Glucose is utilized in some muscle cells from the lactate dehydrogenase reaction. This process is known as the Cori cycle. In most teleosts thus far studied, the Cori cycle is simply insufficient to account for much lactate clearance; the capacity of the liver gluconeogenic arm seems to take place in situ from the lactate formed in the preceding exercise period (Weber et al. 1986; Milligan and McDonald 1988; Schulte et al. 1992; Moyes et al. 1979), we considered the possibility that in this species liver glucose; liver glucose back to muscle glycogen. This intertissue cycling of carbon (glycogen-derived muscle lactate to liver glucose; liver glucose back to muscle glycogen) is termed the Cori cycle. In most teleosts thus far studied, the Cori cycle is not considered to contribute significantly to postexercise lactate clearance; the capacity of the liver gluconeogenic arm of the Cori cycle is simply insufficient to account for much lactate clearance. Instead, most (and maybe all) muscle glycogen synthesis seems to take place in situ from the lactate formed in the preceding exercise period (Weber et al. 1986; Milligan and McDonald 1988; Schulte et al. 1992; Moyes et al. 1979). Since tuna may maintain high body temperatures and have higher than usual enzymatic activities (see Guppy et al. 1979), we considered the possibility that in this species the Cori cycle could be more important in lactate clearance than in other teleosts. For this reason, we attempted to evaluate liver gluconeogenic capacities directly by studying isolated tuna hepatocytes. Specifically, our goal was to determine the relative capacities of gluconeogenesis from the amino acid alanine and from alanine to glycogen in liver hepatocytes isolated from the skipjack tuna (Katsuwonus pelamis). Can. J. Zool. 70 : 1254–1257.


Des hépatocytes de Thon à ventre rayé (Katsuwonus pelamis) ont été isolés et identifiés comme viables ou non viables par examen de l'exécution de bleu trypan, de la charge énergétique et de la tue de lactate déshydrogénasée. La coloration au bleu trypan et la contamination d'erythrocytes était toujours inférieure à 0,1% et la tue de lactate déshydrogénasée est demeurée inférieure à 1% à 25°C pendant 10 h. La charge énergétique dans des cellules fraîchement isolées se situait entre 0,65 et 0,74. L'alanine (10 mM) a produit des taux plus élevés de gluconeogénesie et d'oxydation que le lactate, soit 3,17 ± 0,65 μmol glucose/g h) h) et 9,1 ± 1,10 μmol CO₂/g h), alors que ces taux étaient de 0,47 ± 0,08 et de 1,8 ± 0,27 en présence de lactate. La phosphoenolpyruvate carboxykinase s'est avérée à 30% d'origine cytosolique et à 70% mitochondrial, a finding that is in agreement with the liver's ability to use both lactate and alanine as gluconeogenic substrates. If hepatic gluconeogenesis is the major route of lactate removal postexercise, it is estimated that recovery would require 3800 h! Therefore, tuna liver is not the major site of glucose resynthesis and lactate clearance following burst exercise.


Tuna are able to swim at 3–5 body lengths/s for prolonged time periods, and for short (1 min) bursts they can achieve speeds of up to 20 body lengths/s (Waters and Fierstine 1964). Burst swimming involves a powerful glycolytic activation leading to lactate accumulation in white muscle of nearly 100 μmol/g (Guppy et al. 1979). More recently, Arthur et al. (1992) measured even higher lactate concentrations in tuna white muscle. Under these conditions, blood lactate concentrations rise to values approaching 50 mM (Guppy et al. 1979; Weber et al. 1986; Arthur et al. 1992). What is the fate of this large lactate load?

The classical picture of postexercise lactate clearance emphasizes two main fates: either (i) the lactate is oxidized at various sites in the body or (ii) it is reconverted to glucose and glycogen via liver gluconeogenesis. Glucose formed this way serves ultimately as the precursor for muscle glycogen. This intersite cycling of carbon (glycogen-derived muscle lactate to liver glucose; liver glucose back to muscle glycogen) is termed the Cori cycle. In most teleosts thus far studied, the Cori cycle is not considered to contribute significantly to postexercise lactate clearance; the capacity of the liver gluconeogenic arm of the Cori cycle is simply insufficient to account for much lactate clearance. Instead, mostly (and maybe all) muscle glycogen synthesis seems to take place in situ from the lactate formed in the preceding exercise period (Weber et al. 1986; Milligan and McDonald 1988; Schulte et al. 1992; Moyes et al. 1992). Since tuna may maintain high body temperatures and have higher than usual enzymatic activities (see Guppy et al. 1979), we considered the possibility that in this species the Cori cycle could be more important in lactate clearance than in other teleosts. For this reason, we attempted to evaluate liver gluconeogenic capacities directly by studying isolated tuna hepatocytes.
lactate, based on [14C]substrate flux and the intracellular distribution of phosphoenolpyruvate carboxykinase (PEPCK).

Materials and methods

Experimental animals
Skipjack tuna, *Katsuwonus pelamis*, were caught by hook and line by local fishermen and transported to the Kewalo Research Facility (National Marine Fisheries Service, Honolulu Laboratory). The fish were quickly transferred from the well to large, well-aerated circulating saltwater holding tanks at 25°C. Fish were maintained for up to 4 days without feeding under the above conditions and were generally used on the 2nd and 3rd days.

Hepatocytes isolation
Fish weighing between 1.5 and 3 kg were rapidly netted and killed with a sharp blow to the head. An incision was made ventrally from the anus to the pectoral girdle to expose the liver. The liver was removed and the hepatic portal vein quickly cannulated. The liver was perfused at 4 mL/min for 10 min with solution A, consisting of 196 mM NaCl, 4 mM KCl, 0.9 mM NaHPO₄, 10 mM sodium pyruvate (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), and 10 mM NaHCO₃ at pH 7.2-7.5. It was then perfused for another 10 min with solution B (solution A with 129 U/mL collagenase, Sigma Lot 39F6804). The composition of the perfusion media was determined from the blood osmolite profiles of Bourke et al. (1987). All solutions were prebuffered with 99% O₂ and 1% CO₂. Following perfusion the digested liver was placed in ice-cold solution A and gently teased apart with two razor blades. The resultant cell suspension was not rigorously determined but was estimated to be greater than 50%. Lactate dehydrogenase (LDH) leakage was measured by centrifuging a 1 mL aliquot of cells at 10,000 × g for 10 min in a refrigerated bench-top Jouan (MR 14.11) centrifuge. The supernatant was centrifuged in a refrigerated bench-top Jouan (MR 14.11) centrifuge for 10 min at 300 × g to remove precipitated KCIO₃, a volume was taken for ATP, ADP, and AMP measurement by an LKB 2152 high-performance liquid chromatograph fitted with a 7-µm Aquapore AX-300 weak ion exchange column (Brownlee Labs), using a procedure described by Schulte et al. (1992).

Gluconeogenic rate determination
The rate of gluconeogenesis from radiolabelled precursors by isolated hepatocytes has been previously described (French et al. 1981), and the experiments described here were conducted in the same fashion. The incubations were carried out at 25°C with 10 mM substrate and 0.2 µCi (1 Ci = 37 GBq) of [14C]substrate for intervals of up to 3 h. Radiolabelled samples were counted in a Packard Tri-Carb 4640 liquid scintillation counter.

Total enzyme activity and compartmentation
Two 0.5-g liver samples were obtained in less than a minute after capture, wiped dry with a paper towel, and weighed. The known weight of tissue was quickly immersed in 5 vols. ice-cold homogenization medium (25 mM Tris HCl, 2 mM EDTA, and 0.5% Triton X-100, pH 7.5, solution D) and homogenized with an Ultra-Turrax homogenizer by being ground 2 times for 10 s each and then sonicated 2 times for 10 s each. Homogenates were pipetted into 1.5-mL centrifuge tubes and centrifuged for 10 min at 15,000 × g. A portion of the supernatant was taken for assay immediately, while another was frozen in liquid N₂ and stored on dry ice. The second sample was placed in 5 vol. ice-cold 250 mM sucrose, 2 mM EDTA, and 20 mM Tris HCl buffer at pH 7.2. The tissue was minced with the tip of a fine pair of scissors and homogenized for 10-15 s with an Ultra-Turrax homogenizer at a medium setting. The homogenate was centrifuged in a refrigerated bench-top Jouan (MR 14.11) centrifuge for 10 min at 300 × g to remove large cellular debris (discarded). The supernatant was then centrifuged for another 10 min at 8000 × g to bring down the mitochondria. The supernatant from the last centrifugation was decanted and assayed immediately for PEPCK, citrate synthase (CS), LDH, malate dehydrogenase (MDH), and glutamate dehydrogenase (GPT) activities. The pellet was resuspended in homogenizer buffer (solution D), sonicated 2 times for 10 s each, centrifuged, and assayed for the same enzyme activities. The relative amounts of CS found in the cytosol and LDH found in the matrix could be used to correct for contamination of either compartment.

Enzymes were assayed using a Perkin–Elmer Lambda 2 spectrophotometer and data collected via an IBM computer loaded with a Perkin–Elmer computerized spectroscopy software program (PECSS version 3.2). All assays were tested for saturating substrate concentrations and coupling enzyme activities. Coupling enzymes added as precipitates in ammonium sulphate were centrifuged for 1 min at 15,000 × g and resuspended in assay buffer. The following enzymes were assayed in 50 mM imidazole, pH 7.5, at 25°C. LDH: 1 mM pyruvate (omitted for control), 0.2 mM NADH. MDH: 10 mM oxaloacetate (omitted for control), 0.15 mM NADH. GPT: 200 mM alanine (omitted for control), 10 mM α-ketoglutarate, 0.025 mM pyridoxal 5-phosphate, 0.15 mM NADH, excess LDH. PEPCK was assayed in 50 mM Tris HCl, 20 mM NaHCO₃, and 1 mM MgCl₂, (from 50 mM stock solution), pH 7.5, at 25°C (Persescu et al. 1979), with the addition of the following: 0.5 mM phosphoenolpyruvate, 0.2 mM deoxyglucose 5'-diphosphate (omitted for control), 0.1 mM NADH, and excess MDH. CS was assayed in 50 mM Tris HCl, pH 8.0, at 25°C, with the addition of the following: 0.5 mM acetyl-CoA, 0.5 mM oxaloacetate (omitted for control), and 0.1 mM dithio-bis (nitroreba- zoic acid).

Chemicals, enzymes, and radioisotopes
Uniformly labelled [14C]lactate and alanine were obtained from ICN Biomedicals, Inc. (Irvine, Calif.). Radioisotopes were dried under nitrogen and dissolved in Solution C (without BSA) and used immediately. Amyloglucosidase and acetyl-CoA were obtained from Boehringer Mannheim, Canada, Inc. (LaVal). All other chemicals and enzymes were purchased from Sigma Chemical Co. (St. Louis).

Results
In a group of animals separate from those for hepatocyte isolation the proportion of liver to total body weight was determined to be 1.8% (n = 4). RBC contamination and trypan blue staining were less than 0.1% in all experimental preparations. The supernatant of freshly isolated cells always had less than 0.5% of the LDH activity (n = 5) and after a 10-h incubation at 25°C, the LDH leakage was less than 1% (n = 2). The EC of freshly isolated cells used in gluconeogenic experiments ranged from 0.65 to 0.75. A comparison of the rates of gluconeogenesis from alanine.
and lactate over a 3-h time course is shown in Fig. 1. For both substrates the rate of gluconeogenesis became linear after 1 h of incubation. The rate of gluconeogenesis from alanine was approximately 7 times greater than from lactate: 3.17 ± 0.65 and 0.47 ± 0.08 mmol glucose/g wet weight packed cells·h, respectively. A similar relationship held for the oxidation of these substrates; however, the rate was linear throughout the 3-h time course, and alanine was oxidized at a rate 5 times that of lactate: 9.1 ± 1.2 and 1.8 ± 0.27 mmol CO₂/g wet weight packed cells·h, respectively (n = 5 in both cases, data not shown). The oxidative rates obtained from uniformly labelled substrates can only be qualitatively interpreted due to possible randomization of label.

The intracellular localization of PEPCK was estimated and is shown in Table 1. The activities of the two marker enzymes, CS and LDH, in the two compartments demonstrate a clean separation of matrix from cytosol, with the cytosol being contaminated by only 6% CS activity and the matrix space by only 11% of the total LDH activity. PEPCK was found in both compartments, approximately 70% mitochondrial and 30% cytosolic (corrected for contamination from the respective compartments), or 5.7 and 3 units/g of tissue (Table 1), respectively. This represents 190 times more activity in the cytosol and 360 times more activity in the intramitochondrial space than required by the measured gluconeogenic rates. LDH, the first enzyme in gluconeogenesis from lactate, has a total activity of 26 units/g wet weight tissue. MDH and GPT, two necessary enzymes in the gluconeogenic pathway from alanine, have total activities of 992 and 75 units/g wet weight, respectively. Most of the MDH activity is located in the cytosol (87%), whereas GPT was determined to be 94% cytosolic.

**Discussion**

On the basis of the viability criterion employed, the hepatocyte preparation could be considered a healthy one. LDH leakage occurred at a very low rate, RBC contamination and trypan blue staining were minimal, and a high EC of 0.65–0.74 was observed. Atkinson (1977) points out that an EC less than this results in cell death. The cells were also shown to be gluconeogenic. This is a complex two-compartment pathway and clearly demonstrates the metabolic competence of the hepatocytes. Moreover, the oxidation of alanine and lactate emphasizes the competence of the mitochondrial compartment. Taken together, these data suggest that the hepatocyte preparations were metabolically competent.

In Fig. 1 the rate of gluconeogenesis is shown to become linear after 1 h of incubation. The highest rates of gluconeogenesis and oxidation were obtained from alanine. Interestingly, the rates are similar to those found in trout, but the fuel preference is reversed. That is, the gluconeogenic rate from 10 mM lactate in the trout is approximately 4.2 mmol glucose/(g·h) (corrected for temperature, French et al. 1981) whereas in the tuna it is 0.47 mmol glucose/(g·h). In contrast, 10 mM alanine supports a rate of 3.17 mmol/(g·h) in the tuna and only 0.24 mmol/(g·h) in the trout. All of these gluconeogenic values are very much lower than those measured in the rat, in which the rates from alanine and lactate have been measured to be 64 and 107 mmol glucose/(g·h) at 25°C, respectively (Elliot et al. 1976). Interestingly, the approximately 10-fold difference in gluconeogenic rates from lactate between the trout and tuna coincides with a 10-fold difference in liver LDH, the trout having 251 U/g (Mommsen 1986). Although LDH is usually thought to be a near-equilibrium reaction in vivo, it is possible that the combined effects of pH and substrate concentrations may limit its activity in the required direction in tuna liver gluconeogenesis.

Based on the gluconeogenic rate from lactate (above) one can calculate the length of time required to clear a bolus of lactate generated by the white muscle mass of a tuna. In a 2-kg tuna the white muscle mass is estimated to be 1.3 kg (approximately two-thirds of body weight, Graham and Diener 1978) and the liver comprises 1.8% of the body weight, or 0.036 kg. Lactate concentrations in white muscle have been measured to be as high as 100 mmol/g (Guppy et al. 1979) and would therefore generate 130 mmol lactate. At the measured maximum gluconeogenic rate the liver would require 3800 h to clear the lactate produced by the white muscle! Tuna white muscle has been shown to reduce lactate concentrations to preexercise glycogen repletion and lactate clearance in tuna as in other teleosts.

The intracellular distribution of PEPCK (Table 1) is consistent with the hypothesis that alanine is a good gluconeogenic
substrate. Interestingly, gluconeogenesis from lactate is consistently greater than the alanine rate in most other teleost fishes investigated (for review see Suarez and Mommsen 1987). When the rates of gluconeogenesis measured in other teleosts are considered, the tuna liver is not unusual in its ability to deal with lactate loads, but the tuna is unusual in its ability to generate high lactate loads in white muscle. The metabolic arrangement in tuna may represent a specialization to rapidly convert dietary protein to glucose.

In summary, viable skipjack tuna hepatocytes were prepared, and greater rates of gluconeogenesis were obtained from alanine than from lactate. The distribution of PEPCK in both the cytosolic and matrix compartments is consistent with the liver’s ability to use both amino acids and lactate as gluconeogenic substrates. The maximum rate of gluconeogenesis is not sufficient to implicate the liver as a significant participant in white muscle recovery, but it does suggest that tuna liver plays a more important role in synthesizing glucose from dietary amino acids than from lactate generated in burst exercise.

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