Aspects of the ecology and pathology of *Stephanochasmus baccatus* (Nicoll, 1907) (Digenea; Stephanochasmidae)

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by

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The work presented in this thesis is the result of my own investigations and has neither been accepted nor is being submitted for any other degrees.

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ABSTRACT

The digenean Stephanochasmus baccatus (Nicoll, 1907) is a parasite found in marine flatfish which are of importance to the mariculture industry in Scotland. In the area of study, Loch Ewe, on the North-west coast of Scotland, the first intermediate hosts are the gastropods Buccinum undatum (L.) and Neptunea antiqua (L.). Several species of teleost fish of the Order Heterosomata act as second intermediate host and the definitive host is Eutrigla gurnardus (L.) or Myoxocephalus scorpius (L.). Aspects of the host/parasite relationships at all the host levels were investigated.

Samples of B. undatum and N. antiqua were collected from May to September and the incidence of the natural infections of the major digenean species were recorded. In some samples, height, weight and copulatory organ length were measured with a view to finding an indicator of parasitism.

The histopathology of S. baccatus in the molluscan host was described and seasonal changes discussed in relation to observed variations in the infectivity of cercariae to second intermediate hosts.

Groups of 1+ fish were experimentally infected with cercariae at intervals from May to September when no more S. baccatus infections were found in the molluscs. Differences in the infectivity of the cercariae during these months were demonstrated. The pattern of glycogen deposition in both the

molluscan digestive gland and the intra-molluscan larvae were studied in an attempt to explain the loss of infectivity in cercariae in July and August.

A reduction in infectivity of cercariae after ageing was demonstrated experimentally by infecting fish with cercariae which were freshly dissected out and with cercariae kept for 10 hours at 12°C. This loss of infectivity was correlated with loss of glycogen from cercarial tails.

A variety of I-group intermediate host species were infected experimentally and the distribution of cysts in the body, fins, skin and muscle were compared with the turbot, Scophthalmus maximus (L.) and the plaice, Pleuronectes platessa L. These were shown to differ significantly.

Four species of intermediate host which are of culturable significance, S. maximus, P. platessa, the common dab, Limanda limanda (L.) and the Dover sole, Solea solea (L.) were experimentally infected and the tissue responses to invasion and encystment of S. baccatus were examined sequentially. The inflammatory response and metacercarial growth in each species of fish were found to differ in some important aspects which affected the subsequent viability in the definitive host. The viability of metacercariae from P. platessa and S. maximus were tested by controlled infection of the definitive host M. scorpius. A number of wild caught definitive hosts were examined for natural infections.

An attempt was made to describe the seasonal cycle of events in the life history of S. baccatus based on the information from this and other studies. The relative significance of different host species in different geographic locations was discussed.

ASPECTS OF THE ECOLOGY AND PATHOLOGY OF *STEPHANOCHASMUS BACCATUS* (Nicoll, 1907) (Digenea; Stephanochasmidae)

C O N T E N T S

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All micrographs have been enlarged x 1.25 unless otherwise stated.

LIST OF ABBREVIATIONS

a : amoebocyte
bv : blood vessel
c : cercaria
cc : columnar cell
cf : collagen fibres
cnt : connective tissue
ct : ctenidium
d : digestive gland
dc : degenerating cells
df : dermal fibres
dm : degenerating myofibres
dt : degenerating tubule
ex : excretory bladder
f : foot
fr : fibrocytic replacement tissue
g : gonad
gb : germ ball
gc : giant cell
gl : glycogen
h : heart
i : intestine
IL : Inner Layer
knu : karyorhectic nucleus
l : leukocyte
m : metacercaria
ma : macrophage
ML : Middle Layer
n : nerve
nu : nucleus
nt : normal tubule
OL : Outer Layer
os : oral sucker
pd : parasite debris
pf : proliferating fibroblasts
pnu : pyknotic nucleus
pp : PAS positive material
pm : paracyst membrane
r : redia
rbc : red blood cell
ri : redial intestine
rw : redial wall
s : siphon
Sc : scale
sc : secretory cell
t : digestive gland tubule
td : tubule debris
tp : tunica propria
v : vacuole
vct : vesicular connective tissue

SECTION I

INTRODUCTION

INTRODUCTION

The aquaculture industry in Scotland has been established on a limited scale for a number of years but currently is undergoing a period of expansion. Not only is more capital being invested in existing fish farming systems but there is a considerable interest in the development of techniques for the culture of new species in both the marine and freshwater environments. While in Britain, freshwater aquaculture has been concerned with farming salmonids, marine aquaculturists have investigated the production of less expensive species as well as high cost fish. Some species of flatfish come into this category and the techniques for the culture of plaice, Pleuronectes platessa L., are now well established. The present study was initiated at a time when these fish were thought to be showing economic potential and more detailed knowledge of their diseases was necessary.

The West of Scotland has a coastline and climate well suited for the purpose of mariculture. With this view, studies of the ecology of the natural fauna of these areas are being carried out. Edwards & Steele (1968) investigated the ecology of the plaice population in the Loch Ewe area on the North-West Coast. Larval flatfish are pelagic but after metamorphosis they settle on the sea bottom to feed. In the Loch Ewe area, plaice settlement was found to occur from April to June. They remained in the nursery ground for two years feeding mainly from the siphons of the lamelli branch, Tellina tenuis, Da Costa. The same authors showed that the mortality rate for plaice was 50% per month for the first six months after settlement. Although towards the end of this period the decline in population was partly due to emigration to deeper waters,

in the first few months the decline was a result of mortalities. It is possible that many of these mortalities were related to high parasite burdens or selected predation on heavily infected fish. Numerous studies such as those of Sinderman & Rosenfield (1954), Steele (1966) and MacKenzie (1968) have suggested that intermediate stages of digenean parasites of wild fishes could be important in this respect.

Most Digenea have a life cycle involving three hosts, the first intermediate host of which is a mollusc and the definitive host a vertebrate. In the present study, the second intermediate host of the parasite investigated, is a teleost fish. Since each host harbours a different stage of the life cycle with a different morphology and biology, the effects on the host differ. Digenea are normally well-adapted to their molluscan hosts and appear rarely to be the direct cause of mortalities (Wright, 1966). They may, however, be of much greater pathological significance to the second intermediate host. Cercariae may invade the second intermediate host through the integument or through the wall of the alimentary canal and the resultant traumatic damage can be severe, especially during the migration through the tissues to the site of encystment. (Erasmus, 1972; Matthews, 1973b) Subsequent damage depends on many aspects of the host/parasite relationship.

The systems used for the culture of marine flatfish vary in the degree to which the fish are in contact with members of the natural fauna which may act as first intermediate hosts for digenean parasites. The "ranching" system involves the minimum amount of interference in the natural environment. Similarly, the enclosed pond system does not permit, to any great extent,

the separation of second intermediate host from first intermediate host. In this system, used at Ardtoe on the West Coast of Scotland, a site is selected with a narrow inlet which allows some control of tidal water exchange. Cages are also used which can be moored at selected sites, either floating in midwater or lying on the sea-bed. The greatest separation of fish from the natural environment is the onshore system, such as that used at WFA Hunterston in South-West Scotland where tanks are supplied with heated seawater effluent from adjacent nuclear power station. Even in the latter system, however, the fish stock may be exposed to water which has passed through tanks containing cultured invertebrates which may act as intermediate hosts for many Digenea which infect fish (McVicar & MacKenzie, 1977).

On the nursery grounds juvenile flatfish of a number of species share their habitat with the common or white whelk, Buccinum undatum (L) and the red whelk, Neptunea antiqua (L). These act as first intermediate hosts of the digentic trematode Stephanochasmus baccatus (Nicoll, 1907) [= Stephanostomum baccatum. Following Dollfus (1972), Stephanochasmus Looss is placed in the Family Stephanochasmidae Nicoll, which is characterised by an I-shaped excretory vesicle rather than a Y-shaped one, as in the Acanthacolpidae.]

Previous studies (MacKenzie, 1968, MacKenzie & Gibson, 1970 and MacKenzie & Liversidge, 1975) have shown the pathogenicity of S. baccatus for plaice in the Loch Ewe area but there are gaps in knowledge of several important aspects of the larval biology, ecology and pathology of this parasite.

Recently marine aquaculture developments have expanded to include the rearing of other flatfish species such as Dover sole, (Solea solea L), brill (Scophthalmus rhombus L) and, in particular, turbot (Scophthalmus maximus L). Previous records show that S. maximus is a host of S. baccatus in British waters (Table I) but for the other species no information was available. The study, therefore, expanded to include a variety of flatfish species with potential culturable significance.

The results of this study are also of general interest in the host/parasite relationships of larval Digenea with molluscs and fish. Studies at the first and second intermediate host level have usually been either entirely host orientated, with emphasis on mortality or condition of host, or entirely parasite orientated with little regard to the host, other than as a medium for the growth of the parasite. Few studies have examined the sequential effects of the infection in the intermediate hosts from both an ecological and pathological point of view. The present work has attempted to study aspects of the relationships of a single species of parasite at all the host levels in order to highlight the interrelationships involved and the delicate balance in which they are held. This approach necessitated a study of the intimate association of host and parasite from a histopathological point of view. Histopathology is one of the disciplines of major interest at the Unit of Aquatic Pathobiology and is also a relatively neglected aspect of parasitological studies, particularly of molluscs (Wright, 1966). It is hoped that the work carried out will make a contribution towards bridging the gap between parasi-

tologists with little knowledge of pathology and pathologists with little interest in parasitology.

The scientific names for fish species have been used throughout the text instead of the common name. This is to avoid confusion because of the large number of species which have the same common name in Britain and America but which are of different species and, therefore, have different scientific names.

STEPHANOCHASMUS BACCATUS (Nicoll, 1907)

Stephanochasmus baccatus has been reported many times in British waters although identification in earlier reports is suspect. Prior to 1908, S. baccatus was named Distoma hystrix Dujardin, Stephanostomum hystrix Dujardin, and Distomum valdeinflatum Stossich. The taxonamy of the genus is still confused and is discussed at length in Wolfgang (1955a), Stunkard (1961), MacKenzie (1971) and Dollfus (1972). The life history in different hosts and areas, however, has been described by Wolfgang (1955a), MacKenzie & Gibson (1970) and MacKenzie & Liversidge (1975).

Wolfgang (1954 a and b) carried out extensive observations on infections of S. baccatus in the Winter flounder, (Pseudopleuronectes americanus (Walbaum) in eastern Canadian waters and carried out the basic work on the life cycle as part of his study. However, Stunkard (1961) questioned the validity of some of his experiments anc conclusions, in particular his description of the cercaria and his attempts to infect the definitive host. This prompted the work of MacKenzie & Liversidge (1975). The

life cycle is now established and involves members of the Family Buccinidae as first intermediate hosts, teleosts of order Heterosomata as second intermediate hosts and a variety of definitive hosts which are all piscivorous fish.

In Canadian waters Wolfgang (1955a) found the rediae and cercariae occurred in the common whelk Buccinum undatum and the ten-banded whelk Neptunea decemcostatum (Say) where they occupied the gonads and digestive gland. He stated that cercariae of S. baccatus most closely resembled those of Cercaria neptunea (Lebour, 1911). However, he stated that Lebour (1911) considered her cercaria to be the larval form of Acanthopsolus lageniformis but did not carry out experiments to prove it. This statement is rather surprising since Lebour (1911) described the rediae and cercariae of Acantholopsus lageniformis following her description of C. neptunea. B. undatum and the red whelk, Neptunea antiqua were recorded in the North Sea as natural hosts of C. neptunea by Lebour (1911); and Zelukmann (1966) recorded B. groenlandicum as host to C. neptuni* in Kandaluk Bay. C. neptunea was subsequently shown to be the larva of Stephanochasmus baccatus by MacKenzie (1971).

B. undatum and N. antiqua are large monotocardian prosobranchs of the Sub-order Neogastropoda, Family Buccinidae (Fretter & Graham, 1962). They occur fairly commonly in the sub-littoral zone off the West Coast of Scotland down to 140 metres (Allen, 1962). Their habitat overlaps that of 0-group plaice and other flatfish on the nursery feeding grounds where the molluscs are

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* His spelling

carnivorous, scavenging on a variety of polychaetes, molluscs, crustaceans and fish. Breeding occurs in December for B. undatum and March for N. antiqua (Allen, 1962).

The cercariae of S. baccatus are produced by rediae in the molluscan digestive gland. Young rediae actively feed and contain germ balls; mature rediae have no birth pore and rupture to release mature cercariae. Wolfgang (1955a) described germ ball remnants and mature cercariae as being released together. He also suggested a possible rediae-producing generation of rediae. Zelukmann (1966) said that there were no embryos in rediae containing mature cercariae and he also described a mother sporocyst generation. Howerver, his description differs in other respects from those of S. baccatus, e.g. in the presence of a penetration stylet in the cercariae and his specimen may not be S. baccatus. In any case, it seems that large numbers of cercariae, described by Wolfgang (1955a) as being of the ophthalmoxiphidocercous type, are all released simultaneously from the molluscan host. There are no reports of cercariae having been seen to emerge naturally from the mollusc. Since infection can only be detected by dissection of the host, this imposes a limitation on the experimental techniques. The molluscan hosts in the present study were taken from the Loch Ewe area on the West Coast of Scotland where the cercariae penetrate the skin of flatfish. Table I lists the previous reports of metacercariae of S. baccatus encysted in Heterosomata from British waters.

TABLE 1 Previous reports of S. baccatus meracercariae of S. baccatus encysted in species of Heterosomata from British waters.

Plaice, Turbot,	Pleuronectes platessa L.) Scophthalmus maximus L.)	Kroyer 1938 - 1853
	P. platessa) S. maximus)	Dujardin 1845
Common dab,	Limanda limanda L.	Olsson 1868
	L. limanda)	Johnstone 1905, Irish Sea
Witch	Glyptocephalus cynoglossus L.)	
Lemon sole	Microstomus kitt Walbaum)	Lebour 1908a, 1908b, North Sea
	L. limanda)	
Long rough dab,	Hippoglossoides platessoides Fabricius)	
	L. limanda)	Nicoll & Small 1909, Firth of Clyde
	H. platessoides)	
	P. platessa	Matthews 1968, Irish Sea
	P. platessa)	MacKenzie & Gibson 1970, West Coats of Scotland
	L. limanda)	
	P. platessa	MacKenzie 1971, West Coast of Scotland
	P. platessa)	MacKenzie & Liversidge 1975,
	L. limanda)	West Coast of Scotland

The metacercaria has been described by Wolfgang (1955a) and MacKenzie (1971). They encyst mainly in the fins and musculature of the fish, but, in the case of very small fish, all of the soft tissues may be infested. The cyst can readily be observed, when near the surface, as an opaque white spot giving the name "white spot" or "pearl spot" condition.

The distribution of the cysts in P. platessa was reported by MacKenzie & Gibson (1970) to be asymmetric, with the greater number of cysts occurring on the lower or blind side of the fish. This they related to supposed variations in the structure of the skin on the different surfaces. Wolfgang (1954b) also observed this feature in Pseudopleuronectes americanus (Walbaum) especially in younger fish, and thought it to be a feature of the burrowing behaviour of flatfish into the substrate. MacKenzie (1971) suggested that cercariae were less able to penetrate the skin of older fish because of the increase in its thickness with age. Aged cercariae were less able to penetrate the skins of P. platessa and L. limanda and it was suggested that this was due to exhaustion of the energy resources of the cercariae, which do not feed at this stage.

Wolfgang (1954b) found that parasitism in P. americanus was greater in deeper than in shallow, inshore waters and that older, larger P. americanus bore heavier infections than small ones. He also stated that the infection was not pathogenic and that the parasitism of P. americanus by the digenean did not follow any seasonal pattern. In contrast MacKenzie & Gibson (1970) found that almost all the infection of P. platessa from the West Coast of Scotland occurred in May, June and July of the first year of life and infection thereafter continued at a very low level.

There are few detailed pathological studies of the effect of larval trematode infections in fish and this is also the case for S. baccatus.

MacKenzie & Liversidge (1975) suggested that heavy infections of small 0-group P. platessa could cause mortalities or debilitate the fish to the extent that they were more vulnerable to predation. Arru et al (1968) described myopathy associated with cysts but considered that there was no inflammatory response to Stephanostomum sp. in Mullus barbatus and Mullus surmuletus in the Mediterranean. They concluded that the infections, unless massive, had no serious effect on the condition of the fish. However, adult red mullet are considerably larger than larval plaice and the results are therefore not comparable.

A number of teleost species have been recorded as definitive hosts of S. baccatus and these are shown in Table II.

TABLE II Reports of the definitive hosts of S. baccatus

Fish Species	Location	Report
Halibut, Hippoglossus hippoglossus L.	East coast of Scotland Coast of Maine Canadian Atlantic Waters The Barents Sea Gulf of St. Lawrence	Nicoll (1907, 1915) Manter (1926) Wolfgang (1955a) Polyanski (1958) Ronald (1960)
Sea raven, Hemitripterus americanus Gmelin	Canadian Waters	Wolfgang (1955a)
Wrymouth, Cryptocanthodes maculatus Storer	" "	"
Artic eelpout, Lycodes sp.	" "	"
Rock sole, Lepidopsetta bilineata (Ayres)	Bering Sea	Strelkov (1960)
Yellowfin sole, Limanda aspera (Pallas)	" "	"
Flathead sole, H. elassodon Jordon & Gilbert	" "	"
Yellow gurnard, Trigla lucerna L.	Scottish Coastal Waters	MacKenzie & Gibson (1970)
Grey gurnard, Eutrigla gurnadus L.	" " "	" "
Sea scorpion, Myoxocephalus scorpius L.	Canadian & Scottish Waters	Wolfgang (1955a) MacKenzie & Gibson (1970)

Wolfgang (1955a) found that he could experimentally infect the long horned sculpin, Myoxocephalus octodecimspinosus and the ocean pout, Macrozoarces americanus though natural infections of these had not been found. He found the most common natural host to be the sea raven, Hemitripterus americanus Gmelin, whereas MacKenzie & Gibson (1970) showed experimentally that, in Scottish waters, S. baccatus developed earlier in E. gurnadus than in M. scorpius and concluded from this and observations of natural infections, that E. gurnadus played a more important role in the life history than M. scorpius in Scottish waters.

SECTION II

MATERIALS AND METHODS

MATERIALS AND METHODS

A number of methods were common to all experiments. These are described below. Where more specific methods were employed these are dealt with in the relevant section.

1. MAINTENANCE AND SOURCE OF EXPERIMENTAL ANIMALS

1.1. Fish

Fish were maintained in tanks of circulating sea water employing either System A or System B.

1.1.1 System A

This was the system used in the main zoological aquarium in the University of Stirling and it enabled large numbers of fish to be kept in tanks with the minimum amount of maintenance 8,000 gallons of sea water are stored in underfloor tanks; this is pumped into settling tanks and from there through copper-free pipes into the main aquarium. The water is directed through fibreglass tanks at a controlled rate and outflow pipes return the water through a filter to the storage tanks. Filtration is through gravel or polystyrene filters. The temperature was held relatively constant at 12°C unless otherwise stated in the text.

1.1.2 System B - The isolation aquarium system

Glass tanks 94 x 38 cm or Plastic tanks 70 x 50 cm were held in a constant temperature room with a fixed light/dark regime. Water in individual tanks was recirculated separately using Eheim* seawater aquarium filters. Seawater was changed

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* Eheim Limited

regularly and filter fibre and carbon were also cleaned or renewed, as required.

Although System B required more attention it was found to be the more satisfactory owing to its reliability, and in the event of an accident, only one tank of fish would be affected and losses therefore minimised.

In some experiments fish were kept in plastic tanks at the DAFS Marine Laboratory, Aberdeen. Stock fish were kept in the main aquarium with seawater recirculated from a large storage tank and experimental fish were kept in tanks with individual recirculation of fresh seawater using Eheim or Dynaflo filters. Aquarium temperaturs in the DAFS, Aberdeen Laboratories experiments were maintained at 12°C.

1.1.3 Source

Experimental fish were obtained from a variety of sources although in some cases considerable difficulties were experienced in obtaining an adequate supply of fish at the right time. Availability was governed by both seasonal factors and also whether or not hatchery reared fish were available. When hatchery reared fish were unavailable, wild caught fish were used. The sources of fish are listed in Table III.

All fish used as second intermediate hosts were One-group.

All fish were fed regularly using a prepared pelleted diet (WFA6) or chopped squid.

TABLE III Source of wild and hatchery reared fish

Fish Species		Source
Plaice	(Pleuronectes platessa L.)	Hatchery reared at W.F.A. Hunterston
Plaice Flounder Lemon sole Dover sole Turbot Brill	(Platichthys flesus L.) (Microstomus kitt Walbaum) (Solea solea L.) (Scophthalmus maximus L.) (S. rhombus L.)	Wild caught from coastal regions of N.W. Scotland, e.g. Loch Ewe, Tralee Bay (nr. Oban), Luce Bay (Galloway), New England Bay (Galloway), Aberdeen Bay, St. Andrews and Fife coast.
Turbot		Many were wild caught as young O-group and subsequently reared at W.F.A. Hunterston or Ardtoe or Lowestoft.
Grey gurnard Yellow gurnard Red gurnard Scorpion fish	(Eutrigla gurnardus L.) (Trigla lucerna L.) (Aspitrigla cuculus L.) (Myoxocephalus scorpius L.)	All wild caught by the Aberdeen fishing boats or by Dr. J. Gordon of the SMBA Laboratory, Oban.

1.2 Molluscs

1.2.1 Buccinum undatum and Neptunea antiqua were captured in lobster pots by fishermen in the Loch Ewe area of North West Scotland. They were held in tanks at the DAFS Loch Ewe Field Station for a few days until it was convenient to transport them to the Marine Laboratory, Aberdeen, in tanks of wet seaweed, where they were held in tanks of circulating seawater until they were examined. Some were then transported to Stirling and examined there. Buccinum do not transport easily and several batches were dead on arrival. It is probable that they are sensitive to even small increases in temperature and, therefore, particularly vulnerable in the summer months when they were required.

When the Loch Ewe Buccinum became scarce in the summer of 1975 other sources were tried such as Millport, Loch Striven and the South Coast of England, off Portsmouth. Similar difficulties were encountered in transporting these. Both the Baccinum and Neptunea were fed on chopped squid.

2 EXAMINATION OF MOLLUSCS FOR INFECTION

Baccinum undatum and Neptunea antiqua

The environmental factors which stimulate the release of Stephanochasmus baccatus cercariae from B. undatum and N. antiqua into the environment are not known. There are no external physical features of the molluscan host to indicate that an

individual is parasitised. The only method, therefore, which could be used to assess the degree of parasitisation of the molluscan host was by direct examination of the digestive gland, although this had the considerable disadvantage in that the mollusc had to be killed and the shell removed. This was achieved by severing the head and foot and destroying the cerebral ganglion which lies in the posterior region of the head. The shell was then crushed and the digestive gland removed. The whole gland was examined under a low power stereo microscope and, in the event of an infection being observed, a small piece was removed and teased out in a drop of seawater. The tissue was then examined under high power in order to identify the parasite species.

3. EXPERIMENTAL INFECTIONS

3.1 Experimental Infections of the Second Intermediate Hosts

A suspension of cercariae obtained from the macerated digestive gland of an infected B. undatum was passed through a sieve into water containing the second intermediate hosts. All hosts were I-group.

3.1.1 Infections for Histopathological Study

Infections were carried out in plastic tanks (46 x 31 x 15 cm) containing a layer of fine sand and 9 litres of fresh seawater. This was not recirculated but well aerated and evenly illuminated. The temperature was controlled by conducting the infections in a constant temperature room set at 12°C. The duration of exposure

of fish to cercariae was $1\frac{1}{2}$ or 2 hours. At the end of the exposure the fish were removed and held through two changes of seawater before returning them to a tank in an aquarium employing System B.

3.1.2 Seasonal Infections

The fish were infected in plastic tanks (64 x 64 x 35 cm) containing a layer of fine sand and fresh seawater at 12°C in the DAFS, Aberdeen Marine Laboratory. After addition of the cercariae suspension, the fish remained in these tanks for 10 hours with good aeration.

After 10 hours the recirculation was operated as in System B and the fish remained in these tanks for a further 30 days. Thereafter, they were either sacrificed or removed to the aquarium at Stirling, operating System A.

3.2 Experimental Infections of the Definitive Host

Live Myoxocephalus scorpius were obtained from Aberdeen Bay between November and January. These were maintained in the DAFS Marine Laboratory (Aberdeen) aquarium until January when they were brought to Stirling and kept under similar conditions at 12°C. They were fed on pieces of squid until they were required.

Cysts of S. baccatus were obtained from experimental infections set up in July and August of the previous year in I-group fish. Cysts were excised carefully from the intermediate host tissue and placed in pieces of squid muscle. However, owing to the very small size of some of the cysts from S. maximus, it was difficult to do this without causing damage to the parasite.

Subsequently the parasite cysts were fed to M. scorpius while in situ in the host tissue. The pieces of muscle or fin containing the cysts were then enveloped in pieces of squid muscle and forced into the stomach of M. scorpius using a pair of long, blunt forceps. This operation was facilitated by the large mouth and short oesophagus of this animal.

The M. scorpius were observed for approximately half-an-hour after force-feeding to ensure that regurgitation did not occur. Regurgitation was avoided by placing the meat beyond the cardiac sphincter muscle.

Fish were marked using individually numbered plastic tags tied with nylon monofilament to the dorsal fin.

The length of each M. scorpius was measured and three groups formed containing both small and large fish to avoid any distribution differences due to size. One group was fed cysts from S. maximus and another group was fed cysts from P. platessa. A third group was fed squid only.

The fish were held in shallow plastic tanks containing well-aerated seawater during the feeding operation. This enabled the fish to be observed closely. After half-an-hour they were returned to large fibreglass tanks with recirculating seawater in an aquarium employing System A. They were subsequently fed only chopped squid.

4. EXAMINATION OF FISH FOR METACERCARIAE

The fish were sacrificed by a single blow to the head followed by pithing. Where possible, fish were examined fresh but otherwise fixed whole in 10% buffered formol saline. Where the experiment permitted, infected fish were left for at least 4 weeks before examination. During this time the metacercariae had grown sufficiently to be recognised fairly easily in the fish tissues. Very young larvae were extremely difficult to see because of their small size and the pigmentation of the fish skin. A stereo microscope with magnifications x 6.3, x 10, x 16, x 25 and x 40 was therefore necessary.

Fins were removed individually from the fish and each was examined by removal of the skin and inspection of finely dissected tissue. Small areas of skin removed from the body, where possible without muscle, were then examined. All the skin was removed in this way from both upper and lower surfaces. Finally myotomal, pterygiophoral and mandibular muscles were dissected finely to reveal cysts.

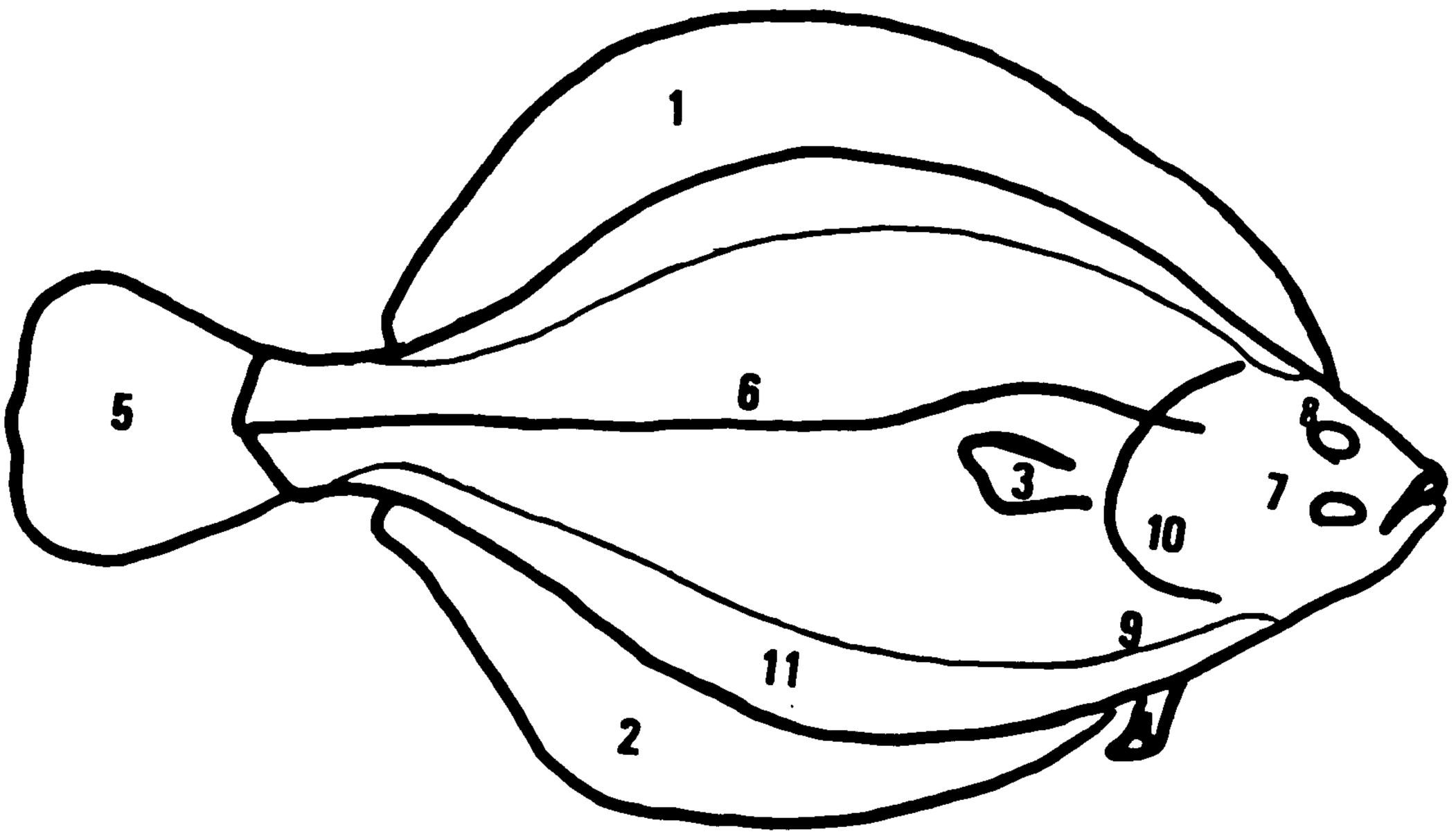
Separate collations were made of the numbers of parasites found in the dorsal fin, ventral fin, tail fin, anal fins and pectoral fins. The body was identified as upper (eyed) surface and lower (blind) surface. Notes were made of cysts in the skin, body muscle and fin muscle of each of these areas, as shown in Figure 1.

Figure 1.

Diagram of a flatfish showing the areas of the body recorded as a site of encystment of S. baccatus

1. Dorsal fin
2. Anal fin
3. Pectoral fin
4. Pelvic fin
5. Caudal fin
6. Upper body
* Lower body
7. Head
8. Eye
9. Visceral cavity
10. Operculum
*. Skin
*. Somatal muscles
11. Pterygiophoral muscles

* not shown in diagram


1
5
6
8
3
7
10
9
11
2

5. EXAMINATION OF FISH FOR ADULT STEPHANOCHASMUS BACCATUS

Most of the fish examined for adult worms were dead (a Triglidae taken from deep water, usually dies on capture). Live fish were sacrificed by a single blow to the head. The abdomen was opened and the intestine cut at the pyloric sphincter and at the external meatus. Some adult S. baccatus were frequently found just inside the anus and careful handling was necessary to prevent loss of these worms.

Intestines were cut into 2 cm lengths and very short ones into 1 cm lengths. Each piece was opened and the contents scraped onto a slide, and examined under the microscope. The flattened gut wall was then also examined for worms attached between folds. Particular attention was given to the rectum which, it is believed, is the favoured site for this species. (MacKenzie pers. comm.)

6. HISTOLOGICAL TECHNIQUES

6.1 Fish

Fish were fixed in 10% buffered formalin after being cut into suitably sized blocks. Prior to processing most blocks were decalcified for approximately 30 minutes in RDC (Bethlehem Instruments Ltd.). Processing of tissue blocks took place in an automatic tissue processor* over a 24 hour cycle, as follows.

The processing schedule was as follows: Table IV

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* Shandon Elliot Limited

TABLE IV The Processing Schedule

50%	Methylated spirits	1 hour
80%	Methylated spirits	2 hours
8%	Phenol/Methylated spirits	3 hours
8%	Phenol/Methylated spirits	3 hours
8%	Phenol/Methylated spirits	2 hours
	Absolute alcohol	2 hours
	Absolute alcohol	1 hour
	Chloroform	1 hour
	Chloroform	1 hour
	Wax	2 hours
	Wax	2 hours
	Wax	5 hours

Blocks were cut out at 5 or 6 mu on a rotary microtome*. Each wax section was examined unstained until a metacercaria was seen and the cyst was then serially sectioned and stained.

Sections were stained routinely in Haemotoxylin and Eosin (H & E). Other stains used were Periodic Acid Schiffs (PAS) with Haemotoxylin and Tartrazine counter stains. One of the trichrome stains, Martius Scarlet Blue (MSB), Picro Mallory or Masson's Trichrome stain was also employed on each section.

6.2 Molluscan Tissue

Pieces of the digestive glands of all infected molluscs were examined during the sampling period from May to September. At the same time pieces of uninfected glands were similarly collected. The uninfected glands were treated in exactly the same way as the infected glands. Tissues were fixed in either 10% buffered formol saline, Bouin's fixative or Gendre's fluid. Processing was as for fish, with omission of the decalcification. Sections were stained routinely with H & E and connective tissue and other structures were stained using the trichromes Martius Scarlet Blue (MSB), Picro Mallory or Masson's Trichrome stain.

Periodic Acid Schiffs stain (PAS) was used to demonstrate carbohydrate complexes.

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* Leitz

Alcian Blue was used for acid mucopolysaccharides. Best's carmine stain and PAS were used with salivary diastase controls to demonstrate glycogen. Controls were incubated for at least 30 minutes with diastase at 35-40°C and sections of rabbit liver were used with each staining batch as positive controls.

SECTION III

RESULTS

PART 1 THE FIRST INTERMEDIATE HOST

Natural digenean infections with particular reference to Stephanochasmus baccatus.

A. Incidence of infections in the molluscan host.

B. Pathology of S. baccatus infection in the mollusc.

NATURAL DIGENEAN INFECTIONS, WITH PARTICULAR REFERENCE TO STEPHANOCHASMUS BACCATUS

Samples of Buccinum undatum and Neptunea antiqua were examined between May and October and the incidence of the three major digenean infections was recorded. The three major species were Stephanochasmus baccatus, Zoogonoides viviparus (Olsson) and Cercaria buccini Lebour.

One sample was retained over winter in the aquarium with constant environmental conditions from November to the following May and then examined.

Because of the difficulty in establishing whether or not a mollusc was infected (the animal had to be killed and the shell smashed in each case), it was decided to see if any external variable could be used to detect infection with reliable results. The two variables examined were copulatory organ length and weight/height ratio as it was thought that there might be a significant different in one or both variables in normal and infected molluscs. The height of the shell, the total weight and the length of the male copulatory organ were measured in one large sample. In other samples, the shell height and length of the male copulatory organ only were measured.

Specimens of B. undatum and N. antiqua infected with S. baccatus were studied in more detail. The infected mollusc tissue was fixed, sectioned and stained for histological examination. Uninfected molluscs were selected as represenative of normal tissues and treated similarly.

A. INCIDENCE OF INFECTIONS IN THE MOLLUSCAN HOST

Table V shows the seasonal incidence of the digeneans found in B. undatum and N. antiqua from Loch Ewe and other sources. The incidence of S. baccatus was observed to diminish from 0.6% in September to zero in October. The high figure obtained for 29 July seemed unusual and an enquiry revealed that the fisherman had set creels in Gruinard Bay and not Loch Ewe on that occasion, owing to bad weather.

The incidence of the three major digenean species is plotted on an appropriate time scale in Figure 2 which compares the trends of the parasite incidence in the mollusc.

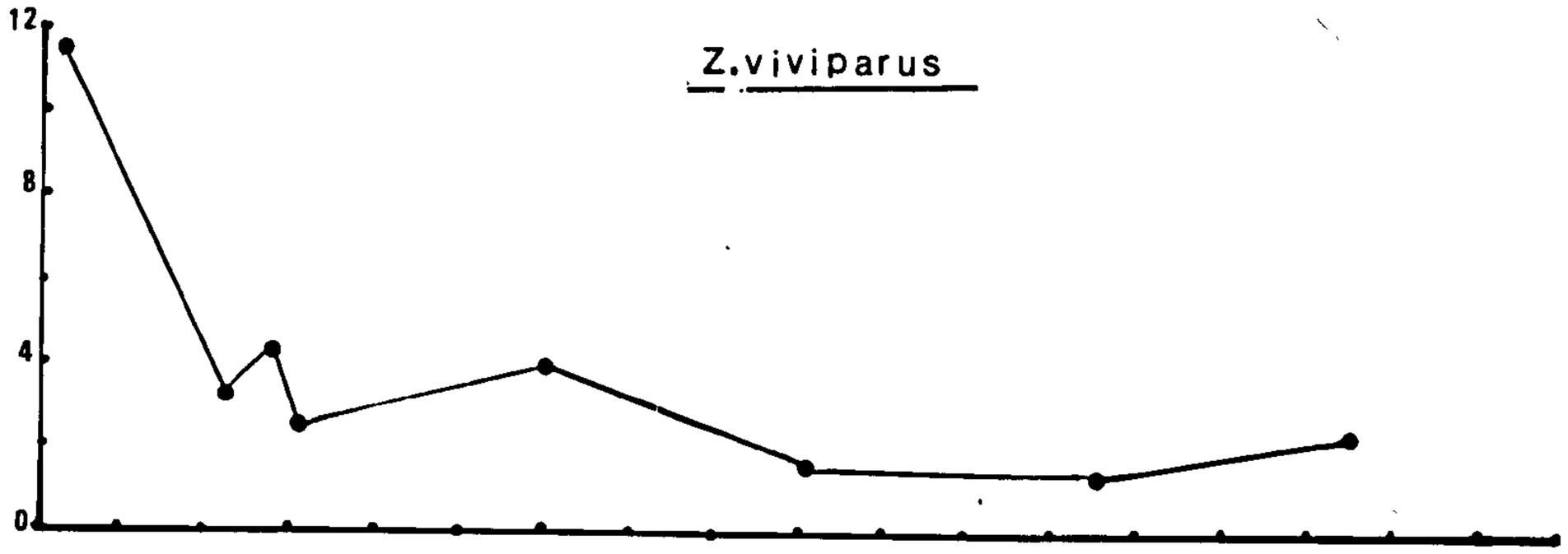
The incidence of C. buccini and Z. viviparus was variable but did not diminish in September and C. buccini over wintered in the molluscs retained from November to May in the aquarium.

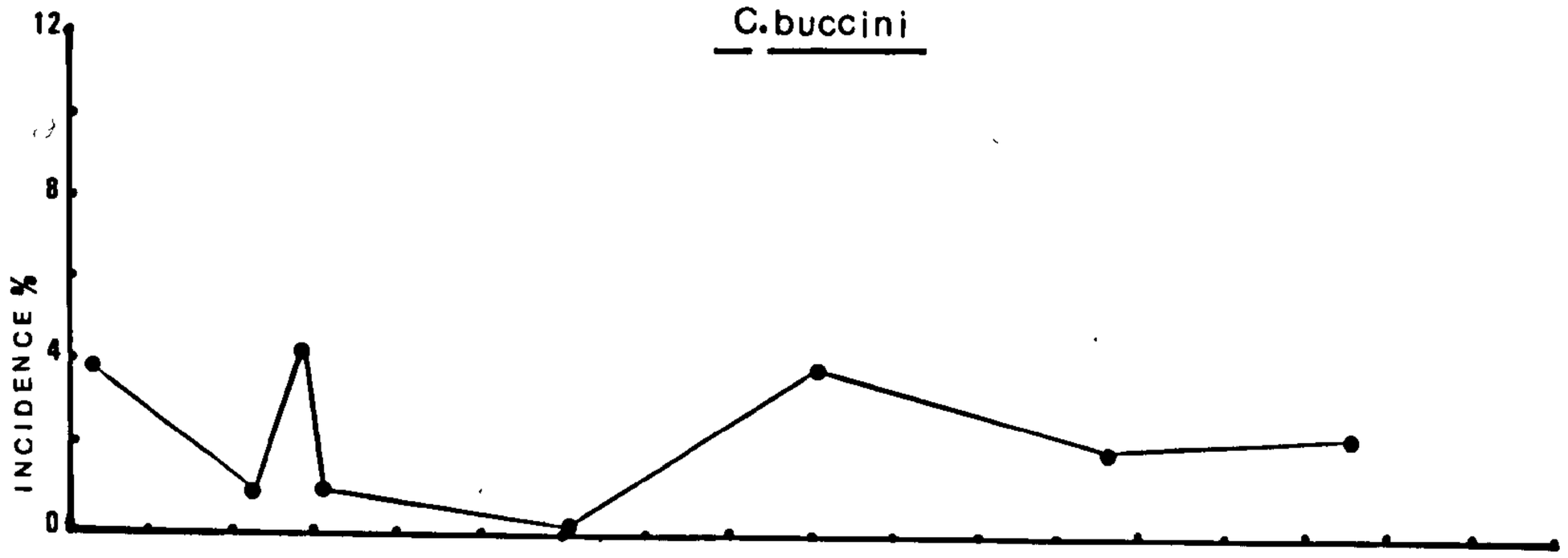
The number of N. antiqua in each sample was small and there were not enough to justify separate figures for each species of mollusc. They were, therefore grouped together in all cases.

Figure 2.

Comparison of the incidence of the three major larval digenean infections in B. undatum and N. antiqua from Loch Ewe.

o: sample from Gruinard Bay


Z.viviparus
12
8
4
0


C.buccini
12
8
4
0
INCIDENCE %

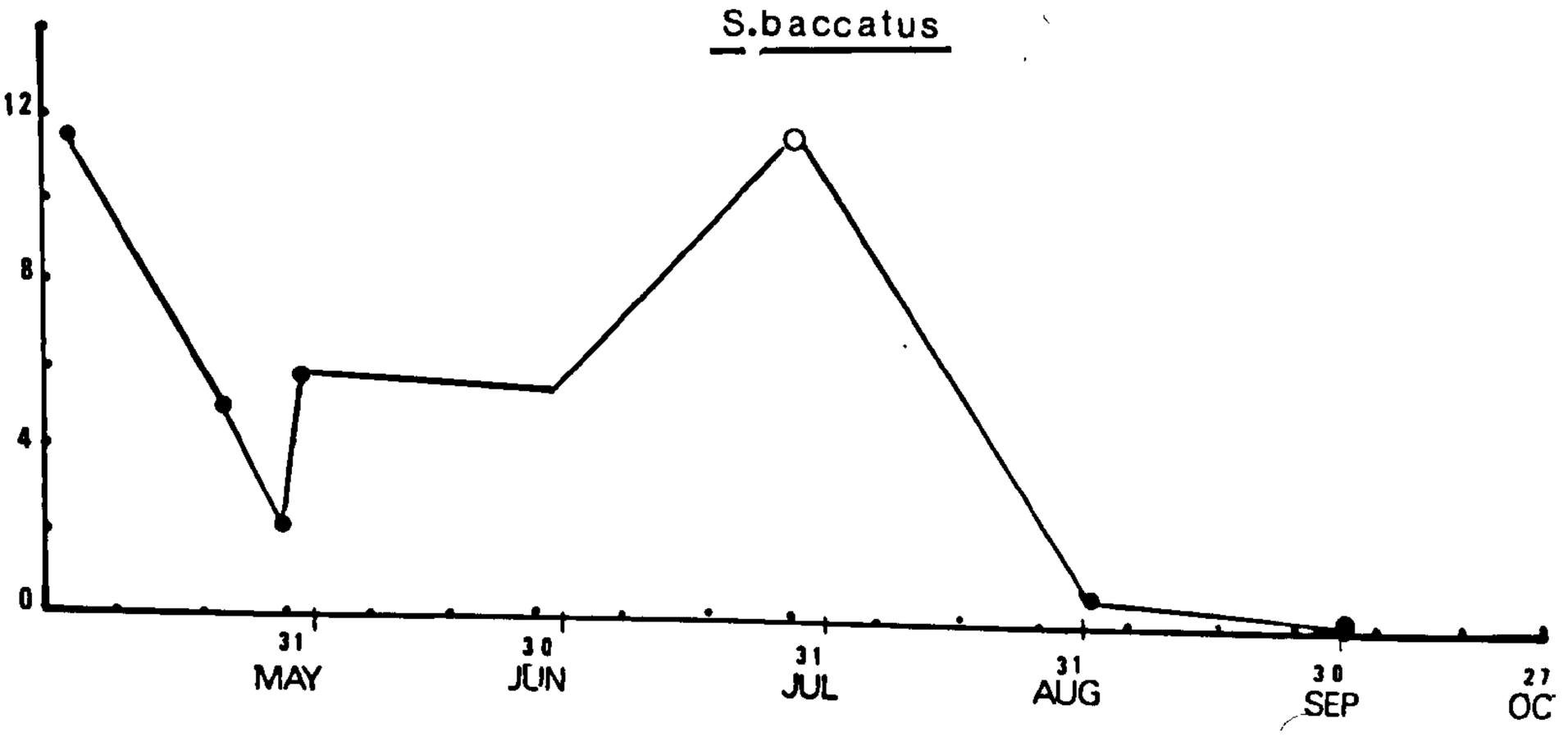

S.baccatus
12
8
4
0
31
MAY
30
JUN
31
JUL
31
AUG
30
SEP
27
OC

TABLE V Incidence (%) of larval trematode infections in Buccinum undatum and Neptunea antiqua

Source	Date of sample	S. baccatus	C. buccini	Z. vivparus	unidentifiable	Sample size
Loch Ewe	3 May 1973	11.5	3.8	11.5		26
	22 May 1974	5.0	0.8	3.4		119
	28 May 1974	2.1	4.2	4.2		48
	31 May 1974	5.8	0.8	2.5		121
	29 June 1974	5.4	0	3.9		129
Gruinard Bay	29 July 1974	11.8	3.7	1.5		136
Loch Ewe	4 Sept. 1974	0.6	1.8	1.2	1.23	163
	Oct. 1973	0	2.1	2.1	4.26	47
	Nov. 1973 examined May 1974	0	4.5	0		22
Off Portsmouth	August 1975	0	0	3.1		128
Loch Striven	28 July 1975	0	8.3	0		24
Millport	11 August 1975	1.1	5.6) 2.2) Double infection	8.9		90

Table VI shows the data obtained for the height, weight and length of the copulatory organ in the males for 5 samples. The figures given for normal (i.e. uninfected) molluscs is the mean of the sample whereas the figures given for infected molluscs are individual measurements. This is because of the relatively small number of infected individuals. Individual measurements for all molluscs are given in the Appendix tables.

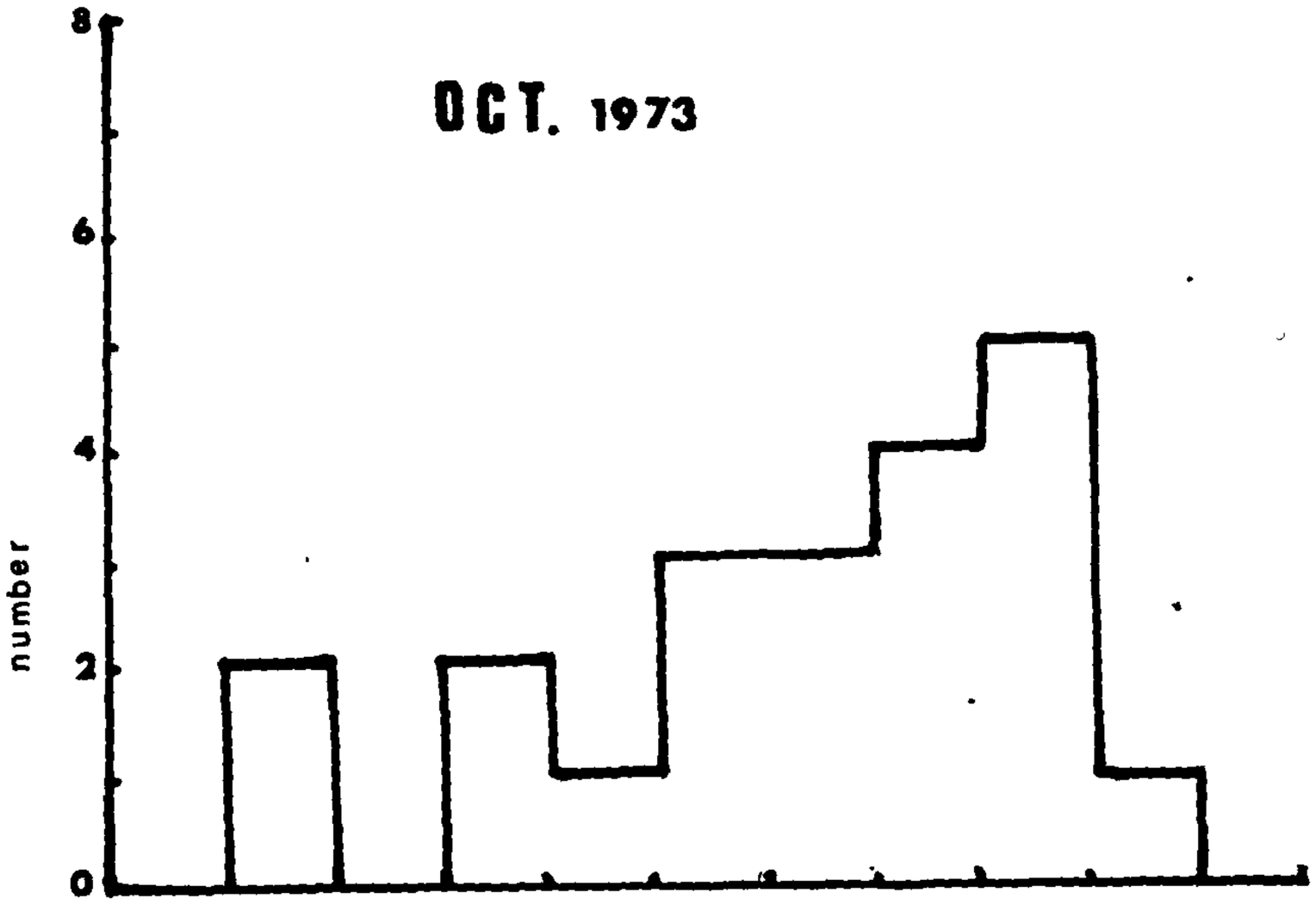
The copulatory organ length of normal molluscs was spread fairly evenly over the range 1 - 8, whereas the uninfected molluscs were 0.6, 3.5, 3.8, 3.9 and 4.9, (taking the samples for May 1973 and 1974) i.e. spread over the lower half of the range. There was no statistically significant difference between the penis lengths of infected and uninfected molluscs.

Similarly, the weight/height ratios for infected animals were 6.1, 6.2 and 6.9 which were well within the range 4 and 8 for normal molluscs. These results indicated that it was unlikely that either copulatory organ length or weight/height ratio would be a useful indication of infection, but the numbers of infected molluscs found were too small for any definite conclusions to be drawn. The weight/height ratios of normal and S. baccatus infected molluscs were not significantly different at the 10% level.

Figure 3 shows the frequency distribution of the lengths of the copulatory organ in May and October. The histogram illustrates that there is seasonal development of the organ, that is, there is an increase in size in October. The mean length of the organ in October 1973 was 6.32 cm $\pm$ 2.17 (21 molluscs) whereas in May 1974, the mean length was 4.84 $\pm$ 2.03 cm

Figure 3.

Size frequency distribution of the male copulatory organs in May and October samples


OCT. 1973
8
6
4
2
0
number

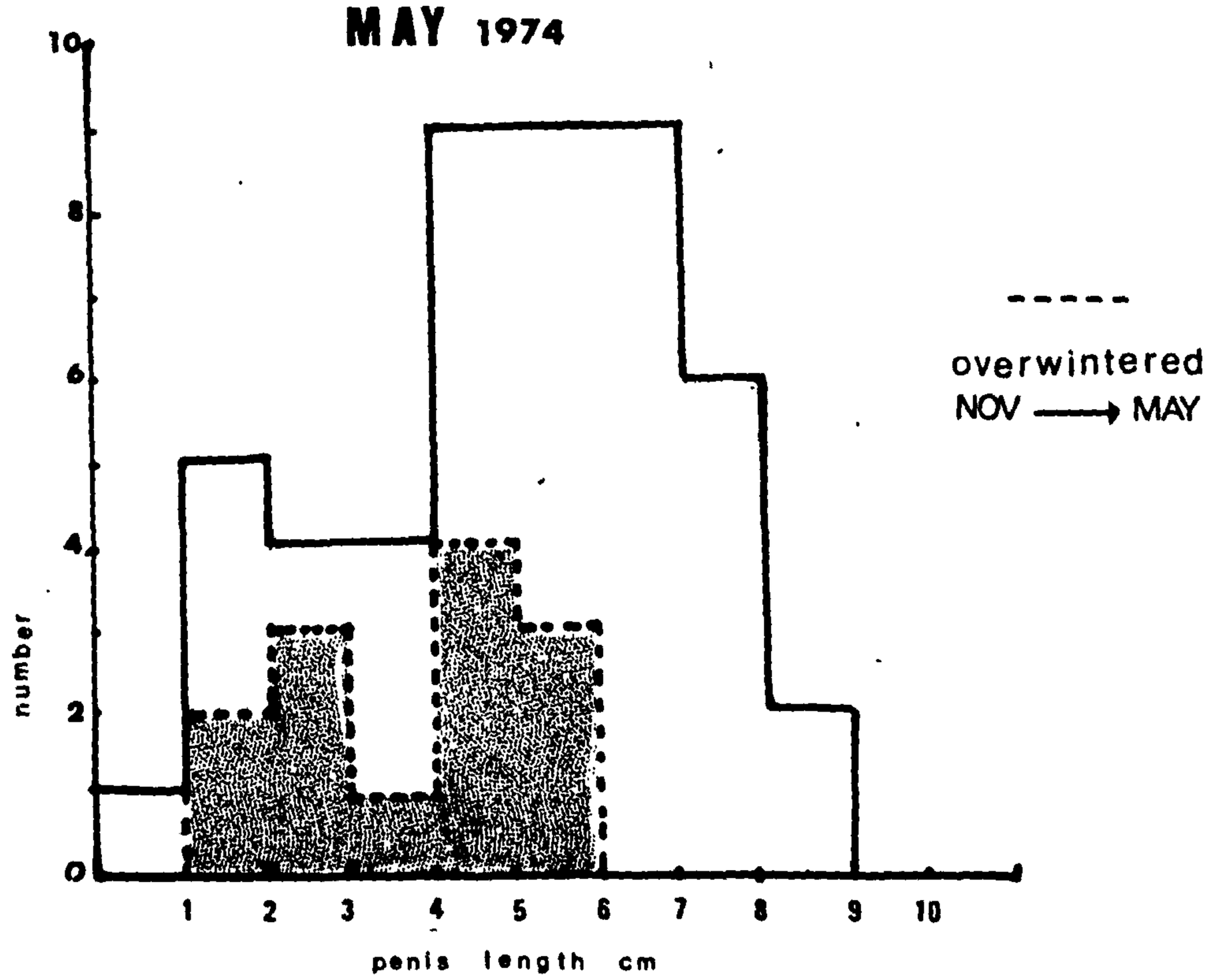

MAY 1974
10
8
6
4
2
0
number
overwintered
NOV ⟶ MAY
1 2 3 4 5 6 7 8 9 10
penis length cm

TABLE VI Showing measurements for height, weight and penis length for 5 samples. Figures for normal molluscs are means of the sample and those for infected molluscs are individual measurements.

Date		Normal		S. baccatus		C. buccini		Z. viviparus	
		M	F	M	F	M	F	M	F
3 May 1973 Sample size 26	Wt	58.1160	59.3410	(66.3640) (52.7600)	58.2460	108.8400		(44.4500) (68.8200)	40.0150
	Ht	9.1700	9.1125	(9.0000) (8.5000)	9.5000	11.4000		(11.4) (9.0)	8.0
	Pl	3.29		(0.6) (3.8)		4.1000		(1.000) (1.4000)	
	Wt/Ht	6.1960	6.4281	(6.9293) (6.2071)	6.1312	9.5474		(4.9389) (7.6467)	5.0019
	Ht/Pl	3.1735		(15.0000) (2.2368)		2.7805		(9.0000) (6.4286)	
	Pl/Wt	19.2900		103.9400 13.8842	-	22.5463		(44.45) (68.82)	
28 May 1974 Sample size 48	Ht	10.8714	9.3055		7.0000		(11.1000) (12.4000)	8.5000	4.4000
	Pl	4.512						0.9000	
	Ht/Pl	3.0314						9.4444	

Contd/...

TABLE VI Continued

Date		Normal		S. baccatus		C. buccini		Z. viviparus	
		M	F	M	F	M	F	M	F
22 May 1974 Sample size 119	Ht	7.6184	7.8646	(8.2000) (7.5000) (8.5000)	(9.6000) (8.2000) (8.1000)			(9.1000) (7.8000) (6.0000)	7.5
	Pl	4.8388		(3.9000) (4.9000) (3.5000)				(4,8000) (5.5000) (0.9000)	
	Ht/Pl	1.9862		(2.1026) (1.5306) (2.4286)				(1.8958) (1.4182) (6.6667(	
May 1974 Overwintered Sample size 22	Ht	8.5385	8.7750			8.2000			
	Pl	3.6848				1.0000			
	Ht/Pl	2.7965				8.2000			
Oct 1973 Sample size 47	Ht	9.1333	9.3636				(9.9000) (9.8000) (10.8000)		8.4000
	Pl	6.3238							
	Ht/Pl	1.7461							

Wt : Weight in grams (gms)
Ht : Height in centimetres (cms)
Pl : Copulatory organ length in cms.

M : Males
F : Females

Individual measurements for Normal and S. baccatus infected molluscs are contained in Appendix Tables 2,4,5.

(49 molluscs). The difference between the means is significant at the 1% level. The increase in the organ size in October coincides with gametogenesis at this time in preparation for spawning in the winter.

The measurements for the group which was over wintered in the aquarium and examined in May fit most closely to those of the molluscs in May in the natural habitat (Figure 3)

B. THE PATHOLOGY OF STEPHANOCHASMUS BACCATUS INFECTIONS

The Normal Digestive gland of Buccinum undatum *

Gross morphology

The digestive gland of B. undatum was found to occupy almost the whole of the visceral spire. It was brown-green or yellowish in colour. The gonad lies over the posterior part of the digestive gland and was attached to the digestive gland tubules by connective tissue (Figure 4). The entire visceral mass was enclosed by a membrane, the tunica propria. The digestive gland itself was tubular and formed by repeated branching of closed ducts opening from the stomach.

Histology

The main histological feature of the digestive gland of B. undatum was the mass of digestive gland tubules which were embedded in a stroma of fine connective tissue (Figure 5). The connective tissue was more extensive between the visceral mass and the bounding membrane and there was a particularly dense fascia binding the gonad to the digestive gland. The latter area was particularly well endowed with arteries derived from the dorsal aorta. The intertubular connective

* The findings for Neptunea antiqua were the same as for Buccinum undatum with respect to both normal and infected animals unless otherwise stated.

Figure 4.

General body plan of B. undatum and N. antiqua

ct - ctenidium
d - digestive gland
f - foot
g - gonad
h - heart
o - operculum

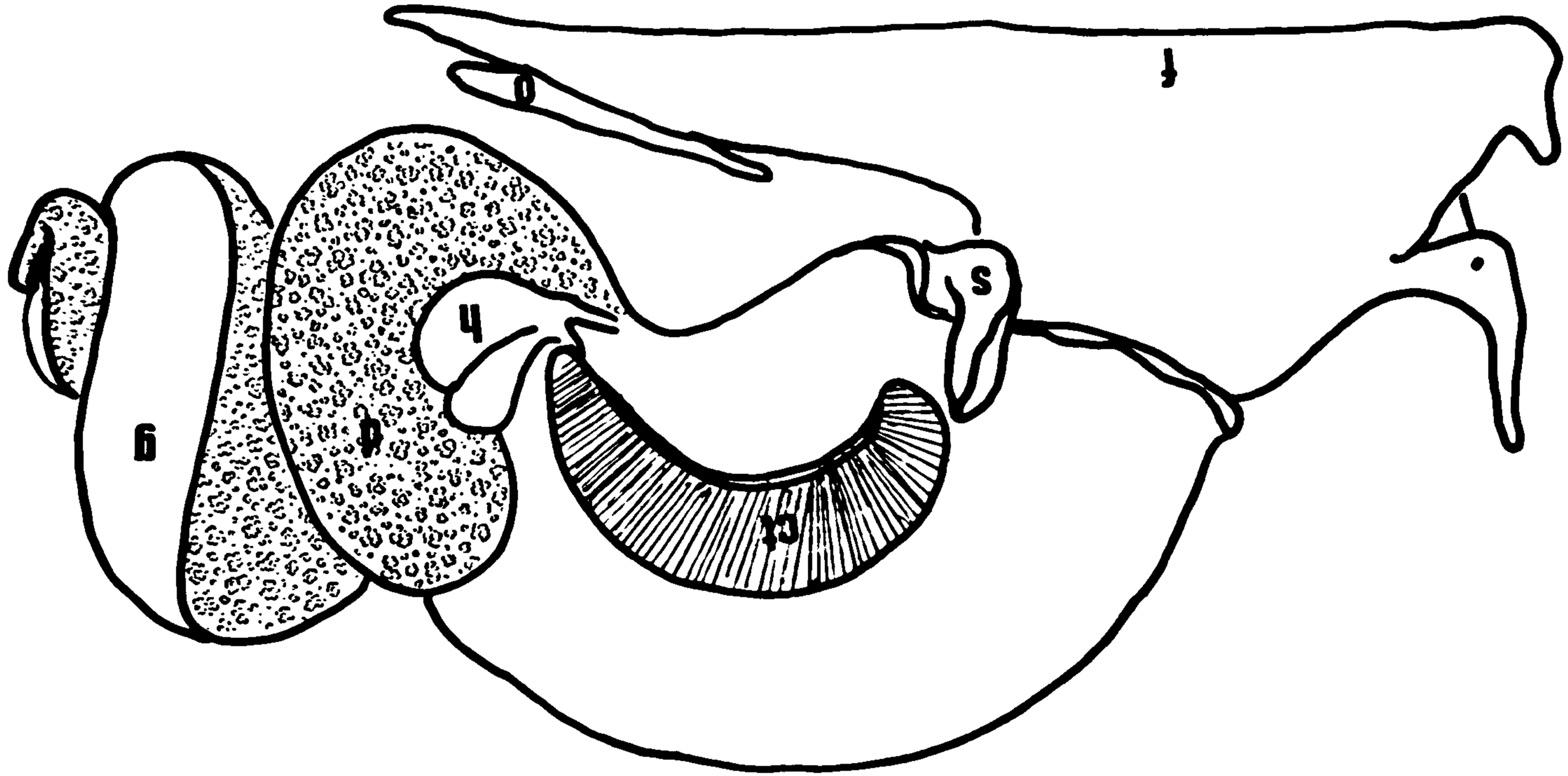

1
4
5
6
7
13

Figure 5. B. undatum; Normal digestive gland in May showing tubules embedded in a fine stroma of connective tissue.

(a) An artery is surrounded by vesicular connective tissue H.E. (x 100)

(b) Absorptive cells packed with acidophilic globules obscuring cell structure M.S.B. (x 100)

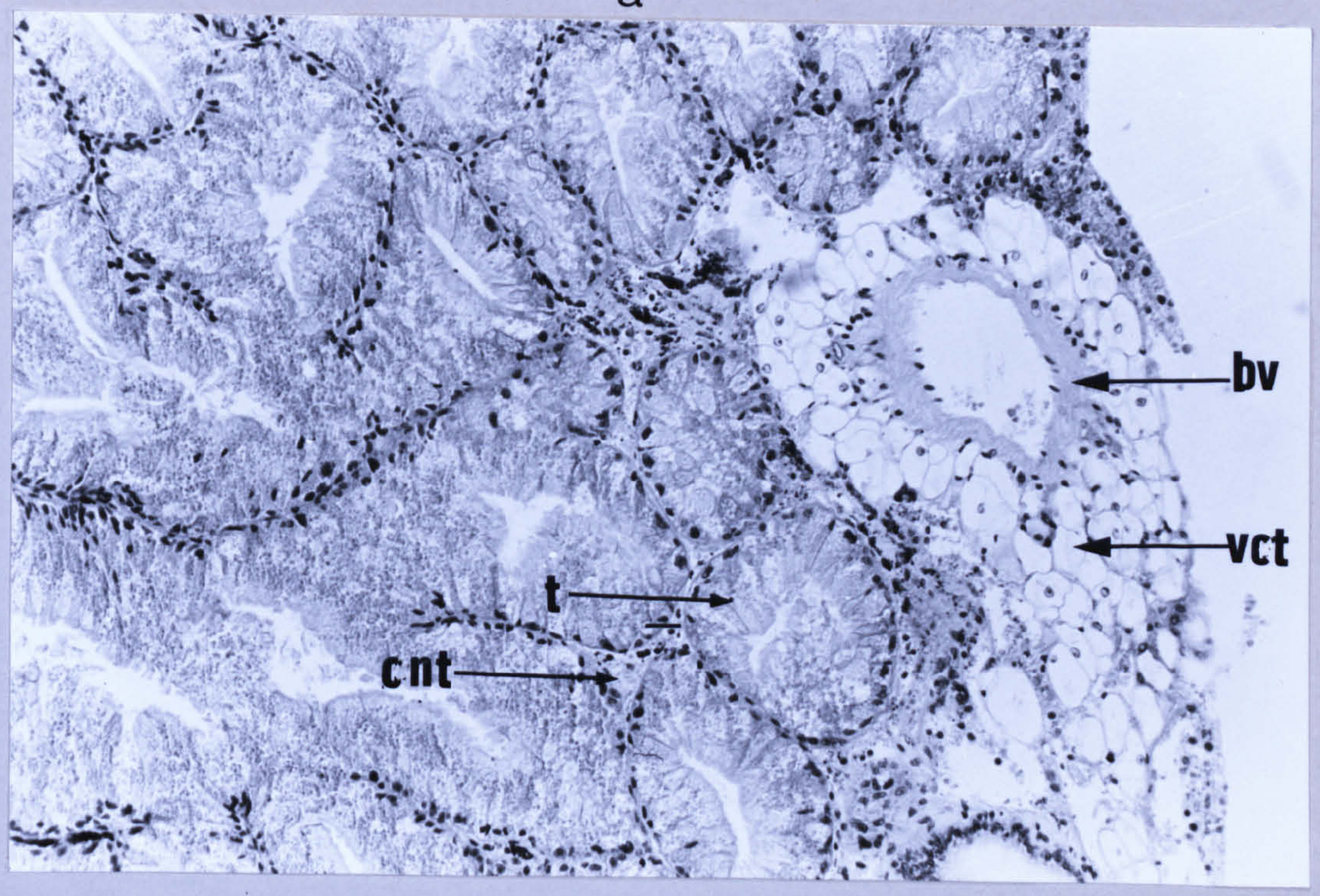
bv : blood vessel

cnt : connective tissue

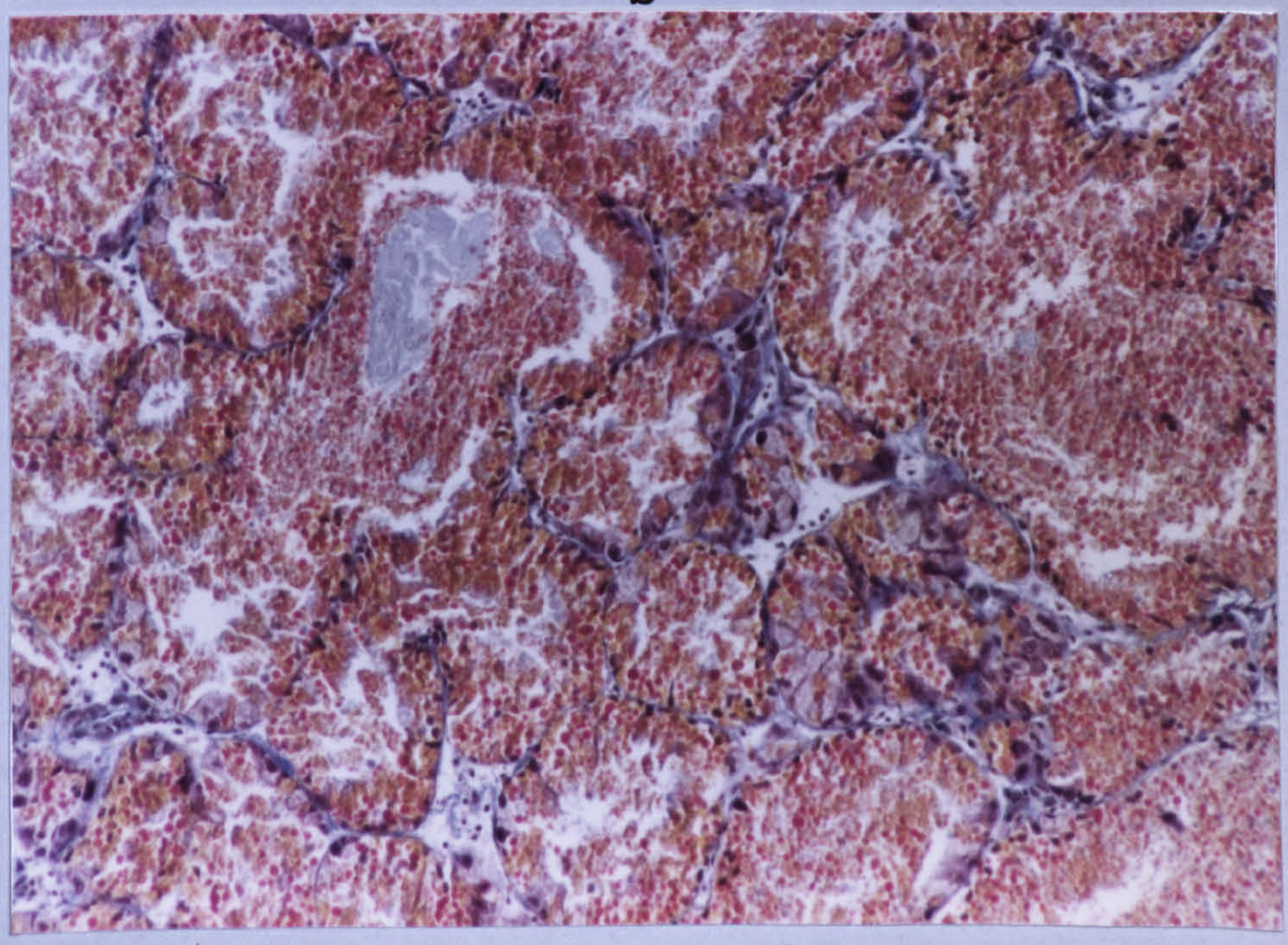
t : digestive gland tubule

vct : vesicular tissue

a



b



tissue was also well vascularised.

The digestive tubules consisted of a single layer of epithelial cells on a prominent basement membrane, circumscribed by a sleeve of connective tissue in which were distributed occasional myofibrils although there was no distinct muscle layer. The epithelial lining of the tubules comprised two distinct cell types. The more frequently observed form was the columnar, absorptive cell which had a basal nucleus and contained numerous globules demonstrating varying degrees of acidophilia. In an uninfected gland taken in May these absorptive cells were so packed with globules that details of cell structure were obscured; lateral walls were not distinguishable in some sections (Figure 5). The free surface of the absorptive cell frequently appeared to be ciliated. These cells corresponded to the absorptive cells described by several authors (Fretter & Graham, 1962) and may have secretory, absorptive, phagocytic and food storage functions. There is presently no consensus of opinion as to their function and indeed they may perform either all or only some of these functions, depending on the species of mollusc and the state of feeding.

The second type of cell was clearly distinguishable from the columnar absorptive cell, being triangular in shape with the wide base lying on the basement membrane and the apex of the triangle pointing towards the gut lumen though not necessarily opening to the lumen (Figure 9). They occurred singly or in groups. The proportions of this type of cell and absorptive cells varied considerably between parts of the same gland and also between individual molluscs. Their cytoplasm was consistently

basophilic and was usually reticulated. Commonly it contained fine dark pigment granules. The nucleus was large and very basophilic. These cells correspond to the secretory cells described by Fretter & Graham (1962) who suggest that they are adapted for uptake of material from the blood and for elaborating it into a secretion which is shed into the lumen. This is supported by the finding of Pal (1971) who examined the fine structure of the basophil cell of Mya arenaria L. and showed it capable of protein synthesis and secretion. These cells appeared to be more numberous in some tubules than others.

A third type of cell was seen but only in the intestinal component of the digestive epithelium. This was eosinophilic, Alcian Blue positive and was seen to discharge its contents into the intestinal lumen.

The tubule lumina in uninfected glands were very small and appeared to reflect a well-fed condition (Figure 5). Digestion in B. undatum, in common with other carnivorous prosobranchs, was considered to be largely extra-cellular in the stomach, followed by absorption of the products of digestion by the columnar cells of the digestive tubule epithelium.

The infected digestive gland of B. undatum

Clinico-pathologically the infected digestive gland was recognised by its swollen and grey appearance. There was normally a large amount of transudate present and the eye spots of cercariae gave the gland a "peppered" appearance. The tunica propria was under pressure and the gland was friable. Most of the digestive glands examined in May to August carried heavy infections but never sufficiently heavy for all the digestive

gland tissue to be destroyed. The level of infection varied considerably between individual hosts.

Histology of the S. baccatus larvae

In all cases the larval stages occurred within the intertubular area of the digestive gland. The distribution of the larvae varied within the gland from occasional individual parasites in the interstices of the tubules to large focal areas consisting exclusively of S. baccatus larvae (Figure 6). The rediae varied considerably in size but rediae of all sizes contained germ balls and cercariae in various stages of development. Wolfgang (1956) described the small rediae of S. baccatus as immature and larger ones, which contained only cercariae in the brood sacs, as mature. However, in the present study many smaller rediae contained mature cercariae although these were always fewer in number. A count of a sample of whole mounts of large rediae contained 13 - 16 cercariae whereas small rediae contained 5 - 7 cercariae.

Mature cercariae were lying free in the intertubular spaces intermingled with rediae. However, some sections showed large aggregations of mature cercariae laying immediately below the tunica propria (Figure 7). These cercariae which were located among the rediae were fixed in varying states of contraction and appeared to be actively migrating towards the aggregates of mature cercariae below the tunica propria.

The rediae possessed a large nuscular pharynx and the gut was frequently seen to contain material which stained yellow/orange in MSB and in Massons Trichrome stained preparations.

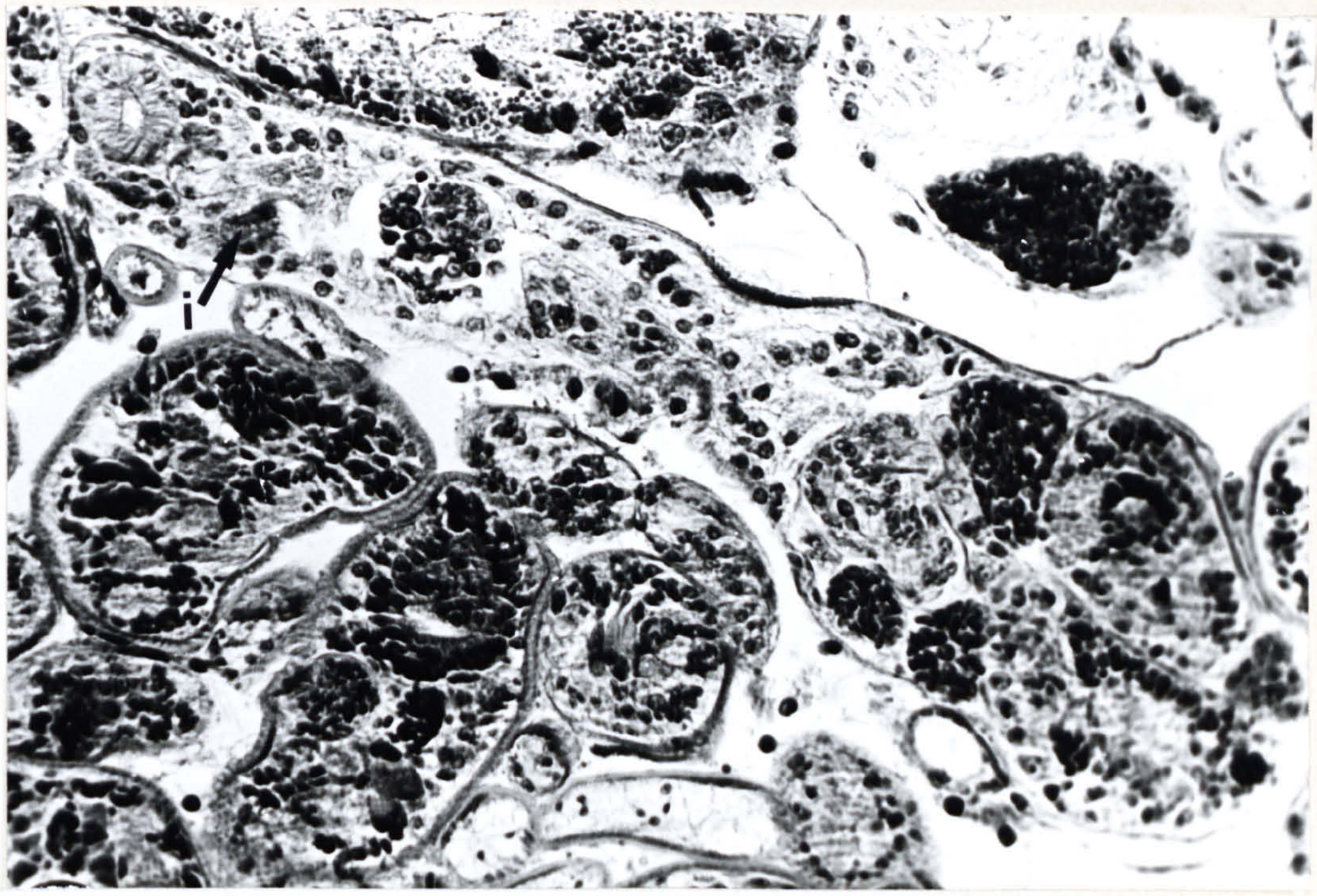
Figure 6. B. undatum; Infected gland in July showing large rediae in heavy infections. Note the oral sucker, the short intestine and the microvilli of the tegument in the anterior region of the redial body.

(a) M.S.B. (x 400)

(b) Masson's Trichrome (x 250)

i : intestine

a



b

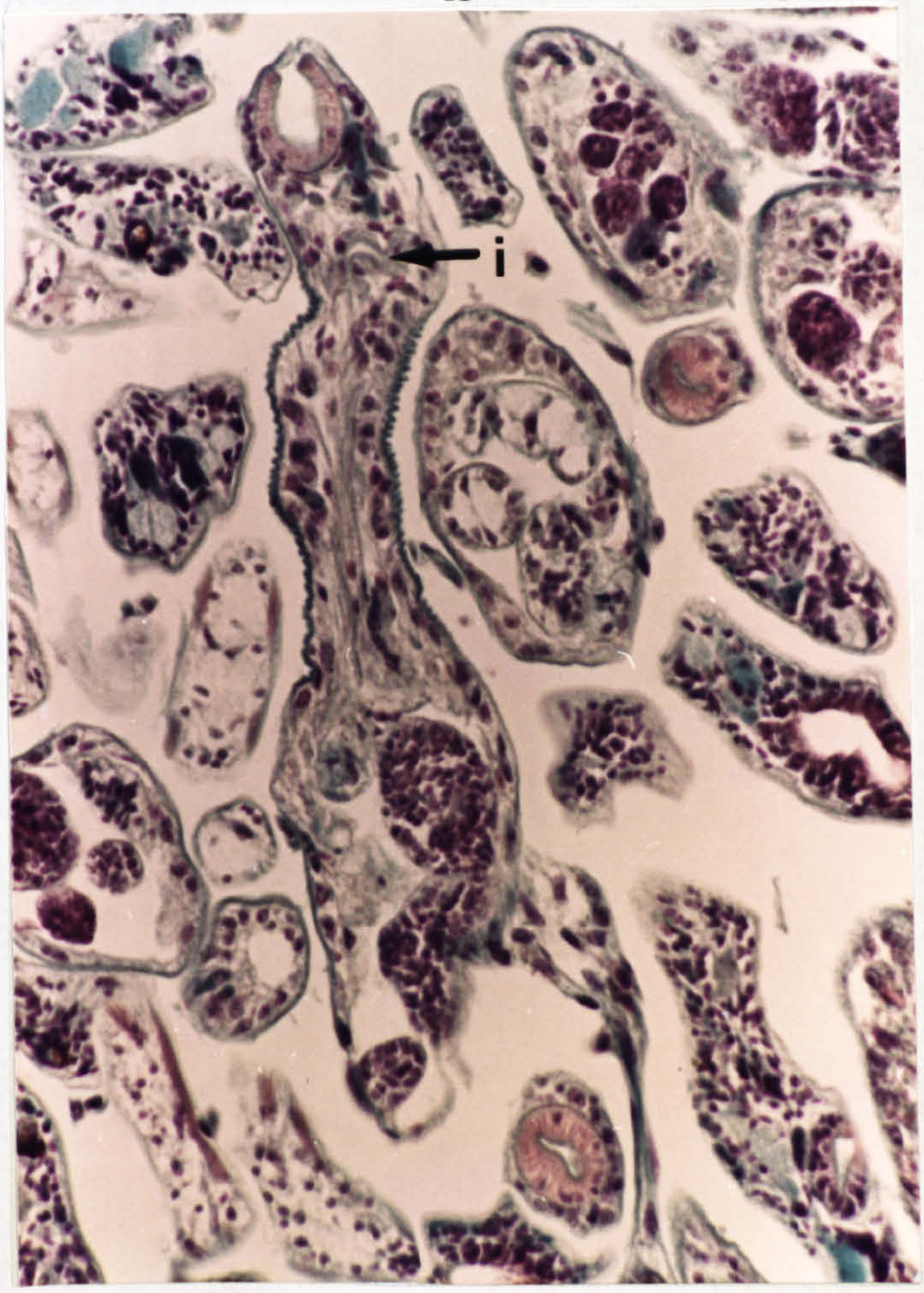


Figure 7. B. undatum; Infected digestive gland in May showing

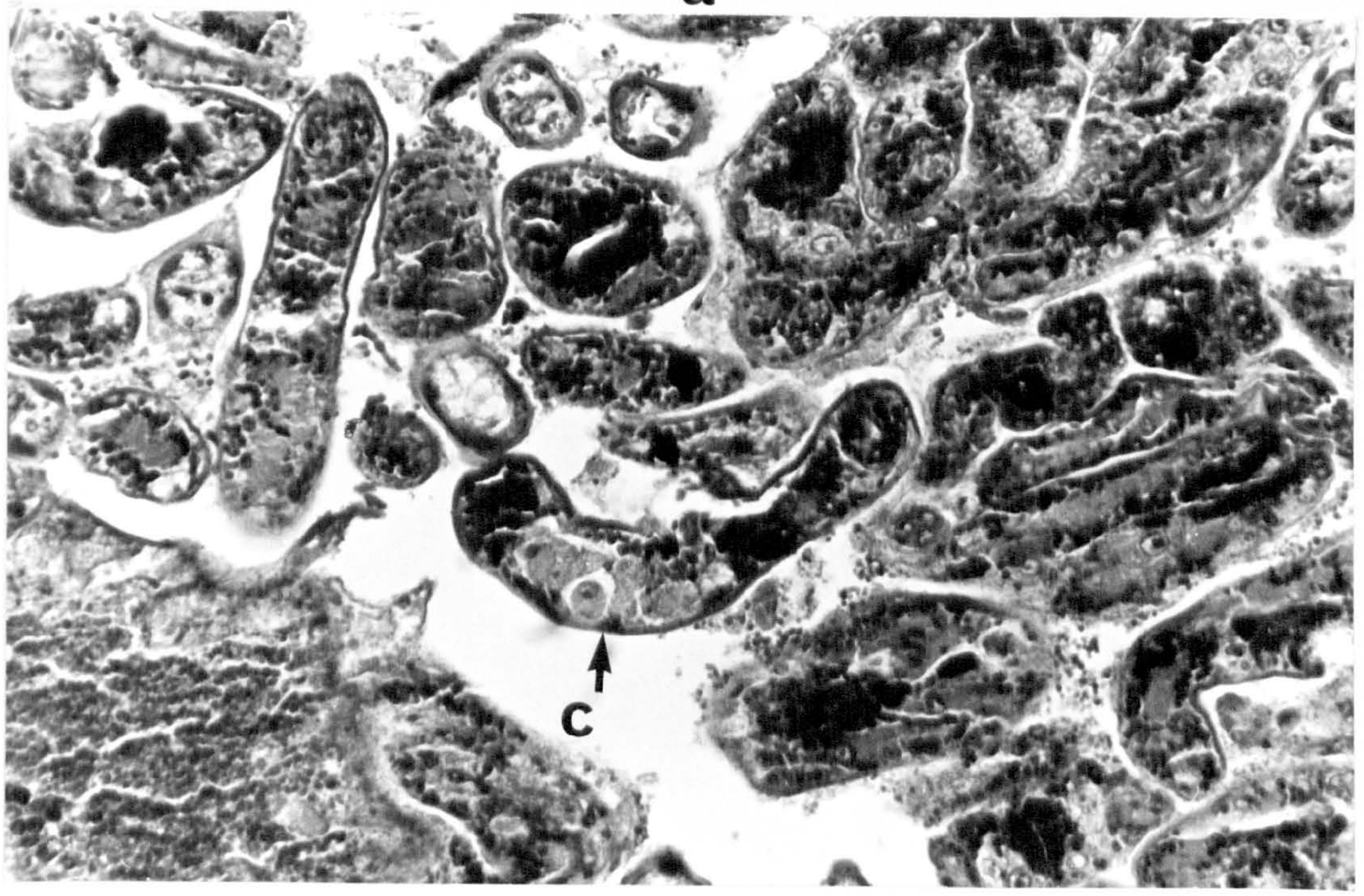
(a) actively migrating cercariae M.S.B. (x 250)

(b) focal aggregations of cercariae below the tunica propria (x 50 approx.)

c : cercaria

tp : tunica propria

a



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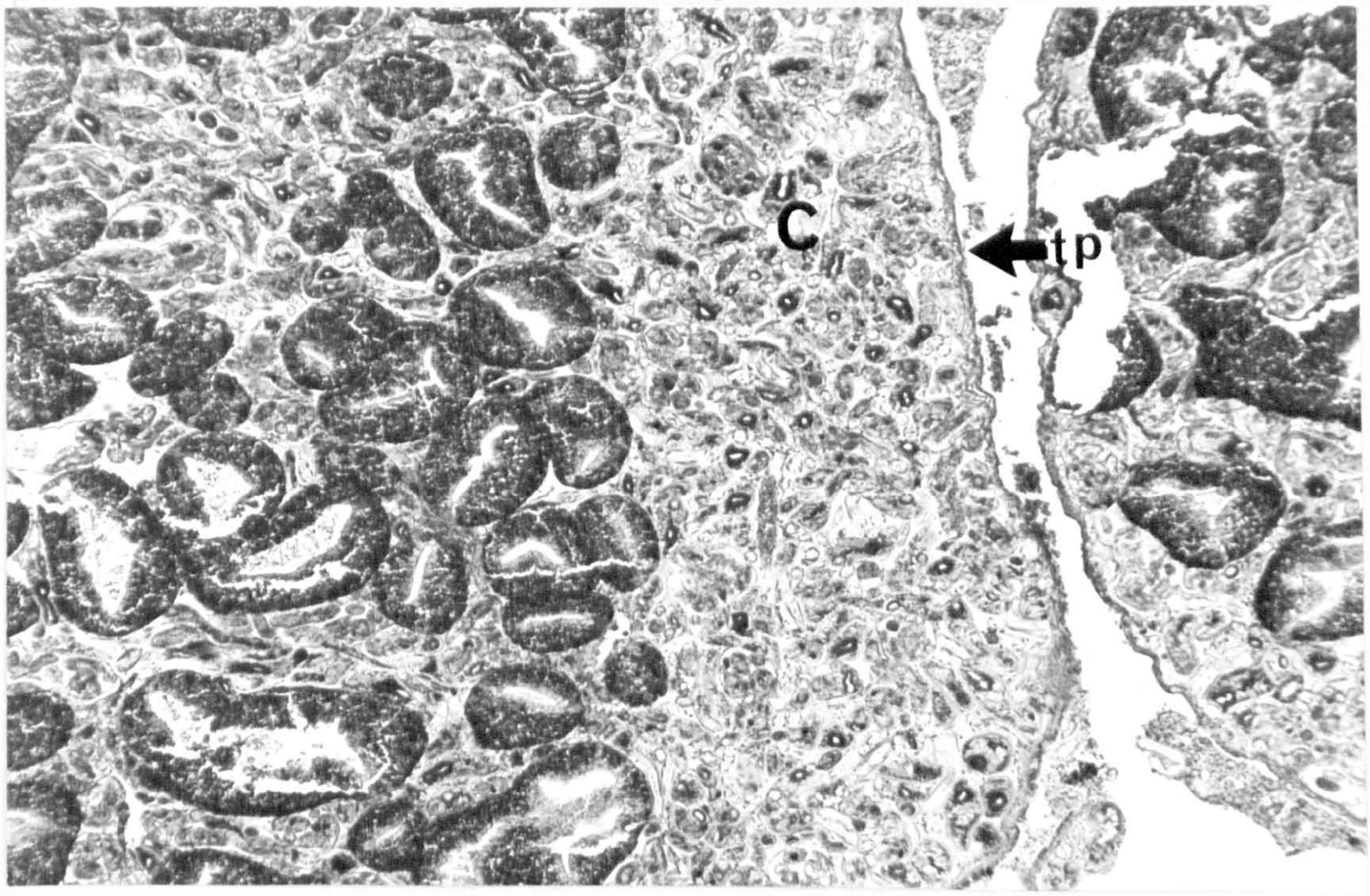


This gut material consisted of refractile globules of irregular shape. In H & E sections it was strongly eosinophilic and it was also Sudan Black B positive indicating a high level of glycerides (Figure 19d). This could be semi-digested host material but it is more likely that it was rejected waste material since these globules were occasionally to be seen outside the redial body. It is not known to what extent this could be artifactual. In some cases it appeared to be crystalline. Only occasionally did the gut contents of the rediae resemble cellular material.

Other tissues of the molluscan host were not studied in detail but occasionally parasites were observed in the adjacent tissues of the gonads and kidney. The rediae observed in the kidney were all empty of germ balls and it is possible that development was retarded in that organ. Parthenitae in the gonad, however, all showed similar stages of development to those in the digestive gland.

The pathogenesis of *Stephanochasmus baccatus* infections in the digestive gland of the host

In most cases large areas of intertubular tissue were occupied by parthenitae. The tubules were reduced in diameter and number; the intertubular area was greatly enlarged and tubules were less branched. Two main types of lesion could be distinguished. In type I, degenerating changes were confined to individual tubules and neighbouring tubules remained unaffected. In type II, large areas of the gland appeared to be undergoing necrosis involving all the tissues in that area. The type II lesion was very severe and appeared to be associated with a massive increase in larvae at the start of the molluscan cycle of infection and was typified by two very heavy infections in May in which large sections of the molluscan digestive gland were destroyed (Figure 12).

Type I Lesion

The typical response to the presence of the parasite in the digestive gland (type I lesion) consisted of tubular damage and separtion of tubules by the masses of parthenitae, which by their migration, feeding and reproduction induced progressive focal tubular degenerative changes as follows. The tubule diameter became progressively smaller, there was a reduction in the number of globules contained in the cytoplasm of the columnar cells and lumina were progressively occluded. The lateral walls of the columnar cells then appeared broken down, leading to complete collapse and resultant deposition of the cytoplasm, nuclei and globules. The cellular elements undemarcated by cell membrane were lying free within the "lumen" bounded by the basement membrane of the tubule epithelium (Figs 8a-d). The secretory cells seemed to be the last to lose their integrity but, in some areas, they eventually became deformed and lay free within the lumen area of the tubule. Ultimately, there was complete necrosis of the epithelium and only an agglomerate of cellular debris remained in the vicinity of the rediae.

The intertubular area was greatly enlarged and filled with transudate owing to the destruction of the tubules. The connective tissue normally present there was recognisable only as scattered cells and collagen fibres seen in the spaces surrounding the rediae. The intertubular arteries were still recognisable but were very compressed, which may help to explain the massive fluid transudate (Figure 13b).

In view of the association of the intracellular vacuolation of the glandular epithelium with pathological change in other conditions, close attention was paid to this in studying sections of infected molluscs and controls but, although vacuoles were

Figure 8. B. undatum; Infected digestive glands in May showing type I lesions.

(a) Showing a degenerating tubule. Note the secretory cells remain entire while columnar cells are disorganised M.S.B. (x 250)

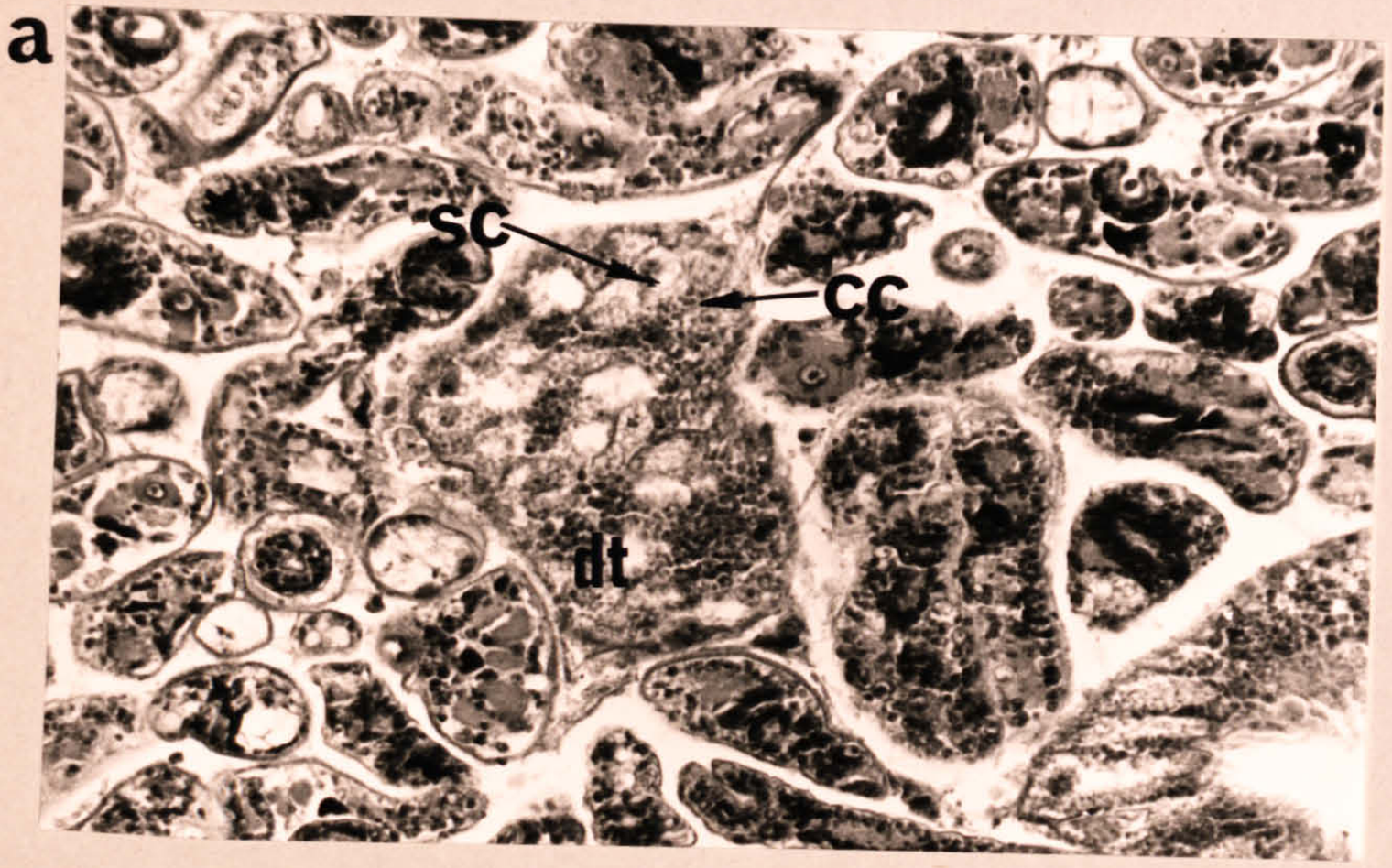
(b) Partial degeneration of a digestive gland tubule while part remains normal M.S.B. (x 250)

(c) Migrating cercariae amongst tubular debris and columnar cell nuclei M.S.B. (x 250)

(d) Normal tubules occur in the gland adjacent to degenerating tubules M.S.B. (x 400)

c : cercaria
cc : columnar cell
dt : degenerating tubule
nt : normal tubule
sc : secretory cell
td : tubular debris

a



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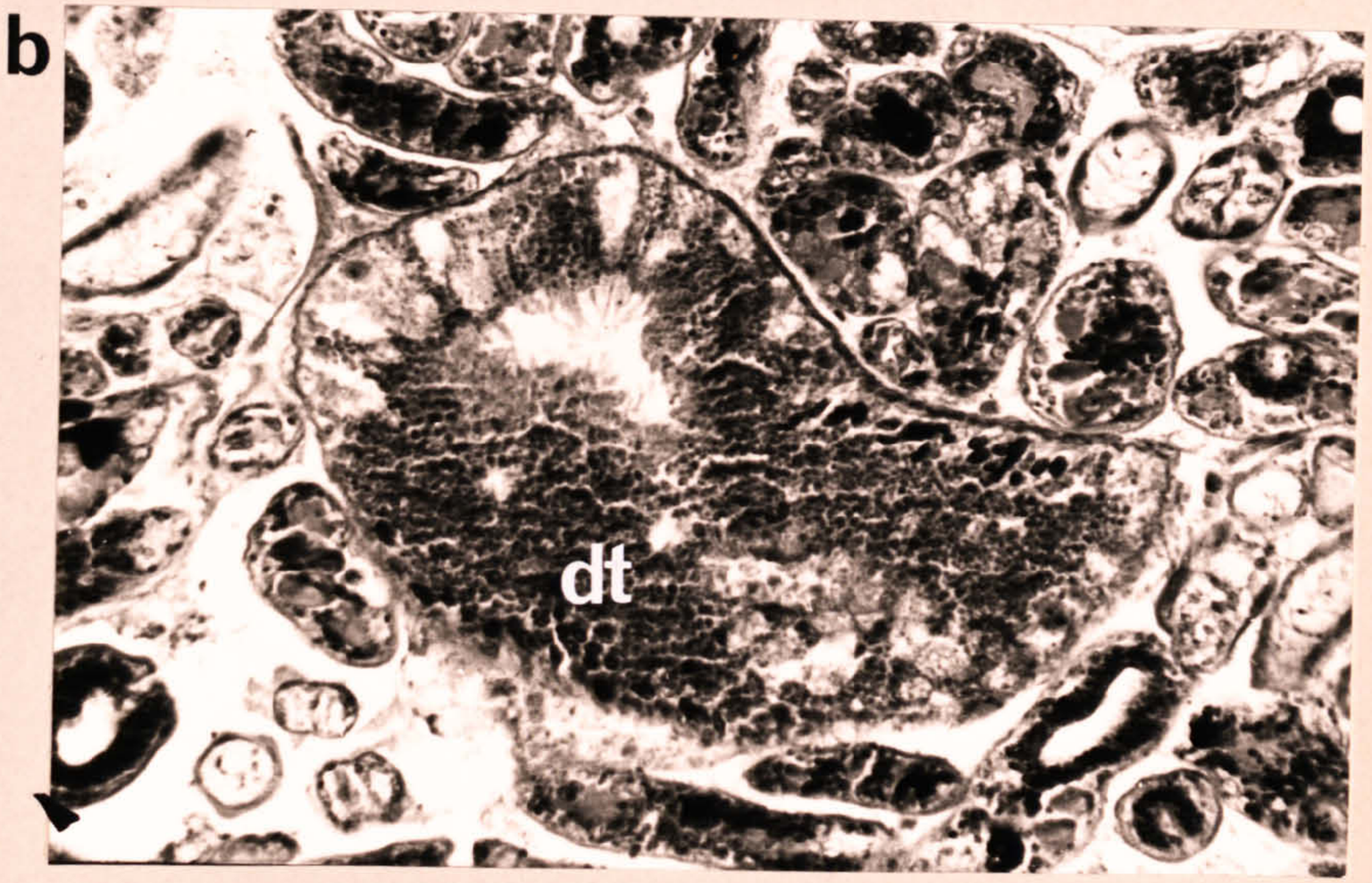
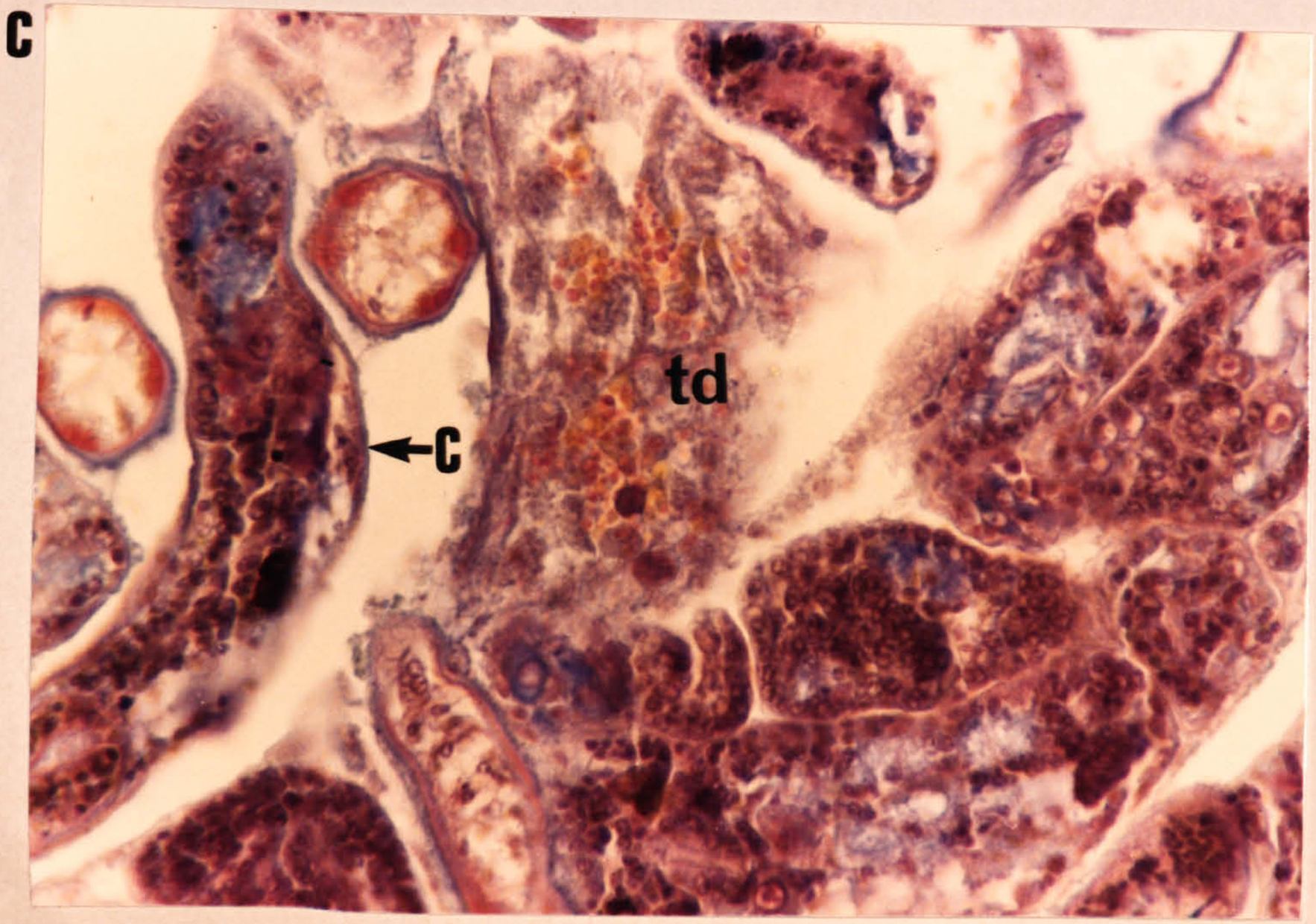


c



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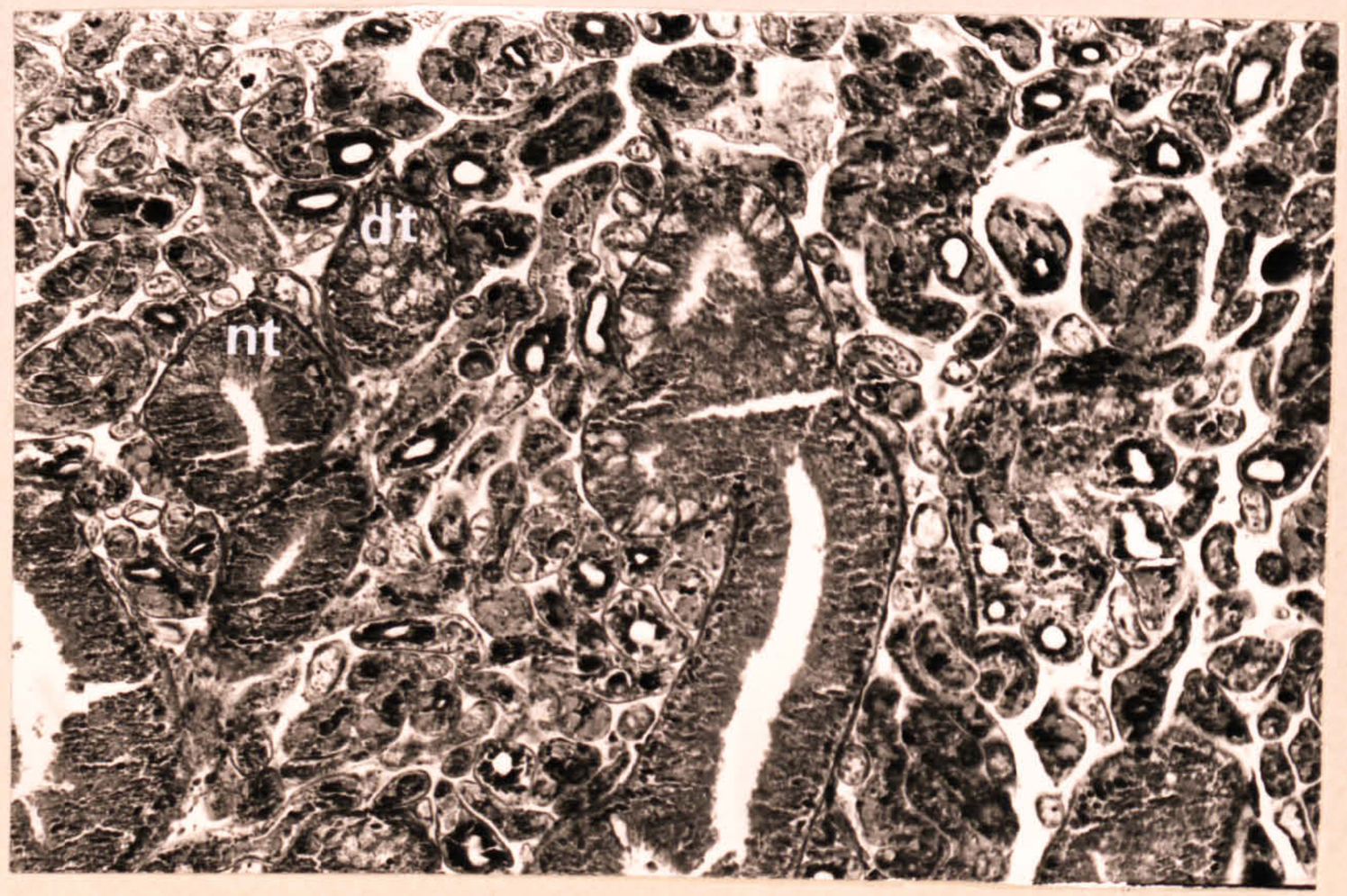


occasionally seen within the columnar cells, there was no correlation with infection.

A feature of the infected gland was the reduction in the number of acidophilic globules within the cytoplasm of columnar absorptive cells (Figure 9). Occasionally the columnar epithelium was also reduced in height so that the lumen of these otherwise normal tubules (Figure 10a) was considerably enlarged and often contained secretory material. An extreme stage of this reduction in tubular height in very heavy infections occasionally resulted in the epithelium being represented over the tubular basement membrane merely as an irregular squamose margin (Figure 10b). In very heavy infections tubules were destroyed by direct feeding by the parasites and appeared as fragmented tubules in close apposition to rediae. In some cases sloughing of the epithelium occurred in otherwise normal tubules (Figure 11).

An occasional feature observed in the digestive glands of both infected molluscs and controls were small areas of replacement fibrosis which may have represented a previous locus of infection with some other pathogen, but, in view of its occasional association with regressing S. baccatus infection in the late summer, it seems possible that when observed earlier in the year it may represent the resolution of a site of infection of the previous year.

Type II Lesion

The type II lesion was only infrequently observed and was characterised histologically by the development of large foci of severe acute liquefactive necrosis in a gland which otherwise manifested the features of the type I lesion (Figure 12).

Figure 9. B. undatum; Infected gland in August showing a reduced number of globules occurring in the columnar cells of the tubule epithelium. Note the triangular, basophilic secretory cells which are very evident in this tubule section M.S.B. (x 250)

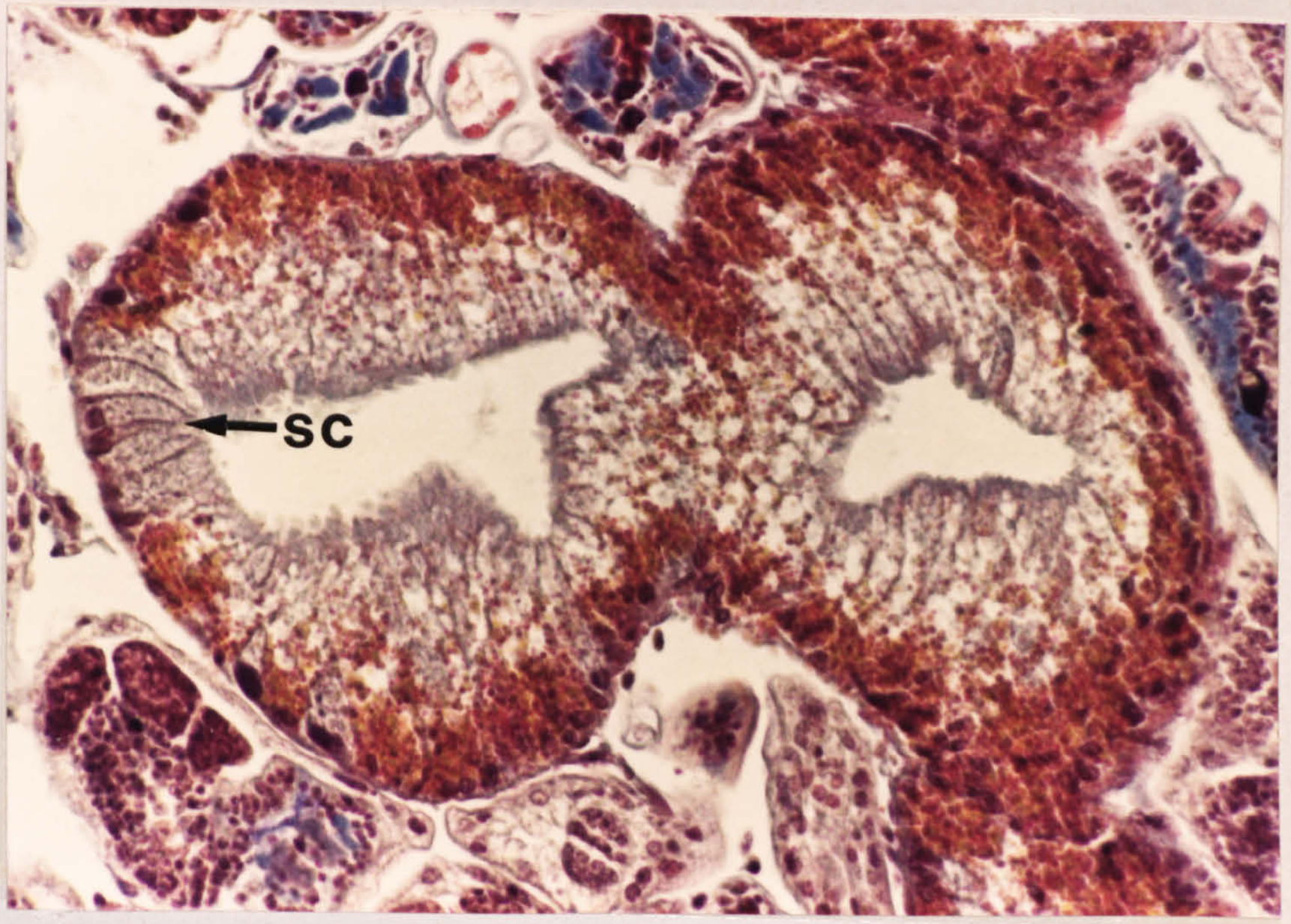
sc : secretory cell

Figure 10. B. undatum; Infected digestive gland in July showing

(a) a reduction in the height of the columnar cells in some tubules M.S.B. (x 50 approx.)

(b) the cells are greatly reduced and appear as squamose cells lining a large tubular lumen M.S.B. (x 400)

cc : columnar cell



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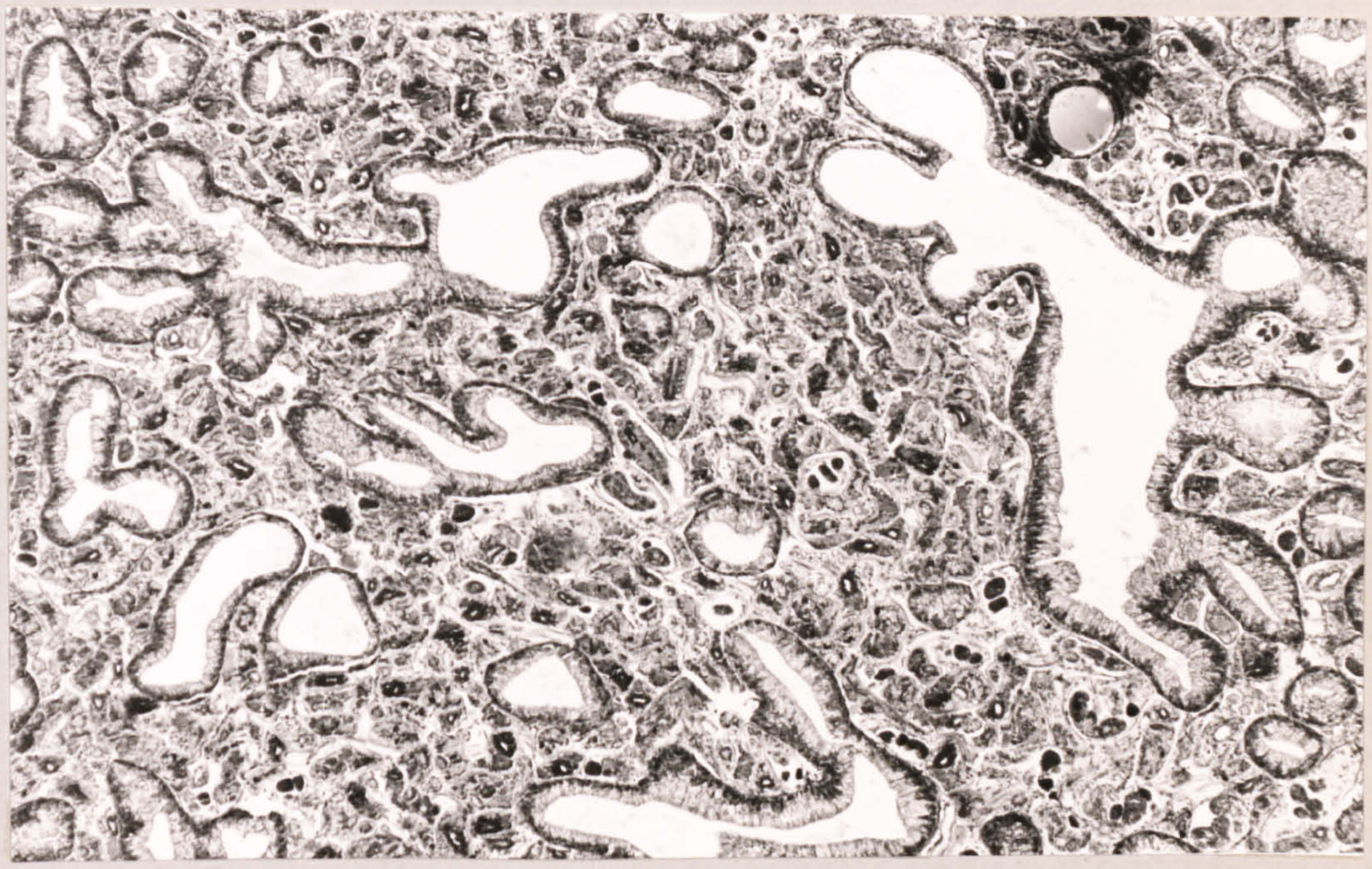

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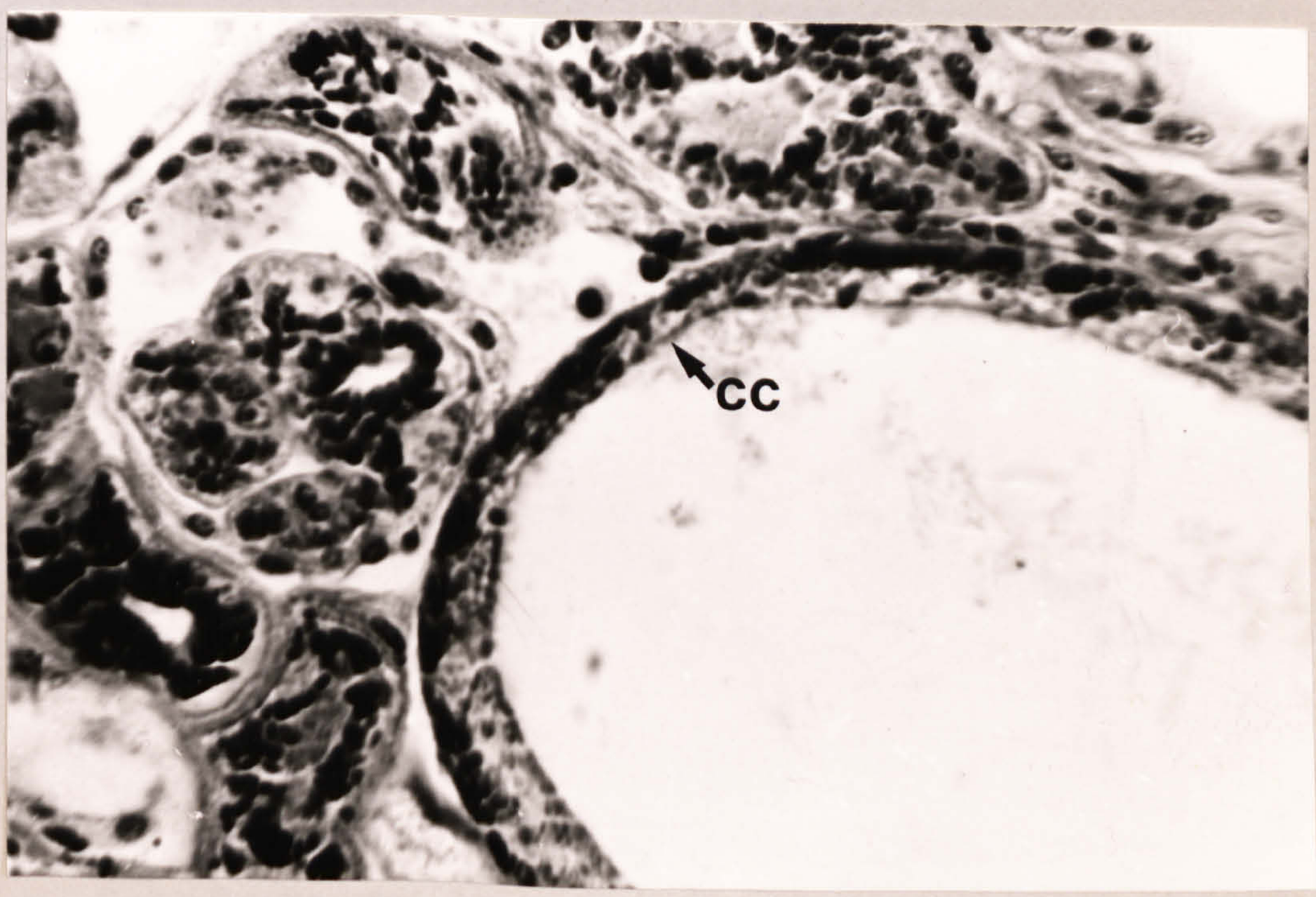


Figure 11. B. undatum; Infected digestive gland in July showing fragmentation and sloughing of epithelium cells into the lumen of the digestive gland tubule in a heavy infection M.S.B. (x 400)

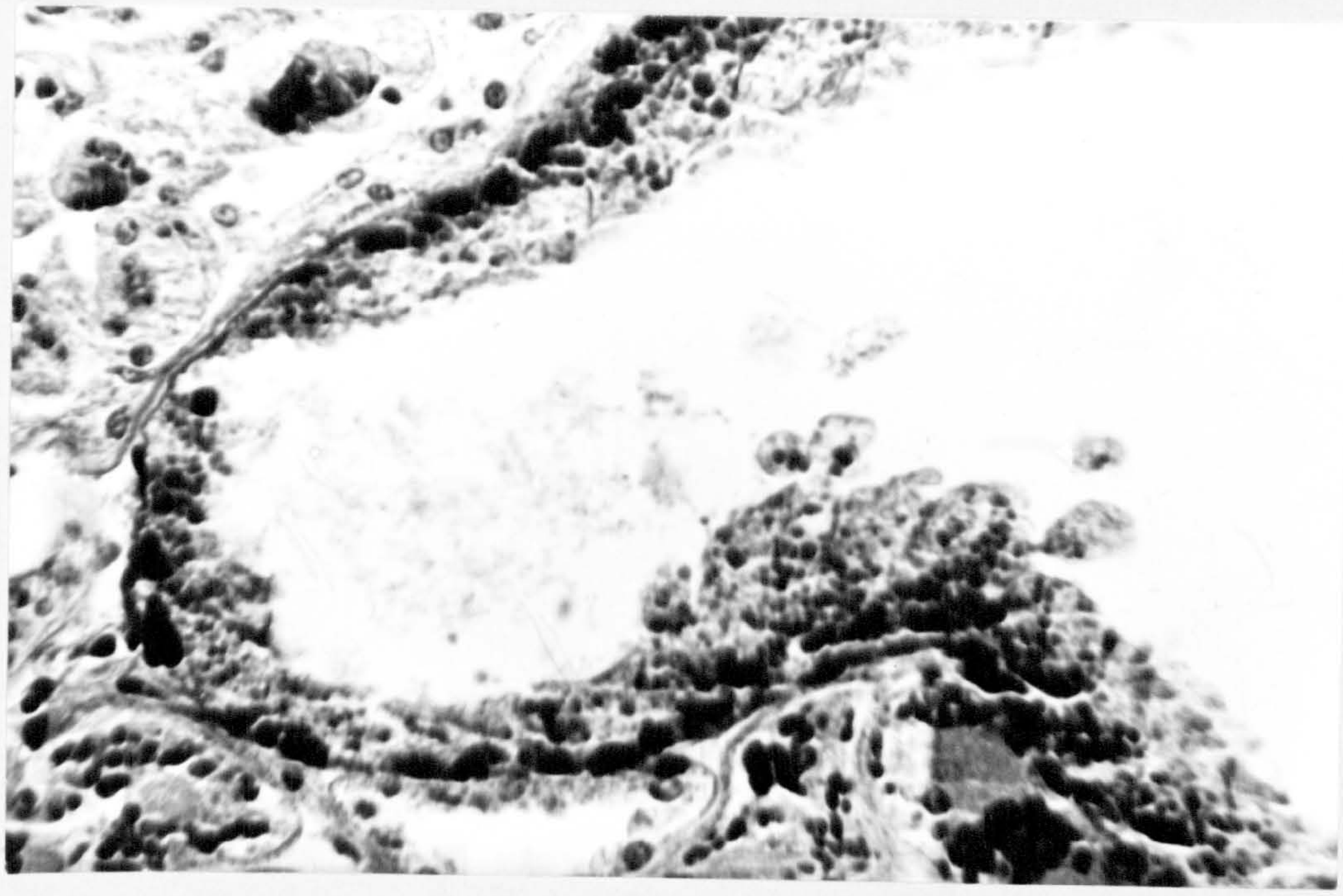


Figure 12. B. undatum; Infected digestive gland showing examples of the type II lesion. Note the disorganisation of the tubular elements in which the parasite larvae are embedded. Amoebocytes have invaded the surrounding tissue.

(a) H.E. (x 50 approx.)

(b) M.S.B. (x 250)

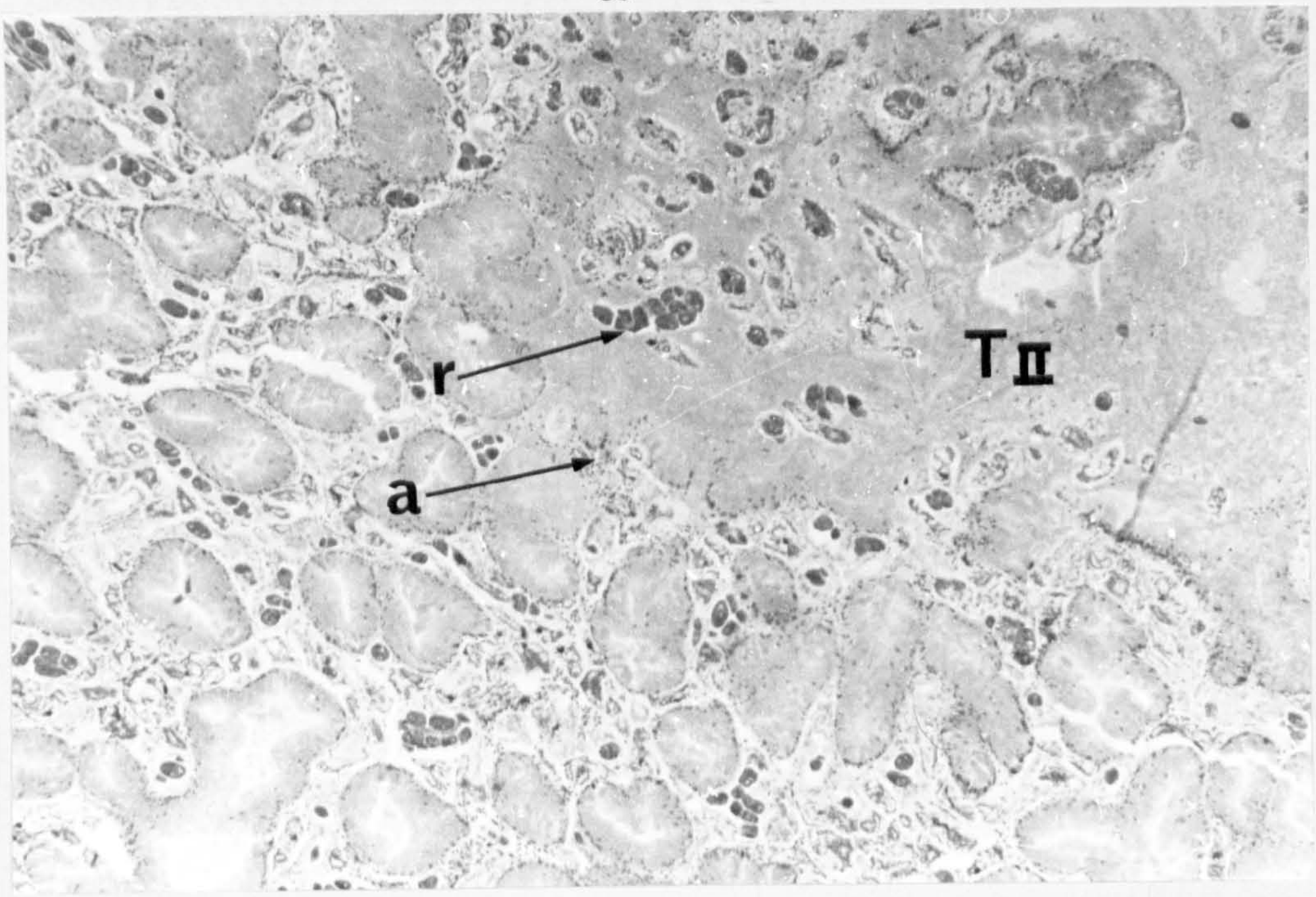
a : amoebocyte(s)

dt : degenerating tubule

r : redia

TII : Type II lesion

a



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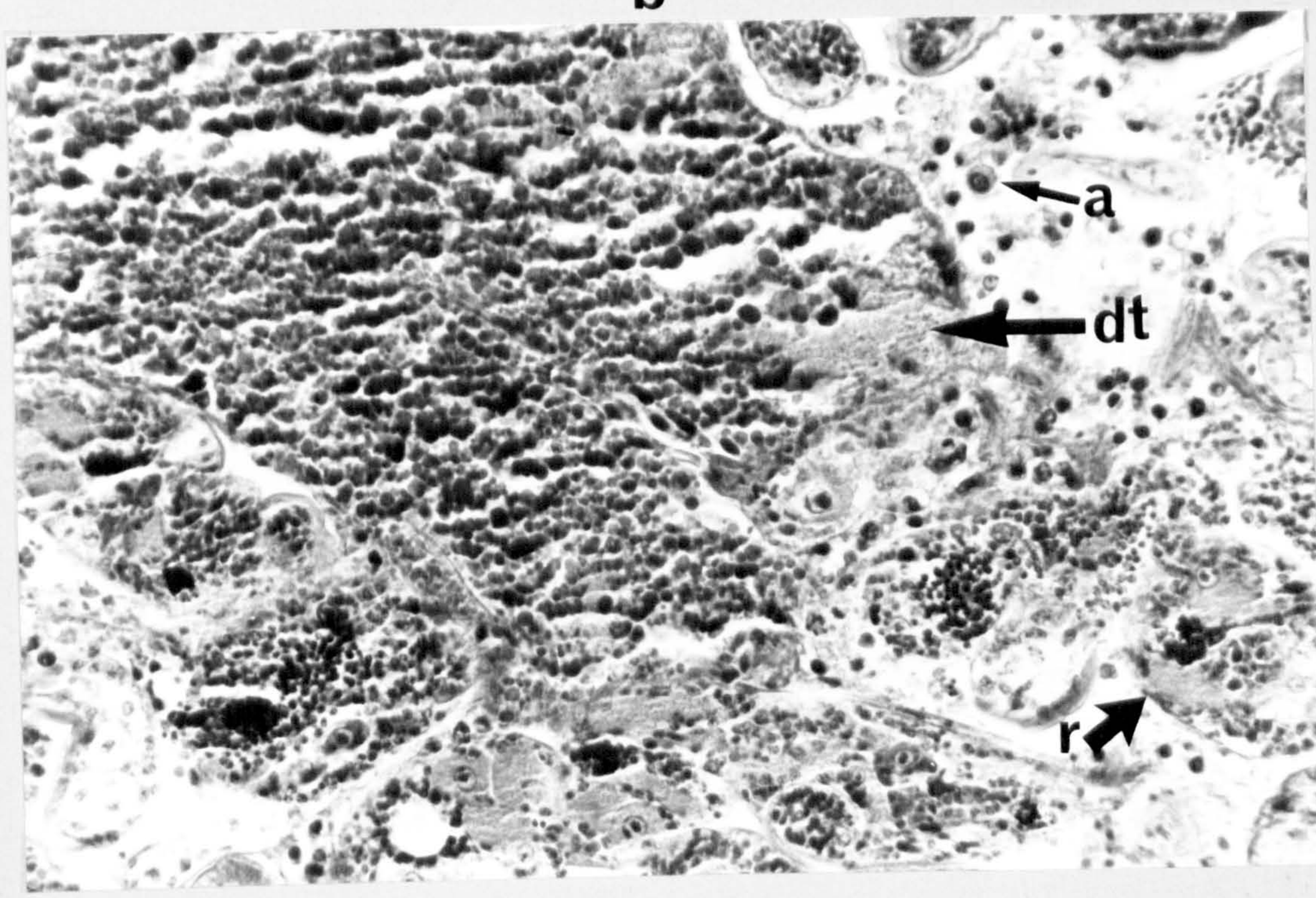


The presence of a type II lesion always coincided with the occurrence of massive diapedesis of leukocytes from the intertubular arteries (Figure 13). The complete necrosis of all the cellular elements within the area of a type II lesion resulted in its appearing as a congery of unorganised columnar absorptive cell globules within which were deposed clusters of highly degenerate rediae and cercariae. Secretory cells appeared more resistant to the necrotising process and could be seen remaining virtually intact, within the affected areas. Although the number of type II lesions seen was small, it was nevertheless apparent that they were consistently associated with heavy infections. The areas affected were always closely opposed to the collumnella and its muscle, suggesting the possibility that the tissue swelling associated with the migration of the parasites had resulted in mechanical rupture of the tubules. This condition lead to an acute infiltrative cellular response aided by autolytic necrosis as a result of the release of proteolytic enzymes from the ruptured digestive cells.

Seasonal changes observed in infected digestive glands

Early season infections

The earliest infections examined were taken in May and were characterised by large numbers of mature, tailed cercariae lying free in the interbubular area and by large aggregations occurring below the tunica propria (Figure 7b). Almost all the May infections were heavy and the damage to the digestive gland was also very severe and a higher proportion of specimens showed the type II lesions than those of the later season. The early

Figure 13. B. undatum; Infected digestive glands in May showing the type II lesion

(a) Leucocytosis in the affected area. M.S.B. (x 100)

(b) Showing compression of blood vessels M.S.B. (x 250)

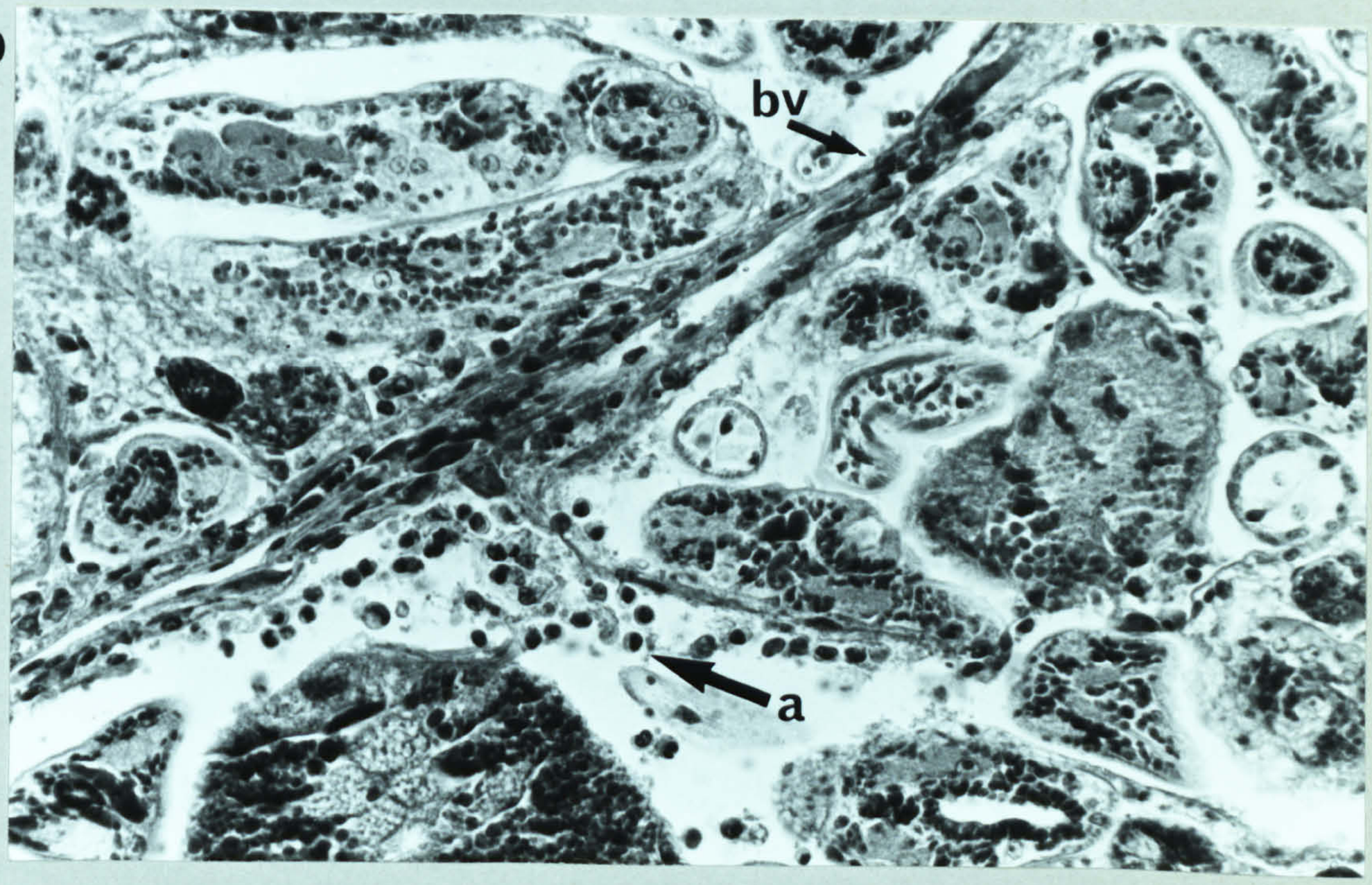
(c) Diapedesis from an inter-tubular artery M.S.B. (x 100)

a : amoebocyte(s)

bv : blood vessel

a

b



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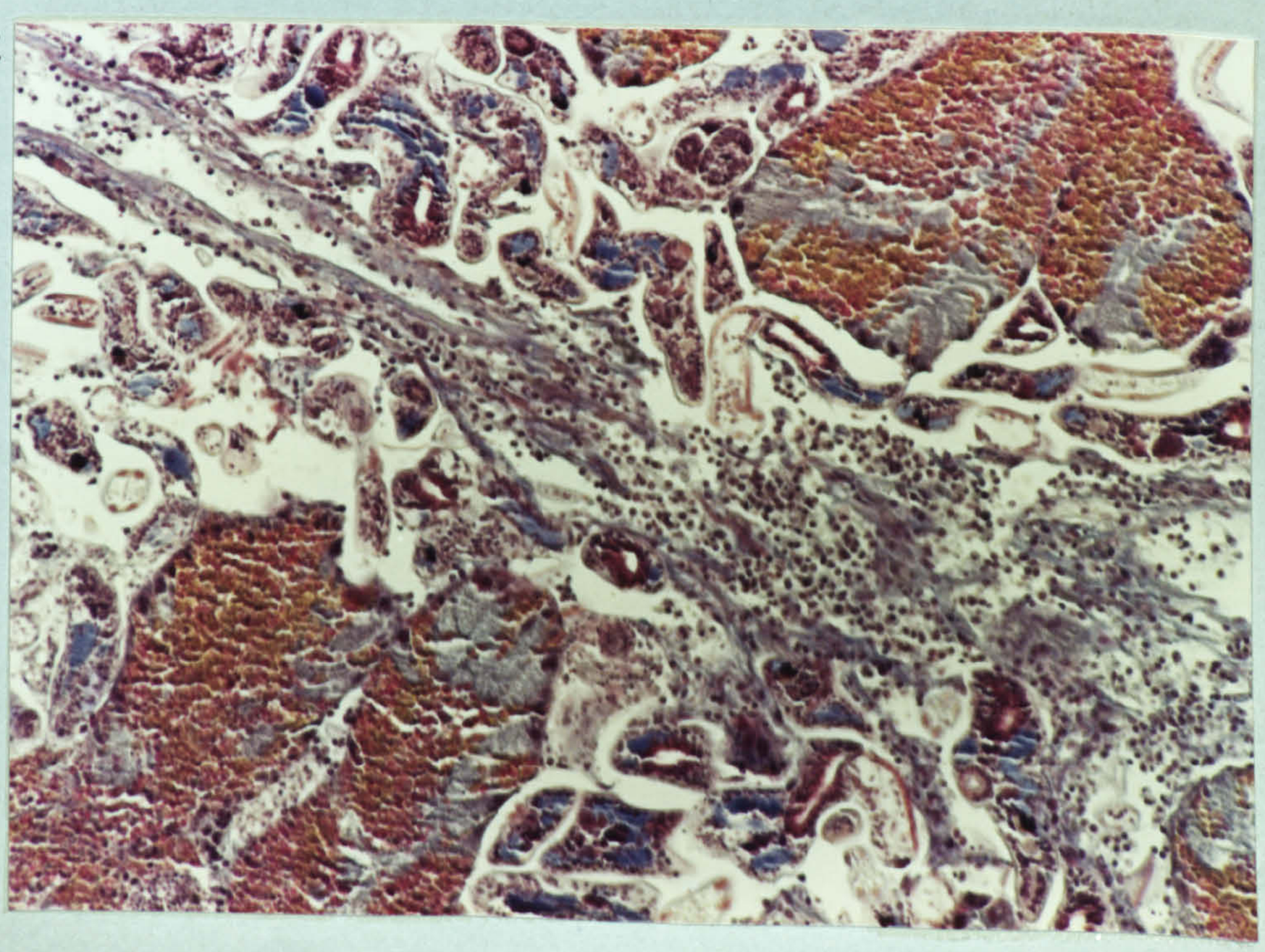


damage may be attributable to large numbers of larva migrans at this time. Specimens in the June/July sample had similar levels of infection and showed the typical type I lesion.

Mid Season

By July and August the aggregations of migrating cercariae below the tunica were rarely seen. Infections consisted largely of rediae containing germ balls and developing cercariae. Many speciments of the July/August sample showed a complete lack of cercariae (Fig.ure 14). However; rediae with food in the gut were seen throughout the period May to August indicating that feeding was continual even though cercarial production had ceased in some cases.

There was a general decrease in the number of globules contained in the columnar cells of infected molluscs but uninfected glands showed no difference between the May and August period. The reduction in height of the columnar cells was first observed in the June/July sample but was more common in the samples of July/August. This may be related to a decrease in the available food material.

Late Season

In September only one gland was identifiable grossly as infected with S. baccatus. In histological section of this specimen few cercariae were seen but the parasite was largely represented by rediae in a state of disintegration. The cells of the redial walls were vacuolated and appeared to be undergoing cytolysis (Figure 15a). The sub-tunical connective tissue was also degenerative and was represented by patches of cellular debris. Focal deposits of fine amorphous material were seen in

Figure 14. B. undatum; Infected digestive glands

(a) Infected gland in May consisting largely of cercariae H.E. (x 50 approx.)

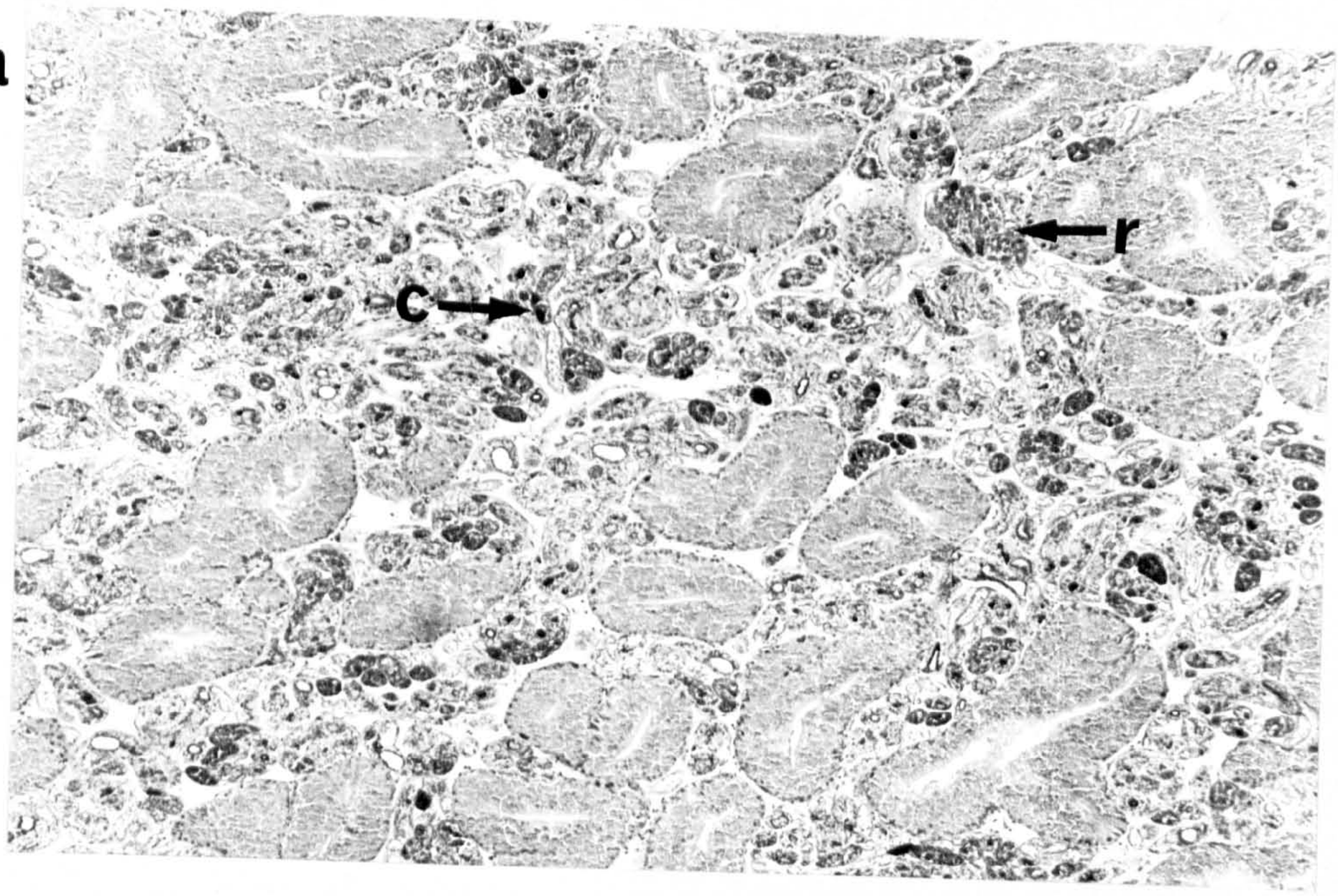
(b) Infected gland in July where rediae with germ balls predominate H.E. (x 50 approx.)

(c) Infected gland in August consisting of rediae with germ balls which are all at a similar stage of development H.E. (x 100 approx

c : cercaria

r : redia

a



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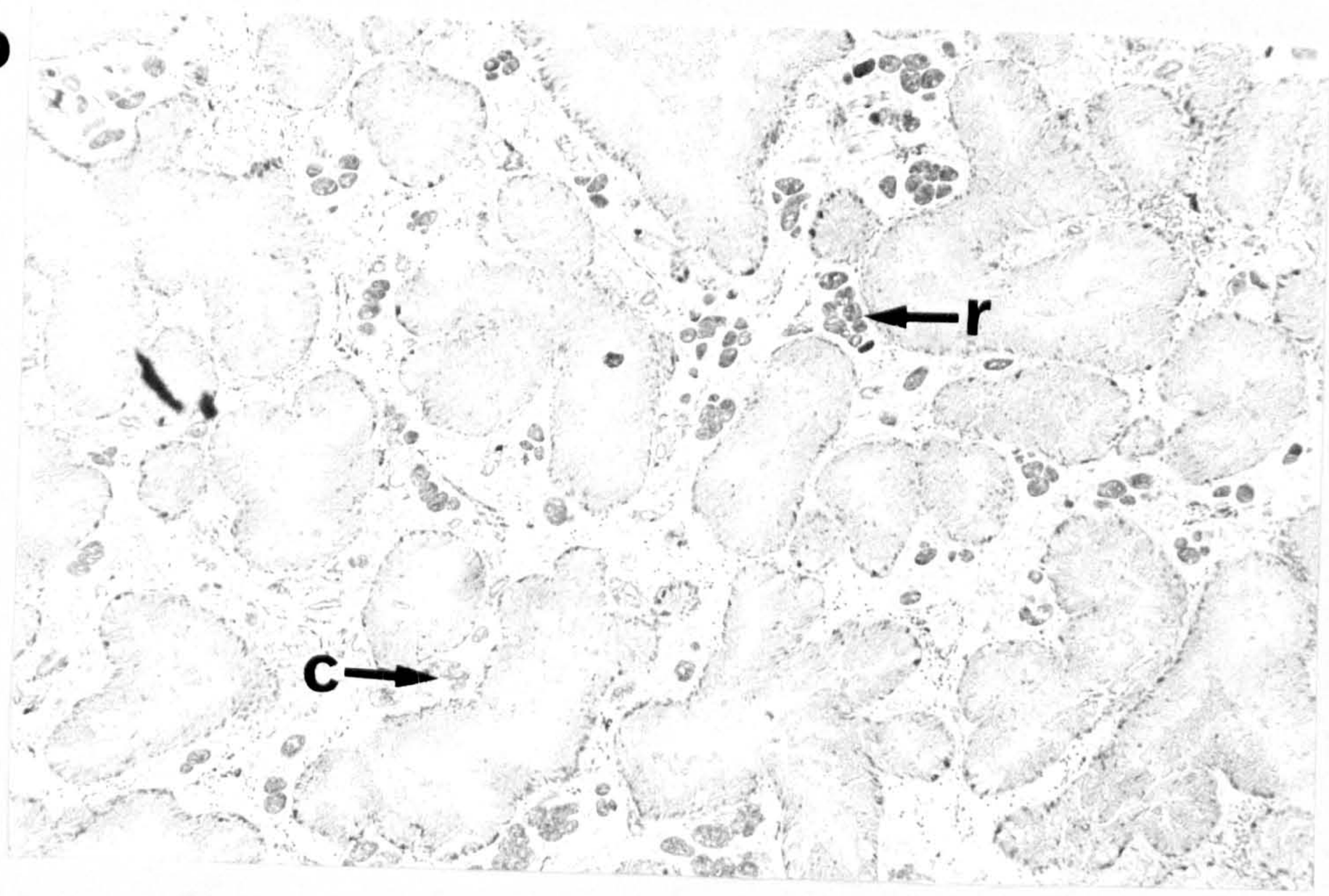

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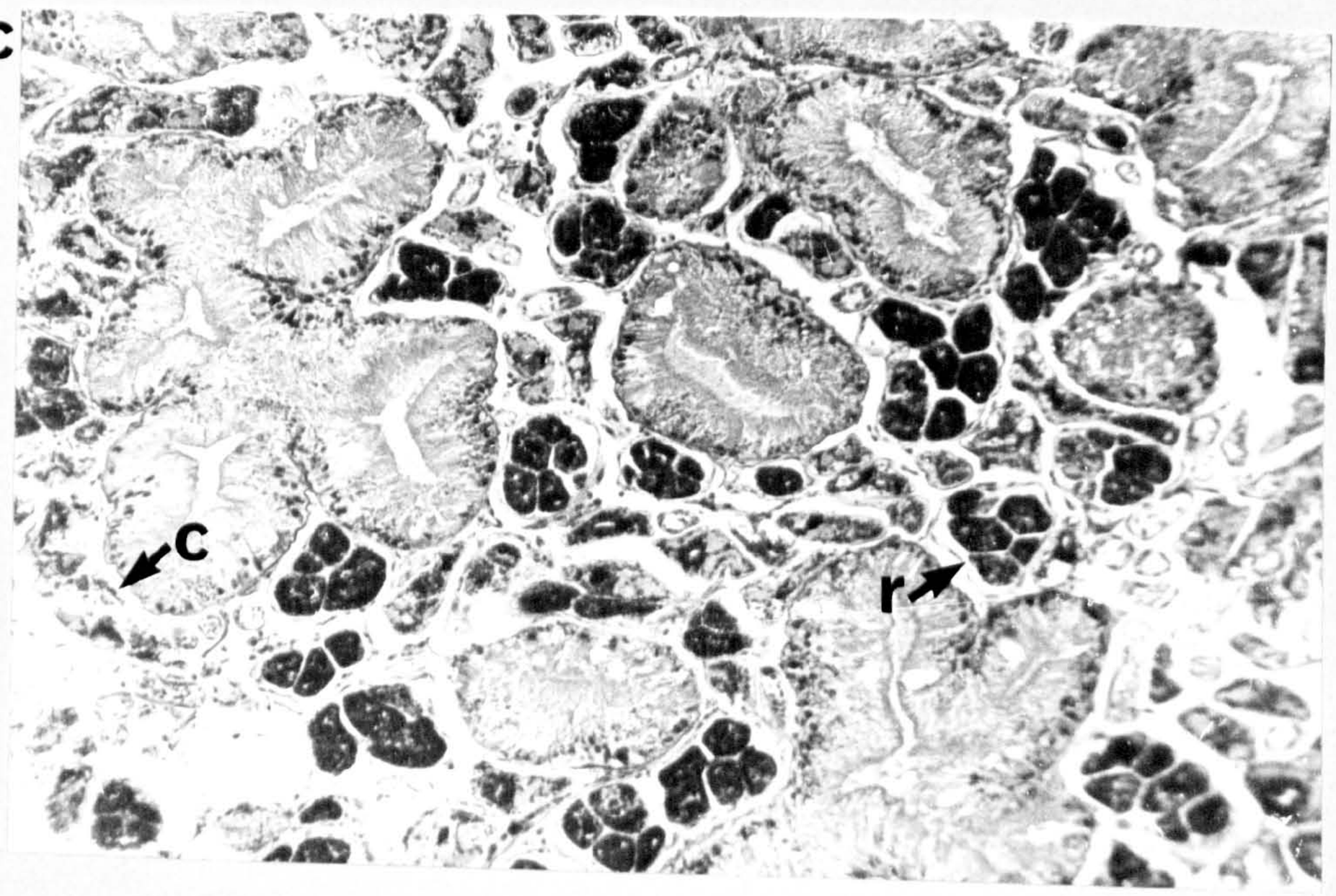


Figure 15. B. undatum; Infected glands showing degenerating rediae.

(a) Redial tegument of August infection showing cytolysis of the redia. Note the vacuolation of the redial wall and swollen nuclei M.S.B. (x 400)

(b) Showing necrosis of parthenitae and resulting stimulation of amoebocytes. Note pyknosis of nuclei in parthenitae M.S.B. (x 250)

(c) Showing degeneration of rediae in September infection. Redial tegument is breaking down and naked germ balls are exposed M.S.B. (x 250)

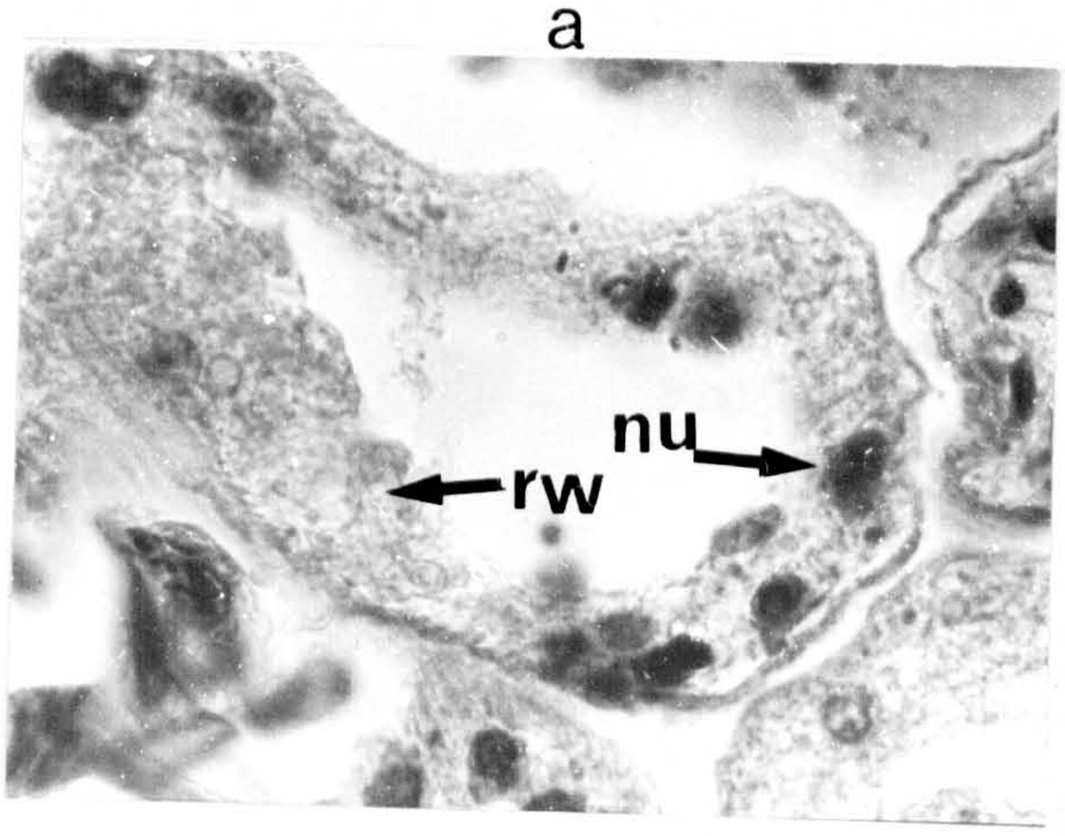
(d) The redial walls have broken down and naked germ balls lie free in the intertubular area M.S.B. (x400)

(e) September infection showing parasite debris in the intertubular area and focal aggregates of amoebocytes M.S.B. (x 250)

(f) Resolution of infection as shown by the fibrosis of the digestive gland intertubular area. Occasional germ balls may still be seen. (x 100)

a : amoebocyte(s)
fr : fibrous replacement tissue
gb : germ ball
nu : nucleus
pd : parasite debris
pnu: pyknotic nuclei
rw : redial wall

a



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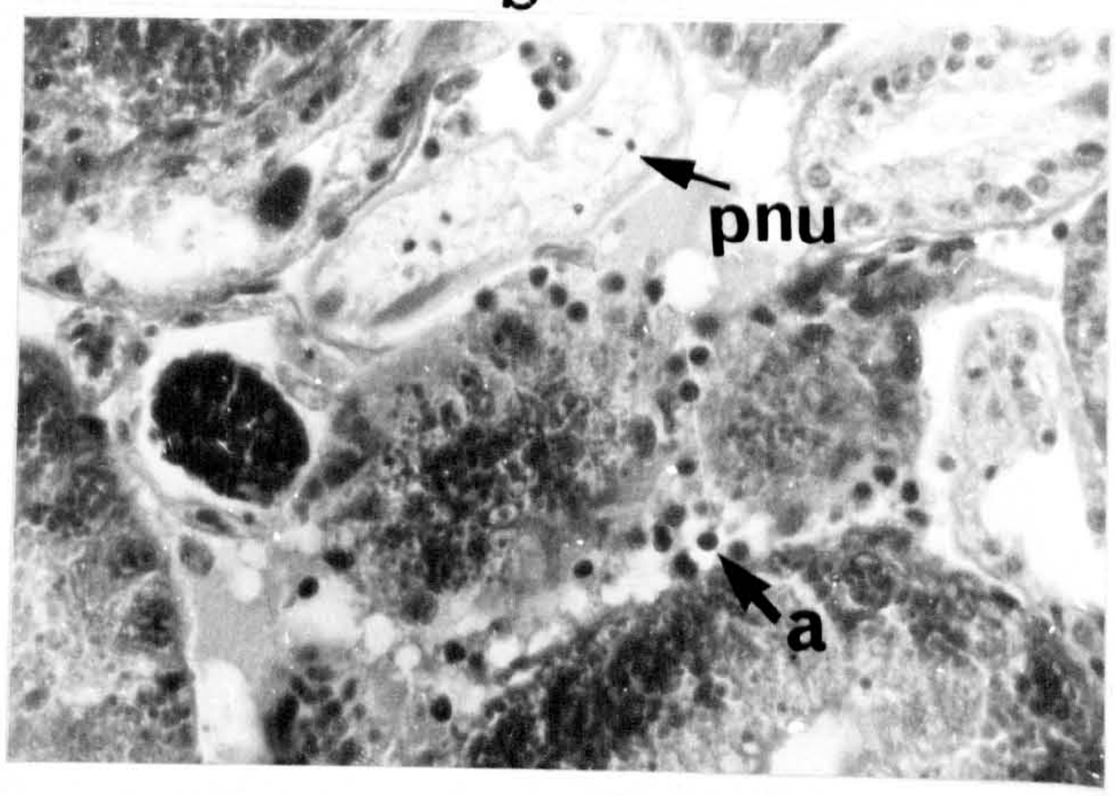

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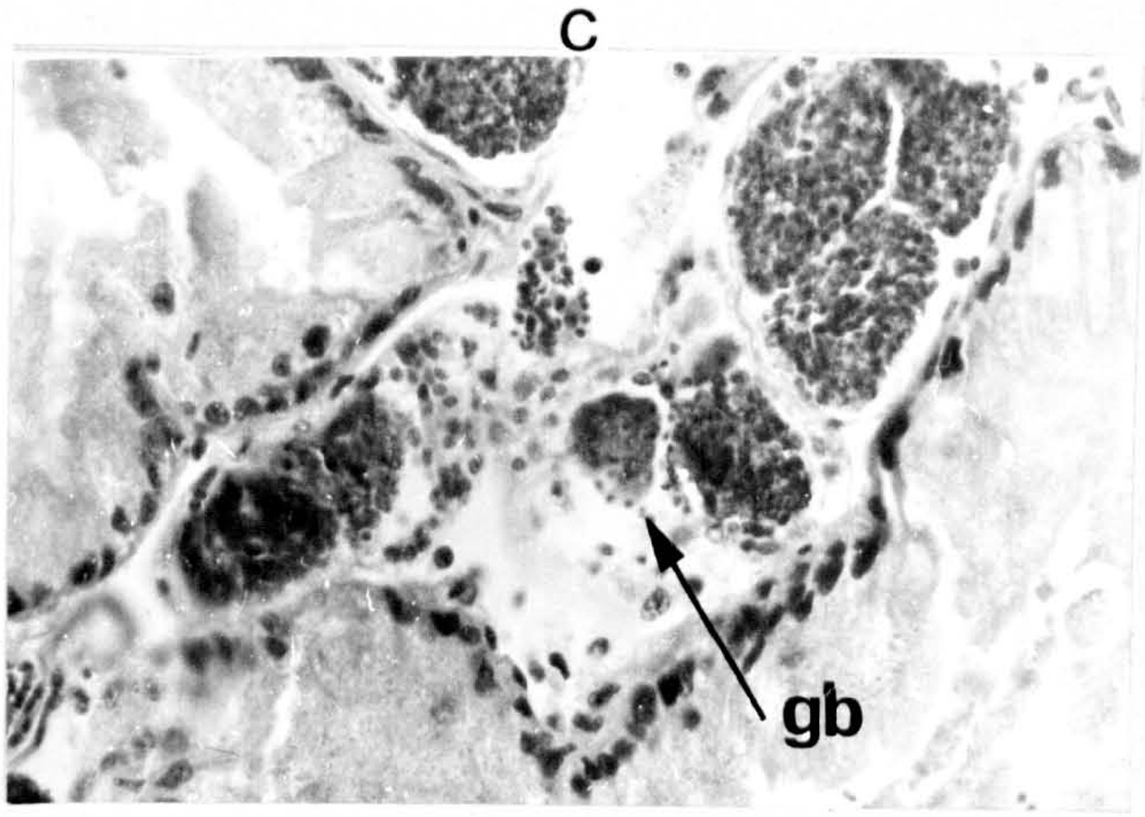

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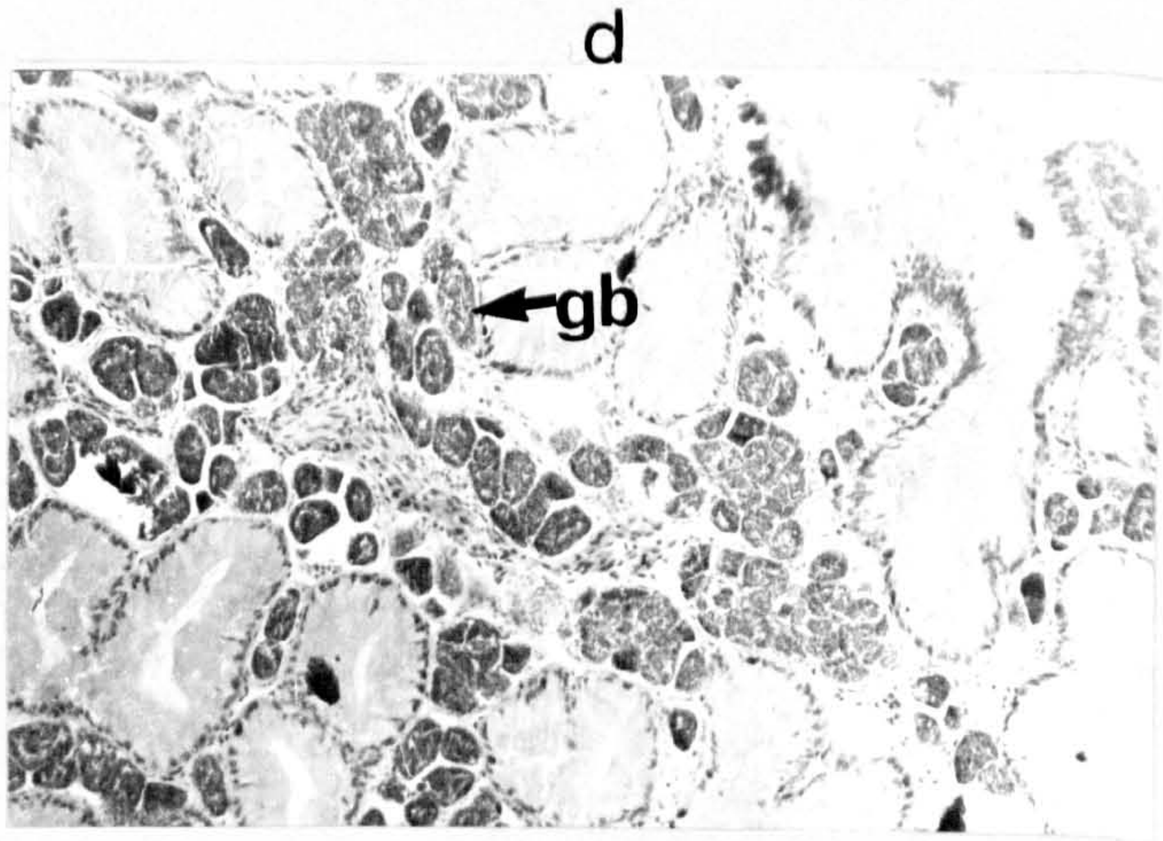

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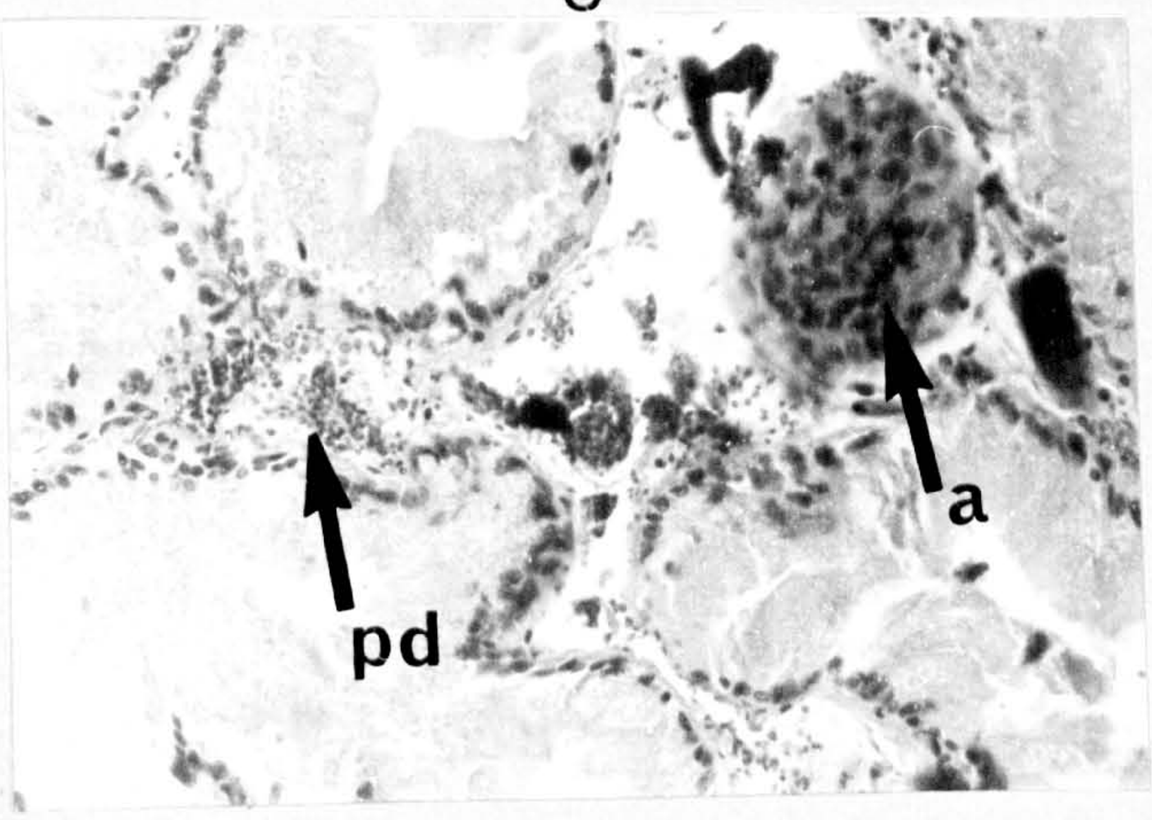

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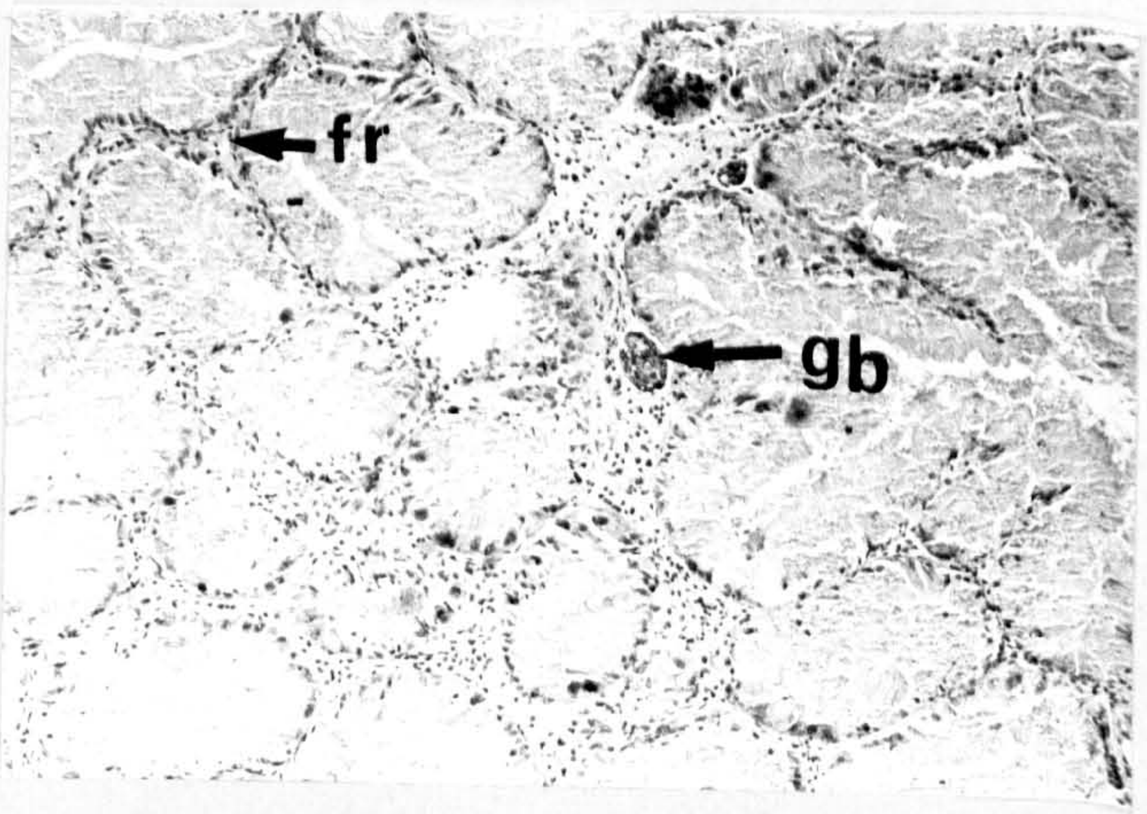


the haemocoelic spaces. Some tubules were completely collapsed. There were areas of replacement fibrosis suggesting a reparative process was taking place (Figure 15f).

Two other glands sampled in September contained grossly unidentifiable parthenitae. In histological section one of these glands contained many rediae with undifferentiated germ balls. These were indistinguishable from those of S. baccatus. No cercariae were seen and many of the rediae had disintegrated or were in the process of disintegration (Fig 15b,c).

A third gland of the same sample contained grossly unidentifiable parthenitae. On subsequent mocroscopical examination it contained merely germ balls and no rediae or redial walls were present. Their cells were in various stages of degeneration. Large numbers of host amoebocytes were present (Fig 15d, e).

Healthy parasites were never seen to be encapsulated by the host. However, in the digestive glands of B. undatum examined in September, a layer of amoebocytes appeared to encapsulate individual moribund rediae and germ balls (Fig 15e, f).

The appearance of the September infections was one of regression of the infection resulting in the eventual death of the parasite and recovery of the gland tissue of the host.

Glycogen Studies

Since glycogen is known to be an important energy storage material in molluscan digestive glands, it was thought that an examination of the glycogen in parasite and host would be worthwhile.

Considerable difficulty was encountered in the demonstration of glycogen. The PAS method showed a great deal of PAS positive material in the gland tissue and within the parasites. This meant the salivary diastase - controls could not be distinguished from the test sections and so the method was of no use. Bests' Carmine staining method on the other hand, which is highly specific for glycogen, showed very little positive material in the digestive gland epithelial cells. Various alternative procedures for the Bests' staining method were tried and alternative fixatives used. These also failed to show the expected large amounts of glycogen in the digestive gland epithelial cells. Well fed B. undatum, fixed immediately in alcoholic Bouins' fixative (Gendres modification) showed no difference from those which had been fixed in buffered formalin. (Rabbit liver controls were always positive for glycogen.) It can only be concluded that the epithelial cells of the digestive gland tubules do not contain high levels of glycogen. Glycogen rarely occurred in the tubular cells and when present was in very small amounts, normally as fine granules localised in a few columnar cells. Occasionally the tubule lumina contained an amorphous glycogen positive material.

Most of the glycogen of the digestive gland was localised in the connective tissue cells, in particular the vesicular connective tissue cells which surrounded the larger blood vessels. Here it was deposited as fine granules and clumps (Figure 16). It also occurred in small patches and fine granules dispersed amongst the connective tissue and muscle below the tunica propria. In these sites there were no consistent difference between infected and uninfected mulluscs. No consistent seasonal differences

Figure 16. B. undatum; Normal gland in May showing glycogen localised in the vesicular connective tissue. Best's carmine (x 250)

d : digestive gland

g : gonad

vct: vesicular connective tissue

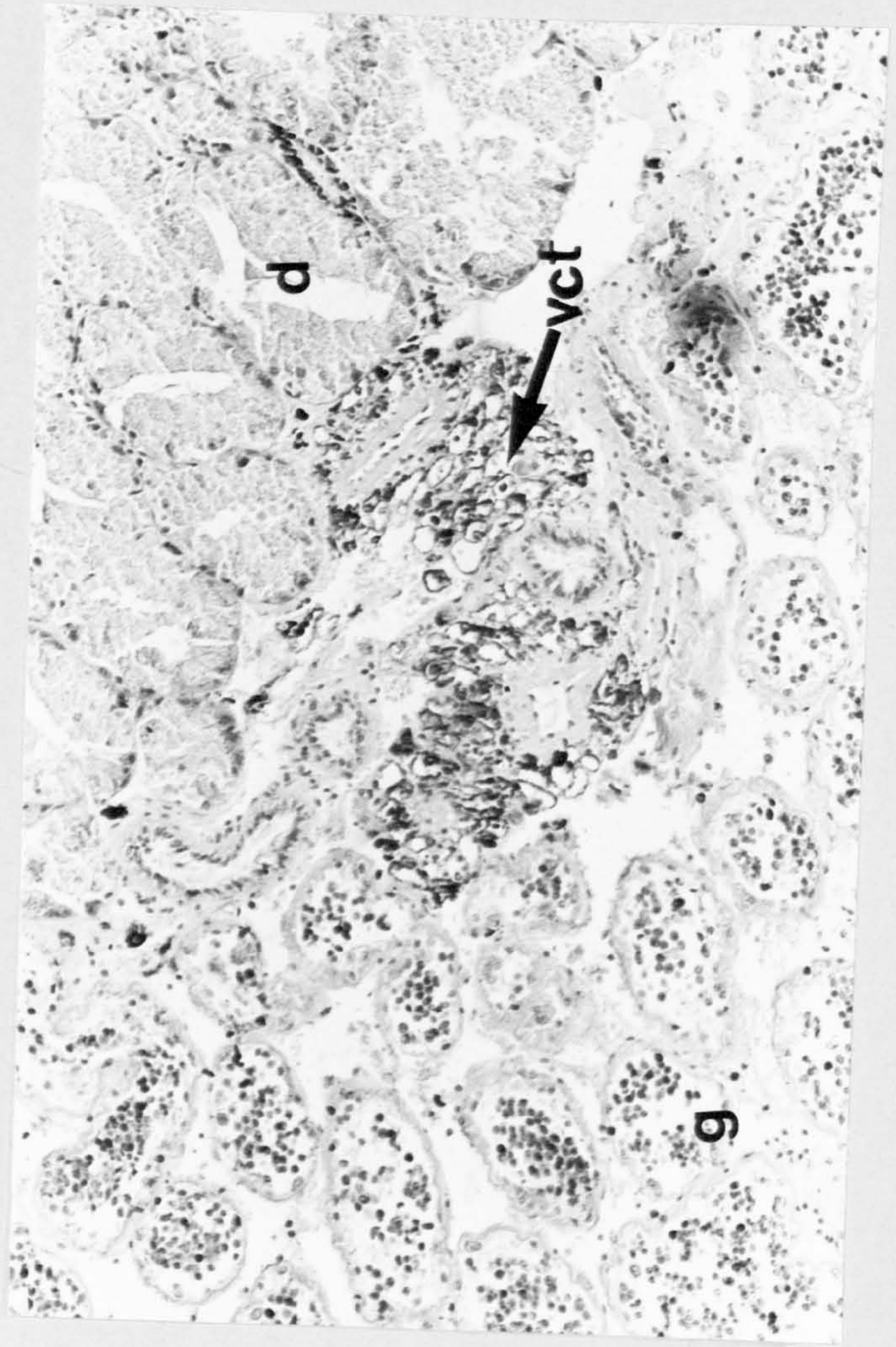
Figure 17. B. undatum; Infected gland in May showing cercariae with large amounts of glycogen in the caudal parenchymal cells. Best's carmine (x 400.)

gl : glycogen

Figure 18. B. undatum; Infected gland in August showing heavy deposits of glycogen in the redial tegument but little or none in the cercarial tails Best's carmine (x 250)

c : cercaria

r : redia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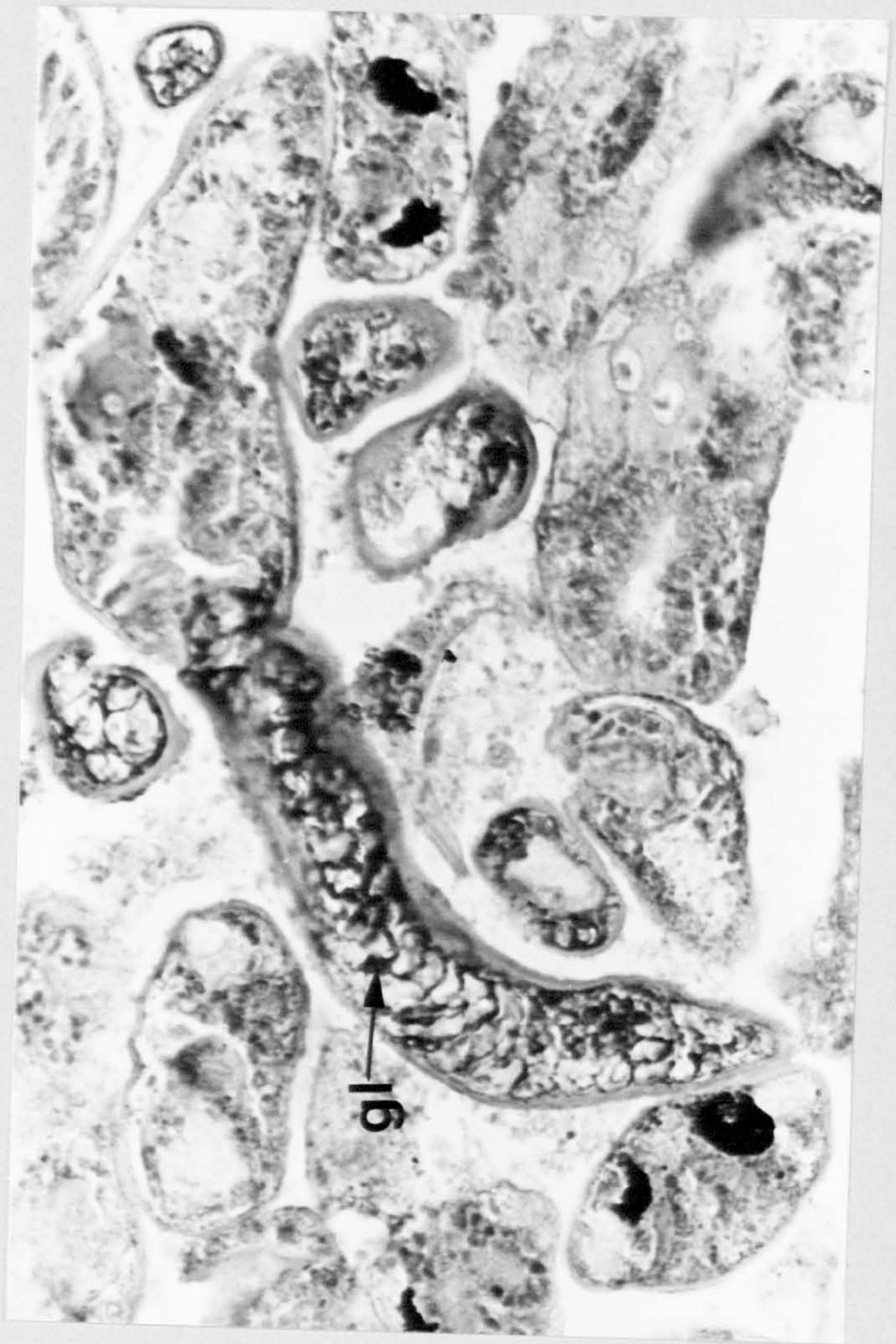
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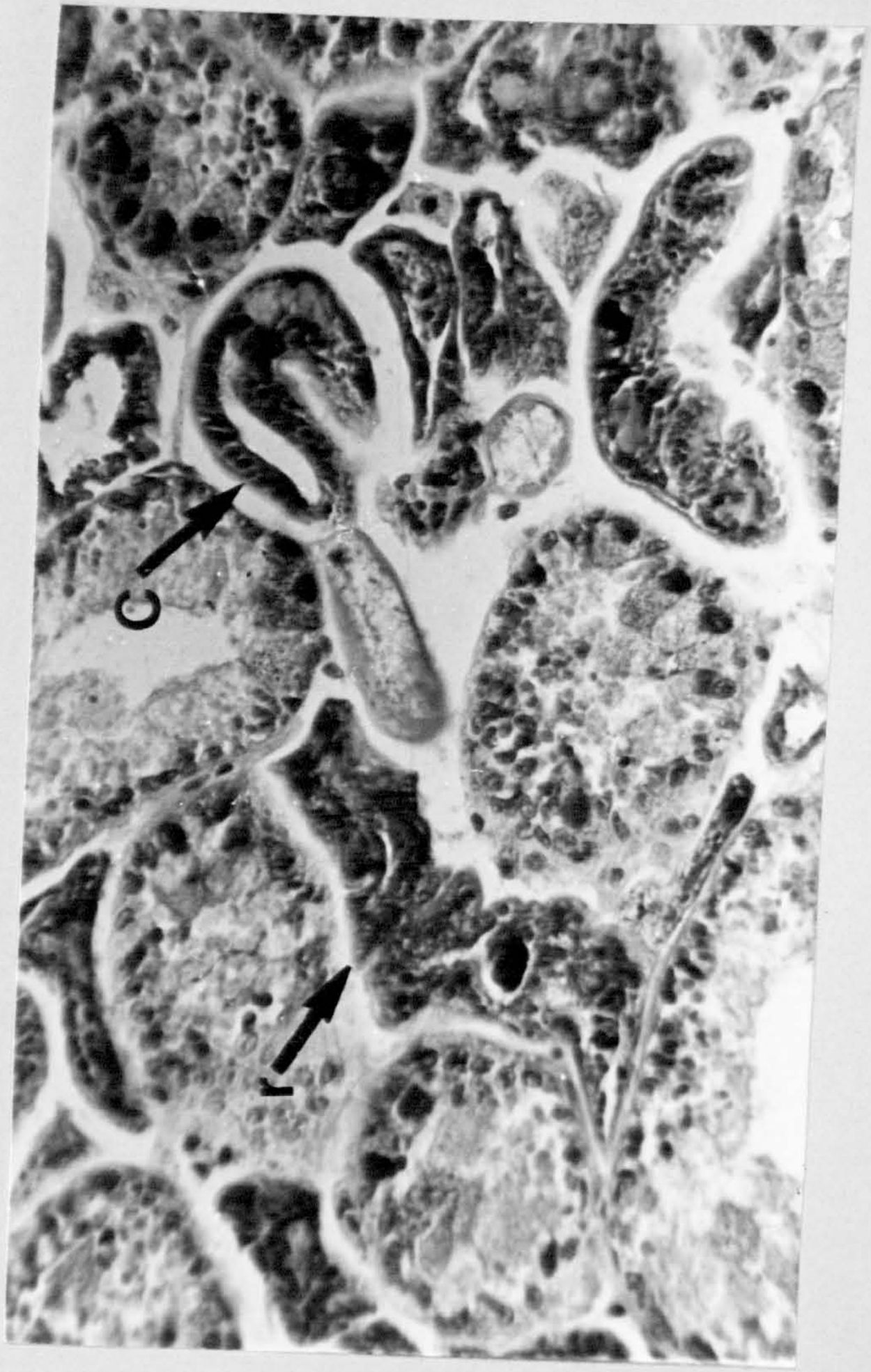
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L

were noted in the presence/absence or amount of glycogen in these glycogen deposits of the vesicular connective tissues.

Differences were seen in the glycogen content of the parasite larvae. In the infected glands of the May period there were many mature cercariae. The glycogen of the cercariae was far more abundant in the tails than any other part. In the tail the glycogen was located in all the large parenchymatous cells (Figure 17). The tails of immature cercariae within the rediae did not have any glycogen. Fine granules were seen in the bodies of both the mature and immature cercariae, but not in germ balls. In mature cercariae it was notably present in a thin layer immediately below the tegument as well as scattered in the parenchymatous tissues. Where there were larger numbers of mature cercariae there was noticeably less glycogen in the rediae. The glycogen in the rediae was in the parenchymal cells, in the subtegumentary cells and in the oral sucker. The rediae with developing cercariae had very heavy deposits both as fine granules and large clumps.

Very small amounts of glycogen were first seen to occur in the columnar cells of the digestive gland epithelium in June and July. By August glycogen was seen in these cells and the lumina more frequently than in earlier infected glands. Variable amounts occurred in the haemocoelic spaces and connective tissue but in all cases heavy deposits occurred in the rediae until August (Figure 18). Presence of glycogen was variable in parasites within the same gland after July, i.e. some individuals had heavy deposits, some little and some none. This was unrelated to the numbers of mature cercariae present.

Lipid Studies

In view of the apparent absence of glycogen as an energy source within the gland, sections of normal and infected glands from the May collections were stained for total lipids. Oil Red O positive material occurred as finely dispersed granules in the digestive gland columnar cells. The Sudan Black B stained sections were strongly positive and globules occurred at the proximal ends of the cells (Fig ure 19). Lipid staining was also observed irregularly scattered throughout the inter-tubular connective tissue. The secretory cells showed no lipid positive material. The haemolymph within the intertubular arteries also demonstrated a strongly positive reaction to lipid stains.

Although infected digestive glands showed a similar pattern of lipid distribution to the normal ones there was, in addition, heavy lipid deposits within the redial walls. As they developed germ balls contained increasing amounts but mature cercariae contained less than other stages. The redial intestine contained strongly lipid positive material (Figure 19d).

It had been hoped to classify the lipids in the digestive gland using the stains Sudan Black B, Oil Red O and Nile Blue Sulphate. However, little difference was seen between the distribution of positive material for the first two stains. (Nile Blue Sulphate distinguishes Neutral fats-red and Fatty acids-blue.) The staining with NBS was relatively heavier than the other two stains and only rarely was there any red-positive material; this consisted of a few small droplets in the tubule cells in two sections only. No fatty acids, therefore were demonstrated in any quantity in the digestive gland.

Figure 19. B. undatum; Infected glands in May showing tissues stained with Sudan Black B for lipids.

(a) Digestive gland in May (x 100)

(b) Heavy lipid deposits in the rediae (x 400)

(c) Showing lipid deposits in developing cercariae (x 400)

(d) Showing redia with lipid material in the redial intestine (x 400)

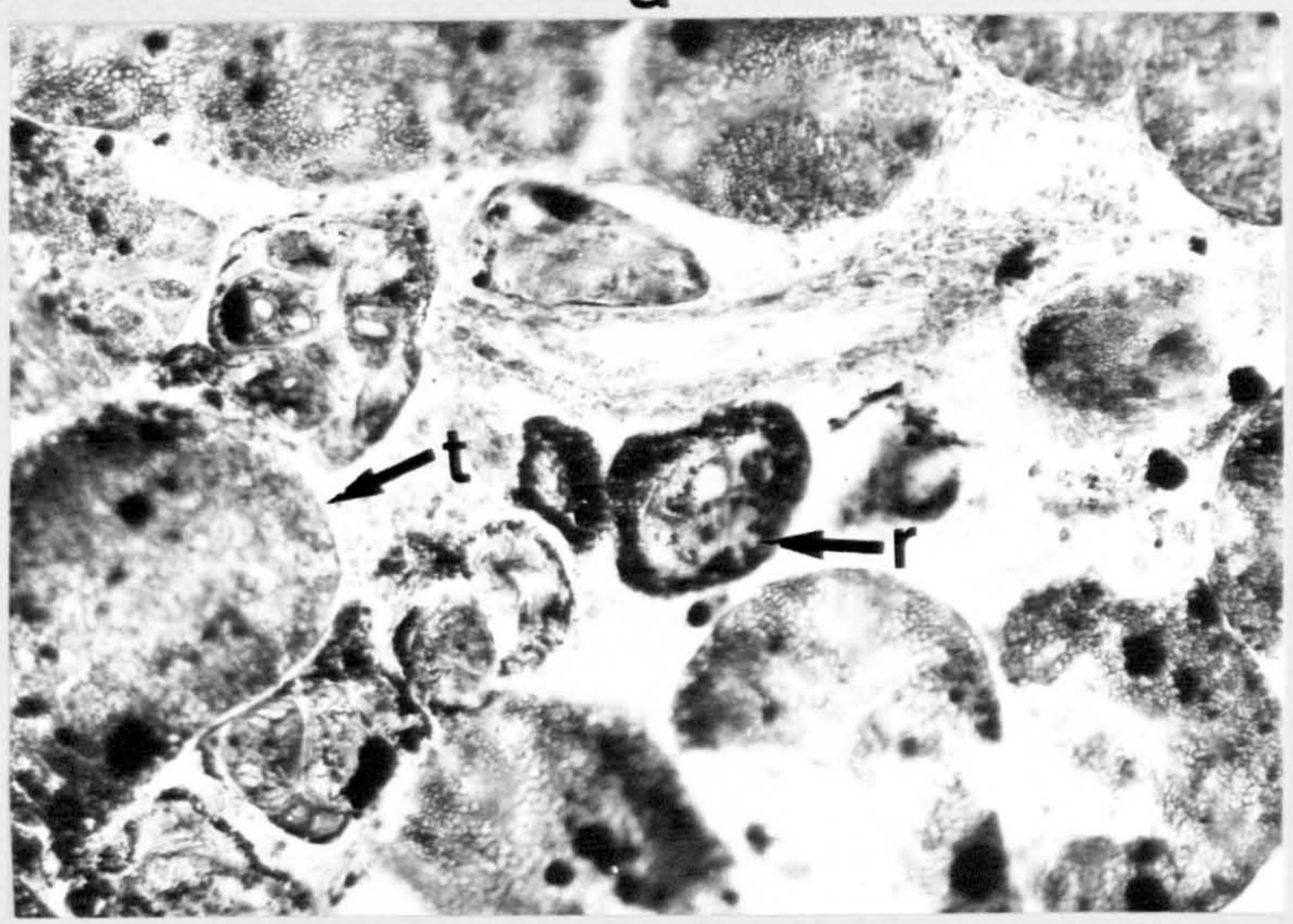
c : cercaria

r : redia

ri : redial intestine

t : digestive gland tubule

a



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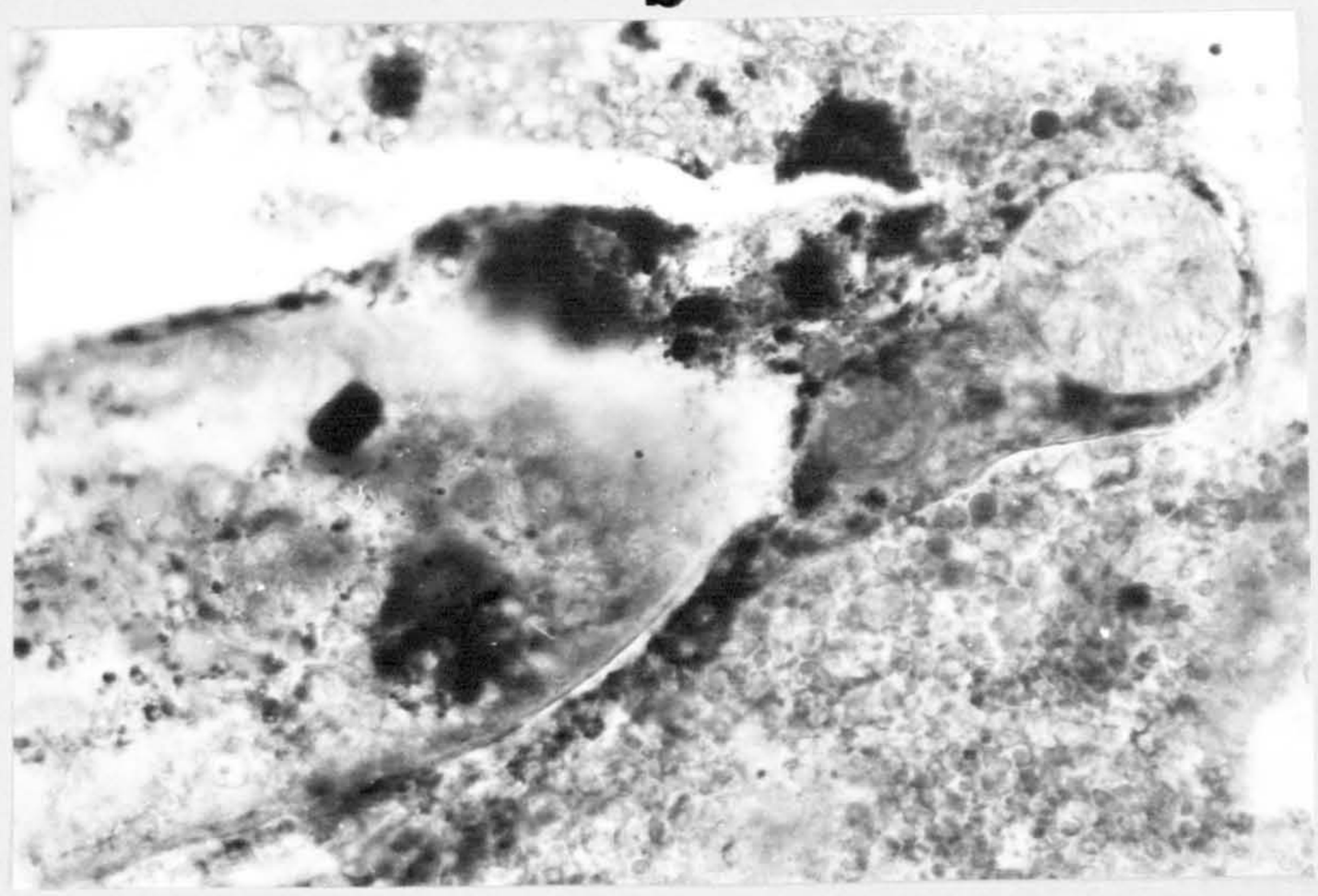

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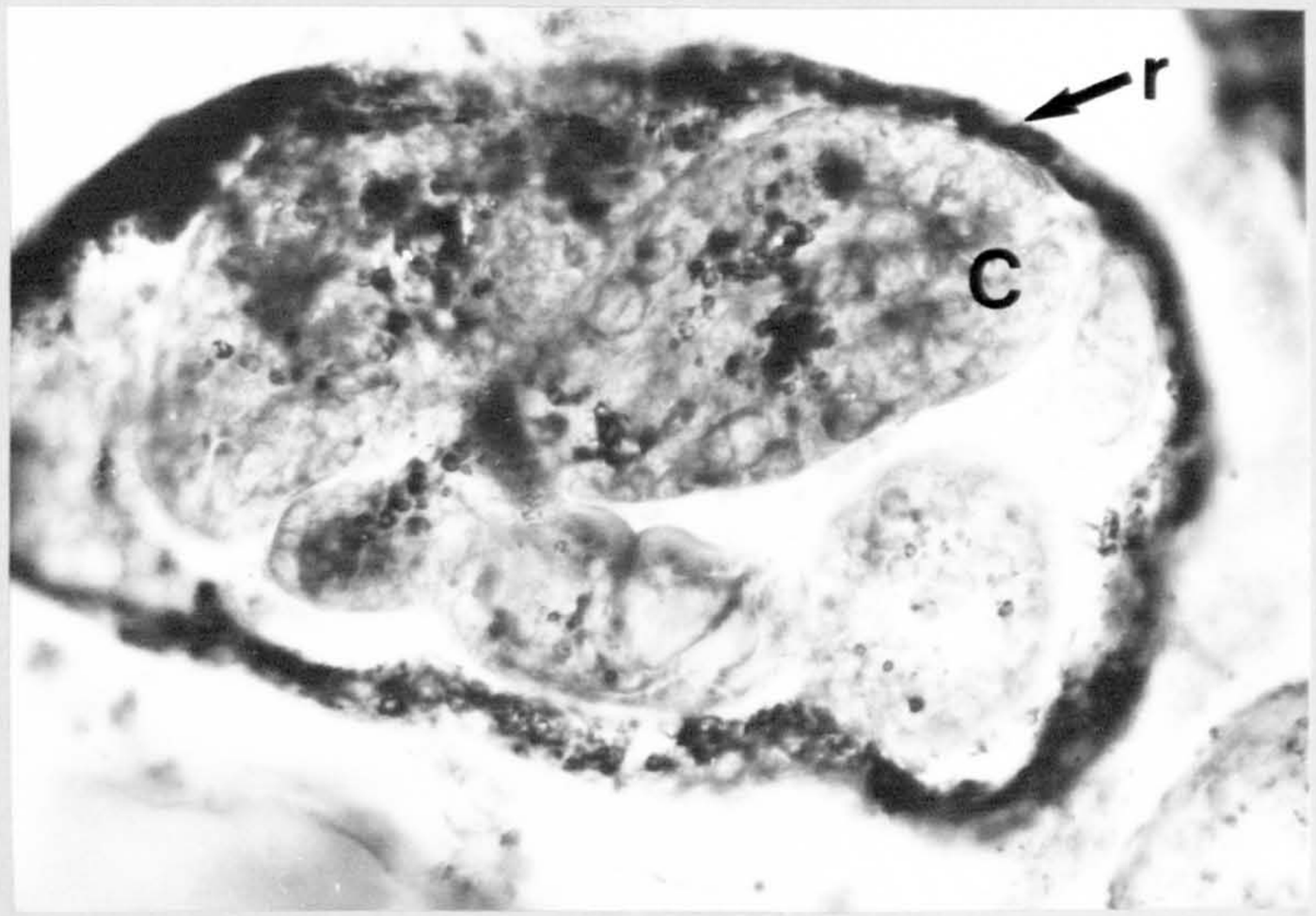

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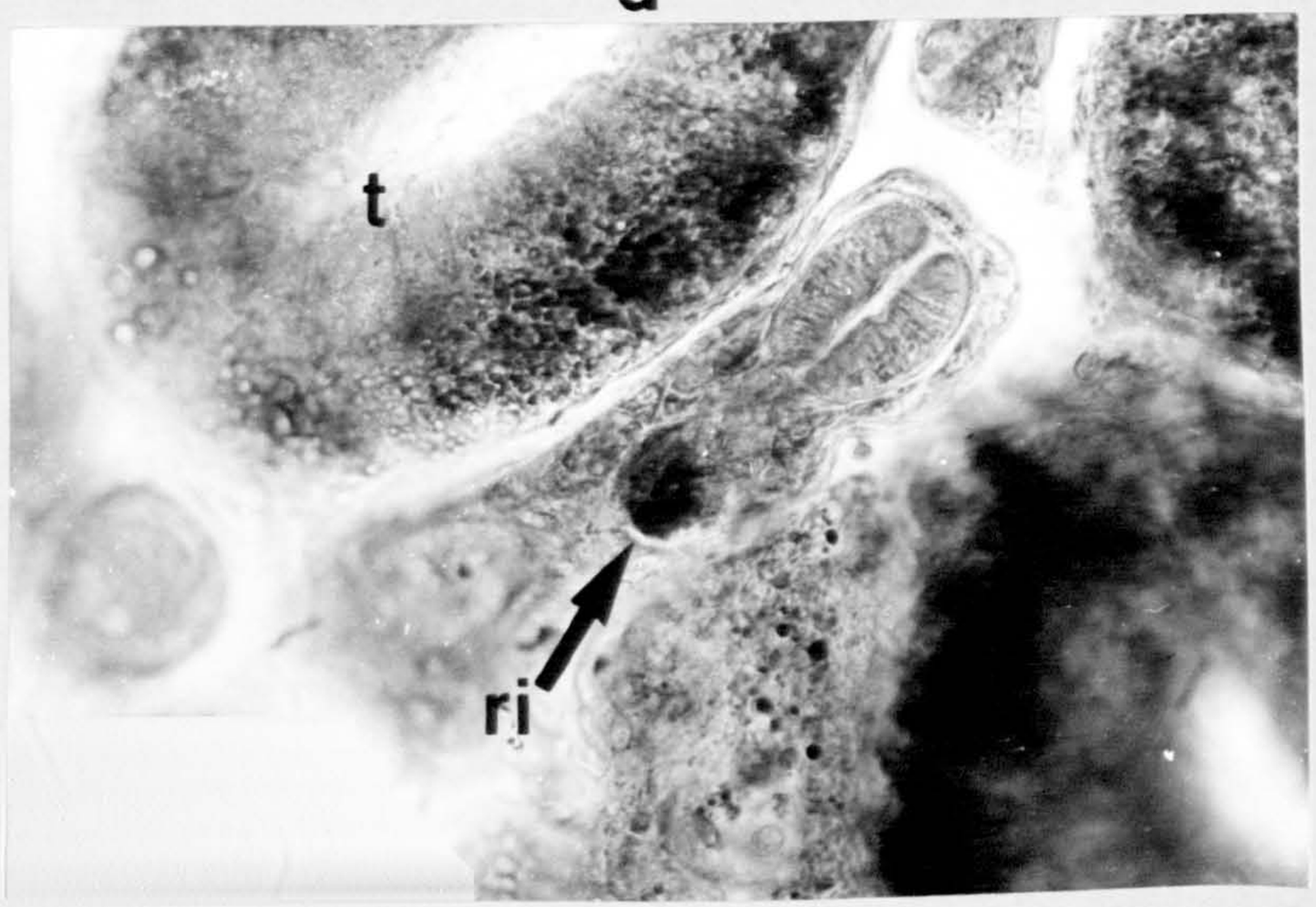


DISCUSSION

A. Incidence

The records of incidence indicate that S. baccatus infection of B. undatum and N. antiqua occurs in Spring, persists during the Summer from May to August and thereafter declines. No S. baccatus infection was found after September and those of September were of rediae without cercariae. Unsuccessful attempts were made to obtain mollusc samples before May as S. baccatus infections are certainly found in April (MacKenzie & Liversidge, 1975). No S. baccatus were seen or have been reported to occur during other months of the year. Wolfgang (1954a) found infected molluscs during the Summer months and MacKenzie (1971) found S. baccatus infections in June only. Lebour (1911, 1918) recorded infections in May from Plymouth and July from Northumberland. However, none of these authors made seasonal collections.

The evidence, therefore indicates a seasonal cycle of infection in the first intermediate host from Loch Ewe broadly from April to August. Depending on temperature and climatic conditions, the pattern may differ or incidence may reach a different level in other localities. The single sample from Gruinard Bay illustrates this; the high incidence in that sample may reflect the different conditions at Gruinard Bay compared with those at Loch Ewe.

Seasonal influences in larval digenean infection in molluscs have been recorded by Miller & Northup (1926) and McDaniel & Coggins (1972) on Nassarius obsoletus (Say). Robson & Williams

(1970) showed that a seasonal peak of incidence of infection of Littorina littorea (L) with Cryptocotyle lingua (Creplin) at Scalby Rocks, Yorkshire, England, occurred from July to November. However, Sindermann & Farrin (1962) found that the period of greatest incidence of the same parasite in the same host species was December to April at Boothbay Harbour, Maine, reflecting, perhaps, the different ecological factors controlling mollusc infections with larval digeneans at different latitudes.

Køie (1969) recorded the incidence of parasites in B. undatum from Øresund and the Gullmar Fjord, Sweden. She did not find S. baccatus infection at this location but the other larval digenean infections, while showing monthly fluctuations, did not show any recognisable seasonal pattern. Cercaria buccini and Zoogonoides viviparus showed a similar pattern as illustrated in this study during the summer months (Figure 2). MacKenzie & Liversidge (1975) suggested that seasonal cycles may be a feature of the acanthocolpid group to which S. baccatus is related.

Unfortunately, the measurements of variables, which it was thought might provide indications of parasitic infection, were too few in number to be conclusive. Køie (1969) found a reduction in the length of the copulatory organ of B. undatum infected with larval digeneans and it may be that a larger sample size of infected molluscs might show this to be the case for the population of B. undatum from Loch Ewe.

The specimens of B. undatum which were overwintered in the aquarium had normal copulatory organ measurements even though their overall height was low. The reduced height for the sample can be attributed to aquarium feeding which is minimal compared to the mollusc's natural diet. Even so, these molluscs spawned in the aquarium at the expense of increased body size.

B. Histopathology

Interpretation of the various phenomena observed in the tissues of the digestive gland of B. undatum, indeed of any mollusc, is difficult since the basic knowledge of changes occurring in the normal digestive cycle of activity is lacking in all but a few cases. This is compounded by the enormous variation in both morphology and physiology of the molluscan digestive system. Much of the available information is concerned with bivalves and, in particular, commercially interesting species. Work on gastropods has been largely concentrated on the herbivorous species. Marine carnivorous prosobranchs such as B. undatum have commanded little attention. Care is necessary therefore, in the interpretation of the observed phenomena so as not to confuse pathological features with normal cellular changes of the digestive cycle.

An indication of the sort of problems which the cyclic changes in the molluscan digestive gland can pose for the histopathologist is presented by the study of McQuiston (1969) on the diphasic digestive cycle in the digestive diverticular of the marine bivalve Lasaea rubra (Montagu). About half the tubules comprising the diverticula take part in digestion during each

tidal cycle. Cellular regeneration takes place in the other half. The newly formed cells take part in digestion during the following tidal cycle while the spent cells are replenished. These cycles affect the size and maturity of the digestive gland epithelial cells. After digestion is complete the cells disintegrate and slough effete matter and cell debris into the lumen in a fashion resembling cytolytic necrosis. This highlights the danger there-for of ascribing a pathological effect to changes which may be purely physiological.

Vacuolation of the cells of the digestive tubules and intestine is also a case in point. Secretory cells were at times more vacuolated than others giving a more reticular appearance and it is believed that this reflects normal cycles of secretory activity. The vacuolation seen occasionally in the columnar cells consisted of a single large vacuole at the distal end of the cell and occupying approximately one third of it. It is more difficult to explain but was also seen in an uninfected gland. Such vacuoles have been described by Cheng & Snyder (1962) in their review of pathological effects of digeneans in molluscs but certainly in the present study no pathological significance could be ascribed to them.

The variety of genuinely pathological effects of which can be found in S. baccatus infections of the digestive gland of B. undatum can be summarised as follows:-

(a) Reduction in the number and size of tubules.
(b) Complete destruction of individual tubules.
(c) Necrosis of large areas of the digestive gland.
(d) Fragmentation of columnar cells.

(e) Sloughing of parts of the tubule epithelium.

(f) Reduction in the number of globules in the columnar cells.

(g) Loss of lateral walls of the columnar cells.

(h) Reduction in height of the columnar cells.

(i) Increase in the intertubular area.

(j) Destruction/fragmentation of intertubular connective tissue.

(k) Compression of blood vessels.

Not all infections showed these features, much depending on the intensity of the infection and its age.

Histological changes in the digestive gland tubules of molluscs have been described by many authors including Faust (1920), Agersberg (1924), Rees (1936), Cheng & James (1960), James (1965), Wright (1966), Patnaik & Ray (1966), James & Bowers (1967) and Mohandas (1974). The type of change described varies and has been reviewed by Cheng & Snyder (1962) and Wright (1966). Changes such as those observed in the present study have been found in other infected molluscs, though not all in the same host. Two features which had little significance in B. undatum but which are major features of parasitism in may molluscs are

(a) the formation of a blocking layer by larger numbers of parasites which results in starvation autolysis and atrophy in the distal part of the gland (James, 1965; Rees, 1936), and

(b) the reduction in the columnar cells to a squamous or cuboidal form.

(b) is thought to be a result of the amount of food available in the mollusc either for storage or normal metabolic processes. It was recorded in the later part of the summer in B. undatum and it may be a result of increasing demands of the developing host gonad and the subsequent diversion of nutrients to that tissue. Those species in which epithelial cell size reduction

has been considered significant are all either grazing herbivores or particulate feeders, e.g. Littorina sp., Lymnaea sp., which have intra-cellular digestion by, e.g. Rees (1936) and Mohandas (1974). In these molluscs the removal of waste products of digestion involves a process whereby a new cell wall develops, cutting off the distal end of the cell where the waste products have accumulated. These are then released into the lumen of the tubule and taken to the rectum. In B. undatum a carnivorous prosobranch, digestion is extra-cellular and removal of waste in this way will not occur, which may explain why the reduction in the cell height is not so marked.

Much discussion in the literature has concentrated on the relative significance of mechanical and physiological damage to the host tissues by the parasite. James (1965) concluded that the significance is a function of many factors including the size and the nature of the germinal sacs, their rate of development in relation to the life span of the host, the time of the initial infection, the mobility of the germinal sacs and the specificity (host resistance) of the parasite.

In the present study the pathological effects on the host were considered to be due to the following factors:

(i) Compression of host tissue by the growth and movements of parthenitae

(ii) Lysis of host cells by parasite enzymes, as a result of either their feeding activity or egestion of waste material (containing digestive enzymes) from the redial intestine

(iii) Toxic action of waste materials from parasitic metabolite excretion

(iv) Starvation owing to competition for food between the parasite, the gonad and the digestive gland.

The evidence for seasonal differences in parasite development suggests that the maximal production of cercariae occurs before June in the area of N.W. Scotland from which the molluscs were sampled. Molluscs containing large numbers of mature cercariae are less frequent in July and August due to a halt in cercarial production; i.e. large numbers of rediae with germ balls could be seen but further development of these was retarded, although occasional cercarial production was found. By September the infection was regressing with the subsequent death and disintegration of the immature rediae.

Whereas the healthy parasites produced no infiltrative response in the molluscs the death of the parasites induced leukocytosis and the moribund parasites became encapsulated. The infected molluscs of this period had several areas of replacement fibrosis. The type II lesion which resulted in death of parasites was seen early in the season with associated leukocytosis. There was no evidence of overwintering in S. baccatus infection in B. undatum.

Glycogen

The examination of the digestive gland for glycogen was carried out in the hope that it would help to explain the loss of infectivity of cercariae in the latter part of the season as revealed by the experimental infections (vide supra), since experimental infections attempted in July, August and September failed to produce the usual large numbers of metacercariae in the flatfish intermediate hosts. Seasonal fluctuations in cercarial emergence are a common phenomenon and those which

have been investigated show a direct temperature relationship e.g. Sindermann & Farrin (1962) demonstrated this relationship with Cryptocotyle lingua (Creplin 1825). However, in the case of S. baccatus there is a marked loss of infectivity long before the cercarial production reduces significantly.

It has been shown (Part 2 A) that one of the factors affecting infectivity is the amount of stored glycogen in the cercariae, in particular, in the tail. It seems reasonable to suggest that the cercariae produced later in the season may not have access to sufficient nutrient for their needs, possibly owing to competition with the developing gonad. Kendall (1949) compared production of mature cercariae of Fasciola hepatica (L) in Lymnaea truncatula which were either well fed or starved and found that the amount of food available to the rediae was a major factor controlling the number of cercariae which matured in a given time. Magalhaes & de Almeida (1956) showed that desiccation of Australorbis glabratus (Say) resulted in a decrease in the glycogen content of different organs. In the digestive gland, stomach and ovotest the glycogen dropped to less than 80% after 15 days out of water. Simoeo & Coelho (1955) reported that the larvae of Schistosoma mansoni Sambon stopped developing when the host A. glabratus was kept out of water but development resumed after re-hydration.

Robson & Williams (1971) also found a reduced glycogen content in the digestive gland and foot of Littorina littorea (L) when the snails were starved for 166 days. Cheng & Snyder (1962) reviewed the literature on the influence of host glycogen on the emergence of cercariae which, they claimed, suggested that

inhibition of normal development of S. mansoni correlated with the amount of glycogen in the host's digestive gland.

However, in the present study the amount of glycogen seen in the digestive gland epithelium cells of B. undatum was so small that it cannot be a significant source of energy for the parasite. Change in the amount of glycogen in this site is, therefore, unlikely to be a factor controlling cercarial production.

Most of the workers studying the digestive gland and its relationship with larval Digenea have described rich deposits of glycogen in the tubule epithelium cells. The molluscan hosts studied were either herbivorous or particulate feeders e.g. Cheng & Snyder (1962), Cheng (1963a), Snyder & Cheng (1966) on Helisoma trivolvis (Say); Faust (1920) on Planorbis guadelupesis, Physopsis africana, Planorbis trivolvis (Say) and Physa sp. and Reader (1971) on Bithynia tentaculata (L). Conversely, the most important deposits of glycogen in B. undatum (a carnivore) digestive gland are the vesicular connective tissue cells and not the digestive gland epithelial cells. Even taking this into account there is far less glycogen present in the digestive gland tissues than would have been anticipated. Cheng (1963a), Cheng & Burton (1966), Von Brand & Files (1947), Reader (1971) and Negus (1969) described the occurence of glycogen in the connective tissue cells but only Meuleman (1972) considered this to be the major site of glycogen deposition (in Biomphalaria pfeifferi (Krauss)). Reid, (1969) stated that the bivalve Tresus capax (Gould) contained no glycogen in the digestive diverticula but did not mention any other glycogen-rich tissue

apart from the gonad. The small amounts of glycogen in the B. undatum tubule cells are probably sufficient for intra-cellular activity.

It has been established by a number of workers that the digestive gland glycogen is reduced in molluscs which have infections of larval Digenea (Faust (1920), Cheng (1963a), Snyder & Cheng (1961), Cheng & Burton (1961), James & Bowers (1967) and Reader (1971)). Similar results were found by Von Brand & Files (1947) and Robson & Williams (1971) using biochemical analyses. This reduction has generally been attributed to the utilisation of the glycogen of the digestive gland by the parasites. However, there was no observable change in the glycogen of the digestive gland tubule or connective tissue cells in B. undatum infected with S. baccatus.

It is possible that significant changes in glycogen levels occurred in other tissues of the mollusc since Robson & Williams (1971) found that the foot glycogen of L. littorea was reduced as well as the glycogen in the digestive gland. Von Brand & & Files (1947) found greater quantities in the muscles of the foot and head region and in those adjacent to the lingual ribbon in Australorbis glabratus. However, in these studies there was always a significant loss of the glycogen from the tubules of the digestive gland as well as from other tissues. In the present study a brief examination of other tissues of B. undatum was made for glycogen and, while many tissues, especially the muscle of the foot, showed a finely granular, evenly dispersed, glycogen positive stain, none of these showed large deposits

which might indicate a major store of glycogen. Moreover, Negus (1968), who found more glycogen in the peripheral connective tissues cells of Turritella communis Risso than than in cells at the centre of the visceral hump, found no difference in the glycogen content of the connective tissue cells of Turritella both uninfected and infected with C. doricha Rothschild. He concluded that the sporocysts do not remove glycogen from connective tissue cells of the host even though they contained large amounts of glycogen within their own tissues. Meuleman (1972) also found no change in glycogen content of connective tissue cells in infected tissue.

Food uptake in Rediae

Despite the fact that the glycogen in the digestive gland epithelium was negligible and often absent, there were considerable amounts of glycogen within the parasites. In particular, the subtegumental and parenchymatous cells were loaded with aggregate glycogen granules indicating that it is a significant component of the redial metabolism.

The rediae can obtain food, per os which is digested in the redial intestine. The contents of the intestine frequently appeared as large globules similar to those described by Køie (1971) who considered them to be waste material but never saw them outside the redia. However, the globules seen in the S. baccatus redial intestine were occasionally seen in groups outside the rediae, in the intertubular space. Extra-corporeal digestion almost certainly takes place as well as ingestion. Tissues surrounding the parasites had often been lysed and Køie (1971) suggests that the acid phosphatase and other hydro-

lytic enzymes produced by the redial intestine are regurgited into the host tissue to be subsequently ingested via the mouth with their hydrolysed substrate. There is also a growing weight of evidence to suggest that nutrients are absorbed through the tegument of rediae. Sporocysts which have no intestine absorb nutrients through the tegument (James, Bowers & Richards, 1966; Cheng & Snyder, 1963). Ultrastructural studies of the tegument of sporocysts and rediae of various other digenean species have shown that they are essentially the same and capable of nutrient transport. Køie (1971), Rees (1956), Krupa, Bal & Cosineau (1967) examined the ultrastructure of the redia of Cryptocotyle lingua and thought the folds on the redial surface to be important for the pinocytic incorporation of nutrients. They also cite observations made by McDaniel & Dixon (pers comm) that these rediae and those of Parochis acanthus Nicoll, readily incorporate substantial quantities of exogenous glucose ^{14}C into polysaccharide. Reader (1971) in his description of digenean infections of Bithynia tentaculata found only a slight increase in the phosphatase activity of digestive gland cells associated with redial infections whereas this increase was marked with sporocyst infections. He concluded that absorption of carbohydrates from intact host cells of rediae was of secondary important to direct ingestion. Contrary to these findings, ultrastructural studies by Køie (1971) concluded that the mouth of the redia of Neophasis lagenifomis (Lebour) (closely related to S. baccatus) had only a limited function compared to the tegument.

Cheng (1963a) performed experiments which suggested that the tegument of the sporocyst of Glyphthelmis pennsylvaniensis

Cheng did not secrete a glycolytic enzyme but a substance which stimulated the host's glycolytic enzymes.

The redial tegument of S. baccatus was found to be thrown into numerous folds and micro-villi which would increase the surface area for the nutrient uptake, but there was no evidence as to the source of the carbohydrate utilised. Blood glucose is a possible carbohydrate source for the rediae, and larval Digenea which occupy other sites lacking in food material have been described as utilising this source of energy (Cheng 1963b).

Whatever the source and method of uptake of nutrients by the rediae in B. undatum it seems very likely that incorporation of nutrients through the redial wall is of some importance. The degree of importance may be age dependent, since young rediae were commonly seen with a full intestine but this was more difficult to see in older rediae because the brood chamber, packed with cercariae, caused the compression and displacement of the intestine. The folding of the tegument and micro-villi were more obvious in the mature rediae especially at the anterior part behind the pharynx. They are also less active and the tegumental source of nutrient may well be more important at this stage.

Lipids

Lipids seem to be more important in the digestive gland cell metabolism than glycogen since appreciable amounts were to be found in the gland. Stickle (1966) studied the nutrient depots of the intertidal prosobranch Thais lamellosa (Gemlin) and concluded that "Prosobranchs thus appear to have a lipid oriented metabolism, but pulmonates are carbohydrate oriented". T. lamellosa is a carnivore as is B. undatum. Martin (1961) in

a review of the carbohydrate metabolism of mulluscs reported that the predatory cephalopods store little glycogen and thought this to be due to their ability to secure food regularly and frequently, and their metabolic systems which interconvert food stuffs readily. Buccinum undatum is an active predator and it may well be a feature of carnivorous molluscs that lipids are a more important metabolite. Relatively little is known about the lipid metabolism of larval Digenea. They were consistently heavily deposited in rediae of S. baccatus but but there is some doubt as to whether they represented a nutrient store or accumulation of waste products.

A full examination of lipid distribution in infected and uninfected glands throughout the season was not part of the present study. It would seem surprising, however, if, in the absence of large amounts of glycogen in the immediate vicinity of the rediae, they did not directly utilise the abundant fats.

Cyclical changes in the molluscan host metabolism

Cyclic physiological changes are known to occur in molluscs and are related to environmental factors and reproductive activity. The changes involve the relative quantities of stored nutrients in mollusc tissues. The operation of a carbohydrate/lipid storage cycle in marine bivalves was reviewed by Gabbott (1975); the cycle consisted of a depletion of the stored glycogen reserves of the digestive gland and a corresponding increase in the lipid content of developing eggs. There is a minimum amount of food reserve in the non-gonad tissue just prior to spawning and the food reserves are then built up again after spawning; this may coincide with the period of maximum food availability. This is then followed

by a slow decline in food reserves beginning with the onset of gametogenesis and culminating in the minimum prior to spawning. This very generalised pattern is sometimes complicated by periods of winter dormancy and possibly other factors. Webber (1970) found a similar pattern in the abalone, Haliotis sp. where the greatest carbohydrate loss was from the foot whereas the greatest lipid loss was from the digestive gland.

The lipid changes in the digestive gland of Tresus capax (Gould) was described by Reid (1969) as relatively constant, falling only from 13 to 8% in the winter; he did not measure the foot lipid. The only study on seasonal changes in food reserve of a marine prosobranch is that of Robson & Williams (1971) on Littorina littorea and a similar pattern of pronounced seasonal changes which were directly related to gonad development and spawning was found. The minimum amount of glycogen found co-incided with the mature spawning condition. Loss of glycogen occurred in the digestive gland as well as the foot.

It has been shown that the digestive gland epithelial cells of B. undatum are not important in the storage of glycogen and it seems more probable that the metabolism of this carnivorous prosobranch is more lipid oriented. Whatever their nature, it seems more than likely that B. undatum undergoes seasonal fluctuations in nutrient reserves, as occurs in other molluscs.

Spawning in B. undatum is said to occur in the latter part of the winter in Plymouth (Thorson G 1946). This is supported by the figures obtained for penis length in this study. Table VI (page 35) shows that the mean length obtained in October is double that for May, reflecting the development of this organ which parellels

gametogenesis. Gabbot (1975) stated that the cyclic nature of gametogenesis and the cycle of storage and utilisation of reserves were more pronounced in northern populations. It would therefore be expected that food reserves of B. undatum would start to run down in the summer to a minimum in the winter and would subsequently build up through the spring and early summer.

The available energy in infected B. undatum was inadequate since it was observed that the gonads of the infected molluscs failed to develop at the same rate as those of the uninfected. Gabbot & Bayne (1973) found that in Mytilus edulis L. the body reserves were used for gonad development as well as energy metabolism when the food available is less than the maintenance requirement. Some evidence of starvation in B. undatum was recorded in July/August where there was a shrinkage of collumnar cells.

The evidence, therefore, does indicate that there is a depletion in the food reserve of the mollusc which coincides with the loss of infectivity of the cercariae. This would also explain the variability in the glycogen content of the tails of mature cercariae found in the digestive glands of the later season. The loss of infectivity of cercariae in the middle and late summer could therefore be related to endogenous physiological changes in B. undatum in response to environmental changes in the habitat occupied by the host.

Erasmus (1972) discusses the factors affecting cercarial emergence and regrets that in many studies the physiology of the snail is ignored since this represents such a particularly

close host/parasite relationship that variations in the physiology of one are bound to affect the other and both are dependent on environmental variables.

The infection of B. undatum with S. baccatus is clearly a case where a greater knowledge of the host's physiology will shed more light on the factors affecting the maturity and infectivity of cercariae.

PART 2 THE SECOND INTERMEDIATE HOST

A. Aspects of the biology of infection in the fish intermediate host.

B. Tissue responses to invasion and encystment in some species of Heterosomata.

A. RESULTS OF EXPERIMENTAL INFECTIONS WITH S. BACCATUS

Experimental infections of a group of mixed flatfish were attempted at the end of June 1973. The fish were killed and examined after three months for cysts of S. baccatus. The number of cysts found in each fish was recorded in Table VII. The sizes ranged from 7.3 to 12.1 cm.

TABLE VII Numbers of metacercariae recovered from I-group fish infected with S. baccatus cercariae.

Fish	No. of cysts	Fish	No. of cysts	Fish	No. of cysts
P. platessa 1	19	S. maximus 1	0	S. rhombus 1	0
" 2	3	" 2	0		
" 3	2	" 3	0		
" 4	6	" 4	0		
" 5	10	" 5	0		
Mean	8		0		0

Table VII shows that only the P. platessa became infected and no S. maximus or S. rhombus were infected. There was, overall, a much smaller number of cysts than was expected. This experiment, together with other evidence of experimental S. baccatus infections which failed to produce large numbers of metacercariae (Sommerville, unpublished) suggested that a seasonal cycle is involved in the infection of the first intermediate host B. undatum with S. baccatus and consequently infections of the second intermediate host.

Further experiments were carried out in order to investigate this possibility.

1. SEASONAL INFECTIONS

All fish used in the seasonal experiments were I-group flatfish and were obtained in April and May from a variety of sources. They were maintained in constant conditions in the aquarium of DAF's Marine Laboratory, Aberdeen, at 12°C. Fish selected for each of the monthly infection experiments always included some of the largest and smallest of the stock.

The size ranges were as follows:

P. platessa	7.5 - 18.0 cm
S. maximus	5.4 - 16.7 cm
S. rhombus	9.5 - 15.1 cm
Platichthys flesus	9.4 - 14.8 cm

Samples of Buccinum undatum containing individuals infected with S. baccatus were obtained at monthly intervals from the same source. Each experimental infection was identical.

The timing of the infections was largely controlled by the availability of B. undatum from the fishermen of Loch Ewe and were as follows:-

	B. undatum collection;	Experimental infection
1.	29 May	31 May
2.	29 June	3 July
3.	29 July	1 August
4.	4 September	No infections

It was hoped that infections could be carried out earlier in the spring but bad weather prevented the fishermen from setting creels and some of the earlier April and May samples of B. undatum died from transportation or temperature stress. Only half the

infected digestive gland was used in each case, and experiments were as far as possible identical.

The results of the seasonal infections can be seen in Table VIII

TABLE VIII Number of metacercarial cysts of S. baccatus recovered from infected hosts at monthly intervals.

Fish sp.	Number of Cysts			
	31 May	3 July	1 August(A)	1 August (B)
P. platessa	601 319 166 941 180 <1000	3 30 1 2 0	15 4 21 21 9	67 5 6 42 29
Mean per fish	534.5	7.2	14.0	29.8
S. maximus	<1000 <1000 540 718 184 143 396	3 17 16 1 10 1	6 5 5 3 1	16 12 29 93 10
Mean per fish	568.7	8.0	4.0	32.0
P. flesus	4 515	0		
S. rhombus	145	0 0 13	5 6	2 5
Mean per fish			5.5	3.5
M. kitt		0		

Clearly, there is a considerable reduction in the numbers of metacercariae recovered from experimental infections carried out after 31st May in all the fish hosts employed.

The mean number of metacercariae found in the August infection was slightly greater than the mean of the July infections but this was probably a result of the low numbers involved.

There were also small differences between individual molluscan infections. For example, the infections of the August experiment A and B were identical except that the S. baccatus cercariae came from two separate individuals and serve to illustrate the difference between two molluscan infections. However, these differences are small and of the same order, whereas those of May range from 143 to greater than 1,000 metacercariae.

Estimates of numbers of cercariae were made for each infection but it was felt that the use of these numbers was invalid because the cercariae did not emerge naturally and, therefore, mature and immature cercariae would be counted together.

2. INFECTIVITY OF AGED CERCARIAE

Procedure

An infected Buccinum undatum digestive gland was divided into two equal parts. Each part was finely teased in seawater in order to release the cercariae. One half was used to infect a group of mixed species of fish hosts and the other half was kept in a beaker of seawater at a constant temperature of 12°C for 10 hours. After 10 hours it was used to infect a tank of fish. Each experiment was identical except that the cercariae in the first tank were freshly released (0 hours) and in the second tank the cercariae had been aged for 10 hours.

The fish, all of which were I-group, were maintained in identical conditions for 2 months when they were killed and examined for metacercarial cysts.

The results of these infections can be seen in Table IX.

TABLE IX Numbers of metacercariae recovered from I-group fish infected with freshly emerged and aged cercariae of S. baccatus

Experiment	P. platessa length cm.	Cyst No	S. maximus length cm.	Cyst No	P. flesus length cm.	Cyst No	S. rhombus length cm.	Cyst No
31 May 0 Hours	14.5	601	10.2	718	14.8	4	10.8	145
	8.0	319	9.0	540	9.4	515		
	7.5	166	12.5	143				
	8.4	941	11.3	184				
	10.5	180	13.3	396				
	8.1	<1000	5.4	<1000				
	5.8	<1000	6.8	<1000				
MEAN	9.0 cm	601	9.8 cm	568:7				
31 May 10 Hours	6.7	20	7.5	16	14.0	0	10.8	1
	6.8	103	8.5	76	8.9	9		
	7.9	30	13.6	8				
	7.1	7	11.8	15				
	13.0	19	10.9	57				
	9.1	2	6.8	19				
	9.0	26	8.7	14				
MEAN	8.5 cm	18:5	9.7 cm	5:7				

Table IX shows quite clearly that aging the cercariae for 10 hours reduces their infectivity considerably.

Small numbers of cercariae were mounted on slides at 0 and 10 hours. These were stained with Best's carmine which is specific for glycogen. While most of the cercariae at 0 hours had strongly positive carmine staining in their tails, those stained after 10 hours showed very little carmine staining and most of them contained no glycogen in their tails at all.

3. VARIATION BETWEEN HOST SPECIES

3.1 Intensity of infection

The number of metacercarial cysts recovered from each species of fish host is recorded in Table VIII. The intensity of infection in S. maximus and P. platessa was comparable in each of the experimental infections, i.e. P. platessa and S. maximus showed more or less equal chances of becoming infected with S. baccatus. S. rhombus was also susceptible to infection, although there were not enough of this species to compare the intensity of infection with P. platessa and S. maximus. Platichthys flesus was found to be susceptible to infection but, again, the number of individuals was too small to allow comparison with other species. The P. flesus in Table VIII, May infection, which had 515 cysts was small, only 9.4 cm., while the P. flesus with 4 cysts was 14.8 cm. The number of S. baccatus cercariae infecting the P. flesus of 9.4 cm. was comparable with P. platessa and S. maximus of similar size, i.e. 940 and 540 respectively. However, a P. platessa of 14.5 cm. had 601 metacercariae and an S. maximus of 13.3 cm. had 396 metacercariae. The small number of cysts found in the P. flesus of 14.8 cm. may be a chance occurrence or the difference in susceptibility of the larger P. flesus may be a reflection of changes which occur with increase in size in this particular species.

An attempt was made to infect specimens of Microstomus kitt, but only two fish were available in the June/July infection and no cysts were recovered from either of these specimens.

3.2 Cyst distribution in the second intermediate host

Recordings were made of the position of each cyst in every fish examined.

Table X shows the percentage of cysts found in the fins and body of S. maximus and P. platessa. The figures are the means of the percentages for the individual fish rather than mean numbers of cysts because this avoids bias caused by particularly heavily infected fish.

TABLE X Distribution of cysts on the fins and body.

Fish	Fins	Body
P. platessa (22 fish)	16%	84%
S. maximus (22 fish)	53%	47%

The table shows that there was a small proportion of cysts on the fins of P. platessa than was found in S. maximus. Assuming that both species of fish have the same fin/body area ratios, a statistical test (Appendix p200) showed that there was a significant difference between the percentage of cysts found on the fins of these I-group P. platessa and S. maximus. This difference was signficant at the 0.1% level. The same is, therefore, true of the cysts on the body of both species.

The body cysts were further divided into upper and lower sides of the body, (the upper body is the eyed side) and these are recorded in Table XI.

TABLE XI Distribution of cysts on the Upper and Lower body (means of the percentages for individual fish)

Fish	Upper Body	Lower Body
P. platessa (15 fish)	28%	72%
S. maximus (9 fish)	93%	7%

Table XI shows that, of the cysts found on the body of P. platessa, by far the majority occurred on the lower surface, whereas for S. maximus the reverse was true. The difference between the two species was significant at the 0.1% level.

The figures were obtained from the data of both May and August infections. These data are separated in Table XII, which shows that there was little difference in the distribution of cysts between the upper and lower body of S. maximus in May and August. Statistically, this is not significant at the 5% level. However, with P. platessa a distinct bias towards the lower side was seen in August which was less apparent in May. The difference in the distribution of cysts in P. platessa between May and August was significant at the 1% level (Appendix p.200).

TABLE XII Showing seasonal distribution of metacercarial cysts (means of the percentages for individual fish)

Fish Species	Month	Upper Body	Lower Body
P. platessa	May (5 fish)	47%	53%
	August (10 fish)	19%	81%
S. maximus	May (5 fish)	89%	11%
	August (4 fish)	98%	2%

The position of the cysts on the body was recorded and cysts were found to occur either in the skin or muscle. Occasionally cysts were found in the visceral cavity and in the eyes. Muscle cyst locations were separated into somatal muscles and pterygiophoral muscles as MacKenzie (1968) found that the greatest number of cysts occurred in the pterygiophoral muscles in 0-group P. platessa.

Table XIII shows that by far the majority of the P. platessa cysts on the body occur in the muscles and fewer cysts occur in the skin. The somatal muscles were more frequently occupied than the pterygiophoral muscles.

TABLE XIII Distribution of cysts on the body of S. maximus and P. platessa

Fish	Total Muscle	Somatic Muscle	Pterygiophoral Muscle	Skin
P. platessa (15 fish)	89%	57%	32%	12%
S. maximus (15 fish)	21%	10%	11%	79%

In S. maximus, on the other hand, cysts occurred largely in the skin and, the 21% which found their way into the muscle were divided more or less equally between the somatal and the pterygiophoral muscles. The difference in distribution of cysts on the body between the two fish species was significant at the 0.1% level using a chi-square test on the actual numbers of cysts. (Appendix p.201).

This distribution in S. maximus was consistent when the May and August infections were examined but P. platessa showed a difference in distribution in May and August infections as seen in Table XIV.

TABLE XIV Seasonal distribution of metacercariae on the body of P. platessa and S. maximus

Fish	Month	Somatal Muscle	Pterygiophoral Muscle	Skin
P. platessa	May (5 fish)	31%	37%	32%
	August (10 fish)	69%	30%	2%
S. maximus	May (5 fish)	14%	10%	76%
	August (4 fish)	5%	13%	82%

The somatal muscles, pterygiophoral muscles and skin of P. platessa shared a similar burden of cysts in May whereas in August there appeared to be a greater number of cysts in the somatal muscles and only 2% in the skin.

A chi-square test performed on the total numbers of cysts in May and in August showed that the difference in distribution between the two months was very significant in the case of P. platessa (0.1% level) whereas in S. maximus the difference was less significant (1% level).

In summary, S. baccatus was generally found in the lower body muscles in P. platessa and, in August, there was an unequal distribution of the cysts in the body muscles such that, the somatal muscles of the lower body were more heavily infected than the pterygiophoral muscles.

In S. maximus, as many S. baccatus were found on the fins as on the body but, of those on the body, the skin on the upper surface was more heavily parasitized.

3.3 Size of Cysts

Examination of second intermediate host species for metacercarial cysts of S. baccatus revealed that cysts dissected from

different host species varied in a number of features. The most obvious was that of size. The cysts from P. platessa and P. flesus were large and the larva easily excised from the thin parasite cyst membrane. Those from S. maximus and S. rhombus, however, were very small and sometimes barely visible except for the pigmented eyespots, which were very prominent. The larvae also lacked the large, white opaque excretory bladder which was very characteristic of the larvae seen in P. platessa. The metacercariae from S. maximus could not be removed from the parasite cyst and the host capsule and parasite cyst could not be separated. The appearance of many of these minute cysts suggested that the cercaria within was dead.

Measurements were made of the dimensions of the cysts in both P. platessa and S. maximus in order to compare the relative sizes for the same age after infection. In the case of S. maximus, the cysts were difficult to measure since the margin of the parasite cyst and the inner host capsule edge were difficult to distinguish. In some cases, therefore, the size of the cysts from S. maximus may be over-estimated.

The cysts which appeared to be dead were not measured.

Table XV shows the mean dimensions of cysts from P. platessa and S. maximus individuals at time intervals post infection.

All measurements of living cysts were made in water and without coverslip pressure.

TABLE XV Mean dimensions of cysts from P. platessa and S. maximus (in microns)

P. platessa			S. maximus		
Age in Days	Mean Lengths	Mean Widths	Age in Days	Mean Lengths	Mean Widths
27	347.7 (49)	318.8	26	270.6 (24)	241.7
46	429.0 (28)	391.6	42	219.1 (24)	202.5
54	442.1 (43)	403.6	50	400.0 (18)	353.2
55	451.2 (48)	416.7			
60	618.5 (14)	553.0	60	176.5 (7)	158.0
60	470.6 (3)	427.5	60	317.7 (15)	309.0
60	750.7 (26)	710.0	60	180.9 (8)	166.2
60	800.0 (15)	758.8	60	188.2 (4)	179.4
			60	202.4 (5)	169.4
			60	307.0 (11)	282.4
Mean of 60 day old cysts	756.1 N=55	713.0		249.3 N=50	232.5

(Figures in brackets indicate the number of individual cysts measured.)

From Table XV it can be seen that differences are apparent in the size of the cysts from S. maximus and P. platessa even at the earliest measurements at 26 and 27 days. The difference increases and at 60 days the cysts from S. maximus are only one third of the size of cysts from P. platessa.

A comparison of the mean lengths of cysts for individual fish at 60 days showed that the length of cysts from P. platessa differ significantly (0.1% level) from the lengths of cysts from S. maximus.

DISCUSSION

1. Seasonal Infections

The experimental infections carried out from May to September support the hypothesis that there is a defined period during the year when the majority of infection of the second intermediate hosts by S. baccatus occur. The greatest infectivity of cercariae was in the May infections and the infectivity was thereafter reduced until September when the larval stages in the molluscan host are lost. This hypothesis is supported by the work of MacKenzie & Gibson (1970), who showed that there was an enormous increase in the incidence of infection of S. baccatus in 0-group P. platessa at this time. MacKenzie & Gibson collected P. platessa for their study from the P. platessa nursery grounds in Loch Ewe, i.e. the same source as for B. undatum in this study. They found that the majority of infections in P. platessa occurred during the first two or three months after metamorphosis in May, June and early July. The reason for this, therefore, seems to be the loss of infectivity of the cercariae in the period following this peak and the absence of any cercariae in the environment after September.

There was no apparent reduction in the numbers of cercariae found in the molluscan infections from July onwards. This was a surprising feature since it might be expected that there would be a gradual decline in the number of cercariae produced to account for the reduced number of cysts found in the intermediate hosts in July and August. No assessment could be made of the state of maturity of cercariae upon dissection and it may have been that the majority of them were immature. This aspect is investigated histologically in the next section.

Seasonal infections of larval digeneans have been shown to occur in the closely related mollusc, Nassarius obsoletus Say Miller & Northup (1926) and McDaniel & Coggins (1972). Wolfgang (1954b) however, collected molluscs infected with S. baccatus from June to September but, as far as can be ascertained from his work, experimental infections were made in June when his major collections of infected molluscs were made. MacKenzie & Liversidge (1975) also made their major collections in June and experimental infections were set up at this time. Variations from year to year may occur, depending on climatic conditions and temperature, but there is no evidence that infections occur outwith this early summer period.

2. Infectivity of Aged Cercariae

The effect of aging cercariae for 10 hours had a dramatic effect on their infectivity when subsequently exposed to the host under identical conditions. In S. maximus, the number of successful cercariae was 3,981 when freshly released from the mollusc compared with 205 after having aged for 10 hours. In P. platessa these numbers were 4,207 and 207 respectively. All the cercariae were from the same host and these differences, therefore, could not be a result of variation in the state of maturity of the cercariae.

These results support those of MacKenzie & Liversidge (1975) who aged cercariae for 11 and 22 hours and found a reduced number of metacercariae became established. Their experimental infections differed in that they did not use the same molluscan digestive gland for the infections at 0 hours and those with aged cercariae

and their figures were not, therefore, directly comparable. They also used fish from a variety of age groups whereas, in this study, all the fish were One-group. The same authors suggested that the decline of infectivity with age may be due to the reduction in the cercarial glycogen reserves. This was confirmed by the staining of representative samples of aged cercariae with specific glycogen staining techniques. Erasmus (1958) also demonstrated that the caudal bodies of Cercaria X Baylis were negative for iodine 24 hours after emergence and concluded that these reserves were utilised during the free swimming period. Miller & McCoy (1930) showed that the infectivity of Cercaria floridensis McCoy was maintained until 11 hours after emergence and it was not until after this time that infectivity gradually dropped until 35 - 38 hours, approximately 13% per hour. They saw active, but tailless cercariae at this time and assumed that it was their ability to reach a host which was reduced rather than their ability to penetrate it. Cercariae of S. baccatus do have considerable amounts of glycogen in their bodies as well as in their tails (see Part 1 B) and it is conceivable that these reserves are utilized in penetration, whereas the tail glycogen contribution may be solely for the purpose of host location. This could probably be tested by setting up infections after inducing tail loss in freshly emerged cercariae.

The loss of infectivity at this stage in the life of the cercariae seems surprising considering that the maximum life-span at 16 - 18°C is about 72 hours (Wolfgang, 1955a). Anderson & Whitfield (1975) showed that the effective time during which Transversotrema patialensis (Soparker) can infect Brachydanio

revi Hamilton-Buchanan is only about half the maximum life-span of the cercarial stage. The time span during which the cercariae were infective closely correlated with the period during which they exhibited active swimming behaviour. Bryant & Williams (1962) showed that the presence of glucose in the external medium resulted in increased longevity of the miracidium of Fasciola hepatica. It would be interesting to attempt to increase the longevity of the cercariae by ageing them in glucose solution. Infections could be set up using cercariae of artificially increased longevity to see what effect this might have on infectivity.

The evidence from these experiments does indicate the close biological association necessary between the first and second intermediate hosts of S. baccatus for the successful promotion of the life cycle.

3. Variations between Host Species

3.1 Intensity of Infection

There was no difference to be found between the intensities of infection in S. maximus and P. platessa and, although there were fewer numbers of other species the intensity of infection in S. rhombus and P. flesus fell within the range for the other two species. S. baccatus infection has not previously been reported from P. flesus and MacKenzie & Liversidge (1975) were unable to infect large P. flesus. MacKenzie & Gibson (1970) suggest that this may be due to the fact that P. flesus are usually found in fresh or brackish water at the time when 0-group

P. platessa become infected. This study supports the hypothesis that the lack of natural infection in P. flesus is a result of ecological separation rather than host resistance. S. solea has not previously been reported as a host for S. baccatus and this species was infected successfully in later experiments (see Part 2 B). Attempts to infect M. kitt failed and this may be because of the large amounts of mucus covering skin. However, the only specimens available were large (19.3 and 24.5 cm.) and it would have been interesting to try to infect small M. kitt of comparable size to those of the other species used. Lebour (1908) reported S. baccatus in M. kitt. Wolfgang (1954a) was able to report infection in a further six species of flatfish and it seems that cercariae of S. baccatus are able to penetrate a large number of species of Heterosomata.

Table XVI summarises the reports of S. baccatus infections in different species of intermediate hosts.

TABLE XVI Intermediate host range of S. baccatus based on previous reports and the present study

Plaice	Pleuronectes platessa	Kroyer	(1838-53)
		Dujardin	(1845)
		Matthews	(1968)
		MacKenzie	(1970)
		MacKenzie & Gibson	(1970)
		MacKenzie & Liversidge	(1975)
		Present study	
	P. pallasii	Zhukov	(1963)
Winter flounder	Pseudopleuronectes americanus	Stafford	(1904)
		Wolfgang	(1954a)
Common Dab	Limanda limanda	Olsson	(1868)
		Johnstone	(1905)
		Lebour	(1908a,b)
		Nicoll & Small	(1909)
		MacKenzie & Gibson	(1970)
		Present study	
Yellowtail	L. ferruginea	Wolfgang	(1954a)
		Ronald	(1960a)
Turbot	Scophthalmus maximus	Kroyer	(1838-53)
		Dujardin	(1845)
		Present study	
Windowpane	S. aquosus	Wolfgang	(1954a)
		Ronald	(1960b)
	Pseudorhombus pentophthalmus	Yamaguti	(1937)
Long rough dab	Hippoglossus plattessoides	Lebour	(1908a,b)
		Huntsman	(1902)
		Nicoll	(1910)
		Wolfgang	(1954a)
Flathead sole	H. elassodon	Strelkov	(1960)
Witch	Glyptocephalus cynoglossus	Johnstone	(1905)
		Wolfgang	(1954a)
		Ronald	(1960b)
Smooth flounder	Liopsetta putnami	Wolfgang	(1954a)
Lemon sole	Microstomus kitt	Lebour	(1908a,b)
Dover sole	Solea solea	Present study	
Flounder	Platychthes flesus	Present study	
Brill	S. rhombus	Present study	

3.2 Cyst Distribution

The majority of the cysts found in I-group P. platessa occurred in the tissues of the body rather than in the fins. However, the proportion of cysts on the fins might be expected to increase with age as shown by MacKenzie & Liversidge (1975). Wolfgang (1955b) and MacKenzie & Gibson (1970) found a greater proportion of cyts on the left or blind side of naturally infected P. americanus and P. platessa respectively. This was also found to be the case in experimental infections of P. platessa in the present study. However, this heavier burden on the lower or blind side of the body seemed to be more marked in infections later in the season (August). MacKenzie & Liversidge (1975) suggested that the asymmetrical distribution of cysts may be due to differences in the thickness of the skin and scale organisation on upper and lower sides of the fish. Histological examination of P. platessa and other species infected with S. baccatus suggest that the upper skin does not show any features which make it more difficult to penetrate than the lower skin. Cercariae appeared to penetrate with ease skins which were more liberally scaled than that of P. platessa, e.g. Limanda limanda (Part 2 B). There is, as yet, no satisfactory explanation for this phenomenon but a study of the behaviour of both cercariae and host might provide more evidence.

The findings for S. maximus were the reverse of those for P. platessa; that is, the majority of the cercariae were found to penetrate the fins of S. maximus and, of the cercariae which penetrated the tissues of the body, by far the greater proportion was on the upper or eyed side. After metamorphosis the eyes of

S. maximus migrate to the left side whereas in P. platessa the eyes migrate to the right side. The majority of the metacercariae on the body of both species occurred on the left side. This may, however, be coincidence as there is no known physiological difference between the left and right sides of fish generally and it seems much more likely to be a result of behavioural differences.

Edwards & Steele (1968) have shown that the main constituent of the diet of wild 0-group P. platessa in Loch Ewe is the siphon of the sand-burrowing bivalve Tellina tenius Da Costs indicating, perhaps, that they feed predominently from the sea bottom. Jones (1973) studied food organisms of young S. maximus from Borth Bay, Wales, and found mysids and polychaetes to be important, although a great variety of food was taken. It appears that S. maximus may be more mid-winter feeders and the differences in feeding behaviour may account for the differences in cyst distribution.

The two species again contrast in that the cysts of S. baccatus found in the body tissues of P. platessa occurred mainly in the somatal myotomes whereas those in S. maximus occurred mainly in the skin. MacKenzie (1971) found that the pterygiophoral muscles were the most common site of S. baccatus metacercariae in 0-group P. platessa.

Miller & McCoy (1930) found differences in the relative infection of the fins of Haemulon sciurus by Cercaria floridensis. McCoy and Evans & Mackiewicz (1958) found more metacercariae on the body than the fins of 35 species of fish and Erasmus (1972)

stated that Cercaria X penetrates at any point along the body of Gasterosterus sp. It is difficult to draw comparisons however, between the round fish of these authors and the flat-fish because their swimming behaviour and orientation are so different.

3.3 Cyst Size Differences

The size of cysts excised from S. rhombus and S. maximus were very small and measurements of cyts from S. maximus were only one third the size of those from P. platessa at 60 days. Wolfgang (1954b) noted that cysts in the fins of heavily infected Pseudo pleuronectes americanus were not as large as cysts from light infections and suggested that this size difference may have been a result of crowding. However, cysts in this study from P. platessa and S. maximus with similar intensities of infection were compared and the size difference was noted in both heavy and light infections. The size difference established in this study was thought to be a result of a fundamental difference in the development and potential viability of the metacercariae from P. platessa and S. maximus and this was investigated further as described in Part 2 B and Part 3 of this study.

B. TISSUE RESPONSES TO INVASION AND ENCYSTMENT OF STEPHANOCHASMUS BACCATUS IN THE SECOND INTERMEDIATE

All second intermediate hosts were I-group fish maintained at 12°C.

Fish were examined at regular intervals after infection but, for convenience, only those stages which show different features from the preceding ones will be described.

Certain terms are used variously by different authors. In this study the term 'paracyst' is used to denote the parasite and cyst of parasite origin. Encystment refers to the process of formation of the paracyst.

The term 'capsule' is used for the host cells formed around the parasite in the process of encapsulation by the host.

The term 'cyst' refers to the parasite, paracyst and host capsule altogether. The terminology of Heterosomatid blood cells will follow that established by Ellis (1976) and Ferguson (1976) and is based on comparisons made with mammalian blood cell form and function.

PLAICE Pleuronectes platessa (L)

Site

In the majority of cases the larvae examined were from the fins. Most typically these occurred adjacent to the fin rays at the proximal end of the fin. Less often larvae were examined from the dermis or connective tissue in the region of the pterygiophoral muscles. Only occasionally were they examined in the

somatal myotomes and their superficial tissues.

Tissue response after 6 hours: At 6 hours after infection larvae were seen in the epidermis and lying on the outer scales. By their extended shape they appeared to be still in the process of migration and low power observations of penetrating larvae showed that the cercariae migrated laterally after penetration (Figure 20). No obvious damage to the epidermis was seen which could definitely be attributed to the parasite. Since the larva migrates for some distance from the point of entry, it is possible that this point would not occur in the same histological section as the larva. The only change occasionally seen at this stage was the development of small plaques of acantholisis, which could have been caused by the passage of the parasite between the Malpighian cells with the consequent rupture of the desmosones. Some larvae at this time had fragments of a mucoid substance attached to the tegument which may have represented penetration gland secretions. There were no signs of haemorrhage in the tissues but numerous red blood cells were often seen lying over the outside skin surface directly above the parasite. (Figure 21) Since there was no evidence of haemorrhage in the tissues, it is likely that these RBC's were released from the fish during the post-mortem procedure. The reason for adhesion only to these particular sites is not clear but may be due to the increased "stickiness" of the fish skin as a result of either adhesive secretions of the cercariae or to changes in the mucus of the cuticle. There was no parasite cyst evident at this time.

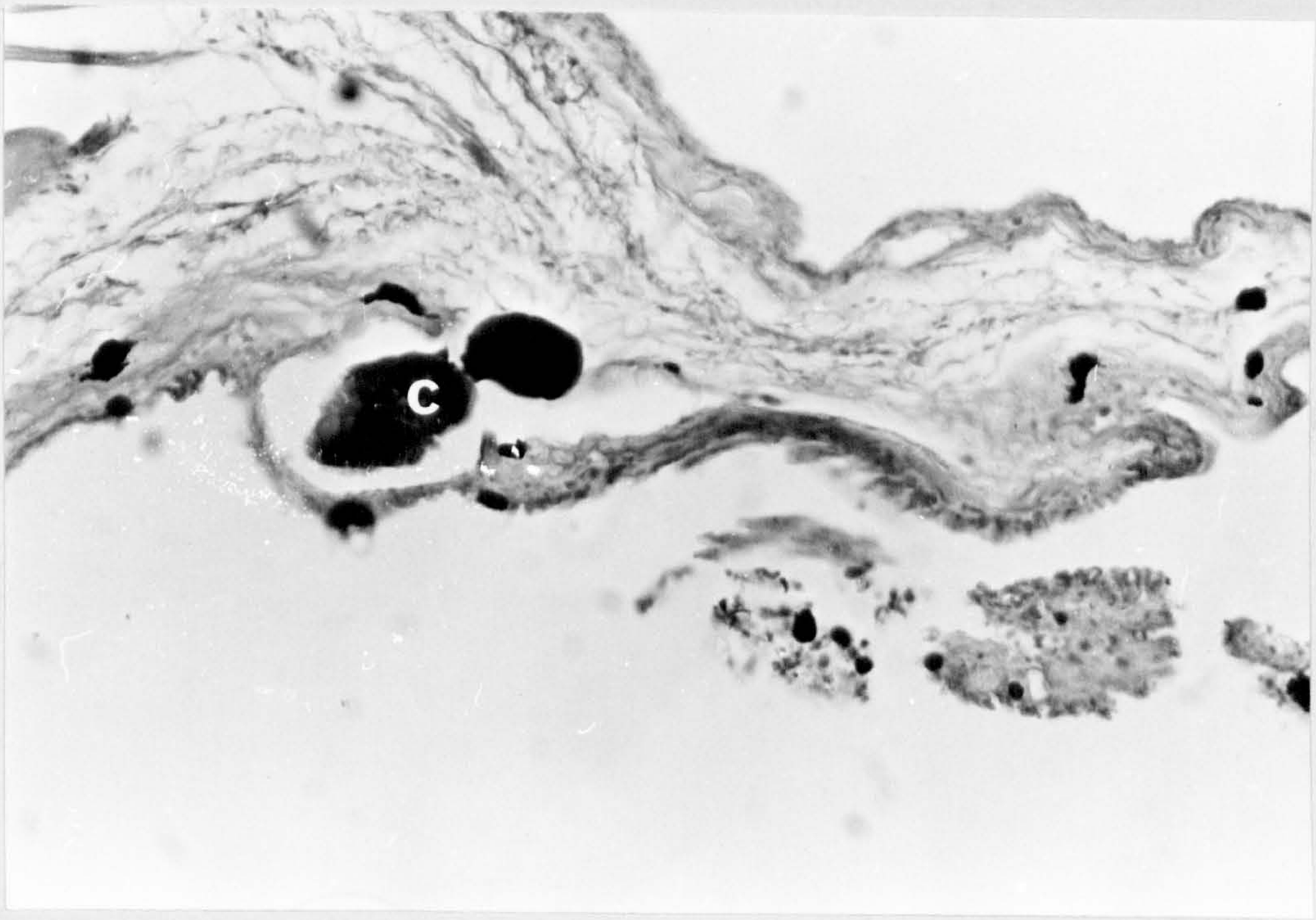
Tissue response at 19 hours - 24 hours: Many larvae were seen to be encysting, as evidenced by the presence of a thin

Figure 20. P. platessa 6 hours post infection showing a cercaria penetrating the basement membrane of the epidermis H.E. (x 250).

c : cercaria

Figure 21. P. platessa 6 hours post infection showing aggregation of red blood cells lying over the outside of the skin surface above the parasite M.S.B. (x 250).

rbc: red blood cells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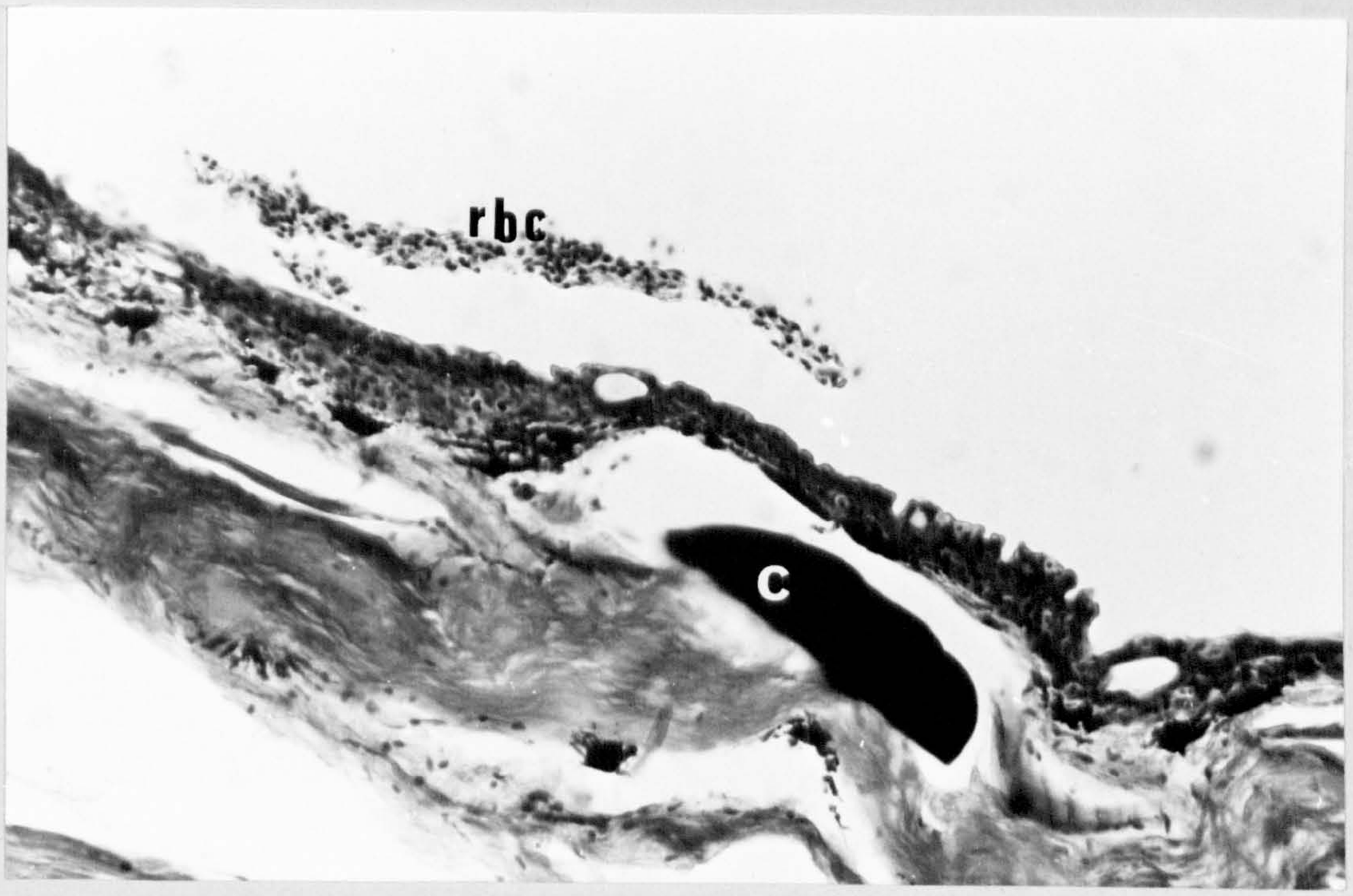

rbc
C

fragile membrane around the curled larvae. A few were still unencysted but encystment was complete by 48 hours. Larvae which had penetrated and subsequently migrated laterally along the stratum compactum tended to cause disruption of the connective tissue fibres and the superficial muscle.

Tissue response after 48 hours: There was no sign of inflammatory processes at 24 hours but by 48 hours there was evidence of an inflammatory cellular infiltrate. The inflammatory cells were mainly macrophages, some with pale and some with very strongly PAS positive granules. Individual cells could be seen penetrating the blood vessel walls and others were in the vicinity of the cyst. (Figure 22) A few cells were seen scattered along the paracyst and forming the beginning of the capsule. (Figure 23) These cells were larger, had no PAS positive granules and had an eccentric nucleus. They were idenfitied as tissue macrophages.

There was also an increase in size of the larva at this stage due to an incrĕase in diameter of the cells in the posterior region particularly those cells in the area around the excretory bladder. These cells usually contained a prominent vacuole which did not take up any stain. This appearance continued throughout the experiment. (Figure 23)

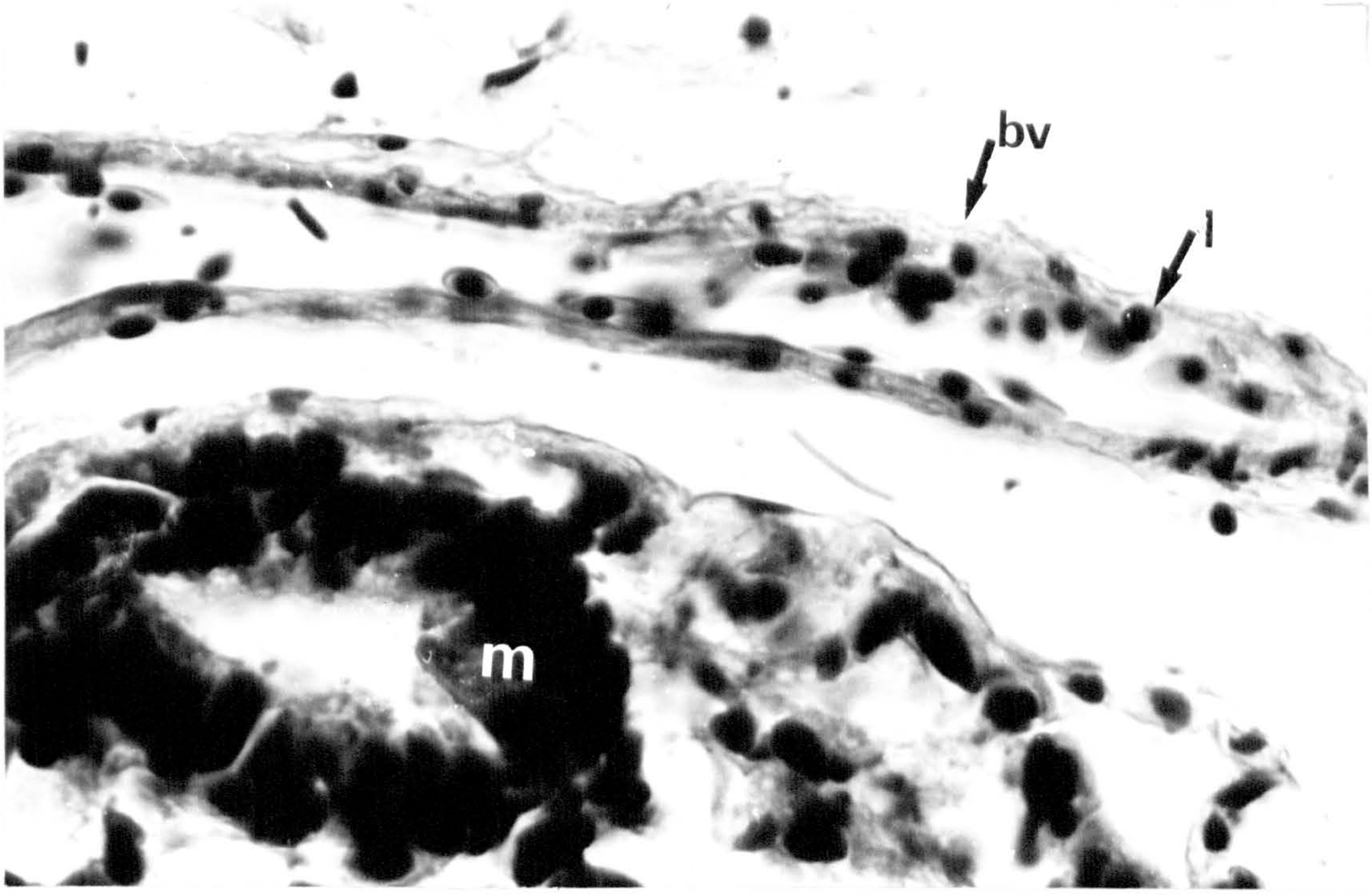
Tissue response after 125 hours: There was a distinct host capsule formed around the paracyst at this stage and the infiltration of macrophages into the area continued. These cells formed the innermost cellular layer of the host capsule which was one or two layers thick.

Figure 22. P. platessa 48 hours post infection shows leukocyte accumulation in a blood vessel adjacent to the metacercaria H.E. (x 1000)

bv : blood vessel
l : leukocyte
m : metacercaria

Figure 23. P. platessa 48 hours post infection showing the onset of diapedesis and the appearance of 'vacuoles' in the parenchymal cells of the larvae H.E. (x 400)

v : vacuole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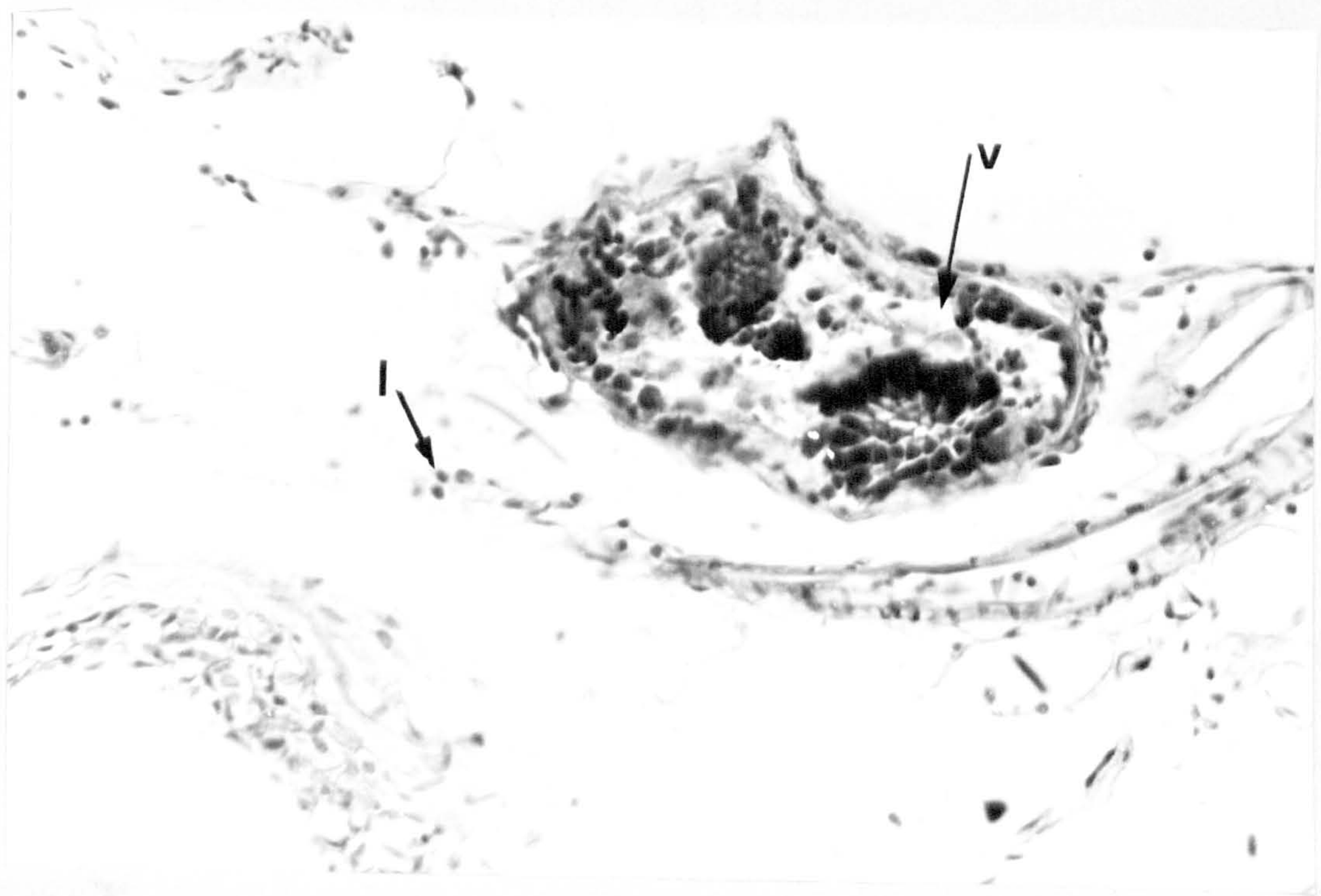
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l

Tissue response after 8 days: The inflammatory reaction by this time was very marked. There was a proliferation of fibroblasts in the vicinity of the cyst which appeared to be forming an outer cellular layer. (Figure 24)

The cells of the capsule were loosely packed and macrophages predominated. There was a conspicuous accumulation of inflammatory cells at the poles of the long axis of the cyst so that the ratio of the thickness of the polar capsule to the sides was 2:1. (Figure 24) There was no evidence of fibroplasia at this stage but a few lymphocytes were occasionally observed within the inflammatory tissue.

Tissue response after 11 days: The capsule appeared to be more compact from 11 days onwards and the inner layer of cells showed signs of degenerative change. The fibroblasts in the outer layer of the cyst had begun to polymerize collagen locally and large macrophages were more evident in this area. (Figure 25)

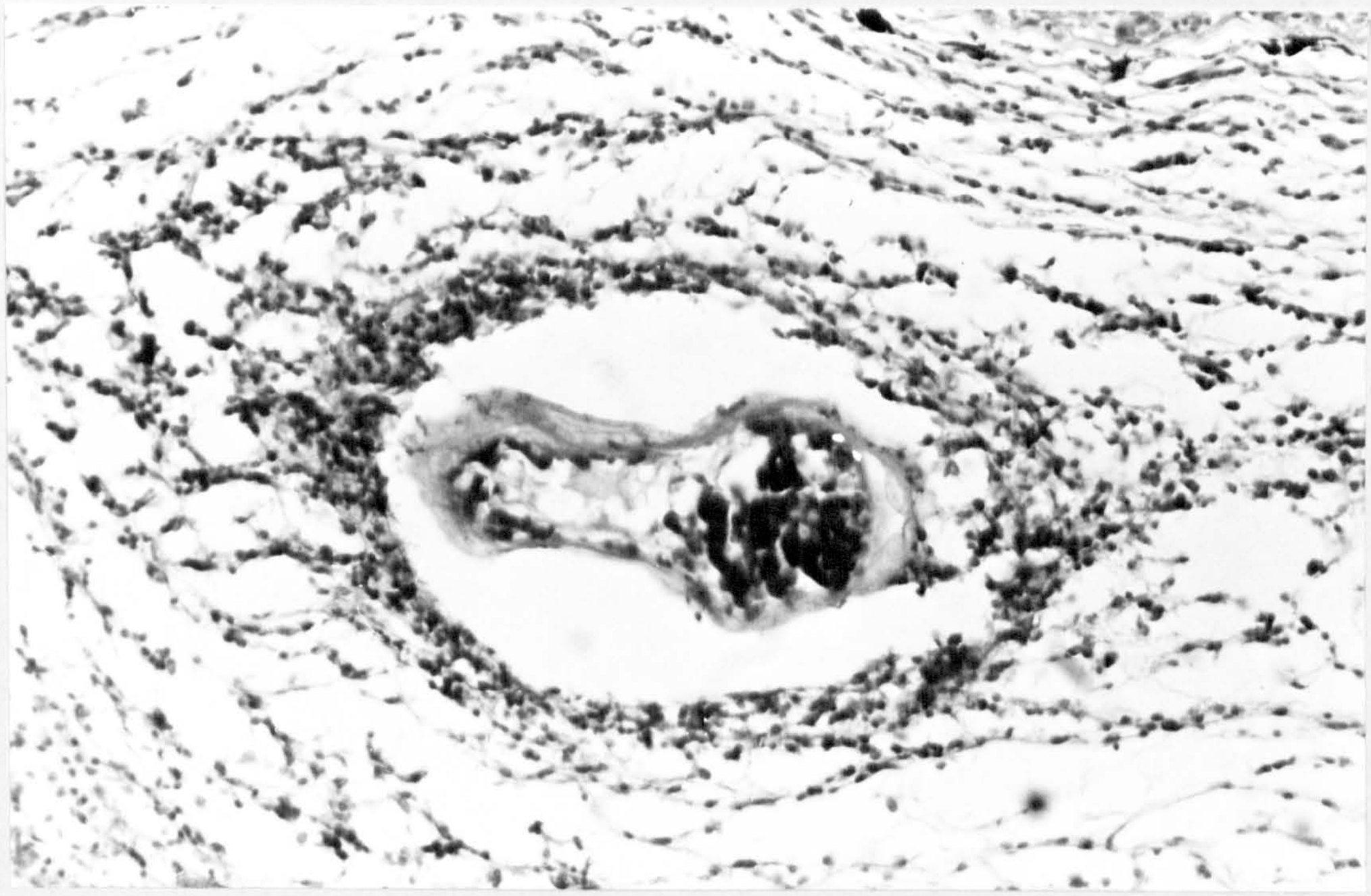
Tissue response after 13 days: At this stage the capsule became more compact and the innermost layer continued to degenerate further so that in some sections it appeared as a dense ground substance containing pyknotic and karyorhectic nuclei. The component marcophages of the second layer became epithelioid and in some instances these macrophages took on a foamy appearance. In other parts individual epithelioid cells fused to form a syncitium.

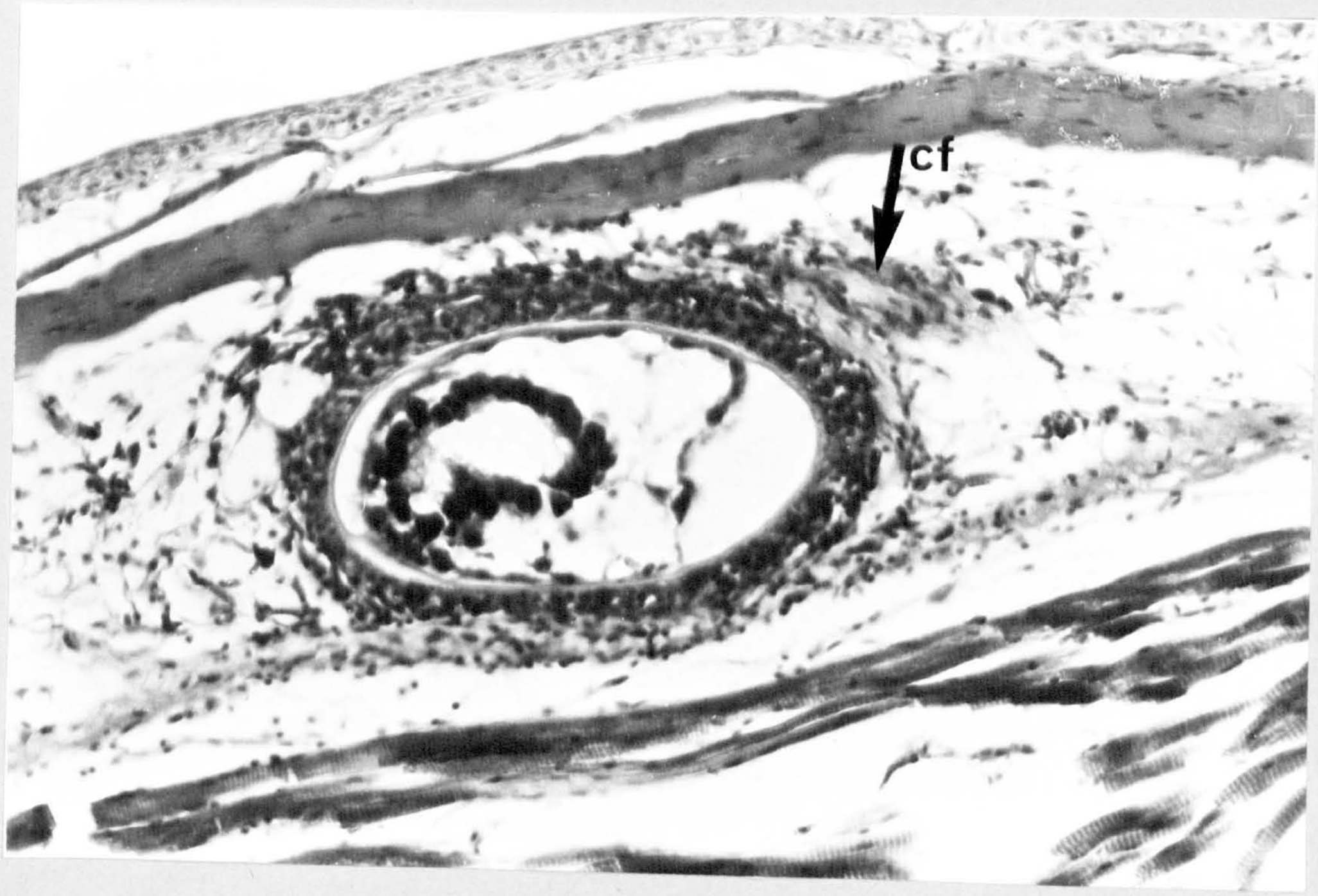
The outermost layer now consisted of elongated fibroblasts in concentric layers around the cyst interspersed between the strands of their fibrous secretion and thus forming a discrete boundary layer.

Figure 24. P. platessa 8 days post infection showing the massive proliferation of fibroblasts in the vicinity of the cyst. Note the greater accumulation of cells at the poles of the long axis of the cyst H.E. (x 250)

Figure 25. P. platessa 11 days post infection showing a well defined, compact capsule and the appearance of collagen fibres in the outer layers M.S.B. (x 250)

cf : collagen fibres

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Tissue response at 16 days: During this period the epithelioid layer became more marked and contained small foci of melanin. The innermost layer of degenerating cells was clearly demarcated and was about 4 mu in thickness, more acidophilic and less cellular. (Figure 26)

Tissues response at 23 days: The picture at this time was dominated by the extensive degeneration of epithelioid tissue in the middle and inner parts of the capsule. (Figure 27) This was difficult to explain, certainly there was no evidence that it was a reaction to a cell mediated response as lymphocytes were rare in the tissues.

Tissue response after 28 days: The capsule had taken on the form of an extensive chronic granuloma with the appearance at this stage of giant cells. The giant cells were on average 20 x 12 mu and varied in form with both Langhan's and foreign body types occurring. (Figure 28) These giant cells appeared to be formed from the most recent macrophages situated in the outer capsule which otherwise consisted largely of fibroblasts (Figure 29)

The other additional feature at this stage was the vascular infiltration of the outer capsule. In many cases it was difficult to decide if such vessels were newly formed since the fin connective tissue was already well endowed with blood vessels and capillaries. The new capillaries were evident in the part of the capsule abutting the dermis. The dermis, of this area, especially the stratum compactum, was much more cellular than normal.

Figure 26. P. platessa 16 days post infection shows a section through the host capsule in which the innermost layer consists of degenerating cells. The epithelioid tissue of the middle layer shows some vacuolation at its centre H.E. (x 400)

dc : degenerating cells

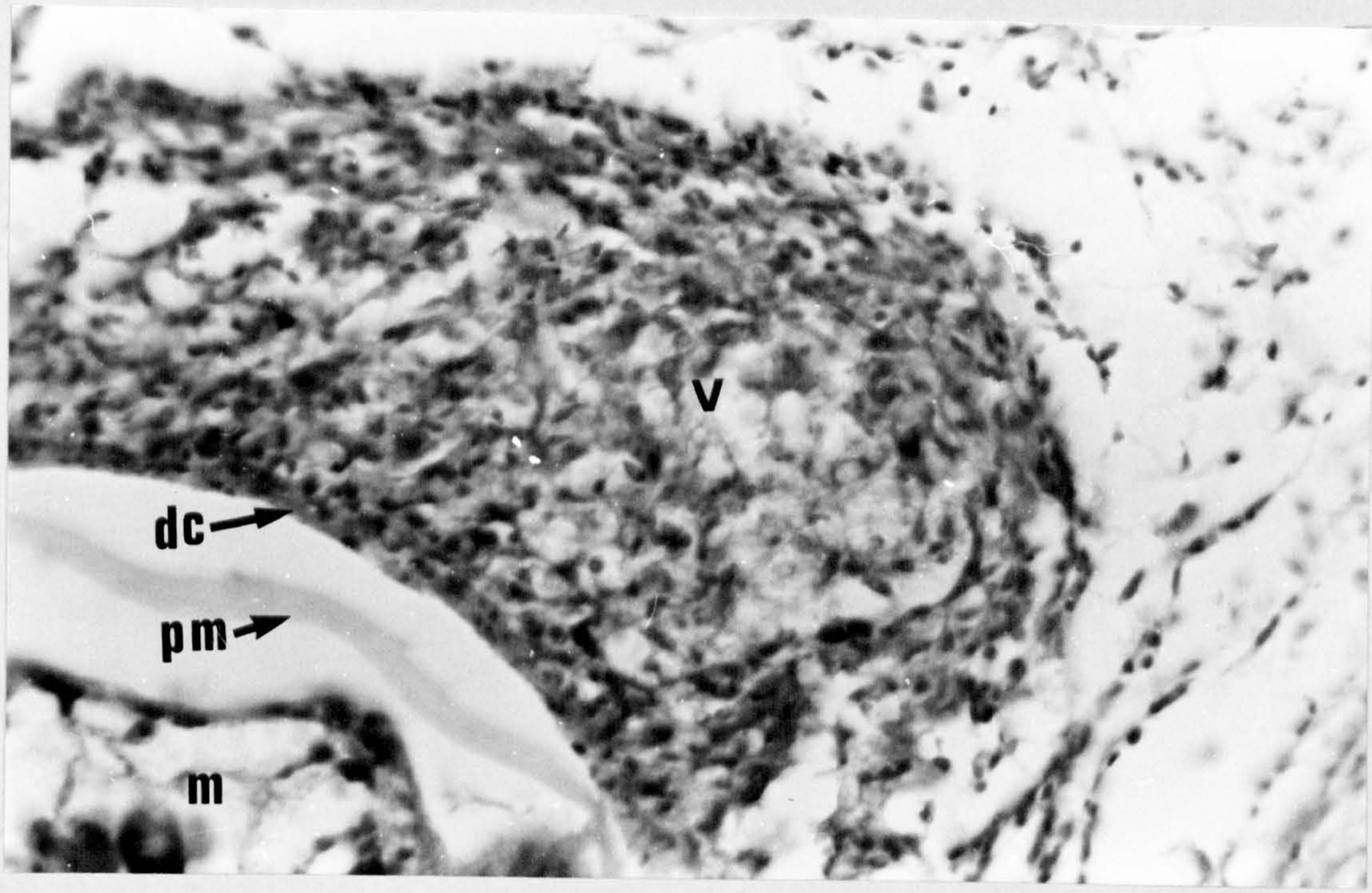
m : metacercaria

pm : paracyst membrane

v : vacuole

Figure 27. P. platessa 23 days post infection. A section through the epithelioid tissue of the capsule showing extensive cellular degeneration. Note the karyorhectic muclei in the region H.E. (x 1000)

knu : karyorhectic nucleus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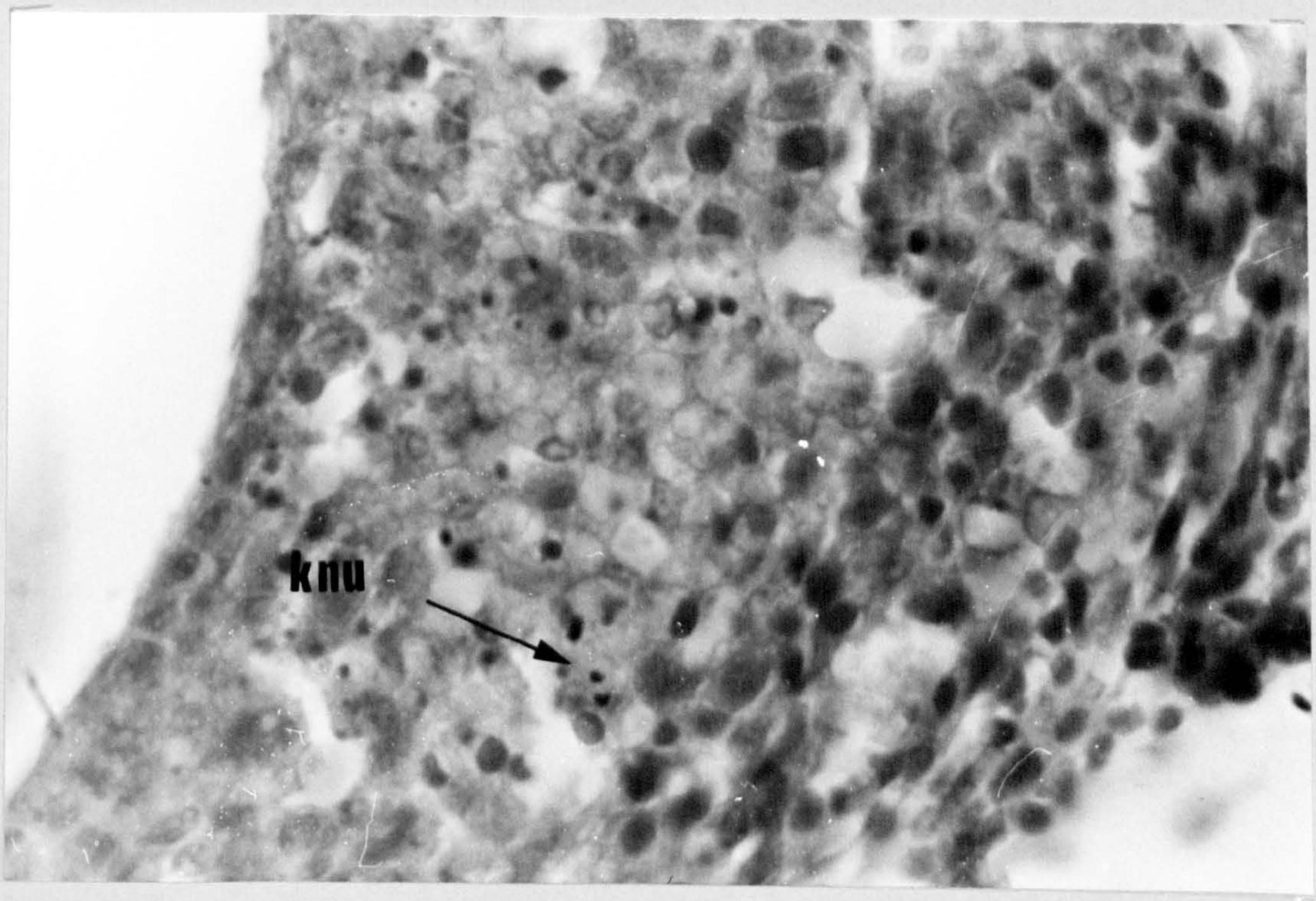

knu

Figure 28. P. platessa 28 days post infection. Section through the middle and outer layer of the capsule showing Langhan's type giant cells in this region H.E. (x 500)

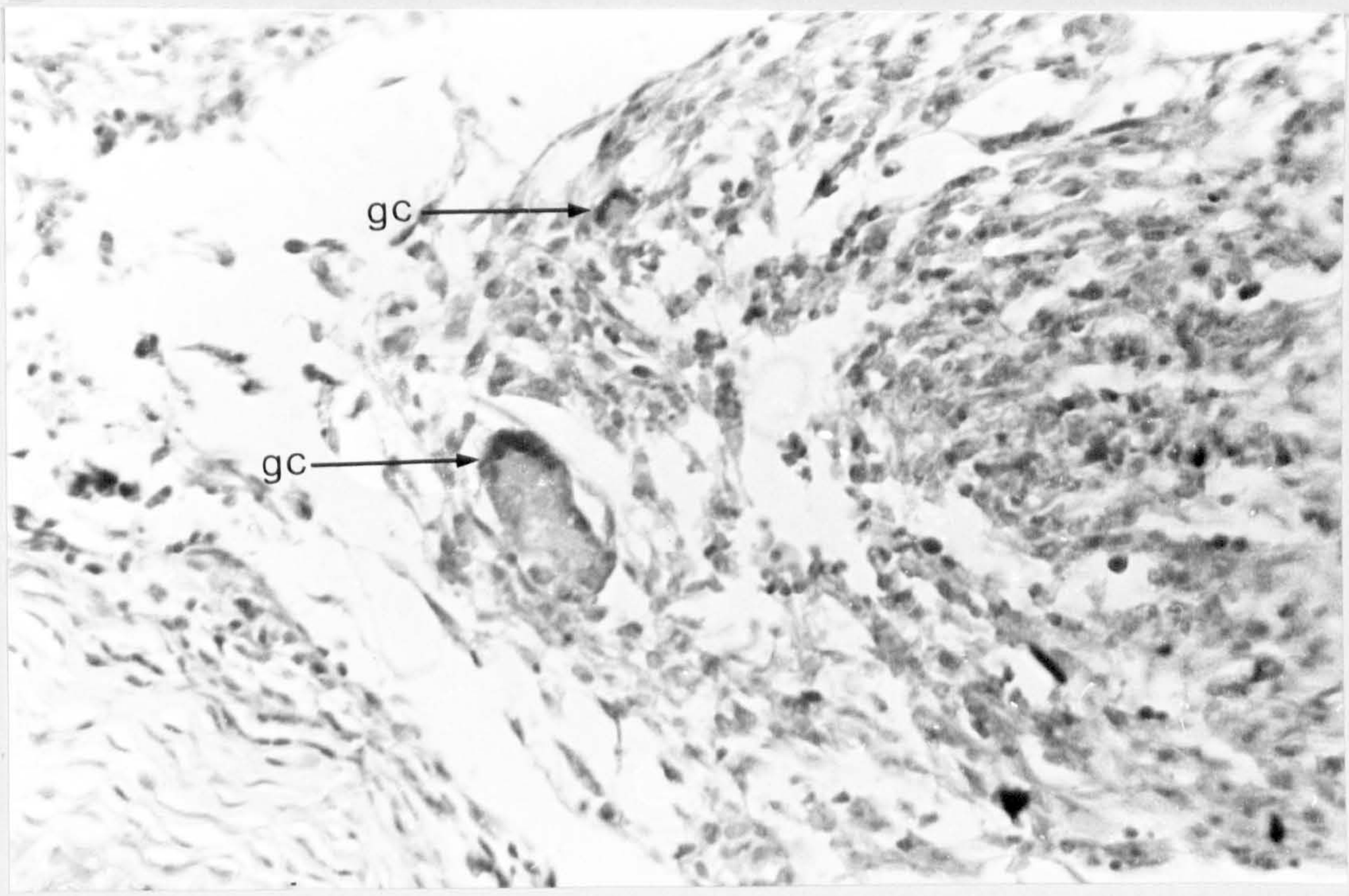
gc : giant cells

OL : Outer Layer

ML : Middle Layer

IL : Inner Layer

Figure 29. P. platessa 28 days post infection. Section through the capsule showing the three layers; the inner layer consisting of a matrix of necrotic ground substance in which are embedded karyohectic nuclei; a middle layer composed of degenerating epithelioid tissue on the periphery of which occur Langhan's and foreign body giant cells; the outermost layer is comprised largely of fibroblasts and collagen H.E. (x 250)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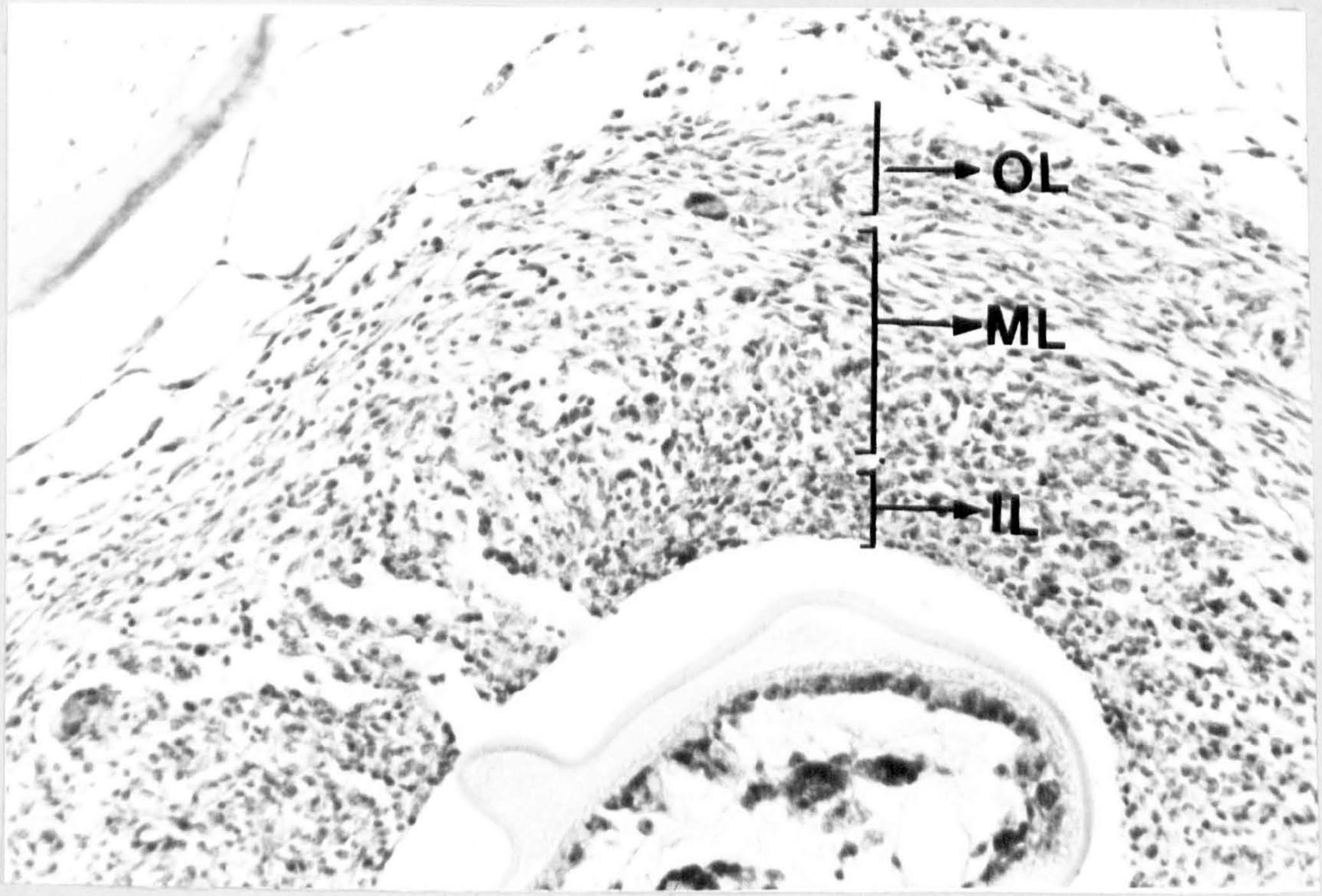
gc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ML
IL

Tissue response after 40 - 45 days: The capsule at 40 days was much more fibrous but despite this, even at 45 days, when the last cysts were examined, there was still a considerable number of migratory cells at the periphery of the capsule. Where a lesion was located within a superficial muscle, myophagia of necrotic fibrils was still extant. At the same time, individual regenerating fibres recognised by their bluish cytoplasm and dark sarcoplasmic nuclei were seen.

General observations on the mature cyst

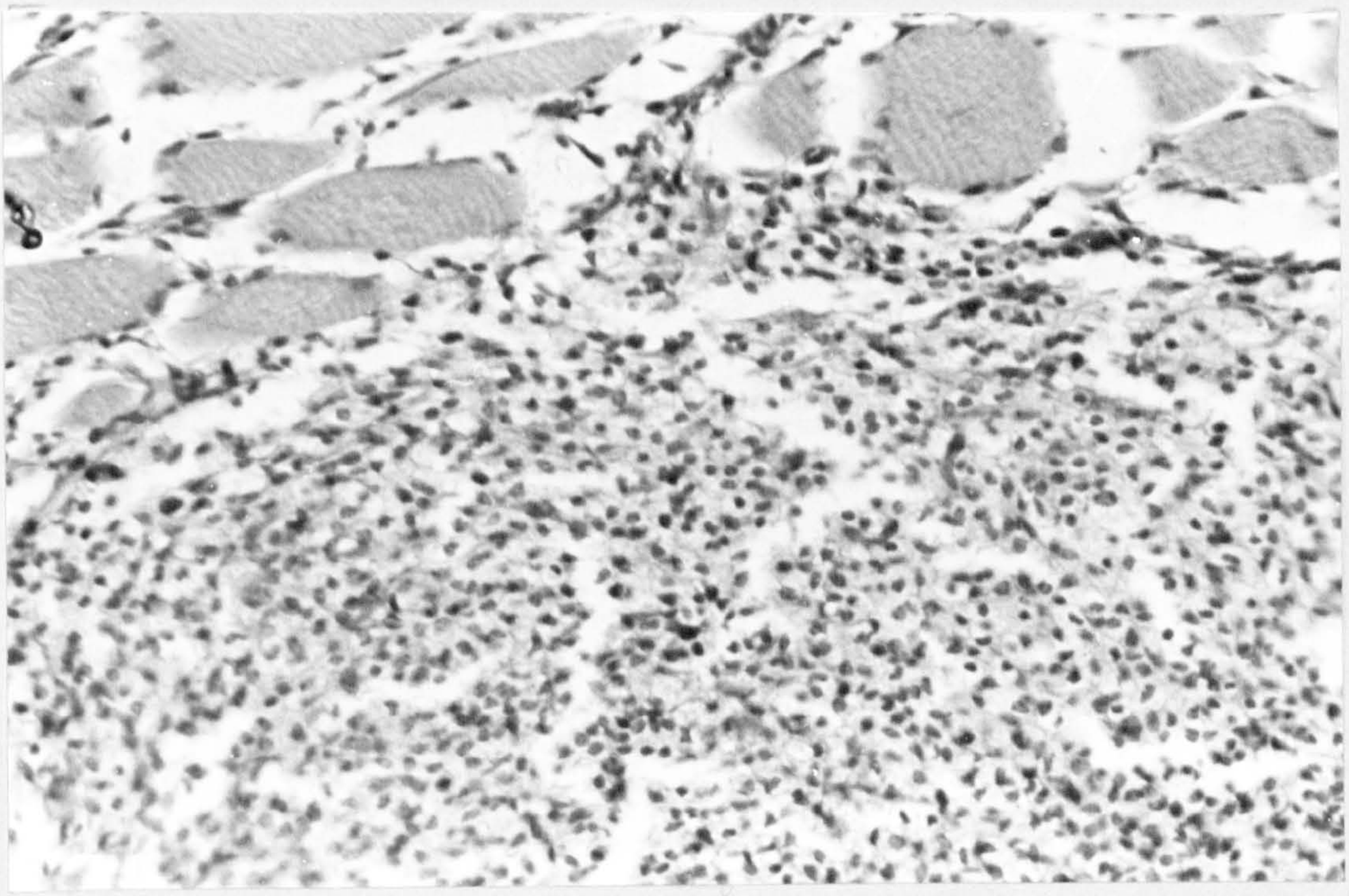
The typical fin cyst at 28 days consisted of 3 layers. The innermost layer abutting the paracyst was by now very much less cellular and consisted of a matrix of necrotic ground substance. The middle layer was composed of degenerating epithelioid tissue in which the nuclei were pyknotic and the cytoplasm was becoming vacuolated. It was on the periphery of this layer that the giant cells occurred. The outermost layer was comprised largely of fibroblasts with increasing amounts of collagen.

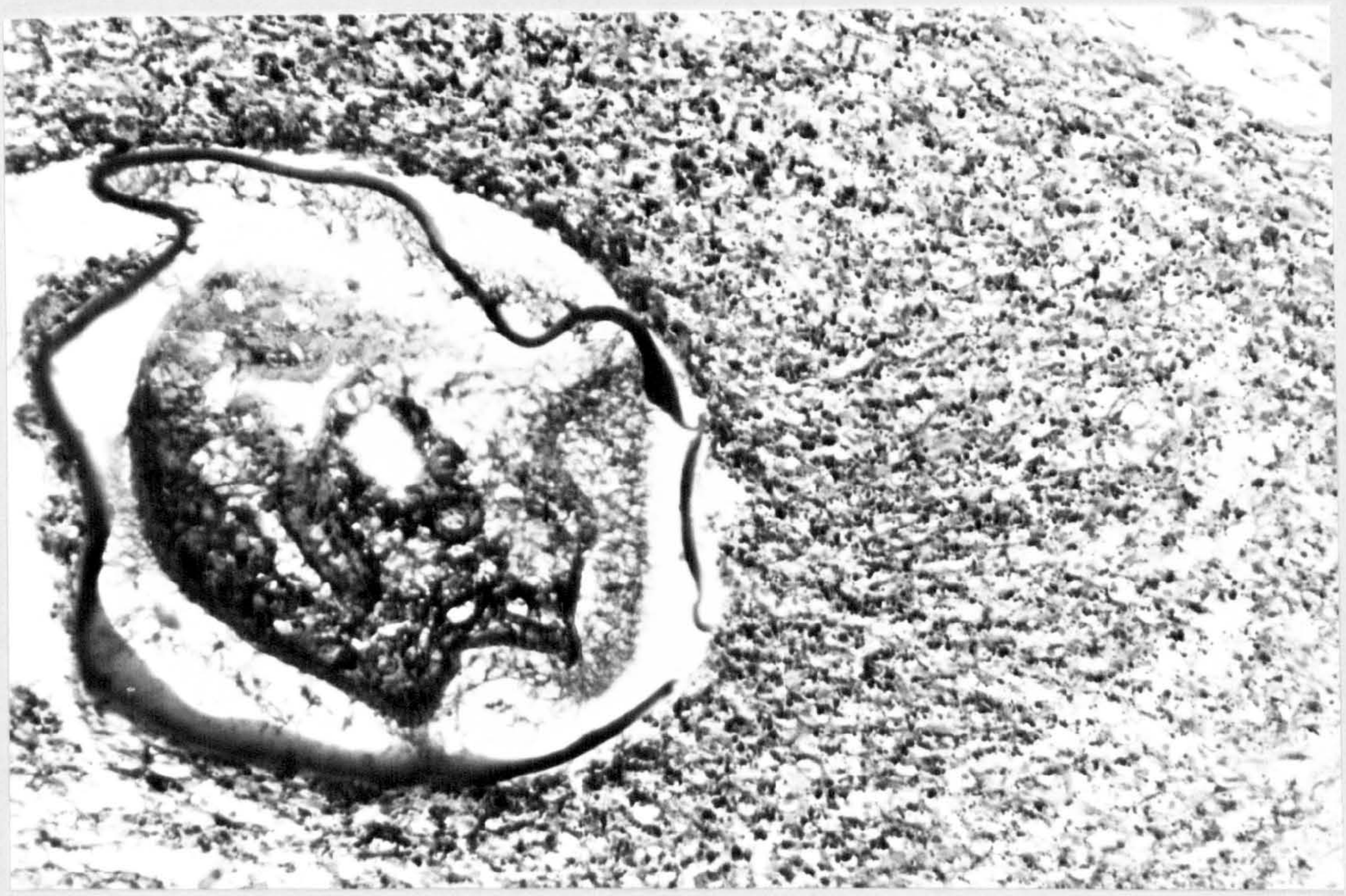
The muscle cysts differed from the fin cysts in that the fibroblast layer was not so extensive and the innermost layer was less distinct. In the middle layer of the muscle cyst the nuclei of the epithelioid cells were more oval and less necrotic, and their cell walls were more easily defined, perhaps indicating a less advanced granulomatous condition. (Figure 30)

All well-formed capsules showed a dense covering of PAS positive granules which were most densely dispersed over the inner layer and becoming less dense towards the outer layer. (Figure 31 The source of these granules is not certain but they appeared to accumulate as a result of the breakdown of the macrophages and

Figure 30. P. platessa 45 days post infection. Section through the capsule of a muscle cyst showing the epithelioid tissue in a less advanced stage of necrosis than in capsules of fin cysts H.E. (x 400)

Figure 31. P. platessa 45 days post infection. Section through a paracyst and capsule showing the covering of PAS positive granules P.A.S. (x 250)





consequent dispersal of their cellular contents.

The contents of the paracyst were very PAS positive. (Figure 32) Generally there was very little functional interference apparent as a result of the parasite's presence although where the cyst occurred in the muscle there was some evidence of compression necrosis as a result of the increasing size of the parasite and host capsule. (Figure 33) In a few cases, where the larva was lying in the hypodermis, there was a slight tumefaction of the skin above the cyst. This occasionally resulted in erosion of the epidermis in that region in the early stages. (Figure 34) However, this effect was not seen in older skin cysts. The epidermis above the cysts however, did show some distension.

Figure 35 illustrates the development of the cyst in P. platessa for 28 days post infection.

THE COMMON DAB Limanda limanda (L)

The early tissue response: Sites of encystment of S. baccatus in the L. limanda were the same as those observed in P. platessa. The cercariae were still penetrating the superficial layers of the skin in the earliest specimens examined at 6 hours post infection. L. limanda skin contains closely overlapping scales laid down in a brickwork fashion (i.e. two layers of scales with the upper scale overlying the area between the two lower scales). Its appearance suggested that it might be a barrier to penetration by cercariae but there was no indication that this was the case. Cercariae were occasionally seen to cause disturbance of scales. (Figure 36)

Figure 32. P. platessa 28 days post infection. Section through cyst showing PAS positive paracyst membrane and PAS positive material within the membrane. Large amounts of PAS positive material occurs within the cells of the larva P.A.S. (x 250)

pm : paracyst membrane

pp : PAS positive material

Figure 33. P. platessa 45 days post infection showing compression of muscle resulting from the increased size of cyst M.S.B. (x 100)

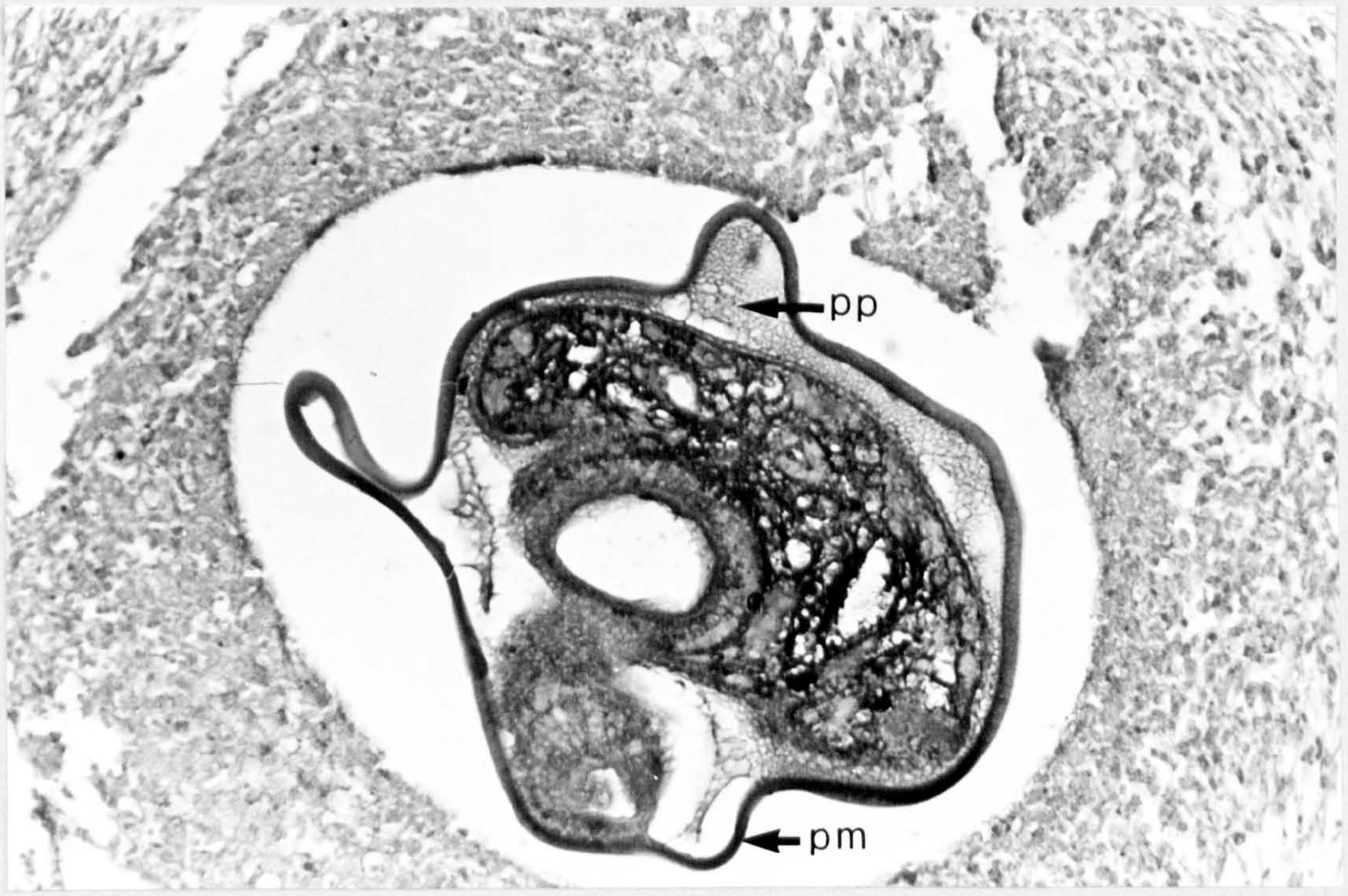

pp
pm

Figure 34. P. platessa 77 hours post infection showing erosion of the epidermis above the metacercaria M.S.B. (x 250)

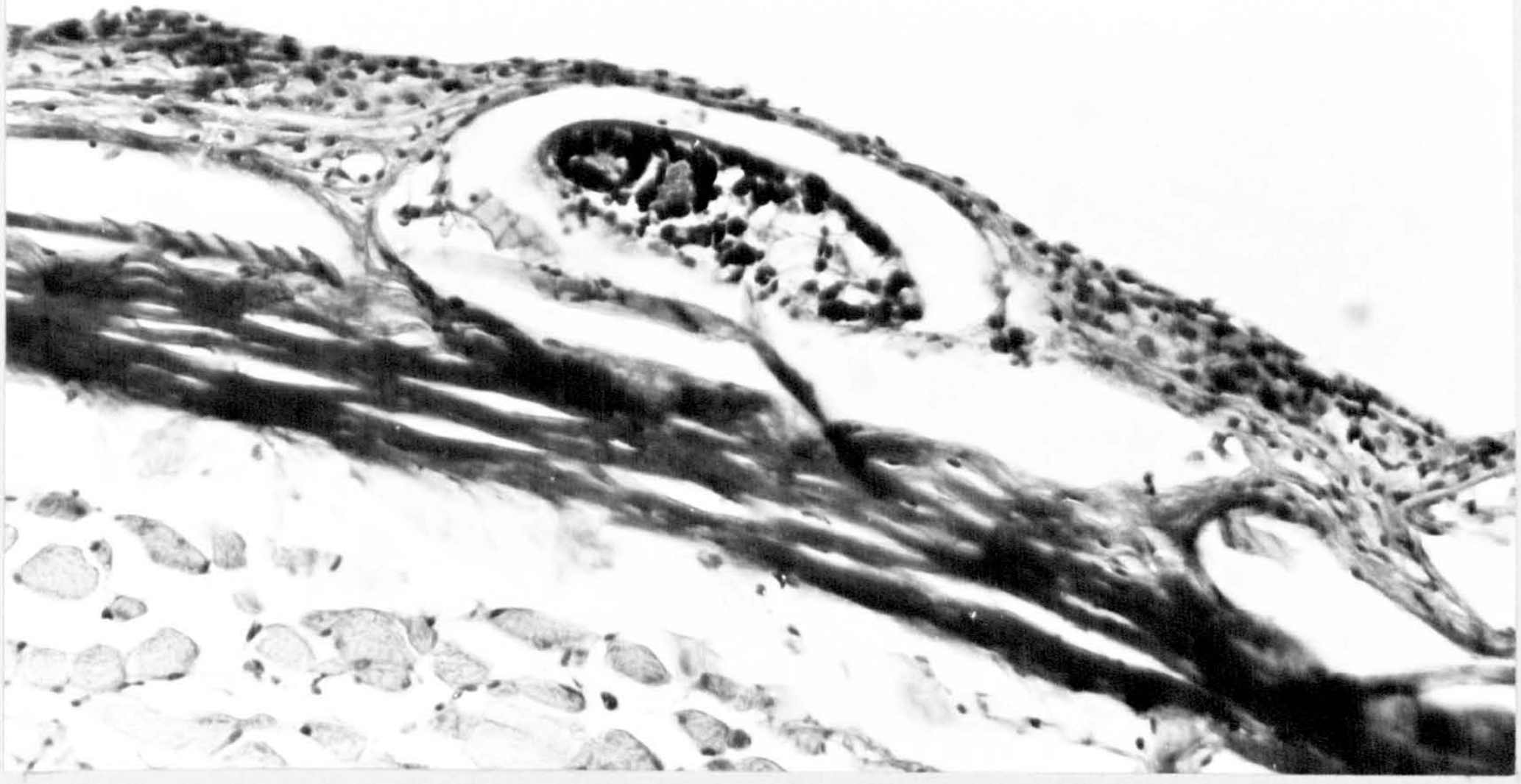


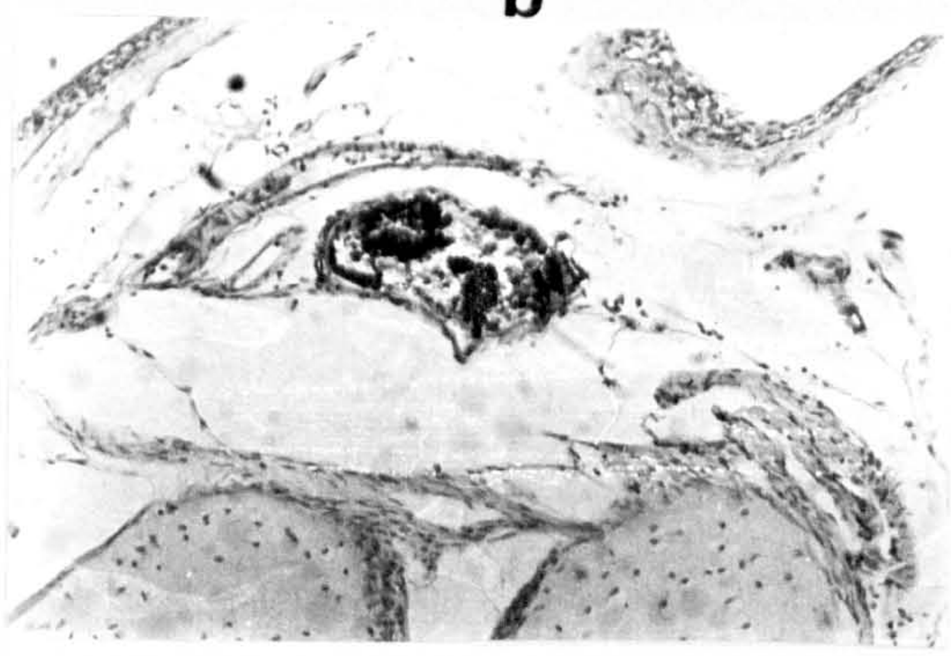
Figure 35. Development of S. baccatus cysts in P. platessa

(a) 6 hours (x 250)
(b) 48 hours (x 250)
(c) 125 hours (x 250)
(d) 8 days (x 250)
(e) 11 days (x 250)
(f) 13 days (x 250)
(g) 16 days (x 100)
(h) 23 days (x 100)
(i) 28 days (x 100)

All photographs have the same visual magnification of x 150

a

b



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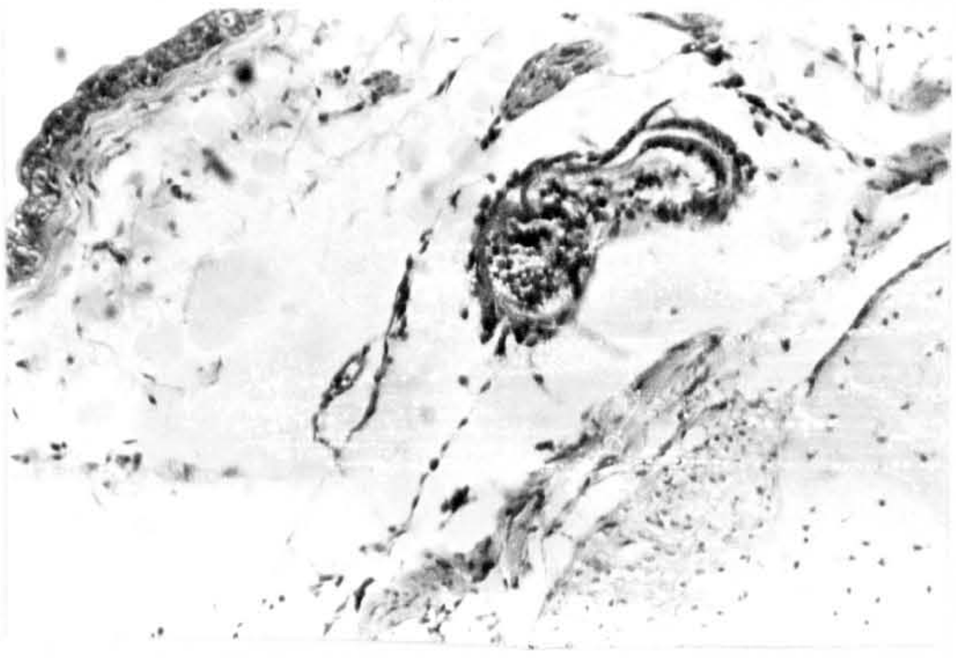

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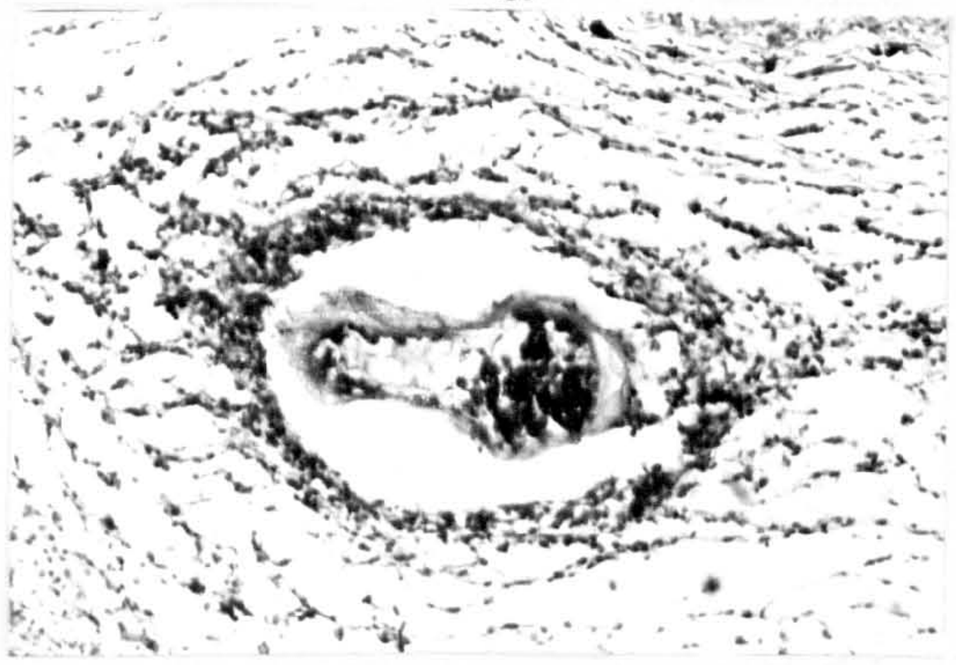

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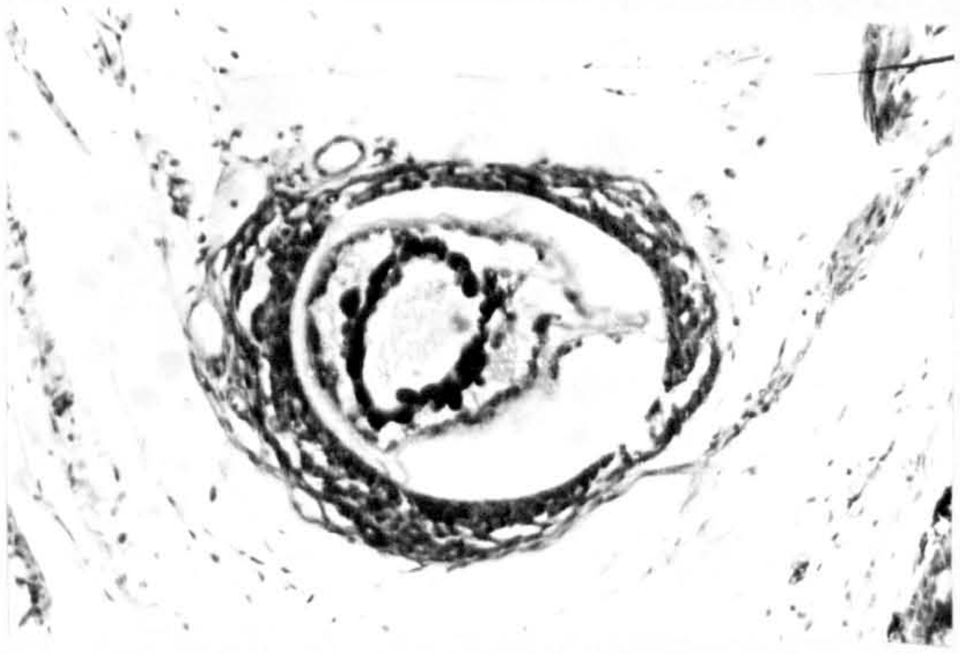

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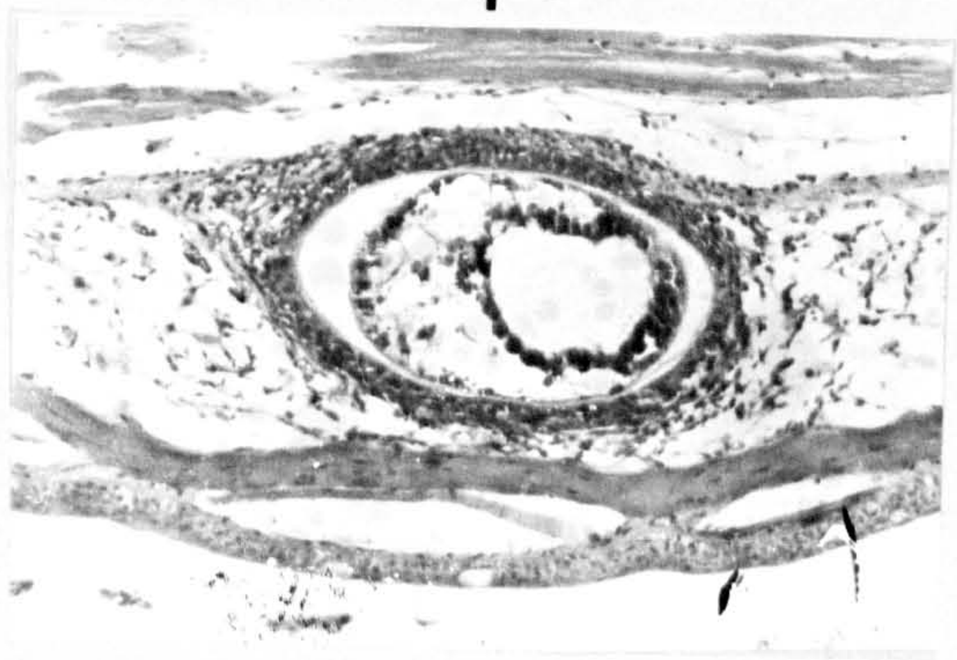

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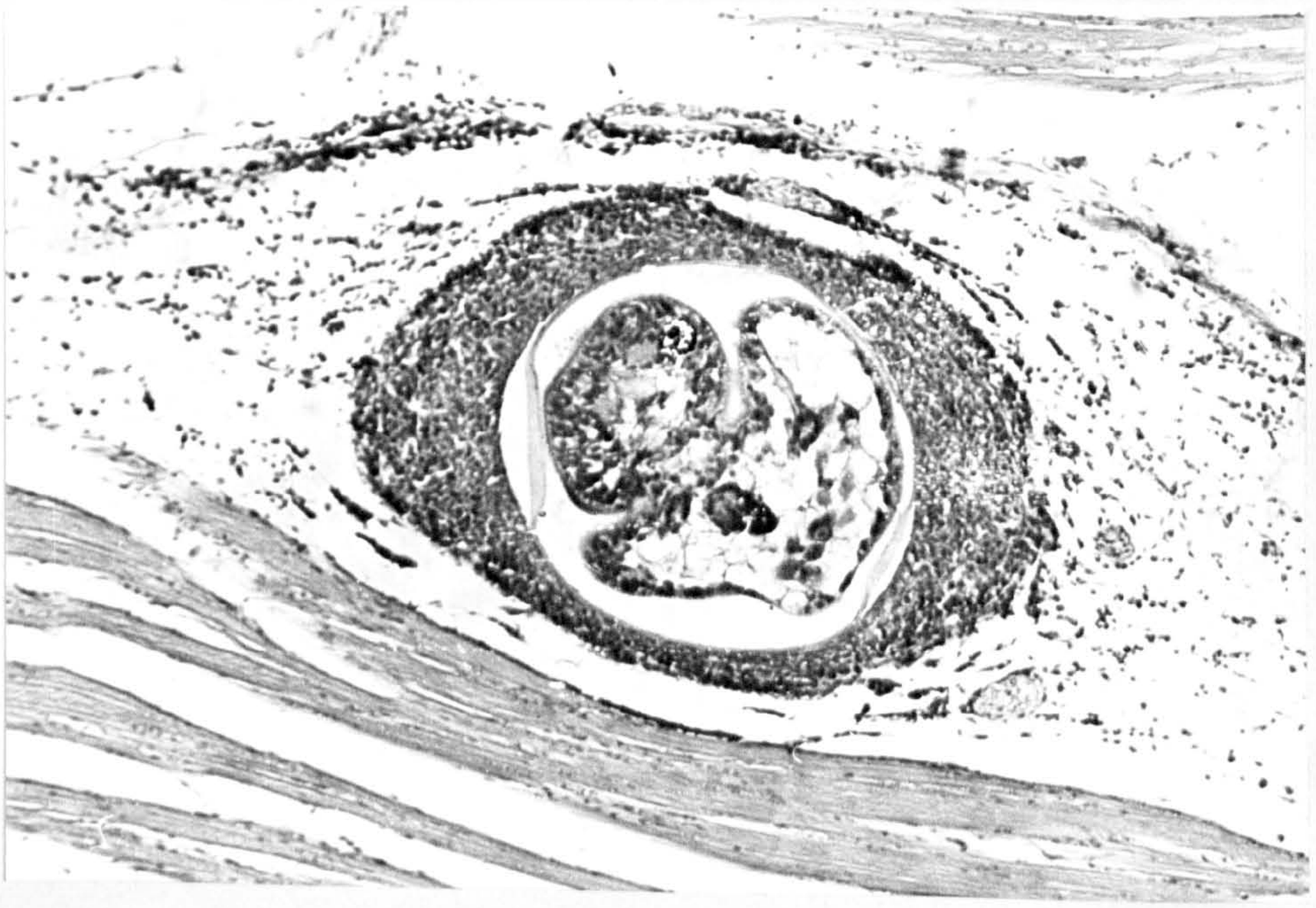

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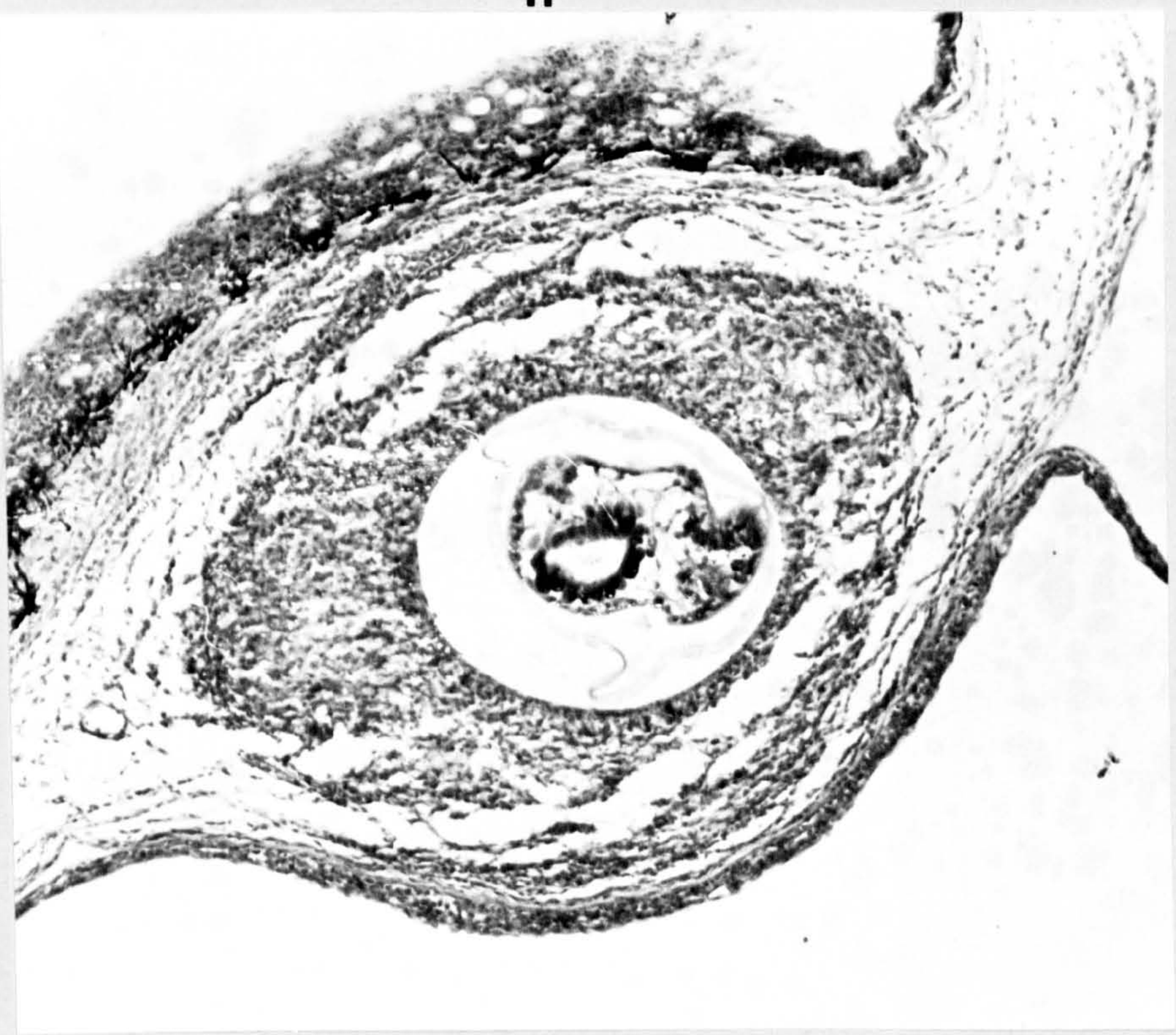
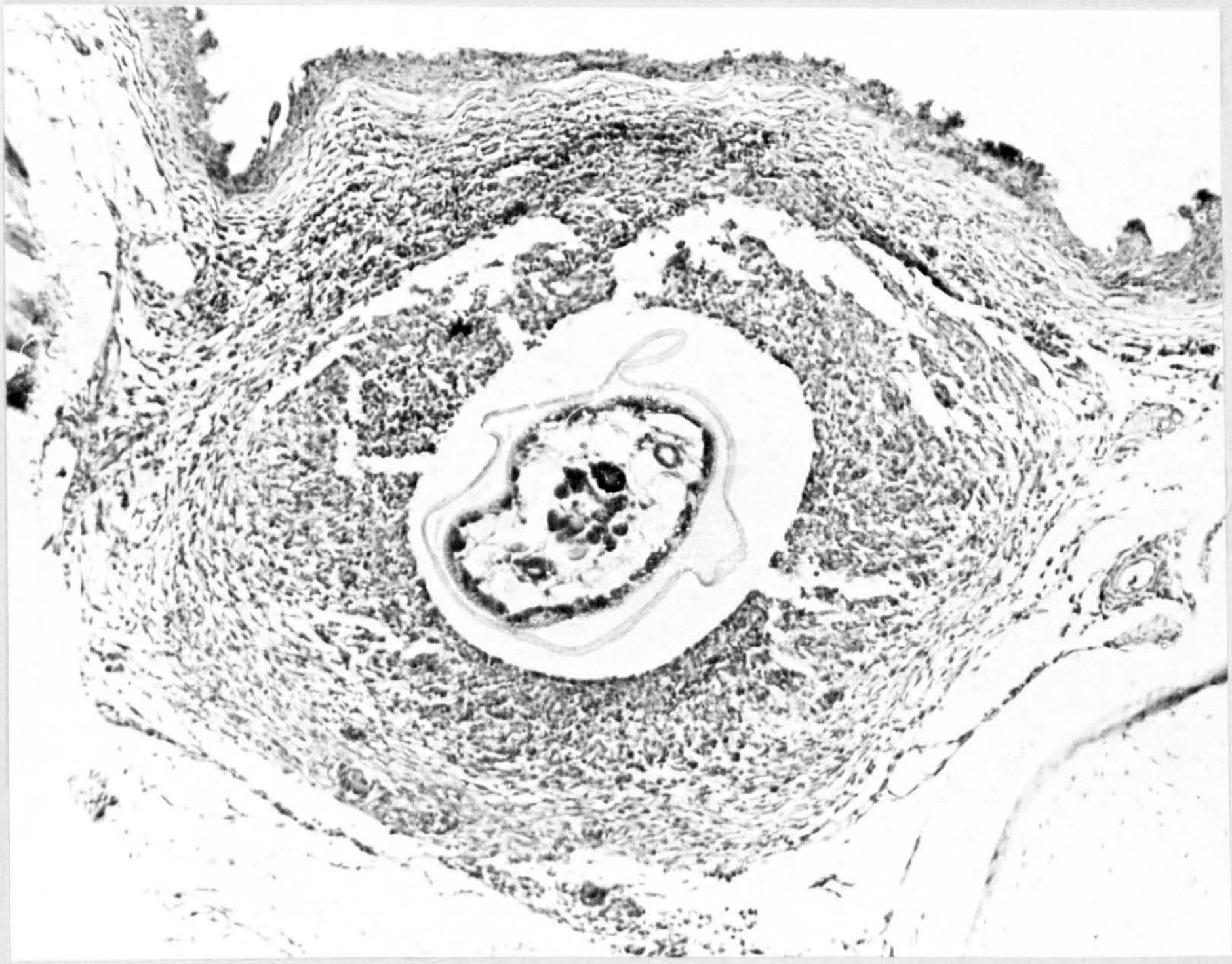


i



Larvae had penetrated the dermis by 19 hours and the beginning of paracyst membrane formation was observed at 24 hours. At the same time a finely granular PAS positive deposit was dispersed in the connective tissue surrounding the cyst. (Figure 37)

Cellular infiltration was not seen until 48 hours when a few macrophages were observed in the area and others were migrating along the connective tissue fibres in the region of the cyst. One or two layers of macrophages were closely applied to the paracyst to form the early stages of the capsule. (Figure 38) However, no leukocyte migration from blood vessels was seen at this time. Muscle cysts showed little or no response, even at 12 days. (Figure 39) The metacercariae showed the vacuolation of parenchymal cells at 48 hours.

Tissue response after 23 days: The host capsule continued development and by 23 days was more compact. It was composed of granulation tissue which was considerably thicker at the poles of the cyst. (Figure 40) There were, by this time, fewer inflammatory cells in the vicinity of the cyst, perhaps indicating a loss of inflammatory stimulus. Occasional giant cells were observed, though these were not as well defined as in P. platessa capsules. Cysts in the fins had become thicker than those in the somatal muscles. The capsules of muscle cysts were composed predominently of fibroblasts but were, by this time, less cellular and more fibrous than had been seen in earlier cysts.

The presence of the parasite had caused degeneration of some of the myofibrils in the immediate vicinity of the cyst and at 23 days there was muscle regeneration and replacement of necrosed myofibres by fibroblasts. (Figure 41)

Figure 36. L. limanda 6 hours post infection showing cercaria migrating through the dermis. Note the disturbance of the overlying scales H.E. (x 100)

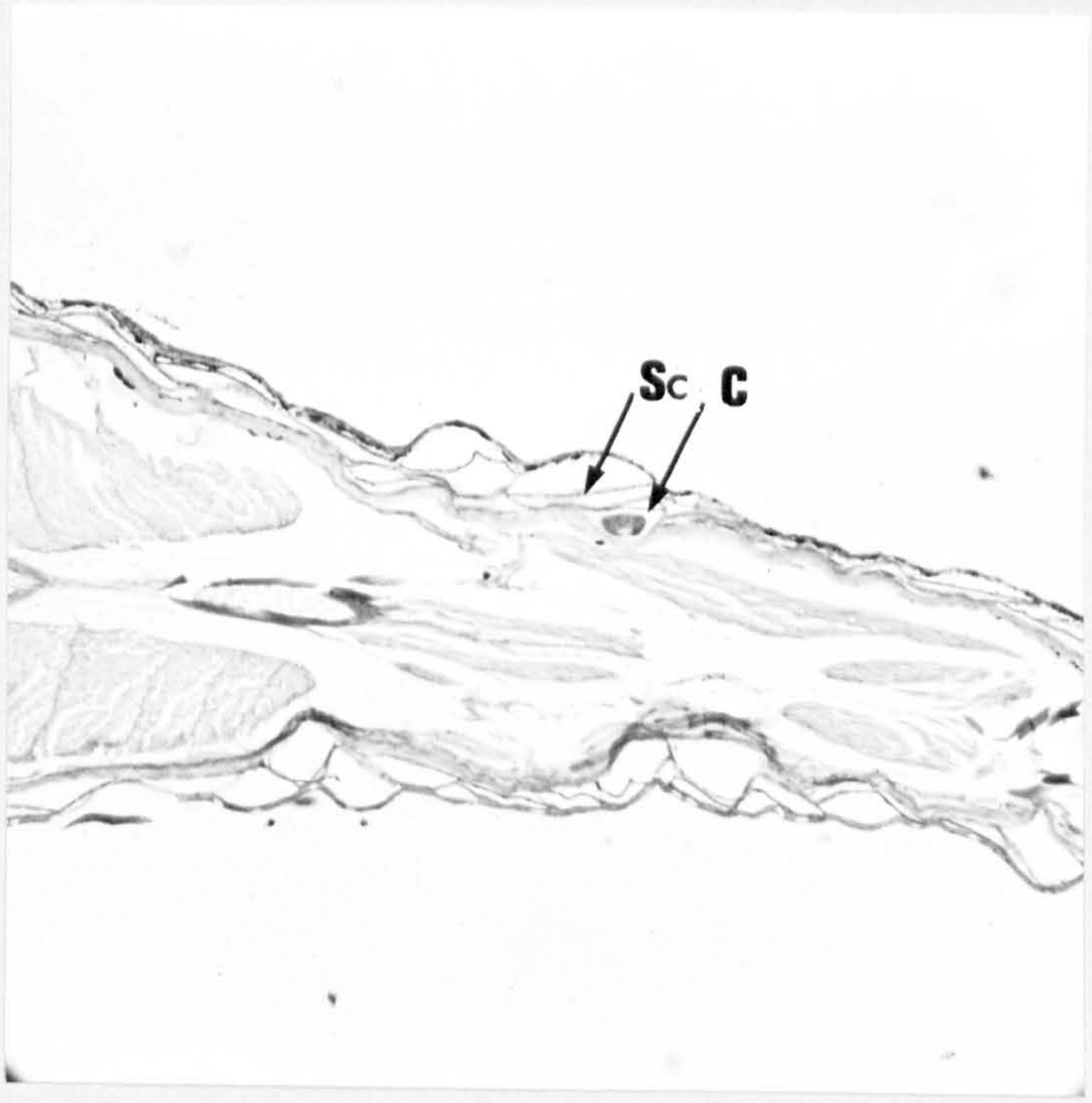
c : cercaria

sc : scale

pm : paracyst membrane

pp : PAS positive material

Figure 37. L. limanda 24 hours post infection. Section through encysted larva in which the formation of the paracyst membrane is in its early stage. Note the deposit of fine PAS positive granules in the area P.A.S. (x 400)


Sc
C

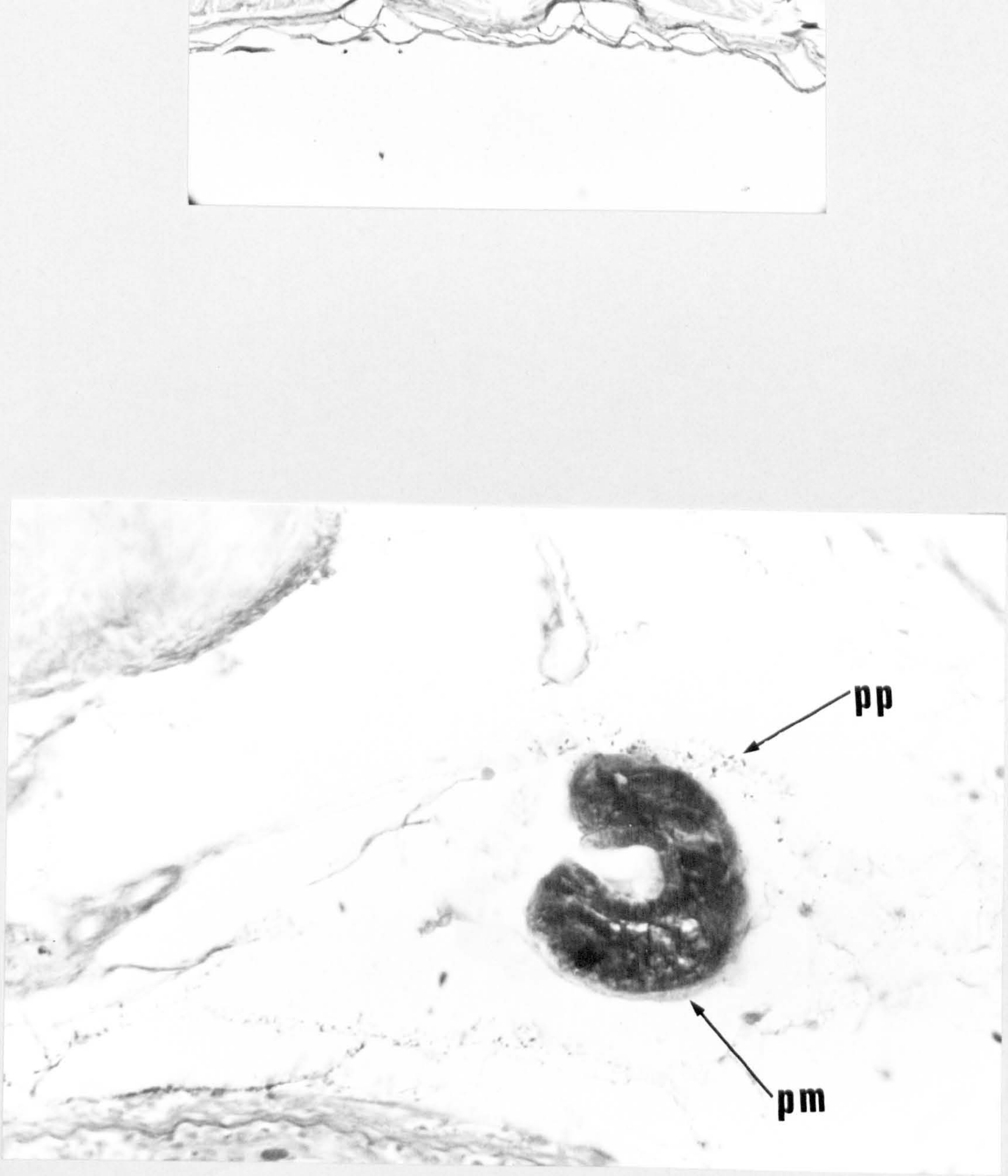
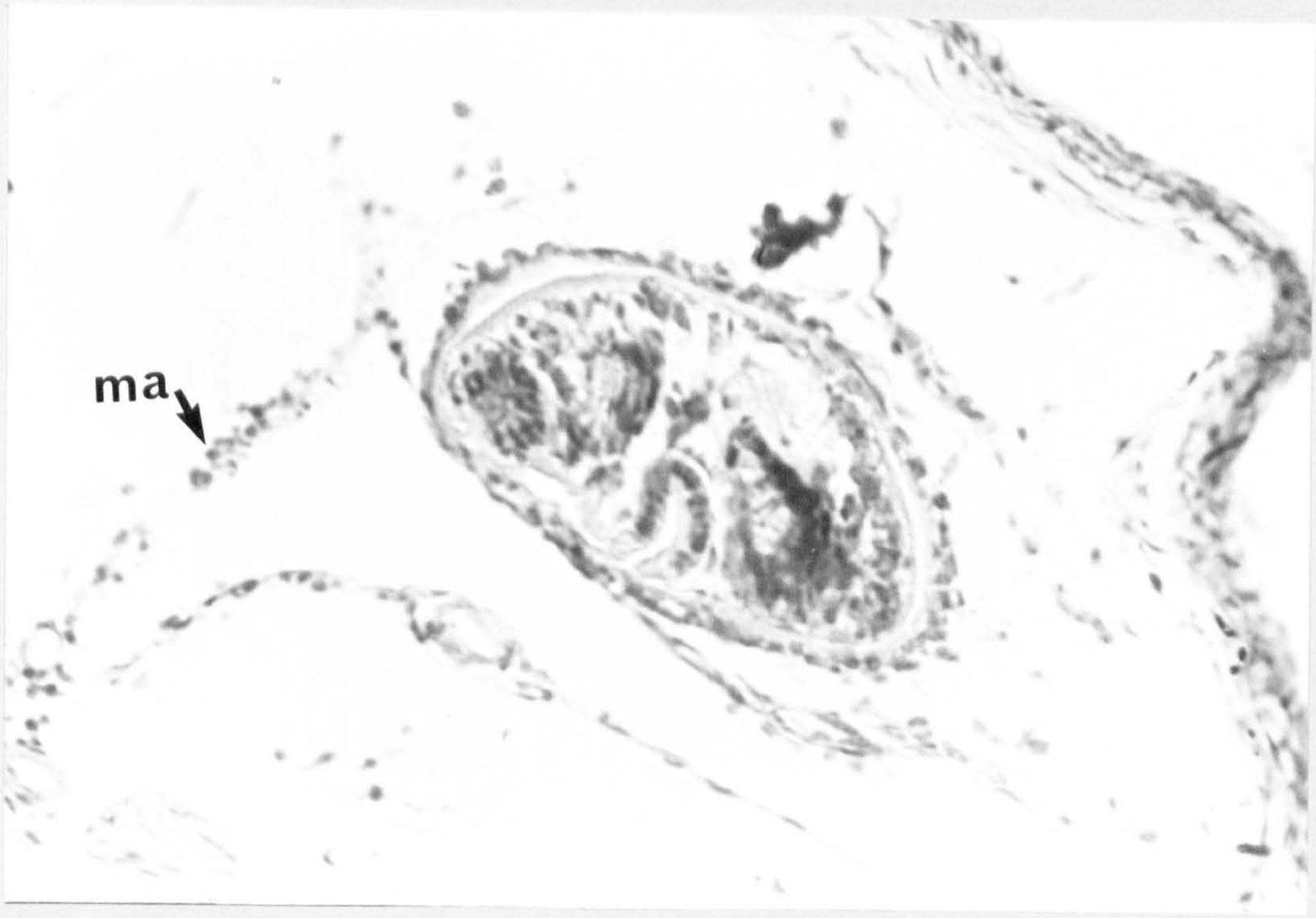

pp
pm

Figure 38. L. limanda 48 hours post infection showing the early stages of capsule formation. Note the migration of macrophages in the vicinity of the cyst H.E. (x 400)

ma : macrophages

Figure 39. L. limanda 12 days post infection. Section through a cyst in muscle showing the lack of response. Note the large vacuoles in the parenchymal cells of the metacercaria which first appeared at 48 hours. H.E. (x 250)

v : vacuole


ma

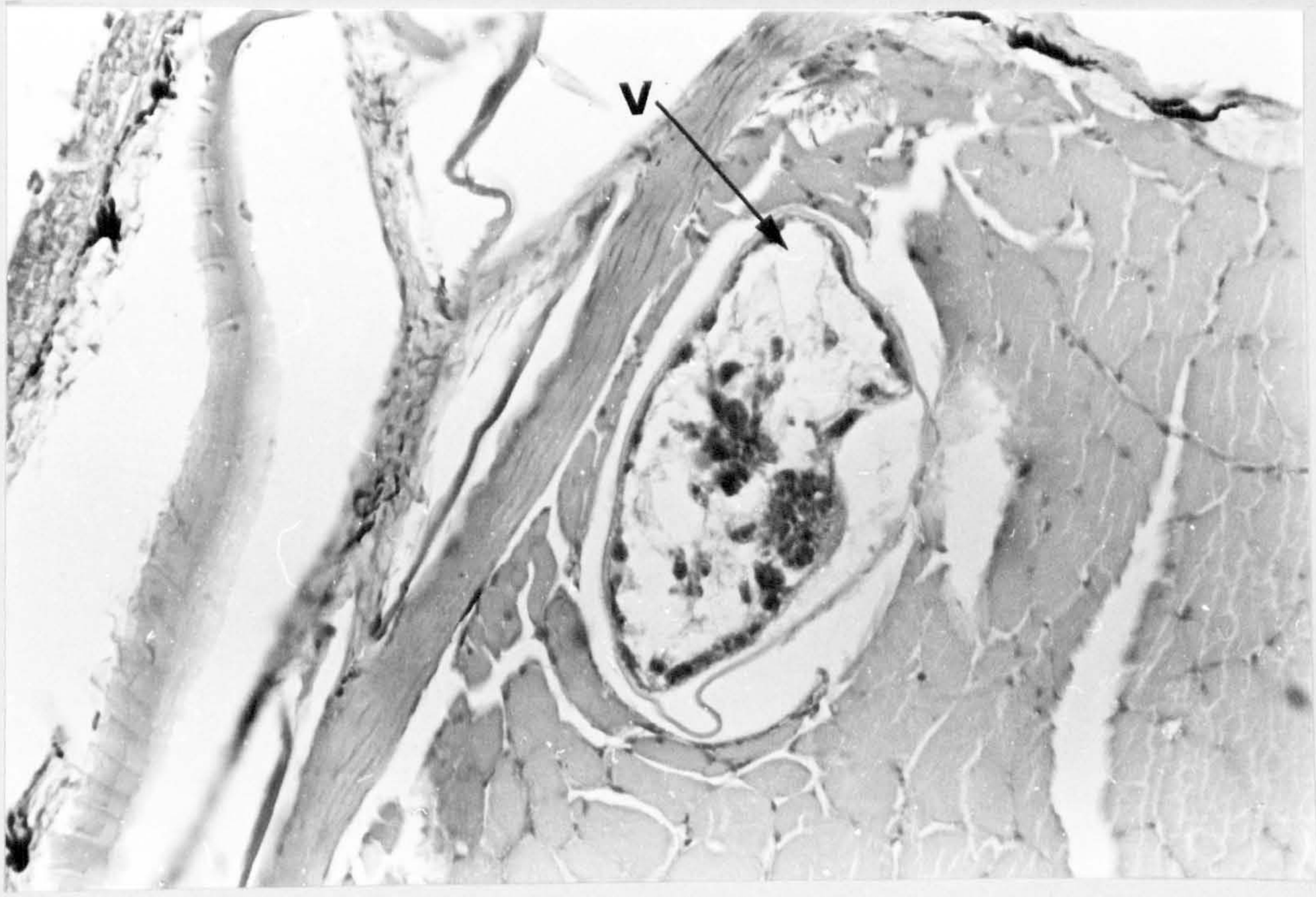
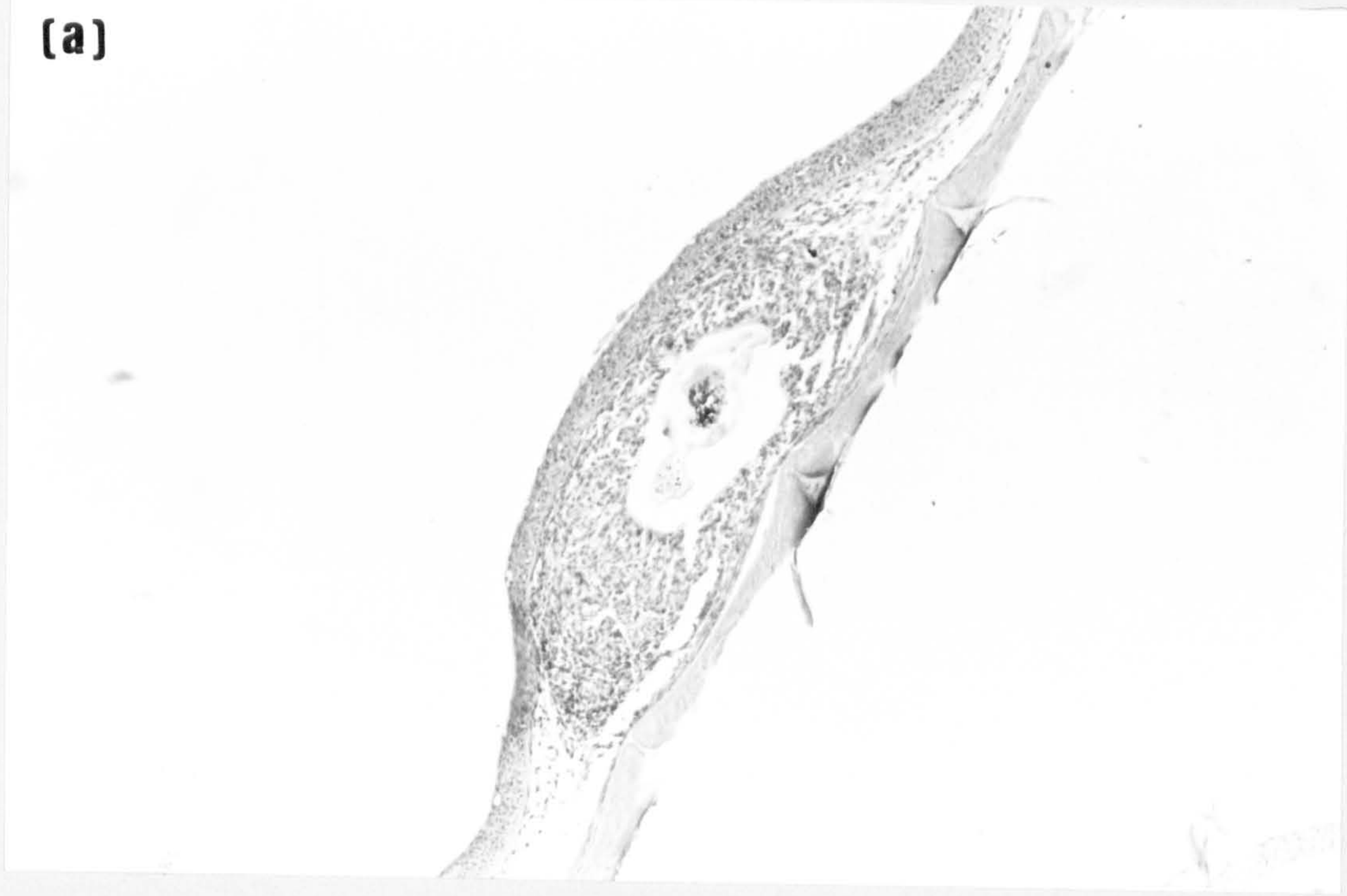

V

Figure 40. L. limanda 23 days post infection.

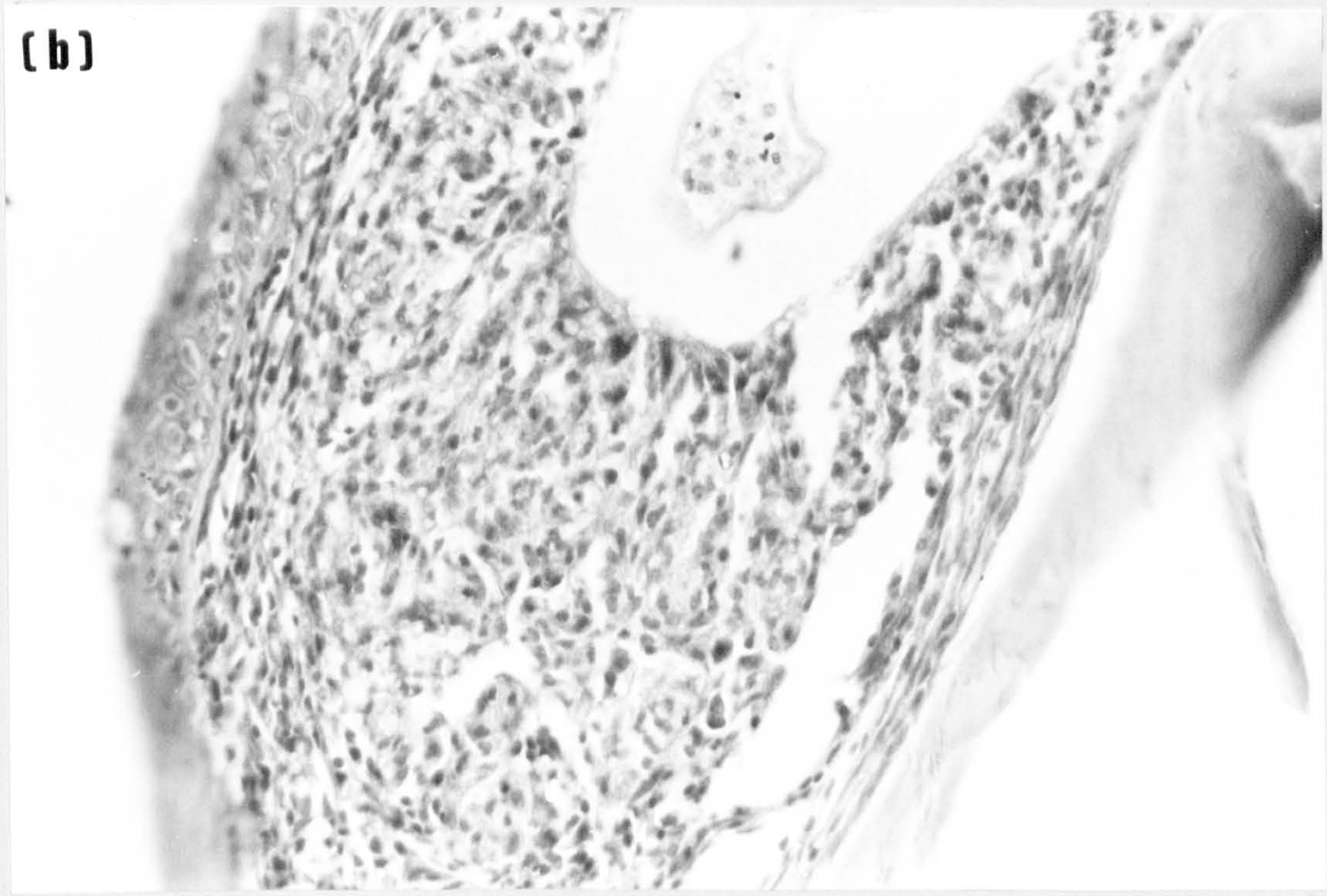
(a) Section through a well-developed cyst in the fin. Note the capsule is thicker at the poles of the cyst H.E. (x 100)

(b) High power, section through the capsule of cyst showing degenerating epithelioid tissue H.E. (x 400)

(a)



(b)



Tissues response after 55 days: The capsule of the muscle cyst did not form a discrete capsule since the cellular components were continuous with the fibrocytic replacement tissue in the muscle surrounding the cyst. (Figure 42) The capsule tissue had become epithelioid and this was showing signs of degeneration (pyknosis, karyorhexis and vacuolation of the cells).

The fin cyst was morphologically similar but the degeneration of epithelioid tissue was more advanced and more collagen was present in the outer capsule. Some lymphocytes were also observed. The capsule thickness was similar in both muscle and fin cysts but the accumulation of cells at the poles was greater (141 mu) in the fin than the muscle (57 mu). Since the capsule size of the fin was greater, it raises the question of how much influence the host capsule has on the growth of the parasite.

General observations on cyst development

The inner cyst diameters paralleled very closely those of P. platessa paracysts but the three distinct layers seen in the P. platessa capsule were not evident in the L. limanda capsule. The L. limanda capsule had more homogenity and the reaction had an overall appearance of restraint compared with P. platessa.

Figure 43 illustrates the development of the S. baccatus cyst up to 23 days.

THE DOVER SOLE Solea solea (L)

Tissue response during 7 days post infection: Cysts of S. baccatus were found in similar situations to those described for P. platessa and L. limanda. However, a greater proportion of

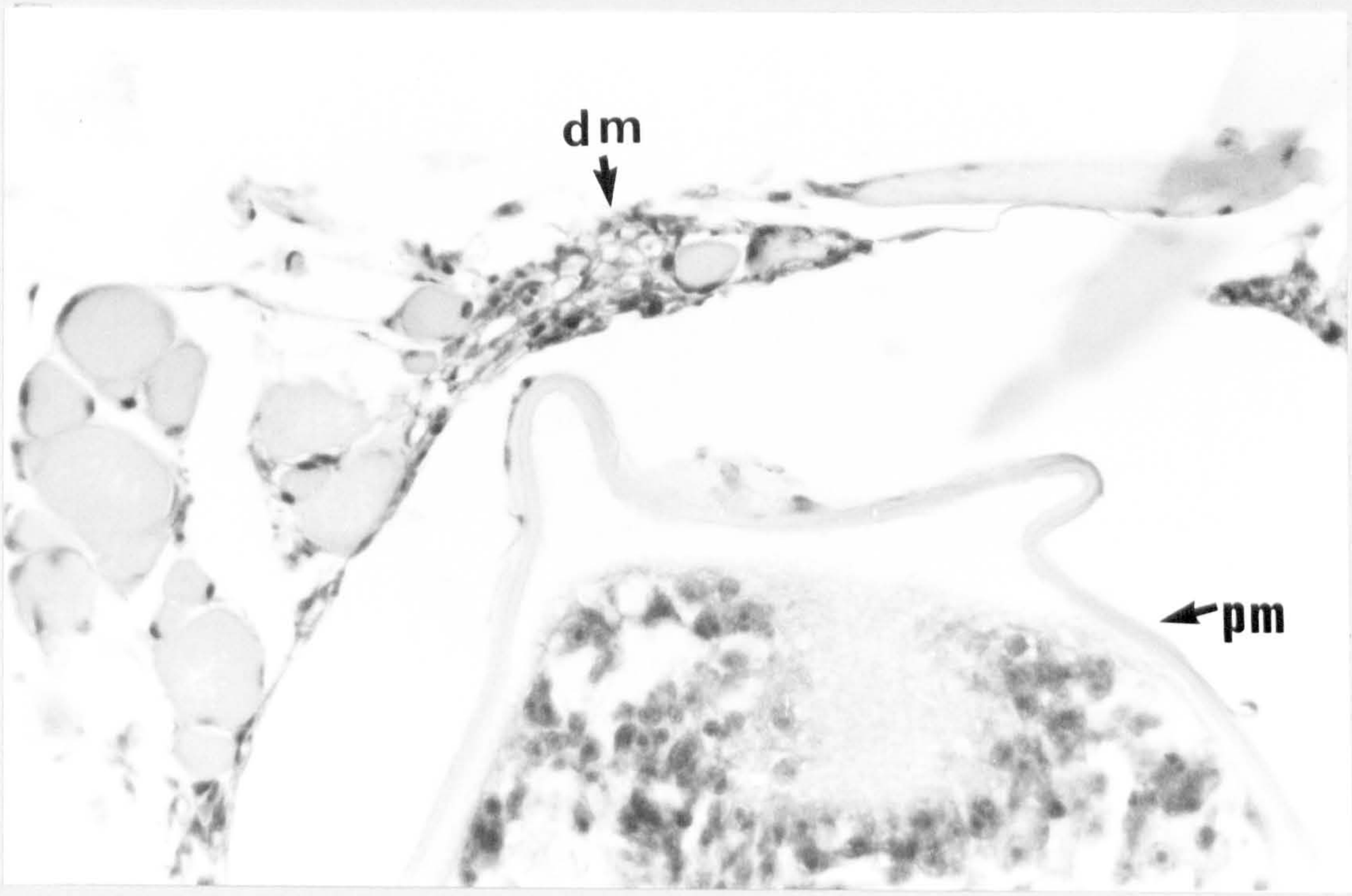
Figure 41. L. limanda 23 days post infection. Section through a muscle cyst showing focal degeneration of myofibrils in the immediate vicinity of the cyst and the proliferation of fibroblasts in the area. Note the lack of response compared with the cysts in the fins H.E. (x 400)

dm : degenerating myofibrils

pm : paracyst membrane

Figure 42. L. limanda 55 days post infection. Section through a muscle cyst showing the cellular components of the capsule continuous with the fibrocytic replacement tissue in the surrounding muscle H.E. (x 100)

fr : fibrocytic replacement tissue


dm
pm

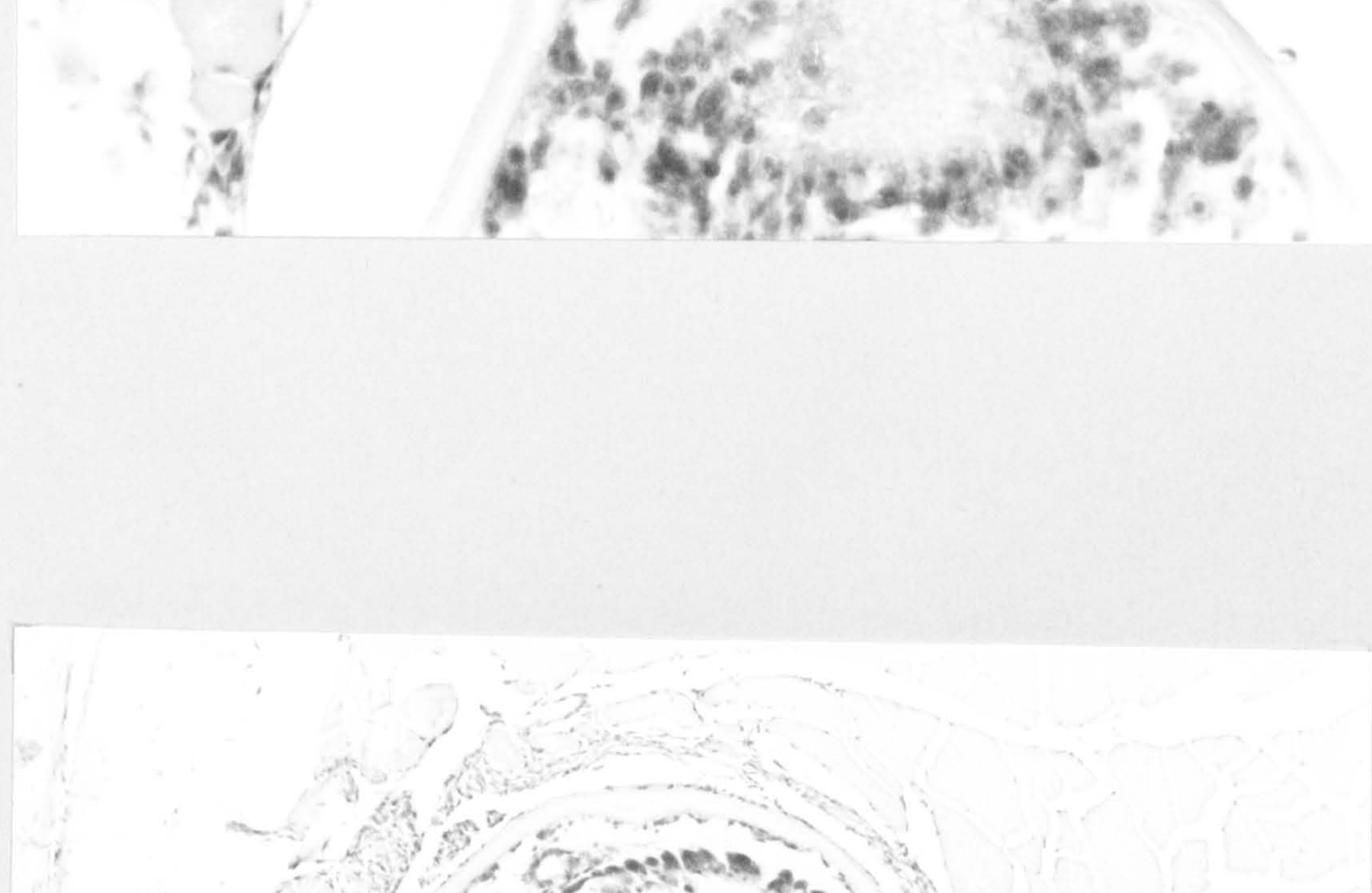

fr

Figure 43. Development of S. baccatus cysts in L. limanda

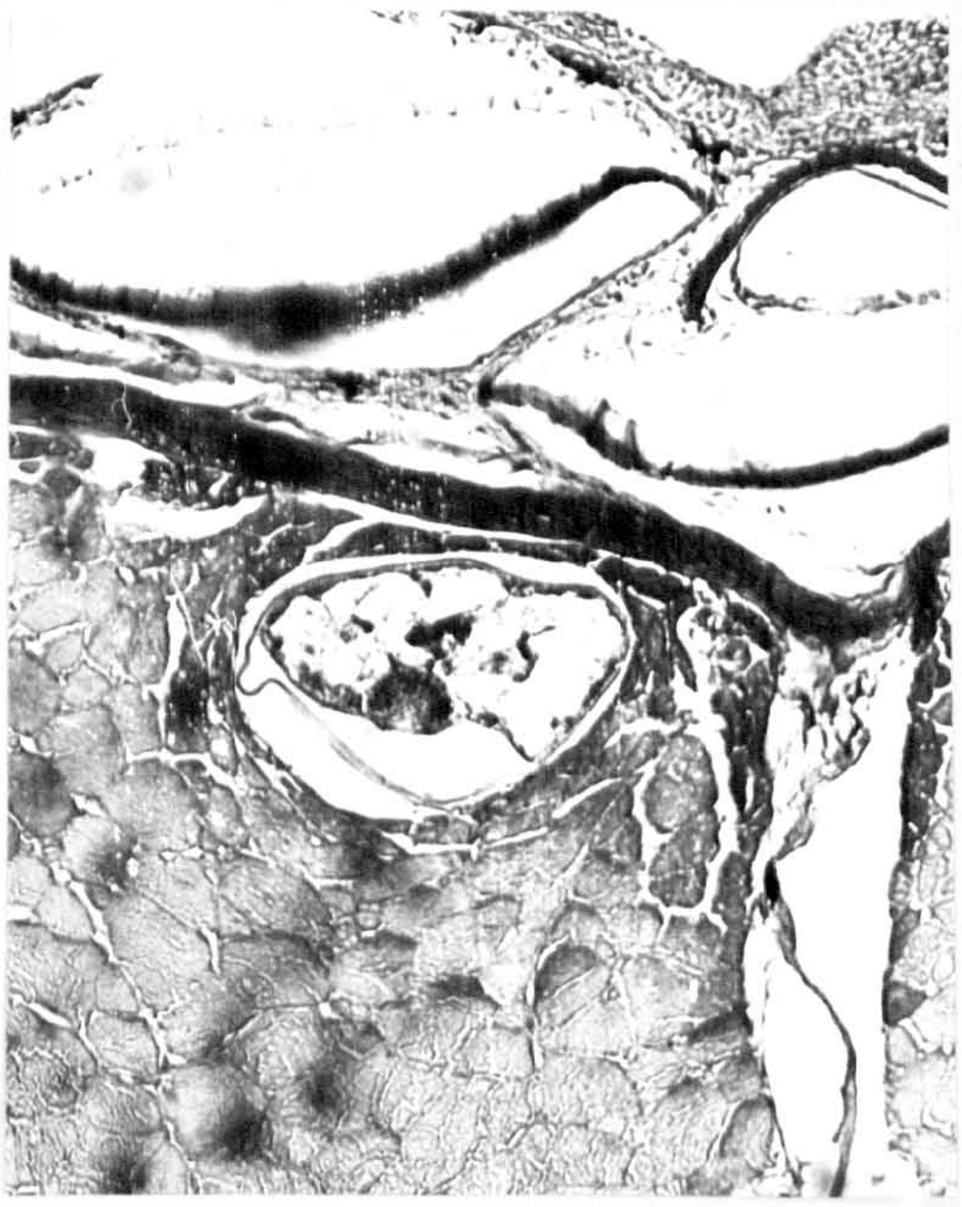
(a) 6 hours (x 100),
(b) 48 hours (x 100)
(c) 12 days (x 100)
(d) 23 days (x 100)

All photographs have the same visual magnification of x 150

a

b

c



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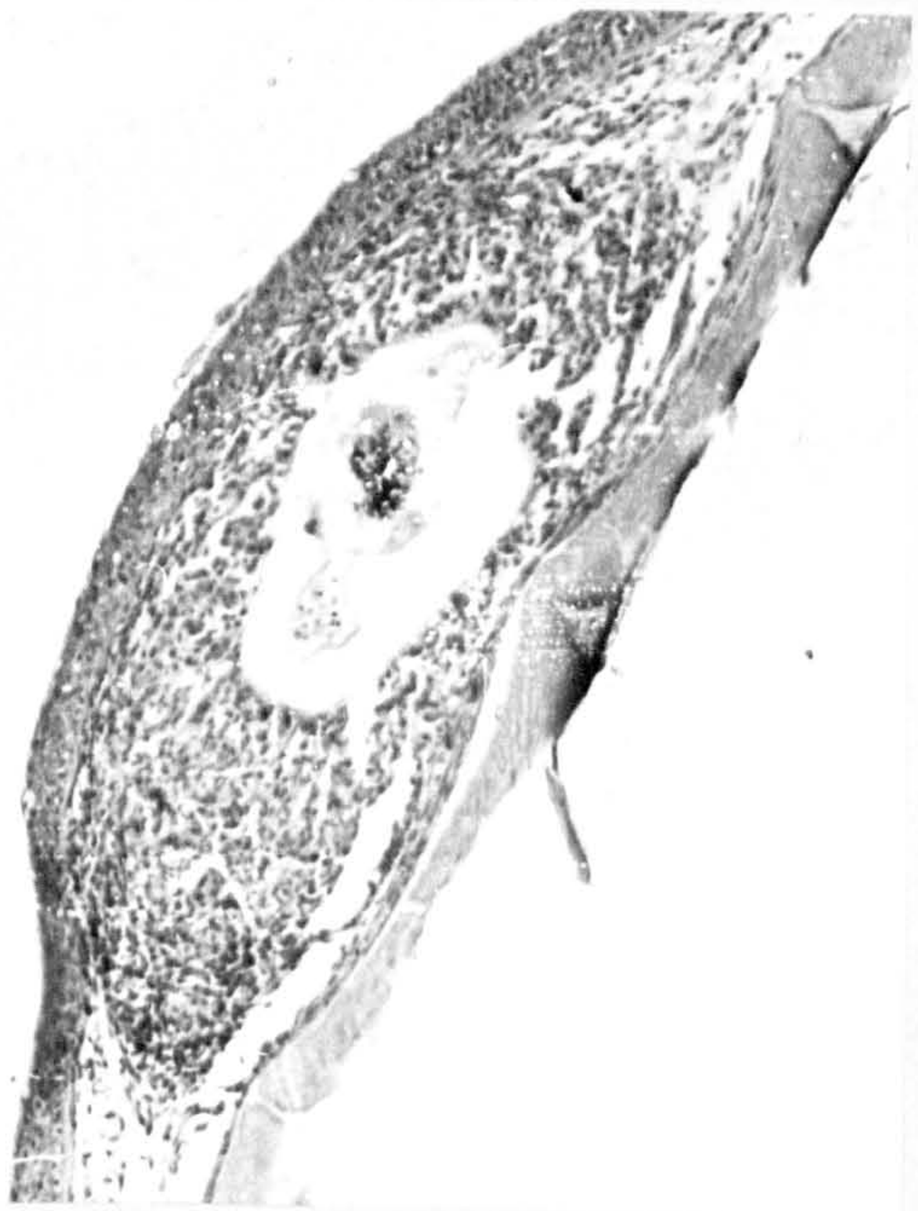


the cysts observed were in the body and fewer in the fins. Occasionally larvae penetrated deeper into the muscles of the body than was seen in the other host species. The path of migration was evident by the appearance of disrupted dermal fibres and local areas of fragmented muscle. (Figure 44) By 27 hours, the cercariae had encysted, although the vacuolated appearance of the parenchymal cells of the metacercariae was not seen until 55 hours.

The earliest signs of capsule formation were evident at 27 hours and consisted of a few fibroblasts applied to the paracyst with fibroblasts and one or two small tissue macrophages in the peripheral connective tissue. (Figure 45) Little change occurred until 96 hours when a number of leukocytes could be seen in the blood vessels throughout the fin and there were signs of the clustering of cells at the poles of the cyst. Even at 7 days the rather loose host capsule was formed from only 1 - 2 layers of cells. This was in contrast to the marked response seen in the P. platessa at this time. (Figure 46)

Tissue response from 17 - 21 days: There was considerable variation seen in the responses to different paracysts. In some cysts, particularly those in the muscle, there was little response, even at 14 days muscle cysts could be seen to produce no response at all. (Figure 47) However, in some fin cysts the capsule was 14 - 40 mu and consisted of a loose aggregation of leukocytes and histiocytes. (Figure 48) Some degeneration of these was taking place in the inner capsule layer at 14 days and some collagen fibres were present. (Figure 49) Connective tissue in the vicinity contained numerous macrophages, some of which had phagocytosed melanin. Macrophages were also seen in blood vessels

Figure 44. S. solea 12 hours post infection. Section through a metacercaria in the somatal myotomes. Note the disrupted dermal fibres and myofibres in the penetration path H.E. (x 250)

df : dermal fibres

dm : disrupted myofibres

m : metacercaria

Figure 45. S. solea 27 hours post infection. Section through a cyst showing the beginning of capsule formation by fibroblast proliferation and a few tissue macrophages M.S.B. (x 400)

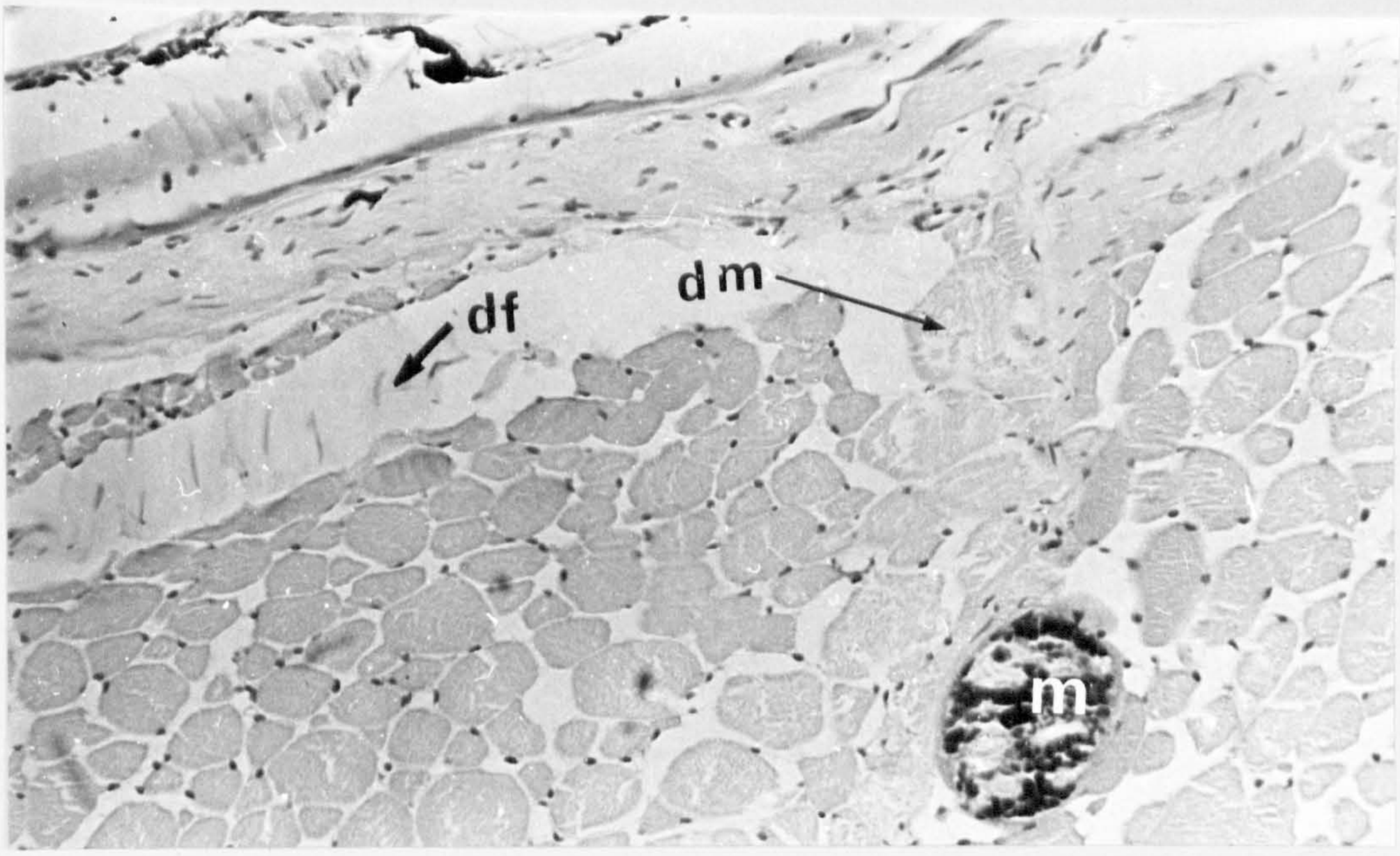
ma : macrophage

pf : proliferating fibroblasts

Figure 46. S. solea 7 days post infection. Section through a metacercaria showing only a thin host capsule formed at this stage. Note the vacuolation of parenchymal cells and the large excretory bladder of the larva M.S.B. (x 400)

ex : excretory bladder

v : vacuole


df
dm
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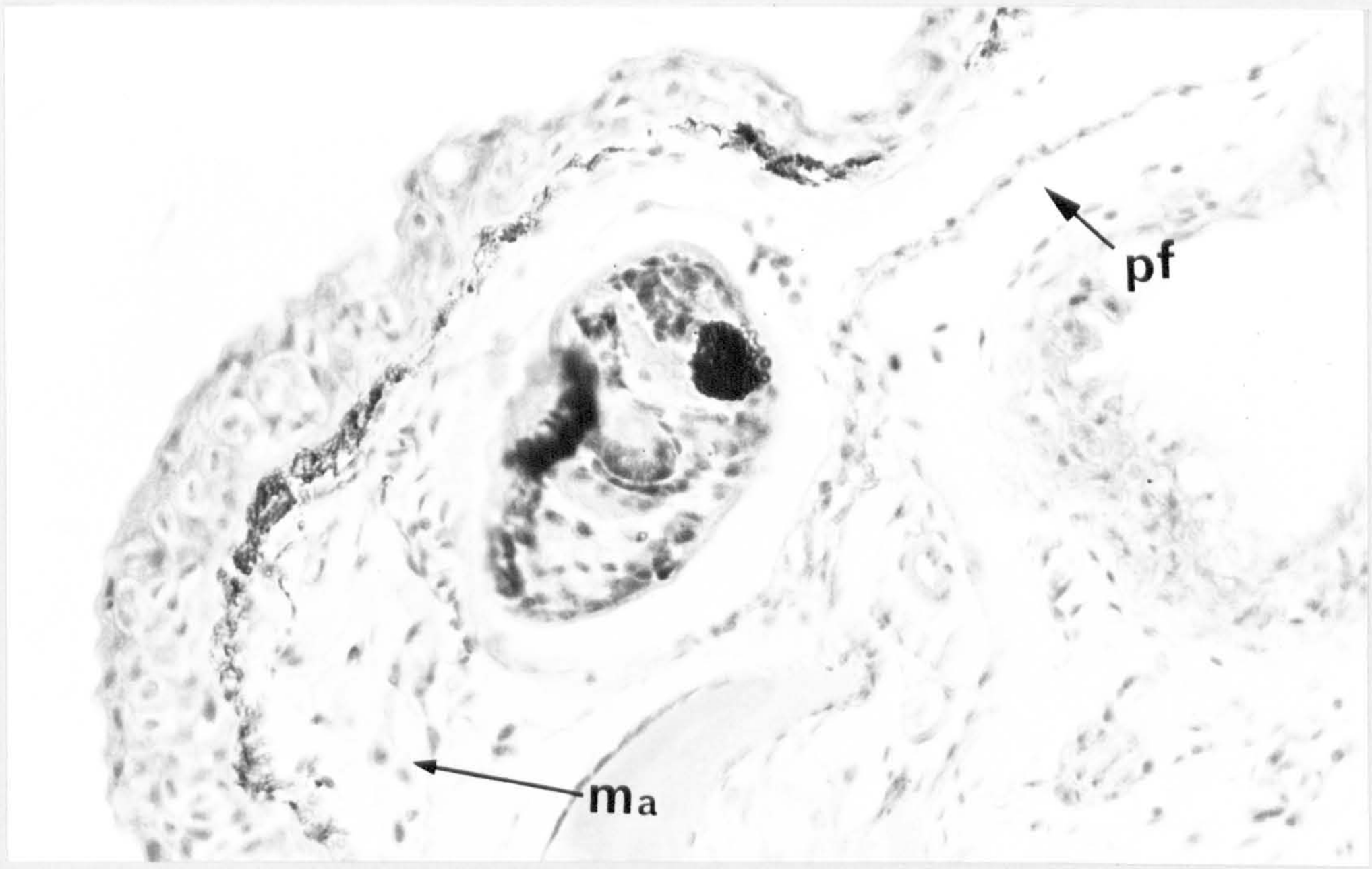
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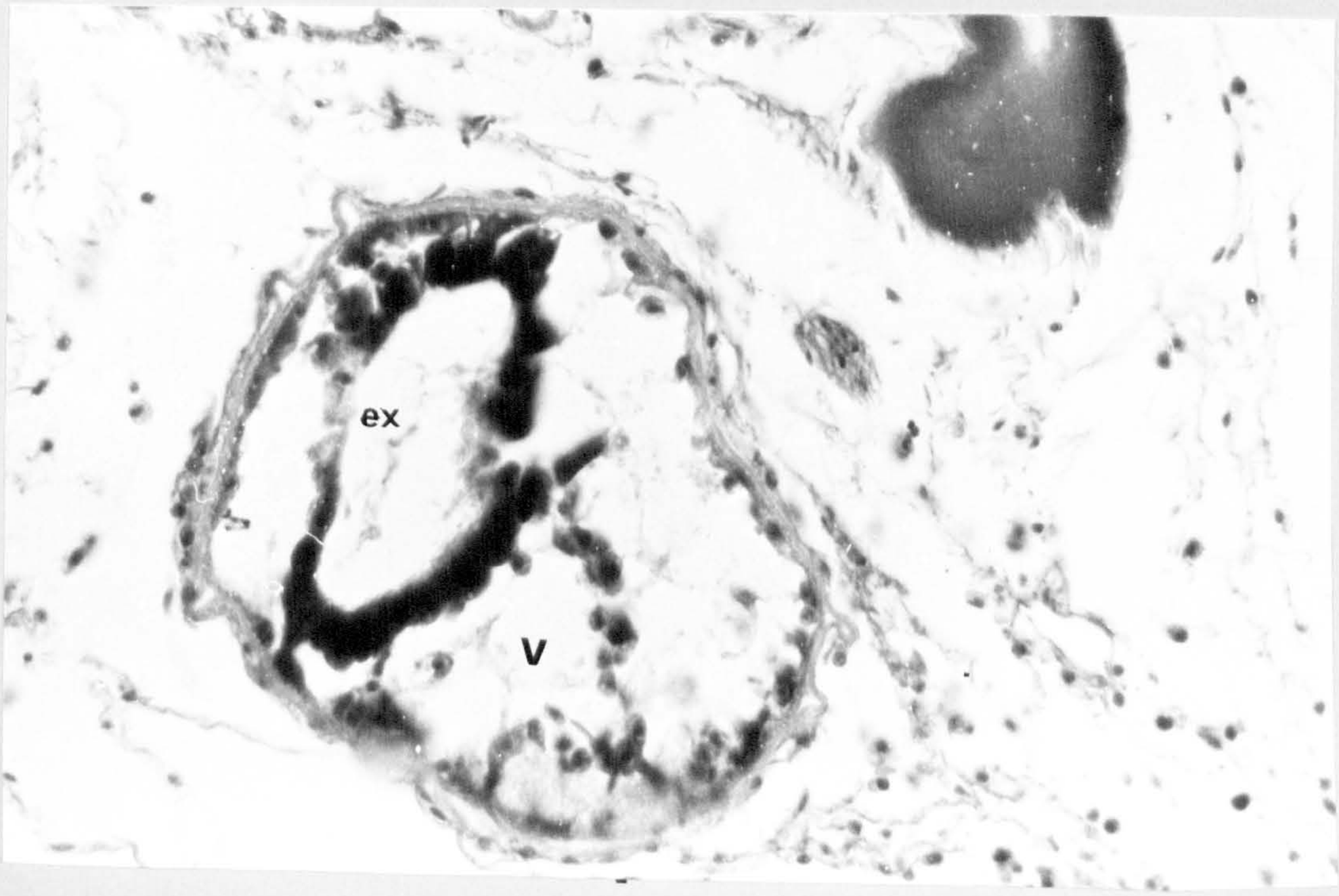
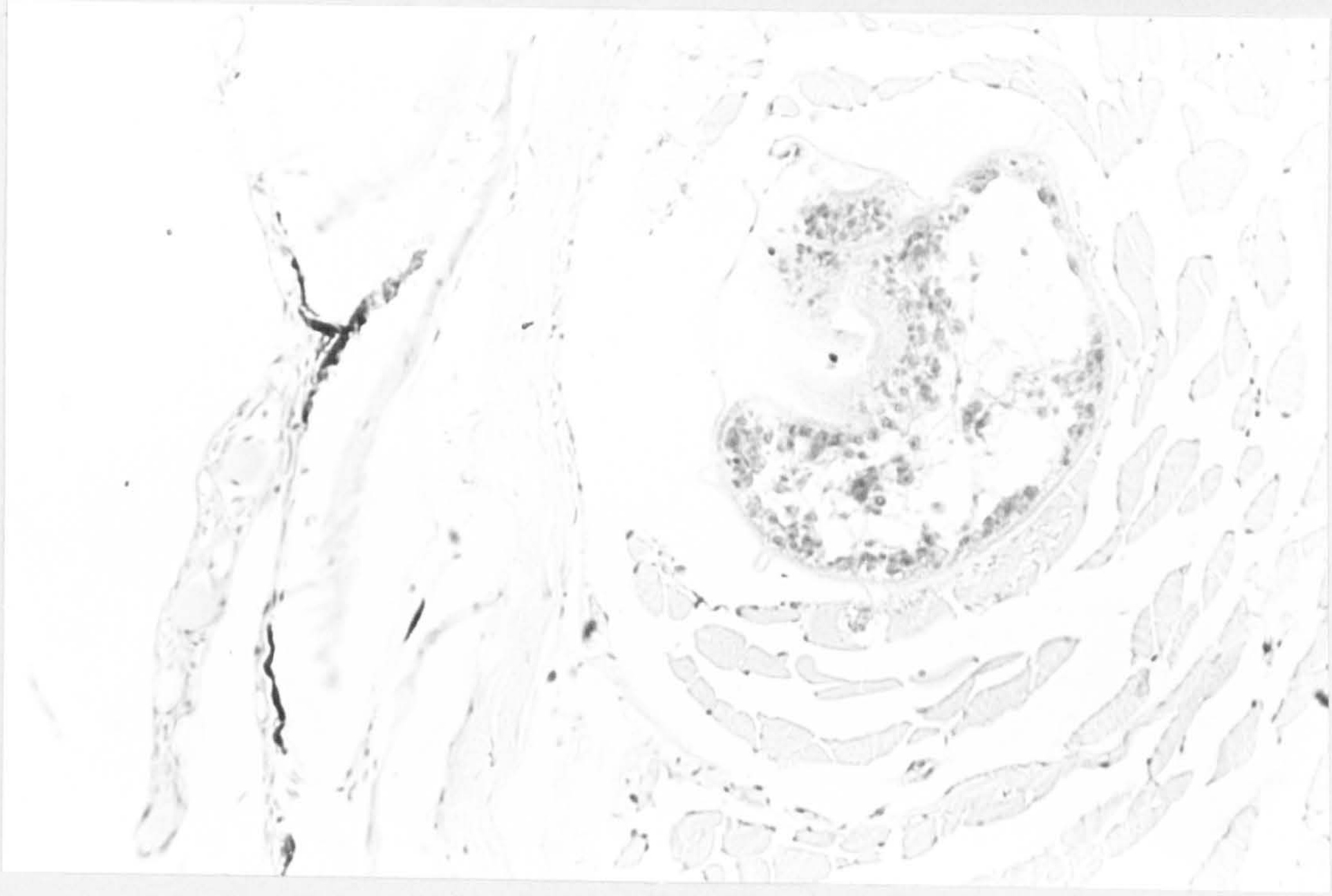
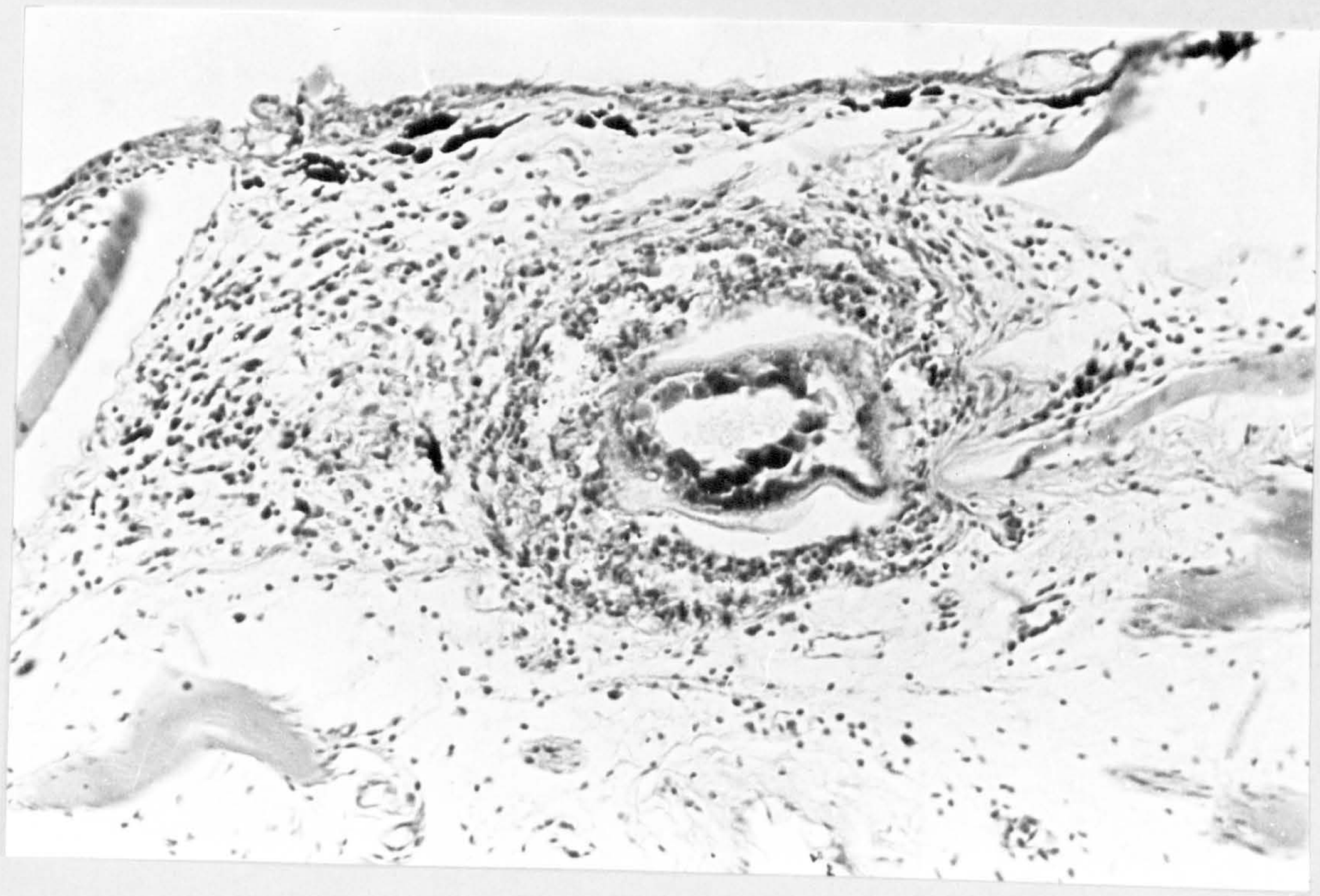

ex
v

Figure 47. S. solea 14 days post infection. Section through a muscle cyst illustrating the lack of host response even at this stage M.S.B. (x 250)

Figure 48. S. solea 14 days post infection. Section through a cyst in the fin of the same fish host as above. The capsule consists of a loose aggregation of leukocytes and histiocytes M.S.B. (x 250)





in the locality of the cyst.

Tissue response from 21 - 31 days: Capsules surrounding paracysts in the fin had now taken on an epithelioid appearance and numerous macrophages were still to be seen in the neighbouring blood capillaries. Muscle cysts, even in the same fish, had thinner capsules which were composed largely of fibroblasts probably derived from the perimysial membrane. By 28 days the muscle cyst capsule had become more compact and the epithelioid tissue was showing signs of degeneration. Muscle degeneration was seen at 21 days but at 28 days there were small regenerating myofibres.

General observations on cyst development

The cysts in the fin frequently caused distortion of the fins (Figure 50) and, in one case (at 14 days), this had resulted in severe erosion of the epidermis such that the capsule was exposed. More often, however, the skin appeared normal except for a slight attenuation of the epidermal cell over the cyst. No hyperplasia was seen and often the capsule was thinner on the side adjacent to the dermis or epidermis.

The S. solea response to infection by S. baccatus appeared to be less well organised than in the P. platessa with a non-specific stimulation of macrophages of the whole tissue around the parasite rather than a localised focal inflammatory response directed at the parasite per se. Fibroblast activity seemed to be greater than seen in P. platessa and L. limanda lesions.

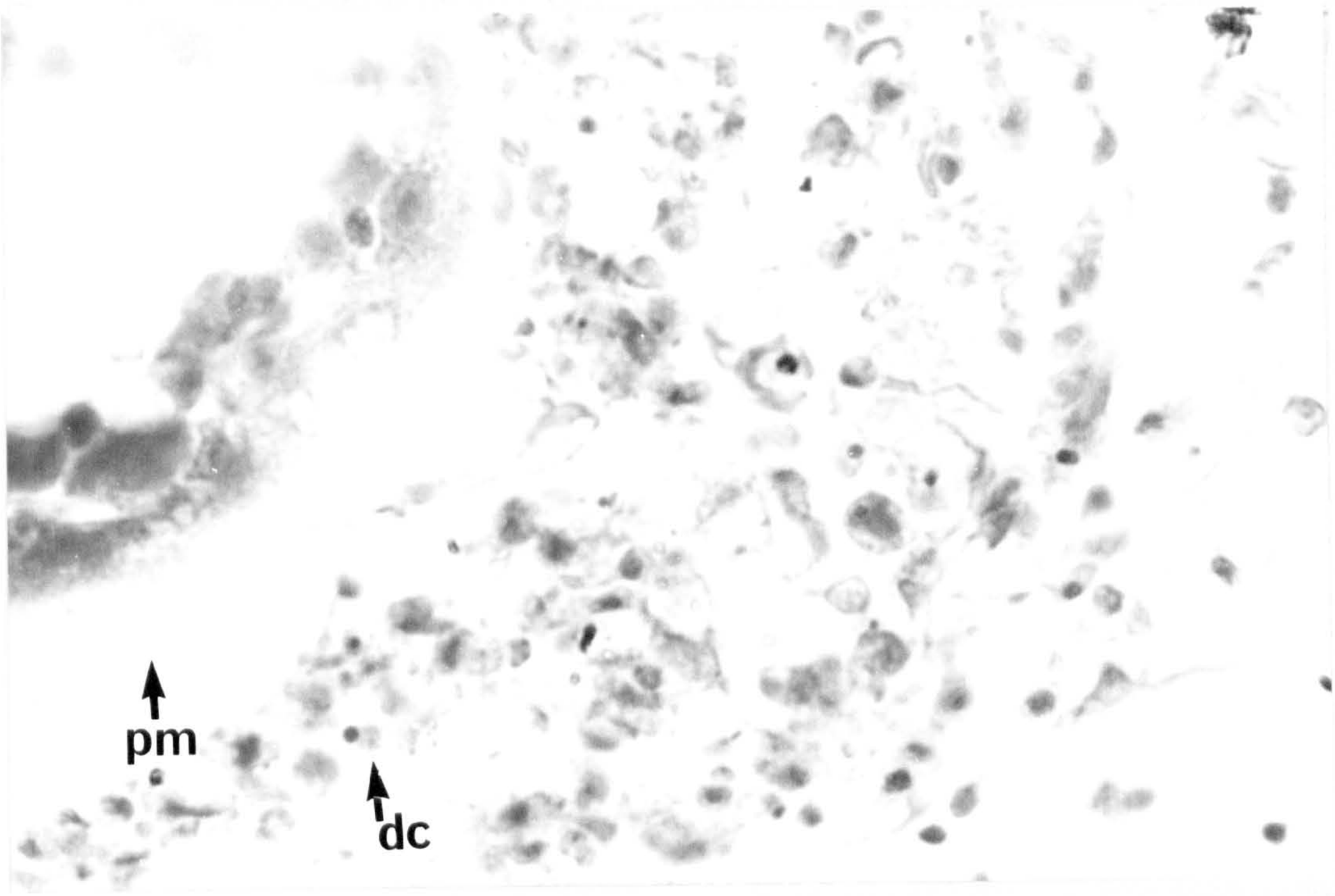
The development of the cyst in S. solea is illustrated in Figure 51.

Figure 49. S. solea 14 days post infection. Section through the capsule showing early degeneration of the epithelioid tissue of the early layers. H.E. (x 100)

dc : degenerating cells

pm : paracyst membrane

Figure 50. S. solea 21 days post infection. Section through a cyst in the fin which has caused a marked distortion of the fin profiles. H.E. (x 100)


pm
dc

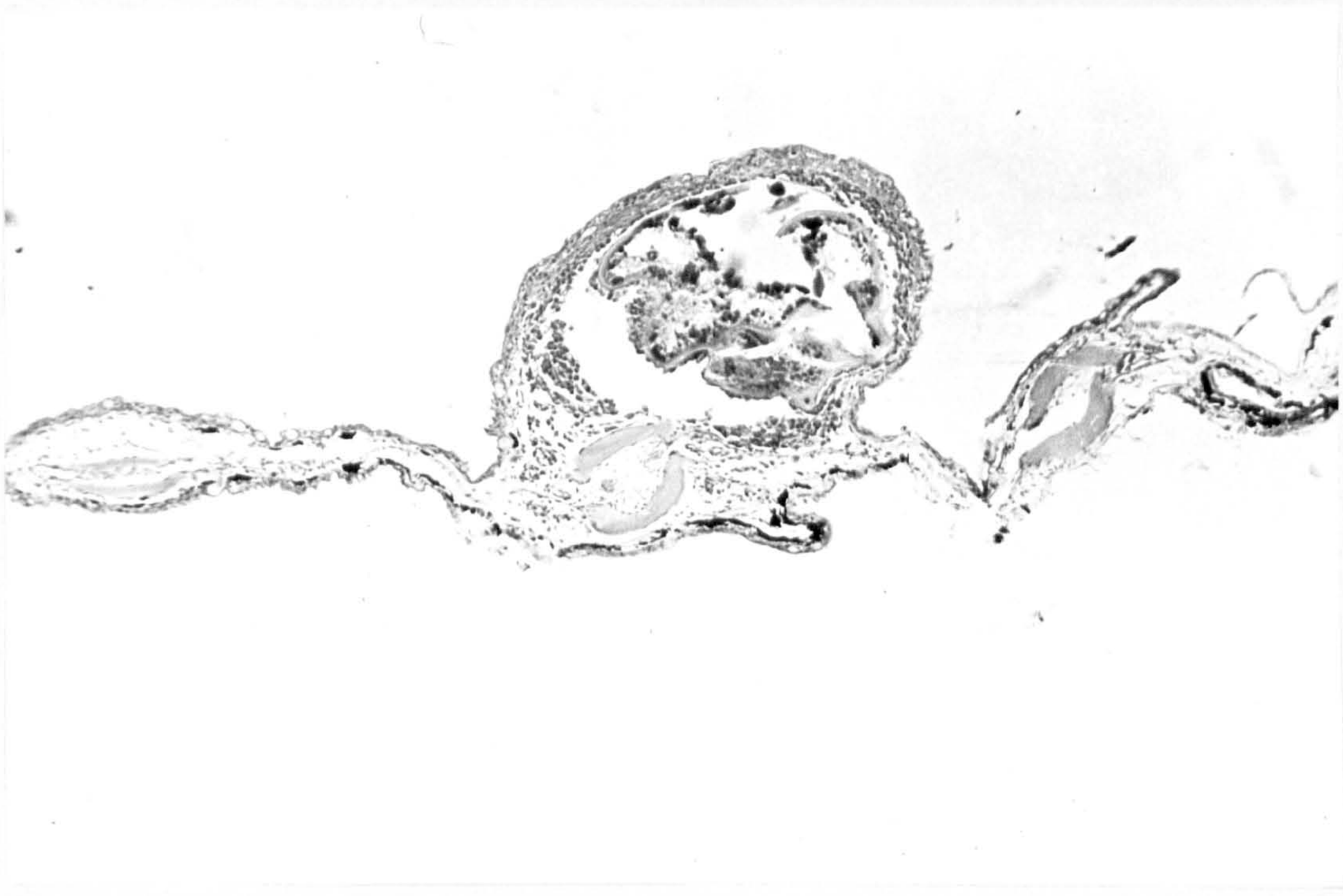
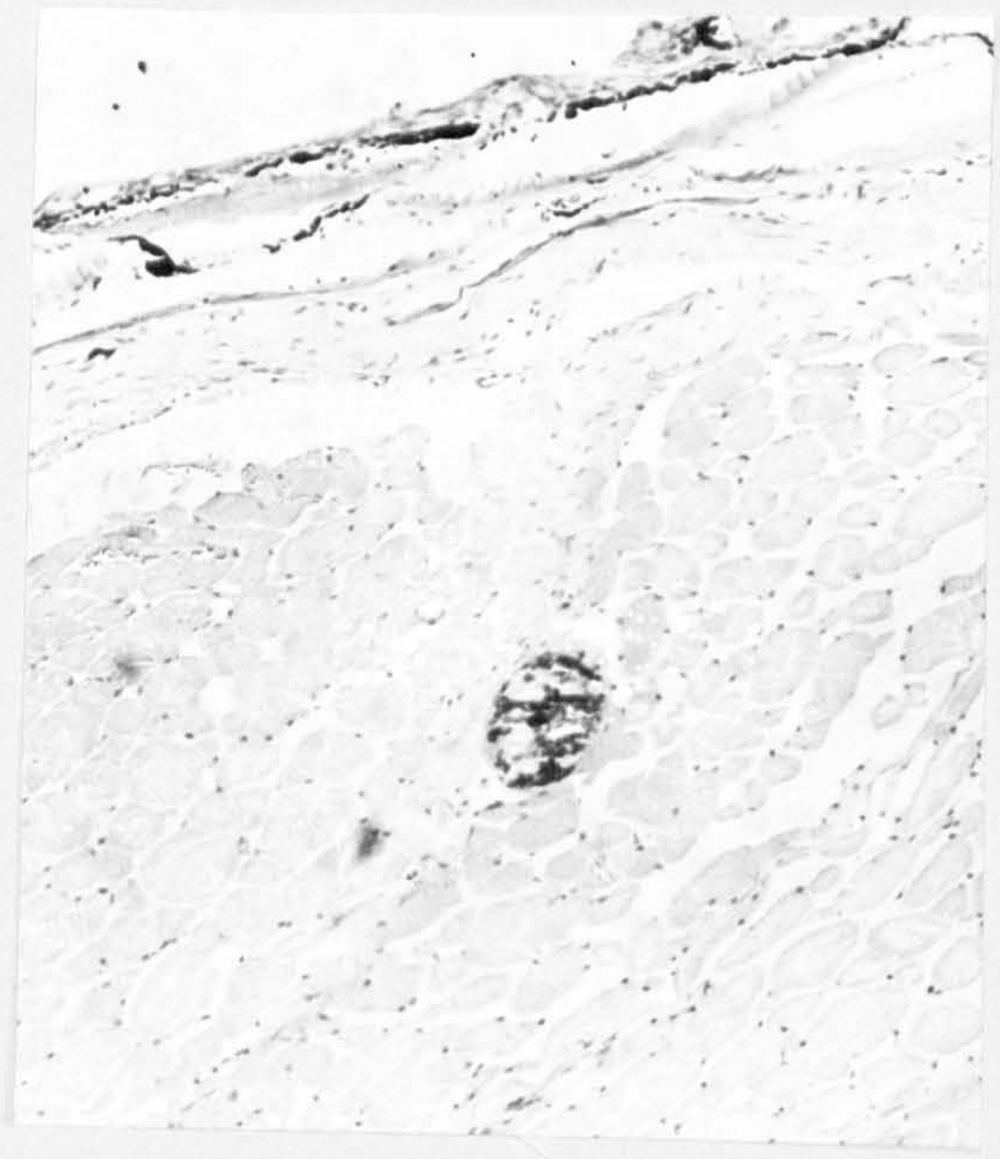


Figure 51. Development of S. baccatus cysts in S. solea

(a) 12 hours (x 100)
(b) 27 hours (x 100)
(c) 7 days (x 100)
(d) 14 days (x 100)
(e) 21 days (x 100)
(f) 28 days (x 100)

All photographs have the same visual magnification of x 150.

a



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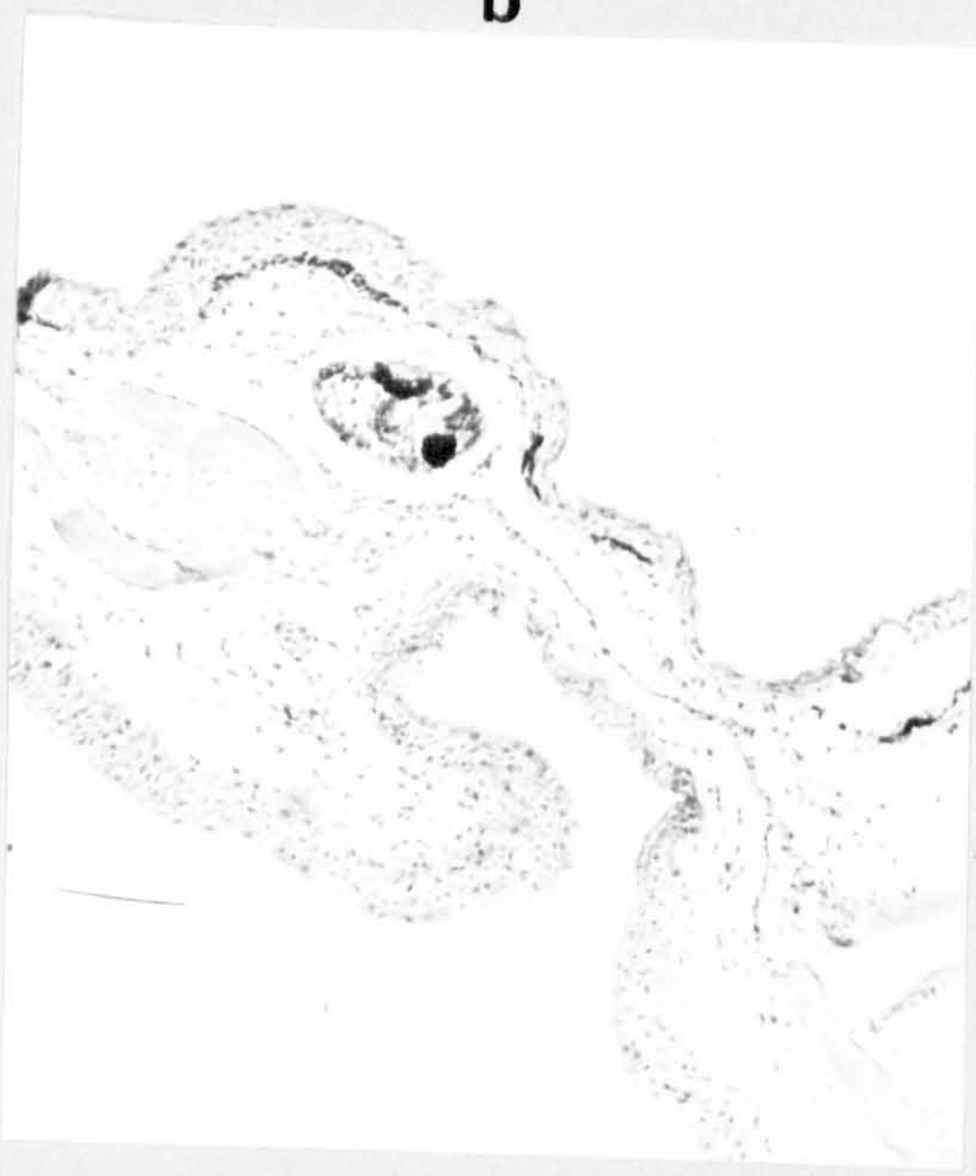

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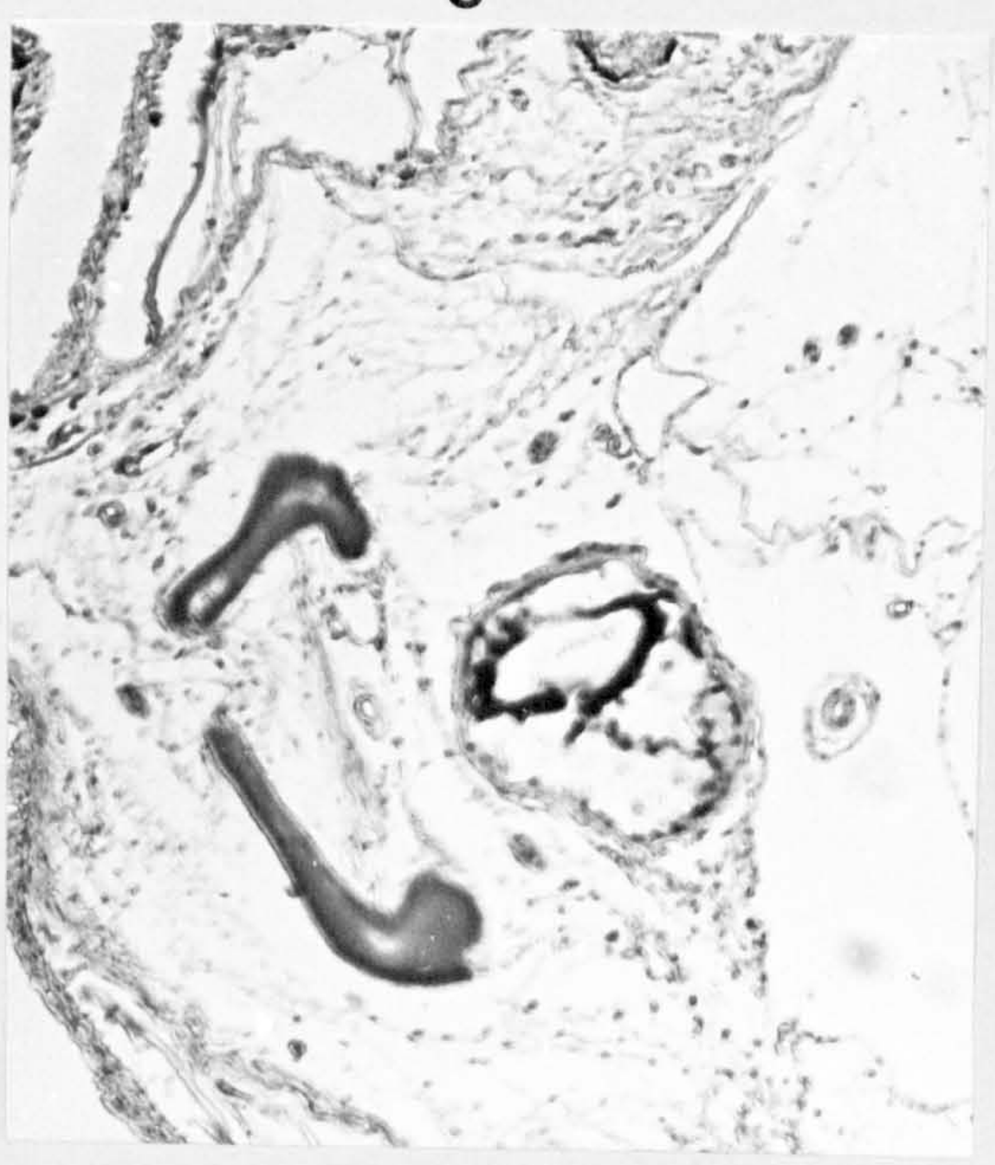

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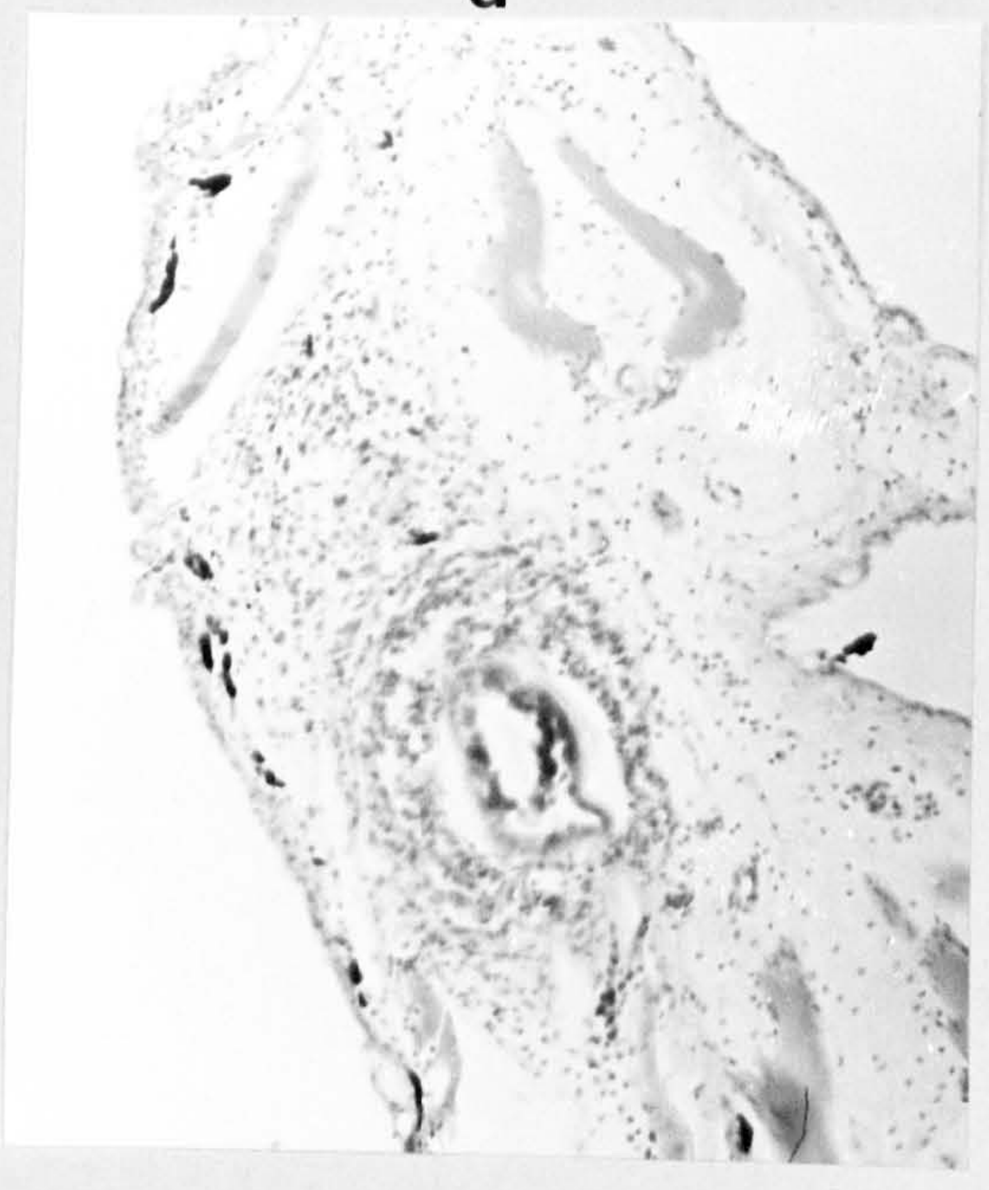

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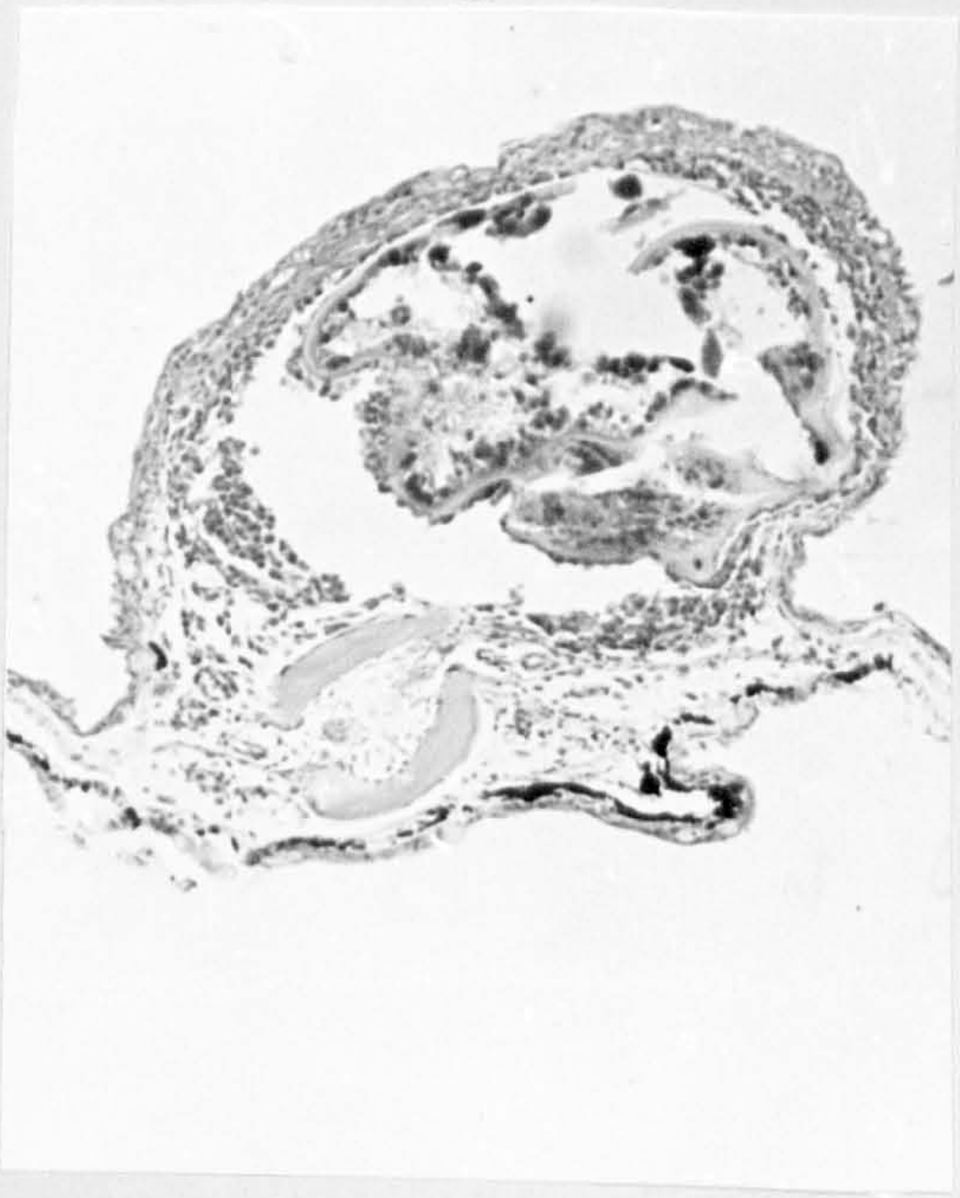

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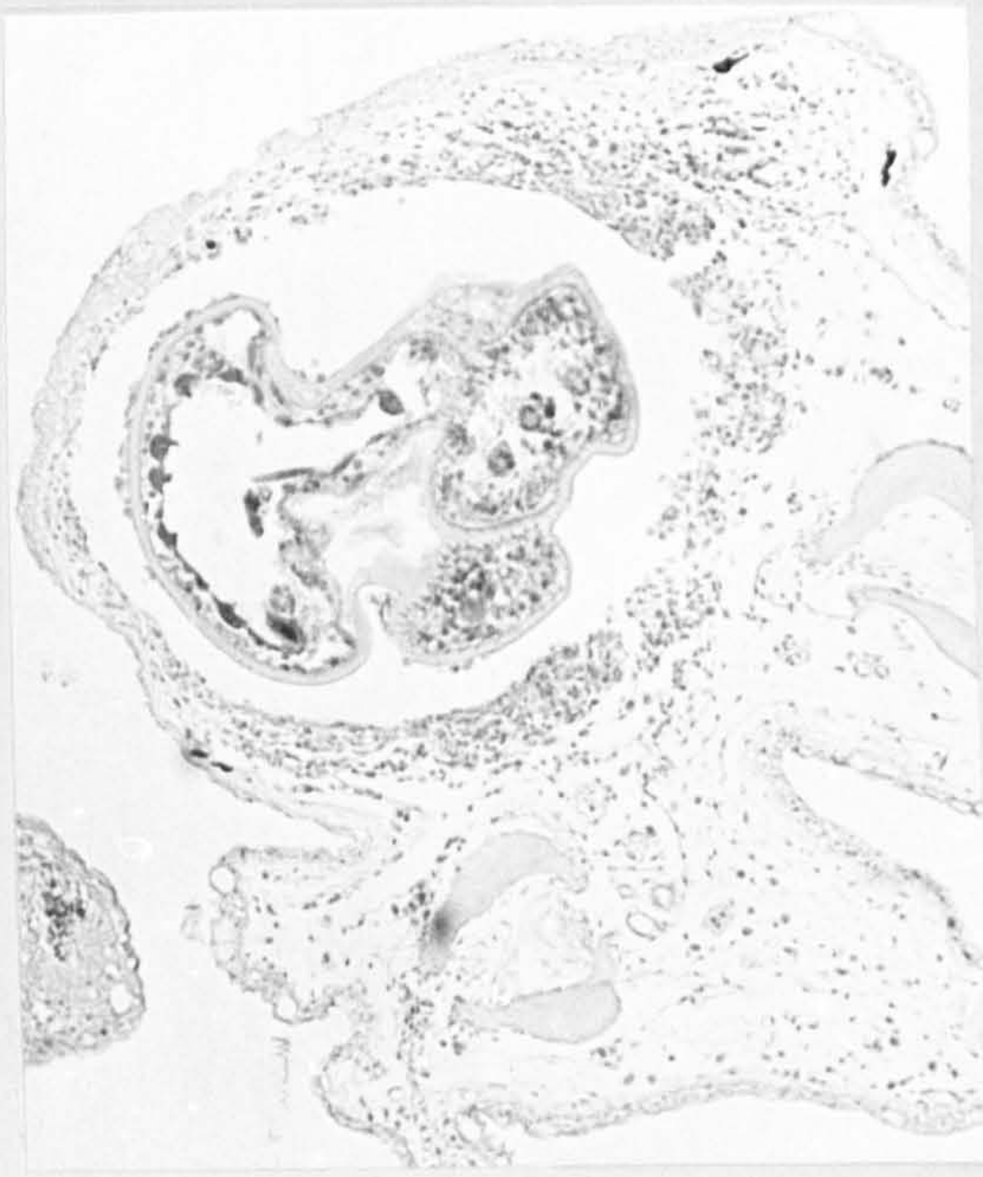


THE TURBOT Scophthalmus maximus (L)

Site

S. baccatus cysts were found to occur most frequently in the fins and all those examined histogically were situated in the loose connective tissue adjacent to the fin rays or in the fin membrane. Cysts in the skin were so small that they were impossible to see without dissection and, therefore, destruction of the skin.

Tissue response at 2 hours: As in the other fish studied, the cercariae were still penetrating the superficial layers of the skin at this time. All of the cercariae observed were in the epidermis although one individual was in the act of penetrating the basement membrane. (Figure 52) The cells of the epidermis appeared normal though in some specimens the Malpighian cells were separated, as shown by a loss of normal tessellation. (Figure 53)

Tissue response after 5 hours: Many cercariae had penetraded into the dermis, but had not yet encysted. There was no apparent effect on the host and tissues surrounding the larva appeared intact.

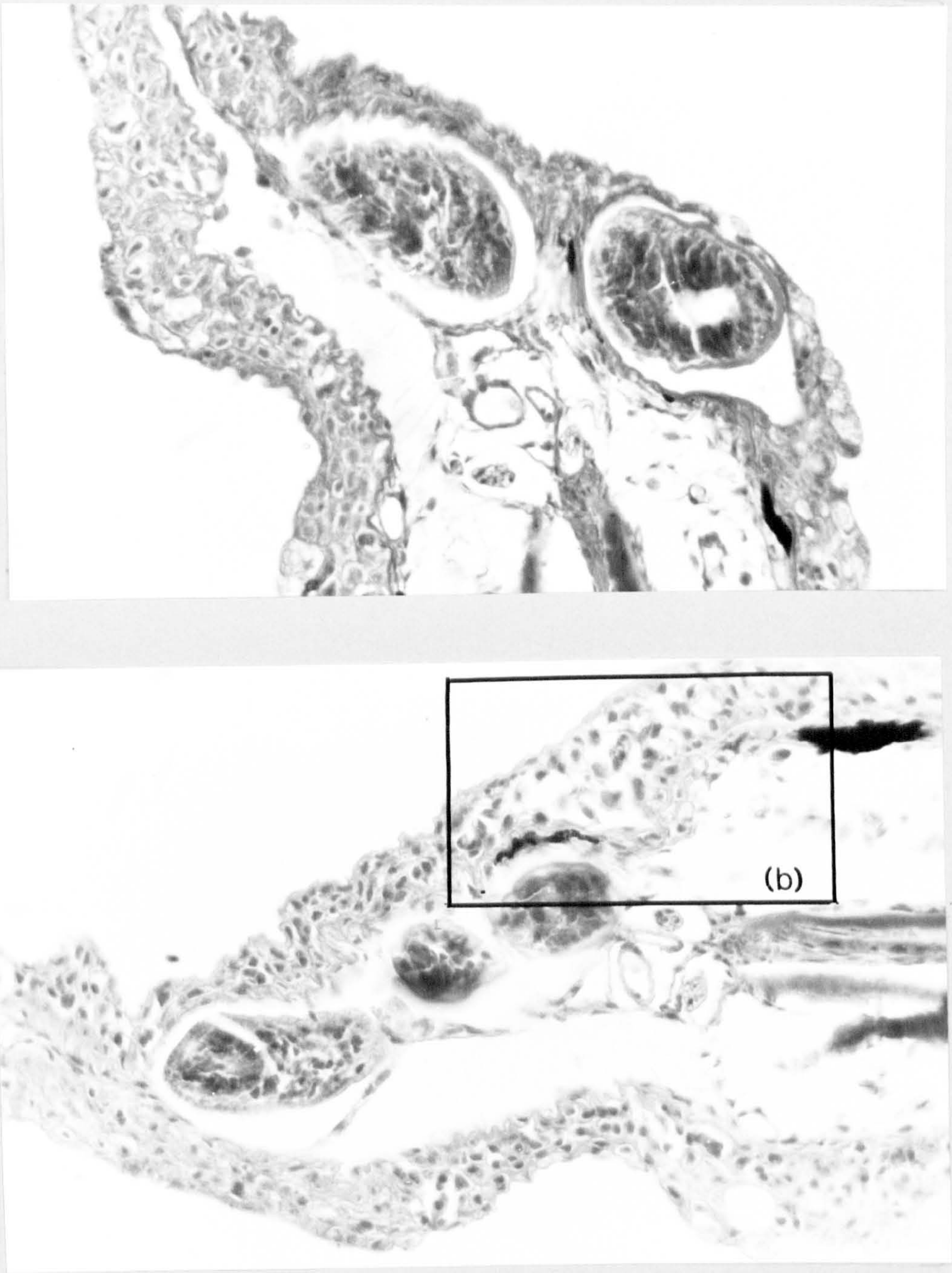
Tissues response after 10 hours: Some of the larvae had encysted and a very thin paracyst membrane was present. Already, at 10 hours, there was a small host capsule forming, represented by individual cells, mainly macrophages, applied to the paracyst. (Figure 54) These cells were probably of tissue origin since it is unlikely that a significant vascular response would be activated at this stage. There was no evidence of active tissue damage by the migrating parasite.

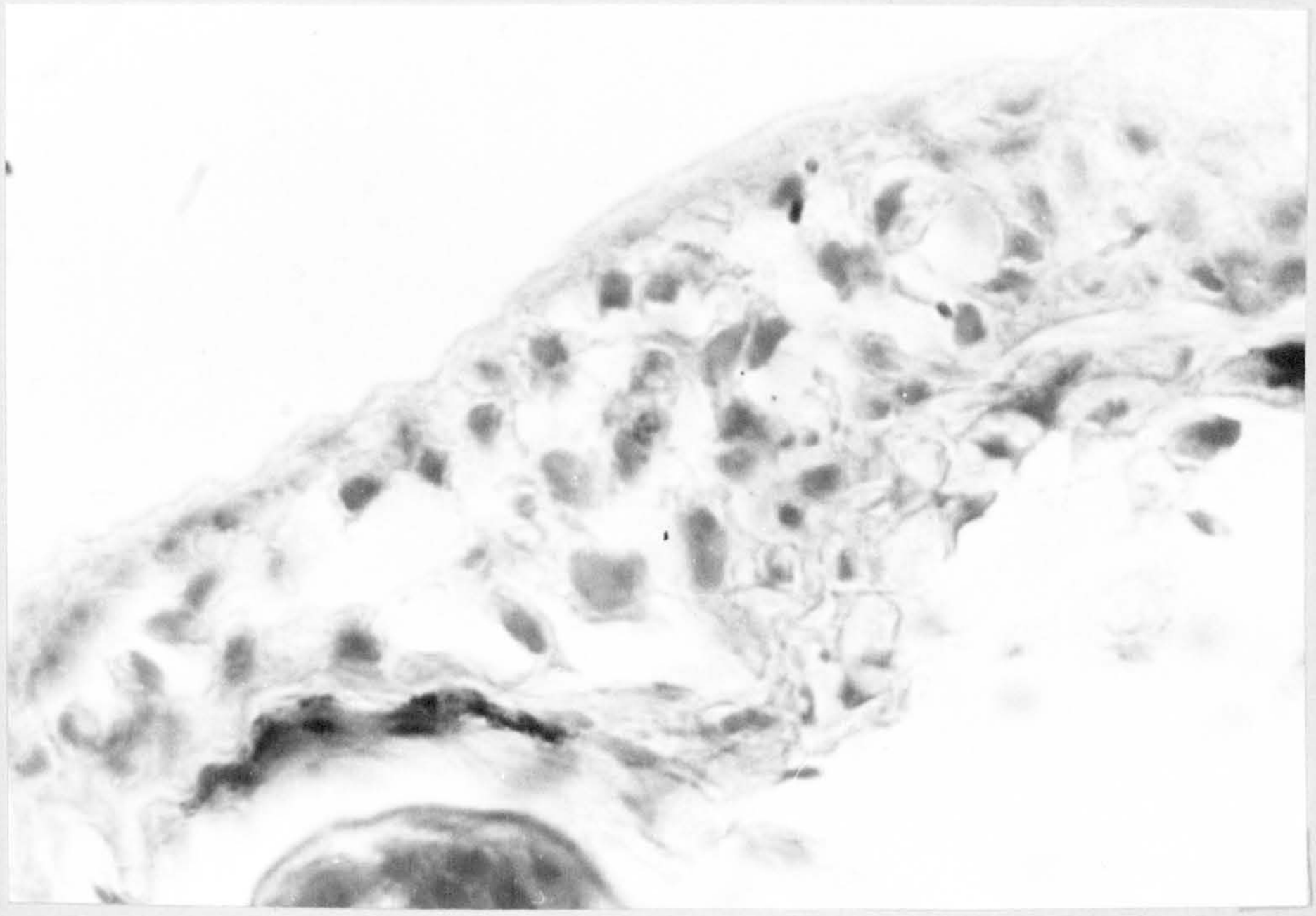
Figure 52. S. maximus 2 hours post infection. Section showing the penetration of the basement membrane by a cercaria P.A.S. (x 400)

Figure 53. S. maximus 2 hours post infection.

(a) Cercaria penetrating the dermis of the fin M.S.B. (x 400)

(b) High power view of the epidermis showing the separation of the Malpighian cells in the penetration path, at the posterior of the cercaria M.S.B. (x 1000)


a
(b)


b

Tissue response after 24 hours: The host capsule was already well defined and consisted of a single layer of host cells. These cells formed a compact wall such that their individual characteristics were indistinguishable. (Figure 55) However, the faint PAS positive staining of the cytoplasm indicated that the capsule was formed, at least in part, from the macrophages. These also occurred in the peripheral connective tissue and in the neighbouring blood vessels.

The paracyst membrane was much more clearly defined than at 10 hours.

Tissue response after 48 hours: By this time the capsule was several cells thick and very compact with the outer cells more flattened and lying in concentric layers. (Figure 56) The outer capsule was more fibrous than previously seen. Diapedesis continued from adjacent blood vessels. (Figure 58)

In one particular case the presence of the parasite appeared to have incited an extremely severe inflammatory response with the result that the paracyst, which had ruptured, was surrounded by host cells with the resulting death and disorganisation of the metacercaria. (Figure 57) The cells of the parasite were not distinguishable from the invading host cells. One macrophage in the lesion was loaded with melanin and this may have originated from the pigmented eyespot of the metacercaria.

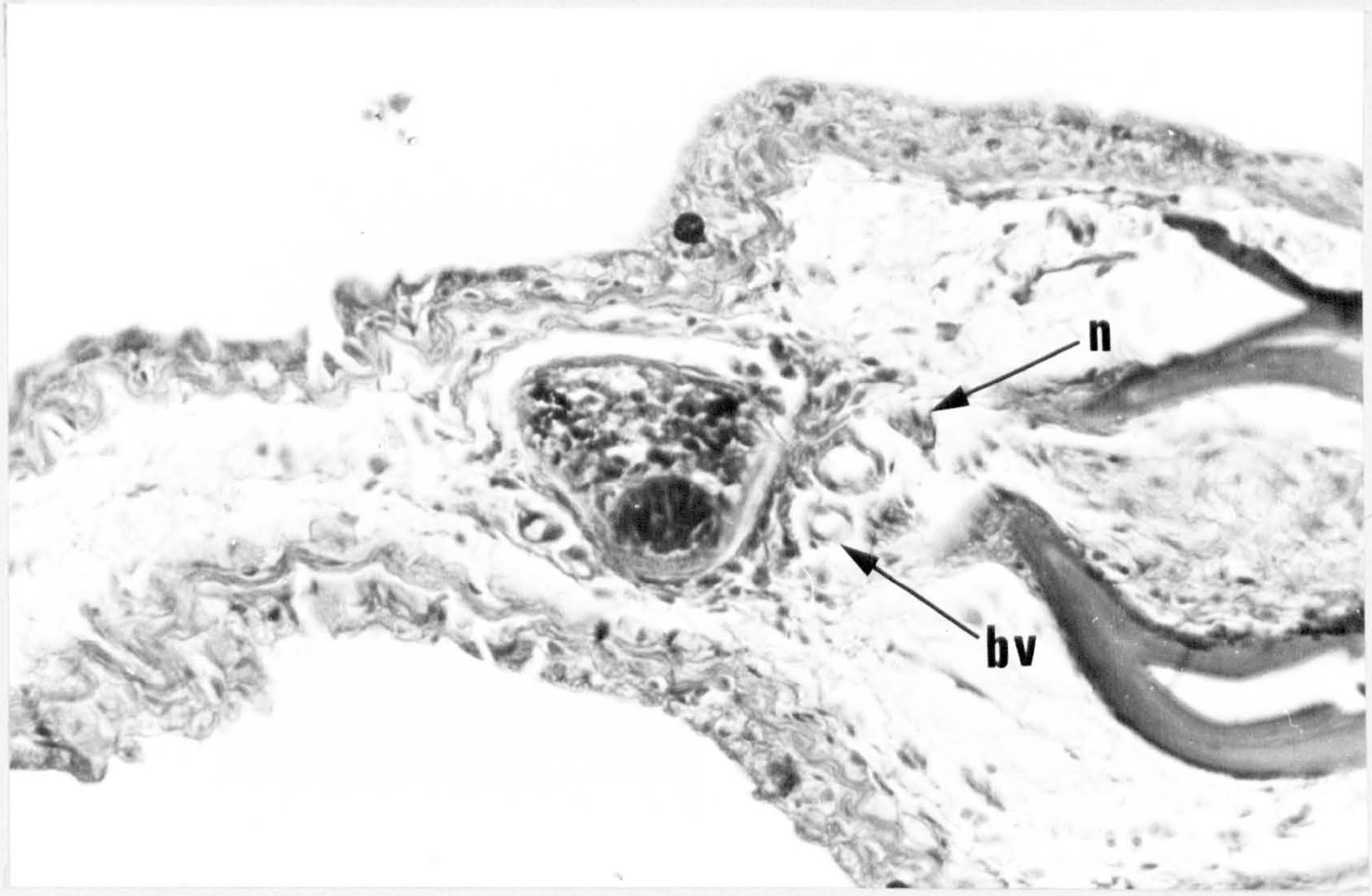
Tissue response after 96 hours: The host capsule was 5 cells thick and the inner layer of cells approximately 5 - 6 mu was losing its cellular appearance, becoming a dense matrix in which were embedded the nuclei, some of which were karyorhectic. The middle

Figure 54. S. maximus 10 hours post infection. Section through a newly encysted metacercaria which, even at this early stage, shows the formation of a small host capsule. Note the proximity of the cyst to the blood vessels and nerves in this region. This site was typical of fin cysts P.A.S. (x 400)

bv : blood vessel

n : nerve

Figure 55. S. maximus 24 hours post infection. Section showing the already well-defined host capsule formed from a compact wall of inflammatory cells P.A.S. (x 1000)


n
bv

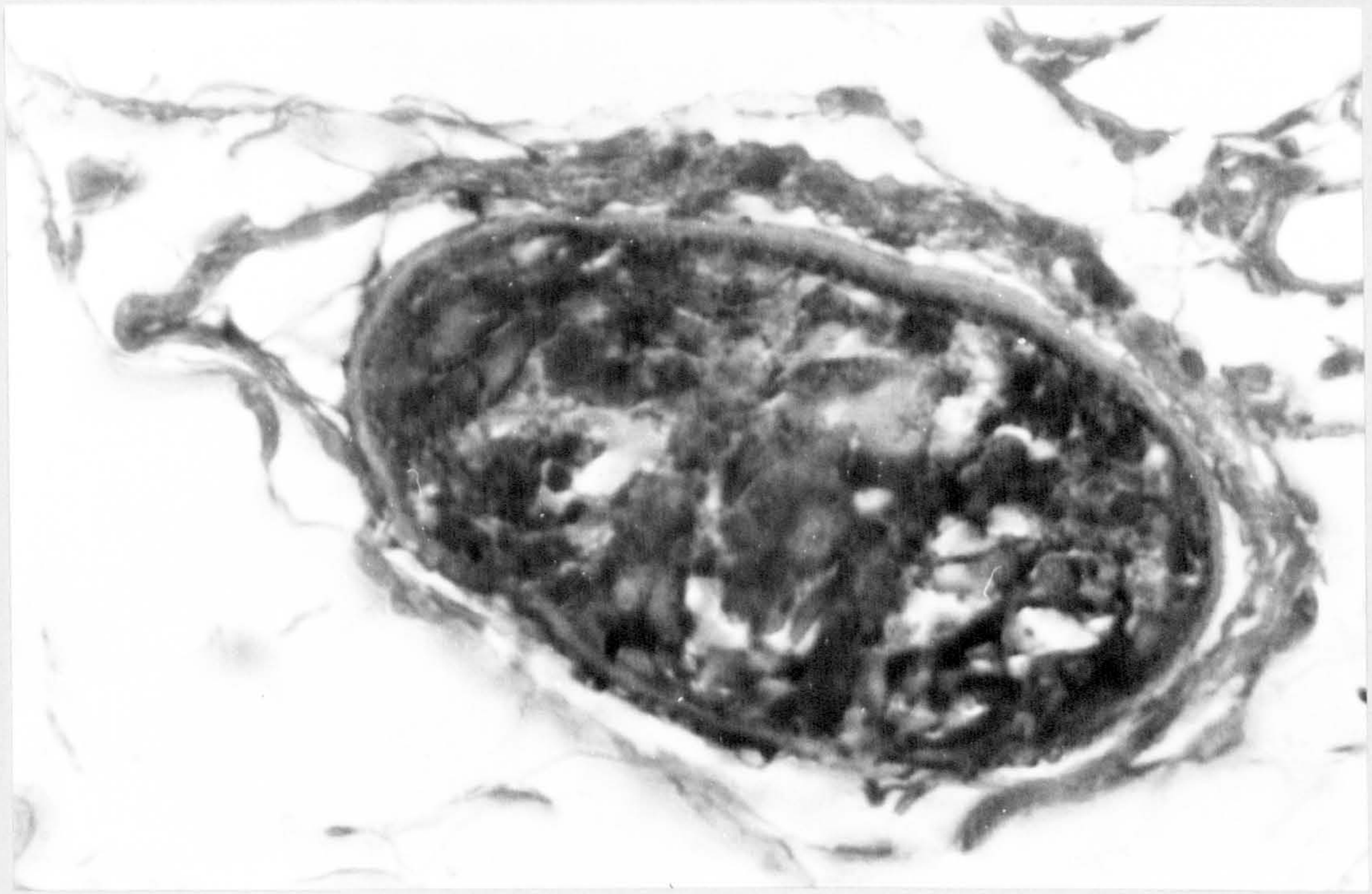


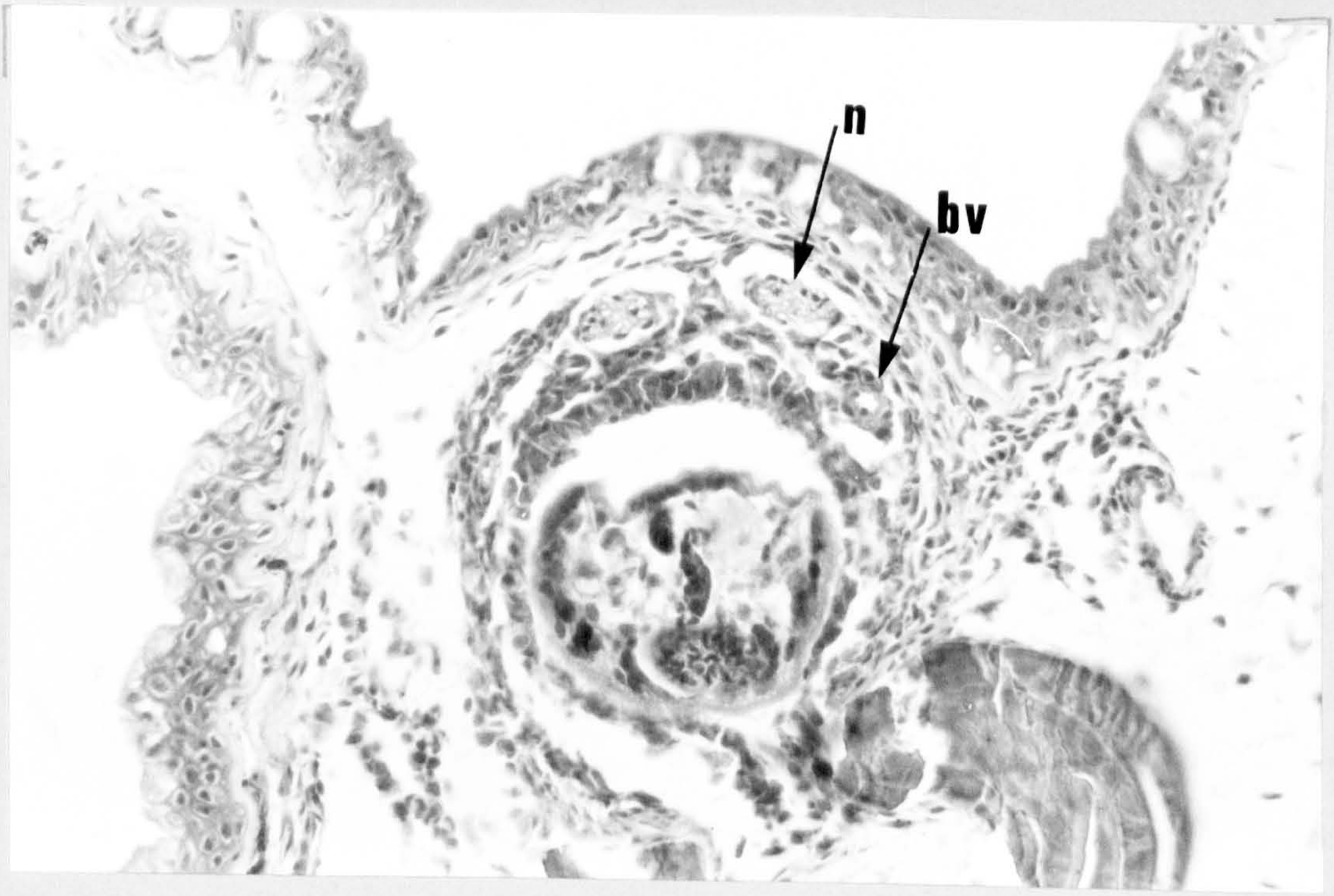
Figure 56. S. maximus 48 hours post infection. Section of a cyst with a compact capsule several cells thick. In the outer capsule fibroblasts lie in concentric layers. Note the incorporation of nerves and blood vessels in the capsule H.E. (x 400)

bv : blood vessel

n : nerve

Figure 57. S. maximus 48 hours post infection. Section through a cyst in which the paracyst has ruptured and the host inflammatory cells have surrounded the larva resulting in complete disorganisation and death of the metacercaria H.E. (x 100)

m: metacercaria


n
bv

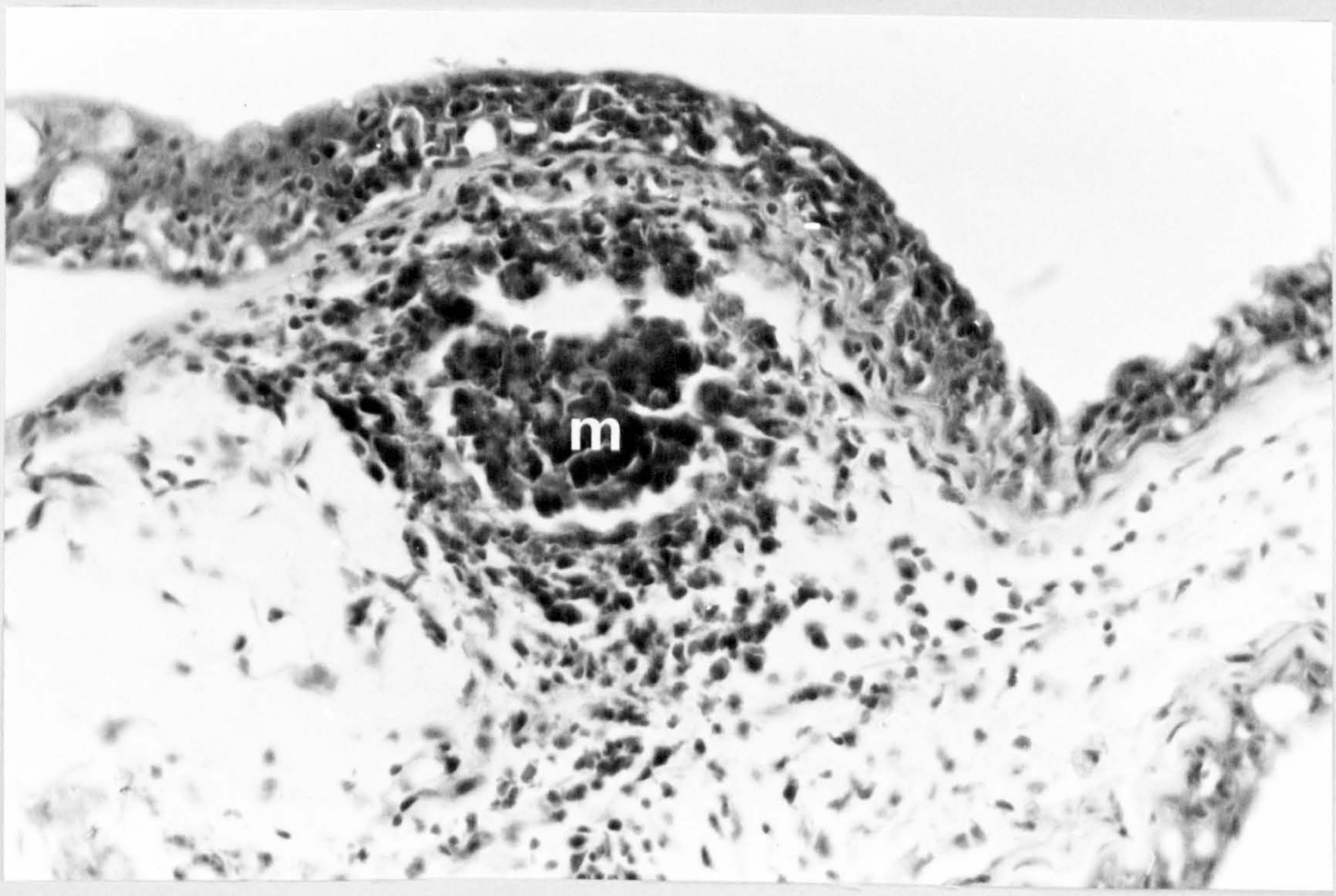
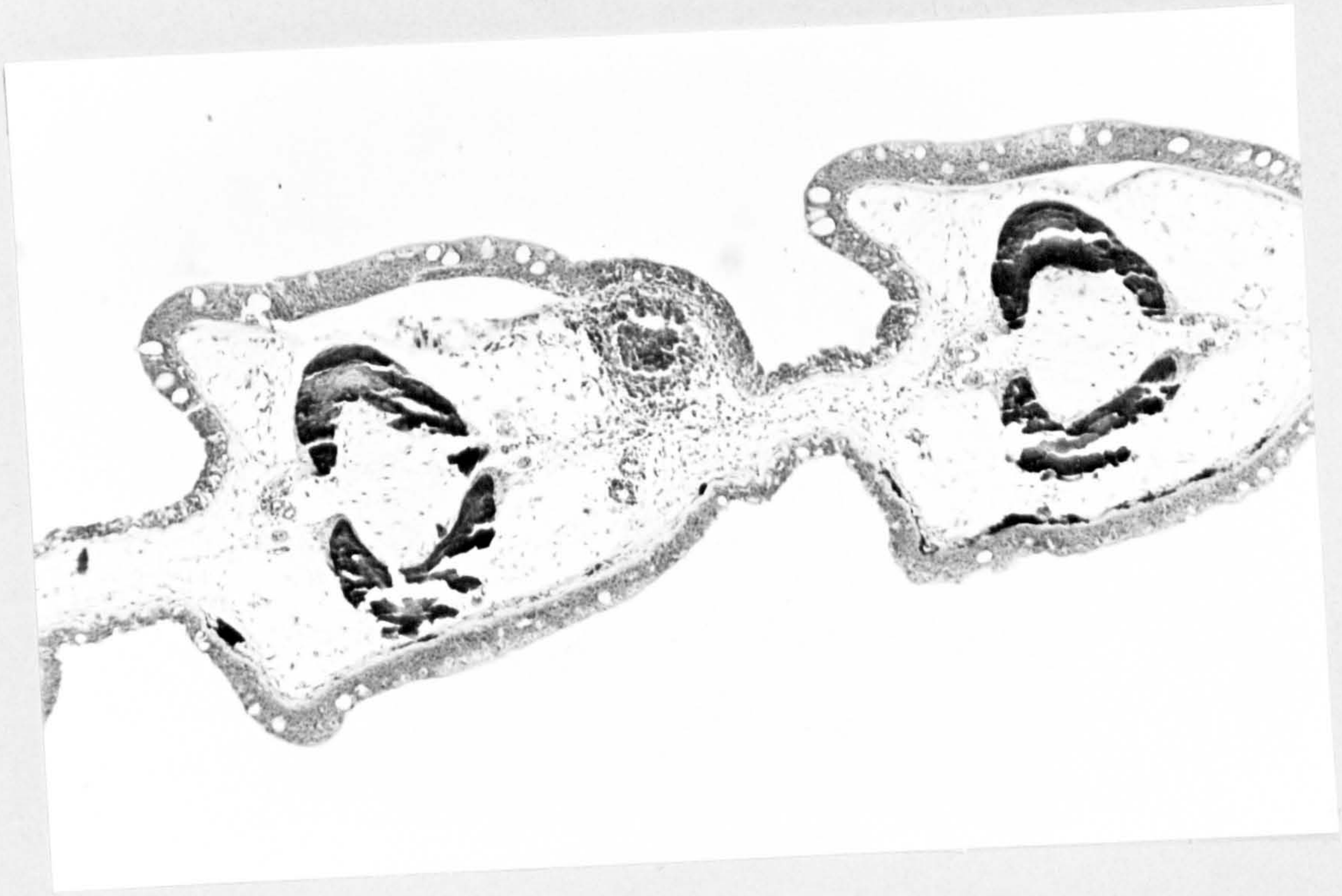
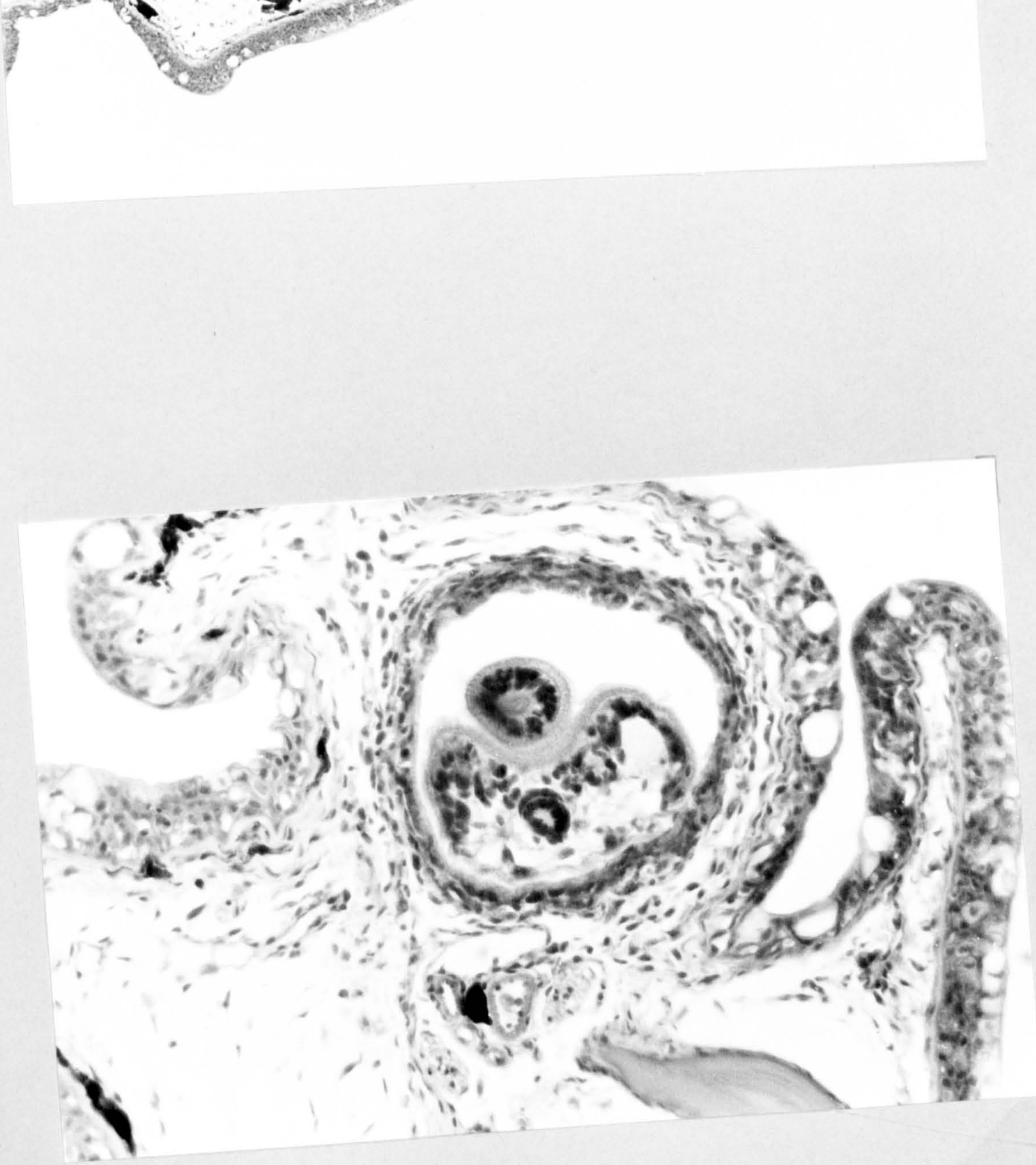

m

Figure 58. S. maximus 48 hours post infection showing migration routes of inflammatory cells into the area of the metacercarial cyst H.E. (x 100)

Figure 59. S. maximus 96 hours post infection. Section showing the advanced capsule at 4 days. Comparable with the capsule of P. platessa at 11 days H.E. (x 400)





layer had an epithelioid appearance while the outer layer consisted of concentric layers of elongated fibroblasts. (Figure 59) It was at this stage that the S. maximus cyst appeared most similar to that of P. platessa but at 4 - 7 days earlier than the comparable state of development in that species.

Tissues response after 5 days: The capsule was similar to that of 96 hours but pyknosis and karyorhexis was more evident in its epithelioid cell component. Diapedesis continued and in some cases there was still a good deal of peripheral cellular activity. Fibres were more evident in the capsule and occasional foci of melanin were also found. Degenerating metacercariae were also seen at this stage and were embedded within a focus of granulomatous tissue.

The metacercaria did not show the vacuolated appearance, found in P. platessa at 48 hours, until 4 - 5 days and this was never as marked as it was in the other hosts studied.

Tissue response after 6 - 10 days: Degenerating metacercariae were again prominent and surviving parasites were embedded within an inner capsule layer now well developed and surrounded by an outer fibroblast layer. (Figure 60) The proliferation of fibroblasts was particularly marked in some cysts. (Figure 61)

Tissue response after 13 days: The capsules were now somewhat thicker (Table XVII) and had a less compact appearance, probably due to the further degeneration in the cellular structure of the leukocytes of the inner capsule. Diapedesis of macrophages from adjacent vessels was not seen after 8 days.

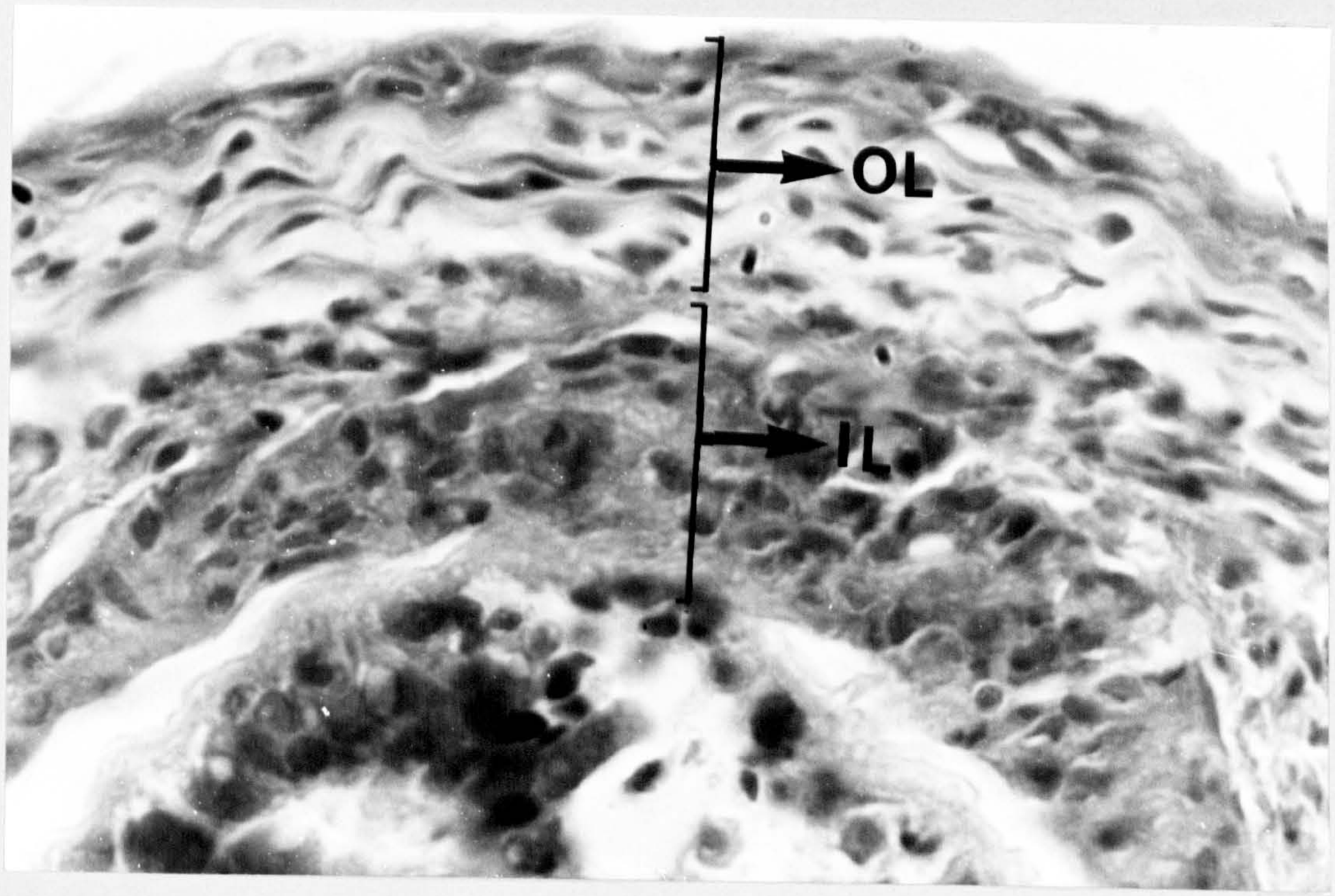
Figure 60. S. maximus 6 days post infection. Section showing the layers of the capsule at this stage. The inner layer is a compact wall of epithelioid tissue and this is surrounded by an outer wall of concentric layers of elongated fibroblasts H.E. (x 1000)

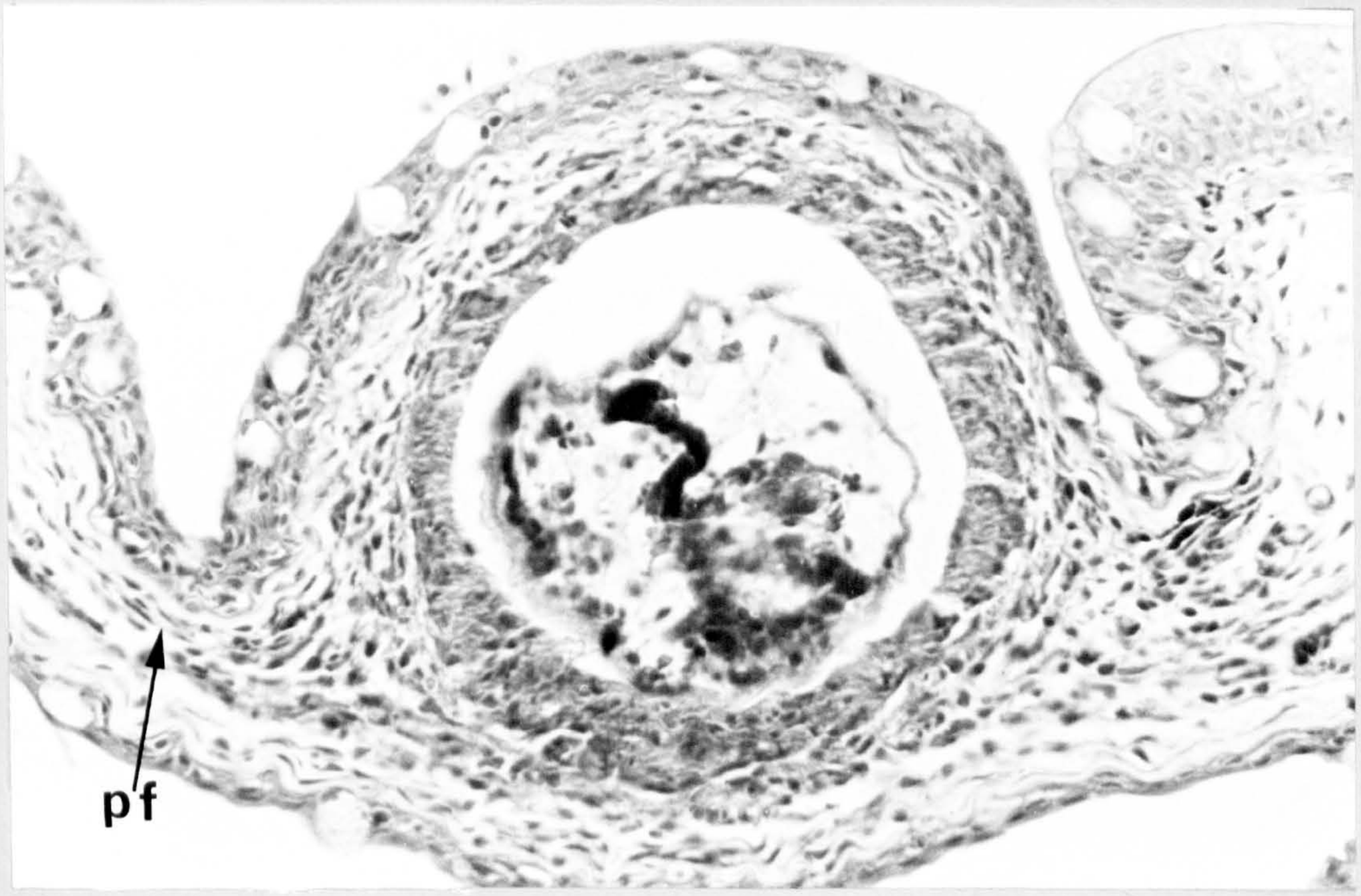
OL : Outer Layer

IL : Inner Layer

Figure 61. S. maximus 6 days post infection. Section through a cyst showing a marked proliferation of fibroblasts H.E. (x 400)

pf : proliferating fibroblasts


OL
IL


pf

Tissue response after 17 days: Connective tissue elements in the outer part of the cyst were more evident by this stage and the whole lesion had taken on an appearance of maturity and stability.

Tissue response after 21 days: The parasite still remained small and cercaria-like in form even at this late stage. It failed to elongate or develop the oral spines which were a feature of S. baccatus larval cysts within P. platessa of this age. The cercariae were still apparently alive but morphologically unchanged from their infective form, even the cercarial pigmented eyespots remaining prominent. (Figure 62) The paracyst remained intact and there was the same PAS positive material condensed between the paracyst and parasite tegument.

In certain cysts the parasite was apparently dead. Sometimes the central core of the cyst appeared as discrete focal accumulations of epithelioid cells surrounded by concentric layers of fibrocytes. The centre of the epithelioid nodule was either vacuolated or contained very basophilic material which was considered to be the remains of chromatin from the pyknotic muclei of the parasite. Both forms of cyst could sometimes be seen in the same section of the fin. (Figure 63)

Occasionally there was extraneous vascularisation of the capsules at this stage and small giant cells could also be seen in the peripheral areas which were not as large or well defined as those observed in P. platessa.

Figure 61. S. maximus 21 days post infection. Longitudinal section through a cyst showing the metacercaria which still has the appearance of a cercaria. Note the prominent eyespot. Picro-Mallory (x 250)

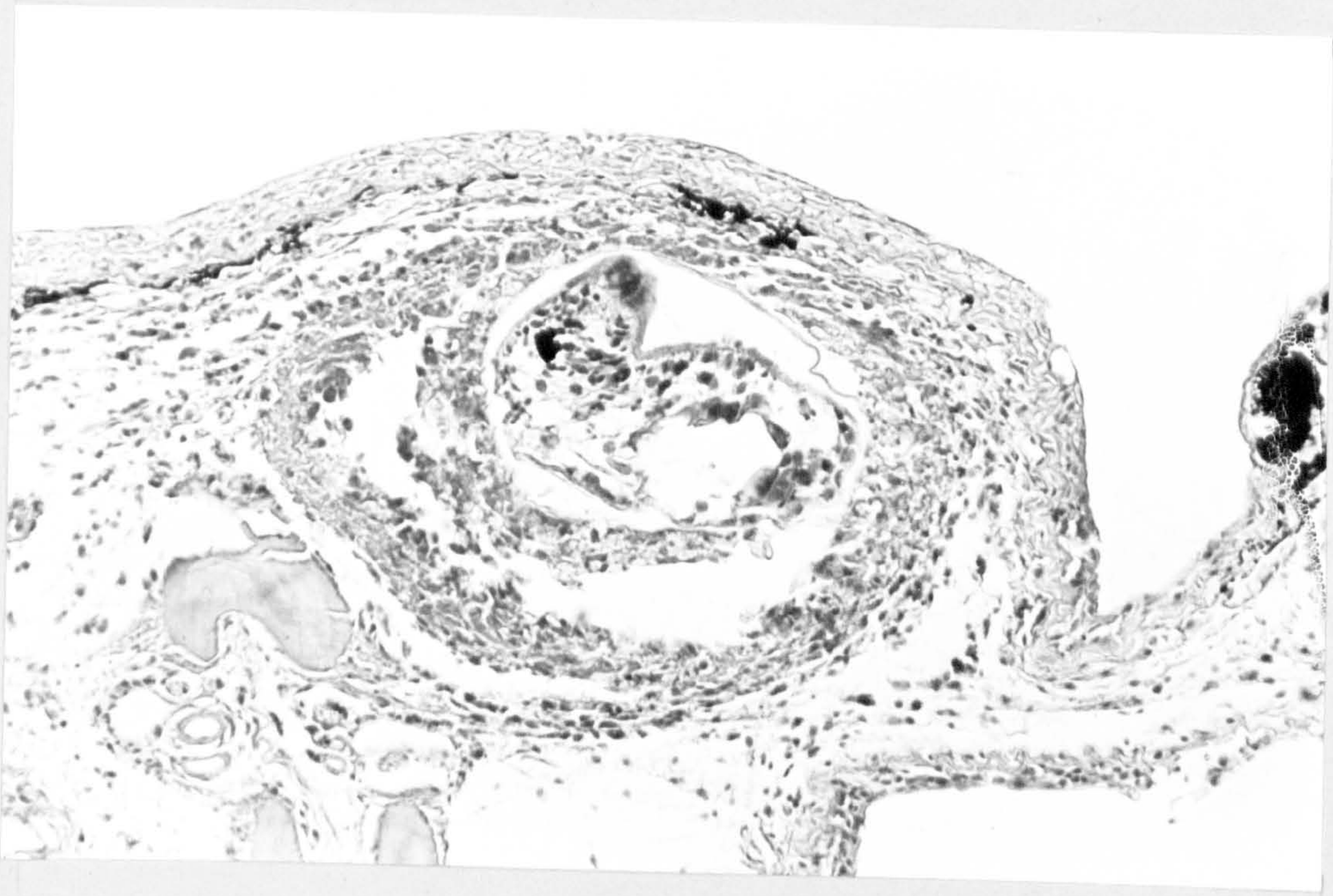
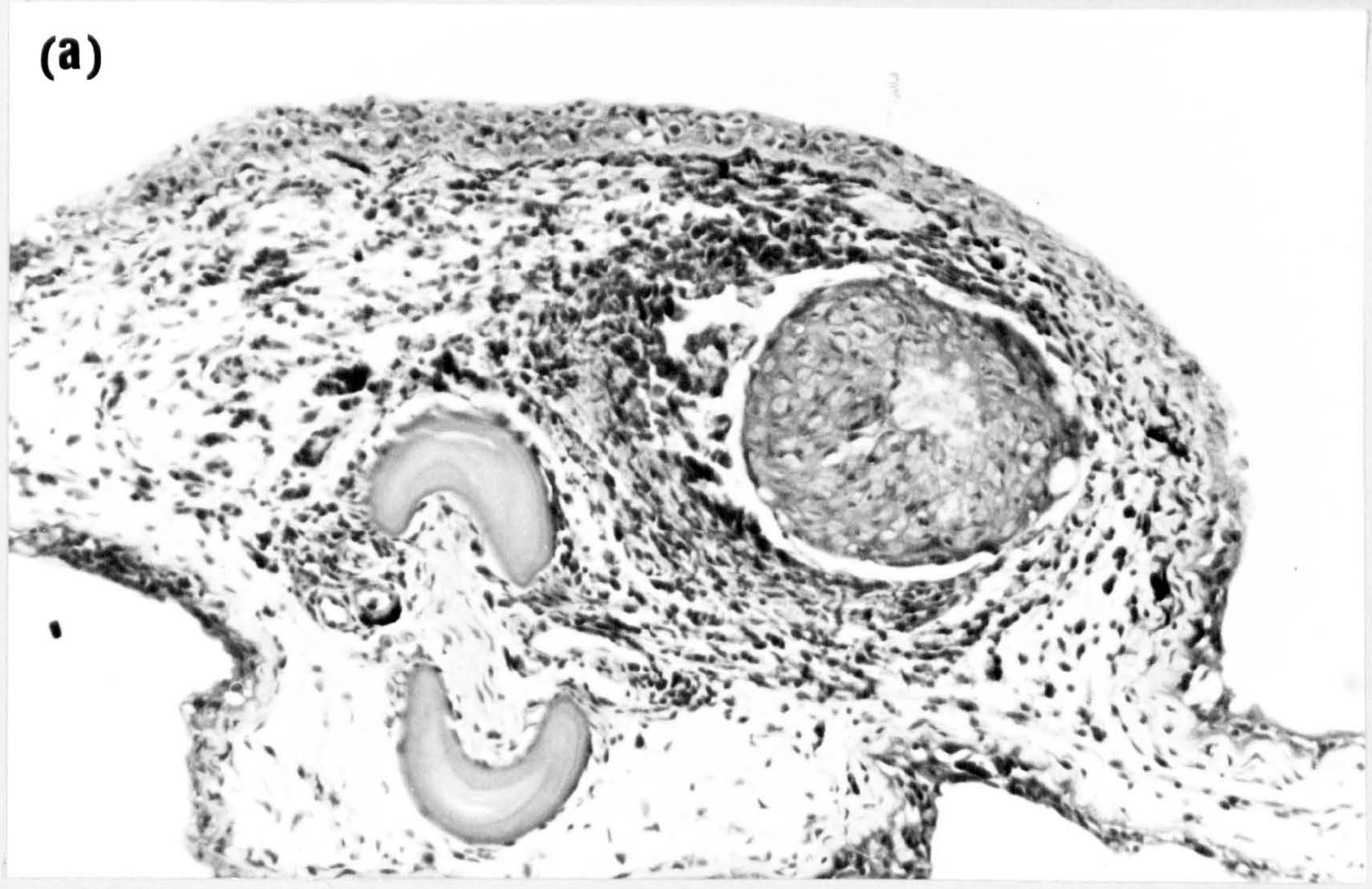
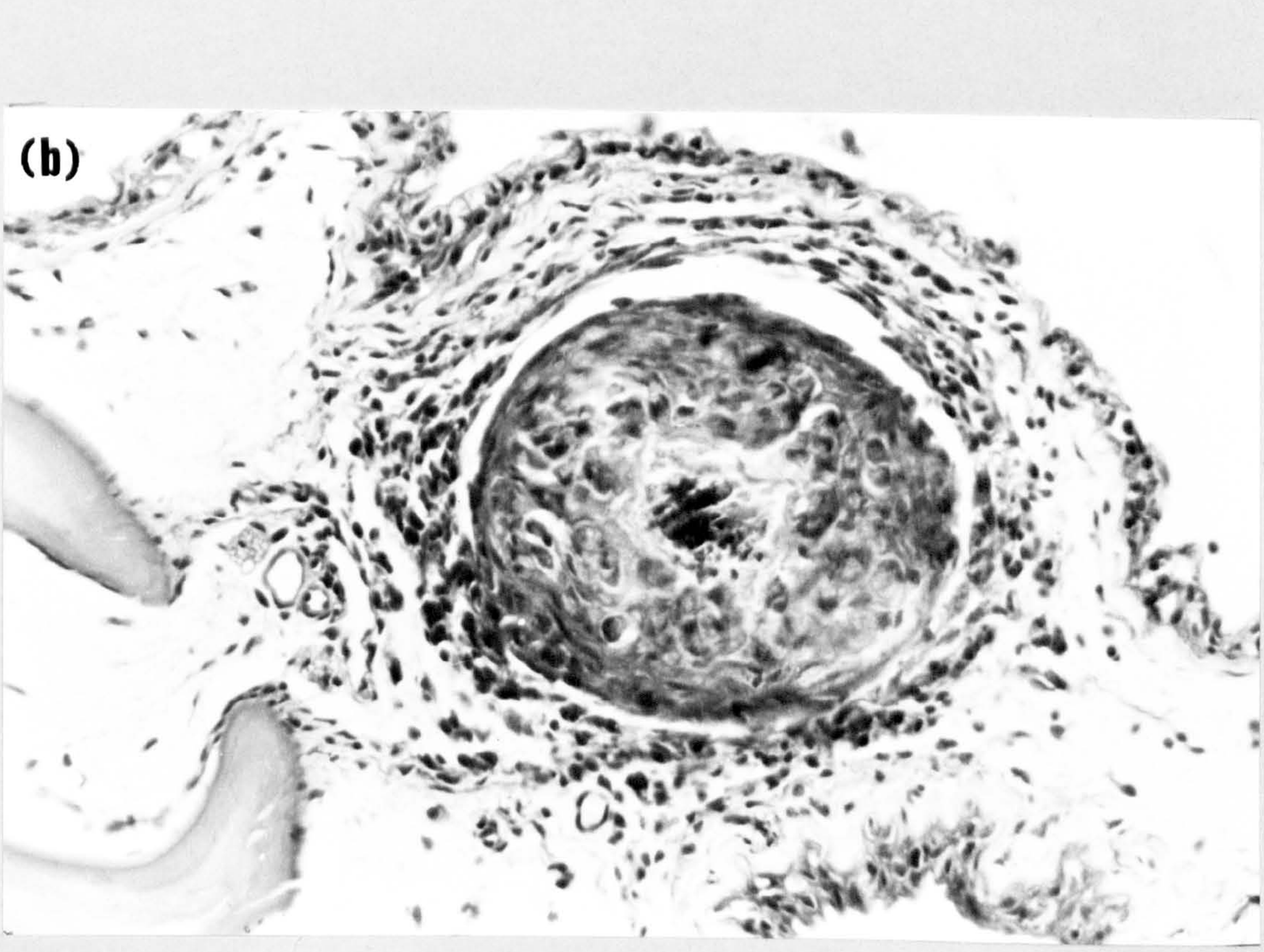


Figure 63. S. maximus 21 days post infection. Section through cysts showing focal accumulations of epithelioid cells surrounded by concentric layers of fibroblasts. The centre of the nodule is vacuolated in (a) and in (b) basophilic material occurs which appears to be the remains of chromatin from pyknotic nuclei H.E. (x 400)


(a)


(b)

Tissues response after 27 - 31 days: There was continued loss of cellular structure in the inner layer of the capsule which became a strongly eosinophilic, granular layer of cytoplasm. The outer layer of fibroblasts underwent further compression and more collagen fibres were laid down. Cellular activity in the area of the cyst was now reduced but occasional macrophages were still present within the connective tissue stroma of the fins.

Cysts containing degenerate parasites were seen in later specimens but the capsule showed little change except for increased nuclear degeneration of the inner layer of the capsule.

General observations on cyst development

All cysts found in S. maximus during histological study were located in the fin, typically in the fin ray. This was particularly well endowed with blood vessels and nerves and during the process of encapsulation of the metacercaria these often became incorporated within the stroma of capsule cells. (Figure 56) Small capillaries were often seen in the region which contributed to the vascularisation of the capsule. A few cysts which occurred in the dermis caused considerable distension of the skin margin immediately overlying them. In early specimens the epidermis was merely attenuated over the cysts but, as they increased in size subsequently, they caused marked distortion of the skin profile. This tumefaction was subsequently subject to mechanical erosion which, in a few cases, resulted in thickening of the epidermis in this area. This was first seen at 10 days. Owing to the exposed position of some of these swellings and the extent of the erosion there was often ulceration of the epidermis. (Fig 64 a-d)

Figure 64. S. maximus 10 days post infection.

(a) Proliferation of epidermal cells in the region of a cyst P.A.S. (x 100)

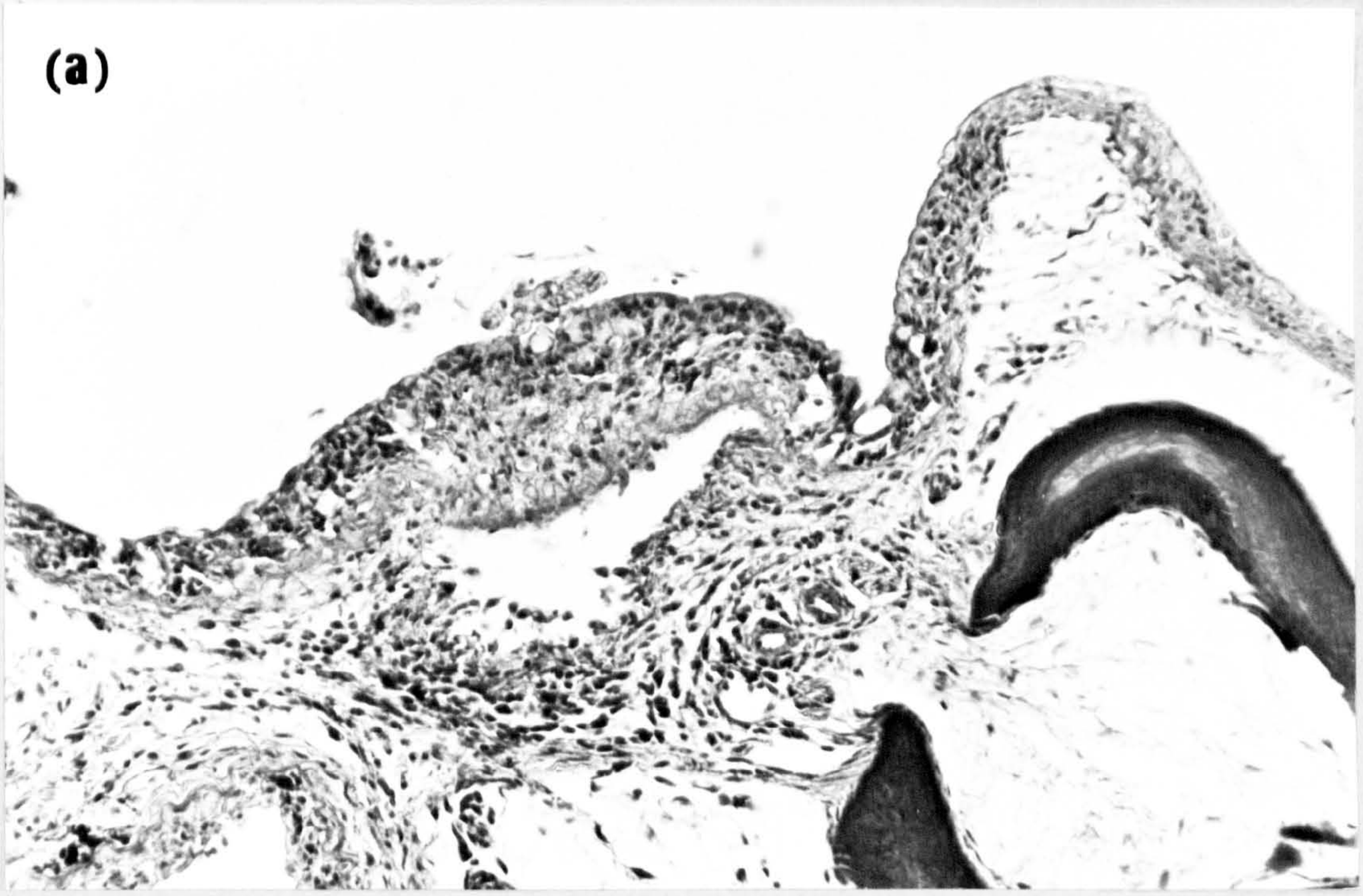
(b) Erosion and ulceration of the epidermis at the site of a cyst Picro-Mallory (x 400)

S. maximus 21 days post infection showing

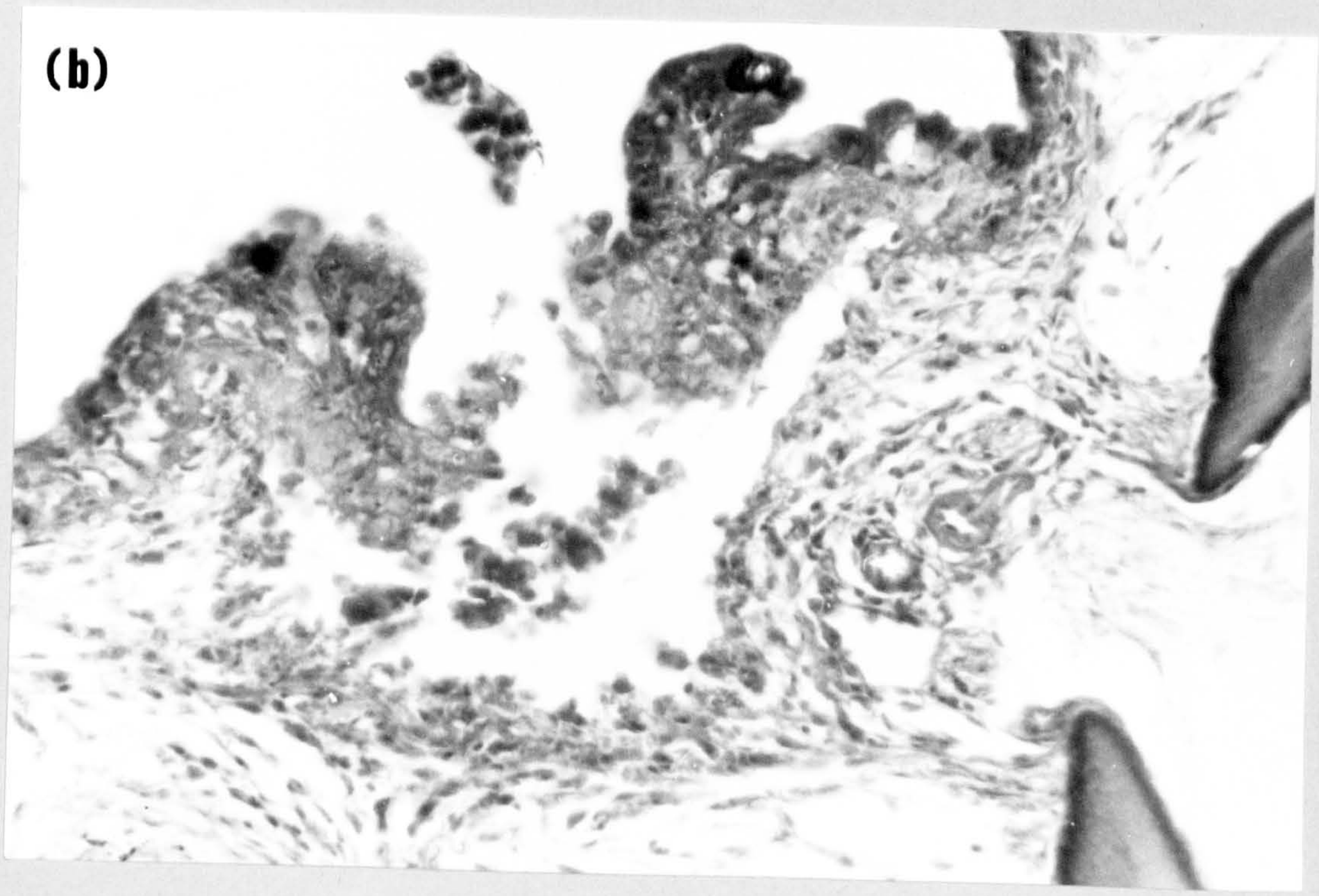
(c) Epidermal thickening at the site of a cyst H.E. (x 250)

(d) Ulceration resulting in herniation of the cyst H.E. (x 100)

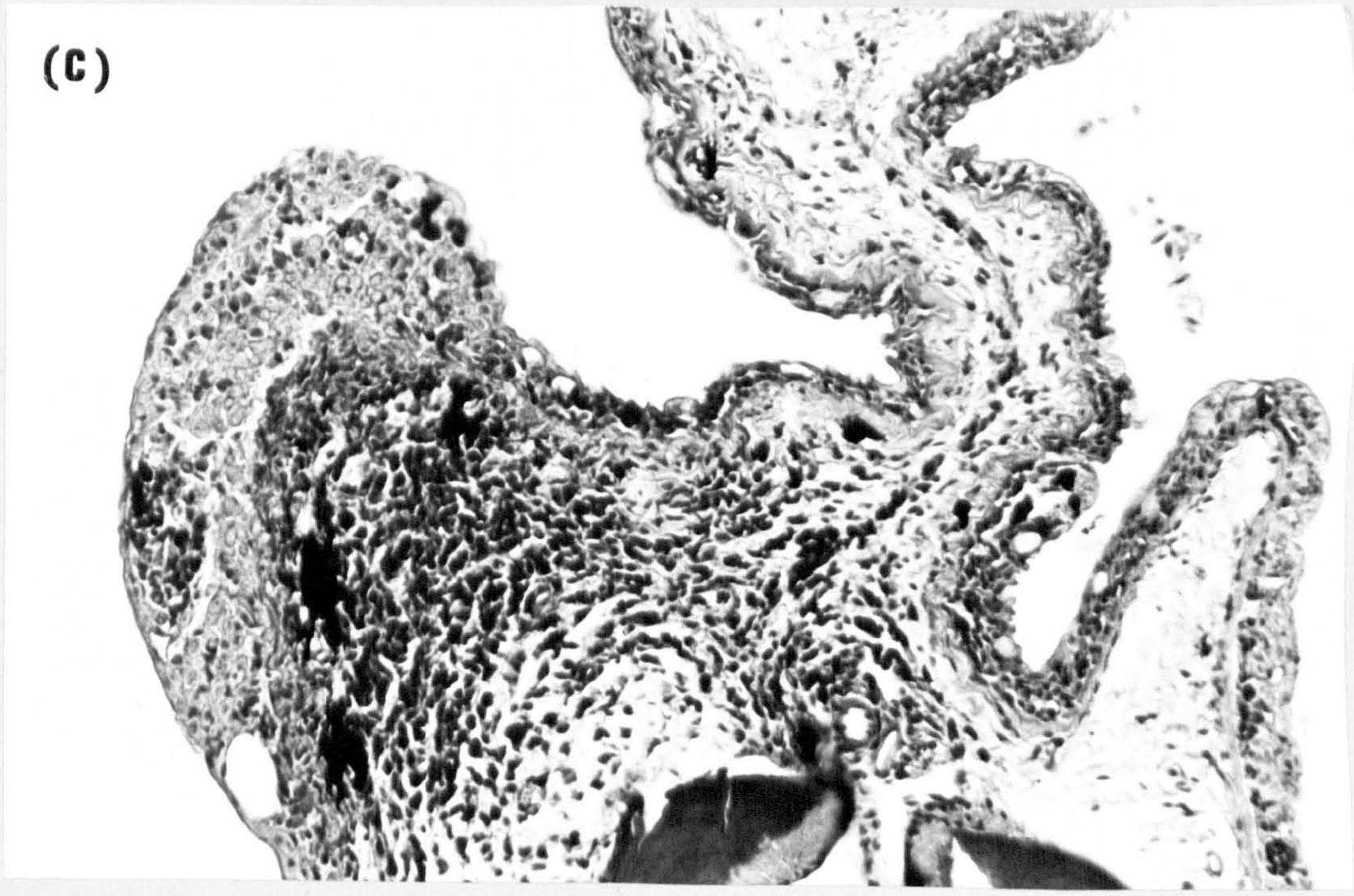
(a)



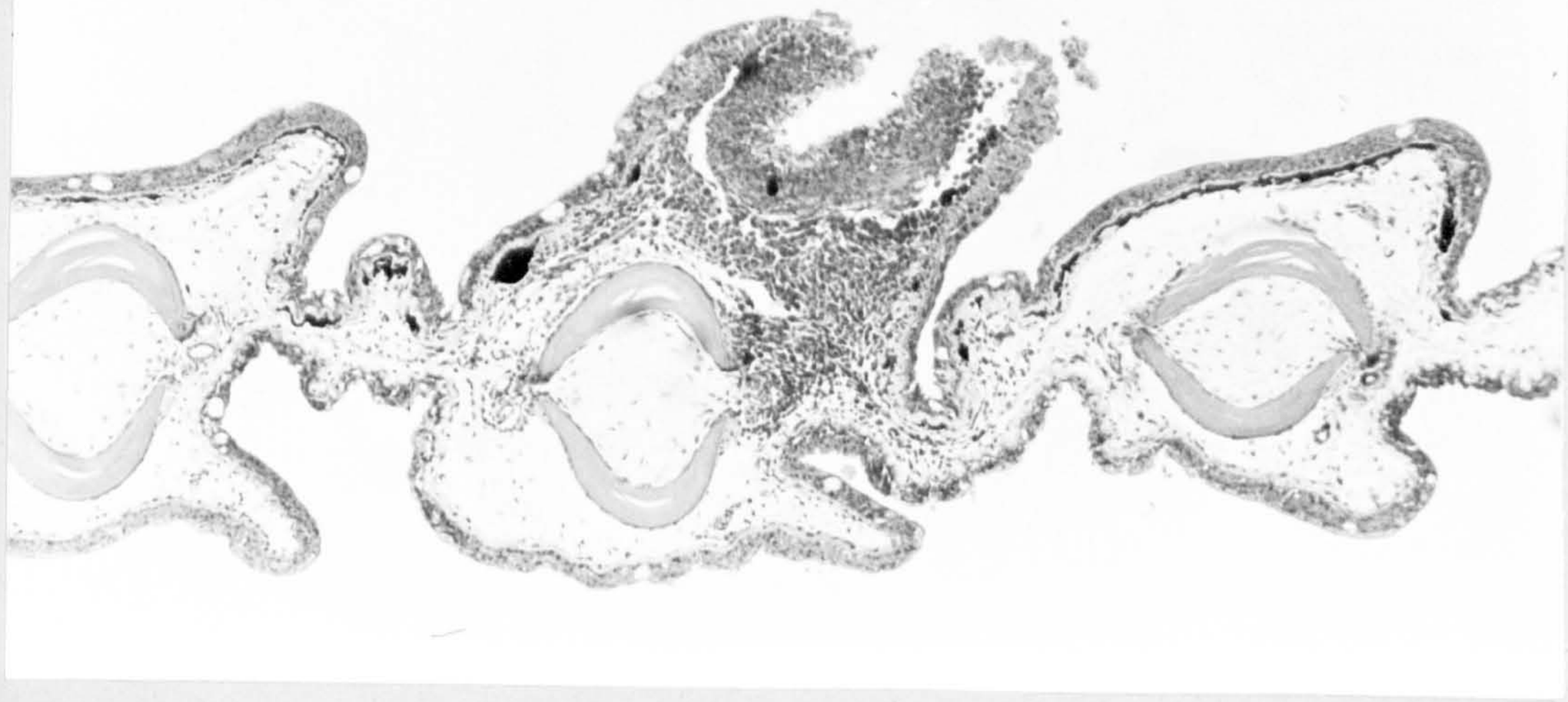
(b)



(c)



(d)



The development of the cyst in S. maximus is illustrated in Figure 65 which shows some of the stages up to 21 days post infection.

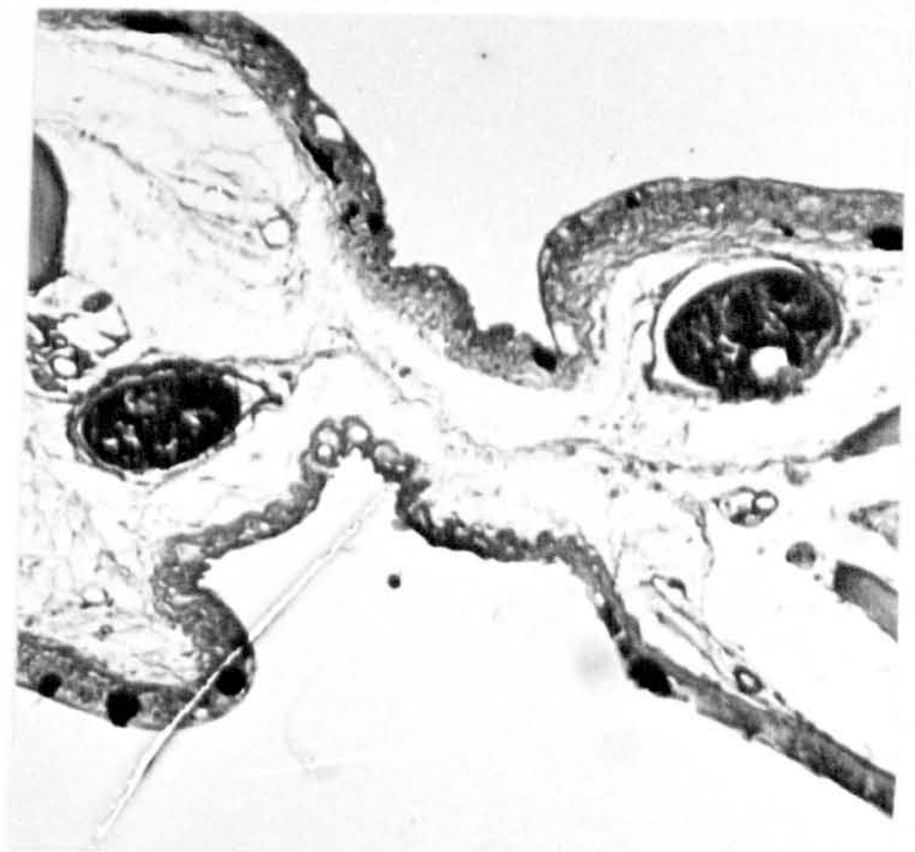
Figure 65. Development of S. baccatus cysts in S. maximus

(a) 10 hours (x 100)
(b) 24 hours (x 100)
(c) 48 hours (dead) (x 100)
(d) 48 hours (x 100)
(e) 96 hours (x 100)
(f) 6 days (x 100)
(g) 21 days (x 100)

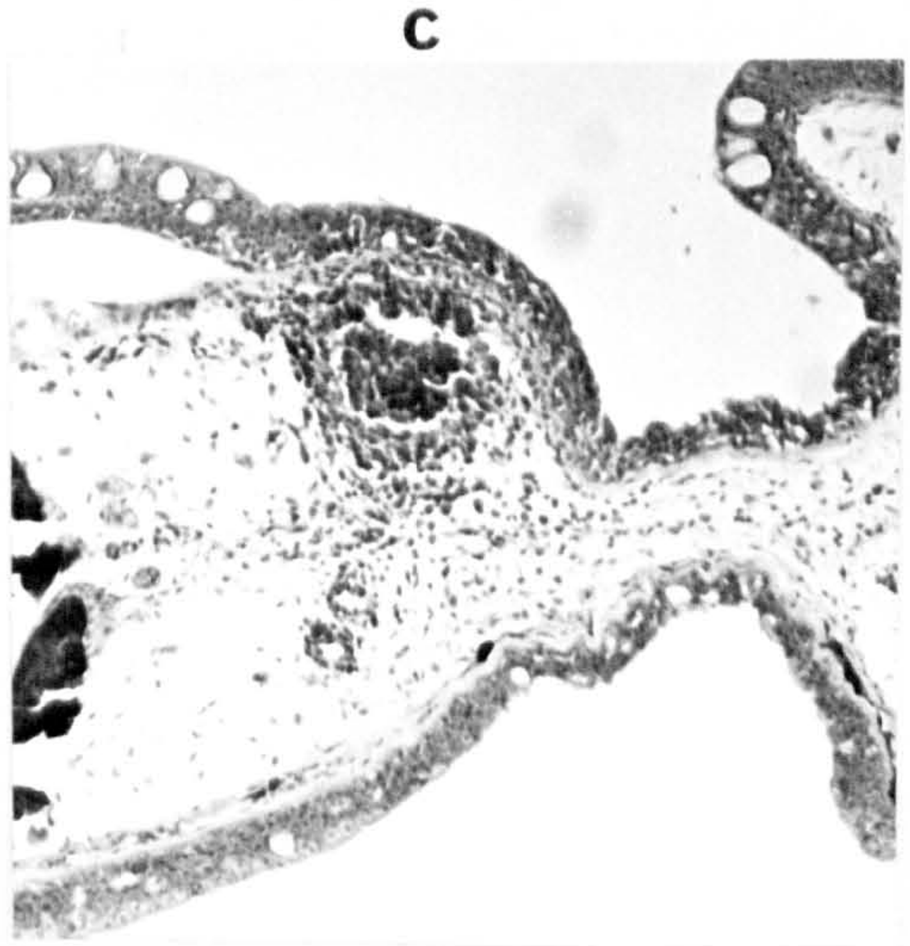
All photographs have the same visual magnification of x 150.

a

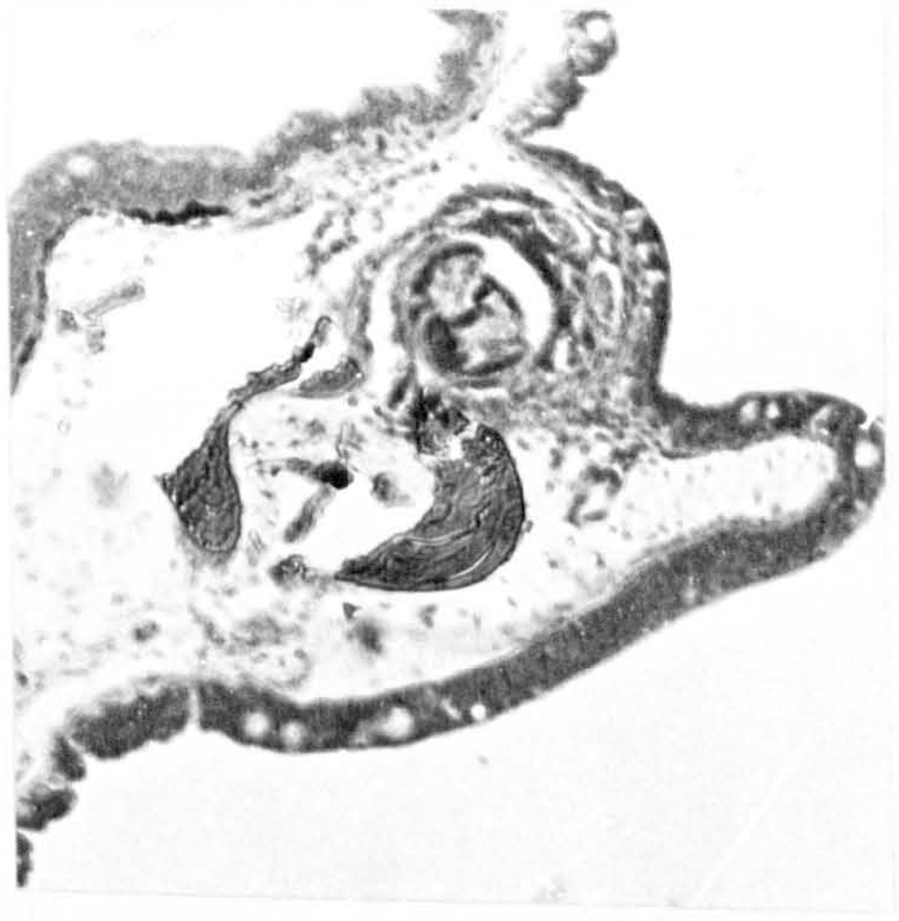
b



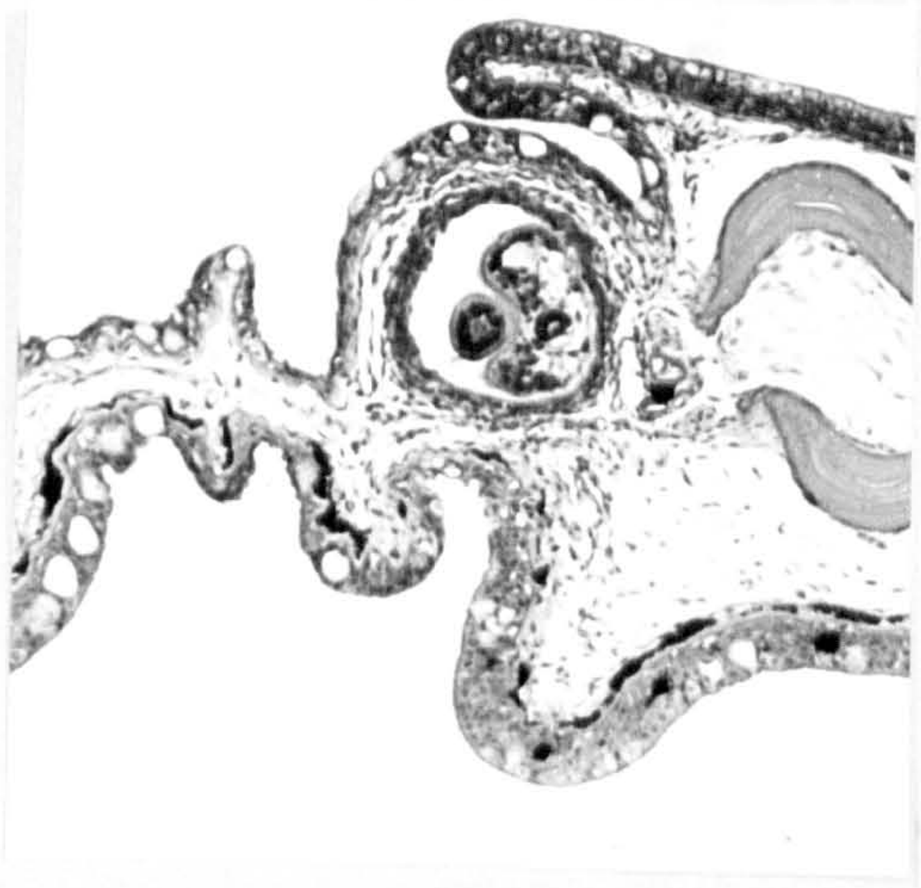
c



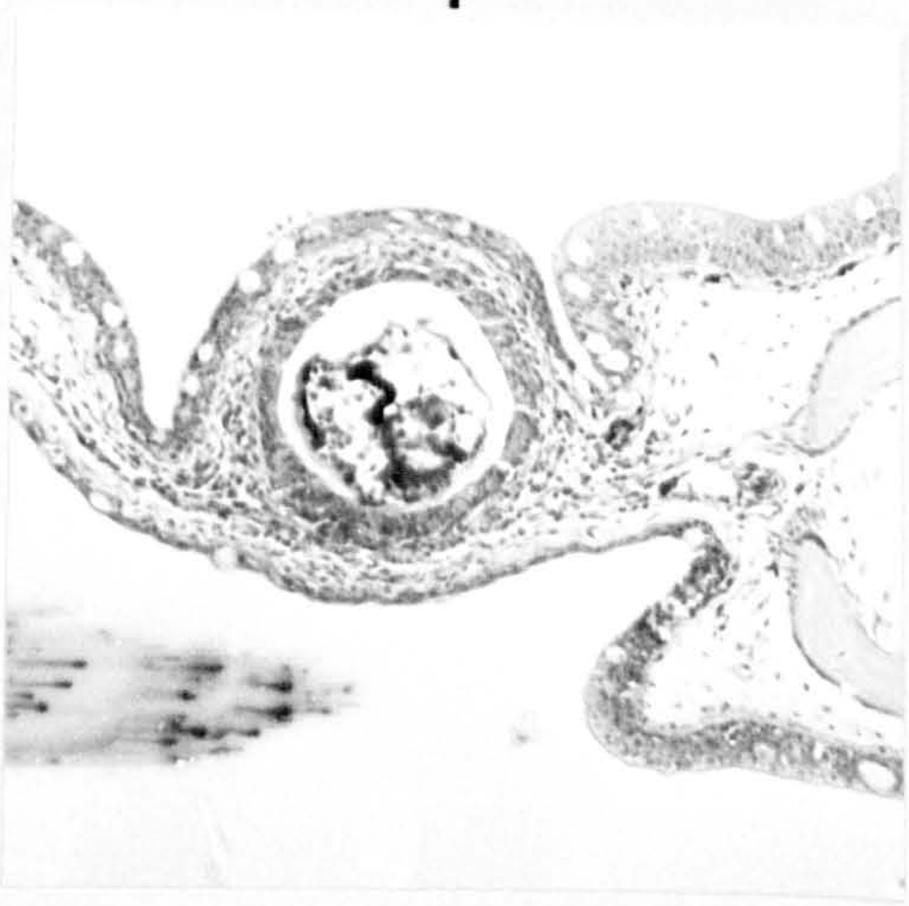
d



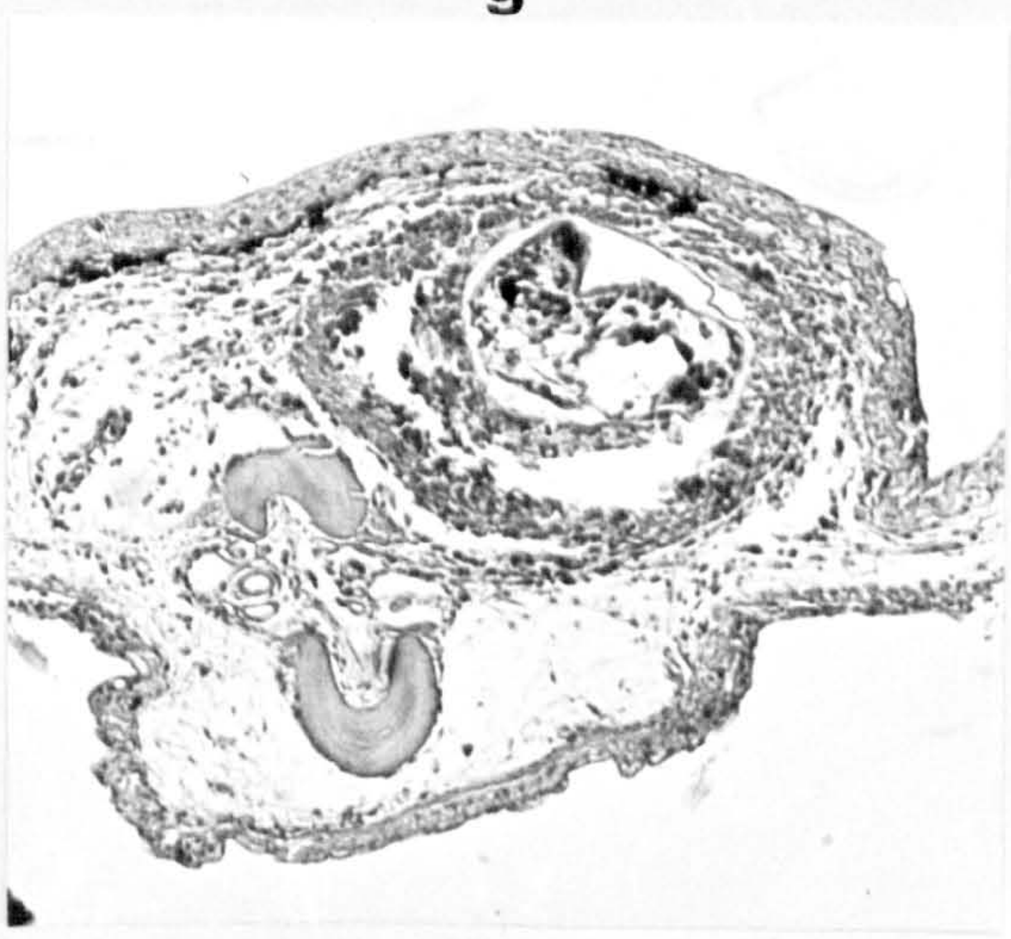
e



f



g



COMPARATIVE DEVELOPMENT OF CYSTS IN DIFFERENT HOST SPECIES

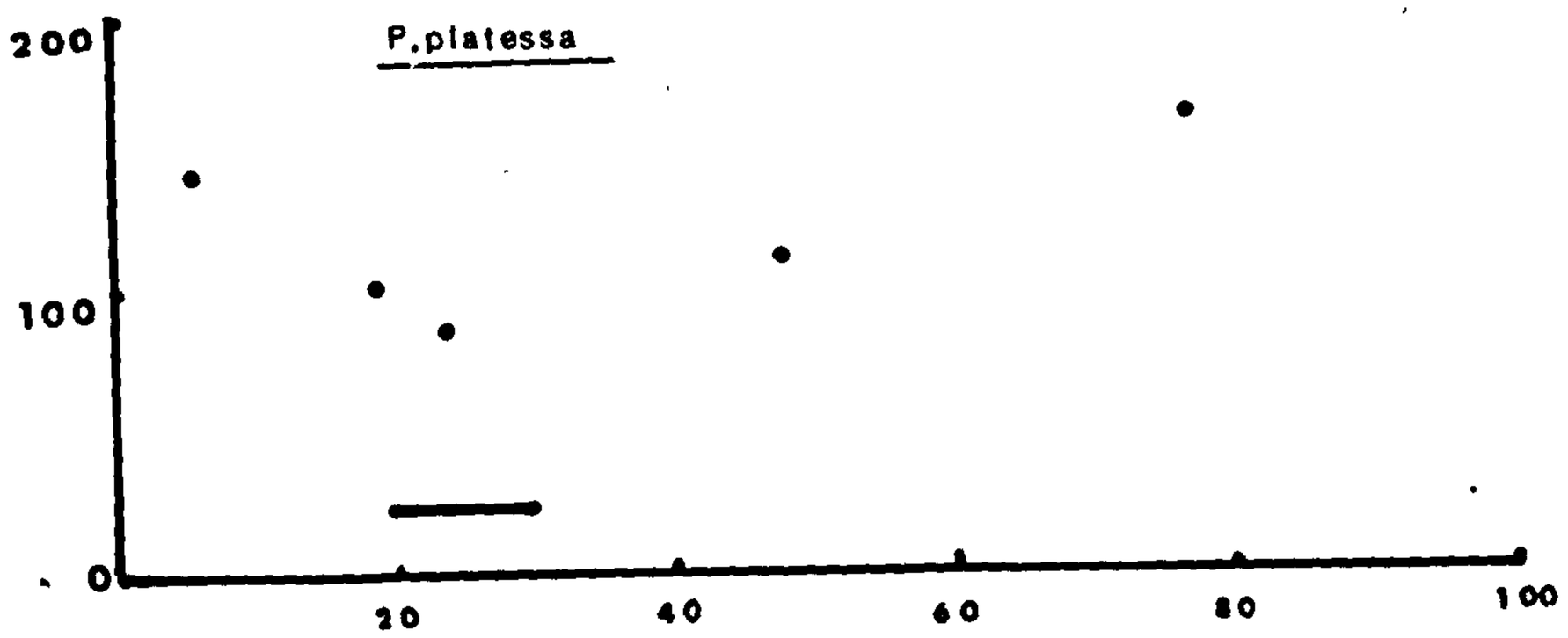
Measurements were made of the length and width of the paracyst (exclusive of host capsule). The figures for the 100 hours post infection are plotted in Figure 66 . The troughs in the curves for all fish indicate the period of encystation since the larva curls prior to production of the paracyst. Figure 67 shows the same measurements for up to 40 days post infection for P. platessa and S. maximus. The increase in size of P. platessa parasites over this period can be compared with the retarded growth of the S. maximus parasites. The curve for S. maximus appears to divide; the reason for this is that some parasites survive and show a little growth whereas the others became reduced in size and will probably die.

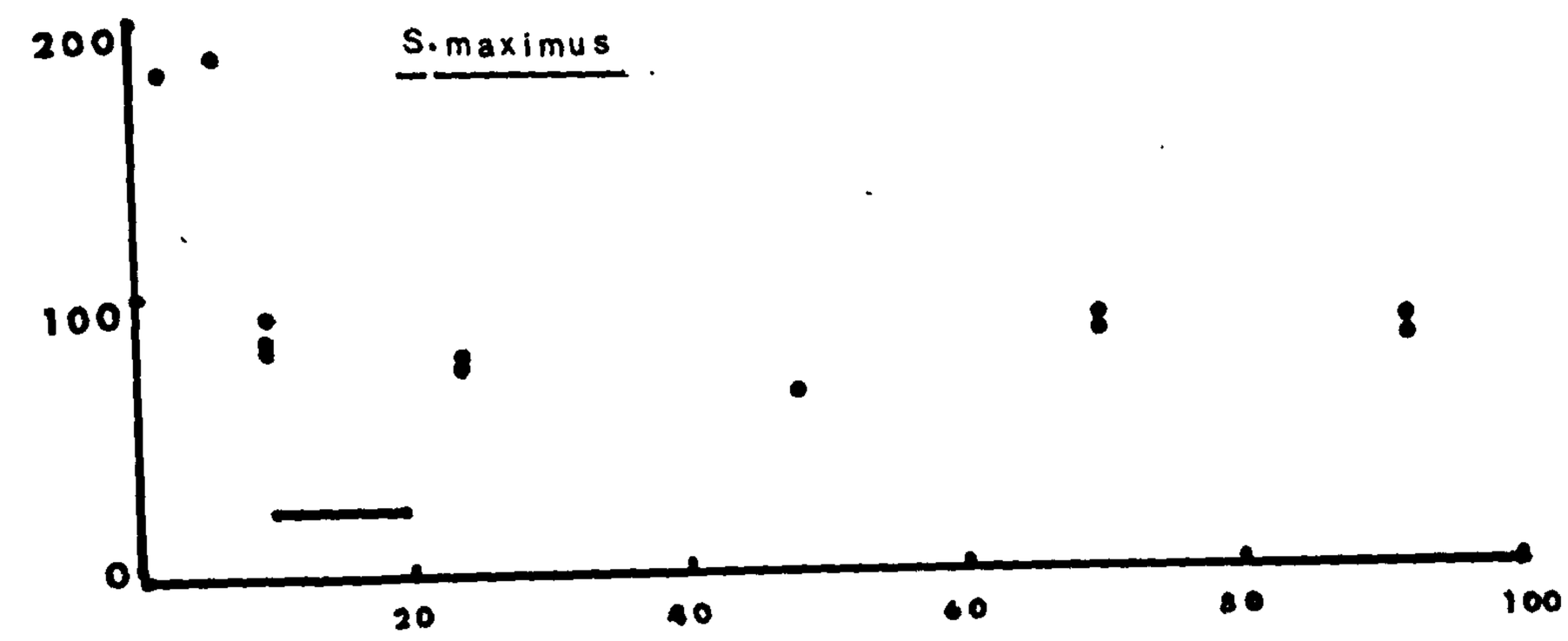
Capsule sizes were noted for some cysts and can be seen in Table XVII. The small size of the S. solea capsules contrast with those of P. platessa and L. limanda. The size of S. maximus capsules was comparable with P. platessa in the later stages of the experimental term. However, the greater thickness of the capsule at the poles which was very obvious in P. platessa was not so apparent in S. maximus.

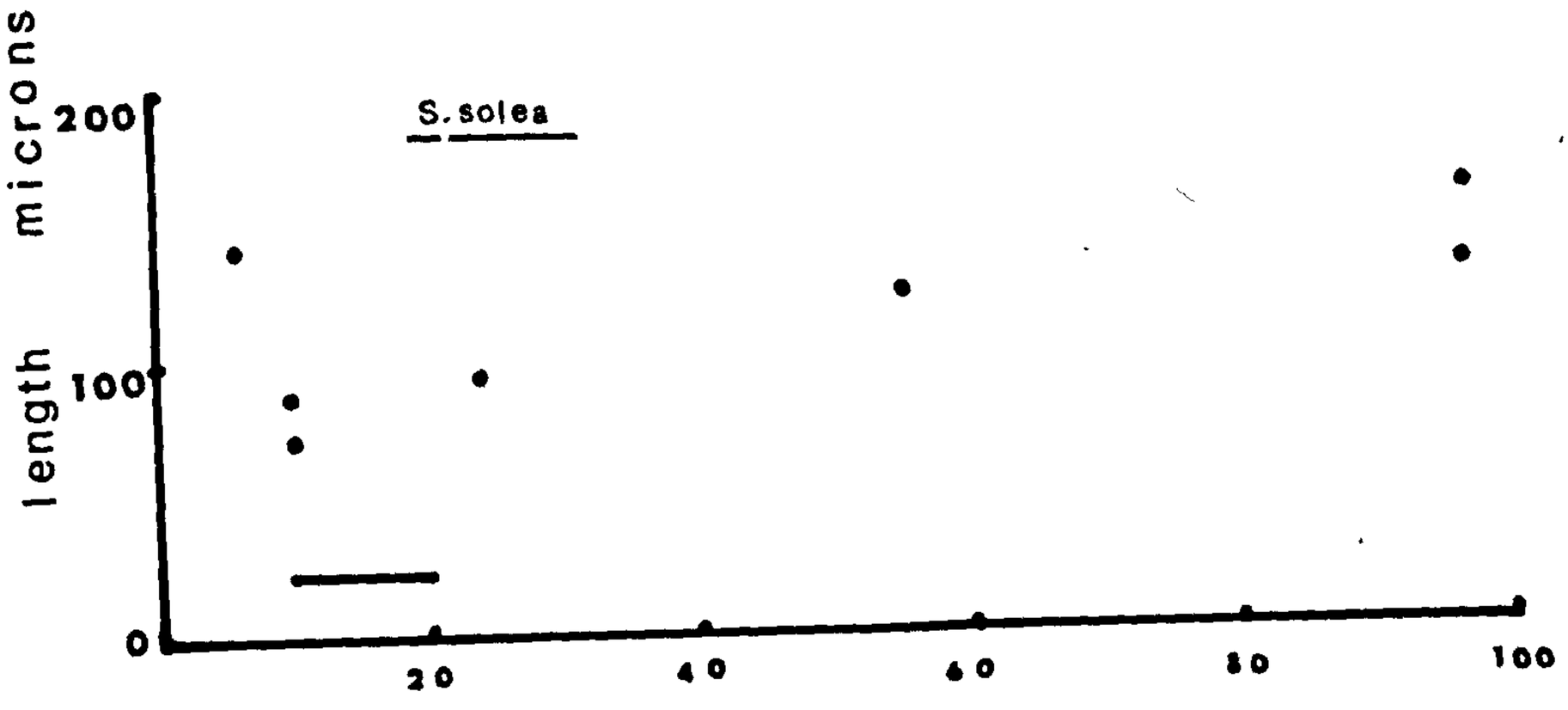
A comparison of the relative size of the paracyst in each of the 4 species of teleost is made in Table XVIII. The P. platessa, L. limanda and S. solea cysts were similar in size to each other throughout the study, whereas those from S. maximus, although comparable in the earliest stages, from 48 hours grow progressively more slowly as they age.

Figure 66. Length of paracyts up to 100 hours post infection in P. platessa, S. maximus, L. limanda and S. solea (measurements in microns)

▬▬ appearance of paracyst membrane


P.platessa
200
100
0
20
40
60
80
100


S.maximus
200
100
0
20
40
60
80
100


S.solea
length micron
200
100
0
20
40
60
80
100

L.limanda
200
100
0
20
40
60
80
100
hours post infection

Figure 67.

Length of paracysts up to 40 days post infection
(measurements in microns)

□ paracysts in P. platessa
● paracysts in S. maximus

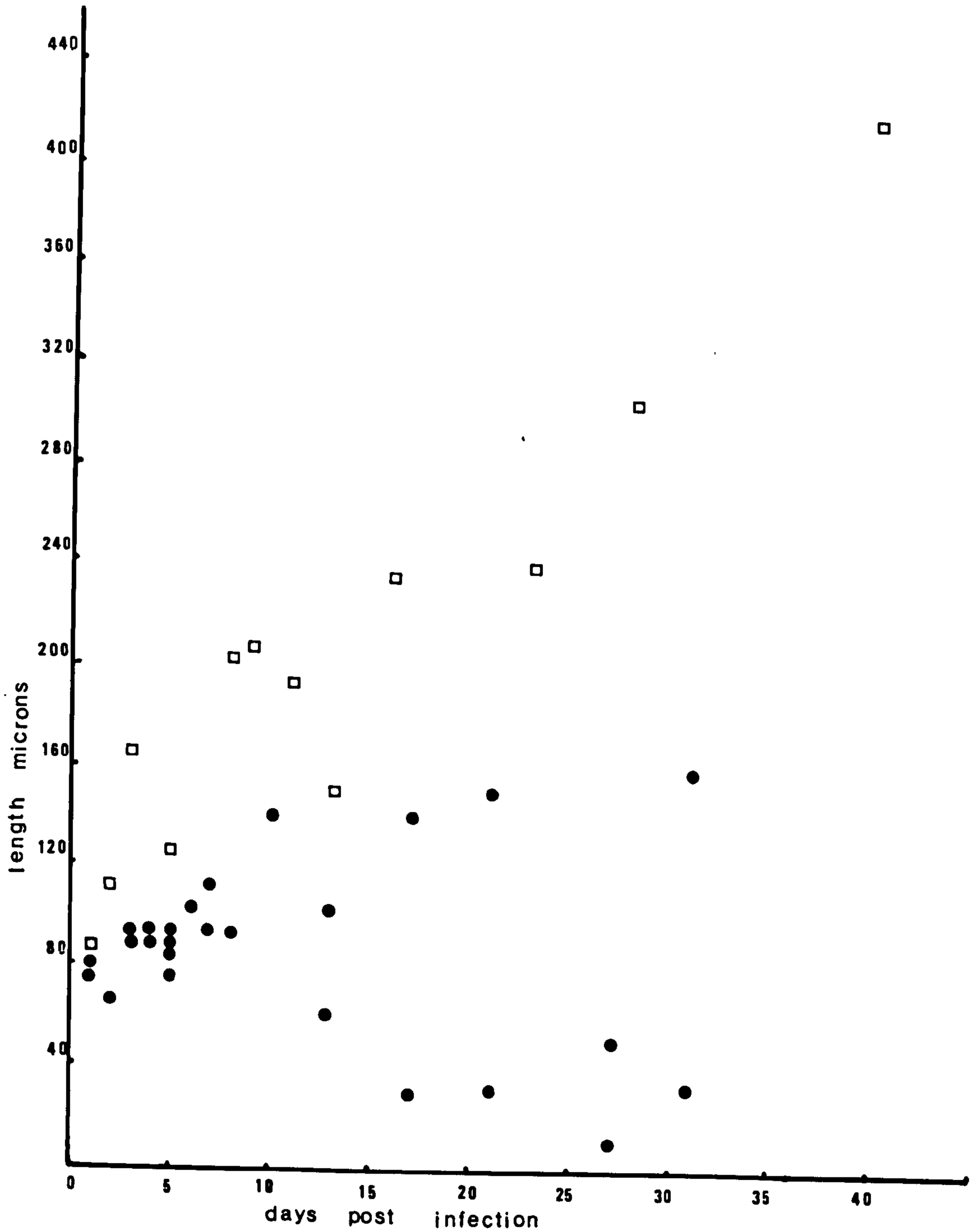

440
400
360
320
280
240
200
160
120
80
40
length microns
0
5
10
15
20
25
30
35
40
days post infection

TABLE XVII Thickness of host capsule developed in second intermediate hosts in response to S. baccatus metacercariae. All measurements are in microns and taken from histological sections.

Time after infection	S. maximus	P. platessa	L. limanda	S. solea
10 Hours	A few cells only			
24	1-2 layers of cells	A few cells only		A few cells only
48	Parasite dead		1-2 layers of cells	
55				A few cells only
96	9-14	1-2 layers of cells		1-2 cells
5 Days	Dead, 5-19, 19-24			
6	14-28			
7	5, 5-9	99-24		1-2 cells
8	9-28		1 layer of cells	
9		5-33		
10	5-19			
11		14-19 (28 at poles)		1 cell thick
12			No reaction	
13	19	14-33 (47 at poles)		
14				No response (muscle 14-38)
16		52 (127-160 at poles)		
17	28-33			1-2 layers
21				14-38, 1-2 cells
23		24-47 (94 at poles)	1-2 layers muscle cyst 28 (207 at poles) fin cyst	
27	9-188			
28		151 (236 at poles)		5 (57 at poles) 4-9
31	57-151			9-19
40		9-24 (113 at poles)		
45		28-33 (72-90 at poles)		
55			9-57, 9-141	

TABLE XVIII Dimensions of the paracysts of S. baccatus metcercariae in the second intermediate host at intervals after infection. All measurements in microns and taken from histological sections.

Time after infection	S. maximus	P. platessa	L. limanda	S. solea
2 Hours	184x 42			
6	188x 47	146x 28	56x 38	141x 38
10	94x 47, 80x 80, 85x 80			
12				71x 52, 89x 47
19		104x 75	94x 38	
24	75x 47, 80x 52	89x 61	75x 52	
27				99x 66
48	66x 66	113x 56	108x 52	
55				127x 99
77	89x 89, 94x 47	165x 56		
96	89x 89, 94x 47			137x 84,165x104
5 Days	94x 89, 89x 61, 75x 66, 85x 66	127x 52		
6	104x104			
7	113x 75, 94x 71			141x118
8	94x 80	203x137	179x 75	
9		207x146		
10	141x 85			
11		193x104		170x122
12			179x113	
13	61x 56,103x 85	151x146		
14				198x160,193x184 104x 25
16		236x203		
17	30x 22,141x104			236x165
21	32x 30,151x141			
23		240x236	254x236,193x 94	
27	11x 14, 52x 66			193x 75,234x165
28		306x231		330x306,302x236
31	34x 34,160x160			302x-
40		419x330		
45				
55			506x412	

DISCUSSION AND CONCLUSIONS

The majority of encysted metacercariae in all fish examined were found in the dermis and loose connective tissue of the fins. Those which occurred on the body were situated in the dermal and hypodermal connective tissues overlying the somatal myotomes. Only occasionally were larvae found in the muscle except in 0-group P. platessa. A few specimens of 0-group P. platessa experimentally infected with large numbers of S. baccatus were fortunately available. Owing to the small size of these fish, the cercariae were able to penetrate throughout the body as well as the somatic muscle and fins. The relatively large size of the metacercarial cysts caused extensive muscle degeneration and consequent invasion and proliferation of inflammatory cells. The capsules after 8 weeks were morphologically similar to those seen in I-group P. platessa but were only half the size of the I-group cysts at the earlier stage of 6 weeks. The growth of the metacercariae was comparable with those infecting I-group P. platessa. There is no doubt that such a massive infection at this early stage of P. platessa development would impede normal functioning of the fish. Certainly their locomotory activity would be affected and there was evidence of compression of vital organs such as liver and kidney caused by the increased size of the cysts at 12 weeks. The responsibility of S. baccatus for heavy mortalities in 0-group P. platessa (MacKenzie, 1971), though significant in the wild, would not occur in the aquaculture situation since such small fish would be reared in enclosed tanks ecologically separated from the primary molluscan host. However, in an aquaculture system which employs the natural habit for rearing of, e.g. the enclosed pond system, 0-group P. platessa may be more exposed to this parasite.

There was some variation in the intensity of the host response in different I-group fish of the same species and the intensity of response occasionally differed between parasites in the same fish, even though they occupied similar tissues. Differences, therefore, must occur in the ability of individual cercariae to stimulate the host response, or in the ability of different sites within the fish to respond.

There was surprisingly little damage to the host tissue. The only effect of cercarial penetration through the epidermis was the occasional local acantholisis and this was thought to be very transient. The loss of cohesion of malpighian cells probably never involved all the desmosomes of any one cell, and those affected would be swiftly renewed. Anderson & Roberts (1975) found that epithelial covering of small wounds in Atlantic salmon was achieved in 2 hours at 20 - 25°C and it therefore seems likely that many of these minute lesions would be repaired very quickly. There was never any evidence of necrosis of the epidermal cells which might be expected if the parasite had assisted penetration by the secretion of histolytic enzymes as reported by Lewert & Lee (1954) and Stirewalt & Evans (1960) for schistosome penetration through mammalian skin. However, Stirewalt & Kruidenier (1961) have shown that the contents of different sets of gland cells of cercariae differ in their histochemical characteristics and suggested that one of the functions of the post-acetabular gland secretions of Schistosoma mansoni is lubricative. In view of the little effect on the epidermis it is more likely that penetration by S. baccatus is achieved by separation of epidermal cells aided by a parasite-secreted lubricant/adhesive.

The most significant effect on the epidermis was the distension caused by the growth of the cyst and capsule in the superficial dermis which resulted in erosion, hyperplasia and, ultimately ulceration in a few cases. It was also possible for the cyst to herniate under these circumstances.

The damage to myofibrils, when observed. was minimal except in 0-group P. platessa where the large size of the parasite in relation to the small size of the fish caused extensive areas of muscle degeneration and distortion by the cysts.

Hoffman & Putz (1965) reported that Uvulifer ambloplotis (Hughes) caused haemorrhages in Lepomis machrochiris. Haemorrhages were also seen by Lee & Cheng (1970) after penetration of Stellantchasmus falcatus into Mugil cephalus. However, no haemorrhage attributable to S. baccatus invasion were seen in any of the fish species studied and cercariae were never seen to penetrate blood vessels.

No evidence of bacterial invasion as described by McQueen et al (1973) was seen but the 0-group P. platessa, 8 weeks post infection, showed an extensive infection of Glugea sp. The possibility of introduction of microsporidian spores by cercariae cannot be excluded.

Encystment and encapsulation

The encystment of S. baccatus cercariae occurred within approximately 24 hours. Wolfgang (1955a) in his study of S. baccatus metacercariae did not report the time of encystment and this seems to be a variable feature of different species of Digenea in fish (Erasmus, 1972). It is probably related to physiological aspects

of the host/parasite relationship. Hoffman (1956) found that the paracyst membrane of Crassiphalia bulboglossa Van Haitsma did not appear until 15 - 16 days after penetration at 21 - 26°C in a variety of hosts and Cryptocotyle lingua encysts within 4 hours in P. platessa (Sommerville, unpublished). The formation of the paracyst membrane is temperature dependent. Hoffman & Putz (1965) showed that Uvulifer ambloplotis (Hughes) produced a paracyst membrane at about 43 days when the host, Lepomis macrochirus, was kept at room temperature but this was reduced to 22 - 24 days at 24°C. In the present study paracyst membranes were observed as early as 10 hours in S. maximus but in P. platessa, L. limanda and S. solea, the paracyst membrane did not appear until 20 - 30 hours and, since temperatures were similar in all cases, this difference was thought to be host induced (possibly due to the encountering of an unfavourable environment in the case of S. maximus.

Encystment was accompanied by a reduction in length of the cysts due to the contraction and curling of the larva at this stage. Following the appearance of the paracyst there was a sharp increase in the size of the cyst and at the same time the larvae took on a vacuolated appearance. It was believed that this increase in size was due to the uptake of fluids rather than an increase in cell number. Sections through Cryptocotyle lingua in similar tissues never demonstrated this phenomenon (Sommerville, unpublished). Hoffman (1956) described the development of C. bulboglossa, a strigeid, in a variety of fish hosts and stated that, at 16 days it appeared 'vacuolated' and Hoffman & Dunbar (1963) in a similar study of Neogogatea kentuckiensis (Cable) described the metacercaria as having a "blown up" appearance after 1 - 2 days, as if the parenchyma had become oedematous. It is believed that these may be observations of the same phenomenon seen in the present study. It would seem to be related to the development of

the paracyst membrane and may reflect osmotic or other physiological changes in the development of the metacercaria.

The composition of the host capsule was qualitatively similar in all the hosts studied and consisted of an inner layer composed of leukocytes, primarily of the macrophage series. These cells underwent epithelioid transformation which was more obvious in the larger capsules of P. platessa and S. maximus. The cellular structure of this tissue gradually degenerated from the inner (oldest) layer of the capsule to form a dense eosinophilic ground substance in which was embedded nuclear debris. The outer layer of the capsule consisted of fibroblasts and reticulin collagen fibres. Reports of connective tissue capsules surrounding encysted helminths are fairly common, e.g. Hoffman & Dunbar (1963), Mikhailova et al (1964), Wood & Yasutake (1956), Williams (1967), McQueen et al (1973) and Erasmus (1972), but few have studied the involvement of inflammatory cells. Most of the interest in metacercarial cysts has been related to the structure and histochemistry of the paracyst, and the nature of the host capsule tends to be loosely described. For example, both Hunter & Hunter (1942) and Hoffman (1956) described host capsules which consist of two layers; an inner, dense, unpigmented layer and an outer loose connective tissue layer which contained melanophores. In both cases the parasite encysted in sites similar to those used by S. baccatus in the hosts studied here. It is conceivable that the inner layer they described is composed of degenerate inflammatory cells as shown here for S. baccatus. The present study supports Bogitsch' assessment that the host capsules described by Hunter

& Hunter (1940) were most likely derived from the reticulo-endothelium system, rather than modified liver cells. Bogitsh's (1962) histochemical study on the cyst and capsule of Postho-diplostomum minimum showed that the layer adjacent to the para-cyst demonstrated a faint PAS positive staining reaction while some cells contained intensely staining PAS positive granules. It would seem that this layer is similar in composition to that described in the present study.

Matthews (1973a) studied the metacercaria of the gastero-stome Prosorhynchus crucibulum (Rudolphi) which secreted an hyaline cyst within 3 - 4 hours following penetration. This membrane subsequently breaks down so that the larva lies free in the host tissues. He stated that there is a strong localised host reaction which results in a fibrous capsule at three months. However, he did not describe the nature of the reaction.

The inflammatory response in fish was reviewed by Finn (1970), G. Timur (1975) and Roberts & Bullock (1976). The latter authors paid particular attention to the integumentary pathology of fish. The inflammatory response observed in the present study is in general agreement with the chronic granulomatous inflammatory reaction as induced by particulate resistant foreign matters described by these authors. The appearance of macrophages in the P. platessa lesion at 24 hours and the onset of fibroplasia at approximately 8 days corresponds with the findings of Finn & Neilson (1971) for rainbow trout muscle traumatized with heat-killed Staphylococci and kept at 15°C. The appearance of epithelioid cells at 16 days and the giant cells seen at 28 days in P. platessa in the present study agrees with the findings of G. Timur (1975) for P. platessa lesions at 10°C. (P. platessa

cysts were not examined between 24 and 28 days and the first occurrence of these may have been earlier than 28 days but not before 24 days). Lymphocytes were only recognised in the later stages of the P. platessa inflammatory lesion (9 and 23 days) and it would seem that they either have a very minor role in these metacercarial infections or that their responses may be delayed.

The only other histological study of Stephanochasmus sp. in fish is that of Arru et al (1968) who described sub- epidermal cysts in Mediterranean red mullet by an unspecified member of the genus Stephanochasmus. They described an absence of any inflammatory response but they observed muscle degeneration in the cyst region which they attributed to compression caused by the parasite. At the poles of the cyst the muscle had been replaced by adipose tissue. No adipose tissue developed in this study but no experiment was continued beyond 55 days and adipose replacement tissue may occur at a later date. Their description, however, is lacking in a number of details such as the age and maturity of the cysts they found. Since no experimental infections were carried out, they must have seen a range of developmental stages. They present figures showing a metacercaria in an apparently mature host capsule which they describe as a "parasitic nodule". It seems likely that his capsule represents the granulomatous tissue derived from inflammatory cells in the early stages of infection in a similar manner to that described in this study.

Wolfgang (1954b) described a layer of opaque white connective tissue around the transparent parasite shell which commenced immediately after encystment of S. baccatus in the winter flounder

(Pseudopleuronectes americanus). He states that a local irritation of host muscle was the only change seen on histological sectioning of the cyst.

Comparison of different hosts

(i) Metacercarial development

Since it is well recognised that the rate of development of metaceracariae and the rate of capsule formation is temperature dependent (Hoffman & Putz, 1965; McQueen et al, 1973) care was undertaken in the present study to ensure all experimental infections were maintained at comparable temperatures. Nevertheless, differences were observed between the host fish species. Although P. platessa, L. limanda and S. solea were similar in the rate of development of the metacercariae, S. maximus metacercariae were retarded. No only were the cyst dimensions smaller in S. maximus (Table XVIII) but the larvae observed were still recognisable as cercariae, i.e. without the development of the metacercarial features, e.g. oral spine formation. Dead larvae in S. maximus were seen as early as 48 hours while these were never seen in the other fish hosts.

Hoffman (1956) attempted the infection of a number of freshwater fish hosts with the cercariae of C. bulbolgossa and found that, although the brook stickleback Eucalia inconstans became infected, the parasite did not attain its full development. It lived up to 81 days and eventually died. He stated that this phenomenon may be unusual since he was not aware of such a phenomenon in any other digenean infections. It would seem that the findings in this study are a further demonstration of this phenomenon. Hoffman considered E. inconstans to be an abnormal host.

Matthews (1973a) found that the cercariae of Prosorhynchus squamatus Odhner and P. crucibulum successfully penetrated many species of fish but failed to develop on entry and eventually died. He did not state how long they took to die or if any development at all took place but it seems that they may differ from S. baccatus in S. maximus and C. bulboglossa

in E. constans in that, in these latter cases, a considerable period of time elapsed before death during which some development did take place.

(ii) Host response

The relative size and duration of development of the layers of the capsule varied between individuals and different species, but were broadly similar in P. platessa, L. limanda and S. solea. The most intense and prolonged reaction was seen in the P. platessa where the capsule was thicker and the epithelioid appearance more marked. Giant cells were seen in the P. platessa lesion (and to a less marked extent in S. maximus and L. limanda) but not in S. solea. Giant cells have not previously been reported in tissue responses to metacercariae in fish, although they have been reported to occur in response to other inflammatory agents. Giant cells in fish were reviewed recently by G. Timur (1975) who induced giant cell formation in P. platessa using a variety of stimuli.

The S. solea capsule was generally much thinner than comparable capsules of P. platessa and this is a similar situation to that found by Sogandares-Bernal & Lumsden (1964) where there were differences in thickness of capsules of Cyprinodon sp. and Mollienesia sp. in response to the same parasite Ascocotyle leighi Burton. The rate of response in S. solea and L. limanda was slow and of generally low intensity.

No qualitative differences in the inflammatory response was seen which could account for the death and retardation of larval development in S. maximus, but the rate of response in S. maximus was accelerated. The appearance of the paracyst membrane was earlier (10 hours compared to 48 hours in P. platessa) and diapedesis was first observed at 24 hours, while this was not notable until 48 hours in P. platessa. It may be that the local tissue histiocytes were more responsive in S. maximus. The delay in the "vacuolated" or "blown up" appearance of the larvae in S. maximus may also have been the first sign of the retardation of the parasite in this host.

Subsequent capsule formation was comparable in S. maximus and P. platessa, the only possible difference being the shape of the capsule which was more oval in P. platessa with distinctly thicker capsular poles.

The cause of retardation of development and, in some cases, the death of the parasite in S. maximus was not very clear. The successful inhibition of the exchange of essential metabolites by the capsule could be considered to be a factor but the nature and size of the capsule was not significantly different from that of P. platessa, in which the parasite survived well. The advancement of the deposition of the first layers of the capsule by at least 38 hours compared to P. platessa may have interfered with development at a critical stage, whereas in the P. platessa the metacercariae may have been able to avoid encapsulation until this critical period had passed. Howell (1973) showed that metacercariae of Stictodora laevi managed to avoid the encapsulation by Gambusia affinis until 21 - 23 days after infection. He demonstrated that host material was associated with the paracyst wall although the paracyst components were antigenically different from the fish. He concluded that either the paracyst disguises itself as fish tissue by its association with fish substances, or the physiochemical properties of the cyst wall protect it. If the former were true for S. baccatus then the metacercariae would be able to contaminate its surface with S. maximus material. There is not enough evidence to come to any conclusions involving the avoidance of encapsulation by physiocochemical properties, of the paracyst wall and this merits further investigation.

A further possible way in which parasites may avoid the inflammatory reaction of this host is incorporation within the

parasite's genome of a range of codes so that antigens of the particular host encountered may be synthesised on infection. If this were the case here then S. baccatus would have to be ecologically separated from the host so as not to have infected it frequently enough to adapt. This seems unlikely since 0-group P. platessa and S. maximus are commonly found together, although S. maximus does tend to occur in a shallower water zone than P. platessa. A third possibility is antigenic mimicry where the evolutionary process enabled the parasite to develop similar antigens to the host. However, the evidence of previous studies of metacercariae such as that of Harris & Cottrell (1974) demonstrated precipitating antibody of P. platessa to the antigens of parasitic Digenea. Cottrell (1977) found that 0-group and adult P. platessa infected with Cryptocotyle lingua and Rhypidocotyle johnstonei produced precipitating antibodies to the antigens of these parasites. However, even where antibody production to helminth parasites has been demonstrated, protection against the disease has not been obvious. There is no evidence to suggest an effective humoral antibody attack on S. baccatus in S. maximus but, without any further evidence, this cannot be ruled out. Antigenic mimicry would restrict the number of hosts available to those with similar antigens and further work would be necessary to ascertain the antigenic relationships of the hosts susceptible to S. baccatus.

Adaptation of S. baccatus may have evolved to the Family Pleuronectidae to which P. platessa and L. limanda belong and the Family Soleidae to which S. solea belongs, but not to the Family Bothidae of which S. maximus is a member. Further evidence of this is found in Part 2 A of this Section where attempts to infect Scophthalmus rhombus, also a member of the Family Bothidae,

resulted in dead and retarded metacercariae. It seems that the L. limanda may be the normal host for S. baccatus in the location from which the molluscan host was obtained. (MacKenzie pers. comm.) In this case adaptation will have taken place to this species. Infection of the members of the Family Bothidae may be unsuccessful owing to their phylogenetic difference and consequent physiological difference from the Pleuronectidae and Soleidae.

The adaptive process often results in a dependence by the parasite on the host for some essential factor or complex of factors. In this particular host/parasite relationship, the factors may be simple or many and varied but the lack of these does not prevent invasion of the host tissues by the cercariae, since large numbers can be found after penetration. There is no evidence of a lack of food since the parasites can still be seen to be alive in many cases (at least until 45 days). What seems to be missing is a growth stimulating factor similar to that discussed by Chandler (1932). Scott (1928) described experimental infections of mammals, e.g. the laboratory rats which were abnormal hosts of the nematode Ancylostoma caninum. The larvae lived for 21 days and when transferred to a susceptible host showed unaltered infectivity. A similar pilot experiment performed during this study showed that a metacercaria from S. maximus of 2 days post infection grew normally when transferred into the peritoneal cavity of P. platessa. This presents interesting possibilities for further work, in particular, a full study involving transference of cysts between susceptible and unsusceptible hosts thereby giving more insight into the interesting phenomenon of host resistance.

PART 3 THE DEFINITIVE HOST

A. Natural infections in wild fish

B. Metacercarial viability studies

INFECTION IN THE DEFINITIVE HOSTS

A. NATURAL INFECTIONS OF WILD CAUGHT HOSTS

It was hoped that eggs from adult Stephanochasmus baccatus could be used to set up laboratory infections in the primary host Buccinum undatum. A large number of potential hosts were examined from a variety of sources. The details of these are given in Table XIX.

Table XIX Results of the examination of wild caught hosts of adult S. baccatus from Scottish Waters.

Host Species	Date of Capture	Source	Number Examined	Size in c.m.	No. of adult S. baccatus
Eutrigla gurnardus	5 Dec	nnear Oban	2	16.5, 17.0	0
E. gurnardus	14 Dec	Tobermoray	6	13.5 - 30.0	0
Trigla lucerna	13 Feb	Deep water off Shetland	70	29.0 - 44.0	1
Aspitrigla cuculus	21 Feb	West Coast of Scotland	64		0
Myoxocephalus scorpius	28 Feb	Aberdeen Bay	26		0
T. lucerna	"		1		0
E. gurnardus	22 Mar	Tiree (Live)	1		0
M. scorpius	Jan-Feb	Aberdeen Bay	13		0
		TOTAL	183		

Unfortunately, only one specimen of S. baccatus was found from all the fish examined. This adult worm was mature and contained three eggs. The eggs were fed (enveloped by a piece of squid) to a large healthy specimen of B. undatum in an aquarium at 12°C.

After 42 days the mollusc was killed, fixed and serially sectioned. There was no evidence of infection by any digenean species.

B. METACERCARIAL VIABILITY STUDIES

The evidence from the experimental infections of species of Heterosomata with S. baccatus and the studies of the histopathology of the metacercarial infections in these hosts suggests that there is a qualitative difference between the metacercariae developed in the S. maximus and S. rhombus and those which develop in P. platessa, Platichthys flesus, L. limanda and S. solea. This difference may affect their infectivity. Metacercariae from P. platessa and S. maximus were used as representatives of those differences in order to study their viability.

The cysts were first examined for movement to try to establish whether larvae from S. maximus were at all active. Generally metacercariae can be stimulated into activity by heat, irritants, etc., but all techniques used on S. baccatus failed to produce movement of any kind. However, no activity could be induced in the encysted larvae from P. platessa and it seems that there may be a dormancy period for these S. baccatus metacercariae.

The only certain method of assessment of the viability of such metacercariae was to attempt to produce adult S. baccatus in the final host and, therefore, experimental infections were carried out using cysts from P. platessa and S. maximus.

The details of these experimental infections are given in Materials and Methods, page 20.

Metacercariae of S. baccatus, do not become infective in M. scorpius until 5 - 7 months old and metacercariae of 8 -15 months old showed optimum development in that host species (MacKenzie, 1971).

The metacercariae used in the experimental infections of this host were, therfore, allowed to mature for 9 - 10 months in P. platessa and S. maximus.

The number of cysts fed to each fish varied from 9 - 59 (Table XX) but all those administered to any one fish were given at the same feed so that the age of the parasite in the final host was known.

Thirteen M. scorpius with a length range 16.5 - 36.0 cm had been fed only on peices of squid and were found to be completely free of S. baccatus infection.

The details of the numbers of cysts fed to individual fish and the numbers of adult worms recovered can be seen in Table XX.

The table shows quite clearly that the majority of metacercariae from S. maximus were unable to develop into adult worms. Of a total of 159 cysts from S. maximus only one adult worm was recovered. In the other group of M. scorpius, 35% of the metacercariae became established in the host intestine.

It had been hoped to continue the experiment until the worms had matured. Unfortunately the conditions in the aquarium made it impossible to continue to the full term.

Table XX Metacercarial viability study

Number of metacerariae fed to M. scorpius and the number of adult worms recovered.

	M. scorpius size in cm	Age of S. baccatus in M. scorpius in Days	Number of metacercariae fed to M. scorpius	No. of adult S. baccatus recovered
Metacercariae from PLAICE	28.5	10	35	15
	29.0	25	18	9
	32.0	15	20	17
	35.0	7	22	7
	17.0*	32	21	2
	16.5	33	43	5
Total			159	55
Metacercariae from TURBOT	29.0	27	59	0
	27.5	13	15	1
	25.5	56	38	0
	20.0	19	16	0
	18.2	13	14	0
	17.0	12	9	0
Total			151	1

* The small number recovered from this M. scorpius may be because this fish died prematurely and had undergone some degeneration before it was examined. Some worms may, therefore, have been lost.

DISCUSSION

The experimental infection of B. undatum would greatly assist the study of the biology of S. baccatus and it is felt that this would best be achieved by obtaining eggs from adults in experimentally infected laboratory hosts such as M. Scorpius. Infections of M. scorpius by metacercariae from P. platessa was relatively easily achieved. Unfortunately the maturation of S. baccatus metacercariae is a slow process (8 - 15 months), thus setting up the life cycle in the laboratory would take some time to complete.

The failure of the infection of B. undatum with eggs from the adult worm found in T. lucerna may be due to the type of eggs which were found. Wolfgang (1955b) described two types of eggs. One type was ovoid, smooth-shelled and slightly flattened at the opercular end. The other type was an hexagonally ribbed form. Both types of eggs may be found in the same specimens. He thought that the ribbed eggs, which were more common in the spring and summer than the winter collections, were infertile since they were transparent and underwent no development in the laboratory. There were not enough eggs found in the present study to carry out a study of the relative infertility of ribbed and smooth eggs, but the findings here do support, to some extent, Wolfgang's suggestion.

The infected T. lucerna which was host to the specimen of adult S. baccatus must have acquired its infection in the autumn of the previous year when it moved inshore to spawn. The fact that the worm was found in the month of February might indicate, therefore, that it was an old infection within the definitive host. It might be possible to speculate that more infertile eggs are produced towards the end of the life of the worm.

The eggs were administered to the mollusc orally in food because it seemed possible that the eggs needed to be ingested by the mollusc through its scavenging activities. However, it may be that the egg normally hatches in the water and actively seeks the next host, although Wolfgang (1955b) produced no miracidia. Not enough specimens were collected to test this hypothesis. Erasmus (1972) states that egg capsules which are undifferentiated when deposited generally take from 10 days to 3 weeks to mature depending on the temperature and the species. Below 10°C little development takes place. He also suggested that egg capsules may over winter at low temperatures and then hatch in the spring when conditions are more favourable.

The temperature may be the key factor in the case of S. baccatus but the factor may also be a temperature-related phenomonon, day length changes, etc.

It is, therefore, possible that the eggs administered to the mollusc were not infective but required a period of time in the external environment in order to embryonate and possibly hatch into miracidia.

METACERCARIAL VIABILITY STUDY

The experimental definitive host infections demonstrate that the vast majority of metacercariae from I-group S. maximus were non-viable in the final host M. scorpius. This is also interesting since two of the earliest records of S. baccatus metacercariae in European waters were those of Kroyer (1838-1853) and Dujardin (1845) who reported them in P. platessa and S. maximus.

The recovery of one adult worm of the 151 metacercariae fed to M. scorpius does indicate that normal development of S. baccatus in S. maximus can occur but is apparently rare. Since no 0-group S. maximus were subjects of experimental infections, there is also the possibility that normal development may occur in the first year of life in S. maximus and they are subsequently resistant to infect.

One of the purposes of this study was to investigate the effect of S. baccatus infection on fish hosts of culturable potential and thus of importance to the flatfish aquaculture industry. It is the simultaneous penetration and migration of large numbers of larval Digenea through the tissues of fish which is frequently the most damaging aspect of the fish/parasite relationship. Small 0-group fish are particularly vulnerable and it is the practice of fish farms to maintain fish of this age in covered concrete or fibreglass tanks, isolated from the first intermediate hosts. At a certain size they are put into cages in the open sea where they may be exposed to infection by S. baccatus if they are retained in cages lying on the sea bottom (MacKenzie, McVicar & Waddell, 1976). None of the host species studied, with the exception of the M. kitt, were able to resist penetration by large numbers of cercariae of S. baccatus and all species are therefore at risk from these effects. However, there is no evidence that smaller numbers of invading cercariae of S. baccatus have any effect on fish health and S. maximus was shown to have a better chance of recovery from infection and would suffer no subsequent damage from the growth and development of the parasite in the muscles, fins and subcutaneous tissues.

SECTION IV

CONCLUSIONS AND GENERAL DISCUSSION

CONCLUSIONS AND GENERAL DISCUSSION

Studies of the natural infections in the molluscan host showed a seasonal cycle of infection which began in April and May and continued until August. No infections occurred in September and there was no evidence of overwintering of S. baccatus in the mollusc. This seasonal cycle was not dependent on the presence or absence of cercariae because, although cercariae were produced after July, they showed a reduced infectivity. The number of cercariae in the mollusc was reduced in August and September when rediae showed signs of degeneration.

Reduced cercarial infectivity correlated with the lack of glycogen seen in many cercarial tails after July. Glycogen was shown to be related to infectivity in aged cercariae. Cercariae which had been aged for 10 hours showed a reduced infectivity and this corresponded with a reduction in glycogen in their tails during the free swimming period. Rediae were found to contain large amounts of glycogen but the digestive gland tubules of the mollusc did not contain significant deposits, although lipids were abundant. Gametogenesis was evident in the mollusc in September and competition for energy resources was thought to occur.

Two species of Heterosomata not hitherto recorded as second intermediate hosts, were successfully infected with S. baccatus: the flounder, Platichthys flesus and the Dover sole, Solea solea. Differences were shown to occur in the distribution of metacercarial cysts between S. maximus and P. platessa. These differences were thought to be related to behavioural differences, since no

differences in skin structure were found to account for them.

Different species of second intermediate host showed variations in the rate and intensity of the inflammatory response. S. maximus and P. platessa showed a greater intensity of reaction to S. baccatus metacercariae than L. limanda and S. solea, and S. maximus response occurred at an accelerated rate. The response had the appearance of an extensive chronic granuloma in which inflammatory giant cells were found 24 - 28 days post infection. Giant cells were most prominent in P. platessa capsules.

Differences in the rate of development of the metacercariae between host species were discovered. Metacercariae from S. maximus and S. rhombus were much smaller than those from P. platessa at the same time after infection. No differences were seen in the development of the metacercariae between L. limanda, S. solea, P. flesus and P. platessa. Morphologically, the metacercaria from S. maximus appeared to have developed very little and when specimens of the final host were infected with metacercariae from P. platessa and S. maximus, by far the majority of larvae from S. maximus never established as adults in the definitive host's intestine.

S. maximus and S. rhombus, both members of the Family Bothidae, were, therefore, considered to be abnormal hosts of S. baccatus to which the parasite was not adapted.

There was no evidence to suggest that small numbers of S. baccatus cysts in any of the fish in the one year age group were affecting their viability. However, 0-group P. platessa were

severely affected because of the large size of the cysts in relation to the body size of the fish which resulted in extensive muscle degeneration. Cercariæ penetrated the skin of all the fish species studied with the exception of Microstomus kitt and therefore, all fish hosts would be vulnerable if subjected to large numbers of cercariae penetrating simultaneously, such as in an enclosed pond system.

The major conclusions regarding the pathology of the infection of S. baccatus in the first and second intermediate hosts have been made in the relevant sections and no further discussion need therefore be made here. However, it is appropriate to synthesize some of these findings in order to allow further consideration of the general ecology of S. baccatus.

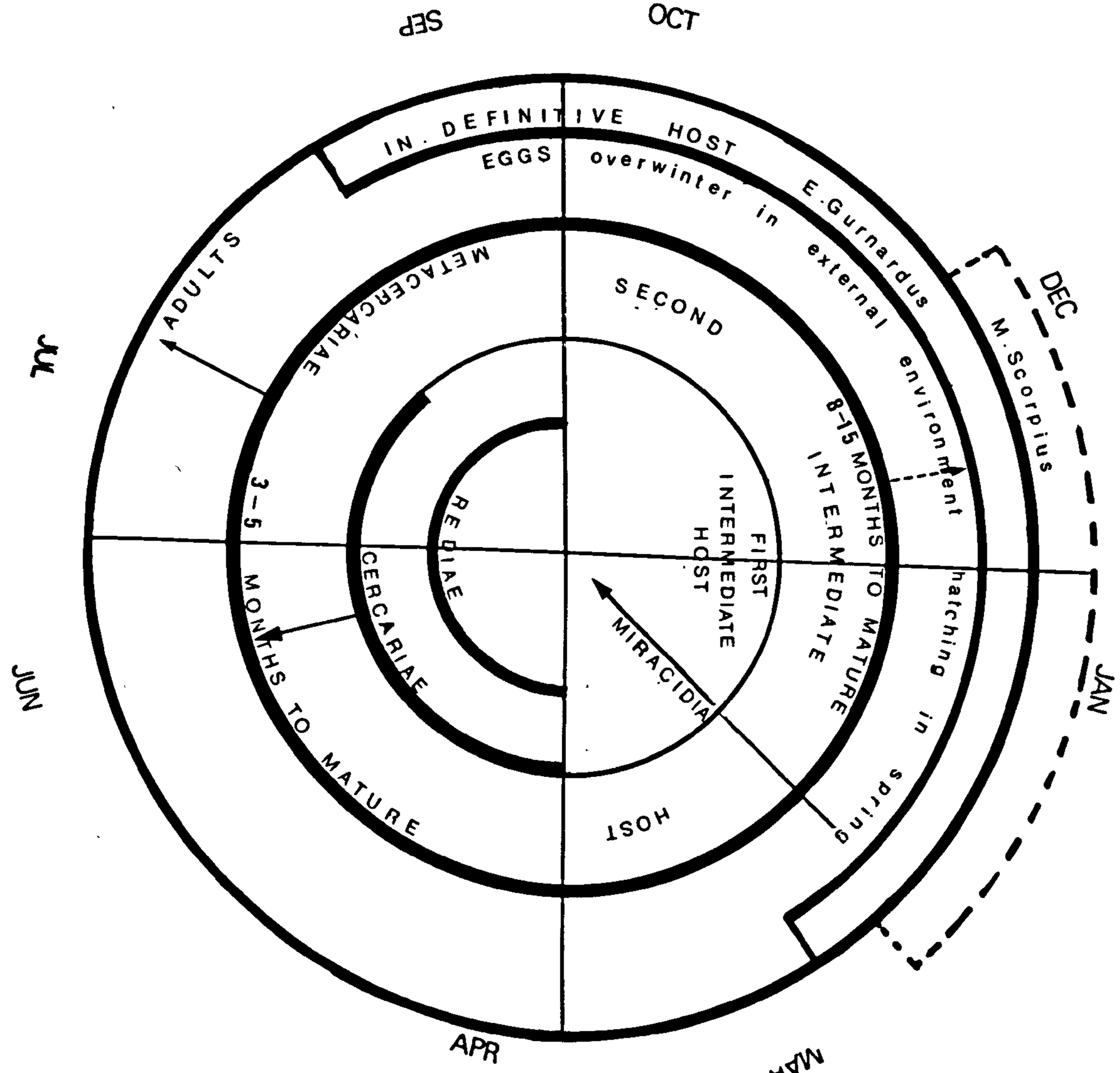
Wild caught definitive hosts showed a very low incidence of infection which may be related to the seasonal cycle of infection. Information from this and previous studies make it possible to describe the seasonal cycle for S. baccatus in the Loch Ewe area. This is represented diagrammatically in Figure 68 .

B. undatum and N. antiqua begin to release cercariae in April and May and there are no more cercariae after September (present study). 0-group P. platessa acquire their infections in May, June and July (MacKenzie & Gibson, 1970). There is a reduction in the intensity of infection in I-group fish in July and August (present study).

The metacercariae from P. platessa take a long time to mature in the second intermediate host and will not infect M. scorpius for 8 - 15 months (MacKenzie & Gibson, 1970) that is, before December or February.

Figure 68.

Diagramatic representation of the seasonal cycle of S. baccatus in Loch Ewe


SEP
OCT
IN. DEFINITIVE HOST
EGGS
overwinter in external environment
E.Gurnardus
DEC
M.Scorpius
ADULTS
METACERCARIAE
SECOND
8-15 MONTHS TO MATURE
INTERMEDIATE
hatching in spring
JAN
3-5 MONTHS TO MATURE
REDIAE
CERCARIAE
FIRST INTERMEDIATE HOST
MIRACIDIA
HOST
JUN
APR
MAY

Maturation of the adult S. baccatus in M. scorpius takes 32 - 36 days when the metacercariae have matured in the second intermediate host for 8 - 15 months; metacercariae younger than this take 6 - 9 weeks to mature in M. scorpius (MacKenzie & Gibson, 1970). Eggs, therefore will be released at the earliest from February onwards, which must lead to infection of B. undatum and N. antiqua and mature sufficiently to produce cercariae in April or May.

The cycle involving E. gurnadus as definitive host however differs because the maturation time in this host is shorter. Metacercariae of 3 - 5 months old are able to infect E. gurnadus and the maturation time in the intestine of this species is only 24 days (MacKenzie & Gibson, 1970). E. gurnadus come inshore to spawn in late summer and autumn at which time juvenile flatfish must be incorporated into their diet and the infection by S. baccatus will take place. E. gurnadus capture data from Upper Loch Torridon (North West Scotland) in 1974 showed that no E. gurnadus were taken in the first six months of the year (Johnstone, pers comm.). The first record of capture was on the last day of July. Occasional catches were then recorded until the end of November. (Bad weather prevented trawling from December until March of the following year when only 2 specimens were caught in 10 fishing expeditions.)

Infection in E. gurnadus probably occurs from August to November and possibly some later than this, but not after March of the following spring. Eggs will be available from September at the earliest.

Nothing is known about the mode of infection of the mollsuc. However, it is suggested that the egg may need a period of development in the external environment before it becomes infective. Only a small number of eggs are apparently produced by S. baccatus adults; only three were found in the specimens of the present study, MacKenzie (1970) reported 1 - 20 in experimental infections and the greatest number reported by Wolfgang (1955b) was 32, but small numbers occurred more commonly. This small production of eggs presupposes a high host finding success rate for miracidia. Hancock (1963) tagged populations of B. undatum and concluded that movements of these animals were very limited between populations. In these circumstances a mobile miracidium would enhance the chances of the parasite of locating the molluscan host. It seems likely, therefore, that the miracidium hatches and undergoes a free swimming period in the external environment.

The length of time required for development of the egg to miracidium is almost certainly temperature dependent (by analogy with other digenean species) and may slow down considerably over the December to February period. The temperature of the shallow water in which E. gurnadus were found (below 20 metres) is falling at this time. The development may thus be more rapid in eggs released from adult S. baccatus in M. scorpius from February onwards possibly owing to the longer day length. Erasmus (1972) suggests that light is an important factor influencing the hatching of eggs in the external environment.

It is possible that the timing of the cycle also differs according to the species of fish acting as the second intermediate host. MacKenzie & Gibson (1970) suggested that L. limanda are

more commonly found as hosts of S. baccatus in Loch Ewe. L. limanda larvae do not settle until June (Edwards & Steele, 1968) and, therefore, the seasonal cycle of infection would begin 1 - 2 months later in this species. Gurnards may feed on young Hippoglossoides platessoides further offshore at different seasons and eggs may then be released when the gurnards migrate inshore.

The relative importance of different host species varies according to geographical location. The most significant hosts in Canadian waters have already been described. The location used in the present investigation may represent the lower southern limits of S. baccatus range. The reports of its occurrence and the clear cut season of infection in the mollusc in these latitudes suggest that it might be a predominently Boreal or Arctic species, where sharply demarcated seasons are characteristic.

Different host species might be expected to be important in these colder waters. Both MacKenzie (1970) and Wolfgang (1954b) found Neptunea sp. to have a higher incidence of infection than B. undatum. Attempts were made to find details of the distribution of Neptunea sp. without success but it is generally believed to have a more northerly distribution than B. undatum. It breeds in early spring (Fretter & Graham, 1962) which is usually a sign of a northerly adapted species (B. undatum breeds in December) and it is not found in South and South West England or South Wales (Fretter & Graham, 1962).

Further, the long rough dab, Hippoglossoides platessoides is consistently heavily infected in Arctic waters (MacKenzie, pers. comm. from observations made at sea on Arctic cruises). Halibut, H. hippoglossus may be the most significant definitive host in Boreal waters. H. hippoglossus has a subarctic distribution and, further northwards, the range may be taken over by the Greenland halibut, Reinhardtius hippoglossoides (Walbaum 1792) which is found North of Faroe in Iceland and Greenland waters to the polar icecaps (Wheeler, 1969). E. gurnadus is the most northerly ranging of the gurnards and is found as far north as Northern Norway and Iceland and therefore overlaps the range of H. hippoglossus and H. platessoides. M. scorpius is a Boreal species and has a range which overlaps H. hippoglossus and E. gurnadus.

In subarctic latitudes the life cycle would therefore involve Neptunea sp., H. platessoides and H. hippoglossus as first intermediate, second intermediate and definitive hosts respectively with R. hippoglossoides extending the range of this parasite into the Arctic waters. However, R. hippoglossoides has not yet been recorded as a host for S. baccatus.

SECTION V

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REFERENCES

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SECTION VI

APPENDIX

A P P E N D I X

TABLE 1 B. UNDATUM sample 3 May 1973

Mollusc	Height cm	Weight gm	Penis length cm	Weight/ Height	Height/ Penis length	Penis length/ Weight
Males Normala	9.3	51.2	3.2	5.0	2.9	17.1
	9.5	70.8	4.1	7.5	2.3	17.3
	8.9	63.0	3.6	7.1	2.5	17.5
	9.0	54.5	1.3	6.1	6.9	41.9
	9.2	59.7	2.4	6.5	4.6	24.9
	10.8	87.3	4.0	8.1	2.7	21.8
	8.2	31.6	3.3	3.9	2.5	9.6
	8.5	44.0	2.9	5.2	2.9	15.2
	9.4	61.1	4.3	6.5	2.2	14.9
	9.3	57.9	4.2	6.2	2.2	13.8
Mean	9.2	58.1	3.3	6.2	3.2	19.3
Females	10.1	66.9		6.6		
Normal	8.3	52.4		4.8		
	8.2	40.1		5.2		
	10.1	42.8		7.5		
	10.8	75.4		8.2		
	9.0	88.8		7.0		
	7.5	62.8		4.9		
	9.5	36.9		7.1		
		67.9				
Mean	9.1	59.3		6.4 ±0.36		
Males						
S. baccatus	52.8	8.5	3.8	6.2	2.2	13.9
S. baccatus	62.4	9.0	0.6	6.9	15.0	103.9
Z. viviparus	44.5	9.0	1.0	4.9	9.0	44.5
Z. viviparus	68.8	9.0	1.4	7.6	6.4	68.8
C. buccini	108.8	11.4	4.1	9.5	2.8	26.5
Females						
S. baccatus	9.5	58.2		6.1		
Z. viviparus	8.0	40.0		5.0		

TABLE 2 B. UNDATUM sample October 1973

Mollusc	Males			Females
	Height cm	Penis length cm	Height/ Penis length	Height cm
Normal	8.4	2.2	3.8	8.9
	11.5	8.7	1.3	10.1
	10.5	8.0	1.3	8.5
	10.1	7.9	1.3	7.4
	10.5	9.3	1.1	8.9
	7.5	5.9	1.3	8.8
	10.5	7.9	1.3	11.0
	6.6	3.9	1.7	8.2
	10.1	7.5	1.3	7.9
	9.2	6.3	1.5	8.4
	8.4	5.0	1.7	10.5
	10.8	8.3	1.3	9.0
	10.8	3.9	2.8	7.9
	7.3	6.8	1.1	11.3
	7.7	6.3	1.2	6.6
	7.5	4.5	1.7	10.8
	8.3	7.4	1.2	8.1
	8.1	5.0	1.6	6.1
	6.8	1.2	5.7	10.7
	10.4	8.2	1.3	9.7
	10.8	8.6	1.3	7.4
				10.9
Mean	9.1	6.3 ±2.17	1.7	9.4
Infected				
Z. viviparus				8.4
C. buccini				10.8
Unidentifiable				9.9
"				9.8
No infected males were found in this sample				

TABLE 3 B. UNDATUM sample 28 May 1974

Mollusc	Males			Females
	Height cm	Penis length cm	Height/ Penis length	Height cm
Normal	11.0	5.9	1.9	9.3
	11.4	7.5	1.5	10.6
	9.5	6.8	1.4	10.0
	10.2	7.9	1.3	11.5
	10.0	6.5	0.2	10.6
	11.5	7.2	1.6	11.5
	9.5	5.8	1.6	9.2
	9.3	4.9	1.9	7.3
	8.3	1.6	5.2	10.2
	10.0	5.7	1.8	10.2
	8.2	1.8	4.6	7.9
	7.8	1.4	5.6	9.2
	8.1	5.9	1.4	13.0
	7.6	5.3	1.4	4.6
	11.1	6.6	1.7	13.6
	11.2	1.8	6.2	4.2
	8.9	1.4	6.4	4.0
	8.7	6.5	1.3	10.6
	7.0	0.9	7.8	
	9.2	6.6	1.4	
	7.5	1.0	7.5	
	5.4	0.9	6.0	
	11.5	4.0	2.9	
	11.6	5.6	2.1	
	4.4	3.3	1.3	
	10.9	4.5	3.0	9.3
Infected				
Z. viviparus	8.5	0.9	9.4	
S. baccatus				7.0
C. buccini				11.1
				12.4
Z. viviparus				4.4

TABLE 4 B. UNDATUM sample 22 May 1974

Mollusc	Males			Females
	Height cm	Penis length cm	Height/ Penis length	Height cm
Normal	8.4	7.0	1.2	8.5
	6.1	5.5	1.1	9.2
	7.6	6.4	1.2	8.5
	6.9	4.5	1.5	8.7
	8.8	7.0	1.3	9.7
	8.0	2.5	3.2	8.9
	8.0	6.0	1.3	8.0
	7.7	5.7	1.4	7.9
	9.4	8.5	1.1	7.2
	7.8	5.5	1.4	7.5
	9.0	7.9	1.1	6.7
	8.1	8.0	1.0	7.3
	8.1	7.0	1.2	6.6
	8.3	6.5	1.4	6.4
	8.0	6.5	1.2	10.2
	7.5	4.1	1.8	9.1
	8.3	5.8	1.4	7.9
	7.3	3.0	2.4	8.5
	8.0	5.4	1.5	7.3
	7.0	5.4	1.3	9.4
	8.3	6.0	1.4	7.5
	6.5	1.6	4.1	7.6
	8.1	6.2	1.3	7.6
	7.1	3.6	2.0	9.4
	8.4	7.5	1.1	9.2
	7.8	5.2	1.5	9.0
	8.0	3.8	2.1	8.4
	7.2	5.6	0.1	8.4
	8.9	4.0	2.2	9.0
	8.7	4.1	2.1	9.5
	6.3	1.1	5.7	7.5
	6.5	4.5	1.4	7.3
	7.2	6.2	1.2	9.8
	8.0	5.7	1.4	7.6
	6.8	1.7	4.0	6.8
	5.9	1.6	3.7	9.8
	7.1	2.8	2.5	7.7
	9.6	7.5	1.3	6.1
	6.5	4.5	1.4	7.9
	8.7	6.0	1.5	7.8
	7.5	3.0	2.5	8.8
	8.0	4.6	1.7	8.4
	7.6	6.9	1.1	6.5
	8.1	2.7	3.0	9.1
	6.2	1.6	3.9	7.1
	5.2	0.7	7.4	7.2
	7.4	4.1	1.8	8.3
	6.7	2.2	3.0	6.4
	7.5	4.4	1.7	8.5

Contd/...

TABLE 4 (Continued)

Mollusc	Males			Female
	Height cm	Penis length cm	Height/ Penis length	Height cm
Normal				6.1
				8.4
				7.4
				6.6
				7.0
				7.3
				4.8
				7.5
				6.3
				6.1
Mean	7.6	4.8 ±2.03	1.9	7.9
Infected				
S. baccatus	8.2	3.9	2.1	
S. baccatus	7.5	4.9	1.5	
S. baccatus	8.5	3.5	2.4	
	9.1	4.8	1.9	
Z. viviparus	7.8	5.5	1.4	
	6.0	0.9	6.7	
S. baccatus				9.6
S. baccatus				8.2
S. baccatus				8.1
Z. viviparus				7.5
C. buccini				9.5

TABLE 5 B. UNDATUM sample May 1974 overwintered in Aquarium

Mollusc	Males			Females
	Height cm	Penis length cm	Height/ penis length	Height cm
Normal	7.7	2.1	3.7	8.7
	9.4	4.2	2.2	9.1
	8.7	5.5	1.6	8.8
	8.5	4.4	1.9	6.9
	10.4	5.4	1.9	3.9
	6.6	4.9	1.3	8.5
	9.2	1.2	7.7	13.0
	9.4	3.1	3.0	11.3
	8.5	2.9	2.9	
	8.4	5.1	1.6	
	8.4	2.6	3.2	
	8.0	2.4	3.3	
	8.2	4.5	1.8	
Mean	8.5	3.7	2.8	8.8
Infected *C. buccini*	8.2	1.0	8.2	

STATISTICAL TESTS PERFORMED ON B. UNDATUM DATA

1. Copulatory organ length in May and October.

Comparing means of samples October 1973 and 22 May 1974.

October 1973: Mean 6.32, S.D. 2.17, No. = 21

22 May 1974 : Mean 4.84, S.D. 2.03, No. = 49

t = 2.69

The difference between the two samples is significant at 1% level.

2. Copulatory organ length in infected and uninfected males.

Comparing means in the sample May 1973 and 22 May 1974 combined.

Infected : Mean 3.34, S.D. 1.60, No. = 5

Uninfected: Mean 4.58, S.D. 1.97, No. = 59

t = 1.63

There is no significant difference between the two means.

3. Weight : Height ratio in infected and uninfected

Comparing means in the sample of May 1973.

Infected : Mean 6.40, S.D. 0.36, No. = 3

Uninfected: Mean 6.30, S.D. 1.32, No. = 18

t = 0.27

There is no significant difference between the means.

TABLE 6. Distribution of cysts in P. platessa.

Experiment	Fish	Fins	Body	Distribution of body cysts				
				Upper Body	Lower Body	Somatal muscles	Fin muscles	Skin
May	1	237	364	176	188	89	97	167
0 Hours	2	48	271	111	160	69	87	115
	3	10	156	81	75	25	47	82
	4	129	812	498	314	313	295	203
	5	13	167	54	113	83	62	15
MEAN		87.4	354.0	184.0	170.8	115.8	117.6	116.4
August	1	0	15	5	10	12	2	0
0 Hours	2	0	4	4	0	4	0	0
A	3	0	21	1	20	10	10	1
	4	1	20	0	20	8	11	1
	5	3	6	0	6	5	1	0
MEAN		0.80	13.2	2.0	11.2	7.8	4.8	0.4
August	1	0	67	14	53	32	27	3
0 Hours	2	0	5	3	2	2	3	0
B	3	4	2	0	0	1	0	0
	4	15	27	2	25	19	5	1
	5	1	28	10	18	17	11	0
MEAN		4.0	25.8	5.8	19.6	14.2	9.2	0.8
May	1	4	16					
10 Hours	2	20	83					
	3	0	30					
	4	1	6					
	5	4	15					
	6	0	2					
	7	0	26					
MEAN		4.14	24.6					

TABLE 7. -Distribution of cysts in S. maximus

Experiment	Fish	Fins	Body	Distribution of body cysts				
				Upper Body	Lower Body	Somatal muscles	Fin muscles	Skin
May	1	236	482	391	91	91	114	258
	2	147	393	341	52	182	0	191
0 Hours	3	59	84	67	17	0	0	79
	4	112	72	70	2	0	1	32
	5	749	147	145	2	0	36	111
MEAN		260.6	235.6	202.8	32.8	54.8	30.2	134.2
August	1	4	2	2	0	0	0	2
	2	4	1	1	0	0	0	1
0 Hours	3	1	4	4	0	0	4	0
	4	0	3	3	0	0	0	3
A	5	1	0	0	0	0	0	0
MEAN		2.0	2.0	2.0	0	0	0.8	1.2
August	1	3	13	13	0	2	0	11
	2	5	7	7	0	0	1	7
0 Hours	3	11	18	17	1	0	3	15
	4	63	30	30	0	2	6	22
B	5	10	0	0	0	0	0	0
MEAN		18.4	13.6	13.4	0.2	0.8	2.0	11.0
May	1	1	15					
	2	40	36					
10 Hours	3	5	3					
	4	15	0					
	5	41	16					
	6	9	10					
	7	8	6					
MEAN		17.0	12.3					

TABLE 8. Total numbers of cysts found in different sites on the body of P. platessa and S. maximus.

Fish species	Month	Skin	Somatal muscles	Fin muscles	Sample
P. platessa	May	520	579	65	5 fish
	August	6	110	70	10 fish
S. maximus	May	673	274	151	5 fish
	August	60	4	14	10 fish

STATISTICAL TESTS EMPLOYED ON CYST DISTRIBUTION DATA

t tests were performed on mean percentages rather than number to avoid bias caused by particularly heavily or lightly infected fish.

1. Comparison of distribution of cysts on P. platessa and S. maximus

(i) Proportion of fin cysts on P. platessa and S. maximus

It is assumed that S. maximus and P. platessa have the same fin/body ratio.

	P. platessa	S. maximus
MEAN	16%	53%
S.D.	17.3	28.0
N.	22	22

t = 5.29

The percentage of cysts found on the fins of P. platessa was significantly different from the percentage of cysts on the fins of S. maximus at 0.1% level.

(ii) Proportion of cysts on the Upper body of S. maximus and P. platessa.

	P. platessa	S. maximus
MEAN	28%	93%
S.D.	21.3	8.0
N.	15	9

t = 10.66

The difference between the proportion of cysts on the upper body in the two species is significant at the 0.1% level.

(iii) Seasonal distribution of cysts on the Upper body of P. platessa and S. maximus.

	P. platessa		S. maximus	
	May	August	May	August
MEAN	47%	19%	89%	98%
S.D.	8.1	19.2	8.6	2.6
N.	5	10	5	4
	t = 3.94		t = 2.25	

The difference between the cyst distribution in May and August for P. platessa is significant at the 1% level.

The difference between the cyst distribution in May and August for S. maximus is not significant at the 5% level.

(iv) Distribution of cysts in the body tissues of P. platessa and S. maximus.

A chi-squared test was performed on the total numbers of cysts found in each of the sites examined for all the fish in the sample. For totals see Appendix table

Tissues compared: Skin, Somatal muscle, Pterygiophoral muscle

Ho - no relationship exists between the types of fish and location of cysts, i.e. type of fish does not affect location.

H_1 - there is a relationship

$$\chi^2 = 396.1$$

Ho is rejected at 0.1% significance level.

(v) Distribution of cysts in the body tissues of P. platessa and S. maximus.

P. platessa

A Chi-squared was performed on the total numbers of cysts in each of the sites examined for all the fish in the sample. Totals in Appendix Table.

Ho - no relationship between month and location of cysts, i.e. month does not affect location.

H_1 - there is a relationship

$$\chi^2 = 76.4$$

Ho is rejected at 0.1% significance level.

S. maximus

As the number of cysts found in the somatal muscle in August was only 4, and as a Chi-squared test cannot be performed with less than 6 in a cell, for this test somatal muscle was amalgamated with pterygiophoral muscle.

Ho - no relationship between month and location of cysts, i.e. month does not affect location.

H_1 - there is a relationship

$$X^2 = 7.2$$

Ho is rejected at 1% significance level but accepted at the 0.1% level.

2. Comparison of mean of means for individual 60 day old fish. Means are used rather than actual individual cyst figures because it avoids bias caused by individuals with unusually heavy or light infections

	P. platessa	S. maximus
MEAN	660	228.8
S.D.	127.9	59.7
N.	4	6

$$t = 6.3$$

The difference between the means is significant at 0.1% level.