# Inter relationships between acid base, ionic and water balance in Rainbow Trout (*Salmo gairdneri*) exercised until fatigue occurred

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ABSTRACT

*Rainbow trout* (Salmo gairdneri) *were exercised at greater than* 3.5 l/sec *until fatigue occurred. Severe exercise induced marked disturbances in acid base, ionic and water balance. Immediately after fatigue the drop in* pH *was due to the combined effects of increased* $P_{CO_2}$ *and metabolic acid. But the bulk of the increase in* $H^+$ *buffered by blood was caused by an increase in metabolic acid. The peak in this contribution, occurring at* 1 *hour post exercise corresponded to the peak lactate concentration. However, there was a considerable quantitative discrepancy between* $|H^+|$ *and* $|L^-|$. *At* 2 *hours post exercise* $|L^-|$ *exceeded* $|H^+|$ *by a maximum volue of* 10.4 *mequiv/l.*

*A rapid increase in plasma volume during recovery following fatigue was accompanied by increases in total* $Na^+$ *and* $Cl^-$ *contents of over* 50% *of the respective resting values. This increase in* $Na^+$ *and* $Cl^-$ *contents occurred presumably via branchial* $Na^+/H^+$ *and* $Cl^-/HCO^-_3$ *ion exchange mechanisms.*

*It seems most likely that a large portion of the metabolic protons found in deficit in blood during recovery was eliminated into the environmental water through appropriate adjustment of* $Na^+ / H^+$ *and* $Cl^- / HCO_3^-$ *exchanges functioning at the gill epithelium. The rest of the metabolic protons contributing to the deficit were most likely exchanged for intracellular* $K^+$.

## 1. Introduction

High values of lactic acid concentrations upto 16mmol/l have been measured in the blood of various freshwater teleosts following severe muscular

activity (Black, 1957; Black *et al*, 1960, 1966; Miller *et al*, 1959). It is evident that lactic acid formation implies the generation of equivalent amounts of $H^+$ and lactate ions. In fact, measurements in the blood of man during and after exercise have indicated that the decrease of base excess or increase of metabolic acid load is about equal to increase of lactate concentration (Keul *et al*, 1967; Turrel and Robinson, 1942; Bouhuys *et al*, 1966).

In the invertebrates and lower vertebrates the situation is thought to be quite different. The quantity of lactate in the blood following activity has been found to be either in excess (e.g. dogfish - Piiper *et al*, 1972; crab-McDonald *et al*, 1979; Wood and Randall, 1981) or in deficit (e.g. flounder-Wood *et al*, 1977; *Cryptobranchus*-Boutilier *et al*, 1980; *Bufo* - Mc Donald *et al*, 1980) to the quantity of metabolic protons buffered by the blood.

The deficit of $H^+$ in blood was attributed to preferential proton retention by the muscle. The accumulation of metabolic protons in excess of lactate in blood was thought to be due to a more rapid efflux of protons from the muscle or the presence of a metabolic acid other than lactic acid.

Apart from the changes in acid base balance, exercise may also have an effect on water and electrolyte balance. Exercise in fish is accompanied by increased functional surface area of the gills, which facilitates greater respiratory gas exchange which in turn cause a concomitant increase in ion and water transfer across the gills. Therefore in the present experiments, the problem of the post exercise discrepancy between metabolic $H^+$ and lactate ions were studied in the context of water and electrolyte balance.

## 2. Materials and Methods

### 2.1 Experimental animals

Experiments were carried out on rainbow trout (*Salmo gairdneri* ) of weight 200-300g. and length 23-35cm. They were maintained in dechlorinated, aerated tapwater at 15 ± 1°C. The fish were fed on commercial trout pellets until two days prior to the experiments. Fish were anaesthetized by immersion in water containing 1g/l tricaine methanesulphonate (MS-222). The dorsal aortic catheter was implanted as described by Smith and Bell (1964). This consisted of a 30cm length of PP60 (Portex, polypropylene tubing) terminating in a 1.5cm

length of huber point 21 G needle. After cannulation the fish were left undisturbed for atleast 24 hours to recover (Hunn and Willford 1970,). 12-14 hours before the start of an experiment a fish was transferred to the swim tunnel.

## 2.2 **Experimental protocol**

Individual fish were exercised at greater than 3.5 l/sec until fatigue occurred. The fish was considered fatigued when by repeated efforts it could no longer hold itself off the grid at the downstream end of the swim tunnel. Blood samples were taken at rest, immediately after fatigue and 1, 2, 3, 4, 6, 8 and 24 hours during recovery. Blood samples were obtained by holding heparinized capillary tubes to the end of the cannula and allowing blood to flow freely into them. In one series of experiments (n=6) approximately 200$\mu$l of blood was removed at each sampling time for measurement of blood acid base parameters; pH, total $CO_2$ and lactate concentrations. In the second series of experiments (n=6) approximately 150$\mu$l of blood was removed at each sampling time for determination of blood volume, haematocrit and plasma $Na^+$, $K^+$ and $Cl^-$ concentrations.

## 2.3 **Analytical procedure**

Acid base balance

Blood pH was determined using 30$\mu$l samples with a Radiometer blood microsystem (type BMS 2b) connected to a Radiometer acid base analyser (PHM 71). Blood and plasma total $CO_2$ measurements were made using an electrode and cuvette system described by Cameron (1971). Plasma was obtained by centrifugation (5000g for 3min) of blood samples in heparinized capillary tubes using a Hawksley haematocrit centrifuge. Lactate analyses were performed on 100$\mu$l blood samples using Sigma reagent kit (no. 826-UV). Assays were carried out on a Cecil spectrophotometer.

Ionic and water balance

Plasma volume was measured using $^{51}Cr$ (sodium chromate, Amersham International Ltd.) labelled erythrocytes as the radioactive marker in the dilution technique. $Na^+$ and $K^+$ concentrations of plasma were determined using a Corning 400 flame photometer. $Cl^-$concentrations were measured by potentiometric titration (Ramsay *et al*, 1955).

## 2.4 Calculations

$P_{CO_2}$ of blood was calculated for each blood sample from, the Henderson-Hasselbalch equation using plasma $|HCC^{-}_{3}|$ and pH values. $|HCO^{-}_{3}|$ of plasma was calculated from the formula : $|HCO^{-}_{3}| = C_{CO_2} - \alpha CO_2 PCO_2$ where $C_{CO_2}$ is plasma $CO_2$ content. Values for $\alpha CO_2$ at the experimental temperature were taken from tabulated figures (Albers, 1970; Severinghaus *et al* 1955a,b). Buffering capacity ($\beta$) at various haematocrit (Ht) values were estimated from the following regression equation.

$$\beta = -0.258\,Ht - 4.018$$

To evaluate the relative contribution of alterations in $P_{CO_2}$ ($|\Delta H^{+}_{c}|$) and metabolic acid ($|\Delta H^{+}_{m}|$) to the total pH change observed, an approach similar to that outlined by Davenport (1969) and Wood *et al* (1977) was adopted.

The plasma volume at each sampling time was obtained from blood volume and the haematocrit (Ht) by the following formula.

$$\text{blood volume} = \text{plasma volume} \times \frac{100}{100-Ht}$$

Ion contents of plasma were obtained from the following relationship,

ion content = plasma volume x ion concentration

Ion contents and plasma volumes are presented as percentage changes from the resting values. When finding the changes, the resting values ($A_o$) were normalized to 100 and the results at various experimental times ($A_i$) were expressed as percentage changes from the resting values.

$$\text{Change in ion content or plasma volume} = \frac{A_i}{A_o} \times 100$$

## 2.5 Treatment of data

Results are generally presented as mean $\pm$ standard error of the mean. Differences between the resting values and values at various experimental times

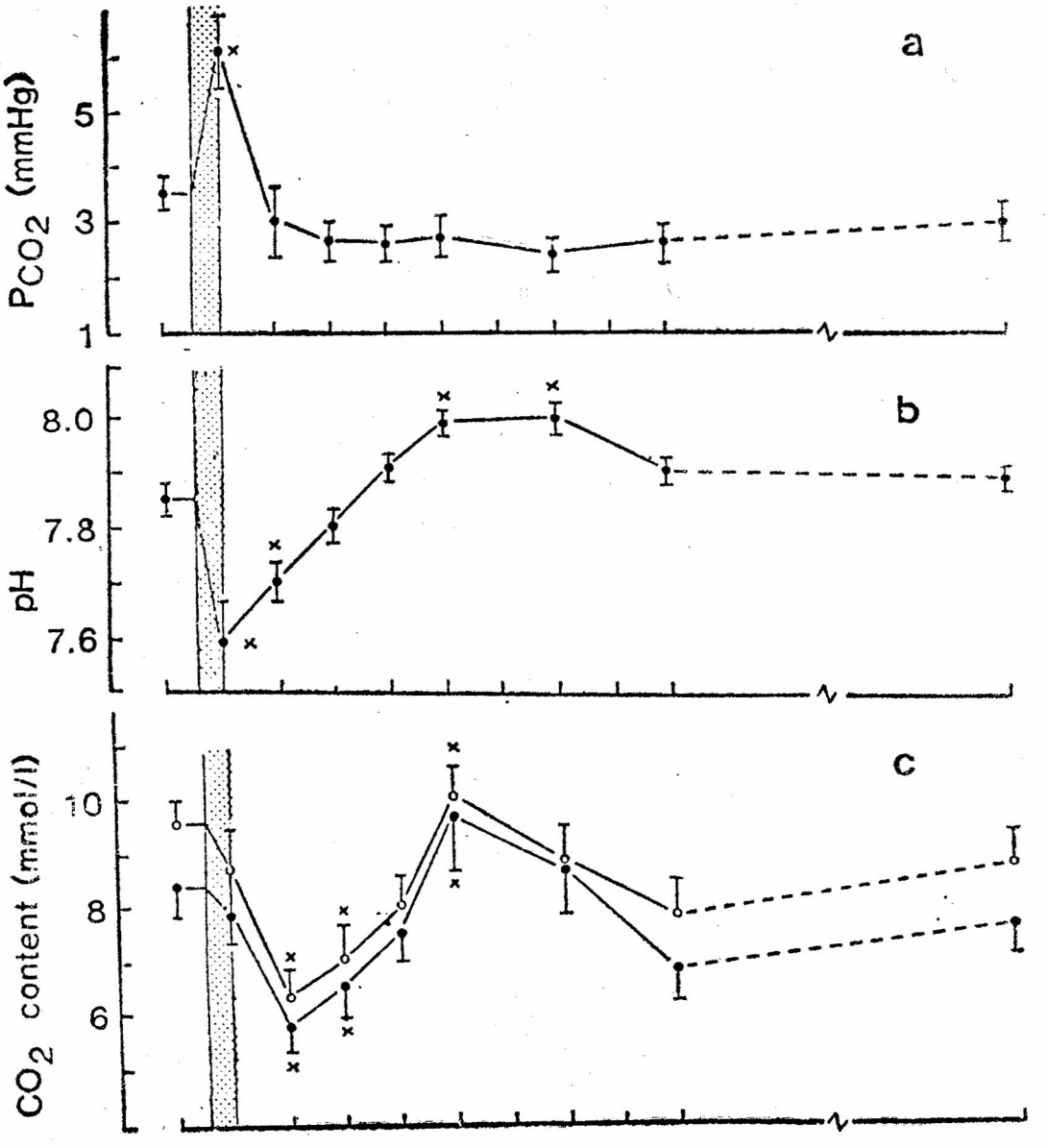


*Fig.* 1 (a) $PCO_2$; (b) pH: (c) CO content of plasma (o) and blood ( ● ) and (d) lactate concentration at rest and during recovery. R = resting sample. Time O = immediately after fatigue. Shaded area = exercise period. X indicates experimental values significantly different ( P0.05 ) from the resting value.

were determined using the one tailed t-test for paired variates. In this analysis each experimental sample is paired with the initial sample at rest which serves as the control. Thus the experimental and control values for the 6 fish in an experiment constitute a set of paired variates. For each set variation between the experimental and initial means are tested.

## 3. Results

Severe exercise in rainbow trout caused marked disturbances in acid base, ionic and water balance.

### 3.1 Acid base balance

The mean velocity of swimming at which fatigue occurred was 4.1 $\pm$ 0.27 l/sec in the first series of experiments where blood samples were obtained for analysis of acid base parameters. The mean duration of swimming was 24.1 $\pm$ 6.8 minutes. The mean changes in pH, total $CO_2$ content, $P_{CO_2}$ and lactate concentration in arterial blood at rest and during recovery from severe exercise leading to fatigue are shown in fig. 1.

In fig. 2 mean $|HCO_3^-|$ of plasma are plotted against pH in a Davenport diagram for each blood sampling time. Immediately after fatigue the drop in pH was due to the combined effects of increased $P_{CO_2}$ and metabolic acid. The $|HCO_3^-|$ represent the sum of two opposing processes, i.e. an increase in $|HCO_3^-|$ accompanying the elevation of $P_{CO_2}$ and a decline in $|HCO_3^-|$ resulting from the addition of metabolic acids to the blood. After 1 hourof recovery $|HCO_3^-|$ continued to fall as a result of lactic acid concentration increasing considerably. During further recovery $|HCO_3^-|$ increased along the $P_{CO_2}$ 2.6 mmHg isopleth which led to pH values higher than those seen in resting animals after 4 hours. Thereafter plasma $|HCO_3^-|$ and pH fell along the same $P_{CO_2}$ isopleth until 8 hours post exercise. After 24 hours, recovery had gone further with pH, $P_{CO_2}$ and $|HCO_3^-|$ levels approaching resting levels.

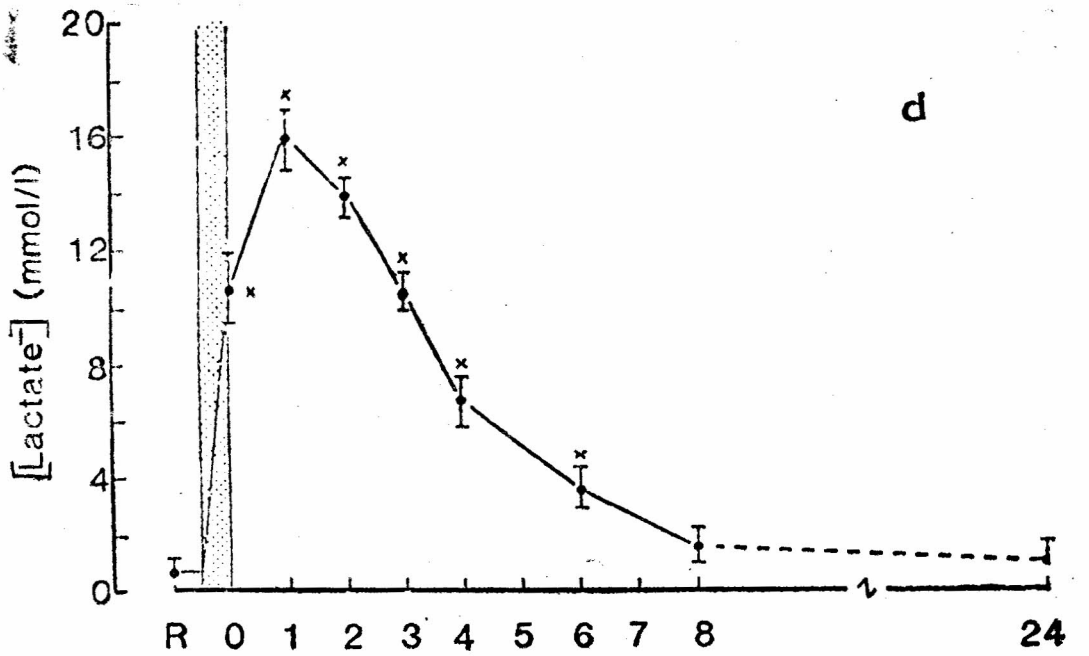


*Fig.* 1 (d) lactate concentration at rest and during recovery. R = resting sample. Time O = immediately after fatigue. Shaded area = exercise period. X indicates experimental values significantly different ( $p < 0.05$ ) from the resting value.

Respiratory and metabolic acid components of the post exercise acidosis were quantified as described in the methods. Fig. 3 shows the changes in lactate concentration ($|\Delta L^-|$), buffered metabolic acid ($|\Delta H_m^+|$) and carbonic acid ($|\Delta H_c^+|$). The contribution of $H^+$ to the total of buffered hydrogen ions was only 30.8% immediately after fatigue and had fallen to zero by 1 hour post exercise. The bulk of the increase in $H^+$ buffered by blood was caused by an increase in metabolic acid. The peak in this contribution, occurring at 1 hour post exercise, corresponded to the peak lactate concentration. However there was a considerable quantitative discrepancy between $|\Delta H_m^+|$ and $|\Delta L^-|$. At 1 hour post exercise $|\Delta L^-|$ exceeded $|\Delta H_m^+|$ by 6.4 m.equiv/l. This difference reached a maximum value of 10.4 m. equiv/l after 2 hours of recovery. Subsequently the levels of both declined and $|\Delta H_m^+|$ had fallen to zero by 4 hours post exercise but appeared again in blood in similar quantity to $|\Delta L^-|$ after 8 hours.

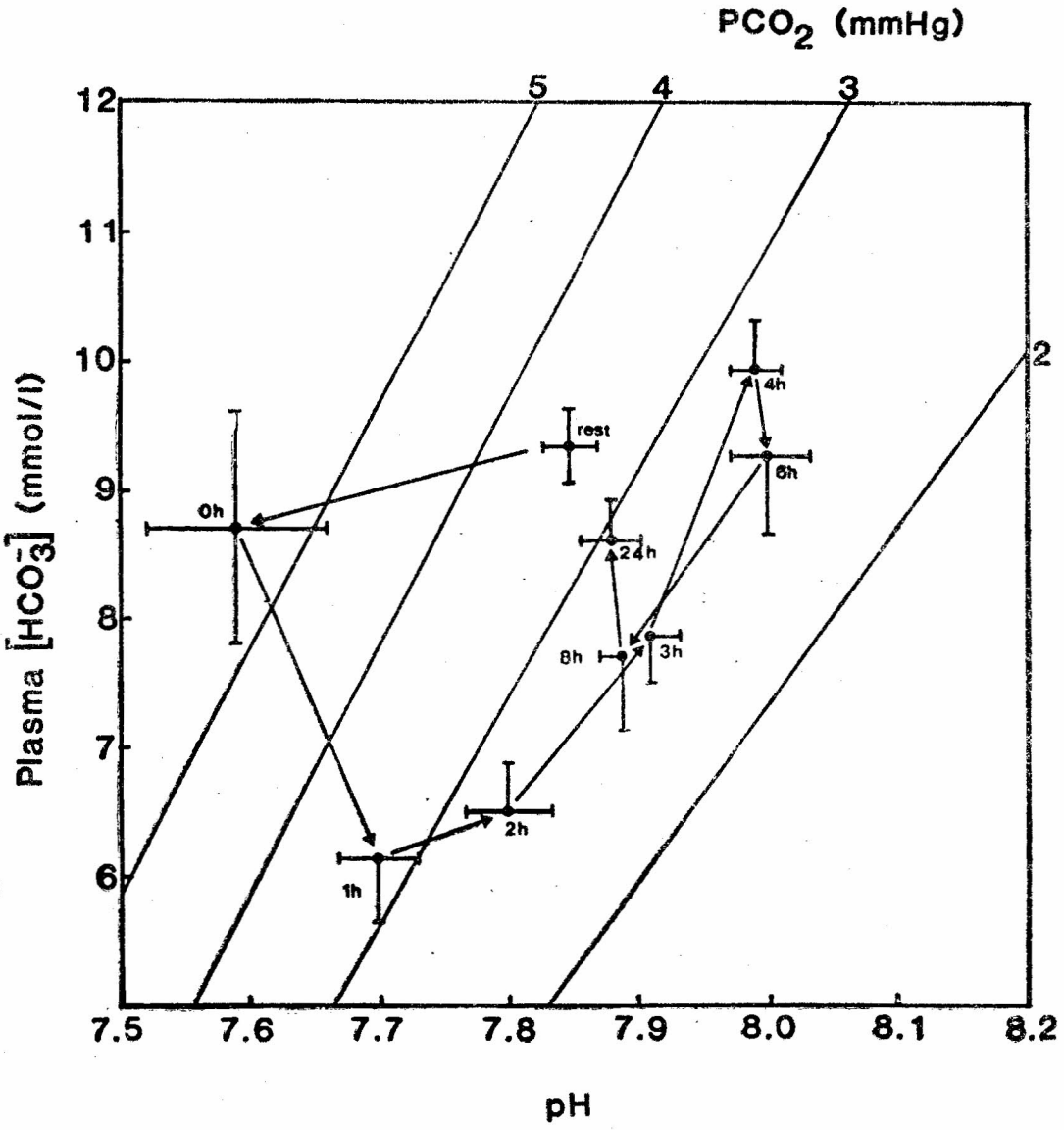


*Fig.* 2 Davenport diagram showing arterial blood acid base changes during recovery in the 6 animals exercised until fatigue (mean ± s. e. n=6). Time O = immediately after fatigue.

### 3.1 Ionic and water balance

The mean speed at which fatigue occurred in the second series of fish whose blood was analysed for ionic and water balance was 4.1 ± 0.23 l/sec. The mean duration of swimming was 25 ± 5.6 minutes. The mean changes in $Na^+$, $K^+$ and $Cl^-$

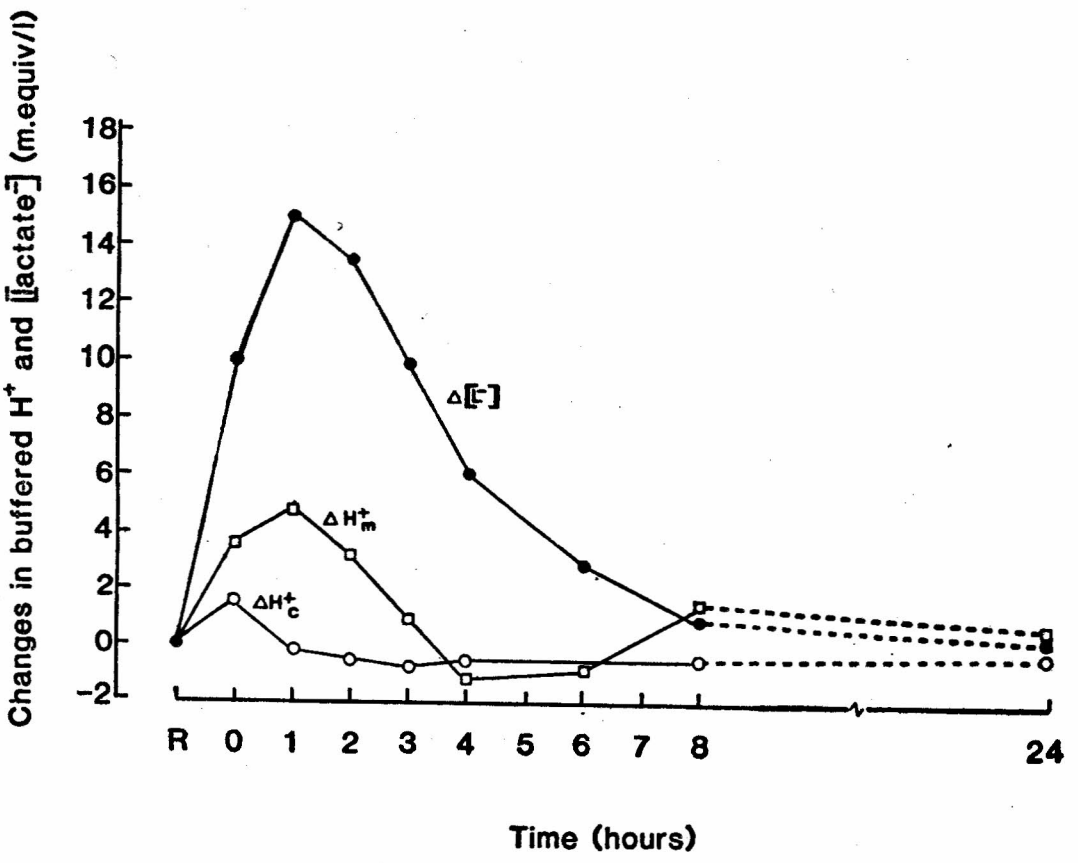


*Fig.* 3 Changes in lactate and buffering of $H+$ in arterial blood following exercise until fatigue $\Delta H^+_c$ is the changes in buffering of $H^+$ due to $P_{co_2}$. $\Delta H^+_m$ is the changes in buffering of $H^+$ due to metabolic acid and $|\Delta_{L^-}|$ is the changes in lactate anion concentration (mean of 6 fish).

concentrations in arterial blood at rest and during recovery from severe exercise leading to fatigue are shown in fig. 4a. Fig. 5 shows the mean changes in plasma volume and the ion contents during the experimental period.

The sum of $|Na^+|$ and $|K^+|$ in excess of the $|Cl^-|$ was calculated at each blood sampling time (fig. 4b). The concentration of $(Na^+ + K^+ - Cl^-)$ was $21.8 \pm 2.03$ mmol/l at rest. This value increased as a result of exhausting activity, reaching a maximum value of $34.0 \pm 2.1$ mmol/l ($p<0.05$) after 2 hours of recovery. This increase in $(Na^+ + K^+ - Cl^-)$ concentration was taken to match the $H^+$ deficit observed in the first series of experiments.

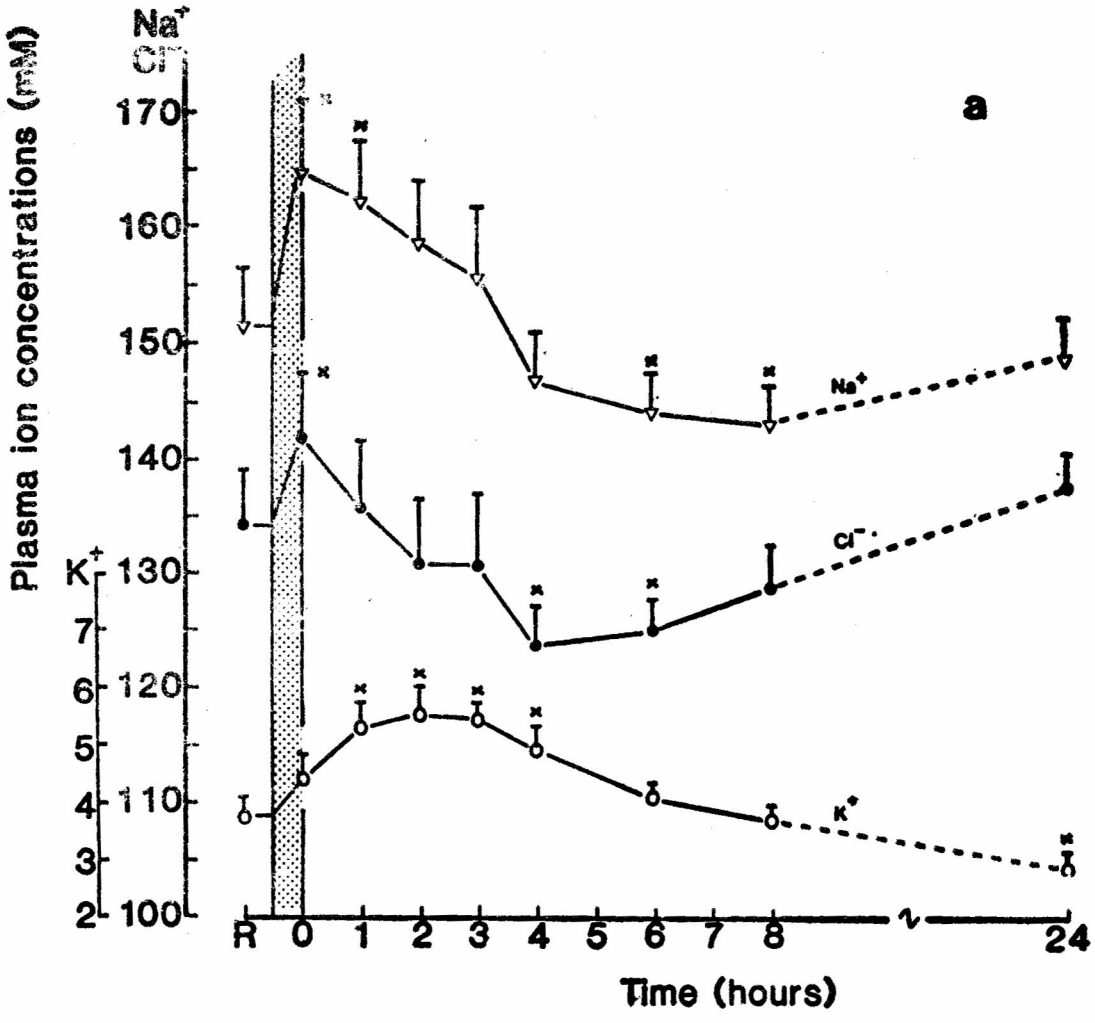


*Fig.* 4 (a) Plasma $Na^+$, $K^+$ and $Cl^-$ concentrations and (b) (see next page) Plasma ($Na^+ + K^+ - Cl^-$ concentration at rest and during recovery. X indicates experimental values significantly different ($P0.05$) from the resting values. R = resting value, Time O = immediately after fatigue. Shaded area = exercise period.

## 4. Discussion

Strenuous exercise provoked increases in aerobic and anaerobic metabolism resulting in the addition of respiratory and metabolic acids to the blood. Although $1\Delta H_c^+$ increased by 1.5 mequiv/l immediately after fatigue the fish was able to regulate this acidosis within 1 hour of its appearance in blood. The increase in $P_{CO_2}$, after fatigue, 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 and Polhemus, 1974).

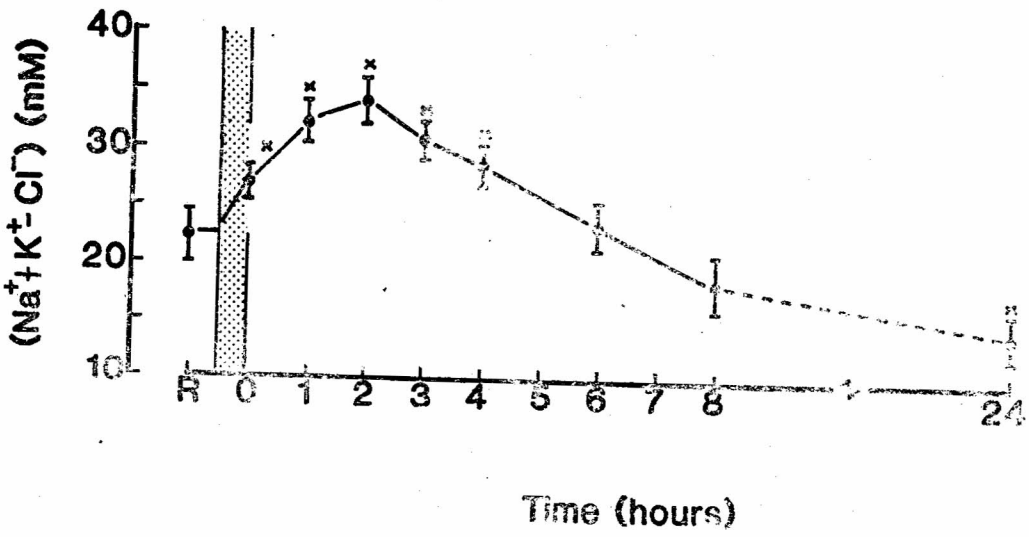


*Fig.* 4 (b) Plasma ($Na^+ + K^+ - Cl^-$) concentration at rest and during recovery X indicates experimental values significantly different (P 0.05) from the resting values. R = resting value, Time O = immediately after fatigue. Shaded area = exercise period.

The total metabolic and carbonic acid in blood reaches a maximum value Immediately after fatigue and this coincides with the maximum depression of pH. After 2 hours of recovery pH of blood reaches values not significantly different from the resting values although $|\Delta H_m^+|$ is 3.1 mequiv/l above the resting value. In fig. 3 it can be seen that $|\Delta H_m^+|$ reaches a peak value I hour after $|\Delta H_c^+|$ The separation of these two effects may be of survival importance to the fish. $|\Delta H_m^+|$ decreases gradually from the peak value reaching zero by the fourth hour. This decrease in $|\Delta H_m^+|$ may be due to utilization of the $H^+$ or uptake of $HCO_3^-$. During recovery from I to 4 hours the rates of disappearance of $|\Delta L^-|$ and $|\Delta H_m^+|$ are approximately similar, suggesting that $H^+$ buffered in the blood disappear in the metabolic consumption of lactate either by conversion to carbohydrate or by oxidation to $CO_2$ and water. Kobayashi and Wood (1980) found that only 2% of an added lactate load was excreted in rainbow trout following infusion of lactic acid into the blood stream. During recovery from 4 to 8 hours, the decrease in $|\Delta L^-|$ was not accompanied by a similar decrease in $|\Delta H_m^+|$ indicating addition of $H^+$ to blood.

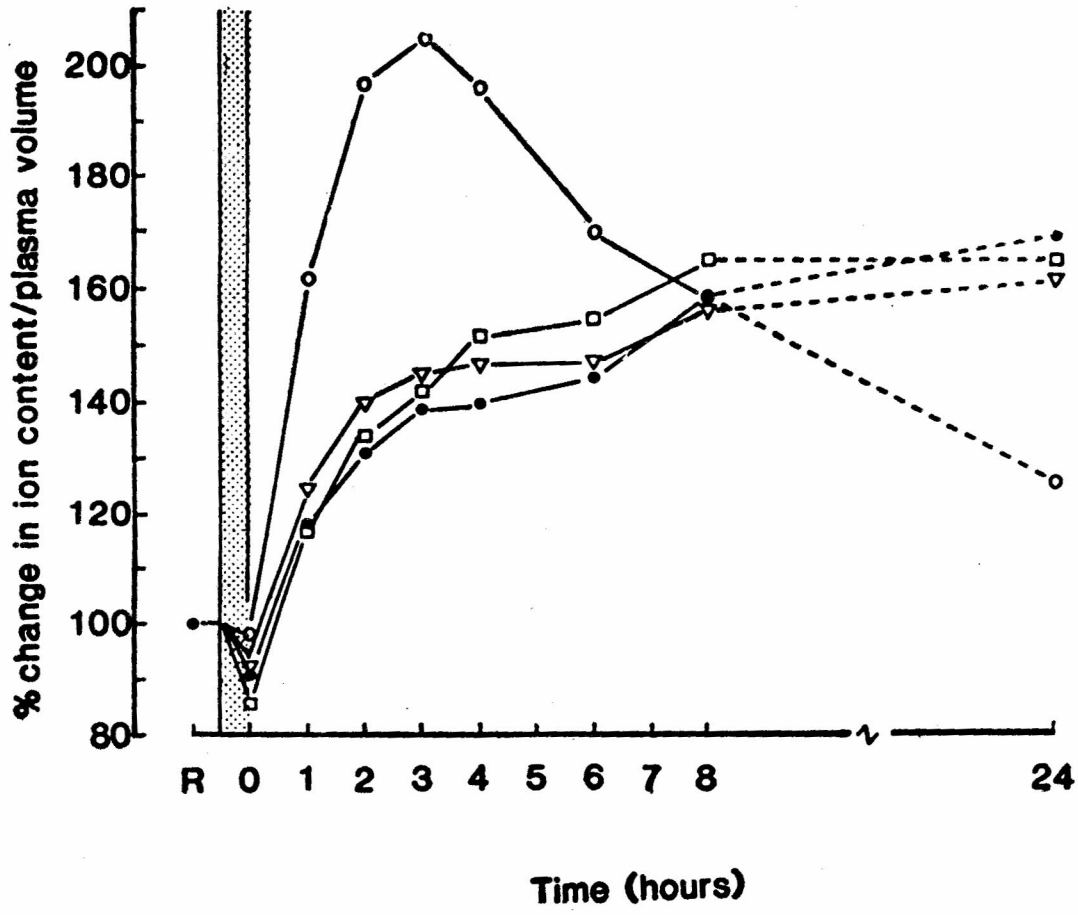


*Fig.* 5 The mean percentage changes in total plasma ion contents and plasma volume at rest and during recovery.
$Na^+$ △, $K^+$ o, $Cl^-$ ●, plasma volume □
Resting ion contents : $Na^+$ = 1.47 mmoles, $K^+$ = 0.037 mmoles, $Cl^-$ = 1.3 mmoles.
Resting plasma volume = 9.67 ml.
R = resting value, Time 0 = immediately after fatigue.
Shaded area = exercise period.

From fig. 3 it is observed that a marked discrepancy between buffered metabolic acid and measured lactate levels in the blood are apparent in the post exercise recovery period. The occurrence of a more gradual increase and decrease in $|\Delta H^+_m|$ than the corresponding changes in lactate levels suggests that

the $H^+$ produced in the tissues in equivalent amounts with lactate enter the blood more slowly than lactate or are eliminated in to the environment. Pliper *et al* (1972) also observed a similar discrepancy between buffered metabolic acid and measured lactate levels in the elasmobranch (*Scyliorhius stellarnis*) and they attributed this to the intracellular retention of $H^+$ since the $H^+$ elimination rate was too small to account for the difference between lactate and $H^+$ levels in the blood. In the trout the discrepancy between $|\Delta H\ m_m^+|$ and $|\Delta L{-}|$ lasts for much shorter period of time than in the elasmobranch. In trout equilibrium between $|\Delta H_m^+|$ and $|\Delta L{-}|$ is reached by 8 hours post exercise whereas in the elasmobranch 22 hours is necessary.

The $H^+$ deficit in this experiment may be due to both, intracellular buffering of $H^+$ and $H^+$ elimination in to the environment.

## 4.1 Intracellular buffering of $H^+$

If $H^+$ were buffered intracellularly while lactate moved into blood, the maintenance of electroneutrality would require the simultaneous displacement of other cations or / and anions. The movement of $Na^+$ - and $K^+$ in to tissue from blood or/and an exchange of blood $Cl^-$ for tissue lactate seem to be the most likely of the possible exchanges. The changes in $K^+$ were different from those in $Na^+$ and $Cl^-$. The latter closely paralleled changes in plasma volume so that the osmotic concentration of the extracellular fluid was maintained since the two ions concerned are the predominant osmotically active solutes. The maximum increase in $|K^+|$ above the resting value was 1.8 mmol/l after 2 hours of recovery. ($Na^+$–$Cl^-$) concentration increased by a maximum value of 10 mmol/l from the resting value after 2 hours of recovery. The increase in ($Na^+$–$Cl^-$) concentration may be due to movement of $Na^+$ from muscle into blood and / or exchange of $Cl^-$ for lactate ions. Thus the average increase in ($Na^+ + K^+$–$Cl^-$) concentration of 11.8 mmol/l corresponds well with the $H^+$ deficit of 10.4 mmol/l observed in the first series of experiments.

After 24 hours of recovery a $H^+$ excess of 1 mmol/l was observed in the first series of experiments (fig 3). But a significant decrease ($p < 0.05$) in ($Na^+ + K^+ - Cl^-$) concentration of 7.5 mmol/lbelow the resting value is evident (fig. 4b). This decrease in the sum of $Na^+$ and $K^+$ concentration over the $Cl^-$ concentration may be due to addition of cations other than $Na^+$ and $K^+$ into, and/or removal of anions other than $Cl^-$ from, blood.

## 4.2 $H^+$ elimination into the environment

A $H^+$ deficit in the blood could also have occurred due to net transfer of $H^+$ into the environmental water through the gills and kidney, though some studies have indicated that disturbances of acid base balance were not compensated by changes in renal excretion (Kobayashi and Wood, 1980; Cameron and Kormanik, 1982). It is very likely that the marked increases in $Na^+$ and $Cl^-$ contents during recovery after severe exercise (fig 5) was due to uptake of these ions from the environmental water rather than from muscle tissue (Gordon, 1959). Evidence for $NH_4^+/Na^+$ and $HCO_3^-/Cl^-$ exchanges at the gill epithelium as part of the mechanism of $Na^+$ and $Cl^-$ accumulation has been found in rainbow trout (Kerstetter and Kirschner, 1972).

If such exchange mechanisms were involved in the present fish the increase in $Na^+$ and $Cl^-$ contents of plasma after fatigue indicates elimination of $HCO_3^-$ and $NH_4^+$ into the environmental water. If $Na^+//NH_4^+$ and $Cl^-/HCO_3^-$ exchanges were taking place at the same rate, there is no net $H^+$ transport across the membrane. When the rate of uptake of $Na^+$ increases above $Cl^-$, probably due to $Na^+/NH_4^+$ exchange rate exceeding $Cl^-/HCO_3^-$ exchange, there is net loss of $H^+$. The content of $Na^+$ over $Cl^-$ (fig. 5) reaches a maximum value after 2 hours of recovery. Seven hours after recovery the situation is reversed, $Cl^-$ content increasing over $Na^+$ content probably due to $Cl^-/HCO_3^-$ exchange rate exceeding $Na^+/NH_4^+$ exchange rate, resulting in a net uptake of $H^+$.

There is no evidence for $K^+$ being involved in $H^+$ elimination through the gills and the increase in I $K^+$ I by 1.82 mmol/l from the resting value after 2 hours of recovery perhaps reflects an exchange of $H^+$ with muscles. However the present experiments do not allow the precise relationships of $H^+$ exchange between muscles, blood and environmental water to be established.

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