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THE BIOLOGY OF

HENNEGUYA EXILIS

by

William L. Current

A DISSERTATION

Presented to the Faculty of

The Graduate College in the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

School of Life Sciences

Under the Supervision of Professor John Janovy, Jr.

Lincoln, Nebraska

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TITLE

THE BIOLOGY OF

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BY

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INTRODUCTION

Organisms within the class Myxosporidia are primarily parasites of lower vertebrates, particularly fishes. Because of the work of Grassé (1960), Lom and de Puytorac (1965a, 1965b), as well as work in our laboratory, we now know that the Myxosporidia should no longer be considered as true protozoans due to the multicellular origin of their spores. However, for practical reasons they are still treated together with other protozoan groups (Lom, 1970). Although myxosporidians are not well understood biologically, they are nevertheless an extremely important group because of the potential threat they pose to the rapidly-expanding fish culture industry (McCraren et al., 1975). To date almost 300 species of myxosporidians have been reported from fishes, and the distribution of this class of parasites generally parallels that of fishes. Infected fishes have been found in habitats ranging from high altitude streams to deep-sea basins approaching 4,000 m in depth (Lom, 1970; Noble, 1966).

The major character of the class Myxosporidia is a spore equipped with one to 6 polar capsules with extrusible polar filaments and a germ cell, the sporoplasm, all of which are surrounded by a shell composed of 2 or more valves. Myxosporidians parasitizing fish are in the order Myxosporida which is characterized by a spore whose outer shell is composed of 2 valves. According to Kudo (1966) there are 10 families within the order Myxosporida. Family characteristics include such features as the number of polar capsules and the presence and absence of such structures as an iodinophilous vacuole. Characters which are used to

separate approximately 26 different genera of Myxosporida are associated with features of overall spore shape and morphology. Sub-generic characters include such features as spore shapes and measurements, and in some instances, non-morphological attributes such as geographical range, host specificity, and organ specificity. The validity of using non-morphological attributes for recognition of myxosporidan species has been discussed by Meglitsch (1957).

Aside from the taxonomic characters listed above, Myxosporida can be divided into 2 major types, coelozoic and histozoic, according to their site of development within a host. Plasmodia (spore producing stages) of coelozoic species develop within the lumen of such organs as the gall bladder, urinary bladder and uriniferous tubules of the kidney, while plasmodia of histozoic species are found in various tissues of different organs. Coelozoic species are considered to be more primitive (Kudo, 1966) and Schulman (1966) has gone so far as to suggest that the gall bladder is probably the site where most primitive Myxosporida originally settled and from where they passed into other organs and hosts. The site of infection varies among different species of histozoic Myxosporda and plasmodia have been found in almost every tissue and organ of host fishes. The gills and gall bladder are most frequently parasitized by myxosporidan species infecting freshwater fishes while species of Myxosporida infecting marine fishes are most often found in the gall bladder and urinary bladder (Kudo, 1966; Lom, 1970).

The nomenclature used to describe life cycle stages and developmental events of Myxosporida is somewhat confusing in that many terms are not presently used in the same context as when they were applied to descriptions of sporozoa for which they were originally intended. A

classic example of how confusion of terms has led to misinterpretation of data is seen in a recent paper by Hulbert et al. (1977). Examples of misused terminology by these authors include the use of: sporogony instead of sporogenesis, germative cells instead of generative cells, pansporoblast in place of enveloping cell, mitotic division rather than envelopment, sporant which is substituted for both sporont and sporoblast, and sporocyst which is used in place of both generative cells and sporont progeny. Therefore, to minimize ambiguity, the following definitions, adopted from Lom (1969), Lom and de Puytorac (1965a, 1965b), Schubert (1968) and Spall (1973) will be used in this paper:

Capsulogenic cell - a differentiated cell type within a sporoblast and committed to the production of a polar capsule.

Cyst - the growing tissue stage of a histozoic myxosporidan in which spores are produced.

Enveloping cell - a non-dividing cell whose role has been established. An enveloping cell envelops (phagocytizes) a sporont and functions to compartmentalize subsequent events of sporogenesis.

Generative cell - any of many small undifferentiated cells within a plasmodium resulting from acquisition of a plasma membrane, cytoplasm, and organelles by a generative nucleus.

Generative nucleus - any of many free nuclei within the cytoplasm of a plasmodium.

Plasmodium - equivalent to "cyst", applies to both histozoic and coelozoic species.

Sporoblast - any of a number of multicellular stages of sporogenesis (2-11 cells) which will produce one (monosporoblast) or more (pansporoblast) spores.

Sporogenesis - the process by which spores are produced within a Myxosporidan plasmodium.

Sporoplasm - the infective diplokaryotic germ cell within a sporoblast or mature spore.

Sporont - a generative cell which has been internalized (enveloped) by an enveloping cell. The sporont and its progeny will divide and then differentiate to produce different cell types involved in sporogenesis.

Sporont progeny - structurally undifferentiated cells in a sporoblast which are division products of the sporont.

Trophozoite - sporoplasm after it is released from the spore and while it is invading host tissues.

Valvogenic cell - differentiated cell type within a sporoblast which is committed to the production of a valve.

For many years it has been supposed that life cycles of Myxosporida were direct from fish to fish, that the route of infection was oral, and that the infectious stage was the spore (Halliday, 1976; Hoffman et al., 1962; Kudo, 1930; Plehn, 1904). According to the traditional hypothesis, after ingestion of spores by a suitable host, polar filaments extrude and adhere to the microvillus border of the intestine. Sporoplasms are then released, penetrate the intestinal mucosa, enter the lymphatics and/or

circulatory system, and are carried to the site of infection (cf. Olsen, 1974). However, none of these latter points have been verified experimentally. Auerbach (1910), Erdman (1912), and Shiba (1934) claimed to have produced such infections in the laboratory but none of these workers used fish which were shown to be parasite-free at the start of their experiments. Successful oral transmission of myxosporidans to laboratory-reared, non-infected fish was reported by Bond (1939) and Uspenskaya (1963, pers. comm. in Hoffman and Putz, 1969), however, transmission by routes other than oral could not be ruled out. Subsequent attempts to duplicate this work have failed (Fryer, 1971; Hoffman and Putz, 1968; Schafer, 1968; Spall, 1973; Walliker, 1968).

The only reproducable transmission experiments involving histozoic myxosporidans have been conducted with Myxosoma cerebralis (Hofer) Kudo, 1933, and Ceratomyxa shasta Noble, 1950, both parasites of salmonids. Experimental infections have been obtained by exposing young salmonids to bottom mud from earthen ponds which had previously contained infected fish (Halliday, 1973, 1974; Schaperclaus, 1931), by placing fish in screened cages into streams, lakes, and ponds where the disease occurred (Fryer, 1971; Hoffman and Putz, 1968; Schafer, 1968), or by placing fish into aquaria containing bottom mud and infective spores (Hoffman and Putz, 1969; Putz and Hoffman, 1966). The latter studies, conducted over a period of 8 years and involving 26 different attempts (Hoffman and Putz, 1970) revealed that spores of M. cerebralis had to be aged in the presence of bottom mud for 3 to 6 months before they were infective. This "aging process" has been confirmed by Putz (1970) and Putz and Herman (1970).

In addition to demonstrating that aging of M. cerebralis spores for at least 3 months in the presence of bottom mud was critical for transmission, the above studies suggest routes other than oral may be important for transmission of histozoic Myxosporida. Putz and Hoffman (1966) and Putz and Herman (1970) reported successful transmission of M. cerebralis to embryonic trout and prefeeding sac fry by placing them into aquaria containing "aged" spores overnight and for at least 40 min, respectively. Also, Schafer (1968) demonstrated that infection by C. shasta did not depend on ingestion of food organisms. In addition, Hoffman (1976) reported that he and Marsha Landolt found parasites, which may have been M. cerebralis, in the epithelial cells of trout. Later, Daniels et al. (1976) presented an ultrastructural description of a similar parasite in epithelial cells of trout previously exposed to M. cerebralis spores. Although these studies did not prove the relationship of this parasite to M. cerebralis, they nevertheless suggested the possibility of an intracellular stage prior to the appearance of spore-producing tissue stages.

Thus, previous research over the past 30 years has demonstrated that some myxosporidan infections can be produced in the laboratory but technical difficulties have hindered resolution of many of the basic questions concerning life cycles within this group. Some of the more important questions that remain unsettled and which are presently the subject of active research are: (1) Is transmission direct or does it require an intermediate or concentrating host? (2) Is the route of infection oral? and (3) Is there an intracellular stage prior to appearance of plasmodia? Since spores are vehicles through which myxosporidans are transmitted, it appears that a better understanding of spore biology may be the key that will unlock the answer to these as well as other important questions.

As early as 1895, Thélohan recognized the importance of spores in myxosporidan life cycles. His early studies involved testing the ability of a variety of chemicals to induce polar filament extrusion in 11 species of Myxosporida. Since that time no substantial progress has been made in elucidating biological mechanisms of polar filament extrusion. As it now stands, a variety of treatments are known to produce polar filament extrusion, however, the harsh nature of these treatments makes it difficult to ascribe biological significance to the process itself. Some of the treatments which have been successful in inducing filament extrusion in a variety of myxosporidan species include: alkalies (KOH, NaOH), sodium hypochlorite, hydrogen peroxide, and urea (Halliday, 1976; Hoffman et al., 1965; Kudo, 1930; Lom, 1964). To date, treatments to which one could ascribe biological significance have been shown to be unsuccessful and included incubation of spores in solutions of trypsin, pepsin, bile salts, and artificial and natural gastric juices (Halliday, 1976; Hoffman et al., 1965; Lom, 1964). Thus, it is apparent that additional studies are needed which are designed to reveal biological mechanisms responsible for not only polar filament extrusion but also exporulation and establishment of the sporoplasm within a host.

Although very little progress has been made toward an understanding of myxosporidan spore biology, there has been some progress toward a more thorough understanding of tissue stages, particularly at the ultrastructural level.

Ultrastructural studies of myxosporidans began in 1960 when Grassé described plasmodia of Sphaeromyxa sp. as having "microvillosities" on the surface and a highly vacuolated endoplasm with numerous generative cells. In the years following, several papers appeared which revealed

structural details of myxosporidan polar capsules and polar filaments (Cheissin et al., 1961; Lom, 1964; Lom and de Puytorac, 1965b; Lom and Vavra, 1964). These studies presented a generalized scheme for myxosporidan polar capsule formation, pointed out striking similarities between myxosporidan polar capsule formation and nematocyst differentiation in coelenterates, and demonstrated that solid polar filaments within polar capsules became hollow and larger in diameter after extrusion. Lom and de Puytorac (1965a, 1965b) presented structural details of myxosporidan plasmodia and reconstructed some of the major events of sporogenesis from various sporogenic stages of species of Henneguya, Myxobolus, Zschokkella, Myxidium, and Chloromyxum. Subsequent studies by Schubert (1968), Lom (1969), Uspenskaya (1969) Spall (1973), Schubert et al. (1975) and Hulbert et al. (1977) have confirmed and supplemented myxosporidan spore development and structure as presented by Lom and de Puytorac (1965a, 1965b). The above studies revealed that there are 2 major types of myxosporidan plasmodia with respect to their surface architecture. Plasmodia of coelozoic species have an outer unit membrane which forms numerous microvilli-like projections while plasmodia of most histozoic species have an outer unit membrane which folds inward to form numerous pinocytic canals. In addition, these studies have demonstrated that the cytoplasm of myxosporidan plasmodia consists of 2 major zones. The ectoplasmic zone contains generative nuclei, generative cells, early sporoblast stages, numerous mitochondria and pinocytic vesicles, as well as a variety of other organelles, e.g., Golgi bodies, smooth and rough endoplasmic reticulum, microtubules and microfilaments, and vacuoles of various sizes and with a variety of contents. The endoplasmic zone contains mature spores and late sporoblast stages as