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Spontaneous Tuberculosis in Fishes and in Other Cold-blooded Vertebrates with Special Reference to *Mycobacterium fortuitum* Cruz from Fish and Human Lesions

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(Plates I-VI)

Introduction

Interest in tuberculosis in cold-blooded animals was stimulated by a report by Bataillon, Dubard & Terre in 1897 on the disease in carp in a pond contaminated with dejecta from tubercular persons. It was later recognized that the acid-fast bacillus causing tuberculosis in the carp was a distinct species for which the name *Mycobacterium piscium* was given by Bataillon, Moeller & Terre (1902). The fact that this disease is found in fish led to speculations along the following lines: (1) that fish may be carriers of human tuberculosis organisms, (2) that fish, amphibians and reptiles could be used for the transmutation of human tuberculosis bacillus by serial passage, and (3) that these, or the acid-fast bacilli from cold-blooded animals, could be used for the treatment and prevention of human tuberculosis. The controversies engendered by discussions on these topics were reviewed by Vogel (1956, 1958) and by Parisot (1958).

The relatively recent discoveries of atypical human pathogenic species, *Mycobacterium fortuitum* Cruz (see Gordon, 1957) and *Myco. balnei* Linell & Norden (1954), have revived interest in this subject.** These atypical forms, which are also found in fish and in water, together with the acid-fast bacilli causing tuberculosis in the lower vertebrates, show certain basic similarities in morphological, cultural and biochemical characteristics. Of particular interest is the report by Ross & Brancato (1959) that one of the strains of acid-fast bacilli isolated in our laboratory (Nigrelli, 1953) from the Neon Tetra (*Hyphessobrycon innesi*) is the same as *Mycobacterium fortuitum*, a pathogenic species first isolated from human and cattle lesions in South America.

This paper deals with further information on the Neon Tetra strains of mycobacteria, tuberculosis in other fishes in the New York Aquarium and with reports of the disease in fishes, amphibians and reptiles by other investigators.

Tuberculosis in Fishes in the New York Aquarium

Routine examinations of fish in the New York Aquarium and a search of the literature show that tuberculosis in poikilothermic animals is much more prevalent than is generally suspected. The host species are listed in Table IV. Table I lists those host species that were found infected in the New York Aquarium and from which the acid-fast bacilli were isolated and cultured.

Pathology of Tuberculosis in Fishes

Tubercular lesions are found in gills, skin, muscle, heart, kidneys, spleen, liver, pancreas, mesenteries, gonads, eyes and brain. Lepromatous-like macular and necrotic skin and fin lesions are characteristic of tuberculosis in the Three-spot or Blue Gourami (Pl. V, fig. 9). The disease in the Neon Tetra is recognized externally by yellowish discoloration of the usually brilliant red markings on both sides of the hind part of the body (Pls. I & II, figs. 1, 2, 3). In most species there is no external evidence but the disease is recognized internally by the presence of extensive, yellowish adhesions or by numerous miliary-like tubercle bodies in various organs (Pls. II, III, V & VI, figs. 4, 5, 10, 11).

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**See also Clark, H. Fred, & Charles C. Shepard, "Effect of Environmental Temperature on Infection with *Microbacterium marinum (balnei)* of Mice and a Number of Poikilothermic Species. Jour. Bact., 86 (5): 1059-1069. Nov., 1963.

TABLE I. HOSTS FROM WHICH ACID-FAST BACILLI HAVE BEEN ISOLATED
(Cultures maintained at the New York Aquarium)

Species	Common Name	Habitat
Tropical Freshwater spp.		
Hyphessobrycon innesi	Neon Tetra	Peruvian Amazon
Trichogaster trichopterus	Three-spot or Blue Gourami	Tropical Far East
Toxotes jaculator	Archerfish	Philippines & Far East
Tropical Marine spp.		
Plectorhynchus sp.	Sweet-lip	Pacific coral reefs
Premnas aculeatus	Spiny Clownfish	Pacific coral reefs
Amphiprion percula	Common Clownfish	Pacific coral reefs
Amphiprion akallaopsis	Skunk Clownfish	Pacific coral reefs
Amphiprion xanthurus	Chocolate Clownfish	Pacific coral reefs
Amphiprion laticlavius	White-saddle Clownfish	Pacific coral reefs

In the Climbing Perch and in the Goldfish the lesions appear as numerous pearl-like bodies in the liver, kidneys and mesenteries (Pls. V & VI, figs. 10, 11). Emaciation, exophthalmia, lordosis and other body abnormalities may or may not be associated with the disease.

Histopathologically, tuberculosis in fishes resembles the tubercular picture in warm-blooded animals but with the following differences: milder or no inflammatory reactions, greater fibrous development, absence of typical giant cells and little or no caseation (Pls. II & IV, figs. 3, 4, 7, 8). The term "hard tubercle" (Pls. V & VI, figs. 10, 11) appears appropriate for the lesions in the Climbing Perch, Goldfish and several other species. These tubercles are formed by coalescence of several smaller units (Pl. VI, fig. 11). In some freshwater fishes some degree of caseation may occur but typically the tissue reaction consists of loosely organized masses of semi-necrotic cells with groups of acid-fast bacilli in the core of epitheloid-like elements. (Pls. II & IV, figs. 3, 7, 8).

The term "mycobacteriosis" was suggested by Parisot & Wood (1960) as being more appropriate for tuberculosis in fish. The suggestion was based on the absence of typical inflammatory responses to the infection in salmonoid fishes. However, since this is not true for fishes generally and since typical tubercles and other classical tissues reactions are present, tuberculosis is a valid term for the disease in fish as well as in other cold-blooded animals.

TRANSMISSION

Tuberculosis in cold-blooded vertebrates can be induced by parenteral injections of mycobacterial suspensions. However, it is generally conceded that the natural mode of infection is by ingestion of the organisms directly from the water, by eating infected tissues or contaminated feed. Such origins of the infective organisms have been experimentally demonstrated for tuberculosis in tadpoles (Nonidez & Kahn, 1934, 1937)[1], in the Mexican platyfish (Baker & Hagen, 1942) and in snails (Michelson, 1961)[2]. The increase in incidence of tuberculosis in hatchery-maintained salmon and trout is directly related to the increased usage of infected salmon carcass as feed for young salmon, a deterimental hatchery practice of which fishery biologists were unaware until recently (Wood & Ordal, 1958). The possibility of transovarian transmission or of the spread of the infection by contamination of sperms and eggs during stripping of mature stock fish or the spawning run fish was also considered. Although the results on the salmon studies were inconclusive (Ross & Johnson, 1962), our observations of tuberculosis in embryonic platyfish and guppies certainly suggest transovarian transmission as a definite possibility. In addition, the entry of infective organisms through lesions of the skin and gills caused by parasites or by mechanical injury should also be considered.

There is no information on the mechanism of spread of tuberculosis in fish. If the portal of entry is through lesions in the skin, the route must be through the lymphatic system or through the blood stream. Since the preponderance of evidence indicates that the infection is brought about by ingestion, the spread must then take place through the gastro-intestinal tract. Just how this is accomplished has not been determined, especially since we have not observed tubercular lesions in this organ (Pl. II, fig. 4).

[1]Jose F. Nonidez & Morton C. Kahn. Tuberculosis induced in the tadpole by feeding. Proc. Soc. Exp. Biol. & Med., 31: 783. Experimental tuberculosis infection in the tadpole and the mechanism of spread. Amer. Rev. Tuberc., 36: 191.

[2]Edward H. Michelson. An acid-fast pathogen of fresh water snails. Amer. J. Trop. Med. & Hyg., 60: 423.

Isolation and Cultural Procedures

Isolation of the acid-fast bacilli from pathological materials in fishes is relatively simple. In the Neon Tetra, for example, the disease is readily recognized by the yellowish discoloration of the red markings. The fish is anaesthetized with urethane (methyl carbamate) or with MS-222 (tricaine methanesulphonate, Sandoz), and the lateral body wall in front of the vent is cauterized with a red-hot scalpel; the flesh adhering to the blade results in a raised flap, exposing the body cavity. A sterile bacteriological needle is plunged into the lesions, preferably into those in the kidneys, and the material is then transferred to slants of media ordinarily used to culture mycobacteria. In our laboratory, slants of Dorset's or Petroff's egg agar is used successfully. It is our experience that with this simple technique pure cultures of acid-fast bacilli are usually obtained. If difficulty is encountered, however, a portion of the disease tissue is digested with 4% sodium hydroxide for 15-30 minutes to destroy the contaminating organisms. The digest is neutralized with HCl and centrifuged. The sediment is then inoculated on one of the media suggested above. The digestion time with NaOH can be varied, depending on the sensitivity of the bacilli and the quantity of tissue to be digested. Diseased tissue can also be treated with trisodium phosphate, oxalic acid or with 6% sulfuric acid. Other techniques and media for handling tubercular fish tissue are suggested by Westgard (1959), and those found in any standard reference on methods in pathology and bacteriology may also be used. Since contamination of fish material may reach a high level in a short time, it is suggested that moribund fish be sacrificed and used for the isolation procedures.

Some Characteristics of Mycobacteria Isolated from Fishes in the New York Aquarium

The mycobacteria are easily demonstrated in smears of the skin and organ lesions by the Ziehl-Neelsen staining method (Pls. I & III, figs. 2, 5). The bacilli, which may or may not be seen within macrophages, are pleomorphic slender rods varying in length from 3 to 7 microns and show the bead-like constituents in the cell when stained, or when seen in electron microscope preparations (Pl. III, fig. 6). The organisms grow slowly as raised colonies on glycerol and egg agar slants when kept at room temperature. However, once growth is established, usually within one to three weeks, subcultures will grow more rapidly. Mycobacteria from tropical fishes grow well at 28°C. and subcultures become luxuriant even at 37°C. (Pl. VI, fig. 12) The degree of pigment production, which may develop either in light or in the dark, varies with the strain and age of the culture. The mycobacteria isolated from freshwater tropical fishes grown on Dorset's or Petroff's media vary in color from cream to yellowish-green; bacilli from stenohaline tropical fishes vary from light to bright yellow color.

Classification of Mycobacteria of Cold-blooded Vertebrates

Table II list the species of mycobacteria from fishes, amphibians and reptiles that have been studied in detail; some of these are recognized as valid species and are included in Bergey's Manual of Determinative Bacteriology.

The growth and nutritional requirements, antigenic structure, pathogenicity, source and habitat of *Mycobacterium piscium, Myco. marinum, Myco. ranae, Myco. thamnopheos* and *Myco. friedmanni* are summarized by Reed (1948). Gordon (1957) recognizes and characterizes only *Myco. marinum. Myco. platypoecilus, Myco. thamnopheos* and *Myco. fortuitum.* The type of culture of *Mycobacterium piscium* is apparently lost while *Myco. ranae* and *Myco. friedmanni,* together with certain fish strains (*e.g.,* from Halibut and Halibut roe), are considered to be identical with *Myco, smegmatis* and/or with *Myco. fortuitum* (Gordon & Smith, 1955). The information on *Mycobacterium anabanti* Besse (1949a) and *Myco. salmoniphilum* Ross (1960) was not available at the time to Gordon and her co-workers for evaluation. However, Gordon & Mihm (1959) reported that certain of the strains from trout and salmon are identical with *Myco. fortuitum.*

Except for the Neon Tetra strains, the mycobacteria of the fish listed in Table I have not been further characterized. Ross & Brancato (1959) considered the Neon Tetra strain 9-21H, which is a subculture of our H-strain, to be identical with *Mycobacterium fortuitum.* Some differences in the utilization of several substances as carbon source are noted between strain 9-21H and strains H and N as analyzed by Vogel (1959). These are shown in Table III and compared with *Mycobacterium marinum, Myco. fortuitum* from mammals and with the several strains of *Myco. salmoniphilum* which were recognized as *Myco. fortuitum* by Gordon and Mihm (1959).

Discussion

As pointed out by Fregnan, Smith & Randall (1961), a great deal of attention has been

TABLE II. SPECIES OF MYCOBACTERIA OF POIKILOTHERMS

Mycobacteria	Host
	I. Fishes
Mycobacterium piscium (see Reed, 1948)	*Cyprinus carpio*, European Carp
Mycobacterium marinum Aronson, 1926	*Abudefduf mauritii*, Sergeant Major *Micropogon undulatus*, Croaker *Centropristis striatus*, Sea Bass (Philadelphia Aquarium)
Mycobacterium platypoecilus Baker & Hagen, 1942	*Playtpoecilus maculatus*, Mexican Platyfish (Cornell Univ.)
Mycobacterium anabanti Besse, 1949a	*Macropodus opercularis*, Paradisefish (France)
Mycobacterium fortuitum Cruz (see Gordon, 1957) Neon Tetra Strain 9-21 H (Ross & Brancato, 1959)	*Hyphessobrycon innesi*, Neon Tetra (New York Aquarium)
Mycobacterium salmoniphilum Ross, 1960	*Oncorhynchus tschawytscha*, Chinook Salmon *Salmon gairdneri*, Steelhead Trout (Hatcheries, Oregon & Washington)
	II. Amphibians
Mycobacterium ranae (Küster, 1905) (see Reed, 1948)	European Frogs
	III. Reptiles
Mycobacterium friedmanni Holland, 1920 (see Reed, 1948)	*Chelone corticata*, Loggerhead Turtle (European Zoo)
Mycobacterium thamnopheos Aronson, 1929	*Thamnophis sirtalis*, Garter Snake (U.S.A.)

directed towards the difficult problem of differentiation and classification of saprophytic and pathogenic mycobacteria ever since the discovery of the tubercle bacillus by Robert Koch in 1882. No entirely satisfactory method has yet been developed and all attempts so far have led to considerable confusion. This is especially true for the attempts to classify the mycobacteria causing tuberculosis in fishes, amphibians and reptiles. For example, Gordon & Smith (1955) and Gordon & Mihm (1959) reported that several strains of mycobacteria from cold-blooded vertebrates were identical either with *Mycobacterium smegmatis or Myco. fortuitum*. Gordon (1957) considers *Myco. marinum*, *Myco. platypoecilus* and *Myco. thamnopheos* to be valid species. However, the discovery of *Mycobacterium balnei* in swimming pools and in human lesions (Linell & Norden, 1954; Swift & Cohen, 1962) has added to the confusion. Bojalil (1959) considers this species to be the same as *Myco. marinum* while McMillen & Kushner (1959) report that *Myco. marinum*, *Myco. platypoecilus* and *Myco. balnei* represent a single species which, by priority, should be *Myco. marinum* Aronson. In addition, McMillen (1960) indicates that *Myco. fortuitum* is a mutation or adaptation of *Myco. marinum*.

If the above reports are valid, then all strains of mycobacteria from marine and freshwater fishes, regardless of ecological and other biological factors, belong to either *Mycobacterium marinum* originally described from Atlantic Coast fishes or to *Mycobacterium fortuitum* first reported from cattle and human abscesses. It is difficult to believe that either species is a ubiquitous pathogen of fish with no host specificity.

In any event, it is quite apparent that tuberculosis in cold-blooded animals, and especially in fishes, is much more widespread than is generally suspected. Although tuberculosis has been found mainly in fishes kept in aquaria, hatcheries and in fish holding ponds, cases of tuberculosis in feral populations have been reported (Nigrelli, 1953). Of particular interest is the epizootics in salmonoids in the Pacific Northwest, both in hatchery-reared fishes and in migrating populations (Wood & Ordal, 1958; Wood, 1959; Ross, 1959; Parisot & Wood, 1960). Tuberculosis in these species (Table IV), the presence of which was first reported by Earp, Ellis & Ordal (1955), is therefore of great economic importance. The disease is also prevalent in a large variety of tropical fresh water fishes. The members of the families Characidae and Cyprinidae, which are highly valued by fish hobbyists, are especially susceptible and commercially available to the microbiologist interested in this problem.

SUMMARY

A survey of fishes in the New York Aquarium and a search of the literature show that tuberculosis in these animals and in other poikilotherms is more prevalent than is generally suspected.

TABLE III. CARBON SOURCES UTILIZED BY MYCOBACTERIA FROM FISH

Carbon Source	Neon Tetra Strains[1]			*Myco. fortuitum*[2]		*Myco. marinum*[3]		*Myco. salmoniphilum*[4]			
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Glucose	—	—	x	x	x	—	—				
Mannose	x	x	x	x	x	x	—	x	x	x	x
Trehalose	x	x	x	x	x	x	—	x	x	x	x
Fructose	x	x	x	—	x	x	x	x	0	0	0
Mannitol	0	x	0	(occasional)	0	0	—	x	0	0	0
Xylose	(slight)	0	0	(occasional)	0	0	—	0	0	0	0
Sorbitol	x	x	0	(rarely)	0	(slight)	0	0	0	0	0
Sucrose	x	0	0	—	0	0	—	0	0	0	0
Rhamnose	0	0	0	0	0	0	—	0	0	0	0
Dextrose	x	x	—	—	—	x	—	x	x	x	x
Dulcitol	0	0	0	0	0	0	—	0	0	0	0
Arabinose	0	(slight)	0	(occasional)	0	0	x	0	0	0	0
Lactose	0	(slight)	0	0	0	0	—	0	0	0	0
Galactose	—	—	0	(occasional)	0	—	0	0	0	0	0
Raffinose	(slight)	(slight)	0	(rarely)	0	0	—	0	0	0	0
Inositol	0	0	0	(occasional)	0	0	—	0	0	0	0
Maltose	(slight)	x	0	(rarely)	0	(slight)	—	0	0	0	0
Inulin	0	0	—	—	—	0	—	—	—	—	—
Erythritol	0	0	—	(occasional)	—	0	—	—	—	—	—
Amygdalin	(slight)	(slight)	—	—	—	(slight)	—	—	—	—	—
Salicin	x	0	—	—	—	0	—	—	—	—	—
Starch	—	—	—	(hydrolyzed)	—	—	—	—	—	—	—

[1]I, H-strain, II, N-strain, Vogel (1959); III, 9-21H strain, Ross & Brancato (1959).

[2]IV, Gordon (1957); V, Ross & Brancato (1959).

[3]VI, Vogel (1959); VII, Gordon (1957) (Gordon & Mihm (1959), 80 strains examined.

[4]VIII, CC-strain from steelhead trout in Chamber Creek Hatchery; IX, OR-strain from steelhead trout in Oak Ridge Hatchery; X, CAR-strain from chinook salmon in Carson Hatchery; XI, SC-strain from Chinook salmon in Spring Creek Hatchery, Ross (1960) (see also Gordon & Mihm, 1959).

TABLE IV. FISHES, AMPHIBIANS AND REPTILES IN WHICH TUBERCULOSIS HAS BEEN REPORTED

Species	Common Name	Reference
I. Teleostomi		
1. Salmonidae		
(1) *Oncorhynchus gorbuscha*	Humpback Salmon	47, 64
(2) *Oncorhynchus keta*	Dog Salmon	47
(3) *Oncorhynchus kisutch*	Silver Salmon	47, 64
(4) *Oncorhynchus nerka*	Blueback Salmon	47, 64
(5) *Oncorhynchus tschawytscha*	Chinook or King Salmon	15, 41, 47
(6) *Salmo gairdneri*	Steelhead, Rainbow Trout	47, 64
2. Osmeridae		
(7) *Osmerus mordax*	American Smelt	62
3. Umbridae		
(8) *Umbra pygmaea*	Mud-Minnow	51
4. Characidae		
(9) *Aphyochorax rubripinnis* (*Aphiocharax rubrepinis)*	Bloodfin	NYA, 11, 27
(10) *Gymnocorymbus ternetzi*	Black Tetra	NYA, 11, 27, 32
(11) *Hemigrammus rhodostomus*	Rummy-Nosed Characin	NYA, 32
(12) *Hemigrammus unilineatus*	Feather Fin	11
(13) *Hemigrammus erythrozona* (*Hyphessobrycon gracilis)*	Glowlight Tetra	11
(14) *Hemigrammus ocellifer*	Head-and-Tail Light	2
(15) *Hyphessobrycon bifasciatus*	Yellow Tetra	27
(16) *Hyphessobrycon flammeus*	Flame Characin	NYA, 11
(17) *Hyphessobrycon cardinalis*	Cardinal Tetra	NYA, 11
(18) *Hyphessobrycon innesi*	Neon Tetra	NYA, 11, 38, 39, (also Conroy 1963)*
(19) *Hyphessobrycon pulcher*		32
(20) *Hyphessobrycon rosaeus* (*= ornatus)*	Rosy Barb	32
(21) *Hyphessobrycon callistus* (*= serpae)*	Serpa Tetra	NYA, 2, 27, 32
(22) *Moenkhausia pittieri*	Pittier's Moenkhausia	NYA, 32
(23) *Pyrrhulina rachoviana*	Rachow's Pyrrhulian	27
(24) *Pristella riddlei*	Riddle's Pristella	2
5. Cyprinidae		
(25) *Notemigonus crysoleucas*	Dace or Roach	62
(26) *Barbus fluviatilis*	European Barb	51
(27) *Brachydanio albolineatus* (*Danio albolineatus)*	Pearl Danio	NYA, 11, 27, 38
(28) *Brachydanio analipunctatus*	Tail-Spot Danio	NYA, 38
(29) *Brachydanio nigrofasciatus*	Spotted Danio	NYA
(30) *Brachydanio rerio* (*Danio rerio)*	Zebra Danio	NYA, 11, 32, 38
(31) *Danio malabaricus*	Giant Danio	NYA, 11, 27, 32
(32) *Carassius auratus* (*C. oratus)*	Goldfish	11, 27, 32
(33) *Carassius carassius*	Cruscian Carp	32
(34) *Cyprinus carpio*	Common Carp	7, 8, 32
Cyprinus carpio	Hi-Goi or Golden Carp	23
(35) *Puntius conchonius* (*Barbus conchonius)*	Rosy Barb	NYA, 11
(36) *Puntius lineatus* (*Barbus lineatus)*	Lined Barb	32
(37) *Puntius nigrofasciatus* (*Barbus nigrofasciatus)*	Black Ruby Barb	32
(38) *Puntius phutunio* (*Barbus phutunio)*	Dwarf or Pygmy Barb	32
(39) *Puntius semifasciolatus*	Chinese Barb	NYA

*David W. Conroy, Univ. Nac. de Buenos Aires, Lab. de Bact., Inst. de Biol. Marino; Microbid. Españ., 16: 47-54.

TABLE IV. FISHES, AMPHIBIANS AND REPTILES IN WHICH TUBERCULOSIS HAS BEEN REPORTED *(Continued)*

Species	Common Name	Reference
(40) *Puntius tetrazona* (*Barbus sumatranus*)	Sumatra Barb	NYA, 32
(41) *Puntius partipentazona*	Five-Banded Barb	32
(42) *Puntius ticto* (*Barbus ticto*)	Ticto Barb	32
(43) *Rasbora einthoveni*	Brilliant Rasbora	32
(44) *Rasbora heteromorpha*	Red Rasbora	NYA, 11
(45) *Rasbora lateristriata*	Striped Rasbora	NYA
(46) *Rasbora leptosoma*	Slender-Bodied Rasbora	32
(47) *Rasbora trilineata*	Scissors-Tail	NYA
(48) *Tanichthys albonubes*	White-Cloud Mountain Fish	NYA, 38
(49) *Idus melanotus*	Golden Orf	32
(50) *Tinca tinca*	Tench	10
6. Siluridae		
(51) *Silurus glanis*	Wels or European Catfish	23
7. Bagridae		
(52) *Rhamdia sapo*		32
8. Clariidae		
(53) *Clarias dumerili*		27, 32
9. Loricariidae		
(54) *Plectostomus punctatus*		32
10. Gadidae		
(55) *Gadus collarias*	Atlantic Codfish	1, 28
11. Cyprinodontidae		
(56) *Aphyosemion australe*	Lyretail	11
(57) *Oryziae latipes* (*Aplocheilus latipes*)	Medaka	10, 11
(58) *Aplocheilus panchax* (*Panchax panchax*)	Panchax	10
(59) *Rivulus cylindraceus*	Cuban Rivulus	11
(60) *Cynolebias wolterstorffi*	(So. American Annals)	Roy L. Walford Aug. 1963† (Personal Communication)
(61) *Cynolebias adeoffi*		Roy L. Walford Aug. 1963† (Personal Communication)
(62) *Cynolebias elongatus*		Roy L. Walford Aug. 1963† (Personal Communication)
12. Poeciliidae		
(63) *Lebistes reticulatus*	Guppy	NYA, 11
(64) *Xiphophorus helleri*	Swordtail	NYA, 11, 27, 32
(65) *Platypoecilus maculatus*	Platyfish	NYA, 6, 11, 38
(65a) *Molliensia sphenops*	Mollie	43
13. Belonidae		
(66) *Belone belone*	European Garfish	44
14. Holocentridae		
(67) *Holocentrus ascensionis*	Squirrelfish	62
15. Atherinidae		
(68) *Melanotaenis nigrans*	Australian Rainbow Fish	11
16. Serranidae		
(69) *Centropristis furvus*		62
(70) *Centropristis striatus*	Black Sea Bass	3
(71) *Epinephelus adscenionis* (*Epinephelus ascenionis*)	Rock Hind	62
(72) *Epinephelus guttatus*	Red Hind	62
(73) *Epinephelus morio*	Red Grouper	62
(74) *Epinephelus* sp.	Gray Grouper	62
(75) *Epinephelus* sp.	Queen Grouper	62
(76) *Epinephelus striatus*	Nassau Grouper	62
(77) *Morone americana*	White Perch	62
(78) *Morone labrax*	European bass	44

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TABLE IV. FISHES, AMPHIBIANS AND REPTILES IN WHICH TUBERCULOSIS HAS BEEN REPORTED *(Continued)*

Species	Common Name	Reference
(79) *Mycteroperca bonaci* (*Epinephelus* var.)	Black Grouper	62
(80) *Mycteroperca falcata* (*Mictoperca phenax)*	Scamp	62
(81) *Roccus saxatilis* (*Roccus lineatus)*	Striped bass	NYA, 3, 62
17. Centrarchidae		
(82) *Lepomis gibbosus* (*Eupomotis gibbosus)*	Pumpkinseed	11
(83) *Micropterus dolomieu*	Smallmouth Bass	62
18. Percidae		
(84) *Lucioperca lucioperca* (*Lucioperca sandra)*	European Pike-Perch	23
(85) *Perca flavescens*	Yellow Perch	62
19. Carangidae		
(86) *Trachinotus carolinus*	Common Pompano	62
(87) *Vomer setapinnis*	Moonfish	62
20. Lutianidae		
(88) *Ocyurus chrysurus*	Yellow-tailed snapper	62
(89) *Lutianus apodus*	Schoolmaster	62
(90) *Lutianus griseus*	Gray snapper	3, 62
(91) *Lutianus jocu*	Dog snapper	62
(92) *Lutianus synagris*	Lane (Spot) snapper	62
21. Sciaenidae		
(93) *Cynoscion regalis*	Weakfish or Gray Squeteague	62
(94) *Leiostomus xanthurus*	Spot	62
(95) *Micropogon undulatus*	Atlantic Croaker	3, 62
(96) *Pogonias cromis*	Black Sea Drum	62
22. Pomadasyidae		
(97) *Bathystoma* sp.	Red-Mouth Grunt	62
(98) *Plectorhynchus* sp.	Sweet Lip	NYA
(99) *Anisotremus surinamensis*	Black Margate	62
(100) *Anisotremus virginicus*	Porkfish	62
23. Toxotidae		
(101) *Toxotes jaculator*	Archerfish	NYA
24. Kyphosidae		
(102) *Kyphosus secatrix*	Bermuda Chub	62
25. Sparidae		
(103) *Archosargus probatocephalus*	Sheephead	62
(104) *Sargus sargus* (*Sargus rondeleti)* (*Diplodus sargus)*	Sargo	32
(105) *Cantharus lineatus*	Oldwife or Black Sea Bream	32
26. Maenidae		
(106) *Spicara argus* (*Smaris alcedo)*	Picarel Martin Pècheur	44
27. Scatophagidae		
(107) *Scatophagus argus*	Scat	32
(108) *Monodactylus argentus*	Silver Angelfish	32
28. Cichlidae		
(109) *Apistogramma ramirezi*	Ramirez's dwarf cichlid	43, 45
(110) *Cichlasoma facetum*	Chanchita	32
(111) *Cichlasoma biocellatum*	Jack Dempsey	32
(112) *Cichlasoma festivum* (*Cichlasoma insignis)*	Festive Cichlid	32
(113) *Cichlasoma meeki*	Firemouth	43, 45
(114) *Aequidens portalegrensis*	Port	NYA, 2
(115) *Aequidens curviceps*	Flag Cichlid	2
(116) *Haplochromis multicolor*	Egyptian Mouth-Breeder	2, 27
(117) *Hemichromis bimaculatus*	Jewelfish	11
(118) *Nannacara anomala*	Golden-Eyed Dwarf Cichlid	2, 27

TABLE IV. FISHES, AMPHIBIANS AND REPTILES IN WHICH TUBERCULOSIS HAS BEEN REPORTED *(Continued)*

Species	Common Name	Reference
(119) *Pterophyllum scalare*, or *Pterophyllum eimekei*	Angelfish or Scalare	NYA, 11, 32
(120) *Symphysodon discus*	Disc Cichlid	NYA, 43, 45
29. Pomacentridae		
(121) *Abudefduf saxatilis* *(Abudefduf marginalis)* *(Abudefduf mauritii)*	Sergeant Major	3, 62
(122) *Amphiprion percula*	Clownfish	NYA
(123) *Amphiprion laticlavius*	White-Saddled Clownfish	NYA
(124) *Amphiprion xanthurus*	Chocolate Clownfish	NYA
(125) *Amphiprion akallaopsis*	Skunkfish	NYA
(126) *Dascyllus auranus*	White-Tailed Puller	NYA, 32
(127) *Pomacentrus leucostictus* *(Eupomacentrus leucostictus)*	Beau Gregory	62
(128) *Premnas biaculeatus*	Spiny Clownfish	NYA
30. Chaetodontidae		
(129) *Pomacanthus arcuatus*	Black Angelfish	62
(130) *Angelichthys isabelita*	Blue Angelfish (common Angelfish)	62
31. Labridae		
(131) *Lachnolaimus maximus*	Hogfish	62
(132) *Tautog onitis*	Tautog	62
32. Acanthuridae		
(133) *Acanthurus coeruleus* *(Teuthis coeruleus)*	Blue Tang	62
33. Anabantidae		
(134) *Anabas testudineus*	Climbing Perch	NYA, 27, 32
(135) *Betta splendens*	Siamese Fighting Fish	NYA, 11
(136) *Colisa lalia*	Dwarf Gourami	NYA, 11, 27
(137) *Macropodus opercularis*	Paradisefish	NYA, 11
(138) *Trichogaster trichopterus* *(Trichopodus trichopterus)*	Three-Spot (Blue) Gourami	NYA, 11, Conroy, 1963
(139) *Trichogaster leeri*	Pearl Gourami	NYA, 32, Conroy, 1963
(140) *Trichopsis vittatus* *(Ctenops vittatus)*	Croaking Gourami	11
34. Triglidae		
(141) *Prionotus carolinus*	Common Sea Robin	62
35. Pleuronectidae		
(142) *Hippoglossus hippoglossus* *(Hippoglossus vulgaris)*	Atlantic halibut	29, 56
36. Bothidae		
(143) *Lophopsetta maculata*	Windowpane	62
(144) *Paralichthys dentatus*	Fluke or Summer Flounder	62
37. Monacanthidae		
(145) *Alutera monacanthus*	Filefish	62
38. Balistidae		
(146) *Balistes vetula*	Queen triggerfish	62
(147) *Balistes carolinensis*	Common triggerfish	62
39. Diodontidae		
(148) *Chilomycterus schoepfi*	Spiny Boxfish or Burrfish	62
(149) *Diodon hystrix*	Porcupine fish	62
(150) *Sphaeroides maculatus*	Northern puffer	62
40. Batrachoididae		
(151) *Opsanus tau*	Northern Toadfish	62
II. Amphibia		
1. Ambystomidae		
(1) *Siredon mexicanus* *(= Ambystoma mexicanus)*	Axolotl	23, 43

TABLE IV. FISHES, AMPHIBIANS AND REPTILES IN WHICH TUBERCULOSIS HAS BEEN REPORTED *(Continued)*

Species	Common Name	Reference
2. Pseudidae		
(2) *Pseudis paradoxa*	Paradox Frog	22, 23
3. Ranidae		
(3) *Rana catesbeiana*	American Bullfrog	5
(4) *Rana* spp.	European Frogs	31, 30, 33, 50
(5) *Rana tigrina*		26
4. Leptodactylidae		
(6) *Leptodactylus pentadactylus*	Robber Frog	13
(7) *Pleurodema cinere*		35
(8) *Pleurodema marmoratus*		35
(9) *Ceratophrys americana*	South American Horned Frog	26
5. Bufonidae		
(10) *Bufo spinulosus*		35
6. Pipidae		
(11) *Xenopus laevis*	South African Clawed Frog	52
III. Reptilia		
1. Chelonidae		
(1) *Chelone corticata* (= *Caretta caretta)*	Loggerhead Turtle	17
2. Trionychidae		
(2) *Trionyx gangeticus*	Indian Softshell Turtle	53
(3) *Trionyx triungis*	Nile Softshell Turtle	11, 23
3. Alligatoridae		
(4) *Caiman sclerops*	Spectacled Caiman	22, 23, 24, 53
4. Iguanidae		
(5) *Ctenosaura multipinnis* (= *Ctenosaura acanthura)*	Spiny-tailed Iguana	53
5. Lacertidae		
(6) *Lacerta viridis*	Green Lizard	9
6. Boidae		
(7) *Boa constrictor*	Boa Constrictor	18
(8) *Python molurus*	Indian Rock Python	50
(9) *Python reticulatus*	Reticulated Python	25
(10) *Python sebae*	African Rock Python	24, 53
(11) *Python* sp.		18, 23, 26, 54
(12) *Python spilotes*		26
7. Colubridae		
(13) *Coluber longissimus* (= *Elaphe longissimus)*	Aesculapian Snake	23, 53
(14) *Lampropeltis getulus holbrooki*	Speckled King Snake	23, 24
(15) *Tropidonotus natrix* var. *murorum* (= *Natrix natrix)*	European Grass Snake	55
(16) *Natrix piscator*	Checkered Keelback	23, 24
(17) *Coluber catenifer* (= *Pituophis catenifer)*	Gopher Snake	4
(18) *Tarbophis fallax*	Cat Snake	22
(19) *Thamnophis sirtalis*	Eastern Garter Snake	4
(20) *Agkistrodon piscivorus*		26
8. Elapidae		
(21) *Naja naja*	Cobra	NYA, 58, 59
9. Crotalidae		
(22) *Crotalus exsul* (= *Crotalus ruber)*	Red Rattlesnake	23, 53
10. Viparidae		
(23) *Bitis arietans*	Puff Adder	23, 53
11. Anguidae		
(24) *Anguis fragilis*	European Slowworm	8

The disease has been found in 151 species of fishes, 11 species of amphibians and 24 species of reptiles.

In the New York Aquarium, tuberculosis was present in 40 species of fishes, especially in tropical freshwater forms belonging to the families Characidae, Cyprinidae and Poecilidae. Of special interest is the report that the acid-fast bacillus isolated from the Neon Tetra *(Hyphessobrycon innesi)* is identical with *Mycobacterium fortuitum,* a human pathogen. The disease is also found in a group of stenohaline, Pacific coral reef species commonly called clownfishes, members of the family Pomacentridae.

The pathology of tuberculosis in the fishes studied in the Aquarium is described and the taxonomy of mycobacteria from cold-blooded vertebrates is briefly discussed.

Stock cultures of mycobacteria isolated from the Neon Tetra, Blue Gourami, Archerfish, Sweet-lip (*Plectorhynchus* sp.) and from the clownfishes are maintained in the laboratory of the New York Aquarium.

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EXPLANATION OF THE PLATES

Plate I

Fig. 1. Neon Tetra infected with tuberculosis. Note various degrees of emaciation. A yellowish discoloration of the usually brilliant red markings on the lateral-posterior area is an external manifestation of the disease. Slightly larger than natural size. Photo by S. C. Dunton, New York Zoological Society.

Fig. 2. Typical acid-fast organisms in smear from discolored area of the skin of the Neon Tetra. Ziehl-Neelsen's stain. 650 ×.

Plate II

Fig. 3. Section through the body wall and muscle of the Neon Tetra showing numerous solitary and coalescent tubercles. Hematoxylin-eosin. 100 ×.

Fig. 4. Section through the body of an infected Neon Tetra showing a characteristic condition of tuberculosis in tropical freshwater fish. Upper left is the liver; the intestine is shown slightly to right of center; in between is the mesentery in which the diffuse exocrine pancreas is present. Hematoxylin-eosin. 50 ×.

Plate III

Fig. 5. Stained smear of the internal organs of infected Neon Tetra showing the typical acid-fast mycobacteria, some of which occur within macrophages. Ziehl-Neelsen's stain. 650 ×.

Fig. 6. Electrophotomicrograph of acid-fast bacillus from a culture of the Neon Tetra Strain-N. 16,300 ×.

Plate IV

Fig. 7. Liver of the cyprinodon *Cynolebias* sp. showing the epitheloid-like tubercles in the parenchyma. Hematoxylin-eosin. 450 ×.

Fig. 8. Higher magnification of the central area of a tubercle shown in fig. 7. Note the typical acid-fast bacilli. Ziehl-Neelsen. 650 ×.

Plate V

Fig. 9. Three-spot or blue gourami with lepromatous-like macular and necrotic skin and fin lesions. ½ natural size.

Fig. 10. Hard tubercles ("pearl bodies") in liver and other organs of the Climbing Perch, *Anabas testudineus*. Slightly larger than natural size.

Plate VI

Fig. 11. Section in region of the kidney of a goldfish showing coalescent and some degree of caseation of the tubercles. Note lymphocytic infiltration and fibrous development. Hematoxylin-eosin. 300 ×.

Fig. 12. Luxuriant subculture of *Mycobacterium*, Neon Tetra Strain-H, originally isolated from the kidney; grown on Dorset's egg agar at 37°C. A subculture of this strain has been identified as *Mycobacterium fortuitum* by Ross & Brancato (1959).

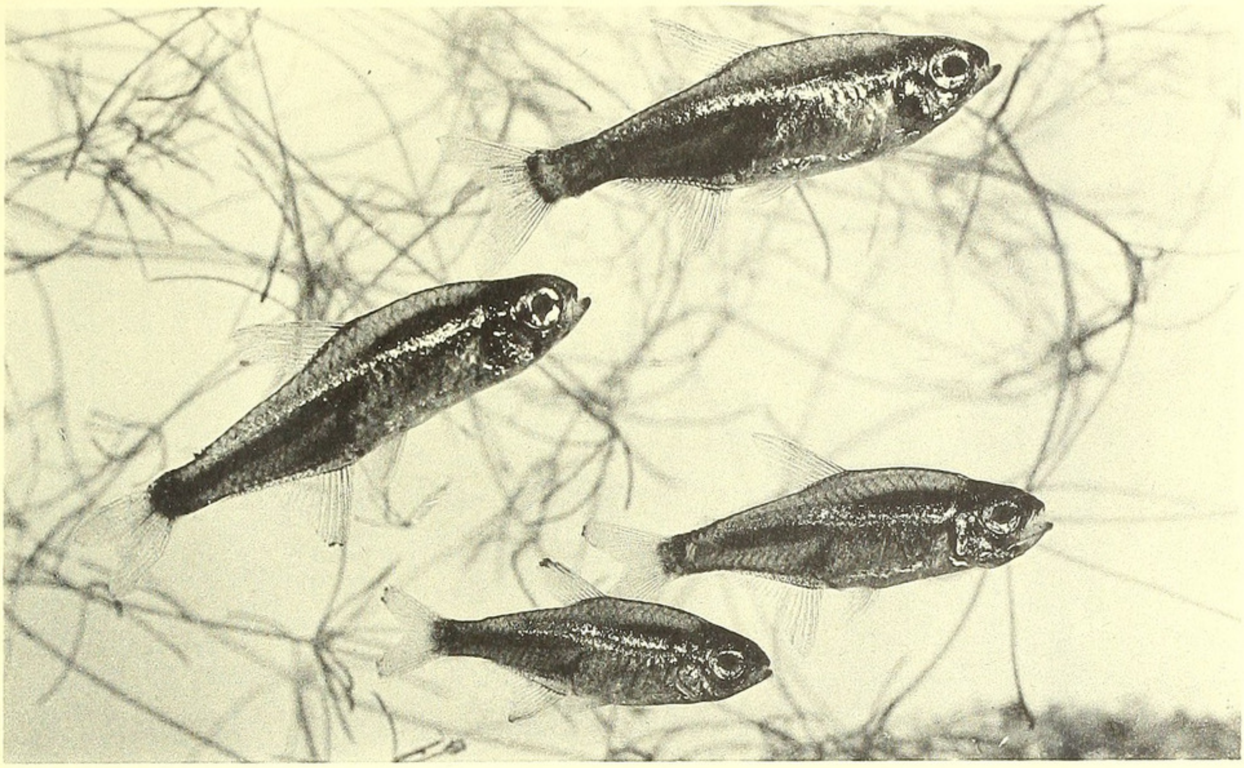

FIG. 1

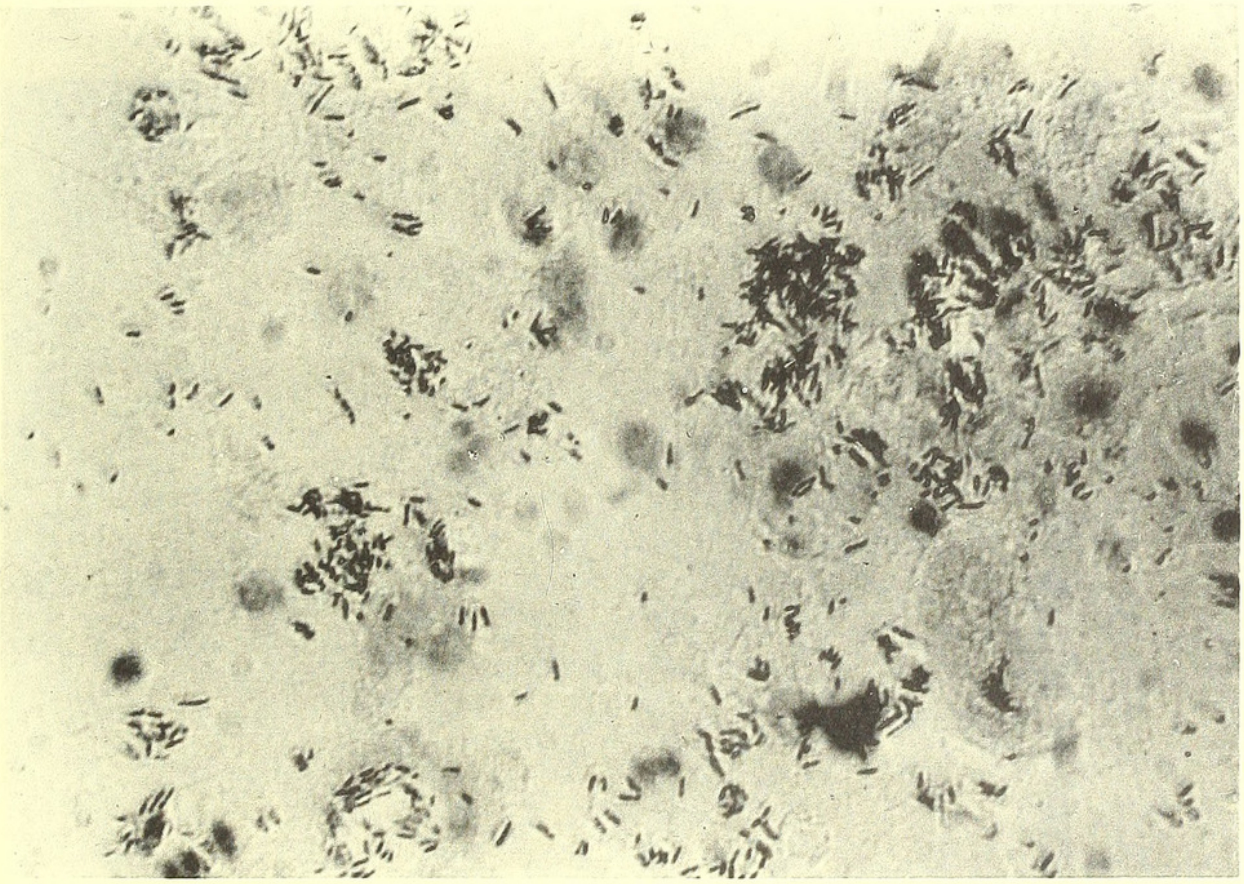

FIG. 2

SPONTANEOUS TUBERCULOSIS IN FISHES AND IN OTHER COLD-BLOODED VERTEBRATES WITH SPECIAL REFERENCE TO MYCOBACTERIUM FORTUITUM CRUZ FROM FISH AND HUMAN LESIONS

FIG. 3

FIG. 4

SPONTANEOUS TUBERCULOSIS IN FISHES AND IN OTHER COLD-BLOODED VERTEBRATES WITH SPECIAL REFERENCE TO MYCOBACTERIUM FORTUITUM CRUZ FROM FISH AND HUMAN LESIONS

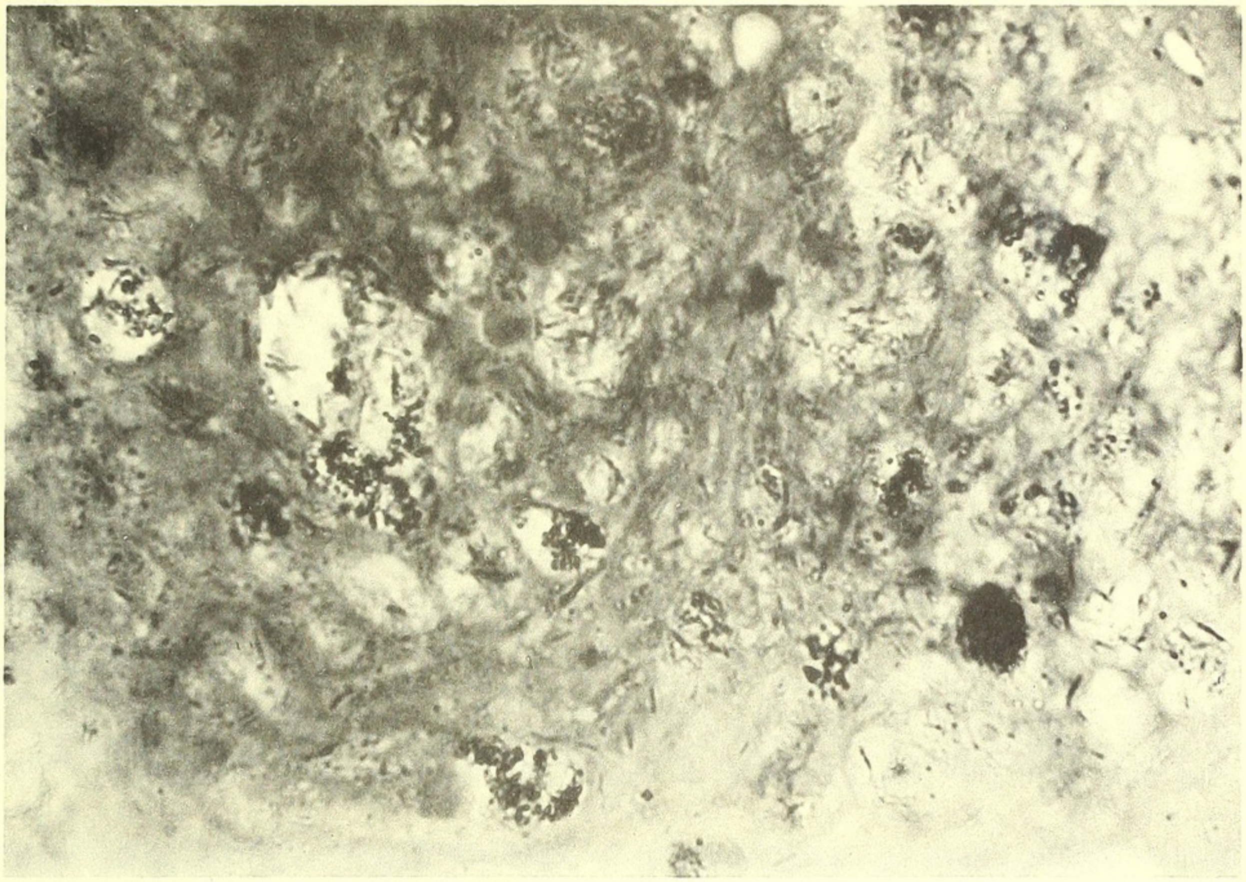

FIG. 5

FIG. 6

SPONTANEOUS TUBERCULOSIS IN FISHES AND IN OTHER COLD-BLOODED VERTEBRATES WITH SPECIAL REFERENCE TO MYCOBACTERIUM FORTUITUM CRUZ FROM FISH AND HUMAN LESIONS

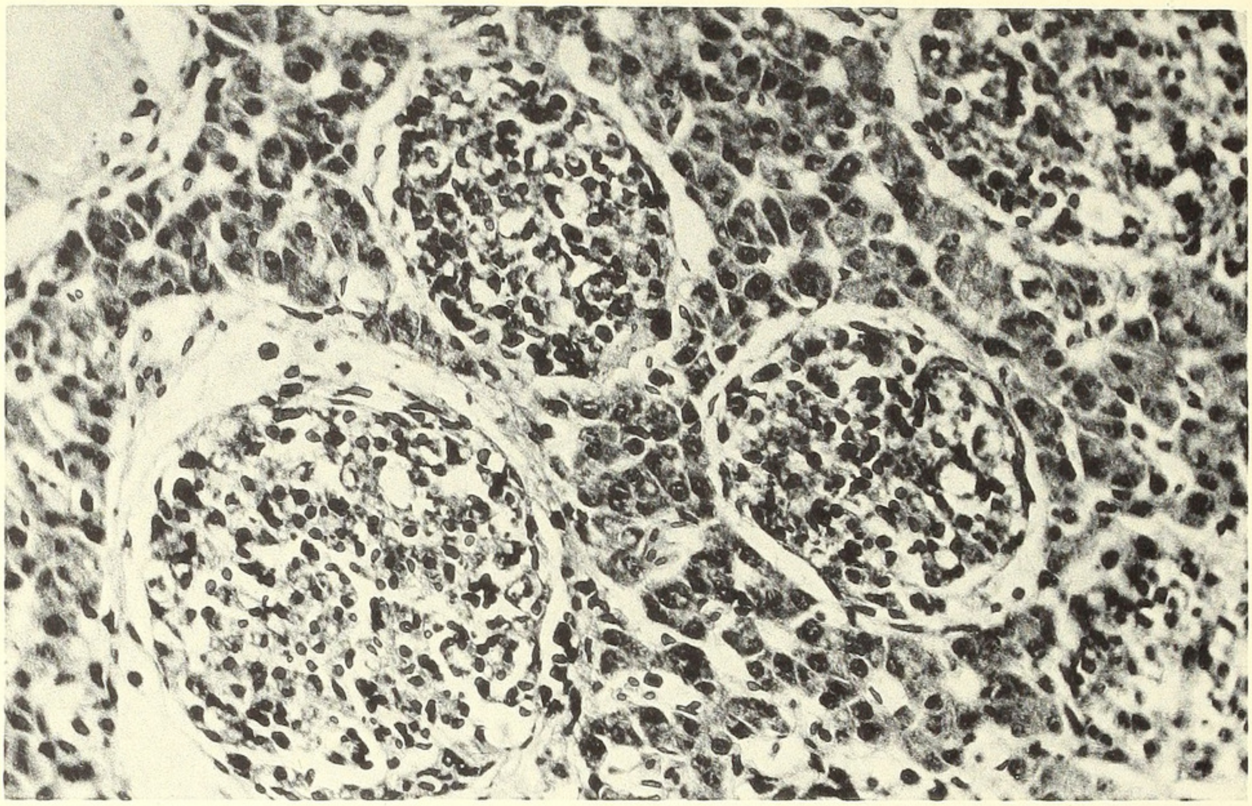

FIG. 7

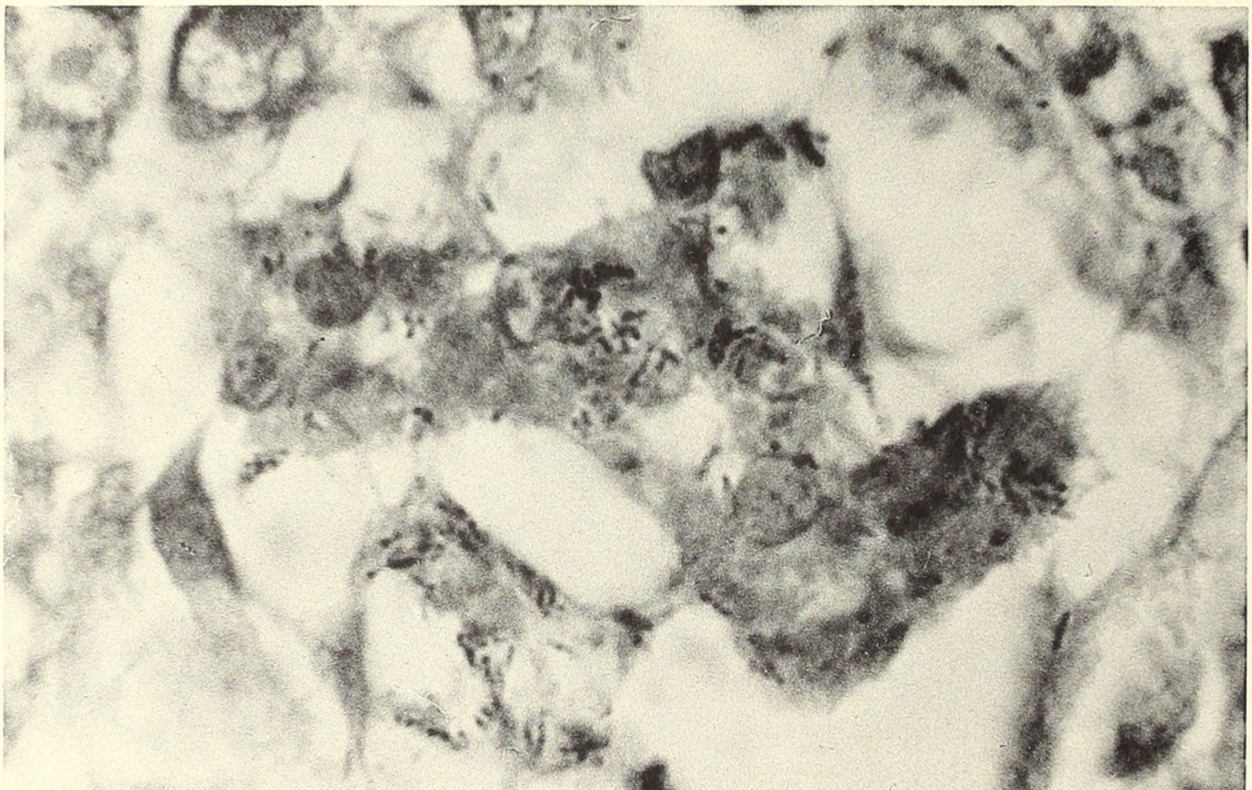

FIG. 8

SPONTANEOUS TUBERCULOSIS IN FISHES AND IN OTHER COLD-BLOODED VERTEBRATES WITH SPECIAL REFERENCE TO MYCOBACTERIUM FORTUITUM CRUZ FROM FISH AND HUMAN LESIONS

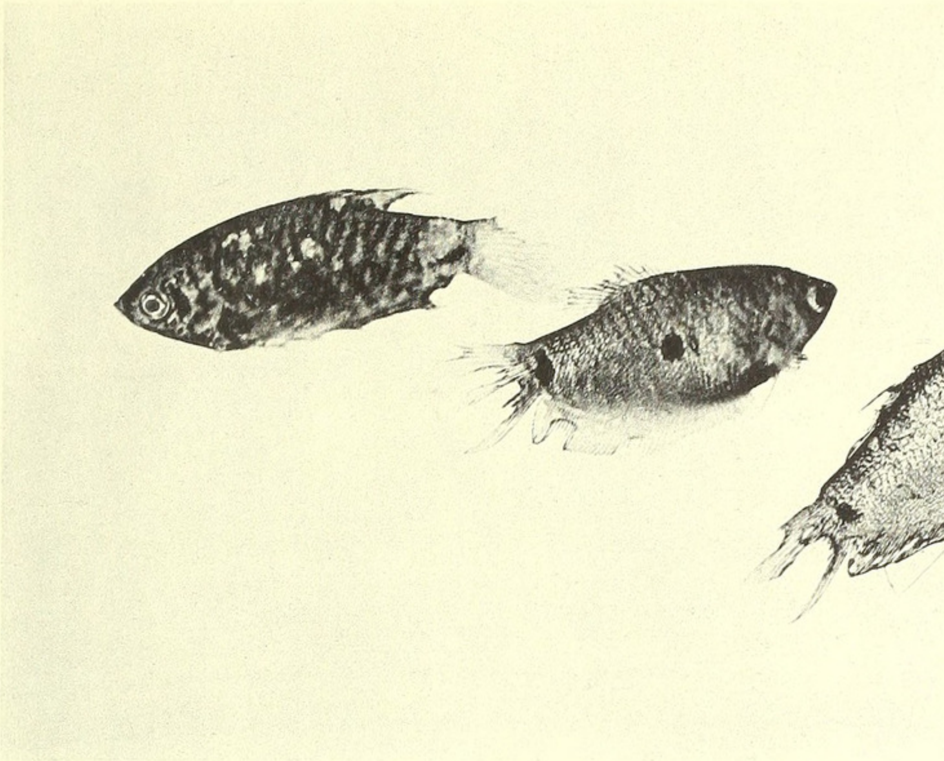

FIG. 9

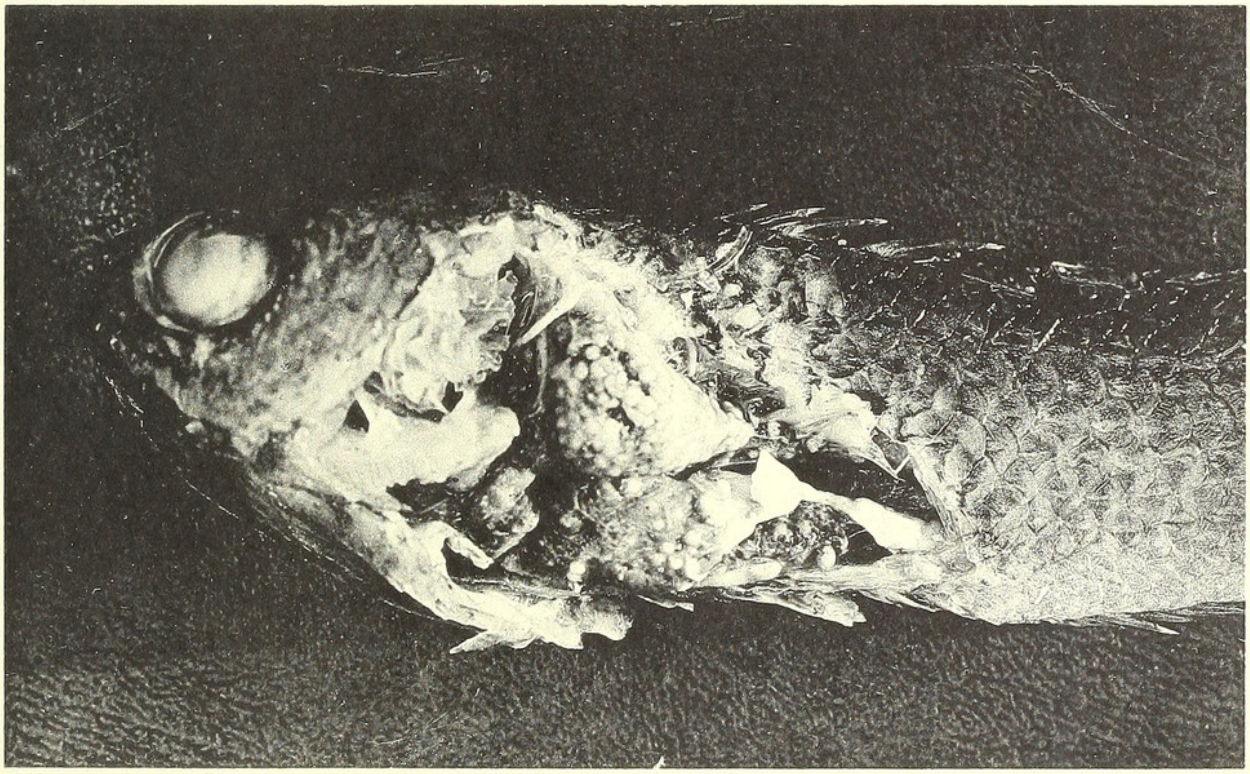

FIG. 10

SPONTANEOUS TUBERCULOSIS IN FISHES AND IN OTHER COLD-BLOODED VERTEBRATES WITH SPECIAL REFERENCE TO MYCOBACTERIUM FORTUITUM CRUZ FROM FISH AND HUMAN LESIONS

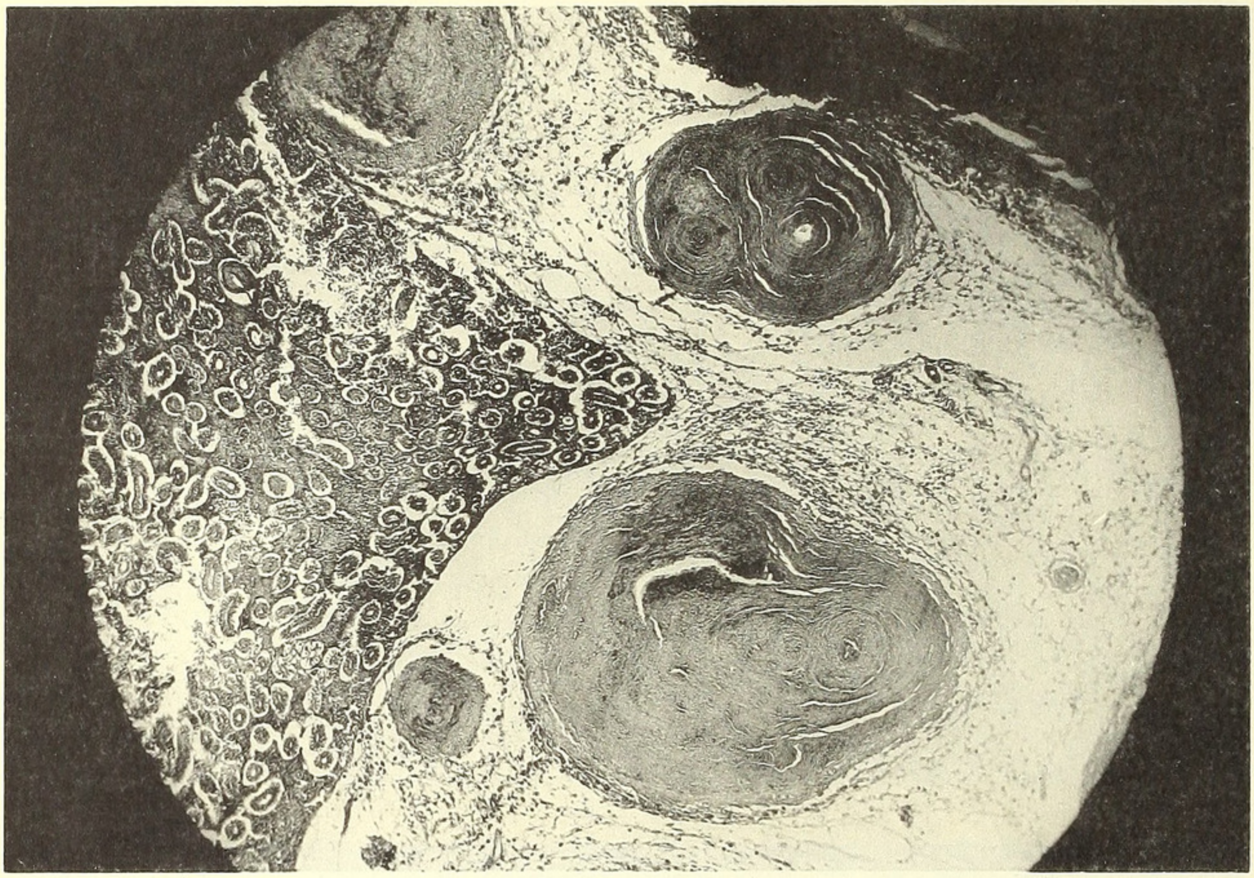

FIG. 11

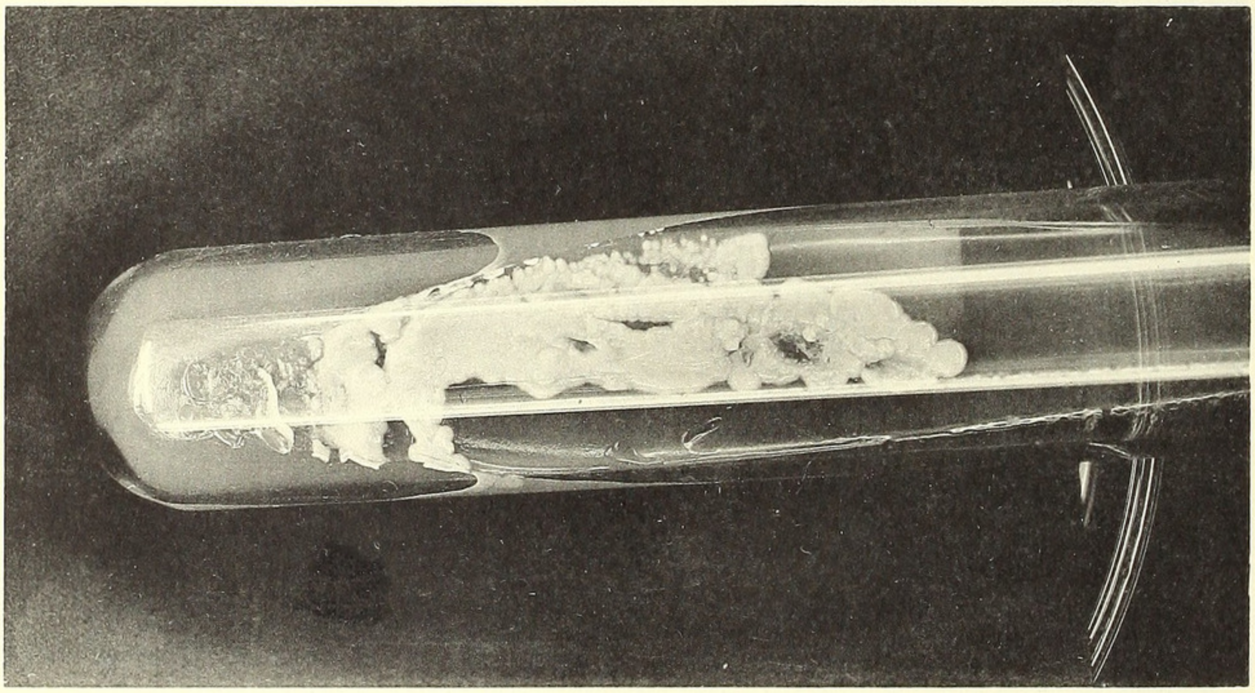

FIG. 12

SPONTANEOUS TUBERCULOSIS IN FISHES AND IN OTHER COLD-BLOODED VERTEBRATES WITH SPECIAL REFERENCE TO MYCOBACTERIUM FORTUITUM CRUZ FROM FISH AND HUMAN LESIONS