In a recent review Redfield (1) has called attention to the atypical manner in which the blood of certain birds and fish combines with oxygen. This matter aroused our interest because in such remotely related animals as *Urechis* (2), the skate (3), four reptiles (4–6), and the dog (7) the form of the oxygen dissociation curve is roughly similar to that of human blood. Accordingly, experiments have been carried out on six species of birds, including those studied by Wastl and Leiner (8). In order to make a complete study on a single specimen of blood, enough birds were decapitated to yield at least 100 cc. of blood. Such a specimen with heparin added remains virtually unchanged during 48 hours storage at 0°; actually our experiments were usually completed within 24 hours.

There is a slight but appreciable error if the usual method of equilibration is applied to blood having nucleated red cells. Oxygen is being utilized continuously; hence, a moving rather than a stationary equilibrium is reached. We sought to avoid this error by the use of hemolyzed red cells. With human blood one thus obtains a solution which is fairly homogeneous, yields very little sediment on centrifugation, and gives an oxygen dissociation curve almost identical in form and position with that of whole blood if proper pH adjustment is made. Bird blood thus treated yields a heterogeneous mass which separates into two phases on

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1 Blood was centrifuged, supernatant plasma and white cells were removed, and the red cells washed three times with 1 per cent NaCl solution. The addition of 2 volumes of distilled water at room temperature soon completes hemolysis. The further addition of 1.2 mg. of NaHCO₃ to each ml. of solution restores the available base to a value about equal to that in the original blood.
centrifugation. The upper half or two-thirds is a homogeneous solution of hemoglobin; the lower portion is a reddish jelly. On washing the jelly with distilled water, its volume increases and some hemoglobin appears in the wash water.

Oxygen dissociation curves have been derived for the supernatant homogeneous solution as well as for the intact whole blood. It was found, however, that such a solution has much less affinity for oxygen than blood, even when pH is taken into account.

![Graph]

**Fig. 1.** Hemoglobin solution from blood of the domestic fowl at 37.5°. Left-hand figure, log $pO_2$ as a function of log $R$ where $R = 100 \text{ Hb/HbO}_2$. Right-hand figure, the relation between the position of the oxygen dissociation curve and pH. The unit of measure for $pO_2$ is mm. of Hg.

The evidence for this is found in Figs. 1 and 2. The dissociation curves for hemoglobin solution at three pH values are given in Fig. 1, together with the relation between pH and position of the curves at one-half saturation. Similar experiments were carried out on whole blood, and in Fig. 2 a comparison is made between hemoglobin solution and whole blood, the pH of the solution as well as of the red cells being 7.1.

Various hypotheses may be advanced to explain this curious
behavior. A plausible one is that two hemoglobins are present in intact red cells of birds and that a partial separation is effected by our procedure. The existence of two hemoglobins in mammalian blood has been suggested by Brinkman, Wildschut, and Wittermans (9) and in the blood of the young domestic fowl by Hall (10). Another possibility is that partial denaturation or coagulation has occurred. However the phenomenon is explained, the fact remains that from the physiological point of view the nature of the oxygen dissociation curve can best be studied in whole blood.

![Oxygen dissociation curves](http://www.jbc.org/)

**Fig. 2.** Oxygen dissociation curves of blood and hemoglobin solutions at 37.5°C. (The data for the blood of the domestic fowl will be published at another time.)

The presentation of data on blood has been simplified by converting all points to a pH_e value of 7.1. In order to accomplish this, the blood was equilibrated at pCO_2 values of 10, 40, and 100 mm. and total CO_2 as well as total O_2 determined for each point. The distribution of bicarbonate between cells and serum was found to be about the same as in human blood. The ionic strength also is about the same; hence it has been assumed that the pK' values calculated for mammalian red cells by Stadic and Hawes (11) are applicable.

It is natural to wonder whether this assumption could be sufficiently in error to account for the difference between the curves
for blood and hemoglobin. This seems improbable, for in our hemoglobin solutions the \( pK' \) was experimentally determined, with the glass electrode, and found to be within a few hundredths of the expected value; a difference of 0.3 would be required to bring the oxygen curves together.

Fig. 3. Oxygen dissociation curves of blood at 37.5°. The broken line corresponds to the curve of Wastl and Leiner (8) for the pigeon blood at 42° and \( pCO_2 \) 40 mm. of Hg.

Fig. 4. Oxygen dissociation curves of blood at 37.5°. The broken line corresponds to the curve of Wastl and Leiner (8) for duck blood at 42° and \( pCO_2 \) 40 mm. of Hg.
The oxygen dissociation curves for four other species of birds are shown in Figs. 3 and 4. These all differ from the curve characteristic of human blood in two respects: there is much less affinity for oxygen, as Wastl and Leiner report (8), and the first increments in oxygen pressure appear to be associated with virtually no uptake of oxygen. This last observation is presumably a consequence of the methodical error referred to above, for in this part of the curve its effect is most evident.

Aside from the lower portion of the curves there is nothing unusual about their form; they all may be considered typical oxygen dissociation curves. The peculiarities found by Wastl and Leiner (8) for duck and pigeon blood are not supported by our study of the same species. We are of the opinion that their results are complicated by the fact that observations were made on two or more specimens of blood. Although \( pCO_2 \) was held constant, there was no control of the alkaline reserve in the different specimens and no assurance, therefore, of constant pH.

**SUMMARY**

The product of hemolysis of bird erythrocytes with water is not homogeneous. The supernatant solution of hemoglobin obtained from this mixture by centrifugation has much less affinity for oxygen than whole blood, even if proper pH adjustment is made.

Bird blood has a lower affinity for oxygen than the blood of man, but the form of the oxygen dissociation curve is much the same.

**BIBLIOGRAPHY**

Oxygen Dissociation Curves of Blood

OXYGEN DISSOCIATION CURVES OF BIRD BLOOD

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