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SOLUBILITY OF NITROGEN AND ARGON IN EEL WHOLE BLOOD AND ITS RELATIONSHIP TO pH

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SUMMARY

The solubility coefficients (α) for the inert gases, nitrogen (N_2) and argon (Ar), were measured by mass spectrometry in whole blood of the freshwater-adapted European eel, *Anguilla anguilla*, at varied pH and at two temperatures, 5 and 15 °C. The pH was altered either by varying P_{CO_2} (0·75–75 mmHg; 1 mmHg = 133·3 Pa) or by adding fixed acid (HCl or lactic acid).

No dependence of α on pH (range 5·5–8·4) or on lactate concentration (range 0·2–25 mmol l^{-1}) was detectable. Average values (±S.D.) for α (μmol l^{-1} $mmHg^{-1}$) were: $\alpha_{N_2} = 1{\cdot}25 \pm 0{\cdot}01$, $\alpha_{Ar} = 2{\cdot}60 \pm 0{\cdot}05$ at 5 °C and $\alpha_{N_2} = 1{\cdot}09 \pm 0{\cdot}03$, $\alpha_{Ar} = 2{\cdot}12 \pm 0{\cdot}07$ at 15 °C. These data yield values for Q_{10} of 0·87 for nitrogen and 0·82 for argon, and for activation energy, E_a (kJ mol^{-1} K^{-1}), of −9·2 for nitrogen and −13·4 for argon. The results do not support earlier reports on significant pH dependence of α in eel blood and suggest, in contrast, that no fundamental differences exist in respect of inert gas solubility between whole blood of the eel and of other vertebrates.

INTRODUCTION

High inert gas pressures have been found in the swim bladder gas of a number of teleost species caught from deep sea (Alexander, 1966; Wittenberg, Wittenberg & Wittenberg, 1981). Since gas partial pressures in the sea increase little with depth (Enns, Scholander & Bradstreet, 1965), much attention has been paid to the gas concentrating mechanisms. It is generally assumed that gas transfer into the swim bladder occurs passively along a partial pressure gradient, and it appears that the counter-current arrangement of the capillaries in the rete mirabile of the swim bladder plays a central role in achieving high gas partial pressures in the swim bladder vessels (see Fänge, 1983). In the swim bladder tissue lactic acid is produced and released into the blood, even at high O_2 levels. An increase in P_{O_2} occurs by way of the Root effect (Root, 1931; Bridges, Hlastala, Riepl & Scheid, 1983), and an increase in the partial pressure of any gas, such as inert gases, by the salting-out effect which then results in high gas partial pressures in the counter-current flow of the rete (Kuhn & Kuhn, 1961; Kuhn, Ramel, Kuhn & Marti, 1963; Steen, 1963*b*).

Key words: fish, swim bladder, salting-out effect, solubility.

Steen (1963*a*) presented data to suggest that reduction of pH from about 7·8 to about 7·6 leads to a sharp decline in the solubility of nitrogen (N_2) and argon (Ar) in eel whole blood at 6·5 °C, an effect which he did not observe in trout or cod blood. A 4 % reduction in N_2 solubility per unit of pH was also found by Abernethy (1972) in whitefish, *Coregonus clupeaformis*, blood, but this reduction was rather continuous with pH. In contrast to these studies, Douglas (1967; cf. Gerth & Hemmingsen, 1982) found no influence of pH on N_2 solubility.

In view of these conflicting results, in this study we investigated the relationship of N_2 and Ar solubility to blood pH at two different temperatures in the European eel *Anguilla anguilla*. We also measured the influence on solubility of increases in blood lactate concentration, which were expected to occur in rete blood during gas secretion into the swim bladder.

MATERIALS AND METHODS

Animals and collection of blood

Specimens of the freshwater-adapted European eel (body mass 400–800 g) were purchased from a local supplier and kept in a freshwater aquarium at 12–14 °C. Following a strike on their heads, the animals were decerebrated and the spinal cord was removed. Blood was withdrawn *via* a catheter inserted in the bulbus arteriosus. Catheter tubing, syringes and glassware were heparinized [250 i.u. heparin ml^{-1} of fish Ringer, which contained (in mmol l^{-1}) NaCl, 124; KCl, 5; $MgSO_4$, 0·9; $CaCl_2$, 1·1; $NaHCO_3$, 10; and glucose, 5. A blood pool of 12–15 ml could be collected from one or two animals.

Analytical procedures

The extraction method of Meyer & Scheid (1980) was used to determine the solubility coefficients, α. In this method blood is equilibrated at known partial pressures of inert gases, and a sample is transferred to a gas-tight extraction vessel where it re-equilibrates with the gas phase, which is initially free of the inert gas. The partial pressure after re-equilibration, measured by mass spectrometry, allows calculation of α. The solubility was determined thus in blood over a wide range of pH, adjusted either by varying the P_{CO_2} of the equilibrating gas or by addition of fixed acid (HCl or lactic acid). Experiments with lactic acid allowed investigation of a possible dependence of α on lactate concentration.

Haematocrit was determined using a microhaematocrit centrifuge (M 1100, Compur, München, FRG) and pH by using electrodes (G 299, Radiometer, Copenhagen, Denmark). Haemoglobin concentration was measured spectrophotometrically in 50-μl blood samples according to the method described by Robin & Harley (1964). Whole blood samples of 100 μl were deproteinized (with perchloric acid) and neutralized for biochemical analysis. [Lactate] was measured according to Gutmann & Wahlefeld (1974), and [ATP] and [GTP] as described by Albers, Götz

& Hughes (1983). Plasma osmolality was determined with a semi-micro osmometer (Knauer, Bad Homburg, FRG). Chloride was determined coulometrically (CMT 10 chloride titrator, Radiometer, Copenhagen). Sodium was assayed with a flame photometer (Eppendorf, Hamburg, FRG).

Experimental protocol

A 1·5-ml sample from the blood pool was equilibrated for 25 min (tonometer model 273, IL, Paderno Dugano, Italy) with gas mixtures containing 20 % O_2, 0·3 % CO_2 in the test gas (N_2 or Ar). These mixtures were provided by precision gas-mixing pumps (Wösthoff, Bochum, FRG). These samples are referred to as untreated blood. For measurement of solubility in acidified blood over a wide pH range, blood was either equilibrated with gas mixtures containing CO_2 up to 10 % (20 % O_2, rest N_2 or Ar) or acidified by addition of HCl or lactic acid before equilibration with 20 % O_2, 0·3 % CO_2 in N_2 or Ar. For acidification, the 1·5-ml sample from the blood pool was centrifuged, and 5–30 % of the plasma was replaced by the same quantity of 0·1 mol l^{-1} HCl or 0·05 mol l^{-1} lactic acid (in fish Ringer) before resuspension.

At the end of equilibration, pH was measured in the untreated or acidified blood, and a 1·0-ml sample was transferred with a calibrated syringe into a gas-tight vessel (volume, 56·3 ml for N_2 and 104·4 ml for Ar) which had previously been flushed with a gas mixture that contained O_2 and CO_2 at the same concentration as the equilibrating mixture, but in which N_2 replaced Ar and *vice versa*. In the gas-tight vessel, which was rotated, blood was re-equilibrated for 15 min, at the end of which N_2 or Ar partial pressures were determined in the gas phase using a sensitive respiratory mass spectrometer (redesigned M3, Varian MAT; see Scheid, 1983). For calibration, a 1-ml sample of the equilibrating gas was injected into a separate, large gas-tight vessel (volume 2341·3 ml), flushed initially with the same gas mixture as the gas-tight blood vessel, and the partial pressure of N_2 or Ar measured with the mass spectrometer after 15 min of re-equilibration.

Equilibration and extraction in the gas-tight vessels were performed at a constant temperature of either 5 or 15 °C.

The solubility coefficient was calculated from the relationship (Meyer & Scheid, 1980):

$$\alpha = \beta g \left[1 - \frac{V}{V_2} \right] \Big/ \left[\frac{P_2}{P_1} \times \frac{V_2}{V_1} - \frac{V}{V_1} \right], \quad (1)$$

in which βg is the capacitance coefficient (55·6 and 57·6 μmol l^{-1} $mmHg^{-1}$ at 15 and 5 °C, respectively; Piiper, Dejours, Haab & Rahn, 1971), V is the blood sample volume (1 ml), V_1 is the volume of the extraction vessel for blood, V_2 is the volume of the gas vessel used for calibration, and P_1 and P_2 are partial pressures in blood or gas vessels after re-equilibration.

Table 1. *Mean values (±S.D.) of blood variables in untreated blood samples*

Variables	Unit	Argon		N_2	
		5°C	15°C	5°C	15°C
Haematocrit	vol%	28·3 ± 6·5	29·0 ± 3·3	27·2 ± 3·3	29·6 ± 4·0
[Haemoglobin]	mmol l^{-1}	4·50 ± 1·36	4·75 ± 0·50	5·54 ± 0·29	5·57 ± 0·94
[Lactate]	mmol l^{-1}	0·97 ± 0·50	0·89 ± 0·57	2·08 ± 0·52	0·87 ± 0·40
Osmolality	mosmol kg^{-1}	254 ± 24	268 ± 31	285 ± 10	302 ± 16
[Na^+]	mmol l^{-1}	116·8 ± 11·4	116·0 ± 15·0	115·1 ± 4·5	119·5 ± 5·9
[Cl^-]	mmol l^{-1}	91·3 ± 13·0	93·4 ± 7·0	104·7 ± 5·1	122·5 ± 5·6
[ATP]	mmol l^{-1}	0·68 ± 0·41	0·41 ± 0·12	0·69 ± 0·09	0·80 ± 0·08
[GTP]	mmol l^{-1}	1·90 ± 0·67	1·61 ± 0·40	1·70 ± 0·31	2·00 ± 0·16
N*		5	6	4	6

* Number of measurements.

Using corresponding values of α at 5 and 15°C, $\alpha(5)$ and $\alpha(15)$, allowed calculation of Q_{10} as:

$$Q_{10} = \alpha(15)/\alpha(5) . \quad (2)$$

Similarly, the activation energy, E_a, was calculated as:

$$E_a = -2{\cdot}303R \times \frac{\Delta \log x}{\Delta(1/T)} , \quad (3)$$

where T is the temperature in Kelvin, R is the gas constant (=8·314 J mol^{-1} K^{-1}) and x is the temperature-dependent variable under study.

RESULTS

Untreated blood

Average pH in untreated blood after equilibration was 7·8 at 15°C. Table 1 shows mean values of relevant blood constituents for the four groups of samples used for determination of Ar or N_2 solubility at 5 or 15°C. It is evident that there were no major differences in those parameters expected to influence solubility, e.g. haemoglobin concentration and osmolality.

Table 2 shows mean values for N_2 and Ar solubility in untreated whole blood of the eel and in fish Ringer's solution. Values in blood were about 3% above those in Ringer's solution. In both blood and Ringer, α_{Ar} was about twice the value for α_{N_2}.

Dependence of solubility on pH and lactate concentration

Fig. 1 shows an example of results obtained with three blood pools out of which 29 samples were equilibrated, some after acidification with CO_2, HCl or lactic acid for measurement of α_{N_2} at 15°C in the pH range between 5·5 and 8·2. There was no significant dependence of α_{N_2} on pH, irrespective of the mode of acidification. Hence, all values were averaged, and the mean in this example was

Table 2. *Mean values (±S.D.) of solubility for argon and nitrogen in untreated eel blood and in Ringer's solution*

Test gas	Liquid	Temperature (°C)	α (μmol l^{-1} mmHg^{-1})	N
Argon	Ringer's solution	5	2·61 ± 0·04	2
		15	2·10 ± 0·05	7
	Blood	5	2·66 ± 0·08	5
		15	2·16 ± 0·08	6
Nitrogen	Ringer's solution	5	1·29 ± 0·01	2
		15	1·07 ± 0·01	6
	Blood	5	1·25 ± 0·05	4
		15	1·10 ± 0·04	6

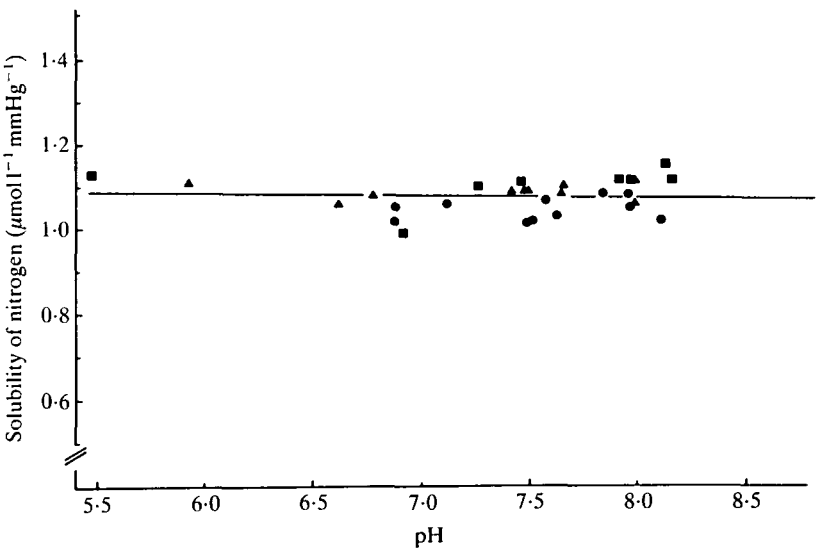

Fig. 1. Solubility of N_2 in whole blood of the eel at 15°C and varying pH. The pH was varied with CO_2 (●), HCL (▲) or lactic acid (■).

1·08 μmol l^{-1} mmHg^{-1} (S.D. = 0·04). Lack of significant dependence upon pH was also found at 5°C and for α_{Ar} at both temperatures.

Mean values from individual experiments, in which all values obtained with a given mode of acidification were averaged, are given in Table 3. There were no significant differences among the types of acidification, and the overall means correspond well with those in untreated blood (Table 2).

Overall mean values for solubilities, obtained by averaging corresponding individual values, irrespective of whether they were acidified or not, are given in Table 4. Values of Q_{10} and activation energy, E_a, were calculated from these overall mean data.

Table 3. *Mean values (±S.D.) for solubility of argon and nitrogen in eel blood obtained in the pH ranges indicated*

Test gas	Temperature (°C)	pH range	Acidification	α (μmol l^{-1} mmHg^{-1})	N
Argon	5	6·22–8·00	HCl	2·63 ± 0·03	6
		6·49–7·94	LA	2·55 ± 0·05	5
		6·44–8·10	LA	2·57 ± 0·06	7
			Overall mean	2·58 ± 0·04	
Argon	15	7·22–8·13	CO_2	2·16 ± 0·02	7
		7·10–8·24	CO_2	2·00 ± 0·03	10
		5·57–8·29	HCl	2·10 ± 0·02	9
		6·31–8·34	HCl	2·11 ± 0·04	8
		6·46–8·04	LA	2·18 ± 0·03	9
			Overall mean	2·11 ± 0·07	
Nitrogen	5	6·60–8·22	HCl	1·24 ± 0·02	6
		6·41–8·31	LA	1·25 ± 0·03	
			Overall mean	1·25 ± 0·01	
Nitrogen	15	6·90–7·98	CO_2	1·04 ± 0·02	6
		6·88–8·12	CO_2	1·05 ± 0·02	11
		5·92–8·02	HCl	1·09 ± 0·02	10
		6·82–8·23	HCl	1·11 ± 0·03	6
		5·47–8·16	LA	1·12 ± 0·02	8
			Overall mean	1·08 ± 0·04	

Mean values were calculated by averaging all measurements in a given blood pool, irrespective of pH.

Overall mean is the average from individual means.

LA, lactic acid.

Table 4. *Overall mean values for solubility of argon and nitrogen in eel whole blood at 5 and 15°C*

Test gas	Temperature (°C)	α (μmol l^{-1} mmHg^{-1})	N	Q_{10}	E_a (kJ mol^{-1})
Argon	5	2·60 ± 0·05	23	0·82	−13·4
	15	2·12 ± 0·07	49		
Nitrogen	5	1·25 ± 0·01	18	0·87	−9·2
	15	1·09 ± 0·03	47		

Calculated values for Q_{10} and activation energy, E_a.

The blood parameters listed in Table 2 were not significantly different for the acidified samples except for haematocrit and lactate concentration. Haematocrit was about 15 % higher at pH 6·0 than at pH 7·8. [Lactate] was obviously elevated in those

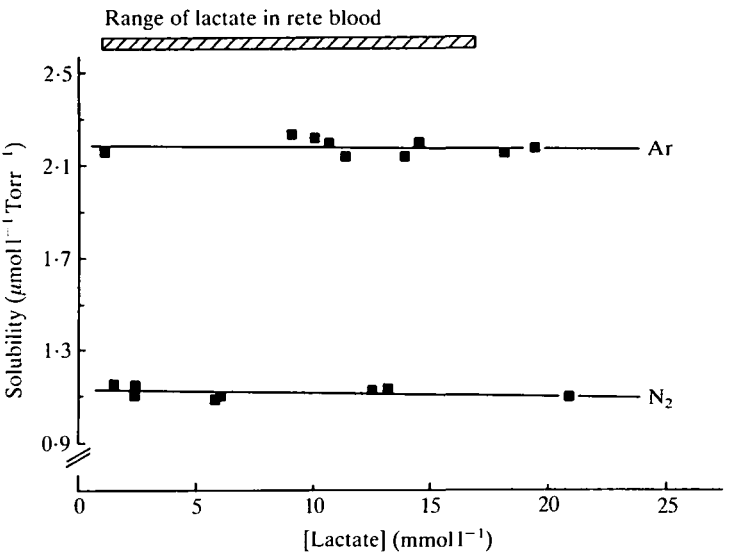

Fig. 2. Solubility of N_2 and Ar at varying lactate concentrations. The lactate concentration in rete blood was measured directly in blood samples collected from the anaesthetized eel during secretion (H. Kobayashi, B. Pelster & P. Scheid, unpublished results).

samples in which lactic acid has been used for acidification. This enabled us to investigate a possible dependence of α on lactate concentration.

Fig. 2 shows data from two experiments in which α_{Ar} and α_{N_2} were measured after acidification with lactic acid. The lactate concentration in untreated blood was between 1 and 2 mmol l^{-1} and was increased to above 20 mmol l^{-1} when the pH was lowered to 6·46 for Ar and 5·47 for N_2 measurements by adding lactic acid. It is evident that lactate, in the concentration range used, did not affect solubility of either gas. This is the basis for averaging data as in Table 3.

DISCUSSION

Dependence of solubility on pH and solute concentration

Our data showed no dependence of argon or nitrogen solubility on pH between 5·5 and 8·4 in eel whole blood. This confirms the earlier results of Douglas (1967) but contradicts Steen (1963*a*), who suggested haemoglobin was responsible for the effect since he did not observe it in plasma. We have no simple explanation for this apparent discrepancy. Our data do not even show the change of solubility with pH reported by Abernethy (1972), who found a reduction of nitrogen solubility in whitefish red cell suspensions of 4 % per unit pH. Abernethy did not observe this reduction in either the yellow pike or the catfish.

In the range tested there was no dependence of solubility on lactate concentration in our experiments. This is not in conflict with the salting-out effect, i.e. the reduction in solubility when the concentration of ions in the solution is increased

Table 5. *Published values for solubility (μmol l^{-1} $mmHg^{-1}$) of argon and nitrogen in whole blood of several vertebrates*

Test gas	Animal	α (μmol l^{-1} $mmHg^{-1}$)	Temperature (°C)	Reference*	α‡ (15°C)
Argon	Rabbit	1·58	37	1	2·44
	Dog	1·33	37	2	2·06
	Human	1·55	37	3	2·40
	Human	1·58	37	4	2·44
	Eel	2·31	6·5	5	1·95
	Eel	2·12	15	6	2·12
Nitrogen	Rabbit	0·73	37	1	0·99
	Ox	0·76	38	7	1·05
	Cod	0·97	6·5	5	0·86
	Trout	1·02	6·5	5	0·91
	Eel	0·87†	20	5	0·93
	Eel	1·06†	6·5	5	0·94
	Eel	1·09	15	6	1·09

*1, Ohta, Ar & Farhi (1979); 2, Meyer, Tebbe & Piiper (1980); 3, Edwards, Velasquez & Farhi (1963); 4, Hlastala, Meyer, Riepl & Scheid (1980); 5, Steen (1963*a*); 6, present study; 7, van Slyke, Dillon & Margaria (1934).

†pH above 7·8.

‡Calculated using Q_{10} values from Table 4.

(Gerth & Hemmingsen, 1982). The range of lactate concentration in our experiments was 0·5–25 mmol l^{-1}, and the maximum change in Na^+ and Cl^- concentration when plasma was replaced by HCl was approximately 10 mmol l^{-1}. Published data for the salting-out effect in water (Gerth & Hemmingsen, 1982; Enns, Douglas & Scholander, 1967) would predict a maximum reduction of α by 1 % or even less in our acidification experiments owing to increasing lactate or salt concentration. The sensitivity of our technique for determining α is too low to detect these changes (Meyer & Scheid, 1980; Hlastala, Meyer, Riepl & Scheid, 1980).

Comparison with published data

Table 5 summarizes published data for Ar and N_2 solubility in blood of ectothermic vertebrates and of mammals. For ease of comparison, we have recalculated these data to 15 °C using the Q_{10} values given in Table 4. Apart from apparent interspecific differences, our data appear to be in the range of those reported by others.

The somewhat higher solubility in whole blood than in Ringer's solution found by us for both Ar and N_2 is in agreement with reports on the influence of protein and red cell concentration on solubility (Yeh & Peterson, 1965; Christoforides & Hedley-Whyte, 1969; Young & Wagner, 1979). However, the range of haemoglobin concentration was too small in our experiments to warrant a quantitative analysis of this dependence.

Physiological significance

The pH and lactate ranges tested by us are expected to occur under physiological conditions in the blood vessels of the swim bladder when gases are secreted (Steen, 1963*b*; Kuhn *et al.* 1963; Enns *et al.* 1967). Our measurements and those of Gerth & Hemmingsen (1982) and Enns *et al.* (1967) predict a maximum change in solubility in this range of only about 1 %. It is of interest to investigate whether this solubility change is sufficient to explain a significant counter-current concentration of inert gases in the rete mirabile vessels on the basis of the salting-out effect.

The calculations of Gerth & Hemmingsen (1982) and Enns *et al.* (1967) predict a maximum transfer of inert gas by virtue of the salting-out effect in the rete mirabile of 10^{-5}–10^{-6} $cm^3\,min^{-1}$. Significant rates of change in swim bladder volume would, therefore, take unrealistically long periods of time. Similar conclusions can be derived from the study of Sund (1977). It thus appears that neither pH changes nor the salting-out effect can explain short-term changes in swim bladder volume on the basis of the present models, which may, however, be valid for long-term adaptations. To explain short-term transfer of inert gases, if they occur at all, would require either modifications in the present models of counter-current in the rete mirabile or would have to invoke mechanisms that are hitherto unknown.

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