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PATHOLOGY OF PARASITIC INFECTIONS IN MARINE TELEOSTS

by

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DECLARATION

This work has not been accepted in substance for any other degree, and is not concurrently being submitted for any other degree.

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This to certify that the work submitted here was carried out by the candidate herself. Due acknowledgement has been given to any assistance received.

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ABSTRACT

The cellular responses of Crenimugil labrosus and Pleuronectes platessa to tissue parasites have been investigated using metacercariae of Cryptocotyle lingua and Rhipidocotyle johnstonei, and the myxosporidian Myxobolus exiguus. The study was based on fish naturally infected with the parasites, and in addition experimental infections of C. lingua were established in grey mullet. The most intense response was induced by R. johnstonei in plaice, and was composed mainly of cells of the macrophage series, which included epithelioid and giant cells. Fibroblasts and collagen deposition formed the periphery of the lesion which was interpreted as a granuloma. Eggs released by this progenetic metacercaria did not produce separate granulomata.

Fibrotic encapsulation and associated melanin deposition formed the host response to the encysted metacercariae of C. lingua in naturally infected plaice and mullet. Experimental infections of mullet produced only a fibrotic capsule. Sub-dermal cysts of M. exiguus produced a slight fibrosis and associated melanin deposition. Other sites produced little response, slight hyperplasia associated with interlamellar cysts.

Haematological studies on mullet and turbot formed a basis for the interpretation of the cellular responses to the parasites. Lymphocytes, monocytes, thrombocytes and granulocytes were identified from both species, and additionally plasma cells from mullet. Phagocytic capacity of circulating monocytes was demonstrated in turbot. Experimental infections of C. lingua in mullet were used additionally to study the formation of the parasitic cyst wall and related changes in the tegument during the metamorphosis from the cercarial to metacercarial stage. The two acellular layers of the parasitic cyst were formed from secretion bodies from the tegument and cystogenic gland cells. Breakdown of the tegument of the early metacercaria preceded replacement by the fully formed metacercarial tegument, which was initially microvillous and later contained secretion bodies. Variation in the structure of the plasmodial wall of M. exiguus occurred with site of development, although sporogenesis was similar to other species. A hyperparasitic microsporidian Nosemoides sp. was found in some plasmodia.

CONTENTS

INTRODUCTION

Research into marine fish farming has been chiefly directed toward the technical problems of hatching and rearing fish in systems suitable for commercial exploitation. In the United Kingdom feasibility studies by the White Fish Authority and establishment of efficient husbandry practice has attracted major British industries including I.C.I., Unilever, and British Oxygen Co., to invest in fish farms many of which are now well established. A variety of marine species are now being cultured in Europe including flatfish, mullet, bass and eels. Two of these fish were used in the present study, namely the thick-lipped grey mullet, Crenimugil labrosus and the plaice, Pleuronectes platessa.

Disease is a major factor in the success of these ventures, and although knowledge of fish disease has advanced considerably in the last decade, parasites which may be present in small numbers in wild fish, and have little effect; can become pathogenic in the high-density, stressed conditions of farmed fish (McVicar, 1979). The need for research in all aspects of fish parasitology in the marine environment is therefore apparent in order to develop methods of diagnosis control and treatment.

The present study is concerned with the cellular reactions of fish to selected parasites, including the metacercarial stages of Digenea, namely Cryptocotyle lingua and Rhipidocotyle johnstonei and the myxosporidian Myxobolus exiguus. These all develop within the fish tissues and would be expected to initiate a marked host reaction. The combination of these parasites and hosts enabled a comparison of the

effects of one parasite in different host species; the effect of unrelated parasites in a single host, and the relationship of site on parasite development.

The inflammatory response of fish to parasitic infection has received particular attention here as relatively little has been documented on this aspect of disease in comparison with mammals. Studies which have been undertaken on the chronic inflammatory response in teleosts however, have noted a qualitative similarity, modified by temperature (Timur, Roberts & McQueen, 1977). Chronic inflammation may develop when an organism is unable to remove an irritant and tissue destruction and inflammation are concurrent with attempts at healing.

The term 'granuloma' may be applied to a chronic inflammatory reaction containing a predominance of cells of the macrophage series (Walter & Israel, 1974). Although granulomas in fish have been reported in response to a variety of agents (reviewed by Finn, 1970; and Timur G. 1975); few studies of the cellular mechanisms involved have been undertaken. Hard, Williams & Lee (1979), have recently drawn attention to the need for such specific studies of host tissue responses in fish to inflammatory agents, to avoid confusion with cancerous lesions.

As a basis for the interpretation of cellular responses to the parasites employed in the present study, haematological studies of the grey mullet and turbot were undertaken.
A study of the tissue responses, and granuloma formation to parasitic infection also requires a knowledge of the structure of the parasite surface. It was necessary therefore to

describe the tegument and associated glands of both C. lingua and R. johnstonei, and also the plasmodial wall of M. exiguus. The discovery of a microsporidian hyperparasite, Nosemoides sp., of M. exiguus was of interest, and is described for the first time.

REVIEW

Past work relevant to the present study is reviewed under two main headings, namely: parasites, and host tissue reactions.

1. PARASITES

Myxosporidia

The order Myxosporida are common parasites of marine and freshwater fish. The classification used by Mitchell (1977), Subphylum Myxospora, Sprague 1969, Order Myxosporida , Butschli, 1881, is adopted here. For much of their life cycle myxosporidians exhibit multicellularity which has led to several workers (Grassé, 1960, Grassé & Lavette, 1978), to question their inclusion in the Protozoa.

Certain species are known to be serious pathogens of commercial fishes, the most widely studied being Myxosoma cerebralis, the causative agent of whirling disease in salmonid fish. The biology and control of this parasite has recently been reviewed by Halliday (1976).

The literature on other species of myxosporidians is reviewed here in chronological order. Most of the present information is related to the morphology and development of the spore - the most conspicuous stage in the life cycle.

Early workers were concerned with recording and describing new species based on spore characteristics. Thelohan (1891) recorded two new sporozoans found in the muscles of Cottus scorpio and Callionymus lyra. Gurley (1893) discussed the classification of known species and later in 1894, reviewed the diseases of fish caused by these parasites. Thelohan (1895) published a general description of myxosporidian parasites; followed by Tyzzer (1900) who discussed general features of the myxosporidian life cycle. Laveran and

Mesnil (1902) described the process of budding in the plasmodium of Myxidium lieberkuhni and concluded that it was a separate process from spore formation. Mercier (1906) examined the development of Myxobolus pfeifferi spores and suggested a sexual process occurred during development of the spores. Parisi (1910) described Sphaerospora caudata from the trophozoite and spore. Kudo (1916) described myxosporidians from fresh water fish in Japan recording three new species. In the same year Georgevitch (1916) gave descriptions of several species of myxosporidians from marine fish at Roscoff.

Hahn (1917a) using a more experimental approach to myxosporidian infections described the life cycle of Myxobolus musculi from the gills and muscle of Fundulus from Woods Hole. He found (1917b), higher rates of infection in captive fish and concluded that higher water temperatures may increase the infection.

Davis (1917) in a description of myxosporidia from the Beaufort region suggested that susceptibility of fish to infection varied with age; and cited the example of Myxidium bergense parasitising Gadus virens, in which only middle-aged fish were infected.

He concluded also that histozoic species of myxosporidia were associated with the freshwater environment being absent from truly marine fishes. Whereas in marine fish myxosporidians are chiefly coelozoic occurring commonly in gall bladders and urinary bladders. He found no group of fish to be free from myxosporidian parasites and some appear to have an annual cycle as Myxidium lieberkuhni from the urinary bladder of

the European pike which has spores in the summer months and trophozoites in the winter.

Kudo (1920) reviewed all 237 known species at that time. He found approximately equal numbers of species in fresh water and marine fish, and proposed a new classification and included a list of parasites, hosts, and geographical distribution. Kudo(1918a), carried out several experiments to investigate exsporulation and found that hydrogen peroxide in the presence of weak alkali effectively induced polar filament extrusion.

Kudo (1918b), described myxosporidian infections from fish at Woods Hole, including a new species, namely Myxosomia funduli from the branchial lamellae and gill filaments of Fundulus heteroclitus and F. majalis.

Southwell and Prashad (1918) described myxosporidia from fresh water fish in India and Ward (1919) claimed the first record of myxosporidian of fish from Alaskan waters. Dunkerley (1921) listed myxosporidian parasites from fish at Plymouth.

Kudo (1921) investigated the chemical nature of Henneguya salmonicola and found the spore coat to be composed of a substance related to chitin, and that the distinct iodinophilous vacuole of Myxobolidae to be similar in reaction to glycogen.

Jameson (1929) described twelve new species of marine myxosporidians from California. All parasites were found in the gall bladders. In 1931 he found similarity with fish from southern California. Flat fish and Elasmobranchs were

most commonly infected and infection was rare among surface feeders.

Kudo (1929), described histozoic species of Myxosporidia from fresh water fishes of Illinois including four species of Myxobolus. Herrick (1936), described two more new species of Myxobolus from the small mouth black bass from Lake Eerie. Kudo (1933), further reviewed the classification of the Myxosporidea.

Bond (1937a), undertook studies on the host specificity of Myxosporidia from Fundulus heteroclitus and concluded that parasites were confined to fish of the genus Fundulus.

Bond (1937b), found the spore coat nuclei of myxosporidia to degenerate with increasing maturity of the spore; and resistance of spores to be limited outside the host with Myxobolidae showing a slightly longer survival time, (Bond 1938). Nigrelli and Smith (1938), described the spore of Myxobolus lintoni and showed that the sporont gave rise to a single spore. Fish (1939), made observations on Henneguya salmonicola Ward, a myxosporidian causing 'tapioca' disease in Pacific salmon.

Noble (1941), considered the relationship between Californian tide pool fishes and their myxosporidia. He found that the same host taken from different environments may be distinguished by the parasite fauna. Fish living in the littoral zone are generally infected with a wide variety of parasites and a heavy infestation of one type of parasite usually excludes others.

Fantham and Porter (1943), described the morphology and life history of Sphaerospora periophthalmi from African

and Indian mudskippers. Dispersal of the spores was found to be by evacuation with the faeces and on decay of hosts. Infection was by the alimentary tract, gill chambers and tail skin. Uninfected fish were found to live longer than infected ones. Rice and Jahn (1943), described myxosporidian parasites of the gills of fish from the Okoboji region and Otto and Jahn (1943), described myxosporidian infections from the same region.

Noble (1943), described the nuclear cycles in Myxidium gasterostei and on reviewing the life cycles in 1944 concluded that the myxosporidia do not undergo true schizogony.

Tripathi (1948), recorded new myxosporidia from Plymouth and proposed a new classification of the order.

Laird (1950), described Henneguya vitiensis from a Fijian marine fish Leiognathus fasciatus Lacèpede 1803, found in heart blood smears, and noted that only three previous records of myxosporidians had been described from this location.

Tripathi (1953), described 15 new species and one new genus of myxosporidians from Indian fish.

Meglitsch (1952), discussed the myxosporidian fauna of fresh water and marine fish. He confirmed that marine fish harbour a higher proportion of coelozoic species and concluded that these were the most primitive forms, the myxosporidia evolving in marine fishes.

He found the physical character of the environment to play a part in determining the distribution of the species.

Noble (1957), discussed seasonal variations between fish and their protozoan parasites. In Callionymus lyra

myxosporidian seasonal changes were found to occur, heavier infections occurring in winter. He found an active predatory habit and a taste for a wide variety of food predisposes a heavy infestation.

Grassé (1960), made one of the first ultrastructural studies of myxosporidians describing Sphaeromyxa sabrezesi from the gall bladder of Hippocampa. He also claimed myxosporidians are multicellular.

Kabata (1961), described five new species of Myxosporidia from fish of the North Sea. Bhatt and Siddiqui (1964), described four new species of Myxosporidia from Ophicephalus punctatus an Indian fish.

Lom and Vavra (1962), discussed the subphylum Cnidospora, and according to these researchers the uniting feature is the presence of nematocyst-like cells found in the Myxosporidia, Microsporidia and Actinomyidae. Honiberg et al (1964), revised the classification of the Protozoa the myxosporidians lying within subphylum III the Cnidospora, Doflein 1901. Sprague (1966), suggested the presence or absence of a filament be rejected as a criterion for separating different groups of sporozoans.

Lom (1964), suggested the polar capsules have a protein stopper and that the polar filament is probably for anchoring the spore and observed it to be sticky. Extruded capsules were found to have a smaller volume than intact ones and extruded filaments were found to be hollow tubes. Lom and Vavra (1965), described the development of the polar capsules of Henneguya psorospermica Thelohan, and concluded the external tube is identical with that participating in the development of the nematocyst of Hydra, supporting the idea that myxosporidians are metazoans.

Lom and de Puytorac (1965b), made ultrastructural studies on Henneguya psorospermica, Myxobolus sp. Zschokkela nova, Chloromyxum cristatum and Myxidium lieberkuhni. They found variation among the trophozoites, which may be limited by a smooth or bristled membrane and may exhibit pinocytotic activity. Sporogenesis within the plasmodium was described and the morphogenesis of the polar capsules. In a note on the ultrastructure of trophozoites, Lom and de Puytorac (1965a), found intense pinocytotic activity in certain plasmodia which varied with the site of development of the trophozoites. Uspenskaja (1966), demonstrated acid phosphatase activity at the periphery of the plasmodium and concluded the parasite must absorb nutrients from the host urine over the whole plasmodial surface by pinocytosis.

Guilford (1963), described new species of myxosporidia from perches collected from Lake Michigan. Parasites were found in the neural tissue, sclerotic cartilage and on the gill filaments.

Noble (1966), described myxosporidia from deepwater fish and found feeding habits, population numbers and reproductive and migratory habits of fish were considered important factors in the acquisition of parasites.

Davies (1968), recorded six species of myxosporidia of fish from the River Lugg in Hertfordshire. No seasonal variation in occurrence, spore shape or size was found for Myxobolus muelleri but spore size varied depending on the host and site of infection. Although myxosporidian spores are used as the basis of taxonomy they were found to vary in size depending on the host and type of tissue infected, and at different times of the year. Manifestation of the same infection may vary according to the host infected.

Walliker (1967), in a study of protozoan parasites from the Amazon region of Brazil and the British Isles carried out experimental infections of fish with myxosporidians. He found spores germinated under the influence of gastric fluid but may require ageing before they can become infective. He also drew attention to the fact that the iodinophilous vacuole can not be used as a taxonomic criterion in the Myxosporidia and the genus Myxosoma should be synonymous with the genus Myxobolus (Walliker, 1968).

Schubert (1968), described the ultrastructure of the plasmodium and developing spore of Henneguya pinnae Schubert. The plasmodium was found to be enclosed within an epitheloid formed by the host.

Lom (1969), described the ultrastructure and sporoblast development in fish - parasitising myxosporidia of the genus Sphaeromyxa.

Meglitsch (1970), described the trophic stages and spores of Sphaerospora undulans from host fish taken from New Zealand.

Iversen, Chitty and van Meter (1971), described Myxosoma cephalis from the brain, meninges, jaw bone, crop tissue and buccal cavity of Mugil cephalus Linnaeus, of South Florida. The parasite may have been responsible for a previous epizootic.

Morrison & Pratt (1973), described Sphaeromyxa maiyai from the Pacific tomcod Microgadus proximus and used scanning electron microscopy to reveal the surface striations of the spore.

Lom and Hoffman (1971), distinguished Myxosoma cerebralis from Myxosoma cartilaginis by scanning electron microscopy.

Siau (1974), described Myxobolus exiguus from the gills and intestine of Mugil ramanda. Scanning preparations clearly distinguished it from Myxobolus muelleri. He later (Siau, 1979), cultured the sporoplasm of M. exiguus in trout cell culture, and found the vegetative nucleus unlike generative nuclei. Yoshino & Noble (1973), surveyed the parasites of Macrourid fishes of the North Atlantic. Myxosporidians and Microsporidians were often found together.

Podlipaev (1975), discussed polysaccharides in spores of the family Myxobolidae. He found the number of polysaccharides increased during spore formation and was gradually used outside the host. The function of the iodinophilous vacuole in systematics was supported.

Paperna (1975), recorded Myxobolus exiguus from Mugil cephalus and Mugil auratus, cysts being found on the internal organs.

Paperna and Zwerner (1974), described Kudoa cerebralis from the Striped Bass Morone saxatilis Walbaum found in the connective tissue of the nervous system.

Hine (1975), described three new species of Myxidium parasitic in Anguilla australis and A. dieffenbachi Gray.

Moser and Love (1975), described Henneguya sebasta from Californian rockfish. The parasite was found on the bulbus and truncus arteriosus and in the heart chamber. It may be a potential pathogen to the rockfish, an important sport and commercial fish.

Schubert, Sprague and Reinboth (1975), described the ultrastructure of Unicapsula sp. from Maena smaris. Moser (1976a), discussed interspecies associations of myxosporidians found together in double infections in deep sea Macrourid fishes and found they occurred together more frequently than could be attributed to chance. He also (Moser, 1976b), described Ceratomyxa anoplopoma from the sablefish Anoplopoma fimbria.

Moser & Noble (1976), recorded myxosporidia of the genus Leptotheca from macrourid fishes and sablefish, and Moser, Love & Jensen, (1976), described the myxosporidia from the Californian rockfish, Sebastes spp. Hine, (1976), recorded three new species of Mysidium parasitic in Anguilla australis and A. dieffenbachii. In a histological study of diseases from the branchial region of fishes Honma & Tamura (1976), reported a myxosporidian of the genus Thelohanellus from the fork tongue goby, Chaenogobius urotaenia.

Uspenskaja (1976), discussed the nuclear cycle of myxosporidia and Current & Janovy (1976), made an ultrastructural study of interlamellar Henneguya exilis, from the channel catfish. Moser & Noble (1977a), described the genus Myxoproteus from macrourid fishes, and the genera Auerbachia, Sphaerospora, Davisia and Chloromyxum from macrourid fishes and the sablefish (Moser & Noble, 1977b). Sankurathri (1977), described Conispora renalis from the kidney of Pacific hake Merluccius productus, and suggested the use of the suture line as a taxonomic criterion.

The fine structure of sporogony of Myxidium zealandicum was described by Hulbert et al. (1977), who found that the plasmodium breaks down when the spores are mature.

George, Harrison and Hadley (1977), recorded the incidence of Myxobolus dentium Fantham from the muskellunge Esox masquinongy Mitchell and from the Niagara river and found infected fish had a poorer condition. Incidence of infection was correlated with water temperature and fish 3+ to 5+ years old had a higher infection rate than fish 6+ to 8+ years old.

Current and Janovy (1978), in a comparative ultrastructural study of interlamellar and intralamellar forms of Henneguya exilis Kudo, from the channel catfish; found similarities in spore structure and sporogenesis but differences in plasmodium structure and relationship with the host cells.

Nakajima & Egusa (1978), described Kudoa pericardialis from the pericardial cavity of cultured yellow tail Seriola quinqueradiata Temminck et Schlegel. Wold & Iverson (1978), described Myxobolus latipinnacola from the sailfinn molly, Poecilia latipinna Lesuer.

Desser & Paterson (1978), made an ultrastructural and cytochemical study of sporogenesis of Myxobolus sp. from the common shiner, Notropis cornutus.

Wyatt (1979), described Facieplatycauda pratti and two new species of Myxobolus from the Klamath Lake sucker Catostomus luxatus (Cope).

Current (1979), described the ultrastructure of the plasmodium wall and sporogenesis of Henneguya adiposa Minchew from the channel catfish. The importance of the structure of the plasmodium wall as a taxonomic feature was noted. Weidner & Overstreet (1979), described sporogenesis of Fabespora vermicola a myxosporidian with motile spores.

Current, Janovy & Knight (1979), described the ultrastructure of the plasmodium wall and sporogenesis of Myxosoma funduli Kudo infecting the gills of Fundulus kansae (Garman). Plasmodia were located within lamellar sinuses. The plasmodium wall consisted of a single unit membrane with a surface coat.

Sadler, K. (1979), infected European eels (Anguilla anguilla L.) with Myxidium giardi, and found spores developed a diffuse histozoic infection of the kidney.

Microsporidia

Microsporidia are protozoa of the order Microsporidia Balbiani 1882, the unicellular spores probably being the smallest eukaryotic cells. They are obligate intracellular parasites and common in fishes with many species known to be pathogenic. General aspects of the biology of these parasites have recently been reviewed in a comprehensive publication by Bulla and Cheng (1976). There are few records of microsporidian hyperparasites of protozoa, although they are common hyperparasites of other groups. Vivier (1975), cites two examples of microsporidian hyperparasites of myxosporidea, including Nosema marionis Stempell 1919 a parasite of the myxosporidian Leptotheca coris, itself a parasite of the fish Coris julius and Coris giofredi. The myxosporidian spores apparently developing normally. However Nosema notabilis a parasite of the myxosporidia Sphaerospora polymorpha itself a parasite of the urinary bladder of fishes Opanus tau and O. beta from Florida; severely restricts spore formation of the myxosporidian in heavy infestations.

Digenea

Metacercariae of two species of Digeneans were used in the present study. These were Cryptocotyle lingua and Rhipidocotyle johnstonei. In order to understand problems in the collection, maintenance and transmission of these fish parasites it is appropriate to briefly review past studies on their life cycles and biology.

(a) Cryptocotyle lingua

The life cycle of Cryptocotyle lingua, a Heterophyid digenean, was described by Stunkard, (1930). The first intermediate host is usually the edible winkle, Littorina littorea, although it has been recorded from Littorina saxatilis by James, (1968); from Littorina obtusata by Iddon (1973), and from Hydrobia ulvae and H. ventrosa by Ginetsinskaya (1961). The metacercariae show a low specificity to marine teleosts which serve as hosts. Rothschild (1939), at Plymouth found that Cryptocotyle lingua infects almost any fish although some species are more susceptible. It has been recorded from a range of fish (Linton, 1940; Hsiao 1941; Christensen and Roth 1949; Sinderman and Rosenfield 1954) although Christensen and Roth (1949), were the first to record the metacercariae in Pleuronectes platessa and also found it in another Pleuronectidae, namely Platichthys flesus. Another infection of P. flesus with C. lingua was recorded by Mawdesley-Thomas and Young (1967). The adult parasite has been recorded from the small intestines of gulls, terns and other fish-eating birds and mammals.

Rothschild (1942), has noted the longevity of C. lingua infections in winkles and the cercariae of C. lingua have

been shown by Stunkard and Shaw (1931), to tolerate conditions of low salinity, enhancing the range for contacting the first intermediate host. The ultrastructure of C. lingua rediae and cercariae has been described by Krupa, Bal & Cousineau (1967), and Krupa, Cousineau & Bal (1966). More recently Rees (1974), described the ultrastructure of the body wall of the cercaria and Rees & Day (1976), have described the origin and development of the epidermis of the cercaria. Rees (1975a), has also described the photoreceptors of the cercaria and (1975b), the ultrastructure of the cercarial tail. Van der Broek (1979), described infections of estuarine fish with C. lingua and found metacercariae were retained for several years, and infections were most intense in fins and gills of pleuronectids.

(b) Rhipidocotyle johnstonei

This Gasterostome parasite has received less attention than Cryptocotyle lingua due probably to its molluscan host being unknown.

The life cycle and progenetic metacercaria has been described by Matthews (1968), and Cottrell (1975), has demonstrated a temperature dependent response to the parasite in plaice.

Other species of Rhipidocotyle have been described by Stunkard (1974), Kniskern (1952), and Krull (1934).

Parasite Surfaces

The tissue parasites studied here, including both myxosporidians and digenea are dependent on the intertransference of materials through their surfaces. They may therefore both be expected to exhibit structural surface modifications reflecting this function.

The structure of the trematode tegument has been the subject of comprehensive reviews by Lee (1966, 1972),

He found the tegument to exhibit topographical features indicating an absorptive capacity. Experimental evidence supporting this suggestion (Bjorkman and Thorsell 1964), has been criticised on the grounds that manipulative treatment may damage the tegument (Halton and Arme 1971).

From structural observations on myxosporidian plasmodia several authors have suggested it to be capable of active pinocytosis (Lom and de Puytorac 1965a, Uspenskaja 1966, Schubert 1968, Lom 1969 and Current and Janovy 1976). A recent review on the subject of endocytosis has been published by Silverstein, Steinman and Cohn, (1977).

A carbohydrate surface coat or glycocalyx may be important in both trematode and myxosporidian surface function. Lumsden (1975), emphasised its importance to parasitic helminths in permeability control and ionic regulation and protection of the parasite against host enzymes and involvement in antigen antibody reactions. Threadgold (1975), illustrated techniques for ultrastructural demonstration of the glycocalyx.

Erasmus (1977), discussed the host-parasite interface of trematodes and Pappas and Read (1975), suggested the term interfacial space to include the site of digestion and absorption of host material.

2. HOST TISSUE REACTIONS

The humoral and cellular response of fish to parasitic infestations has received considerable attention in recent times. It has been recognised that there is an essential similarity in tissue responses, including inflammatory responses to adverse stimuli and pathogens whether they

occur in mammals or fish (Finn and Nielsen, 1971).

The cyst produced by myxosporidians and larval digeneans may be composed of both parasitic and host cell elements. Circulating leucocytes of infected fish may contribute to the elaboration of the host response; and the literature on fish leucocytes has recently been reviewed by Ellis (1977), who drew attention to the need for a more functional approach to the interpretation of these blood cells.

The following section is concerned with the host reaction associated with the development of myxosporidian and digenean cysts in fish.

Myxosporidia

Studies on the effects of myxosporidian infections in fish have been less numerous than descriptions of the parasites, and the pathogenicity of the majority of species in fish is questionable. Fantham and Porter (1912), described the effects of myxosporidian parasites on the gall bladders of marine teleosts and elasmobranchs and found infections generally produced thickening of the gall bladder and changes in the colour and composition of the bile. Degeneration of the epithelium was recorded in Myxidium sp. infected gall bladders of Gadus pollachius. Hahn (1913), described tumours in Fundulus heteroclitus resulting from an infection of Myxobolus musculi where the muscle fibres became degenerated and later bacterial infection occurred. Thompson (1916) described a similar sporozoan infection of the muscle fibres of halibut producing a condition known as 'wormy' halibut. Hahn (1917), described a myxosporidian, namely Chloromyxum clupeidae infecting the muscle of young herring causing white

cysts between the fibres which he suggested made the fish more susceptible to predators. In the same paper he also described the occurrence of Myxobolus pleuronectidae from Pseudopleuronectes americanus producing a sore with degenerated muscle fibres. Davis (1924), described Unicapsula muscularis which caused the condition known as 'wormy' halibut. Only in late stages of development did the muscle fibres show signs of injury when swelling and hyaline degeneration occurred. He also described a similar condition in the muscles of barbel produced by Myxobolus pfeifferi. Development of myxosporidians in the muscle fibres however, was thought to be rare, the spores having been transported there via the blood capillaries.

Kudo (1929), reported a hyperplastic growth of the gill epithelium of the channel catfish Ictalurus punctatus infected with Henneguya exilis. Parasites were encysted on the gill lamellae and the surrounding tissue responded by an enormous hyperplastic growth of the adjacent epithelium together with an inflammatory response.

Fantham (1930), in a description of myxosporidia from marine fish of South Africa, found tumours in barbel and carp caused by Myxobolus ovidalis, and found the infection to be more pathogenic in barbel. Spores pass out of the pustules, and he suggested the polar filaments anchor the spores to another fish and the amoebula creeps between the scales.

Kudo (1929), noted tumour formation due to Myxobolus notatus Mavor in the connective tissue and tail muscles of Pimecephales notatus and Leuciscus rutilis. The tissue surrounding the trophozoite is composed of modified connective tissue cells.

Nigrelli and Smith (1938), discussed the tissue responses of Cyprinodon variegatus to the myxosporidian Myxobolus lintoni. The parasite produced two types of tumours; a flattened type with the overlying skin ulcerated and pigmented and a large pendant type. The tumours had an external layer of melanophores beneath which trophozoites and spores were embedded in a meshwork of fibroblasts. In deeper parts a mild leucocytosis occurred and partial destruction of scales in diseased regions occurred. The tumours were not encapsulated as an irregular infiltration occurred along the margins. They concluded that little is known about myxosporidian histopathology but coelozoic species seem to have little effect whereas parasites localised in the fins, skin or musculature may produce tumour-like growths.

Nigrelli and Smith (1940), described the disease affecting barbels Ameirus nebulosus (Leseur), caused by Henneguya ameiurensis. Although the parasites were encapsulated by host connective tissue, pathological changes were also produced adjacent to the encapsulated organisms: hyperplasia of the overlying epithelium and melanophores was common.

Nigrelli (1948), described prickle cell hyperplasia in the snout of the Redhorse sucker Moxostoma aureolum associated with an infection by the myxosporidian Myxobolus moxostomi. The most noticeable pathological changes occurred in regions immediately above and adjacent to the site of infection. Fibrous growth was thickened and vascularised, and in some areas the connective tissue appeared to give rise to a supporting stroma of hypertrophied stratified epithelium. The cellular elements of the latter being mainly prickle cells and macrophages. He concluded that intercellular and intracellular cnidosporidians elaborate proteolytic enzymes and other chemicals which may elicit the tissue response from the host.

Nigrelli (1953), reviewed the tumours from temperate freshwater fish of North America, including those attributed to myxosporidian infections.

Kabata (1957), described Myxobolus aeglefini from the cranial cartilage of Pleuronectes platessa and found the cartilage to be hypertrophied. He found that sporozoan parasites tend to be tissue rather than host specific.

Kabata (1960), described Kudoa clupeidae from the topknot Zeugopterus punctatus. The fish was found to have cysts in the flesh along the long axes of the muscle fibres.

Lewis and Summerfelt (1964), described Myxobolus notemigoni from the golden shiner where cysts were attached to the underside of the scales. The cyst wall was composed of spindle-shaped cells similar to fibrous tissue. This infestation was of commercial importance as it diminished the appearance of an important bait fish.

Lewis (1968), described Myxobolus argentatus from Notemigonus crysolencas from Illinois and Arkansas. Cysts were found in the subdermal connective tissue and most frequently in the branchial chamber, but also on the surface of the fish where they appeared as small golden elevated spots. The golden colour was due to pigmentation of the overlying tissues, and mature cysts were found to open through the integument allowing numerous spores to escape into the water. Ward (1919), had previously described the cyst of Myxobolus aureatus having a golden colour due to pigmentation of the cyst wall. In M. argentatus the pigmentation is due to the pigmentation of the integument of the host.

Deslisle (1972), described Henneguya sp. as a contributory cause of death of Esox niger, the chain pickerel, in Brome Lake, Quebec. An extensive gill filament infection severely reduced the respiratory surface.

McCraren, Landolt, Hoffman, and Meyer (1975), described infections in the channel catfish Ictalurus punctatus produced by various species of Henneguya. Two types of gill infections are known an intralamellar form where spore development occurs within capillaries of gill lamellae, and an interlamellar form which develops among the basal cells between the gill lamellae. The interlamellar form has caused massive losses among young cultured fish. Almost complete loss of respiratory function accompanies infection.

Cutaneous forms of infection occur with papillomatous growths on the dorsal, anal and caudal fins of cultured fish.

Haemorrhages were often associated with lesions and growths consisted of hyperplastic squamous epithelium. A mandibular form was found in wild channel catfish and adjacent tissue was found to be necrotic.

A gall bladder form was found in cultured fish and gall bladders were enlarged and the bile duct obstructed. These fish were also infected with the intralamellar branchial form.

Komourdijan et al (1977), described Myxidium zealandicum in Anguilla rostrata from the gills and in the kidneys, the latter causing the least damage. The kidney cyst was formed outside the renal capsule whereas the gill cyst was formed within the secondary lamellae; the infection spreading until the entire primary filament was ensheathed in cysts, and the epithelium destroyed.

Neuman (1977), also described myxosporidiosis in Anguilla rostrata the parasites found in the intestine and gills, and the integument. The integumental lesions revealed complete loss of epidermis and haemorrhagic areas.

Dykova and Lom (1978), described the histopathological changes in fish gills infected with myxosporidian parasites of the genus Henneguya. He found that during growth of the vegetative stage the gill tissue may undergo hyperplasia of the epithelium and displacement of the lamellae. After the myxosporidian cyst, surrounded by a host tissue capsule, has reached maturity the inflammatory reaction may reduce the cyst to granulomatous tissue.

Hau & Chang (1977), described the pathology of Myxidium matusii infections and found spore filled cysts were accompanied by chronic inflammation. Huang et al (1977), found a Myxidium sp. in the eel kidney may be a causal factor in swollen kidney disease.

Digenea

Metacercarial stages occurring in fish have recently been reviewed by Higgins (1977), who classified the cysts according to the origin and number of the various layers. Matthews (1974), suggested the degree of host cellular reaction associated with metacercariae of six species of gasterostome may be due to the presence or absence of a cyst wall and with the site of infection, in different species of marine fish. The amount of connective tissue at the site of encystment may affect the host response. Rhipidocotyle johnstonei found in the muscle layers feeding directly on host tissue initiates an intense host reaction. Cryptocotyle lingua also develops superficially and has a thick connective tissue capsule which may be melanised (Hsiao, 1941, Chapman and Hunter, 1954, Rothschild, 1939).

Pathological effects of C. lingua based on histological observations by McQueen et al. (1973) and Iddon, (1973), have shown epidermal lesions, myofibrillar necrosis and swelling of the intermuscular septa. Haemorrhage and muscular necrosis have also been reported associated with the metacercaria of Stellantchasmus falcatus Onji and Nishio 1915, by Lee and Cheng, (1970). The metacercariae of Stephanostomum sp. in the Mediterranean red mullet have been found to cause fragmentation and atrophy of the muscle fibres, (Arru, et al. 1968).

Ultrastructural studies on metacercariae in fish have been confined to species encysting in the liver, heart or kidney. Descriptions include those of Ascocotyle chandleri (Lumsden, 1968), A. pachycystis (Stein and Lumsden, 1971). A. leighi (Stein and Lumsden, 1971) and Posthodiplostomum minimum (Mitchell, 1974; Mitchell and Crang,1976). None of these were found to evoke a significant host reaction.

MATERIALS AND METHODS

1. COLLECTION AND MAINTENANCE OF HOSTS

(a) Fish

The following species of fish were used in the study: thick-lipped grey mullet, Crenimugil labrosus (Risso, 1826), thin-lipped grey mullet, Liza ramanda (Risso, 1826); and golden grey mullet, Liza auratus (Risso, 1810); plaice Pleuronectes platessa (Linnaeus, 1758) and turbot, Scophthalamus maximus (Linnaeus, 1758). These were collected from various sites in the South-West of England and from Cardigan Bay, Wales.

Mullet were caught with the aid of a seine net and '0' group flatfish with a Riley push net. Following capture fish were transported to the laboratory without delay in containers of well-aerated sea water.

Fish were maintained in aquaria, sea water being recirculated through an Eheim biological filtration unit and Paxman cooler. Constant temperatures being established by means of a heater, thermostat and relay system.

75 gallon aquaria supported approximately 200 '0' group flatfish or up to 12 adults at one time. Perspex tanks 33.0cms x 21.0 cms x 22.0cms of aerated sea water were used for '0' group mullet only and stocked at levels of up to 10 fish.

Sea water was obtained from the Marine Biological Association of the United Kingdom at Plymouth where it had been monitored to ensure high quality. Partial water changes were made in circulating aquaria systems at two-week intervals and complete

changes of small perspex tanks were undertaken every week.

Plaice and turbot were fed on the following diet which was recommended by the White Fish Authority:

Minced beef or fish (100 parts)
A synthetic binder - (3-4 parts)
Vitamin premix "Beta" Animal feed supplements (1-2 parts)
Red Shrimp meal (10 parts)

The food was dispensed as simulated worms produced by extruding the food through a 5 ml syringe. This method was convenient for feeding '0' group flatfish during summer and early autumn, although once weaned small lumps of food could be dispensed into aquaria to simplify the process.

Fish were fed every two days and waste food removed 1 hour after introduction into the tank to prevent water contamination.

Mullet were conveniently fed on commercial dried trout pellets as manufactured by 'Pond Pride'.

(b) Winkles

The edible winkle Littorina littorea, was collected at low water from the Yealm Estuary at Newton Ferrers and Noss Mayo, Devon.

Infected specimens were maintained in the laboratory in a polypropylene tank at a room temperature of $12^{o}C$ - $15^{o}C$.

The tank contained aerated sea water to a depth of 5 cm and several large stones. The winkles were fed on green algae, either Ulva sp. or Enteromorpha sp. Sea water in the tank quickly became contaminated and was changed several times each week.

2. HANDLING TECHNIQUES FOR FISH

(a) Handling

Fish are prone to damage and stress if handled without due care. In routine procedures including necessary visual screening and latex marking small fish could be held gently on moist tissue paper or within a soft aquarium net, and large fish held in a damp towel.

For any treatment which might produce undue stress the fish were placed in a solution of 1 part MS222 in 1000 parts sea water until anaesthetised. Procedures were carried out as rapidly as possible and fish immediately returned to aerated sea water tanks.

(b) Marking

Fish were marked either by latex injection or liquid nitrogen branding. Latex injection was the preferred method for flatfish. The latex was introduced subepidermally on the ventral surface using a 1 ml syringe and 25 gauge needle. The four colours available, red, green, yellow and blue enable numerous combinations for individual fish.

Liquid nitrogen was more suitable for marking grey mullet. A metal wire loop was dipped into liquid nitrogen and touched on the skin for 2 seconds. A darkened mark appeared on the skin within an hour.

(c) Infection

Cercariae of C. lingua were used for experimental infection within 4 hours of emergence from Littorina littorea. These were concentrated using a spot light source, and estimations per unit volume made by counting the numbers present in three 0.1 ml samples and taking an average value.

Mullet were exposed to known numbers of cercariae in 150 ml glass beakers of sea water at room temperature.

Two other methods of exposing fish to infection were investigated in an attempt to concentrate parasites at a known site in the fish. In the first case small plastic tubes made from the disposable covers to syringe needles were bonded to plaice skin using Eastman 910 adhesive with simplex detail repair material. Cercariae were then pipetted into the well so-formed.

Secondly, small grey mullet were suspended in tubes of sea water with the tail fin immersed in a cercarial suspension as described by Ratanarat,(1969).

Neither method proved sufficiently successful to warrant its routine application.

(d) Blood samples

Fish blood was taken from the caudal vein using a 25 gauge needle and 1 ml syringe, without anticoagulants. In turbot the caudal vein was located to the left of the lateral line when viewed from the topographical dorsal aspect and was clearly seen if the fish was held on an illuminated white glass sheet. In grey mullet the needle was inserted toward the caudal fin adjacent to the anus.

Up to 0.2 ml of blood could be taken from '0' group turbot and 0-2 group grey mullet, and up to 1 ml from adult fish.

3. MEASUREMENT OF PHAGOCYTIC ACTIVITY

Preliminary studies on the rate of clearance of colloidal carbon particles from the circulating blood were

carried out on turbot as a basis for comparison of phagocytic rates in fish exposed to parasitic infections.

Water-based colloidal carbon (Reeves Ltd) was tested with 1 ml of turbot serum to check the stability of the colloid when added to the circulating blood. The weight of carbon per ml was estimated by drying 1 ml of the colloid on aluminium foil in an oven at 60°C and weighing the residue.

A dosage of 16 mg/100 g fish was chosen as a suitable concentration. The carbon was diluted in plaice Ringer (Cobb et al. 1973) in 1% gelatin, and injected using a No. 30 gauge needle into the caudal vein. The fish were returned to a tank of aerated sea water between treatments.

0.25 ml samples of blood were withdrawn from the caudal vein at the selected time intervals. A fresh syringe and needle were used for each sample taken and the blood added immediately to 2 ml of 0.1% sodium carbonate in a Bijou bottle, to lyse the erythrocytes. A reading of optical density was made on a Pye Unicam Spectrophotometer at 675 nm wavelength. Lysed whole blood was used as the control and readings taken against a standard curve.

4. EXAMINATION OF HOSTS FOR PARASITES

(a) Screening living hosts

Fish Live fish were usually screened for metacercariae or myxosporidian cysts with the naked eye or with the aid of a binocular microscope, when the position of the parasite was seen through the body wall. The process was enhanced by placing the fish over an illuminated translucent polypropylene tank.

Metacercariae of C. lingua were visible as cysts along fin rays in plaice and mullet whereas R. johnstonei metacercariae were visible from the white area of host cells surrounding the parasite. It was usually possible to confirm the presence of metacercariae by their movement and structure when viewed under a binocular microscope. Fish were usually anaesthetised prior to screening.

Myxosporidian cysts of M. exiguus were visible as white round or thread-like cysts on the gill lamellae and black or brown cysts in the skin and scales.

Winkles These were screened for infection with Cryptocotyle lingua as follows: Several hundred winkles were collected, as previous investigators have estimated an infection rate of 5%. These were allowed to dry overnight and then washed thoroughly with running tap water to remove all mud. Groups of four were then placed in beakers of sea water and covered with glass plates to prevent the winkles escaping. The beakers were illuminated with fluorescent lamps and after several hours infected winkles could be isolated directly or subscreened from beakers containing actively swimming cercariae of C. lingua. The cercariae, visible to the naked eye, were readily concentrated with a point light source.

Infected winkles did not always show the effect reported by Willey and Gross (1957), in having dark brown or yellow feet, although infected individuals were often larger.

(b) Post mortem

Fish were killed by an exposure to an overdose of MS222 or by pithing. Metacercarial and myxosporidian cysts were

removed immediately by dissection from the tissues, or with the surrounding host tissue and processed as appropriate. It was sometimes found convenient to store freshly killed fish for future examination. These were stored in sealed polythene bags at -20°C.

Post mortem examination of mullet was necessary in order to screen tissue infection and distribution within the host. A total of 972 grey mullet representing the three species caught locally were examined.

After thawing at room temperature each fish was measured, species recorded and examined for parasites. The opercular cover was lifted and each gill arch examined for the white plasmodia of M. exiguus. The body cavity was opened and the intestinal tract examined for cysts.

5. HISTOLOGY

Histological investigations were carried out on Cryptocotyle lingua, Rhipidocotyle johnstonei and Myxobolus exiguus and on surrounding host tissue. Parasites and host tissue, excised from freshly killed grey mullet and plaice with the aid of a Wild M5 Stereomicroscope were rinsed in sea water prior to fixation.

Examination of myxosporidian spores was carried out by rupturing plasmodia from the gills. Spores released were either examined fresh in sea water under phase contrast or smeared on a clean glass slide for fixation and staining.

Fish blood for smear preparations was taken from live fish using a 1 ml syringe and 25 gauge needle.

Polar filaments of myxosporidian spores were extruded in a saturated solution of urea for 1 hour at 4°C, in a 1:1 spore suspension in urea. After centrifugation in microhaematocrit tubes the spores were ready for fixation.

Light Microscopy

(a) Tissue sections

Fish tissues and parasites were fixed in Bouin's fluid or 10% neutral formalin. Material was dehydrated by upgrading through an alcohol series, cleared in xylene and embedded in paraffin wax.

For general histological studies sections of 8 µm thickness were stained by one of the following methods:-

Heidenhain's Iron Haematoxylin with Eosin after Pantin (1964), Weigert's Haematoxylin with Van Gieson's stain after Culling (1963) and Mallory's triple stain after Gray (1954).

Resin-embedded 1 um sections were stained with 1% toluidine blue in 1% borax and used for routine location and identification of tissue parasites.

(b) Smears

Thin films of fish blood were fixed in methanol and stained in Giemsa (B.D.H.) in a concentration of 1:20 in phosphate buffer at pH 7.2 for 20 minutes. The smears were rinsed with tap water, dried and examined under oil immersion. Air dried smears of myxosporidian spores were fixed in methanol and stained with either Giemsa, PAS technique for glycogen from Pearse (1968) or iodine.

Electron Microscopy

(a) Transmission

Fixation was carried out at 4°C for 1 - 2 hours in 4% paraformaldehyde neutralised with 1N NaOH in 3% glutaraldehyde in 0.2M sodium cacodylate buffer at pH 7.2. After rinsing in buffer tissue was post-fixed in 1% osmium tetroxide in 0.2M cacodylate buffer, dehydrated in a graded ethanol series, treated with propylene oxide or acetone and embedded in Spurr embedding resin.

Penetration of metacercarial cysts with resin often required several days in resin/acetone mixtures, prior to embedding.

Sections were cut at 600Å on a Porte-Blum MT 2-B ultramicrotome using glass knives. Sections were mounted on uncoated copper grids and stained with saturated aqueous uranyl acetate for 10 minutes rinsed in distilled water and counter-stained with Reynolds (1963) lead citrate for 5 minutes and finally washed with several changes of distilled water.

Sections were examined using a Phillips EM 300 transmission microscope at an accelerating voltage of 80 kV.

(b) Scanning

Material required for scanning electron microscopy was fixed and post-osmicated, as for transmission electron microscopy, dehydrated to pure acetone and critically point dried with liquid carbon dioxide using a Polaron 300 critical point unit. Tissue was then mounted on metal stubs with double-sided tape. Material was then coated with gold in an EMScope sputter coating unit and examined with a Phillips 500 scanning electron microscope by courtesy

of Pye Unicam Cambridge.

Photography

Photographic records of ultrastructural investigations were made on glass plates, cut film or 35 mm film. Kodak DG10 developer at 20°C was used for plates and cut film and Kodak D163 developer at 20°C for 35 mm film. KB14 35 mm film was used for light microscopy and developed in Acutol. Kodak Unifix fixer was used throughout.

Prints were made using a Rapidoprint processor or dish developed in D163 at 20°C. An enlargement of X2.5 was used for plates and cut film and X7.0 for 35 mm film. These prints were used for routine observations and measurements. Suitable micrographs were selected and printed on Kodak bromide paper in a dish of D163 at 20°C. Prints were trimmed and mounted on cardboard using Cow Gum and labelled with Letraset sheet No. 118.

A negative was made of the plate and printed on Ilford Ilfospeed medium weight glossy paper using Ilfospeed developer and fixer. After washing for 2 minutes the prints were dried.

RESULTS

TELEOST BLOOD

A study of the structure of the blood cells of turbot, Scopthalmus maximus; and the thick-lipped grey mullet, Crenimugil labrosus was undertaken at both the light and electron microscope level, as a basis for the interpretation of haematological changes associated with parasitic infections. In addition phagocytosis was investigated in Scopthalmus maximus both qualitatively from ultrastructural observations and quantitatively by measurement of the rate clearance of intravenously injected colloidal carbon.

Structure

Scopthalmus maximus

Four types of leucocytes were identified, namely: thrombocytes, lymphocytes, monocytes and a neutrophil type granulocyte.

Thrombocytes These elongated oval cells were clearly recognised in Giemsa stained blood smears, whereas few were found in ultrastructural preparations, a feature which might be attributed to the preparative techniques used for blood. Four morphological types are described and these closely resemble those recorded in plaice by Ellis (1976).

(i) Spiked. An elongated form, with the cytoplasm rounded at one pole and drawn out into a tapering spike at the other. The cytoplasm stained light blue grey with the oval nucleus densely staining purple. The cell measured 8.8 μm x 3.5 μm (Pl. 1D).

(ii) Spindle. This form is oval measuring 6.9 x 10.0 μm and characterised by a nucleus with an irregular outline. (Pl. 1A).

(iii) Ovoid. Similar to the spindle form but with a regular oval nucleus bordered by a smooth distinct margin of cytoplasm. The cell measured 3.0 μm x 10.1 μm. (Pl. 1A,F).

(iv) Lone nucleus. These had the appearance of a naked densely staining nucleus, occasionally with a small amount of grey-blue cytoplasm attached. (Pl. 1B,E). These cells may be difficult to distinguish from small lymphocytes.

Only the ovoid form was detected in E.M. preparations. The large nucleus was bordered by a narrow margin of cytoplasm containing a few vesicles, scattered ribosomes, smooth endoplasmic reticulum and a prominent centriole. (Pl. 2A).

Lymphocytes. These cells were characterised in Giemsa preparations by the high nucleus to cytoplasm ratio. Sizes varied from 3.5 to 8.1 μm diameter, the majority being 4-6 μm diameter. The narrow margin of basophilic cytoplasm was often drawn out into pseudopodia (Pl. 1A) although cells with a regular margin of cytoplasm were present. (Pl. 1C).

Ultrastructural studies confirmed these findings, the large nucleus bordered by a narrow margin of cytoplasm (Pl. 2E). The indented or horse-shoe shaped nucleus sometimes appeared as two nuclei in some planes of section, (Pl. 2C) with the chromatin denser peripherally. The cytoplasm contained small vesicles, scattered ribosomes, smooth E.R. and a few large elongated mitochondria. (Pl. 2B).

Monocytes. These cells are most clearly identified at E.M. level by their phagocytic activity. The most prominent feature of the cytoplasm were the numerous pseudopodia and phagocytic vacuoles containing ingested haemaglobin and in one cell part of a granulocyte (Pl. 3D). The cytoplasm contained smooth E.R., Golgi stacks, mitochondria and ribosomes. The nucleus was ovoid to reniform with the chromatin denser peripherally. Monocytes measured from 4.6 x 2.3 µm to 5.1 x 3.5 µm.

Granulocyte. Only one type of granulocyte was identified and tentatively classified as a neutrophil on the basis of the ultrastructure of the granules. These resemble one – granule of the plaice neutrophil (Ferguson 1976).

The cell was irregular in outline, but without evidence of phagocytic activity. The cell measured 5.6 x 3.6 µm to 7.4 x 4.2 µm and the numerous round to oval membrane-bound cytoplasmic granules were fibrillar. The granules showed varying degrees of organisation, the fibrils running parallel or irregularly within the granule. Other cytoplasmic inclusions were Golgi profiles and numerous electron lucent vesicles. (Pl. 2F,G,H). The nucleus was round to oval in outline and without lobes.

Crenimugil labrosus

The structure of the peripheral leucocytes were investigated from Giemsa stained blood smears and from electron microscopy using two fixatives.

Six types of cells were differentiated and included: thrombocytes, lymphocytes, plasma cells, monocytes, and an eosinophil, and neutrophil granulocyte.

Thrombocyte. As with the turbot preparations these cells were most readily separated from the other leucocytes in the Giemsa stained smears. Thrombocytes were similar in appearance to the turbot cells and the following morphological forms were present:

(i) The spiked form (Pl. 4E), with the cytoplasm at one pole drawn out into a tapering spike. This cell was up to 13.0 um in length and averaged 3.0 µm in width.

(ii) The spindle cell (Pl. 4B) an elongated cell rounded at both poles measured 10.7 µm x 2.3 µm.

(iii) The ovoid form (Pl. 4E) measured 10.0 µm x 3.0 µm.

The lone nucleus was not encountered and probably reflects the unstressed conditions under which the blood sample was taken. The whole range of morphological forms of the thrombocyte encountered in blood smears from the turbot and the grey mullet reflect the fragility and easily deformable nature of the cytoplasm of this cell, a feature necessary to its function as the clotting agent (Wardle, 1971).

Ultrastructural studies revealed an elongated cell measuring 8.5 µm x 1.8 µm with a large nucleus with the chromatin separated into light and dark phases (Pl. 5E). The tapering cytoplasm contained large electron lucent vacuoles.

Lymphocytes. From blood smears lymphocytes had large densely staining nuclei with the peripheral cytoplasm often drawn out into numerous pseudopodia (Pl. 4D) or as a narrow regular margin (Pl. 4I). Lymphocytes measured from 4.6 μm to 7.7 μm in diameter.

The ultrastructure of this cell showed the high nucleus to cytoplasm ratio, the nucleus probably being dome-shaped as it appeared as a ring in some planes of section (Pl. 5A,C). The chromatin had definite dark and light phases. Cytoplasmic inclusions were electron lucent vacuoles, mitochondria and scattered ribosomes. Some cells had the cytoplasm extended into fine pseudopodia (Pl. 5A,B) while in others the cell margin was regular (Pl. 6A,B). Large lymphocytes were also present, measuring 6.1 μm x 7.7 μm with numerous mitochondria and limited amounts of rough endoplasmic reticulum.

Plasma Cells. These cells were differentiated from ultrastructural preparations by the large nucleus and extensive rough endoplasmic reticulum cisternae (Pl. 5F,G and Pl. 6H). The cell measured 7.7 μm x 4.7 μm and was not identified in blood smears. Beside the extensive rough E.R. the cytoplasm also included mitochondria and scattered ribosomes and exhibited features of a cell engaged in active protein synthesis.

Monocytes. From Giemsa stained blood smears monocytes were distinguished by their vacuolated cytoplasm, and irregular shaped nucleus with dark and light areas of chromatin. These cells were the largest peripheral leucocytes and measured on average 12.3 μm in diameter. Monocytes were distinguished

from the ultrastructural study by their vacuolated cytoplasm being less electron dense than the lymphocytes, containing Golgi profiles and scattered ribosomes (Pl. 5D).

Eosinophil. From Giemsa stained blood smears the eosinophil was distinguished by the reniform nucleus and pale cytoplasm containing refractile orange staining granules. The cell measured 9.3 μm to 11.5 μm in diameter. These observations were confirmed by electron microscopy when the reniform nucleus was apparent (Pl. 6E) and the cytoplasm contained the amorphous round granules measuring 0.5 μm in diameter together with scattered ribosomes and limited rough E.R. cisternae (Pl. 6D,G).

Neutrophil. From Giemsa stained blood smears the neutrophil appeared as a pale staining cell with a blue granular cytoplasm and epicentric nucleus (Pl. 4H), and measured 10.7 μm in diameter. This cell was not well fixed in ultrastructural preparations and only part of a cell with a fibrillar granule was identified (Pl. 6F).

Phagocytosis

The ultrastructural studies revealed the phagocytic capacity of the circulating monocytes of turbot, and preliminary studies were therefore carried on a 2 group fish in order to compare possible variations in phagocytic activity of fish exposed to parasitic infections. The fish weighing 154.5 gms was injected intravenously with 25 mgs of colloidalcarbon (Reeves Ltd.) and 0.25 ml samples taken from the blood, to measure rate of clearance. The results are expressed in Fig 2

Fig. 0

Rate of clearance of carbon from the blood of 2 group turbot

Period after injection (mins)	5	15	25	35	50
Optical density (nms)	2.04	1.46	1.10	0.8	0.32

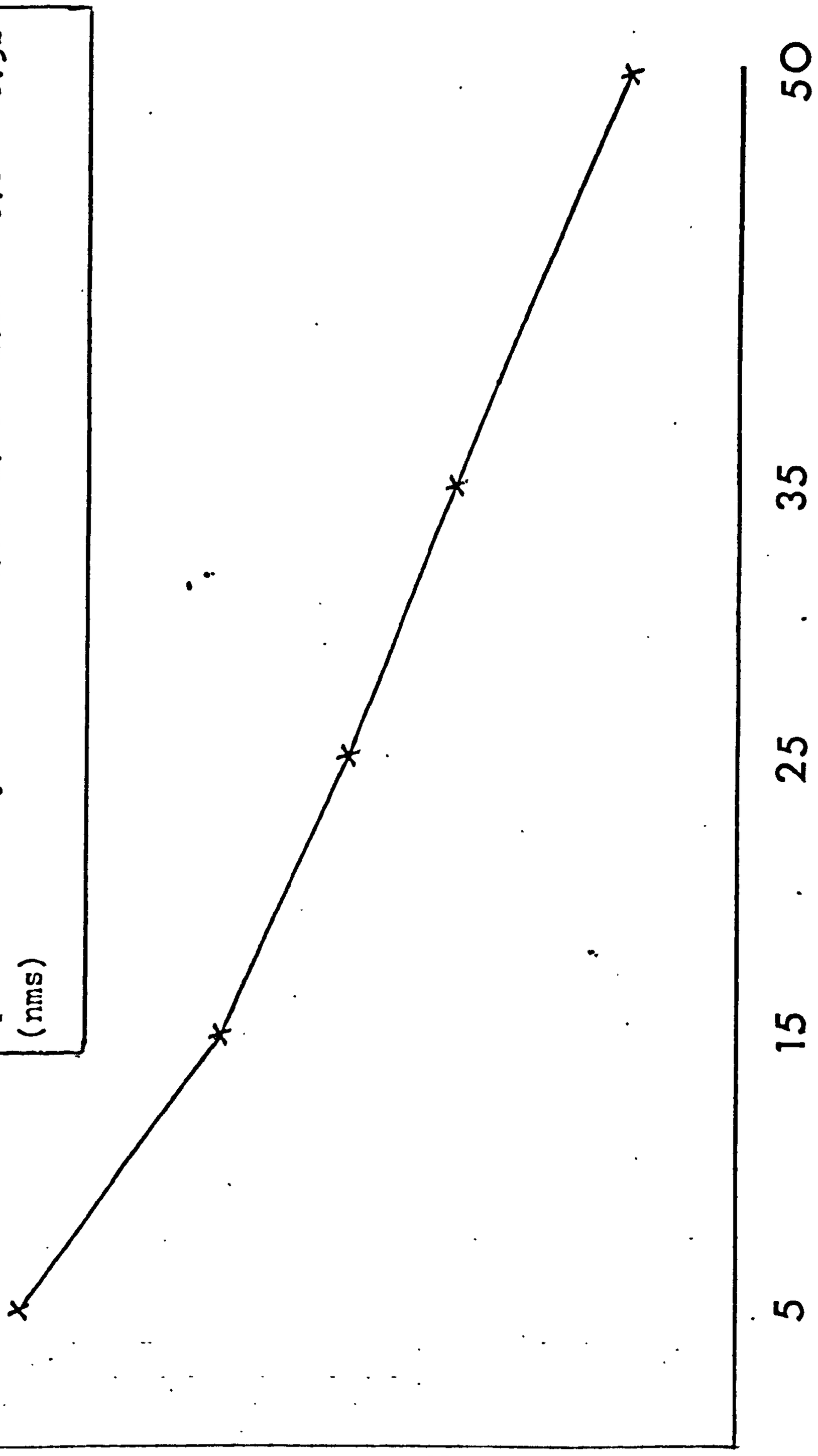


PLATE I

Light microscopy of turbot leucocytes from the peripheral blood, stained with Giemsa.

A. Lymphocyte with large purple staining nucleus and pinky grey cytoplasm. Two thrombocytes at bottom right have the same staining properties. Two morphological forms present include T2 the spindle cell and T3,the ovoid cell.
X I,586

B. Lymphocyte and T2, spindle cell and T4, lone nucleus thrombocytes.
X I,586

D. Two thrombocytes : TI, spiked form and T4, lone nucleus form.
X I,586

C. Lymphocyte with large nucleus and narrow margin of cytoplasm.
X I,586

E. Clump of thrombocytes include TI,and T4 types.
X I,586

F. Thrombocyte of T3, ovoid form.
X I,586

N.B. No monocytes or granulocytes were distinguished from the Giemsa stained preparations examined.

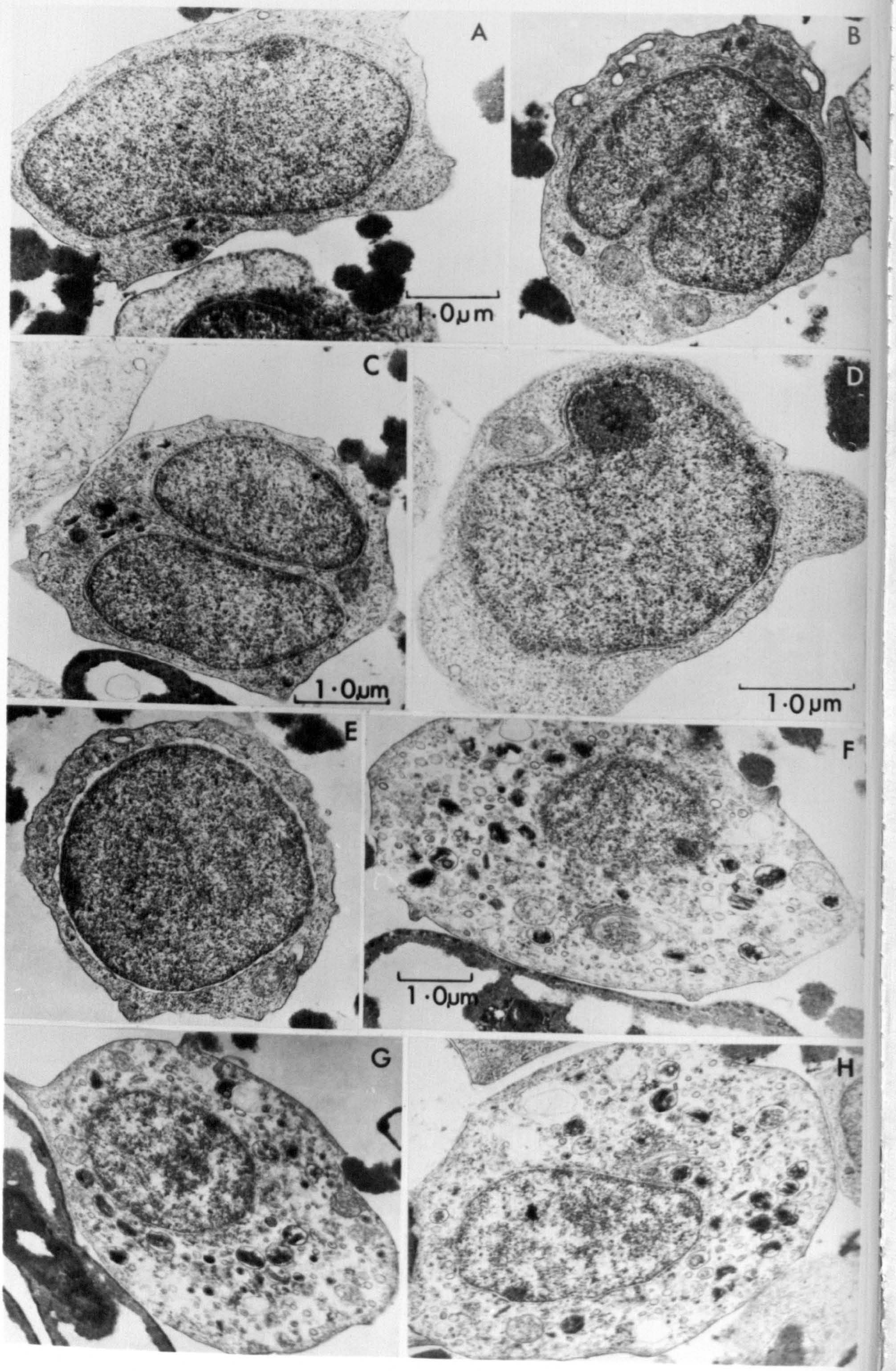

A
1·0µm
B
C
1·0µm
D
1·0µm
E
F
1·0µm
G
H

PLATE 2

Ultrastructure of turbot (Scopthalmus maximus) leucocytes from the peripheral blood.

A. Thrombocyte with large nucleus in an elongated spindle cell.
X I7,I36

B. Lymphocyte with horseshoe shaped nucleus and narrow margin of cytoplasm of cytoplasm containing large mitochondria.
X I3, I04

C. Lymphocyte with horseshoe shaped nucleus, appearing as two nuclei in plane of section.
X I7,I36

D. Lymphocyte with large nucleolus in nucleus.
X 2I, 672

E. Classic small lymphocyte with large nucleus and narrow margin of cytoplasm .
X I3,I04

F. Granulocyte of the neutrophil type, with cytoplasm containing Golgi profiles and fibrillar granules in a vesicular cytoplasm.
X I3,I04

G. Granulocyte of the neutrophil type.
X I3,I04

H. Granulocyte of the neutrophil type.
X I3,I04

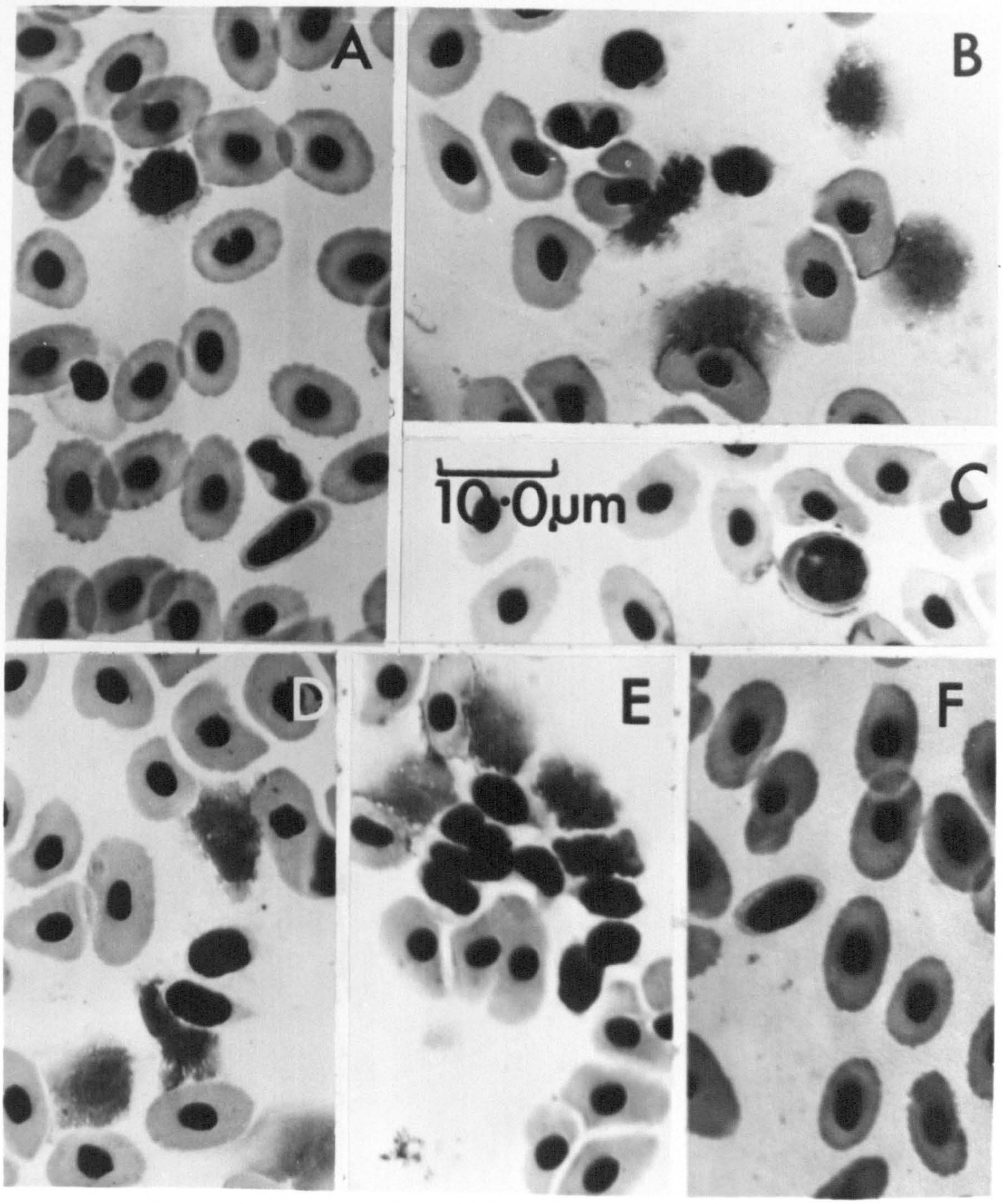

A
B
10·0μm
C
D
E
F

PLATE 3

Ultrastructure of turbot (Scopthalmus maximus), monocytes

A. Monocyte actively phagocytosing haemoglobin from lysed erythrocytes.Large pseudopodia engulf the haemoglobin,and cytoplasm contains many vesicles and vacuoles.
X 21,736

B. Monocyte with phagocytosed haemoglobin within a cytoplasmic vacuole.
X 19,760

C. Monocyte with numerous pseudopodia extending from the cell surface.
X 19,760

D. Monocyte with cytoplasm containing much ingested material ,including part of a neutrophil granulocyte within a double membrane bound vacuole. Ingested haemoglobin, profiles of Golgi apparatus and numerous vacuoles and vesicles also present in the cytoplasm.
X 21,736

E. Detail of pseudopodia showing formation of phagocytic vacuole with haemoglobin being ingested.
X 27,360

F. Two pseudopodia extending from the monocyte cytoplasm to form the phagocytic vacuole.
X 34,200

hg. haemoglobin from lysed erythrocytes
m. mitochondrion
p. pseudopodia
va. vacuole of phagocytosed material

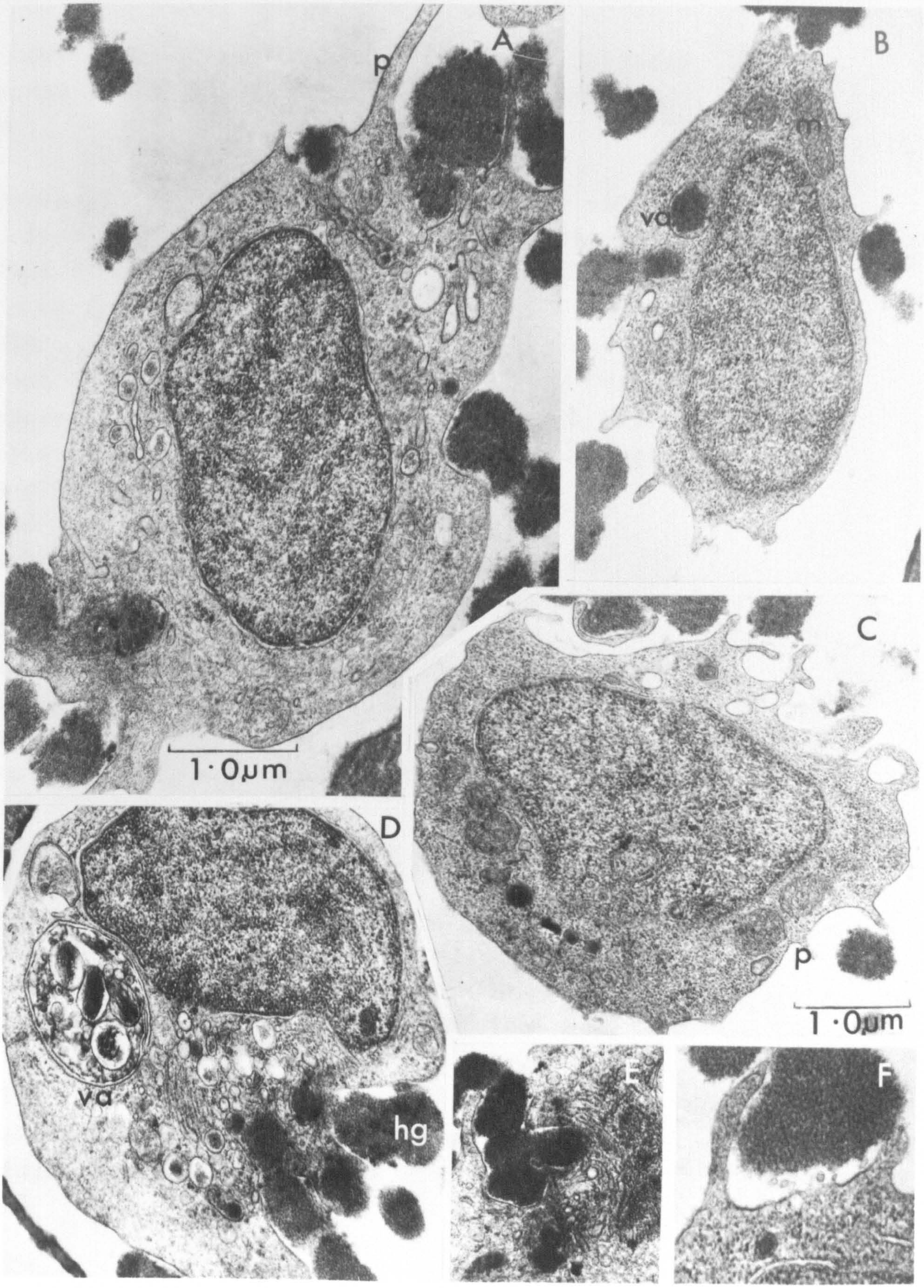

A
p
1·0µm
B
m
va
C
p
1·0µm
D
va
hg
E
F

PLATE 4

Light microscopy of grey mullet, Crenimugil labrosus leucocytes from the peripheral blood, stained with Giemsa

A. Thrombocytes of the spiked TI, spindle T2 and ovoid T3, forms; and three small densely staining lymphocytes Nuclei stain deep purple and cytoplasm of thrombocytes grey and lymphocytes blue.
X I,3I6

B. Thrombocytes of spindle T2, and ovoid T3, forms lymphocyte and eosinophil granulocyte with dense purple staining reniform nucleus and blue-grey cytoplasm with pinkish fine granules.
X I,3I6

C. Lymphocytes, spiked TI, form thrombocyte and monocyte with pinky grey cytoplasm with vacuoles.
X I,3I6

D. Two lymphocytes with purple staining nuclei and blue cytoplasm.
X I.3I6

E. Spiked from TI, and spindle cell T2 thrombocyte.
X I,3I6

F. Monocyte with vacuolated pinky grey cytoplasm.
X I,3I6

G. Two monocytes and eosinophil type granulocyte.
X I,3I6

H. Granulocyte of neutrophil type with blue grey cytoplasm and fine blue granules.Nucleus oval and stains purple.
X I,3I6

I. Small lymphocyte with large nucleus and blue staining peripheral margin of cytoplasm.
X I,3I6

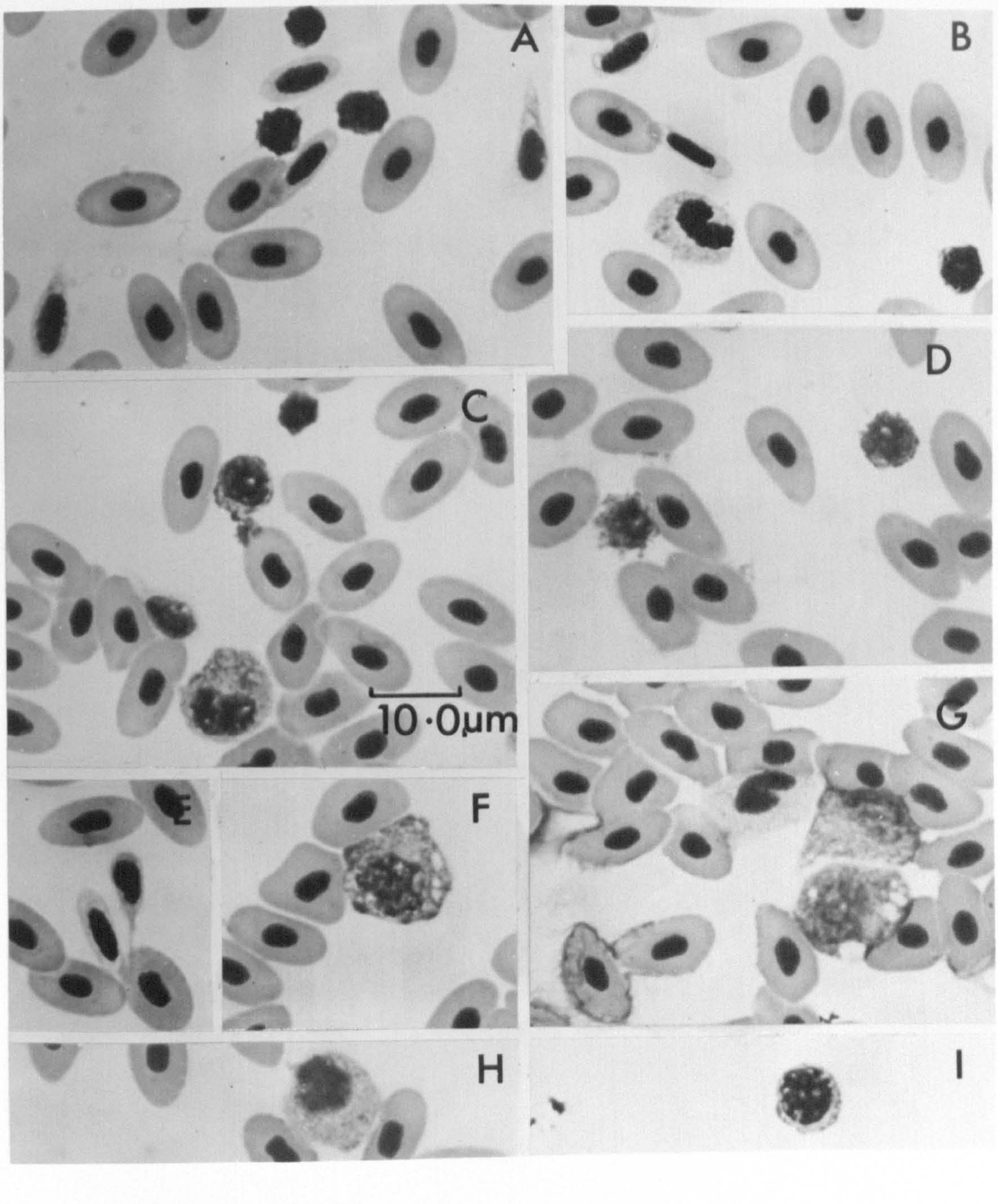

A
B
C
D
10·0µm
E
F
G
H
I

PLATE 5

Ultrastructure of grey mullet (Crenimugil labrosus) peripheral leucocytes, fixed in Karnovsky's (I963), fixative in teleost ringer

A. Two lymphocytes with numerous fine pseudopodia. Dome shaped nucleus, appearing as a ring in plane of section Nuclear chromatin condensed into distinct dark and light phases.
X 29,I60

B. Small lymphocyte with large nucleus and narrow margin of cytoplasm extending into many fine pseudopodia.
X I6,800

C. Larger lymphocyte with dense cytoplasm containing many ribosomes.
X I7,820

D. Monocyte with pale vacuolated cytoplasm containing Golgi profiles, smooth ER and vacuoles and vesicles.
X I6,800

E. Thrombocyte, an elongated cell with a large nucleus and vesiculated cytoplasm.
X I4,256

F. Plasma cell with typical extensive rough ER cisternae.
X I4,256

G. Detail of cytoplasm of plasma cell showing rough ER cisternae and numerous free ribosomes.
X 38,880

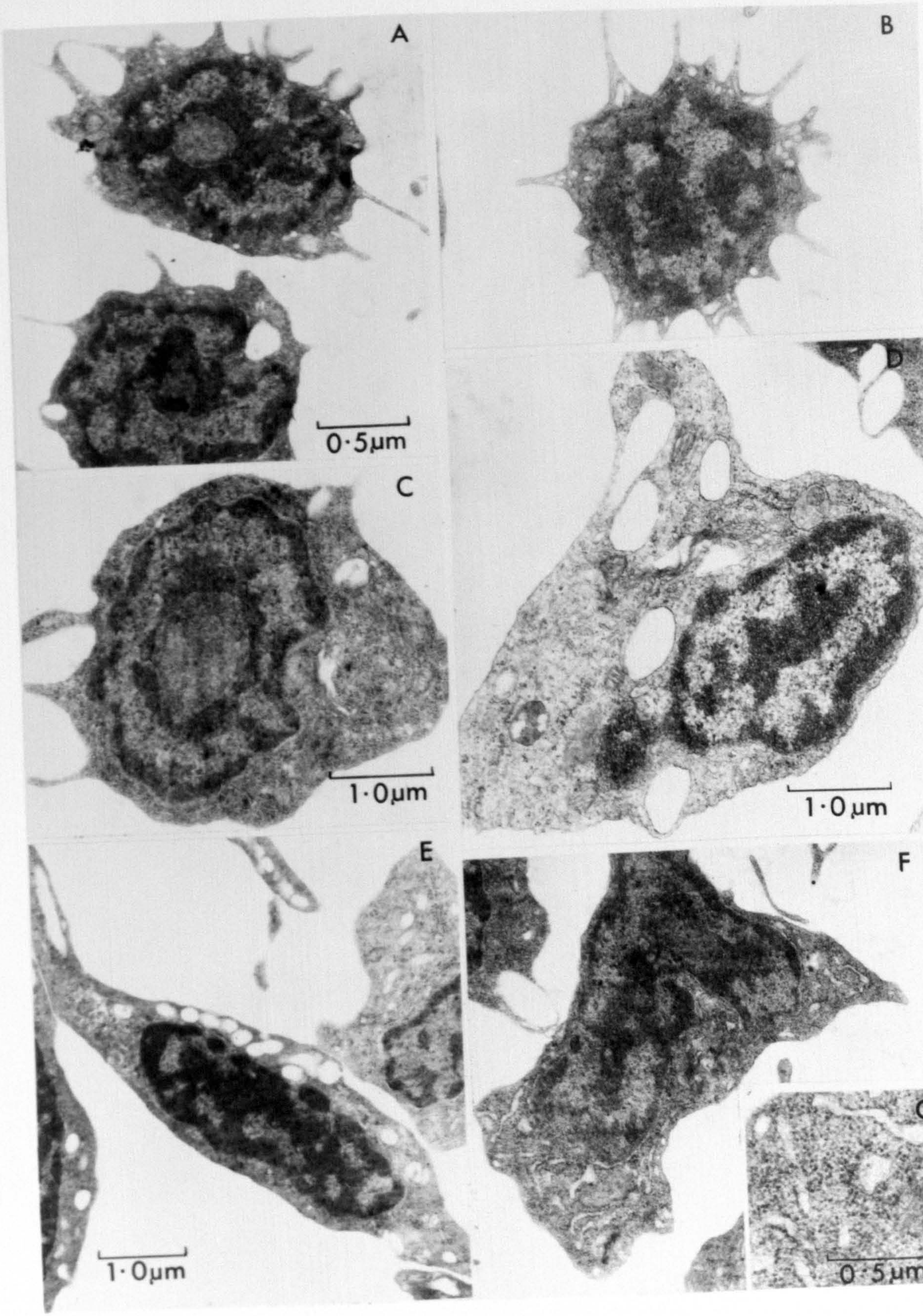

A
0·5μm
B
C
1·0μm
D
1·0μm
E
1·0μm
F
G
0·5μm

PLATE 6

Ultrastructure of grey mullet (Crenimugil labrosus) peripheral leucocytes fixed with 5% glutaraldehyde in cacodylate buffer

A. Small lymphocytes with poorly fixed cytoplasm.
X 9,360

B. Small lymphocytes with nuclear chromatin clearly defined into light and dark phases. Narrow margin of cytoplasm containing mitochondria, ribosomes and vesicles.
X 9,360

C. Large lymphocyte, cytoplasm containing ribosomes and mitochondria.
X I5,300

D. Part of an eosinophilic granulocyte , with small homogeneous granules of medium electron density and rough ER cisternae.
X 9,360

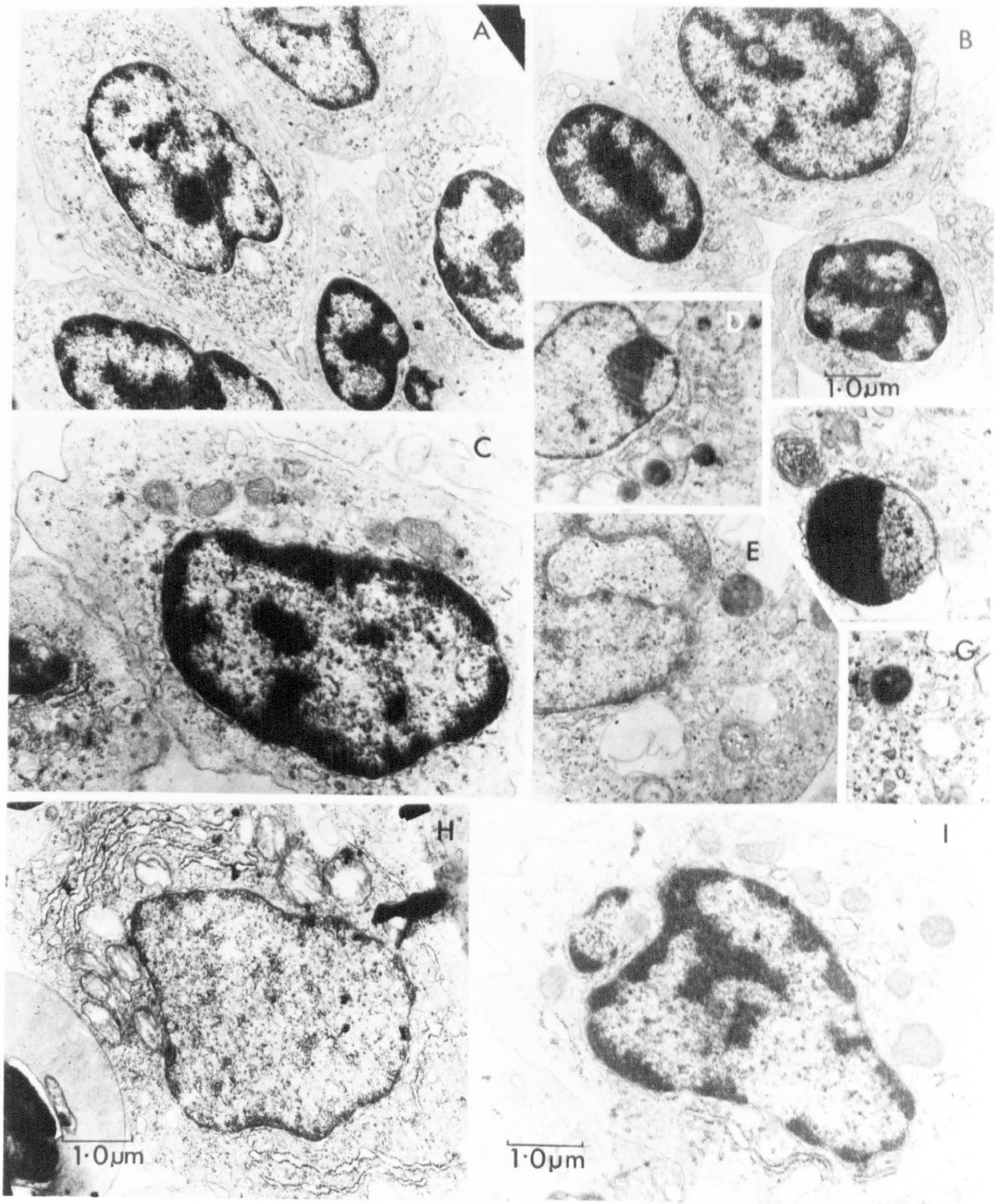
E. Part of eosinophilic granulocyte with reniform nucleus and homogeneous granules.
X II,700

F. Part of second type of granulocyte with fibrillar granules and nucleus with extremely electron dense zone.
X 9,360

G. Granule from eosinophil type granulocyte.
X II,700

H. Developing plasma cell with distinct rough ER cisternae and large mitochondria .
X II,700

I. Monocyte.
X II,700


A
B
1·0μm
C
D
E
G
H
1·0μm
I
1·0μm

METACERCARIAE

The development of Cryptocotyle lingua metacercariae in Crenimugil labrosus and Pleuronectes platessa; and Rhipidocotyle johnstonei metacercariae in P. platessa were studied in order to determine the role of parasite and host components in cyst formation and encapsulation.

Changes at the host parasite interface during the course of infection

(a) Cryptocotyle lingua

Ultrastructural investigations were made on the parasite epidermis and surrounding host tissues during the course of infection of C. lingua in the thick-lipped grey mullet Crenimugil labrosus. Metacercarial stages were examined at approximately the following time intervals after cercarial penetration: 15 mins; 30 mins; 90 mins; 2 hours; 3 hours; 1 day; 14 days; 21 days; 38 days and 55 days. The major changes associated with penetration, migration, encystment and encapsulation are summarised in Figs 1,2 & 3

It was first necessary to investigate the structure of the cercarial epidermis and associated gland cells as a basis for comparison with the metacercaria throughout its development. During the course of this work a similar study was published by Rees (1974), and Day (1976), and the results described below confirm the findings of these researchers.

The epidermis is typical of other digeneans in being an outer syncytial layer connected by protoplasmic processes to nucleated sub-epidermal cell bodies (Pl. 8). The epidermis contains conical backwardly directed spines, clearly seen in scanning preparations (Pl. 7B, C). These

rest on the inner plasma membrane with their apices protruding beyond the surface, (Pl. 11E) yet covered by the outer plasma membrane. They are absent from the lining of the oral sucker, the tail surface and the ventral sucker rudiment. Modified spines occur at the anterior extremity, near the pores terminating the penetration gland cells, and form a piercing apparatus.

Five types of membrane bound secretion bodies are found in the epidermis and their classification after Rees (1974), is given as follows:- Type Sb1, large elongated granular bodies orientated at right angles to the surface arranged in groups in the lobes between the spines and terminating close below the outer plasma membrane; type Sb2, small elongate bodies with fibrillar contents; type Sb3, oval or rounded bodies with fibrillar contents; type Sb4, large round or oval homogeneous electron dense bodies; type Sb5, similar homogeneous electron lucent bodies.

Histochemical studies by Rees and Day (1976), show types Sb1, Sb2 and Sb3 to contain acid mucopolysaccharides or mucoid substances and types Sb4 and Sb5 to contain mucoproteins or neutral mucopolysaccharides.

Connected directly with the epidermis by protoplasmic processes, each containing a large mitochondrion, were the cystogenic gland cells (Pl. 10A). The mitochondrion prevents the secretion bodies from passing into the epidermis prematurely.

Five types of membrane bound secretion bodies were identified by Rees (1974), in the cystogenic gland cells,

denoted by Sbc here, and include types Sbc1; large round or oval electron dense bodies, larger than those in the epidermal cell body; Sbc2 small electron dense bodies; Sbc3, large round or oval electron lucent bodies, larger than those in the epidermal cell body; Sbc4, small electron lucent bodies; Sbc5, small spherical granular bodies.

The penetration gland cells occupy the central region of the body, and a duct continuous with the cell membrane passes forward and through the epidermis to a surface pore. Only one type of membrane bound oval or dumb bell shaped secretion bodies were found within the microtubule-lined ducts (Pl. 10C).

The secretion bodies present within the epidermis, penetration glands and cystogenic glands provide the cercaria with the materials necessary for the processes leading to encystment within the fish second intermediate host. The sequence of events from initial contact with the fish surface to full development of the metacercaria and associated host reaction are subdivided into the following stages:

1. Penetration and Migration
2. Pre-encystment
3. Encystment
4. Growth and Encapsulation

1. <u>Penetration and Migration</u>: On contact with the fish cercariae attach to the skin by means of the tail stem, assisted by spines on the body surface. The oral sucker is applied closely to the fish skin, and through the combined mechanical action of the modified spines and penetration gland secretions the cercaria penetrates the epidermis.

Although the nature of the secretions have not been investigated it is generally assumed that hyaluronidases and collagenases are present. Cercarial extract of C. lingua have been shown to have lytic action on fish fins by Hunter and Hunter (1937).

Damage to the fish tissue during penetration and migration was consistent with lytic necrosis, haemorrhages occurring when small capillaries were damaged.

Ultrastructural studies 15 minutes post penetration revealed lysed connective tissue cells and isolated nuclei (Pl. 9D, H) around the metacercaria. Secretion bodies of types Sb2, Sb3 and Sb4 were aligned in two layers in the epidermis. Types Sb2 and Sb3 were close to the outer plasma membrane, and type Sb4 beneath, close to the basement lamina, (Pl. 10B, E). The metacercarial epidermis no longer contained type Sb1 secretion bodies characteristic of the cercaria, and it is probable that these are released on penetration to form a protective and lubricatory surface coat of acid mucopolysaccharide or mucoprotein.

A failure to identify secretion bodies Sb5 at this stage may be attributed to differences in interpretation or fixation as they are differentiated from types Sb4 only on the basis of electron density (Rees, 1974).

Release of the epidermal secretion bodies occurs by fusion of the membrane of the secretion body with the outer plasma membrane of the epidermis, and release of the contents to the outside. The sites of release are indicated by cup-shaped depressions in the outer plasma membrane (Pl. 10E). Release of type Sb3 secretion bodies, which contain acid

mucopolysaccharides or mucoid substances (Rees & Day, 1976), may also contribute to protection and lubrication of the metacercarial surface during migration.

Small numbers of secretion bodies remained within the penetration gland ducts (Pl. 10C) and these secretions may be utilised in the enlargement of the terminal region of migratory tracts forming a cavity prior to encystment.

2. Pre-encystment: Migration was usually completed within 30 minutes of penetration, the parasite attaining distances of approximately 3 mm in this time. Parasites penetrating fins often migrated along a path parallel with the fin rays. On completion of migration the metacercaria began circular movements, enlarging the cavity for encystment. Metacercariae were extremely active at this stage and ultrastructural studies 30 minutes post penetration revealed extensive areas of eroded cytoplasm and isolated nuclei with a large fluid-filled lumen between the parasite surface and host cells (Pl. 11A, C, N). The remaining secretions from the penetration gland cells presumably assist in lysis of the surrounding cells enlarging the cyst cavity.

Numerous depressions in the epidermis at the outer plasma membrane contained membranous or flocculent material in the process of being released from the epidermis (Pl. 11J, K, L, M). Secretion bodies of types Sb2 and Sb3 are released from the epidermis before those of the cystogenic glands and it is possible that their function is concerned with protection of the parasite surface, enlargement of the precystic space or may act as precursors for cyst formation. Morphological changes occurred in these secretions, while still within the epidermis. (Pl. 11G).

During pre-encystment behaviour and cavity enlargement the cystogenic secretions remained within their cells; the mitochondrion in the protoplasmic process blocking their route into the epidermis, 90 minutes post penetration the epidermis was almost devoid of other recognisable secretory products (Pl. 12), only a few of type Sb2 and Sb3 remaining. The cytoplasm contained glycogen, few mitochondria and vacuoles. The cyst cavity contained whorls of membranous material. Secretion bodies were still retained within the prominent cystogenic gland cells (Pl. 12B, C, F).

3. Encystment: The cyst wall of parasitic origin was first evident 2.0 hours post penetration forming adjacent to the host tissues (Pl. 13).

The following types of secretion bodies were present in the epidermis at this stage: Sb2, Sb3 and Sb4 which may have been replenished from the epidermal cell bodies (Pl. 13A,C) and may account for their absence from the previous metacercarial stage examined. Alternatively this may reflect individual differences in rates of encystment.

Secretion bodies derived from the cystogenic gland cells were also present for the first time; Sbc5 (Pl. 13G) in the epidermis, and Sbc4 (Pl. 13D) in the cyst lumen. Maximum concentrations of these secretions were present in the epidermis and cyst cavity 3.0 hours post penetration (Pl. 14). The epidermis containing numerous Sbc5 secretion bodies (Pl. 14H) while Sbc4 secretion bodies were found within the cyst lumen (Pl. 14E, F). It appears that the Sbc4 secretion bodies form the outer (layer 1), of the parasitic cyst by coalescence of

individual secretion bodies. New material also appeared to fuse with the inner surface of the cyst (Pl. 14E, F) which was very convoluted at this stage (Pl. 14D).

During the process of cyst formation the metacercaria is highly active, constantly turning, a feature which may ensure a cyst wall of uniform thickness and maximum size to accommodate future growth of the parasite. The fully formed parasitic cyst is composed of two layers of acellular material. The outer more electron dense layer 1 measures 0.03 µm in thickness, and the inner, more granular layer 2, measures 0.57 µm in thickness at one day (Pl. 15). These layers increase to 0.04 µm and 1.48 µm by day 55 (Pl. 12).

Numerous vacuoles and vesicles, and amorphous material was also present within the cyst lumen during the initial period of encystment (Pl. 14D, F). Some of this may represent lysed fish cells which become enclosed within the parasitic cyst and may serve as an initial source of nutrient for the parasite. The secretion of layer 2 of the parasitic cyst may also be occurring simultaneously (Pl. 14B), but could not be related directly to a particular secretion body. It seems likely that all the secretion bodies from the cystogenic gland cells contribute to the biochemical changes leading to the fully-formed parasitic cyst.

In addition to identified secretions from the cystogenic gland cells, large flocculent inclusions up to 0.4 µm in diameter were found in the epidermis during this stage (Pl. 14H). Their origin and function were unknown but may represent changes in secretions from the cystogenic gland cells prior to their release, or changes within the epidermis.

Fundamental changes in the structure of the epidermis were observed during encystment. These included complete breakdown of the outer epidermal layer exposing the body musculature, within the cyst lumen (Pl. 16A,D,H). Secretion bodies from the cystogenic gland cells of types Sbc1, Sbc3 and Sbc5 continued to move into the epidermis and cyst lumen during this period of reorganisation (Pl. 15G, H) and may contribute to its breakdown (Pl. 16) and resynthesis of a secondary absorptive epidermal layer (Pl. 17).

The host reaction at these early stages included the formation of microfilaments on the outer surface of the parasitic cyst first observed 3.0 hours post infection (Pl. 14F). These probably represent protocollagen filaments, the initial stages of a healing reaction. Lymphocytes and monocytes characteristic of an inflammatory response, were also associated with the parasitic cyst one day post infection, (Pl. 16F,G,H).

4. Growth and Encapsulation: The metacercaria examined 14 days post infection had a microvillous epidermis (Pl. 17B,D), a feature reflecting a phase of nutritive dependance on the host. Iddon (1973), found that metacercariae increased tenfold in size during development in plaice. In view of the parasite's isolation within the cyst wall all intertransference of materials is likely to be through the epidermis in small molecular form.

The 2 layered parasitic cyst conformed to the structure described previously. In addition an electron dense deposit of granular material was first evident adhering to the outer surface of layer 1 (Pl. 17E).

The host component of the cyst was clearly represented by an encapsulating layer of collagen fibres measuring 2.0 μm in depth (Pl. 17C,E,F). These fibres originated from associated active fibroblasts, which were identified by their extensive rough ER cisternae and peripheral margin of cytoplasm. Protoplasmic processes extended from the fibroblasts and interdigitated with the collagen fibres. (Pl. 17C,E,F).

The microvillous structure of the metacercarial epidermis was retained for 3-4 weeks and coincided with the growth phase of the metacercaria. Further changes in the structure were then evident which were associated with the future function of this layer in the definitive host. The outer surface became more rounded in appearance and two new types of secretion bodies were present 38 days post infection. These were designated types Sb6 and Sb7. Secretion bodies of type Sb6 were first found in the epidermis 21 days post infection and were oval, granular and measured 0.05 μm in diameter (Pl. 18E). Type Sb7 measured 0.1 μm in diameter and had an electron dense core with radiating spores of electron dense material giving a cartwheel appearance (Pl. 19F, I). Both types were found within one epidermal cell (Pl. 19B). Mitochondria containing electron dense granules formed the other epidermal inclusions (Pl. 19I). Replacement spines were again present in the epidermis 21 days post infection (Pl. 18F) and represent the spines of the future adult. The cercarial spines break down (Day 1976), and were absent from the epidermis 14 days post infection (Pl. 17B,D).

The structure of the parasitic cyst remained unchanged, although further deposition of the electron dense material externally was observed in later stages (Pl. 19J,K), similar material being observed within the parasitic cyst (Pl. 19J).

Encapsulation of the parasitic cyst with collagen progressed through the infection from 3.3 µm at 21 days, 3.5 µm at 38 days up to the final stage examined at 55 days when the capsule was 5.0 µm in width (Pl. 20A).

Although melanised cysts were found in naturally infected grey mullet, no melanin was found associated with the host capsule in the experimentally infected fish up to day 55 (Pl. 20). Melanin was however characteristic of the host capsule of naturally infected plaice examined for comparison (Pl. 21). The melanin was deposited as free membrane bound granules (Pl. 21D,G) and within melanophores (Pl. 21F). The melanin was deposited outside the collagen fibril layer which measured 14.0 µm in depth. The electron dense deposit on the outer surface of the parasitic cyst was also present. (Pl. 21A).

Fig. 1

Metacercarial surface of Cryptocotyle lingua during penetration, migration and pre-encystment.

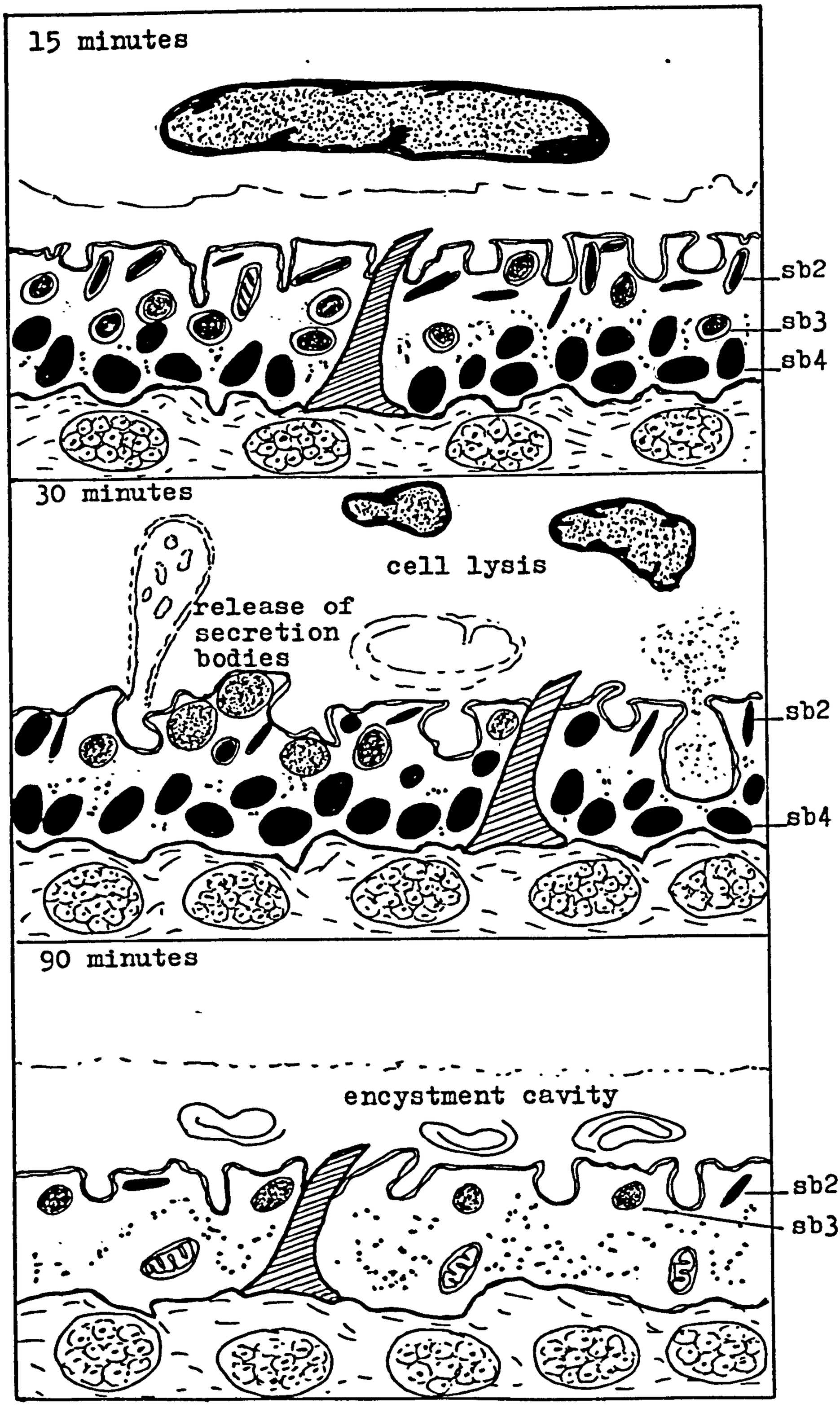


Fig. 2

Metacercarial surface of Cryptocotyle lingua during encystment.

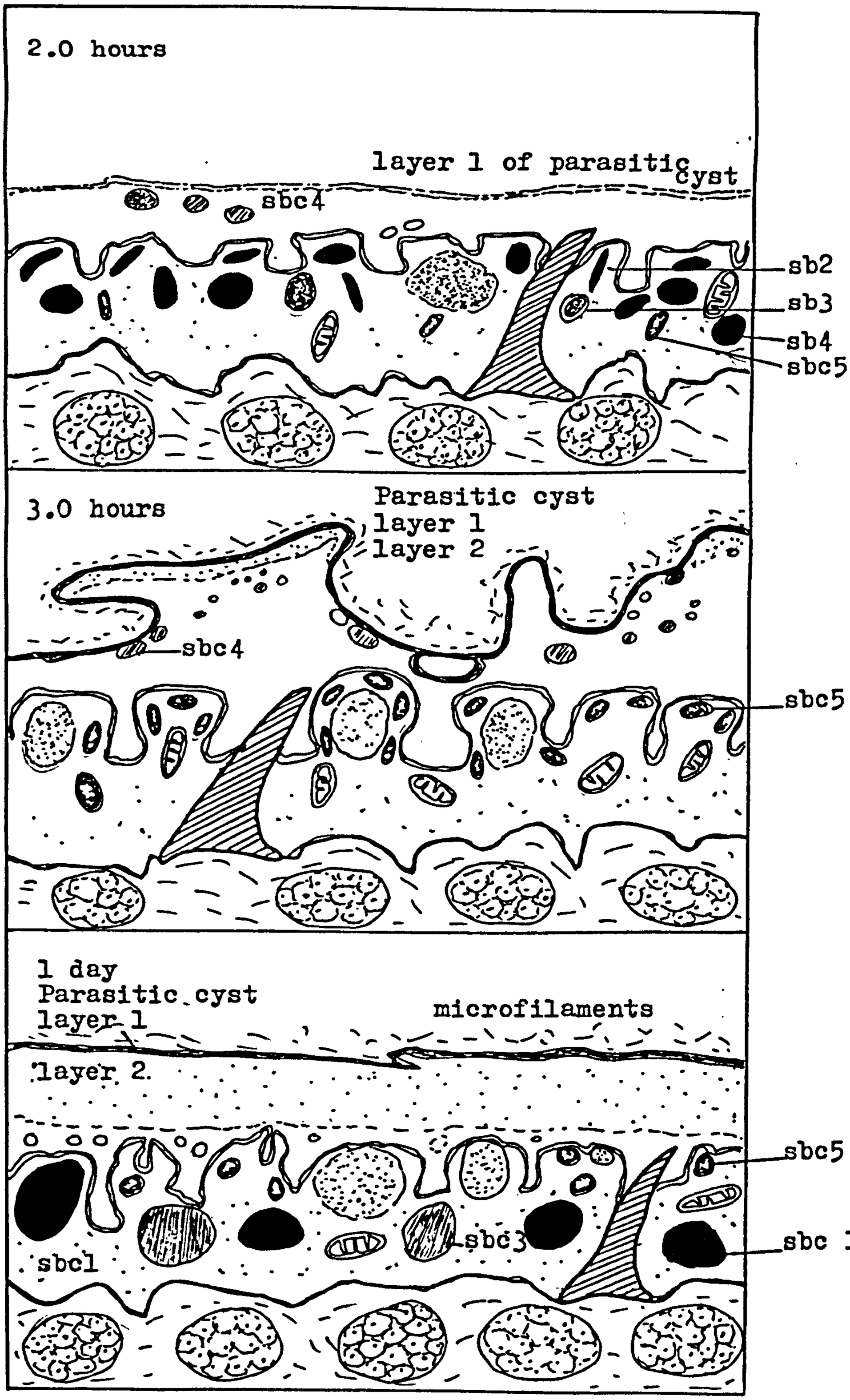


Fig. 3

Metacercarial surface, parasitic cyst and host reaction to Cryptocotyle lingua during encapsulation

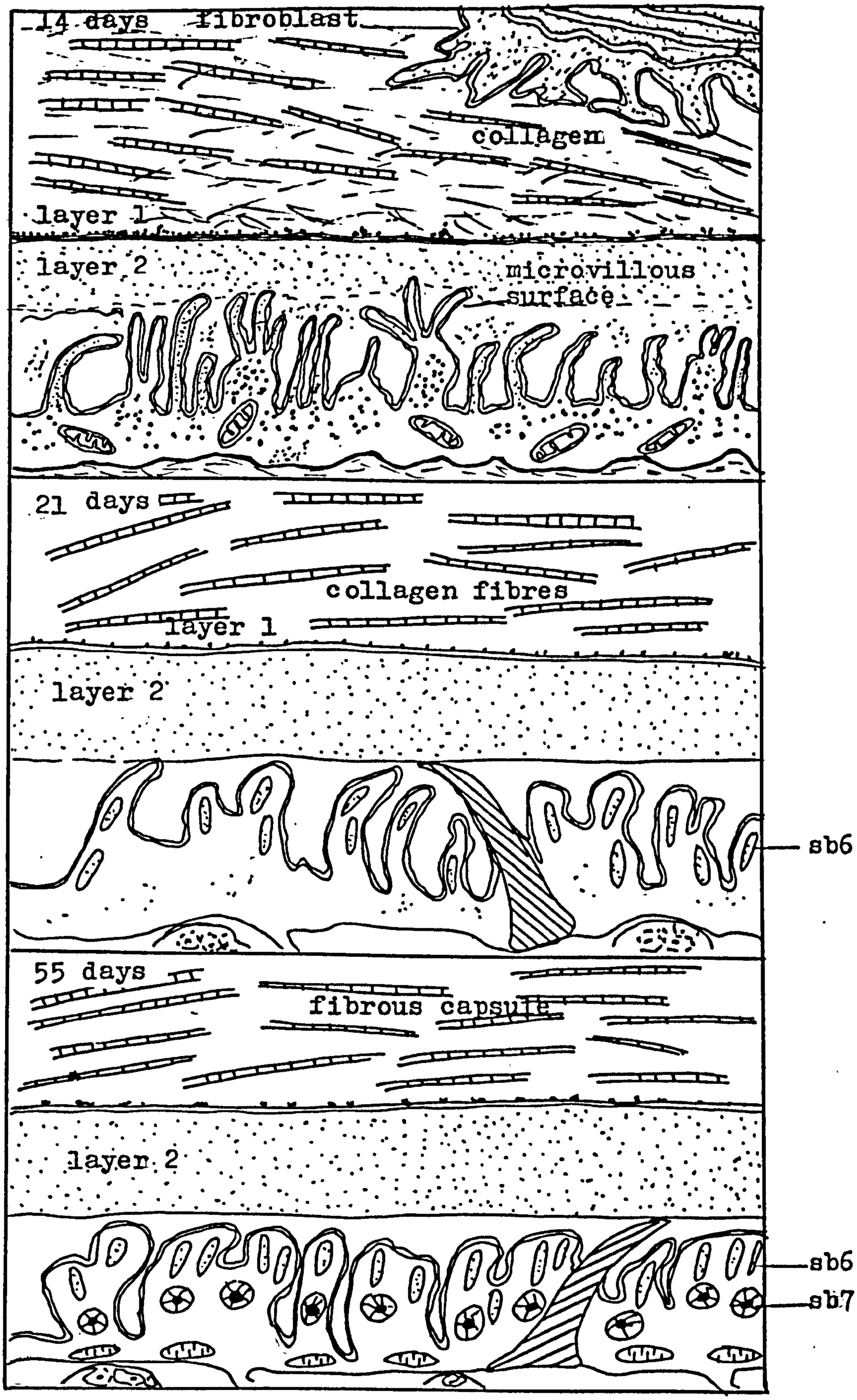


PLATE 7

Scanning electron microscopy of cercariae of Cryptocotyle lingua, and light microscopy of the metacercarial cyst from plaice

A. Cercariae of C.lingua, showing the head with the oral depression and the tail.
X 440

B. Anterior head region of the cercaria showing the dense armature of backwardly directed spines, which may assist in attachment and penetration of the fish intermediate host.
X I2,I50

C. Detail of the spines from the cercaria of C. lingua.
X I4,580

D. Mature metacercaria from a naturally infected plaice, the metacercaria enclosed within the parasitic cyst surrounded with the host capsule comprising the inner layer of collagen and the outer melanophores.
X I06

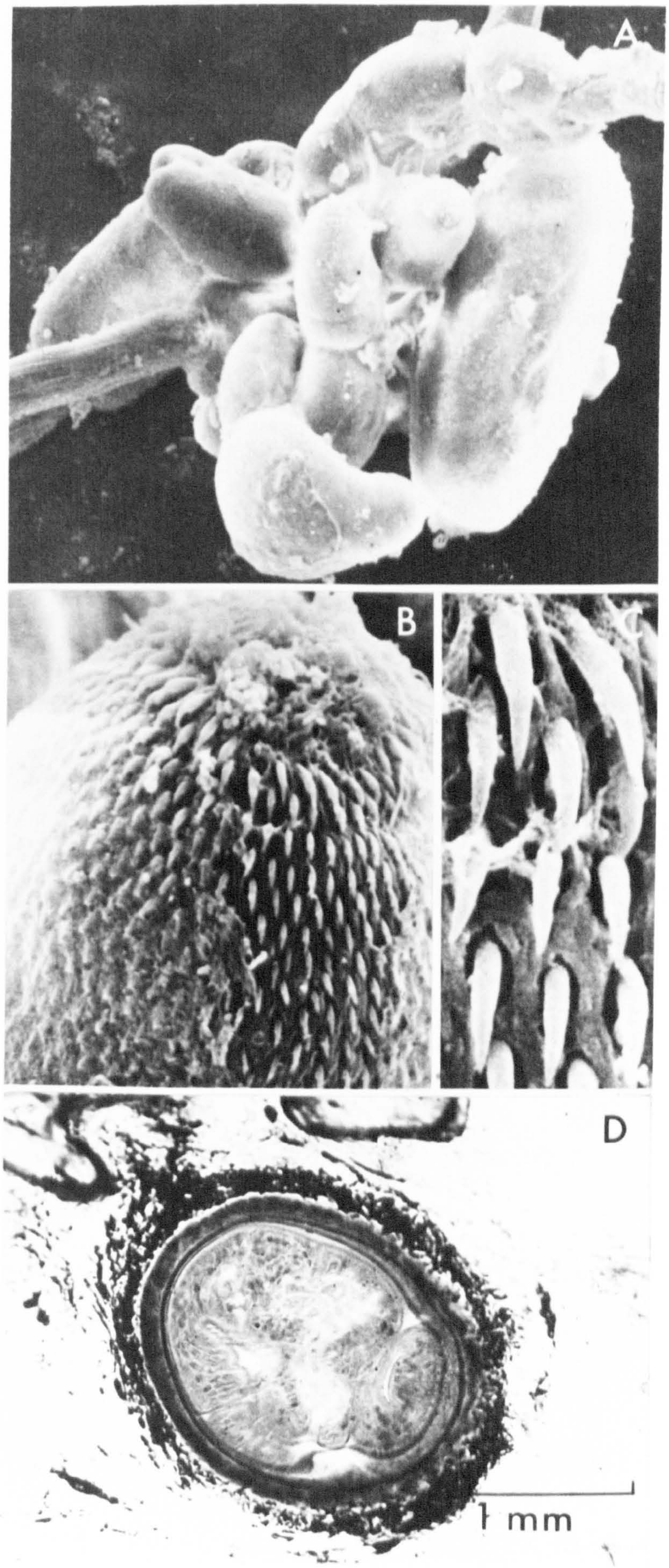

A
B
C
D
1 mm

PLATE 8

Ultrastructure of the epidermis of Cryptocotyle lingua and associated secretion cell bodies, I5 minuites post infection.

A. Epidermis contains three types of membrane bound secretion body, sb2, sb3 and sb4. The epidermal cell bodies (ec), also contain these secretion bodies The cystogenic gland cells (cc), also contain secretion bodies , four types being identified, including sbcI, sbc2, sbc3 and sbc4. The cytoplasm contains large areas of granular endoplasmic reticulum (rer), indicative of an actively protein-synthesising cell.

cc. cystogenic gland cell

ec. epidermal cell body

cm. circular muscle

my. myoblast

n. nucleus

sb2. epidermal secretion body , small0.2μm X0.05μm elongate bodies with fibrillar contents.

sb3. oval or round0.05μm X0.07μm , body with fibrillar contents. -0.13μm

sb4. large oval or round0.34μm X0.13μm homogeneous electron dense bodies.

sbcI. cystogenic gland secretion body, large0.55μm X 0.4 μm electron dense body

sbc2. small0.2 μm X0.13μm electron dense body

sbc3. large0.8 μm X0.8 μm electron lucent body.

sbc4. small0.3 μm X0.4 μm electron lucent body.

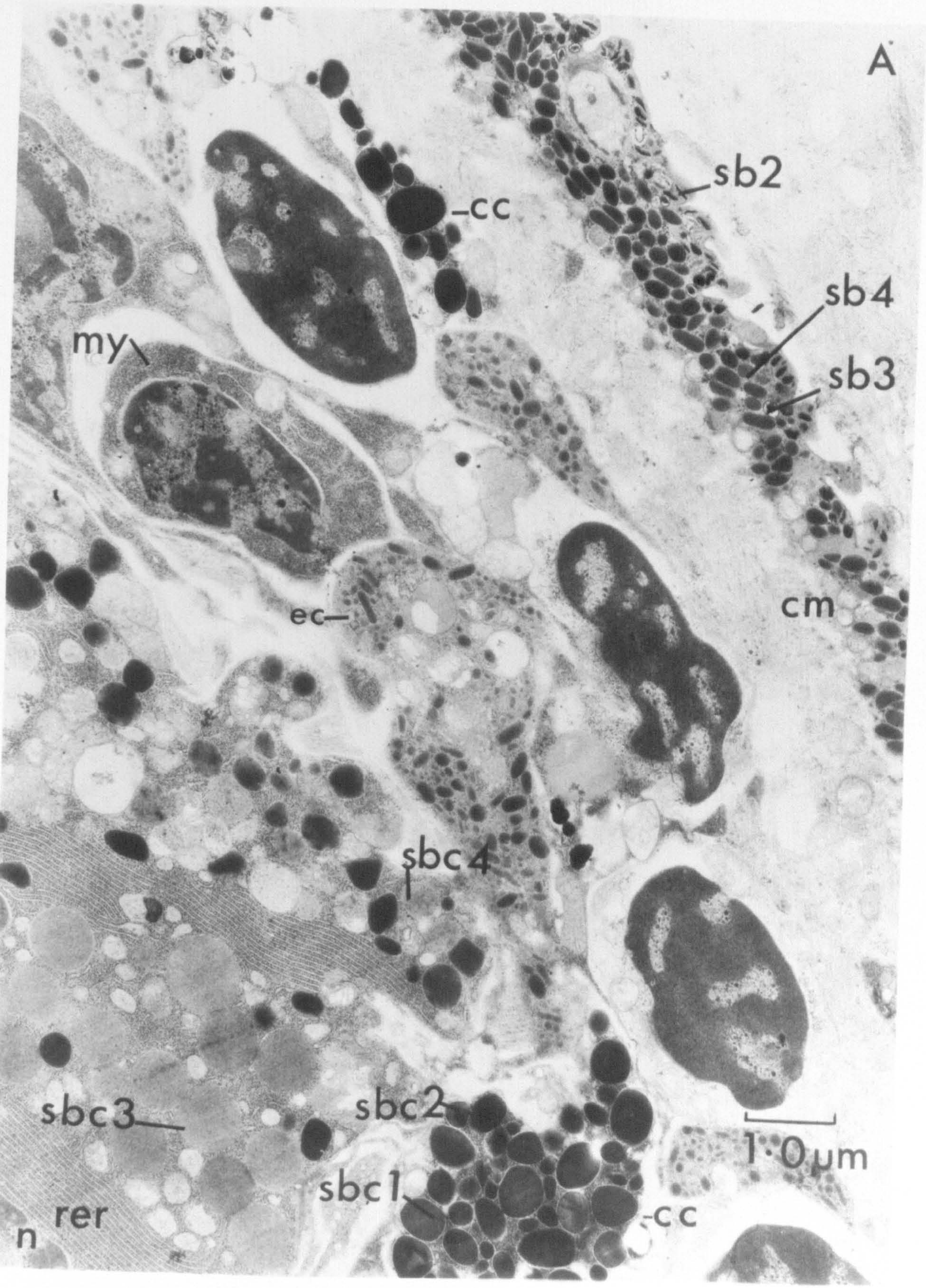

A
sb2
cc
sb4
sb3
my
ec
cm
sbc4
sbc3
sbc2
sbc1
cc
n
rer
1·0 µm

PLATE 9 (continued)

h. host cell
lm. longitudinal muscle
mt. microtubules
my. myoblast
pgd.penetration gland duct
sb. secretion body

PLATE 9

Ultrastructure of the epidermis of C.lingua and the associated fin connective tissue of Crenimugil labrosus I5 minuites after penetration.

A. Tegument of the metacercaria and cystogenic gland cells (cc); the metacercaria lying within the connective tissues of the dermis of the fin rays.
X 2,672

B. Tegument (te), containing the secretion bodies, the cystogenic gland cells (cc), and externally the host connective tissue(h).
X 8,552

C. Tegument and a cystogenic gland cell connected by a protoplasmic process containing a mitochondrion (m).
X 8,552

D. Tegument with secretion bodies, musculature of the body wall and epidermal cell bodies (ec).
X 8,552

E. Section through the penetration gland cells with their microtubule lined ducts and the irregular secretion bodies.
X 8,552

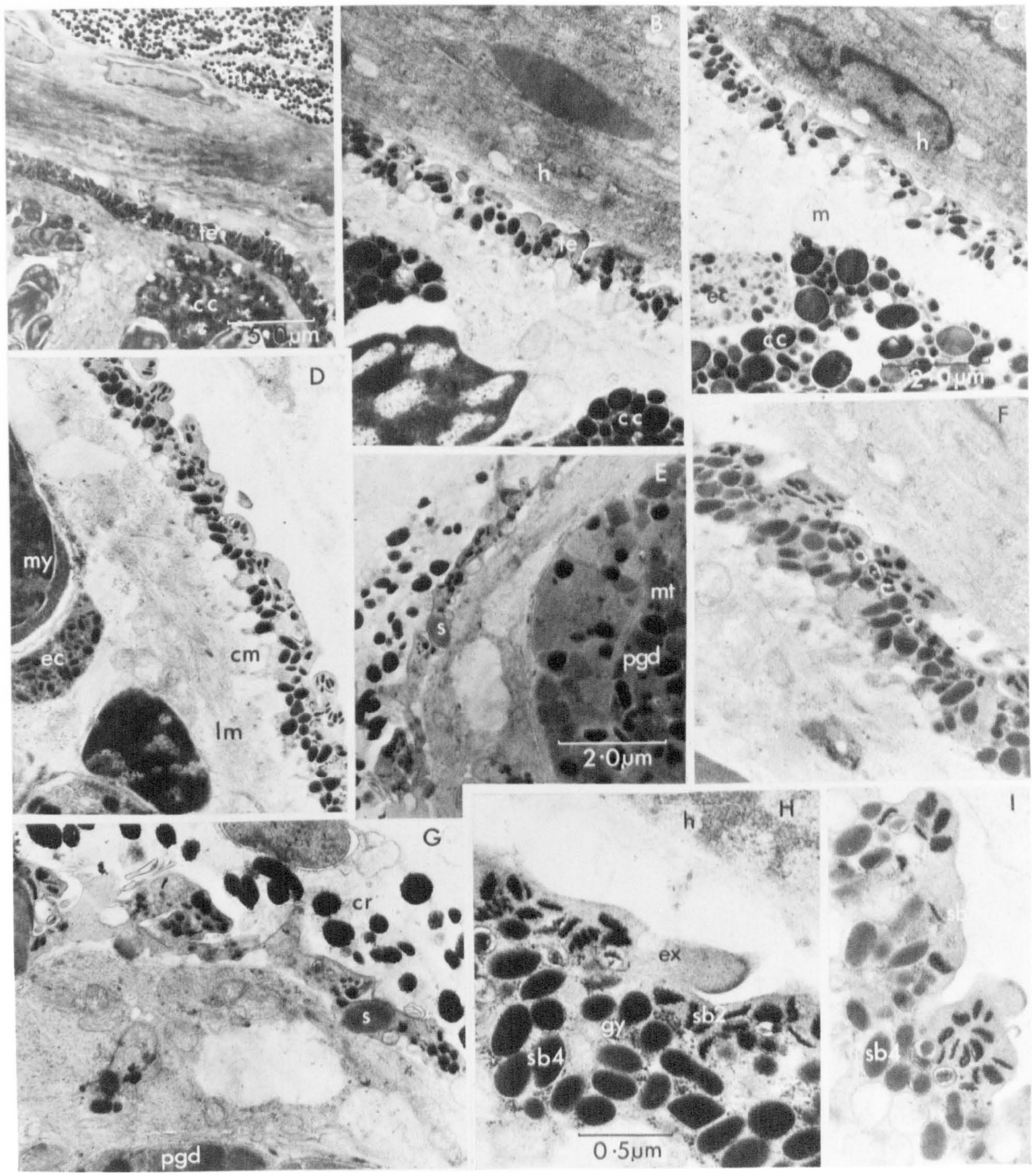
F. Tegument with the packed secretion bodies.
X I3,980

G. Lysis of a chromatophore (cr),associated with the metacercaria.
X I3,980

H. Detail of the tegument containing the sb2 and sb4 and glycogen.
X 28,784

I. Tegument with sb2, sb3 and sb4 secretion bodies.
X 22,204

cc.cystogenic gland cells
cr. chromatophore
cm. circular muscle
ec. epidermal cell body
ex. site of release of secretion bodies


A
fe
cc
5·0μm
B
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C
h
m
ec
cc
2·0μm
D
my
ec
cm
lm
E
s
mt
pgd
2·0μm
F
G
cr
s
pgd
H
h
ex
gy
sb4
sb2
0·5μm
I
sb
sb1

PLATE I5

Ultrastructure of the epidermis and cyst wall I day post infection .

A. Metacercaria enclosed by the parasitic cyst.
X 6,8I0

B. Metacercarial epidermis with spines enclosed by the two layered parasitic cyst wall.
X 6,8I0

C. Metacercaria within the parasitic cyst.
X II,498

D. Epidermis of the metacercaria with spines and sbc5 secretion bodies, resting on the circular and longitudinal muscles of the body wall.
X I9,0I6

E. Vacuolated tegument enclosed by the two layered parasitic cyst.
X I9,0I6

F. Thin epidermal layer containing sbc5 secretion bodies.
X I9,0I6

G. Two layered acellular parasitic cyst and epidermis with spines and sbc5 secretion bodies.
X 38,9I8

H. Epidermis packed with secretion bodies from the cystogenic gland cells, including sbcI,sbc3 and sbc5 types. Microfilaments (mf), adhering to the outer surface of layer I of the parasitic cyst.
XI5,036

PLATE I5 (continued)

I. Thin epidermal layer containing sbc5 secretion bodies and microfilaments adhering to the outer surface of the parasitic cyst.

X I9,0I6

J. Epidermis with large flocculent inclusions, which may represent changes in secretion bodies from the cystogenic gland cells prior to their release.

X 30,958

cm. circular muscle

lm. longitudinal muscle

mf. microfilaments

s. spine

sbc. secretion body from cystogenic gland cell

te. tegument or epidermis

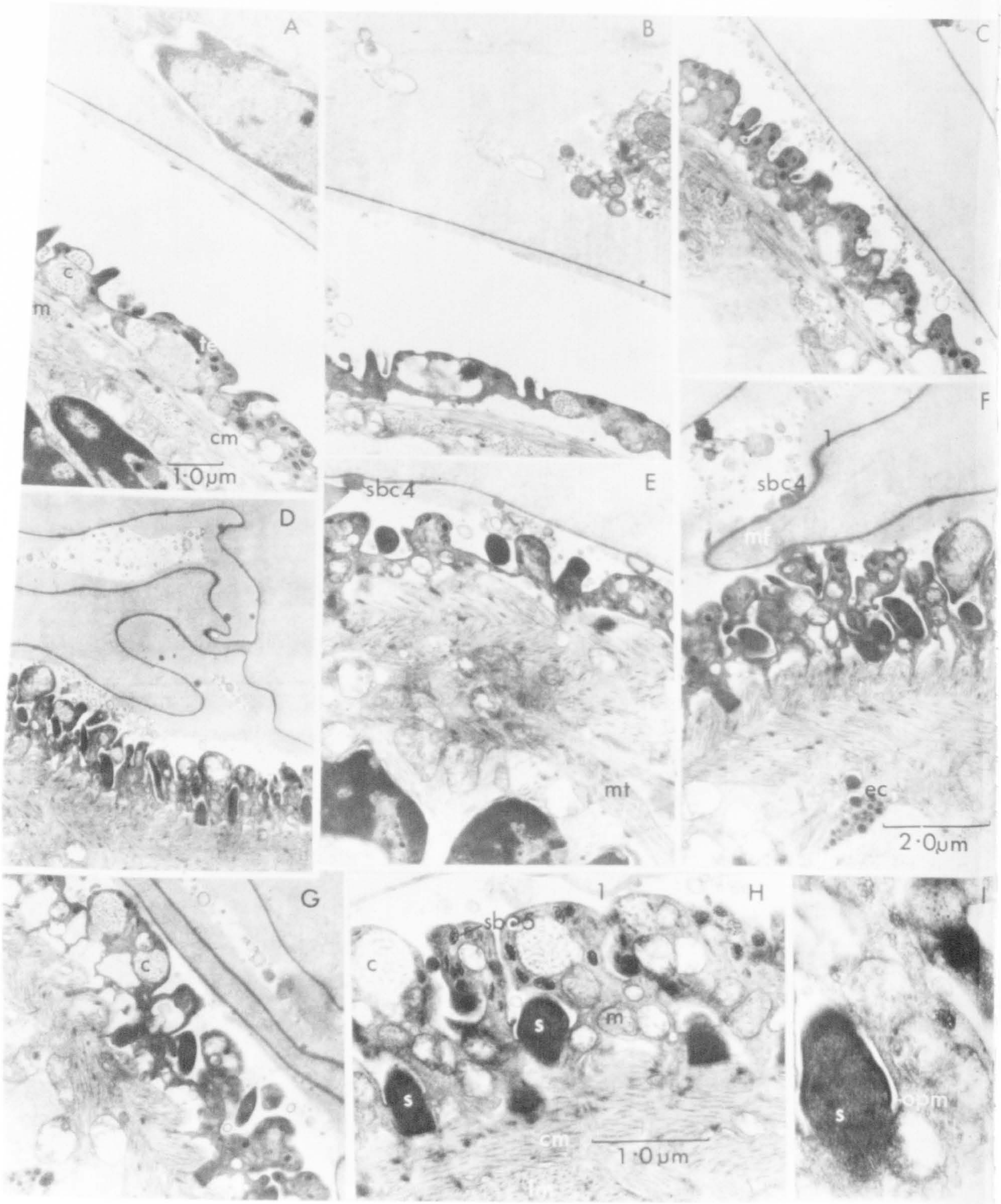

A
B
C
c
m
cm
1·0μm
D
E
sbc4
F
sbc4
mt
mt
ec
2·0μm
G
c
H
I
sbc4
c
s
m
s
cm
1·0μm
s
0·5μm

PLATE I4

Ultrastructure of the epidermis of C. lingua and the parasitic cyst 3 hours post infection

A. Epidermis with many large flocculent inclusions (c), and the clear demarcation of the metacercaria within the parasitic cyst.
X 8,238

B. Vacuolated tegument with spines and flocculent inclusions; the metacercaria clearly encysted within the two layered parasitic cyst.
X 8,238

C. Secretion of the inner acellular layer 2 of the parasitic cyst with numerous vacuoles and vesicles forming this layer.
X 8,238

D. To show the extremely folded nature of the outer parasitic cyst.
X 4,846

E. The sbc4 secretion bodies from the cystogenic gland cells appear to be released in tact from the the tegument and fuse with the inner surface of the parasitic cyst, layer I.
X 8,238

F. Fusion of sbc4 secretion bodies with the inner surface of layer I of the parasitic cyst and microfilaments adhering to the outer surface.
X 8,238

G. Epidermis with large flocculent inclusions and the folded

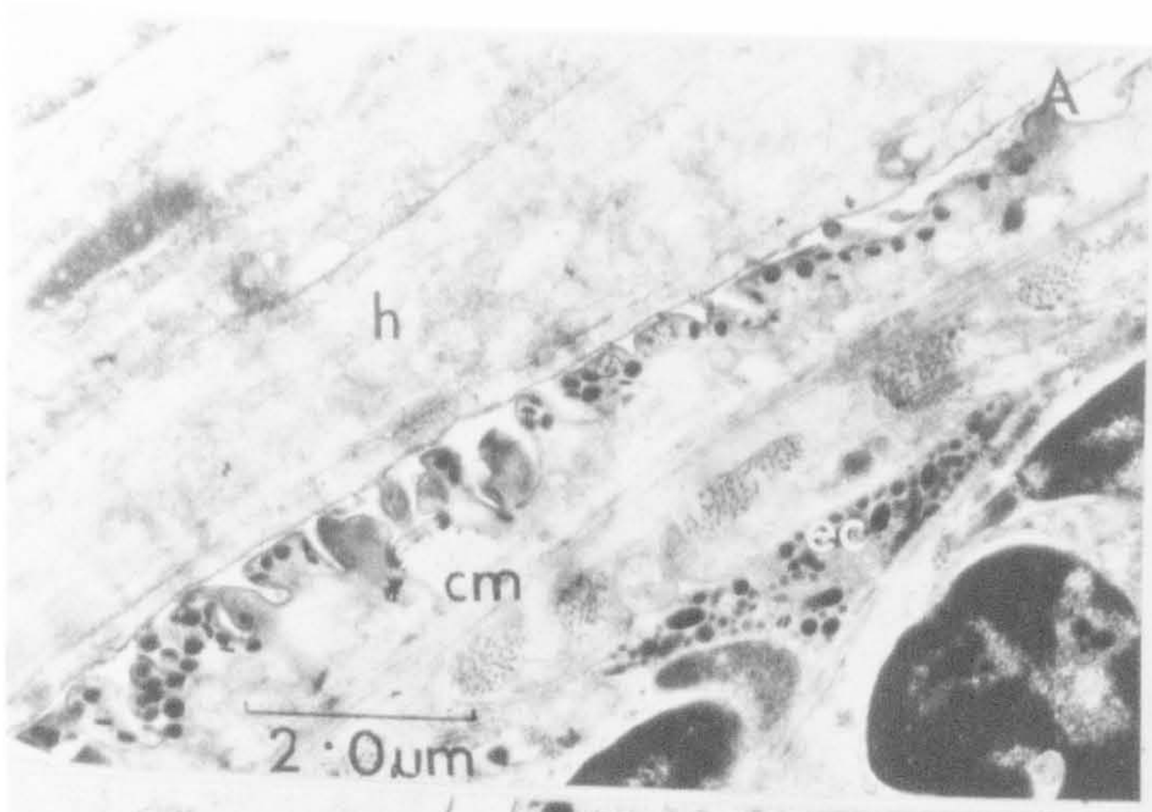
PLATE I4 (continued)

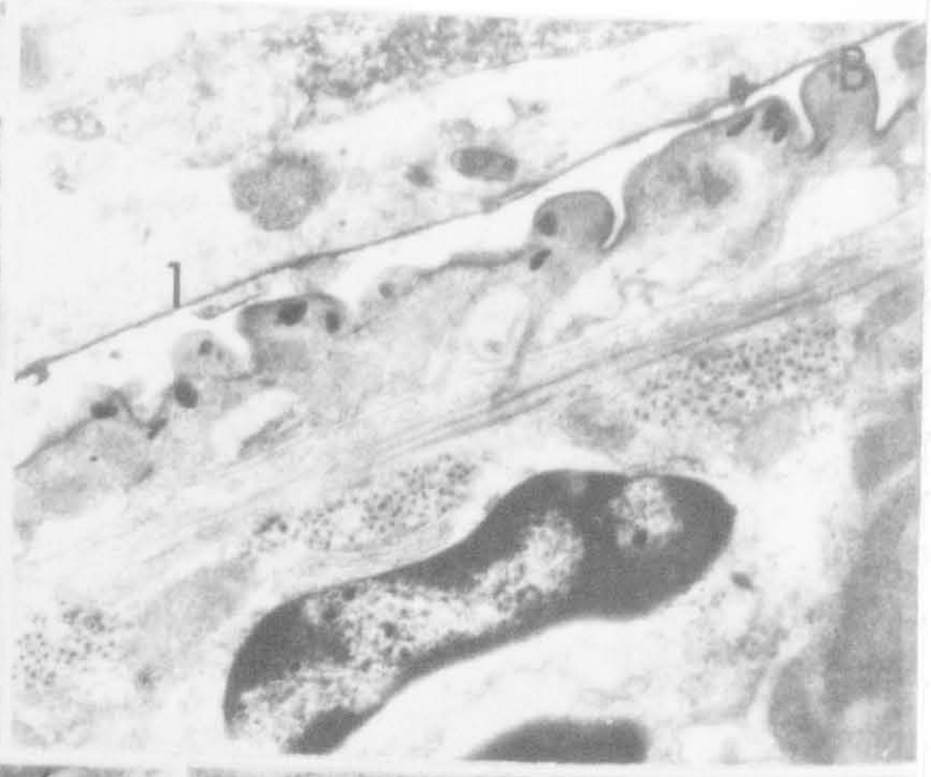
G. parasitic cyst.
X 8,238

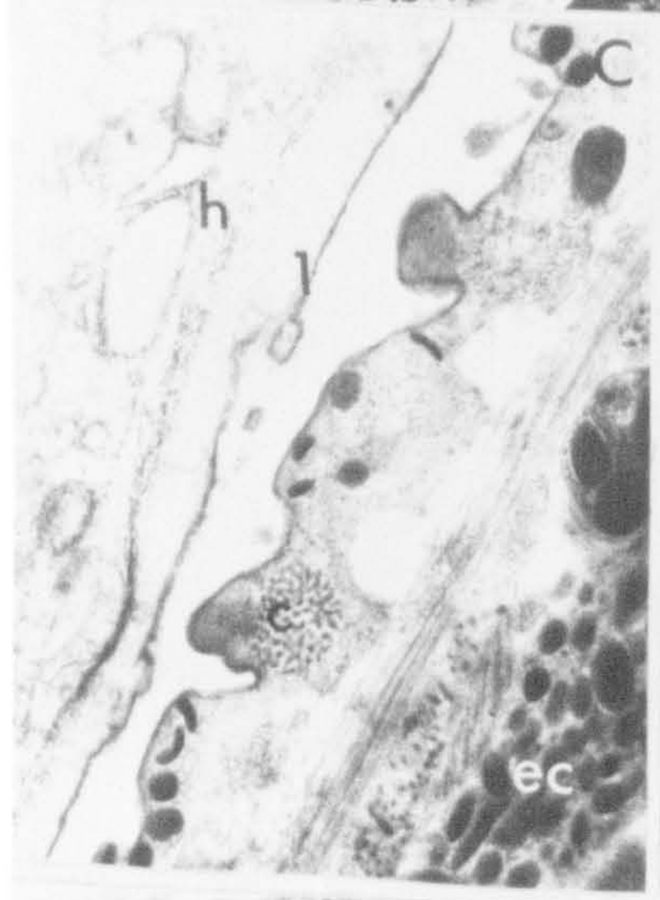
H. Detail of the epidermis showing the secretion bodies to be sbc5 type originating from the cystogenic gland cells.
X I7,03I

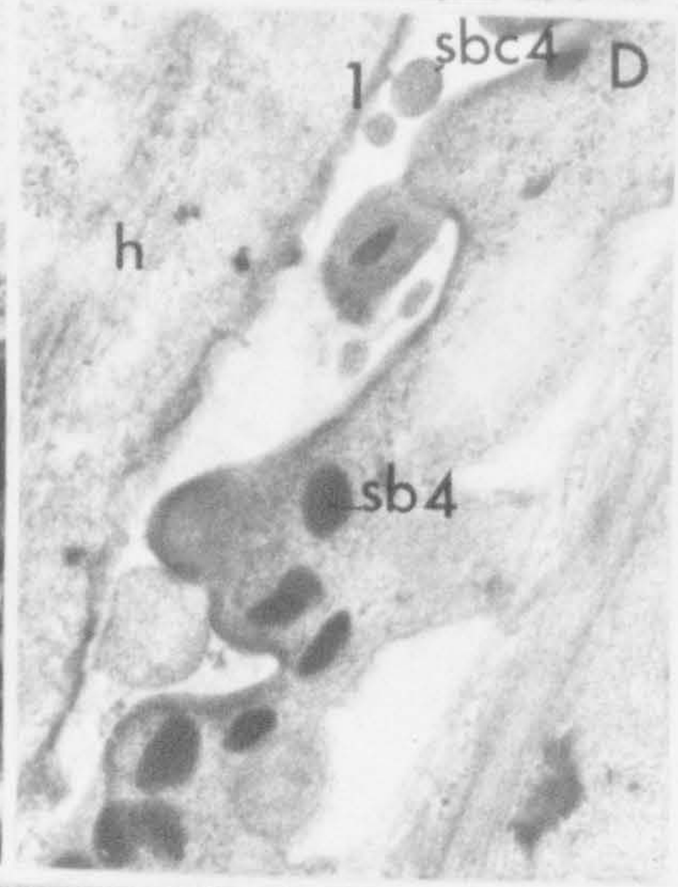
I. Spine showing crystalline structure, covered by the outer plasma membrane of the epidermis.
X 33,85I

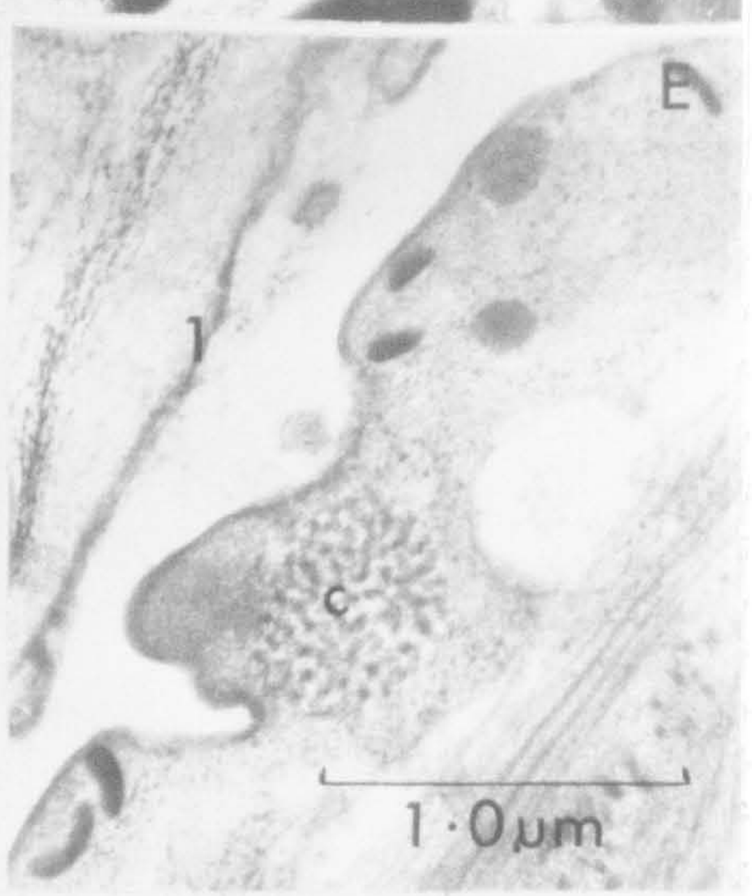
c. flocculent inclusion in epidermis
cm. circular muscle
ec. epidermal cell body
h. host cell
lm. longitudinal muscle
m. mitochondria
mt. microtubules
mf. microfilaments
opm. outer plasma membrane
s. spine
sb. secretion body
I. outer parasitic cyst
2. inner parasitic cyst

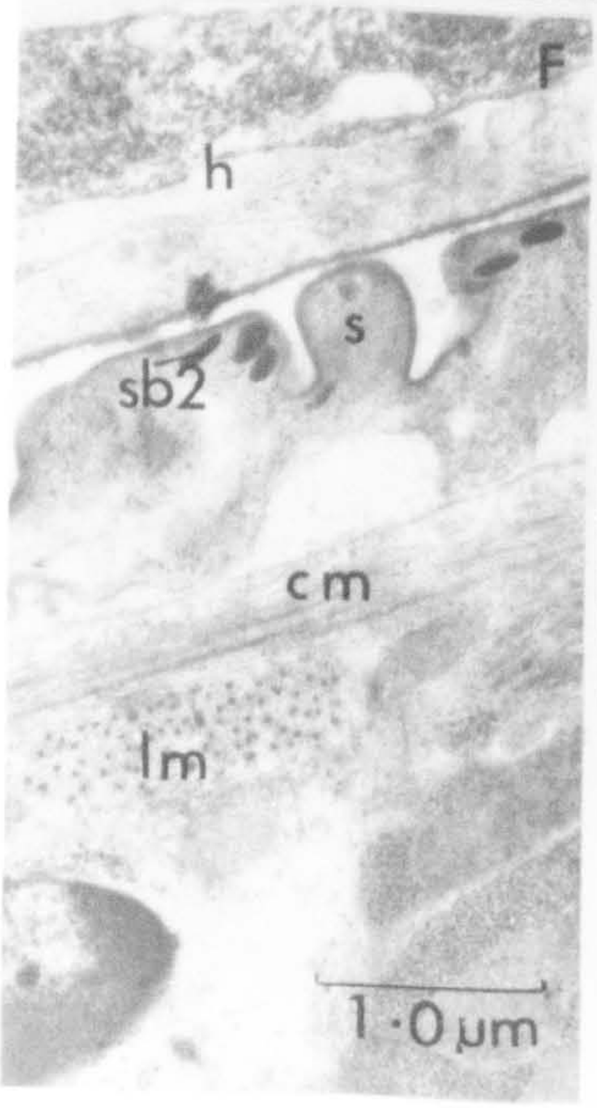

A
h
cm
2·0µm

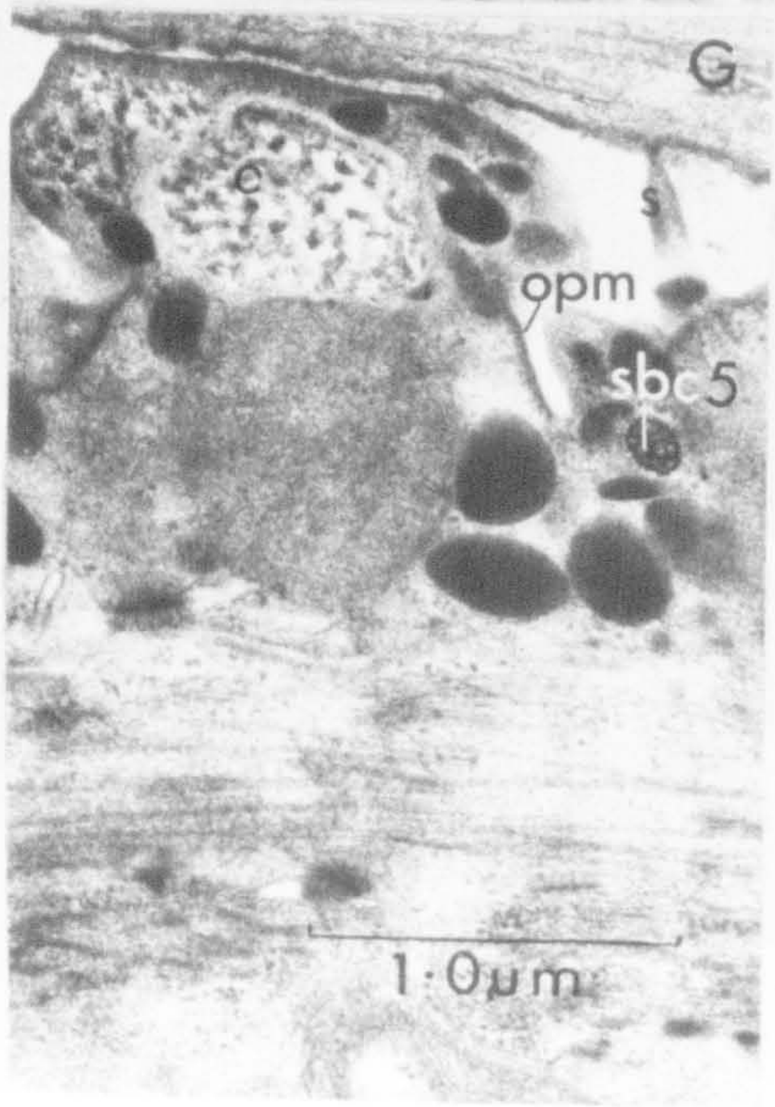

B
l


C
h
l
c
ec


sbc4
D
l
h
sb4


E
l
c
1·0µm


F
h
s
sb2
cm
1m
1·0µm


G
c
s
opm
sbc5
1·0µm

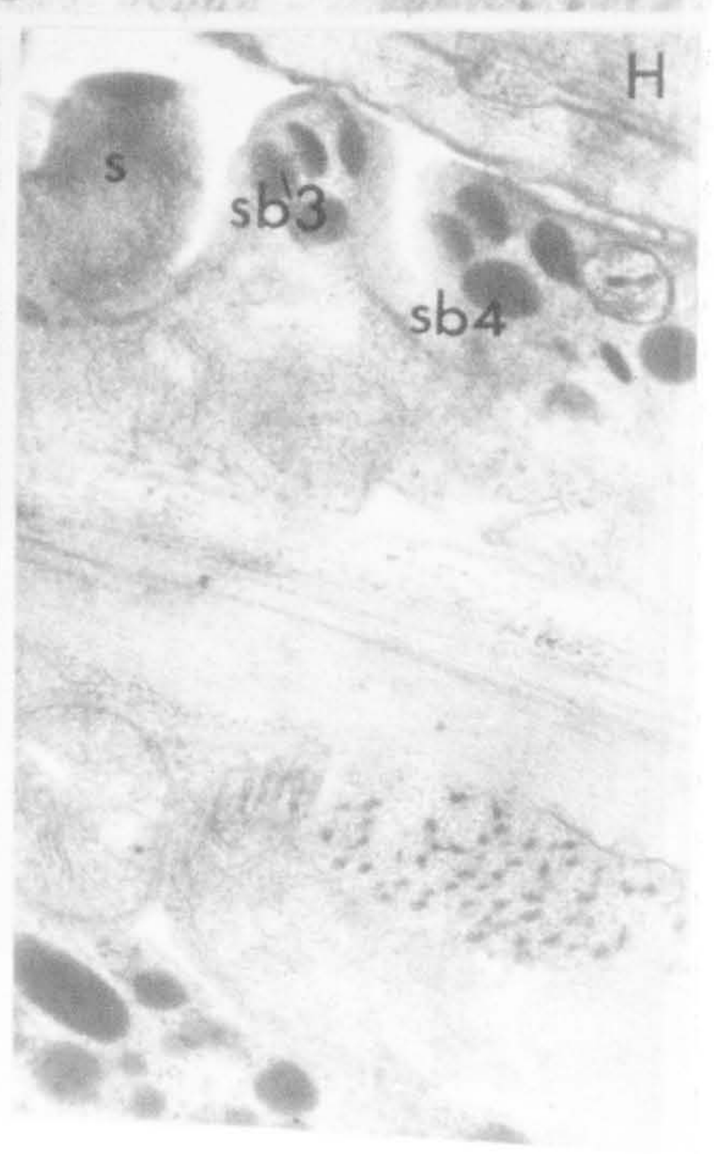

H
s
sb3
sb4

PLATE I3

Ultrastructure of the epidermis of C. lingua and the associated host tissue 2 hours post infection.

A. Epidermis containing secretion bodies and underlying epidermal cell body (ec).
X 8,245

B. Epidermis with few secretion bodies and initial thin parasitic cyst (I), present.
X II,I36

C. Epidermis with few secretion bodies and initial thin parasitic cyst at the junction with the host cells.
X I3,920

D. Epidermis with sb4 secretion bodies and sbc4 secretion bodies within the parasitic cyst.
X 28,9I2

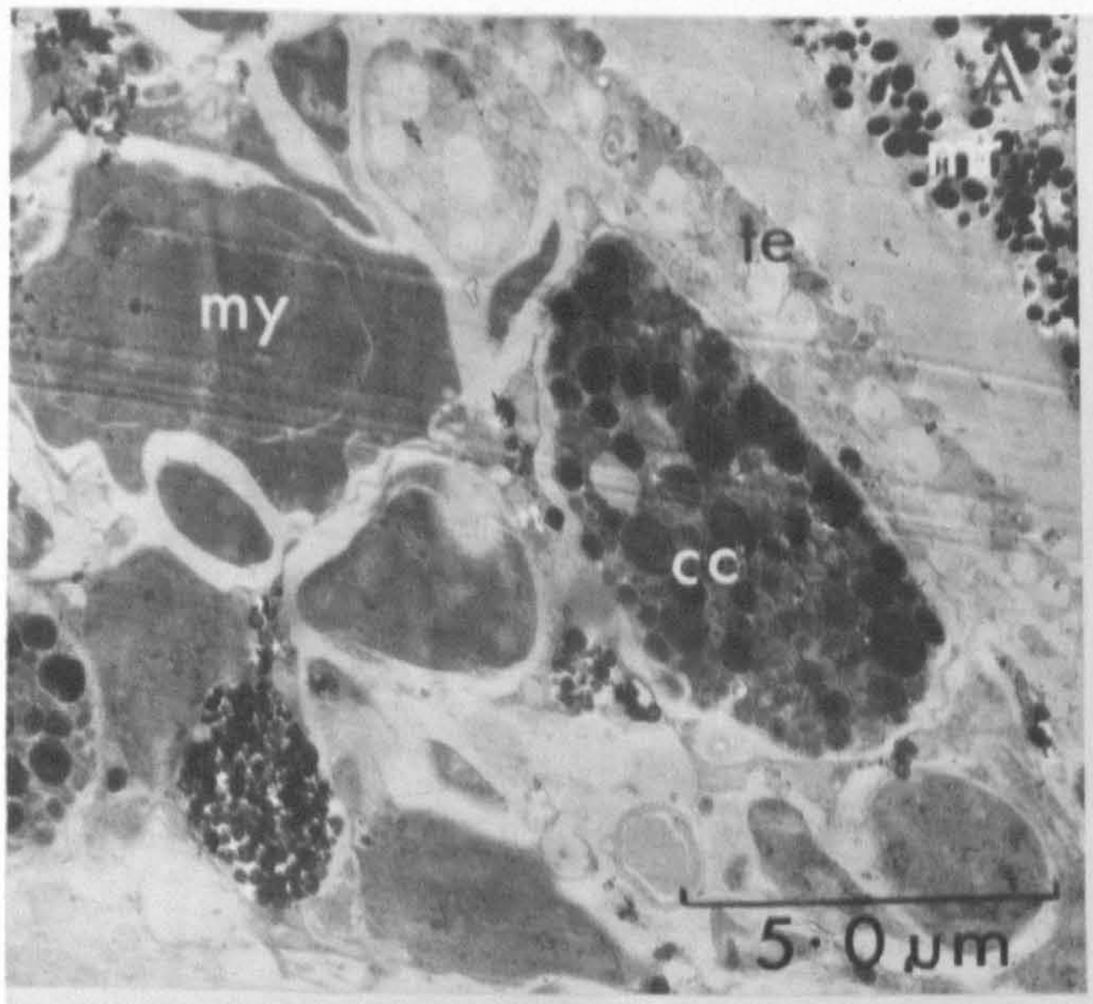
E. Epidermis with large flocculent inclusion.
X 28,9I2

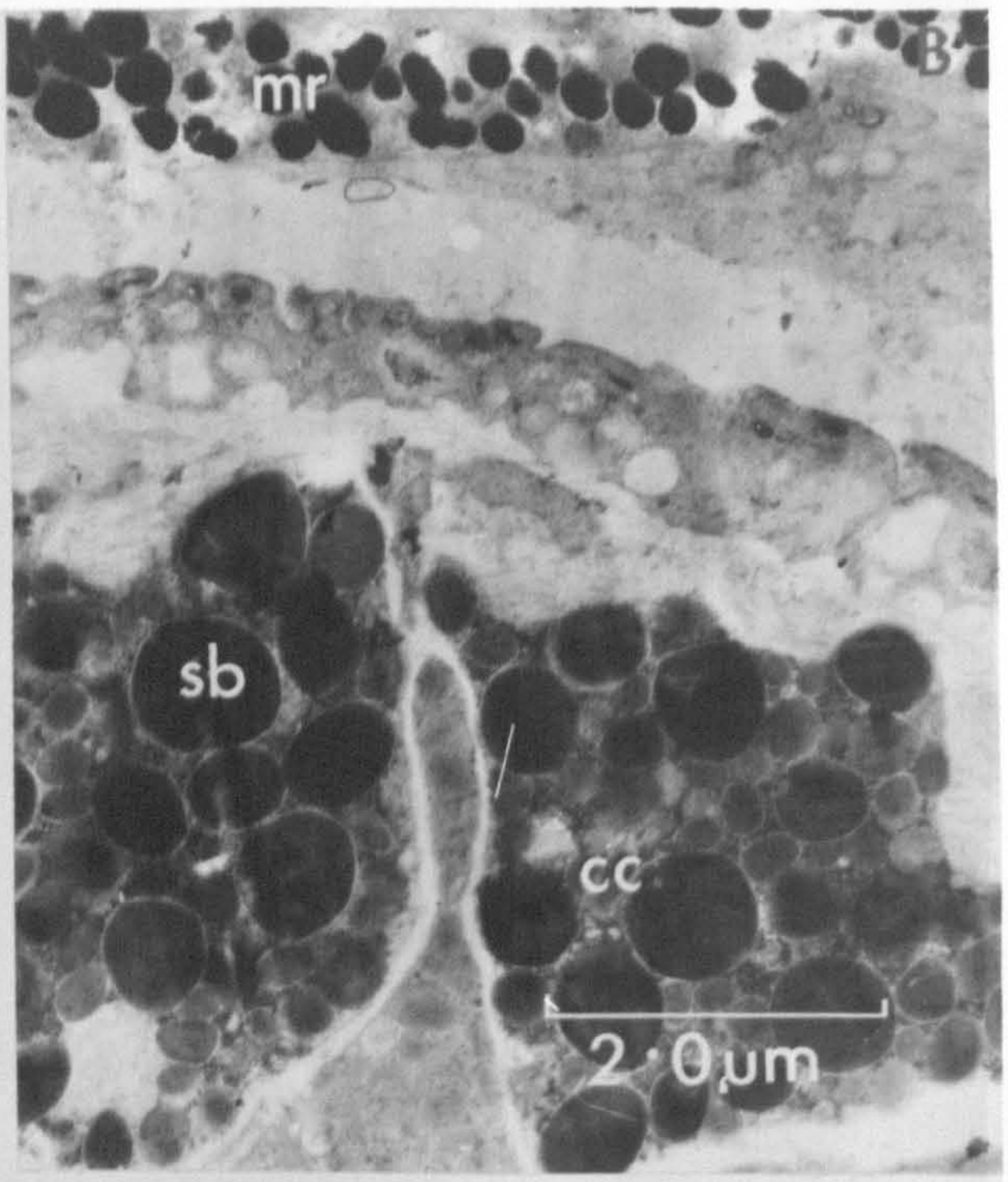
F. Epidermis with sb2 secretion bodies and the underlying circular and longitudinal muscles of the body wall.
X 28,9I2

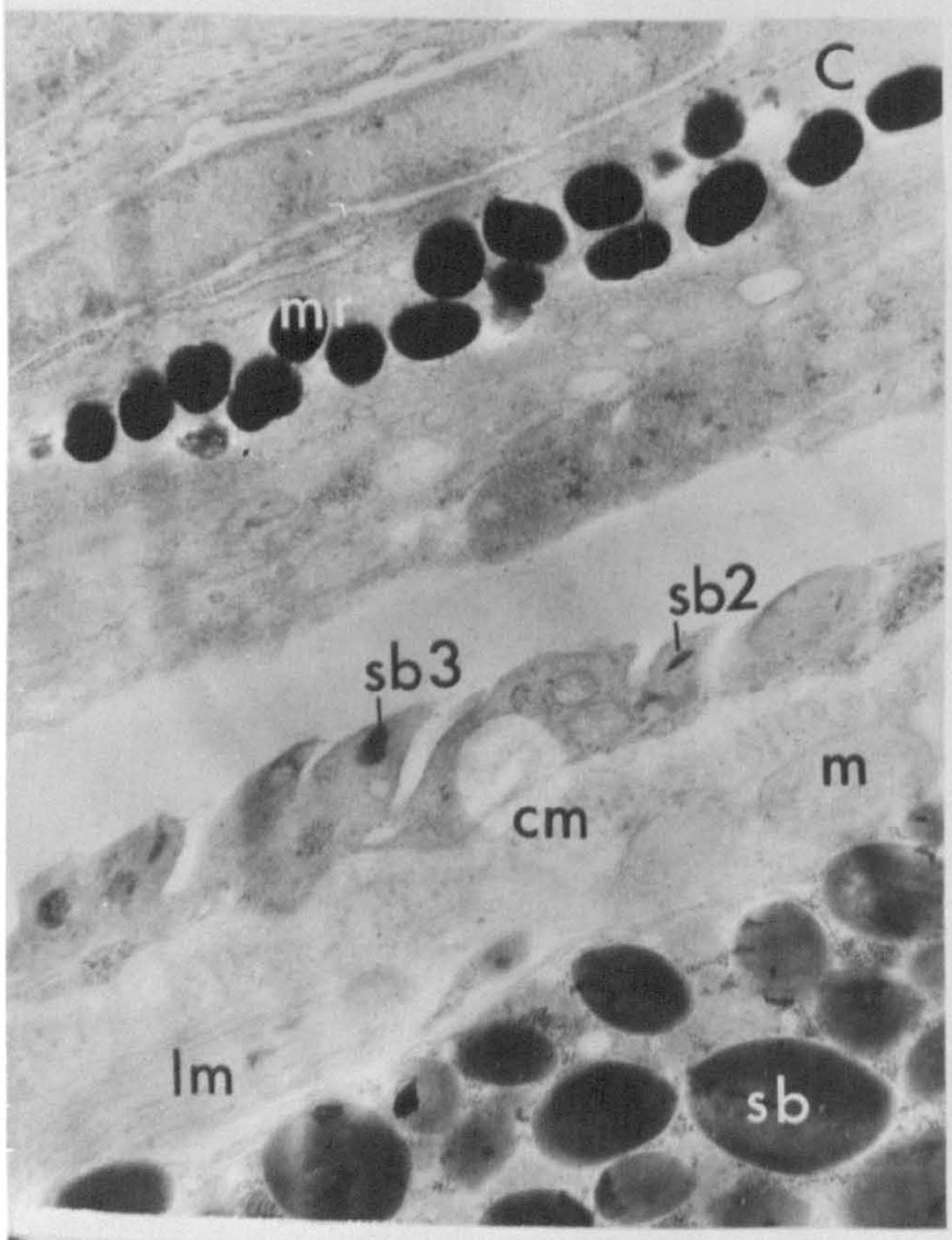
G. Epidermis with sb4 and sbc5 secretion bodies.
X 28,9I2

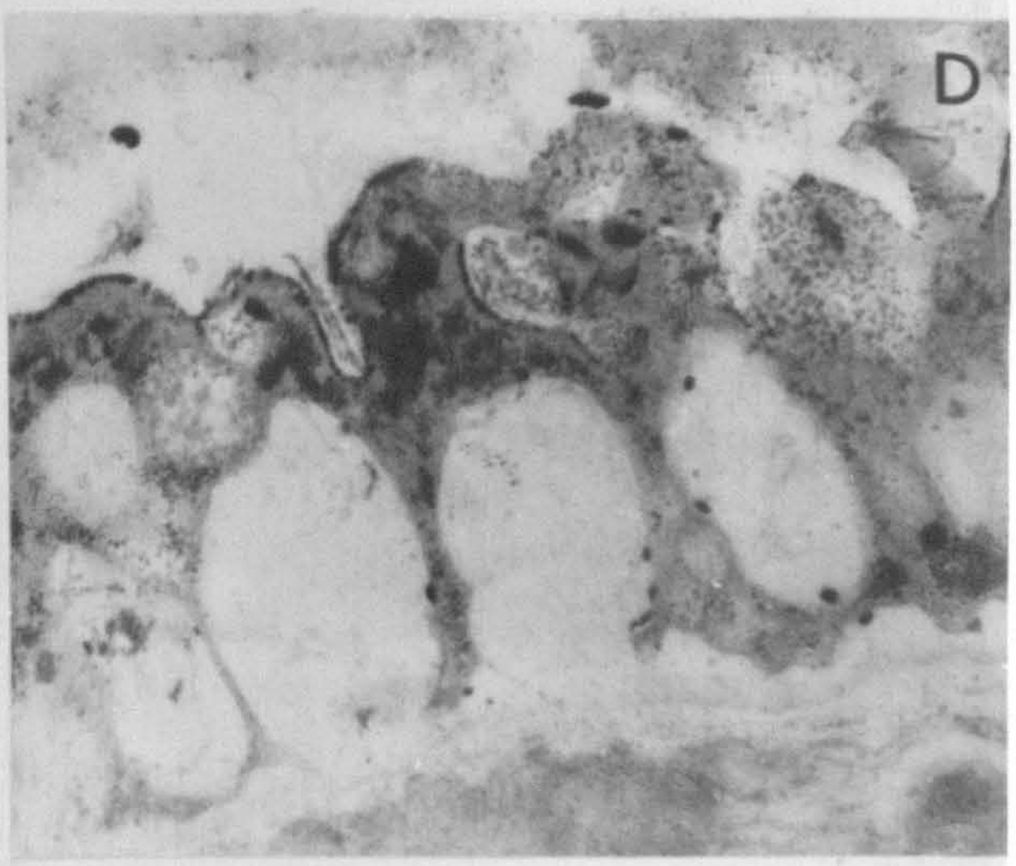
H. Epidermis with sb3 and sb4 secretion bodies.
X 28,9I2

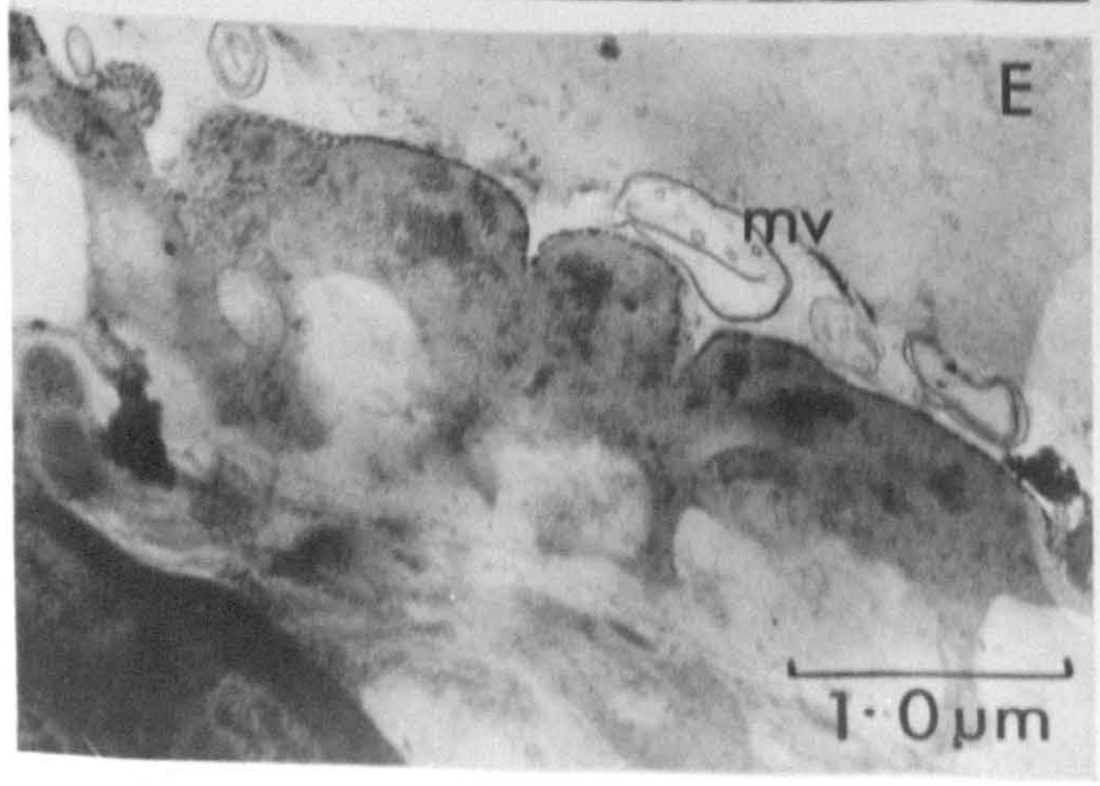
c. flocculent inclusion in epidermis
cm. circular muscles
ec. epidermal cell body
lm. longitudinal muscle
opm. outer plasma membrane
sb. secretion body


A
my
le
cc
5·0 µm


B
mr
sb
cc
2·0 µm


C
m
sb2
sb3
cm
m
lm
sb


D


E
mv
1·0 µm

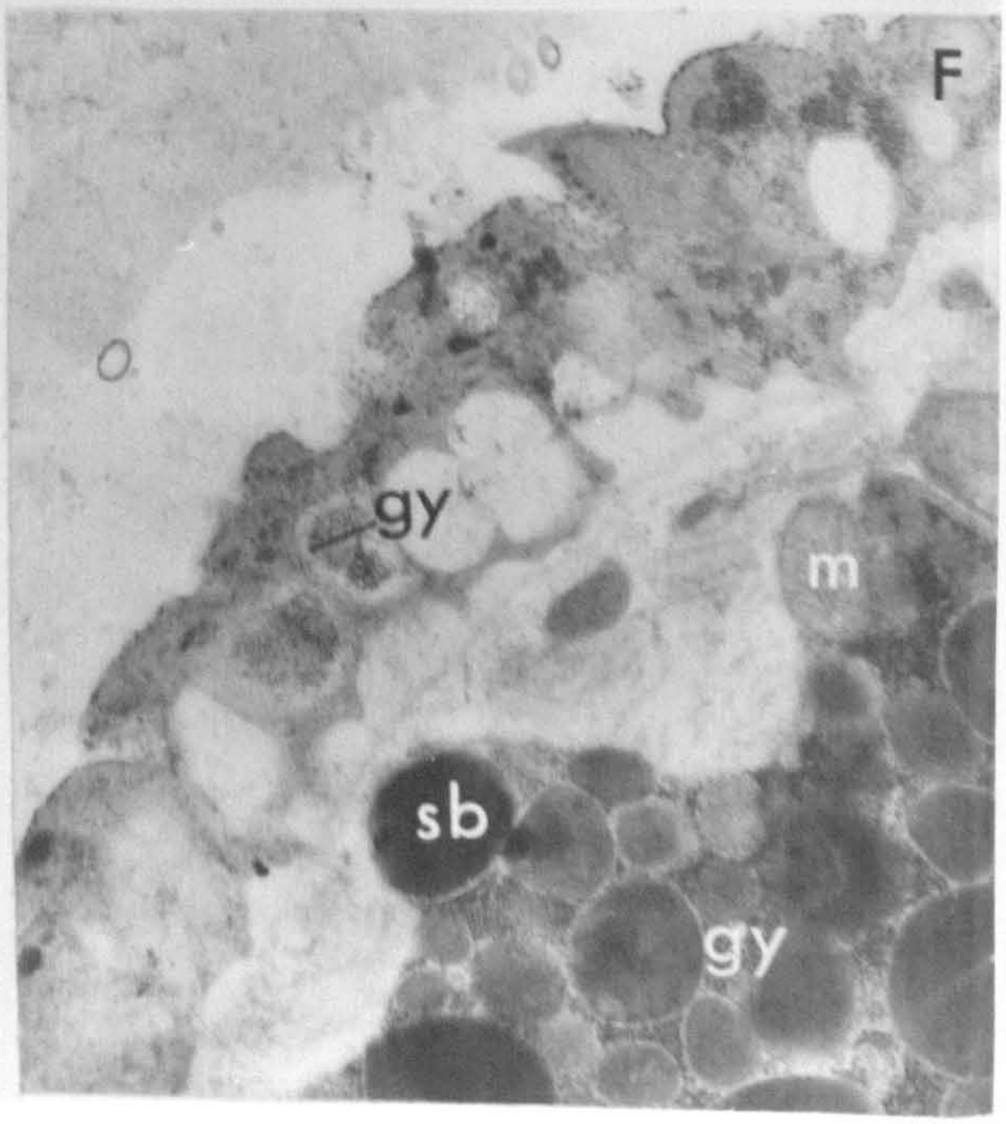

F
gy
m
sb
gy

PLATE I2

Ultrastructure of the tegument and associated structures and host tissues 90 minuites post penetration.

A. Tegument almost devoid of secretion bodies. Underlying cystogenic gland cells contain packed masses of electron dense secretion bodies.
X 5,647

B. Tegument and part of cystogenic gland cells with secretion bodies.
X I2,529

C. Detail of the tegument with sb2 and sb3 secretion bodies . Lysis of host cells has left a space between the surface of the metacercaria and the melanophore (mr).
X 20,482

D. Tegument devoid of secretion bodies but containing glycogen.
X 25,903

E. Tegument with no secretion bodies but some glycogen. Whorls of membranous material (mv), lie in the fluid filled space between the tegument and the host cells.
X 20,482

F. Tegument without secretion bodies but containing some glycogen (gy),underlying cystogenic gland cells contain the large homogeneous secretion bodies.
X 20,482

cc. cystogenic gland cells
gy. glycogen
m. mitochondria
mr. melanophore
mv. membranous vesicles

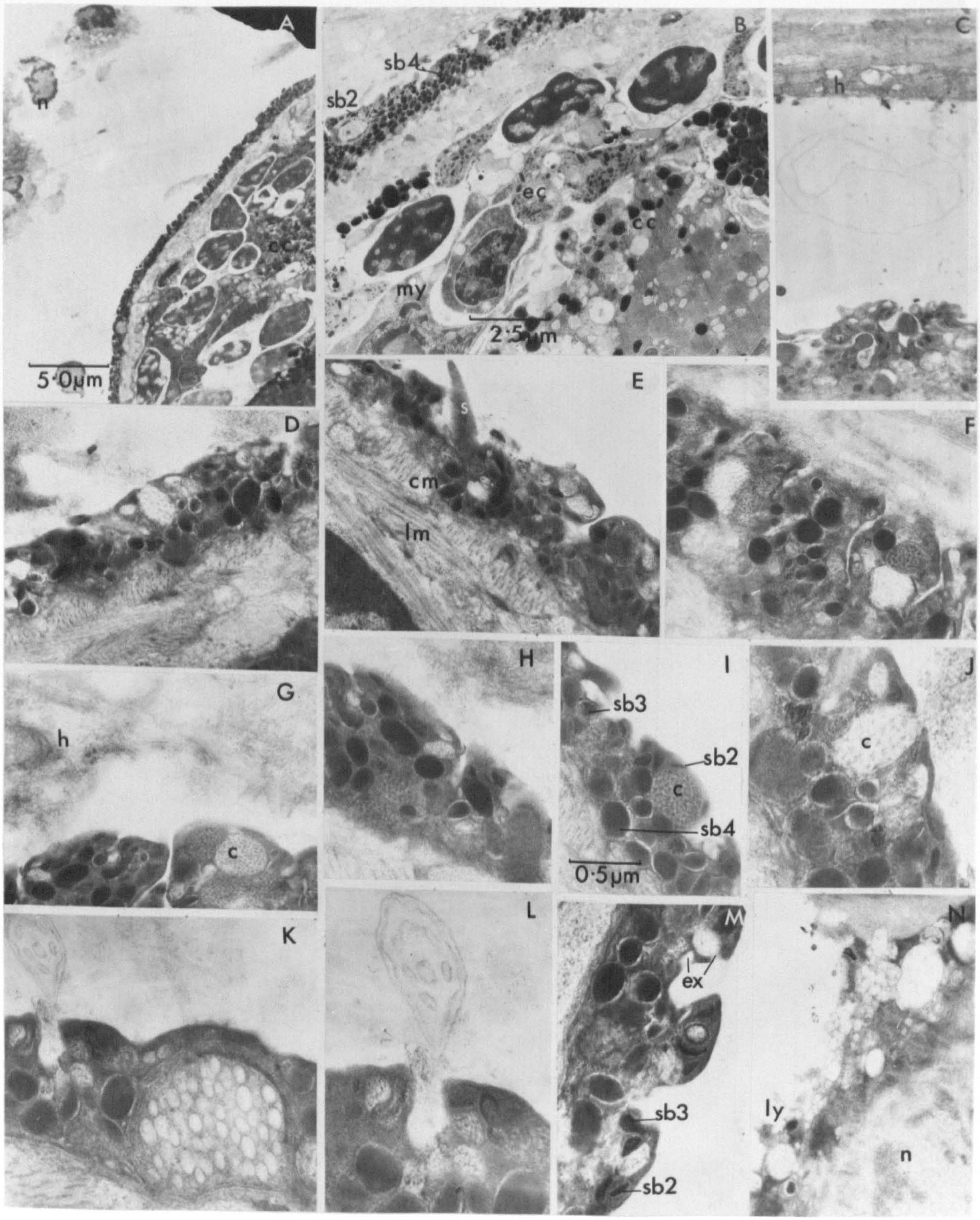

A
n
cc
5·0μm
B
sb4
sb2
ec
cc
my
2·5μm
C
h
D
E
s
cm
lm
F
G
h
c
H
I
sb3
sb2
c
sb4
0·5μm
J
c
K
L
M
ex
sb3
sb2
N
ly
n

PLATE II

Ultrastructure of the metacercarial surface and the associated fin connective tissue 30 minuites after penetration.

A. Section through the metacercaria and the associated connective tissue of the host showing the evidence of cell lysis with isolated nuclei at some distance from the metacercarial surface.
X 2,636

B. Section of the body wall of the metacercaria showing the tegument packed with predominately sb4 secretion bodies and a few peripherally located sb2 secretion bodies. Beneath the tegument and the muscles of the body wall are the epidermal cell bodies (ec), cystogenic gland cells (cc), myoblasts (my), and parenchyma cells.
X 5,IIO

C. Cavity formed around the metacercaria by lysis of the fish cells (h).
X IO,545

D. Tegument with secretion bodies and depression in the surface with flocculent contents, representing release of a secretion body.
X I7,440

E. Tegument with spine and many flocculent bodies derived from a change in secretion bodies prior to their release.
X I7,440

F. Tegument with sb4 secretion bodies and flocculent bodies
X I7,440

G. Tegument eith flocculent bodies (c), and associated lysis of host cells (h).
X IO,545

H. Tegument with secretion bodies.
X 2I,902

I. Detail of the tegument with a few peripherally located sb2 and sb3 secretion bodies , flocculent bodies (c),

PLATE II (continued)

I. and sb4 secretion bodies.
X 21,902

J. Release of contents of flocculent body from the tegument.
X 35,692

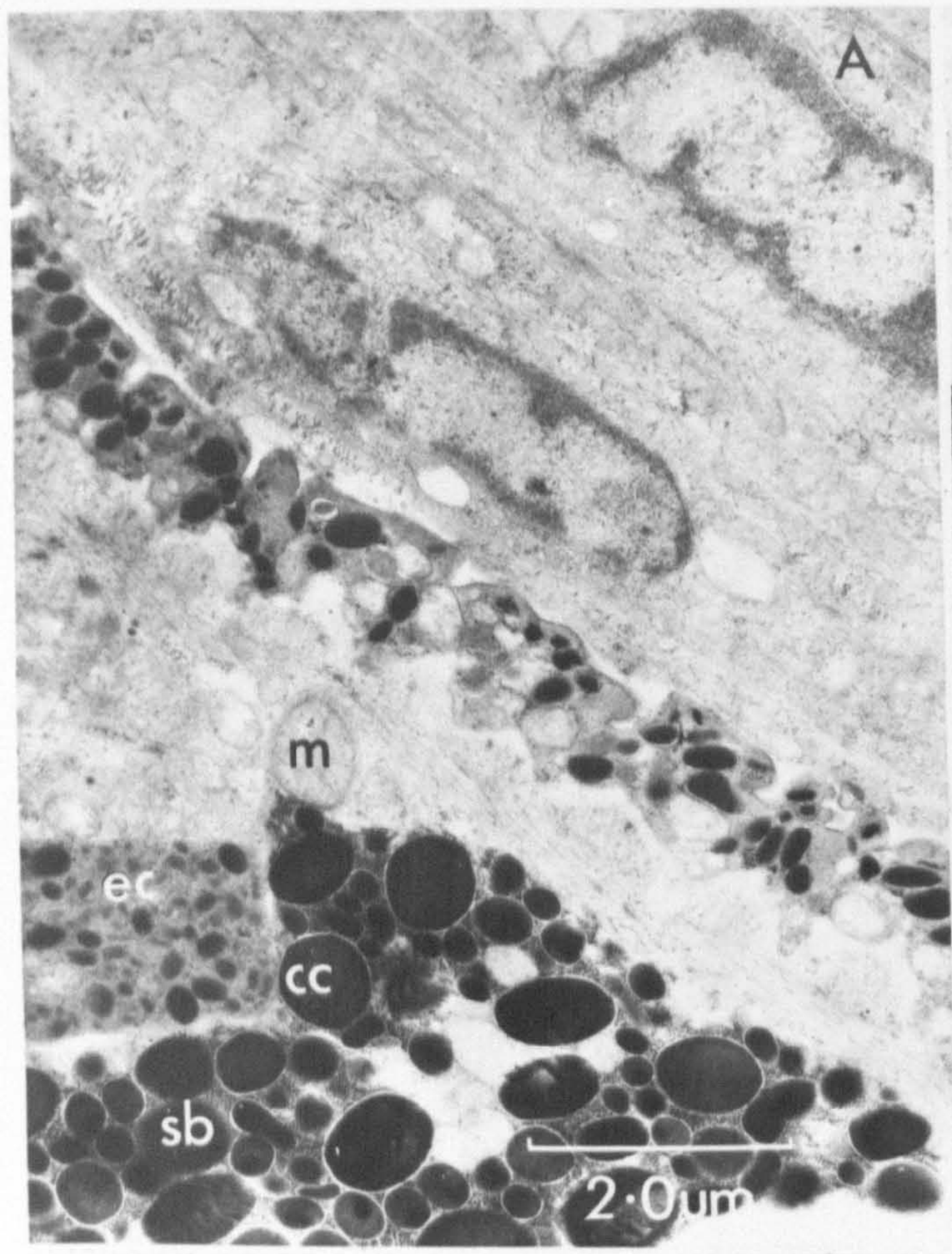
K. Large multivesiculated electron lucent body.
X 28,392

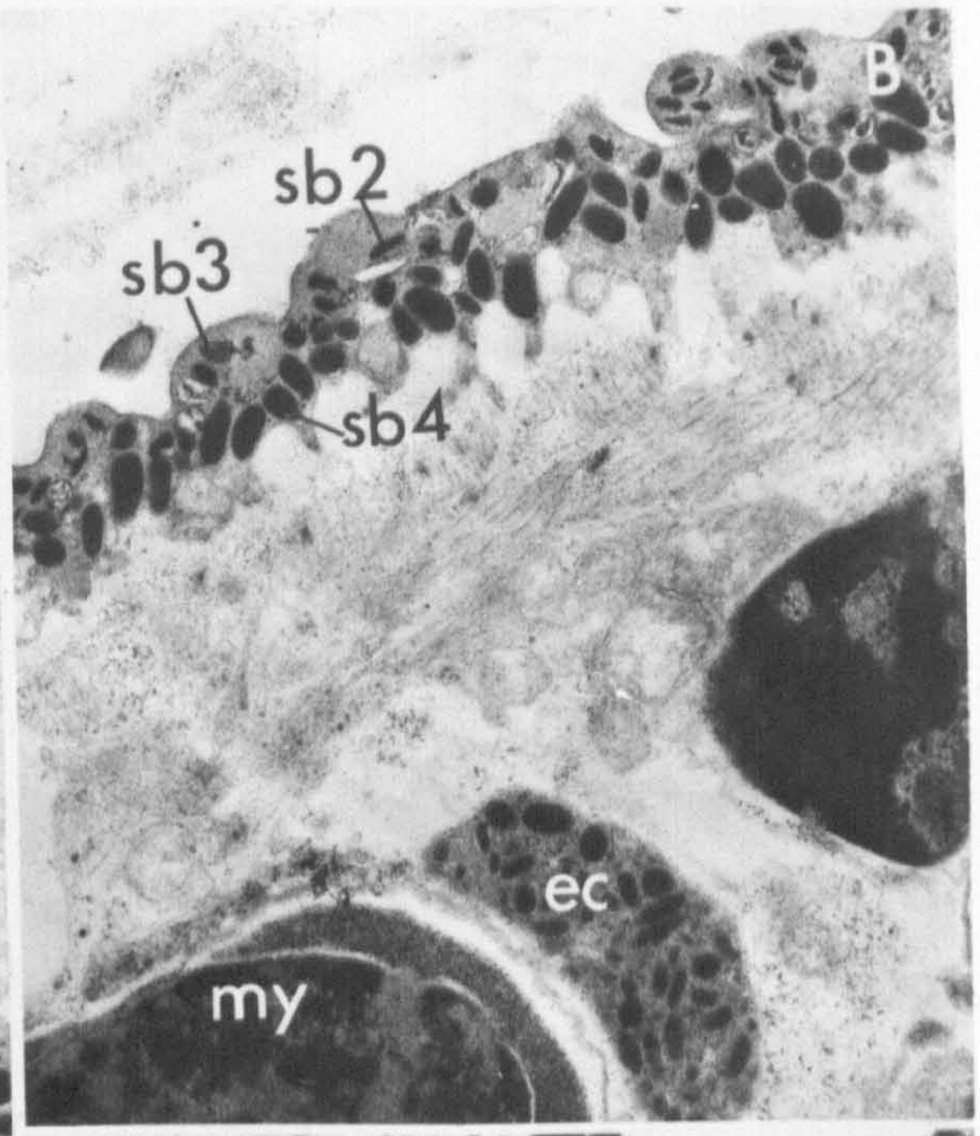
L. Release of membranous material from the tegument.
X 43,804

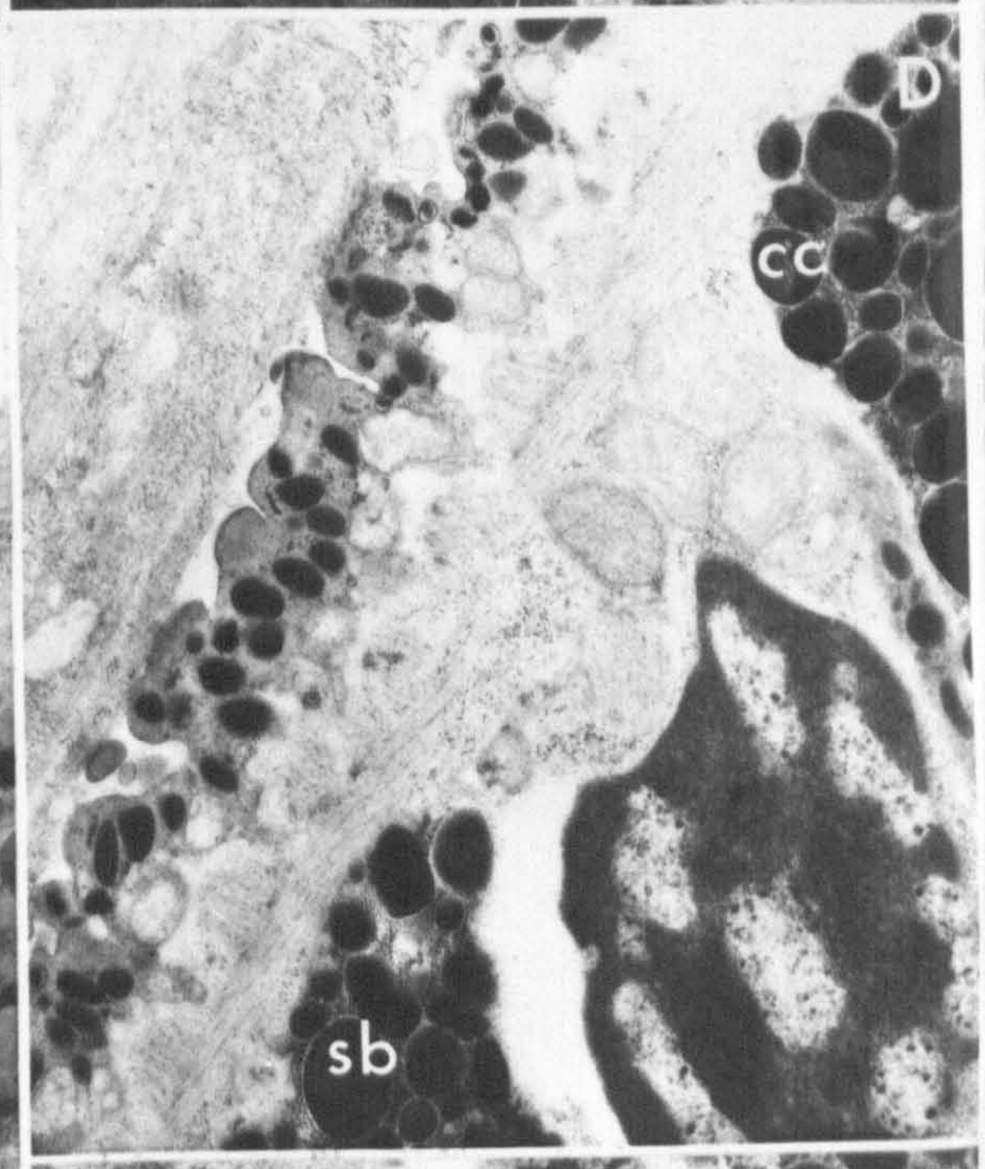
M. Depressions in the tegument where secretion bodies released (ex), and few peripherally located sb2 and sb3 secretion bodies.
X 28,392

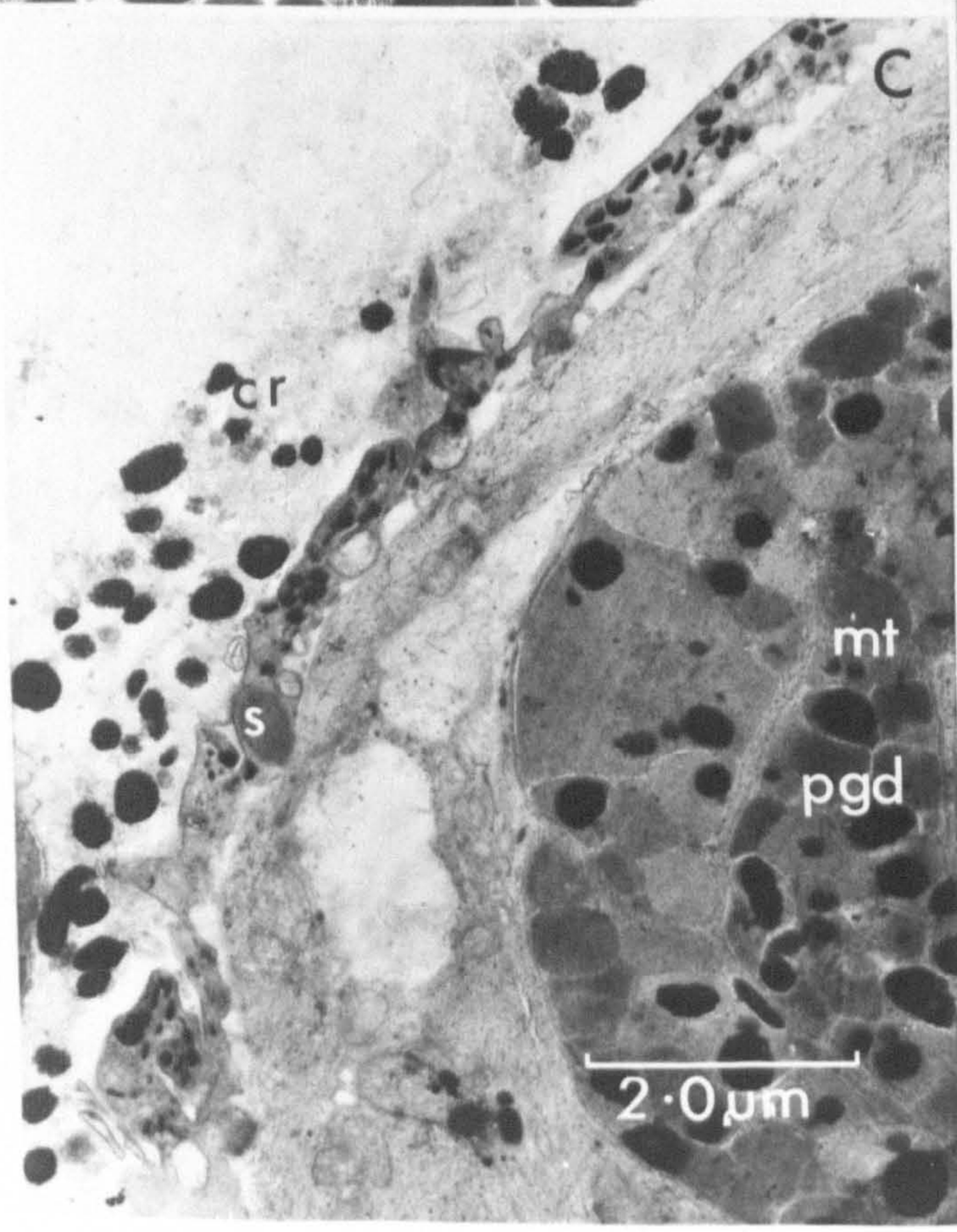
N. Lysis of fin connective tissue cells, cytoplasm being extremely vacuolated.
X 28,392

c. flocculent body
cc. cystogenic gland cell
cm. circular muscles
ex. point of release of secretion body from tegument
h. host cells
lm. longitudinal muscles
my. myoblast
n. nucleus
s. spine
sb. secretion body


A
m
ec
cc
sb
2·0um


B
sb2
sb3
sb4
ec
my


D
cc
sb


C
cr
s
mt
pgd
2·0µm

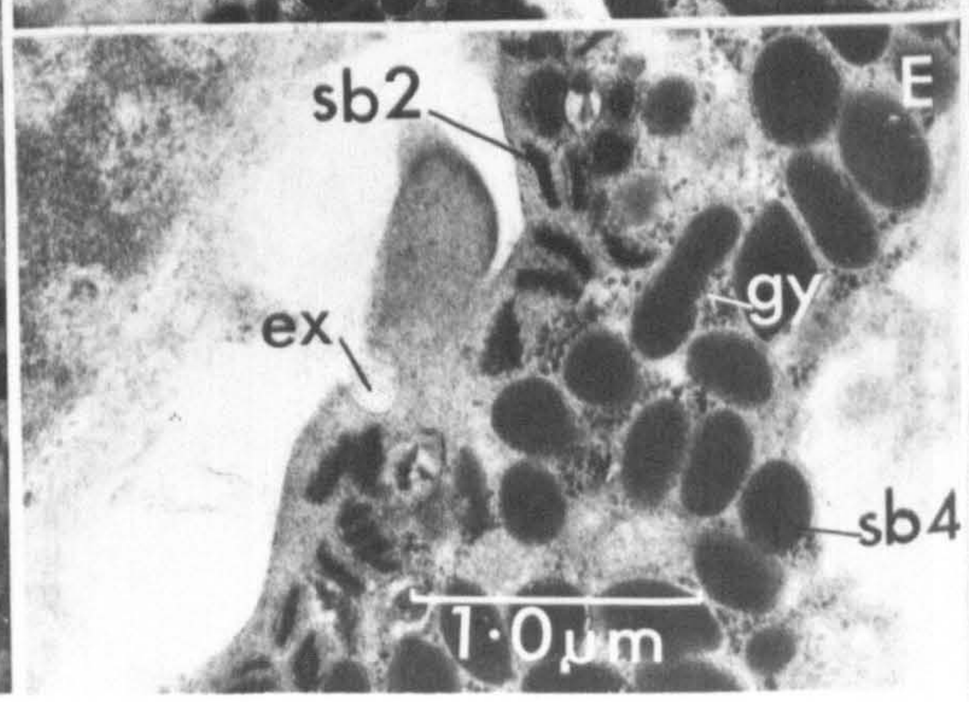

E
sb2
ex
gy
sb4
1·0µm

PLATE IO

Ultrastructure of the metacercarial surface and associated gland cells I5 minuites after penetration.

A. Tegument containing membrane bound secretion bodies and the underlying epidermal cell bodies (ec), and cystogenic gland cells with their contained secretion bodies (sb), connected with the epidermis by a protoplasmic process containing a mitochondrion.
X I2,646

B. Tegument containing the three types of membrane bound secretion bodies sb2,sb3, and sb4.The tegument rests on the circular and longitudinal muscles of the body wall below which are the myoblasts (my), and the epidermal cell bodies.
X I2,646

C. Section through the anterior metacercaria with the microtubule lined penetration gland ducts.The chromatophore (cr), associated with the metacercarial surface shows the lytic effects of the metacercarial tegumental and glandular secretions.
X I2,646

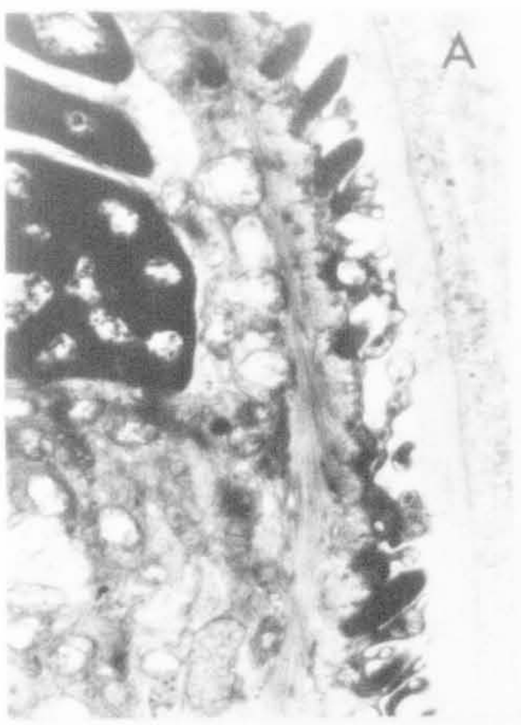
D. Tegument with secretion bodies and part of cystogenic gland cells.
X I2,646

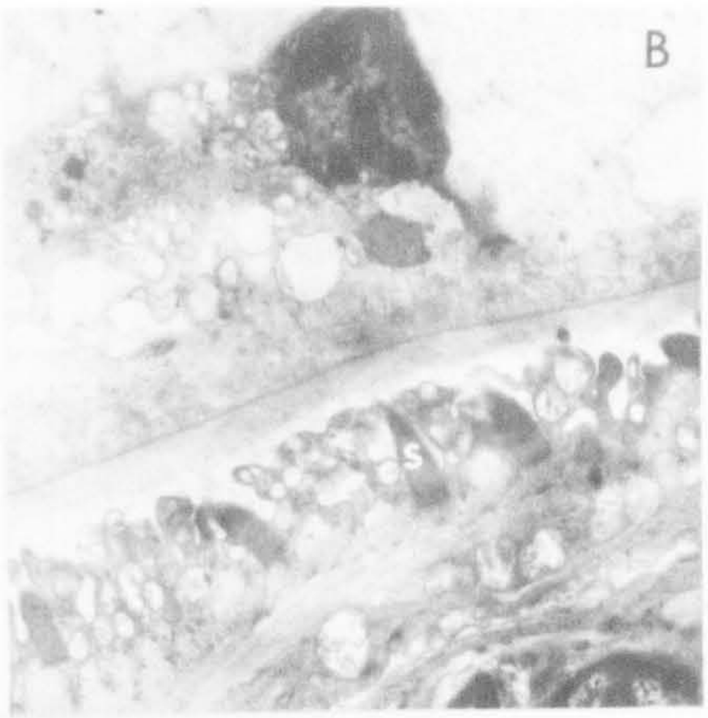
E. Detail of the tegument with the sb2 and sb3 secretion bodies aligned near the outer plasma membrane and the sb4 secretion bodies near the inner plasma membrane. Glycogen was also present among the secretion bodies, and the outer plasma membrane showed depressions (ex), in the surface at points where secretion bodies had fused with the outer plasma membrane.
X 20,672

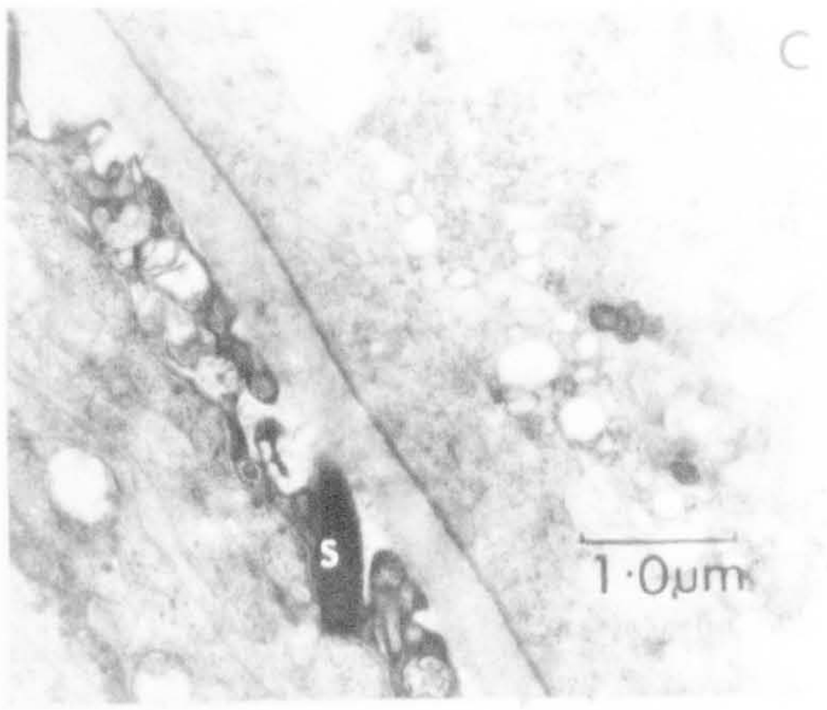
cc. cystogenic gland cells
ec. epidermal cell body
ex. point at which secretion body fused with membrane

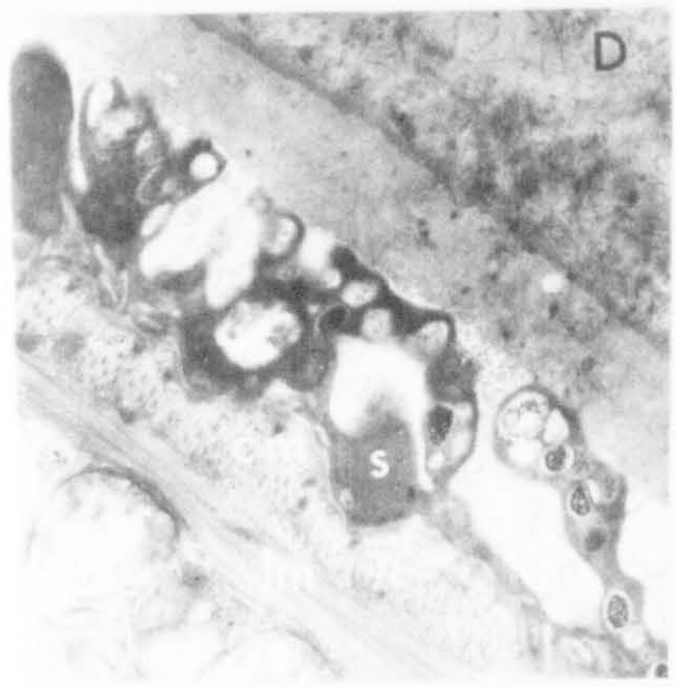
PLATE IO (continued)

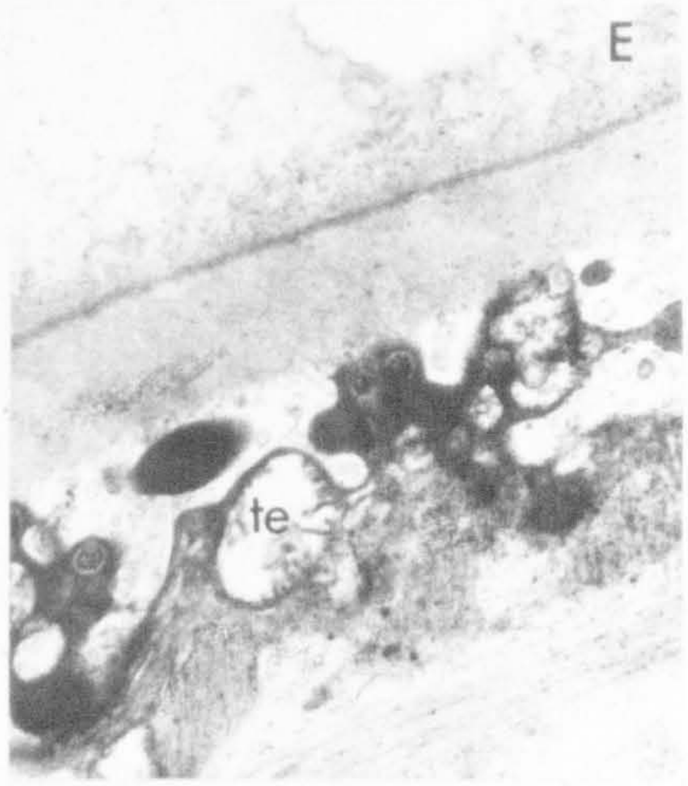
gy. glycogen
m. mitochondrion
my. myoblast
mt. microtubules
pgd.penetration gland cell
sb. secretion body
sb2.small elongated bodies with fibrillar contents
sb3.oval or rounded bodies with fibrillar contents
sb4.oval homogeneous electron dense bodies.

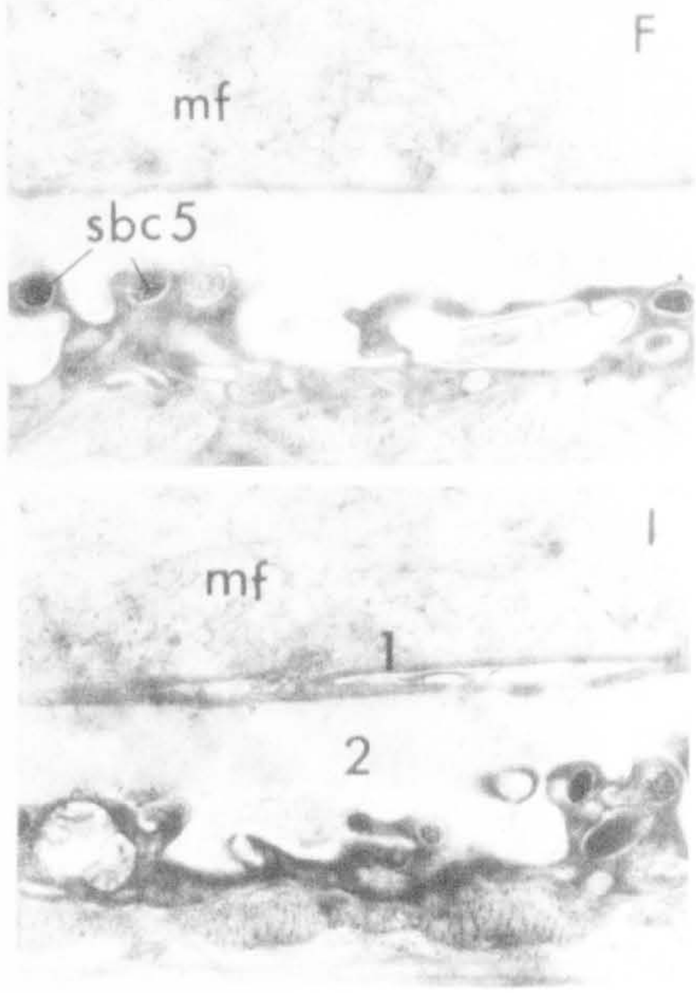

A

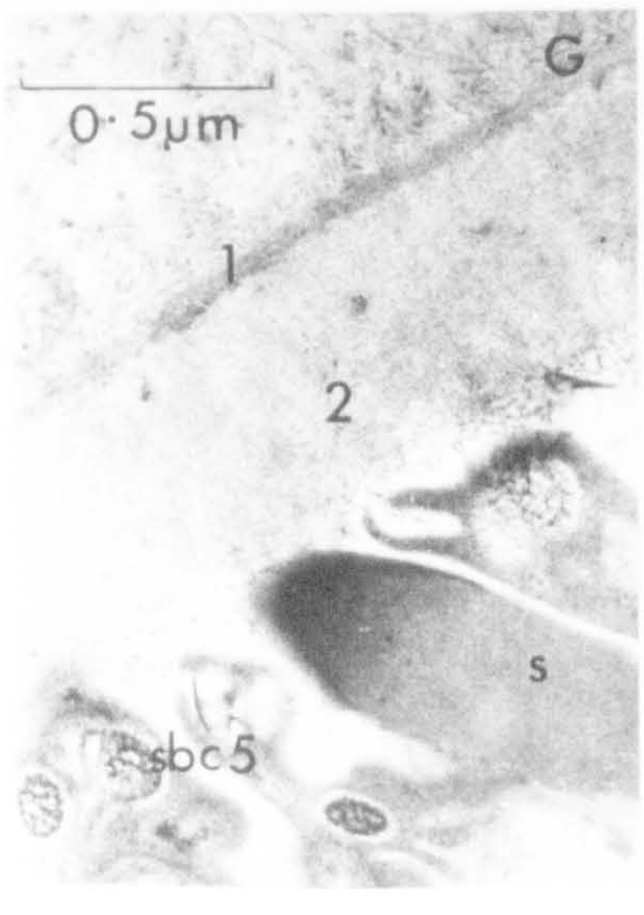

B
s

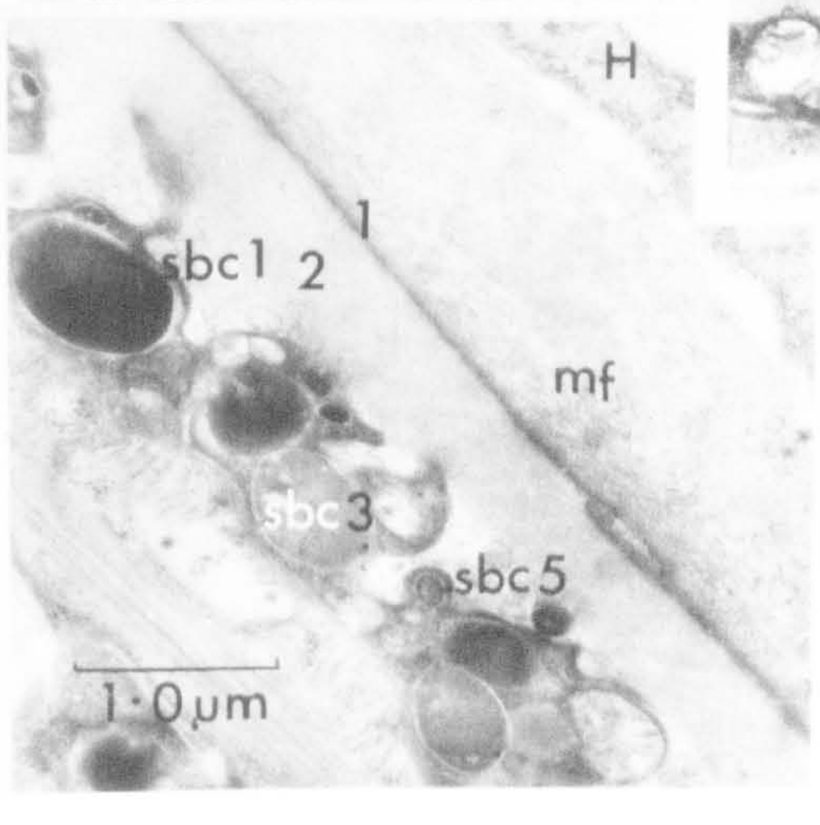

C
s
1·0µm


D
s


E
te


F
mf
sbc 5
I
mf
1
2


G
0·5µm
1
2
s
sbc 5


H
1
sbc 1
2
mf
sbc 3
sbc 5
1·0µm

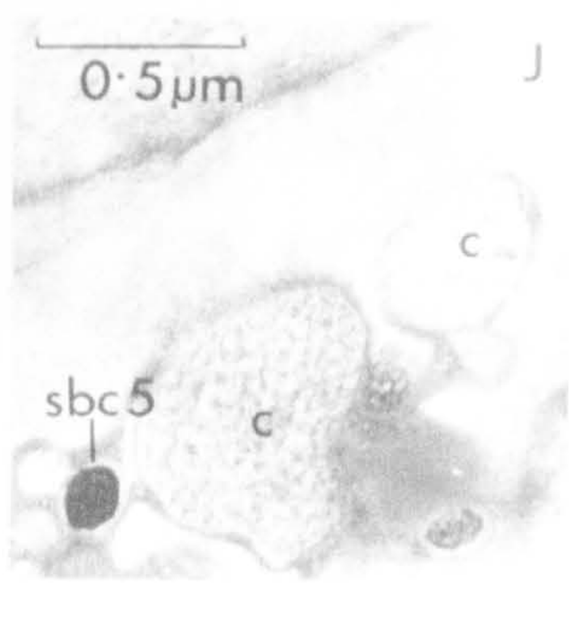

0·5µm
J
c
sbc 5
c

PLATE I6 (continued)

J. Detail of the inner layer of the parasitic cyst with sbc5 secretion body moving through it.
X 27,302

h. host tissue
le. leucocyte
mf. microfilaments
mr. melanophore
pcs.parasitic cyst space
ps. metacercarial surface
sbc.secretion from cystogenic gland cell
my. myoblast
ve. fusion of material with parasitic cyst.

PLATE I6

Ultrastructure of the metacercarial surface I day post penetration, showing breakdown of the tegument.

A. Metacercaria lying within the parasitic cyst the tegument having broken down.
X 2,699

B. Surface of the metacercaria (ps), completely disorganised within the parasitic cyst.
X 3,286

C. Part of the metacercarial surface with the tegument still intact. Secretion bodies from the cystogenic gland cells (sbc), are moving into the tegument.
X 4,044

D. Tegument almost completely broken down with the underlying muscles of the body wall exposed yet enclosed within the parasitic cyst.
X 5,056

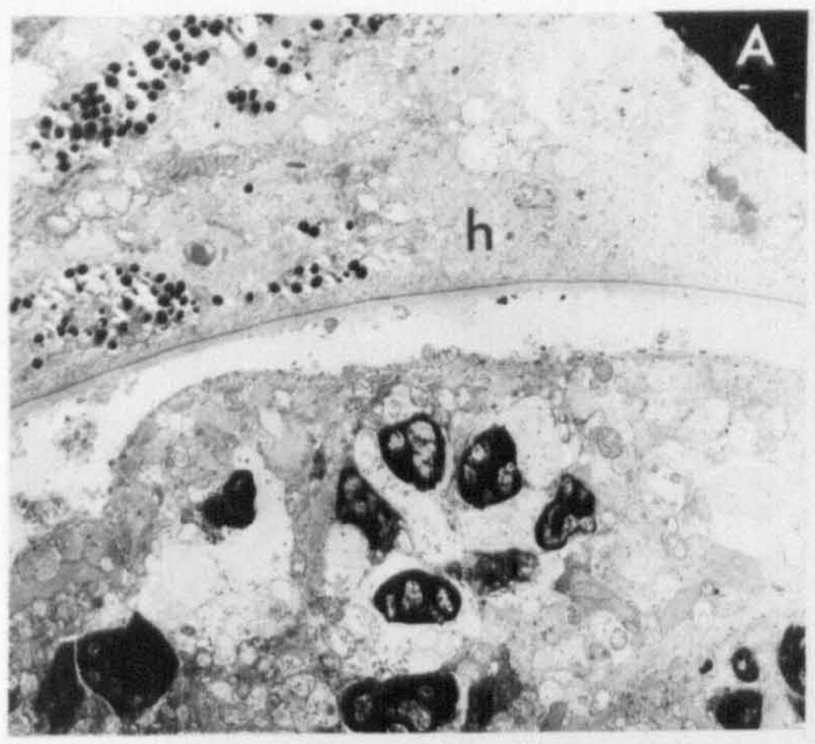
E. Parasitic cyst containing the metacercaria and host leucocytes associated with the parasitic cyst externally
X 4,044

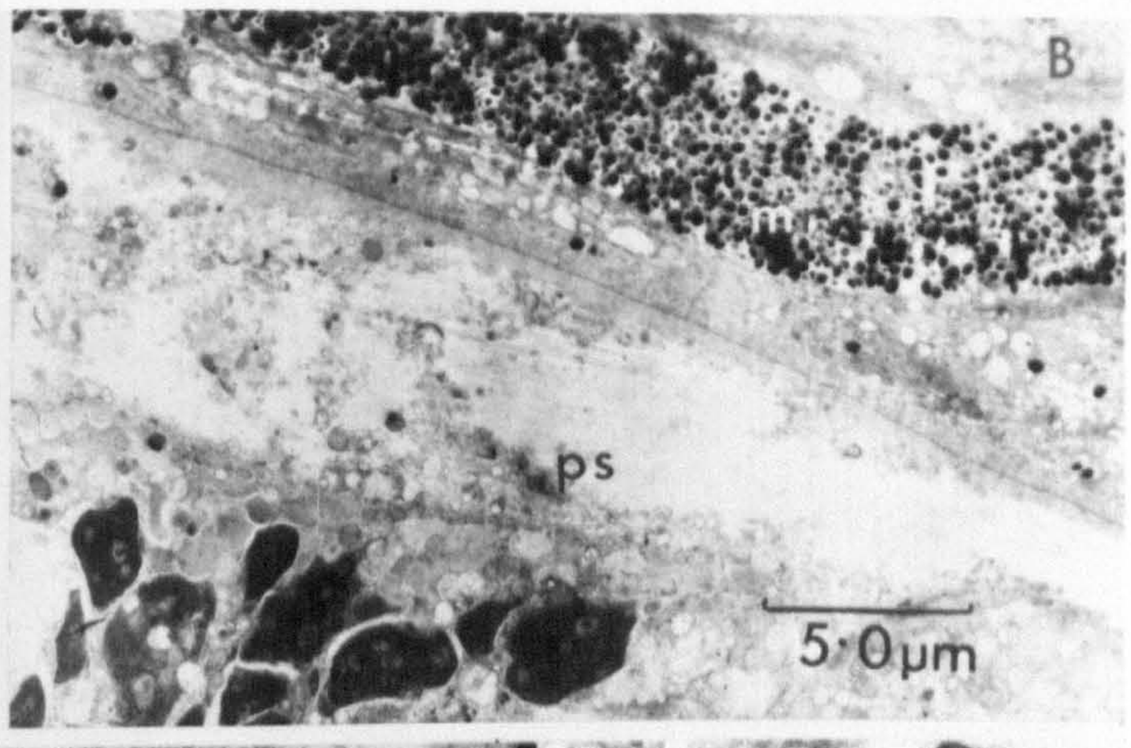
F. Metacercaria within the parasitic cyst and host leucocytes migrating towards it.
X 4,044

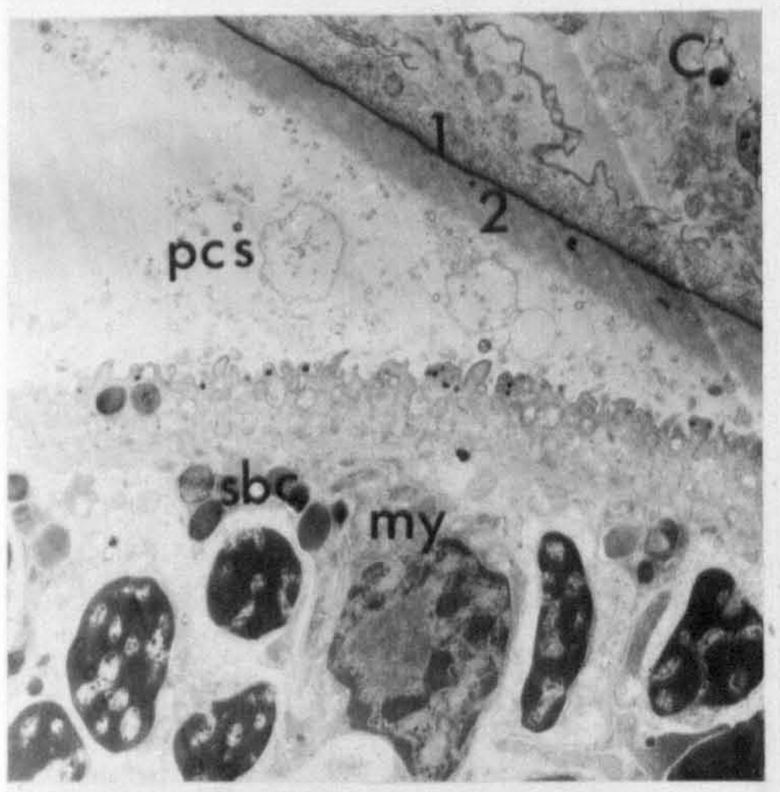
G. Detail of the surface of the metacercaria with sbc3 secretion bodies from the cystogenic gland cells moving into the ill defined tegument. Material appears to be added to the inner surface of the parasitic cyst forming loops on the inner surface. Host leucocyte adhering to the outer surface of the parasitic cyst.
X 7,786

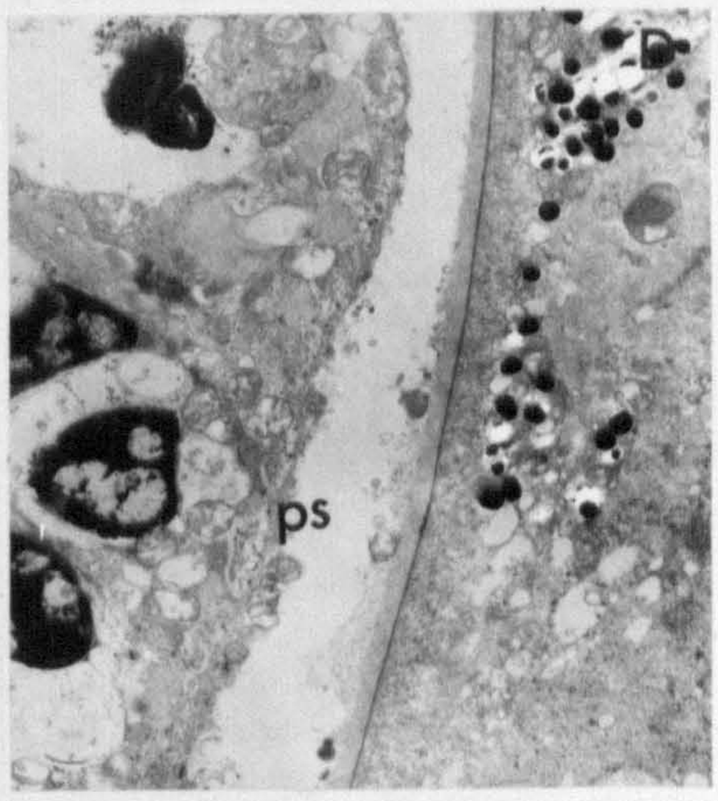
H. Metacercarial surface denuded of the tegument . Host leucocytes associated externally with the parasitic cyst.
X 7,786

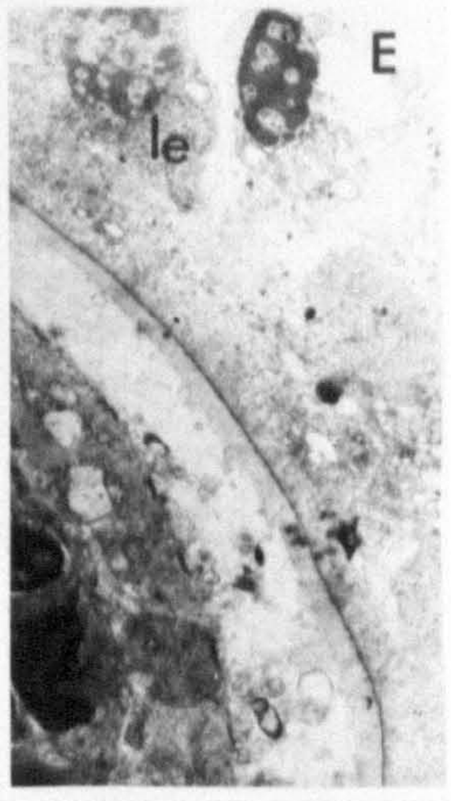
I. Two layered parasitic cyst with microfilaments (mf), adhering to the outer surface of the parasitic cyst.
X 7,786

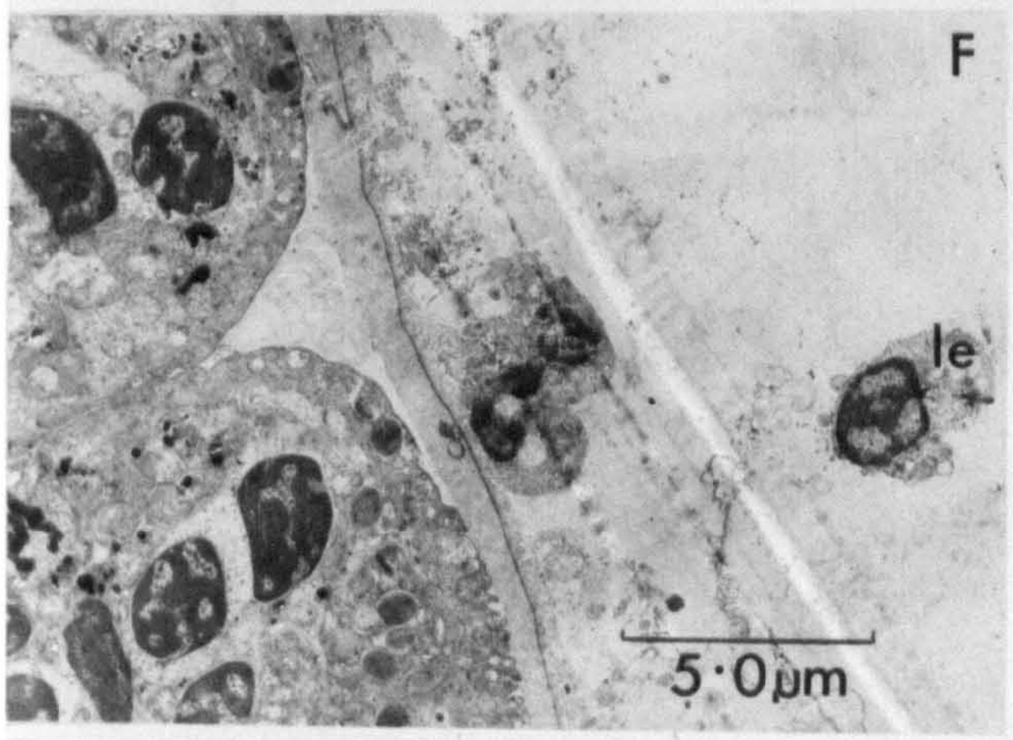

A
h

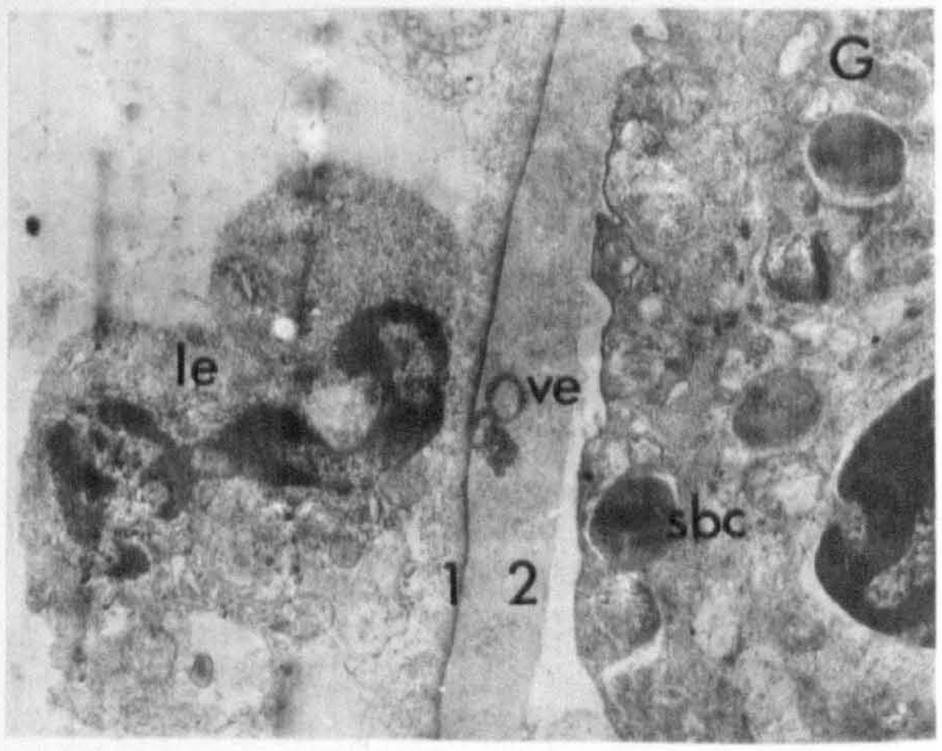

B
ps
5·0μm

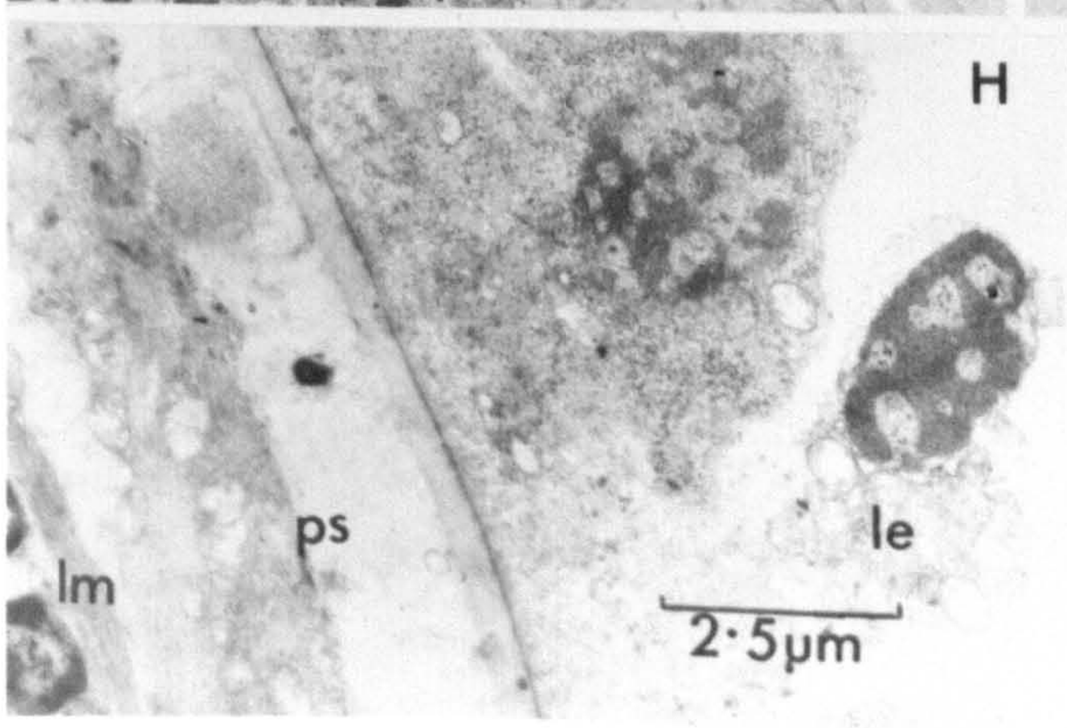

C
1
2
pcs
sbc
my

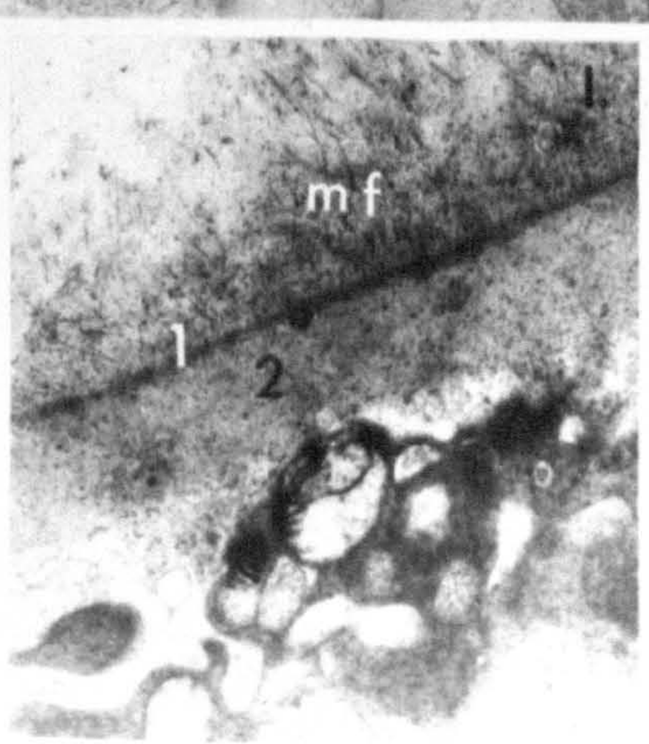

D
ps


E
le


F
le
5·0μm


G
le
ve
sbc
1
2


H
ps
le
lm
2·5μm


I
mf
1
2


1 J
2
te

PLATE I7

Ultrastructure of the metacercarial surface and host reaction I4 days post infection.

A. Section through the metacercarial cyst which is composed of the two acellular layers of the parasitic cyst and externally the layer of collagen fibrils. The metacercarial tegument (te), has numerous fine protoplasmic extensions.
X I0,882

B. Metacercarial surface showing the fine protoplasmic extensions of the tegument.
X I3,606

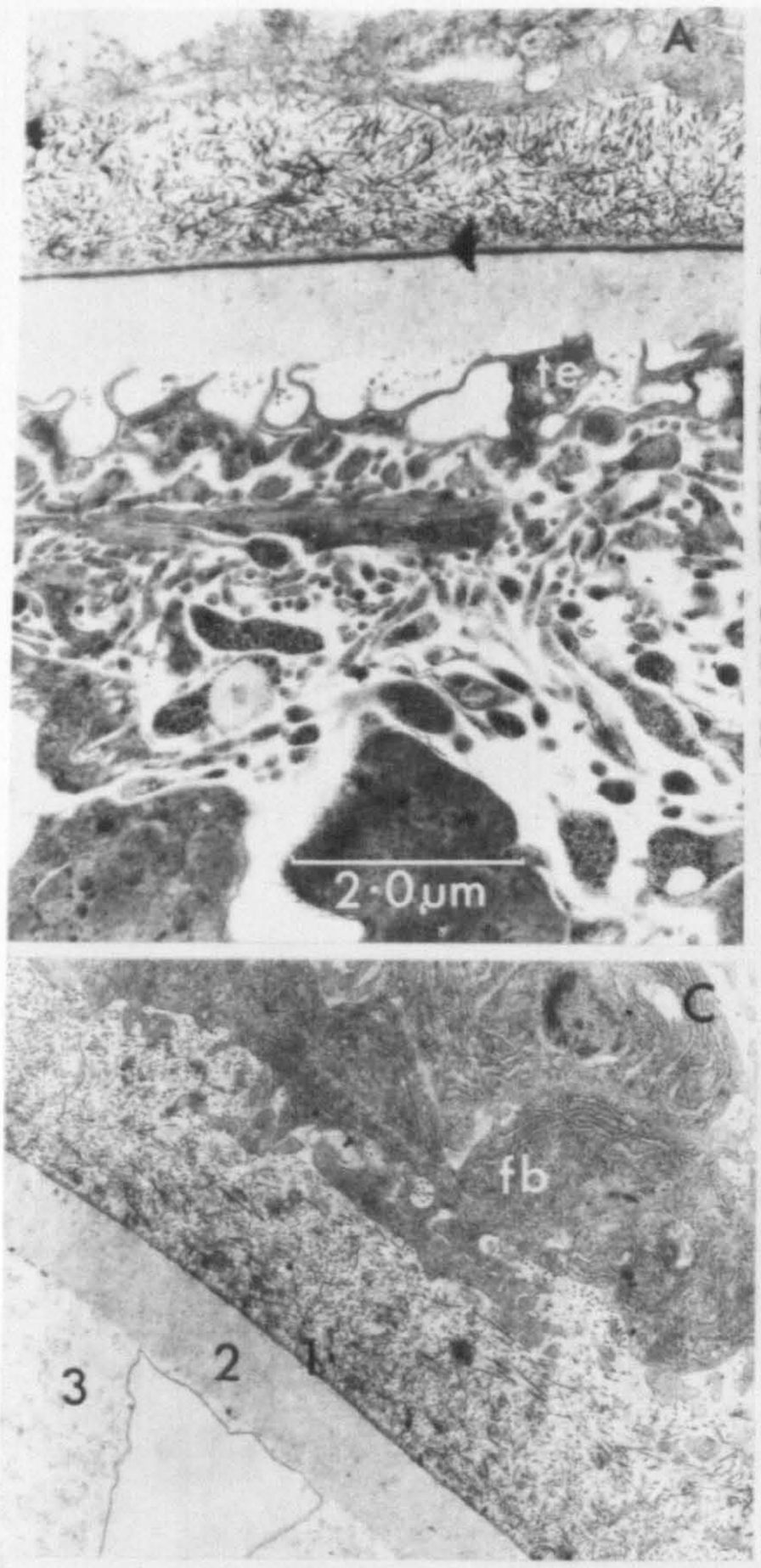
C. Fibroblasts (fb), associated with the external surface of the parasitic cyst, actively synthesising collagen. Parasitic cyst appears three layered the inner flocculent material (3), probably from fixation of the cyst fluid.
X 8,057

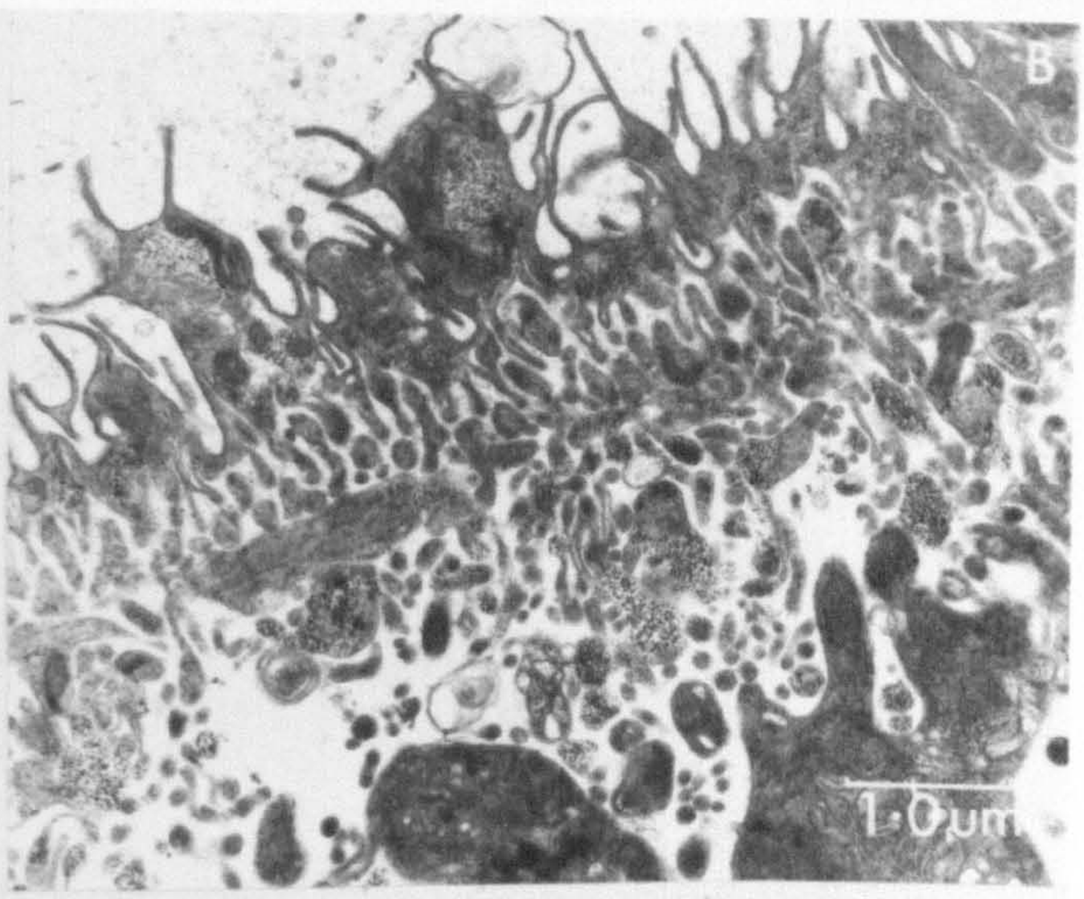
D. Detail of the tegument (te), with fine protoplasmic extensions and containing glycogen (gy), mitochondria (m), but no spines or inclusion bodies. Underlying tissues appear to be undergoing extensive metamorphosis.
X 27, 302

E. Parasitic cyst with the two acellular layers (I,2), and the associated host fibroblasts (fb), and collagen fibres (col).
X 27,302

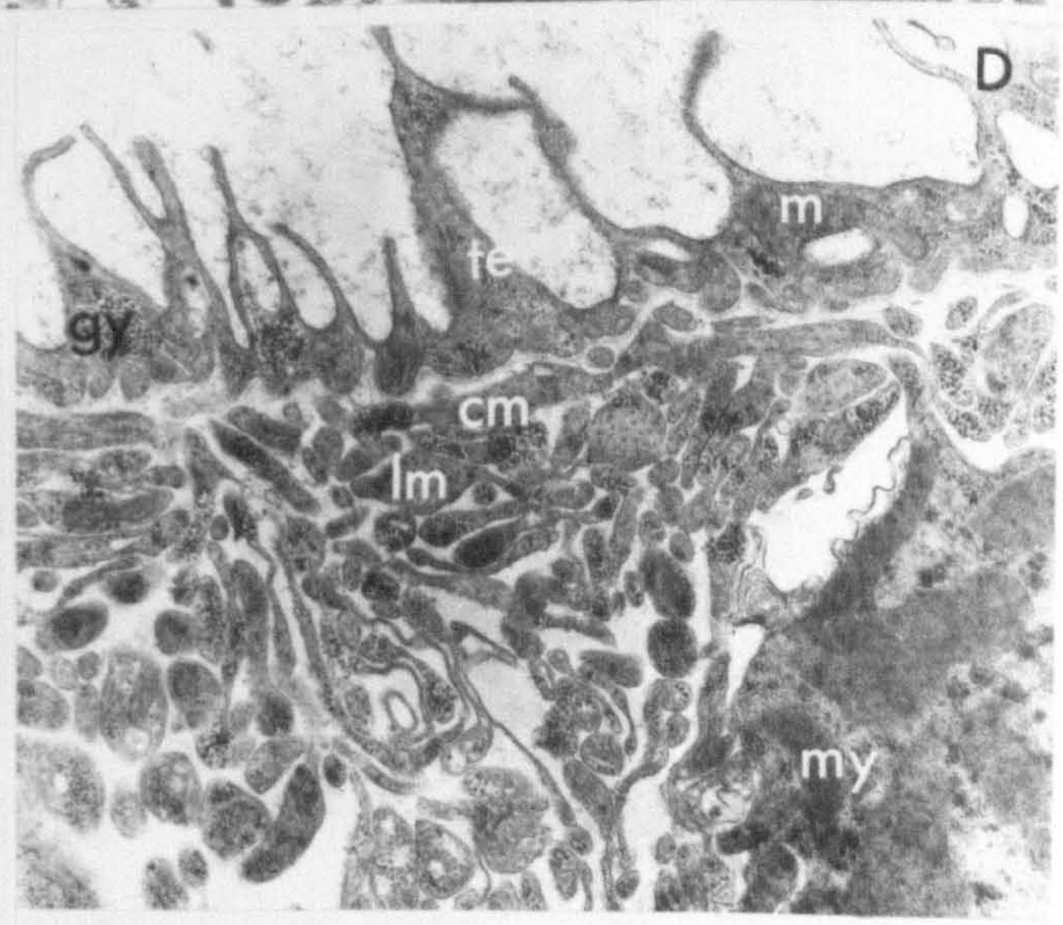
F. Parasitic cyst with externally associated host fibroblast with extensive granular endoplasmic reticulum .
X I3,603

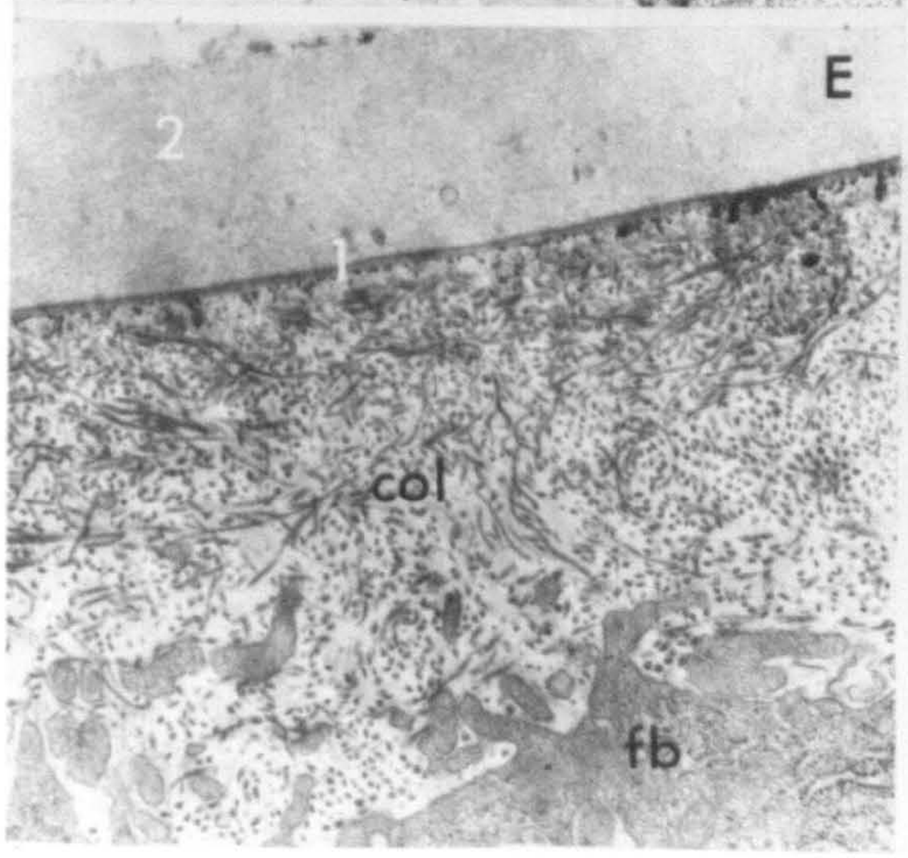
col.collagen fibres
fb. fibroblast
gy. glycogen
te. tegument


A
te
2·0μm


B
1·0μm

C
fb
3
2
1


D
m
te
gy
cm
lm
my


E
2
1
col
fb

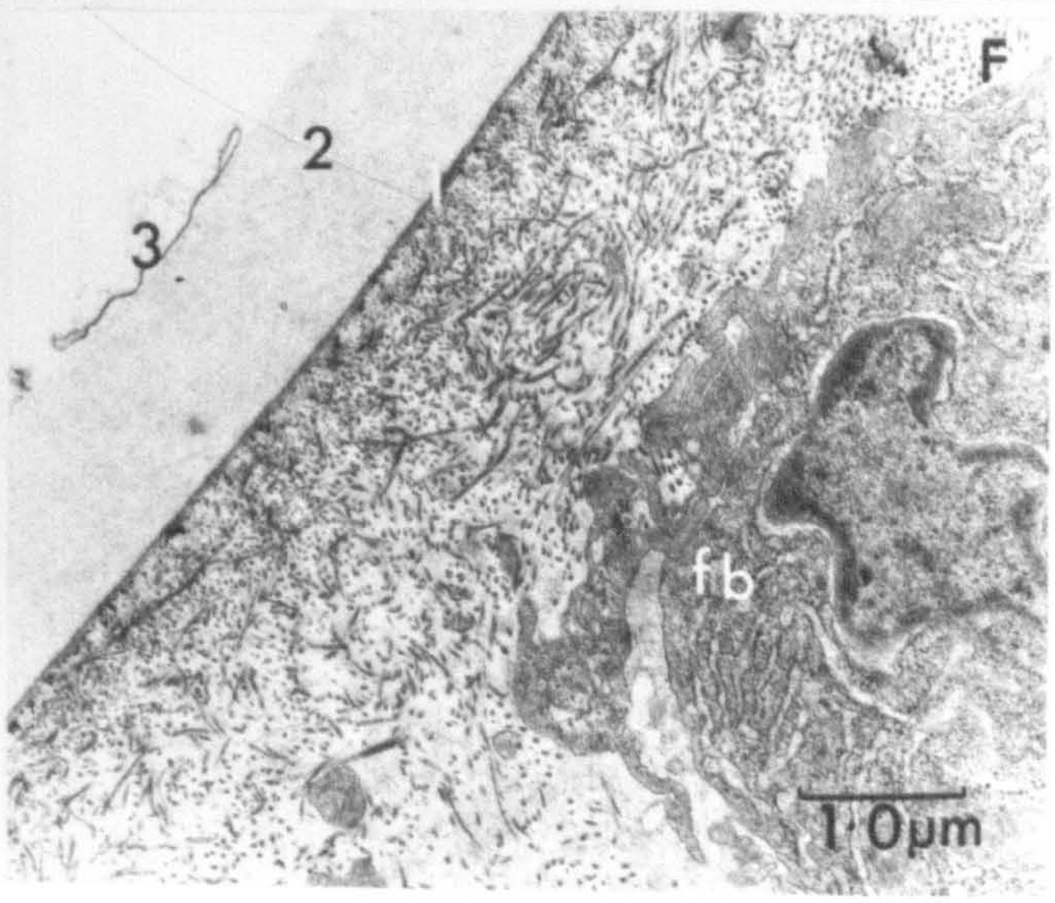

F
2
3
fb
1·0μm

PLATE I8

Ultrastructure of the metacercarial surface and associated host reaction 2I days post infection.

A. Section through the metacercarial cyst showing the metacercaria and the fluid filled space between it and the cyst wall.
X 2,336

B. Metacercarial cyst with the two acellular layers (I,2), and the flocculent inner material (3), which represents the fluid contained within the cyst.
X 4,376

C. Parasitic cyst with the two acellular layers (I,2), the inner flocculent material (3), and the surface of the metacercaria .
X 9,I02

D. Metacercarial cyst with the inner flocculent material containing some electron dense particles. Protoplasmic extensions of the tegument contain secretion bodies designated type sb6.
X I8,8I6

E. Metacercarial surface containing sb6 secretion bodies and spines.
X I4,878

F. Section of tegument containing spines.
X I4,878

G. Parasitic cyst and host reaction of collagen fibres.
X 9,I02

H. Collagen fibres and fibroblasts associated with the external surface of the parasitic cyst.
X II,377

I. Detail of the parasitic cyst showing the granular nature of the inner parasitic cyst and the electron dense deposit on the outer surface of the cyst. This resembles the flocculent material seen within the cyst and may be excretory material.
X 23,630

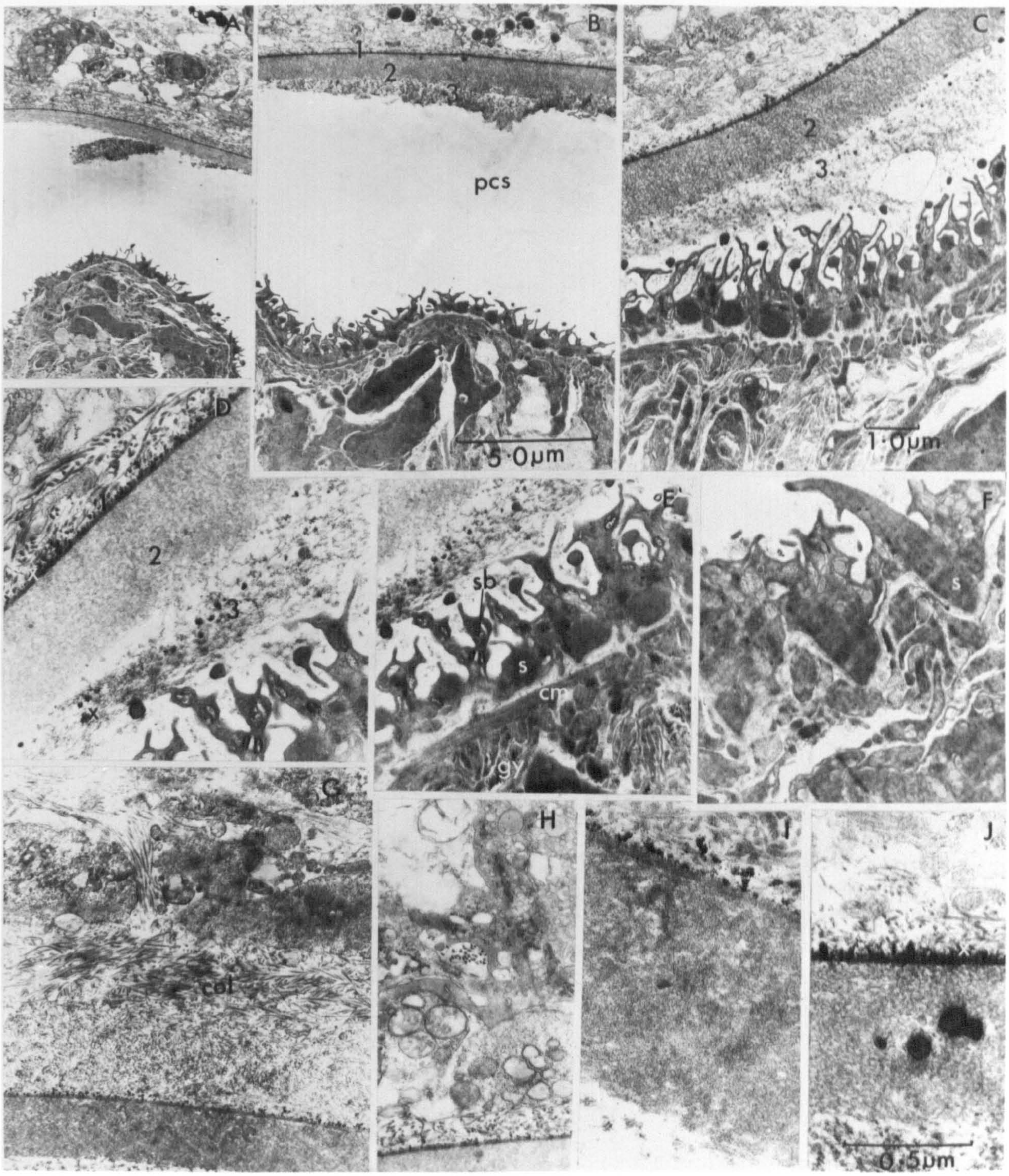

A
B
1
2
3
pcs
5·0μm
C
2
3
1·0μm
D
2
3
x
E
sb
s
cm
gy
F
s
G
col
H
I
J
x
0·5μm

PLATE I9 (continued)

J,K.
lucent layer. The outer surface clearly shows the granular electron dense deposit, which may be excretory material.
X 45,I00

ec. epidermal cell body
ed. electron dense deposit
m. mitochondrion
pp. protoplasmic process
sb6. oval secretion body with irregular electron dense and electron lucent structure
sb7. round secretion body with electron dense core and cartwheel radii
te. tegument

PLATE I9

Ultrastructure of the metacercarial tegument, cyst wall and host reaction 38 days post infection

A. Section through the metacercaria and cyst wall with the two acellular layers (I,2), and the encapsulating collagen deposition.
X 6,43I

B. Section through the metacercarial cyst with the epidermal cell bodies connected with the tegument.
X 6,43I

C. Tegument containing two types of secretion body produced by one type of epidermal cell.
X 2,505

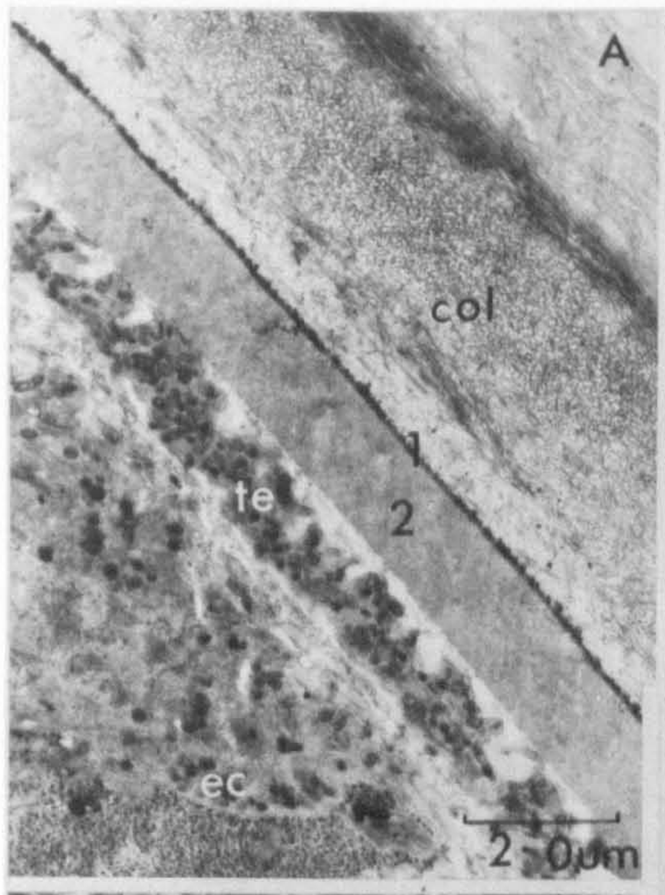
D,E,F.
Structure of the parasitic cyst showing the two acellular layers (I,2), with a deposition of irregular electron dense material on the outer surface of layer I. The tegument rests on fibrous interstitial material (f), and contains two types of secretion body.
X 22,550

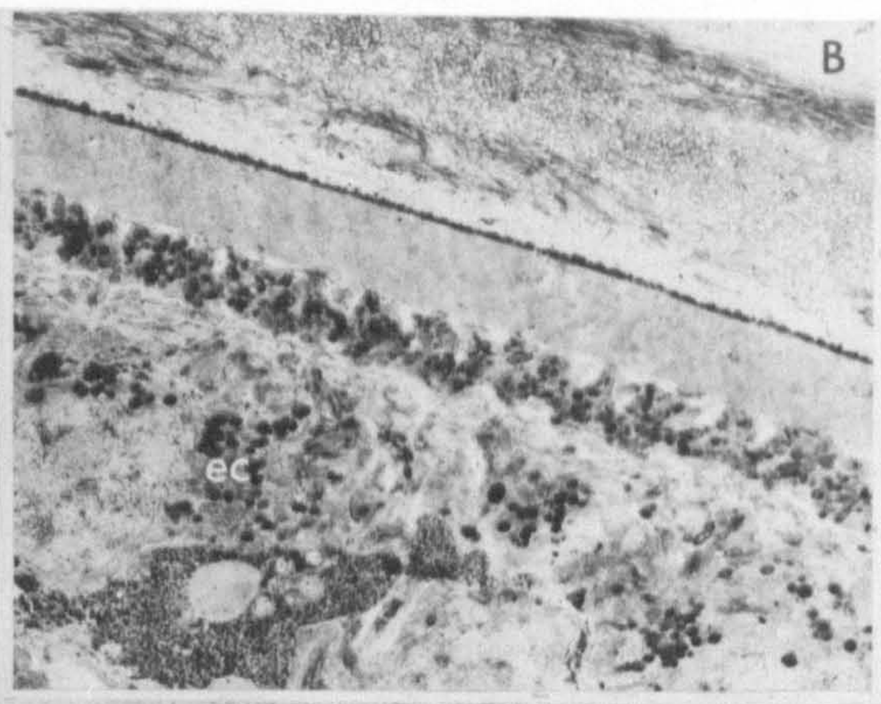
G. Detail of the two acellular layers of the parasitic cyst with the electron dense deposit (ed), on the outer surface.
X 29,232

H. Fibroblast and associated collagen deposition (col), around the parasitic cyst.
X I0,857

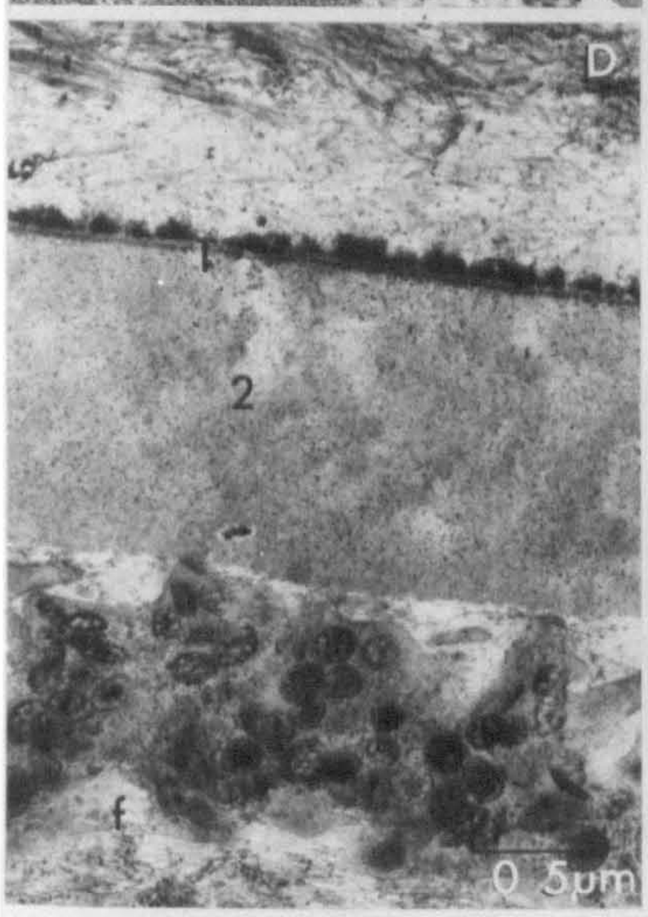
I. Secretion bodies within the tegument are of two morphological types designated sb6 and sb7. Protoplasmic processes connect the tegument with the underlying cell bodies with the tegument.
X 36,748

J,K.
Detail of the outer surface of the parasitic cyst layer I having a trilaminate structure with two more electron dense layers bounding an electron

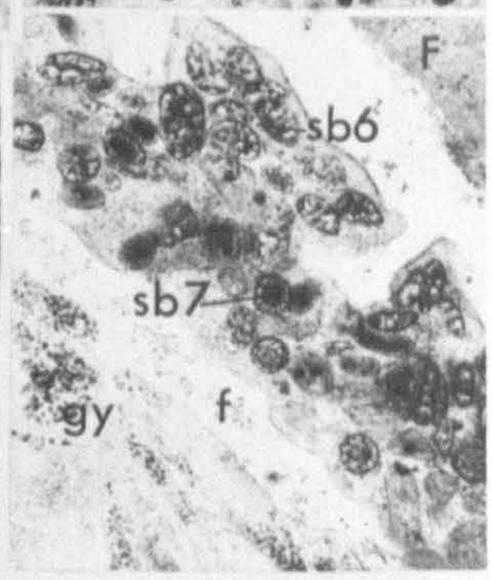

A
col
1
2
te
ec
2·0µm

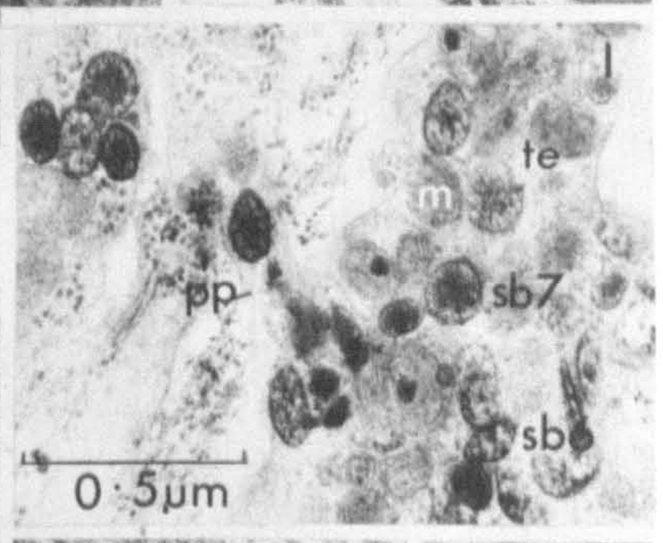

B
ec

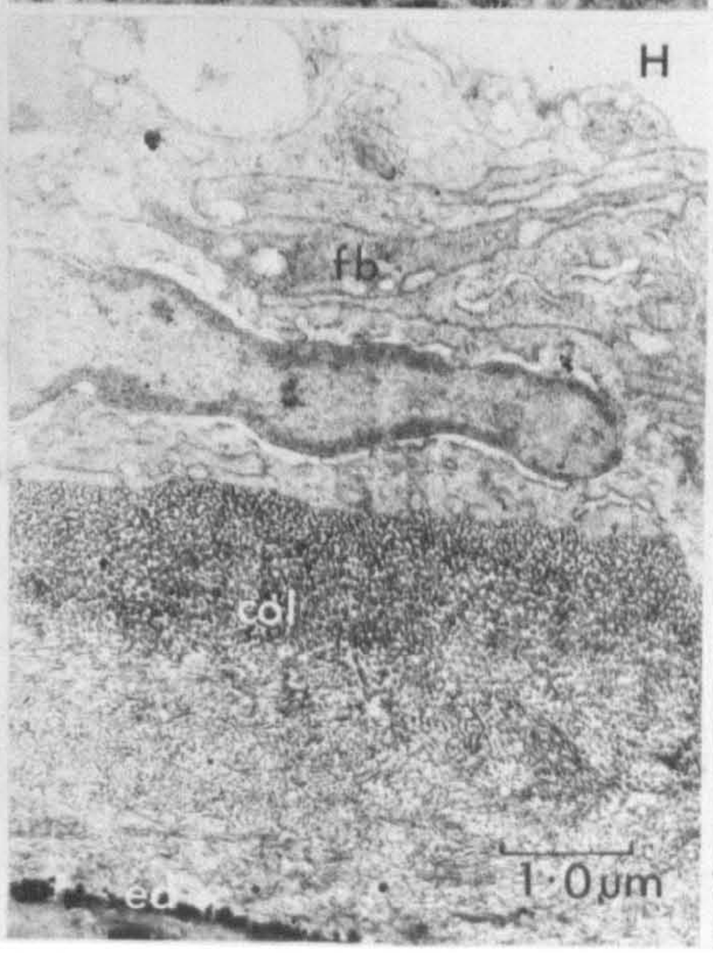

C
ec

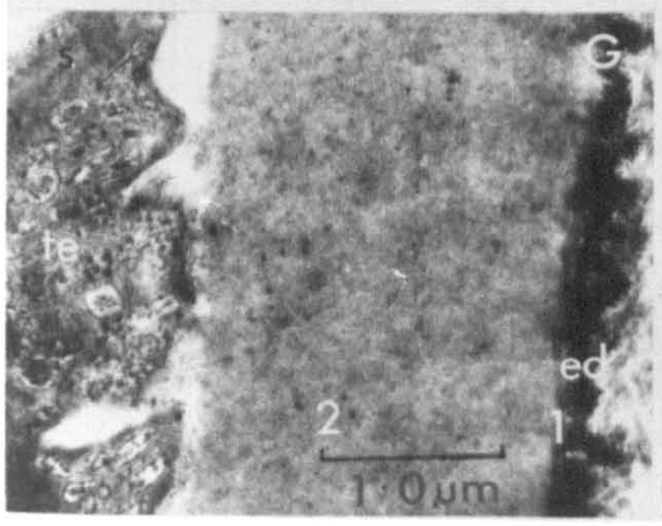

D
1
2
f
0·5µm

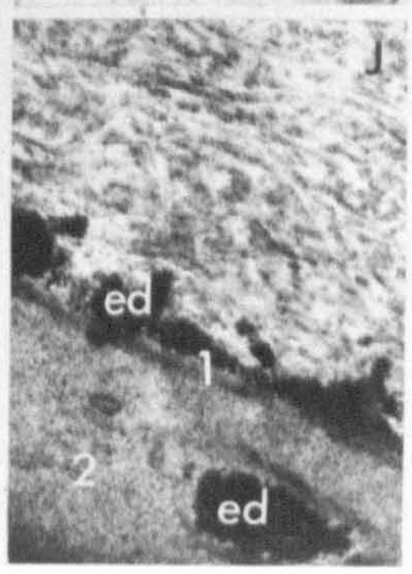

E
bl
f


F
sb6
sb7
gy
f


I
te
m
pp
sb7
sb
0·5µm


H
fb
col
ed
1·0µm


G
te
ed
2
1
1·0µm


J
ed
1
2
ed


K
ed
1
2

PLATE 20 (continued)

K. Part of the secretion cell body containing the packed masses of secretion bodies.
X II,273

col. collagen
ed. electron dense deposit
ec. epidermal cell body
f. fibrous interstitial material
pp. protoplasmic process
te. tegument

PLATE 20

Ultrastructure of the metacercarial surface, parasitic cyst and associated host reaction 55 days post infection.

A. Section of the metacercarial cyst within the fin ray tissue of C.labrosus. The metacercarial cyst is encapsulated with collagen.
X 2,8I8

B. Metacercarial tegument , parasitic cyst and collagenous host capsule.
X 6,677

C. The two acellular layers of the parasitic cyst (I,2), and the flocculent material contained within the cyst (3), and the electron dense deposit on the outer surface of the parasitic cyst.
X II,273

D. Metacercarial tegument connected by a protoplasmic process (pp), to the underlying cell body.
X 9,0I8

E. Tegument clearly showing the lobular surface and the sb6 and sb7 secretion bodies. Mitochondria contain electron dense inclusions.
X I8,644

F. Metacercarial surface and parasitic cyst.
X I4,742

G. Detail of the outer surface of the parasitic cyst with the granular electron dense deposit on the outer surface (ed).
X 38,I56

H. Metacercarial tegument containing the sb6 and sb7 secretion bodies.
X I8,644

I. Parasitic cyst and metacercarial tegument connected by protoplasmic process (pp), to epidermal cell body.
X I8,644

J. Tegument with sb6 and sb7 secretion bodies.
X I8,644

55 days.

Plate 12
Plate 20

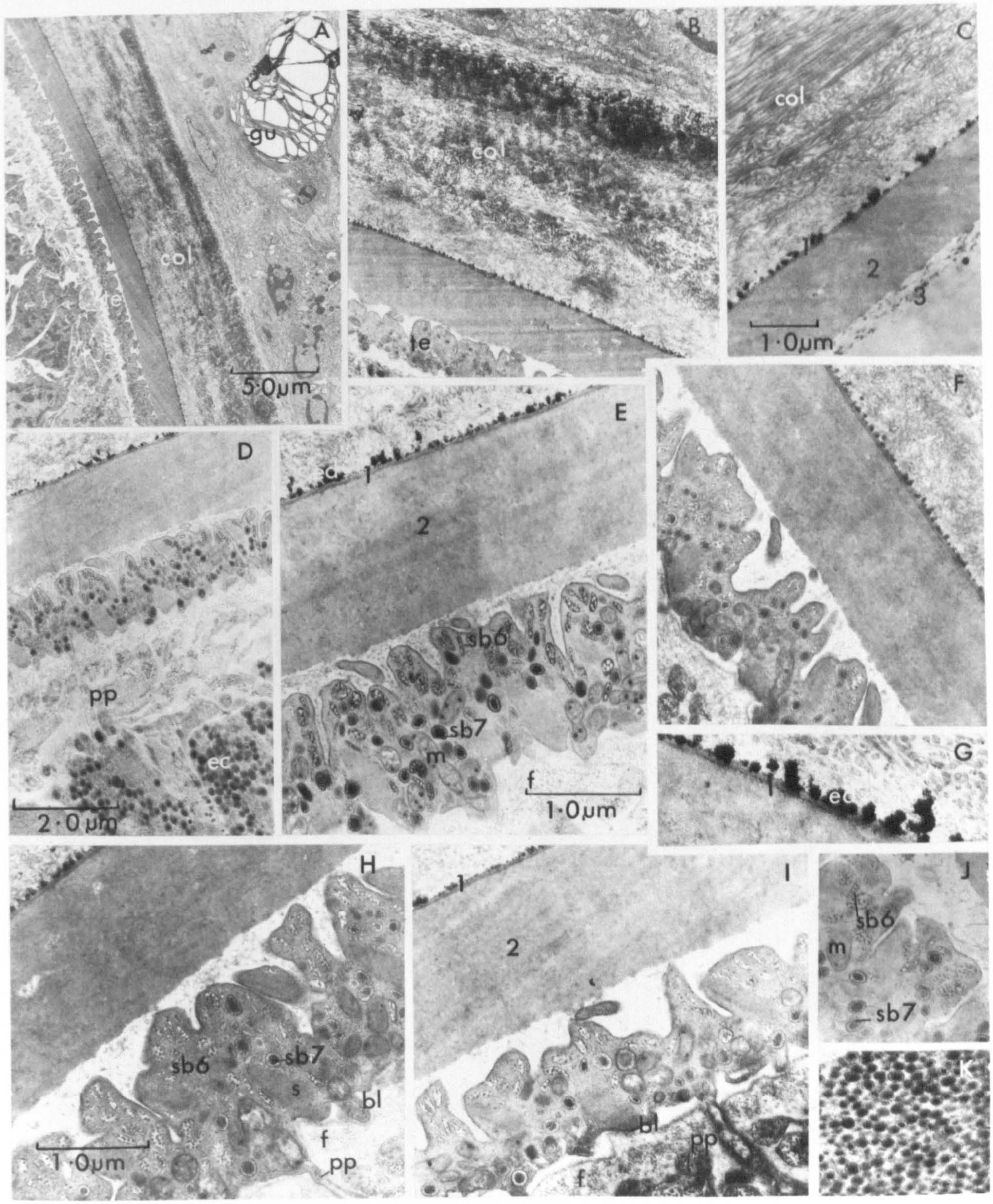


PLATE 2I

Ultrastructure of the metacercarial surface, parasitic cyst and host reaction of naturally infected plaice.

A. Section of metacercarial cyst showing the tegument with spines (s), the sb6 and sb7 secretion bodies, the fluid filled space within the parasitic cyst (pcs), and the acellular cyst wall and the electron dense material on the outer surface.
X IO,6I6

B. Host encapsulation of the metacercarial cyst with the fibrous capsule of collagen fibres and the melanin granules (mg), external to the collagen.
X 3,3I7

C. Structure of the parasitic cyst with the flocculent contents of layer 3, the amorphous acellular layer 2, and the outer acellular layer I which has become detached during preparation.
X 2I, 947

D. Collagen fibres and interspersed melanin granules of the outer host capsule.
X 5,I04

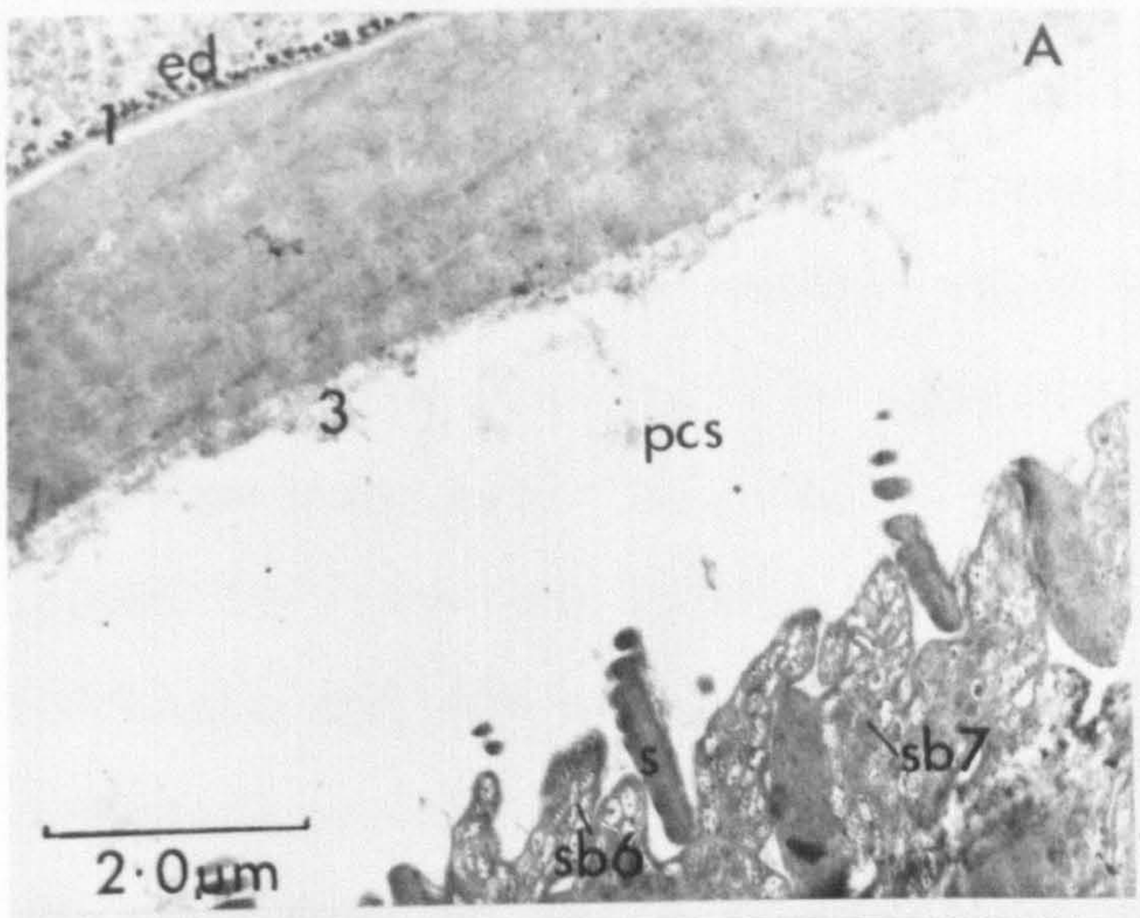
E. Collagen fibres deposited around the parasitic cyst.
X 5,I04

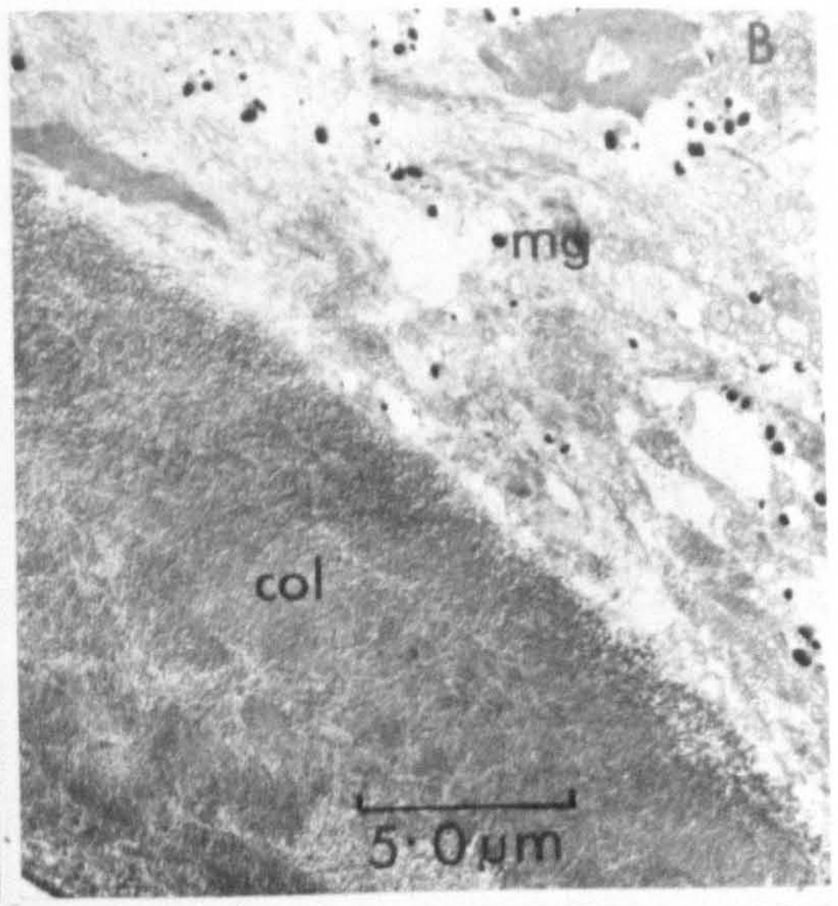
F. Melanophores containing melanin granules from the outer host capsule. Guanophores associated with the melanophores, guanin crystals lost during preparation
X IO,6I6

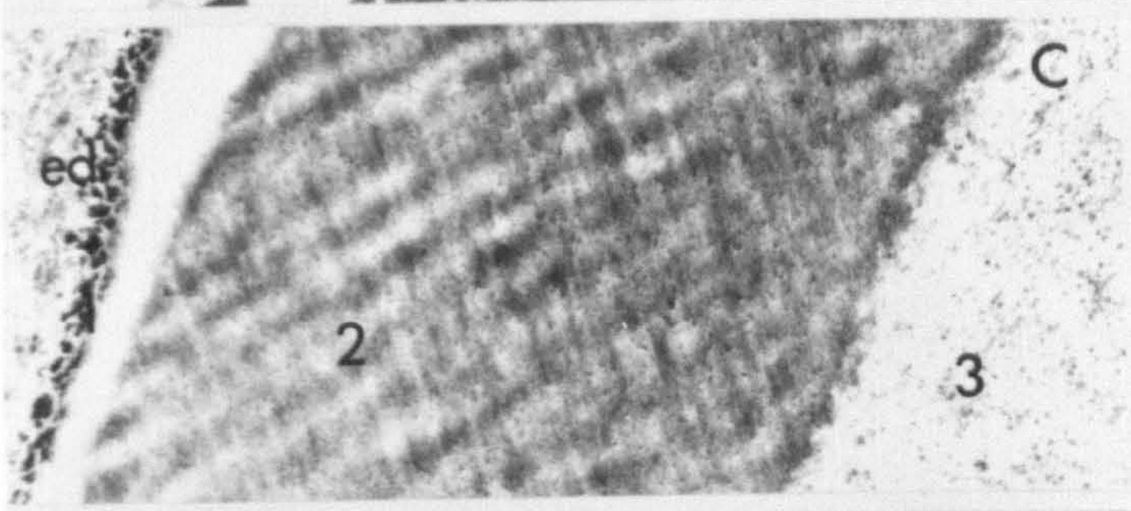
G. Detail of the melanin granules and collagen fibres of the outer host reaction.
X IO,6I6

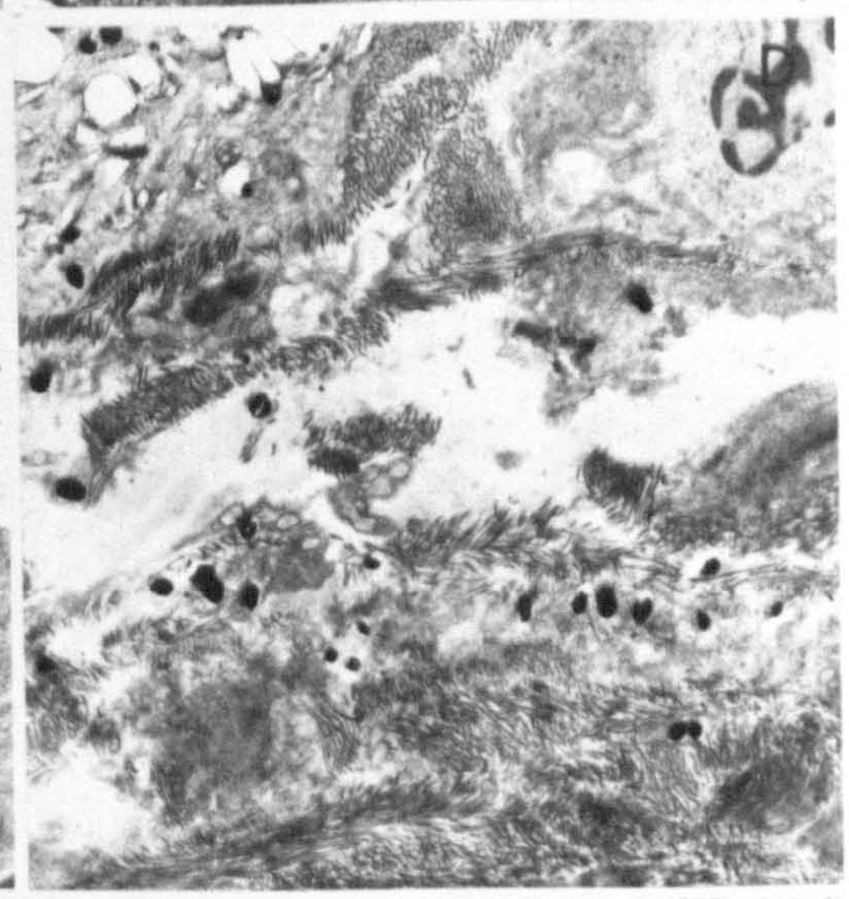
H. Collagen fibres, melanin granules and guanophore of the outer host capsule.
X I7,353

col. collagen
ed. electron dense deposit
gu. guanophore
mg. melanin granule
n. nucleus
pcs. parasitic cyst space
sb. secretion body

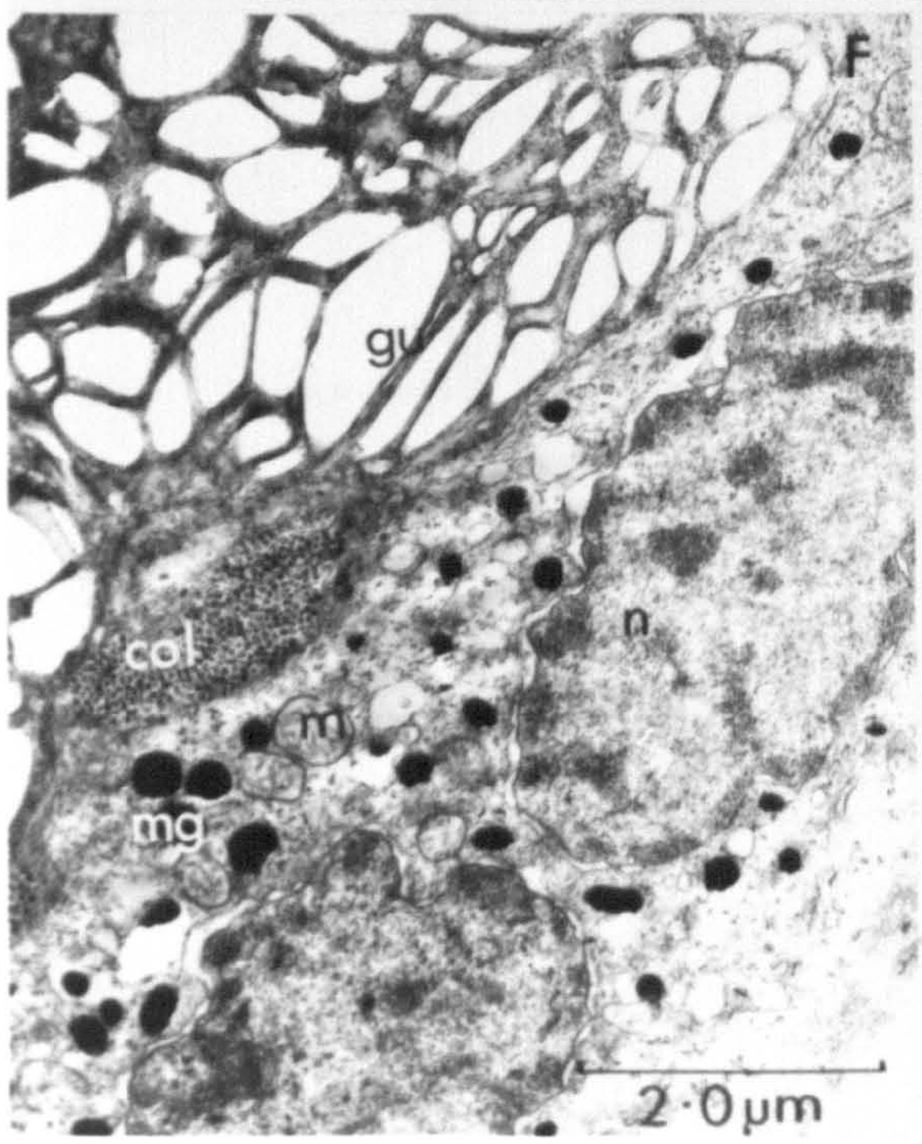

A
ed
3
pcs
s
sb7
sb6
2·0µm

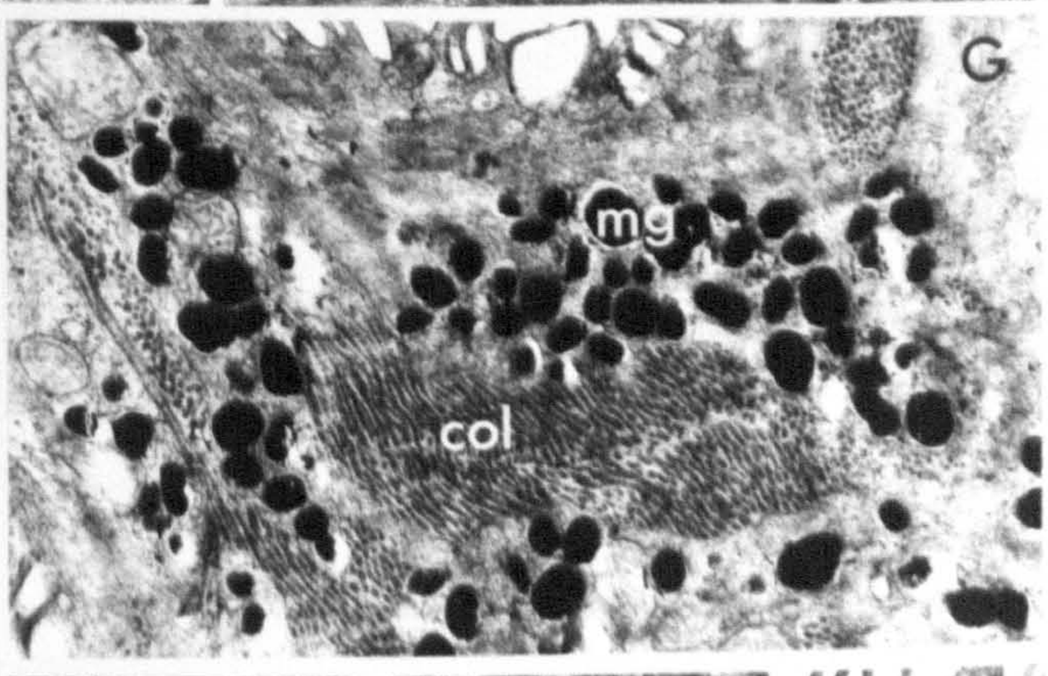

B
mg
col
5·0µm

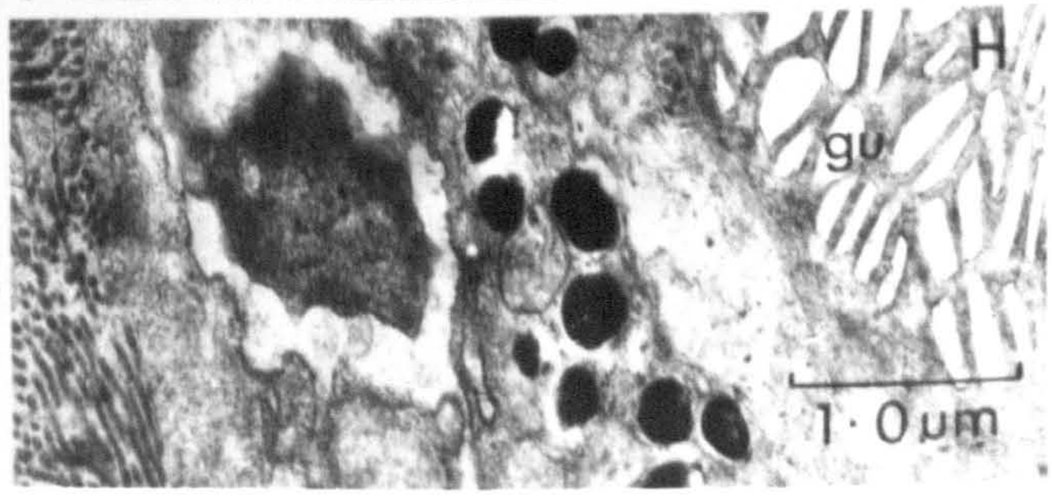

C
ed
2
3


D


E
col
1


F
gu
col
n
m
mg
2·0µm


G
mg
col


H
gu
1·0µm

(b) Rhipidocotyle johnstonei nom. nov.

The metacercariae occur throughout the body musculature and connective tissues, in the interspinal zone at the base of the ventral dorsal and caudal fins of '0' group and in the fins of '1' group plaice. The parasites are clearly visible through the body wall (Pl. 22A). They have previously been described by Johnstone (1905), and Matthews (1968), who found haemorrhages and fin erosion associated with the infection. The parasite survives approximately 10 months and may produce 10,000 eggs some of which escape into the surrounding host tissues Matthews(1968), showed that infections were seasonal, appearing in early June in the '0' group population, off the west coast of Wales and in May from Broadsands Bay, S. Devon.

It was not possible to infect plaice experimentally with R. johnstonei nom. nov. as the first intermediate host in the life cycle was unknown. However '0' group fish become infected shortly after metamorphosis, when as young flatfish they inhabit shallow, sandy inshore zones. It is therefore possible to detect the first infections in the juvenile population by regular netting between May and June.

Fish collected in August during the present study were estimated as having infections of about 3 months duration. At this stage the parasite was adult, producing eggs and completely encapsulated by host tissues, (Pl. 22B) the capsules being white, oval in shape and measuring 1.27 x 0.6 mm. Tubular capsules measuring up to 5.0 x 0.5 mm were occasionally found containing several parasites. The capsules are fluid filled and the parasites are free within them, there being no cyst of parasitic origin. The parasite is therefore able

to feed directly on the surrounding host tissues.

Metacercarial stages examined during the present ultrastructural investigation at 3 and 9 months post-infection revealed changes in the parasite surface and host reaction.

Parasite Surface. The surface of R. johnstonei is typically that of a digenean (Lyons 1977), in being a syncytium connected by protoplasmic processes to cell bodies (Pl. 22C, I). The tegument exhibited regional surface differences in that the epidermis covering the sucker and rhynchus did not bear spines (Pl. 22E, S) whereas the tegument covering the rest of the body surface bore conspicuous backwardly directed spines. They occupied the whole depth of the tegument originating immediately above the inner plasma membrane (Pl. 22P). The spines were serrated or comb-like (Pl. 22N, O) and covered by the outer plasma membrane (Pl. 22P). The spines displayed a crystalline structure and attachment plaques were present at the junction of the inner plasma membrane and basement lamina below the spines, represented by an electron dense zone at the base of the spine (Pl. 22P).

Other inclusions of the epidermis were mitochondria of medium size, some with electron dense inclusions (Pl. 22J). Two types of secretion bodies were identified on the basis of their morphology, and designated types Sb1 and Sb2. The former (Sb1) were electron dense rod-like bodies measuring 0.025 μm x 0.005 μm and orientated at right angles to the surface; while the latter (Sb2) were round to oval granular heterogeneous inclusions (Pl. 22P, R) 0.16 μm x 0.32 μm in size found throughout the epidermis. These secretion bodies originated within the cytoplasm of the same cell (Pl. 22C).

A characteristic feature of the outer plasma membrane of R. johnstonei was a surface coat of electron dense material which measured 0.005 µm in thickness (Pls. 22P; 26A, E) and may represent a glycocalyx.

Although essentially similar in structure the epidermis of the 9-month metacercaria showed certain structural changes, which may be indicative of senility. These included myelin figures in the cytoplasm (Pl. 26E) and only few Sb2 type secretion bodies in the cytoplasm.

Beneath the epidermis the body wall of the metacercariae was composed of the outer circular and inner longitudinal muscle fibres lying below the basement lamina and fibrous interstitial layers (Pl. 22H). The myoblasts lie below the muscle fibres among the secretion cell bodies and parenchyma cells (Pl. 22C, D).

Host Reaction. Ultrastructural examination of the capsule of the 3-month infection revealed cellular debris, lipid and macrophages within the capsule (Pl. 23A, B). The metacercaria was surrounded by macrophages with highly vacuolated cytoplasm suggestive of active phagocytosis (Pl. 23B).

The macrophages measured up to 12.0 µm in diameter and ingested material in various stages of degradation was clearly visible within the cytoplasm (Pl. 23A, B; Pl. 24A, D). The nucleus was eccentric being pushed to one side by the accommodation of the ingested material (Pl. 24A, D).

Other leucocytes present within the capsule included small lymphocytes (Pl. 23E); monocytes, the macrophage precursor (Pl. 23F)

and plasma cells (Pl. 24C).

Lymphocytes and monocytes were identified by comparison with the circulating cells of plaice described by Ferguson (1976), and fish blood cells described in this study.

Plasma cells are characterised by their extensive rough E.R. cisternae (Pl. 24C), and were similar in morphology to those described for the paddlefish by Clawson et al. (1966).

Free macrophages, monocytes, lymphocytes and plasma cells surrounded the parasite within the fluid-filled lumen of the capsule. Toward the periphery of the capsule however free (Pl. 24B), and fixed cells (Pl. 24A), with the morphological features of epithelioid cells were found. These cells were filiform in shape with elongated nuclei characterised by finely dispersed chromatin denser peripherally and large nucleoli. The cytoplasm contained vacuoles and phagosomes of ingested material and lyosomes. (Pl. 24A, B). The cytoplasmic boundaries of the fixed epithelioid cells were difficult to delineate (Pl. 24A). Epithelioid cells have been described from plaice by Timur G (1975), and Timur M (1975), in chronic inflammation.

Most of the fixed tissue cells of the capsule at this early stage of infection were epithelioid cells (Pl. 24A zone 2) with an outer margin of fibroblasts and associated extracellular collagen (Pl. 24A zone 3). Matthews (1968) described the capsule of R. johnstonei to be composed of connective tissue cells.

In the later (approx. 9 months) infection examined the macrophages at the parasite surface had extremely vacuolated 'foamy' cytoplasm containing much ingested lipid (Pl. 25D).

Some of the macrophages associated with the eggs released by the metacercaria, had indeterminate cytoplasmic boundaries and were interpreted as multinucleated 'foreign-body' type giant cells (Pl. 25B), which may have arisen from fusion of existing macrophages. The nuclei were randomly distributed within the extremely vacuolated cytoplasm, containing ingested material. The macrophages and multinucleated giant cells merged into a region of epithelioid cells; elongated, vacuolated cells with indeterminate cytoplasmic boundaries (Pl. 25E, F). The outer capsule was composed of fibroblasts (Pl. 25E), with large quantities of extracellular collagen (Pl. 25G).

The structure of the surface of the metacercaria and associated host cells is represented in Fig. 5.

Fig 4

Parasite surface and host cellular reaction of

Rhipidocotyle johnstonei in plaice

az. = attachment zone

bl. = basement lamina

cd. = cellular debris

cm. = circular muscle

col. = collagen

ec. = epidermal cell body

ep. = epithelioid cell

f. = fibrous interstitial material

fb. = fibroblast

gc. = giant cell

gy. = glycogen

l. = lipid

lm. = longitudinal muscle

m. = mitochondrion

ms. = macrophage

my. = myoblast

n. = nucleus

opm. = outer plasma membrane

pa. = parenchyma cell

pc. = plasma cell

pp. = protoplasmic process

s. = spine

sb. = secretion body

tgc. = tegumental gland cell

1. = macrophage zone

2. = epithelioid cell zone

3. = fibroblast zone

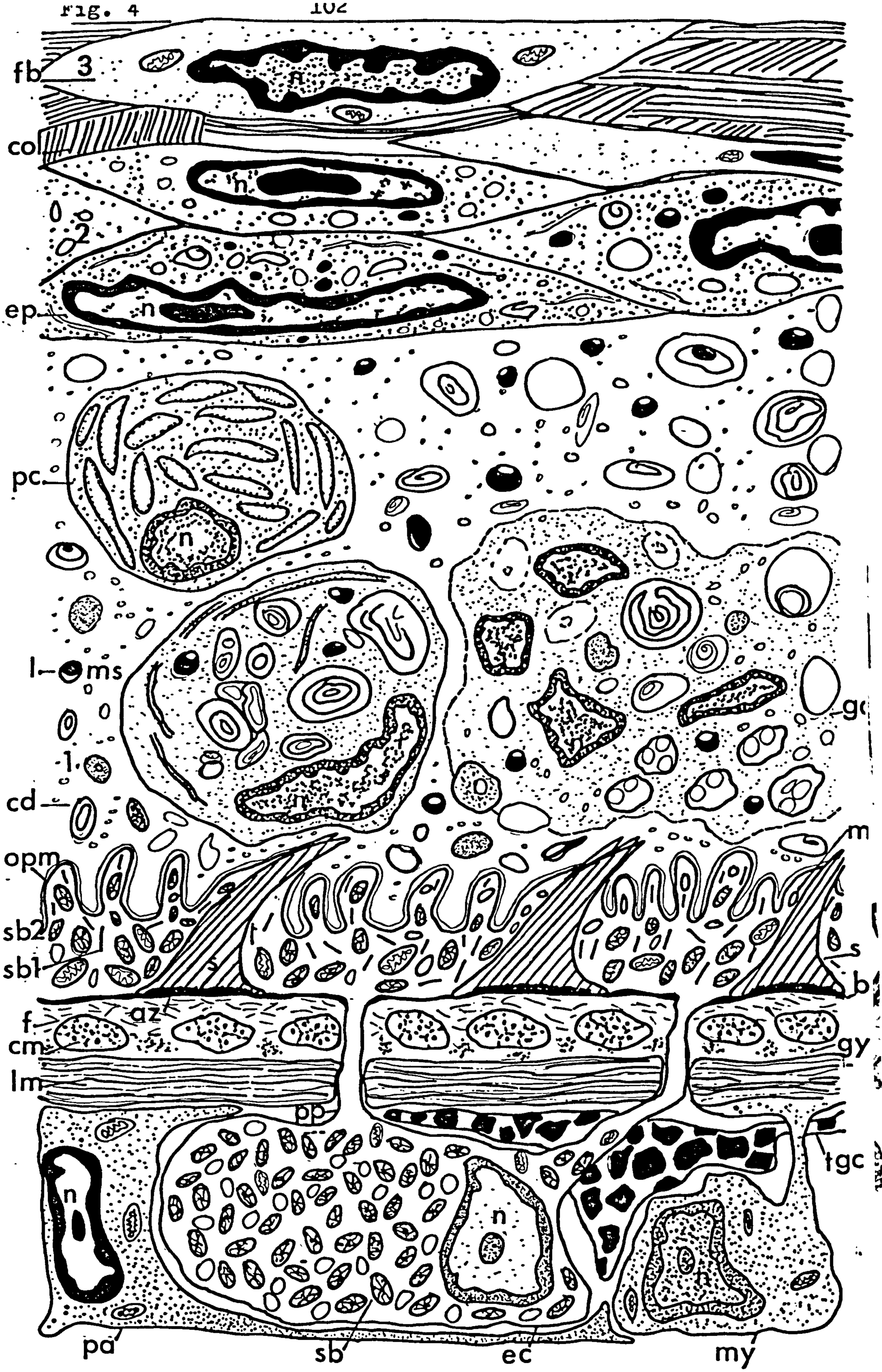

fb
3
A
col
h
0
2
ep
n
pc
n
l
ms
1
cd
opm
sb2
sb1
az
f
cm
lm
pp
n
n
n
pa
sb
ec
my
tgc
gy
m
s
b

PLATE 22 (continued)

bl. basement lamina
cm. circular muscle
ec. epidermal cell body
f. fibrous interstitial material
gy. glycogen
le. plaice cells
lm. longitudinal muscle
m. mitochondrion
mt. microtubules
my. myoblast
pa. parenchyma cell
rj. metacercaria of R.johnstonei
tgc. tegumental gland cell
z. muscle fibres of plaice

PLATE 22 (continued)

I. Folded tegument containing numerous secretion bodies with the associated underlying cell body (ec).
X 6,406

J. Detail of the tegument with the secretion bodies and mitochondria with electron dense inclusions.
X 7,696

K. Gland cell opening through the tegument by a microtubule lined duct. Secretion bodies from the gland cell appear to coalesce at the surface.
X I0,8I6

L. Detail of part of gland cell lined with microtubules (mt), and containing the electron dense secretion bodies.
X 4,I60

M. Tegument with serrated spines and underlying cell bodies of the body wall.
X 5,24I

N. Detail of the serrated surface of the spines.
X 7,696

O. Multipointed spine.
X 8,652

P. Longitudinal section through two spines showing thorn shape, the multipointed shape apparent only in oblique section.Spines rest on the basement lamina (bl), at which point an electron dense junction indicates an attachment plaque.Outer plasma membrane bears an electron dense coat.
X I0,8I6

Q. Detail of the tegument with sb2 type secretion bodies.
X I0,8I6.

R. Detail of the tegument with sbI and sb2 secretion bodies
X I4,I44

S. Tegument of the oral sucker without spines.
X 5,24I

PLATE 22

Metacercarial surface of Rhipidocotyle johnstonei 3 months post infection of 0 group plaice.

A. 0 group plaice infected with R. johnstonei metacercariae, clearly visible through the body wall of the fish.

B. Section through the body wall of resin embedded tissue, showing the parasite within the muscles (z), and completely surrounded with host leucocytes (le), forming a capsule. Eggs are present both within the metacercaria and externally within the host capsule.

X

C. Ultrastructure of the body wall of the metacercaria showing the tegument and underlying epidermal cell bodies (ec), part of a tegumental gland cell (tgc) and myoblast (my).

X4,I60

D. Section through the body wall with the folded tegument with spines and the epidermal cell bodies (ec) and parenchyma cells (pa).

X3,328

E. Section through the parasite at the oral sucker showing the lack of spines, contrasted with the tegument of the body surface.

X I,248

F. Folded tegument with spines and underlying cell bodies.

X 3,328

G. Multipointed spine and part of the underlying tegumental gland cell (tgc).

X8,652

H. Detail of the body wall musculature showing the outer circular and inner longitudinal muscles, (cm,lm); and accompanying glycogen (gy), and mitochondria (m).

X IO,8I6

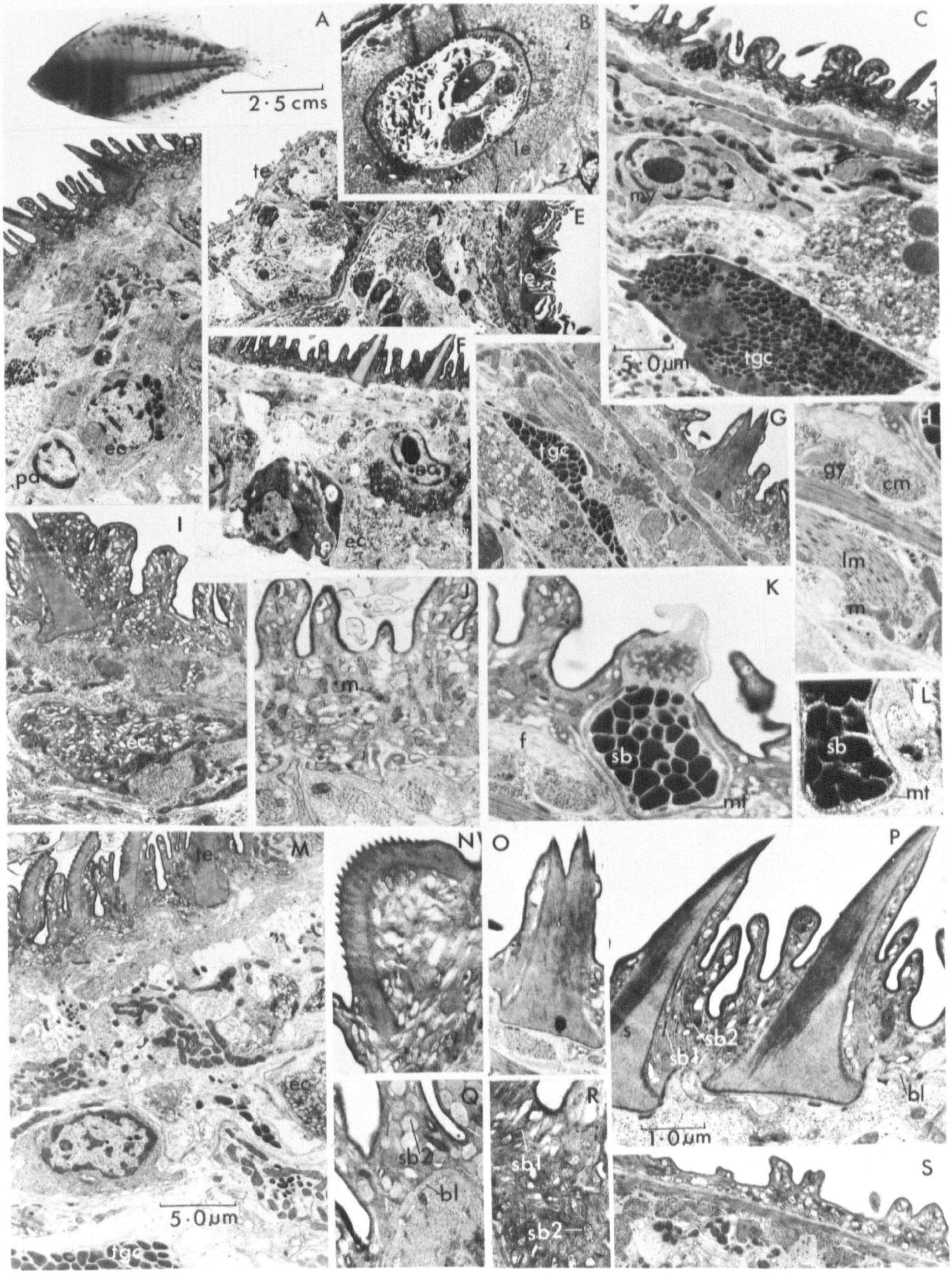

A
2·5 cms
B
rj
le
z
C
my
ec
5·0µm
tgc
D
te
ec
pd
E
te
F
ec
G
tgc
gr
cm
lm
m
H
I
ec
J
lm
K
f
sb
mt
L
sb
mt
M
je
ec
5·0µm
tgc
N
O
P
s
sb2
sb1
bl
1·0µm
Q
sb2
bl
R
sb1
sb2
S

PLATE 23

Ultrastructure of metacercarial surface of R. johnstonei and cellular reaction of plaice, 3 months post infection.

A. Surface of metacercaria and associated plaice macrophages containing membranous phagocytosed material.
X 2,947

B. Folded tegument of R. johnstonei metacercaria with spines (s), and associated plaice monocytes and macrophages comprising the host capsule.
X 3,080

C. Section through the body wall of the metacercaria showing the folded tegument with spines and the underlying cell bodies (ec). Macrophages with large electron lucent vacuoles.
X 3,588

D. Cellular debris within the capsule contains membranous material and lipid (g), or possibly secretory material from the metacercaria.
X 9,328

E. Tegument of R.johnstonei metacercaria and small lymphocyte of plaice.
X 5,520

F. Monocyte from the plaice capsule associated with R.johnstonei metacercaria. Cytoplasm contains numerous vacuoles and vesicles.
X 9,180

G. Monocyte transforming into a macrophage, with the nucleus becoming eccentric and the cytoplasm highly vacuolated.
X 9,180

ec. epidermal cell body
g. lipid or secretory material
ms. macrophages
s. spine
te. tegument

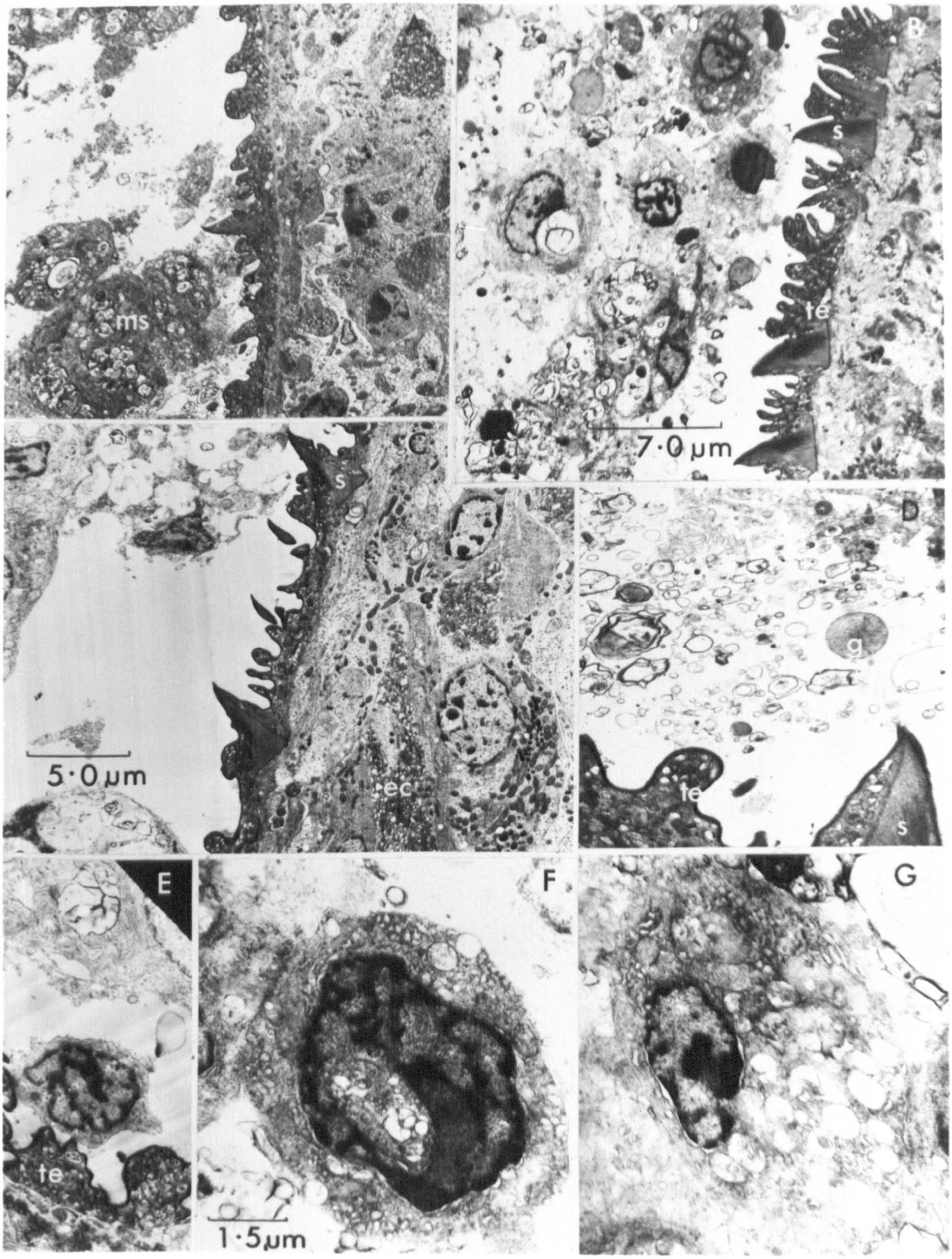

ms
7·0 µm
te
s
C
5·0 µm
ec
D
g
te
s
E
te
F
1·5 µm
G

PLATE 24

The cellular response of plaice to R. johnstonei metacercariae, 3 months post infection.

A. Montage section through the host capsule of R. johnstonei metacercaria. Associated with the surface of the parasite are free macrophages, rounded cells with eccentric nuclei and cytoplasm containing ingested membranous and lipid or secretory material. Much cellular debris surrounds the free macrophages, labelled zone I. The middle zone of the capsule (2), comprised the epithelioid cells with their indefinite cell boundaries and elongate nuclei with large nucleoli.Some evidence of ingested material.The outer region of the capsule (3), composed of fibroblasts with extracellular deposits of collagen.
X 3,772

B. A free epithelioid cell at the periphery of the macrophages.The cell is elongate in shape and the cytoplasm contains vesicles and smooth ER profiles. The elongated nucleus is irregular in outline with the chromatin denser peripherally and the nucleolus large.
X 9,434

C. Plasma cell from the inner host capsule associated with the macrophages.Cytoplasm contains the typical distended rough ER cisternae.
X I8,532

D. Macrophage closely associated with the tegument of the metacercaria. The cell is large with an eccentric nucleus and the cytoplasm filled with phagocytosed membranous material, large electron lucent vacuoles, and rough ER profiles.
X 9,434

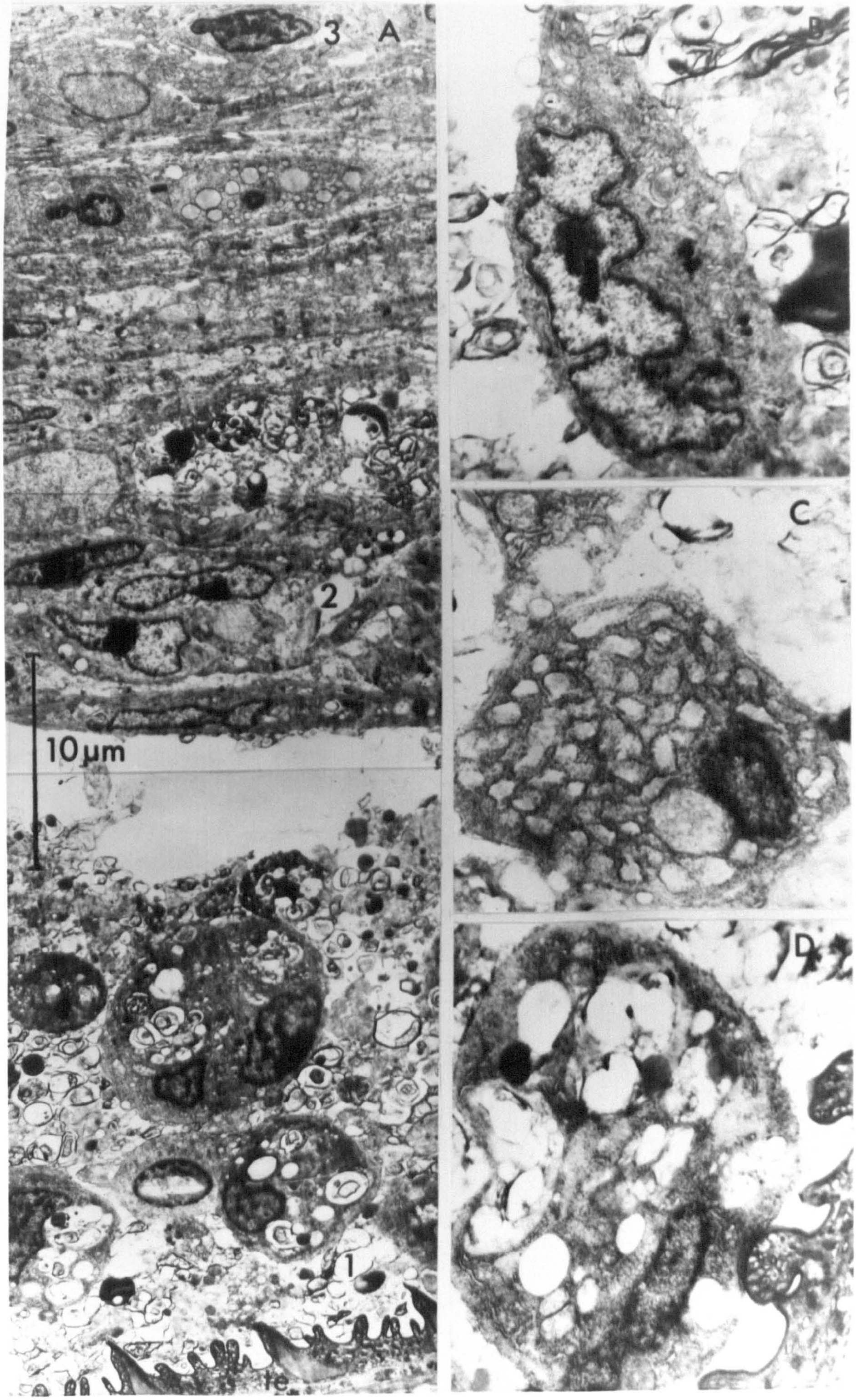

3
A
2
10µm
1
te
C
D

PLATE 25

The cellular response of plaice to R.johnstonei metacercaria, 9 months post infection.

A. Section through the body wall of the metacercaria and the associated plaice cells, which comprise discrete macrophages and indistinct foamy cytoplasm interpreted as part of multinucleated giant cells.
X 3,692

B. Part of an egg released by the parasite into the capsule, surrounded by discrete macrophages (ms) and the foamy cytoplasm of multinucleated giant cells.These may have resulted from the fusion of several macrophages.
X 2,655

C. Surface of the metacercaria with the folded tegument and the associated macrophages containing phagocytosed material.
X 5,680

D. Macrophages within the capsule, with their eccentric nuclei and the cytoplasm filled with phagocytosed material.
X 4,544

E. Epithelioid and fibroblastic layers of the plaice capsule.
X 2,655

F. Epithelioid cells of the middle region of the capsule with vacuolated cytoplasm some containing ingested material.
X 3,976

G. Detail of the outer capsule with the extracellular collagen associated with the fibroblasts.
X 7,653

eg. egg
g. lipid or secretory material
in. epithelioid layer of capsule
ms. macrophage
ou. fibroblast layer of capsule

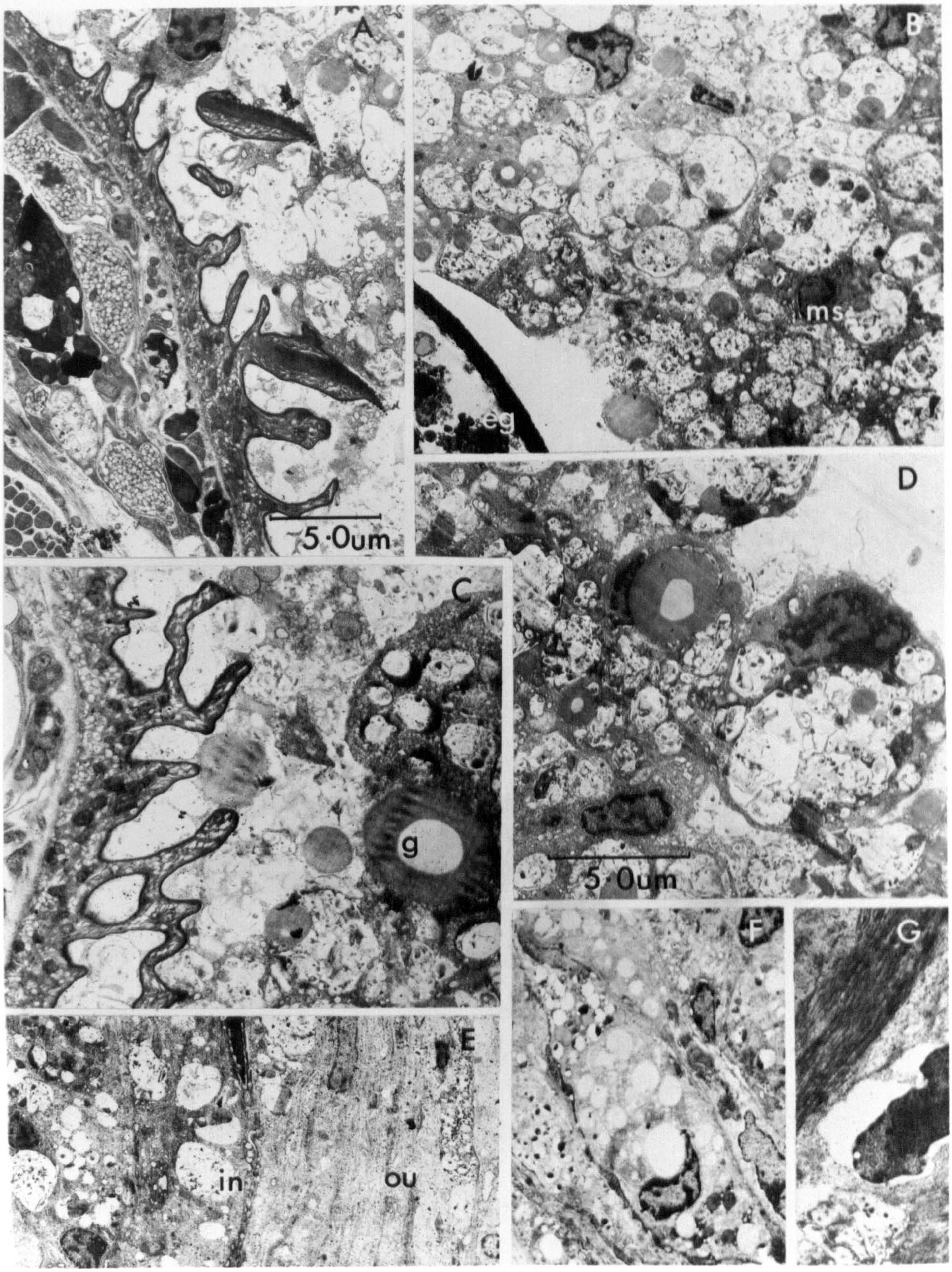

A
5·0um
B
ms
eg
D
C
g
5·0um
F
G
E
in
ou

PLATE 26

Ultrastructure of metacercaria of R.johnstonei 9 months post infection.

A. Section through the body wall showing the tegument with the electron dense coat to the outer plasma membrane.Secretion bodies of both types present in the tegument and the underlying cell bodies.
X 7,056

B. Section through the body wall showing two types of secretory gland cell.
X 7,056

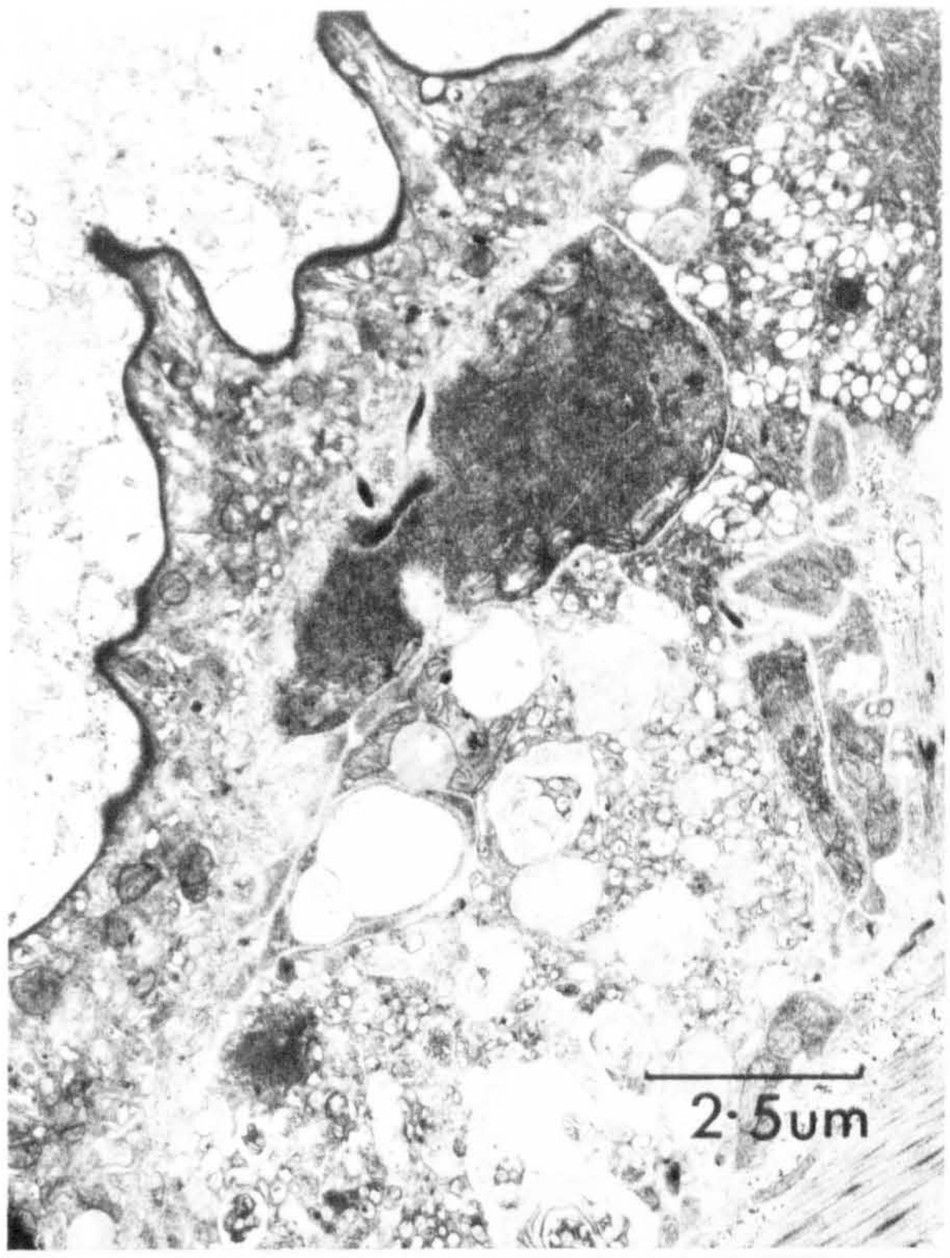
C. Tegument with the electron dense coat to the outer plasma membrane, secretion bodies and mitochondria. Underlying myoblast and secretion cell bodies.
X 5,600

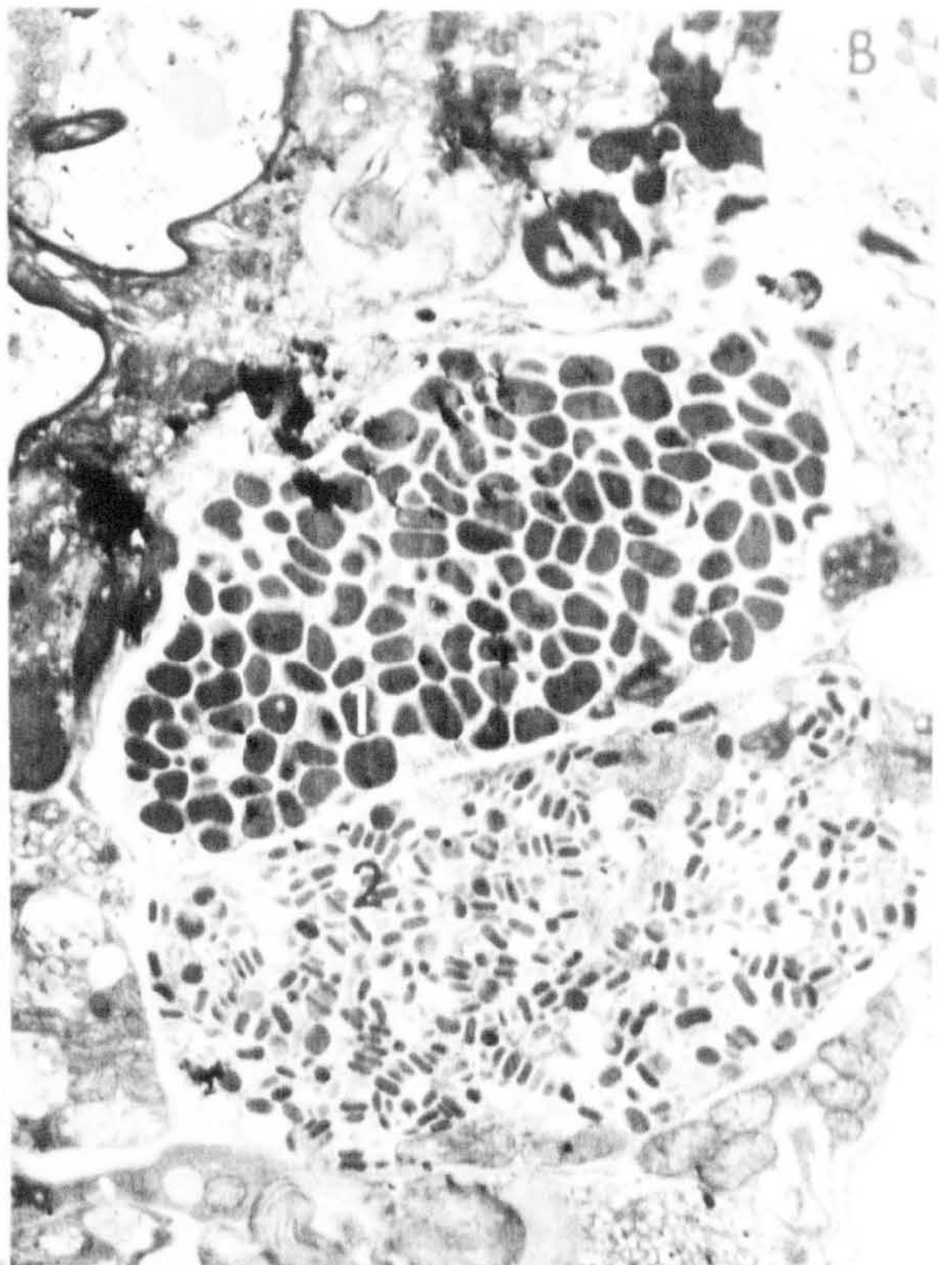
D. Tegument with outer electron dense coat, secretion bodies, vacuoles and mitochondria.
X 3,360

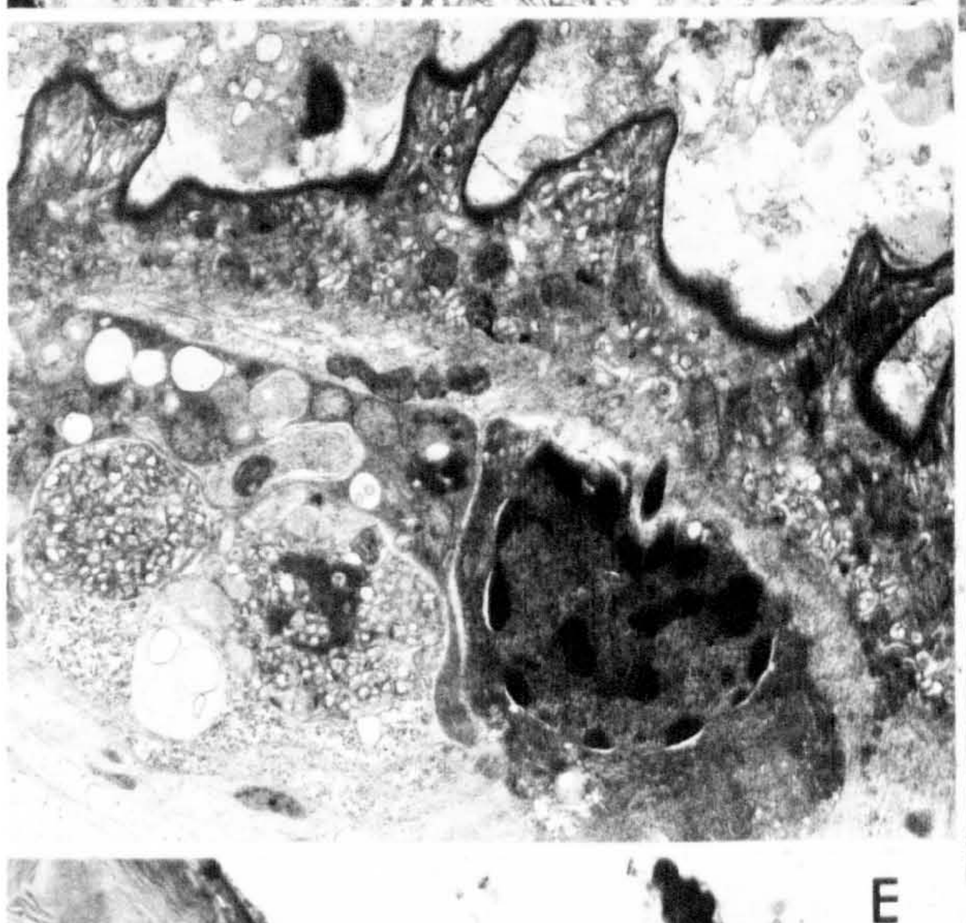
E. Spine with serrated surface and tegument containing myelin figures (my).
X II,648

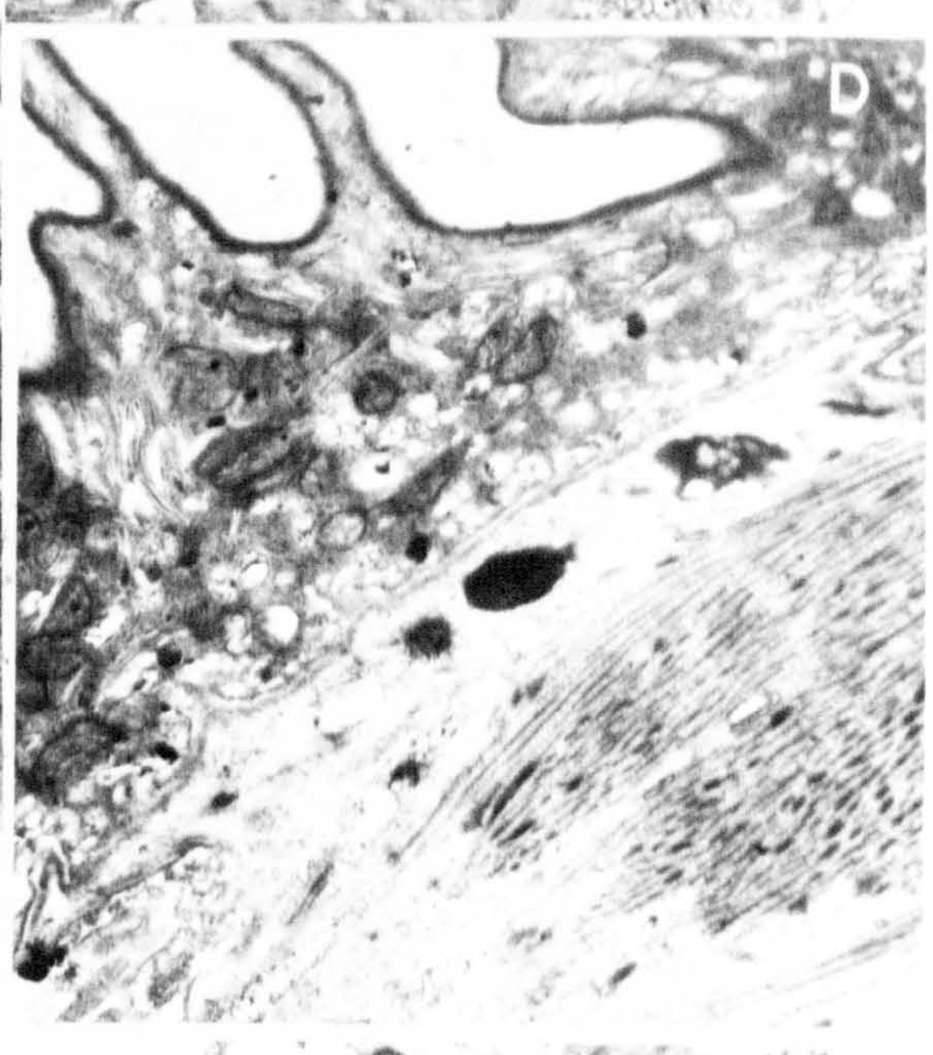
F. Folded tegument with secretion bodies, vacuoles and mitochondria.
X 5,600

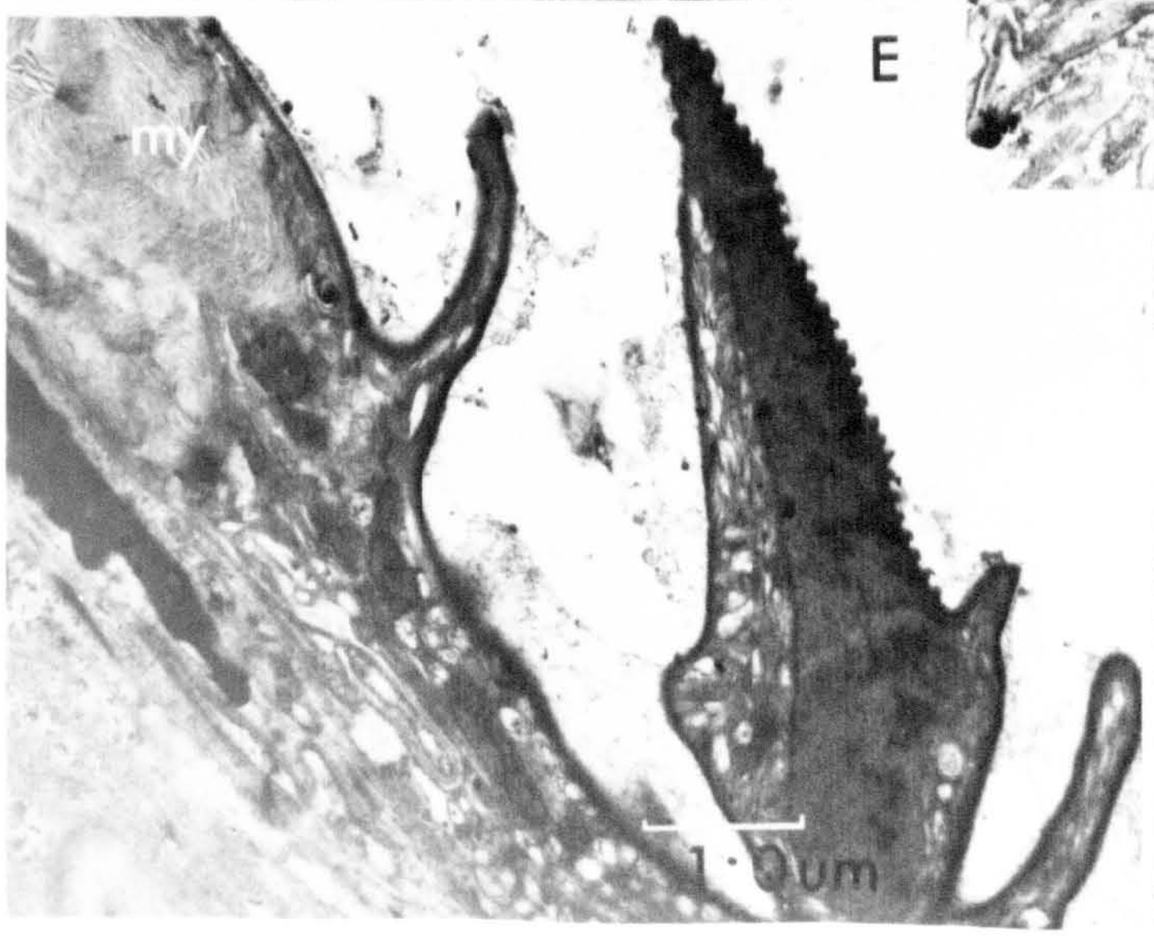
my. myelin figure

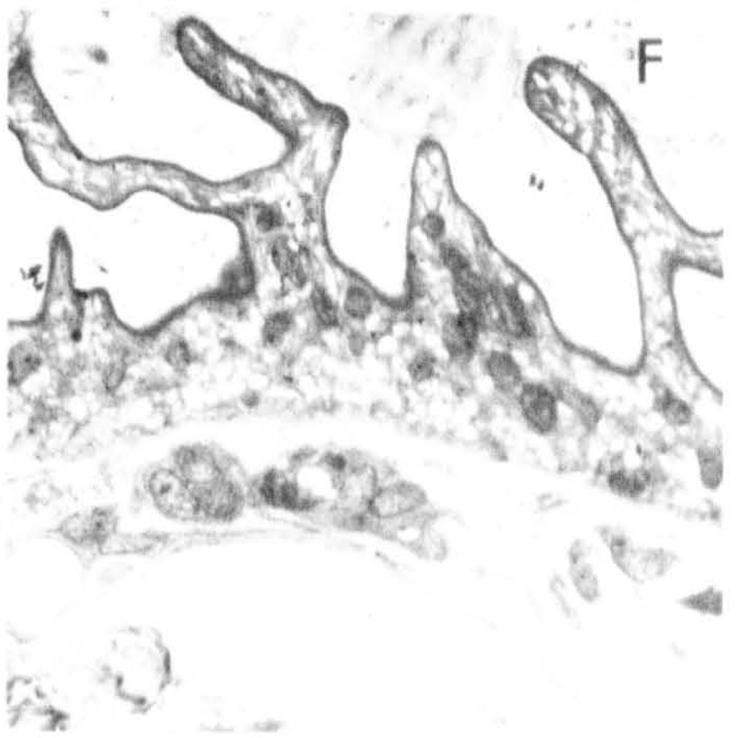

A
2·5um


B
2




D


E
my
1·0um


F

MYXOSPORIDIA

An ultrastructural investigation of Myxobolus exiguus from Crenimugil labrosus was undertaken to determine the host parasite cellular associations and spore development at various sites in the fish. The main study was carried out on the gill plasmodia and compared with development in gut, skin and scales.

Structure

Myxobolus exiguus

The white round or elongate plasmodia of M. exiguus were found in the gill lamellae, wall of intestine and subepidermally in wild fish; the latter site being associated with melanisation of surrounding host tissues (Pl. 27).

Typical myxosporidian spores were identified from squash preparations of excised plasmodia, examined with phase microscopy (Pl. 28A). Spores were also identified in sections of liver and from bile smears. The spores are oval measuring 8.0 μm x 7.0 μm bivalvular with the pair of polar capsules at one pole and the sporoplasm at the other. (Pl. 28A). The polar capsules stained deep purple with Giemsa (Pl. 28B,E) and extruded filaments were uniformly stained (Pl. 28C) or had a banded appearance (Pl. 28D). The filaments measured up to 35 μm in length. The sporoplasm was positive for PAS and Best's carmine and diastase fast even after 24 hours enzyme treatment. No iodinophilous vacuoles were detected although the spore gave a diffuse grey reaction throughout.

Plasmodia. The sac-like syncytial plasmodia contain mature and developing spores. S.E.M. preparations of gill plasmodia measuring 1.3 mms (Pl. 29A) showed the surface to be corrugated (Pl. 29E,F) with gill epithelial cells attached (Pl. 29A,E). The exposed surface of a fractured plasmodium revealed the packed masses of spores (Pl. 29B,D).

In transverse section the plasmodial wall is clearly demarcated into an outer ectoplasm and inner endoplasm (Pl. 30). The ectoplasm is an amorphous layer traversed by blindly ending channels which open at the surface (Pl. 30A). The surface membrane bears a conspicuous 'fuzzy coat' or glycocalyx 0.06 μm in depth (Pl. 30A,B). The endoplasmic layer measured 1.0 μm - 1.4 μm in depth and contained the mitochondria, ribosomes and vacuoles and scattered membrane profiles. (Pl. 30B). The inner endoplasmic layer contains the vegetative nuclei, generative cells and developing sporoblasts (Pl. 31A,G), mature spores filling the lumen of the plasmodium (Pl. 31A).

Generative cells were small cells measuring 1.0 μm x 1.9 μm with a proportionately large nucleus and prominent nucleolus (Pl. 31F). The cytoplasm was bounded by a single plasma membrane and contained free ribosomes, glycogen and several mitochondria. How these cells arise within the plasmodium is not known.

Differences in the structure of the plasmodial wall from the other sites were noted. The gut and skin plasmodia had a vacuolated ectoplasm up to 2.0 μm in depth; the skin plasmodium having long finger-like extensions of the surface (Pl. 35C). The scale plasmodium had a granular ectoplasm

(Pl. 30G) and no organelle were identified in the endoplasm.

Spore Formation. The earliest stage observed was the envelopment of one generative cell by another (Pl. 31G). Subsequent development of the disporous pansporoblast results from the division of the enclosed cell, while the outer or nurse cell persists around the pansporoblast eventually becoming the pansporoblastic membrane. (Pl. 31J).

Each spore developing within the disporous pansporoblast develops from 5 cells: two peripherally arranged valvogenic cells, a pair of capsulogenic cells and a binucleate sporoplasm (Pl. 31H,I).

The capsular primordia develop within the cytoplasm of the nucleated capsulogenic cells (Pl. 33A). At first these are membrane bound oval bodies which are clearly defined into three zones: an outer electron lucent zone, a middle electron dense zone and an inner granular matrix (Pl. 33B,G).

Profiles of the membrane bound external tube measuring 0.3 um in diameter were also present in the cytoplasm of the capsulogenic cell (Pl. 33D,F,L,M). The primordia of the external tube appear to grow out from the proximal pole of the capsular primordia into the cell cytoplasm (Pl. 33H,I,J,K).

Electron dense material is present in the matrix of the capsular primordia and lumen of the external tube (Pl. 33M) and it is generally concluded (Lom & de Puytorac, 1965) that this material forms the mature filament, and develops simultaneously in the capsular primordia and external tube. The external tube was not found in later stages of development when the mature filament was coiled within the capsule (Pl. 33N).

Spore. S.E.M. preparations of spores (Pl. 29C) showed the smooth surface and thickened ridge at the junction of the two valves. In section the valvogenic cells were flattened cells, expanded at their septate intercellular junctions (Pl. 32I). The cytoplasm contained mitochondria, vacuoles, scattered ribosomes and irregular areas of amorphous electron dense material (Pl. 32H,I). The capsule nuclei were often observed in the thickened ridge areas during developmental stages but had degenerated in the mature spore.

The mature polar capsules which develop asynchronously from the capsulogenic cells (Pl. 34A) were oval and measured 3.0 μm x 1.7 μm. The outer electron dense wall 0.01 μm - 0.02 μm in thickness is continuous with the everted filament (Pl. 34B). The tubular filament was hollow measuring 0.02 μm in diameter and forms a helical coil of 4 loops in the granular matrix, the centre of which has a lattice-like appearance (Pl. 34E).

Irregular spores were sometimes encountered within the subdermal plasmodia when the shell valves did not completely enclose the amoebula (Pl. 35G) or an irregular spore shell developed containing three polar capsules (Pl. 35H). An electron lucent zone of 0.2 μm separates the capsule wall and granular cortex (Pl. 34C).

The lumen of the coiled filament is filled with an electron dense material, which was ejected on eversion, the filament appearing hollow (Pl. 34B).

The sporoplasm occupies one third of the spore volume being limited to the distal end of the mature spore (Pl. 32C).

It is binucleate (Pl. 32F) and the cytoplasm contains free ribosomes, mitochondria (Pl. 32B) and numerous spherical electron dense inclusions (Pl. 32J,L) probably lipid. B glycogen particles were scattered within the cytoplasm, (Pl. 32G,K) and in one spore was confined to a particular area (Pl. 32H).

Host Reaction. The presence of the plasmodia in the grey mullet generally elicited little host reaction. The most noticeable response being associated with the subdermal skin cysts which caused distension of the skin surface under which they were developing (Pl. 27A,B,C). Melanisation of the area around the plasmodia in the skin and scales make these plasmodia particularly noticeable. These melanised cysts tended to increase in number in fish kept under laboratory conditions. The melanin was deposited either as free granules (Pl. 36E) or within melanophores (Pl. 36F).

Collagen deposition was also associated with the subdermal skin plasmodia the collagen filaments being associated with the extensive ramifications of the ectoplasm (Pl. 35C).

Slight hyperplasia of epithelial cells associated with the intralamellar plasmodia was noted (Pl. 27H) and tentative evidence of cell lysis associated with gut plasmodia (Pl. 37B).

PLATE 27

Gross morphology and histology of the infection of Crenimugil labrosus with the myxosporidian Myxobolus exiguus

A,B,& C.
Prominent black melanised cysts of M.exiguus in the skin and scales of C. labrosus . 2-3 group fish approximately IO cms. in length.

D. Transverse section through a melanised cyst from the skin. Proliferation of the melanophores (mr), at the junction of the epidermal (ep), and dermal layers (dr) of the skin. Middle of cyst contains the mass of myxosporidian spores.
X 84

E. Parts of the intestines from two fish showing the plasmodia to be either roun or band like. Young fish tend to carry the round cysts (2), whereas older fish had band like plasmodia.

F. Longitudinal section through the intestine of a 2 group fish , showing the superficial location of the plasmodia in the connective tissue. The cyst is packed with spores yet has induced no cellular response by the fish.
X 84

G. Longitudinal section through the gills of an infected fish showing the interlamellar location of the plasmodia the plasmodium is surrounded by a layer of epithelial cells.
X I05

H. Section of the interlamellar cyst containing spores (sps), enclosed by host epithelial and mucous cells.
X 437

dr. dermis
ep. epidermis
he. epithelial cells
mr. melanophores
sps.spores of myxosporidian

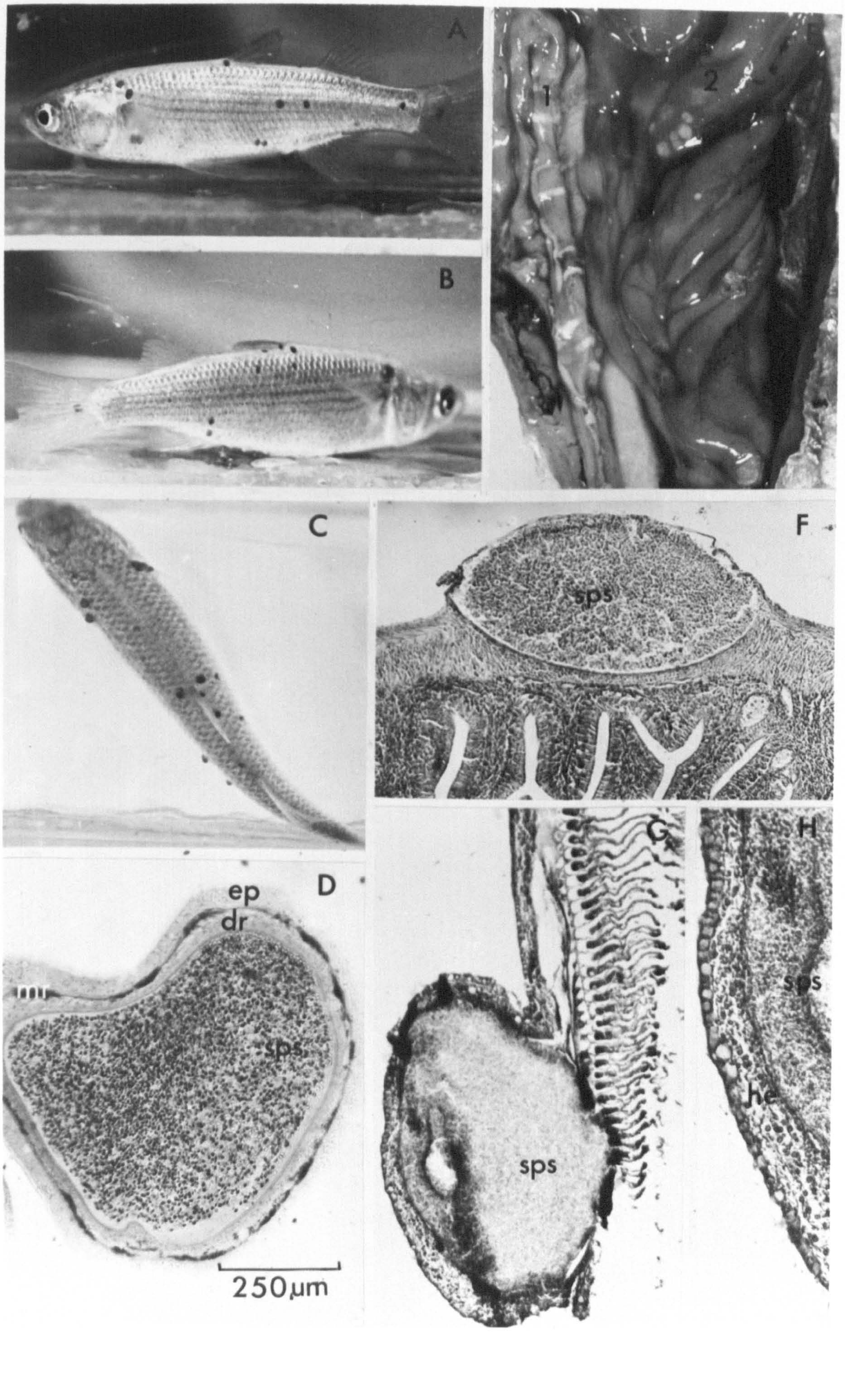

A
B
C
D
ep
dr
sps
250µm
1
2
F
sps
G
H
sps
he
sps

PLATE 28

Light micrographs of spores of Myxobolus exiguus, unstained and stained with Giemsa

A. Phase contrast light micrograph of mature spores of Myxobolus exiguus from the gill plasmodia. Polar capsules clearly visible and extruded polar filament in one spore. Spores measure 9.0 x7.5µm.
X 828

B. Single spore from Giemsa stained smear,showing paired densely stained polar capsules at anterior of spore.
X 2,380

C. Spores from Giemsa stained smear with extruded polar filaments.
X 2,380

D. Spore with extruded polar filament exhibiting a banded appearance, due to spiral twisting of filament on extrusion or uneven adhesion to slide.
X 2,380

E. Pair of extruded polar filaments showing the continuity of the polar capsule with the polar filament on eversion.
X 2,380

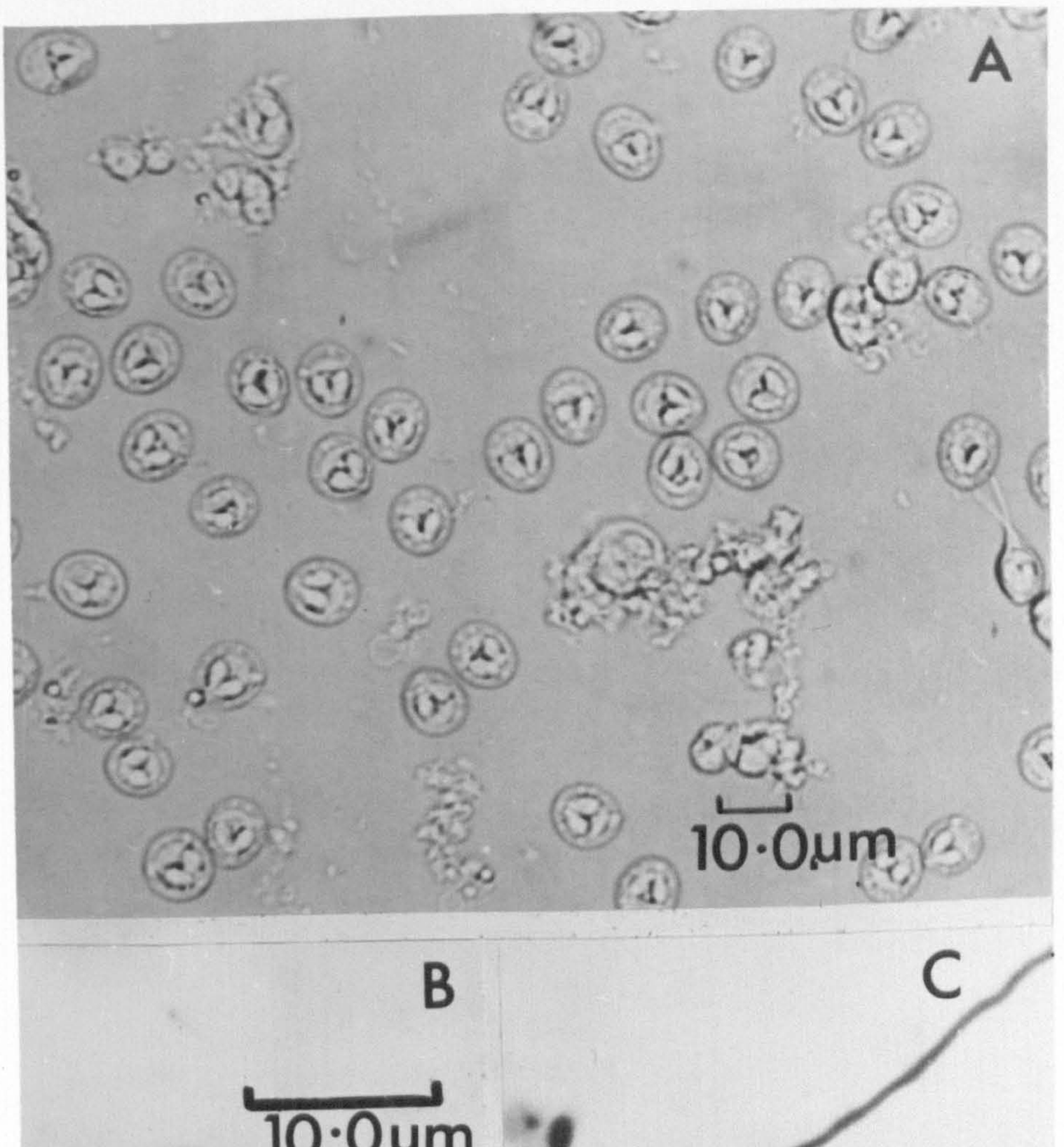

A
10·0µm
B
10·0µm
C
D
E

PLATE 29

Scanning electron microscopy of the gill plasmodia and spores of Myxobolus exiguus

A. Whole sausage shaped plasmodium detached from the interlamellar site in the gill lamellae of Crenimugil labrosus. Gill epithelial cells attached to part of the plasmodial surface.
X II5

B. Broken end of a plasmodium revealing the packed masses of spores within the plasmodium.
X 900

C. Single spore of M.exiguus, with the two shell valves apposed at the thickened rim.
X 7,200

D. Spores within the endoplasm of the plasmodia.
X 3,600

E. Surface of the plasmodium showing the corrugated surface and the attached gill epithelial cells.
X 3,600

F. Detail of the surface of the gill plasmodium showing the deeply corrugated surface, increasing the surface area.
X 7,200

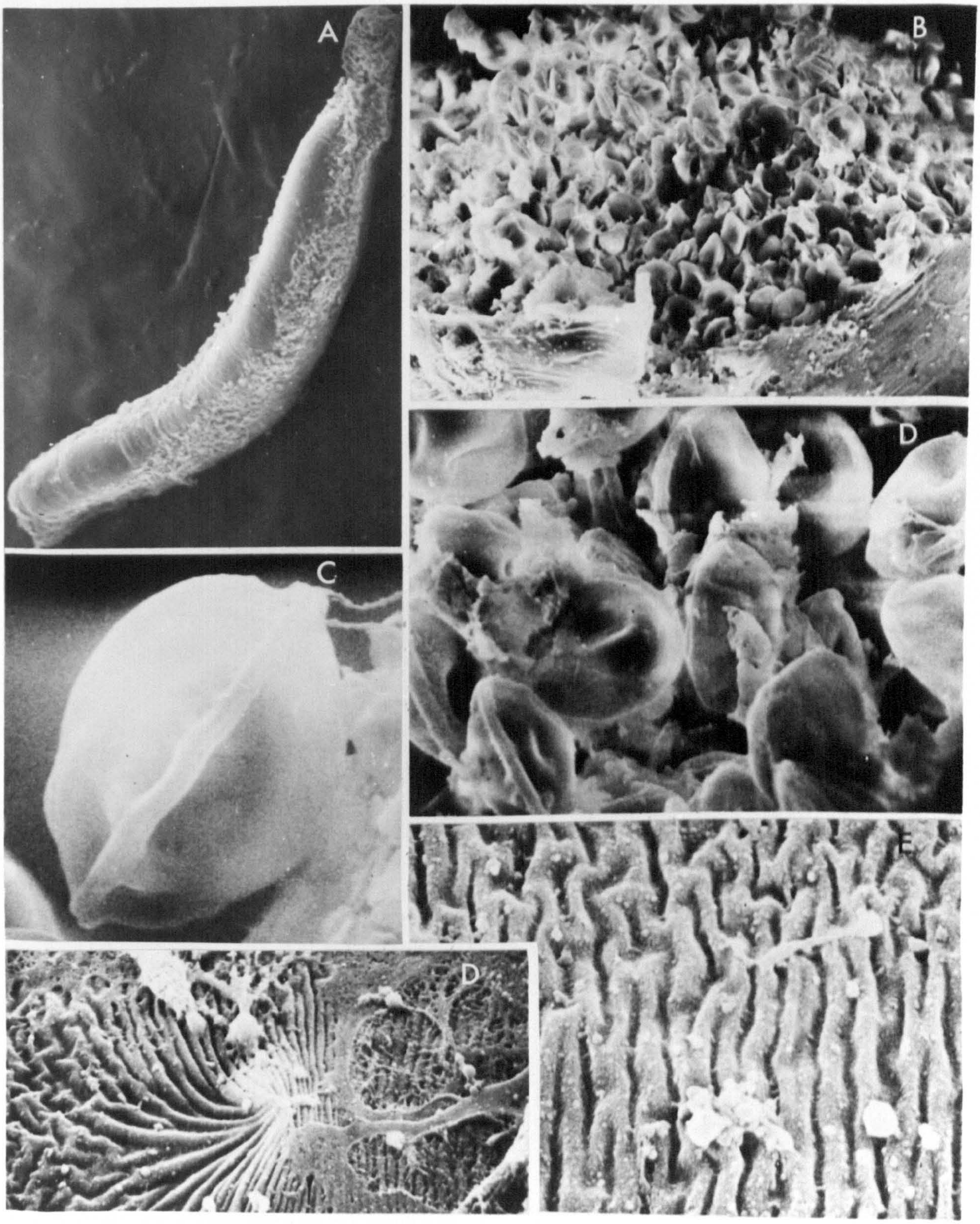

A
B
C
D
D
E
D

PLATE 30

Ultrastructure of the ectoplasm and endoplasm of plasmodia from the gills, gut, skin and scales of C.labrosus.

A. Transverse section of the gill plasmodium ectoplasm of M.exiguus . Deep blindly ending channels traverse the ectoplasm from the surface membrane which bears a filamentous glycocalyx (gx).
X 5I,840

B. Transverse section through the gill plasmodium showing the surface glycocalyx,channels in the ectoplasm, and mitochondria and vacuoles in the endoplasm.
X 44,800

C. Transverse section of plasmodium from the gut, with the more homogeneous ectoplasm with few channels and vacuoles. Surface membrane bears a glycocalyx and is closely associated with the host cell membrane (hcm).
X I7,305

D. Transverse section through the gut plasmodium with large vacuoles in the ectoplasm .
X I0,080

E. Transverse section through the plasmodium from the skin, with the ectoplasm filled with numerous small vacuoles.
X 8,640

F. Detail of the ectoplasm from the skin plasmodium with surface glycocalyx and numerous small vacuoles. The ectoplasm was extended into numerous cytoplasmic extensions among the cells of the dermis.
X 22,464

G. Ectoplasm from the scale plasmodium has a striated appearance with no channels or vacuoles.
X I6,640

ch. channel in ectoplasm

gx. glycocalyx
hcm.host cell membrane

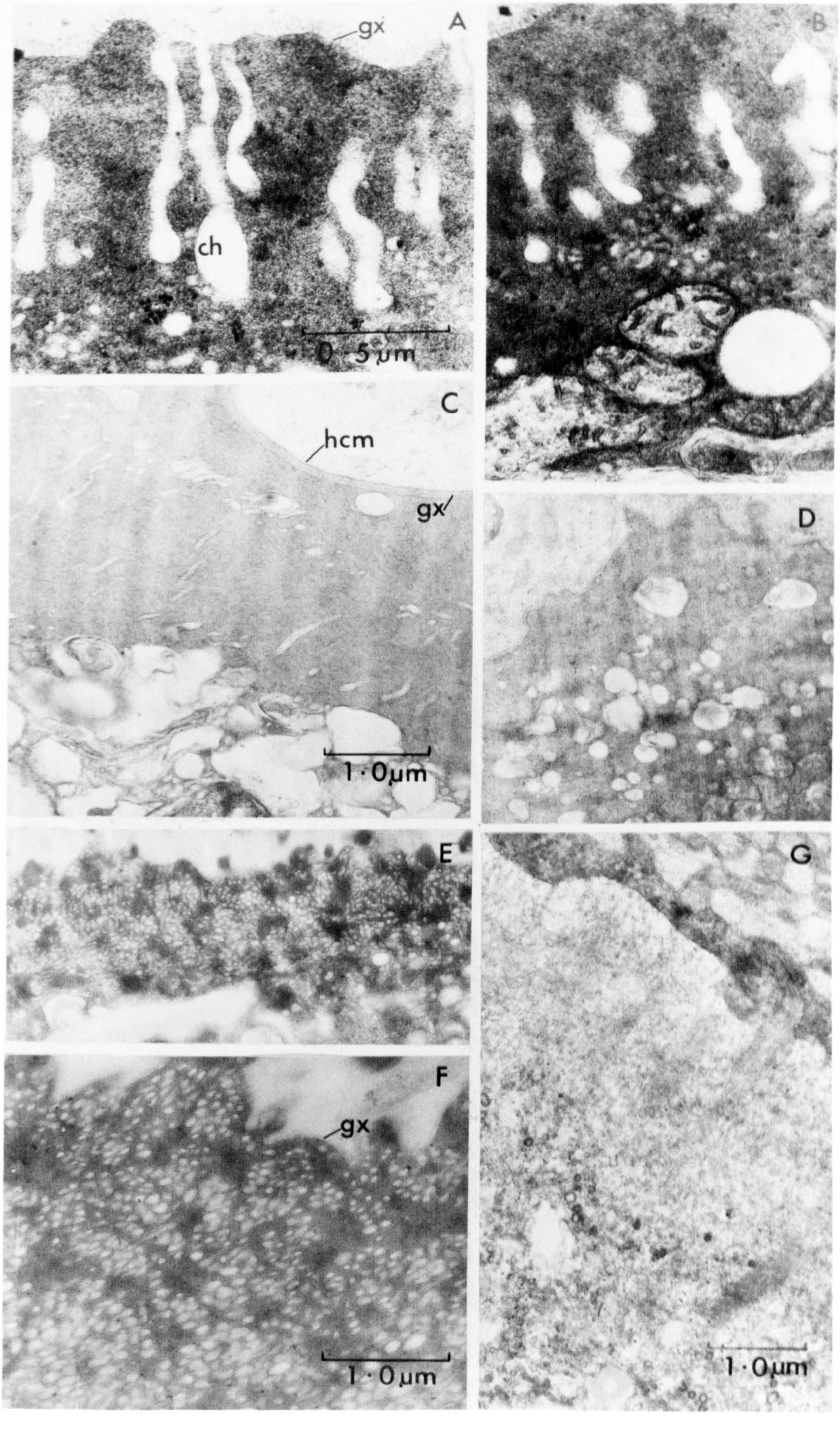

A
gx
ch
0·5 µm
B
C
hcm
gx
1·0µm
D
E
F
gx
1·0µm
G
1·0µm

PLATE 3I (continued)

ch. channels in the ectoplasm
dsr.developing spore
ect.ectoplasm
en. endoplasm
ge. generative cell
h. host gill epithelium
m. mitochondrion
n. vegetative nucleus
psm.pansporoblastic membrane
sr. spore

PLATE 31

Ultrastructural development of the spore of Myxobolus exiguus within the gill plasmodium.

A. Transverse section through the gill plasmodium showing the outer ectoplasm (ect), the vesicular endoplasm (en), containing the vegetative nuclei (n) and developing and mature spores (sr) within the inner endoplasm.
X 4,485

B. Outer endoplasm with the associated gill epithelial cell, (h).
X 6,906

C. Ectoplasm of plasmodium traversed by the blindly ending channels and the numerous mitochondria at the junction of ectoplasm and endoplasm.
X 24,219

D. Numerous mitochondria in the endoplasm.
X 9,328

E. Developing pansporoblast containing four cells within an enveloping cell (ev).
X II,66I

F. Generative cell within the endoplasm.
X I7,940

G. Two celled pansporoblast within the enveloping cell cytoplasm.
X I0,626

H. Two spores developing within the pansporoblast. The developing polar capsule cells are visible, one from each spore. The polar capsule cells are nucleated with the capsular primordiawithin the cytoplasm.
X II,66I

I. Two immature spores within the pansporoblast .
X 9,328

J. Two developing spores (dsr), within the pansporoblastic membrane, derived from the original enveloping cell.
X 9,328

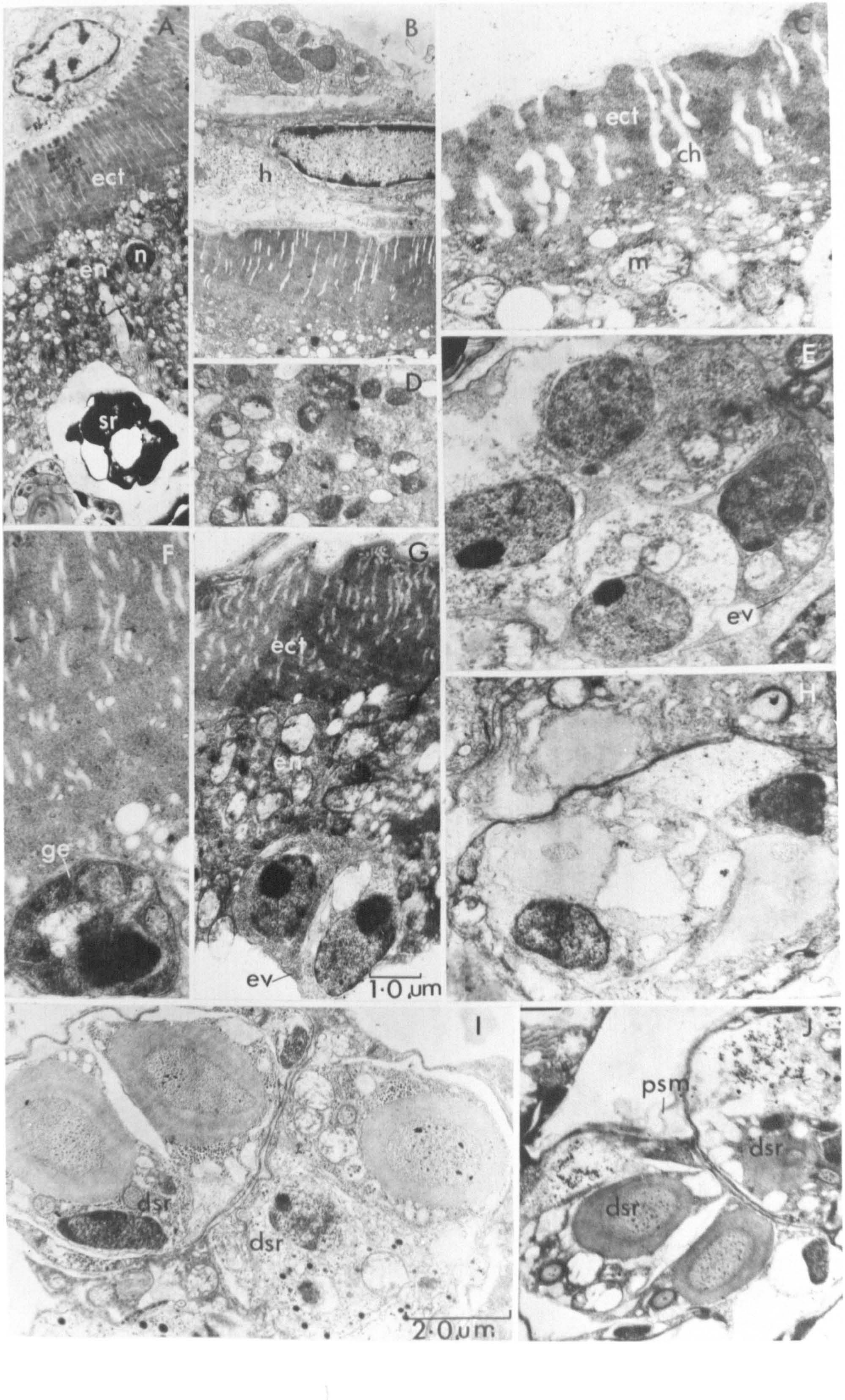

A
ect
en
n
sr
B
h
C
ect
ch
m
D
E
ev
F
ge
G
ect
en
ev
1·0 µm
H
I
dsr
dsr
2·0 µm
J
psm
dsr
dsr

PLATE 32 (continued)

am. amoebula
n. nucleus
pc. polar capsule
sv. shell valves

PLATE 32

Ultrastructure of the mature spore of Myxobolus exiguus

A. Mature spore with one complete polar capsule and part of the other in the plane of the section. The amoebula (am), with one nucleus in section.
X IO,478

B. Mature spore with the paired polar capsules at the anterior of the spore and amoebula at the posterior.
X 8,382

C. Spore with one mature and one immature polar capsule. Part of the other spore with immature polar capsule within the pansporoblast.
X 8,382

D. Section through the polar capsules, showing the thickened rims of the cells forming the shell valves.
X I3,702

E. Section of spore showing one polar capsule in longitudinal section. The shell valve cells become progressively electron dense as they mature.
X IO,478

F. Section of the spore through the amoebula with both the nuclei in the plane of the section.
X 8,382

G. Section of the spore through the amoebula,containing glycogen and electron dense droplets possibly lipid.
X I3,702

H. Spore with the amoebula showing the stored glycogen aggregated into a distinct area of the cytoplasm.
X I3,702

I. Detail of the junction of the two shell valve cells Septate cell junction.
X 39.060

J. Inclusions within the cytoplasm of the amoebula, possibly lipid.
X 39,060

K. Glycogen stored within the cytoplasm of the amoebula.
X 34,720

L. Electron dense inclusions within the amoebula.
X 34,720

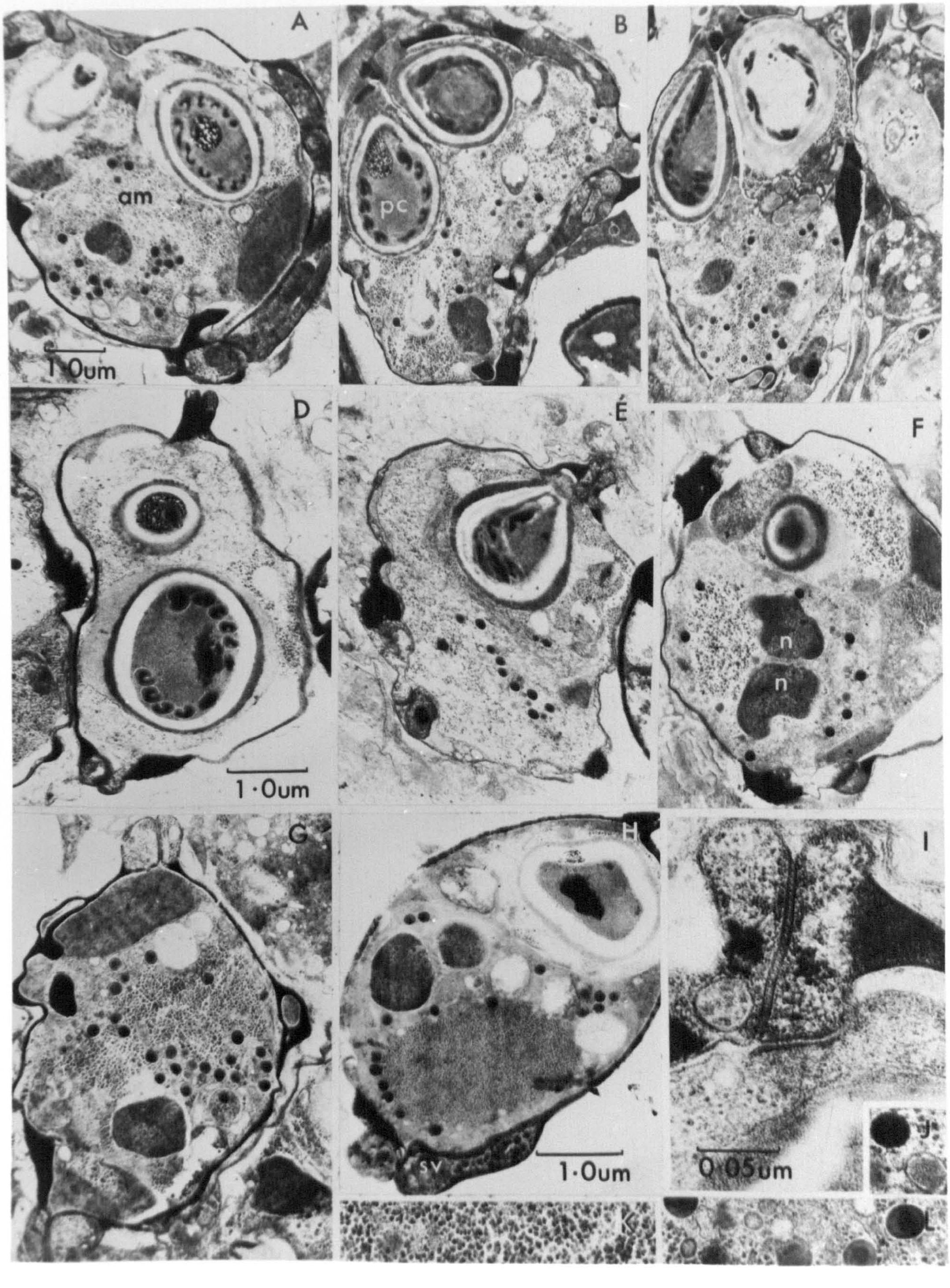

A
am
1·0um
B
pc
D
1·0um
É
F
n
n
G
1·0um
H
sv
I
0·05um
K
L

PLATE 33

Development of the polar capsules of Myxobolus exiguus spores, within the capsulogenic cells

A. Developing spores within the plasmodium showing the capsulogenic cells (cp), the electron lucent capsular primordia (cpr) and the nuclei (n) of the capsulogenic cells.
X 10,335

B. Capsular primordium with electron lucent outer region, median electron dense ring and inner granular matrix.
X 10,335

C. Two capsular primordia (cpr), with profiles of the external tube (ext), within the cytoplasm of the capsulogenic cell.
X 9,646

D. Capsular primordium with outer electron lucent region and inner granular matrix, and longitudinal profile through the external tube.
X 15,953

E. Detail of longitudinal section through the external tube (ext), with the electron dense material developing within the lumen of the tube.
X 15,953

F. Section through the capsular primordium with transverse sections through the external tube showing the electron dense material within the lumen of the external tube.
X 12,614

G. Transverse section through both capsular primordia of one developing spore.
X 5,713

H,I,J,K. Stages in the development of the polar capsule. Outgrowth of the external filament from the capsular primordium to give a three-layered structure having an outer electron dense margin continuous with the polar filament, an electron lucent median layer and electron dense core continuous with the electron dense lumen of the polar filament. In longitudinal section the external tube (ext), is continuous with the capsular primordium. Profiles of the polar filament also occur in the cytoplasm of the capsulogenic cell.
H X 2,226
I X 2,583
J X 15,953
K X 15,953

L. Transverse section through two capsular primordia with the developing polar filament.
X 12,614

M. Longitudinal section through the capsular primordium and external tube with the electron dense inner lumen of the filament developing.
X 15,953

N. Polar capsule with the filaments (pf), inverted and coiled within the inner lumen (3), of the polar capsule. The three-layered structure of the capsule is apparent.
X 25,970

cp = capsulogenic cell
cpr = capsular primordium
ext = external tube
n = nucleus

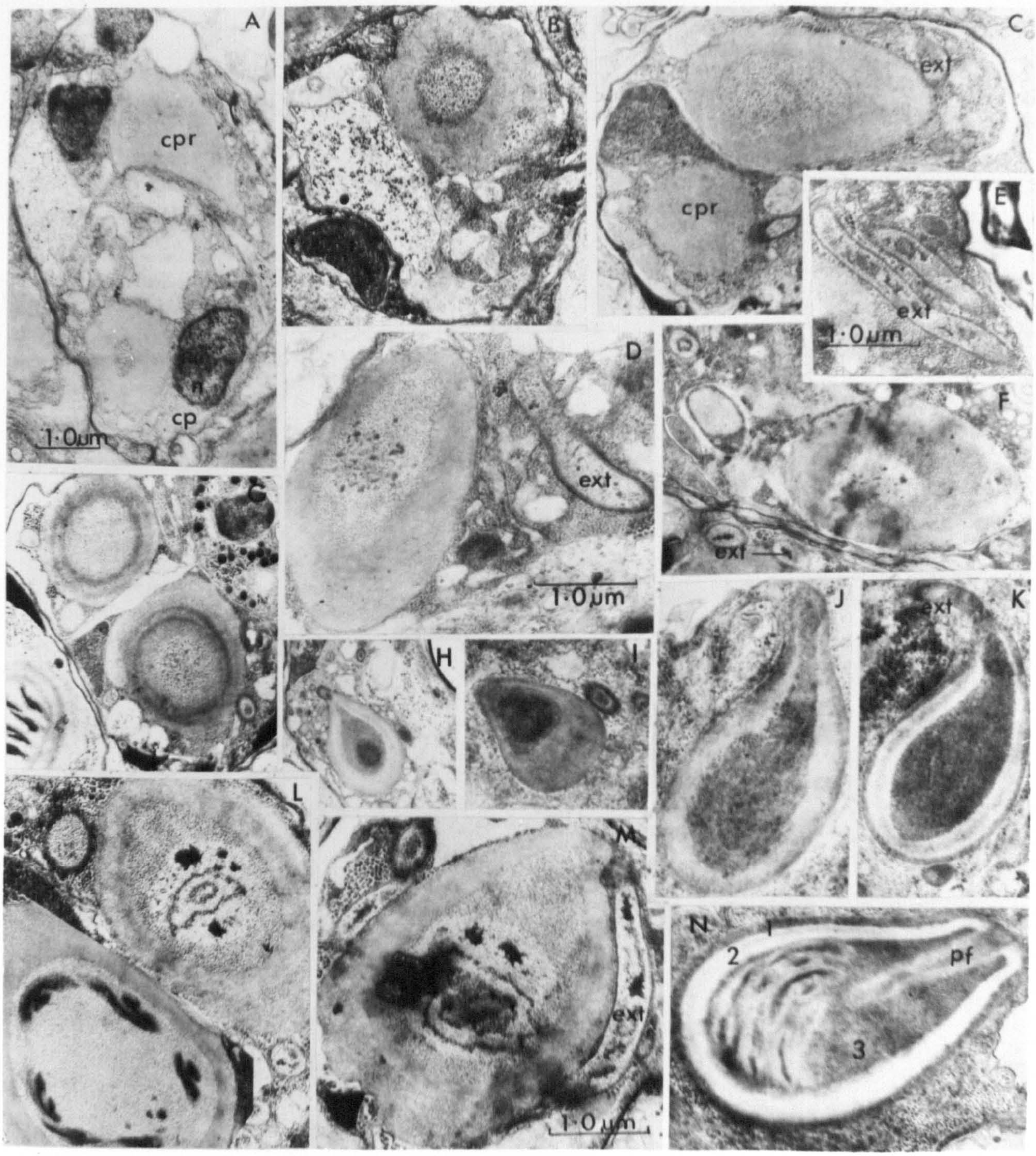

A
cpr
n
cp
1·0µm
B
C
ext
cpr
E
ext
1·0µm
D
ext
1·0µm
F
ext
H
I
J
K
ext
L
M
ext
1·0µm
N
1
2
pf
3

PLATE 34

Ultrastructure of the mature polar capsule of Myxobolus exiguus spores

A. The unequal development of the two polar capsules within the same spore. The capsule on the left is mature whereas the right hand capsule is still developing within capsulogenic cell (cp).
X 18,060

B. The polar capsule after the polar filament has been extruded with urea. The outer electron dense wall of the capsule (1) can be seen to be continuous with the polar filament (pf) and the lumen of capsule and filament are hollow. The filament leaves the spore through the shell valve cell (arrow). The shell valve cells (sv), can also be seen to have separated.
X 20,640

C. The polar capsule cut obliquely showing the coils of the polar filament within the inner granular layer (3). Part of a more coarsely granular inner layer is evident. The filament coils appear as a figure of 8 in cross section due to the helical twisting of the filament.
X 29,400

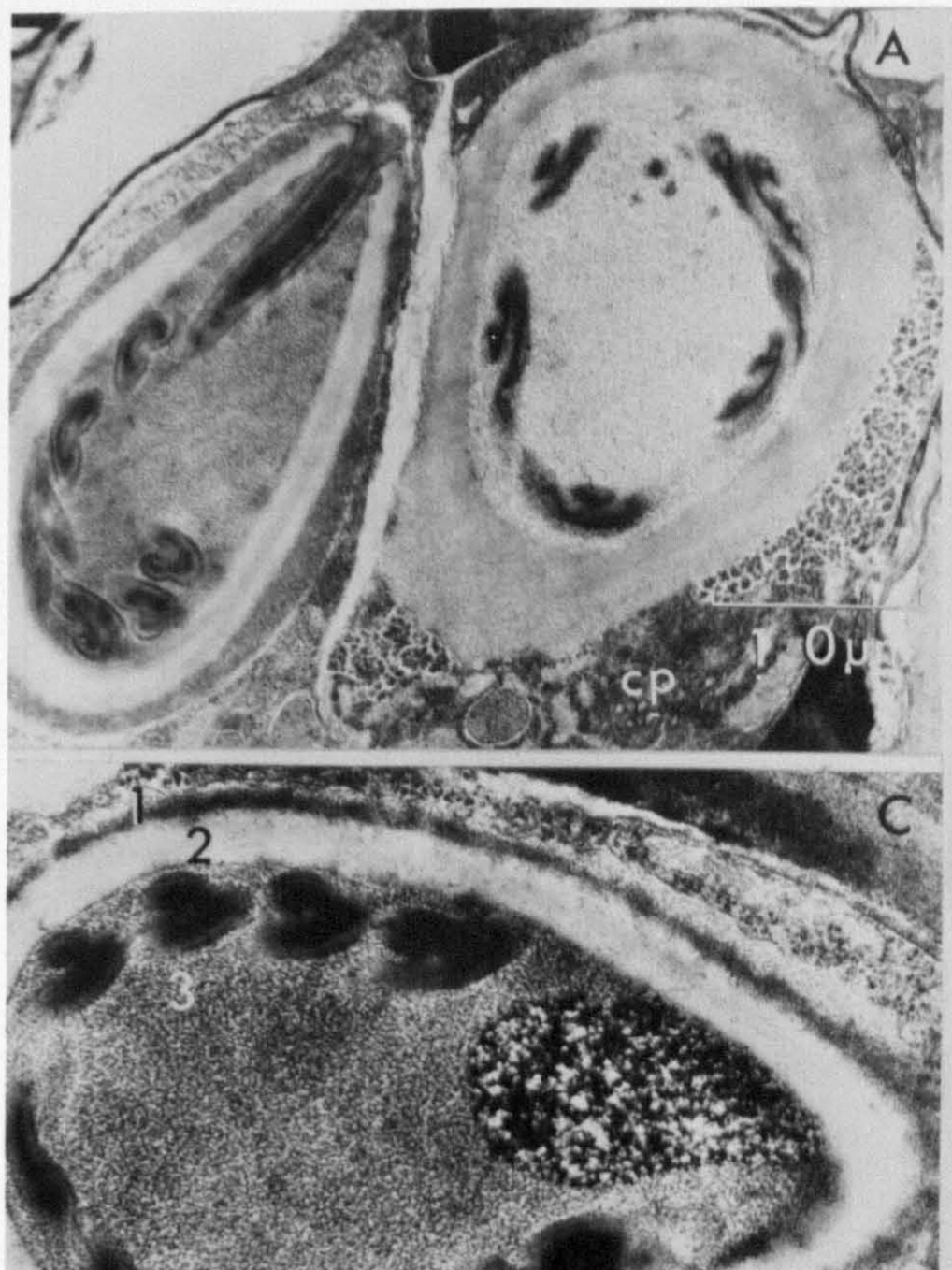
D. Longitudinal section through the polar capsule showing the filament coiled within the inner layer 3 of the polar capsule and within this what appears to be a hollow lumen.
X 29,400

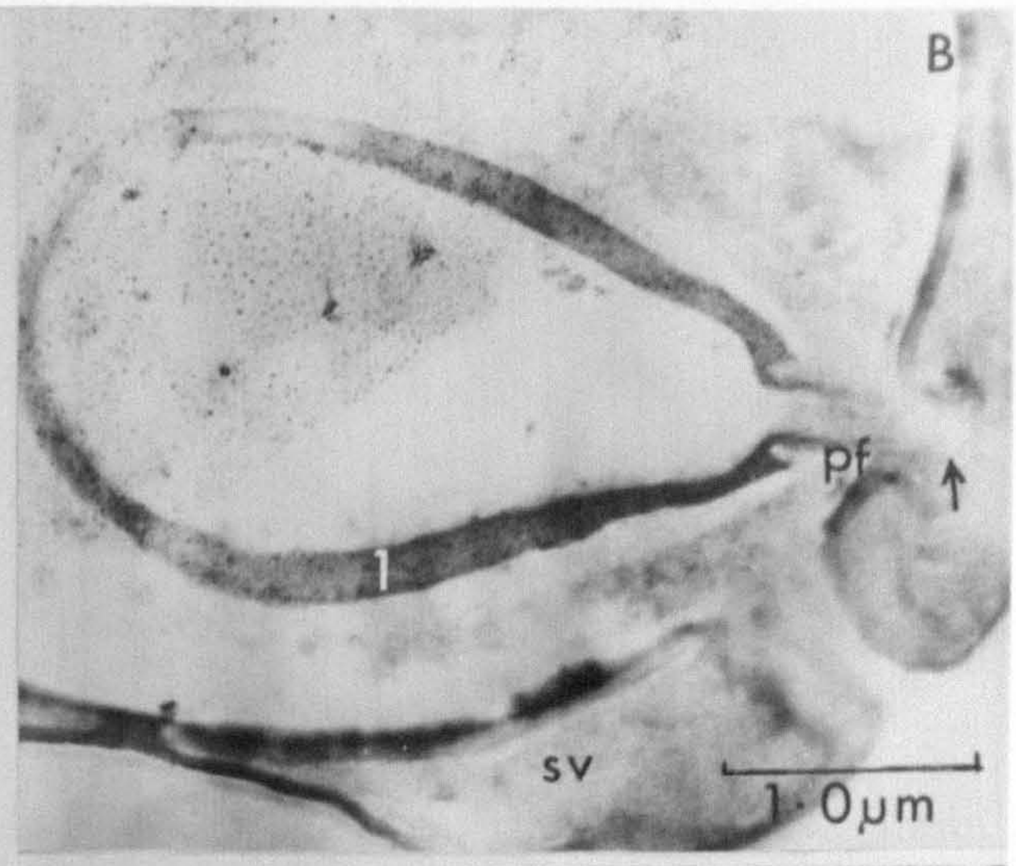
E. Longitudinal section through the polar capsule showing its three layered structure with hollow lumen.
X 29,400

F. Structure of the polar filament with the outer electron dense margin continuous with the outer electron dense wall (1) thickened in the polar capsule. The polar filament lumen is filled with an electron dense homogeneous material, which is lost on extrusion as the extruded filament is hollow (B). The filament is enveloped within a sleeve of the inner granular material of layer 3 of the polar capsule.
X 45,000

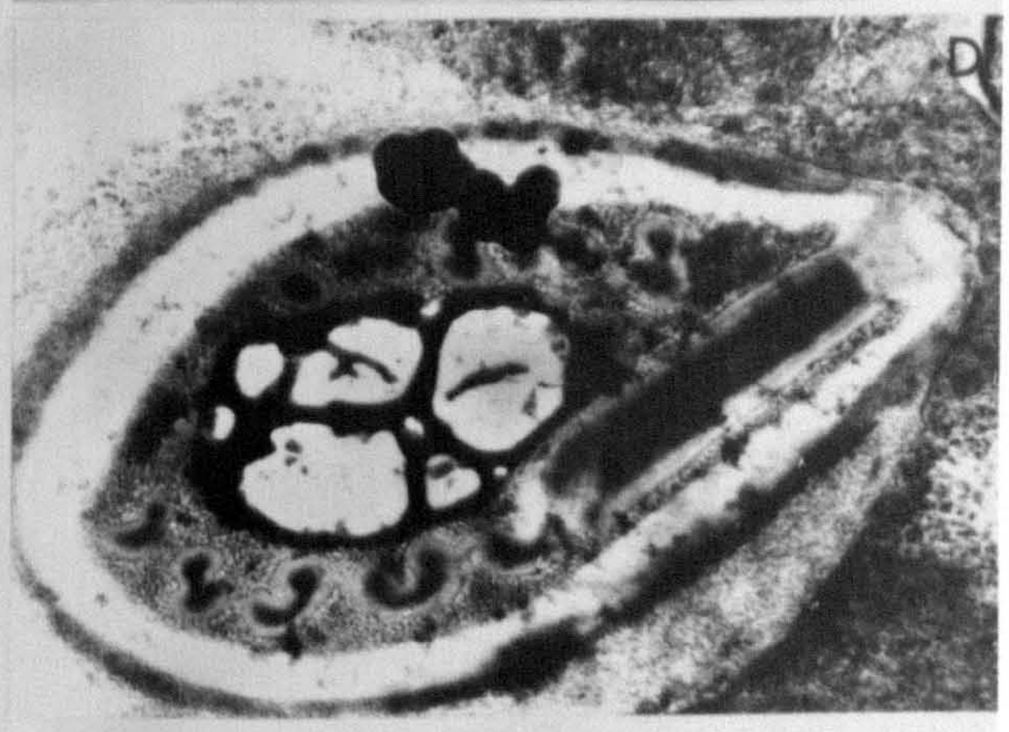
G. Longitudinal section through the polar capsule and the shell valves with the cytoplasm of the capsulogenic cell (arrow) bulging into the shell valve cell at the point at which the filament is extruded from the spore.
X 24,300

cp = capsulogenic cell


A
cp
1·0μ


B
1
pf
sv
1·0μm

C
1
2
3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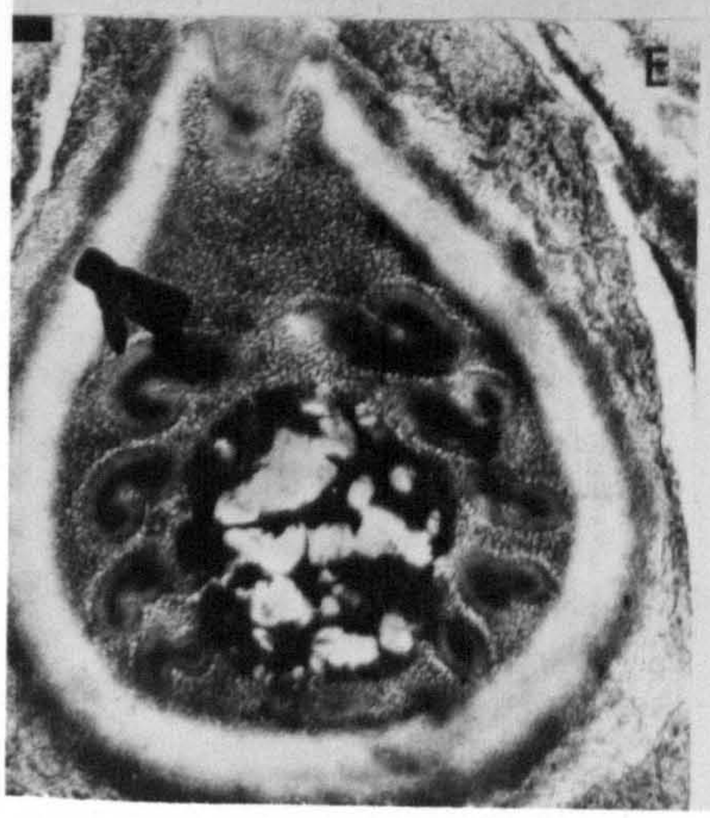
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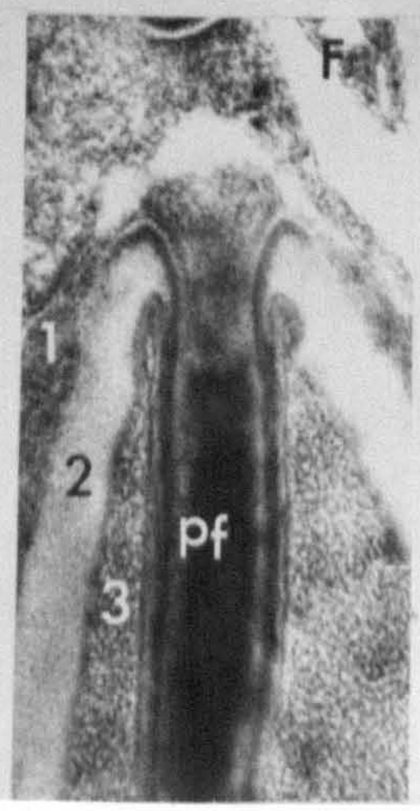

F
1
2
pf
3

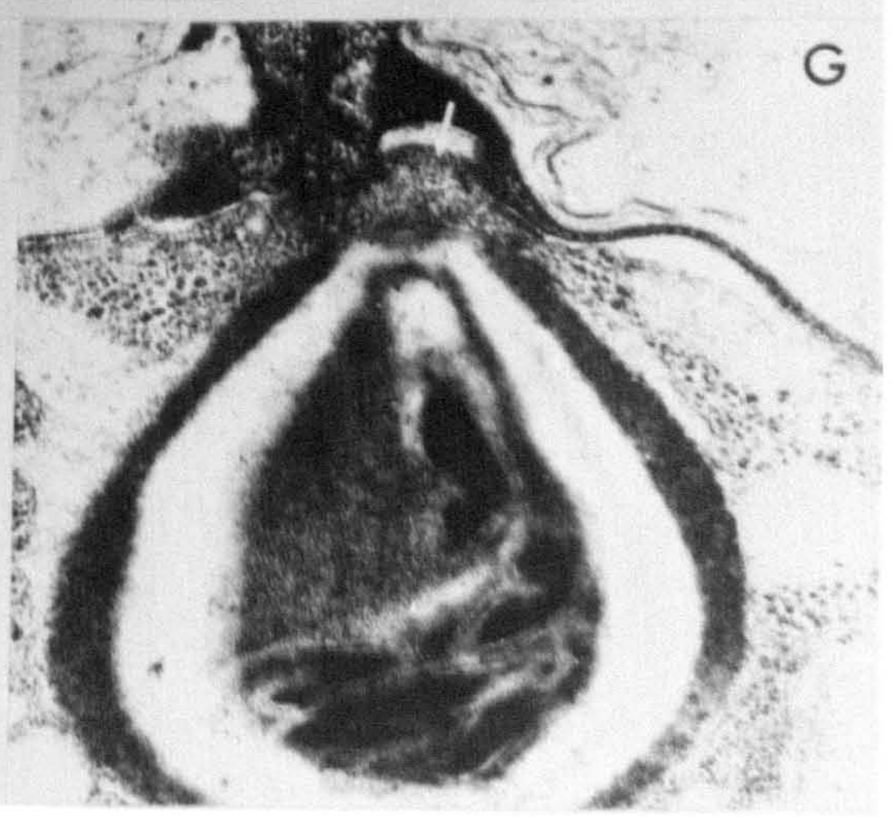

G

PLATE 35

Development of Myxobolus exiguus in the skin of Crenimugil labrosus

A. Transverse section of the plasmodium beneath the dermis showing the numerous protoplasmic extensions of the ectoplasm of the plasmodium (p) into the dermis.
X 4096

B. Transver section through the plasmodium with the outer ectoplasmic and inner endoplasmic layers with the generative cells (cr) and developing sporoblasts (spr).
X 4096

C. Surface of the ectoplasm with the long protoplasmic processes of the ectoplasm extending among the dermal cells. Collagen filaments (fs), form a network among the extensions of the ectoplasm.
X 10,649

D. Ectoplasmic layer of the plasmodium showing the extremely vacuolated nature.
X 17,408

E. Developing pansporoblast (psm), with developing sporoblast cells (spr) within the pansporoblast membrane.
X 10,649

F. Pansporoblasts with two young spores developing within each pansporoblast.
X 4,733

G. Irregular spore with four shell valves with thick fuzzy coat on each surface, shell valves did not form complete spore and the amoebula (am) is not enclosed by the spore.
X 7,884

H. Irregular spore with complete shell with five parts, enclosing three polar capsules but no clearly differentiated amoebula.
X 12,320

am = amoebula
dr = dermis of fish skin
ect = ectoplasm
en = endoplasm
fb = fibroblast
fs = collagen fibrils
ge = generative cell
p = protoplasmic extension ectoplasm
pc = polar capsule
psm = pansporoblast
spr = sporoblast
sv = shell valves
va = vacuole

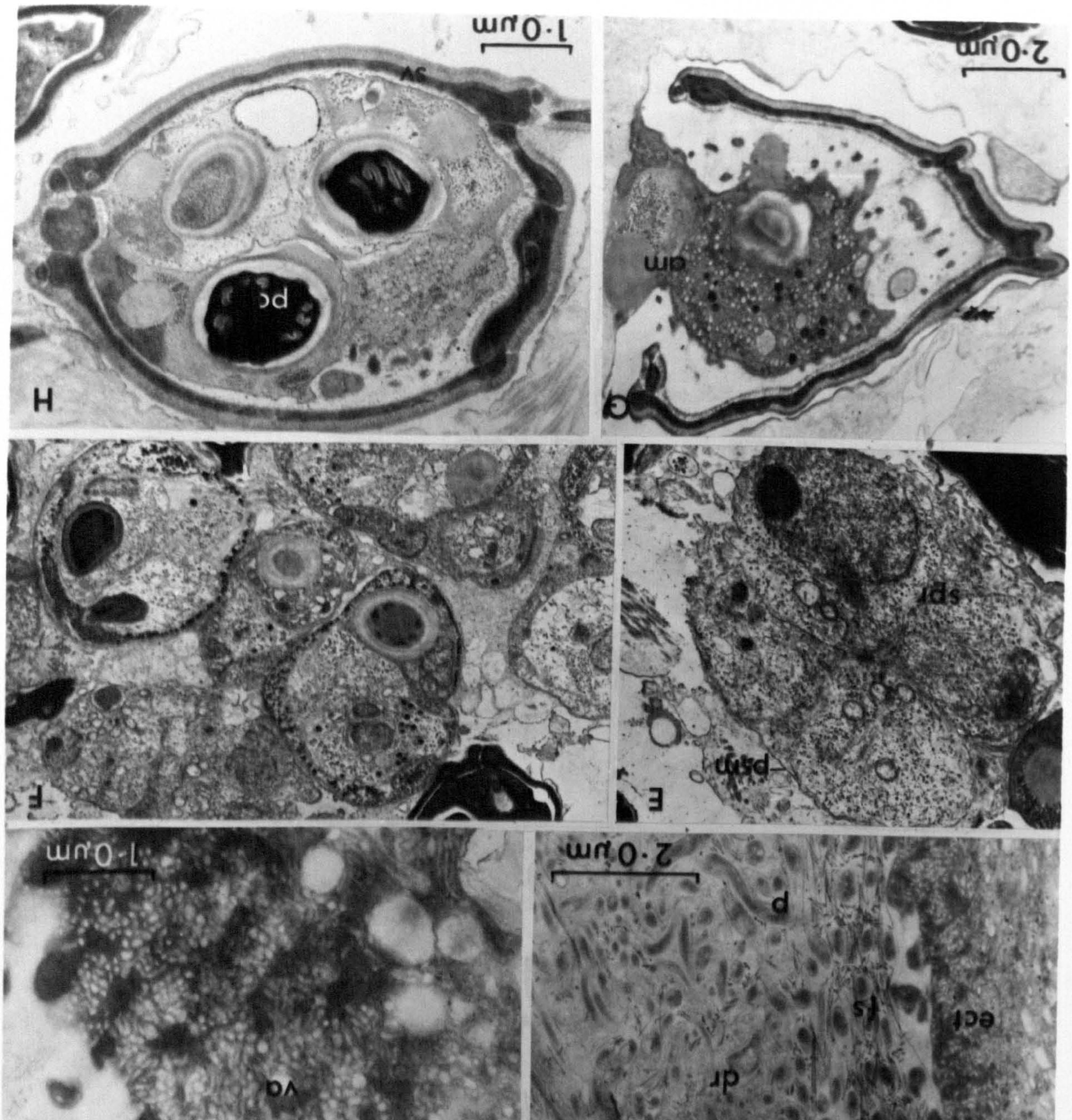

1·0 µm
sv
pc
H
2·0 µm
am
G
F
sp
psm
E
1·0 µm
va
D
2·0 µm
p
dr
fs
ect
C
5·0 µm
spr
ge
en
ect
B
5·0 µm
p
ect
fs
tb
dr
A

PLATE 36

Development of Myxobolus exiguus in the scales of Crenimugil labrosus

A. Plasmodium in the scale tissue.

X 3,825

B. Ectoplasm of the scale plasmodium showing homogeneous structure.

X 7,956

C. Ectoplasm of scale plasmodium and scale connective tissue.

X 9,945

D. Actively synthesising fibroblast associated with plasmodium

X 4,819

E. Deposition of melanin granules around the plasmodium.

X 7,956

F. Melanophores around the plasmodium.

X 4,819

G. Developing pansporoblasts in the endoplasm.

X 7,956

H. Developing pansporoblasts in the endoplasm.

X 7,956

I. Pansporoblast within the pansporoblast membrane.

X 13,005

J. Mature spore, of which there were few in the scale plasmodium.

X 7,956

ect. = ectoplasm
en. = endoplasm
fb. = fibroblast
mg. = melanin granule
mr. = melanophore

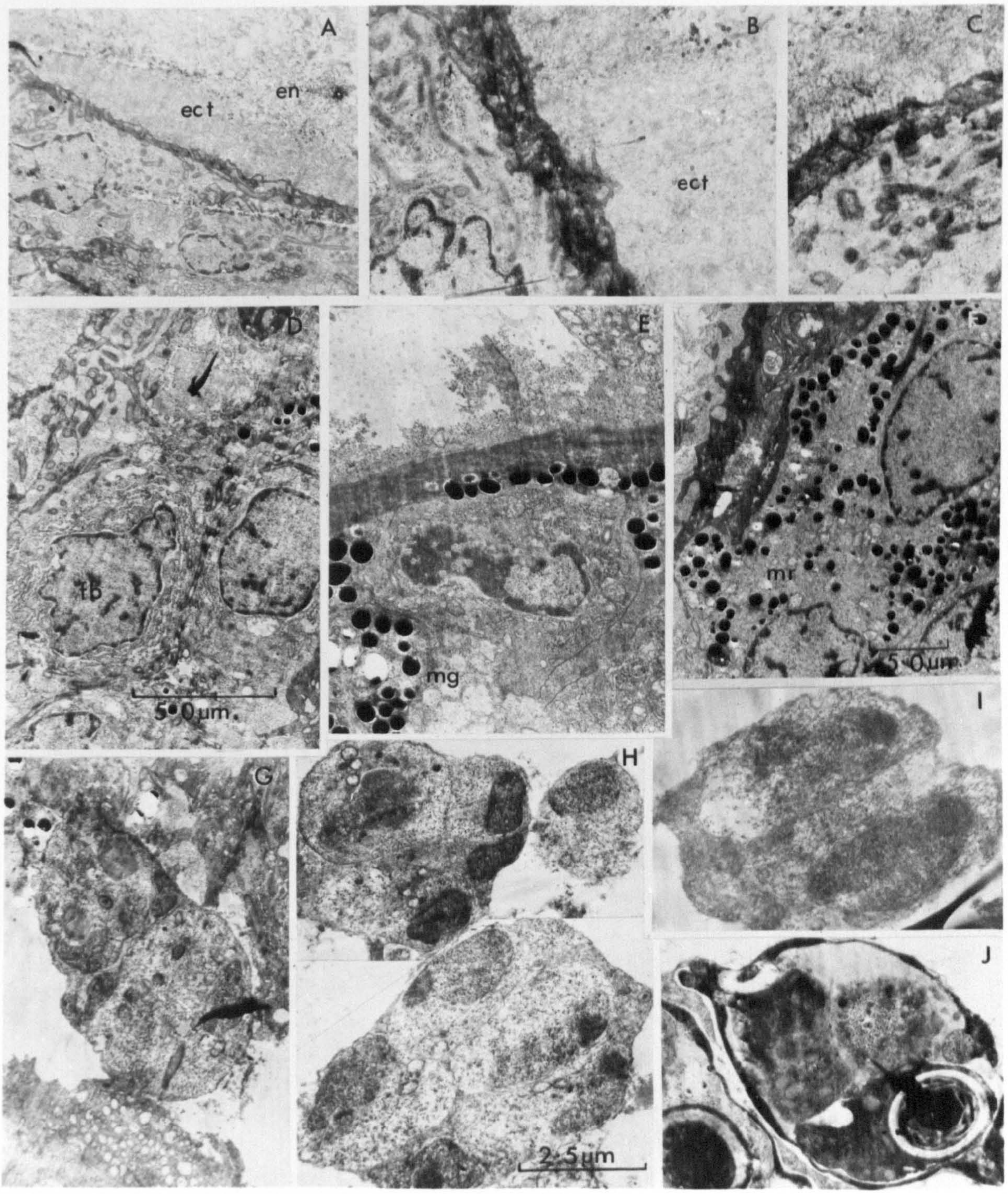

A
en
ect
B
ect
C
D
tb
5·0 µm
E
mg
F
mr
5·0µm
G
H
2·5µm
I
J

PLATE 37

Development of Myxobolus exiguus on the gut of Crenimugil labrosus

A. Plasmodium developing in the gut surface connective tissue.

X 2,082

B. Lysis of gut connective tissue cells in direct contact with the ectoplasmic layer of the plasmodium.

X 3,900

C. Vacuolated nature of the ectoplasmic layer of the Plasmodium

X 4,914

D. Ectoplasm of the plasmodium showing fine channels, (ch), surface glycocalyx, and associated gut cell.

X 8,112

E. Ectoplasm with channels.

X 8,112

F. Vacuolated ectoplasm with generative cells.

X 4,914

G. Developing spores within the endoplasm.

X 6,006

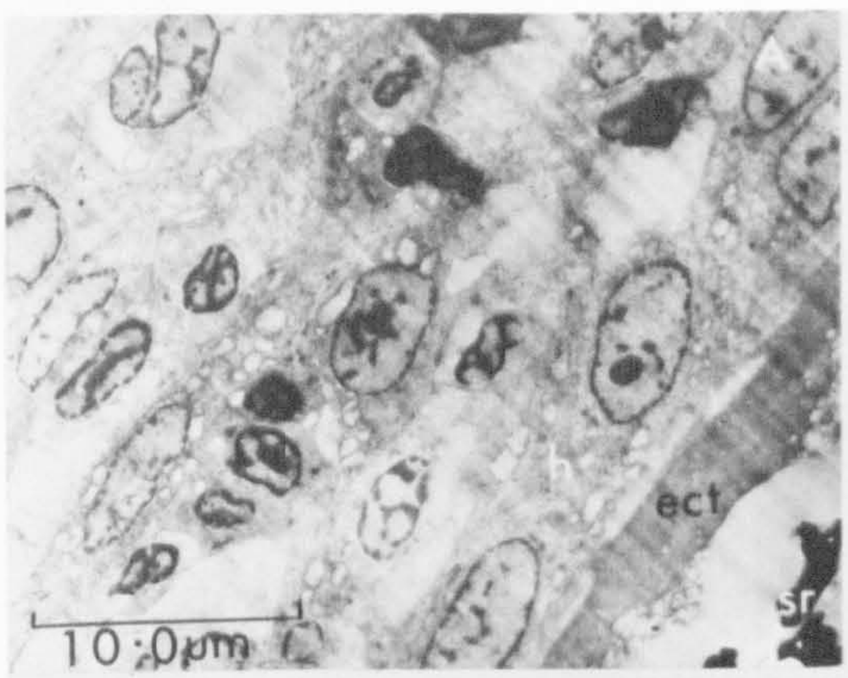
H. Vacuolated ectoplasm with mitochondria and closely associated host cell (h).

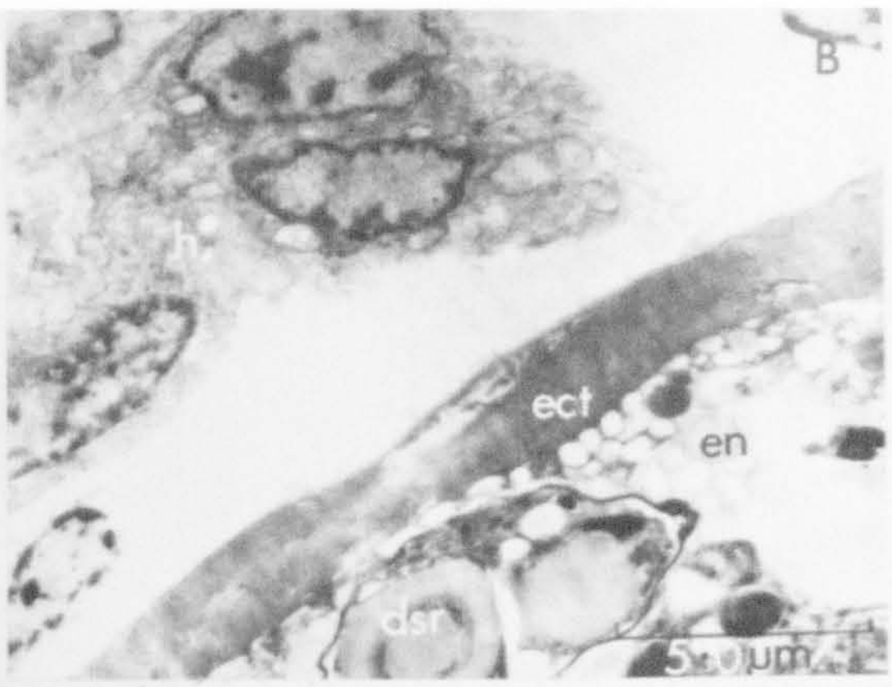
X 8,112

I. Mature spore from the gut plasmodium showing the paired polar capsules and amoebula.

X 10,140

am. = amoebula
ch. = channels in the ectoplasm.
dsr.= developing spore
en. = endoplasm
ect. = ectoplasm
ge. = generative cell
m. = mitochondrion
pl. = polar capsule
sr. = spore
va. = vacuole


10·0µm
h
ect
sr


B
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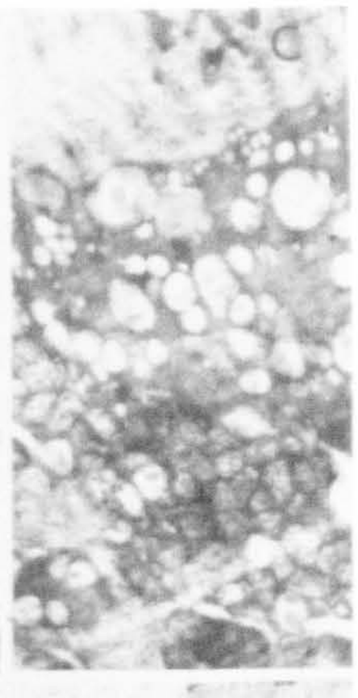
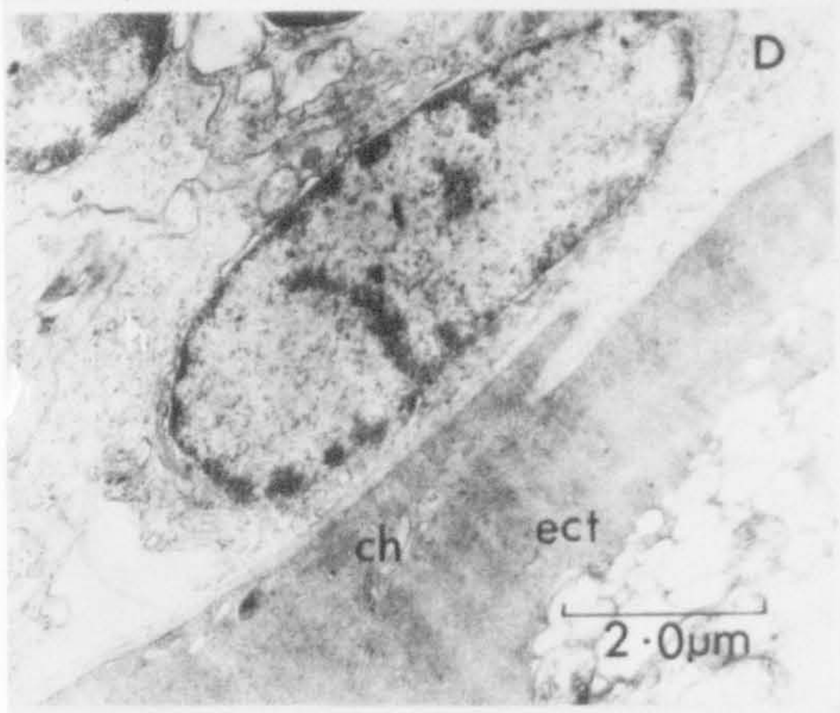
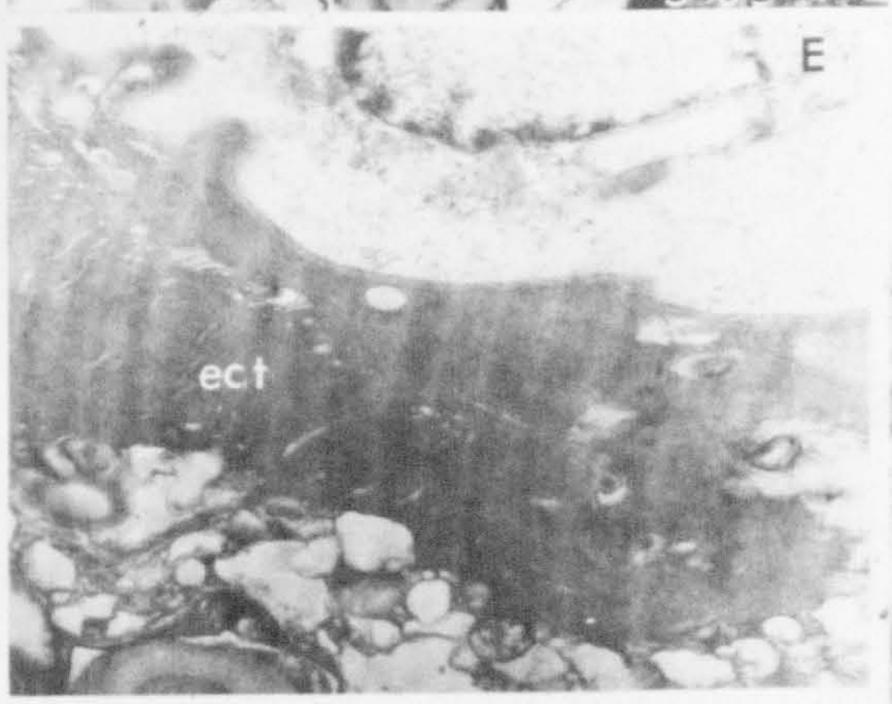

C


D
ch
ect
2·0µm


E
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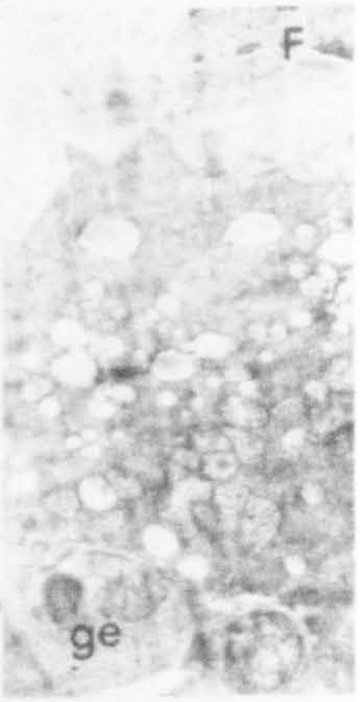
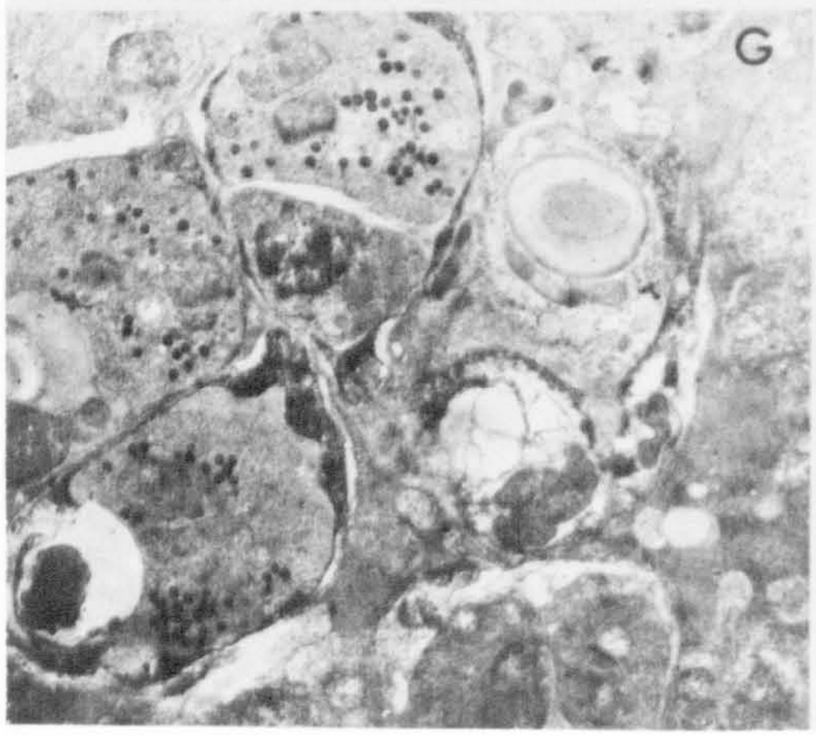

F
ge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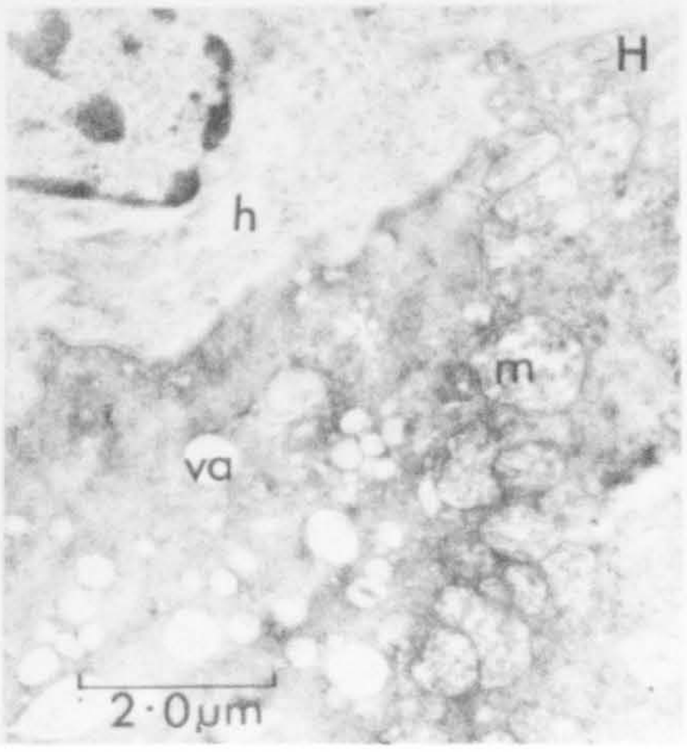

H
h
m
va
2·0µm

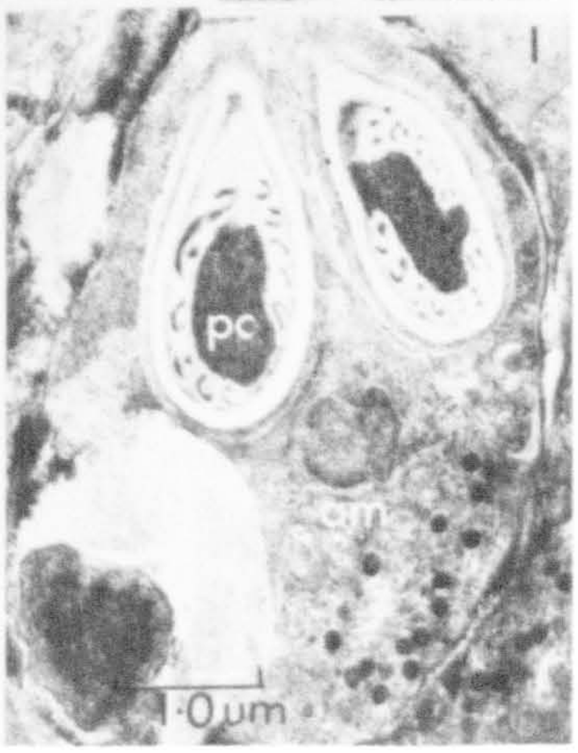
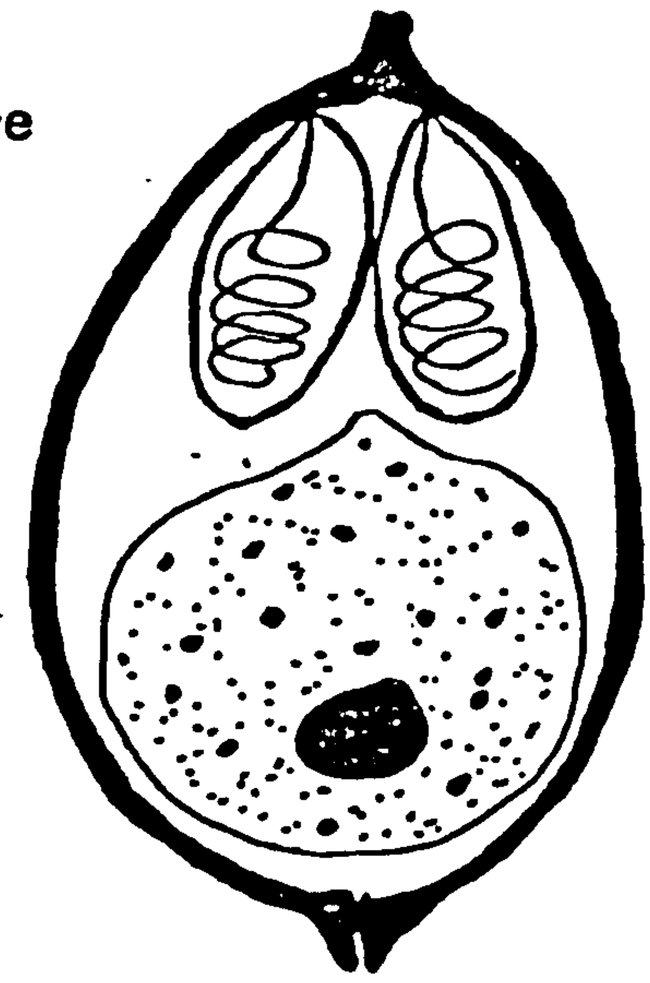

I
pc
1·0µm

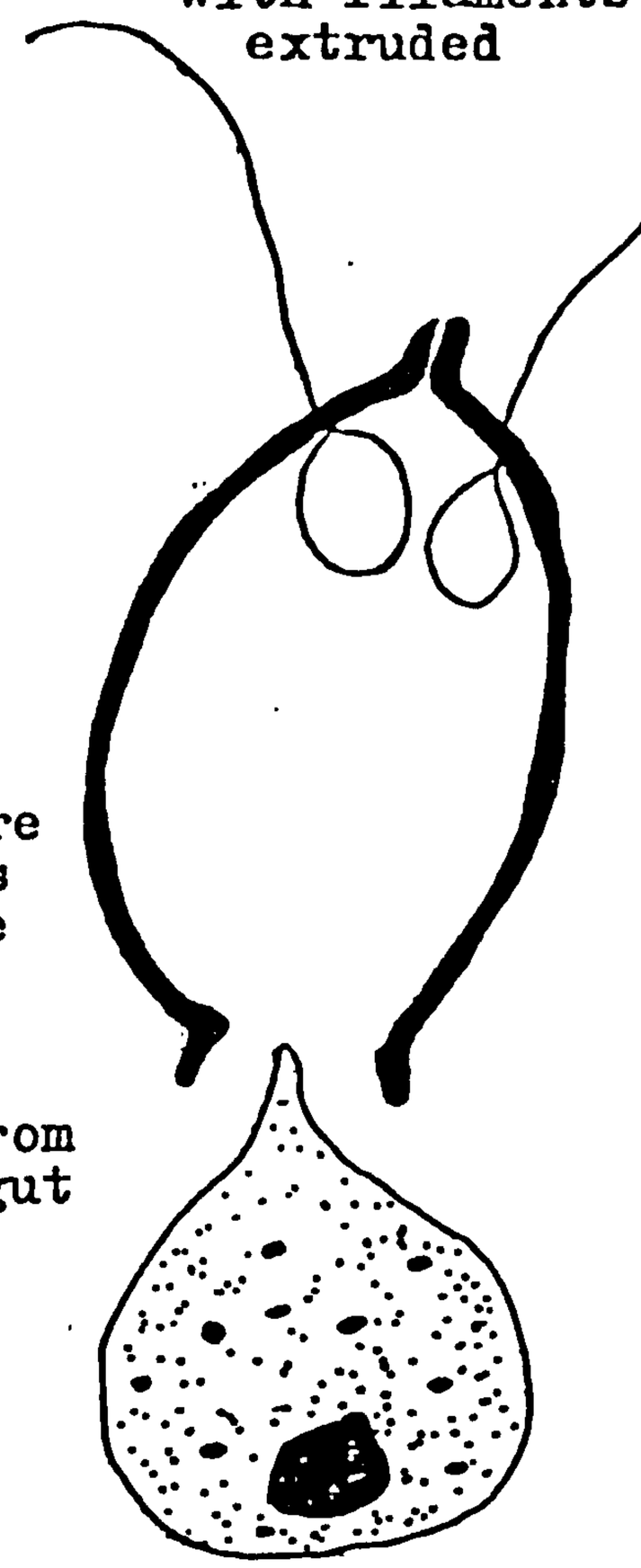
Fig. 5

Life Cycle of Myxobolus exiguus

1. Mature spore



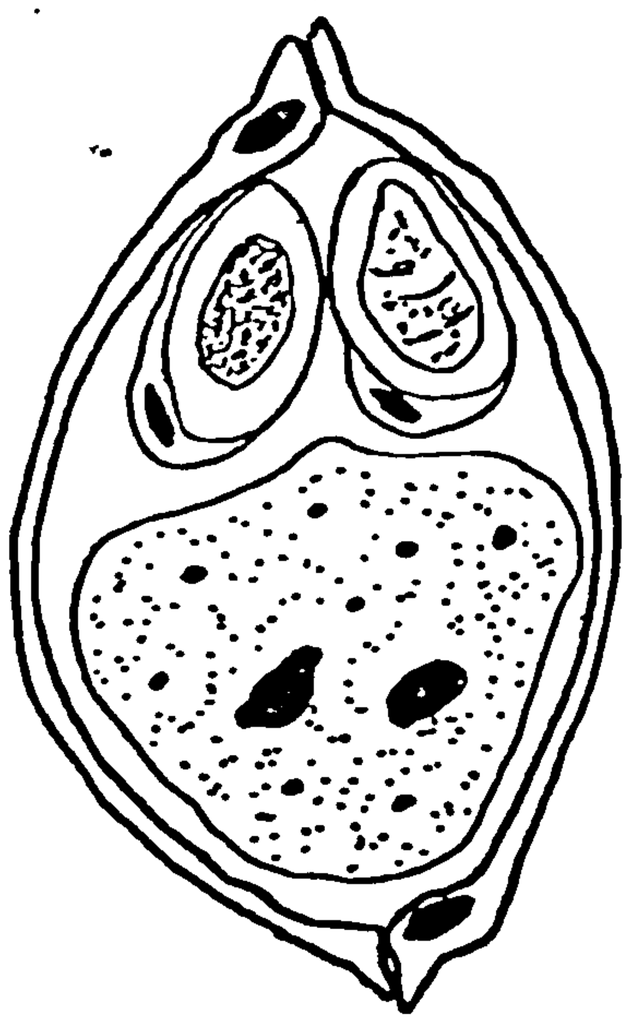
2. Polar capsules with filaments extruded



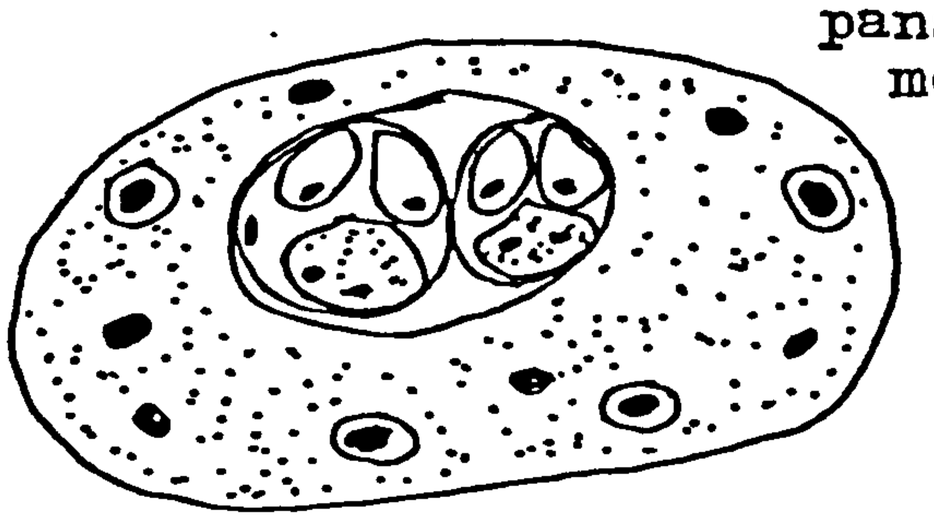
3. Spore valves separate

4. Amoebula escapes from spore in gut of fish.

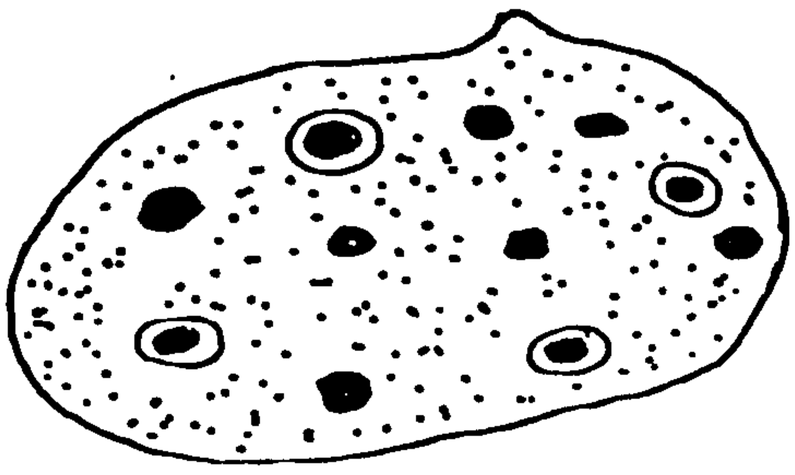
9. Immature spore



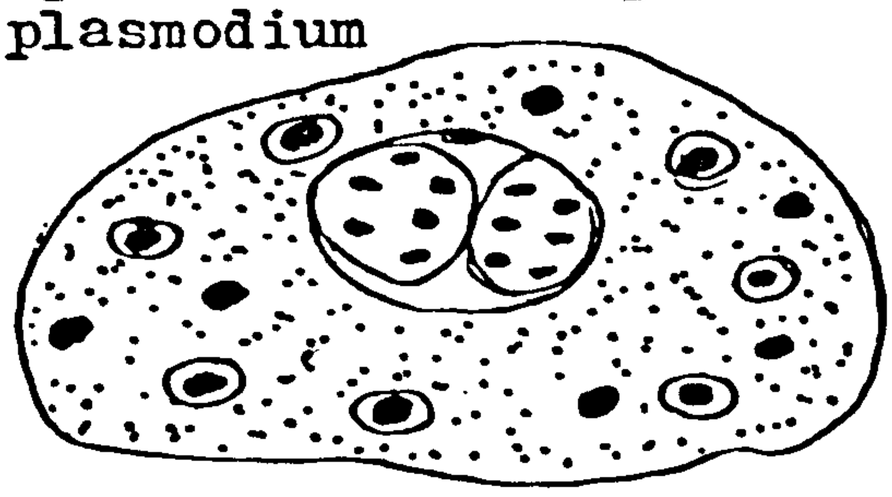
8. Two spores develop within pansporoblast membrane



5. Plasmodium grows in intercellular site in host tissue.



7. Sporogenesis- disporous pansporoblast develops in plasmodium

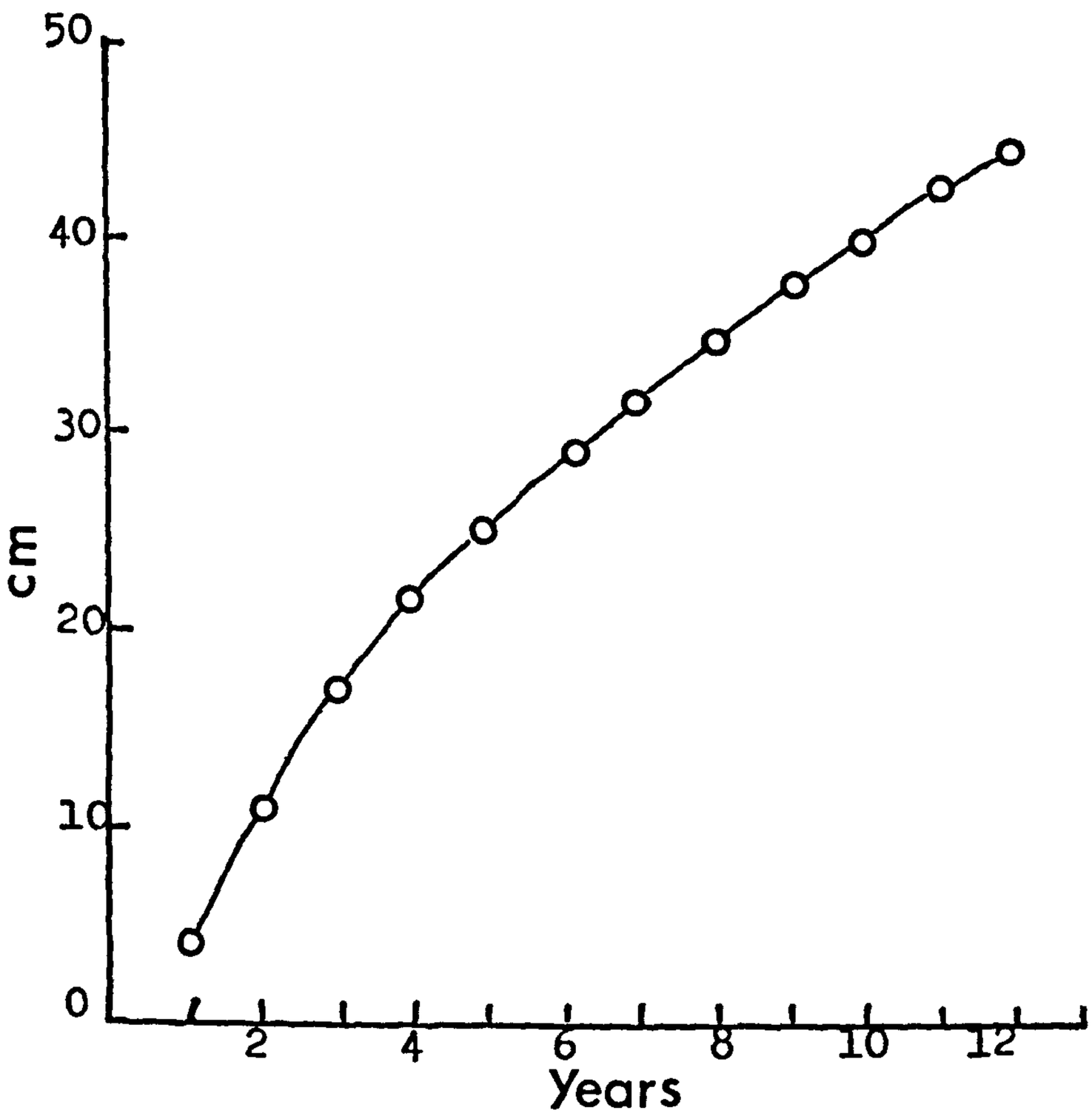


6. Vegetative and generative nuclei present in plasmodium

Fig. 9

Growth of C.labrosus deduced from fork length.

Taken from Hickling (1970).



Summer infections of M. exiguus in grey mullet from the Tamar Estuary

A total of 972 grey mullet of the three species, (Crenimugil labrosus; Liza ramanda; Liza auratus), were sampled from the Tamar Estuary during the period February-August 1975.

C. labrosus was the most common species, comprising 83% of the fish sampled, and was the only species present in all age classes throughout the six month sample period. Fig 6 therefore represents the incidence of M.exiguus in C.labrosus during the sample period. The age classes were determined from the total length measurements of the fish and compared with Hickling's (1970), graph of the growth rates of this slow-growing fish, which he attributed to the poor diet. The annual rings of the scales have been found to be an unreliable method of ageing this fish (Hickling, 1970).

The site of infection was also recorded, and the gut appeared to be the primary site of infection and the only site recorded from 0-1 group fish. The interlamellar site on the gills was the next site infected and infected fish of the 1-2 and 2-3 age classes commonly bore cysts on the gills, although usually accompanied by a gut infection. Cysts on the gills were not usually found in 3-4 group fish, and possibly become detached during feeding activities of this fish, when mud is strained over the gill rakers, (Hickling, 1970).

The connective tissue between the fin rays frequently bore conspicuous cysts in the 3-4 group fish, and histological studies revealed a variety of internal connective tissue sites may be infected.

Summer infections of Grey Mullet from the Tamar Estuary with *Myxobolus exiguus*

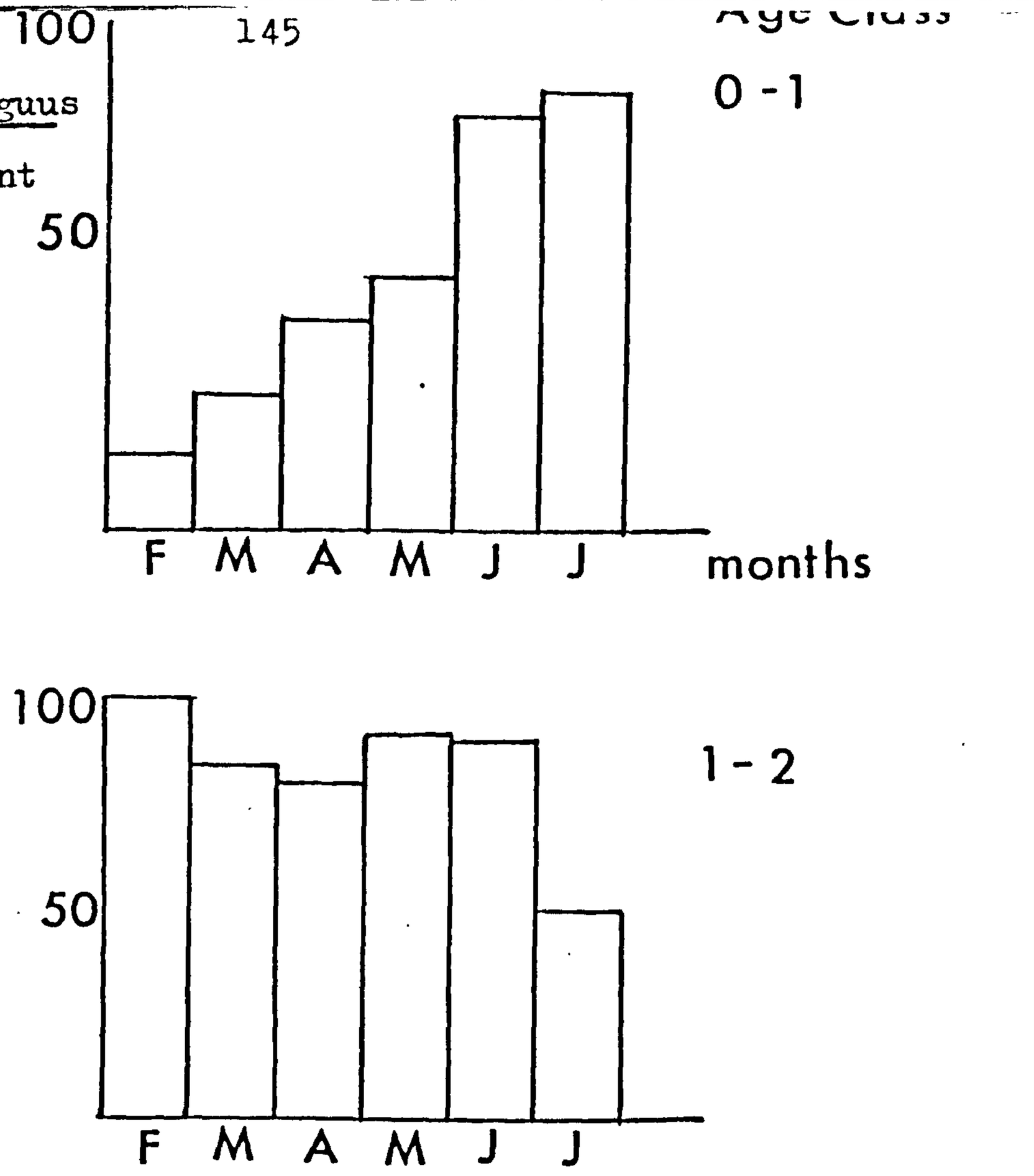
Month	Species	Size Range (cms)	Age Class	Total No. Fish	Infected No.	Infected %	Total No. *C. labrosus*	Infected No.	Infected %	Site
February	C.l.	3.5-5.5	0-1	55	10	19	55	10	19	gut
	C.l.	8.1	1-2	1	1	100	1	1	100	gut & gills
	C.l., L.r.	9.9-12.3	2-3	16	15	94	15	14	94	gut & gills
	C.l., L.a.	16.9-22.0	3-4	4	4	100	3	3	100	gut & connectiv tissue
March	C.l.	3.9-5.9	0-1	50	15	33	50	15	33	gut
	C.l., L.a.	6.1-8.9	1-2	62	53	86	49	41	84	gut & gills
	C.l., L.a., L.r.	10.3-14.0	2-3	35	33	95	30	29	97	gut & gills
	C.l., L.a.	15.0-22.4	3-4	51	41	81	36	30	84	gut & connectiv tissue
April	C.l.	4.0-6.0	0-1	12	5	45	12	5	45	gut
	C.l., L.a.	6.1-8.8	1-2	41	32	80	28	22	80	gut & gills
	C.l., L.a.	8.9-13.0	2-3	51	31	61	27	21	78	gut & gills
	C.l., L.a.	15.0-22.4	3-4	35	32	92	26	26	100	gut & connectiv tissue
May	C.l.	4.1-6.1	0-1	18	9	50	18	9	50	gut
	C.l.	6.2-9.8	1-2	33	30	91	33	30	91	gut & gills
	C.l., L.a.	9.9-13.0	2-3	80	77	97	79	76	97	gut & gills
	C.l., L.a.	16.0-21.5	3-4	20	17	85	8	8	100	gut & connectiv tissue

Month	Species	Size Range (cms)	Age Class	Total No. Fish	Infected No.	Infected %	Total No. C. labrosus	Infected No.	Infected %	Site
June	C.l.	4.4-6.3	0-1	15	12	79	15	12	79	gut
	C.l., L.a.	6.4-9.0	1-2	39		84			89	gut & gills
	C.l., L.a., L.r.	9.3-13.8	2-3	122	79	65	114	72	64	gut & gills
	C.l., L.a.	15.2-23.0	3-4	38	19	50	33	29	88	gut & connecti tissue
July	C.l.	4.3-6.4	0-1	20	16	79	20	16	79	gut
	C.l.	7.0-8.7	1-2	4	2	50	4	2	50	gut & gills
	C.l., L.r.	9.6-15.0	2-3	119	89	75	113	83	74	gut & gills
	C.l., L.r.	15.9-21.9	3-4	26	10	39	10	9	90	gut & connecti tissue
August	C.l.	2.9-3.3	0	25	-	-	25	-	-	-
TOTALS				972			804			

C.l. = Crenimugil labrosus (thick-lipped grey mullet)
L.a. = Liza auratus (golden grey mullet)
L.r. = Liza ramanda (thin-lipped grey mullet)

Fig. 6

Incidence of M.exiguus within the different age classes of C.labrosus during summer months from the Tamar Estuary.



The ultrastructure of Nosemoides sp. a microsporidian hyperparasite of Myxobolus exiguus

Studies on the ultrastructure of M. exiguus revealed an interesting example of hyperparasitism by a microsporidian species of Nosemoides (Vivier 1975). This microsporidian parasite was found in some plasmodia examined from the gills and gut. The species is described here for the first time.

The Spore

Individual spores were found singly in the endoplasm of M. exiguus (Pl. 38A). In general structure and organisation they were typical of Microsporidia as described by Vavra (197). The spore was ovoid in shape measuring 2.7 x 1.4 um, and bounded by a smooth trilaminar wall consisting of the outer electron dense exospore, an electron lucent endospore measuring 0.04 um in width and a membrane bounding the cytoplasmic contents of the spore. These are composed of the sporoplasm and the extrusion apparatus.

The sporoplasm occupied the central region of the spore (Pl. 39A,B), situated between the posterior vacuole and anterior polaroplast. The single nucleus was bounded by a double unit membrane with pores (Pl. 39A) and the surrounding cytoplasm was uniformly granular, with scattered ribosomes and some rough endoplasmic reticulum profiles (Pl. 39A).

The extrusion apparatus, comprising the polaroplast, filament and posterior vacuole occupy most of the spore (Pl. 39A). The anterior third of the spore contained the polaroplast which has a conspicuous structure of tightly packed smooth membrane lamellae. The outer layers were

tightly packed and electron dense whereas the inner layers were of a more loosely arranged vesicular composition (Pl. 39A).

The basal part of the polar filament passes through the centre of the polaroplast terminating at the anchoring disc (Pl. 39Aa). The filament descends from the attachment site along the longitudinal axis of the spore making 15 coils around the sporoplasm in the central portion of the spore. The filament is 0.1 μm in diameter and consists of several concentric layers (Pl. 39F,G) in low resolution micrographs appearing as a double dense ring (Pl. 39F), lined externally by polyribosomes.

The posterior vacuole, occupying the posterior quarter of the spore was limited by a membrane and has flocculent contents. (Pl. 39A,B).

Sporogony

Early stages of sporogony were not found however it is assumed that development is apansporoblastic the sporoblast developing free within the host cytoplasm. Immature spores were found in the gut plasmodia and bounded by a simple unit membrane in early developmental stages (Pl. 38C,E,F). The cell had a round to oval nucleus, bounded by a double unit membrane and the cytoplasm contained ribosomes, endoplasmic reticulum and developing polar filament profiles (Pl. 38E). No mitochondria or reserve substances were present at any stage of development. During maturation of the spore the cell membrane transforms into a thick wall (Pl. 38D) and the inner plasma membrane of the sporoblast remains as the cytoplasmic membrane of the spore (Pl. 38D). The outer dense

coat becomes the exospore and the electron lucent area separating the plasmalemma from the exsporont membrane becomes the endospore (Pl. 38H,I).

These developing spores were often surrounded by microtubules (Pl. 38D,J).

s. mature microsporidian spore
sp. developing sporoblast
pl. plasmodium of myxosporidian
ve. vesicles or filaments surrounding exospore

PLATE 38

Ultrastructure of Nosemoides sp. hyperparasite of Myxobolus exiguus.

A. Mature and extremely electron dense spores of microsporidian, Nosemoides sp. within the endoplasm of gill plasmodium of M. exiguus.
X 8,I53

B. Spore of Nosemoides sp. lying adjacent to amoebula of M.exiguus free within the plasmodium.
X IO,I92

C. Developing sporoblast.
X I5,523

D. Developing sporoblast with thickened exospore surrounded by profiles of the surrounding vesicles or tubules.
X 2I, I68

E. Young sporoblast bound by a single membrane and showing a nucleus and cytoplasm with profiles of the developing polar filament and polaroplast.
X 2I,I68

F. Developing sporoblast and mature spores.
X I2, 230

G. Mature spore with exospore, endospore and membrane profiles of the polaroplast.
X 2I,I68

H. Mature spore with profiles of the polar filament and vesicular area of the developing polaroplast.
X 2I,I68

I. Young spore enclosed by the exospore and endospore and cytoplasm containing many ribosomes and smooth membrane profiles.
X 34,496

J. Mature electron dense spore showing some filament profiles and endospore, exospore and surrounding tubules.
X 27,440

K. Mature electron dense spore with nucleus and longitudinal filament profiles.
X 27,440

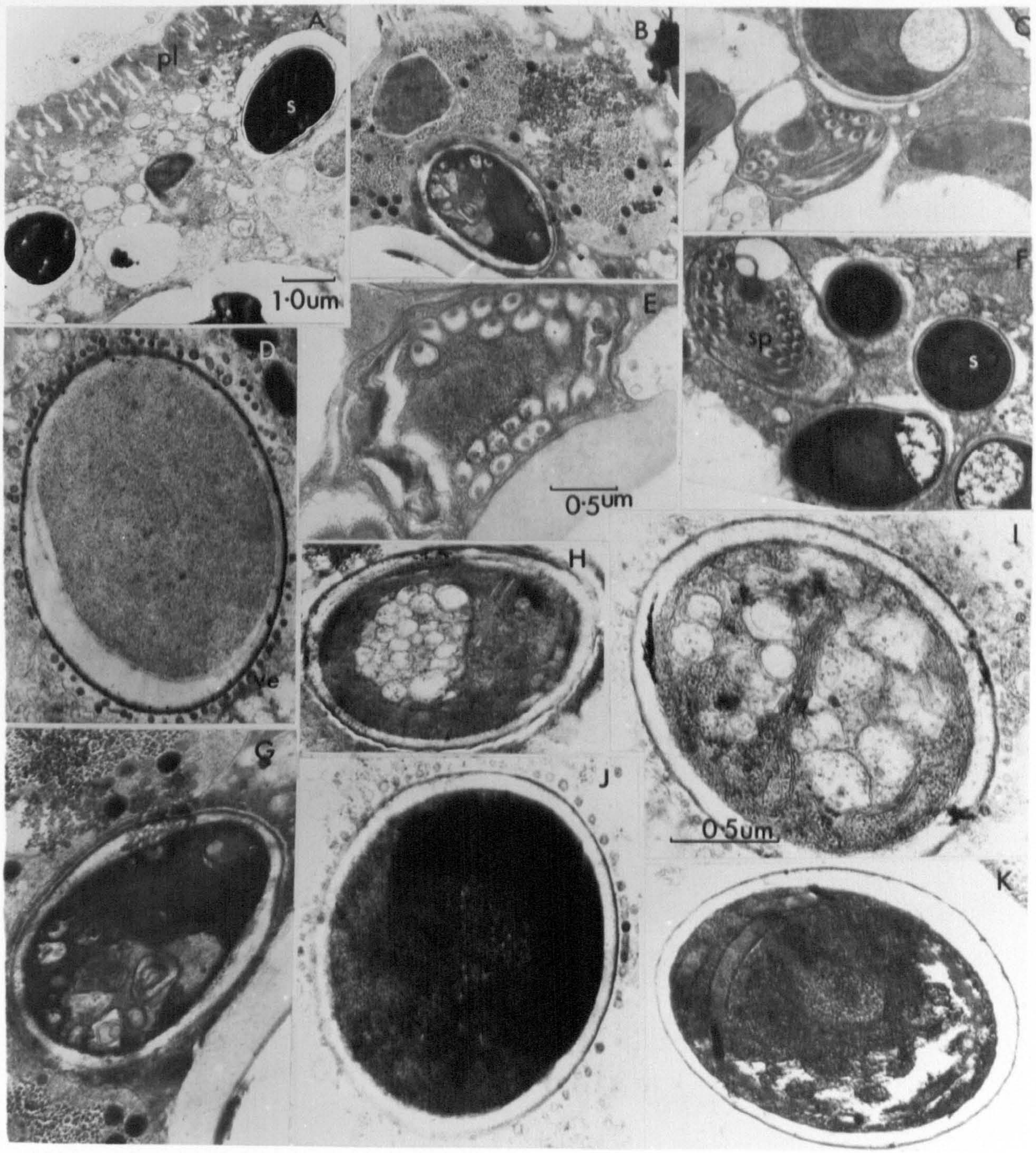

A
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PLATE 39

Ultrastructure of Nosemoides sp. spore

A. Mature spore exhibiting all the features of a typical myxosporidian spore, with the electron dense exospore (ex), and electron lucent endospore (en). The nucleus (n) is bounded by a double membrane and the cytoplasm contains many ribosomes particularly around the polar filament (pf) profiles. The polaroplast (ps) shows the outer electron dense closely applied lamellae, and the inner more loosely arranged lamellae.

X 49,245

a. Detail of the umbrella shaped attachment zone or anchoring disc of the polar filament.

X I8,572

B. Mature spore with nucleus, posterior vacuole, polar filament profiles. The polaroplast represented by an electron dense anterior region.

X 37,989

C. Polar filament penetrating the spore wall and exhibiting a double structure, with an outer filament tube and an inner filament lumen.

X 75,978

D. Young spore with nucleus, cytoplasm with numerous ribosomes, and smooth membrane profiles of the developing polaroplast.

X 30,250

E. Profile of polar filament penetrating the spore wall, possibly by disruption during preparation as the whole filament has not everted but remains as profiles within the young spore. Cytoplasm contains many ribosomes and numerous smooth membrane profiles of the developing polaroplast.

X 64,440

F. Transverse section of the polar filament showing it as a double dense ring.

X 6I,908

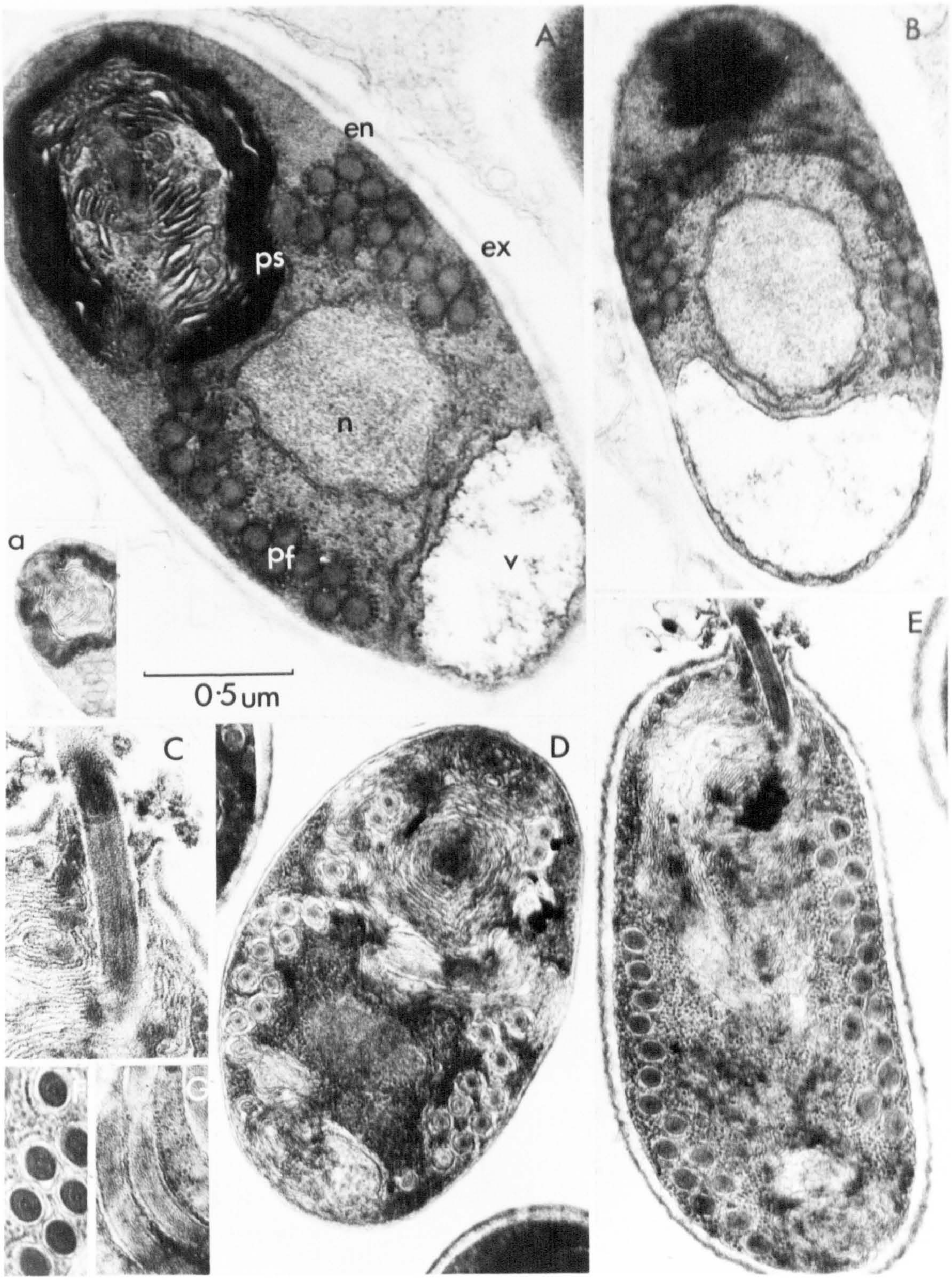
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PLATE 40

Ultrastructure of the plasmodium of M.exiguus containing spores of both myxosporidian and microsporidian Nosemoides sp.

A. Mature spore of M.exiguus and developing sporoblast of microsporidian Nosemoides sp.
X 9,922

B. Mature spores of both myxosporidian and microsporidian showing the great difference in size. The amoebula of the M. exiguus spore contains a membrane bound inclusion (i),not encountered in spores without the associated microsporidian hyperparasite.
X I6,380

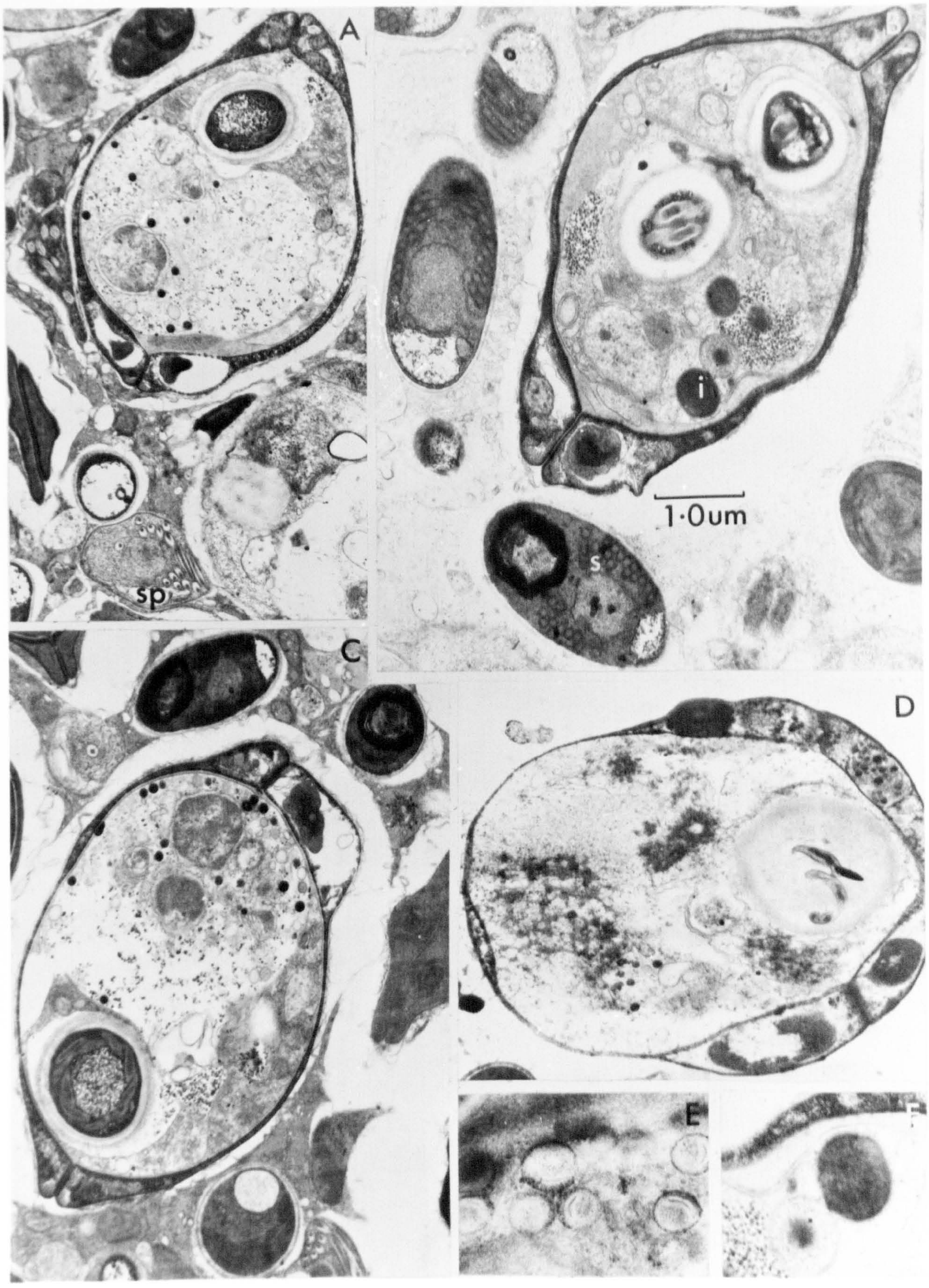
C. Mature spore of M. exiguus and spores of Nosemoides sp.
X I2,577

D. Spore of M.exiguus , from plasmodia with microsporidia
X I6,380

E. Vesicular electron lucent inclusion found in the cytoplasm of some amoebulae associated with the microsporidian hyperparasite
X 63,000

F. Detail of the membrane bound inclusion from the amoebula of M.exiguus
X 20,250

i. membrane bound inclusion from amoebula of M. exiguus
s. mature microsporidian spore
sp. developing microsporidian sporoblast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DISCUSSION

Although the three parasites studied develop within fish connective tissue, the localised host cell response was noticeable different in each case; Rhipidocotyle johnstonei inducing an intense cellular reaction; Cryptocotyle lingua a fibrotic capsule, often melanised and ultimately calcified; and Myxobolus exiguus initiating little or no response. This range of response may be related to the nature and activity of the surfaces of the parasites involved.

The surface layer if all three parasites are membrane-bound syncytia, with structural evidence of intense metabolic activity. In M. exiguus the entire plasmodium is a syncytium and the surface is the only route for the intertransference of materials with the host tissues; whereas metacercarial stages have well-developed digestive and excretory systems. However, it is probable that the tegument plays the major role in the absorption of nutrients in metacercariae, particularly in those like Cryptocotyle lingua which are encysted.

The outer ectoplasm of the plasmodium of M. exiguus was traversed by extensive channels or vacuoles which were interpreted as structural evidence of pinocytotic activity although further studies using tracers would be necessary to confirm this. Similar channels have been observed in the plasmodial walls of all species of myxosporidia examined, (Lom & de Puytorac, 1965a; Desser & Paterson, 1978). However, the structure of the plasmodial wall may partly depend on the specific location of the parasites (Desser & Paterson,1978); a feature demonstrated by M. exiguus plasmodia. They were however all bounded by a single unit membrane, a feature in common with coelozoic species (Lom & de Puytorac, 1965; Lom

1969). Desser & Paterson (1978), however found the plasmodial wall of a Myxobolus sp. from the lamellar region of the gills of the common shiner. like that of Henneguya exilis Kudo from the same location in the gills of the channel catfish (Current & Janovy, 1976), to be bound by a double limiting membrane.

Current & Janovy (1976), suggested that the pinocytotic channels formed by the inner membrane of the plasmodial wall of H. exilis were stable features and that uptake of portions of the host's cell cytoplasm or interstial material occurs when the outer membrane invaginates into these channels. Minchew (1972), claimed that H. exilis enhances its food supply by inducing hyperplasia of the basal cells of the gill lamellae. A similar situation may exist with M.exiguus as slight hyperplasia of gill epithelial cells was observed in the present study.

Uspenskaja (1966), has suggested that the actual process of absorption of nutrients by myxosporidian plasmodia may be similar to the process of contact digestion described in cestodes by Smyth (1972), and in the vertebrate intestine by Crane (1967, 1968, 1969). This process was reviewed by Ugolev (1968), who found that digestion occurs on the membranes dividing the intracellular and extracellular environments, and that the enzymes involved are fixed on the cell membrane.

The metacercarial tegument of C.lingua and R.johnstonei are cytoplasmic layers, essentially similar to other digeneans (Lee 1966, 1972; Lyons, 1977), and secrete a wide range of metabolic products into the host's tissues. This process was most obvious during the encystment process of C.lingua when

membrane-bound secretion bodies were released sequentially from the tegument. These secretion bodies were derived from the epidermal cell bodies and cystogenic gland cells both of which were connected to the tegument by protoplasmic processes. The penetration glands also contained secretion bodies, but these reached the surface via microtubule lined ducts, which would ensure their direction to specific sites.

The secretions produced by the penetration gland cells represents a holocrine secretory process, as once depleted no further secretions are produced. This is of significance to the metacercaria as penetration and migration is limited by this secretory process. Release of material from the tegument has been termed a type of eccrine (merocrine), secretion (Erasmus, 1972).

Rees (1974),described five types of membrane bound secretion bodies in the cercarial tegument . Only three of these types were identified here, namely sb2, sb3 and sb4, from the metacercarial tegument 15 minutes following infection. It is assumed that types sb1 and sb5 were secreted during penetration of the fish epidermis, together with secretions from the penetration glands. Some of these secretions are assumed to be histolytic enzymes as evidence of lytic necrosis in migratory tracts of metacercariae was present in electron micrographs. Similar damage to host tissue from enzyme secretions from pre-acetabular glands of Schistosoma mansoni was recorded by Stirewalt & Dorsey (1974).

Prior to encystment the parasite lies in a fluid-filled space, formed from lysis of the fish cells and release of secretions from the penetration glands and some secretion

bodies. The cystogenic gland cells however, were still replete with their large homogeneous secretion bodies, their route to the tegument being blocked by a mitochondrion in the connecting protoplasmic process. Such a mechanism would ensure the release of the cystogenic glands secretion bodies at the correct time, a factor of crucial importance in the orderly synthesis of the parasitic cyst.

Encystment of C. lingua may be triggered by a factor present in the serum of susceptible fish, which may stimulate release of material from the cystogenic gland cells. Iddon (1973), found the time of exposure to serum may determine the start of encystment. Alternatively MacKenzie & Liversidge (1975), have suggested that depletion of cercarial glycogen reserves may be the signal for encystment. In the present study the site of encystment was usually reached within 30 minutes, but great variations occur in the duration of migration and encystment in different species of trematodes have been recorded by others. The Heterophyiid, Stellantchasmus falcatus taking up to two days (Lee & Cheng,1970); whereas Bucephalus haimeanus may complete cyst formation within ten minutes in post-larval plaice (Matthews, 1973a). During the encystment process the metacercaria of C. lingua is highly active, constantly turning which may assist in the even deposition of cyst wall components and the movement of secretion bodies through the tegument.

The actual release of the secretion bodies from the tegument is sffected by the fusion of the secretion body membrane with the outer plasma membrane of the tegument, and

the subsequent release of the contents to the outside. This process was observed 30 minutes post penetration, indentations in the tegument indicating sites of release of the secretion bodies. Morphological changes within the tegument prior to their release may reflect changes in the chemistry of the contents. Southgate (1971), described similar changes in the morphology of secretion bodies prior to their release, during encystment of Notocotylus attenuatus.

90 minutes post penetration the tegument of C. lingua was almost devoid of secretion bodies except for a few corresponding to types sb2 and sb3. However, the tegument still contained high levels of glycogen which may be used as an energy source during the final stage of encystment.

Synthesis of layer 1 of the parasitic cyst was first observed 2 hours post penetration when a narrow, 0.03µm electron dense line was visible at the junction of the parasitic cyst cavity and the host cells. This was associated with sbc4 secretion bodies within the cyst cavity, derived from the cystogenic gland cells. These apparently expand and fuse to form the outer acellular layer of the parasitic cyst. A change in the physical nature of these secretions may occur in the fluid derived from lysed fish cells and preceeding metacercarial secretions. Southgate (1971), made similar observations on the encystment process of N. attenuatus when contact with water in the external environment, changed the physical bature of secretion granules resulting in expansion and fusion to form the outer cyst wall.

Addition of sbc4 secretion of C. lingua to the outer

(layer 1), of the parasitic cyst was readily demonstrated 3.0 hours post penetration when these secretions were present both within the cyst and fusing or coalescing with the inner surface. The highly folded nature of the parasitic cyst may allow for future growth of the metacercaria, which may increase ten times in volume during maturation (Iddon, 1973).

The inner acellular layer (layer 2), of the parasitic cyst was also being laid down 3 hours post penetration. This granular layer was deposited on the inner surface of layer 1 of the parasitic cyst and by day 1 was complete; the inner layer measuring 0.52μm.

The synthesis of the parasitic cyst is associated with the appearance in the tegument of secretion bodies originating from the cystogenic gland cells. Janoff & Ford (1965), detected acid glycerophosphatase in the cystogenic glands of C. lingua and suggested that this enzyme is autolytic and acts on the gland cells facilitating the rapid release of the cystogenic material. Most secretion bodies appeared to bave been released by 1 day post infection.

The observations on the encystment process of C. lingua confirm that a sequential release of secretion bodies from the tegument takes place during entry, migration and encystment in the second intermediate host. The orderly release of materials into and from the tegument must be of crucial importance to the effective synthesis of the metacercarial cyst. Secretions must be released at the correct time to ensure the cyst is laid down outside the tegument and the release of precursors is synchronised. Similar observations on the secretion and structure of metacercarial cysts have been made by Lumsden (1968),

Stein & Lumsden (1971a,b), Strong & Cable (1972), and Mitchell (1974).

After release of all secretion bodies, breakdown of the tegument occurred in the 1 day old metacercariae of C. lingua . Higgins (1977), similarly found the tegument of Bucephalus haimeanus to almost completely breakdown during encystment. Secretions from the cystogenic gland cells may contribute to this autolysis in C. lingua. The tegument was replaced by the metacercarial tegument, although the replacement mechanism was not observed.

Spines present in the cercarial tegument were also found to break down and to be replaced by new spines. These are probably synthesised on polyribosomes in the tegumental cells as was observed for Fasciola hepatica by Bennet & Threadgold (1975). In the present study spines were absent from the tegument at 14 days post infection but had been replaced by 21 days; whereas Day (1976), found spines to break down at 10 days and to be replaced at 34 days, in a different host fish. Bibby & Rees (1971), described a similar reorganisation in Diplostomum phoxini metacercariae.

The spines of C. lingua and R. johnstonei both had a crystalline structure, and rested on the inner plasma membrane of the tegument. The dark zone at the base of the spine and underlying muscle observed in R. johnstonei may represent an association between muscle and spine. The outer apices of the spines protruded from the surface but were covered by the outer plasma membrane. Cercarial spines probable assist the cercaria in adhering to a passing fish, and during penetration and migration to the site of encystment. The anterior spines of

C. lingua are modified to form a piercing apparatus which supplements the action of the penetration gland cells (Rees 1974). The spines of the unencysted metacercaria of R. johnstonei may abrade host tissues and assist in movements within the fish during feeding.

The highly folded and microvillous nature of the metacercarial surface of C. lingua 14 days post infection is suggestive of an increased surface for absorption during a period of rapid metacercarial growth. This resembled the redial surface of this parasite described by Krupa, Bal & Cousineau (1967), which was found to absorb glucose, (McDaniel & Dixon 1967), and to take up colloidal thorium by pinocytosis (Krupa, Cousineau & Inoue, 1970). Iddon (1973), demonstrated alkaline phosphatase in the cyst of C. lingua, an enzyme which Smyth (1966), considered may be concerned with active transport of glucose. Absorption of all nutrients during growth of the metacercaria must be via the cyst wall and tegument once any nutrients enclosed within the cyst during encystment are depleted. Glycogen found in the tegument of C. lingua 14 days post infection was of the type, consistent with a surface having high energy requirements (Krupa, Bal & Cousineau, 1967).

Both C. lingua and R. johnstonei fully developed metacercariae had two types of secretion bodies present in the tegument. The function of these may be to contribute to the ground substance of the tegument by internal dispersion (Bogitsh, 1968; Wilson & Barnes, 1974a, b); or they may be a source of material added to the inner surface of the cyst wall in C. lingua, or to the surface glycocalyx in R. johnstonei. Although secretion

bodies have been defined on morphological grounds in the present study the necessity of further studies to determine the biochemical nature of the secretions is realised.

The parasitic cyst of C. lingua is composed of carbohydrate and protein (Iddon, 1973), and is quite thin and delicate as it is protected by the host tissues. The complete metacercarial cyst wall is composed of both parasitic secretions and a host capsule, and falls within the first category of metacercarial cysts mentioned by Hunter & Dalton (1939), and reviewed by Higgins (1977).

The functional significance of a parasitic cyst is raised by the present study as R. johnstonei develops free within the host tissues, whereas C. lingua is enclosed by a parasitic cyst. Cottrell (1976), using indirect fluorescent antibody techniques detected antibody to secretory antigens of R. johnstonei but was unable to detect antibody to C. lingua, which is enclosed by a cyst wall. This may support the suggestion of Stein & Lumsden (1971), that the cyst may serve to sequester metacercarial antigens. Reichenback-Klinke (1954), suggests that the cyst wall may partition the parasite from the potentially destructive immune response of the host. The cyst wall of C. lingua may enable the metacercaria to control the immediate biochemical environment and allow the parasite to develop in a wide range of fish hosts, whereas R. johnstonei is specific to plaice. The metacercaria of R. johnstonei may also require a wider range of metabolites than C. lingua as it feeds on ingested host cells; whereas C. lingua would only have access to small molecules such as glucose and amino acids absorbed via the cyst wall.

The progenetic metacercariae of R. johnstonei survive

without a parasitic cyst for the ten months required for the development of the eggs, a feature which may be attributed to the activity of the tegument.

A feature of the plasma membrane of the tegument of R. johnstonei and the plasmodia of M. exiguus is the presence of a glycocalyx. These varied morphologically in being an electron dense zone in R. johnstonei and filamentous in M. exiguus. A wide range of morphological types have been described which may reflect their chemical heterogeneity. The glycocalyx may vary with the type of fixation used (Threadgold, 1974), and may require specific staining to reveal (Threadgold, 1975). The inner granular parasitic cyst of C. lingua which was secreted by the tegument may also be considered analogous to the surface glycocalyces of other trematodes (Lumsden, 1975) and the surfaces of cells (Ito 1969).

The term 'glycocalyx' denotes a region of the plasma membrane even if it appears as a morphologically distinct layer (Lumsden 1975). Glycocalyces may be either attached or unattached (Threadgold 1976a), the former being firmly bound to the plasma membrane, and follows its contours and shape during functional changes such as pinocytosis. The unattached type of glycocalyx in contrast does not move during functional changes in the shape of the underlying membrane. The present study has demonstrated the presence of both types of glycocalyx. The attached type present on the surface of M. exiguus and R. johnstonei, has also been described for other myxosporidian plasmodia (Lom & de Puytorac 1965a, 1966) and trematode surfaces (Threadgold 1976b, Lumsden 1975). This type is also found in most protozoa, on microvilli and on the phagocytic

surface of many cells (Threadgold 1976a) The unattached glycocalyx has been demonstrated on muscle cells of vertebrates, endothelial cells of capillaries and the basal lamina of most epithelia (Threadgold, 1976a). The inner granular layer of the metacercarial cyst of C. lingua is an extension of this type.

Glycocalyces are predominantly mucoproteins and muco-polysaccharides containing highly anionic sugars, i.e. carboxylic acids, sulphate esters or sulphamides and are polyanionic with a strong net negative charge, and can bind cations such as Ca++ K+ and Na+. The glycocalyx is readily pervaded by water ions and small molecules, and probably exerts a regulatory influence allowing or denying access to the membrane. The sialic acid is involved in the release of proteins from the cell and the transport of K+ and contributes to the net negative charge. Since it is the cell layer immediately in contact with the environment it probably exerts a decisive regulatory influence, allowing or denying access to the membrane. The glycocalyx acts therefore as a filter being a hydrolytic barrier to large molecules. It is also a protective layer against mechanical abrasion, host enzymes, and viral and bacterial infections.

Structural evidence of pinocytotic activity was found in the plasmodium of M. exiguus and the tegument of R. johnstonei a process in which the glycocalyx may be involved. Brandt & Pappas 1960), found that binding to the glycocalyx may be a prerequisite for uptake of nutrients in the amoeba Chaos chaos. Colloidal particles and proteins were bound to the cell surface prior to uptake, which may increase the permeability of the plasma membrane and allow molecules such as glucose to enter the cell (Brandt & Freeman 1967). Higgins (1977), found the

metacercarial stage of Bucephalus haimeanus was capable of

absorbing dissolved nutrients through the tegument by an active process. A similar process may occur in the absorption of nutrients through the tegument by the metacercaria of C. lingua.

The glycocalyx or surface coat influences the absorptive properties of the surface membrane and may also have a protective function, especially in metacercariae which do not form cysts, (Harris & Cheng, 1973). Mucopolysaccharides may contribute to the surface coat and have been recorded from the surface of R. johnstonei (Matthews, 1968); and C lingua is thought to secrete a mucopolysaccharide or mucoprotein coat on penetration of the fish (Rees,1974). The secretion bodies present in the cercarial epidermis have been shown to contain mucopolysaccharides (Rees & Day, 1974). Mucopolysaccharides have also been recorded from the epidermis of other cercariae (Dixon, 1966; Belton & Harris, 1967); adult digenea, (Lee, 1966) and cestodes (Monne. 1959), and may protect against host enzymes.

The surface coat may also mask the membrane's potential antigenicity, and in schistosomes there is evidence that host or host-like antigenic determinants are incorporated into the surface membrane and prevent immune recognition, (Clegg, 1972). Stein & Lumsden, (1973), considered that a similar surface electronegative charge density found on schistosomules and host leucocytes may minimise adherence of these cells to the parasite. Berezntsev, (1975), related an evolutionary selection in helminth parasites for the ability to inhibit the protective leucocyte response of the host. Similar factors may explain the lack of leucocytes attached to the surface of R. johnstonei, or the C. lingua cyst.

The present study demonstrated that the metacercarial surface appears to release a far greater quantity of antigenic material than the myxosporidian plasmodia. The most intense

cellular response was induced by the metacercaria of R. johnstonei in plaice, movements of the parasite causing mechanical abrasion of the host tissues. The metacercaria does not secrete a cyst and its surface is in direct contact with the plaice tissues. The host reaction is characterised by a massive infiltration of macrophages, together to a lesser extent with other leucocytes and eventual encapsulation by fibroblasts.

Pronounced infiltration by leucocytes was not evident in C. lingua infections in plaice or mullet although host response included fibrosis with encapsulation of the matacercaria and associated melanisation and calcification. C.lingua remains encysted throughout its development in the fish, a feature which might account for the more limited response compared to that of R. johnstonei, an observation which agrees with Hoffman, (1956), who found that encysted metacercaria elicit less host response than unencysted species. It is also possible that C. lingua incorporates fish material into the cyst wall during encystment, thereby disguising the parasitic cyst. Howell, (1973), found that Strictodora lari cysts which encysted in vivo had fish material associated with them.

The earliest stage of R. johnstonei examined was approximately 3 months after infection when the host response was well developed and comprised lymphocytes, monocytes, plasma cells, macrophages and epithelioid cells. Macrophages were by far the most common cell type, containing large quantities of phagocytosed material in the cytoplasm, mainly cellular debris associated with the activities of the parasite.It is assumed that these macrophages are derived from the circulating monocytes which have migrated from the circulation. Ellis, (1977), considered that macrophages in fish are typically found in

connective and other tissues and not normally as a component of the circulating leucocytes, although the monocyte may be considered a macrophage precursor. This agrees with the definition of the mononuclear phagocyte system of Van Furth et.al. (1972).

Phagocytosis is the first line of defence against invading microbes and is the defining characteristic of monocytes and macrophages. It was demonstrated in the present study by the circulating monocytes of turbot, by ingestion of erythrocyte fragments and clearance of colloidal carbon from the circulation. Engulfment of erythrocyte fragments occured by the fusion of two pseudopodia to form a phagosome. This differed from the ultrastructural observations of McKinney et. al.(1976), who found that in phagocytosis of yeasts by gar monocytes, only a single pseudopodium was formed which wrapped around the yeast. Clearance of the intravenously injected colloidal carbon occured rapidly during the observations on turbot. This method could provide a technique for determination of the possible increase in phagocytic cells associated with parasitic infections. The carbon may have been taken up by the circulating monocytes and by macrophages in the spleen, kidney, heart and other tissues. Ellis, Munro & Roberts, (1976), demonstrated uptake of carbon particles by macrophages in these sites in plaice. Macrophages which phagocytosed carbon particles formed aggregates in association with lymphoid cells, often within the existing nodules of melanin containing cells called melanomacrophages, (Roberts, 1975).

The scavenging function of fish macrophages is known, as in the infiltration of necrotic tissue in trout infected with IPN virus (Wolf & Quimby, 1969); but it is not known whether

macrophages in fish process antigen as a preliminary stage to antibody production. Klontz, (1972), however found antigen containing macrophages in the antibody producing organs of fish.

In mammals the macrophage has two functions in the handling of antigen: it is concerned with catabolism and elimination of potentially immunogenic molecules and the concentration of a small amount of immunogen. The macrophage thus processes antigen prior to presentation to lymphocytes, and is a cell essential to the functioning of lymphocytes, but which is not antigen specific. Antibody responses, cell-mediated responses and mitogen responses all require the presence of the macrophage, (Uanue & Cerottini, 1970). The presence of macrophages together with lymphocytes and plasma cells in the 3 month capsule of R. johnstonei in plaice suggests that macrophages and lymphocytes may co-operate in a cell mediated response.

Macrophages may therefore be immobilised within the R. johnstonei host capsule by the release of macrophage migration inhibition factor, (MIF), by T lymphocyte equivalents which have migrated to the site. Timur G (1975) and Timur M (1975), have demonstrated the production of MIF-like factors in plaice kidney cells in response to specific stimulation with antigen. Macrophages may be inhibited from movement, yet powers of phagocytosis may be enhanced and ingestion and processing of antigen may occur. Subsequent presentation of the processed antigen to lymphoid cells may lead to antibody production by transformed B cell equivalents.

Ellis (1977), suggested that much of the Ig in fishes may be produced by stimulated lymphocytes which do not undergo full

differentiation into plasma cells. These cells were however found in the host capsule of R. johnstonei in plaice, and this may be correlated with the demonstration of humoral response to secretory antigens of this parasite by Cottrell (1975). Plasma cells are characterised by their well-developed rough surfaced endoplasmic reticulum, and large amounts of intra-cytoplasmic Ig. These cells have not been widely recognised among fishes although they were described from the paddlefish by Clawson, Finstad & Good (1966) and the circulating leucocytes of C.labrosus in the present study.

Further cells of the monocyte line were found surrounding the macrophages, lymphocytes and plasma cells in the R. johnstonei capsule. These cells were morphologically similar to epithelioid cells, which have been described from chronic inflammatory lesions in plaice to a variety of irritants by Timur G (1975), Timur M (1975), and Timur, Roberts & McQueen (1977).The epithelioid cells in the R. johnstonei capsule had cytoplasmic inclusions in common with the macrophages but differed in their elongated shape and their indefinite boundaries, in those forming contact with adjacent cells, aa finding in common with Timur G (1975). From studies on mammals epithelioid cells were originally named because of a superficial similarity to epithelial tissue cells (Carr, 1973). They have also been refered to as'nonsense'cells due to the active appearance of their nuclei, Golgi and E.R., yet their function is unclear although they are a feature of many types of granulomatous inflammation.

Papadimitriou & Spector (1971), have suggested that these cells develop from macrophages when the influx of macrophages into a lesion exceeds the number required to digest an

indigestible irritant; or when the irritant is digestible, not acutely toxic to the macrophage but continuously available, so that new macrophages live long enough to develop epithelioid characteristics. Epithelioid cells have lost the powers of phagocytosis but can take up material by pinocytosis (Walter & Israel, 1974); and may form a barrier between the macrophages and host tissue and may ingest subparticulate material or secrete enzymes (Papadimitriou & Spector, 1971).

The 9 month infection of R. johnstonei also included Foreign-body type giant cells together with the macrophages in the capsule. These were identified by their scattered nuclei and vacuolated cytoplasm, and may have arisen from the fusion of macrophages. Timur M (1975), found both Langhans and Foreign-body type giant cells in response to carageenin in plaice. Previous reports of the occurrence of this cell type in fish have been reviewed by Timur G (1975). It seems that this cell is produced in long lasting chronic lesions in response to indigestible materials. From this study it seems likely that macrophages, epithelioid cells and giant cells are all derived from the monocyte precursor as has been demonstrated from in vitro studies on chicken monocytes by Sutton & Weiss, (1966). They suggested that the function of the monocyte is synthesis and storage of lysosomes, while the macrophage and epithelioid cell function as phagocytes and the multinucleated giant cell may engage in active transport in addition to sequestering foreign particles.

The absence of distinctive lysosomes from the macrophages, epithelioid and giant cells participating in the response to

R. johnstonei in plaice corresponds to a similar observation by Timur, Roberts & McQueen (1977); on the macrophages in the carageenin granuloma of plaice, although the circulating monocytes contain lysosomes (Ferguson, 1976). Lysosomal granules however, have recently been used as criteria for distinguishing macrophages, epithelioid cells and giant cells in foreign-body granulomas in an in vivo ultrastructural study of rat macrophages (van der Rhee, van der Burgh-de-Winter, & Daems, 1979).

All the features of chronic inflammation described in response to R. johnstonei metacercariae in plaice are typical of a granulomatous lesion. Roberts (1978), has defined granulomata in fish as chronic inflammation with the development of a proliferative lesion progressing to fibrosis. The lesion is called a granuloma and is a characteristic of many fish diseases, and may be caused by silicaceous diatoms, bacteria or fungi. The chronic lesion, develops as a central zone of necrotic cell material containing the initiating agent, with a surrounding layer of macrophages and other inflammatory cells, together with a proliferation of fibroblasts. The lesion appears yellow or white with a cheesy or hard consistency and may be calcified.
Descriptions of granulomata in fish produced in response to a variety of agents have been included by Corbel, (1975); Timur, M, (1975); Timur, G, (1975); Timur et al. (1977) and Roberts, (1978).

It is interesting that the eggs of R. johnstonei did not induce their own granulomata, although became incorporated within the granulomatous lesion of the parasite. The egg

capsule composed of scleretin appears to be immunologically inert and the absence of response suggests also that it is impermeable to miracidial antigen. This contrasts with schistosomes which are able to escape from the host tissues by the release of miracidial enzymes which induce intense granulomata, the major factor in the development of hepatosplenic disease, (Warren, 1968). The schistosome egg granuloma is a manifestation of delayed hypersensitivity, (Smithers & Terry, 1976), and although the granuloma contributes significantly to the pathology of the disease it seems also to protect the host liver from the secretions of the eggs.

Many references to granulomata exist including those produced in response to Fasciola eggs and dead flukes (Murray & Rushton, 1975); and to nematode parasites reviewed by Poynter, (1966). Dunsford et.al. (1974), believed that granulomas are formed in response to a sustained release of antigen, which would normally be toxic to the host, but is sequestered in situ by the host phagocytes. The response is basically a walling off of an antigen containing space (Lichtenberg & Mekbel, 1962). This may explain the survival of plaice with quite large numbers of R. johnstonei metacercariae, with little apparent effect.

Fibrosis is a feature of granuloma formation, and results in encapsulation. Fibroblasts synthesising collagen were present in the 3 month R. johnstonei granuloma, surrounding the epithelioid cells and amounts of collagen had increased in the 9 month capsule. Metacercariae of C. lingua in mullet and plaice also became encapsulated with collagen. This encysted metacercaria did not however exhibit any of the macrophage response characteristic of the R. johnstonei capsule. The presence of the parasitic cyst of C. lingua may reduce the antigenicity of this parasite.

Fibrotic encapsulation of C. lingua began very soon after encystment and at three hours post infection randomly arranged microfilaments were associated with the surface of the parasitic cyst. Iddon (1973), found similar filaments associated with the metacercarial cyst in plaice and drew attention to their similarity to tonofilaments (Wellings et. al. 1967; Roberts, Young & Milne, 1971; Roberts, Bell & Young, 1973). However, it seems more likely that these extracellular filaments are protocollagen in view of the associated fibroblasts.

From mammalian studies it seems that the fibroblast is derived from local connective tissue cells (Spector, 1973); and not from the mononuclear cells of the inflammatory exudate, (Ross et. al. 1970). However some workers believe monocytes may differentiate into fibroblasts (Ham, 1969); and in vitro studies have suggested a transformation of monocytes and macrophages into fibroblasts, (Stirling & Kakkar, 1969; Vernon-Roberts, 1972). Macek, Hurych & Smetana,(1973), demonstrated fibroblast differentiation in long term cultivation of foetal peripheral blood cells. The problems of fibroblast origin and differentiation are by no means resolved from mammalian studies and work on fish tissue is still in its infancy.

Although the mechanisms which activate fibroblast proliferation in chronic granulomas are not known (Silver, 1973); it is generally assumed that fibroblasts represent attempts at healing. Collagen is also involved in the final stage of repair in wound healing responses in fish (Roberts, Ball, Munro & Shearer, 1971; Anderson & Roberts, 1975; Phromsuthirak, 1977); and mammals, (Ross & Benditt, 1965; Ross, 1968).

Spector noted that fibroblasts appear in granulomas when macrophages with phagocytosed irritant lie in proximity to

locally derived fibroblasts. He suggested that the macrophages release substances, possibly of lysosomal origin, which cause fibroblasts to proliferate and synthesise collagen and mucopolysaccharides. Since the presence of irritant seems to hinder final fibrosis, it is likely that macrophages release substances which interfere with collagen deposition e.g. collagenase. This secretory activity may be attributed to epithelioid cells (Papadimitriou & Spector, 1971).

Actively synthesising fibroblasts were found with associated extracellular collagen, around the metacercarial cyst of C. lingua at 14 days post infection. These active fibroblasts had extensive rough ER cisternae with a peripheral margin of cytoplasm, a feature which differentiates them from plasma cells. Since collagen is deposited and functions extracellularly the cell must provide a means of transport for the transit of the newly synthesised protein through the cytoplasm and across the cell membrane.

Procollagen is synthesised on membrane-bound ribosomes on the rough endoplasmic reticulum. The protein may then either pass to the Golgi complex where glycosylation of peptidyl hydroxylysine may occur; or alternatively, vesicles may bud off directly from the RER, by-passing the Golgi. Finally procollagen may gain access to the exterior of the cell by direct intermittent communication of the cisternae of the RER with the extracellular space. Microtubules may be responsible for the transcellar movement of procollagen from the site of synthesis to a more peripheral location in the cell.

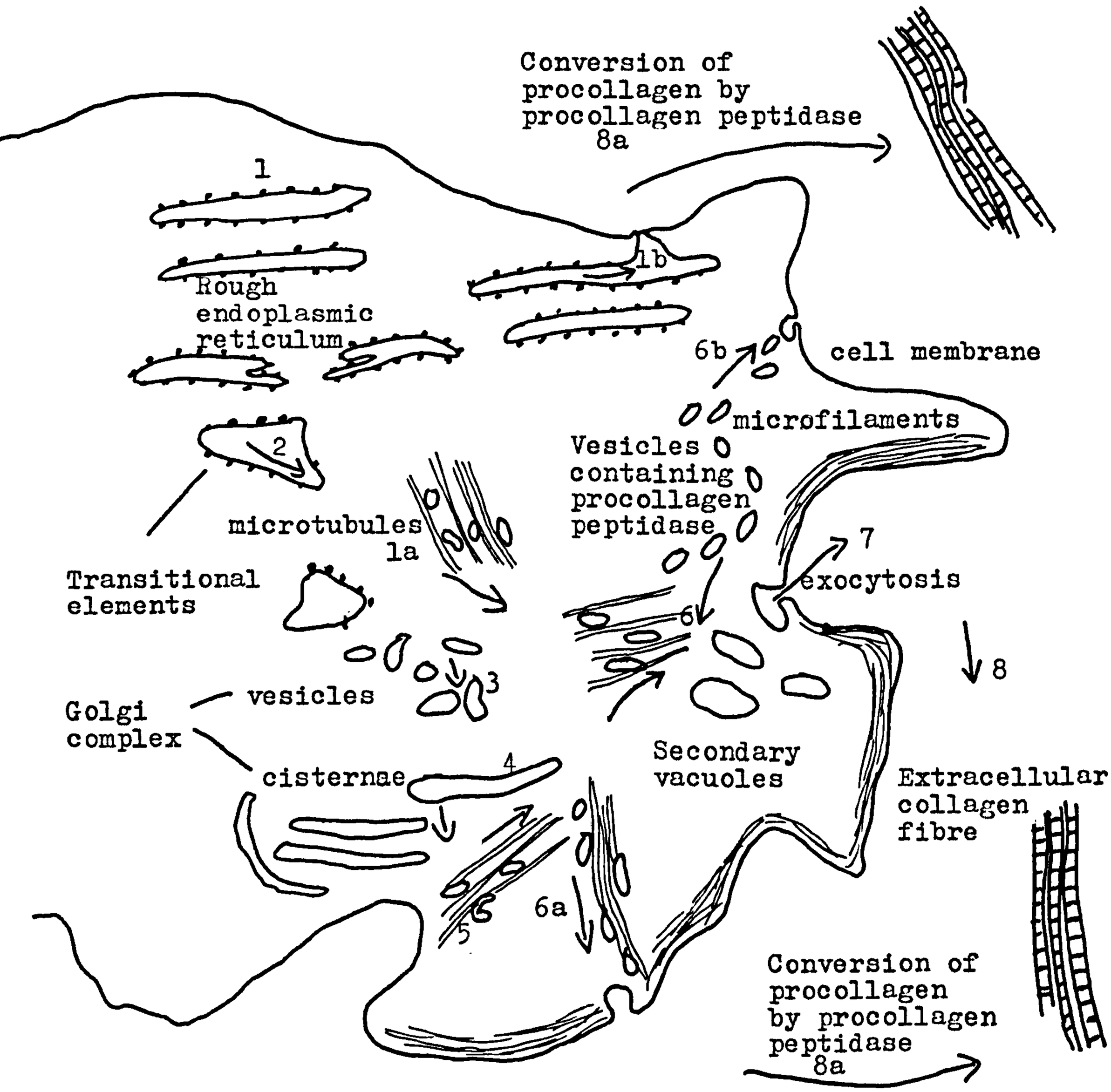
Extrusion of procollagen may occur in one of atleast two

ways: vesicles containing procollagen may fuse with the cell membrane and procollagen is then converted extracellularly by procollagen peptidase. Alternatively, fusion of vesicles containing the enzyme may occur intracellularly, the result of which is extruded by an exocytotic process. In either scheme polymerisation of collagen to form fibres occurs extracellularly, (Bornstein & Ehrlich, 1973). These events are summarised in Fig.

Collagen fibres are composed of fibrils, which are made up of tropocollagen molecules. The newly synthesised procollagen consists of a molecule larger than tropocollagen because of the presence of cystine-containing extensions at the NH_2 terminal ends of the polypeptide chains. When these extensions are removed aggregation occurs, and the fibres are formed by the lateral alignment of the molecules. The first-formed fibres have little strength, but with the formation of cross linkages tensile strength develops. As maturation proceeds the fibres increase in size and the amount of surrounding ground substance is reduced, (Walter & Israel, 1974). Collagen fibres deposited around the metacercarial cysts of C. lingua and R. johnstonei granuloma displayed the characteristic banding with a periodicity of 64nm. Melanisation of the fibrotic capsule was a feature of the metacercarial cyst developing in plaice.

Melanised cysts of M.exiguus also occured in sub-epidermal locations in the grey mullet, but were not found in the experimental infection of C. lingua maintained at 15°C in the laboratory. Iddon, (1973), found melanin containing cells appeared in experimentally infected plaice maintained at 15°C 11-12 days after exposure, and after 47 days when kept at 12°C.

Fig 7
Taken from Bornstein & Ehrlich (1973)



Alternate schemes for the intracellular translocation and secretion of collagen. The following pathways are considered: Procollagen, synthesised in the rough endoplasmic reticulum (1) is transferred via the transitional elements(2)to the Golgi complex (3,4). It is then transported in vesicles by microtubular (5) and is transferred to the extracellular space either directly (6a) or via secondary vacuoles formed by fusion with vesicles containing procollagen peptidase (6,7). In the former case conversion to collagen by procollagen peptidase would occur extracellularly (8a) Alternatively procollagen may bypass the Golgi (1a) and then follow one of the above two sequences. Finally procollagen may gain access to the extracellular space by direct intermittent communication of the cisternae of the rough endoplasmic reticulum (1b). In this instance conversion to collagen by procollagen peptidase (8a) would also occur extracellularly.

The inhibiting effect of low temperature on melanin containing cell production was confirmed by McQueen et. al. (1973); and modification by temperature is a general feature of the poikilotherm inflammatory response (Finn & Nielson, 1971).

The melanin found associated with the fibrous capsule of C.lingua in plaice, and the skin and scale plasmodia of M. exiguus was either intracellular or deposited as granules extracellularly. The latter probably resulted from the rupture of melanin-containing cells due to pressure exerted by the growing parasite, an observation also made by Hunter & Dalton (1939), in melanisation of Clinostomum marginatum in the banded sunfish.

The melanin-containing cells associated with the cyst of C. lingua and the plasmodia of M. exiguus probably arise from the migration of melanocytes from foci around adjacent blood vessels or from the stratum spongiosum fo the dermis, rather than from an in situ melanin synthesis. Evidence of this comes from the fact that pigmented cysts of C. lingua are found in unpigmented sites such as the ventral surface of pleuronectid fish, and the cornea. From histological studies of metacercarial cysts in the cornea, Roberts (1975), found melanocytes migrating into the cyst - the melanocyte-stimulating factor probably being a secretion produced by the parasite. An earlier suggestion made by Hunter (1941), that melanin production is due to a DOPA released by the parasite which stimulates DOPA deficient cells to melanin synthesis, does not account for the presence of pigmented cysts in sites where no melanin precursor cells exist.

The species of fish may also affect the development of pigment formation associated with the metacercariae. In the spotted goby the cysts of C. lingua cause no pigment formation while those of Cryptocotyle jejuna are heavily pigmented (Rothschild, 1939).The reason for the lack of melanin associated with the experimentally infected grey mullet is difficult to explain in view of the fact that wild fish often bore pigmented cysts, and melanocytes were associated with the M. exiguus infections. It may be that melanocytes do not become associated with the cyst until later than the 57 days of the experimental period examined.

Cryptcotyle lingua has been reported to cause a general proliferation of melanin-containing cells over the whole body producing a completely blackened fish (Hsiao, 1942, Mawdesley-Thomas & Young, 1967), a condition similar to melanosis produced by X-irridation (Smith, 1935).

The melanin-containing cells of teleost fish include melanocytes, melanophore and melanin-macrophages. The melanocytes are cells which synthesise a special melanin-containing organelle - the melanosome. Melanophores are a type of melanocyte, which by intracellular displacement of the melanosomes contribute to colour changes . The melanin-macrophage is a large phagocytic cell, containing melanin, found in the haematopoietic tissue, (Roberts, 1975).

Melanocytes are dendritic cells situated in the stratum spongiosum and the hypodermis and are DOPA and tyrosinase positive, indicating that they are responsible for synthesis of melanin in the melanosomes. These cells are also found in small groups associated with the larger veins and

lymphatics, a site which may represent a primitive analogue of the peripheral lymphoid nodules of mammals and birds, (Roberts, 1975).

Melanophores are the pigment cells which are immediately sub-epidermal and are asteroid cells which are under nervous or humoral control. By adjustment of their melanin granules the contribution made by melanin pigments to the total colour of the fish is altered. Disease processes affecting nervous control of pigment cells produces a blackening of fish skin. In whirling disease, Myxosoma cerebralis infection of rainbow trout, compression damage to cartilage of the head and vertebrae, results in loss of control of posterior chromatophores and intense blackening of the tail area, (Wolf & Markiw, 1976).

Melanophores are also involved in healing lesions of fish, melanosomes released from dacaged pigment cells are engulfed by macrophages which migrate through the epidermis and dehisce on the surface (Roberts, 1975). In certain ulcerative conditions such as U.D.N. of salmon and furunculosis of Cyprimidae melanocytes grow into the epithelialised scar tissue as it heals and develop into melanophores, (Mawdesley-Thomas, 1969, Roberts et. al. 1971).

Melanin containing cells of the melanin macrophage centres are a feature of teleost haematopoietic tissue. The pigment franules in the melanin-macrophages appear to be contained in groups suggesting that they have been phagocytosed. Experimentally it has been shown that they are capable of concentrating circulating Salmonella

organisms as well as carbon particles and haemosiderin; and the function of melanin macrophage centres may be as a repository for metabolically inert material or material required for recycling, (Agius, 1979). It has been suggested that melanin macrophage centres should be considered a part of the reticulo-endothelial system, and part of the defensive system of the fish against microbial attack, (Roberts, 1974). Thorpe & Roberts,(1972), described the displacement of melanin containing macrophages from the kidney and spleen into the circulation during severe septicaemia in Salmo trutta.

Edelstein, (1971), has suggested that melanins may have a defensive role in many organisms, as a source of quinone-free radicles and in association with peroxidase as a bactericidal system. Melanin can oxidise NADH to produce hydrogen peroxide (Van Woert & Palmer, 1969), which might be employed in a bactericidal process similar to that which occurs in polymorphs.

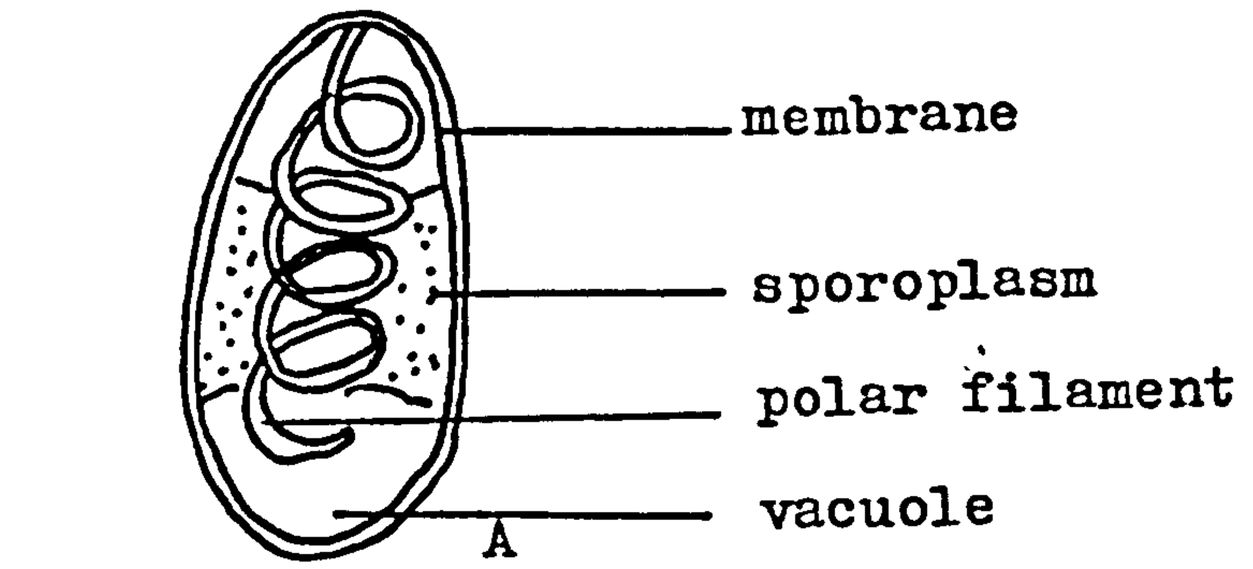
Apart from melanisation of the skin and scale cysts and slight hyperplasia in the interlamellar cyst M. exiguus induced little response in infected fish. Histopathological studies on myxosporidians are rare, although histozooic forms are generally considered the most damaging to fish. Hyperplasia and encapsulation are the usual responses, although some species appear to elicit no response, (Rogers & Gaines. 1975). Roberts & Elson (1970), found no cellular response to Myxosoma cerebralis in salmonids, and Aisa, (1972) found only mechanical pressure effects, with deformation or atrophy of the secondary lamellae in an infection of Myxobolus ellipsoides braemaeformis in

the gills of tenches Tinca tinca. A preference for the melanin-macrophage tissue of the spleen and kidney of its host the roach is shown by Myxobolus pseudo dispar, where melanisation may be greater than in uninfected tissue, (Roberts, 1975).

The site of development may be very important in the relative pathogenicity of the myxosporidian parasite. Henneguya sp.develop inter- and intra- lamellar cysts in the channel catfish (McCraren, Landolt, Hoffman & Meyer, 1975); and only the intralamellar form is seriously pathogenic, causing extensive hyperplasia, (Current & Janovy, 1978).

Dykova & Lom, (1978), have found that elevated temperature produces a granulomatous inflammatory response to the gill cysts of Henneguya psorospermica, in perch and ruff. They suggested that the tissue response of fish to myxosporidian infections may have two phases: during growth and maturation of the plasmodia leading to spore production, displacement, atrophy and hyperplasia of the tissues occurs. When the cyst is full of mature spores a chronic inflammatory reaction results in replacement of the cyst by granulomatous tissue. It would be interesting to investigate the effects of temperature on the development of a laboratory infection of M. exiguus. Lucky, (1970), found that the tissue response to Myxobolus ellipsoides only started when the parasite had reached a minimum size.

The spore is the infective agent in the life cycle of both myxosporidian and microsporidian parasites, and the present study allows the comparison of both types. This is the only known ultrastructural description of this



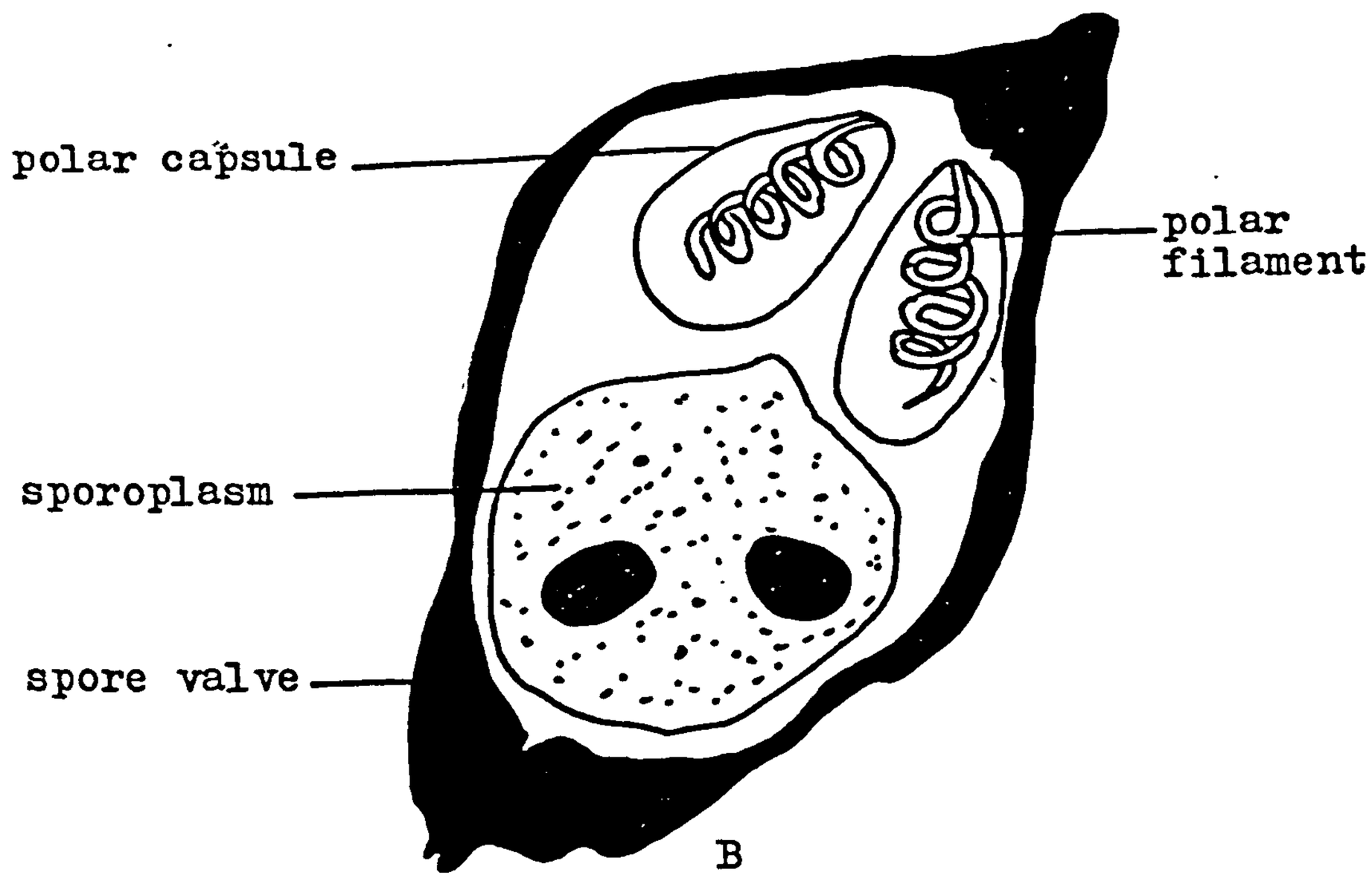


Fig. 8

Generalised structure of a microsporidian (A), and myxosporidian (B), spore.

hyperparasitic association, and the spores of both parasites have certain features in common. This led to their association in many modern taxonomic treatments, in that both were cnidocyst producing protozoans. They are now however considered separate groups, (Mitchell,1977).

The spores of M. exiguus and the hyperparasitic Nosemoides sp.both have a thickened spore wall, which is composed of two valves in M. exiguus; both have an infective amoebula or sporoplasm and both have an extrusion apparatus. The microsporidian spore however develops from a single cell whereas five cells are involved in the development of the myxosporidian spore. Sporogenesis of M. exiguus may occur in a similar way to that described for Sphaeromyxa elegini by Uspenskaja, (1976). He found that only six nuclei in the pansporoblast undergo meiotic division to give rise to two capsulogenic, two valvogenic and two sporoplasm nuclei; the other two remaining as residual nuclei. Six of the nuclei are surrounded by cytoplasm to give two valvogenic, and two capsulogenic cells and one binucleate sporoplasm. Each spore of M. exiguus probably arises in a similar way.

Both the polar capsule cells of M. exiguus and the spore of Nosemoides sp. contain internally coiled filaments, the myxosporidian filament also being spirally twisted along its length giving a figure of eight cross section. Both filaments are extruded by eversion, and artifically extruded filaments of M. exiguus polar capsules showed the filaments to be hollow and continuous with the capsule wall Lom & de Puytorac,(1965b), made similar observation on several

myxosporidian species. The function of the myxosporidian filament is probably to anchor the spore while the amoebula while the escapes; whereas the microsporidian filament may be the route through which the sporoplasm leaves the spore to infect a new host cell, (Lom & Vavra, 1963; Weidner, 1972). The development of the polar filament in the capsulogenic cells of M. exiguus agrees with previous descriptions (Lom, 1969; Lom & de Puytorac, 1965b; Schubert, 1968); but has yielded no further information on how the external tubule developing in the cytoplasm of the capsulogenic cell is withdrawn into the capsule; or of the cytoplasmic origin of the capsular promordia and the external tubule. Recent observations suggest that the granular E.R. is involved in the synthesis of the capsular primordia and the external tubule, (Desser & Paterson, 1978).

The similarity between the formation of myxosporidian polar capsules and nematocysts of anthozoan and hydrozoan coelenterates have been noted by several authors, (Lom & Vavra, 1964; Lenz, 1965; Westfall, 1966). The fact that several cells are involved in the formation of myxosporidian spores have led to the suggestion that Myxosporidia be excluded from the Protozoa (Grasse, 1960; Lom, 1969; Grasse & Lavette, 1978).

From the observations on the incidence of infection of grey mullet with M. exiguus, it appears that fish become infected during their first year in the estuary, as an increase in the number infected was noted from February (19%), to July (94%). None of the 0 group sample caught in August were found to be infected although the difficulties

in detecting the early infection in fish 3.0cms. in length are considerable.

Spawning of grey mullet occurs at sea, near the Scilly Isles and youngfish are found in Plymouth Sound in May (Hickling, 1970). It is unlikely that fish are infected at sea, the estuarine habitat with the shallow water and muddy substrates seems the most likely for infection to occur. The feeding habits of the grey mullet may enhance the chances of the infective spores being ingested. The fish sucks up the algal felts from the substrate and the organic material and sand is ground up in the gizzard-like stomach (Hickling,1970). The food material is passed through the extremely long intestine, and it seems that this is the primary infection site as young fish commonly had cysts in this site.

It is likely that spores are carried to the secondary infection sites via the circulation, as spores are within the size range (10μm), of fish blood cells. The range of secondary tissue sites infected tend to support this supposition. Fish in the 2-3 group were commonly infected with interlamellar gill cysts, whereas fish in the 3-4 group infrequently bore gill cysts. This may be attributed to the feeding activities of the fish when a strong jet of water is passed over the gills, and cysts of a certain size may become detached. 3-4 group fish more commonly had cysts in fin connective tissue.

No adverse affects on infected fish were noted and the parasite induced very little cellular response. However in

adverse environmental conditions it may act as a contributory factor, causing death of fish. Schulman (1957), reported deaths of grey mullet Mugil cephalus infected with M. exiguus. Petrushevski & Schulman (1961), found infected gill filaments were swollen and haemorrhaged. Myxobolus exiguus has also been reported from Mugil cephalus in Peru, (Armas, 1979).

The metacercarial stages of C. lingua and R. johnstonei studied here may also produce haemorrhages in infected fish, and in the case of R. johnstonei in young plaice may also affect the swimming performance and increase risks of predation, (Matthews, 1968).

In summary, therefore, the cellular response of the fish to the parasites has been shown to resemble the mammalian inflammatory response. The metacercaria of R. johnstonei induced a granuloma in plaice, qualitatively similar to the mammalian chronic inflammatory lesion. The degree of response has further been demonstrated to be related to the nature and activity of the parasites surface.

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