

Anisakis and Anisakiasis

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I. INTRODUCTION

An enormous literature has accumulated on the general biology of *Anisakis* Dujardin, 1845, and on the "accidental" infection of man and other animals with this nematode, a condition called anisakiasis. Certain other genera of

marine anisakine nematodes, including *Phocanema* (= *Terranova* = *Porrocaecum*) and *Contracaecum*, have also been implicated in human infection (see, for example, Jackson, 1975, Williams and Jones, 1976). These conditions have also been called anisakiasis (Cheng, 1976). However, this review is concerned only with *Anisakis sensu stricto* and with accidental infections associated with this genus.

This review has been restricted to *Anisakis* not only because members of this genus appear to be the most abundant of the marine anisakines but also because current knowledge indicates that they may be the most important with regard to human infection, at least on a worldwide basis.

That various developmental stages of *Anisakis* and other marine anisakines often occur together in the same individual hosts during the course of "normal" life histories has resulted in considerable taxonomic and other confusion. For this reason it has proved impossible in this review to avoid mention of other genera, but this may be rewarding in that available information on a related genus may help towards an understanding of the biology of *Anisakis*.

Larval stages of anisakine nematodes were probably first recognized in fish hosts as early as the 13th century (Myers, 1976). The period since 1945 has seen a vast increase in the output of relevant literature, for which there appear to be two main reasons.

First, economic anxiety concerning the occurrence of larval nematodes in the flesh of cod caught in Western North Atlantic waters and elsewhere led to scientific investigation of the biology and life history of anisakines in relation to populations of final hosts, with particular reference to *Phocanema* and seals (see, for example, Myers, 1960, and references therein).

Secondly, and most significantly with regard to the increase in the literature during the past 15 years or so, this period saw the first reports by Dutch workers (e.g. Van Thiel *et al.*, 1960) of a larval nematode from herring in the gastrointestinal tract of man in The Netherlands. At first the worm was incorrectly identified as "*Eustoma rotundatum*" but later corrected to *Anisakis* by Van Thiel (1962). This discovery prompted the formation in 1960 in The Netherlands of an "*Anisakis* Committee" to co-ordinate research (Ruitenberg, 1970). Subsequently an anisakiasis problem in man in Japan was recognized with the formation in that country in 1965 of a "research group on parasitic granuloma" which worked for three years with government aid (Oshima, 1972).

With the literature explosion that has accompanied these research activities it is not surprising that several review-type papers dealing wholly or partly with *Anisakis* and related genera have appeared in recent years, including those by Dollfus (1970), Ruitenberg (1970), Oshima (1972), Euzéby (1973), Van Thiel (1976) and Williams and Jones (1976). Certain aspects of anisakiasis in man, especially pathology and immunology, were reviewed by Areán (1971), Bissereu (1975) and Soulsby (1976). Five papers (i.e. those by Jackson, 1975; Myers, 1975; Cheng, 1976; Norris and Overstreet, 1976; Bier, 1976) were developed from presentations at a symposium held in New York in 1974 entitled "Anisakiasis: a new disease from raw fish."

The aim of this review is to bring together the many and varied aspects of

the extensive world literature on *Anisakis* and anisakiasis (*sensu stricto*), including use of the nematode as a biological tag in applied fishery science. Selectivity has been inevitable because the relevant literature is so enormous but we hope that no important information has been omitted.

II. CLASSIFICATION OF *ANISAKIS*

The classification scheme for nematodes of the superfamily Ascaridoidea proposed by Hartwich (1974), based largely on features of the "excretory system", is adopted in this review. Keys to the higher taxa shown in the following complete classification of *Anisakis* are given by Anderson *et al.* (1974).

Class Nematoda

Subclass Secernentea

Order Ascaridida

Superfamily Ascaridoidea

Family Anisakidae

Subfamily Anisakinae

Tribe Anisakinea

Genus *Anisakis* Dujardin, 1845.

A clue to some aspects of the taxonomic confusion that exists in the literature regarding *Anisakis* Dujardin, 1845, lies in the following list of synonyms given by Hartwich (1974) for this genus:

Anisakis (*Skrjabinisakis*) Mozgovoi, 1951; *Capsularia* Zeder, 1800, *nec* Modeer, 1793; *Conocephalus* Diesing, 1861, *nec* Thunberg, 1812; *Filocapsularia* Deslongchamps, 1824, *nom. oblit.*; *Peritrachelius* Diesing, 1861; *Stomachus* Goeze in Zeder, 1800, *nom. nud.*

III. TAXONOMY AND ADULT MORPHOLOGY OF *ANISAKIS*

In a revision of the genus *Anisakis*, Davey (1971a) gave the following generic diagnosis:

Three lips each bearing a bilobed anterior projection which carries the single dentigerous ridge; interlabia absent; excretory gland with duct opening between ventrolateral lips; oesophagus with anterior muscular portion (preventriculus) and posterior ventriculus, the latter being oblong and sometimes sigmoid or else as broad as long; no oesophageal appendix or intestinal caecum; vulva in middle or first third of body; spicules of male unequal; preanal papillae numerous; postanal papillae including a group of three or four pairs set close to the tip of the tail on the ventral side.

Habitat: Stomach and intestine of marine mammals.

Genotype: *Anisakis simplex* (Rudolphi, 1809, det. Krabbe, 1878).

Confronted with the descriptions, some very poor, of 21 species of the genus *Anisakis*, Davey (1971a) undertook critical examination of the extent of individual variation in taxonomic characters; he concluded that there are only three valid species (with 14 synonyms between them) and retained four others as *species inquirendae* for lack of sufficient data, as follows:

Valid species

1. *A. simplex* (Rudolphi, 1809, det. Krabbe, 1878) (with 10 synonyms).
2. *A. typica* (Diesing, 1860) (with one synonym).
3. *A. physeteris* Baylis, 1923 (with three synonyms).

Species inquirendae

1. *A. dussumierii* (van Beneden, 1870), from a dolphin in the Indian Ocean near the Maldiv Islands.
2. *A. schupakovi* Mozgovoï, 1951, from Caspian seal in the Caspian Sea (redescribed and regarded as valid by Delyamure *et al.*, 1964—see also below).
3. *A. alexandri* Hsü and Hoeppli, 1933, from *Sotalia sinensis* in the (?) China Sea.
4. *A. insignis* (Diesing, 1851) from *Inia geoffrensis* in South American rivers (redescribed and regarded as valid by Petter, 1972—see also below).

The key provided by Davey (1971a) for the identification of adult *A. simplex*, *A. typica* and *A. physeteris* contains an error—two references are made to “oesophagus” when “ventriculus” is clearly intended. Davey claimed that the morphological characters in order of importance for identifying the three species named in his key are the spicules, the postanal papillae, the form of the ventriculus, the position of the vulva, and the form of the lips. These species are said to be satisfactory when discussed in geographical and ecological terms; the marine mammalian hosts are discussed in section V.

Kurochkin (1975) and, as mentioned earlier, Delyamure *et al.* (1964) redescribed *A. schupakovi* Mozgovoï, 1951, from Caspian seal (*Pusa caspica*) and regarded it as a valid species. Apparently Davey (1971a) was not acquainted with this redescription of *A. schupakovi* at the time of his revision of the genus; however, Kurochkin (1975) stated that “later Davey (personal communication) confirmed the validity of this species”. On ecological grounds alone, *A. schupakovi* would appear to be valid in that its host (*P. caspica*) is a geographically isolated relict species confined to the Caspian Sea. Mokhayer (1974) referred to larval *A. schupakovi* in young sturgeons in the Caspian Sea.

Davey (1971a) did not refer to *Anisakis pacificus* Skryabin, 1959, from a sperm whale and other cetaceans in the northwestern Pacific Ocean; examination of the description of this species in relation to Davey's revision of the genus suggests that *A. pacificus* is a synonym of *A. simplex* (Dr. D. I. Gibson, personal communication). Oshmarin and Demshin (1972) described *A. rugocephalus* from “dolphins” in Vietnamese waters; the validity of this species is also doubtful and its possible synonymy with *A. simplex* should be investigated.

As mentioned above, *A. insignis* was regarded as a valid species by Petter (1972); her illustrated redescription, based on male and female specimens from *Inia geoffrensis* in the Rio Ibaré (Bolivia), ascribed certain morphological features to this species which have interesting implications for the generic

diagnosis given above and for the taxonomic criteria of Davey (1971a). These features as well as certain other aspects warrant further discussion.

According to Davey (1971a) the spicules of *Anisakis* spp. are unequal, the right one being consistently shorter than the left, and their ratio is useful taxonomically. However, Petter (1972) stated that the two thin alate spicules of *A. insignis* are identical. Furthermore, Petter referred to two cuticular folds forming two "superposed collars" at the base of the lips in *A. insignis*, distinctly visible in adult specimens but barely formed in young ones; this feature does not appear to have been reported for other species.

Despite Davey's (1971a) valuable discussion and revision of the genus, there still appear to be problems concerning the taxonomic distinction between *A. simplex* and *A. typica*. For example, Oshima (1972) reported that he was unable to distinguish the females of *A. typica* in blue-white dolphins (*Stenella caeruleoalba*) containing males of both *A. simplex* and *A. typica*. Furthermore, the possibility that eggs obtained from these female worms represent different "strains" adds further confusion (see Bier, 1976; and section IV. B).

Whilst Young and Lowe (1969) referred in the text of their paper to *A. typica* from marine mammals in British waters, confusion may arise if readers overlook a short erratum slip attached to this paper stating that "taxonomic studies by Davey (in press) suggest that the adult specimens of *Anisakis* recorded in this paper are probably *Anisakis simplex* and not *Anisakis typica*".

Nomenclatural instability has also added to problems of the taxonomic status of *A. simplex*. Van Thiel (1966) proposed the name "*Anisakis marina* (Linnaeus, 1767)" for the species occurring in marine mammals in the North Sea and South Atlantic and this name occurs in more recent literature (e.g. Ruitenbergh, 1970). However, following the arguments put forward by various workers, including Khalil (1969) and Davey (1971a), Van Thiel (1976) agreed to use the name *A. simplex* (and not *A. marina*) to avoid confusion.

With regard to adult morphology, Grabda (1976) has recently provided adequate illustrated descriptions of adult male and female *A. simplex*, including cross-section morphology at different levels, so the following account is restricted to certain morphological features which, among others, may have received inadequate attention.

Inglis (1964a) described and illustrated the cephalic septum and lips of *A. simplex*, *A. physeteris* and other ascaridoids; he argued that the septum enables lip movements to be controlled largely by muscles of the oesophagus with some assistance from the body-wall muscles which, in *Anisakis*, are of the polymyarian—coelomyarian type.

A comparative study of the complex structure of the basically three-layered cuticle in *A. simplex*, *A. physeteris* and other ascaridoids was also made by Inglis (1964b). The cuticles of the two *Anisakis* species differed in several details including topography of the outer surface. In *A. simplex*, the outer surface (apart from the anterior tip of the body) is highly modified by the development of large posteriorly overlapping, flanged annulations; this feature is not apparent in *A. physeteris*.

Davey (1971a) referred to a pair of cervical papillae near the anterior end of the body as characteristic of all species of *Anisakis*, whilst Petter (1972) described two lateral deirids slightly below the nerve ring in *A. insignis*. Beverley-Burton *et al.* (1977) mentioned lateral cervical papillae about 0.3 mm behind the nerve ring in larval *A. simplex*. McLaren (1976) stated that cervical papillae and deirids are different names for the same sense organs and speculated that they may be responsible for determining whether or not the nematode can pass successfully through a restricted space.

A mucron (tail spine) at the tip of the tail has been claimed to be "a larval feature normally lost at the final moult" (Davey, 1971a) but not all "larval types" of *Anisakis* appear to possess one (see section IV. E). According to Beverley-Burton *et al.* (1977) the mucron of larval *A. simplex* (a type (I) larva) is retractable and it is tempting to suggest, therefore, that it functions in penetration but evidence is lacking. Davey (1971a) stated that adult female *Anisakis* sometimes possesses what looks like a mucron, sunk back into a fold of cuticle, but that adult males never have such a structure.

With regard to certain aspects of the anterior part of the alimentary tract of adult *Anisakis*, Davey (1971a) reported that the junction of the preventriculus with the ventriculus (component parts of the oesophagus) is suggestive of a sphincter-like arrangement in all species; the feeding mechanism of *Anisakis* has not been studied in detail so the function of the supposed sphincter is unclear. According to Davey (1971a), the ventriculus in *A. simplex* is often, but not invariably, sigmoid and always longer than it is broad; that of *A. typica* is essentially similar. The ventriculus of *A. physeteris* is short, never sigmoid and may be broader than long; the short form of the ventriculus appears to have given rise to the assumption that *Anisakis* larva (II) develops into this species (see also section IV. E2).

IV. LIFE HISTORY, GENERAL DEVELOPMENT AND LARVAL MORPHOLOGY OF *ANISAKIS*

A. GENERAL LIFE-HISTORY PATTERN

According to various authors (e.g. Chitwood, 1974) the life history of a "typical" nematode shows the following course of development characterized by four moults:

Egg—First stage larva—(1st moult)—Second stage larva—(2nd moult)—Third stage larva—(3rd moult)—Fourth stage larva—(4th moult)—Fifth stage: adult.

The term "larva" is used here because of its extensive usage in the literature, although the terms "juvenile" (Hyman, 1951) or "nymph" (Chitwood, 1974) may be more appropriate. Various adaptations or modifications to this basic sequence are seen in different groups of nematodes and such is the case with *Anisakis* and other ascaridoids.

Before embarking on a discussion of the life history of *Anisakis* and its developmental stages, it should be pointed out that problems of semantics and terminology feature among the many difficulties associated with interpretation

of the literature on this nematode genus and its relatives. The fourth-stage larva of *Anisakis* is often referred to as a "preadult", whilst McClelland and Ronald (1974b) in an account of *Contracaecum osculatatum* refer to this stage as a "subadult" and use "preadult" for the fifth stage (presumably an immature adult). Other terms used loosely in the relevant literature include "exsheathment", and the "transfer", "transport", "paratenic" and "intermediate" host concepts but it would be invidious to particularize. Odening (1976) drew attention to the need for clarification of concepts and terminology of hosts and parasites and attempted this in a lengthy account.

Field observations indicate that crustaceans (especially euphausiids), squid and fish (especially teleosts) are hosts of *Anisakis* larvae, whilst marine mammals (especially cetaceans) harbour larvae and egg-producing adults. The highly speculative scheme for the life history of *Anisakis* given in Fig. 1 is based on an appraisal of the relevant literature; it is intended as a companion to the following discussion and is offered with due reserve. The reader's attention is also directed to the pictorial representations of the life history given by Oshima (1972) and by Williams and Jones (1976).

The hosts referred to in Fig. 1 may be regarded as "natural" hosts featuring in the course of the "normal" life history of *Anisakis*. Other animals, including man, may be regarded as "accidental" hosts which acquire infection by eating fish or squid harbouring live *Anisakis* larvae; such larvae either do not develop at all or may develop to the preadult (fourth) stage—in either case they ultimately die and represent a loss to the parasite population. Various other aspects of accidental infections are dealt with elsewhere in this review.

With regard to the "normal" life history, there are difficulties in assigning a role to the various hosts named in Fig. 1 and to assessing the relative importance of each, particularly in view of the paucity of field observations and the lack of experimental evidence.

The first moult evidently takes place within the egg; the larva which hatches is second stage and bears the cuticle of the first stage as a sheath (Smith, 1971; Van Banning, 1971; Oshima, 1972). There is evidence that *Anisakis* undergoes two moults in the marine mammalian final host (Kagei *et al.*, 1967; Kikuchi *et al.*, 1967; Jacobsen and Berland, 1969; Gibson, 1970), presumably the 3rd and 4th. If these observations are correct, three of the four moults of a "typical" nematode are accounted for (i.e. the 1st, 3rd and 4th moults) but what of the remaining (2nd) moult? It has been assumed by authors that it is the third-stage larva of *Anisakis* which is infective to the final host and that this stage occurs in fish and squid. This implies that a second moult has taken place, but in which host? Some Japanese workers (e.g. Oshima, 1972) are convinced that a moult occurs in a crustacean (euphausiid) host but experimental proof is lacking. Other workers (e.g. Kagei, 1969) indicated that the moult takes place in fish but again without experimental demonstration. Yet others are non-committal or obscure on this point. Larvae from crustaceans, squid and fish collected in the field are morphologically similar but detailed morphological comparisons of larvae from these different sources are lacking.

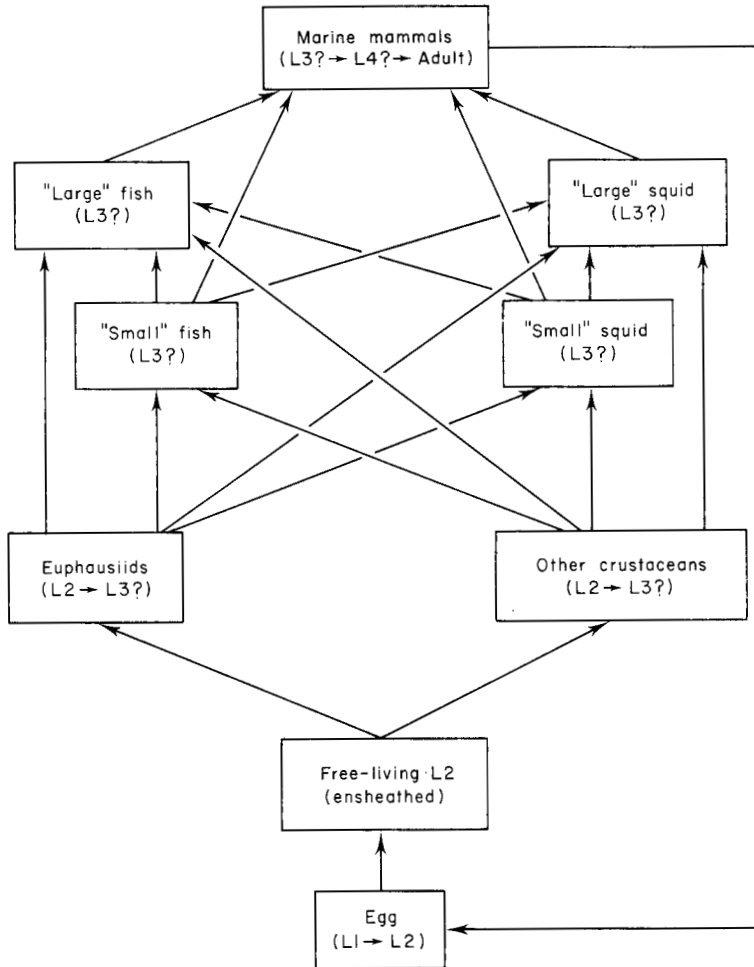


FIG. 1. Speculative life-history pattern of *Anisakis*.

What little evidence there is suggests that larvae in crustaceans (or at least in euphausiids) grow from less than 0.33 mm up to 30 mm or more in length and it might be argued that such length increase with its accompanying increase in girth is unlikely to occur without the intervention of a moult.

If a moult does occur in crustaceans then these are "intermediate" hosts, whilst fish and squid are "paratenic" hosts. If this is the case, then larvae from crustaceans might be expected to be directly infective to final hosts (a possibility not shown in Fig. 1)—unless it is argued that "conditioning" of larvae in fish or squid is required before they are infective.

Experimental evidence that larvae are capable of transferring from fish to fish in prey-predator relationships without apparent morphological change (Smith, 1974) perhaps indicates that a moult does not occur in fish. Sluiter (1974) reported that *Anisakis* larvae from the stomach lumen of herring were consistently smaller than those recovered from the body-cavity of this host; this observation and Sluiter's conclusion that larvae grow in herring require confirmation. In-vitro observations by, for example, Sommerville and Davey (1976) and Grabda (1976) on larvae from fish indicate that this stage does not feed; apparently feeding does not begin until after the moult to the preadult (fourth) stage.

Published evidence concerning the life histories of certain other aquatic ascaridoid nematodes is not especially helpful in interpreting that of *Anisakis*. For example, McClelland and Ronald (1974a,b) compare the results of their in-vitro culture work on *Terranova* (= *Phocanema*) *decepiens* and *Contracaecum* *osculatum* with the field observations of other workers; eggs were used to initiate the cultures. They suggest that these nematodes may develop "neotenually" from second stage to infective stage under natural conditions—the infective stage being third stage in the case of *C. osculatum* but fourth stage in the case of *P. decepiens*. Intriguing but unresolved problems of this nature are discussed by Cheng (1976) and the reader's attention is also directed to the life history accounts by Norris and Overstreet (1976) for *Thynnascaris* spp. of fish and by Huizinga (1966, 1967) for *Contracaecum* spp. of piscivorous birds.

Work with *Anisakis* in vitro has been restricted largely to cultures initiated with larvae from fish (see section VI). It would be of great value to undertake culture work starting with the egg and to couple this approach with a study of experimental infection in crustaceans.

Information on the morphology and general biology of the egg, second-stage larva and of the larval stages of *Anisakis* in crustaceans, squid and fish is given in the following sections.

B. EGG

Adult female *Anisakis* recovered from blue-white dolphins in Japanese waters by Kobayashi *et al.* (1966, 1968a) were associated with male worms, most of which were identified as *A. simplex* but some as *A. typica*. According to Oshima (1972) the female worms were of two morphological types, one slender bodied with the vulva in the anterior half of the body, the other stout bodied with the vulva in the posterior half. Both female types yielded morphologically indistinguishable ellipsoidal eggs measuring $45.5\text{--}58.1 \times 41.3\text{--}53.2\text{ }\mu\text{m}$ which, in terms of time required for hatching at various temperatures from 2 to 27°C, appeared to represent either of two "strains"—one adapted to high temperatures, the other to low temperatures. As Bier (1976) has indicated, this finding "clouds the already confused taxonomic distinction between *A. simplex* and *A. typica* which Davey (1971a) had seemingly resolved by associating *A. typica*, the species of the stout females, with warmer climates".

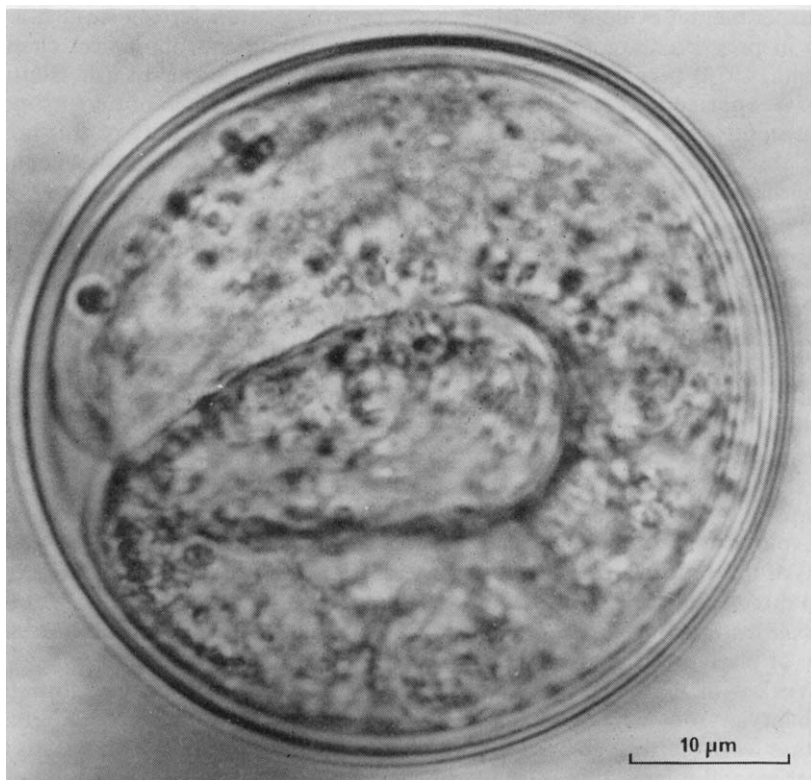


FIG. 2. Embryonated egg of *Anisakis* (bar scale, 10 µm).

The worms cultured *in vitro* by Van Banning (1971) and subsequently identified as *A. simplex* by Pippy and Van Banning (1975) produced eggs round to oval in shape and measuring 40×50 µm on average. These eggs hatched in 4–8 days at 13–18°C and in 20–27 days at 5–7°C.

The eggs are apparently passed unembryonated with the faeces of marine mammals and subsequently embryonate in sea water (Fig. 2). According to Sluiter (1974) the eggs of *Anisakis* sink in sea water and may reach the seabed by the time of hatching; however, the rate of sinking will depend on local conditions, e.g. upwelling of currents, and the hatch time evidently depends on temperature.

C. SECOND-STAGE LARVA

The free-living larva which emerges from the egg is apparently a second stage and ensheathed in the cast cuticle of the first moult which evidently takes place within the egg (Fig. 3). The larval body narrows posteriorly, shows a nerve ring and bears an anterior boring tooth, whilst the alimentary

tract is poorly differentiated (Van Banning, 1971; Oshima, 1972) but further detailed information is lacking. Average length without the sheath is given as 230 μm by Van Banning (1971) and as 286 μm by Oshima (1972); average length with the sheath is 355 μm according to Van Banning. Smith (1971) gives a length range of about 0.22–0.29 mm without the sheath, and of about 0.33–0.37 mm with the sheath. Larvae are very active and survive without exsheathing in sea water for three to four weeks at 13–18°C, and for six to seven weeks at 5–7°C. Increasing mortality was seen at temperatures above 20°C and 34°C was lethal (Van Banning, 1971).

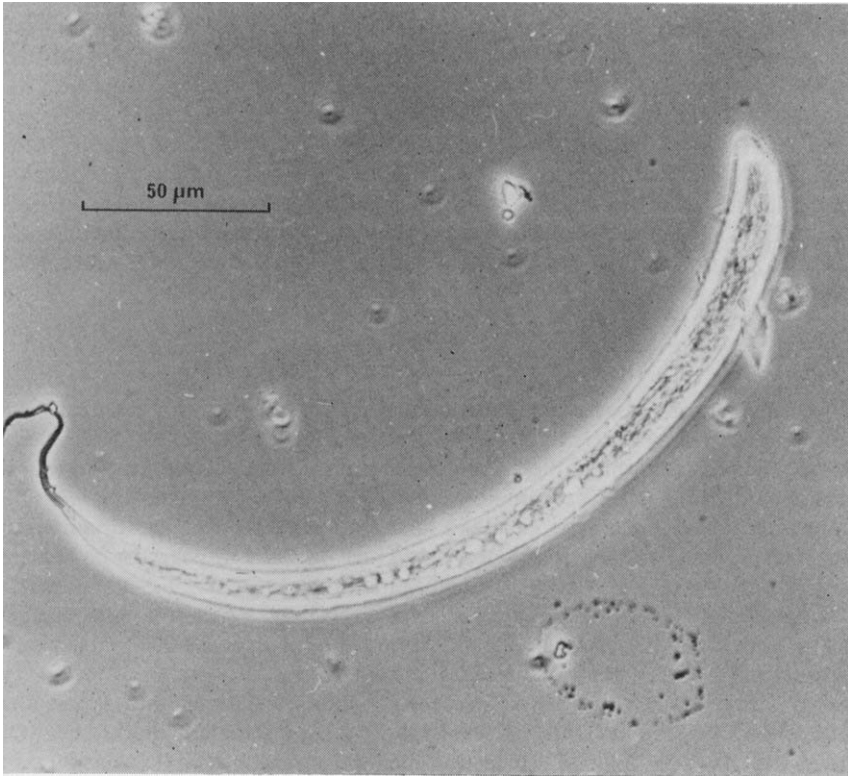


FIG. 3. Free-living, ensheathed second-stage larva of *Anisakis* (bar scale, 50 μm).

With regard to the fate of hatched larvae, workers have been influenced by a) the apparent importance of crustaceans in the life histories of other aquatic ascaridoids and b) the importance of crustaceans in the diet of fish heavily infected with *Anisakis* larvae. These factors have led to field surveys for larval *Anisakis* in marine crustaceans (see section IV. D). However, there is little experimental evidence concerning the mode of entry into crustaceans or subsequent development of the ensheathed larvae and further work is

necessary. Japanese workers (Oshima, 1969; Oshima *et al.*, 1968; Kagei, 1969) have attempted experimental infection of euphausiids, and their results are summarized by Oshima (1972). In two experiments, ten *Euphausia similis* and six *E. pacifica* were exposed singly to large numbers of hatched larvae and killed at intervals of 1–8 days after exposure. The eggs from which the larvae were hatched originated from female worms associated with males identified as *A. simplex* (mostly) with some *A. typica*. Interpretation of the tabulated results suggests that six of the 16 euphausiids became infected with a total of 18 larvae, of which nine had exsheathed. The larvae were found in the haemocoel of the euphausiids and the data indicate that at least some multiple infections occurred. The lengths and widths of the larvae, which had spent varying periods up to eight days in euphausiids, fell within the range of those for freshly-hatched larvae; the data are not strictly comparable because different methods of fixation were used. The observations could not be continued because no euphausiid survived beyond eight days. Lasker and Theilacker (1965) drew attention to the problems of maintaining euphausiids alive in the laboratory for long periods. They suggest a method for capturing undamaged euphausiids which are then nurtured individually in the laboratory and each offered several thousand newly hatched *Artemia salina* nauplii. Such an approach may yield suitable experimental material for work with *Anisakis*.

D. LARVAE IN MARINE CRUSTACEANS

As indicated above, field surveys of marine crustaceans for *Anisakis* have been conducted by several authors with some success (Table 1). The crustaceans referred to in this table are all of the subclass Malacostraca and represent the following orders: Euphausiacea (*Thysanoessa*, *Meganyctiphanes* and *Euphausia*), Decapoda (the caridean shrimp, *Pandalus* and the brachyuran crab, *Hyas*) and Amphipoda (*Caprella*). Thus *Anisakis* appears to be loosely host specific even at this stage of the life history.

Table 1 refers only to surveys which have yielded positive results. Kagei (1974) tabulated the results of an examination of over 67 000 crustaceans (representing three different orders) collected in different years from 19 widely scattered localities in the Antarctic Ocean, waters around Japan, the East China Sea, Sea of Okhotsk, Northern North Pacific and Bering Sea. Of these, only two euphausiids from one locality harboured larval *Anisakis*. In the smaller area of the Northern North Sea and European North Atlantic, Smith (1971) found a patchy distribution of larval *Anisakis* in euphausiids with infection apparently absent at many localities. Collard (1970) stated that "Brinton (pers. comm.) in his exhaustive studies of the Euphausiacea . . . noted that nematode larvae are not abundant in these caustaceans (*sic*), and the author failed to find them in any of several thousand examinations". Noble (1973) remarked that he "recently examined 983 euphausiids taken from Californian coastal waters and not one was infected with nematodes or any other metazoan parasite".

The few surveys conducted so far indicate a low prevalence of infection in

TABLE 1
Anisakis larvae in marine crustaceans

Locality	Hosts	No. examined	No. infected (% infected)	References
Barents Sea	<i>Caprella septentrionalis</i> <i>Hyas araneus</i> <i>Thysanoessa raschii</i>	855 990 ?	1 (0·117) 1 (0·101) 1	Uspenskaya (1963)
European North Atlantic and Northern North Sea	<i>Thysanoessa inermis</i> <i>T. longicaudata</i> <i>Meganycitiphanes norvegicus</i>	2730 950 3178	18 (0·659) 3 (0·316) 1 (0·031)	Smith (1971)
Bristol Bay (Bering Sea) and Northern North Pacific	<i>Thysanoessa raschii</i> <i>T. longipes</i> <i>Euphausia pacifica</i>	121 438 54000	3 (2·479) 3 (0·685) 1 (0·002)	Shimazu and Oshima (1972)
East China Sea	<i>Euphausia pacifica</i>	28219	2 (0·007)	Kagei (1974)
Sea of Japan	<i>Pandalus borealis</i>	5046	5 (0·099)	Shiraki <i>et al.</i> (1976)
Sea of Okhotsk	<i>Pandalus kessleri</i>	724	2 (0·276)	Shiraki <i>et al.</i> (1976)

crustaceans (Table 1) and only one larva per infected host; this may not be inconsistent with the known abundance of *Anisakis* larvae in fish for the following reasons. With regard to euphausiids, for example, the population density is often high and they are eaten in enormous quantities by fish (Mauchline and Fisher, 1969), which may therefore acquire large numbers of larvae despite the low prevalence of infection in euphausiids. Furthermore, larvae are apparently long-lived in fish and therefore tend to accumulate with increasing host age (e.g. Bishop and Margolis, 1955; Davey, 1972a) and larvae may transfer from fish to fish in prey-predator relationships (Smith, 1974). It should be stressed that whilst present evidence suggests that euphausiids are of importance among the crustacean hosts of *Anisakis*, further work is necessary to assess the relative importance of these and other invertebrates in the life history. No detailed morphological study of larvae from crustaceans is available. The 41 larvae reported so far ranged in length from 5.1 mm (Smith, 1971) to 32.7 mm (Oshima *et al.*, 1969). In addition to these 41 larvae, Sluiter (1974) referred to an *Anisakis* larva, 9.4 mm long, which was apparently emerging from a euphausiid, *Thysanoessa raschii*, in the stomach of a North Sea herring. In general morphology (especially in relation to characteristics of the ventriculus and tail) all 42 larvae appear to resemble *Anisakis* larva (I) of Shiraki (1974) and *Anisakis* sp. larva (I) of Berland (1961) from fish and squid (see section IV. E1).

Reimer *et al.* (1971) referred to larvae of *Anisakis* from chaetognaths in the North Sea as being (in translation) "typical for the genus in the structure of the powerful oesophageal bulb without caeca"; this brief description lacks an illustration and may fit that of some other nematode genus (possibly not even an ascaridoid). These authors also stated that "Uspenskaya (1963) indicates that larvae of *Anisakis* are found in euphausiids and chaetognaths" but we can find no reference to larval *Anisakis* in chaetognaths in Uspenskaya (1963) or in Uspenskaya (1955, 1960).

In Uspenskaya (1963) there is an illustrated description of a larval "*Eustoma rotundatum* (Rudolphi, 1819)" from the anomuran decapod *Lithodes maja* in the Barents Sea which appears to resemble larval *Anisakis*. Vivares (1972/73, 1973) also referred to *Eustoma rotundatum* (without illustration) in three decapod crustaceans in the Mediterranean. The status of these forms is uncertain, especially since the names "*E. rotundatum*" or "*E. rotundata*" are implicated in the confused taxonomy and biology of another ascaridoid genus, namely, *Pseudanisakis* (see, for example, Williams and Richards, 1968; Gibson, 1973, 1974).

E. LARVAE IN MARINE FISH AND SQUID

The abundant literature on larval anisakids (and other ascaridoids) in marine fish and squid is complicated by a variety of taxonomic and nomenclatural problems, many of which remain unresolved. The state of this confusion with regard to larvae in fish may be judged from the papers by, for example, Punt (1941), Baylis (1944), Dollfus (1953, 1970), Templeman *et al.* (1957), Berland (1961) and Myers (1975) and with reference to the

many earlier papers cited by these authors. Although a detailed account of these problems is outside the scope of this review, it may be helpful to point out that complications have arisen from attempts to identify larval nematodes to genus or even species on somewhat tenuous morphological grounds, despite lack of knowledge of the life history and the absence of experimental evidence.

According to Myers (1975) the literature since 1767 contains over 100 descriptions of anisakine larval stages, the classification of which is too often based on arbitrary measurements. On the basis of anterior digestive tract formation and position of the "excretory" pore, Myers (1975) recognized five "larval types" to which generic names are applied loosely, viz. *Anisakis* (*sensu lato*) larvae, *Phocanema* (*sensu lato*) larvae, *Contracaecum* (*sensu lato*) larvae, *Raphidascaris* (*sensu lato*) larvae and *Multicaecum* (*sensu lato*) larvae.

Hartwich (1974) proposed a classification of adult nematodes of the superfamily Ascaridoidea based largely on the "excretory system". If the Hartwich scheme reflects phylogenetic relationships (as it is claimed to do), then the groupings and subgroupings of genera under the "larval types" proposed by Myers appear highly artificial in that genera from different families, subfamilies or tribes are associated together. For example, with regard to the topic of this review, under *Anisakis* (*sensu lato*) larvae is a subgroup comprising *Anisakis* (Anisakidae: Anisakinae: Anisakinea), *Paranisakiopsis* (Anisakidae: Raphidascaridinae: Paranisakinea) and *Acanthocheilus* (Acanthocheilidae), characterized by a ventriculus lacking both intestinal caecum and with the "excretory" pore opening at the base of the subventral lips. (It is relevant to mention that Gibson and Taylor in 1976 suggested transfer of *Paranisakiopsis* to Anisakinae.)

In a detailed account of the morphology and morphometrics of larval anisakids in fish and squid from northern coastal waters of Japan, Shiraki (1974) recognized four "larval types" of *Anisakis*, namely *Anisakis* larva (I), (II), (III) and (IV), along with other larval types representing *Terranova* (= *Phocanema*), *Contracaecum*, *Raphidascaris* and *Thynnascaris*; much of the Japanese work in this field during the past decade or so was reviewed.

Shiraki's "larval types" of *Anisakis* fall into the *Anisakis* (*sensu lato*) larvae group (*Anisakis*—*Paranisakiopsis*—*Acanthocheilus* subgroup) of Myers (1975) but, in view of the problems outlined above, further comment is not possible. The following discussion of Shiraki's "larval types" in relation to the *Anisakis* larvae reported by European and other workers is presented with some reserve.

The diagrammatic representations of the anterior and the posterior end of an *Anisakis* larva given in Fig. 4 are intended to facilitate discussion. The descriptive terms used for the anterior part of the alimentary tract are those of Myers (1975). With regard to descriptive terms, the following variations are among those which occur in the literature and which may lead to confusion: oesophagus (= pharynx); preventriculus (= muscular oesophagus); ventriculus (= glandular oesophagus). Furthermore, the measurements given for the oesophagus by some workers appear to exclude the ventriculus.

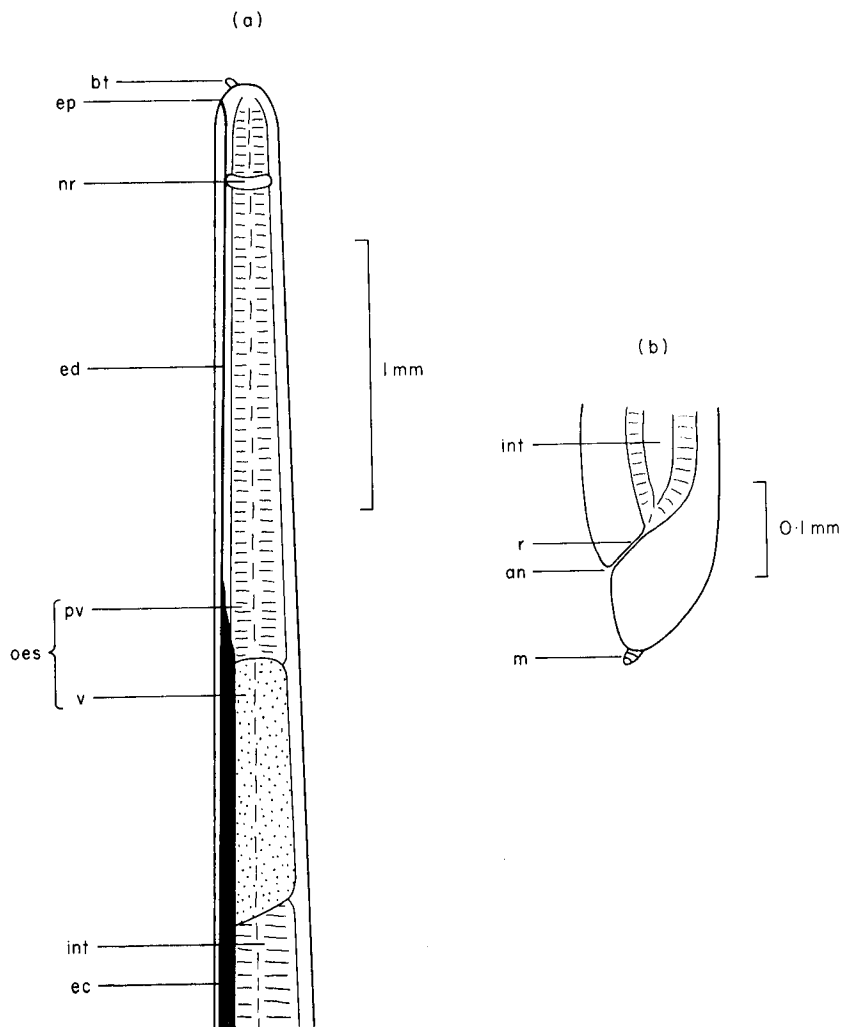


FIG. 4. Diagram of larval *Anisakis* from fish (a) anterior end (bar scale, 1 mm), (b) posterior extremity (bar scale, 0.1 mm)—anal glands omitted. Anus, an; boring tooth, bt; "excretory" cell, ec; "excretory" duct, ed; "excretory" pore, ep; intestine, int; mucron, m; nerve ring, nr; oesophagus, oes; preventriculus, pv; rectum, r; ventriculus, v.

Japanese and other workers have given detailed ratios of the length of various parts of the body to total body length for larval *Anisakis* (and other larval anisakids); such ratios are not considered in this review because they may be of value only if account is taken of allometric growth in nematodes (Inglis, 1954) and if appropriate morphometric analyses are undertaken (see, for example, the approach used by McClelland and Ronald, 1974a,b).

1. *Anisakis larva* (I)

This larval type is described by Shiraki (1974), Oshima (1972) and Koyama *et al.* (1969) as possessing a relatively long ventriculus with an oblique ventricular-intestinal junction, and a rounded tail bearing a mucron.

The head bears one dorsal and two ventrolateral lips; between the ventrolateral lips is an anteroventrally directed boring tooth, just behind which opens the "excretory" pore. Some morphometric data are given in Table 2.

Japanese workers regard this larval type as identical to *Anisakis* sp. larva (I) of Berland (1961) from teleosts in Norwegian waters. Such larvae from herring in the northern North Sea were cultured *in vitro* to the adult stage by Van Banning (1971) and identified as *Anisakis simplex* (Rudolphi, 1809, det. Krabbe, 1878) by Pippy and Van Banning (1975).

The recent illustrated description of the larva by Beverley-Burton *et al.* (1977), including a comparison with the earlier descriptions by Berland (1961), Grainger (1959), Punt (1941) and earlier workers, provides some interesting new information on aspects of morphology; unfortunately, comparable detailed information is not available for other larval types. The "excretory cell" shows anterior extensions of the nucleus and a bifurcation of the duct in its narrow posterior part; neither of these features was mentioned by Lee *et al.* (1973) in their ultrastructural study of the "excretory system" of unspecified *Anisakis* larvae, or by Mueller (1927) in his study of the adult *A. simplex* system. The "excretory cell" lies ventrally and somewhat to the left side in the anterior region of the pseudocoel; it is elongate to oval in cross section, except at the extremities where it is rounded, and is contiguous to hypodermal cells, alimentary canal and, occasionally, somatic musculature. In the intestinal region the cell shows a connection with the left lateral chord. The presence of one dorsal and two small ventrolateral oesophageal glands confirms Hsü's (1933) observations on adult *Anisakis*. The terminal tail spine (mucron) is said to be retractable. The observation of three rectal (anal) glands (two dorsal, one ventral) is interesting in relation to the two rectal glands reported by Sluiter (1974) in an *Anisakis* larva (lacking a mucron and apparently moulting) which appeared to be emerging from a euphausiid in a herring stomach. Sluiter's observations are relevant to the controversy surrounding the timing and place of the supposed second moult in *Anisakis* (see also Section IV. A) but require confirmation; the position of rectal glands as illustrated by some authors indicates a possible confusion with the four epithelial cells which, according to Beverley-Burton *et al.* (1977), externally line the short intestinal-rectal valve.

That larvae from herring and salmon at widely separated localities in the North Atlantic and North Sea are morphologically similar suggested to Beverley-Burton *et al.* (1977) that Type I larvae represent a single species. However, genetic heterogeneity (acid phosphatase polymorphism) among the different samples suggested the presence of either more than one species or more than one population of the same species. Involvement of larval *A. physeteris* was discounted because "larval *A. physeteris* (= *Anisakis* Type II larvae) are likely morphologically different from Type I larvae (Oshima,

TABLE 2

Some morphometric features of four "larval types" of Anisakis from northern coastal waters of Japan. Mean: range measurements (in mm) in each case. (Taken from Shiraki, 1974)

Larval type	No. of specimens	Total length	Maximum Width	Preventriculus length	Ventriculus length	Tail length	Host(s)
(I)	9	28.4:23.0-31.7	0.49:0.45-0.52	2.06:1.76-2.33	1.30:1.08-1.46	0.12:0.10-0.13	Teleosts and squid
(II)	13	25.7:21.6-31.9	0.61:0.53-0.71	2.15:1.80-2.51	0.58:0.47-0.71	0.28:0.21-0.38	Teleosts and squid
(III)	19	28.9:23.8-38.4	0.84:0.65-0.97	1.83:1.46-2.33	0.57:0.47-0.73	0.16:0.10-0.20	Teleosts
(IV)	9	20.1:14.8-26.4	0.54:0.41-0.67	1.59:1.24-2.18	0.38:0.30-0.59	0.15:0.11-0.19	Teleost (<i>Theragra chalcogramma</i>)

1972)". Larval *A. simplex* and *A. typica* may be morphologically and morphometrically similar but from a consideration of the supposed geographic range of the two species (see Davey, 1971a) in relation to the provenance of their samples, Beverley-Burton *et al.* (1977) conclude that only one species (*A. simplex*) is represented by Type I larvae in herring and salmon in the North Atlantic but that more than one population of this species may exist.

If *Anisakis* larva (I) of Japanese workers and *Anisakis* sp. larva (I) (= *A. simplex*) are identical, then this species is abundant in a great variety of fish and in squid in many localities (see section V).

The length range (23.0–31.7 mm) given for this larval type by Shiraki (1974) may be conservative; from juvenile herring in the North Sea the writers (unpublished observations) have recovered larvae 8.8 mm long and upwards, whilst Koyama *et al.* (1969) referred in a table to larvae up to 36.0 mm long.

2. *Anisakis larva (II)*

According to Shiraki (1974), Oshima (1972) and Koyama *et al.* (1969) this larval type is characterized by a short ventriculus with horizontal ventricular-intestinal junction, and a long, conical, tapering tail lacking a mucron. The head is similar to that of larva (I) but the boring tooth is said to be larger and directed anteriorly. For some morphometric data see Table 2.

Japanese workers regard this larval type as identical to *Anisakis* sp. larva (II) of Berland (1961). Some workers (e.g. Oshima, 1972) claim that it may be the larva of *A. physeteris* but proof is lacking; culture of the larva *in vitro* may be rewarding.

Larva (II) does not appear to be as common as larva (I) at least in Japanese waters (see section V). Berland's (1961) specimens of *Anisakis* sp. larva (II) came from *Lampris guttatus* in Oslo Fjord (Norway). According to Wheeler (1969), *L. guttatus* is uncommon in northern European waters; it seems to be most common in middle depths of the open ocean (especially south of Europe) and, perhaps significantly, feeds extensively on pelagic squid.

3. *Anisakis larva (III)*

This larval type is described by Shiraki (1974) as possessing a stout body, short ventriculus with horizontal junction, and a short, rounded tail without mucron (although Oshima, 1972, and Otsuru *et al.*, 1968, 1969, referred to a short, conical tail with a small mucron). The head is apparently similar to that of larva (II). Some morphometric data are given in Table 2.

Shiraki (1974) reported larva (III) in three teleost species in Japanese waters (see section V). The relationship of this larval type to an adult remains unknown; in-vitro culture does not appear to have been attempted.

4. *Anisakis larva (IV)*

According to Shiraki (1974) and Otsuru *et al.* (1969) this larval type is relatively small, has a short ventriculus with horizontal junction, and a short,

conical, pointed tail without mucron. The head is apparently similar to that of larva (II). For some morphometric data see Table 2.

Larva (IV) was recovered from *Theragra chalcogramma* in Japanese waters (Shiraki, 1974) and, like larva (III), nothing appears to be known of its relationship to an adult.

5. Morphology of *Anisakis* larval types in cross section

With the aim of identifying anisakid larvae in tissue sections of pathological specimens resected from the human alimentary tract, Japanese workers (e.g. Oshima, 1972; Shiraki, 1974) have provided illustrated descriptions of such features as the number of muscle cells in a quadrant of the body, number of intestinal cells, maximum body width and shape of the lateral chords as seen in transverse section at different levels. Discrepancies occur in the information on certain diagnostic features given by Oshima (1972) and by Shiraki (1974) for at least *Anisakis* larva (I) and larva (II), which tends to cast doubt on the merits of this system. Myers (1975) also expressed reservations about some of the criteria but suggested that further study may show morphology of the lateral chords in cross sections to be a valuable characteristic for diagnosis.

V. GEOGRAPHICAL AND HOST DISTRIBUTION OF *ANISAKIS*

The geographical distribution of *Anisakis* and its distribution among host species is a complex and, as yet, poorly understood subject. As a genus *Anisakis* has a worldwide distribution occurring in all major oceans and seas but individual species are in some cases more restricted in distribution.

Anisakis simplex is probably the most cosmopolitan species, occurring worldwide in 23 cetacean and 11 pinniped species according to Davey (1971a). An additional three cetacean and one pinniped species were listed as hosts of *A. simplex* by Dailey and Brownell (1972). Records of *A. simplex* from marine mammals are most frequent in colder temperate and polar waters (Davey, 1971a). Within a given area there may be different populations of *A. simplex*, as suggested by Beverley-Burton *et al.* (1977) for the North Atlantic (see also section IV. E1).

Anisakis typica is listed from only eight cetacean species by Davey (1971a) who also noted that records of this species are restricted to warmer waters between 40°N and 36°S, a region where *A. simplex* is apparently rare. However, the taxonomic distinction between *A. simplex* and *A. typica* is problematical (see also section III and IV. B).

The relative importance of different cetacean species as hosts for *A. simplex* and *A. typica* is impossible to determine due to the lack of data on the population sizes of the hosts and the extent of their infection with *Anisakis*. It is likely that there are significant differences between geographical areas. Thus, Kagei and Kureha (1970) did not find *Anisakis* sp. in *Balaenoptera borealis* and *B. physalus* from the Antarctic Ocean although these species are known to be infected in other regions such as the North Pacific and North Atlantic (Klumov, 1963; Young, 1972).

Whilst pinnipeds have been widely recorded as hosts for *Anisakis simplex*

(Davey, 1971a) they are probably of limited importance in this role. In British waters, for example, *A. simplex* may be numerous in grey seals (*Halichoerus grypus*) but as only a small proportion mature successfully in this host (Young, 1972; Wootten, unpublished observations) it is thought that seals contribute little to the general abundance of the species.

Anisakis physeteris has been recorded from only four cetacean species according to Davey (1971a) and most records are from the sperm whale, *Physeter catodon*. The distribution of *A. physeteris* is worldwide (Davey, 1971a) paralleling that of its major final host.

Of the other species of *Anisakis* which may be regarded as valid, *A. insignis* is restricted to South American rivers, the habitat of its host the Amazon River Dolphin, *Inia geoffrensis* (Petter, 1972) and *A. schupakovi* is found only in the Caspian seal, *Pusa caspica*, from the Caspian Sea (Kurochkin, 1975).

Larval *Anisakis* have an extremely wide distribution in fish and squid both in terms of the range of host species and geographical distribution. The exact distribution of different *Anisakis* species is confused because no larva of *A. typica* has as yet been positively identified and also because many authors do not distinguish between "larval types".

Kagei (1970), Oshima (1972) and Shiraki (1974) listed the occurrence of *Anisakis* larvae by type in fish and squid in Japanese waters. *Anisakis* larva (I) is apparently the most abundant larval type, occurring in 123 fish species and in one squid species (*Todarodes pacificus*) (Oshima, 1972). High rates of infections with larva (I) were found in Pacific pollock (*Theragra chalcogramma*), cod (*Gadus morhua macrocephalus*), common mackerel (*Pneumatophorus japonicus japonicus*), herring (*Clupea pallasii*), horse mackerel (*Trachurus japonicus*), salmon (*Oncorhynchus* spp.) and squid (*Todarodes pacificus*).

Type (II) larvae are less abundant than type (I) larvae in Japanese waters, occurring in 25 fish species and two squid species (*T. pacificus* and *Doryteuthis bleekeri*) (Oshima, 1972).

In Japanese waters, type (III) larvae occur in the teleosts *Theragra chalcogramma*, *Gadus morhua* and *Synphobranchius affinis*, whilst type (IV) larvae have been recovered apparently only from *T. chalcogramma* (Shiraki, 1974).

The occurrence of *Anisakis* larvae in fish and shellfish from Taiwan was listed by Yamaguchi *et al.* (1970) and Chen (1971), and in fish from the Philippines by Jueco *et al.* (1971). Hewitt and Hine (1972) listed 57 fish species as hosts for *Anisakis* larvae in New Zealand waters, including 10 species of elasmobranchs (in which, however, larvae were found only in the stomach); these authors suggested that the New Zealand larvae are probably *A. simplex*.

The Russian literature contains a number of records of anisakid larvae in fish from different oceans of the world. For example, Polyanski (1966) found *Anisakis* larvae in 28 species of fish from the Barents Sea; cod (*Gadus morhua*), saithe (*Pollachius virens*), redfish (*Sebastes marinus*), catfish (*Lycichthys denticulatus*) and bullhead (*Myoxocephalus scorpius*) were particularly heavily infected. Kurochkin and Leont'eva (1970) reported widespread infection with anisakid larvae of fish from the Indian and Pacific Oceans. Larval *Anisakis* were found in 19 species of fish from the Indian Ocean by Parukhin (1971),

members of the family Sauridae being especially heavily infected. Kurochkin (1972) referred to *Anisakis* sp. larvae in squid *Todarodes pacificus* in the Ussuriiskii Gulf (USSR).

Anisakis larvae are relatively common in some mesopelagic and bathypelagic fish, particularly myctophids (Collard, 1970; Noble and Collard, 1970). Larval *Anisakis* were widely but discontinuously distributed in mesopelagic fish but apparently absent from cold undiluted water masses (Noble and Collard, 1970). Larvae were also common in a number of macrourid species caught at depths of up to 4000 m from different areas (Noble, 1973). The means by which such deep-water benthic fish become infected with *Anisakis* is not clear. Collard (1970) suggested that mesopelagic myctophids acquire larvae in the epipelagic or surface waters during their diurnal vertical migrations by feeding on infected invertebrates. Myctophids could then in turn be preyed upon by bathypelagic species of fish and squid to which parasites might transfer. Collard did not believe that nematodes could survive such a transfer but subsequent research has shown that *Anisakis* larvae can in fact successfully transfer between fish (Smith, 1974). A downward transfer of *Anisakis* larvae from the epipelagic zone might account for at least part of the observed infections of deep-water benthic fish. On the other hand, invertebrates present at such depths, including euphausiids, which form part of the diet of macrourids (Noble, 1973), might act as intermediate hosts becoming infected by eggs and larvae descending through the water column from the epipelagic or mesopelagic zones where cetaceans are found.

Anisakis larvae have been recorded occasionally from purely freshwater fish. Thus Bassleer *et al.* (1973) found larvae in cultured trout in Belgium and Wootten and Smith (1975) reported larvae in trout from a British reservoir. In these cases trout had most probably become infected through being fed on untreated marine fish offal. Trout found infected in Ireland (Kane, 1966) and in Scotland (Kennedy, 1974) may have acquired larvae by similar means.

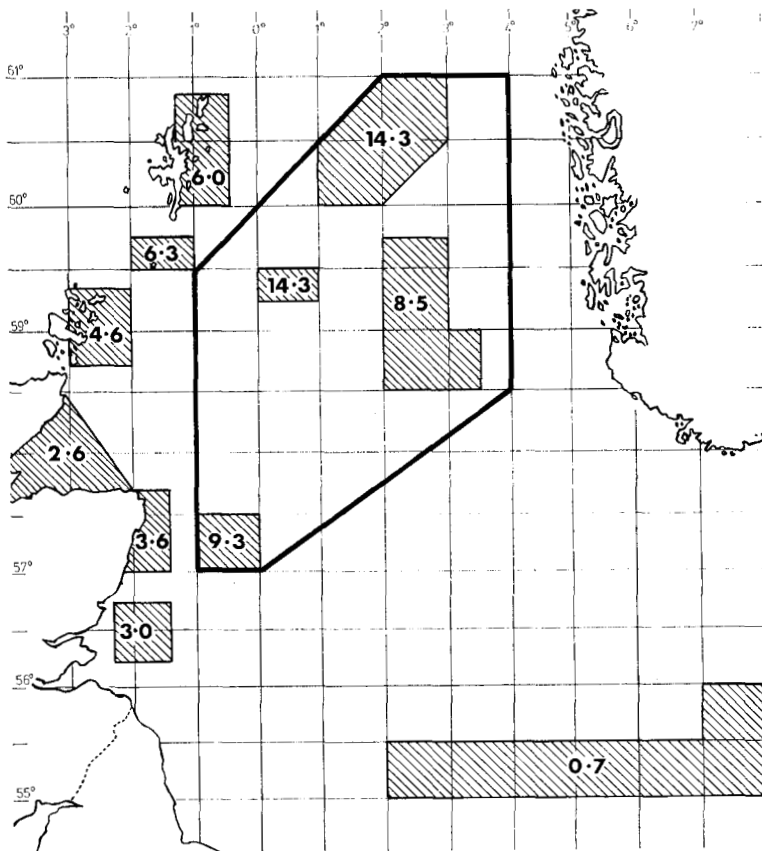
Anisakis larvae were recorded from seven species of fish in ponds and lakes in Dagestan (USSR) by Aligadzhiev and Altaev (1973) and in four species from the Tisa River basin by Ergens *et al.* (1975). It is not clear where these larvae originated but they may have been introduced with stocked fish fed on offal and subsequently spread to other species by predation and scavenging.

Except in the most general terms it is extremely difficult to determine the factors governing the distribution and abundance of *Anisakis* in a given geographical area. This is mainly due to the difficulties associated with adequate sampling of the wide range of hosts existing in any area in order to determine their relative importance in the life history of the parasite. Within the northern North Sea a number of studies have provided information on the biology and distribution of *Anisakis simplex* mainly in relation to the infection of food fish.

The major intermediate hosts of *A. simplex* in the northern North Sea are possibly euphausiids of the genus *Thysanoessa* (Smith, 1971). The highest prevalence of infection of *Thysanoessa* occurs within the central area of the northern North Sea with levels of up to 4% recorded at individual localities.

The average prevalence of infection of *Thysanoessa* spp. with *Anisakis* larvae is 0.3% which is a high figure given the abundance of these organisms and the quantity consumed by fish.

The high levels of euphausiid abundance and *Anisakis* infection in the central northern North Sea corresponds fairly well with the abundance of larvae in fish. The mean numbers of larval *A. simplex* in the musculature of whiting is much higher in this area than in waters off the Scottish East coast, the Shetlands and more southerly areas of the North Sea (Fig. 5) (Wootten and Waddell, 1977). The number of larvae in the musculature is positively correlated with the total number of larval *Anisakis* present in whiting. A similar geographical distribution of larvae has been observed in cod (Young, 1972; Wootten and Waddell, 1977), juvenile haddock and Norway Pout (Wootten, unpublished observations). Which fish species are of the greatest



importance in the transmission of *Anisakis* to the mammalian final hosts is uncertain. Although larger cod and whiting can be very heavily infected they may not be as significant in transmission as, for example, clupeoids (especially herring) or small gadoids which are an important source of food for the porpoise (*Phocaena phocaena*) (Rae, 1965) and possibly other cetaceans in the North Sea.

The abundance and distribution of cetaceans in the northern North Sea is little known. Porpoise, various dolphin species (including *Delphinus delphis*, *Tursiops truncatus*, *Lagenorhynchus albirostris* and *L. acutus*) and pilot whales (*Globicephala melaena*) are apparently the most abundant species (Evans, 1976). The relative importance of these cetaceans as hosts for *A. simplex* in the North Sea is unknown although small numbers of some species have been examined (Van Thiel, 1966; Young, 1972); among these, *P. phocaena*, *T. truncatus* and *L. albirostris* may contain large numbers of adult *A. simplex*. Cetaceans are found throughout the northern North Sea and there is no evidence that they are more abundant in the central area than in any other region. Seals are not significant hosts of *A. simplex* in the North Sea although a few adult parasites occur in young grey seals (*Halichoerus grypus*) on the Scottish East coast (Young, 1972; Wootten, unpublished observations). Such evidence as exists suggests that the abundance and distribution of the euphausiid hosts may be major factors determining the relative abundance of *A. simplex* in the northern North Sea.

The abundance of *Anisakis* may fluctuate quite dramatically with time. In the northern North Sea there appears to have been a large increase in the abundance of larval *A. simplex* in the musculature of whiting in recent years; in 1960–63 the prevalence of infection was less than 1.5% in over 9000 fish examined, but in 1971–74 the prevalence was 60% or more (Wootten and Waddell, 1977). There has also been a large increase in recent years in the infection of cod from Scottish waters with larval nematodes (Rae, 1963, 1972). Rae identified larvae as *Phocanema* but more recent work (Wootten and Waddell, 1977) has shown that larval *Anisakis* is very common in cod from some areas, notably the northern North Sea; it is probable that the increases in infection observed by Rae were due, at least in part, to *Anisakis*. Such apparent increases in abundance are probably due to changes in the populations of one or more of the host species but no obvious explanation is possible at the present time. There is no evidence of any increase in cetacean numbers in recent years. Possibly there has been a change in the abundance of euphausiids and/or the relative importance of euphausiids as a food item for fish. There have been large-scale changes in the plankton populations of the North Sea since 1950 (Cushing, 1975; Longhurst *et al.*, 1972) and, whilst some organisms such as the copepod *Pseudocalanus* have declined sharply in abundance, euphausiids appear to have at least held their own if not increased. As zooplankton is a major item in the diet of many fish species it is tempting to speculate that with a diminution in the availability of some organisms there has been a tendency for fish to feed more heavily on euphausiids and in this way to acquire heavier infections of larval *Anisakis*. This might lead to an increase in the over-all abundance of *Anisakis*. Many fish populations, especially

gadoids, have been at a high level in the northern North Sea since about 1960 (Longhurst *et al.*, 1972) and possibly this increase in "paratenic" host populations has also had an effect on the over-all abundance of the parasite.

VI. IN-VITRO CULTURE OF *ANISAKIS* IN RELATION TO *IN VIVO* OBSERVATIONS

Anisakis larvae from fish have been used by different workers to initiate cultures *in vitro* using various techniques and with varying degrees of success.

Grainger (1959) placed larvae from cod in an Na_2HPO_4 -citric acid buffer solution at pH 2.2 and 37°C. Addition of 1% pepsin assisted excapsulation but was not essential. The presence of pieces of fish for 24 h towards the beginning of the culture period was essential for the moult to preadult, which then occurred in three to five days; pepsin was not essential for the moult. Various morphological differences between larvae and preadults were noted. Attempts to rear adults were unsuccessful.

Khalil (1969) reported that larval *Anisakis* from herring moulted after four to five days at 36°C in a mixture of liver extract, horse serum and Medium 199. Size increase was seen from the seventh day but all died by 40 days in culture. Females developed better than males but the egg-laying stage was not reached.

Van Banning (1971) succeeded in culturing larvae from North Sea herring, mackerel and Norway haddock through to egg-laying adults in a digested liver extract (pH 2.0) with added beef blood. The most suitable temperature was between 34 and 37°C. At 10–11°C larvae did not moult but remained at this stage for up to at least 80 days. High mortality occurred at 38°C, and at higher temperatures up to 40°C development to adult was not completed. At 34°C the first moult occurred within four days. That pieces of cuticle were frequently found when the medium was renewed suggested that there are two or three moults before the worm matures (but this aspect is not elaborated). The preadult stage varied in duration from at least 26 days to a maximum of 98 days. The phase to full maturation was short at about seven days. Male worms measured 3.5–7.0 cm, females 4.5–15.0 cm; they were subsequently identified as *A. simplex* by Pippy and Van Banning (1975). Mature males were placed in tubes with just maturing females; fertilization took place and viable eggs were obtained which hatched satisfactorily. Grabda (1976) cultured larvae from Baltic herring through to the adult stage using a modification of Van Banning's technique with essentially similar results; again the adults were identified as *A. simplex*.

Schulz (1974) cultured larval *Anisakis* from herring to preadult in a medium comprising CEE, liver extract, protein hydrolysate and pig serum. At 39°C in a gas phase of 10% CO_2 in air, moulting was first seen on the third day; on average, 50% survived for 18 days but no further development was seen.

Sommerville and Davey (1976) have shown that development of third-stage *Anisakis* larvae (from barracouta, *Leionura atun*) and the moult to preadult can take place in a far simpler medium than any used by the above workers; this paper contains the essential results given by Sommerville (1974) and

Sommerville and Buzzell (1974). These authors found that larvae can form a new cuticle and moult to preadult *in vitro* at 37°C in about three to five days but that relative success depended on certain conditions. In a culture medium of Krebs–Ringer balanced salt solution (BSS) under 5% CO₂ in air, relatively few moulted and survival was poor. More larvae moulted and survival was enhanced in Medium 199 even if the gas phase was air, but the presence of 5% CO₂ led to a quicker moult. The benefits of high CO₂ concentrations with both BSS and Medium 199 accrued only if this gas was present during the first 40 h of incubation. Worms were harmed at temperatures near 40°C. The pharynx of moulted worms (fourth stage) usually contracted vigorously whilst that of third stage larvae was invariably immobile. These observations and studies on uptake of serum conjugated with rhodamine suggested that worms do not feed until after they have moulted, a conclusion also reached by Yasuraoka *et al.* (1967) and Koyama *et al.* (1967). That external physico-chemical stimuli may trigger internal regulatory mechanisms leading to development of *Anisakis in vitro* is related by Sommerville and Davey to similar observations on stronglyloid nematodes (e.g. *Haemonchus*).

There is always the possibility of abnormality in growth and development during *in-vitro* culture of a parasite. However, in several respects the above results obtained for *Anisakis* fit fairly well with observations *in vivo* on the course of events in experimentally infected laboratory animals, especially that concerning the moult to preadult. For example, Gibson (1970) studied development of *Anisakis* larvae from herring in rats. He described and illustrated morphological changes in the head, ventriculus, gut wall and cuticle that accompanied the moult from larva to preadult. Larvae showed no signs of sexual development but after three to four days in the rat they moulted and some showed a developing vulva, vagina and coiled uteri; development of a male system was not seen. Some preadults, particularly those which had spent 11 days or more in rats, showed cuticular separation especially at the anterior end which suggested that another moult was about to take place. Gibson's observations *in vivo* are in general agreement with those obtained *in vitro* by other workers for the moult to preadult.

Apart from the observations recorded by Van Banning (1971) and Grabda (1976) during their culture work *in vitro*, little information is available on the preadult to adult phase of development. *In-vivo* studies with laboratory animals offer little insight into this aspect and it is unfortunate that investigation of the natural final hosts is fraught with practical difficulties. Marine mammals are large animals, difficult and expensive to maintain in captivity; captive animals have commercial value as a public spectacle and this and other factors, including aesthetic ones, may preclude experiments on them. Marine mammals shot in the wild or found dead or dying have been used for study, but post-mortem changes may lead to difficulties of interpretation. Therefore, the following conclusions reached by different workers from observations on wild-caught animals should be viewed with caution.

Kagei *et al.* (1967), according to Oshima (1972), observed *A. simplex* or *A. typica* in blue-white dolphins. During the moult to the fourth stage (preadult) these authors noted loss of the boring tooth and mucron and

acquisition of distinct transverse striations to the cuticle more than 17 μm apart. Worms more than 30 mm long had moulted to the fifth (adult) stage, showing an open vagina in females and paired spicules and caudal papillae in males; the cuticular striations were 23–42 μm apart.

Kikuchi *et al.* (1967) studied *Anisakis* sp. in dolphins (*Tursiops gilli*), and suggested that third-stage larvae invade the gastric mucosa and submucosa and grow to a length of 20–27 mm before moulting. The preadults are then said to return to the stomach lumen and to attach themselves to its wall; after further development and at a length of 28–35 mm they are said to undergo another moult to achieve the adult stage. Whilst the observation of two moults in the final host may be correct, there are reasons to doubt deep tissue penetration and growth to 20–27 mm within tissues before the moult to preadult. The work of Vik (1964) and Young and Lowe (1969) suggests that development and moulting occur during superficial attachment to the alimentary tract in the final host. Furthermore, Gibson (1970) observed a preadult only 13 mm long in experimentally infected rats.

A wide range in length has been recorded not only for stages recovered from wild crustaceans, fish and squid but also for adult males and females obtained from final hosts and *in vitro*; it may be unrealistic, therefore, to expect any correlation between length and the timing of a particular moult.

VII. PHYSIOLOGY AND BIOCHEMISTRY OF *ANISAKIS*

A wealth of detailed information is available on the nutrition, digestion and general metabolism of nematodes in general, as is evident from the reviews of Brand (1966), Bryant (1975) and Lee and Atkinson (1976), and of the various contributors to chapters in Florkin and Scheer (1969). However, the available information on these aspects for *Anisakis* is fragmentary and often relates only to larvae removed from fish.

Metabolic studies by Hamajima *et al.* (1969) on *Anisakis* sp. larvae indicated that the Embden-Meyerhof pathway might act as an energy supply from several carbohydrates. Krebs' tricarboxylic acid cycle oxidation and a succinoxidase system also appeared to be present. Kawai (1968) separated long-chain fatty acids from various organs of larvae by gas chromatography.

Anisakis larval emulsions were examined for lactate dehydrogenase isozymes by Nagase (1968a) using cellulose acetate chromatography; the two bands that appeared were identified as LDH-1 and LDH-2 known from human sera.

The amylase activity in extracts of *Anisakis* larvae reported by Taniguchi (1967) was active over a pH range of 4.0 to 9.0, with two maxima (at pH 4.0 and at pH 7.0). With regard to isozymes, electrophoretic and zymogram analyses revealed six distinct protein fractions showing amolytic activity.

Ruitenbergh (1970, 1972) and Ruitenbergh and Loendersloot (1971a,b) reported on enzyme histochemical tests on *Anisakis* sp. larvae taken from herring; various organs and tissues were studied, in some cases with the larvae penetrated (experimentally) into rabbit stomachs. The main results are summarized below.

The cuticle showed weak activity by only three enzymes (5-nucleotidase, adenosine triphosphatase and phosphorylase). In the musculature, moderate to strong activity was shown by various oxidative enzymes. Alkaline phosphatase was absent from the brush border of the intestinal tract, but here other phosphatases (acid phosphatase, 5-nucleotidase and adenosine triphosphatase) showed moderate to strong activity. Leucine aminopeptidase activity was also strong in the brush border. Intracellularly, the epithelial cells of the intestinal tract showed strong PAS activity shown to be glycogen; of the phosphatases, only adenosine triphosphatase showed some activity. Polyacrylamide gel electrophoresis showed only one band of acid phosphatase activity, i.e. isozymes were absent.

The "excretory organ" of larvae showed slight to strong activity by 18 enzymes. Non-specific esterase activity was especially strong in the lateral part of the organ, with the central area showing moderate activity. Some leucine aminopeptidase activity was also present.

Protein-rich substances, possibly histolytic enzymes, may be contained in the membrane-bound secretory granules noted by Lee *et al.* (1973) in their ultrastructural study of the "excretory system" of *Anisakis* larvae; these granules occupied much of the gland. Mueller (1927) also referred to secretory granules in the ventral gland of the "excretory system" of adult *Anisakis simplex*. The various roles that have been ascribed to the so-called "excretory system" of nematodes in general include excretion, osmoregulation, larval exsheathment, penetration of host tissues and extracorporeal digestion (see, for example, Wright and Newall, 1976); these matters are controversial and the position regarding *Anisakis* in particular is not clear.

Little is known of the tissue penetration mechanism(s) of *Anisakis* in either cold-blooded or warm-blooded hosts. Matthews (1977) examined enzymic activity in larval *Anisakis* (from herring) using an azocoll dye release technique. Secretions showing azocoll positive activity were released at temperatures above 10°C but the optimum temperature both for release and for activity against the substrate appeared to be about 37°C, the body temperature of marine mammalian final hosts. This result is somewhat anomalous in that more extensive larval migration appears to occur in fish than in marine mammals; further work is in progress.

Okuno (1968, 1969) reported 18 amino acids in hydrolysed tissues of *Anisakis* sp. larvae. Proline, alanine, glycine and glutamic acid were most common, whilst tryptophan, lysine, histidine, arginine and cystine were found in very small amounts. Oishi *et al.* (1972a) reported 17 amino acids in larval *Anisakis* from Alaska pollack and recorded differences in the abundance of certain of these depending on the locality of host capture (Okhotsk Sea and Pacific Ocean). Moreover, larvae from pollack contained three or six times more aspartic and glutamic acid than larvae from mackerel, but less tyrosine, histidine and arginine.

The amino-acid composition of the cuticle and of the TCA supernatant and precipitated fractions of the reproductive organs and perienteric fluid of adult male and female *Anisakis physeteris* (from sperm whales) was reported by Vigliierchio and Görtz (1972a). The amino-acid nitrogen of the cuticle

accounted for about 96% of the total nitrogen; over 50% of the cuticle protein hydrolysate consisted of proline, glycine and arginine. In reproductive organ fractions, lysine, glutamic acid, glycine, valine, leucine and histidine accounted for about 62% of total nitrogen. Interesting differences were recorded in the amounts of certain amino acids present in corresponding tissues of male and female worms, and in different tissue fractions from worms of the same sex: there was more serine in female than male cuticle; there was more proline in the protein of female than male reproductive organs; methionine was present in cuticular protein and in reproductive organ protein and non-protein fractions but absent from perienteric fluid; cystine was present in cuticular protein but not in other fractions. From an increase in the ammonia content of stored untreated perienteric fluid, Viglierchio and Görtz (1972a) suggested tentatively that ornithine cycle and amino-acid oxidase activity may operate *in vivo*.

Suzuki *et al.* (1974) reported on the haemoglobin extracted from the perienteric fluid of larval *Anisakis* as an antigen in immunodiagnosis but no information on its spectral or other characteristics was given. Viglierchio and Görtz (1972b) found that a partially purified haemoglobin from the perienteric fluid of adult *Anisakis physeteris* differed in spectral and reactive properties from the haemoglobin and myoglobin of both the sperm whale host and the fin whale. Deoxygenation with sodium dithionite of the worm haemoglobin and the cetacean haemoglobins and myoglobins took less than one minute. Oxidation with potassium ferricyanide of the cetacean pigments was also rapid, but t_{50} values for oxidation of worm haemoglobin were high: 450 s for pigment from male worms, 660 s for that from females. Another sex difference in worms was found in the iron and copper content of the haemoglobin—respectively, 3.90 and 0.195 mg 100 ml⁻¹ for males, and 3.00 and 0.180 mg 100 ml⁻¹ for females. *Anisakis* haemoglobin may function more in the transport of oxygen than in its storage but further work is needed.

Williams and Jones (1976) recounted experiments at Aberdeen University the unpublished results of which showed that larval *Anisakis* can survive for at least eight days at 12°C in dilutions of sea water from 0% (distilled water) to 100% (normal sea water). Various other approaches including weight loss/gain in different dilutions and a silver staining technique suggested that the cuticle is relatively impermeable; this might explain the survival of larval *Anisakis* in a wide range of hosts but comparable studies on preadults and adults have not been conducted.

VIII. PATHOLOGY OF ANISAKIASIS

The following account of pathology associated with *Anisakis* infection deals with various developmental stages of the nematode in various animals, be they “natural”, “accidental” or experimentally infected hosts. For convenience the animal groups are treated in ascending phylogenetic sequence. Squid (molluscs) are omitted because no relevant reports appear to be available. The pathology of anisakiasis of man is dealt with in section IX. B.

A. CRUSTACEANS

No direct observations of pathology in crustaceans have been reported. It is interesting to note, however, that whilst multiple infection of euphausiids with freshly-hatched second-stage larvae of *Anisakis* was achieved experimentally by Oshima (1972) (see also section IV. C), all reports of natural infections in crustaceans refer to only one larva per infected host (see section IV. D). It is tempting to speculate that multiple infection eventually kills hosts, and that only hosts infected with a single larva are able to survive. On the other hand, it is possible that one or more mechanisms operate to control the parasite number during the course of larval development; Halvorsen and Williams (1968) discussed some of the literature on this phenomenon in relation to their observations on *Gyrocotyle* in *Chimaera*. Clearly, comparative studies on the histology of infected and uninfected crustacean hosts and an experimental approach are required.

B. FISH

The effects of *Anisakis* larvae on fish are not clear. Several workers have suggested that larvae can adversely affect the condition of infected fish but many of these studies lack firm evidence and seem rather subjective in nature. The literature on the effect of larval *Anisakis* on fish has been reviewed previously by Margolis (1970) and Cheng (1976).

Larval *Anisakis* are found encapsulated throughout the viscera and musculature of fish although the actual distribution within the tissues varies between fish species. For example, in cod from Scottish waters, less than 12% of the total number of *Anisakis* larvae present are found in the musculature whereas in the whiting over 50% occur in this site (Wootten and Waddell, 1977). Within the viscera, larvae are found mainly on the surface of the liver or among the mesenteries, especially those surrounding the gut. Polyanski (1966) gave data on the occurrence of larval *Anisakis* in these sites in 28 species of fish from the Barents Sea. Larvae may be found free in the gut lumen of the host or may be seen at different stages of penetration through the gut mucosa (Sluiter, 1974); presumably such larvae have been only recently acquired by ingestion of infected intermediate hosts.

Encapsulated larvae are normally tightly coiled in a spiral (Fig. 6). The process of encapsulation and the nature of the capsule in the liver of fish were studied by Kahl (1938), Mikhailova *et al.* (1964) and Prusevich (1964). The capsule is of host connective tissue origin and three-layered. The inner layer is thin and consists of damaged cellular elements; this layer originally surrounds each coil of the larva. The middle layer is made up of a large proportion of partly degenerated fibroblastic elements, whilst the outer layer consists of loose connective tissue and may be slightly vascularized. The capsule is continually eroded from within and built up on the outside. The thickness of the capsule depends on the quality of host connective tissue nearby and on the age of the capsule. Older capsules in which the nematode has died are reduced

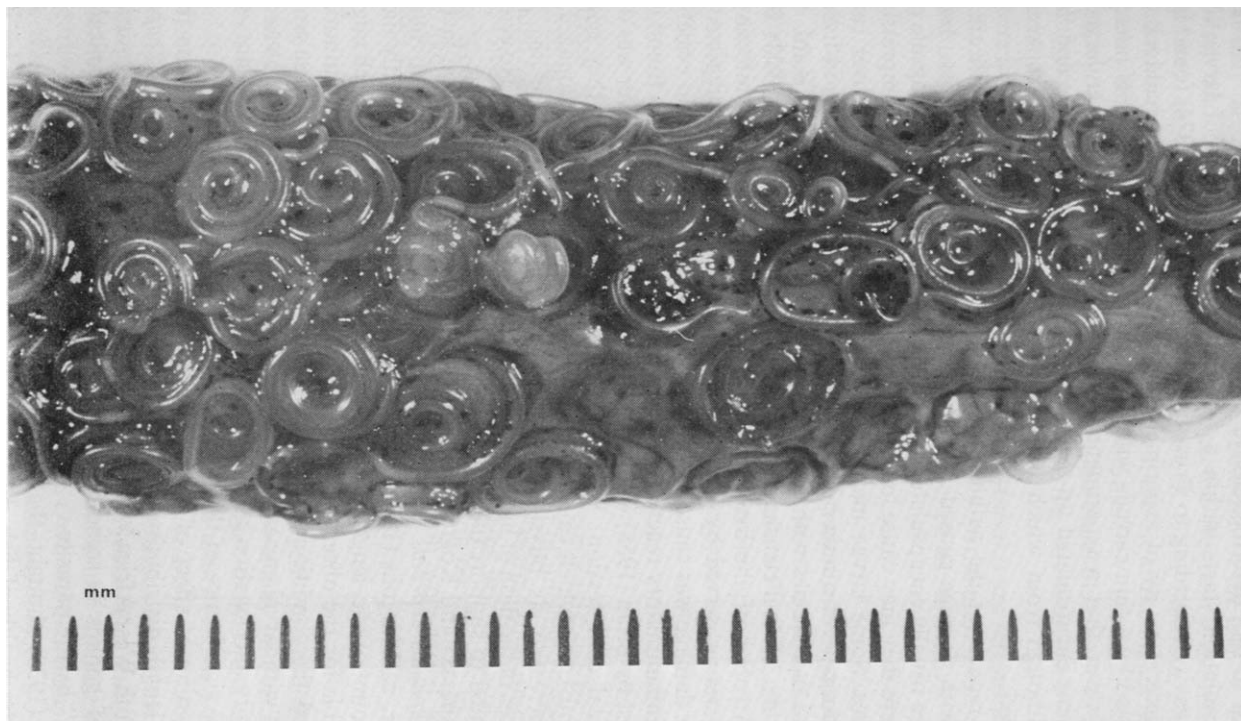


FIG. 6. Portion of liver from a gadoid fish heavily infected with encapsulated *Anisakis* larvae.

in size and may become calcified. Kahl (1938) compared the structure of capsules from different sites in several fish species and, in all cases, found it to be similar to that described above.

Encapsulation starts within a few hours of the arrival of larvae in the visceral cavity according to Prusevich (1964), and Smith (1974) found larvae in the visceral cavity of experimentally infected whiting and haddock to be surrounded by a thin capsule within 24 hours of infection. It is of interest to note, however, that in experimentally infected trout in fresh water, larvae had not become encapsulated up to 24 days after infection (Wootten and Smith, 1975) although trout which had been infected for some months were encapsulated.

Most reports of the pathogenic effect of larval *Anisakis* in fish are concerned with damage caused by the larvae to the liver of the host. *Anisakis* larvae are usually encapsulated just under the connective tissue capsule of the liver where they may cause local damage, probably mainly by compression of the tissues. Some larvae may penetrate more deeply into the liver causing greater damage. A number of authors have reported a reduction in liver size in several fish species with heavy infections of larval *Anisakis* (Kahl, 1938; Akhmerov, 1951; Petrushevski and Kogteva, 1954; Remotti, 1933; Brian, 1958). Atrophy of the liver of hake in heavy but superficial infections of *Anisakis* was observed by Remotti (1933) but Guiart (1938) suggested that this liver damage was caused by toxins secreted by the worm. No evidence of general inflammatory reaction or degenerative liver changes was found in the same host by Brian (1958) who concluded that atrophy of the liver was due to mechanical compression. When larvae died Brian suggested they released "thanatotoxins" which caused local liver damage and that undamaged liver tissue increased its physiological activity, thus accounting for the slight differences in the flesh of hake with infected and uninfected livers. No evidence is presented to support these latter views.

Petrushevski and Kogteva (1954) reported a fall in the condition factor of *Myoxocephalus scorpius* with increasing liver infections of larval *Anisakis*. However, as Margolis (1970) pointed out, these authors apparently did not take account of age, length or spawning condition of the hosts which might be responsible for the observed differences in condition. The same authors also compared the condition factor of cod and *M. scorpius* in relation to their infection with larval anisakids (including *Anisakis*) but made no allowance for the identity and location of larvae in different hosts. Both these factors could affect the significance of the observed infections (Margolis, 1970).

No significant effect of larval *Anisakis* infection on the condition factor, average length distribution or gonad weight of anchovy (*Engraulis capensis*) from South West Africa was recorded by Hennig (1974); the "underdevelopment" of gonads in some fish was thought to be due to "mechanical suppression" by the parasite.

Kahl (1938) thought that severe infections with larval *Anisakis* could impair the health of fish. In some cod, Kahl found large tubercles in the stomach wall containing many degenerating and live larvae. Heavy infections in the body cavity of redfish (*Sebastes marinus*) caused visceral adhesions.

Anisakis larvae in ulcerous cavities in the stomach wall of severely emaciated *Ophiodon elongatus* were recorded by Arai (1969) who considered that the larvae may have been responsible for the condition of the fish.

Bishop and Margolis (1955) found that the frequency distributions of *Anisakis* sp. larvae in Pacific herring were skewed. Using this data, preliminary calculations by Lester (1977) showed that truncation at the higher end of the frequency distributions did not affect the parameter *N*, the total numbers of fish expected to be present according to the theoretical distributions involved; this suggested that little or no mortality of herring was associated with larval *Anisakis* infection.

There is little conclusive evidence that larval *Anisakis* are serious pathogens of fish; there is a need for carefully controlled experimental infections to be carried out to determine with certainty the effect of larvae on fish.

C. AMPHIBIANS

According to Oishi *et al.* (1969), larval *Anisakis* were administered to bullfrogs by Hirao and Yamaguchi (1964). Larvae quickly migrated into the abdominal cavity but haemorrhages were observed in the stomach wall two days after infection. After five days larvae were becoming encysted, but most were still alive 30 days after infection and were capable of reinfecting other bullfrogs.

D. REPTILES

Farmed green turtles (*Chelonia mydas*) may become infected by "worms" acquired in fish feeds (Clayton, 1975). The growth of infected turtles may be "stunted" and Clayton pointed out the danger of worms migrating into the flesh. Given the ability of larval *Anisakis* to transfer between hosts (Smith, 1974; Wootten and Smith, 1975) and their common occurrence in fish, it is quite likely that the worms referred to by Clayton were larval anisakids, possibly including *Anisakis*.

E. BIRDS

There are surprisingly few records of *Anisakis* in piscivorous birds in view of its abundance in marine fish. The paucity of records may only reflect a lack of parasitological studies on piscivorous birds rather than a real scarcity of *Anisakis* in these hosts. Kreis (1958) records an immature female *Anisakis* sp. larva in the oesophagus of a fulmar (*Fulmarus glacialis*), whilst Bakke and Baruš (1975) found *A. simplex* larvae in the oesophagus and ventriculus of common gulls (*Larus canus*) from Norway. In the North-West Pacific Ocean, *Anisakis* sp. larvae were recorded in *Larus argentatus* and *Lunda cirrhata* by Belogurov *et al.* (1968) and in *Larus crassirostris*, *Oceanodroma monorchis* and *Phalacrocorax ussuriensis* by Alekseev and Smetanina (1968).

On the east coast of Britain, ulceration of the proventriculus of fulmars caused by *Anisakis* sp. larvae and preadults (probably *A. simplex*) was described by Riley (1972). Clusters of nematodes were found at the centre of

ulcerous swellings, with each worm lying at the centre of a necrotic area. Histolysis was present around the anterior ends of the worms. The nematodes had penetrated into the submucosa and affected the muscularis. The proventricular glands were extensively vacuolated in the region of penetration. Riley (1972) suggested that as only a small proportion of the very large proventriculus of the fulmar was destroyed by the ulcers it was unlikely that the functioning of the organ would be seriously affected. Secondary infection of the ulcers may occur.

F. MARINE MAMMALS

One of the earliest records of pathology in marine mammals caused by *Anisakis* was apparently that of Murie (1868) who described ulceration of the fundic stomach of the walrus caused by "*Ascaris bicolor*" (= *A. simplex*) which he believed led to the death of the host. Subsequently a number of authors have described ulceration of the stomach of pinnipeds and cetaceans by *Anisakis* spp.

A detailed description of lesions from the stomach of grey seals (*Halichoerus grypus*) and porpoises (*Phocaena phocaena*) was given by Young and Lowe (1969). These authors found groups of 50–100 larval and "immature" *Anisakis* associated with inflammatory areas 1–6 cm in diameter in the fundic stomach of the grey seal. Such aggregations of *Anisakis* were found only in juvenile grey seals. The anterior ends of the nematodes were embedded in the mucosa and submucosa at the centre of the lesions and were surrounded by fibrin and inflammatory cells. The lips of the nematodes were surrounded by an amorphous eosinophilic substance and fibrin layer which was in turn enclosed by an area of massive infiltration. In some parts of the lesions inflammatory areas surrounding cast nematode cuticles were found in the submucosa. A large amount of granulation tissue was found around each inflammatory focus in the mucosa and produced a raised inflammatory area.

In the porpoise Young and Lowe (1969) found lesions mainly in the first stomach (Figs 7 and 8) but they also occurred in the second and third stomachs. In most lesions only "immature" *Anisakis* were found but in some cases adult *A. simplex* also occurred. The histology of the lesions from porpoise resembled that in grey seals although there were many more inflammatory areas with cast cuticles in the former host. These areas were often calcified. Large masses of granulation tissue were formed around the lesions which greatly thickened the submucosa and, in some cases, also the epithelial and muscular layers of the stomach wall.

Similar lesions were described by Vik (1964) in the stomach of porpoise caused by clusters of *Anisakis* larvae. Cowan (1967) reported a severe inflammatory response in the peptic stomach of pilot whales (*Globicephala melaena*) to infection by groups of *Anisakis*. Inflammatory reactions and ulceration caused by *Anisakis* were found in the bottlenose dolphin (*Tursiops gilli*), pacific white-sided dolphin (*Lagenorhynchus obliquidens*), Risso's dolphin (*Grampus griseus*) and rough-toothed dolphin (*Steno bredanensis*) by Kikuchi *et al.* (1967) and in the blue-white dolphin (*Stenella caeruleoalba*),

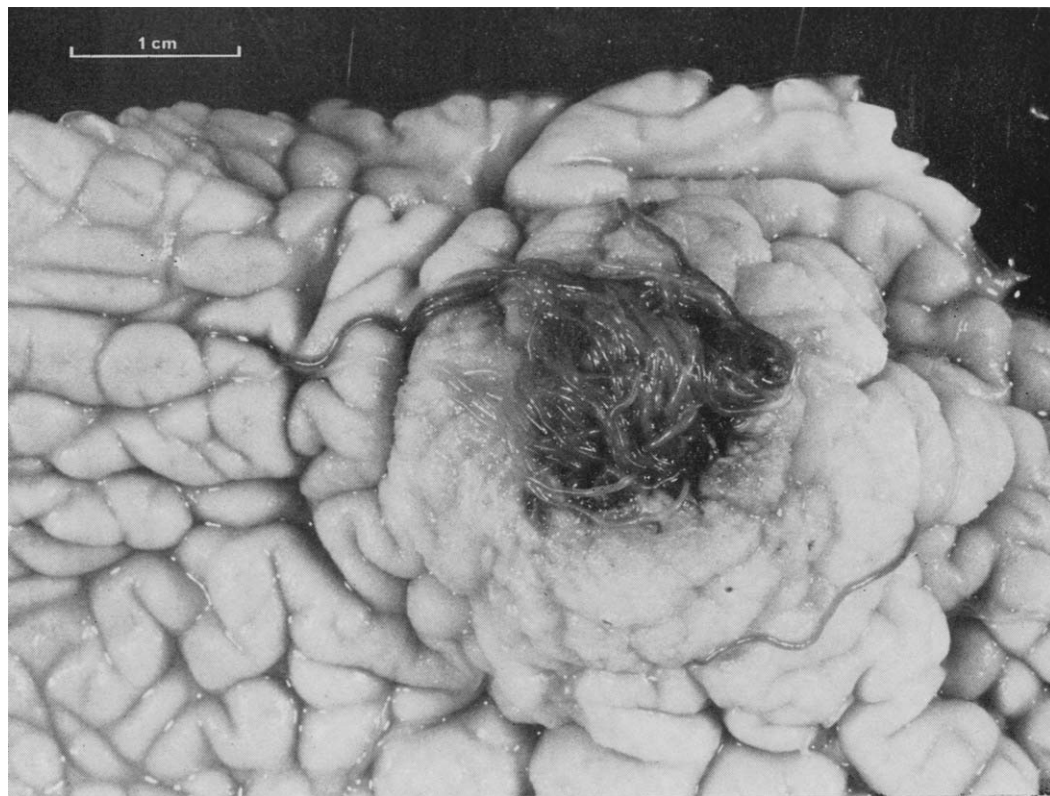


FIG. 7. First stomach of porpoise (*Phocaena phocaena*): lesion containing a cluster of larval and preadult *Anisakis*.



FIG. 8. First stomach of porpoise (*Phocaena phocaena*): bisected lesion, more severe than that shown in Fig. 7, containing a large cluster of "immature" and adult *Anisakis*. (Courtesy D. Lowe.)

Dall's porpoise (*Phocaenoides dalli*) and *Phocaena phocaena* by Kagei *et al.* (1967). Testi *et al.* (1971) described ulceration caused by *Anisakis* sp. in a common dolphin (*Delphinus delphis*) whilst Mikagi *et al.* (1971) suggested that gastric ulcers in the California sea-lion (*Zalophus californianus*) were due to *Anisakis* sp.

The severity of gastric lesions in marine mammals is presumably due to the mechanical irritation of large numbers of *Anisakis* larvae penetrating the stomach wall at one point. Toxic secretions from the parasites may also have some effect on the stomach wall. The stimulus causing the aggregation of *Anisakis* larvae at one point is unknown but it may be that the initial attachment of only a few parasites will cause a lesion which then provides a more suitable site for other individuals.

According to Riley (1972), Hoeppli (1933) found that tissues around the anterior end of *Anisakis* sp. in the stomach wall of the walrus were necrotic and suggested that they were liquified by secretions from the oesophageal glands of the nematodes. In addition, Hsü (1933) described a positive correlation between the size of the dorsal oesophageal gland and extracorporeal digestion in some ascaridoids, including *Anisakis*. The glandular secretions of a large cluster of *Anisakis* sp. may produce a partially digested mass of tissue upon which the nematodes may feed (Riley, 1972). Repeated infections of larval *Anisakis* or presensitization in experimentally infected mammals may cause an increased host response (see also section X. B) and it is possible that such an effect occurs in naturally infected marine mammals.

The cast cuticles observed in lesions by some authors may be those of larvae which have invaded the mucosa before moulting to the fourth stage. Adult *Anisakis* are normally only superficially attached to the stomach wall (Kikuchi *et al.*, 1967; Young and Lowe, 1969) and thus do not cause ulceration. The location of ulcers within the stomach of marine mammals was suggested by Young and Lowe (1969) to reflect the area where larvae are activated after ingestion by the host. Ronald (1960, 1962, 1963) suggested that in the related genus *Terranova* (= *Phocanema*) the stimulus causing movement of larvae ingested with fish is the high temperature of mammalian stomach fluids. Larval anisakids may penetrate into the gut wall in the first area where food is macerated regardless of any other stimuli. This area is the fundic stomach of pinnipeds and the first stomach of cetaceans. Lesions reported to be caused by *Anisakis* and other anisakid nematodes predominate in these regions.

Blessing and Peitz (1970) described ulcers caused by *Anisakis* in the Atlantic bottlenosed dolphin (*Tursiops truncatus*) and *Zalophus* sp. and suggested that ulcers healing spontaneously may produce stenoses in the pyloric region which contribute to the death of the host. In a similar case, Gembardt *et al.* (1971) reported that dilatation of the stomach wall in a sea-lion (*Mirounga leonina*) caused by a pyloric stenosis, due to inflammation and ulceration resulting from an *Anisakis* infection, led to chronic congestion in the abdominal venous system and eventually to the death of the animal by circulatory decompensation. Anisakid nematodes in the stomach wall of the California sea-lion (*Zalophus californianus*) may on occasion perforate the gut wall and be found in the peritoneal cavity (Griner, 1974).

Gastric nematodes of marine mammals kept in captivity have been successfully removed by anthelmintic drugs. Ridgway (1972) recommended thiabendazole at a dose rate of 45 mg kg⁻¹ body-weight repeated after three months and then at six-month intervals. In severe cases, an initial dose of 70 mg kg⁻¹.

can be administered followed by a further dose of 45 mg kg⁻¹ in 1–3 weeks. Sweeney and Ridgway (1975) suggested a dose rate of 73 mg kg⁻¹ for thia-bendazole. The same authors reported moderate effectiveness of piperazine at a dosage of 55 mg kg⁻¹ and good effectiveness of levamisole at 8–11 mg kg⁻¹. If reinfection of captive mammals is prevented by the use of a frozen fish diet, nematodes are lost and ulcers heal within six months (Griner, 1974).

G. OTHER MAMMALS (EXCLUDING MAN)

Various mammals have been used as experimental animals to elucidate the pathology of *Anisakis*, particularly in relation to the problem of the response in sensitized hosts (see also section X. B). Larvae administered orally to rabbits mainly penetrated the stomach and caused lesions similar to those recorded from man (see section IX. B). Ruitenbergh *et al.* (1971) found that where a number of larvae had invaded the gastric mucosa at a single point an ulcer surrounded by haemorrhages was present. Three days after infection, larvae were surrounded by necrotic tissue with granulocytes including many eosinophils. After five days, plasma cells were present and fibroblasts appeared at the periphery of the inflamed area. By ten days the degenerating larvae were surrounded by extensive granulation tissue outside which were large numbers of fibroblasts. Larval remnants eventually became surrounded by young connective tissue which, in turn, was enclosed by extensive mononuclear infiltration. After some months the larval remnants showed signs of calcification. Normally, the host reaction around a single larva penetrating the stomach wall was slight but such infections could produce a severe reaction.

Japanese workers found that sensitized rabbits showed a much stronger reaction to invading *Anisakis* larvae, including the formation of eosinophilic granulomata, than did non-sensitized animals in which the "foreign-body" type of reaction was observed (Oishi *et al.*, 1969). The reaction of experimental hosts to invading larvae was strong in rabbits, less so in dogs and only slight in cats. In sensitized dogs, in contrast to rabbits, infiltration of neutrophils occurred but there was no formation of granulomata, whilst in cats only slight neutrophil infiltration occurred. In dogs, the ratio of larvae invading the stomach and intestine was 1:1 but in rabbits the ratio was 17.8:1 (Oishi *et al.*, 1969).

In smaller mammals, such as rats and guinea-pigs, larvae mainly attack the stomach wall and a large proportion pass right through the wall and migrate amongst the viscera. Larvae are capable of migrating through the stomach wall of rats within 24 h of infection (Oishi *et al.*, 1969). Larvae penetrating the stomach wall of rats were surrounded by a thick layer of eosinophils and lymphocytes after 24 h; large numbers of red blood cells were present in the submucosa (Young and Lowe, 1969). Four days after infection larvae and cast sheaths were surrounded by numerous eosinophils and lymphocytes in which macrophages and fibroblasts were scattered. The submucosa showed a general eosinophilic infiltration and numerous lymphocytes. Reinfected rats showed similar cellular responses but these were both more rapid and more intensive. A more intense reaction to invading larvae in sensitized rats was

also seen by Japanese workers (Oishi *et al.*, 1969). Larvae do not survive for much more than seven days after they have penetrated the gut wall (Young and Lowe, 1969). According to Oishi *et al.* (1969), necrotizing larvae were found in localized granulomata in the body cavity, but after 40 days no traces of larvae could be seen. Okumura (1967) found that *Anisakis* larvae from different fish hosts showed different patterns of penetration of the alimentary tract of rats and survived for varying periods of time in this host.

Myers (1963) found that larvae fed to guinea-pigs penetrated the stomach wall and migrated around the viscera where they could be found alive for up to five days but had disappeared after six days. Macroscopically, inflammation and haemorrhage could be seen at the point of invasion of the gastric wall and infiltration of leucocytes and macrophages occurred around larvae.

The pathology of *Anisakis* larvae in pigs has recently been described by Japanese and American authors. Ashizawa *et al.* (1973a,b) and Usui *et al.* (1973) found three types of gross lesion in the stomachs of pigs which had become infected with *Anisakis* larva (I) by feeding on fish offal in Japan. Type I lesions were primary mechanical ones, the severity of which was proportional to the numbers of larvae present. Haemorrhage and ulceration of the mucosal and submucosal layers occurred but the muscular and serosal layers were unaffected. In Type II lesions there was an Arthus-type reaction with oedema and cellular infiltration in the mucosal and submucosal layers, connective tissues and muscle layers. Type III lesions were characterized by marked degeneration of the mucous membrane layers, necrosis, ulceration and cellular infiltration. Abscesses were evident in the submucosal layers and slight but diffuse proliferation of connective tissues occurred. Giant cells were seen around dead larvae. The pathology of *Anisakis* larvae in pigs generally resembles that found in other mammals, including man, but differs in the small numbers of granulomata seen and in the presence of allergic oedema in the submucosal layer.

Jackson and Bier and their co-workers have recently investigated the pathology of experimental infections of *Anisakis* larvae in miniature pigs. The majority of larvae penetrated the stomach and were eventually found entirely within the submucosa (Jackson *et al.*, 1976). Ulceration occurred where *Anisakis* penetrated the stomach wall; that there were more ulcers than penetrating larvae suggested to Bier *et al.* (1976a) that larvae could withdraw and repenetrate elsewhere. The area of penetration was haemorrhagic and oedematous and these severe conditions persisted for at least one month. Portions of larvae within the submucosa were surrounded by an acellular cast, probably a nematode secretion. Bier *et al.* (1976b) investigated the blood picture of infected pigs and found a mild eosinophilia one day after infection which receded by day seven. Increased blood levels of amylase, lactic dehydrogenase and bilirubin were also found.

Infection of monkeys, including the Taiwan monkey (*Macaca cyclopis*), was reported by Hsieh and Chen (1970), Wu (1970) and Yamaguchi *et al.* (1970). A brown bear from Alaska was found infected with larval *Anisakis* by Davey (1971b).

In conclusion, mention may be made of the studies of Kitayama *et al.*

(1967) on parasitic granulomata in stray dogs in Hokkaido, Japan. The small granulomatous foci were often caused by foreign bodies or *Toxocara canis* larvae but in some cases unidentified nematode larvae were found. Since all infected dogs were from seaside areas it is possible that *Anisakis* larvae were involved. One case studied in detail revealed *Terranova* (= *Phocanema*) and *Contracaecum* larvae in stomach lesions.

IX. ANISAKIASIS OF MAN

A. GEOGRAPHICAL DISTRIBUTION AND EPIDEMIOLOGY

Anisakis has only relatively recently been recognized as an agent of disease in man (see historical account in Jackson, 1975). Human infection with *Anisakis* was classified by Sprent (1969) into one of four categories of anthro-pozoonosis; for an appraisal of this classification and of the approach of other authors to the concept of "zoonoses" see Williams and Jones (1976).

Hitchcock (1950) found "ascarid" larvae in the stools of 10% of over 100 Alaskan Eskimos examined, one specimen was "with reasonable certainty" identified as *Anisakis*. The first recorded case of a gastrointestinal lesion in man attributable to *Anisakis* occurred in The Netherlands in 1955 (Van Thiel *et al.*, 1960). From 1955 to 1968 a total of 160 confirmed or highly probable cases of anisakiasis were reported in The Netherlands, but since the introduction of freezing regulations for green herring in 1968 (see section XI) only a few new cases have been observed (Van Thiel, 1976). In Japan, anisakiasis was first reported in 1965 (Asami *et al.*, 1965) and subsequently some hundreds of cases have been detected (Oshima in Van Thiel, 1976). Individual cases have also been described from Korea (Kim *et al.* 1971), Denmark (Andreassen, 1970), France (Doby *et al.*, 1975), Belgium (Fain *et al.*, 1969) and the USA (Pinkus *et al.*, 1975).

Anisakiasis might occur in any country where the population at large or ethnic minorities traditionally eat raw or inadequately cooked marine fish or squid harbouring live *Anisakis* larvae. Jackson (1975) reviewed this aspect in a public health context and, with regard to the USA, discussed the possibility of increased risk of infection through increasing consumer interest in "foreign dishes" and the "natural foods movement" with its emphasis on not overcooking food.

Particular eating habits in countries whose people are traditionally at risk may have a bearing on the actual occurrence of anisakiasis. Thus Chen (1971) reported no human cases of anisakiasis in Taiwan and offered the interesting suggestion that the Chinese, unlike the Japanese, eat raw fish at the end of a meal when their stomachs are full and that this may reduce the chances of infection.

Many cases of anisakiasis have been diagnosed from sections of nematodes in tissues resected from the gastrointestinal tract. Given the difficulty of positively identifying anisakine larvae in histological sections it should be stressed that not all of these cases may have been due, in fact, to *Anisakis*; it is possible that one or more of several other anisakine genera were involved.

Where entire larvae recovered from man have been definitely identified as *Anisakis* they appear to fit the description of type (I) larvae (see section IV. E1). In some cases there are indications of a moult to the preadult (fourth) stage.

In Japan, most cases of anisakiasis have occurred in patients aged 20 to 50 years (Oshima, 1972). In both Japan and The Netherlands, recorded rates of infection are higher in men than in women (Ruitenbergh, 1970; Oshima, 1972). Tentative suggestions have been made that this sex difference is due to the greater propensity of men to eat raw sea food during the day with alcoholic beverages but no firm conclusion is possible.

In Japan over half the recorded cases of anisakiasis have occurred in the stomach whilst in The Netherlands most cases are intestinal. The reasons for the difference in the localization of larvae are not clear but some suggestions are discussed by Van Thiel (1976). These include differences between the two countries in the quantity of seafood eaten at any one time, the frequency of consumption, stomach fullness, rate of stomach emptying, methods of diagnosis and acidity of gastric juices. Climatic conditions are known to influence gut complaints and, particularly, gastric acidity, so this factor alone may account for the observed differences (Van Thiel, 1976).

B. CLINICAL AND PATHOLOGICAL ASPECTS

As mentioned above, both gastric and intestinal anisakiasis occur in man. The clinical presentation of the two conditions differs in some respects. In gastric anisakiasis sudden stomach pains, nausea and vomiting occur 4–6 hours after the ingestion of raw sea food. Such cases are rarely correctly diagnosed and become chronic with similar manifestations lasting for more than a year. Eosinophils range from 4–41% in more than half the recorded cases but leucocytosis is absent or slight (Oshima, 1972). Yokogawa and Yoshimura (1967) found occult blood in the gastric juices and stools of 70% of cases. Anacidity or hypoacidity occurred in many patients; possibly reduced or enhanced gastric secretion enhances the ability of the larvae to penetrate the stomach wall (Asami and Inoshita, 1967).

Intestinal anisakiasis occurs usually within seven days of the ingestion of raw seafood. There is severe pain in the lower abdomen with nausea, vomiting, fever, diarrhoea and occult blood in the stools (Van Thiel *et al.*, 1960; Ishikura *et al.*, 1967; Oshima, 1972; Pinkus *et al.*, 1975). Eosinophilia is not usually apparent but in many cases there is a marked leucocytosis. Within the peritoneal cavity 200–500 ml of straw-coloured ascites may be present (Ishikura *et al.*, 1967). The affected region of the small intestine, usually the ileum, is often covered with a fibrous exudate and oedematously thickened causing partial obstruction and proximal distension of the intestine (Van Thiel *et al.*, 1960; Ishikura *et al.*, 1967; Oshima, 1972; Pinkus *et al.*, 1975). Larvae are occasionally found protruding through the gut wall into the abdominal cavity (Ishikura *et al.*, 1967; Van Thiel and Van Houten, 1967). In addition, larvae can be found at other sites in the alimentary tract including the caecum, appendix, colon and rectum, or outside the gut including lymph

nodes, pancreas, greater omentum and mesenteries (Otsuru *et al.*, 1965; Van Thiel and Van Houten, 1967; Yokogawa and Yoshimura, 1967). There have also been some cases of larvae penetrating the wall of the pharynx (Morishita and Nishimura, 1965; Tanaka *et al.*, 1968; Kim *et al.*, 1971).

Japanese workers have classified the lesions found in anisakiasis into five types, the following descriptions of which are based largely on the account of Oshima (1972).

1. A "foreign body response" reaction can occur in the stomach causing benign clinical symptoms with infiltration and proliferation of neutrophils associated with some eosinophils and foreign body giant cells. There may be granulomatous change around the invading larva.

2. In acute intestinal anisakiasis a "phlegmonous reaction (Arthus type)" is frequently seen. There is oedematous thickening of the intestinal wall with massive eosinophilic infiltration of all layers accompanied by other cellular elements. Haemorrhage and fibrin exudation may occur. The larva, which is usually alive or intact, is surrounded by a layer of eosinophils, neutrophils and histiocytes. The larva may be visible in an ulcer in the mucosa but it is often buried within the submucosa, where the reaction is centred, with no ulceration. The mucosa may be oedematous with obliteration of the mucosal folds (Pinkus *et al.*, 1975). The lymphatic channels, including regional lymph nodes, may be involved in the eosinophilic granulomatous response (Pinkus *et al.*, 1975).

3. In chronic gastric and intestinal anisakiasis an "abscess type" lesion may be found. A marked abscess with many eosinophils accompanied by histiocytes and lymphocytes is seen around the degenerating larva and this in turn is surrounded by a granuloma. At the inner layer of the granuloma, necrosis and haemorrhage with eosinophilic infiltration and fibrin exudation or fibrinoid degeneration are seen.

4. In cases of gastric anisakiasis of more than six months duration, an "abscess-granulomatous" type lesion is sometimes found in which a degenerating larva is situated in a reduced abscess surrounded by granulation tissue with slight collagenization. Eosinophilic infiltration of the granuloma is less intensive than in the abscess type lesion and indeed lymphocytes may be dominant instead of eosinophils. The degenerating larva is invaded by eosinophils and surrounded by foreign body giant cells.

5. The most advanced lesion, the "granulomatous" type, occurs when the abscess is completely replaced by granulomatous tissue with eosinophilic infiltration. Only larval debris may be apparent. This type of lesion is seen only occasionally in long standing cases of gastric and intestinal anisakiasis.

C. DIAGNOSIS

The diagnosis of anisakiasis is difficult and in most cases is only correctly made after post-operative pathological examination of the affected part of the gut. Gastric anisakiasis is often diagnosed as stomach ulcer, cancer, tumour or polyp, whilst intestinal anisakiasis is diagnosed mainly as acute appendicitis or as acute regional enteritis (Crohn's disease) (Oshima, 1972). A clinical

diagnosis of gastric anisakiasis can be achieved by fibergastroscopy when the living larva can be observed directly penetrating the stomach wall. Larvae can sometimes be seen by X-ray using the "mucal relief method" (Oshima, 1972). In intestinal anisakiasis the symptoms are alarming and patients are normally operated on within a few days of the onset of disease. Post-operative diagnosis is made by identification of whole larvae or, less reliably, by examining cross sections of larvae in histological sections.

The difficulty of accurate diagnosis of anisakiasis may account for its recent recognition as a disease in man. Before the disease was recognized it seems probable that cases were misdiagnosed, as described above. Thus, according to Oshima (1972), of 1531 cases diagnosed initially as acute regional enteritis, 140 were due to helminths mostly thought to be *Anisakis* whilst 622 were a phlegmonous reaction (Arthus type) with eosinophilia possibly caused by *Anisakis*. The possibility that *Anisakis* was responsible for at least some cases of eosinophilic granulomata in the alimentary tract in the UK and Norway is discussed by Ashby *et al.* (1964) and Jacobsen and Berland (1969). The number of cases of anisakiasis reported from Japan is low in relation to the size of the population at risk and it is suggested by Oshima (1972) that this is due to the difficulty that larvae have in penetrating a healthy human stomach or intestine.

An account of the search for a suitable immunodiagnostic test for anisakiasis in man is given in section X.D.

D. TREATMENT

Because of the difficulty of making a correct diagnosis of anisakiasis and because of the often alarming nature of the symptoms, especially in the acute intestinal condition, treatment is usually by surgery with resection of the affected part. In diagnosed cases of acute gastric anisakiasis, larvae may be removed by means of a gastrofiberscope. In chronic gastric anisakiasis the affected area should be removed surgically in order to avoid possible future allergic exacerbation of the lesion (Oshima, 1972).

If a correct pre-operative diagnosis can be made in intestinal anisakiasis a conservative treatment is recommended with administration of antibiotics and isotonic glucose solution (Oshima, 1972). Prognosis of anisakiasis is good providing post-operative peritonitis is avoided. Van Thiel *et al.* (1960) reported that two patients died due to post-operative enteritis after surgery for intestinal anisakiasis.

X. IMMUNOLOGY AND IMMUNODIAGNOSIS OF ANISAKIASIS

A. GENERAL REMARKS

It has proved difficult to form a coherent view of the extensive literature on this general aspect and it may be helpful to offer some insight into the ways in which this situation may have arisen.

Before a humoral or cell-mediated antibody response can be elicited it may be presumed that at least some worm penetration of the tissues must occur.

Oshima (1972) suggested that *Anisakis* larvae may have difficulty in penetrating the healthy human gastrointestinal tract. However, Asami and Inoshita (1967) experimented with guinea-pigs and regarded the penetration capacity of the larvae as more important than host susceptibility; they noted, as did Lee and Chyu (1970), that some anterior halves of larvae were capable of penetration. Penetration of larvae through the alimentary tract into the body-cavity was recorded in guinea-pigs by Myers (1963) and Asami and Inoshita (1967), and in rats by Nagase (1968b), Young and Lowe (1969), Gibson (1970) and Hashiguchi and Takei (1975). On the other hand, larvae were apparently not recovered from the body-cavity of rabbits by Oyanagi (1967), Ruitenbergh (1970, 1971) or Ruitenbergh *et al.* (1971). Other examples could be cited but are there real differences between experimental hosts in this regard and can experience with any of them be extrapolated to the situation in man?

Williams and Jones (1976) drew attention to these problems in their interesting review of marine helminths (including *Anisakis*) and human health, and advocated an experimental approach using a far wider range of experimental terrestrial animals of which the physiology or, at least, natural feeding habits are similar to those of man.

In addition to these problems, difficulties of interpretation with regard to immunological and other work on *Anisakis* have arisen due to a combination of one or more of the following factors:

- (a) Different workers have used different fish hosts as sources of larval *Anisakis* in different zoogeographical areas.
- (b) One or more species of *Anisakis* may have been used, given the difficulties of specific determination of larvae.
- (c) Larvae have been subjected to different storage techniques and times, with varying degrees of handling and possible damage.
- (d) Some workers have treated their experimental hosts with antiperistaltic drugs, fed them special diets, or starved them before infection.
- (e) The mode of infection has varied.
- (f) There is often inadequate information on whether or not a moult to the fourth stage has occurred and, if so, in which habitat.

Bier (1976) also noted difficulties of this sort and emphasized that establishment of laboratory life cycles is necessary to provide experimental material in the quantities needed for reproducible results.

With the above reservations in mind, there follows a review of work on the immunopathology and immunodiagnosis of anisakiasis.

B. "HYPERSENSITIVITY" IN RELATION TO PATHOGENESIS

Secondary exposure to antigen of a primed or sensitized animal may result in boosting of the immune response but it may also cause tissue-damaging reactions. These are described as hypersensitivity reactions which may be classified into four types (Coombs and Gell, 1968) to which a fifth type was added by Roitt (1971). The classification is as follows: Type I reaction (anaphylactic, reagin-dependent); Type II reaction (cytotoxic); Type III

reaction (damage by toxic complexes); Type IV reaction (delayed, tuberculin-type, cell-mediated); Type V reaction (stimulation hypersensitivity). Types I, II, III and V are immediate-type reactions which involve humoral antibodies, whilst Type IV involves antibodies bound to the surface of lymphocytes. Specialists in this field do not appear to agree on definitions and mechanisms involved in hypersensitivity reactions. In these circumstances it is difficult to offer a clear picture of the position regarding anisakiasis, especially in view of the fragmentary and equivocal nature of much of the evidence. Type I and Type III reactions at least may be involved.

No special studies have been made of the immune response to infection with *Anisakis* in those animals which feature in the "normal" life cycle, such as crustaceans, squid, fish or marine mammals. Some information on this aspect may be deduced from the histopathological studies that have been made of infected fish and marine mammals (see sections VIII. B and F) but most of the available information comes from investigations on the humoral antibody response of man and other mammals to accidental or experimental infection. Recent work from Japan (Nakamura *et al.*, 1974; Saeki *et al.*, 1973, 1975) suggests that some attention is now being paid to cell-mediated immunity; these brief reports are in the Japanese language and in the form of "abstracts of meetings" so no further comment is possible here.

Rodenburg and Wielinga (1960) suggested the possible "allergic" nature of anisakiasis of man and since that time a number of Dutch and Japanese workers have discussed this aspect from observations not only in man but also in other mammals including experimentally infected pigs, guinea-pigs, cats, dogs and rabbits.

In an attempt to explain the apparent rareness of anisakiasis of man, several workers have investigated the "double-hit theory" apparently first postulated by Roskam (see Ruitenbergh, 1970). The view is that within a certain period a second *Anisakis* larva needs to penetrate the gastrointestinal tract at approximately the same site where a first larva has already penetrated in order to produce severe reaction. In a series of papers Kuipers and co-workers (Kuipers, 1962, 1964; Kuipers *et al.*, 1963) investigated this suggestion of a local hypersensitivity resulting from primary infection using experimentally infected rabbits. From these studies and observations on the proximity of lesions in resected tissue samples from man, Kuipers (1964) concluded that the "double-hit theory" was valid. Ruitenbergh and co-workers (Ruitenbergh, 1970, 1971; Ruitenbergh *et al.*, 1971) reviewed the evidence and conducted further studies on experimentally infected rabbits. Ruitenbergh (1971) concluded that a local hypersensitivity may be produced by a previously penetrated larva and that another larva penetrating at this site may provoke severe reaction with necrosis, oedema and haemorrhage. Significantly, however, he found that the same reaction could be induced by one larva in a previously uninfected rabbit; he suggested, therefore, that human anisakiasis may be caused by a single larva.

As an alternative to the controversial "double-hit theory", Oshima (1972) discussed the "exacerbation theory" of Kozima *et al.* (1966) to explain severe reaction in man. Exudate from a dead larva may react with the newly formed

granuloma which has been previously sensitized by the metabolic products of the living larva. Similarly, from experiments with rabbits, Oyanagi (1967) suggested that the causative agents of tissue reaction may be the excretions and secretions (ES) of living larvae or the decomposition products of dead larvae. Oshima (1972) pointed out that the "exacerbation theory" does not explain the "phlegmonous type (Arthus type) reaction" seen in acute intestinal anisakiasis of man and discussed this problem further. Discussion of a supposed local hypersensitivity in the gastrointestinal tissues of man appears to founder on uncertainty as to how long the hypersensitivity takes to develop and, importantly, for how long it persists.

Pinkus *et al.* (1975) noted that the marked oedema, eosinophilia and granuloma formation in anisakiasis appear out of all proportion to the small size of the worm; they suggested that spreading lymphangitis may be involved in pathogenesis. In a human case studied by Pinkus and co-workers, the eosinophilic and granulomatous response involved lymphatic channels including the regional lymph nodes. Antigens released by the degenerating larva may be carried through the lymphatics of tissue which has already been sensitized by material from the live parasite. As these substances progress along the course of lymphatics, they may elicit an eosinophilic granulomatous thrombolympangitis. Obstruction of lymphatic channels by this mechanism may contribute to the marked oedema.

From intradermal tests using somatic or ES antigen of larval *Anisakis*, Kobayashi *et al.* (1968b) reported higher skin sensitivity in persons predisposed to allergic diseases (e.g. asthma, urticaria, eczema and dermatitis) than in "normal" persons. Kobayashi *et al.* (1968c) reported that the ES antigen showed a higher specificity than the somatic antigen in skin tests. Suzuki *et al.* (1970, 1971) used a haemoglobin extract of larval *Anisakis* as antigen in intradermal tests on man; tests on over 1000 Japanese showed positive reactions to be more common among people living by the sea than in those inhabiting mountainous regions. From these results and special observations on clinical symptoms and pathology in 35 cases, Suzuki *et al.* suggest that anisakiasis may be divided into two forms, mild and fulminant: the mild form shows negative intradermal reaction and may be the result of primary infection; the fulminant form shows positive intradermal reaction and may be an allergic reaction induced by secondary infection. However, conclusions arising from the use of skin tests should be viewed with caution; false positives may arise because antigenic material itself may be capable of degranulating mast cells and releasing histamine, without there being any need for it to react with the specific antibodies found only in infected individuals (Terry, 1968).

C. ANTIBODY RESPONSE

Ascaridoid infections in general are characterized by a good response of all major classes of immunoglobulins. A distinctive feature is the unusually high levels of IgE and IgG antibodies that are induced in man and animals. High

levels of specific IgE are produced not only by active ascaridoid infection but also by sensitization with either crude extracts or semi-purified preparations from the nematodes. The ascaridoids also show a marked degree of antigenic cross-reactivity between different species and genera (Soulsby, 1976).

These general statements may apply to accidental infection with *Anisakis* in man and some support for this view has come from animal experiments. Taniguchi (1970a) injected phosphate-buffered saline extracts of larval or adult *Anisakis* with Freund's complete adjuvant into the foot pads of rabbits. After six days homocytotropic antibody was found in three of five rabbits and persisted for 14 to 20 days. In rabbits infected with three oral doses of 60 larvae at two-day intervals, homocytotropic antibody was detected after ten days in two of three rabbits and persisted for about ten days. The maximum titre for homologous passive cutaneous anaphylaxis (PCA) was reached after a latent period of 72 hours and was present at 144 hours. Homocytotropic activity was heat labile and inactivated by reduction and alkylation. From their work on mice experimentally infected with larval *Anisakis*, Kobayashi *et al.* (1972, 1974) concluded that the immunoglobulin responsible for the observed mast cell degranulation was mostly reaginic in nature. Reagins are generally recognized as belonging to the IgE class (Eisen, 1974).

Sato *et al.* (1975) reported on possible aetiological mechanisms that operate in anisakiasis. Their work is interesting with regard to the mast cell degranulation studies of Kobayashi *et al.* (1972, 1974) and to immediate-type hypersensitivity (anaphylactic) reactions in relation to IgE described by Eisen (1974). Sato *et al.* (1975) sensitized guinea-pigs with larval *Anisakis* and examined, *in vitro*, the anaphylactic response of segments of ileum. Segments contracted vigorously when treated with whole worm extract of larval *Anisakis* but there was no response when the treatment was repeated. A lower response was obtained with larval extracts of *Terranova* (= *Phocanema*) and *Contracaecum* (? *Thynnascaris*).

The eosinophil leucocyte with granuloma formation is a regular feature of many helminth infections including *Anisakis* in man and animals. Poynter (1966) discussed evidence which suggests that the eosinophil is attracted by, and concerned with, histamine liberated as a result of tissue damage. More recently, Warren (1974, in discussion on schistosomiasis) briefly discussed the "eosinophil chemotactic factor of anaphylaxis" in relation to granuloma formation and IgE but the picture is not yet clear.

The formation of precipitate in the head region of larval *Anisakis* incubated in human sera may not be an immune phenomenon since it was observed in both "immune" and "control" sera (Van Thiel, 1967). The precipitate may involve non-specific esterase, high concentrations of which occur in the "excretory gland" but which does not appear to be antigenic (Ruitenbergh, 1970; Ruitenbergh and Loendersloot, 1971a, b).

D. IMMUNODIAGNOSIS

Search for a sensitive and reliable immunodiagnostic test for anisakiasis of man has been hampered largely by the common problem of cross-reactivity

with other genera and species in the superfamily Ascaridoidea and even with other, less closely related, helminths. Cross-reactivity has been a feature of a variety of tests (intradermal, complement fixation, immunofluorescent, haemagglutination) using various antigens; most of the published information has been adequately reviewed by Ruitenber (1970), Oshima (1972) and Williams and Jones (1976).

Antigenic analyses, conducted largely by Japanese workers, also reveal evidence of cross-reactivity. For example, from tests using heterologous and homologous antisera, Taniguchi (1966) reported cross-reactivity between larval *Anisakis*, adult *Toxocara* and adult *Ascaris*. Starch gel electrophoretic analysis of larval *Anisakis* and adult *Ascaris* suggested that only one component of more than 20 from each of the nematodes was species specific (Suzuki, 1968).

Taniguchi (1970b) sensitized rabbits with saline extracts of either larval *Anisakis* (from mackerel) or adult *Anisakis* (from dolphin); in serological tests, higher precipitation titres occurred with the adult extract. Electrophoresis demonstrated more than six antigenic components in the larval extract and more than five in the adult extract; Ouchterlony tests failed to distinguish between adult and larval extracts. Tsuji (1975) used immunoelectrophoresis to study homologous and heterologous antibody-antigen reactions of the antigens from various helminths; he stated that specific bands were identified for larval *Anisakis* (from mackerel and dolphin) and that larval and adult antigens of *Anisakis* are different.

Perhaps the most promising immunodiagnostic test to have been reported is that of Suzuki *et al.* (1974) using an indirect fluorescent antibody test with haemoglobin extracted from larval *Anisakis* as antigen. The haemoglobin antigen is claimed to be stable and sensitive; the extraction technique and antigenic characteristics were reported in a series of earlier papers (Suzuki *et al.*, 1969, 1970, 1971; Sato *et al.*, 1973, 1974; Shiraki *et al.*, 1973). The method used is essentially that of the soluble antigen fluorescent antibody test of Toussaint and Anderson (1965), with coupling of the protein to fine agarose beads as suggested by Porath *et al.* (1967). The haemoglobin is extracted from perienteric fluid of larval *Anisakis* by ammonium sulphate precipitation and DEAE cellulose column chromatography. Coupling of the water-soluble haemoglobin to cyanogen bromide-activated Sepharose 4B eliminated difficulties associated with removing non-reacting test serum, and hence conjugate, from the cellulose acetate matrix. The antigen-coupled gel retained its antigenicity for over two months at 4°C; greater stability was obtained by lyophilizing the material in a 2% saccharose and 0.5% BSA solution. In cross-reactivity experiments with *Ascaris* and *Toxocara* infections in rabbits, the *Anisakis* haemoglobin antigen was more specific than whole worm antigen. The success of this or any other immunodiagnostic technique for general use will depend on the free availability of antigen in sufficient quantities and the careful standardization of reagents.

XI. PREVENTION OF ACCIDENTAL INFECTION WITH *ANISAKIS*

The importance of *Anisakis* as a human pathogen has resulted in much research into methods of locating larvae in fish, particularly in the flesh, and killing them. Larvae are colourless and normally tightly coiled in a spiral some 3 mm in diameter which makes them extremely difficult to find within the flesh. In the fish processing industry, candling of fish fillets has been widely used to detect larval nematodes. Candling involves examining fillets over a ground glass screen illuminated from below by a fluorescent light. Although candling is a reasonably efficient method of locating the larger and more highly coloured larvae of *Phocanema* it is, in our experience, of only limited value in the detection of *Anisakis* larvae. In fish with dark-coloured flesh it is virtually useless. An improvement in the candling technique was suggested by Power (1961) who sliced fillets longitudinally before candling; such a method can be of only limited commercial value.

Most larval *Anisakis* within the flesh of fish are located within the hypaxial muscles which surround the body cavity (see, for example, Templeman *et al.*, 1957; Novotny and Uzmam, 1960; Smith and Wootten, 1975). The practice of block filleting in which the epaxial muscles alone are removed from the fish will result in only a relatively small proportion of the *Anisakis* larvae in a fish remaining in the final product.

A number of other methods of locating nematode larvae within the flesh of fish has been developed but these are of value only in scientific investigations or in small-scale samplings of commercial catches. The simplest method is dissection of the flesh but the most efficient and reliable technique is digestion of the flesh using a mixture of pepsin and hydrochloric acid (Stern *et al.*, 1958; Smith and Wootten, 1975). Larval *Anisakis* are extremely resistant to digestion even at temperatures up to 50°C presumably because of the tough nature of the cuticle. The superiority of a digest method over dissection was demonstrated by Stern *et al.* (1958) who found that digest recovered 21.9% more *Anisakis* larvae from the flesh of chum salmon (*Oncorhynchus keta*) than manual dissection. Similarly, low numbers of larval *Anisakis* in the flesh of herring reported by Khalil (1969) and Davey (1972a) compared with those given by Smith and Wootten (1975) are certainly due to the use of a digest technique by us.

Oishi *et al.* (1971) used several techniques to determine the number of larval *Anisakis* in the flesh and viscera of a number of fish and squid species. In contrast to other workers they did not find enzymatic digestions suitable; bacterial decomposition was also unsuitable. Larvae were easily detected by the compression of musculature between two glass plates but this method was less suitable for dark muscle or viscera. Autolysis was a better method for recovering larvae from the viscera.

Because it is impossible to detect all larval *Anisakis* within the flesh of fish without rendering it useless for human consumption, methods must be devised of killing larvae before it is eaten. In countries such as the United Kingdom, where fish products are usually well cooked before consumption

anisakiasis is apparently rare. Larval *Anisakis* are killed very quickly at temperatures above 60°C both in water (Kawada, 1968) and in fish flesh (Davey, 1972b). At temperatures below this the death of larvae cannot be guaranteed.

In some smoking processes, for example the kippering of herring, the temperature of the fish flesh does not rise above 40°C so live larvae may still be present. Khalil (1969) found that cured and smoked split herring contained live larvae, although deboned and block-filleted herring in which the hypaxial muscles are removed contained no larvae. In a review of Dutch smoking procedures, Ruitenbergh (1970) suggested that kippered and golden herring which are heated to about 28°C and 40°C respectively during the smoking process would contain live larvae whereas red herring heated to 60°C would be safe. Hot smoking processes employed in the United Kingdom for the preparation of salmon, white fish and some herring products involve temperatures of up to 80°C which would result in the death of all larvae.

Freezing of infected fish probably represents the only effective way of controlling anisakiasis where fish are eaten raw or lightly salted. Gustafson (1953) showed that at -17°C all larval *Anisakis* were killed within 24 hours. Commercial freezing of 100 lb blocks of herring at about -30°C for 16 hours followed by storage at -12°C killed nearly all larvae within 24 hours and any survivors apparently within one week. In The Netherlands, freezing regulations were introduced to prevent anisakiasis through consumption of lightly cured herring. Under these regulations herring must be frozen to -20°C within 12 hours and kept at not higher than this temperature for at least 24 hours (Ruitenbergh, 1970). Ruitenbergh was unable to infect rabbits with larval *Anisakis* treated in this way. Frozen fish may be safely used for smoking or other processes which in themselves will not kill all larvae. In some fish, notably herring, there is a migration of *Anisakis* larvae from the viscera into the flesh if fish are not gutted immediately after death (Van Thiel *et al.*, 1960; Vik, 1966; Smith and Wootten, 1975). Such migrations could be prevented by shipboard freezing soon after capture. Trash marine fish to be used as feed for cultured fish should also be frozen before use in order to prevent transmission of larval *Anisakis* (Bassleer *et al.*, 1973; Wootten and Smith, 1975).

Larval *Anisakis* are very resistant to curing processes. Van Thiel *et al.* (1960) showed that larvae could survive in a saturated salt solution for 1½ hours whilst Khalil (1969) reported that they survived in such a solution for less than one day. At a concentration of 50 g litre⁻¹, however, larvae survived for a "very long time". In The Netherlands, salted herring may be divided into "heavily salted" (kept in a brine solution of a strength of at least 20° Baumé) and "lightly salted" (kept in a solution of less than 20° Baumé). In the former, larvae are killed within ten days but in the latter larvae can survive for long periods thus necessitating the use of freezing regulations (Ruitenbergh, 1970). Grabda (1973) reported that larval *Anisakis* can survive for 7-35 days in a brine solution of 15°-19° Baumé. Dry salt will kill larvae in less than ten minutes (Khalil, 1969) but a suitable concentration must be reached throughout the body of the treated fish. As an example of this, Khalil (1969) noted

that in lightly cured herring kept in coarse dry salt for six days only 2% of the larvae from females survived but 82% from males were still alive. Khalil attributed this difference to the greater moisture content of soft roes lessening the effect of the salt.

Larval *Anisakis* are also able to withstand the marinating process for herring as used in Europe. Marination involves subjecting herring to an acetic acid and brine mixture in order to denature the proteins. The duration of the process may vary as does the ratio of herring to solution in the baths. This ratio may vary from 1 part herring to 2 parts solution to 2 parts herring to 1 part solution. Ruitenbergh (1970) found that a herring/solution ratio of 1:1 and an initial concentration of 4% acetic acid and 6% sodium chloride would not kill *Anisakis* larvae after 26 days, although earlier Dutch work had shown that marination for 70 days in this solution was effective. The earlier work also showed that with a herring/solution ratio of 2:2:1 and an initial concentration of 7% acid and 15% salt, the ability of larvae to penetrate agar gel *in vitro* was reduced from 100% after six days to only 3% at 30 days. Priebe *et al.* (1973) concluded that at a salt concentration of 5.8% and pH not greater than 4.1, herring should remain in solution for at least 30 days. At 5.0% salt concentration the period should be 35 days. When these conditions cannot be fulfilled, only deep-frozen herring should be used.

Dutch and Japanese workers have experimented with the use of irradiation to render herring safe. Van Mameren and Houwing (1967) found that in aqueous solution all *Anisakis* larvae were killed in a salt concentration of 6% and a radiation dose of 1.0 Mrad (at 0.006 Mrad min⁻¹). Many larvae survived at lower salt concentrations and radiation doses. Larvae in 5% salted vacuum packed herring irradiated at 0.3 and 0.6 Mrad at 0.003 Mrad min⁻¹ survived in large numbers. Higher dosage rates unacceptably altered the flavour of the herring products. Oishi *et al.* (1972b) concluded that the use of irradiation in fish processing is not practicable because decomposition of fish products occurs at doses over 0.6 Mrad.

Anisakis larvae are remarkably resistant to a wide variety of chemicals, spices and food additives (Oshima, 1972; Oishi *et al.*, 1974) many of which are used in the preparation of raw fish dishes in Japan. Kato (in Oshima, 1972) found that larvae were killed in two hours by a 5% solution of commercial wasabi powder, a Japanese fish seasoning, but suggested that larvae in fish would not be killed during the usual conditions of use of wasabi.

In many countries, particularly in the Far East, the practice of eating raw or very lightly salted fish is widespread and deeply rooted in the way of life of the population. For these reasons, public health education programmes designed to draw attention to the dangers of anisakiasis will be unlikely to achieve great success. In such countries it would be extremely difficult to apply preventive legislation such as compulsory freezing of fish products because the fishing industry is often made up of widely scattered small units of production and the variety of fish used for human consumption is very great. It is probable that in some of these countries the elimination of anisakiasis cannot be separated from their economic and social development which may eventually enable effective preventive measures to be undertaken.

XII. *ANISAKIS* AS A BIOLOGICAL TAG FOR FISH

As the need for a more rational use of the dwindling fish resources of the world increases the accurate distinction of fish stocks becomes of paramount importance. Fisheries scientists have relied mainly on mechanical tagging procedures to determine the movements of fish but such methods are time consuming and expensive with a very poor return rate of tagged fish. In recent years increasing use has been made of parasites as biological tags for fish.

The use of parasites in this manner depends on the fact that different stocks of a fish species may have significantly differing rates of infection of one or more parasite species. Thus, parasitological examination of a sample of fish may reveal from which stock it is derived. If a quantitative assessment of the infection rate of different stocks can be made, it is at least theoretically possible to identify the individual components of mixed stocks.

A number of conditions which preferably must be fulfilled before a parasite can be successfully used as a biological tag were listed by Kabata (1963). In summary these are as follows:

1. A parasite should be common in one stock but rare or absent in other stocks of fish.
2. The parasite should include only the host species under study in its life cycle.
3. Infections of the host fish with the parasite must be of reasonably long duration.
4. The infection of the parasite must remain relatively stable within and between years.
5. Environmental conditions throughout the area studied must be within the physiological range of the parasite.

The use of larval *Anisakis* as a biological tag for fish may be criticized in relation to the second of these conditions. Kabata (1963) acknowledged that the usefulness of a parasite with two or more hosts in its life cycle will depend on the distribution and abundance of all the host populations. *Anisakis* has crustacean, squid, fish and mammalian hosts, and within any given area there may be a number of species of each group acting as hosts. It is doubtful that there is any geographical area where the circulation of *Anisakis* amongst its different hosts is adequately defined, so the value of the parasite as a tag is reduced.

Few workers who have used larval *Anisakis* as biological tags have attempted a specific identification of the larvae. The possibility always exists that at least two species of *Anisakis* occur in the host under investigation and this greatly complicates the use of the parasite as a tag. The incidence of infection of larval *Anisakis* in fish probably varies greatly over a relative short period (Wootten and Waddell, 1977) and this also might affect the usefulness of the parasite as a tag in the long term.

Large differences in the abundance of larval *Anisakis* in a fish species from different areas are well known and not surprisingly, therefore, larvae have

been used as biological tags, either alone or in conjunction with other parasites, by a number of workers.

Bishop and Margolis (1955) found that the intensity of infection of Pacific herring (*Clupea pallasii*) with larval *Anisakis* differed significantly between geographical areas off British Columbia. Within the Gulf of Maine, Sindermann (1961a) found that Atlantic herring (*Clupea harengus*) spawning on Georges Bank and off Nova Scotia were distinguishable by differences in the prevalence of larval anisakines and cestodes. In juvenile herring from the coast of Maine, Sindermann found that one-year-old fish from eastern Maine were more heavily infected with larval anisakines than fish from western Maine. This difference persisted in two-year-old herring although "western" fish showed a proportionally greater increase in infection which indicated a possible westward movement of herring that had spent their first year off the east coast of Maine. It is possible that the herring examined by Sindermann were parasitized by both larval *Anisakis* and *Thynnascaris*.

Considerable differences in the incidence of infection with larval *Anisakis* of herring from the Gulf of St. Lawrence and around Newfoundland were noted by Parsons and Hodder (1971). Herring fished near the Magdalen Islands in spring and autumn and near Gaspé in summer had a very similar level of infection to those fished off south-western Newfoundland in winter, which suggested that all these fisheries were on a common stock of herring. A similar incidence of infection with larval *Anisakis* in herring from north-eastern Nova Scotia and the Banquereau-Sable Island areas suggested an inshore-offshore migration of fish between these areas.

From a study of the level of infection with *Anisakis simplex* larvae in addition to differences in otolith type and gonad maturity, Grabda (1974) was able to distinguish between stocks of herring in the southern Baltic. Only those spring-spawning herring which spawned in this area from November to May were heavily infected with larvae. These fish acquired their infections in the North Sea during the summer months. Herring which had spent all their lives within the Baltic were only occasionally infected.

The use of *Anisakis* larvae as a biological tag may be of value in the separation of adult herring stocks around the British Isles. The level of infection of herring from these waters with larval *Anisakis* varies greatly between different localities (Khalil, 1969; Davey, 1972a). In general, the abundance of larval *Anisakis* decreases from north to south in these waters.

In the northern North Sea, MacKenzie and Wootten (1973) showed that the level of infection of whiting (*Merlangius merlangus*) with larval *Anisakis* (probably *A. simplex*) and the plerocercoids of the trypanorhynch cestode *Gilquinia squali* was highest in central offshore areas and lowest off the Scottish East coast, with an intermediate level of infection around the Shetland Isles. These observations are not inconsistent with the results of mechanical tagging which indicate distinct inshore and offshore whiting populations in the northern North Sea with an area of mixing around the Shetlands (Hislop and MacKenzie, 1976).

The use of parasites, including larval anisakines, as biological tags for red-fish (*Sebastes marinus*) was suggested by Sindermann (1961b), who found

larvae to be much less abundant in fish from offshore areas along the coasts of Maine and Nova Scotia than in those from inshore areas. A comparison of the parasite fauna of redfish from different fishing grounds in the general area suggested little mixing of stocks.

Anisakis larvae have also been used as biological tags for fish populations in limited inshore areas. Gibson (1972) used a number of parasites of flounders (*Platichthys flesus*), including *Anisakis* sp. larvae, as biological tags to distinguish between populations in a small area off the Scottish East coast. Flounders from the estuaries of the Rivers Dee and Ythan could be distinguished from each other and from fish caught in the purely marine habitat of Aberdeen Bay. *Anisakis* sp. larvae were much more abundant in the flounders caught in the open sea, probably because more infected crustacean hosts were available in this area. Kilambi and De Lacy (1967) were able to distinguish between spawning populations of surf smelt (*Hypomesus pretiosus*) on the coast of Washington State and in Puget Sound by the levels of infection with larval *Anisakis*. Fish from the outer coast were more heavily infected than those in Puget Sound, from which it was deduced that they had fed in the open sea where the cetacean hosts were abundant.

The distinction between Atlantic salmon (*Salmo salar*) of European and North American origin in the high seas fishery at Greenland has assumed great importance in recent years as a result of the demand for conservation of this species. Nyman and Pippy (1972) found that *Anisakis simplex* larvae, in conjunction with the abundance of the cestode *Eubothrium crassum* and biochemical features of the host fish, could be used as a biological tag. In biochemically identified salmon of North American origin caught at Greenland there appeared to be significantly fewer *Anisakis* larvae than in European fish. The numbers of larvae in mechanically tagged and biochemically identified N. American salmon were similar. In an earlier publication, Pippy (1969) used parasites, including larval *Anisakis*, to distinguish between salmon populations caught off the Canadian East coast. It appeared to be possible also to distinguish between salmon which had migrated to Greenland and those which had not. Konovalov (1967) used a number of parasites including larval *Anisakis* sp. to identify local stocks of Pacific salmon (*Oncorhynchus*) in the eastern USSR.

Sindermann (1961a) suggested that parasites will seldom provide definite answers to problems of the identification of fish populations and migrations but that in conjunction with other techniques, such as mechanical tagging and meristic studies (to which must now be added serological studies), they may provide a significant contribution. Within these limitations *Anisakis* larvae may be useful as a biological tag.

XIII. CONCLUSIONS

This review of the literature on *Anisakis* and anisakiasis (*sensu stricto*) has revealed many gaps in our knowledge to which attention has been drawn in various sections. The aim of the following paragraphs is to highlight only certain of the aspects which appear to warrant further investigation.

Reference to *Anisakis* in the more recent literature as "herring-worm" is misleading and should be avoided in future; in at least the European North Atlantic, recent work suggests that whilst larval *Anisakis* is common in herring it is equally, if not more, abundant in other fish species, including blue whiting (*Micromesistius poutassou*) (Wootton and Smith, 1976, and work in progress).

Despite Davey's (1971a) revision of the genus *Anisakis* there are still taxonomic problems, including the distinction between *A. simplex* and *A. typica*. The generic diagnosis of *Anisakis* should be re-examined in the light of Petter's (1972) redescription of *A. insignis*.

Anisakis larva (I) from North Atlantic waters has been cultured *in vitro* and shown to develop into *A. simplex*. There is a need to culture the other "larval types" in order to confirm that they do, in fact, represent *Anisakis* and, if so, to determine which species they represent.

Whilst euphausiids appear to be important crustacean hosts of *Anisakis* at least in offshore areas, further work may show other crustaceans to be significant, possibly in inshore waters. Further investigation may show squid to be of greater significance in the transmission of *Anisakis* to final hosts than the literature indicates, bearing in mind the importance of squid in the diet of many cetacean species (Clarke, 1966). Several aspects of development remain obscure. For example, the course of events in crustacean hosts has not been fully established, including whether or not the second moult occurs in these hosts.

There is a dearth of information on the behaviour, physiology and biochemistry of different stages in the life history of *Anisakis*. Such information as exists is concerned mainly with the larval stage from fish. Rather more physiological work has been done on the related genus *Phocanema* and similar experimental approaches to *Anisakis* may prove to be rewarding.

For example, detailed studies by K. G. Davey and co-workers on the moulting process in *Phocanema* have provided evidence that an "ecdysial hormone" released from neurosecretory cells of the ganglia associated with the nerve ring may act on the "excretory gland", causing activation and release of "leucine aminopeptidase" and other enzymes (Davey, 1976, and references therein).

Certain volatile ketones and alcohols are produced by larval *Phocanema* and the intriguing suggestion has been made that these may function as local anaesthetics as the worm burrows through fish muscle (Ackman and Gjelstad, 1975; Ackman, 1976).

In view of the taxonomic importance of the "excretory system" in ascariid nematodes (Hartwich, 1974) it is unfortunate that its physiological functions are so poorly understood; *Anisakis* may prove to be a useful experimental model for investigation of these functions.

With regard to accidental infection of man with *Anisakis*, further study might be made of a possible allergic predisposition to severe gastrointestinal reaction.

In addition to *Anisakis*, certain other marine anisakine genera (e.g. *Phocanema*, *Contracaecum*) have been implicated as causative agents of human

anisakiasis (*sensu lato*). In these circumstances there is a need for reliable methods of differential diagnosis.

There is much scope for future investigation.

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