A MYXOSPORIDIAN FROM THE MUSCULATURE OF SPRING CHINOOK SALMON

by

Ellis Junior Wyatt

A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1961

ACKNOWLEDGMENTS

Much of the research for this thesis was done by the author while in the employ of the Oregon State Fish Commission. I would like to express my grateful appreciation to this organization for permission to use the data gathered while in their employ. Special thanks is due to the research and hatchery personnel of the Hatchery Biology Section, who made some of the observations reported here, and for the collection of some of the fish used in this study.

My appreciation and thanks are also extended to the reference librarians of Oregon State University for obtaining the many interlibrary loans needed to complete this thesis.

The writer expresses appreciation to Dr. Ivan Pratt for his helpful encouragement, and critical review of this manuscript.

APPROVED:

Redacted for Privacy

Professor of Zoology

In Charge of Major

Redacted for Privacy

Chairman of the Department of Zoology

Redacted for Privacy

Chairman of School Graduate Committee

Redacted for Privacy

Dean of Graduate School

Date thesis is presented May 15, 1961

Typed by : Beverly Thayer

TABLE OF CONTENTS

LIST OF FIGURES

A MYXOSPORIDIAN FROM THE MUSCULATURE OF SPRING CHINOOK SALMON

INTRODUCTION

The classification of the myxosporidia was first attempted by Thélohan in 1892 (17, pp. 165-178). This classification was based on the characteristics of the spore (11, p. 52).

The work of Doflein (5, pp. 361-379;6), Auerbach (1, 261 pp.), Parisi (15, pp. 283-290), and Poche (16, pp. 125-321) again attempted the classification of this group. These attempts were directed toward a classification on the basis of the trophozoite (18, p. 115).

Davis (4, pp. 201-243) went back to the classification based on the characteristics of the spore and included the concept of the site of the infection to separate members of this group.

The excellent monograph of Kudo (11, pp. 1-265) brought together all the then known forms of the myxosporidia and provided a new scheme of classification using the form of the spore to separate taxonomic groups. Kudo in 1933 published a revision of his classification (12, pp. 195-216).

Tripathi (18, pp. 110-118) offered still another scheme of classification which has been neglected in favor of the scheme

presented by Kudo (12, pp. 195-216; 13, pp. 643-667).

The parasite dealt with in this thesis is found to be in the family Myxobolidae (17, pp. 165-178) and in the genus Myxobolus (3, pp. 590-603). Kudo (12, pp. 195-216) lists 70 species from this genus. Tripathi (19, pp. 63-88) provided a check list which included 112 species. A survey of the literature indicates that at the present time there are about 128 species described as being from this genus.

Kudo (13, pp. 658-660) defined the genus to include all forms having spores which are oval or ellipsoidal and flattened, with 2 polar capsules at the anterior end; sporoplasm with an iodinophilous vacuole; sometimes with a posterior elongation of the shell and exclusively histozoic in fresh water fish or amphibians.

The myxosporidian discussed here is from the musculature of spring chinook salmon, Oncorhynchus tshawytscha (Walbaum). A survey of the literature indicates only one species of the genus Myxobolus as being described from the genus Oncorhynchus. This was Myxobolus kisutchi from the spinal cord of the coho salmon, O. kisutch (20, p. 635).

A description of the morphology, effect on the host, and the relationship of this form to other species will be given.

MATERIALS AND METHODS

Spring chinook salmon fingerlings weighing about 25 fish to the pound and ranging from 99 mm. to 152 mm. were studied. These fish were from the Oregon State Fish Commission hatcheries on the South Santiam River in Linn County and the Willamette and McKenzie Rivers in Lane County. Observations were also made on fish that originated at the McKenzie River hatchery but were used for diet experiments at the Oregon State Fish Commission Research Laboratory in Clackamas, Oregon. Further observations were made on fish that were shipped to the Metolius River hatchery in Jefferson County from the Willamette and South Santiam hatcheries. The Willamette River fish were not found to be infected with Myxobolus sp.

Material used for sectioning was fixed in Bouin's fixative. Ten micron paraffin sections were cut and stained with Heidenhain's iron hematoxylin and eosin. Smear preparations of infected musculature were fixed in Schaudinn's fluid and stained with Giemsa's stain. Smear preparations fixed in alcohol formol were stained with Bauer's modified Feulgen reaction for glycogen; this and fresh spores subjected to Lugol's iodine, both demonstrated the iodinophilous vacuole. Extrusion of the polar filaments was under the influence of NaOH. Air dried smears of infected muscle tissue stained with Ziehl-Neelson's carbol fuchsin and Löffler's

methylene blue provided permanent preparations of spores with extruded polar filaments (Fig. 6). Measurements were made of fresh spores as well as Giemsa stained material. Drawings were made with the aid of a Leitz drawing lens. Photomicrographs were taken with a 35 mm. Leica camera mounted on a Leitz microscope.

HISTORY OF OBSERVATIONS and GENERAL EFFECTS ON THE HOST

The Hatchery Biology Section of the Oregon State Fish Commission Research division has the responsibility for monitoring and dealing with deviations in the normal state of health of fish being reared in Fish Commission hatcheries. This service is either conducted as periodic visits or upon the request of hatchery superintendents.

The parasite dealt with in this thesis was first seen prior to 1956 in the course of routine visits to the McKenzie River hatchery by members of the Hatchery Biology Section.

In July of 1958 the author had occasion to examine microscopically small bits of kidney and gill tissue of a number of fingerling salmon from the McKenzie River hatchery. Isolated spores were seen in both tissues. Observations of a similiar nature were made on different occasions throughout the rearing periods of 1958, 1959, and 1960 by other members of the Hatchery Biology Section and the author. It was also observed that these fish exhibited a "fungusing" in the early fall of each of these years, but the cause of this was not understood.

The 1958 brood South Santiam spring chinook fingerlings were closely monitored during their hatchery life, due to the initiation of the use of a new type of food the "Oregon Pellet"

at this hatchery in May of 1959. Personnel observing the nutrition noted a general deterioration in the blood picture of these fish in early August. In the months of September, October, November, and December a series of visits was made to this hatchery because of the continued deterioration of the health of these fish. It was observed that many fish were exhibiting frayed fins and tails. Also observed were slightly swollen circular areas on the sides of the body which were devoid of scales. Many dark, lethargic fish were congregated at the tail ends of the ponds. Fish exhibiting the above symptoms invariably had pale gills, low hematocrit and hemoglobin values. Also observed were spores having a similiar form as those observed in McKenzie River fish. Because no myxosporidian had been implicated in conditions of this nature in the past, this parasite was not connected to the symptoms at that time.

A deterioration of the blood picture occurred in October in South Santiam fish that had been shipped to the Metolius River hatchery in July 1959. In late November typical symptoms noted at South Santiam were also noted in the South Santiam fish transplanted to the Metolius.

In August 1959, Willamette River fish were also sent to the Metolius River hatchery for rearing and placedin a separate pond from those sent from the South Santiam. The Willamette River fish maintained a relatively normal state of health throughout their

rearing period. Examination of bits of musculature, gill, and kidney tissue of these fish never revealed the presence of the parasite, while the parasite was seen in those of South Santiam origin.

In May of 1959 a group of McKenzie River spring chinook fingerlings were sent to the Oregon State Fish Commission Research Laboratory in Clackamas, Oregon, and were subjected to experimental diets. At the close of these experiments early in 1960, control lots of these fish were frozen. The author obtained 29 of them for examination. The musculature, kidney, and gill tissue of each of these fish contained spores. Twenty-one of the 26 livers examined contained spores and 15 of the 18 spleens were found to contain spores.

On a visit to the South Santiam hatchery in December of 1959, the author placed fish in Bouin's fixative for later study.

DATA

Examination of sections of the musculature of fish showing the typical symptoms noted earlier were found to be heavily parasitized with numerous cysts each containing many spores of a myxosporidian from the genus Myxobolus. Many fish not exhibiting the overt symptoms were found to be similarly parasitized.

The cysts are oriented parallel to the long axis of a bundle of muscle fibers and they replace these fibers for some length (Fig. 8). In some cases 2/3 of the length of a myotome is involved. They are seen in the epaxial and hypaxial musculature in about equal numbers. In a piece of tissue 8 mm x $1\frac{1}{2}$ mm. x 10 microns, an average of 19 cysts per cross section were observed. This fish showed the typical symptoms described above.

The relationship of the swollen circular areas, which are devoid of scales, to the cysts, was substantiated by observations of sections through these necrotic areas (Fig. 7). The corium is absent and the area is covered only by the regenerating epidermis. Cysts are seen lying in the bundles of muscle fibers immediately below the connective tissue under the regenerating epidermis. In certain areas where no necrosis had occurred, the epidermis and corium seem to be elevated. Associated with this are cysts located in the outermost bundles of muscle fibers. It is thought that these areas are sites where new external lesions

will appear.

Most cysts are intact, but in certain cases free spores can be observed in the connective tissue surrounding the bundles of muscle fibers (Figs. 7, FS). These cases occur in both the deeper muscle masses and near the corium.

Gross observations of swollen kidneys, cream colored livers, and enlarged spleens were made. Examination of histological sections of these tissues confirm abnormal cytological conditions.

DESCRIPTION OF THE SPORE

In front view, the spore is oval with the anterior end slightly attenuated (Figs. 1, 4, 5, 6). In side view it is pyriform (Fig. 2). In end view the spore is broadly lenticular (Fig. 3). The polar capsules are pyriform and when viewed in the sutural plane, the ends are seen to be reflected outward so that each lies on opposite sides of the sutural ridge (Fig. 2). The coiled polar filaments are distinct in fresh spores and those treated with Lugol's iodine (Figs. 4, 5). Glycogen or glycogen-like material is seen in the area between the polar capsules. There is no intercapsular appendix. The shell is moderately thick and becomes slightly thinner at the anterior end of the spore. The valves are unmarked. The sutural ridge is distinct, but a sutural line is not seen. The ridge has a marked curvature as it follows the spore contours. In fresh spores the sporoplasm is almost homogenous with the iodinophilous vacuole, and is poorly defined (Fig. 4). The vacuole stains deeply with Lugol's iodine (Fig. 5) and with Bauer's modified Feulgen reagent. Either 1 or 2 nuclei (Fig. 1) are seen in the sporoplasm of most spores stained with Giemsa. Infrequently Giemsa-stained spores may show 1 or 2 residual nuclei associated with the polar capsules.

See Table 1 for biometric data.

VEGETATIVE FORM

The cysts are spindle shaped (Fig. 8) having average dimensions of .079 x .142 x .674 mm. Most cysts contain only mature or nearly mature spores. A few trophozoites were observed. Disporoblastic development only was seen. Whether development can occur otherwise was not determined. The cysts are polysporus.

DISCUSSION

The date of shipment of the spring chinook fingerlings from the McKenzie River hatchery to the Research Laboratory in Clackamas, Oregon, was on May 30, 1959. Because the water supply used for the diet experiments at Clackamas is free of fish it is certain that these fish were infected before the date of shipment.

It seems possible that those spring chinook adults returning to the McKenzie River to spawn and die, above the hatchery water supply could provide the means for the infection of the young fish at the hatchery. It is less clear as to the possible origin of the infection of the South Santiam fingerlings as the water supply for this hatchery is not derived from the stream where returning adults spawn.

The McKenzie and South Santiam hatcheries have dirt ponds for the rearing of fish. The Myxobolus sp. discussed in this thesis has only been seen at these two hatcheries. Fantham (7, p. 386) reported the finding of spores entangled in the scum on the water in which fish infected with Myxobolus ovoidalis were kept. It is possible that dirt hatchery ponds may somehow provide a better environment for the transmission of this Myxobolus sp. than do concrete raceway ponds. Fish (8, p. 177) pointed out the fact that rearing of fish under artifical conditions with the concomitant crowding, provides an ideal

situation for the dissemination of a parasite with no intermediate host involved in its life history. This is true of the myxosporidia.

The exact effect of the Myxobolus sp. on the host is complicated by the fact that the diets being fed the South Santiam hatchery fish and those shipped from the South Santiam to the Metolius River hatchery for rearing, were known to contain high levels of rancid material. These fish were found to be infected. The Willamette fish transfered to the Metolius did not receive a rancid diet and were never found to be infected. In comparing the South Santiam and Willamette transfers it would appear that this parasite had considerable influence. The fish originating at the South Santiam hatchery showed the typical symptoms of low hematocrits and hemoglobins, cream colored livers and enlarged spleens. The Willamette fish were normal. This evidence does not stand up however, in that infected fish from the McKenzie River hatchery which were known to have received a non-rancid died did not exhibit the symptoms noted above. Until an experiment is run in which rancid material is fed to non-infected fish it is impossible to determine the relative effects of the parasite and the diet.

Observations of "fungusing" of the McKenzie River spring chinook fingerlings in the late summer and early fall of several years preceding this study might indicate a relationship to this parasite. The loss of scales plus the development of small areas

of necrosis could be the factors which would allow the invasion of the fungus.

In studying the sections of liver, kidney and spleens of South Santiam fish, spores were observed only in the kidney of one fish. In this instance only two spores were seen. However, wet mount examination of small bits of these same tissues plus gill filaments of McKenzie River fish disclosed spores in each of these tissues. All spores in the gill filaments were within the gill capillaries. This might be used as evidence to suggest that the spores seen in other tissues are derived from the musculature and arrive in these tissues via the blood stream.

Most spores are seen to lie within cysts located within a bundle of muscle fibers. However, free spores lying in the intermuscular connective tissue are also seen. In some cases bundles of muscle fibers are obviously missing in these areas. Nigrelli (14, p. 45) has suggested that myxosporidians elaborate proteolytic enzymes that might account for the above observations.

The 1958 brood spring chinook fingerlings at the South Santiam hatchery did not suffer excessively high mortalities. However, the author was present at the hatchery at the time of release of part of these fish in late December 1959. It was observed that after the handling necessary in the weighing out of these fish prior to liberation many of these fish were quite exhausted so that their successful migration to the sea seemed doubtful.

Yasutake and Wood (20, p.636) also make note of the possible

consequences of myxosporidian infections in hatchery reared fish. It is their contention that certain infections could be of considerable significance in the life histories of these fish and contribute to the cases where adults return in less than expected numbers.

Hahn (9, pp. 193-214) described Myxobolus musculi from the muscle and gills of Fundulus heteroclitus and F. major. This form was renamed Myxobolus funduli by Kudo (11, pp. 151-152), in that the former name had already been used by Keysselitz in 1908 (10, pp. 452-453). Myxobolus funduli differs from the form described in this thesis in the following respects. The width of the spore is narrower; the polar capsules are shorter; the spore shell is very thin and almost invisible, and the spore is more rounded anteriorly. The lesions formed in fish infected with Myxobolus funduli differ from those observed in Myxobolus sp. Furthermore, no cysts are formed by Myxobolus sp. except in the body musculature, while cysts are produced in the connective tissue of the gill by Myxobolus funduli.

Keysselitz (10, pp. 452-453) described Myxobolus musculi from the muscle of the main body, rarely that of the fins and operculum, and the kidney of Barbus fluviatilis. Spores were also observed in the liver, kidney, spleen and ovary in a state of diffuse infiltration (11, p. 148). The general nature of this infection relative to sites of spores in tissues other than the ovary, might

appear similar to the Myxobolus sp. described here. The size and general morphological features of this form differ markedly from Myxobolus sp. described in this thesis.

Kudo (11, p. 155) described Myxobolus koi from the connective tissue of the gill filaments of Cyprinus carpio. In morphology Myxobolus koi resembles Myxobolus sp. except in the thickness of the spore. The site of the infection, the host, and the size and shape of the cyst also differ.

SUMMARY AND CONCLUSIONS

A myxosporidian from the body musculature of spring chinook salmon fingerlings from the Oregon State Fish Commission hatcheries on the South Santiam and McKenzie Rivers is described. Spores were also seen in the gill capillaries, liver, spleen, and kidney of these fish. The primary site of the infection is the body musculature.

Circular necrotic areas which are devoid of scales occur on the sides of infected fish. These areas were shown to be associated with the presence of cysts. Fish not showing these areas of necrosis were also found to be infected.

Infected fish receiving a diet containing rancid materials showed low hematocrit and hemoglobin values, swollen kidneys and spleens, and cream colored livers. Infected fish known to have received a non-rancid diet did not exhibit these symptoms. Thus, the exact effect of this parasite on the host is not known.

The parasite discussed in this thesis is in the genus, Myxobolus. A table of biometric data is included. A survey of the literature indicates that this genus now contains about 128 species. This Myxobolus sp. does not appear to be closely related to any form thus far described in the literature, when compared in terms of the host, site of the infection, and specific morphology.

BIBLIOGRAPHY

1. Auerbach, M. Die Cnidosporidien (Myxosporidien, Actinomyxidien, Microsporidien). Eine monographische Studie. Leipzig, W. Klinkhardt. 1910. 261 p. (Cited in 11, p. 7; 18, p. 115)

2. _____ Unsere heutigen Kenntnisse über die geographische Verbreitung der Myxosporidien. Zoölogische Jahrbücher, Abtheilung für Systematik 30:471-494. 1911. (Cited in 18, p. 115; 11, p. 200)

3. Bütschli, O. Myxosporidia. In: H.G. Bronn's Klassen und Ordnungen des Thier-reichs. vol. 1. Protozoa. Leipzig, C.F. Winter'sche Verlagshandlung, 1882. p. 590-603. (Cited in 11, p. 128, 201)

4. Davis, H.S. Myxosporidia of the Beaufort Region. A Systematic and biologic study. Bulletin of the United States Bureau of Fisheries 35:201-243. 1917.

5. Doflein, F. Fortschritte auf dem Gebiete der Myxosporidienkunde. Zusammenfassende Uebersicht. Zoologisches Zentralblatt 6:361-379. 1899. (Cited in 18, p. 115)

6. _____ Die Protozoen als Parasiten und Krankheitserreger nach biologischen Gesichtspunkten dargestellt. Jena, G. Fischer. 1901. (Cited in 18, p. 115)

7. Fantham, H.B. Some parasitic protozoa found in South Africa. XIII. South African Journal of Science 27:376-390. 1930.

8. Fish, F.F. Notes on Myxobolus inornatus, n. sp., a myxosporidian, parasitic in the black bass Huro floridana (LeSuer). Transactions of the American Fisheries Society 68:173-177. 1938.

9. Hahn, C.W. Sporozoön parasites of certain fishes in the vicinity of Woods Hole, Massachusetts. Bulletin of the United States Bureau of Fisheries 33:193-214. 1915.

10. Keysselitz, G. Ueber durch Sporozoen (Myxosporidien) hervorgerufene pathologische Veränderungen. Verhandlungen der Gesellschaft Deutscher Naturforscher und Ärzte 79: 452-453. 1908. (Cited in 11, pp. 148-149)

11. Kudo, R.R. Studies on myxosporidia. A synopsis of genera and species of myxosporidia. Illinois Biological Monographs 5 (3/4): 1-265. 1920.

12. _____ A taxonomic consideration of myxosporidia. Transactions of the American Microscopical Society 52: 195-216. 1933.

13. _____ Protozoology. Springfield, Illinois, C.C. Thomas. 1954. 966 p.

14. Nigrelli, R.F. Prickle cell hyperplasia in the snout of the redhorse sucker (Moxostoma aureolum) associated with an infection by the myxosporidian Myxobolus moxostomi sp. nov. Zoologica 33:43-46. 1948.

15. Parisi, B. Primo contributo alla distribusione geografica dei missosporidi in Italia. Atti della Società Italiana di Scienze Naturali 50:283-290. 1912. (Cited in 18, p. 115)

16. Poche, F. Das System der Protozoa. Archiv für Protistenkunde 30:125-321. 1913. (Cited in 18, p. 115)

17. Thélohan, P. Observation sur les myxosporidies et éssai de classification de ces organismes. Société Philomathique de Paris. Bulletin 4:165-178. 1892. (Cited in 18, p. 115)

18. Tripathi, Y.R. Some new myxosporidia from Plymouth with a proposed new classification of the order. Parasitology 39: (1/2): 110-118. 1948.

19. _____ Studies on parasites of Indian fishes 1. Protozoa myxosporidia, together with a check list of parasitic protozoa described from Indian fishes. Records of the Indian Museum 50:63-88. 1953.

20. Yasutake, W.T. and E.M. Wood. Some myxosporida found in Pacific Northwest salmonids. The Journal of Parasitology 43:633-637. 1957.

APPENDIX

TABLE 1

FRESH SPORES

CHARACTER	MEAN (MICRONS)	STANDARD DEVIATION	RANGE (MICRONS)	NO. EXAMINED
Spores				
Valve Length	15.03	.90	12.8 to 17.28	100
Valve Width	10.26	.63	8.96 to 11.52	100
Thickness	7.50	.62	6.4 to 8.96	56
Polar Capsules				
Length	8.76	.73	7.04 to 10.24	100
Width	3.29	.32	2.56 to 4.48	100
Vacuole (Lugol's Iodine)				
Length	2.79	.40	1.92 to 3.84	50
Width	3.66	.59	2.56 to 5.12	50
Polar Filaments				
Length (NaOH) Extruded	63.48	3.22	55.68 to 70.40	32

FIXED AND STAINED SPORES
(Schaudinn's and Giemsa)

Spores				
Valve Length	13.18	.58	12.16 to 14.72	100
Valve Width	7.59	.50	7.04 to 8.96	100
Polar Capsules				
Length	7.35	.42	6.40 to 8.32	100
Width	2.59	.30	1.92 to 3.20	100

Figure 1

Giemsa stained spore showing the usual position of sporoplasm nuclei.

Figure 2

Formalin preserved spore in the sutural view.

Figure 3

Formalin preserved spore in end view.

Figures 1, 2, and 3 were made with the aid of a Leitz Drawing Lens.

FIG.1

FIG.2

FIG.3

Figure 4

Photomicrograph of a fresh spore showing the poorly defined iodinophilous vacuole (x 900).

Figure 5

Photomicrograph of a fresh spore after treatment with Lugol's iodine (x 900).

Figure 6

Photomicrograph of spores stained with Ziehl-Neelson's carbol fuchsin and Löffler's methylene blue showing the extruded polar filaments (x 900).

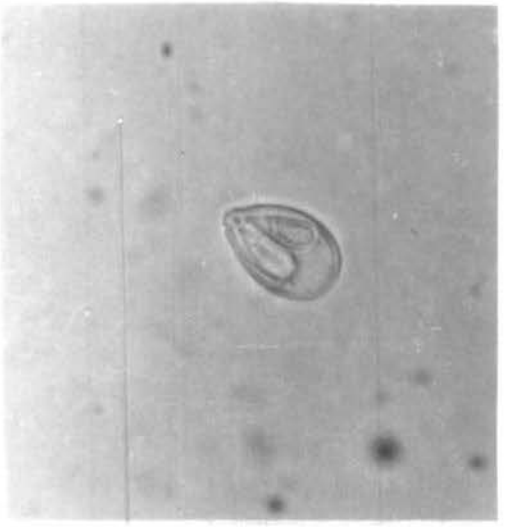

FIG.4

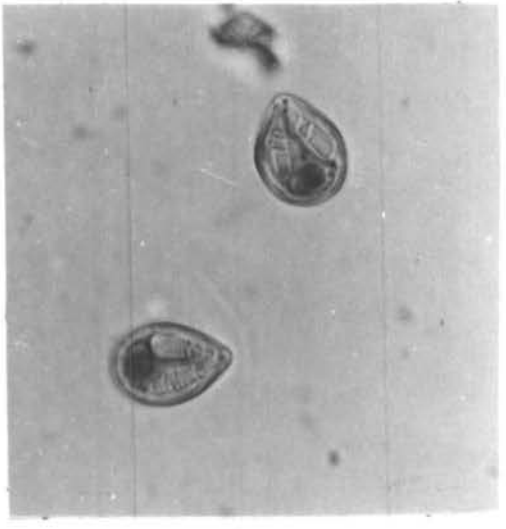

FIG.5

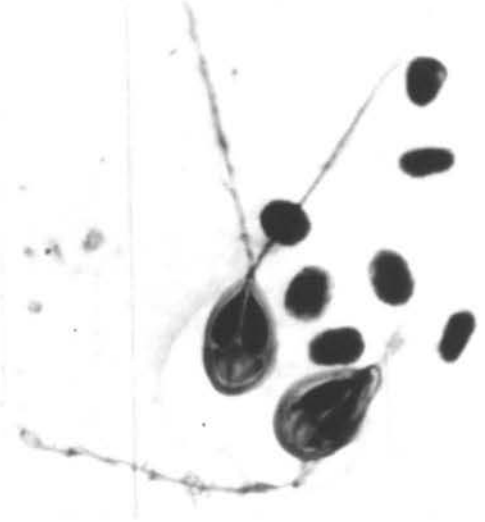

FIG.6

Figure 7

Photomicrograph of a cross section of infected muscle (x 153).

C - Cyst
EC - End of Corium
FS - Free Spores
RE - Regenerating Epidermis
T - Trophozoite

Figure 8

Photomicrograph of a longitudinal section of infected muscle (x 144).

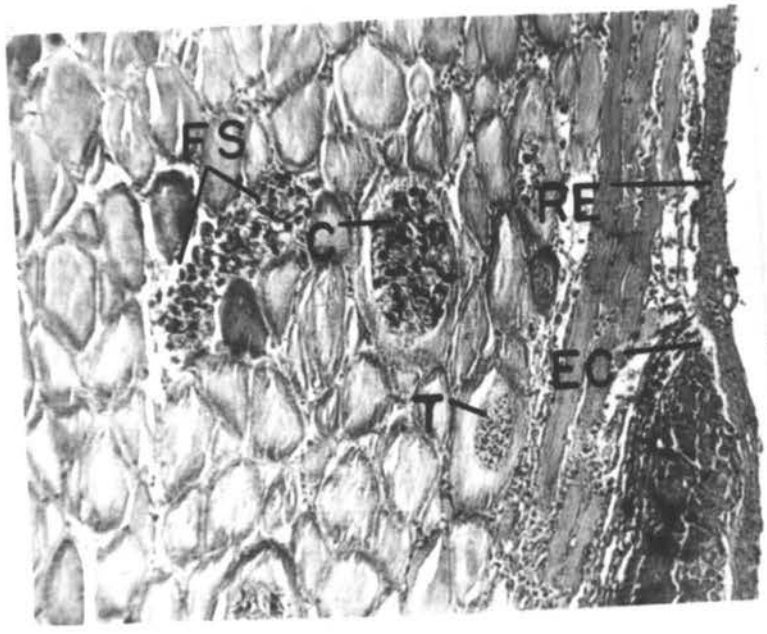

FIG.7

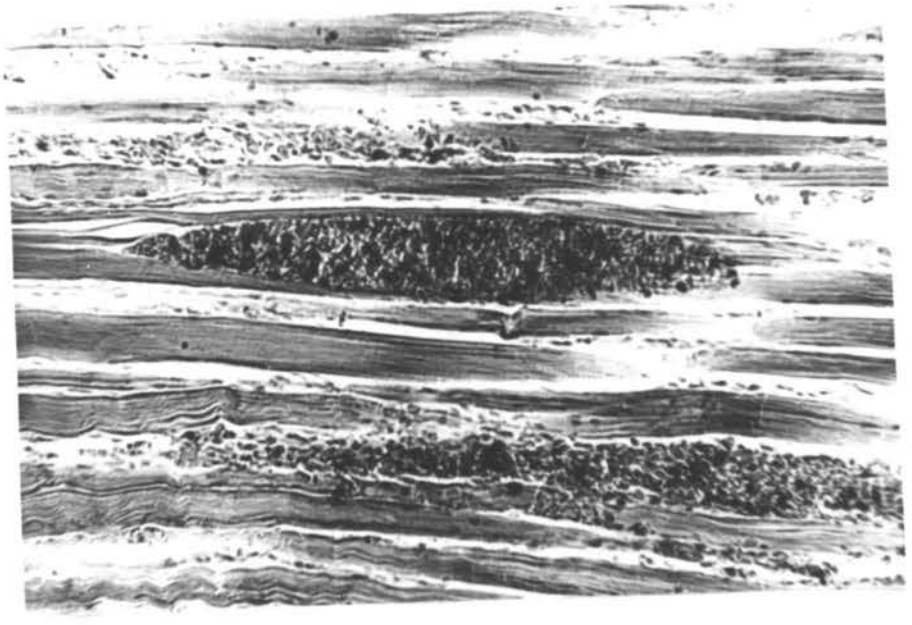

FIG.8