The Active Transport of Oxygen and Carbon Dioxide into the Swim-Bladder of Fish

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ABSTRACT The active transport of oxygen and carbon dioxide into the swim-bladder of fish is discussed. The rete mirabile is a capillary network which is involved in the gas secretion into the bladder. The rete is regarded as a counter-current multiplier. Lactic acid which is produced in the gas gland generates in the rete single concentrating effects for oxygen and carbon dioxide; i.e., for equal partial pressures the concentrations of the gases in the afferent rete capillaries are higher than those in the efferent ones. The single concentrating effects were calculated from measurements of sea robin blood (Root, 1931). The multiplication of these effects within the rete for different rete lengths and different transport rates was numerically evaluated. The calculated O_2 and CO_2 pressures in the bladder are in good agreement with the experimental results of Scholander and van Dam (1953). The descent velocities at equilibrium between bladder pressure and hydrostatic pressure are discussed for fishes with different rete lengths.

INTRODUCTION

During the experiments with their bathyscaphe *Pérès*, Picard, and Ruivo (1958) at a depth of 2200 m observed fishes (*Halosaurus johnsonii*) which floated. These fishes have therefore the same density as the surrounding water. The density of a fish without a gas-filled swim-bladder is practically independent of depth and it is a few percent higher than the density of the surrounding water (Denton and Marshall, 1958). A fish with a gas-filled and closed bladder, however, is as a Cartesian diver at a depth in an unstable equilibrium of floating. According to Borelli (1679) fishes should control the volume of the swim-bladder by muscular activity. But this view is in contrast to several experimental findings (Moreau, 1876; Jacobs, 1930, 1932, 1940). Morphological studies (Jaeger, 1903; Marshall, 1960) showed that the rete mirabile (Delaroche, 1809; Woodland, 1911; Krogh, 1922; Scholander, 1954; Wittenberg et al., 1964), the gas gland (Müller, 1840), and the oval (Winterstein, 1921; Ledebur, 1929) of the bladder are organs which may be involved in the secretion (Bohr, 1894; Jacobs, 1932) and the resorption (Moreau,

1877; Franz, 1937) of gas. The exact mechanism of the regulation of the bladder volume has been, until now, not known (Rauther, 1923; Meesters and Nagel, 1934; Jacobs, 1940; Fänge, 1953).

The gas concentrations in the sea water are practically independent of depth and the partial pressures of the dissolved gases in the sea water are close to the equilibrium values at the surface. The gases are actively transported from low partial pressures in the sea water to high partial pressures in the swim-bladder (Biot, 1807; Hüfner, 1892; Schloesing and Richard, 1898; Saunders, 1953; Tait, 1956; Scholander et al., 1956; Wittenberg, 1958 and 1961). For the generation and maintenance of the gas pressures in the swim-bladder free energy must be supplied and theories which postulate a mere exchange between blood and swim-bladder fail to describe the facts (Dela-roche, 1809; Hüfner, 1892). The rete mirabile whose highly ordered structure consists of many arterial and venous capillaries in close diffusion contact was early considered as an organ with a high exchange capacity. But a pressure gradient is only built up and maintained within the rete, if a single concentrating effect exists between the efferent and afferent capillaries. In the case of oxygen reversible single concentrating effects are the Bohr and the Root effect (Scholander and van Dam, 1954; Green and Root, 1933), e.g. a lowering of the oxygen concentration in the blood at constant oxygen partial pressure with increasing acid content; in the case of carbon dioxide a shift of the bicarbonate equilibrium with pH changes. For all dissolved gases (O_2 , CO_2 , N_2 , A) the salting-out effect (Koch, 1934) or the Steen effect (Steen, 1963 *a*) enables an active secretion into the bladder. But, with oxygen bubbles nitrogen and argon may be passively transported into the bladder (Wyman et al., 1952; Wittenberg, 1958). During the gas secretion an acid (Hall, 1924), e.g. lactic acid (Ball et al., 1955; H. J. Kuhn et al., 1962; Steen, 1962), is produced within the gas gland thus generating a single concentrating effect between the afferent and the efferent capillaries, which may be multiplied in the rete (Woodland, 1911; Haldane, 1922; Hall, 1924; Jacobs, 1930; Scholander and van Dam, 1954; W. Kuhn et al., 1964). The theory of the multiplication of the single concentrating effect in the rete is related to the theory of counter-current multiplication (W. Kuhn and Ryffel, 1942; Scholander, 1954).

COUNTERCURRENT MULTIPLICATION OF OXYGEN AND CARBON DIOXIDE

The striking structure of the rete led to the idea of a countercurrent exchanger. In short we can say that the blood derived from the a.coeliaca arrives through the a.ductus pneumatici at the rete. Here the artery splits into a large number of parallel capillaries, the afferent capillaries of the rete. Then the capillaries are assembled again leading the blood to the gas gland capillaries in the bladder wall. Another capillary network coating the interior surface of the

wall is built up. The gas gland capillaries are in close diffusion contact with the inside swim-bladder wall and enable a fast gas exchange between blood and bladder. The blood runs back from the gas gland to the rete. Here the vein splits into parallel capillaries and forms with the arterial ones a checker-board pattern (Krogh, 1922). The venous capillaries assemble again into a vein which is connected with the portal vein leading the blood to the liver.

The function of the rete as a countercurrent multiplier (Hargitay and W. Kuhn, 1951) is shown schematically in Fig. 1. The afferent channel S_a and the efferent channel S_e , each of height a , length L , and width b , correspond to an afferent and an efferent limb of the rete capillary system. The channels are separated by a membrane, M , which is assumed to be nonpermeable to all substances in the blood with the exception of the dissolved gases. Of course, real membranes do not exhibit this behavior (Steen, 1963 *b*), but

FIGURE 1. Schematic representation of the rete as a countercurrent multiplier (details in text).

practically the assumption of a specific gas permeability does not affect the gas secretion into the bladder (W. Kuhn et al., 1963). In Fig. 1 G designates the gas gland capillaries which are separated from the swim-bladder B by a membrane M' . Although inert gases, e.g. nitrogen and argon, are in reality secreted into the bladder, we assume in this paper that the membrane M' is only permeable for oxygen and carbon dioxide. The diffusion resistance of these gases (O_2 , CO_2) in the membrane M' is neglected. The total gas pressure P in the bladder is the sum of the partial pressures P_{O_2} and P_{CO_2} . Lactic acid, A , is produced in the gas gland, and is added to the blood of the gas gland capillaries. The mean velocities of the blood in the afferent and efferent channels are u_a and u_e . $P_{a,x}^{O_2}$ and $P_{a,x}^{CO_2}$ are the partial pressures of O_2 and CO_2 in the afferent channel at the length x of the rete (Fig. 1); $C_{a,x}^{O_2}$ and $C_{a,x}^{CO_2}$ are the corresponding concentrations of these gases at equilibrium with the given partial pressures.

It is well known that the reaction $H_2CO_3 \rightleftharpoons H_2O + CO_2$ is slow (Faurholt,

1924; Meier and Schwarzenbach, 1957), and that this reaction is catalyzed by carbonic anhydrase (Meldrum and Roughton, 1933; De Voe and Kistia-kowsky, 1961). High carbonic anhydrase concentrations in the gas gland and the rete (Leiner, 1940) support the assumption of chemical equilibria for carbon dioxide and water. In a steady state conservation of oxygen leads to a relation between the O_2 mass which is transported with the blood and the O_2 mass which diffuses through the membrane M . With several approximations outlined in earlier papers [mean velocity of blood flow (Kuhn and Ryffel, 1942), mean permeation Γ_{O_2} (Marti, 1963; W. Kuhn et al., 1964), and the back diffusion (Kuhn and Kuhn, 1961)] one obtains for the O_2 concentrations and the O_2 partial pressures in both channels:

$$u_a ab \frac{\partial c_{a,x}^{O_2}}{\partial x} = \Gamma_{O_2} \frac{b}{a} [P_{e,x}^{O_2} - P_{a,x}^{O_2}] \quad (1)$$

$$u_e ab \frac{\partial c_{e,x}^{O_2}}{\partial x} = -\Gamma_{O_2} \frac{b}{a} [P_{e,x}^{O_2} - P_{a,x}^{O_2}] \quad (2)$$

For the carbon dioxide pressures adequate equations exist:

$$u_a ab \frac{\partial c_{a,x}^{CO_2}}{\partial x} = \Gamma_{CO_2} \frac{b}{a} [P_{e,x}^{CO_2} - P_{a,x}^{CO_2}] \quad (3)$$

$$u_e ab \frac{\partial c_{e,x}^{CO_2}}{\partial x} = -\Gamma_{CO_2} \frac{b}{a} [P_{e,x}^{CO_2} - P_{a,x}^{CO_2}] \quad (4)$$

Because the whole blood transport is reversed within the gas gland capillaries, the velocities of the blood flow in the afferent and efferent channels are of equal amount but of opposite direction:

$$u_e = -u_a \quad (5)$$

The membrane M (Fig. 1) between the channels is nonpermeable for the blood. Therefore the velocities u_a and u_e are independent of x . We easily obtain two solutions of the four differential equations (1) to (4): Addition of equations (1) and (2) and subsequent integration lead with (5) to

$$u_a ab (c_{a,x}^{O_2} - c_{e,x}^{O_2}) = E_{O_2} = \text{constant.} \quad (6)$$

E_{O_2} is called the transport rate for oxygen; it describes the moles of oxygen which are transported per second through both channels in the direction of x (Fig. 1). By analogy we get for carbon dioxide

$$u_a ab (c_{a,x}^{CO_2} - c_{e,x}^{CO_2}) = E_{CO_2} = \text{constant.} \quad (7)$$

For the further discussion of the unsolved equations (1) and (3) we consider

the transport rates and the concentrations in the afferent channels as independent quantities and assume a blood transport $\dot{q} = u_{ab}$ of 0.5 ml/hr (Steen, 1963 *b*). For a solution of (1) and (3) we must also know the O_2 and CO_2 partial pressures as a function of their concentrations. The relationships are complicated for any blood. We have, therefore, chosen a defined blood, the blood of sea robin for which numerical data were given by Root (1931).

FIGURE 2. Three dimensional representations of the oxygen concentrations (C^{O_2} , in volume per cent) in the blood of sea robin as a function of the oxygen partial pressure, $\log P^{O_2}$, and the carbon dioxide partial pressure, $\log P^{CO_2}$. The O_2 concentration in the afferent capillaries of the rete corresponds to the surface through the top of the sheets. The surface through the black lines on the sheets gives the O_2 concentration in the efferent capillaries.

Figs. 2 and 3 summarize and extrapolate the experimental values of Root. In Fig. 2 the oxygen concentrations in the afferent capillaries (surface through the top of the white sheets) and in the efferent capillaries (surface through the black lines on the white sheets) are represented as a function of the partial pressures of oxygen and carbon dioxide. The total content of oxygen in 1 ml of blood is the sum of the physically dissolved oxygen and of the oxygen bound to the hemoglobin. It is assumed that the solubility coefficient does not depend on the O_2 partial pressures nor on the lactic acid concentration. In the sea robin blood a marked Root effect is observed: the O_2 capacity of this

FIGURE 3. Three dimensional representations of the carbon dioxide concentrations (C^{CO_2} , in volume per cent) in the blood of sea robin as a function of the oxygen and carbon dioxide partial pressures on a logarithmic scale. The CO_2 concentration in the afferent capillaries of the rete corresponds to the surface through the top of the sheets. The surface through the black lines on the sheets gives the CO_2 concentration in the efferent capillaries.

blood diminishes if an acid, lactic acid, or carbon dioxide, is added to the blood. The O_2 concentration surface which is observed at an elevated lactic acid concentration ($\Delta c^A = 2 \mu M/ml$) is represented in Fig. 2 by the black lines drawn on the white sheets. This lactic acid shift was observed between efferent and afferent capillaries in vitro (Hall, 1924; Ball et al., 1955) and in vivo (H. J. Kuhn et al., 1962; Steen, 1963 *b*). Also shown in Fig. 2 are three lines of constant oxygen concentrations for $c^{O_2} = 5, 10$, and 15 vol. % within the original blood.

In Fig. 3 the CO_2 concentration surfaces for blood of the sea robin are presented (Root, 1931). As independent variables the gas partial pressures P^{O_2} and P^{CO_2} are given on a logarithmic scale. The CO_2 concentration surface for the original blood goes through the top of the sheets. The black lines on the sheets represent the CO_2 concentration of the acidic blood ($\Delta c^A = 2 \mu M/ml$) at varying O_2 partial pressures for constant CO_2 partial pressures. The surfaces are based on measurements by Root of the total CO_2 concentration of reduced and oxygenated blood and were evolved from the O_2 concentration surface by an application of thermodynamic Maxwell relations. The total CO_2 content in the blood is the sum of the physically dissolved carbon dioxide of the bicarbonate ions and of the carbaminic acid. By an elevation of the lactic acid concentration the H_2CO_3/HCO_3^- equilibrium is shifted to carbonic acid.

The relations (1) and (3) between the gas concentrations in the afferent capillaries of the rete and the partial pressure difference over the two capillaries are now discussed with the two c^{O_2} surfaces (Fig. 2) and the two c^{CO_2} surfaces (Fig. 3). First we divide equation (1) by (3):

$$\frac{dc_{a,x}^{O_2}}{dc_{a,x}^{CO_2}} = \frac{\Gamma_{O_2}}{\Gamma_{CO_2}} \frac{P_{e,x}^{O_2} - P_{a,x}^{O_2}}{P_{e,x}^{CO_2} - P_{a,x}^{CO_2}} \quad (8)$$

For animal tissue the quotient $\Gamma_{O_2}/\Gamma_{CO_2}$ has the mean value 0.03 (Krogh, 1919).

The initial point of a solution curve for equation (8) is given by the mean values $P_{a,o}^{O_2} = 150$ mm Hg, $P_{a,o}^{CO_2} = 1$ mm Hg (Denton, 1961). In the steady end state, i.e. for vanishing transport rates, the O_2 and CO_2 concentrations are equal in both channels (equations 6 and 7). The solutions of $c_{e,o}^{O_2} = c_{a,o}^{O_2}$, $c_{e,o}^{CO_2} = c_{a,o}^{CO_2}$ give the initial partial pressures in the afferent channel ($P_{a,o}^{O_2} = 310$ mm Hg, $P_{a,o}^{CO_2} = 1.3$ mm Hg) and we obtain at the afferent entrance of the rete $dc_{a,o}^{O_2}/dc_{a,o}^{CO_2} = 15.2$. The integration of equation (8) is accomplished with the polygon course approximation. For the steady end state the solution curve of equation (8) is shown in Fig. 3 as a black line on the CO_2 surface.

Transcription of equation (1) and integration lead to

$$\int_{c_{x=0}^{O_2}}^{c_{x=l}^{O_2}} \frac{dc_{a,x}^{O_2}}{P_{e,x}^{O_2} - P_{a,x}^{O_2}} = \frac{\Gamma_{O_2}}{ua^2} L \quad (9)$$

We are discussing retes of length 0.1 cm (Wittenberg et al., 1964), or 0.24 cm, of capillary radius $a = 5 \cdot 10^{-4}$ cm, and of a permeation $\Gamma_{O_2} = 3 \cdot 10^{-17}$ mole dyne $^{-1}$ s $^{-1}$ cm (Kuhn and Kuhn, 1961). The pressure differences are determined as a function of $c_{a,x}^{O_2}$ along the solution curves of equation (8) and the integration of equation (9) is performed numerically. By such methods we have determined the O_2 and CO_2 concentrations in the glandular capillaries

TABLE I
OXYGEN AND CARBON DIOXIDE PARTIAL PRESSURES IN THE SWIM-BLADDER OF SEA ROBIN, NUMERICALLY CALCULATED AT A STEADY STATE OF THE RETE, FOR DIFFERENT TRANSPORT RATES AND TWO RETE LENGTHS

E^{O_2}	E^{CO_2}	P^{O_2} mm Hg	P^{CO_2} mm Hg	P^{O_2} mm Hg	P^{CO_2} mm Hg
		Rete length 0.1 cm		Rete length 0.25 cm	
ml gas/hr	ml gas/hr				
0	0	23,000	23	120,000	340
0.1	0	13,000	19	92,000	250
0.2	0	9,000	17	45,000	280
0.3	0	250	2.5	1,300	190
0	0.12	17,000	8	110,000	15
0.15	0.06	10,000	13	40,000	85
0	0.24	23,000	3.7	>120,000	~4

The transport rates are given in milliliters of gas under normal conditions, i.e. at 0°C and 760 mm Hg.

and the corresponding O_2 and CO_2 partial pressures in the bladder, which are summarized in Table I for different transport rates.

DISCUSSION

It was mentioned before that the results presented in Table I are only applicable in the case of sea robin blood. The blood of other fish provides different rete functions which can be discussed in the same way that we have for the sea robin blood. We think that there will be only small fluctuations of the partial pressures for O_2 and CO_2 in the swim-bladder for the blood of different fish. It is known that mammalian blood exhibits a different behavior upon the addition of an acid. In spite of such differences it is an important support to the theory outlined in this paper that the countercurrent multiplication of CO_2 and O_2 in bovine hemoglobin solutions is predicted on the basis of the transport equations and of the concentration surfaces of these gases in such solutions (Lesslauer et al., 1966)

In the steady end state, i.e. for vanishing transport rates, the bladder of the sea robin is filled with practically pure oxygen. With a rete length of 0.1 cm (0.25 cm) the bladder contains only 0.1 (0.25) % carbon dioxide at a total pressure of 30 atm. (150). For *Sebastes marinus* caught by Scholander and van Dam (1953) at a depth of 440 m the carbon dioxide content was 0.4% of the oxygen. The same authors found for *Antimora viola* caught at 810 m 1.2% carbon dioxide. These findings provide support for our calculations.

Although the permeation of carbon dioxide is better by a factor of 35 than that of oxygen, it would be wrong to conclude that a high carbon dioxide partial pressure is built up in the rete. The partial pressures of the gases in the bladder can be predicted only after a comprehensive discussion of all the important features: morphological aspects of the rete, blood properties, regulation of the metabolism in the gas gland, regulation of the resorption bladder etc. The results in Table I show that with increasing transport rates the partial pressures in the bladder decrease. If in 1 hr 0.3 ml (normal conditions) oxygen and no carbon dioxide are transported through a rete of length $L = 0.1$ cm into the bladder, the oxygen partial pressure in the bladder is in a steady state, $P^{O_2} = 250$ mm Hg and the carbon dioxide partial pressure is $P^{CO_2} = 2.5$ mm Hg. The transport rate $E_{O_2} = 0.3$ ml/hr is near the oxygen transport rate for which the O_2 partial pressure in the bladder is equal to the O_2 partial pressure in the surrounding water.

High gas pressures in the bladder involve low rates of filling (Table I). A fish which lives at a depth of 110 m has a gas pressure of 12 atm. in the bladder. The gas is in our case practically pure oxygen because we do not take into consideration the inert gas secretion. Therefore, according to Table I and the depth of 110 m the filling rate is 0.2 ml per hour for a fish with a rete length $L = 0.1$ cm. A fish with a weight of 600 g has a bladder volume of about 30 cm³ (Denton, 1961). The time necessary to fill the bladder from the pressure of 1 atm. at the surface to 12 atm. at a depth of 110 m is 70 days. A change of 10 m from 110 to 120 m needs, at a constant bladder volume, a time of 6 days. Fishes with longer retes fill their bladders faster. According to equation (9) one obtains for a constant quotient, $\frac{L}{ua^2} \sim \frac{L}{\dot{q}}$, the same oxygen concentration $c_{x=L}^{O_2}$ at the vertex and therefore the same oxygen partial pressure in the bladder. The filling time for the bladder is reduced by the same factor as the rete length is increased if a constant $\frac{L}{\dot{q}}$ is assumed. Fishes with six retes of a length $L = 2$ cm (*Lionurus filicaudae*; Marshall, 1960) can fill their bladders by a factor of 120 times faster than fishes with one rete of a length $L = 0.1$ cm if the blood transport through each rete is raised by a factor of twenty. Fish of a certain species have the same rete length, but the diameter of the rete capillaries and, as a consequence, the blood transport

through the rete, can be regulated within a wide range (Jacobs, 1930). This mechanism enables the fish to regulate the gas pressure in the bladder and the transport rate into the bladder. For a complete filling of the bladder at a depth of 110 m a fish with six retes of a length $L = 2$ cm instead of one rete with $L = 0.1$ cm and with a blood transport in each rete $\dot{q} = 10$ ml/hr instead of $\dot{q} = 0.5$ ml/hr needs one-half day and for a change from 110 to 120 m only 72 min. These calculated filling times are quite sensible, but for an extremely short rete length ($L = 0.1$ cm) the times are rather long.

The descent velocity for which equilibrium between bladder pressure and hydrostatic pressure exists is 1.7 m per day for a fish with one rete of a length $L = 0.1$ cm and for a fish with six retes of a length $L = 2$ cm, 200 m per day. At higher descent velocities the increase of the mean density for a fish must be balanced by muscle work. By compression the bladder volume can only vanish and the mean density of a descent for a fish can at most increase by 5%. However, a fish which rises at high velocities would inflate, if it has no organ of gas resorption. With the oval and the resorption bladder and with the regulation of secretion and resorption of gas fish are capable of ascending with high velocities.

On the other hand the leakage of the gas through the wall of the secretion bladder must be low. A bladder of 30 ml volume may have a surface of about 60 cm². At a depth of 110 m the O₂ partial pressure difference over the bladder wall (thickness 0.1 cm) is about 12 atm. If the permeation of the bladder wall is $\Gamma_{O_2} = 3 \cdot 10^{-17}$ mole dyne⁻¹s⁻¹ cm, i.e. the mean value of ordinary tissue, an oxygen loss of 17.5 ml (normal conditions) per hour can be calculated. But a fish with a rete length of 0.1 cm at this depth can transport only 0.2 ml O₂ per hr into the bladder, and therefore the permeation $\Gamma_{O_2}^w$ of the bladder wall must be lower than Γ_{O_2} . A fast filling of the bladder demands smaller diffusion losses than the transport rate E^{O_2} . We may assume that the diffusion losses are only 0.5% of the gas transport E^{O_2} , i.e. 10⁻³ ml O₂ (normal condition) per hr. In this case the permeation of the bladder wall must be $\Gamma_{O_2}^w = \Gamma_{O_2} \cdot \frac{10^{-3}}{17.5} = 1.7 \cdot 10^{-21}$ mole dyne⁻¹s⁻¹ cm. We do not know any experimental data for the permeation of the swim-bladder wall. Polyvinylidene chloride is a synthetic foil with a very low permeation $\Gamma_{O_2} = 1.8 \cdot 10^{-21}$ mole dyne⁻¹s⁻¹ cm (Nitsche and Wolf, 1962). From this point of view it would be interesting to know from experimentation the gas permeation of the bladder wall.

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