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A histological study on ancylodiscoidosis in the sheatfish (*Silurus glanis*)

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Summary

A massive infection of two weeks old, 2 cm long sheatfish (*Silurus glanis*) fry with *Ancylodiscoides vistulensis* larvae killed the hosts within a day, owing to attachment of the worms all over the integument. Attachment of 80—120 larvae on the gills caused death of the host in 2—4 days, while less intensive infections resulted in the development of typical ancylodiscoidosis with regressive and progressive lesions of the gills.

Death within 2—4 days was due to the microtraumata inflicted on the cutaneous and gill epithelium cells by the hooks of the parasites. At a later stage also the deeper located tissues of the gill became injured, and tissue necrosis can be accounted for subsequent adhesion of gill filaments. In less rapid cases, in addition to lesions developing at the site of parasites, more general proliferation of the stratified epithelial cells begins progressively involving the entire structure of the gills. Loss of respiratory lamellae and adhesion of adjacent filaments in consequence of proliferation accounted ultimately for death of the host in respiratory trouble.

Key words: gill parasitosis; Ancylodiscoides vistulensis (A. siluri) (Monogenea); sheatfish (Silurus glanis); pathology; histology; experimental infection

Introduction

The sheatfish (*Silurus glanis*) has been noted for fast growth and for palatability as table fish. The possibilities of its propagation and rearing have been explored in Hungary for several years (Antalfi, 1958; Szalay, 1963; Horváth and Tamás, 1976).

Among the diseases affecting sheatfish, ancylodiscoidosis represents a major problem. Its occurrence among cultured sheatfish fry was first observed by Papp (1955), who identified its causal agent as *Ancylodiscoides siluri* (Zandt, 1924). Later Molnár (1968) concluded from detailed investigations into the biological aspects and dynamics of ancylodiscoidosis that *A. vistulensis* (Siwak, 1932) but not *A. siluri* was responsible for this parasitosis of cultured sheatfish. The high pathogenicity of *A. vistulensis* had long ago been recognized by Siwak (1931).

The ancylodiscoidosis of the sheatfish was not previously examined by histological techniques. A histological study on this parasitosis in sheatfish experimentally infected with *Ancylodiscoides* larvae is reported in this paper.

Material and methods

Artificially hatched 2 cm long sheatfish fry, reared in a parasite-free environment, were used. The larvae were derived from a 150 cm long, massively infected brood-fish, transferred from the pond farm to the laboratory for oviposition. The donor fish was kept in an aquarium for 6 days, then it was exterminated and subjected to a detailed parasitological examination. Larvae containing water in the donor's aquarium was later on used for exposure of the fry to larval infection over different periods of time. After exposure the fry were transfered to an aerated aquarium and were fed *Tubifex* until spontaneous death or extermination. The temperature of the rearing aquarium was maintained at 20—22 °C.

As far as possible, infected fry were secured for histological study in the terminal stages of ancylodiscoidosis. The moribund fish were killed and were either fixed *in toto*, or only the gills were used for further study. In the latter case the unilateral hemibranchium was examined in native state under the microscope, while the contralateral gill was fixed in 4 % formaline or Bouin's solution. Paraffine sections were prepared and stained with haematoxylin and eosin, PAS-technique, Wiegert's iron-haematoxylin, or Farkas-Mallory's technique.

Results

Parasitological examination

The donor brood-fish had carried more than ten thousand *Ancylodiscoides* on its gills, but showed no notable gross change of the filaments. Most worms were identified as *A. vistulensis*, and only one in 20 were *A. siluri*. Microscopic examination of the aquarium water showed the presence of 8—12 *Ancylodiscoides* miracidia in each ml. The larva count of the aquarium water remained unchanged for five days removal of the donor fish, from the fifth day it tended to decrease and from the seventh day the fry exposed in the aquarium no longer became infected.

Within a few minutes after transfer of the fry to the aquarium, the *Ancylodiscoides* larvae began to establish themselves on the integument and within two hours they covered the entire body surface of the host, including its barbels. Hosts maintained in the infected aquarium water longer than two hours invariably died within the next 24 hours. Exposure for two hours also resulted in a massive infection, 80—120 larvae established themselves on the gills of each sheatfish and continued their life cycle. These hosts died of ancylodiscoidosis within 2—4 days, depending on the intensity of the infection. Hosts infected by less numerous larvae (i. e. exposed for a shorter time) survived for several weeks, during which their larva burden was increased by the invasion of newly hatched larvae. In the least exposed group the hosts, which grew to 3—3.5 cm length in the meantime, carried on average 120, 130 and 150 parasites, respectively at extermination on days 14, 25 and 35 after infection (Fig. 1). All parasites found on the gills of fry hosts were identified as *A. vistulensis.*

The gills of naturally died fish were packed with parasites, coated by mucus, and had a characteristically pale colour and indefinite structure. From the fourth day after infection, fusion of the gill filaments was also seen.

Histopathological findings

During the first hours after infection the *Ancylodiscoides* larvae had localized chiefly on the body surface and epithelium of orobranchial cavity in the host (Fig. 2 and 3), but from the third day on they were found exclusively on the gill filaments.

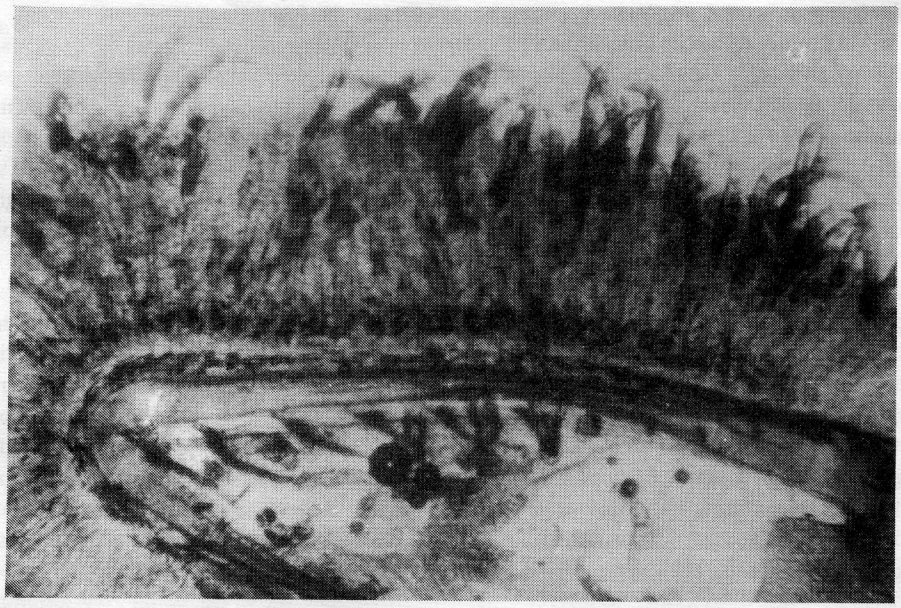

Fig. 1. Adults of *Ancylodiscoides vistulensis* on the gills of sheatfish fry (H. and E.; ×200)

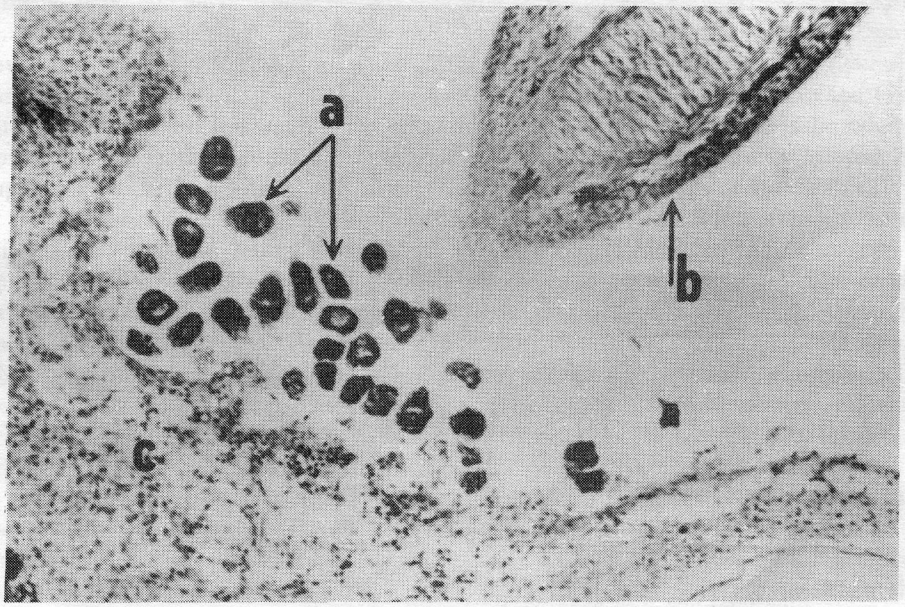

Fig. 2. *A. vistulensis* larvae attached to the skin near to the origin of the barbel:
a) larvae; b) cross section of the barbel; c) injured epidermis (H. and E.; ×200)

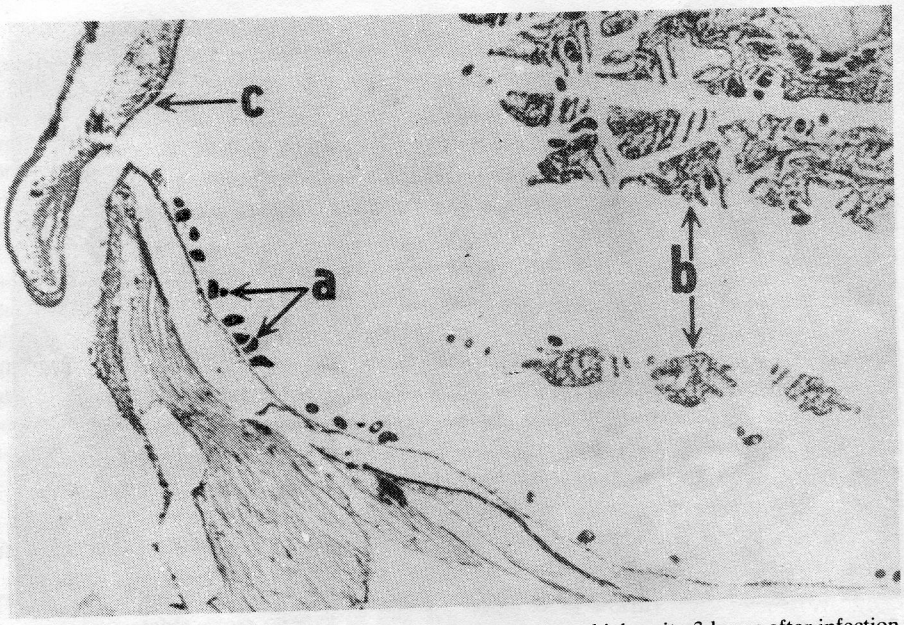

Fig. 3. Larvae attached to the lining epithelium of the orobranchial cavity 3 hours after infection:
a) larvae; b) gill filaments; c) gill cover (H. and E.; ×100)

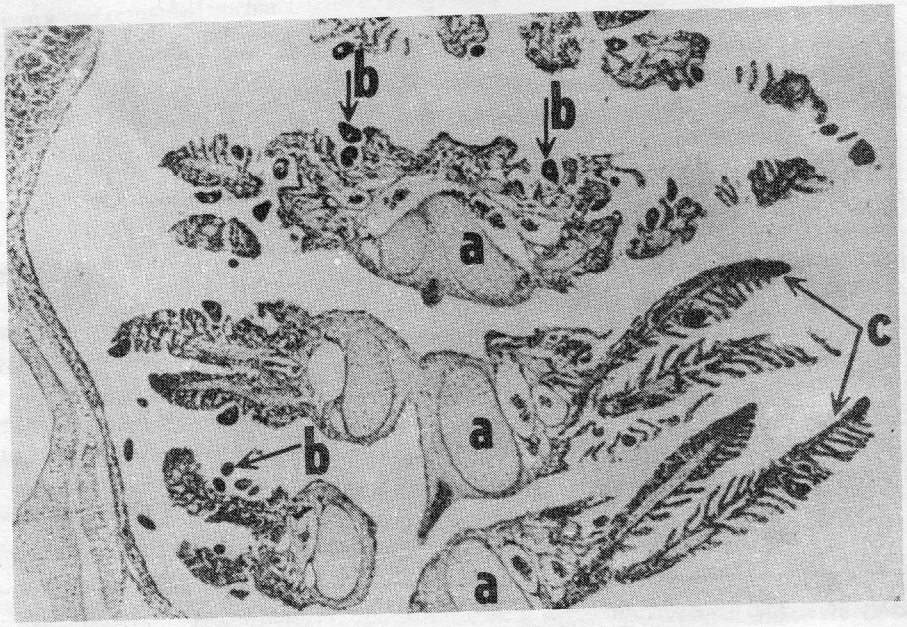

Fig. 4. Larvae attached to the gills:
a) hemibranchia; b) larvae; c) gill filaments (H. and E.; ×100)

Hosts died within one or two days after infection showed the following histological changes: The larvae were loosely attached on the epidermis and gill epithelium, by sinking their hooks and developing ventral anchors superficially into epithelial cells. The stratified epithelium was therefore discontinuous over the larva-invaded skin surface (Fig. 2). The most conspicuous lesions occurred around the gill slits, near to the barbels, and along the origin of the dorsal fins. The larvae attaching to the gills caused minor depressions in the surface tissues (Fig. 4), deformity of the gill lamellae, injury of respiratory and stratified epithelium cells, and minor haemorrhages.

After a few days of infection the ventral anchors of the larvae grew long enough to penetrate into deeper layers of epithelium. Four days after infection a general tissue reaction was developed in addition to local lesions at the attachment sites. The inter-lamellar gill epithelium, normally consisting of 2—3 cell rows, began to proliferate. The epithelium cells, normally characterized by an abundance of nuclear chromatin, amorphus shape of the nucleus and paucity of cytoplasm, showed swollen, round, pale-staining nuclei, and a turbid cytoplasm with signs of vacuolar degeneration. The superficial cells became necrotic, and between the necrotized tissues of adjacent gill filaments loose contact arose (Fig. 5). Part of the gill filaments were fused by adhesion, but still showed a well defined structure. Around the parasites attached on the margins of adhesive filaments, small depressions arose; the epithelium filling out the depressions was discontinuous. Parasites trapped in adhesions of gill filaments were encircled by necrotic tissue.

After 3—5 weeks of infection the parasites localizing on the gill filaments displayed well developed dorsal and ventral anchors, and accounted for three types of hemibranchial lesions:

a) In less advanced cases of lesions the parasites attached on gill filaments still containing lamellae sank their anchors into two adjacent lamellae clinging to one with their dorsal pair of anchors, to the other with the ventral pair. The anchors were often sunk as deep as the cartilagineous supporting structure of the gill filament. At the attachment sites of the parasites the tissue was deficient (Fig. 6), while in adjacent parasite-free sites almost the entire interspace of the lamellae was filled by proliferating tissue. Around the anchors the stratified epithelium, respiratory epithelium and capillary endothelium were equally injured, and capillary haemorrhages were present. Membrane injuries of the superficial epithelial cell layer at the attachment sites accounted for denudation of the cytoplasm and often also of the nucleus. Remnants of lateral hooks were not infrequently found in injured regions of epithelium. The epithelium of the gill lamellae disappeared completely in some places, exposing the endothelium of capillary vessels to direct contact with the outside world. The apical parts of the gill lamellae were seldom coated with epithelium, displaying as a rule obliterated capillaries on their surface. In gill regions not invaded by parasites, the gill lamellae were very low and their interspaces were filled out by masses of turbid appearing epithelium cells with swollen, pale nuclei. Cells with a vacuolized cytoplasm resembling goblet cells were often admixing with this epithelial tissue.

b) In gill filaments showing more advanced lesions (Fig. 7), the lamellae projected from the filament only in places. The proliferating epithelium bulged above the surface of the lamellae, which thus appeared to recede into grooves. The nuclei of the superficial epithelium cells were flat, and were surrounded by a narrow margin of cytoplasm. At the attachment sites of parasites depressions arose in the epithelium; injury and necrosis of the superficial cells were obvious in the depressions and in their surroundings.

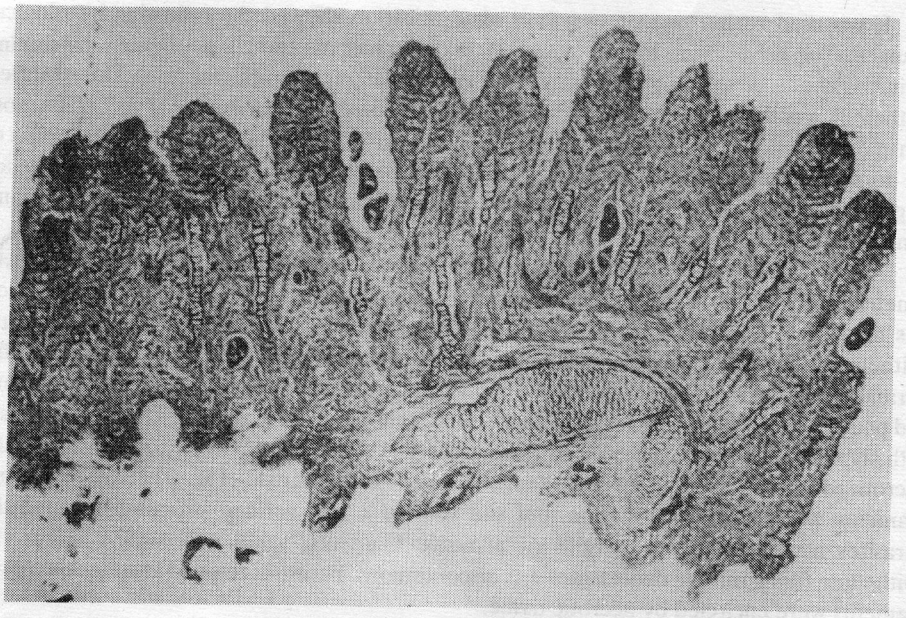

Fig. 5. Detail of the gill of a sheatfish 4 days after infection. The gill lamellae can no longer be identified owing to cell proliferation. Adhesion of the filaments has commenced. Some parasites are trapped within adhesions (H. and E.; ×100)

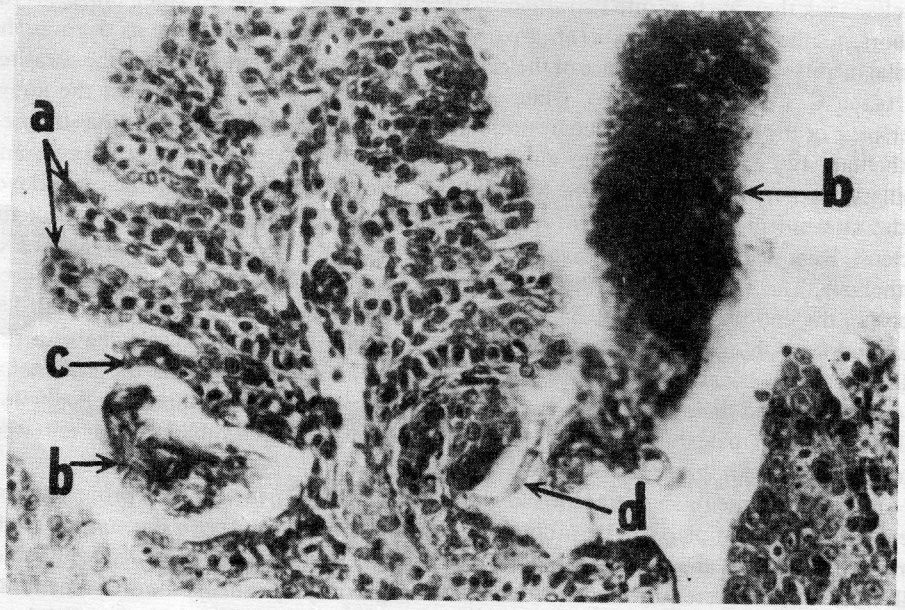

Fig. 6. Gill of a sheatfish 3 weeks after infection: a) fairly preserved gill lamellae; b) parasites; c) lamella devoid of epithelium; d) penetration of the parasite's anchor into the tissue (H. and E.; ×450)

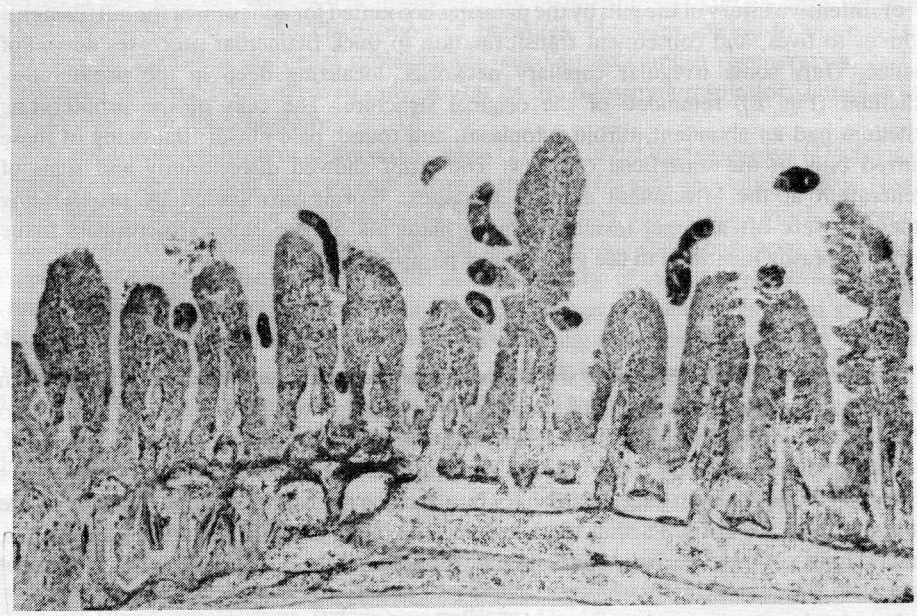

Fig. 7. Gill of a sheatfish 3 weeks after infection. Filaments without lamellae. Note depressions at the attachment sites of parasites (H. and E.; ×100)

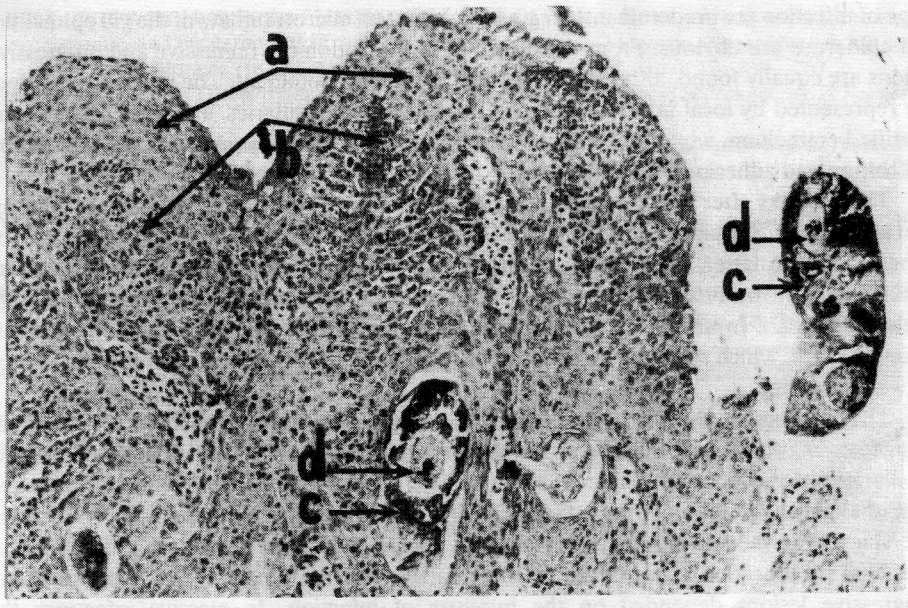

Fig. 8. Adhesion of gill filaments:
a) proliferating epithelial cells with round nuclei; b) remnants of lamellae; c) parasites; d) tissue debris in the gut of the parasite (H. and E.; ×450)

c) Intensive injury of the gills by the parasites accounted for adhesion of the gill filaments by threes to fives, and consequent transformation to thick filamentar processes devoid of lamellae. Only some irregular capillary networks, localizing deep in the proliferating epithelium (Fig. 8), reminded of the original structure. The cells of the proliferating epithelium had an abundant, turbid cytoplasm, and round, pale nuclei; flattening of these occurred only in the superficial cell row. The latter showed discontinuity and signs of degeneration at the attachment sites of parasites. Worms surrounded by proliferating epithelium were often found inside the fused filaments. Incorporated structureless tissue debris were easily identified in the gut of many parasites (Fig. 8).

Discussion

The present experiments have shown that clinically healthy sheatfish may transmit an extraordinarily massive *Ancylodiscoides* infection to their few weeks old offspring. It was also shown that, in accordance with my earlier observations (Molnár, 1968), the species *A. vistulensis* plays the primary role in the gill parasitosis of the sheatfish. Already larval stages are able to kill the 2—3 cm long sheatfish fry by attaching themselves in large numbers on the gills and skin of the hosts. Further to an intensive irritating effect of the attached parasites, a general injury of the superficial epithelium by the hooks of the larvae contributes to the fatal outcome.

Invasion by a lesser number of parasites is also lethal to the 2—3 cm long fry, if 80—120 stages attach themselves to one host. In such cases the direct cause of death is the injury of the gills, but damages of the integument also play an important role. The lesions seen in the early stage of infection are predominantly regressive, above all microtraumata of the gill epithelium and epidermis are obvious. From the fourth day of infection on, regressive and progressive lesions are equally found, although the former are still predominant. The regressive changes are represented by local injury of the gill epithelium and capillaries, and by necrosis of the stratified epithelium, while progressive alterations are indicated by proliferation of stratified epithelium and adhesion of gill filaments.

Three weeks after infection chiefly progressive lesions were found. Epithelial proliferation accounted for disappearence of lamellae from the gill filaments, and in most cases also for adhesion of the latter, giving thus rise to the deformed structures regarded by Wunder (1929), Kollmann (1972) and Lucký (1964) as the most characteristic morphological sign of dactylogyrosis. Injury of the tips of lamellae by the parasite cannot in itself explain their disappearance, which seems to by due primarily to epithelial proliferation in the interspaces between lamellae.

The cytological changes were non-characteristic. Around the anchors cell necrosis, diapedesis of blood cells, injury of respiratory epithelium cells, and denudation of the capillaries were seen. Cytoplasmic degeneration and nuclear hypertrophy frequently occurred in epithelial cells well away from attachment sites. The hypertrophic cells gradually filled the interspaces between lamellae, particularly in a more advanced stage, after the onset of epithelial proliferation. The nature of the changes, and the proportion of regressive and progressive lesions depended on the intensity of infection. In massive infections the degenerative lesions caused death of the host before the onset of proliferation. In cases with a more chronic course of the parasitosis, filaments with intact lamellae, filamentar processes

devoid of lamellae, and adhesion of filaments were variously present on the same hemibranchium depending on spatial distribution of parasites. However, owing to the consistent presence of parasites, epithelial proliferation failed to accomplish regeneration, and the chronically infected hosts survived only as long as the remaining respiratory lamellae were able to cope with their function.

A round-cell infiltration, observed by Prost (1963) in the dactylogyrosis (*Dactylogyrus extensus*) of the common carp, was not found in sheatfish fry with ancylodiscoidosis. Neither was I able to demonstrate an increase of the eosinophilic leucocytes, which is generally characteristic of parasites, nor a goblet cell proliferation, which had been described by Paperna (1964) and Kollmann (1972) as characteristic of carp dactylogyrosis due to *D. vastator*. On the contrary, goblet cells seemed to disappear from the infected gills of the sheatfish fry, and the serous—mucous exudation coating the infected structures seemed to originate from the disintegration of vacuolized, necrotic epithelium cells, as is the case in the dactylogyrosis of the grasscarp (Molnár, 1972). The adhesion of adjacent filaments, reinforced in a later stages by epithelial proliferation, is probably started by the merging of degenerated cells.

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Гистологическое исследование анкилодискоидоза сома обыкновенного (*Silurus glanis*)

Выводы

Массивная инвазия двухнедельного малка сома европейского (*Silurus glanis*) длиной в 2 см личинками *Ancylodiscoides vistulensis* имеет своим следствием гибель хозяина в течение суток,

а именно вследствие прикрепления гельминтов к покрову по всему телу. После прикрепления 80—120 личинок к жаберным листкам, хозяин погибает через 2—4 дня, между тем как менее интенсивные инвазии вызывают возникновение типического анкилодискоидоза с регрессивными и прогрессивными повреждениями на жаберных листках.

Гибель в течение 2—4 дней вызвана микротравмами на эпителиальных клетках кожи и жабр, причиненными крючьями паразита. В более поздней стадии повреждаются ткани жабр, расположенные глубже, а причиной последующего склеивания листков жабр можно считать некроз тканей. В менее острых случаях к повреждениям, развивающимся на месте локализации паразитов, присоединяется довольно обширная пролиферация расслоенных эпителиальных клеток во всей структуре жабр. Потеря респираторных пластинок и склеивание смежных листков вследствие пролиферации представляют в конечном счете причину гибели хозяина с признаками затруднений дыхания.

К. Молнар

Histologisches Studium bei der Ancylodiskoidose des Welses (*Silurus glanis*)

Zusammenfassung

Die massive Invasion einer zweiwöchigen, 2 cm langen Brut des Welses (*Silurus glanis*) durch Larven von *Ancylodiscoides vistulensis* töten den Wirt im Laufe eines Tages infolge der Fixierung der Helminthen an das Integument des ganzen Körpers. Das Anhaften von 80—120 Larven an den Kiemenblättern führt binnen 2—4 Tagen zum Tode des Wirts, während eine weniger intensive Invasion zum Auftreten einer Ancylodiskoidose mit regressiven und progressiven Läsionen an den Kiemenblättern führt.

Das Eingehen im Laufe von 2—4 Tagen wurde durch Mikrotraumen hervorgerufen, die durch die Häkchen der Parasiten an den Epithelialzellen der Haut und der Kiemen verursacht wurden. Im späteren Stadium werden die tiefer gelegenen Kiemengewebe beschädigt und die darauffolgende Verklebung der Kiemenblätter wird durch die Nekrose der Gewebe bedingt. In weniger akuten Fällen reihen sich zu den am Lokalisationsort der Parasiten sich entwickelnden Läsionen, ausgebreitete Proliferationen der in den Schichten anwesenden Epithelialzellen in der gesamten Kiemenstruktur. Der Verlust der Respirationslamellen sowie die Verklebung der anliegenden Blätter infolge der Proliferation werden letzten Endes zur Ursache des Eingehens des Wirtes unter Symptomen von Atmungsschwierigkeiten.

K. Molnár

Histologické štúdium pri ancylodiskoidóze sumca obyčajného (*Silurus glanis*)

Súhrn

Masívna invázia dvojtýždňového plôdika sumca obyčajného (*Silurus glanis*), s dĺžkou 2 cm, larvami *Ancylodiscoides vistulensis* vedie k uhynutiu hostiteľa v priebehu jedného dňa, a to v dôsledku fixovania sa helmintov k integumentu po celom tele. Prichytenie sa na žiabrových lístkoch 80—120 lariev vyvoláva úhyn hostiteľa po 2—4 dňoch, zatiaľ čo menej intenzívne inváziе vedú k vzniku typickej ancylodiskoidózy s regresívnymi a progresívnymi léziami na žiabrových lístkoch.

Uhynutie v priebehu 2—4 dní bolo spôsobené mikrotraumami na epiteliálnych bunkách kože a žiaber háčikmi parazita. V neskoršom štádiu sa poškodzujú hlbšie ležiace tkanivá žiaber a za príčinu následného zlepovania žiabrových lístkov možno pokladať nekrózu tkanív. Pri menej akútnych prípadoch sa k léziám, vyvíjajúcim sa v mieste lokalizácie parazitov, pridružuje rozsiahlejšia proliferácia rozvrstvených epiteliálnych buniek v celej žiabrovej štruktúre. Strata respiračných lamiel a zlepovanie priľahlých lístkov následkom proliferácie predstavuje v konečnom dôsledku príčinu úhynu hostiteľa s príznakmi respiračných ťažkostí.

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