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ON THE *PLISTOPHORA* INFECTION IN EEL

I. Histopathology, Ultrastructure, and Development of *Plistophora anguillarum* in Eel, *Anguilla japonica*

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Wen-Huei T'sui and Chung-Hsiung Wang (1988) On the *Plistophora* infection in eel: I. Histopathology, Ultrastructure, and Development of *Plistophora anguillarum* in eel, *Anguilla japonica*. *Bull. Inst. Zool., Academia Sinica* **27**(3): 159-166. The Microspora, *Plistophora anguillarum* was known to be the causative agent of "beko" disease of Japanese eel, *Anguilla japonica*. This protozoa parasitized mainly in the skeletal muscle of the eel. The infection of *P. anguillarum* caused the necrosis of the muscle resulting the various degree of the body curvature. Mainly, harm done to the eel was by mechanical damage in early infections.

In addition to the histopathology, the ultrastructure and the development of *P. anguillarum* were investigated with the light and the electron microscopes. Several distinct developmental stages of *P. anguillarum* observed in the infected muscle were also described in present study.

Key words: Plistophora, Eel, Histopathology, Ultrastructure, Development.

The Microspora, *Plistophora anguillarum*, was first described by Hoshina in 1951, as the causative agent of "beko" disease of Japanese eel, *Anguilla japonica*. Subsequently, several investigators reported their findings in this disease which include histological changes of infected fish; electron microscopic observations of the spore; condition of extrusion of the polar filament of the spore, fluorescent antibody diagnosis for the infection in eels, as well as the experimental induction of this microsporidiosis in eel (Akada, *et al.*, 1977; Hashimoto, *et al.*, 1976; Hashimoto & Takinami, 1976; Hoshina, 1951; Kano & Fukui, 1982; Kou, *et al.*, 1977; Lin & Hsiao, 1977). All of these studies were attempted to understand this well known harmful disease in eel culture, in order to find some methods for the control and prophylexis. With the same purpose, a series experiments concerning this protozoan disease are carried out in our laboratory. The histopathology of "beko" disease, the ultrastructure and the development of *P. anguillarum* observed with light and electron microscopies will be described in the present report as the first part of these series researches.

MATERIALS AND METHODS

The infected eel, *Anguilla japonica*, was imobilized by ice, then dissected. The infected muscle was isolated and treated with different methods for the following purposes.

(1) Histopathology

The infected muscles were fixed in Zenker's fixative for routine paraffin sections. The sections, 3 um thick, were stained with hematoxylin-eosin; Mallory triple stain;

Masson trichrome stain; Feulgen stain; and PAS stain, then observed with the light microscope.

(2) Transmission electron microscopy

The infected muscles were fixed initially in 2% glutaraldehyde in phosphate buffer, then post-fixed in 1% osmic acid. After several steps of dehydration, the muscles were then embedded in Spurr epon. The ultrathin sections were stained by uranyl acetate and lead hydroxide. Electron micrographs were taken with JEM 100S.

(3) Scanning electron microscopy

The infected muscles were fixed as the method of (2). After dehydration in alcoholic series, the muscles were dried in critical point apparatus. The dried muscles were coated in vacuum with carbon and gold. Micrographs were taken with JSM 15.

OBSERVATIONS

I. External feature

The *Plistophora anguillarum* attacks mainly the muscular tissue of eel. The necrosis of the muscle results the various degree of the body curvature (Fig. 1). The muscle is very soft and looks pale in the depressed sites. The border of myotomes here also become unclear (Fig. 2). These characteristics make "beko" disease easy to be distinguished from those body curvature diseases resulted from the abnormality of skeletal system, in which the color and hardness of the muscle look the same throughout the body.

II. Histopathology

There are many nodules, 20—75×17—57 μm in size, found in the muscle fibers parasitized with *P. anguillarum*, viewed with scanning electron microscope (Fig. 6).

The histological observations reveal that these nodules are actually the cysts of *P. anguillarum* (Fig. 3a-d). The infected muscle

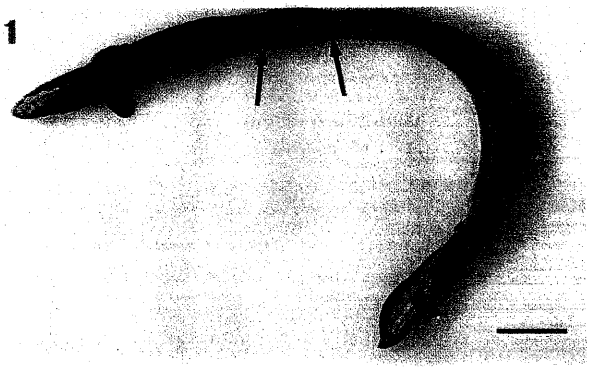

Fig. 1. The eel infected with *Plistophora anguillarum*. Arrows indicate the depressed sites of the trunk. Bar: 4 cm.

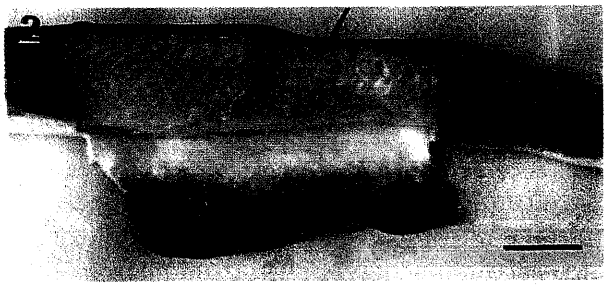

Fig. 2. A *Plistophora* infected eel with skin partially removed. The arrow indicates the pale depressed site of trunk with unclear myotome border. Bar: 2 cm.

TABLE 1
The staining properties of normal striated muscle (NM) and the infected muscle (IM) of *Anguilla japonica* with microsporiliosis

		H-E	Mallory	Masson	PAS
Muscle bundle	NM	pink	red	light pink	light pink
	IM	pink to red	red to pink	light green to bluish	pink to deep red

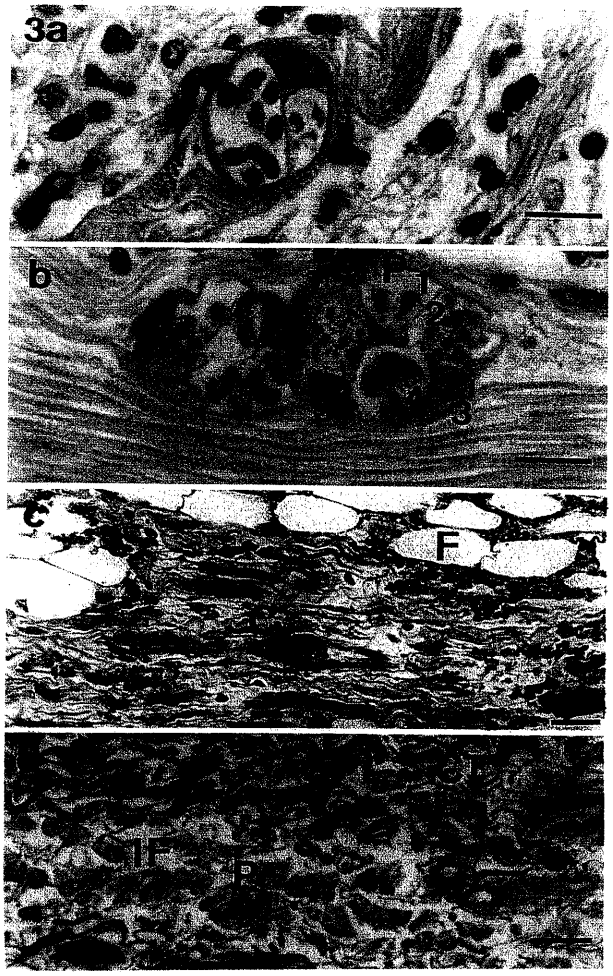

Fig. 3. Micrographs of *Plistophora anguillarum* infected tissues of the eel. a, A cyst in the muscle bundle. Masson trichrome stain. Bar: 10 μm. b, Pansporoblast (1-8) in cysts. Masson trichrome stain. Bar: 10 μm. c, The muscle tissue with some vacuoles resulted from fatty infiltration. Hematoxylin-eosin stain. Bar: 40 μm. d, Infiltration of connective tissue in the area with destructive or dissolved muscle. Mallory triple stain. Bar: 20 μm. CT: connective tissue; Cy: cyst; F: fatty tissue; IF: infective foci; M: muscle; P: pansporoblast

shows some extent of dilation due to inflammatory edema (Fig. 5a, b). The staining properties of infected muscle are changed, which was summerized in Table 1. The most obvious difference between infected and normal muscle is that the infected muscle shows much stronger PAS-positive reaction than that of normal one. Zenker's degeneration of muscle fiber, inflammatory cell infiltration and fatty infiltration are readily seen in the infected area (Fig. 3c, d). In the advanced infection, the muscle fibers are almost disappeared. Instead, the connective fibers proliferate and fill in these sites leading to the formation of infective foci (Fig. 3d).

The ultrastructural changes of the infected muscle include the separation of sarcolemma and muscle fibrls due to the edema, formation of vacuoles in sarcoplasma, destruction of membrane-bound organelles and dissolution of myofibrils (Fig. 5a, b).

III. Development of *P. anguillarum* in muscle of eel

Four distinct development stages of *P. anguillarum* can be observed in muscular tissue, 1. the uninucleated young schizont; 2. the multinucleated schizont; 3. sporont; 4. pansporoblast.

The mononucleated schizont, 10-14$\times$ 4-6.5 μm in size, has a small nucleus, 1-2.5 μm in size, surrounded by cytoplasm with basophilic granules (Fig. 4a). It is highly possible that these schizonts come from autoinfection, by hatching of spores within the hosts in which they are produced. The young schizonts migrate with ameboid movement to the final site of infection, muscle. After getting into the muscle cell the schizont develops between the myofibrils. The schizont, undergoes a process of multiple fission, form multinucleated schizont.

The karyokinesis is followed by cytokinesis to produce 2-8 uninucleated sporonts (Fig. 4b, c). Till this stage, the schizont remains unchanged in size and bounded by a thin plasmalemma which endows the

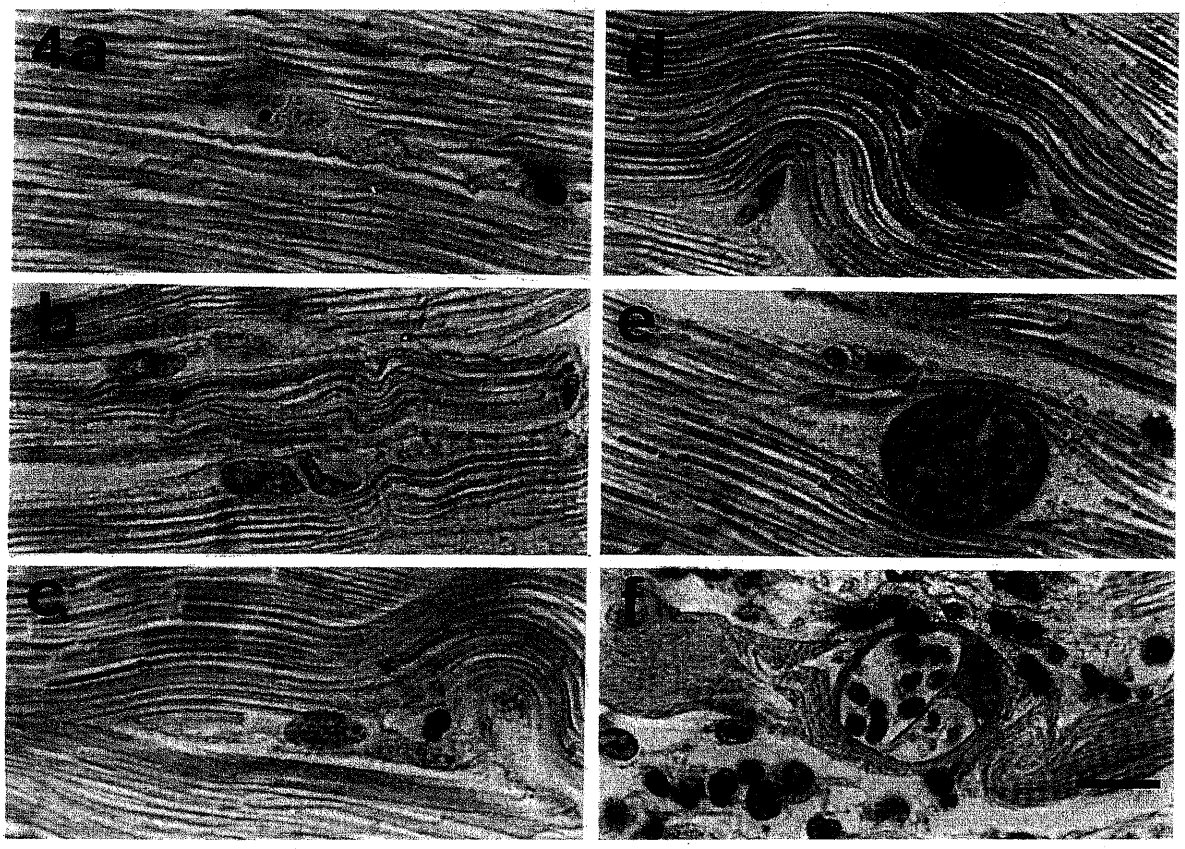

Fig. 4. Developmental stages of *Plistophora anguillarum* in the infected muscle of eel showing (haematoxylin-eosin stain). a, mononucleated schizont. b, c, 3-8 mononucleated sporonts producted by schizont with karyokinesis followed by cytokinesis. d, the formation of the cyst membrane around multinucleated plasmodia. e, cytokinesis of multinucleatedplasmodia. f, pansporoblasts in cyst. Bar: 10 μm.

schizont the ability of free movement. However, changes occur in the surface structure and cytoplasmic organization of the sporont in the following development stages. The characteristics of the surface membrane are changed by addition of some PAS positive materials external to the plasmalemma to form a cyst membrane (Fig. 4d). Inside the cyst, the sporonts undergo sporogony. Each sporont give rise a multinucleated plasmodium with basophilic cytoplasm surrounded by plasmalemma (Fig. 4d). At the later stage, the multinucleated plasmodium subsequently splits through cytokinesis (Fig. 4e, 7). During this period, the additional PAS-positive layers are added to the outside of the plasmalemma of the sporont to form a pansporoblast. Simultaneously, the uninucleated daughters of sporont develop into sporoblasts and then spores (Fig. 4f, 8). At this stage, the parasite becomes a cyst with 4-8 pansporoblast (Fig. 4f, 9). The staining properties of the cyst is summerized in Table 2.

The mature spore is oval-shaped with smooth surface (Fig. 10). At the anterior

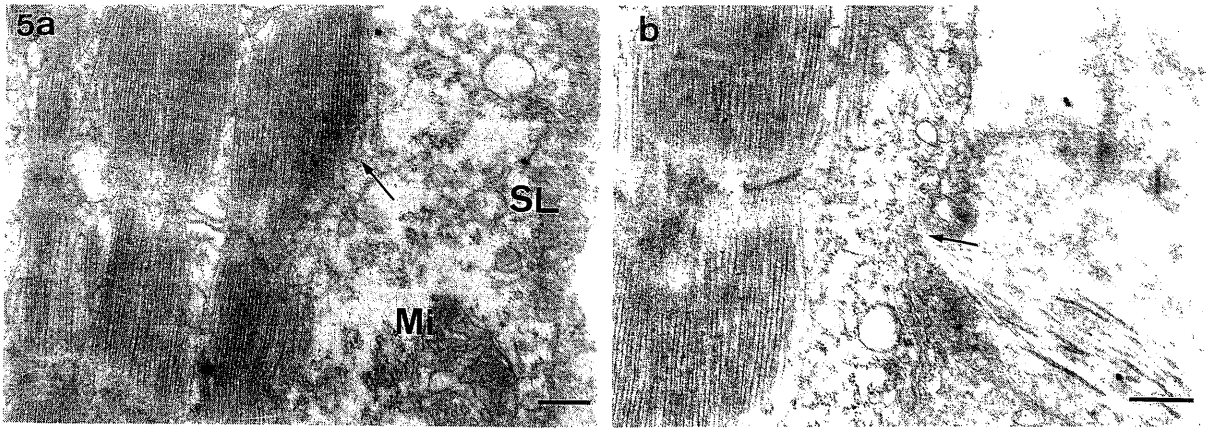

Fig. 5. Electron micrographs of eel muscle tissue infected with *Plistophora anguillarum* showing. a, The fine structure of the disruption of muscle (arrow), abnormality of mitochondria and edema between sarcolemma and myofibrils. b, Completely disruption myofibrils (arrow). Bar: 0.5 μm; Mi: mitochondria; SL: sarcolemma.

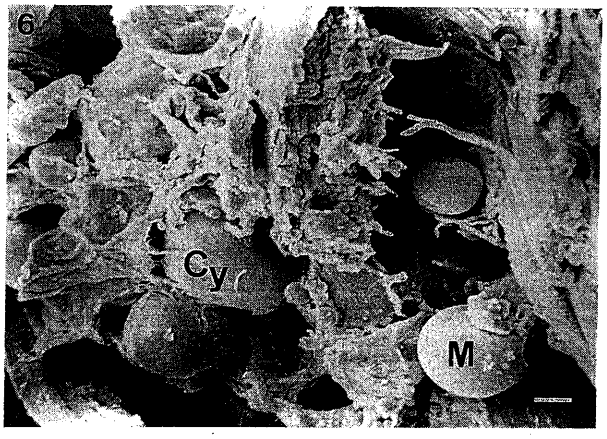

Fig. 6. The scanning electron micrograph of the muscle fibers parasitized with *Plistophora anguillarum*. Bar: 20 μm; Cy: cyst; M: muscle.

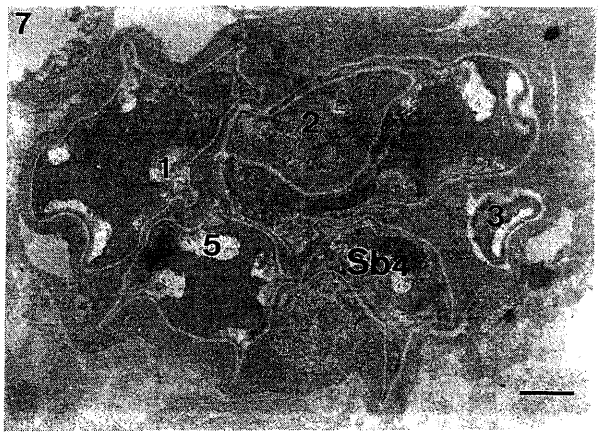

Fig. 7. Electron micrograph of five sporoblasts (1-5) formed by multinucleated plasmodium through cytokinesis. Bar: 1 μm; Sb: sporoblast.

tip, a polar aperture can be observed. The moderate electron dense polarplast, possibly formed by Golgi apparatus, locates at the anterior pole of the spore and contains laminar structure (Fig. 11). There are about 42 polar filament coils within the mature spore. The extreme anterior end of polar filament beneath the polar aperture appears to be anchor-shaped in section. Observations of serial sections show that this anchor-shaped structure is acturally a mushroom-like form. This mushroom-like structure is called polar cap which consisted of two parts, polar sac and polar cork. The remainder of the intrasporal space is occupied by the sporoplasm with sporonucleus.

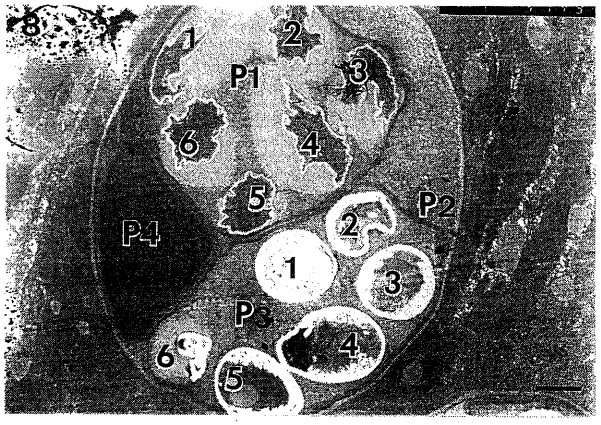

Fig. 8. Electron micrograph of a cyst of *Plistophora anguillarum* showing four pansporoblasts (P1-P4) with 6 sporoblasts in P1 and 6 spores in P3. Bar: 3 μm; M: muscle; P: pansporoblast; Sb: sporoblast.

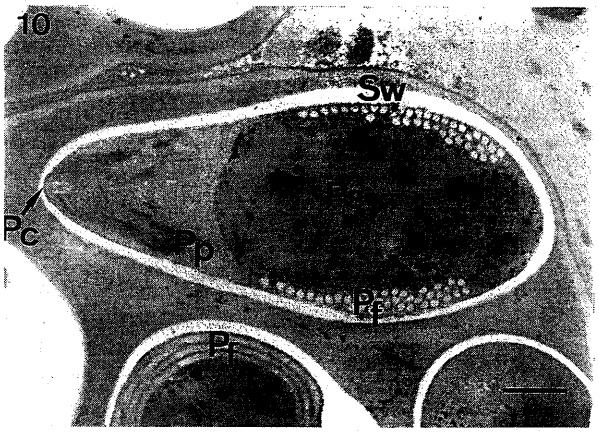

Fig. 10. Electron micrograph of mature spores showing the ultrastructure of the spore. Bar: 1 μm; Pc: polar cap; Pf: polar filament; Pp: polaroplast; Ps: postersome; Sw: spore wall.

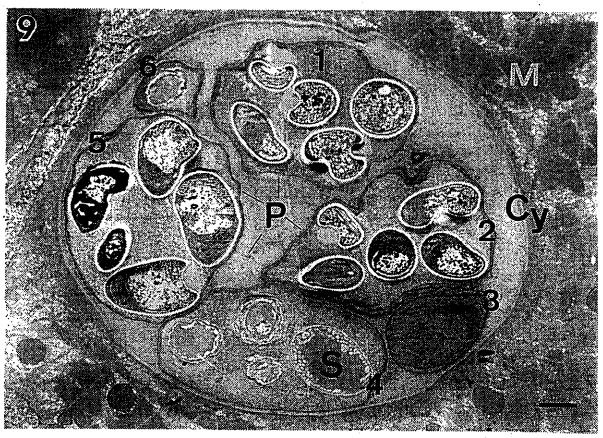

Fig. 9. Electron micrograph of a well-developed cyst of *Plistophora anguillarum* showing spores in pansporoblasts (1-6). Bar: 3 μm; Cy: cyst; M: muscle; P: pansporoblast; S: spore.

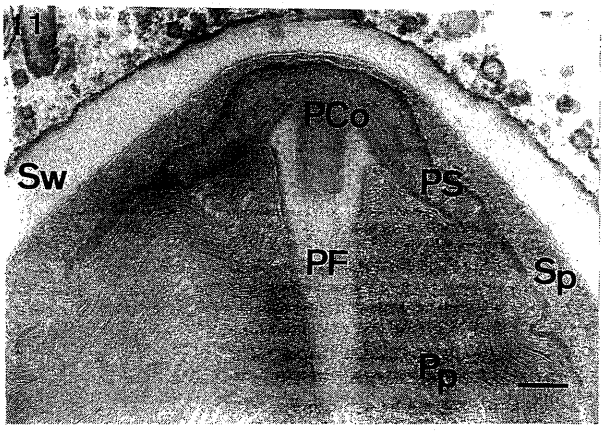

Fig. 11. Electron micrograph of *Plistophora anguillarum* showing the anterior structure of mature spore. Bar: 0.1 μm; PCo: polar cork; PF: polar filament; Pp: polaroplast; PS: polar sac; Sp: sporoplasm; Sw: spore wall.

TABLE 2
The staining properties of *Plistophora anguillarum*

	H-E	Mallory	Masson	PAS
cyst membrane	pink	red	light pink	red
pansporoblast membrane	pink	red	light pink	red
sporoblast:				
cytoplasm	purple	light brown	deep pink to red	deep red
nucleus	blue	orange	blue	—*
spore:				
polar cap	—	—	blue	deep red
polar body	pink	red	red	—
polar filament	pink	red	—	red
posterosome	purple	—	—	pink
nucleus	blue	—	blue	—

*: chromophobic

DISCUSSION

The development of *Plistophora anguillarum* follows the courses of pansporoblastic development in which the sporont devides into sporoblasts within a persisting surface membrane. Canning and Hazard (1982) recommended that the terms, pansporoblast and pansporoblast membrane, be restricted for use in the Myxospora and the term sporophorous vesicle be used for the spore-containing sacs found in the Microspora because it is now accepted that there is no relationship between Microspora and Myxospora. But we think it is better not to change a term that has adopted for a long time. Also it is not surprising that scientists use the same term to describe two analogous structures, for example the "flagellum" is used for both prokaryotic and eukaryotic cells.

The infection of *P. anguillarum* causes the necrosis of the muscle resulting the various degree of the body curvature. Mainly, harm done to the eel is by mechanical damage in early infections and chemical damage in late infections. Chemical damage might involve the release of toxic substances from the ruptured cyst. The muscle is eventually destroyed, apparently by a chemical histolytic action on the muscle. In late infections, the infiltration of a large number of these protozoan parasites into the proliferating connective tissue leads to the formation of infective foci.

There are few papers that describe the ultrastructure of *P. anguillarum*. However Hashimoto and Takinami (1976) had studied the ultrastructure of the spore of *P. anguillarum* but they did not describe in detail about the anterior end of the spore. As indicated by Lom (1972), the exact knowledge of the mechanism of spore hatching of microspora is particular important for better understanding of the invasion process of these protozoan parasites. In this respect the structure and function of the polar filament are of prime importance. Weidner (1972), by studying the spore of *Nosema bombysis* of silkworm, suggested that the glycoprotein matrix of the polar sac acted as permeability barrier and, once broken, instantaneous swelling of the polaroplast membranes caused the filament to evert through the polar sac. The present study indicated that there are polar sac and polar cork located at the extreme anterior end of polar filament. With the morphological evidences, it is proposed that the extrusion of polar filament of *P. anguillarum* may result from the change of permeability of

polar sac and polar cork and expansion of polaroplast. After extrusion of polar filament, the sporoplasm is discharged through the hollow, evaginating polar filament and injected into eel tissue to complete the infection.

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鰻 魚 凹 凸 病 之 研 究

I. 日本鰻凹凸病原蟲之組織病理、超微構造及發育

崔 文 慧　　王 重 雄

鰻魚凹凸病是由鰻魚微孢子蟲 *Plistophora anguillarum*，寄生於魚體的骨骼肌所引起，被感染的肌肉因肌肉壞死糜爛，使魚體表面呈不同程度的凹陷，肌肉感染初期受微孢子蟲胞囊的機械性損傷。

本研究利用光學和電子顯微鏡術，研究罹患凹凸病鰻魚 (*Anguilla japonica*) 之組織病理和病原蟲—鰻魚微孢子蟲 (*P. anguillarum*) 的微細構造。除此之外，於肌肉組織內可見到的幾種不同發育時期的微孢子蟲，亦將在本文中詳細描述。