

## MYCOBACTERIAL INFECTIONS IN BIRDS<sup>1</sup>

### I. A PECULIAR INFECTION WITH ACID-FAST BACTERIA IN WOOD PIGEONS (*COLUMBA PALUMBUS L.*)

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This investigation by workers at the State Veterinary Serum Laboratory, Copenhagen, follows up an observation by Plum (*Ibid.* 32 (1942) 465) who, in a study of avian tuberculosis among wild birds, encountered a peculiar infection in wood pigeons in which acid-fast bacilli were present which could not be grown on culture media. Prior to the present investigations a similar infection had been demonstrated in five wood pigeons.

In the work here reported a total of 230 wood pigeons were examined, in 13 of which the infection was present. On autopsy, material was removed from liver and spleen for smears and cultures. Smears were stained by Ziehl-Neelsen's method and if acid-fast were present, additional material was fixed in four per cent formalin for histological examination. Cultures were made from the liver and spleen of all birds on Loewenstein's and on Besredka's medium. If acid-fast had been found, Dunkin's medium for isolation of *M. paratuberculosis* was also inoculated. (Contains killed *M. phlei*.) Later on, experiments were made with various modifications of these media, including addition of killed tubercle bacilli, or extract of such, to Loewenstein's and extract from the liver of an infected wood pigeon to Dunkin's. The inoculated media were observed for at least three months.

*Pathologic changes.*—Macroscopic changes as a rule were found only in the liver and spleen and were most pronounced in the latter organs. The nutrition of the birds was usually very good and it is emphasized that no pathologic processes were found in the digestive canal. In some cases no macro-

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scopic changes were found and in others only an increase in size and consistency of liver and spleen were detected. Frequently only tiny punctate or greyish-yellow streaks were seen, but sometimes, and usually only in the spleen, there were larger caseated nodules. In certain cases irregular or roundish indistinctly defined nodes were present in both organs consisting of fleshy tissue without areas of necrosis. In extreme cases, both liver and spleen were crowded with such nodes which almost replaced the normal tissue. Smears from organs showing macroscopic changes nearly always showed enormous numbers of acid-fast bacteria, resembling tubercle bacilli, arranged in heaps. In macroscopically unchanged organs in which infection was present the number of acid-fasts was considerably fewer.

Two exceptional cases were found: In an emaciated bird a firm node of walnut size was lying free in the peritoneal cavity, which appeared to have been detached from the liver. The cut surface of this nodule was dry-greyish and presented a concentric pattern. The mesentery of the crop showed several nodules from pin head to almond size, of similar nature to the large one. In the remnants of the liver a single miliary process was observed, and in the myocardium four scattered, miliary yellowish areas surrounded by a greyish zone. Smears from the node in the peritoneal cavity showed numerous acid-fast rods.

In the second case only the head of the pigeon was sent to the laboratory. Below the right eye there was a firm tumor-like node, a little larger than a bean, the overlying skin being bare but intact. A similar node was present, subcutaneously, above the left eye. On section both nodes were firm, putty-like, brown in color, with smooth surface and concentric pattern, surrounded by a loose thin capsule. Smears showed myriads of acid-fast bacteria.

The histological changes suggested those of tuberculosis. Three types are described with no sharp borderline between them. (1) A necrotic caseous mass, surrounded by a dense ring of giant cells, partly of the Langhans' type, partly with nuclei scattered throughout the cell, and partly like foreign body giant cells arranged in palisades (2 illustrations). Other nodules consisted of giant and epithelioid cells with only a small central area of caseation, or without any regressive changes. Lymphocytes were numerous in the loose surrounding

connective tissue. Acid-fasts were present in enormous numbers chiefly in necrotic tissues and in epithelioid and giant cells but also in fewer numbers lying free in connective tissue. (2) Nodular or more diffuse infiltrations with epithelioid and giant-cells. There were some with central areas of necrosis (illustration). In addition the liver showed infiltration with lymphocytes mostly as irregular streaks between epithelioid and giant-cells (illustration). These processes showed enormous heaps of acid-fast bacilli. (3) Another type observed only in the liver consisted of lymphocytic infiltrations in the center of which there were islands of epithelioid and giant-cells. Acid-fast bacilli were found but were scanty in very small infiltrations. In some cases a few acid-fast rods were found in the liver, in absence of any detected tissue changes.

Thus the changes observed were chiefly of a productive nature but caseation was not infrequent. The predominating feature was the infiltrating, large-celled hyperplasia.

*Attempts at transmission.*—Seven domestic pigeons, four very young, were inoculated with liver from infected wood pigeons, two intramuscularly and two intravenously. All were negative when killed five or six months later. Two chickens were inoculated intramuscularly and killed after about six months. Each showed a mass of connective tissue at the site of inoculation, containing several small caseated nodules (pin-head size), smears from which revealed acid-fast rods. Histologically these nodules consisted of epithelioid and giant-cells with central nuclei. Many showed a greater or lesser degree of caseation. Similar nodules were found in the liver. Material from these nodules was injected intramuscularly into two additional chickens which were normal when killed six months later. Two guinea pigs and one rabbit were inoculated subcutaneously and one rabbit intravenously with liver from a naturally infected pigeon with negative results, except that the rabbit inoculated subcutaneously showed scattered miliary nodules in the lungs containing a few acid-fast bacilli. Cultures were negative. Before and after inoculation, tests with avian and bovine tuberculin were made on one rabbit and three guinea pigs. The guinea pigs developed a distinct hypersensitivity to avian tuberculin and the rabbit a weak reaction to bovine and to avian.

The authors note that the infection had not been described previously in wood pigeons (except by Plum). A similar in-

fection had been described in a black grouse (*Tetrao tetrix L.*) by O. Grini (*Norsk Vet. Tidsskr.* 1942, 54:337).

Danish wood pigeons leave Denmark early in October, migrating to Holland, Belgium and France, returning in March. A few may stay in Denmark through the winter. In October a great many pass through Denmark on their southward migration from farther north. The pigeons examined were caught between October and December and were probably remnants of Danish birds besides some from Sweden.

#### AUTHOR'S SUMMARY

Description is given of an infection in wood pigeons (*Columba palumbus L.*) with acid-fast bacteria which morphologically resemble tubercle bacilli, but fail to grow on culture media.

The infection attacks preferably the spleen and liver, where a productive inflammation develops that may lead to the formation of fleshy nodules, and which microscopically greatly resembles tuberculous productive inflammation.

In experiments on various animals, intramuscular and intravenous inoculation of two cockerels and one rabbit, respectively, resulted in tuberculous-like pathological processes; but these could not be transmitted to other animals. No lesion corresponding to the spontaneous infection could be produced experimentally. Inoculation of other cockerels and rabbits as well as domestic pigeons and guinea pigs gave no take. Of the inoculated guinea pigs three were submitted to tuberculin tests and showed a fairly marked sensitiveness to avian tuberculin.

So far this infection has been found accidentally in five wood pigeons sent to this laboratory from various parts of the country, and in 13 cases revealed by a systematic investigation comprising 230 wood pigeons.