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Glucose and lactate kinetics in the fed and
food-deprived American eel,
Anquilla rostrata
(LeSueur).

by

Ila M. E. Cornish

A thesis
presented to the University of Ottawa
in partial fulfillment of the
requirements for the degree of
Master of Science
in
Department of Biology

OTTAWA, Ontario, 1983



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ABSTRACT

Simultaneous infusion of ^3H -6-glucose and ^{14}C -U-lactate was employed to determine glucose turnover, lactate irreversible replacement rate, and rates of carbon exchange in free-swimming American eels, Anquilla rostrata, under fed, 6 month, 15 month, and 36 month food-deprived (maturing), and anaesthetized conditions.

Fed animals exhibited glucose turnover rates, averaging about $1.0 \text{ mg}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$, 200-fold higher than lactate production rates. Thirty-five per cent of the lactate produced was (re)converted to glucose, indicating an active Cori cycle in these animals. However, this represented less than 1% of the total glucose produced.

Food-deprivation for six months did not alter the pattern observed in fed animals, with the exception of increased plasma lactate irreversible replacement rate and lactate gluconeogenesis.

Following fifteen months of food-deprivation, glucose turnover decreased 15-fold, and clearance rates decreased concomitantly, although plasma concentrations were maintained. Lactate production rates were increased significantly in these animals although clearance rates were unaffected. All of these animals exhibited non-DSS conditions with respect to lactate.

Maturing animals deprived of food for 36 months maintained plasma glucose concentrations, turnover and clearance rates, lactate production and clearance similar to 6 month food-deprived immature animals, although diversion of lactate to glucose was not increased above that of fed animals.

Anaesthetized animals (fed and food-deprived) exhibited major alterations apparent in increased plasma glucose concentrations, decreased glucose turnover and clearance rates; increased plasma lactate concentrations, 500-fold and 100-fold in fed and food-deprived animals, respectively. Under both conditions, lactate appearance rates were increased 100-fold or more. In fed animals, lactate disappearance was inhibited. Lactate clearance rates were equivalent, 100-fold less than free-swimming animals. Under both conditions, lactate gluconeogenesis was completely inhibited. The necessity of free-swimming fish to assess the true metabolic pattern of these fish is indicated by these studies.

RESUME

La perfusion simultanée de ^3H -6-glucose et de ^{14}C -U-lactate fut utilisée pour déterminer le renouvellement du glucose, le taux de remplacement irréversible d'acide lactique, et le taux d'échange de carbone chez l'anguille américaine, Anquilla rostrata, nourrie et sous des conditions de jeûne pendant 6, 15 et 36 mois (adultes), et sous anesthésie.

Les animaux nourris montrèrent un taux de renouvellement de glucose, en moyenne de $1.0 \text{ mg} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$, 200 fois plus élevé que les taux de production d'acide lactique. Trente cinq pour cent de l'acide lactique produite fut reconvertie en glucose, ce qui indique la présence d'un cycle Cori actif chez les animaux. Par contre, ceci représente moins de 1% du glucose produit.

A l'exception d'une hausse du taux de remplacement irréversible d'acide lactique plasmiq ue et de la gluconeogenese lactique, le jeûne pendant six mois ne modifia pas les observations faites chez les animaux nourris.

Après 15 mois de jeûne, le renouvellement du glucose diminua de 15 fois et les taux d'écoulement diminuèrent simultanément tandis que les concentrations plasmiq ues demeurèrent les mêmes. Les taux de production d'acide lactique ont

augmenté de façon significative bien que les taux d'écoulement ne furent pas affectés. Chez tout ces animaux des concentrations plasmiqes instables d'acide lactique furent enregistrées.

Chez les adultes jeûnant pendant trente six mois les concentrations plasmiqes de glucose, les taux de renouvellement et d'écoulement, et la production et l'écoulement d'acide lactique sont restés semblables à ceux des animaux jeûnant six mois, bien que le détournement d'acide lactique vers le glucose n'augmente pas au-dessus des niveaux enregistrés chez les animaux nourris.

Les poissons anesthésiés (nourris et jeûnant) montrèrent des augmentations importantes des concentrations de glucose plasmiqes et des diminutions du taux de renouvellement et d'écoulement du glucose. Les concentrations plasmiqes d'acide lactique augmentèrent 500 fois chez les animaux nourris et 100 fois chez ceux jeûnant. Sous ces deux conditions, les taux d'apparition d'acide lactique augmentèrent cent fois ou plus. Chez les animaux nourris, la disparition d'acide lactique fut inhibée. Les taux d'écoulement d'acide lactique étaient équivalents: 100 fois moins que chez les animaux actifs. Sous les deux conditions, la gluconéogénèse fut complètement inhibée. Ces études indiquent le besoin d'utiliser des poissons actifs pour déterminer le vrai modèle métabolique de ces animaux.

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Chapter I
INTRODUCTION

The dependence of omnivorous mammals on carbohydrate as an energy source has long been recognized. It is not yet clear, however, to what extent this is reflected in their piscine predecessors.

Fish generally maintain low metabolic rates, high protein intakes, and little carbohydrate intake. Nonetheless, a very large proportion of fish body mass is white skeletal muscle, considered to be an anaerobic tissue which depends upon carbohydrate as an energy source. This implies a carbohydrate requirement and, in view of the low dietary intake, a necessarily active endogenous production. Studies of enzyme activities and the assessment of available stores as criteria of carbohydrate utilization, yield conflicting results and considerable interspecific variation. Tracer studies to definitively assess glucose utilization are limited (Bever et al., 1977; Lin et al., 1978) and none have assessed lactate turnover or the reincorporation of lactate carbon into glucose.

Anquilla spp. are fish of medium white muscle densities (Greer-Walker and Pull, 1975) and in vitro studies have

shown high gluconeogenic rates (Hayashi and Ooshiro, 1975a,b; Phillips and Hird, 1977; Renaud and Moon, 1980a,b). No attempts, however, have been made to examine in vivo glucose utilization or Cori cycle activity in any eel species.

The intent of this study is to quantify the extent of glucose utilization and lactate turnover in the American eel, Anguilla rostrata. Various experimental conditions including food-deprivation and anaesthetization are investigated. These studies should help assess the carbohydrate dependence of the eel.

1.1 MAMMALIA

1.1.1 Glucose Utilization

The dependence on carbohydrate as a fuel in omnivorous mammals is reflected in the constancy of blood glucose. Homeostatic mechanisms maintain concentrations consistent with energy requirements. Some tissues can obtain energy from the oxidation of free fatty acids or ketone bodies, while others maintain an absolute requirement for glucose. In particular, erythrocytes, leucocytes, brain, renal medulla, retina, intestinal mucosa, and skeletal muscle (under conditions of severe exercise) depend upon glucose as a primary source of energy (Krebs, 1972). Glucose is derived

primarily from exogenous sources under fed conditions. Glycogen storage is minimal, limited to about 5% of liver and 1% of muscle wet weights, or less than one day's caloric requirement. Without exogenous input, de novo glucose synthesis through hepatic and renal cortical gluconeogenesis is immediately increased to ensure adequate blood glucose for these glucose-dependent tissues.

1.1.2 Gluconeogenesis

Gluconeogenesis becomes important to a mammal under the conditions of fasting (or decreased carbohydrate intake, including the post-absorptive state) and recovery from muscular activity. Both conditions are represented by depletion of glycogen stores. Glycerol and amino acids represent the bulk of the gluconeogenic precursors, at least for the first 5 to 6 weeks of fasting. Thereafter, a general metabolic depression and the switch of the nervous system to ketone body oxidation (Cahill et al., 1968; Newsholme, 1976) reduces the requirement for gluconeogenesis. As fat reserves are depleted during prolonged starvation, gluconeogenesis again predominates as indicated by extensive protein depletion and increased urea excretion (Cahill et al., 1968).

During strenuous muscular activity, muscle glycogen is depleted by glycolysis. Liver glycogen is mobilized and the glucose diverted to muscle glycolysis. Increased muscle

lactate concentrations diffuse into the blood during the recovery period, to be reconverted to glucose in the liver. This hepatic-muscular, glucose-lactate cycle is known as the Cori Cycle (Cori and Cori, 1929). Increased gluconeogenesis under these conditions is primarily aimed at clearing plasma lactate and replenishing liver and muscle glycogen, but no net increase in carbohydrate carbon occurs. Issekutz et al. (1976) found that in exercising dogs, 7 to 26% of glucose released into the blood is derived from lactate carbon. This represents 25% of the total lactate carbon produced, 5% more than under resting conditions. Under exercising and resting conditions, however, most of the lactate produced in muscle was oxidized by other tissues. Estimations of the Cori Cycle contribution in fed resting rats are comparable (20-30%) (Preminet and Poyart, 1975). Issekutz et al. (1976) and Hermansen and Vaage (1977), however, have evidence for lactate reversion to glycogen within the muscle itself.

The importance of glucose as a fuel in specific mammalian tissues and its maintenance by increased endogenous production is unquestioned under food-deprived conditions and following the depletion of carbohydrate stores in muscle by exercise. The scenario in fish is not as well understood.

1.2 PISCES

Glucose utilization in fish has traditionally been considered low when compared to mammals (Love, 1970; Walton and Cowey, 1982). Predominantly carnivores, fish maintain high dietary requirements for protein, which can be two-to three-times higher than mammals (Walton and Cowey, 1982; Cowey and Sargent, 1979). Nonetheless, optimal growth requires 20% carbohydrate in the diet. The addition of both carbohydrate and lipid has been found to enhance protein utilization (Cowey and Sargent, 1972; van Waarde, 1983). Decreased dietary carbohydrate intake as compared to mammals may not necessarily reflect a lower dependence on glucose; if fish tissues depend upon glucose, the animal may require a higher dependence upon gluconeogenesis.

Another criterion upon which low glucose utilization has been based is the low metabolism of fish (Love, 1970; Walton and Cowey, 1982). As ectotherms, metabolic rates are approximately 10-fold less than mammals and birds (Bennett, 1978). Allowing body temperature to follow ambient generally reduces energy demand. An overall reduction in energy requirement suggests a proportionally decreased glucose requirement. Fish, however, have a much lower aerobic capacity than birds and mammals, relying to a larger extent on anaerobic metabolism (Bennett, 1978). Glucose is the major if not only fuel available for anaerobic energy production

(Newsholme and Start, 1973). Muscle lactate levels at rest and after electrical stimulation are higher in fish than in higher vertebrates (Phillips and Hird, 1977), supporting the idea of a higher anaerobic metabolism. Substrate availability to support this metabolic pattern is essential.

Despite a generally lower metabolic rate and carbohydrate intake, glucose does appear to represent an important fuel for fish species.

1.2.1 Glucose Utilization

Much of the work on glucose utilization in fish has been indirect. This usually involves the assay of non-equilibrium or rate-limiting enzymes of glycolysis and/or gluconeogenesis (Newsholme and Start, 1973). These pathways are shown in Figure 1.

All of the enzymes of glycolysis have been detected in various fish tissues. Tissue activities have been summarized by Walton and Cowey (1982). Relative glycolytic potentials based on enzyme activities in trout, plaice, and cod are in the order, skeletal muscle > heart; brain > kidney; gill > liver (Knox, et al., 1980). MacLeod (1963), measuring lactate formation from glucose, in tissue preparations from steelhead trout, found glycolytic rates to decrease in the order, heart > muscle > brain > liver > kidney.

The key gluconeogenic enzymes predominate in liver and to a lesser extent in kidney. FDPase is present in muscle, but here its role is not gluconeogenic as PEPCK and PC are absent and these enzymes are necessary for gluconeogenesis from pyruvate-level substrates (Knox et al., 1980; Moon and Johnston, 1980; Walton and Cowey, 1982).

Comparisons of glycolytic and gluconeogenic enzymes in plaice (Pleuronectes platessa) suggest liver metabolism is directed towards glucose production, as in mammals. The activities of the regulatory enzymes, PFK and PK are low, while those of PEPCK, FDPase, and G-6-Pase are relatively high (Moon and Johnston, 1980).

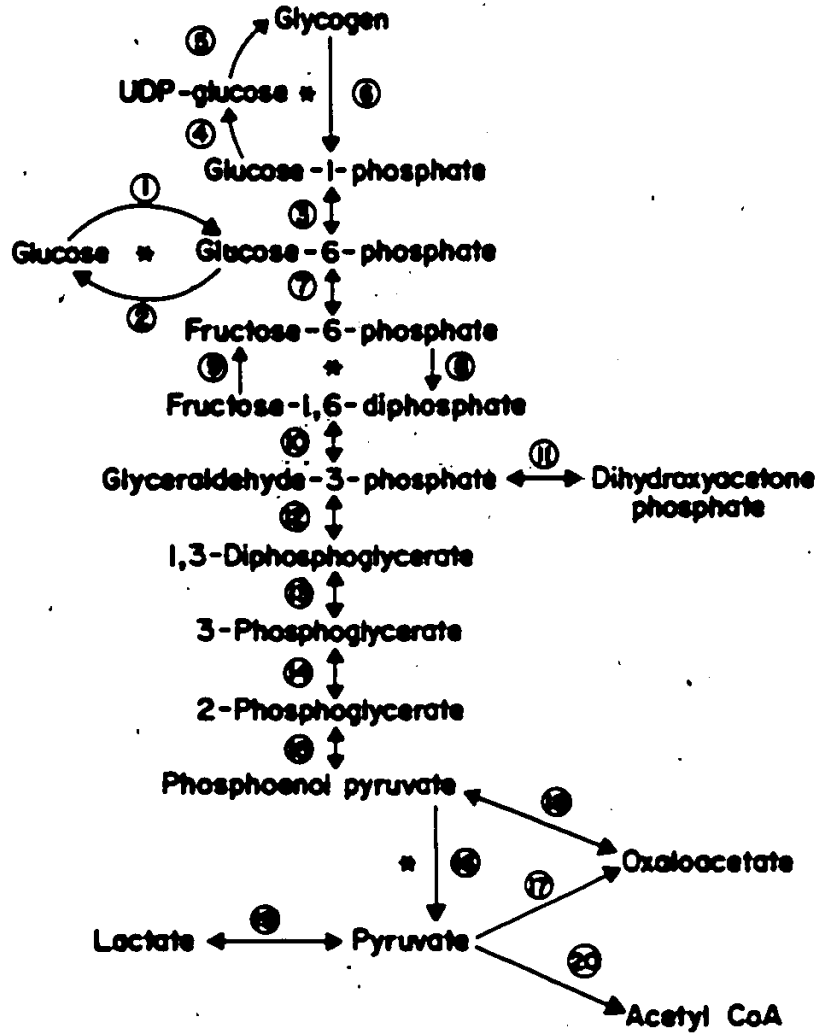
Most mammalian livers possess an inducible glucokinase (GK), a high K_m isozyme of HK. Its activity varies with plasma glucose and insulin concentrations. GK tightly controls plasma glucose concentration in most mammals (Newsholme and Start, 1973). Fish, ruminants, and birds do not possess a glucokinase (Ureta, 1982). Hexokinases, with lower glucose K_m values, are likely saturated at normal plasma glucose concentrations. No apparent enzymic control mechanisms to deal with abnormal glucose loads exist in these animals. This has again suggested to some authors further evidence of low glucose utilization in fish (Palmer and Ryman, 1974). This is not necessarily correct as glucokinase represents an adaptation to a high carbohydrate intake (Ureta, 1982). Rat liver GK activities decrease during fasting

Figure 1: Routes of glucose utilization

1. Hexokinase, HK; Glucose-6-phosphatase, G-6-Pase; 3. Phosphoglucomutase, PGM; 4. UDP-pyrophosphorylase; 5. Glycogen synthetase, GS; 6. Glycogen phosphorylase; 7. Phosphoglucose isomerase, PGI; 8. Phosphofructokinase, PFK; 9. Fructose diphosphatase, FDPase; 10. Aldolase; 11. Triose-phosphate isomerase TPI; 12. Triose phosphate dehydrogenase, TPDH; 13. 3-phosphoglycerokinase, PGK; 14. 2,3-phosphoglycerate mutase, 2,3-PGM; 15. Enolase; 16. Pyruvate kinase, PK; 17. Pyruvate carboxylase, PC; 18. Phosphoenolpyruvate carboxykinase, PEPCK; 19. Lactate dehydrogenase, LDH; 20. Pyruvate dehydrogenase. (adapted from Walton and Cowey, 1982).

'*' denotes a regulatory step.

GLUCONEOGENESIS



GLYCOLYSIS

even though plasma glucose remains normal (Newsholme and Start, 1973), suggesting GK is present to offset large changes in plasma concentrations especially following exogenous input. Fish, which are not naturally exposed to a high oral glucose intake, apparently have not evolved this type of control.

Thus, trout exposed to an oral glucose challenge exhibit a diabetic response (Palmer and Ryman, 1974). This same response is not apparent in free-swimming animals administered smaller glucose loads intra-arterially. Ince and Thorpe (1977) have shown that insulin release increased in direct proportion to increased plasma glucose concentrations from 10 to 100 mg.ml⁻¹ in Anquilla anquilla. Higher glucose loads have no further influence upon plasma insulin concentrations. The eel appears well equipped to respond to changes in plasma glucose concentrations within the range to which they are naturally exposed.

Palmer and Ryman (1974) have proposed that glucose stimulates liver glycogenesis through glycogen synthetase as it does in mammals (Hers et al., 1970). This may represent the principal enzymic control over hepatic glucose uptake in fish species.

Cowey et al. (1975) have studied glucose utilization in plaice after intraperitoneal injection of ¹⁴C-glucose. Their results showed that plaice oxidized glucose far more slowly than values reported for mice (Vrba, 1966). After 18

hours, 12 to 23% (depending upon the dietary regime) of the ^{14}C dose was recovered as CO_2 . This is compared to 30% after 1 hour and 80% after 8 hours in mice. Considering that the plaice were at 5°C and mice near 40°C , and allowing for a two-fold change in reaction rate per 10°C , one would expect a 5-fold difference in rates between mice and plaice with respect to glucose oxidation, not the observed 20-fold. Q_{10} effects, therefore, cannot account for these differences. Plaice apparently depend less upon the aerobic metabolism of glucose than mice (mammals).

In vitro studies with the genus Anquilla, suggest it may show a particularly high glucose metabolism compared with other fish. Hayashi and Ooshiro (1975a,b) reported that the rate of glucose formation from lactate in A. japonica perfused liver was similar to that of rats. Of a number of vertebrates, the eel A. australis occidentalis (after 3 to 4 months of food-deprivation), contained the most liver glycogen (higher than fed rat). In addition, these animals had the highest hepatic PEPCK activity (total and particulate) and incorporated 5-times more ^{14}C -lactate into glucose than rat in isolated perfused liver preparations, exceeded only by the lamprey (Phillips and Hird, 1977). A relatively low muscle glycogen content suggests that Cori cycle activity to refurbish glucose as a fuel for muscular contraction may be essential. Lactate, at physiological concentrations, exhibited the highest gluconeogenic rate in the A. rostrata he-

patocyte preparation, followed by alanine (Renaud and Moon, 1980b). Thus, Anquilla species could be particularly valuable to studies such as the one proposed here.

1.2.2 Food-deprivation

Starvation or fasting and the ensuing effects on enzyme activities and tissue stores has been an active research area of fish metabolism. Many fish undergo natural fasting periods, either annually or associated with a spawning migration. Many species, therefore, are highly tolerant of food-deprivation and this is not considered a stressful situation (Love, 1970). Changes after the removal of exogenous substrate may reflect the necessity of that substrate under normal feeding conditions.

Whereas mammals deplete glycogen reserves within 24 to 48 hrs after cessation of exogenous input (Newsholme and Start, 1973), many fish retain hepatic reserves over much longer durations. Carp maintain blood glucose concentrations and liver glycogen contents unchanged after 22 days of food-deprivation (Nagai and Ikeda, 1971a). Even after 100 days, appreciable liver glycogen (1.5%) remained. Similarly, the European (Larsson and Lewander, 1973), Japanese (Inui and Ohshima, 1966; Hayashi and Ooshiro, 1975a) and American (Renaud and Moon, 1980b) eels all maintain liver glycogen for extended periods (> weeks).

Chang and Idler (1960) observed a two-fold increase in hepatic glycogen of sockeye salmon (Oncorhynchus nerka) after a spawning migration that involved both starvation and considerable muscular effort. Fontaine and Hatey (1953) concluded that fish are well adapted to the regulation of carbohydrate formation from other body constituents. They reported essentially no change in blood glucose concentrations pre- and post-spawning migration in Atlantic salmon (Salmo salar), despite initially low glycogen reserves. Carbohydrate metabolism does contribute to pathways supplying energy for migration.

The basis for liver glycogen retention by fish under starvation conditions is unclear. Tissue mobilization is probably not restricted by a paucity of phosphorylase activity. The total phosphorylase activity in red, white, and cardiac muscle of rainbow trout is much greater than that of the liver; trout liver phosphorylase activities, essentially equal those of rat (Covey and Sargent, 1979). Restricted mobilization may be hormonally mediated. Both epinephrine and glucagon modify brown bullhead phosphorylase activity in vitro (Umminger and Benziger, 1975). The fact that mobilization of glycogen is limited during fish starvation, implies that the demands of any tissues requiring glucose (eg, nervous tissues) must be met by gluconeogenesis rather than glycogenolysis. In the eel hepatocyte preparation (Renaud, and Moon, 1980b), gluconeogenesis was found to be preferen-

tially stimulated over glycogenolysis. The extreme metabolic depletion associated with starvation coupled to spawning migration, suggests glucose production by gluconeogenesis may actually exceed glucose demand, promoting storage of the excess as glycogen (Covey and Sargent, 1979).

Indirect evidence suggests that hepatic gluconeogenesis is enhanced under conditions of starvation. Inui and Ohshima (1966) and Larsson and Lewander (1973) found liver glycogen content rose. Moon and Johnston (1980) found increased gluconeogenic potential in plaice (Pleuronectes platessa). Under fed conditions, plaice liver, as in mammals, is geared towards glucose production. Glycolytic enzymes (PFK and PK) have relatively low activities while gluconeogenic enzymes (PEPCK, FDPase and G-6-Pase) have high activities. High HK activities permit liver competition for plasma glucose. A four month food-deprivation did not radically alter this pattern. HK activity was reduced more than that of G-6-Pase. This decreased the HK/G-6-Pase ratio, thus promoting glucose release from the liver. Liver soluble PEPCK activities increased 8-fold with 4 months of food-deprivation again implying increased gluconeogenesis. Major increases in the activities of liver amino acid transaminases and glutamate dehydrogenase occurred concurrently with skeletal muscle protein degradation. These changes are indicative of the importance of amino acid gluconeogenesis during starvation, and have also been reported for trout

(Morata et al., 1982). Mommsen et al. (1980) reported changes in tissue enzyme and metabolite profiles consistent with a transfer of amino acids from skeletal muscle to be utilized for hepatic gluconeogenesis in migrating sockeye salmon.

Additional evidence for increased amino acid gluconeogenesis derives from studies on rainbow trout, Salmo gairdneri (Covey et al., 1977a). Plasma amino acids decreased as did alanine gluconeogenesis when trout were placed on a high carbohydrate/low protein diet. When dietary carbohydrate is decreased either through administration of a low carbohydrate/high protein diet or complete cessation of exogenous intake for 21 days, alanine gluconeogenesis was increased. This change was reflected in increased gluconeogenic (G-6-Pase, FDPase, and PEPCK) and reduced glycolytic (HK, PK, and PFK) enzyme activities. Given these studies which point to the maintenance of glucose in certain fish species during starvation, by inference, glucose must play an important metabolic role.

Gluconeogenesis is also a means to recover lactate carbon (Cori Cycle activity). In vitro preparations of liver slices or hepatocytes have been used to estimate gluconeogenic rates from various substrates in trout (Covey et al., 1977a,b; Walton and Covey, 1979a,b; French et al., 1981) and eels (Hayashi and Ooshiro, 1975; Phillips and Hird, 1977; Renaud and Moon, 1980a). Lactate has been found to contri-

bute the highest rate of gluconeogenesis, above alanine. Taking temperature into account, gluconeogenic rates from both substrates are similar to those reported for rats at 37°C (Walton and Cowey, 1982). Particularly high lactate-gluconeogenic rates in Anquilla have already been mentioned.

These data suggest, as pointed out by Renaud and Moon (1980a), that Cori Cycle activity may be of major importance to fish in view of the large muscle mass (40 to 60% of body weight; Bone, 1978) which by necessity depends upon carbohydrate under both fed and food-deprived conditions. This is not entirely clear, however, as will be discussed.

There remains a paucity of data on glucose utilization by those tissues known in mammals to be strictly dependent upon carbohydrate as a fuel. Skeletal muscle, however, has been widely studied in fish. Although no quantitative data is available on glucose utilization by this tissue, the data to date implies that this substrate is of primary importance.

1.2.3 Muscle Glucose Metabolism

The locomotory musculature accounts for 40-60% of the total body mass in most fish (Bone, 1978). Two major fibre types are present, red and white. Existing evidence on structural and biochemical properties suggest that these two fibre types play similar roles to their counterparts in mammals (Bilinski, 1974; Bone, 1978). Red muscle, representing

0 to 26% of the muscle mass (Greer-Walker and Pull, 1975), is a highly vascularized, highly oxidative tissue, depending predominantly upon lipid as a fuel though it is also known to use carbohydrate (Johnston and Goldspink, 1973; Driedzic and Hochachka, 1975, 1978; Moon and Johnston, 1980). White muscle, representing most of the muscle mass, is poorly vascularized, depending upon anaerobic glycolysis for energy production. The classic view is that red fibres dominate under slow swimming conditions and white fibres dominate at high velocities involving burst activity. Red fibres contain a higher glycogen content than the white fibres. These distinctions, in particular with respect to swimming speeds, are not abrupt. White muscle can also be used at lower swimming speeds (Bone, 1978) and in the eel, Anquilla anquilla, these fibres show electrical activity at the slowest swimming speeds (Grillner and Kashin, 1976).

The greater the energetic demands placed on muscle, the greater the glycogen depletion and lactate accumulation (Driedzic and Hochachka, 1978). The highest rate of glycogen utilization occurs during burst activity, primarily powered by the white muscle. Glycogen mobilization may be very rapid - up to 40 μ moles of glycogen-derived glucose $\cdot g^{-1} \cdot sec^{-1}$, depleting one-half of the available store in 15 seconds (Stevens and Black, 1966).

This dependence on glucose implies an equal dependence on Cori Cycle activity as a means to recycle carbon back into

glucose. Studies by Black et al. (1962; 1966) on rainbow trout, however, found that Cori Cycle activity may not be of prime importance. Metabolic recovery from exhaustive exercise was considerably slower than in mammals, with muscle lactate concentrations not reaching pre-exercise levels for 6-8 hrs. Blood lactate peaked after 2-4 hrs and returned to normal after 8-12 hrs. Blood glucose and liver glycogen remained unchanged throughout; muscle glycogen remained low for up to 24 hrs. This slow recovery may be associated with acid-base balance. Fish blood buffering capacities are low (Albers, 1970) as are blood volumes (2-10%, see Prosser, 1973). Calculations by Wardle (1978) show that following exhaustive exercise, the transfer of all muscle lactate into the circulation would increase plasma concentrations to 500 mM. Retention within or slow release of lactic acid from the muscle (possibly mediated by catecholamines (Wardle, 1978), in which buffering capacities are sufficient (Castellini and Somero, 1981), would alleviate the potential lethality associated with increased plasma lactate concentrations (Wardle, 1978). Wood et al. (1977) has provided evidence suggesting that following exercise, slow lactic acid release from the muscle allows a temporal separation of metabolic and respiratory acidosis in the starry flounder (Pleuronectes stellatus).

Species-specific differences are also apparent, possibly correlated with activity. Following exhaustive exercise,

white muscle lactate contents in rainbow trout are 3-fold higher than those of the flathead sole, Hippoglossoides assodon (Turner et al., 1983a,b). In the trout (fast swimmer) lactate is quickly (relatively speaking) released and blood lactate increases 20-fold, in excess of proton concentration. In the sole (sluggish swimmer), muscle lactate declines more slowly and blood lactate increases only 4-fold, in this case, exceeded by protons. Similar observations of low blood lactate following exercise have been made for other Pleuronectiformes: Pleuronectes platessa (Wardle, 1978); Pleuronectes stellatus (Wood et al., 1977); as well as the sea raven, Hemitripterus americanus (Louise Milligan, personal communication). The relative retention of lactate in the sole white muscle suggests in situ metabolism. Isolated perfused trout trunk studies (Turner et al., 1983a) indicate that, although lactate is released, this can only account for 10% of the lactate disappearance from muscle. Here also in situ gluconeogenesis has been proposed. A similar situation may exist in the eel which, like the Pleuronectiformes, also has low lactate contents (Renaud and Moon, 1980b). This slow release of muscle lactate may limit Cori cycle substrate and glycogen replenishment.

The hypothesis that fish red muscle acts as a liver to recycle white muscle lactate to glucose has been consistently raised (Wittenberger and Diaciuc, 1965; Wittenberger et al., 1975; Hulbert and Moon, 1978a; Moon and Johnston, 1980)

and agrees with observations of lactate retention. The initial observations in carp (Cyprinus carpio) muscle by Wittenberger proposed a direct transfer of lactate from white muscle, without the aid of the circulatory system. Red muscle would then ensure the essential nutrients for the muscular effort of white muscle: the anaerobic endproducts of glycolysis of white muscle are oxidized in red muscle which stores energy for white muscle. This is consistent with the work of Issekutz et al. (1976) and Hermansen and Vaage (1977) who suggested that working mammalian muscle of mixed fibre types both produces and utilizes lactate. If this hypothesis is correct, Cori cycle activity may be less important.

Batty and Wardle (1979) found that radiolabelled lactate injected into plaice muscle was converted to glucose after exhaustive exercise. However, Moon and Johnston (1980) could not detect PEPCK activities in plaice muscle, an enzyme essential for lactate gluconeogenesis. Thus, little direct evidence supports Wittenberger's proposal. A study of lactate turnover and carbon exchange between lactate and glucose may help to clarify this issue.

Glucose is a necessary substrate for white muscle especially during burst exercise. Fish seldom, however, swim at such speeds. There is some evidence suggesting that white muscle (and, hence glucose) is utilized at less than burst speeds (Grillner and Kashin, 1976; Dreidzic and Hochachka,

1978; Johnston and Moon, 1980). In addition, an enzyme activity and ultra-structure study found considerable variation in the aerobic capacities and principal fuels supporting activity in white fibres of a number of fish species (Johnston and Moon, 1981). Even though capillary density was low, white fibres potentially could oxidize carbohydrate completely in those fish where such fibres are used at slow swimming speeds.

While red muscle predominates at low swimming speeds and has classically been considered to use lipid as its primary fuel (Love, 1970), increasing evidence suggests that carbohydrate can also be used. On a weight basis, the rate of glycogen utilization under severe energy demands by red muscle may be three times that of white (Johnston and Goldspink, 1973). In Atlantic cod, Gadus morhua, lactate concentrations accumulated in both red and white muscles (Fraser et al., 1966) and glycogen utilization occurred at both high and low swimming speeds (Beamish, 1968). Plaice red skeletal muscle has higher HK activities than liver (Moon and Johnston, 1980) and glycogen concentrations are 3.5-times those of white muscle (Johnston and Goldspink, 1973), suggesting that glucose may be an important fuel source for this tissue also.

Glycogen phosphorylase activity in some fish (dogfish, Scylliorhincus canicula, A. anguilla, and S. gairdneri; Crabtree and Newsholme, 1972) is lower in red than white skele-

tal muscle while HK activities are much higher in red. Red muscle, then, may be more dependent upon exogenous plasma glucose than endogenous glycogen (Crabtree and Newsholme, 1972). These enzyme activities may at least give the red muscle a competitive advantage in capturing plasma glucose for storage. PK, an indicator of glycolytic activity, has higher activities in red than white muscle in yellow eel. In the silver eel, activities were three-fold higher in red muscle (Boström and Johansson, 1972).

Red muscle glycogen concentrations are maintained during food-deprivation and HK activities which are considerably higher than those of white muscle, fall only slightly. These results again, suggest that in plaice, carbohydrate may be a major fuel source for red muscle (Moon and Johnston, 1980).

Glucose can have both a catabolic and anabolic role. In fact, Bokdawala and George (1967) found glucose utilization in red muscle was associated with fatty acid synthesis in carp. Aster (1981), however, found little fatty acid synthesis in American eel red muscle.

Carbohydrate utilization in fish tissues, including skeletal muscle, is complex. Definitive evidence exists for white muscle utilizing glucose, but in addition, red muscle and possibly other tissues do as well. Certainly species differences are apparent, but the maintenance of plasma glucose during food-deprivation implies some metabolic role.

The study of glucose and lactate turnover in vivo should provide a better understanding of its role in the American eel.

1.3 TRACERS AND TURNOVERS

Evidence does exist for glucose utilization by fish under various nutritive states and muscular activity. However, the majority of these studies are not definitive. Assessment of enzyme activities indicate the maximum potential for a given metabolic pathway or controls exerted over that pathway (Newsholme and Start, 1973). Most enzymes catalyzing rate-limiting steps are subject to allosteric control by various metabolites. Therefore, maximal in vitro activities do not necessarily reflect in vivo activities. They cannot assess the extent to which a given pathway is employed. Observations of tissue glycogen stores may detail at what point in time these depots are depleted. When contents are stable, however, these observations alone are insufficient. Stability may imply lack of utilization or complete replacement. The application of radioactive tracers allows determination of the rate of use of a given compound and the rate of its conversion to other metabolites.

Radioactive tracers have been employed in assessing turnover rates of glucose or (somewhat less) lactate in a variety of mammalian studies (symposia in Federation Proceedings:

1974, 1977, 1982; Hetenyi et al., 1983). The kinetics of these processes have been examined, and recent studies have found that the site of tracer administration (venous vs arterial) and sampling (venous vs arterial) can alter the results (Katz et al., 1981; Okajima, et al., 1981). In addition to mammals, tracer experiments have been reported for chickens (Brady et al., 1977; Riesenfeld et al., 1981) and penguins (Groscolas and Rodriguez, 1981), and two fish species, the fingerling coho salmon, Oncorhynchus kisutch (Walbaum) (Lin et al., 1978) and the kelp bass, Paralabrax clathratus (Bever et al., 1977, 1981). Estimations of glucose turnover rates in both bass and fingerling salmon, yield values approximately 10-fold lower than that of the post-absorptive rat (normalized with respect to body weight, but not temperature). Both fish species have rather low plasma glucose concentrations compared to mammals, and at least in kelp bass, these are highly variable.

Tracer administration in fingerling salmon was by intraperitoneal injection into anaesthetized fish. Turnover rates are dependent on the duration necessary for tracer distribution within the body. The increased time involved in absorption from the peritoneal cavity (as compared to intravascular administration) could clearly alter the results. Further, anaesthesia is known to have deleterious metabolic and extra-metabolic effects (eg., Mazeaud et al., 1977). Caution must be employed in interpreting data derived under such conditions.

Glucose turnover values for fingerling salmon do, nonetheless, conform with those determined for kelp bass (though body weights of bass were 10-fold higher) and with the lower metabolic rates of these animals compared to mammals. One might have predicted increased glucose utilization more comparable to mammalian values, in view of those aspects discussed in previous sections. (1) Fish have a higher anaerobic scope; glucose is the only source of fuel under anaerobic conditions. (2) Muscle, both red and white, making up 40-60% of body weight, utilizes glucose as substrate. (3) Enzymes of glucose utilizing pathways have comparable activity in trout and rat. (4) Studies of post-exercise and food-deprivation conditions suggest fish and mammals employ similar strategies with respect to carbohydrate metabolism. The major exception of longterm glycogen retention under food-deprivation conditions combined with the naturally low carbohydrate intake under fed conditions, implies fish may show a greater dependency on gluconeogenesis. Many investigators have found fish gluconeogenic rates to be comparable to those of rats. In vivo studies have found gluconeogenic rates of kelp bass to be equal to those of rats (Bever et al., 1981).

1.4 PURPOSE

Further studies of fish carbohydrate metabolism are required. The intent of this study, therefore, is to determine the utilization of glucose and lactate by estimation of their respective turnover rates in the American eel, Anquilla rostrata. Radioactive tracers will be employed to provide a definitive determination of the utilization of these substrates, rather than the indirect estimation by the quantitation of tissue stores and enzyme activities. The extent of re-circulation of carbon between glucose and lactate (Cori cycle activity) will also be assessed.

The choice of species, A. rostrata, is based on (1) in vitro evidence of a relatively high gluconeogenic potential, (2) the high proportion of white fibres compared to red (80-90%; Greer-Walker and Pull, 1975; Hulbert and Moon, 1978b), (3) its ability to tolerate fasting both short-term (annually in response to low temperature) and long-term (spawning migration) (Sinha and Jones, 1975) and (4) its hardiness to experimental manipulation. Metabolic turnover will be assessed in fed animals, employing controls for ports of infusion and duration of laboratory acclimation. The effects of food-deprivation of 6 and 15 months in immature animals and of 36 months in maturing females will be investigated. These determinations will reflect the biochemical strategies of the natural short-term fast and the

limitations of these strategies under long-term food-deprivation. The effect of anaesthesia will be investigated under fed and food-deprived conditions.

These studies should provide a better understanding of carbohydrate utilization and dependency in a fish species.

Chapter II

METHODS AND MATERIALS

2.1 EXPERIMENTAL ANIMALS

Immature female yellow eels (Anquilla rostrata (LeSueur)) were retrieved from the St. Lawrence River at the Saunderson hydroelectric dam, Cornwall, Ontario. Bronzing females, beginning migration from the St. Lawrence basin, were purchased from a commercial fish dealer in Quebec City, Quebec. The animals were maintained in white fiberglass tanks in approximately 300 l of City of Ottawa dechlorinated tap water. The aerated flow-through water system did not fluctuate below 12°C or above 15°C during the experimental period. Fluorescent lighting was regulated on a 12L:12D cycle, with lights on at 8:00 a.m. EST. Fed animals received earthworms three times weekly, averaging one to two worms per fish per feeding. Analysis of this diet has been previously reported and is consistent with the natural high protein, low carbohydrate diet of these animals (Aster and Moon, 1981). Not more than one hundred Cornwall eels were held together in one tank. Quebec City animals were limited to approximately twenty per tank.

2.1.1 Experimental groups

Eight experimental groups were employed (Table 1). Modified parameters involved the state of anaesthesia; the state of food-deprivation; the duration of lab acclimation; and the ports of infusion and sampling. Six groups were allowed to recover from anaesthesia and were maintained free-swimming over the time course of the experiment. Of these six, three groups were fed, three deprived of food. For one fed experimental group, the port of infusion was the caudal vein; the sampling port was the pneumogastric artery (VA). In all other free-swimming animals, these two ports were reversed: fed, 12 month laboratory-acclimated (AV); fed, 6 week laboratory-acclimated (FN); 6, 15, and 36 month food-deprived (F6, F15, F36, respectively). Only F36 was comprised of maturing animals (downstream migrants). All other experimental groups included only immature fish (upstream migrants).

Two groups were anaesthetized during the experiment. In both, the infusion port and sampling port were the pneumogastric vein and artery, respectively. One anaesthetized group was fed (TA) and one deprived of food for 8 months (FTA). The pneumogastric vessel system was selected as it supplies and drains the swimbladder and its occlusion should not markedly affect blood flow, except to this organ.

TABLE 1
Experimental groups

GROUP	MANIPULATION	(N)	BWT (G)	CAUGHT	I	S
VA	FED	6	142±24	08/1981	CV	PA
AV	FED	6	130±16	08/1981	PA	CV
FN	FED	5	127±14	07/1982	PA	CV
F6	6 MONTH FAST	6	116±27	09/1981	PA	CV
F15	15 MONTH FAST	5	100±21	09/1980	PA	CV
F36	36 MONTH FAST	6	191±41	06/1979	PA	CV
TA	FED	6	192±41	09/1980	PV	PA
	ANAESTHETIZED					
FTA	8 MONTH FAST	5	127±24	09/1980	PV	PA
	ANAESTHETIZED					

Note: I, infusion cannula; S, sampling cannula; CV, caudal vein; PA, pneumogastric artery; PV, pneumogastric vein. Body weights (BWT) are expressed as mean ± one standard deviation.

2.2 SURGICAL TECHNIQUE

The fish were anaesthetized in 1.5 g·l⁻¹ MS-222 (ethyl-N-aminobenzoate methane sulphonic acid) buffered with Trizma base (Sigma Chemical Co., St. Louis, MO.) to pH 7.4. At this concentration the time to inactivity was approximately 10 minutes per 100 gram body weight. Maintenance of inactivity was accomplished by a constant flow of aerated, recirculated, buffered MS-222 (0.2 g·l⁻¹) over the gills of the animal by means of a Manostat Ministaltic pump. Temperature was maintained at 12°C for those animals which remained anaesthetized for the entire experiment. Wet paper towel and a small amount of ice kept the anaesthetized animal cool and moist for the duration of the experiment.

2.2.1 Cannulation of the caudal vein

Intramedic PE-10 cannula tubing was filled with a standard fresh water Teleost Ringer's solution (6.42 g NaCl, 0.15 g KCl, 0.22 g CaCl₂ (hydrous), 0.12 g MgSO₄, 0.084 g NaHCO₃, 0.069 g NaH₂PO₄·H₂O per litre). The solution was heparinized (1000 iu·ml⁻¹, porcine heparin, sodium salt, Sigma Chemical Co.) if the venous cannula was to be used for sampling. The inserted end of the cannula was bevelled. A small (approximately 20 mm) piece of PE-50 tubing was flared at one end by flame. This was slid over the bevelled end

to about 3 cm with the flared end distal to the bevelled end of the cannula.

Exposure of the caudal vein involved an incision at a point one third of the way from the end of the tail and 4 or 5 mm ventral to the lateral line. This location minimized destruction of red muscle concentrated in this area and, therefore, avoided excessive hemorrhage. The white muscle was eased away from the fascia surrounding the vertebral column using blunt dissection to avoid destruction of arterioles and venules perfusing the muscle. The fascia above the caudal vein was removed on either side of one vertebral spine. The caudal artery lying dorsal to the vein within the haemal arch was not usually exposed although it was necessary to separate the two vessels.

The vein is durable enough to allow its lifting with fine blunt forceps. In this manner, a serrefine was placed as far craniad to the exposed vertebral spine as possible. A thread run under the vessel on the caudal side was used for leverage and allowed for temporary cessation of blood flow while a 27 gauge hypodermic needle punctured the vessel just cranial to the string. The PE-10 cannula was then inserted through the small hole and slid cranially to the clamp. The clamp was then removed and the cannula was moved gently craniad until 2 to 3 cm was lying within the vein.

To ensure that the cannula was not occluded, a small amount of blood was withdrawn and replaced. With the cannula

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satisfactorily positioned, the flared PE-50 tubing was moved carefully down the cannula into a position just above the entry point into the vessel. A small drop of Krazy Glue (Krazy Glue Inc., Chicago, IL.) was used to secure it around the cannula. Bleeding was usually minimal but as a precaution fibrin (bovine fibrin, Sigma Chemical Co.) was dusted over the exposed vessel.

The cannula was not tied into the caudal vein thereby allowing blood to flow freely past it within the vessel. The flared PE-50 tubing prevented the cannula from being forced out of the vessel by muscular contraction. To further anchor the cannula, it was stitched to the underside of the incision. The stitch was knotted and then both sides of the incision were stitched together. Koban sewing thread (Coats, size 50) was chosen as a suture due to its durable polyester core and non-irritating cotton sheath.

2.2.2 Cannulation of the pneumogastric artery

As with the caudal vein, the Teleost Ringer's solution filling the cannula was heparinized only if the vessel was to be used for sampling. The incision for the pneumogastric cannulation was along the ventral midline beginning 5 or 6 cm behind the pectoral girdle, exposing the apex of the liver, and extending approximately 5 cm caudally. The gut was retracted and the fascia dorsal to it removed to expose the

artery. The vessel was cannulated cranial to its bifurcation in the same manner as the caudal vein except the artery was tied off caudally (at the bifurcation) prior to placing the serrefine, as far cranially as possible in the exposed area. Intramedic PE-50 tubing was used for the cannula and once in place was secured directly within the vessel. As a further precaution, the thread used to initially occlude the vessel was knotted again around the cannula after the cannula was checked for smooth blood flow. If the animal was to be returned to the water for the experimental procedure, the cannula was anchored to the skin and underlying muscle prior to closing the incision.

2.2.3 Cannulation of the pneumogastric vein

This was performed only in those animals to be anaesthetized for the duration of the experiment. In these animals the vein was always the infusion port and the cannula (PE-50), therefore, was filled with non-heparinized Teleost Ringer's.

This cannulation was necessarily performed prior to that of the artery because the vein is closer to the midline and slightly ventral, often embedded in the muscle of the gut at the point of incision. The vessel is very fragile and could not be cleared completely without its destruction. To avoid this, a silk suture (size 6-0, Ethicon, Peterborough, Ont.)

was run through the gut muscle and below the vessel at either extremity of the exposed area. The serrefine was placed cranially as before and the most caudal suture tied off the vein. With the cannula in place, the more cranial suture was used to secure it within the vessel.

2.2.4 . Recovery from surgery

Those animals which were to remain anaesthetized throughout the experiment (TA and PTA groups) were allowed no period of recovery. The infusion was begun immediately and the animal was maintained on $0.2 \text{ g} \cdot \text{l}^{-1}$ MS-222 for the duration of the experiment.

For the majority of the fish this was not the case. The cannulae were heat-sealed and tagged with a piece of labelling tape for easy retrieval the next morning. The animal was then placed in an individual box of clear plexiglass suspended near the surface of the water. A small piece of tygon tubing fitted loosely into the water inflow system of the tank was placed into the animal's mouth to perfuse the gills with fresh water and aid in recovery from anaesthesia. Under these conditions all the animals were revived. Otherwise, this was often not the case.

The boxes, measuring $72 \times 13 \times 10.5 \text{ cm}$ (l X w X h), had a small hole at either end and were fitted with a sliding lid, blackened to avoid visually disturbing the animal during the

experiment. A 1 cm wide slit running the length of the lid allowed the cannulae to flow freely behind the fish. Tygon tubing inserted in one hole acted as a siphon pulling fresh water through the box and out of the tank. Flow rate was controlled by a clamp on the exit tubing.

The experiments were begun between 6:00 a.m. and 10:00 a.m. the following day, allowing a mean recovery time of 20.4 ± 2.9 hr with the exception of two fish, F15-4 and F36-1, which were allowed 49.0 and 47.5 hr recovery time, respectively.

2.3 EXPERIMENTAL PROTOCOL

For experiments carried out using swimming fish, the fused ends of the cannulae were snipped off and hypodermic needles and syringes inserted: 27 gauge in the venous cannula and 23 gauge in the arterial cannula. An initial blood sample of 0.25 ml was withdrawn prior to initiation of infusion to provide a preliminary concentration of glucose and lactate.

The infusate containing D-[6-³H(N)]-glucose (specific activity: 30 Ci·mmol⁻¹) and L-[1-¹⁴C(U)]-lactic acid, sodium salt (specific activity: 164.5 mCi·mmol⁻¹) (New England Nuclear Canada Ltd., Lachine, Quebec) was diluted in fresh water Teleost Ringer's solution to mean concentrations of 2.06×10^7 dpm·ml⁻¹ and 8.1×10^7 dpm·ml⁻¹, respectively. The

exact concentrations varied between experiments. A 0.1 ml priming dose of ^3H -6-glucose equal in radioactivity to two hours of infusion was then injected into the vessel which would be used for infusion. Since the infusion cannula was necessarily about 1 m long to allow free movement without disturbing the infusion pump, the primer could fill the cannula and not in effect be injected into the animal. (The PE-10 cannula held a volume of approximately 0.1 ml, the PE-50 cannula a volume of about 0.25 ml.) The appropriate volume of infusate was, therefore, injected into the cannula to compensate and the infusion was then begun at a constant rate of 0.05 ml per hour using a Sage infusion pump.

A smaller residual volume was present in the sampling cannula (approximately 40 cm long). During each sampling, this volume was removed together with a small portion of blood and placed aside temporarily. A second clean syringe was then used to take the timed sample of 0.4 ml (with the exception of the preliminary sample of 0.25 ml as previously stated). The cannula was stoppered with the first syringe. The blood sample was injected into a previously heparinized Eppendorf centrifuge tube on ice. Ten minutes were required to sample from the caudal vein as compared with two to three minutes from the artery. The sampling time was considered to be the median between beginning and end.

Approximately 20 μl of blood was taken up into a haematocrit tube, stoppered with clay and centrifuged for 10 mi-

minutes in a clinical centrifuge at 2750 g and 8°C. The blood remaining in the Eppendorf tube was centrifuged for 30 sec (Eppendorf centrifuge 5412), and 110 μ l of plasma was pipetted into a 0.5 ml Eppendorf tube placed temporarily on ice. Equal volumes (85 μ l) of plasma and 10% TCA (trichloroacetic acid) were mixed and centrifuged (Eppendorf centrifuge) for 3 minutes to yield a deproteinized plasma sample. The supernatant was divided into two aliquots, 50 μ l for glucose analysis and the remainder for lactate analysis. The three samples (including the untreated plasma sample) were then frozen at -70°C until analysis could be carried out.

During this final centrifugation, the red blood cells and any plasma remaining in the Eppendorf tube were vortexed gently with 50 μ l of fresh water Teleost Ringer's (non-heparinized) solution. Once resuspended, the "blood" was taken up through a heparinized 23 gauge needle and syringe. To this was added the small amount of blood which inevitably remained within the second sampling syringe. This was reinjected into the animal and followed by the mixture of heparinized Ringer's solution and blood in the first syringe. Finally, the cannula was flushed with approximately 0.1 ml of clean heparinized Ringer's solution. The total time from sample removal to reinjection was approximately 3 min.

Heparinization of the centrifuge tubes involved addition of approximately 5 μ l of heparinized Ringer's (1000 iu·ml⁻¹) solution and subsequent evaporation overnight.

Prior to the first and last sample of groups AV and VA (see Table 1), 100 μ l of blood was taken up into a Hamilton syringe. The needle of the syringe which encompasses a very small volume had been filled with heparinized Teleost Ringer's to avoid contact between air and blood. The blood pH was estimated (BMS 3 Mk 2 Blood Micro System and PHM 71 Mk 2 Acid-Base Analyzer, Radiometer, Copenhagen). As this parameter was changed marginally over the course of the experiment (± 0.03 pH units), this measurement was not performed in other fish due to the large amount of blood required for which red blood cells could not be replaced.

Animals anaesthetized for the entire experiment were also infused with ^3H -6-glucose and ^{14}C -U-lactate at rates varying from 23126 to 65308 and from 5634 to 18095 dpm·ml $^{-1}$, respectively, following a priming dose of ^3H -6-glucose equivalent to 0.5 hr of infusion. The rate of infusion was 0.25 ml per hour and hourly samples of 0.25 ml were taken from the pneumogastric artery. Samples were treated, as previously mentioned, although red cells were discarded. A smaller sample was possible because lactate concentrations were high enough to permit the use of only 20 μ l for this determination.

2.4 ANALYTICAL PROCEDURES

2.4.1 Glucose

D-glucose concentrations were measured using a modified version of the Sigma technical bulletin no. 510, an enzymatic colourimetric determination using glucose oxidase (β -D-glucose: oxygen oxidoreductase, EC 1.1.3.4) and peroxidase (donor: hydrogen-peroxide oxidoreductase, EC 1.11.1.7).

Volumes of the "colour reagent" (containing the enzymes) and the H_2SO_4 (7.8 N) used to stop the reaction were halved to 2.0 and 2.5 ml, respectively. Sufficiently high plasma glucose concentrations allowed reduction of sample volume (deproteinized plasma) to 20 μ l. Each sample was run in duplicate. Each time the assay was run, a standard curve from 0 to 2.0 mg/ml was also run in duplicate. Measurements were made at 540 nm on a Pye Unicam SP6-200 spectrophotometer.

2.4.2 Lactate

L--lactate was determined with lactate dehydrogenase (L-lactate:NAD oxidoreductase, EC 1.1.1.27, beef hearts, (Boehringer-Mannheim Canada Ltd.) and nicotinamide dinucleo-

tide (lithium salt crystal, Boehringer-Mannheim Canada Ltd.) using a hydrazine-glycine buffer at pH 9.5, according to Horst (1965), modified slightly to accommodate the small volumes of available plasma.

Deproteinized plasma samples from TA and PTA animals were measured spectrophotometrically (Pye Unicam SP6-200) at 340 nm, the characteristic peak wavelength of nicotinamide dinucleotide, reduced form (NADH). Sample (20 μ l), NAD (50 μ l, 10 mg·ml⁻¹) and 0.75 ml of buffer were added to a 1.0 ml cuvette. The zero reading was taken before addition of 50 μ l of LDH (100 μ g·ml⁻¹); twenty minutes later the final reading was taken. Samples were always run in duplicate as was a standard curve from 0 to 2.0 mg·ml⁻¹.

Plasma samples from other groups of fish could not be read under these conditions. These were read spectrofluorometrically (Turner model 430; excitation 350 nm, emission 468 nm). The same buffer and concentrations of NAD and LDH were employed. A deproteinized plasma sample (100 μ l) was added to 1.8 ml hydrazine-glycine buffer and 50 μ l of NAD in unused 12 X 75 borosilicate culture tubes. Zero readings were necessary prior to addition of 50 μ l LDH. Plasma samples could not be run in duplicate due to the large volume required for this determination. Standard curves from 0 to 200 μ g·ml⁻¹ were run in duplicate using 50 μ l of standard.

2.4.3 Radioactive determination

Separation of glucose and lactate was achieved by column chromatography using methods slightly modified from Reilly (1975) and Exton and Park (1967). The anion exchange resin, AG 1-X8 (200 mesh, chloride ion form; BioRad Laboratories Canada Ltd., Mississauga, Ont.) was packed wet into 0.7 X 4 cm polypropylene Econocolumns (BioRad Laboratories); 6.0 ml of 1.0 M sodium acetate trihydrate was passed through the column, followed by 4.0 ml deionized water. (All solutions involved in this procedure were prepared with deionized water.)

Plasma samples were thawed and deproteinized using 0.1 ml (plasma) $\text{Ba}(\text{OH})_2$ ($47.3 \text{ g}\cdot\text{l}^{-1}$), 0.2 ml ZnSO_4 ($55 \text{ g}\cdot\text{l}^{-1}$), and 0.8 ml deionized water (Exton and Park, 1967). This was vortexed and centrifuged for 10 min in a clinical centrifuge at 2750 g. The supernatant (1.0 ml) was placed on the prepared column, followed by 10.0 ml, then 3.0 ml of deionized water. During this procedure, lactate remains on the column (Reilly, 1975). The total effluent containing glucose and alanine and any other neutral or basic compounds was shaken for 60 min with 1.0 g of AG 3-X4 (200-400 mesh, chloride ion form; BioRad Laboratories) and 0.5 g of Dowex 50W-X8 (200-400 mesh, hydrogen ion form; BioRad Laboratories). This combination of resins has been shown to effectively remove alanine (Exton and Park, 1967). This was verified:

97% of ^{14}C -alanine added to an aqueous sample was removed by this process. After shaking, the resins were allowed to settle, and 8.0 ml of the fluid were transferred to a glass scintillation vial.

Immediately following the emergence of the glucose fraction, 2.0 ml of deionized water followed by 4.0 ml of 0.5 M formic acid were passed through the column. Effluents were discarded. Finally 10.0 ml of 0.5 M formic acid was added to the column. The column was allowed to drip directly into glass scintillation vials. These vials, as well as those containing the glucose fraction, were placed in a drying oven (50°C) until completely evaporated. The vials were then cooled and reconstituted with 100 μl of distilled water. Ten ml of Aquasol (New England Nuclear Canada Ltd.) or 5.0 ml of Aqualyte (Canlab, Ottawa, Ont.) was added and the vials were allowed to stand overnight in the dark at room temperature prior to counting.

Standards of ^3H -6-glucose or ^{14}C -U-lactate, or a combination of both were run on columns in duplicate with non-radioactive plasma undergoing the same procedures just outlined. Standards were run with each set of columns. The efficiency of ^{14}C -lactate removal into the lactate fraction averaged 78%, that of ^3H -glucose, 91%. Two to 3 per cent of the lactate was found in the glucose fraction and the same amount of glucose in the lactate fraction. These "spillovers" were taken into account in calculating the final radioactive glucose and lactate activities.

The samples were counted on an LKB Wallac 1215 Rackbeta liquid scintillation counter for five minutes each, twice. Internal standards were used to determine counting efficiencies.

2.5 CALCULATIONS

Turnover rate was determined according to the equation developed by Stetten et al. (1951) for infusion experiments with or without primer. These equations and their application have been recently reviewed (Hetenyi et al., 1983). The most conventional form (Hetenyi and Norwich, 1974) is

$$R_T = R^* / \overline{SA} \quad (i)$$

$$R_i = R^* / \overline{SA} \quad (ii)$$

R_T is the turnover rate and R_i is the irreversible disposal rate, depending upon the tracer employed (3H or ^{14}C , respectively), in $mg \cdot min^{-1}$. R^* is the rate of infusion of tracer, in $dpm \cdot min^{-1}$. \overline{SA} is the specific activity, once the tracer has reached equilibrium with the tracee, in $dpm \cdot mg^{-1}$. Equilibrium is achieved when the \overline{SA} vs time curve reaches a plateau (\overline{SA}).

Plateaux were determined graphically, and verified in accordance with the model of Zilversmit et al. (cited in Shipley and Clark, 1972). If Δ is determined for each time point as the difference between the plateau value (\overline{SA}) and

the point on the SA curve as it approaches the \overline{SA} value, then a semi-log plot of Δ_{95} time yields a straight line.

^3H -6-glucose is considered the most reliable label to determine glucose turnover, (R_T) because the ^3H is not recycled back to glucose (or the effect is negligible; Okajima *et al.*, 1981) but is irreversibly lost to tritiated water during the conversions of pyruvate to alanine, pyruvate to oxaloacetate, and interconversion of malate and fumarate (Dunn *et al.*, 1976; Bever *et al.*, 1977; Katz, 1979). ^{14}C -lactic acid is not ideal for lactate turnover studies as it underestimates these values by about 50% due to carbon recycling (Okajima *et al.*, 1981). Rather, the ^{14}C -label yields the irreversible disposal rate, the rate of disappearance of carbon atoms from the circulation which do not return to the same compound in the plasma (Shibley and Clark, 1972).

Stetten's equation (i, ii) can only be used for animals in dynamic steady state (DSS), when plasma concentrations of the compound in question are relatively constant. Under these conditions, the turnover rate (R_T) is equivalent to the rate of appearance (R_a) and the rate of disappearance (R_d) of the plasma compound (eg., glucose).

Under conditions of changing plasma concentrations, the two latter rates must be determined individually according to equations developed by Wall *et al.* (1957)

$$R_a = R^* - \frac{pV[(C_2 + C_1)/2][(SA_2 - SA_1)/(t_2 - t_1)]}{(SA_2 + SA_1)/2} \quad (\text{iii})$$

$$R_d = R_a - pV(C_2 - C_1)/(t_2 - t_1) \quad (\text{iv})$$

R_a and R_d are the rates of appearance and disappearance, respectively, of the plasma metabolite, in $\text{mg}\cdot\text{min}^{-1}$. C is the plasma concentration, in $\text{mg}\cdot\text{ml}^{-1}$, and t is the time lapsed from the beginning of the infusion, in min. V is the size of the metabolite pool in ml. Not all of the metabolite in this pool exchanges rapidly with that in the plasma (that compartment of the pool into which tracer is infused and from which samples are collected) (Wall *et al.*, 1957). The pool fraction, p , is the correction factor for this incomplete mixing; it is the proportion of the pool within which the tracer quickly reaches equilibrium. Wall *et al.* (1957) assumed that the proportion was one-half. It was later experimentally determined by Cowan and Hetenyi (1971) to be 0.65 for glucose for dogs in the post-absorptive condition. This value is species- and condition-specific.

These two equations (iii, iv) are based upon the model of the metabolite pool as a single, well-mixed compartment. New molecules of the specific metabolite entering this space

of constant volume, mix instantly with all the glucose already present. The equations are applicable to a linear system, the constraints of which are outlined by Norwich (1977). The glucose system has been found to adhere to these constraints (Norwich and Hetenyi, 1971) and these equations have been experimentally validated for glucose (Radziuk et al., 1974). The validity of the Stetten equation is not restricted to a single compartment model but is applicable to a more general system which allows free movement of molecules by convection or diffusion. It is, however, dependent upon the constancy of SA in the space occupied by the given compound (Norwich, 1977). Its validity in this respect was experimentally proven by Norwich and Hetenyi (1971).

For comparative purposes, the equations of Wall et al. (1957) have also been applied to lactate under conditions when dynamic steady state was not maintained for this metabolite, in accordance with Issekutz et al. (1976). The value V was taken to be equivalent to total body water (71.4 ml·100g⁻¹ for fresh water teleosts; Holmes and Donaldson, 1969) and p to be 0.50 (Steele, 1971).

Metabolic clearance rate, a measure of the efficiency of removal of a metabolite (as opposed to the rate of removal), is independent of concentration. Determinations were made for glucose and lactate applying the formula

$$MCR = R^2/\bar{C}^2$$

(v)

\bar{C}^* , in $\text{dpm} \cdot \text{ml}^{-1}$, is the plateau plasma concentration of tracer, determined graphically and verified for ^3H -glucose and ^{14}C -lactate in the same manner as that described for $\bar{\text{SA}}$.

The rate of production of glucose from lactate (vi) was determined according to Shipley and Clark (1972). The rate of production of lactate from glucose (vii) was determined in a similar manner.

$$R(\text{L} \rightarrow \text{G}) = [\bar{\text{SA}}(^{14}\text{C}\text{-glucose}) / \bar{\text{SA}}(^{14}\text{C}\text{-lactate})] R_T(\text{glucose}) \quad (\text{vi})$$

$$R(\text{G} \rightarrow \text{L}) = [\bar{\text{SA}}(^3\text{H}\text{-lactate}) / \bar{\text{SA}}(^3\text{H}\text{-glucose})] R_i(\text{lactate}) \quad (\text{vii})$$

R is the rate of exchange of carbon atoms from lactate to glucose, (L→G), or glucose to lactate, (G→L), in $\mu\text{g C min}^{-1}$. SA values are represented here in $\text{dpm} \cdot \mu\text{g}^{-1}$ carbon atom. $R_T(\text{glucose})$ is represented as $\mu\text{g carbon} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$. This equation is dependent on steady state conditions of both glucose and lactate and was only used where applicable.

The proportion of glucose carbon turned over, derived from lactate is

$$R(\text{L} \rightarrow \text{G}) / R_T(\text{glucose}) \quad (\text{vi})$$

The proportion of lactate turnover contributing to glucose carbon is

$$R(L \rightarrow G) / R_T(\text{lactate}) \quad (\text{vii})$$

Due to the utilization of ^{14}C -lactate (rather than ^3H -lactate) this value will represent the proportion of the irreversible disposal rate of lactate which is converted to glucose. $R_i(\text{lactate})$ is represented as $\mu\text{g carbon} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$.

If $R(L \rightarrow G)$ and $R_i(\text{lactate})$ are mutually exclusive and there are no other factors contributing to lactate turnover (all carbon returning to plasma lactate does so through glucose); then total lactate turnover would represent the sum of $R(L \rightarrow G)$ and $R_i(\text{lactate})$.

2.6 STATISTICS

Normal distributions were verified by the Kolmogorov-Smirnov goodness of fit test before applying one way ANOVA to determine differences of statistical significance at a level of $p \leq 0.05$. When more than two levels were involved and statistically significant differences were apparent, a Student-Newman-Keuls multiple range test was employed (Campbell, 1967; Zar, 1974).

Chapter III

RESULTS

3.1 FED, FREE-SWIMMING ANIMALS

All fed animals (VA, AV, and FN groups) maintained concentrations of plasma glucose and plasma lactate consistent with a dynamic steady state (DSS) (Fig. 2A, Fig. 3A). Glucose concentrations were generally more stable and from 200 to 400-fold greater than lactate. The variability of venous (AV) concentrations was found to be greater than that of arterial (VA), but no significant differences existed between venous and arterial plasma concentrations, of 6 week (FN) and 12 month (AV) laboratory acclimated animals for either metabolite (Table 2).

Tracer concentrations were allowed to approach equilibrium concentrations (DSS) before the experiments were terminated as evidenced in the specific activity curves (Fig. 2B, C, Fig. 3C).

Glucose turnover ($R_T(\text{glucose})$) estimates using venous specific activities (AV) yielded higher rates, though not significantly, than when arterial specific activities (VA) were employed (Table 2). The same tendency was apparent

Figure 2: Plasma glucose concentrations and specific activities in fed animals under DSS conditions.

Arterial sample, 12 months acclimation (VA- \bullet - \bullet - \bullet - \bullet); venous sample, 12 months acclimation [AV- \square - \square - \square - \square (480 min), (240 min)]; venous sample, 6 weeks acclimation (FN- Δ - Δ - Δ - Δ).

Specific activity is represented as $SA \cdot R^{*1} \cdot 100g^{-1}$, in $min \cdot mg^{-1} \cdot 100g^{-1}$. All values are represented as mean \pm one standard deviation. (n-values as in Table 1 with the exception of FN, n=4).

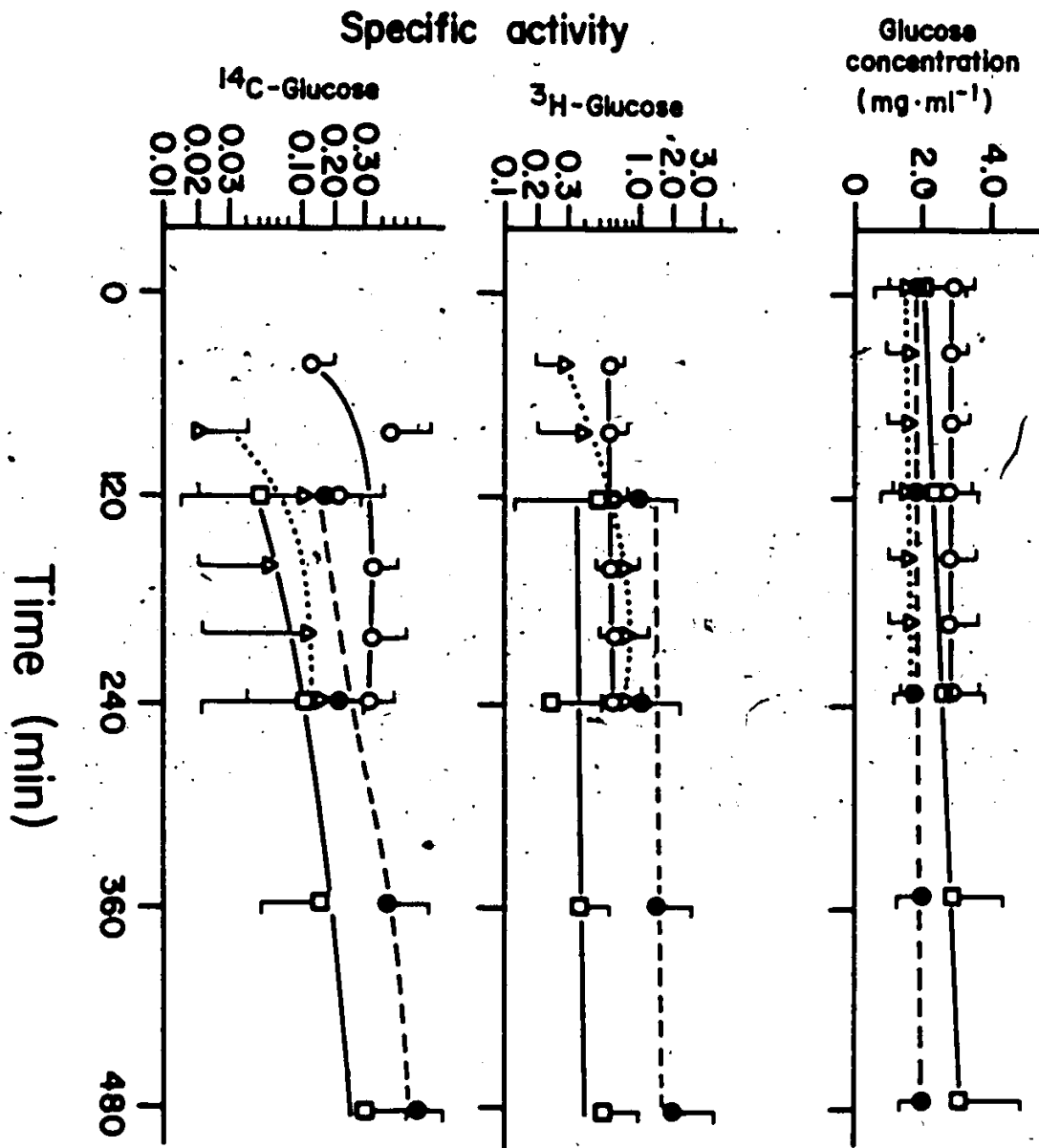
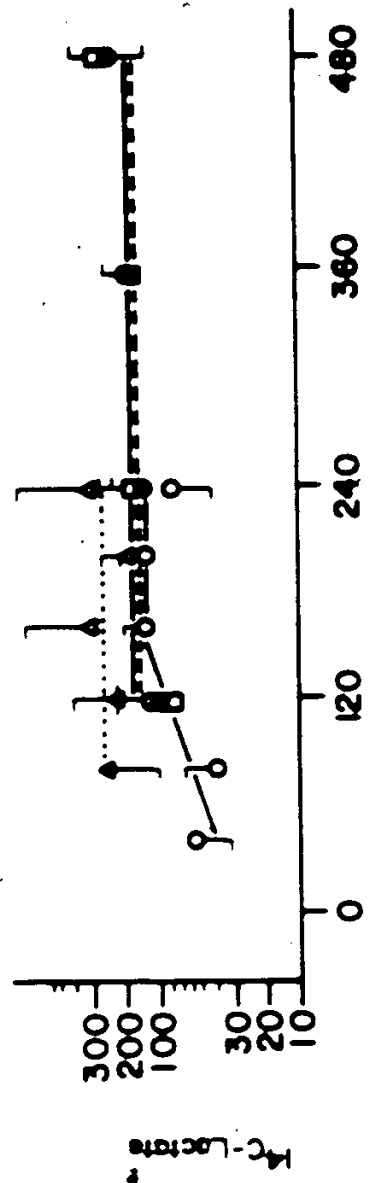
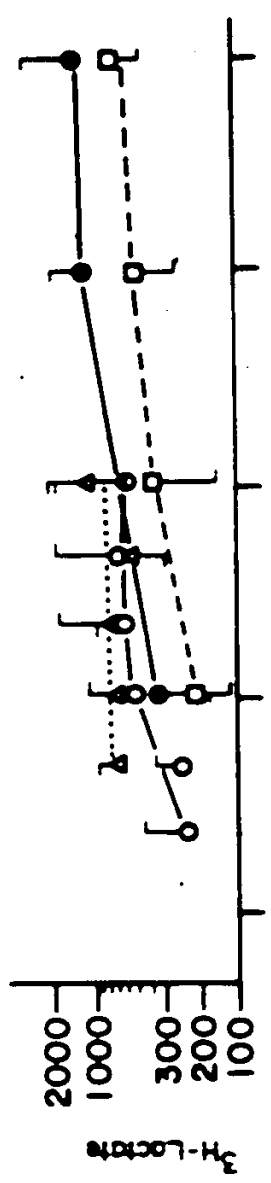
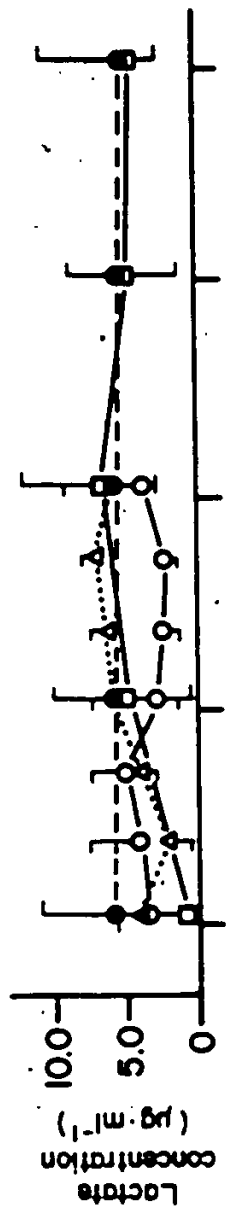


Figure 3: Plasma lactate concentrations and specific activities in fed animals under DSS conditions

Arterial sample, 12 months acclimation (VA- \bullet --- \bullet); venous sample, 12 months acclimation [AV- \square --- \square (480 min), \circ --- \circ (240 min)]; venous sample, 6 weeks acclimation (FN- Δ --- Δ).

Specific activity is represented as $SA \cdot R^{*1} \cdot 100g^{-1}$, in $min \cdot mg^{-1} \cdot 100g^{-1}$. All values are represented as mean \pm one standard deviation. (n-values as in Table 1 with the exception of FN, n=3).



Time (min)

2

TABLE 2

Glucose and lactate kinetics in the fed American eel

PARAMETER	VA (arterial)	AV (venous)	PN (venous)
	(6)	(6)	(4)
GLUCOSE mg·mL ⁻¹	1.43 ± 0.56	2.45 ± 1.11	1.49 ± 0.58
R _y GLUCOSE mg·mL ⁻¹ ·100g	0.69 ± 0.53	1.37 ± 0.80	0.76 ± 0.24
MCR GLUCOSE mL·mL ⁻¹ ·100g	0.58 ± 0.40	0.71 ± 0.37	0.46 ± 0.12
LACTATE μg·mL ⁻¹	5.1 ± 2.1	4.4 ± 2.4	6.1 ± 1.9
R _y LACTATE μg·mL ⁻¹ ·100g	1.71 ± 0.89	3.41 ± 4.14	4.69 ± 1.21
MCR LACTATE mL·mL ⁻¹ ·100g	1.04 ± 0.57	1.74 ± 1.51	1.45 ± 0.25

Notes: R_y glucose = glucose turnover rate; R_y lactate = lactate irreversible replacement rate; MCR, metabolic clearance rate; DSS and for DSS. All values are represented as mean ± the standard deviation. Plasma concentrations are group means of the final 120 minutes of infusion. DSS. N values in parentheses represent DSS animals.

There are no statistically significant differences.

with lactate irreversible replacement rates ($R_i(\text{lactate})$), but the variability of the arterial values may possibly obscure a significant difference between the two values (Table 2). $R_i(\text{lactate})$ values were at least 2 orders of magnitude lower than glucose turnover in accordance with the respective differences in circulating plasma concentrations.

Lab acclimation period did not significantly affect the turnover rate of glucose or the irreversible replacement rate of lactate, although both tended to be lower at 6 weeks than at 12 months (Table 2).

Metabolic clearance rates (MCR) were unchanged regardless of sampling port, or acclimation period for both glucose and lactate, even though mean values differ markedly (Table 2). There were no significant differences between clearance rates of glucose and lactate within a given experimental group. Standard deviations were much smaller in the 6 week acclimated group as compared to the other fed experimental groups, suggesting a closer comparability between individuals.

No significant differences were apparent between fed groups with respect to the rate of conversion of lactate carbon to plasma glucose ($P_L \rightarrow G$) or the proportion of glucose turnover derived from lactate carbon (Table 3). In all cases, less than 0.40% of the glucose turnover was derived from lactate carbon. The rate of production of lactate from glucose ($P_G \rightarrow L$) was indistinguishable between

TABLE 3

Carbon exchange in fed animals

PARAMETER	VA (6)	AV (6)	PN (3)
R(L→G) ($\mu\text{gC}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$)	4.25 ± 2.81	7.40 ± 3.98	6.14 ± 1.77
rate of conversion of plasma lactate carbon to plasma glucose carbon			
R(G→L) ($\text{mgC}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$)	10.27 ± 8.57	15.02 ± 6.58	9.12 ± 3.93
rate of conversion of plasma glucose carbon to plasma lactate carbon			
$\frac{R(L\rightarrow G) \times 100\%}{R_T(\text{Glucose})}$	0.35 ± 0.16	0.29 ± 0.20	0.33 ± 0.07
per cent of glucose turnover derived from lactate carbon			
Estimated total lactate turnover ($\mu\text{g}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$)	4.04 ± 1.66	8.17 ± 5.17	6.99 ± 0.88
Total lactate carbon turned over, converted to glucose	36.2	35.4	33.3
Total lactate carbon turned over, not reconverted to lactate	63.7	64.6	66.7

Note: all values are represented as mean ± one standard deviation of animals in dynamic steady state with respect to both glucose and lactate.

There are no statistically significant differences between experimental groups.

the three experimental groups, in each case, at least three orders of magnitude higher than the reciprocal exchange.

The estimated total lactate turnover for each fed group was approximately 35% higher than the irreversible replacement rate. This value of 35% represented the proportion converted to glucose (Table 3). Large differences between the proportion of glucose turnover derived from lactate and the proportion of lactate turnover converted to glucose, reflected the large differences between plasma glucose and lactate concentrations and their respective turnover rates for these animals.

3.2 FOOD-DEPRIVATION

All animals in the fasted groups (F6, F15, F36) maintained constant plasma glucose concentrations (Fig. 4A), except F6-5 (Fig. 6A). Lactate concentrations were far more variable. Half of the 6 month and all of the 15 month food-deprived groups increased lactate concentrations over the experimental period (Fig. 6C). Maturing eels, deprived of food for 36 months, maintained the lowest and most constant plasma lactate concentrations (Figs. 3B and 4C). No significant differences were apparent between either the fed (AV) and food-deprived immature eels (F6, F15) or between F6 and F15 with respect to either glucose or lactate in those eels in DSS (Table 4). As in the fed experimental groups,

Figure 4: Plasma glucose concentration and specific activities in food-deprived animals

6 month food-deprived (F6-●—●, n=5); 15 month food-deprived (F15-○—○, n=5); and 36 month food-deprived maturing animals (F36-▲—▲, n=6)

Specific activity is represented as $SA \cdot R^* \cdot 100g^{-1}$, in $min \cdot mg^{-1} \cdot 100g^{-1}$. All values are represented as mean \pm one standard deviation.

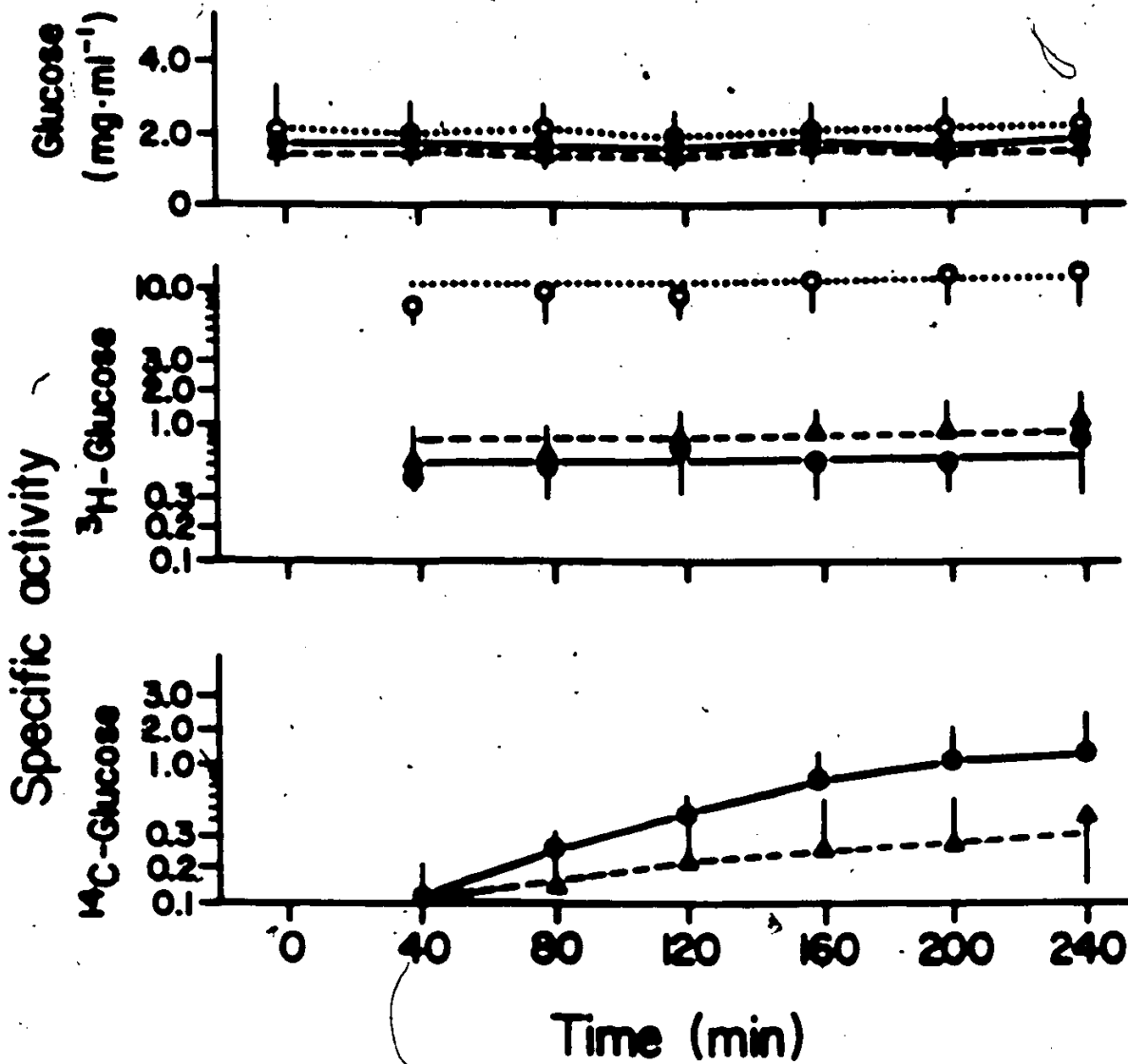


Figure 5: Plasma lactate concentration and specific activities in food-deprived animals

6 month food-deprived (P6-●—●, n=5); 36 month food-deprived maturing animals (P36-△—△, n=6).

Specific activity is represented as SA-R² · 100g⁻¹, in min mg⁻¹ · 100g⁻¹. All values are represented as mean ± one standard deviation.

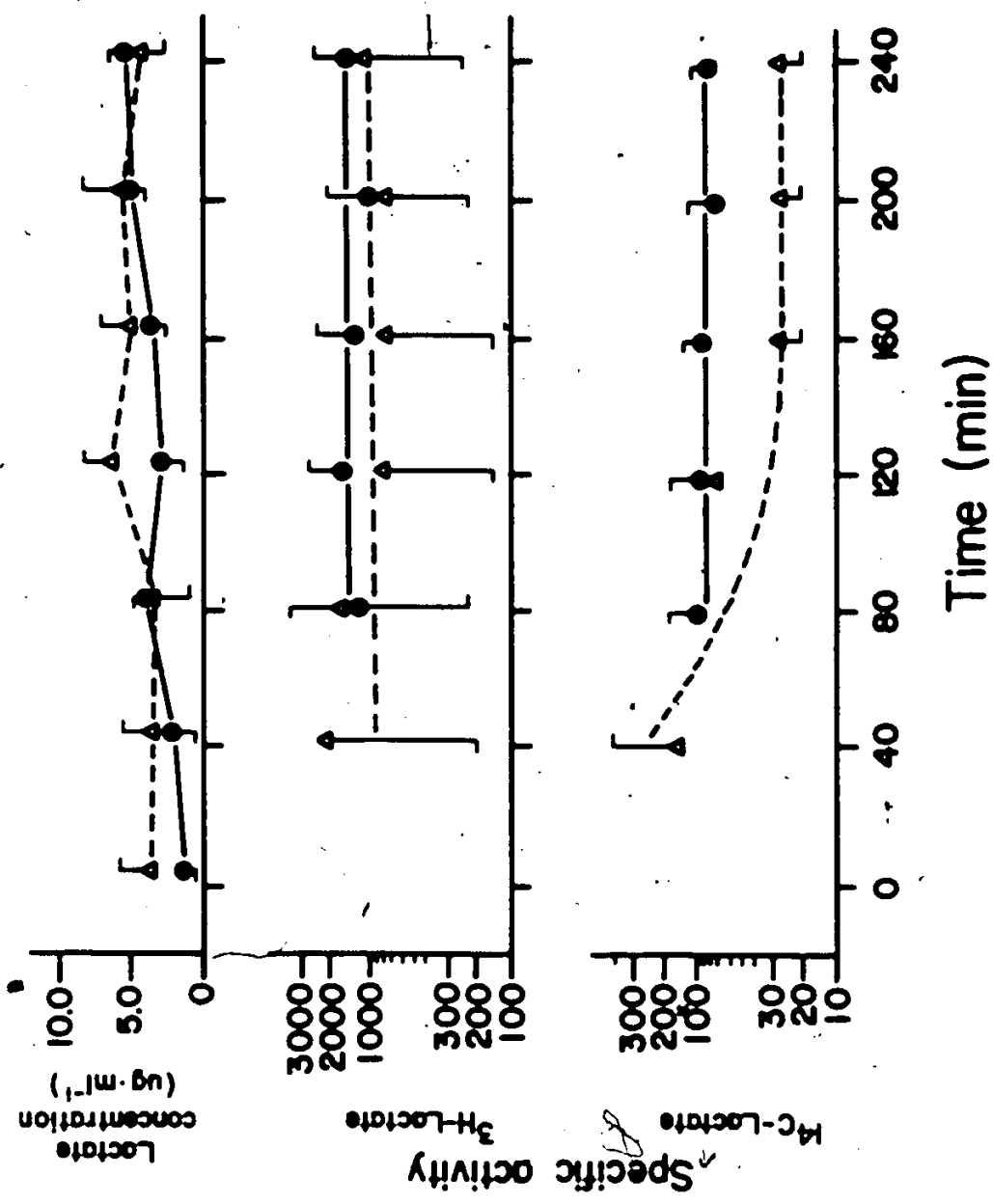


Figure 6: Plasma concentrations and specific activities of food-deprived animals out of steady state

Glucose (F6-5, ●—●) and lactate: 6 month food-deprived (●—●, n=3), 15 month food-deprived (○—○, n=5), and two 36 month deprived animals, shown individually (F36-1, ▲—▲; F36-6, Δ—Δ).

Specific activity is represented as $SA \cdot R^* \cdot 100g^{-1}$, in $min \cdot mg^{-1} \cdot 100g^{-1}$. F6 and F15 (lactate) groups are represented as $mean \pm one \text{ standard deviation}$.

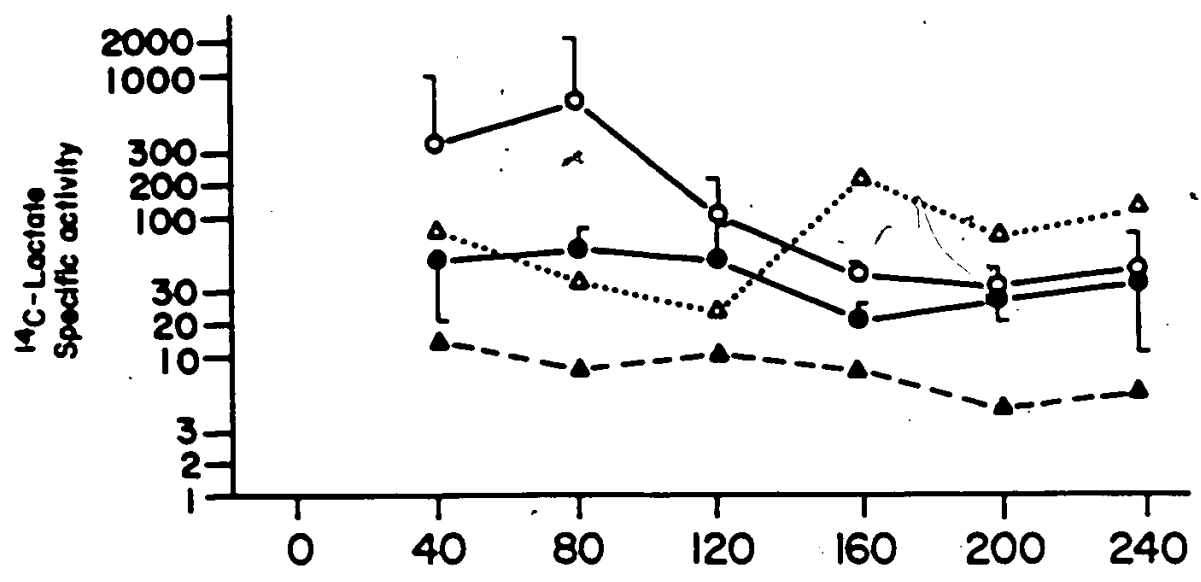
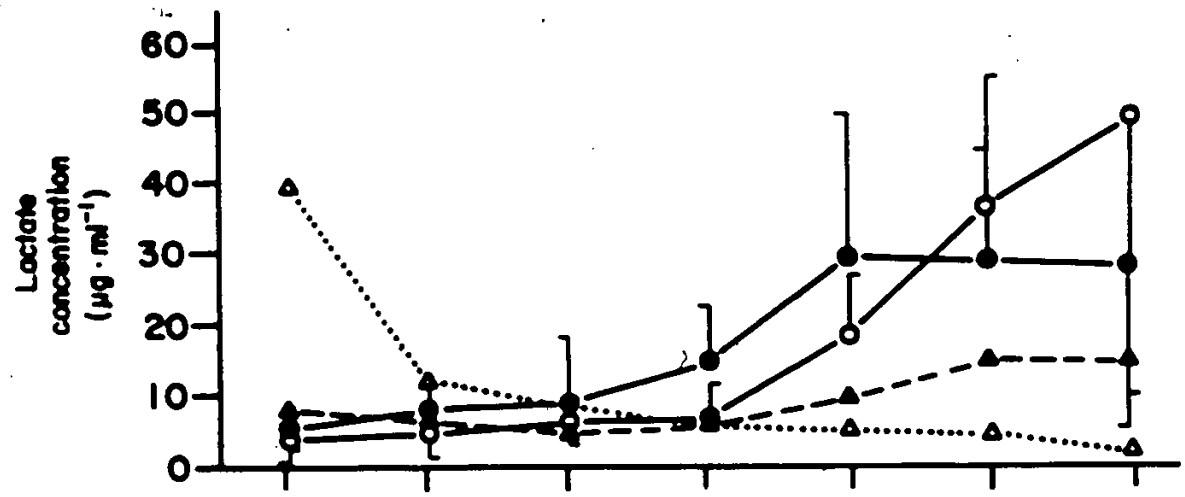
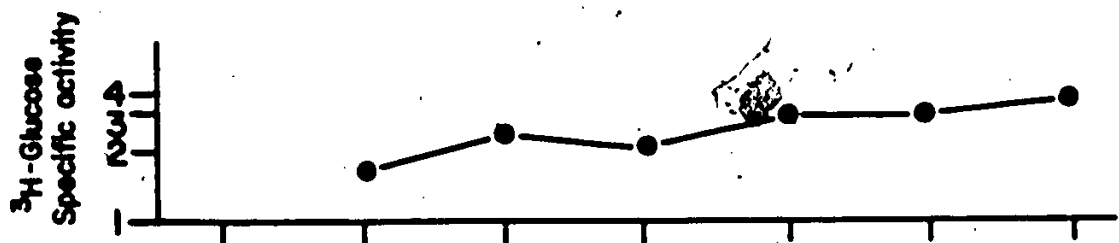
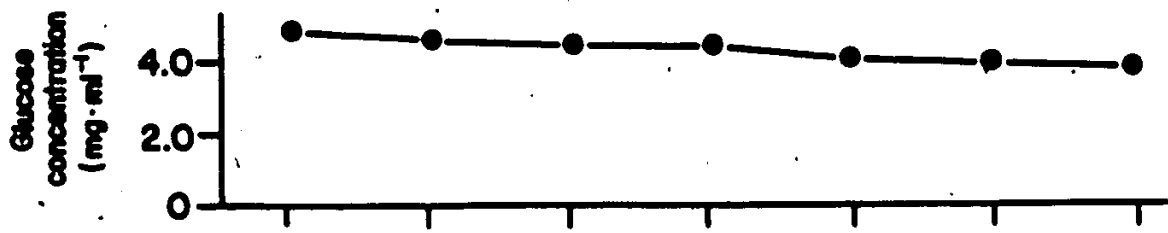


TABLE 4

Glucose and lactate kinetics in the food-deprived American eel

PARAMETER	AV	F6	F15	F36
(n)		(5)	(5)	(6)
GLUCOSE (mg·ml ⁻¹)	2.45	1.72 ± 0.42	2.04 ± 0.82	1.68 ± 0.65
R _T (Glucose) (mg·min ⁻¹ ·100g ⁻¹)	1.37	1.38 ± 0.47	0.09 ± 0.02* **	0.57 ± 0.35
MCR (Glucose) (ml·min ⁻¹ ·100g ⁻¹)	0.71	0.67 ± 0.35	0.04 ± 0.01* **	0.52 ± 0.40
(n)		(3)		(4)
LACTATE (μg·ml ⁻¹)	4.4	4.8 ± 0.8		5.2 ± 1.9
Ri(Lactate) (μg·min ⁻¹ ·100g ⁻¹)	5.40	12.51 ± 2.05*		10.03 ± 5.03
MCR(Lactate) (ml·min ⁻¹ ·100g ⁻¹)	1.74	2.31 ± 0.74	1.26 ± 0.37**	2.20 ± 0.97

Note: R_T(Glucose), glucose turnover rate; Ri(Lactate), lactate irreversible replacement rate; MCR, metabolic clearance rate (DSS and non-DSS); n values in parentheses represent DSS animals. All values are represented as mean ± one standard deviation. Plasma concentrations are group means of the final 120 minutes of sampling time (DSS). AV values are taken from Table 2.

*' denotes significant difference (p ≤ 0.05) from controls.
 '**' denotes significant difference (p ≤ 0.05) between F6 and F15 groups.

food-deprived eels maintained plasma glucose 200 to 400-fold higher than plasma lactate concentrations.

Mean specific activity curves approached plateau values for those animals in DSS in terms of the respective cold metabolites (Fig. 4B, C, Fig. 5C). Where plasma glucose or lactate concentrations deviated from DSS, plasma specific activities necessarily deviated. This was most apparent in the 15 month fish where specific activities decreased markedly as plasma lactate increased (Fig. 6D).

Glucose turnover (R_T) was not affected by 6 months of food-deprivation (Table 4). The fifteen month value decreased by 15-fold, significantly different from both fed and 6 month food-deprived individuals, even though minor changes occurred with respect to glucose concentration. Mean R_T (glucose) for 36 month food-deprived animals was approximately half the rate of fed immature fish, and mean plasma glucose concentrations tended to decrease.

The irreversible replacement rate of lactate was significantly increased at both 6 months and 36 months of food-deprivation, compared to the fed group (Table 4). R_i (lactate) could not be assessed for the 15 month food-deprived animals as lactate concentrations were not in DSS. Metabolic clearance rates of lactate could be calculated as MCR is independent of concentration.

Metabolic clearance rates of glucose and lactate were unaffected by 6 months of food-deprivation and 15 months of

food-deprivation for lactate (Table 4). MCR(glucose) after 15 months decreased significantly. In all cases, food-deprivation resulted in a significantly depressed MCR(glucose) relative to MCR(lactate).

Six and 15 month food-deprived animals out of DSS showed positive correlations between plasma lactate concentration and R_a (0.84, 0.89, respectively) and R_d (0.80, 0.79, respectively) (Table 5).

The only food-deprived animal considered not to be in DSS with respect to glucose, F6-5, exhibited both decreasing concentrations over the experimental time course (Fig. 6A) and hyperglycemia (Table 5). The rates of glucose appearance (R_a) and disappearance (R_d) for this animal were 55-fold less than the mean R_T (glucose) of the five other 6 month food-deprived animals (Table 4).

Those food-deprived animals showing increasing plasma lactate concentrations also maintained the highest rates of appearance and disappearance of plasma lactate when compared to those animals in DSS (compare Table 4 and 5). F36-6, the only animal of all observed in which plasma concentrations decreased over the experimental time course, maintained the lowest rates. Further, only this animal revealed a rate of disappearance higher than its rate of appearance (Table 5).

The rate of exchange of carbon from lactate to glucose ($R(L-G)$) and the proportion of glucose turnover derived from lactate carbon increased 3-fold after food-deprivation

TABLE 5

Food-deprived animals out of steady state

GROUP	LACTATE ($\mu\text{g}\cdot\text{ml}^{-1}$)	Ra (LACTATE) ($\mu\text{g}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$)	Rd (LACTATE) ($\mu\text{g}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$)
	6.3 \pm 3.2	14.9	15.7
F6	34.4 \pm 2.5	27.8	22.5
	45.6 \pm 26.6	80.7	64.7
	12.0 \pm 2.4	19.1	15.6
	14.2 \pm 1.8	28.8	26.9
F15	42.3 \pm 29.5	32.3	21.2
	42.8 \pm 12.7	42.5	28.4
	65.0 \pm 35.8	82.1	56.3
F36	3.7 \pm 1.3	4.3	5.4
	13.0 \pm 3.0	36.9	34.3
	GLUCOSE ($\text{mg}\cdot\text{ml}^{-1}$)	Ra (GLUCOSE) ($\text{mg}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$)	Rd (GLUCOSE) ($\text{mg}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$)
F6	4.05 \pm 0.16	0.035	0.036

Note: Ra, rate of appearance; Rd, rate of disappearance.

Plasma concentrations are individual means \pm one standard deviation of the final 120 minutes of sampling.

for 6 months (Table 6). Nonetheless, this value represented less than 1% of the total glucose turned over. The reciprocal exchange, $R(G \rightarrow L)$, was increased more than 4-fold in Fe animals (Table 6).

The estimated total lactate turnover after 6 months of food-deprivation was increased about three-fold (Table 6). The increased production of glucose from lactate carbon represented a further 10% of estimated total turnover, still 10% below lactate carbon irreversibly lost. The combination of increased total lactate turnover and increased lactate turned-over diverted towards glucose, reflected an overall increased production of glucose from lactate carbon.

Thirty-six month food-deprived animals showed comparable rates of conversion of lactate carbon to glucose ($R(G \rightarrow L)$) to those of fed immature eels (Table 6). Slightly more glucose turnover was derived from lactate. The rate of production of lactate from glucose ($R(L \rightarrow G)$) was somewhat higher than fed, immature animals (Table 6).

Estimated total lactate turnover in thirty-six month food-deprived animals was approximately 1.5-times that of fed immature animals (Table 6). This was predominantly represented by lactate carbon irreversibly lost. Only 22% was reconverted to glucose.

TABLE 6

Carbon exchange in food-deprived animals

PARAMETER	AV (6)		F36 (4)
$R_{L \rightarrow G}$ ($\mu\text{gC} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$)	7.40	$26.87 \pm 5.76^*$	8.00 ± 4.96
rate of conversion of plasma lactate carbon to plasma glucose carbon			
$R_{G \rightarrow L}$ ($\mu\text{gC} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$)	15.83	$66.27 \pm 24.29^*$	25.53 ± 11.76
rate of conversion of plasma glucose carbon to plasma lactate carbon			
$\frac{R_{L \rightarrow G} \times 100}{R_T(\text{Glucose})}$	0.29	$0.98 \pm 0.37^*$	0.51 ± 0.18
per cent of glucose turnover derived from lactate carbon			
Estimated total lactate turnover ($\mu\text{g} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$)	8.17	$22.58 \pm 3.99^*$	13.03 ± 6.04
Total lactate carbon turned over, converted to glucose	35.4	44.5^*	21.7
Total lactate carbon turned over, not returned to lactate.	64.6	55.5^*	79.3

Note: all values are represented as mean \pm one standard deviation of animals in dynamic steady state with respect to both glucose and lactate.

* represents a significant difference ($p \leq 0.05$) between F6 and controls (AV). AV values are taken from Table 3.

3.3 ANAESTHESIA

Fed and 8 month food-deprived animals under anaesthesia maintained constant arterial plasma glucose concentration (Fig. 7A), which were not significantly different from each other or from fed, free-swimming animals (VA) (Table 7). Plasma lactate concentrations were, however, severely affected by anaesthesia. Both experimental groups (TA, PTA) had plasma lactate concentrations significantly above the fed, free-swimming groups (Fig. 7B, Tables 2, 4, and 8; note- mg·ml⁻¹ in contrast with $\mu\text{g}\cdot\text{ml}^{-1}$). These values constantly increased over the experimental period for both fed and food-deprived anaesthetized groups of fish, indicating the absence of a DSS with respect to lactate (Fig. 7B).

Glucose specific activity curves for both anaesthetized groups reached plateaux (Fig. 7C). Those for lactate decreased following the increased cold concentrations (Fig. 7D).

Haematocrit values were significantly increased in both anaesthetized groups above fed, free-swimming animals ($.40 \pm .05$, $.43 \pm .05$, and $.23 \pm .05$, for TA, PTA, and VA, respectively). Continual sampling resulted in decreases in haematocrit of 50% in these animals, dependent on body weight. No such decreases were apparent in free-swimming animals, where red cells were reinjected.

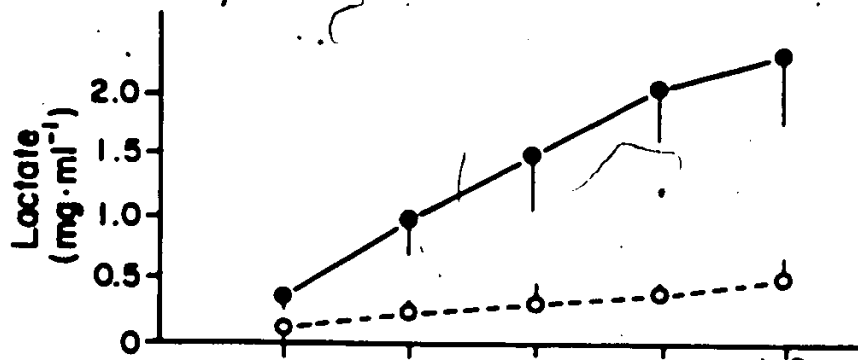
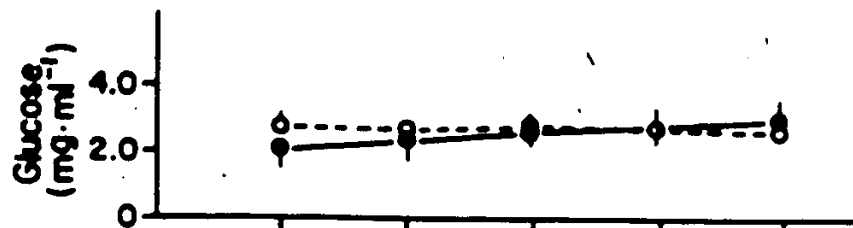
Figure 7: Plasma concentrations and specific activities in anaesthetized animals

Fed (—●—, TA) and 8 month food-deprived (---○---, FTA) animals.

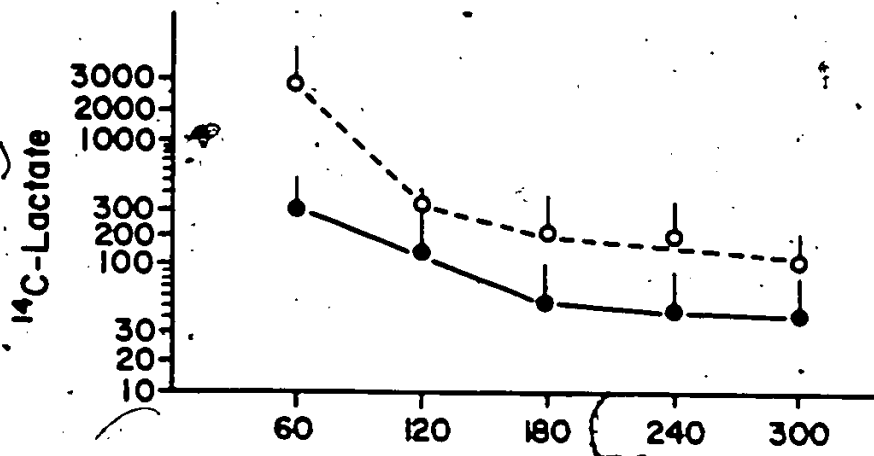
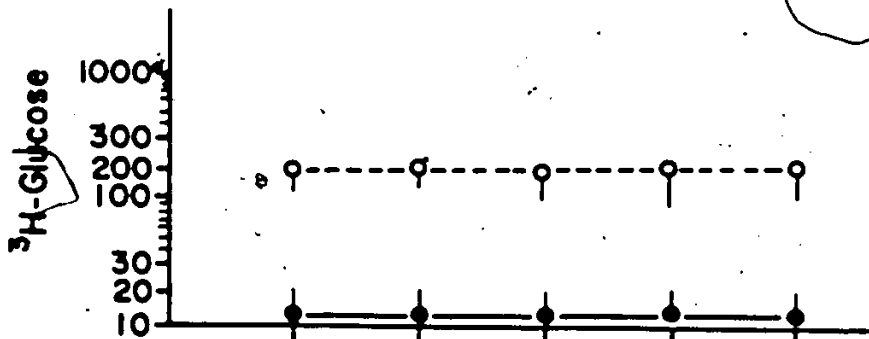
Specific activity is represented as $SA \cdot R^{1/2} \cdot 100g^{-1}$, in $min \cdot mg^{-1} \cdot 100g^{-1}$. All lactate values are out of dynamic steady state.

All values are represented as mean \pm one standard deviation.

Concentration



Specific activity



Time (min)

TABLE 7

Metabolic turnover rates of anaesthetized animals

PARAMETER	VA (6)	TA (6)	F6 (6)	FTA (5)
Glucose (mg·ml ⁻¹)	1.43	2.93 ± 0.94*	1.72	2.75 ± 0.77
R _T (Glucose) (mg·min ⁻¹ ·100g ⁻¹)	0.69	.0390 ± .0210*	1.38	.0031 ± .0008**
MCR(Glucose) (ml·min ⁻¹ ·100g ⁻¹)	0.58	.0210 ± .0210*	0.67	.0031 ± .0003**
MCR(Lactate) (ml·min ⁻¹ ·100g ⁻¹)	1.04	.0085 ± .0030*	2.31	.0080 ± .0010

Note: plasma glucose concentrations are group means of the final 120 minutes of sampling; R_T(glucose), the rate of glucose turnover; MCR, metabolic clearance rate; n in parentheses.

All values are represented as mean ± one standard deviation. '*' denotes significant difference (p ≤ 0.05) of TA from VA. '**' denotes significant difference (p ≤ 0.05) of FTA from VA.

VA and F6 values are taken from Tables 2 and 4, respectively.

Turnover and metabolic clearance rates of glucose were depressed 20-fold in the fed, anaesthetized animal as compared to the fed, free-swimming animal (Table 7). Deprivation of food for 8 months further depressed R_T and MCR by 10-fold, and even more when compared to F6 animals (Table 4).

Metabolic clearance rates of lactate were equivalent in both anaesthetized groups and 100-fold lower than these values in the free-swimming animal (Table 7). In the fed groups, the MCR(glucose) was significantly higher than that of lactate; in the food-deprived group, this was reversed.

Although lactate concentrations were significantly lower in food-deprived than in fed anaesthetized animals, the rate of appearance of lactate was generally higher (with one exception) (Table 8). Disappearance rates were considerably lower in the fed group, even zero.

Fed anaesthetized animals showed no definite correlations between lactate concentrations and either R_a or R_d (lactate) (Table 8). In 8 month food-deprived animals a positive correlation between concentration and R_a (0.88) and R_d (0.82) was apparent. No similar correlation was found for glucose.

TABLE 8

Rates of appearance and disappearance of plasma lactate

GROUP	LACTATE (mg·ml ⁻¹)	Ra (LACTATE) (mg·min ⁻¹ ·100g ⁻¹)	Rd (LACTATE) (mg·min ⁻¹ ·100g ⁻¹)
	0.77 ± 0.19	0.221	0.036
	1.21 ± 0.61	0.254	0.0
TA	1.36 ± 0.35	0.029	0.0
	1.58 ± 0.21	0.053	0.0
	2.36 ± 0.52	0.565	0.120
	3.01 ± 0.21	0.148	0.0
	0.08 ± 0.03	0.997	0.960
	0.49 ± 0.07	0.465	0.242
FTA	0.53 ± 0.16	1.010	0.750
	0.61 ± 0.22	0.469	0.173
	0.70 ± 0.28	0.451	0.155

Note: Ra, rate of appearance; Rd, rate of disappearance.

Plasma concentrations are represented as individual means ± one standard deviation of the final 120 minutes of sampling.

Chapter IV
DISCUSSION

4.1 FED ANIMALS

4.1.1 Plasma Concentrations

4.1.1.1 Glucose

Inter- and intra-specific variation in fish blood glucose can be extensive (Chavin and Young, 1970). Both experimental technique and physiological factors are implicated in these differences.

Generally reported mean plasma concentrations for A. rostrata are close to $1.0 \text{ mg} \cdot \text{ml}^{-1}$ (Butler, 1968; Inui and Yokote, 1975; Epple and Lewis, 1977), although, Bendayan and Rasio (1981) found low temperature acclimation can increase this value to $6.0 \text{ mg} \cdot \text{ml}^{-1}$ or above. Mean concentrations reported in this study (Table 2) range from 1.43 (VA) and 1.49 (FN) to 2.45 (AV) $\text{mg} \cdot \text{ml}^{-1}$. These values were not significantly different, suggesting the duration of laboratory acclimation had no effect on plasma concentrations and that no

apparent differences are maintained between arterial and venous concentrations. Only one 6 month food-deprived animal had plasma concentrations in excess of $4.0 \text{ mg} \cdot \text{ml}^{-1}$ (Fig. 6A). This animal could be considered hyperglycemic.

Individual eels maintained constant plasma concentrations over the duration of the experiments (Fig. 2A). This is in contrast with the kelp bass studies of Bever *et al.* (1977; 1981). Of the 4 animals reported, the least variation over 24 hours was $0.35 \text{ mg} \cdot \text{ml}^{-1}$; one fish exhibited changes of $1.00 \text{ mg} \cdot \text{ml}^{-1}$. No specific diel pattern was apparent. Eel plasma concentrations varied no more than $0.05 \text{ mg} \cdot \text{ml}^{-1}$ within any individual over the duration of the experiment. Diel concentrations monitored in *A. rostrata* over a 33 hour sampling period, remained relatively unchanged and independent of changes in plasma cortisol (Appendix A, Fig. 10). The American eel, in contrast to kelp bass, appears capable of maintaining constant blood sugar levels and as such, a more important role for glucose in the eel would be expected.

4.1.1.2 Lactate

Plasma lactate concentrations, less than $10 \text{ } \mu\text{g} \cdot \text{ml}^{-1}$ (Fig. 3A, Table 2), are about 6- to 10-fold less than values previously reported for this genus (Larsson and Lewander, 1973; Dave *et al.*, 1975; Phillips and Hird, 1977). Blood withdrawal from a patent cannula abolishes any struggle associated with obtaining the sample. Unnecessary muscular contrac-

tions can increase plasma lactate concentrations. Oikari and Soivio (1975) reported that lactate concentrations determined from stunned fish do not represent the resting state. Lactate values obtained in this study from free-swimming animals are probably very close to natural, resting plasma concentrations in the American eel.

No significant differences were apparent between arterial and venous samples (Table 2). Nor did laboratory acclimation affect concentrations. Lactate concentrations were 300- to 500-fold lower than plasma glucose concentrations suggesting that plasma lactate cannot contribute significantly as a gluconeogenic substrate under these conditions.

4.1.2 Glucose Kinetics

The rate of turnover of glucose represents the rate of its appearance in and disappearance from the plasma, hence, it is the rate of utilization of glucose by a given species. Under steady state conditions and without exogenous input, glucose production by the liver and to a small extent, the kidney, must equal glucose demand by the body's tissues. When production equals demand, the proportion of stored carbohydrate (glycogen) is unimportant. Glucose utilization in muscle occurs primarily by glycogenolysis, but turnover rate does not measure this directly. However, hepatic glucose output which necessarily replenishes the depleted muscle

carbohydrate, is accounted for in turnover estimations. The turnover rate, therefore, estimates the total rate of glucose production, whether due to glycogenolysis or gluconeogenesis. In mammals, fasting (24-48 hrs) prior to glucose turnover estimations promotes hepatic glycogenolysis dissociating glucose produced by these two pathways. Eels, however, maintain glycogen content over months of food-deprivation (Renaud and Moon, 1980b), so glucose production primarily estimates gluconeogenesis, even under fed conditions. Further evidence for this is given later (p. 93).

Glucose utilization varies greatly in those species which have been studied (Table 9). The lowest values are in kelp bass and the highest in goldfinches. Within mammals, the highest values are reported in rat, approximately 2- to 3-fold higher than dog, cat, monkey, and pig, and 10-fold higher than sheep and horse. The rate of glucose utilization by A. rostrata reported in this study is similar to rat and more than 10-fold above that of kelp bass and coho salmon (Table 2 and 9).

The large interspecific differences between fish can partially be explained by methodological differences. Studies in coho salmon involved light anaesthesia followed by intraperitoneal injection of tracer (Lin et al., 1978). Turnover rates determined in this manner are inaccurate as discussed in the Introduction. Studies on kelp bass (Bever et al.,



TABLE 9

Interspecific glucose turnover rates

Concentration (Conc) is in $\text{mg}\cdot\text{ml}^{-1}$; Turnover is in $\text{mg}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$; Metabolic clearance rate (MCR) is in $\text{ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$. All animals were post-absorptive unless otherwise indicated.

^3H -6-glucose was employed in all studies with noted exceptions. Metabolic clearance rates were computed as the ratio of turnover to concentration (Norwich, 1977) if not supplied by the author.

Authors: (1) Marsh and Dawson, 1982; (2) Riesenfeld et al., 1981; (3) Groscolas and Rodriguez, 1981; (4) Armstrong et al., 1979; (5) Hall et al., 1979; (6) Katz et al., 1974b; (7) Freminet and LeClerc, 1980; (8) Kettlehut et al., 1980; (9) Issekutz, 1977; (10) Anwer et al., 1976; (11) Trayhurn et al., 1981; (12) Brockman et al., 1975; (13) Lin et al., 1978; (14) Bever et al., 1977, 1981.

SPECIES	CONDITION	CONC.	TURNOVER	MCR	AUTHOR
Goldfinch	³ H-3-glucose				
	30°C	4.05	3.14	0.78	(1)
	-15°C	4.34	2.92	0.61	
Rock chicken		2.04	1.31	0.64	(2)
Emperor penguin		2.81	0.80		(3)
Cynomolgus monkey	64hr fast	0.49	0.40	0.81	(4)
Man	³ H-3-glucose	0.90	0.18	0.20	(5)
Rabbit	³ H-2-glucose	1.07-1.65	0.30-0.65		(6)
Guinea-pig	fed	1.31	0.90	0.69	(7)
	48hr fast	1.31	0.80	0.61	
Rat	³ H-2-glucose				
	HI CHO diet	1.00	1.10	1.10	(8)
	HI PRO diet	0.85	0.96	1.13	
Cat	³ H-2-glucose				
	HI CHO diet	0.90	0.52	0.58	(8)
	HI PRO diet	0.75	0.52	0.69	
Dog		1.20	0.36	0.30	(9)
Horse	fed	1.00-1.21	0.11-0.14		(10)
	72hr fast	0.84-1.02	0.06-0.13		
Pig	24hr fast	0.81	0.37	0.46	(11)
Sheep	fed	0.45	0.14	0.31	(12)
	3d fast	0.36	0.07	0.19	
American eel (PN)		1.49	0.76	0.48	
Coho salmon		0.63	0.043	0.07	(13)
Kelp bass	fed	0.06-1.59	0.035	0.07	(14)
	20-42d fast	0.04-1.00	0.025	0.05	
	72d fast	0.59	0.035	0.06	

1977, 1981) employed intravascular injection known to yield identical results to infusion studies in rats (Katz et al., 1974a) although the latter method is more reliable (Okajima et al., 1981). The large differences in glucose turnover between eels and the kelp bass must be considered inherent interspecific differences. Mammalian differences of this magnitude are apparent (i.e., rat and sheep).

Metabolic clearance rate, the volume of metabolite cleared from the blood per unit time, represents the efficiency of removal of that metabolite. This value represents net clearance of a compound by all routes of disposal: excretion, degradation, and sequestration (Riggs, 1963; Shipley and Clark, 1972).

Clearance is independent of substrate concentration up to some critical concentration beyond which dependency is exhibited. It can be modified by hormones (e.g., insulin; Shipley and Clark, 1972) or redistribution of blood flow (e.g., exercise; Issekutz et al., 1976). Any factor affecting the rate of uptake of a compound out of proportion of its concentration, will affect clearance rate.

Clearance rates for glucose calculated as Rt/\bar{C} (Norwich, 1977) from mean values expressed by the authors are presented in Table 9. The highest values are apparent for rat. Mammalian values range from 0.20 (man) to 1.13 (rat) $\text{ml} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$. Values for eels are intermediate, averaging $0.6 \text{ ml} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ (Tables 2 and 9). Considering the major

differences in turnover rates, clearance rates are relatively constant between species. This implies that tissue uptake mechanisms and control systems are common to all systems. The notable differences include coho salmon and kelp bass, which also have low plasma glucose concentrations and turnover rates (Table 9).

The causal determinants associated with such large discrepancies between eel and bass turnover rates are not apparent. Diet is known to alter glucose production in fish (Covey *et al.*, 1977a,b), but both fish were maintained on high protein diets (earthworms for eels; squid for bass). Rate differences could not be explained by normal temperature effects (Q_{10} values are 2-3 per 10°C change; Prosser, 1973); bass were maintained at 18-20°C, eels at 12-15°C.

Rather, these differences may reflect different lifestyles, different activity levels, or differential use of metabolic stores and biochemical pathways. Kelp bass occupy a rather limited area off the coast of Southern California (Stapleton, 1968) and compared with eels (Sinha and Jones, 1975), they travel little.

Activity differences may result in altered quantities and proportions of red and white muscle (Love, 1970). Oxygen consumption (Gordon, 1972a,b) and enzyme profiles (Johnston, 1977) are quite distinct in these two muscle types and between species in the same muscle type. Thus, distinct inter-specific comparisons of turnover rates may be expected.

The large fluctuations in glucose concentrations exhibited by kelp bass measured over periods of 24 hrs. (Bever et al., 1977) suggest that glucose homeostasis is poor, and that plasma glucose (and hence carbohydrate) may not be an important substrate in these animals. The measured turnover rates confirm this suggestion (Table 9). In contrast, eels maintain constant plasma glucose concentrations (Appendix A, Fig. 10) and high R_T values (Tables 2 and 9). Thus, glucose is an important substrate for eels under these experimental conditions.

The large standard deviations noted for R_T reflect large individual differences (Table 2). Similar differences are reported in mammalian studies. Glucose turnover rates in rabbits average $3.98 \text{ mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ while values span a range of $3.41 \text{ mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ (Katz et al., 1974b). Glucose turnover rates in kelp bass (Bever et al., 1977, 1981) and lactate turnover rates in rats (Okajima et al., 1981) vary in a similar manner. Intraspecific variation is, apparently, a widespread phenomenon in these studies. Unfortunately, it obscures any significant differences between VA and AV or AV and FN fish though mean values vary greatly (Table 2).

Eel glucose turnover rates comparable to rats (Katz et al., 1974b; Kusaka and Ui, 1977) were not expected (Table 9). Weight specific oxygen consumption rates are approximately 10-fold lower in fish than in mammals (Prosser, 1973), but fish are reported to depend to a larger extent on

anaerobic metabolism (Bennett, 1978). Considering the similarity of glucose utilization in A. rostrata and the laboratory rat, but the large difference in metabolic rates, one might assume glucose utilization to be primarily anaerobic. This would agree with low glucose oxidation rates in plaice compared with mite (Covey et al., 1975), but not with the low plasma lactate concentrations and turnover rates noted for the American eel.

4.1.3 Lactate Kinetics

Lactate turnover rates have not previously been estimated in fish, and fewer mammalian species have been studied in this regard than with respect to glucose turnover (Tables 9 and 10). Lactate irreversible replacement rate is lower than glucose turnover rate, for a given species, although lactate turnover rate may be higher, at least for rat. The metabolic clearance rate of lactate is generally higher than that of glucose.

Irreversible lactate replacement rates estimated for A. rostrata are low compared to those of mammals (Tables 2 and 10): 50- to 100-fold lower than dogs; up to 200-fold lower than rats; and 160- to 200-fold lower than glucose turnover rates determined in the eel (Tables 2 and 9).

TABLE 10

Interspecific lactate irreversible replacement rates

SPECIES	CONDITION	CONC.	Ri(LACTATE)	MCR	AUTHOR
Man		0.05-0.12	0.09-0.15		(1)
Rat	(A-VC)	0.056-0.117			(2)
	¹⁴ C-U-lactate		0.62-0.88	6.5-12.3	
	³ H-3-lactate		1.20-2.20	13.5-23.8	
	(VA)	0.064-0.100			
	¹⁴ C-U-lactate		0.45-0.64	5.2-8.7	
	³ H-3-lactate		0.65-1.04	8.3-14.6	
Guinea-pig	fed	0.12	0.38	3.17	(3)
	48hr fast	0.11	0.35	3.18	
Dog	0d fast	0.255	0.32	1.3	(4)
	21d fast	0.101	0.20	2.0	
Sheep	arterial s.	0.05	0.010	0.20	(5)
	venous s.	0.06	0.015	0.25	
American eel (FN)		0.007	0.005	0.45	

Concentration (Conc) is in $\text{mg}\cdot\text{ml}^{-1}$; Turnover is in $\text{mg}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$; Metabolic clearance rate (MCR) is in $\text{ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$. ¹⁴C-U-lactate was employed if not noted. Metabolic clearance rates were computed as in Table 9, if not supplied by the author.

Authors: (1) Kreisberg *et al.*, 1971; (2) Okajima *et al.*, 1981; (3) Freminet and LeClerc, 1980; (4) Brady *et al.*, 1977; (5) Reilly and Chadresena, 1977.

Total lactate turnover rates (as would be determined by infusing ^3H -lactate) were estimated by adding the rate of conversion of lactate to glucose ($R(L \rightarrow G)$) and the irreversible replacement rate ($R_i(\text{lactate})$) (Table 3). Some ^{14}C -lactate may be converted to glucose and not reconverted to lactate (Okajima et al., 1981), which could result in an overestimate of this value. For rats, total lactate turnover (^3H -lactate; Table 10) is 2-fold greater than the irreversible replacement rate (^{14}C -lactate). In eels, the estimate is 1.5-fold greater and possibly close to the real turnover value (compare $R_i(\text{lactate})$, Table 2 with estimated total lactate turnover, Table 3).

The MCR of lactate was also low (Tables 2 and 10), consistent with low plasma concentration and turnover rates, and in agreement with early studies of swimming energetics. Following recovery from muscular activity, blood lactate is cleared very slowly, requiring 12 to 24 hrs to return plasma concentrations to normal (Black, 1957; Black et al., 1962), in contrast to one hr in dogs (Cresticelli and Taylor, 1944). These species differences are reflected in estimated clearance rates (Table 10) with eel values well below those of mammals.

The experimental condition used in this study allowed only slow swimming in these eels. Red muscle, utilized by most fish under these swimming conditions, would totally oxidize metabolic fuels (Bilinski, 1974; Johnston and Moon,

1981). A. anguilla does use white muscle under these conditions (Grillner and Kashin, 1976); lactate production would be expected by the white muscle as a result of its low perfusion (Satchell, 1971) and its primarily anaerobic metabolism (Hulbert and Moon, 1978c).

No data are available to show lactate production under these conditions by eel white muscle. Previous studies, however, have found that muscle lactate release from a number of fish species is very slow (Black, 1957; Black et al., 1962, 1966; Wardle, 1978; Turner et al., 1983a,b). Slow release may be an adaptation to overcome poor plasma buffer capacities in fish, and thus, the lethality of acid flooding the system following exercise (Bennett, 1978; Wardle, 1978). Conversely, fish are known to have high muscle buffering capacities (Castellini and Somero, 1981) and it is possible that muscle lactate could be reconverted to glucose in situ (Wittenberger and Diaciuc, 1965; Wittenberger et al. 1975; Hulbert and Moon, 1978a; Moon and Johnston, 1980; Turner et al., 1983a,b) just as reported for mammalian muscle (Isselkutz et al., 1976; Hermansen and Vaage, 1977).

If reversion does occur in the muscle (red and/or white), lactate would not reappear in the circulation, thus accounting for the low lactate turnover and metabolic clearance rates reported here.

As reversion is not 100% efficient (Hermansen and Vaage, 1977), additional glucose must be added to the meta-

bolite pool of the muscle, thus, partially accounting for the higher than expected rate of glucose turnover and metabolic clearance.

Such speculations await a better experimental understanding of plasma and muscle lactate changes in the resting American eel.

4.1.4 Carbon exchange

The data reported here reveal apparent discrepancies. The lactate production rate ($R_i(\text{lactate})$) suggests limited glycolytic activity, while the rate of production of lactate from glucose ($R(G \rightarrow L)$) indicates a contradictory situation (Tables 2 and 3). To reconcile this paradox, further experiments are necessary and in their absence, a speculative argument is presented based upon studies of widely varying fish species and limited published data.

The slow appearance of lactate into the blood in post-exercise recovery studies for fish (Black, 1957; Black et al., 1962, 1966; Wood et al., 1977; Wardle, 1978; Turner, et al., 1983a,b), may be interpreted as a slow release from producing tissues, rather than low production rates. $R_i(\text{lactate})$ values alone cannot distinguish between the two possibilities. Large discrepancies between glucose and lactate turnover rates would suggest a predominance of aerobic glucose metabolism, in which case, lactate appearance would account

for most if not all lactate production. Metabolic rates, however, do not support high glucose oxidation rates, as shown below, suggesting that $R_i(\text{lactate})$ values do not reflect total lactate production.

The complete oxidation of one mole of glucose requires 6 moles of oxygen (Lehninger, 1975). At 15°, the oxygen consumption rate of fed A. rostrata is 30 ml·hr⁻¹·kg⁻¹ (Walsh et al., 1983). If total oxygen consumption was utilized in the oxidation of glucose, only 8.0% of the glucose turned over could undergo complete oxidation (based on rates of 0.8 mg·min⁻¹·100g⁻¹ (FN); see Appendix C for calculations). This is in better agreement with the general view of fish glucose metabolism (eg, Cowey et al., 1975), but it leaves the fate of glucose in A. rostrata unsolved. Low lactate production suggests low anaerobic catabolism and metabolic rates suggest minimal complete oxidation. Glucose utilized may be primarily involved in biosynthesis.

Lipid synthesis in immature A. rostrata is known to be slow, though continual (Aster, 1980) and probably little glucose is directed towards triglyceride biosynthesis. Further, Nagai and Ikeda (1971a) reported that dietary amino acids in carp were the best precursors for lipid synthesis.

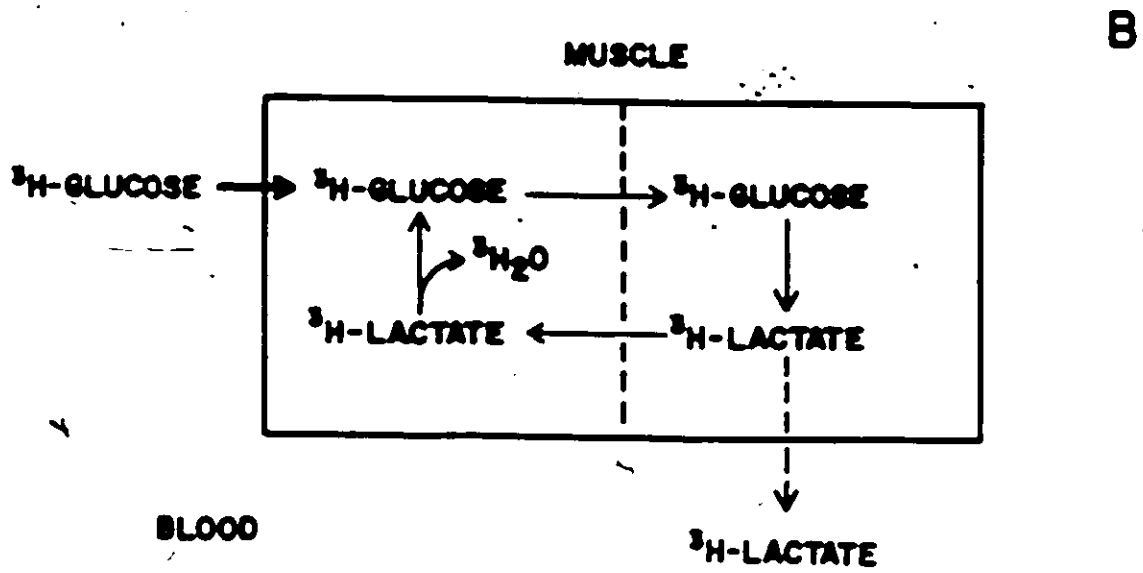
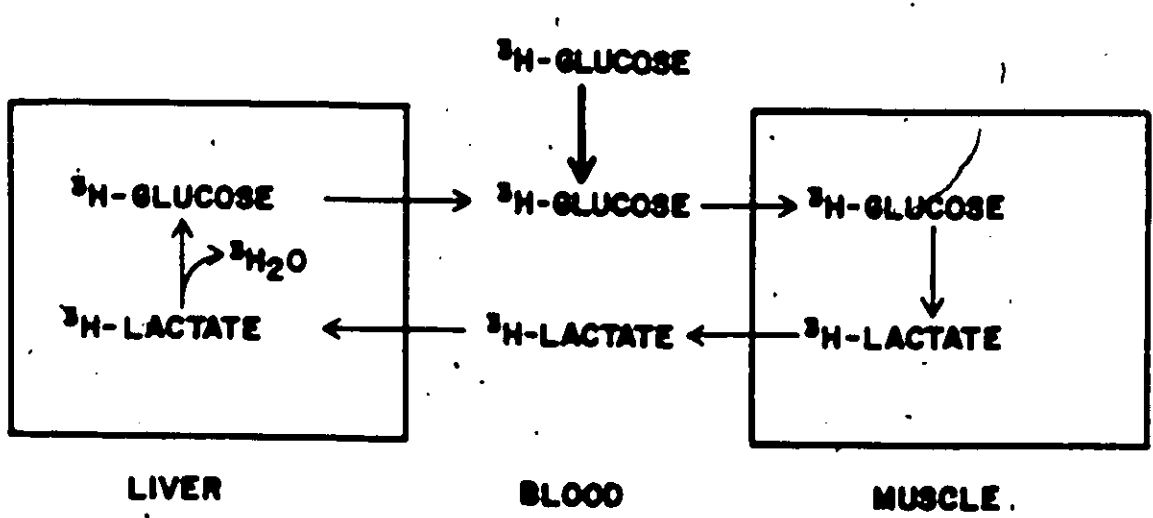
Lactate production rates from glucose ($R(G-L)$, Table 3) suggest high glycolytic rates in these animals, not reflected in lactate production rates ($R_i(\text{lactate})$). However, there is a large discrepancy between ³H-specific activities

of glucose and lactate in the plasma which cannot be explained based on known concepts of tracer kinetics and carbon exchange between these two metabolites. Applying equation (vii) to these data (with the appropriate changes), the percentage of glucose turned over, contributing to lactate would be 385% (PN values). An apparent concentrating of ^3H in plasma lactate has occurred, resulting in higher specific activities than those of plasma glucose. In the conventional mammalian system (depicted, Fig. 8A), this is impossible. Any rise in ^3H -lactate specific activity, due to recycling (Okajima et al., 1981) would also be apparent in ^3H -glucose, the latter always maintaining a higher specific activity. This occurs because lactate enters the blood prior to (re)conversion to glucose. This is the expected trend, though, to the author's knowledge, no exchange between ^3H -glucose and ^3H -lactate has been previously reported.

To account for the discrepancy in specific activity, substantial recycling without metabolite release into the blood stream is required. It is possible that lactate/glucose recycling within the muscle (Wittenberger and Diaciuc, 1965; Wittenberger et al., 1975; Hulbert and Moon, 1978a; Moon and Johnston, 1980; Turner et al., 1983a,b), coupled with slow lactate release from this tissue (Wood et al., 1977; Wardle,

Figure 8: Tracer exchange

- A: Conventional mammalian pathway.
- B: Proposed Anguilliform pathway.



1978; Turner et al., 1983a,b) might possibly account for the high ^3H -lactate specific activity (Fig. 8B). Recycling of ^3H between glucose and lactate, coupled to continual uptake of ^3H -glucose into the muscle to replenish loss due to metabolic inefficiencies might partially resolve this discrepancy.

The differential rates of proton and lactate release noted by Turner et al. (1983b) may be related to the excess of ^3H determined in eel plasma. However, the ^3H would have to originate from C-1 of lactate (C-3 or C-4 of glucose) to be extruded as protons, which cannot be reconciled in view of known sites of tracer loss (Katz, 1979).

The calculation of the rate of recycling necessary to account for the increased lactate specific activity requires far too many assumptions. The presence of the system could, however, be assessed by determining ^3H -glucose infusion over successive time courses. The specific activity of lactate and other glycolytic intermediates in muscle should successively increase, eventually exceeding plasma glucose specific activity.

If this pathway (or recycling in any other tissue) occurs, lactate production from glucose may be much higher than the lactate irreversible replacement rates indicate, though $R(\text{G} \rightarrow \text{L})$ would overestimate the value due to ^3H recycling. Slow release of lactate from the muscle falsely lowers $R_i(\text{lactate})$ estimations. Further, low plasma lactate

concentrations disallow high glucose production rates from ^3H -lactate in other tissues (eg, liver). Hence, first, plasma glucose is primarily derived from precursors other than lactate and, second, in view of this, plasma ^3H -glucose specific activity is probably not falsely increased (or only marginally so), so that glucose turnover rate estimations should not be affected. These tentative suggestions involve considerable metabolic activity given the resting state of the animal. This may, however, be accounted for by the large skeletal muscle mass.

Muscle glycogen turnover at rest has not been determined in fish, with the exception of qualitative estimations (Wittenberger, 1972), and swimming energetics have not been assessed in an Anguilla spp. Total muscle glycogen content in these animals is approximately 140 mg, based on determinations by Moon (1983a) and red and white muscle proportions in Anguilla spp. (Greer-Walker and Pull, 1975; Hulbert and Moon, 1978b) Data reported by Johnston and Goldspink (1973) for the coalfish (Gadus virens) indicated that at swimming speeds of $1 \text{ body length} \cdot \text{s}^{-1}$, 12% of glycogen in red and 7% in white muscle is consumed. Assuming (1) similar swimming energetics in eels, (2) an average of 10% glycogen utilized at speeds of $1 \text{ bl} \cdot \text{s}^{-1}$, and (3) swimming speeds of $0.001 \text{ bl} \cdot \text{s}^{-1}$, probably consistent with the activity observed, $0.85 \text{ mg glucose units} \cdot \text{min}^{-1}$ would be consumed (see Appendix C). These calculations suggest that a large proportion of the

glucose utilized may be associated with glycogen biosynthesis (possibly in conjunction with lactate recycling), replacing carbohydrate utilized in muscular contraction even at minimal swimming speeds.

Although there is no direct evidence to suggest that this is the case, this could account for high glucose utilization rates, low lactate production rates, and the apparently excessive specific activity of ^3H -lactate. The high rate of glucose production, however, is unresolved. What precursor(s) can account for the high rates of glucose production?

In agreement with low rates of lactate appearance, the rates of conversion of lactate to glucose are also low, $7.4 \mu\text{g C}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$. Proportionately, however, this represents 35% of the lactate released (Table 3). This value is almost two-fold higher than mammalian values. In fed rats at rest (Kusaka and Ui, 1977), only 19% of the lactate produced is converted to glucose; dogs at rest showed similar values (Issekutz *et al.*, 1976; Hetenyi *et al.*, 1980). Following exercise, this value is increased to 30-38% in dogs (Issekutz *et al.*) or values similar to resting eels.

The Cori cycle could represent an important means of carbon retrieval at rest in the eel as previously suggested by Renaud and Moon (1980b). The increased lactate production associated with muscular activity may enhance this role in the eel as reported in mammals (Issekutz *et al.*, 1976). The *in vitro* liver slice studies of Mosse (1980) support this

contention. Snapper (Chrysophrys auratus) held without exercise decreased the contribution of lactate to glucose 12-fold, and both black bream (Ancanthopagrus butcheri) and snapper increased this rate 4-fold when forced to swim continuously for 14 days. Both these species at rest had significantly higher rates of lactate incorporation into glucose than more sluggish species (sand flathead, Platycephalus bassiensis, and greenback flounder, Rhombosolea tapirina). Australian salmon, Arripis trutta, incorporated no lactate into glucose. Alanine gluconeogenesis was not significantly affected by activity nor did it vary interspecifically. It is not clear whether increased gluconeogenic rates were associated with increased plasma concentrations, as these were not reported. These studies strongly support physical activity as a determinant of gluconeogenesis, but no mechanism(s) was proposed.

Sanchez-Medina et al. (1972) found that rat kidney gluconeogenesis (lactate, pyruvate, glutamine, and glutamate) increased with exercise. They proposed that the acidosis resulting from increasing lactate concentrations was the effective stimulant. Bicarbonate infusion inhibited this gluconeogenic increase.

Although a large proportion of the lactate carbon turned over in the eel was converted to glucose, this represents quantitatively little glucose. Glucose production rates are at least 160-fold greater than lactate production rates.

Thus, less than 1% of glucose turned over was derived from lactate. Considering tracer dilution by exchange of ^{13}C for ^{14}C within the oxaloacetate pool, this value could increase at most by 2-fold (Hetenyi, 1982). Similar values in mammals are: man, range from 10 to 33% (Cahill et al., 1966); rat, 20% (Kusaka and Ui, 1977) to 50% (Okajima et al., 1981); and dog, 9% (Issekutz et al., 1976), 14% (Hetenyi et al., 1980) and 20% (Wolfe et al., 1977). These discrepancies may be related to activity state, hormones, or plasma lactate concentrations.

Whereas mammals divert significant amounts of lactate to glucose, essentially all of the glucose produced in eels at rest is derived from some other source(s). Bever et al. (1977) found essentially no carbon recycling between glucose and lactate. Lin et al. (1978), however, reported recycling in coho salmon to be 19%. This implies species-specific differences, but it may be related only to lactate concentrations. In vitro incubations of liver tissue slices or isolated hepatocytes report that lactate supports high rates of glucose production in Anquilla (Hayashi and Ooshiro, 1975a,b; Phillips and Hird, 1977; Renaud and Moon, 1980b). Renaud and Moon (1980b) found[?] lactate to be a better glucose precursor than selected amino acids in the eel hepatocyte. Similar results have been reported for trout hepatocytes (French et al., 1981). The present in vivo study found that this is not the case in the free-swimming ani-

mal. In vitro assessments may be affected by the absence of extrinsic control, abnormal substrate concentrations, and the presence or absence of other substrates.

Other studies have demonstrated that the contribution of a precursor to glucose production can be modified by other substrates. Examples are lactate (Issekutz et al., 1976) and glycerol (Steele et al., 1971) in mammals. Amino acids were shown to increase gluconeogenesis from pyruvate and leucine progressively increased gluconeogenic rates from alanine, pyruvate, and lactate in eel hepatocytes (Hayashi and Ooshiro, 1979). Nagai and Ikeda (1972) found increased plasma glucose concentrations following glutamate injection. Such studies reflect the problems of interpretation of in vitro gluconeogenic rate determinations using high concentrations of a single precursor in the absence of physiological substrate concentrations of other precursors. Lactate may not be preferentially utilized in the presence of other substrates. Determinations in the free-swimming animals suggest this is the case (Table 3), at least under conditions of low plasma concentrations.

If lactate is not a major contributor to glucose production, what other possibilities could explain the high production rates noted here? Assuming a hepatic glycogen content of 10 mg·100mg⁻¹ wet weight and a hepatosomatic index of 1.0 (Renaud and Moon, 1980b), at a glucose production rate of 1.0 mg·min⁻¹·100g⁻¹, a resting 100g eel could sup-

port glucose production for about 1.5 hrs by hepatic glycogenolysis. (This is not unlike the situation in rat, with similar production rates (Katz et al., 1974b) and hepatic glycogen contents (Newsholme and Start, 1973).) Hepatic glycogen, therefore, cannot represent a major source of plasma glucose. Omnivorous animals can derive all of the required carbohydrate exogenously (Newsholme and Start, 1973). Dietary sources of carbohydrate available to the eel, however, are low. Based on daily ration and the glycogen content of earthworms, a diet of similar composition to that of the natural habitat (Aster and Moon, 1981), only 24 mg glucose units are available from the diet, representing less than a half hour requirement. Gluconeogenesis must represent the primary source of plasma glucose even under a feeding condition.

Studies by Nagai and Ikeda (1971b) reported an active gluconeogenic pathway in the carp, Cyprinus carpio. Following intraperitoneal injection of ¹⁴C-6-glucose, ¹⁴C randomization into carbons 1-5 was assessed. Fifty-five per cent of the ¹⁴C remaining in glucose was attached to carbon atoms other than C-6 in animals fed a high carbohydrate diet. Control or low carbohydrate dietary conditions increased this percentage and plasma glucose concentrations. Blood glucose concentrations increased with brief (10-15 days) starvation, without changes in hepatic glycogen concentration. These data support gluconeogenesis as the primary

source of plasma glucose in carp (Nagai and Ikeda, 1971a). A similar situation apparently exists in A. rostrata.

The major mammalian gluconeogenic precursors are amino acids and glycerol. Although many amino acids are potential precursors, only alanine (primarily), serine, threonine, and glycine give rise to significant amounts of glucose (Exton, 1972).

Despite this contention, Hetenyi et al. (1980, 1983) found that the proportion of glucose derived from alanine and glycerol is always less than that from lactate. Further, more than 80% of the glucose carbon remains unaccounted for in dog (Hetenyi et al., 1980). The proportion of glucose derived from alanine in mammals varies interspecifically. Values vary from about 2% (sheep) to a high of 15% (rat), with humans being intermediate (see Hetenyi et al., 1983). Age, hormones and fasting influence these values.

Renaud and Moon (1980b) reported that at 1 mM concentrations, alanine was about half as effective as lactate as a gluconeogenic substrate. Gluconeogenesis from aspartate was lower. At 10mM concentrations (Renaud and Moon, 1980a), glycerol was the most effective gluconeogenic substrate, followed by alanine, then lactate. These data suggest that at elevated concentrations, both glycerol and alanine are better gluconeogenic precursors than lactate.

French et al. (1981) compared gluconeogenesis in hepatocytes from fed and 3 week starved/exercised (low hepatic

glycogen) trout. Lactate contributed equally under both conditions, and was the primary substrate for glucose production. Serine was the most effective amino acid followed by asparagine and alanine. Contributions from alanine were ten-fold lower than those of lactate. Unfortunately, substrate concentrations were 10 mM for lactate and 2 mM for the amino acids. Walton and Cowey (1979a,b) also found lactate to be the primary contributor, though only 2-fold better than alanine. Serine contributed slightly less again. Glucogenic rates in trout hepatocytes were found to be much lower than the corresponding values for the eel system (Renaud and Moon, 1980b).

Bever et al. (1981) also found gluconeogenesis in kelp bass occurred primarily from alanine followed by glutamate. Rates were as high as that of rats and increased further with 10 weeks of food-deprivation. Rates from aspartate were low, and lactate was not studied.

The results presented here strongly suggest lactate is not a prime contributor to gluconeogenesis in the fed American eel. In view of the high protein diet (Aster and Moon, 1981) and low lipid utilization (Moon, 1983a) of these animals, it does not seem unreasonable to suggest that amino acids may be important gluconeogenic substrates under fed conditions. Confirmation awaits further study.

4.1.5 Tracer Administration

Recent studies in rats by Katz et al. (1981) and Okajima et al. (1981) have revealed that the ports of sampling and infusion may alter the derived turnover rate for certain compounds. Infusion of ^3H -lactate into the aortic arch (A) and sampling from the vena cava (VC) yields turnover rates nearly 2-fold larger than the reverse (15.2 vs 8.7 $\text{mg}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$). Metabolic clearance rate is affected to the same extent (1.75-fold), although the ranges overlap in the two modes. Irreversible replacement rates (^{14}C -lactate) differ to a lesser extent (1.32-fold).

This difference in turnover rate for lactate is also apparent for alanine (Golden et al., 1981; Katz, 1982), but not for glucose (McGuire et al., 1976; Katz et al., 1981; Okajima et al., 1981).

The basis for these discrepancies is thought to involve differences in turnover rate (lactate > glucose), and the distribution of the two substances within the body (glucose, primarily extracellular; lactate, extra- and intracellular). These factors may allow a more uniform specific activity throughout the glucose pool, and hence eliminate sampling and infusion artifacts for this substrate (Katz, 1982).

The choice of infusion and sampling ports in the eel was based more on accessibility and convenience than in view of tracer kinetics. The circulatory system of a fish varies

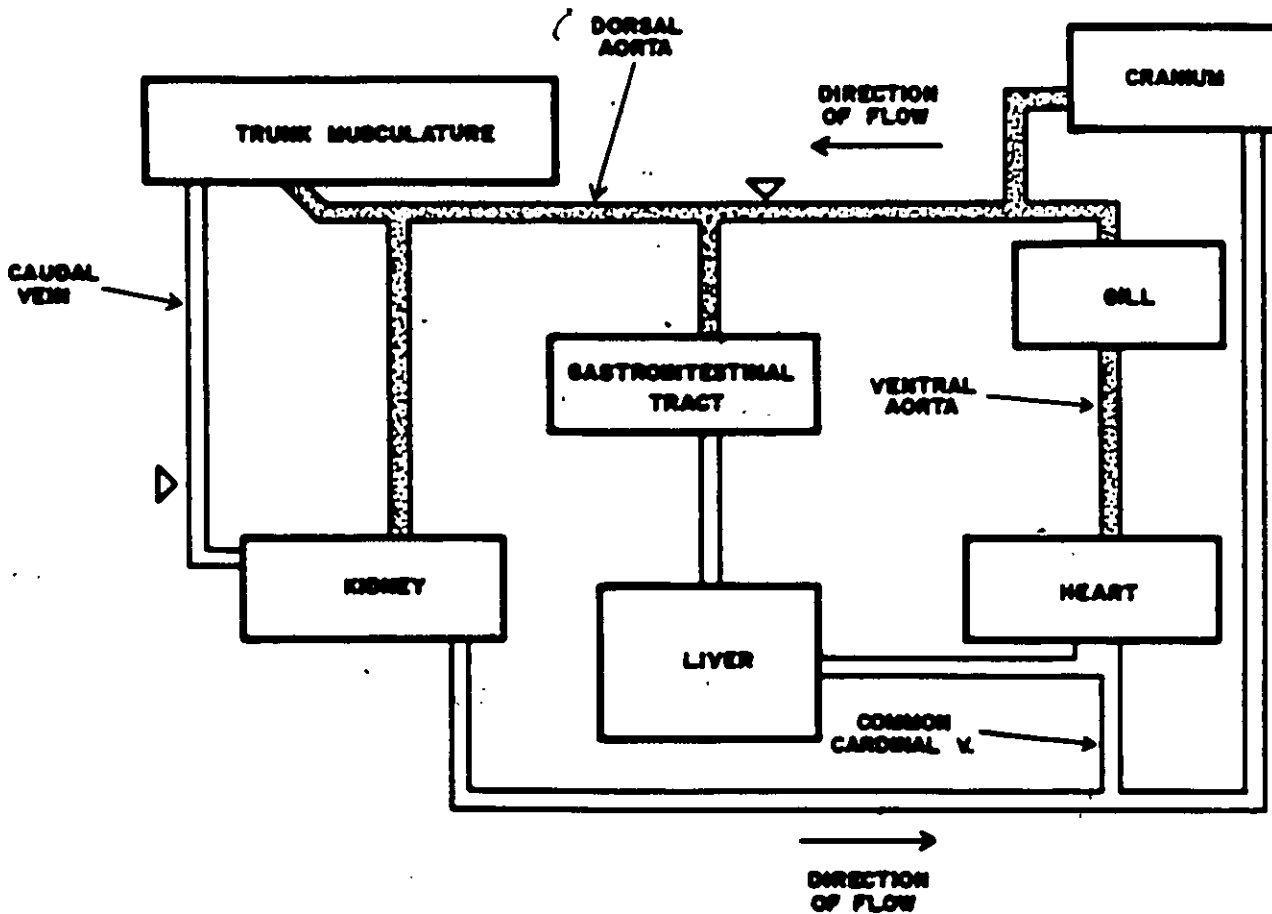
significantly from the mammal, having only one heart which receives only venous blood. A schematic diagram is presented (Fig. 9). To mimic the A-VC mode of mammals in fish, the site of infusion would be the dorsal aorta and, that of sampling, the common cardinal vein or ventral aorta. The cardinal vein is not easily accessible. The ventral aorta is accessible but the increased blood pressure would introduce technical problems not easily circumvented. As a compromise to both the animal and the technique, access points were chosen as indicated in the diagram: the dorsal aorta (through the pneumogastric artery) and the caudal vein, to represent AV and VA modes.

No significant differences were apparent in any estimated parameters between AV and VA modes for either lactate or glucose (Table 2). A larger variance in the AV mode was apparent as reported for rats (Katz et al., 1981; Okajima et al., 1981). Mean glucose concentrations and turnover rates and lactate turnover rates and clearance were approximately 2-fold higher in the AV mode. Glucose clearance was slightly higher and lactate concentration, essentially unchanged. The rate of production of glucose from lactate (R(L G)) was also increased, but this reflected differences in concentration (Table 3).

Figure 9: Schematic diagram of the piscine circulatory system

Adaptation from Smith and Bell, 1975.

Arrows indicate ports of infusion or sampling (see text).



Major differences (Table 2) between the two modes involved sampling before (VA) and after (AV) the skeletal muscle mass of the tail. If these mean differences were to remain given larger sample sizes, the classic view of muscle utilizing glucose and producing lactate, and liver utilizing lactate and producing glucose, would have to be re-examined for the American eel. As these differences were not statistically significant, there is no necessity to make such speculations. Simultaneous sampling of both vessels and activity control would be required to establish the significance of these observations.

Based on higher mean rates of turnover and clearance of both lactate and glucose in the AV mode, this mode was employed for other fed and food-deprived groups (not the anaesthetized group). With subsequent production by successive tissues or utilization of a metabolite, specific activity can only decrease. In view of this, one cannot overestimate turnover or clearance rates. Although values were not statistically significant between the AV and VA modes, increased mean values in the AV mode, suggested this as the more appropriate administration scheme.

4.1.6 Laboratory Acclimation

Many metabolic experiments require (or convenience dictates) that animals be held in laboratory facilities for ex-

tended periods of time. In comparison with the natural habitat, the laboratory conditions tend to restrict normal animal activity patterns. The difference in activity levels may produce experimental artifacts, as reported by Mosse (1980). Differences between FN and AV fish, although not statistically significant, suggested that lengthy laboratory acclimation of one year (AV) tended to increase venous plasma sugar concentration, glucose turnover and clearance rates, and lactate clearance rates. Lactate concentrations, irreversible replacement rates, and the conversion rates of lactate to glucose ($R(L \rightarrow G)$) remained virtually unchanged (Tables 2 and 3). FN animals, acclimated for 6 weeks, were subjected to identical conditions as those acclimated for one year. Variation between individuals in the FN group was much smaller for all parameters determined than that of AV animals as a proportion of the mean. Dietary variations are known to affect metabolism (Cowey *et al.*, 1975; 1977a,b; Walton and Cowey, 1979a,b) and overall body composition (Cowey and Sargent, 1972). The diet of these animals was identical during their laboratory period, but recent studies suggest an earth worm diet may not represent a complete fish diet (Hilton, 1983).

Many other factors could be associated with differences in mean values and the extent of the standard deviation. Activity decreases (Mosse, 1980) and the obliteration of seasonality could modify normal hormonal variations. Baker

3

and Rance (1980) reported that diel cortisol fluctuations can be entrained by light.

These studies, although not statistically significant, do not strongly support the lack of an acclimation phenomenon. Future studies are required to look more closely at this possibility.

4.1.7 Summary: Glucose Metabolism in Fed Eels

These studies of fed animals have assessed the importance of glucose as an energy source to the American eel, A. ros-trata. These animals produce, utilize, and clear glucose at rates mimicing those of mammals (Tables 2 and 9). Considering the low aerobic metabolism of fish, one would expect this high glucose utilization rate to be primarily anaerobic, resulting in lactate production. Anaerobic scope may be the causal factor demanding high glucose production rates as carbohydrate represents the only utilizable substrate under such conditions (Newsholme and Start, 1973). Low lactate production rates, up to 200 times lower than glucose production rates indicate that anaerobic glucose utilization represents only a small fraction of total catabolism (Table 2). It is possible, however, that anaerobic glucose metabolism exceeds indications, associated with low tissue lactate release.


Nervous tissue utilizes the major amount of glucose in mammals at rest followed by red cells, renal cortical tissue, and skeletal muscle (at rest) all of which use approximately the same amount of glucose, and one-fourth that of the brain (Newsholme, 1976). The nervous system of the fish is considerably smaller and less complex than that of the mammal, and probably utilizes considerably less substrate. Fish generally have low blood volumes and haematocrits, and their red cell aerobic metabolism probably requires less substrate (Prosser, 1973). Renal metabolism is poorly studied in fish. Oxidation rates in isolated trout heart (Lanctin et al., 1981) show that lactate is the preferred substrate over glucose. Considering the skeletal muscle mass (Bone, 1978) and its high glycolytic potential (Knox et al., 1982), it seems likely that most of the glucose turnover is associated with this tissue (red and white).

Under free-swimming conditions a high glucose turnover rate in A. rostrata may provide support for muscular contraction. This may involve primarily aerobic glucose utilization in red muscle or anaerobic lactate production associated with contraction in white muscle. In view of the very low proportional circulatory perfusion of white muscle or active retention, lactate so produced would be released slowly into the blood, in accordance with studies by Wood et al. (1977), Wardle (1978), and Turner et al. (1983a,b).

A large proportion of the lactate so produced (up to 35% of the lactate turned over) is converted to glucose (Table 3). This value is high with respect to comparable rates in mammals and occurs at plasma lactate concentrations well below those of mammals. In vitro studies by Renaud and Moon (1980a,b) suggest that lactate gluconeogenesis may be concentration dependent, as reported in mammals (Issekutz et al., 1976). Cori cycle activity does represent an important recovery pathway of lactate carbon, but at the low lactate concentrations noted here, it plays a negligible role in total glucose production (Table 3). Under conditions of increased muscular work, this contribution could be substantially increased.

Discrepancies between lactate irreversible replacement rate (R_i) and the rate of lactate production from glucose ($R(G \rightarrow L)$) may be related to lactate retention within the muscle and in situ recycling to glucose.

Essentially all of the glucose released into the plasma was derived from metabolites other than lactate. Most in vitro studies have reported amino acids and glycerol can contribute to gluconeogenesis, although no more than 50% of glucose production has been accounted for in such studies (Hetenyi et al., 1983). In view of high dietary protein intake, and maintenance of lipid with 6 months of food-deprivation (Moon, 1983a), amino acids probably assume an important gluconeogenic role.



Although these studies can not entirely explain the discrepancy between glucose and lactate kinetics, the results support the importance of these metabolites in the eel. This is further confirmed by the alterations in these parameters during food-deprivation.

4.2 FOOD-DEPRIVATION

4.2.1 Concentration

4.2.1.1 Glucose

The well documented, rapid (hours) decline in plasma glucose concentrations with fasting in mammals (Hall et al., 1977; Brady et al., 1977; Kusaka and Ui, 1977) has not been demonstrated in fish. Most fish maintain blood glucose for months in the absence of food, including Anquilla spp. (Larsson and Lewander, 1973; Dave et al., 1975; Renaud and Moon, 1980b).

Results reported in Table 4 confirm this general pattern. Glucose concentrations varied from 1.7 to 2.0 mg·ml⁻¹ in F6, F15, and F36 eels, compared to 1.4 to 2.5 mg·ml⁻¹ in fed animals (Table 2). Glucose concentrations were unchanged during the perfusion period (Fig. 4A) except for one F6 eel which exhibited a gradual plasma decrease (Fig. 6A). It is

apparent that food-deprivation has little effect on glucose homeostasis in these animals.

Different species show varying responses to starvation. Anquilla rostrata appears to be the only species to show significant increases in plasma glucose over the course of food-deprivation (Renaud and Moon, 1980b) with maintenance of normal concentrations for up to 15 months (Fig. 4A, Table 4), suggesting that glucose may be more important to this species than to others and that A. rostrata is highly tolerant of food-deprivation as suggested by Moon (1983a).

4.2.1.2 Lactate

Few studies have monitored lactate concentrations during fasting. Values in dogs initially decrease then return to normal over a 3 week fast (Brady et al., 1977). Studies with A. anquilla reported either little change (Larsson and Lewander, 1973) or progressive increases (Dave et al., 1975) after 145 days, depending upon holding conditions.

In this study, 6 months of food-deprivation did not alter plasma lactate concentrations of those animals which maintained DSS during the experiment (Fig. 5A, Table 4). The lability of lactate, however, increased with fasting, especially in the F15 group. Half of the 6 month and all of the 15 month experimental groups exhibited increasing plasma lactate concentrations during infusion (Fig. 6C, Table 5), suggestive of a stress response. The progressive increase

from time zero (Fig. 6) suggested these animals were not stressed prior to tracer infusion but the stress was related to either the infusion or sampling. A stress effect is likely considering that lactate did not initially and subsequently decrease, but progressively rose.

An incomplete recovery from surgery and the remaining hormone titre may have rapidly promoted increased lactate concentration. Alternatively, the increased susceptibility to stress with food-deprivation may be related to initially higher hormonal titres (pre-surgery). Dave *et al.* (1975) found that basal cortisol levels increased significantly with duration of food-deprivation. Assuming an unchanged threshold concentration is necessary to elicit a stress response, an increase in basal cortisol would reduce the distance from that threshold.

Plasma lactate concentrations of maturing animals deprived of food for 36 months were indistinguishable from fed and 6 month food-deprived immature animals (Fig. 5A, Table 4). Two animals responded abberently. One (F36-1), exhibited an initial spike in plasma concentrations returning to normal values after 80 minutes (Fig. 6C), and it may have been disturbed in preparation for infusion. The second animal (F36-6; Fig. 6C) exhibited a stress response resembling that of F15 animals in which lactate progressively increased, although to a smaller extent, reaching a final mean plasma concentration of 13.0 ug ml^{-1} (Table 5).

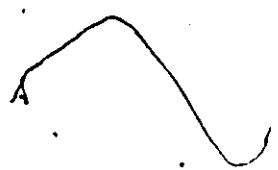
Maturing animals were less susceptible to the stress of prolonged food-deprivation than immature animals. Immature animals were upstream migrants of about 120g (Table 1) and stored reserves are quantitatively smaller and qualitatively different from the maturing downstream animals (Aster, 1981). A 15-month fast would be considered an extreme metabolic stressor for these immature animals (Moon, 1983b).

4.2.2 Glucose Kinetics

Food-deprivation in mammals decreases glucose turnover rates in a manner which parallels changes in plasma concentrations. This is exhibited in dogs as an immediate (1-2 day) decline followed by a progressively slow decrease to an unstable plateau of 69% after one or more weeks (Cowan et al., 1969; Brady et al., 1977).

Consistent with maintenance of glucose concentrations, food-deprivation of 6 months in A. rostrata had no effect on turnover or metabolic clearance rates (Table 4). A similar situation existed for kelp bass deprived of food for 70 days, although, as noted previously, turnover rates were very low in comparison to those of A. rostrata (Bever et al., 1981).

Anquilla spp. annually undergo natural fasts of 6 months (Sinha and Jones, 1975; Moon, 1983a) and one would not expect major metabolic alterations over this time period as



would be expected for mammals. However, these annual fasts are associated with decreased ambient temperatures, decreased activity, and decreased metabolic rates (Bertin, 1965; Tesch, 1977).

Ambient temperatures in this study were maintained at 12-15°C. Metabolic rates have been reported to decline by about 10% at 15°C without food (Walsh et al., 1983). The maintenance of glucose kinetics in the 6 month food-deprived eel compared to fed animals implies a considerable depletion of gluconeogenic precursors to maintain production rates equal to those of fed animals. Hepatocyte studies by Renaud and Moon (1980b) support an increased gluconeogenesis at high lactate concentrations (10mM) and a general increase from alanine, following food-deprivation. Cowey et al. (1977b) reported no change in alanine gluconeogenesis with fasting in trout. Glucose apparently represents an important energetic substrate to the fasted eel considering the metabolic expense of its synthesis. One might expect some compensation associated with decreased substrate availability, metabolic depression and activity decreases; carbohydrate utilization is, however, not compromised.

In contrast to 6 months of food-deprivation, following 15 months, animals exhibited visible signs of depletion. The muscle mass of the tail had decreased and the fish were generally less responsive to netting. Significant changes in myofibrillar protein content have been reported in eels at 6

months (Moon, 1983a). Despite maintenance of plasma glucose, turnover rate and MCR were decreased 15-fold below fed and 6 month food-deprived animals (Table 4). Immature eels, deprived of food for 10 months exhibited decreased hepatic glycogen contents and decreased total hepatocyte glucose production (Renaud and Moon, 1980b), and after 12 months, major ultrastructural modifications were apparent (Moon, 1983b). Long term food-deprivation resulted in a necessary compromise of glucose production. A decreased MCR implies tissue uptake of glucose was also limited. This process occurs by facilitated-diffusion in mammals (Newsholme and Start, 1973), and if so in fish, this process must have been inhibited. Decreased tissue hexokinase activities could account for this change, and this enzyme has been reported to decrease in trout with fasting (Covey *et al.*, 1977a).

All 15 month fasted eels demonstrated plasma lactate increases, implicating a stress response. Plasma glucose concentrations, however, were unaffected. Possibly glucose production was limited by the lack of carbohydrate stores and insufficient gluconeogenic precursor concentrations. It is also possible that the stressor was not sufficient to affect plasma glucose concentrations. Plasma lactate increases primarily in response to a stressor (Oikari and Soivio, 1975).

The large seaward migrating eels (F36), food-deprived for 36 months, exhibited no major differences in glucose kinet-

ics in contrast to the immature 15 month food-deprived animals (Table 4). Effects of depletion were more visible in these animals than 15 month food-deprived immature animals. The tail musculature had clearly undergone a large extensive degradation. As seaward migrants, these eels begin food-deprivation with large lipid reserves (Lovern, 1938; Bertin, 1965; Lewander et al., 1974), and probably depend predominantly on this store to sustain them under laboratory conditions for 36 months. Food-deprivation, therefore, may impose a metabolic stress of lower proportion than in the immature animals. Although mean glucose production rates were reduced, the change in this parameter over the 36 months is not known. Lipid may have been burned initially and only subsequently, glucose turnover increased. Nonetheless, following 3 years without exogenous input, these animals were capable of sustaining glucose production rates comparable to mammalian values (Tables 4 and 9), and did not change their MCR for glucose. Biochemical differences between maturing and immature animals are discussed further in another section (4.2.5.2).

The importance of assessing in vivo turnover estimates is exemplified in these studies. In the entire F15 group, plasma glucose concentrations were maintained as high as fed and 6 month food-deprived animals (Fig. 4A, Table 4). In view of this fact, one might expect that glucose production and utilization rates would be equivalent between the 3

groups. Turnover rates were found to be 15-fold lower in F15 animals than the other two groups.

One animal deprived of food for 6 months (F6-5) was found to be hyperglycemic with a plasma concentration more than 2-fold higher than mean values for the rest of the group (Fig. 6A). Rates of appearance (Ra) and disappearance (Rd) were 39-fold below the mean turnover rate for the rest of the experimental group (Table 5). Unlike the other animals in this group, this fish exhibited a glucose-response to stress. Catecholamines and cortisol are associated with the response to stress (Mazeud et al., 1977) and both are known to increase plasma glucose concentrations in fish (Thorpe and Ince, 1974; Mazeud et al., 1977). Since plasma glucose concentrations decreased over the duration of the experiment, this fish may have been recovering from hormonally induced effects.

4.2.3 Lactate Kinetics

Lactate turnover (irreversible replacement rate) increased with food-deprivation in both maturing (F36) and immature (F6) animals (Table 4). Irreversible replacement rate increased two-fold with 6 months of food-deprivation. Total estimated turnover rate was almost three-fold higher than fed animals (Table 5). This may suggest an increased utilization of anaerobic metabolism. Unlike the marine

flatfish (Johnston, 1981a) and migrating salmon (Mommsen et al., 1980), A. rostrata does not preferentially degrade white muscle over red muscle protein under conditions of food-deprivation, although, locomotory function apparently declines in white muscle with long-term food-deprivation of immature eels (Moon, 1983a,b). In view of this, one might expect decreased lactate production rates. Changes in membrane permeability to metabolites as suggested by histological studies (Moon, 1983a) may be responsible for increased release of lactate.

Immature animals which did not maintain DSS increased lactate appearance and disappearance rates as expected with higher plasma concentrations (Tables 4 and 5). There was a positive correlation ($R=0.85$ and 0.83 for P6 and F15 animals, respectively) between the final plasma concentration and the rate of appearance sustaining it. This type of relationship has also been observed under exercising conditions in dogs (Issekutz, et al., 1976).

Food-deprivation did not significantly alter lactate clearance rates with respect to fed animals (Table 5), although the MCR of 15 month food-deprived animals was significantly lower than those of 6 month food-deprived animals. Prolonged food-deprivation is associated with decreased tissue weights as stores are mobilized. The hepatosomatic index decreases and red and white muscle water contents increase with food-deprivation (Moon and Johnston, 1980; Moon,

1983a). Decreased cell sizes might be expected to decrease plasma clearance of any metabolite not actively transported. This may, however, be counter-balanced by concentration gradients if blood volume and tissue circulatory perfusion is unchanged. Unchanged clearance rates suggest this may be the case.

Maturing animals, deprived of food for 36 months demonstrated clearance rates similar to those of 6 month food-deprived immature animals (Table 4). Tissue depletion which must have been extensive in these animals did not affect their ability to clear lactate.

4.2.4 Carbon Exchange

There is some indication in mammals for both an increase in gluconeogenesis and a greater contribution of certain precursors to gluconeogenesis with fasting (lactate, Kusaka and Ui, 1977; and glycerol, Hall et al., 1977).

American eels, food-deprived for six months increased glucose production rates from lactate nearly 4-fold, representing a 3-fold increase in the proportion of glucose produced derived from lactate (Table 6) and a 10% increase in the amount of lactate produced diverted towards glucose. Such increases agree with in vitro work by Renaud and Moon (1980b). With respect to glucose production, however, these increases were largely insignificant. As in fed animals,

essentially all of the glucose was derived from other sources, with only 1% of the glucose produced derived from lactate (Table 7). In addition, more than half of the lactate produced was not diverted towards glucose.

A number of studies have reported increased activity of specific gluconeogenic enzymes in food-deprived fish. Both pyruvate and aspartate aminotransferase activities increased in the liver of the Japanese eel, A. japonica (Inui and Yokote, 1975). PEPCK activities increased in bass, Dicentrarchus labrax (Zammit and Newsholme, 1979), and plaice, Pleuronectes platessa (Moon and Johnston, 1980). Liver and kidney transaminases, PEPCK, FDPase, and G6Pase activities increased after 60 days in rainbow trout (Morata et al., 1982). Coupled with increased blood ammonia, increased amino acid gluconeogenesis was proposed. Large decreases in total fat reserves, however, suggested that lipid was the main energy source under these conditions in trout.

Kelp bass deprived of food for 72 days increased alanine and glutamate carbon incorporation into glucose at rates higher than fasted rats (Bever et al., 1981).

White muscle acts as the primary source of amino acid mobilization in sockeye salmon during migration. Mommsen et al. (1980) reported that alanine is the major amino acid released and the enzymes associated with amino acid mobilization were preferentially conserved by these salmon. Patterson et al. (1974) found that white muscle contractile

proteins were used preferentially over those of red muscle in plaice deprived of food for 30 weeks. Other studies (Moon and Johnston, 1980; Johnston, 1981a) also suggest this trend, and an ultrastructural study (Johnston, 1981b) provided evidence for increased muscle lysosomal enzyme activities.

These studies suggest that in many species of fish, glucose production is important and endogenous production increases with food-deprivation, primarily from amino acid precursors.

Studies by Moon (1983a,b) found plasma glucose and hepatic glycogen unaffected after 6 months of food-deprivation in immature American eels. Quantitative changes in enzyme activities were minor when compared to those in plaice (Patterson et al., 1974; Moon and Johnston, 1980) and migrating salmon (Mommsen et al., 1980), suggesting the eel is a fasting-tolerant species. Combined with the knowledge that in vitro gluconeogenic rates increased with food-deprivation (Renaud and Moon, 1980b) and in vivo rates match glucose production of fed animals (Table 5), enzyme activities are probably not limiting to gluconeogenesis in the eel (Moon, 1983a). In addition, unlike many species (Love, 1970), significant depletion of lipid was not apparent. In fact, lipid content increased, predominantly in white muscle. Triglycerides apparently did not act as an energy source or as a source for glucose production (glycerol). In fact, some

of the glucose produced may be diverted towards triglyceride formation. This is in contrast to the European eel (Larsson and Levander, 1973; Dave et al., 1975). In view of this and the low contributions from lactate (Table 6), amino acids from myofibrillar protein degradation (Moon, 1983a,b) must represent important gluconeogenic precursors under food-deprived conditions.

In view of the studies discussed above and that of Kettlehut et al. (1980), it seems likely that gluconeogenesis is the main source of glucose production under fed and fasting conditions. Kettlehut et al. found that plasma glucose concentrations and gluconeogenic rates in rats (omnivores) and cats (carnivores) were unaffected by three days of food-deprivation if, prior to fasting, the animals had been maintained on high protein diets. High carbohydrate diets, however, resulted in decreased plasma glucose and major hepatic glycogenolysis. Glucose production rates were unchanged under both dietary fed conditions suggesting that under conditions of high protein dietary intake, gluconeogenic rates are increased.

Walton and Cowey (1979a) found similar results in fish. ¹⁴C-alanine incorporation into glucose in trout hepatocytes was unchanged in animals deprived of food for 21 days and those maintained on a high protein diet. Further, these rates were 6-fold greater than those of trout fed a diet of high carbohydrate content.

Dietary history, then, may establish the extent of metabolic perturbations during subsequent food-deprivation. A. rostrata were fed diets from which 65.4% of the energy content was derived from protein. Enzymic compensation with respect to carbohydrate metabolism does not appear to occur after 6 months (Moon, 1983a). Amino acid gluconeogenic rates may represent an equal support of plasma glucose under fed conditions and up to 6 months of food-deprivation. High gluconeogenic rates under fed conditions may reflect the high tolerance of A. rostrata to food-deprivation, the large muscle mass of the animals representing an important available energy store. Necessarily, sufficient depletion must eventually result in decreased glucose production. Fifteen months of food-deprivation is apparently sufficient.

The discrepancy between $R(\text{lactate})$ and $R(\text{G} \rightarrow \text{L})$ (see section 4.1.4) is augmented under food-deprived (maturing and immature) conditions (Table 6). This suggests increased anaerobic glucose utilization. The basis for this is not clear.

Maturing, food-deprived animals did not increase glucose production from lactate above fed immature animals, although lactate turnover rates were increased. As a result, only 22% of the total estimated lactate produced acted as gluconeogenic substrate. As in the case of fed and food-deprived immature animals, the contribution of lactate to glucose production is insignificant, representing less than 1% (Table 6).

The lifestyle of the eel precludes the availability of fully mature animals necessitating the study of the transition phase (Larsson and Fange, 1969; Boström and Johansson, 1972; Johansson et al., 1974; Lewander et al., 1974; Dave et al., 1975; Hulbert and Moon, 1978a,b,c) or artificial induction of maturation via hormonal injections (Pankhurst 1982a,b). Under both conditions, red muscle content increased (Boström and Johansson, 1972; Lewander et al., 1974; Pankhurst, 1982a). The oxidative capacity (hexose monophosphate shunt and tricarboxylic acid cycle enzyme activities) is increased twofold in both red and white muscle in the silvering (A. anguilla) eel in comparison with the immature yellow eel. Glycolytic enzyme activities, however, decrease to one third in red muscle (Boström and Johansson, 1972). Plasma triglycerides and free fatty acids increase (Lewander et al., 1974) as did red muscle lipid and glycogen content (Hulbert and Moon, 1978c). Red muscle is also proportionately increased by about 2-fold (Hulbert and Moon, 1978c; Pankhurst, 1982a).

The effects of food-deprivation in these maturing eels have not been investigated. In view of enzymic alterations and lipid stores, this has been considered the major energy source for migration (Love, 1970). One would predict lipid to represent the sustaining source of energy permitting the survival of the long-term food-deprived laboratory animal. High glucose production rates after 36 months, however, sug-

gest that glucose remains an important substrate to these animals.

4.2.5 Summary: Glucose Metabolism

4.2.5.1 Immature animals

The American eel, A. rostrata, exhibits distinctly different strategies from mammals during food-deprivation. Following six months without exogenous input, glucose turnover rates are not compromised (Table 4) confirming data for fed animals that glucose represents an important fuel source.

Increased lactate production rates (Table 4) suggest an increased anaerobic catabolism of glucose, possibly an adaptation allowing carbon recovery. Concomittant increases in lactate gluconeogenesis, however, remain insignificant with respect to glucose production (Table 6). The potential for high lactate gluconeogenic capacities indicated by in vitro studies (Hayashi and Ooshiro, 1975a,b; Phillips and Hird, 1977; Renaud and Moon, 1980b) was not realized in the free-swimming animal. As in fed animals, essentially all of the glucose produced was derived from other sources, presumably amino acids. Plasma concentrations may represent a limiting factor. Lactate gluconeogenesis may increase with increased availability as is the case in mammals (Issekutz et al., 1976).

Food-deprivation up to 15 months, led to the expected compromise in glucose production. Glucose turnover rates were decreased 15-fold, although plasma concentrations were maintained (Table 4). The rising lactate concentrations in all animals deprived of food for 15 months (Fig. 6C) implied a hormonally mediated stress response. Plasma glucose concentrations may have also been affected. Reduced glucose clearance rates indicated that any increases would be cleared slowly (Table 4).

The discrepancy between concentration and turnover rates of glucose emphasizes the importance of in vivo turnover assessment. Maintenance of plasma concentrations suggested that production and utilization rates might also be maintained. In fact, turnover values are decreased 10-fold below those of fed animals (Tables 2 and 4).

Decreases in glucose production rates rendered these values similar to lactate production rates (Tables 4 and 5), suggesting that the contribution of lactate to glucose production may be significant after 15 months of food-deprivation. Unfortunately such determinations were precluded as the DSS was not attained during infusion.

4.2.5.2 Maturation

Maturing animals are less affected by food-deprivation. The parameters estimated in the 36 month food-deprived eels closely resembled immature animals which sustained these

conditions for only 6 months. In contrast to immature animals deprived of food for less than half the period (F15), glucose turnover and clearance rates were equivalent to fed, immature animals (Table 4). The biochemical changes associated with maturation are important preparatory adaptations for their migratory fast as exhibited by their tolerance to food-deprivation.

Unlike the immature eel, lipid probably is the largest energy reserve. Glucose turnover studies, however, suggested that carbohydrate remained an important fuel. Carbon may be derived from triglycerides (glycerol) or amino acids. Maturing animals show a higher metabolic capacity (Bostrom and Johansson, 1972) and red muscle is more active at this time (Pankhurst, 1982a). Hulbert and Moon (1978a) demonstrated that red muscle has the potential to metabolize lactate over a wide pH range and suggested that red muscle may be more important for metabolite recycling (Wittenberger et al., 1975) than contractility.

An enhanced dependence upon white skeletal muscle for motility may explain the maintenance of glucose turnover and increased lactate production after 36 months of food-deprivation. If so, during the natural migration, lipid would be mobilized initially with some diversion of triglycerides to glucose production. Amino acids associated with the structural proteins of white muscle would be mobilized only secondarily. There are no records reporting the time necessary for eels to complete their spawning migration.

Increased lactate production (Table 4) does support increased use of white skeletal muscle in these animals. Here, as in other fish, lactate release may be limited (Wood et al., 1977; Wardle, 1978; Turner et al., 1983a,b). Decreased reincorporation of lactate carbon into glucose (Table 6) may reflect larger gluconeogenic precursor pools in these animals compared with immature food-deprived animals.

4.3 ANAESTHESIA

4.3.1 Plasma Glucose and Lactate

Anaesthesia resulted in major disturbances in both glucose and lactate homeostasis, affecting all estimated parameters (Fig. 7, Table 7 and 8).

Plasma glucose concentrations were relatively constant (Fig. 7A) but approximately 2-fold higher than those of free-swimming animals (Table 7). Generally, anaesthesia to immobilization does not increase plasma glucose concentrations (Houston et al., 1971a,b; Oikari and Soivio, 1975; Appendix B, Table 11). Hyperglycemia is, however, known to be associated with struggle which often accompanies anaesthesia (Chavin and Young, 1970; Houston et al., 1971a,b). Increased concentrations may be associated with gill perfusion of anaesthetic. After 60 minutes, concentrations had in-

creased significantly above determinations following 15 minutes of submersion (Appendix B, Table 11). Surgery also may act as a stressor although serial samples following surgery during gill perfusion did not further increase plasma glucose concentrations above those initially observed (Appendix B, Fig. 11). Cortisol concentrations changed inconsistently under the various anaesthetized conditions (Appendix B: Table 11, Fig. 11), suggesting that cortisol did not play a primary role in this glucose response. Catecholamines were not estimated but may be responsible for variations in plasma concentrations. LeBras (1982) observed increases in noradrenaline and, to a lesser extent, adrenaline for 3 hrs following surgery in A. anguilla. Catecholamines are known to stimulate hepatic glucose release in mammals (Exton, 1972) and fish (Thorpe and Ince, 1974).

A number of investigators have noted haemoconcentration associated with MS-222 anaesthesia (Houston et al., 1971a; Soivio et al., 1977; Smit et al., 1979a,b; Nieminen et al., 1982). Haematocrits determined here were increased 2-fold, (.40 and .23 in anaesthetized and free-swimming animals, respectively). However, red blood cells were not reinjected in these animals resulting in haematocrit decreases of up to 50% over the course of the experiment. As glucose concentrations were determined in plasma, haemoconcentration does not play a role. The constancy observed (Fig. 7) is real as allowances were made for continual haemodilution due to infusion and sampling.

In contrast to plasma glucose, increased lactate concentrations have generally been associated with the effects of anaesthesia (Oikari and Soivio, 1975; Soivio et al., 1977; Wedemeyer, 1970; Nieminen et al., 1982). The mechanism(s) involved, however, has not been defined. Soivio et al. (1977) found that the increased lactate concentrations which occurred with anaesthesia, declined immediately following the return of rainbow trout to fresh water, unlike that produced with exercise (Black, 1957; Black et al., 1962, 1966). The struggle associated with transfer can further augment the effect (Houston et al., 1971a,b). Wardle (1978) found that propranolol (β -adrenergic blocker) injection stimulated massive increases in blood lactate concentration. MS-222 may exert a similar action. Increasing plasma concentrations in this study (Fig. 7B, Table 8) may be associated with the initial struggle, augmented by MS-222 effects. A compounding factor, decreasing haematocrits, mentioned above, presumably limited oxygen delivery to the tissues. This may have enhanced anaerobic metabolism.

Plasma lactate concentrations increased 5-fold in fed animals and 4-fold in food-deprived animals over the 5 hour experimental period (Fig. 7B). These concentrations were up to 500-fold above those of free-swimming animals (compare Figs. 2B and 5B). Fed anaesthetized animals increased concentrations to a mean of $2.5 \text{ mg} \cdot \text{ml}^{-1}$ in contrast with a peak of $0.5 \text{ mg} \cdot \text{ml}^{-1}$ in food-deprived animals. It is not clear

what effect nutritional status had in this respect. One would not expect food-deprived animals to be less susceptible to stress and, in fact, just the opposite might be proposed (see p. 108-110). These animals, however, may not be capable of reacting as quickly to a stressor. Wardle (1978) has suggested that increased catecholamines cause muscle to actively retain lactate. Titres may be increased in food-deprived animals resulting in decreased plasma concentrations. Alternatively, decreased available carbohydrate in the food-deprived animals may have limited lactate production.

Lactate concentrations are probably affected by something other than that which mediated increased plasma glucose concentrations as the two parameters did not increase to the same degree and the increase in lactate was continual during infusion. Plasma lactate production rates generally exceed glucose production rates more than 2-fold (Tables 7 and 8) suggesting that glycogen is being mobilized to produce lactate.

4.3.2 Turnover

Plasma glucose turnover rates measured in anaesthetized fish were markedly reduced compared to free-swimming animals (Table 2); approximately 20-fold in fed animals and 200-fold in 8-month food-deprived animals (Table 7). It is not clear what factors are associated with these decreases.

Hyperglycemia in mammals inhibits hepatic glucose release mediated by glucose, insulin, and to some extent catecholamines, diverting amino acids into protein, and away from glucose (Exton, 1972). This may also be occurring here. Food-deprived animals are either more susceptible to this effect, or simply show very reduced glucose production rates related to their nutritive state. Free-swimming animals deprived of food for 6 months did not alter glucose production rates, but after 15 months these rates decreased to $0.09 \text{ mg min}^{-1} \cdot 100\text{g}^{-1}$ (Table 4). Animals deprived of food for 8 months under free-swimming conditions presumably produce glucose at some intermediate rate. Assuming anaesthesia affects turnover rates in both fed and food-deprived animals to the same extent, mean glucose turnover rates in free-swimming, 8 month food-deprived animals would be expected to fall near $0.06 \text{ mg} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$, within the same range as the 15 month food-deprived animals. The decreased glucose production and utilization noted in the latter group may occur between 6 and 8 months of food-deprivation. Alternatively, glucose production rates in food-deprived animals may be more responsive to increased plasma glucose concentrations or other hormonal effectors. It is clear from these studies as it was with the 15 month food-deprived free-swimming animals, that increased concentrations are not necessarily associated with increased production rates, again emphasizing the importance of in vivo assessment of turnover rates to ascertain utilization of a given compound.

The rate of increase of plasma lactate is much greater in fed animals as depicted by the difference in slopes (Fig. 7B). Mean plasma appearance rates in food-deprived fish, however, were about 3-fold higher than fed animals (individual values are given in Table 8). The food-deprived experimental group maintained its ability to retrieve lactate from the blood, commensurate with appearance rates, while, disappearance rates in fed animals were very low, less than $1 \mu\text{g}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ in 4 of 6 cases. This also could be interpreted in light of decreased carbohydrate reserves as discussed below. The significance of the difference between fed and food-deprived animals in this respect is unclear. The similar trends in plasma lactate (Fig. 7B) and cortisol (Appendix B, Fig. 11) suggest this hormone has a stimulatory role (Mazeaud et al., 1977).

4.3.3 Clearance

Glucose clearance rates are also markedly affected by anaesthesia (Table 7). Both turnover and clearance rates were depressed to the same extent within either experimental group when compared to fed, free-swimming controls. This suggests that a single factor or the same group of factors is responsible for both turnover and clearance decreases, though the effect is augmented in the food-deprived group. Factors associated with changing clearance rates have been

detailed previously (section 4.1.2). Factors associated with decreased production rates (increased glucose concentrations or catecholamines) may also be associated with vasoconstriction, decreasing uptake. Decreased cardiac output may also play a role.

Lactate clearance rates are decreased to the same value in both fed and food-deprived anaesthetized animals even though lactate concentrations are not increased to the same extent (Table 7). Considering the high plasma concentrations relative to free-swimming animals (Tables 2 and 8), decreased clearance rates may be associated with saturation of the cellular transport systems. This cannot totally account for the discrepancy, however, as MCR is equal in both anaesthetized groups, while plasma lactate concentrations differ by 5-fold. Factors other than concentration must be affecting clearance. Differentially decreased blood flow between the two groups may be involved; some hormonal effector may have been released; a pH change may be involved. Soivio et al. (1977) found a decrease of 0.3 units following 15 minutes of anaesthesia. Similar decreases have been associated with increased plasma lactate concentrations following exhaustive exercise and also with increased pCO_2 (Wood et al., 1977). MS-222 does stimulate increased pCO_2 , but it should be reduced with the neutralized solution used here (Smit and Hattingh, 1979; Smit et al., 1979a,b). Gill perfusion of the anaesthetic, however, may augment this effect.

The lack of red cell replacement in these experimental animals, possibly affecting oxygen delivery, may have induced anaerobic metabolism, increasing production of lactic acid. Increased concentrations are known to affect blood pH (Wood *et al.*, 1977), inducing further metabolic disturbances and possibly cellular transport. Food-deprived animals may have limited glycogen stores. Therefore, glycolysis and lactate production could not be increased to the same extent as it could in fed animals. If increased concentrations inhibit tissue uptake of lactate (due to pH or concentration), then, it is the limited glycogen stores which, in effect allow food-deprived animals to maintain high R_d , comparable to R_a values (Table 8).

4.3.4 Summary: Glucose Metabolism

It is clear from this study that the changes observed during anaesthesia are not associated simply with depressed metabolic rates. If this were true, within each experimental group, production and clearance rates of both glucose and lactate would be affected to the same degree, and this does not occur (Table 7). Glucose production rates were impaired while those of lactate were massively increased, despite the decreased metabolism associated with anaesthesia (Houston *et al.*, 1971); cortisol is possibly implicated. In food-deprived animals lactate disappearance rates are in-

creased concomitantly with appearance rates, although to a lesser extent. In fed animals, lactate production rates were increased, but there were no associated increases in lactate disappearance, resulting in major alterations in plasma concentrations (Fig. 7B, Table 8). Further, under both experimental conditions, no ^{14}C was found associated with glucose, suggesting gluconeogenesis from lactate was impaired. pH decreases possibly associated with decreased oxygen delivery (Wood et al., 1977) may have inhibited this energy requiring pathway. It is also possible that any ^{14}C produced was diluted by increasing lactate concentrations so that lactate gluconeogenesis, shown to be low in fed free-swimming animals (Table 3), could simply not be detected. Anaesthetized fish were the only animals examined in this study in which plasma concentrations of lactate and glucose were similar. If high gluconeogenic rates from lactate occur in vivo as reported in vitro (Renaud and Moon, 1980b), high rates would have been expected in this group. Obviously factors other than lactate concentration affect gluconeogenic rates.

It is apparent from these studies that anaesthesia markedly alters both glucose and lactate metabolism, and that nutritive state alters the pattern. These studies stress the difficulty of extrapolating data to the in vivo situation and emphasize that the study of anaesthetized fish only allows one to examine the altered biochemistry associated with anaesthesia.

Chapter V

SUMMARY

5.1 FED ANIMALS

Experiments on fed animals show large (200-fold) discrepancies between plasma glucose and lactate concentrations. Turnover rates of these metabolites reflect these differences. However, the data support the importance of glucose as a metabolic substrate to A. rostrata and further, that glucose is metabolized primarily anaerobically.

The large library of data with regard to muscle metabolism in fish, in contrast to a paucity of information on glucose utilization in other major tissues with which it is associated in mammals, has restricted interpretations of the data presented here. Considering the extent of the muscle mass of the animal, however, major metabolic turnovers are most likely associated with this tissue.

High glucose utilization and low lactate production rates are probably associated with aerobic catabolism in red muscle and anaerobic metabolism in white muscle coupled with low lactate release from white muscle. The dependence of muscle on plasma glucose as a substrate implies an active

Cori cycle. Although proportionately large amounts of lactate turnover are diverted to glucose, the contribution is negligible compared to total glucose production. Recycling within the muscle may decrease the necessary contribution of the Cori cycle to glucose production. In view of high dietary protein intake, it is suggested that amino acids represent the major gluconeogenic precursors in these animals.

Metabolic clearance rates, like turnover rates, of glucose are equivalent to mammalian values, suggesting similar mechanisms of tissue uptake. Lactate clearance rates are low in accordance with other studies of fish. This may represent a limiting factor in lactate gluconeogenesis.

Laboratory acclimation time had no significant influence on any parameter studied, nor did reversal of sampling and infusion ports. Results in fed, free-swimming animals, in contrast to many in vitro studies, emphasize the importance of in vivo assessments of substrate utilization and carbon exchange.

5.2 FOOD-DEPRIVED ANIMALS

Food-deprivation for 6 months did not alter carbohydrate metabolism significantly. Plasma glucose concentrations, turnover and clearance rates were unchanged with respect to fed controls. Maintenance of these parameters reflects the importance of glucose as a fuel to these animals and also their tolerance of food-deprivation.

Lactate production rates and conversion of lactate to glucose were increased proportionately indicating an increase in anaerobic metabolism of glucose which may be associated with carbon recovery. The increase in glucose derived from lactate remained, as in fed animals, insignificant with respect to glucose production. Amino acids, again, probably represent the major gluconeogenic precursors in fasted animals.

Fifteen months of food-deprivation had major effects on both glucose and lactate metabolism. Increasing lactate concentrations imply a stress response. The stress may also have provoked high plasma glucose concentrations, but turnover rates were depressed.

Fifteen months without exogenous input necessitated major changes in glucose turnover rates, which were decreased 15-fold. Clearance rates decreased concomitantly, reflecting a decreased utilization of glucose under these conditions.

Maturing animals deprived of food for 36 months did not show similar compensations with respect to carbohydrate metabolism. Plasma glucose concentrations, turnover, and clearance rates are maintained similar to fed and 6 month food-deprived immature animals. Lactate production is increased though no proportionate increase in lactate gluconeogenesis occurred. The incredible tolerance of food-deprivation exhibited by these animals is probably associated

with the increased lipid content which accompanies maturation. Glucose production may be maintained by triglyceride and/or amino acid gluconeogenesis.

5.3 ANAESTHETIZED ANIMALS

Anaesthesia provoked major metabolic effects in both fed and food-deprived animals. Plasma glucose concentrations were increased, although stable during perfusion. Glucose turnover and clearance rates were decreased 20-fold in fed animals and a further 10-fold following 8 months of food-deprivation. Lactate clearance rates were also significantly depressed; lactate production rates were increased massively. Plasma lactate concentrations continuously rose in the plasma. No lactate carbon was recovered in glucose suggesting a complete inhibition of lactate gluconeogenesis.

Glucose and lactate effects were distinct and varied with the nutritive state of the animal. Changes in glucose parameters were not reflected in cortisol titres, implicating catecholamines, which have been also associated with the primary stress response in fish. Both cortisol and catecholamines may be associated with increased lactate production. The increased lactate concentrations, may initiate a feed-forward effect which stimulates further increases in plasma concentrations and in fed animals, inhibits tissue uptake. These studies further emphasize the importance of assessing carbohydrate metabolism under free-swimming conditions.

Appendix A

DIEL CORTISOL AND GLUCOSE RHYTHMS

Experiments were performed to establish if any relationship existed between plasma cortisol and glucose concentrations or between the duration of the experiment and plasma cortisol concentration.

The pneumogastric artery and caudal vein of 4 fed, immature animals were cannulated according to the procedures described in the Methods and Materials. The arterial cannula was cut so, as to leave only about 2 cm exposed and cauterized; the venous cannula was not altered.

Experiments were carried out on the day following surgery, beginning at 6:00 am (lights on: 7:00 am EST; L:D=12:12). Samples of 0.15 ml were taken from the venous cannula every 3 hrs for 33 hrs and red cells were reinjected according to procedures outlined in the Methods and Materials. Plasma (30 μ l) was deproteinized with an equal volume of 10% trichloroacetic acid. This, and the remaining plasma, were frozen at -70° until analyses were performed.

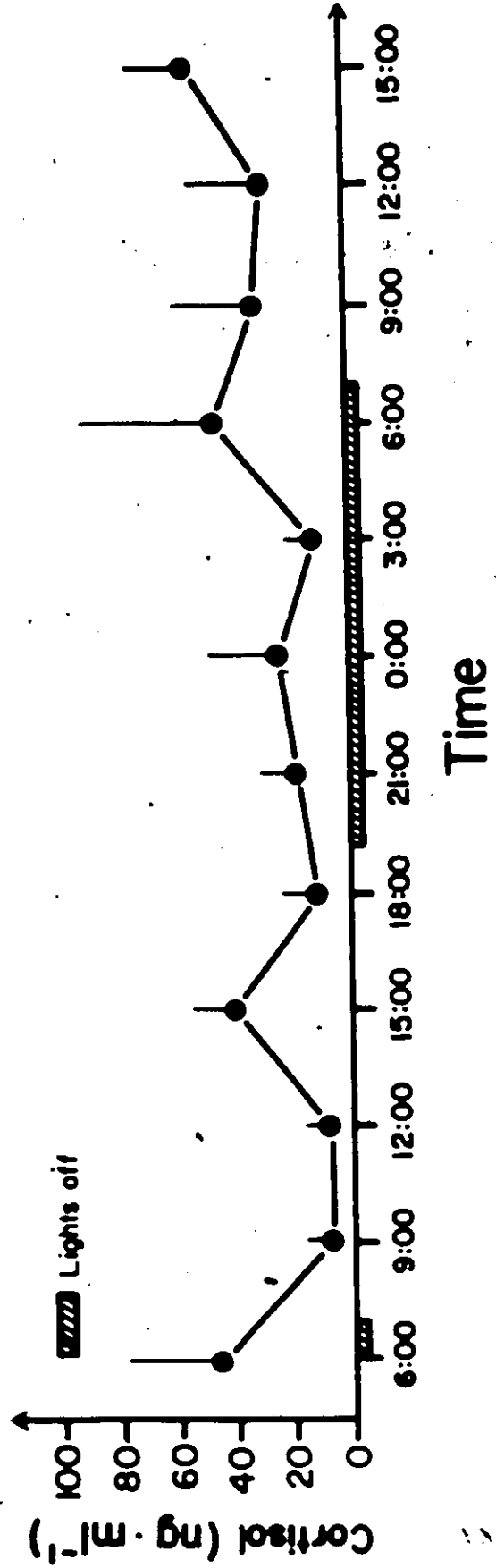
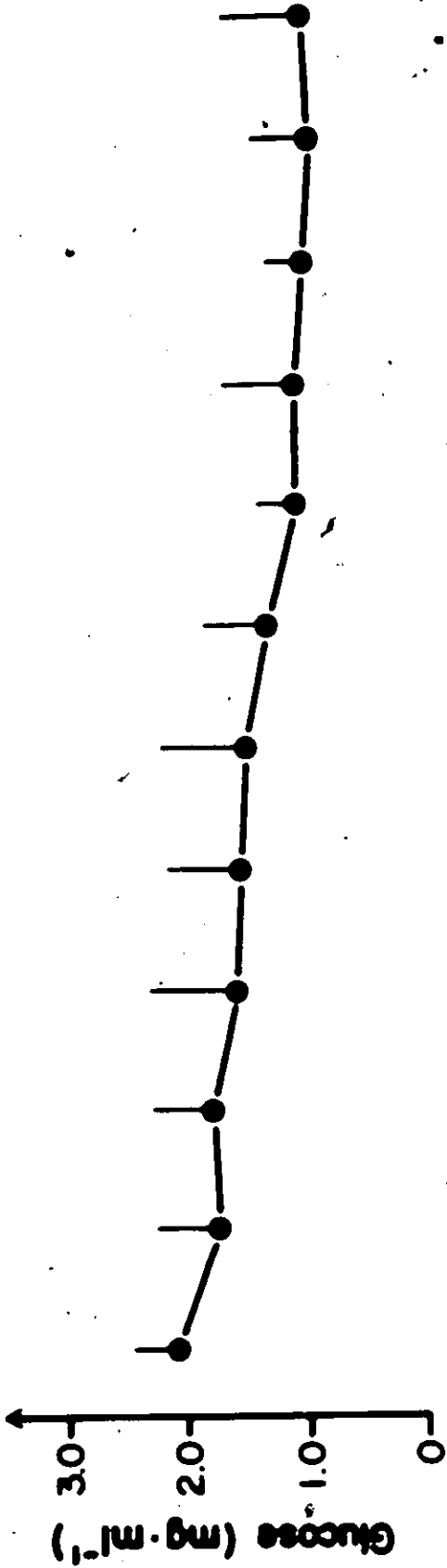
Deproteinized plasma was assayed for glucose according to the procedures outlined in the Methods and Materials. Plasma cortisol was determined in whole plasma in duplicate using the Cortisol (125-I) Radioimmunoassay kit (Immophase, Corning Medical and Scientific, Medfield MA).

Plasma glucose concentrations fell slowly during the experimental period, apparently independent of cortisol changes (Fig. 10). This trend to decrease can be at least partly explained by the continued dilution of the blood by sampling and cannular flushing. Although cortisol fluctuates, peak mean values do not exceed $60 \text{ ng}\cdot\text{ml}^{-1}$, within the range of diel patterns determined for Cyprinus carpio and Carassius auratus (Redgate, 1974; Peter et al., 1978). A possible diel cortisol rhythm with peaks at 0600 and 1500 hrs appeared to be present.



Figure 10: Diel plasma glucose and cortisol concentrations

All values are represented as mean \pm one standard deviation.



Appendix B

EFFECTS OF ANAESTHETIZATION ON PLASMA GLUCOSE AND CORTISOL CONCENTRATIONS

To assess the effect of MS-222 (ethyl-m-aminobenzoate methane sulphonate) and sampling technique on cortisol and glucose, four groups of animals were subjected to various experimental designs. Group A included animals decapitated without exposure to the anaesthetic. Group B, animals were anaesthetized in 1.0 g·l⁻¹ MS-222 buffered to pH 7.0, until no tail reflex was apparent (10 - 15 min). Blood samples were again obtained through decapitation. Group C was identical to Group B, except that blood samples were obtained by heart puncture. Group D involved anaesthesia and gill perfusion, but without cannulation. Perfusion was continued for 60, 120, and 180 minutes. Blood was collected by heart puncture. Group E, animals were anaesthetized, the gills perfused, and the pneumogastric artery and vein cannulated (duration of surgery was less than one hour); the artery was used for sampling. Cortisol and glucose concentrations were determined according to the techniques described in Appendix A.

No statistically significant differences were apparent in glucose or cortisol concentrations between animals subjected

to 10 to 15 min immersion in MS-222 or decapitation without anaesthetization (Table 9).

Plasma glucose concentrations are significantly higher ($p \leq 0.05$) in animals subjected to gill perfusion. There was, however, no correlation between glucose and cortisol concentrations. This was also apparent in cannulated animals. Small peaks in cortisol concentration did not reflect changes in plasma glucose titres. This was apparent under anaesthetized (Fig. 11) and free-swimming (Fig. 10) conditions.

TABLE 11

Alterations in plasma glucose and cortisol under various conditions of anaesthesia

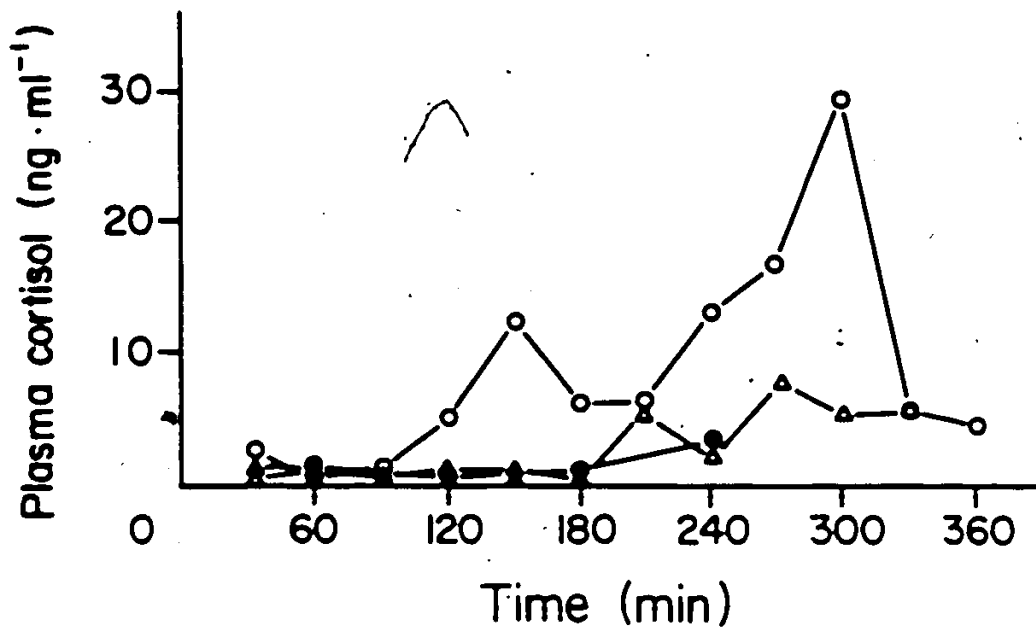
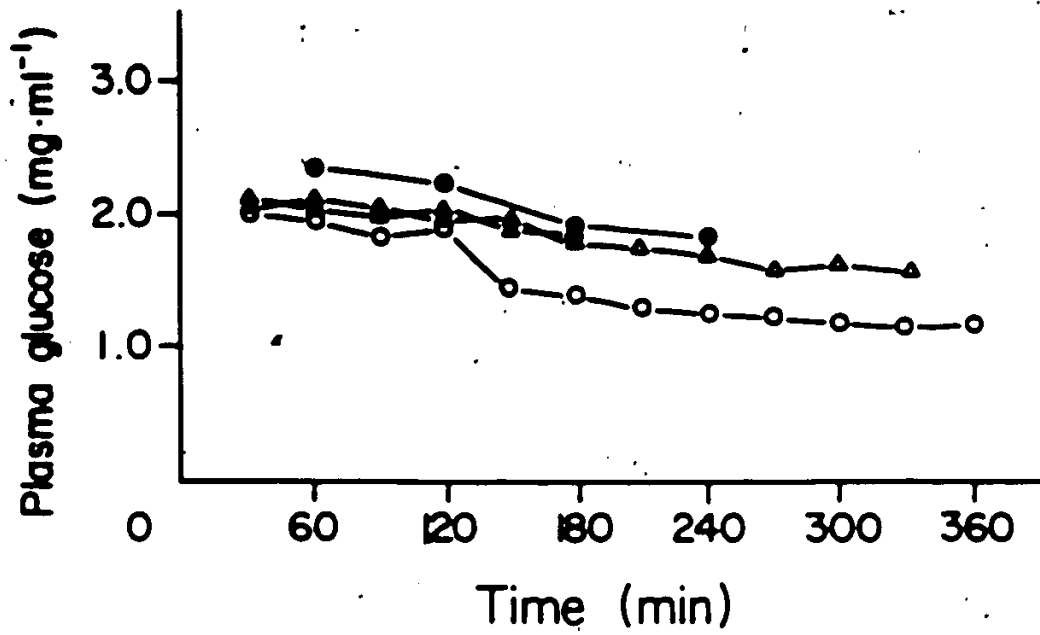
GR	ANAESTHESIA	TECHNIQUE	N	BWT (g)	GLUCOSE (mg·ml ⁻¹)	CORTISOL (ng·ml ⁻¹)
A	NONE	DECAPITATION	11	111±17	0.93±0.50	108±84
B	1.0g·l ⁻¹	DECAPITATION	5	136±17	0.82±0.05	301±219
C	1.0g·l ⁻¹	HT PUNCTURE	7	97±15	1.32±0.29	60±37
D	1.0g·l ⁻¹	HT PUNCTURE				
		60 min	4	86±10	2.09±0.27	29±19
	plus gill					
	perfusion	120 min	4	96±26	2.18±0.31	100±78
		180 min	2	153±18	2.01±0.07	31±17

Note: GR, experimental group; BWT, body weight.

All values are represented as mean ± one standard deviation.

Figure 11: Arterial glucose and cortisol concentrations of anaesthetized animals

Blood samples were obtained from the cannulated pneumogastric artery, under anaesthetized conditions.



Appendix C
CALCULATIONS

Total Glucose Oxidation

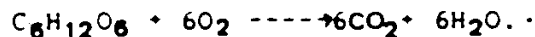
The calculation of the proportion of total glucose oxidation, based on oxygen consumption rates was based on a similar calculation by van Waarde (1983). The oxygen consumption rate for A. rostrata was reported to be 30 ml·hr⁻¹·kg⁻¹ (Walsh et al., 1983).

A 1 kg eel consumes 30 ml·hr⁻¹ oxygen.

A 100g eel consumes 3 ml·hr⁻¹ or 0.13 mmol·hr⁻¹.

A 100g eel utilizes 0.8 mg glucose·min⁻¹ or 0.27 mmol·hr⁻¹.

Carbohydrate is oxidized according to the equation



Complete oxidation of 0.27 mmol·hr⁻¹ would require 6 X 0.27

= 1.62 mmol O₂·hr⁻¹.

If all oxygen consumed (0.13 mmol·hr⁻¹) was utilized to completely oxidize glucose, the percentage of glucose completely oxidized would be (0.13/1.62) X 100% = 8.0%.

At a glucose turnover rate of 0.8 mg min^{-1} and an oxygen consumption rate of $3 \text{ ml}\cdot\text{hr}^{-1}$, 8% of the glucose utilized could be completely oxidized.

Glycogen Turnover

The coalfish, Gadus virens, swimming at $1 \text{ bl}\cdot\text{s}^{-1}$, depletes 12% of its red muscle glycogen and 7% white muscle glycogen in one second. Assuming that the average depletion of total muscle glycogen is 10% per second at $1 \text{ bl}\cdot\text{s}^{-1}$, and that this rate varies proportionately with speed up to that point, swimming at $0.001 \text{ bl}\cdot\text{s}^{-1}$, 0.01% total glycogen would be consumed.

Total muscle glycogen content in the American eel was determined by Moon (1983a):

Red muscle glycogen - $0.25 \text{ mg}\cdot 100\text{mg}^{-1}$ wet weight.

White muscle glycogen - $0.21 \text{ mg}\cdot 100\text{mg}^{-1}$ wet weight.

Total muscle makes up 60% of the animal by weight (Bone, 1978) and 8% of the muscle is red (Greer-Walker and Pull, 1975), hence, a 100g animal has 4.8% red and 55.2% white muscle. A 100g eel, then, has

$$(0.25 \text{ mg}\cdot 100\text{mg}^{-1}) \cdot (10) \cdot (4.8 \text{ g}) \cdot (1.1)$$

$$+ (0.21 \text{ mg}\cdot 100\text{mg}^{-1}) \cdot (10) \cdot (55.2 \text{ g}) \cdot (1.1)$$

= 141 mg glucose units.

(1.1 is the conversion factor of glycogen to glucose units.)

Assuming the eel consumes a proportion of glycogen comparable to that of the coalfish, a 100g eel, swimming at 0.001 bl.s⁻¹ will consume 0.0141 mg.s⁻¹ or 0.85 mg.min⁻¹ glucose. A glucose production rate of 0.8 mg.min⁻¹ (PN) could not entirely support this swimming speed.

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