J. exp. Biol. (1979), **82**, 303–320
Printed in Great Britain

METABOLIC SOURCES OF HEAT AND POWER IN TUNA MUSCLES

II. ENZYME AND METABOLITE PROFILES

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(*Received* 26 *October* 1978)

SUMMARY

Tuna appear able to maintain their muscles at 5–10 °C above ambient by balancing heat produced *in situ* and conserved by a counter-current heat exchanger with heat lost to the sea. Metabolite profiles under three different activity states (rest, burst swimming, and steady state swimming during feeding frenzies at sea) were used to identify which metabolic processes in white and red muscles could account for observed excess temperatures.

During burst swimming, transient changes in metabolite levels indicate that the metabolism of both red and white muscle contributes to powering burst swimming; red muscle work is sustained mainly by oxidative metabolism while white muscle work depends upon an intense anaerobic glycolysis. The rate of metabolism in red muscle is easily high enough to account for the measured (10 °C) increase in temperature at this time. However, in white muscle, anaerobic glycolysis can account for only about a 2 °C maximum rise in temperature.

The highest sustained swimming speeds and the highest muscle temperatures in skipjack are found during feeding frenzies at sea. As in burst swimming, during steady-state swimming red muscle temperatures can be accounted for by oxidative metabolism. In the case of white muscle, the lactate measurements indicate that anaerobic glycolysis could only lead to a 0·3 °C temperature rise. However, if the fraction of utilized glycogen that is not fermented (about 60 %) is assumed to be fully oxidized, enough heat is generated to raise white muscle temperatures by over 10 °C. The observed excess temperature at this time is about 8–10 °C, showing that areobic carbohydrate metabolism in white muscle is probably the major heat source during feeding frenzies.

These interpretations are fully consistent with enzyme profiles of red and white muscles in tuna. They do not, however, explain why tuna have warm muscles. The latter problem is briefly discussed.

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INTRODUCTION

Euthynnus pelamis, the skipjack tuna, have warm bodies and can swim extremely fast, up to an absolute velocity of 30 mph (Dizon, Brill & Yeun, 1978). Various physiological and anatomical properties are now known to contribute to their exceptionally capable swimming and to the maintenance of warm bodies (see Sharp & Pirages, 1978; Carey *et al.* 1971 for reviews). Although the actual sites of thermogenesis and energy production for swimming clearly are the locomotory muscles (Carey *et al.* 1971), the metabolic sources of heat and power have not been adequately explained.

In red muscle, the situation seems straightforward. Tuna red muscle is similar to that in other vertebrates in most properties examined (Hulbert *et al.* 1979) and displays the high oxidative (mainly fat-based) metabolic potential that would have to be developed to allow high, sustained swimming abilities (George & Stevens, 1978). That too implies equally high thermogenic capacities (Hulbert *et al.* 1979, see Discussion below).

The difficulty arises when considering white muscle. Tuna white muscle displays properties implying a high potential for anaerobic glycolysis (Guppy & Hochachka, 1978) along with significant oxidative capacity, indicated, for example, by relatively numerous mitochondria and ample capillarity (Hulbert *et al.* 1979). Although some intracellular fat is present in tuna white muscle, the predominant fuel is undoubtedly carbohydrate (Hulbert *et al.* 1979). Thus, in metabolic terms, the question of white muscle participation in different kinds of swimming and in thermogenesis seems to depend upon the relative roles of anaerobic and aerobic carbohydrate metabolism. For these reasons, we focused our attention in this study primarily upon carbohydrate, rather than fat, metabolism and primarily upon white muscle, data on red muscle being included mainly for contrast. We found that enzyme profiles of white and red muscle are consistent with our earlier ultrastructural studies. Metabolite data indicate that red muscle contribution to swimming at all speeds is totally aerobic, burning either fat or carbohydrate or both. During feeding frenzies (steady-state swimming) at sea, when white muscle is known to heat up by nearly 10 °C, white muscle contribution to swimming is supported mainly by aerobic glucose-based metabolism, whereas burst swimming is supported almost totally by the most intense anaerobic glycolysis thus far known in nature.

MATERIALS AND METHODS

Experimental animals

All experimental animals were obtained from local fishermen working out of Honolulu. Groups of 10–20 tuna (1–2 kg body weight) were transferred from bait wells on commercial fishing vessels to large holding tanks at the Kewalo Laboratories of the National Marine Fisheries in Honolulu, Hawaii. For most of the experiments, the animals were used during the second and third days of captivity; typically they were not used in the first day of captivity, nor after the third day.

Enzyme extraction

Tissues were excised from freshly killed fish and homogenized with a Polytron PCM-2-110, in 19 volumes of 50 mM imidazole buffer, pH 7·0 containing 20 mM-$MgSO_4$, 200 mM-KCl and 1 mM EDTA (see list of abbreviations, p. 306). Mercapto-ethanol (20 mM) was added for the preparation of hexokinase and phosphofructokinase. The well stirred homogenates were spun at 12 000 *g* for 20 min and the supernatants used for determining enzyme levels.

Enzyme assay

Enzyme activities were monitored in 1 ml cuvettes (1 cm light path) using a Unicam SP 1800 recording spectrophotometer. The reaction cuvettes were held in cell holders thermally equilibrated with a constant temperature bath and circulator. The rate was determined by the decrease in absorbence of NADH at 340 nm (in the case of citrate synthetase, the rate was determined by the increase in absorbence of DTNB at 412 nm). Standard conditions with saturating substrate concentrations were used for each enzyme as described elsewhere (Hochachka *et al.* 1978). All assays were done at 25 °C.

The comparison of enzyme activities between different species is frought with dangers of artifacts arising from differing extraction efficiencies, differing enzyme stabilities, the pelleting of some enzyme activity, and so forth (see Scrutton & Utter, 1968; Opie & Newsholme, 1967). For that reason, in interpreting the data in Table 1, more emphasis is placed on enzyme levels that appear unusually high (rather than vice versa) and on a direct comparison of red and white muscles, since extraction and assay conditions for both were identical.

Definition of activity states

Muscle samples were taken from fish that were performing at one of three different levels of activity, termed resting, burst swimming, or steady-state swimming. Samples from resting tuna were obtained from animals swimming laps in a circular pool at approximately 1–2 body lengths/s. For tuna, this is the only approximation to basal metabolism that exists, since, unlike many teleosts with swim-bladders, the skipjack tuna lose hydrodynamic equilibrium and fall out of the water column if their cruising speed decreases below about 1·2 lengths/s (Dizon, personal communication). In terms of metabolite concentrations therefore, we are dealing with a working equilibrium system not directly comparable to 'resting' mammalian muscles.

Burst swimming for periods of 7–10 min was obtained by attaching a line and hook to the lower mandible and releasing the tuna 'on line' into a circular tank, 5 m in diameter, 1 m deep. Under these conditions, bursts of up to 20 lengths/s can be achieved (Dizon, personal communication).

Samples from tuna in high velocity, steady-state swimming associated with feeding frenzies were obtained through the cooperation of local fishermen. These fish were hooked at sea at the stern of the boat and immediately hauled on board for sampling. Sampling time was about 15 s longer than for the other two experimental groups. During such feeding frenzies, skipjack tuna are thought to be in one of their most active states with muscles at their highest temperatures (Stevens & Fry, 1971; Gulbert *et al.* 1979).

Preparation of tissue for metabolite assays

Upon netting, the fish was instantly killed by spinalectomy and a thin steak (less than 1 cm thick) was quickly cut from the area of the leading edge of the dorsal fin. In the 1976 experiments small pieces of red and white muscle (less than 1 g) were excised from the steak and immediately frozen in liquid nitrogen; this procedure took less than 30 s. In the 1977 experiments, the steak was freeze clamped with Wollenburger tongs cooled in liquid nitrogen; this procedure also took about 20 s. The frozen tissues was powdered in a mortar and pestle, previously cooled by liquid nitrogen and then an aliquot of the powdered tissue was placed into a glass tube (cooled in a dry ice-ethanol bath). Immediately before and after the addition of the powder, the tube was weighed to the nearest milligram. Approximately 4 parts (by weight) of 8% (v/v) $HClO_4$ in 40% (v/v) ethanol was added to the cold powder, mixed quickly with a spatula and homogenized for 1 min, at dry ice-ethanol temperatures, with a Polytron. The resulting supernatant was centrifuged at 25 000 ***g*** for 10 min to produce supernatant 'a'; the precipitate remaining in the homogenizing tube was rehomogenized in two volumes of 6% (v/v) $HClO_4$. This homogenate was added to supernatant 'a' and spun at 25000 ***g*** for 10 min. The second supernatant was adjusted to pH 6·0 by the slow addition of 3 M-K_2CO_3 containing 0·5 M triethanolamine base, and then spun at 25000 ***g*** for 10 min to remove the precipitated $KClO_4$. The final supernatant was measured to the nearest 0·1 ml and either assayed immediately or stored at −20 °C.

Metabolite assay techniques

All metabolities were measured enzymically and were based on the absorbence of the pyridine nucleotides at 340 nm. Assays were carried out on a Unicam SP 1800 dual-beam recording spectrophotometer using conditions described elsewhere (Hochachka *et al.* 1978). Glycogen was isolated by the method of Osterberg (1929) and assayed using the Sigma Kit no. 510 (Sigma Chemical Co., St Louis).

ABBREVIATIONS USED

EDTA	ethylene diamine tetraacetic acid
NADH	nicotinamide adenine dinucleotide (reduced)
NAD^+	nicotinamide adenine dinucleotide (oxidized)
DTNB	5,5′-dithiobis-(2-nitrobenzoic acid)
G3P	glyceraldehyde-3-phosphate
G6P	glucose-6-phosphate
CrP	creatine phosphate
F6P	fructose-6-phosphate
FBP	fructose biphosphate
DHAP	dihydroxy acetone phosphate
αGP	α-glycerophosphate
u.	units of enzyme activity (μmol. substrate utilized/min)
αKG	α-ketoglutarate
AMP, ADP, ATP	adenosine mono-, di-, and triphosphate

RESULTS AND DISCUSSIONS

Muscle enzyme profiles

The activities of a variety of enzymes in skipjack red and white muscle are given in Table 1. In view of the noted differences in red and white muscle fine structure, the enzyme profiles observed are as expected. Thus, all the glycolytic enzymes (with the exception of hexokinase) are between 5–10-fold more active in white muscle than in red. In contrast, the activity of citrate synthase catalysing the entry step into the Krebs cycle and thought to be a control site (Tischler *et al.* 1977), is 7-fold higher in red muscle than in white. Similarly, the activity of glutamate dehydrogenase, catalysing the entry of glutamate into the Krebs cycle, also occurs in substantially higher activities in red than in white muscle (Table 1).

When compared to other fish red skeletal muscles, which on the whole have enzyme profiles that do not differ greatly from those in mammalian skeletal muscles, the skipjack red muscle appears typical with perhaps the exception of lactate dehydrogenase and glutamate-oxaloacetate transaminase. The activities of these two enzymes are somewhat higher in skipjack red muscle than in other red muscles (Alp, Newsholme & Zammit, 1976; Crabtree & Newsholme, 1972; Beatty & Bocek, 1970; Hochachka *et al.* 1978).

In contrast to red muscle, tuna white muscle is rather unusual. Most of the values obtained for glycolytic enzymes, assayed at 25 °C, occur in substantially higher levels than in rat skeletal muscle, assayed at 37 °C; included in this series are aldolase, triose phosphate isomerase, phosphoglycerate kinase, enolase, pyruvate kinase, and lactate dehydrogenase (see Scrutton & Utter, 1968 for extensive literature on mammalian values).

Pyruvate kinase activities are about 3-fold higher in skipjack white muscle than in other fish white muscles (Hochachka *et al.* 1978; Beatty & Bocek, 1970). The activity of lactate dehydrogenase, kinetically specialized for lactate production, is the highest so far found in any tissue in any organism (Guppy & Hochachka, 1978). From these data we anticipated that phosphofructokinase levels might also be higher than usual. However, the enzyme was too unstable in tuna muscles to be accurately measured.

Most estimates of fish and mammalian muscle glycogen phosphorylase are about 50 u./g, but the rat and rabbit have higher activities at around 100 u./g (Scrutton & Utter, 1968; Crabtree & Newsholme, 1972; Beatty & Bocek, 1970). Tuna red muscle displays lower activities than found in other vertebrates, while tuna white muscle activities are as high as in any mammalian muscle thus far studied and are higher than in other fish muscles (Table 1). Since glycogen phosphorylase regulates the initial mobilization of glycogen, the large activity differences between red and white muscle correlate with the abundant glycogen present in white muscle (Hulbert *et al.* 1979; see also Table 4).

Because of an obviously high capacity for anaerobic glycogen metabolism, the surprising feature of tuna white muscle is its aerobic potential. This is indicated in a number of enzyme levels. Glutamate-oxaloacetate transaminase, for example, occurs at about 1·5–2-fold higher levels than found in other fish white muscles (Hochachka *et al.* 1978). Citrate synthase follows the same trend, although the differences here are not as large. Srere (1969) has noted that citrate synthase is better solubilized after

Table 1. *Enzyme profiles in skipjack muscles*

(Units of enzyme activities (μmol product formed/min.g wet weight) are at 25 °C, and optimal substrate, cofactor, and H^+ levels; $n = 4$, with one standard deviation given in parentheses.)

Enzyme	Red muscle	White muscle
Phosphorylase	22·0 (0·64)	106·2 (11·14)
Hexokinase	1·2 (0·78)	0·78 (0·45)
Phosphoglucomutase	31·3 (6·04)	152·8 (46·48)
Phosphoglucose isomerase	84.4 (14·00)	426·0 (157·73)
Glucose-6-phosphate dehydrogenase	0	0
Phosphofructokinase	Unstable	Unstable
Aldolase	35·5 (10·05)	269·2 (38·62)
Triose phosphate isomerase	1414·6 (2 values)	9886·0 (1763·23)
Phosphoglycerate kinase	371·1 (34·8)	1982·7 (433·01)
Enolase	77·7 (9·23)	522·4 (156·68)
Pyruvate kinase	195·2 (37·0)	1294·9 (249·5)
Lactate dehydrogenase	514·4 (74·25)	5492·3 (154·73)
α-Glycerophosphate dehydrogenase	21·7 (2·77)	104·5 (9·24)
Citrate synthase	20·6 (0·00)	2·1 (0·87)
Glutamate dehydrogenase	5·9 (0·58)	3·0 (1·15)
Malate dehydrogenase	723·4 (30·95)	718·0 (160·85)
Glutamate-oxaloacetate transaminase	101·9 (6·18)	43·0 (3·41)
Glutamate-pyruvate transaminase	7·7 (2·25)	2·0 (2·31)
Creatine phosphokinase	554·2 (268·7)	516·4 (101·21)
Myokinase	381·8 (23·21)	946·7 (102·02)

freezing and thawing muscle tissue; in tuna white muscle prepared this way, citrate synthase occurs at 7–8 u./g (Hochachka, Hulbert & Guppey, 1978), a value approaching that in some mammalian muscles (Holloszy *et al.* 1970). Interestingly, malate dehydrogenase occurs in similar activities in red and white muscles. Because mitochondria are far more numerous in red muscle, these values may imply a higher ratio of cytoplasmic/mitochondrial malate dehydrogenase activity in white muscle, as occurs in hypoxia-adapted Amazon fishes (Hochachka *et al.* 1978).

The activity of α-glycerophosphate dehydrogenase (over 100 u./g) in tuna white muscle is also unusually high. Although activities of this enzyme in white muscle usually are higher than in red (Crabtree & Newsholme, 1972; Blanchaer, 1964), a typical activity in vertebrate white muscle is 5–25 u./g; i.e. one twenty-fifth to one quarter the value in tuna white muscle (Hochachka *et al.* 1978; Beatty & Bocek, 1970; Crabtree & Newsholme, 1972). This enzyme, when in high activities, usually functions as the cytoplasmic arm of the α-glycerophosphate cycle, which balances redox during aerobic carbohydrate metabolism (Sacktor,1976; Hochachka & Guppy, 1976; Storey & Hochachka, 1975).

In this context, the values for tuna muscle hexokinase are also instructive. Whereas in the tuna hexokinase is equally active in both red and white muscle (Table 1), in mammals and other fishes, red muscle usually has more hexokinase activity than white (Peter, Jeffrees & Lamb, 1968; Crabtree & Newsholme, 1972; Pette, 1966; Burleigh & Schimke, 1968). This result is consistent with a relatively high aerobic glucose metabolic potential in tuna white muscle.

Finally, a brief comment should be made on the levels of creatine phosphokinase and myokinase in tuna muscles. Both enzymes occur in very substantial activities in both red and white muscles, implying that neither creatine phosphate mobilizatio

Table 2. *Metabolite concentrations (μmol/g wet weight) in red and white muscle during three different activity states*

(Experiments done in 1976. Each value is an average of at least 4 (usually 5) experiments; values in parentheses are 1 standard deviation.)

	Red muscle			White muscle		
	Rest	Burst	Steady state	Rest	Burst	Steady state
Glucose	1·83 (0·39)	1·70 (0·62)	0·18 (0·09)	0·34 (0·14)	2·35 (0·51)	0·16 (0·05)
G6P	1·75 (0·13)	2·16 (0·68)	1·97 (0·30)	1·57 (0·26)	3·87 (1·06)	2·70 (1·02)
F6P	0·25 (0·14)	0·46 (0·07)	0·58 (0·12)	0·33 (0·16)	0·78 (0·14)	0·80 (0·19)
FBP	0·35 (0·04)	0·41 (0·03)	0·28 (0·07)	0·35 (0·10)	0·69 (0·11)	0·35 (0·14)
DHAP	0·38 (0·06)	0·33 (0·03)	0·23 (0·12)	0·32 (0·05)	0·30 (0·08)	0·12 (0·05)
G3P	0·22 (0·04)	0·18 (0·05)	0·11 (0·03)	0·15 (0·05)	0·14 (0·04)	0·13 (0·07)
Pyruvate	0·14 (0·03)	0·19 (0·04)	0·18 (0·10)	0·35 (0·08)	1·10 (0·22)	0·35 (0·19)
Lactate	12·30 (2·10)	18·20 (4·30)	10·47 (1·82)	13·05 (2·73)	84·00 (10·40)	20·18 (5·43)
αGP	1·83 (0·39)	3·16 (0·92)	1·73 (0·25)	2·00 (0·54)	0·37 (0·34)	1·65 (0·24)
Citrate	0·49 (0·06)	0·57 (0·09)	0·59 (0·11)	0·25 (0·09)	0·20 (0·04)	0·25 (0·03)
αKG	0·14 (0·02)	0·12 (0·04)	0·14 (0·06)	0·12 (0·04)	0·05 (0·03)	0·06 (0·01)
Malate	0·42 (0·06)	0·55 (0·08)	0·96 (0·36)	0·19 (0·22)	0·31 (0·06)	0·25 (0·045)
CrP	3·40 (0·56)	1·67 (0·15)	0·73 (0·12)	14·25 (2·70)	1·35 (0·48)	1·65 (0·059)
ATP	4·20 (0·39)	3·33 (0·32)	3·55 (0·44)	5·50 (2·14)	2·90 (1·51)	5·45 (0·73)
ADP	1·17 (0·29)	1·11 (0·1)	0·49 (0·07)	0·72 (0·10)	0·63 (0·12)	0·46 (0·03)
AMP	0·16 (0·005)	0·17 (3 values)	0·16 (1 value)	0·09 (0·03)	0·11 (0·05)	0·16 (0·05)

nor adenylate equilibration are likely to be limiting functions. The activity of myokinase in red muscle is in the mammalian range, but in white muscle it is substantially higher (Criss, 1971). Creatine phosphokinase activities are not unusually high (Newsholme *et al.* 1978). It is not clear why the ratios of the two activities are different between red and white muscle. However, it should be remembered that creatine phosphokinase occurs in cytoplasmic and mitochondrial forms and that some of the cytoplasmic form may be specifically bound to the M-line of the myofibrils, a situation that may be expected to lead to differences between red and white muscle (Eppenberger *et al.* 1975).

Muscle metabolite profiles during rest and work

Levels of various glycolytic and Krebs cycle intermediates, the adenylates, creatine, creatine phosphate, and glycogen in red and white muscles are given in Tables 2, 3, 4 and 7. The data in Table 2 are from 1976 when tissue samples were simply dropped into liquid nitrogen. The data in Table 3 are from 1977, when freeze-clamping with Wollenberger tongs was employed to quick-freeze the tissues in case the relatively longer freezing time in 1976 had led to artifacts. A comparison between Tables 2 and 3 demonstrates only small differences (discussed below).

Glycogen and fat

Glycogen levels in the skipjack white muscle at rest are high compared to other teleost white muscle values (1000 versus 400 mg %) and are somewhat higher than red muscle values (Table 4) which is contrary to the situation seen in other teleosts (Walker & Johansen, 1977; Johnston, Davison & Goldspink, 1977; Pritchard, Hunter & Lasker, 1971; Johnston & Goldspink, 1973). The skipjack values are in fact similar to mammalian values which tend to be around 1000 mg % with smaller differences

Table 3. *Metabolite concentrations (μmol/g wet weight) in red and white muscle during th different activity states*

(Experiments done in 1977. Each value is an average of at least 4 (usually 5) experiments; values in parentheses are 1 standard deviation.)

	Red muscle			White muscle		
	Rest	Burst	Steady state	Rest	Burst	Steady state
Glucose	0·36 (0·09)	2·03 (0·43)	0·52 (0·20)	0·24 (0·05)	2·68 (0·45)	0·48 (0·08)
G6P	0·82 (0·13)	2·50 (0·41)	1·48 (0·16)	0·71 (0·26)	5·00 (2·21)	3·34 (0·54)
F6P	0·14 (0·05)	0·38 (0·09)	0·27 (0·09)	0·12 (0·04)	0·97 (0·50)	0·57 (0·06)
FBP	0·19 (0·05)	0·20*	0·11 (0·05)	0·18 (0·08)	0·13 (0·05)	0·28 (0·06)
Pyruvate	0·22 (0·20)	0·23 (0·05)	0·14 (0·007)	0·20 (0·07)	1·47 (0·46)	0·69 (0·22)
Lactate	3·10 (1·17)	7·20 (1·86)	5·82 (2·26)	5·14 (1·17)	68·50 (21·13)	18·70 (3·10)
Citrate	0·96 (0·46)	1·51 (1·20)	0·62 (0·25)	0·30 (0·17)	1·15 (0·49)	0·10†
Malate	0·45 (0·18)	0·97 (0·59)	0·59 (0·13)	0·12 (0·05)	0·47 (0·31)	0·18 (0·06)
CrP	4·76 (0·31)	2·86 (1·06)	1·31 (0·47)	30·70 (13·20)	3·45 (2·06)	6·30 (1·34)

* Average of two values. † All other values were 0·0.

between red and white fibres (Baldwin *et al.* 1973; Ahlborg *et al.* 1967). Barrett & Conner (1964) found similar glycogen levels in white muscle of freshly caught skipjack. Glycogen levels in the white muscle are lower during sustained swimming and drop most markedly after burst swimming. Myofibrillar glycogen granules and glycogen in glycogen bodies are both depleted under the latter conditions (Hulbert *et al.* 1979). Figures for total glycogen and lactate (Tables 4, 5) indicate that during burst swimming all of the glycogen depleted appears as lactate, whereas during steady-state swimming at sea, only about 40 % appears as lactate; most of the rest is assumed to be fully oxidized.

Glycogen levels in the red muscle do not change during feeding and the burst swimming levels are variable (Table 4). However, values for the total glycogen and lactate pool (Table 5) are similar for all three states and point out that glycogen carbon is either in glycogen or lactate and thus that there is no aerobic metabolism of glycogen in red muscle under these conditions.

Scanning of many EM grids indicates that triglyceride droplets in red muscle are depleted during burst swimming (Hulbert *et al.* 1979). This surprising lability of intracellular fat is taken to indicate that it would also be utilized during feeding frenzies; unfortunately, no tissues from fish in this state could be obtained for electron microscopic studies.

Glucose

In both muscles glucose levels are highest during burst swimming. In white muscle at rest or during feeding, glucose levels are low. That too is observed in red muscle during feeding, but red muscle glucose levels are high in resting tuna in the 1976 experiments. Not enough information is available on blood glucose levels to be certain of transient profiles; however, preliminary data suggest that red and white muscle glucose pools equilibrate with the blood during burst swimming, but not during rest or steady-state swimming (Hochachka, Hulbert & Guppy, 1978).

Table 4. *Individual glycogen and lactate levels (μmol/g wet weight) in red and white muscles during the three activity states (1977 experiments)*

(Each value is an average of at least 4 (usually 5) experiments; values in parentheses are 1 standard deviation. Means of glycogen values are expressed as μmol glucose/g (above) and as mg% (below).)

	Red muscle		White muscle	
Fish no.	Lactate	Glycogen	Lactate	Glycogen
Rest				
1	4·7	36·3	4·0	41·8
2	2·1	30·5	3·8	44·5
3	2·0	34·1	5·9	41·5
4	3·9	—	5·6	58·0
5	2·8	40·4	6·4	60·5
Mean	3·1 (1·17)	35·3 (4·1)	5·1 (1·17)	49·2 (9·2)
		635·0		885·6
Feeding				
1	9·3	42·4	19·2	41·2
2	6·5	40·1	15·4	28·2
3	5·6	46·0	23·7	29·2
4	3·6	25·9	17·4	26·4
5	4·1	44·2	17·9	38·2
Mean	5·8 (2·26)	39·7 (8·0)	18·7 (3·10)	32·6 (6·6)
		714·6		586·8
Burst				
1	9·6	6·9	84·5	21·6
2	8·1	18·3	80·6	19·1
3	6·0	43·6	32·0	59·1
4	7·5	50·1	75·6	9·6
5	4·8	49·0	69·6	22·6
Mean	7·2 (1·86)	33·6 (19·7)	68·5 (21·13)	26·4 (19·0)
		604·8		475·2

Table 5. *Total glycogen and lactate, in terms of glucose equivalents (μmol/g wet weight) from glycogen and lactate in skipjack red and white muscle under the three activity states*

(If all glycogen were to appear as lactate, the values during the two swimming levels would be the same as in the resting animals. Each value is an average of at least 4 (usually 5) experiments. Values in parentheses are 1 standard deviation.)

	Rest	Feeding	Burst
Red muscle	36·3 (4·2)	41·9 (12·6)	37·6 (17·6)
White muscle	51·9 (9·5)	41·7 (7·0)	60·0 (11·7)

Hexose phosphates

Glucose-6-phosphate occurs at higher concentrations than either fructose-6-phosphate or fructose biphosphate (Tables 2, 3). The significance of concentration changes of these intermediates is discussed below.

Pyruvate

Pyruvate concentrations in red muscle remain remarkably constant through different activity states (Tables 2, 3). The notable difference in white muscle is a large increase in pyruvate levels (to over 1 μmol/g) during burst swimming.

Lactate

Lactate levels in resting tuna are about 3-fold lower in both muscles in the 1977 experiments. Apart from this, the trend is the same. There is a slight increase in lactate levels in the red muscle during burst swimming, and in the white muscle, lactate levels increase by 3–6-fold during feeding and by a maximum of 20-fold during burst swimming. The 70–90 μmol/g lactate concentrations produced in the white muscle after 10 min of burst work (Tables 2, 3) are to our knowledge the fastest rate of lactate production known, reaching the highest levels recorded for muscular work. The jack mackerel can develop 70 μmol/g lactate in 8 min, but control values were 40 μmol/g (Pritchard *et al.* 1971). In carp and coalfish exercised to fatigue, the lactate concentration increases to 12 and 20 μmol/g respectively (Driedzic, unpublished; Johnston & Goldspink, 1973) and it has been repeatedly shown that the level of lactate in salmonids can approach 30–50 μmol/g (Black *et al.* 1962; Stevens & Black, 1966; Hammond & Hickman, 1966; Bilinsky, 1974). A variety of mammals, exercised to exhaustion, were found to accumulate 10–30 mM lactate in 10 min (Seeherman, Taylor & Maloiy, 1976). Blood lactate in the skipjack tuna is not in equilibrium with the tissues under feeding or burst conditions (Hochachka, Hulbert & Guppy, 1978), which at least in the latter situation is characteristic of teleosts (Black *et al.* 1962).

Alpha-glycerophosphate

Alpha-glycerophosphate levels approximately double during burst swimming in both tissues (Table 2) which is in accordance with other studies on fish, insects, and rats (Driedzic & Hochachka, 1976; Edington, Ward & Saville, 1973; Ford & Candy, 1972).

Citrate, malate, and α-ketoglutarate

The concentrations of citrate, malate, and α-ketoglutarate in red and white muscle do not change dramatically in different activity states, which may imply that there is no major augmentation of the Krebs cycle pool of intermediates during different swimming activities. In any event, the lack of any major change in citrate concentration is important since it rules out potential regulatory roles of this metabolite at the level of phosphofructokinase. In other systems, citrate inhibits glycolysis at this locus (see Scrutton & Utter, 1968, for example).

Adenylates and creatine-phosphate

There is some fluctuation in the levels of the adenylates in both tissues under the different excerise states, the most dramatic one being the fall of ATP levels in white muscle during burst swimming. The energy charge and pool size are higher in the white muscle. The energy charge is highest during feeding and lowest during burst swimming in both tissues. The adenylate pool size is highest during rest in both tissues and lowest during feeding and burst work in the red and white muscles respectively (Tables 2 and 6). Resting creatine-phosphate levels in both experiments are at least 4-fold higher in the white muscle and drop (most markedly in the white muscle) in both tissues with exercise (Tables 2 and 3).

Table 6. *The energy charge ($ATP + \frac{1}{2}ADP/ATP + ADP + AMP$) and adenylate pool size (the sum of the concentrations of ATP, ADP, and AMP) during the three activity states. Calculated from mean values given in Table 2*

	Red muscle			White muscle		
	Rest	Feeding	Burst	Rest	Feeding	Brust
Energy charge	0·86	0·90	0·84	0·93	0·94	0·88
Adenylate pool	5·57	4·20	4·57	6·42	6·12	3·64

Mass action ratios

The metabolic implications of metabolite data sometimes can be best displayed by comparing observed mass action ratios with those predicted from equilibrium constants. As expected from other systems (Scrutton & Utter, 1968), hexokinase and phosphofructokinase are far from equilibrium and thus appear as regulatory sites in tuna red and white muscle. Phosphoglycoisomerase is included for comparison and is very close to equilibrium (Table 7). Although glucose assays can be in error (Newsholme & Start, 1973), this does not change the conclusion that the hexokinase reaction is out of equilibrium. The mass action ratio is at least 4 orders of magnitude removed from equilibrium implying that glucose levels would have to be in error by at least 4 orders of magnitude in order that the hexokinase reaction be in equilibrium.

The degree to which the mass action ratio of regulatory enzymes differs from the expected equilibrium will vary, depending upon the activation/inhibition state of the enzyme. Thus in the rat heart, phosphofructokinase is farther from equilibrium under aerobic conditions when glycolysis is inhibited than under anaerobic conditions when glycolysis is activated (Williamson, 1965). In the skipjack tuna muscles, there is no change in the mass action ratio of the phosphoglucose isomerase reaction as the activity state varies, nor in the mass action ratio of the hexokinase and phosphofructokinase reactions between rest and burst swimming. However, upon transition from rest to feeding, the hexokinase reaction mass action ratio moves closer to equilibrium, indicating an activation of hexokinase, consistent with an activation of aerobic glycolysis. At the same time the mass action ratio for the phosphofructokinase step moves further from equilibrium indicating limiting phosphofructokinase function.

Lactate/pyruvate ratios are not consistent between the 1976 and 1977 experiments. The freeze-clamp data (1977) however indicate a definite increase in the white muscle lactate/pyruvate ratio upon burst swimming and this is also the case for the red muscle in the 1976 data. An increase in this ratio assuming that lactate dehydrogenase is a reaction which is close to equilibrium (Merrill & Guynn, 1976; Tischler *et al.* 1977) signifies a pH drop or that the $NAD^+/NADH$ couple is becoming more reduced. Both occurrences have been reported in working and/or anaerobic tissue (MacDonald & Jobsis 1976; Steenbergen, Deleeuw & Williamson, 1977).

Sources of power for burst swimming

From the above data, it should be possible to summarize at least qualitatively the nature of carbohydrate metabolism in tuna muscles during different activity states.

Table 7. *Mass action ratios for the hexokinase, phosphoglucoseisomerase and phosphofructokinase reactions during the three activity states. Expected equilibrium values taken from Newsholme & Start* (1973)

	Expected equilibrium constant	Rest	Feeding	Burst
Hexokinase				
76 Red	$3.9–5.5 \times 10^3$	0.29	1.5	0.43
76 White		0.59	1.4	0.36
Phosphoglucose isomerase				
76 Red	0.36–0.47	0.14	0.29	0.21
77 Red		0.17	0.18	0.15
76 White		0.21	0.3	0.2
77 White		0.17	0.17	0.19
Phosphofructokinase				
76 Red	$0.9–1.2 \times 10^3$	0.4	0.06	0.31
76 White		0.13	0.04	0.19

During burst swimming, the following metabolite concentration changes seem particularly relevant.

(1) Creatine-phosphate levels in white muscle decrease by a factor of 10, in red muscle by about a half.

(2) Particularly in white muscle, the adenylate pool size and ATP levels decrease, leading to a slight drop in the energy charge.

(3) Glycogen drops markedly in white muscle.

(4) Lactate accumulates in white muscle to levels 7–20 times higher than in the resting state and pH thus probably drops; in red muscle, lactate levels increase, but modestly (1.5-fold).

(5) Intracellular fat droplets are depleted in red muscle (Hulbert *et al.* 1979).

These data, in the absence of any other, unequivocally identify anaerobic glycolysis as the predominant contribution to white muscle metabolism during burst swimming. While glycogen is the predominant fuel for the process, glucose appears to be 'spared' through hexose phosphate inhibition of hexokinase (see Katzen & Soderman, 1975, for literature on hexokinase regulation). Thus, glucose levels actually rise in white muscle during burst swimming. Although other sites may also be involved in regulation (Scrutton & Utter, 1968; Newsholme & Start, 1973), an important element of control resides at the level of phosphofructokinase, which is held from equilibrium (Table 7), despite deinhibition due to creatine phosphate depletion (Storey & Hochachka, 1974) and the obviously large increase in carbon flow that must occur through this site during burst swimming.

In contrast, the data for red muscle show metabolism to be largely aerobic during burst swimming. That red muscle metabolism is activated under these conditions is indicated (1) by the fall in triglyceride (Hulbert *et al.* 1979), ATP, and creatine-phosphate levels, (2) by the increase in concentration of the hexose phosphates, and (3) by the large rise in temperature (Hulbert *et al.* 1979). Also, direct electrophysiological studies (Rayner & Keenan, 1967; R. Brill & W. C. Hulbert, unpublished data) indicate that red muscle is active during burst swimming.

In summary then, the metabolism of both red and white muscle contributes to burst swimming; red muscle work is sustained mainly by aerobic metabolism whereas white muscle work depends upon an intense anaerobic glycolysis.

Sources of power for steady-state swimming

Does a similar division of metabolic function between red and white muscle occur during the steady-state swimming associated with prey capure at sea ? During such feeding frenzies, when the highest sustained activity levels of skipjack tuna are thought to be reached, the known metabolic changes in red muscle can be summarized as follows:

(1) Creatine-phosphate levels drop dramatically, but ATP concentrations are sustained well within the normal range.

(2) Glucose concentrations are low while hexose phosphate levels rise, indicating a hexokinase activation; at the same time, lactate levels remain in the normal range (Table 2).

(3) Temperature rises by up to 10 °C (Hulbert *et al.* 1979).

These data are taken to mean (*a*) that red muscle is contributing to frenzy swimming, and (*b*) that the red muscle contribution is aerobic, powered in part by glucose oxidation and probably in part by fat oxidation (as in burst swimming).

By comparison, in white muscle:

(1) Creatine-phosphate levels drop but ATP concentrations are maintained in the normal range.

(2) Glucose levels remain low, but G6P and F6P concentrations are high, consistent with a flow of glucose carbon into the glycolytic path through hexokinase activation. Glycogen levels also drop. Some glucose and glycogen carbon appears in lactate, which accumulates to levels one-fifth to one quarter those observed in burst swimming; the rest presumably is fully oxidized.

(3) Temperature rises by 8–10 °C (Hulbert *et al.* 1979).

These data are taken to mean that white muscle is contributing to frenzy swimming and that this contribution, as in the case of red muscle, is largely aerobic, with only a minor anaerobic component. As in the case of burst swimming, an important element of control resides at the level of phosphofructokinase, which moves even further from equilibrium during feeding (Table 7).

Sources of heat for red and white muscle

Since heat is a byproduct of metabolism, knowing how much is produced should allow us to confirm and refine our interpretations of the above metabolic data. Three potential sources of heat are clearly available to tuna red and white muscles:

(1) anaerobic glycolysis, yielding 47 kcal/mol of glucose fermented;
(2) glucose oxidation, yielding about 686 kcal/mol of glucose fully oxidized; and
(3) fat oxidation, yielding 9·3 kcal/g (or 2340 kcal/mol palmitate) fully oxidized.

During burst swimming, the red muscle temperature increases by 10 °C in 10 min (Hulbert *et al.* 1979). Basal oxygen uptake measurements of 2 mg oxygen/g.h (Neill,

Chang & Dizon, 1976) would raise red muscle temperature by 1.4 °C/kg. 10 min if carbohydrate only were oxidized. About a 7-fold increase in heat production would therefore be necessary to raise the red muscle temperature 10 °C in 10 min. Considering that the above figure for oxygen uptake is for basal metabolism, that it is for the whole body, not just red muscle, and that lipid oxidation is not considered, it is well within the capabilities of the red muscle to produce enough metabolic heat in 10 min to raise its temperature by 10 °C.

During 10 min of burst swimming, deep white muscle temperatures can also rise, by 5 °C on average under our conditions (Hulbert *et al.* 1979). At such time lactate accumulates to levels of almost 100 μmol/g. If we make the limit assumption of a quantitative conversion with no heat loss to the outside, this process could raise the temperature of a kilogram of white muscle by about 2·3 °C. That is, despite the astonishing anaerobic glycolysis that can be activated at this time, it is energetically too inefficient to account for the observed thermogenesis in the white muscle. The additional heat in the white muscle must either come from conduction from the red muscle or from the aerobic metabolism of blood-borne glucose.

During steady-state swimming in feeding frenzies at sea, the situation is similar. Again, the red muscle is the hottest tissue and the same metabolic processes as before can account for the heat produced. By comparison, white muscle heats up somewhat more than during burst swimming; average excess temperatures for 'deep' white muscle observed under these conditions are about 8–10 °C (Hulbert *et al.* 1979). If we assume that 60% of the glycogen depleted (equivalent to about 22 μmol glucose/g) during this kind of swimming is fully oxidized, enough heat would be generated to raise the temperature of 1 kg of white muscle by over 11 °C. From lactate measurements under these conditions, anaerobic glycolysis in contrast could only generate enough heat to raise muscle temperature about 0·3 °C. It is evident therefore that the metabolic versatility of the tuna myotomal muscle is easily adequate to account for the amount of heat formed under feeding conditions.

Why tuna get hot

There have been many hypotheses advanced on the function of the warm muscle in tuna; most have been biased toward the 'high' temperature aspect of these muscles, and only one (Neill *et al.* 1976) makes use of the fact that temperature may be less variable in tuna muscles. A combination of these characteristics, a constant and higher-than-ambient temperature, describes the basis of endothermy in many organisms. If we can explain why these two features appear in other organisms, perhaps we can explain why tuna also have developed them.

In metabolic terms, the advantages of a constant body temperature are readily explained. Most biochemical processes and structures underlying metabolism are dependent for their integrity upon the sequential formation (or breakage) of weak, non-covalent bonding. The list of such functions is far beyond the purpose of this paper; the interested reader is referred to the literature (see, for example, Low & Somero, 1976; Hochachka & Somero, 1973; Fersht, 1977; Holbrook *et al.* 1975). The important point to emphasize, however, is that such weak bonds are differentially affected by temperature, which is why it is more difficult for organisms to cope with

thermal change than with a given absolute (high or low) temperature. Thus, as pointed out by Heinrich (1977), a good strategy for highly active organisms is the tailoring of enzymes for specific temperatures and the regulation of body temperature. But why choose temperatures higher than ambient?

One explanation for the high 'set point' in terrestrial endotherms (see Heinrich, 1977 and Crompton, Taylor & Jagger, 1978) centres on the need for controlled heat loss. If the body temperature is above ambient, heat loss can readily occur by mechanisms (such as conduction and radiation) not involving significant water loss. But if the body temperature is not above ambient, evaporative heat loss is the only effective way of holding down the body temperature. Particularly for small terrestrial organisms, this process necessitates detrimentally large losses of water. Thus the set point in endotherms appears to be a compromise between the *disadvantage* of the metabolic costs of heating up to well above ambient and the *advantage* of having a high body temperature to facilitate heat loss to the environment. Set points are thus related to the upper range of environmental temperatures, which is why some primitive mammals, such as the hedgehog, are active at night (at lower environmental temperatures) and have lower set points (Crompton *et al.* 1978).

We propose by analogy that the tuna is an endothermic teleost somewhat akin to the hedgehog in terms of thermal regulation. Since the tuna is continuously active, muscle metabolism serves as an excellent heat source; the sea serves as an excellent heat sink. Thus, the only barrier to endothermy is a way to dampen water temperature fluctuations which would be transmitted through the gills to the musculature in 'normal' teleosts. This is the role of the rete, a drastic, but effective way of avoiding complete thermal equilibration at the gills. As in the hedgehog, a primitive mammalian homeotherm with about a 30 °C body temperature (Crompton *et al.* 1978), the tuna balances heat production and heat loss to maintain a body temperature 5–10 °C above ambient. If body temperature were set at ambient, temperaturere gulation by controlled heat loss would be more difficult. If body temperature were set much higher, the metabolic costs would become prohibitive; even with the current arrangement, the metabolic rate of the tuna is many times higher than that of other teleosts, being similar to that of mammals of comparable body size (Dizon *et al.* 1978).

Because the tuna is probably more active at sea than in captivity, we assume that the resting temperatures observed in captivity are never found normally. That is, at sea the muscle temperature of tuna is probably as stable as in primitive mammals (± 3 °C or so). Indeed, F. G. Carey and E. D. Stevens (unpublished data) have recorded body temperatures of tunas at sea and found them to be remarkably constant. Thus, the key combination of a constant and higher-than-ambient body temperature typical of other endotherms seems also to be well expressed by the tuna.

This work was supported by an operating grant from the National Research Council of Canada (to P.W.H.). Especial thanks are due to Dr Andrew Dizon, Richard Brill, and other staff members of the National Marine Fisheries in Honolulu. Especial thanks are due to the director, R. Shomura, for broadly interpreting our experimental needs, particularly during Tuna-Tora-Tuna. A John Simon Guggenheim Fellowship was held (by P.W.H.) during a part of this study.

REFERENCES

AHLBORG, B., BERGSTROM, J., EKULUND, L. G. & HULTMAN, E. (1967). Muscle glycogen and muscle electrolytes during prolonged physical exercise. *Acta physiol. scand.* **70**, 129–142.

ALP, P. R., NEWSHOLME, E. A. & ZAMMIT, V. A. (1976). Activities of citrate synthetase and NAD-linked and NADP-linked isocitrate dehydrogenase in muscles from vertebrates and invertebrates. *Biochem. J.* **154**, 689–701.

BALDWIN, K. M., REITMAN, J. S., TERJUNG, R. L., WINDER, W. W. & HOLLOSZY, J. O. (1973). Substrate depletion in different types of muscle and in liver during prolonged running. *Am. J. Physiol.* **225**, 1045–1050.

BARRETT, I. & CONNER, A. R. (1964). Muscle glycogen and blood lactate in yellowfin (*T. albacares*)and skipjack (*Katsuwonus pelamis*) following capture and tagging. *Bull. inter-Am. trop. Tuna Commun Bull.* **9** (4), 219–252.

BEATTY, C. H. & BOCEK, R. M. (1970). Biochemistry of the red and white muscle. In *The Physiology and Biochemistry of Muscle as a Food*, 2 (ed. E. J. Briskey, R. G. Cassens and B. B. Marsh). The University of Wisconsin Press.

BILINSKI, E. (1974). Biochemical aspects of fish swimming. In *Biochemical and Biophysical Perspectives in Marine Biology*, vol. 1 (ed. D. C. Malins and J. R. Sargent), Academic Press pp. 239–288.

BLACK, E. C., ROBERTSON, C. A., LAM, K. C. & CHU, W. G. (1962). Changes in glycogen, pyruvate and lactate in rainbow trout (*Salmo gairdneri*) during and following muscular activity. *J. Fish. Res. Bd Can.* **19**, 409–436.

BLANCHAER, M. C. (1964). Respiration of mitochondria of red and white skeletal muscle. *Am. J. Physiol.* **206**, 1015–1020.

BURLEIGH, A. J. K. & SCHIMKE, R. T. (1968). On the activities of some enzymes concerned with glycolysis and glycogenolysis in extracts of rabbit skeletal muscle. *Biochem. biophys. Res. Commun.* **31**, 831–836.

CAREY, F. G., TEAL, J. M., KANWISHER, J. W. & LAWSON, K. D. (1971). Warm-bodied fish. *Am. Zool.* **11**, 137–145.

CRABTREE, B. & NEWSHOLME, E. A. (1972). The activities of phosphorylase, hexokinase, phosphofrucktokinase, LDH, and α-GPDH in muscles from vertebrates and invertebrates. *Biochem. J.* **126**, 49–58.

CRISS, W. E. (1971). Relationship of ATP : AMP phosphotransferase isozymes to tissue respiration. *Archs Biochem. Biophys.* **144**, 138–142.

CROMPTON, A. W., TAYLOR, C. R. & JAGGER, J. A. (1978). Evolution of homeothermy in mammals. *Nature, Lond.* **272**, 333–336.

DIZON, A., BRILL, R. & YUEN, H. (1978). Respiratory physiology of skipjack tuna. In *Physiological Ecology of Tuna* (ed. G. Sharp and A. Dizon) Academic Press.

DRIEDZIC, W. C. & HOCHACHKA, P. W. (1976). Control of energy metabolism in fish white muscle. *Am. J. Physiol.* **230**, 579–582.

EDINGTON, D. W., WARD, G. R. & SAVILLE, W. A. (1973). Energy metabolism of working muscle, concentration profile of selected metabolites. *Am. J. Physiol.* **224**, 1375–1380.

EPPENBERGER, H. M., WALLIMANN, T., KUHN, H. J. & TURNER, D. C. (1975). Localization of creatine kinase isozymes in muscle cells: physiological significance. In *Isozymes II. Physiological Function.* (ed. C. L. Markert) Academic Press.

FERSHT, A. (1977). *Enzyme Structure and Function.* San Francisco: W. H. Freeman & Company.

FORD, W. C. I. & CANDY, D. J. (1972). The regulation of glycolysis in perfused locust flight muscle. *Biochem. J.* **130**, 1101–1112.

GEORGE, J. C. & STEVENS, E. D. (1978). Fine structure and metabolic adaptation of red and white muscles in tuna. *Env. Biol. Fish.* **3**, 185–191.

GUPPY, M. & HOCHACHKA, P. W. (1978). Controlling the highest lactate dehydrogenase activity known in nature. *Am. J. Physiol.* **234** (3), R136–R140.

HAMMOND, B. R. & HICKMAN, C. P. (1966). The effect of physical conditioning in the metabolism of lactate, phosphate, and glucose in rainbow trout, *Salmo gairdneri. J. Fish. Res. Bd Can.* **23**, 65–83.

HEINRICH, B. (1977). Why have some animals evolved to regulate high body temperature? *Am. Nat.* **111** (980), 623–640.

HOCHACHKA, P. W. & SOMERO, G. N. (1973). *Strategies of Biochemical Adaptation*, pp. 179–270. Philadelphia: W. B. Saunders Company.

HOCHACHKA, P. W. & GUPPY, M. (1977). *Variation on a Theme by Embden Meyerhof and Parnas. Oxygen and Physiological Function* (ed. F. J. Jobsis), pp. 292–314. Professional Information Library, Dallas, Texas, U.S.A.

HOCHACHKA, P. W., GUPPY, M., GUDERLEY, H. E., STOREY, K. B. & HULBERT, W. C. (1978). Metabolic biochemistry of water vs air breathing fishes: Muscle enzymes and ultrastructure. *Can. J. Zool.* **56** (2), 736–750

HOCHACHKA, P. W., HULBERT, W. C. & GUPPY, M. (1978). The tuna power plant and furnace. In *The Physiological Ecology of Tuna* (ed.) G. S. Sharp and A. Dizon), pp. 153–174. Academic Press.
HOLBROOK, J., LILJAS, A., STEINDEL, S. & ROSSMAN, M. (1975). Lactate dehydrogenase. In *The Enzymes*. (ed. P. D. Boyer), XI, A, 191–292.
HOLLOSZY, J. O., OSCAI, L. B., DON, I. J. & MOLE, P. A. (1970). Mitochondrial citric acid cycle and related enzymes: adaptive response to exercise. *Biochem. biophys. Res. Commun.* **40**, 1368–1372.
HULBERT, W. C., GUPPY, M., MURPHY, B. & HOCHACHKA, P. W. (1979). Metabolic sources of heat and power in tuna muscles. I. Muscle fine structure. *J. exp. Biol.* **82**, 289–301.
JOHNSTON, I. A. & GOLDSPINK, G. (1973). A study of glycogen and lactate in the myotomal muscles and liver of the coalfish (*Gadus virens* L.) during sustained swimming. *J. mar. Biol. Ass. U.K.* **53**, 17–26.
JOHNSTON, I. A., DAVIDSON, W. & GOLDSPINK, G. (1977). Energy metabolism of carp swimming muscle. *J. comp. Physiol.* **114**, 206–216.
KATZEN, H. M. & SODERMAN, D. D. (1975). The hexokinase isozymes: Sulfhydral considerations in the regulation of the particle bound and soluble states. In: *Isozymes II. Physiological Function*. (ed. C. C. Markert). pp. 797–817. Academic Press.
LOW, P. S. & SOMERO, G. N. (1976). Adaptation of muscle pyruvate kinase to environmental temperatures and pressures. *J. exp. Zool.* **198**, 1–11.
MACDONALD, V. W. & JOBSIS, F. F. (1976). Spectrophotometric studies on the pH of frog skeletal muscle. pH change during and after contractile activity. *J. gen. Physiol.* **68**, 179–195.
MERRILL, D. K. & GUYNN, R. W. (1976). Electroconvulsive seizure: An investigation into the validity of calculating the cytoplasmic free NAD/NADH ratio from substrate concentrations of brain. *J. Neurochem.* **27**, 459–464.
NEILL, W. H., CHANG, R. & DIZON, A. (1976). Magnitude and ecological implications of thermal inertia in skipjack tuna, *Katsuwonus pelamis* (Linaeus). *Env. Biol. Fish.* **1**, 61–80.
NEWSHOLME, E. A., BEIS, I., LEECH, A. R. & ZAMMIT, V. A. (1978). The role of creatine kinase and Arginine kinase in muscle. *Biochem. J.* **172**, 533–537.
NEWSHOLME, E. A. & START, C. (1973). *Regulation in Metabolism*. London: John Wiley and Sons.
OPIE, L. H. & Newsholme, E. A. (1967). The activities of fructose 1,6-diphosphatase, phosphofructokinase and PEP carboxykinase in white muscle and red muscle. *Biochem. J.* **103**, 391–399.
OSTERBERG, A. E. (1939). The estimation of glycogen in small amounts of tissue. *J. biol. Chem.* **85**, 97–108.
PETER, J. B., JEFFREES, R. N. & LAMB, D. R. (1968). Exercise: Effects on hexokinase activity in red and white skeletal muscle. *Science, N.Y.* **160**, 200–201.
PETTE, D. (1966). Mitochondrial enzyme activities. In *Regulation of Metabolic Processes in Mitochondria* (ed. J. M. Tager, S. Papa, E. Quagliariello and E. C. Slater). Amsterdam: Elsevier.
PRITCHARD, A. W., HUNTER, J. R. & LASKER, R. (1971). The relation between exercise and biochemical changes in red and white muscle and liver in the jack mackerel, *Trachurus symmetricus*. *Fishery Bulletin U.S.* **69**, 379–386.
RAYNER, M. D. & KEENAN, M. J. (1967). Role of red and white muscles in the swimming of skipjack tuna. *Nature, Lond.* **214**, 392–393.
SACKTOR, B. (1976). Biochemical adaptation for flight in the insect (ed. R. M. S. Smellie and J. F. Pennock) *Biochemical Society Symp.* **41**, 111–131.
SCRUTTON, M. C. & UTTER, M. F. (1968). The regulation of glycolysis and gluconeogensis in animal tissues. *Annual Review of Biochemistry* **37**, 249–302.
SEEHERMAN, H. J., TAYLOR, C. R. & MALOIY, G. M. O. (1976). Maximum aerobic power and anaerobic glycolysis during running in lions, horses and dogs. *Fed. Proc.* **35**, 3262.
SHARP, G. & PIRAGES, S. W. (1978). The distribution of red and white swimming muscle, their biochemistry and the biochemical phylogeny of selected scombrid fishes. In *The Physiological Ecology of Tunas* (ed. G. Sharp and A. Dizon), pp. 41–78. Academic Press.
SRERE, P. W. (1969). Citrate synthase. *Methods Enzymol.* **13**, 3–16.
STEENBERGEN, C., DELEEUW, G. & WILLIAMSON, J. R. (1977). Tissue oxygen gradients in whole-tissue ischmia in rat heart. *Fed. Proc.* **36**, 1215.
STEVENS, E. D. & BLACK, E. C. (1966). The effect of intermittent exercise on carbohydrate metabolism in rainbow trout, *Salmo gairdneri*. *J. Fish. Res. Bd Can.* **23**, 471–485.
STEVENS, E. D. & FRY, F. E. J. (1971). Brain and muscle temperatures in ocean caught and captive skipjack tuna. *Comp. Biochem. Physiol.* **38**A, 203–211.
STOREY, K. B. & HOCHACHKA, P. W. (1974). Activation of muscle glycolysis: A role for creatine phosphate in phosphofructokinase regulation. *FEBS Lett.* 46, 337–339.
STOREY, K. B. & HOCHACHKA, P. W. (1975). The kinetic requirements of cytoplasmic alpha-glycerophosphate (α-GP) dehydrogenase in muscle with active α-GP cycles. *Comp. Biochem. Physiol.* **52**B, 175–178.
BÜCHLER, M., FRIEDRICHS, D., COLL, K. & WILLIAMSON, J. R. (1977). Pyridine nucleotide distributions

and enzyme mass action ratios in hepatocytes from fed and starved rats. *Arch. Biochem. Bioph*[illegible] **184**, 222–236.

WALKER, R. M. & JOHANSEN, P. H. (1977). Anaerobic metabolism in goldfish (*Carassius auratus*). *Can. J. Zool.* **55**, 1304–1311.

WILLIAMSON, J. R. (1965). Metabolic control in the perfused rat heart. In *Control of Energy Metabolism* (ed. B. Chance, R. W. Estabrook and J. R. Williamson), pp. 333–346. Academic Press.