J. exp. Biol. (1982), 96, 221–237
With 11 *figures*
Printed in Great Britain

THE INFLUENCE OF EXPERIMENTAL ANAEMIA ON BLOOD ACID–BASE REGULATION *IN VIVO* AND *IN VITRO* IN THE STARRY FLOUNDER (*PLATICHTHYS STELLATUS*) AND THE RAINBOW TROUT (*SALMO GAIRDNERI*)

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(*Received* 27 *March* 1981)

SUMMARY

Severe experimental anaemia caused a rise in P_{CO_2} and an associated fall in pH (respiratory acidosis) in arterial and venous blood of both flounder and trout *in vivo*. In some trout, but not in flounder, there was also a rise in blood lactate, indicating metabolic acidosis. *In vitro*, blood buffer capacities declined with haematocrit, a factor which contributed to the extent of the acidoses *in vivo*. However, haematocrit did not influence the pK^1 of the plasma HCO_3^-/H_2CO_3 system or the actual measurement of blood pH. The Donnan ratio for HCO_3^- varied linearly with pH over the range 7·0–7·6, indicating a passive distribution of HCO_3^- across the trout erythrocyte. The present data, together with other recent results, indicate that the teleost red blood cell does play a role in plasma HCO_3^- dehydration and CO_2 excretion, and therefore opposes the theory of Haswell & Randall (1978) that the erythrocyte is functionally impermeable to HCO_3^-.

INTRODUCTION

Current theory holds that CO_2 excretion at the teleost gill occurs via the dehydration of plasma HCO_3^- by carbonic anhydrase within the gill epithelium. Erythrocytic carbonic anhydrase is thought not to be involved in this process because the red blood cell appears functionally impermeable to plasma HCO_3^- due to the presence of an unknown plasma inhibitor (Haswell & Randall, 1976, 1978; Haswell, Zeidler & Kim, 1978; Haswell, Randall & Perry, 1980). An important piece of evidence presented in support of this theory was that blood pH and P_{CO_2} levels *in vivo* were unaffected by massive experimental depletion of the red cells in the freshwater rainbow trout, *Salmo gairdneri* (Haswell & Randall, 1978; Haswell *et al.* 1980). However, in the seawater starry flounder, *Platichthys stellatus*, we have recently shown that similar anaemia produces significant decreases in both arterial and venous

blood pH levels (Wood, McMahon & McDonald, 1979*b*). The aim of the present study was to explore the basis of this apparent difference between trout and flounder by examining the *in vivo* and *in vitro* characteristics of the blood during severe experimental anaemia in the two species. The conclusions cast some doubt on the Haswell & Randall (1978) model of CO_2 excretion in teleost fish.

SYMBOLS

Symbols employed for respiratory parameters follow the system of Dejours (1975) and have been defined in Wood *et al.* (1979*a*, *b*).

MATERIALS AND METHODS

I. *Experimental animals*

Starry flounder (*Platichthys stellatus* Pallas; 300–800 g) were collected and acclimated for at least 10 days to running sea water (salinity = 27 ± 1‰, temperature = 9 ± 1 °C) at Friday Harbor Laboratories, University of Washington, as described previously (Wood *et al.* 1977, 1979*a*). Rainbow trout (*Salmo gairdneri* Richardson; 100–350 g) were purchased from Spring Valley Trout Farm, Petersburg, Ontario, and acclimated for at least 2 weeks to running dechlorinated fresh water (temperature = 13 ± 1·5 °C) at McMaster University. Flounder were fitted with caudal artery and/or caudal vein catheters (Watters & Smith, 1973; Wood *et al.* 1977, 1979*a*) while under 1:15000 MS-222 anaesthesia. After surgery, the flounder were allowed to recover for at least 72 h in individual chambers (30 × 30 × 15 cm deep) which were filled to a depth of 6 cm with fine beach sand and shielded from the investigators. The chambers were continually flushed at > 500 ml/min with sea water (P_{I,O_2} = 125–155 torr, P_{I,CO_2} < 1·0 torr) at acclimation temperature. During the whole experimental period, the flounder remained buried in the sand with only the eyes and mouth exposed. Trout were fitted with either dorsal aortic or ventral aortic cannulae (Smith & Bell, 1964; Holeton & Randall, 1967) while under similar anaesthesia, and allowed to recover for 36–48 h before sampling. During recovery and subsequent experimentation, trout were held in darkened Plexiglas or wooden boxes (40 × 6 × 7 cm deep) supplied with fresh water (P_{I,O_2} = 145–175 torr; P_{I,CO_2} = 2·0–3·5 torr) at > 300 ml/min.

II. In vivo *experiments*

(i) *Platichthys stellatus*. Two experimental series were performed. In the first (N = 6), flounder fitted with venous catheters only were rendered progressively anaemic in 4–6 stages by sequential bleeding and plasma reinfusion as described by Wood *et al.* (1979*b*). The final Ht was ≃ 1%. The fish were allowed to stabilize for at least 24 h at each new Ht before a set of measurements (pH_e, Ht, blood lactate), was taken. In the second series (N = 12) fish representing a range of Ht from normal to severely anaemic and bearing arterial and/or venous catheters were examined. The range in Ht was obtained from a combination of natural variation (Wood *et al.* 1979*b*), sequential bleeding (as above), and the blood loss accompanying

repetitive sampling. Again animals were allowed to stabilize for at least 24 h at each Ht before sampling. Samples were analysed for pH, C_{CO_2}, P_{CO_2}, and Ht. While Ht was determined on every sample, generally only one or two of the other parameters were measured simultaneously. In total, between 2 and 11 samples were taken from each fish.

(ii) *Salmo gairdneri.* Two series were again performed. In the first ($N = 17$), trout fitted with dorsal aortic catheters were subjected to progressive anaemia in a manner identical to that described for flounder. The final Ht was 1–5%. Only pH_a was measured on most samples, though blood lactate and P_{a,CO_2} were also determined in a few cases. In the second series, fish bearing either dorsal aortic ($N = 11$) or ventral aortic catheters ($N = 4$) were compared at their normal Ht and during severe anaemia. The anaemia was again induced by sequential bleeding and plasma reinfusion at 24 h intervals in at least four stages. Samples were analysed for Ht, pH, P_{CO_2}, P_{O_2}, C_{CO_2} (whole blood), C_{p,CO_2} (true plasma), and blood lactate simultaneously; P_{I,O_2} and P_{I,CO_2} were also recorded.

III. In vitro *experiments*

The acid–base and CO_2-combining characteristics of blood of different Ht's from both species were studied by *in vitro* tonometry. Blood was obtained by blind needle puncture of the caudal artery of anaesthetized flounder and from the dorsal aortic catheters of unanaesthetized, fully recovered trout. After heparinization at $\simeq$ 100 i.u./ml, the blood from several animals was pooled, well mixed, centrifuged very briefly to separate the red blood cells, and then made up at several different Ht's by mixing of appropriate fractions of plasma and erythrocytes. Aliquots (volume = 5 ml) were placed in separate large tonometer shaker flasks (individual flask volume = 25 or 50 ml) at the acclimation temperature and gassed for at least 2·0 h with several humidified mixtures of CO_2 in air supplied by Wösthoff mixing pumps or by analysed gas cylinders. At each P_{CO_2}, the pooled blood was analysed for Ht, pH and C_{CO_2} (whole blood) in the flounder study, and for Ht, haemoglobin concentration ([Hb]), pH, C_{CO_2} (whole blood) and C_{p,CO_2} (true plasma) in the trout study. When a number of different equilibration P_{CO_2} were employed, the total tonometry time was up to 30 h, but no haemolysis, variation in $C_{O_2}^{max}$ (checked in flounder only) or change in haemoglobin concentration (checked in trout only) was detected. Furthermore, in the trout blood experiments, the initial equilibration gas was routinely repeated at the end of the 30 h run. Agreement with the initial values was within the error of the measurements. For this reason, we believe there was no significant deterioration during extended tonometry.

IV. *Analytical procedures*

The analytical techniques for Ht, pH, C_{CO_2} (whole blood), P_{CO_2}, $C_{O_2}^{max}$, and blood lactate in the flounder study have been described by Wood *et al.* (1977, 1979*a*). Similar procedures were employed in the trout work, but with the following modifications. $C_{O_2}^{max}$ was not measured. Ht was determined in sodium-heparinized rather than ammonium-heparinized capillary tubes, lactate analyses were performed on 150 μl rather than 250 μl of blood, and the recommendations of Boutilier, Randall,

Shelton & Toews (1978) with respect to pre-equilibration of the electrode and sample replacement during P_{CO_2} measurements were followed. As a result, P_{CO_2} determinations were more precise on trout (reproducibility $\simeq \pm 5\%$) than on flounder ($\simeq \pm 15\%$). P_{O_2} (Radiometer microelectrode), [Hb] (cyanomethaemoglobin method using Hycel reagents), and C_{p,CO_2} (true plasma) were determined only in the trout study. For C_{p,CO_2} the sealed haematocrit tube was broken, the plasma aspirated anaerobically into a Hamilton syringe, and the CO_2 content determined via the Cameron (1971) method, as with whole blood C_{CO_2}. Blood and plasma [HCO_3^-]'s were calculated as:

$$C_{CO_2} - \alpha_{p,CO_2} . P_{CO_2},$$

where α_{p,CO_2} is the appropriate solubility coefficient for CO_2 in blood plasma (Severinghaus, 1965).

Statistical comparisons have been made on the basis of an experimental design in which each animal (at its normal Ht) is used as its own control (paired Student's two-tailed t test). Values are expressed as mean $\pm$ 1 S.E. (N).

RESULTS

I. In vivo *experiments*

(i) *Platichthys stellatus*. In flounder of the first series ($N = 6$) fitted with only a venous cannula, a highly significant ($P < 0.001$) fall in pH_v occurred as the fish were rendered progressively anaemic (Fig. 1A). On average, pH_v declined from 7.921 ± 0.009 at Ht = $14.2 \pm 2.0\%$ to 7.792 ± 0.011 at Ht = $0.7 \pm 0.1\%$. The effect became apparent at an Ht of 5–10% and thereafter became progressively greater. Blood lactate was very low at normal Ht (< 0·30 mM) and did not increase during anaemia, indicating no development of lactacidosis (Fig. 1B). The pH data mirror our earlier findings in flounder encumbered with a number of cannulae, electrodes, and a ventilation mask (cf. Fig. 6 of Wood *et al.* 1979*b*), confirming that the phenomenon is not an artifact of experimental disturbance.

The second series of flounder ($N = 12$) demonstrated that the fall in pH induced by anaemia was associated with an increase in P_{CO_2} (Fig. 2A, B). The effect occurred in both arterial and venous blood, but was more pronounced in the latter. In both cases, the slopes of the regression relationships of P_{CO_2} on Ht were significantly different from zero ($P < 0.05$), but the relationships did not appear to be linear. For this reason, the lines have been fitted by eye. In venous blood, major increases in P_{CO_2} clearly occurred only at Ht's below 5%. There was a suggestion of a similar trend in arterial blood, but the data were unconvincing. In venous blood, C_{CO_2} also tended to increase during anaemia (Fig. 3B; regression slope significantly different from 0, $P < 0.05$) but remained approximately stable in arterial blood (Fig. 3A). In agreement with the lactate data (Fig. 1B), this clearly demonstrates the absence of a metabolic acidosis, which would have reduced [HCO_3^-] and therefore C_{CO_2}.

Fig. 4 illustrates the strong negative correlation ($r = -0.789$, $P < 0.001$) between pH and log P_{CO_2} for all simultaneous *in vivo* measurements of the two parameters in this series, indicating a respiratory acidosis due to CO_2 retention. This relationship is still highly significant ($r = 0.712$, $P < 0.001$) even when the three venous point

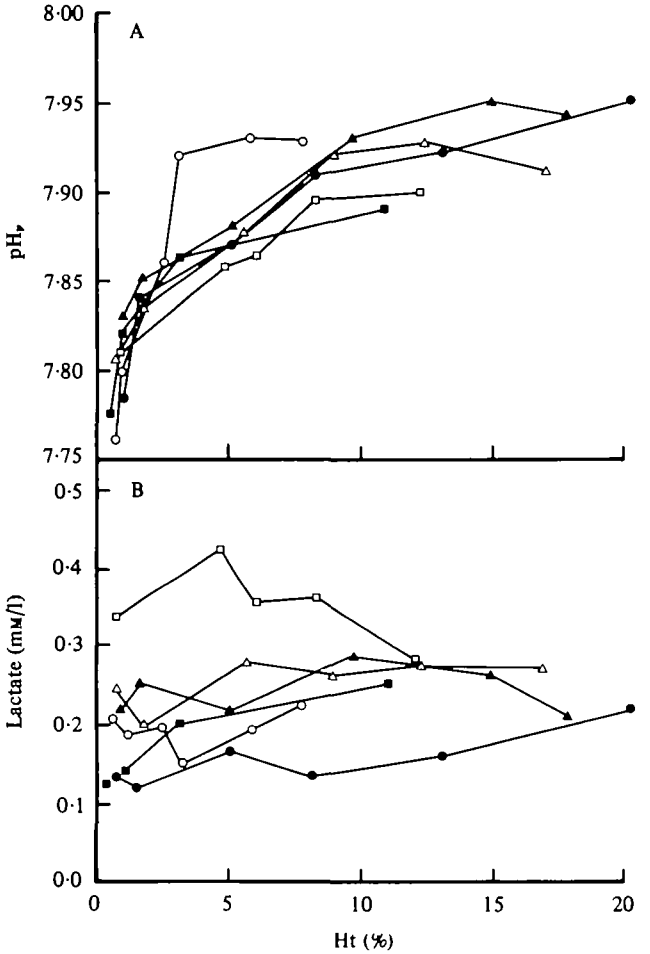

Fig. 1. The relationships between haematocrit (Ht) and: (A) venous pH (pH_v); (B) venous blood lactate concentration in six starry flounder rendered progressively anaemic. Each symbol represents data from a single animal.

at very low pH from severely anaemic fish are omitted. Theoretically, slightly curvilinear relationships are expected between pH and log P_{CO_2} (e.g. Figs. 7, 10A) in blood of a fixed Ht. The *in vivo* relationship will become even more curvilinear as the fish becomes progressively anaemic and acidotic, because the slopes of the log P_{CO_2} *vs.* pH lines decrease with Ht (e.g. Figs. 7, 10A). Consequently, a linear regression relationship has not been fitted to the data.

(ii) *Salmo gairdneri*. Of the 17 trout in the first series, pH_a declined during severe anaemia in 14. For the sake of clarity, the data from individual animals have been averaged over 5% Ht intervals in Fig. 5. The overall fall ($N = 17$) from $7{\cdot}861 \pm 0{\cdot}008$ at Ht = $24{\cdot}9 \pm 1{\cdot}2$% to $7{\cdot}758 \pm 0{\cdot}017$ at Ht = $2{\cdot}6 \pm 0{\cdot}3$% was highly significant ($P < 0{\cdot}001$). The final Ht in trout was not as low as in flounder because death commonly occurred at Ht's less than $\simeq 2$%. As with flounder (Fig. 1A), the inflexion point of the pH_a *vs.* Ht relationship was generally at an Ht of 5–10%, though there was much greater variability in the data (Fig. 5). Measurements of

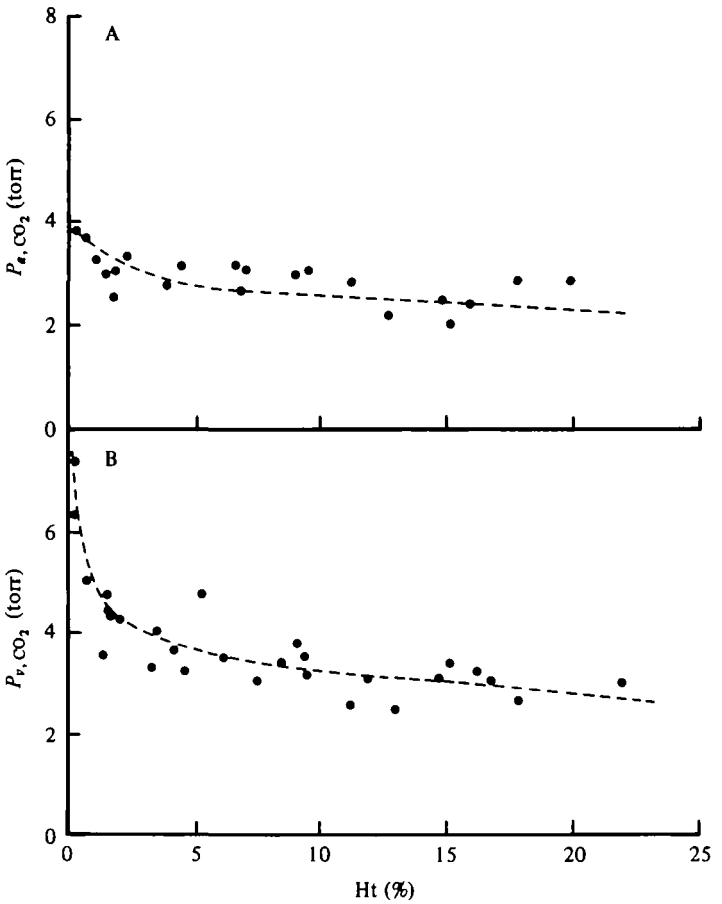

Fig. 2. The relationships between haematocrit (Ht) and: (A) arterial CO_2 tension (P_{a,CO_2}); (B) venous CO_2 tension (P_{v,CO_2}) in starry flounder at a range of Ht's from normal to severely anaemic. Data were taken from 12 fish. The lines have been fitted by eye.

P_{a,CO_2} and blood lactate in a few of these fish suggested an upward trend in both parameters during severe anaemia. The influence of these factors on blood acid–base status during anaemia was therefore examined in detail in a second experimental series.

Severe experimental anaemia again caused a significant acidosis in both arterial (Table 1; $N = 11$) and venous blood (Table 2; $N = 4$) of trout in this second series. In both cases, there were small but significant increases in P_{CO_2} (Tables 1, 2), indicating a definite respiratory acidosis. This effect occurred in the face of a constant P_{I,CO_2} (Tables 1, 2). As an independent check, P_{CO_2} values were also calculated from C_{p,CO_2} (plasma) and pH measurements and tabulated values of pK^1 (Albers, 1970) and α_{p,CO_2} (Severinghaus, 1965) using the Henderson–Hasselbalch equation (cf. Albers, 1970). While these calculated values were slightly different from the measured values for reasons outlined in Reeves (1977), increases of comparable magnitude again occurred during anaemia. In about half the fish, a definite lactacidosis also occurred; mean blood lactate levels increased in both groups, although with great variability. The overall change was significant only in the arterial group which had

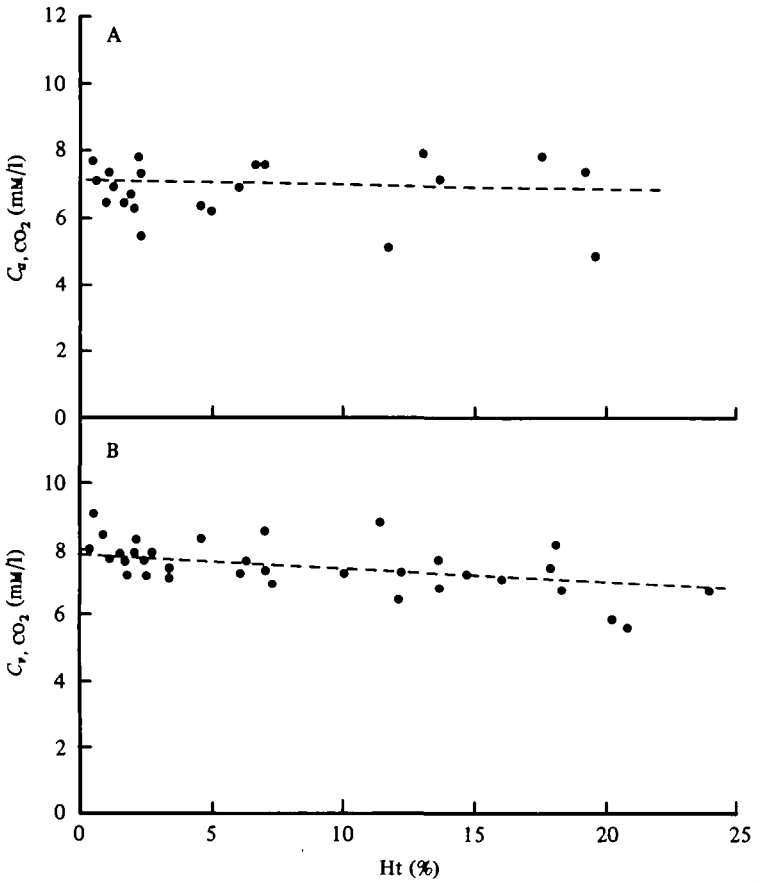

Fig. 3. The relationships between haematocrit (Ht) and: (A) arterial whole blood CO_2 content (C_{a,CO_2}); (B) venous whole blood CO_2 content (C_{v,CO_2}) in starry flounder at a range of Ht's from normal to severely anaemic. Data were taken from the same 12 fish as in Fig. 2, though the measurements were not all simultaneous with those in Fig. 2. The lines have been fitted by eye.

a larger N number than the venous group (Tables 1, 2). Nevertheless, C_{CO_2} (whole blood) and C_{p,CO_2} (plasma) declined significantly in the venous group, providing indirect evidence of metabolic acidosis (Table 2). The overall picture appears to be that of respiratory acidosis compounded to a highly variable extent by lactacidosis.

II. In vitro *experiments*

(i) *Salmo gairdneri*. The studies with trout blood were more extensive than those with flounder blood, and so will be dealt with first. Fig. 6 illustrates typical linear relationships between pH and $[HCO_3^-]$ in whole blood (Fig. 6A) and true plasma (Fig. 6B) for bloods of different Ht's made up from a homogeneous pool and equilibrated at a range of P_{CO_2}'s. Fig. 7 illustrates, for the same samples, the expected curvilinear relationships between log P_{CO_2} and pH. Blood buffer capacities were obviously functions of Ht, $-\Delta HCO_3^-/\Delta pH$ (Fig. 6) and $-\Delta \log P_{CO_2}/\Delta pH$ (Fig. 7) both increasing with red blood cell concentration. These results emphasize that for a given change in metabolic acid (e.g. lactic) or P_{CO_2}, a greater change in pH will

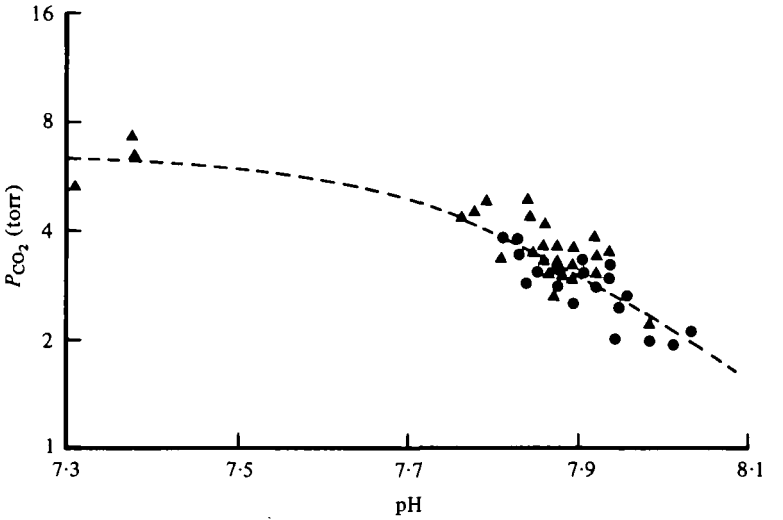

Fig. 4. The relationship between blood carbon dioxide tension (P_{CO_2}, logarithmic scale) and blood pH for all simultaneous measurements of these two parameters in the same 12 starry flounder as in Figs. 2 and 3. ●, Arterial; ▲, venous. Measurements were made at a range of haematocrits from normal to severely anaemic. P_{CO_2} tended to rise and pH tended to fall as haematocrit declined. The correlation coefficient (r) between pH and log P_{CO_2} was $-0{\cdot}789$ ($N = 46$, $P < 0{\cdot}001$).

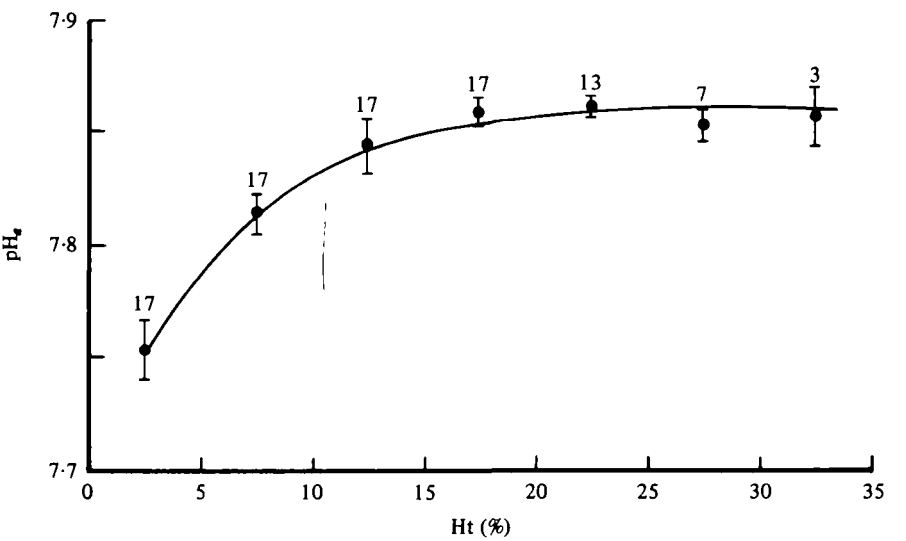

Fig. 5. The relationship between haematocrit (Ht) and arterial pH (pH_a) in 17 rainbow trout rendered progressively anaemic. For the sake of clarity, the data from individual animals have been averaged over 5% Ht intervals, and the resultant means ± 1 S.E. (N) plotted at the midpoints of the intervals.

occur in anaemic blood than in blood of normal Ht. This factor must have contributed to the extent of the acidoses *in vivo*, where P_{CO_2} and lactate both increased (Tables 1 and 2).

For any given set of incubations, the buffer lines for true plasma from different Ht's intersected at a common point where the P_{CO_2}, pH, and $[HCO_3^-]$ values were the same for all Ht's (Fig. 6B, 7). For whole blood of different Ht's, the intersection point was more diffuse than that for true plasma and always occurred at

Table 1. *The influence of severe experimental anaemia on blood gas and acid–base regulation in the arterial blood of rainbow trout* in vivo. *Means* ± 1 S.E. (N)

	(N = 11)		
	Control	Anaemia	P*
P_{I,CO_2} (torr)	2·47 ± 0·08	2·45 ± 0·09	n.s.
P_{I,O_2} (torr)	161·5 ± 2·7	165·3 ± 0·9	n.s.
Haematocrit (%)	24·3 ± 1·7	3·3 ± 0·8	0·001
pH_a	7·887 ± 0·018	7·808 ± 0·021	0·01
P_{a,CO_2}-measured (torr)	3·47 ± 0·18	4·10 ± 0·24	0·05
P_{a,CO_2}-calculated (torr)	3·91 ± 0·29	4·56 ± 0·29	0·05
C_{a,CO_2}-blood (mM)	9·08 ± 0·47	10·50 ± 0·60	0·01
C_{a,CO_2}-plasma (mM)	11·23 ± 0·39	11·07 ± 0·64	n.s.
Lactate-blood (mM)	0·97 ± 0·08	3·54 ± 0·97	0·05
P_{a,O_2} (torr)	131·6 ± 4·0	144·9 ± 2·4	0·02

NOTE. Each animal was examined at normal Ht and in severe anaemia, thereby serving as its own control.

* Significance of difference by Student's paired two-tailed t test.

Table 2. *The influence of severe experimental anaemia on blood gas and acid-regulation in the venous blood of rainbow trout* in vivo. *Means* ± 1 S.E. (N)

	(N = 4)		
	Control	Anaemia	P*
P_{I,CO_2} (torr)	2·61 ± 0·01	2·71 ± 0·06	n.s.
P_{I,O_2} (torr)	155·0 ± 2·3	153·0 ± 1·9	n.s.
Haematocrit (%)	20·6 ± 4·0	4·3 ± 0·4	0·05
pH_v	7·795 ± 0·026	7·666 ± 0·027	0·001
P_{v,CO_2}-measured (torr)	4·69 ± 0·21	5·54 ± 0·44	0·05
$P_{v\,CO_2}$-calculated (torr)	4·98 ± 0·62	5·82 ± 0·90	0·05
$C_{v\,CO_2}$-blood (mM)	9·61 ± 0·71	8·66 ± 0·67	0·05
$C_{v\,CO_2}$-plasma (mM)	11·26 ± 0·51	9·17 ± 0·81	0·05
Lactate-blood (mM)	1·45 ± 0·81	4·62 ± 2·40	n.s.
P_{v,O_2} (torr)	33·1 ± 6·1	23·2 ± 3·1	n.s.

NOTE. Each animal was examined at normal Ht and in severe anaemia, thereby serving as its own control.

* Significance of difference by Student's paired two-tailed t test.

lower pH (compare Fig. 6A with 6B) because of the unequal distribution of HCO_3^- across the erythrocyte membrane (see below). If the original blood sample was not pre-equilibrated prior to tonometry, the intersection point for true plasma buffer lines of different Ht corresponded closely to the expected *in vivo* acid–base status of arterial true plasma (Table 1), which would represent the conditions in the sample prior to processing. If, on the other hand, the original blood sample was pre-equilibrated to a higher P_{CO_2} before the whole separation and reconstitution procedure and its acid–base status were recorded (as in the example in Figs. 6 and 7) then the intersection point was shifted to this new, more acidotic position. Thus the intersection point represented the pH, P_{CO_2}, and plasma HCO_3^- levels of the original blood at the time of separation.

In other words, homogeneous trout bloods of different Ht's but the same levels of P_{CO_2} and plasma HCO_3^- will have identical pH's. This means that Ht does not ffect the pH value (actually plasma pH) which can be read on a whole blood sample

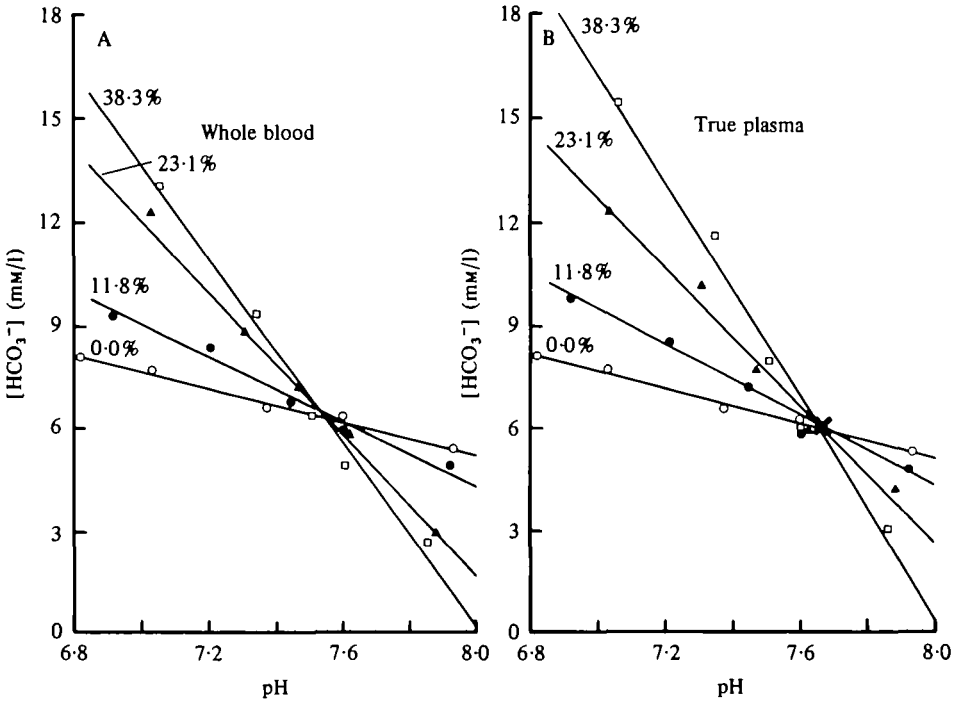

Fig. 6. Typical *in vitro* relationships between pH and bicarbonate concentration ($[HCO_3^-]$) for (A) whole blood and (B) true plasma in homogeneous rainbow trout blood pooled from four animals and made up to different haematocrits. Note the common intersection point for the straight-line relationships between true plasma $[HCO_3^-]$ and pH at different haematocrits (Fig. 6B). Prior to separation and reconstitution, the original blood in this example was equilibrated to P_{CO_2} = 4·25 torr, producing pH = 7·640 and true plasma $[HCO_3^-]$ = 5·99 mM (indicated by X) which closely approximated the intersection point after separation, reconstitution, and tonometry.

with a glass electrode. More importantly, it also means that Ht does not influence the pK^1 of the plasma HCO_3^-/H_2CO_3 system. Neither of these factors therefore contributed to the acid–base disturbance during anaemia observed *in vivo* (Fig. 5; Tables 1, 2). Another way of proving this point is to calculate pK^1 at a range of pH's for each Ht using the $[HCO_3^-]$, pH, and P_{CO_2} data and the Henderson–Hasselbalch equation (Albers, 1970). This has been done in Fig. 8 for the data from the incubations in Figs. 6B and 7; similar relationships were seen in other incubations. Clearly, pK^1, while showing the expected negative relationship with pH (Albers, 1970), was independent of Ht, and variation in the calculated pK^1's was a random function of measurement errors.

At any given Ht, the concentrations of HCO_3^- for whole blood were always less than those for true plasma, indicating a lower $[HCO_3^-]$ inside than outside the erythrocyte (compare Figs. 6A and 6B). A similar effect was seen *in vivo* (cf. C_{CO_2} values in Tables 1, 2). However, for any given Ht the whole blood $[HCO_3^-]$ progressively approached the true plasma $[HCO_3^-]$ as pH fell *in vitro*, indicating that HCO_3^- distribution was influenced by pH. As a consequence, the slope of the $[HCO_3^-]$ *vs.* pH relationship, which represents the non-bicarbonate buffer capacity of the system ($\beta = -\Delta HCO_3^-/\Delta pH$), was always lower for whole blood than for

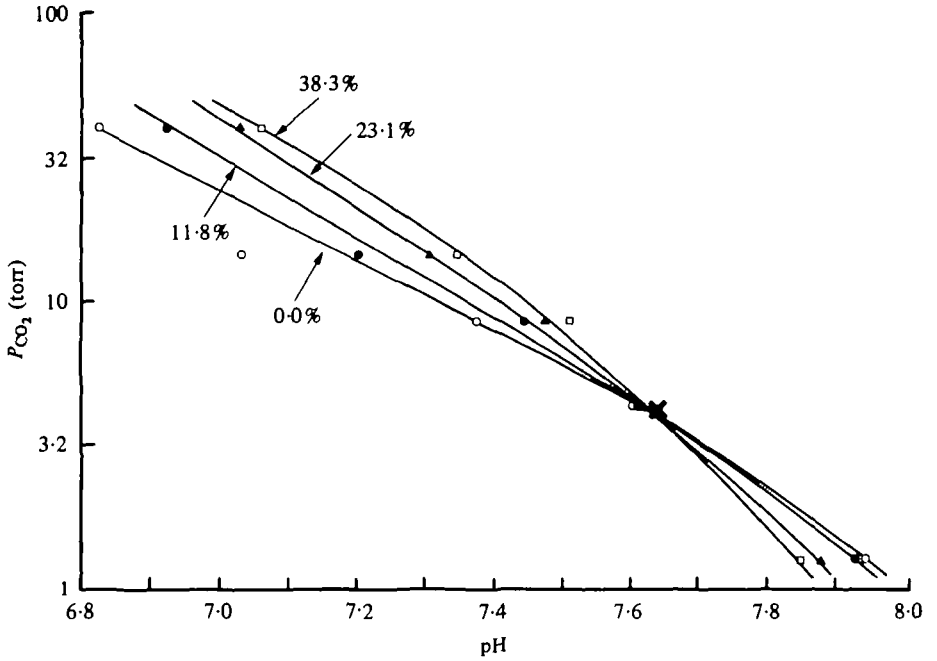

Fig. 7. Typical *in vitro* relationships between pH and CO_2 tension (P_{CO_2}, logarithmic scale) in homogeneous rainbow trout blood at different haematocrits. Data are from the same example as Fig. 6; see legend of Fig. 6 for further details. Again, note the close proximity of the intersection point to the original equilibration acid–base status (X).

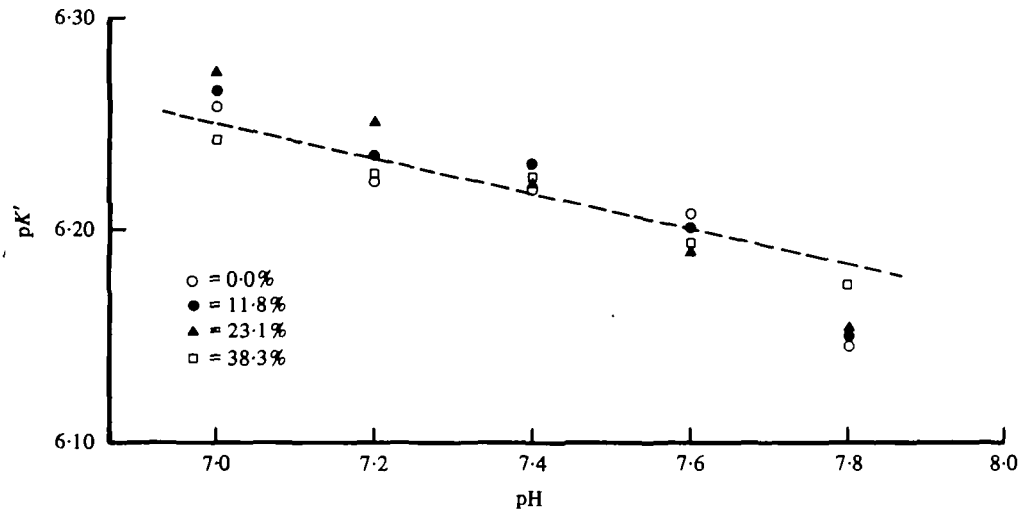

Fig. 8. Typical *in vitro* relationship between pH and pK^1 in true plasma from homogeneous rainbow trout blood at different haematocrits. Data are from the same example as Fig. 6; see legend of Fig. 6 for further details. Note the complete lack of effect of haematocrit on pK^1. The dotted line represents the relationship for mammalian true plasma at this temperature (14 °C) reported by Albers (1970) from the data of Severinghaus, Stupfel & Bradley (1956).

Table 3. *The regression relationships between blood haematocrit (Ht*) or haemoglobin concentration [Hb]† and the non-bicarbonate buffer capacity (β‡) of whole blood and true plasma in trout and flounder* in vitro

Salmo gairdneri	
Whole blood	β‡ = −28·35 Ht* − 2·59
(N = 8)	r = 0·98 P < 0·001
True plasma	β‡ = −33·97 Ht* − 2·39
(N = 8)	r = 0·99 P < 0·001
Whole blood	β‡ = −1·073 [Hb]† − 2·48
(N = 8)	r = 0·98 P < 0·001
True plasma	β‡ = −1·271 [Hb]† − 2·31
(N = 8)	r = 0·97 P < 0·001
Platichthys stellatus	
Whole blood	β‡ = −16·51 Ht* − 2·89
(N = 8)	r = 0·74 P < 0·05

* Ht expressed as a decimal.
† [Hb] expressed in g/100 ml.
‡ β expressed in slykes (= mmol.L^{-1}.pH^{-1}).

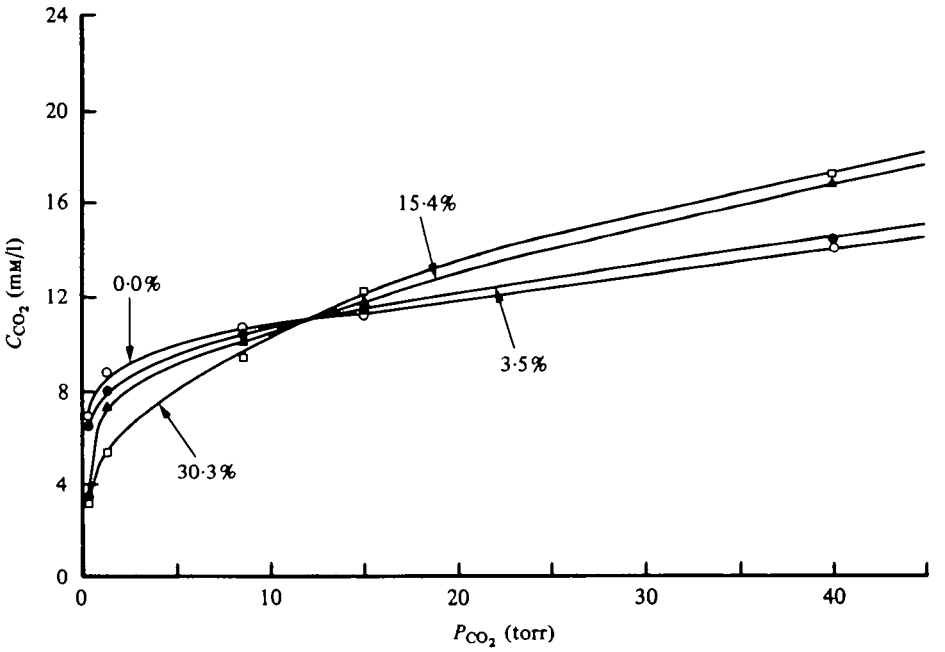

Fig. 9. Typical *in vitro* relationships between CO_2 tension (P_{CO_2}) and whole blood CO_2 content (C_{CO_2}) (i.e. 'CO_2-combining curves') for homogeneous rainbow trout blood pooled from three animals and made up to different haematocrits. Data are from a different example from that shown in Figs. 6, 7, 8 and the original blood was not equilibrated prior to separation and reconstitution.

true plasma (compare Fig. 6A and B). This difference is clearly evident in the slopes of the linear regression relationships of whole blood and true plasma β against both Ht and [Hb] (Table 3).

A representative set of CO_2-combining curves for homogeneous whole blood of different Ht's is shown in Fig. 9. The data are from a different experiment than in

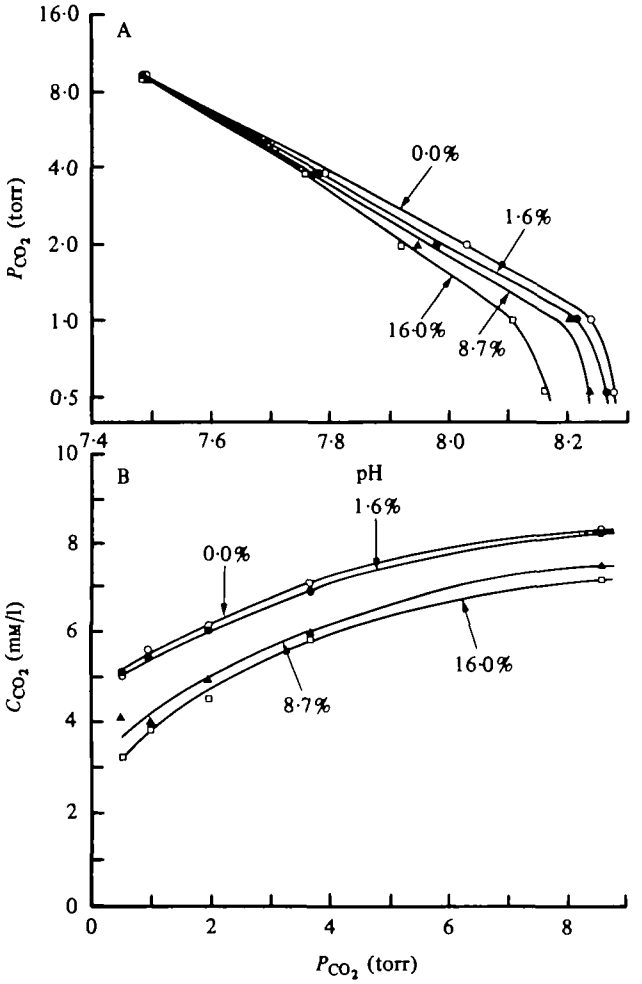

Fig. 10. Typical relationships between (A) pH and CO_2 tension (P_{CO_2}, logarithmic scale) and between (B) P_{CO_2} and whole blood CO_2 content (C_{CO_2}) (i.e. 'CO_2-combining curves') for homogeneous starry flounder blood made up to different haematocrits. The data in (A) and (B) are taken from the same set of equilibrations. The original blood was not equilibrated prior to separation and reconstitution; the low pH and high P_{CO_2} of the common intersection point in Fig. 9A reflected the *in vivo* conditions at the time of blood sampling.

Figs. 6 and 7. The curves typically crossed over so that low Ht blood had a higher C_{CO_2} at low P_{CO_2} (physiological range), with the reverse at high P_{CO_2} (Fig. 9). The crossover resulted from three interacting factors: the change in relative HCO_3^- distribution across the erythrocyte with pH, the greater buffer capacity of high Ht blood which thereby lessened the pH change, and the greater erythrocytic contribution to total volume with high Ht whole blood. The crossover was therefore often not as discrete as in Fig. 9. Furthermore, the position of the crossover on the P_{CO_2} axis was influenced by the original P_{CO_2} of the blood at the time of separation (although always considerably higher than this value). Finally, the position of the curves on the C_{CO_2} axis was a function of the $[HCO_3^-]$ of the original blood.

(ii) *Platichthys stellatus*. While the flounder *in vitro* studies were less extensive and dealt only with whole blood, they showed similar basic trends as the trout

data. Fig. 10 presents typical results. Blood buffering was again a function of Ht (Fig. 10A, Table 3) though the slope of the regression relationship of β against Ht was much lower than in trout (Table 3). The intersection point of the buffer curves was always at a very high P_{CO_2} (8–12 torr; e.g. Fig. 10A), reflecting the P_{CO_2} of the original blood taken from the caudal vein of anaesthetized, air-exposed flounder. The CO_2-combining curves (Fig. 10B) were only measured in the physiological range of P_{CO_2}, and showed an inverse relationship between Ht and C_{CO_2}, as in trout blood (cf. Fig. 9).

DISCUSSION

The present study has shown that severe experimental anaemia induces a rise in P_{CO_2} and a resultant drop in pH (respiratory acidosis) in the arterial and venous blood of both *Platichthys stellatus* and *Salmo gairdneri*. In the latter, the pH depression is augmented by lactic acid release (metabolic acidosis) in some animals. In both, the buffer capacity of the blood falls with anaemia, an effect which must exacerbate the acidosis. The two species therefore respond to anaemia in a similar way, and differences are only quantitative.

It is difficult to explain the disagreement between the present results and those of Haswell & Randall (1978) on trout, for very similar methodology was used. The most notable difference is that Haswell & Randall (1978) simply recorded P_{a,CO_2} and pH_a in different fish at a variety of Ht's while we employed a paired experimental design which allowed each fish (at normal Ht) to serve as its own control for the effects of anaemia. The actual changes in acid–base status occurring during anaemia in trout are relatively small, and therefore our technique had a better chance of detecting them. Nevertheless, using the approach of Haswell & Randall (1978), we were still able to detect the acid–base disturbance during anaemia in the flounder. Additionally, the data of Cameron & Randall (1972) on trout also show a clear negative correlation between Ht and P_{a,CO_2}, though these authors do not comment on the trend.

The results therefore indicate that the teleost erythrocyte does play a role in CO_2 excretion, presumably via dehydration of plasma HCO_3^- by erythrocytic carbonic anhydrase. This implies that the red cell is permeable to HCO_3^-. Our *in vitro* studies on trout blood, showing a change in HCO_3^- distribution across the erythrocyte with pH, lend qualified support to this position. From measurements of whole blood and true plasma C_{CO_2} and Ht, it is possible to calculate the Donnan ratio for HCO_3^- ($r_{HCO_3^-}$) across the red blood cell membrane (cf. McDonald, Boutilier & Toews, 1980). From plots such as those in Fig. 6, this calculation has been performed at a range of pH for the five trout blood samples with highest Ht; the higher the Ht, the greater the reliability of the estimate. Interpolation of the buffer lines to common pH (7·0, 7·2, 7·4, 7·6, 7·8) has been employed to allow averaging of the results. The major assumptions are that the water content of plasma is 95% and that of the erythrocyte is 70% (Eddy, 1974), that the physical solubility of CO_2 is the same in plasma and erythrocytic fluid, and that carbamino-CO_2 is negligible. Nothing concrete is known about the latter two assumptions in fish (Albers, 1970; Eddy, 1974); errors here will affect the absolute value of the $r_{HCO_3^-}$ but should have minor influence on any relationship with pH.

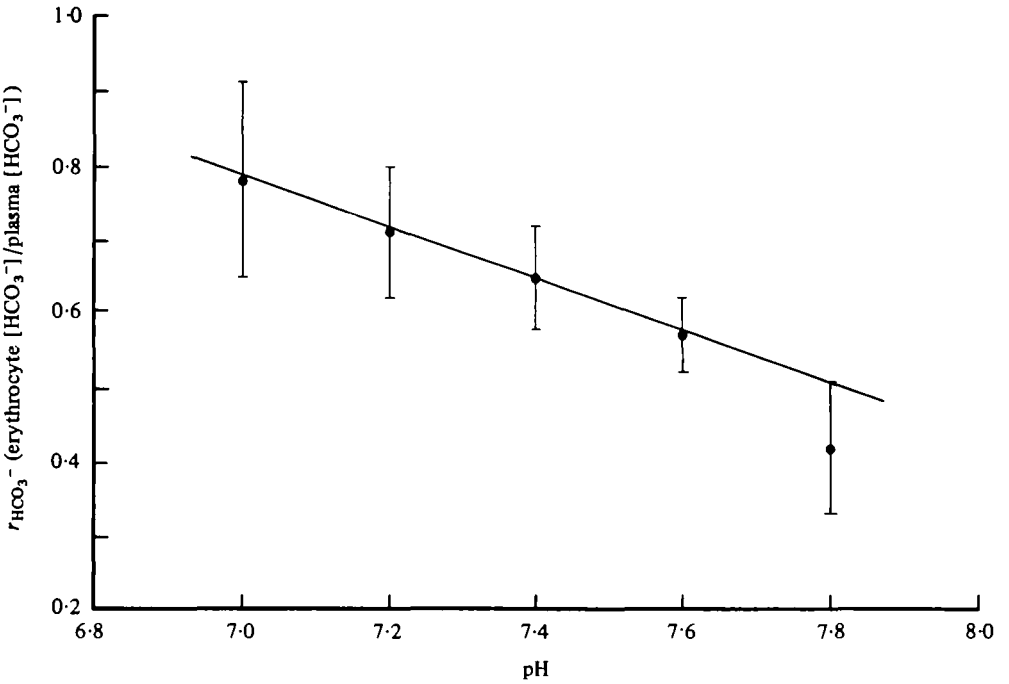

Fig. 11. The relationship between pH and the Donnan ratio ($r_{HCO_3^-}$; means ± 1 S.E.) for HCO_3^- distribution across the erythrocyte membrane in the blood of rainbow trout *in vitro*. The line fitted is the regression relationship for the data from pH 7·0 to 7·6: $r_{HCO_3^-} = -0{\cdot}345$ pH $+3{\cdot}196$ ($N = 4$, $r = 0{\cdot}99$, $P < 0{\cdot}001$). See text for additional details.

Over the range of pH from 7·0 to 7·6, the results show a linear variation of $r_{HCO_3^-}$ with pH (Fig. 11), which would indicate a passive distribution of HCO_3^-, and therefore permeability of the erythrocyte to HCO_3^- (Siggaard-Andersen, 1974). This evidence says nothing about the physiological timing of the event because the tonometry period was 2 h, adequate for the establishment of the HCO_3^- distribution with or without catalysis by carbonic anhydrase. Unfortunately at higher, more physiological pH's, the results are unclear. At pH = 7·8, the calculated value is below the relationship defined by the other points. It is impossible to tell whether this deviation reflects measurement error or the start of true curvilinearity, though we favour the former explanation. At pH's above 7·8, there was so little HCO_3^- in the blood as to produce occasional spurious negative values for erythrocytic [HCO_3^-], and therefore render meaningless the calculation of $r_{HCO_3^-}$.

The present data are in rough quantitative agreement with the early measurements of $r_{HCO_3^-}$ in trout blood by Ferguson & Black (1941), but not with those of Eddy (1974). The reason for this difference is unknown. Nevertheless, all three investigations show a rise in $r_{HCO_3^-}$ as pH falls. In the present study, the slope of the variation with pH ($-0{\cdot}34$) and the absolute value of the Donnan ratio (0·64) at the usual reference pH of 7·4 are virtually identical to those in mammalian systems (Siggaard-Andersen, 1974; Reeves, 1976).

Cameron (1978) has shown that there is a classical chloride shift in teleost blood which can be completely blocked by the carbonic anhydrase inhibitor acetazolamide. Haswell *et al.* (1978) have found that chloride is passively distributed across the

teleost red cell membrane. Obaid, Critz & Crandall (1979) have demonstrated a Cl^-/HCO_3^- exchange across the elasmobranch red blood cell and catalysis of plasma [HCO_3^-] to CO_2 by dogfish erythrocytic carbonic anhydrase. Combination of these results with the present findings that red cell removal causes CO_2 retention *in vivo*, and that HCO_3^- seems to be passively distributed *in vitro*, provides a strong argument against the theory of Haswell & Randall (1978).

Two pieces of evidence remain in favour of the concept that the teleost red cell is functionally impermeable to HCO_3^- and therefore plays no role in CO_2 excretion. The first is that Haswell & Randall (1978) found no effect of severe anaemia on net CO_2 excretion in trout. The weakness here is that the measurements were made 24 h after the induction of anaemia. It seems likely that anaemia would cause a transitory disturbance of net CO_2 excretion. The rise in blood P_{CO_2}, together with the increased delivery rate of physically dissolved CO_2 to the gills by the elevated cardiac output during anaemia (Cameron & Davis, 1970; Wood *et al.* 1979*b*) would quickly return CO_2 excretion to normal. For example, Randall & Wood (1981) found that while carbonic anhydrase was important in CO_2 excretion in crabs, no change in resting CO_2 excretion could be detected 12–14 h after blockade of the enzyme with acetazolamide because of a compensatory rise in the diffusion gradient. The second piece of evidence is that both trout and *Tilapia* red blood cells incubated with homologous plasma *in vitro* failed to dehydrate added HCO_3^- (Haswell & Randall, 1976; Haswell *et al.* 1978). This evidence is difficult to discount, because rat red blood cells did provide normal catalysis in the same assay. Further work will be needed to solve this problem. However, we believe the weight of evidence shows that the fish red cell catalyses CO_2 excretion in the standard mammalian fashion. This does not mean that catalysis within the gill epithelium (Haswell *et al.* 1980) is unimportant; indeed, it seems likely that both sites are involved, providing the system with considerable flexibility. This may explain why blood P_{CO_2} and pH levels were not disturbed until rather severe levels of anaemia in the present study.

The *in vitro* results on acid–base and CO_2-combining characteristics of trout and flounder blood at different Ht's deserve little further comment, for they follow the standard pattern defined for mammalian blood (Siggaard-Andersen, 1974). However, one point which is clearly emphasized by the data is the importance of knowing, and if possible standardizing, the acid–base characteristics and Ht (or [Hb]) of the original blood stock prior to tonometry. These factors can so markedly influence the position, form, and slope of buffer and CO_2-combining curves as to make comparisons virtually meaningless unless this information is known.

The regression relationships between β and Ht or [Hb] in trout and flounder (Table 3) are presented in the hope they may be of use to other workers performing Davenport (1974) diagram-type analyses of acid–base changes in these species (e.g. Wood *et al.* 1977; McDonald, Hōbe & Wood, 1980; McDonald, Boutilier & Toews, 1980). The present trout relationship of β *vs.* Ht determined on pooled blood is similar to but probably more accurate than that reported by McDonald, Hōbe & Wood (1980) for individual blood samples from the same species. The two relationships give very similar values of β over the normal physiological range of Ht. However in our experience Ht can fluctuate greatly due simply to swelling of the erythrocytes with acid–base disturbance, so the use of [Hb] as an index of β is to be preferred in future studies.

We thank the Director, Dr A. O. D. Willows, and staff of Friday Harbor Laboratories, University of Washington, for their assistance and hospitality. Financial support was provided by grants from the National Research Council of Canada and the Natural Sciences and Engineering Research Council of Canada to B.R.M. and C.M.W.

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