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METABOLIC RESPONSES OF TROUT (*SALMO GAIRDNERI*) TO ACUTE ENVIRONMENTAL HYPOXIA

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SUMMARY

Rainbow trout were subjected to 1 and 3 h of environmental hypoxia (20 Torr, 4 °C), after which samples of blood, heart, brain, liver, and red and white muscle were removed for metabolite determination. The heart, brain and white muscle all showed signs of glycolytic activation. High-energy phosphate stores in the liver were greatly depleted, although there was no measurable decline in liver glycogen content. Glycolytic activation in the white muscle is argued to have a major impact on the hypoxia tolerance of trout, as this tissue produces the bulk of the lactate. These responses of the trout are contrasted with those of the African lungfish, a fish which is relatively tolerant of hypoxia.

INTRODUCTION

When a cell becomes hypoxic, the rate of oxygen delivery is less than that which is required for oxidative metabolism to supply the energy needs of the cell. The result is a reorganization of metabolic processes which are designed to compensate for the lack of oxygen. Numerous studies indicate that such reorganization tends to follow one of two generalized patterns: either the rate of anaerobic ATP production increases (Pasteur effect) or the ATP turnover rate declines (De Zwaan & Wijsman, 1976; Dunn, 1985; Hochachka & Somero, 1984; Pamatmat, 1979; Robin, 1980; van den Thillart, 1982). In most vertebrates, the first pattern involves glycolytic activation, with glucose or glycogen as the substrates and lactate as the product. In the second pattern, that of metabolic depression, the strategy is to balance reduced rates of ATP production with the reduced rates of ATP utilization. In hypoxia, as in all other metabolic states (Atkinson, 1977), a controlled coupling must be maintained between ATP production and utilization (Dunn, 1985).

One indication that the strategy of metabolic depression is being utilized is the lack of glycolytic activation when oxygen supply declines (Dunn, 1985; Hochachka & Somero, 1984; Hochachka & Dunn, 1983, 1985). If the metabolic rate of the whole body is depressed, then one or more tissues in the body must be responding to a decline in oxygen delivery with metabolic depression (i.e. a lack of a Pasteur effect).

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During hypoxia, the African lungfish exhibits a reduced metabolic rate (Lahiri, Szidon & Fishman, 1970) and its white muscle fibres are capable of preventing glycolytic activation (Dunn, Hochachka, Davison & Guppy, 1983). Although the lungfish would not be capable of surviving hypoxia without the capacity for glycolytic ATP production in the heart and brain, it is probable that the capacity for metabolic depression in the white muscle is a very significant adaptation to hypoxia in these fish (Dunn, 1985; Dunn *et al.* 1983).

Trout differ from lungfish in that they do not exhibit a decline in metabolic rate during acute hypoxia until oxygen tensions fall below about 40 Torr; rates of oxygen uptake may actually increase when the animal is exposed to reduced environmental oxygen tensions (Holeton & Randall, 1967; Hughes & Saunders, 1970; McKim & Goeden, 1982). Also, salmonids are among the most sensitive of fishes to oxygen deficiency (Doudoroff & Shumway, 1970), whereas lungfish are relatively tolerant to hypoxia (Lahiri *et al.* 1970). From this we predicted that the Pasteur effect in trout tissues, specifically the white muscle, would be much more apparent than in the lungfish. To test this hypothesis, we analysed the inter-tissue metabolic responses to hypoxia in trout and examined metabolite changes in heart, brain, blood, red muscle, white muscle and liver during imposed hypoxia.

MATERIALS AND METHODS

Experimental animals

Rainbow trout, (*Salmo gairdneri* Richardson), were obtained from Sun Valley trout farms (Mission BC), and kept in large outdoor tanks with a flow-through water system and a natural photoperiod. They were fed midday with commercial trout pellets until 2 days before the experiment. The fish had a mean mass of 320 g. The experiments were carried out in early spring when the water temperature was 4 °C.

Experimental procedure

The fish were placed in a black Plexiglas holding box 1 day before experimentation. The box had six slatted compartments, each of which held one fish. Oxygen tensions were adjusted by bubbling nitrogen into a central mixing chamber at the front of the box. Water flowed from this chamber directly into each of the fish compartments. Oxygen tension was monitored by a Radiometer oxygen electrode connected serially to an amplifier and a chart recorder. Water and gas flow rates were adjusted to obtain a reproducible decline in oxygen tension in the box. The oxygen tension declined gradually to 20 Torr after 20 min and was maintained there for the remainder of the exposure.

Preliminary tests were performed varying time of initiation, the duration of exposure, the availability of surface access and the level of oxygen tension. This was done to determine a procedure for hypoxic exposure which would result in a reproducible increase in blood [lactate] with no fish mortality. Plasma [lactate] in non-stressed fish, in fish exposed to hypoxia for 3 h beginning at 06.30 h, and in fish exposed for 3 h beginning at 10.30 h were $0{\cdot}68 \pm 0{\cdot}40\,\mathrm{mmol\,l^{-1}}$, $4{\cdot}00 \pm 0{\cdot}75\,\mathrm{mmol\,l^{-1}}$

and $6{\cdot}85 \pm 0{\cdot}65\,\mathrm{mmol\,l^{-1}}$, respectively ($\bar{x} \pm$ S.D., $N = 5$). The time chosen for initiation of the experiment was 10.30 h. Fish were denied access to the surface by floating wood in the chambers since it was found that this produced higher and more reproducible blood [lactate].

In the major metabolite experiment, samples were taken from eight control fish (without access to the surface but not subjected to hypoxia) and seven fish exposed for 3 h at 20 Torr. Table 3 includes data from four fish subjected to 1 h of hypoxia (to make the data distribution more uniform for a correlation analysis). Timing began concurrently with the initiation of declining oxygen tensions.

Metabolite preparation and assay

Tissues were removed from beheaded fish and frozen at −196 °C by clamping with aluminium tongs which had been cooled in liquid nitrogen. The order of freezing was blood, heart, brain, liver, red muscle and white muscle. The heart and brain were frozen within 60 s and the total time for freezing all tissues was roughly 150 s. The order of tissue clamping was chosen to ensure that tissues with the highest metabolic rate were frozen first. Freezing times are unlikely to have allowed serious metabolic changes since muscle [creatine phosphate], a rapidly mobilized metabolite, is high (Dunn *et al.* 1983; van den Thillart, Kesbeke & van Waarde, 1980; van Waarde, van den Thillart & Kesbeke, 1983). In addition, the lag time in freezing some tissues is unlikely to affect the analysis since both control and hypoxic samples were treated identically. Muscle samples were removed from two locations. The anterior samples were taken from the region just caudal to the gills and the posterior samples were taken from the region between the dorsal fin and the caudal peduncle. Blood was withdrawn from the trunk *via* caudal vessel puncture and placed directly into 1 volume of ice-cold 6 % perchloric acid (PCA). Tissues were ground to a fine powder using a mortar and pestle resting on dry ice and further cooled by flushing with liquid nitrogen. About 0·5 g of powder was homogenized in 5 volumes of $1{\cdot}4\,\mathrm{mol\,l^{-1}}$ PCA using a Polytron homogenizer–sonicator. Two samples of 100 μl were removed and frozen at −80 °C for subsequent glycogen analysis. The homogenate was spun at 10 000 g for 15 min and the pellet discarded. The supernatant was neutralized to pH 6·7 with $3\,\mathrm{mol\,l^{-1}}$ K_2CO_3 and spun again. All metabolites, except glucose, glycogen and lactate, were measured within 9 h of neutralization. The order in which metabolites were assayed was kept constant with ATP and creatine phosphate (CrP) being measured first.

Metabolites were measured by linking them enzymatically to reactions using $NADH/NAD^+$, or $NADPH/NADP^+$, and following the reaction at 340 nm on a Unicam SP-1800 spectrophotometer. Glycogen was measured using the amyloglucosidase technique (Keppler & Decker, 1970). The remaining metabolites were measured with the techniques of Hochachka, Hulbert & Guppy (1978) and Bergmeyer (1974).

Data were compared using one-way analysis of variance (ANOVA) with a significance level of $P < 0{\cdot}05$.

RESULTS

Preliminary experiments indicated that both time of day and the availability of access to the surface affected the metabolic response to environmental hypoxia in the trout (see Materials and Methods). The following data are from trials begun at 10.30 h with fish that were denied access to the surface.

There was very little change in the concentration of metabolites in the brain during hypoxia. Glycogen concentrations showed a declining trend ($P = 0{\cdot}06$) (Table 2). The total pool of creatine and CrP was maintained, as was the energy charge (EC) (Table 1).

The heart showed significant declines in glycogen content while [lactate] increased. The concentrations of ATP and CrP, and the total adenylate pool declined. The energy charge did not change.

Neither glycogen content, nor [glucose] changed in the liver during hypoxia, while the concentrations of glucose-6-phosphate (G6P) and lactate increased. The levels of ATP and CrP fell, as did the total pools of creatine+CrP and of adenylates (Tables 1, 2). The EC declined, indicating that a metabolic stress had occurred (Vetter & Hodson, 1982).

Although [G6P] and [lactate] rose in red muscle, there was no change in [glucose] or glycogen content. The only significant change in the high-energy phosphate compounds was a fall in [CrP]. The concentrations of CrP were higher in the tail region of the control fish, while [G6P] was higher in the anterior samples.

In white muscle, [creatine] increased and [CrP] declined. The concentrations of G6P and lactate rose significantly in the tail. In control fish, CrP concentrations were higher in the tail. Glycogen content in the tail showed a declining trend ($P = 0{\cdot}06$) (Table 2).

Table 3 shows the results of correlations performed between blood and tissue lactate and glucose concentrations. Individual data points (not mean values) were used to calculate the correlation coefficient. Data from four fish killed at 1 h are included in order to make the data distribution more uniform. The brain was the only tissue where [lactate] did not correlate with the blood [lactate]. In all other tissues, blood concentrations of glucose and lactate correlated with those in the blood.

The available supply of substrates for glycolysis and the amount of lactate produced are listed in Table 4 (anterior and posterior data are pooled). Although the liver was the main glycogen store in terms of absolute concentration (133 $\mu\mathrm{mol\,g^{-1}}$), the white muscle contained the greatest supply of carbohydrate of all the tissues. G6P was present in large enough amounts in the white muscle to warrant inclusion into calculations of available carbohydrates. The pools of glycogen, glucose and G6P are shown in Fig. 1 to stress the fact that white muscle, not liver, was the major potential store of glycolytic substrates.

Just as the white muscle contained most of the substrate, it also contained most of the end-product – lactate (Fig. 2). The greatest percentage changes in [lactate] were in the heart, brain and blood.

Table 1. *Concentrations of adenylates, creatine phosphate and creatine and the energy charge in trout tissues*

Experimental condition	*N*	ATP	ADP	AMP	EC	Total adenylates	Creatine	Creatine phosphate	Total creatine
Brain									
Control	7	0·73 ± 0·18	0·58 ± 0·14	0·39 ± 0·20	0·61 ± 0·11	1·70 ± 0·25	9·68 ± 1·37	0·98 ± 0·53	10·66 ± 0·98
3 h	8	0·70 ± 0·23	0·73 ± 0·20	0·26 ± 0·11	0·63 ± 0·09	1·69 ± 0·39	8·55 ± 0·93	1·03 ± 0·46	9·57 ± 0·81
Heart									
Control	7	2·43 ± 0·65	0·72 ± 0·27	0·17 ± 0·14	0·84 ± 0·07	3·33 ± 0·60	3·87 ± 0·78	4·63 ± 0·54	8·51 ± 0·82
3 h	8	1·68 ± 0·58*	0·76 ± 0·33	0·13 ± 0·08	0·80 ± 0·08	2·57 ± 0·65*	4·55 ± 1·48	2·93 ± 1·23*	7·48 ± 1·61
Liver									
Control	7	1·35 ± 0·21	0·55 ± 0·38	0·24 ± 0·08	0·76 ± 0·07	2·14 ± 0·34	1·20 ± 0·54	0·28 ± 0·22	1·48 ± 0·49
3 h	8	0·53 ± 0·23*	0·67 ± 0·09	0·28 ± 0·06	0·58 ± 0·09*	1·48 ± 0·24*	1·03 ± 0·41	0·02 ± 0·02*	1·04 ± 0·41
Anterior red muscle									
Control	7	2·85 ± 0·97	0·68 ± 0·22	0·15 ± 0·03	0·86 ± 0·08	3·69 ± 0·84	12·43 ± 1·91	5·74 ± 2·93†	18·16 ± 3·51
3 h	8	2·65 ± 0·44	0·82 ± 0·12	0·13 ± 0·05	0·85 ± 0·03	3·61 ± 0·40	13·76 ± 4·44	3·57 ± 1·59	17·33 ± 4·19
Posterior red muscle									
Control	7	3·73 ± 0·90	0·76 ± 0·16	0·15 ± 0·06	0·88 ± 0·05	4·64 ± 0·79	11·65 ± 1·97	9·28 ± 2·56	20·93 ± 4·22
3 h	8	3·21 ± 0·43	0·83 ± 0·09	0·11 ± 0·04	0·88 ± 0·02	4·16 ± 0·51	12·76 ± 2·47	4·72 ± 2·01*	17·48 ± 2·72
Anterior white muscle									
Control	7	7·42 ± 0·71	1·01 ± 0·20	0·10 ± 0·03	0·93 ± 0·02	8·54 ± 0·60	25·71 ± 4·94	20·77 ± 2·10†	46·48 ± 5·81
3 h	8	6·78 ± 0·44	1·07 ± 0·16	0·10 ± 0·06	0·92 ± 0·01	7·95 ± 0·49	34·19 ± 4·06*	13·65 ± 4·96*	48·55 ± 2·02
Posterior white muscle									
Control	7	7·41 ± 0·86	0·91 ± 0·19	0·09 ± 0·05	0·93 ± 0·02	8·42 ± 0·71	24·47 ± 4·92	26·94 ± 5·20	51·41 ± 6·27
3 h	8	6·94 ± 0·35	1·11 ± 0·32	0·11 ± 0·06	0·92 ± 0·02	8·15 ± 0·46	34·92 ± 4·06*	13·63 ± 4·96*	48·55 ± 2·02

* Significantly differs from normoxia ($P < 0·05$).

† Significantly differs from posterior sample ($P < 0·05$).

Values are means ± 1 S.D. in $\mathrm{mmol\,l^{-1}}$.

EC, energy charge.

The ratios of blood [lactate] to tissue [lactate] are listed in Table 5. White muscle is the only tissue where [lactate] is consistently higher than it is in the blood. In the remainder of the tissues, the ratio changes during hypoxia, with blood [lactate] becoming higher than it is in the respective tissues.

Table 2. *Selected trout glycolytic metabolites at rest and during acute hypoxia*

Experimental condition	*N*	Glycogen	Glucose	G6P	Lactate
Brain					
Control	7	3·69 ± 1·78	5·04 ± 3·72	0·08 ± 0·07	2·12 ± 1·23
3 h	8	1·83 ± 1·61	4·99 ± 2·14	0·13 ± 0·05	3·91 ± 1·21
Heart					
Control	7	36·28 ± 19·13	8·82 ± 5·02	0·24 ± 0·07	0·71 ± 0·23
3 h	8	19·27 ± 10·32	7·53 ± 2·48	0·28 ± 0·17	6·15 ± 3·52•
Liver					
Control	7	133·5 ± 68·66	11·9 ± 9·04	0·15 ± 0·14	0·95 ± 0·16
3 h	8	142·8 ± 58·3	12·2 ± 4·19	1·21 ± 0·43•	5·40 ± 1·55•
Anterior red muscle					
Control	7	14·56 ± 6·01	1·71 ± 1·32	0·44 ± 0·11†	2·34 ± 1·14
3 h	8	16·52 ± 8·79	1·65 ± 0·33	1·29 ± 0·41•	4·35 ± 2·03•
Posterior red muscle					
Control	7	15·26 ± 9·04	1·60 ± 1·05	0·25 ± 0·13	1·73 ± 1·05
3 h	8	15·80 ± 8·10	1·55 ± 0·39	1·02 ± 0·30•	4·15 ± 2·21•
Anterior white muscle					
Control	7	15·89 ± 5·04	0·74 ± 0·47	0·80 ± 0·44	6·77 ± 4·85
3 h	8	12·12 ± 4·94	0·72 ± 0·22	1·40 ± 0·61	10·90 ± 3·88
Posterior white muscle					
Control	7	18·00 ± 7·58	0·70 ± 0·48	0·57 ± 0·34	5·78 ± 3·67
3 h	8	12·28 ± 4·30	0·76 ± 0·24	1·36 ± 0·47•	11·39 ± 2·92•
Blood					
Control	7		12·51 ± 9·45		0·37 ± 0·27
3 h	8		7·30 ± 3·07		6·93 ± 1·98•

• Significantly differs from normoxia ($P < 0·05$).
† Significantly differs from posterior sample ($P < 0·05$).
Values are means ± 1 S.D. in μmol g^{-1} wet weight.
G6P, glucose-6-phosphate.

Table 3. *Correlations between tissue and blood metabolite concentrations in trout*

	Lactate		Glucose	
Tissue	*P*	*r*	*P*	*r*
Brain	0·0859	0·416	<0·0001	0·876
Heart	<0·0001	0·895	<0·0001	0·857
Liver	<0·0001	0·973	<0·0001	0·906
Anterior red muscle	0·0003	0·750	<0·0001	0·838
Posterior red muscle	0·0014	0·693	<0·0001	0·838
Anterior white muscle	0·0052	0·629	<0·0001	0·797
Posterior white muscle	0·0018	0·683	0·0004	0·742

Probability that $r = 0$; $N = 19$.

DISCUSSION

This discussion begins by examining tissue responses to hypoxia and concludes with a more integrated discussion of whole-body metabolism.

Brain

The observation that there are no significant changes in the concentrations of high-energy phosphate compounds or lactate in the brain indicates that this tissue is still receiving most of its oxygen requirement. The only observed metabolite change is a declining trend in glycogen content ($P = 0{\cdot}06$).

It has been previously noted that stored glycogen content may correlate with the relative hypoxia tolerance of the tissue (Daw, Wenger & Berne, 1967; Dawes, Mott & Shelley, 1959; Kerem, Hammond & Elsner, 1973). Since glycogen content in trout brain is less than half that of lungfish (Dunn *et al.* 1983), it is possible that the trout brain does not have as great a capacity for anaerobic ATP generation as does the lungfish brain.

The following calculation indicates that, even if all of the glycogen was fermented to lactate, the ATP produced probably could supply the brain for only about 10 min. The brain from a 500-g fish requires about $5\,\mu\mathrm{mol}\,O_2\,h^{-1}$, or $30\,\mu\mathrm{mol}\,\mathrm{ATP}\,h^{-1}$ (McDougal *et al.* 1968). Trout brain glycogen content (glucosyl units) is $3{\cdot}7\,\mu\mathrm{mol}\,g^{-1}$ and so the organ in total contains only 1·6 μmol of glucosyl units. This could produce only 4·8 μmol of ATP *via* glycolysis.

The above points indicate that the brain is not likely to produce a significant proportion of its ATP requirement from glycogen fermentation. At the same time,

Table 4. *Glycolytic metabolite stores in trout*

Tissue	Glycogen	Glucose	Glucose-6-phosphate	Lactate
Liver				
Control	594 ± 305	53 ± 40	0·7 ± 0·6	4·2 ± 0·7
3 h	635 ± 259	54 ± 19	5·4 ± 1·9*	24 ± 6·9*
White muscle				
Control	5592 ± 2106	238 ± 155	227 ± 117	2017 ± 1394
3 h	4025 ± 1491	244 ± 64	455 ± 161*	3677 ± 1087*
Red muscle				
Control	373 ± 176	41 ± 29	8·6 ± 2·5	51 ± 24
3 h	404 ± 209	40 ± 7	29 ± 8·3*	106 ± 52*
Others				
Control	19 ± 10	328 ± 299	0·15 ± 0·05	11 ± 8
3 h	11 ± 4	225 ± 96	0·19 ± 0·05	213 ± 61*

* Significantly differs from control ($P < 0{\cdot}05$).

Others includes the blood (where applicable), as well as the heart and the brain.

Tissue weights (% of body weight) used for calculations are as follows: brain, 0·087; heart, 0·072; liver, 0·089 (this study); red muscle, 5·0; blood, 6 (Daxboeck, 1981); white muscle, 66 (Stevens, 1968).

Values are means ± 1 S.D., in μmol/500-g fish.

the concentrations of energy-linked metabolites are maintained. For these reasons we conclude that brain metabolism remains largely aerobic and that this tissue is protected from hypoxia by other mechanisms.

Heart

The heart, in contrast, exhibits an intermediate response to hypoxia. Although there is no significant change in the EC, significant declines in the total adenylate pool, [ATP], [CrP] and glycogen content indicate that anaerobic ATP synthesis is occurring in the heart.

Since the source of glucosyl units for glycolysis is probably endogenous (liver glycogen content does not decline), one may calculate the amount of ATP produced by anaerobic glycolysis. Taking mean values, 17 μmol g^{-1} wet weight of glycogen (glucosyl units) are mobilized in the heart. This translates to the utilization of 1·22 μmol of glucosyl units in 3 h, or the anaerobic production of 1·22 μmol ATP heart^{-1} h^{-1}.

The rates of oxygen uptake in ocean pout and sea raven hearts are 76 and 92 nmol g^{-1} dry weight s^{-1} (Driedzic, 1983). These values translate to the production of approximately 10 μmol ATP 0·072 g^{-1} wet weight h^{-1}. If trout hearts

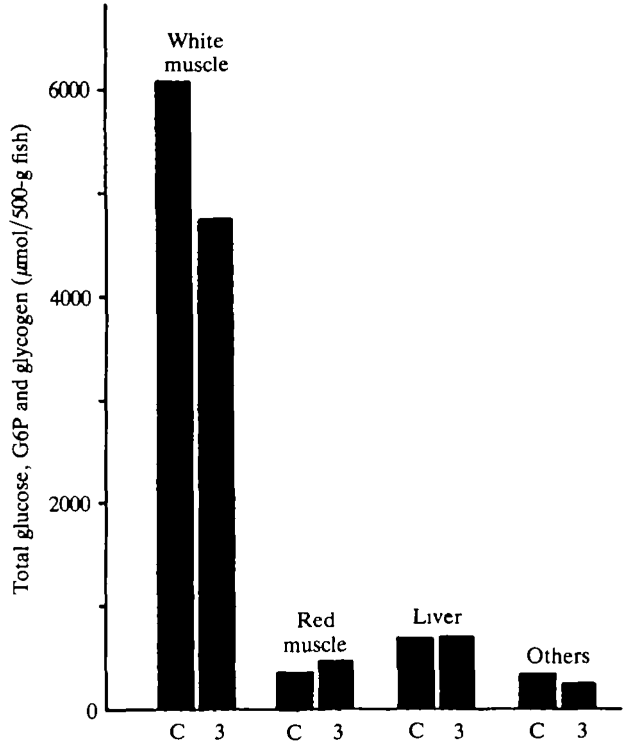

Fig. 1. Total glucose, glycogen and glucose-6-phosphate (G6P) stores in trout. The others are the brain, heart and blood. C indicates control values and 3 indicates values from trout exposed to hypoxia for 3 h. Tissue weights used in the calculations are listed in Table 4.

have a similar metabolic rate, then the heart is supplying roughly 10% of its ATP using anaerobic glycolysis.

If all of the lactate produced were to remain in the heart, the lactate concentration would have to increase by 33 μmol g^{-1} wet weight of tissue. Since the actual

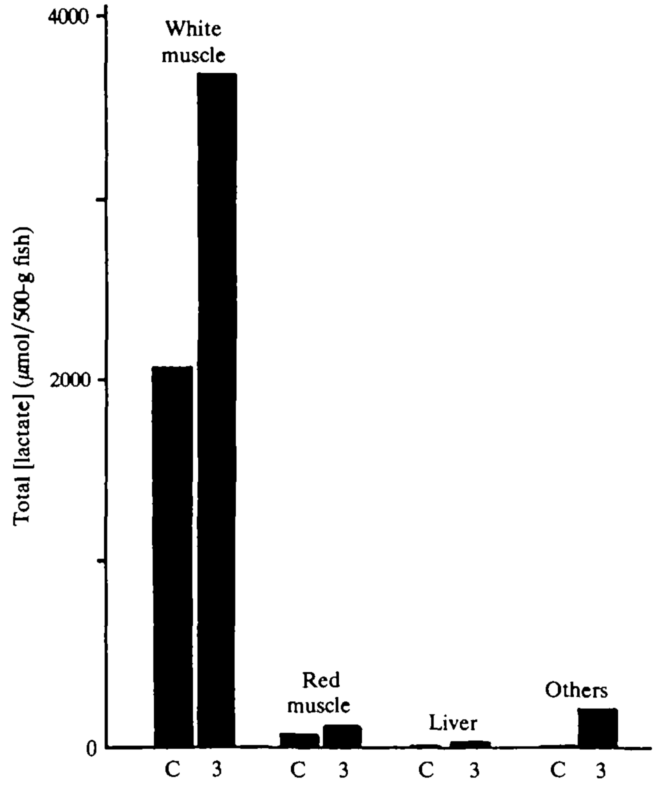

Fig. 2. Total lactate stores in trout. The others are the brain, heart and blood. C indicates control values and 3 indicates values from trout exposed to hypoxia for 3 h. Tissue weights used in the calculations are listed in Table 4.

Table 5. *The ratios of blood lactate to tissue lactate in trout*

Tissue	Control	3-h exposure	Change in mean ratio
Brain	0·264 ± 0·270	1·960 ± 0·871	+1·696
Heart	0·551 ± 0·384	1·348 ± 0·589	+0·797
Liver	0·409 ± 0·313	1·298 ± 0·184	+0·889
Anterior red muscle	0·195 ± 0·141	1·823 ± 0·692	+1·628
Posterior red muscle	0·283 ± 0·182	2·055 ± 0·908	+1·772
Anterior white muscle	0·103 ± 0·105	0·656 ± 0·134	+0·553
Posterior white muscle	0·099 ± 0·081	0·627 ± 0·192	+0·528

All changes between control and 3 h are significantly different at $P < 0{\cdot}05$.
Values are means ± 1 S.D.

concentration increase is only 5·4 μmol g^{-1}, the heart is probably a net lactate exporter during the hypoxic conditions of an experiment. That the heart is not surviving the hypoxic stress well, is indicated by the fact that concentrations of high-energy phosphate compounds decline even though glycolysis appears to be activated.

Liver

This tissue shows the most drastic metabolite changes during the hypoxic stress. The EC, [ATP], total adenylate content and [CrP] all decline significantly. The low concentrations of these metabolites indicate that the resynthesis of glucose (or glycogen) from 3-carbon molecules (i.e. lactate or amino acids) is probably inhibited by a lack of chemical energy. Such severe declines in EC during hypoxia have also been observed in flounder, goldfish and eel (Jorgensen & Mustafa, 1980; van den Thillart *et al.* 1980; van Waarde *et al.* 1983).

Since the conversion of glycogen to glucose does not require high-energy phosphate compounds, the liver may still be releasing glucose to the blood. However, liver glycogen content does not change during hypoxia. This contrasts with the report that liver glycogen content fell from 70 μmol g^{-1} to less than 5 μmol g^{-1} (glucosyl units) in *Salmo clarki* exposed to hypoxic conditions for 1 h (Heath & Pritchard, 1965).

The response of liver glycogen phosphorylase to hypoxia seems to be variable. Metabolite measurements from the livers of lungfish (Dunn *et al.* 1983) and flounder (Jorgensen & Mustafa, 1980) indicate a decline in liver glycogen content, carp show no change (Johnston, 1975*a*), while tench liver glycogen content declines only for a short time (Demael-Suard *et al.* 1974). It appears that the regulation of fish liver glycogen phosphorylase awaits further investigation.

Red muscle

Metabolic conditions in the red muscle appear to be similar to those in the brain, in that both tissues are protected during hypoxia (as indicated by the lack of change in metabolite concentrations).

CrP concentrations in posterior red muscle decline during hypoxia, but anterior samples show no such change. It is possible that this change is due more to exercise than to hypoxic dysoxia.

The rational for this statement is that posterior regions may exhibit more marked concentration changes due to struggling than would anterior samples. Somero & Childress (1980) found a small increase in lactate dehydrogenase (LDH) activity in white muscle of *Paralabrax clathratus* just caudal to the dorsal fin. The present experiments show that CrP concentrations in the red and white muscle of trout are higher posterior to the dorsal fin than in the anterior samples. These data suggest that the posterior myotome may differ metabolically from the anterior regions in the capacity to produce ATP using rapid anaerobic processes. Since the trout is not particularly tolerant to environmental hypoxia, this metabolic potential is likely to be used during exercise.

The fact that control posterior samples display a higher [CrP] than do anterior samples, and that the decline in [CrP] is seen only in the posterior samples, suggests that the store of CrP is being mobilized for work rather than anaerobic augmentation of oxidative metabolism.

During hypoxia, [lactate] and [G6P] increase. These changes may reflect glycolytic activation, but they may also indicate an influx of lactate due to increased blood concentrations, and a lack of phosphofructokinase (PFK) activation when hexokinase or glycogen phosphorylase is supplying G6P. Although it is uncertain whether glycolysis has been activated, the maintenance of high-energy phosphate compound levels indicates that this muscle has not been greatly stressed. This is a similar observation to that made by Johnston (1975*b*). If the muscle is not oxygen limited, then it is likely that red fibres are utilizing lactate, since this metabolite is readily oxidized (Bilinski & Jonas, 1972; Hochachka, 1985) and lactate turnover increases during hypoxia (J. F. Dunn & P. W. Hochachka, in preparation).

White muscle

As mentioned above, endogenous glycogen content may reflect the relative capacity to synthesize ATP *via* glycolysis. Using this argument, trout muscle glycolytic capacity exceeds that of the lungfish and is similar to that of the goldfish (Dunn *et al.* 1983; van den Thillart *et al.* 1980).

During hypoxia, white muscle glycogen content shows a declining trend ($P = 0{\cdot}06$), [CrP] declines and [lactate] increases. Although it is possible that the lactate is synthesized elsewhere in the body and delivered to the white muscle *via* the blood, there are two reasons why such a scenario is unlikely.

Firstly, white muscle is the only tissue where the ratio of blood to tissue lactate concentrations remains less than one throughout the experiment (Table 5). Since a cell is relatively negative inside (Prosser, 1973), this indicates that white muscle is synthesizing lactate and that the electrochemical gradient is from tissue to blood. Secondly, comparisons of the total quantity of lactate produced in the body with the quantity of substrate available which is *not* contained in the white muscle, indicate that there is barely enough substrate available to account for the increase in [lactate] (Table 4; Figs 1, 2). Thus, we conclude that white muscle glycogen is being mobilized and is the source of the observed rise in [lactate]. Unlike the situation in lungfish (Dunn *et al.* 1983), the above evidence (and Johnston, 1975*b*) supports the premise that the glycolytic rate increases in trout white muscle when the muscle becomes hypoxic (a standard Pasteur effect).

Although increases in anaerobic metabolism are not sufficient to maintain [CrP], the EC and [ATP] remain constant. This capacity of white muscle to maintain a high 'energy status' during oxygen limitation is common in fish, having been observed in lungfish, flounder, goldfish and eel (Dunn *et al.* 1983; Jorgensen & Mustafa, 1980; van den Thillart *et al.* 1980; van Waarde *et al.* 1983).

Tissue–tissue interactions

Which tissue or tissues are the main sources of the lactate, and what inferences can be made about the metabolic rate of trout and its component organs during hypoxia?

Liver glycogen deposits are used during hypoxia in many animals as a source of glucose for catabolism (Hochachka & Somero, 1984; van den Thillart *et al.* 1980; van Waarde *et al.* 1983). During this experiment, however, liver glycogen content was unchanged. In addition, the turnover rates of glucose did not rise during a comparable hypoxic exposure (J. F. Dunn & P. W. Hochachka, in preparation), which indicates that there is minimal increase in glucose flux from the liver to peripheral tissues. If liver glycogen is not being mobilized to fuel glycolysis in other tissues, then the source must be endogenous glycogen stores. However, the heart is the only tissue in which glycogen content declines significantly. It is obvious that this small store cannot account for all of the lactate produced.

The problem with glycogen budgets is that the individual variation is high, making it difficult to detect changes. If mean values are used, however, then the total pool of glycogen, glucose and G6P in the body of a 500-g fish falls from 7474 μmol to 6128 μmol (a decline of 2692 μmol of 3-carbon units). At the same time, lactate rises from 2137 μmol to 4020 μmol (an increase of 1883 μmol). This means that two-thirds of the decline in substrates can be accounted for as lactate, a reasonable estimate since lactate may be oxidized or metabolized elsewhere (Bilinski & Jonas, 1972).

The calculation can be advanced one step further to determine which tissue is the greatest contributor to the total lactate pool. The net change in total lactate is 1882 μmol/500-g fish. If all of the substrates in all of the tissues, except the white muscle, were converted to lactate, there would be barely enough to account for the rise in lactate concentrations (Figs 1, 2). It is therefore apparent that the white muscle substrate stores must be depleted to account for the resultant lactate increase.

Since glucose-6-phosphatase is not present in white muscle, and therefore cannot release glucosyl units into the blood, it follows that G6P formed from glycogen breakdown must be utilized *in situ*. This statement is supported by the observation that 85 % of the observed increase in whole body lactate stores occurs in the white muscle (Fig. 2).

These calculations emphasize the strong influence of white muscle upon whole body metabolism during hypoxia. This tissue provides the major source of fuel for glycolysis, and it utilizes that fuel *in situ*. This causes a significant and deleterious impact upon the metabolism of the rest of the body by producing a large lactate load.

This is not a general response to hypoxia in fish. The hypoxia tolerance of African lungfish may be due to the capacity of white muscle to prevent glycogen mobilization and a Pasteur effect during hypoxia (Dunn *et al.* 1983). In goldfish, glycogen stores in liver and red muscle are the major sources of carbohydrate, while there is no glycogen depletion in white muscle (van den Thillart *et al.* 1980). The final option is that glycogen is depleted in both the liver and the white muscle, a pattern which is observed in the European eel (van Waarde *et al.* 1983).

In summary, all of the tissues examined in the trout exhibit a response to acute hypoxia. Metabolite measurements indicate that liver is the most stressed. Although lactate concentrations increase in all of the tissues, it appears that the white muscle is the major source of this metabolite. Even though the mass-specific metabolic rate of the non-working white muscle is small compared to other tissues, it is proposed that the metabolism of white muscle profoundly affects the metabolic status of the whole animal.

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