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HISTOLOGICAL CHANGES IN THE SWIMBLADDER WALL OF EELS DUE TO ABNORMAL LOCATION OF ADULTS AND SECOND STAGE LARVAE OF *Anguillicola crassus*

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In severe *Anguillicola crassus* infection of eels, adult helminths and 2nd stage larvae staying in the swimbladder lumen may occasionally get, through minor lesions of the tunica interna, into the subserosa of the swimbladder wall where they die and disintegrate. A thin connective tissue capsule is formed around the helminths that behave as foreign bodies in intercellular location, while the lacunas of the surrounding loose connective tissue comprise melanin-containing macrophages. In the environment of the 2nd stage larvae the formation of giant cells is a typical finding.

Key words: *Anguillicola crassus,* Nematoda, eel, swimbladder, abnormal location, histopathology

Since *Anguillicola crassus* was introduced into Europe in the mid-1980s, (Neumann, 1985) numerous papers have dealt with its prevalence (Peters and Hartmann, 1986; Taraschewski et al., 1987; Hartmann, 1987; Dupont and Petter, 1988; Belpaire et al., 1989; Koops and Hartmann, 1989; Kennedy and Fitch, 1990; Koie, 1991; Székely et al., 1991; Moravec, 1992), life cycle (De Charleroy et al., 1990; Haenen and van Banning, 1991; Höglund and Thomas, 1992; Thomas and Ollevier, 1992), seasonal occurrence (van Willigen and Dekker, 1989), and pathogenic effect exerted on the host (Mellergaard, 1988; Haenen et al., 1989; Boon et al., 1989; Boon et al., 1990*a,b,c*; van Banning and Haenen, 1990; Molnár et al., 1991; Möller et al., 1991; Sprengel and Lüchtenberg, 1991; Höglund et al., 1992). At the same time, data on the histopathological changes caused by the parasite can be found only in the works of Haenen et al. (1989), van Banning and Haenen (1990), and Molnár et al. (1993). The latter authors gave a detailed description of the general lesions produced by adult worms and by larvae in the mucous membrane of the swimbladder and in the subserosa, but only incidentally mentioned the mechanical injury caused to the swimbladder wall. At the same time, Liewes and Schaminee-Main (1987) and Kamstra (1990) graded by severity the swimbladder lesions caused by anguillicolosis in eels. They regarded

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as the most severe lesion the rupture of the swimbladder wall and the appearance of a brownish-blackish substance consisting of nematode debris in the swimbladder wall that had been replaced by a thick layer of connective tissue.

This paper presents the histopathological changes resulting from the anguillicolosis-induced discontinuity of the swimbladder wall, and provides data on the histogenesis of the granuloma-like lesions caused by helminths and larvae abnormally entering the swimbladder wall, as well as on the nature of the pigmentation which is seen in some cases.

Materials and methods

The material used in this study comprised eels that had been derived from Lake Balaton and dissected in 1992 (Molnár et al., 1993). In 1993, complementary studies were carried out: of 344 eels caught from different regions of Lake Balaton, only those specimens which exhibited changes resulting from swimbladder lesions (helminths and 2nd stage larvae located in the swimbladder wall, or intensive pigment formation) were processed for histology. In contrast to the earlier paper, here we do not follow Dorn's nomenclature, and refer to the loose connective tissue surrounded by the serosa and the muscular layer by the name of subserosa, as part of the tunica externa, rather than by the name of submucosa. According to the classification adopted by us, the tunica interna comprises the muscular layer and the mucosa.

Swimbladders intended for histological processing were placed into Bouin's solution in their entirety for some minutes, then were cut through at the affected part or transversely in the middle, and the smaller parts were again fixed in Bouin's solution for 4 hours. The materials were embedded in paraffin wax and cut into 4 μm thick sections. The preparations were stained with haematoxylin-eosin for general information, with picrosirius stain to study collagenic fibres, by Brown-Brenn and by Ziehl-Neelsen for bacteria, by Perls for haemosiderin, and by the periodic acid–Schiff (PAS) reaction for mucous cells. Some sections were treated with 10% H_2O_2 solution for one hour, then stained also by the method of Perls and by the Oil-red procedure.

Electron microscopic examination involved the subsequent processing of material that had been previously fixed in Bouin's solution and embedded in paraffin wax.

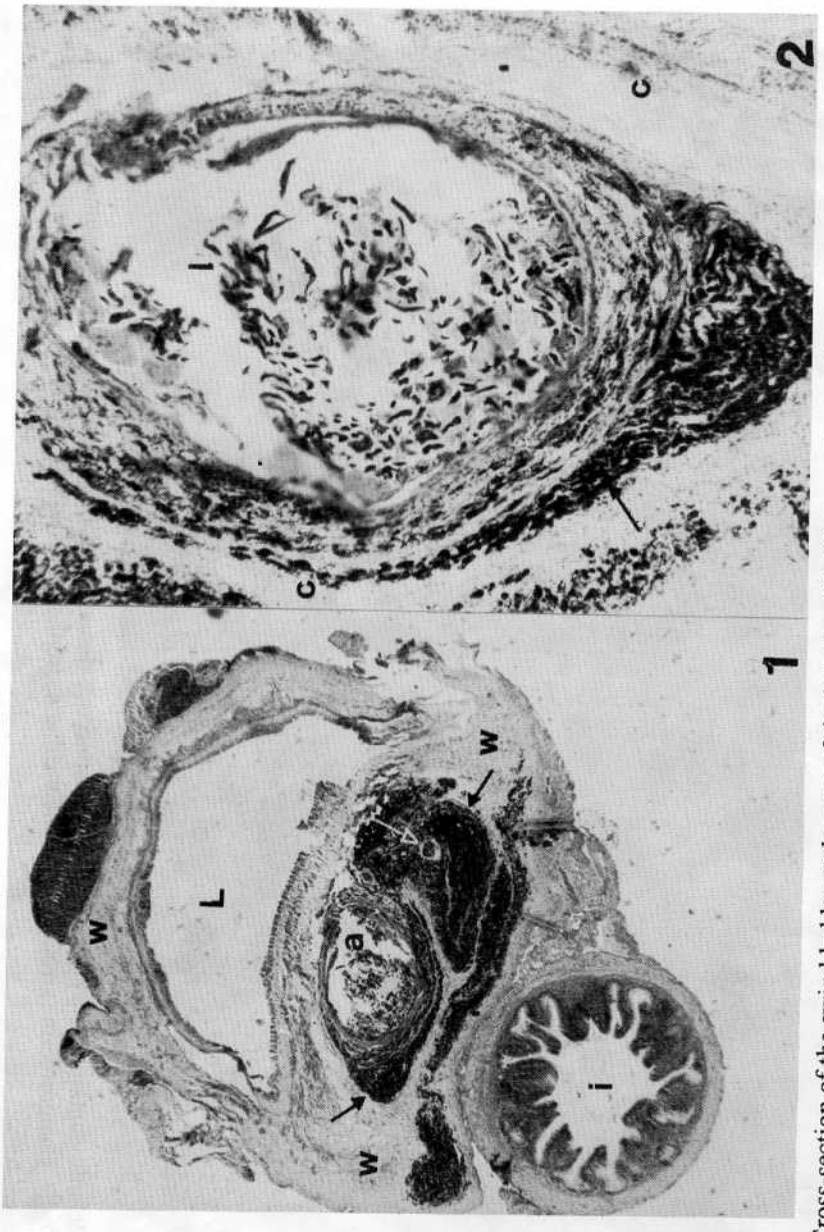

Fig. 1. Cross-section of the swimbladder and a part of the intestine (i). Note an abnormally located female *Anguillicola crassus* (a) in the wall (w) of the swimbladder. The worm was translocated here from the lumen (L) due to the rupture of the muscular layer and mucosa. An adult (a) and a 4th stage larva (empty arrow) are surrounded by melanomacrophages (arrow). Haematoxylin and eosin (H. and E.), × 35

Fig. 2. Enlarged picture of the extraluminal *A. crassus* female shown in Fig. 1. Second stage larvae (l) fill the body of the helminth. The connective tissue of subserosa (c) around the worm is infiltrated by melanomacrophages (arrow). H. and E., × 130

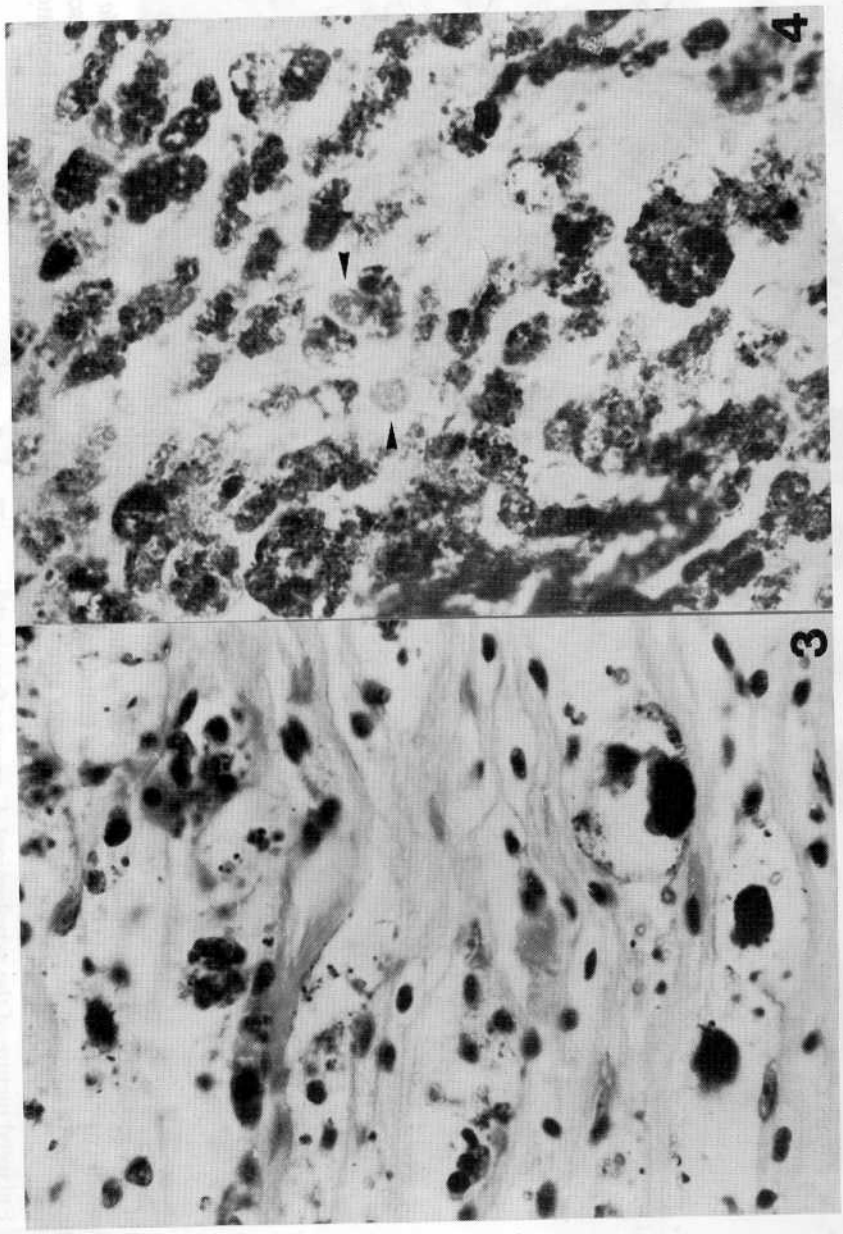

Fig. 3. Melanin-containing macrophages among connective tissue cells filling the lacunas of the subserosa. H. and E., × 1200
Fig. 4. Haemosiderin (arrow-head) in the melanomacrophages. Perls staining after bleaching with H_2O_2. × 800

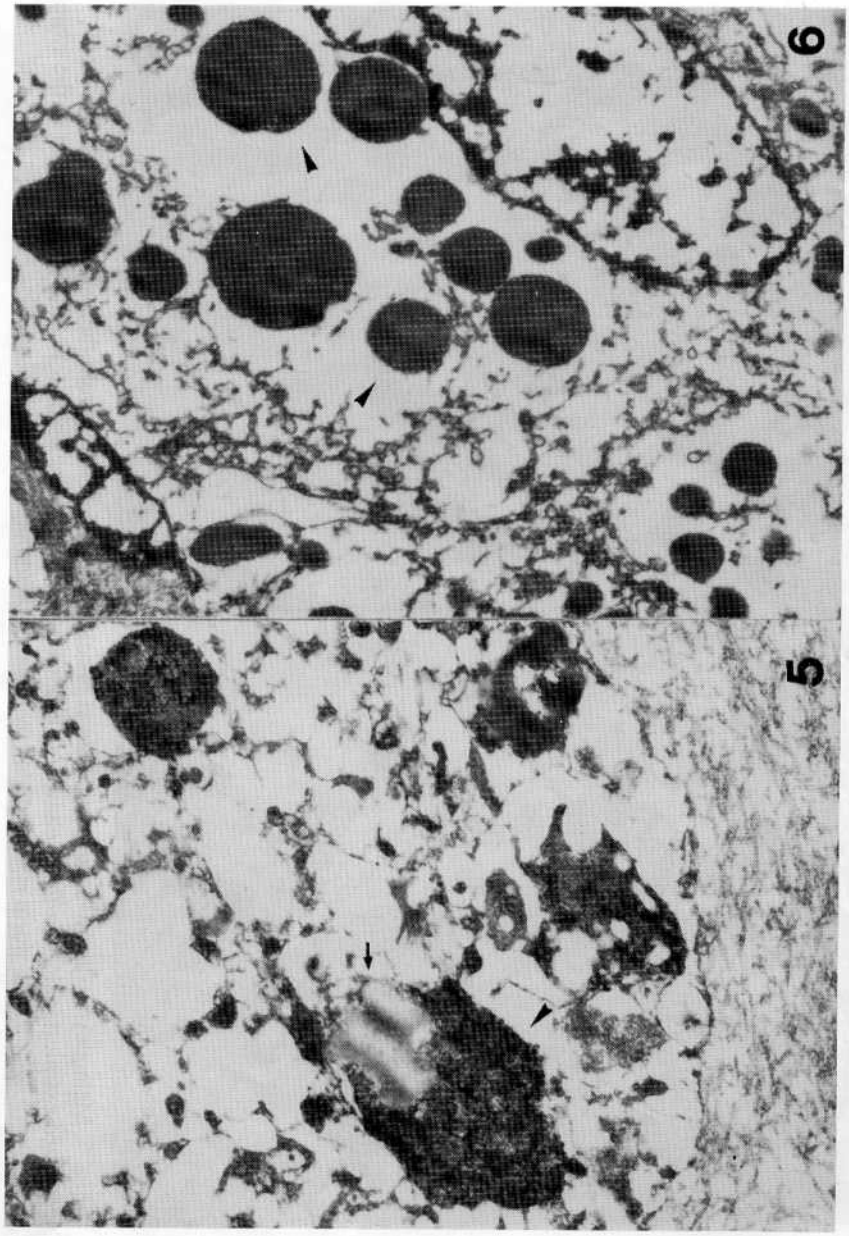

Fig. 5. Electron microscopic section of a macrophage. Inside the cytoplasm note amorphous bodies containing haemosiderin (arrow-head) and lipoid material (arrow). The round body is built up of pigment. × 12,800

Fig. 6. Electron microscopic section of a melanomacrophage. Round-shaped melanin-pigmented material (arrows) is located in the cytoplasm. × 12,800

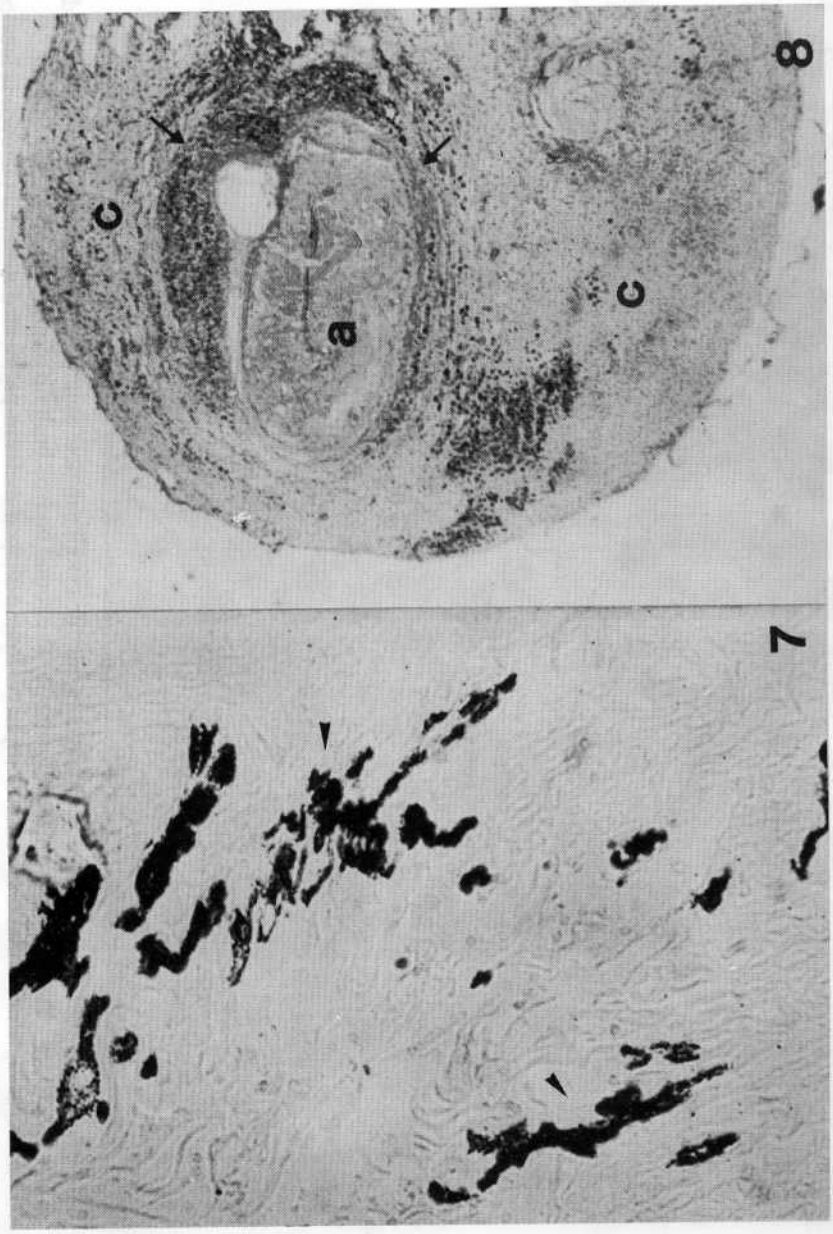

Fig. 7. Melanocytes (arrow-head) in unstained histological section made from the swimbladder serosa. × 800

Fig. 8. Necrotized extraluminal *Anguillicola* adult (a) located in the connective tissue (c) of the posterior part of the swimbladder. The worm is surrounded by melanomacrophages (arrow). H. and E., × 28

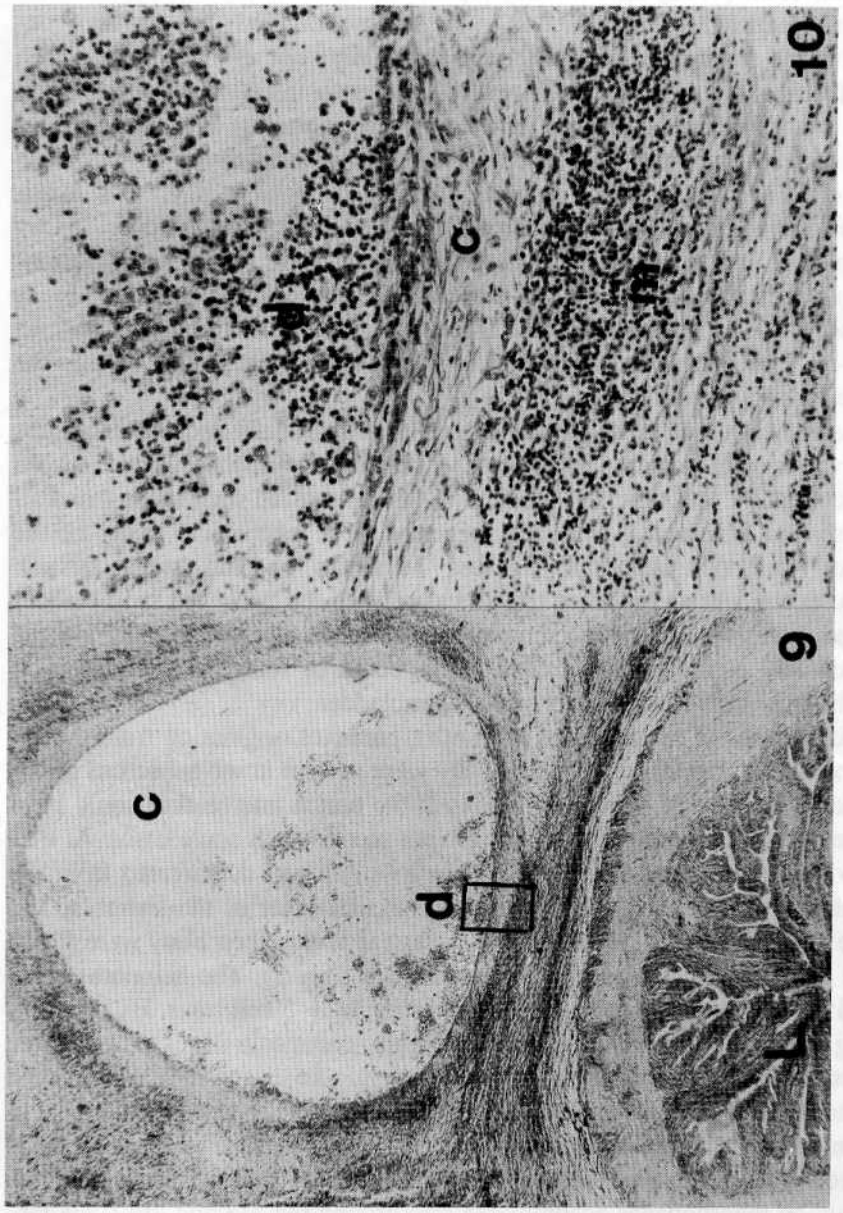

Fig. 9. Remnants of an extraluminal female *A. crassus*. In the cyst (c) the place of the lysed parasite is filled by serum and cell debris (d) of parasite origin. The lumen (L) of the swimbladder is inapparent. H. and E., × 25

Fig. 10. Enlarged part of Fig. 9. No cuticle of the worm is seen. Cell debris (d) of the worm is in direct contact with connective tissue of the subserosa. The latter has been infiltrated by mononuclear cells (m). H. and E., × 250

No bacteria were seen in the connective tissue layer surrounding the parasites using Brown-Brenn and Ziehl-Neelsen stains. By Perls staining, no haemosiderin was detectable. Some of the brown-black pigment stained red with Ziehl-Neelsen and dark red with PAS. Hydrogen peroxide treatment bleached the pigment contained by macrophages surrounding the parasite. Subsequently part of the bleached pigment stained red with oil-red, and haemosiderin was detected in some macrophages by Perls stain (Fig. 4).

Electron microscopy revealed granules composed of haemosiderin and a lipoid material in the cytoplasm of some macrophages, and melanin granules were also detected (Figs 5 and 6).

Black pigment was found not only in macrophages but also in melanocytes (Fig. 7). These cells were located in the blood vessel walls, in the tunica propria and in the serosa either individually or in groups. They differed from the macrophages (Fig. 3) both in shape and in the fine-grained black pigment they contained which did not stain by Ziehl-Neelsen nor by PAS, was not affected by hydrogen peroxide treatment, and was negative even by the Perls stain.

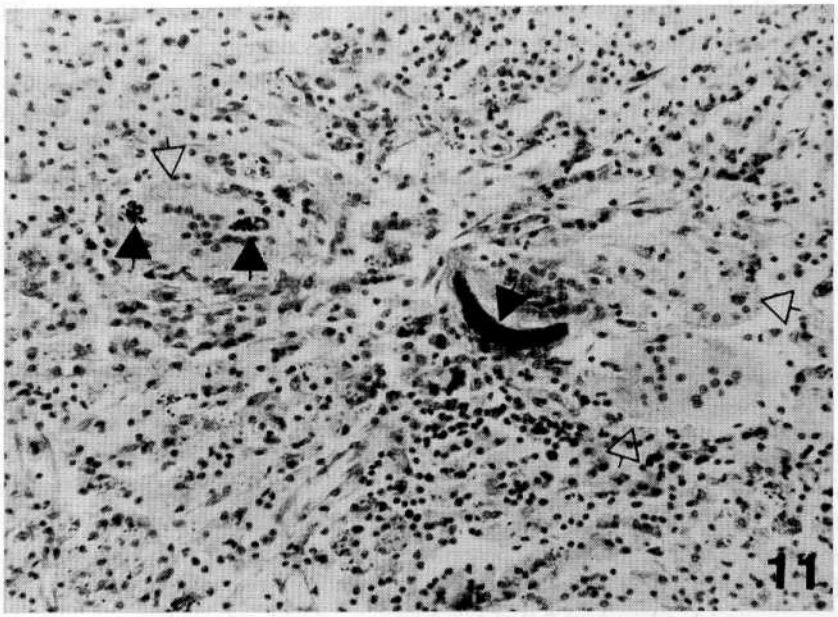

Fig. 11. Subserosa of the swimbladder. Giant cells of foreign body type (empty arrows) have been formed around 2nd stage larvae (arrow) penetrating this layer from the lumen through the damaged tunica interna. H. and E., × 250

In more advanced cases the helminth was located in the caudal part of the swimbladder, distant from the lumen (Fig. 8). These helminths had already died; both their cuticle and their inner organs had completely degenerated and their former structure was completely unrecognizable. Areas containing necrotic helminths were surrounded by a compact connective tissue consisting of 4–5 pale-staining layers. On the side of the demarcating connective tissue facing the helminth there was nuclear debris, while on its outer surface masses of mononuclear cells were seen. That layer was surrounded by a broad pigmented zone also in that case, which — as in the case described above — consisted of loose connective tissue containing masses of melanomacrophages that had entered the serous lacunas. That pigment also stained as described earlier.

In the most advanced cases, evidence for the involvement of the abnormally located helminths could be inferred only from the presence of worm debris in the connective tissue capsule or from the cyst that developed in the helminth's place (Fig. 9). In those cases the lumen of the swimbladder was completely collapsed and contained no air. The subserosa contained a cyst that had an enormous lumen and a wall consisting of 6–8 layers of compact connective tissue. Besides the amorphous and pale-staining stroma, the cyst contained probably helminth-derived cells of degenerated cytoplasm and nuclear debris (Fig. 10). Here and there, the outlines of helminth eggs were still discernible in the lumen of the cyst. The innermost layer of the connective tissue capsule was degenerated. Numerous mononuclear elements had appeared in the compact connective tissue, and these cells could be seen in both the subserosa and the serosa. Macrophages appeared also in the interstitial spaces; however, these contained only brown pigment. The pathological process was aggravated by the penetration into the serosa and subserosa of numerous 2nd stage larvae which were surrounded by macrophages, mononuclear cells and also by giant cells (Fig. 11). The pigment showed staining properties similar to those reported for the pigment contained by the macrophages.

Discussion

These cases demonstrate that healing lesions of a recovering severe anguillicolosis are frequently accompanied by complications associated with pronounced damage to the swimbladder wall. Such lesions may develop primarily in the wake of the swimbladder wall rupture described by Liewes and Schaminee-Main (1987) and Kamstra (1990). Thomas and Ollevier (1992) frequently observed thickened (fibrotic) swimbladders but failed to observe swimbladder rupture and helminths freely located in the abdominal cavity. In the cases examined in this study, we did not observe the swimbladder rupture or atrophy, as did Liewes and Schaminee-Main (1987) and Kamstra (1990). However, based upon the adult helminths seen in extraluminal position and the presence of 2nd stage larvae in the tissues, we as-

sume that the local injury of the swimbladder epithelium and muscular layer is much more common than indicated from our dissections. Tissue injury and degeneration of the tunica interna of the swimbladder probably enable helminths, eggs and larvae situated in the lumen to penetrate into the loose, extensible connective tissue, driven by the enhanced intraluminal pressure. Accompanied by the penetrating helminths are tissue and parasite debris and other inflammatory breakdown products which are also translocated into intercellular position. Initially only the tissue debris while later on also the necrotic parasites, 2nd stage larvae and eggs behave as extraneous material, and elicit a foreign-body type giant cell reaction. This stimulates the eel's immune response to segregate and then remove the foreign materials. Tissue enzymes are likely to play the main role in the breakdown of helminths. The first sign of these reactions is the formation of a limiting connective tissue layer around the parasites, with the presence of pigment-containing macrophages that assemble to resorb and remove the foreign material. As the formation of the collagenic fibres takes at least 7–9 days, the pathological processes observed must have started earlier than that. However, parasites could not have penetrated the swimbladder wall more than 8–10 weeks earlier, as during that amount of time connective tissue tends to completely fill up tissue gaps in the destroyed area.

The formation and nature of the pigment deposited in the macrophages are not known in sufficient detail. In agreement with Roberts (1975) we believe that cells containing black pigment in fish (and thus also in the eel) can be classified into at least two types. The black pigment found in the serosa covering the swimbladder, in the blood vessel walls and in the tunica propria is situated in melanocytes, cells which get to these sites as a result of processes associated with aging. In contrast, the brown-black pigment found in the vicinity of the parasites results from the activity of macrophages involved in resorption and removal. That pigment mainly consists of phagocytosed lipoid-like substances, lipofuscin and a small amount of haemosiderin. The degree of pigmentation depends on how advanced the process is. In new haemorrhagic processes always the haemosiderin-containing macrophages while in more chronic cases those containing a darker pigment are encountered more frequently. The latter probably contain more or less oxidized lipofuscin, as suggested by Roberts (1975). As a result of the host reaction induced by the adult helminths and by the 2nd stage larvae, in such cases the 3rd and 4th stage larvae migrating in the swimbladder also undergo necrosis and become surrounded by pigment-containing macrophages. In all likelihood, immunobiological events may be involved in the processes described; this hypothesis, however, requires further study. Although areas infiltrated by mononuclear cells can undoubtedly be found also around the helminths, the appearance of these cellular elements indicates primarily the presence of 2nd stage larvae that have penetrated the tissues. In severe infection, foreign body type giant cells appear in the areas infiltrated by mononuclear cells. These cells indicate injury of the limit-

ing host cells. However, the possible involvement of bacterial activity can neither be ruled out, even if no bacteria could be demonstrated by Braun-Brenn and Ziehl-Neelsen staining.

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