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TISSUE REACTION TO GLUGEA PLECOGLOSSI INFECTION IN ITS NATURAL HOST, PLECOGLOSSUS ALTIVELIS

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Abstract. The microsporidian *Glugea plecoglossi* induces in its fish host *Plecoglossus altivelis* a hypertrophic growth of the infected cells, resulting in formation of large xenomas. Early thin-walled xenomas did not elicit any reaction of the surrounding tissue. Later, however, coinciding with the mature xenoma being filled up with a growing mass of spores, proliferative inflammatory reaction sets in, changing gradually the xenoma into a granuloma. The granulation tissue grows into the spore mass. The spores are eliminated by macrophages and the granuloma undergoes involution. A complete functional reparation of heavily infected organs is hardly possible. Essentially, the tissue reaction was found to follow the pattern known in *G. anomala* infections in sticklebacks and in some other *Glugea* species initiating the formation of large xenomas.

Microsporidians are among the most important protozoan parasites affecting fish. Most literature concerning microsporidians of fishes, however, deals with taxonomic and life cycle problems. The existing data on the pathology of microsporidian infections in fishes were summarized by Canning (1976). Her review indicates that most of the observations concern macroscopical changes and mortality rate.

The present paper is the result both of the effort to fill the gaps existing in the understanding of the fish tissue reactions to microsporidian infections and of the interest in microsporidian species found to be serious pathogens in conditions of intensive fish husbandry. It deals with histopathological changes provoked by *Glugea plecoglossi* Takahashi et Egusa, 1977 in tissues of the ayu (*Plecoglossus altivelis* Okada). The ayu is a commercially very important cultured freshwater fish in Japan.

MATERIAL AND METHODS

Tissue samples of internal organs (digestive tract, liver, spleen, ovary and testes), of trunk musculature and fins of spontaneously infected *Plecoglossus altivelis* were fixed partly in 10% formalin, partly in Bouin's fluid. Paraffin sections 5 to 7 μm thick were stained with haematoxylin-eosin, trichrome stain according to Masson and treated with the PAS reaction for polysaccharides.

RESULTS

Xenomas were found in the intestine, ovary, testes, spleen, in body cavity attached to peritoneum and in the fins. They differed in size and stage of development. Small, thin-walled xenomas filled as a rule with cylindrical developmental stages of the parasite were situated in the subepithelial connective tissue and lamina muscularis of the intestine. Xenomas situated in all other organs were much larger and in more advanced, but unequal stages of development, so that in the same organ there were:

a) xenomas with a distinct wall appearing in the light microscope as a homogeneous, refractile and PAS-positive layer, with a relatively wide marginal cytoplasmic zone containing numerous nuclei of the host cell and developmental stages (cylindrical schizonts, sporogonial vacuoles) while their center was occupied by mature spores;

b) xenomas with a still distinct wall, filled with spores, while their marginal cytoplasmic zone was reduced to a very thin granular layer without host cell nuclei;

c) xenomas changed to parasitary granulomas.

Only the early, thin-walled xenomas with cylindrical schizonts about 50 μm in size observed in the intestine, and more advanced xenoma with a distinct wall found in the testes did not elicit any reaction of the neighbouring tissue (Plate I, Figs. 1, 2).

The large xenomas (up to 3 mm in size) provoked a proliferative inflammation of the tissue and formation of a granulation tissue in differing stages of maturation. Some of the large xenomas were encased only with concentrically arranged fibrous connective tissue or with a thin margin of maturing granulation tissue (Plate II, Fig. 1).

A probably more advanced stage was represented by large xenomas surrounded by a wide zone of newly formed, richly infiltrated granulation tissue which included also multinucleate giant cells. In these cases the xenoma wall became less stainable and seemed to undergo oedematous changes turning the wall into a wide layer of fibrous appearance (Plate II, Fig. 2, Plate III, Fig. 1).

In close vicinity of the xenomas, where the granulation tissue matures into a concentrically arranged fibrous connective tissue, eosinophilic amorphous masses could be detected as an evidence of hyalinisation of the latter.

The layer of granulation tissue was absent in xenomas situated in subcutis of the fins. However, xenomas of a corresponding stage of development situated in other organs evoke formation of granulation tissue. In the former, the tissue reaction was manifested by proliferation or fibroblasts and histiocytes, while infiltrating cells were absent.

In the testes, tissue reaction to large xenomas (-containing developmental stages or only mature spores) was represented only by the formation of a relatively very thin layer of concentrically arranged connective tissue sharply delimited from the surrounding tissue (Plate III, Fig. 2, Plate IV, Fig. 1).

The most advanced stage of the tissue reaction was observed in the ovary. The xenomas has changed into an enormous granuloma, the xenoma wall completely disappeared, at the periphery there was a granulation tissue growing into the spore mass. Spores were dispersed also between the proliferating fibroblasts, while in the central mass they were gradually engulfed by macrophages (Plate IV, Fig. 2).

All affected organs of *Plecoglossus altivelis* were markedly damaged by pressure atrophy due to large xenomas and by the development of tissue reactions. The xenoma can be completely eliminated by the granulomatous inflammatory reaction with subsequent phagocytosis of the spores; however, a complete functional restitution of a heavily invaded organ is hardly possible.

The material available thus far from the natural infection does not permit us to state to which extent the changes of the structure of the xenoma are linked with the development of the tissue reaction and if the oedematous changes of the xenoma wall are in any direct relation to the formation of the granulation tissue. Different character of the tissue reaction in different host organs made it difficult to see to what extent the developmental stage of the xenoma determines the kind of tissue response.

DISCUSSION

Glugea plecoglossi, described by the Japanese authors Takahashi and Egusa in 1977, was recognized as the causative agent of one of the most serious diseases of its host, *Plecoglossus altivelis*. Known in Japan under the vernacular name of ayu, this fish is one of the most important species of food fish in Japan — the culture of which may spread to other countries, too. In this respect it is quite urgent to collect data on the development and pathogenicity od *G. plecoglossi* infection as one the prerequisites of effective control measures.

The development of *G. plecoglossi* follows essentially the pattern (Takahashi and Egusa 1977a) of *G. anomala* infection of sticklebacks (see Débaisieux 1920 and Weissenberg 1968). Incidental to this is the low organ specificity and close resemblance of the host tissue reaction. Presumably xenomas originate by transformation of the same type of host cells, i.e., mesenchymal cells. The type of xenomas is more or less identical in all body organs. This seems to be the same in other *Glugea* species, too, (e.g., *G. hertwigi* — Delisle, 1972) with the exception of *G. atherinae*, in which intestinal xenomas supersede all others in their huge size and thickness of the xenoma wall (Berrebi 1978). A preliminary comparison of *G. plecoglossi* with the action of other xenoma-inducing *Glugea* species infecting most varied hosts indicates that the character of the tissue reaction is most probably determined in the first place by the stage of development of the xenoma. There are striking differences in the tissue reaction to an early, thin-walled xenoma, to a full grown xenoma still with proliferating developmental stages but a small central mass of spores, and to a "mature" xenoma packed full with spores; this is the stage when the full vigour of the host reaction sets in.

Along with the deployment of the proliferative inflammation it is the change in the structure of the xenoma wall which makes itself first noticeable. The changes in xenoma wall — oedematous swelling, pervasion by host's tissue elements — were aptly described as early as in 1920 by Débaisieux. At present, it is difficult to decide if it is the advance of the proliferative inflammation itself which initiates the xenoma wall changes or whether — a more plausible explanation — the onset of the inflammation is provoked by light-microscopically undetectable changes of the xenoma contents and its wall. A signal triggering the inflammation may be given on a metabolic or biochemical level.

The sole clue to explanation of differences in the intensity of inflammatory changes will be to follow in detail the influence of time factor and of the age classes of the host in experimental infections and further, to compare the reactions in tissues differing in the moiety of connective tissue they contain, e.g., parenchymatous organs vs. the intestine.

G. plecoglossi, in addition to its economic importance, is an ideal model organism for studies of this type of host-parasite relationship. The existing papers dealing with this species (Takahashi and Egusa 1976, 1977a, b) left this problem untouched.

ТКАНЕВАЯ РЕАКЦИЯ РЫБЫ *PLECOGLOSSUS ALTIVELIS* ПРИ ЗАРАЖЕНИИ МИКРОСПОРИДИЕЙ *GLUGEA PLECOGLOSSI*

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Резюме. Микроспоридия *Glugea plecoglossi* вызывает у естественного хозяина *Plecoglossus altivelis* гипертрофический рост зараженных клеток, который заключается образованием больших ксеномов. У молодых тонкостенных ксеномов ответной тканевой реакции хозяина не наблюдается. Впоследствии, ксеномы в содержимом которых преобладают уже зрелые споры, вызывают пролиферативное воспаление с образованием грануломов. Гранулярная ткань прорастает в массу спор, которые постепенно ликвидированы фагоцитозом. Вследствие того происходит инволюция грануломов. При значительной интенсивности инвазии невозможно предполагать осуществление полной функциональной репара-

ции. Ответная тканевая реакция хозяина в основном соответствует тканевой реакции, обнаруженной после заражения колюшек видом *G. anomala* и реакциям после заражения другими видами рода *Glugea*, образующими большие ксеномы.

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N. V. Tupikova, L. V. Komarova: Principy i metody zoologicheskogo kartografirovaniy (Principles and methods of zoological cartography). *Publ. House Moskovsk. Univers., Moskva 1979, 189 pp., 29 Figs., 14 Tables. Price 0.60 R.*

The drawing of maps, on which the occurrence and distribution of particular animal species and groups are plotted, constitutes an integral part of certain type of zoological research. The present publication is a methodological manual encompassing the problems encountered in the cartography of zoological objects. It is divided into five chapters entitled as follows: Common problems of animal cartography; Preparatory work, collecting, registration and processing of material, formulation of legend; Classification of zoological objects and phenomena; Drawing of map; Methods of marking zoological objects and phenomena on a map. The titles show the range of problems discussed in relevant parts of the text. On the basis of abundant factographic material from the territory of the Soviet Union and using numerous examples the reader will be informed about the entire process of preparation and the mapping of zoological objects.

Of special interest to the parasitologist are chapters about the mapping of the occurrence of ticks and different mammals — reservoirs of infection. The chapters are thus concerning the problems solved within the framework of studies on natural focality of diseases. It is a valuable and topical publication and its authors are to be warmly congratulated on providing a practical guide to this field of research.

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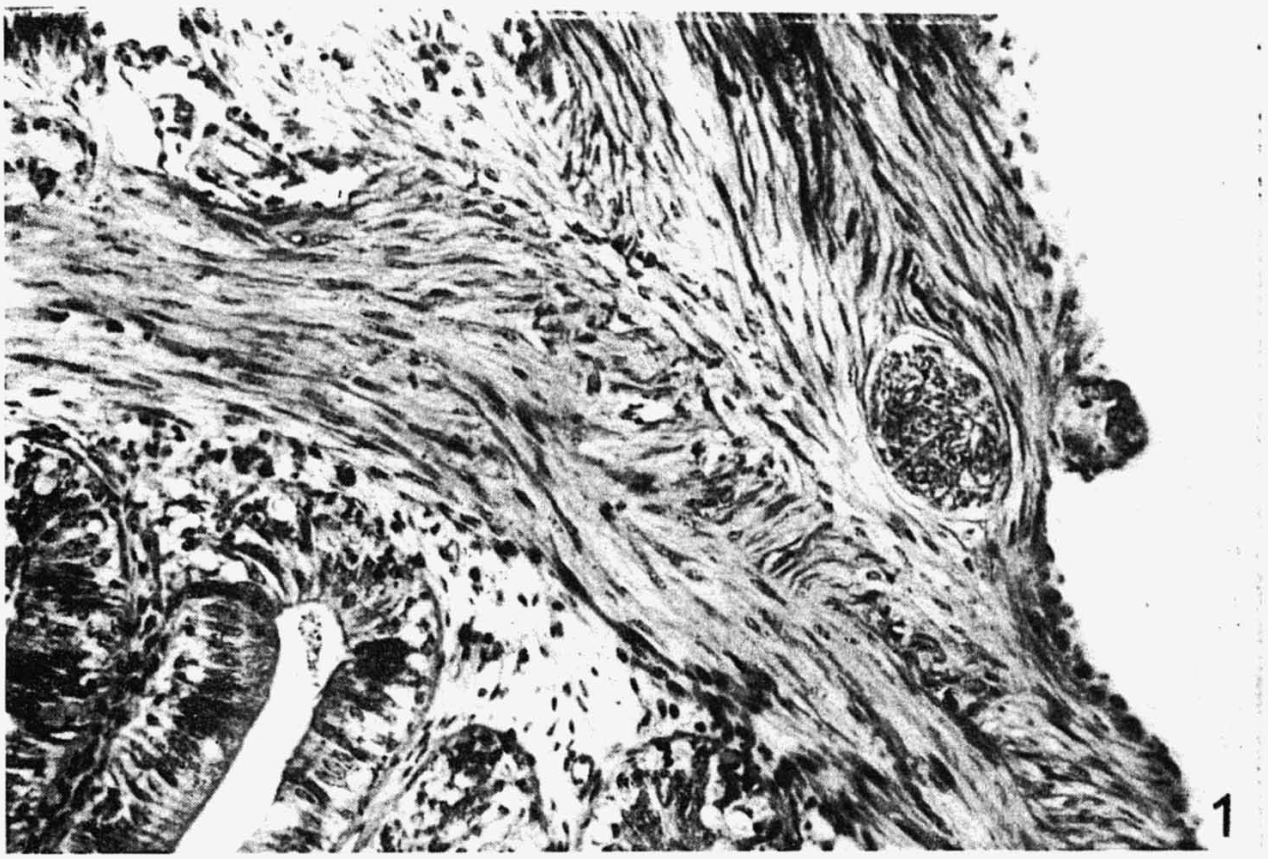


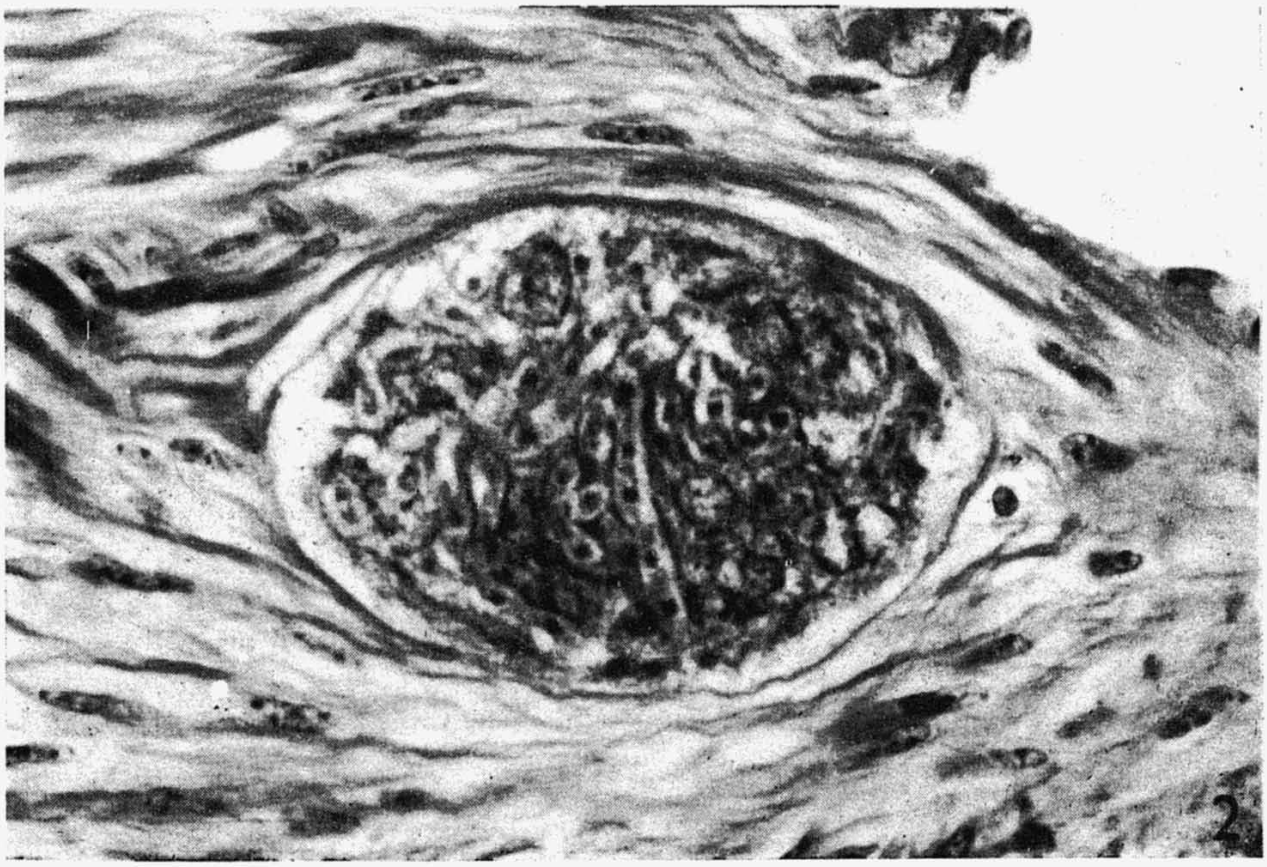


Fig. 1. Xenoma in lamina muscularis of the intestine. HE (× 330.)

Fig. 2. A higher magnification of Fig. 1. (× 1200.)

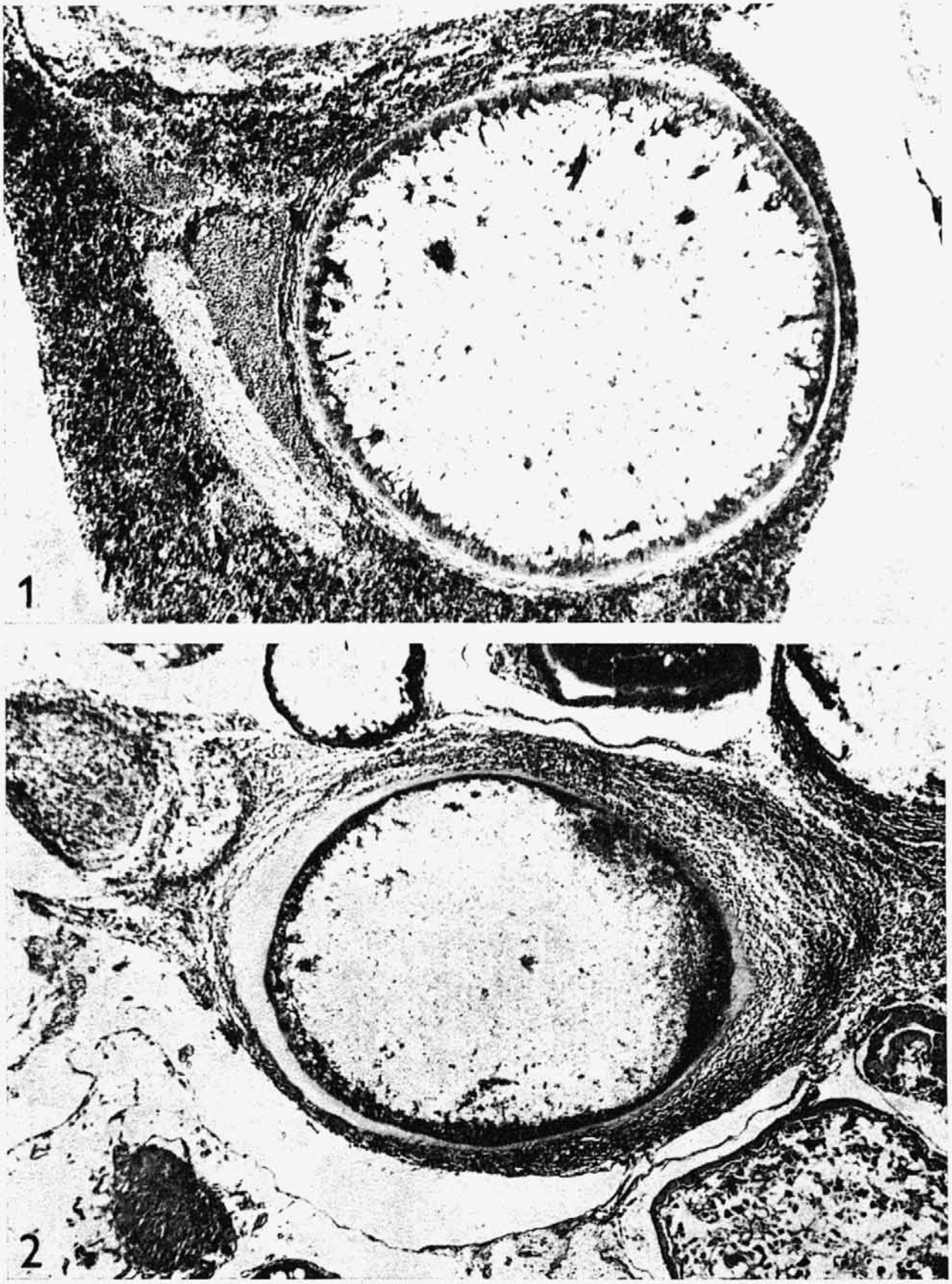
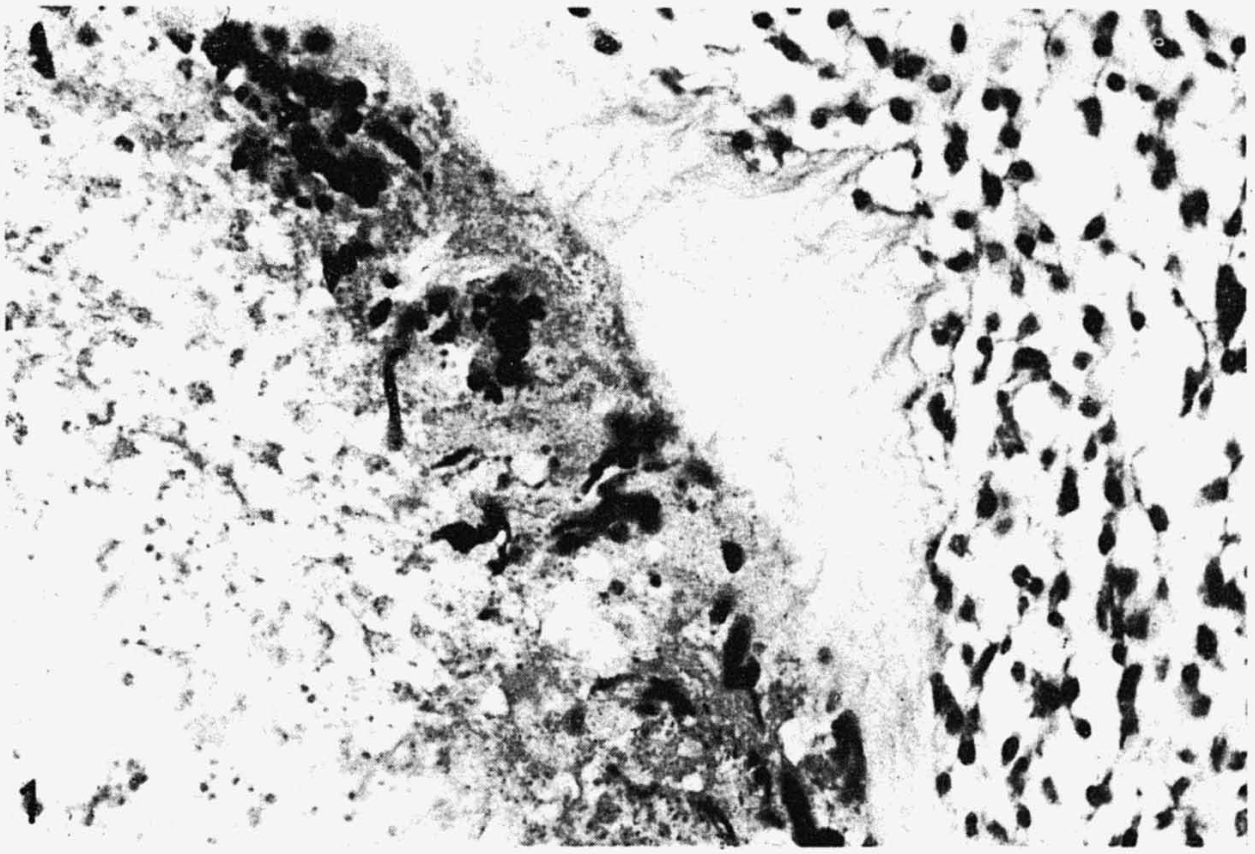


Fig. 1. Xenoma in the spleen, surrounded with only a thin layer of connective tissue. Cytoplasmic layer with developing stages still present. HE (× 80).

Fig. 2. Xenoma encased with a wide zone of granulation tissue. Ovary. HE (× 80).



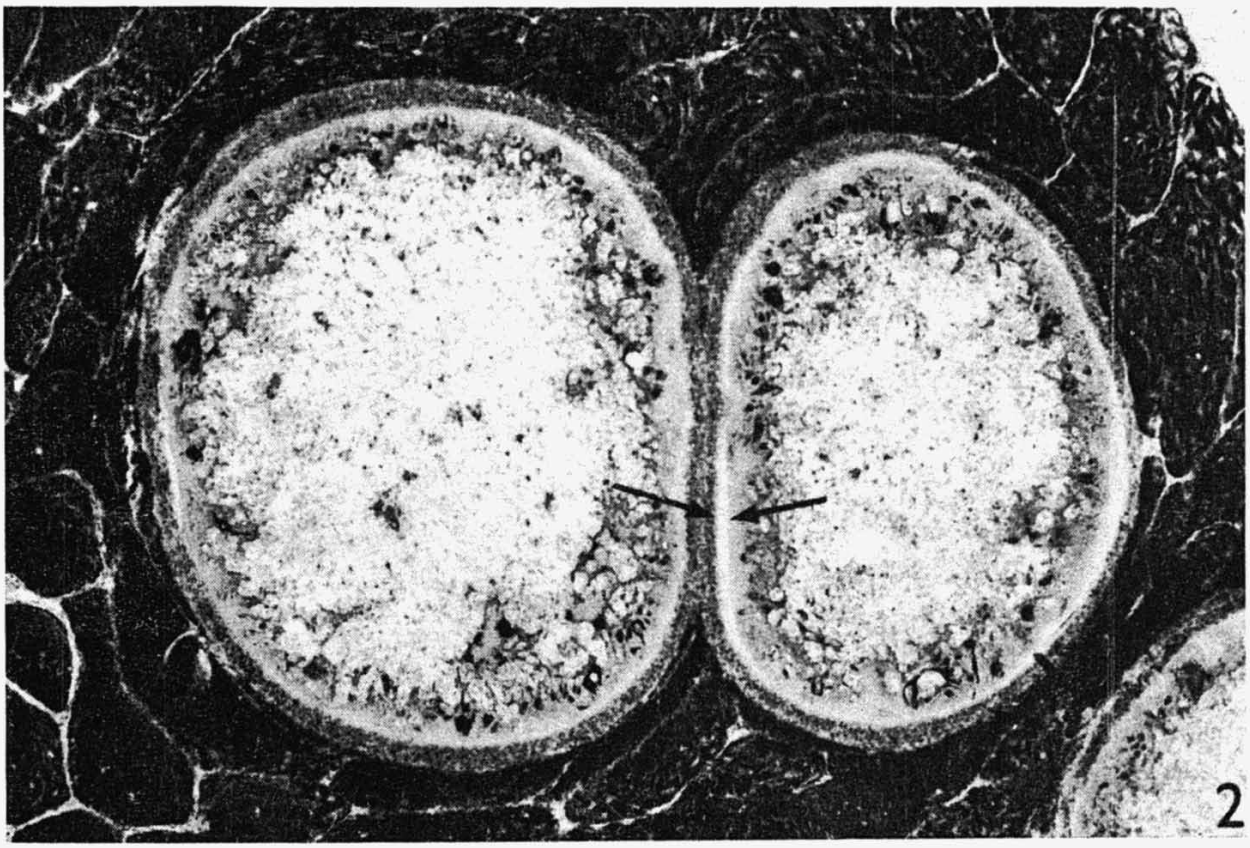
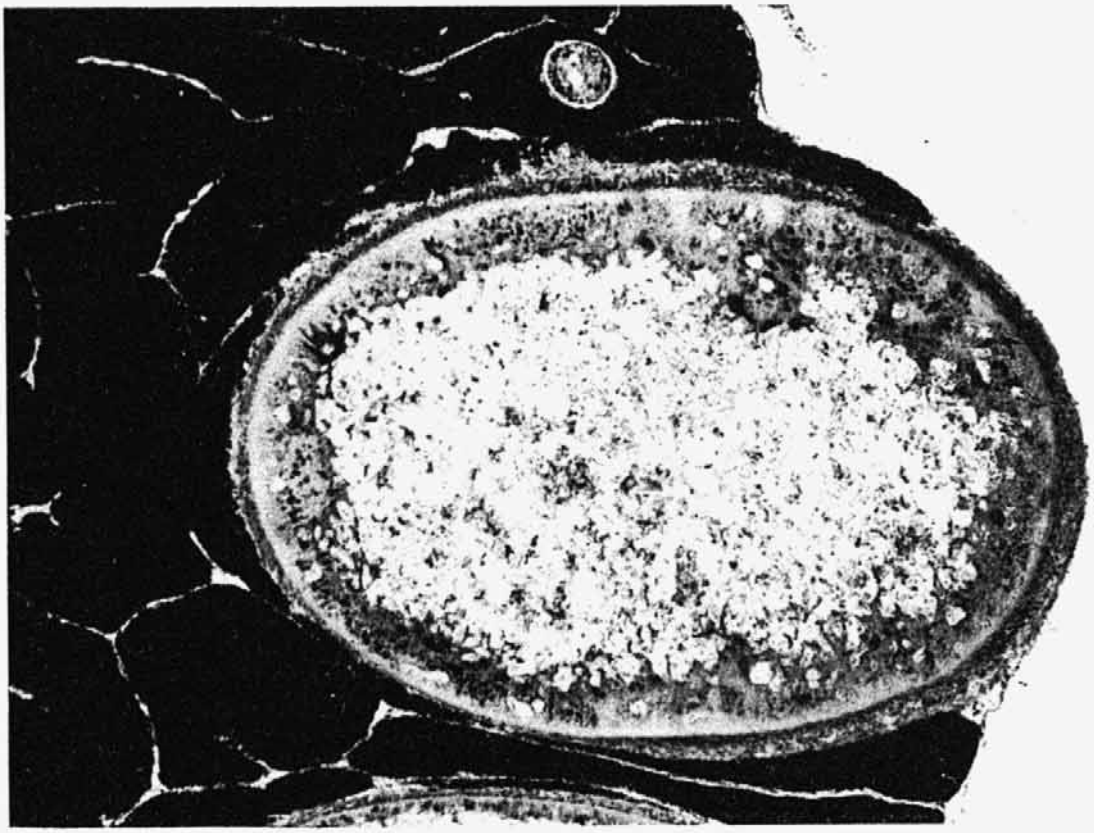


Fig. 1. Oedematous changes in the membrane of a xenoma encased with a wide zone of granulation tissue. HE (× 1200).

Fig. 2. Two xenomas in testes; arrow points at the xenoma wall. HE (× 70).



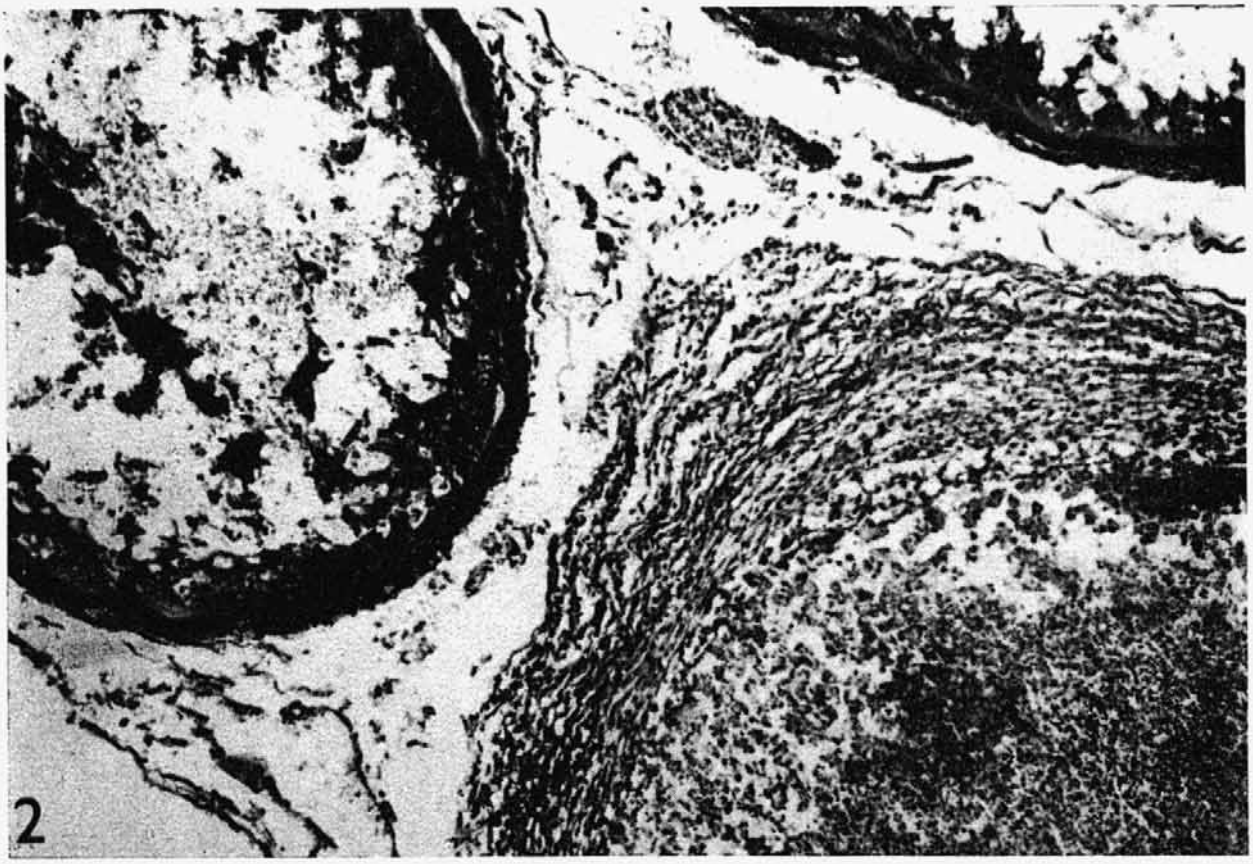


Fig. 1. Two xenomas of different size in testes; there is no host reaction to the small one. HE (× 85).
Fig. 2. Granuloma with a central mass of spores (bottom right) facing two developing xenomas. Ovary. HE (× 85).