STUDIES ON THE ANÆMIA IN CHICKS INFECTED WITH P. GALLINACEUM

BY A. S. RAMASWAMY, R. RAMA RAO AND N. N. DE

(Pharmacology Laboratory, Indian Institute of Science, Bangalore)

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The significance of anæmia in malaria has been emphasized by many investigators (Young, 1937; Terzian, 1941; Taliaferro and Kluver, 1940; Hewitt, 1942). In the ducks infected with P. lophuræ the degree of anæmia is proportional to the number of parasites in the peripheral blood. In most cases of P. lophuræ infection in ducks a marked drop in the total number of red blood cells occurs as the peak of parasitæmia is reached; the number of red blood cells and the level of hæmolglobin return to normal within 3–5 days following the peak of infection (Hewitt, 1942). In an untreated acute infection, death usually occurs on the 10–12 day. Hill (1942) has concluded from her studies on pigeons infected with P. relictum that death results from anæmia. Rigdon and Varnadoe (1945) have recently shown that life may be prolonged in ducks infected with P. lophuræ by repeated injection of normal duck blood.

Apparently few observations have been made on the erythrocytes in the chick and the changes they show in severe anæmia. Hewitt (1942) and Hewitt, Richardson and Seager (1942) have shown in their study of the morphology of red blood cells of malaria-infected ducks that varying degree of polychromasia occurs. The nuclei are larger than those of the mature cells and these cells may be round or elliptical. Binucleated and anucleated forms and deeply basophilic erythroblasts may also be found in the peripheral blood during severe infections. Taliaferro and Kluver (1940) have reviewed the subject and have extended our knowledge of the hæmatology of malaria in panamanian monkeys. They emphasized the occurrence of anæmia and a decrease in the amount of hæmolglobin. Normoblasts and anisocytosis were found in the peripheral blood during malarial infections in monkeys.

The physiological studies made in this laboratory owing to the similarity of the course of malaria infection in chicks infected with P. gallinaceum (De and Ramaswamy, 1948) have caused us to study further the changes which may occur in the erythrocytes of chicks in malaria.
Methods and Materials

Chicks, 8-12 weeks old, were used for the purpose of this study. The strain of the malaria parasite, *P. gallinaceum*, was originally obtained from the King's Institute, Guindy, and was passed by blood inoculation through chicks of different ages and breed before being transferred to the present series under experiment. Intra-muscular inoculations have been used exclusively throughout the present work. Donor blood was drawn by cardiac puncture from two or three birds and pooled and an equal amount of citrated saline (2·0 per cent., sodium citrate, 0·9 per cent. saline) has been used as diluent.

Blood smears were stained with Giemsa and Leishmann stain. The parasitæmia was determined by counting the number of parasitized cells per 500 red cells. 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 outline in comparison to the more elliptical shape of adult red cells.

The same blood smears used for determining the degree of parasitæmia were used to measure the size of different red cells. About 100 cells were measured each day during the infection to establish their average size. Measurements were made with an ocular micrometer calibrated in the usual way. A Price-Jones curve was drawn as is done in the case of elliptical erythrocytosis in man (Winston Evans, 1943). The diameters of 100 cells were measured as also the distribution of 100 cells were measured and the distribution of the cells was plotted according to the area (\(\pi/4\) times the product of the long and short axis, *i.e.*, the area of an ellipse). Standard technics were used for counting the red cells. Hayem's fluid was used for the diluent. Hæmoglobin determinations were made by using Sahli's hæmoglobinometer. Smears of the spleen, liver and the bone marrow from the femur, were taken from the infected as well as normal birds and stained in the usual way.

Experimental

The degree of parasitæmia and the severity of the accompanying anæmia varied with the age of the bird and the size of the inoculum. The course of a typical infection which ended in death is shown in Fig. 1. The degree of the anæmia is found to be proportional to the parasitæmia. Accompanying this anæmia there is a simultaneous increase in the erythroblasts in the peripheral blood. In the case of chicks the peak of parasitæmia was reached on the sixth or seventh day and then the number of parasites in peripheral blood rapidly decreased until only a few were present on the ninth day. In
those that survived, the haemoglobin and red cell count steadily increased from the tenth day onwards. The fall in haemoglobin and colour index run parallel with the total number of erythrocytes in the peripheral blood (Fig. 2).
All the infected birds showed a marked increase in the percentage of young red cells of erythroblastic type. Near the time of death immature forms of erythrocytes enter the peripheral circulation in large numbers. Apart from this there is a variation in the size and shape of the erythroblasts found in the peripheral blood during a course of infection. These immature cells were at first elliptical in shape and slightly smaller than the adult red blood cells, but as the anaemia progressed and great strain was placed on the bone marrow, the peripheral blood was flooded with even younger cells; many of these being very small and almost spherical in shape. Typical cells seen in the peripheral blood were photographed at different intervals during the course of the infection to show the variations in their size and shape (Fig. 3).

The late erythroblasts possess a round nucleus and the particles of chromatin are larger and more compact than they are in the young type of cell. There is also a larger amount of cytoplasm which stains bluish purple. There was a marked haemopoietic response of the bone marrow, liver and spleen, but the greatest proliferation was in the bone marrow. The bone marrow of some of the chicks showed a preponderance of early erythroblasts. These cells are large and round. The nucleus filling up the greater portion of the cell leaving only a thin rim of cytoplasm. Chromatin of the nucleus is fine and loosely packed. The cell measuring usually about 12-15 microns in diameter. This type of cell being very seldom seen in the peripheral blood as contrasted with the young red cell usually present in peripheral circulation. It is very interesting to note that the early erythroblasts do not have parasites with in their cytoplasm, excepting a few of the more mature erythroblasts in the peripheral blood.

The results obtained with the Price-Jones curves in chick malaria have proved somewhat anomalous. Using Leishmann stained blood films we have examined Price-Jones curves in chicks with severe malaria at a time when parasites were present in the blood. The data concerning them are given in the table below (I).

**TABLE I**

*Data regarding Price-Jones curves in chick malaria (P. gallinaceum)*

<table>
<thead>
<tr>
<th>Case type</th>
<th>No. of birds</th>
<th>Day of infection</th>
<th>R.B.C. per cmm. in millions</th>
<th>Price-Jones measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M.C.A. µm</td>
</tr>
<tr>
<td>Normal</td>
<td>.. 20</td>
<td>..</td>
<td>4-4.5</td>
<td>53.44</td>
</tr>
<tr>
<td>Infected</td>
<td>.. 20</td>
<td>5 days</td>
<td>1.8-2</td>
<td>54.42</td>
</tr>
</tbody>
</table>

*M.C.A. = Mean red cell area; σ = Standard deviation; ν = Coefficient variability*
DISCUSSION

The results of the observations made in this study are essentially comparable to those reported by Hewitt (1942) and Rigdon and Rostorfer (1947) working on *P. lophurae* infection in ducks. The bizarre forms of red cells that may be observed in the peripheral blood may be due to pathological cells that are formed in the bone marrow finding way into the peripheral circulation. The presence of spherical erythroblasts in the blood indicates the morphological forms that a normal erythroblast of the chick undergoes before it assumes elliptical shape as a fully formed red blood cell. The bone marrow studies indicate that young red cell is round at first and the normal elliptical shape is assumed as it grows into a mature cell.

In anaemia certain physical properties of blood alter. Hamburger (1895)—Reference from Haden (1934)—observed that the erythrocytes of the horse and dog decreased in diameter as the plasma was diluted with water. Blood smears showing rouleaux formation are unsuitable for both diffraction methods (Eve's Halometer) and Price-Jones measurements but in the case of the chick these complications are not evident. So it was thought in this series, in the absence of rouleaux formation the corpuscles may not be behaving quite normally on fixation and staining. Fixation with methyl alcohol in the Leishmann stained films was for only $\frac{1}{2}$ to 1 minute, whereas in Giemsa it was in contact with methyl alcohol for 2 minutes. It may be possible that shrinkage of the corpuscle is greater on this account. It was noted in a few of the profound anaemias, where films were made simultaneously with Leishmann and Giemsa stains that the Price-Jones curves showed no marked difference. These observations are not sufficiently extensive for us to state that the anomalous results obtained are due to abnormal shrinkage of the corpuscle in stained blood films, and also a study of the red cells in the chick infected with *P. gallinaceaum* is rendered difficult by the transitory nature of the hæmopoetic response. The abnormal cells are present for only a short time in the peripheral circulation and either complete recovery or death follows from the infection. Erythrocyte measurements made on normal chicks vary in size from 8·8 to 12 $\mu$ (Major axis). The nuclei of these cells were 2·8 $\mu$ to 6·4 $\mu$ (Major axis). The normal appearing erythrocytes in the peripheral blood of chicks infected with malaria are 9·6 $\mu$ to 12 $\mu$ and their nuclei 3·6 $\mu$ to 5·6 $\mu$. The more mature erythrocytes in the peripheral circulation are elliptical in shape in chicks with a low grade infection. Some of the late erythroblasts are smaller and almost spherical at the time the peak of parasitaemia is reached in the highly parasitised birds. As the degree of anaemia diminishes the late erythroblasts again become
larger and approach the shape of an elliptical body. These small round erythroblasts in chicks are found in significant numbers for 24–48 hours only among the birds that survive a severe malarial infection. It is interesting to note that the young types of erythroblasts in the peripheral blood did not have any parasites in their cytoplasm. Apparently these plasmodia do not prefer late erythroblasts to adult red cells. It is suggested therefore that one significant factor in the mechanism by which rapid decrease in the parasitæmia occurs in highly parasitized birds following the peak of infection is the result of an absence of mature erythrocytes for the parasites to enter.

Histological studies on the spleen and liver of ducks infected with *P. lophura* (Rigdon, 1944) have shown a proliferation of cells that were considered to be hæmopoietic 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 red blood series. The changes that are seen in the type of red cells in peripheral blood, of course, reflect what is going on with in the blood-forming tissues. The spleen, liver and bone marrow supply young erythrocytes for the peripheral blood. Further, greatest number of red cells are formed in the bone marrow in comparison with the liver and the spleen.

**Summary**

1. The study of the anæmia in chicks produced by *P. gallinaceum* shows the following characteristics. They are: (1) a slightly lowered colour index, (2) Hyperplasia of the bone marrow corresponding with a drop in the red cell count, (3) and slightly raised mean red cell area.

2. Small late erythroblasts which are spherical occur in the peripheral blood when the maximum strain is placed on the hæmopoietic tissues and the normal elliptical erythrocytes are found in the peripheral blood as the bird recovers from the acute infection.

3. It is interesting to note that the rapid reduction in the number of parasites following the peak of the parasitæmia may be related to the character of the parasites apparently preferring the erythrocytes to the erythroblasts.

**Acknowledgements**

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REFERENCES

Hill, Claire McDowell . . . Ibid., 1942, 36, 362.

EXPLANATION OF PLATE

The morphological variations in the type of red blood cell that occur in the peripheral blood of a Chick that survives a severe malarial infection (P. gallinaceum) × 1,050. A. Normal erythrocytes as observed on the third day following infection. B. On the fifth day some of the erythrocytes are larger and others are smaller than a normal adult red cell. C. & D. The peak of parasitemia occurs on the 6-7 day of infection. Small round erythroblasts are present at this period. The cells on the 7th day are frequently larger and some are elliptical in shape, while others are spherical in shape. The cytoplasm stains deep blue than it does in normal erythrocytes. E. The erythrocytes on the 9th day are not distinguishable from normal red cells. F. The normal erythrocytes appearing in large numbers on the 10-12 day of infection.