SPONTANEOUS AND EXPERIMENTAL INFECTION OF PIGEONS WITH B. AERTRYCKE *

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INTRODUCTION

During the course of some experiments upon blood formation in pigeons, which had first been undernourished for varying periods of time and then maintained upon diets consisting mainly of fetal beef tissues, an occasional bird became critically ill, developing diarrhea, grave anemia, and high leucocytosis with many young myeloid cells in the peripheral blood. Death invariably occurred within a few days after the onset of these symptoms. Such pigeons, though greatly emaciated, showed at autopsy marked enlargement of the liver, spleen and kidneys, as well as extreme myeloid hyperplasia of the bone marrow. Throughout the liver and kidneys, innumerable small yellowish gray points and irregularly shaped zones were grossly visible. Upon microscopic examination these areas were found to consist mainly of large numbers of myelocytes grouped about the blood vessels in these organs. The spleen showed a marked increase of large, clear, mononuclear, phagocytic cells and almost complete absence of lymphoid tissue, but contained no such accumulations of myelocytes as were seen in the liver and kidneys.

These changes presented a striking contrast to the fatty bone marrow and atrophied organs of the other equally poorly nourished pigeons of our series dying or killed after identical experimental procedures. Though it appeared that the exceptional pigeons were dying of some unusual complication, possibly of bacterial origin, we were unfamiliar with any variety of microorganism which had been shown to be capable of producing such lesions. Therefore, the changes in the blood and bone marrow, as well as the extensive extramedullary formation of myeloid tissue, seemed to us to present a problem worthy of further study.

* Received for publication March 27, 1931.

PRELIMINARY EXPERIMENTS

No bacteriological studies were made upon the first three birds exhibiting the unusual features just mentioned, but cultures of the blood, liver, spleen, kidney and bone marrow of the fourth bird (Pigeon 86) to die under these conditions all gave pure growths of a small bacillus which was identified by Dr. L. T. Webster of the Rockefeller Institute as *Bacterium aertrycke*.

This organism, which varies from 1 to 2.5 microns in length and from 0.3 to 0.4 of a micron in thickness, is a Gram-negative, motile rod with rounded ends. In smears prepared from young cultures, the bacillus varies in morphology from rod to almost coccoid forms and its body stains evenly, though individual organisms with more deeply staining ends are usually present. Numerous long, peritrichal flagella are revealed by appropriate stains. In cultures, both acid and gas are produced in dextrose, maltose, mannite, levulose, xylose, galactose and dulcitol, the reactions being more marked in the last four sugars than in the three former ones. Saccharose and lactose are not affected, even after several days' incubation. Indol is not formed. Litmus milk is rendered slightly acid within the first twenty-four hours but the reaction later changes to alkaline. Milk is not clotted. On potato the growth is very poor, forming only a faintly visible, grayish white pellicle. Coagulated serum and gelatin are not liquefied. In broth the growth produces even cloudiness, but after several days a grayish white sediment is usually formed. Cultures on lead acetate medium show that H₂S is produced. On meat infusion agar slant the organism forms a thin, transparent, confluent growth, a characteristic of all bacteria belonging to the paratyphoid group. On blood agar plates the growth is luxuriant; large, round, flat colonies with pale centers are formed in twenty-four hours. On China blue rosolic acid medium the colonies are small, round, slightly elevated and of distinctly pinkish color.

The immunological behavior of this organism strongly suggests that it represents a separate species. Cultures are agglutinated by immune pigeon and rabbit sera in high dilutions, the usual titre being 1:2560. *B. typhosus* and *B. paratyphosus B* are agglutinated by anti-aertrycke serum in dilutions of 1:40 and 1:10 respectively. Rabbit sera rendered immune to *B. typhosus*, *B. paratyphosus A*, *B*, and *C* usually agglutinate *B. aertrycke* in dilutions up to 1:40. The

blood of normal pigeons rarely contains agglutinins for *B. aertrycke*, and even on these occasions the bacilli are not agglutinated in dilutions greater than 1:20.

Inasmuch as we have subsequently used the culture of organisms isolated from the liver of Pigeon 86 to produce all experimental infections, we submit the following complete protocol of this bird.

TABLE I
Protocol of Pigeon 86

Diet	Full grain (Apr. 24-26)	Starvation (Apr. 27- May 3)	5 gm. fetal liver daily (May 4-16)					Food refused	
Dates blood examined. . . .	April 25	May 2	May 6	7	10	13	14	15	16
White blood cells (thousands per cmm.)	14	4	11	9	14	55	69	133	187
Granulocytes %	40	54	78	86	64	87	88	91	85
Myelocytes %	0	0	0	0	0	0	0	0	5
Lymphocytes %	42	44	12	4	26	1	5	4	4
Monocytes %	18	2	10	10	10	12	7	5	6
Red blood cells (millions per cmm.)	3.7	2.6	3.3	3.1	3.1	2.5	2.2	1.9	1.2
Thrombocytes (thousands per cmm.)	75	16	25	36	37	26	59	33	37
Weight (gm.)	425	355	320	320	300	280	265	245	230 Died

The *liver* and *kidneys* were found to be greatly swollen and studded with massive accumulations of myelocytes intermingled with smaller, basophilic, poorly differentiated, mononuclear cells. Many large mononuclear phagocytes and bacteria were found in the sinuses of the liver. The *spleen* was also much enlarged, due mainly to the presence of large, mononuclear phagocytes, but showed no such accumulations of myeloid cells as were seen in the liver and kidneys. The heart, lungs, alimentary tract, reproductive organs and brain were unaltered.

At the time of autopsy the blood as well as emulsions of the various organs of Pigeon 86 were injected into a series of normal birds. Of three pigeons, each injected intraperitoneally with 1 cc. of blood, none developed any symptoms of disease. When these birds were killed for study a month later, no anatomical changes were found, but *B. aertrycke* was grown from the livers of two of them. Of seven

pigeons injected intraperitoneally with 2 cc. of a thick saline emulsion of liver, kidney, or bone marrow, two which had received liver became acutely ill within twenty-four hours and died forty-eight hours and five days respectively after inoculation. Shortly before death one of these birds exhibited a leucocytosis of 75,000 cells per cmm., of which 3 per cent were myelocytes. The other showed only a slight increase in total number of leucocytes, 21,000 per cmm., but of this number 71 per cent were myelocytes. These two pigeons showed acute fibrinopurulent peritonitis, as well as the same types of anatomical changes seen in Pigeon 86, and *B. aertrycke* was recovered from the liver of each of them in pure culture. None of the remaining five birds showed any symptoms of disease following injection, or any macroscopic lesions* when killed a month later, but *B. aertrycke* was grown from the livers of three of them at this time. Cultures made from the livers of the other two birds were sterile.

Three normal pigeons were injected intraperitoneally with 0.1 cc., 0.5 cc., and 1 cc. respectively of a pure 24 hour broth culture of *B. aertrycke*, originally isolated from the liver of Pigeon 86. The first bird showed no manifestations of disease and no anatomical changes when killed a month later. The other two birds, however, died four days and thirty-six hours respectively after inoculation, showing all of the clinical and most of the anatomical characteristics of the disease under study. In addition, fibrinopurulent peritonitis was present in both instances. No myelocytic infiltrations were found in the liver and kidneys of the bird dying thirty-six hours after inoculation. From the livers of all three pigeons *B. aertrycke* was isolated in pure culture.

It therefore seemed highly probable that infection with *B. aertrycke* was the cause of the unusual lesions and death of certain of our birds but, inasmuch as each pigeon was kept in a separate cage and cultures of their water and food failed to reveal the presence of this organism, the source of the infection was not clear. However, since it had been demonstrated experimentally that small doses of *B. aertrycke* had little or no effect upon normal pigeons, and, moreover, that these bacteria often survived within the body for at least a month without producing symptoms of disease or anatomical

* In the removal of this work from New York to Peiping the microscopic preparations made from the tissues of these birds were unfortunately lost.

changes, it did not appear unlikely that certain pigeons naturally harbored minimal numbers of this organism and developed generalized infection when subjected to a state of malnutrition. At this point it is of interest to note that a single previously normal pigeon (No. 94), after a period of eight days' starvation, became unduly ill, totally refused food and died within forty-eight hours, showing all of the lesions accompanying infection with *B. aertrycke*. This organism was recovered in pure culture from the liver.

In order to determine whether or not pigeons dying of malnutrition, but showing no evidence of bacterial infection, ever harbored *B. aertrycke* in their tissues, cultures were made of the livers of twenty such birds which had been kept under experimental conditions identical with those of the pigeons dying of infection with this organism. Sixteen of these cultures showed no growth of bacteria, but from four birds *B. aertrycke* was recovered. Though none of these four pigeons showed changes in the blood before death which would suggest infection with *B. aertrycke*, in two of them the characteristic lesions accompanying infection with this organism were found. The other two showed only the effects of malnutrition.

The results of these preliminary studies may be summarized as follows: In a series of fifty-two pigeons, which first had been subjected to a period of starvation and then maintained for varying periods of time on fetal beef tissues, seven cases of an acute, fatal disease associated with marked anemia and enormous activity of myeloid cells occurred. From three of these birds, which were studied bacteriologically, *B. aertrycke* was isolated in pure culture, and in the tissues of the other four an abundance of bacteria morphologically identical with this organism were demonstrated. Four additional instances of the presence of *B. aertrycke* were encountered in twenty routine bacteriological examinations of pigeons of this series showing no clinical evidence of bacterial infection. Two of these showed anatomical changes attributable to the infection with *B. aertrycke*, while the other two did not.

Although it was demonstrated that a disease process could be initiated in normal pigeons by inoculation of tissue from an infected bird as well as by inoculation of cultures of *B. aertrycke*, the nature of the lesions, in both the spontaneous and experimental infections, caused us to have some doubt as to their exclusive relationship to this organism. The lesions in which the bacteria were found,

consisting of aggregations of mononuclear phagocytes and polymorphonuclear leucocytes, were readily explained. However, such massive myeloid hyperplasia of the bone marrow, the frequent appearance of large numbers of myelocytes in the peripheral blood, and the striking heterotopic formation of myeloid tissue in the liver and kidneys presented a picture of myeloid activity hitherto unrecorded in the course of bacterial infections. Furthermore, since *B. aertrycke*, in several instances, had been found in pigeons which showed no symptoms or anatomical evidence of active infection with this organism, and also had been shown experimentally to survive in the tissues of pigeons for considerable periods of time without producing disease, it was obvious that further facts must be sought before the relationship of this organism and the interesting lesions with which it was frequently associated in pigeons could be understood.

METHODS

Each bird, upon entering the laboratory, was placed in a separate cage and observed for a period of two weeks, during which time the weight was followed and the blood examined several times. Only those pigeons which appeared normal during this time were used for experimentation.

Supravital staining with neutral red and Janus green,¹ which facilitates a ready distinction of all the cellular elements of the blood, was used routinely in making the differential cell counts. Striking changes in the blood, such as the appearance of numerous young myeloid forms, were always checked in fixed films. The total counts of the red and white cells were made according to the method of Forkner,² which we have found to be thoroughly reliable.

In order that experimental infection might be more nearly comparable to infection naturally acquired, we have always introduced the bacteria by mouth, slowly feeding 1 cc. of a 24 hour pure broth culture, drop by drop, with a small pipette. After infection, the blood was studied at frequent intervals until death or recovery. Autopsies, as well as bacteriological and serological studies, were done promptly after death, an occasional moribund bird being killed in order to secure perfectly fresh tissues.

To test our cultures for the presence of some filterable form of microorganism or other substance which might have been respon-

sible for the excessive growth of myeloid tissue regularly accompanying infection with *B. aertrycke*, normal pigeons were fed or infected with sterile filtrates prepared from this organism.

To serve as controls for our other observations, bacteriological, serological and anatomical studies were made upon a series of apparently normal pigeons.

THE BLOOD OF NORMAL PIGEONS

The blood of normal pigeons has been the object of study of only a few investigators,^{3, 4, 5} but all of those who have dealt with it have reported marked fluctuations in number of all types of blood cells.

During the course of our experience, based upon 182 apparently healthy pigeons bought at random in the markets of New York and Peiping, we have found that the red blood cells remain unusually constant in individual birds, fluctuations of as much as 400,000 cells being quite exceptional. When first brought into the laboratory, most pigeons show erythrocyte counts of 3,500,000 to 4,000,000 cells per cmm. Only an occasional bird shows a slightly higher count, while erythrocyte counts lower than 3,000,000 almost invariably have been found to be associated with some obvious disease.

The white blood cells, however, have shown considerably greater fluctuations in number than have the erythrocytes. Though the total number of white cells was generally found to lie between 8,000 and 20,000 per cmm., an occasional bird, in which no disease was apparent, was studied which presented a count far above or below these levels. We have seen a few such pigeons with leucocyte counts of 50,000 cells per cmm., for which there was no obvious explanation. However, after being kept in the laboratory for a few days, the white cells in such instances have invariably fallen to normal levels and later on have shown no tendency to rise to their original height.

The percentages of the different varieties of blood cells also have been found to vary greatly in different birds. They have remained quite constant in individuals, though the total white cell counts of these same pigeons have varied as much as 10,000 cells from day to day. White counts made in rapid succession upon the same bird almost always agree closely.

The morphological characteristics of the various elements of the pigeon's blood, as far as we know, are nowhere adequately illus-

trated, though Forkner² has recently presented a well illustrated study of the blood of normal fowls, which in most essential features resembles that of the pigeon. We have, therefore, devoted most of the accompanying Plate 67, which illustrates supravitality stained cells from the blood of pigeons, to the early myelocytic types encountered in the circulation in our experimental birds.

The granules of the main group of polymorphonuclear leucocytes (Figs. 1 and 2) of the pigeon, as in the fowl, are rod-shaped but exhibit a decidedly more brilliant and darker yellowish red color when stained either supravitality with neutral red or in fixed films with Wright's stain. We have found no cells in the pigeon's blood closely corresponding to the "pseudoeosinophils" found in small numbers in the fowl's blood by Forkner, but we have observed regularly a few myeloid cells, not present in the blood of fowls, with pleomorphic or round nuclei and large, brilliantly eosinophilic granules in their cytoplasm. Such cells, which generally exist only in small numbers, less than 5 per cent, are found to be much more numerous in occasional birds, sometimes comprising 50 per cent of the total number of leucocytes. The relationship of these cells to the other cells of the myeloid series is not clear. They apparently tend to remain high in individual birds which show no other abnormality but, if for any reason a leucocytosis occurs, their numbers have never been observed to increase. We have looked upon such cells as true eosinophils and at first thought that their large numbers were perhaps associated with parasitic infections. Though this association has existed in a few instances, such is frequently not the case. Only recently we have seen an apparently normal pigeon with the duodenum and ileum greatly distended with a solid mass of small nematodes in which the total white cell count was 15,000 per cmm., of which only 2 per cent were eosinophilic myeloid cells with round granules. The granules of the basophils of the pigeon are very small, round and stain deep crimson with neutral red. The monocytes (Figs. 12, 13), lymphocytes (Fig. 14), erythrocytes, and thrombocytes show only very slight variations in morphology from those of the fowl. The general appearance of the myelocytes of the two species is about the same, though slight differences in color of the granules exist in both supravitality stained (Figs. 5 to 11) and fixed preparations.

In the following table are summarized the results of our studies

of the blood of normal pigeons. Only birds which were apparently free from disease have been included. The figures given represent average values.

TABLE II
Blood Counts on Normal Pigeons

	New York	Peiping	Total
Number of Pigeons	100	82	182
Number of Blood Counts	238	139	377

	New York		Peiping	
	Total cells per cmm.	Percentage	Total cells per cmm.	Percentage
Erythrocytes	3,813,000	..	3,803,000	..
Leucocytes	13,100	..	15,200	..
Thrombocytes	34,060	..	42,520	..
Eosinophilic leucocytes with rods ...	5,371	41	5,320	35
Eosinophilic leucocytes with round granules	917	7	760	5
Basophilic leucocytes	393	3	304	2
Lymphocytes	5,109	39	7,144	47
Monocytes	1,310	10	1,672	11

THE EXPERIMENTAL INFECTION OF PIGEONS WITH *B. AERTRYCKE*

After two weeks preliminary observation, during which time their blood was studied, seventeen apparently normal pigeons were each fed 1 cc. of a 24 hour broth culture of *B. aertrycke*. All of these pigeons became acutely ill within thirty-six hours, most of them developing diarrhea and showing loss of weight, although given food and water in unlimited amounts. Nine of them died within ten to twenty-five days after infection; the remaining eight recovered.

Changes in the Blood: After feeding *B. aertrycke* the total number of leucocytes, which had varied in different birds under normal conditions from 6,000 to 29,000 cells per cmm., rose sharply to levels of 46,000 to 160,000 cells per cmm. (Chart 1). Most of the pigeons which died succumbed at a point near the peak of their leucocytosis, but in two birds, after only moderately high leucocytoses of 46,000 and 48,000 cells per cmm., the total number of white cells suddenly fell to 2,000 and 6,000 cells per cmm. respectively. Examination of the bone marrow of these two birds after death showed very few myeloid forms but did reveal a striking degree of hyperplasia of the erythrocytic cells. Extensive myeloid infiltration was present, however, in kidneys and liver of each bird. This particular observation

is suggestive of the two types of leukemic manifestation, erythro-leucosis and myeloid leucosis, reported as occurring in different fowls inoculated with identical material from an animal dying of a typical myeloid leukemia. That is, with apparently the same underlying stimulus, two different responses may be elicited, depending upon the individual bird. It is not known at present what the nature of the factors governing the individual response may be.

The leucocytosis following infection with *B. aertrycke* was clearly due primarily to an increase in number of the eosinophilic polymorphonuclear leucocytes with rod-shaped granules (Chart 2). Within three to five days after infection mitochondria became visible in the motile granulocytes. The delicately shaped rods of these cells became greatly swollen (Figs. 1, 2, 4), assuming an oval or rounded form though they still stained brilliantly, and atypical microcytes (Fig. 3) were found. After a week many of these younger adult forms of the eosinophilic rod cells, as shown by their slightly indented nuclei and numerous mitochondria, were seen regularly in the peripheral blood. The motility of all granulocytes was always strikingly decreased during the entire course of the infection. Actual myelocytes, frequently very young forms, appeared in practically all pigeons of this group in numbers varying from 2 to 10 per cent of the total number of leucocytes, but in no instance of experimental infection have we observed such a great number of myelocytes in the peripheral blood as frequently occurred in undernourished pigeons dying from infection with *B. aertrycke* naturally acquired. Fig. 7 represents the late myelocytes "C," ³² with the complement of specific preleucocytic granules nearing the maximum. The myelocytes of the bird have spherical granules throughout the period of maturation in bone marrow, the change to rods occurring coincident with the change in staining reaction, lobing of the nucleus and the acquisition of motility, which immediately precede the extramedullary circulatory appearance and function of these cells. Under normal conditions only fully mature cells with none or very occasional mitochondria, and only rod-shaped granules appear in the circulation. Figs. 5, 8, and 10 represent the stage known as myelocyte "B," midway between myelocyte "C" and the earliest myeloid cells showing specific granulation, namely myelocytes "A" (Figs. 6, 9 and 11). No changes in the polymorphonuclear leucocytes with round eosinophilic granules or in the basophils were noted.

The monocytes showed considerable increase in total number after infection with *B. aertrycke*, tending to reach their highest point before the fifteenth day. Many young monocytes with dense, homogeneous cytoplasm and numerous mitochondria appeared regularly during the infection (Chart 3).

The lymphocytes exhibited a general tendency to fall during the course of the infection. Especially in those birds which succumbed, the diminution of lymphocytes was most marked (Chart 4).

Changes in the Viscera: The changes observed in the viscera of pigeons following oral administration of a large dose of *B. aertrycke* have been almost identical in all birds dying as the result of infection. Therefore, since individual birds showed only minor variations in the degree of lesions commonly present, these changes will be discussed collectively. By far the most interesting are those of the liver, kidney, bone marrow and spleen.

The *liver* is always enlarged, at times reaching a weight of 20 gm. It has an opaque, swollen appearance, is generally of pale, brownish red color, and throughout the organ small, round or irregularly shaped patches of opaque, yellowish gray tissue are seen. Microscopically, the liver cells are found to be greatly swollen and the sinuses filled with large, pale, phagocytic cells containing cellular debris, hemosiderin and occasionally red blood cells. Such phagocytic cells, together with a few polymorphonuclear leucocytes, are often found in solid masses, showing varying degrees of necrosis in their central areas where clumps of small Gram-negative bacilli are usually present (Figs. 15, 16). Many such bacilli are scattered in smaller number throughout the liver sinuses. In addition to these lesions, which clearly seem to be due directly to the bacteria which are found within them, each portal and hepatic vein is found to be surrounded by varying numbers of myelocytes intermingled with lesser numbers of slightly smaller, basophilic, mononuclear cells, which contain no granules in their cytoplasm (Figs. 17, 18, 19). In early infections these latter cells have occasionally outnumbered the myelocytes with which they were associated. The myeloid tissue usually present is quite excessive, frequently forming areas visible to the naked eye. Bacteria have never been found in these lesions.

The *kidneys* are always considerably swollen and of pale, yellowish brown color. Large foci of myelocytes and basophilic, mononuclear cells apparently arising about the blood vessels, are regu-

larly present which closely correspond to the lesions seen in the liver (Fig. 20). In some instances there are also small collections of mononuclear, non-granular cells scattered throughout the interstitial tissue of the kidneys. The epithelium of the tubules is generally much swollen.

Of all the changes occurring in pigeons after infection with *B. aertrycke* those found in the *bone marrow* are perhaps the most extensive. The radius, ulna and femur, which were examined regularly, were filled with solid, firm masses of grayish red, opaque marrow, which literally burst forth from the cracks in the shaft during the process of removal. On microscopic examination one sees a solid mass of myeloid cells in which young forms predominate. The capillaries are collapsed by pressure of the proliferating myelocytes and erythrogenesis, so conspicuous in the marrow of normal pigeons, is seen only in certain very limited areas (Figs. 21, 22). Scattered throughout the marrow are found in many instances large and small aggregations of pale, mononuclear cells similar to those seen in the liver. Occasionally, bacteria may be demonstrated in these areas. Capillaries filled with fresh thrombi and bacteria are seen frequently.

The *spleen* is enlarged from one to three times its normal size, is of very soft consistency and pale pink or yellowish brown in color. Microscopically, the most striking alteration is found to be a marked proliferation of large, clear mononuclear cells. This change, which takes place in the follicles as well as the pulp, is attended with almost total disappearance from the entire spleen of cells which can be identified as lymphocytes. Within the sinuses and throughout the pulp many such phagocytic cells, frequently grouped in nodules with a few polymorphonuclear leucocytes about cellular debris and bacteria, are present. Though fairly numerous polymorphonuclear leucocytes and, in a few instances, occasional myelocytes are seen scattered throughout the spleen, no collections of myeloid tissue occur in the spleen which are in any way comparable to those seen regularly in the liver and kidneys. In the case of only one pigeon, which was shown to be ill with acute *B. aertrycke* infection when brought into the laboratory, were large areas of young myelocytes found in the spleen.

The *lungs*, in most instances where infection has been induced by mouth, have shown lobular pneumonia of an unusual type. Situated only in the dependent (anterior) portion of the lungs, about bronchi

plugged with necrotic exudate, were caseous, often coalescing areas of consolidation which were sharply demarcated from the surrounding tissue. Microscopically, such areas, within which were myriads of Gram-negative bacilli, were found to be composed of necrotic lung tissue and exudate well encapsulated by a thick zone of mononuclear phagocytes and polymorphonuclear leucocytes. *B. aertrycke* was recovered regularly from such patches of pneumonia. The constant, characteristic distribution of these lesions strongly suggests that they were caused by aspiration of a portion of the culture of bacilli with which the birds were fed. No lesions of any kind have occurred in the lungs of pigeons naturally infected with *B. aertrycke*.

We have never found any significant lesions in the *intestine* of pigeons following natural or experimental infection with *B. aertrycke*. Upon being introduced by mouth, these organisms may be recovered from the stool during the following three or four days in about 50 per cent of the birds. After this time, however, they tend to disappear from the intestine. Intestinal contents and mucosa have been cultured in twenty-six pigeons dying from five to twenty-five days after oral administration of *B. aertrycke*, but in only six instances was the organism recovered. Pure cultures of *B. aertrycke* were grown from the liver of each of these birds and morphologically identical organisms demonstrated in sections of other organs.

The *heart*, *pancreas*, *adrenals*, *thyroid*, *ovaries*, and *testes* have shown no lesions worthy of note. We have examined the *brain* in only four birds infected with *B. aertrycke*, but no lesions were found in these instances.

EXPERIMENTS WITH BACTERIA-FREE FILTRATES PREPARED FROM CULTURES OF *B. AERTRYCKE*

Although the oral administration to pigeons of what seemed to be pure cultures of *B. aertrycke* was followed regularly by the disease which we have described, the deduction might not follow that all of the anatomical changes present were caused by this organism. It was clear that the lesions containing bacteria were the direct result of infection with *B. aertrycke*, but the possibility still existed that our cultures were contaminated with a filterable microorganism or contained some filterable agent which was responsible for the changes in the myeloid cells. Accordingly it was decided to test upon pigeons

the effect of bacteria-free filtrates prepared from virulent cultures of *B. aertrycke*.

From a 200 cc. flask of a 24 hour broth culture of *B. aertrycke*, 100 cc. was passed through a VV Berkefeld filter. To test its sterility 40 cc. of this filtrate was immediately inoculated into a fresh flask of sterile broth, and this flask, together with the flask containing the remaining 60 cc. of filtrate, incubated overnight. No growth of bacteria occurred in either of these two flasks. Each of six apparently normal pigeons, whose blood had been found free of natural agglutinins to *B. aertrycke*, was then fed 2 cc. of this sterile *B. aertrycke* filtrate. To test the potency of the organism from which the filtrate was made, 1 cc. of the original broth culture of *B. aertrycke*, then 36 hours old, was fed to each of six other normal pigeons.

As shown in Chart 5, no appreciable changes occurred within twenty-one days in the blood of the six pigeons fed with filtrate, nor did they show any loss of weight or other evidence of disease. When they were killed at the end of this time, no agglutinins for *B. aertrycke* were found in their blood, cultures of liver were sterile in each instance, and no gross or microscopic lesions suggestive of infection with *B. aertrycke* were found in any of their tissues.

All of the six pigeons fed with the original culture of *B. aertrycke* from which the filtrate was made became acutely ill. Four died; two recovered. Autopsies upon the four which died showed all of the characteristic lesions of infection with *B. aertrycke* and this organism was recovered from the organs in each case. The sera of the two birds which recovered agglutinated cultures of *B. aertrycke* in dilutions of 1:320 and 1:2560 respectively one month after infection.

This experiment has since been repeated in a slightly different form. Another sample of filtrate, prepared as outlined above, was fed in amounts of 2 cc. to each of five normal pigeons and also injected intravenously in amounts varying from 0.2 to 1.5 cc. into each of five others. At the same time, as controls, two normal birds were injected intravenously with 1 and 2 cc. of sterile broth, respectively, and each of two others fed with 2 cc. of the same material. The virulence of the culture of *B. aertrycke* from which the filtrate was made was tested by feeding 1 cc. to each of two normal birds.

None of this series of ten pigeons, either fed or intravenously inoculated with *B. aertrycke* filtrate, or the four controls receiving

sterile broth showed any change in blood picture, loss of weight or other evidence of disease during the ensuing month after administration of these substances. When killed at this time no gross or microscopic changes in any way resembling the lesions occurring in *B. aertrycke* infection were seen. Cultures of the liver made upon blood agar plates were universally sterile, and the blood in each case showed no agglutinins for *B. aertrycke*. Both of the controls, upon which the virulence of the culture of *B. aertrycke* was tested, died within ten days. They showed all of the characteristic symptoms and lesions produced by this organism, which was recovered from the livers of both birds.

Therefore, inasmuch as two different series of pigeons, in all numbering sixteen birds, either fed with or intravenously injected with large, single doses of broth filtrate prepared from virulent cultures of *B. aertrycke* have shown no clinical, anatomical, bacteriological or serological evidence of disease, it seems at least highly probable that all aspects of the disease-picture regularly occurring in pigeons after experimental infection with *B. aertrycke* are due entirely to that organism or its products. However, the possibility that a virus or other substance may be concerned in the alterations of the myeloid tissue is still to be kept in mind until the question has been studied further.

STUDIES UPON NORMAL PIGEONS

In order to gain some idea of the frequency with which pathological changes occur within the tissues of apparently normal pigeons, and to serve as a general control for our observations upon pigeons infected with *B. aertrycke*, we have studied sixteen birds which gave the appearance of being entirely free of disease. The results are summarized in Table III.

All of the six pigeons, which are stated to have been under observation for ten months, were kept in separate cages but in close proximity to the cages of pigeons infected with *B. aertrycke*. During this time their bloods were examined at frequent intervals but no significant changes were observed; none of them showed any loss of weight or other evidence of disease. The remaining twelve birds observed for one week before being killed for study were well nourished, took their food well, and their blood cells, upon two examinations, showed no pathological changes.

TABLE III

Summary of Pathological Changes in Sixteen Normal Pigeons used as Controls for Pigeons Infected with B. Aertrycke

No.	Period of observation	Cultures of liver	Agglutination <i>B. Aertrycke</i>	Pathological changes in tissues
7	10 months	No growth	1:64	Moderate increase in small, round cells about blood vessels of liver and kidney. In a few instances round masses of these cells resembling germinal centers of lymph glands seen
8	10 months	No growth	o	Three small groups of adult polymorphonuclear leucocytes with an occasional myelocyte among them seen adjacent to portal veins
10	10 months	No growth	o	Small round cells (lymphocytes?) slightly increased about vessels of liver
11	10 months	No growth	1:32	Enormous increase of lymphocytes about vessels of liver. Moderate increase of such cells in kidney
12	10 months	No growth	o	No lesions
13	10 months	No growth	o	No lesions
72	1 week	No growth	o	Very slight increase of round cells about vessels of liver
73	1 week	No growth	o	Considerable increase round cells in liver. Small healing necroses in liver and also small nodules consisting of central giant cells and debris surrounded by large, clear monocyctic cells and lymphocytes. No tubercle bacilli demonstrable. These lesions do not resemble those of tuberculosis
75	1 week	No growth	o	No lesions
77	1 week	No growth	o	Liver shows nodules as in Pigeon 73. Moderate hyperplasia of bone marrow
79	1 week	No growth	o	Liver shows 1 nodule as in Pigeon 73
81	1 week	No growth	o	Moderate increase of round cells in liver, grouped about which are a few adult polymorphonuclear leucocytes and an occasional myelocyte. Many small basophilic cells seen about vessels and in interstitial tissue of kidney. Moderate hyperplasia of bone marrow
82	1 week	No growth	o	Periportal round cells of liver moderately increased. Also a few polymorphonuclear leucocytes and an occasional myelocyte seen in these areas. No increase of round cells in kidney but 1 small patch of myelocytes seen
84	1 week	No growth	o	No lesions
85	1 week	No growth	o	No lesions
88	1 week	No growth	o	No lesions

As seen in the table, cultures of the liver made upon blood agar plates were universally sterile, but the sera of two of the pigeons which had been kept for a long period of time in close proximity to birds infected with *B. aertrycke* were found to agglutinate *B. aertrycke* in dilutions of 1:64 and 1:32 respectively. No agglutinins were present in the bloods of any of the other birds. Attention is called to the frequency with which pathological changes are found in the liver and kidneys of apparently normal pigeons, also to the fact that occasional myelocytes are present in the tissues of such birds. No noteworthy changes of any kind were found in the bone marrow, lungs, spleen, testes, ovaries and intestine.

STUDIES UPON PIGEONS SURVIVING EXPERIMENTAL INFECTION WITH *B. AERTRYCKE*

Bacteriological, serological and anatomical studies have been made upon eight pigeons which survived experimental infection with *B. aertrycke* for periods of time varying from five to nine months. All of these pigeons were well nourished and showed no alterations in the peripheral blood or other manifestations of disease when killed for study. Each of these birds had, however, become clinically ill, had lost weight and shown marked increase of the myeloid elements of the blood for a period shortly after oral administration of *B. aertrycke*.

Bacteriological cultures made routinely from the liver on blood agar plates were universally sterile, and cultures of the intestinal contents and mucosa failed to show pathogenic organisms of any kind.

The results of the serological studies are shown in Table IV.

The changes found in the tissues of these pigeons, though of considerable extent, cannot be fully understood until the histogenesis of the lesions produced by *B. aertrycke* has been studied further. Briefly stated, they consisted almost entirely of the healing of the destructive lesions caused by the bacteria. Pigeon 15, killed nine months after infection, showed only one small, encapsulated, caseous area in the lung, the remains of a small patch of pneumonia, no changes of any kind being present in any of the other tissues. The other seven pigeons, killed five months after infection, showed many large phagocytic cells in the liver and spleen loaded with iron

pigment, numerous healing necroses in the liver, and many circumscribed caseous areas in the lungs, each surrounded by a wall of giant cells and connective tissue. These latter lesions, obviously the result of the characteristic lobular pneumonia produced by *B. aertrycke*, varied from microscopic size to as much as 7 mm. in diameter. The smaller ones were identical with the larger healing necroses found in

TABLE IV
Agglutination Titer of Blood Serum to B. Aertrycke

No.	Before infection	After infection		
		1 month	5 months	9 months
15	0	..	1:1280	0
16	0	0	1:160	..
20	0	1:2560	0	..
22	0	1:80	0	..
25	0	1:1260	0	..
36	0	0	0	..
38	0	1:320	0	..
42	0	1:320	1:80	..

the liver and also bore close resemblance to the nodular lesions frequently found in the liver of apparently normal pigeons. There was almost no trace left of the myeloid hyperplasia which may have existed during the phase of acute disease in this series of pigeons. In three birds, quite numerous small groups of adult polymorphonuclear leucocytes were seen about the blood vessels in the liver. The bone marrow had returned to its normal appearance in six of the pigeons, but in the two remaining birds gelatinous degeneration of the marrow of the radius was present.

DISCUSSION

A number of previous workers have already called attention to unusually high leucocytoses varying from 200,000 to 600,000 cells per cmm. which characteristically occur in birds during the course of certain bacterial infections. Among these may be mentioned Smith and Moore,⁶ who described a disease of fowls caused by an organism to which they gave the name *B. sanguinarium*, Burckhardt,⁷ and Hirschfeld and Jacoby,^{8,9} who studied fowls infected with

avian tuberculosis. So great was the number of myeloid cells in the blood that Smith and Moore considered the disease which they studied to be a form of myeloid leukemia, while Burckhardt expressed the view that myeloid leukemia in fowls was a manifestation of tuberculosis. Hirschfeld and Jacoby oppose Burckhardt's opinion. We can find no blood studies upon pigeons infected with tuberculosis but have ourselves observed a pigeon dying of naturally acquired tuberculous infection whose blood contained 185,000 leucocytes per cmm., of which 92 per cent were mature polymorphonuclears. Cultures of the blood and organs of this bird on blood agar plates and broth were sterile. Though the bone marrow showed marked myeloid hyperplasia, the cells present were mostly adult leucocytes or myelocytes well advanced toward maturity. No myelocytes were seen in the blood and there was no infiltration of other organs with myeloid cells. The mouth, pharynx and bones of the paranasal sinuses showed large caseous lesions, while the liver and spleen contained countless small tubercles. In all of these lesions masses of acid-fast bacilli were present.

None of the observers who have studied these hyperleucocytoses of birds caused by bacteria has reported the presence of an appreciable number of immature white cells in the peripheral blood or the occurrence of infiltrations of such cells in the viscera. We therefore agree with Opie,¹⁰ who recently expressed the view that those investigators reporting the experimental production of diseases closely related to leukemia by means of bacterial infection have failed to justify their claim.

In the case of both natural and experimental infection of pigeons with *B. aertrycke*, however, the situation seems to us to be somewhat different from that of other bacterial infections of birds hitherto reported, though we are by no means prepared to state that this disease is in any way fundamentally related to myeloid leukemia of birds, mammals or human beings. At the present stage of our studies of *B. aertrycke* infection in pigeons we can call attention only to the common occurrence of large numbers of immature myeloid cells in the peripheral blood and the constant appearance of extensive myeloid foci in the liver and kidneys of birds infected with this organism. These changes, to our knowledge, have not been observed to occur in other bacterial infections. We wish to emphasize the fact that, in addition to the hyperplasia and heterotopia of the myeloid tissue

here described, in which no bacteria were found, other widely spread lesions of a totally different character, consisting of accumulations of mononuclear phagocytic cells, containing *B. aertrycke* in abundance, occurred with regularity.

Inasmuch as we have not yet had the opportunity of studying fowl leukemia we can make no definite statement concerning the relationship of this disease to *B. aertrycke* infection in pigeons, though one would be inclined to consider the two conditions as separate entities. Most of our knowledge of fowl leukemia is derived from the studies of Ellermann and Bang,¹¹ Ellermann,^{12, 13, 14, 15} Hirschfeld and Jacoby,^{8, 9} and Schmeisser,^{16, 17} who, in general, agree that the disease is caused by a filterable microorganism. Ellerman and Bang, who first advanced this theory, did so upon the ground that they were able to transmit the disease to a certain percentage of normal fowls by the injection of bacteria-free filtrates prepared from the organs of a fowl dying of the disease spontaneously acquired. These authors, however, do not appear to have searched very thoroughly for bacteria. No mention is made of cultures made directly from the organs of the birds spontaneously or experimentally infected, nor were the tissues of these birds examined microscopically for bacteria. The only proof offered for the sterility of the filtrate with which the disease was experimentally produced is the statement that it was clear and gave rise to no growth when cultured in several types of media. The amount of filtrate cultured was not mentioned, but it was stated that cultures of the emulsion of tissues from which the infectious filtrate was made gave a rich growth of bacteria. No further study was made of these organisms. Though it is possible that the filtrate with which Ellermann and Bang claimed to have produced leukemia in fowls was actually free of bacteria, one does not feel convinced that such was the case from the account of their experiments. Hirschfeld and Jacoby,⁹ who attempted to repeat the experiments of Ellermann and Bang,¹¹ began their studies with a leukemic fowl obtained directly from the latter workers and were apparently successful in transmitting the disease to other fowls by injections of whole organ emulsion prepared from the diseased bird. However, their stock of fowls proved to be heavily infected with spontaneously acquired tuberculosis. The results of their experiments are not altogether clear, though they studied great numbers of birds in their attempt to separate the effects of these two diseases. It is

worthy of note that Hirschfeld and Jacoby did not succeed in producing leukemia in any of their fowls by the injection of bacteria-free filtrates made from the organs of leukemic birds. Schmeisser,^{16, 17} has studied a disease of fowls considered to be myeloid leukemia, which he found to be transmissible to normal birds by injection of organ emulsions made from both spontaneously and experimentally infected birds. He has written a good description of the changes in the blood and other organs but in neither of his papers has he mentioned any bacteriological studies whatever.

Just as this paper was completed the publications of Furth and Stubbs,^{23, 24} appeared, confirming once more the filterable nature of the etiological agent in fowl leukemia. While the similarity is striking in certain phases of the hemopoietic response, it would seem that entirely different causative agents were at work in fowl leukemia and *B. aertrycke* infection in pigeons. Though all of these investigators who have studied fowl leukemia have described lesions apparently identical with the changes in the myeloid tissue occurring in pigeons infected with *B. aertrycke*, none has reported the presence of the widely spread collections of monocytic cells frequently grouped about bacteria which we have regularly observed. It seems unlikely that such lesions would be overlooked. Furthermore, whereas the incubation period of fowl leukemia is stated to be approximately two months, our pigeons infected with large doses of *B. aertrycke* became ill within two days, and small doses of these bacteria produced no symptoms of disease at all. In relation to this latter point may be mentioned the work of Winternitz and Schmeisser,¹⁸ who studied a series of fowls experimentally infected with *B. sanguinarium*. As previously found by Smith and Moore, their birds developed high leucocytoses but showed no extramedullary accumulations of myelocytes. A single bird, however, developed changes in the blood characteristic of myeloid leukemia several weeks after injection of the bacteria and was found to show extensive infiltration of the liver and kidneys with myelocytes. No bacteria of any kind were recovered from this bird and these investigators were inclined to conclude that the case was one of true fowl leukemia. They suggested the possibility that leukemia may be produced in the fowl by graded doses of *B. sanguinarium*, but did not pursue the matter further.

Aside from its possible relationship to fowl leukemia, the question

of *B. aertrycke* infection in pigeons is, in itself, a matter of considerable interest. Our observations, though inadequate as absolute proof, would point strongly to the assumption that pigeons frequently harbor minimal numbers of this organism which, under ordinary conditions, cause them no harm. However, under adverse conditions, such as severe malnutrition, it would seem that these bacteria might multiply and frequently become sufficiently pathogenic to produce disease.

That *B. aertrycke* infection is a natural disease of pigeons is shown by observations recently made upon a bird noted to be seriously ill when brought into the laboratory. Immediate examination of the blood showed 2,900,000 erythrocytes and 108,000 leucocytes per cmm., eosinophilic leucocytes 64 per cent, myelocytes 2 per cent, lymphocytes 20 per cent, and monocytes 14 per cent. A culture of the blood made at the same time was positive for *B. aertrycke*, which was identical in all of its characteristics with the strains isolated from spontaneous cases of *B. aertrycke* infection developing in pigeons in the laboratory in New York. On the following day a second blood culture was also positive for *B. aertrycke*. This pigeon was isolated from the other birds and observed for eight days, during which time it remained critically ill, continued to lose weight, and the granulocytes of the blood increased to 83 per cent. After death on the eighth day, all of the lesions characteristic of *B. aertrycke* infection were found in advanced degree and the organism was recovered from the liver, kidney, bone marrow and blood. Intraperitoneal injection of saline emulsion made from the liver, as well as pure cultures of the bacteria, produced both symptoms and lesions in normal pigeons identical with those occurring after experimental infection with other strains of *B. aertrycke*.

We have not yet studied the effect of oral administration of *B. aertrycke* upon animals other than pigeons, but have found these organisms to be highly pathogenic for chickens, mice, guinea pigs and rabbits when injected in fairly large doses. The animals died of septicemia within a few days but did not show myeloid alterations in their organs such as those seen in pigeons infected by the oral route.

Though *B. aertrycke* is generally mentioned in the literature in association with food poisoning in man, it also has been observed to cause disease in animals. The system of nomenclature for the group

of bacteria related to *B. aertrycke* is so inaccurate, however, that relatively few of the investigators who have reported such observations appear to have been dealing with the same organism. Otte¹⁹ has reviewed the many studies upon natural and experimental infection of birds with bacteria of this type, and Beck and Meyer²⁰ have studied a disease of pigeons, widespread in Germany, which was caused by a bacillus considered to be *B. aertrycke*. No blood studies were made by any of these observers. Infiltrations with mononuclear cells, similar to those we have observed in pigeons, were of common occurrence but no changes in the myeloid cells were reported.

Beaudette and Edwards²¹ have reported an epidemic in canaries caused by this organism, Beaudette²² an epidemic in young pigeons, and Doyle²³ has described an epidemic among young chicks. Meyer and Matsumura²⁴ state that a considerable percentage of wild rats are carriers of *B. aertrycke*. Petrie and O'Brien²⁵ described an epidemic in guinea pigs which they thought was due to a filterable microorganism, but regularly cultured *B. aertrycke* from the dead animals. This organism was highly pathogenic for normal guinea pigs when injected subcutaneously, but of low virulence when administered by mouth. Topley and Ayrton,²⁶ working with white mice, found the greatest variation in the behavior of different strains of *B. aertrycke*. Some strains were excreted regularly from the intestine after oral administration, while others appeared only transiently, in some instances fatal infection taking place without the organisms ever appearing in the feces. These workers also showed that *B. aertrycke* frequently remained in the tissues of mice without causing disease. Topley,²⁷ at a later date, demonstrated *B. aertrycke* of unaltered virulence in the spleens of mice actively immunized to lethal doses of this organism. Edington²⁸ has recently made a very thorough study of another guinea pig epidemic caused by *B. aertrycke* in which this organism was recovered regularly from the blood, intestine, gall-bladder, liver, spleen and urine. Blood studies showed a leucopenia varying from 2,000 to 4,000 cells per cmm. with no striking change in the differential count. The lesions found were empyema of the gall-bladder, necrosis in the spleen and liver, hyperemia of the intestine and occasional small ulcerations in the lymphoid follicles of the mucosa, accumulations of phagocytes with necroses in the mesenteric lymph nodes, catarrhal inflammation of urinary

bladder, purulent endometritis, infrequent pneumonia and cloudy swelling of kidneys. No mention was made of the bone marrow.

Though the literature contains several references,^{29, 30, 31} other than instances of food poisoning, to human cases of fatal infection with *B. aertrycke*, none of them has resembled the disease caused by this organism which we have studied in pigeons. From the accounts of such cases it seems that *B. aertrycke* was recovered only from the blood, and was never proved to be the cause of any of the lesions present.

SUMMARY AND CONCLUSIONS

The apparently spontaneous development of a fatal disease in undernourished pigeons is reported which is characterized by anemia, 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 myeloid tissue. In addition to these myeloid changes, large, nodular, often necrotic masses of mononuclear phagocytic cells are frequently found scattered throughout the liver, spleen, kidneys and bone marrow.

A small, Gram-negative bacillus, regularly recovered in pure culture from the blood, liver, kidney, spleen, and bone marrow of these cases, has been identified as *B. aertrycke*. In sections, the bacteria are found to be present in the foci of mononuclear cells, but do not occur within the collections of myelocytes.

Disease has been produced experimentally in normal pigeons by the intraperitoneal injection of liver emulsion made from naturally infected birds, intraperitoneal injection of *B. aertrycke* derived from the same source, and also by oral administration of single large doses of broth cultures of this organism.

Bacteria-free filtrates of broth cultures of *B. aertrycke* have had no demonstrable effect upon normal pigeons when injected or administered orally in single large doses.

Attention is called to the frequency with which pathological changes occur in the tissues of apparently normal birds.

NOTE: The authors wish to extend their warmest thanks to Dr. L. T. Webster, of the Rockefeller Institute, and Dr. T. J. Kurotchkin, of the Department of Bacteriology of the Peiping Union Medical College, for their kind assistance with the bacteriological and serological studies reported in these experiments.

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DESCRIPTION OF PLATES

PLATE 67

Cells stained supravitaly with neutral red and Janus green from the peripheral circulation of pigeons in this experimental series.

- FIG. 1. Young adult eosinophilic leucocyte showing mitochondria and a mixture of spheres and rods making up the specific granules. These are the first changes to appear in the circulating granulocytes of the pigeon under stress. Pigeon 70, April 16, 1929, size 9×12 microns.
- FIG. 2. Young adult eosinophilic leucocyte with mitochondria. Pigeon 65, peripheral blood, April 3, 1929, size $9 \times 9\frac{1}{2}$ microns.
- FIG. 3. Atypical micro-eosinophil with swollen rods not infrequently found in the circulation as an indication of bone marrow stimulation. Pigeon 65, April 3, 1929, size 8×9 microns.
- FIG. 4. Adult eosinophilic leucocyte showing the incomplete transition of spherical granules to rods and indicating a premature delivery of this cell to the circulation. Pigeon 65, April 3, 1929, size 10×15 microns.
- FIG. 5. Eosinophilic myelocyte "B" with round nucleus, mitochondria and a moderate number of specific, spherical granules. Pigeon 65, April 3, 1919, size 10×10 microns. See erythrocyte for relative size.
- FIG. 6. Eosinophilic myelocyte "A" with many mitochondria and few specific granules. Size 9×10 microns. Note the different stages of maturation found within the eosinophilic group on the same day from Pigeon 65, April 3, 1929.
- FIG. 7. Eosinophilic myelocyte "C," showing mitochondria and spherical granules arranged about the centrosphere, a not infrequent arrangement in the myelocytic stage. The slightly indented achromatic nucleus contains one nucleolus. Pigeon 50, March 22, 1929, size $10\frac{1}{2} \times 12$ microns.
- FIG. 8. Myelocyte "B" contains approximately one-half the final concentration of specific granules found in the fully mature eosinophil and many mitochondria. The area about the centrosome is clear of granules. The nucleus shows very little chromatin structure at this stage of development. Pigeon 50, March 22, 1929, size 15×15 microns.
- FIG. 9. This myelocyte "A" has relatively few specific granules, several vacuoles, and many mitochondria. The nucleus contains two nucleoli and very little chromatin. Size $12 \times 13\frac{1}{2}$ microns. Cells 7, 8 and 9 show the various stages of myelocytic maturation found on the same day in the blood of Pigeon 50, March 22, 1929.
- FIG. 10. Myelocyte "B." Pigeon 65, April 4, 1929, size 9×12 microns.
- FIG. 11. Myelocyte "A." Pigeon 65, April 4, 1929, size 10×14 microns.
- FIG. 12. Monocyte from the peripheral blood of Pigeon 50, March 21, 1929. Note the slightly different shade of neutral red reaction in the vacuoles of this cell as contrasted with the granules in the myelocytes of Figs. 7, 8 and 9 from the same bird. See accompanying erythrocyte for relative size.
- FIG. 13. Monocyte from Pigeon 65, April 4, 1929. Contrast this cell with the myelocytes of Figs. 10 and 11 taken from the same bird on the same date. Size $9 \times 10\frac{1}{2}$ microns.
- FIG. 14. Small lymphocyte with many mitochondria and a few scattered neutral red vacuoles. Pigeon 67, April 4, 1929, size 9×9 microns. See accompanying erythrocyte for relative size.

PLATE 68

- FIG. 15. Liver of Pigeon R714, naturally infected with *B. aertrycke*, showing unusually large collection of monocytic cells, in which there is a necrotic area containing Gram-negative bacilli. Hematoxylin-eosin stain. $\times 62$.
- FIG. 16. Smaller collection of monocytic cells in liver of Pigeon 24, experimentally infected with *B. aertrycke*. There are bacteria and a minute area of necrosis in the center about which is heavy infiltration with adult polymorphonuclear leucocytes. Hematoxylin-eosin stain. $\times 300$.
- FIG. 17. Low power picture of liver of Pigeon 14, experimentally infected with *B. aertrycke*, showing general distribution of infiltrations of myeloid cells. $\times 62$.
- FIG. 18. Small branch of portal vein with surrounding zone of myelocytes. Experimental infection *B. aertrycke*, Pigeon 14. Hematoxylin-eosin stain. $\times 300$.

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Cash and Doan

Infection of Pigeons with *B. Aertrycke*

PLATE 69

FIG 10. Myelocytes surrounding portal vein. A small portion of the vessel filled with erythrocytes may be seen in the upper left-hand corner. Natural infection with *B. aertrycke*. Pigeon 14. Hematoxylin-eosin stain. $\times 1300$.

FIG. 20. Infiltration of kidney with myelocytes. Experimental infection with *B. aertrycke*. Pigeon 14. Hematoxylin-eosin stain. $\times 158$.

FIG. 21. Bone marrow from central portion of shaft of radius of Pigeon 30 dying of experimental *B. aertrycke* infection. The partially collapsed capillaries are marked out by the characteristic nuclei of erythrocytes. All of the remaining cells are myelocytes of varying degrees of maturity. The more mature forms appear darker than the others due to their greater number of granules. The degree of myeloid hyperplasia may be appreciated by bearing in mind the fact that the capillary network of this portion of the marrow of normal pigeons contains only fat among its meshes. Hematoxylin-eosin stain. $\times 300$.

FIG. 22. Oil-immersion picture from same marrow as Fig. 7, illustrating the pale staining, myeloid forms with only a few granules in their cytoplasm. A few mature myelocytes with their full quota of granules are also seen. Hematoxylin-eosin stain. $\times 1300$.

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