OBSERVATIONS ON THE ANEMIA IN DUCKS INFECTED WITH P. LOPHURAE*

By R. H. Rigdon, M.D. and H. H. Rostorfer, Ph.D

The studies of many investigators have emphasized the significance of the anemia in malaria. The degree of anemia in the duck infected with P. lophurae is proportional to the number of parasites in the peripheral blood. In most cases of P. lophurae infection in ducks a marked drop in the total number of red cells occurs as the peak of parasitemia is approached; the number of red cells and the level of hemoglobin return to normal within 3 to 5 days following the peak of infection. Hill concluded from her studies on pigeons infected with P. relictum that death results from the anemia. Rigdon and Varnadoe have recently shown that life may be prolonged in ducks infected with P. lophurae by the frequent injection of normal duck blood.

Apparently few observations have been made on the erythrocytes in the duck and the changes they may show in a severe anemia. Hewitt has contributed many hematological observations on both the normal and malarial infected ducks. He has emphasized the occurrence of an anemia and the fall in hemoglobin in P. lophurae infected birds. Hewitt has called attention to the varying degrees of polychromemia in the red cells; the fact that the nuclei are larger than those of mature red cells; and also, that they may be round rather than elliptical. Binucleated red cells, anucleated forms, and deeply basophilic erythroblasts may be found in the peripheral blood during severe infections.

Taliaferro and Kluver have reviewed the subject and supplemented our knowledge of the hematology of malaria in Panamanian monkeys. They emphasized the occurrence of anemia and a decrease in the amount of hemoglobin. Normoblasts and anisocytosis were found in the peripheral blood during malarial infections in monkeys.

The physiological studies made in this laboratory on the oxygen-carrying capacity of the blood in experimental malaria and the effect of high altitudes on the course of P. lophurae infection in ducks have caused us to study further the changes which may occur in the duck's erythrocytes in malaria.

METHODS AND MATERIALS

White Pekin ducks, 2 to 4 weeks of age, infected with P. lophurae were used for this study. Blood for smears was obtained by puncturing a vein in the legs. They were stained with a combination of Giemsa's and Wright's stains. The parasitemia was determined by counting the number of parasitized cells per 500 red cells. The number of both the young and adult red cells also was determined per 500 red cells.

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The erythroblast was differentiated from the erythrocytes in this study by the bluish staining of the cytoplasm and by the spherical shape of the nucleus and cell body in comparison to the more elliptical shape of the mature red cells.

The same blood smears used for determining the degree of parasitemia were used to measure the size of the different red cells. Twenty-five to 100 cells were measured each day during the infection to establish their average size. Measurements were made with an ocular micrometer. The maximum and minimum lengths of both the cell and the nucleus were recorded. Standard technics were used for counting the erythrocytes. Hayem's fluid was used for the diluent.

Blood for the determination of hemoglobin, hematocrit, color index, and cell volume was obtained by cardiac puncture from two to three birds and pooled. Liquaemine* in a dilution of one to nine was used as the anticoagulant. The functional hemoglobin was calculated from the oxygen capacity, which was determined by equilibrating the blood with air at 38°C and analyzing it on the Van Slyke manometric apparatus. The percentage of functional hemoglobin was obtained by allowing the colorimetric hemoglobin to represent 100 per cent and considering the hemoglobin determined from the oxygen capacity as a percentage of the colorimetric hemoglobin. The latter was determined by the method of Schultze and Elvehjem.11 The color index was calculated by dividing the hemoglobin in grams obtained from the oxygen capacity, by the erythrocyte count. The hematocrit was obtained by centrifuging 10 cubic centimeters of blood at 3000 r.p.m. The cell volume was obtained by dividing the volume of cells in 10 cubic centimeters of blood by the erythrocyte count. Smears were prepared from the femur bone marrow, the spleen, and the liver from malarial infected and control birds. A small portion of the tissue was placed in a drop of duck plasma on a glass slide and carefully teased out. Smears were made from this preparation and were stained similarly to the blood.

EXPERIMENTAL

The degree of parasitemia and the severity of the accompanying anemia varied with the age of the duck and the number of parasites injected. The course of a typical malarial infection in a fatal case is shown in figure 1. The degree of the anemia usually is proportional to the parasitemia. Accompanying this anemia there is a progressive increase in the number of erythroblasts in the peripheral blood. In these ducks the peak of the parasitemia is reached on the fifth day and then the number of parasites rapidly decreases until only a few are present on the eighth day (fig. 2). The most severe anemia is present approximately 24 hours following the peak of the parasitemia. In the ducks that survive the total number of red cells rapidly increases and reaches approximately normal levels by the tenth day following inoculation. The level of hemoglobin, the color index, and the hematocrit all decrease at a parallel rate with the decrease in the total number of erythrocytes; also, they increase correspondingly with the increase in the number of red blood cells in the peripheral circulation (fig. 2).

* Supplied through the courtesy of Roche-Organon, Inc., Nutley, N. J.
This represents the normal development of the parasitemia and the anemia in young ducks infected with *P. lophurae*. Usually there occurs a decrease in the degree of the parasitemia preceding the time of death.

Accompanying the anemia in *P. lophurae* infections in ducks there is a decrease in cell volume, hemoglobin, and hematocrit. With a return in the number of erythrocytes to normal there is a corresponding increase in the cell volume, hemoglobin and hematocrit.
The proportion of young red cells to adult erythrocytes during the infection is indicated by the graph in figure 3. There is a variation in the size and shape of the adult and young red cells and their nuclei are measured in both their maximal and minimal diameters and the average is plotted on the lower portion of this figure. The areas between the maximum and minimum measurements of both the cells and their nuclei are indicated on the diagram. The adult erythrocyte with its nucleus maintains a uniform size and shape during the course of the infection, while the young erythrocyte with its nucleus varies both in size and shape during this time. At the time of the greatest number of young cells in the peripheral blood many of the young erythrocytes and their nuclei are small and almost spherical. With the subsequent decrease in the number of young cells the young erythrocytes increase in size and their shape approaches that of an elliptical body as indicated by the diagram in the lower half of this figure.

The figure in the upper half also indicates the rapid increase in the number of parasitized cells with a corresponding diminution in the number of adult erythrocytes that occurs until the peak is reached on the fifth day of the infection. There is an increase in the number of young erythrocytes until approximately 24 hours following the peak of the parasitemia, at which time the number of young cells rapidly decreases. This is accompanied by a corresponding increase in the number of adult types of erythrocytes in the peripheral blood.

erythroblasts in the peripheral blood during the course of the infection as shown by the diagrams in figure 3. Only typical adult erythrocytes are included in the group
Fig. 4. This shows the variations in the type of erythrocyte that may occur in the peripheral blood of a duck that survives a severe malarial infection. A, normal erythrocytes as observed on the third day following inoculation; B, on the fifth day some of the erythrocytes are larger and others are smaller than a normal adult red cell. The cytoplasm stains a bluish purple color. C, few normal-appearing adult erythrocytes are present on the sixth day. Small round erythroblasts are conspicuous at this time. D, the peak of the parasitemia occurs on the fifth day. The cells on the seventh day are frequently large and some are elliptical in shape while others are spherical-like in shape in the stained smears. The cytoplasm usually stains a deeper blue than it does in adult erythrocytes. E, the erythrocytes on the ninth day are indistinguishable from normal red cells. F, the erythrocytes are normal on the tenth day following inoculation.
indicated as "Adult Red Cells" in figure 3. It is obvious from these data that this cell remains uniform in size and shape throughout the period of infection. In contrast to the "Adult Red Cell," the erythroblasts vary widely in both size and shape during the course of the infection as shown by the diagram in figure 3 indicated as "Young Red Cells." These young cells at first are elliptical in shape and slightly smaller than the adult erythrocyte; however, at the time of the greatest number of erythroblasts in the circulation many of these young cells are small and almost spherical in shape. These small round cells are found in significant number only in those ducks that have a very high degree of parasitemia, a severe anemia, and survive the infection. Two to three days following the peak of parasitemia the erythroblasts become larger and approach the shape of an elliptical body.

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* A: Young red cells—illustrated in fig. 5B.
B: Middle-aged red cells—illustrated in fig. 5C.
C: Youngest type of red cells—illustrated in fig. 5D.
† Counted 337 normal adult erythrocytes in peripheral blood.
‡ Counted 100 hepatic cells in this smear.

Since no satisfactory grouping could be made for measuring the young red cells within the peripheral blood, typical cells were photographed at different intervals during the course of the infection to show the variations in their size and shape (fig. 4). It is obvious from these photographs that the shape of a young erythroblast in a stained smear approaches that of a sphere. Furthermore, many of the cells are small on the fifth and sixth days of the infection at a time when the greatest strain is put upon the hematopoietic system. Erythrocytes in the peripheral blood, bone marrow, spleen, and liver are classified as to age and the results are given in table 1. All ducks with malaria show a marked increase in the percentage of young cells of the erythrocytic series. Near the time of death many of the immature forms of erythrocytes enter the peripheral circulation. The hematopoietic responses of the femur bone marrow, spleen, and liver are similar in that each shows a hyperplasia of the erythroblastic tissue. Apparently the greatest number of red cells are formed in the bone marrow in comparison with the liver and the spleen.
The tissue from 5 ducks was taken immediately following death from malaria and that from 3 ducks the day following the time of the peak of the infection. In each of these 8 ducks the bone marrow, liver, and spleen showed a marked increase in the number of young erythrocytes when compared with the control ducks of similar age (table 1). The bone marrow of some of the ducks showed a predominance of "very young erythroblasts." These cells are large and round (fig. 3D). The nucleus fills the greater portion of the cell. The chromatin of the nucleus is fine and loosely packed. The cytoplasm is represented by a narrow band of a blue staining material. The larger of these "very young erythroblasts" are approximately 12–15 microns in diameter. The cell classified in this study as a "middle-aged erythroblast" is a round cell, smaller than the so-called "very young erythroblast" de-
scribed above. The nucleus is round and the particles of chromatin are larger and more compact than they are in the younger type of cell. There is also a larger amount of cytoplasm in these cells than in the younger cells. The cytoplasm stains a bluish purple (fig. 5C). In the cytoplasm of many of these cells there are large, clear unstained areas. Frequently the cytoplasm is almost completely replaced by these unstained areas (fig. 5C). The oldest type of young red cells present in the hematopoietic tissue is an elliptical-shaped cell with a similarly shaped nucleus. The cytoplasm stains bluish pink. The chromatin of the nucleus is coarse and more compact than it is in the younger cells (fig. 5B). In some of the ducks the bone marrow, liver, and spleen show a predominance of so-called "very young erythroblasts" while others show a majority of the "middle-aged type of erythroblasts." The cells in the peripheral blood of these ducks with malaria are primarily like those described above as "young red cells." Occasionally, however, few of the younger types of erythroblasts are present in the peripheral blood. Ducks dying from the disease may have a large number of the youngest forms of erythrocytes in the peripheral blood.

The youngest types of erythroblasts do not have parasites within their cytoplasm and only a few of the more mature erythroblasts have parasites within their cytoplasm. The absence of malarial parasites within these young cells is most conspicuous especially when these cells are present in the peripheral circulation.

DISCUSSION

The results of the observations made in this study on the development of the anemia and the determination of the amount of hemoglobin in ducks infected with P. lophurae are essentially the same as those reported by Hewitt.4 The bizarre forms of erythrocytes that may be observed in the peripheral circulation emphasize the fact that pathological cells may develop in the blood-forming tissue and escape into the circulation. The presence of a large number of erythroblasts in the circulating blood which are more spherical than elliptical in shape indicates the morphological forms through which a normal erythroblast of the duck must pass to reach maturity. Smears from the bone marrow confirm the opinion that a young red cell is round and subsequently becomes elliptical in shape.

Young red cells in malaria usually are spherical when they enter the blood stream and apparently become elliptical as they grow older. This observation would indicate that there is no fundamental difference in the cell structure of these young cells which are produced in excessive numbers in malaria and the young cells that reach the peripheral blood under normal conditions. This change in the development of erythrocytes from a spherical form in the hematopoietic tissues to an elliptical-shaped cell may be the result of a variation in the environment. Hamburger12 observed that erythrocytes of the horse and dog decreased in diameter as the plasma was diluted with distilled water. He pointed out the fact that the cells so treated become globular. Haden13 has called attention to many of the problems associated with any study on the variations in cell diameter and cell volume. Any study on the red cells in the duck infected with P. lophurae is difficult to follow since the hematopoietic response is so rapid and the interval is so short between
ANEMIA IN DUCKS INFECTED WITH P. LOPHURAE

the time of appearance of abnormal numbers of atypical cells in the peripheral blood and either complete recovery or death from the infection.

Magath and Higgins\(^4\) found the erythrocytes in normal tame mallard ducks to vary in size from 9.9 to 13.4 microns long (average 11.2) by 5.9 to 8.9 microns wide (average 6.7). The nuclei of these cells were 5.0 to 7.0 microns long by 1.5 to 2.5 microns wide. The normal-appearing erythrocytes in the peripheral blood of young white Pekin ducks infected with malaria are 11.3 by 7.1 microns and their nuclei 5.6 by 2.9 microns. The more mature erythrocytes in the peripheral circulation are elliptical in shape in ducks with a low parasitemia. Some of the erythroblasts, however, are smaller and almost spherical at the time the peak of parasitemia is reached in the highly parasitized birds. These small erythroblasts occur at a time when the greatest number of young cells are present in the circulation. With a diminution in the degree of the anemia the young erythroblasts again become larger and approach the shape of an elliptical body. Apparently when the hematopoietic system is forced to the point of maximum production the erythroblasts in the ducks may leave the hematopoietic centers as small round cells. Normoblasts are usually larger than erythrocytes when observed in the circulation in acute anemia in man. It is suggested that this difference in man and duck may be explained by the probability that life in man is not compatible with such an acute and severe strain on the hematopoietic system. In chronic anemia in man we have microcytes in the peripheral blood. These small round erythroblasts in ducks are found in significant numbers for 24 to 48 hours only in the few birds that survive a severe malarial infection.

It is of interest to observe that the small type of erythroblast is present in the circulation in the ducks in significant numbers for only a short time. The question arises: Do these small young cells develop into the larger type of erythroblasts and then subsequently become mature red cells? The rapidity of the recovery from the anemia might suggest that such occurs. These young cells are more easily broken by centrifugation than the adult red cell. It is known that the microcytes in congenital hemolytic anemia are more fragile than normal red cells. In this human disease there is a varying percentage of microcytes and macrocytes in the peripheral blood at different times. The microcytes apparently appear in greater numbers during the periods of crisis when the hematopoietic tissues no doubt are put under the greatest strain. Apparently the hematopoietic tissues of the duck when placed under a severe strain also respond with the escape of small erythroblasts into the blood stream. As far as we know there are no studies on the oxygen-carrying capacity of the microcytes in diseases in man such as congenital hemolytic anemia. It has been shown, however, that duck blood with a high percentage of young erythrocytes is a poor carrier of oxygen.\(^1\) If a corresponding phenomenon does occur in man then we may have an explanation for the disproportion between the clinical manifestations and the degree of anemia frequently observed in this disease.

The rapid increase in the number of erythroblasts preceding the peak of parasitemia in \(P.\ lophurae\) infection in ducks is very different from the observations of Terzian on \(P.\ lophurae\) infection in chicks. Terzian says that "in view of the rapid
cell destruction taking place during the course of an infection, one would expect an immediate reticulocyte response. It is of interest to note, however, that such a response is singularly lacking and does not occur until some time after the numbers of parasites have begun to decline. . . . Once the animal has succeeded in clearing the circulation of the parasites it is able to regenerate its blood supply very rapidly. Figure 3 shows that the number of erythroblasts in the peripheral blood of ducks is increased with the diminution in the number of erythrocytes within the circulation; furthermore, young cells appear in the peripheral blood before the peak of parasitemia is reached.

Previous experimental studies have shown that the oxygen-carrying capacity of duck blood is decreased when there are many erythroblasts in the circulation. The present study also shows that the amount of hemoglobin in duck blood with a high percentage of erythroblasts is less than that in normal blood. Furthermore, the ratio of hemoglobin to cell volume is not proportional to the number of red cells, following the time of the peak of the infection. This, of course, indicates a disproportion between the size of the red cell and a normal amount of hemoglobin for the cell. The illustrations in figure 4 show some large erythroblasts in the peripheral blood following the time of the peak of parasitemia. The percentage of such large cells, however, was not determined in this study.

Histological studies on the spleen and liver of ducks infected with *P. lophurae* have shown a proliferation of cells that were considered to be hematopoietic tissue. The present study of the cells in the liver and spleen would indicate that these hyperplastic foci are formed primarily by cells of the erythrocytic series. The changes that occur in the type of red cell in the peripheral blood, of course, are indicative of the processes occurring within the blood-forming tissue. In some of the birds dying from the infection only very young forms of the erythroblasts are present in significant numbers within the hematopoietic tissues. This, of course, would suggest that the need for red cells in the peripheral circulation in these birds is greater than the ability of the host to produce them. It would seem that ducks with *P. lophurae* infection might not succumb if the hematopoietic tissue always could supply an adequate number of mature erythrocytes. At the time of death young erythroblasts may be present in the peripheral blood. The ducks that have a high parasitemia at the time of death show a large number of erythroblasts with one or more parasites within their cytoplasm. Apparently these plasmodia do not prefer young erythrocytes to adult cells; however, when the adult cells are absent they will enter the younger forms. It may be significant that *P. lophurae* does not prefer the young erythrocytes since the rapid decrease in the degree of parasitemia occurs at the time when the peripheral blood has the maximum number of these young cells. It is suggested therefore that one significant factor in the mechanism by which a rapid decrease in the parasitemia occurs in highly parasitized birds following the peak of infection is the result of an absence of mature erythrocytes for the parasites to enter.

The increase in the color index of the blood from normal ducks given copper and iron would suggest that the anemia in the duck may be influenced favorably by the
administration of large quantities of these elements. However, since it requires approximately two weeks for the blood of normal ducks to show any significant increase in the color index it would be almost useless to give copper and iron to ducks after they are infected with *P. lophurae* in an attempt to influence the anemia during the acute phase of the infection.

The spleen, liver, and bone marrow supply young erythrocytes for the peripheral blood. The data given in table 1 show that the greatest number of the youngest forms of erythrocytes are present in the bone marrow, and furthermore, the young cells in the spleen and liver usually are more mature than those in the bone marrow. In this connection it would be of interest to know if myeloblasts, myelocytes, and polymorphonuclear leukocytes develop similarly in the spleen, liver, and bone marrow in myeloid leukemia in man.

**SUMMARY**

This study of the anemia produced by *P. lophurae* in ducks emphasizes the significance of a decrease in the number of red cells in this disease, and furthermore, it suggests that the rapid diminution in the number of parasites following the peak of the parasitemia may be directly related to the character of this anemia since these parasites apparently do not prefer young erythroblasts to mature erythrocytes.

The observations made in this study show that erythrocytes in the ducks first appear as spherical bodies in stained smears and when mature they are elliptical in shape. Under abnormal conditions such as may occur with a severe parasitemia, erythroblasts appear in the peripheral blood in large numbers, sometimes almost completely replacing the adult type of red cell. Both microcytes and macrocytes appear in the blood stream. The amount of hemoglobin within some of these young erythrocytes is small as indicated by the hemoglobin determinations and the staining reaction. Small young erythroblasts with little hemoglobin appear in the peripheral blood when the maximum load is placed upon the hematopoietic tissues.

**REFERENCES**

R. H. RIGDON AND H. H. ROSTORFER

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