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# ROLE OF GLYCOLYSIS IN ADENYLATE DEPLETION AND REPLETION DURING WORK AND RECOVERY IN TELEOST WHITE MUSCLE

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## SUMMARY

Measurements of metabolite concentrations before and immediately after swimming of trout to exhaustion indicate that all three potential endogenous fuels of anaerobic metabolism [glycogen, phosphocreatine (PCr) and adenosine triphosphate (ATP)] are utilized during anaerobic white muscle work. Lactate, $H^+$, creatine, $P_i$, $NH_4^+$ and inosine monophosphate (IMP) are formed in the process. Glycolysis is considered to be functionally (if loosely) coupled to adenylate depletion by setting up conditions favouring AMP-deaminase-catalysed formation of IMP and $NH_3$. During recovery under these experimental conditions, glycolysis appears to outcompete oxidative metabolism as an ADP acceptor; therefore, in this kind of white muscle, glycolysis is also linked to IMP reconversion to AMP and thus to adenylate replenishment. The net process generates $H^+$, which is why ATP replenishment must be completed before PCr concentrations can be returned to pre-exercise values.

## INTRODUCTION

Although high-intensity (anaerobic) work by teleost white muscle is known to involve the utilization of three endogenous fuels [glycogen, phosphocreatine (PCr) and adenosine triphosphate (ATP)], the interactions between the metabolic pathways for mobilizing them are not well explored (Hochachka, 1985). How does PCr hydrolysis affect glycolysis? How does glycolysis influence adenylate depletion and *vice versa*? The answers to questions such as these are unknown, and our dearth of knowledge about the interactions of metabolic pathways involved in replenishing these fuels during post-exercise recovery periods is even greater. What is the temporal sequence of replenishment of ATP, PCr and glycogen? Is that sequence determined by interactions of these pathways of recovery metabolism? Why is white muscle glycogen replenishment, known to require up to a day or so (Black, Connor, Lamb & Chiu, 1962), such a slow process? Answers to these questions may be found by analysing depletion of these fuels during exhaustive work and their replenishment

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during early phases of recovery, when temporal ordering of metabolic events may be accentuated. Thus we arranged an experimental exercise protocol for trout which, over a long (about 45 min) period, gradually depleted almost all available white muscle glycogen, PCr and ATP. We analysed how the metabolic pathways for utilization of these fuels during work interacted, then how the pathways for replenishing these three fuels interacted during early phases of recovery, in order to develop a coherent interpretation of both processes. This analysis showed that glycolysis plays an important role in adenylate depletion during work and, surprisingly, that ATP replenishment can be linked to sustained glycolytic function during early phases of recovery, in this way delaying the onset of glycogen repletion.

## MATERIALS AND METHODS

### *Fish*

Rainbow trout (*Salmo gairdneri*) of both sexes weighing 60–100 g were obtained from the Sun-Valley Trout Farm, Mission, BC Canada. Fish were fed *ad libitum* and maintained in outdoor tanks with a continuous supply of fresh, aerated, dechlorinated tap water. The water temperature was $9 \pm 1$ °C.

### *Swimming protocol to complete exhaustion and recovery*

Fish were exercised in a Brett-type swim tunnel (Brett, 1964). After familiarizing each fish to the swim tunnel for 30 min at 1·5–2·0 body lengths $s^{-1}$, the exhaustive swim protocol began at the highest maximum water velocity (90 cm $s^{-1}$ or about 5 body lengths $s^{-1}$). As each fish started to fatigue (i.e. could no longer maintain its swimming position in the swim tunnel at 90 cm $s^{-1}$), the water velocity was decreased. After a few minutes at this reduced swim velocity, the speed was increased again to minimize recovery of the anaerobic ATP-generating systems in white muscle. By continually oscillating the speed control in this way, fish were completely exhausted at both high and low swimming velocities. The procedure was halted when fish could no longer swim at 1–2 body lengths $s^{-1}$. The average time to complete exhaustion was 45 min. The term 'exhaustion' means the inability of trout to maintain a swimming velocity equivalent to 1–2 body lengths $s^{-1}$ with the subsequent loss of postural support and orientation. In this highly specific physiological state, animals were completely flaccid with no apparent signs of rigor.

Exhausted fish were randomly assigned to 0, 10, 20, 45 or 120 min recovery periods. Trout were transferred by net from the swim tunnel to individual black holding boxes. The holding boxes had a continuous supply of circulating, well-aerated water and were maintained at a water temperature of $9 \pm 1$ °C. Control (pre-exercise) fish were kept in the holding boxes for 3 days prior to muscle sampling.

### *Muscle dissection and blood sampling procedures*

Fish were stunned by a sharp blow to the head. Blood was obtained percutaneously from the caudal vessels and placed on ice (average time 10 s). White epaxial muscle

Table 1. *High-energy phosphates in 'resting' rainbow trout freeze-clamped after specific numbers of tail flaps (average values in μmol g$^{-1}$ white muscle)*

| | Phosphocreatine | ATP | Ratio* | N |
|---|---|---|---|---|
| Pre-exercise | 14·84 | 7·43 | 0·50 | 2 |
| 1–2 tail flaps | 8·38 | 6·44 | 0·77 | 2 |
| 3–4 tail flaps | 4·27 | 5·36 | 1·26 | 2 |

* Ratio [ATP]/[phosphocreatine].

was quickly excised from a site posterior to the dorsal fin. Samples were immediately freeze-clamped in liquid nitrogen and stored at −70°C for 3 days prior to analysis. Liver was removed and freeze-clamped, followed by red muscle. The average times between stunning the fish and freeze-clamping the tissues were about 30, 45 and 100 s for white muscle, liver and red muscle, respectively. Whole blood was either acid-extracted, as described below for tissue, or was centrifuged at 10 000 rev. min$^{-1}$ for 2 min; in the latter case, the plasma was removed, frozen in liquid nitrogen and stored at −70°C until required.

*Muscle sampling technique and high-energy phosphates*

A major problem in most invasive studies on muscle metabolism in exercising animals is devising an appropriate muscle sampling method that minimizes concentration changes occurring in highly labile metabolites such as phosphagen and adenylates. Table 1 shows the effect of sampling time on the concentrations of phosphocreatine (PCr) and ATP in white muscle of trout as a function of tail flaps. It is clear that the PCr concentration decreases by 45 % and 70 % after 1–2 and 3–4 tail flaps, respectively, while [ATP] predictably undergoes smaller percentage decreases (about 10–30 %). The results of this experiment, which have been frequently observed in our studies (W. S. Parkhouse & G. P. Dobson, unpublished data), set the time limits required for sampling white muscle, in resting and in pre-exercise trout. Exhausted trout were so flaccid that artefacts due to tail flapping did not arise.

*Tissue homogenization, extraction and neutralization*

Tissue was powdered under liquid nitrogen using a pre-cooled mortar and pestle. About 500 mg of powdered tissue was transferred to a pre-cooled, pre-weighed vial containing a known volume of ice-cold 0·6 mol l$^{-1}$ perchloric acid (PCA) and then accurately re-weighed. A further volume of PCA was added and the powder homogenized at intermediate to high speed for 15 s at 0°C using a Polytron homogenizer. The homogenization procedure was repeated and the sides of the vial were washed down with a further volume of PCA. The suspension was stirred at low speed and duplicate 100-μl samples were removed for glycogen determination. The remaining suspension was centrifuged at 8000 rev. min$^{-1}$ for 10 min at 0°C. Samples of the supernatant were neutralized with 2 mol l$^{-1}$ $K_2CO_3$. The neutralized extracts were centrifuged and the supernatants removed, placed on ice, and used immediately for metabolite analysis.

### *Metabolite assays*

All metabolites were measured in a Pye-Unicam SP8-100 UV-VIS spectrophotometer at 340 nm using the routine NADH/NADPH-coupled enzymatic procedures described elsewhere (Murphy, Zapol & Hochachka, 1980). Each assay was validated with the appropriate standard(s). All procedures were performed in duplicate. Free fatty acids were determined enzymatically using fatty acyl-coenzyme A synthetase as described by Shimizu, Inoue, Tani & Yamada (1979).

### *Reagents*

Enzymes, coenzymes and substrates for metabolite determinations were obtained from Boehringer–Mannheim. Other chemicals and buffer reagents were obtained from Sigma Chemical Company.

## RESULTS

### *Metabolite levels in white and red muscle*

The results of the effect of exhaustive exercise and recovery on muscle metabolites in rainbow trout are summarized in Tables 2 and 3. It is clear from the data that at exhaustion, glycogen, the major energy source of white muscle, was essentially depleted (by 90 %). Associated with this decrease was a four-fold increase in [lactate] (6·62 to 23·38 $\mu$mol $g^{-1}$ wet mass), a two-fold decrease in muscle [glucose] (1·56 to 0·66 $\mu$mol $g^{-1}$), a large fall in [ATP] (5·24 to 0·45 $\mu$mol $g^{-1}$), a four-fold rise in [AMP] (0·15 to 0·41 $\mu$mol $g^{-1}$) and consequent rise in [ammonia] (1·03 to 5·80 $\mu$mol $g^{-1}$). Total ADP concentration did not change significantly during the exercise bout. In parallel studies, the decline in the total adenylate pool was always reflected in a rise in [IMP] (Dobson, 1986; Parkhouse, 1986), a stoichiometry that is assumed throughout this paper. [Phosphocreatine] decreased from 17·5 to 1·40 $\mu$mol $g^{-1}$ wet mass with a corresponding rise in [creatine] (Table 1). At exhaustion, [alanine] increased two-fold in white muscle (1·19 to 2·07 $\mu$mol $g^{-1}$ wet mass) while [malate] increased three-fold (0·11 to 0·35 $\mu$mol $g^{-1}$ wet mass).

At selected times during the 2-h recovery period, there was no evidence of glycogen replenishment (Table 2). White muscle [glucose] increased two-fold from 0·66 to 1·10 $\mu$mol $g^{-1}$ wet mass after 10 min of recovery but then decreased back to levels observed in the exhausted state. This decrease in [glucose] was significant and a further reduction was noted in fish swimming aerobically at 2 body lengths $s^{-1}$ for 45 min (Table 2). During the first 20 min of the 2-h recovery period, the group of fish remaining quiescent in the black box showed no change in white muscle lactate concentrations; then, after 45 min, [lactate] rose nearly two-fold (22·41 to 37·11 $\mu$mol $g^{-1}$). Recovery while swimming for 45 min had only a modest effect on these lactate profiles.

During recovery of white muscle adenylates, ATP and AMP were fully replenished within about 45 min (Table 3). Associated with the replenishment of the adenylates was a two-fold increase in [PCr] (1·4 to about 3·0 $\mu$mol $g^{-1}$ wet mass) after 10 min, but this pool showed no further net change in the remaining 2-h recovery

Table 2. *White muscle metabolite concentrations in trout before and after strenuous exercise (in $\mu mol\,g^{-1}$ wet mass ± S.E.M.)*

| | Glycogen | Glucose | Lactate | Alanine | Malate | *N* |
|---|---|---|---|---|---|---|
| Rest | 9·90 ± 0·87 | 1·56 ± 0·20 | 6·62 ± 0·95 | 1·19 ± 0·02 | 0·11 ± 0·01 | 6 |
| Exh. | 0·62 ± 0·08 | 0·66 ± 0·13 | 23·38 ± 0·51 | 2·07 ± 0·28 | 0·35 ± 0·05 | 5 |
| 10 min r | 0·80 ± 0·31 | 1·10 ± 0·17 | 22·41 ± 2·33 | 2·21 ± 0·20 | 0·49 ± 0·06 | 5 |
| 20 min r | 0·94 ± 0·25 | 0·76 ± 0·19 | 23·99 ± 1·22 | 1·86 ± 0·16 | 0·73 ± 0·08 | 5 |
| 45 min r | 0·69 ± 0·18 | 0·65 ± 0·05 | 37·11 ± 1·64 | 2·11 ± 0·12 | 0·97 ± 0·03 | 5 |
| 120 min r | — | 0·94 ± 0·17 | 31·12 ± 1·59 | 1·61 ± 0·19 | 0·66 ± 0·08 | 5 |
| 45 min s | 0·99 ± 0·19 | 0·32 ± 0·02 | 31·36 ± 1·83 | 1·38 ± 0·31 | 0·81 ± 0·07 | 4 |

Exh. = exhausted state; r = recovery time; s = recovery while swimming as described in Materials and Methods.

Table 3. *White muscle metabolites in rainbow trout before and after strenuous exercise (values in $\mu mol\,g^{-1}$ wet mass ± S.E.M.)*

| | Creatine | PCr | ATP | ADP | AMP | Ammonia | *N* |
|---|---|---|---|---|---|---|---|
| Rest | 27·52 ± 3·85 | 17·47 ± 0·78 | 5·24 ± 0·13 | 0·82 ± 0·03 | 0·15 ± 0·02 | 1·03 ± 0·04* | 5 |
| Exh. | 43·97 ± 1·31 | 1·39 ± 0·07 | 0·45 ± 0·03 | 0·97 ± 0·03 | 0·41 ± 0·02 | 5·80 ± 0·07 | 5 |
| 10 min r | 42·42 ± 0·96 | 2·61 ± 0·42 | 1·16 ± 0·20 | 1·00 ± 0·05 | 0·30 ± 0·03 | 4·99 ± 0·42 | 5 |
| 20 min r | 40·15 ± 3·30 | 2·70 ± 0·30 | 4·17 ± 1·27 | 0·84 ± 0·07 | 0·22 ± 0·04 | 5·16 ± 0·32 | 5 |
| 45 min r | 36·78 ± 1·23 | 2·98 ± 0·17 | 5·70 ± 0·94 | 0·68 ± 0·01 | 0·14 ± 0·02 | 5·27 ± 0·10 | 5 |
| 120 min r | 30·22 ± 1·65 | 3·78 ± 0·25 | 9·70 ± 1·81 | 0·65 ± 0·04 | 0·06 ± 0·01 | 4·96 ± 1·45 | 5 |
| 45 min s | 38·20 ± 1·98 | 3·61 ± 0·75 | 8·81 ± 1·45 | 0·64 ± 0·02 | 0·12 ± 0·02 | 4·77 ± 0·48 | 5 |

PCr, phosphocreatine.
* Results from an additional experiment in Dobson (1986).
Exh. = exhausted state; r = recovery time; s = recovery while swimming.

period (Table 3). Because the sum of the adenylates plus IMP never changed under these conditions (Dobson, 1986; Parkhouse, 1986), we assume that despite stabilized ammonia concentrations of about $5{\cdot}0\,\mu mol\,g^{-1}$ wet mass during recovery, IMP concentrations decreased in a stoichiometric manner with the replenishment of ATP and AMP. Alanine levels during this time changed only slightly, while [malate] continued to increase, with the most pronounced increase (two-fold) occurring after 45 min recovery in the black box.

Concentration changes in PCr, ATP, AMP and ADP in red muscle before and following exhaustive exercise were in sharp contrast to those found for white muscle (Table 4). The concentration of PCr decreased only 10% at exhaustion with no appreciable change in the adenylates. During the recovery period, however, [PCr] decreased to 40% of its pre-exercise value; [ATP] also fell somewhat but [ADP] and [AMP] did not change in recovery.

### *Metabolite levels in whole blood, plasma and liver*

The concentrations of several metabolites in whole blood (glucose, lactate, creatine, ammonia and alanine) prior to and following swimming to exhaustion are

Table 4. *Red muscle metabolites in rainbow trout before and after strenuous exercise (values in $\mu mol\,g^{-1}$ wet mass ± S.E.M.)*

| | PCr | ATP | ADP | AMP | *N* |
|---|---|---|---|---|---|
| Rest | 0·50 ± 0·03 | 1·67 ± 0·20 | 0·64 ± 0·05 | 0·90 ± 0·04 | 6 |
| Exh. | 0·46 ± 0·02 | 2·05 ± 0·18 | 0·62 ± 0·01 | 1·05 ± 0·05 | 5 |
| 10 min r | 0·29 ± 0·17 | 0·97 ± 0·11 | 0·62 ± 0·02 | 1·02 ± 0·06 | 5 |
| 20 min r | 0·41 ± 0·09 | 1·33 ± 0·17 | 0·59 ± 0·04 | 1·01 ± 0·06 | 5 |
| 45 min r | 0·59 ± 0·22 | 1·70 ± 0·82 | 0·56 ± 0·04 | 1·18 ± 0·04 | 5 |
| 120 min r | 0·60 ± 0·08 | 1·63 ± 0·29 | 0·58 ± 0·04 | 1·19 ± 0·09 | 5 |
| 45 min s | 0·71 ± 0·06 | 1·60 ± 0·19 | 0·46 ± 0·03 | 1·10 ± 0·03 | 4 |

PCr, phosphocreatine.
Exh. = exhausted state; r = recovery time; s = recovery while swimming.

Table 5. *Metabolite concentrations in perchloric acid extracts of whole blood from rainbow trout at rest and after strenuous exercise (in $\mu mol\,ml^{-1}$ blood ± S.E.M.)*

| | Glucose | Lactate | Creatine | Ammonia | Alanine | *N* |
|---|---|---|---|---|---|---|
| Rest | 19·45 ± 1·45 | 1·55 ± 0·36 | 0·47 ± 0·06 | 0·45 ± 0·03 | 0·42 ± 0·06 | 6 |
| Exh. | 8·17 ± 0·77 | 7·80 ± 0·71 | 0·33 ± 0·06 | 0·53 ± 0·10 | 0·59 ± 0·05 | 5 |
| 10 min r | 4·44 ± 0·32 | 7·49 ± 0·43 | 0·31 ± 0·05 | 0·42 ± 0·05 | 0·57 ± 0·02 | 5 |
| 20 min r | 5·06 ± 0·74 | 9·00 ± 0·70 | 0·44 ± 0·08 | 0·45 ± 0·03 | 0·63 ± 0·06 | 5 |
| 45 min r | 5·86 ± 0·69 | 9·28 ± 0·47 | 0·52 ± 0·10 | 0·45 ± 0·04 | 0·56 ± 0·05 | 5 |
| 120 min r | 7·29 ± 0·98 | 11·11 ± 1·04 | 0·33 ± 0·02 | 0·36 ± 0·04 | 0·54 ± 0·05 | 5 |
| 45 min s | 5·85 ± 0·36 | 8·24 ± 0·44 | 0·25 ± 0·04 | 1·25 ± 0·13 | 0·58 ± 0·05 | 4 |

Exh. = exhausted state; r = recovery time; s = recovery while swimming.

shown in Table 5. During exercise to exhaustion, blood [glucose] fell (19·45 to 8·17 $\mu mol\,g^{-1}$ wet mass), [lactate] increased (1·55 to 7·8 $\mu mol\,g^{-1}$ wet mass), but concentrations of creatine, ammonia and alanine changed only slightly. During the 2-h recovery period, blood [glucose] remained at reduced levels. Blood lactate concentration increased slightly during the recovery period, with no drastic differences evident in those fish swimming for 45 min at 2 body lengths $s^{-1}$ (Table 5). Blood ammonia levels remained unchanged during recovery in the black box but increased about three-fold (0·45 to 1·25 $\mu mol\,ml^{-1}$ plasma) during recovery while swimming (Table 5). At exhaustion, plasma [free fatty acid] decreased by 35% (1·15 to 0·73 $\mu mol\,ml^{-1}$ plasma); there was little change in the plasma triglyceride concentrations (Table 6), but plasma glycerol concentration during this time nearly doubled (Table 6). In the subsequent recovery period, plasma [free fatty acid] remained similar to values at exhaustion while plasma [triglyceride] fell (3·20 to 1·73 $\mu mol\,ml^{-1}$ plasma) then increased to slightly higher values than in the pre-exercise state (Table 6). Similarly, there was a decrease in plasma glycerol concentration after 10 min of recovery but this level was maintained throughout the remaining recovery period.

The concentrations of several liver metabolites (PCr, ATP, ADP and AMP) all underwent modest concentration changes during and following exhaustive exercise

Table 6. *Plasma metabolites in rainbow trout before and after strenuous exercise (in μmol ml$^{-1}$ plasma ± S.E.M.)*

| | Free fatty acids | Glycerol | Triglycerides | *N* |
|---|---|---|---|---|
| Rest | 1·15 ± 0·18 | 0·22 ± 0·02 | 2·88 ± 0·74 | 6 |
| Exh. | 0·73 ± 0·03 | 0·40 ± 0·04 | 3·20 ± 0·85 | 5 |
| 10 min r | 0·64 ± 0·01 | 0·29 ± 0·02 | 1·73 ± 0·47 | 5 |
| 20 min r | 0·77 ± 0·06 | 0·32 ± 0·03 | 3·71 ± 0·51 | 5 |
| 45 min r | 0·66 ± 0·04 | 0·27 ± 0·05 | 4·51 ± 0·87 | 5 |
| 120 min r | 0·80 ± 0·02 | 0·30 ± 0·03 | 3·40 ± 0·62 | 5 |
| 45 min s | 0·79 ± 0·02 | 0·28 ± 0·03 | 3·81 ± 0·52 | 4 |

Exh. = exhausted state; r = recovery time; s = recovery while swimming.

Table 7. *Liver metabolites in rainbow trout before and after strenuous exercise (in μmol g$^{-1}$ wet mass ± S.E.M.)*

| | Creatine | PCr | ATP | ADP | AMP | Ammonia | *N* |
|---|---|---|---|---|---|---|---|
| Rest | 1·65 ± 0·17 | 0·17 ± 0·03 | 1·32 ± 0·11 | 0·86 ± 0·07 | 0·90 ± 0·07 | 5·90 ± 0·49 | 5 |
| Exh. | 1·25 ± 0·17 | 0·22 ± 0·05 | 0·72 ± 0·08 | 0·62 ± 0·04 | 1·09 ± 0·06 | 3·80 ± 0·23 | 5 |
| 10 min r | 1·54 ± 0·09 | 0·14 ± 0·05 | 0·69 ± 0·10 | 0·78 ± 0·06 | 1·08 ± 0·08 | 3·50 ± 0·13 | 5 |
| 20 min r | 1·47 ± 0·25 | 0·14 ± 0·04 | 0·71 ± 0·09 | 0·75 ± 0·06 | 1·23 ± 0·05 | 3·11 ± 0·30 | 5 |
| 45 min r | 1·50 ± 0·11 | 0·09 ± 0·02 | 0·61 ± 0·08 | 0·69 ± 0·05 | 1·33 ± 0·08 | 3·94 ± 0·58 | 5 |
| 120 min r | 1·57 ± 0·08 | 0·04 ± 0·01 | 0·77 ± 0·05 | 0·82 ± 0·03 | 1·23 ± 0·09 | 3·22 ± 0·48 | 5 |
| 45 min s | 1·11 ± 0·18 | 0·07 ± 0·01 | 0·62 ± 0·05 | 0·78 ± 0·01 | 1·13 ± 0·09 | 2·55 ± 0·27 | 5 |

PCr, phosphocreatine.
Exh. = exhausted state; r = recovery time; s = recovery while swimming.

(Table 7). At exhaustion, no appreciable change in the concentration of PCr was observed. [ATP] decreased by 45 % from 1·32 to 0·72 μmol g$^{-1}$ wet mass, [ADP] fell by about 30 % from 0·86 to 0·62 μmol g$^{-1}$ wet mass, and [AMP] increased only slightly from 0·9 to 1·09 μmol g$^{-1}$ wet mass. During the recovery period [PCr] continued to fall with a corresponding increase in liver creatine concentration (Table 7), whereas [ATP] during this time remained fairly stable. The concentrations of ADP increased during recovery back to pre-exercise levels, while [AMP] remained largely unchanged over the 2-h period.

Liver [glycogen] decreased by 20 % but [glucose] did not change much during the exercise bout. [Lactate], however, increased from 1·58 to 5·25 μmol g$^{-1}$ wet mass and continued to increase during the 2-h recovery period (Table 8). Liver glycogen concentration was restored to the pre-exercise level after 2 h and it is noteworthy that swimming at low speed for 45 min during recovery facilitated liver glycogen repletion. Liver [glucose] followed a different pattern in recovery from that expected. Despite little change following exhaustive exercise, [glucose] decreased 50 % after 10 min of recovery, then stabilized at this value. Liver [alanine] increased three-fold from 1·06 to 3·55 μmol g$^{-1}$ wet mass at exhaustion and increased slightly during the early stages of recovery. The concentration of malate did not change with exercise but decreased slightly during the recovery period.

Table 8. *Liver metabolites in trout before and after exhaustion (in $\mu$mol $g^{-1}$ wet mass ± S.E.M.)*

| | Glycogen | Glucose | Lactate | Alanine | Malate | *N* |
|---|---|---|---|---|---|---|
| Rest | 53·84 ± 6·24 | 19·75 ± 1·17 | 1·58 ± 0·21 | 1·06 ± 0·42 | 0·98 ± 0·08 | 6 |
| Exh. | 41·01 ± 6·65 | 18·88 ± 0·64 | 5·25 ± 0·45 | 3·55 ± 0·53 | 1·07 ± 0·12 | 5 |
| 10 min r | 42·66 ± 13·63 | 11·08 ± 1·94 | 5·41 ± 0·52 | 4·47 ± 0·74 | 0·57 ± 0·05 | 5 |
| 20 min r | 44·73 ± 10·40 | 11·11 ± 1·00 | 6·57 ± 0·46 | 4·61 ± 0·63 | 0·43 ± 0·08 | 5 |
| 45 min r | 36·70 ± 12·67 | 12·47 ± 1·63 | 5·42 ± 0·38 | 3·95 ± 0·80 | 0·79 ± 0·13 | 5 |
| 120 min r | 50·20 ± 10·29 | 15·09 ± 1·23 | 6·56 ± 0·81 | 2·95 ± 0·83 | 0·98 ± 0·09 | 5 |
| 45 min s | 63·70 ± 9·18 | 13·45 ± 0·46 | 4·73 ± 0·48 | 4·41 ± 0·58 | 0·73 ± 0·17 | 4 |

Exh. = exhausted state; r = recovery time; s = recovery while swimming.

## DISCUSSION

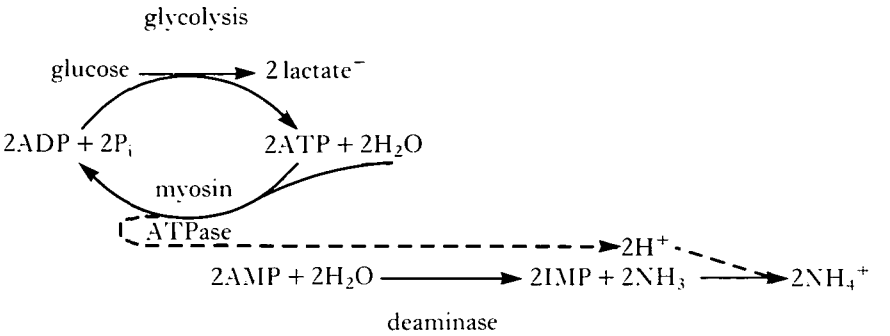
### *Fuel use and end product formation in white muscle*

Assuming a 1:1 stoichiometry between lactate and $H^+$ formation (Hochachka & Mommsen, 1983), the lactate concentrations indicate net $H^+$ production in white muscle during exhaustive work; previous studies, however, did not predict that the process of $H^+$ production should continue for at least the first hour or two of recovery, as indicated by our lactate data. These two phases of net lactate and $H^+$ production correlate with periods of ATP depletion (during work) and ATP replenishment (during early recovery) and both coincide with periods of active glycolytic flux in white muscle. Although these results are perplexing at first glance, we shall argue below that they help to explain why PCr and glycogen are only modestly or not at all replenished during the first 2 h of recovery. To understand the argument, it is necessary to examine closely the metabolic pathways operating at this time.

While these studies cannot define oxidative pathways of white muscle metabolism, they indicate that in this tissue three potential fuels for anaerobic energy generation (glycogen, PCr and ATP) are fully utilized when the organism is driven to exhaustion: glycogen drops to about 15% of pre-exercise levels, PCr to 8%, and ATP to 12% (Table 3). The extensive use of white muscle glycogen and PCr reserves during exhaustive swimming has been observed before (for example see Driedzic & Hochachka, 1976) and is not surprising. It may be emphasized, however, that the relationship between glycogen depleted and lactate accumulated has almost a 1:2 stoichiometry, while for PCr and creatine it is about 1:1 as required for metabolic systems behaving as if largely in isolation. Whereas almost total depletion of glycogen and PCr is not unexpected, the decline in ATP to below 1 $\mu$mol $g^{-1}$ is unusual, particularly by mammalian standards, where ATP levels appear to be 'defended' at about 2·5 $\mu$mol $g^{-1}$ during exhaustive muscle work (see for example Shoubridge, Briggs & Radda, 1982).

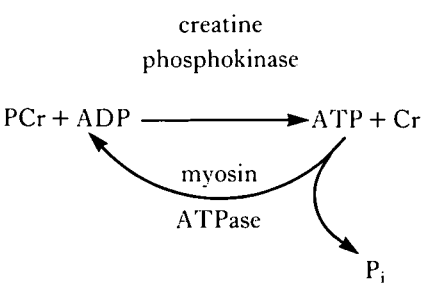
From the anaerobic mobilization of glycogen, PCr and ATP, at least six metabolites can be viewed as major anaerobic end products: lactate anions, protons, $NH_4^+$ and creatine and, in addition, inorganic phosphate ($P_i$) and IMP accumulate under these conditions (Dobson, 1986; Parkhouse, 1986). How are these six end

products formed? As discussed elsewhere (see Hochachka, 1985), the origin of IMP is the AMP deaminase reaction; on release of IMP and $NH_3$, protons are absorbed from the medium to form $NH_4^+$. Since protons are formed during anaerobic muscle work, the overall metabolic coupling can be summarized as follows:




If the two pathways (glycolysis + AMP→ IMP conversion) were stoichiometrically coupled, lactate and $NH_4^+$ would accumulate in a 1:1 ratio; at exhaustion, the observed ratio is in fact about 5:1 (Table 3). Therefore, $[H^+]$ should, and indeed does, rise, as indicated by several different criteria (Dobson, 1986; Parkhouse, 1986; Wood & Perry, 1985). Three glycolytically dependent processes presumably contribute to activation of AMP deaminase at this time: (i) a drop in energy charge and in absolute [ATP], (ii) a coincident drop in [GTP], which is a necessary result of the equilibration of GTP and ATP (see below), and (iii) a drop in pH, as AMP deaminase displays an acidic pH optimum of about pH 6·5 (Dudley & Terjung, 1985*a*,*b*).

In addition to the above five end products, it is now well known, that in mammalian muscles working largely anaerobically (Kushmerick, 1985), $P_i$ accumulates in proportion to PCr depletion. This has also now been demonstrated in trout muscle (Parkhouse, 1986; Dobson, 1986), but in this tissue it is further augmented by the large ATP depletion, and the summed process can be written as follows:




The accumulation of at least three of these end products may be mechanistically significant since two of them, $P_i$ and $NH_4^+$, are known activators of phosphofructokinase (Dobson, Yamamoto & Hochachka, 1986), while IMP is a possible activator of glycogen phosphorylase *b*. That is, as these three compounds are formed, they

may increase their own rates of production and thus autocatalytically increase glycolytic flux at appropriate times (when anaerobic ATP production is needed for sustaining burst swimming).

Finally, it should be mentioned that alanine and malate may represent minor anaerobic end products (Hochachka, 1985); another possibility is that their accumulation is due to augmentation of the Krebs cycle pool of intermediates and is indicative of minor oxidative contribution to ATP turnover at this time. As in red muscle, this could be fuelled by some endogenous substrates or by exogenous triglyceride, free fatty acid and glucose (Table 6).

*Glycogen and glucose profiles during recovery*

Glycogen replenishment in fish white muscle is known to take up to a day (Black *et al.* 1962) with little or no recovery evident in the first few post-exercise hours. Under our protocol, the absence of continued [glycogen] decrease in recovery, in fact, could be perplexing because lactate continues to accumulate during this time (Table 2). What is the carbon source for this continued lactate production and why is it occurring? Clearly, the carbon source is not endogenous glycogen, since its concentration does not change at this time; besides, its availability may already be prohibitively low. Because white muscle constitutes such a large fraction of the body mass, no other tissues or organs could store enough lactate to explain the observed augmentation of the white muscle pool. Thus, exogenous lactate can also be ruled out as a source of white muscle lactate at this time. A more probable source is liver-derived plasma glucose, for high perfusion rates under these conditions (Randall & Daxboeck, 1982) and high substrate availability (Tables 5, 8) would favour glucose flux to white muscle in the trout during recovery; high perfusion and adequate availability are preconditions for high flux rates of any plasma metabolite during exercise or recovery (see for example Weber, Brill & Hochachka, 1986). This interpretation is favoured by metabolite data for the liver, where the main catabolic end product during exercise appears to be glucose (Table 8). Although glucose does not accumulate in the liver at any time, storage glycogen is mobilized to a far greater extent than can be accounted for by lactate accumulation. Thus, the usual metabolic fate for liver glycogen is assumed to be conversion to glucose: liver glycogen → liver glucose → plasma glucose → muscle metabolism. In this regard, it is interesting that [glucose] in white muscle increases significantly during the first 10 min of recovery, then stabilizes at slightly lower levels (Table 2). Throughout this period, effective 'downhill' concentration differences are maintained between liver and plasma glucose compartments and between plasma and muscle (Tables 2, 5, 8), which would favour glucose-primed metabolism at a time (post-exercise) when muscle becomes more freely permeable to glucose. Whatever the source of lactate, be it liver-derived glucose or endogenous substrates or a combination of both, the question of its role remains. We suggest that the function of this glycolytic activation is to participate in, and complete, the ATP replenishment process, but, to appreciate why, it is important to review how ATP reserves are regained in white muscle tissue under these conditions.

*Glycolysis and the purine nucleotide cycle during recovery*

Since the nucleotide end product formed during depletion of the adenylates is IMP, the question of how ATP supplies are replenished can be rephrased in terms of how IMP is reconverted back to AMP, ADP and ATP. Some time ago, Lowenstein and his coworkers emphasized that the AMP ↔ IMP interconversions in vertebrate muscles, when summed, operate as a purine nucleotide cycle (Lowenstein, 1972). However, in teleost white muscle (Driedzic & Hochachka, 1976) it is evident that the reaction pathways constitute a cycle only in a formal sense. *In vivo*, AMP deaminase catalyses an effectively irreversible reaction that functions as a metabolic pathway *during work*, while the back arm of the cycle functions as another metabolic pathway bypassing AMP deaminase *during recovery*, thus returning IMP to the adenylate pool as AMP (see pathway in detail below). In trout white muscle, at least under our conditions, complete 'filling' of the adenylate pool appears to coincide with further lactate formation and thus to require coupling IMP → AMP conversion with glycolysis. As the first step in this arm of the cycle requires GTP, which occurs at only low concentrations in trout white muscle (Parkhouse, 1986; Dobson, 1986), the reaction sequence can be illustrated as follows (written for alkaline conditions, at about pH 8, with nucleotides unprotonated and for acidic conditions, about pH 6, with nucleotides fully protonated).

**pH 8·0**

$$GDP^{3-} + ATP^{4-} \rightarrow ADP^{3-} + GTP^{4-}$$

$$IMP^{2-} + \text{aspartate}^{-} + GTP^{4-} \rightarrow GDP^{3-} + P_i^{2-} + \text{adenylosuccinate}^{4-} + 2H^{+}$$

$$\text{adenylosuccinate}^{4-} + H_2O \rightarrow \text{fumarate}^{2-} + AMP^{2-}$$

$$AMP^{2-} + ATP^{4-} \rightarrow 2ADP^{3-}$$

$$1{\cdot}5\,\text{glucose} + 3ADP^{3-} + 3P_i^{2-} \rightarrow 3\,\text{lactate}^{-} + 3ATP^{4-}$$

Net:

$$IMP^{2-} + \text{aspartate}^{-} + 1{\cdot}5\,\text{glucose} + 2P_i^{2-} \rightarrow 3\,\text{lactate}^{-} + ATP^{4-} + \text{fumarate}^{2-} + 2H^{+}$$

**pH 6·0**

$$HGDP^{2-} + HATP^{3-} \rightarrow HADP^{2-} + HGTP^{3-}$$

$$HIMP^{-} + \text{aspartate}^{-} + HGTP^{3-} \rightarrow HGDP^{2-} + H_2PO_4^{-} + \text{H-adenylosuccinate}^{3-} + H^{+}$$

$$\text{H-adenylosuccinate}^{3-} + H_2O \rightarrow \text{fumarate}^{2-} + HAMP^{-}$$

$$HAMP^{-} + HATP^{3-} \leftrightarrow 2HADP^{2-}$$

$$1{\cdot}5\,\text{glucose} + 3HADP^{2-} + 3H_2PO_4^{-} \rightarrow 3\,\text{lactate}^{-} + 3HATP^{3-} + 3H^{+}$$

Net:

$$HIMP^{-} + \text{aspartate}^{-} + 1{\cdot}5\,\text{glucose} + 2H_2PO_4^{-} \rightarrow 3\,\text{lactate}^{-} + \text{fumarate}^{2-} + HATP^{3-} + 4H^{+}$$

In this view, glycolysis can serve as the main ADP acceptor during recovery of fully exhausted trout white muscle, allowing for complete replenishment of ATP pools, either because it simply outcompetes the meagre oxidative capacity of this tissue, or because $O_2$ availability to mitochondrial metabolism remains limited.

*Proton stoichiometry of IMP→AMP and of net IMP→ATP conversion*

The above metabolic sequence not only explains why continued lactate formation in trout white muscle coincides with augmentation of the adenylates, it also suggests that recovery of the ATP pool may aggravate tissue acid–base problems, *because the IMP→AMP arm of the purine nucleotide cycle proceeds with the net release of $H^+$ at all physiologically reasonable intracellular pH values*. From the reaction sequence as written above, it is evident that at pH 6, IMP conversion to AMP proceeds with the generation of a mole of $H^+$ per mole of IMP consumed. At higher pH, the number of moles of $H^+$ per mole of IMP increases (to a value of 2 when all nucleotides are unprotonated). When the pathway is coupled with glycolysis (as a source of high-energy phosphate), the proton stoichiometry of the summed sequence also depends upon pH. Although intracellular pH in recovery in white muscle under our conditions is not accurately known, the maximum range clearly falls between pH 6 and pH 8 (Wood & Perry, 1985). At pH 6, IMP conversion to ATP coupled with glycolysis proceeds with a net yield of $4H^+$ per ATP formed while at pH 8, $2H^+$ are formed per ATP synthesized; *in vivo* the stoichiometry lies somewhere between these two extremes. Evidently, the proton yield must vary as normal intracellular pH is established, yet it clearly is always at least slightly in excess of $2H^+$ per ATP synthesized. That is why in teleost white muscle one would expect adenylate replenishment to proceed with coincident $H^+$ generation (which is implied by the lactate data) or with a continued export of $H^+$ into the circulation [which may be favoured in some teleosts or even in trout under more modest swimming stresses (Wood & Perry, 1985)]. Additional evidence for this expectation comes from measured lactate/ATP concentration ratios.

*Lactate and ATP stoichiometry in white muscle in recovery*

An important conceptual insight arising from the above pathway analysis is that during adenylate regeneration in trout muscle, lactate and ATP are, for a short time, key end products of metabolism which should accumulate in a theoretical ratio of 3:1. Over the first 45 min of the recovery period, during which time the ATP pool is essentially fully replenished (from about 0·5 to 5·7 $\mu mol\,g^{-1}$), white muscle lactate increases from 24 to 37 $\mu mol\,g^{-1}$ (Table 2); the observed ratio is satisfyingly close to the 3:1 lactate/ATP concentration ratio predicted from the above metabolic sequence and can be taken as additional evidence for our interpretation.

*Why ATP and PCr replenishment occur separately*

The proton stoichiometry of net IMP→ ATP conversion also helps to explain one of the more striking observations of this study: PCr replenishment is only partial, even after 2 h (Table 3), which is unlike the situation in mammalian muscles of

mixed composition where PCr replenishment following work may be fairly rapid (Harris *et al.* 1976). Since, in principle, the catalytic potential of creatine phosphokinase (CPK) is high enough to use up all resting amounts of PCr in a matter of seconds, why is that same catalytic potential not used to restore PCr equally rapidly to these same resting levels? The simple reason why this does not occur is because $H^+$ production necessarily rises simultaneously with rising [ATP], in effect preventing the reaction equilibrium

$$H^+ + PCr + ADP \leftrightarrow ATP + Cr$$

from shifting towards the left (i.e. preventing the more complete phosphorylation of Cr to PCr). Although recent studies (Meyer, Brown & Kushmerick, 1985; Dobson *et al.* 1986) clearly establish that $H^+$ *per se* probably does not determine when fatigue sets in, it is evident that, in a system such as teleost white muscle, regaining $H^+$ homeostasis must precede complete PCr replenishment. Yet because of the continued net yield of $H^+$ during IMP $\rightarrow$ ATP conversion, $H^+$ homeostasis may not be achievable until ATP pools are fully recharged (i.e. until flux through the above metabolic sequences becomes negligible). That is why ATP replenishment precedes the complete recharging of PCr reserves (Table 3).

*Why glycogen and ATP replenishment occur separately*

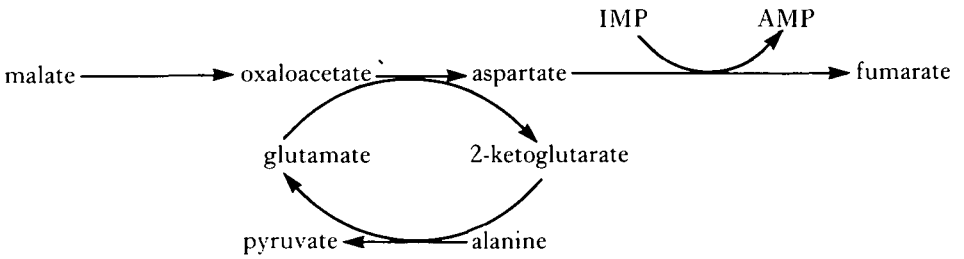
Although it has been known for many years that glycogen repletion in fish white muscle requires long time periods, the above metabolic sequence, showing the coupling of IMP $\rightarrow$ AMP conversion with glycolysis, explains for the first time why this should be so: because in vertebrate white muscle, much if not all glycogen replenishment depends upon lactate reconversion to glycogen *in situ* (Shiota, Golden & Katz, 1984). Reversed glycolysis is the most probable pathway by which this occurs (Shiota *et al.* 1984), and net flux in this direction obviously cannot proceed while the pathway is being used in the forward direction to recharge cellular ATP reserves. That is why ATP replenishment necessarily precedes glycogen replenishment and why, during early phases of recovery, no increase in white muscle [glycogen] is observed (Table 2), a situation in sharp contrast with that in the liver.

Since a large adenylate replenishment is not required in the liver (and acidification seems much more modest), the main recovery process in this tissue seems geared towards glycogen repletion which is complete within 2 h (Table 8). Glucose is probably not the precursor for the glycogen reformed in recovery because at this time glucose availability is not yet normal, presumably due to continued uptake and fermentation in white muscle (see above). Although this is consistent with current concepts of glucose as an inefficient substrate for liver glycogen (Newgard, Hirsch, Foster & McGarry, 1983), we would be less hasty in arriving at such a conclusion if two other potential glycogen precursors were not available. However, as indicated in Table 8, liver [alanine] and [lactate] are both high at exhaustion. Whereas [alanine] does not increase further during recovery, [lactate] continues to rise [apparently due to uptake from a plasma pool which contains lactate at even higher concentrations (Table 5)]; its ready availability should make it an ideal precursor for glycogen

(Newgard *et al.* 1983). We assume that lactate and $H^+$ may enter liver cells *via* a symport system, or lactate and $OH^-$ may exchange *via* an antiport system, so that the CPK equilibrium is shifted slightly to the right, a process which may underlie the modest adjustments noted in these intermediates during recovery and which is consistent with current concepts of lactate transport (see Johnson *et al.* 1980; Koch, Webster & Lowell, 1981; Hochachka & Mommsen, 1983).

*Source of aspartate for IMP→AMP conversion*

Although the above interpretation explains many of our data, a perplexing problem, which has thus far eluded resolution, concerns the origin of white muscle aspartate, the acknowledged nitrogen donor for the IMP→AMP conversion (Dudley & Terjung, 1985*a*,*b*). The amount required is considerable (about $5\,\mu mol\,g^{-1}$), while the amount available in salmonid white muscle is about $0{\cdot}1$–$0{\cdot}2\,\mu mol\,g^{-1}$ (Mommsen, French & Hochachka, 1980). Thus it seems necessary to postulate the existence of an endogenous or exogenous precursor. Liver malate is a possible metabolite candidate under the recovery conditions in this study. Liver [malate] changes only modestly during exercise (Table 8) but, interestingly enough, decreases during early recovery (Table 8) while [malate] in white muscle increases (Table 2). A possible scheme that could account for sparking the flow of IMP to AMP is:




In this event, white muscle [alanine] should drop during recovery; unfortunately, this is not convincingly demonstrable (Table 2), either because the above process does not occur, or because alanine precursors, such as glucose (Table 2), are mobilized as fast as alanine is used. Under recovery conditions favouring oxidative metabolism in white muscle, $NH_4^+$ fixation *via* the glutamate dehydrogenase reaction could serve as the nitrogen source for IMP→AMP conversion (T. P. Mommsen & P. W. Hochachka, unpublished data). Clearly, further work is needed to clarify this important question.

*Other roles for $H^+$ during recovery*

Because of the pervasive effects of change in $[H^+]$ on catalysis and metabolic regulation (Hochachka & Somero, 1984), a final and equally perplexing question concerns why nature should design a muscle metabolism which generates $H^+$ during early phases of recovery. We can think of two possible advantages. In the first place, $H^+$ accumulation during recovery may facilitate lactate efflux from white muscle. As

mentioned above, current evidence on lactate movements across cell membranes cannot distinguish between a symport system (lactate and $H^+$ moving together) and an antiport system (lactate exchanging for $OH^-$). In either event, an increase in intracellular $[H^+]$ should facilitate lactate efflux; if this effect is not offset by corresponding changes in passive diffusion (due to changes in dissociation of lactic acid), it may represent enough of an advantage to help explain the origin of this novel metabolic arrangement.

Another advantage of continued $H^+$ accumulation during early phases of recovery may arise for $NH_4^+$ retention within white muscle cells. A modest rise in $[H^+]$ at physiological pH ranges leads to quite large increases in the $NH_4^+/NH_3$ concentration ratio, which in turn should facilitate $NH_4^+$ retention in white muscle (D. J. Randall, personal communication). Because of the large mass of white muscle relative to total body mass, equilibration of $NH_4^+$ between plasma and intracellular fluid could, in effect, flood the organism with ammonium ions at concentrations some five-fold higher than actually observed (Table 5). Whether or not $NH_4^+$ at about 5 mmol $l^{-1}$ is detrimental to the teleost central nervous system or to other tissues is not known; however, such concentrations would be highly undesirable in mammals. In such event, the advantage of $NH_4^+$ retention may be added to selective forces favouring utilization and development of the IMP → AMP conversion sequence in the first place.

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