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Fish Pathology

HIGH MORALITIES IN THE PERCH, *PERCA FLUVIATILIS* L. ASSOCIATED WITH EXTENSIVE *GRANULOMA* FORMATION

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Mass moralities were ancountered in *Perca fluviatilis* from Axwell Park Pond in Northumberland. It was know from a previous study (Ali 1984) that this population has a high incidence of "liver cysts". At the time of this morality fish had numerous "cysts" in and on the liver, the kidneys, apleen and heart and within the mesenteric tissues. Histological studies showed that these cysts were granulomas, with loci the nature of which could not be determined. At this time the infection was clearly of an epizootic nature, the high incidence approximately 60% falling to 2–3% after a population crash.

Since the original description granulomas have been observed at two other locations in perch and dace. The cyclical nature of the infection, the unpolluted and widaly separated habitats and the immunological natura of the response with secondary granulomas being produced suggest that the stimulus is biological in origin.

INTRODUCTION

In a survey of the parasites of perch from Northumberland and Co. Durham, fish from Axwell Park Pond and Rothley Lake were found to contain structures described as "Liver Cysts" by Ali (1984). These "cysts" which were pale cream in colour were clearly visible on the surface of the liver and varied in size from 360–520μ in diameter. Their incidence was high in fish from Axwell Park (53.6%), lower at Rothley Lake (21%) and not reported from fish in other study areas.

Andrews (1977) also recorded "capsules" in the livers of perch from Llyn Tegid. From

their description Ali assumed that these structures were the same as those he had reported. Both workers speculated on their possible origin considering mycobacteria, microsporidia or encysted stages of various helminths to be responsible. No conclusions were reached concerning their nature or origin.

In February 1984 a report was received of a high mortality of perch in Axwell Park Pond. Precise numbers were unobtainable but enquiries indicated that over several days a few hundred fish may have died, many having been picked up by birds but subsequently discarded uneaten in the surrounding environment. It was decided to re-examine fish from this lake in order to establish, if possible the cause of these mortalities.

MATERIALS AND METHODS

Fish obtained following the reported high mortality were maintained in aquaria; a number of these were clearly in poor condition and deteriorated in a matter of hours. These were examined immediately; the livers contained numerous large cysts as did other tissues, particularly the anterior mesenteric tissues. Accordingly livers, the mesenteric tissue, adjacent digestive tract and other major organs were removed and fixed in neutral buffered formalin. Material for routine microscopical examination was embedded in Fibrowax (Gurr), sectioned at 6μ and stained with H & E and Mallory's Triple Stain. Subsequently sectioned material was stained by the following methods PAS, PTAH, PAF/Van Giesson, Ziehl Nielson, Grams, Lendrum's acid picro-mallory.

RESULTS

General Observations

Several distinct types of distribution of cysts were observed. The most common condition, found over a period of several months was the presence of 1–2 cysts on the surface of the liver. These were pearly cream in colour and their maximum diameter did not exceed 2 mm. These were encountered in fish 2–5 years of age; in the younger fish the "cysts" were loosely attached to the serosal coat of the liver and were readily detached. In older fish the cysts had become recessed into pits but were also easily detachable, fig. 1. In this category cysts were not encountered in other parts of the anatomy.

Male fish 6 years of age or more were found in which a single cyst had become totally enclosed in a fluid filled space within the liver. These fish again appeared normal in other respects.

Fish obtained at the time of the high mortalities had numerous cysts on and embedded within their livers, the latter being usually associated with the hepatic blood vessels. Numerous small cysts were apparent in the anterior mesenteric tissues, on the surface of

the kidneys (Fig. 2) and on the heart. Tissues such as the gut walls and body musculature appeared to be free from cysts.

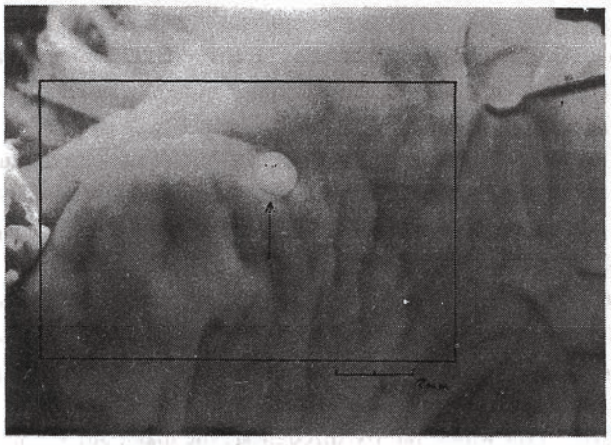

Fig. 1. Partially recessed granuloma (arrowed) in ventral surface of liver.

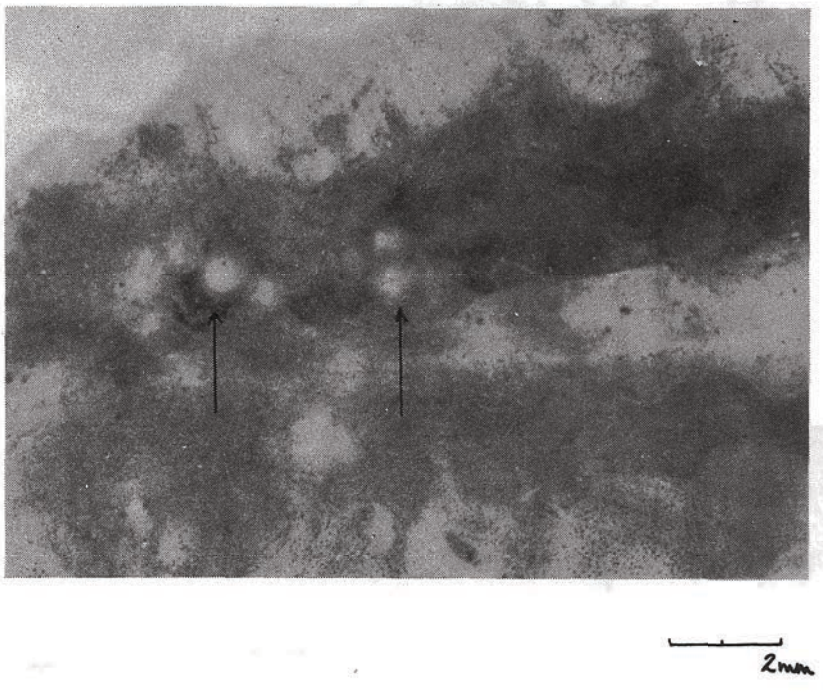

Fig. 2. Numerous small granulomas (arrowed) on surface of kidney after removal of the peritoneum.

Microscopic and Histological Examination

Microscopic and Histological Examination

Sections of cysts from the liver demonstrated that they were granulomas fig. 3 & 4. Fig. 2 demonstrates their superficial nature in fish which did not have granulomas in other tissues while fig. 3 a horizontal section from a fish. obtained during the high mortalities shows that the granulomas have become numerous, are partially embedded in the liver and several are multilocular.

The organisation of the granulomas is complex but remarkably similar in all cases examined and this can be seen in Fig. 4. There is a central locus (a) which has invariably become necrotic surrounded by a number of layers of collagen or collagen derivatives. Layer (b) is divisible into two zones on the basis of its staining reactions, Lendrum's acid picro-mallory indicates that it consists of collagen and fibrin. Three narrow layers (c) (d) and (e) are mostly collagen though (d) also contains fibrin. Layer (f) is the widest, lightly staining and is basically collagen; the outermost layer (g) is primarily composed of fibrin. Sections stained with PAS, PTAH or Gordon and Sweet's reticular stain demonstrated the presence of reticular fibres which ramify throughout the mass, but are more numerous in the central area.

The layered organisation of the granulomas is demonstrated most clearly by staining with H & E1,& Mallory's trichrome & fibre types by the other staining procedures

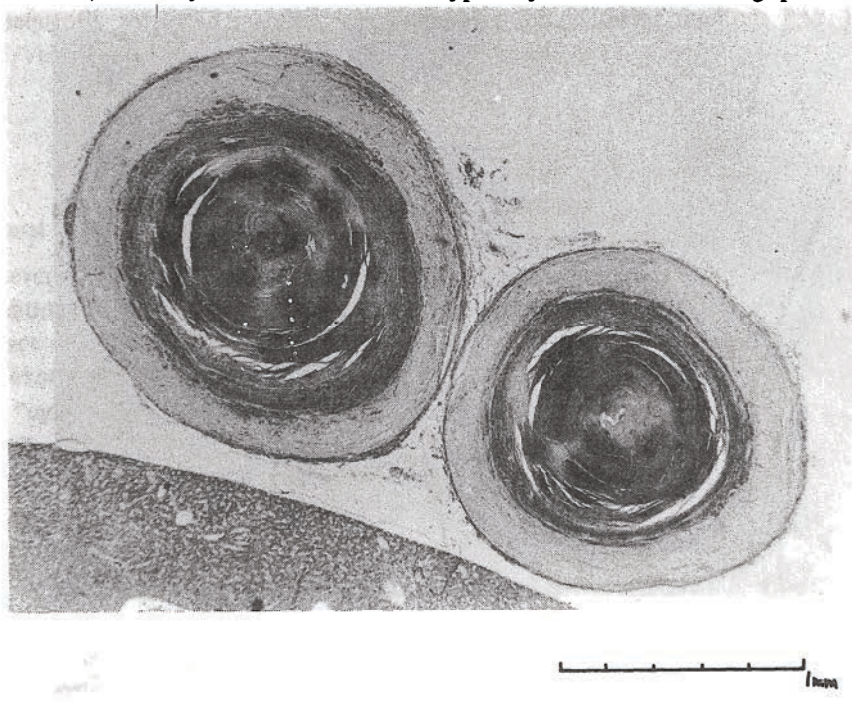

Fig. 3. Vertical section of two superficial granulomas on ventral surface of liver, H & E.

employed. The most marked differentiation is seen after the use of PTAH, see fig. 5 in which layer (f) is clearly demarked from the other layers in that it stains lightly pink indicating that it is recent and unmodified collagen: Reticulin and older collagen staining brown/red is abundant in layer (g) ramifying through (f) into the central mass.

Sections stained with Zeigh Neilson and Grams did not demonstrate the presence of any types of bacteria. Similarly PAS did not demonstrate the polar capsules of microsporidians. Other staining procedures e.g. Giemsa did not show the presence of protozoans.

Sections of the anterior mesenteric tissues taken from fish in which the condition had become acute reveal the presence of numerous small granulomas, (fig 6) all having the same constant organisation. In fish usually at the point of death the mesenteric granulomas had become multilocular and large necrotic areas had developed fig. 7. The granulomas are relatively incompressible considerable pressure being necessary to cause rupture, when they shear at the interface of layers (e) and (f). The outer layers of the

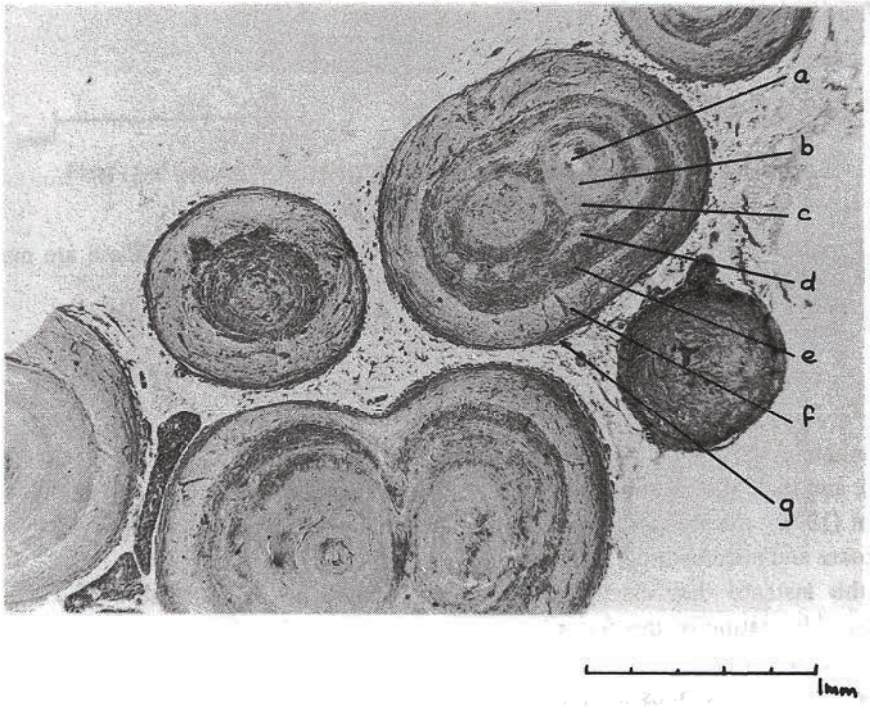

Fig. 4. Horizontal section of partially recessed granulomas on liver surface from a heavily infected fish, H & E.

a – necrotic locus; b – first connective tissue layer; c – first complex layer, d – second connective tissue layer; e – second complex layer; f – thick compound layer; g – connective tissue capsule.

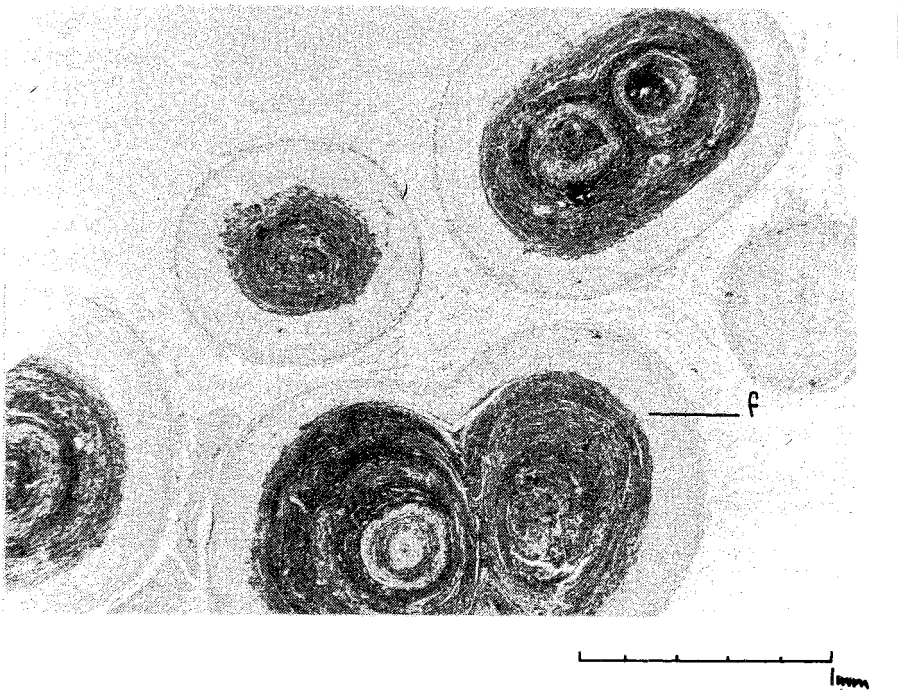

Fig. 5. As Fig. 3, stained with PTAH to show differentiation of outer layer (f).

central mass so released contained numerous small secondaries fig. 8. These are much more numerous in older granulomas.

DISCUSSION

Granulomas are frequently encountered in both marine and freshwater fish, Amlacher (1976), and may arise in response to a wide range of stimuli, synthetic diets, Dunbar and Herman (1971); silicaceous diatoms, mycobacterium tuberculosis, fungi Roberts (1978); sporozoans and microsporidia Amlacher (1976).

In this instance they clearly have a central focus which has illicited a very strong response. The nature of this focus is not apparent since it has degenerated and in moore advanced granulomas has become highly nectrotic. Sectioned material stained by the PAS and Ziehl Neilsen methods did not demonstrate the presence of either microsporidia on mycobacteria. A nonbiological origin seems unlikely however since the condition was observed from two widely separated and different types of habitat. Additionally since the original observation of Ali (1984) we have encountered these types of granulomas in perch and dace from other habitats in Northumberland.

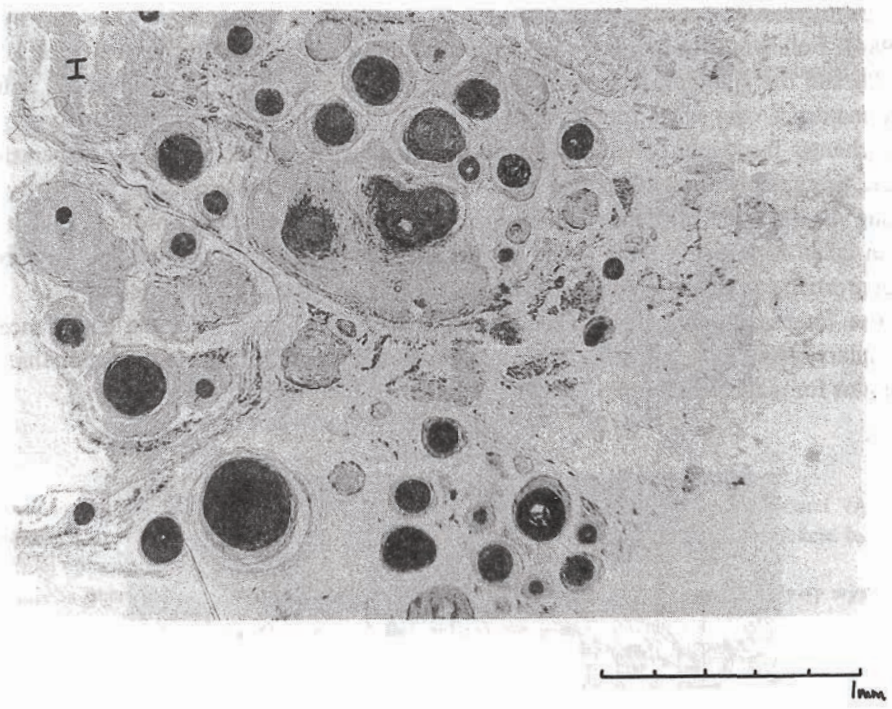

Fig. 6. Section of anterior mesenteric tissue showing numerous small granulomas, H & E. I – wall of intestine.

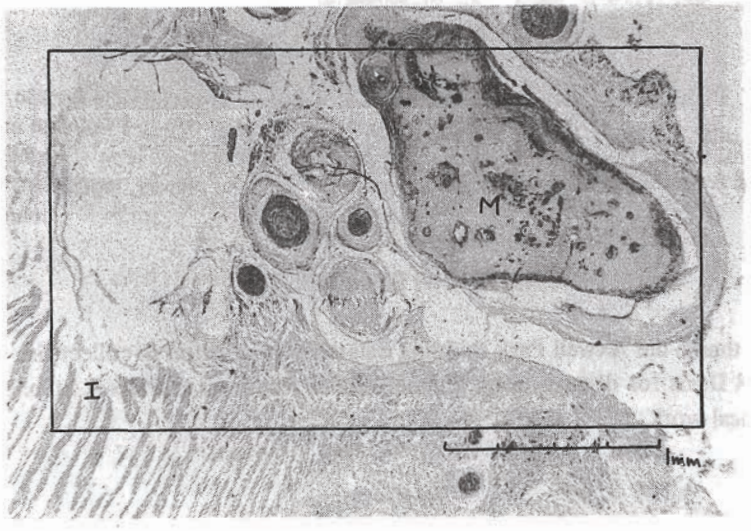

Fig. 7. Section of anterior mesenteric tissue from fish in terminal stages of disease. Granulomas have largely replaced mesenteric tissue and have become multilocular and necrotic, H & E. M – multilocular granuloma, I – wall of intestine

The incidence of the original occurrence at Axwell Park was reported to be 58% by Ali (1984). Following the period when high mortalities were reported the incidence fell to 2–3% and the granulomas were of the first type i.e. one or two of a superficial nature. The incidence of a single totally enclosed granuloma in older fish more than 6 years did not change throughout the study period, although the sample size was comparatively small; presumably these older fish have successfully isolated the inducing factor. By the spring of 1987 the incidence had risen to 12% but recent samples indicate that it has again fallen to a low level. It would appear that the condition is cyclical and produces a high mortality in the perch population.

The intense immunological type of response shown by the fish, the occurrence of secondary granulomas and the cyclical nature of the condition would suggest that the stimulus for granuloma formation is in fact biological in origin.

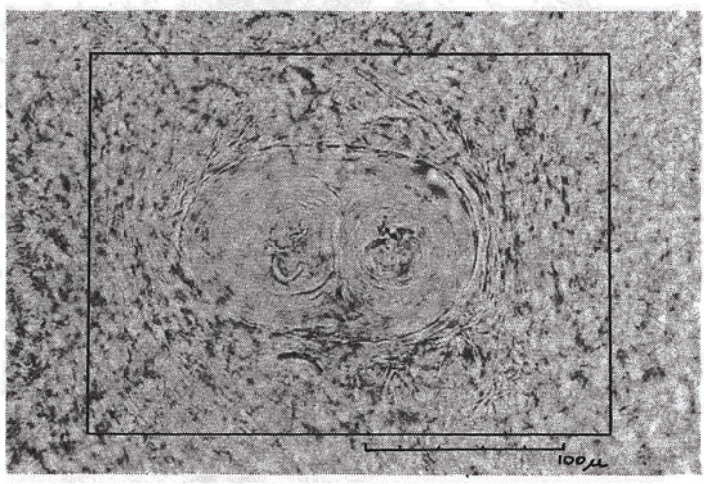

Fig. 8. Formation of secondary granulomas within layers of primary, unstained.

ACKNOWLEDGEMENTS

We wish to thank the Axwell Park Building Estate for permission to collect fish from the pond; Dr BR Davis for discussion on granulomatous responses and Mrs V Stephenson for the histological work.

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STRESZCZENIE

Autorzy opisują silne zarażenie okoni w stawach Axwell Park w Northumberland (Anglia), dochodzące do 60% osobników a nawet doprowadzające do 2–3% śmiertelności. Infekcja była już wcześniej opisywana przez Ali (1984).

Choroba objawia się występowaniem różnej wielkości cyst, głównie w wątrobie. Cysty występują na powierzchni jak również i we wnętrzu, w miąższu. Poza tym w nerkach, śledzionie, sercu a nawet na otrzewnej. Histologicznie wykazują koncentryczną budowę ziarniaków pasożytniczych, w których już niepodobne określić czynnika patogennego. Niewątpliwie są one pochodzenia epizootycznego, wszystko wskazuje na ich biologiczny charakter. Dotychczas nie udało się stwierdzić ich pochodzenia grzybiczego ani pierwotniaczego. Wielkość cyst dochodzi 2 μm średnicy.

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