*Acta Veterinaria Academiae Scientiarum Hungaricae, Tomus 22 (1), pp. 9—24 (1972)*

# STUDIES ON GILL PARASITOSIS OF THE GRASSCARP (CTENOPHARYNGODON IDELLA) CAUSED BY DACTYLOGYRUS LAMELLATUS ACHMEROV, 1952

## IV. HISTOPATHOLOGICAL CHANGES

By

K. MOLNÁR

Veterinary Medical Research Institute (Director: D. DERZSY) of the Hungarian Academy of Sciences, Budapest

(Received May 10, 1971)

Investigation of microscopic lesions of fish gills is often hampered by the inadequacy of existing information about the anatomy and histology of the normal gill. In order to facilitate an understanding of the pathohistology of *Dactylogyrus lamellatus* infections in grasscarp *(Ctenopharyngodon idella)*, the first part of this paper is devoted to a description of the gill structure determined in healthy specimens of the species.

The gill anatomy of cyprinids has been studied by PLEHN (1901), SCHÄPERCLAUS (1954), BYCZKOWSKA-SMYK (1959) and HARDER (1964). Histological details have been dealt with by fewer authors (e.g. BINET and VERNE, 1931) and have usually been examined in connection with pathological changes, although KUHN and KOECKE (1956) and LANG (1968) studied the normal histology of the gill lamellae of *Carassius carassius* for diagnostic purposes. The acidophilic cell elements of the gills have been observed by LEINER (1937) and HOLLIDAY and PARRY (1962).

Microscopic gill lesions have chiefly been studied in connection with carp dactylogyrosis; WUNDER (1926, 1929). KULWIEC (1929), SPICZAKOW (1930), IVASIK (1953), SCHÄPERCLAUS (1954), BAUER (1959) and PAPERNA (1964) have dealt with the damages of *D. vastator* and BAUER and NIKOLSKAYA (1954) and PROST (1963) with those of *D. extensus*.

WILDE (1937) investigated gill lesions caused in the tench by *D. macracanthus*.

Observations on gill parasitosis of herbivorous fishes have as yet been reported only by BAUER et al. (1959).

### Materials and methods

Grasscarps of various ages and body dimensions were used in the examinations. The majority were procured from pond farms, the rest originated from a laboratory stock. On extermination of the fish, the gills were divided into two parts: one half was examined in untreated state for gross and microscopic lesions, the other half was fixed immediately in 10% formalin for histological processing. The fixing solution was diluted to 4% after 4—24 hours and the fixed specimen was embedded in paraffin after 1—8 weeks. The sections were stained with haematoxylin and eosin or by Farkas—Mallory's technique. Most sections were cut longitudinally so that the branchial lamellae were sectioned parallel to their longitudinal axis (Fig. 2). Exceptionally, sections were cut in the plane of the lamellae or transversally.

## Results

### *I. Anatomical structure of the gill lamella of grasscarp*

A row of branchial lamellae is attached to either side of the gill arch. A cartilaginous ray runs along each lamella; this consists of a thick axis and two lateral plate-like processes that extend on either side toward the edge of the lamella (Fig. 1). The axis lies closer to the inner than to the outer edge of the lamella; the inner edge is understood as that near the contralateral lamella. In transversal section the inner platelike process is shorter than the outer one

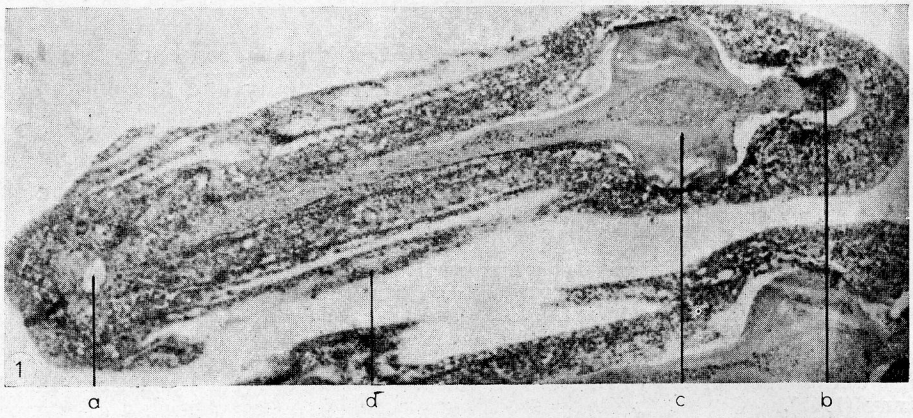


*Fig. 1.* Branchial lamellae of a 3-summer grasscarp in cross section: a, arteria laminae branchialis efferens; b, arteria laminae branchialis afferens; c, axis of cartilaginous ray; d, respiratory plates in cross section

and its length corresponds with the diameter of the axis, whereas the outer process is 6—7 times longer. The arteria laminae branchialis efferens passes along the edge of the outer plate, and is continuous with the arteria laminae branchialis afferens running along the edge of the inner plate.

The respiratory plates (foliae) are perpendicular to the plane of the branchial lamellae; they extend from the axis of the cartilaginous ray to the a. laminae branchialis efferens and are not more than one-third as thick as they are long. The edges of the lamellae are free of respiratory plates on both sides.

### *II. Normal histology of the gill lamella of grasscarp*

The cell types found in the gill lamella of the grasscarp correspond, for the most part, with those observed by KUHN and KOECKE (1956) in the gills of *Carassius carassius.*

The cartilaginous supporting structure of the branchial lamellae is surrounded by connective tissue. This forms a single layer in young fishes but is multilayered and fibrous in older ones. The constituent cells and their nuclei are elongated. Fibrous connective tissue surrounds the afferent and efferent arteries and a few cells are present along the arterioles passing into the respiratory plates.

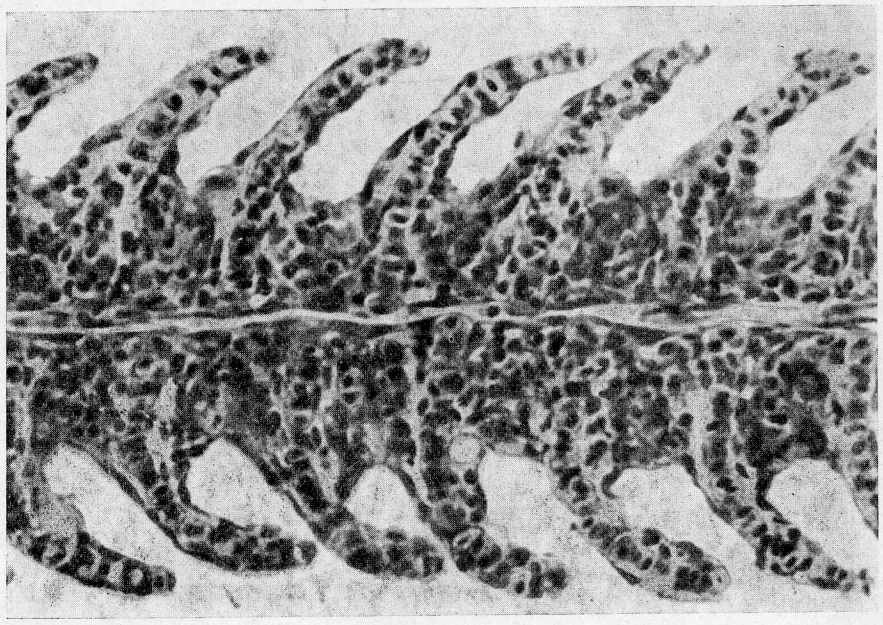

*Fig. 2.* Branchial lamella of a one-summer grasscarp in longitudinal section

The branchial lamellae are covered by a stratified epithelium which is continuous over the edges but interrupted where the respiratory plates arise from the lamella (Fig. 2). The epithelium is composed chiefly of cuboidal cells, which, although of common origin, represent two different morphological types (Fig. 3). One cell type forming the bulk of the branchial epithelium in adult fishes, but appearing only in the superficial epithelial layers in young ones, is characterized by large, round or oval nuclei which stain lightly and have 1—3 chromatin granules. The cytoplasm, also large and pale-staining, encloses vacuoles of various sizes and has confluent margins. The other cells form the bulk of the branchial epithelium in young fishes, but in older ones are seen only in the deep layers; they are relatively small and, like their nuclei, irregularly polygonal. The nuclear chromatin stains easily and encloses one or two nucleoli. The cytoplasm is dense, with confluent margins. Several transitory forms exist between the two cell types.

The acidophilic cells described by LEINER (1937) in *Carassius* are regularly encountered in the deep layers of the stratified epithelium. These are very large and have a disc-like or elongated nucleus at one end. The cytoplasm contains small eosinophilic granules and many small non-staining vacuoles. In some cells the granules are clumped.

Other normal components of the stratified branchial epithelium are the goblet cells, most of which are found at the surface or at the edges of the branchial lamella, although some between the respiratory plates. An additional

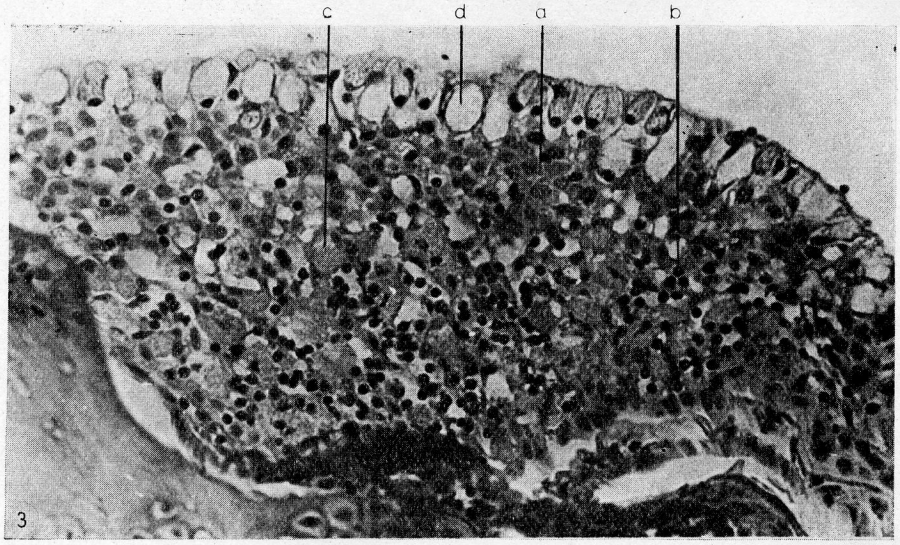


*Fig. 3.* Stratified gill epithelium of the grasscarp: a, type 1 cuboidal cells; b, type 2 cuboida cells; c, acidophilic cells; d, goblet cells

cell type occurring at the edges of the lamellae is oval in shape and one-third of the size of goblet cells. The nucleus of these cells lies on the epithelial side and eosinophilic stripes pass from the nucleus to the cell surface. Nothing is known about the function of these cells, but similar ones occur in the urinary tract mucosa (Figs 3, 4).

The respiratory plates attach to the lateral surfaces of the branchial lamellae. The supporting structure of the plates is composed of a meshwork of modified endothelial cells, the so-called pilaster cells. The respiratory plates are covered by respiratory epithelium. The pilaster cells, which form endothelial walls of the capillary network, are oval or round endothelial cells with flattened nuclei (Figs 5, 6).

The epithelial cells coating the respiratory plates are probably of the same origin as the cuboidal cells of the branchial epithelium, but both they and their nuclei are flatter, particularly in younger fish and they attach to the basement

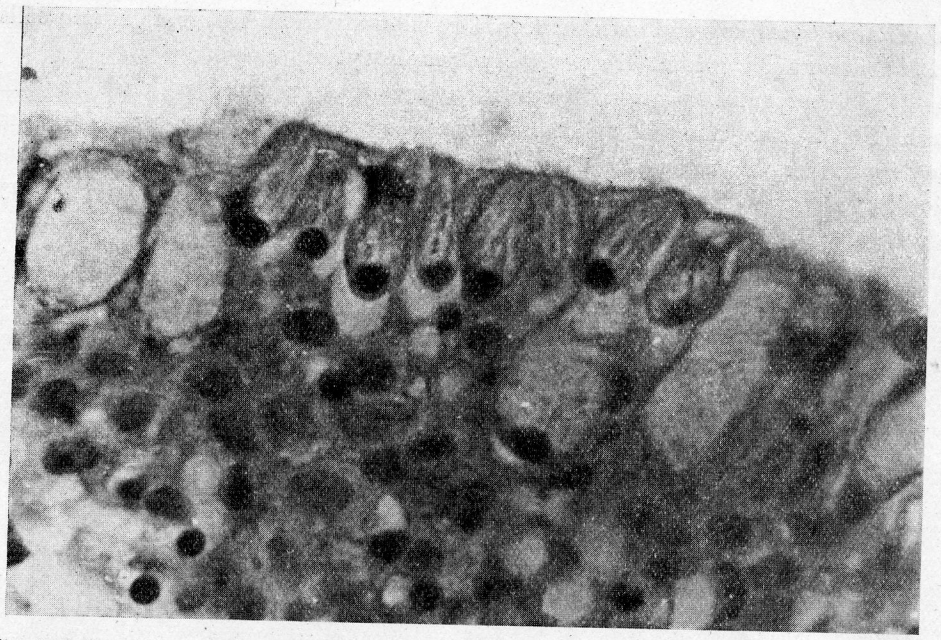

*Fig. 4.* Cells with eosinophilic striation and of an unknown function between goblet cells

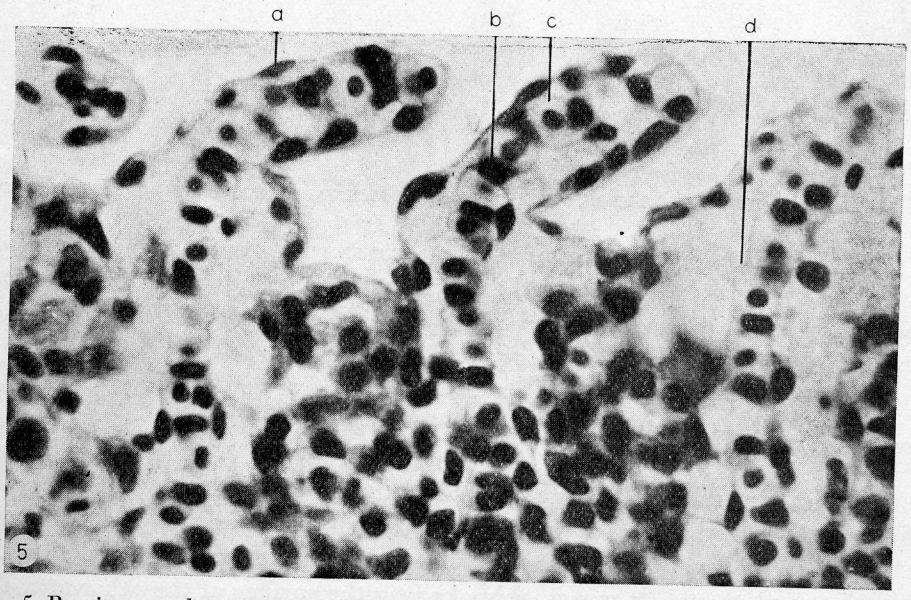


*Fig. 5.* Respiratory plates in transversal section: a, epithelial cells; b, endothelial cells; c, red blood cells; d, basement membrane

membrane overlying the endothelium. Occasionally there are small, round cells with large nuclei, which are probably lymphocytes.

In interpreting sections for routine examination cut parallel to the gill arch (Fig. 2) care must be taken not to confuse the picture that results from any inaccuracy in the plane of section with that presented by true "denudation" of the tips of the branchial lamellae, as in both cases the respiratory plates are absent in whole or in part.

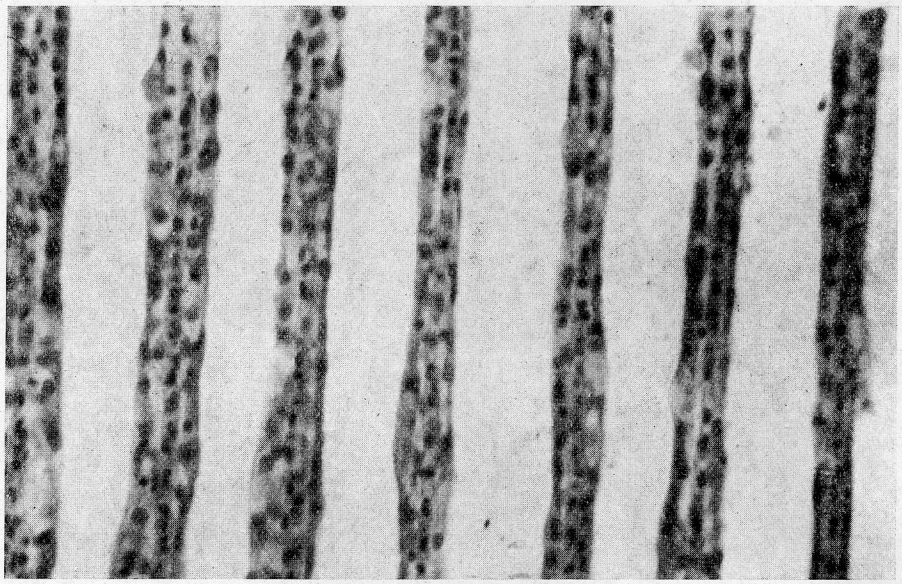

*Fig. 6.* Respiratory plates in longitudinal section

### *III. Histological examination of gills from grasscarps infected with D. lamellatus*

The sections of gill specimens from grasscarps with dactylogyrosis contained many parasites and showed two kinds of changes: local lesions developing in the immediate surroundings of the parasite, and general lesions involving either the entire gill or its greater part.

#### *Local lesions*

These are relatively slight, as PAPERNA (1964) and PROST (1963) also observed in dactylogyrosis of the common carp. The parasite usually settles in the space between two respiratory plates, sinking its central hooks into the base of one plate and its marginal hooks into the surface of the other one. The tip of the central hook either penetrates the cells or pierces an artery; it may

even pass completely through the plate (Fig. 7). The marginal hooks sink into the cytoplasm of the coating epithelial cells and tear it. The central hooks cause epithelial erosions and minor haemorrhages, but no tissue proliferation.

*General gill lesions*

These comprise degeneration, tissue defects, haemorrhages, necrosis, atrophy and cell proliferation. The nature of the lesions depends on the duration of the disease, on the number of parasites established on the gills and on the extent of regeneration.

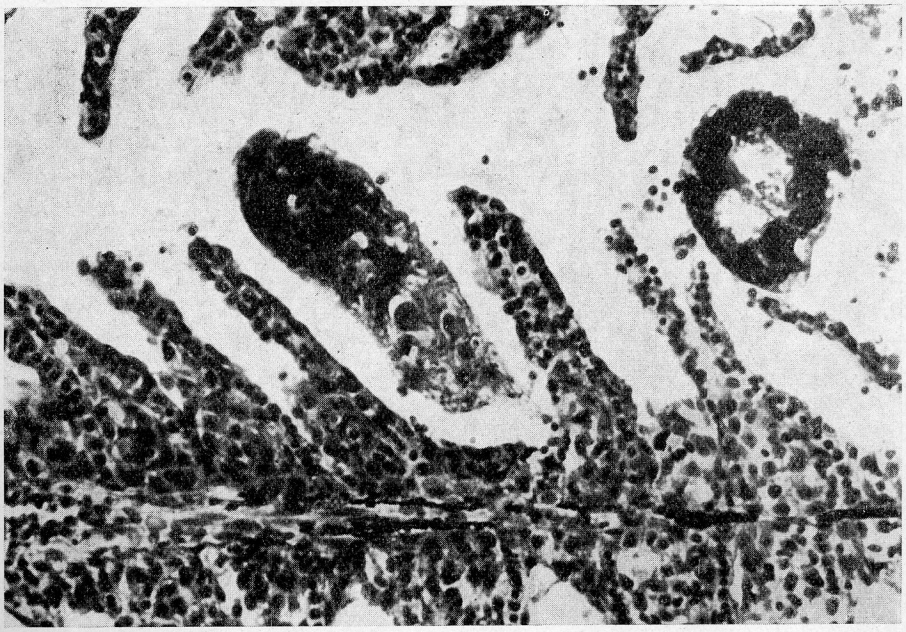

*Fig. 7. D. lamellatus* sinking its hooks into the base of the respiratory plate

1. An abrupt invasion of the host by many *D. lamellatus* parasites results in a rapid course of dactylogyrosis. This form of the disease ends fatally before the characteristic symptoms and lesions can develop.

Grossly, the gills are pale and anaemic and examination under the stereomicroscope reveals the presence of many parasites and loss of definition of the gill structure. Microscopically, degenerative changes appear to be predominant. The epithelial cells of the respiratory plates are damaged or may be entirely absent in places, leaving the endothelial surface exposed. The remaining epithelial cells are ruptured or degenerated (Fig. 8). The type 1 cuboidal cells of the stratified epithelium between the plates also degenerate or necrotize.

Extravasated red blood cells and eosinophilic patches of plasma are seen between the branchial lamellae and respiratory plates.

2. Typical acute dactylogyrosis is manifested 2—3 weeks after invasion of the parasites. By this time the gill lesions become grossly visible. The massively infested gills are paler than normal and may assume a mosaic-like appearance if there are haemorrhages. Copious mucus covers the lamellae. The latter appear deformed and carry thick greyish bodies and processes of various sizes which may form adhesions by fusion. Some break off, leaving visible defects.

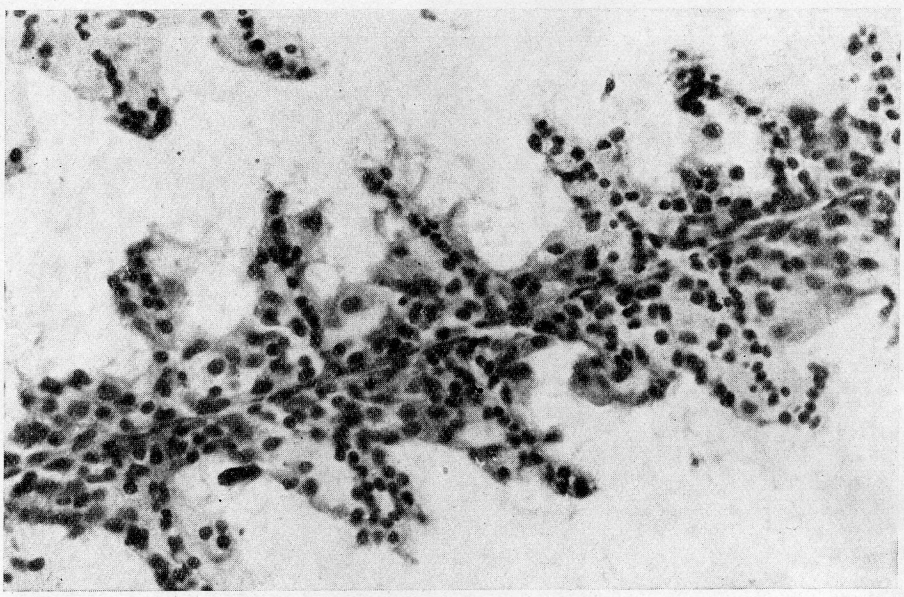

*Fig. 8.* Degeneration and necrosis of the respiratory plate epithelium

This form of dactylogyrosis is characterized by proliferation of the gill epithelium. The proliferation originates from the cuboidal cells, which grow larger and increase in number, causing a thickening of the lamella. The epithelial cells between the respiratory plates enlarge, bulging above the level of the plates and eventually producing adhesions between the lamellae.

The cells of the proliferative tissue have a foamy cytoplasm and a large, round, clear nucleus which encloses one or two lightly staining nucleoli. The cytoplasmic vacuoles are small and granular in the deeply seated cells, large and vesicular in cells of the upper layers. The cell margins are indistinct. Some of the superficially localized cells necrotize and undergo cytoplasmic disintegration and karyolysis (Fig. 9).

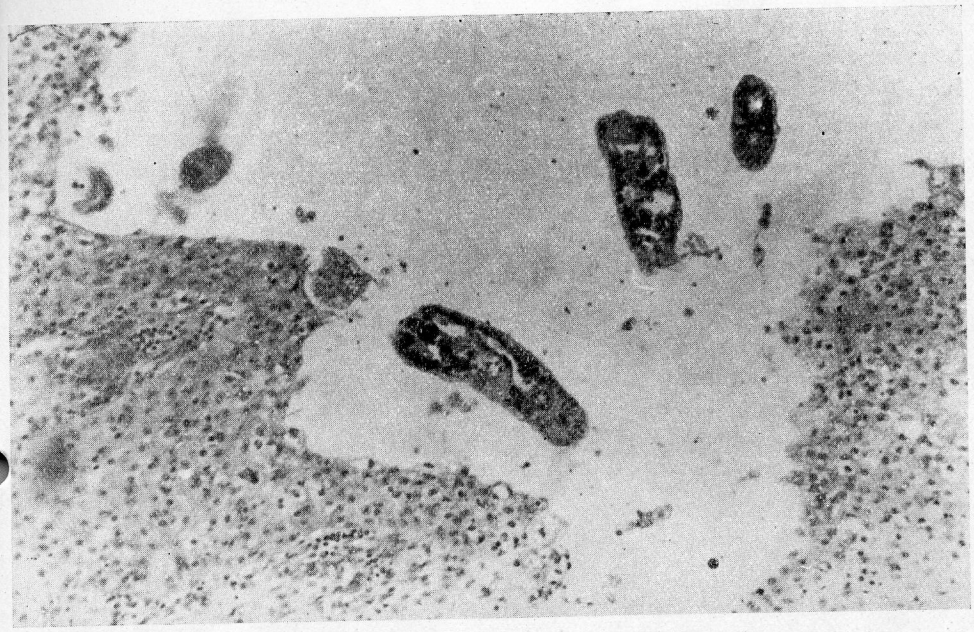

*Fig. 9.* Ruptured epithelial cells around the site of attachment of the parasite

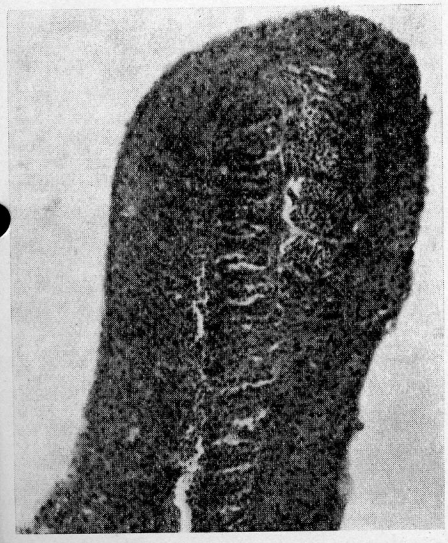

*Fig. 10.* Adhesion of branchial lamellae and lacunar haemorrhages

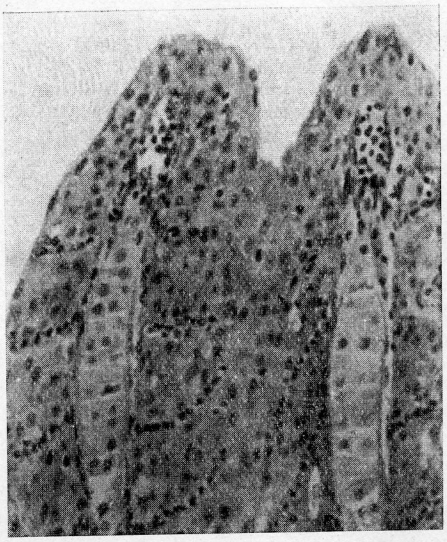

*Fig. 11.* Fusion of branchial lamellae as a result of cell proliferation

In moderately severe cases of dactylogyrosis, the normal structure can be recognized at the base of the lamellae, but in the central part of the swollen lamella degenerated cuboidal cells fill the spaces between the respiratory plates, pushing the plate epithelium toward, and the capillary net downward, deep into the proliferative tissue. At the tip of the lamellae, several layers of proliferative tissue overgrow the remains of the capillaries, thus giving rise to the respiratory-plate-free "lamellar processes" observed by WUNDER (1929).

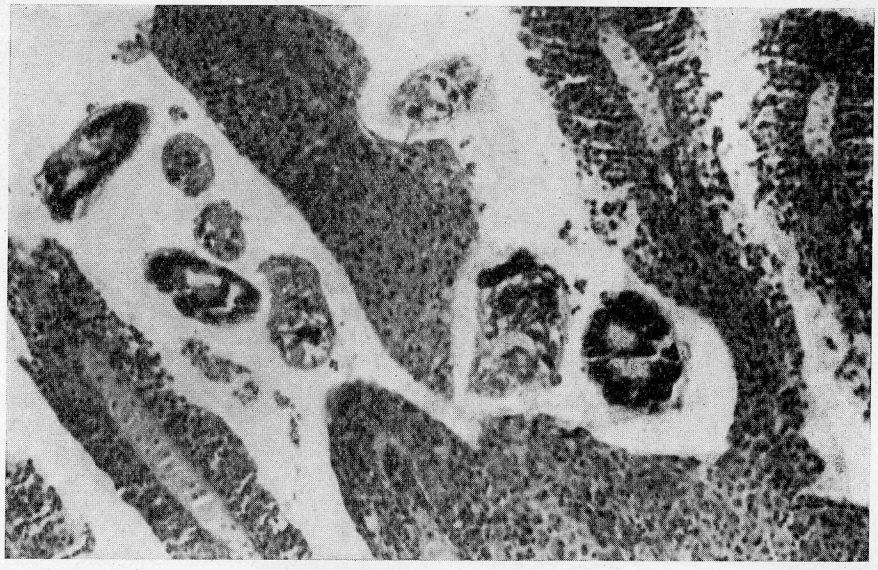

*Fig. 12.* Parasites established at the tips of the branchial lamellae

In severe cases, 3—5 or even more gill lamellae may coalesce with one another (Figs 10, 11), usually at the tips, but occasionally along their entire length. The original structure of the lamellae is hardly perceptible within the intricate pattern of the proliferative tissue. The capillary network, pushed into the deep epithelium by proliferation, can be recognized in places, but elsewhere it is seen only in the form of lacunas consisting of endothelial and red blood cells. The proliferative tissue occasionally contains mononuclear cells. Connective tissue cells are seen only around the vessels or along the deformed cartilaginous supporting structure of the gill lamellae.

The cells of the proliferative tissue degenerate and necrotize on severely deformed gill lamellae. In the softened, necrotic tissue the endothelial network is retained in a relatively intact state until the epithelial cells disappear. Necrosis later involves the cartilaginous parts and arteries and affected lamellae break off, leaving grossly visible gill defects.

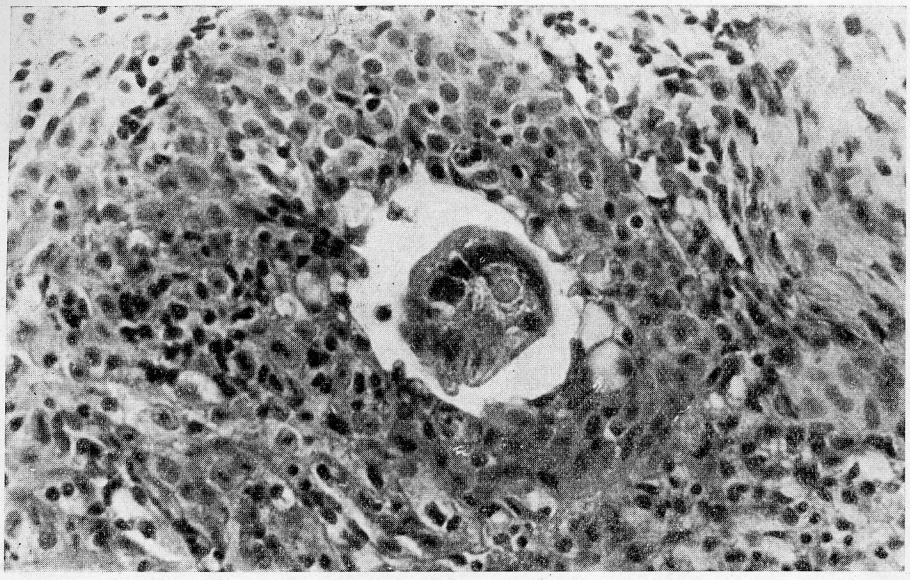

*Fig. 13.* Parasite encased by proliferative tissue in transversal section

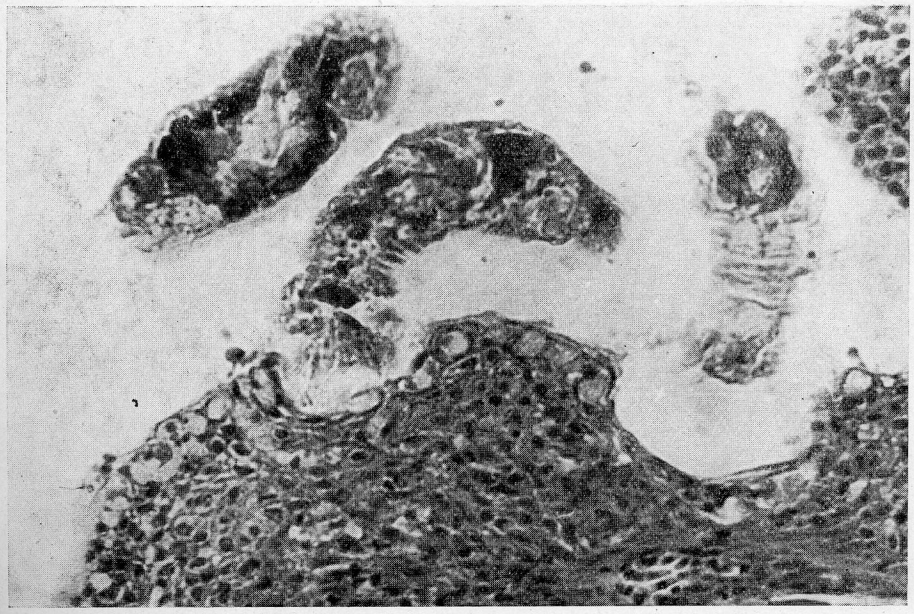

*Fig. 14.* Characteristic depressions at the attachment sites of the parasites

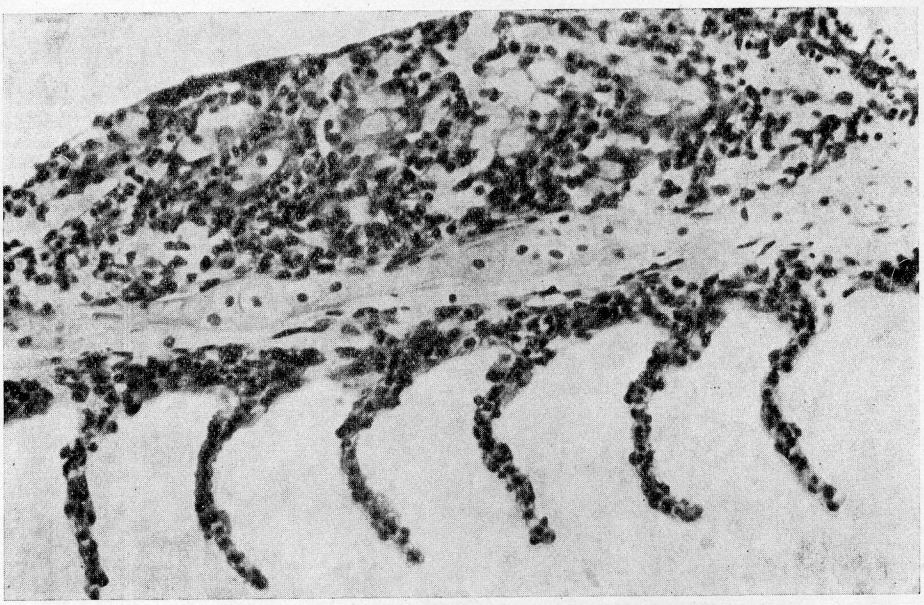

*Fig. 15.* Local tissue proliferation in chronic dactylogyrosis

*Fig. 16.* Epithelial atrophy in chronic dactylogyrosis

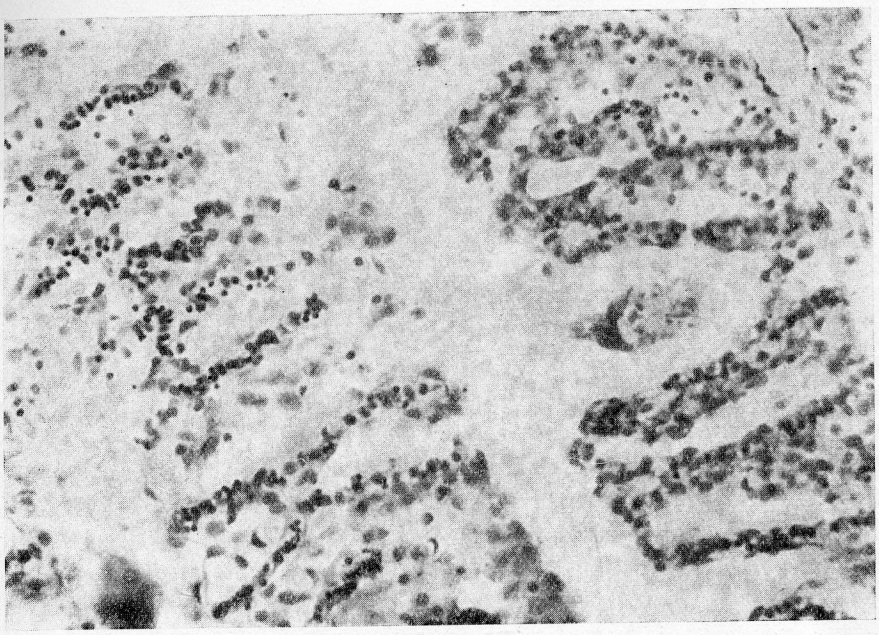

*Fig. 17.* Necrosis of cuboidal cells

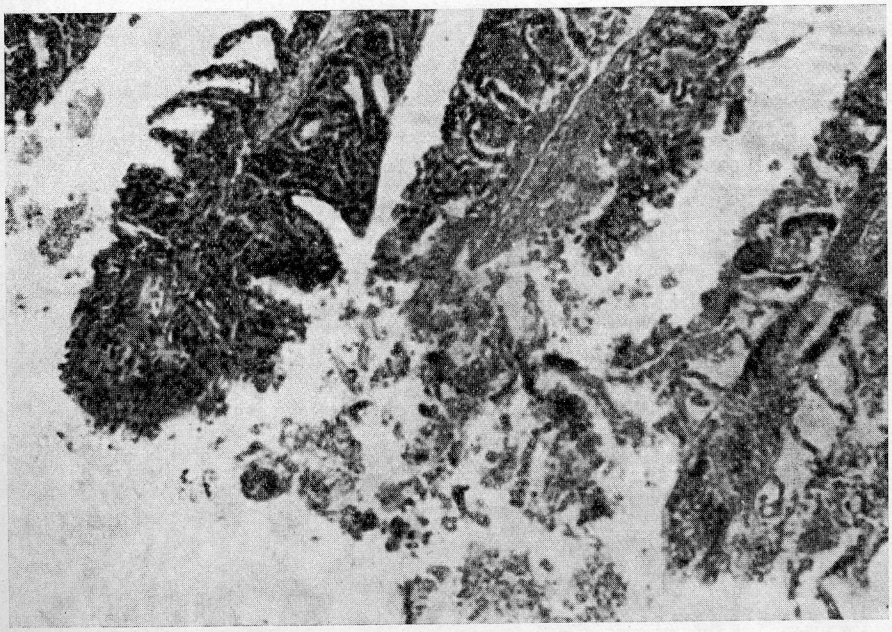

*Fig. 18.* Necrosis of the tips of the lamellae

The parasites expelled from their localizations by the proliferative tissue either reattach to the stratified epithelium at the tips and edges of the lamellae (Fig. 12) or are retained between the lamellae and become encased by proliferative tissue (Fig. 13). A characteristic depression arises around the haptor of parasites fixing themselves to the stratified epithelium (Fig. 14).

The microscopic picture of chronic *D. lamellatus* infection is predominated by degenerative lesions. The epithelial coat of the respiratory plates disappears, leaving them either denuded or fringed with cell fragments hanging from the basement membrane. In less affected areas, the proliferative tissue fills the spaces between the respiratory plates but without causing adhesions (Fig. 15). A more characteristic change is the reduction of epithelium between the plates; only 2—3 layers cover the underlying cartilage and connective tissue and the upper layer is damaged (Fig. 16). In certain cases the greater part of the gills becomes necrotic; necrotic lamellae held together only by the cartilaginous part of the gill arches alternate with sound ones. In such areas, cell necrosis takes place by both karyolysis and karyorrhexis (Figs 17 and 18).

## Discussion

The gross and microscopic lesions caused by *D. lamellatus* resemble the damages of *D. vastator* (WUNDER, 1929; PAPERNA, 1964; USPENSKAYA, 1961), *D. macracanthus* (WILDE, 1937) and *D. extensus* (BAUER and NIKOLSKAYA, 1954; PROST, 1963). Lesions caused by *D. vastator* and *D. macracanthus* are characterized by tissue proliferation and those by *D. extensus* by superficial epithelial damage.

The type of the predominant microscopic lesion depends on the body dimensions of the host, on the number of the invading parasites and on the duration of invasion. If the fish is small, fewer parasites are required to cause a gill dysfunction than in a larger fish. If the infestation is massive, symptoms and gill damages appear earlier than after a mild invasion. While the size of the host and the number of the parasites determine the pathogenicity and the severity of the degenerative processes, the duration of invasion has a decisive influence on the nature of the microscopic lesions.

An abrupt, massive larval invasion chiefly affects the respiratory epithelium. Degeneration or desquamation of the epithelium diminishes the oxygen intake, but more damage is done by extravasation of blood plasma across the denuded endothelium as in young 2—3 cm long grasscarps this results in an osmotic dysbalance of the blood.

If larval invasion takes place over a longer period, the host organism tries to compensate the degenerative changes by regenerative phenomena. Tissue proliferation is typical of acute dactylogyrosis. Proliferation involves

the cuboidal epithelium (PAPERNA, 1964) but not connective tissue cells. The initial phase of regeneration is characterized by swelling of the cuboidal cells and their nucleus proliferation begins only later with the enlarged cells rising above the level of the respiratory plates and overgrowing them. Cells at the surface gradually degenerate and mucus accumulates in their cytoplasm.

Epithelial cell proliferation prevents denudation of the endothelium and hence extravasation of blood plasma, but it isolates the capillaries from their surroundings thereby inhibiting oxygen exchange. In mild cases of dactylogyrosis, when the unimpaired gill area is large enough to provide for sufficient oxygen uptake, cell proliferation can compensate the degenerative lesions caused by the parasite. If, however, the gill lesions are extensive, respiratory function ceases and the fish dies of asphyxia. A certain amount of mucus is present on normal gills, through the activity of the goblet cells localized on the surface of the epithelium and between respiratory plates, but copious secretion of mucus by impaired gill cells interferes with respiration. The increased secretion of mucus is not, however, related to hyperfunction or multiplication of goblet cells (PAPERNA, 1964) because most of these deteriorate along with the superficial epithelium. In all probability it originates from cuboidal cells which undergo a mucoid degeneration prior to disruption. This process takes place primarily in the superficial layers of the gill epithelium.

Interestingly, the acidophilic cells of the stratified epithelium do not notably multiply during the gill disease, although this is usual in other pathologic processes. Aggregation of the cytoplasmic granules is, however, more common than the normal finely granular appearance.

In chronic dactylogyrosis, the compensatory mechanism is limited to the substitution of the deteriorated tissues and no cell proliferation takes place. General weight loss due to parasitosis results in atrophy of the gill tissues, and degenerative phenomena are seen at the sites where the parasites attach, as well as in the superficial cell layers.

Necrosis of certain gill areas takes place both in acute and chronic dactylogyrosis. After the acute condition has subsided, the lamellar tips which have become fused by tissue proliferation necrotize and fall off. In chronic dactylogyrosis, necrosis of the lamellae very likely takes place in consequence of the impairment and obliteration of the supplying vessels. After detachment of the necrotic parts a complete restitution is possible.

## SUMMARY

Gills of healthy grasscarps *(Ctenopharyngodon idella)* and specimens infected with *Dactylogyrus lamellatus* were examined. The anatomical and histological structure of normal branchial lamellae is described to aid diagnostic comparison.

Establishment of *D. lamellatus* parasites on the gill lamellae gives rise to local and general lesions. The local lesions comprise erosions, endothelial impairment and minor areas of cell degeneration around the site of attachment of the parasite. The general gill lesions include

degeneration, tissue damage, haemorrhages, necrosis, atrophy and cell proliferation. The nature of the lesions depends on the duration of the disease, the number of parasites established on the gills and the extent of regeneration.

If the infection takes a rapid course, the typical lesion is degeneration of the respiratory plate epithelium. In acute dactylogyrosis this is compensated by proliferation of the cuboidal cells of the lamellar epithelium which results in the disappearance of respiratory plates, process-like transformation and adhesion of the lamellae. If the condition is chronic, the respiratory epithelium becomes damaged, the epithelium atrophies and the lamellae necrotize but there is only a local degeneration.

## REFERENCES

Бауер, О. Н.: ДАН СССР **78** (1951), 825—827. IDEM: Изв. ГосНИОРХ **49** (1959), 5—203. Бауер, О. Н., Мусселиус, В. А. and Стрелков, Ю. А.: Болезни прудовых рыб. Москва, 1969. Бауер, О. Н. and Никольская, Н. П.: Тр. пробл. и темат. совещ. ЗИН **4** (1954), 99—109. BINET, L. and VERNE, J.: J. Physiol. Path. Gen. **29** (1931), 689—694. BYCZKOWSKA-SMYK, W.: Acta Biol. Cracov. Ser. Zool. **2** (1959), 73—88. HARDER, W.: Anatomie der Fische. Stuttgart, 1964. HOLLIDAY, F. G. T. and PARRY, G.: Nature (London) **193** (1962), 192. Ивасик, В. М.: Тр. Н.-и. Инст. Пруд. и Оз.-реч. рыб. Хоз. **9** (1953), 85—112. KUHN, O. and KOECKE, H. U.: Z. Zellforschung **43** (1956), 611—643. KULWIEC, Z.: Arch. hydrobiol. rybactwa **4** (1929), 227—281. LANG, W.: Arch. Fischereiwiss. **18** (1968), 25—45. LEINER, M.: Zool. Anz. **117** (1937), 322—325. PAPERNA, I.: Bamidgeh. **16** (1964), 129—141. PLEHN, M.: Zool. Anz. **24** (1901), 439—443. PROST, M.: Acta parasit. polon. **11** (1963), 17—47. SCHÄPERCLAUS, W.: Fischkrankheiten. Berlin, 1954. SPICZAKOW, T.: Arch. hydrobiol. rybactwa **5** (1930), 1—52. Садковская, О. Д.: Работы по гельминт. к 80-летию акад. К. И. Скрябина. Москва, 1958, 320—321. Успенская, А. В.: Зоол. Журнал **40** (1961), 7—12. IDEM: ДАН СССР **112** (1962), 1212—1215. WILDE, J.: Z. Parasitenkunde **9** (1937), 203—236. WUNDER, W.: Biol. Zbl. **46** (1926), 44—53. WUNDER, W.: Z. Fischerei **27** (1929), 511—541.

Address of the author: Dr. Kálmán MOLNÁR, Budapest XIV., Hungária krt 21, Hungary