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KINETICS OF OXYGEN UPTAKE AND RELEASE BY RED BLOOD CELLS OF CHICKEN AND DUCK

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SUMMARY

The specific conductance (G) for O_2 transfer by red blood cells (RBCs) of chicken and muscovy duck was measured using the experimental (stopped-flow) and analytical techniques (RBC model) previously applied to human RBC (Yamaguchi, Nguyen Phu, Scheid & Piiper, 1985). Avian RBCs behaved similarly to human RBCs: G values were of similar magnitude; G for O_2 uptake decreased with time and increasing O_2 saturation; G for O_2 release at high levels of dithionite decreased slightly with decreasing O_2 saturation; G for O_2 release was higher than G for O_2 uptake. The deoxygenation kinetics of oxyhaemoglobin in solution was similar for both avian species.

The G measured for O_2 release at high dithionite concentration, considered to represent a good approximation to intra-erythrocyte O_2 diffusion conductance, averaged (in $mmol\,min^{-1}\,Torr^{-1}\,ml^{-1}$ RBC) 0·33 for chicken and 0·25 for duck (at 41 °C, pH of the suspension = 7·5, O_2 saturation range 0·4–0·8). These species differences can be explained by differences in cell size, the RBC volume averaging 104 μm^3 in the chicken and 155 μm^3 in the duck. Compared with human RBCs, the G estimates for avian RBCs are somewhat smaller than would be predicted from size differences, which can be explained by the discoid shape of mammalian RBCs which constitutes an advantage compared with the ovoid avian RBC.

INTRODUCTION

In recent years, the analysis of O_2 transfer kinetics of red blood cells (RBCs) using data obtained by stopped-flow techniques has been shown to be limited by resistance to O_2 diffusion in the medium surrounding the RBCs (Gad-el-Hak, Morton & Kutchai, 1977; Coin & Olson, 1979; Rice, 1980; Vandegriff & Olson, 1984). This realization has led to a general criticism of the stopped-flow method, but has also stimulated refinements in technology and modelling (Holland, Shibata, Scheid & Piiper, 1985; Yamaguchi *et al.* 1985).

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Key words: duck, chicken, birds, blood O_2 dissociation curve, red blood cells, diffusion, oxygen uptake, oxygen release.

In the present study the O_2 kinetics of avian RBCs has been studied for the first time to find out if the technique is suitable for avian RBCs and if there are major differences between avian and mammalian RBCs. Such differences could be expected on the basis of shape and size differences, the nucleated duck and chicken RBCs being larger than human RBCs and more spherical with a smaller surface area to volume ratio. These differences are expected to yield slower O_2 transfer kinetics for the avian, compared with the human, RBC.

The need for information on the kinetics of avian RBCs in O_2 transfer has arisen with the progress in the analysis of gas exchange in bird lungs (reviewed by Scheid, 1979), in particular in connection with studies aimed at analysis of gas–blood equilibration of O_2 (Scheid & Piiper, 1970; Burger, Meyer, Graf & Scheid, 1979; Geiser, Gratz, Hiramoto & Scheid, 1984) and the morphometry of gas exchange structures in avian lungs (Abdalla *et al.* 1982; Maina & King, 1982; Maina, 1984).

MATERIALS AND METHODS

Blood was obtained by venipuncture from three ducks (domestic form of the muscovy duck, *Cairina moschata*, average body mass 1·9 kg) and three domestic hens (*Gallus domesticus*, average body mass 1·9 kg), was anticoagulated with heparin (5 i.u. ml^{-1} blood Vetren®, Promonta, Hamburg, FRG) and immediately stored on ice. Experiments were completed within 12 h after withdrawal of the blood.

Since the methods were the same as those described previously (Yamaguchi *et al.* 1985), only a brief account will be given.

Blood parameters

The O_2 capacity of blood was determined from the O_2 content measured with a Lex-O_2-Con (Lexington Instrument Corporation, Waltham, MA, USA) in blood equilibrated with 50% O_2, subtracting physically dissolved O_2. Haematocrit was determined by centrifugation for 5 min in a microcentrifuge (M 1100, Compur Electronic GmbH, Munich, FRG). The O_2 capacity of RBCs (mmol O_2 l^{-1} RBC) was calculated as O_2 capacity of blood divided by haematocrit. The RBC volume (mean corpuscular volume) was calculated from haematocrit and red cell number determined by an electronic cell counter (Coulter Counter®, Model ZBI, Coulter Electronics, Luton, England).

O_2 kinetics of RBCs

The measurements of O_2 saturation kinetics of RBC suspensions were performed using a stopped-flow apparatus attached to a dual-wavelength spectrophotometer (Sigma-ZWS-II, Biochem, Munich, FRG) measuring the extinction difference between two wavelengths (560 and 577 nm). Each kinetic curve used for calculation of the specific O_2 conductance of RBCs, G, was based on eight successive measurements, which were averaged by a signal averager (Nicolet 1070, Nicolet Instruments Corp., Offenbach, FRG).

The measurements were performed at 41 °C, the average body temperature of the experimental animals.

O_2 uptake at varied initial S_{O_2}

Aliquots of RBC suspension obtained by diluting (1:50) whole blood in isotonic saline–phosphate–bicarbonate buffer were equilibrated with water vapour-saturated gas mixtures of a range of O_2 concentrations (0 %, 5 %, 6 % and 8 % for measurements in duck blood and 0 %, 6 %, 8 % and 10 % for measurements in chicken blood). Aliquots of the same buffer were equilibrated with gas mixtures of O_2 concentrations varied from 50 % to 70 % in such a manner as to achieve a similar final P_{O_2} after mixing and stopped-flow equilibration. For all equilibrations, gas of constant CO_2 concentration of 5 % was used to yield P_{CO_2} close to 35 Torr and pH close to 7·5, corresponding to the values measured in arterial blood of the unanaesthetized, undisturbed chickens and ducks (Kawashiro & Scheid, 1975). For calibration, the initial and final values of S_{O_2} were reproduced by mixing red cell suspension with buffer solution of identical P_{O_2}. For an accurate measurement at $S_{O_2} = 0$, RBCs suspended in buffer containing sodium dithionite were used. Using the same technique, RBC suspensions and buffer equilibrated at identical P_{O_2}, between 14 and 100 Torr, were mixed and S_{O_2} was recorded to construct the actual blood O_2 dissociation curve which is required for the calculation of G.

O_2 release in the presence of 40 mmol l^{-1} sodium dithionite

RBC suspension was equilibrated with a gas mixture containing 50 % O_2 and 5 % CO_2 (in N_2). This suspension of completely oxygenated RBCs was mixed with deoxygenated isotonic buffer solution containing 80 mmol l^{-1} sodium dithionite ($Na_2S_2O_4$) the pH of which was titrated to 7·5 using 1 mol l^{-1} NaOH at P_{CO_2} = 35 Torr.

Calculations

The specific O_2 transfer conductance G [mmol O_2 min^{-1} $Torr^{-1}$ ml^{-1} RBC], defined as the amount of O_2 taken up by, or released from, 1 ml of RBCs in 1 min for 1 Torr P_{O_2} difference between the RBC interior and the medium, was calculated for any S_{O_2} of the kinetics curve from the relationship (Yamaguchi *et al.* 1985):

$$G = C_{Hb} \times \frac{\Delta S_{O_2}/\Delta t}{P_m - P_{eq}}, \quad (1)$$

where $\Delta S_{O_2}/\Delta t$ (min^{-1}) is the rate of change in S_{O_2} as read from the recording; C_{Hb} (mmol O_2 ml^{-1} RBC) is the O_2 capacity of RBCs; $P_m - P_{eq}$ (Torr) is the momentary O_2 partial pressure difference between the medium (P_m) and the RBC haemoglobin (P_{eq}, corresponding to S_{O_2} on the actual O_2 dissociation curve). In O_2 release measurements, P_m was zero because of the presence of dithionite. In the case of O_2 uptake, P_m was estimated from the known initial medium P_{O_2} and the mass balance, whereby the amount of O_2 transfer between medium and RBCs was measured as the change in S_{O_2} (Yamaguchi *et al.* 1985).

Table 1. *Blood data*

	Chicken	Duck	Man
O_2 capacity (mmol l^{-1} blood)	6·1 ± 0·6	8·5 ± 0·4	9·3
Haematocrit	0·33 ± 0·03	0·44 ± 0·02	0·47
RBC concentration ($\times 10^{12} l^{-1}$ blood)	3·1 ± 0·3	2·9 ± 0·1	5·3
RBC O_2 capacity* (mmol l^{-1} RBC)	18·3	19·3	19·8
Mean RBC volume, V_{RBC}† (μm^3)	104 ± 3	155 ± 1	87

Mean values (± S.E.) for the chicken and duck of this study, together with the relevant data for human blood as measured by Yamaguchi, Nguyen Phu, Scheid & Piiper (1985).

* Calculated as the ratio of O_2 capacity and haematocrit.

† Calculated as the ratio of haematocrit and RBC concentration.

Kinetics of haemoglobin deoxygenation

Oxygenated RBC suspension was haemolysed by addition of saponin and mixed with buffer solution containing 80 mmol l^{-1} dithionite. The rate constant k was calculated from the slope of the logarithmic decrease of S_{O_2}:

$$k = -d(\ln S_{O_2})/dt. \quad (2)$$

RESULTS

Haematological parameters

Table 1 contains mean experimental values for O_2 capacity, haematocrit and RBC concentration in chicken and duck, together with values obtained with the same technique in human blood (Yamaguchi *et al.* 1985). Derived from these measurements are the RBC O_2 capacity and mean RBC volume.

The mean values for P_{50} and for Hill's n in the range $0{\cdot}3 < S_{O_2} < 0{\cdot}9$ are 51·1 Torr and 3·3 for the chicken, and 49·0 Torr and 3·3 for the duck.

Kinetics of O_2 uptake by RBCs

Average G values for O_2 uptake by chicken and duck RBCs are plotted against time after flow stop in Fig. 1, and against S_{O_2} in Fig. 2. In these figures, as in Fig. 3, the standard deviation is omitted for clarity; however, the maximum difference between corresponding measurements in different animals of one species was less than 10%. The following features are evident.

(1) The initial G values are not clearly dependent on initial S_{O_2}.

(2) The G values decrease markedly with time or with increasing S_{O_2}.

(3) There is a tendency for the decline of G with time or with increasing S_{O_2} to be steeper with higher initial S_{O_2}.

(4) The initial G values are higher for chicken RBCs than for duck RBCs, the ratio for G at corresponding initial S_{O_2} values, 1·13 ± 0·04 (mean ± S.E.), being significantly different from unity ($P < 0{\cdot}01$).

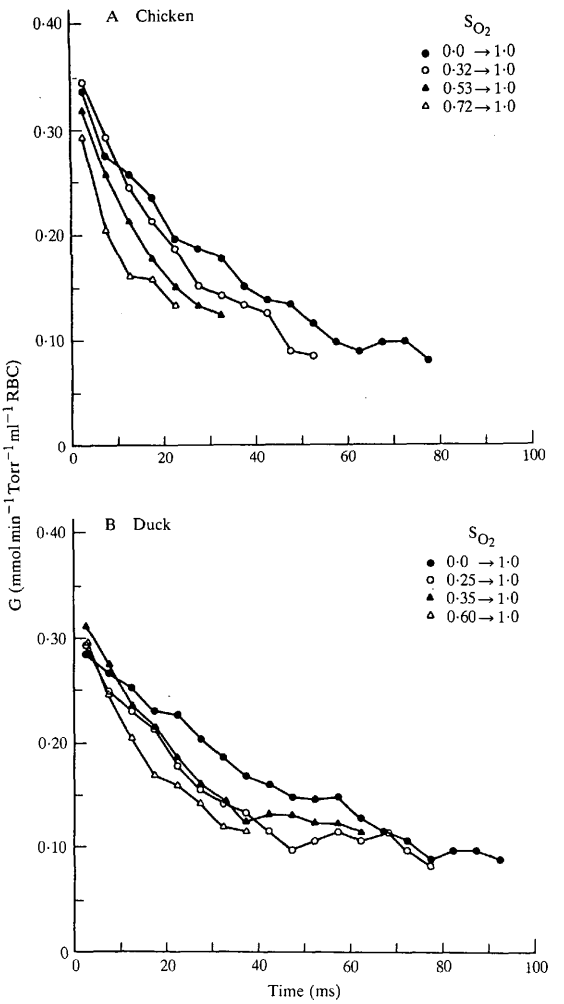

Fig. 1. Specific O_2 conductance, G, for oxygenation kinetics of chicken (A) and duck RBC suspension (B) starting from different fractional O_2 saturation levels (S_{O_2}). Each symbol represents an average from three animals, the standard deviation being less than 10%. Abscissa is time after flow stop.

Kinetics of O_2 release by RBCs

The G values for O_2 release from RBCs into 40 mmol l^{-1} dithionite are plotted against S_{O_2} in Fig. 3 (the plots against time are similar).

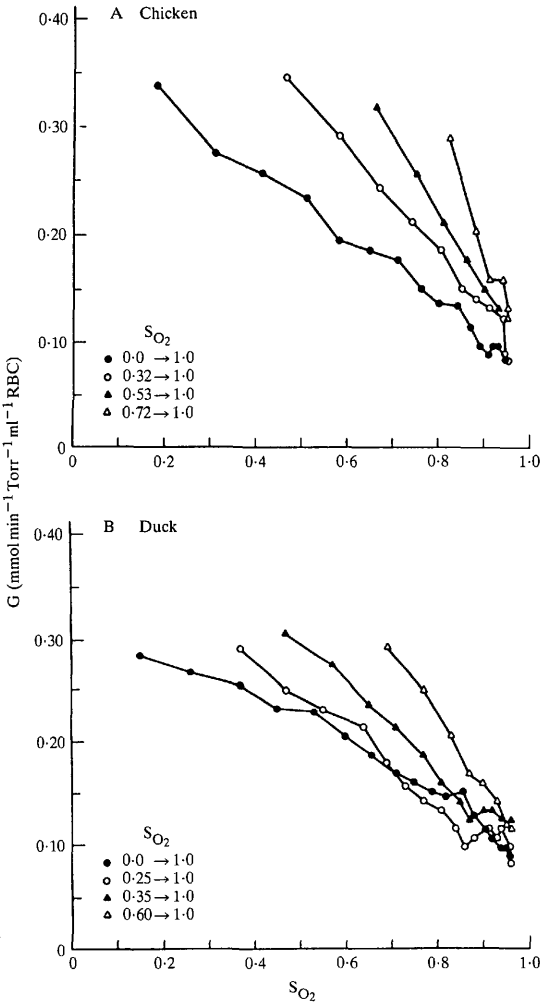

Fig. 2. Data of Fig. 1 plotted against O_2 saturation (S_{O_2}).

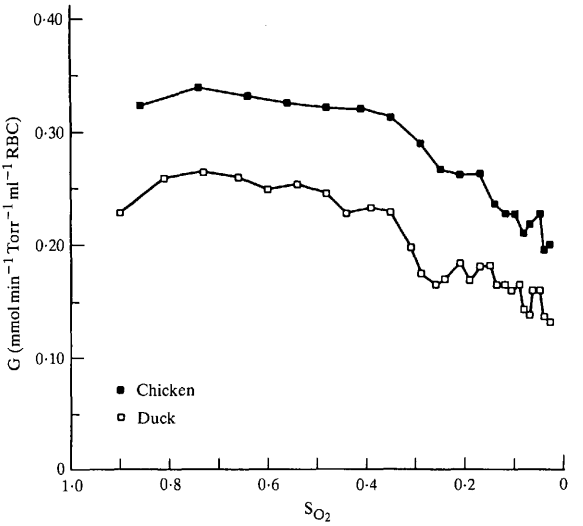

Fig. 3. Specific O_2 conductance, G, for deoxygenation kinetics into buffer containing 40 mmol l^{-1} dithionite, plotted against S_{O_2}. Data are mean values for three chicken and three ducks, respectively.

(1) There is a tendency for G initially to increase with decreasing S_{O_2}. This is followed by a plateau and a decrease to about 60 % of the plateau value at the end of desaturation.

(2) The G values for chicken are higher than those for duck. In the plateau range ($0{\cdot}4 < S_{O_2} < 0{\cdot}8$) G (mmol min^{-1} Torr^{-1} ml^{-1} RBC) for chicken averages 0·33, for duck 0·25, and their ratio over the entire S_{O_2} range is $1{\cdot}29 \pm 0{\cdot}01$ (mean ± S.E.M.). The mean G values are plotted in Table 2 together with human data.

Deoxygenation kinetics of haemolysate

The deoxygenation kinetics of duck and chicken haemoglobin solution show time courses which are almost identical, and yield the following values for the rate constant k (s^{-1}): chicken, 172; duck, 186. For human haemoglobin solution (at 37 °C) the time course is very similar, k averaging 185 s^{-1}.

DISCUSSION

Haematological values and O_2 dissociation curve

The values for O_2 capacity, haematocrit, RBC number and mean RBC volume (Table 1) for the chicken are within the range of values reported in the literature

(Christensen & Dill, 1935; Morgan & Chichester, 1935; Chiodi & Terman, 1965; Sturkie, 1976; Baumann & Baumann, 1977; Steel, Petersen, Blanks & Smalley, 1977). Our O_2 capacity value for the duck is close to that previously reported by us (Scheid & Kawashiro, 1975; Holle, Meyer & Scheid, 1977; Meyer, Holle & Scheid, 1978). The mean RBC volume of the Pekin duck, about 180 μm^3 (Sturkie, 1976; Gaehtgens, Schmidt & Will, 1981) is somewhat higher than our value for the muscovy duck.

The P_{50} value for chicken blood (51·1 Torr) is close to that reported by others (Chiodi & Terman, 1965; Baumann & Baumann, 1977; Hirsowitz, Fell & Torrance, 1977). The P_{50} value for the duck (49·0 Torr) is within the wide range of values reported for Pekin and muscovy ducks (cf. Meyer *et al.* 1978).

The Hill coefficient values reported for chicken and duck blood range from 2·6 to 3·3 (Christensen & Dill, 1935; Morgan & Chichester, 1935; Bartels, Hiller & Reinhardt, 1966; Scheipers, Kawashiro & Scheid, 1975; Wells, 1976), our values being at the upper margin. There was a definite tendency of Hill's n to increase with S_{O_2}.

Kinetics of O_2 uptake and release by RBCs

The characteristics of the kinetics of O_2 uptake and release by RBCs as well as the deoxygenation kinetics of oxyhaemoglobin are in every respect similar to those reported for human RBCs by Yamaguchi *et al.* (1985). These authors concluded from the analysis of their results that O_2 transfer of RBCs was limited by diffusion both within RBCs and in the medium around the RBCs. The increase of the effective O_2 diffusion pathway by progressive depletion of the medium surrounding the RBCs was held to be responsible for the decrease of G with time during O_2 uptake. But even the initial value, determined for the time interval 0–5 ms after flow stop, was considered to be influenced by the extracellular O_2 gradient.

The important finding in human RBCs that with increasing dithionite concentration the O_2 release kinetics approached an upper limit (Yamaguchi *et al.* 1985) indicates that O_2 release into a medium with a sufficiently high dithionite concentration is not limited by diffusion or reaction kinetics (of $Na_2S_2O_4$ with O_2) in the medium. Thus in these conditions, diffusion inside the RBC is most probably limiting O_2 release.

C. Hook, K. Yamaguchi, J. Piiper & P. Scheid (in preparation) use the deoxygenation kinetics of human haemoglobin solution in model calculations and conclude that diffusion plays a more significant role in O_2 transfer in the RBC interior than in the reaction. This result is further supported in experiments in which the temperature and pH dependence of G are measured (K. Yamaguchi, J. Glahn, P. Scheid & J. Piiper, in preparation).

It appears justifiable to apply these conclusions to the avian RBC as well. The dependence of G on S_{O_2}, particularly when using 40 mmol l^{-1} dithionite, is very similar to that of human RBCs (Yamaguchi *et al.* 1985). Second, the deoxygenation kinetics of avian haemoglobin solution is not much slower than for human haemoglobin solution, even if the human k value is extrapolated to that expected

at 41 °C using the Q_{10} of K. Yamaguchi, J. Glahn, P. Scheid & J. Piiper (in preparation) whereby a value of $k = 300\,s^{-1}$ is estimated, compared with the avian estimate of about $180\,s^{-1}$.

Thus, the plateau values for G for O_2 release may be considered as the best approximations to diffusive O_2 conductance of the RBC and to be valid also for O_2 uptake in a medium without external O_2 diffusion limitation, i.e. in a sufficiently stirred medium. These values are listed in Table 2 together with the corresponding value for human RBCs (Yamaguchi *et al.* 1985).

In the mammalian RBC, the cell membrane appears to play no important limiting role in diffusive O_2 transfer (Kreuzer & Yahr, 1960; Kutchai & Staub, 1969; Rotman *et al.* 1980; Huxley & Kutchai, 1985). Although experimental evidence is lacking, there is no reason to assume that this is not also true for the avian RBCs.

Comparison of O_2 kinetics of RBCs (duck vs *chicken* vs *man)*

Table 2 shows a sequence in G values: G(duck) < G(chicken) < G(man). In this comparison, differences in the cell volume, and thus the number of RBCs per ml, must be considered. It is hence of interest to calculate and compare O_2 transfer conductance for a single RBC, g, which is obtained as $G \times V_{RBC}$. The values of g in Table 2 show less variation among species than the G estimates. Can the remaining differences be attributed to differences in cell shape and size?

Assuming diffusion to be the main rate-limiting process in O_2 exchange, the g values should be proportional to the cell surface area, i.e. to $(V_{RBC})^{2/3}$, and inversely related to the cell thickness, i.e. to $(V_{RBC})^{1/3}$. Hence,

$$g \propto (V_{RBC})^{1/3}. \quad (3)$$

Table 2 yields a ratio of experimental values: g(chicken)/g(duck) = 0·87, and equation 3 using the V_{RBC} data of Table 1, predicts a g ratio of 0·88, which is an excellent agreement.

On the other hand, the experimental ratio g(man)/g(chicken) = 1·09, whereas the predicted ratio, using equation 3 and V_{RBC} from Table 1, is 0·94. This shows that the O_2 exchange conductance of human RBCs is about 15 % larger than would be predicted from the avian data on the assumption of isomorphy between avian and human RBCs.

Table 2. *O_2 exchange conductance for 1 ml of RBC, G, and for a single RBC, g*

	Chicken	Duck	Man
G (mmol min^{-1} $Torr^{-1}$ ml^{-1} RBC)*	0·33	0·25	0·43
g (fmol min^{-1} $Torr^{-1}$)†	34	39	37

Mean values. Chicken and duck, this study; man, from Yamaguchi, Nguyen Phu, Scheid & Piiper (1985).

* Corrected from experimental temperature, 37 °C, to 41 °C using the temperature dependence of K. Yamaguchi, J. Glahn, P. Scheid & J. Piiper (1986).

† Calculated as the ratio of G and the number of RBCs per 1 ml which equals $1/V_{RBC}$.

On the other hand, differences in experimental O_2 exchange kinetics among mammalian species appear to be related to size differences of RBCs. Thus, Holland & Forster (1966) found the velocity constants of the initial rate of O_2 uptake in mammalian RBCs of widely differing size, to decrease as cell volume increases, and Jones (1979) obtained the best correlation of his data with the surface area to volume ratio, i.e. $(V_{RBC})^{1/3}$. Thus, both for a comparison between mammalian species and between avian species the difference in g appears to be predictable on the basis of size differences in isomorphous bodies. This prediction appears, on the other hand, to be invalid for a comparison between mammalian and avian RBCs.

There are some differences between avian and mammalian RBCs which may have relevance to their O_2 exchange conductance. First, in comparison with the discoid mammalian cell, the shape of the avian RBC is more spherical, so that the surface area for a given volume, and hence the conductance g, is smaller.

Second, the presence of the nucleus in the avian red cell is expected to affect g in at least two ways. On the one hand, the haemoglobin concentration in the cytoplasm is higher than that calculated on the basis of the RBC volume, and therefore the O_2 diffusivity may be expected to be reduced. In fact, the values for O_2 capacity of avian RBCs must be corrected for the nucleus volume, 22% in the chicken (Abdalla *et al.* 1982) and 19% in the duck (Maina & King, 1982), which gives cytoplasmic haemoglobin concentrations of 23·5 and 23·8 mmol l^{-1} cytoplasm, which are thus about 20% larger than the mammalian value.

On the other hand, for a given cytoplasm volume, the cell surface area, and thus the area available for O_2 diffusion, is increased by the presence of the nucleus. In fact, the avian RBC may be modelled as a spherical shell containing haemoglobin, with a haemoglobin-free nucleus. C. Hook, K. Yamaguchi, J. Piiper & P. Scheid (in preparation) have shown that the g value for the spherical shell model is superior to that of the sphere (of equal volume). These differences show that in fact the avian RBC cannot simply be considered as isomorphous with the mammalian RBC, and that quantitative predictions of differences in g are difficult to obtain.

Combined membrane/blood O_2 conductance

In the analysis of the diffusion aspect in pulmonary gas transfer involving blood, it is often useful to consider gas transfer as a two-step process consisting of diffusion through a barrier ('membrane') and diffusion (+ chemical reaction) in blood. This simple model was introduced by Roughton & Forster (1957) for application to CO uptake in mammalian lungs; thereafter, the equation was also applied to O_2 exchange (Staub, Bishop & Forster, 1962):

$$\frac{1}{D_{tot}} = \frac{1}{D_m} + \frac{1}{Q_c \times \theta}, \tag{4}$$

where D_{tot} is the total pulmonary conductance or diffusing capacity; D_m is the diffusing capacity of the gas/blood tissue barrier; Q_c is capillary volume and θ is the specific conductance of blood for gas transfer.

θ refers to blood, whereas G, introduced by Yamaguchi *et al.* (1985) and used in this report, refers to RBCs. Therefore, their relationship is determined by the fractional RBC volume or haematocrit (h)

$$\theta = h \times G. \tag{5}$$

Substitution of equation 5 into equation 4 yields:

$$\frac{1}{D_{tot}} = \frac{1}{D_m} + \frac{1}{Q_c \times h \times G}. \tag{6}$$

It should be noted that $Q_c \times h$ is the (functional) total RBC volume in the gas exchanging organ.

Use of G instead of θ, and of equation 6 instead of equation 4, has important advantages, particularly in comparative physiology. Whereas θ refers to a normal, standardized haematocrit, G accounts for the fact that the haematocrit is highly variable in an individual animal, according to the physiological state, and between individuals and species. Therefore, it is useful to single out the haematocrit, h, as a separate variable in the report of blood O_2 kinetics. The same objective would be attained by considering G for a single cell, g (see above), with the RBC count as a measured parameter.

Abdalla *et al.* (1982) have estimated the total volume of RBCs in chicken pulmonary capillaries as $Q_c \times h = 2{\cdot}2\,cm^3$. Using the estimate of the maximum D_{tot} for the chicken lung of Scheid & Piiper (1970), 67 μmol min^{-1} Torr^{-1}, in equation 6 yields an estimate for D_m of about 74 μmol min^{-1} Torr^{-1} compared with the blood conductance, $Q_c \times h \times G = 730\,\mu$mol min^{-1} Torr^{-1}. These estimates suggest that the gas/blood membrane of the parabronchial air capillaries offers a sizeably larger resistance to O_2 transfer than does the blood. For the duck, $Q_c \times h = 2{\cdot}2\,cm^3$ has been measured by Maina & King (1982), and D_{tot} about 100 μmol min^{-1} Torr^{-1} by Burger *et al.* (1979), yielding an estimate of 550 for $Q_c \times h \times G$ and of 120 μmol min^{-1} Torr^{-1} for D_m. Again, the major resistance appears to reside in the membrane. These results are in contrast to the conclusion of the morphometrists (Abdalla *et al.* 1982; Maina, 1984). It should, however, be appreciated that morphometric and physiological techniques do not necessarily measure the same functional parameters. The morphometric estimates of diffusing capacity are based on functional parameters, like Krogh's diffusion constant for O_2 in lung tissue and blood O_2 transfer kinetics which are unknown or were unknown to Maina & King (1984) and to Maina (1982) at the time of their study. On the other hand, measurements of D_{tot} are influenced by 'functional inhomogeneities' in the lungs (e.g. unequal distribution of ventilation to perfusion). If not taken into account, the inhomogeneities lead to an apparently decreased D_{tot}, as shown for avian lung models by Burger *et al.* (1979). Thus the calculated high $Q_c \times h \times G/D_m$ ratios may be overestimated due to underestimation of D_{tot}.

The most important result of the present study is that the O_2 uptake and release kinetics of chicken and duck RBCs is similar to that of human RBCs. As in mammals

the O_2 kinetics of avian RBCs appear to be size-dependent. For the single cell, the O_2 transfer conductance is smaller for the chicken than for the duck red cell, and this difference is expected since the ratio surface area/radius, which is important for O_2 diffusion in the cell, is smaller for the chicken RBC. For whole blood, the specific O_2 transfer conductance (i.e. O_2 conductance per unit RBC volume) is larger in the chicken than in the duck, and this is because the larger number of small cells in blood of a given haematocrit more than compensates for the smaller conductance of the single cell.

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