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Descriptive Pathology of Controlled Infections of the Ciliate Ectoparasite *Ichthyophthirius multifiliis* Fouquet, 1876 in *Oreochromis mossambicus* (Peters) Fry

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Abstract

Thirty-day-old *Oreochromis mossambicus* (Peters) fry were subjected to sublethal infections of 200-300 tomites per fish of *Ichthyophthirius multifiliis* Fouquet, 1876 under controlled conditions and the sequential pathology was studied. Hyperplasia, epithelial cell hypertrophy and mild degenerative changes only were observed. No sloughing of dermal epithelium was evident, nor was spongiosis, oedema, nor underlying muscular hypertrophy. It was concluded that *O. mossambicus* needs a greater number of parasites to produce the histopathological changes described by previous workers.

Introduction

Investigations into the pathology of *Ichthyophthirius multifiliis* Fouquet, 1876 infections in cultured fishes have recently gained considerable attention. Since the critical study of the sequential pathology in common carp (*Cyprinus carpio* L.) carried out by Hines and Spira (1974), McLay (1985) has investigated the ultrastructural pathology in experimentally infected *Salmo gairdneri* Richardson and

Ventura and Paperna (1985) studied the histopathology in a wide range of host fishes, both naturally and experimentally infected, which they obtained from diverse geographical regions. Apart from a few naturally infected fingerlings of Mossambique tilapia (*Oreochromis mossambicus* (Peters)) which they examined, there nave been no other reports on the pathology of controlled infections of *I. multifiliis* in this important cultured species. The fry of this species are highly susceptible to *I. multifiliis* infections (Subasinghe and Sommerville 1986). The present study was designed to describe the histopathology of *I. multifiliis* infections in fry of *O. mossambicus*.

Materials and Methods

Thirty-day-old fry of genetically pure Mossambique tilapia (as identified by McAndrew and Mujumdar 1983) were used in this investigation. They were kept in well aerated glass aquaria at a temperature of 27° ± 1°C and fed twice a day with ground commercial trout pellets (Ewos Baker Ltd., Bathgate, Scotland) at a particle size of 500-1,000 µm.

The *Ichthyophthirius* isolate denoted RS/CM/84 (isolated from a male firemouth cichlid, *Cichlasoma meeki* (Gunther) in March 1984, and kept at 27° ± 1°C) was used for this study. Only tomites of age 6-12 hours were used for the infection trials. An estimated dose level of 200-300 tomites per fish was used in the infection trials. The method developed by Dickerson et al. (1981) for standardized quantitative infections of *I. multifiliis* was adopted. The method used for the preparation of tomites for infection is given in Subasinghe and Sommerville (1986).

Duplicate infection trials were carried out in 1-l glass beakers covered with black polyethylene. The number of fish used in each beaker was 20. The exposure time was standardized to 3 hours. After exposure each group of fish was placed in a separate 12-1 perspex aquarium containing 8 l of aerated water. Aquaria were individually aerated and a controlled temperature of 27° ± 1°C was maintained. Fish were observed daily for a period of 15 days for survival. At days 4, 8 and 12 of the course of experiment, half of the water from each aquarium was changed with aerated water. An identical uninfected group of 20 fish were also observed as a control. All fish were fed as previously outlined.

A random sample of four fish was removed after 12 hours, 1, 2, 4, 6, 8 and 12 days post-exposure. A similar sampling procedure was adopted for the uninfected controls. Fish were killed by severing the spinal cord and fixed in 10% phosphate buffered formalin followed by routine processing to paraffin wax. Histological sections were cut at a thickness of 10 μm and stained with hematoxylin and eosin.

Results

Clinical and Gross Changes

All fish exposed to a dose level of 200-300 tomites/fish in this experiment were found to be visibly infected by day 4 post-exposure. The fish were never observed to be severely stressed, i.e., the extreme flashing behavior, lethargy and avoidance of feeding, as typically shown by heavily infected fish, were absent. Feeding and swimming behavior was normal and was similar to that of the controls.

By the end of day 4 and the beginning of day 5 post-exposure, the number of visible white spots (trophonts) on the fry markedly decreased. During this period, and the following 24-hour period, the fish became comparatively less active than the controls.

Dermal Histopathology

The thickness of the epidermis of the control fish was variable, depending on the area of the body. In the dorsal and ventral regions of the body, including the head, the thickness varied from six to ten cells, whereas in the lateral regions it was less than six cells thick; sometimes only two to three layers of cells were observed. Goblet-shaped mucous cells were evident within the upper layers of the epidermis. The density of the mucous cells was visibly higher in the buccal epithelium.

The tightly arranged epidermis lay above the thin basement membrane. The dermis, comprised of the stratum spongiosum and stratum compactum, was usually three to four times thicker than the epidermis (Fig. 1).

After 12 hours of exposure, the parasites were located only a few cells below the surface of the thicker layers of epidermis (Fig. 2). In the areas where the epidermis was thin, the parasites were present

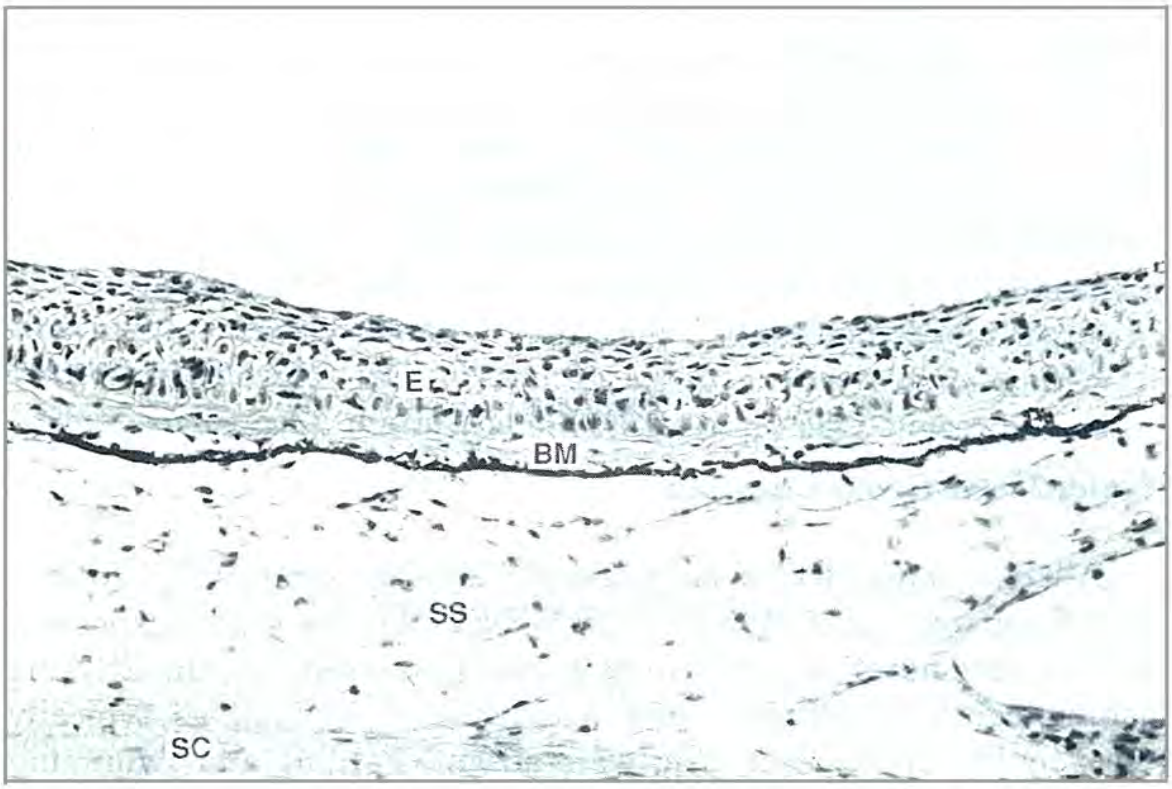

Fig. 1. Photomicrograph of a histological section of epidermis of an uninfected fish. 250X magnification; hematoxylin and eosin.
E : Epidermis
BM: Basement membrane
SS : Stratum spongiosum
SC : Stratum compactum

close to the basement membrane. In some specimens where invasion by large numbers of tomites was evident, the superficial epithelium was abraded. No host tissue response was evident around the perimeters of the parasites nor was there any sign of a path of entry into the inner epidermal layers.

By 24-hour post-exposure, parasites were most frequently located on the basement membrane, surrounded by an empty space. In these cases the ciliature of the parasites was clearly visible. No sign of any inflammatory response or host tissue reaction was evident.

After 48 hours of exposure, individual fish showed varying degrees of response to infection. Mild hyperplasia was evident, but only in some samples. Generally, the overlying epidermal cells directly above the growing trophonts were somewhat compressed and flattened. No sign of extensive tissue changes was evident.

By day 4 post-infection the growing parasites were found to protrude out into the overlying epithelium, resulting in a nodular-like projection at the site of the parasite. As a result, displacement of

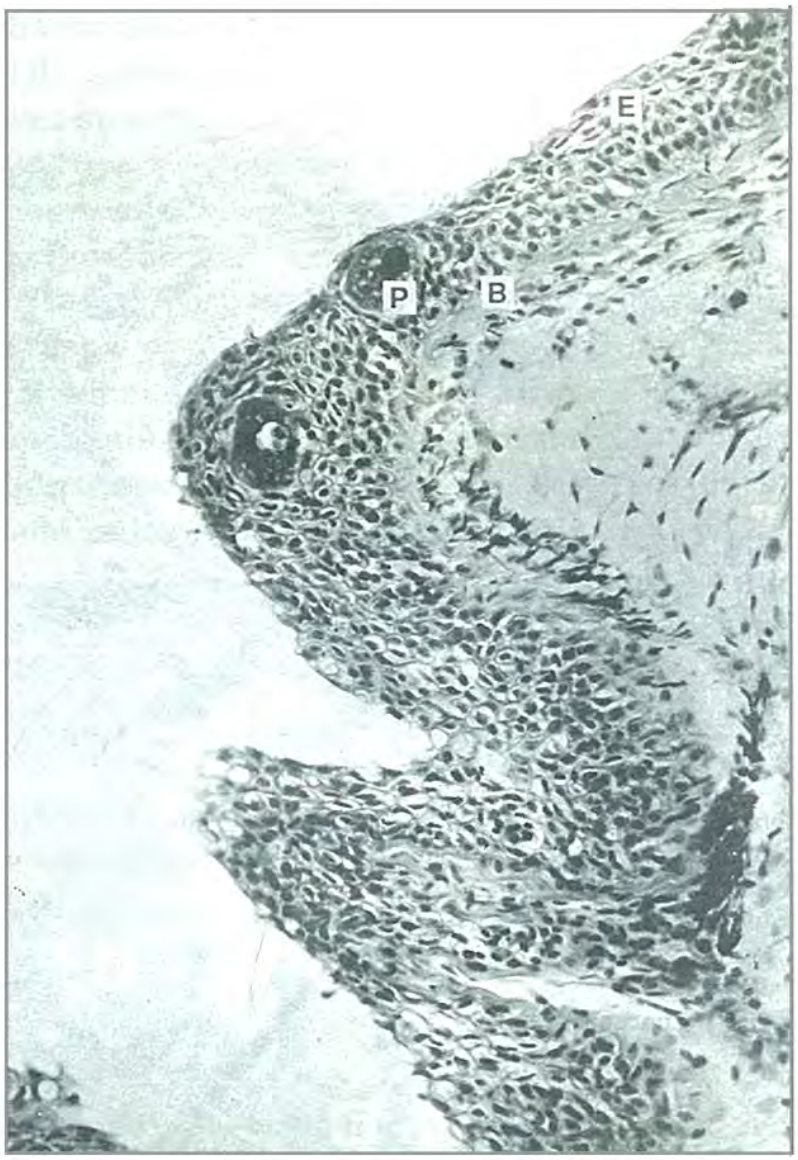

Fig. 2. Photomicrograph of a histological section of epidermis of a fish at 12-hour post-exposure to *I. multifiliis*. 250X magnification; hematoxylin and eosin.
E : Epidermis
P : Parasite
B : Basement membrane

overlying epidermal layers was frequently visible. Moderate hyperplasia of the epithelium was also evident near the perimeter of the parasites.

The expansion of the growing trophonts into the epithelial layers resulted in a flattening and stretching of the overlying epithelial cells to accommodate the parasites. However, in areas where the thickness of the epithelium was high this flattening and stretching was not very obvious (Fig. 3).

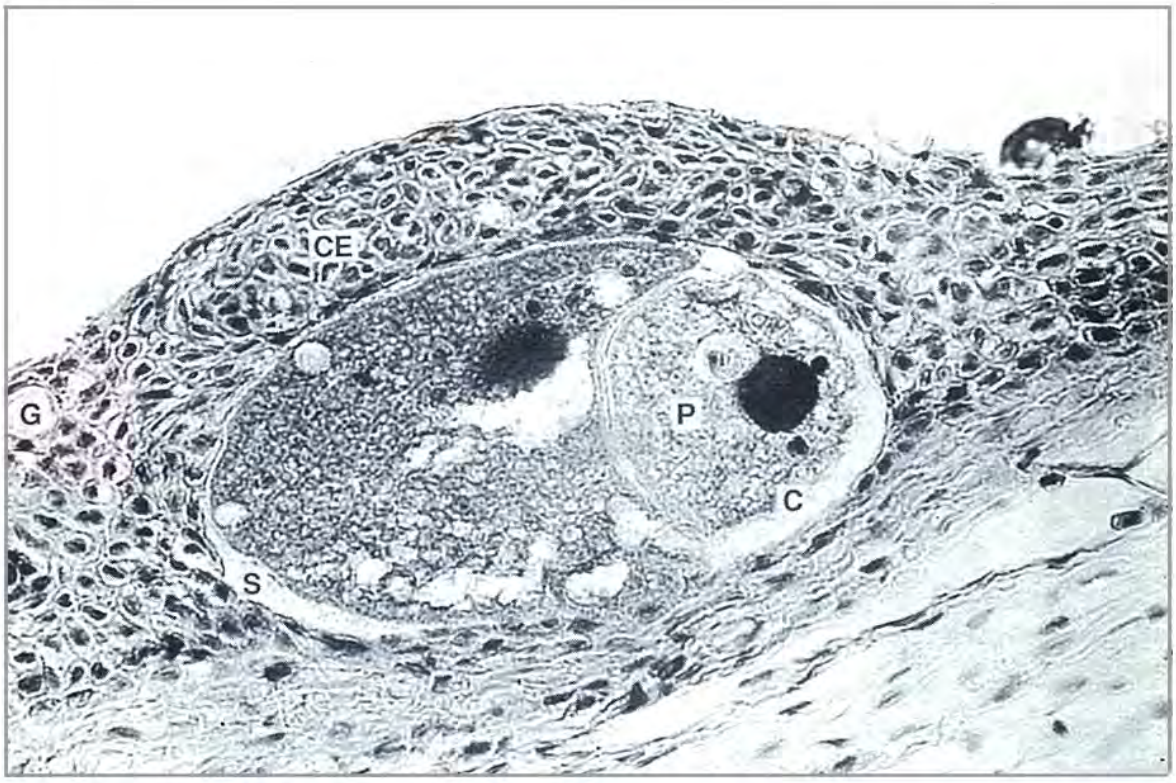

Fig. 3. Photomicrograph of a histological section of epidermis of a fish at 96-hour post-exposure to *I. multifiliis* showing relative lack of histopathological changes. 250X magnification; hematoxylin and eosin.
CE : Relatively less compressed epidermal cells
P : Parasite
C : Cilia
S : Space around the parasite
G : Goblet cell

In samples taken at days 8 and 12 post-exposure, even in the areas where parasite invasion was high, generalized hyperplasia was prevalent. However, these hyperplastic areas were confined to the perimeter of the settlement sites of the parasites. Moderate numbers of necrotic epithelial cells were present around the perimeter of the parasites in some samples. General desquamation and degeneration of the superficial layers of the epidermis were evident to a lesser degree.

Branchial Histopathology

In the control fish, the epithelium of the gill filament was squamose, especially at the base of the origin of the gill lamellae, and was generally two to four cells thick.

At 12 hours post-exposure, small parasites were visible within the epithelial layers of the gill filament. No sign of host tissue disruption was evident.

After 24 hours exposure, the parasites were found on the basement membrane within a vacuole. Otherwise, the tissues were histologically normal.

By 2 to 4 days post-exposure, generalized hyperplasia, together with hypertrophy of filamental and lamellar epithelial cells, was visible in individual fish carrying heavy infections. In contrast, mildly infected individuals showed no sign of tissue damage. The parasites settled within intercellular spaces, displacing the interlamellar epithelium and causing expansion of intercellular spaces. (Fig. 4).

Fig. 4. Section of gill of a fish at 3 days post-exposure to *I. multifiliis*. Note expansion of intercellular spaces of the epithelium. 250X magnification; hematoxylin and eosin.
E : Expansion of intercellular spaces
P : Parasite

At 8 to 12 days post-exposure, extensive hyperplasia and fusion of secondary lamellae were more frequently observed and eosinophilic granular cells were present in such areas. Necrosis, as shown by nuclear pyknosis and karyorrhexis, was evident in some samples. However, these cellular changes were not common. Trophonts were sometimes observed immediately above the basement membrane of the lamellae or, in some specimens, the parasites were found above the basement membrane, leaving an empty space underneath.

Discussion

Hyperplasia, epithelial cell hypertrophy and mild degenerative changes were observed in the histological material examined in this study. Although these features were described by Hines and Spira (1974) and McLay (1985), the extent of the damage observed during the present study was not as severe as that described by these authors. Ventura and Paperna (1985) reported that the pathological conditions described by Hines and Spira (1974) occurred only in extremely heavy infections induced experimentally or in particularly severe epizootics. Hines and Spira (1974) in their study used a 24-day lethal dose level of 400 trophonts/fish, while McLay (1985) employed a 30-day lethal infection level of 5,000 tomites/l. In contrast, the present study utilized a dose level of 200-300 tomites/fish, and a 15-day sublethal infection level. Although direct comparison of the dose levels used is not possible as a result of differences in measures employed, it is clear that the dose level used in the present study was considerably lower than that employed by Hines and Spira (1974) and McLay (1985). The absence of several histopathological changes, such as spongiosis and inflammation of underlying musculature, as observed by Hines and Spira (1974) and McLay (1985), may be associated with these differences in dose levels.

Roberts and Bullock (1976) and Pickering and Richards (1980) in their reviews on fish epidermis reported that conditions such as inadequate water quality, hormonal stimuli and microbial infections are associated with hyperplastic responses in teleost fishes. Ellis (1981) considered hyperplasia to be a nonspecific protective response, which minimizes the changes of epidermal disruption in the presence of irritant conditions. In ichthyophthiriasis where the parasites are generally located on the basement membrane, this hyperplastic response could be of extra importance to the parasite. Especially in areas where the epidermis is thin (e.g., one to three cells thick), the growth of the parasite could cause rupture of the epidermis, exposing the parasite to the external environment. This could be detrimental to some stages of the parasite. Therefore, even though hyperplasia may be considered a nonspecific protective response by the fish, in ichthyophthiriasis, the parasite seems to benefit from it. Perhaps this may indicate an advanced nature of parasitism which is capable of taking advantage of the host response, for better survival.

Hines and Spira (1974) observed pathological changes in the liver such as gradual devacuolization of cytoplasm of the hepatocytes

and focal areas of early necrosis. They attributed these changes to the mobilization of fat reserves, perhaps as a result of starvation. In contrast, McLay (1985) attributed the observed degeneration of hepatic tissues to an increase in secondary lysosomal bodies in the hepatocytes, resulting in impaired lysosomal function. The present study did not reveal any histopathogical changes in the liver tissue of experimentally infected fry. No mobilization of fat or glycogen reserves was necessary, as all fish continued feeding until the experiment was terminated. If the level of infection used had been very high, similar histopathological changes as described by Hines and Spira (1974) and McLay (1985) might have been expected.

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