PROPERTIES OF THE BLOOD OF THE DOMESTIC FOWL

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The development by Henderson and his associates of a physico-chemical method of describing blood and its subsequent application to the human species has opened up a new field in comparative physiology. This paper is based on an investigation of a portion of that field which has hitherto been rather neglected. Previous investigators (1-6) have confined themselves to particular aspects of a large number of species within the class (Aves), while we shall attempt a general description of one variety within a species, namely the Rhode Island Red fowl. Necessarily, it is understood that this isolated species, especially because it is domesticated, may not be wholly representative of the class.

Before going further it is to be noted that each of the experiments (with a very few exceptions) upon which this paper is based has been carried out upon a composite mixture of blood and not on that of individual birds. This has the effect of producing a more general description of the species and, at the same time, introducing certain inconsistencies in the nomogram which will be pointed out when that is discussed. In addition to this it should be noted that all equilibrations have been maintained at a temperature of 40°, which is the approximate body temperature of birds.

Composition of Cells and Serum—A comparison of the composition of cells and serum in the chicken, dog (7), and man (7) is made in Table I. The ion concentrations given have been determined according to the methods used by Dill, Talbott, and Edwards (8).

A marked difference is seen in the low value found for serum protein. On the other hand, cell hemoglobin is only slightly lower in chicken than in dog or man. Fritsch (1) gives a mean value for the hemoglobin content of hen cells even lower than that found in
our specimens. This is also confirmed by Dukes and Schwarte (5). Our value seems to lie in the upper limits of the range found by these investigators. Fritsch also states (1) that although the erythrocyte count is smaller in chickens the hemoglobin content is actually higher (per cell) than in some mammals (horse, cow, and dog).

The values for proteinate in both cells and serum are noticeably lower than in either man or dog (7), as would naturally be expected from the differences in protein content. The value for serum proteinate has been calculated according to the equation developed by Van Slyke, Hastings, Hiller, and Sendroy (9) for base bound by serum protein with the substitution of our own experimental value for the slope (α) of the buffer curve. However, that equation is based on human serum where the albumin to globulin ratio is evidently different so that the value of the constant (5.08) may not be correct for chickens. The value for cell proteinate involves

| Table I |
|---------------------------------
| Comparison of Ion Distribution in Oxygenated Blood at pH 7.45 |
| Concentrations are in units per liter of serum and of cells, respectively. |
|                              | Serum  |                   | Cells  |                   |
|                              | Dog    | Man    | Chicken | Dog    | Man    | Chicken |
| H2O, cc                      | 942    | 939    | 963     | 736    | 726    | 692     |
| Na, m.-eq                    | 146.5  | 139.3  | 158.0   | 110.5  | 14.9   | 25.6    |
| K                            | 3.7    | 3.3    | 5.7     | 6.0    | 93.7   | 92.8    |
| Ca                           | 5.2    | 4.7    | 6.1     |        |        |         |
| Mg*                          | 2.0    | 2.0    | 2.0     |        |        |         |
| Σ cations, m.-eq             | 157.4  | 149.3  | 171.8   | 116.5  | 108.6  | 118.4   |
| Cl                           | 118.3  | 106.8  | 117.8   | 59.7   | 51.3   | 53.4    |
| HCO3-                        | 18.6   | 24.0   | 21.1    | 11.0   | 12.7   | 12.2    |
| Proteinate                   | 16.4   | 17.0   | 12.1    | 51.8   | 52.6   | 46.4    |
| HPO42- + H2PO4- , m.-eq      | 2.3    | 2.2    | 4.2     | 1.2    | 1.1    | 2.4     |
| Lactate, m.-eq               | 3.0    | 1.2    | 7.8     | 1.6    | 0.8    | 3.1     |
| Σ anions                     | 158.0  | 151.2  | 101.0   | 125.3  | 118.5  | 117.5   |
| Protein, gm                  | 66.7   | 68.8   | 43.3    |        |        |         |
| Total Hb, mm                 |        |        |         | 20.1   | 20.4   | 18.4    |

* The value for magnesium in the serum is assumed.
the assumption of a value for $pK_\text{f}$ given by Stadie and Hawes (10), although this was determined in erythrocytes lacking a nu-
cleus. The cell proteinate value was then calculated from the
equation of Van Slyke, Wu, and McLean (11). In calculating dis-
solved CO$_2$ account has been taken of the equilibration tempera-
ture, 40°, and of the water contents of cells and serum. A study of
the $pK_\text{f}$ of separated chicken serum was also carried out. De-
terminations of pH, total CO$_2$ in the liquid, and of $p$CO$_2$ in the gas
phase following equilibration were performed in the usual way over
a range of temperature from 20–43° and at various CO$_2$ pressures
from about 6 to 100 mm. of mercury. The data so obtained to-
gether with our mean values from a number of determinations for
serum water gave, by use of the Henderson-Hasselbalch equation,
values of $pK_1$.

In order to compare our technique with that of previous investi-
gators a few determinations were also carried out on human serum
and on a bicarbonate solution, with the same methods as with
chicken serum.

The average value so obtained for chicken serum at 40° was 6.08
with a temperature coefficient of $-0.005$ unit of $pK_\text{f}$ per degree
over a range from 20–40°. These results are in close agreement
with those of Robinson, Price, and Cullen (12) and of Hastings,
Sendroy, and Van Slyke (13).

The values for cell sodium and potassium approach those of man
more closely than those of the dog; however, the value for sodium
is larger in the chicken than in man (7). The phosphate deter-
minations given are higher than those found in less complete ana-
lyses and the evidence seems to show that there is little significant
difference between those in the chicken and either those of the dog
or man.

Lactate, at any time a variable quantity, is more questionable
here because of the lapse of time (2 to 3 hours) between collection
and equilibration of the blood and the nucleate nature of its cells.
However, the blood was collected directly in a glass container (with
heparin) immersed in cracked ice in a vacuum jug and was kept
in this condition until the analyses were completed. Furthermore,

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1 The pH determinations were made on a glass electrode through the
kindness of Dr. A. A. Green.
Determinations of lactic acid on two samples of venous blood and one of arterial blood obtained by cardiac puncture yielded values from 2.9 to 3.5 milli-equivalents per liter. When these values for whole blood are compared with those given in Table I for cells and serum, it is evident that there was some increase in lactic acid before analysis. In general, our values agree with those found by Dyer and Roe (14).

Distribution of Anions between Cells and Serum—Van Slyke, Wu, and McLean (11) have developed a method of calculating the ratio \( r \) from the values of the cell and serum protein and base found in a given blood. The relationship is as follows:

\[
r = 1 - \frac{(BP)_{s} + (Hb)}{2(B)_{s} - (BP)_{s} + (Hb)} + \frac{(BP)_{s}}{2(B)_{s} - (BP)_{s}}
\]

It is readily seen that if, as in the case of chicken blood, the concentrations of the cations, \((B)_{c}\) and \((B)_{s}\), as well as the cell protein, \((Hb)_{c}\), are comparable to those found in human blood, while serum protein concentration is quite different, there should be a significant difference in the value of \( r \) found experimentally. A more detailed analysis of the ratio \( r \) from the standpoint of the Donnan equilibrium theory shows further that this deviation from the value of \( r \) in human blood may appear in either the chloride or the bicarbonate ratio or in both. Our data indicate that the difference lies mainly in the bicarbonate ratio (at least in oxygenated blood). If hemoglobin combines with \( CO_2 \), such a result would suggest that chicken hemoglobin binds less \( CO_2 \) than does that of human blood. This conclusion has been arrived at from other considerations.

Because great difficulty was experienced in obtaining consistent results with determinations of the ratio for either chloride or bicarbonate ions, experiments were made on five separate composite blood samples. Estimates of the bicarbonate ratio were further augmented by calculations based on three sets of \( CO_2 \) dissociation curves and mean values assumed for \((H_2O)_{c}\) and \((H_2O)_{s}\). The most probable straight lines\(^2\) determined by these values as func-

\(^2\) The formula used for the calculation of the most probable straight line towards which a series of values tends is as follows: \( y = Rx - R\bar{x}m + \bar{ym} \); where \( R = (N\Sigma xy - \Sigma x\Sigma y)/(N\Sigma x^2 - (\Sigma x)^2) \), \( N \) = number of observations, \( \bar{x}m \) = mean value of \( x \), and \( \bar{ym} \) = mean value of \( y \).
tions of the total CO₂ of whole blood and of the state of oxidation were calculated. Then with the relationship established between pH₄ and total CO₂ of whole blood, the equations for the ion ratios (r) were calculated with the following results.

\[
\begin{align*}
\text{r}_\text{HCO}_3^- (\text{oxygenated}) &= 2.333 - 0.220 \text{pH}_4 \\
\text{r}_\text{HCO}_3^- (\text{reduced}) &= 2.365 - 0.216 \\
\text{r}_\text{Cl}^- (\text{oxygenated}) &= 1.697 - 0.144 \\
\text{r}_\text{Cl}^- (\text{reduced}) &= 2.444 - 0.236
\end{align*}
\]

At a pH₄ of 7.45 in oxygenated blood, these equations give values of 0.694 for \text{r}_\text{HCO}_3^- and 0.624 for \text{r}_\text{Cl}^- as compared with 0.750 and 0.637, respectively, for man (7). The relationship between the distribution ratios for the two species is as follows:

At pH₄ 7.45,

\[
\begin{align*}
\text{r}_\text{Cl}^- &= 0.800 \text{r}_\text{HCO}_3^- = 0.901 \text{r}_\text{theory (man (15))} \\
\text{r}_\text{Cl}^- &= 0.901 \text{r}_\text{HCO}_3^- = 0.86 \text{r}_\text{theory (chicken)}
\end{align*}
\]

**Buffer Value of Serum**—Henderson, Dill, Edwards, and Morgan (16) have defined the buffering power of serum by the equation

\[
\frac{d(C_{BP})}{d(\text{pH}_4)} = aC_p,
\]

where a may be considered a convenient measure of the buffer value of serum protein. They have found values of 0.110 for man, while Dill, Edwards, et al. (7) have found a mean value of 0.093 for the dog. Our mean value for chicken serum protein from six determinations is 0.118. In the chicken, as in the dog, the results seem to be more variable than in man.

The value of a for chicken serum protein is about one-thirteenth greater than for human serum protein. However, the protein concentration of human serum is one-half greater, so that per unit volume of serum, human serum has about one-third greater buffer value than that of chicken. If, now, we consider equal volumes of chicken and human whole blood, since the serum volume is markedly greater in the former, the effective buffering value of serum protein in whole blood is approximately the same for both species.

**Buffer Values of Oxygenated Whole Blood**—It is possible by the use of the empirical chart developed by Henderson and his associates (17) to compare the buffer value of chicken blood with that of human blood of the same alkaline reserve and oxygen
Properties of Blood of Domestic Fowl

capacity. Such a comparison is made in Table II and it is seen that per unit volume chicken blood has only slightly less (98 per cent) buffering power than human blood. Since the buffer value of a given volume of chicken serum is less than that of human serum, it follows that the buffering value of the chicken cells must be somewhat greater.

Oxygen Dissociation Curves—The oxygen dissociation curves shown in Fig. 1 are derived from smoothed data, as follows: With experimental data from three or four different samples of composite blood equilibrated at the three CO₂ pressures indicated and varying oxygen pressures, a rough plot is made of per cent oxygen saturation against pO₂. Since the carbon dioxide pressures are never exactly 10 mm., 40 mm., or 100 mm. of Hg, the necessary corrections are applied on the theory that over a small range of oxygen pressure the corrections are linear. From the curves so plotted and corrected, log (100Hb/HbO₂) and log pO₂ are determined and plotted against one another. All previous work on mammalian blood tends to show that curves at different pCO₂ values plotted in this way yield parallel lines. In our work these lines had a pronounced curvature, whereas it is usually found that

<table>
<thead>
<tr>
<th>Date</th>
<th>Serum protein gm. per l.</th>
<th>HbO₂ capacity m.-eq. per l.</th>
<th>CO₂ capacity at pCO₂ 40 mm. m.-eq. per l.</th>
<th>ΔCO₂(m-x) Observed m.-eq. per l.</th>
<th>ΔCO₂(m-x) Calculated from human blood m.-eq. per l.</th>
<th>Ratio</th>
</tr>
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<tr>
<td>Nov. 29</td>
<td>48.31</td>
<td>7.80</td>
<td>20.44</td>
<td>5.30</td>
<td>5.42</td>
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<tr>
<td>&quot;</td>
<td>52.22</td>
<td>5.38</td>
<td>21.92</td>
<td>4.81</td>
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<td>1.000</td>
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<tr>
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<td>50.81</td>
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<td>17.25</td>
<td>5.12</td>
<td>5.44</td>
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<td>4.85</td>
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<tr>
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<td>4.27</td>
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<tr>
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<tr>
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<td>18.15</td>
<td>5.80</td>
<td>5.43</td>
<td>1.068</td>
</tr>
</tbody>
</table>

Mean ratio................................................................................................................................. 0.975
they are more nearly straight. This, however, does not affect their use in the present instance. Taking account of this fact that the curves should be parallel, the best fitting sets are drawn through the points. In the particular case under consideration, the curve at 40 mm. \( pCO_2 \) being the best defined, the others were drawn parallel to it. Finally, from the logarithmic plot so ob-

![Fig. 1. Oxygen dissociation curves from smoothed data](image)

Fig. 1. Oxygen dissociation curves from smoothed data

tained the values for \( HbO_2 \) in per cent and \( pO_2 \) in mm. of Hg were read off and plotted to give the smoothed curves shown in Fig. 1.

It has been previously stated in this paper that the buffering values of chicken and human hemoglobin seem much the same. A comparison of the effect of changing \( pCO_2 \) on the oxygen dissociation curves of the two species confirms this observation.
In Fig. 2 a comparison of the oxygen dissociation curves of man (7), dog (7), and chicken has been made at pH 7.1. The equilibration temperatures are different, but since the comparison is made at approximately normal body temperature for each animal, it is, we believe, valid. The displacement of the oxygen dissociation curves to the right has been noticed in other birds. Wastl and Leiner (6) report it in the case of ducks, geese, and pigeons. The greatest displacement is found in the chicken and the least in

Fig. 2. Comparison of oxygen dissociation curves at pH 7.1. Equilibration temperatures for man and dog were 37.5° and for chicken, 40.0°.
the goose, which is close to that for man. These authors attribute 
the displacement in part to the decrease in oxygen-combining 
capacity of the hemoglobin due to the rise in temperature and also 
to a definitely smaller affinity of the hemoglobin for oxygen.

In Fig. 3 the effect of acid change on the affinity of the blood 
for oxygen is shown. The effect is evidently much the same in 
dog, man, and chicken, but the smaller affinity of the chicken

\[
\text{FIG. 3. Position of oxygen dissociation curves (log } O_2 \text{ when } HbO_2 = 50 \text{ per cent) in relation to change in pH.}
\]

hemoglobin is shown in the distance its curve lies above those for 
the mammals. For chicken blood the relation within physiological limits is \( pO_2 = 5.76 - 0.57 \text{ pH.} \)

Nomogram—The alignment chart shown in Fig. 4 has been con-
structed in accordance with Henderson's method (15). It presents 
a roughly quantitative picture of chicken blood. On such a chart 
any straight line cuts the plotted scales of the variables in simul-
taneously occurring values. On account of certain inconsistencies,
such as the fact that the line representing oxygen pressure is based on an oxygen capacity of about 13.5 volumes per cent of O\textsubscript{2} taken from a mean value for the blood samples used in constructing the

Fig. 4. Blood of Rhode Island Red chicken. (HCO\textsubscript{3}), is given per unit of serum.

smoothed O\textsubscript{2} dissociation curves (Fig. 1), this is not entirely true of our nomogram. All other variables except the serum volume and the chloride and bicarbonate ion ratios are from a single day's
experiment in which the oxygen capacity was 11.93 volumes per cent of O₂. Hence any pO₂ values read from the chart will be about 10 per cent too high.

The line labeled \( r_{\text{theory}} \) was calculated according to the relationship developed by Van Slyke, Wu, and McLean (11) for \( r \) as a function of the base bound by protein and the protein concentrations in cells and serum with our experimental data on chicken blood for these values.

The construction of the lines representing \( pO₂ \) deserves some further comment. Of all the variables concerned in the nomographic picture of the blood, the oxygen pressure is the least amenable to such treatment. It is almost impossible to obtain it as a simple linear function, and since the nomogram is accurate only for such linear relationships or those which are approximately so, the introduction of such a variable is quite inaccurate. To overcome the difficulty the oxygen pressure has been plotted for three different carbon dioxide pressures. The great difference between the curves serves to emphasize our previous statement. In reading a value for oxygen pressure, then, it must be interpolated from these curves at the corresponding \( pCO₂ \).

The data for the placing of the arterial and venous curves were obtained from blood taken by cardiac puncture on nine different birds. In all cases attempts were made to obtain both arterial and venous samples from the same bird but usually this was impossible. The values of the oxygen capacity, oxygen content, and carbon dioxide content were determined on each sample. The venous oxygen capacities were usually found to be about 1 volume per cent less than the arterial. In some cases a sample was equilibrated with CO₂ to find the approximate position of the CO₂ dissociation curves. The value for the CO₂ content of venous blood used in the construction of the curve was calculated on the assumption that the respiratory quotient of chickens is about 0.9 because of their largely carbohydrate diet. The calculated value agrees roughly with those found on direct analysis.

With the values for CO₂ contents and the CO₂ dissociation curve plotted from the equilibrations performed, and with a knowledge of the usual slope of such curves obtained from previous work, the approximate arterial \( pCO₂ \) was calculated as 34 mm. of mercury and that of mixed venous blood as 45 mm. This indicates
that a pressure head of about 10 mm. exists between venous and arterial blood for the removal of CO₂.

The percentage utilization of oxygen in the resting bird is evidently much greater than in the human being. In most cases our birds were quiet while the cardiac punctures were made, so that the results are probably close to the resting values and the increased oxygen utilization is real. Arterial blood is only about 88 per cent saturated with oxygen as compared with the value of 95 per cent found in man; the venous saturation is very much lower, reaching an average of about 40 per cent. This greater oxygen utilization is possibly connected with the relatively greater activity of birds and might be even more pronounced in those species that retain the use of their wings for flying.

Miscellaneous Observations—The red cell count of chicken blood was determined in seven cases and values from 2.45 to 3.67 millions with a mean of 2.94 were found. This is lower than in human blood to almost the same degree as the cell volume. Fritsch (1) gives mean red cell counts of his own and various authors varying from 2.62 to 4.28 millions for different species of chickens. Wastl and Leiner (6) give mean cell counts of 3.075 millions for pigeons and 2.75 millions for ducks. We also made one leucocyte count which was about 10,000.

In many cases we experienced difficulty in obtaining consistent values for oxygen capacity. It was finally found that unless the blood was rotated continuously in a rather large container (relative to the quantity of blood used) with frequent changes of air during the time samples were being withdrawn, equilibrium could not be reached. This is evidently due to the very high rate of metabolism (oxygen consumption) of the nucleated cells as compared with the anucleate mammalian type. Tipton (18) has shown that this high rate of respiration exists in chicken erythrocytes and has found that it falls off rapidly with diminishing temperature. Indeed, if the rate at 20° is taken as a reference, there is a falling off of about 240 per cent as the temperature drops from 38° to 20°. This would indicate (although the temperature coefficient is probably different over the range from 20–0°) that, under the conditions in which our blood samples were kept after collection, metabolism was slight.
SUMMARY

A physicochemical study of the blood of a domesticated bird, the Rhode Island Red chicken, has been made. The buffering values of the serum proteins and of the cells are about the same as for man, if the relative concentrations are taken into account. The oxygen dissociation curves are the familiar sigmoid shape but are displaced further to the right than is the case with any bird previously reported. A nomographic picture of the blood as a whole is given.

Physiologically the very high utilization of oxygen by the tissues as compared to that in man is striking.

The authors are greatly indebted to Professor L. J. Henderson and Dr. D. B. Dill for their interest and advice, without which this work would have been impossible. We also wish to thank the other workers in the Fatigue Laboratory for their valuable technical assistance throughout the course of the research.

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