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Starvation and the Activities of Glycolytic and Gluconeogenic Enzymes in Skeletal Muscles and Liver of the Plaice, *Pleuronectes platessa*

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Summary. Changes in body index parameters and liver, red muscle and white muscle enzyme profiles have been determined in fed and four month starved plaice, *Pleuronectes platessa*. The results are compared to other vertebrates to estimate specific tissue metabolic patterns and changes in these patterns with starvation.

1. Liver demonstrates the lowest glycolytic but highest gluconeogenic capacity of the three tissues. Red muscle has little, if any, gluconeogenic potential, based upon low activities of phosphoenol pyruvate carboxykinase and glucose-6-phosphatase and no detectable activities of pyruvate carboxylase. Plaice white skeletal muscle has the highest glycolytic potential of the tissues studied.

2. Plaice starved for four months demonstrate significant reductions in liver-somatic index and red muscle-somatic index, and increases in tissue water contents (Table 1). Enzyme activities generally decline in both muscle types, but are maintained in the liver (Table 2). Activities of liver soluble phosphoenol pyruvate carboxykinase increase by approximately 8-fold, suggesting that the enzymic response to starvation in plaice is similar to that of mammals.

3. These results suggest that starvation in plaice is associated with both a decrease in spontaneous activity and metabolic capacity of skeletal muscles, and an enhanced potential for liver gluconeogenesis. Also, it is possible that the precursors for liver gluconeogenesis do not form pyruvate as an intermediate step.

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Abbreviations: FDPase, fructose 1,6-bis phosphatase; *G-6-Pase,* glucose-6-phosphatase; *pNPPase,* p-nitrophenyl phosphatase; *PC,* pyruvate carboxylase; *PEPCK,* phosphoenol pyruvate carboxykinase; *PFK,* phosphofructokinase; *PK,* pyruvate kinase

Introduction

Many species of fish undergo natural periods of depletion due to seasonal fluctuations in food supply and/or spawning migration. A number of metabolic responses to starvation have been documented and it is clear that these effects vary between species and according to the length of the fast (see Love, 1970). The particular metabolic strategy employed appears to be related in part to the amount and sites of storage of body lipids (Love, 1970). "Fatty fish", such as herring, have high concentrations of lipid in the muscle tissue. In these species there is little protein breakdown even after several months starvation (Inui and Ohshima, 1966; Love, 1970). In contrast, species with less extensive lipid reserves, the so-called "non-fatty fish", tend to maintain carbohydrate stores at the expense of peripheral protein by active gluconeogenesis (Stimpson, 1965; Butler, 1968; Renaud and Moon, 1980). For example, during severe starvation in flounder (Templeman and Andrews, 1956) and plaice (Patterson et al., 1974) a major part of white muscle contractile protein becomes utilised resulting in water contents in excess of 95%. A common finding, at least under laboratory conditions, is that starvation is also associated with general decline in metabolic and locomotory activity (Love, 1970).

In those species which maintain carbohydrate stores at the expense of peripheral protein, little is known about either the metabolism and transport of amino acids from muscle or the tissues involved in gluconeogenesis. However, contractile proteins appear to be mobilized to a much greater extent from white than red muscles (Johnston and Goldspink, 1973; Patterson and Goldspink, 1973).

Wittenberger and co-workers have proposed that red muscle plays a role in whole body carbohydrate balance and a lactate-glucose exchange with white

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muscle (Wittenberger, 1973; Wittenberger et al., 1975). This suggestion is derived from an original idea by Breakkan (1956) that fish red muscle may have analogous functions to that of the liver in higher vertebrates. Implicit in these and other studies (e.g. Wittenberger and Diaciuc, 1965; Driedzic and Hochachka, 1975; Hulbert and Moon, 1978) is a potential for glucose synthesis in fish red muscle.

In mammals, gluconeogenesis is confined primarily to the liver, although kidney can produce glucose at an equivalent rate during prolonged starvation (Owen et al., 1969). Isotopic studies on the Australian eel (Phillips and Hird, 1977), the American eel (Renaud and Moon, 1980) and rainbow trout (Cowey et al., 1977a, b; Walton and Cowey, 1979a, b) suggest that lactate and certain amino acid precursors can be converted to glucose in fish liver. Few studies, however, have examined activities of the critical gluconeogenic enzymes of this tissue, and no studies have compared these activities in red muscle with those in liver.

The present study compares the activities of key gluconeogenic and glycolytic enzymes in the liver and muscles of plaice (*Pleuronectes platessa*) under fed and four month starved conditions. Preliminary account of part of this work has been presented to the Biochemical Society (Johnston and Moon, 1979; Moon and Johnston, 1979).

Materials and Methods

Animals

Plaice (*Pleuronectes platessa*), 150 to 200 g body weight, were netted by commercial fishermen in the Firth of Forth, Scotland, during the months August to November, 1978. Fish were brought immediately to the laboratory and maintained in recirculated, filtered sea water at 15±2 °C. Fed fish were used within two weeks of capture and the starved group was food-deprived for 4 months. All fish were visibly healthy at the time of sacrifice.

Tissue and Enzyme Preparation

Fish were stunned by a blow to the head and decapitated. The liver, both dorsal and ventral red muscle masses, and a sample of white muscle were rapidly dissected and placed on ice. Care was taken to avoid contamination of red muscle samples with the adjacent white muscle. Tissues were minced with scissors and homogenized at 0 °C with an 'Ultraturrax' homogenizer for three periods of 10 s in 5 volumes of extraction medium.

Two extraction media were employed; medium A for all enzymes except phosphorylase; and medium B for phosphorylase. Medium A: 50 mM Tris, 5 mM EDTA, 2 mM $MgCl_2$, 1 mM dithiothreitol, 225 mM mannitol, 75 mM sucrose, adjusted to pH 7.5 with HCl. Medium B: 100 mM Tris-maleate buffer, 20 mM NaF, 1 mM EDTA, 0.5 mg/ml bovine serum albumin, 10 mM dithiothreitol, adjusted to pH 6.8.

Homogenates were centrifuged at 600 *g* for 15 min at 0 °C (Beckman J-21B). These supernatants were further fractionated by 15 min centrifugation at 12,000 *g*. The pellet, which contained 95% of the total cytochrome oxidase activity, was suspended in approximately 10 volumes of extraction medium A less mannitol-sucrose. A high speed or microsomal pellet was prepared from the 12,000 *g* supernatant by 60 min centrifugation at 120,000 *g* (Beckman Spinco L2-65B, type 50 rotor). This pellet was suspended in approximately 10 volumes of extraction medium A less mannitol-sucrose.

Enzyme Assays

Measurements of enzyme activity were performed at 15±0.5 °C with appropriate enzyme and reagent banks using a Beckman model 24 recording spectrophotometer. Preliminary experiments established the optimal conditions for each enzyme with respect to pH, and substrate and co-factor concentrations in the various tissue sub-cellular fractions. Enzyme activities expressed as units (μmoles/min) per g dry weight are based on activities in 600 *g* tissue supernatants; for activities bound to sub-cellular fractions (mitochondria and microsomes), on the per cent dry weight of each fraction as estimated from the total fraction protein content. Tissue water content was determined by drying tissues for 48 h at 85 °C, and protein was estimated by a phenol-Folin technique (Maddy and Spooner, 1970). Optimal assay conditions for individual enzymes were as follows:

Phosphorylase (EC 2.4.1.1). Phosphorylase was assayed in the physiological direction according to Burleigh and Schimke (1968): 50 mM phosphate buffer, pH 7.0, 1 mM EDTA, 10 mM NaF, 8 mM $MgCl_2$, 0.2 mg/ml fructose 1,6-bisphosphate, 0.4 mM NADP, 0.3% bovine serum albumin, 0.5% purified muscle glycogen, 1 mM AMP and excess phosphoglucomutase and glucose-6-phosphate dehydrogenase (1 ml final volume).

Hexokinase (EC 2.7.1.1). Hexokinase was assayed using an ATP regenerating system in a medium containing 85 mM Tris-HCl, pH 7.5, 8 mM $MgCl_2$, 0.8 mM EDTA, 1 mM glucose, 2.5 mM ATP, 0.4 mM NADP, 10 mM phosphoryl creatine, 100 μg creatine phosphokinase and 100 μg glucose-6-phosphate dehydrogenase (1 ml final volume).

Glucose 6-Phosphatase (EC 3.1.3.9). G-6-Pase was assayed according to Baginski et al. (1974) using 25 mM cacodylic buffer, pH 6.5, 25 mM glucose-6-phosphate and 0.1 ml extraction medium A (0.4 ml final volume). Reactions were terminated by adding 0.4 ml 10% trichloroacetic acid and the inorganic phosphate produced estimated according to Rockstein and Herron (1951). Nonspecific phosphatase activities were estimated with 25 mM p-nitrophenol phosphate replacing glucose 6-phosphate in the reaction medium. Inorganic P_i produced from substrate and enzyme banks was subtracted from the total P_i to give enzyme-specific activities.

Phosphofructokinase (EC 2.7.1.11). PFK was assayed in a medium containing 50 mM Tris-HCl, pH 7.5, 4.5 mM fructose-6-phosphate, 3 mM ATP, 25 mM KCl, 6 mM $MgCl_2$ 0.15 mM NADH and excess aldolase, triose phosphate isomerase and α-glycerophosphate dehydrogenase (1 ml final volume).

Fructose 1,6-Bisphosphatase (EC 3.1.3.11). FDPase was assayed in a medium containing 70 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 0.2 mM fructose 1,6-bis phosphate, 0.4 mM NADP, and excess phosphoglucoseisomerase and glucose-6-phosphate dehydrogenase (1 ml final volume).

Pyruvate Kinase (EC 2.7.1.40). PK was assayed in a medium containing 50 mM Tris-HCl, pH 7.5, 1.5 mM phosphoenol pyruvate, 2 mM ADP, 4 mM $MgCl_2$, 77 mM KCl, 0.32 mM NADH and excess PK free purified lactate dehydrogenase (1 ml final volume).

Pyruvate Carboxylase (EC 6.4.1.1). PC was assayed according to Crabtree et al. (1972) using 50 mM Tris-HCl, pH 7.5, 9.4 mM $MgCl_2$, 3 mM ATP, 10 mM pyruvate, 10 mM $KHCO_3$, 0.75 mM acetyl CoA, 5 mM phosphoryl creatine, 100 µg creatine phosphokinase, 2 mM DTNB (5,5′-dithiobis-2-nitrobenzoic acid) in 40 mM phosphate, pH 8.0), and excess citrate synthetase (1 ml final volume). Reactions were started by the addition of pyruvate and monitored at 412 nm.

Phosphoenol Pyruvate Carboxykinase (ED 4.1.1.32). PEPCK was assayed according to Pogson and Smith (1975) using 100 mM Tris-HCl, pH 8.1, 15 mM NaF, 18 mM $MgCl_2$, 1 mM glutathione, 3 mM ITP and 4 mM oxaloacetate. Reactions were terminated with 0.1 ml potassium borohydrate (50 mg/ml), 10 µl octan-2-ol added, cooled and 0.2 ml 30% perchloric acid added. Samples were neutralized with triethanolamine containing KOH, and an aliquot was used to estimate phosphoenol pyruvate produced by the pyruvate kinase reaction noted above (excluding PEP, but including purified PK enzyme).

Results

Changes in Body Components

Starvation significantly modified condition index, liver-somatic index and red muscle-somatic index (Table 1). The 50% decrease in liver-somatic index suggests major decreases in liver size, and these changes were noticeable during dissection. Liver colour changed from brick red to pale pink with starvation and also bile volume increased (see Love, 1970).

Tissue water content increased in liver and red and white muscles, with the greatest increase occurring in the liver and white muscle (Table 1). These increases were particularly obvious in white muscle, where one value approached 95% water. The results indicate that major changes occur in composition of plaice tissues during starvation.

Table 1. Change in body index parameters as a function of four months starvation in the plaice, *Pleuronectes platessa.* Mean ± SEM of six animals

	Fed	Starved
Body weight (g)	176.7±7.2	192.8±9.2*
Body length (cm)	25.6±0.4	34.2±2.2**
Tissue water (%)		
Liver	73.8±1.4	81.7±0.3**
Red muscle	76.9±1.6	82.8±1.3*
White muscle	77.8±0.5	85.2±1.4**
Condition index ([body wt × 100]/length^3)	10.5±0.3	5.4±1.1**
Liver-somatic index ([liver wt × 100]/body wt)	1.1±0.1	0.54±0.04**
Red muscle-somatic index ([red muscle wt × 100]/body wt)	2.3±0.2	1.6±0.1**

*, ** Significantly different from fed controls at $^*P < 0.05$ or $^{**}P < 0.01$

Enzyme Profiles

Key glycolytic/gluconeogenic enzyme couples were examined in tissues of plaice to establish possible changes in metabolite flows with starvation. Since significant increases in tissue water content were noted, all enzyme activities are based on activity units per g dry weight. In general, the results (Table 2) indicate decreases in most enzyme activities with starvation, with a few important exceptions.

Tissue phosphorylase activity remains unchanged (Table 2). Neither red muscle nor white muscle contain significant G-6-Pase activity, and that which is present could be accounted for by non-specific phosphatase activities (Table 2). Liver G-6-Pase activity is maintained during starvation although the slight increase in non-specific phosphatase in this tissue decreases total activity slightly (Table 2). Similarly, hexokinase levels tend to drop in all three tissues, with the greatest decrease in white muscle (Table 2).

Major decreases are noted for the other glycolytic enzymes PFK and PK especially in the red and white skeletal muscles (Table 2). Based upon these two enzymes, liver has a poor glycolytic potential, the latter being 8 to 12 times less than in red muscle, which is less active than white muscle. The changes in red muscle PFK and PK are quantitatively smaller than those in the white muscle indicating glycolysis is preferentially maintained in the red muscle with starvation. FDPase activity drops substantially in both muscle types, but is unchanged in liver (Table 2). Starvation results in no change in the liver FDPase/PFK ratio, but a two-fold decrease in this ratio in the two muscle types.

The most significant enzyme change noted during starvation in plaice is the increase in liver PEPCK (Table 2). This increase is associated with the soluble, not the bound, fraction (Tables 2 and 3), as reported for mammals (Tilghman et al., 1976). Similarly, red muscle PEPCK increases with starvation, especially in the soluble fraction, but the total enzyme activity is low relative to the liver value (Tables 2 and 3). No activity could be detected in the white muscle. Pyruvate carboxylase activities were restricted to the cytochrome oxidase-enriched pellet (mitochondria) of liver, but no other tissues demonstrated assayable lev-

Table 2. Activities (µmoles/min·g dry wt) of selected enzymes in fed and four month starved plaice *(Pleuronectes platessa)* liver, red muscle and white muscle. Mean ± SEM (number of animals); where SEM is not given, either sample size is small (phosphorylase) or some samples gave zero activity. See Materials and Methods for analyses

Enzyme	State	Fraction	Liver	Red muscle	White muscle
Phosphorylase	fed	crude	4.45 ± 0.15 (4)	3.92 ± 0.08 (4)	4.48 ± 0.10 (4)
	starved	crude	3.29	3.71	4.17
Hexokinase	fed	crude	1.34 ± 0.08 (6)	1.72 ± 0.12 (6)	0.26 ± 0.03 (6)
	starved	crude	0.87 ± 0.17 (6)	1.55 ± 0.52 (6)	0.09 ± 0.004 (5)
Phosphofructokinase	fed	crude	4.70 ± 0.54 (6)	76.7 ± 11.2 (6)	130.8 ± 6.4 (6)
	starved	crude	4.16 ± 0.93 (5)	40.4 ± 5.9 (5)	56.7 ± 7.8 (5)
Pyruvate kinase	fed	crude	15.2 ± 3.3 (6)	452.5 ± 62.8 (6)	651.2 ± 127 (6)
	starved	crude	12.2 ± 2.2 (6)	128.7 ± 26.8 (6)	162.1 ± 17.6 (4)
Glucose-6-phosphatase	fed	micro[a]	1.04 ± 0.10 (6)	0.26 (6)	0.23 (6)
	starved	micro	1.16 ± 0.26 (6)	0 (6)	0.45 (6)
Non-specific phosphatase	fed	micro	0.40 ± 0.03 (6)	0.35 ± 0.12 (6)	0.06 (6)
	starved	micro	0.64 (2)	0 (2)	0 (2)
Fructose-1,6-bis-phosphatase	fed	crude	9.18 ± 0.61 (6)	4.88 ± 1.4 (6)	4.21 ± 0.47 (6)
	starved	crude	7.92 ± 1.7 (5)	1.20 ± 0.33 (5)	0.96 ± 0.18 (5)
Phosphoenol pyruvate carboxykinase	fed	mito[b]	0.73 ± 0.07 (6)	0.02 ± 0.003 (6)	0
	starved	mito	0.67 ± 0.19 (4)	0.04 (4)	0
	fed	sol[c]	0.02 ± 0.004 (6)	0.001 (6)	0
	starved	sol	0.46 ± 0.06 (4)	0.006 ± 0.002 (4)	0

[a] microsomes; [b] mitochondria; [c] soluble

Table 3. Organ specific activities (nmoles/min·organ weight) of phosphoenol pyruvate carboxykinase from the soluble and mitochondria fractions of fed (6 estimates) and four month starved (4 estimates) plaice, *Pleuronectes platessa*

Fraction	Liver		Red muscle	
	Fed	Starved	Fed	Starved
Soluble	171.3 ± 27.6	1,269.9 ± 277.1***	4.5 ± 1.8	14.2 ± 4.5*
Mitochondria	533.5 ± 112.6	225.0 ± 51.5*	1.0 ± 0.2	3.5 ± 1.3*
Total	704.9 ± 106.6	1,494.9 ± 326.4**	5.5 ± 1.7	17.7 ± 5.1**
Red muscle as a % of the liver capacity	–	–	0.8%	1.2%

*, **, *** Significantly different from fed controls at * $P < 0.1$, ** $P < 0.05$ or *** $P < 0.01$

els. Activity of this fraction is 5.6 ± 1.3 nmoles/min·mg protein; however, no activity could be detected in the starved plaice liver, even though major activity increases were seen for PEPCK.

Cytochrome oxidase activity decreases in liver, remains unchanged in red muscle, and increases in white muscle (Table 2). This enzyme is enriched in the mitochondrial fraction of all tissues, although substantial activity is detectable in the red muscle 12,000 *g* supernatant. Such a tissue difference may indicate that red muscle mitchondria are more fragile than those of the other tissues.

Enzyme Localization

Many of the glycolytic and gluconeogenic enzymes are localized to specific cellular components. The most extensively studied is hexokinase (see Ottaway and Mowbray, 1977). Plaice tissue hexokinase is primarily soluble in the liver and white muscle, but bound to either the 12,000 *g* or 120,000 *g* pellets in red muscle (Table 4). Starvation alters this distribution, especially in red muscle where the pattern is the reverse of the fed situation. Both liver and white muscle hexokinase change in a similar reverse manner

Table 4. The distribution of hexokinase and phosphoenol pyruvate carboxykinase between the soluble and total particulate fractions (bound = mitochondria + microsomes) of plaice, *Pleuronectes platessa*, liver and red and white muscles. Values are % of total activity; numbers of animals given in brackets. ND, none detected

	Soluble	Bound
Fed:		
Hexokinase (6)		
Liver	55.7 ± 12.1	44.3 ± 12.1
Red muscle	39.0 ± 3.6	61.0 ± 3.6
White muscle	69.3 ± 5.7	30.7 ± 5.7
PEP carboxykinase (6)		
Liver	18.5 ± 4.4	81.7 ± 4.3
Red muscle	34.8 ± 13.3	65.7 ± 13.2
White muscle	ND	ND
Four months starved:		
Hexokinase (6)		
Liver	34.5 ± 7.6	65.5 ± 7.6
Red muscle	83.4 ± 2.6	16.6 ± 2.6
White muscle	42.9 ± 9.6	57.1 ± 9.6
PEP carboxykinase (4)		
Liver	82.4 ± 1.2	17.6 ± 0.9
Red muscle	53.2 ± 15.4	46.8 ± 15.4
White muscle	ND	ND

to the fed plaice. Many factors could be responsible for these changes (Ottaway and Mowbray, 1977) and whether such differences are significant remains to be proven.

As noted above, both PEPCK and PC are localized principally in the mitochondrial fraction in fed plaice liver. Starvation results in a large increase in both the total liver PEPCK activity (Table 4) and the fraction specific to the cytoplasm (Tables 3 and 4). Red muscle activities are essentially equally distributed between both fractions (Table 4) but significant increases do occur in total PEPCK activities and the amount in each fraction with starvation.

Discussion

It has been suggested that determinations of maximal in vitro activities of non-equilibrium enzymes provides a useful semiquantitative estimate of maximal metabolic flux and the nature of the principal fuel supporting metabolic activities in different tissues (see for example, Newsholme et al., 1978). Enzymes catalysing non-equilibrium reactions can be identified by measurements of mass action ratios and they usually have among the lowest activities in the pathway being regulatory steps in the control of metabolism. Non-equilibrium or regulatory steps in glycolysis include phosphorylase, hexokinase, phosphofructokinase and pyruvate kinase (Simon and Robin, 1972; Newsholme et al., 1978). The indicator enzyme for gluconeogenesis is often taken as fructose 1,6-bisphosphatase (Bloxham and York, 1976) even though this activity generally exceeds that of either phosphoenol pyruvate carboxykinase or pyruvate carboxylase by a large amount (Söling and Kleineke, 1976).

It would appear that as in mammalian liver, plaice liver carbohydrate metabolism is geared to glucose production. Activities of PFK and PK are low, but PEPCK, FDPase and G-6-Pase are relatively high. High hexokinase values in the fed state allow the liver to compete for blood glucose and maintain its role as a "glucostat", just as in mammals (Stalmans, 1976). This apparently is not the case in either the lamprey (Larsen, 1978) or the hagfish (Inui and Gorbman, 1978) where hepatectomy does not alter blood glucose. Starvation reduces liver hexokinase values, which coupled to a slight increase in G-6-Pase, increases the G6-Pase/hexokinase ratio from 0.75 to 1.3. Even though the liver-somatic index falls, this increased ratio may allow for the maintenance of glucose release in the starved liver. Interestingly, no similar change is seen at the FDPase/PFK step. Unlike mammals (Söling and Kleineke, 1976), no glucokinase activity was detected in the plaice liver (activation of glucose phosphorylation at 100 mM compared to 10 mM glucose which was saturating for plaice hexokinase). O'Neil and Langslow (1978) found no glucokinase activity in chicken liver, and they concluded that changes in glucose-6-phosphate and G-6-Pase control liver glucose flux; a similar possibility exists for plaice liver.

The most significant change reported in this paper is the increase in liver PEPCK activity (Table 3). Of the four "classic" gluconeogenic enzymes, the activity and distribution of PEPCK vary the most between different animals (Söling and Kleineke, 1976). The plaice responds to starvation in a manner much like the mammal (Tilghman et al., 1976) since both total and soluble PEPCK activities increase. To the authors' knowledge only one other study has been reported in a lower vertebrate, *Xenopus*, and here the enzyme responds in a similar manner (Woof and Janssens, 1978).

Although plaice liver PEPCK increases, no parallel increase in PC occurred. This result may possibly reflect either a change in liver metabolites with starvation thus altering the responsiveness of the enzyme to specific effectors (see Söling and Kleineke, 1976), or a change in the precursors for liver gluconeogenesis with starvation. Alanine and lactate are the major mammalian liver gluconeogenic precursors and both require PC and PEPCK activities to promote glucose

synthesis (Söling and Kleineke, 1976). These precursors are derived from muscle proteolysis and anaerobic glycolysis, respectively. A major decrease in spontaneous activity was noted in starved plaice and skeletal muscle glycolytic potential decrease simultaneously (see below), so lactate production will decline. Little is known of amino acid release from the skeletal muscles of fish, although recently, Leech et al. (1979) reported that alanine release from dogfish tail muscle decreased after two weeks of starvation. If so, PC activities need not necessarily change, since pyruvate may not be a direct precursor for glucose. More work is necessary to understand muscle proteolysis and liver gluconeogenesis in fish.

It must also be pointed out that PK activities are at least 10-times higher than PEPCK activities (Table 2). If substrate cycling at this point is to be prevented (see Söling and Kleineke, 1976), inhibition of PK is necessary. However, fish liver PKs are generally non-regulatory in contrast to the mammalian enzyme (see Moon and Hulbert, 1979) which may make this step critical to gluconeogenic control.

Thus, plaice liver is principally a gluconeogenic organ, with little glycolytic potential. The precise nature of gluconeogenic regulation in this tissue is unknown, but it is possible that non-pyruvate producing amino acids are the major precursors of glucose.

Both plaice muscle types have a high glycolytic capacity, being of the order seen in heart and skeletal muscles of other vertebrates (Newsholme and Start, 1973). It is *unlikely* that gluconeogenesis occurs in plaice red muscle as evidenced by the very low activities of PEPCK and G6-Pase and no detectable activities of PC. This does not support the suggestion that fish red muscle has analogous functions to the liver in higher vertebrates (see Introduction). Newsholme and co-workers have reached similar conclusions from a study of a variety of other vertebrate muscle types (Opie and Newsholme, 1967; Crabtree et al., 1972). The presence of PEPCK in muscle tissues may be related to the presence of either a malate-oxaloacetate cycle (Opie and Newsholme, 1967) or more likely to catalyse a reaction whereby certain amino acids are either oxidized or converted to alanine (Newsholme and Williams, 1978). Similarly, muscle FDPase is generally accepted to be involved with metabolic cycling at the FDPase/PFK locus, and more specifically with AMP amplication of glycolysis (Newsholme et al., 1978). Fish skeletal muscles generally have higher activities of both PFK and FDPase than other vertebrates which may reflect the necessity for rapid activation of a low standard metabolic level to a state of high activity (Newsholme and Start, 1973; Johnston, 1977). Certainly the presence of a modulator-sensitive muscle PK supports this hypothesis (Johnston, 1975; Zammit et al., 1978; Moon and Hulbert, 1979).

Plaice red skeletal muscle has a high aerobic capacity possessing comparable cytochrome oxidase activity to other teleost red muscles (Fig. 1 in Johnston and Moon, 1980). The presence of relatively high levels of hexokinase (Table 2), plus its distribution within the cell (Table 4), suggests that glucose is an important fuel source. Johnston and Goldspink (1973) report glycogen concentrations 3.5-times higher in red than white skeletal muscle of plaice, indicating that these high hexokinase activities provide the tissue with a competitive advantage to procure blood glucose. Since hexokinase levels fall only slightly with starvation, this advantage is maintained, as is tissue glycogen (Johnston and Goldspink, 1973). Carbohydrate appears to be of major metabolic importance to this tissue, even though it is generally assumed that lipid provides the chief fuel to support activities of this aerobic tissue (see Love, 1970).

No major differences were noted in the activities of plaice white muscle enzymes when compared to other vertebrate white muscles (Newsholme and Start, 1973). This tissue is generally assumed to be glycolytic and in fish is used during period of burst swimming, although some species recruit white fibres for sustained activity (see e.g. Johnston and Moon, 1980).

Starvation in plaice is accompanied by a general decline in muscle enzyme activities, although the change is quantitatively larger in white than red muscle. Since the white muscle accounts for at least 75% of the total fish weight, small decreases in tissue reserves could result in significant changes in body metabolite balance. An increase in phosphatase activities (Table 2) may be associated with increases in white muscle autolysis (see Love, 1970), and the increase in red muscle PEPCK activities (Table 3) to enhanced amino acid metabolism. The general decline in enzymes in muscle tissue is probably associated with the decrease in spontaneous activity reported for many fish during starvation (Love, 1970).

Thus, the major starvation strategy of the plaice consists of at least four components: one, a generalized decline in overt activities associated with decreases in tissue metabolism and enzyme levels in skeletal muscle; two, a mobilization of skeletal muscle and liver reserves as indicated by significant decreases in tissue indices (Table 1); three, the maintenance of liver metabolic integrity more than any other tissue; and four, an enhanced potential for liver gluconeogenesis probably from precursors other than those generating pyruvate. Further, plaice red muscle apparently has no gluconeogenic capacity under either experimental condition employed in this study. The use of the enzyme approach to starvation studies has

allowed us to follow more closely the metabolic changes occurring during this period. However, more work is necessary to pinpoint those specific steps which control the individual metabolic pathways and thereby determined the flow of carbon during starvation.

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