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# OXYGENATION AND DEOXYGENATION KINETICS OF RED CELLS IN ISOLATED LAMELLAE OF FISH GILLS

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SUMMARY

1. The kinetics of oxygen transfer across the water–blood barrier in fish gills were studied by measuring oxygenation and deoxygenation of single red blood cells contained within isolated secondary lamellae using a microphotometric technique.

2. Recordings of the overall time courses show that both oxygenation and deoxygenation are more rapid in the carp than in the eel. In both species the half-time for deoxygenation is about five times slower than for oxygenation.

3. It is shown that the resistances to oxygenation and deoxygenation are identical; the differences in the shapes of the $O_2$ *v.* time curves recorded being attributable to the influence of the $HbO_2$ dissociation curve upon the driving force for diffusion.

4. It is concluded that a diffusional as opposed to a chemical reaction resistance provides the main barrier to oxygen transfer in gill secondary lamellae.

## INTRODUCTION

Kinetic measurements of the association of oxygen with haemoglobin *in vitro* have shown that this reaction is so rapid that oxygenation of a red cell should be complete long before the cell has resided in the pulmonary capillary for its normal sojourn (Roughton, 1964). Dissociation is also rapid (Roughton, 1964). It can thus be argued that the rates of oxygenation and deoxygenation of the blood are not limited by the reaction between oxygen and haemoglobin, but by diffusion.

The oxygenation of human and avian red cells, *in vivo*, has been shown to be five to ten-fold faster than deoxygenation, (Mochizuki, Tazawa & Ono, 1973). This difference may be explained by the non-linear nature of the oxyhaemoglobin dissociation curve (Tazawa, Ono & Mochizuki, 1976).

In this paper we examine the *in vitro* oxygenation kinetics of red cells in the secondary lamellae of gills of carp and eel using the technique employed by Mochizuki *et al.* (1973) and developed by Ono & Tazawa (1975). The technique measures the oxygen

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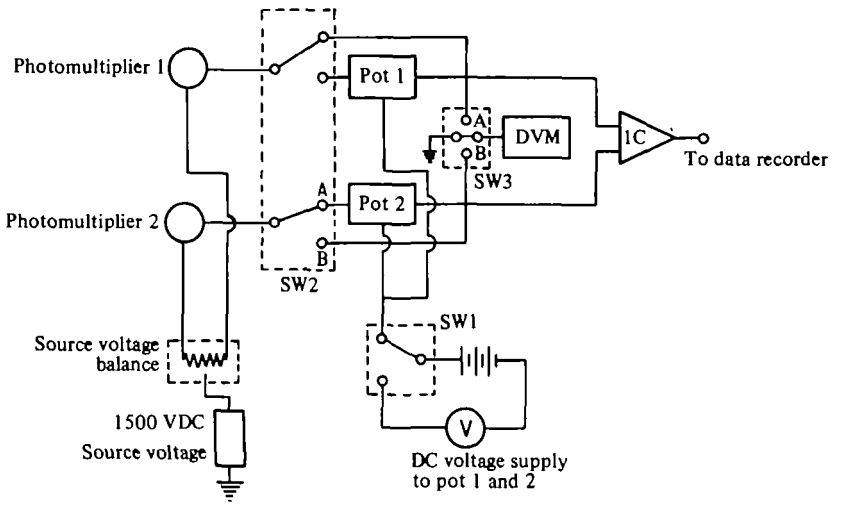


Fig. 1. Diagram of electric circuit for the microphotometric apparatus. Light passes to two photomultipliers (PMT) via interference filters (599 and 572 $\mu$m). Sensitivities of the two PMTs are equalized by controlling the photomultiplier source voltage using Pot 1 and Pot 2 and SW3 to measure voltages on the digital voltmeter (DVM). SW2 remains in either position A or B. For efficient detection of voltage changes when the blood cell is exposed to different gas mixtures, output signals must be well balanced by equalizing the pedestal voltages of the two PMTs by setting SW3 to positions A and B alternatively. Pot 2 is effective when SW3 is in position A. The output of PMT1 shown on the DVM is made equal to that of PMT2 by turning Pot 2. If it is insufficient, SW2 must be set to B and Pot 1 can then be used to equalize the signals. After completion of these procedures SW3 must be set to position C, the balanced output signal i.e. zero volts is amplified by the integrated amplifier (IC).

associated with haemoglobin by measuring changes in the absorption of transmitted light by the pigment, and can easily be applied to the secondary lamellae. A preliminary report has been published (Hughes & Koyama, 1974). An interesting by-product of the present analysis has been a method for estimating the thickness of the water–blood barrier which is far more rapid than that described by Hughes & Perry (1976) and Hughes (1972).

## MATERIALS AND METHODS

### *Preparation of secondary lamellae*

Four common carp (*Cyprinus carpio*) and four Japanese eels (*Anguilla japonica*) weighing 200–500 g were obtained from commercial dealers. Fish were confined in a part of an aquarium and pieces of gill filament less than 1 mm in length were cut from the filament tips. These small pieces of filament were placed in saline on a microscope slide and individual secondary lamellae isolated by means of a micro-scalpel. (In the carp and eel there are respectively about 19 and 17 secondary lamellae/mm on each side of each gill filament.) Several isolated lamellae were placed in a small region of the cuvette cover glass, moistened with a 1% solution of glycerine. About five other droplets of this solution were placed in the cuvette and served to maintain the humidity of the moist chamber.

*Experimental procedure*

The method for following the oxygenation state of a red cell was essentially that described by Ono & Tazawa (1975). The reaction cuvette with the mounted secondary lamella was placed on the microscope stage and a single red blood cell was focused upon (magnification ×400). The red cells in these fish are relatively large (carp 14 × 8 $\mu$m, eel 13·7 × 9·7 $\mu$m) and filled most of the field. Care was taken to include as little of the nucleus as possible. A period of a few minutes was then allowed for output from the photomultiplier (see below) to become stable before an experiment. Gas mixtures were delivered from syringes and were passed over the preparation as described by Mochizuki *et al.* (1973). Operation of a syringe resulted in an immediate change in the composition of the gas surrounding the lamellae. Rate of gas flow was determined from the rate of fall of the syringe plunger. Controls showed that the passage of gas across the preparation had no effect when the gas composition remained the same. Since each secondary lamella had one side fixed to the slide, gas exchange could only occur from the exposed surface of the secondary lamella.

Experiments were carried out at room temperature (20 °C). Composition of the gas mixtures was determined using a 0·5 ml Scholander gas analyser. The results reported in this study refer to normal air (0·03 % $CO_2$), but experiments carried out with 2·2 %, 5·75 % and 12·6 % $CO_2$ showed no significant difference.

*Apparatus*

Light transmitted through the red cell was passed through interference filters to two photomultipliers as described by Mochizuki, *et al.* (1973) and Ono & Tazawa (1975). One photomultiplier monitored light absorbed by reduced haemoglobin, at a wavelength of 572 nm. The other monitored absorption by oxyhaemoglobin, at 599 nm. Differences in the output of these two photomultipliers give a measure of the $O_2$ saturation of the haemoglobin in a single red blood cell. Because of the small changes involved, it was essential to ensure adequate balance in the two photomultipliers. The electric circuit is shown schematically in Fig. 1.

## RESULTS

Experiments were carried out using 24 red blood cells in 7 secondary lamellae from eels, and 30 cells from 6 lamellae of carp. Both oxygenation and deoxygenation were found to be faster in the carp than in the eels. Oxygenation was appreciably faster than deoxygenation in both species. The mean half-time for deoxygenation in carp was 1·35 s (S.D. 0·36) compared with 0·32 s (S.D. 0·06) for the same 30 red blood cells. In the 24 samples from the eels, the mean half-time for deoxygenation was 3·1 s (S.D. 1·3) compared with 0·50 s (S.D. 0·24) for oxygenation to a $P_{O_2}$ of 155 mmHg at a $P_{CO_2}$ of 0·03 mmHg and 20 °C. Thus oxygenation of the *in situ* red cell was faster than deoxygenation by a factor of 4·5 for carp and 6·2 for eel. Typical time courses for the elimination and uptake of oxygen by the red cell in the secondary lamella are shown in Fig. 2 for carp and Fig. 3 for eel.

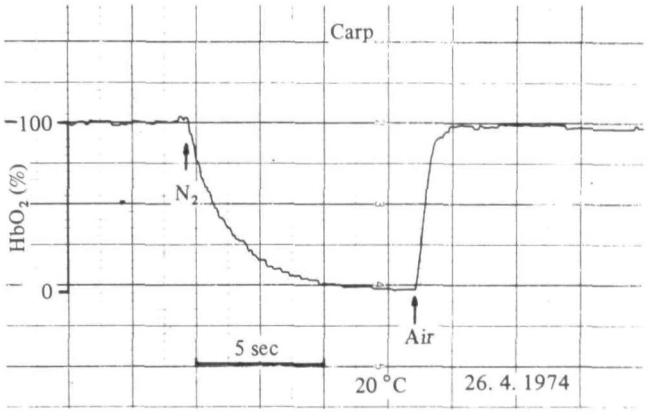


Fig. 2. *Cyprinus carpio*: recording of time course for deoxygenation and oxygenation of single red blood cell in a gill secondary lamella.

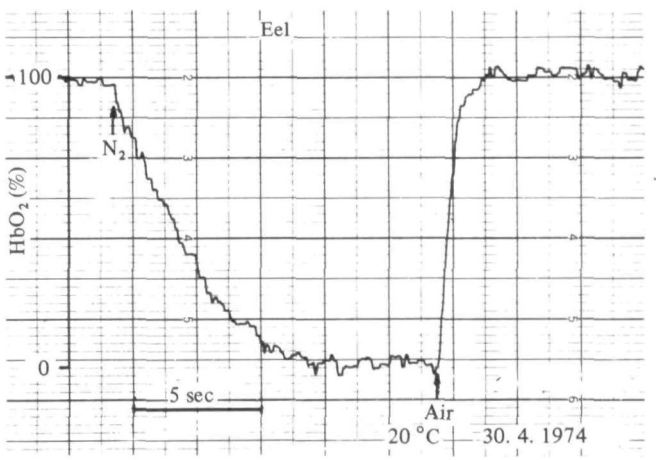


Fig. 3. *Anguilla japonica*: recording of time course for deoxygenation and oxygenation of single red blood cell in a gill secondary lamella.

*Analysis of data*

The oxyhaemoglobin dissociation curve shows a steeper gradient at lower $P_{O_2}$, so a given rise in blood $P_{O_2}$ involves more oxygen than an equivalent fall in $P_{O_2}$. Quantitative allowance for this inherent asymmetry has been made by incorporating a Bohr integration into an analysis in which all resistance is diffusional and is the same in both directions. This can be described mathematically by a very simple model in which uptake and elimination are reduced to a common basis by defining an oxygen exchange integral ($I$) in applying the Bohr integration to both situations as follows.

The Fick diffusion equation can be expressed (Comroe, 1962) in the following modified form:

$$\Delta S_c = K(P_0 - P_c)\,\Delta t, \qquad (1)$$

Where $\Delta S_c$ is the increase in $O_2$ saturation in the time element ($\Delta t$) during uptake

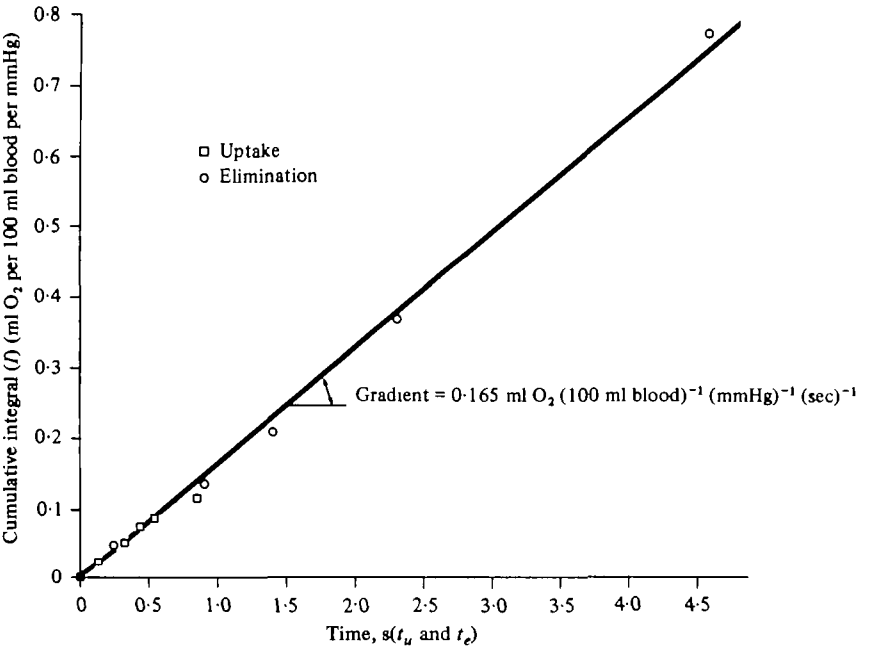


Fig. 4. A typical experiment from which the integral ($I$) for oxygen transfer in an eal gill secondary lamella is plotted against the uptake time ($t_u$) for oxygenation of a red cell *in situ* and elimination time ($t_e$) for deoxygenation. $I$ essentially represents the $O_2$ flux per unit driving force and thus allows for the shape of the $HbO_2$ dissociation curve as seen by its definition – equation (2). This expression is used to determine the points shown by simple graphical integration of experimental data. The gradient represents the overall resistance to oxygen transfer which therefore appears to be the same for uptake as for elimination.

$K$ is the proportionality constant for diffusive conductance of oxygen between a gas phase at an $O_2$ partial pressure of $P_0$ and a red cell at $P_c$ as estimated from $S_{O_2}$ using the appropriate blood $O_2$ dissociation curve.

Summation over the entire exchange period ($t$) enables us to define the oxygen exchange integral $I$ as:

$$I = \Sigma \frac{S}{(P_0 - P_c)}, \tag{2}$$

where $t$ can be the uptake period ($t_u$) or elimination period ($t_e$) provided $I$ is computed according to its mathematical definition. Thus:

$$I = K.t. \tag{3}$$

$I$ has been evaluated according to equation 2 using the very convenient technique of graphical integration. When the values of $I$ computed from uptake data and elimination data are plotted against $t_u$ and $t_e$ respectively, it can be seen in Fig. 4 that:

1. Both plots are straight lines, confirming that $K$ is a constant in equation 3 and;
2. $K$ is the same for both uptake and elimination, i.e. for both oxygenation and deoxygenation.

Fig. 4 refers to one 'typical' run, but the above relationships were found to hold for all samples of both carp and eel gills.

## DISCUSSION

The time courses of oxygenation and deoxygenation of the red cell in the secondary lamellar channels of the carp and eel closely resemble those reported for human and avian red blood cells (Mochizuki *et al.* 1973). Moreover, the half-times for deoxygenation exceed those for oxygenation by similar ratios, calculated as 4·5 for carp and 6·2 for the eel. These similarities between warm and cold-blooded animals support the view that delays imposed by chemical kinetics are not so important.

Analysis of the data indicates that both uptake and elimination are limited by diffusion, for the integral ($I$) is linearly related to uptake time and elimination time. Rate of elimination was the same as for uptake.

The analysis can also explain the more pronounced shoulder in the uptake curve compared with that for elimination. This can be appreciated from the manner in which $I$ for uptake does not change greatly after haemoglobin has exceeded a $P_{O_2}$ of about 50–60 mmHg, while $I$ for elimination continues to increase at a steadier rate as the $P_{O_2}$ approaches 0.

### *The diffusion barrier*

In Fig. 4 it can be seen that data for both uptake and elimination in the eel give the value of 0·165 ml $O_2$ (100 ml blood)$^{-1}$ (mmHg.)$^{-1}$ (s)$^{-1}$ for the gradient of $I$ *v.* $t$, giving:

$$K = 1{\cdot}65 \times 10^{-3} \text{ ml } O_2 \text{ ml}^{-1} \text{ (mmHg)}^{-1} \text{ (s)}^{-1}. \quad (4)$$

If we regard the diffusion barrier simply as a flat sheet of area $A$ and thickness $x$, then, under steady-state conditions, the rate of transfer of oxygen to blood ($\dot{q}$) is given by Fick's law of diffusion as:

$$\dot{q} = \alpha D A(P_0 - P_c)/x, \quad (5)$$

where $\alpha$ is the solubility of oxygen in tissue and $D$ is the diffusion coefficient. $\dot{q}$ also determines the rate of saturation ($\Delta S_c/\Delta t$) of a volume ($V$) as:

$$\dot{q} = V(\Delta S_c/\Delta t). \quad (6)$$

Substituting $V = Ax$ and eliminating ($\Delta S_c/\Delta t$) and $\dot{q}$ from equations 1, 5 and 6 gives:

$$x^2 = \alpha D/K. \quad (7)$$

Substituting for $K$ according to equation 4 and the following values for $\alpha$ and $D$:

$$D = 10^{-5} \text{ cm}^2 \text{ s}^{-1}$$
$$\alpha = 4{\cdot}6 \times 10^{-5} \text{ ml. } O_2 \text{ (ml)}^{-1} \text{ (mmHg)}^{-1},$$

for oxygen in water at 20 °C, equation 7 gives

$$x = 5{\cdot}3 \ \mu\text{m}.$$

This estimate of the effective thickness of the diffusion barrier is close to the value of 6 $\mu$m estimated morphometrically for the eel (Steen & Berg, 1966).

Thus, not only does microphotometry of the *in situ* red cell confirm that th

limitation to oxygenation and deoxygenation is imposed by a physical diffusion barrier, but it provides a particularly useful means of quantifying the water-to-blood barrier in the gill or air-to-blood barrier in the lung. Measurements may be carried out relatively easily and on tissues soon after their removal from the whole animal. The technique would be useful for comparative studies between different species and between individuals living under varying conditions which might affect the barrier to gas transfer as a result of environmental influences, including the use of pollutants. Such a method would be far less laborious than those involving morphometry (Hughes, 1976); although consideration must be given to the cost and technical problems, including the involvement of complex electronics.

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