G testing is verified by its successful use throughout this investigation.

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Tuberculosis Induced in the Tadpole by Feeding.*

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Aronson[1] isolated a strain of tubercle bacillus from an iguana which was found dead in the Philadelphia Zoo. A number of tubercles were seen in the lung of this animal and also in the liver. The spleen contained no gross tubercles. A single tubercle was found in the upper pole of the left kidney. In guinea pigs injected intracutaneously with this culture Aronson found that a small ulcer occurred at the site of inoculation. The glands became enlarged and succulent but went on to healing. The organism was found to be pathogenic for the chameleon and salamander and also for the frog. It was not pathogenic for the snake. As regards the frog Aronson's findings have been confirmed by Holly[2] and by the writers with a transfer of this organism which Aronson sent to us sometime ago for further study.

Examination of stained slide preparations reveals the organism to be acid- and alcohol-fast. Ziehl-Neelsen stained vertical sections from growing colonies made with a technique reported by us[3] show some non-acid-fast rods in addition, but the tiny non-acid-fast rods and granules so conspicuous in vertical sections of human and bovine tubercle bacillus colonies are entirely lacking. It would seem then that this organism multiplies principally by simple fission. According to Henderson and Aronson[4] the organism is culturally and serologically identical with *Mycobacterium marinum* which was isolated from salt water fish.

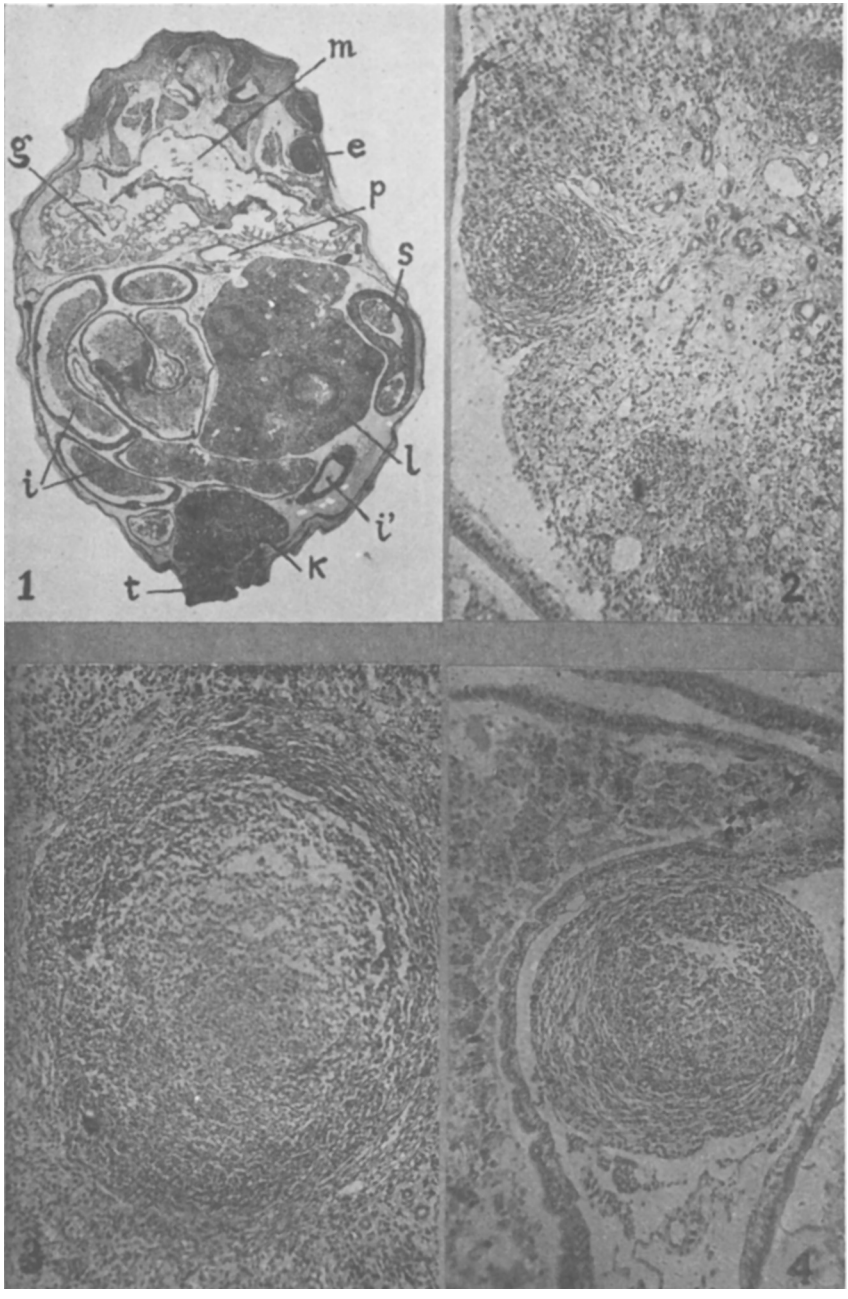
* This study was made in cooperation with the Research Board, National Tuberculosis Association.

1 Personal Communication, Dr. Joseph Aronson, Phipps Institute, Philadelphia, Pa.

2 Personal Communication, Miss Pearl Holly, New York City Department of Health.

3 Kahn and Nonidez, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 577.

4 Personal Communication.



All figures are unretouched photomicrographs from sections stained with hematoxylin and eosin.

FIG. I. Frontal section of infected tadpole (tail not included) showing large

In view of the pathogenicity of this organism for frogs it occurred to the writers to determine whether a similar situation existed for tadpoles. If this proved to be the case the tadpole would make a valuable experimental animal for certain problems as the entire animal may be sectioned and all of the organs examined in the same creature at the same time. It would also permit investigations concerning the reaction of embryonic tissue toward infection by a member of the *Mycobacterium* group.

Tadpoles of the leopard frog *(Rana pipiens)* were kept in sterile aquarium water contained in Stender dishes having a diameter of 10 cm. and a depth of 4.5 cm. A large loopful of a 5-day-old culture was scraped from a slant of Petroff's egg medium and deposited in the water. Possibly in view of the bright orange color no difficulty was experienced in inducing the tadpoles to feed upon this material. After feeding on the bacteria the tadpoles were removed to other Stender dishes also containing sterile aquarium water.

The infected tadpoles were fixed in alcohol or in a mixture of alcohol-chloral hydrate-formaldehyde, which latter mixture gave better fixation. The tails were cut off after fixation and the whole body dehydrated, embedded in paraffin and cut into frontal serial sections. (7-10μ thick). The entire animal with the exception of the tail was thus included on the slides. (Fig. 1.)

The sections were stained with hematoxylin-eosin (for histological details) or with the Ziehl-Neelsen technique.

Tadpoles given the bacteria as their only food for several days were soon overwhelmed without showing lesions of tuberculosis although numerous acid-fast organisms were found in liver and lung. Those fed twice with an interval of several days between feedings developed typical tuberculosis in the liver and intestine.

In one of the experiments 5 tadpoles were fed tubercle bacilli for one day; they were given yolk from a hard-boiled egg the 4 following days. On the fifth day they were fed tubercle bacilli again. One tadpole died during the night and could not be preserved for histological study. The other 4 were killed as follows: No. 1, killed 10 days after first, 5 days after second feeding. No. 2, killed 12 days after first, 7 days after second feeding. Nos. 3 and 4, killed 29 days after first, 24 days after second feeding.

tubercles in liver (l). e, eye; g, gills; i, small, and i′, large intestine; k, kidneys (mesonephroi); m, mouth cavity; p, transition from pharynx into oesophagus; s, stomach; t, root of the tail.

FIG. 2. Early tubercles at the portal of the liver. The small ducts appearing in cross section are hepatic ducts.

FIG. 3. Advanced tubercle with necrotic center, liver.

FIG. 4. Tubercle developed in submucosa of the small intestine.

While only 2 feedings of bacteria were given it is possible that the tadpoles ingested these organisms from the feces, for the organisms seemingly reproduce in the intestinal tract as they remain there for a long time after feeding them has been discontinued.

Study of the sections of the 4 tadpoles revealed the following conditions: No. 1, tubercle bacilli present in liver; first stages of formation of the tubercles already seen. Also larger tubercles in submucosa of intestine. No. 2, numerous tubercles present in liver, a few with beginning necrosis. No. 3, early, and advanced tubercles with necrotic centers in liver. No. 4, mostly early tubercles, a few with beginning necrosis in liver. A few tubercles also seen in the spleen, containing bacilli.

The structure of the tubercles does not differ in any essential point from those found in mammals. In the largest ones the center appears necrotic and contains acid-fast bacteria. (Fig. 3.) Around the necrotic center there are numerous epithelioid cells, as in the mammal, but no giant cells. The smaller tubercles, whether in the liver (Fig. 2) or in the intestine (Fig. 4) also contain acid-fast bacteria enclosed within phagocytic cells not yet degenerated. The area of the intestinal wall close to the tubercles shows marked changes in its cells and an abundance of tissue phagocytes.

Aside from the intestinal wall, the liver and the lungs, no other organ shows either lesions or bacteria. The stomach wall appears intact and organisms do not occur within the glands, which have wide ducts opening on the surface of the mucosa. Bacteria were also absent in the spleen (except in tadpole No. 4), pancreas, kidneys (mesonephroi), thymus and gonads.

Of special interest are the large numbers of acid-fast organisms found in the alveoli and central cavity of the lungs. The latter in the amphibia are much simpler than the corresponding organs of birds and mammals. Each is a sac with a wide lumen and diverticula representing the alveoli. In the tadpole the lungs extend from the glottis (representing the larynx) to the posterior end of the body and are in contact with the organs of the abdominal cavity since there is no diaphragm.

That the bacilli present within the lungs were not inhaled by the tadpoles is shown by the following facts: 1. Numerous bacilli were found in the tadpoles in which the lungs had not reached full development, the respiratory exchanges taking place through the gills. In these young tadpoles the glottis is closed and no air enters the lungs. 2. Our observations indicate that the tubercle bacilli present in the lung are brought there by migrating phagocytes. Accordingly,

the bacilli occur among degenerated cells most of which are phagocytes, though there are also a few desquamated epithelial cells. Phagocytes containing tubercle bacilli were observed crossing the walls of the lung prior to their entrance in the lung cavity. 3. In older tadpoles, breathing through their lungs, the cellular contents of the lung cavity are much reduced; free cells and bacteria are presumably eliminated through the movement of the cilia of the epithelial cells lining part of the walls of the central cavity of the lung. The alveolar walls are much thinner in this case, and still contain large numbers of acid-fast bacteria.

Another feature of great interest from the standpoint of the path followed by the tubercle bacilli during the infection is their total absence from the blood. We have carefully examined the contents of the heart and of the larger veins, especially the inferior vena cava and the branches of the portal vein in the liver. In no case have we been able to identify free tubercle bacilli in the plasma or enclosed within leucocytes. Furthermore, in some areas in which extravascular leucocytes were present (submucosa of the intestine in the vicinity of tubercles) they did not contain any bacteria.

From our observations it follows that phagocytosis of the bacilli is accomplished only by tissue phagocytes similar to, or identical with, the histiocytes of the mammals.

A point now being investigated by the writers is the exact mode of passage of the bacilli from the intestinal cavity to other organs of the tadpole. Once the intestinal barrier has been surmounted the indications are that they follow the lymphatics or enter the peritoneal cavity, and in the latter case they may enter the organs by crossing through their outer serous coat.

In concluding we wish to emphasize the main point of the present communication, that is, that typical tuberculous lesions may be produced by feeding, and that at least in the case of tadpoles still breathing through their gills large numbers of tubercle bacilli may reach the lungs by an indirect route, being dumped there, so to speak, possibly to facilitate their elimination from the body. The presence of bacilli in the lungs of the tadpoles cannot be attributed, therefore, to direct inhalation of bacilli.