae each.

Huizinga reported that the larva sheds its sheath in the copepod gut and penetrated the hosts gut wall within 15 to 30 min. Larvae removed from the hemocoel of a copepod after 7 days had a mean length of 350 mm. In comparison, the free-living L<sub>2</sub> had an average length of 329 mm. Mozgovi et al. (1965) reported growth from 420 to 460 in 2 to 3 weeks in copepods.

It is of interest to note that free-swimming L<sub>2</sub>s are also infective for fish, although apparently with less frequency than for copepods. Moreover, free-swimming L<sub>2</sub>s are less infective to fish than those that occur in copepods. For example, Huizinga (1966)

stated that if guppies, Lebaistes reticulatus, ingested L<sub>2</sub>s of C. spiculigerum, the infection rate is low. Specifically, of 200 guppies fed "large numbers" of such larvae, only 7 became infected. These larvae were recovered from the fish host's intestinal mucosa and mesenteries as motile, exsheathed L<sub>2</sub>. Ensheathed, dead, L<sub>2</sub>s were encountered in examination of the fecal strands of the guppies. In comparison, if guppies and killifish, Fundulus heteroclitus, were fed copepods harboring exsheathed L<sub>2</sub>s, all became infected. This information suggests that ingestion, exsheathment, and subsequent migration within the first intermediate host greatly enhances the infectivity of these L<sub>2</sub>s to fish.

Mozogovoi et al. (1965) unsuccessfully attempted to infect Daphnia, Gammarus, dragonfly larvae, chironomids, oligochaetes, molluscs, tadpoles, and baby fish with C. spiculigerum larvae hatched from eggs. However, the larvae were infective to dragon flies of the genera Coenagrion and Agrion after as little as 24 hr in the copepod host. The intensity of infection in dragonflies fed infected copepods was 30-60 larvae per individual, with the L<sub>2</sub> usually located in the middle body segments of the dragon fly larva. The nematodes were motile for several days but subsequently became coiled and quiescent. The growth rate of C. spiculigerum was more rapid in dragonfly larvae after 1 day in a copepod and 10 days in a dragonfly than in copepods alone. The larvae were 690 to 880  $\mu\text{m}$

long after 11 days in two hosts compared to 420 to 460  $\mu\text{m}$  maximum attained in copepods alone. The authors found that some larvae from copepods were also infective to fish, suggesting that the dragonfly larva act, as a paratenic host.

According to Huizinga (1966), when an  $L_2$  was ingested by a fish host, it penetrated the intestinal wall and became encapsulated by host tissues. In spite of this, growth occurred and the larva attained a mean length of 1262  $\mu\text{m}$  after 18 days in the fish. At this time the second cuticle was deposited without complete ecdysis. The cuticle of the  $L_2$  ensheathed the  $L_3$ .

Huizinga also found that the avian definitive host became infected with C. spiculigerum when fish harboring ensheathed  $L_3$  were ingested. Within the definitive host, the nematode not only casts its sheath but also, presumably, molted twice prior to developing into the adult. The course of infection in the definitive host was not followed in detail.

Mozgovoi et al. (1965) found that larvae from dragonflies were also infective to fish. Cormorants, their natural definitive host, were be infected by larvae that grew and matured for 10 days in cyclops and 44 days in a dragonfly larva, but not with larvae that had been retained for 10 days in a cyclops and 30 days in the fish host, the bream. The only report which addresses itself to the minimum infective size of larvae Contracaecum is that of McClelland (1975) on in vitro cultured larvae of C. osculatum which

required growth to at least 6 mm at 15°C in order to molt when the cultivation temperature was raised to 35°C.

A MULTI-HOST FORM: Phocanema decipiens. McClelland (1978) reported that the primary development of P. decipiens larvae occurred in the hemocoel of harpacticoid and cyclopoid copepods. Development continued in amphipods. The nematode larvae became infective to fish after as little as 4 weeks in invertebrate hosts. Table 1 lists the genera of invertebrates and the specific and common names of fish which were used as experimental hosts for P. decipiens. All the copepods were previously undescribed. In addition, nudibranch molluscs have also served as second intermediate hosts.

When copepods ingested ensheathed larval P. decipiens, the parasites molted in the copepod's gut and penetrated the gut wall. Penetration of the gut wall was accomplished by the larval nematodes pressing on one side the gut with their tail and penetrating with the anterior end through the opposite side of the intestine. Growth in the hemocoel of a copepod was temperature dependent. For example, in Tisbe spp., larvae grew from an average of 200 µm at time of infection to an average of 325 µm in length after 6 weeks at 5°C or after 1 week at 15°C. These measurements were taken from living worms, fixed larvae were about 20% shorter. Further growth was not observed in copepods after longer periods of incubation.

McClelland normally infected adult amphipods by allowing them to browse on infected copepods. However, he reported that smaller larval amphipods were susceptible to infection with free living L<sub>2</sub> larvae, when such an infection occurs, there was one invertebrate host only is needed before the P. decipiens larvae became infective to fish. Adult amphipods were not susceptible to infection with ensheathed L<sub>2</sub> larvae of P. decipiens. McClelland attributed the lack of infectivity to the larger gut lumen of adult amphipods; the lumen being too large for larvae to span by bracing themselves against the other side in order to penetrate.

Larvae from invertebrates became infective for fish after they reached 4 mm in length. They grew to this size in as little as 4 weeks in invertebrate hosts maintained at 15°C. After attaining infective size, larvae continued to grow in the invertebrate and after 12 weeks the average length was 7 mm (max 9.8 mm). A greater growth rate was achieved in vertebrates. For example, in smelt, larvae grow from 4 mm upon ingestion to 27 mm in 8 weeks when the host is maintained at 15°C. The growth rate is similar in cod but less in killifish and flounder.

Fish-to-fish transmission of larval P. decipiens was demonstrated experimentally by McClelland (1978). After 1 week in killifish, Fundulus spp., larvae were infective to cod and smelt that they ingested the infected killifish. The infectivity of younger or older larvae was not tested.

### Anisakinae.

INVERTEBRATE INTERMEDIATE HOSTS. A variety of invertebrates have been described as intermediate hosts of anisakine nematodes. Table 1 lists those that have been reported as naturally infected with anisakine nematodes. Table 2 lists those hosts which served as intermediate hosts under experimental conditions. It should be noted that three phyla are represented in the experimental host list and six phyla in the list of natural hosts. These data suggest a lack of host specificity in larval anisakine nematodes. Conditions which influence intermediate host susceptibility to anisakine nematodes have not been investigated except for McClelland's (1975) observation on the effect of gut size.

As previously mentioned, Mozgovoï et al. (1965) reported that the larvae of C. spiculigerum grow more rapidly in dragonfly larvae after an initial period of development in copepods.

McClelland (1978) reported that the growth rate of larval P. decipiens in gammaridian amphipods was dependent upon the intensity of infection. The larvae grew to an average length of 6 mm in 8 weeks in amphipods infected with 1 or 2 larvae, but to only two-thirds this size in amphipods with heavier infections. McClelland suggests that the rate of growth is dependent on the quantity of nutrients available. Larger hosts with similar infection rates can provide the developing larvae with more available nutrients than small hosts; therefore, the more rapid growth rate in larger invertebrates would be expected. Comparison

of McClelland's (1974, 1975) in vitro and in vivo growth data suggest this is the case eliminating the possibility that other factors in heavily infected hosts influence the growth rate.

FISH INTERMEDIATE HOSTS. When larvae of anisakines are found in naturally infected fish, it is not always possible to distinguish whether the host is a true intermediate host or a paratenic host. For example, Thomas (1937a,b) has reported that L<sub>3</sub> of Contracaecum spiculigerum could be transmitted from guppies, Lebistes reticulatus, to larger predatory fish in which they become encapsulated. Similarly, Huizinga (1967) experimentally transmitted L<sub>3</sub> of C. multipapillatum in guppies to the large mouth bass, Micropterus salmoides, and recovered encapsulated L<sub>3</sub> larvae in the bass on necropsy 7 days later. Scott (1954) and McClelland (1976) have demonstrated that Phocanema decipiens may be transmitted from fish to fish and Khalil (1969) recently reported this is also possible with Anisakis sp.

The possible existence of paratenic hosts in the life cycles of anisakines was first suggested by Agersborg (1918). This investigator observed the bluefish, Gadus (= Pollachius) virens, and the Norwegian inshore cod, Gadus callarias, and concluded that L<sub>3</sub>s of this parasite were accumulating in inshore fishes as the result of dumping discarded viscera from processing plants into shallow water where the worms embedded in viscera were ingested by the young bluefish and cod inhabiting these waters.

It could be argued that larvae in fishes that are true intermediate hosts are not usually encapsulated and may be distinguished from those in paratenic hosts on this basis. However, insufficient experimental data are available to support this thesis. In other words, it is not known that nematodes in true intermediate hosts are always were encapsulated and those in paratenic hosts are always encapsulated.

DEFINITIVE HOSTS. When a fish intermediate or paratenic host harboring L<sub>3</sub>s was ingested by the definitive host, which may be another fish, an amphibian, a reptile, a bird, or a mammal, the parasite may undergo one or two additional molts and eventually develop into the adult. Details of development in the definitive host phase of the life cycle of anisakines have remained largely unknown.

According to Rausch (1953) and Schiller (1954) who studied Phocanema (= Porrocaecum) decipiens, the L<sub>3</sub>, upon being ingested by the sea otter, became free in the small intestine and burrowed into the mucosa where it molted and developed into a L<sub>4</sub>. The L<sub>4</sub> migrated from the host's intestinal mucosa, up the alimentary tract, and entered the stomach where it becomes attached to the gastric mucosa. At this site, the larva undergoes the fourth molt to become the adult. Young adults remained attached to the host's stomach wall until reaching sexual maturity, at which time they moved into the intestinal lumen.

Scott (1953) found that harbor seals Phoca vitulina, which had been exposed experimentally to larval Phocanema decipiens passed eggs in their feces as early as day 17 and usually by day 22 post-exposure. McClelland (1976) reported detection of eggs around day 20 and that patency lasted 35 to 40 days in primary infections in harbor seals. In one harbor seal, which retained 54.7% of the infective dose of 1000 larvae to day 30, the maturation of the nematodes was delayed; however, each adult from the infestation was as large or larger than those individuals from lighter infections in this particular host. The patency period of females in challenge infections was reduced to 15 days, indicating the possibility of an acquired immune response. McClelland (1976) also reported that some seals lost a significant portion of their infections by regurgitation. He attributed regurgitation to causes other than the P. decipiens infections. Usually less than 10% of the nematode dose reached patency in this study of harbor seals. However, in gray seals about 50% of the force fed worms reached patency, and the individual nematodes were larger and females contained correspondingly more eggs in this host. This suggested that the gray seal is a better host than the harbor seal. Gray seals are also more numerous (Mansfield and Beck, 1977), 25,000 compared to 2000 for harbor seals, in the Atlantic Maritime Provinces of Canada. McClelland considers the grey seal to be the major source of P. decipiens eggs in the northwestern Atlantic Ocean.

### Host Pathology

INVERTEBRATES. Practically nothing is known about the pathogenicity of larval anisakines in their first intermediate hosts. Dogiel et al., (1958) stated that infected crustaceans swam more slowly than noninfected ones. This, of course, rendered them more vulnerable to predation. McClelland (1975) reported that infections with three or more larvae of P. decipiens often were fatal to copepods. He has also noted differences in the degree of host cellular response to the larvae. These gross observations have not been analyzed microscopically.

McClelland (1976) also reported, as preliminary findings, the heavy infection in gammarid amphipods with Phocanema decipiens. The amphipods did not show increased mortality, lower growth rates, or impaired reproductive capability compared to uninfected controls. However, the amphipods moved sluggishly, had depleted reserves of both carbohydrates and fats, and "milky" precipitates in their hemolymph.

FISH. Agersborg (1918) noted that larval anisakines cause a decrease in the overall quality of marketable fish in Norway, especially the bluefish and inshore cod. He did not identify the larval nematodes. However, Punt (1941) and Berland (1961) have since examined fish caught in the same area and are of the opinion that Agersborg studied fish parasitized by an Anisakis sp. and a Contracaecum sp.

Bazikalova (1932), working with gadoid fishes from the Barrents Sea in the USSR, has implicated larval Contracaecum spp. as the cause of deleterious effects on the fish. Her conclusions have been supported by several subsequent Russian investigators (Markowski, 1937; Shulman, 1948; Shulman and Shulman-Albova, 1953; Petrushevsky and Kogtiva, 1954; Getysevichyute, 1955; and others). These investigators unanimously concluded that the absolute size and weight of the liver, the total weight of the fish, and the coefficient of condition, (Coefficient of condition (K) =  $\frac{W \times 100}{L^2}$  where W = weight in grams and L = length, in cm of the fish), is based on the gutted weight, i.e., the weight without the internal organs), of infected fish all decrease as a function of larval Contracaecum density.

Numerous other observations on the pathology caused by anisakines in fish hosts are available. The following synopsis presents two views on the mechanism of the pathogenicity.

Remotti (1933a) found Anisakis larvae on the surface of the atrophied liver of Merluccius merluccius. Guairt (1938) used this finding and his own of Contracaecum capsularia on the liver's surface of other moribund fish to support his view that the damage to fish was due to toxic rather than mechanical damage. This hypothesis had been suggested earlier by Bazikalova (1932). Rosenthal (1967) has studied the effect in cultures of larval Contracaecum on larval herring, Culpea harengus, the worms were observed in situ through the body wall in cultures of the herring by

use of cinemicroscopy. He reported that the locomotor activity of the Contracaecum larvae increased, beginning about 7 days post-infection. The elasticity of the host intestinal tissue was reduced and remained in this pathologic condition. The damaging effects persist and the young herring died about 11 days post-infection, after undergoing a period of violent, uncoordinated swimming. The larvae usually migrated to the heart region of the dead host, and then left the body by penetrating the body wall. The escaped larvae remained alive, but inactive, for about 3 days in sea water. Rosenthal attributed most of the pathologic effects of Contracaecum larvae to mechanical damage, but did not discount the possibility of metabolic waste products acting as toxins. It should be mentioned that this study was carried out on herring larvae under 20 mm in length.

Experimental studies which mention pathology in fish are limited, for example: Thomas (1940) reported that guppies heavily infected with Contracaecum spiculigerium became emaciated and died. McClelland (1976) reported a 30% mortality among Phocanema decipiens-infected Fundulus heteroclitus. He also noted a proliferation of fatty tissue in the mesenteries and peritoneum of infected fish.

DEFINITIVE HOSTS. The invasion by adult members of the Anisakinae into the alimentary tracts of fishes may cause pathological changes. For example, Linton (1900) reported that when Acanthocheilus nudifex occurred in the submucosal crypts of a shark (Galeocerdo cuvieri) stomach, the underlying serosa became hardened

and the associated mucosa was hemorrhagic and heavily infiltrated with leucocytes. Williams and Richards (1968) described the infiltration by eosinophils around the anterior end of Pseudanisakis rotunda in the submucosa of the elasmobranch Raja radiata. Eosinophilic infiltration was also noted around the sloughed cuticle of this parasite.

Murie (1868) reported that large numbers of Anisakis rosmari (= A. bicolor) were associated with stomach ulcers in a walrus. He concluded that the ulcers probably caused the death of the animal. Murie's description of the lesions follows:

"On opening the stomach, which was of moderate size, I was much surprised to find that it contained small, round worms, a species of Ascaris, in such quantities that, when these were turned out, there was altogether about half a pailful. They occupied the entire interior of the viscus, but were in greatest abundance at the bend of the peculiar siphon-like stomach.

"The entozoa swarmed between the rugae, and in many cases were firmly attached to the membrane. The mucous membrane lining the interior was of an intense red hue; but here and there were somewhat paler patches. More rigid examination showed that these last were extensive ulcerations, the mucous membrane being entirely eroded, and only the muscular and a very thin lining of submucous tissue remained, preventing perforation of the walls of the stomach. The chief ulcerations were some four in number, and varied in size and situation.

"One, nearly circular, three-fourths of an inch in diameter, occupied the anterior wall at a distance of between 5 and 6 inches from the cardiac end. Another, somewhat diamond-shaped, 2 1/2 inches by 1 1/2 inches at widest, also existed on the anterior wall of the viscus and about its middle. In the ulcerated erosion, the mucous coat was in some parts so excavated underneath as to leave one-half inch of an overhanging lappet of membrane. On a section being made vertically, the submucous tissue was seen to be absent, the muscular and serous coats alone prevent perforation of the wall. At this part of the wall the stomach had a thickness of only 0.1 of an inch, although it seemed as if the muscular fibers were slightly increased in numbers here, possibly from the effects of the irritation going on in the neighborhood and within. A third ulcer, of an elliptical form, 2 1/2 inches long, and with more regular edges than the preceding, had been eaten away on the anterior wall, close to the lesser curvature of the stomach and between 5 and 6 inches from the pylorus. Between the second and third erosions here described, but on the posterior wall of the stomach, another very extensive patch of ulceration had taken place. This ulcer stretched between the greater and lesser curvatures. It had a semilunar figure, was rather more than 4 inches long, possessed irregular borders, and varied from one-half to 1 inch in width. The mucous coat around had been undermined in a manner similar to that described above as occurring in the second ulcer. To the right and lying parallel

with this large excavation were a series of small circular and ovoid spots, which had been eroded in like manner with those already described. The spots just spoken of varied in size from about a three-penny piece to a shilling, and they evidently were fast running into one single, long ulcer, resembling that upon the left side. Only a very few worms were found here and there in the intestinal tract; some were observed to have passed previously after the horse fat having been given."

Hoepli (1932) reported an ulcerous lesion in the stomach wall of a walrus, Rosmarus rosmarus, infected with Anisakis sp. which was identified by Hsu and Hoepli (1933) as Anisakis alata but which subsequent authors have regarded as being A. rosmari. The ulcerous lesion was characterized by host necrotic tissues surrounding the anterior end of the worm. In fact, Hoepli reported a zone of tissue liquifaction of about 0.5 mm in diameter surrounding the anterior end of the parasite. He attributed this liquifaction to esophageal secretions of the parasite.

Rausch (1953) studied the sea otter, Enhydra lutris, from the Aleutian Island of Amchitka that were infected with Phocanema (= Terranova = Porrocaecum) decipiens. He noted that no externally visible symptoms occur in infected otters until they are near the terminal stages of illness, before dying they became weak and depressed. The specimens examined were obtained from dead or moribund otters, some of which had been captured and thus unusually stressed.

Healthy sea otters do not contain much subcutaneous or abdominal fat, hence an overall deterioration of the body's condition in parasitized specimens is not readily evident. The cause of death of animals with P. decipiens appeared to be a generalized peritonitis resulting from penetration of the gut wall by larval parasites. Numerous P. decipiens were found on the surface of the greater omentum, the body cavity contained a thin bloody fluid, and intestinal coils and serous membranes were coated with a fibropurulent exudate. The intestinal coils adhered to one another and to the greater omentum in inflamed areas. If the omentum had been penetrated, it was thickened and often adhered to internal organs which were invaded by larval nematodes. The areas surrounding intestinal perforations were inflamed and greatly congested. The nematodes often protruded into the abdominal cavity and dense aggregations of L<sub>3</sub> nematodes could be found attached to the intestinal wall around the perforations. The stomach showed dense aggregations of P. decipiens embedded in the mucosa, but no stomach perforations were noted. Older L<sub>4</sub> and immature adult nematodes were predominant in the stomach lesions. These could be differentiated by their darker color in contrast to the translucent younger stages.

Microscopically, Rausch determined that the anterior ends of the nematodes in the stomach were deeply embedded in the gastric

mucosa. The gastric mucosa was eroded to the level of the muscularis mucosa in the areas of parasite attachment and occasional invasions into the muscularis externa were noted. Tissues were infiltrated with monocytes and the general areas was hyperemic. In the case of the small intestine, the areas showing pathological manifestations were those directly surrounding the sites of invasion. The mucosa in such an area had epithelial desquamation, but it was otherwise unaltered. Groups of larval nematodes were found at each focus which was infiltrated mainly by segmented neutrophils. Fibroblastic proliferation also occurred.

The omentum near perforations was coated with a fibropurulent exudate, although the serosal epithelium was usually intact. The cellular reaction consisted mainly of infiltration by segmented neutrophils. When nematodes were found embedded in inflammatory tissue, liquifaction of host tissues around the anterior end of the worm was observed. The invaded spleen showed marked fibrosis, adherent omentum, and hyperemia in the regions of the parasites. The cellular exudate consisted of segmented neutrophils, macrophages, and plasma cells in decreasing order of abundance.

Vik (1962) has described briefly, two lesions in the stomach walls of porpoises caused by Anisakis larvae. The lesions were circular, one was 10 mm and the other 22 mm in diameter, and they contained 15 and 50 nematodes, respectively. These larvae corresponded morphologically to those described by Berland (1961)

and those described by van Thiel et al. (1960) from fish and man, respectively.

Young and Lowe (1969) described gross lesions in two of nine gray seals from the Orkney Islands. The lesions, elevated inflammatory areas about 3 cm in diameter, were located in the fundus of the stomach and contained dense clumps of Contracaecum sp. larvae. Each larva had penetrated deeply into the submucosa and destroyed the overlying mucosa. The anterior, buried parts of the nematodes were surrounded by fibrin and inflammatory cells. The lips of each nematode were surrounded by an eosinophilic, amorphous substance which was itself surrounded by degenerating nuclei and fibrin; outside this layer of necrosis there was a massive region of cellular infiltration. Similar areas of infiltration were encountered in other parts of the lesion but no nematodes, only cast cuticles, were apparent.

The average Orkney seal contained 3,000 nematodes, 75.9% were Contracaecum sp. and 23.9% were Phocanema (= Terranova) sp., while the remaining nematodes were Anisakis sp., 21% of the nematodes were fully mature. Seals from eastern Scotland had fewer nematodes (an average of 600/seal) and the proportions of each genus differed: Phocanema sp. 1.6%, Contracaecum sp. 26.2%, and Anisakis sp. 72% and, furthermore, 3% of the nematodes were mature. These lesions were present in nine of the 12 seals examined. Lesions in eight of the seals were associated with Anisakis sp. larvae while the remaining lesion was associated with Contracaecum sp.

These authors also reported finding gross lesions in four of seven porpoises examined from eastern Scotland. The stomach of the porpoise is divided into five chambers; each chamber is numbered, starting at the esophagus, and termed a stomach. The lesions were associated with Anisakis simplex infections and Anisakis sp. larvae in all but one case, a lesion in the second stomach that contained nematode cuticles but no living nematodes. The other large nodular lesions, 2-5 cm in depth, had a crown of tightly clustered Anisakis sp. (40 to 400 individual nematodes, a large portion of which were adults). Histologically, these lesions were similar to those in grey seals; but there were more numerous inflammatory areas in the submucosa associated with recently cast cuticles and there were no apparent intact nematodes. These areas were often calcified, and the eosinophils and neutrophils present showed degenerative changes. Lesions were found in the first, second, and third stomachs. The mass of granulation tissue was largest in the first stomach where the submucosa had increased to 4 cm in thickness compared to a normal thickness of 2 mm. The stratified squamous epithelial and muscular layers were also of increased thickness.

The lesions in the other two stomachs were similar, but less severe. In the second stomach there was calcification of a muscularis externa area between the longitudinal and circular layers.

### Anisakine as Experimental Animals

As experimental animals, the anisakine nematodes have distinct advantages and disadvantages. They are plentiful if, and only if, one has had direct access to their natural hosts. The stomach of one Weddell seal may supply as many as 500 adult nematodes, and a single female worm contains an estimated 750,000 eggs. A single cod fillet may contain more than 50 infective larvae.

Laboratories not near habitats of natural hosts are at a disadvantage because the material does not store or travel well. Numerous poor or negative results in physiological experiments can be attributed to the use of shipped parasites.

To date, no complete life cycle of an anisakine nematode has been established in convenient experimental hosts. Yet, the few laboratories that do have ready access to fresh material may take advantage not only of the relatively large size and abundance of the parasites, but of their wide temperature tolerance, of the variety of hosts in which the worms may develop, of interesting evolutionary divergences among anisakine species and genera, and if one has the freshest material to start with, of successful culture attempts compared to those with most other nematode parasites of mammals.

Some of the experiments done with anisakines have basic information as their goal. Carbohydrate and amino acid analyses of tissue and fluids (Fairbairn, 1958; Viglierchio and Gortz, 1972), behavioral characteristics and adaptations (Ronald, 1960),

cultivation of the adult and larval stages (Townesley et al., 1963; McClelland and Ronald, 1970). Other studies have practical aims, such as prevention or diagnosis of human disease.

Reviewed herein are two aspects of experimental work: anisakine development in cultures and determination of the worm's temperature tolerances. To begin with, however, the need for establishing anisakine life cycles in the laboratory must be stressed.

EXPERIMENTAL HOSTS. The quality of all experimental data obtained with anisakines will be improved when the complete life cycles of members of several genera from different definitive hosts become established in the laboratory. At minimum, achievement of this goal will remove uncertainties associated with the species, stage, and age of experimental materials derived from the field. It may also define the role of the various anisakines as etiologic agents of human disease, add comparative information to the establishment of taxa, and reduce the temporal delays and unpredictable quantities associated with the collection of field material.

Worm penetration studies are just one example of the inadequacy of field material for experimental work, especially at distant laboratories. Houwing (1969) at Ijmuiden, Holland, has reported that 100% of the fresh Anisakis marina would penetrate agar butts in vitro. Ruitenbergh (1970) reported that, at most, 43% of these

larvae from the same source would penetrate. Ruitenberg worked at an inland laboratory to which the worms had to be shipped. He also selected his material, using only rapidly moving larvae. It is possible that such selection may have provided him with larvae that are the least able to adapt to their environment, rather than with those that are resting successfully through a period of stress. When laboratory cycled material becomes available, one will at least be able to test whether the experimental material is still infective.

#### Cultivation

All stages of the anisakine life cycle i.e., egg, larvae, and adults, have been used to initiate cultures.

ANISAKINE EGGS. Complex culture media are not needed for egg development; fresh water or sea-water is sufficient. In the laboratory it may be necessary to obtain the eggs free of microbial contaminants. Phocanema decipiens eggs, in common with the eggs of other ascarids, were sticky and adhere firmly to container walls. This characteristic makes them difficult to wash in order to dilute contaminants to extinction. Moreover, axenizing solutions such as 0.12% gluteraldehyde, 0.45 formalin, 1.33% Zephiran Chloride (Winthrop Laboratories trade mark for a mixture of alkyl dimethylbenzylaminium chlorides, alkyl groups range from C<sub>8</sub> to C<sub>18</sub> or 0.05% sodium hypochlorite) inhibited egg development.

With respect to their tolerance for chemical disinfectants, anisakines were more fragile than some other ascarids. Their eggs developed in the following antibiotics at one and two times the recommended concentration for tissue culture: gentamycin, penicillin, streptomycin, neomycin, amphotericin B, and mycostatin (Bier, 1976).

Egg development, hatching time, and optimal incubation temperatures vary among species and strains. Kobayashi et al. (1966, 1968), as reported in Oshima (1972), found that an unspecified female Anisakis in the stomach of the blue-white dolphin, that also contained males specified as A. simplex, produced eggs which would develop in sea-water or physiological saline but not in 0.5% formal agar. Eggs from individual female worms were incubated separately at temperatures from 2° to 27°C. Table 3 shows the time required for hatching at various temperatures. Two strains were evident: one adapted to high temperatures and one adapted to lower temperatures. Two morphologically distinct types of female worms had yielded these eggs. One type of female had slender bodies, with the vulva in the anterior portion of the body. The other was stout by comparison, with the vulva in the posterior half of the body. However, both strains of eggs were derived from each morphological type of female worm. This finding further clouds the

already confused taxonomic distinction between A. simplex and A. typica which Davey (1971a) had seemingly resolved by associating A. typica, the species with stout females, with warmer climates.

HATCHED LARVAE. Some cultivation experiments have been started with anisakine larvae hatched from eggs. McClelland and Ronald (1970, 1974, 1974) and McClelland (1971) reported the long term cultivation, growth, and molting of the hatched larvae of Phocanema decipiens (= Terranova decipiens) and Contracecum osculatum in a mixture of Eagle's minimal essential medium and 20% fetal calf serum. Table 4 indicates the extent of the in vitro larval growth of P. decipiens. The average length of larvae at hatching was 140  $\mu\text{m}$ ; the average length after 52 weeks at 15°C was 31.1 mm. Growth commenced soon after spontaneous ecdysis of the first stage larval cuticle. The intestinal caecum first appears in larvae that are 6 weeks old and approximately 2 mm in length. There is no development of the genital primordium, yet there has been overall growth as indicated by an almost constant ratio of the anterior extremity of the genital primordium to the overall length of the nematode. No molts were observed during these 52 weeks. These observations from hatching through 8 weeks of cultivation at 15°C have been confirmed (Bier, 1976).

Freshly hatched larvae of C. osculatum average 382  $\mu\text{m}$  long. They were induced to exsheath with 0.05% sodium hypochlorite (Davey, 1969). Their growth pattern is documented in Table 5. The larvae lengthened to 6.5 mm in 32 weeks, the venticular appendix was present at hatching, the intestinal caecum had developed after 2 weeks of cultivation and the extent of the overall growth of the nematode was approximately 20 fold in 22 weeks.

The appearance of the venticular and intestinal appendages so early in the development of these two species supports Janiszewska's (1938) claim that she could identify larval forms of anisakines from fish as to genus, but not as to species. McClelland (1971) differentiated the position of the excretory pore, a characteristic of taxonomic importance, as early as the time of hatching in both C. osculatum and P. decipiens.

FISH-BORNE LARVAE. Numerous workers have removed anisakine larvae from fish and tried to cultivate them to adulthood using conditions that might simulate necessary stimuli from the definitive hosts.

Martin (1921) reported attempts to grow ascaroid larvae with an intestinal caecum which had been obtained from the flesh of the smelt, Osmerus eperlenus. These larvae were probably Phocanema decipiens (= Ascaris capsularia). Two specimens molted at 38°C in a medium of raw fish, pepsin, and HCl. Grainger (1959) cultured P. decipiens and an Anisakis sp. under similar conditions. The worms

that molted did so 4-7 days after they had fed. The major change reported in both species were the differentiation of the lip mass into three distinct labia. McClelland (1971) noted additional changes in P. decipiens: the doubling of four lip papillae and the development of the genital primordia to produce a rudimentary ovary, uterus, and vagina. No vulva was produced by females in his cultures.

Promising results were obtained by Townsley et al. (1963) using larval P. decipiens from fresh cod (Gadus morhua). They reported the maturation of both the sexes and the development of eggs in females. The medium used was human tissue culture Medium 199, beef embryo extract, glucose, and fresh liver extract. The development of gonadal tissue began 2 weeks after molting in female worms and was accompanied by rapid size increase, approximately one-third the worm's overall length. The males did not show the size increase, but the tail recurved and became dorsoventrally flattened. Copulation was not observed and the viability of the eggs produced in vitro was not tested.

A brief report by Van Banning (1975) described the most successful rearing of A. marina to date. The cultivation medium consisted of an acid digest of liver extract and citrated beef blood. The larvae molted within 4 days after introduction into this culture medium. The final maturation of the nematodes took 1 week and was accompanied by a thickening of the worms; this maturation

occurred 26 to 98 days after molting. A problem, insufficient fertilization, was overcome by adding older cultured mature males to cultures containing just maturing females. The length of the mature males was between 3.5 and 7 cm and of the females between 4.5 and 15 cm. Eggs produced in vitro hatched and yielded viable ensheathed larvae.

The major similarity between the successful cultures of Van Banning (1975) and Townsley's (1963) group is that both used absolutely fresh fish as a source of anisakine larvae. Those workers reporting molting without maturation worked with admittedly aged material or have not attested to its freshness.

A series of culture reports on Phocanema decipiens from cod fillets, K.G. Davey (1965, 1966), Davey and Kañ (1967, 1968), and Kan and Davey (1968a,b) described in detail the cuticle, molting, and ecdysis. Also, they postulated that ecdysis is controlled by a neurosecretory mechanism. The larval cuticle consists of a single layer of longitudinal fibers resting on a basal lamella, a matrix with two faintly osmophilic bands in a less osmophilic medium, and an inner and outer cortical layer. The new cuticle formed during molting was produced by the hypodermis and had arisen through three successive waves of condensation at its outer edge. The central layer splits into three osmophilic matrix. Some of the positive histochemical reactions of larval cuticle and of cuticle formed in vitro are summarized in Table 6. From these reactions, it is

evident that collagenase-labile protein was the major constituent of both cuticles. Protection against digestion is gained by the formation of disulfide bonds. The cuticle was not digested by trypsin, pepsin, or papain, and no evidence of tanning was found.

The secretion of new cuticle was accompanied by typical changes of secretory cells. The hypodermal and muscle cells showed enlargement of nuclei, nucleoli, mitochondria, and an increase of cytoplasmic and nucleolar RNA. Osmophilic material from the muscle cells appeared to be secreted into the hypodermis during cuticular deposition. Formation of a new cuticle occurred in the absence of nutrients in a saline solution at 35°C; however, a richer growth medium was necessary for ecdysis.

Ecdysis followed cuticular deposition, after 3.5 to 6 days for culture in a complex medium. Histochemically, ecdysis was monitored by testing for leucine aminopeptidase (LAP), an enzyme which was found in the excretory gland and then is released with the so-called exsheathing fluid that separated the old and new cuticles. In saline-incubated controls, LAP was released on day 3, but release was not accompanied by synthesis of enzyme, nor did ecdysis occur. On the other hand, nematodes incubated in complex culture medium showed an increase of LAP activity in the excretory gland on the first 2 days of cultivation, with release and appearance of LAP

activity between the old and new cuticle. Neurosecretory product was demonstrable by staining with paraldehyde-fuchsin; it was produced by cells of the dorsal and ventral ganglia of the nerve ring, being more apparent in the ventral ganglionic cells. This synthesis reached peak activity on the third day of cultivation. The release of neurosecretory product coincided with the release of LAP by the excretory gland and was also thought to increase LAP synthesis by the excretory gland. To test this hypothesis, nematodes were treated surgically. With their excretory gland partially exposed, they were placed in culture medium and treated with extract of the heads (including the nerve ring) of nematodes cultured for varying periods. The head extract of worms cultured for 3 days produced a marked increase in LAP production over a 3 hr period. Head extracts of worms cultured for 1, 2, 4, or 5 days, or extracts of body wall produced only a weak reaction. Davey (1971) reported that saline-incubated nematodes were induced to ecdyse after 2.5 days if juvenile hormone or farnesyl methyl ether at high concentration was added to the saline. Nematodes treated with these compounds in normal cultivation medium did not ecdyse due to the premature release of LAP. Evidence was given that these compounds act by stimulating the neuroendocrine system to produce paraldehyde-fuchsin positive material.

In summary, Davey and Kan (1968) defined histochemically the structure and composition of P. decipiens cuticle in larvae from

fish and from cultures, demonstrating that the processes of cuticle deposition and ecdysis are independent. They postulated and provided evidence for a neurosecretory mechanism that control ecdysis; and, they have shown that this system can be stimulated by an insect hormone and by a synthetic analog.

## MATERIALS AND METHODS

### Epidemiological Studies

FISH SAMPLING. One-thousand-ten whole and apparently fresh fish were bought in retail markets in the Washington, D.C. area. Most purchases were made during a three year period, 1972-1974, but two small additional samples were obtained in 1975. As far as could be determined, the fish had been refrigerated but not frozen, and the majority had been caught in the Chesapeake Bay or adjacent waters. After purchase, they were taken to the laboratory as quickly as possible and either examined for nematodes immediately or stored until examined. The stored samples were kept at ca 5°C in a food refrigerator.

IDENTIFICATION. Fish were selected by common name and gross features at the time of purchase. Prior to dissection, they were identified by the criteria of Hildebrand and Schroeder (1928) and classified using current nomenclature (Bailey *et al.*, 1970).

RECOVERY OF NEMATODES. As a first step, fish being examined were eviscerated; the viscera and the eviscerated portions of each fish were examined separately. The eviscerated portion, consisting mostly of edible flesh, was dissected grossly. Then, specimens were either dissected minutely to expose parasites, or digested for 4 hr at 35-37°C in a 1% pepsin solution that had been adjusted to pH 3 with 3N HCl after the addition of 200 g of fish per liter, or candled (Power, 1958). The nematode recovery efficiency of the three methods could not be compared because of the apparently few

parasites in the eviscerated portions (see Results). When nematodes were found in these examinations, they were washed in Ringer's solution and placed in clean Ringer's for viability and infectivity testing.

For isolating the nematodes from fish viscera, each set of viscera was placed on an 8 x 8 cm wire grid made of size 6 x 6 mm mesh. The grid was suspended in a funnel with a top diameter of 15 cm. Funnels had been closed at the bottom with tubing and a clamp. They were filled with ca 600 ml of Ringer's solution to cover the viscera on the grid. Nematodes from the viscera settle to the funnel's stem during several hours at controlled room temperature (ca. 24°C). It had been shown early in the survey that with a settling time of 4 hr almost all nematodes could be recovered live in the bottom 50 ml of liquid in a funnel. Longer settling times, i.e., 8 and 12 hr, increased the recovery of nematodes slightly but decreased their viability drastically. Consequently, 4 hr became the standard settling time. The 50 ml of liquid were drained into a container by opening the clamp at the bottom of the funnel. Aliquots of 50 ml were then removed from the container, placed in Petri dishes, and examined grossly as well as microscopically. The nematodes in each dish were counted and washed in clean Ringer's solution. They were tested for viability by their movement in clean Ringer's at room temperature, and for potential infectivity in a 35-37°C incubator (Healy et al., 1975).

RECOVERY OF OTHER PARASITES. Besides nematodes, endoparasites recovered and observed frequently during these examinations included cestodes, acanthocephalans, and digenetic trematodes. Observed as ecto-parasites, were monogenetic trematodes and copepods. No attempt was made to detect protozoa.

IDENTIFICATION. After being recovered, washed, and tested for viability plus infectivity, the nematodes were fixed in hot (55°C) 70% ethanol or in formalin buffered with phosphate to pH 7 at room temperature (ca 24°C). Specimens fixed in the ethanol were stored in 70% ethanol with 10% glycerol. Specimens fixed in the formalin were also stored in formalin. Storage containers were tightly closed screwcapped glass vials.

For microscopical examination, the nematodes were poured from the vial into a Petri dish containing a clearing solution of 70% ethanol with 10% glycerol. (Specimens fixed and stored in formalin were transferred into the Petri dish through 30% and 50% ethanol.) Petri dishes were placed in a vacuum dessicator at ca. -1 atmosphere to evaporate the alcohol and water. The refractive index of the remaining glycerine, 1.473 at 20°C, renders visible the following morphological features of nematodes: digestive tract, reproductive organs, and nerve ring on the interior, and cuticular striations, cuticular appendages, sensory papillae, and lips on the exterior. Not easily or usually visible in glycerine are the internal excretory duct or its surface opening (pore). Since the position of

the pore is important for classification, nematodes were recleared in phenol (Mallinckrodt loose crystals liquefied by the addition of 10 ml of 95% ethanol per 100 g of the crystals). In this phenol solution with a refractive index of 1.501 at 26°C, the excretory duct and pore are visible, as are other structures visible in glycerine, except for the nerve ring. An advantage of viewing nematodes in glycerine is their pliability in this medium. It permits easy manipulation. Specimens are brittle in phenol or other heterocyclic hydrocarbons such as xylene (refractive index = 1.505 at 20°C).

Cleared nematodes in a small amount of clearing solution were placed on microscope slides and covered with a coverglass. Morphological examinations were conducted at various magnifications using bright field, phase contrast, and interference contrast microscopy. The general morphological criteria used for identifying the specimens were those of, Baylis (1920), York and Maplestone (1926), Chitwood and Chitwood (1950), Hoffman (1970), Hartwich (1957, 1974) and Myers (1975). During the course of this study, certain criteria were amended, as stated in the Results section of the text.

After examination, the nematodes were washed overnight in 70% ethanol and returned to their storage vials in glycerine or in 70% ethanol with glycerine.

Information gathered during the microscopical examination was added to that already on the paper slips in the vials. Samples of the collection will be deposited with the U.S. National Museum (Animal Parasite Institute, U.S. Department of Agriculture's Research Center, Beltsville, Maryland 20705, U.S.A.).

Experimental Studies

INFECTIVE MATERIAL. Anisakis sp. larvae and Phocanema decipiens larvae were collected from fish markets or vessels in areas of parasite abundance and shipped by air to Washington, D.C. Sebastes paucispinus (bocaccio rockfish) infected with Anisakis sp. larvae were purchased from fish wholesalers in Seattle, Washington. The

fish were eviscerated and the viscera were placed in appropriate sized vials, covered with 0.9% saline, capped, and shipped on wet ice to Washington, D.C. by air freight. These larvae were received within 1 day after purchase. One shipment of 25 Anisakis sp. larvae (Table 24) was obtained from the viscera of Gadus morrhua (cod) from Halifax, Nova Scotia, Canada. It was received within 4 days after shipment. Upon arrival larvae were isolated from the viscera by dissection in saline, selected larvae were transferred to Petri dishes, counted, and placed in the refrigerator overnight.

P. decipiens larvae were obtained from fish processors in Halifax, Nova Scotia, Canada, in two forms: (1) as isolated larvae mechanically removed from cod fillets and when available (2) , in heavily infected fillets purchased from the processors. Isolated larvae and fillets were transferred to double plastic garbage bags (isolated larvae were covered with saline), sealed and refrigerated overnight. The next day the bags were packed with ice and shipped to Washington, D.C. via air express or air freight. Shipping times varied from 2 to 14 days (air express) and 2-5 days (air freight).

Upon arrival, larvae were dissected from the fillets, selected, and isolated in Petri dishes of saline and placed in the refrigerator (2°C) overnight. The next morning larvae were transferred to polyethylene tubes (9.5 mm internal diameter walls 1.6 mm) with fish paste (fish flesh:water, blended for ca 1 min at

25000 rpm). Tubes were then closed with 00 rubber stoppers and stored on ice for transfer to the Special Purpose Animal Laboratory (SPAL) Beltsville, Maryland.

PIG CARE. The housing and diet of the pigs was described by Earl and Taylor (1967). Pigs were farrowed in the open and on arrival at SPAL were treated with Algard 02.42 mg/kg (Shell Chemical Company, Dallas, Texas) twice at biweekly intervals.

For infection, pigs were restrained in a trough, their mouth was secured open with a jaw spreader and an unstoppered tube was passed down their throats into their stomachs. The nematodes were force fed by removal of a second stopper and addition of 50 ml of fish paste followed by 50 ml of water added to the stomach tube with the aid of a syringe.

CLINICAL TESTING. For clinical testing, venous blood was obtained from the heart of a restrained pig with a syringe equipped with a 20-gauge needle. For hematological tests, 5 ml of blood was collected in a tube containing EDTA and immediately mixed by inversion (5X) (B.D. Vacutainer, address Becton Dickinson, Rutherford, N.J.). For clinical chemistry determinations, 5 ml blood were collected in a plain glass tube. Tubes of blood were transported on ice to the Oscar Hunter Memorial Laboratory for clinical analysis. If pigs were necropsied, the tubes and ice were stored in a cold room (40°C) until transported to the laboratory. In all cases the tubes were delivered to the clinical laboratory within two and a half hours after collection.

The clinical tests performed are listed in Tables 15 and 16. Hemoglobin was determined colorimetrically, white blood cell counts (WBC) and red blood cell counts (RBC) were made with a Coulter counter (Coulter Electronics Inc., Hialeah, Florida), hematocrits were determined by centrifugation, and differential counts were made microscopically by counting 100 white blood cells. Amylase activity was determined by the Somogyi (1938) method and lipase activity by the method of Cherry and Crandall (1932). The other 12 chemical determinations were made on an SMA 12 channel autoanalyzer (Technicon Corp., Tarrytown, New York).

#### NECROPSY PROCEDURE.

Pigs were rendered unconscious by electrocution and placed on the autopsy table. About 500 ml of venous blood was collected from the neck. Each limb was released from the body by cutting along the ventral and lateral surfaces and disjuncting from the girdle. The pulmonary and cardiac cavity was opened by severing the ribs to the right side of the midline. The abdominal cavity was opened by extending the previous cut first down the right side of the abdomen ca. 250 mm from the midline and then down the left side. The entire digestive tract from the esophagus to the anus was then removed. The stomach was separated and cut open along the lesser curvature. If present, the nematodes were counted and gross pathological changes were recorded. The entire stomach, or portions exhibiting pathology, were fixed in 10% buffered formalin. Concurrently, the remainder of the intestinal tract was straightened by removal of the

mesenteries, records were made of the nematodes or pathological conditions observed. The intestine was then opened with autopsy shears and examined for parasites and sections prepared for pathological study were fixed as above. The remaining organs i.e., the liver, kidney, pancreas, heart, and lungs, were sliced in ca 3 mm sections and examined grossly for lesions. The body wall, diaphragm, bladder, uterus and ovaries were examined by visual inspection.

#### HISTOLOGICAL STUDIES

Nematodes, if 10 mm or smaller, were punctured (2 x) with an 00 insect needle in length; longer nematodes were cut into 10 mm pieces. These tissues were processed by dehydration, clearing, and infiltration with 56-58°C Paraplast (Sherwood Inc., St. Louis, MO.) by the method of Galigher and Kozloff (1964). Fixed stomach tissues were cut into blocks of appropriate size to include the lesion or cross section of the lesion. The maximum size of a block in any direction was 25 mm and most were ca. 10 x 10 x the thickness of the stomach wall. Blocks were prepared using Lab Tek (Miles Laboratories, Naperville, Illinois) embedding rings and molds of appropriate size (16x16 x 6.5 or 31x25 x 13 mm). Serial sections were cut at 4-7  $\mu$ m and placed on slides using the albumin flotation technique (Grey, 1958). Slides were dried on a slide warmer adjusted to 42°C. Most of the slides were stained with Azure A-Eosin B by the method of Lillie (1965), others were stained with Methyl Green Pyronin Y (Lillie, 1965), a modified Shorrs' stain

(Vetterling and Thompson, 1972) or hematoxylin and eosin (Lillie, 1965).

INACTIVATION STUDIES. To determine the extent to which anisakine nematodes resist freezing temperatures, fish were placed in the freezing compartment of a household freezer adjusted to  $-20^{\circ}\text{C}$ . Each fish was placed on an aluminum plate at the bottom of the freezer. This method of freezing is similar to the commercial freezing process used by seafood processors. Fish were removed from the freezer and thawed at room temperature (ca  $22^{\circ}\text{C}$ ) prior to examination for parasites. Viability of recovered anisakines was determined by spontaneous movement.

## RESULTS

### Taxonomic Studies: Diagnoses

The subfamily Anisakinae and the genera encountered in this study are redefined as follows, utilizing the new information obtained in this work.

SUBFAMILY ANISAKINAE. Usually long  $>2$  cm and stout  $>1$  mm diameter ascarids, the mouth is surrounded by a dorsal and two subventral lips, dentigerous ridges and interlabia present or absent. Cuticular collar or ornamentation behind lips present or absent. The mouth leads to an elongated primarily muscular, esophagus (pharynx) with a triradiate lumen. The muscular esophagus joins a primarily glandular ventriculus which is globular to cylindrical in shape. The ventriculus may have one or more solid, ventral appendages termed ventricular appendix(ices) usually

directed posteriorly. Both lumens of the muscular esophagus and that of the glandular ventriculus are lined with cuticle. A sphincter is present at the ventricular intestinal junction. At this junction, the intestine may give rise to one or more anteriorly directed appendages, termed intestinal caecum. The dorsal intestinal caecum may terminate in a ligament attached to the body wall on the left side. The intestine is elongate with more than 20 cells in cross section. The intestine terminates near the posterior end of the body with a cuticle-lined rectum separated from intestine by a sphincter. Anal glands are adjacent to the rectum.

The excretory system is one of three types. A bifid tubular system consisting of a central lumen surrounded by a layer of tissue and attached to the lateral cords. The tube and tissue more developed on the left side; the tubes unite to form a ventral, cuticle-lined excretory duct which leads to the surface just posterior to the nerve ring. The second type of excretory system consists of a relatively broad band of tissue attached to the left lateral cord behind the nerve ring. This band of tissue extends ventrally around the worm and may reach the level of the right cord; there are large nucleui; the cuticle-lined excretory duct extends through the nerve ring and opens at the base of the subventral lips. The third system is a combination of the two preceding types; the left lateral cord is associated with a broad band of tissue while the right lateral cord is associated with tubular element and tissue; elements of each side join together posterior to the nerve

ring to form an excretory duct which opens near the level of the nerve ring, or at the base of the lips.

The body and orifices are covered by a syncytial cuticle; below the cuticle hypodermal nuclei in a syncytium condensed along each side of the body as lateral cords. The nervous system is centered in a group of ganglia surrounding anterior muscular esophagus. Major nerve trunks are located in the lateral cords with some anterior nerve trunks are located dorsally and ventrally located. Lip papillae may be divided into an inner and outer circle. Papillae distribution: two double papillae are located, on the outer margins of the dorsal lip and one on each subventral lip toward ventral margins and 2 single papillae are located on the dorsal margin of each subventral lip adjacent to the amphid. Paired cervical papillae (dirides) located near level of the nerve ring on lateral surfaces of worms. Phasmids on the lateral surface of the tail about midway between the anus and tail tip.

Female genital system is usually paired and coiled tubular ovaries are connected to the uterus by oviducts. The vagina leads to the vulva on the ventral surface in middle half of body.

The male reproductive system of coiled testes expand and coil posteriorly as vas deferens, enlarge as a straight, posteriorly directed, seminal vesicle that joins a vas efferens and ejaculatory duct united with rectum on ventral surface to form a cloaca.

Male proctodeum usually gives rise to two spicules (one in Heligmus) and possibly a gubernaculum (in certain Porrocaecum spp.) in addition to dorsal pouches which contain the spicules. Male tail and posterior body support papillae which may be termed preanal adanal, or postanal depending on their positions.

GENUS Phocanema Myers, 1959. Anisakinae. Body elongate, adults minimally 30 mm long x 1.5 mm diameter. Lips longer than broad with dentigerous ridges and without interlabia. Muscular esophagus elongate and claviform, ventriculus cylindrical, intestinal caecum shorter than ventriculus. Three pairs of rectal glands present. Excretory pore at base of lips, excretory gland ventrolateral extending from posterior to nerve ring to near midbody as a broad band attached to the left lateral cord. Excretory gland nucleus large adjacent to anterior intestine. Thick cuticle with striations and punctations without lateral alae. Vulva of females near midbody, eggs subspheroid. Males with 2 nearly equal spicules, broad caudal alae, more than 100 pair preanal papillae, paired ventrolateral postanal papillae except terminal median papillum. Mature in marine mammals (seals). Adult (Fig 1-4, 40-44). Immature (Fig 5-6). Larvae (Fig 70-71, 96-101).

GENUS Contraecum Railliet and Henry, 1912. Anisakinae. Body length variable over 20 mm as adults and at least 1 mm in diameter. Lips elongate with dentigerous ridges and interlabia. Elongate muscular esophagus, ventriculus subspheroid to cylindrical with ventral posteriorly directed ventrolateral appendix, dorsolateral

intestinal caecum variable in length attached to the left body wall by a ligament. Excretory pore at base of subventral interlabium, excretory duct extending ventrally to just posterior to nerve ring where it passes toward the left lateral cord to become a band attached to the left lateral cord extending to the middle third of the body. Large excretory gland nucleus over anterior intestine posterior to ventriculus. Cuticle expanded behind the lips to form a collar, lateral alae on body of both sexes. Cuticle marked by transverse striae and lateral ridges. Vulva near midbody in females. Eggs spherical to subspherical. In males, alate spicules equal or subequal, preanal papillae paired and positioned ventrolateral to lateral pedunculate postanal paired papillae. Adults in marine mammals and birds. Adults. (Fig 7-10, 45-48)  
Larvae (Fig 78-82.)

GENUS Anisakis Railliet and Henry, 1912. Anisakinae. Large and stout nematodes, adults 35 mm x 2 mm. Elongated lips with dentigerous ridges without interlabia. Claviform muscular esophagus, ventriculus cylindrical without appendix, may be straight or folded upon itself. Intestine without caecum, rectal glands paired. Excretory pore ventral between base of subventral lips. Excretory duct passes ventral posterior to nerve ring where it joins the excretory gland attached to the left lateral cord. Excretory gland ribbon-like occupying ventrolateral portion of pseudocoelom below the digestive tract. Excretory gland extends posteriorly

beyond midbody. Large excretory gland nucleus at level of anterior intestine. Cuticle without lateral alae with transverse longitudinal striae. In females vulva opens in middle 1/3 of body. Eggs subspherical. Males with two equal or subequal spicules; preanal papillae ventrolateral on posterior 1/2 of body, postanal paired papillae pedunculate. Adults in marine mammals. Adults (Fig 11-14). Larva (Fig. 73).

GENUS Paranisakiopsis Yamaguti, 1941. Anisakinae. Medium to large nematodes, adults minimally 10 x 1 mm. Lips elongate with fine dentigerous ridges and well developed interlabia. Muscular esophagus claviform, ventriculus cylindrical without appendix. Intestine without caecum. Rectal glands paired. Excretory pore near base of ventral interlabium. Ventral excretory duct extends beyond nerve ring where it joins a band type excretory gland associated with the left lateral cord. Excretory gland may extend posteriad beyond midbody. Excretory gland nucleus near level of ventriculus. Cuticle with transverse and longitudinal striae, somewhat expanded behind the base of the lips. Lateral alae present, vestigial or absent in each sex. Female vulva near midbody. Eggs subspherical. Male with nearly equal spicules; preanal papillae on posterior 1/3 of body. Postanal paired papillae on short peduncles. Adults in macrurid fish. Adult (Fig. 15-16, 62-64).

GENUS Sulcascaris Hartwich, 1957. Anisakinae. Adults large 20 x 1.2 mm. Lips elongate with dentigerous ridges and interlabia. Claviform muscular esophagus, cylindrical ventriculus without appendix. Intestinal caecum variable in length usually not exceeding length of ventriculus, occasionally two caeca are present. Excretory pore near base of ventral interlabium. Excretory duct ventral, extending beyond ventriculus where it branches to the lateral cords, on the right side an excretory tubule. The posterior extent of the tubule variable from the level of the ventriculus to beyond midbody. Ventrolateral band type excretory gland present associated with the lateral cord and extending to near midbody. Excretory gland nucleus at level of ventriculus or anterior intestine. Cuticle with transverse and longitudinal striae, cuticular fold (collar) at base of lips may hide excretory pore. Lateral alae present on body. Female vulva located near midbody, eggs subspherical. Male spicules nearly equal, preanal papillae ventrolateral on posterior third of body. Postanal papillae pedunculate and usually paired. Adults in marine turtles.

GENUS Thynnascaris Dollfus, 1935. Anisakinae. Adults variable in size with elongate body. Lips variable elongate to compressed with dentigerous ridges or dentate modifications, interlabia present. Muscular esophagus claviform, subspherical ventriculus with a solid posteriorly directed appendix of variable length.

Lumenate intestinal caecum of variable length present and attached to the body wall by a ligament. Paired rectal glands present. Excretory pore posterior to but near level of the nerve ring, branched excretory duct leading to each lateral cord which contains excretory tubule; both the tubule and tissue are more developed on the left side than on the right. Excretory tubule on left extends beyond midbody to near the tail. Excretory tubule on right variable may end near level of ventriculus. Excretory tubule nucleus on left side adjacent to esophagus near level of ventriculus. Tubule nucleus on right side not observed. Cuticle with transverse and longitudinal striae, in addition transverse cuticular rings may be present. Caudal end of tail with protrusable tip which may be armed with spines or other ornamentation. Lateral alae present, vestigial, or absent. In females, vulva located in the middle 1/3 of the body and eggs subspherical. Male spicules equal or unequal, preanal papillae usually confined to the posterior 1/3 of the body, postanal papillae are paired. Adults in fish,. Adults (Fig 17-28, 49-61). Larvae (Fig. 74-77, 83-87).

GENUS Goezia Zeder, 1800. Anisakinae. Body stout and of variable size. Lips flattened with dentigerous ridges without interlabia. Muscular esophagus claviform, subspherical ventriculus with ventral posteriorly directed appendix of variable length. Lumenate intestinal caecum present, connected to left lateral body wall. Rectal glands paired. Excretory pore just posterior to nerve

ring, excretory duct leading from pore to tubules contained in the lateral cords. Posterior extent of excretory system and tubule nuclei not observed. Cuticle armed with posteriorly directed spines decreasing posteriorly, tail with an extendable "finger-like" projection. In females the vulva is located in the middle 1/3 of the body and eggs subspherical. Preanal papillae of males confined to posterior 1/3 of body, postanal papillae on short peduncles, spicules variable unequal to subequal. Adults in fish and crocodiles. Adults (Fig. 29-32).

GENUS Raphidascaris Railliet and Henry, 1915. Anisakinae. Body elongate and variable in length. Lips generally elongate with dentigerous ridges and interlabia. Claviform esophagus. Ventriculus gives rise to a ventral posteriorly directed appendix of variable length. Intestine without appendix, rectal glands paired. Excretory pore posterior to nerve ring, excretory duct branches to lateral cords. Excretory tubules contained in the lateral cords with the left more developed than the right. Both tubules extend to near the tail. Tubule nuclei at level of muscular esophagus. Cuticle with transverse and longitudinal striae. Lateral alae united ventrally between the nerve ring and base of lips. Female's vulva near midbody and eggs subspherical. In males, alate spicules equal or subequal. Preanal papillae ventrolateral on posterior 1/3 of body, postanal papillae pedunculate and paired. Adults in fish, usually freshwater fish. Adults (Fig 33-36). Larvae (Fig 68-69, 91-95).

GENUS Porrocaecum Railliet and Henry, 1912. Anisakinae. Body elongate of variable size. Lips variable from elongate to compressed, with dentigerous ridges and interlabia. Muscular esophagus claviform, ventriculus cylindrical, lumenate intestinal caecum of variable length attached to body wall by a ligament, anal glands apparently paired. Cuticle with marked transverse striae, longitudinal striae and alae absent. Excretory pore posterior to nerve ring, excretory gland a small band of tissue associated with the left lateral cord, excretory gland nucleus not observed, no tubular element observed in the right lateral cord. In females, the vulva opens in the middle one-third of the body, eggs spherical to subspherical. In males, spicules variable equal to unequal, preanal papillae confined to posterior one-third of the body; paired ventrolateral postanal papillae are raised on small peduncles. Adults in birds and rats. Adult (immature) (Fig 37-39). Larval (Fig 65-67, 88-90).

#### Epidemiological Studies

MARKET FISH. Roundworms occurring in the edible fish from U.S. waters are currently of concern. That health problems could be associated with some of these parasitic nematodes became apparent in the 1950s (Hitchcock, 1950; Buckley, 1951; Straub, 1955). Certain anisakine nematode larvae were discovered in patients, several of whom had acute abdominal pain, and in remaining portions of fish they had eaten raw (Ruitenbergh, 1970). During the following decade there were increasingly frequent reports of this "new" human

disease, anisakiasis. Cases were recognized in Europe, especially in the Netherlands (Ruitenbergh, 1970), as well as in Japan (Oshima, 1972). In the U.S.A., raw and semiraw fish recipes appeared to be gaining popularity with consumers (Jackson, 1975) but, at the time, comprehensive nematodes surveys of seafoods had not been done with either market samples or fresh catch. This situation prompted new work on the anisakines and anisakiasis, of which the Washington, D.C. area samplings presented here are a part.

As survey and experimental work were beginning, the publication of what were (Kates et al., 1973; Little and MacPhail, 1972; Little and Most, 1973) believed to be the first reports of verified human infections in North America gave additional reason to the research effort. The earlier reports (Pfaff, 1867, Buckley 1951; Hitchcock 1953, J.T. Davey, 1971) of human infections were lost in the literature until later.

One thousand and ten fish belonging to 14 families, 20 genera and 23 species were examined for parasitic nematodes, a total of 6,547 nematodes were found.

The survey included: (1) Bothidae: 134 Paralichthys dentatus (summer flounder); (2) Clupeidae: 76 Alosa aestivalis (blueback herring), 13 Alosa sapidissima (American shad) 2 Clupea harengus harengus (Atlantic herring); (3) Gadidae: 1 Gadus morhua (Atlantic cod), 1 Melanogrammus aeglefinus (haddock), 149 Urophycis regius (spotted hake, ling cod); (4) Ictaluridae: 8 Ictalurus catus (white catfish), 3 Ictalurus punctatus (channel catfish); (5) Lutjanidae; 3

Lutjanus campechanus (red snapper); (6) Mugilidae: 1 Mugil cephalus (striped mullet); (7) Percichthyidae: 28 Morone americana (white perch), 255 Morone saxatilis (striped bass, Chesapeake Bay rockfish); (8) Percidae: 28 Perca flavescens (yellow perch); (8) Pomatomidae: 5 Pomatomus saltatrix (bluefish); (10) Sciaenidae: 61 Cynoscion regalis (weakfish), 4 Leiostomus xanthurus (spot), 184 Micropogon undulatus (Atlantic croaker), 1 Sciaenops ocellata (red drum); (11) Scombridae: 12 Scomber scombrus (Atlantic mackerel); (12) Serranidae: 36 Centropristis striata (black sea bass); (13) Sparidae: 2 Stenotomus chrysops (scup); (14) Stromateidae: 3 Peprilus triacanthus (butterfish).

Categories and Numbers of Recovered Nematodes. All nematodes recovered in the fish survey (Table 1) were assigned to one of 14 categories.

Anisakine Nematodes. Anisakis sp. (Dujardin, 1845). Eleven larvae, each with boring tooth, met the criteria for this genus. They could be identified more precisely than Myers' (1975) sensu lato type, which includes 9 genera. By morphological criteria (Tables 12, 13), the specimens were differentiated from larvae of the genera Viverranisakis, Paranisakis, Ichthyanisakis, Belanisakis, Acanthocheilus, Pseudanisakis and Paranisakiopsis. By a physiological criterion (Healy et al. (1976), survival for 24 hr or more at 35-37°C, these larvae were judged not to belong to the morphologically somewhat similar but incompletely described genus Heligmus, which matures in ectothermic hosts and presumably can not

survive for long at and above 35°C. No marked differences were noted among the 11 larvae, and it is thought that they may belong to a single Anisakis sp. These larvae are indistinguishable from Berland's (1961) Anisakis type-1 larva.

Porrocaecum sp. Railliet and Henry, 1912. Forty-one boring tooth-bearing larvae could have been members of the genera Paradujardinia, Dujardinascaris, Pseudoterranova, Porrocaecum, or Aliascaris by the employing criteria listed in Table 3. However, the genera Paradujardinia and Dujardinascaris have at least two additional characteristics that disqualify the specimens from the genus: the spherical shape of the ventriculus and the extreme narrowness of the caecum (Myers, 1975). The caeca of my specimens were much longer than the ventriculus, hence they were not members of the genus Pseudoterranova, the members of which have a caecum that is only slightly longer than the ventriculus. The genus Aliascaris is characterized by cuticular cordons and "trilobed wart-like" structures all along the body. Thus, the characteristics of these 41 boring tooth larvae are those described for the genus Porrocaecum.

It was observed that Porrocaecum sp. larvae lacked alae and had a boring tooth more prominent and cuticularized than that of Phocanema sp. larvae from Canadian cod. Cuticularization gives the tooth a darker brown color than that of Phocanema, and this color is noticeable even at low magnifications. Since no significant differences were noticed among the 41 larvae, they are thought to

belong to the same species of Porrocaecum. Tested specimens of the Porrocaecum sp. did not survive incubation at 35-37°C for more than 4 hr.

Thynnascaris spp. Dollfus 1935, and sensu lato type. Three thousand two hundred twenty-one nematodes, adults as well as larvae, were identified as belonging to the genus Thynnascaris or to Myers' (1975) larval type. They were recovered from 15 species of fish. There was more than one type of toothed infective larva, suggesting the possibility that there are different species. The finding (Beacham and Haley, 1976) in white perch of a "Contraecum sp.", may be a misidentified Thynnascaris sp.

The genus Thynnascaris cannot be distinguished easily from the genera Lappetascaris, Goezia, Pulchrascaris, or Heterotyphlum by the criteria listed in Table 3. There are, however, additional criteria. The genus Heterotyphlum has a very small ventriculus (and older stages have no interlabia); the genus Lappetascaris has a long ventricular appendix that extends almost to midbody; there are spines on the cuticle of the genus Goezia; the separation of ventriculus and ventricular appendix is definite in Thynnascaris but not in Pulchrascaris.

Goezia sp. As mentioned, cuticular spines are the simplest way of distinguishing the genus Goezia from the genus Thynnascaris and from other related anisakine nematodes. Twenty-one larval specimens of what is probably a single Goezia sp. were recovered in this survey. Striped bass was the principal host.

Because of its spines and flattened lips, some authorities (Hartwich 1957, 1974; Myers 1975) do not group the genus Goezia with the anisakines. It is argued that no other anisakines have these features. However, there are cuticular rings not only on some larvae but also on some adults which are clearly seen in the genus Thynnascaris. This, I think, justifies the reinstatement of this "anisakine-like" nematode genus with an ornamented cuticle among the "true" anisakines.

Raphidascaris acus. Only the older larvae and adults of the genera Raphidascaris and Raphidascaroides can be distinguished by the criteria listed in Table 3. The 1220 specimens recovered in this survey all had a boring tooth, thus were infective larvae i.e., younger stages. Initially they were designated as Raphidascaris sensu lato, which includes Raphidascaris and Raphidascaroides spp; however, Raphidascaris acus is the only species of either genus which has a long ventricular appendix that extends past the nematode's midbody. Since all the specimens had such an appendix, they appear to be R. acus. They were recovered from most species of fish that were also parasitized by Thynnascaris spp.

Unidentified ("U") Anisakines. 225 nematodes could be identified as anisakines but not in greater detail because of damaged or missing parts or because of degenerative changes prior to fixation.

Non-Anisakine Nematodes. Spinitectus spp. Seventy-one adult and larval nematodes were classified as belonging to the genus Spinitectus by the criteria of York and Maplestone (1926). These nematodes, which mature in fish, were recovered from spotted hake, striped bass, and summer flounder.

Bulbodactinis sp. One hundred-four adult and larval nematodes were classified as Bulbodactinis sp. according to the criteria of York and Maplestone (1926). There is only one species in their genus. These nematodes, which mature in fish, were found mostly in the spotted hake.

Metabronema sp. One hundred eight nematodes were classified according to the criteria of York and Maplestone (1926) as a species in the genus Metabronema. Members of this genus mature in fish. My specimens, mostly adults, were recovered primarily from summer flounder and Atlantic croaker.

Spirurinae. One hundred and eleven larval nematodes were identified by the general criteria of York and Maplestone (1926) as belonging to the subfamily Spirurinae. The principal fish sources were the summer flounder and the Atlantic croaker.

Unidentified (+ "U") Larva. Four hundred and forty-seven similar specimens of small, fairly featureless and therefore apparently still young nematode larvae were categorized as Larva "U". They could not be anisakines because they lack a boring tooth. Atlantic croaker, spot and summer flounder were the principal source fishes for this category of nematode.

Philometra sp. Six hundred and sixty-two adult and immature nematodes from striped bass were classified by the criteria of Hoffman (1970) as belonging to the genus Philometra. There appeared to be only one species of Philometra in the striped bass (Bier et al., 1974).

Capillaria sp. A single specimen was classified according to Hoffman's (1970) criteria as a Capillaria sp. It was a larva recovered from the weakfish. Nematodes of this genus mature in fish.

Unidentified ("U") Nematodes. There were 294 nematodes that could not be classified because of missing or damaged parts or degenerative changes prior to fixation.

#### Viability

The vast majority of nematodes recovered from these whole, unfrozen fish were viable when first observed. Only a few ( 1%) nematodes from fish that had begun to decay appeared moribund although active nematodes were also recovered from these fish. Many of the nematode specimens reported as broken or damaged still appeared to be alive, and their injuries probably occurred during the isolation process.

#### Distribution in Fish

The vast majority of nematodes recovered from the whole, unfrozen fish in this survey were located in the viscera. Only two were isolated from the eviscerated portion consisting primarily of edible flesh. Both were Anisakis sp., occurring in Atlantic herring and spotted hake.

## Experimental Studies

### PIG ANISAKIASIS

#### Course of Infection

With Phocanema decipiens larvae. The highest rate of infection observed in was slightly over 50% of the larvae fed (see Table 17). The remainder of the nematodes were probably digested as they were not recovered from the feces or found dead in the gut contents. Table 17 summarizes the course of infection of pigs fed Phocanema decipiens larvae, additional observations are recorded below.

During the first 2 days post-feeding, the nematodes found were active at necropsy. The larvae would coil and uncoil at random, coils formed resembled a tightly wound spring. On days 4 and 5 post-feeding most of the nematodes recovered were inactive, showing no spontaneous movement. Upon stimulation by touch, these inactive nematodes would form loose coils. From days 6-11, the nematodes returned to their more active state and spontaneously formed tight coils. After day 11, the recovered nematodes were mainly inactive but moved upon stimulation by touch.

The first inactive period was correlated with the molt from L<sub>3</sub> to L<sub>4</sub>. Prior to day 3 all recovered nematodes examined possessed a boring tooth and an ill-defined lip region. On day 4 post-feeding, some (2) larvae were found to have well defined lips and to lack a boring tooth. An active larvae recovered on day 6 post-feeding retained a partially shed L<sub>3</sub> cuticle which was attached to the posterior half of the body. No evidence of a second molt was observed in the specimens recovered later.

The larval Anisakis sp. also showed a similar pattern of movement to that observed in P. decipiens larvae. The larvae observed during the first 2 days after infection moved spontaneously. On days 3 and 4 larvae moved only when touched and then formed only loose coils. Lipped larvae were first recovered on day 4 of post-infection. On days 6 and 7, the observed Anisakis sp. larvae had migrated completely into the stomach wall. These larvae were found at the base of the mucosa and in the upper layer of the submucosa, indicating the larvae had penetrated the muscularis mucosa. Larvae were not recovered or observed after seven days in pigs fed Anisakis sp. Additional data on the courses of Anisakis sp. infections in pigs is presented in Table 24.

Variation in movement was noted in P. decipiens larvae from various shipments. Most larvae from some shipments moved with smooth undulating movements or were coiled and remained motionless; whereas most larvae from other shipments exhibited constant jerky movements and did not coil. The former larvae produced higher rate of initial infection and infections of longer duration. Individual Anisakis spp. larvae showed similar differences in movements, but no individual shipments were dominated by smooth moving, tight coiling larvae.

Gross Pathology. Data on the gross pathology observed in each pig fed Phocanema decipiens larvae is summarized in Table 18. The following provides additional details on the gross pathology observed in selected pigs.

Each Phocanema decipiens larva was surrounded by a slight swelling (ca. 1-1.5 mm high extending 1.5 to 2.5 mm laterally from the nematode). These lesions will be termed "typical" and appeared as though a small doughnut had been slipped just under the mucosa. When a nematode was removed, a ca. 1 mm diameter bloody ulcerous crater remained in the center of the doughnut. Nematodes had penetrated 5 to 8 mm into the mucosa. Hemorrhagic spots beneath attached nematodes were observed from the outside (lamina propria side) of the stomachs of pigs 8062 and 7513. Small lesions similar to those from which nematodes were purposely removed existed on the fundus of all pigs with nematode larvae. The number of these small lesions varied from 1.5 to 5 times the number of nematodes present in the stomach.

At 2 days post-infection one lesion 12 mm in diameter was observed with a nematode at the center. The stomach wall surrounding this lesion was edematous, smaller lesions were also present in this pig.

At 4 days post-infection gross pathological alterations ranged from the typical lesions to a general edema involving the entire fundus. In pig 8852 with four nematodes recovered from the mesenteries, serous fluid was present in the body cavity. Lesions in the digestive tract were the typical small type. The only nematode recovered 5 days post-infection was located in the crater of an ulcer; the crater was irregularly shaped and the ulcer was 1.5 mm across in one direction and 1.2 cm at right angles to the first.

The ulcers extended into the submucosa; no blood was observed in the stomach or the intestine.

At 7 days post-infection the nematodes were found mainly in two groups, one of 15 nematodes and the other of 10 nematodes; others (8) apparently were attached randomly to the fundus wall and each was surrounded by a typical lesion. Each of the clusters was located in a bleeding ulcer; the stomach contained coagulated blood and edema occurred in the tissues surrounding the clusters. Clustering was not observed 8 days post-infection; however, in a pig from which no worms were recovered a bleeding ulcer was present, and clotted blood was found in the stomach. The six nematodes recovered 17 days post-infection were clustered in a roughly round bleeding ulcer of ca. 1 cm diameter.

The small lesions observed around each attached larva during the first 4 days of infection were similar to those observed in Phocanema-infected pigs. The ulcerous craters left on removal of the Anisakis larvae averaged 0.9 mm in diameter, reflecting the smaller diameter of the larvae. The average thickness of the fundus wall in control pigs was 0.75 to 1 cm. The generalized edema of the fundus observed between days 4 and 52 in one infection was dramatic. Maximum edema was observed on day 4 post-infection. The gross pathological data on individual pigs is tabulated in Table 24.

Anisakis sp. larvae were not observed in the tissues at the base of the larger ulcers observed on days 7 and 18 or associated with the small ulcers present 52 days post-infection. Larvae were

observed in the tissues at the base of some small (1-2 mm) fundic ulcers observed on day 7.

Microscopic Pathology. Phocanema decipiens usually penetrate the stomach wall 5-7 mm to the depth of the muscularis mucosa (Fig. 102). Occasional penetrations through the muscularis mucosa (Fig 103) were observed. The cellular reaction consisted of a dense cellular infiltration surrounding the anterior end of each nematode. The reaction is more diffuse as the distance from the worm increases (Fig 104). The tissues adjacent to the anterior end of the nematodes showed hemorrhage and edema. Surrounding the anterior end of each nematode is an attachment cap or anchor. It consists of an eosinophilic substance (Fig 105). As early as day 4, cells resembling fibroblasts may be observed in the mucosa and submucosa adjacent to the attachment cap (Fig 106). The cellular reaction, however, was predominated by eosinophils remained similar throughout the course of infection. Fig 107 illustrates the reaction at 15 days post-infection. Eosiniphils remain the major constituent of the reaction.

The tissue response to Anisakis sp. larvae were similar to those observed in P. decipiens-infected pigs throughout the course of infection. The dense infiltration of eosinophils adjacent to the larvae and the more diffuse reaction away from the larvae are illustrated (Fig 108, 109). The attachment cap is shown in Fig 110. When the nematodes had completely penetrated the gut wall (day 7) the tissue reaction remained similar (Fig 111).

Clinical Data. The hematology tests and units of measurement are listed in Table 15. Table 16 lists the serum chemistry tests, abbreviations, and units of measurements. Pigs fed Phocanema decipiens larvae and tested are identified in Table 17. Tables 18, 20, and 22 present the results of the hematological tests from pigs fed P. decipiens larvae. Tables 19, 21, and 23 present the results of the serum chemical tests from pigs fed P. decipiens larvae.

Pigs fed Anisakis sp. larvae on which clinical tests were performed are identified in Table 24. Tables 25, 27, and 29 present the results of the hematological tests from pigs fed Anisakis sp. larvae. Tables 26, 28 and 30 present the results of serum chemical tests from pigs fed Anisakis sp. larvae.

Hematological data on control pigs is presented in Tables 31 and 33. Serum chemical data on control pigs is presented.

#### INACTIVATION

Table 7 presents data on survival of isolated anisakine larvae at temperatures above 40°C. Data in Table 8 are derived from larvae heated in fish flesh. Table 9 presents data on survival of Anisakis-type larvae in fried fish fingers. Table 10 presents data on the maximum survival time of anisakine larvae at freezing temperatures.

### DISCUSSION AND CONCLUSIONS

#### Epidemiological Studies

MARKET FISH. Six thousand five hundred and forty-seven nematodes were recovered from 703 of 1010 market fish bought in the

Washington, D.C. area. The mean nematode burden is 6.48 roundworms per fish, and the overall nematode infection rate of the fish is 69.60%.

Averages, however, tend to erase differences. Both the intensity and the distribution of the nematode burden differ among different types of fish, and a variety of nematodes cause the infections. Because of these differences, it is necessary to discuss the incidence of particular nematodes in the various fish groups.

Nematodes of the anisakine genera Thynnascaris and Raphidascaris were the parasites most frequently recovered in the survey. No fish species of which a minimum of 25 specimens was examined, proved to be free of a Thynnascaris sp. and only one, a white perch, was free of Raphidascaris acus. These nematodes have not been incriminated as pathogens for human consumers of raw or semiraw fish. There are at least two possible reasons for this. First, specimens of these genera are not usually found in what are considered to be the edible portions of fish. Second, if swallowed live by mammals, the infective larvae recovered in this survey die without attaching to the digestive mucosa and are evacuated with the feces. Only some Thynnascaris sp. larvae from warm waters are known to survive long enough to attach to host tissue at mammalian temperatures (Norris and Overstreet, 1976). Maturation of Thynnascaris sp. and Raphidascaris sp. occurs only in fish hosts.

Overall, 3,221 Thynnascaris spp. specimens were recovered from 486 fish belonging to 15 species (Table 11). The majority were larval forms; adults were found with greatest frequency in Atlantic mackerel and weakfish. Of the Rhapidascaris sp., 1,220 specimens were recovered from 128 fish belonging to 11 species; no adult nematodes of the genus were found in fish examined for this survey.

Anisakis sp. and Porrocaecum sp. were the two genera of anisakines identified in the survey which include potential pathogens. Actual cases of human infection with some anisakine species are no longer considered to be unusual (Juels et al., 1975). As expected, the Anisakis sp. larvae recovered from our samples did survive the 24 hr/35-37°C digestion test; the Porrocaecum sp. larva did not; presumably it is a species of the genus that matures in fish rather than in birds or mammals. Consequently, the Anisakis sp. larva but not the Porrocaecum sp. larva is considered to be a pathogen.

Few specimens of these genera were found. There were eleven Anisakis sp. larvae from 7 fish belonging to 3 species (spotted hake, Atlantic herring, Atlantic mackerel). Only two specimens occurred in the edible portions of fish. Forty-one Porrocaecum sp. larvae were found in the viscera of 12 fish belonging to 4 species, i.e., spotted hake, striped bass, weakfish, and Atlantic croaker.

Other than the specimens of one anisakine genus, Anisakis, no other nematode identified in the survey are considered to be pathogenic for human consumers. The seasonal periodicity of the Philometra sp. life cycle in striped bass (Chesapeake Bay rockfish) has been documented (Bier et al., 1976). Because many fish species were not uniformly available year round or, if available, were caught at different locations depending on the time of year, one can not make a valid analysis of seasonal fluctuation in nematode incidence other than for striped bass.

The "unidentified larva" is probably a heterogeneous collection of very young, undifferentiated nematodes; even if some belonged to potentially pathogenic species, they would not be directly infectious for mammals so early in the life cycle.

Among fish species of which 25 or more were examined, spotted hake was the most "wormy" and white perch the least wormy - both in terms of the percentage of infected fish and of worm numbers per fish (Table 14). Spotted hake was also the largest source of pathogenic anisakines.

The "true" or Atlantic herring, Clupea harengus, deserves special mention although few specimens were examined. What is sold as "herring" in D.C. markets is usually a mixture of species. The majority are Alosa spp. but occasional Clupea harengus occur (> 2% of lots). The only pathogen (one specimen of the Anisakis sp. larva) recovered from a herring in this survey was from a true herring.

The incidence of pathogenic nematodes in market fish of the Washington, D.C., area, 11 Anisakis sp. larvae of 6547 nematodes, is "low" in two respects. There were few compared to the number of nonpathogenic nematodes in the same fish. Also, there were few compared to the number of potentially pathogenic nematodes in fish from various locations along the U.S. Pacific Coast (Myers 1976, Stern et al., 1976) and in fish from more northern Atlantic coastal waters (Cheng, 1976; Margolis, 1977). This infrequency of potential pathogens is attributed to the paucity in the Chesapeake Bay and adjacent waters of marine mammals that are the definitive hosts for most of the anisakines that can infect humans. Along the U.S. Pacific Coast there is a substantial population of marine mammals close to shore. Correspondingly, many of the fish there are infected with pathogens of the genera Anisakis sp. and Phocanema sp. larvae. Seals occur along the Atlantic coast north of Cape Cod and account for the high incidence of Phocanema decipiens, the pathogenic "codworm" anisakine, in the catch of those waters.

Because of the absence of marine mammals in the Chesapeake Bay, why should there be even one pathogenic Anisakis sp. larva in fish from Washington, D.C. area markets? There are three possible explanations. First, some fish may not have been caught locally, contrary to the assurances of the fish vendors. (Red snapper is definitely not from the area and Atlantic cod probably not.) Second, fish from elsewhere may have migrated into the area accidentally or been swept in. This probably happens to the occasional Atlantic "true" herring that join schools of Alosa spp. Third, migrating whales, that usually travel off North America's eastern continental shelf, come close to shore on occasion and are transient sources of infection.

Although the prevalence of pathogenic anisakines in fish is determined by the distribution of the definitive hosts of the parasites and the feeding and migrating habits of the fish intermediate hosts, none of these are invariable patterns. Chances for human infection with an anisakine nematode from sashimi or ceviche may be greater in Seattle, but there is some risk even in D.C. For example, whereas only 0.05% of spotted hake in the major collection during 1972-74 were infected with the Anisakis sp. larva, 0.2% were infected in a small 1975 survey. Changes of climate, and in offshore fishing limits and marketing practices, could change the future number of pathogenic nematodes found in market fish of Washington, D.C. area.

## Taxonomical Studies

### SUBFAMILY ANISAKINAE

The newer classification systems of Hartwich (1975) and Osche (1958) were rejected primarily because it was thought they were premature. The system of Baylis' (1923) as adapted here has the three major disadvantages: (1) the large number of genera included in the subfamily make it unwieldy; (2) it is not a natural grouping and probably includes several stocks of different origins; (3) it does not attempt to evaluate the taxonomic importance of the excretory system and other lip morphology, cuticular alterations or natural history data.

There are, however, three major advantages to Baylis' adapted system: (1) gaps in our knowledge are made vividly apparent, (2) the subfamily name is widely recognized and accepted; and (3) morphological criteria are not given undue weight prior to a detailed analysis of their variation within each genus.

In Table 13, the genera included are commonly accepted Anisakinae with the following exceptions. Heterochielus and Goezia were separated by Baylis (1920) from the others. My reexamination of Goezia shows basic similarities with the other anisakines. The following characteristics were not known to exist in other anisakines when Baylis separated the subfamilies: (1) laterally compressed lips, (2) finger-like projections on the tail, and (3) cuticle with spines and ridges. Thynnascaris inquires (Linton,

1901) Rasheed, 1965 and Thynnascaris ocogocephali (Olsen 1953), both have prominent cuticular rings (Figs 18) and a tendency towards shorter flatter lips (Figs 18,19). Finger-like projections on the tail similar to those in the genus. Goezia have since been observed in the genus Thynnascaris (Fig 25,26). The basic similarities in the digestive tract and excretory system of Goezia and other members of the subfamily are considered to be unifying and the distinctions are considered of generic importance only. The genus Heligmus is accepted because evidence of its relation to other nematodes or the Anisakinae has not been established. A literature search, however, has not uncovered any reference to fresh specimens of this genus since Dujardin's (1845) original description. The genus is recognizable due to the unique character of possession of only one spicule. The genus Lappetascaris Rasheed 1960 is accepted in my interpretation of the subfamily Anisakinae. This genus has a band of cuticular ornamentation immediately behind the lips; in this characteristic it is unlike other members of the subfamily. The cuticularized lips are armed with semilunar plates (Fig 17 and 20), a feature shared with some members of the genus Thynnascaris, and Porrocaecum, during at least at some stages of their development (Osche, 1958).

The digestive system is similar to that found in Thynnascaris and Contraecum. The excretory pore is located posterior to the nerve ring as it is in those anisakines with a tubular excretory system. Rasheed placed this genus in the family Anisakidae as the

only member of a new subfamily Lappetascaridinae. It would, therefore, be included in the subfamily Anisakinae as described here.

Of the genera redescribed in this work, Phocanema, Contracecum, Anisakis and Paranisakiopsis have a broad band type excretory gland associated with the left lateral cord. The excretory pore is located ventrally just posterior to the lips. Prior to the finding of this type of excretory system in Terranova and Paranisakiopsis the broad band type excretory gland was associated with endothermic hosts. The digestive tract of Anisakis and Paranisakiopsis has an oblong ventriculus and no appendages. Contracecum and Phocanema have an intestinal caecum. Contracecum also has a ventricular appendix. Contracecum and Paranisakiopsis have interblabia and some members of both genera have lateral alae on the body. Dentigerous ridges are found in Anisakis, Paranisakiopsis, Phocanema and Terranova. Coleoascaris and Phocascaris are the only other members of the Anisakinae for which this type excretory system has been described and both mature in mammals. The excretory system consisting of a band on the left and a tube on the right side was found in the genus Salcascaris, a species which matures in marine turtles. Pseudanisakis, from elasmobranchs, also has this type of excretory system, but differs from Sulcascaris in that the excretory pore is at the level of the nerve ring. This type excretory system is so far reported only from ectothermic hosts.

Thynnascaris, Goezia, Raphidascaris, and Porrocaecum described in this work have the third type excretory system consisting of

tubular elements associated with the lateral cords. Porrocaecum spp. are reported from both ectothermic and endothermic hosts. The other genera mature in ectothermic hosts.

Of the 30 genera listed in Table 13 the excretory system has been described in detail for only the 13 genera discussed above. Further study is needed to see if any clear correlations exist between the type of excretory system and the structure of other systems such as the digestive system or the type of definitive host.

The discussion of the presence and absence of other structures in genera with the band type excretory systems indicates that the relationship is complex. More detailed analysis of the digestive tract, cuticle, excretory system, and reproductive system may provide a means of assigning the genera included here in the Anisakinae to smaller more closely related groups. At present there is not enough data to distinguish between forms that are diverging and those that are converging.

#### DEVELOPMENT IN LARVAL ANISAKINES

Digestive Tract. In hatched ensheathed second stage larvae, the anterior digestive tract is divided into distinct sections. The glandular ventriculus is distinct from the more muscular esophagus. The intestine is incomplete, but consists of a solid mass of cells located behind the ventriculus ending at the base of the tail where the anus will form. The intestinal caecum is not yet formed in either Phocanema decipiens or Contracecum osculatum (McClelland, 1971). By the time C. osculatum or P. decipiens larvae have grown

to 1 mm in length the intestinal caecum is developed (McClelland, 1975). C. osculatum possesses a ventricular appendix at hatching (Davey, 1969; McClelland, 1975). Small (2-4 mm) Raphidascaris larvae from fish, possess a well developed ventricular appendix. Larvae have been identified to genus on the assumption that digestive development proceeds in the other members of the genus at the same rate as in those documented. All larvae encountered in this study with the possible exception of Raphidascaris, have developed to the stage where the intestine shows numerous cells in cross section.

McClelland (1974) reports the early development of rectal glands in cultured P. decipiens and C. osculatum. Rectal glands were present in all larvae recovered from fish in this study.

Genital Primordia. Development of genital primordia occurs at different times in anisakine larvae. McClelland and Ronald (1974) reports that the genital primordia of Contracaecum osculatum does not appear to increase in cell number from the time the larvae hatch until the larvae molted at increased temperature (35°C). The genital primordia of Phocanema decipiens, however, increased in number of cells and size during cultivation at 15°C (McClelland 1974). There are similar reports of the absence of cell division and marked growth in C. spiculigerum (Huizinga, 1966). Likewise, development of the genital primordium does not occur in Anisakis sp. larvae. Stefanski (1936) noted the development of the genital primordium in Thynnascaris aduncum in fish. Some Thynnascaris spp.

and all Raphidascaris acus and Goezia sp. larvae in fish have well developed genital primordia. Fig 18 shows the development of the genital primordium in Raphidascaris acus larvae. The extent of the development of the genital primordia in the larvae of other genera is not known.

The only boring toothed larvae that had extensively developed genital primordia were those which develop in ectothermic hosts. The genital primordia were not developed in all larvae with a Thynnascaris sp. boring toothed encountered.

Cuticle. Transverse striations were present on the cuticle of all larvae encountered in this study. The significance of these markings is limited for two reasons: (1) there is almost no data on cuticular striae of larval forms (especially anisakines) available; and (2) these markings change significantly upon molting (Lichtenfels, 1977). The prominent cuticular spines of Goezia sp. are unique enough to allow generic diagnosis, when coupled with digestive tract formation. I consider lateral alae to be of specific significance on the basis of current observations. Raphidascaris acus has lateral alae as an adult and they are present on the larvae encountered in this study. Lateral alae are developed, vestigial or absent in the genus Paranisakiopsis (Bier, et al. in preparation). The Thynnascaris spp. larvae encountered in this study with lateral alae presumably have alae as adults. Caudal alae in male P. decipiens do not develop until the final molt (McClelland, 1978).

The development described for larva of anisakines is frequently sufficient for the identification of larvae to genus, and in some cases to species.

Excretory Gland. The position of the excretory pore is established by the time larvae hatch in P. decipiens and C. osculatum (McClelland, 1971). One of the outstanding features of larval C. osculatum larvae is the excretory gland nucleus. The position of the excretory pore could be ascertained near the level of the nerve ring in the smallest specimens of Raphidascaris acus and Thynnascaris spp (ca 2 mm in length) encountered in this study.

Sensory Structures. Outlines of the larger lip papillae were observed in larvae with a boring tooth recovered from fish. These papillae are on the lateral surface of each lip. The two papillae on the dorsal lip are doubled (Fig 21) and the more dorsal papillum of the subventral lip is double while the other, adjacent to the amphid in adults is single. The amphids were not observed in larvae. The cervical papillae of Thynnascaris spp., Phocanema decipiens, Anisakis sp. and Contracecum osculatum larvae were observed in outline. Phasmids were not observed on the tail of larvae with a boring tooth. They were observed in lipped larvae of Thynnascaris spp., Sulcascaris sp. and Contracecum osculatum. Male caudal papillae are found only in adults.

## Experimental Studies

### PIG ANISAKIASIS

Course of infection. McClelland (1977) recently reported that P. decipiens undergoes two molts in seal definitive hosts. The first molt occurs between days 3 and 5 and the second between days 7 and 10. The nematodes may become fully mature by 17 days post-feeding. In pigs, P. decipiens larvae undergo a molt during the first 4 days. The lipped larvae that emerge from this molt are easily distinguished from the larvae with a boring tooth which were isolated from fish and fed to the pigs. The adults from the second molt in seals are distinguished from the lipped larvae morphometrically, i.e., lip size, tail size, relative distance to nerve ring and ventriculus, and by sexual development; the females have an open vulva, and the males bear caudal papillae. No specimens recovered from pigs showed this sexual differentiation. The larvae in pigs only molted once. The majority of larvae molted on day 4 some molted as early as day 3 and a few as late as day 7. This timing is within the range of occurrence of the first molt in the seal host.

McClelland (1977) reported clustering of immature adult P. decipiens in seals at 15 days post-infection. This phenomenon was observed in pigs earlier; however, in other respects it is similar in both hosts. In both cases, the clusters were associated with deep craters.

Diet has been shown to influence the course of nematode infections in other hosts (Lincicome, 1972). Pigs used in this study were kept on the SPAL grain based diet. P. decipiens from natural infections were found intermingled with the ingesta (Scott, 1953, McClelland, 1971; and others); when the stomach was empty nematodes were attached. In these experimental infections, nematodes were seldom found free in the lumen of the stomach in pigs, although the stomach was never completely empty of food. That there are more attachment sites than the number of nematodes present indicates that the larvae detach temporarily and, then, reattach.

K.G. Davey (1971) has shown in vitro that P. decipiens larvae will undergo the molt observed in pigs in nonnutritive medium if they are chemically stimulated at the proper time. The lack of growth throughout the course of infection in pigs indicates that the nutritional needs of P. decipiens may not have been satisfied by the experimental conditions. Growth and further development in pigs on a fish diet would indicate the inadequacy either in nutrition or proper stimulatory factors of the grain based-diet.

Infectivity and duration of infection of individual nematode shipments were variable. P. decipiens larvae appear less vulnerable than Anisakis sp. larvae to the stress involved in transport or handling as evidenced by their higher initial infectivity and longer duration in the pig host.

Comparison Gross Pathology. During the first 7 days post-infection (pf) lesions produced by both Phocanema decipiens and Anisakis sp. are similar. The lesions in individual pigs are described in Tables 18 and 24. Tables 18 and 24 shows that most lesions were found on the fundus. Each attached nematode had penetrated to a depth of 4-7 mm., and the surface of the mucosa was swollen to about 1 mm. in a 1-2 mm. diameter ring around each attached nematode. This lesion appeared as if a small doughnut was inserted just beneath the surface of the mucosa. If a nematode was removed, blood was observed at the base of the lesion. Similar lesions without nematodes were observed on the fundus; these lesions exceeded the number of nematodes present. Some of these "ulcers" had a bloody crater at their center which appeared similar to the lesions from which the nematodes were removed manually. Other lesions consisted of the doughnut shaped swelling with pink tissue at its center; some doughnut shaped swellings were filled with tissue which extended into the lumen as small knobs (2-3 mm). The small areas of hemorrhage (2-3 mm diameter) that were occasionally visible from outside of the stomach occurred so infrequently that it is not significant that similar lesions were not found in Anisakis sp. exposed pigs.

Gross edema was noted throughout the course of infection in only one set of infections with Anisakis sp. and Phocanema decipiens larvae. There is not sufficient data to choose among the following explanations. All pigs in which the generalized edema was observed

were approximately the same age and belonged to two litters which were raised together: (1) the parents of these pigs could have genetically predisposed them to this type of tissue reaction; (2) the pigs may have all been sensitized by an agent (biological or chemical) that predisposed them to a generalized tissue reaction; (3) the nematodes used for these infections could have been either more virulent or more viable. (Increased viability was not observed in overall infectivity rates).

Infectivity. Whatever shortcomings the pig has as a substitute host for development of Phocanema decipiens under experimental conditions, the initial infectivity may equal the infectivity in the normal definitive hosts. The initial infectivity and longevity of the larvae varied with the individual shipments of larval nematodes. Some larvae did not appear healthy, as judged by irregular and jerky movements when isolated in saline. These larvae did not produce high rates of infection. Larvae that were either immobile or moved with smooth undulations produced high initial rates of infection and were recovered longer after infection. The source of the larvae (fish processors in Nova Scotia) introduced uncontrollable variables in the treatment of the larvae. Another uncontrollable variable was introduced when the larvae were shipped by commercial air express or air freight. Transit time varied from two days to 14 days. To test the effect of storage time on infectivity a pig was fed larvae stored for 30 days at 4°C in fish fillets. The infection rate in this pig at 48 hours was 20%

compared to 29% at 24 hrs when the nematodes were fed on arrival from Canada.

In addition to diet, miniature pigs differ from seals in normal body temperature. Ronald (1960) showed 35°C (compared to 33° and 37°C) was optimal for in vitro survival of P. decipiens larvae from fish. The internal body temperature of seals is 35.5°C and the normal resting body temperature of miniature pigs is 39°C. With exercise, the body temperature of pigs rises to 42.3°C (Hoernicke, 1966). The confined area in which the pigs for this study were housed did limit their exercise. Body temperatures are, therefore, assumed to be close to the resting body temperatures. Ronald (1960) found 100% mortality of P. decipiens after nine days at 37°C and after 5.7 hours at 40°C. Some P. decipiens larvae showed more thermal tolerance in vivo than was observed by Ronald in vitro. However, thermal stress could account for the curtailed development of P. decipiens in miniature pigs. This hypothesis could be tested if a reliable method of cultivation were available.

The initial infectivity of Anisakis sp. larvae in pigs is similar to that of Phocanema decipiens larvae. The infectivity of Anisakis sp. larvae to pigs cannot be compared with infectivity for natural hosts due to lack of data. Anisakis spp. mature in whales and dolphins.

Peptic Ulcers. The most common ulcers in swine occur in the nonglandular esophagogastric region of the stomach (Perry et al., 1966). McIntosh (1897) was apparently the first to report ulcers in

pig stomachs. He described the ulcers as being of variable shape and being filled with a greyish brown substance, "the root or base extends through the stomach forming a hard, bluish, purple tumor...." Chronic ulcers were differentiated from acute by the degree of fibrosis. Although McIntosh did not describe the location of these ulcers, their description is comparable with ulcers in the esophagogastric region. Various etiologic agents have been blamed for causing stomach ulcers in pigs.

McIntosh associated ulcers with pigs that had experienced hog cholera, the association of viral illness and increased prevalence of ulcers has been reiterated by Hooper and Haeltermah (1966) among others. Rosenow (1923) isolated Streptococcus from ulcers in pigs and other domestic animals. He found these bacteria would induce ulcers when reintroduced through the circulatory system. Fungi were associated with ulcers in pig stomachs by Gitter and Austwick (1949). Multicellular parasites were present in an ulcer case reported by Andress and Connelly (1944). In addition, the incidence of ulcers has also been associated with the diet of pigs.

Gelatinized grain was shown to increase the incidence of peptic ulcers.

The previous factors have all been associated directly with the stomach, Muggenberg et al. (1966) induced ulcers by subcutaneous administration of histamine in mineral oil and beeswax mixture. Gaafar and Keittivati (1972) reported experimental induction of esophagogastric ulcers in pigs by the second administration of

Ascaris suum larvae, but not resulting from the first exposure. Finding stomach tissues that were negative for Ascaris lead the authors to postulate a histamine related mechanism.

Degranulation of eosinophils is sequentially and theoretically (Engelfield, 1972) associated with the release of histamine. The finding of a tissue response consisting almost entirely of eosinophils suggests this model for further evaluation of the role of histamine release in the formation of ulcers and as a model for the elucidation of the relationship between eosinophil degranulation and histamine release.

The ulcers described from pigs fed larval Anisakis sp. and P. decipiens fed pigs are differentiated from the other types of ulcers just discussed by their location. The "anisakine" ulcers were found in the acid secreting portion of the fundus and glandular portion of the cardiac stomach. These areas are not normal locations for gastric ulcers. Anderson and Scotti (1972) stated that ulcers occur in the areas adjacent to but never in the acid secreting portions of the stomach. The ulcers in Anisakis sp. and P. decipiens resemble acute ulcers except for their location. They are similar in size and shape and involve mainly the mucosa. Only superficial layers of the submucosa are frequently hemorrhaged. Microscopically, the ulcers induced by Anisakis sp. and Phocanema decipiens larvae are distinguished from typical acute ulcers by the preponderance of eosinophils in the cellular response.

The small lesions without nematodes seen in both P. decipiens and Anisakis sp. infections were observed in various states associated with healing; open and hemorrhaging or filled with granulation tissue, or filled with connective tissue. Larger ulcers in P. decipiens fed pigs examined 15 or more days post-infection were also observed in these three stages.

Clinical Data. Table 18 and 24 indicate the variation in infectivity rates and pathology observed in this study. The clinical laboratory data reported in Tables 19-23 and 25-34 was gathered in hopes of finding a test or group of tests which could aid in the diagnosis of anisakiasis. The results of the first experiment indicated that changes occurred in the levels of some serum components (Tables 19 and 25) and in the relative numbers of circulating blood cells (Tables 18 and 26). Changes are defined as follows: lactate dehydrogenases values above 535 when the baseline data is below this level; amylase levels at least 100 above baseline values; lipase values 1.0 or more, and eosinophils more than 9 if higher than baseline. Gross pathology is defined by the finding of more than 20 larvae, presence of ulcers larger than individual penetrations, generalized edema of the fundus, or finding of serous fluid in the body cavity.

The prefeeding baselines were extended in the experiments conducted in the spring of 1976 (Tables 20, 21, P. decipiens pigs; Tables 29, 30 Anisakis sp. pigs). Control data from this prefeeding period and that presented in Tables 33 and 34 indicate

that the previously observed postfeeding changes fall within the range of variation of population and may not be attributed to anisakine infections, alone.

Miniature pigs fed Anisakis sp. or Phocanema decipiens on which clinical laboratory data was gathered may be divided into the following four groups: (1) those pigs in which gross pathology and clinical changes were observed compared to prefeeding levels; (2) those pigs in which gross pathology was observed but had stable clinical results after feeding; (3) those pigs in which pathology was not observed; (4) those pigs in which pathology was not observed but changes in clinical results were observed. Testing on larger numbers of animals may reveal a tendency for pigs exposed to anisakine nematodes to fit into one of these groups.

#### INACTIVATION

Work done to assure the safety of fish eaten by the consumer includes study of the temperature tolerance of anisakine larvae. Most, but not all, fish is heated prior to eating in the United States. In The Netherlands, herring that is to be eaten raw must now be frozen. Yet, neither country is totally free of anisakiasis.

Heat. Visthum (1933) reported the emergence of apparently alive ascaroid nematodes from a marinated cod fillet boiled for 20 minutes. Since his report, no one else has confirmed the ability of anisakine larvae to withstand temperatures over 60°C. Tables 7 and 8 summarize the reliable data on the temperature tolerance of

Anisakis-type and Phocanema-type larvae from fish. This evidence was obtained by placing the isolated nematodes in incubation solutions and then applying heat, or by adding the nematodes to already heated solutions, or by heating the nematodes in fish. Ronald (1960) also reports similar data on larvae in moist chambers and evaluates the insulative properties of fish flesh.

The data in Tables 7 and 8, obtained by different methods, are closely comparable. They place an upper limit of 60°C for 1 min as the minimum necessary to kill all anisakine larvae.

Table 9 summarizes the data of J.T. Davey (1972) on the temperature tolerance of Anisakis marina larvae in fried fish fingers. Larvae and a thermistor were implanted in a cavity of a fish "finger", covered with fish flesh, sealed with batter, and refrigerated or frozen overnight. Then, the "fingers" were fried in shallow fat until the thermistor reached a predetermined temperature. The nematodes were removed immediately to warm saline and observed for one hour; they were classified as dead, sluggish or active. This data from fried fish fingers further supports the other temperature studies.

Examination of the tabular data leads to a recommendation that fish ought to be cooked so that the internal temperature reaches or exceeds 60°C for at least 1 min; this will assure the consumer that all anisakine larvae are killed.

Cold. Anisakine larvae are capable of surviving extended periods of refrigeration that easily exceed the useful shelf-life of fresh fish. Ronald (1960) reported that larvae kept refrigerated at 0°C survive for 133 days in a physiological solution.

Table 10 shows the maximum survival time of anisakine larvae exposed to freezing temperatures in situ in fish flesh or on the mesenteries. Survival at -20°C duplicates the temperatures of home freezers or modern refrigerator freezing compartments, and is similar to the temperature of the common commercial block freezing apparatus. Ronald (1960) uses the criterion of fluorescence under UV light to determine viability; all other workers used the criterion of motility after thawing and recovering the larvae.

Ruitenbergh (1970) has translated The Netherland's Green Herring Law: "All herring to be sold as lightly salted herring must be frozen in such a way that the herring has reached a temperature of -20°C within 12 hours. Furthermore, the herring so frozen should be stored during a period of 24 hrs in such a way that this herring is kept at a temperature of not less than -20°C during this period". The data upon which this statement is based indicates a mistake in translation. The last part should read... this herring is kept at a temperature of not more than -20°C during this period.

New data (Table 10) indicate that this regulation may not be sufficient to inactivate the variety of living anisakines found in the fish products of North America.

Smoking and Marination. Other methods of preserving fish such as smoking and pickling have been studied. The review by Oshima (1972) covers the marinating and pickling of fish in Japan and Ruitenberg (1970) reviews marinating and smoking procedures in The Netherlands. Comparison of data is difficult due to the different methods used in each country. In general, smoking and marination procedures are not sufficient to kill anisakine larvae. The exception are dry salting, which will kill anisakines if the salt reaches all parts of the flesh in concentrated form, or hot smoking, which will kill anisakines if the internal temperature exceeds 60°C for 1 min.

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## APPENDIX I

Human Anisakiasis: Phocanema larva from a California man.

On February, 27, 1975, a 44-year-old man from Marin County, California, coughed, felt something in the back of his throat, and then manually extracted a wriggling worm from his mouth. About 10 days before coughing up the worm, the man and his wife had eaten sashimi which was prepared at home from raw white sea bass brought at a fish market in Marin County. The bass had reportedly been caught off the northern Mexico or southern California coast and immediately shipped refrigerated (not frozen) to the market. The couple usually eats raw fish only three or four times a year. The man had no other symptoms associated with this event. His wife remained asymptomatic.

After the patient removed the nematode from his throat, it was placed in water for approximately 2 hr prior to fixation in 10% formalin. The specimen was then submitted to the Marin County Health Department for identification. It was then forwarded to the California State Health Department, the Communicable Disease Center and finally to the Laboratory of Parasitology of the Food and Drug Administration (FDA). Prior to arrival at the FDA the specimen was transferred to 70% ethanol. For examination, the specimen was cleared in phenol liquified with 95% ethanol.

The day after coughing up the nematode, the man's blood count revealed a mild eosinophilia. The white blood count was 8200

cells/mm<sup>3</sup> (differential count: segmented neutrophils 62%, lymphocytes 26%, monocytes 3%, basophils 1%, and eosinophils 8%). The total eosinophil count was 774 cells/mm<sup>3</sup> (normal 50-500 cells/mm<sup>3</sup>). No abnormalities were noted on chest x-ray or a single stool examination for parasites, eggs of parasite, occult blood, and bacterial pathogens. A repeat blood count 2 weeks later showed 8900 white blood cells/mm<sup>3</sup> (differential count: segmented neutrophils 68%, lymphocytes 25%, monocytes 6%, and eosinophils 1%). The total eosinophil count was 216 cells/mm<sup>3</sup>. An upper gastrointestinal and small bowel series (with a 24-hr follow-up film), done 2 weeks after discovery of the nematodes, were normal.

#### Description of Parasite

The recovered nematode is 28.9 mm long, with a maximum width of 1.0 mm, and in poor condition. There are three fleshy lips with barely apparent denticular ridges (a boring tooth and interlabia are absent). The cuticle of the subventral lips overlaps the lateral margin of the dorsal lip as in Kates et al. (1973). There are two papillae on the dorsal lip, and each subventral lip has a single papilla. The papillae appear to be double. The excretory pore is located at the base of the subventral lips and has an adjacent cuticular projection (hook) and palisade (Figs 1, 2). The mouth is prominent; the esophagus consists of a muscular portion the preventriculus (3.72 mm long) and a ventriculus (0.91 mm long). The intestine has a caecum (0.85 mm long) projecting in the anterior direction. The tail appears to be distorted, but of the two anal lips the anterior is the more prominent. Reproductive organs were

thought to be semideveloped but are mostly obscured by an opaque material.

#### Identification

Because of the presence of a ventriculus, anteriorly projecting intestinal caecum and excretory pore at the level of the lips, the nematode can be classified as a Phocanema type larva (Myers, 1975). Members of two genera, Phocanema and Terranova, possess this type of larva. The presence of three fleshy lips, further identifies the nematode as a member of the genus Phocanema. Lips of Terranova spp. are poorly differentiated from the neck region. That this larvae molted in the patient and is more mature than the Phocanema type larvae commonly found in fish was indicated by a well developed lip and mouth region, absence of sheath, or new cuticle, and by the apparently semideveloped state of the reproductive organs.

The fishborne larval stages of, at least, those anisakine nematodes that mature in mammals are able to infect human consumer when fresh or insufficiently frozen fish is eaten in a raw, raw-marinated or undercooked state. Full development of these larvae does not occur in the human host, but the parasite may live long enough to cause a variety of symptoms from mild to severe.

At least four types of human anisakiasis can be distinguished. In lumenal anisakiasis, the mildest form, there is no evidence of tissue penetration by the parasite. Infections may become manifest when living nematodes are voided by coughing, vomiting, or in the stool. The irritation that prompts the vomiting or coughing is

though to be due to mechanical friction at the luminal surface or, possibly, caused by the anisakine's secretory and excretory products. In gastric and intestinal anisakiasis, the nematodes penetrate the respective mucosa and attach to or become embedded in the submucosa; the posterior portion of attached anisakines may be free in the stomach or intestinal lumen. In mesenteric anisakiasis, the parasites perforate the digestive tract and migrate onto or into the mesenteries and other parenteral tissues.

The first confirmed case of human anisakiasis occurred in 1955 in The Netherlands (Straub, 1960) although a suspected case was reported as early as 1867 (Pfaff). The Netherlands (Ruitenbergh, 1970) and Japan (Oshima, 1972) have the largest number of reported cases. In North America there are two suspect cases (Chitwood, 1970; Pfaff, 1867), a group diagnosis based on a stool examination that is difficult to interpret (Hitchcock, 1950), and seven confirmed instances of human infection with an anisakine nematode (Davey, 1971; Little and MacPhail, 1972; Kates *et al.*, 1973; Chitwood, 1975; Little and Most, 1973; Richman and Lewicki, 1973; Lichtenfels and Brancato, 1976). The present case is the eighth confirmed case in North America, the fourth from the west coast.

This infection with a Phocanema sp. larva was apparently an instance of luminal anisakiasis. However, because of the patient's slight and transitory eosinophilia, a temporary tissue phase cannot be dismissed.

To date, Phocanema sp. larvae have been recovered from most North American cases of human anisakiasis. In one case a penetrated parasite was identified as an Anisakis sp. larva (Richman and Lewicki, 1973). Davey (1971) reported an Anisakis sp. larva in an Alaskan Eskimo's vomitus. Only one Phocanema sp. larva has been recovered from parenteral human tissues in North America (Little and MacPhail, 1972). The others, as in the present instance, were voided with feces or extricated through the mouth.

## APPENDIX II

### Personal Data:

Jeffrey W. Bier Born: Detroit, Michigan - 16, April 1942

### Parents:

Dorothy Elizabeth Root Bier and Carl Bier

### Educational Background:

Undergraduate	Roanoke College	B.S. 1965
Graduate	Lehigh University	M.S. 1971

### Honors and Awards:

Antarctic Service Medal, 1966. Received for overwintering as a civilian scientist at McMurdo, Station.

Bier Point, Antarctica. Dedicated 1966 for contribution to U.S. Antarctic Research Program.

### Special Invitations:

W.A. Jones Cell Science Center, Tissue Culture Association, Inc.  
"Application of Invertebrate Culture Systems to the Study of Bio-Sciences. I. Nematode Cell Culture." "Animal Parasites." October, 1973.

New York Society of Tropical Medicine. "Anisakiasis" A New Disease from Raw Fish." "Experimental Anisakiasis" Cultivation and Temperature Tolerance Determinations." March, 1974. Published 1976.

Helminthological Society of Washington. "Course of Infection and Microscopic Pathology of *Phocanema decipiens* in natural and experimental hosts." December 1977.

### Membership in Professional Societies:

American Association for the Advancement of Science.  
American Society of Parasitologists.  
Helminthological Society of Washington.  
Tropical Medicine Society of Washington.

### Offices and Committee Assignments Held in Professional Societies:

Executive Committee, Helminthological Society of Washington - 1978 and 1979.

### Participation in National Scientific Meeting, Technical Conferences, Workshops, etc.

Participant. "International Workshop on Biological Control of Vectors." Lehigh University, Bethlehem, PA., June, 1972.

Participant. "Systemic Parasitology Past, Present, Future," Special Interest Group of International Congress of Parasitology, III. Munich, Germany, August, 1974.

Co-organizer. "Discussion Group on Anisakine Nematodes."  
International Congress of Parasitology, III. Munich, Germany,  
August, 1974.

Demonstration. "New Information on Excretory System Leads to  
Classification of Larval Nematode from Shellfish." Second Annual  
Fish Health Workshop. Easton, MD, May, 1977.

Demonstration. "New Information on Excretory System Leads to  
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Publications:

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Payne, C.R. Wilson. Survey of microflora and microfauna associated  
with the Moroccan food snail, Helix aspersa. *Proceedings Third  
International Congress of Parasitology* 3:1601-1602.

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Journal of Tropical Medicine and Hygiene* 24:942-949.

1976. Bier, Jeffrey W. Experimental anisakiasis: cultivation and temperature tolerance determinations. *Journal of Milk and Food Technology*. 39:132-137. (Review).
1976. Jackson, G.J., J.W. Bier, and W.L. Payne. Experimental anisakiasis in pigs; course of infection with larval Anisakis sp. and Phocanema sp. nematodes from fishes. *Transactions American Microscopical Society* 95:264.
1976. Bier, J.W., G.J. Jackson, F.L. Earl and W.G. Knollenberg. Experimental anisakiasis: gross and microscopic pathology with larval Anisakis and Phocanema spp. nematodes from fishes. *Transactions American Microscopical Society* 95:265.
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In preparation:

1978. Bier, J.W., J.R. Lichtenfels, R.A. Campbell, and P.A. Madden. A redescription of Paranisakiopsis lintoni (Linton, 1907) with notes on the genus.

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TABLE 1. Invertebrates Reported Naturally Infected with Anisakine Nematodes

Host	Parasite	Stage	Locality
<b>Coelenterata</b>			
<b>Hydrozoa</b>			
<u>Phialidium sp.</u>	<u>Contracaecum</u>	L	North Sea
<u>Polyorchis penicillatus</u>	<u>Thynnascaris</u>	3,4	C.E. North
<b>Scyphozoa</b>			
<u>Ceriantharia</u>	<u>Thynnascaris</u>	L	N.E. South
<b>Ctenopora</b>			
<u>Ctenophore</u>	... "worm in shape resembling a filaria"	N.G.	N.E. North
<u>Pleurobranchia pileus</u>	<u>Agamonema capsularia</u>	L	North Sea
<u>Pleurobranchia pileus</u>	<u>Thynnascaris sp.</u>	L	N.W. South
<u>Pleurobranchia pileus</u>		L	North Sea
<b>Chaetognatha</b>			
<u>Sagitta bipunctata</u>	<u>Contracaecum</u>	L	N.E. North
<u>Sagitta bipunctata</u>	<u>Thynnascaris sp.</u>	L	N.W. South
<u>Sagitta elegans</u>	<u>Contracaecum sp.</u>	L	North Sea
<u>Sagitta elegans</u>	<u>Contracaecum sp.</u>	L	North Sea
<u>Sagitta elegans</u>	<u>Contracaecum sp.</u>	L	White Sea
<u>Sagitta euxina</u>	<u>Contracaecum</u>	N.G.	Black Sea

ed with Anisakine Nematodes

	Stage	Locality	Authority	Date
	L	North Sea	Wulker	1929
	3,4	C.E. North Pacific	Lichtenfels	1974
	L	N.E. South Atlantic	Schuermans-Stekhoven	1941
resembling a filaria"	N.G.	N.E. North Atlantic	Forbes	1839
ia	L	North Sea	Mortensen	1912
	L	N.W. South Pacific	Boyle	1966
	L	North Sea	Norris and Overstreet	1976
	L	N.E. North Atlantic	Lebour	1917
	L	N.W. South Pacific	Boyle	1966
	L	North Sea	Reimer <u>et al.</u>	1971
	L	North Sea	Smith	1971
	L	White Sea	Kulachkova	1970
	N.G.	Black Sea	Elfan	1960

TABLE I. Invertebrates Reported Naturally Infected with Anisakine Nematodes (Continued)

Host	Parasite	Stage	Locality	Authority	Date
<u>Sagitta friderici</u>	<u>Thynnascaris</u>	L	C.E. North Atlantic	Furnestin	1957
<u>Sagitta hispida</u>	<u>Thynnascaris</u> sp.	L	N.C. Gulf of Mexico	Norris and Overstreet	1976
<u>Sagitta inflata</u>	<u>Thynnascaris</u> sp.	L	Mediterranean area	Ghirardelli	1950
<u>Sagitta setosa</u>	<u>Thynnascaris</u> sp.	L	C.E. North Atlantic	Vitello <u>et al.</u>	1970
<u>Sagitta setosa</u>	Nematode	L	English Channel	Russell	1932
<u>Sagitta setosa</u>	<u>Contraecum</u> sp.	L	North Sea	Reimer <u>et al.</u>	1971
<u>Sagitta</u> sp.	<u>Thynnascaris</u> sp.	L	C.E. North Atlantic	Vitello <u>et al.</u>	1970
<u>Sagitta</u> sp.	<u>Contraecum</u> sp.	L	Mediterranean Sea	Pierantoni	1914
<u>Sagitta</u> sp.	<u>Contraecum</u> sp.	L	North Atlantic	Hulton	1964
<u>Sagitta</u> sp.	<u>Contraecum</u> sp.	L	North Sea	Muiker	1929
<u>Sagitta</u> sp.	<u>Contraecum</u> sp.	L	Serdolik Bay	Ass	1961
<u>Sagitta tennis</u>	<u>Thynnascaris</u> sp.	L	N.C. Gulf of Mexico	Norris and Overstreet	1976
Mollusca					
Gastropoda					
<u>Buccinum</u> sp.	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960
<u>Busycon canaliculata</u>	<u>Paranisaktopsis</u> sp.	3,4	C.W. North Atlantic	Lichtenfels <u>et al.</u>	1976
<u>Cantharus cancellarius</u>	<u>Thynnascaris</u> sp.	L	N.C. Gulf of Mexico	Norris and Overstreet	1976
<u>Cyclonassa neritea</u>	<u>Thynnascaris aduncum</u>	L	Black Sea	Dolgikh	1966
<u>Littorina</u> sp.	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960

TABLE I. Invertebrates Reported Naturally Infected with Anisakine Nematodes (Continued)

Host	Parasite	Stage	Locality
<u>Todarodes pacificus</u>	<u>Terranova sp.</u>	L	C.W. North Pa
Annelida			
Polychaeta			
<u>Enoe nodosa</u>	<u>Thynnascaris sp.</u>	L	White Sea
<u>Gattiana cirrosa</u>	<u>Thynnascaris aduncum</u>	2,3,4	White Sea
<u>Gattiana sp.</u>	<u>Thynnascaris aduncum</u>	2	White Sea
<u>Harmothoe imbricata</u>	<u>Thynnascaris aduncum</u>	2,3,4	White Sea
<sup>142</sup> <u>Harmothoe sp.</u>	<u>Thynnascaris aduncum</u>	2	White Sea
<u>Lepidonotus sp.</u>	<u>Thynnascaris aduncum</u>	2	White Sea
<u>Lepidonotus squamatus</u>	<u>Thynnascaris aduncum</u>	2,3,4	White Sea
<u>Nereis helgolandica</u>	<u>Thynnascaris aduncum</u>	L	White Sea
<u>Nereis pelagica</u>	<u>Thynnascaris aduncum</u>	L	White Sea
<u>Nereis sp.</u>	<u>Phocanema decipiens</u>	2	N.W. North At
<u>Nereis sp.</u>	<u>Thynnascaris aduncum</u>	2	White Sea
Sea mouse	<u>Phocanema decipiens</u>	2	N.W. North At
<u>Tomopterus helgolandioa</u>	<u>Contracaecum sp.</u>	L	North Sea
Oligochaeta			
<u>Eiseniella tetraedra</u>	<u>Porrocaecum semiteres</u>	3	Poland
<u>Lumbricus terrestris</u>	<u>Porrocaecum semiteres</u>	2,3	

ith Anisakine Nematodes (Continued)

Stage	Locality	Authority	Date
L	C.W. North Pacific	Orihara <u>et al.</u>	1968
L	White Sea	Papova	1967
2,3,4	White Sea	Papova and Valter	1968
2	White Sea	Papova <u>et al.</u>	1964
2,3,4	White Sea	Uspenskaya	1963
2	White Sea	Papova <u>et al.</u>	1964
2	White Sea	Papova <u>et al.</u>	1964
2,3,4	White Sea	Papova and Valter	1965
L	White Sea	Papova and Valter	1976
L	White Sea	Papova and Valter	1976
2	N.W. North Atlantic	Myers	1960
2	White Sea	Papova <u>et al.</u>	1964
2	N.W. North Atlantic	Myers	1960
L	North Sea	Reimer <u>et al.</u>	1971
3	Poland	Moravec	1971
2,3		Igis	1967

TABLE I. Invertebrates Reported Naturally Infected with Anisakine Nematodes (Continued)

Host	Parasite	Stage	Locality
<u>Lunatia heros</u>	<u>Paranisakiopsis lintoni</u>	4	C.W. North
<u>Massa reticulata</u>	<u>Thynnascaris aduncum</u>	L	Black Sea
<u>Thais haematostoma</u>	<u>Thynnascaris sp.</u>	L	N.C. Gulf of
Pelecepada			
<u>Mya arenaria</u>	<u>Phocanema decipiens</u>	2	N.W. North
<u>Mytilus edulis</u>	<u>Phocanema decipiens</u>	2	N.W. North
<u>Pecten</u>	<u>Paranisakiopsis lintoni</u>	4	C.W. North
<u>Pecten</u>	<u>Sulcascaris sp.</u>	4	C.W. North
<u>Spisula solidissima</u>	<u>Sulcascaris sp. lintoni</u>	4	C.W. North
Cephalapoda			
<u>Dorytenthis bleckeri</u>	<u>Anisakis sp. II</u>	L	C.W. North
<u>Illex argentinus</u>	<u>Anisakis sp.</u>	L	C.W. South
<u>Illex illecebrosus</u>	<u>Anisakis sp.</u>	L	N.W. North
<u>Illex illecebrosus</u>	<u>Contracaecum sp.</u>	L	N.W. North
<u>Lolliguncula brevis</u>	<u>Thynnascaris</u>	L	N.C. Gulf of
<u>Ommatostrephes sagittatus</u>	<u>Anisakis sp. I</u>	L	North Sea
<u>Todarodes pacificus</u>	<u>Anisakis I</u>	L	C.W. North
<u>Todarodes pacificus</u>	<u>Anisakis sp. I</u>	L	C.W. North
<u>Todarodes pacificus</u>	<u>Anisakis sp. II</u>	L	C.W. North
<u>Todarodes pacificus</u>	<u>Contracaecum sp.</u>	L	C.W. North

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with Anisakine Nematodes (Continued)

Stage	Locality	Authority	Date
4	C.W. North Atlantic	Lichtenfels <u>et al.</u>	1976
L	Black Sea	Dolgikh	1966
L	N.C. Gulf of Mexico	Norris and Overstreet	1976
2	N.W. North Atlantic	Myers	1960
2	N.W. North Atlantic	Myers	1960
4	C.W. North Atlantic	Cobb	1930
4	C.W. North Atlantic	Hutton	1964
4	C.W. North Atlantic	Lichtenfels <u>et al.</u>	1976
L	C.W. North Pacific	Kato <u>et al.</u>	1968
L	C.W. South Atlantic	Threlfall	1968
L	N.W. North Atlantic	Threlfall	1970
L	N.W. North Atlantic	Brown and Threlfall	1968
L	N.C. Gulf of Mexico	Norris and Overstreet	1976
L	North Sea	Berland	1961
L	C.W. North Pacific	Yamaguchi	1966
L	C.W. North Pacific	Kobayashi <u>et al.</u>	1963
L	C.W. North Pacific	Kato <u>et al.</u>	1969
L	C.W. North Pacific	Shiraki <u>et al.</u>	1969

TABLE I. Invertebrates Reported Naturally Infected with Anisakine Nematodes (Continued)

Host	Parasite	Stage	Locality	Authority	Date
Brachiopoda					
Cladocera					
<u>Leptodora kinultii</u>	<u>Raphidascaris sp.</u>	L	Japan	Kataoka and Momma	1932
Copepoda					
<u>Calanus finmarchicus</u>	<u>Contracaecum sp.</u>	L	Barents Sea	Apstein	1911
<u>Calanus finmarchicus</u>	<u>Contracaecum sp.</u>	L	North Sea	Wulker	1929
<u>Calanus finmarchicus</u>	<u>Contracaecum sp.</u>	L	North Sea	Uspenskaya	1963
<u>Calanus sp.</u>	<u>Contracaecum sp.</u>	L	North Sea	Apstein	1911
Copepod	<u>Ascaris sp.</u>	L	Mediterranean Sea	Pferantoni	1917
<u>Diaptomus sp.</u>	<u>Goezia spinulosa</u>	N.G.	C.W. South Atlantic	Freitas and Lent	1946
<u>Euchoela sp.</u>	<u>Contracaecum</u>	L	North Sea	Apstein	1911
<u>Pseudocalanus elongatus</u>	<u>Contracaecum</u>	L	North Sea	Apstein	1911
<u>Temora longicornis</u>	<u>Thynnascaris aduncum</u>	L	White Sea	Papova and Valter	1969
Malacostraca					
Mysidacea					
<u>Erythrops erythrophthalma</u>	<u>Contracaecum sp.</u>	L	N.W. North Atlantic	Scott	1957
<u>Mysis mixta</u>	<u>Contracaecum sp.</u>	L	N.W. North Atlantic	Scott and Black	1957
<u>Mysis mixta</u>	<u>Phocanema</u>	L	N.W. North Atlantic	Scott and Black	1960
<u>Mysis retracula</u>	<u>Phocanema decipiens</u>	L	N.W. North Atlantic	Myers	1960
<u>Mysis stenolepsis</u>	<u>Contracaecum sp.</u>	2	N.W. North Atlantic		

TABLE I. Invertebrates Reported Naturally Infected with Anisakine Nematodes (Continued)

Host	Parasite	Stage	Locality	Authority	Date
<u>Mysis stenolepis</u>	<u>Phocanema</u>	L	N.W. North Atlantic	Scott and Black	1960
<u>Mysis stenolepis</u>	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960
<u>Neomysis americana</u>	<u>Contracaecum</u> sp.	L	N.W. North Atlantic	Scott	1967
Unidentified mysid	<u>Phocanema</u>	L	N.W. North Atlantic	Scott and Black	1960
Unidentified mysid	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960
Isopoda					
<u>Iaera albitrons</u>	<u>Thynnascaris aduncum</u>	2	White Sea	Valter	1968
<u>Idothea</u> sp.	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960
Amphipoda					
<u>Anonyx nugax</u>	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960
<u>Ampelisca</u> sp.	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960
<u>Cumacea</u>	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960
<u>Caprella septentrionalis</u>	<u>Anisakis</u> sp.		Uspenskaya	Uspenskaya	1963
<u>Caprella septentrionalis</u>	<u>Thynnascaris aduncum</u>		White Sea	Valter	1963
<u>Gammarus</u> sp.	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960
<u>Onosimus plautus</u>	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960
<u>Rachotropis aculeata</u>	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960
<u>Syrrhoe crenulata</u>	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960
Euphausiacea					
<u>Euphausia pacifica</u>	<u>Anisakis</u> sp.	L	C.M. North Pacific	Shimazu and Oshima	1970

TABLE I. Invertebrates Reported Naturally Infected with Anisakine Nematodes (Continued)

Host	Parasite	Stage	Locality	Authority	Date
<u>Euphausia pacifica</u>	<u>Contracaecum</u>		C.W. North Pacific	Shimazu and Oshima	1969
<u>Meganctiphanes noruogica</u>	<u>Anisakis sp.</u>	2,3	North Sea	Sluifers and Smith	1972
<u>Myctiphanes couchii</u>	<u>Contracaecum sp.</u>	L	N.E. North Atlantic	Reimer	1971
<u>Thysanoessa inermis</u>	<u>Contracaecum sp.</u>	2	North Sea	Smith	1971
<u>Thysanoessa inermis</u>	<u>Anisakis sp.</u>	3	North Sea	Sluifers	1972
<u>Thysanoessa inermis</u>	<u>Anisakis sp.</u>	2	North Sea	Smith	1971
<u>Thysanoessa longicaudata</u>	<u>Anisakis sp.</u>	L	N.? North Pacific	Kagei	1969
<u>Thysanoessa longipes</u>	<u>Anisakis sp.</u>	2	North Sea	Smith	1971
<u>Thysanoessa raschii</u>	<u>Anisakis sp. I</u>	L	C.W. North Pacific	Oshima <u>et al.</u>	1969
<u>Thysanoessa raschii</u>	<u>Anisakis sp. I</u>	L	N.E. North Pacific	Oshima <u>et al.</u>	1969
<u>Thysanoessa raschii</u>	<u>Anisakis sp.</u>	3	North Sea	Sluifers	1972
<u>Thysanoessa raschii</u>	<u>Anisakis sp.</u>	L	White Sea	Uspenskaya	1960
<u>Thysanoessa raschii</u>	<u>Contracaecum</u>	L	N.C. North Pacific	Oshima and Shimazu	1971
<u>Thysanoessa raschii</u>	<u>Contracaecum</u>	2	North Sea	Smith	1971
<u>Thysanoessa raschii</u>	<u>Contracaecum</u>	L	N.M. North Atlantic	Scott and Black	1960
Decapoda					
<u>Argis sp.</u>	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960
<u>Cancer irroratus</u>	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960
<u>Clibanarius vittatus</u>	<u>Thynnascaris sp.</u>	L	N.C. Gulf of Mexico	Norris and Oversreest	1976
<u>Crago septempinosus</u>	<u>Contracaecum sp.</u>	L	N.M. North Atlantic	Scott and Black	1960

TABLE I. Invertebrates Reported Naturally Infected with Anisakine Nematodes (Continued)

Host	Parasite	Stage	Locality	Authority	Date
<u>Sicyonia dorsalis</u>	<u>Thynnascaris</u> sp.	L	C.W. North Atlantic	Hutton <u>et al.</u>	1962
<u>Sicyonia typica</u>	<u>Thynnascaris</u> sp.	L	C.W. North Atlantic	Hutton <u>et al.</u>	1962
<u>Solenocera atlantidis</u>	<u>Thynnascaris</u> sp.	L	C.W. North Atlantic	Hutton <u>et al.</u>	1962
<u>Spiranotocaris groenlandica</u>	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960
<u>Trachypenaeus constrictus</u>	<u>Thynnascaris</u> sp.	L	C.W. North Atlantic	Hutton <u>et al.</u>	1962
<u>Trachypenaeus similis</u>	<u>Thynnascaris</u> sp.	L	C.W. North Atlantic	Hutton <u>et al.</u>	1962
<u>Xiphopeneus kroyeri</u>	<u>Thynnascaris</u> sp.	L	C.W. North Atlantic	Hutton <u>et al.</u>	1962
Insecta					
Chironomidae	<u>Raphidascaris acus</u>		Poland	Moravec	1970
Diptera					
<u>Prodiamesa olivacea</u>	<u>Raphidascaris acus</u>	2	Czechoslovakia	Moravec	1970
Echinodermata					
Asteroidea					
<u>Lucidia clathrata</u>	<u>Thynnascaris</u> sp.	L	N.C. Gulf of Mexico	Norris and Overstreet	1976
Starfish	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960
Echinoidea					
Seaurchin	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960
Holothurioidea					
Sea cucumber	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960

NG = Not given. L = larval but no stage assigned. A = adult.

TABLE I. Invertebrates Reported Naturally Infected with Anisakine Nematodes (Continued)

Host	Parasite	Stage	Locality	Authority	Date
<u>Emerita talpoida</u>	<u>Thynnascaris</u> sp.	L	N.C. Gulf of Mexico	Norris and Overstreet	1976
<u>Homarus americanus</u>	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1963
<u>Hyas araneus</u>	<u>Anisakis</u> sp.	L	White Sea	Uspenskaya	1963
<u>Pagurus kroyeri</u>	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960
<u>Pandulus borealis</u>	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960
<u>Pandulus</u> sp.	<u>Phocanema decipiens</u>	2	N.W. North Atlantic	Myers	1960
<u>Pandulus borealis</u>	<u>Thynnascaris aduncum</u>	L	Barents Sea	Uspenskaya	1960
<u>Pandulus borealis</u>	<u>Thynnascaris</u> sp.	A	N.W. North Atlantic	Margolis and Butler	1954
<u>Penaeus aztecus</u>	<u>Thynnascaris</u> sp.	L	C.W. North Atlantic	Hutton	1964
<u>Penaeus aztecus</u>	<u>Thynnascaris</u> sp.	L	N.E. Gulf of Mexico	Kruze	1959
<u>Penaeus brasiliensis</u>	<u>Thynnascaris</u> sp.	L	C.W. North Atlantic	Hutton et al.	1962
<u>Penaeus californiensis</u>	<u>Contracaecum</u> sp.	L	S.E. North Pacific	Norris and Overstreet	1976
<u>Penaeus duorarum</u>	<u>Thynnascaris</u> sp.	L	C.W. North Atlantic	Hutton et al.	1962
<u>Penaeus duorarum</u>	<u>Thynnascaris</u> sp.	L	N.E. Gulf of Mexico	Kruze	1959
<u>Penaeus setiferus</u>	<u>Thynnascaris</u> sp.	L	C.W. North Atlantic	Hutton et al.	1962
<u>Penaeus setiferus</u>	<u>Thynnascaris</u> sp.	L	N.E. Gulf of Mexico	Kruze	1959
<u>Penaeus setiferus</u>	<u>Thynnascaris</u> sp.	L	N.C. Gulf of Mexico	Overstreet	1973
<u>Penaeus stylirrostris</u>	<u>Thynnascaris</u> sp.	L	S.E. North Pacific	Hutton et al.	1962
<u>Penaeus vannamei</u>	<u>Contracaecum</u> sp.	L	S.E. North Pacific	Norris and Overstreet	1976
<u>Penaeus vannamei</u>	<u>Thynnascaris</u> sp.	L	S.E. North Pacific	Norris and Overstreet	1976
Sea Crabs	<u>Thynnascaris iniquis</u>	L	Indian Ocean	Kalyankar	1972

TABLE 2: Experimental Invertebrate Intermediate Hosts of Anisakine Nematodes.

Host	Parasite	Stage	Authority	Date
<b>Mollusca</b>				
<b>Gastropoda</b>				
<u>Buccinum</u> sp.	<u>Phocanema decipiens</u>	2	Myers	1960
<u>Littorina</u> sp.	<u>Phocanema decipiens</u>	2	Myers	1960
<u>Margarites groenlandicus</u>	<u>Thynnascaris aduncum</u>	N.G.	Valter	1968
<b>Nudibranch</b>	<u>Phocanema decipiens</u>	2	McClelland	1977
<b>Palaeopoda</b>				
<u>Mya arenaria</u>	<u>Phocanema decipiens</u>	2	Myers	1960
<u>Mytilus edulis</u>	<u>Phocanema decipiens</u>	2	Myers	1960
<b>A Polychaeta</b>				
<u>Harmothoe fabricata</u>	<u>Thynnascaris aduncum</u>		Valter	1968
<u>Harmothoe</u>	<u>Phocanema decipiens</u>	2	Myers	1960
<u>Lepidonotus</u> sp.	<u>Phocanema decipiens</u>	2	Myers	1960
<u>Lepidonotus</u> sp.	<u>Thynnascaris aduncum</u>		Popova <u>et al.</u>	1964
<u>Lepidonotus squamatus</u>	<u>Thynnascaris aduncum</u>		Valter	1968
<u>Nereis</u> sp.	<u>Phocanema decipiens</u>	2	Myers	1960
Sea mouse	<u>Phocanema decipiens</u>	2	Myers	1960
<b>Oligochaeta</b>				
<u>Allobophora</u> spp.	<u>Porrocaecum crassum</u>	2, 3	Supryaga	1972
<u>Criodrilus lacuum</u>	<u>Porrocaecum crassum</u>	2, 3	Supryaga	1972
<u>Criodrilus lacuum</u>	<u>Porrocaecum ensicaudatum</u>		Iygis	1972
<u>Criodrilus lacuum</u>	<u>Raphidascaris acus</u>		Supryaga-Kosinova	1969
Earthworm	<u>Porrocaecum crassum</u>	2, 3, 4?	Mozgovoi	1952
<u>Eisenia foetida</u>	<u>Porrocaecum semiteres</u>		Iygis	1967
<u>Eisenia</u> spp.	<u>Porrocaecum crassum</u>	2, 3	Supryaga	1972
<u>Eisenia ukrainae</u>	<u>Raphidascaris acus</u>		Supryaga-Kosinova	1969
<u>Eiseniella teraedra</u>	<u>Porrocaecum crassum</u>	2, 3	Supryaga	1972
<u>Eophila montana</u>	<u>Porrocaecum crassum</u>	2, 3	Supryaga	1972
<u>Lumbricus herculeus</u>	<u>Porrocaecum talpae</u>		Osche	1955

TABLE 2. Experimental Invertebrate Intermediate Hosts of Anisakine Nematodes (Continued)

Host	Parasite	Stage	Authority	Date
<u>Lumbricus rubellus</u>	<u>Porrocaecum semiteres</u>		Mozgovoï and Bishaeva	1959
<u>Lumbricus rubellus</u>	<u>Porrocaecum semiteres</u>		Igis	1967
<u>Lumbricus terrestris</u>	<u>Porrocaecum ensicaudatum</u>	3,3	Levin	1957
<u>Lumbricus terrestris</u>	<u>Porrocaecum ensicaudatum</u>		Kysavy	1959
Naidae	<u>Raphidascaris acus</u>		Engashe	1964
<u>Nais</u> sp.	<u>Raphidascaris acus</u>		Engashe	1965
<u>Octolasion lacteum</u>	<u>Porrocaecum ensicaudatum</u>	2,3	Levin	1957
<u>Tubifex</u>	<u>Raphidascaris acus</u>	2,3	Engashe	1965
Tubificidae	<u>Raphidascaris acus</u>		Engashe	1964
Arthropoda				
Copepoda				
<u>Acartia bifilosa</u>	<u>Thynnascaris aduncum</u>		Markowski	1937
<u>Acartia longiremis</u>	<u>Thynnascaris aduncum</u>		Popova & Valter	1965
<u>Alteutha</u> sp.	<u>Phocanema decipiens</u>	2	McClelland	1977
<u>Agaira</u> sp.	<u>Phocanema decipiens</u>	2	McClelland	1977
<u>Amphiascus similis</u>	<u>Contracaecum osculatum</u>	2	Davey	1969
<u>Cyclops</u> sp.	<u>Contracaecum microcephalum</u>	2,3	Mozgovoï et al.	1965
<u>Cyclops</u> sp.	<u>Contracaecum micropapillatum</u>		Semenova	1972
<u>Cyclops</u> sp.	<u>Contracaecum spicaligerum</u>	2,3	Mozgovoï et al.	1965
<u>Cyclops strenuus</u>	<u>Contracaecum spiculigerum</u>	2,3	Mozgovoï et al.	1968
<u>Cyclops vernalis</u>	<u>Contracaecum multipapillatum</u>	2	Huizinga	1967
<u>Cyclops vernalis</u>	<u>Contracaecum spiculigerum</u>	2	Huizinga	1966
<u>Danielsennia</u> sp.	<u>Phocanema decipiens</u>	2	McClelland	1977
<u>Diaptomus</u>	<u>Contracaecum micropapillatum</u>		Semenova	1971
<u>Diaptomus castor</u>	<u>Goezia ascaroides</u>	2,3	Mozgovoï et al.	1971
<u>Diaptomus gracilis</u>	<u>Contracaecum spiculigerum</u>	2,3	Mozgovoï et al.	1968
<u>Diosaccidae</u> sp.	<u>Phocanema decipiens</u>	2	McClelland	1968
<u>Enhydrosona</u> sp.	<u>Phocanema decipiens</u>	2	McClelland	1977
<u>Eurytemora affinis</u>	<u>Thynnascaris aduncum</u>		Markowski	1937
<u>Halectinosoma</u> spp.	<u>Phocanema decipiens</u>	2	McClelland	1977

Table 2. Experimental Invertebrate Intermediate Hosts of Anisakine Nematode (Continued)

Host	Parasite	Stage	Authority	Date
<u>Idya fuscata</u>	<u>Contracaecum osculatum</u>	2	Davey	1969
<u>Macrocyclus albidus</u>	<u>Contracaecum spiculigerum</u>	2,3	Hozgovi et al.	1968
<u>Macrocyclus fuscus</u>	<u>Contracaecum spiculigerum</u>	2,3	Hozgovi et al.	1968
<u>Macrocyclus</u> sp.	<u>Contracaecum microcephalum</u>	2,3	Hozgovi et al.	1965
<u>Macrocyclus</u> sp.	<u>Contracaecum micropapillatum</u>	2,3	Semenova	1971
<u>Macrocyclus</u> sp.	<u>Contracaecum spasaki</u>	2	Hozgovi et al.	1965
<u>Macrocyclus</u> sp.	<u>Contracaecum spiculigerum</u>	2,3	Hozgovi et al.	1965
<u>Macrocyclus</u> sp.	<u>Contracaecum spiculigerum</u>	2,3	Hozgovi et al.	1968
<u>Macrocyclus leuckarti</u>	<u>Contracaecum micropapillatum</u>	2,3	Hozgovi et al.	1968
<u>Mesocyclops</u> sp.	<u>Thynnascaris aduncum</u>	2,3	Semenova	1971
<u>Microsetella norvegica</u>	<u>Thynnascaris aduncum</u>		Valter	1968
<u>Pseudocalanus elongatus</u>	<u>Thynnascaris aduncum</u>		McClelland	1977
Robertgurney (affinity n.s.n. sp.)	<u>Phocanema decipiens</u>		Papova and Valter	1968
<u>Tanora longicornis</u>	<u>Thynnascaris aduncum</u>		Huizinga	1967
<u>Tigriopus californicus</u>	<u>Thynnascaris aduncum</u>	2	Huizinga	1966
<u>Tigriopus californicus</u>	<u>Contracaecum multipapillatum</u>	2	Huizinga	1966
<u>Tigriopus californicus</u>	<u>Contracaecum spiculigerum</u>		Fenner	1941
<u>Tigriopus californicus</u>	<u>Contracaecum spiculigerum</u>		McClelland	1977
<u>Tisbe</u> spp.	<u>Contracaecum spiculigerum</u>	2		
<u>Tisbe</u> spp.	<u>Phocanema decipiens</u>	2		
Malacostraca				
Myadacea				
<u>Mysis mixta</u>	<u>Phocanema decipiens</u>	2	Myers	1960
<u>Mysis raticulata</u>	<u>Phocanema decipiens</u>	2	Myers	1960
<u>Mysis</u> sp.	<u>Phocanema decipiens</u>	2	Myers	1960
<u>Mysis stenolepsis</u>	<u>Phocanema decipiens</u>	2	McClelland	1977
<u>Mysis stenolepsis</u>	<u>Phocanema decipiens</u>	2	Myers	1960
Isopoda				
<u>Isaera albitrons</u>	<u>Thynnascaris aduncum</u>		Valter	1968
Amphipoda				
<u>Ampelisca</u>	<u>Phocanema decipiens</u>	2	Myers	1960
Amphipod unidentified	<u>Contracaecum micropapillatum</u>	2,3	Semenova	1972
Amphipod unidentified	<u>Thynnascaris bidentatum</u>	M.G.	Geller and Babich	1953
Cumacea				
<u>Gammarus lawrencianus</u>	<u>Phocanema decipiens</u>	2	Myers	1960
<u>Gammarus</u> sp.	<u>Phocanema decipiens</u>	2	McClelland	1977
<u>Gammarus</u> sp.	<u>Contracaecum micropapillatum</u>	2,3	Semenova	1972
<u>Gammarus</u> sp.	<u>Phocanema decipiens</u>	2	Myers	1960

Table 2. Experimental Invertebrate Intermediate Hosts of Anisakine Nematodes (Continued)

Host	Parasite	Stage	Authority	Date
<u>Rachotropis aculeata</u>	<u>Phocanema decipiens</u>	2	Myers	1960
<u>Unicola sp</u>	<u>Phocanema decipiens</u>	2	McClelland	1977
<b>Euphausiacea</b>				
<u>Euphausia pacifica</u>	<u>Anisakis simplex</u>	2	Oshima <u>et al.</u>	1968
<u>Euphausia similis</u>	<u>Anisakis simplex</u>	2	Oshima <u>et al.</u>	1968
<b>Insecta</b>				
<b>Diptera</b>				
<u>Aedes communis</u>	<u>Raphidascaris acus</u>	3	Yakalovich	1975
<b>Chironomidae</b>				
<u>Chironomus sp.</u>	<u>Raphidascaris acus</u>	2,3	Engasher	1964
<u>Chironomus sp.</u>	<u>Contracaecum microcephalum</u>		Mozgovi. <u>et al.</u>	1968
<u>Chironomus spp.</u>	<u>Contracaecum micropapillatum</u>	2	Semenova	1973
<u>Culicoides</u>	<u>Raphidascaris acus</u>	2,3	Engasher	1964
<u>Pelopia sp.</u>	<u>Raphidascaris acus</u>	2	Engasher	1964
<u>Pelopia sp.</u>	<u>Raphidascaris acus</u>	2	Engasher	1965
<u>Procladius</u>	<u>Raphidascaris acus</u>	2,3	Engasher	1965
<b>Neuroptera</b>				
<u>Sialis</u>	<u>Raphidascaris acus</u>		Engasher	1965
<b>Odonata</b>				
<u>Agrion</u>	<u>Contracaecum microcephalum</u>	N.G.	Mozgovi <u>et al.</u>	1968
<u>Agrion</u>	<u>Contracaecum micropapillatum</u>	N.G.	Semenova	1973
<u>Agrion</u>	<u>Contracaecum spasskii</u>	N.G.	Mozgovi <u>et al.</u>	1965
<u>Agrion</u>	<u>Contracaecum spiculigerum</u>	N.G.	Mozgovi <u>et al.</u>	1965
<u>Anax</u>	<u>Contracaecum microcephalum</u>	N.G.	Mozgovi <u>et al.</u>	1968
<b>Coenagrion</b>				
<u>Coenagrion</u>	<u>Contracaecum microcephalum</u>		Mozgovi <u>et al.</u>	1968
<u>Coenagrion</u>	<u>Contracaecum micropapillatum</u>	3	Semenova	1973
<u>Coenagrion</u>	<u>Contracaecum spasskii</u>	N.G.	Mozgovi <u>et al.</u>	1965
<u>Coenagrion</u>	<u>Contracaecum spiculigerum</u>	N.G.	Mozgovi <u>et al.</u>	1965
<u>Damselfly</u>	<u>Raphidascaris canadensis</u>	N.G.	Thomas	1940
<u>Dragon fly</u>	<u>Contracaecum microcephalum</u>	N.G.	Mozgovi <u>et al.</u>	1965
<u>Dragon fly</u>	<u>Raphidascaris canadensis</u>	N.G.	Thomas	1940

TABLE 2. Experimental Invertebrate Intermediate Hosts of Anisakine Nematodes (Continued)

Host	Parasite	Stage	Authority	Date
Odonata	<u>Contracaecum micropapillatum</u>	2	Semenova	1972
Coleoptera <u>Berosus</u> sp.	<u>Raphidascaris acua</u>	2	Engashev	1965

\*N.G. = No stage given by author. L = larval.

Table 3

Variance in Temperature Tolerance of Anisakis eggs

°C	DAYS TO HATCHING	
	Low T <sup>o</sup> Strain	High T <sup>o</sup> Strain
2	34	nd
7	14	nd
17	5	11
27	nd	3
37	nd	nd

nd = no development or hatching

Compiled from Oshima (1972)

TABLE 4

The In Vitro Growth Of Phocanema decipiens

	0	1	2	4	6	8	12	18	26	36	52
WEEKS:											
Total Length	140	190	412	977	2,380	3,780	6,270	11,300	15,700	21,900	30,100
Esophagus Length	37	44	87	213	395	536	705	929	1,170	1,410	1,960
Ventriculus Length	15	23	48	103	203	283	390	522	629	778	1,050
Caecum Length					43	74	129	202	270	335	702
Maximum Diameter	14	19	29	35	70	93	149	244	354	475	632
Genital* Primorida Position	0.58	0.64	0.64	0.65	0.64	0.61	0.58	0.57	0.52	0.50	0.52
	0.58	0.64	0.64	0.65	0.64	0.61	0.58	0.57	0.52	0.58	0.57

Adapted from McClelland (1970). ALL MEASUREMENTS IN MICROMETERS. \* Distance from anterior end as ratio of total length.

TABLE 5

The In Vitro Growth Of Contracecum osculatum

WEEKS:	0	1	2	4	6	10	16	22
Total Length	382.	542.	901.	1,399.	2,020.	2,980.	4,180.	6,000.
Esophagus Length	77.	105.	160.	231.	376.	507.	579.	722.
Ventriculus Length	13.	16.	24.	34.	50.	63.	81.	80.
Appendix Length Vent.	70.	81.	140.	224.	351.	506.	589.	693.
Caecum Length			15.	79.	168.	231.	283.	387.
Maximum Diameter	19.	26.	41.	62.	91.	148.	187.	243.
Genital* Primorida Position	0.61	0.59	0.59	0.59	0.62	0.62	0.57	0.52

Adapted from McClelland (1970). ALL MEASUREMENTS IN MICROMETERS. \* Distance from anterior end as ratio of total length.

TABLE 6. Some Positive Histochemical Reactions of Phocanema decipiens Cuticle

Histochemical Tests	Larvae from Cod Muscle			Cuticle formed in vitro at 35 C			
	Cortex <sup>1</sup>	Matrix	Fiber Layer	Basal Lamella	Cortex	Matrix	Basal Lamella
PAS <sup>2</sup>	±	±	+	±	±	±	±
Hyaluronidase	FAST	FAST	FAST	FAST	FAST	FAST	FAST
Hg BPP <sup>3</sup>	Blue	Blue/red	FAST	Red	Blue	Blue/red	Red
DMAB-Nitrile <sup>4</sup>	-/+	-	-	-	-/+	=	-
Sakagucho <sup>5</sup>	++/+	++	++	++	+	++	+
Van Gieson <sup>6</sup>	+	++	+	++	+	++	+
Collagenase	Fast/Labile	Labile	Labile	Labile	Fast/Labile	Labile	Labile
DDD <sup>7</sup>	+++/>++	++	+	++	+++	+++	++
PFAB <sup>8</sup> -Keritization	+++/>++	+	+	+	++/>+	+	+
Osmim fixation	++/>+++	+	++	+	++/>+	+	+
Sudan Black B	++	+	++	+	+	+	+
Pyr. Ext. Sudan Black B <sup>9</sup>	++	+	++	+	+	+	+

1. The symbols to the left of the / indicate outer cortex; those to the right inner cortex where the reactions are different. 2. Periodic acid Schiff Reaction - the conclusion of Kan and Davey from these results & other carbohydrate tests is that only the matrix of the fiber layer contains any appreciable carbohydrate. 3. Mercury brom phenol blue is a general protein stain. 4. Dimethylaminobenzaldehyde-nitrate method detects tryptophan. 5. A specific test for arginine. 6. A specific test for -SH groups. 7. Dihydroxy-dinaphthyl-disulfide a specific test for the presence of S-S bonds. 8. Pyridine extracted Sudan Black B - a confirmative test for lipids. 9. Pyridine extracted Sudan Black B - confirmative test for lipids. - = negative, + = weakly reactive, ++ = more reactive, +++ = highly reactive. Adapted from Davey and Kan, 1968.

TABLE 7

## Heating Anisakine Larvae

°C	MAXIMUM LARVAL SURVIVAL	GENUS	REPORTER
60	1 sec.	<u>Anisakis</u>	Kawada (1968)
55	10 sec.		Houwing (1969)
50	10 sec.	<u>Anisakis</u>	Van Thiel <u>et al.</u> (1960)
45	78 min.		Kawada (1968)
			Khalil, (1969)
40	57 hrs.	<u>Phocanema</u>	Ronald (1960)
45	30 min.		
50	10 min.		
60	1 min.		

TABLE 8.

## Heating Fish With Anisakine Larvae

°C	MAXIMUM LARVAL SURVIVAL	GENUS	REPORTER
50	10 sec.	<u>Anisakis</u>	Van Thiel <u>et al.</u> , (1960)
55	10 sec.		Houwing, (1969)
40	57 hrs.	<u>Phocanema</u>	Khalil, (1969)
45	30 min.		Ronald, (1960)
50	10 min.		
60	1 min.		

TABLE 9

Anisakis-Type Larvae In Fried Fish Fingers

INITIAL °C	FINAL °C	TIME	NUMBER OF LARVAE		
			DEAD	SLUGGISH	ACTIVE
-13	55	4 min	10	0	0
-10	55	5	3	3	4
-7	55	4.25	7	3	
4	>60	5.45	10	0	

Adapted from J.T. Davey (1972)

TABLE 10

## FREEZING FISH WITH ANISAKINE LARVAE

°C	MAXIMUM LARVAL SURVIVAL	GENUS	REPORTER
-5	144 hrs.		
-10	288	<u>Anisakis</u>	Gustafson, (1953)
-17	10		
-5	96		
-10	17	<u>Phocanema</u>	Ronald, (1960)
-20	16.5		
-20	52	<u>Contracecum</u>	Bier (1976)



Addendum to Table 11.

<u>Scientific Name</u>	<u>Common Name</u>
<u>Paralichthys dentatus</u>	summer flounder
<u>Alosa aestivalis</u>	blueback herring
<u>Alosa sapidissima</u>	American shad
<u>Clupea harengus harengus</u>	Atlantic herring
<u>Gadus morhua</u>	Atlantic cod
<u>Melanogrammus aeglefinus</u>	haddock
<u>Urophycis regius</u>	spotted hake
<u>Ictalurus catus</u>	white catfish
<u>Ictalurus punctatus</u>	channel catfish
<u>Lutjanus campechanus</u>	red snapper
<u>Mugil cephalus</u>	striped mullet
<u>Morone americana</u>	white perch
<u>Morone saxatilis</u>	striped bass
<u>Perca flavescens</u>	yellow perch
<u>Pomatomus saltatrix</u>	bluefish
<u>Cynoscion regalis</u>	weakfish
<u>Leiostomus xanthurus</u>	spot
<u>Micropogon undulatus</u>	Atlantic croaker
<u>Sciaenops ocellata</u>	red drum
<u>Scomber scombrus</u>	Atlantic mackerel
<u>Centropristis striata</u>	black sea bass
<u>Stenotomus chrysops</u>	scup
<u>Peprilus triacanthus</u>	butterfish

TABLE 12. General Characteristics of Anisakine Nematodes\*

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- 1) 3 lips
  - a) no lip ornaments known
  - b) may have interlabia
- 2) esophagus in 2 or more distinct parts
  - a) part 1: anterior muscular tube
  - b) part 2: posterior glandular sack, the "ventriculus"
  - c) ventricular appendices: 0 - several
- 3) excretory system
  - a) the gland is a band, tube or both but not a normal Renette cell
  - b) excretory pore opens at base of subventral lips or on ventral surface just posterior to nerve ring
- 4) female genital opening in anterior half of body
- 5) host requirements
  - a) larval anisakines probably require an invertebrate as intermediate host (unlike their "terrestrial" ascarid relatives)
  - b) a second intermediate host, invertebrate or vertebrate, may be required
  - c) additional paratenic or transport hosts are often involved in the life cycle

\*Emendation of Baylis (8)

TABLE 13. Morphological Differentiation of the Anisakine Nematodes <sup>a</sup>

Genera	Mouth		Neck Modifications behind Lips	Body Alae	Excretory System		Digestive Appendix Ventriculus		
	Interlabia	Dentigerous Ridges			Pore	Nerve Ring		Gland Band Tube	
Anisakis	-	+	-	-	+	-	+	-	-
Viverranisakis	+	+	?	?	?	?	?	?	-
Paranisakis	+	-	-	-	-	+	?	?	-
Ichthyanisakis	+	-	+	+	?	?	?	?	-
Belanisakis	+	-	-	+	?	?	?	?	-
Heligmus	?	?	?	?	?	?	?	?	-
Acanthocheilus	-	+	-	-	-	+	?	?	-
Pseudanisakis	-	+	-	-	-	+	L	R	-
Paranisakiopsis	+	+	-	<u>+</u>	+	-	+	-	-
Paradujardinia	+	-	-	-	-	+	?	?	-
Dujardinascaris	+	+	-	<u>+</u>	-	+	?	?	-
Phocanema	-	+	-	-	+	-	+	-	-
Terranova	-	+	-	+	+	-	+	-	-
Sulcascaris	+	+	-	-	+	-	L	R	-
Pseudoterranova	-	+	-	?	-	+	?	?	-
Porrocaecum	+	+	-	<u>+</u>	-	+	-	+	-
Alfascaris	-	?	-	+	-	+	?	?	-
Cloeoascaris	-	+	+	?	?	?	+	-	+
Pelicanascaris	+	+	+	-	+	-	?	?	+
Phocascaris	-	+	-	?	+	-	+	-	+
Contraecum	+	-	-	<u>+</u>	+	-	+	-	+
Lappetascaris	-	-	+	-	-	+	?	?	+
Goezia	-	-	-	-	-	+	-	+	+
Pulchrascaris	-	+	-	-	?	?	?	?	?
Thynnascaris	+	+	-	<u>+</u>	-	+	-	+	+
Heterotyphlum	-	-	-	?	-	+	?	?	+
Raphidascaris	-	-	-	+	-	+	-	+	+
Raphidascaroides	+	<u>+</u>	-	?	-	+	?	?	+
Multicaecum	+	?	-	?	-	+	?	?	4
Polycacum	-	?	+	?	-	+	?	?	5

of the Anisakine Nematodes <sup>a</sup>

Neck Modifications behind Lips	Body Alae	Lip Base	Excretory System			Digestive System Appendages		Reproductive System		Female Vulva; anterior
			Pore	Nerve Ring	Gland Band	Tube	Ventriculus	Intestine	Male Spicules	
-	-	+	-	+	-	-	-	2	-	+
?	?	?	?	?	?	-	-	2	-	+
-	-	-	+	?	?	-	-	2	+	+
+	+	?	?	?	?	-	-	2	-	+
-	+	?	?	?	?	-	-	2	+	+
?	?	?	?	?	?	-	-	1	-	+
-	-	-	+	?	?	-	-	2	-	+
-	-	-	+	L	R	-	-	2	-	+
-	+	+	-	+	-	-	-	2	-	+
-	-	-	+	?	?	-	+	2	-	+
-	+	-	+	?	?	-	+	2	+	+
-	-	+	-	+	-	-	+	2	-	+
-	+	+	-	+	-	-	+	2	+	+
-	-	+	-	L	R	-	+	2	-	+
-	?	-	+	?	?	-	+	2	+	+
-	+	-	+	-	+	-	+	2	+	+
-	+	-	+	?	?	-	+	2	-	+
+	?	?	?	+	-	+	+	2	-	+
+	-	+	-	?	?	+	+	2	-	+
-	?	+	-	+	-	+	+	2	-	+
-	+	+	-	+	-	+	+	2	-	+
+	-	-	+	?	?	+	+	2	-	+
-	-	-	+	-	+	+	+	2	-	+
-	-	?	?	?	?	?	+	2	-	+
-	+	-	+	-	+	+	+	2	-	+
-	?	-	+	?	?	+	+	2	-	+
-	?	-	+	?	?	4	+	2	+	+
+	?	-	+	?	?	5	+	?	?	+

Addendum to Table 13: Anisakine Nematodes Authors and Definitive Hosts

Genera	Definitive Host
Anisakis (Dujardin, 1845; Davey, 1969)	Marine mammals
Viverranisakis (Soota and Chaturvedi, 1971) <sup>a</sup>	Land mammals
Paranisakis (Baylis, 1923; Myers, 1975)	Elasmobranchs
Ichthyanisakis (Genre, 1928; Myers, 1975)	Fish
Belanisakis (Maplestone, 1930; Myers, 1975)	Birds
Heligmus (Dujardin, 1845; Myers, 1975)	Fish
Acanthocheilus (Molin, 1858; Gibson, 1973) <sup>b</sup>	Sharks
Pseudanisakis (Layman and Barokova, 1926; Gibson, 1973) <sup>b</sup>	Skates & Rays
Paranisakiopsis (Yamaguti, 1941) <sup>c</sup>	Macrourid fish
Paradujardinia (Travassos, 1933; Myers, 1975)	Sirens
Dujardinascaris (Baylis, 1947; Myers, 1975)	Reptiles
Phocanema (Myers, 1960, 1975)	Marine mammals
Terranova (Leiper and Atkinson, 1914; Hartwich, 1957)	Elasmobranchs & Crocodiles
Sulcascaris (Hartwich, 1957; Lichtenfels et al., 1978)	Marine turtles
Pseudoterranova (Mozgovoï, 1950; Myers, 1975)	Marine mammals
Porrocaecum (Railliet and Henry, 1914; Myers, 1975)	Fish, Birds & Rodents
Aliascaris (Kalyankar, 1971) <sup>d</sup>	Marine fish
Cloeoscaris (Baylis, 1923; Hartwich, 1957)	Otter

Paradujardinia (Travassos, 1933; Myers, 1975)	Sirens
Dujardinascaris (Baylis, 1947; Myers, 1975)	Reptiles
Phocanema (Myers, 1960, 1975)	Marine mammals
Terranova (Leiper and Atkinson, 1914; Hartwich, 1957)	Elasmobranchs & Crocodiles
Sulcascaris (Hartwich, 1957; Lichtenfels et al., 1978)	Marine turtles
Pseudoterranova (Mozgovoi, 1950; Myers, 1975)	Marine mammals
Porrocaecum (Railliet and Henry, 1914; Myers, 1975)	Fish, Birds & Rodents
Aliascaris (Kalyankar, 1971) <sup>d</sup>	Marine fish
Cloeoascaris (Baylis, 1923; Hartwich, 1957)	Otter
Pelicanascaris (Ali and Farooqui, 1970) <sup>e</sup>	Birds
Phocascaris (Host, 1932) <sup>f</sup>	Marine mammals
Contraecum (Railliet and Henry, 1912; Hartwich, 1957) <sup>g</sup>	Birds, and marine mammals
Lappetascaris (Rasheed, 1965)	Fish
Goezia (Zeder, 1800; Hartwich, 1957)	Fish, (?) reptiles
Pulchrascaris (Vincente and Santos, 1972)	Fish
Thynnascaris (Dollfus, 1933; Hartwich, 1957)	Fish, (?) prawn (42)
Heterotyphlum (Spaul, 1927; Myers, 1975) <sup>h</sup>	Fish, (?) sea snakes
Raphidascaris (Railliet and Henry, 1915; Hartwich, 1957)	Fish
Raphidascaroides (Yamaguti, 1941; Myers, 1975)	Fish
Multicaecum (Baylis, 1923; Myers, 1975)	Reptiles
Polycaecum (Maplestone, 1930; Myers, 1975)	Reptiles

Legend Table 13.

<sup>a</sup>Definitions: Interlabia (raised structures between lips); dentigerous ridges (raised structures on lips' inner surface); modifications behind lips (vertical or horizontal cuticular structures, but not cuticular folds as described for Phocanema sp.); alae (longitudinal cuticular wings); excretory pore (surface opening of excretory system); excretory gland (tube type gland is associated with lateral cords and only the nuclear region extends into pseudocoelom; band type gland fills ventral pseudocoelom and contains a tubule plus a large nucleus); ventricular appendage (solid projection of posterior esophagus); intestinal appendage or caecum (anterior projection of the intestine with lumen, ending blindly at a ligment to body wall); spicule (movable accessory male structure, normally paired); gubernaculum (immobile accessory male structure), vulva (surface opening of female genitalia).

<sup>b</sup>Viverranisakis is the only genus reported exclusively from land mammals (civet cat, mongoose). Acanthocheilus can be distinguished by its round ventriculus and "small number of distinct teeth" from Pseudanisakis which has an oblong or oval ventriculus and "one to two complete rings of denticles on a vellum surrounding the mouth." Givson, 1973. Metanisakis Mozgovoy, 1950 is a synonym of Pseudanisakis Hartwich, 1957. Bier Lichtenfels and Campbell are preparing a manuscript which describes the excretory system of Paranisakiopsis as shown in this table. Aliascaris is distinguished from other anisakine genera by cordons and "trilobed papilla-like"

structures in longitudinal rows (Kalyankar, 1971). Pelicanascaris lacks a ventriculus according to the text of Ali and Furooqi, 1970 but not according to their illustration. Phocascaris not as redefined by Berland, 1963. The genus Cerascaris Cobb, 1929 is rejected for Contraecaecum because the major distinguishing characteristic is a larval feature, the boring tooth. The subgenus Acollaris is also rejected because the genus Thynnascaris has priority for these distinguishing characteristics. Heterotyphlum is distinguished from Paraheterotyphlum Johnston and Mawson, 1948 only by spicule proportions and host. Hortwich, 1957 synonymizes these genera but Schmidt and Kahtz, 1973 resurrected Paraheterotyphlum. The original author of the genus and a reference to a recent redescription follow the generic names. Symbols are as follows: +(existence of a feature); -(absence of a feature); (+) conflicting reports of existence of a feature); ? not mentioned; L(exists on left side); R (exists on right side); 1 (only one of a pair reported); 2 (pair reported; 4 (2 anterior and 2 posterior; 5 (2 anterior and 3 posterior).

TABLE 14. Nematode Infection Rates of Principal\* Fish In  
The Washington, D.C. Area Market Survey

infected fish	%	nematodes per examined fish	
		No.	
spotted hake	95	12.4	spotted hake
striped bass	74	8.9	summer flounder
blueback herring	72	7.3	Atlantic croaker
weakfish	71	6.9	yellow perch
black sea bass	67	4.8	blueback herring
Atlantic croaker	66	4.6	black sea bass
summer flounder	63	3.4	striped bass
yellow perch	46	3.3	weakfish
white perch	18	0.4	white perch

\* a minimum no. of 25 fish were examined.

TABLE 15. Hematology Test And Units Of Measurements

Test	Units
Hemoglobin	g hemoglobin/100 ml whole blood
Hematocrit	% packed red cells in whole blood
Red Blood Cell Count	red blood cells/ml whole blood
White Blood Cell Count	white blood cells/ml whole blood
Differential Count	
Neutrophils	number/100 white blood cells
Lymphocytes	number/100 white blood cells
Eosinophils	number/100 white blood cells
Basophils	number/100 white blood cells

TABLE 16. Serum Chemistry Tests, Abbreviations And Units Of Measurements

Calcium	Ca	mg/100 ml
Phosphorus	P	mg/100 ml
Blood Urea Nitrogen	BUN	mg/100 ml
Uric Acid	UA	mg/100 ml
Glucose	Glucose	mg/100 ml
Cholesterol	Cho	mg/100 ml
Total Protein	TP	g/100 ml
Albumin	Alb	g/100 ml
Bilirubin	Br	mg/100 ml
Alkaline Phosphatase	AP	mU/ml
Lactate Dehydrogenase	Ldh	mU/ml
Serum Glutamate Oxaloacetic Transaminase	SGOT	mU/ml
Amylase	Amy	Somogyi units/ml
Lipase	Lip	Cherry-Crandall Units/ml

1. International milliunit equals 1 micromole product formed per minute per milliliter serum multiplied by 1000.

TABLE 17. Synopsis Of Infection Data On Pigs Fed Phocanema decipiens Larvae

Pig No.	Dose	Days	Location	Free	Attached	Clinical Test	Other Parasites	Gross Pathology
9332	100							Intubated necropsy.
8062	100	1	Fundus	0	49	yes		Local test
			Cardiac	0	1			hemorrhage
			Duodenum	0	1			<u>P. decipiens</u>
			Large Intestine	0	1			
8777	100	1	Fundus	0	29	yes		Local test
			Ileum	1	0			diameter 0
8222	100	1	Fundus	0	8	yes		Local test
			Duodenum	1	4			
			Large Intestine	5	2			
8849	90	1	Fundus	0	14	no	<u>Ascaris suum</u>	Local test
			Large Intestine	2	1			
7573	100	1	Fundus	2	10	no	<u>Ascaris suum</u> <u>Trichuris suis</u>	Local test
								hemorrhage
								larval <u>P.</u>
8942	100	2	Fundus	0	5	no		Local test
								by a 12 mm
9335	100	2	Fundus	0	49	no		Lesion at
			Cardiac	0	6			
7683	100	3		0	0	no		Hemorrhage
8061	100	4	Fundus	0	23	yes		Local test
			Large Intestine	0	1			abdominal
								Hemorrhage
								points of

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decipiens Larvae

Clinical Other Parasites  
Test

Gross Pathology

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		Intubated in lungs. Pig died and was incinerated without necropsy.
yes		Local lesions at penetration sites - ca. 5mm. diameter hemorrhage visible through serosa-corresponding to larval <u>P. decipiens</u> .
yes		Local lesions at penetration sites-fundus with 2 ca. 5mm. diameter ulcer with nematodes.
yes		Local lesion at penetration sites.
no	<u>Ascaris suum</u>	Local lesions at penetration sites.
no	<u>Ascaris suum</u> <u>Trichuris suis</u>	Local lesions at penetrations sites-ca. 4mm. diameter hemorrhage visible through serosa corresponding to larval <u>P. decipiens</u> attachment.
no		Local lesions at penetration sites. 1 worm surrounded by a 12 mm. diameter zone of edema.
no		Lesion at penetration sites. Ulcer.
no		Hemorrhagic area in fundus.
yes		Local lesions at penetration sites. Serous fluid in abdominal cavity. Nematodes on mesenteries unencapsulated. Hemorrhagic area in duodenum ca. 150 mm. below attachment points of <u>P. decipiens</u> larvae .

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7623	100	10		0	0	no	<u>Ascaris suum</u>	No gross
9343	100	10	Fundus	0	5			Local 1 brown 1
214	100	11	Fundus	1	22	no	<u>Ascaris suum</u>	2 local of 3-4-
256	100	11	Fundus	0	2	no	<u>Ascaris suum</u>	Local 1 ulcers
8814	100	11	Fundus	0	6	yes		Local 1 bleeding
8931	100	0	Fundus	0	0	yes		Irregular
32	100	14	Fundus	0	5	no	<u>Ascaris suum</u>	Local 1 ulcers of fundus. 6
203	100	14	Fundus	0	4	no	<u>Ascaris suum</u>	Local 1 edema ad
9331	100	14	Fundus	0	4	no		Larvae f healed f
8941	100	15	Fundus	0	0	yes	<u>Ascaris suum</u>	No gross
8913	100	15		0	0	yes		Partially
31	100	17		0	0	no	<u>Ascaris suum</u> <u>Trichuris suis</u>	18 white
9664	100	17		0	0	no	<u>Ascaris suum</u> <u>Metastrongylis apris</u>	Partially
9701	100	17		0	0	no	<u>Ascaris suum</u>	Partially
9723	100	17		0	0	no	<u>Ascaris suum</u>	Partially
9985	100	18	Stomach	0	1	no	<u>Ascaris suum</u>	45 white

no	<u>Ascaris suum</u>	No gross pathology observed.  Local lesions at penetration sites. Fundus with a brown layer between mucosae and submucosae.
no	<u>Ascaris suum</u>	2 local lesions on individual penetration sites. 6 clusters of 3-4- nematodes in uclers.
no	<u>Ascaris suum</u>	Local lesions around individual penetration sites. 3 small ulcers in cardiac region of stomach.
yes		Local lesion at penetration site 2 and 3 large nematodes in bleeding ulcers. 1 bleeding ulcer without nematodes.
yes		Irregular 5 mm diameter ulcer in fundus.
no	<u>Ascaris suum</u>	Local lesions at 3 penetration sites- 2 larvae in bleeding ulcers ca. 17 and 20 mm. diameter 7 additional ulcers in fundus. Generalized edema in fundus.
no	<u>Ascaris suum</u>	Local lesions at penetration sites. 8 ulcers in fundus, edema adjacent to ulcers.
no		Larvae in cluster in 7 X 6 mm. ulcer. 6 ulcers partially healed in fundus.
yes	<u>Ascaris suum</u>	No gross pathology observed.
yes		Partially healed ulcer on fundus.
no	<u>Ascaris suum</u> <u>Trichuris suis</u>	18 white bumps on fundus.
no	<u>Ascaris suum</u> <u>Metastrongylis apris</u>	Partially healed ulcer on fundus.
no	<u>Ascaris suum</u>	Partially healed ulcer on fundus.
no	<u>Ascaris suum</u>	Partially healed ulcer on fundus
no	<u>Ascaris suum</u>	45 white bumps.

8061	100	4	Fundus Large Intestine	0 0	23 1	yes		Local les slight ed
8875	100	4	Fundus Duodenum	0 3	13 3	no		Local les
8852	100	4	Fundus Mesenteries Duodenum	0 4 0	8 0 3	no		Local les abdominal Hemorrhag points of
8891	100	4	Fundus	0	3	no	<u>Metastrongylis apri</u>	Local les with tiss
7681	100	5	Fundus	0	1	no	<u>Ascarops strongylina</u> <u>Ascaris suum</u>	Bleeding 2 white n
7682	100	5		0	0	no	<u>Ascarops strongylina</u> <u>Ascaris suum</u>	No gross
9314	100	6	Fundus Cardiac	9	15			Local
39	100	7	Fundus	0	39	no	<u>Ascaris suum</u> <u>Trichuris suis</u>	Local les worms slig
8082	100	7	Fundus	0	33	yes		Local les clusters of cluster of
8847	100	8	Fundus	0	6	no		Local les
8811	100	8	Fundus	0	29	no		Local les fundus.
8926	100	8		0	0	no	<u>Ascaris suum</u>	Bleeding u
9984	100	8	Fundus	0	19	no	<u>Ascaris suum</u>	Local les of 5, 5, 6
33	100	9	Fundus	0	2	no	<u>Ascaris suum</u>	Larvae in fundus, 4

yes		Local lesions at penetration sites. Hemorrhage and slight edema in fundus.
no		Local lesions at penetration sites.
no		Local lesions at penetration sites. Serous fluid in abdominal cavity. Nematodes on mesenteries unencapsulated. Hemorrhagic area in duodenum ca. 150 mm. below attachment points of <u>P. decipiens</u> larvae.
no	<u>Metastrongylis apri</u>	Local lesions at penetration sites. Lungs hemorrhagic and with tissue condensation around <u>Metastrongylus apri</u>
no	<u>Ascarops strongylina</u> <u>Ascaris suum</u>	Bleeding ulcer surrounding 15 X 12 mm. around <u>P. decipiens</u> 2 white nodules 3 mm. diameter on lungs.
no	<u>Ascarops strongylina</u> <u>Ascaris suum</u>	No gross pathology observed.
		Local
no	<u>Ascaris suum</u> <u>Trichuris suis</u>	Local lesions at penetration sites. 5 clusters of 2 and 3 worms slightly edema of entire fundus.
yes		Local lesions at penetration sites of individual larva. clusters of 15 larvae in 9 X 16 mm. bleeding ulcer cluster of 10 larvae in 10 X 12 bleeding ulcer.
no		Local lesions at penetration sites.
no		Local lesions at penetration sites. Slight edema in entire fundus.
no	<u>Ascaris suum</u>	Bleeding ulcers ca. 2 mm. diameter on fundus.
no	<u>Ascaris suum</u>	Local lesions at penetration sites of 2 larvae. Clusters of 5, 5, 6 larvae in ulcers. 7 ulcers without nematodes.
no	<u>Ascaris suum</u>	Larvae in ucler about 1 cm in diameter 35 other ulcers on fundus, 4 bleeding.

314	100	18	Fundus	0	2	no	<u>Ascaris suum</u>	Local te ulcers o
9644	100	18		0	0	no	<u>Ascaris suum</u>	No gross
9702	100	18		0	0	no	<u>Ascaris suum</u>	2 pearly
9721	100	18		0	0	no		2 pearly

no	<u>Ascaris suum</u>	Local lesions at penetration sites. 18 white bumps and ulcers on fundus.
no	<u>Ascaris suum</u>	No gross pathology observed.
no	<u>Ascaris suum</u>	2 pearly spots on liver.
no		2 pearly spots on liver.

TABLE 18. Hematology Data From Pigs Fed Phocanema decipiens

Pig No.	Date	Hemo-globin	Hemato-crite	Red Blood Cell Count	White Blood Cell Count	Neutro-philis	Lympho-cytes	Mono-cytes	Eosinophilis	Baso-philis
8061	4/25/75	11.4	43	7,000,000	22,000	34	62	4	0	0
8062	4/25/75	15.4	46	7,900,000	27,600	26	66	8	0	0
8075	4/25/75	13.7	40	7,000,000	22,000	38	60	2	0	0
8081	4/25/75	15.2	45	6,900,000	23,400	26	70	4	0	0
8082	4/25/75	15.6	47	7,600,000	20,700	40	54	4	2	0
8061	4/28/75	-	-	-	-	-	-	-	-	-
8062	4/28/75	15.0	43	7,700,000	26,000	23	75	0	2	0
8075	4/28/75	13.2	39	6,900,000	22,000	45	53	2	0	0
8081	4/28/75	14.7	43	6,900,000	24,000	20	72	1	7	0
8082	4/28/75	14.3	42	7,100,000	21,000	37	60	0	3	0
8061	4/29/75	13.9	43	6,900,000	23,400	30	66	0	4	0
8062	4/29/75	15.0	44	7,600,000	28,600	45	53	0	2	0
8075	4/29/75	13.3	40	6,800,000	22,500	30	54	0	16	0
8081	4/29/75	14.1	41	6,400,000	25,400	18	66	2	14	0
8082	4/29/75	14.2	42	7,000,000	27,200	29	50	2	19	0
8061	5/2/75	13.7	41	6,800,000	23,200	32	58	5	5	0
8075	5/2/75	13.9	41	7,400,000	25,500	36	56	4	4	0
8081	5/2/75	13.9	42	6,800,000	28,400	26	64	4	6	0
8082	5/2/75	14.6	44	7,500,000	26,500	26	65	8	1	0
8075	5/5/76	13.6	41	7,500,000	18,500	34	56	3	7	0

8081	5/5/75	14.0	42	6,600,000	23,600	16	80	2	2	0
8082	5/5/75	14.5	44	7,500,000	26,000	46	50	2	2	0
8075	5/12/75	13.7	41	7,400,000	25,000	44	40	8	7	1
8081	5/12/75	13.7	41	6,800,000	19,000	26	69	4	1	0
8075	5/19/75	15.1	43	7,600,000	20,100	48	45	3	4	0
8081	5/19/75	14.7	44	7,200,000	28,400	24	73	3	0	0
8081	6/9/75	13.9	42	6,700,000	25,200	24	73	3	0	0

TABLE 19. Clinical Chemistry Data From Pigs Fed Phocanema decipiens

Pig No	Date	Ca	P	Bun	U A	Glu	Cho	TP	Alb	Br	A P	L Dh	S G T	Amy	Lip
8061	4/25/75	11.4	9.8	5.0	1.0	110	90	7.0	3.5	0.2	139	370	51	914	0.2
8062	4/25/75	10.8	10.2	3.0	1.0	95	80	7.0	2.9	0.1	105	410	67	1,205	0.2
8075	4/25/75	10.2	9.2	6.0	1.1	106	80	6.9	2.3	0.2	186	493	63	1,118	0.2
8081	4/25/75	10.6	8.9	5.0	1.0	112	81	6.8	3.1	0.2	148	451	75	1,188	0.1
8082	4/25/75	10.4	8.9	5.0	1.0	140	110	6.7	2.7	0.2	139	385	66	1,120	0.2
8061	4/28/75	12.1	10.5	9.0	0.2	167	30	7.3	2.8	0.1	133	440	48	890	0.4
8062	4/28/75	11.2	9.9	8.0	0.2	125	25	6.8	3.0	0.1	107	390	59	1,725	0.2
8075	4/28/75	11.3	9.8	10.5	0.2	150	3	7.0	2.1	0.1	175	520	58	1,810	0.1
8081	4/28/75	11.2	8.2	10.0	0.1	150	72	7.0	3.1	0.2	146	430	70	780	0.1
8082	4/28/75	10.8	9.4	9.0	0.1	125	-44	7.0	2.5	0.1	122	400	53	1,795	0.1
8061	4/29/75	11.0	9.5	6.0	0.2	114	320	7.0	3.0	0.2	140	445	51	3,136	0.3
8062	4/29/75	10.4	7.3	10.0	0.1	131	235	7.2	2.6	0.1	164	420	73	4,989	0.4
8075	4/29/75	10.3	8.5	7.0	0.2	105	180	7.4	2.3	0.2	167	575	78	4,195	0.2
8081	4/29/75	11.3	8.6	6.0	0.1	122	170	7.1	3.1	0.1	144	595	78	3,136	0.2
8082	4/29/75	10.4	7.8	5.0	0.2	95	196	6.5	2.8	0.2	114	455	66	4,205	0.2
8061	4/29/75	11.8	10.8	12.0	0.3	132	157	6.7	3.2	0.3	130	615	62	1,953	0.4
8075	4/29/75	10.6	11.4	13.0	0.2	106	99	6.6	2.3	0.2	142	750	92	3,280	0.4
8081	4/29/75	10.7	10.5	8.0	0.2	110	107	6.7	2.6	0.2	135	690	78	1,963	0.4
8082	4/29/75	10.2	10.5	7.0	0.2	105	100	6.8	2.3	0.2	101	609	77	3,318	0.4

8842	4/23/76	17.8	51	9,500,000	17,600	25	70	3	2	0
8848	4/23/76	16.9	49	8,500,000	16,600	24	60	0	16	0
8846	4/27/76	16.7	50	8,400,000	11,400	31	65	0	4	0
8842	4/27/76	17.2	52	9,400,000	13,600	25	63	0	2	0
8848	4/27/76	17.2	51	8,900,000	16,200	30	62	0	8	0
<hr/>										
8846	4/29/76	16.7	52	8,800,000	13,300	40	55	0	5	0
8842	4/29/76	17.5	56	10,000,000	13,900	45	53	0	2	0
8848	4/29/76	16.6	52	9,200,000	18,700	27	50	0	23	0
8846	5/3/76	16.0	49	8,100,000	13,700	21	70	8	1	0
8842	5/3/76	16.5	51	9,600,000	16,900	41	52	6	1	0
8848	5/3/76	16.8	52	9,000,000	17,400	24	60	9	7	0

Line indicates exposure to 100 Anisakis sp. larvae.

TABLE 20. Hematology Data On Pigs Fed *Phocanema decipiens* larvae

Pig No.	Date	Hemo-globin	Hemato-crit	Red Blood Cell Count	White Blood Cell Count	Neutro-phils	Lympho-cytes	Mono-cytes	Eosino-phils	Baso-phils
8814	1/27/76	15.9	45	7,600,000	15,300	17	74	3	6	0
8812	1/27/76	-	-	-	-	-	-	-	-	-
8814	1/30/76	16.0	46	7,500,000	22,800	42	54	4	0	0
8812	1/30/76	15.2	45	7,500,000	22,000	33	65	2	0	0
8814	2/5/76	16.5	50	8,400,000	20,800	38	60	0	2	0
8812	2/5/76	15.3	47	8,000,000	25,900	31	65	0	4	0
8814	2/10/76	13.4	41	7,200,000	18,300	24	71	2	3	1
8812	2/10/76	15.0	46	8,000,000	19,400	43	55	1	1	0
8814	2/13/76	12.5	37	6,200,000	22,900	25	70	0	5	0
8812	2/13/76	13.7	43	7,200,000	20,400	29	67	0	4	0
8814	2/17/76	12.3	39	6,200,000	28,900	34	63	0	3	0
8812	2/17/76	13.8	43	7,500,000	26,300	38	60	0	2	0
8814	2/20/76	13.3	41	6,600,000	30,100	15	85	0	0	0
8812	2/20/76	-	-	-	-	-	-	-	-	-
8814	2/23/76	14.3	43	7,100,000	26,000	16	80	2	2	0
8812	2/23/76	15.9	48	8,000,000	18,400	23	69	6	2	0
8814	2/26/76	15.1	45	7,100,000	27,700	32	59	9	0	0
8812	2/26/76	14.5	44	7,100,000	19,700	59	32	8	1	0
8814	2/27/76	14.1	42	6,700,000	18,300	31	64	4	1	0
8812	2/27/76	15.9	47	7,800,000	17,700	38	52	8	2	0

8814	3/1/76	14.9	44	7,000,000	22,100	24	67	7	2	0
8812	3/1/76	15.5	45	7,500,000	15,900	22	69	8	1	0
8814	3/5/76	15.7	46	7,000,000	19,800	34	61	5	0	0
8812	3/5/76	16.4	50	8,200,000	16,000	34	53	7	6	0
8812	3/9/76	17.0	52	8,600,000	21,700	27	67	1	5	0
8812	3/12/76	16.6	49	8,100,000	24,800	35	57	0	8	0

Line indicates exposure to 100 Phocanema decipiens larvae.

TABLE 21. Clinical Chemistry Data On Pigs Fed Phocanema decipiens larvae

Pig No.	Date	Ca	P	BUN	U A	Glu	Cho	T P	Alb	Br	A P	L Dh	S G O T	Amy	Lip
8814	1/27/76	11.9	7.8	9.9	0.7	85	125	7.0	3.3	0.2	138	210	78	222	0.2
8812	1/27/76	13.0	7.3	5.2	0.2	115	105	6.4	2.8	0.1	111	618	75	171	0.2
8814	1/30/76	11.0	8.4	10.2	0.3	96	90	6.9	3.1	0.1	160	520	52	2,864	0.3
8812	1/30/76	11.7	9.9	7.0	0.2	94	85	6.7	3.1	0.1	135	545	55	2,500	0.2
8814	2/5/76	10.6	7.5	13.8	0.1	127	113	6.7	2.8	0.1	146	460	47	4,333	0.2
8812	2/5/76	11.0	7.6	9.0	0.5	135	90	6.4	3.1	0.2	123	440	40	3,325	0.1
8814	2/10/76	10.5	9.5	14.5	0.1	125	104	6.7	2.5	0.1	120	460	65	4,105	0.1
8812	2/10/76	11.3	11.1	11.0	0.4	135	100	7.3	3.3	0.1	115	480	53	3,425	0.1
8814	2/13/76	10.8	9.9	12.5	0.1	140	111	6.4	2.5	0.1	125	525	30	2,940	0.1
8812	2/13/76	11.6	10.8	8.0	0.1	145	140	6.6	2.5	0.1	145	460	45	3,315	0.1
8814	2/17/76	10.9	10.2	11.5	0.2	115	85	6.5	2.6	0.1	125	570	82	5,185	0.1
8812	2/17/76	10.5	9.9	10.6	0.6	122	90	6.4	3.0	0.2	131	384	58	5,220	-
8814	2/20/76	12.8	10.8	19.0	0.5	135	112	6.5	2.6	0.2	185	440	65	5,585	0.1
8812	2/20/76	12.6	11.4	19.0	0.1	150	100	6.8	2.6	0.1	180	415	57	5,190	0
8814	2/23/76	11.7	9.3	9.7	0.1	130	95	6.9	2.6	0.1	172	460	51	5,210	0.1
8812	2/23/76	11.5	10.3	9.6	0.1	132	105	7.1	3.1	0.1	190	535	59	4,808	0.1
8814	2/26/86	10.7	11.0	12.4	0.2	125	96	6.4	2.8	0.1	183	1,030	81	5,075	0.1
8812	2/26/76	11.0	10.5	11.0	0.3	133	115	6.3	2.6	0.2	154	575	77	3,127	0.1
8814	2/27/76	11.3	9.0	14.0	0.7	125	108	6.7	2.8	0.2	165	473	77	4,335	0.1

8812	2/27/76	11.7	9.6	11.4	0.3	120	129	7.0	2.8	0.2	145	487	69	4,100	0.1
8814	3/1/76	12.6	9.0	17.4	0.2	125	128	6.8	2.6	0.2	200	420	50	2,755	0.1
8812	3/1/76	12.4	9.9	12.3	0.2	140	135	7.2	2.8	0.2	139	430	54	1,560	0.1
8814	3/5/76	11.7	9.5	14.0	0.1	125	77	6.5	2.8	0.1	-177	355	47	3,835	0.1
8812	3/5/76	11.4	9.4	11.0	0.4	120	71	7.3	3.3	0.2	129	380	47	2,650	0.1
8812	3/9/76	12.3	10.1	9.5	0.2	121	69	7.8	3.1	0.2	120	474	49	1,659	0.2
8812	3/12/76	11.5	9.5	8.1	0.2	129	61	7.4	2.9	0.2	114	490	49	4,611	0.2

Line indicates exposure to 100 Phocanema decipiens larvae.

TABLE 22. Hematology Data On Pigs Fed Phocanema decipiens Larvae

Pig No.	Date	Hemo globin	Hemo crit	RBC	WBC	Neut	Lymp	Mono	Eos	Bas	Total Eosin
8931	5/18/76	13.7	44	7,900,000	17,500	38	60	0	2	0	340
8941	5/18/76	15.5	47	8,300,000	14,800	35	63	0	2	0	280
8913	5/18/76	16.5	52	9,900,000	18,800	27	69	4	0	0	720
8931	5/21/76	13.2	39	7,500,000	19,800	38	61	1	0	0	50
8941	5/21/76	15.1	42	7,800,000	18,200	29	69	1	1	0	175
8913	5/21/76	16.4	46	9,200,000	19,300	32	65	3	0	0	180
8931	5/24/76	12.4	40	7,600,000	20,100	39	51	10	0	0	130
8941	5/24/76	14.1	43	7,900,000	18,900	30	62	5	3	0	530
8913	5/24/76	15.3	50	10,000,000	21,700	25	69	5	1	0	220
8931	5/25/76	12.5	37	7,000,000	19,900	35	61	2	2	0	400
8941	5/25/76	13.9	39	7,200,000	18,500	28	70	0	4	0	770
8913	5/25/76	15.8	46	9,200,000	19,800	32	65	1	2	0	380
8931	5/28/76	12.5	37	6,900,000	15,700	43	55	0	2	0	40
8941	5/28/76	15.0	43	7,700,000	14,600	23	70	0	7	0	100
8913	5/28/76	16.3	46	9,200,000	17,100	37	60	0	3	0	60
8931	6/1/76	13.5	38	7,300,000	14,600	29	70	0	1	0	150
8941	6/1/76	15.4	43	7,800,000	16,300	37	56	0	7	0	1100
8913	6/1/76	17.1	49	9,200,000	16,500	29	65	5	1	0	155
8931	6/4/76	13.0	39	7,200,000	18,400	37	60	3	0	0	55
8941	6/4/76	15.2	44	7,500,000	15,700	39	55	6	0	0	100
8913	6/4/76	16.2	47	8,800,000	14,400	41	55	4	0	0	85
8941	6/8/76	14.6	41	7,200,000	16,300	30	60	10	0	0	1600
8913	6/8/76	16.4	48	8,600,000	18,500	33	65	0	2	0	400

Line indicates exposure to 100 Phocanema decipiens larvae.

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TABLE 23. Clinical Chemistry Data From Pigs Fed Phocanema decipiens Larvae

Pig No.	Date	Ca	P	Bun	UA	Glu	Cho	TP	Alb	Br	AP	LDH	SGOT	Amy
8931	5/18/76	11.9	8.4	12.0	0.5	100	65	7.1	2.6	0.1	90	470	60	4620
8941	5/18/76	12.0	8.4	6.5	4.0	100	76	6.7	2.6	0.1	75	430	63	6040
8913	5/18/76	12.8	8.4	13.0	0.5	108	80	7.2	2.6	0.1	77	525	50	6742
8931	5/21/76	10.6	7.2	10.0	0.1	105	100	7.0	2.7	0.1	90	320	61	4935
8941	5/21/76	11.0	7.7	8.0	0.3	115	175	6.6	3.1	0.1	65	290	62	2445
8913	5/21/76	11.1	7.2	9.0	0.1	105	83	7.0	3.0	0.2	71	400	88	5095
8931	5/24/76	11.0	8.3	9.0	0.1	95	85	7.1	2.6	0.1	90	490	50	5445
8941	5/24/76	11.2	9.0	0.6	0.1	90	75	7.0	2.7	0.1	60	420	48	3770
8913	5/24/76	12.0	8.4	10.0	0.1	115	70	7.2	2.7	0.1	75	495	57	5425
8931	5/25/76	11.4	7.9	11.3	0.2	110	101	7.5	2.7	0.2	102	570	76	5810
8941	5/25/76	11.8	8.2	8.9	0.3	103	96	7.1	2.3	0.1	65	467	67	4116
8913	5/25/76	12.5	8.5	10.1	0.1	105	83	7.4	2.7	0.1	83	660	90	6795
8931	5/28/76	11.4	7.9	11.0	0.3	120	70	7.1	2.5	0.1	85	395	55	8540
8941	5/28/76	11.3	7.6	7.0	0.4	115	65	6.8	3.0	0.2	62	400	82	3245
8913	5/28/76	13.0	8.1	12.0	0.3	125	55	7.6	3.0	0.1	76	490	58	4335
8931	6/1/76	11.4	7.2	11.0	0.1	111	82	7.0	2.8	0.1	90	413	57	5609
8941	6/1/76	11.3	7.6	7.0	0.2	100	68	7.1	3.1	0.2	67	374	55	6320
8913	6/1/76	11.6	7.6	9.5	0.1	110	45	7.0	2.9	0.2	75	460	63	5639
8931	6/4/76	11.2	8.5	9.5	0.1	98	95	7.3	2.8	0.2	99	417	47	4502
8941	6/4/76	11.3	8.3	7.8	0.1	90	85	7.2	3.1	0.1	70	380	59	3170
8913	6/4/76	11.5	7.8	12.2	0.1	112	58	7.2	2.8	0.2	82	473	64	3748
8941	6/8/76	11.7	8.4	6.0	0.8	100	62	6.8	2.9	0.1	67	305	45	3560
8913	6/8/76	12.7	8.6	9.5	0.7	118	49	7.3	3.0	0.1	85	420	47	5222

Line indicates exposure to 100 Phocanema decipiens larvae.

TABLE 24. Synopsis of Infection Data On Pigs Fed Anisakis sp. Larvae

Pig No	Dose	Days P.E.	Location	Free	Attached	Clinical Test	Other Parasites	Gross Pathology
8063	100	1	Fundus Cardiac Ileum Large Intestine	3 2	48 1	yes		Local lesions at penetration sites & hemorrhagic paths in stomach.
8851	100	1	Fundus Large Intestine	3 2	4	no	<u>Ascaris suum</u>	Local lesions at penetration sites.
7535	25	2	Fundus		5	no		Local lesions at penetration sites.
8361	100	3	Fundus	4	51	no		Local lesions at penetration sites.
7693	200	4	Fundus Ileum	4 3	99 3	no		Local lesions at penetration sites. Generalized edema involving entire fundus, average thickness 2.6 cm.
7245	100	4	Fundus	15	30	no		Local lesions at penetration sites. Generalized edema involving entire fundus, average thickness 2.6 cm.
8322	100	4	Fundus Mesenteries Duodenum		7 3 3	no		Local lesions at penetration sites
8072	100	4	Fundus Cardiac		35 13	yes		Local lesions at penetration sites. Generalized edema & hemorrhage of fundus. Fundus surface had "blood blister" 5 X 3 X 3 mm. oval.
8821	100	6				no		No pathology observed.
8842	100	6				yes		No pathology observed.
8846	100	6	Jejunum	1		yes	<u>Ascaris suum</u>	Fundus with white nodules and some edema.
8848	100	6				yes	<u>Ascaris suum</u>	No pathology observed.
8365	100	6	Fundus	1		yes		Local lesions at penetration sites.

8074	100	7	yes	Fibrosis of submucosa $\neq$ .
8323	100	7	yes	Serous fluid in body cavity. 7 X 9 X 3 mm. bleeding ulcer on fundus containing pus.
8362	100	7	yes	Ulcer.
8364	100	7	yes	4 ulcers each ca. 3 mm. diameter with pus.
8366	100	7	yes	No pathology observed.
8392	100	7	yes	No pathology observed.
6742	100	7	no	Stomach edematous, average thickness 2.7 cm., with 110 1-2-mm. ulcers on fundus, 15 on pyloric and 35 on cardiac region.
6657	100	18	no	2 ulcers on fundus and gross edema 2.5 cm. average thickness.
8073	100	21	yes	No pathology observed in intestinal tract. 3 spots on liver.
8079	100	42	yes	Serous fluid in abdominal cavity, 2 2mm. diameter hemorrhagic spots on cardiac portion of stomach.
6638	100	52	no	4 small ulcers on lesser curvature of stomach - edema fundus, average thickness 1.5 cm.

TABLE 25. Hematology Data From Pigs Fed *Anisakis* sp. Larvae

Pig No.	Date	Hemo-globin	Hemato-crit	Red Blood Cell Count	White Blood Cell Count	Neutro-phils	Lympho-cytes	Mono-cytes	Eosino-phils	Baso-phils
8063	4/25/75	13.7	40	7,000,000	20,800	40	54	4	2	0
8072	4/25/75	18.0	46	7,600,000	26,200	48	46	6	0	0
8073	4/25/75	14.0	42	7,000,000	28,000	44	42	6	8	0
8074	4/25/75	14.9	43	7,500,000	26,900	40	52	4	4	0
8079	4/25/75	14.6	42	6,900,000	24,000	48	43	6	3	0
8063	4/28/75	13.4	39	7,000,000	20,400	23	70	0	7	0
8072	4/28/75	14.8	42	7,300,000	24,000	49	45	2	4	0
8073	4/28/75	13.7	40	6,900,000	25,800	38	48	3	11	0
8074	4/28/76	14.4	42	7,500,000	20,700	40	57	0	3	0
8079	4/28/75	13.6	39	6,600,000	22,600	32	60	1	7	0
8063	4/29/75	12.7	38	6,700,000	25,200	41	47	0	12	0
8072	4/29/75	14.8	44	7,500,000	26,300	45	52	2	1	0
8073	4/29/75	13.4	40	6,800,000	27,400	36	46	0	18	0
8074	4/29/75	13.9	40	7,000,000	23,300	56	41	0	3	0
8079	4/29/75	13.1	38	6,300,000	22,700	46	44	0	10	0
8072	5/2/75	16.1	46	8,100,000	25,900	36	52	8	4	0
8073	5/2/75	13.9	41	7,100,000	27,900	36	50	2	12	0
8074	5/2/75	13.1	39	7,100,000	23,900	41	53	4	2	0
8079	5/2/75	12.5	38	6,200,000	25,000	48	43	4	5	0
8073	5/2/75	13.2	40	7,000,000	24,300	44	50	3	3	0

8074	5/2/75	13.5	40	7,200,000	22,100	37	43	3	17	0
8079	5/2/75	13.9	41	6,700,000	22,300	49	47	1	3	0
8073	5/12/75	13.9	41	7,200,000	21,200	40	51	5	4	0
8079	5/12/75	14.5	42	7,200,000	21,200	37	58	5	0	0
8073	5/19/75	16.8	46	8,000,000	28,800	33	59	5	3	0
8079	5/19/75	15.5	47	7,600,000	21,800	42	53	5	0	0
8079	6/7.75	-	-	-	-	-	-	-	-	-

Line indicates exposure to 100 Anisakis sp. larvae.

TABLE 26. Clinical Chemistry Data From Pigs Fed Anisakis sp. Larvae

Pig No.	Date	Ca	P	Bun	U A	Glucose	Cholesterol	T P	Alb	Br	A P	L Dh	S G O T	Amy	Lip
8063	4/25/75	10.3	9.2	7.0	1.0	108	100	6.5	2.4	0.1	150	425	76	968	0.2
8072	4/25/75	11.0	10.2	7.0	1.0	130	115	6.4	2.6	0.1	140	380	57	1,170	0.2
8073	4/25/75	10.5	8.3	6.0	1.0	115	72	6.8	2.6	0.	106	354	56	961	0.1
8074	4/25/75	10.2	9.3	5.1	1.0	99	75	6.3	2.6	0.1	151	406	70	1,125	0.2
8079	4/25/75	10.2	8.3	8.0	1.1	150	86	6.6	2.7	0.2	131	430	61	950	0.2
8063	4/28/75	10.3	9.8	12.0	0.1	145	0	6.5	3.0	0.1	144	440	62	429	0.2
8072	4/28/75	11.6	11.1	10.5	0.1	145	0	6.4	2.5	0.1	142	395	38	1,075	0.2
8073	4/28/75	10.8	9.4	9.0	0.2	160	10	7.1	2.6	0.1	95	400	43	1,500	0.2
8074	4/28/75	11.2	9.4	10.0	0.1	155	3	6.6	2.5	0.1	148	435	55	755	0.1
8079	4/28/75	10.8	9.6	9.5	0.1	122	55	6.8	2.3	0.1	117	575	52	1,824	0.1
8063	4/29/75	10.3	8.1	7.0	0.1	117	145	6.7	2.2	0.1	126	420	67	2,753	0.3
8072	4/29/75	11.0	10.5	6.0	0.2	117	142	6.9	2.8	0.1	155	450	60	3,233	0.2
8073	4/29/75	10.3	9.3	8.5	0.2	97	170	7.1	2.6	0.1	106	470	66	3,263	0.3
8074	4/29/75	10.6	9.4	5.0	0.2	120	170	6.8	2.5	0.2	158	490	66	4,180	0.8
8079	4/29/75	10.4	7.9	7.5	0.3	114	210	6.7	2.6	0.2	120	535	63	3,746	0.2
8072	5/2/75	10.8	12.0	9.0	0.3	105	115	7.1	2.7	0.2	155	705	67	1,725	0.4
8073	5/2/75	10.5	10.8	8.5	0.3	95	150	7.0	2.6	0.3	130	720	79	2,462	1.3
8074	5/2/75	10.5	10.5	11.0	0.3	92	152	7.1	2.1	0.2	180	780	90	2,382	0.4
8079	5/2/75	10.3	10.5	11.0	0.3	95	109	6.2	2.0	0.2	134	780	87	3,126	0.3

8073	5/5/75	10.6	8.1	8.0	0.5	93	75	6.9	3.0	0.3	127	485	70	1,287	0.6
8074	5/5/75	11.1	8.3	11.5	0.4	100	70	7.0	2.3	0.2	154	600	65	1,150	0.2
8079	5/5/75	10.8	8.3	11.0	0.3	105	100	6.6	2.3	0.2	155	575	75	1,375	0.2
8073	5/12/75	10.0	9.7	12.0	0.2	125	60	6.5	2.5	0.2	110	450	68	2,245	0.1
8079	5/12/75	10.1	10.0	11.0	0.5	110	110	5.9	2.3	0.2	175	500	64	2,845	0.1
8073	5/19/76	10.7	10.0	11.0	0.9	90	85	6.7	2.8	0.1	120	385	69	2,600	1.0
8079	5/19/76	11.1	10.5	10.0	0.5	94	106	6.1	3.0	0.1	226	406	70	2,406	0.1
8079	6/7/75	10.0	10.8	6.0	0.3	100	85	6.2	2.1	0.2	192	478	84	2,320	0.2

Line indicates exposure to 100 Phocanema decipiens larvae.

TABLE 27. Hematology Data From Pigs Fed Anisakis sp. larvae

Pig	Date	Hemo- globin	Hemato- crit	Red Blood Cell Count	White Blood Cell Count	Neutro- phils	Lympho- cytes	Mono- cytes	Eosino- phils	Baso- phils
8323	9/19/75	13.1	38	7,000,000	26,200	33	65	1	1	0
8362	9/19/75	13.8	38	7,000,000	3,200	35	57	8	0	0
8364	9/19/75	11.9	34	6,200,000	27,000	23	71	6	0	0
8365	9/19/75	-	-	-	-	-	-	-	-	-
8366	9/19/75	11.3	33	6,400,000	23,400	33	66	0	1	0
8392	9/19/75	13.6	39	7,300,000	11,700	42	56	2	0	0
8323	9/26/75	14.5	41	7,500,000	20,500	22	76	2	0	0
8362	9/26/75	12.3	34	6,000,000	26,300	28	67	2	3	0
8364	9/26/75	12.9	37	6,500,000	28,800	38	58	2	2	0
8365	9/26/75	12.4	36	6,600,000	30,000	16	78	6	0	0
8366	9/26/75	11.8	34	6,200,000	17,700	46	51	2	1	0
8392	9/26/75	13.6	42	7,900,000	10,800	36	59	5	0	0
8323	9/30/75	14.7	41	7,900,000	21,400	30	67	3	0	0
8362	9/30/75	14.6	41	7,300,000	20,300	16	77	3	4	0
8364	9/30/75	14.7	41	7,400,000	25,200	17	81	2	0	0
8365	9/30/75	13.8	39	7,500,000	22,800	22	75	3	0	0
8366	9/30/75	12.8	36	6,700,000	22,000	25	68	4	3	0
8392	9/30/75	14.9	41	7,900,000	13,200	19	76	5	0	0
8323	10/2/75	14.8	43	8,000,000	19,400	31	62	4	3	0

8362	10/2/75	13.5	38	6,700,000	19,600	42	54	4	0	0
8364	10/2/75	14.9	42	7,400,000	22,600	32	62	5	1	0
8366	10/2/75	12.6	36	6,500,000	21,900	19	77	4	0	0
8392	10/2/75	14.0	39	7,500,000	15,100	18	71	9	2	0
8323	10/3/75	14.0	40	7,800,000	19,900	57	40	3	0	0
8362	10/3/75	12.0	33	6,000,000	23,700	54	40	6	0	0
8364	10/3/75	12.8	38	6,900,000	24,500	46	42	2	10	0
8366	10/3/75	11.7	34	6,200,000	30,700	50	43	2	5	0
8392	10/3/75	12.8	38	6,900,000	19,000	40	40	20	0	0

TABLE 28. Clinical Chemistry Data from Pigs Fed Anisakis sp. larvae.

Pig No.	Date	Ca	P	BUN	U A	Glu	Cho	T P	Alb	Br	A P	L Dh	S G O T	Amy	Lip
8323	9/19/75	10.6	9.5	11.5	0.5	127	127	5.8	2.1	0.2	175	350	58	2,503	0
8362	9/19/75	10.3	8.6	13.0	0.3	107	68	5.9	1.6	0.1	136	365	58	2,453	0
8364	9/19/75	10.6	9.6	12.0	0.3	158	98	6.0	1.7	0.1	203	480	77	2,992	0
8365	9/19/75	10.7	9.2	10.5	0.3	163	93	5.5	1.7	0.1	192	455	70	2,527	0
8366	9/19/75	10.4	9.3	10.0	0.3	165	78	5.8	1.7	0.1	156	371	70	2,465	0
8329	9/19/75	11.0	10.5	8.0	0.2	164	75	5.7	2.1	0.1	150	362	43	1,766	0
8323	9/23/75	10.8	9.2	8.0	0.2	150	103	6.1	2.2	0.1	170	350	64	2,000	0.3
8362	9/23/75	11.7	11.4	9.0	0.2	210	90	6.0	1.9	0.1	155	380	90	1,330	0.3
8364	9/23/75	10.9	9.4	12.0	0.2	95	134	6.4	2.9	0.1	220	360	19	2,055	0.3
8365	9/23/75	10.8	9.7	7.0	0.2	150	125	5.8	2.2	0.1	242	450	85	1,610	0.2
8362	9/23/75	9.7	9.7	9.0	0.1	138	85	5.5	2.1	0.1	172	363	75	1,072	0.2
8392	9/23/75	10.6	9.4	7.0	0.1	95	82	6.4	2.9	0.1	115	367	67	1,705	0.3
8323	9/30/75	10.3	8.7	7.0	0.3	115	66	5.8	1.3	0.3	148	316	60	3,335	0.1
8362	9/30/75	11.4	9.9	13.0	0.4	115	116	6.7	1.3	0.2	150	440	55	2,645	0.2
8364	9/30/75	11.0	3.8	12.0	0.3	105	138	7.1	1.5	0.2	202	490	73	5,305	4.5
8365	9/30/75	10.9	3.4	7.0	0.2	130	135	6.1	1.4	0.2	220	490	47	2,560	0.2

8366	9/30/75	10.6	9.1	8.0	0.2	105	75	5.8	1.2	0.2	134	575	95	2,955	0.1
8392	9/30/75	10.3	9.4	11.0	0.3	94	135	6.9	1.8	0.5	105	440	70	2,656	0.1
8323	10/2/75	10.9	10.2	11.0	0.3	160	105	5.8	1.9	0.1	160	358	55	2,900	0.1
8362	10/2/75	10.9	9.6	11.0	0.3	95	60	5.7	1.9	0.1	155	245	57	2,595	0.1
8364	10/2/75	11.8	9.3	14.0	0.5	110	94	6.7	2.6	0.2	210	280	67	4,730	0.2
8366	10/2/75	10.2	9.3	11.0	0.2	140	80	6.0	2.1	0.1	148	235	107	2,490	0.1
8392	10/2/75	10.5	8.2	7.0	0.1	105	68	6.0	2.2	0.1	95	445	67	2,315	0.1
8323	10/3/75	11.3	9.1	12.5	0.3	140	105	5.6	2.2	0.1	145	370	55	3,920	0.2
8362	10/3/75	10.5	8.3	11.0	0.3	110	72	5.8	2.2	0.2	136	425	65	2,520	0.3
8364	10/30/75	11.1	8.2	10.0	0.4	99	80	6.2	2.4	0.1	187	438	79	4,741	0.1
8366	10/30/75	10.7	8.7	10.0	0.3	145	90	5.9	2.2	0.1	138	555	95	2,725	0.2
8392	10/30/75	10.5	8.2	8.0	0.4	105	96	6.0	2.4	0.2	185	450	79	2,455	0.1

Line indicates exposure to 100 Anisakis sp. larvae.

TABLE 29. Hematology Data on Pigs Fed Anisakis sp. Larvae

Pig No.	Date	Hemo-globin	Hemato-crit	Red Blood Cell Count	White Blood Cell Count	Neutro-phils	Lumpho-cytes	Mono-cytes	Eosino-phils	Baso-phils
8846	1/27/76	-	-	-	-	-	-	-	-	-
8846	1/30/76	13.7	40	6,900,000	12,500	21	79	0	0	0
8846	2/5/76	14.0	42	7,500,000	15,000	28	70	0	2	0
8842	2/5/76	14.8	45	8,200,000	20,300	40	58	0	2	0
8846	2/10/76	13.2	43	7,500,000	13,500	35	63	1	1	0
8842	2/10/76	13.1	42	7,900,000	17,100	44	55	0	1	0
8848	2/10/76	12.6	40	7,300,000	14,800	15	84	1	0	0
8846	2/13/76	12.5	41	7,100,000	13,600	32	63	2	3	0
8842	2/13/76	12.7	41	7,500,000	14,700	33	67	0	0	0
8848	2/13/76	12.2	37	6,400,000						
8846	2/17/76	13.0	41	6,900,000	14,200	46	54	0	0	0
8842	2/17/76	12.8	40	7,000,000	17,700	35	60	0	5	0
8848	2/17/76	12.2		6,000,000	14,000	38	60	0	2	0
8846	2/20/76	13.2	41	6,600,000	14,000	32	65	0	3	0
8842	2/20/76	13.2	41	7,300,000	13,500	38	60	0	2	0
8848	2/20/76	13.5	42	6,700,000	17,400	29	66	0	5	0
8846	2/23/76	13.8	42	6,800,000	12,900	29	63	6	2	0
8842	2/23/76	13.9	42	7,400,000	12,700	31	65	4	0	0
8848	2/23/76	13.7	41	6,600,000						
8846	2/26/76	13.7	42	7,200,000	15,500	29	63	8	0	0
8842	2/26/76	15.4	47	8,200,000	16,200	42	54	4	0	0

8848	2/17/76	12.8	40	7,000,000	17,700	35	60	0	5	0
8848	2/17/76	12.2		6,000,000	14,000	38	60	0	2	0
8846	2/20/76	13.2	41	6,600,000	14,000	32	65	0	3	0
8842	2/20/76	13.2	41	7,300,000	13,500	38	60	0	2	0
8848	2/20/76	13.5	42	6,700,000	17,400	29	66	0	5	0
8846	2/23/76	13.8	42	6,800,000	12,900	29	63	6	2	0
8842	2/23/76	13.9	42	7,400,000	12,700	31	65	4	0	0
8848	2/23/76	13.7	41	6,600,000						
8846	2/26/76	13.7	42	7,200,000	15,500	29	63	8	0	0
8842	2/26/76	15.4	47	8,200,000	16,200	42	54	4	0	0
8846	2/27/76	14.8	44	7,200,000	12,900	44	47	8	1	0
8842	2/27/76	13.8	42	7,400,000	10,600	41	56	3	0	0
8842	2/27/76	14.1	42	7,000,000	12,300	27	62	10	1	0
8846	3/1/76	15.7	46	-7,500,000	12,200	31	66	2	1	0
8842	3/1/76	14.6	43	7,600,000	12,800	37	55	6	2	0
8848	3/1/76	14.4	42	3,200,000	12,600	21	75	3	1	0
8846	3/5/76	15.2	45	7,300,000	15,900	38	53	8	1	0
8842	3/5/76	15.6	46	8,200,000	14,100	38	56	6	0	0
8848	3/6/76	13.8	41	6,900,000	15,000	33	63	2	2	0
8846	3/9/76	15.6	46	7,700,000	14,000	31	68	0	1	0
8842	3/9/76	15.5	49	8,700,000	13,100	50	48	2	0	0
8848	3/9/76	14.7	45	7,400,000	12,000	23	47	0	0	0
8846	3/12/76	15.6	45	7,500,000	15,700	33	65	0	2	0
8842	3/12/76	15.8	48	8,500,000	13,700	28	68	0	4	0
8848	3/12/76	14.6	43	7,100,000	14,300	32	65	0	3	0

8846	3/19/76	16.7	53	8,600,000	12,200	24	72	3	1	0
8842	3/19/76	16.8	55	9,500,000	15,800	28	63	7	2	0
8848	3/17/76	16.5	51	8,400,000	14,100	29	64	4	3	0
8846	3/23/76	17.1	50	8,200,000	15,900	32	63	0	3	0
8842	3/23/76	17.0	53	9,300,000	11,900	45	55	0	0	0
8848	3/23/76	15.9	48	8,600,000	14,400	31	67	0	2	0
8846	3/26/76	16.2	49	8,100,000	13,100	24	70	5	1	0
8842	3/26/76	15.6	50	8,900,000	12,600	39	58	3	0	0
8848	3/26/76	15.5	49	8,200,000	14,500	38	55	6	1	0
8846	3/30/76	15.9	48	8,000,000	12,700	27	70	2	1	0
8842	3/30/76	16.4	50	8,700,000	13,300	29	70	1	0	0
8848	3/30/76	15.1	46	7,800,000	11,700	20	74	4	2	0
8846	4/12/76	16.3	51	8,600,000	13,200	15	82	1	2	0
8842	4/12/76	15.3	49	8,700,000	14,100	37	61	2	0	0
8848	4/2/76	15.8	47	8,500,000	13,700	29	68	3	1	0
8846	4/6/76	16.6	51	8,400,000	13,100	28	71	1	0	0
8842	3/6/76	17.4	53	9,400,000	12,600	19	78	1	2	0
8848	4/6/76	16.3	50	8,400,000	18,800	17	83	0	0	0
8846	4/9/76	16.7	51	8,700,000	14,500	26	68	5	1	0
8842	4/9/76	17.2	56	10,500,000	15,200	24	67	5	4	0
8848	4/9/76	16.1	50	8,700,000	14,000	21	72	2	5	0
8846	4/13/76	16.7	52	9,000,000	14,000	39	49	8	4	0
8842	4/13/76	16.9	53	9,600,000	14,200	40	60	0	0	0
8848	4/13/76	16.6	51	8,900,000	13,900	26	69	2	3	0

8842	3/26/76	15.6	50	8,900,000	12,600	39	58	3	0	0
8848	3/26/76	15.5	49	8,200,000	14,500	38	55	6	1	0
8846	3/30/76	15.9	48	8,000,000	12,700	27	70	2	1	0
8842	3/30/76	16.4	50	8,700,000	13,300	29	70	1	0	0
8848	3/30/76	15.1	46	7,800,000	11,700	20	74	4	2	0
8846	4/12/76	16.3	51	8,600,000	13,200	15	82	1	2	0
8842	4/12/76	15.3	49	8,700,000	14,100	37	61	2	0	0
8848	4/2/76	15.8	47	8,500,000	13,700	29	68	3	1	0
8846	4/6/76	16.6	51	8,400,000	13,100	28	71	1	0	0
8842	3/6/76	17.4	53	9,400,000	12,600	19	78	1	2	0
8848	4/6/76	16.3	50	8,400,000	18,800	17	83	0	0	0
8846	4/9/76	16.7	51	8,700,000	14,500	26	68	5	1	0
8842	4/9/76	17.2	56	10,500,000	15,200	24	67	5	4	0
8848	4/9/76	16.1	50	8,700,000	14,000	21	72	2	5	0
8846	4/13/76	16.7	52	9,000,000	14,000	39	49	8	4	0
8842	4/13/76	16.9	53	9,600,000	14,200	40	60	0	0	0
8848	4/13/76	16.6	51	8,900,000	13,900	26	69	2	3	0
8846	4/16/76	17.2	53	9,100,000	12,400	30	70	0	0	0
8842	4/16/76	16.6	50	9,100,000	15,300	30	68	0	2	0
8848	7/16/76	16.6	52	8,900,000	16,300	83	60	0	7	0
8846	4/20/76	16.2	46	7,900,000	13,000	30	66	0	4	0
8842	4/20/76	18.0	53	9,900,000	14,400	31	65	0	4	0
8848	4/20/76	16.8	50	8,900,000	15,500	19	69	0	12	0
8846	4/23/76	16.1	47	8,100,000	14,700	15	80	0	5	0

8842	4/23/76	17.8	51	9,500,000	17,600	25	70	3	2	0
8848	4/23/76	16.9	49	8,500,000	16,600	24	60	0	16	0
8846	4/27/76	16.7	50	8,400,000	11,400	31	65	0	4	0
8842	4/27/76	17.2	52	9,400,000	13,600	25	63	0	2	0
8848	4/27/76	17.2	51	8,900,000	16,200	30	62	0	8	0
8846	4/29/76	16.7	52	8,800,000	13,300	40	55	0	5	0
8842	4/29/76	17.5	56	10,000,000	13,900	45	53	0	2	0
8848	4/29/76	16.6	52	9,200,000	18,700	27	50	0	23	0
8846	5/3/76	16.0	49	8,100,000	13,700	21	70	8	1	0
8842	5/3/76	16.5	51	9,600,000	16,900	41	52	6	1	0
8848	5/3/76	16.8	52	9,000,000	17,400	24	60	9	7	0

Line indicates exposure to 100 Anisakis sp. larvae.

TABLE 30. Clinical Chemistry Data On Pigs Fed Anisakis sp. Larvae

Pig No.	Date	Ca	P	Bun	U A	Glu	Cho	T P	Alb	Br	A P	L Dh	S G O T	Amy	Lip
8846	1/27/76	10.4	7.7	6.0	0.2	90	57	6.3	3.0	0.1	103	726	84	147	0.2
8846	1/30/75	9.9	9.4	6.0	0.3	91	110	5.9	3.0	0.2	113	545	62	2,679	0.1
8846	2/5/76	10.4	7.3	9.0	0.1	100	120	5.9	2.8	0.1	87	485	43	4,500	0.1
8842	2/5/76	10.5	7.4	9.0	0.2	110	114	6.4	2.7	0.1	107	500	33	3,250	0.1
8846	2/10/76	10.7	9.9	15.0	0.1	120	95	6.2	2.5	0.1	76	520	60	4,620	0.2
8842	2/10/76	10.8	10.5	12.0	0.1	115	100	6.5	2.5	1.0	117	510	55	3,445	0.2
8848	2/10/76	12.0	10.5	14.0	0.1	137	115	6.3	2.5	0.1	187	550	58	4,903	0.1
8846	2/13/76	10.3	11.7	13.0	0.3	113	115	6.0	1.6	0.2	100	455	55	3,647	0.1
8842	2/13/76	10.5	10.0	9.0	0.1	103	108	6.2	2.4	0.1	127	490	67	2,837	0.1
8848	2/13/76	10.3	10.5	14.0	0.1	120	50	5.8	2.3	0.1	100	470	58	3,040	0.1
8841	2/17/76	10.1	9.6	11.0	0.2	107	90	5.9	2.6	0.1	114	515	77	5,233	0.1
8842	2/17/76	10.2	9.9	8.0	0.2	103	105	6.4	1.6	2.7	141	466	63	4,595	0.1
8848	2/17/76	9.8	10.2	9.4	0.2	100	92	6.0	2.8	0.1	123	488	77	5,240	0.1
8846	2/20/76	11.9	10.2	13.0	0.1	125	80	6.2	2.6	0.1	146	450	67	5,215	0.1
8842	2/20/76	11.2	9.5	13.0	0.1	125	87	6.4	2.5	0.1	187	445	56	5,415	-
8848	2/20/76	11.5	10.8	20.0	0.5	120	89	6.0	2.6	0.1	132	400	65	4,480	0
8846	2/23/75	10.7	8.6	8.2	0.1	116	85	6.4	3.0	0.1	140	505	57	4,824	0
8842	2/23/76	10.5	8.7	6.0	0.1	120	93	6.9	2.6	0.1	170	515	57	4,480	0.1
8848	2/23/76	10.5	9.2	9.0	0.5	116	107	6.3	3.0	0.2	120	480	60	5,224	0.1
8846	2/26/76	11.3	9.7	13.0	0.5	120	90	6.7	2.8	0.2	185	368	50	4,100	0.1
8842	2/26/76	11.7	8.6	0.7	0.6	115	178	6.7	2.8	0.3	155	360	59	3,245	0.1
8846	2/27/76	10.8	8.7	9.0	0.2	100	135	6.5	2.8	0.2	122	478	57	5,540	0.1

8842	2/27/76	10.7	8.9	6.8	0.2	115	90	6.5	2.6	0.1	132	366	57	3,045	0.1
8848	2/27/76	11.1	9.7	12.0	0.2	110	124	6.6	2.6	0.1	109	440	55	4,490	0.1
8846	3/1/76	11.5	9.9	12.0	0.2	125	135	6.7	2.8	0.1	116	525	50	2,630	0.1
8842	3/1/76	11.9	10.2	11.0	0.1	134	115	6.7	2.6	0.1	144	395	43	1,166	0.5
8848	3/1/76	11.0	9.2	12.0	0.5	130	144	6.5	3.1	0.2	113	440	54	2,630	0.5
8846	3/5/76	10.9	9.4	9.0	0.1	105	89	6.4	3.0	0.1	99	460	46	4,495	0.1
8842	3/5/76	11.8	9.7	11.0	0.1	120	85	6.8	3.1	0.1	126	355	41	3,040	0.1
8848	3/5/76	11.1	9.9	15.0	0.1	115	89	5.0	2.8	0.1	91	430	50	4,485	0.1
8846	3/9/76	11.7	9.5	10.1	0.1	115	76	6.6	2.9	0.1	96	489	49	2,155	0.2
8842	3/9/76	11.6	9.3	9.1	0.1	120	60	6.8	2.9	0.1	135	535	54	1,350	0.2
8846	3/12/76	10.6	8.9	10.0	0.4	99	95	6.5	2.9	0.2	95	516	45	4,781	0.1
8842	3/12/76	10.8	8.8	6.0	0.1	115	74	6.7	2.9	0.1	131	501	39	2,185	0.1
8848	3/12/76	11.1	9.3	10.1	0.2	104	85	6.5	2.6	0.1	95	540	42	3,106	0.1
8846	3/19/76	11.0	8.2	9.0	0.1	95	100	6.8	3.0	0.2	90	555	52	4,045	0.2
8842	3/19/76	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1
8848	3/17/76	11.2	8.4	9.0	0.3	100	100	6.8	3.3	0.1	87	505	60	3,740	0.1
8846	3/23/76	12.3	9.3	12.0	0.3	95	102	7.3	3.0	0.2	100	395	48	4,505	0.4
8842	3/23/76	10.7	7.7	16.0	0.2	120	60	6.5	3.0	0.2	120	500	65	2,440	0.2
8848	3/23/76	11.0	9.1	10.0	0.1	105	95	6.3	2.5	0.1	85	490	85	3,250	0.3
8846	3/26/76	9.9	9.1	10.0	0.2	100	109	6.3	3.1	0.2	89	545	55	2,900	0.1
8842	3/26/76	10.4	8.5	0.8	0.1	105	95	6.9	2.9	0.2	98	415	52	1,855	0.1
8848	3/26/76	10.0	9.2	0.8	0.1	100	105	6.6	2.8	0.2	85	479	68	2,400	0.1
8846	3/30/76	11.8	8.3	9.0	0.1	117	83	6.6	3.3	0.2	68	505	47	4,750	0.4
						105	80	6.8	3.1	0.2	100	482	42	4,190	0.2

8846	3/9/76	11.7	9.5	10.1	0.1	115	76	6.6	2.9	0.1	96	489	49	2,155	0.2
8842	3/9/76	11.6	9.3	9.1	0.1	120	60	6.8	2.9	0.1	135	535	54	1,350	0.2
8846	3/12/76	10.6	8.9	10.0	0.4	99	95	6.5	2.9	0.2	95	516	45	4,781	0.1
8842	3/12/76	10.8	8.8	6.0	0.1	115	74	6.7	2.9	0.1	131	501	39	2,185	0.1
8848	3/12/76	11.1	9.3	10.1	0.2	104	85	6.5	2.6	0.1	95	540	42	3,106	0.1
8846	3/19/76	11.0	8.2	9.0	0.1	95	100	6.8	3.0	0.2	90	555	52	4,045	0.2
8842	3/19/76	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1
8848	3/17/76	11.2	8.4	9.0	0.3	100	100	6.8	3.3	0.1	87	505	60	3,740	0.1
8846	3/23/76	12.3	9.3	12.0	0.3	95	102	7.3	3.0	0.2	100	395	48	4,505	0.4
8842	3/23/76	10.7	7.7	16.0	0.2	120	60	6.5	3.0	0.2	120	500	65	2,440	0.2
8848	3/23/76	11.0	9.1	10.0	0.1	105	95	6.3	2.5	0.1	85	490	85	3,250	0.3
8846	3/26/76	9.9	9.1	10.0	0.2	100	109	6.3	3.1	0.2	89	545	55	2,900	0.1
8842	3/26/76	10.4	8.5	0.8	0.1	105	95	6.9	2.9	0.2	98	415	52	1,855	0.1
8848	3/26/76	10.0	9.2	0.8	0.1	100	105	6.6	2.8	0.2	85	479	68	2,400	0.1
8846	3/30/76	11.8	8.3	9.0	0.1	117	83	6.6	3.3	0.2	68	505	47	4,750	0.4
8842	3/30/76	11.0	9.3	6.0	0.1	105	80	6.8	3.1	0.2	100	482	42	4,190	0.2
8848	3/30/76	10.9	9.2	9.9	0.1	90	103	6.6	3.0	0.2	77	545	65	4,930	0.2
8846	4/2/76	11.3	9.2	9.0	0.1	102	100	6.9	2.6	0.1	75	495	54	6,498	0.2
8842	4/2/76	10.8	8.6	5.2	0.1	110	83	6.6	2.6	0.1	103	389	43	3,750	0.1
8848	4/2/76	10.3	8.6	9.2	0.1	97	100	6.6	2.4	0.1	80	480	62	5,240	0.1
8846	4/6/76	11.0	8.4	11.5	0.2	110	88	6.4	2.8	0.1	72	450	53	3,900	0.1
8842	4/6/76	10.9	8.7	7.9	0.2	116	70	6.5	2.8	0.1	95	395	53	2,584	0.1
8848	4/6/76	10.6	9.8	11.1	0.5	102	90	6.8	3.1	0.2	76	410	57	3,158	0.1
8846	4/9/76	10.7	8.8	8.8	0.1	97	91	6.5	2.6	0.1	72	415	59	5,823	0.2

TABLE 31. Hematology Data on Control Pigs

Pig No.	Date	Hemo-globin	Hemato-crite	Red Blood Cell Count	White Blood Cell Count	Neutro-phils	Lympho-cytes	Mono-cytes	Eosino-phils	Baso-phils
8321	9/19/76	11.6	36	6,700,000	25,000	65	27	5	3	0
8391	9/19/76	13.7	39	6,800,000	11,800	20	69	6	5	0
8321	9/26/76	12.0	35	6,500,00	29,000	32	66	1	1	0
8391	9/26/76	13.0	38	6,700,000	9,200	31	67	2	0	0
8321	9/30/76	11.8	34	6,300,000	30,200	39	53	4	4	0
8391	9/30/76	13.1	36	6,500,000	13,600	19	77	1	3	0
8321	10/2/76	12.5	37	6,800,000	23,500	27	71	1	1	0
8391	10/2/76	13.6	38	6,800,000	13,100	33	64	3	0	0
8321	10/3/76	12.3	35	6,700,000	27,200	45	50	0	5	0
8391	10/3/76	11.4	32	5,700,000	15,800	46	50	0	4	0
8321	10/10/76	13.7	40	7,400,000	21,400	18	78	4	0	0
8391	10/10/76	13.5	41	6,800,000	13,700	33	64	3	1	0

TABLE 32. Clinical Chemistry Data on Control Pigs

Pig No	Date	Ca	P	Bun	U A	GLu	Cho	T P	Alb	Br	A P	L Dh	S G O T	Amy	Lfp
8321	9/19/75	9.4	7.8	8.5	0.3	110	60	6.3	1.5	0.1	98	260	50	2,520	0
8391	9/19/75	11.4	10.0	11.5	0.2	150	97	6.0	2.2	0.1	197	440	63	2,600	0.1
8321	9/26/75	10.6	9.7	7.5	0.2	85	90	6.3	1.9	0.1	120	383	104	3,265	0.7
8391	9/26/75	10.9	9.9	11.0	0.1	85	92	6.4	2.7	0.1	147	415	83	2,065	0.3
8321	9/30/75	10.0	8.7	5.0	0.2	75	90	6.2	1.2	0.2	124	470	83	3,985	0.1
8391	9/30/75	10.7	8.6	10.0	0.2	105	95	6.3	1.6	0.2	110	515	89	3,145	0.1
8321	10/2/75	10.8	9.1	9.0	0.2	100	80	6.3	1.9	0.1	150	427	63	3,960	0.1
8391	10/2/75	11.2	9.3	12.0	0.2	139	75	6.2	2.6	0.1	122	485	78	2,590	0.1
8321	10/2/75	10.0	7.9	10.0	0.3	90	80	6.1	2.2	0.1	134	450	77	4,910	0.2
8391	10/3/75	11.2	8.4	14.0	0.3	110	84	4.8	2.6	0.1	110	505	86	2,640	0.1
8321	10/10/75	10.7	8.8	8.0	0.5	125	74	6.5	3.0	0.2	119	390	56	4,075	0.1
8391	10/10/75	11.7	9.5	14.0	0.1	110	79	6.2	3.3	0.2	106	470	65	2,950	0.1

TABLE 33. Hematology Data On Control Pig

Pig No.	Date	Hemo-globin	Hemato-crit	Red Blood Cell Count	White Blood Cell Count	Neutro-phil	Lympho-cytes	Mono-cytes	Eosino-phil	Baso-phil
8873	2/5/76	17.5	54	9,200,000	29,000	27	70	0	3	0
8873	2/10/76	15.4	49	9,400,000	8,100	26	73	0	1	0
8873	2/13/76	13.6	44	8,200,000	20,400	36	60	2	2	0
8873		14.4	44	7,800,000	21,000	22	75	0	3	0
8873	2/20/76	14.7	44	8,100,000	21,400	27	71	0	2	0
8873	2/23/76	15.1	42	7,800,000	21,000	21	73	6	0	0
8873	2/27/76	16.4	50	9,100,000	24,300	28	62	8	2	0
8873	3/1/76	16.4	48	8,900,000	19,400	24	73	3	0	0
8873	3/5/76	16.9	51	9,200,000	22,300	33	60	7	0	0
8873	3/9/76	17.2	53	9,700,000	21,200	32	66	2	0	0
8873	3/12/76	18.1	57	9,000,000	26,500	32	63	0	5	0
8873	3/19/76	18.5	59	11,000,000	22,500	29	61	8	2	0
8873	3/23/76	18.7	59	10,200,000	26,300	24	74	0	2	0
8873	3/26/76	17.8	52	9,300,000	24,900	24	71	3	1	1
8873	3/26/76	17.7	50	9,200,000	22,500	30	64	6	0	0
8873	4/2/76	18.2	59	10,800,000	18,500	24	73	3	0	0
8873	4/6/76	17.4	55	9,800,000	27,700	25	72	1	1	1
8873	4/9/76	18.6	58	11,000,000	23,500	26	67	3	4	0
8873	4/13/76	18.7	59	11,100,000	25,500	22	75	2	1	0

8873	3/23/76	18.7	59	10,200,000	26,300	24	74	0	2	0
8873	3/26/76	17.8	52	9,300,000	24,900	24	71	3	1	1
8873	3/26/76	17.7	50	9,200,000	22,500	30	64	6	0	0
8873	4/2/76	18.2	59	10,800,000	18,500	24	73	3	0	0
8873	4/6/76	17.4	55	9,800,000	27,700	25	72	1	1	1
8873	4/9/76	18.6	58	11,000,000	23,500	26	67	3	4	0
8873	4/13/76	18.7	59	11,100,000	25,500	22	75	2	1	0
8873	4/16/76	18.7	58	10,600,000	22,700	27	70	3	0	0
8873	4/20/76	17.9	54	10,100,000	20,500	20	75	0	5	0
8873	4/23/76	18.7	54	10,800,000	19,200	25	66	3	6	0
8873	4/27/76	19.2	58	9,900,000	19,700	30	68	0	2	0
8873	4/29/76	18.6	59	10,700,000	23,100	32	65	0	3	0
8873	5/3/76	-	-	-	-	-	-	-	-	-

TABLE 34. Clinical Chemistry Data On Control Pigs

Pig No.	Date	Ca	P	Bun	UA	Glu	Cho	IP	Alb	Br	AP	L Dh	SGOT	Amy	Lfp
8873	2/5/76	10.1	7.1		0.4	88	121	6.4	2.5	0.3	175	477	35	4,142	0.2
8873	2/10/76	12.0	10.5	14.0	0.1	137	115	6.3	2.5	0.1	187	550	58	4,903	0.1
8873	2/13/76	11.2	11.7	14.0	0.1	125	120	6.0	2.5	0.1	186	490	48	3,735	0.1
8873	2/17/76	11.4	10.0	12.0	0.2	110	98	6.7	3.0	0.1	165	460	57	5,230	0.1
8873	2/20/76	12.2	10.5	20.0	0.2	130	82	6.2	2.5	0.1	192	192	56	5,410	0.1
8873	2/23/76	11.1	9.1	13.0	0.6	125	92	6.4	3.0	0.1	182	450	47	5,075	0.1
8873	2/27/76	11.7	9.6	15.0	0.2	100	140	6.8	2.8	0.2	175	490	55	5,240	0.1
8873	3/1/76	12.1	9.1	19.2	0.1	122	120	6.8	2.8	0.1	160	460	44	1,658	0.4
8873	3/5/76	12.0	9.5	15.2	0.1	100	85	6.5	3.0	0.1	140	425	40	5,440	0.1
8873	3/9/76	11.9	9.2	10.1	0.1	110	85	6.9	2.9	0.2	140	540	45	2,850	0.2

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TABLE 34. Clinical Chemistry Data On Control Pigs (Continued)

8873	3/12/76	12.6	9.6	10.0	0.4	115	96	7.0	3.1	0.2	146	474	28	4,625	0.2
8873	3/19/76	12.7	8.0	11.0	0.1	90	80	7.3	3.3	0.6	110	490	46	4,530	0.2
8873	3/23/76	11.1	9.1	10.0	0.2	100	95	6.7	2.6	0.1	94	475	50	5,100	0.3
8873	3/26/76	11.1	8.8	0.7	0.1	85	90	7.2	2.8	0.2	72	370	44	2,115	0.1
8873	3/30/76	11.9	8.6	9.0	0.2	84	107	7.7	3.3	0.3	61	335	31	5,140	0.1
206 8873	4/2/76	11.7	8.1	8.0	0.2	95	105	7.8	2.6	0.1	83	364	43	6,045	0.1
8873	4/6/76	11.6	8.5	10.3	0.2	90	90	7.5	2.8	0.2	95	382	47	4,650	0.2
8873	4/9/76	12.1	8.5	9.0	0.4	104	87	7.5	2.8	0.1	115	365	42	9,656	0.2
8873	4/13/76	12.5	8.2	10.0	0.2	96	102	7.5	3.1	0.1	123	330	43	3,355	0.4

TABLE 34. Clinical Chemistry Data On Control Pigs (Continued)

8873	4/16/76	11.5	7.8	11.1	0.1	100	90	7.2	2.6	0.1	113	417	51	4,500	0.3
8873	4/20/76	11.1	8.2	12.0	0.2	90	115	7.4	2.4	0.2	-	425	50	6,050	0.1
8873	4/26/76	12.0	7.6	14.0	0.2	110	103	7.4	2.6	0.1	83	540	48	5,090	0.2
8873	4/27/76	12.1	7.7	11.0	0.1	90	75	7.6	3.0	0.1	85	401	37	4,510	0.1
8873	4/29/76	12.1	7.7	11.0	0.1	88	95	7.3	3.1	0.1	93	450	34	4,791	0.2
8873	5/3/76	12.0	8.0	0.8	0.2	98	85	7.4	1.8	0.2	105	455	48	3,462	0.1

Fig 1. Lateral view of anterior end of Phocanema sp. Larva from mouth of a Marin County, California, man, cleared in phenol. Interference microscopy.

Fig 2. En face view of head of Phocanema sp. Larva from the throat of a Marin County, California, man, cleared in phenol. Interference microscopy.

Bar = 0.1 mm; dr = dentigerous ridge; ed = excretory duct; p = pallisade-like projection.

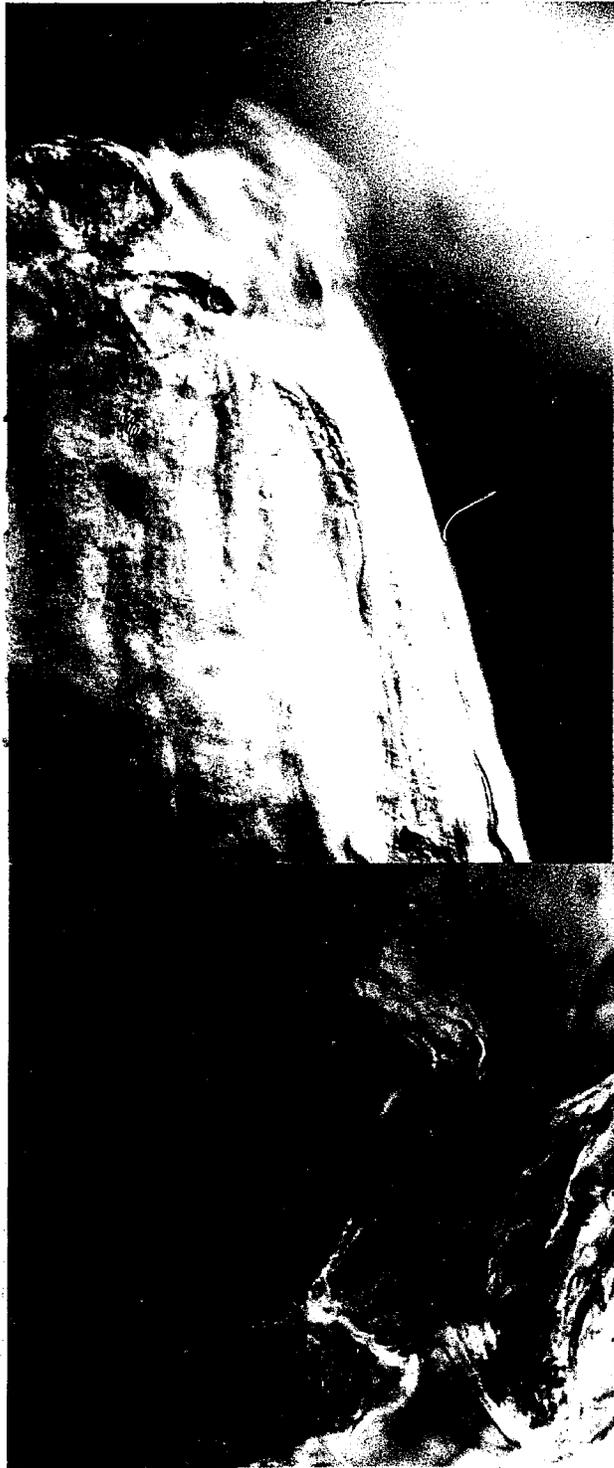


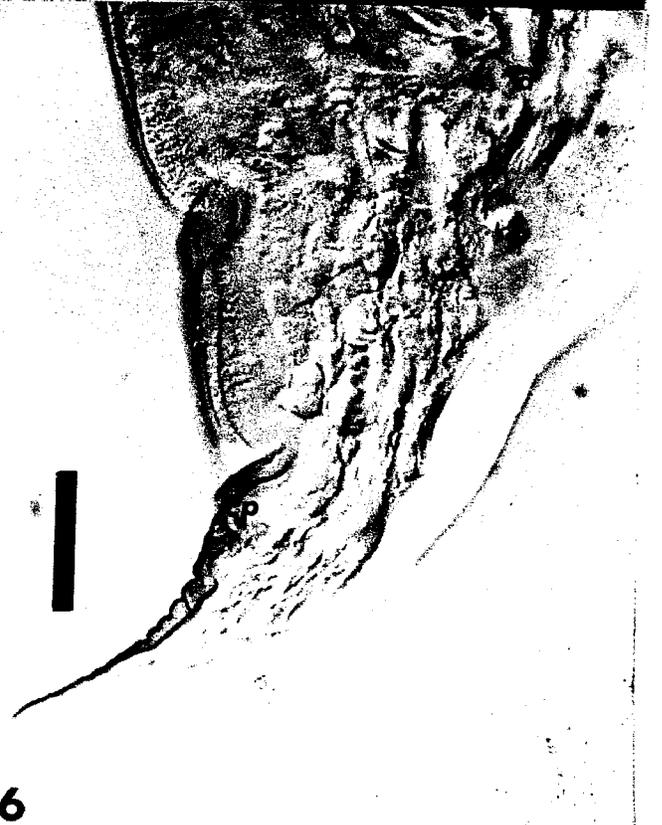
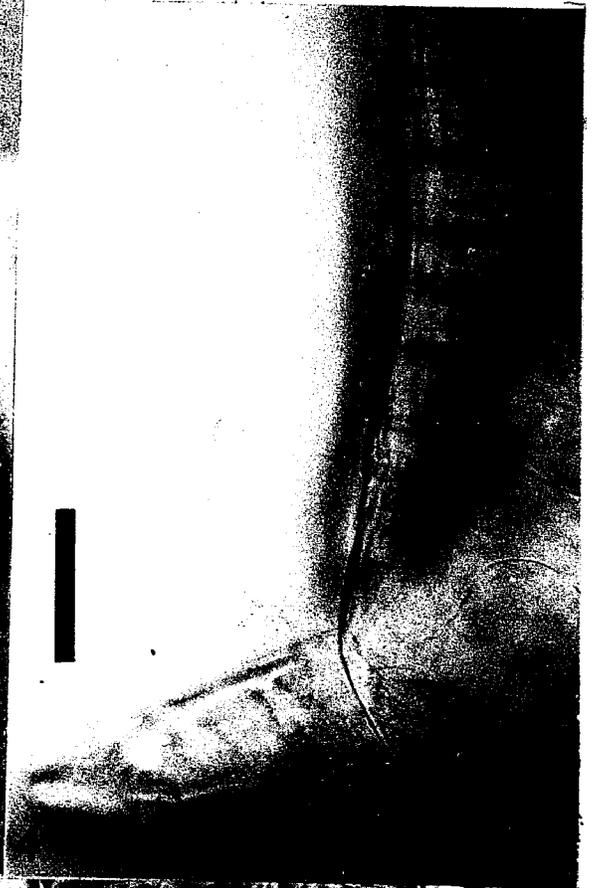
Fig 3. Lateral view of anterior end of adult Phocanema decipiens. Cleared in glycerin. Interference microscopy.

Fig 4. Lateral view of tail of adult male Phocanema decipiens. Cleared in glycerin. Interference microscopy.

Fig 5. Semilateral view of anterior end of Phocanema sp. United States National Museum-66550, identified as Pseudoterranova kogeii cleared in phenol. Note excretory pore at base of lips; by definition, the excretory pore of Pseudoterranova is at the level of the nerve ring. Brightfield microscopy.

Fig 6. Semilateral view of twisted tail of Phocanema sp. United States National Museum-66550 identified as Pseudoterranova kogeii, cleared in phenol. Interence microscopy.

Bar = 0.1 mm; cc = cuticular collar; cp = caudal papillum; dl = dorsal lip; e = excretory pore; svl = subventral lip.



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Fig 7. Lateral view of anterior end of adult Contracaecum hoffmani. United States National Museum-8943c. Cleared in phenol. Brightfield microscopy. Note crystalline material in excretory duct.

Fig 8.. Semilateral view of anterior end of adult Colntracaecum quadracuspa. United States National Museum-50650. Cleared in phenol. Brightfield microscopy.

Fig 9. Lateral view of anterior end of immature Contracaecum osculatum. Cleared in phenol. Interference microscopy.

Fig 10. Dorsal view of anterior end of immature Contracaecum osculatum. Phase microscopy.

Bar = 0.1 mm; cp = cervical papilum; e = excretory pore; ed = excretory duct; il = interlabium.

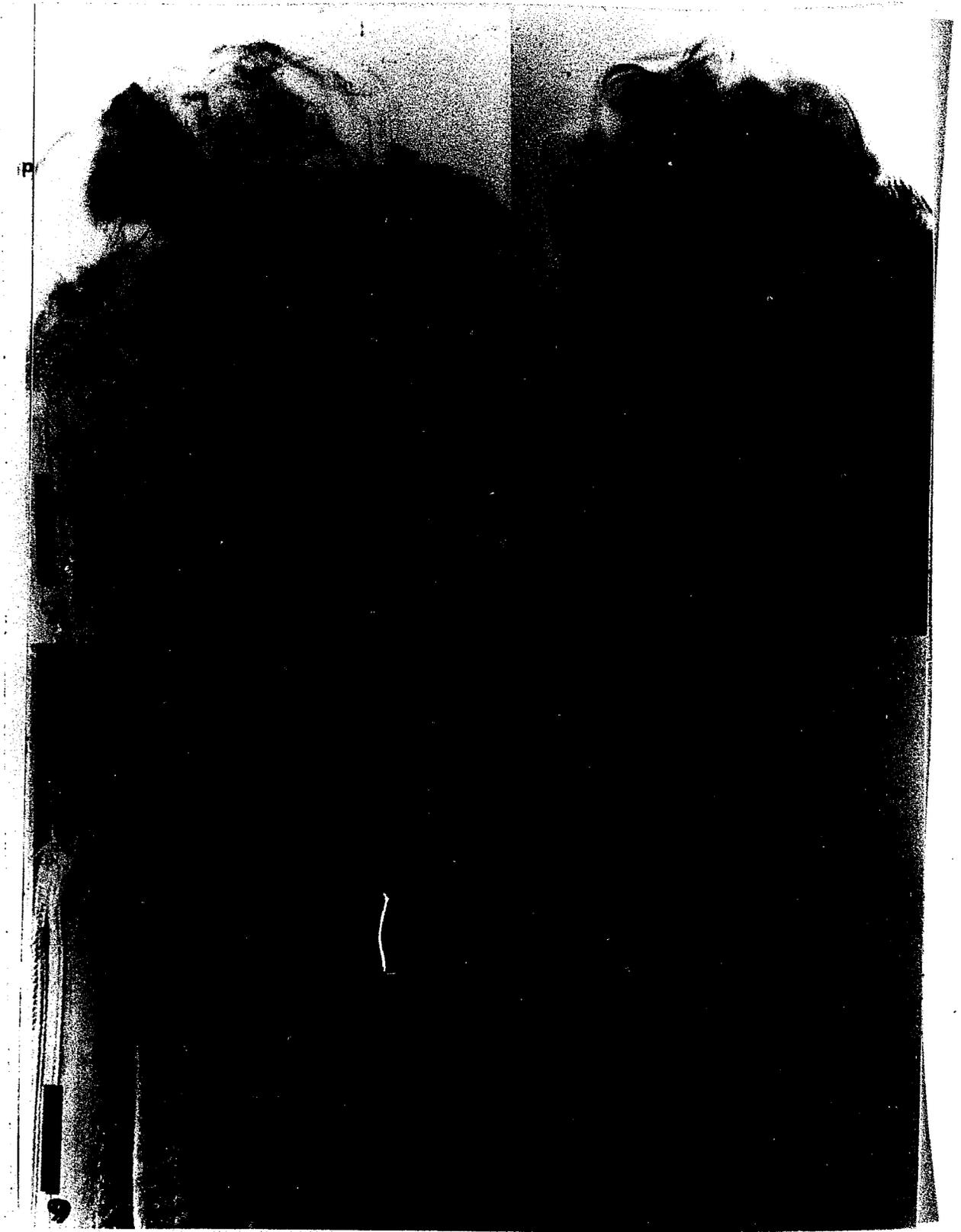


Fig 11. Lateral view of subventral lip of adult Anisakis simplex. United States National Museum-61303, cleared in phenol. Interference microscopy.

Fig 12. Lateral view of immature Anisakis simplex. United States National Museum-61303, at level of cervical papillae (dirides), cleared in phenol. Bright field microscopy.

Fig 13. Lateral view of Anisakis simplex. United States National Museum-61303 at level of ventriculus cleared in phenol. Bright field microscopy. Note width of ventriculus in relation to body.

Fig 14. Lateral view of immature female Anisakis simplex. United States National Museum-61303, at level of vulva, cleared in phenol. Interference microscopy.

Bar = 0.1 mm; cp = cervical papillu; dr = dentigerous ridge; e = muscular esophagus; ep = excretory pore; i = intestine; u = uterus; v = vulva.



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Fig 15. Lateral view of subventral lip of adult Paranisakiopsis lintoni. Cleared in glycerin. Interference microscopy.

Fig 16. Lateral view of caudal end of adult male Paranisakiopsis lintoni. Cleared in phenol. Brightfield microscopy.

Bar = 0.1 mm; ep = excretory pore; svl = subventral lip.



Fig 17. Lateral view of the anterior end of adult Thynnascaris incurum. United States National Museum-60051. Cleared in phenol.

Fig 18. Lateral view of anterior end of adult Thynnascaris inquires. United States National Museum-6622. Cleared in phenol. Brightfield microscopy.

Fig 19. Dorsal view of dorsal lip of adult Thynnascaris aduncum. United States National Museum-39531. Cleared in phenol. Brightfield microscopy.

Fig 20. En face view of lips of Thynnascaris clavatum. Cleared in phenol. Bright field microscopy.

Bar = 0.1 mm; cr - cuticular rings; e = esophagus; d = denticles; dl = dorsal lip; svl = subventral lip.



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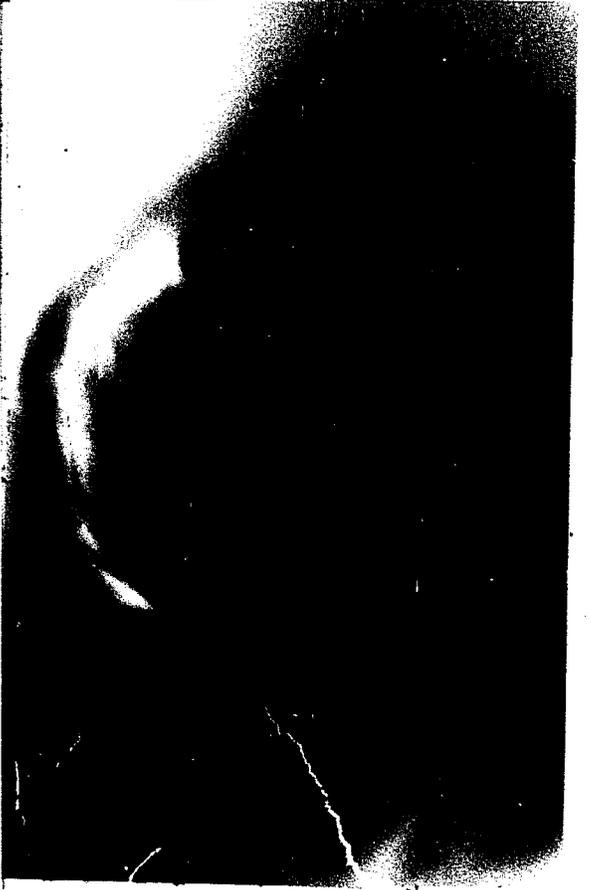


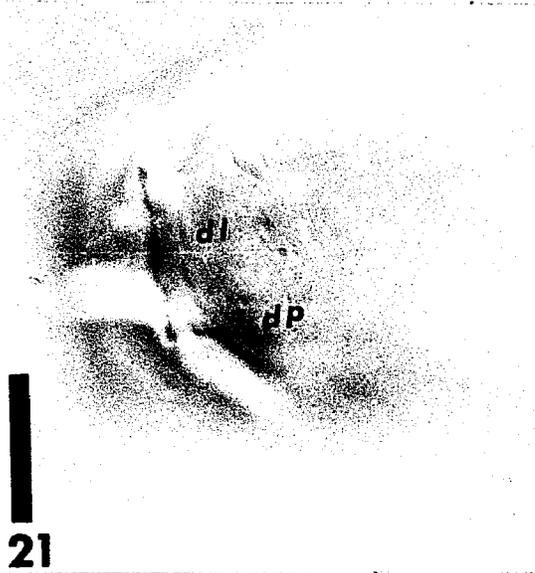
Fig 21. Anterior view of dorsal lip of Thynnascaris clavatum. Cleared in glycerin jelly. Brightfield microscopy.

Fig 22. En face view of lips of immature Thynnascaris clavatum. Cleared in glycerin. Brightfield microscopy.

Fig 23. Lateral view, near nerve ring of Thynnascaris habena. United States National Museum-47684. Cleared in phenol. Brightfield microscopy.

Fig 24. Lateral view, venteral surface of Thynnascaris habena. United States National Museum-47684. Cleared in phenol. Interference microscopy.

Bar = 0.1 mm; cr = cuticular ring, dl = dorsal lip; dp = double papillae; e = esophagus; ep = excretory pore.



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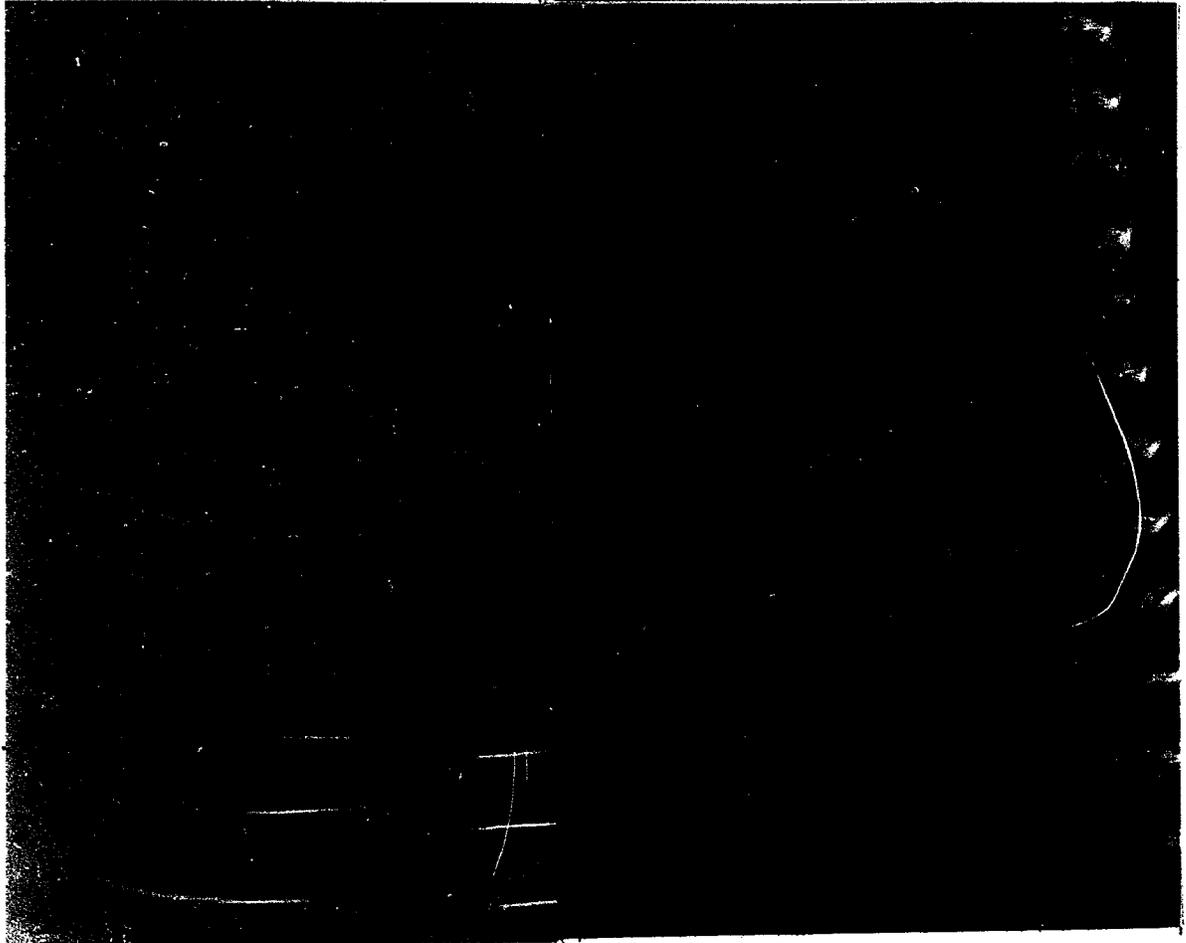


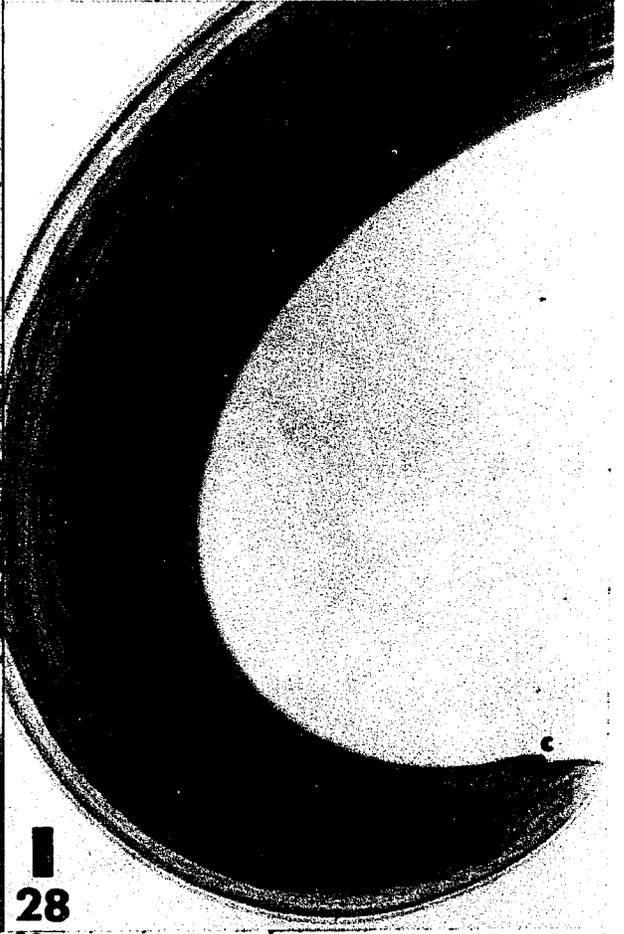
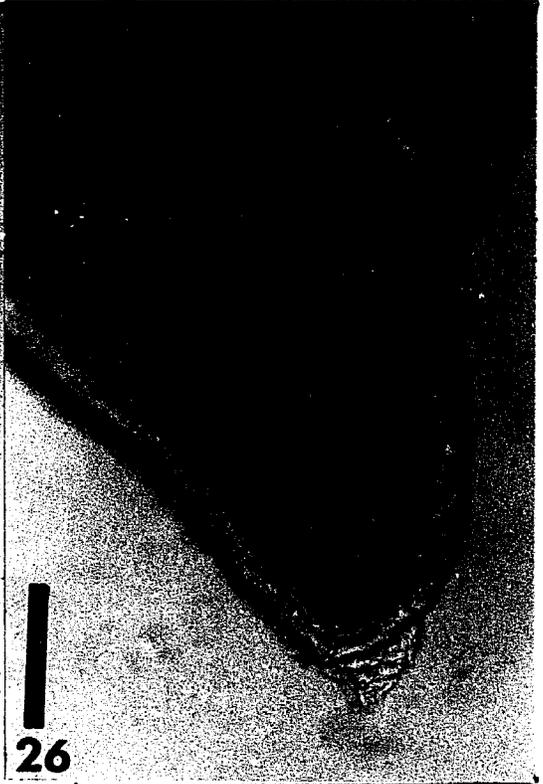
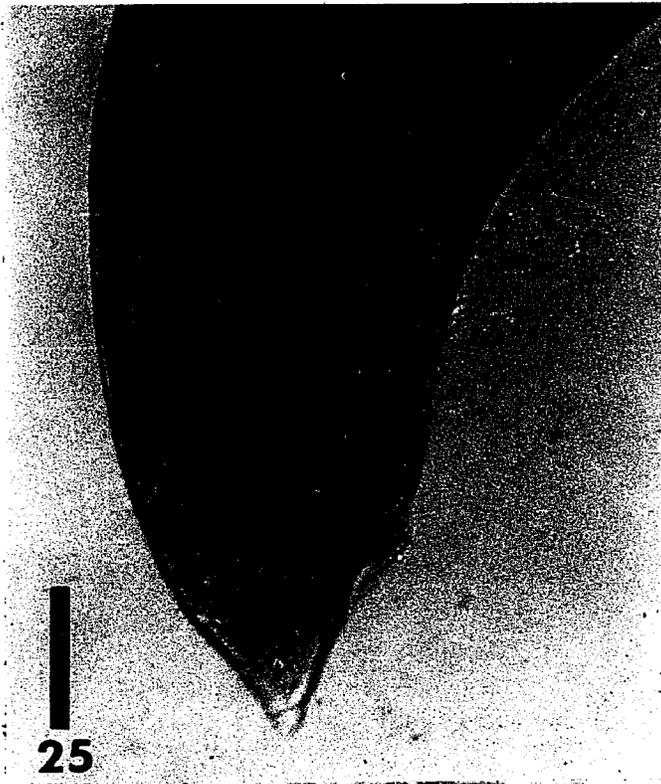
Fig 25. Lateral view of tail of adult male Thynnascaris clavatum. United States National Museum-42155. Cleared in phenol. Interference microscopy.

Fig 26. Lateral view of tail of adult female Thynnascaris increscens. United States National Museum-6640. Cleared in phenol. Interference microscopy.

Fig 27. Lateral view of the tail of adult female Thynnascaris clavatum. Cleared in phenol. Phase microscopy.

Fig 28. Lateral view of the tail of adult male Thynnascaris aduncum. United States National Museum-39537. Cleared in phenol. Brightfield microscopy.

a = anus; bar = 0.1 mm; c = cloaca; ed = ejaculatory duct; r = rectum; rg = rectal glands; s = spiculas; st = spined tail



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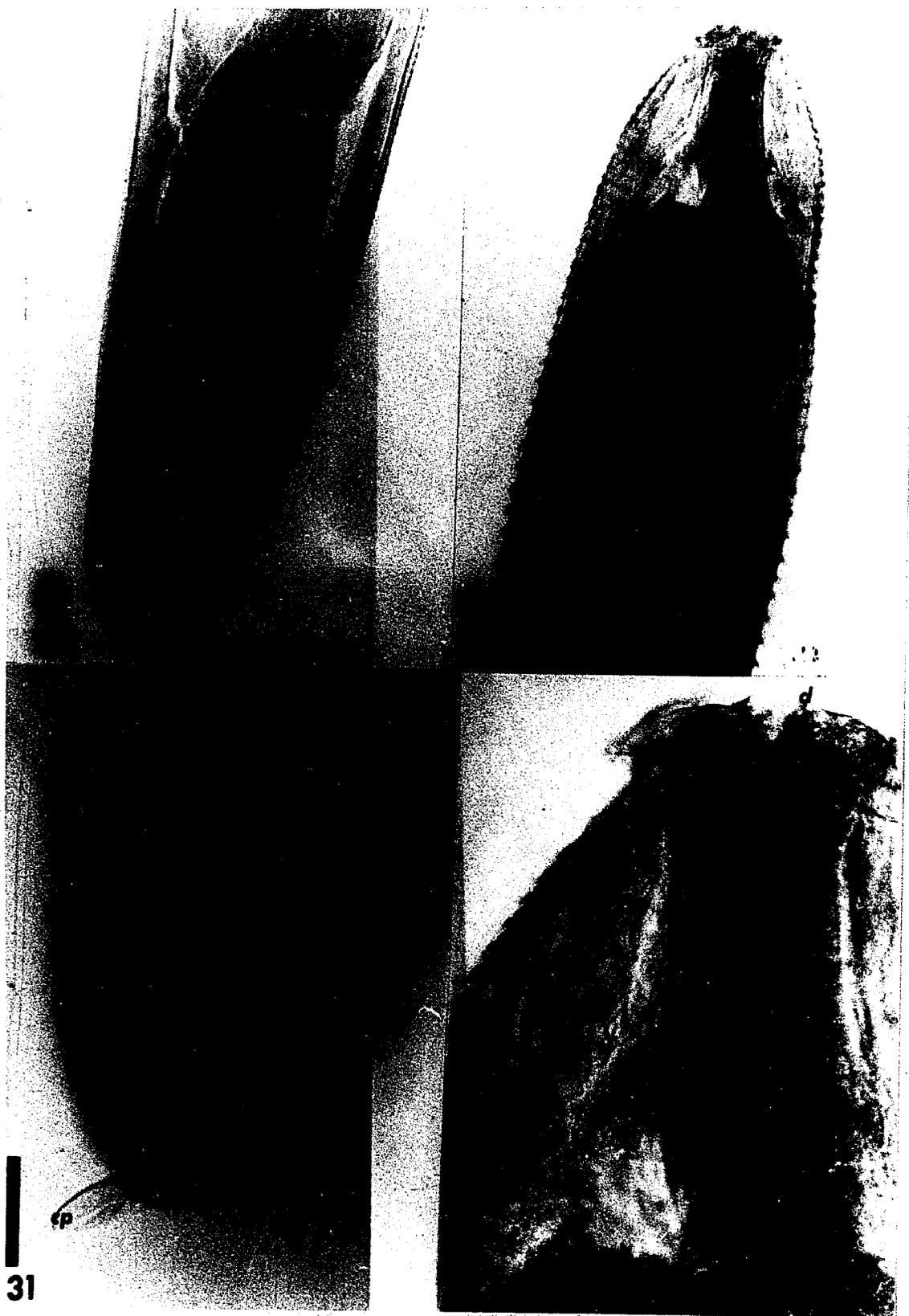
Fig 29. Semiventral view of the male tail of Goezia minuta. United States National Museum-39542. Cleared in phenol.

Fig 30. Lateral view of anterior end of Goezia minuta. United States National Museum-39542. Cleared in phenol. Brightfield microscopy.

Fig 31. Semiventral view of the male tail of Goezia minuta. United States National Museum-39542. Cleared in phenol. Brightfield microscopy.

Fig 32. Lateral view of the head of Goezia minuta. United States National Museum-39542. Cleared in phenol. Brightfield microscopy.

Bar = 0.1 mm; d = denticle; e = esophagus; ed = ejaculatory duct; p = caudal papillum; s = spicule; t = testis.



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Fig 33. Lateral view of the subventral lip of adult Raphidascaris acus. United States National Museum-38820. Cleared in phenol. Brightfield microscopy. Note cuticular expansions at the edge of the lip.

Fig 34. Lateral view of head of adult Raphidascaris acus. United States National Museum-38820. Cleared in phenol. Interference Microscopy.

Fig 35. Lateral view of the posterior end of adult male Raphidascaris acus. United States National Museum-38820. Cleared in phenol. Brightfield microscopy.

Fig 36. Lateral view of the tail of adult female Raphidascaris acus. United States National Museum-38820. Cleared in phenol. Brightfield microscopy.

Bar = 0.1 mm; a = junction of lateral alae on ventral surface; c = cloaca; p = palp or fleshy part of lip; s = spicule; svl = subventral lip.

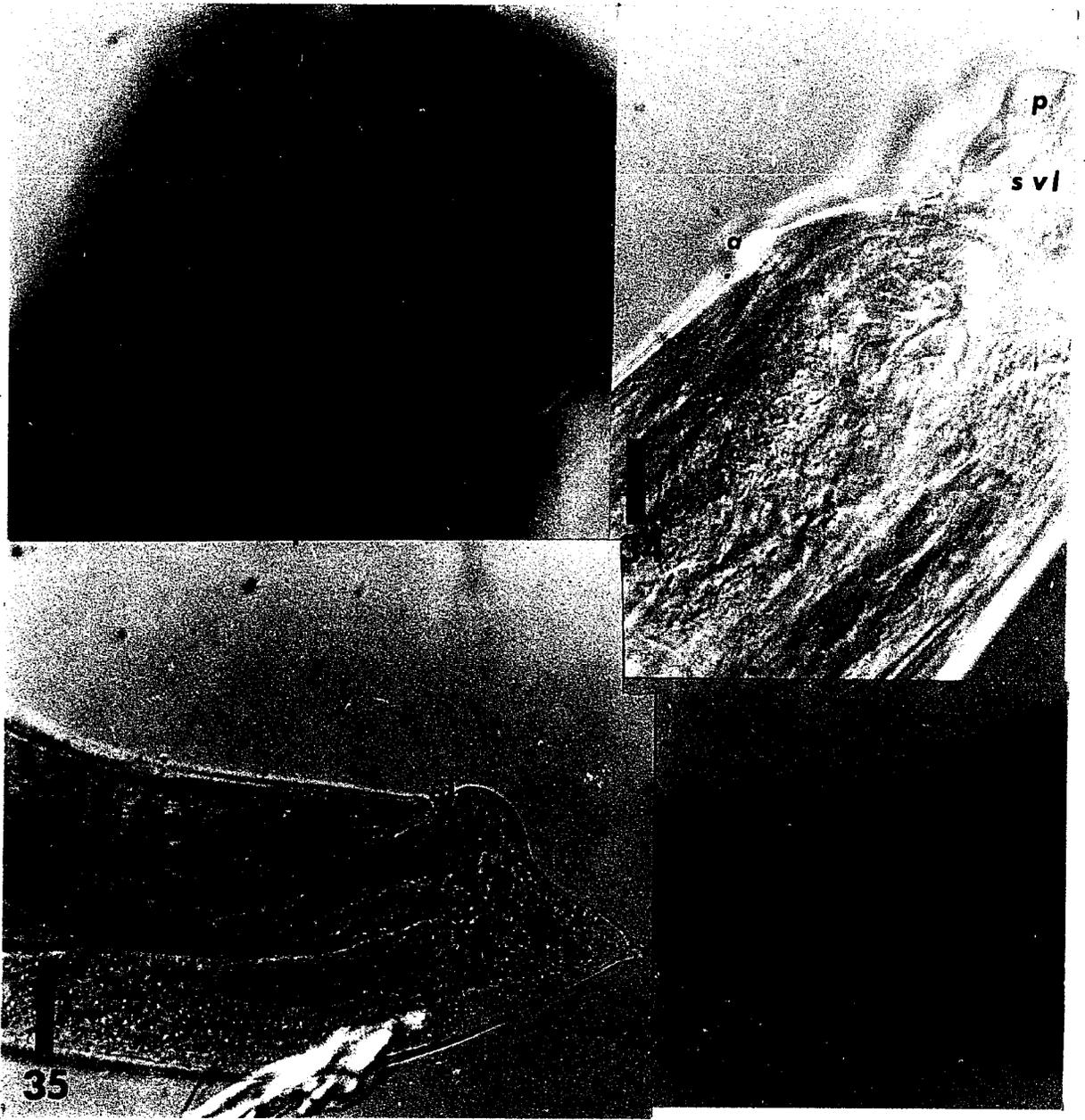
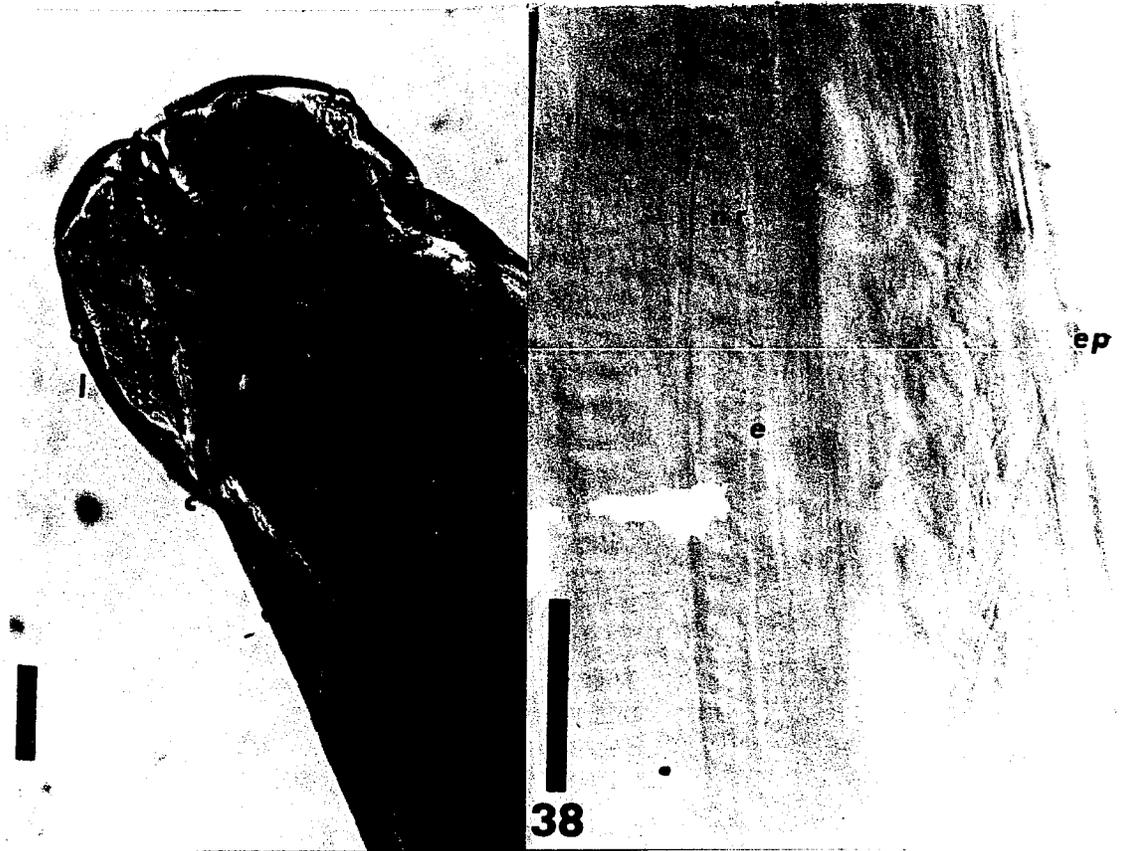


Fig 37. Lateral view of anterior end of immature Porrocaecum sp. Cleared in phenol. Brightfield microscopy.

Fig 38. Lateral view of near level of nerve ring, of immature Porrocaecum sp. Interference microscopy.

Fig 39. Lateral view of the tail of immature Porrocaecum sp. United States National Museum-27173. Cleared in phenol. Brightfield microscopy.

Bar = 0.1 mm; a = amphid cell body; c = cuticular collar; e = esophagus; ep = excretory pore; l = subventral lip; nr = nerve ring; rg = rectal gland.



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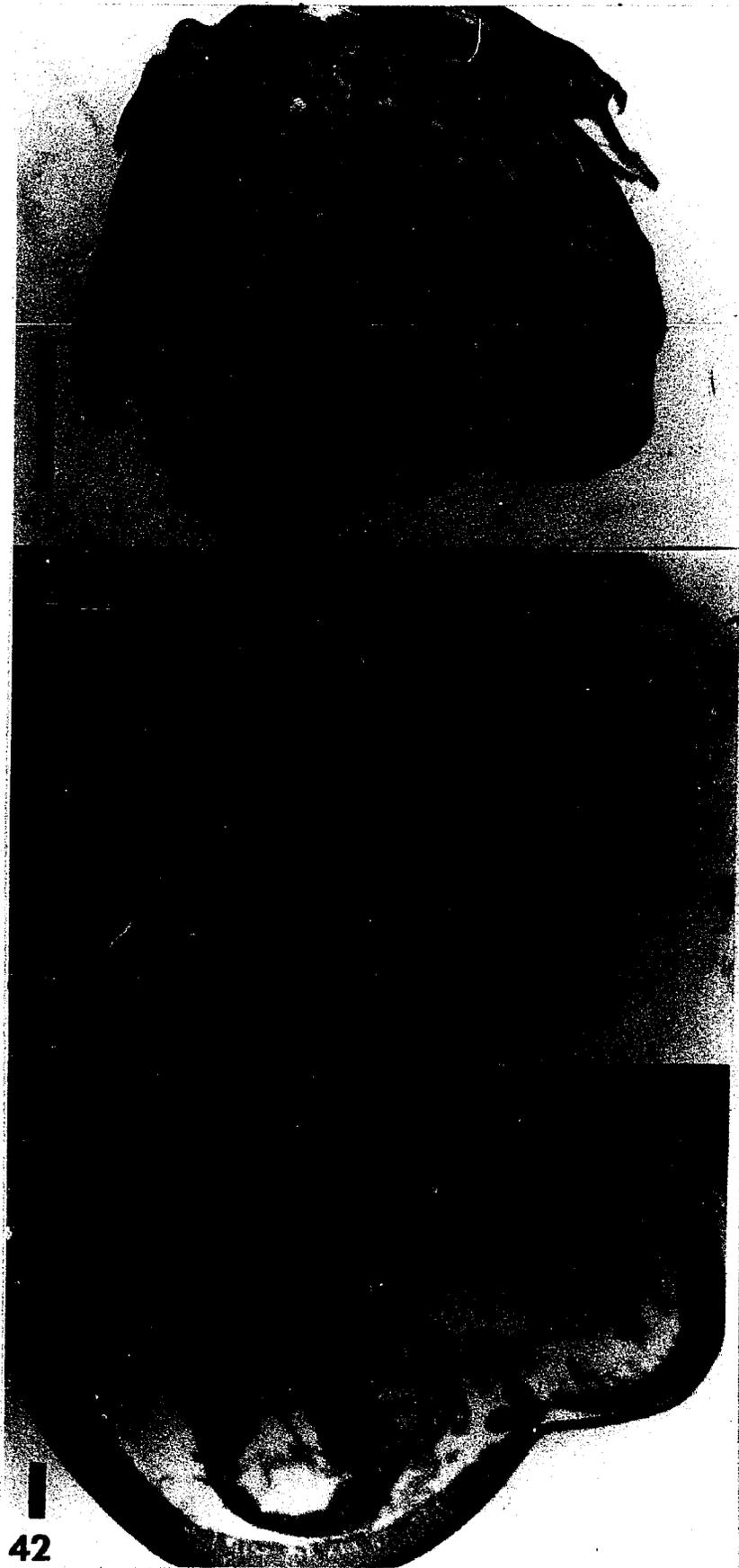
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229

Fig 40. Cross section through the center of esophagus of adult Phocanema decipiens. Stained with hematoxylin and eosin.

Fig 41. Cross section through the esophagus at edge of nerve ring of an adult Phocanema decipiens. Stained with hematoxylin and eosin.

Fig 42. Cross section through the ventriculus of an adult Phocanema decipiens. Stained with azure A and eosin B.

Bar = 0.1 mm; e = esophagus; ed = excretory duct; eg = excretory gland; ic = intestinal caecum; v = ventriculus.



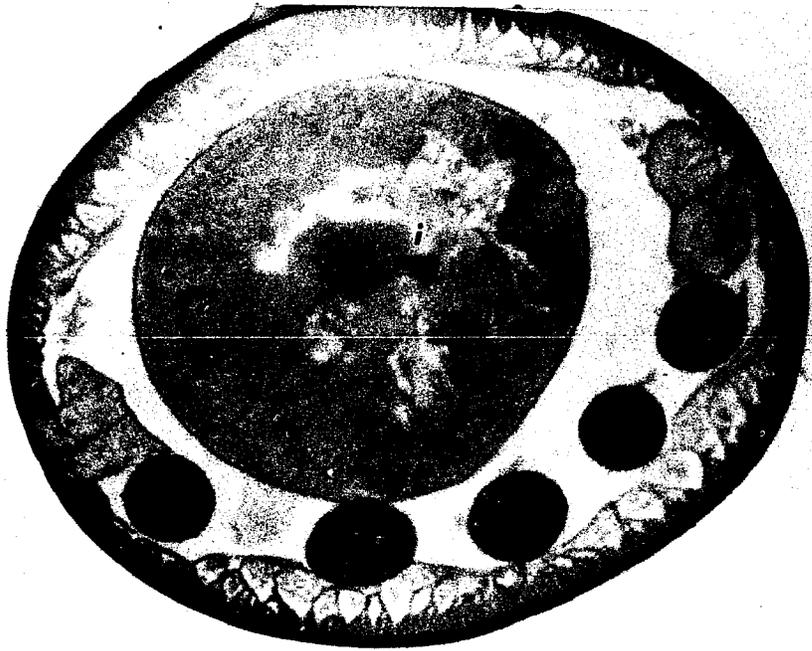
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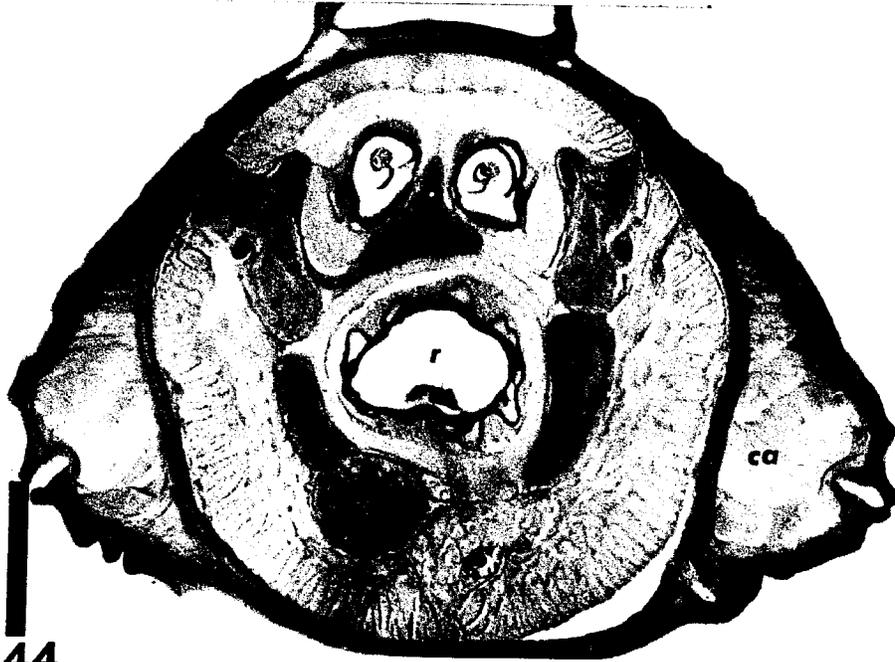
Fig 43. Cross section through the intestine of an adult male Phocanema decipiens. Stained with azure A and eosin B.

Fig 44. Cross section through the tail of an adult male Phocanema decipiens. Stained with azure A and eosin B.

Bar = 0.1 mm; ca = caudal ala; ed = ejaculatory duct; i = intestine;  
lc = lateral cord; r = rectum; s = spicule.



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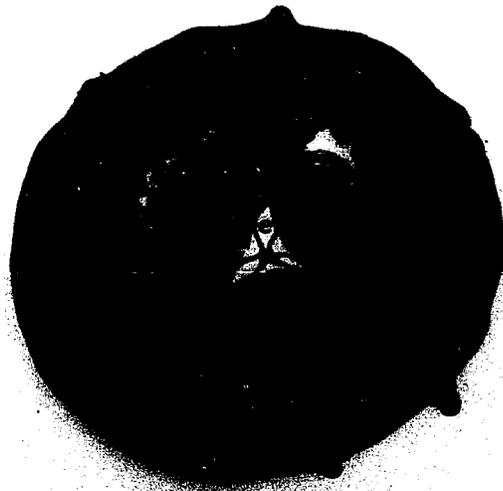
Fig 45. Cross section through the anterior esophagus of an adult Contracaecum spiculigerum. United States National Museum-52082. Stained with hematoxylin and eosin.

Fig 46. Cross section through the esophagus of adult Contracaecum spiculigerum. United States National Museum-52082. Stained with hemotaxylin and eosin.

Fig 47. Cross section through ventricular-intestinal junction of adult Contracaecum spiculigerum. United States National Museum-52082. Stained with hematoxylin and eosin.

Fig 48. Cross section through the ventricular appendix of adult Contracaecum spiculigerum. United States National Museum-52082. Stained with hematoxylin and esoin.

Bar = 0.1 mm; e = esophagus; ed = excretory duct; eg = excretory gland; lc = lateral cord; v = ventriculus; va = ventricular appendix.



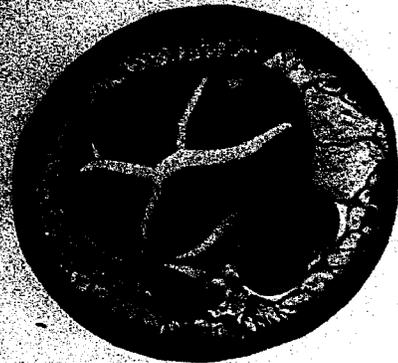
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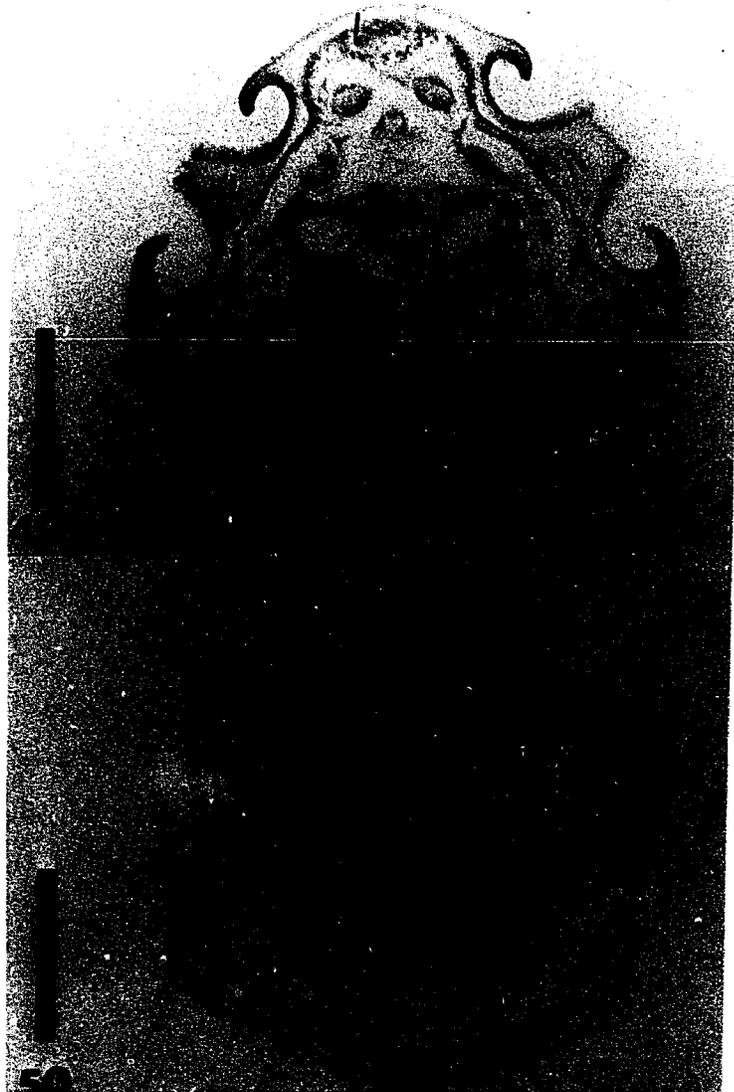
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Fig 49. Cross section through the base of the lips of adult Thynnascaris sp. Stained with azure A and eosin B.

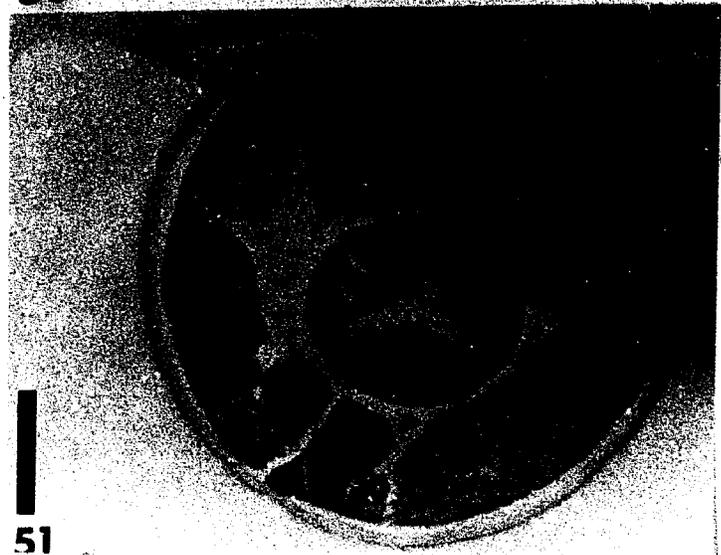
Fig 50. Cross section through the anterior esophagus of adult Thynnascaris sp. Stained with azure A and eosin B.

Fig 51. Cross section through the esophagus of an adult Thynnascaris sp. Stained with azure A and eosin B.

aeg = anterior esophageal gland; Bar = 0.1 mm; e = esophagus; ee = excretory canal; ic = intestinal caecum; il = interlabium; l = lip.



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Fig 52. Cross section through the esophagus of adult Thynnascaris sp. Stained with azure A and eosin B.

Fig 53. Cross section through the esophagus of adult Thynnascaris sp. Stained with azure A and eosin B.

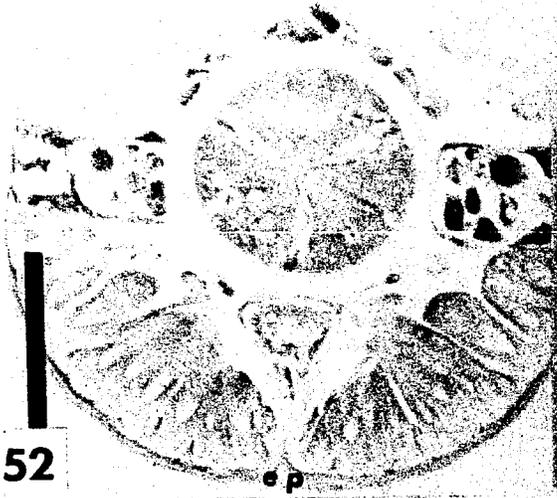
Fig 54. Cross section through the esophagus of adult Thynnascaris sp. Stained with azure A and eosin B.

Fig 55. Cross section through the esophagus of adult Thynnascaris sp. Stained with azure A and eosin B.

Fig 56. Cross section through the esophagus of adult Thynnascaris sp. Stained with azure A and eosin B.

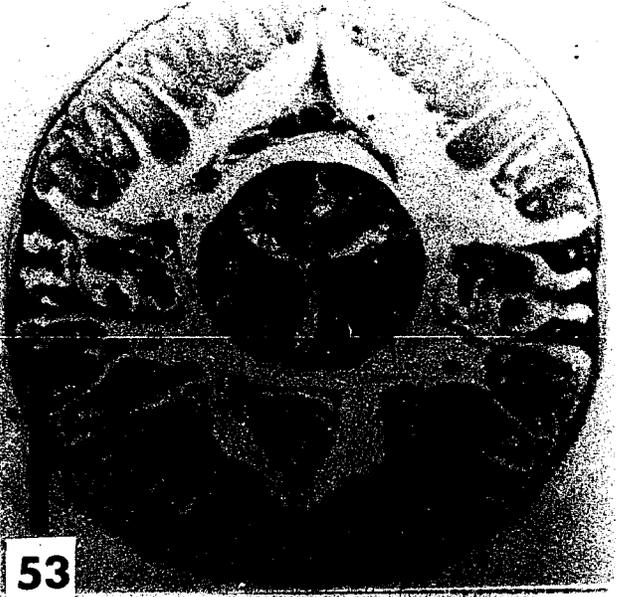
Fig 57. Cross section through the esophagus of adult Thynnascaris sp. Stained with azure A and eosin B.

Bar = 0.1 mm; e = esophagus; ed = excretory duct; ep = excretory pore; lc = lateral cord; m = body wall muscle; n = nucleus; led = left excretory duct; red = right excretory duct.

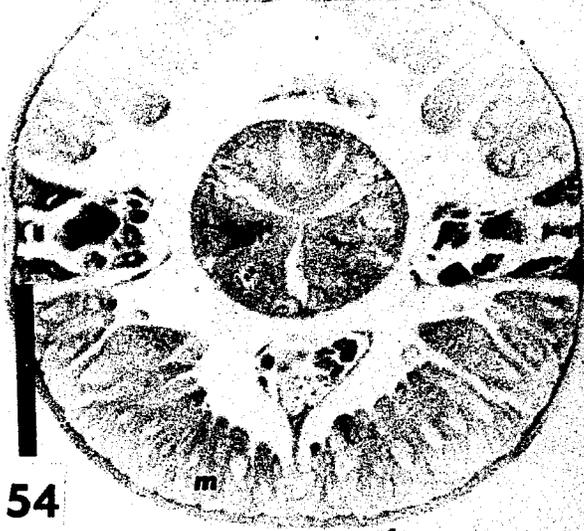


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ep

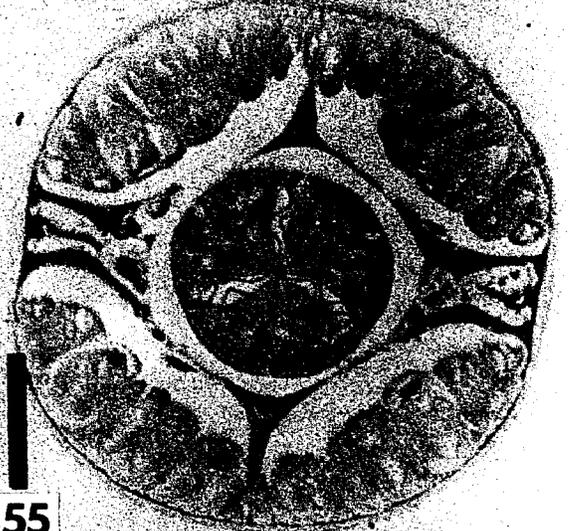


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m

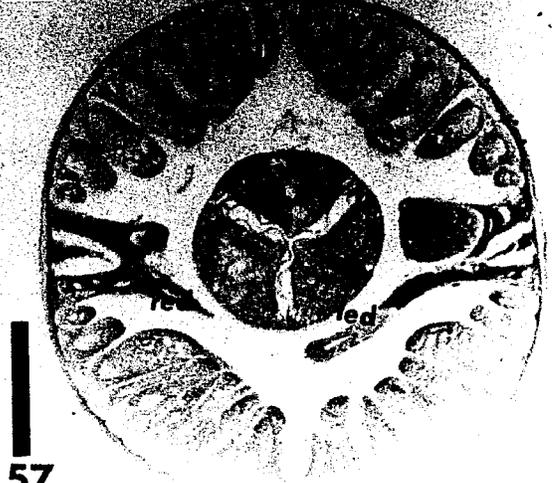


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ed

Fig 58. Section through ventricular appendix of adult Thynnascaris sp. Stained with azure A and eosin B.

Fig 59. Section through the intestine of adult Thynnascaris sp. Stained with azure A and eosin B.

Fig 60. Section through the tail of an adult Thynnascaris sp. Stained with azure A and eosin b.

Bar = 0.1 mm; ed = ejaculatory duct; eg = excretory gland; i = intestine; lc = lateral cord; p = caudal papillum; r = rectum; va = ventricula appendix.

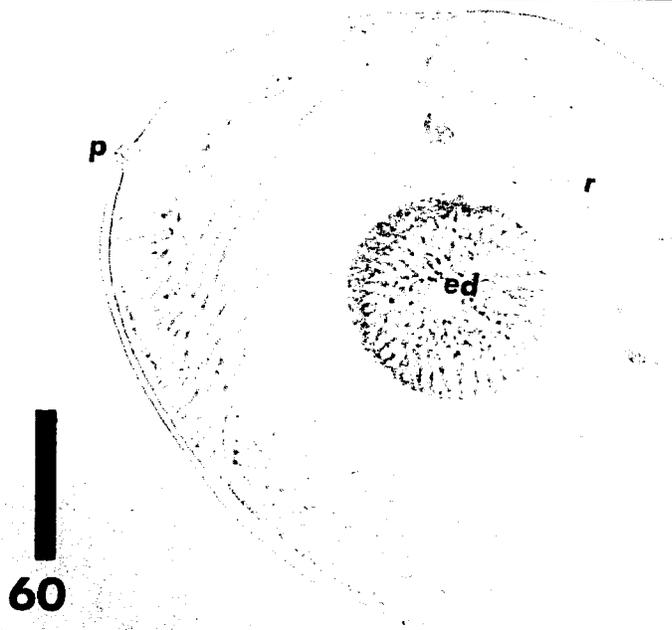
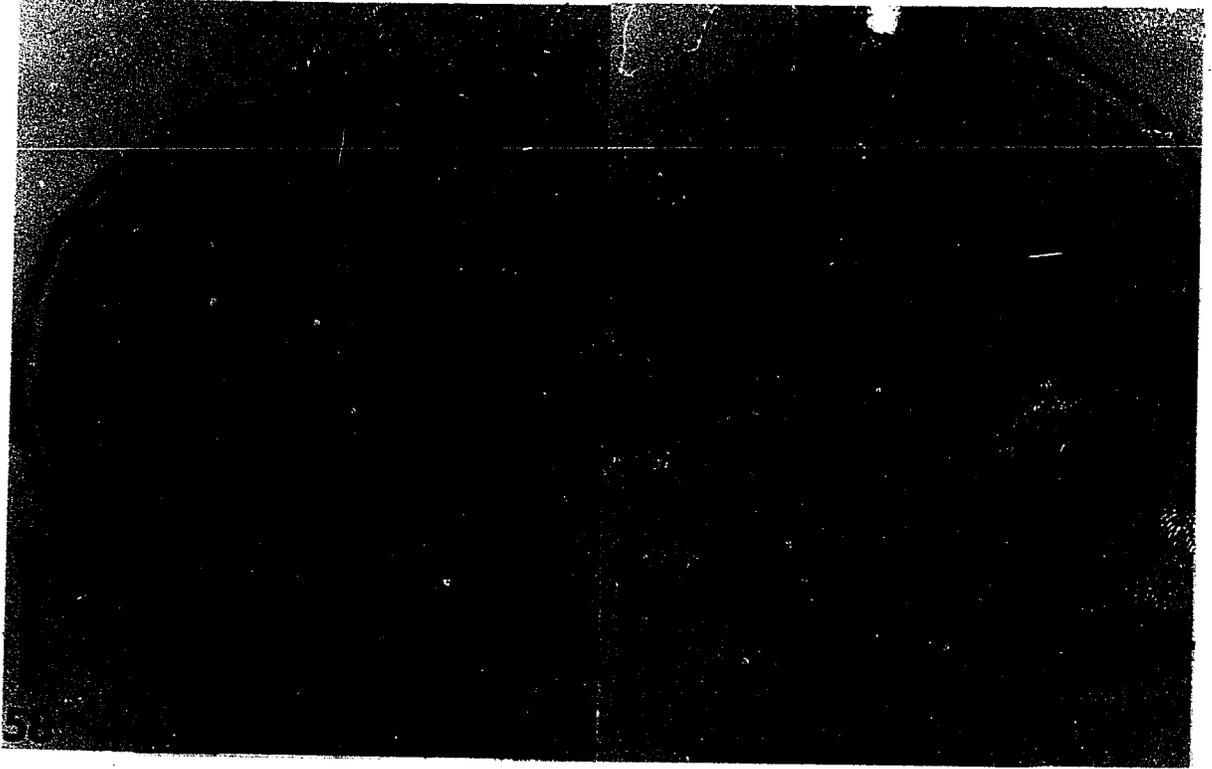


Fig 61. Section through the body wall at level of the mid-intestine of adult Thynnascaris clavatum. Stained with azure A and eosin B. Interference microscopy.

Bar = 0.01 mm; cs = cuticular spines.

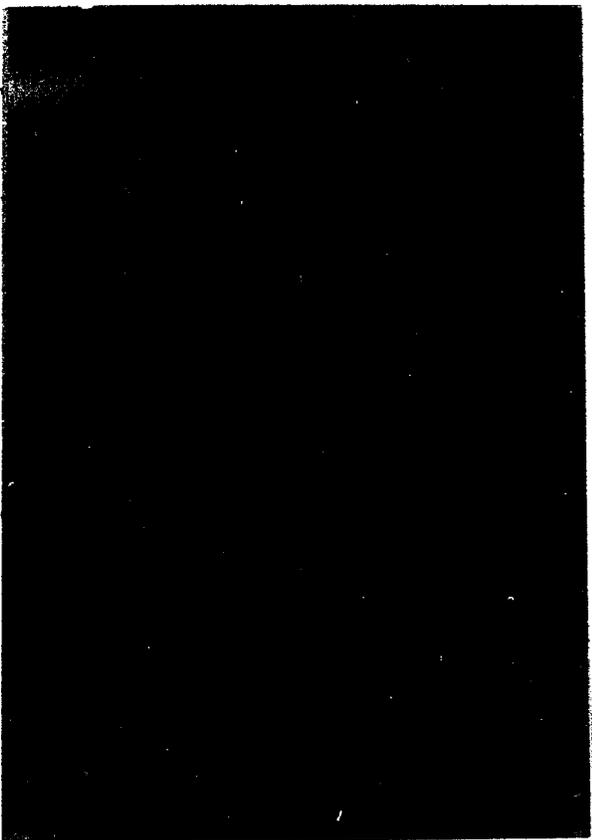


Fig 62. Section through the esophagus of an adult Paranisakiopsis lintoni. Stained with azure A and eosin B.

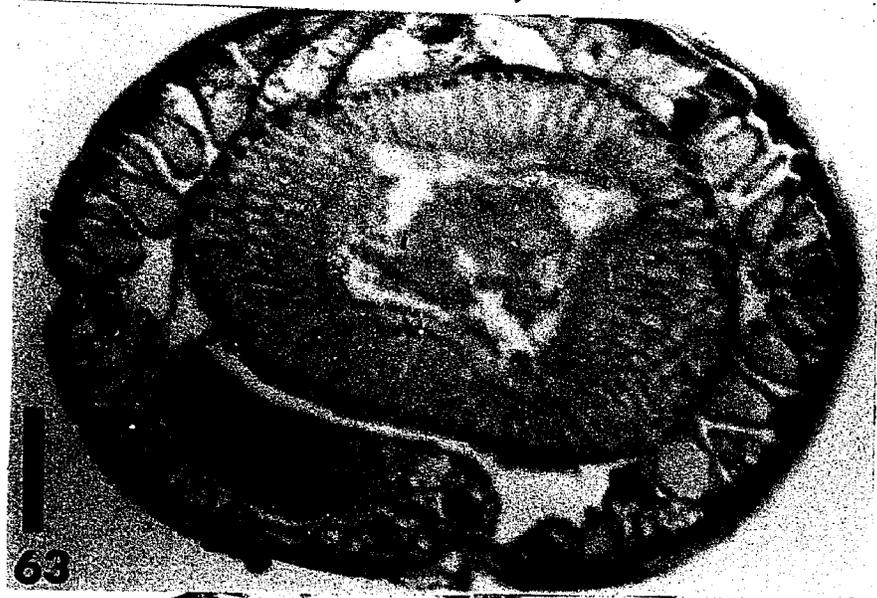
Fig 63. Section through the anterior intestine of an adult Paranisakiopsis lintoni. Stained with azure A and eosin B.

Fig 64. Section through the mid-intestine of an adult Paranisakiopsis lintoni. Stained with a azure A and eosin B.

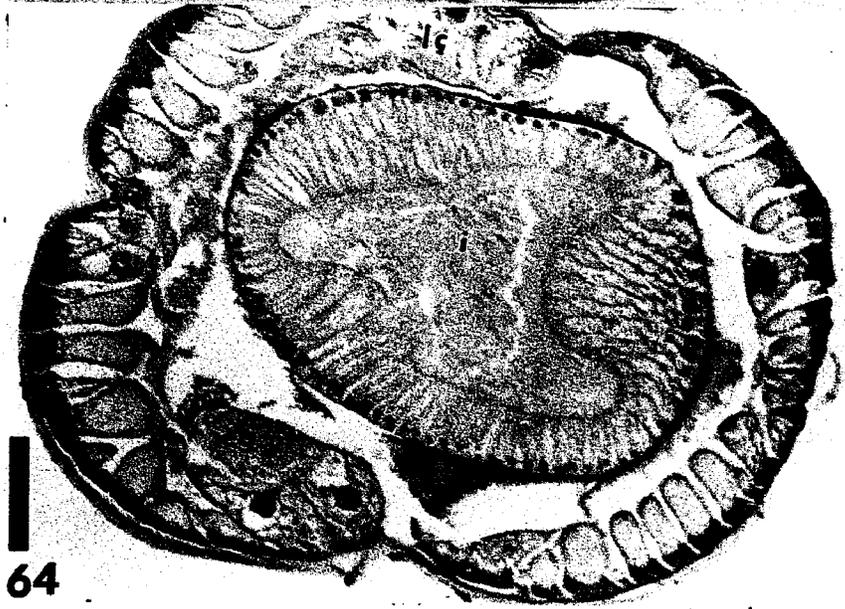
a = ala; e = esophagus; ed = excretory duct; eg = excretory gland; i = intestine; lc = lateral cord.



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Fig 65. Lateral view of anterior end of larval Porrocaecum sp. Cleared in phenol. Phase microscopy.

Fig 66. Lateral view of larval Porrocaecum sp. at level of nerve ring. specimen cleared in phenol. Interference microscopy.

Fig 67. Lateral view of the tail of larval Porrocaecum sp. Cleared in phenol. Interference microscopy.

a = anus; Bar = 0.1 mm; e = esophagus; ep = excretory pore; ic = intestinal caecum; nr = nerve ring; rg = rectal gland.

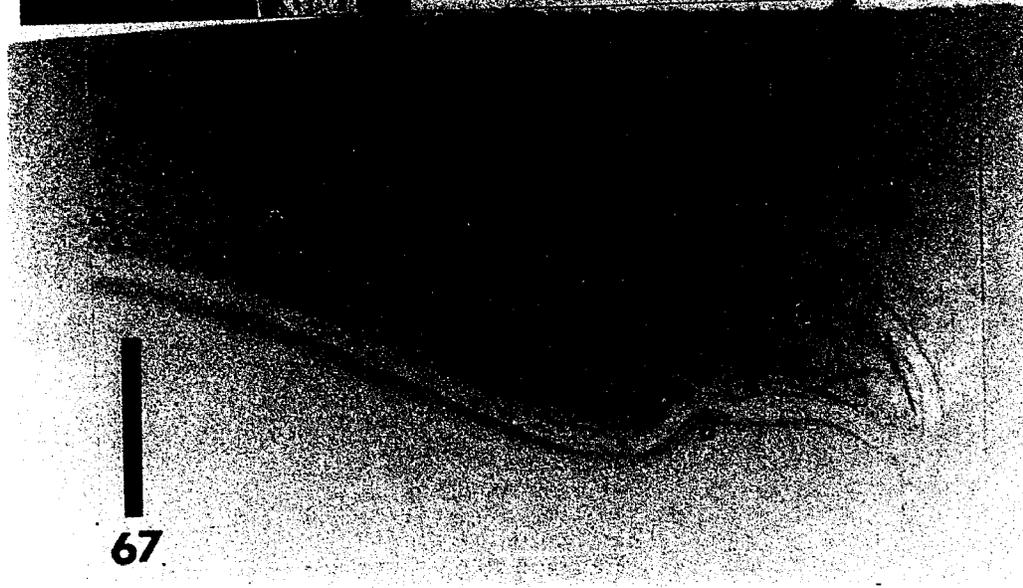
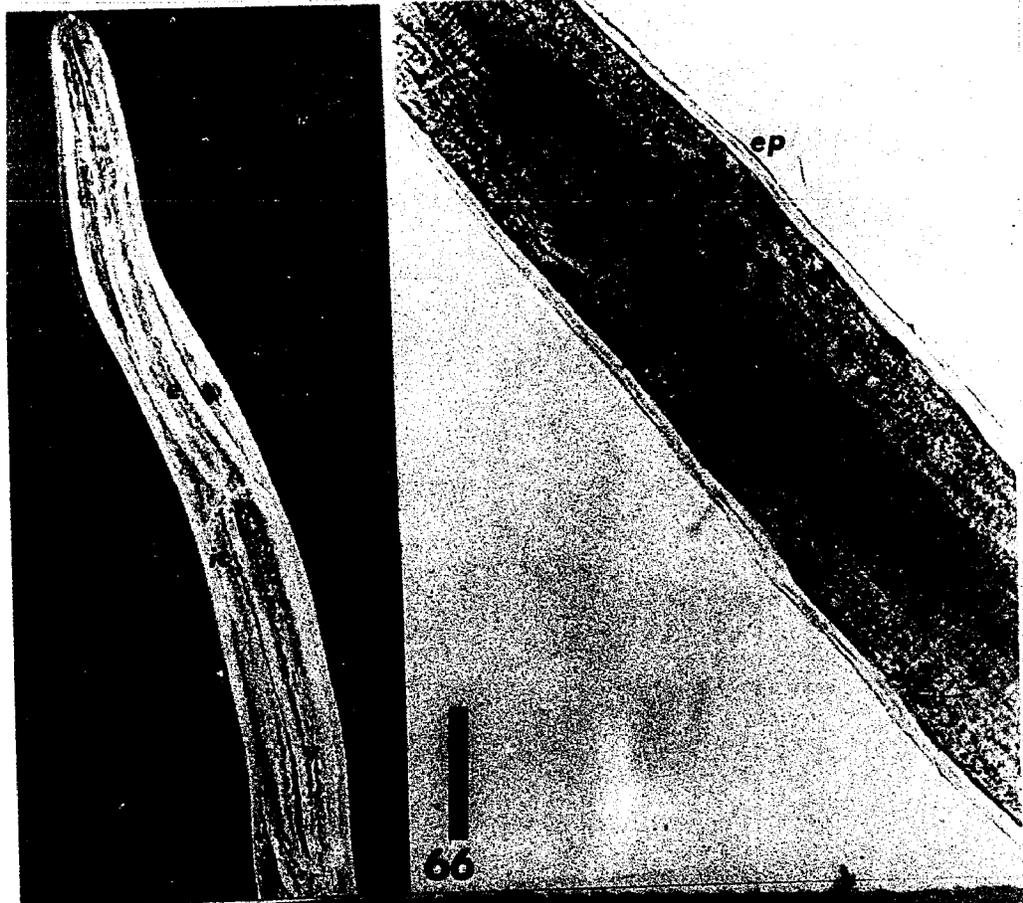


Fig 68. Lateral view of genital primordium in larval Raphidascaris acus. Cleared in glycerin. Interference microscopy.

Fig 69. Lateral view of posterior end of larval Raphidascaris acus. Cleared in glycerin. Interference microscopy.

a = anus; Bar = 0.01 mm; u = uterus; o = ovary.

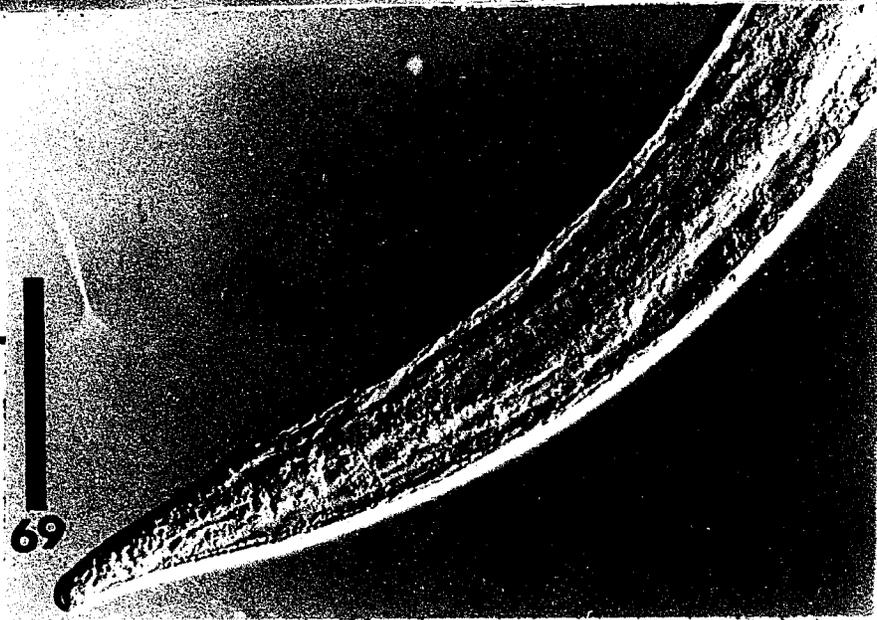
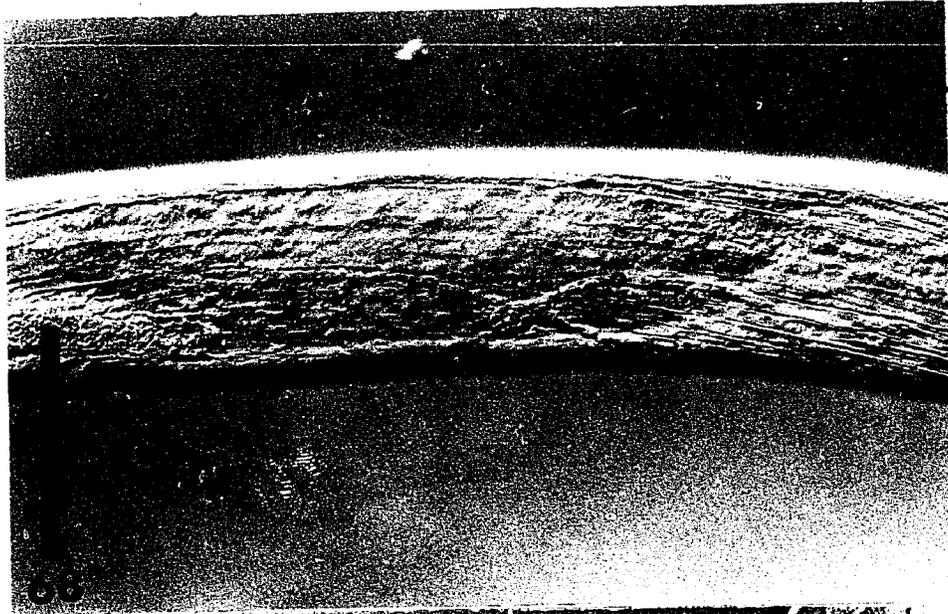


Fig 70. lateral view head of larval Phocanema decipiens. Cleared in glycerin. Brightfield microscopy.

Fig 71. Dorsal view of head of larval Phocanema decipiens. Cleared in phenol. Interference microscopy.

Fig 72. Lateral view of ventriculus of larval Phocanema decipiens. Cleared in phenol. Brightfield microscopy.

Bar = 0.1 mm; bt = boring tooth; e = esophagus; ep = excretory pore; i = intestine; ic = intestinal caecum.

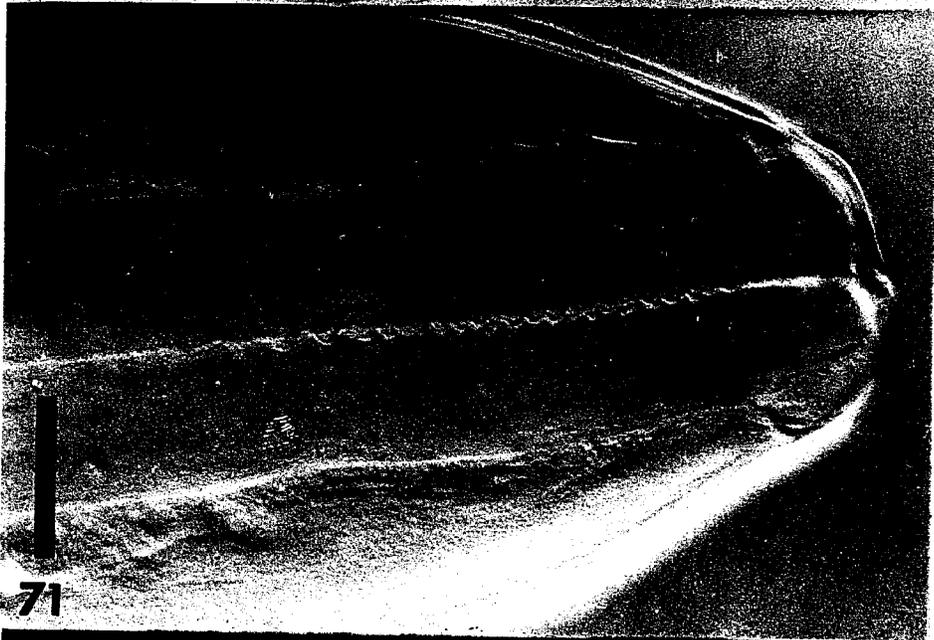
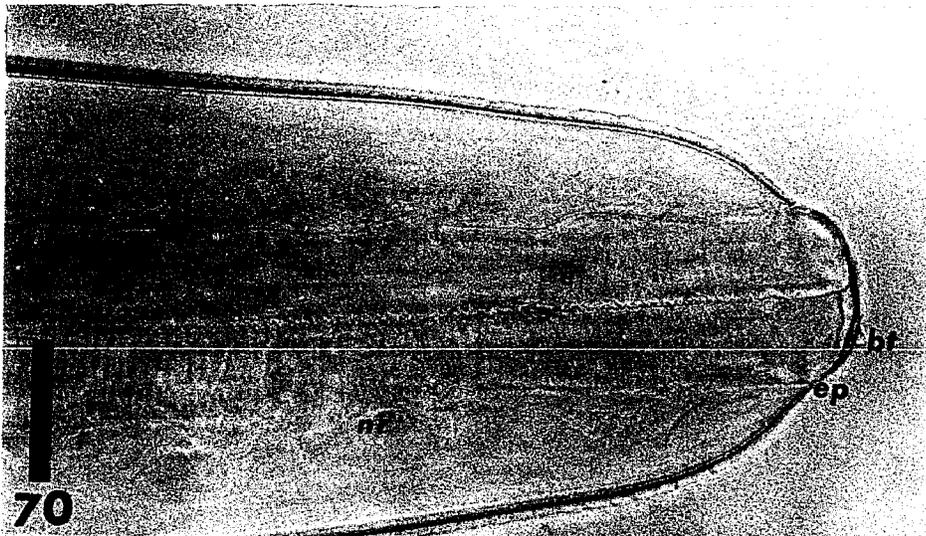


Fig 73. Dorsal view of the anterior end of a larval Anisakis sp.  
Cleared in phenol. Brightfield microscopy.

Bar = 0.1 mm; bt = boring tooth; e = esophagus.

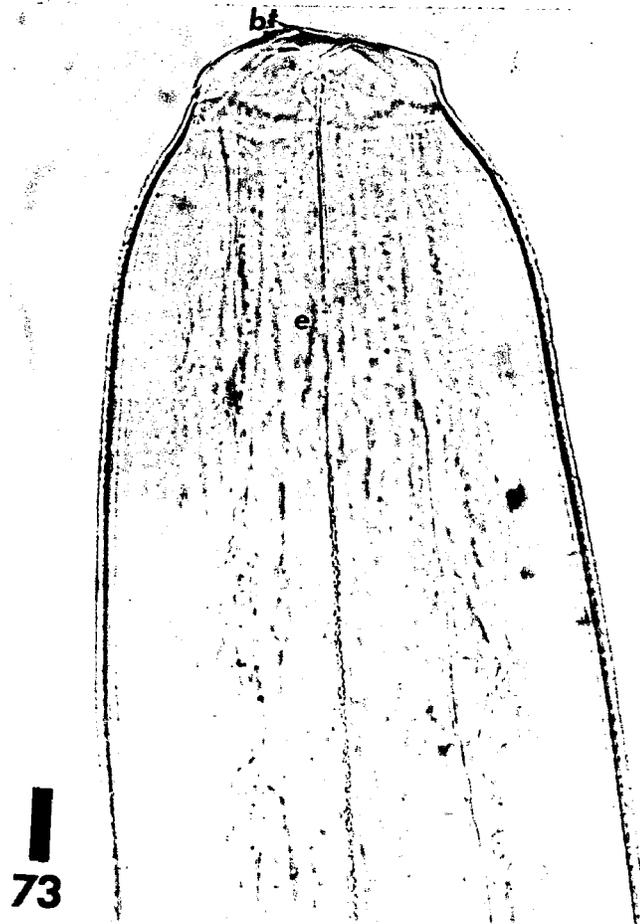


Fig 74. Lateral view of the anterior end of a Thynnascaris sp. larva. Cleared glycerin. Brightfield microscopy.

Fig 75. Lateral view of the anterior end of a Thynnascaris sp. larva. Cleared in glycerin. Brightfield microscopy.

Fig 76. Lateral view of the tail of a Thynnascaris sp. larva. Cleared in phenol. Interference microscopy.

Fig 77. Lateral view of the tail of a Thynnascaris sp. larva. Cleared in phenol. Interference microscopy.

a = anus; c = cloaca; Bar = 0.1 mm; e = esophagus; i = intestine; ic = intestinal caecum; mr = nerve ring; s = caudal spine; v = ventriculus; va = ventricular appendix.

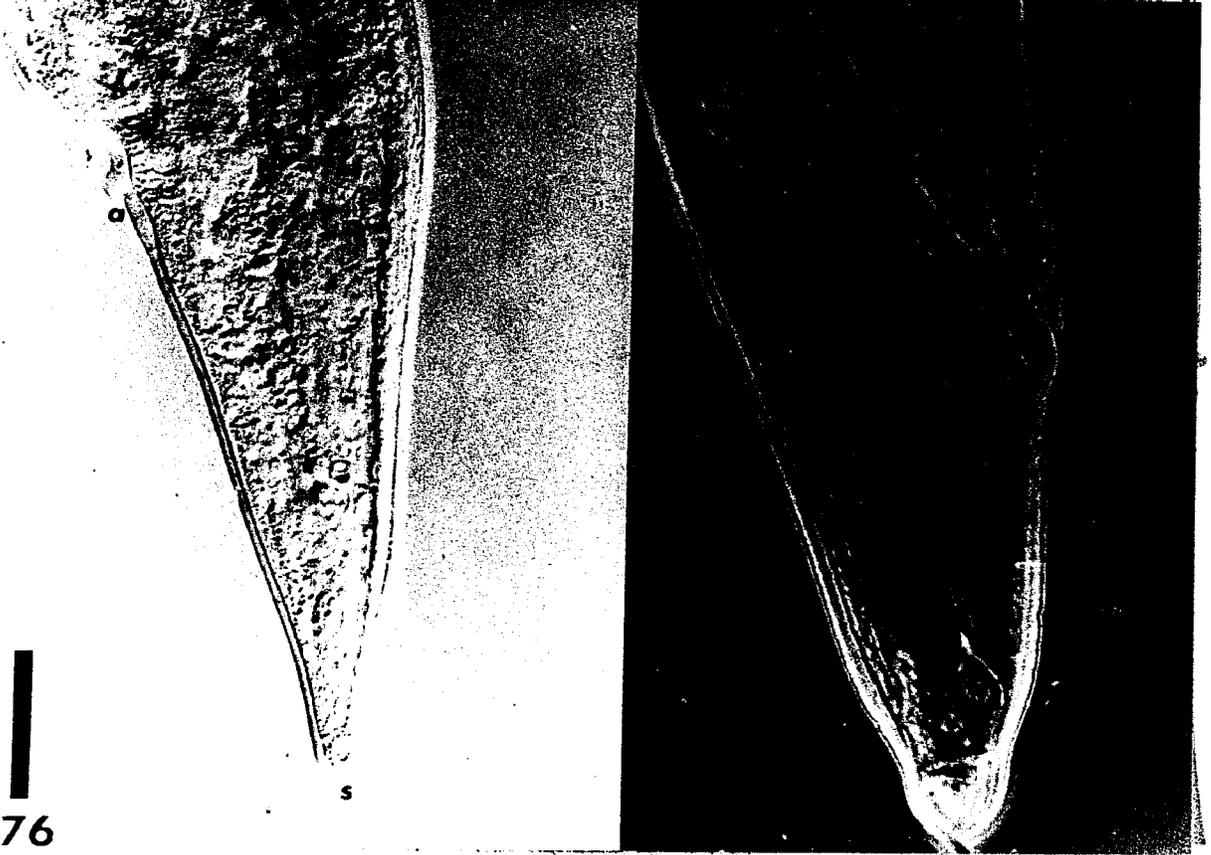


Fig 78. Dorsal view of the head of larval Contracaecum robustum. United States National Museum-39534. Cleared in phenol. Interference microscopy.

Fig 79. Lateral view of anterior end of larval Contracaecum robustum. United States National Museum-39534. Cleared in phenol. Brightfield microscopy.

Fig 80. Lateral view of the posterior end of larval Contracaecum robustum. United States National Museum-39534. Cleared in phenol. Brightfield microscopy.

a = anus; Bar = 0.1 mm; bt = boring tooth; e = esophagus; i = intestine; ic = intestinal caecum.

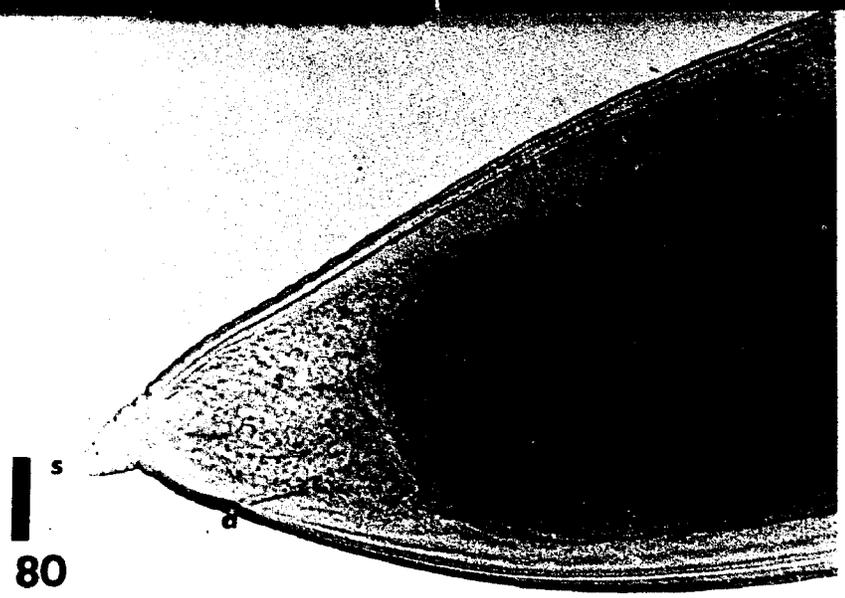
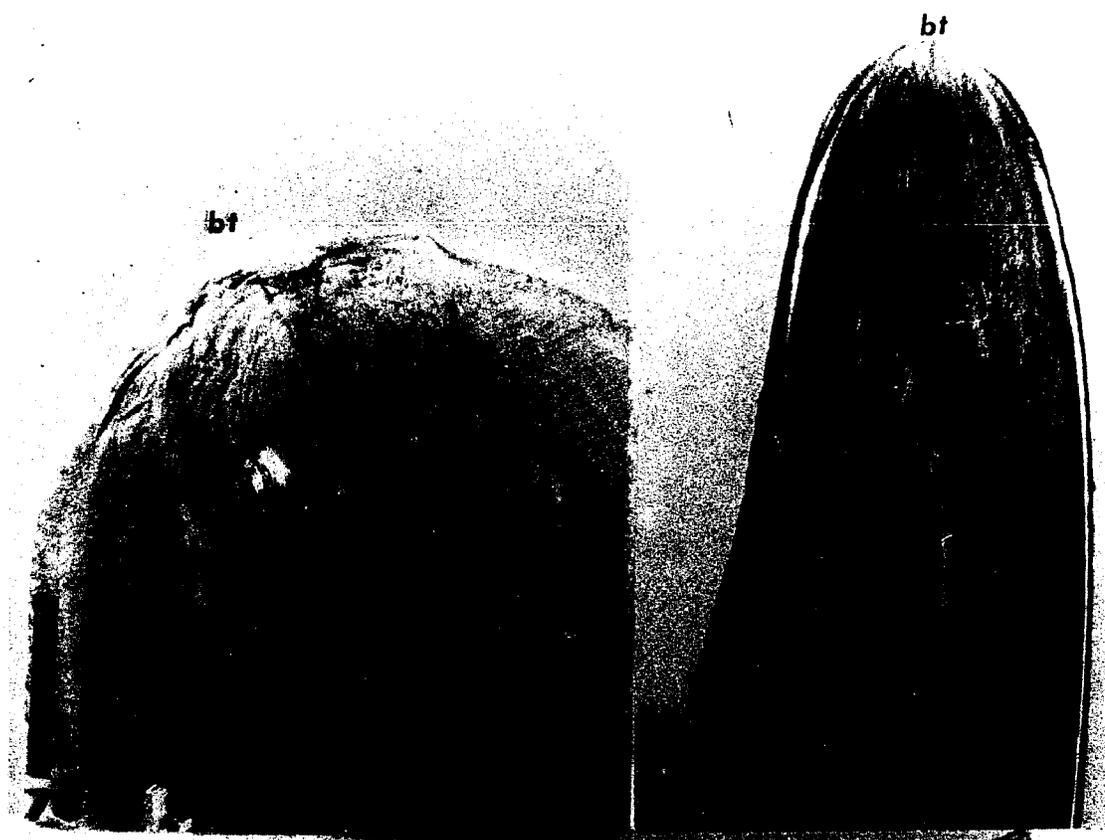


Fig 81. Lateral view of ventricular region of larval Contracaecum osculatum. Cleared in glycerin. Brightfield microscopy.

Fig 82. Lateral view of anterior intestine of larval Contracaecum osculatum. Cleared in glycerin. Interference microscopy.

Bar = 0.1 mm; e = excretory gland nucleus; i = intestine.

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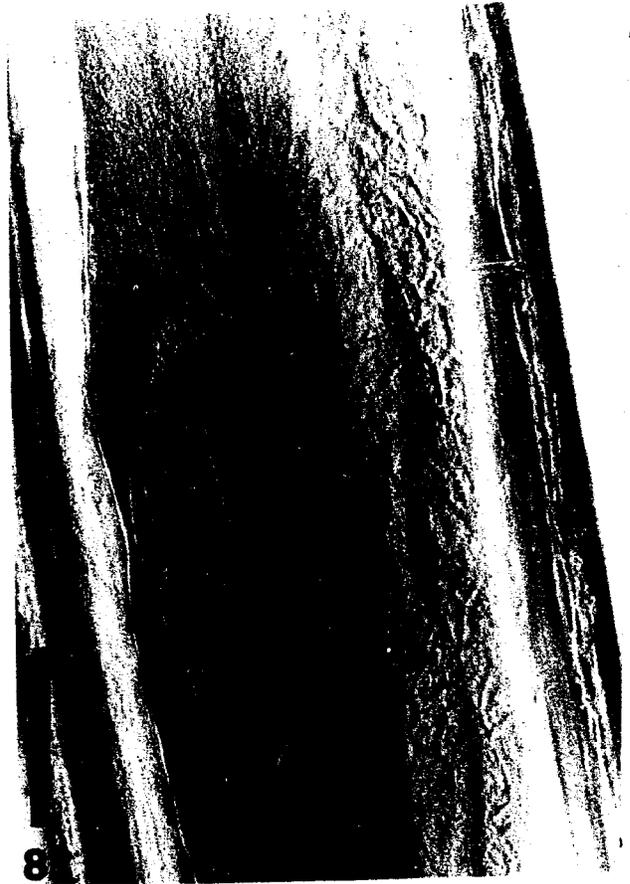


Fig 83. Cross section through the anterior end of a larval Thynnascaris sp. Stained with azure A and eosin B.

Fig 84. Cross section through the nervering of a larval Thynnascaris sp. Stained with azure A and eosin B.

Fig 85. Cross section through the esophagus of larval Thynnascaris sp. Stained with azure A and eosin B.

Fig 86. Cross section through the ventriculus of larval Thynnascaris sp. Stained with azure A and eosin b.

Fig 87. Cross section through the intestine of larval Thynnascaris sp. At level of the mid-intestine. Stained with azure A and eosin B.

Bar = 0.1 mm; e = esophagus; eg = excretory gland; i = intestine; ic = intestinal caecum; l = lip base; lc = lateral cord; nr = nerve ring.

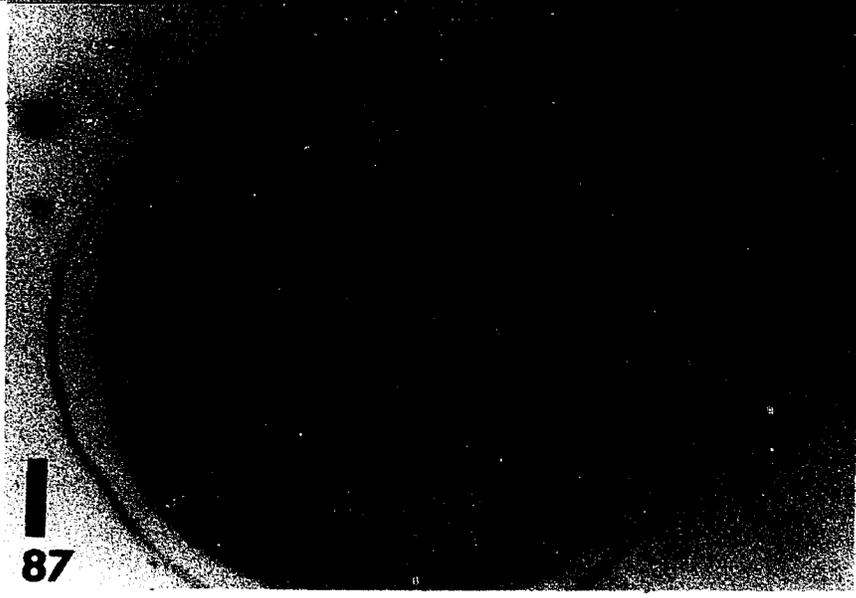
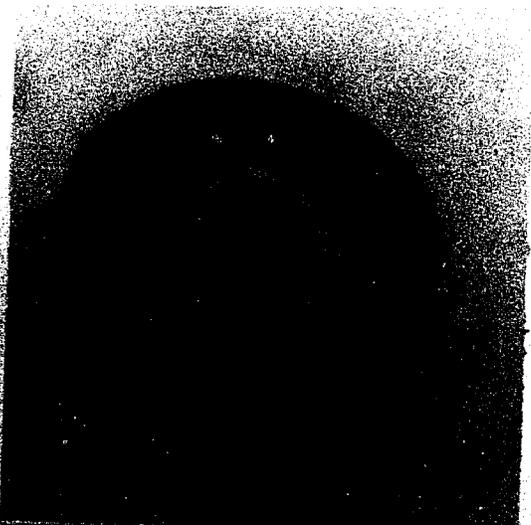
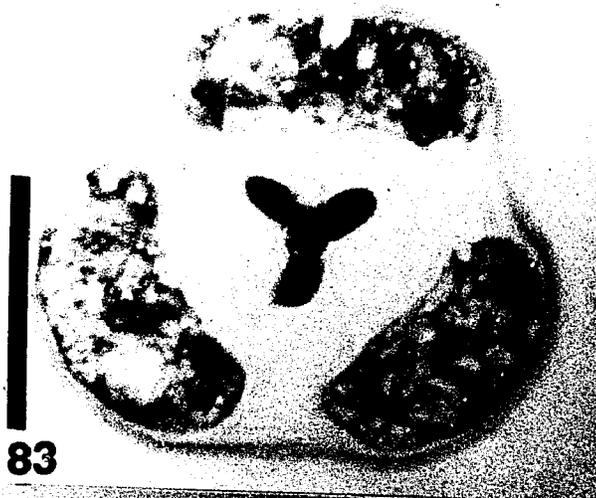
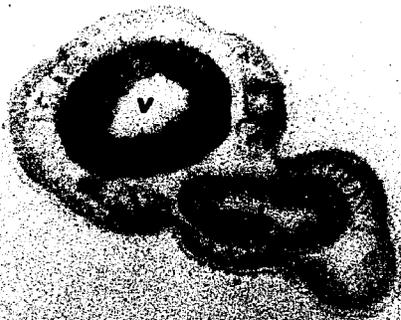


Fig 88. Cross section through the ventriculus of a larval Porrocaecum sp. Stained with azure A and eosin B.

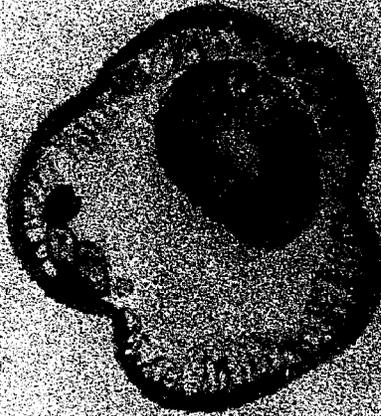
Fig 89. Cross section through the anterior intestine of a larval Porrocaecum sp. Stained with azure A and eosin B.

Fig 90. Cross section through the mid-intestine of a larval Porrocaecum sp. Stained with azure A and eosin B.

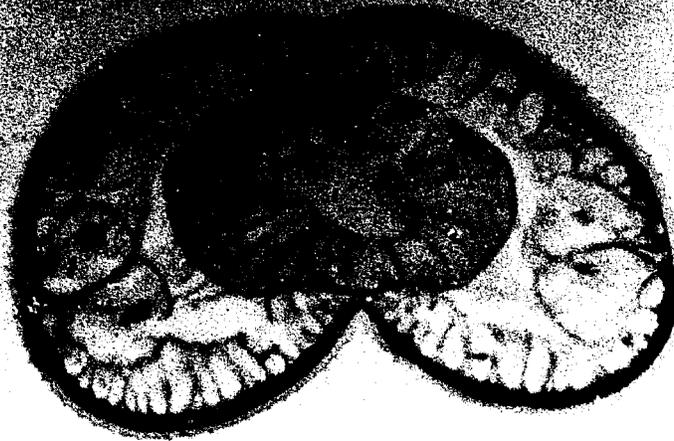
Bar = 0.1 mm; g = excretory gland; i = intestine; l = lateral cord; v = ventriculus.



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Fig 91. Cross section through the lips of a larval Raphidascaris acus. Stained with azure A and eosin B.

Fig 92. Cross section through the nerve ring of a larval Raphidascaris acus. Stained with azure A and eosin B.

Fig 93. Cross section through the esophagus of a larval Raphidascaris acus. Stained with azure A and eosin B.

Fig 94. Cross section through the ventriculus of a larval Raphidascaris acus. Stained with azure A and eosin b.

Fig 95. Cross section through the mid-intestine of a larval Raphidascaris acus. Stained with azure A and eosin b.

Bar = 0.01 mm; i = intestine; l = lateral cord; lp = lip primordium; nr = nerve ring; v = ventriculus; va = ventricular appendix.

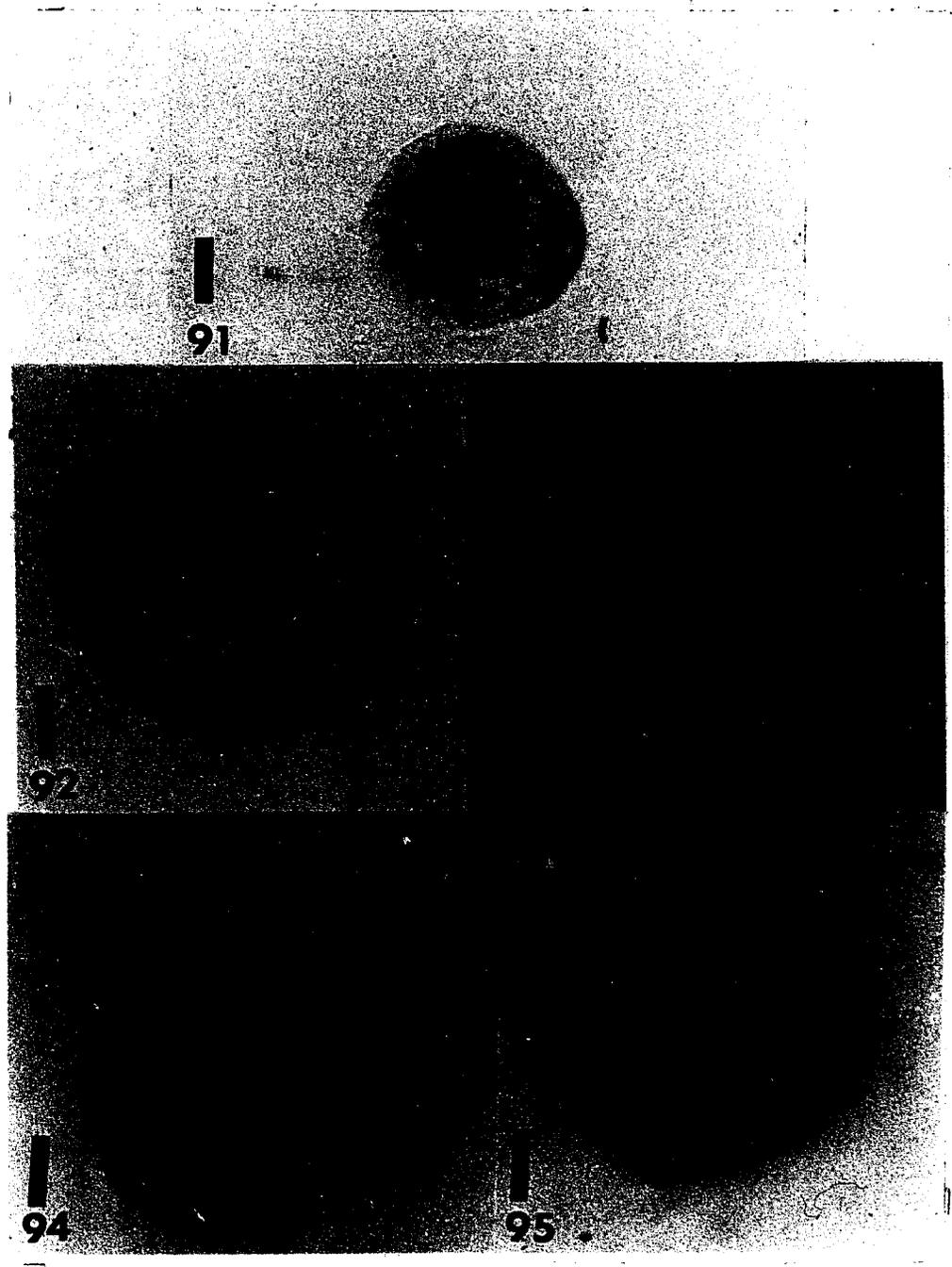


Fig 96. Cross section through the base of the lips of a larval Phocanema decipiens. Stained with hematoxylin and eosin.

Fig 97. Cross section through the esophagus of a larval Phocanema decipiens. Stained with hematoxylin and eosin.

Fig 98. Cross section through the ventriculus of a larval Phocanema decipiens. Stained with hematoxylin and eosin.

Bar = 0.1 mm; e = esophagus; ed = excretory duct; g = excretory gland; i = intestine; ic = intestinal caecum; v = ventriculus.



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Fig 99. Cross section through the ventricular intestinal junction of a larval Phocanema decipiens. Stained with hematoxylin and eosin.

Fig 100. Cross section through the intestine of a larval Phocanema decipiens. Stained with hematoxylin and eosin.

Fig 101. Cross section through the rectum of a larval Phocanema decipiens. Stained with hematoxylin and eosin.

Bar = 0.1 mm; i = intestine; l = lateral cord; g = excretory gland; r = rectum; rl = rectal ligament; v = ventriculus.



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Fig 102. Azure A-eosin B stained section through a Phocanema decipiens lesion at the level of the muscularis mucosā. Lesion in the fundus of a pig fed 100 larvae 2 days prior to necropsy.

Fig 103. Azure A-eosin B stained section through a Phocanema decipiens. Located in the submucosa of the fundus of a pig fed 100 larvae 7 days prior to necropsy.

ac = attachment cap; Bar = 0.1 mm; c = nematode cuticle; m = mucosa; mm = muscularis mucosa; i = dense infiltrate.

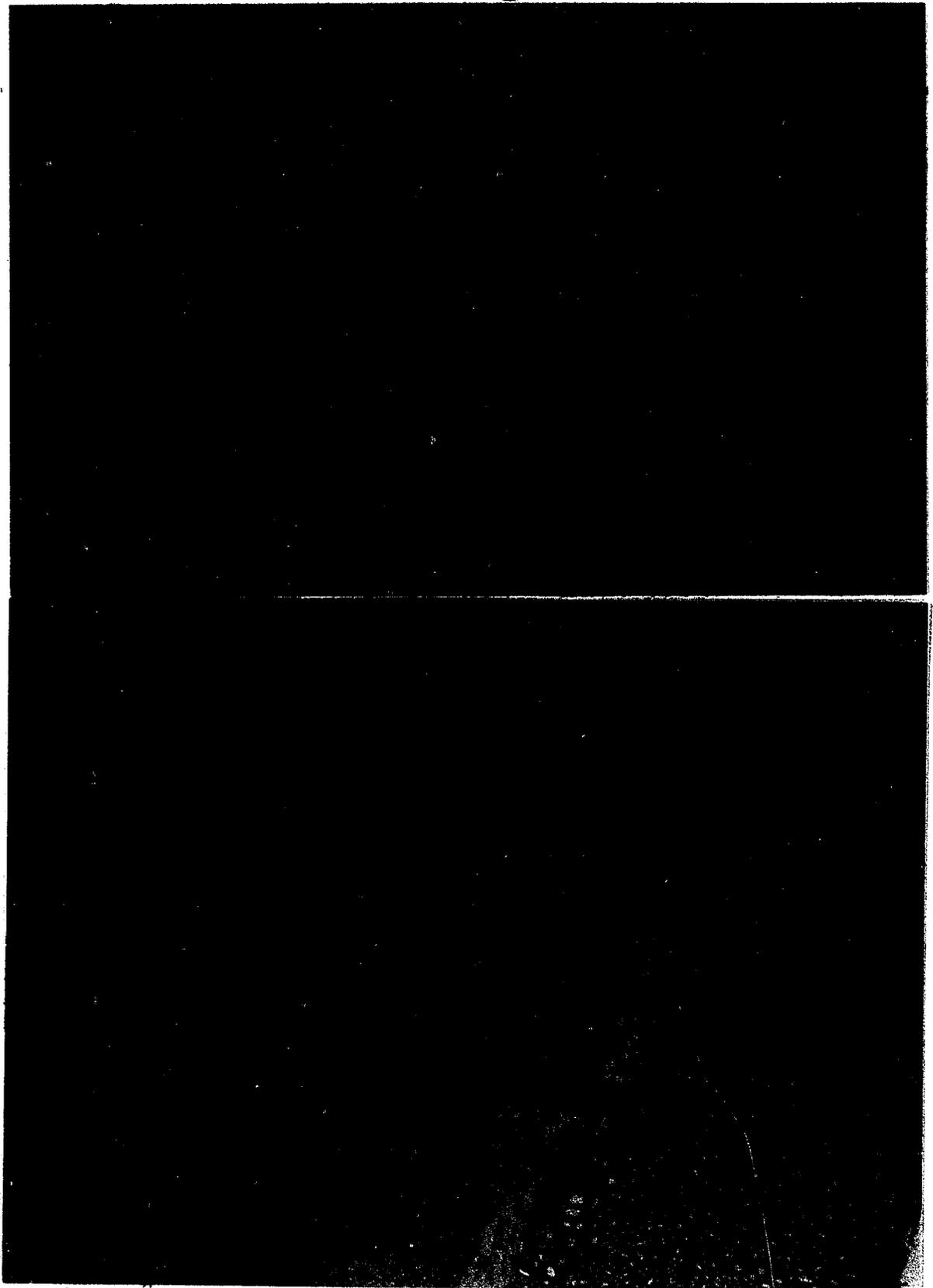


Fig 104. Azure A-eosin B stained section of the fundic submucosa. Pig was fed 100 Phocanema decipiens larvae 7 days prior to necropsy. A larva was located at the center of the dense infiltration.

Fig 105. Azure A-eosin B stained section through the anterior end of a Phocanema decipiens larva and attachment cap located at the level of the muscularis mucosa in the fundus region of a pig stomach. The pig was fed 100 P. decipiens larvae 1 day prior to necropsy.

ac = attachment cap; Bar = 0.1 mm; es = esophagus; e = eosinophil; m = mucosa; mm = muscularis mucosa; sm = submucosa.

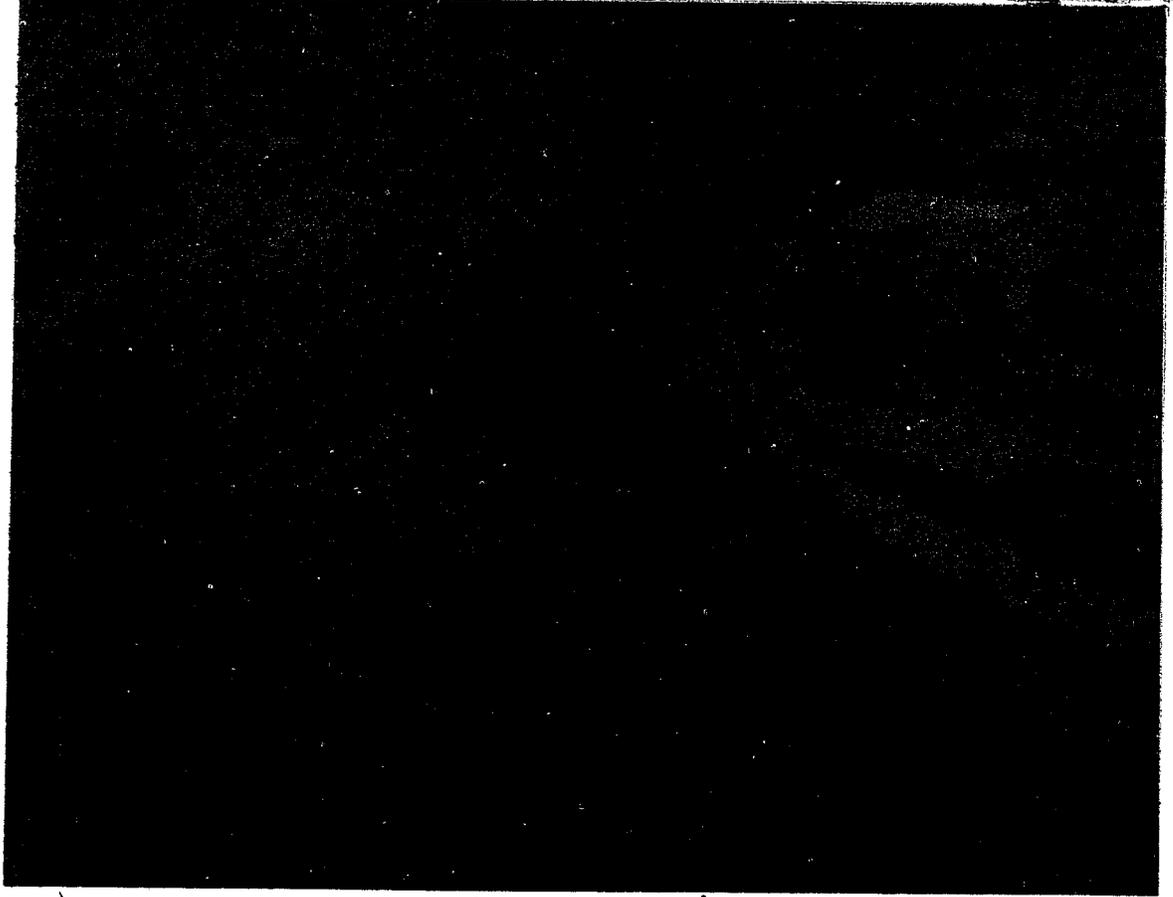
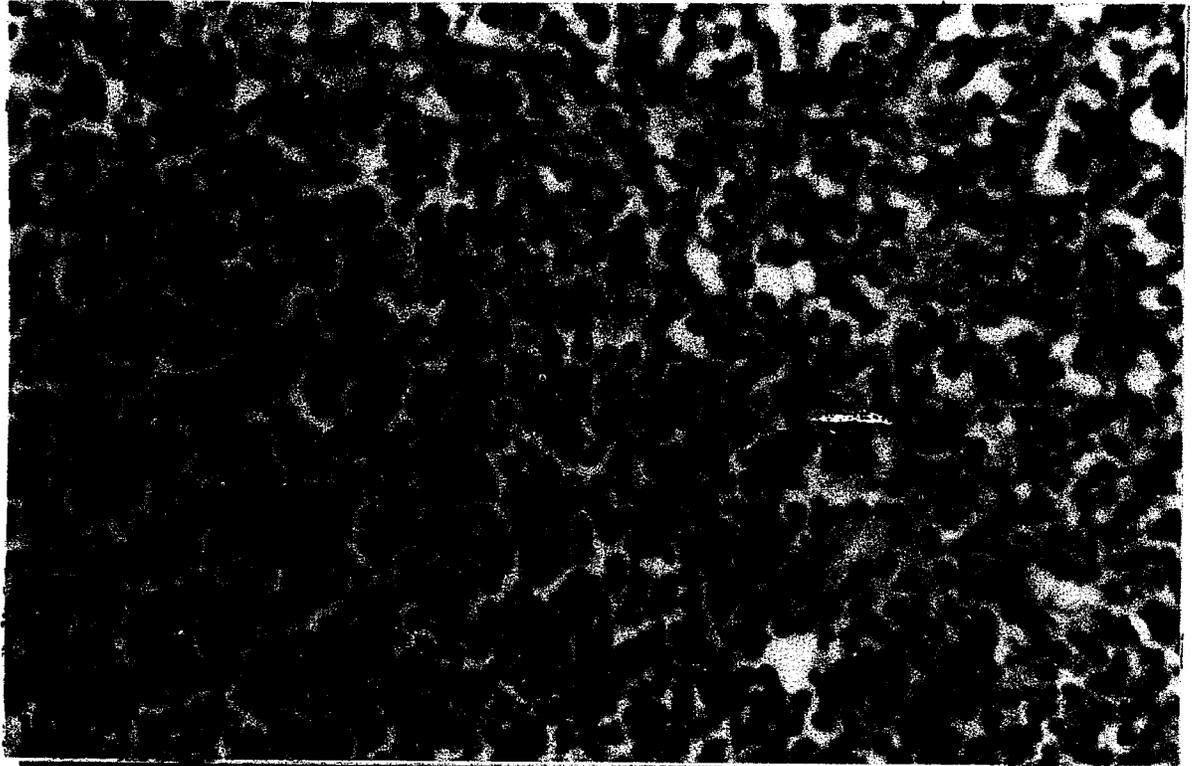


Fig 106. Azure A-eosin B stained section at level of the mucosa in the fundus region of a pig stomach. The pig was fed 100 Phocanema decipiens larvae 4 days prior to necropsy. Fibroblast-like cells are present adjacent to the nematode.

Fig 107. Azure A-eosin B stained section of the fundus region of a pig stomach at the level of the submucosa. The pig was fed 100 Phocanema decipiens larvae 15 days prior to necropsy. A lesion containing a cluster of 4 nematodes was present in the adjacent tissues.

Bar = 0.1 mm; e = eosinophil; f = fibroblast-like cell; l = Phocanema decipiens larva; sm = submucosa.

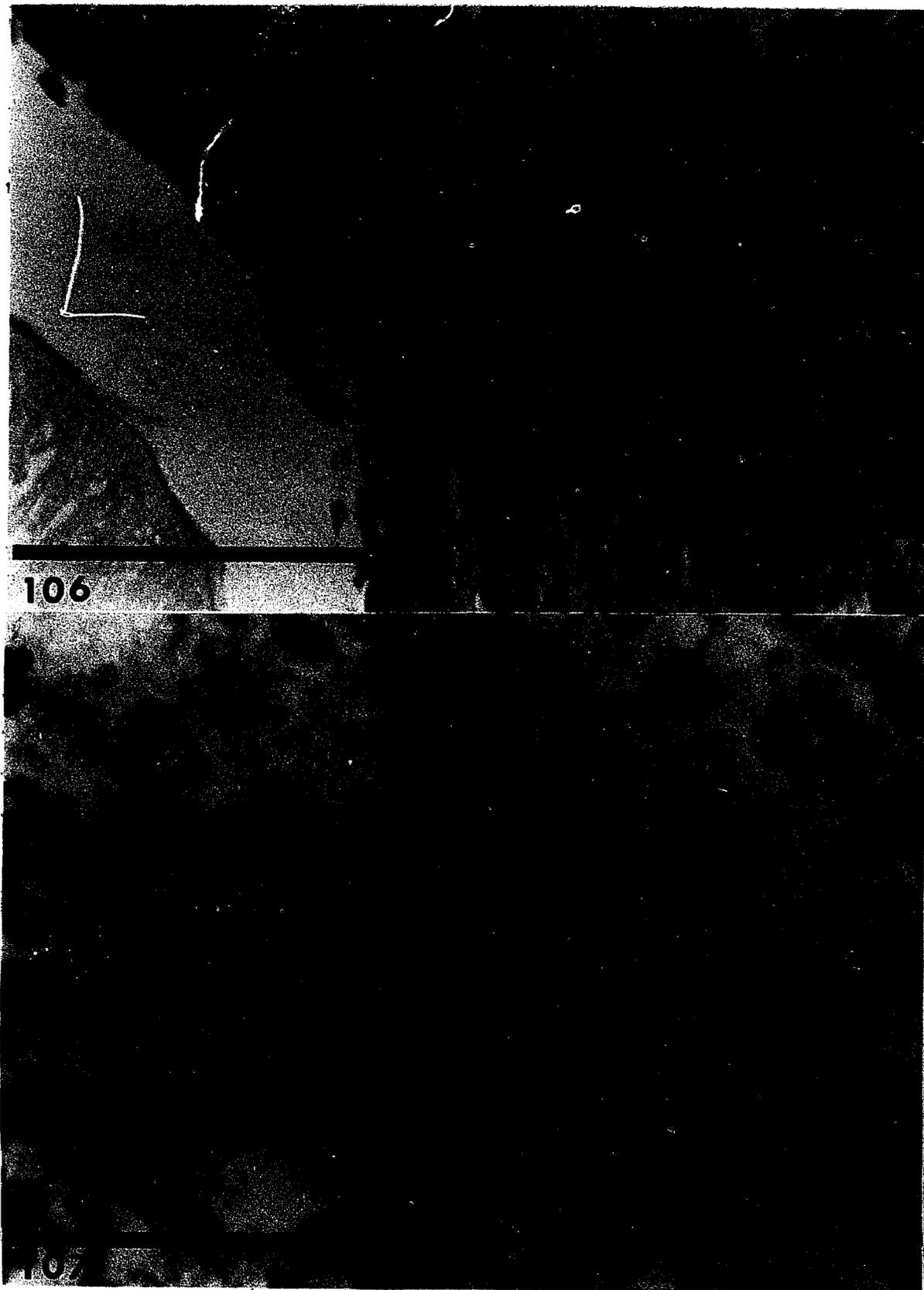


Fig 108. Azure A-eosin B stained section of the fundus region of a pig stomach with a cross section of an Anisakis sp. larva in the submucosa. The pig was fed 100 Anisakis sp. larvae 4 days prior to necropsy. The tissue is heavily infiltrated by eosinophils adjacent to the larva with diffuse infiltration surrounding this area.

Fig 109. Azure A-eosin B stained section of the submucosa from the fundus region of the stomach of a pig fed 100 Anisakis sp. larvae 4 days prior to necropsy. A larva was located 1.5 mm from the edge of the region photographed, labeled l. Eosinophils are less dense here than adjacent to the larva.

Bar = 0.1 mm; e = eosinophil; l = direction of larva; la = larval Anisakis sp; m = mucosa; mm = muscularis mucosa; sm = submucosa.

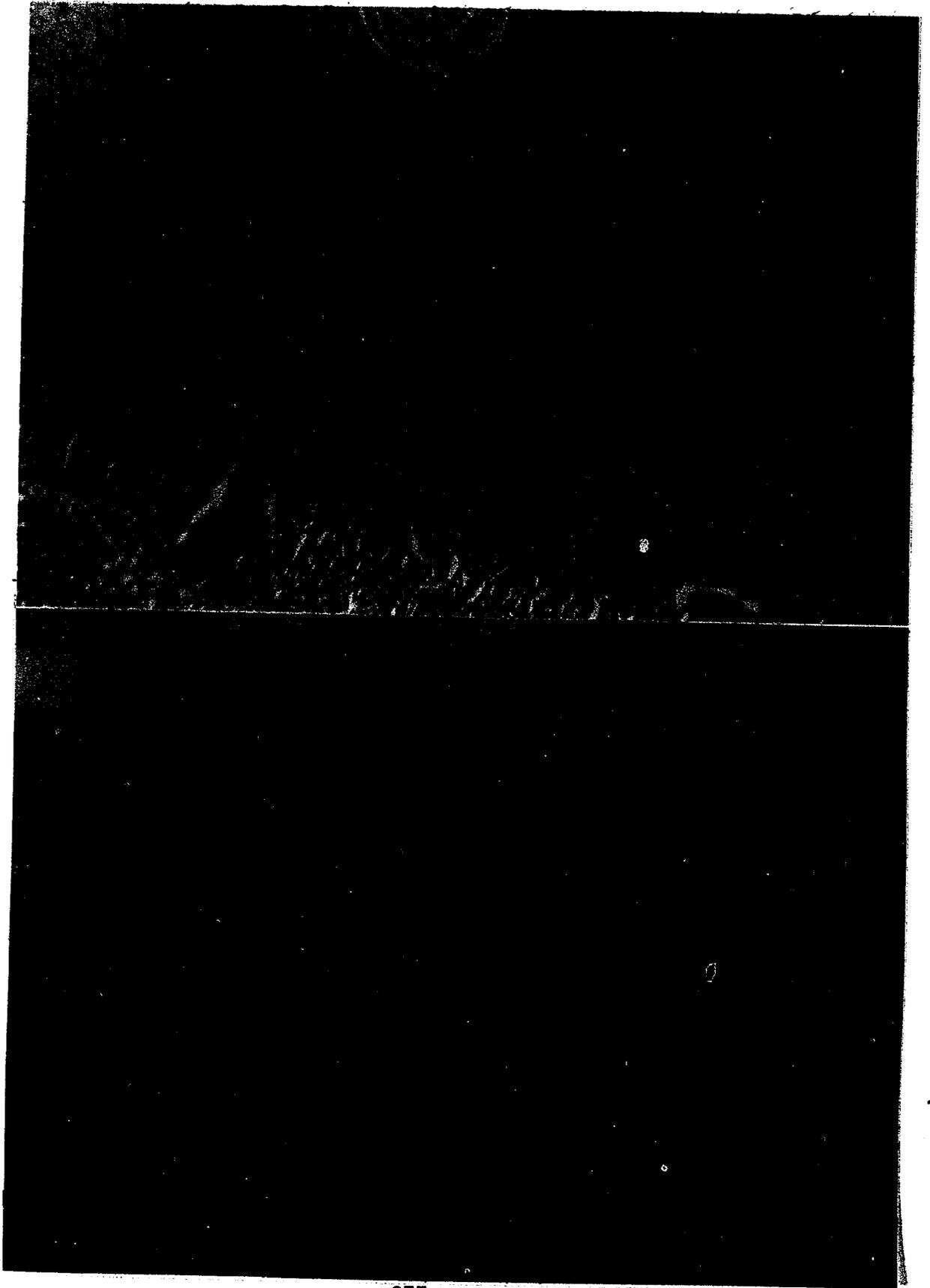


Fig 110. Azure A-eosin b stained section of an attachment cap in the mucosae of the fundus region of the stomach. Pig fed 100 Anisakis sp. 3 days prior to necropsy.

Fig 111. Azure A-eosin B stained section through an Anisakis sp. larvae completely embedded in the submucosae of the fundus region of the pig stomach. One hundred larvae (100) were fed 7 days prior to necropsy. The cellular infiltrate consists almost entirely of eosinophils in this area.

ac = attachment cap; Bar = 0.1 mm; e = eosinophil; la = larval Anisakis sp; m = mucosa; sm = submucosa.

