TUBERCULOSIS OF POIKILOTHERMS*

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INTRODUCTION

The name tuberculosis is used to describe a chronic disease of Man and other animals caused by certain species of bacteria belonging to the genus *Mycobacterium*. Such bacteria are termed 'acid-fast' due to their property of resisting the decolorizing action of various mineral acids and of alcohol.

The disease is typically characterized by the presence of tubercles, or cellular lesions resulting from the irritating action of the bacteria at the site where they are ingested and held by the phagocytes. The tubercle itself may undergo a process of progressive caseation, which commences in the centre and extends outwards. It is quite common to find cases where the tuberculous part has been subsequently infected by pyogenic organisms which produce a suppuration. Where this occurs, an abscess is formed, which results in an ulcer in the event of its occurring on the surface of an organ. The bacteria may be liberated into the bloodstream from the primary tubercular focus, by means of which they reach other bodily organs such as the spleen, kidney, and liver. This condition is known as miliary tuberculosis.

Since the discovery of *Mycobacterium tuberculosis* as the causative organism of human tuberculosis by KOCH in 1882, a number of workers have observed diseased conditions of cold-blooded animals caused by other acid-fast bacteria. The purpose of this paper is thus to briefly present these findings in the form of a short review.

There exists a considerable difference between the pathology of the disease as seen in Man and that observed in poikilotherms, as also in the morphology of the causative agents. This difference has led PARISOT and WOOD (1960) to propose the name 'fish mycobacteriosis' in place of 'fish tuberculosis'. Whilst such a change may be correct for purely technical reasons, the older terminology has been maintained herein since it is felt that it may be more familiar to the reader.

TUBERCULOSIS IN FISH

a) FRESHWATER FISH

The disease was described for the first time in freshwater fish by BATAILLON, DUBARD, and TERRE (1897). These workers observed it in a carp (*Cyprinus carpio*) living in a pond contamina-

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ted by the sputa of a human subject suffering from tuberculosis. They described the presence of a tumour roughly the size of a pigeon's egg, greyish-white in colour, situated on the ventral surface of the abdominal wall, near to the ovary. This tumour showed all the signs of being a tubercle, and was seen to contain numerous acid-fast bacilli. By means of experimental studies, it was shown that the organism isolated was pathogenic to the snake, lizard, and frog (in which it led to the production of a disseminated disease), but not to the carp, from which the original strain had been isolated. The morphological characteristics of this organism, together with the source from which it had been obtained, led to its being classified as *Mycobacterium piscium* by Bataillon, Moeller, and Terre (1902).

Von Betegh (1910a, b), in a study of the action of *Mycobacterium piscium* to marine fishes, showed that it was non-pathogenic to them. An interesting result obtained by this worker was that it is pathogenic to the eel (*Anguilla vulgaris*), a catadromous species which migrates to the sea to spawn. In the eel, *Myco. piscium* produced a local lesion of short duration, from which it was not possible to re-isolate the organism. Von Betegh suggested therefore that the eel may constitute an intermediate vector.

Mae (1922) isolated an acid-fast bacillus from the saps of Lausanne, and named it *Mycobacterium aquae*. He studied its action on goldfish (*Carassius auratus*). Schreitmüller and Lederer (1930) observed a case of tuberculosis in the same species of fish maintained in the Frankfort Zoo, and noted that the eyes, intestine, and liver were the principal organs affected.

Jahnel (1940) found cases of tuberculosis in various species of fish, observing acid-fast bacilli in the middle of tubercular granules. These granules may necrotize in time to form yellowish (or at times black) bodies which appear to be composed of epithelial cells. It is of interest to note that in smears stained by the method of Ziehl-Nielsen, these necrotic bodies may be seen even where acid-fast bacilli are not observed.

Baker and Hagan (1942) studied a case of tuberculosis in the tropical fish *Platypoecilis maculatus*, where the symptoms shown by the diseased individuals varied between emaciation, listlessness, and loss of colour and appetite, to definite ulceration of the mouth and dorsal fin regions. Smears prepared from these ulcers, as also from the liver, spleen, and gills, showed the presence of acid-fast bacilli, and cultures were obtained from the kidney and spleen. The bacterium isolated was pathogenic for the goldfish, and was described as *Mycobacterium platypoecilis*.

Nigrelli (1943, 1953) described tuberculosis as a disease of the neon tetra (*Hyphessobrycon innesi*), in which there was a slight inflammatory response in the affected fish. A more recent study of this isolate by Ross and Brancato (1959) has shown that it is a strain of *Mycobacterium fortuitum*.

Besse (1949) made a thorough study of fish tuberculosis, and observed a turbidity of the eyes and a tendency to melanism in the body. This same worker (Besse, 1949, 1952) has differentiated five distinct varieties of the disease, in accordance with the symptoms observed. These varieties are:

a) *Macropodus*-type;
b) ascitic type of *Betta splendens*;
c) exophthalmos of *Brachydanio*;
d) caseous granuloma in the Cyprinidae;
e) emaciation in the Poeciliidae;

Of a total of 288 exotic fish which he examined, Besse observed acid-fast bacilli in 243 of them.
or in 80% of all those which he studied. He believed furthermore that certain families are more susceptible to the disease than others, giving as an example the Anabantidae, Atherinidae, Centrarchididae, Characidae, Cyprinidae, Cyprinodontidae, and Poeciliidae. The species which he considered to be the most susceptible to tuberculosis are *Hyphessobrycon flammeus* and *Pristella riddlei*.

Besse isolated an acid-fast bacillus from the Paradise Fish (*Macropodus opercularis*) which he named *Mycobacterium anabanti*. It was shown experimentally that this organism was pathogenic to the rainbow perch (*Eupomotis gibbosus*).

Earp, Ellis, and Ordal (1953) encountered acid-fast bacilli in some fifty Chinook salmon (*Oncorhynchus tshawytscha*), and it is now known with certainty that the pink salmon (*Oncorhynchus gorbuscha*), Chum salmon (*Oncorhynchus keta*), silver salmon (*Oncorhynchus kisutch*), blue-back salmon (*Oncorhynchus nerka*), and the rainbow trout (*Salmo gairdneri*) are also subject to the disease. Many of the strains isolated from salmonids up to the present have been studied by Ross (1960), by whom certain of them were placed into the new species *Mycobacterium salmoniphilum*.

Ross (1959) showed that infections of salmon by acid-fast bacilli are of wide distribution among hatchery reared stocks, reaching a proportion of 62% of all fish examined. He also demonstrated that such infections may occur in wild stocks and in natural conditions, although this conclusion was not reached by Wood (1959).

Parisot (1958) has given a comprehensive description of the symptoms to be observed in infected salmonids. The disease is manifest by a general softening of the kidney, greyish-white lesions in the liver (which at times may present atypical miliary picture), and under-development of the genital organs. A study of the histopathology of piscine tuberculosis has been made by Parisot and Wood (1960) with reference to the salmonids. In the young fish, a massive infection of the adipose and fibrous tissues is to be seen, which is even more prominent in the pancreas. In the liver, spleen, and kidney, the infection is generally intracellular in its distribution, and the bacteria may be observed in masses of general distribution throughout the organs. No acid-fast bacteria were to be seen in the gill tissues of the young fish.

In the adult salmon, the same workers found massive lesions, at times accompanied by a marked caseous necrosis, which affected both the heart and the gills. The liver, spleen, and kidney suffered considerable damage, but no inflammatory reaction was observed.

Bandlow (1959) studied piscine tuberculosis, with particular reference to the symptoms of the disease in tropical varieties. He observed that the swim bladder may be of a whitish colour, and filled with a serous liquid in such a way as to strongly resemble the typical 'swim bladder disease'.

Amlacher (1961) has described the principal symptoms of fish tuberculosis as being:

a) emaciation;
b) loss of appetite;
c) loss of colour;
d) slight raising of the scales;
e) exophthalmos;
f) deformities of the body and vertebral column.

Conroy (1963a) observed a case of the disease in the neon tetra (*Hyphessobrycon innesi*). The diseased fish showed symptoms of emaciation, loss of colour and appetite, and in many cases the presence of cutaneous ulcers was noted in the region of the dorsal fin. Internally, the liver was
covered with minute greyish-white tubercles, and the kidney was completely softened. Acid-fast bacteria were observed in smears from the ulcers, kidney, and liver, and the organism isolated was provisionally considered to be a strain of *Mycobacterium* sp. (Conroy and Valdés, 1963). Further more detailed studies of this isolate by Gordon (1963a) have indicated however that the organism is a strain of *Nocardia asterioides*, and it is believed to be the first occasion on which a strain of this bacterium has been isolated from diseased fish (Gordon, 1963b).

Other cases of the disease have been observed in *Trichogaster trichopterus* and *T. leeri* (Conroy, 1964). On this occasion small lesions were observed in the dorsal fin region, and around the mouth, and the caudal peduncle showed lesions in certain instances. The presence of acid-fast bacilli was demonstrated in smears from the ulcers, kidney, and liver, and the organism isolated in pure culture from each of these organs. Whilst the taxonomy of this bacterium is at present undergoing more detailed studies, it is quite possible that it is a strain of *Mycobacterium* sp.

**b) MARINE FISH**

Bertarelli and Bocchia (1910) examined a number of the sea-fish offered for sale on the Italian markets with a view to determining whether or not the disease is present in the sea. They failed to detect acid-fast bacteria however, for which reason they supposed that tuberculosis among marine fish is either extremely rare or not present.

Alexander (1913) and Johnstone (1913) observed the disease in the cod (*Gadus callarias*). The former worker described the presence of a darkish patch on the skin from which he isolated acid-fast bacilli in culture. Due to the fact that the isolate was only able to grow in the presence of a coccus however, the same was never fully identified. Johnstone found typical lesions of a tuberculous nature in histological sections prepared from the diseased fish. Numerous acid-fast bacilli were observed in the lesions.

A further case of tuberculosis in marine fish was described by Sutherland (1922) who observed it in the halibut (*Hippoglossus vulgaris*). The bacteria were widely distributed in the viscera, and the organism was isolated in pure culture. It was shown to be non-pathogenic to the frog.

Aronson (1926) made a study of the causes of death of various marine fishes maintained in the Philadelphia Zoo. He isolated acid-fast bacilli from the liver and spleen of croakers (*Micropogon natalus*), sea-bass (*Centropristes striatus*), and sergeant-major (*Abudefduf mauritii*). This isolate was highly pathogenic to the frog, and was designated *Mycobacterium marinum*.

Johnstone (1927) encountered a case of tuberculosis in the halibut (*Hippoglossus vulgaris*), in which the kidney was considerably swollen and covered with tubercles of a yellowish colour and somewhat granular in appearance. The ovary was small and markedly underdeveloped, and was shown to contain numerous acid-fast bacteria.

Reichenbach-Klinke (1955) has observed the disease in a number of marine fish, among which are included the garfish (*Belon belon*), the white perch (*Morone labrax or americana*), and Spicara alcedo. Acid-fast bacilli were isolated from all of these.

Conroy (1963b) has described tuberculosis for the Horse Mackerel (*Trachurus picturatus*) from Argentina. The disease was of a caseous type, and acid-fast bacteria were observed in smears taken from the affected parts.

**TUBERCULOSIS IN AMPHIBIANS**
LE DOUX-LEBARD (1900) described a case of tuberculosis in frogs, wherein the liver was covered with soft lesions. The same worker concluded that the disease had been caused by the 'piscine type' of KOCH's bacillus.

WEBER and TANTE (1905), during the course of a study on spontaneous tuberculosis of frogs, were able to isolate an acid-fast bacillus of the cold-water type from the water and moss of the tank in which the animals were housed. They concluded therefore that such acid-fast bacteria may exist as saprophytes in close relation with amphibians.

KÜSTER (1905) examined some 500 frogs, and obtained positive cultures of acid-fast bacilli from the livers of three of them. In the diseased animals, the liver was covered with greyish-white lesions. The organism isolated was described as *Mycobacterium ranae*.

BAERTHELEIN and TOYADA (1913), as also LICHTENSTEIN (1920), described cases of the disease in frogs as being caused by acid-fast bacilli, and further described the reactions of their isolates.

BACHMANN (quoted by FIDANZA, 1944) has observed a case of tuberculosis in the salamander (*Salamandra* sp.) in Argentina. The peritoneal cavity of the animal was full of liquid, and the liver covered with typical tubercles. An acid-fast bacterium was isolated, but not identified.

ELKAN (1960) has observed the presence of tuberculosis in the common toad (*Sapo sapo*), for which he described two cases. All of the internal organs were affected, but the disease was most noticeable in the intestine and testes.

The same author similarly observed the disease in the South African clawed toad (*Xenopus laevis*), a single specimen of which was seen to have a small irregular tumour in the coccygeal region. The kidney presented tuberculous symptoms, and caseation was present. Acid-fast bacilli were isolated from this case, and subsequently identified as *Mycobacterium xenopei* by SCHWABACHER (1959).

DARZINS (1950), in Brazil, has described a disease resembling tuberculosis in *Leptodactylus pentadactylus*. An acid-fast organism isolated therefrom was described as *Mycobacterium giae* (VON STUCKRAD, 1955), but more recent work on the same has shown that it is a strain of *Mycobacterium fortuitum* (GORDON and SMITH, 1955).

TUBERCULOSIS IN REPTILES

SIBLEY (1889, 1890) is credited with having been the first to observe tuberculosis in poikilotherms. This worker observed it in Italian grass snake (*Tropidonatus natrix*) which had died in captivity. The animal had a number of small sub-cutaneous tumours, from which acid-fast bacteria were isolated. The organism was named *Mycobacterium tropidonatus*.

FRIEDMANN (1903), whilst performing an autopsy on a tortoise maintained in a sea water tank in the Berlin Zoo, observed whitish lesions of the lung from which he isolated acid-fast bacilli. Experimentally, this organism produced a miliary tuberculosis following injection into the tortoise, lizard, snake, and frog, and was at first called *Mycobacterium testudinis*. Later workers have referred to this as *Mycobacterium freundii*, but it is now more generally known as *Mycobacterium chelonii*.

A naturally occurring case of the disease was observed by ARONSON (1929) in the garter snake (*Thamnophes butleri*), from which an organism described as *Mycobacterium thamnophi* was isolated.

GRIFFITH (1929, 1930) has described a number of cases of tuberculosis in wild animals maintained in captivity. He isolated *Mycobacterium marinum* from four estuarine caymans, and *Mycobacterium marinum* from four estuarine caymans, and *Mycobacterium marinum* from four estuarine caymans, and *Mycobacterium marinum* from four estuarine caymans, and *Mycobacterium marinum* from four estuarine caymans, and *Mycobacterium marinum* from four estuarine caymans, and *Mycobacterium marinum* from four estuarine caymans, and *Mycobacterium marinum* from four estuarine caymans, and *Mycobacterium marinum* from four estuarine caymans, and *Mycobacterium marinum* from four estuarine caymans, and *Mycobacterium marinum* from four estuarine caymans, and *Mycobacterium marinum* from four estuarine caymans, and *Mycobacterium marinum* from four estuarine caymans, and *Mycobacterium marinum* from four estuarine caymans, and *Mycobacterium marinum* from four estuarine caymans, and *Mycobacterium marinum* from four estuarine caymans, and *Mycobacterium marinum* from four estuarine caymans, and *Mycobacterium 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piscium from one freshwater snake. The strains pathogenic to cold-blooded animals were divided by him into three main groups on a basis of their pathogenic action. These groups comprise:

- snake-strain;
- cayman-strain;
- turtle-strain;

The third one attacks principally the lungs, whereas the other two produce granular lesions of the spleen, liver, and skin.

Cases of tuberculosis are not uncommon in reptiles maintained in Zoos (Fiennes, 1961), and a strain isolated from diseased terrapins has been identified as *Mycobacterium fortuitum* by Gordon and Smith (1955).

**DISCUSSION**

The foregoing data serve to demonstrate the fact that tuberculosis is fairly widespread among poikilotherms. It is becoming increasingly realized that the disease is of great importance in pisciculture, as demonstrated by the work of Ross, Earp, and Wood (1959). No attempt has been made in this present paper to describe the morphological and culture characteristics of the acid-fast bacteria isolated from cold-blooded animals, as this is a field which is receiving much attention at this moment.

Any reader who wishes to consult the earlier reviews on the disease is well advised to read the work of Herzog (1902), Alexander (1913), Klofstoek (1920), Küster (1928), Griffith (1930), and Aronson (1938). More recent work on the subject is that of Parisot (1958), and Vogel (1958), both of which authors have made a very comprehensive summary of the disease with special reference to fish.

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ADDENDA

Transmission of tuberculosis in fishes

With reference to the transmission of tuberculosis in fishes, PARISOT & WOOD (1960) observed that heavy infections with acid-fast bacteria were present in young chinook salmon (Oncorhynchus tshawytscha) containing yolk remnants, and they considered the concentration of organisms to be such as to follow closely the anatomical distribution of the yolk. From this observation they concluded that this may be a primary site of infection. ROSS & JOHNSON (1962) investigated the possibility that mycobacterial infections in chinook salmon might be transmitted by the transovarian route or by contact during the time of fertilization. Their results were not conclusive, although they believed that this means of transmission may be a possibility.

CONROY (1966) presented evidence that in Mexican platys Platypoecilus maculatus, a viviparous species, tuberculosis is transmitted by transovarian route. Transverse and longitudinal sections through well-formed but unborn embryos in the ovary of an infected female showed the presence of acid-fast bacilli in the pharyngeal region, and it was concluded that the infection had been acquired via the intra-ovarian fluid. The organism was isolated in culture from the embryos, and shown to be identical with that isolated from the female itself.

Chemotherapy

The literature on fish tuberculosis contains very few references to treatment of the disease with antibiotics and other chemotherapeutic substances. REICHENBACH-KLINKE (1955) attempted to treat the disease in pearl gouramies (Trichogaster leeri) by means of an ointment containing penicillin. This was rubbed onto the surface wounds of the skin, and the condition is reported to have healed completely. It may be that such a treatment is applicable in cases where the disease manifests itself as lepromatous-like skin lesions, as in gouramies, but it would be impractical in other species where it presents a different symptomatology.

VAN DUIJN (1961) described an instance when an epizootic of tuberculosis in black widows (Gymnocorymbus ternetzi) was controlled by means of terramycin. The antibiotic in the form of Terramycin, Animal Formula was added to the aquarium water at a concentration of 227 mgm. crude powder/litre. The fish were treated for three days, when the water was changed. Following the first treatment they became normal in colour, regained their appetite, and swam in a normal manner: the same treatment was repeated twice more, changing the water after each application, and the symptoms finally disappeared.

CONROY & SOLAROLO (1965) demonstrated that a number of strains of acid-fast bacteria isolated from cases of tuberculosis in fish are sensitive in vitro to kanamycin sulphate in relatively low concentrations. Spontaneous cases of the disease were successfully treated by CONROY (1966) using kanamycin, the antibiotic being incorporated into a farinaceous food at a level of 0.01%. The administration of this medicated food led to a rapid and permanent recovery in all of the fishes.
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