

Spring 1969

THE LIFE HISTORY, AND
ULTRASTRUCTURAL DEVELOPMENT OF
THE EPIDERMIS, OF A MARINE
TREMATODE, LEPOCREADIUM
SETIFEROIDES

MARGARET MAGENDANTZ

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**MAGENDANTZ, Margaret, 1941-
THE LIFE HISTORY, AND ULTRASTRUCTURAL
DEVELOPMENT OF THE EPIDERMIS OF A
MARINE TREMATODE, LEPOCREADIUM
SETIFEROIDES.**

**University of New Hampshire, Ph.D., 1969
Zoology**

University Microfilms, Inc., Ann Arbor, Michigan

THE LIFE HISTORY, AND ULTRASTRUCTURAL DEVELOPMENT OF THE EPIDERMIS, OF A
MARINE TREMATODE, LEPOCREADIUM SETIFEROIDES

BY

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B.A., Smith College, 1963

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A THESIS

Submitted to the University of New Hampshire

In Partial Fulfillment of

The Requirements of the Degree of

Doctor of Philosophy

Graduate School

Department of Zoology

June 1969

This thesis has been examined and approved

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Date June 19, 1969

ACKNOWLEDGEMENTS

This study was directed by Professor Wilbur L. Bullock. I am grateful for Dr. Bullock's many suggestions and patient guidance during my doctoral studies. In addition, Dr. Bullock's courses and research have been of value to me in their emphasis on both the many biological questions posed by parasitology and the relationships of parasitology to animal and human populations throughout the world.

I am grateful to the Department of Zoology for the experience gained as a Graduate Teaching Assistant and for materials supplied for my research.

I wish to thank the other members of my doctoral committee, Dr. Arthur C. Borrer, Dr. Robert A. Croker, Dr. Arthur C. Mathieson, Dr. Lorus J. Milne and Dr. Samuel C. Smith, whose diverse fields of interest and questions widened the scope of the present study. I am grateful for their criticisms also during the writing of this thesis.

I wish to thank Dr. Peter H. Cooke for instruction in electron microscopy and for numerous suggestions concerning this area of my research.

I am grateful to Mr. Emory C. Clippert for instruction in the use of the electron microscope and accessory equipment.

Finally I wish to thank the many Zoology graduate students who helped me seining on the mudflats.

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ABSTRACT

THE LIFE HISTORY, AND ULTRASTRUCTURAL DEVELOPMENT OF THE EPIDERMIS, OF A MARINE TREMATODE, LEPOCREADIUM SETIFEROIDES

by

MARGARET MAGENDANTZ

The life cycle postulated for Lepocreadium setiferoides Miller and Northup, 1926 in the Great Bay region of New Hampshire is related to seasonal environmental changes and movements of the hosts in a salt marsh depression and creek. It involves the gastropod Nassarius obsoletus, the spionid polychaete Polydora ligni and the Smooth Flounder (Lipsetta putnami). The life stages of this marine digenetic trematode are further elucidated, and the miracidia, young rediae and metacercariae described for the first time. By means of light and electron microscopy the development of L. setiferoides is followed and the modifications of its epidermis are related to external conditions affecting the three hosts and internal differences in the host's tissues.

The percentage of snails, 15-25mm long, infected and releasing cercariae is low (a maximum of 7%), possibly due to the unusually high incidence of another trematode Zoogonus rubellus, and to the low incidence of gravid worms in the definitive host, L. putnami.

A likely second intermediate host of L. setiferoides is the spionid, P. ligni, which is naturally infected with L. setiferoides and lives in mucous tubes on the shells of the first intermediate host from June through September. This period corresponds with the time of cercarial release. Owing to the transparency of P. ligni, it affords an unusual opportunity to observe the development of a metacercaria in vivo. Experimental infections suggest that ingestion of infected P. ligni

is an effective means of transmitting this parasite.

Adult worms are found in the anterior intestine of L. putnami, the Winter Flounder (Pseudopleuronectes americanus) and the Shorthorn Sculpin (Myoxocephalus scorpius), and, on the basis of adult and egg morphological similarities, it is tentatively concluded that they are all L. setiferotbs. Sexual maturation of adults in L. putnami occurs in late fall and spring.

The epidermis of the rediae consists of superficial syncytial and lower nucleated cellular regions. Its surface area is increased by (1) microvilli (2) tortuous invaginations of the body wall and (3) narrow inpocketings of the body wall lined with numerous microvilli. Young rediae bearing epidermal microvilli are surrounded by a clear space in the host tissue, whereas older rediae have a degenerate epidermis lacking microvilli and are closely embedded in host tissue.

In the cercarial germinal balls the epidermis appears to develop from upward cytoplasmic extensions of nucleated epidermal cells below the muscle layers.

The characteristic features of the epidermis of cercariae, metacercariae and adults are (1) division into superficial syncytial and lower nucleated regions connected by cytoplasmic processes (2) spherical (sDB) and rod-like (rDB) membrane-bound dense inclusions (3) mitochondria (4) invaginations of the apical and basal plasmalemma (5) a dense zone beneath the apical plasmalemma and (6) Golgi complexes and spherical and oval bodies often with embedded sDB's.

Changes occurring in the epidermis during development from the cercarial to adult stage which may be related to changes in absorption and secretion processes are (1) tripling of the number of mitochondria in the superficial epidermis, (2) concentration of the mitochondria in the distal half of the superficial epidermis (3) up to a three-fold decrease

in the diameter of the mitochondria, and (4) orientation of the mitochondrial cristae towards the apical surface and parallel to the rDB's.

The predominant secretion process in the cercarial, metacercarial and adult epidermis involves the sDB's and rDB's, the latter of which appear to merge with the dense zone beneath the apical plasmalemma bearing a coating of fine filaments. The inclusions appear to form in the lower epidermis possibly in association with the Golgi complexes and/or spherical and oval bodies. The sDB's and rDB's reach the superficial epidermis via the cytoplasmic processes, and the latter become elongated in close proximity to the mitochondria as they approach the apical dense zone.

SECTION I

INTRODUCTION

The Digenea, endoparasitic flatworms with complex life cycles involving two, three or more hosts are found in many phyla of the animal kingdom. Thus their host-parasite relationships may be examined in a wide variety of internal and external environments. In addition, owing to the peculiar nature of their life cycles, they provide material for studies of some unusual types of tissue development. One involves the sequential modifications of tissues during development from the cercarial to the adult stage, in response to a series of hosts, host tissues and sometimes external environments. The other involves the complete ontogeny of tissues in each of the three early larval stages, the miracidia, rediae and cercariae, with differences related to varying modes of nutrition, locomotion and protection.

Digenetic trematodes in about 54 families complete their adult life in fish (Yamaguti, 1958). Most studies have indicated only the incidence of the parasites. However, Sindermann and Farrin (1962) have examined the interrelationships between the life histories of trematodes and marine fish. Marine trematodes, in addition to their possible economic importance, may exhibit life cycles that reveal correlations with features of the marine environment which are not seen in freshwater and terrestrial species. Marine fish trematodes may also be compared with trematodes which complete their adult life in homeotherms.

Trematodes of marine fish pass their early larval stages in invertebrates. The interrelationships between trematodes and marine mollusks have been reviewed by Cheng (1967). An understanding of

pathological effects of trematodes such as those described by Cheng and Burton (1966) and Hopkins (1957) on the oyster Crassostrea virginica is of importance to commercial fisheries. In addition, trematode larval stages present biological questions concerning the conditions which allow for development to the cercarial stages and for the release of cercariae without reinvasion of the mollusk host.

An outline of the digenetic trematode life cycle follows in order to familiarize the reader with the form and behaviour of the diverse life stages, and to indicate how and when embryogenesis occurs during the life cycle. Adult digenetic trematodes are hermaphroditic and reproduce sexually in vertebrates, which thus serve as definitive hosts. The fertilized eggs escape from the definitive host via the feces, urine or sputum, and develop into the first larval stage, the miracidium, which is often free-swimming. The life cycle involves both agamic and sexual reproductive phases, hence the name Digenea, meaning two generations. Development of the larval stages, including transformation of the miracidium occurs in one or more intermediate hosts. The first intermediate host usually is a mollusk. After establishment in the first intermediate host, the miracidium loses its ciliated epidermal plates and transforms into the first generation of germinal sacs, either sporocysts or rediae. These and subsequent larval stages contain germinal cells, which are believed to descend from the fertilized ovum (Cort, 1944). The major systems, integumentary, digestive, excretory and nervous systems as present in the adult, are formed during development of the cercariae. Free-swimming cercariae may penetrate into a second intermediate host. The latter, with contained metacercariae, may then be ingested by the definitive hosts. In summary, miracidia

develop from fertilized ova, and cercariae from germinal cells. In contrast, first generation rediae develop by a transformation of miracidia. Similarly, metacercariae and adults do not develop from germinal masses, but rather by a series of modifications of the cercarial larvae.

The development and modifications of the trematode "cuticle" or epidermis, as it is more properly termed (Lee, 1966) are of interest, because the epidermis is in direct contact with host tissue, and may be involved in nutrient absorption, secretion of digestive enzymes and excretion of some metabolic wastes. It may also be the site of action of some anti-helminthic drugs and of secretion of substances protecting the parasite from digestion by host enzymes. The development of the epidermis from the fertilized ovum to the adult stage in a single species has not been followed, except in one cursory study (Bils and Martin, 1966). There is considerable information on the adult epidermis of several trematode species, but little on the epidermis of larval stages, especially concerning possible changes, both morphological and functional, in the epidermis during the transformation of the larval stages into the adult. Electron microscopic studies may further light microscopic investigations on epidermal development, such as those of Cheng and Provenza (1960), and provide answers for such disputed questions as the origin of the flatworm epidermis and the phylogenetic relations between the various flatworm groups.

Larval stages of the marine digenetic trematode presumed to be Lepocreadium setiferoides Miller and Northup, 1926 are commonly observed among the trematode fauna of the gastropod Nassarius obsoletus Say from salt marshes in southeastern New Hampshire. Dr. Wilbur Bullock of the

Department of Zoology at the University of New Hampshire found (pers. comm.) adult trematodes of Lepocreadium, believed to be either L. setiferoides or L. trullaforme in the intestines of the Smooth Flounder (Liopsetta putnami), the Longhorn Sculpin (Myoxocephalus octodecimspinosus) and the Winter Flounder (Pseudopleuronectes americanus). Miller and Northup (1926) first described Cercaria setiferoides from N. obsoletus at Woods Hole, Massachusetts. Martin (1938) partly worked out the life cycle of L. setiferoides and described the larval stages in N. obsoletus and the adult stages in flounders from Woods Hole. He did not observe the miracidium. Dr. Bullock suggested the study of L. setiferoides and L. trullaforme and the family Lepocreadiidae for the subject of my thesis.

The Family Lepocreadiidae Nicoll 1934 includes at least 10 genera of trematodes of marine and fresh-water fishes. The following life histories of species in the genus Lepocreadium have been worked out in part. L. album (Stossich, 1890) Stossich, 1904 was described by Palombi (1934 and 1937) in the Gulf of Naples; that of L. setiferoides was described by Martin (1938) and that of L. pegorchis (Stossich, 1900) was described by Bartoli (1967) at Marseilles, France. In these life cycles the first larval stage in the first intermediate host is a simple redia containing developing cercariae. No daughter rediae have been described. The cercariae are of the ophthalmotrichocercous type with large eyespots and groups of setae on the tail (setiferous-tailed). There are no studies on the modes of nutrition, growth and release of the larval stages of Lepocreadium. The cercariae "develop" into metacercariae in a variety of second intermediate hosts, including bivalves (L. pegorchis and L. album), annelids and flatworms (L. setiferoides) and opisthobranchs (L. album). Despite the designation metacercaria, it

is not clear from the reports what, if any development beyond the cercarial form, does occur in the second intermediate host. Nor has it been determined whether the latter is essential in the Lepocreadium life cycle. Furthermore, there are discrepancies in the descriptions regarding the supposed encystment of the metacercariae. The miracidial stage has been observed in only one species of Lepocreadium, that of L. album by Palombi (1937).

The objectives of the present study were to:

1. further elucidate the life stages of Lepocreadium setiferoides, particularly in relation to migrations of its hosts.
2. follow the development of L. setiferoides in the laboratory and to examine the temperature conditions affecting this development in order to postulate a life cycle.
3. compare the eggs and adult worms of Lepocreadium taken from different fish hosts in relation to the problems of speciation in Lepocreadium.
4. follow by light and electron microscopy the development and modifications of the epidermis of the life stages of L. setiferoides, in order to elucidate:
 - (a) the morphological and possible functional differences between the redial epidermis and the cercarial-metacercarial-adult epidermis in relation to the interactions between the parasite and host tissues.
 - (b) the morphological changes which occur during transformation of the cercaria into the adult and the possible functional significance of these changes with respect to parasite-host interrelations.

SECTION II

LITERATURE REVIEW

Part 1. The Parasitic Platyhelminth Epidermis

The Nature of the Epidermis. The term cuticle in reference to animal tissues was used until recently to describe the outer covering of parasitic flatworms and other helminth groups. It is the diminutive form of the Latin word "cutis" meaning skin, and is generally used to describe a nonliving layer secreted by an underlying epidermis or hypodermis. A true cuticle fitting this definition is characteristic of the phyla Nematoda, Annelida and Arthropoda. For many years the parasitic flatworms, Trematoda and Cestoda, were thought to have a nonliving cuticle. However, the electron microscopic studies of Threadgold (1963a and b) on the fluke Fasciola hepatica showed that the outer layer of the body wall is actually a living cytoplasmic layer bounded externally and internally by a plasmalemma with connections to nucleated portions beneath the muscle layers. Hence the term cuticle is no longer appropriate in descriptions of parasitic flatworms. Some authors retain the term cuticle to refer to the outer nonnucleated portion while others prefer the terms tegument, integument or subtegument. In Lee's review (1966) of the parasitic flatworms, the term epidermis is introduced. In the present paper this term will be used, since all recent electron microscopic studies indicate that the outer layer of the trematode body wall is a cytoplasmic layer with similarities to the epidermal tissues of other animals, and totally unlike the cuticle of nematodes, annelids and arthropods.

The epidermis of trematodes, unlike that of mammals and birds is not keratinized. However, like the epidermis of invertebrates and cold-blooded vertebrates it is exposed to external osmotic and ionic changes. Since the epidermis of trematodes and cestodes resembles other epithelial tissues, it can be examined with respect to secretion, absorption and intracellular digestive processes characteristic of these tissues.

The Development and Functions of the Trematode Epidermis. From the little work done on the development of the parasitic platyhelminth epidermis two main hypotheses have evolved: (1) that the epidermis represents a sunken primary epidermis with the nucleated portions beneath the muscle layers and embedded in the parenchyma and (2) that the epidermis represents a secondary epidermis of parenchymal (mesodermal) origin.

That the adult and cercarial trematode epidermis is a primary epidermis of ectodermal origin has been suggested by the work of Cheng and James (1960), Baer and Joyeux (1961), and Bils and Martin (1966) on the development of the epidermis in the cercarial germinal balls. Indirect evidence that the epidermis is derived from an ectoderm is its similarity to the epidermis of some acoelous turbellarians (Dorey, 1965), in which the basal parts of epidermal cells form narrow projections through the muscle layers into the deeper parenchyma. But to my knowledge no one has determined whether the separation of the nucleated portions from the surface layer of the epidermis results from a sinking down of the former or of a lifting up of the latter.

Support for the hypothesis that the epidermis is of parenchymal origin is the work of Cheng (1963b) in which the injured body surface of a trematode was seen to be renewed by parenchymal beta cells.

The epidermis of each of the larval (miracidium, sporocyst, redia and cercaria) and adult stages of trematodes will be briefly reviewed below.

The epidermis of the miracidium, unlike that of all other life stages, consists of ciliated nucleated epidermal plates.

The outer layer of sporocysts was formerly thought to be a cuticle. But ultrastructural studies (Bils and Martin, 1966) have indicated that it is a cytoplasmic layer bearing microvillous-like projections. Indirect evidence of the outer layer's cytoplasmic nature is the increase in fatty acids and neutral fats in host tissues resulting from the sporocyst's lipases or those of the host tissue (Cheng and Snyder, 1962b). Cheng and Snyder (1963) found glucose on the inner and outer surfaces of the sporocyst body wall, suggesting that it was absorbed through the latter. Hyaluronidase and alkaline and acid phosphatase enzymes involved possibly in histolytic and absorptive processes have also been demonstrated on these surfaces (James and Bowers, 1967).

Only recent electron microscopic studies have indicated the cytoplasmic nature of the outer redial body wall and the microvilli on its apical surface (Bils and Martin, 1966; Rees, 1966; Ginetsinskaya, 1967; Krupa et al., 1967 and Krupa et al., 1968). The redia may be formed either directly by transformation of the miracidium, or from germinal balls within the brood chambers of sporocysts or miracidia. Only the latter process has actually been observed by Stunkard and Cable (1932) and by Rees (1940). Rees reported that the redial wall of Parorchis developed from somatic cells of the redial embryo within the miracidium. The transformation of miracidia directly into rediae has

not been described, but it may occur, since some life cycles do not include a sporocyst life stage. In the transformation of miracidia into sporocysts Chen (1937) observed that the ciliated epidermis was cast off and the miracidium transformed into an elongated sac. Campbell and Todd (1955) observed, in vitro, the metamorphosis of a miracidium into a sporocyst.

The redia, unlike the sporocyst, possesses a gut and actively feeds. Cheng (1963a) observed that the rediae of Echinoparyphium caused extensive damage by ingesting snail host tissue and presumably by excreting waste materials. Cheng (1964) and Krupa et al. (1968) detected phosphatases in the redial body wall. Aminopeptidases also located there were thought to cause lysis of host hepatopancreatic tissue (Cheng and Yee, 1968).

Cercariae arise from germinal balls. The cercarial body covering was first studied at the ultrastructural level by Cardell (1962), who maintained that it was a secreted cuticle. Rees (1967), Belton and Harris (1967) and McAnally (1969) found the cercarial body covering to be a cytoplasmic layer without microvilli. Large amounts of glycogen and fatty acids were observed in cercariae by Cheng and Snyder (1962a and b). They suggested that the fatty acid could either be absorbed or synthesized from glycogen and could be used during the free-swimming stage, since most of it had disappeared by the adult stage.

The metacercarial epidermis has been described in species which form cysts. Since in the present study L. setiferoides did not encyst, these papers will not be reviewed here.

Senft et al. (1961), in the first ultrastructural study of an adult trematode body covering, concluded that it was an amorphous

cuticle, Threadgold (1963a and b) first described the cytoplasmic nature of the trematode body covering and its basic division into two regions, an outer syncytium connected to an inner nucleated portion termed the tegumental cells.

The trematode epidermis has been examined most extensively in liver flukes (Threadgold, 1963a and b; Björkman and Thorsell, 1964) and blood flukes (Lee, 1966; Smith et al., 1969; McAnally, 1969), and to a lesser extent in lung flukes (Burton, 1964; Threadgold, 1968), bladder flukes (Burton, 1966) and intestinal flukes (Malton and Dermott, 1967). There has been little attempt to compare the trematode epidermis in relation to the different locations of trematodes in host tissues.

Histochemistry and Biochemistry of the Epidermis. The characteristic form of respiration in endoparasites including trematodes, is anaerobic or aerobic glycolysis (von Brand, 1966). Malton (1967b) demonstrated in trematodes large glycogen deposits, predominantly in parenchymal and muscle tissues. Cestode parenchymal tissue also has large stores of glycogen (Lumsden, 1966). Autoradiography of an adult trematode in which the gut was ligated, demonstrated rapid uptake of glucose in the superficial epidermis and subepidermis (Nollen, 1968).

Evidence for a typical Krebs citric acid cycle in parasites is not complete (von Brand, 1966). Furthermore, it is not known how well the electron transport chain which utilizes energy derived from Krebs citric acid cycle can function in endoparasites under conditions of low oxygen tension. For instance, it is not understood how DPNH of the electron transport chain can continue to be dehydrogenated. Breeding (as quoted by von Brand, 1966) suggested that DPNH may be dehydrogenated anaerobically in the production of succinate by reduction

of fumarate. There is indirect evidence that aerobic oxidative phosphorylation in trematodes is limited as compared to that in other free-living animals. Lumsden (1967) has suggested that the abundance of mitochondria but the poor development of their cristae indicates a limited involvement in aerobic respiration, but a possible role in other processes.

Acid and alkaline phosphatases occur in bone marrow, kidney tubules and intestinal mucosa where glucose is transported, suggesting that they may function in glucose transport. Bullock (1949), Stadtman (1961), Rothman (1966), Lumsden et al. (1968) and Morris and Threadgold (1968) have demonstrated phosphatases in the cuticular and subcuticular tissues of various helminths.

Recent high magnification electron micrographs and autoradiographs have shown that the plasmalemma of the trematode epidermis may be involved in absorption processes. Bogitsh (1968) described a thin coating resembling a glycocalyx on the apical plasmalemma of a trematode epidermis. The term glycocalyx was introduced by Bennett (1963) to designate the polysaccharide coating characteristic of the outer surface of cell membranes in a variety of cell types. On the apical plasmalemma of epithelial brush borders the glycocalyx has the form of fine filaments which give a positive periodic acid Schiff reaction (Revel, 1965). Amino acid absorption has been demonstrated in the epidermis of cestodes (Read et al., 1963) and in Fasciola hepatica (Pantelouris and Gresson, 1960 and Kurelic and Ehrlich, 1963). Protein, in the form of ferritin, has also been found to be absorbed by E. hepatica (Björkman and Thorsell, 1964). Although the epidermis of adult cestodes and trematodes can absorb carbohydrates, amino acids, proteins and possibly lipids, there is the question of whether these are available in usable form for the adult worm. Many

trematodes as adults live in the lumen of blood vessels, hepatic ducts or intestines, regions generally with high concentrations of dissolved nutrients. Threadgold (1968) suggested that dense inclusion bodies in the epidermis may have a digestive function and Bogitsh (1968) suggested that they might give rise to the glycocalyx.

Part 2. Ecology of Marine Larval Trematodes Including Those Infecting Nassarius obsoletus.

Conditions allowing development and release of larval trematodes from marine gastropods and for infection of the subsequent host are related in part to migratory patterns of these hosts and to their location in the littoral zone.

N. obsoletus occurs both subtidally and intertidally (Dimon, 1905), but in fall it moves below low tide level (Sindermann, 1956).

At least nine different species of larval trematodes have been described in N. obsoletus. McMermott's thesis (1951) includes descriptions of trematodes found in N. obsoletus in New Jersey.

Several trematode species use N. obsoletus as first intermediate host and infect marine fish in their metacercarial or adult stage. Possible life cycles of these are tabulated below. That of Z. rubellus was

Trematode Species	2nd Intermediate Host	Definitive Host
<u>Zoogonus rubellus</u>	<u>Nereis virens</u>	<u>Anguilla rostrata</u>
<u>Hepocreadium setiferoides</u>	<u>Polydora ligni</u>	<u>Liopsetta putnami</u>
<u>Stephanostomum tenue</u>	<u>Menidia menidia</u>	<u>Sphaeroides maculata</u>

postulated by Stunkard (1938a), that of Stephanostomum by Martin (1939) and that of L. setiferoides in the present study.

Migratory movements of marine fish may determine in part the life cycles of some trematodes. Gambino (1959) found that the incidence of Z. rubellus in N. obsoletus rose sharply in March at Woods Hole, coinciding with anadromous migrations of young eels (Bigelow and Schroeder, 1953).

Migrations of marine birds may affect the life cycles of their trematodes. N. obsoletus harbors the schistosome Austrobilharzia variglandis, which as an adult infects the Herring Gull (Larus argentatus) and as a cercaria is the causative agent of swimmer's or clam-digger's itch on the Atlantic and Pacific coasts. In Connecticut Penner (1953) found that the incidence of A. variglandis in N. obsoletus occurred in wintertime, coinciding with the time when the Red-breasted Merganser (Mergus serrator) was resident on Connecticut shores. In contrast, on the Maine coast the highest incidence of A. variglandis occurred in the fall just before N. obsoletus migrated into deeper water and at the time when the Red-breasted Merganser, the Lesser Scaup (Aythya affinis) and the Black Duck (Anas rubripes) were migrating (Sindermann, 1960). Sindermann (1956) and Vernberg and Vernberg (1963) also suggested that the higher incidence of infection might be caused in part by the trematode infection preventing the migration of N. obsoletus.

The early larval stages of Himasthla quissetensis, another trematode of Herring Gulls, are found in N. obsoletus (Stunkard, 1938b). Trematodes of marine shore birds also infect other species of marine gastropods. Sindermann and Farrin (1962) in observing the larval stages of Cryptocotyle lingua in Littorina littorea on the Maine coast found that the migration of the second intermediate host, the Herring (Clupea harengus) into the bays between May and November coincided with the

period of cercarial emergence. In Littorina neritoides, infected with several cercarial species, Rothschild (1941) found that the larger snails had a higher incidence of infection, and suggested that the trematode infections slowed down the growth rate, so that the time required for the snails to reach a certain size was increased, thus allowing for a greater percentage of infection.

Part 3. The Family Lepocreadiidae

The Lepocreadiidae includes digenetic trematodes which, as adults, parasitize the gut of marine and freshwater fish, and as early larvae, the tissues of gastropods.

Stossich (1903) erected the Family Allocreadiidae, a family of digenetic trematodes of freshwater and marine fish, in which the adult was characterized morphologically by (1) extensive vitellaria (yolk glands) (2) oral and ventral suckers (3) testes posterior to the ovary and (4) a uterus with only an ascending limb. Prior to 1934 species of the genus Lepocreadium were assigned to the Family Allocreadiidae. The classification outlined in the Zoological Record of 1934 and attributed to Nicoll, the section editor, designated a Family Lepocreadiidae, including the genus Lepocreadium distinct from the Family Allocreadiidae. Although Yamaguti (1958) lists the genus Lepocreadium under the Subfamily Lepocreadiinae of the Family Allocreadiidae, most authors adhere to the classification of Nicoll (1934).

Cable and Hunninen (1941) separated the Allocreadiidae and Lepocreadiidae by larval as well as by adult characters. The Allocreadiid cercariae have a cotylomicrocercous tail (short and cup-shaped), whereas the Lepocreadiid cercariae have a trichocercous tail (with spines or

bristles) and conspicuous eyespots. LaRue (1957) separated the digenetic trematodes into two suborders, the Epitheliocystida and the Anepitheliocystida, on the basis of the type of formation of the excretory bladder. His superfamily Allocreadioidea, including both the Lepocreadiidae and Allocreadiidae, contains a variety of cercarial types, hence is probably not a natural grouping. Cable's classification (1956) is based on both morphological and ecological criteria. He distinguishes three superfamilies, the Allocreadioidea, Lepocreadioidae and the Opecoelioidae. The Lepocreadioidae are mostly marine and develop in prosobranch gastropods. Their cercariae are biocellate and bear variably ornate tails (some are trichocercous) and they lack a stylet. The Allocreadioidea parasitize marine and brackish water fish. Their cercariae develop in lamellibranchs and are of the ophthalmoxiphidial type with eyespots, stylet and simple tails. The Opecoelioidae are found in fresh-water and marine fish and their cercariae, developing in prosobranch gastropods, have no eyespots nor body spines, and possess a stylet and a short glandular tail.

SECTION III

MATERIALS AND METHODS

Collection Sites. Initial surveys of N. obsoletus were made at the following locations in Great Bay: Crommet Creek, Adam's Point, Beard's Creek, Jackson's Landing and Emerson's Beach. The seasonal migrations of N. obsoletus and P. ligni, and the seasonal incidence of L. setiferoides in N. obsoletus were studied at Johnson's Creek.

Smooth Flounders were collected with a Common sense minnow seine of $\frac{1}{4}$ " in summer and fall, 1968 and spring, 1969 at the following locations: Emerson's Beach, Johnson's Creek, Crommet Creek and Adam's Point. During January and February, 1969 Smooth Flounders were collected in smelt nets under the ice in the Oyster River by Mr. Philpott Paine

A few Winter Flounder were obtained in an eel trap below the Oyster River dam by Dr. Gilbert Samuel.

In Portsmouth Harbor during fall, 1968 some Winter Flounder and 25 Sculpins (Family Cottidae) including the Grubby (Myoxocephalus aeneus), the Longhorn Sculpin (M. octodecimspinosus) and the Shorthorn Sculpin (M. scorpius) were collected on hook and line by Mr. Joseph Bettencourt. Finally, one sample of 25 Winter flounder was obtained from deep water off the coast of Portsmouth in February, 1969 by Mr. Larry Grant.

Light and Electron Microscopy. N. obsoletus, releasing cercariae of L. setiferoides, were either dissected in the laboratory after collection, or they were held in running sea water for one to three months. Portions of the digestive gland, gonads and reproductive ducts

were excised, and rediae and cercariae were isolated from snail tissue and immediately fixed (see below). Eight infected snails were examined.

Polydora ligni, after removal from the N. obsoletus shells were examined for natural infections of L. setiferoides metacercariae. For studies of metacercarial development, P. ligni were exposed to cercariae at room temperature (25-26° C) for 24 hours and then transferred to fresh water. They were prepared for epon embedding at 6, 22 and 40 hours and 23 and 42 days after exposure to cercariae. Six P. ligni containing 10-20 metacercariae were examined.

Adult L. setiferoides, taken from the anterior intestine of Liopsetta putnami, and excised pieces of the intestine were prepared for epon-embedding.

Tissues of N. obsoletus to be prepared for paraffin embedding were fixed in Bouin's, Zenker's or Davidson's fixatives. Paraffin sections were cut on a Spencer Rotary microtome at 8u and stained with Ehrlich's Hematoxylin and Eosin. Trematodes and host tissues to be embedded in epon were usually prefixed in glutaraldehyde. Two groups of cercariae and adult worms were fixed in osmium tetroxide alone without prefixation. The following procedure gave the best preservation of both the superficial and lower nucleated portions of the trematode epidermis. Tissues were washed for five minutes in .35M sucrose and postfixed in .10M sodium phosphate buffered 1% osmium tetroxide for two hours. All solutions were buffered to a pH of 7.5 and contained CaCl_2 in a concentration just short of saturation. Tissues were dehydrated in ethanol to propylene oxide, transferred to a mixture of one-half propylene oxide and one-half epon, and placed in a vacuum desiccator for 48 hours. They were then transferred to a small amount of epon in gelatin capsules and placed in a desiccator for 24 hours. Thick sections (.9u) for light

microscopy were cut on a Porter-Blum MT-2 ultramicrotome and stained with 1% methylene blue containing 1% borax, or with 2% toluidine blue. Thin sections were cut with glass knives, stained with 2% aqueous uranyl acetate for 20 minutes, and lead citrate (Reynolds, 1963) for two minutes, and observed on the Akashi Tronscope 80 at 80KV at initial magnifications of 3,000-24,000x. (Magnifications given in figure legends are total magnifications).

SECTION IV

FIELD OBSERVATIONS AND LABORATORY EXPERIMENTS ON THE LIFE CYCLE

The First Intermediate Host. N. obsoletus Say is the only gastropod reported to harbor the rediae and cercariae of L. setiferoides. The snail is abundant in tidal marsh mudflats in New Hampshire. N. trivittatus Say, the local congener, is usually restricted to sand beaches and subtidal locations.

The greatest number of large snails (20-25mm in shell length) was found in a depression above the level of Johnson's Creek. The depression retained an inch or two of water over its surface at low tide, while at high tide it was flooded with one to two feet of water. Such large snails were rarely found at any other location. Snails collected at Beard's Creek on a mud-sand substrate averaged approximately 15mm long. The depression contained a matting of the green algae Ulva and Cladophora on which Nassa fed.

Snails were first observed migrating into this depression on March 30, 1967. They were abundant there until the end of October. The first snail egg capsules were found on eel grass (Zostera), which grows in the Creek but not in the depression, on June 30, 1967. Presumably some female snails either move down temporarily via the run-off channels from the depression into the Creek to deposit their egg capsules, or the gravid females do not ascend into the depression until after depositing them.

Snails (15-25mm long) from Johnson's Creek were examined for the incidence of larval trematodes. Johnson's Creek snails were chosen for this study, since initial surveys had shown that snails less than about

15mm long did not release cercariae, and that the greatest number of large snails above 15mm occurred at Johnson's Creek. Snails were isolated in jars of sea water at room temperature (25-26°C) for two days, two feet below a 60 watt light bulb. Table I indicates not the total incidence of larval trematode infections, but rather the incidence of snails releasing cercariae. Snails less than about 15mm long, in which cercariae were not released, were not counted. The highest incidence of infection (up to 6%, or 9% of infected snails) with L. setiferoides occurred in snails from Johnson's Creek. At Johnson's Creek there was also a high incidence of larval Zoogonus (up to 70% of snails 15-25mm long). The other trematodes found in N. obsoletus at Johnson's Creek were Himastha quissetensis, Stephanostomum sp. and an unknown species of parapleurolophocercous type, similar to McDermott's (1961) Species E.

Discussion

The following aspects of the life cycle of L. setiferoides with respect to the first intermediate host will be discussed: (1) the growth of N. obsoletus on various substrates, (2) the time of year when N. obsoletus becomes infected with L. setiferoides, (3) the incidence of L. setiferoides in relation to the incidence of other trematodes, (4) the age of the snails infected with L. setiferoides, and (5) the time of year when the cercariae are released and infective for the second intermediate or definitive hosts.

The large size of snails in the Johnson's Creek depression and their small size in Beard's Creek may be related to the greater amount of organic matter in the fine mud substrate in the former and the lesser amounts in the sandy mud substrate in the latter. In measurements of the

TABLE I

Incidence of larval Trematodes in Nassarius obsoletus (15-25mm long)

at Johnson's Creek

Date	No. of snails examined	Total % of snails infected*	% of snails infected by each trematode family	Trematode family
9/24/66	290	71	56.6 8.3 6.8	Zoogonidae Echinostomidae Lepocreadiidae & Acanthocolpidae
6/19/67	142	78	70.4 5.0 2.1	Zoogonidae Echinostomidae Lepocreadiidae
7/27/67	70	78	60.0 4.3 5.7 2.9 2.9	Zoogonidae Echinostomidae Lepocreadiidae Acanthocolpidae Unknown species
8/11/67	57	55	43.9 10.5 5.2	Zoogonidae Echinostomidae Lepocreadiidae
8/22/67	133	54	36.8 4.5 6.0 3.0 2.3	Zoogonidae Echinostomidae Lepocreadiidae Acanthocolpidae Unknown species
8/31/67	116	96	53.4 5.2 4.3 3.4	Zoogonidae Echinostomidae Lepocreadiidae Acanthocolpidae
10/9/67	290	48	32.8 4.5 5.5 3.4 2.1	Zoogonidae Echinostomidae Lepocreadiidae Acanthocolpidae Unknown species
10/22/67	100	79	70.0 2.0 3.0 4.0	Zoogonidae Echinostomidae Lepocreadiidae Unknown species

* infected= releasing cercariae

organic content in various substrates, ranges of 0.39-1.63% carbon/lgm of sediment in a mudflat and 0.16-0.38% in a sandflat were found (Gordon, 1966; George, 1966). N. obsoletus may obtain some organic nutrients from the substrate; Jenner (1956) reported it passed much sand through the digestive tract.

N. obsoletus probably becomes infected with E. setiferoides sometime between early April and late October. This hypothesis is based on the following observations: (1) eggs are released in the feces of the Smooth Flounder, L. putnami, in both spring and fall, (2) the eggs do not develop into miracidia below about 14°C as demonstrated by their inability to develop in the laboratory at a temperature of 14-16°C, thus it is unlikely that they can develop into miracidia in Johnson's Creek before late May. (The water temperature in the depression may be above 14°C during low tide by April, but it is unlikely that eggs are deposited there, since flounders were never seen in the depression. The trematode eggs may be dropped when the flounders are burrowed in the mud of the Creek, thus reducing the possibility of their being transported into the depression. The eggs may even remain undeveloped but viable over winter. However, to my knowledge there are no studies on the longevity of trematode eggs in the external environment). (3) since the eggs develop into miracidia and hatch in sea water, N. obsoletus probably becomes infected by penetration of the miracidia rather than by ingestion of the eggs; thus the time when N. obsoletus is infected is dependent on the time when the eggs can hatch into free-swimming miracidia, (4) N. obsoletus was observed migrating from deep water in late March and early April. Thus, even if they can become infected by ingestion of E. setiferoides eggs this probably does not occur until they begin feeding at the onset of their migration.

N. obsoletus, releasing cercariae of L. setiferoides, ranged from 14-25mm in length. Scheltema (1964) found that the mean length of three-year old mature snails (second year class) was about 12-15mm at Woods Hole. Thus the snails releasing L. setiferoides in Johnson's Creek are probably at least three years old. Smaller snails harbor the infection, but possibly their immaturity prevents the maturation and release of cercariae.

The total incidence of infection (including all trematode species) in snails, 15-25mm in length, averaged about 75% with a maximum of 96%. Of the infected snails a maximum of 9% were infected with L. setiferoides and 89% with Zoogonus sp. Gambino (1959) found a total incidence of infection of 40% in N. obsoletus, 15-25mm in length in Rhode Island. At Woods Hole the percentage of infected snails with L. setiferoides was 70% in July, but in all other months Z. rubellus constituted over half of the infections (Miller and Northup, 1926). In New Jersey McDermott (1951) found 26.5% of the infected snails with L. setiferoides and only 13-15% with Z. rubellus. In the present study the low percentage of L. setiferoides infections may be due to the extremely high incidence of Z. rubellus (on the average 75% of the larger snails as compared to 13-15% in McDermott's study). The limited ability of L. setiferoides to reach maturity and produce eggs in L. putnami (see Section IV- Definitive Hosts) in Great Bay may also be a factor determining the low incidence of L. setiferoides in N. obsoletus.

The Second Intermediate Host. In June, 1967 penetration of a Lapocreadium setiferoides cercaria into a spionid polychaete was observed for the first time. I identified the polychaete as Polydora ligni Webster, 1879 (Family Spionidae) according to the description of Pettibone (1964). Its smooth mucous tubes were found in the spiral grooves and occasionally in holes in the shells of N. obsoletus. About

50% of P. ligni removed from shells were naturally infected with metacercariae of L. setiferoides.

The highest incidence of P. ligni on N. obsoletus (about 10% of the snails, 15-25mm long) occurred in June, July and August. The snail shells bore one, and occasionally two or three P. ligni tubes. The tubes also contained the eggs and larvae of P. ligni. Another polychaete, identified as Hypaniola grayi, Pettibone, 1953 (Family Ampharetidae), was abundant (up to 15/ft²). Its tough mucous, in which were embedded bits of debris, were about three times the length of the worms and were never found on N. obsoletus shells. H. grayi was not infected in the field or after 24 hours exposure to cercariae.

The presence of P. ligni coincided with cercarial release from N. obsoletus, when water temperature in the depression exceeded 17 C° in June, July and August.

To demonstrate transfer of L. setiferoides metacercariae to L. putnami and their development to the adult stage, L. putnami were kept in trays with several P. ligni. (See Table II for an outline of the six experiments). To reduce the natural infections of L. setiferoides in experimental fish, they were kept in a cold room aquarium (13 ± C) without food for 6 to 48 days prior to the experiments. The controls in which the duration of natural infections in starved fish was followed (see Table III) indicated that starvation for as short as 12 days was effective in removing infections from many, but not all fish. The possibility therefore remains that the adult L. setiferoides recovered from experimental fish originated from natural infections. In at least one fish in each of the six experiments adult worms were recovered in greater numbers than in the controls. Gravid worms were recovered in only one experiment, in which the metacercariae had

FIGURE I

Seasonal Distribution of Polydora ligni on N. obsoletus Shells in the
Depression at Johnson's Creek

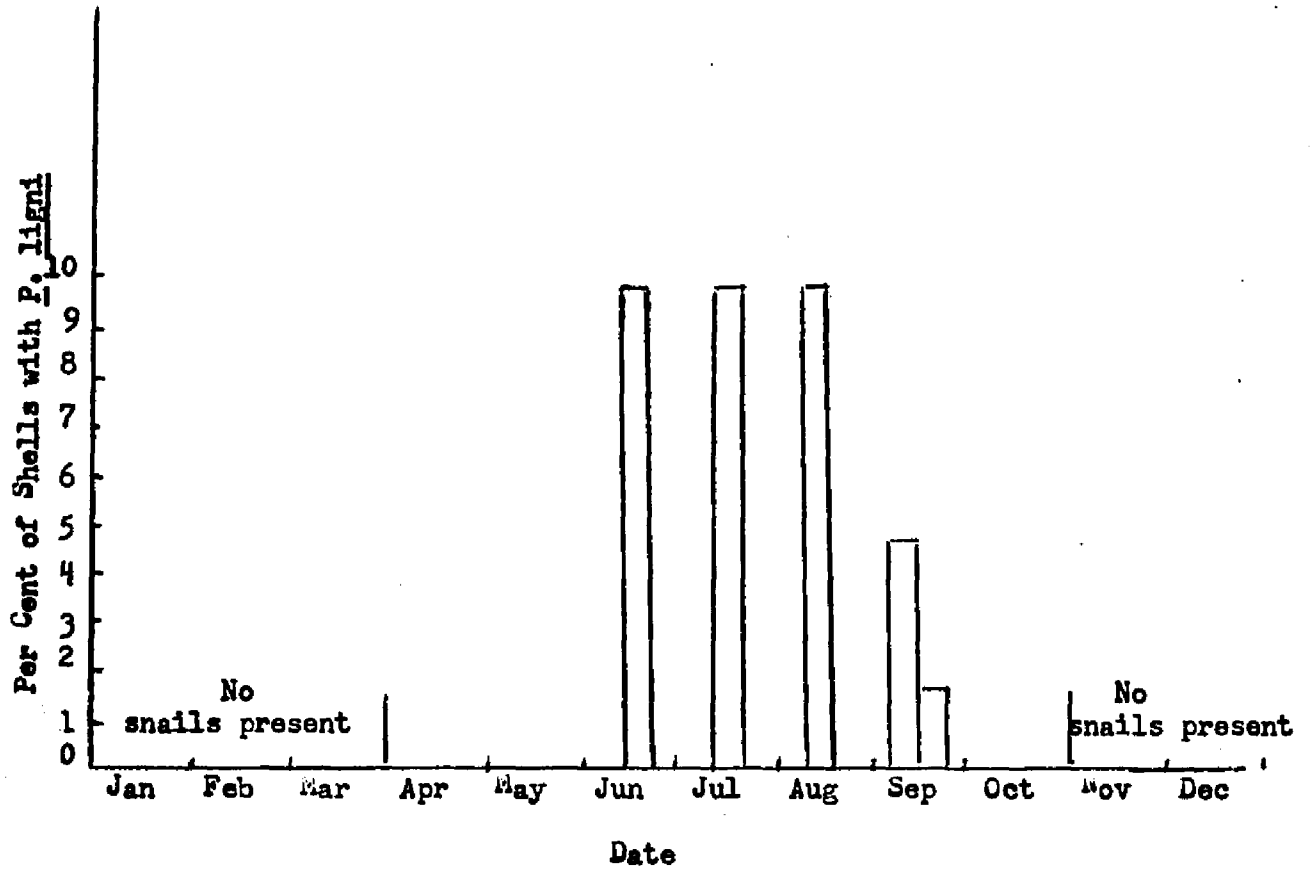


TABLE II

Infection of L. putnami by Ingestion of P. ligni Containing Metacercariae

Experiment	Time <u>L. putnami</u> not fed (days)	Time Metacerc. in <u>P. ligni</u> (days)	Day <u>L. putnami</u> dissected after ingesting <u>P. ligni</u>	No. of <u>L. putnami</u> infected of total ()	No. of <u>L. setiferoides</u> recovered
1	48	15	37th	1(5)	30 immature & 10 gravid
2	6	12	9th	1(3)	20+immature
3	11	13	8th	1(6)	20+immature
4	19	20	16th	1(1)	50+immature
5	32	34	8th	1(5)	15+immature
6	24	48	32nd 43rd	1(3) 2 dead fish	30+immature

TABLE III

Duration of Natural Infections of L. setiferoides in L. putnami

Day <u>L. putnami</u> dissected	No. of <u>L. putnami</u> infected of total ()	No. of adult <u>L. setiferoides</u> recovered
60th	0(1)	0
39th	1(1)	6 immature
34th	0(3)	0
12th	0(3)	0
19th	0(3)	0
11th	1(1)	100 immature & 7 gravid
13th	1(4)	20+ immature
32nd	1(5)	3 immature

developed for only 15 days in P. ligni.

Discussion

There are several interesting problems concerning the second intermediate host and the metacercariae of L. setiferoides: (1) the time of year when the second intermediate host, P. ligni becomes infected; (2) the possibility that P. ligni is the natural second intermediate host; and (3) the time of year when L. putnami, the definitive hosts becomes infected by ingesting P. ligni.

P. ligni was found on N. obsoletus shells from June through September. Since the period of cercarial release (April to October) overlaps, P. ligni may become infected throughout its duration on shells. There are no reports of this spionid on N. obsoletus shells, but it is known to form mud blisters on the inside of oyster shells (Mortenson and Galtstoff, 1944).

L. setiferoides undergoes no development of its reproductive organs in P. ligni. Lack of metacercarial development is also characteristic of L. album (Palombi, 1934) and L. pegorchis (Bartoli, 1967), thus cannot be used as an argument against P. ligni being a natural second intermediate host. Metacercariae remain free and unencysted in P. ligni for at least three months, suggesting that P. ligni is not detrimental to metacercariae and may provide nutrients for their sustenance, if not for growth. P. ligni has a prominent dorsal blood vessel, providing the metacercariae contact with a good blood supply.

Sexually mature worms were recovered in only one of the six experiments in which L. putnami were fed infected P. ligni. The failure to obtain mature worms more often may have been due to the inability of poorly fed fish to supply enough nutrients for development of the parasites, rather than to the absence in the second intermediate host of

some factor involved in metacercarial development. Though sexually mature worms were recovered in only one case, the recovery of immature worms as long as 37 days after ingestion and in larger numbers as compared to their recovery in control fish, suggests that L. putnami can be infected by ingesting P. ligni.

The location of P. ligni on large shells, though advantageous for infection with L. setiferoides, would also appear to aid in transfer of metacercariae to the definitive host, L. putnami. L. putnami infected with L. setiferoides are as small as 42mm in length and thus cannot ingest snails 15-25mm in length. However, possibly larger L. cutnami, 100+ mm long may ingest N. obsoletus bearing P. ligni in fall when the snails migrate into deeper water. Also, L. cutnami may be infected by browsing on shells or by ingesting P. ligni which have left shells and have either burrowed into the mud, or, in the case of larvae, have become planktonic, and are in shallow water. P. ligni has a planktonic larval stage from July to the end of October in Sweden (Hannerz, 1956). Possibly early larval stages before leaving the mucous tubes to become planktonic are infected with L. setiferoides cercariae. Finally, L. putnami may ingest cercariae released on the mudflats and transported to deeper water in the summer.

The Definitive Hosts. The collections of fish provide some new information on the seasonal and age distribution of Smooth and Winter Flounders, the only two flatfish (Family Pleuronectidae) encountered in the Great Bay region. Winter Flounders from the Oyster River ranged from 9.5-14.0cm and those from deep water off the coast of Portsmouth from 11.0-40.0cm. Smooth Flounders in shallow water were most abundant in July and August (10-15 per 50yd. seine haul). By mid-September they were scarce (one per 50yds.). Flounders were not found at Emerson's Beach in

early December, but nine were collected at Crommet Creek from a total of 15 seine hauls. For the size and seasonal occurrence of Smooth Flounders see Table IV and Figure II. They ranged from 2.0-19.0cm in length. Spawning males and females were found in November, December and January, and post-gravid females in February. The smallest mature female was 5.8cm and the smallest mature male 6.1cm. Small just-metamorphosed larvae of H. putnami were not seen.

For the incidence of Lepocreadium in the intestine of sculpins and flounders see Table V. Twenty M. scorpius were examined in fall, 1968. Only one was infected, with about 20 gravid Lepocreadium. Lepocreadium was not found in two M. aeneus and eight M. octodecimspinosus examined.

Two of the four Winter Flounders, 9.9-14.0cm long, collected in the Oyster River, were infected with gravid Lepocreadium. Winter Flounders from Portsmouth Harbor and from off the coast were not infected.

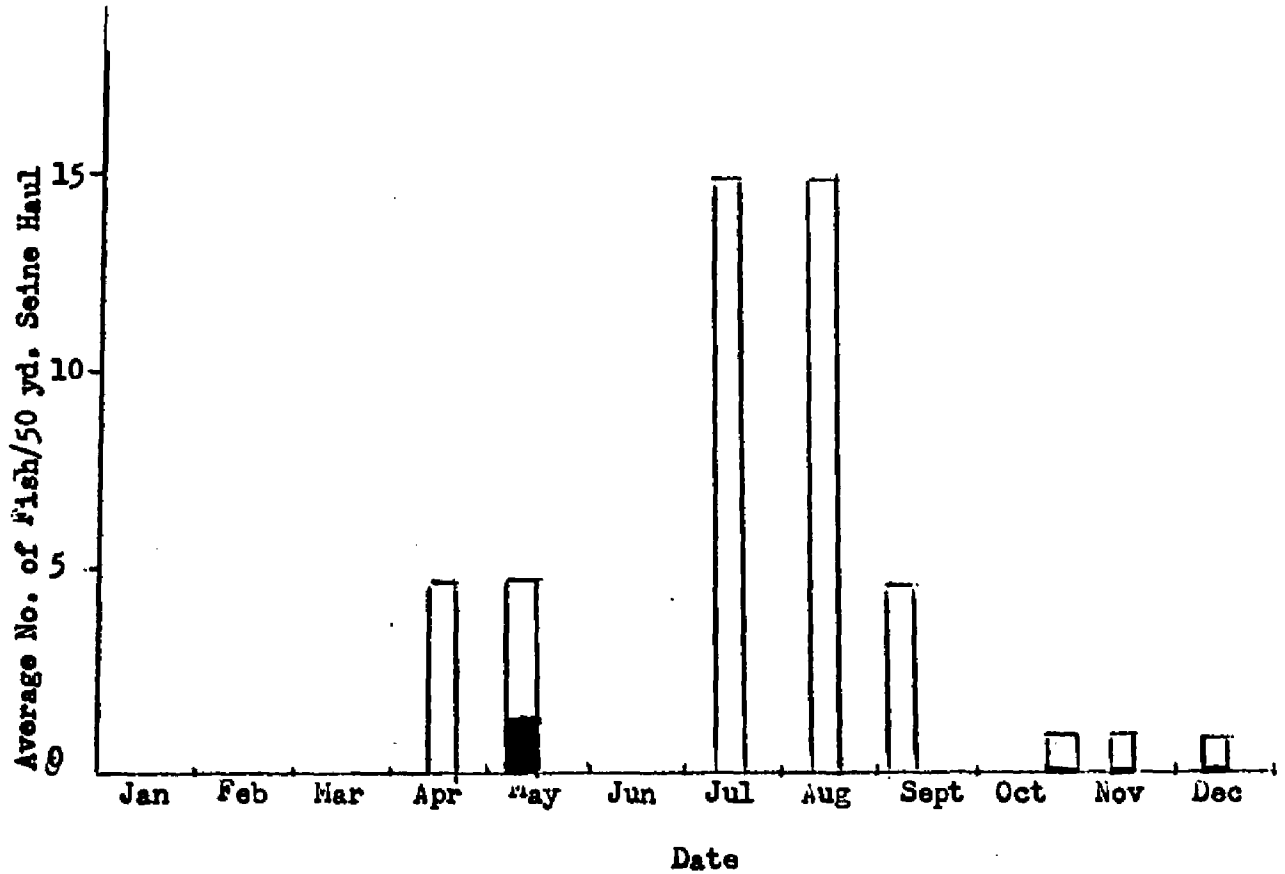
In May 25% of H. putnami contained gravid L. setiferoides. This was the highest incidence of gravid worms during the year. The heaviest infections (50-100worms/fish) and the highest incidence of infection (50-90% of fish) occurred in July and August, but no gravid worms were present. Infected fish ranged from 2.8-19.0cm in length, thus all age classes harbored the infection. In October, November and December 11% of L. putnami contained gravid worms, infections were light (10-15 worms) and on the average only 50% of the fish were infected. By January and February the incidence of infection had dropped to about 10% and the infections were very light (1-10 worms).

Discussion

L. putnami is abundant from May through mid-September in shallow creeks of Great Bay, whereas P. americanus, rare in shallow waters of the Bay, is more common in coastal waters. However, there may be local populations

FIGURE II

Seasonal Distribution of L. putnami at Emerson's Beach and Crommet Creek, and Percentage Infected with Gravid L. setiferoides



Note: Portion of blocks darkened =% of L. putnami/month with gravid L. setiferoides

TABLE IV

Size Distribution of L. putnami in Shallow Water in the Great Bay Region

No. of Fish per Size Class	Date										
	June	July	August	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April
2.0- 2.9		3	2								
3.0- 3.9		3									
4.0- 4.9		13	13	2	1						
5.0- 5.9	1		4	7	3	8	2	56	4		1
6.0- 6.9		1	1	2	3	10	3	7	2		
7.0- 7.9		1	3	2			1	7	2		
8.0- 8.9	1	2	5		5	1	1	5			1
9.0- 9.9		2	2		2	2	2	5			4
10.0-10.9	3	1	2	3		1		2	3		
11.0-11.9		1	2			1		1	2		1
12.0-12.9		1							2		
13.0-13.9				1	1				2		
14.0-14.9						1		1	1		
15.0-15.9									1		
16.0-16.9		1									
17.0-17.9											
18.0-18.9											
19.0-19.9				1							

TABLE V

Incidence of Lepocreadium in L. putnami, P. americanus and M. scorpius

Location	Date	Size of Fish (cm)	<u>L. putnami</u>			<u>P. americanus</u>			<u>M. scorpius</u>		
			N	#I	#E	N	#I	#E	N	#I	#E
2	5/3	5.0-16.0	3	8	2						
2	7/8	2.6-12.2	15	9	0						
7	7/13	11.3				1	0	0			
6	7/15	2.0- 9.5	16	8	0						
7	7/26	18.0-23.3				3	0	0			
6	8/6	2.4-10.5	22	12	0						
5	8/12	4.2-11.9	14	13	0						
6	9/6	4.8- 8.5	12	10	0						
3	9/19	9.5				1	0	00			
7	9/30	23.0-23.4									
1	9/30	6.0-13.7	5	0	0						
3	10/3	8.5- 9.2	2	0	0						
2	10/4	5.6- 8.7	4	0	0						
7	10/6	36.8							1	0	0
7	10/8	29.2-30.8							2	0	0
7	10/12	32.4							1	0	0
7	10/13	36.3	4	1	1				1	0	0
6	10/13	5.5- 9.2									
7	10/14	21.1-29.2							2	0	0
7	10/15	29.9-34.9							3	0	0
7	10/16	37.6							1	0	0
7	10/18	27.9-28.5							1	0	0
1	10/18	4.7-13.1	5	2	1						
3	10/19	14.0				1	1	1			
6	10/20	5.5- 8.1	3	2	1						
3	10/22	9.5				1	0	0			
7	10/26	30.9-34.0							2	0	0
7	10/29	30.9-35.0							4	0	0
1	11/3	5.4-14.0	3	3	1						
3	11/16	7.2	1	1	1						
3	11/17	5.9- 6.6	3	3	0						
6	11/19	11.2	1	0	0						
3	11/19	9.9				1	1	1			
7	11/23	30.4-31.4							2	0	0
1	11/23	5.1-10.4	16	11	2						
1	11/29	5.8- 9.6	2	2	0						
1	12/4	5.7- 9.7	9	7	1						
3	1/3	5.7-13.9	18	4	0						
3	1/31	6.0-14.0	10	0	0						
8	2/1	11.0-40.0									
3	2/21	5.7-14.0	15	1	0	23	0	0			
6	4/17	5.6-11.8	10	3	0						

Locations

- (1) Crommet Creek
- (2) Adam's Point
- (3) Oyster River below dam
- (5) Johnson's Creek
- (6) Emerson's Beach
- (7) Portsmouth Harbor
- (8) Portsmouth off coast

N = Number of fish examined
 #I = Number of fish infected
 #E = Number of fish with gravid worms

of Winter Flounder. Trawling in deeper water in the Bay will be necessary to determine the distribution of flounders at all depths.

The breeding season of L. putnami in the Bay extends from November to January, and thus is similar to that of Smooth Flounders in Salem, Mass. (Bigelow and Schroeder, 1953).

Winter flounders in the Oyster River are half the size of those reported by Bigelow and Schroeder(1953) in shallow waters of the Gulf of Maine.

Data in Table IV indicate that Smooth Flounders in the Bay grow 50-60mm (about 2 inches) in the first year (0 year class), since the smallest fish in January reach this size, and the larvae produced in late fall and winter have not settled.

The presence of L. setiferoides in L. putnami in October, November and December but not in July, August and September, suggests a correlation between the breeding season of L. putnami and the maturation of L. setiferoides. However, gravid worms were also recovered in May. There are two other possible explanations for the occurrence of gravid worms in late fall collections: (1) L. putnami may be more likely to ingest cercariae released from N. obsoletus, and P. ligni containing metacercariae, when these hosts are migrating from the mudflats into deeper water in late summer and fall. (2) The maturation of the parasite may take several months. The heavy infections with immature adults in L. putnami in July and August possibly result from direct ingestion of cercariae. During the remainder of the summer and in fall many worms may drop out of the gut, but some may develop to sexual maturity, resulting in light infections and occasional gravid worms in late fall and winter.

The extremely low incidence of gravid adults in L. putnami in 1968 may indicate (1) a low incidence for this particular year, or (2) that L. putnami is not the usual definitive host in the life cycle of L. setiferoides.

Possibly it matures more readily in P. americanus; however, because of the few P. americanus obtained in the present study, this possibility could not be confirmed. In further studies the P. americanus population from all depths should be examined for the incidence of L. setiferoides.

The marked decline in incidence of L. setiferoides in L. putnami in January and February in all age classes, is probably not due to cessation of feeding, since many fish contained polychaetes in their gut at this time. Since the incidence of infection is high in all year classes during the summer, probably flounders which are initially infected in their first summer and lose the infection in late winter can become infected again in the second year.

Description of the Life Stages of L. setiferoides, Rediae in N. obsoletus consist of a single generation, containing the cercarial germinal balls (figs. 2-3). Younger rediae, .14-.21mm in length and .04-.08mm in width, contain only a few germinal cells, or small germinal balls. Cercariae leave the rediae in an immature state, with a short tail lacking setae, and complete maturation free in the host tissue. Both rediae and cercariae are located in the gonads, digestive gland and connective tissue of the oviduct and digestive tract. Cercariae emerge in the presence of light and are photopositive.

The free-swimming cercaria (fig.4) has two prominent eyespots, a tail longer than the body, bearing lateral bunches of setae, and an excretory bladder (fig. 5) containing refractile spheres. The following are measurements on live, lightly compressed cercariae; body length, .21-.28mm; pharynx length, .023-.028mm; oral sucker width, .060-.082mm; ventral sucker width, .040-.059mm; diameter of eyespots, .018-.023mm; tail length, .35-.74mm; tail width at base, .023-.035mm.

Metacercariae are found, in both natural and experimental infections,

in the coelom of P. ligni (fig. 6). In no case were they encysted, even after three months. Due to the transparency of the host's body wall living metacercariae could be observed in the coelom (fig. 7). The number of metacercariae in a single P. ligni ranged from 1-30, with an average of about 20 in a single P. ligni. Metacercariae did not appear to affect the host adversely. When exposed to large numbers of cercariae during experimental infection, P. ligni lashed vigorously aiding their contact with the polychaete body wall.

The morphology of living adult L. setiferoides (figs. 8-9) taken from L. putnami was studied. Except for their larger size the specimens agree with the description of L. setiferoides by Martin (1938); thus excepting some comments on the reproductive system, they are not redescribed here. The distribution of vitellaria, from the mid-level of the pharynx to the posterior end of the body agrees with that of Martin's specimens. The single right ovary is emptied by a conspicuous ciliated oviduct not described by Martin. Distal to the testes an external seminal vesicle leads by a narrow duct into the internal seminal vesicle within the cirrus pouch. Martin did not use the term external seminal vesicle, but described a dilation of the distal end of the ductus deferens. The internal seminal vesicle is followed by the pars prostatica glandular tissue (which Martin also described), surrounding the cirrus. The latter extends to just anterior to the ventral sucker.

Dr. Bullock's specimens of Lepocreadium from P. americanus, 17.5-25.5cm in length, show the following features: (1) the anterior extent of the vitellaria near the pharynx depends on the degree of contraction of the specimens, and (2) the posterior distribution of the vitellaria is variable, in some specimens overlapping the posterior testis and in others not at all. Furthermore, the egg dimensions vary

considerably between specimens in the same host (e.g. egg length ranges from at least .089-.130mm) as well as between specimens from different hosts of the same species.

Discussion

L. trullaforme was described as a new species from Longhorn Sculpins (Myoxocephalus octodecimspinosus), the type host and from Winter Flounders (Pseudopleuronectes americanus), Hogchokers (Achirus fasciatus), White Perch (Morone americanus), Kingfish (Menticirrhus saxatilis) and Cunners (Tautoglabrus adspersus) at Woods Hole by Linton (1940). His description was made without reference to L. setiferoides from flounders, the life cycle of which had been described in part by Martin (1938) at Woods Hole. Stunkard (1969) has recently pointed out an error in Linton's description, in that the holotype of L. trullaforme was actually from the Cutlassfish Trichiurus lepturus rather than from M. octodecimspinosus. Sogandares-Bernal and Hutton (1960) continued to recognize the two species and redescribed L. trullaforme from T. lepturus, but did not compare the latter with Martin's specimens of L. setiferoides.

Comparing Martin's description (1938) of L. setiferoides and Sogandares-Bernal and Hutton's description (1960) of L. trullaforme, the two supposedly differ in (1) the anterior extent of the vitellaria: in L. setiferoides extending to the anterior margin or mid level of the pharynx, in L. trullaforme extending only to slightly posterior to the pharynx; and (2) the posterior distribution of the vitellaria: in L. setiferoides the vitellaria not coalescing over the posterior testis, whereas in L. trullaforme the vitellaria overlapping the ovary on the right and coalescing over the posterior body including the posterior testis.

In the past there was a tendency amongst parasitologists to

describe as separate species adult trematodes from different hosts showing morphological differences. Many species description were based entirely on adult worm morphology without reference to the life cycle. The validity of many of these species is questionable, for as Stunkard (1957) pointed out, a single species may vary depending on which host species it inhabits, and on differences in physiological conditions of hosts. In addition, Stunkard (1969) has demonstrated the importance of interrelating studies of larval stages, developmental processes and adult morphology with taxonomic studies.

The following are some tentative conclusions concerning the identification of Lepocreadium in flounders and sculpins on the New Hampshire coast. (1) The specimens of Lepocreadium from L. putnami collected in Great Bay have been identified as L. setiferoides, because of the similarities in life cycle and larval and adult morphology between these worms and those described as L. setiferoides from flounders at Woods Hole by Martin (1938). (2) The life cycle of Lepocreadium in M. scorpius and P. americanus is not known, except for the development of the miracidium from the former. It is probably justifiable to describe Lepocreadium from M. scorpius and P. americanus as L. setiferoides on the basis of their similarities to adult worms and eggs from L. putnami. L. setiferoides and L. trullaforme are probably synonymous, but further life cycle studies will be necessary to determine how closely related are individuals of Lepocreadium from L. putnami, P. americanus and M. octodecimspinosus. The following studies are suggested:

- (1) Experimentally infect N. obsoletus from Great Bay with miracidia developed from eggs in M. scorpius at Portsmouth Harbor.
- (2) Identify the larval stages of Lepocreadium in N. obsoletus from Portsmouth Harbor in locations where M. scorpius and P. americanus are

known to occur.

(3) Experimentally infect L. putnami from Great Bay either with cercariae of Lepocreadium from N. obsoletus from Portsmouth Harbor or by ingestion of P. ligni infected with cercariae from Portsmouth.

(4) Investigate possible second intermediate hosts, including P. ligni in Portsmouth Harbor.

(5). Compare the development of Lepocreadium eggs from the three fish hosts. (This was begun in the present study).

The Miracidia. Gravid adult Lepocreadium were recovered from the intestine of three species of marine fish: L. putnami, P. americanus and M. scorpius. The number of L. putnami with gravid L. setiferoides was extremely low (10 out of 183 fish dissected).

The Lepocreadium eggs from L. putnami and M. scorpius were similar in size, while those from P. americanus were somewhat smaller (mean egg lengths: from P. americanus, .108mm; from L. putnami, .139mm and from M. scorpius, .138mm). All eggs had a length greater than the diameter of the ventral sucker. Unfortunately it was not possible to compare the development of the eggs from the three different hosts: only those from M. scorpius developed into mature ciliated miracidia. Eggs from L. putnami developed for 24 days at which time an eyespot, but no cilia, was visible, (figs. 13-14).

Eggs are released from adult worms prior to (fig. 11) or in early cleavage (fig. 12). They develop in sea water at 25-26°C, but not at 14°C. By the 27th day some eggs from M. scorpius had developed into elongated miracidia with a conspicuous eyespot and a covering of ciliated plates (fig. 15), with the head end directed towards the operculum. By the 38th day some miracidia had hatched. I was unable to collect free-swimming miracidia for experimental infections of the first intermediate host, N. obsoletus.

Discussion

The miracidium has been described for only one other species of Lepocreadium, L. album, by Palombi (1934 and 1937), but the developmental time was not stated. According to his descriptions and drawings L. album miracidia have no eyespots and no operculum. Both are present in Lepocreadium miracidia from E. putnami and M. scorpius.

The long period (38 days at 26° C) observed here for miracidial development in L. setiferoides may not be required under natural conditions. Stunkard (1969) found that miracidia of Neopechona pyriforme (Family Lepocreadiidae), which infect the gastropod Anachis avara of the lower littoral zone emerge in sea water in 9-10 days. On the other hand, the long developmental period of L. setiferoides miracidia may be advantageous in retarding the hatching time of miracidia until N. obsoletus migrates from deeper water in April and May.

SECTION V

THE LARVAL AND ADULT LIFE STAGES AND DEVELOPMENT OF THE EPIDERMIS

The Redia. The radial germinal sacs, in which the cercariae develop, are found, in some infected N. obsoletus, predominantly in the connective tissue of the gonad and oviduct. In other snails they occupy both the gonadal connective tissue and spaces between the tubules of the digestive gland. Up to 20 larvae per 1mm of host tissue section was observed. In uninfected snails the average distance between the hepatopancreatic tubules is about .08mm, hence young rediae, about .04mm in diameter, may migrate between these tubules without injuring them. As the germinal balls develop within them, the rediae increase in size up to $4\frac{1}{2}$ times to a diameter of .5mm, presumably press on the walls of the tubules, and may cause breakdown of some of them. However, many tubules appear to remain intact. Extensive breakdown occurs in the connective tissue of the gonad. Young rediae have a large muscular pharynx which is often filled with host tissue cells, (fig. 17), and a saccular gut bearing projections. They appear to ingest host tissue cells, and in so doing, procure a path for their migration. A trail of disrupted tissue is often seen posterior to them. The gonadal connective tissue may repair itself; older rediae are often surrounded closely by intact connective tissue cells, (fig. 21). Possible indirect evidence of radial digestion of host tissue cells are the many granules (0.3-3.0u in diameter) stained metachromatically with methylene blue, seen in both the host tubule cells and in the radial parenchyma.

Rediae of various sizes are found in close proximity. In contrast, the maturation of the cercariae is more uniform; in some infections none

or very few cercariae are seen free in the host tissue, while in others there are many cercariae with eyespots and tails outside of the rediae. (For further discussion on cercarial maturation see Section V - The Cercaria).

Young rediae, containing only a few separate germinal cells or a small germinal mass and a prominent sac-shaped gut, measure .14-.21mm in length and .04-.08mm in width, (fig. 18). The miracidia were not observed during their invasion nor during their presumed transformation into rediae. In the smallest rediae the body wall is smooth, but in rediae about .18mm or more in length, portions of the body wall are folded, (fig. 18). In addition, the body wall of young rediae displays two types of surface modifications (1) tortuous invaginations up to half the diameter of the redia (figs. 20 and 22) and (2) narrow inpocketings bearing numerous microvilli (fig. 27). The superficial epidermis of young rediae is thin (.5 μ) with a prominent basal lamina constituting about one-third of the thickness of the epidermis, (figs. 22-27). There are few mitochondria, about 1 per 1 μ length of 60 μ thick section of epidermis. Spherical, electron-dense, membrane-bound granules, henceforth called secretory granules, .1 μ in diameter, occurred in the epidermis of all young rediae examined (figs. 22-27). They occasionally appear to pass to the outside of the epidermis, but not to merge into a dense zone beneath the apical plasmalemma. Cytoplasmic nucleated regions below the muscle layers in the radial wall contain similar granules; in a few places cytoplasmic projections from these regions extend through the muscle layers to the superficial epidermis (fig. 25). There is an outer circular and an inner longitudinal layer of muscle. The muscle consists of thick (200-300A in diameter) and thin (60A in diameter) myofilaments (fig. 23). These are not arranged in

A and I bands.

Long thin projections (microvilli) extend from the apical surface of the radial epidermis. These vary considerably in length in any given region of the epidermis and between different rediae. The longest microvilli range from about .26-.96 μ in length. Their width is more uniform, from .04-.05 μ . They are often swollen distally; occasionally vesicles appear to be pinched off (fig. 23). The material of the secretory granules may be extruded by the pinching off of these vesicles. At the interface between the epidermis of young redia bearing microvilli and the host digestive gland tissue, there is a clear area (fig. 26). In older rediae, .30-.54mm in length and .09-.15mm in width, the germinal cavity becomes packed with developing germinal balls. The radial wall surrounding this cavity appears to be stretched and the basal lamina of the epidermis is not folded. In places the radial epidermis and its microvilli are lacking, presumably disintegrated or worn-off (fig. 29).

Large (up to 0.8 μ in diameter) non-membrane bound lipid-like inclusions are numerous in the snail digestive tubule cells. In the radial parenchymal tissue there are alpha particles (or rosettes) of glycogen and large electron-dense spheres bounded by numerous membranes (fig. 28).

Discussion

The occurrence of various sizes of rediae in close proximity in N. obsoletus tissues suggests that several infections with miracidia at different times may occur. In contrast, maturation and release of cercariae may be uniform; in some infections there are numerous cercariae outside the rediae, whereas in others there are few or none. Possibly some

internal host factor or an external factor such as temperature, initiates the release of cercariae from the rediae. Dinnik and Dinnik (1964) found that low temperatures prevented the development of the cercariae but not that of the daughter rediae of Fasciola gigantica.

The redial epidermis of E. setiferoides, consisting of a cytoplasmic syncytium bearing microvilli, is similar to that of other species, e.g. Bills and Martin (1966), Rees (1966), Ginetsinskaya (1967) and Krupa et al., (1967 and 1968). However, my findings suggest that functional changes occur in the epidermis during development of the redia.

The effects of L. setiferoides on the host tissues may be compared with ultrastructural changes observed in the epidermis. Young rediae with microvilli are seen in portions of the gonads where extensive histolysis has occurred. The microvilli and secretory granules in the young redial epidermis may cause, at least in part, the histolytic effects. Older rediae, lacking epidermal microvilli and secretion granules, may not cause lysis since they are often closely embedded in host tissue. In older rediae the epidermis may degenerate, allowing for a restoration of the host tissue.

The presence of epidermal microvilli suggests that the redial epidermis of L. setiferoides can absorb nutrients. Microvilli are found on cells which have an absorptive function (e.g. intestinal epithelial cells, Granger and Baker, 1950). Nutrients may be made available by the histolytic processes of young rediae. However, autoradiographic studies, for instance, with labeled glucose, will be necessary to demonstrate absorption processes directly. To the author's knowledge there have been no such studies on rediae. The presence of glycogen and lipid inclusions in the redial parenchyma is also indirect evidence of the absorption of

nutrients, but without autoradiographic studies it cannot be said whether these originate from absorption through the epidermis or through the gut. The small number of mitochondria in the radial epidermis might suggest that absorption processes requiring metabolic energy are limited. However, with an ample supply of carbohydrates in the snail host tissue, the rediae probably derive considerable metabolic energy from glycolysis.

To what extent the radial epidermis of L. setiferoides is involved in absorptive processes is not known. The deep invaginations of the radial wall and the narrow inpocketings bearing numerous microvilli have not been described in other radial species. They appear to be an effective means of increasing the absorptive surface of the redia. Several studies support the present hypothesis that the radial epidermis has histolytic and absorptive functions. The studies of Cheng and Snyder (1962a and b; 1963) on the sporocysts and cercariae of Glythelmins in Helisoma showed an increase in fatty acids, neutral fats and glucose and a decrease in glycogen in the infected hepatopancreas, the presence of fatty acids and glucose in the sporocyst wall, and glycogen and fatty acids in the cercariae. These studies suggest that the sporocysts cause the breakdown of hepatopancreatic glycogen to glucose and of triglycerides to fatty acids, and that they absorb glucose which is converted into glycogen in the cercariae. Phosphatase enzymes, which may be involved in glucose absorption (Stadtman, 1961) have been demonstrated in the radial epidermis of Cryptocotyle lingua (Krupa et al., 1968).

The large muscular pharynx, often filled with host tissue, in L. setiferoides redia suggests that the gut is an important site of nutrient uptake.

In summary, there is indirect evidence in the present study that the redial epidermis undergoes changes during the maturation of rediae. The young redial epidermis with microvilli and secretory granules, appears to break down host tissue, as evidenced by the clear areas around these rediae, the ruptured host cell membranes and the release of inclusions of the host cells. In older rediae the epidermis appears to degenerate, thus allowing for restoration of host tissue.

The Cercaria. Single germinal cells and small germinal balls lie posteriorly in the germinal sac (fig. 18). Apparently mitotic divisions of cells within the same germinal balls are synchronous. Occasionally mitotic figures of the metaphase stage are seen in young germinal balls. During early anaphase six chromosomes occur in each daughter cell (fig. 16), hence L. setiferoides has a diploid complement of 12 chromosomes.

Electron micrographs suggest that the epidermis develops from the upward extension of cytoplasmic regions of large cells located beneath the developing body wall muscles (fig. 29). The nuclei of these cells were never seen on the surface of the germinal balls. In the earliest stage in which the surface epidermis is visible with mitochondria and a basement membrane, it is about .25 μ in thickness (as compared to the 2-3 μ thick mature cercarial epidermis. Its basement membrane is interrupted by cytoplasmic projections from the lower nucleated region of the epidermis (fig. 29).

Cercariae apparently emerge from rediae in an immature state, since in dissections of large rediae, mature cercariae were never found.

The epidermis is described below, followed by brief observations on the excretory bladder epithelium and the mitochondria of the tail, for the purpose of comparison.

The epidermis of emerged cercariae is between 2.5-3-5 μ in thickness, and is organized as in adult trematodes. It is divided into a superficial syncytial layer connected by narrow cytoplasmic projections to a nucleated cellular region beneath the muscle layers. The apical surface is extensively folded and the crypts are as deep as one-half the thickness of the superficial epidermis. The latter is bounded on its apical and basal surfaces by a plasmalemma. Directly beneath the apical surface is a distinct narrow dense zone (.04-.05 thick) (fig. 30-31). This dense zone is not seen in the bladder and caecal epithelia. The basal lamina is interrupted (fig. 30) by epidermal cytoplasmic projections, about .25 μ in width extending from the nucleated region of the epidermis between the circular and longitudinal muscle bundles.

In the superficial epidermis the "cuticular" spines appear to be fused at their base with the basal plasmalemma, and at this site of fusion there is an increased density (fig. 30). The spines show fine longitudinal striations with a periodicity of 200A (fig. 31). The superficial epidermis contains many clear vesicles and tiny invaginations of the basal plasmalemma, about .04 μ in width. About six mitochondria per μ length occur in 60-80 μ sections of the superficial epidermis. They are spherical and from .13-.50 μ in diameter, whereas the mitochondria of parenchymal cells range from .48-1.00 μ in diameter. The cristae are plate-like.

Two types of membrane-bound dense inclusions occur in the epidermis. Some are spherical, .107-.114 μ in diameter, and others are rod-like. Henceforth these are abbreviated as sDB and rDB respectively. The latter are most numerous just beneath the apical surface where they are oriented at right angles and reach their maximum elongation of .3 μ and minimum width of .13 μ . The rDB's are also often expanded at their distal ends into bulb-like structures (fig. 31), which appear to merge with the

dense zone beneath the apical plasmalemma.

The lower nucleated region of the epidermis lies below the body wall muscles at a distance of 6-8 μ from the basal plasmalemma. In the nuclei of the epidermal cells the nucleoplasm has a conspicuously light electron density but contains large clumps of chromatin. The epidermal cell cytoplasm is commonly packed with DB's similar to those in the surface epidermis (fig. 32). Agranular endoplasmic reticulum and mitochondria are also prominent components of the epidermal cells (fig. 32). Where the DB's are numerous there are often large less dense spherical or oval bodies, 0.9-1.6 μ in diameter, containing clear areas about the size of the sDB's (fig. 32). Occasionally the latter are lodged in these bodies.

Dense glycogen-like particles, about 150A in diameter, are dispersed in the lower epidermal cells, but in the adjacent parenchymal cells they are in heavy accumulations (fig. 32).

The epidermis of the suckers is about 1.8 μ thick (fig. 33). Two features distinguish it from that of the body wall. Thin projections, about .60 μ long and .084 μ in diameter, project from the basal plasmalemma through half the thickness of the epidermis and contain extensions of the basal lamina as a central core (fig. 33). Large dorso-ventral muscle bundles attach to cylindrical projections of the lower surface of the basal lamina.

The epithelium of the excretory bladder is about .8 μ thick (fig. 34). Long leaf-like projections, bounded by a plasmalemma, extend from the apical surface. They average .6 μ in diameter and 1.2 μ long. Large osmiophilic inclusions occur in the underlying parenchymal cells. Living specimens of cercariae, metacercariae and adult worms

contain refractile spheres in the excretory bladder (figs. 4-5, 7 and 8). In electron micrographs these spheres are seen either free in the lumen or enmeshed in the projections of the bladder epithelium. They consist of numerous dense concentric layers (fig. 34).

The cercarial tail whips rapidly and continuously. It contains bundles of myofibers arranged in four groups. Between these groups are masses of mitochondria arranged in a broad cross pattern (fig. 35). The mitochondria average 1.8 μ in length, as compared to the maximum length of .5 μ of the epidermal mitochondria. The cristae are more numerous in the tail mitochondria than in the epidermal and parenchymal mitochondria.

Discussion

In the morphogenesis of cercarial germinal balls of L. setiferoides, the epidermis may be of parenchymal origin; it appears to develop from upward extensions of large cells located beneath the body wall muscles. This observation is in contrast to the conclusion of Cheng and James (1960), who found that the cercarial epidermis of Crepidostomum was derived from the ectoderm.

The cercarial epidermis comprises a superficial syncytial layer connected by cytoplasmic projections to the inner nucleated epidermal cells proper beneath the body muscle layers.

The cercarial epidermis displays many of the ultrastructural features of the adult epidermis, including a thin dense zone directly beneath the apical plasmalemma, mitochondria and DB's in both the superficial and nucleated portions of the epidermis, and the absence of endoplasmic reticulum and Golgi complexes in the superficial epidermis. A major question is whether the DB's and mitochondria are functional in the cercarial stage, or whether they are merely present

in preparation for the metacercarial and adult stages. However, only autoradiographic studies (e.g. to measure the rate of uptake of substances into the epidermis) and biochemical fractionation studies (e.g. to isolate the DB's) can give direct evidence of metabolic processes.

The mitochondria in the cercarial epidermis are sparsely scattered throughout the epidermis. Their cristae are few compared to those in the tail mitochondria. Despite their poor development the epidermis may absorb carbohydrates and lipids. These are the primary nutrients in molluscan hepatopancreas and gonads (Cheng and Snyder, 1962a and b). Both glycogen and lipid inclusions are probably present in the cercarial parenchyma. The size and dense accumulations of the 150-250A particles, indicate that they are probably glycogen deposits, similar to those described in mammalian tissues by Fawcett (1966). Their accumulation in the parenchyma resembles glycogen distribution in other trematodes (e.g. in Bucephalus in Crassostrea described by Cheng and Burton, 1966). Particles, similar in size but in much smaller concentrations in the epidermal cells may be either free ribosomes or dispersed glycogen particles. The absence, or at least smaller amounts of glycogen particles suggests that in epidermal cells glucose is either (1) transported to parenchymal cells or (2) immediately used up in the synthesis of the dense inclusion bodies, which may be acid mucopolysaccharides. (See further discussion on DB's in Section V - Comparison of Epidermis). Since the cercariae complete maturation outside the redia in the snail tissue, during this period they may absorb nutrients through the oral sucker.

In the excretory bladder the spherical excretory spheres formed in the cercaria and present in the metacercaria and adult may be either

(1) retained in the metacercaria and adult after their initial formation in the cercaria or (2) formed anew in each stage. Erasmus (1967) described the fine structure of the excretory reserve bladder of a strigeoid trematode. The L. setiferoides bladder resembles it in its possession of epithelial cell apical filaments and concentrically layered spheres. The latter contain lipids according to Erasmus. However, Martin and Bils (1964) called these spheres calcareous concretions. It would be of interest to study (1) the chemical nature of the spheres, particularly since they are present in the cercaria, metacercaria and adult stages, in order to determine whether they contain the same substances throughout their diameter (if they are retained through the 3 life stages), and (2) the manner of deposition of materials on the spheres, whether by precipitation of substances in solution in the excretory bladder or by secretion of substances from the epithelial cells while the spheres are still in contact with them.

The Metacercaria. Living metacercariae were observed for three months through the transparent body wall of P. ligni. The prominent excretory spheres were present throughout this time. At no time was a cyst of parasite origin secreted around the metacercariae. Ultrastructural studies on the 42 day-old metacercariae are not complete and thus will not be described here.

Figure 36 is the epidermis of a metacercaria approximately 40 hours after penetration into the second intermediate host. The mitochondria are still spherical. Both sDB's and the rDB's are present, and the latter appear to merge occasionally with the dense zone beneath the apical plasma-lemma. In the muscle and parenchymal tissue there are numerous single and clumped glycogen-like particles, 120-160A in diameter. The only marked

difference in the metacercarial epidermis as compared to the cercarial epidermis is a flattening of both the apical and basal surfaces of the former.

The caecal epithelium (fig. 37) bears little resemblance to the epidermis. It contains large dense nuclei. Continuous with the apical plasmalemma are long thin microvilli, about 3.2 μ long and .05 μ in diameter, containing a central core, about 70A in diameter. In the epithelial cytoplasm the most conspicuous components are the granular endoplasmic reticulum bearing particles, 130-180A in diameter, and numerous mitochondria.

Discussion

The metacercarial epidermis will be discussed in Section V - Comparison of the Epidermis.

Microvilli have been observed in the gut of a number of trematodes (Thorsell and Björkman, 1965; Halton, 1966; Dike, 1967 and Halton et al., 1968). Thorsell and Björkman demonstrated by autoradiographic methods the absorption of amino acids. Intracellular formation of insoluble hematin suggests that hemoglobin can be taken up directly by the gut (Halton et al., 1968). The presence of granular endoplasmic reticulum in the above and present studies suggests that the gut epithelium is involved in secretory functions. Fawcett (1966) states that a well-developed granular endoplasmic reticulum is characteristic of protein-secreting glandular cells. The gut of L. setiferoides may be the principal site of amino acid absorption. Ingested host protein may be hydrolyzed in the gut lumen, and the smaller peptides and amino acids then absorbed. Thorsell and Björkman (1965) demonstrated the secretion of lytic substances in the trematode gut.

The Adult. The adult is attached by its oral sucker to the intestinal mucosa of the definitive host (the Smooth Flounder, Liopsetta putnami), and is embedded in the folds of the mucosa.

The adult epidermis (figs. 38-42) like that of the cercaria and metacercaria, has a superficial layer connected by cytoplasmic projections (fig. 39) to a nucleated region beneath the muscle layers. The surface of the former bears irregular folds, ranging from 0.5-1.0u in width, formed by invaginations of the apical surface projecting through up to one-half the thickness of the superficial epidermis. The basal portion of the superficial epidermis is also deeply folded. The outer leaflet of the apical plasmalemma is coated with fine filaments (fig. 41). Beneath the apical plasmalemma is a distinct narrow dense zone similar to that in the cercarial and metacercarial epidermis (figs. 40-41). In the superficial epidermis the most conspicuous elements are mitochondria and DB's. Granular endoplasmic reticulum and Golgi complexes are absent.

The mitochondria (about .11-.18u in length and .08-.15u in diameter) are smaller than those of the adult parenchymal cells, which average .5u in length, and smaller than mitochondria in the cercarial epidermis (about .13-.5u in diameter). The mitochondria are oriented with their longitudinal axis towards the distal surface, (figs. 40-41). Their cristae are parallel to this axis and thus at right angles to the distal surface. This arrangement of the cristae is independent of methods employed in preparation of the material.

The number of mitochondria in the superficial epidermis is greater in the adult than in the cercaria, i.e. at least 20 per 1u length of epidermis in sections 60-80mu in thickness in the former as compared to about six in the latter. In addition, in the adult the mitochondria show

a distinct concentration in the distal half of the superficial epidermis.

sDB's and rDB's like those in the cercarial and metacercaria epidermis, are present in the adult epidermis (fig. 40). The rDB's vary from an oval rod to an elongated dumbbell. They reach their greatest elongation (.36u) and minimum width (.04u) where they are close to the mitochondria, and often follow the contours of the mitochondria. At the apical surface the distal ends of the rDB's are often swollen and appear to merge with the dense zone.

The lower nucleated region, the epidermal cells proper, below the body wall muscles, varies considerably in its distance (1.5-4.5u) from the basal plasmalemma of the superficial epidermis. The epidermal cell cytoplasm resembles that of the cercaria. The DB's are seen in heavy accumulations and there are Golgi complexes and some agranular endoplasmic reticulum. The Golgi complexes consist of flattened cisternae and, at the tips of these, Golgi vesicles (fig. 42). The rDB's resemble the Golgi cisternae in shape and size. Agranular endoplasmic reticulum is more visible in regions where there are few DB's. The number of rDB's and sDB's in some cells appears to be about equal (fig. 42), whereas in others, the sDB's predominate (fig. 38). The maximum elongation of the rDB's in the lower epidermal cells is about .25u in contrast to that of .36u in the superficial epidermis. The epidermal cells are uninucleate, and separated by cell membranes (fig. 38). Small glycogen-like particles (110-160A in diameter) are found in the lower epidermal cells, but in much denser accumulations in the surrounding parenchymal cells and between the muscle bundles (fig. 38 and 42).

Comparison of the Epidermis of Adult, Metacercarial and Cercarial Epidermis. The epidermis of the adult, metacercaria and cercaria are similar in having (1) beneath the apical plasmalemma a dense zone similar in density to the dense inclusions (DB's), (2) sDB's and rDB's in both

the superficial and lower epidermis (3) Golgi complexes and spherical and oval bodies associated with the DB's (4) no granular endoplasmic reticulum (5) heavy deposits, presumably of glycogen, in the parenchyma, and (6) dispersed 150A particles, which may be either ribosomes or glycogen particles in the epidermal cells.

As the epidermis of the adult develops from that of the cercaria and metacercaria, the number of mitochondria in the superficial epidermis triples, the mitochondria concentrate in the distal half of the superficial epidermis, they are reduced in diameter three-fold, the mitochondrial cristae orient at right angles to the apical surface, and the number of DB's in the superficial epidermis increases.

Discussion

Two problems will be considered concerning the epidermis: (1) the conditions which may effect morphological and functional changes in the adult epidermis and (2) the involvement of the epidermis in absorption and secretion processes.

The differences observed between the cercarial and adult epidermis are assumed to be fairly constant throughout the parasite's body length; however, in further studies care should be taken to compare the same region of the epidermis in the three life stages.

L. setiferoides in transferring from the first or second intermediate host to the definitive hosts is exposed to differences between the invertebrate tissue or the coelom and the vertebrate gut. I suggest that a greater change in metabolic rate, oxygen consumption and nutrient absorption, hence possibly a greater development of the mitochondria would occur in a trematode, such as F. hepatica, which transfers from an invertebrate to a mammal, than in a trematode such as Zoogonus or Lepocreadium which transfers from an invertebrate to a cold-blooded definitive host. If the rate of metabolism and nutrient absorption of some adult trematodes

is greater than that of their larval stages, due to the higher body temperature of the definitive hosts, one might expect a greater development of the adult epidermis for absorption processes. But despite a lack of temperature change in the transferral of L. setiferoides from the intermediate to the definitive hosts, there were considerable differences between the adult epidermis and that of the cercaria and metacercaria. In other species of trematodes it would be of interest to determine whether changes in the adult epidermis are due to the increased body temperature of the definitive hosts or if they are due to other factors. There are apparently no species of trematodes nor cestodes which naturally infect both cold-blooded and warm-blooded vertebrates. However, it might be possible in experimental infections to procure the development of a cold-blooded fluke in an experimental warm-blooded definitive host.

In the coelom of Polydora ligni one would expect metacercariae of L. setiferoides to have a greater supply of oxygen than do the cercariae in the snail hepatopancreas. The spionid is small (1-5mm long) with a thin body wall, thus the oxygen tension probably is close to that of sea water. Furthermore, P. ligni has well-developed respiratory branchiae and a circulatory system, in close proximity to the coelom, facilitating rapid diffusion of oxygen. Since no development or growth occurs, which would require absorption of large amounts of nutrients, its oxygen requirements are probably quite low. Thus oxygen tension would not appear to be a limiting factor in the development of the mitochondria. Further studies are needed to determine whether if after longer periods in the aerobic coelom of P. ligni there are changes in the epidermal mitochondria of metacercariae.

The adult of L. setiferoides is attached to the intestinal mucosa of various fish hosts. The oxygen tension of the lumen of the vertebrate gut

is low. However, since small blood vessels are located in the submucosa, the oxygen tension in the mucosal cells, to which L. setiferoides is attached, is probably considerably higher than that in the gut lumen proper. The adults of various species of trematodes live in different tissues (e.g. the gut, lungs, blood and hepatic ducts). S. mansoni, a blood fluke, has few mitochondria in its epidermis (Morris and Threadgold, 1968; McAnally, 1969). The oxygen tension in mesenteric veins is higher than that in the hepatic ducts and gut. F. hepatica in the hepatic ducts and veins has a large number of epidermal mitochondria (Threadgold, 1963a and b), whereas Megalodiscus, in the frog intestine, has apparently no epidermal mitochondria (Bogitsh, 1968). From this cursory review it appears that in comparing different species of trematodes, the number of epidermal mitochondria is not proportional to the oxygen tension. The relationship between mitochondrial production and oxygen tension has been studied in yeast. Plattner and Schatz (1969) have shown by freeze-etching electron microscopic techniques that mitochondria, (which they call protomitochondria) are present in anaerobically grown yeast cells and that they contain inner membranes, but lack a functional electron transfer chain and oxidative phosphorylative system. Thus, at least in some organisms, oxygen tension may not control the production of mitochondria nor the presence of their cristae but may determine their functional capacity. In the present work, the functioning of the epidermal mitochondria has not been studied at the biochemical level; however, it has been found that the increase in the number of mitochondria is independent of the presumed changes in oxygen tension (i.e. the digestive gland of the snail and the gut of the flounder are quite anaerobic, whereas the coelom of the spionid is aerobic).

The increased size of the adult H. setiferoides may be a factor in part responsible for the changes in the adult epidermis. Theoretically

with an increase in body volume there is a decrease in surface-volume ratio. Thus in the adult L. setiferoides the increased folding of the adult epidermis and the greater number of mitochondria may be a means to compensate for a decreased surface area.

Sexual maturation in the adult may also account for differences between the epidermis of adult and larval stages. Development of the vitellaria or yolk glands and the large eggs undoubtedly requires increased nutrient uptake, Bogitsh et al. (1966) found in the cestode Hymenolepis the highest concentration of glycogen in immature proglottids and the lowest amounts in mature ones. Possibly in sexually maturing trmeatodes and cestodes glycogen, both that stored and that from the continual uptake of glucose, is broken down to pyruvic acid, which in turn is used in the synthesis of lipid materials of the yolk glands.

That the epidermis is involved in absorption and secretion processes is suggested by the structure of the apical plasmalemma and dense zone beneath it, and by the changes in the number and structure of the mitochondria. The apical plasmalemma has a trilaminate structure and in addition a filamentous coating on its outer leaflet, which resembles a glycocalyx. (See further discussion of the glycocalyx under the discussion of secretion processes). The apical plasmalemma appears to be a specialized membrane with a filamentous apical coat and with the inner layer of the trilaminate membrane closely adhering to the underlying dense zone. This specialization of the apical plasmalemma is in line with the view of Porter et al. (1967), that cell membranes are highly variable structures. The material composing the dense zone is concentrated beneath the apical plasmalemma, and thus may more readily diffuse through the plasmalemma to the exterior.

In the transformation of the cercaria into the adult the mitochondria show several changes. One change is a three-fold decrease in their diameter. If the mitochondria were perfect spheres there would be a concomitant reduction in volume of about 27 times. However, since the mitochondria are somewhat elongate (about .11-.18 μ in length and .08-.15 μ in diameter), in the adult epidermis, the reduction in diameter is thus compensated for to some degree. Theoretically a decrease in volume would be accompanied by a reduction in the depth of infolding, (and thus surface area) of the cristae, but only in the case where the cristae had extended through the diameter of the mitochondria initially. In the present study, though the cristae have a different orientation (parallel to the long axis) in the adult epidermal mitochondria, there is not a marked difference in the degree of elaboration of their cristae between the three life stages, as there is between the epidermal and tail mitochondria.

The smaller mitochondria in the adult epidermis might suggest that in the epidermis of this stage there is a lesser requirement for oxidative phosphorylation. However, their smaller size is probably compensated for by their greater abundance. In addition in the adult epidermis the mitochondria are concentrated in the distal half of the adult epidermis and are somewhat elongated, with their long axis directed towards the apical surface. The question may be asked whether these mitochondrial changes are indicative of changes in oxidative phosphorylation and in the processes dependent on it. First, however, it should be asked whether mitochondria in parasitic platyhelminths do in fact carry out a functional Krebs citric acid cycle. According to von Brand (1966) there is good evidence for a functional Krebs citric acid cycle in parasitic flatworms. Even if such occurs in L. setiferoides, the question remains whether an electron transport chain and oxidative phosphorylation can occur in L. setiferoides

in the digestive gland of N. obsoletus, where anaerobic conditions may exist. Vernberg (1963) has shown that some larval trematodes from N. obsoletus can utilize oxygen at very low oxygen tensions. Though mitochondrial oxidative phosphorylation may be limited in the epidermis of L. setiferoides, there may be alternative anaerobic pathways for the synthesis of ATP in mitochondria. Von Brand (1966) has suggested that a pathway involving reduction of fumarate to succinate and the dehydrogenation of DPNH may also involve the synthesis of ATP. Ornithine transcarbamylase, a mitochondrial enzyme involved in the formation of citrulline in the urea cycle, has been found in cestodes. The subsequent anaerobic breakdown of citrulline may allow for the nonoxidative generation of ATP (Campbell and Lee, 1963). In summary, mitochondrial changes may reflect not only changes in oxidative phosphorylation but also changes in other biochemical processes.

The concentration of the mitochondria in the distal half of the adult epidermis suggests that a greater degree of absorption and/or secretion processes occurs in the adult than in the cercarial or metacercarial epidermis. The elongation and position of the mitochondria in the adult folded epidermis resembles that in the kidney convoluted tubule cells described by Fawcett (1958), where the basal plasmalemma is greatly folded and where, in the folds mitochondria are lodged, suggesting that they supply energy for the transport processes of the kidney tubule membranes. Active transport of amino acids and glucose has been demonstrated in the epidermis of cestodes (Murrell, 1968; Read et al., 1963). The orientation of the mitochondrial cristae parallel to the rDB's in the adult epidermis of L. setiferoides may be indicative of mitochondrial involvement in the secretion processes of the elongated rDB's. Since the enzymes of the electron transport chain and of oxidative

phosphorylation are located on the mitochondrial cristae, the orientation of the cristae towards portions of the cell where ATP is needed may be significant. Studies on the mitochondria of mammalian tissues suggest that the number and complexity of structure of mitochondria is related to the energy requirements for specific cell functions (Fawcett, 1966). For example, in muscle tissue and in tissues where fats are being synthesized from carbohydrates, mitochondria are generally well-developed. Possibly then, the changes in number, size and distribution of mitochondria in the adult epidermis of L. setiferoides facilitate nutrient absorption by the adult for its growth and egg production. Adult worms may absorb larger amounts per unit of surface area, because of their small body size relative to large egg size.

Secretion processes in the epidermis of L. setiferoides are suggested by the numerous dense inclusions in both the superficial and lower nucleated layers. The apparent mergence of the rod-like inclusions with the dense zone beneath the apical plasmalemma indicates that they have a secretory function. The substance of the dense zone may diffuse through the apical plasmalemma and contribute to the filamentous coating on its surface. Revel (1965) found the glycocalyx to consist of acid mucopolysaccharides that were resistant to mucolytic agents. Ultrastructural studies for the detection of acid mucopolysaccharides and glycoproteins in L. setiferoides are planned. The increased number of rDB's in the adult epidermis may indicate an increased rate of replacement of the dense zone beneath the apical plasmalemma, possibly in response to the digestive action of secretions of the fish gut mucosa.

In L. setiferoides the DB's appear to form in the lower epidermal cells, in association with the Golgi complexes and/or the spherical

and oval bodies, both of which do not occur in the superficial epidermis. That they form in the Golgi complexes is suggested by the small vesicles similar to the sDB's seen at the ends of the Golgi cisternae. This hypothesis is in agreement with several descriptions of mammalian epithelial tissues, such as those of Palay (1958) and Fawcett (1966) on exocrine and endocrine tissues of the pancreas and that of Neutra and LeBlond (1969) on mucous-secreting goblet cells. Cell-coating substances generally consist of glycoproteins (proteins combined with carbohydrates). Neutra and LeBlond found that the substances forming the carbohydrate-rich surface coating of intestinal columnar cells are actually synthesized in the Golgi complexes. The DB's may form in the spherical and oval bodies, in which the sDB's are occasionally seen embedded, or from Golgi cisternae which become separated from the Golgi complexes. There is no support from other studies for these last two hypotheses. Possibly the latter could be tested by fractionation and biochemical assaying, if a separate Golgi complex and DB fractions could be obtained.

Granular endoplasmic reticulum is absent from both the epidermal cells and the superficial epidermis, suggesting that the epidermis is not involved in the synthesis of proteins to be secreted externally. The DB's in the epidermis may consist primarily of carbohydrates; Fawcett (1966) stated that the synthesis of substances which are not proteinaceous but which consist rather of carbohydrates, may occur in the Golgi complexes. Granular endoplasmic reticulum may be present in early stages (not observed) of the secretory cycle of the epidermal cells, when the components of the DB's are being synthesized. Presumably only late stages in the secretory cycle, when many DB's had already formed, were observed in the present study.

The Golgi complexes and spherical and oval bodies with embedded DB's are confined to the lower epidermis, thus probably the DB's migrate

by some means via the cytoplasmic projections up into the superficial epidermis. However, the DB's may form in the dense zone from substances obtained from the exterior.

The following are hypotheses concerning the means by which DB's, if formed in the lower epidermis, may migrate to the superficial epidermis.

(1) They may become so numerous in the lower epidermal cell cytoplasm, that they are eventually forced by mechanical processes into the cytoplasmic processes. (2) They may move by contracting and extending. Their intimate association with mitochondria and the orientation of the mitochondrial cristae parallel to the rDB's might facilitate such a contraction process. Swelling and "contraction" processes have been described in some subcellular organelles e.g. mitochondria, Lehninger (1964). A possible experiment to test the second hypothesis would involve the removal of the superficial epidermis (leaving the cytoplasmic processes and lower epidermal cells intact), and observations at intervals of the renewal of the superficial epidermis and the passage of subcellular organelles to the surface. A second experiment would entail the application of mitochondrial inhibitors of oxidative and glycolytic phosphorylation, to determine whether the elongation of the rDB's could be prevented.

It would be interesting to determine the composition and functions of the DB's, because of their occurrence in most trematodes, and their location near the host-parasite interface. Also, they may demonstrate differences dependent on their location in host tissues. One histochemical study at the ultrastructural level has indicated that the DB's of F. hepatica do not contain acid mucopolysaccharides nor acid phosphatases (Threadgold, 1967). Because of the close phylogenetic relationships between the free-living and parasitic flatworms, there may be similarities in the

formation of subepidermal rhabdites of free-living flatworms and the DB's of trematodes. Rhabdites are large proteinaceous inclusions visible with the light microscope. During the period of maximum protein synthesis the granular endoplasmic reticulum and Golgi complexes are extensive, but subsequently they decline (Lentz, 1966). Fractionation and biochemical assay studies on the DB's, similar to those of Siekevitz and Palade (1958) on the pancreatic zymogen granules could be employed to determine their composition and functions. Also, autoradiographic studies with radioactively labeled glucose measured at intervals may be of value in determining whether the Golgi complexes in the lower epidermis take up glucose for the synthesis of mucopolysaccharides or glycoproteins, and subsequently form the DB's.

SECTION VI

SUMMARY AND CONCLUSIONS

The Life Cycle. The larval stages of Hepocreadium in N. obsoletus from the Great Bay region of New Hampshire were identified as L. setiferoides because of their similarity to those of L. setiferoides from Woods Hole, Massachusetts. The percentage of infected snails, 15-20mm long, with L. setiferoides was low (a maximum of 9%) as compared to the percentage infected with Zoogonus rubellus (up to 89%). The high incidence of Z. rubellus and the infrequency with which L. setiferoides becomes gravid in the definitive host (Liopsetta putnami) may account, in part, for the low incidence of L. setiferoides in Johnson's Creek as compared to that at Woods Hole, where L. setiferoides may account for up to 70% of the total incidence of trematode infections in N. obsoletus in July.

A possible second intermediate host of H. setiferoides, the Spionid polychaete, P. ligni, was found naturally infected with metacercariae. It occurred in mucous tubes on shells of the first intermediate host, N. obsoletus, from June through September. This period corresponds approximately with the time of cercarial release from the snails. There was no development of the reproductive organs in the metacercaria. Sexually mature worms were recovered in only one out of six experimental infections in which the Smooth Flounder (Liopsetta putnami) was fed infected P. ligni. This low recovery may have been due to the poor nutritional state of the flounder under experimental conditions rather than to the inability of metacercariae from P. ligni to develop in L. putnami. The larger numbers of adult worms found in experimental fish than in control fish suggests that ingestion of infected P. ligni (either planktonic, burrowed in mud, or on large snail shells)

is an effective means of infection of L. putnami.

Adults of Lepocreadium were found in the anterior intestine of three fish, L. outnami, the Shorthorn Sculpin (Myoxocephalus scorpius) and the Winter Flounder (Pseudopleuronectes americanus). Because of the similarities in morphology and life cycle between Lepocreadium from L. putnami and L. setiferoides described by Martin (1938) at Woods Hole, the former has been identified as L. setiferoides. The life cycle of Lepocreadium adults from M. scorpius and P. americanus was not worked out. It is probably justifiable to describe them as L. setiferoides on the basis of their similarities to adults and eggs from L. putnami, and to synonymize L. setiferoides and L. trullaforma. However, further life cycle studies are needed to compare the development of individuals of Lepocreadium from L. outnami, P. americanus and M. scorpius.

L. putnami had the heaviest infections of L. setiferoides (50+ worms/fish in July and August, lighter infections (15-20 worms) from September through December and very light infections (1-5 worms) from January through April. The highest incidence of infection (50-90% of L. putnami) also occurred in July and August and may have resulted from direct ingestion of cercariae, when both the Smooth Flounder and N. obsoletus were located in shallow water, at which time the water temperature exceeded 17°C and cercariae were released from snails. The decline in incidence of infection to near zero in January and February, and the high incidence in all age classes in summer suggests that flounders may lose the infection in winter but may be receptive to reinfection. The presence of gravid worms in late fall suggests that (1) the maturation of worms ingested in summer takes several months or that (2) L. putnami is particularly susceptible to infection in fall when N. obsoletus and P. ligni move into deeper water.

The miracidium of Hepocreadium from M. scorpius and L. putnami has been described for the first time. Its developmental time (38 days at 26° C) is comparatively slow. Eggs do not develop into miracidia at 14° C or lower. The slow developmental time may be an advantage in delaying hatching of miracidia until N. obsoletus migrates from deeper water in April and May.

Development of the Epidermis as Related to the Life Cycle. The young redial germinal sacs have a body wall folded into lobes and displaying two types of surface modifications: (1) tortuous invaginations and (2) narrow inpocketings. The epidermis, including that lining the surface modifications, bears numerous microvilli and contains membrane-bound granules, which do not merge into a dense zone beneath the apical plasmalemma. The lower nucleated region of the young redial epidermis, below the muscle layers, is continuous with the superficial layer and contains dense masses of membrane-bound granules (secretory granules). The young rediae are often surrounded by a clear space and lysed host cells, whereas older rediae are often closely surrounded by host cells. The epidermis of older rediae lacking microvilli and secretory granules appears degenerate, and may allow for restoration of host tissue. Thus the redial epidermis, which in early stages contains microvilli and secretory granules and is capable of lysing host tissues, loses these morphological and functional features in later stages.

In the development of the cercarial epidermis in the germinal balls, the superficial layer appears to form from cytoplasmic projections which extend up from nucleated epidermal cells below the muscle layers onto the surface of the germinal balls.

The major features in the epidermis and parenchyma which the cercaria, metacercaria and adult have in common are (1) a dense zone

beneath the apical plasmalemma similar to the dense inclusions (DB), (2) spherical and rod-like membrane-bound dense inclusions (sDB and rDB) in both superficial and lower epidermis, (3) Golgi complexes and spherical and oval large bodies in which sDB's are embedded, (4) glycogen deposits in the parenchyma tissue closely surrounding the lower epidermal cells, and (5) dispersed particles, 150250A in diameter, which may be either single glycogen particles or free ribosomes.

Changes occurring during development of the adult epidermis from the cercarial and metacercarial epidermis are: (1) tripling of the number of mitochondria in the superficial epidermis, (2) concentration of the mitochondria in the distal half of the superficial epidermis, (3) up to a three-fold decrease in the diameter of the mitochondria (4) orientation of the mitochondrial cristae towards the apical surface and parallel to the rDB's, which orientation is not seen in the cercarial or metacercarial mitochondria, and (5) an increase in the number of DB's in the superficial epidermis.

These changes do not appear to be correlated with temperature and oxygen conditions, but may be related to (1) the increased size of the adult, (2) a supply of nutrients available to adult worms larger than that available to metacercariae in the coelom of the second intermediate host and to cercariae in sea water, (3) a greater rate of nutrient absorption in the adult as the vitellaria and eggs develop, and (4) an increased rate of replacement of the dense zone beneath the apical plasmalemma, possibly in response to the digestive action of secretions of the fish gut mucosa.

The features of the epidermis of L. setiferoides which suggest involvement in absorption processes, particularly in the adult, can be summarized as follows: (1) folding of the epidermis, (2) pinocytotic invaginations of the plasmalemma, (3) increase in the number of mitochondria

In adult epidermis, and (5) the filamentous coating on the external surface of the apical plasmalemma.

The features of the epidermis which suggest involvement in secretion processes can be summarized as follows: (1) the filamentous coating on the apical plasmalemma, (2) the dense zone beneath the apical plasmalemma, (3) the spherical (sDB) and rod-like (rDB) membrane-bound dense inclusion bodies, the latter merging with the dense zone, (4) the association of the DB's with the Golgi complexes, (5) the greater elongation of the rDB's in the superficial epidermis than in the lower epidermis, (6) increase in the number of rDB's in the adult epidermis, (7) the proximity of the rDB's to the mitochondria in the adult epidermis, and (8) concentration of the mitochondria towards the apical surface.

The following problems concerning development and modifications of the epidermis could be further examined in L. setiferoides and other digenetic trematodes: (1) development of the ciliated miracidial epidermis, (2) transformation of the miracidial epidermis into the redial epidermis, (3) composition and functions of the DB's by autoradiographic tracing of glucose uptake at intervals into the lower epidermal cell Golgi complexes and spherical and oval bodies, and by biochemical fractionation and assaying, (4) changes in the metacercarial epidermis after long periods in the second intermediate host, and (5) the apparent movement and extension and contraction of the rod-like inclusions. These phenomena could be examined by removing the superficial epidermis, leaving the cytoplasmic connecting processes and lower epidermis intact, and by observing at intervals with the electron microscope the regeneration of the superficial epidermis and the effects, if any of mitochondrial inhibitors on extension and contraction of the DB's.

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APPENDIX

Figure 1. Anterior end of a live redial germinal sac of L. setiferoides containing cercarial germinal balls. 300x.

Figure 2. Cercarial germinal balls removed from live redia. 1700x.

Figure 3. Older cercarial germinal ball showing primordium of tail (T). 1400x.

Figure 4. Live cercaria of L. setiferoides. Ventral view showing oral sucker (OS), ventral sucker (VS), eyespots (E), excretory bladder (EB) and setae (S) on tail. The swelling midway along tail is not a constant feature. 120x.

Figure 5. The excretory bladder of the cercaria showing refractile spheres. 1200x.

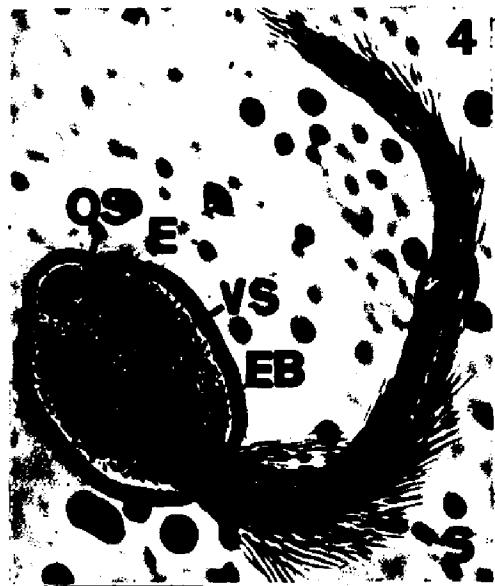
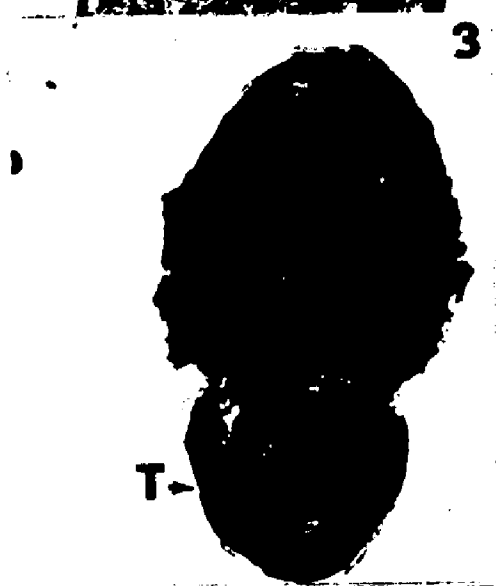
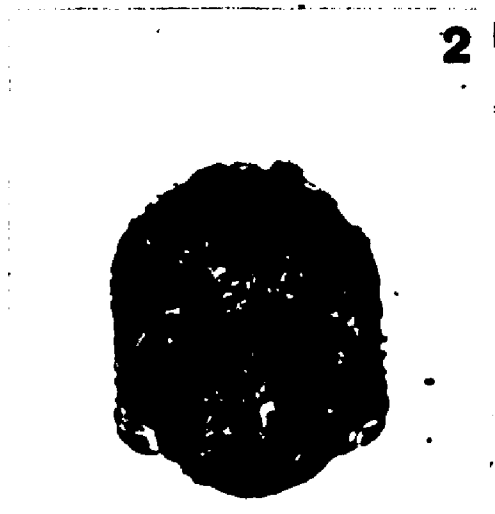


Figure 6. Living Polydora ligni, a second intermediate host of L. setiferoides, removed from its mucous tube on a Nassarius obsoletus shell. 200x.

Figure 7. The metacercaria of L. setiferoides seen through the transparent body wall of a living P. ligni. Note the excretory spheres (Ex S) in the excretory bladder. 400x.

Figure 8. Live immature adult L. setiferoides (.30mm in length) from the Smooth Flounder (Liopsetta putnami). Ventral view showing eyespots and excretory bladder with excretory spheres. 260x.

Figure 9. Live adult L. setiferoides with protruded cirrus from L. putnami. 260x.

Figure 10. Epidermis of a live adult L. setiferoides with prominent spines. 1600x.

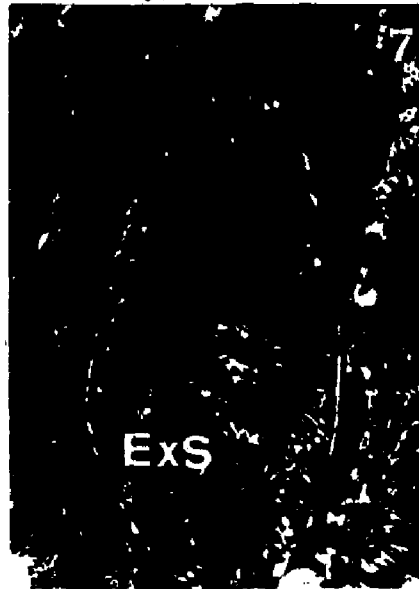


Figure 11. Uncleaved egg just released by an adult Lepocreadium taken from Myoxocephalus scorpius. length= .08mm; width= .04mm. 700x.

Figure 12. Egg in an early stage of differentiation when released by an adult Lepocreadium taken from M. scorpius. 700x.

Figure 13. 13 day-old miracidium, still in the egg shell, developed from an egg of an adult L. setiferoides taken from L. putnami. 1000x.

Figure 14. 27 day-old miracidium with an eyespot (E) developed from an egg of an adult Lepocreadium taken from M. scorpius.

Figure 15. 38 day-old miracidium, still in the egg shell, with an eyespot and epidermal ciliated plates (C) developed from an egg of an adult Lepocreadium taken from M. scorpius. 1000x.

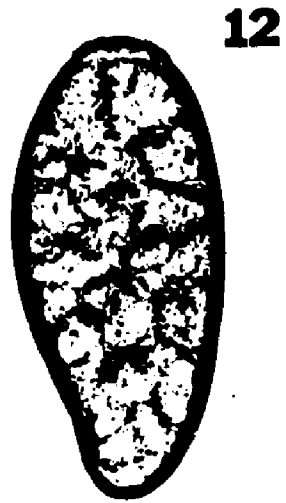
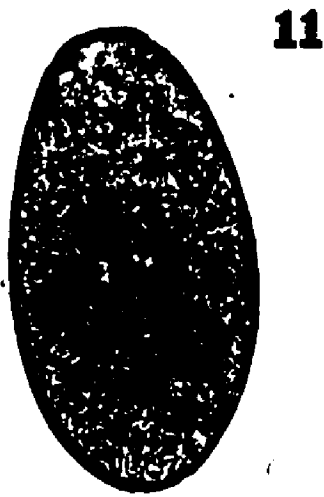


Figure 16. Section of a redia containing germinal balls in which the cell nuclei show various mitotic figures. One cell near the top is in early anaphase with 6 chromosomes in each daughter cell. 3000x

Figure 17. Young redia in gonadal connective tissue of N. obsoletus. Note the open pharynx (Ph) with ingested host tissue and the absence of germinal balls. 300x.

Figure 18. Young redia with young germinal balls (GB), germinal cells (GC) and a folded redial body wall (RW). 600x.

Figure 19. Young redia with microvilli (Mv) on the epidermis of the body wall. 3000x.

Figure 20. A tortuous invagination (I) of the redial body wall. 2800x.

Figure 21. Mature redia in gonadal connective tissue. It contains germinal balls (GB) and its body wall (RW) is bounded closely by the connective tissue. 380x.

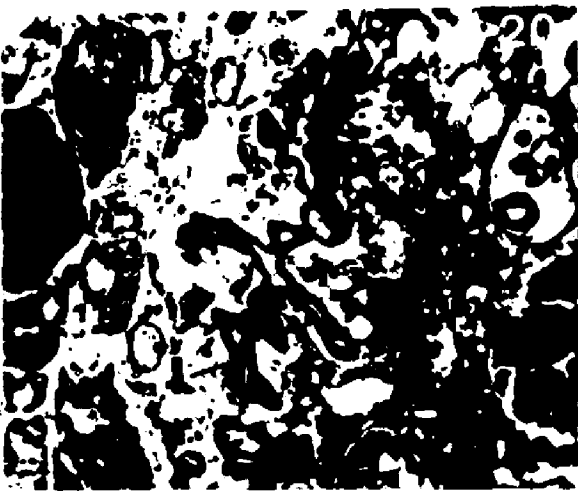
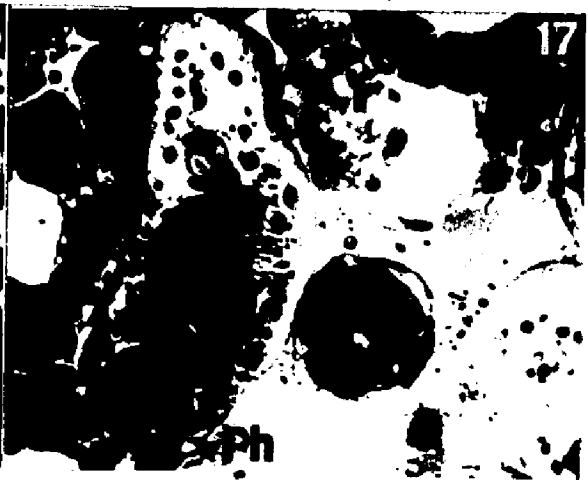
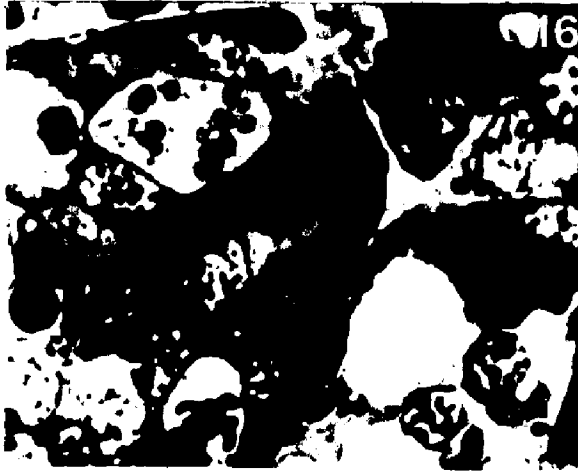
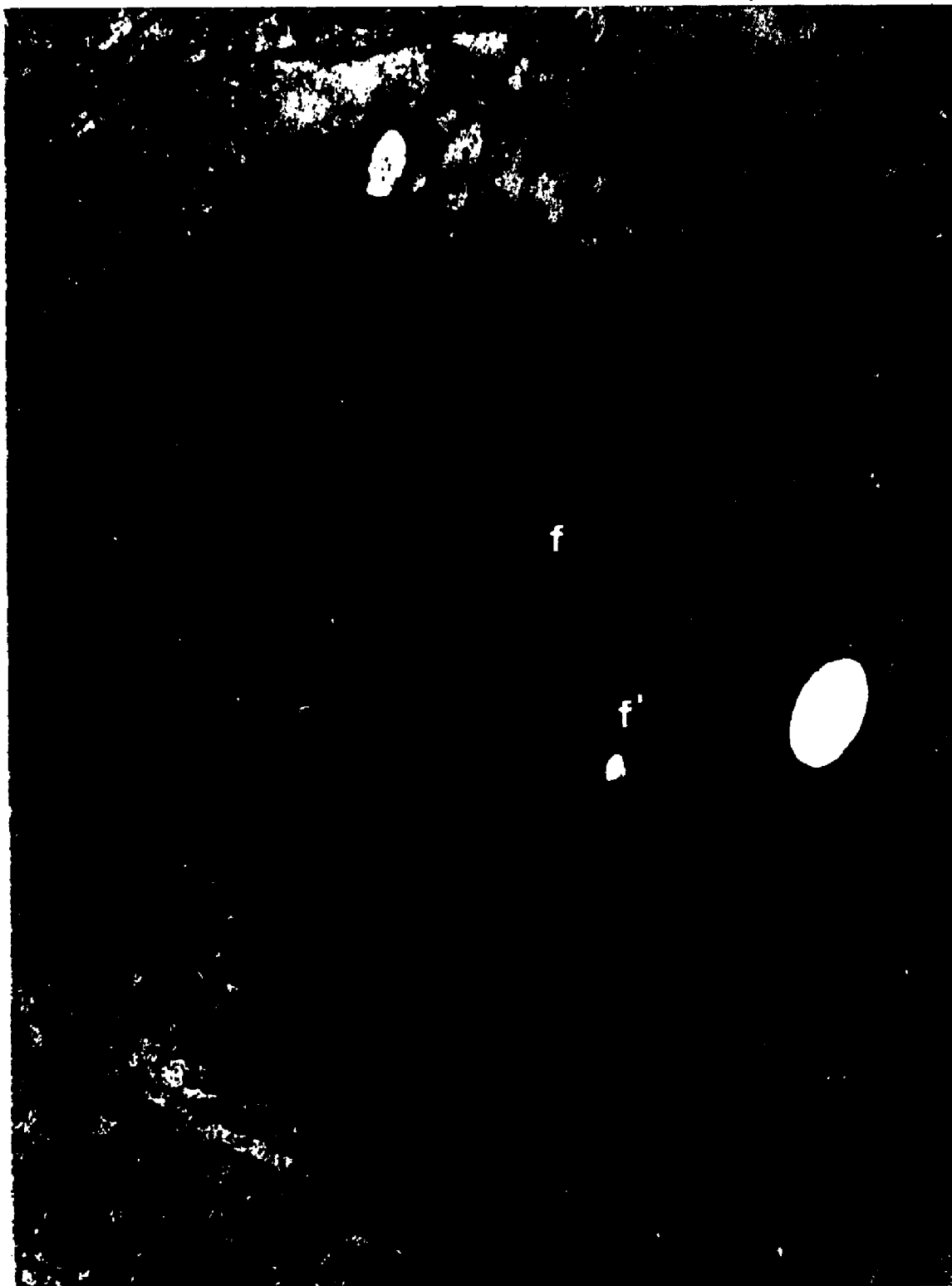


Figure 22. A tortuous invagination of the radial body wall. The wall is folded into lobes containing a bundle of circular muscle (CiM) beneath which is the longitudinal muscle (LM). Thick and thin myofilaments are evident in the circular muscle. The superficial epidermis (Sc) bears microvilli (Mv) and is bordered on its lower surface by the basal lamina.(BL). 20,800x



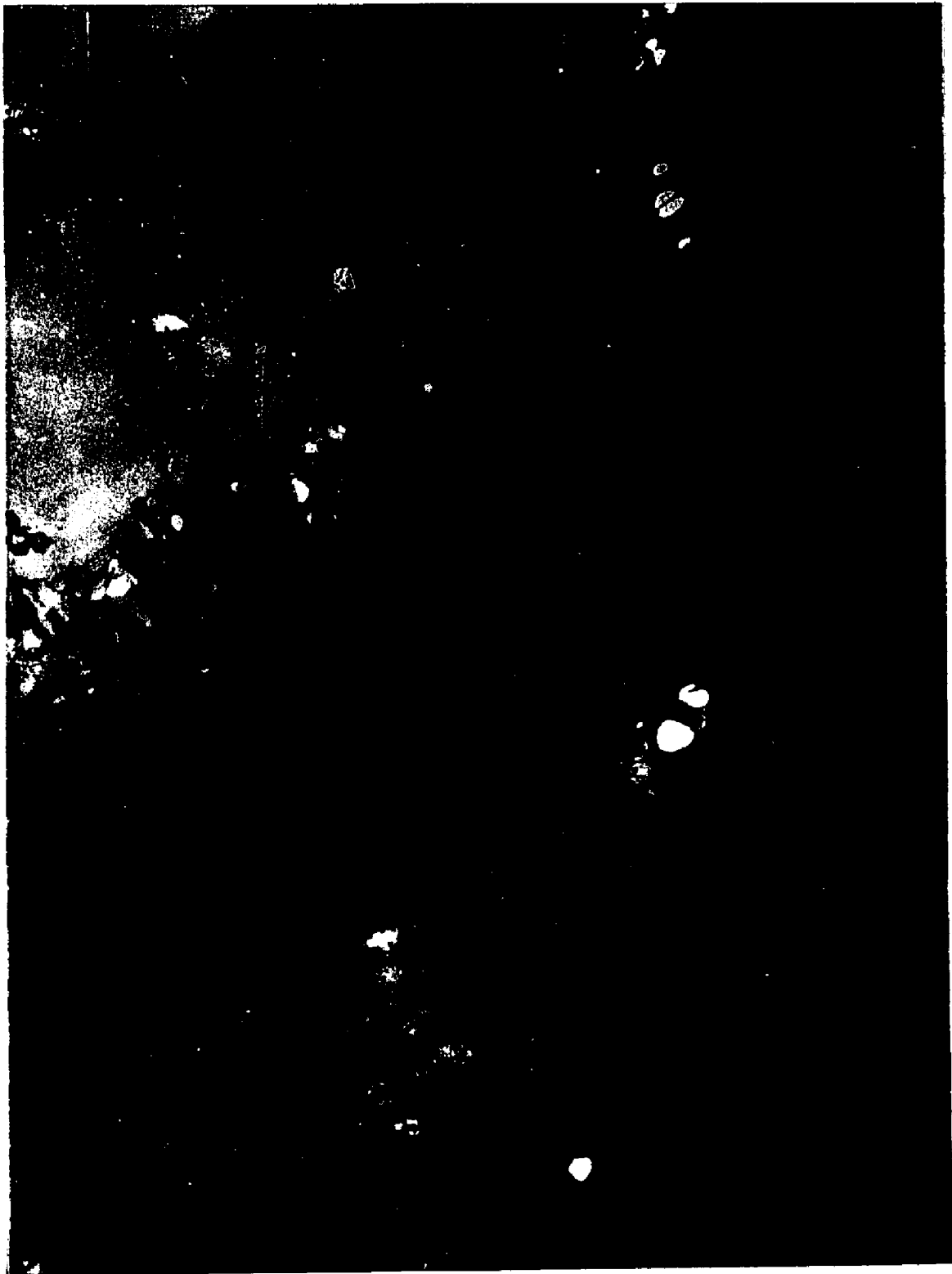
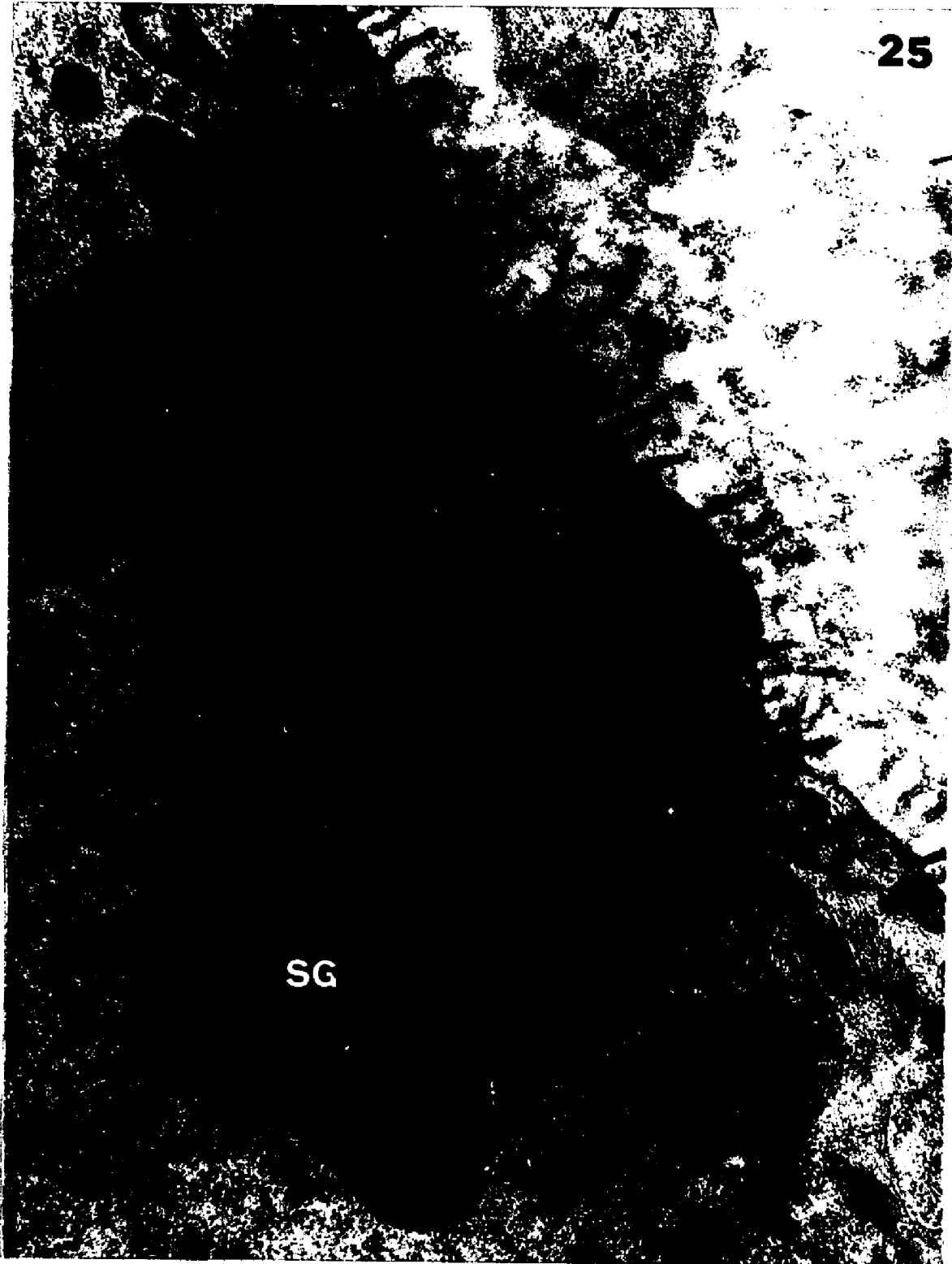


Figure 23. Portion of radial body wall folded into lobes. Long (.8u) microvilli (Mv) project from the superficial epidermis (SE). There is a thick basal lamina (BL), bundles of circular muscle (CiM) in each lobe and longitudinal muscle (Li). The arrow points to vesicles appearing to pinch off from the tips of some microvilli. 25,800x

Figure 24. Two radial body walls with a thin epidermis (SE) bearing numerous microvilli (Mv). The lower epidermal region (LE) contains numerous dense secretory granules (SG). 6,300x





25

SG

Figure 25. Superficial (SE) and lower regions (LE) of a radial epidermis showing microvilli (Mv), secretory granules (SG) and a cytoplasmic process (CP) connecting the two portions of the epidermis. 19,200x.

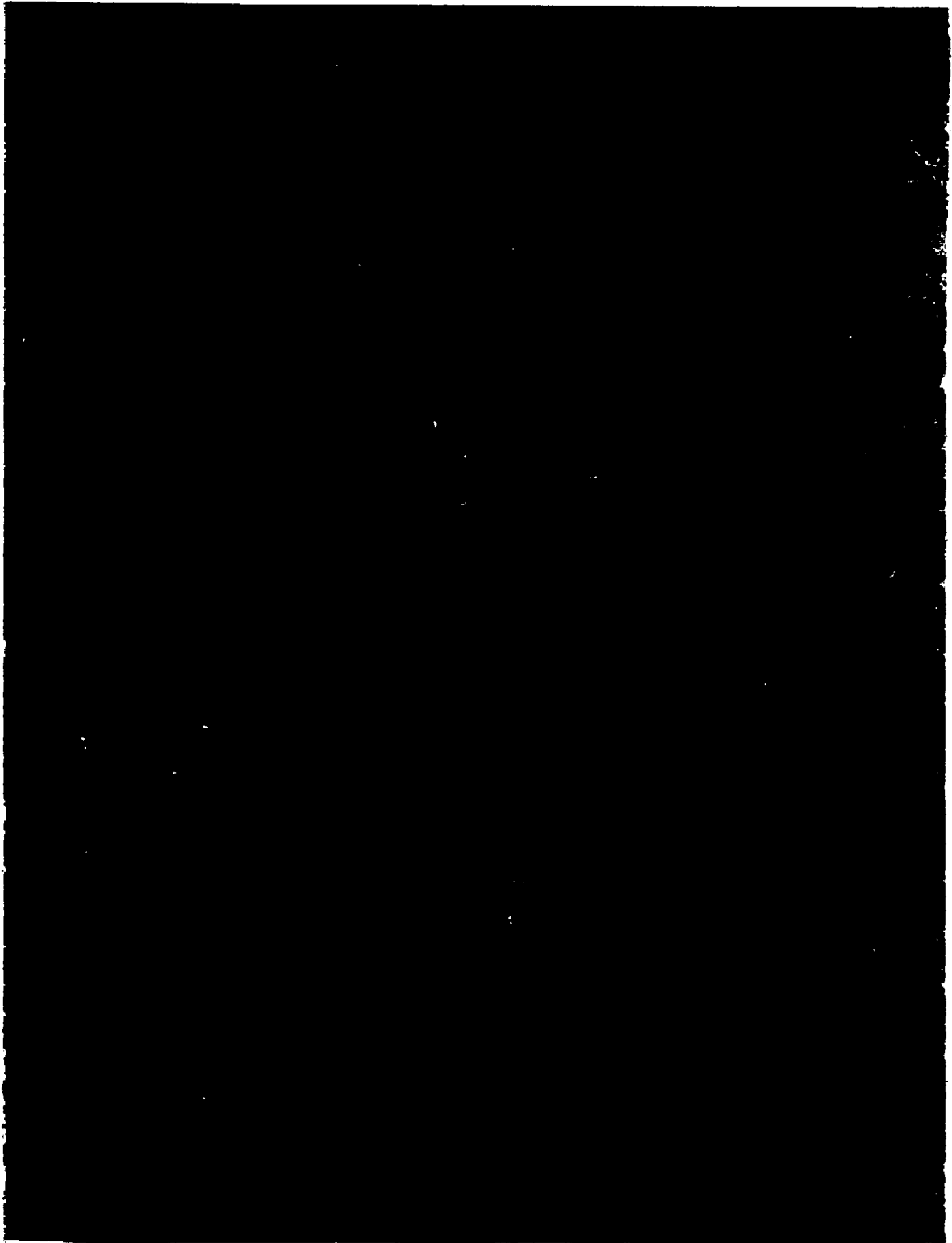
Figure 26. A redia within the hepatopancreas of N. obsoletus. The epidermis (E) bears microvilli (Mv), and there is a small space between the epidermis and host tissue (HT). 20,000x





Figure 27. A narrow inpocketing (I) of the radial body wall. Numerous microvilli (Mv) project from the epidermis in the inpocketing. The epidermis on the surface of the radia also bears microvilli and contains secretory granules (SG). 15,300x

Figure 28. Parenchyma tissue of a redia. The large lipid-like dense inclusions (L) are bounded by whorls of membranes. Glycogen rosettes (GL) averaging 500A in diameter, have a subunit structure of 160A particles. 33,100x.



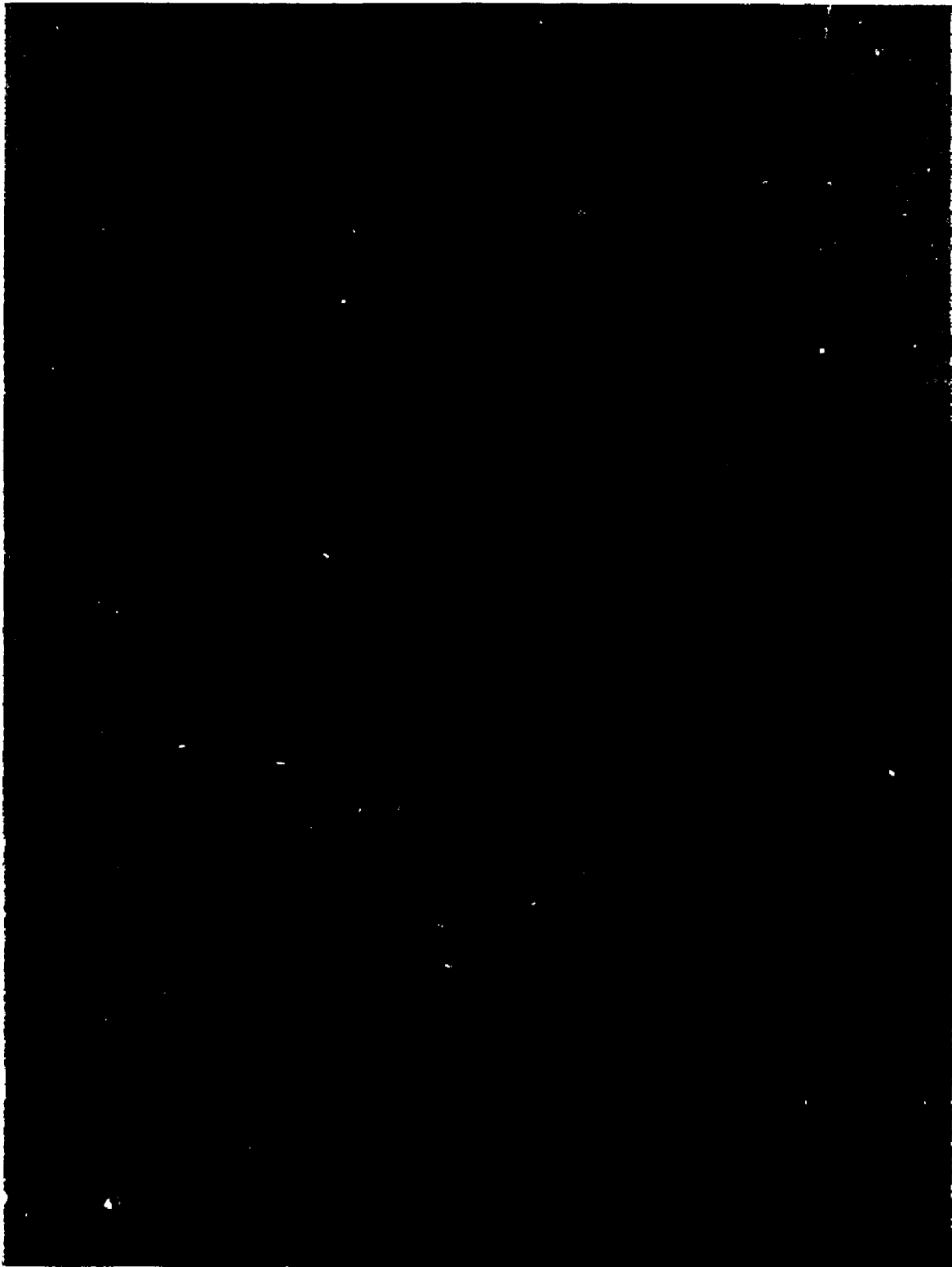
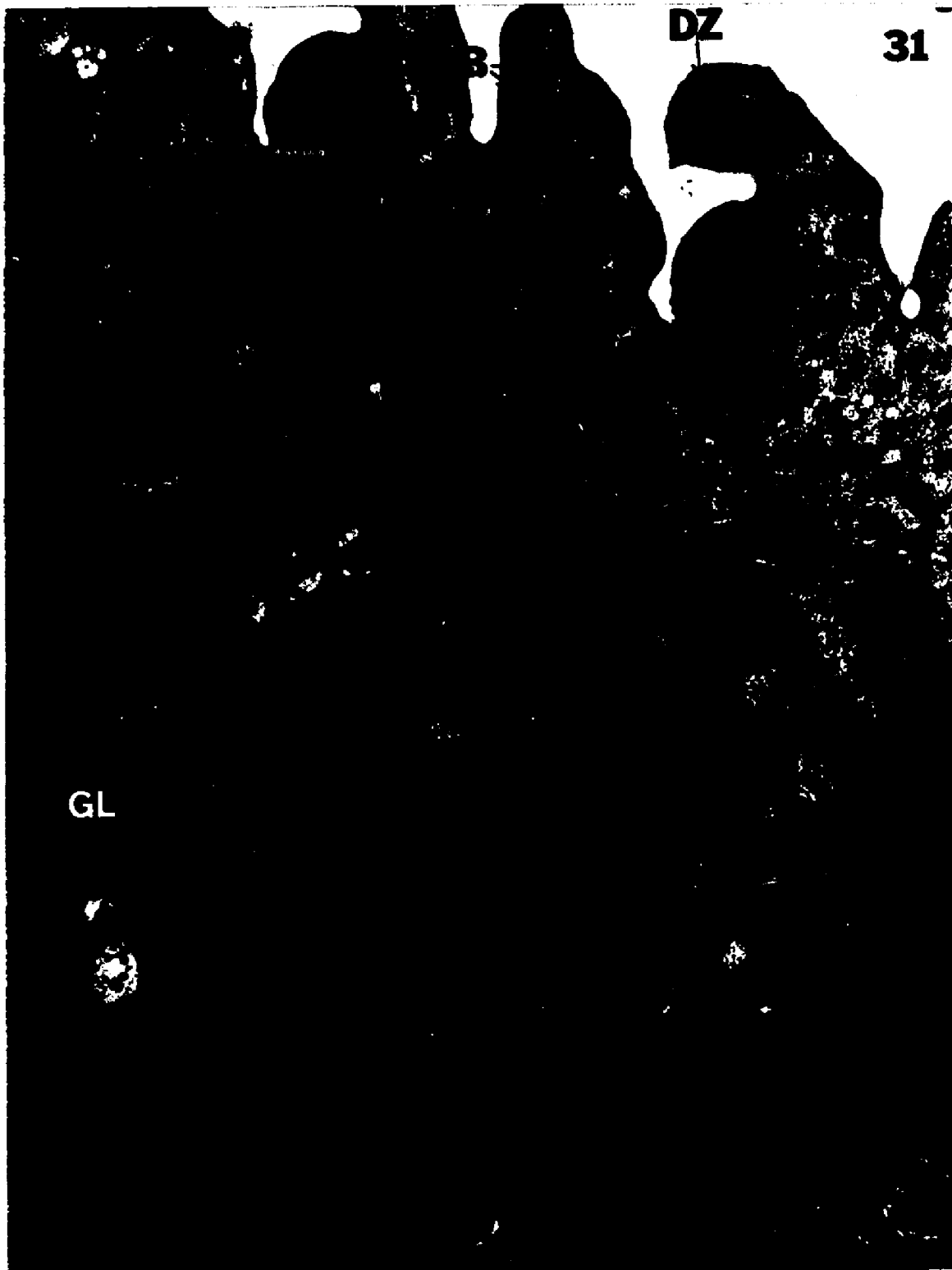


Figure 29. Body wall (RW) of an older redia near the germinal cavity. Note the absence of the epidermis and folds in the wall. Within the germinal cavity is a developing cercaria with a thin epidermis (E) and a cytoplasmic process (CP) continuous with an epidermal cell containing a nucleus (N). 11,300x.

Figure 30. Transverse section of superficial epidermis of a cercaria. Beneath the apical surface are a thin dense zone (DZ) and rod-like dense bodies (rDB). Mitochondria (M) are sparsely scattered amongst clear vesicles (V) in the syncytial cytoplasm. The basal plasmalemma (bPl) and basal lamina (BL) are interrupted by a cytoplasmic process (CP), the cell membranes of which are distinct. It penetrates through the circular muscle (CiM) and longitudinal muscle (LiM) layers to the underlying parenchyma. Spines (S) are visible in the superficial epidermis and show an increased density (arrow) in their apparent fusion with the basal plasmalemma. Note the small size of the mitochondria in the epidermis as compared to that of the parenchymal mitochondria (M_{pa}).
19,100x.





DZ

31

GL

Figure 31. Tangential section of superficial epidermis of a cercaria. Beneath the apical surface is a dense zone (DZ) into which the rDB's occasionally appear to merge. Spherical mitochondria (M) are scattered throughout the epidermis. The spine (S) on the right shows a periodicity of 200A. Dense accumulations of glycogen-like particles, averaging 240A in diameter, are deposited in the underlying muscle tissue. 19,600x.

Figure 32 (A). Superficial layer (SE) and lower cellular region (LE) of cercarial epidermis. Some portions of the epidermal cells, with nuclei (N) contain masses of DB's amongst which there are large less dense spherical and oval bodies (x). In other portions where there are few DB's, agranular endoplasmic reticulum (aER) is clearly seen. 6,600x.

(B). Higher magnification of part of the lower epidermis similar to that seen in the insert above, showing the large spherical and oval bodies (x) surrounded by DB's. 70,000x

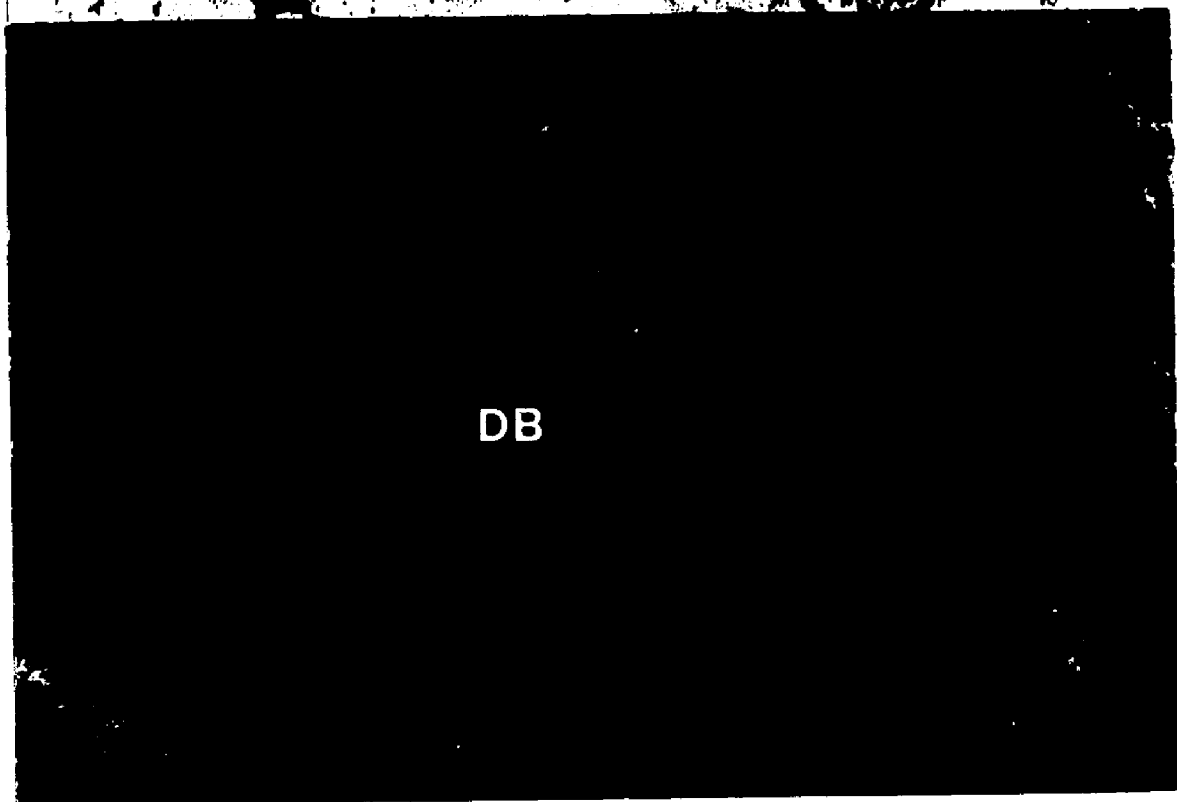
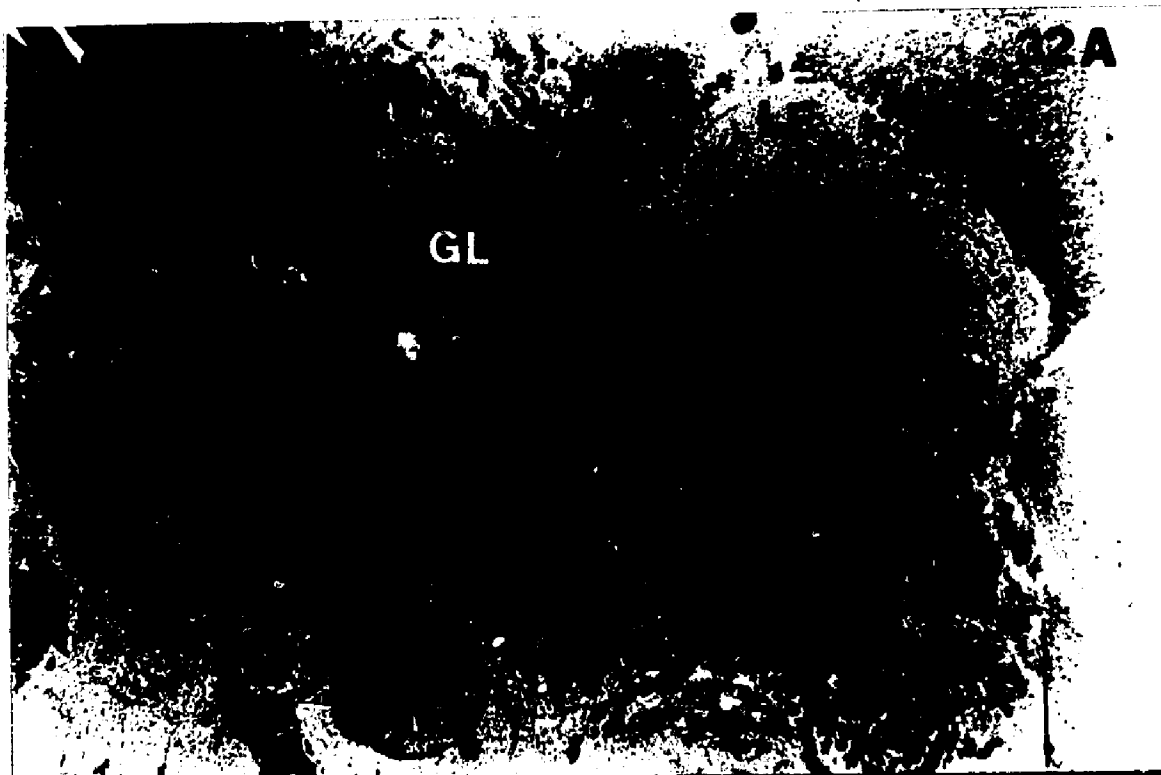




Figure 33. Epidermis of the oral sucker of a cercaria. Extending from the basal plasmalemma are long projections (Pr) with a central core of basal lamina (BL) material. Dorso-ventral sucker muscle fibers (dvm) appear to attach to cylindrical projections of the basal lamina (arrows). 38,200x

Figure 34. Excretory bladder of the cercaria with concentrically-layered excretory spheres. The excretory epithelium bears projections (P) and the underlying tissue contains lipid-like inclusions (L). 21,400x.

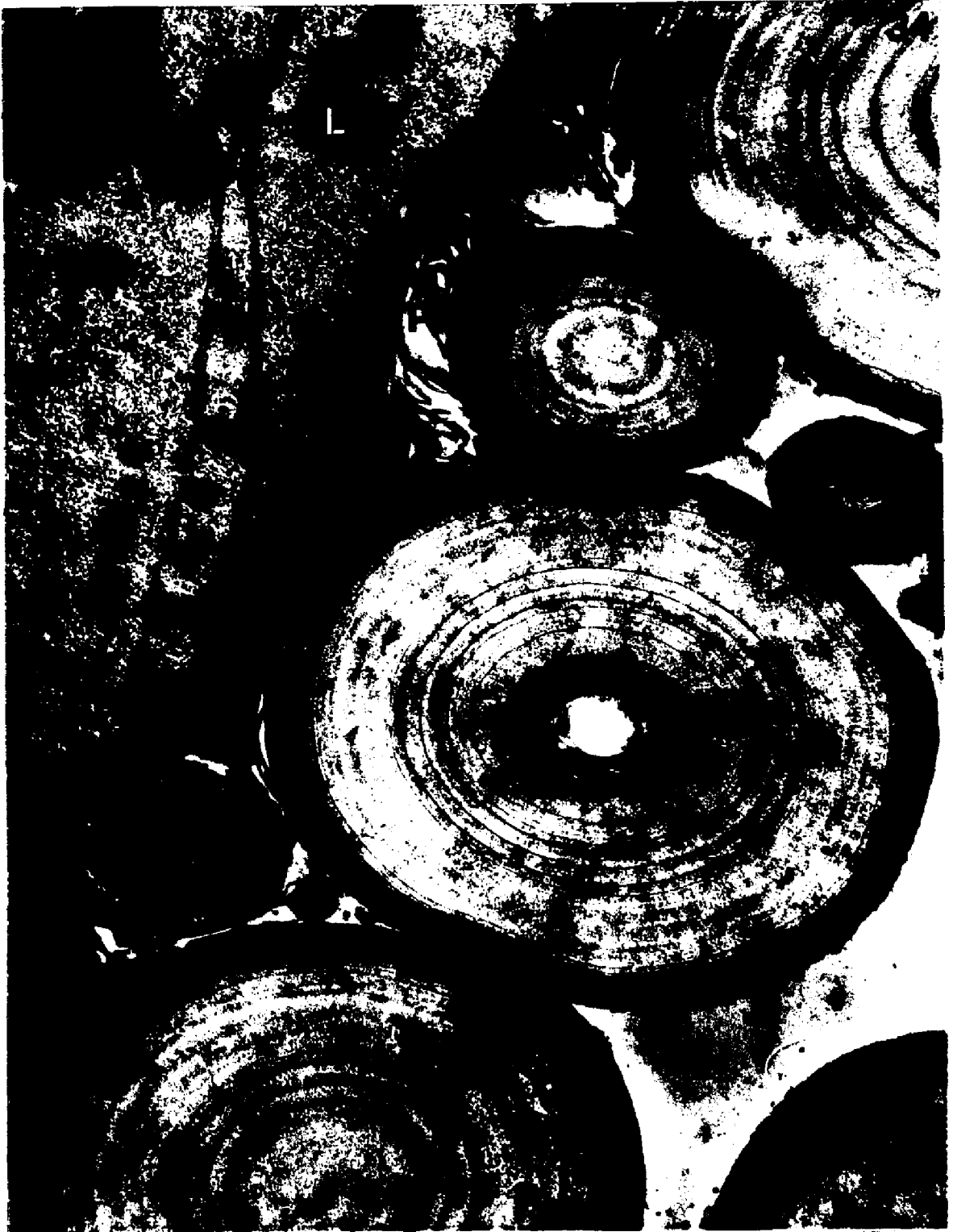




Figure 35. Transverse section of a cercarial tail in which groups of muscle fibers (My) are separated by a mass of mitochondria (M) arranged in a broad cross pattern. 18,000x.

Figure 36. Superficial epidermis, underlying muscle layers and parenchyma of the metacercaria within the coelom of the second intermediate host. Both rDB's and sDB's are scattered throughout the superficial epidermis. Just beneath the apical plasmalemma is a dense zone (DZ) into which the rDB's appear to merge (arrows). The larger mitochondria are .36-.40u in diameter. The basal plasmalemma displays small invaginations (arrows). A cytoplasmic process (CP) projects through the basal plasmalemma and basal lamina. Beneath the superficial epidermis are circular (CM) and longitudinal muscle (LM) bundles. The numerous dense clumps of glycogen particles (GL) in the parenchymal tissue average 120-160A in diameter. 19,200x



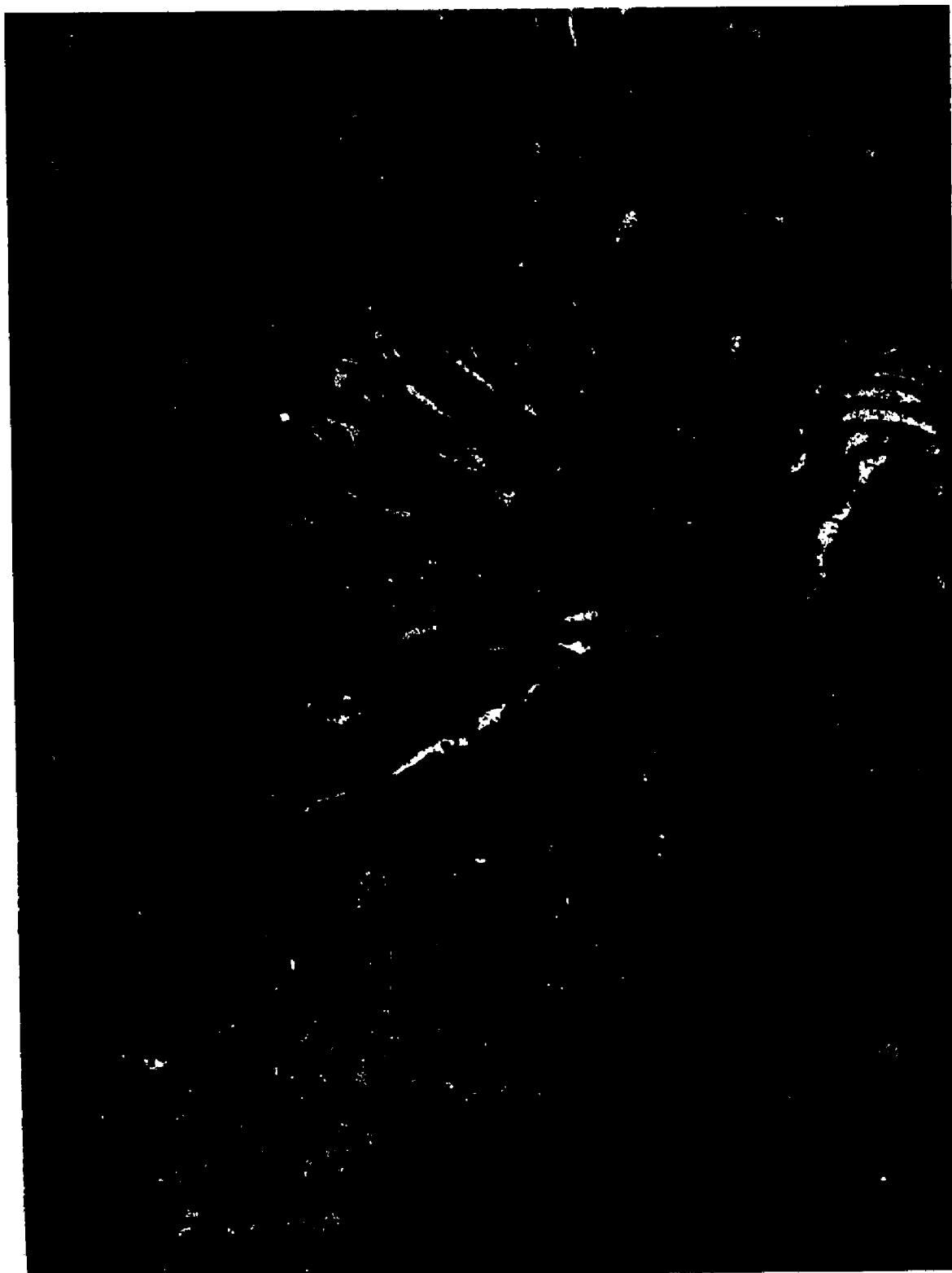


Figure 37. Caecal epithelium of a metacercaria with long microvilli (Mv) projecting from the apical surface, granular endoplasmic reticulum (gER) and mitochondria (M). 20,900x

Figure 38. Adult epidermis including the superficial syncytial layer (SE) and lower epidermal cells (LE) with nuclei (N) and cell membranes (CM). In the epidermal cells there are numerous sDB's. Small glycogen-like (GL) particles, 160-180A in diameter are scarce in epidermal cells but abundant in the surrounding parenchymal cells. 33,200x

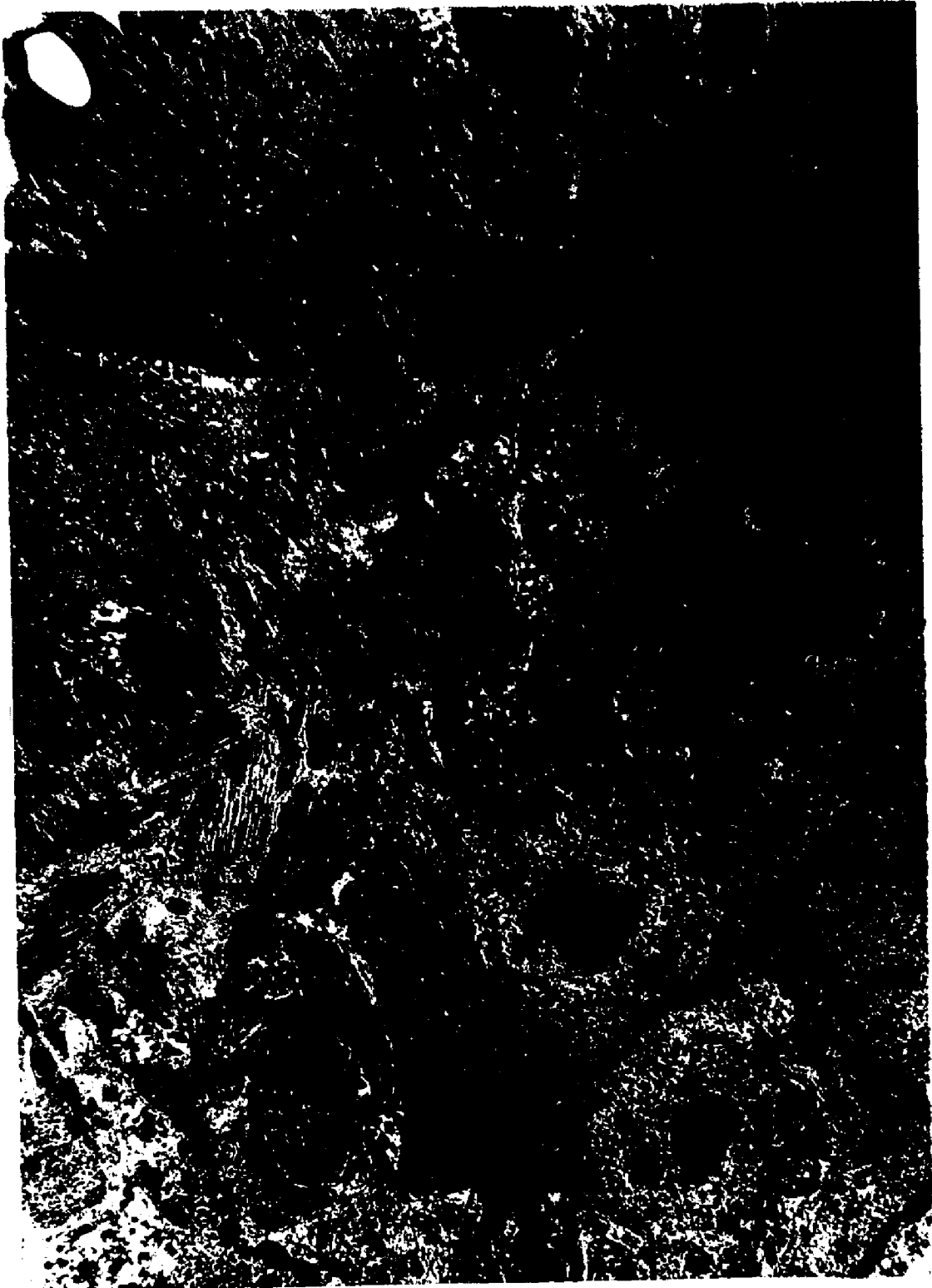




Figure 39. Adult epidermis with narrow cytoplasmic processes (CP) leading up from a lower nucleated epidermal cell (LE) to the superficial layer (SE). 17,800x

Figure 40. Superficial layer of adult epidermis with numerous rDB's, sDB's and mitochondria (M). The mitochondria are oriented with their long axis at right angles to the apical surface, and the rDB's are attenuated particularly near the latter. 31,000x





Figure 41. Higher magnification of the superficial layer of adult epidermis. The mitochondria (M) are oriented with their long axis and cristae at right angles to the apical surface and parallel to the rDB's. The apical plasmalemma bears a coating of fine filaments (F). 39,800x.

Figure 42. Portion of the lower epidermis of the adult, with rDB's, sDB's, mitochondria (M), Golgi complexes (G) and less dense regions (x) showing a periodicity of amongst the DB's. Glycogen-like particles (GL) are sparse in the dermal cells but in dense accumulations in the parenchymal cells. 72,000x

