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CONTRIBUTION OF NET ION TRANSFER MECHANISMS TO ACID-BASE REGULATION AFTER EXHAUSTING ACTIVITY IN THE LARGER SPOTTED DOGFISH (*SCYLIORHINUS STELLARIS*)

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SUMMARY

Specimens of the larger spotted dogfish (*Scyliorhinus stellaris*) were electrically stimulated to exhaustion in a closed seawater recirculation system. The production of large quantities of lactic acid by anaerobic metabolism and the resultant efflux of the dissociation products, H^+ and lactate, from the white musculature resulted in severe acid-base disturbances and in increases in plasma lactate concentration, the two effects having extremely different time courses. Plasma pH and bicarbonate were maximally depressed 15–30 min after exercise, whereas peak lactate concentrations of up to 30 mM were not attained before 4–8 h after exercise. The acid-base status was restored to normal 10–14 h after exercise, long before the aerobic processing of surplus lactic acid was complete 22–30 h after exercise.

This behaviour can be explained on the basis of an interaction of transfer rates, buffer values and equilibria between intracellular and extracellular compartments with the transient net transfer of surplus H^+ ions to the environmental water. About half of the original quantity of H^+ was transferred net to the environment via the branchial epithelium during the first 8–10 h, and it was later taken up again at the rate of aerobic lactic acid processing in the metabolism of the fish, whereas a transfer of lactate was not observed at any time during the experiment. As a result, the distribution patterns of H^+ and lactate differed from each other and varied with time elapsed after anaerobic exercise, leading to the apparent 'H^+ ion deficit' which has been observed in the blood of several fish species during lactacidosis.

Net transfer of H^+ ions to the environment facilitates rapid normalization of the acid-base status long before the original stress, lactic acid, is removed from the organism and thus represents an effective regulatory mechanism for the defence of the internal milieu in fish.

INTRODUCTION

The normal cruising and positioning activity in fish is considered to be performed exclusively with aerobic energy production by the well perfused red musculature. In

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Key words: Acid-base regulation, ion transfer, muscular activity, fish.

contrast, during emergency situations and predacious activities, recruitment of the poorly perfused white musculature results in production of large quantities of lactic acid as the metabolic end product of anaerobic glycolysis. Up to 84 mmol/kg tissue weight can be accumulated in white muscles of fish (Wardle, 1978).

At the physiological pH in intracellular compartments of tissues and in the extracellular space (e.g. Heisler, Weitz & Weitz, 1976*a*,*b*; Randall & Cameron, 1973; Janssen & Randall, 1975; Eddy, Lomholt, Weber & Johansen, 1977; Heisler, Neumann & Holeton, 1980; for reference Heisler, 1980, 1982*a*), lactic acid, because of its low pK value, is almost completely dissociated to equimolar quantities of H^+ and lactate ions, and thus represents a considerable stress for the acid-base regulation of the animal.

The dissociation products of lactic acid are gradually eliminated from the muscle cells, increasing the lactate concentration in plasma to peak values of up to 30 mM within 2–8 h after strenuous activity (Secondat & Diaz, 1942; Black, 1957*a*,*b*,*c*; Piiper, Meyer & Drees, 1972; for review see Heisler, 1982*a*). The acid-base disturbances induced by the H^+ ion efflux from muscle cells peak much earlier than the lactate concentration. Plasma pH and bicarbonate concentration are maximally depressed in usually less than 1 h (in most cases 15 min) after exercise (see Heisler, 1982*a*). Piiper *et al.* (1972) however, pointed out that, in spite of the apparently much faster efflux kinetics of H^+ ions from muscle cells, the excess of lactate present in the extracellular space in comparison to the control conditions exceeds the surplus H^+ ions several fold. This apparent 'H^+ ion deficit' may in part be attributed to different distributions of surplus H^+ and lactate ions between various intracellular and extracellular body compartments of the fish (Piiper *et al.* 1972). Another mechanism to explain the observed discrepancy would be net transfer of surplus H^+ ions to the environmental water, a mechanism which has been shown to be used in acid-base regulation in fish during hypercapnia (Heisler *et al.* 1976*b*), after temperature changes (Heisler, 1978; Cameron & Kormanik, 1982*a*), and after infusion of nonvolatile acids and bases (Cameron & Kormanik, 1982*b*).

The aim of the present study was therefore to evaluate the contributions of transepithelial ion transfer mechanisms in gills, kidneys and rectal gland of the larger spotted dogfish (*Scyliorhinus stellaris*) to acid-base regulation during recovery from lactacidosis resulting from exhausting muscular activity.

Parts of the results of this study have been published in abstract form (Holeton & Heisler, 1978; Heisler & Holeton, 1979).

MATERIALS AND METHODS

Specimens of larger spotted dogfish (*Scyliorhinus stellaris*, weight 1·5–3·5 kg, mean 2·37 ± 0·35 kg) were caught in the Bay of Naples and kept for time periods between 2 weeks and several months at temperatures between 14 and 17 °C in large, well-aerated, seawater aquaria (>800 l/fish). The water in the aquaria was replaced by new sea water at an approximate rate of more than 500 l/(fish · day). The animals were fed on a diet of small fish until 3–5 days before the experiments. At least 1 week before experimentation the fish were transferred into smaller temperature-controlled

tanks ($16 \pm 0.5\,°C$, volume about 60–100 l/fish) which were flushed with new sea water at a rate of >400 l/(fish · day).

At least 24 h (in most cases more than 36 h) prior to the experiments the fish were anaesthetized in aerated urethane/sea water solution (20 g/l) until reactivity ceased, and then placed ventral side up on an operating rack. Anaesthesia and oxygen supply were maintained during surgery by irrigating the gills with oxygenated sea water containing 3 g/l urethane. After opening the body cavity by a mid-ventral incision, a polyethylene catheter (PE 60) was introduced through a splenic artery into the dorsal aorta and connected to a larger thick-walled and unkinkable PVC tubing (1 mm i.d., 1·8 mm o.d.). The PVC catheter was fed out of the body cavity through an additional small mid-ventral incision in the caudal third of the body cavity. In order to sample urine a thick-walled PVC catheter with multiple holes in the sides of the tip was introduced into the urinary papilla, secured by a circular atraumatic suture around the papilla and fastened to the body wall by additional sutures. Fluid secreted from the rectal gland and released from the abdominal pores was collected by means of the thumb of a rubber glove glued around the cloaca of female specimens with Histacryl Blue tissue adhesive (Braun, Melsungen, F.R.G.), a procedure made impossible by the claspers of male specimens.

After completion of surgery, the fish gills were flushed with fresh sea water until the fish became active and showed apparently normal ventilation after introduction into the experimental chamber. The chamber was flushed with fresh sea water for at least 24 h before the system was closed for experimentation.

Experimental apparatus

The experiments were conducted in a closed seawater recirculation system (volume 14–20 l) consisting of a fish chamber slightly larger than the fish, a pump, and an oxygenator with bubble trap (Heisler, 1978). The water was thermostatted to $16 \pm 0.2\,°C$ and aerated in the oxygenator at a rate of >8 l/min with CO_2-stripped air and recirculated at a rate of about 6 l/min. By this procedure, water P_{O_2} was kept higher than 130 mmHg, and water P_{CO_2} lower than 0·65 mmHg, throughout the experiments.

For continuous measurement of net bicarbonate transfer* water from the recirculation system was pumped by a roller pump through a series of three glass columns, fitted with bottoms of fritted glass discs, and therein equilibrated at constant temperature ($40 \pm 0.05\,°C$) with an extremely constant gas mixture of 1% CO_2 in nitrogen, provided by a selected gas mixing pump (Wösthoff, Bochum, F.R.G.). Before being returned to the fish system the water thus standardized for temperature and P_{CO_2} was fed over an electrode chain consisting of a spherical pH glass electrode and a double electrolyte bridge Ag/AgCl reference electrode with sleeve diaphragm, which was especially treated and selected for long term stability (drift less than 0·002 pH units/48 h in sea water equilibrated with a humidified gas of constant P_{CO_2}). This 'Δ-bicarbonate measurement system' (Heisler, 1978) was calibrated in absolute terms by the addition of known amounts of HCl or $NaHCO_3$.

* The term 'bicarbonate transfer' is descriptively used, although the mechanisms may involve transfer of OH^- ions or transfer of H^+ in the opposite direction; they are indistinguishable by the methods used.

Procedure

After recovery from anaesthesia the seawater recirculation system was closed and the control values of HCO_3 and ammonia release were determined for 8–18 h. When steady excretion occurred and three blood samples taken at 30 min intervals showed steady state conditions of plasma acid-base parameters, the fish were prodded and stimulated to vigorous activity in the chamber by mild electric shocks (a.c., 8–10 V, 50 Hz) for a period of 10 min. After this period the fish were exhausted and capable of only weak avoidance movements. The end of the stimulation period was defined as time zero. Blood samples (~0·3–0·4 ml) were withdrawn into heparinized syringes from the dorsal aorta and water samples (~2 ml) were taken from the recirculation system 15 and 30 min, 1, 1·5, 2, 3, 4, 6, 8, 10, 12, 20, 22, 24, 26 and 30 h after time zero. The dorsal aortic catheter was subsequently flushed with 1 ml heparinized dogfish Ringer. The blood was immediately analysed for plasma pH, P_{CO_2} and P_{O_2} using appropriate microelectrodes (Radiometer) as described previously (Heisler *et al.* 1976*a*; Heisler, 1978). Plasma bicarbonate concentration was calculated by application of the Henderson-Hasselbalch equation using pK'_1 values as a function of pH (Albers & Pleschka, 1967) and CO_2 solubility, α_{CO_2} (Pleschka & Wittenbrock, 1971) determined in plasma of *Scyliorhinus stellaris*. Plasma was separated from the red cells by centrifugation (16000 *g*) for 1 min. Samples of whole blood, plasma and sea water were deproteinized by addition of perchloric acid and the supernatant decanted and stored for later lactate analysis according to the method described by Benadé & Heisler (1978). The ammonia concentration of the water was measured in alkalinized (pH > 12) water samples using gas-sensitive ammonia electrodes (Orion Research Inc., Cambridge, Mass., U.S.A.) calibrated with alkalinized NH_4Cl standards. Urine and fluid from rectal gland and abdominal pores were collected for each 6 h during the control period and from time zero to 5 h, from 5 h to 10 h, from 10 h to 22 h, and from 22 h to 30 h after time zero, and analysed for titratable acidity, bicarbonate and ammonia concentration.

Non-respiratory blood buffer curves were established on blood samples pooled from three specimens (haematocrit of 17·5 %). Two samples (60 μl) of the pooled blood were equilibrated with a gas mixture of 0·196 % CO_2 in CO_2-free air (P_{CO_2} = 1·45 mmHg) and plasma pH was measured. Then a known amount of 1 N-HCl was added to the blood pool, another two samples equilibrated and pH measured again. This procedure was repeated with several known additions of HCl. A similar buffer curve was established by equilibration with a gas mixture of 0·654 % CO_2 in CO_2-free air (P_{CO_2} = 4·85 mmHg).

Model calculations

In order to establish a semiquantitative distribution pattern of surplus H^+ ions and lactate ions throughout the recovery period from strenuous exercise, the experimental system was treated as a three-compartment system consisting of 'intracellular space', 'extracellular space' and the environmental water (including the quantitatively unimportant urine).

Assuming that the diffusion resistance between extravascular and intravascul

extracellular space was small as compared to that of cell membranes, and that tissue perfusion was not a limiting factor for the H^+ and lactate transfer between interstitial space and plasma, and that the compartment volume remained constant, the following relationships were applied to estimate the quantity of surplus H^+ (ΔH^+_e) and lactate ions ($\Delta Lact^-_e$) present in the extracellular body compartment (including blood):

$$\Delta H^+_e = \underbrace{\Delta [HCO_3^-]_{pl} \cdot F \cdot (V_e - V_{pl})}_{A} + \underbrace{\Delta pH_{pl} \cdot \beta_{NB\,int} \cdot (V_e - V_{pl})}_{B} + \underbrace{\Delta H^+_b \cdot V_b}_{C} \quad (1)$$

$$\Delta Lact^-_e = \underbrace{\Delta [Lact^-]_{pl} \cdot F \cdot (V_e - V_{pl})}_{D} + \underbrace{\Delta [Lact^-]_b \cdot V_b}_{E} . \quad (2)$$

A represents the amount of surplus H^+ ions indicated by the change in bicarbonate concentration in the extravascular extracellular space,

B represents the amount of surplus H^+ ions buffered by the interstitial non-bicarbonate buffers,

C is the amount of surplus H^+ ions in the blood according to Fig. 4,

D represents the amount of surplus lactate in the extravascular extracellular space,

E is the amount of surplus lactate in the blood,

V_e is the total extracellular space of *Scyliorhinus*, measured to be 26·8 % of body water (Heisler, 1978),

V_{pl} is the plasma volume, calculated from haematocrit and blood volume,

V_b blood volume, assumed to be 5 % of the body weight,

$\beta_{NB\,int}$ is the non-bicarbonate buffer value of interstitial fluid, estimated to be 0·5 meq/(pH·l) according to the phosphate concentration of the interstitial space,

F is the Donnan factor, assumed to be 1·02 according to a plasma protein concentration of about 2·5 to 3 g %,

ΔH^+_b is the quantity of surplus H^+ ions in the blood, determined for the measured pH and P_{CO_2} values from the blood buffer curves (Fig. 4), intermediate P_{CO_2} values were interpolated.

The amount of H^+ ions released from the intracellular compartment was taken as the sum of the amount buffered in the extracellular space (ΔH^+_e) and the net amount transferred to the environmental water ($\Delta H^+_{e \to sw}$):

$$\Delta H^+_{i \to e} = \Delta H^+_e + \Delta H^+_{e \to sw} . \quad (3)$$

The quantity of surplus H^+ and lactate ions in the intracellular compartment is the difference between the total quantity in the experimental system at time t ($\Delta H^+_{tot} = \Delta Lact^-_{tot}$) and the amount present in the extracellular space and environmental water:

$$\Delta H^+_i = \Delta H^+_{tot} - \Delta H^+_{i \to e} \quad (4)$$

and, as lactate was not transferred to the environmental water at any time during the experiment:

$$\Delta Lact^-_i = \Delta Lact^-_{tot} - \Delta Lact^-_e \quad (5)$$

Since total body lactate was not measured in these experiments, it was estimated on the basis of the total amount of surplus H^+ ions in the system at certain times after exercise. When the extracellular acid-base parameters have reattained control values, it can be assumed that the intracellular body compartments also lack any surplus H^+ ions. Then the surplus H^+ ions which have been transferred to the environment are equivalent to the quantity of lactic acid still surplus in the whole system. Such conditions are found in the present experiments from 14 or 12 h after exercise to the end of the experiment for the high or low lactate groups respectively. The rate of H^+ recovery from sea water then reflects the lactic acid disappearance from the whole system due to aerobic metabolic processing. Assuming this rate to be the same and constant during the first period of the experiment*, extrapolation of the linear parts of the net $\Delta H^+_{e \to sw}$ curves ($\Delta H^+_{e \to sw: t\text{-}control}$) (Fig. 3) between 14 and 22 h, and between 12 and 18 h to time zero for high or low lactate groups, respectively can yield approximate values for the total quantity of lactic acid originally produced and the rate of lactic acid removal by aerobic metabolic processing.

RESULTS

The imposed strenuous exercise resulted in a considerable fall of plasma pH immediately after the end of the activity period with a concomitant fall of plasma bicarbonate concentration and a rise in dorsal aortic P_{CO_2} (Figs 1, 2). Lactate concentration rose more slowly and attained peak values not before 4–6 h after exercise (Figs 1, 2). Since all other variables appeared to be correlated with the amount of lactate released to the blood, and since the most appropriate parameter, the quantity of energy produced anaerobically, could not be measured, the experiments were divided according to the fortuitous formation of two similar sized groups ('high' and 'low' lactate groups) with peak lactate concentrations attained averaging about 9 and 22 mM respectively.

In the high lactate group (Fig. 1) the acidosis was characterized by a sharp drop in arterial plasma pH from 7·8 to 7·2 and a decline in plasma bicarbonate concentration from about 7 to 3 mM within about 30 min. After 2 h plasma bicarbonate started gradually to return towards the pre-exercise level during the following 10 h (Fig. 1). Restoration of pH took several hours more due to still increased arterial P_{CO_2}. Plasma lactate rose to about 20 mM after 8 h. In the low lactate group (Fig. 2), where maximum lactate levels reached only about 9 mM after 4 h, the general pattern observed was similar to the high lactate group. Duration and intensity of the acidosis, however, were not as severe. Recovery of plasma bicarbonate was faster and complete within 8 h. Also, in this group the restoration of arterial pH took a few hours longer. In both groups arterial lactate concentration rose much more slowly than pH and bicarbonate concentration fell and was still considerably elevated when plasma pH and bicarbonate were already restored. This fast recovery of the acid-base status in the plasma of *Scyliorhinus* before the lactic acid has been aerobically processed further

* The results of the present experiments i.e. the constant rate of H^+ recovery from sea water during the second phase of the recovery period, strongly suggest a constant rate of aerobic lactate processing, which may be due to rate limitation of metabolic pathways. This is not contrary to exponential disappearance curves of lactate from the extracellular space, which are often found in higher vertebrates (see: Di Prampero, 1981). These are largely affected by exponential redistribution of lactate between exercised and non-exercised tissue compartments.

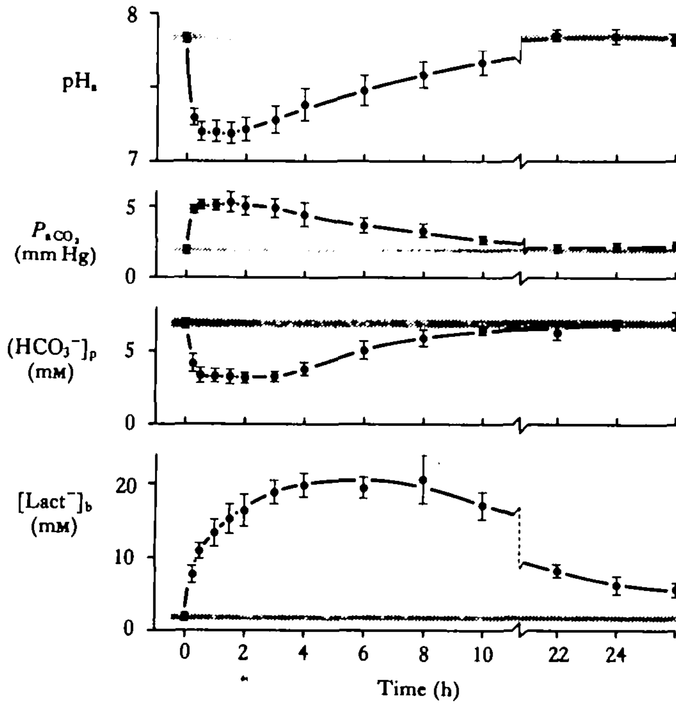

Fig. 1. Plasma pH, P_{CO_2}, bicarbonate concentration and lactate concentration in dorsal aortic blood of *Scyliorhinus* as a function of time after exhausting activity (High lactate group, $\bar{x} \pm$ S.E., $N = 9$).

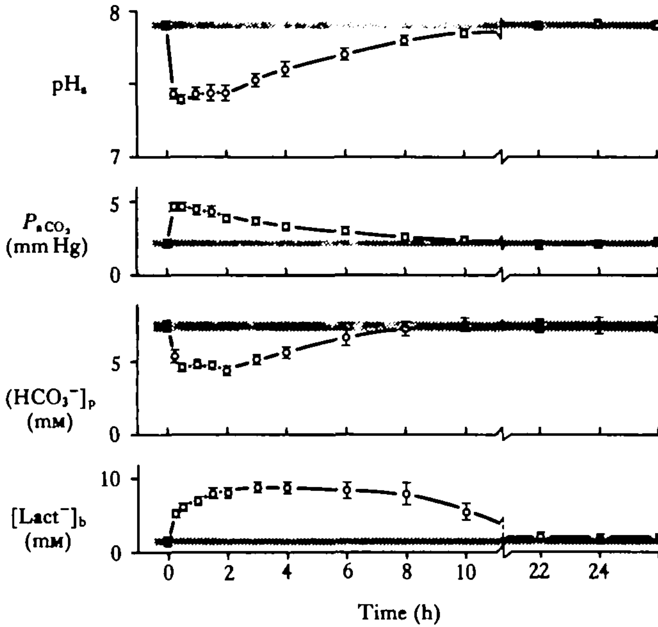

Fig. 2. Plasma pH, P_{CO_2}, bicarbonate concentration and lactate concentration in dorsal aortic blood of *Scyliorhinus* as a function of time after exhausting activity (Low lactate group, $\bar{x} \pm$ S.E., $N = 11$).

can be attributed to transepithelial ion transfer processes between environmental water and the extracellular space of the fish.

During the control period the fish released ammonia at a rate of 0·64 μmol/(min · kg body water), of which about two thirds were balanced by the bicarbonate release (OH^- release, or equivalent H^+ uptake) of 0·44 μmol/(min · kg body water) (Fig. 3). The resultant net H^+ excretion (ammonia minus bicarbonate excretion) was thus small (0·2 ± 0·22 μmol/(min · kg body water)) and, relative to the rate of oxygen uptake (34·8 μmol/(min · kg body water); Randall, Heisler & Drees, 1976), in the same range (0·57 % of $\dot{V}_{O_2}$) as in higher vertebrates (Heisler, 1982*a*).

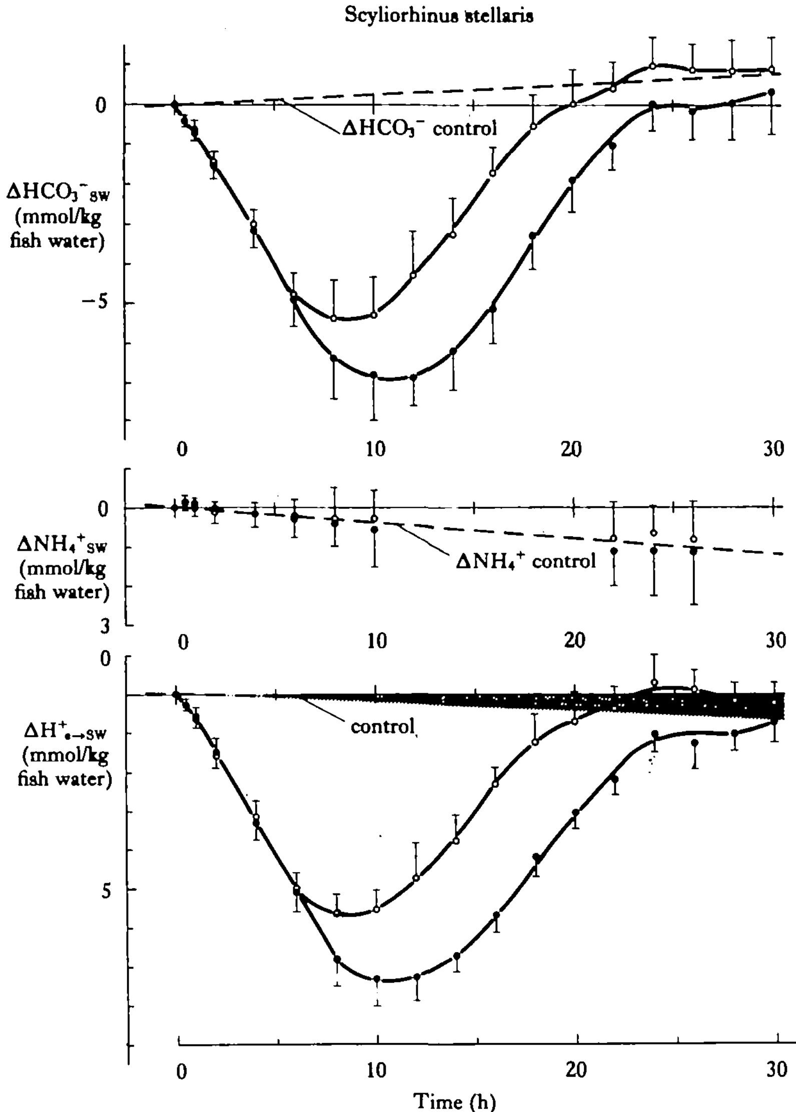

Fig. 3. Changes in bicarbonate and NH^+_4 content in the environmental water of *Scyliorhinus* after exhausting activity. $\Delta H^+_{e\rightarrow sw}$ (lower panel) represents the difference of the above two ions and is the net amount of H^+ ions excreted by the animal ($\bar{x}$ ± S.E., N = 9 and 11 for high lactate, closed circles, and low lactate groups, open circles, respectively).

After strenuous exercise the net H^+ excretion rate increased markedly (Fig. 3), exclusively as a result of net bicarbonate uptake from the environment, whereas the ammonia release remained unaffected. After the first 2 h the uptake rate was virtually constant until control plasma bicarbonate concentrations were approached (~10 h for high lactate group, ~8 h for low lactate group). After a delay of a few hours, the net H^+ excretion was reversed to a net H^+ gain and returned to the control range of cumulative net H^+ excretion 20 or 30 h after the exercise in the low or high lactate groups respectively (Fig. 3), at about the same time as plasma lactate returned to control levels (Figs 1, 2). In contrast to H^+ ions, lactate was not found to be transferred to the sea water at any time of the experiment.

In the low and high lactate groups, the maximal rates of net H^+ extrusion are about the same (14·3 or 15·7 μmol/(min · kg body water), respectively), and the average uptake rates for the first 6 h after exercise are identical (~14 μmol/(min · kg body water)), in spite of the rather different extent of acidosis in the dorsal aortic blood. This suggests that the mechanisms involved may be rate-limited.

Most of the net H^+ extrusion to the environmental water during the first hours of recovery from exercise (7·2 or 5·2 mmol/kg body water for high or low lactate groups, respectively) was performed at the branchial epithelium. The contribution of the kidneys was insignificant and exceeded 1 % of the quantity transferred in only 2 of 20 experiments (1·2 % and 2·3 %, both experiments of the low lactate group). Urine pH was rather constant (range 5·45–5·98) and not significantly different between control and post-exercise periods. Urine flow rates were quite variable (0·1–1·8 ml/(h · kg body weight)) and loosely correlated with the volume of dogfish Ringer administered during flushing of the dorsal aortic catheter after blood sampling. Also, the net H^+ transfer via fluid secreted from the rectal gland and the abdominal pores was insignificant and well below 1 % of the total quantity eliminated to the environmental water.

The non-respiratory buffer curves of *Scyliorhinus* blood determined at two different P_{CO_2} values are presented in Fig. 4. Slope and position of the curves are largely determined by the bicarbonate concentration in the blood. The non-bicarbonate buffer value component of these curves, obtained by stripping the curves of the bicarbonate component, is slightly higher (+25 %) than the value of 8 meq/(l · pH) reported by Piiper *et al.* (1972) for *Scyliorhinus* blood, which can partly be attributed to the higher haematocrit in our experiments (+15 %). Haematocrit averaged 16·8 ± 2·6 % ($\bar{x} \pm$ S.D., $N = 72$, of 20 specimens) and was not significantly affected by the sampling procedure during the experiments. The fractional water content (F_{H_2O}) of the blood, determined in 27 samples of 15 specimens, was related to the haematocrit (range 10–21 %) according to the linear regression:

$$F_{H_2O} = 0{\cdot}263\,Ht + 94{\cdot}6 \quad (r = 0{\cdot}98)$$

The lactate concentration in whole blood water averaged 0·94 ± 0·03 ($\bar{x} \pm$ S.D.) of the concentration in plasma water.

DISCUSSION

The results of the present experiments confirm the observation of previous authors that in fish after strenuous exercise the plasma pH and bicarbonate concentration

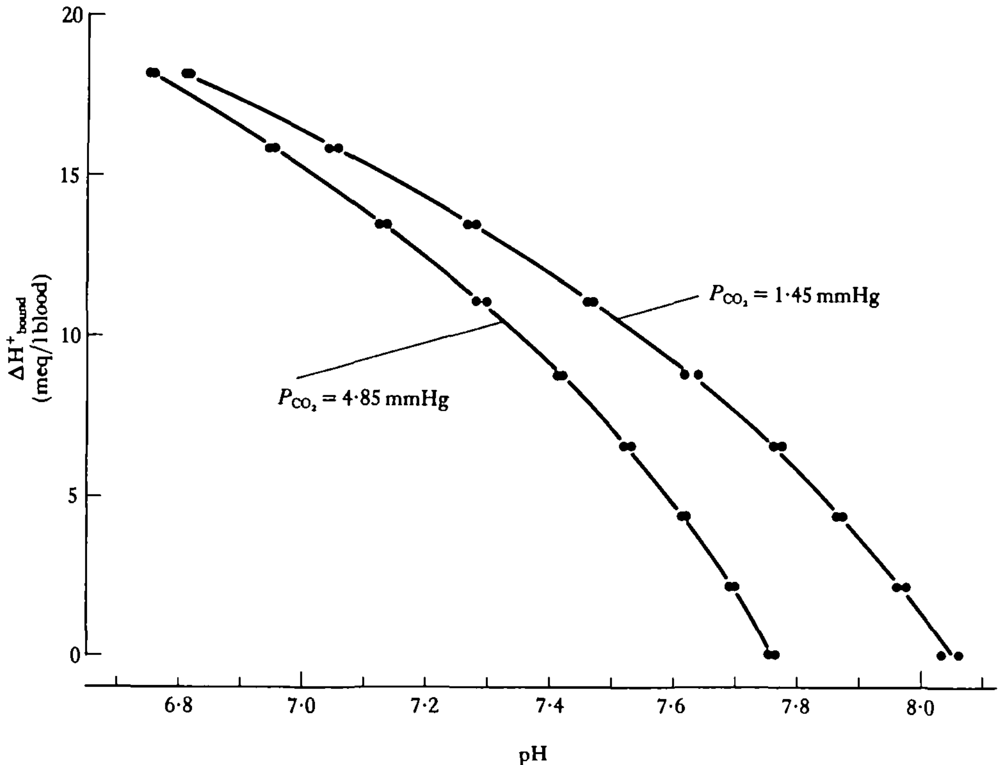

Fig. 4. Nonrespiratory buffer curves of *Scyliorhinus* blood, determined by titration with nonvolatile acid. Upper curve: at P_{CO_2} = 1·45 mmHg, lower curve: at P_{CO_2} = 4·85 mmHg.

attain maximal depression much earlier than the peak plasma lactate values are reached (for review see Heisler, 1982*a*). Accordingly the rate constants (*k*) for accumulation of surplus H^+ ions and lactate in the extracellular compartment (calculated according to equations 1 and 2) are different by a factor of about 12 (Fig. 5). Since H^+ ions are transferred from the extracellular space to the environment at the same time, the discrepancy between the rate constants for the efflux of H^+ and lactate from the muscle cells must be expected to be even larger.

The quantity of lactate present in the extracellular space in surplus, however, far exceeds the quantity of H^+ ions buffered in the same compartment (Fig. 7, middle panel), a result which has also been obtained in several previous studies (see Heisler, 1982*a*). This difference, termed 'hydrogen ion deficit' by Piiper *et al.* (1972), suggested that the hydrogen ion elimination from the major site of production, the white muscle cells, was much slower than the comparable lactate efflux, being just sufficient to account for the aerobic metabolic processing of lactic acid in liver, heart and red muscle. Of all previous investigators, only Piiper *et al.* (1972) attempted to measure, but could not demonstrate, a net H^+ transfer to the environment, probably because of an extremely high resting H^+ excretion rate in their experiments.

The results of the present study indicate that a sizeable component of the 'hydrogen ion deficit' is due to temporary net transfer of H^+ ions to the ambient water (Fig. 3). Taking this into account, estimates of the total quantities of H^+ and lactate released from the intracellular body compartments show that only during the first period aft

exercise and only in the high lactate group, was there a greater net transfer of lactate across the cell membranes into the extracellular space than of H^+ ions to the extracellular space and, to a certain extent, to the water (Fig. 6). At all other times, the quantity of H^+ ions released from the intracellular space generously exceeds the amount of lactate present in the extracellular compartment.

A precise assessment of the distribution of surplus H^+ ions and lactate ions among intracellular and extracellular body compartments and the environmental water can be based only on total body lactate analyses at various times of the recovery period. An approximate value for the total amount of surplus lactic acid in the system can be derived indirectly from the rate of disappearance of H^+ ions from the sea water when extracellular and intracellular compartments no longer contain surplus H^+ ions (see methods: model calculations):

High lactate group:

$$\Delta H^+_{tot} = \Delta Lact^-_{tot} = -0{\cdot}655t + 15{\cdot}8 \ (r = 0{\cdot}998) \ \text{(mmol/kg body water)} \quad (6)$$

Low lactate group:

$$\Delta H^+_{tot} = \Delta Lact^-_{tot} = -0{\cdot}606t + 11{\cdot}9 \ (r = 0{\cdot}995) \ \text{(mmol/kg body water)} \quad (7)$$

These values, clearly only rough estimates, show that the total amounts of lactic acid

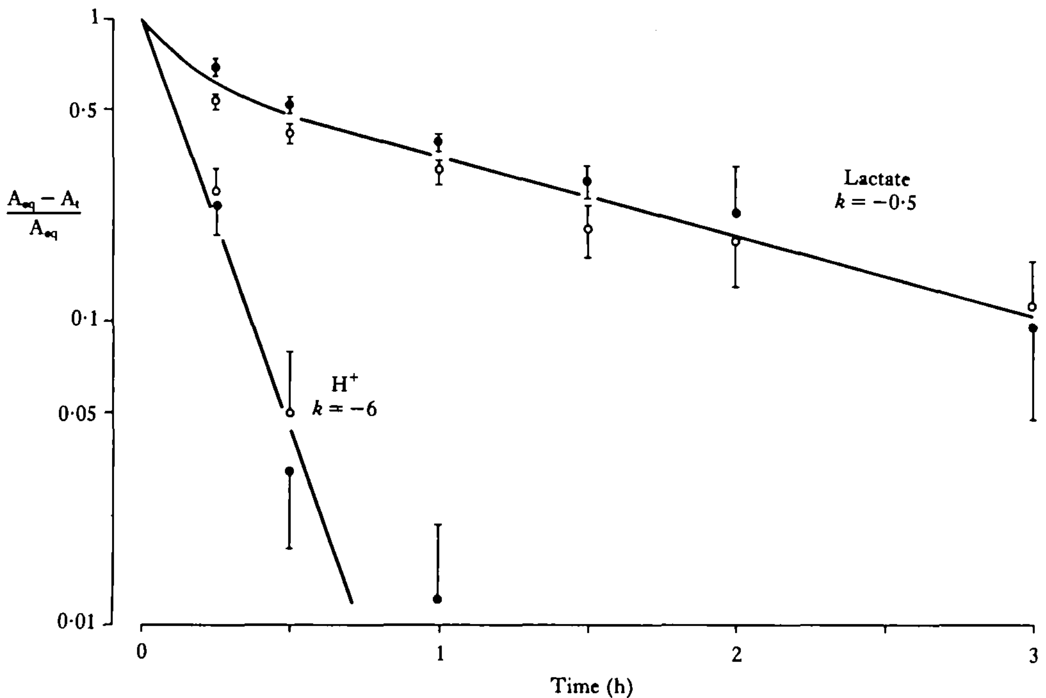

Fig. 5. Semi-logarithmic plot of the H^+ and lactate accumulation in the blood of *Scyliorhinus*. The difference between the maximal amount surplus in the blood (A_{eq}) minus the amount at time t (A_t) divided by the maximal amount has been plotted.

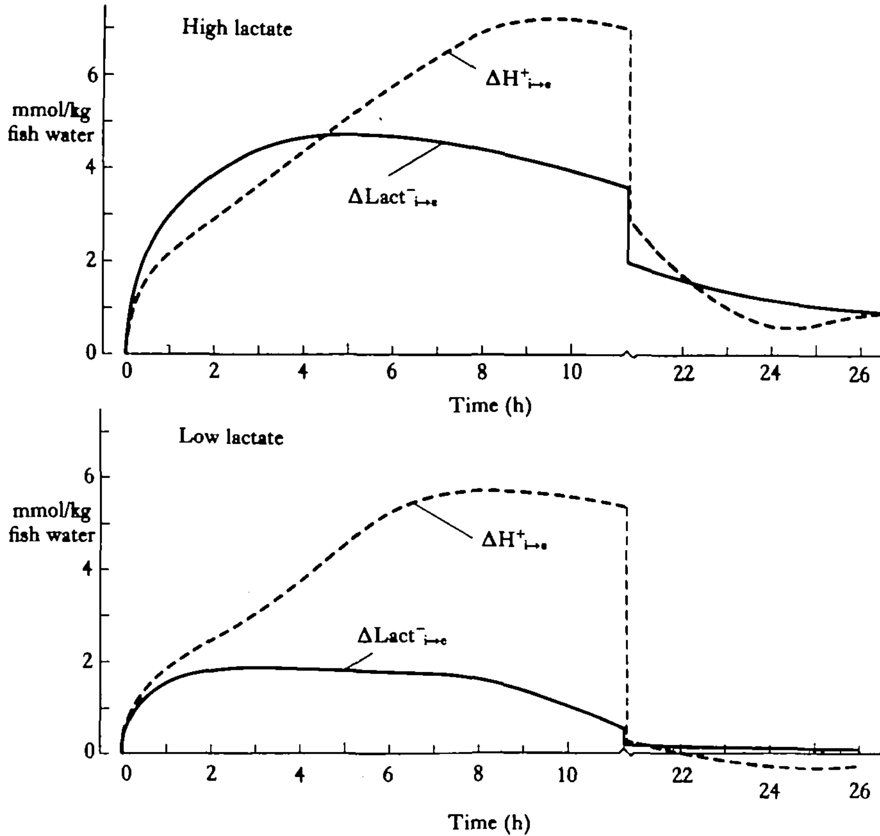

Fig. 6. The quantity of H^+ and lactate ions transferred from the intracellular space to other compartments as a function of time after strenuous exercise (model calculations according to equations 3 and 2).

produced as a result of the anaerobic exercise (about 16 or 12 mmol/kg body water for high or low lactate groups respectively) were not as different between the two groups as could be expected from the average peak lactate concentrations attained in the arterial blood (20·5 and 8·6 mM, respectively, see Figs 1 and 2).

Based on these estimates, and the volume and fractional extracellular space of white muscle in *Scyliorhinus* (Heisler, 1978; Heisler *et al.* 1976*a*; Heisler *et al.* 1980), the muscle lactate concentration at the end of the exercise can be estimated at about 45 or 34 mmol/kg tissue water for high or low lactate groups, respectively. Such levels are in good accordance with literature data (30–84 mmol/kg tissue weight, Wardle, 1978) and may in fact represent underestimates, if significant quantities of H^+ were still stored in the intracellular compartment after restoration of the extracellular acid-base parameters or if the aerobic lactate processing rate was concentration-dependent instead of being constant.

After the end of exercise, the dissociation products of lactic acid, H^+ and lactate ions, are released from the intracellular compartment with evidently different time courses (Fig. 7), which is attributable to different kinds of limitations.

At the beginning of the efflux period, the initial rapid release of only a minor quantity of H^+ ions results in apparent saturation of the extracellular fluid (Fig.

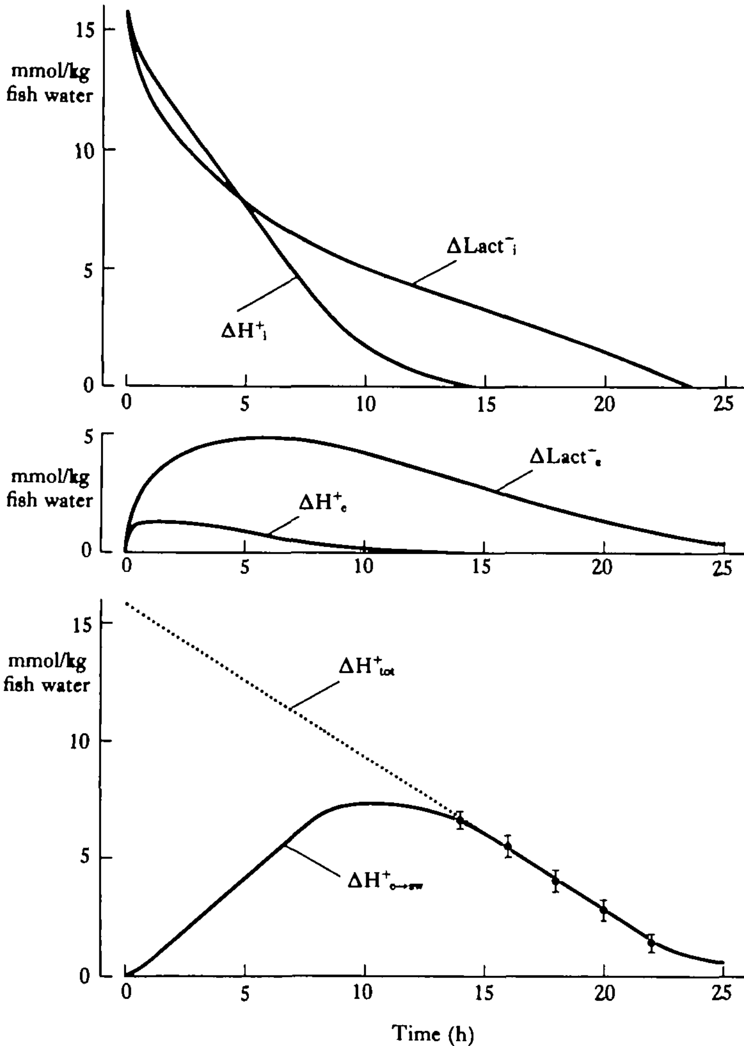

Fig. 7. Model calculations of the distribution of H^+ and lactate ions between intracellular and extracellular body compartments of *Scyliorhinus* and the environmental sea water for the high lactate group (see also Methods: equations 2, 3, 4, 5 and Discussion).

ΔH^+_e). This may be explained by the existence of an 'equilibrium limitation' for further transfer of H^+ ions across the cell membrane. According to the relatively small volume and the low buffer value of the extracellular space as compared to the intracellular muscle compartment (Heisler & Neumann, 1980), transfer of only a small fraction of the H^+ ions from the intracellular space is sufficient to lower the extracellular pH (or bicarbonate concentration) so much that a new equilibrium between intracellular and extracellular pH (or bicarbonate concentration) of the muscle cells is attained, thus eliminating the driving force for further net H^+ transfer. Only removal of H^+ ions from the extracellular space by excretion into the environment (Fig. 7, $\Delta H^+_{e\rightarrow sw}$), as well as aerobic metabolic processing of lactic acid, results further H^+ efflux from the muscle cells, these two mechanisms then determining

the rate of H^+ elimination from the intracellular compartments (Fig. 7, ΔH^+_i betwee
0·5 and 14 h).

Lactate efflux from the intracellular space is governed by other factors than the H^+ ion efflux. At equilibrium, lactate is distributed across the cell membrane either according to the membrane potential, or according to the intracellular/extracellular pH difference, depending on the form in which lactate is predominantly transferred across the cell membrane (ionized or non-ionized). In both possible cases, at equilibrium the extracellular concentration will exceed the intracellular concentration by far. Thus, in contrast to the conditions for H^+ ions, a large fraction of the lactate produced intracellularly can be transferred to the extracellular space before equilibrium is attained. These conditions are expressed in the experimental data by the relatively large transfer of lactate ions during the first hours of the experiment, exceeding the magnitude of H^+ ion efflux (Fig. 7) in spite of the much higher efflux time constant for H^+ ions (Fig. 5). As lactate is not released to the environmental water, the pattern is later reversed, when all surplus H^+ ions are quantitatively eliminated from the organism and transiently 'stored' in the sea water (Fig. 7). Lactate can then be further eliminated from the intracellular space only at the rate of aerobic metabolic processing.

The observed dissociation in the distribution of H^+ and lactate ions between various fluid compartments has to be considered as the result of differences in equilibria, in capacities (buffering properties) of various compartments, and in transfer rates and transfer mechanisms for these ions. The interaction of these factors is rather complex and is reflected by the applied three-compartment model only in its general pattern. It is evident, however, that transepithelial net transfer of H^+ ions to the environmental water is a dominating factor in determining the distribution pattern in the fish and plays a most important role in acid-base regulation after strenuous exercise. Kidneys, rectal gland and abdominal pores contribute only insignificantly to this transfer, which has been found similarly in other stress situations and for different fish species (e.g. Cross *et al.* 1969; Hodler, Heinemann, Fishman & Smith, 1955; Heisler *et al.* 1976*b*; Heisler, 1978; Cameron & Wood, 1978; Heisler, 1982*b*; for review see Heisler, 1980, 1982*a*) so that the observed transfer has to be attributed almost exclusively to the branchial epithelium. The net transfer rates during the H^+ extrusion period (0–6 h) are relatively constant and independent of the changes in extracellular H^+ load during this period and of the load differences between high and low lactate groups. This suggests that carrier-mediated, rate-limited ion transfer mechanisms, presumably ion exchange mechanisms for H^+ against Na^+ and HCO_3^- against Cl^- are involved (e.g. Kerstetter, Kirschner & Rafuse, 1970; Payan & Maetz, 1973; Maetz & Garcia-Romeu, 1964; De Renzis & Maetz, 1973; De Renzis, 1975; Kerstetter & Kirschner, 1972; Kormanik & Evans, 1979; for review see Maetz, 1974; Evans, 1980).

Based on the present data it cannot be decided which single species or which combination of ions (H^+; or HCO_3^-, OH^- in opposite direction) are transferred. Only a contribution of NH_4^+ excretion to the net H^+ transfer can be excluded, since ammonia release was low and was not increased in response to lactacidosis. To what extent this behaviour is peculiar to the ureotelic dogfish and whether ammoniotelic fish species utilize the ammonia mechanism to a larger extent during exercise-induced lactacidosis remains as a subject for further studies.

CONCLUSION

Strenuous muscular activity leads in *Scyliorhinus* to the production of large quantities of lactic acid as a metabolic endproduct of anaerobic glycolysis. The dissociation products of lactic acid, H^+ and lactate ions, are eliminated from the muscle cells with different kinetics and according to different limitations, resulting in different, and continuously changing, distribution patterns of surplus H^+ and lactate between various compartments during the recovery period. Surplus H^+ ions are transiently net transferred to the environmental water, presumably via branchial HCO_3^-/Cl^- and H^+/Na^+ ion exchange mechanisms, whereas lactate is not released from the organism. This net transfer of H^+ ions enables restoration of extracellular, and presumably also intracellular, pH and bicarbonate concentration at a time when not more than half of the originally produced lactic acid has been resynthesized to glycogen or further oxidized to CO_2. This important mechanism allows an early normalization of the acid-base status after anaerobic muscular activity.

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