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**Spontaneous and Experimental Infection of Pigeons with
B. Aertrycke.**

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In the course of some studies upon blood-formation in pigeons, which had first been rendered anemic by fasting and then maintained upon various diets, several birds showed total white blood-cell counts varying from 60,000 to 187,000 cells per cmm. In the blood of these pigeons, myelocytes, varying from 2% to 70%, appeared. Coincident with the development of this striking change of the myeloid elements of the blood, these pigeons became acutely ill, rapidly developed an extreme degree of anemia (1,200,000 to 1,900,000 erythrocytes per cmm.) and died within less than a week after the apparent onset of their illness. In a series of 52 pigeons, 7 birds died in this manner.

Autopsies showed marked enlargement of the liver, spleen, and kidneys and massive hyperplasia of the bone-marrow. The liver and kidneys were studded with extensive infiltrations of myelocytes in which extremely young forms, frequently in mitosis, were present. In addition, large numbers of mononuclear phagocytes, frequently forming nodules, were found in the liver, spleen, kidneys, and bone-marrow. In these latter lesions numerous small, Gram-negative bacilli were regularly present, but no bacteria were ever demonstrated in the accumulations of myelocytes seen in the liver and kidneys. In none of the other 45 pigeons of the series were changes like these noted.

No adequate bacteriological studies were done upon the first 3 birds showing these lesions, but cultures of the blood, liver, kidney, and bone-marrow of the fourth bird (Pigeon 86) all gave pure growths of a small, Gram-negative bacillus, identified by Dr. L. T. Webster as *B. aertrycke*. An identical organism was recovered from the other 3 birds spontaneously developing the same disease.

The blood and tissues of Pigeon 86 were inoculated into 8 well-nourished, apparently normal pigeons. Three birds received 1 cc. each of blood given intravenously, two 2 cc. each of a heavy saline emulsion of liver, two 2 cc. each of a similar emulsion of bone-marrow, and a single bird was injected with 2 cc. of emulsion prepared from the kidney. One of the pigeons which had been inoculated

with the liver-emulsion became ill within 24 hours and died on the fifth day after inoculation. Though the total leucocyte count of this bird never exceeded 35,000 cells per cmm., the myelocytes varied from 36% to 71% during the course of its illness. At autopsy, lesions identical with those of Pigeon 86 were found and *B. aertrycke* in pure culture was recovered from the liver. None of the other 7 pigeons showed any evidence of disease, although they were observed for a month before being killed for study. It is of interest, however, that *B. aertrycke* was grown from the livers of 3 of them, although no lesions were present. Cultures made from the livers of the other 3 remained sterile.

Broth cultures of the organism (*B. aertrycke*) originally isolated from the liver of Pigeon 86 have proven highly pathogenic for pigeons when injected intraperitoneally, 0.5 cc. of a 24-hour culture causing death within 48 hours. Five such birds all developed moderately high leucocytosis but showed no myelocytes in the peripheral blood. At autopsy, intense fibrinopurulent peritonitis, acute splenic tumor, cloudy swelling of the liver and kidneys, and early, but well-marked, myeloid hyperplasia of the bone-marrow were found; *B. aertrycke* was readily recovered from all 5 birds.

Seven normal pigeons, whose blood previously had been studied and also found to contain no agglutinins for *B. aertrycke*, were fed with 24-hour broth culture of the original strain of *B. aertrycke*, 1 cc. being administered to each bird, drop by drop, from a small pipette. Within 48 hours, all developed loss of appetite, diarrhea, and leucocytosis. After an illness lasting from 9 to 27 days, 4 died and 3 recovered. During this time the individual birds developed leucocytoses varying from 60,000 to 162,000 per cmm., due mainly to an increase in number of myeloid cells but also in part produced by a well-marked rise in monocytes. Myelocytes appeared in numbers varying from 1% to 10%. There was a moderate reduction in number of lymphocytes. Those birds which died had developed a much greater degree of anemia than those which recovered, but in other respects no well-marked difference was apparent in the blood-pictures of the 2 groups. At autopsy, the 4 pigeons which died showed enormous myeloid hyperplasia of the bone-marrow, extensive infiltrations of myelocytes throughout the liver and kidneys, as well as all of the other lesions which have been described above in birds spontaneously developing what appeared to be the same disease. In addition, 3 of these pigeons showed lobular pneumonia which was clearly due to bacteria aspirated at the time of feeding. No lesions were present in the intestines of any of them. *B. aertrycke*

was isolated in pure culture from the liver of all 4 birds, from the pneumonic lesions of each of the 3 cases in which they occurred, but from the intestine of only one bird. Cultures of the intestine of the other 3 gave colon bacilli. All cultures were made immediately after death.

One month after oral administration of *B. aertrycke*, the sera of the 3 birds which recovered were tested for agglutinins to this organism. None was found in one case, but the other 2 agglutinated the bacteria in dilutions of 1/1280 and 1/2560 respectively. At the end of 2 months the blood pictures and weights of all 3 pigeons had returned to normal. They have been observed now for 5 months and have shown no manifestations of disease.

Although it seemed highly probable that the organism with which we were dealing was responsible for these striking disturbances of the myeloid cells, it was obviously necessary to rule out the presence of some filterable forms of microorganism in our cultures before drawing this conclusion. Accordingly, each of 6 normal pigeons was fed 2 cc. of a bacteria-free filtrate prepared by passing a 24-hour broth culture of *B. aertrycke* through a VV Berkefeld filter. To test the sterility of this filtrate, 40 cc. of it, taken from that with which the birds were fed, was inoculated into 100 cc. of sterile broth. No growth took place within 48 hours. None of these 6 pigeons showed any loss of weight, changes in the blood or other manifestations of disease for one month after oral administration of filtrate. They were then killed for study but no gross or microscopical lesions other than certain minor changes frequently observed in apparently normal pigeons were found. No agglutinins for *B. aertrycke* were found in the blood of any of them; cultures of the liver were sterile in all instances and no pathogenic organisms were isolated from the intestine. The pathogenicity of the broth culture of *B. aertrycke* from which the filtrate was prepared was tested by feeding it in amounts of 1 cc. to each of 5 normal pigeons. All 5 birds developed characteristic symptoms of infection with this organism. Two of them died, showing all of the lesions which have been described and *B. aertrycke* was readily recovered in both instances. The other 3 survived but showed high agglutination titers to *B. aertrycke* a month after having been fed with this organism.

We therefore offer these experiments as evidence of the following points: 1. The spontaneous development in under-nourished pigeons of a fatal disease associated with marked myeloid hyperplasia of the bone marrow, striking increase of the myeloid elements of the blood, and extensive infiltration of the liver and kidneys with mye-

loid tissue. 2. The cultivation of a bacillus from such pigeons, which has been identified as *B. aertrycke*. 3. The experimental production in pigeons of apparently the same disease and identical lesions by injection of liver-emulsion of an infected bird or the oral administration of cultures of this organism. 4. Bacteria-free filtrate of *B. aertrycke* produces no demonstrable effect when fed in large, single doses to normal pigeons.

Though the relationship of this disease to fowl leucemia is one of considerable interest, we can offer no further comment on this question since we have not yet had the opportunity of studying the latter disease. Though the literature contains many reports of unusually high leucocytoses in birds, we have been unable to find any instance in which large numbers of myelocytes in the peripheral blood or heterotopic formation of myeloid cells, as we have observed, have resulted from bacterial infection. Further studies now in progress upon this subject will be reported at a later date.