

AN ANALYSIS OF CHANGES IN BLOOD pH FOLLOWING EXHAUSTING ACTIVITY IN THE STARRY FLOUNDER, *PLATICHTHYS STELLATUS*

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SUMMARY

Exhausting activity results in a marked and immediate drop in blood pH which gradually returns to normal over the following 6 h. The acidosis is caused largely by elevated P_{CO_2} levels, which vary inversely with pH. Blood lactate concentration increases slowly, reaching a maximum at 2-4 h post-exercise, and contributes significantly to the acidosis only late in the recovery period. The slow time course of lactic acid release into the blood permits temporal separation of the peak metabolic acidosis from the peak respiratory acidosis. Evidence is presented that a metabolic acid other than lactic also makes a modest contribution to the pH depression during the recovery period.

INTRODUCTION

In man, strenuous exercise results in the accumulation of lactic acid in the blood stream. The change in blood lactate concentration is rapid; a peak is reached within a few minutes post-exercise, and resting levels are re-established in about 1 h (Bang, 1936; Crescitelli & Taylor, 1944). The accompanying disturbance in the acid/base status of the blood appears largely attributable to this lactacidosis. There is a strong correlation between blood lactate concentration and pH, and the increase in base deficit can be almost entirely accounted for by the rise in lactic acid (Laug, 1934; Visser, Kreukniet & Maas, 1964; Keul, Keppler & Doll, 1967). Hyperventilation during exercise causes a slight decrease in $P_{\text{a,CO}_2}$, even in the face of a small increase in $P_{\text{v,CO}_2}$ due to elevated tissue metabolism, but the influence of these P_{CO_2} alterations on blood pH is minimal (Astrand & Rodahl, 1970).

In fish, the levels of blood lactate ultimately attained after exercise are similar to those in mammals, but the time course of the response is much slower (Secondat & Diaz, 1942; Auvergnat & Secondat, 1942; Black *et al.* 1959; Black, Manning & Hayashi, 1966; Piiper, Meyer & Drees, 1972). Peak concentrations are reached in 2-4 h, and up to 24 h may be required for a complete return to resting levels. In general, blood pH reaches a minimum before the lactate peak and returns to normal

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over a faster time course. Relationships between blood $[\text{HCO}_3^-]$ and lactate appear variable. Despite these inconsistencies, previous workers have offered an essentially mammalian interpretation of their data - i.e. the increase in lactic acid causes a drop in pH and a decrease in $[\text{HCO}_3^-]$. The possible influence of changes in blood P_{CO_2} has been ignored. Nevertheless, evidence is available that both $P_{\text{a,CO}_2}$ and $P_{\text{v,CO}_2}$ may increase during and after swimming activity in fish (Stevens & Randall, 1967; Piiper *et al.* 1972), although the absolute changes are small (a few mmHg). There is a linear/log relationship between pH and P_{CO_2} ; changes in P_{CO_2} around 2 mmHg (fish levels) will have a marked effect on pH compared with changes of the same magnitude around 40 mmHg (mammalian levels) (Randall & Cameron, 1973).

The aim of the present investigation was, therefore, to examine the relative roles of lactic acid and P_{CO_2} levels in changing blood pH following strenuous activity in a teleost fish. *Platichthys stellatus* was chosen for study as it can be quickly exercised to exhaustion by manual chasing, and can be chronically cannulated without difficulty. In lactate studies, the importance of blood sampling via indwelling cannulae (i.e. without disturbance to the animal) has recently been documented (Driedzic & Kiceniuk, 1976).

MATERIALS AND METHODS

Starry flounders (*Platichthys stellatus* Pallas; 300–800 g) were collected by otter trawl from East Sound of Orcas Island and held in large sand covered tanks for at least 10 days before use at Friday Harbor Laboratories, University of Washington. The acclimation conditions were those employed in subsequent experiments: running sea water, salinity = $27 \pm 1\text{‰}$, temperature = $9 \pm 1^\circ\text{C}$.

I. In vitro experiments

In vitro determinations of CO_2 combining curves and buffer capacities were performed on heparinized whole blood taken by blind puncture of the haemal arch. Samples were placed in 50 ml tonometer shaker flasks (5 ml/flask) at $9 \pm 0.5^\circ\text{C}$ and gassed for at least 2.5 h with humidified mixtures of CO_2 in air supplied by Wosthoff mixing pumps fed from analysed gas cylinders.

II. In vivo experiments

For all *in vivo* experiments, the fish ($N = 6$) were anaesthetized on an operating table with 1:15000 MS-222 and the caudal vein chronically catheterized with Clay-Adams PE 50 as described by Watters & Smith (1973). In one animal, the caudal artery was similarly cannulated. The wound was dusted with the fish antibiotic 'Furanace' (Nifurpironol, Dainippon Pharmaceutical) and closed with silk sutures. The animals were then transferred to individual chambers (30 × 30 cm and 15 cm deep) and allowed to recover for at least 72 h. The chambers were shielded from the investigators and were filled to a depth of 6 cm with fine beach sand. As in the main holding tanks, the flounder in the experimental chambers remained typically buried with only the eyes and mouth exposed. The use of sand was important, for fish held in bare tanks exhibited a good deal of spontaneous activity which would have confounded the objectives of this study.

Several initial blood samples, totalling about 1.0 ml, were drawn from the cathet

Before the imposition of swimming activity. The animal was then quickly transferred to a larger tank (100 × 100 cm and 20 cm deep) and exercised by manual chasing for 10 min. By the end of the exercise period, all fish became completely refractory to stimulation and were unable to right themselves, indicating that this procedure produced exhaustion. A blood sample (0.5 ml) was taken immediately post-exercise (time 0), and the flounder returned to its individual compartment. Additional samples (0.5 ml) were removed at 20 min, 1 h, 2 h, 4 h, 6 h, and 24 h. Blood samples were taken without any apparent disturbance to the animal.

III. Analytical procedures

Blood was drawn anaerobically. All *in vitro* samples were analysed for pH, total CO_2 , and haematocrit. All *in vivo* samples were assayed for pH, lactate, and haematocrit. The initial blood samples from each animal were analysed for P_{CO_2} and total CO_2 . In two fish, total CO_2 measurements were also performed on the post-exercise series of samples.

Lactate analyses were performed on 250 μl blood samples immediately deproteinized in 500 μl ice-cold perchloric acid. The supernatant was analysed enzymatically (lactic dehydrogenase) for L-lactate with Sigma reagents (see Sigma bulletin no. 826-UV). Blood pH's were determined by injecting 50 μl aliquots directly into a Radiometer micro-electrode connected to a Radiometer PHM 71 acid-base analyser and thermostatted to the experimental temperature. Measurements of blood P_{CO_2} were performed on 200 μl samples with a thermostatted Radiometer CO_2 electrode fitted with a thin silicone membrane and connected to the PHM 71. P_{CO_2} determinations were relatively imprecise due to the necessity of using high meter gain and a long response time (8–10 min) for the low P_{CO_2} levels in fish blood at 9 °C. Each sample was bracketed by calibration gas standards from analysed cylinders, and multiple determinations were performed on each fish. Nevertheless, error may have reached $\pm 20\%$. Blood total CO_2 levels (25–50 μl samples) were assayed by the micro-method of Cameron (1971), using a Teflon membrane on the P_{CO_2} electrode for greater stability and bracketing each unknown by sodium bicarbonate standards. Haematocrits were measured by centrifuging 50 μl blood samples in ammonium heparinized capillary tubes at 5000 g for 5 min.

IV. Theoretical approach

To evaluate the relative contributions of alterations in P_{CO_2} and lactic acid to the total pH change observed, an approach similar to that outlined by Woodbury (1965) and Davenport (1974) was adopted. A Davenport diagram (e.g. Fig. 1), which relates pH to $[\text{HCO}_3^-]$ at various P_{CO_2} 's was constructed using the Henderson-Hasselbalch equation:

$$\text{pH} = \text{pK}^1 + \log \frac{[\text{HCO}_3^-]}{\alpha \text{CO}_2 \cdot P_{\text{CO}_2}},$$

where pK^1 is the negative logarithm of the apparent first dissociation constant of carbonic acid and αCO_2 is the physical solubility of CO_2 in plasma. $[\text{HCO}_3^-]$ equals the difference between total CO_2 concentration and $\alpha \text{CO}_2 \cdot P_{\text{CO}_2}$. As the ionic activity of fish plasma is fairly close to that of mammalian plasma (Albers, 1970), values for

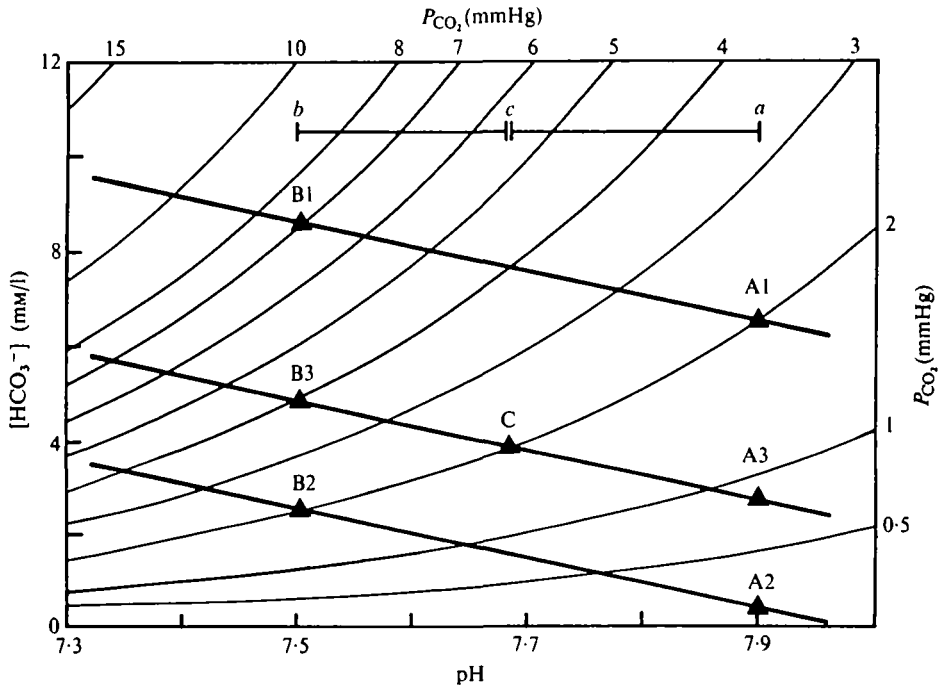


Fig. 1. Davenport diagram for flounder blood relating $[\text{HCO}_3^-]$, pH, and P_{CO_2} (isopleths in mmHg). Parallel lines A_1B_1 , A_2B_2 , and A_3B_3 represent buffer lines of slope β as determined *in vitro*. The figure illustrates the method employed in determining the fraction of a total pH drop (horizontal distance ab) which would have been due to lactic acid changes alone (ac/ab) and the additional fraction due to P_{CO_2} changes (bc/ab). A_1 = initial point at rest. A_1B_1 : pH drop is due solely to P_{CO_2} increase. A_2B_2 : pH drop is due solely to lactic acid increase. A_3B_3 : pH drop is due to both factors. See text for further details.

pK^1 and αCO_2 at the experimental temperature and pH were taken from tabulated mammalian figures (Severinghaus, 1965; Albers, 1970).

Blood is a complex buffering system containing both non-bicarbonate (protein) and bicarbonate buffers. A change in P_{CO_2} will titrate only the non-bicarbonate buffers; the slope of this buffer line ($\beta = \Delta[\text{HCO}_3^-]/\Delta\text{pH}$) can be determined *in vitro* by equilibrating whole blood at various P_{CO_2} 's (Piiper *et al.* 1972). However, the addition of any non-carbonic acid (e.g. lactic acid) to blood will titrate both the carbonic acid and protein buffers; the amount of added acid will be proportional, but not equal, to the decrease in $[\text{HCO}_3^-]$.

The initial *in vivo* blood sample from the resting animal is plotted as point A_1 (Fig. 1). A buffer line with slope β is plotted through A_1 . If a post-exercise decrease in pH (horizontal distance ab) is due solely to a change in P_{CO_2} , then the blood will simply be titrated along the buffer line to point B_1 . On the other hand, if the same post-exercise change in pH (ab) is due solely to an increase in lactic acid, then the blood will be titrated along a constant P_{CO_2} isopleth to B_2 . A second buffer line with slope β may be constructed through B_2 . At the original pH on this second buffer line (point A_2), the vertical distance A_1A_2 equals the amount of lactic acid added (in $[\text{HCO}_3^-]$ equivalents). The vertical distance A_1B_2 (the actual decrease in $[\text{HCO}_3^-]$) is the amount buffered by the carbonic acid system, and A_2B_2 ($\Delta\text{pH} \times \beta$) is the amount buffered by proteins.

However, it is more probable that *in vivo*, a pH change (*ab*) will have both a lactic acid and a P_{CO_2} component. Metabolically, L-lactate and H^+ ions are produced and removed in equivalent quantities (Piiper *et al.* 1972) so the amount of added lactic acid added can be considered equal to that of the measured L-lactate ion. This measured change in lactic acid can be plotted (in $[\text{HCO}_3^-]$ equivalents) as vertical distance A_1A_3 . Another buffer line with slope β can be plotted through A_3 ; this line will intersect the final pH at point B_3 . The intersection of this buffer line with the original P_{CO_2} isopleth indicates the point (C) which would have been reached due to the increase in lactic acid alone in the absence of a P_{CO_2} change. The additional effect of a P_{CO_2} rise causes the pH to decrease further to B_3 . Therefore *ac* is the pH drop which would have been caused by the rise in lactic acid alone, and *bc* the additional decrement due to the P_{CO_2} increase.

If the observed point B_3 does not agree with the point B_3 predicted by the above procedure, then the situation is more complex than the assumptions of the analysis. For example, acids other than lactic may be entering the blood (observed B_3 below predicted B_3), or the blood may be losing and/or accumulating HCO_3^- and H^+ ions at differential rates so as to either raise pH (observed B_3 above predicted B_3) or lower pH (observed B_3 below predicted B_3) at the *in vivo* P_{CO_2} . Nevertheless, *ac/ab* will still equal the fraction of the total pH change which would have been caused by the increase in lactic acid alone.

The above approach was originally designed for the mammalian system. However, it depends only on physico-chemical principles which should be identical in fish and mammalian blood. Therefore its application to the fish system appears valid, subject to the accuracies of the values of pK^1 and αCO_2 employed.

RESULTS

I. In vitro experiments

CO_2 combining curves in the physiological range of P_{CO_2} for fish were determined on five different batches of whole blood ranging in mean haematocrit from 1% to 16%. Results for the 16% haematocrit (pooled blood from four animals) are shown in Fig. 2A and the form is typical of all experiments. The only variation was a tendency for upward displacement of the curve (i.e. an increase in CO_2 combining capacity) as haematocrit decreased. Within each batch of blood, haematocrit increased with P_{CO_2} ; in the example shown (Fig. 2A), haematocrit rose from 14.5% to 17.4% between $P_{\text{CO}_2} = 0.52$ and 8.64 mmHg.

For each batch of blood, the non-bicarbonate buffer value (β) was determined as the slope of the line relating $[\text{HCO}_3^-]$ to pH (e.g. Fig. 2B). β varied with haematocrit – i.e. $\beta = -3.6 \text{ mm l}^{-1} \text{ pH}^{-1}$ at 1% haematocrit, $-4.2 \text{ mm l}^{-1} \text{ pH}^{-1}$ at 9%, and $-5.2 \text{ mm l}^{-1} \text{ pH}^{-1}$ at 16%. The latter value ($\beta = -5.2 \text{ mm l}^{-1} \text{ pH}^{-1}$) was the figure employed in the subsequent analysis of the *in vivo* results, as the mean initial and overall haematocrits of the animals in these experiments were $16.1 \pm 1.2\%$ (6) [$\bar{x} \pm 1 \text{ S.E. (N)}$] and $14.2 \pm 1.0\%$ (6) respectively (Fig. 3). The temporal changes in haematocrit were small enough to render the use of a single value for β an insignificant source of error. The log/linear relationship between P_{CO_2} and pH in flounder blood

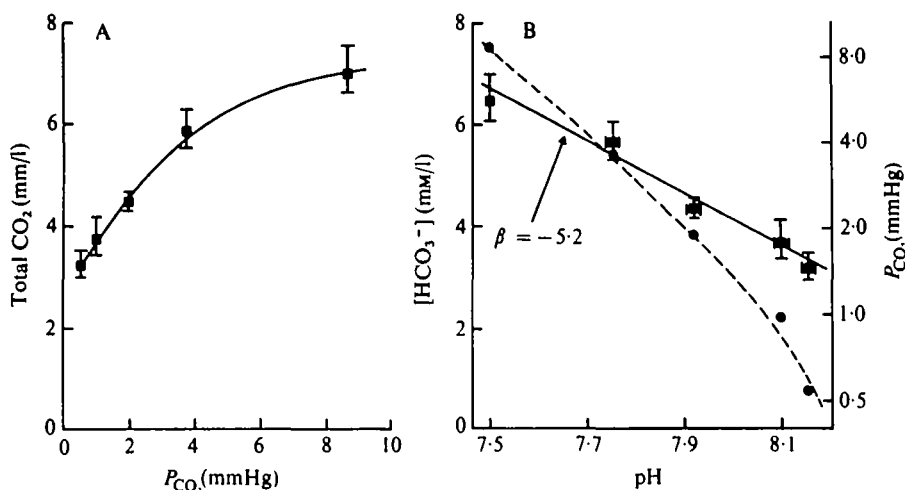


Fig. 2. (A) CO₂ combining curves and (B) buffer lines for [HCO₃⁻]/pH (■) and log P_{CO₂}/pH (●) in flounder blood *in vitro*. Pooled blood from four animals. Mean haematocrit = 16.0%. Means and range shown for 3–4 determinations (total CO₂, [HCO₃⁻]) or 2–3 determinations (pH) at each point. The slope of the [HCO₃⁻]/pH buffer line ($\beta = -5.2 \text{ mM l}^{-1} \text{ pH}^{-1}$) was used in the subsequent analysis of the *in vivo* results.

is also shown in Fig. 2B, and illustrates the marked effect on pH of P_{CO₂} variations in the physiological range.

II. *In vivo* experiments

The initial lactate concentration in the venous blood of resting flounder ($N = 6$) was extremely low ($0.28 \pm 0.06 \text{ mM l}^{-1}$) and after 10 min of exhausting exercise had risen only about 1.8 fold (Fig. 3D). However, lactate levels continued to rise during recovery to a peak of 6–7 times the resting level at 2–4 h. By 24 h, the resting concentration was re-established.

Venous pH exhibited a very different pattern, dropping dramatically from 7.900 (6) to 7.516 (6) immediately post-exercise (Fig. 3C), and then slowly increasing towards the resting value during recovery, despite the accompanying rise in lactate. At 6 h, pH remained slightly depressed, but had returned to normal at 24 h. In the one fish with both arterial and venous cannulae, arterial pH varied in a similar pattern to venous pH, although the absolute changes were smaller (Fig. 3C). Consequently, the $a-v$ difference in pH increased from 0.030 at rest to about 0.180 at 0 and 20 min, and then gradually returned to normal at 6 and 24 h. Arterial blood was analysed for lactate at time 0, 20 min, and 4 h. Within the error of the assay, no differences could be detected between arterial and venous lactate concentrations.

Despite the diluting effect of repetitive blood sampling, haematocrit actually increased slightly at 0 and 20 min after exercise (Fig. 3E). Haematocrit declined at subsequent sample times during recovery.

The initial venous P_{CO₂} in resting flounder was 2.88 ± 0.20 (6) mmHg as determined directly with a P_{CO₂} electrode and the initial total venous CO₂ content was 7.26 ± 0.13 (6) mm l⁻¹. Using the Henderson–Hasselbalch equation, it was also possible to calculate P_{CO₂} from the measured values of total CO₂ and pH for each fish. The resulting estimate, 2.21 ± 0.14 (6) mmHg was lower than the direct measurement, but the

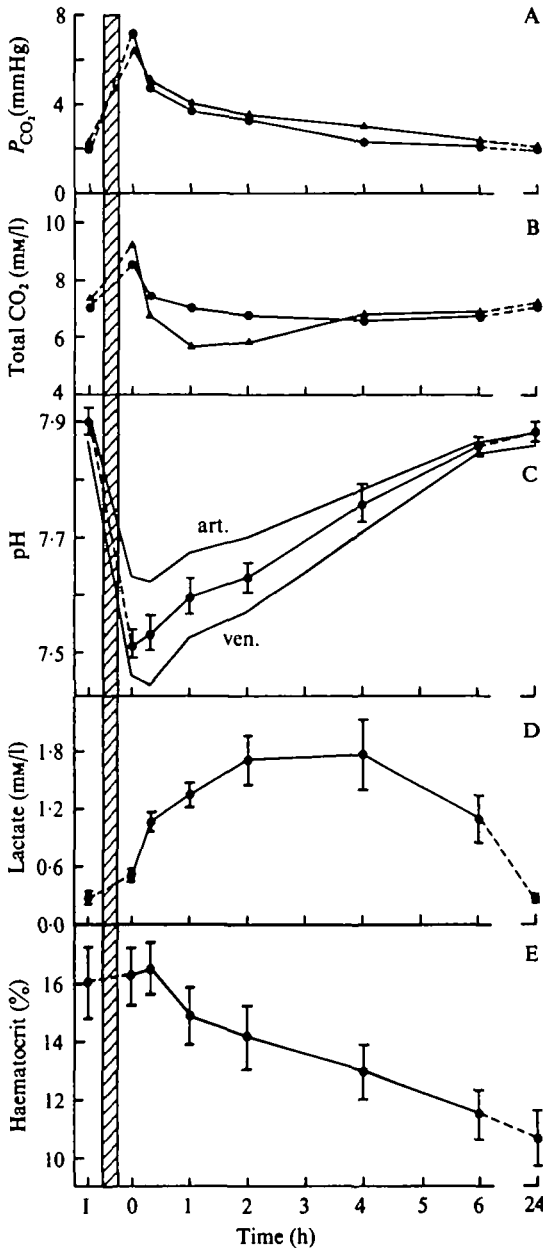


Fig. 3. (A) P_{CO_2} (as calculated by the Henderson-Hasselbalch equation) and (B) total CO_2 concentration in the venous blood of two flounders (● = 800 g, ▲ = 450 g); average (C) pH, (D) lactate concentration, and (E) haematocrit in the venous blood of six flounders (means \pm 1 S.E.) before and after 10 min of exhausting activity. In (C) simultaneous measurements of arterial and venous pH in a single flounder (330 g) are indicated by plain lines. Activity = bar. I = initial resting sample. Time 0 = immediately post-exercise.

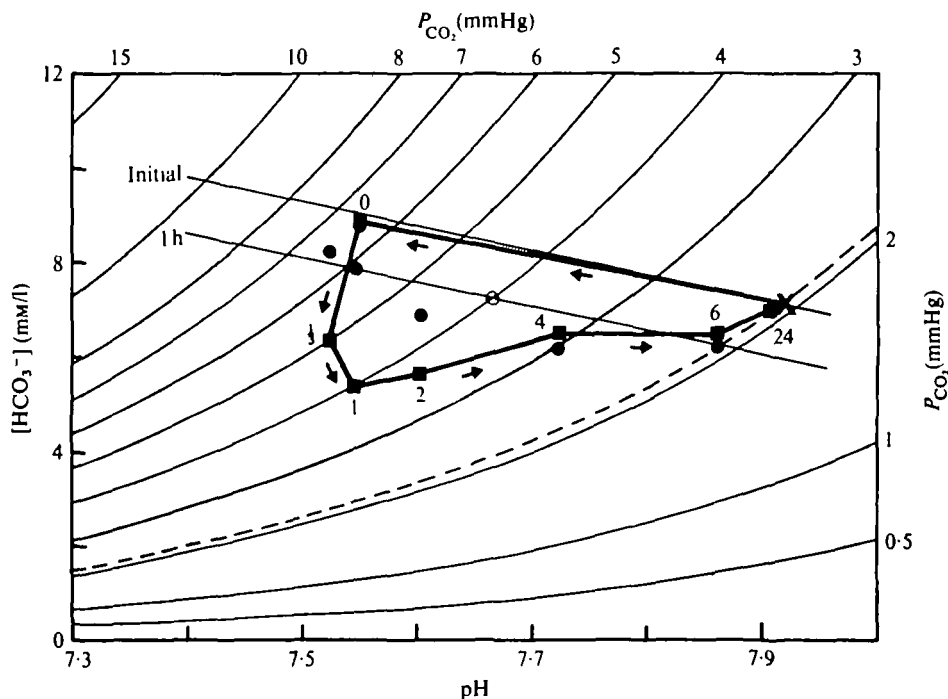


Fig. 4. Davenport diagram display of temporal changes in venous blood acid/base status after 10 min of exhausting activity in a single flounder (weight = 450 g). ■ = observed points; ● = points predicted by the analysis. Note good agreement between observed and predicted points at 0, 4, 6 and 24 h, but not at $\frac{1}{2}$, 1 and 2 h. X = initial resting point. Broken line indicates initial $P_{CO_2} = 2.1$ mmHg isopleth. For illustrative purposes, the initial position of the buffer line and its position at 1 h as predicted by the analysis have been included. ○ indicates point on $P_{CO_2} = 4$ mmHg isopleth which would have been reached at 1 h if the pH depression at this time were solely due to the measured lactic acid concentration and a $P_{CO_2} = 4$ mmHg. See text for further details.

difference was of marginal significance for both statistical ($0.05 < P < 0.10$ by paired Student's two-tailed t -test) and methodological reasons (the errors involved in direct P_{CO_2} determination in fish blood at low temperatures). Thus Henderson-Hasselbalch calculations based on measured pH and total CO_2 appear to give reasonable estimates of P_{CO_2} in flounder blood. On this basis, P_{CO_2} 's were computed at each sample time for the two fish in which total CO_2 contents were determined throughout the experimental period.

The results were similar for the two animals (Fig. 3 A, B). Venous P_{CO_2} increased markedly from about 2.0 mmHg, at rest, to 6.5 mmHg immediately after activity. P_{CO_2} levels gradually declined during recovery, but remained slightly elevated even at 6 h. By 24 h, they had returned to normal. The time course of changes in pH appeared to be inversely correlated with variations in P_{CO_2} , and not with those in lactate. Total CO_2 in venous blood was elevated at time 0 in concert with the large P_{CO_2} increase, but subsequently decreased below resting levels despite continuing high P_{CO_2} levels. This drop in total CO_2 appeared to be at least partially due to the slow post-exercise rise in lactic acid, and was more pronounced in the fish demonstrating higher lactate concentrations. The depression of total CO_2 persisted slightly at 6 h, but had disappeared by 24 h.

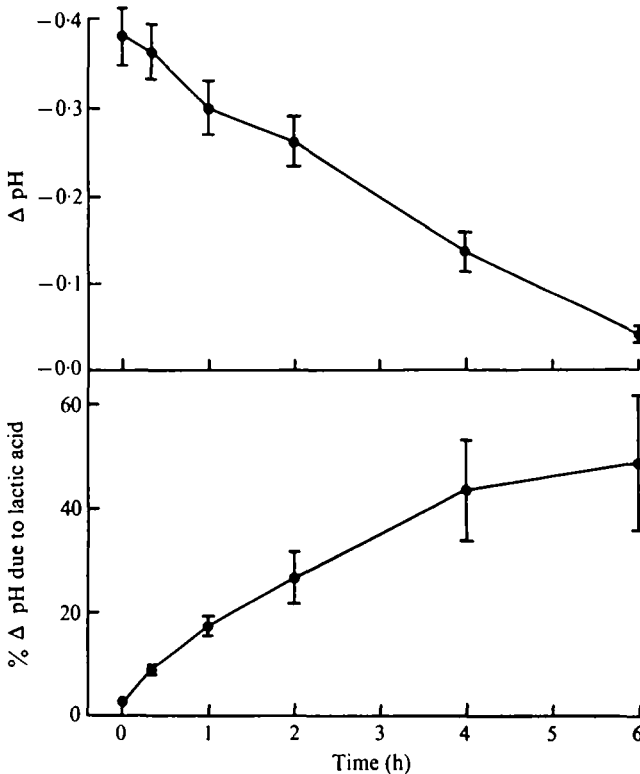


Fig. 5. Total change in venous pH from initial resting value and percentage of this change due to the measured increase in lactic acid concentration alone between 0 and 6 h after 10 min of exhausting activity in *Platichthys stellatus*. Means \pm 1 S.E. $N = 6$.

The complete Davenport diagram analysis, as outlined in Methods (Fig. 1), was applied to these two fish for which values for P_{CO_2} and total CO_2 (and thus $[HCO_3^-]$) were available in addition to lactate and pH data throughout the experimental period (Fig. 4). Again, results were very similar for the two animals. Immediately after exercise (time 0) and later in the recovery period (4 and 6 h), there was good agreement between the observed values of $[HCO_3^-]$ and those predicted by the analysis on the basis of an acidosis resulting entirely from the combined effects of blood P_{CO_2} and lactic acid alterations (Fig. 4). At time 0, when the lactate increase was slight (Fig. 3 D), this factor caused almost none of the large drop in pH ($< 3\%$; i.e. ac/ab of Fig. 1). The abrupt rise in P_{CO_2} at this time was responsible for virtually all of the acidosis (i.e. bc/ab). The animal simply moved up the buffer line to the new P_{CO_2} (Fig. 4), so the increase in HCO_3^- was about equal to the product of Δ pH and β . At intermediate times (20 min, 1 h, 2 h) the contribution of lactic acid (ac/ab) to the total pH change remained modest ($< 26\%$) but the observed values of $[HCO_3^-]$ were considerably lower than the predicted figures (Fig. 4). Under these circumstances, the remainder of the pH change (bc/ab) could not be attributed unequivocally to P_{CO_2} variation alone (further consideration is given to this discrepancy in the Discussion). Nevertheless, calculations indicate that the prolonged elevation of P_{CO_2} above resting levels (Fig. 3 A) remained responsible for at least 50% of the total pH depression at these sample times. By 4 and 6 h, the pH decrement was

much reduced, but the rise in lactate (Fig. 3 D) and relative fall in P_{CO_2} (Fig. 3 A) mean that the contribution of lactic acid (*ac/ab*) to this reduced acidosis had become significant (e.g. 51% at 4 h in the example shown in Fig. 4), although P_{CO_2} remained important.

In the other four fish (for which complete P_{CO_2} and total CO_2 data were lacking) agreement between observed and predicted $[\text{HCO}_3^-]$'s could not be examined. Nevertheless, initial P_{CO_2} and total CO_2 figures were available for these animals, so it was still possible to determine that fraction of the total pH change due to lactic acid alone (*ac/ab*) at each sample time. The results were generally similar to those for the first two fish, and the averaged data for all six are presented in Fig. 5. The mean lactate contribution to the total pH change increased from $2.3 \pm 0.5\%$ at time 0 to $48.3 \pm 12.8\%$ at 6 h. However, the magnitude of the total pH change markedly decreased over this period (Fig. 5), so the overall influence of lactic acid on blood pH was minor. The large variability at 4 and 6 h reflects the fact that the absolute pH change, on which the calculations were based, had become relatively small by these times (Figs. 3 C, 5).

DISCUSSION

As Piiper *et al.* (1972) have pointed out, use of the whole blood buffer value (β), as in the present study, rather than the true plasma buffer value, is to be preferred for a quantitative analysis of H^+ ion balance in whole blood. Lactate concentrations were also measured in whole blood. On the other hand, the Henderson-Hasselbalch equation, on which the Davenport diagram is based, is designed for use with true plasma data. The negative relationship between CO_2 combining capacity and haematocrit *in vitro* suggests that as in the trout (Eddy, 1974), $[\text{HCO}_3^-]$ inside the erythrocyte of the flounder is low compared with that in plasma (Wood, McMahon & McDonald, unpublished results). Corrections on this basis would result in improved agreement between the initial measured P_{CO_2} 's and those calculated by the Henderson-Hasselbalch equation, but would have negligible quantitative effect on the conclusions of the study (i.e. the percentage of the total pH drop due to lactic acid (Fig. 5)).

The post-exercise fall in blood pH of the flounder is clearly of different genesis from that of the mammal. In the latter, the metabolic component (i.e. lactic acid) is of prime importance, whereas in the flounder, the respiratory component (i.e. P_{CO_2}) is dominant, at least in venous blood. The overall contribution of lactic acid is small, and becomes significant only late in recovery when elevated P_{CO_2} 's have declined.

The occurrence of a marked respiratory acidosis immediately after exercise may explain the strategy behind the slow time course of post-exercise changes in blood lactic acid in fish. This delay appears to be due to slow transfer of lactate between the bloodstream and the major glycolytic site, white muscle (Black *et al.* 1962; Stevens & Black, 1966). The circulation to white muscle may actually be impaired following strenuous activity in fish (Hayashi, 1961, in Black *et al.* 1962; Wood & Randall, 1973). If the lactate release into the bloodstream were rapid as in mammals, then the peak metabolic acidosis would coincide with the peak respiratory acidosis, resulting in a possibly fatal depression of blood pH. By temporally separating the two phenomena, the fish is able to maintain blood H^+ ion activity within tolerable limits. These considerations are especially important in view of the generally low buffer capacities of fish blood (Albers, 1970).

Both the resting and post-exercise maximum levels of lactate in *Platichthys stellatus* were considerably lower than previously reported values in other fish (e.g. Secondat & Diaz, 1942; Black *et al.* 1959, 1962, 1966; Stevens & Black, 1966; Piiper *et al.* 1972). These differences could be of methodological origin or reflect real inter-species variation. In the present study, care was taken to ensure that the animals were completely quiet both before and after exercise, and blood samples were drawn without disturbance via indwelling cannulae (cf. Driedzic & Kiceniuk, 1976).

The procedure used to exhaust the flounder would have had little effect on many other teleosts, and one wonders why the exercise capacity is so low. Inadequacy of O_2 transport does not appear to be the primary factor, for there was little difference in exercise performance and post-exercise lactate levels between normal and severely anaemic flounder (Wood, McMahon & McDonald, unpublished results). The answer may be that the drastic rise in P_{CO_2} during activity quickly reduces pH to a level which limits vital metabolic reactions. High P_{CO_2} and low pH may cause secondary problems in O_2 delivery – e.g. the Bohr and Root effects (Riggs, 1970), decreased myocardial contractility (Poupa & Johansen, 1975), and increased blood viscosity associated with erythrocytic swelling (Ferguson & Black, 1941; Eddy, 1974). This swelling was clearly seen in the present study, for haematocrit increased with high P_{CO_2} *in vitro*, and *in vivo* at the times of maximum P_{CO_2} (Fig. 3 E).

The pH data for the single animal in which both arterial and venous measurements were made (Fig. 3 C) strongly indicate that P_{a,CO_2} varied in similar fashion to P_{v,CO_2} , although the absolute changes were smaller. From the log P_{CO_2} /pH relationship of Fig. 2 B, it can be roughly estimated that P_{a,CO_2} rose from 2.1 to 5.5 mmHg immediately after exercise, and P_{v,CO_2} from 2.4 to 9.0 mmHg, resulting in an increased $a-v$ pH difference. P_{a,CO_2} also increased after exhausting activity in the dogfish (Piiper *et al.* 1972).

The large elevation of P_{v,CO_2} levels after activity in the flounder is explicable by high rates of tissue CO_2 production. Because the CO_2 combining curve of flounder blood markedly flattens above about 4 mmHg P_{CO_2} (Fig. 2 A), rather small increases in total CO_2 content will be associated with large increases in P_{v,CO_2} . An increase in P_{a,CO_2} after exercise may reflect a limitation of CO_2 excretion due to a decrease in blood residence time at the gills associated with an elevated cardiac output (Cameron & Polhemus, 1974).

In Results, it was noted that at 20 min, 1 h and 2 h, the observed $[HCO_3^-]$'s were considerably lower than those predicted by the analysis (Fig. 4). A number of possible explanations exist. Firstly, it is conceivable, though unlikely, that the addition of lactic acid to the blood may have altered β , so that the predicted $[HCO_3^-]$ was in error (Davenport, 1974). However, trout blood showed no change in β after addition of comparable amounts of hydrochloric acid (Eddy, 1976). A second possibility would be a selective removal of the lactate ion from the bloodstream unaccompanied by an H^+ ion; we are aware of no evidence that this ever occurs. Manipulation of H^+ and/or HCO_3^- fluxes between the blood and the tissues or external environment (branchial, urinary fluxes) so as to adjust pH in the face of elevated P_{CO_2} 's is another explanation (Piiper *et al.* 1972; Cameron, 1976; Randall, Heisler & Drees, 1976). However, this also seems most improbable because the net effect appears disadvantageous – e.g. in Fig. 4 at 1 h the pH (7.545) at the observed

Table 1. *Changes in the concentration of lactic acid and a hypothetical metabolic acid^a at 0-6 h after exhausting activity in Platichtys stellatus*

Time (h)	Δ Lactic acid (mm l ⁻¹)	Δ Hypothetical acid (mm l ⁻¹)
0	+0.23	+0.35
$\frac{1}{2}$	+0.70	+1.39
1	+0.96	+1.52
2	+1.28	+0.99
4	+1.26	-0.04
6	+0.69	-0.14

Mean values for the two animals of Fig. 3A, B. Δ Hypothetical acid was calculated as the difference between the observed $[\text{HCO}_3^-]$ and the $[\text{HCO}_3^-]$ predicted by the Davenport diagram analysis at the measured pH (see Figs. 1, 4).

$P_{\text{CO}_2} = 4$ mmHg was lower than it would have been at this P_{CO_2} had there been no such adjustment (7.668). The most likely explanation is that some other metabolic acid(s) besides lactic was entering the blood in significant amounts at these sample times, resulting in depression of the buffer line and an additional base deficit. On this assumption, the concentration of this hypothetical acid in the blood at each sample time has been calculated as the difference in base deficit (Table 1).

The substance apparently occurs in similar amounts to lactic acid, but enters and leaves the blood over a more rapid time course (Table 1). A peak is reached at about 1 h, and the substance is undetectable by 4 h. As with lactic acid, its contribution to the acidosis is modest, and raised P_{CO_2} levels remain responsible for at least 50% of the total pH depression at 20 min, 1 h and 2 h. The identity of this hypothetical acid(s) is unknown, but it is interesting to note that Driedzic & Hochachka (1976) have recently reported evidence for the release of NH_4^+ ions from white muscle after strenuous activity in the carp. Ammonium chloride produces metabolic acidosis in mammals (Davenport, 1974).

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