COMPLEX LIFE CYCLES IN HETEROPHYID TREMATODES: STRUCTURAL AND DEVELOPMENTAL DESIGN IN THE ASCOCOTYLE COMPLEX OF SPECIES

MARK H. ARMITAGE, M.S.
587 VENTU PARK ROAD, SUITE 304
THOUSAND OAKS, CA 91320

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ABSTRACT
Microscopic heterophyid trematode parasitic worms of the Genus *Ascocotyle* infect certain amnicolid and hydrobiid snails and certain cyprinodont and poeciliid estuarine fishes as first and second intermediate hosts. Adult trematode worms are found to mature in the intestines of particular definitive hosts, most often piscivorous birds, but also certain mammals. A survey of these parasites, harvested from fish hearts and gills collected in Mississippi, Texas and California, shows that they are are obligated to complex life cycles requiring at least three disparate and different hosts to achieve fecundity. Methods of infection, host infection site and host specificity are often unique to each different species of these parasites. Additionally, *Ascocotyle* worms demonstrate highly specialized structures such as HCl resistant cysts, HCl sensitive penetration glands and sensory organs which may serve to guide them to the specific infection site. These heteroecious life cycles and specialized structures are shown to be too complex to have developed by chance, therefore, evolutionary mechanisms appear insufficient to explain them. A creationist design argument for the presence of such parasites is promulgated.

INTRODUCTION

Arthur Looss [1] erected the genus *Ascocotyle* with *A. coleostomum* (Looss, 1896) as the genotype for the *Ascocotyle* complex. Excluding synonyms, or identical organisms with differing names, there are at present over 30 named species comprising the *Ascocotyle* complex as defined per Travassos [2], Stunkard and Uzmann [3], Burton [4], Sogandares-Bernal and Bridgman [5], and Sogandares-Bernal and Lumsden [6]. The adult worms (Fig #1) are non-pathogenic, intestinal parasites of piscivorous, or fish-eating, birds and mammals [7].

It is in the intestines of these definitive hosts that the parasite matures and produces eggs which are passed with feces into the marsh or estuary [8]. Because these organisms are hermaphroditic, or self-fertilizing, one parasite can populate a marsh with eggs [9]. Each fertilized egg has been shown to be able to produce a redia capable of growing 6000 swimming larvae over a one year period [10]. The first intermediate hosts are amnicolid and hydrobiid snails, which take up the eggs while feeding over the bottom surface of the estuary [11]. Infection within the particular snail type specifically depends upon the particular species of *Ascocotyle* present, and cross infection has been shown not to occur in many experimental trials [12, 13, 14, 15]. The eggs develop into a redia or brood stage (Fig #2), and often travel from the the snail digestive gland to the gonads or hepatopancreas where further development ensues. The redia grows in length and bears up to 50 cercaria in various stages of development. When mature, these cercaria (Fig #3,4) leave the redia through a birth pore, mostly during daylight hours (but often at dawn), and swim out of the snail towards illuminated areas of the water [16, 17]. The second intermediate hosts are centrarchiid, cyprinodontid, mugilid, and poeciliid fishes, and, in at least one instance, anuran tadpoles [18]. Cercaria swim near these fish and are taken up in the respiratory current. Some *Ascocotyle* will, at this point, attach to and penetrate the gill fillaments of the fish, dropping their tails before or soon after penetration [19, 20, 21]. Some species will immediately encyst in the gill, by the production of a multi-layered hyaline, collagen-like cyst [22, 23], while others will search for and enter the efferent blood vessels removing blood from the gills [24, 25]. The metacercaria will then travel within the blood system, either against or with blood flow, to the heart, liver, brain and other organs, where cysts will be produced and further development is arrested [ibid]. Other species of cercaria do not attach to the fish gills, but rather, are swallowed by the fish. They produce cysts in the stomach and intestine or penetrate the intestine and encyst in the liver and mesentary [26, 27].

As mentioned previously, host infection and even organ infection site is *Ascocotyle* species characteristic [28, 29, 30, 31]. It is at this point in the life cycle, that predation upon the infected fish by herons, egrets , or raccoons and other mammals, must occur in order that the definitive host can become infected and the worm can sexually mature. Metacercarial cysts, which are HCl resistant, successfully pass the definitive host stomach and dissolve only within the intestine, freeing the worm to fertilize and eject eggs. These life cycles are typically completed in brackish water estuarine marsh habitats, but some second intermediate hosts have been shown to swim out to sea [32]. In this paper, specific, complex life cycles and morphological structures of *Ascocotyle leighi, A. pachycystis and A. diminuta*, collected from fish hearts in Mississippi, Texas and California are evaluated in the light of the creation/evolution paradigms.

MATERIALS AND METHODS

Sheepshead minnow, (*Cyprinodon variegatus*), Sailfin Molly, (*Poecilia latipinna*), Mosquitofish (*Gambusia affinis)* (Fig #5) and Killifish (*Fundulus parvipinnis*) (Fig# 6) specimens were collected by seine, dip net and trap method from Pine Island, Mississippi, South Padre Island, Texas, and Newport Beach and Point Mugu, California, and of 60 fish collected, only 7 were uninfected. The hearts, gills and livers of these fish were harvested and examined under a dissecting microscope for the presence of metacercarial cysts, indicating a possible *Ascocotyle* infection. Some of the hearts and gills were fixed in glutaraldehyde, embedded in plastics and thin-sectioned [33]. Some cysts were removed from the heart and gill tissue for mechanical and enzymatic excystment and further study under scanning electron microscopy [34], (Fig# 7). Worms which were enzymatically excysted only exited their cysts under conditions of a 7.4 PH adjusted mixture of saline, RPMI media and trypsin in an incubator at 37^0C for 3-5 hours. Using fine needle forceps, other cysts were forcibly popped open releasing the live metacercaria for observation under high magnification light microscopy. Whole mounts were made of some of these excysted worms (Fig# 8). 2-5 micron thick sections of fish hearts and gills were cut with diamond and glass knives, stained with Methylene Blue and Azure II and coverslipped (Fig# 9, 10, 11).

Trematodes for SEM study were processed through osmium, thiocarbohydrazide and a graded series of alcohols to absolute alcohol. Dehydration by the critical point drying method was attempted resulting in the loss of many specimens . Air dried worms were transferred by hand under the dissecting microscope to SEM stubs and were sputter coated for 4 minutes at 30mA deposition. Stubs were then transferred to a Jeol JSM 35 Scanning Electron Microscope and were observed and photographed at 100-2000X magnification at 15 and 25 KV.

DISCUSSION

These parasites are an evolutionary enigma for several reasons and their presence raises more questions than are answered. The evolutionary paradigm for **r-strategists** (or organisms which are small, fast-growing and which have short, highly-populated generation times), calls for them to employ a rapdily developing, independent life cycle which allows them to exploit their environment quickly, achieve sudden fecundity and bear the most possible offspring with a minimum of exposure to survival hazards and expenditure of energy. In the light of that definition, *Ascocotyle* breaks all the rules. For one, *Ascocotyle* manifests a life cycle which may take up to a year or much more to complete, if the second intermediate host (bearing many such parasites) can escape predation or death. As mentioned previously, many examples exist of infected first and second intermediate hosts being kept alive in laboratory aquaria for long periods of time with no ill-effect, showing that the parasite is capable of surviving (or enduring) a lengthy hiatus before it can pass on its genes. This type of life cycle is uncommon for many microscopic organisms which typically bear offspring quickly, and do not expend energy on many intermediate stages. This may run counter to some thinking that evolution selects those r-strategists which develop into fitter populations faster than others in order to exploit the available environmental resources [35]. Conversely, some authors feel that evolutionary selection may run along a continuum from r-strategists to **K-strategists** (slow-growing organisms with long generation times and few offspring), where, "In the ecological void the optimal adaptive strategy channels all possible resources into survival and production of a few offspring of extremely high competitive ability." [36] Clearly ascocotylids are not k-strategists in the sense that many cercaria can be produced from one snail bearing a redia. By placing most of those cercaria into one or a few hosts which may or may not become predated upon, *Ascocotyle* certainly seems to minimize its chances for success.

Secondly, *Ascocotyle* is anything but independent, being strictly an obligate parasite. In the world of parasites, this feature is not unique as many completely depend upon other organisms to survive (many organisms would vanish tomorrow if the host population upon which they live died out).

The enigma for a 'survival of the fittest' interpretation of life cycles is that *Ascocotyle,* like most trematodes is obligated to **three hosts**, and therefore its chances of reaching sexual maturity are several times smaller than other, more independent organisms. Again, this does not seem to square with the typical r-strategy scenario under which ascocotylids should operate.

This parasite requires a snail as the first intermediate host, but not just any snail will do. Some 160 types of snails inhabit these estuaries [37], yet the redia do not develop in most of them. What is it about the "right" snail which causes the egg to develop into a redia, and what happens to eggs which are ingested by snails within which no development takes place? It would seem best to develop an evolutionary plan which would allow many types of intermediate hosts to serve as appropriate vehicles for development.

It also appears, based on the life histories referenced herein that the redia only develop in a certain part of the snail [38]. What mechanism guides the redia to the hepatopancreas or for that matter the gonads for completion of that stage?

These parasites require a 2nd intermediate fish host and not just any fish. As mentioned previously, laboratory studies have shown that *Ascocotyle* is very selective about the fish hosts which it infects.

What is not clear is what mechanism guides these cercaria to the proper fish for encystment. Do redial and cercarial spines and sensory papillae around the oral sucker and along the ventral tegument (Fig 12) play a role in host/organ detection and emplacement? When one would expect sibling species to be most alike, why do some sibling species (like *A. leighi* and *A. pachycystis*) only infect certain, but not the same fish in the same locality?

In addition, ascocotylids are not only host specific but they are organ specific within the 2nd intermediate host. Some metacercaria will travel with blood flow within the 2nd intermediate host and always end up in the same organ or site, others swim against the efferent flow and encyst within the heart. What mechanism or sensory apparatus indicates to the parasite which organ they are in or how to get there? Since they are generally non pathogenic and since a heavy parasite burden (even to the heart) has a minimal impact upon the 2nd intermediate host [39] one wonders if these parasites may confer some advantage to the host.

Finally, they require a 3rd intermediate host, which really begs the evolutionary question. Concern over this is expressed by some authors, in an attempt to supply an evolutionary explanation [40, 41]. Here the definitive host must digest the fish, while the cyst must pass this process unscathed. If the goal is to quickly survive and reproduce, why tie survival to the (potentially lethal) digestive process of a mammal or bird? It would seem to be "safer" to infect, say the shell of a shellfish which may be discarded by a raccoon or a bird after the meal is complete.

A. sexidigita and *A. mcintoshi* both appear to go the digestive route [42,43], not once, but twice; first within the fish and then the bird. What allows this cercaria to resist digestion within the fish stomach if it does not encyst within a protective capsule before burrowing through the intestinal submucosa to the visceral organs?

Cysts ingested by the definitive host also do not dissolve in HCl which is found normally within the host stomach, but only break down in a 7.4 pH adjusted solution of saline and media (nutrient broth) with trypsin (a digestive enzyme) at a temperature of 37^0C. These are very close to the conditions found within the definitive host intestine. Biochemical and ultrastructural cyst studies have shown that there is a small collagen content to the cyst wall, but of more value is the lipid-protein complex, which would definitely assist the passage of the metacercaria through the digestive tract. [44, 45, 46, 47, 48, 49, 50]. *A. mcintoshi* cercarial penetration glands, which assist the cercaria to enter an encystment site, do evert for penetration in weak solutions of HCl, as shown by Leigh [51] but otherwise do not. This indicates a mechanism which is sensitive to gastric juices which would be encountered when swallowed by the fish.

The question which must be asked at this point, is: "What if these relationships, behaviors and specialized structures were designed?" If they were designed, what would constitute a design feature or structure for *Ascocotyle* or any other parasite, and could we recognize it if we saw it? Michael Behe, in his seminal book, [52], goes to great lengths to show this by illustrating the bewildering complexity of the bacterial flagella, the chemical-electrical basis for vision, and the cascade system of blood clotting. Using the phrase "irreducibly complex", he deftly shows that these complex systems are comprised of components, or sub-parts, any of which, if not present, would prevent the entire system from working, making it worthless.

He also shows the utter foolishness of expecting that a gradualistic, Darwinian mechanism could have produced such elegant systems, by chance, using the trial and error method, "The impotence of Darwinian theory in accounting for the molecular basis of life is evident not only from the analysis in this book, but also from the complete absence in the professional scientific literature of any detailed models by which complex biochemical systems could have been produced...the scientific community is paralyzed. No one at Harvard University, no one at the National Institutes of Health, no member of the National Academy of Sciences, no Nobel prize winner - no one at all can give a detailed account of how the cilium, or vision, or blood clotting, or any complex biochemical process might have developed in a Darwinian fashion."

That Michael Behe can recognize intelligent design in bacteria, blood and vision is evident, "There is an elephant in the roomful of scientists who are trying to explain the development of life. The elephant is labeled 'intelligent design.' To a person who does not feel obligated to restrict his search to unintelligent causes, the straightforward conclusion is that many biochemical systems were designed. They were designed not by the laws of nature, not by chance and necessity; rather they were **planned**." (Emphasis in the original). If Behe can see intelligent, planned design in a bacterial flagella, then clearly he would see intelligent, planned design in the HCl sensitive cercarial penetration glands and the HCl resistant, yet trypsin and pH sensitive metacercarial cyst of *Ascocotyle* which requires it to be HCl resistant at one point in its fantastic voyage, and yet precisely trypsin and pH sensitive at another. These and other features found within the members of the *Ascocotyle* complex can be no less objects of intelligent, planned design than Behe's bacterial flagella.

Finally, the "limitations" of hermaphrodism as understood by an evolutionary system which would seek to amplify genetic mixing to every extent possible does not seem to fit the ascocotylids. Every member of this diverse group is a hermaphrodite, yet many significant differences between species exist. Often these difficulties are so dramatic that many new Genus and subgenus levels have been established and discarded [53, 54, 55].

CONCLUSION

Complex obligate life cycles, as shown within the *Ascocotyle* complex require that all of the special structures and features be in place or the system will fail and the organism will not live to bear offspring. Eggs must be ingested by a snail which will not digest them, but rather, which will provide sanctuary for the cercaria to develop in close proximity to the next host in the life cycle. The second intermediate host must as well, readily accept the infection and also be able to support a large parasite burden with no ill effects. The cercaria must be able to penetrate that host tissue, with a minimum of tissue response, and find the appropriate organ for encystment. Some cercaria must have the ability to resist gastric digestion on their way to encystment within the intestinal lining. The metacercaria must produce an HCl resistant, yet trypsin sensitive cyst. Finally, the hermaphroditic adult must be able to survive in the definitive host and produce eggs which will fall exactly where the first host may ingest them. Chance alone cannot account for this system or the structures it displays. An argument from intelligent design, however, might be made on the basis of the "irreducible complexity" of the structures and features found in this group.

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FIGURES:
Figure 1. *Ascocotyle mcintoshi*, after Lumsden, 1963. Scale bar = 100 microns
Figure 2. Typical redia, after Stein, 1968. Scale bar = 10 microns
Figures 3, 4. Typical cercaria, after Stein, 1968. Scale bar = 10 microns
Figure 5. *Gambusia affinis*, with penny for reference.
Figure 6. *Fundulus parvipinnis*, with penny for reference.
Figure 7. *A. diminuta*, SEM micrographs.
Figure 8. *A. angrense*, whole mount, brightfield.
Figure 9. *A. leighi*, encysted in fish heart, thin section.
Figure 10. *A. pachycystis*, encysted in fish heart, thin section.
Figure 11. *A. diminuta*, encysted in fish gill, thin section.
Figure 12. *A. pachycystis*, showing tegumental papillae.

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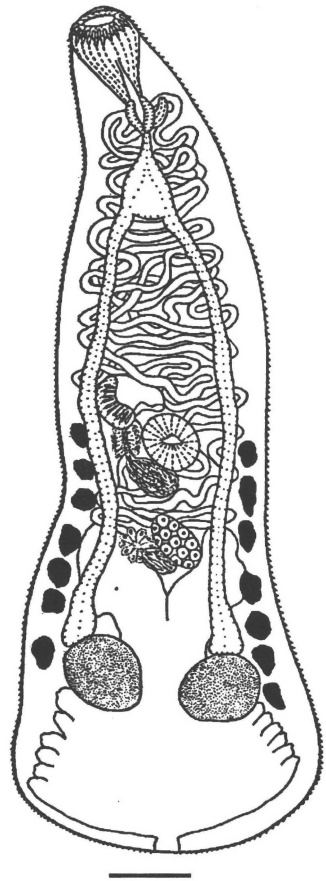

Figure 1
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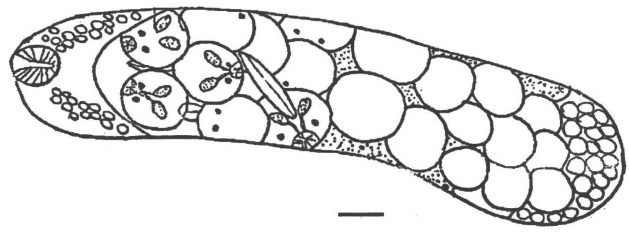

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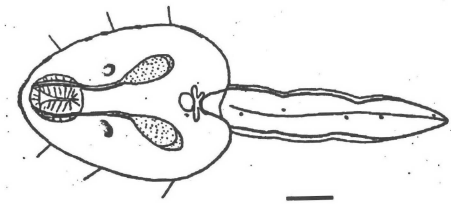

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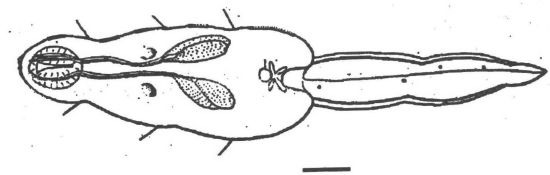

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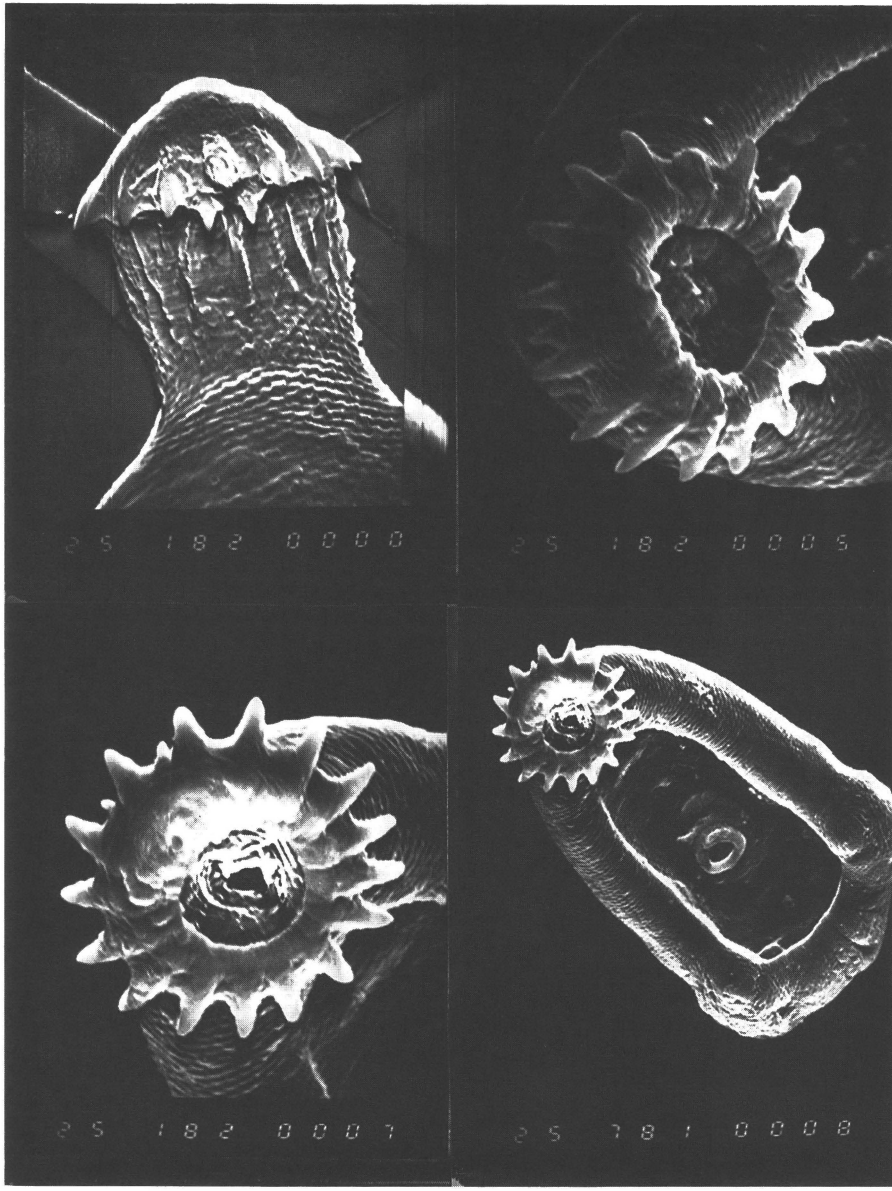

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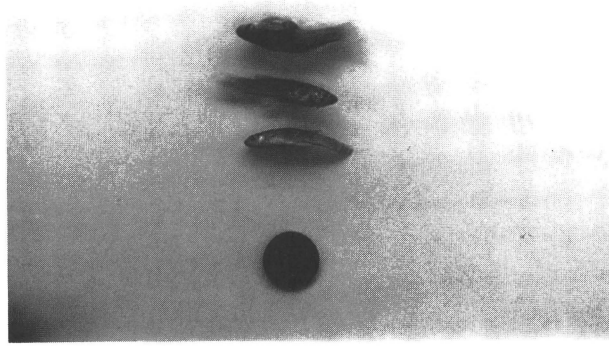

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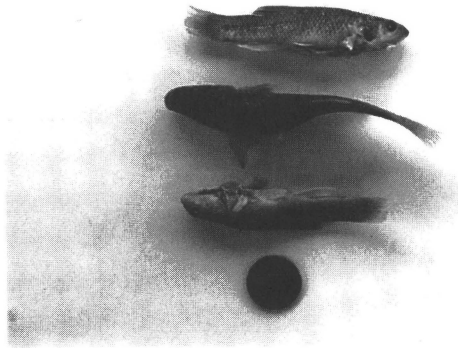

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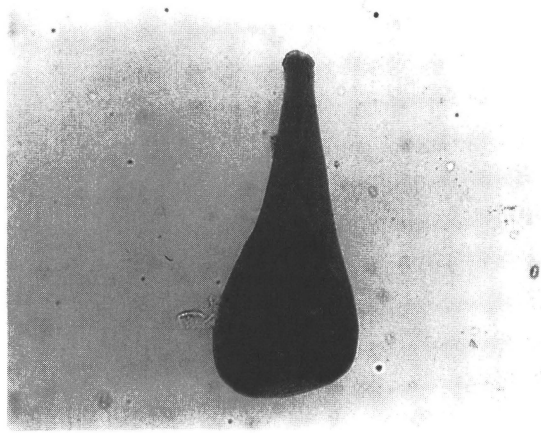

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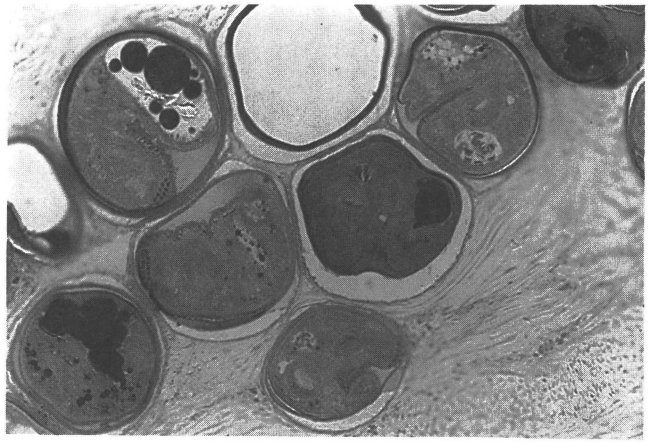

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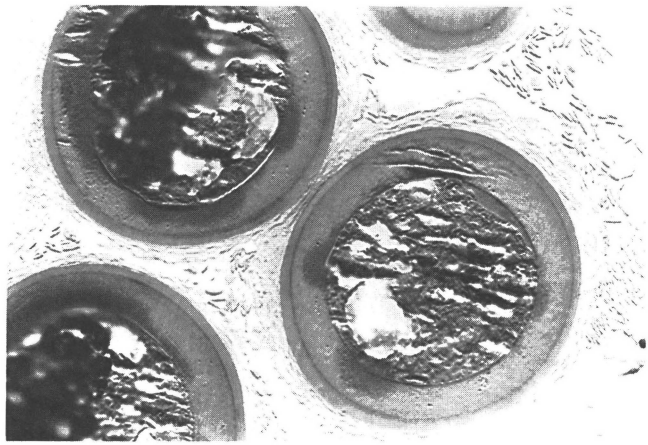

Figure 10

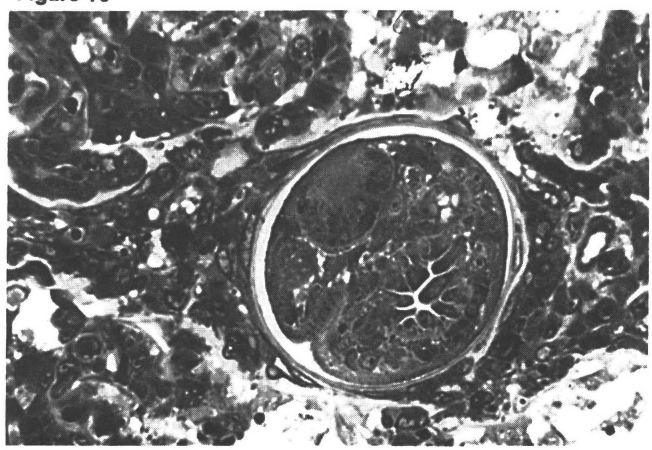

Figure 11

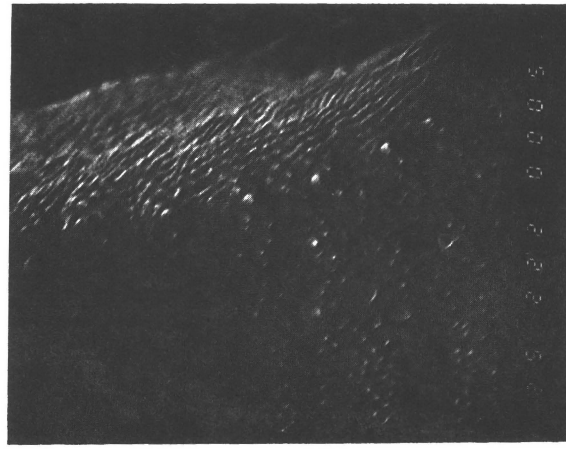

Figure 12