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FACTORS AFFECTING LACTATE AND PROTON EFFLUX FROM PRE-EXERCISED, ISOLATED-PERFUSED RAINBOW TROUT TRUNKS

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Glycolysis in muscle during the anaerobic component of severe exercise rapidly produces lactate (La^-) and metabolic protons (H^+_m) in equal amounts, some of which may enter the blood (Krebs, Wood & Alberti, 1975). *In vivo* experimentation using strenuously exercised fish has yielded two patterns: La^- accumulating in blood either in greater or lesser amounts than H^+_m. It was rare to find both species at the same concentration, as is the usual case in man (Keul, Keppler & Doll, 1967). In the dogfish (Piiper, Meyer & Drees, 1972) and rainbow trout (Turner, Wood & Clark, 1983*a*) after exercise, ΔLa^- in blood greatly exceeded ΔH^+_m. The opposite discrepancy occurred in the starry flounder (Wood, McMahon & McDonald, 1977), muskellunge (Beggs, Holeton & Crossman, 1980), and flathead sole (Turner, Wood and Hōbe, 1983*b*), where ΔLa^- was consistently lower than ΔH^+_m. These discrepancies could be caused either by differential removal rates of the two ionic species from the blood space or by differential release rates from the myotome. We have recently argued that the latter is the dominant process (Turner *et al.* 1983*a*,*b*). However, this is extremely difficult to prove with *in vivo* methodology, as measurements in a single body compartment with periodic sampling give little information about the dynamic equilibrium between production, metabolism and fluxes between compartments. *In vitro* muscle preparations can be used to overcome many of these difficulties (e.g., Mainwood & Worsley-Brown, 1975; Benadé & Heisler, 1978). Isolated-perfused rainbow trout trunks have been employed to examine the physiology of systemic vascular resistance control (Wood & Shelton, 1975; Wood, 1977) and substrate utilization by muscle (Moen & Klungsoyr, 1981). In the present study, by strenuously exercising intact trout, then rapidly preparing them as isolated-perfused trunks, it was possible to determine net H^+_m and La^- movement from muscle to perfusate during recovery while simultaneously monitoring muscle [La^-] by repetitive biopsy.

The trout was first subjected to 6 min of severe burst exercise (cf. Turner *et al.* 1983*a*) and then within 2 min made into an eviscerated trunk preparation identical to that of Wood (1977). Constant flow perfusion through the dorsal aorta duplicated the technique of Wood & Shelton (1975) except that trunks were maintained in air at 15 °C and the total venous effluent was collected in a graduated cylinder. Inflow pressure was maintained at 20–40 cmH_2O with a flow of 6·0–8·0 $ml\,kg^{-1}$ fish weight min^{-1}

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from a Buchler peristaltic pump. Six successive 30-min flux periods were used. Inflow (i) and outflow (o) perfusate were analysed for pH, $[HCO_3^-]$, and P_{CO_2} using standard acid-base techniques described in Turner *et al.* (1983*a*). ΔH_m^+ between inflow and outflow was calculated by the method of McDonald *et al.* (1980):

$$\Delta H_m^+ = [HCO_3^-]_i - [HCO_3^-]_o - \beta(pH_i - pH_o),$$

where β is the non-bicarbonate buffer capacity of the perfusate. This technique measures only the 'fixed' or metabolic acid component; any variations in P_{CO_2} (i.e. respiratory acid) which might occur during effluent collection will not affect the accuracy of the H_m^+ determination. White muscle biopsies (~100 mg) were taken at the beginning of the experiment and at the end of each flux period and La^- concentrations were determined on deproteinized muscle homogenates and perfusates as described by Turner *et al.* (1983*a*) using enzymatic analysis (Sigma, 1977).

The perfusate was glucose-free Cortland salmonid saline (Wolf, 1963) to which was added 55 g l^{-1} haemoglobin (bovine type II), 40 g l^{-1} polyvinylpyrrolidone, and 50 000 i.u. l^{-1} ammonium heparin (all Sigma). *In vitro* tonometry at a physiological range of P_{CO_2} established the non-bicarbonate buffer capacity (β) to be −8·89 slykes, a typical value for trout blood (Wood, McDonald & McMahon, 1982).

Oxygenation with CO_2 in O_2 mixtures maintained P_{CO_2} at desired levels; $[HCO_3^-]$ was then adjusted to required values with NaOH or HCl. As the haemoglobin preparation used had a high methaemoglobin content, O_2 was used as the mixing gas to maximize O_2 delivery to the preparation. Measured O_2 content was ~5·1 vol% as compared to 7·8 vol% in normal trout blood (Holeton & Randall, 1967). The perfusion flow rate of 6·0–8·0 ml kg^{-1} min^{-1} was chosen as a compromise between O_2 delivery requirements and measurement accuracy for ΔH_m^+ and ΔLa^- between inflow and outflow. Randall & Daxboeck (1982) calculate that blood flow to this largely white muscle mass would range from ~5 to ~10 ml kg^{-1} min^{-1} in trout *in vivo* during rest and exercise; therefore the compromise does not appear unreasonable. Tripling the perfusion rate in a control experiment had no effect on measured ΔLa^- or ΔH_m^+ effluxes, or muscle La^- levels. Oxygen consumption, as calculated for one control trunk using the Fick principle, from measurements of perfusion flow rate and inflow and outflow O_2 content, represented approximately 1/6 the consumption of an intact trout (Cameron & Davis, 1970) and remained constant during the experimental period. Effluent P_{O_2} generally remained above 200 Torr; therefore error in the H_m^+ analysis due to the Haldane effect was negligible.

For the control experiments ($N = 5$), a perfusate closely simulating resting arterial acid-base status of rainbow trout at 15 °C (cf. Turner *et al.* 1983*a*) was employed with pH = 7·832 ± 0·007, P_{CO_2} = 2·30 ± 0·05 Torr, and $[HCO_3^-]$ = 5·56 ± 0·06 mequiv l^{-1} ($\bar{x} \pm 1$ S.E.M.). An acidotic perfusate ($N = 4$) was formulated with pH = 7·339 ± 0·011, P_{CO_2} = 6·82 ± 0·27 Torr, and $[HCO_3^-]$ = 4·80 ± 0·11 mequiv l^{-1}; this was of approximately similar composition to acidotic arterial blood in immediately post-exercise animals (cf. Turner *et al.* 1983*a*). In a third series ($N = 4$), 10^{-4} M-SITS (4-acetamido-4′-isothyocyanato-stilbene-2,2′-disulphonic acid; BDH) was added to the control perfusate. SITS has been shown greatly to reduce anion transport in many systems including red blood cells, both human (Cabantchik & Rothstein, 1972; Halestrap, 1976) and fish (Haswell, Zeidler & Kim, 1978) and fish gills (Per[illegible]

Table 1. *Net lactate (La^-) and metabolic proton (H_m^+) efflux (μequiv min^{-1} kg^{-1}) ($\bar{x} \pm 1$ S.E.M.) from muscle to perfusate, and muscle lactate concentrations (mequiv kg^{-1}) during recovery from 6 min of severe exercise in the isolated-perfused trunk of rainbow trout*

	Control pH=7·832 ± 0·007 (N=5)		Acidotic pH=7·339 ± 0·011 (N=4)		SITS 10^{-4} M pH=7·762 ± 0·012 (N=4)		Muscle
	Net La^- efflux	Net H_m^+ efflux	Net La^- efflux	Net H_m^+ efflux	Net La^- efflux	Net H_m^+ efflux	$[La^-]$† (N=13)
0–30 min	20·2 ± 5·0	13·9 ± 2·1	19·7 ± 6·0	6·1 ± 1·5$^{\Delta}$	37·5 ± 8·8	17·3 ± 3·6	36·6 ± 3·4
30–60 min	15·1 ± 5·3	11·8 ± 2·6	14·2 ± 4·0	5·1 ± 1·4$^{*\Delta}$	44·8 ± 5·0$^{\Delta}$	20·7 ± 3·9	38·6 ± 4·3
60–90 min	10·4 ± 5·2	12·0 ± 2·9	14·8 ± 2·9	4·5 ± 2·0$^{*\Delta}$	37·6 ± 2·7$^{\Delta}$	17·8 ± 2·0	39·9 ± 3·5
90–120 min	14·1 ± 5·1	8·4 ± 1·8	13·6 ± 2·6	5·6 ± 1·2	35·5 ± 5·0$^{\Delta}$	21·4 ± 4·1$^{\Delta}$	36·8 ± 5·5
120–150 min	6·4 ± 2·5	11·5 ± 1·5	12·2 ± 4·8	5·4 ± 2·3	35·2 ± 3·6$^{\Delta}$	22·8 ± 3·5$^{\Delta}$	43·9 ± 4·4
150–180 min	5·1 ± 1·9	8·6 ± 1·2	15·2 ± 4·9	3·6 ± 2·9	36·3 ± 6·2$^{\Delta}$	21·6 ± 4·4$^{\Delta}$	43·0 ± 5·0

* Indicates significant difference ($P \leq 0{\cdot}05$) between lactate and proton efflux rates (by paired 't' test).
$^{\Delta}$ Indicates significant difference ($P \leq 0{\cdot}05$) relative to comparable control rate (by unpaired 't' test).
† Muscle lactate levels at end of each 30-min period, all treatments combined.

Haswell, Randall & Farrell, 1981). SITS was employed here to assess the impact of anion transport blockade on lactate efflux dynamics from the myotome.

At the start of perfusion (0 min), the immediately post-exercise [La^-] in white muscle was 36·65 ± 3·39 (13) mequiv kg^{-1}, a value equal to that seen *in vivo* (Turner *et al.* 1983*a*). Over the following 180 min of perfusion, muscle [La^-] rose very gradually; these data are combined in Table 1 as there were no significant differences between treatments. *In vivo*, a 45 % decline occurs over this period. The reason for this difference is unknown. Deficiency in O_2 supply, while possible, seems unlikely (see above) and an artifact of intracellular dehydration cannot be implicated as the trunks gained 6·7 ± 2·0 (13) % in weight during the experiments. As none of the trunks flexed during the experiment or biopsy, further lactate production from muscular contraction can be eliminated.

The flux data for individual periods are reported in Table 1, and in Fig. 1 they are condensed into total effluxes over the entire 180 min experiment to emphasize the overall effects observed. When pre-exercised trunks were perfused with control perfusate of acid-base status similar to that of resting blood, net efflux rates of La^- and H_m^+ were not significantly different. The reason for the decreases in efflux with time remain unknown. When an acidotic perfusate, simulating immediately post-exercise blood acid-base status, was employed, H_m^+ efflux decreased sharply relative to

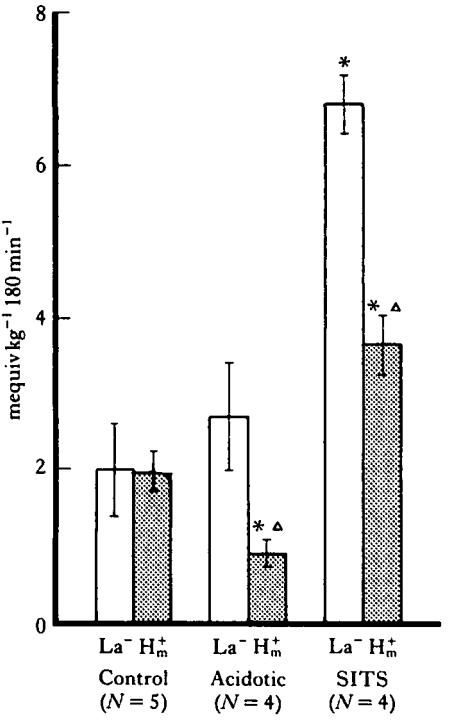

Fig. 1. Total net lactate (La^-) and metabolic proton (H_m^+) efflux ($\bar{x} \pm 1$ S.E.M.; N) over the 180-min experiment from pre-exercised rainbow trout trunks perfused with control, acidotic, or SITS-containing media. * Indicates a significant difference ($P \leq 0{\cdot}05$) from the control level (by unpaired t-test). Δ indicates a significant difference ($P \leq 0{\cdot}05$) between H_m^+ and La^- fluxes in the same treatment (by paired t-test).

Table 2. *A comparison of the measured blood concentrations of lactate (La^-) and metabolic protons (H^+_m) in rainbow trout* in vivo *0·5 h after severe exercise (Turner, Wood & Clark, 1983a) with values calculated from the measured net efflux rates of La^- and H^+_m from the perfused trunk preparations* in vitro.

	In vitro				*In vivo*
	Measured efflux rate (μequiv min^{-1} kg^{-1})	Time (min)	Blood volume ($l\,kg^{-1}$)	Concentration (mequiv l^{-1})	Concentration (mequiv l^{-1})
La^-	19·70 ± 6·0	30	0·0558	10·59 ± 3·23	11·27 ± 0·71
H^+_m	6·13 ± 1·5	30	0·0558	3.30 ± 0·81	6·90 ± 0·57

It is assumed that blood volume is 55·8 ml kg^{-1} (Milligan & Wood, 1982), the efflux rates for the acidotic perfusate apply, and that there is no excretion or utilization of either species from the blood over the first 30 min post-exercise.

controls, while La^- efflux remained unaltered. Conversely, perfusion with SITS in control media caused a pronounced 2·5-fold increase in La^- efflux. H^+_m efflux was not affected to the same extent, but was significantly elevated in three of the six flux periods. In both the acidotic and SITS treatments, overall La^- and H^+_m efflux rates were significantly different from each other.

We therefore advance the hypothesis that movements of La^- and H^+_m from ICF to ECF in the trout trunk are dissociated processes (cf. Mainwood & Worsley-Brown, 1975; Benadé & Heisler, 1978), H^+_m efflux being affected by the ECF acid-base status (perhaps a simple H^+ gradient effect) while La^- efflux remains unaltered. The results are consistent with the hypothesis that a differential release of H^+_m relative to the La^- from the myotome is one component responsible for the observed differences between ΔLa^- and ΔH^+_m in the blood following exercise in the trout (Turner *et al.* 1983*a*). While the differential elimination of H^+ may certainly occur (Heisler, 1982), this is not the only cause of the discrepancy found in the blood after exercise. Indeed the predicted discrepancy calculated solely from *in vitro* efflux rates from muscle to blood (Table 2) closely approximates to the actual measured *in vivo* discrepancy. The inhibitory effect of extracellular acidosis on H^+_m efflux provides a simple negative feedback mechanism for limiting extracellular acidosis. This may be particularly important in lessening the extent of blood metabolic acidosis immediately after exercise, the time at which respiratory acidosis (elevated Pa_{CO_2}) is at its peak (Turner *et al.* 1983*a*), thereby preventing possibly fatal pH depressions in poorly buffered fish blood (Wood *et al.* 1977).

While the possibility of non-specific effects should never be overlooked in drug experiments, elevation, rather than reduction, of La^- efflux caused by SITS suggests that there is *inward* transport of La^- from ECF to muscle ICF. Thus after exercise, intracellular La^- would diffuse down its concentration gradient to the ECF, but the majority would be transported back into the muscle cells. When this transport is inhibited by SITS, the outwards diffusion of La^- is left unopposed and net La^- efflux increases markedly. While this idea is somewhat novel, it is entirely in accord with the interpretation of Wardle (1978) and Batty & Wardle (1979) that La^- is actively tained in the white muscle of plaice and with our own similar conclusions about

trout and sole (Turner *et al.* 1983*a*,*b*). Such a mechanism would keep the majority of La^- in the myotome for metabolism *in situ*. This contrasts with the traditional view that the La^- is largely exported to the blood for aerobic processing in other tissues such as liver and heart (e.g. Heisler, 1982). Indeed only ~10% of the observed La^- disappearance from muscle *in vivo* (Turner *et al.* 1983*a*) can be accounted for by the La^- efflux rates measured *in vitro* in the present study. However, it must be remembered that muscle [La^-] did not decline with time *in vitro* as it did *in vivo*, so the perfused preparation does not entirely duplicate the situation in the living animal.

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