Biochem. J. (1979) **184**, 313–322
Printed in Great Britain

Activities of Enzymes of Fat and Ketone-Body Metabolism and Effects of Starvation on Blood Concentrations of Glucose and Fat Fuels in Teleost and Elasmobranch Fish

By Victor A. ZAMMIT and Eric A. NEWSHOLME
Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

(*Received* 8 *May* 1979)

1. Activities of 3-oxo acid CoA-transferase and carnitine palmitoyltransferase together with tri- and di-acylglycerol lipase were present in red and heart muscles of the teleost fish. However, D-3-hydroxybutyrate dehydrogenase activity was not detectable. These results suggest that the heart and red muscles of the teleosts should be able to utilize the fat fuels triacylglycerol, fatty acids or acetoacetate, but not hydroxybutyrate. The muscles from the elasmobranchs differed in that D-3-hydroxybutyrate dehydrogenase and 3-oxo acid CoA-transferase activities were present, but carnitine palmitoyltransferase activity was not detectable. This suggests that ketone bodies are the most important fat fuels in elasmobranchs. 2. The concentrations of acetoacetate, 3-hydroxybutyrate, glycerol, non-esterified fatty acids and triacylglycerols were measured in blood or plasma of several species of fish (teleosts and elasmobranchs) in the fed state. Teleosts have a 10-fold higher concentration of plasma non-esterified fatty acids, but a lower blood concentration of ketone bodies; both acetoacetate and 3-hydroxybutyrate are present in blood of elasmobranchs, whereas 3-hydroxybutyrate is absent from that of the teleosts. 3. The effects of starvation (up to 150 days) on the concentrations of blood metabolites were studied in a teleost (bass) and an elasmobranch (dogfish). In the bass there was a 60% decrease in blood glucose after 100 and 150 days starvation. In dogfish there was a large increase in the concentration of ketone bodies, whereas in bass the concentration of acetoacetate (the only ketone body present) remained low (<0.04mM) throughout the period of starvation. The concentration of plasma non-esterified fatty acids increased in bass, but decreased in dogfish. These changes are consistent with the predictions based on the enzyme-activity data. 4. Starvation did not change the activities of ketone-body-utilizing enzymes or that of phosphoenolpyruvate carboxykinase in heart and red skeletal muscles of both fish, but it decreased markedly the activity of phosphoenolpyruvate carboxykinase in white skeletal muscle of both fish. However, in the liver of the dogfish, starvation resulted in a twofold increase in the activities of 3-hydroxybutyrate dehydrogenase and acetoacetyl-CoA thiolase, whereas in bass liver it decreased the activity of acetoacetyl-CoA thiolase and increased that of 3-oxo acid CoA-transferase. The activity of phosphoenolpyruvate carboxykinase was increased twofold in the liver of bass, but was unchanged in that of the dogfish. 5. The difference in changes in concentrations of blood metabolites and enzyme activities in the two fish support the suggestion that, in starvation, ketone bodies, but not non-esterified fatty acids, are an important fuel for muscle in elasmobranchs, whereas non-esterified fatty acids, but not ketone bodies, are an important fuel in teleosts. The results are discussed in relation to the evolution of a discrete lipid-storing adipose tissue in teleosts and higher vertebrates.

In mammals and birds the reserve lipid fuel triacylglycerol is stored primarily in a discrete adipose tissue. When it is necessary to mobilize this fuel it is released from the adipose tissue as non-esterified fatty acids and glycerol, but some of the non-esterified fatty acids are converted into ketone bodies in the liver (see Newsholme & Start, 1973). In some lower animals lipid is stored primarily in a tissue that, from a functional point of view, can be described as a liver (e.g. the fat-body of the insect). In the locust (see Tietz, 1967), and probably in insects in general (see

Abbreviations used: PEPCK, phosphoenolpyruvate carboxykinase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA.

Crabtree & Newsholme, 1972), this lipid is mobilized as diacylglycerol rather than non-esterified fatty acids or ketone bodies. However, in fish, the more primitive elasmobranchs store most of their triacylglycerol in the liver, whereas the more advanced teleosts store a considerable proportion of their triacylglycerol in a discrete abdominal tissue and/or in adipocytes which are distributed throughout the muscle fibres (Love, 1970). Consequently, these two classes of fish offer a unique opportunity to investigate the type of fat fuel that is mobilized from the two different storage tissues in animals from the same phylum.

Two experimental approaches have been used to investigate the fat fuels utilized by these fish. First, the activities of key enzymes involved in fatty acid (Crabtree & Newsholme, 1972) and ketone-body (Beis, 1978) oxidation have been measured in muscles of three teleosts and four elasmobranchs. Secondly, the blood concentrations of glucose, non-esterified fatty acids, ketone bodies, lactate, glycerol and triacylglycerol were measured in bass (a teleost) and dogfish (an elasmobranch) as soon as possible after capture and after feeding or starvation in captivity. At the same time, samples of liver were taken for assay of the activities of the enzymes of ketone-body metabolism (acetoacetyl-CoA thiolase, EC 2.3.1.9; 3-oxo acid CoA-transferase, EC 2.8.3.5; HMG-CoA synthase, EC 4.1.3.5; and 3-hydroxybutyrate dehydrogenase, EC 1.1.1.30) and a gluconeogenic enzyme, PEPCK (EC 4.1.1.32). The results are reported and discussed in this paper.

Materials and Methods

Chemicals and enzymes

Scintillation materials and L(−)-carnitine were obtained from Koch–Light Laboratories, Colnbrook, Bucks. SL3 0BZ, U.K. D(+)-Carnitine was a gift from Otsuka Pharmaceutical Laboratory, Naruto, Tokushima, Japan. Glycerol tri[1-^{14}C]oleate was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Acetoacetyl-CoA was synthesized from CoA and diketene as described previously (Sugden & Newsholme, 1973). All other biochemicals and enzymes were obtained from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey KT2 7BH, U.K. All inorganic chemicals were obtained from BDH Chemicals, Poole, Dorset BH12 4NN, U.K.

Source and treatment of animals

All the fish used in this work were caught by trawl off Plymouth, U.K., by using boats of the Marine Biological Association of the United Kingdom, Plymouth. For the starvation experiments, the dogfish and bass were brought to the laboratory as quickly as possible (approx. 1–2h after capture) and they were transferred to outside tanks (capacity 4m^3) with continuously running sea water. The experiments were started after a period of 2 weeks, during which the fish were allowed to acclimatize to the new environment and feeding routine. Food consisted of chopped bibs (*Triscopterus minutus*) given to the fish every third day. The fish were fed to satiation. The experiments were carried out between March and September 1977. During this time the temperature of the sea water in the holding tanks varied between 10 and 15°C. When the fish were required for experiment they were rapidly caught by net, stunned by a blow on the head (bass) or restrained from moving (dogfish) and a sample of mixed venous and arterial blood was taken with a syringe by puncturing the caudal blood vessels at the level of the anal fin. The syringe contained 10mg of EDTA/ml of sampled blood to prevent coagulation. The procedure was completed within 2min, after which the fish were killed and samples of muscle were dissected as described below. Preliminary experiments indicated that there were no significant differences in blood metabolite concentrations and tissue enzyme activities between male and female animals, except in those animals that were sexually gravid. Consequently male and non-gravid female animals were used indiscriminately for experimentation.

Preparation of blood samples

For the measurement of concentrations of glucose, glycerol, acetoacetate, 3-hydroxybutyrate and lactate, 1.0ml of blood was mixed immediately after sampling with an equal volume of cold 10% (w/v) $HClO_4$. The precipitated protein was removed by centrifugation at 3000*g* for 15min. Samples of the supernatant were neutralized with 10M-KOH to pH7.0 (for assay of acetoacetate and glucose) or pH9.0 (for assay of 3-hydroxybutyrate, lactate and glycerol). The $KClO_4$ precipitate was removed by centrifugation for 10min at 3000*g*. The supernatant was either used immediately for assay (see below) or stored under liquid nitrogen until required (within 1 month).

For measurement of concentrations of triacylglycerol and non-esterified fatty acids, blood plasma was obtained by centrifugation of whole blood at 3000*g* for 15min. It was found that haemolysis was minimal unless coagulation occurred. The plasma so obtained was stored under liquid nitrogen until used for assay (see below), usually within 1 month.

Measurement of metabolite concentrations

The concentrations of the following metabolites were assayed enzymically as described previously: acetoacetate and 3-hydroxybutyrate (Williamson *et al.*, 1962), glucose (Bergmeyer *et al.*, 1974), lactate (Gutman & Wahlefeld, 1974), glycerol (Garland & Randle, 1962) and triacylglycerols (Wahlefeld, 1974). The concentration of non-esterified fatty acids was

measured colorimetrically by a modification of the method of Duncombe (1963). Before colorimetric determination, non-esterified fatty acids were extracted from the total lipid extract of plasma (Dole, 1958) as described by Borgström (1952). This was necessary because the addition of the $CuSO_4$ reagent to the total lipid extract of fish blood resulted in precipitation of non-polar lipids, which interfered with subsequent colour development. Preliminary experiments established that 95–98% of total non-esterified fatty acids were extracted and measured by this procedure. Solutions containing known amounts of palmitic acid were subjected to the same procedure and were used as standards.

Dissection of muscles and preparation of homogenates

Fish were stunned with a blow on the head, then heart and white and red trunk muscles were rapidly dissected. In dissecting trunk muscles, care was taken always to take samples from a selected group of myotomes at the level of the anterior edge of the posterior dorsal fin. This procedure was followed in order to minimize variations in enzyme activities owing to longitudinal differences in enzyme content of trunk muscles. The tissues were kept on ice until used, usually within 1 h.

Homogenates were made with a hand-operated all-glass homogenizer kept at 0°C. For lipase assays tissues were homogenized in 200 mM-triethanolamine, pH 7.4. For assay of carnitine palmitoyltransferase the extraction medium contained 100 mM-Tris/HCl and 1 mM-EDTA at pH 7.4. The extraction media for all other assays were as described elsewhere (Alp *et al.*, 1976; Zammit & Newsholme, 1976; Beis, 1978). All homogenates were sonicated for two 15 s periods at 0°C in a 150 W MSE sonic disintegrator working at maximum intensity.

Assay of enzyme activities

Carnitine palmitoyltransferase activity was assayed spectrophotometrically as described by Bieber *et al.* (1972). The assay medium contained 60 mM-Tris/HCl, 1.5 mM-EDTA, 1.25 mM-L(−)carnitine, 0.25 mM-5,5′-dithiobis-(2-nitrobenzoic acid) and 35 μM-palmitoyl-CoA, in a total volume of 0.5 ml at pH 8.0. The reaction was started by addition of 5–10 μl of homogenate. The increase in A_{412} was followed on a Unicam SP. 800 recording spectrophotometer. Controls, in which D(+)-carnitine replaced L(−)-carnitine, were run concurrently.

Triacylglycerol lipase activity was measured radiochemically by measuring the release of [^{14}C]-oleate from glycerol tri[1-^{14}C]oleate (Bilinski & Lau, 1969). The reaction mixture contained 0.1 mM-glycerol tri[1-^{14}C]oleate (2 μCi/mmol) as 0.1 ml of a suspension in 30% (w/v) poly(vinyl alcohol) (mol.wt. 14000) in 200 mM-triethanolamine, pH 7.4 (prepared as described by Crabtree & Newsholme, 1972), and 0.1 ml of homogenate. The reaction was started by addition of homogenate. The reaction was stopped by the addition of 0.8 ml of a mixture of 0.5 M-H_2SO_4, iso-octane and isopropyl alcohol (1:10:40, by vol.), and total lipid was further extracted as described by Carter (1967). Non-esterified fatty acids were isolated from the iso-octane phase by the alcoholic NaOH-partition method of Borgström (1952) after addition of carrier oleate (10 μmol). The final iso-octane phase, containing the fatty acids, was evaporated to dryness under nitrogen in scintillation vials. Toluene scintillant [3 g of 2,4-diphenyloxazole and 0.1 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene in 1 litre of sulphur-free toluene] was then added and radioactivity measured in a Packard LS-500 scintillation counter. The channels-ratio method was used for quench correction (Baillie, 1960). Preliminary experiments established that fish phospholipid had no effect on lipase activity (see Bilinski & Lau, 1969). Diacylglycerol lipase activity was measured as described previously (Crabtree & Newsholme, 1972) by monitoring the release of glycerol from glycerol dioleate by the method of Newsholme & Taylor (1968).

The maximal activities of acetoacetyl-CoA thiolase, 3-oxo acid-CoA transferase, HMG-CoA synthase and 3-hydroxybutyrate dehydrogenase were measured spectrophotometrically as described elsewhere (Sugden & Newsholme, 1973; Clinkenbeard *et al.*, 1975; Beis, 1978). The activity of PEPCK was measured spectrophotometrically as described by Zammit & Newsholme (1976). All enzyme assays were performed at 10°C and spectrophotometric assays were carried out in a Unicam SP. 800 recording spectrophotometer. Full details of these assays and the tests carried out to ensure that maximum activities were measured are given by Zammit & Newsholme (1976), Beis (1978) and Zammit *et al.* (1979).

Results and Discussion

Activities of the enzymes of fat-fuel metabolism in muscle

The activities of 3-oxo acid CoA-transferase and acetoacetyl-CoA thiolase are similar in the muscles of the three teleost fish (mackerel, plaice and bass) to those in the muscles of the four elasmobranchs (smooth hound, spur dog, ray and dogfish) (Table 1). However, carnitine palmitoyltransferase activity was not detectable in all the muscles of the four elasmobranchs investigated, whereas it was present in those of the teleosts (Table 1), (carnitine palmitoyltransferase activity was present in the livers of the elasmobranchs investigated; 0.04 and 0.07 μmol/min per g for dogfish and ray, respectively). Similarly, the lipase activities were in general much lower in the muscles of the elasmobranchs. These results

Table 1. *Maximal activities of* D-3-*hydroxybutyrate dehydrogenase, 3-oxo acid CoA-transferase, acetoacetyl-CoA thiolase, carnitine palmitoyltransferase and di- and tri-acylglycerol lipase in muscle of teleosts and elasmobranchs*

Enzyme activities were measured as described in the Materials and Methods section. The tissues were extracted usually 3–4 h after capture and assayed as soon as possible (within 1 h) of extraction. The activities are presented as means, with the ranges and numbers of separate animals used in parentheses.

Animal		Enzyme activities (μmol of substrate utilized/min per g fresh wt. at 10°C)				Enzyme activities (nmol of fatty acid produced/min per g fresh wt. at 10°C)	
		D-3-Hydroxybutyrate dehydrogenase	3-Oxo acid CoA-transferase	Acetoacetyl-CoA thiolase	Carnitine palmitoyl-transferase	Triacylglycerol lipase	Diacylglycerol lipase
Teleosts							
Plaice (*Pleuronectes platessa*)	Heart	<0.01 (3)	13.5 (12.1–14.7) (4)	4.1 (3.4–4.8) (3)	0.20 (0.17, 0.23) (2)	0.90 (0.75–1.0) (3)	37.3 (25.1–47.3) (4)
	Red	<0.01 (2)	8.9 (6.7–10.1) (6)	4.8 (2.9–6.2) (6)	0.07 (0.03–0.12) (3)	3.7 (2.0–5.4) (2)	21.0 (18.9–22.1) (2)
	White	<0.01 (1)	<0.01 (2)	<0.01 (2)	—	0.1 (3)	1.0 (1.0) (2)
Bass (*Dicentrarcus labrax*)	Heart	<0.01 (2)	17.6 (15.9–19.2) (4)	3.7 (3.5–3.8) (3)	0.60 (0.60, 0.60) (2)	2.8 (1.4–4.6) (6)	56.1 (44.7–58.9) (4)
	Red	<0.01 (1)	18.3 (18.1–18.5) (3)	5.1 (4.9–5.2) (4)	0.75 (0.57–0.96) (3)	1.8 (1.2–3.4) (4)	27.2 (22.7–32.6) (3)
	White	—	0.01 (0.01) (3)	—	—	1.0 (0.7–1.2) (4)	1.7 (1.3–1.9) (3)
Mackerel (*Scombrus scombrus*)	Heart	<0.01 (3)	13.3 (10.5–16.5) (3)	7.8 (1.5–8.4) (6)	0.40 (0.32–0.52) (3)	13.1 (10.7–15.3) (4)	146 (120–170) (4)
	Red	<0.01 (3)	11.1 (8.7–13.4) (4)	8.5 (7.1–10.3) (5)	0.60 (0.44–0.72) (3)	4.5 (1.4–13.7) (5)	204 (170–236) (3)
	White	<0.01 (2)	0.05 (0.02–0.07) (4)	<0.01 (3)	—	0.7 (0.6–0.8) (3)	12.1 (9.2–15.1) (3)
Elasmobranchs							
Dogfish (*Scylliorhinus canicula*)	Heart	1.2 (1.0–1.3) (3)	9.9 (7.7–11.4) (4)	6.7 (5.4–8.7) (4)	<0.01 (2)	1.3 (1.0–1.9) (3)	13.7 (11.1–15.0) (3)
	Red	1.3 (1.2–1.4) (4)	15.3 (13.9–16.1) (4)	5.7 (5.0–6.2) (3)	<0.01 (3)	<0.2 (3)	<1.0 (3)
	White	0.02 (0.02) (2)	<0.01 (3)	0.02 (0.01–0.02) (3)	<0.01 (2)	—	—
	Branchial	0.65 (0.59–0.69) (3)	7.8 (7.1–8.9) (3)	2.3 (1.8–2.7) (4)	<0.01 (3)	—	—
Ray (*Raja clavata*)	Heart	0.72 (0.6–0.86) (4)	37.5 (36.0–38.1) (4)	4.5 (3.2–5.7) (3)	<0.01 (3)	1.1 (0.7–1.5) (4)	—
	Red	0.37 (0.32–0.41) (3)	27.9 (21.3–35.7) (5)	6.5 (4.6–9.8) (4)	<0.01 (3)	0.54 (0.30–0.75) (3)	—
	White	—	<0.01 (2)	—	—	0.40 (0.10–0.72) (3)	—
Spur dog (*Squalus acanthias*)	Heart	0.21 (0.17–0.26) (3)	9.4 (8.5–9.8) (3)	3.2 (1.8–3.7) (4)	<0.01 (3)	1.3 (1.1, 1.5) (2)	—
	Red	0.64 (0.45–0.87) (3)	6.41 (4.3–9.6) (3)	4.3 (3.7–4.7) (3)	<0.01 (3)	<0.1 (2)	—
Smooth hound (*Mustelus asterias*)	Heart	0.27 (0.18–0.29) (3)	11.5 (10.1–12.7) (3)	3.6 (3.4–3.7) (3)	<0.01 (2)	—	—
	Red	0.84 (0.75–0.92) (3)	22.7 (19.7–24.1) (3)	14.6 (13.8–15.1) (3)	<0.01 (2)	—	—

suggested that fatty acids, which require carnitine palmitoyltransferase activity for oxidation, are not important as a fat fuel in the elasmobranchs. Since D-3-hydroxybutyrate dehydrogenase activity was not detectable in muscles of the teleosts, and since D-3-hydroxybutyrate is known to be the quantitatively important ketone for oxidation in starvation in mammals, this finding suggested that ketone bodies may not be an important fuel in the teleosts during starvation. For this reason the concentrations of ketone bodies, fatty acids, glycerol and triacylglycerol were measured in the blood of teleosts and elasmobranchs.

Concentrations of fat fuels in the blood of elasmobranchs and teleosts

An investigation of several species of fish in the fed state indicated that there were marked differences in the concentrations of fat fuels in the blood of teleosts and elasmobranchs: concentrations of non-esterified fatty acids were about 10-fold higher in teleosts; 3-hydroxybutyrate was present in the blood of elasmobranchs, but could not be detected in teleosts; and glycerol was detected in teleosts, but not in the elasmobranchs (Table 2). These results provided further support for the views that non-esterified fatty acids were unimportant as a fat fuel in elasmobranchs and that ketone bodies were unimportant in the teleosts. Therefore a study was undertaken to investigate the effects of prolonged starvation on the concentrations of fat fuels and glucose in representatives of the two classes of fish, bass (a teleost) and dogfish (an elasmobranch).

In this work it has been assumed that an increase in the concentration of the fat fuels in the blood or plasma is indicative of increased rates of utilization and oxidation.

Effects of stress of capture on concentrations of metabolites in blood of fish

In order to investigate the effects of stress during capture and subsequent transport to the laboratory, blood samples were taken from the fish immediately on arrival at the laboratory (2–3 h after capture). In Table 3 the concentrations of glucose, glycerol, acetoacetate, 3-hydroxybutyrate, lactate and non-esterified fatty acids in blood of freshly caught fish are compared with the concentrations of these metabolites in the blood of fish that had been kept in captivity and fed for up to 150 days (see the Materials and Methods section). Since stress on fish during re-capture from tanks was minimal, it was assumed that concentrations of metabolites in blood of animals after 40 or 100 days represent basal, steady-state values for the fed animals. (However, these results must be interpreted with some caution, since maintenance of fish in laboratory tanks may produce a different metabolic state from that pertaining to the fed animals in the wild.) Stress during capture results in almost a 3-fold increase in blood glucose in both fish and a 5- and 10-fold increase in blood lactate in bass and dogfish respectively (Table 3). The increase in blood glucose is probably due to stress-stimulation of hepatic glycogenolysis (see Patent, 1970; Thorpe & Ince, 1974; Umminger *et al.*, 1975). The increase in lactate is probably due to stimulation of glycolysis in the muscles and a slow rate of removal of lactate from the blood of the fish (Chavin & Young, 1970; Driedzik & Kicenicek, 1976). There was a 30% increase in non-esterified fatty acids concentration and a similar increase in glycerol concentrations in the bass, but they were not statistically significant. It is suggested that this is due to stimulation of the adipose-tissue lipase by stress. However, in the dogfish, there was no change in the concentrations of glycerol or non-esterified fatty acids, which is consistent with the suggested inability of this animal to mobilize its fat stores as non-esterified fatty acids.

Effect of starvation on the concentration of fuels in blood

The concentration of glucose in the fed bass is 5-fold higher than that in blood of fed dogfish. Although starvation for 40 days caused no change in blood glucose concentration in either fish, it was decreased after 100 and 150 days starvation in the bass (see Table 3). Both acetoacetate and 3-hydroxybutyrate were detected in blood of dogfish, whereas only acetoacetate was detectable in that of the bass (and other teleosts; see Table 2): hydroxybutyrate was not detectable in the bass even after 150 days starvation. This finding is consistent with the lack of detectable 3-hydroxybutyrate dehydrogenase activity in liver of marine teleost fish (Beis, 1978). The concentration of acetoacetate was similar in the blood of both species in the freshly caught animals. However, the concentration in the dogfish increases by 6-fold during starvation, whereas in the bass, even after 150 days starvation, the concentration was only 0.04 μmol/ml. The concentration of hydroxybutyrate in the fed dogfish 150 days after capture was 0.01 mM, whereas, after starvation for this period, the concentration rose to 1.86 mM; the concentration of total ketone bodies increased from approx. 0.1 mM in the fed state to approx. 2 mM in the starved state (2.28 mM after 150 days starvation). The mean 3-hydroxybutyrate/acetoacetate concentration ratio increased from a mean of 0.57 for fed dogfish to a mean of 4.9 in the starved animal (Table 3).

Starvation of the dogfish for 40 and 150 days decreased the plasma non-esterified fatty acids concentration by about 30%, although these changes were not statistically significant. In bass, starvation for 40 days increased the plasma non-esterified fatty acids concentration by about 65%. However, after 150 days starvation the concentration is decreased by

Table 2. *Concentrations of ketone bodies, glycerol, non-esterified fatty acids and triacylglycerols in the blood or plasma of selected teleosts and elasmobranchs*

Concentrations were measured as described in the Materials and Methods section. Concentrations are given in μmol/ml of blood, except for non-esterified fatty acids and triacylglycerols which are μmol/ml of plasma. They were measured 2–3 h after capture. The results are reported as means, with ranges and numbers of animals used in parentheses.

	Fuel or metabolite concentration in blood or plasma (μmol/ml)				
	Acetoacetate	3-Hydroxy-butyrate	Glycerol	Non-esterified fatty acids	Triacylglycerol
Teleosts					
Bass (*Dicentrarcus labrax*)	0.04 (0.028–0.066) (8)	<0.001 (8)	0.02 (0.001–0.048) (8)	1.10 (0.447–1.53) (8)	4.56 (2.32–5.78) (8)
Red mullet (*Mullus surmuletus*)	0.01 (0.007–0.014) (4)	<0.001 (3)	0.15 (0.091–0.228) (4)	1.42 (1.31–1.76) (4)	1.84 (1.35–2.22) (3)
Mackerel (*Scombrus scombrus*)	0.05 (0.040–0.062) (4)	<0.001 (3)	0.01 (0.001–0.020) (4)	1.22 (0.085–1.43) (3)	1.24 (1.05–1.47) (3)
Elasmobranchs					
Dogfish (*Scylliorhinus canicula*)	0.07 (0.045–0.109) (8)	0.06 (0.013–0.140) (8)	<0.001 (8)	0.15 (0.107–0.241) (8)	0.85 (0.635–1.028) (8)
Spur dog (*Squalus acanthias*)	0.13 (0.078–0.156) (3)	0.2 (0.173–0.287) (4)	<0.001 (3)	0.15 (0.130–0.183) (3)	0.39 (0.360–0.420) (3)

about 36%. Glycerol could not be detected in the blood of either the fed or starved dogfish. In the bass, starvation increased the concentration of glycerol 3–7-fold. Thus, contrary to that of fatty acids, the concentration of glycerol remained elevated at 150 days starvation.

The blood concentration of non-esterified fatty acids in the bass after 40 and 100 days starvation is similar to that in the rat after 2 days and in the human after 6 days starvation (for review, see Newsholme, 1976). The increase in the blood concentration of glycerol in the bass suggests that the increased non-esterified fatty acids concentration represents increased mobilization from the distinct adipose-tissue stores of triacylglycerols. The decrease in weight of adipose tissue (see Table 4) and the decreased total lipid content of muscle during starvation (from 2.51 ± 0.23 to 2.07 ± 0.19 mg/g of muscle for eight animals in each group) is consistent with this suggestion. Despite this increase in non-esterified fatty acids, there is no statistically significant increase in the concentration of acetoacetate in the bass during starvation, and the total ketone-body concentration remains very low (0.05 μmol/ml; see Table 3). However, in the dogfish the non-esterified fatty acids concentration is decreased, whereas that of ketone bodies is increased at each time interval investigated: at 150 days the ketone-body concentration is increased by 28-fold to reach a value of 2.28 mM, which is similar to that observed in the rat after 96 h starvation (see Hawkins *et al.*, 1971). Furthermore, as in the mammals, the largest concentration increase occurs in 3-hydroxybutyrate (0.01 to 1.86 mM), so that the hydroxybutyrate/acetoacetate concentration ratio increases markedly. These findings suggest that, in the dogfish, hepatic triacylglycerol stores are not mobilized as non-esterified fatty acids but as ketone bodies. This is supported by the absence of detectable glycerol from the blood, since it is probable that the glycerol that is produced from hepatic lipolysis will be phosphorylated within the liver. The reason for the dependence of the dogfish (and presumably all elasmobranchs; see Tables 1, 2 and 3) on ketone bodies as the major fat fuel during starvation is not known. It is possible that, for some unknown reason, non-esterified fatty acids cannot be released from the liver. Since non-esterified fatty acids are not very soluble in aqueous medium, physiologically meaningful quantities of non-esterified fatty acids can only be transported if a transport protein such as albumin is present in the blood. Albumin is not present in the blood of the elasmobranchs, but it is present in that of most teleosts, including the bass (Lauter *et al.*, 1968; Sulya *et al.*, 1961). Once a transport protein for non-esterified fatty acids is present in the blood, triacylglycerols can be mobilized as non-esterified fatty acids and the dependence on ketone bodies is removed. Furthermore, triacylglycerols can now be stored in a tissue specific for this storage rather than

Table 3. *Concentrations of glucose, acetoacetate, hydroxybutyrate, glycerol, lactate, non-esterified fatty acids and triacylglycerol in blood or plasma of fed and unfed bass and dogfish*

Methods of determination and details of capture and feeding are given in the Materials and Methods section. Both starved and fed fish were given food for 14 days after capture. Concentrations are given as means ± S.E.M. in μmol/ml of blood, except for non-esterified fatty acids and triacylglycerols which are μmol/ml of plasma. Numbers of animals used for each determination was eight. Statistical significance (Student's *t* test) of the difference in concentration between fed and starved is indicated by †($P<0.025$), *($P<0.01$) and **($P<0.001$).

		Fuel or metabolite concentration in blood or plasma (μmol/ml)													
		Glucose		Acetoacetate		3-Hydroxybutyrate		Glycerol		Lactate		Non-esterified fatty acids		Triacylglycerol	
Animal	Time after capture	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved
Dogfish	2h	1.42 ±0.54	—	0.07 ±0.034	—	0.06 ±0.040	—	<0.001	—	4.22 ±3.55	—	0.15 ±0.042	—	—	—
	40 days	0.49 ±0.123	0.55 ±0.167	0.04 ±0.029	0.29 ±0.084*	0.03 ±0.018	1.61 ±0.600*	<0.001	<0.001	0.26 ±0.345	0.31 ±0.285	0.13 ±0.039	0.09 ±0.013	—	—
	100 days	0.62 ±0.183	0.41 ±0.173	0.11 ±0.044	0.38 ±0.342	0.09 ±0.009	1.84 ±0.070**	<0.001	<0.001	0.85 ±0.390	0.22 ±0.088	0.15 ±0.035	0.14 ±0.042	0.85 ±0.152	0.68 ±0.135
	150 days	0.50 ±0.139	0.37 ±0.107	0.07 ±0.017	0.42 ±0.104*	0.01 ±0.010	1.86 ±0.710†	<0.001	<0.001	0.31 ±0.297	0.29 ±0.144	0.13 ±0.022	0.09 ±0.016	0.70 ±0.216	0.22 ±0.123
Bass	2h	7.39 ±1.69	—	0.04 ±0.015	—	<0.001	—	0.022 ±0.020	—	4.27 ±2.11	—	1.10 ±0.095	—	—	—
	40 days	2.61 ±0.696	2.63 ±0.280	0.04 ±0.017	0.04 ±0.017	<0.001	<0.001	0.014 ±0.011	0.066 ±0.033	1.38 ±0.48	1.57 ±0.850	0.90 ±0.080	1.5 ±0.292†	6.04 ±2.25	3.03 ±0.78†
	100 days	3.20 ±0.491	1.91 ±0.297†	0.02 ±0.004	0.05 ±0.031	<0.001	<0.001	0.002 ±0.002	0.073 ±0.015**	1.99 ±0.49	2.14 ±0.182	—	1.22 ±0.235	4.56 ±0.64	1.29 ±0.51*
	150 days	2.95 ±0.426	1.06 ±0.028**	0.02 ±0.004	0.04 ±0.022	<0.001	<0.001	0.007 ±0.004	0.043 ±0.029	0.29 ±0.034	1.91 ±0.460*	0.76 ±0.25	0.44 ±0.16	3.3 ±1.09	0.71 ±0.49†

Table 4. *Total body, liver and adipose-tissue weight in fed and starved bass and dogfish*
Results are presented as means ± S.E.M. Number of animals used for each determination was eight. Both starved and fed fish were given food for 14 days after capture.

		Weight (g)					
	Time after capture	Total body		Liver		Adipose tissue	
Animal	(days)	Fed	Starved	Fed	Starved	Fed	Starved
Bass	40	339 ± 146	462 ± 133	6.97 ± 2.82	4.18 ± 1.93	3.72 ± 2.03	5.68 ± 4.62
	100	462 ± 205	362 ± 60	9.20 ± 3.24	2.83 ± 0.94	6.60 ± 3.54	0.43 ± 0.38
	150	533 ± 193	310 ± 129	7.9 ± 3.42	1.61 ± 0.23	12.01 ± 4.44	< 0.01
Dogfish	40	821 ± 287	811 ± 143	54.8 ± 9.8	57.2 ± 18.3	—	—
	100	767 ± 86	642 ± 92	70.0 ± 15.0	36.1 ± 15.0	—	—
	150	735 ± 167	536 ± 111	51.7 ± 13.3	30.7 ± 20.7	—	—

in the liver. Thus the teleosts may represent an important evolutionary position in metabolism in that they are the first group of animals that have developed a specific storage tissue for triacylglycerols and the ability to mobilize and transport non-esterified fatty acids.

The apparent reluctance to convert non-esterified fatty acids into ketone bodies during starvation in the teleost is in marked contrast with higher animals. This poses interesting questions as to why both non-esterified fatty acids and ketone bodies need to be utilized in higher animals during starvation and at what stage in the evolutionary development both non-esterified fatty acids and ketone bodies are available in the blood during starvation. The increasing size of the brain in relation to body size and the need to restrict glucose formation from amino acids in order to conserve body protein may be a possible answer, since ketone bodies, but not non-esterified fatty acids, are utilized in preference to glucose by the brain of higher animals (for reviews see Newsholme & Start, 1973; Newsholme, 1976). Nonetheless, it should be emphasized that the enzymes for the formation of ketone bodies (i.e. HMG-CoA-cycle enzymes) are present in the liver of the teleost fish (Beis, 1978) and that ketone-body-utilization enzymes are present in muscle and brain of such fish (Table 1) (Beis, 1978; Sugden & Newsholme, 1973). It is not known under what conditions ketone bodies are produced and utilized in the teleost fish.

Effect of starvation on enzyme activities

Enzymes for ketone-body metabolism are present in the tissues of both types of fish (Beis, 1978; Sugden & Newsholme, 1973). In view of the emphasis on ketone bodies as a fuel in the dogfish during starvation, it was considered important to study the effects of starvation on the activities of these enzymes in the two fish. There were no marked changes in the activities of the ketone-body-utilization enzymes in the heart or red skeletal muscles of either fish (results not shown). However, the patterns of changes were different in the livers of the two types of fish. In the dogfish, starvation produced no changes in the activities of 3-oxo acid CoA-transferase or HMG-CoA synthase, but it increased those of acetoacetyl-CoA thiolase and hydroxybutyrate dehydrogenase (2–3-fold). In the bass, starvation caused no change in the activity of the HMG-CoA synthase, it increased that of the 3-oxo acid CoA-transferase (by about 2-fold) and decreased that of acetoacetyl-CoA thiolase (see Table 5). The direction of these changes may be important. The fact that the thiolase activity increases in the dogfish liver, when the rate of ketone-body production is probably increased, but the activity decreases in the liver of the bass, in which ketone-body production appears unimportant, suggests that thiolase plays a role in the regulation of the rate of ketogenesis. The presence of 3-oxo acid CoA-transferase activity in the livers of some fish has been previously reported by Phillips & Hird (1977), who suggested that it catalyses the conversion of acetoacetyl-CoA into acetoacetate for the formation of ketone bodies. The present results do not support this view, since the activity of 3-oxo acid CoA-transferase increases in the liver of the bass when there is no increase in blood acetoacetate, but it is unchanged in the dogfish, in which the ketone-body concentration increases dramatically.

In both the dogfish and the bass, starvation did not change PEPCK activity in heart or red skeletal muscle (results not shown). However, although 40 days starvation had no effect on PEPCK activity in white skeletal muscle, it was markedly decreased at both 100 and 150 days starvation (0.18 ± 0.075 and 0.15 ± 0.063 compared with 0.05 ± 0.035 and 0.03 ± 0.029 μmol/min per g fresh wt. for 100 and 150 days of feeding and starvation respectively for eight dogfish in each group, and 0.11 ± 0.029 and 0.07 ±

Table 5. *Activities of 3-oxo acid CoA-transferase, acetoacetyl-CoA thiolase, HMG-CoA synthase, 3-hydroxybutyrate dehydrogenase and phosphoenolpyruvate carboxykinase in livers of dogfish and bass during prolonged starvation*

Enzyme activities were measured as described in the Materials and Methods section and are presented as means ± S.E.M. The number of animals used for each result was eight. Both starved and fed fish were given food for 14 days after capture. The statistical significance (Student's *t* test) of the difference between fed and starved is indicated by *($P<0.05$), **($P<0.01$) and ***($P<0.001$).

Animal	Time after capture	Enzyme activities																	
		3-Oxo acid CoA-transferase				Acetoacetyl-CoA thiolase				HMG-CoA synthase				3-Hydroxybutyrate dehydrogenase				Phosphoenolpyruvate carboxykinase			
		(μmol/min per g)		(nmol/min per mg of protein)		(μmol/min per g)		(nmol/min per mg of protein)		(μmol/min per g)		(nmol/min per mg of protein)		(μmol/min per g)		(nmol/min per mg of protein)		(μmol/min per g)		(nmol/min per mg of protein)	
		Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved
Dogfish	40 days	1.25 ± 0.15	1.05 ± 0.45	63.5 ± 18.0	59.2 ± 22.1	4.43 ± 1.65	7.03* ± 1.99	74.50 ± 22.16	142.96** ± 41.48	0.40 ± 0.06	0.48 ± 0.18	8.24 ± 2.04	9.70 ± 2.42	0.30 ± 0.12	0.59** ± 0.18	6.71 ± 1.83	17.46*** ± 5.49	0.05 ± 0.02	0.074 ± 0.031	1.43 ± 0.94	1.71 ± 0.33
	100 days	1.91 ± 0.51	1.55 ± 0.82	86.0 ± 34.0	69.5 ± 13.5	—	—	—	—	0.40 ± 0.17	0.42 ± 0.10	8.62 ± 1.68	9.04 ± 3.10	0.30 ± 0.15	0.45 ± 0.19	6.35 ± 2.05	13.21*** ± 4.2	0.09 ± 0.05	0.058 ± 0.031	1.94 ± 0.63	1.37 ± 0.64
	150 days	1.85 ± 0.25	2.95 ± 1.35	83.2 ± 11.2	80.0 ± 11.5	4.39 ± 1.24	7.77* ± 3.36	101.2 ± 31.2	199.31* ± 91.33	0.41 ± 0.12	0.29 ± 0.13	9.23 ± 2.61	8.80 ± 2.99	0.37 ± 0.13	1.07*** ± 0.62	7.8 ± 2.8	23.20*** ± 5.85	0.07 ± 0.03	0.058 ± 0.040	1.75 ± 0.43	1.58 ± 0.84
Bass	40 days	12.5 ± 5.9	28.8* ± 9.8	136.2 ± 54.1	220.0 ± 113.8	3.53 ± 0.97	3.97 ± 0.75	34.25 ± 2.99	24.36** ± 3.52	0.18 ± 0.12	0.22 ± 0.09	2.28 ± 0.97	1.94 ± 0.63	—	—	—	—	0.56 ± 0.25	1.74*** ± 0.44	5.37 ± 2.19	10.86*** ± 2.26
	100 days	11.7 ± 3.2	17.8* ± 4.1	128.7 ± 63.1	196.4 ± 49.2	4.55 ± 0.75	—	58.32 ± 18.93	—	0.15 ± 0.04	0.23 ± 0.20	1.49 ± 0.23	1.63 ± 0.21	—	—	—	—	0.44 ± 0.19	1.63*** ± 0.76	5.50 ± 2.62	11.09** ± 4.87
	150 days	12.1 ± 3.4	26.5** ± 8.5	176.6 ± 48.9	263.3 ± 87.3	4.01 ± 0.63	1.85*** ± 0.80	43.2 ± 13.5	15.43*** ± 5.03	0.17 ± 0.04	0.20 ± 0.03	1.60 ± 0.48	1.22 ± 0.39	—	—	—	—	0.47 ± 0.16	1.24*** ± 0.25	5.60 ± 1.81	12.22*** ± 1.87

0.038 compared with 0.07±0.024 and 0.03±0.008 μmol/min per g fresh wt. for 100 and 150 days of feeding and starvation respectively for eight bass in each group). This suggests that the rate of alanine production by white muscle will be decreased in both species of fish during prolonged starvation and may represent part of a mechanism for conservation of body protein (see Newsholme & Williams, 1978). In contrast with this, the activity of PEPCK is increased in the liver of the bass, whereas it is unchanged in that of the dogfish (Table 5). This suggests that gluconeogenesis may be more important during starvation in the teleost than in the dogfish.

We thank Professor R. R. Porter, F.R.S., for his interest and encouragement and Professor E. J. Denton, F.R.S., for use of facilities at the Laboratory of Marine Biological Association, Plymouth. This work was supported by the Science Research Council (grant B/RG/72271).

References

Alp, P. R., Newsholme, E. A. & Zammit, V. A. (1976) *Biochem. J.* **154**, 689–700

Baillie, L. A. (1960) *Int. J. Appl. Radiat. Isotop.* **8**, 1–7

Beis, A. (1978) D. Phil. Thesis, University of Oxford

Bergmeyer, H.-U., Bernt, E., Schmidt, F. & Stork, H. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), pp. 1196–1201, Academic Press, London and New York

Bieber, L. L., Abraham, T. & Helmarth, T. (1972) *Anal. Biochem.* **50**, 509–518

Bilinski, E. & Lau, Y. C. (1969) *J. Fish. Res. Board Can.* **26**, 1857–1866

Borgström, B. (1952) *Acta Physiol. Scand.* **25**, 111–119

Carter, J. R. (1967) *Biochim. Biophys. Acta* **137**, 147–156

Chavin, W. & Young, J. E. (1970) *Comp. Biochem. Physiol.* **33**, 629–653

Clinkenbeard, K. D., Reed, W. D., Mooney, R. A. & Lane, M. D. (1975) *J. Biol. Chem.* **250**, 3108–3116

Crabtree, B. & Newsholme, E. A. (1972) *Biochem. J.* **130**, 675–705

Dole, V. P. (1958) *J. Clin. Invest.* **35**, 150–155

Driedzik, W. R. & Kicenicek, J. W. (1976) *J. Fish. Res. Board Can.* **33**, 173–176

Duncombe, W. G. (1963) *Biochem. J.* **88**, 7–10

Garland, P. B. & Randle, P. J. (1962) *Nature (London)* **196**, 987–988

Gutman, I. & Wahlefeld, A. W. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), pp. 1464–1468, Academic Press, London and New York

Hawkins, R. A., Williamson, D. H. & Krebs, H. A. (1971) *Biochem. J.* **122**, 13–18

Lauter, C. J., Brandenburger, B. & Trams, E. G. (1968) *Comp. Biochem. Physiol.* **24**, 243–247

Love, R. M. (1970) *The Chemical Biology of Fishes*, Academic Press, London and New York

Newsholme, E. A. (1976) *Clin. Endocrinol. Metab.* **5**, 543–578

Newsholme, E. A. & Start, C. (1973) *Regulation in Metabolism*, Wiley Interscience, London and New York

Newsholme, E. A. & Taylor, K. (1968) *Biochim. Biophys. Acta* **158**, 11–24
Newsholme, E. A. & Williams, T. (1978) *Biochem. J.* **176**, 623–626
Patent, C. J. (1970) *Gen. Comp. Endocrinol.* **14**, 215–242
Phillips, J. W. & Hird, F. J. R. (1977) *Comp. Biochem. Physiol. B* **57**, 133–138
Sugden, P. H. & Newsholme, E. A. (1973) *Biochem. J.* **134**, 97–101
Sulya, L. L., Box, B. E. & Gunter, G. (1961) *Am. J. Physiol.* **200**, 152–154
Thorpe, A. & Ince, B. N. (1974) *Gen. Comp. Endocrinol.* **23**, 29–44
Tietz, A. (1967) *Eur. J. Biochem.* **2**, 236–242
Umminger, B. L., Benzinger, D. & Levy, S. (1975) *Comp. Biochem. Physiol. C* **51**, 111–115
Wahlefeld, A. W. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), pp. 1831–1835, Academic Press, London and New York
Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962) *Biochem. J.* **82**, 90–96
Zammit, V. A. & Newsholme, E. A. (1976) *Biochem. J.* **160**, 447–462
Zammit, V. A., Beis, A. & Newsholme, E. A. (1979) *FEBS Lett.* **103**, 212–215