National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

Ottawa, Canada
K1A 0N4

NL-339 (Rev. 8/80)

ABSTRACT

Proteins represent an important potential metabolic reserve which can be recruited in food-deprived fish, and it is the function of aspartate aminotransferase (Asp AT; EC 2.6.1.1) to incorporate the amino acid aspartate into the metabolic framework of all animal cells. This study was designed to investigate the effects of food deprivation on the kinetic and physical properties of Asp ATs isolated and partially purified from liver, red muscle and white muscle of the American eel, Anguilla rostrata LeSueur.

The trends in total tissue homogenate activities of Asp ATs from food deprived eels alluded to but did not reflect the true activity trends. Liver mitochondrial and white muscle cytosol activities increased 10- and 6-fold, respectively, while the other subcellular fractions were essentially unchanged.

An examination of the kinetic and physical properties of the eel tissue fraction Asp ATs showed alterations in Vmax and $Km_{(s)}$ with food deprivation indicating major activity changes at low substrate concentrations. Electrophoretic and isoelectricfocusing studies of the cytosol and mitochondrial enzymes found these changes were associated with alterations in the relative mobilities of the enzymes. Furthermore, the molecular weights of the enzymes changed with food deprivation.

These modifications of eel tissue subcellular fraction Asp ATs were postulated to reflect an enhanced role of this enzyme during food deprivation.

LIST OF ABBREVIATIONS

Ala AT	-	alanine aminotransferase
Asp	-	aspartate
Asp AT	-	aspartate aminotransferase
CoA	-	coenzyme A
CS	-	citrate synthetase
EDTA	-	ethylenediamine tetraacetic acid
EGTA	-	ethyleneglycol-Bis (β -aminoethylether) N, N'-tetraacetic acid
GDH	-	glutamate dehydrogenase
Glu	-	glutamate
HSI	-	hepato-sometic index
LDH	-	lactate dehydrogenase
Lv	-	liver
MDH	-	malate dehydrogenase
NAD	-	β-nicotinamide adenine dinucleotide
NADH	-	the reduced form of NAD
OAA	-	oxaloacetate
PAGE	-	polyacrylamide gel electrophoresis
PEP CK	-	phosphoenol pyruvate carboxykinase
P-5'-P	-	pyridoxal-5'-phosphate
RM	-	red muscle
SGOT	-	serum glutamate-oxaloacetate transaminase
Tris	-	Tris (hydroxy-methyl) aminomethane
WM	-	white muscle
α-KG	-	α-Ketoglutarate

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

This thesis would not have been possible without the aid of the Ministry of Natural Resources and especially Field Biologist Peter Liew and Fisheries and Wildlife Supervisor Bill Lannin at the eel ladder associated with the Saunders Hydroelectric Dam, Cornwall, Ontario.

The funds for this work were generously extended from the operating grant to T.W. Moon from the Natural Sciences and Engineering Research Council of Canada (#A6944).

Intellectual support and guidance came from Dr. J.C. Fenwick and Dr. D.J. Kushner with special mention to Dr. W.C. Hulbert and an immense thank you to Dr. T.W. Moon, a major motivating force and the supervisor of this tome.

In closing this gratutity, the tremendous moral support of my mother, Mrs. E.M. Huta, must be mentioned as well as the patience, understanding and enthusiasm of my wife, Cheryl. I would also like to thank Mrs. L. Cleminson for the typing of the manuscript.

I

INTRODUCTION

INTRODUCTION

GENERAL CONSIDERATIONS

Starvation represents one of the most challenging metabolic perturbations that an organism can experience. Typically the response to food withdrawal is dramatic and affects a host of compensatory cellular mechanisms. These responses have been investigated in only a few species and as yet no consistent paradigm has evolved. Body reserves are mobilized, but the type (carbohydrate, protein or lipid) depends upon the species in question, the extent of food reserves, the duration of food deprivation, the maturity of the organism and probably numerous other parameters yet to be defined.

It is the aim of this thesis to investigate one small aspect of the starvation strategy of the American eel, Anguilla rostrata LeSueur. In particular, proteins represent a potentially important metabolic reserve, and it is the function of the enzyme Aspartate Aminotransferase (Asp AT;EC 2.6.1.1) to introduce the amino acid aspartate into the metabolic framework of all animal cells. Since the enzyme is situated at such a critical metabolic position and amino acids are potentially such an important starvation substrate, Asp AT has been studied in this organism.

STARVATION

Mammals are primarily omnivores with precise plasma glucose homeostasis based on the integration between hormones such as glucagon, insulin and cortisol, and liver function (Cannon et al., 1956; Lundquist and Trygstrup, 1973). Star-

vation in this group is characterized by at least three phases depending upon the length of food deprivation (Ruderman et al., 1976). The first stage is short term occurring during the post absorptive period where liver glycogenolysis (glycogen breakdown) maintains blood glucose. The second or intermediate phase occurs after a period of food deprivation exceeding one day; muscle proteolysis liberates amino acids to the plasma increasing the availability of substrates and enhancing liver gluconeogenesis (glucose production from small carbon precursors). The principal amino acid released is alanine arising from the transamination of amino acids liberated by peripheral muscle tissue proteolysis. This results in a flood of plasma alanine and glutamine, with the former the principal gluconeogenic substrate of the liver (Felig, 1975; Lindsay, 1980).

The plasma amino acids and their corresponding α-keto acids are subject to increased liver uptake during starvation by circulating hormones (Krebs, 1972; Pardridge and Jefferson, 1975). If the starvation period exceeds one week, protein sparing occurs and lipolysis and the resulting ketone bodies provide cellular metabolic fuels. This mechanism arises in part from ketone body control of liver gluconeogenesis by reducing the availability of plasma alanine (Williams, 1980). Although only a few mammals have been studied in this manner, it is likely that much species heterogeneity will be observed in this response to food deprivation especially when mammalian carnivores (e.g., cats)

are examined (Krebs, 1972).

Fish, including the American eel (Anguilla rostrata), are generally considered to be carnivores. The natural diet of most fish, therefore, is low in carbohydrate but high in protein, and this type of diet is necessary to achieve optimum growth in cultured species (Cowey, 1975). The free amino acids in the plasma (of rainbow trout) exhibit much variation but no uniformity after feeding (Schlisio and Nicolai, 1978) except for a rise in glutamate and aspartate which peak 3 to 12 hours after a meal. This effect corresponds with heightened Asp AT activities in the liver when disproportionate amounts of amino acids are fed (Harper et al., 1970). Artificial diets high in carbohydrate reduce the digestability of both carbohydrates and proteins (Singh and Nose, 1967; Inabe et al., 1963), although the requirement for protein does not change (Lee and Putman, 1973).

The regulation of blood glucose in fish is not as precise as observed in mammals (Palmer and Ryman, 1972; Cowey et al., 1977b). Cowey et al. (1976) reported that injected beef insulin reduced the blood glucose level and altered liver gluconeogenic enzyme activities in rainbow trout fed a high carbohydrate but not a high protein diet. These changes paralleled changes in alanine gluconeogenesis as indicated by whole animal uptake studies (Cowey et al., 1977a). While the fed state exhibits a predominant reliance on dietary protein intake to support tissue metabolism, the starved state is geared to either a glycogen-fat metabolism where there is

initial glycogen breakdown, as in the Japanese eel (Inui and Oshima, 1966), or a protein-fat metabolism where glycogen and blood glucose are maintained and gluconeogenesis is inferred to increase (Love, 1970; Cowey et al., 1977a).

In fish there are two natural starvation periods, the first occurs seasonally in response to a decrease in ambient temperature, and the second is a non-feeding spawning migration near the termination of the life cycle of some species. During the winter, the activity level of the American eel (Vladykov, 1955), the European eel (Dave et al., 1975), and the carp (Creach and Serfaty, 1974) fall dramatically. Energy demands would be greatly reduced, probably approaching that required to maintain tissue integrity. In some species (Atlantic cod, Tilapia, goldfish and rainbow trout) carbohydrates are the most readily mobilized substrate during this state (Stimpson, 1965; Black et al., 1966; Kamura, 1966; Swallow and Flemming, 1969). Whether this reduction in carbohydrate plays a particularly important role during food deprivation at low temperature remains controversial (see below). Severe loss of protein was noted in winter starved carp tissues by Creach and Serfaty (1974). However, Ince and Thorpe (1976) found that in the Northern pike, protein content remains normal and there was a significant depletion in lipid after one month of starvation. They also found a decrease in liver glycogen and blood glucose during this period. A similar trend has been reported in the mature Japanese eel during the early stages of starvation (Inui and

Oshima, 1966). In the goldfish, lipid and protein are the main energy sources while the glycogen content of the liver is unchanged after 25 days of starvation (Stimpson, 1965). Jedryckowski and Fischer (1973) suggest that on the basis of excreted nitrogen in the silver eel, the oxidation of protein in the respiratory process is minimal and that lipids are used. Recent studies on the American eel find larger variation in tissue proteins, but significant increases in tissue fats after 6 months of starvation (Moon, 1980; Aster and Moon, 1979). The eel is the only fish reported that increase body fat during starvation (Love, 1970).

Migration as opposed to a low temperature fast is associated with the maintenance of glucose concentrations in the Atlantic and Pacific salmon (Jonas and MacLeod, 1959). At the end of migration, liver and muscle glycogen stores are essentially identical to the pre-migratory values in the Atlantic salmon (Fontaine and Hatey, 1953), while liver glycogen of the Pacific salmon doubles (Chang and Idler, 1960). Liver glycogen is substantially reduced after migration in the lamprey (Savina and Wotzczak, 1977) with no changes in the glycogen content of skeletal muscles. Regardless of the course that carbohydrate reserves take, migration results in a significant loss of fat and/or protein in all fish (Love, 1970).

Green (1919a, b) first suggested that lipids and proteins must be considered important starvation fuels in fish. Although the original studies were done on salmon, Love (1970)

has extended this generalization to all species, based principally on the low concentration of tissue carbohydrate and imprecision of blood glucose homeostasis. Even though carbohydrate is metabolised by skeletal muscles during contraction, carbohydrates *per se* do not appear to be a major source of energy during starvation. During these periods, blood glucose is maintained and liver glycogen stores are either too low (Kamura, 1966) or remain unchanged (Wittenberger and Giurgea, 1973). This apparent low carbohydrate turnover may result from low glucose utilization (Cowey *et al.*, 1975; Cowey *et al.*, 1977a) or an increase in gluconeogenesis from amino acids derived from skeletal muscle proteolysis (Stimpson, 1965; Butler, 1968; Larsson and Lewander, 1975). The evidence of enhanced glucoeogenesis in fish during starvation is principally inferential, being based on reciprocal changes in muscle protein and tissue glycogen. Studies by Cowey *et al.* (1977a) support this hypothesis for six week food deprived trout. However, Leech *et al.* (1979) have recently reported that plasma alanine levels increase during the first six weeks of starvation in the spiny dogfish, but thereafter return to control values. Ultrastructural studies have shown white muscle atrophy in both the cod (Walker, 1971) and the plaice (Johnston, 1980a). Therefore, even though muscle protein by mass alone represents a potentially important source of gluconeogenic precusors (Creach and Serfaty, 1974) and active muscle catabolism occurs during starvation (Siebert *et al.*, 1964; Johnston, 1980a), no generalization

can yet be made concerning fish gluconeogenesis during starvation.

Where starvation mobilizes lipid reserves, the first transition to fat preference in mammalian systems is under tight hormonal and metabolite controls (Drummond, 1971). In fish, the importance of fat and the pattern of mobilization varies, depending on where the metabolic stores are located (Fontaine and Olivereau, 1963).

It is apparent that fish are not easily classified as to their metabolic strategy during food deprivation. In order to approach a possible explanation for this much species heterogeneity, the various fish tissues involved with starvation will be surveyed.

METABOLIC CONSIDERATIONS OF FISH LIVER

Cowey (1975) and Lee and Putman (1973) have demonstrated that fish (rainbow trout) require a high protein diet with an obligatory supplement of simple sugars (Inabe et al., 1963; Singh and Nose, 1967; Palmer and Ryman, 1972) for optimal growth. This high protein diet would be processed by the gut releasing free amino acids into the portal circulation where the liver would have first option on the available substrates. Numerous authors (Braekkan, 1959; Cowey et al., 1977; Renaud and Moon, 1980a) have shown that fish liver is well suited for amino acid metabolism in the fed state. Dietary amino acids could enter the gluconeogenic pathway, be oxidized or utilized for protein synthesis in the liver. Alternatively, these amino acids could pass the liver unchanged into the

general circulation to be picked up by other tissues.

Other nutrients will be available to the liver; dietary carbohydrates, when present, may enter into hepatic carbohydrate metabolism and act to reduce the amino acid flux through the gluconeogenic pathway (Renaud and Moon, 1980a). Fat metabolism in the fed fish liver would be limited to triglyceride transformation (into free fatty acids), esterization, fat storage and probably oxidation (Guynn et al., 1972; Goodridge, 1972; Jacobs et al., 1973; McGee and Spector, 1975). Recent studies on the American eel (Aster and Moon, 1979) indicate that the liver is the primary site for fatty acid synthesis with acetate, probably derived from intestinal bacterial activity, the most active substrate (Aster, personal communication).

In the food-deprived fish, liver glycogen generally declines probably to maintain plasma glucose, yet quantities are limited and starvation can be extensive. This reserve would be rapidly depleted unless replaced through gluconeogenesis (see Renaud and Moon, 1980a, b). Liver triglycerides have been reported to be reduced in starved European eels (Larsson and Lewander, 1973; Dave et al., 1975), but increase when eels mature (Lewander et al., 1974). Thus lipids play a major role in the economy of the liver, probably supplying fuels for other tissues during both food deprivation and migration, as indicated by the general reductions noted in the hepatosomatic index during starvation (see Love, 1970).

Wittenberger and Giurgea (1973) have shown a five-fold

increase in the activity of starved carp liver Asp AT during a winter starvation period. Moon and Johnston (1981) found a similar large increase in plaice liver Asp AT after three months of food deprivation. Larsson and Lewander (1973) reported a two-fold increase in the same enzyme in the mature European eel after 145 days of starvation. They suggested this increase may be necessitated by an enhanced hepatic lipolysis, since to utilize the additional Acetyl CoA produced from β-oxidation of fatty acids, Krebs cycle intermediates must increase and one role for Asp AT is to increase oxaloacetate concentrations (Lehninger, 1975). The liver appears to undergo a major reorganization associated with enhanced amino acid utilization, probably for gluconeogenesis (see Moon and Johnston, 1980). These changes include significant alterations in enzyme activities (Moon and Johnston, 1980) and in the transport of amino acids (Moon and Johnston, 1981) as reported for starved plaice liver. Leech et al. (1979) found a major increase in plasma alanine in the spiny dogfish starved less than two weeks followed by a return to pre-starved levels, suggesting a system very similar to that during mammalian starvation. Assumably alanine would be made available to the liver for gluconeogenesis, but Renaud and Moon (1980b) found that alanine at levels occurring in starved eel plasma did not appreciably contribute to glucose production, at least not during the first 8 months of starvation.

As in mammals, these changes are apparently hormonally

directed (Cannon et al., 1956; Butler, 1968; Butler et al., 1969a,b). Specific details are controversial in fish beyond these generalizations: glucocorticoids are associated with carbohydrate feeding and starvation metabolism (Butler, 1968; Butler et al., 1969b); glucocorticoids and especially cortisol are involved in the provision of muscle amino acids and their availability during starvation (Chang and Idler, 1960; Storer, 1967; Chester Jones et al., 1969); insulin reduces plasma glucose, increases glucose carbon appearing as liver and muscle fats, and increases amino acid incorporation into muscle protein (Ince and Thorpe, 1976); and adrenaline is associated with the availability of plasma free fatty acids (Larsson, 1972).

METABOLIC CONSIDERATIONS OF FISH RED MUSCLE

Vertebrate skeletal muscles are composed of both red and white muscle fibers (see Prosser, 1973; Johnston, 1980b). Red muscle is characterised by an abundance of mitochondria and vascularity, high myoglobin concentrations, abundant triglyoeride droplets and well developed mechanisms for the uptake of fatty acids and glucose (Prosser, 1973; Johnston, 1980b). By virtue of these characteristics, it is expected that this muscle would metabolise aerobically with fats the principal fuel (Fritz et al., 1958; Slater, 1960; Bilinski, 1974) and possibly amino acids as a secondary fuel in the feeding state to energize the slow swimming movements of fish (Bone, 1966; Bilinski, 1974; Driedzic and Hochachka, 1978; Bone et al., 1978; Johnston, 1980b).

This expectation is supported by Braekken's studies (1959) which indicated that fish red muscle has liver-like properties. Although there is some inferred evidence to support this theory (see Hulbert and Moon, 1978b), this may be a metabolic oversimplification. Johnston and Moon (1980 a,b) have found that at least brook trout and salthe, and probably most other fish, maintain a very active anaerobic carbohydrate based red muscle metabolism. The exact affect these results will have on our present understanding of red muscle metabolism remains unknown. Anaerobic glycolysis is not thought to be a significant factor in this tissue as a result of high blood flows and the other characteristics mentioned above (see Bilinski, 1974; Johnston, 1980b), but enzyme profiles certainly predict a substantial anaerobic capacity (Johnston and Moon, 1980, a,b).

Fish starvation is associated with decreases in locomotor activities (Beamish, 1964; Johnston, 1980a) which are drastic in the European eel (Jedryczkowski and Fischer, 1973). This is associated with decreased metabolism as indicated by reduced oxygen consumption (Beamish, 1964) and $^{14}CO_2$ released from labelled glucose (Creach and Murat, 1974).

Red muscle water content does not change significantly during starvation (14 weeks) in the plaice (Johnston and Goldspink, 1973; Patterson et al., 1974) or in the European eel (Wodtke, 1974), although there are ultrastructural changes (e.g., total fiber and mitochondrial volumes decrease; Patterson and Goldspink, 1973). Metabolically, glycogen

levels fall by two-fold, lipids by three-fold and protein not at all in these same experiments and in general all changes tend to be small compared with these similar parameters in white muscle (Johnston and Goldspink, 1973). Starvation in plaice red muscle is also associated with a general decline in glycolytic and gluconeogenic enzyme activities, even through hexokinase, glycogen phosphorylase (total) and oxidative enzyme activities remain unchanged (Moon and Johnston, 1980). These results, plus the change seen in fuel reserves, suggest that, at least for the plaice, lipids and carbohydrates are the major fuels used to support starvation metabolism in red muscle. If amino acids are used, they must be derived from sources other than red muscle.

It is apparent, therefore, that fish red muscle has the potential to utilize amino acids as metabolic fuels, but that fats represent the principal endogenous substrate with carbohydrates possibly being important. Such a metabolic pattern does not appear to be altered even with extensive starvation.

METABOLIC CONSIDERATIONS OF FISH WHITE MUSCLE

Fish white muscle is structurally and metabolically similar to other vertebrate white muscles (see Prosser, 1973). The glycolytic pathway of fish white muscle is qualitatively identical to that described in other systems (Hochachka, 1967; Tarr, 1972) indicating that this muscle maintains an

active anaerobic metabolism based on glycolysis (Love, 1970; Bilinski, 1974; Driedzic and Hochachka, 1978). As such this tissue must depend on glycogen derived from remote carbohydrate sources for the energy of contraction since little muscle glycogen is present (Love, 1970; Prosser, 1973). These sources include liver and potentially red muscle (see Hulbert and Moon, 1978b). Even though glucose/glycogen are metabolized by white skeletal muscle during contraction, carbohydrate does not appear to be a major source of energy for this tissue during food deprivation. In fact, the major decreases in fish activity previously noted which occurs during starvation indicate this tissue may not serve an important contractile role during this period (see below and Hulbert and Moon, 1978b).

Major decreases in white muscle protein content have been reported for a number of fish species during starvation (see Love, 1970) including the eel (Lovern, 1939; Larsson and Lewander, 1973; Dave et al., 1975; Renaud and Moon, 1980b; Moon, 1980). In all cases, water content increases, a trend indicative of protein utilization (Love, 1970). Furthermore, muscle triglyceride levels fall with food deprivation in many fishes (Love, 1970) and the Swedish authors have reported this for the European eel (e.g., Holmberg et al., 1972; Larsson and Lewander, 1973). Tragically, little attempt has been made by these authors to separate red and white muscle; since Hulbert and Moon (1978a) found no major triglyceride deposits in American eel white muscle, the significance of

these studies is unknown. Some fatty fish, such as mature silver eels, store large quantities of triglycerides in muscle which are essentially unavailable to the oxidatively-poor white muscle. Further, Love (1970) finds that the eel is the only fish known to increase white muscle fat content during starvation (unlike the report of the Swedish authors above). Our laboratory has found this in immature American eel white muscle after starvation, but the significance of this observation is unknown.

Behaviourly, during migration (Jedryczkowski and Fischer, 1973) and starvation (Love, 1970), white muscle-directed swimming activity is rarely seen unless the fish are frightened. It has been suggested that the major function of white muscle in starved fish is to provide a storage for amino acids (Creach and Serfaty, 1974) and extracellular lipids (Love, 1970). One should remember that the white muscle mass of a fish is essentially neutrally buoyant in water, so this large reserve of lipid and amino acids represents a minimal metabolic cost to transport. Three studies add some evidence to this postulate. First, Johnston and Goldspink (1973) suggest that the large increase in water content of plaice white muscle during starvation will alter the relationship between adjacent myofibrils reducing the effective tension of the muscle. Secondly, Moon and Johnston (1980) found major decreases in all enzymatic activities assayed (both glycolytic and oxidative) in starved plaice white muscle, again indicating a major decrease in the metabolic

potential of this muscle mass. Thirdly, Johnston (1980a) has observed major deterioration in myofibrillar ultrastructure with long term starvation in the plaice, indicative of proteolytic enzyme attack; Walker (1971) found a similar situation in the cod. In fact, cod muscle activities of cathepsins and peptidases are amongst the highest reported in the vertebrates (Siebert et al., 1964; Love, 1970). The exact fate of these mobilized nutrients is not known, although a number of authors have suggested that they are cycled through metabolic pathways in red muscle (Wittenberger and Giurgea, 1973; Wittenberger et al., 1975; Driedzic and Hochachka, 1978; Hulbert and Moon, 1978b), or other tissues (Moon and Johnston, 1981).

It is apparent that during starvation, fish white muscle may play a reduced contractile role, but an enhanced metabolic participation by providing metabolites for other tissues. The extent of this change in function will probably depend upon such factors as age, level of reserves, species, temperature, etc., but certainly the literature does suggest this occurs. Unfortunately, what controls the change is unknown, as are the specific events leading to the change.

THE EEL - Anguilla rostrata LeSueur

The life history, morphology and metabolism of the Anguilla eels have been well documented in major reviews (Bertin, 1956; Sinha and Jones, 1975; Tesch, 1978). It is generally accepted that both the European eel, A. anguilla, and the American eel, A. rostrata, breed in the proximity

of the Sargasso Sea. The exact breeding grounds within this area are, however, still in dispute (see especially Vladykov, 1964; Sinha and Jones, 1975). Also in dispute is whether the American and the European eels are genetically distinct or whether they arise from the same spawning but follow separate currents (Stasko and Romel, 1974; Sinha and Jones, 1975; Pantelouris et al., 1976).

Despite these uncertainties, American eel larvae develop following fertilization into surface dwelling leptocephali which drift passively with the currents toward the coast of North America. Approximately one year after hatching, they arrive on the continental shelf and enter fresh water river systems where metamorphosis begins, first into the intermediate stage, the glass eel, and finally into a pigmented elver. The transition of the glass eel into an elver is marked by numerous morphologically significant changes; pigmentation develops, the mouth parts reorganize allowing active pursuit feeding to replace planktonic alimentation, the digestive tract and anus are repositioned, and importantly, the general morphology of the elver assumes the shape of the adult (Sinha and Jones, 1975).

From larval hatching to the point elvers begin the upstream freshwater migration is approximately 2-3 years. Again it is generally accepted (although not without dispute) that only the females migrate upstream and the males remain in the brackish estuarian areas (Sinha and Jones, 1975). The eels which do migrate upstream remain in fresh water for

5-10 years during which time the pigmentation gradually changes from a yellow to the characteristic silver (European eel) or bronze (American eel) stage (Vladykov, 1955) in preparation for seaward migration. Other dramatic morphological and physiological changes occur at this time including increases in fat deposits, thickening of the skin, changes in osmoregulatory capacity, development of the gonads, increased endocrine activity, cessation of food consumption, resorption of the digestive tract, and increased quantities of red muscle and alterations in the activities of several oxidative and glycolytic enzymes in both red and white muscles (Boström and Johansson, 1972; Dave et al., 1974; also extensive reviews by Bertin, 1956; Sinha and Jones, 1975).

Importantly, the maturing eel undergoes two natural starvation periods. The first is a seasonal fast in response to decreasing ambient temperatures; once temperatures drop below 10°C, the eel stops feeding and buries itself in the mud of the river bottom (Nyman, 1972; Sinha and Jones, 1975). In north temperate waters, this period can last up to 8 months and usually exceeds 6 months. Second, since the eel ceases to feed upon commencement of seaward migration (Bertin, 1956), the entire spawning migration is conducted using only stored energy, which must suffice for locomotion and gonadal maturation. After spawning it is presumed that the eels die at sea but no adults have ever been captured in the Sargasso Sea area, nor have any adult eels ever been reported return-

ing to the North American coast (Sinha and Jones, 1975). Therefore, the eel lends itself to studies of starvation metabolism since this fish is in a natural state of nutritional deprivation during much of it's life history.

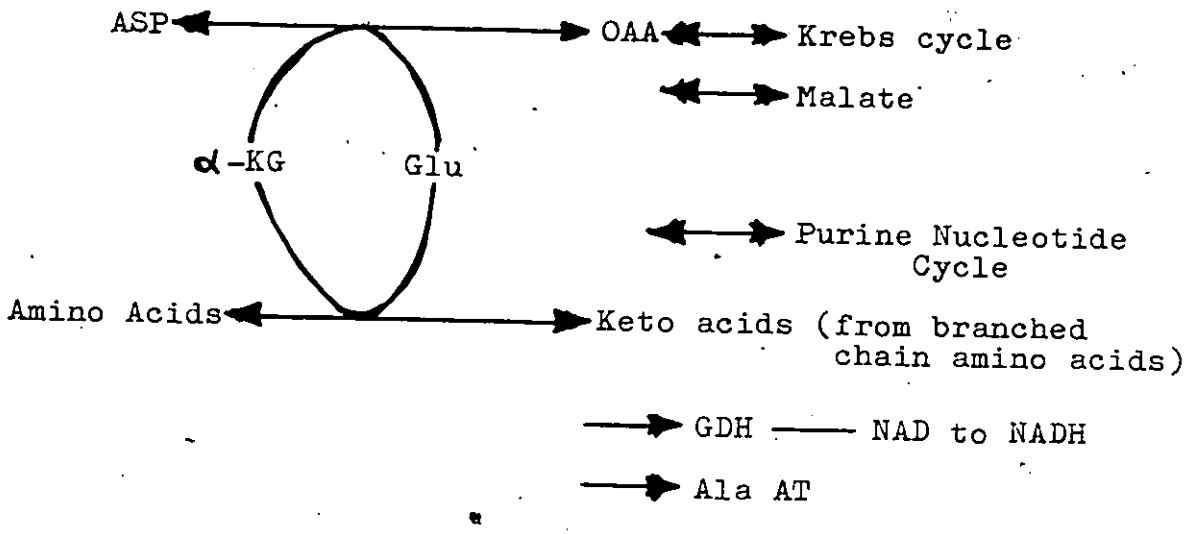
Another important consideration in choosing the eel as an experimental animal is that the musculature is clearly divided into two relatively homogeneous populations of red and white muscle fiber groups (Hulbert and Moon, 1978a). This allows the collection of pure preparations of each muscle type for biochemical analyses. Furthermore, there is a marked increase in the amount of red muscle concurrent with the seaward migration suggesting that the red muscle may play a more important role in migration than at any other time during the life cycle of the eel (Boström and Johanssen, 1972; Sinha and Jones, 1975).

Lastly, the economic value of the eel in Europe has led to a voluminous data base of physiological and biochemical correlates (see e.g., Sinha and Jones, 1975) providing an excellent basis for comparisons.

THE ENZYME - Aspartate Aminotransferase

The enzyme Asp AT or glutamate-oxaloacetate transaminase (GOT) (EC 2.6.1.1) is involved in the conversion of aspartate (Asp) and α-ketoglutarate (α-KG) into oxaloacetate (OAA) and glutamate (Glu). This reaction is reversible (Herbst and Engle, 1934) and both the substrates and products can contribute to elements of the tricarboxylic acid cycle, glycolysis, gluconeogenesis, purine nucleotide

cycle, branched chain amino acid metabolism, glutamate dehydrogenase (GDH) and alanine aminotransferase (Ala AT), as demonstrated in the following diagram:



(see Lehninger, 1975)

As a result of the multiplicity of the functions and the key position Asp AT occupies in metabolism, the enzyme could play a significant role in the metabolism of starved animals, including fish, where the primary source of metabolic energy is from lipids and amino acids.

The determination of serum glutamate-oxaloacetate transaminase (SGOT) or Asp AT has become a diagnostic tool for cardiac, pulmonary and hepatic diseases in humans (Wroblewski and LaDue, 1956; Amador and Wacker, 1962). This enzyme, together with Ala AT, is frequently used by fish biologists to diagnose sublethal insult to liver by pollutants (see D'Apollona and Anderson, 1980) and disease (Bell, 1968). As a result, Asp AT has been extensively studied in

many animal species. Results of these investigations have shown that few consistencies exist between homologous Asp ATs with the exception of the reactants and the products.

In mammalian systems the molecular weight of Asp AT has been reported to be 79,000 daltons (Owen and Hochachka, 1974-dolphin muscle), 90,000 daltons (Martinez-Carrion, et al., 1967-pig heart), 103,000 daltons (Krista and Fonda, 1973-beef brain) and 110,000 daltons (Jenkins et al., 1959-pig heart). All authors report that Asp AT exists as at least two electrophoretically and biochemically distinct isozymes of cytosolic and mitochondrial origin. When these two forms are further characterized, each can produce a single band (Saier and Williamson, 1967), single and multiple bands (Bertland and Kaplan, 1970; Magee and Phillips, 1971) or multiple band patterns (Martinez-Carrion et al., 1965, 1967; Nisselbaum and Bodanski, 1966; Martinez-Carrion and Tiemeier, 1967; Shrawder and Martinez-Carrion, 1973; Chen et al., 1973; John and Jones, 1974) on electrophoresis. Where multiple isozymic forms occurred, each isozyme exhibited similar catalytic and immunochemical properties. pH optima vary throughout the literature from less than 7.5 in the Pacific salmon (Bell, 1968) to 8.0-8.5 in pig heart (Worthington Manual, p.49-50). Filosofova-Lyzlova (1972) found that the cytosolic and mitochondrial isozymes of some lower vertebrates (pike and tench) differed in pH optima; Owen and Hochachka (1974) found a similar situation in dolphin muscle. Jenkins et al. (1959) found that the enzyme binds

two moles of pyridoxal-5'-phosphate (P-5'-P) (pig heart) and Yan-Hwa and Churchich (1975) found that resolved/dissociated Asp AT requires two moles of P-5'-P to restore 95% of the native enzyme activity. Where only one mole is available to the dissociated enzyme, three identifiable forms exist suggesting that the two native enzyme subunits can function independently.

Where the enzyme Asp AT has been kinetically studied, again the results are variable. Krista and Fonda (1973) reported that beef brain cytosolic Asp AT had an apparent $Km_{(Asp)}$ of 2.0 mM and 0.17 mM for the $Km_{(\alpha\text{-KG})}$. They also found significant inhibition of the reaction from 1 to 3 mM α-KG. Owen and Hochachka (1974) found similar apparent Km values in the cytosolic form from dolphin skeletal muscle, but very different values for the mitochondrial form. Again, significant substrate inhibition of the reaction occurred and it was postulated that the enzyme may be controlled by concentrations of substrates and/or products (see Cornell et al., 1974). In the pike and tench, Filosofova-Lyzlova (1972) reported quite different values from those of mammals although substrate inhibition was also noted at least for mitochondrial Asp AT. In the carp and the European eel, food deprivation results in a five-fold and two-fold increase in activity for Asp AT in liver and muscle tissue, respectively (Larsson and Lewander, 1973; Wittenberger and Giurgea, 1973). Similar results have been reported for three month food-deprived plaice (Moon and Johnson, 1981).

One would predict from the literature that Asp AT is an enzyme of approximately 80-110 thousand daltons existing as cytosolic and mitochondrial forms, possessing multiple isozymes, having a requirement for P-5'-P, and possibly exhibiting substrate inhibition. Whether these characteristics are observed for Asp AT isolated from American eel tissues and whether changes in activity occur as a result of food deprivation, are the subject of this thesis.

STATEMENT OF THE PROBLEM

From the preceeding, it is evident that the American eel provides a model for examining the effects of starvation on amino acid metabolism. Since the eel does not feed during both seaward migration and the winter low temperature period, the provision of energy during these periods must be generated from stored reserves. Lipids and proteins (amino acids) are the primary fuel stores in the eel and their metabolism must be critically controlled to enhance the animal's survival potential. With the central position of aspartate aminotransferase to both amino acid and lipid metabolism, it was thought this enzyme would be sensitive to nutrient conditions (see Wilson, 1973). This thesis studies the activities and characteristics of Asp AT in liver, red muscle and white muscle isolated from the American eel and how the enzyme is influenced by six to ten months of food deprivation.

Where there are changes in the apparent activity of the enzyme, an attempt will be made to establish whether this

results from changes in the cytosolic or mitochondrial enzymes, or both. In addition, some attempt will be made to physically characterize the enzyme in order to relate or dissociate any changes in the activities of Asp AT during starvation with these characteristics.

Therefore, two hypotheses will be tested by this study:
1- nutrient deprivation in the American eel, Anguilla rostrata, results in an enhanced amino acid metabolism as reflected by an increase in the activities of tissue Asp AT; and,
2- the specific changes in Asp AT will be greatest in those tissues which contribute the most to amino acid metabolism.

II

MATERIALS AND METHODS

MATERIALS AND METHODS

ANIMALS

American eels, Anguilla rostrata, were collected at Quebec City, Quebec, and Cornwall, Ontario. The eels taken from the St. Lawrence River at Quebec City were netted by a commercial fisherman in the summer of 1974. They were transported to the University of Ottawa laboratory in wooden crates containing ice on an open flatbed truck. Upon arrival the eels were retained in 1000 litre tanks with a continuous supply of aerated declorinated Ottawa city tap water. The photoperiod was regulated at 12:12 (light:dark) and the water temperature was approximately 12°C. The eels averaged 1 kg in weight; since they were in the seaward phase of their migration, they would not respond to feeding attempts (see Sinha and Jones, 1975).

Eels were also removed from the St. Lawrence River at Cornwall from the top of the eel ladder (maintained by the Ontario Ministry of Natural Resources) associated with the Saunders Hydro Electric Dam. These fish were collected with a hand net and transported either in wooden or styrofoam containers with melting ice to keep the animals damp. They were then held in the University of Ottawa laboratory as described above. After five days in the laboratory, the eels were sized and those weighing between 150 and 600 g (i.e., the largest animals) were segregated into groups of approximately 25 fish each. Groups were either fed a diet of chopped beef liver or were deprived of food for the 10 month duration of

the experiment, during which time water temperature was maintained at 12± 2°C and the photoperiod was 12:12. The feeding state was verified by gut contents when the animals were sacrificed.

TISSUE PREPARATION

Quebec City eels were sacrificed after at least six months of starvation; the Cornwall eels were fed or starved for ten months. In all cases, the eels were decapitated followed by exsanguination. The large Quebec eels were frozen whole at -20°C and the enzyme was prepared from isolated tissues within one month of freezing. The smaller Cornwall eels were dissected prior to freezing, the liver was removed, the gut checked to assess the feeding state, and the carcass and the liver frozen in liquid nitrogen. Thereafter, the frozen samples were placed at -60°C (Revco ultra-low temperature freezer) until enzymes were isolated. This procedure assured that all eels from a particular experiment were in the same nutrient state, and the freezing process did not decrease the specific activities of Asp AT (see Results). Also, this procedure made possible the removal of numerous small samples of liver, red and white muscles without the entire piece thawing and altering enzyme activities.

Red muscle adheres tightly to white muscle at the lateral line (see Hulbert and Moon, 1978a), but can be removed without contamination in frozen sections (see Hulbert and Moon, 1978a). The skin was easily stripped from the frozen carcass and the underlying red muscle freed with a scalpel from the larger

white muscle mass. Once the red muscle was removed, the still frozen muscle mass was scraped to remove any adhering red muscle, and a pure white epaxial muscle piece was removed. These frozen pieces, including liver, were weighed prior to homogenization.

ENZYME PREPARATION

Two enzyme preparations were used in these studies. Total tissue Asp AT was assayed from partially purified, whole tissue homogenates; alternatively, the crude homogenate was subjected to differential centrifugation and cytosol and mitochondrial Asp AT activities were estimated.

Samples of liver, red muscle and white muscle were collected from frozen Quebec or Cornwall eels as above, weighed and homogenized on ice with either a Sorvall Omni-mixer or a Brinkman Polytron PUC-2 homogenizer. The homogenization buffer consisted of 100 mM potassium phosphate buffer, pH 7.4 with 10 mM EDTA, 2.5 mM 2-mercaptoethanol, 0.25 mM P-5'-P, and 2.0 mM α-ketoglutarate. The tissue to buffer ratio for liver was 1:4 and 1:5 for the muscles. The crude homogenate was brought to 60°C in a water bath as quickly as possible with stirring. Once this temperature was attained, the homogenate was immediately chilled in an ice bath. The heat treated homogenate was centrifuged at 16,000 xg for 30 min. at 0°C in a Sorvall RC-2B refrigerated centrifuge and the supernatant decanted through a layer of fiber wool to remove cellular debris and congealed fats. Heat treatment resulted in a 15-fold purification of the enzyme with a 60% yield, but

subsequent treatment (including gel exclusion chromatography and salt fractionation with ammonium sulfate) did not significantly increase the purification but dramatically decreased the recovery. Therefore, no further attempts were made to purify the enzyme. Activities in the cleared supernatant were concentrated by volume reduction in the presence of sucrose. The homogenate was pipetted into cellulose dialysis bags and packed in crystalline sucrose at 5°C until the remaining volume was approximately one-third of the original. Asp AT prepared in this manner was used to estimate total tissue Asp AT activities (see Results).

Differential centrifugation was used to separate mitochondrial from cytosol Asp AT according to the methods of Hogeboom et al. (1948), DeRosa and Swick (1975) and Weinbach et al. (1961). Buffer used in this preparation contained 225 mM mannitol, 75 mM sucrose, 10 mM EDTA or EGTA in 0.01 M Tris buffer titrated to pH 7.8 with HCl (Tyler and Gonze, 1967). Tissues were isolated from frozen Cornwall eels, homogenized at low speed with the polytron and mitochondria were prepared according to Moon and Ouellet (1979). Eel liver mitochondria are known to maintain both morphological and biochemical integrity using these techniques (see Moon and Ouellet, 1979). Cytosol Asp AT is determined in the supernatant over the 12,000 xg cell pellet, and this pellet is resuspended in the above buffer less mannitol-sucrose to give mitochondrial Asp AT. Activities in these fractions were not concentrated unless below a level which could be easily determined. These

preparations were not subjected to heat treatment.

Some question was raised as to the suitability of the frozen tissues for the studies of Asp AT localization. Therefore, two additional experiments were undertaken. First, marker enzymes were assayed to monitor the purity of each fraction; activities of lactate dehydrogenase (LDH, EC 1.1.2.7) indicated the cytosol fraction and glutamate dehydrogenase (GDH, EC 1.4.1.2) and citrate synthetase (CS, EC 4.1.3.7) the mitochondrial fraction. Second, fresh tissue was prepared in a manner identical to the frozen tissue (see above), and Asp AT and marker enzyme activities were estimated in each fraction (see Results).

ENZYME ASSAYS

Enzyme activities were recorded as alterations in the absorbance of a chromatogenic substance at its optimal wavelength with a Gilford 2100 recording spectrophotometer. Cuvette temperatures were controlled by coupling the jacketed cuvette holder to a Haake constant temperature water bath. Cuvette temperatures were monitored with a Yellow Springs Telethermometer and did not vary by more than 0.1°C of the indicated value.

Eel Asp AT was assayed in the forward and reverse directions employing a NAD-coupled enzyme system. Specific activities were estimated using the extinction coefficient for NADH of 6.22 $cm^2/\mu mole$. All assays were done at least in duplicate and blanks (no substrate addition) were used to verify the authenticity of the reaction. Where necessary,

these blank values were substracted to give Asp AT activities.

In the forward direction, Asp and α-ketoglutaric acid (α-KG) are transaminated to oxaloacetate (OAA) and glutamate (Glu). This reaction was coupled to OAA reduction by NADH and dialyzed Sigma purified pig heart malate dehydrogenase (MDH) and recorded as a decrease in the absorbancy at 340 nm (see Amador and Wacker, 1962; Karmen, 1955). The standard assay contained the following:

100 mM phosphate buffer, pH 7.5
10.0 mM Asp
2.5 mM α-KG
0.45 mM NADH
excess dialysed Sigma MDH
0.25 mM pyridoxal-5'-phosphate (P-5'-P)
0.5 mM $MgCl_2$

The reaction was initiated by Asp after addition of homogenate. Each of these components was modified in turn according to the specific experiment (see details in Table and Figure legends).

For the reverse reaction, a fairly pure tissue homogenate was required so that the competing enzymes did not interfere with the assay. Starved liver cytosol Asp AT was partially purified by heat treatment followed by sucrose concentration; this preparation was passed through a Sephadex G-100 column twice to reduce activities of the competing enzymes. To assay activity, the elutant had to be extensively sucrose concentrated and dialysed. As above, the activities were monitored at 340 nm as an increase in absorbancy resulting

from the production of NADH in the presence of purified, extensively dialysed, glutamate dehydrogenase (GDH). The standard assay contained the following:

100 mM phosphate buffer, pH 6.9
1.48 mM oxaloacetate
1.84 mM glutamate
0.45 mM NADH
excess dialysed Sigma GDH

The reaction was initiated by OAA after homogenate addition and rates were linear for at least 2 min. Specific experiments altered this standard assay (see Table and Figure legends).

The following represent the standard conditions under which the marker enzymes were assayed:

Lactate dehydrogenase (LDH); in a final cuvette volume of 0.5 ml,

100 mM tris-HCl, pH 7.5
0.45 mM NADH
2.0 mM pyruvate

The reaction was initiated by the addition of from 0.65 to 25 µg of protein and monitored at 340 nm; blanks (no pyruvate) and purified Sigma LDH were used to verify the authenticity of the reaction.

Glutamate dehydrogenase (GDH); in a final cuvette volume of 0.5 ml,

100 mM tris-HCl, pH 7.5
0.45 mM NADH
2.0 mM α-KG
20 mM NH_4Cl

The reaction was initiated by the addition of from 0.65 to 25 µg of protein and monitored at 340 nm; blanks and Sigma purified GDH were used to verify the authenticity of the reaction.

Citrate sythetase (CS): in a final cuvette volume of 0.5 ml,

100 mM tris-HCl, pH 7.5

25 mM 5,5 dithiobis-2-nitobenzoic acid (DTNB)

0.5 mM Acetyl CoA

0.4 mM oxaloacetate

The reaction was initiated with the addition of from 0.65 to 25 µg of protein and blank (no OAA) values were subtracted. The change in absorbancy was read at 412 nm and the activities were calculated using an extinction coefficient of 13.6 $cm^2/\mu mole$.

OTHER ANALYTICAL TECHNIQUES

Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) was carried out using a modification of the technique of Davis (1964) on 5% gels (Hulbert, 1976). Approximately 50 µg of protein was applied to the 0.8 x 9.0 cm gels and run at 6.5 mA/gel until the Bromophenol Blue marker approached 5 mm of the gel end. The gels were stained according to the methods of Decker and Rau (1963) which couples a diazonium salt directly to the OAA produced in the enzymatic reaction. The stain consisted of

500 mg Asp

75 mg ∝-KG

50 mg P-5'-P

200 mg Fast Blue-BB salt

These dry ingredients were added to 100 mls of 50 mM tris-HCl, pH 7.6 just prior to use. The stain was then added to a test tube containing the individual gels and staining continued in the dark until the background colour of the gel was a dirty brown. The bands were distinctly dark blue encircling the gel. Omission of any one ingredient in the stain resulted in no banding.

Isoelectrofocusing

Isoelectrofocusing of the fed and starved liver total homogenates was accomplished using the granulated technique described by Radola (1969). The gel was prepared by swelling 7.5 gms of Sephadex G-75/40 in 100 mls of distilled, deionized water containing 2.5 mls of LKB pH 3-10 ampholine. The bed preparation was degassed under vacuum and spread on a 20 x 20 cm glass plate to give a final thickness, after partial dehydration, of approximately 1.5 mm. The samples were prepared by sucrose concentrating total homogenates to a final protein concentration of approximately 10 mg/ml. About 70 µl of this preparation was then applied to the gel bed 7.0 cm from the anode with the aid of a cover slip. Samples were run at 350 volts for 4 hrs. and then 800 volts for another 4 hrs. Both homogenates were run concurrently and in duplicate. Evaluation of the focused samples was accomplished by removing the Sephadex bed in 0.35 cm widths with a spatula and adding 0.5 ml distilled water to extract the enzyme

proteins. The pH of each fraction was determined with a Radiometer BMS3 MK 2 blood micro system and the enzyme activity determined according to the standard assay.

Molecular Weight Determination

The assay of Asp AT molecular weights in the tissue fractions of fed and starved Cornwall eels was accomplished by following the directions outlined in the Sephadex Molecular Weight Calibration Kit Instruction Manual. 5 grams Sephadex G-100 were swollen in 100 mM phosphate buffer containing 0.1 M NaCl at room temperature. The swollen gel was poured into a 2.5 x 29 cm column and allowed to gravity pack to within approximately 1 cm of the top. Buffer was allowed to drain freely through the column at a pressure head of 20 cm H_2O. The packed, flowing column was then equilibrated to 5°C in a cold room where buffer was allowed to flow for two days prior to calibration of the column. Column outflow was monitored at 254/280 nm with an LKB Uvicord attached to a Fisher Recordall. Aliquots were collected with a LKB Ultrarac Fraction Collector. At no time was the column buffer flow stopped, except to apply samples. Column void volume was established by applying 1 ml Dextran blue to the column bed. Four consecutive void volumes were estimated and the average used to determine K_{av} for the standard proteins. After each standard protein run, buffer was passed through the column to restore the elutant to baseline and the void volume was rechecked to assure that it had not changed. The tissue fractions containing Asp AT were run individually on the column and the

elutant collected as above. Each sample was then assayed for Asp AT activity according to the standard assay (forward direction). The K_{av} was estimated for the peak activity fractions and compared to the standard protein curve to estimate the molecular weight of the particular tissue or fraction Asp AT.

Protein Determination

Where necessary, protein content was estimated either by the method of Lowry et al. (1951) or Layne (1957). Bovine serum albumin was used as a standard and the two methods did not vary by more than ± 0.05 mg/ml.

CHEMICALS

All biochemicals were obtained from Sigma Chemical Co., St. Louis, Missouri. All other chemicals were obtained from local distributors and were of the highest possible purity.

III

RESULTS

RESULTS

CHARACTERIZATION OF Asp AT FROM EEL TISSUES

Initially, these studies were intended to examine both Asp AT and alanine aminotransferase in eel tissue. Unfortunately the latter enzyme was unstable and demonstrated activity levels at least 10-fold lower than Asp AT. The information collected concerning Ala AT was not included in the thesis, but the reason certain conditions were ultimately employed in the study of Asp AT was a direct result of my attempt to stabilize Ala AT. The following experiments were intended to characterize total tissue Asp AT, and the enzymes were isolated from pooled tissues of bronze, seaward migrating Quebec eels and treated as noted in the Materials and Methods.

1. Effect of Buffers

The activity of partially purified eel liver Asp AT was essentially identical in 100 mM phosphate or Tris-HCl buffers between pH 7 and 9. Tris-maleate buffer under the same conditions produced substantially lower activities, being 50% of the other buffers at pH 7.5. Similar results were found using red and white muscle enzyme preparations. Therefore, phosphate buffers have been used in all kinetic studies of Asp AT from eel tissues since it can be easily formulated across a broad pH range (5.5 to 8.2) and it has a low thermal coefficient relative to Tris buffers.

2. Effect of pH

Using the phosphate buffer system, the effect of pH on

eel tissue Asp ATs was studied at substrate concentrations giving both optimal activities (V_{opt}) and one-half optimal activities (i.e., Km or the Michaelis constant). Fig. 1 demonstrates that the profiles for the liver (Lv), white muscle (WM) and red muscle (RM) enzymes are flat with some activation above approx. pH 7.5 (especially WM-Asp AT). This flatness is exaggerated at Km-concentrations of substrates, a level thought to be closer to cellular substrate concentrations (Fersht, 1977).

3. Effect of Substrate Concentrations

Since Asp AT has two substrates (see Introduction), it is necessary to study both in a reciprocal manner. Tables 1 and 2 illustrate the changes in V_{opt} and Km of eel tissue Asp ATs when the two substrates are varied in this manner.

Liver Asp AT at α-KG concentrations of 0.5, 1.0 and 2.0 mM demonstrated Asp saturation curves which were essentially unchanged in both V_{opt} and $Km_{(Asp)}$. Only at a concentration of 0.125 mM α-KG (the approx. $Km_{(\alpha\text{-KG})}$) did V_{opt} and Km change (Table 1). This suggests that Lv-Asp AT in the total homogenate is relatively insensitive to α-KG concentrations once a minimum is present. These curves also exhibited varied degrees of Asp inhibition but no consistent trend was noted. With respect to α-KG saturation (Table 2), Asp concentrations modulated both V_{opt} and $Km_{(\alpha\text{-KG})}$. As Asp was increased, both V_{opt} and $Km_{(\alpha\text{-KG})}$ increased suggesting that Asp concentrations, not α-KG concentrations are important effectors of Lv-Asp AT. Again, substrate inhibition was

Fig. 1.

Total Asp AT activities as a function of pH. Enzymes were partially purified and isolated from starved Quebec eel liver, red muscle and white muscle pooled tissue homogenates and assayed according to the Standard Assay at 20°C.

Symbols:

- ● 10.0 mM Asp, 1.0 mM α-KG
- ○ 5.0 mM Asp, 1.0 mM α-KG
- ▲ 5.0 mM Asp, 2.5 mM α-KG
- △ 0.75 mM Asp, 0.3 mM α-KG
- ■ 10.0 mM Asp, 2.0 mM α-KG
- □ 0.39 mM Asp, 0.125 mM α-KG

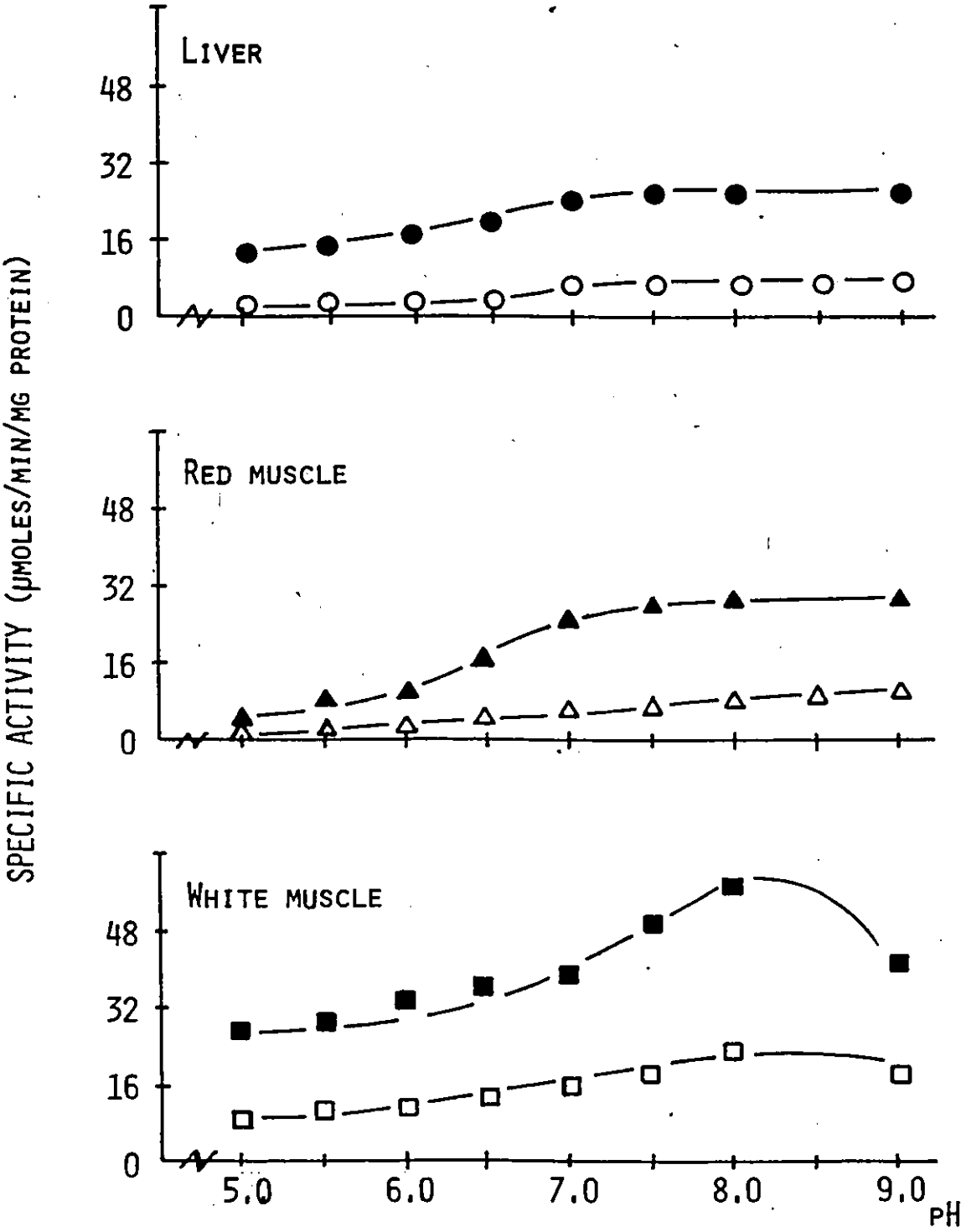

LIVER
RED MUSCLE
WHITE MUSCLE
48
32
16
0
5.0
6.0
7.0
8.0
9.0
pH
SPECIFIC ACTIVITY (μMOLES/MIN/MG PROTEIN)

TABLE 1

The effect of varying concentrations of α-ketoglutarate on the kinetic parameters of Aspartate for eel tissue Asp ATs. Enzymes were isolated from liver, red muscle and white muscle of starved Quebec eels. Homogenates were partially purified whole tissue extracts assayed according to the Standard Assay at pH 7.5 and 20°C.

TISSUE	α-KG (mM)	V_{opt} (μmoles/min/mg)	$Km_{(Asp)}$ (mM)	INHIBITION *
LIVER	0.125	7.0	0.33	0
	0.5	25.5	1.00	+
	1.0	24.5	1.00	-
	2.0	23.6	1.00	+
RED MUSCLE	0.3	38.5	0.77	+
	2.5	16.8	0.77	-
WHITE MUSCLE	0.125	13.5	0.16	0
	2.0	38.5	0.67	0

* Inhibition- Substantial inhibition indicated by +, no appreciable evidence by 0 and - means saturation curve tends to increase at high α-KG.

TABLE 2

The effect of varying the concentrations of Aspartate on the kinetic parameters of α-Ketoglutarate for eel tissue Asp ATs. Enzymes were isolated from liver, red muscle and white muscle of starved Quebec eels. Homogenates were partially purified whole tissue extracts assayed according to the Standard Assay at pH 7.5 and 20°C.

TISSUE	Asp (mM)	V_{opt} (μmoles/min/mg)	$Km_{(\alpha\text{-KG})}$ (mM)	INHIBITION *
LIVER	0.125	3.3	0.078	+
	1.0	12.0	0.178	+
	10.0	29.9	0.208	-
RED MUSCLE	0.75	25.0	1.42	0
	5.0	38.5	1.42	+
WHITE MUSCLE	0.39	38.6	0.16	0
	10.0	19.2	0.25	0

* Inhibition - as per Table 1

observed at least at the low Asp concentrations.

The pooled RM-Asp AT was quite different from the Lv-enzyme. Here, Km for either substrate is unaffected by the other but V_{opt} is either increased (with Asp) or decreased (with α-KG) (Tables 1 and 2).

White muscle Asp AT demonstrated some of the lowest Km values of the three enzymes studied and some of the highest activities (Tables 1 and 2). In general, increases in one substrate increased the Km for the other. Also, no substrate inhibition was observed for this enzyme at any concentration tested.

These alterations in kinetic parameters by substrates may indicate an inherent control by substrate operates in eel tissue Asp ATs. Such a scheme has been previously suggested (see Owen and Hochachka, 1974). However, since these preparations represent total tissue Asp AT, it is possible that changes observed here are a result of competition between cytosol and mitochondrial tissue isozymes. If this is so, these kinetic results could represent artifacts, but they do require further investigation in the respective isolated subcellular fractions (see below).

4. Effect of Temperature

Temperature is a known enzyme modulator, causing changes in both V_{opt} and Km (see Hochachka and Somero, 1973). Furthermore, temperature can be used to distinguish enzyme types, and therefore, eel tissue Asp ATs were compared over a range of temperatures corresponding to those which eels could

encounter in Nature. Arrhenius plots were determined at both V_{opt} and Km levels of substrates by estimating activity values generated from Asp and α-KG saturation curves run at the corresponding temperatures. These plots can be used to estimate the effects of temperature at both concentrations of substrates and secondly, the effects of temperature on enzyme-substrate affinity (see Borgmann and Moon, 1976).

Fig. 2 presents a series of parallel lines, indicating that a) the three tissue Asp ATs have the same temperature sensitivities at maximal activities (Ea, or the Arrhenius number, is approx. 20.8 kcal/mole) and b) the affinity of the enzyme for its substrates is temperature insensitive since slopes at substrate concentrations corresponding to V_{opt} and Km are equivalent. With this high Ea-value and a thermally-insensitive Km, eel tissue Asp AT activities will be greatly depressed at low temperatures (see Hochachka and Somero, 1973), a result not inconsistent with the normal metabolic reduction observed in these animals at low ambient temperatures (Sinha and Jones, 1975), and the effects of temperature observed for other eel tissue enzymes (e.g., Moon and Hulbert, 1980).

5. Other Effectors of Tissue Asp ATs

The requirement for pyridoxal-5'-phosphate (P-5'-P) by Asp AT has been shown by various authors (see Introduction). To verify this for eel tissue Asp ATs, a quantity of eel liver homogenate was exhaustively dialysed against buffer without P-5'-P added and activity was checked at increasing P-5'-P concentrations. Fig. 3A demonstrates that P-5'-P does increase

Fig. 2.

Effect of temperature on eel tissue Asp ATs. Enzymes were partially purified and isolated from food-deprived Quebec eel liver, red muscle and white muscle pooled homogenates and assayed at pH 7.5. Arrhenious plots were determined at both saturating and Km-values of substrate by estimating activity values generated from aspartate saturation curves assayed from 5 to 30°C.

Symbols:

- □ Log V_{opt} white muscle Asp AT
- △ Log V_{opt} red muscle Asp AT
- ○ Log V_{opt} liver Asp AT
- ■ Log V_{Km} white muscle Asp AT
- ▲ Log V_{Km} red muscle Asp AT
- ● Log V_{Km} liver Asp AT

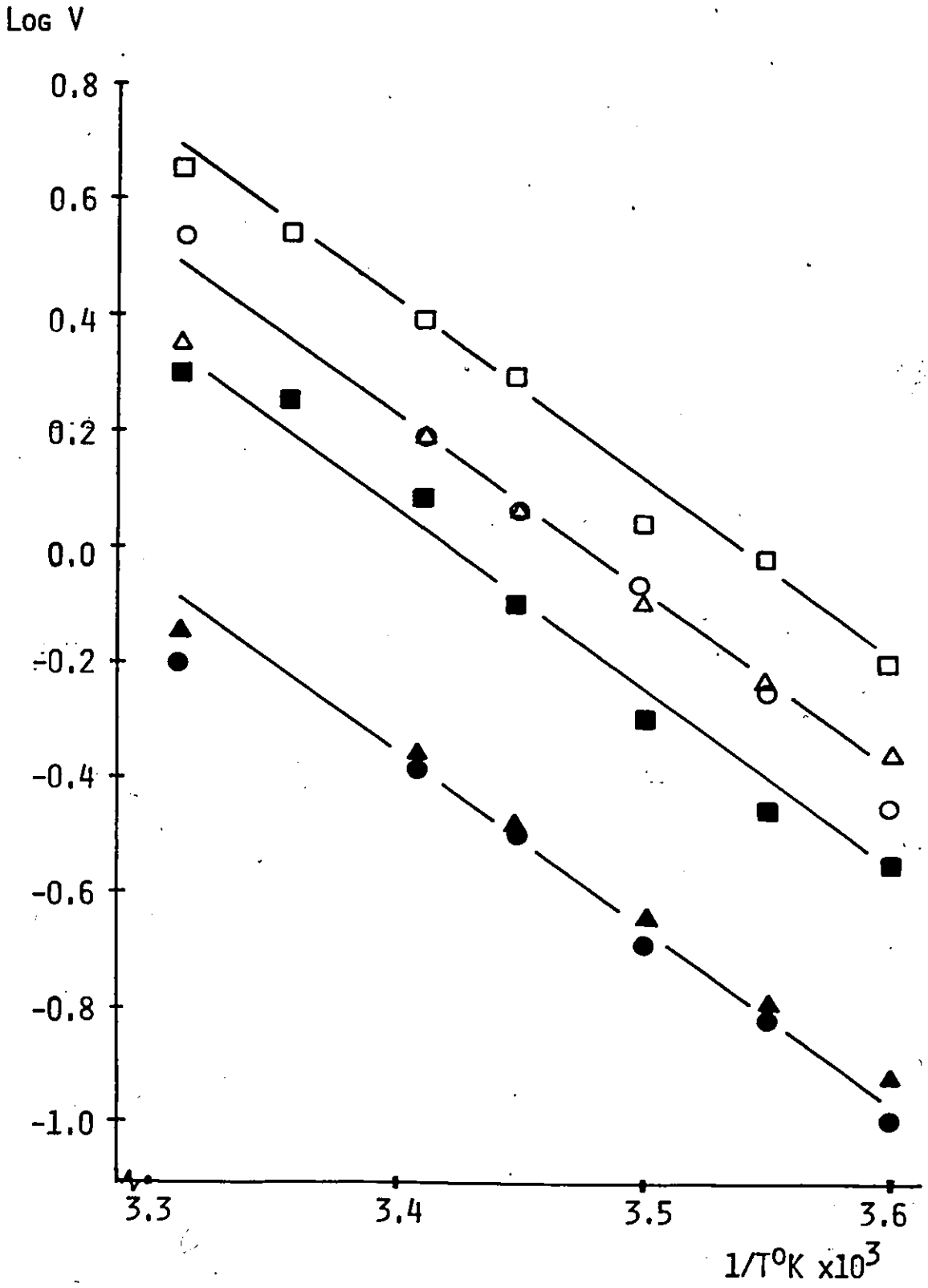
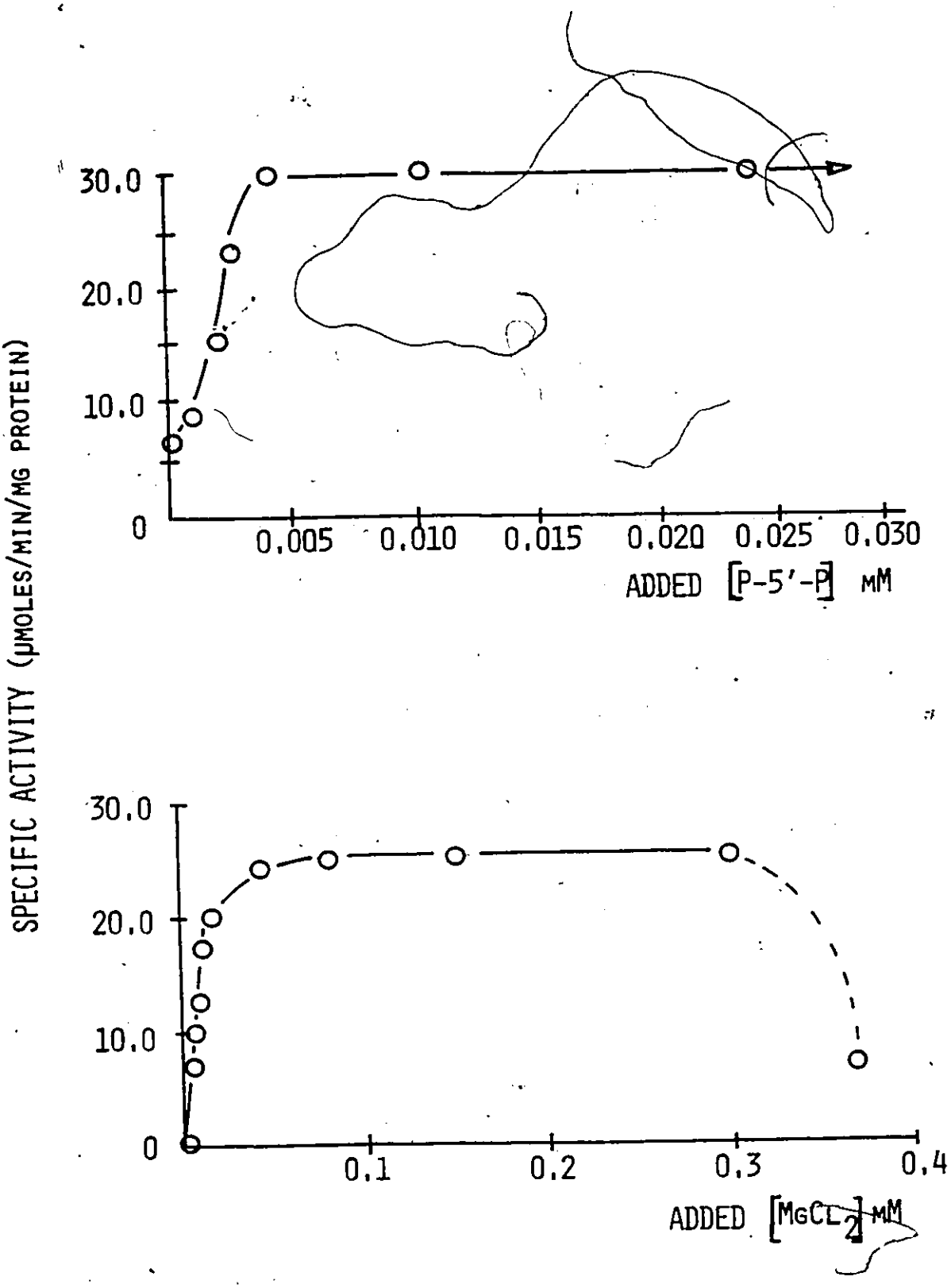

Log V
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
3.3
3.4
3.5
3.6
1/T°K x10³

Fig. 3A.

The effect of pyridoxal-5'-phosphate (P-5'-P) on activity of eel liver mitochondrial Asp AT. The partially purified liver mitochondrial homogenate from food-deprived Cornwall eels was sucrose concentrated to one-quarter the original volume and dialysed against 100 mM phosphate buffer for 18 hrs with constant stirring at 5°C. Activity was assayed according to the Standard Assay as P-5'-P concentrations were varied.

Fig. 3B.

The effect of Mg^{++}-ions on eel liver mitochondrial Asp AT. The partially purified liver mitochondrial homogenate was prepared as in Fig. 3A. Activity was assayed as Mg^{++}-ion concentrations varied according to the Standard Assay.


SPECIFIC ACTIVITY (μMOLES/MIN/MG PROTEIN)
30.0
20.0
10.0
0
0.005
0.010
0.015
0.020
0.025
0.030
ADDED [P-5'-P] mM
30.0
20.0
10.0
0
0.1
0.2
0.3
0.4
ADDED [MgCl2] mM

enzyme activities and that there is a residual quantity bound very tightly to the enzyme which dialysis did not remove; other authors have also reported a high P-5'-P binding tenacity (see Guirard and Snell, 1964; Bell, 1968).

It was also found that Mg^{++}-ions at low concentrations activated liver Asp AT (Fig. 3B). This apparent activation of Asp AT by Mg^{++} has not been reported for any other homologous Asp AT to the author's knowledge. Such activation need not be directly due to Mg^{++}-ions but may represent a general ionic or osmotic effect (see Moon et al., 1977). American eel Asp AT has a small Stokes radius (see Table 7) and by altering the ionic environment the bond configuration of the enzyme may have been affected. Nevertheless, Mg^{++} was added to all assays to produce maximum Asp AT activities.

6. The Reverse Reaction

The forward reaction of Asp AT (i.e., towards OAA) is easily assayed by coupling the system to malate dehydrogenase (MDH). Since the equilibrium constant for MDH lies well towards malate (Lehninger, 1975), any OAA produced will be rapidly reduced to malate by NADH and MDH. The ease of assaying the forward reaction makes the reverse reaction difficult since many enzymes in a partially purified preparation, including MDH, compete for OAA as well as glutamate, the two substrates necessary for Asp and α-KG production from Asp AT. Therefore, it was necessary to extensively treat the homogenate to rid it of competing enzymes which are present at much higher activities so this direction could be assayed

(see Materials and Methods). For this reason, only liver cytosol Asp AT was investigated.

When the reciprocal substrates were at saturating levels, the $Km_{(glut)}$ was 3.3 mM and $Km_{(OAA)}$ was 0.11 mM (Fig. 4). As is apparent from the plots, glutamate shows strong inhibition above 4 mM and OAA only moderate inhibition even at 7 mM which is much above estimated in vivo OAA concentrations (Fersht, 1977). Thus, as is seen for the forward reaction, substrate inhibition could play a role in controlling this reaction direction. Certainly with the low Km-values estimated for both substrates, the proper conditions could direct this reaction in this direction. It should be noted that specific activity in the reverse direction is apparently higher than the forward direction (compare Fig. 4 with Figs. 1 or 5); this is, however, an artifact of the differential purification of the enzyme for the two experiments and may not represent a true phenomenon.

STARVATION AND TOTAL TISSUE Asp AT ACTIVITIES

1. Changes in Index Parameters

Starvation in fish is associated with major changes in body reserves (Love, 1970). Table 3 indicates some of these changes in the immature Cornwall eels used for the remaining enzyme studies. Unfortunately, when animals were selected for these experiments, there was a bias towards larger fish in the depletion group to improve the chances of these animals surviving 10 months of starvation.

It is interesting to note that hepato-somatic index

Fig. 4. The Reverse Reaction.

Food-deprived liver cytosol Asp AT was isolated and extensively treated according to the Materials and Methods. Activity was assayed according to the Standard Assay method at 1.48 mM OAA as glutamate concentrations were varied and at 1.84 mM glutamate as OAA concentrations were varied.

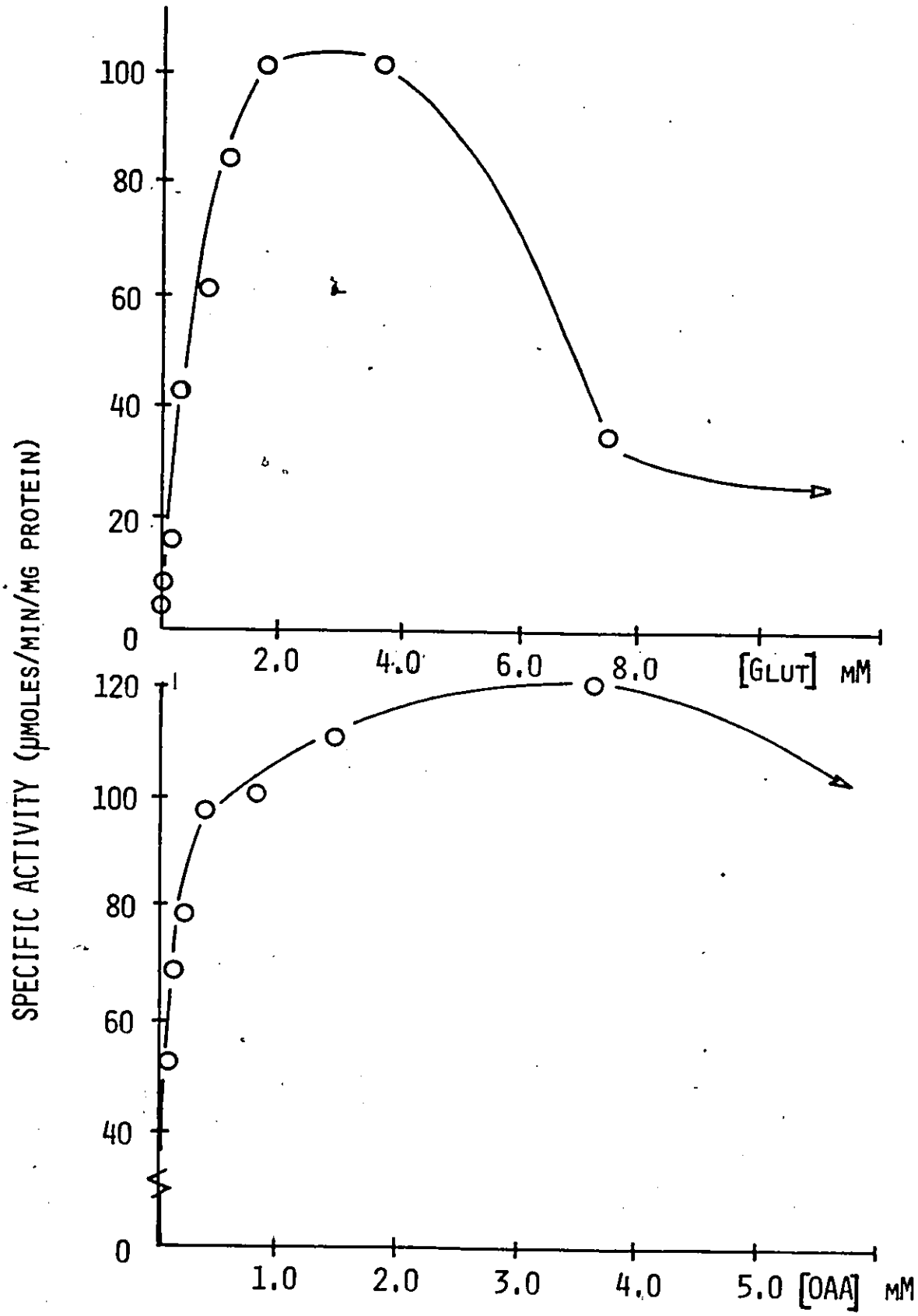

SPECIFIC ACTIVITY (μMOLES/MIN/MG PROTEIN)
100
80
60
40
20
0
2.0
4.0
6.0
8.0
[GLUT] MM
120
100
80
60
40
0
1.0
2.0
3.0
4.0
5.0 [OAA] MM

TABLE 3

The effect of starvation on a number of index parameters and total Asp AT activities in fed and ten month starved American eels collected from Cornwall.

PARAMETER	FED	STARVED
Average animal weight(g)	204±224	591±168
Number of animals	15	7
Average liver weight (g)	1.7±0.3	7.0±0.4
Hepato-somatic index $\left(\frac{\text{Lv Wt.}}{\text{Body Wt.}} \times 100\right)$	0.9±0.7	1.3±0.2
Soluble Protein Content (mg extracted/g wet tissue wt)		
Liver	248±110	188±136
Red muscle	61±1	24±10 *
White muscle	44±9	58±50
Specific Activity (µmoles NADH/min/mg protein)		
Liver	16±8	21±4
Red muscle	66±23	52±9
White muscle	11±3	21±4 *

* Significantly different at $p < 0.05$

between the two groups did not decrease as previously reported for this fish (Aster and Moon, 1975; Renaud and Moon, 1980b) and fish in general (Love, 1970). This may have resulted from the large difference in weight (both total animal mass and liver mass) between the two groups as noted above (Table 3). It would be anticipated that differences in development and relative muscle mass in these two groups could explain this aberrant result.

Extractable tissue protein quantities between the two groups generally declined in liver and red muscle, but white muscle values rose slightly (Table 3). These less than predictable results (especially for white muscle) may again be related to both the size problem and the fact extractable not total tissue protein was estimated. These data do suggest, however, that protein mobilization has occurred in these immature eels during starvation.

These results, although clouded by the selection of eels of widely different size classes, do suggest that changes are occurring in protein-amino acid metabolism during starvation. It is of interest to see whether these changes are reflected by changes in Asp AT, one of the more important enzymes of amino acid metabolism.

2. Changes in Total Tissue Asp AT

Each tissue studied demonstrated a unique pattern of Asp AT specific activities (Table 3). A significant increase in the white muscle enzyme is seen, which coupled to an apparent increase in soluble white muscle protein, suggests

that white muscle Asp AT is spared preferentially to other tissue proteins and possibly even enhanced. Studies in the carp (Wittenberger and Giurgea, 1973; Creach and Serfaty, 1974) found a similar trend and may indicate an increased importance in its metabolic role. Eel liver and red muscle Asp AT activities increase and decrease, respectively, but not significantly. With the major changes seen in extractable (soluble) protein content of these two tissues (Table 3), it seems that this enzyme may subserve an important metabolic role in these tissues and is thus conserved (red muscle) or even slightly enhanced (liver).

The major problem with values of total Asp AT activities is that this enzyme is normally localized to both the mitochondrial and cytosol cellular compartments (see Introduction). As noted above, changes seen here probably represent a composite pattern and could be obscuring important compartmental differences for this enzyme. Thus it was necessary to isolate mitochondria and estimate activities of Asp AT from non-contaminated mitochondrial and cytosol fractions.

PROPERTIES OF ISOLATED EEL TISSUE MITOCHONDRIAL AND CYTOSOL Asp AT

1. General Characteristics of the Preparation

The preparation of all previous enzymes had employed a Sorvall Omni-mixer homogenizer, and there was some question as to whether this rotating flat-blade homogenizer could produce an intact mitochondrial preparation. It was found by Moon and Ouellet (1979) that the Brinkmann Polytron PUC-2

at low speeds produced a biochemically and morphologically intact eel liver mitochondrial preparation. These conditions were duplicated for the preparation of subcellular Asp AT activities in this study.

Furthermore, since frozen tissue was used as a source of enzyme in all experiments (see Materials and Methods), it was necessary to establish whether this treatment could provide preparations with a minimal cross-contamination of either mitochondrial or cytosol Asp AT activities. Therefore, activities of Asp AT were estimated in mitochondrial and cytosol fractions of fresh and frozen eel livers prepared with the Polytron. Appropriate marker enzymes were assayed to indicate the extent of contamination.

Frozen tissue represents a better source of Asp AT with activities enhanced by an average of 10 times over that of fresh tissues (Table 4). When activities were assayed in the two fractions, no apparent difference could be detected between preparations using the Sorvall or Polytron, but Polytron enzyme extracts of the mitochondrial fraction had more than double the activities of the Sorvall preparations. Furthermore, the amount of contamination between fractions was small (usually less than 10%) as indicated by the marker enzymes, but it is just as apparent that the cytosol fraction was always contaminated with some mitochondrial Asp AT. It can also be seen that the total Asp AT reported in the previous studies represents primarily the cytosol activities, since the correlation between total and cytosol activities is

TABLE 4

Activities (μmoles/min/mg protein) of liver Asp AT from the cytosol (cyto) and mitochondria (mito) of fed and starved American eels. Other enzymes used as markers were lactate dehydrogenase (LDH) for the cytosol, glutamate dehydrogenase (GDH) and citrate synthetase (CS) for the mitochondrial fractions. Pooled total tissue isolates were prepared from frozen or fresh Cornwall eels using the Sorvall Omnimixer and the Polytron homogenizer. In order to verify the effect of freezing the experiments were repeated using the Polytron homogenizer with fresh eel liver tissue.

DESCRIPTION		Asp AT	LDH	GDH	CS
SORVALL:	Starved Total	37.0	2.5	4.5	--
	Starved Cyto	24.7	2.3	<1.0	0.1
	Starved Mito	183.0	<1.0	8.3	12.0
POLYTRON:	FROZEN TISSUE				
	Fed Cyto	11.0			
	Fed Mito	35.5			
	Starved Cyto	23.3			
	Starved Mito	462.1			
	FRESH TISSUE				
	Fed Cyto	1.1	--	1.3	0.9
	Fed Mito	6.5	--	35.5	9.0
	Starved Cyto	2.9	--	9.9	1.4
	Starved Mito	15.4	--	52.4	37.0

high compared to total and mitochondrial (Table 4).

These results suggest that although the preparation of subcellular Asp ATs are not pure, certainly the extent of contamination is small and can not account for the effects noted later in this thesis. Also, as can be seen from Table 4, activities of liver mitochondrial Asp AT increase many times above that of the cytosol enzyme after ten months food deprivation. This system, therefore, should permit the detection of changes in tissue Asp AT during starvation, if any occur.

2. Enzyme Characterization

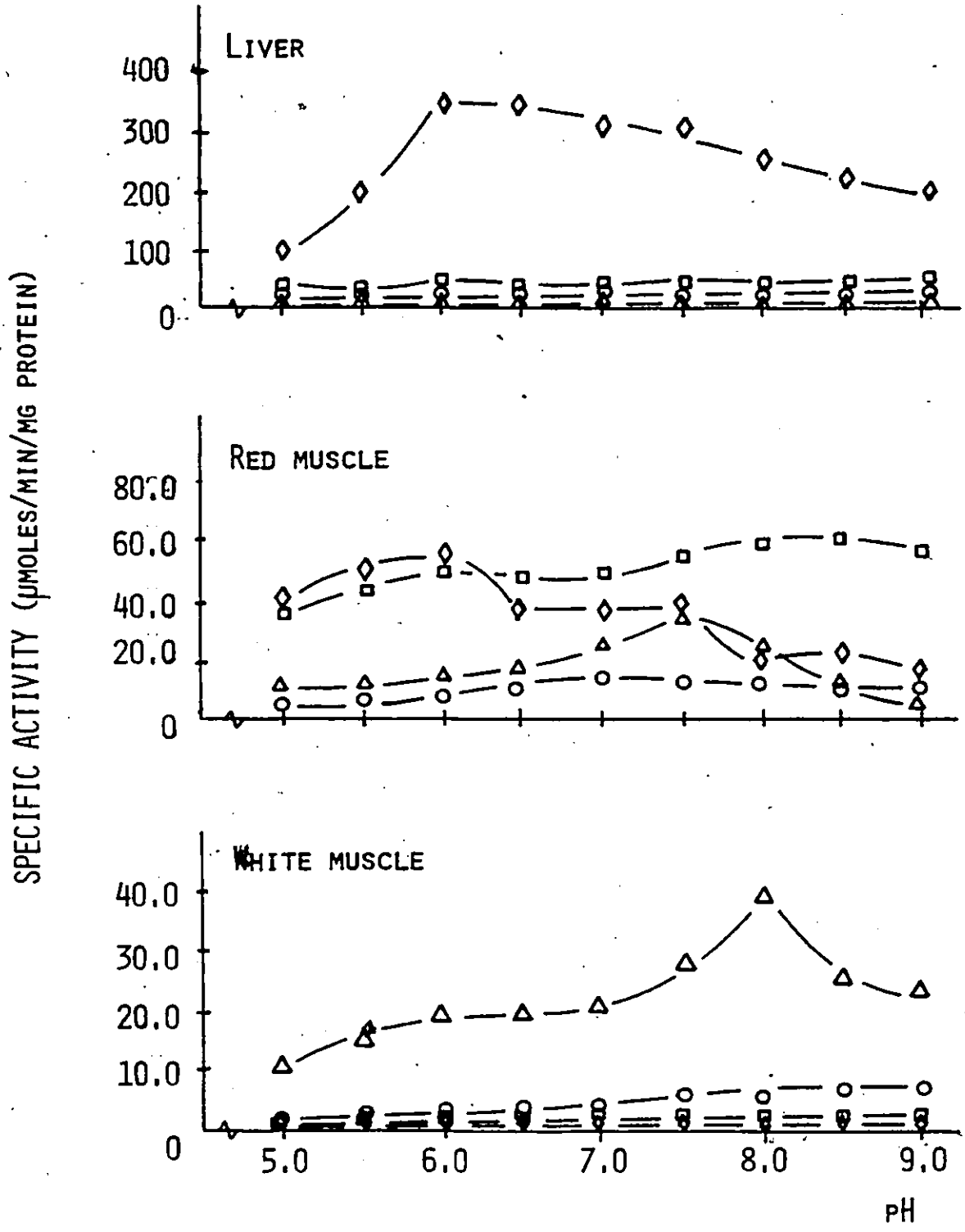
It was previously noted (Fig. 1) that pH had very little effect on total tissue Asp AT. When each fraction was assayed (Fig. 5) this general pattern held, except for the starved liver mitochondrial enzyme. Here, activity peaks at pH 6.0, followed by a general decline at higher pH values. A small peak is also observed for the homologous enzyme from white muscle. The most interesting feature of these data is that for the tissues with possible "liver" or "liver-like" functions (i.e., liver and red muscle-see Breaken, 1959; Hulbert and Moon, 1978), the activities of Asp AT isolated from starved tissue mitochondria were the highest (at least for red muscle at low pH) and the homologous cytosol enzyme were the lowest, with the enzymes from the fed group intermediate between these extremes. The white muscle enzymes are just opposite to this pattern, with the cytosol activities being highest, especially around pH 8.0 and mitochondrial the

Fig. 5.

Tissue fraction Asp AT activities as a function of pH. Enzymes were isolated from fed and food-deprived Cornwall eel liver, red muscle and white muscle. Tissue fraction homogenates were pooled, sucrose concentrated and assayed according to the Standard Assay at 7.35 mM Asp and 1.49 mM α-KG at 20°C.

Symbols:

- ○ Fed cytosol Asp AT
- △ Food-deprived Asp AT
- □ Fed mitochondrial Asp AT
- ◇ Food-deprived mitochondrial Asp AT


LIVER
400
300
200
100
0
RED MUSCLE
80.0
60.0
40.0
20.0
0
WHITE MUSCLE
40.0
30.0
20.0
10.0
0
5.0
6.0
7.0
8.0
9.0
pH
SPECIFIC ACTIVITY (µMOLES/MIN/MG PROTEIN)

lowest. It is apparent from these plots (Fig. 5) that changes in pH will play little if any regulatory role over Asp AT activities.

These data do support, however, the idea that more important changes in Asp AT activities are occurring than suggested by previous assays of total activity (Table 3). Table 5 represents pooled activities of Asp AT from the tissue subcellular fractions and clearly demonstrates a 10-fold increase in starved liver mitochondrial Asp AT specific activities compared to the fed control.

The minor increase in total liver Asp AT seen in Table 3 was a reflection of this major increase, but it was masked by dilution of the mitochondrial fraction by the larger cytosol component. In red muscle there was a slight increase in cytosol Asp AT activity. Again, it would appear that the small decrease in the total homogenate activity created a false impression and that a major decrease in activity in reality had occurred in at least the mitochondrial fraction. The major increase in total while muscle Asp AT noted previously (Table 3) can be seen to reflect an increase in the cytosol fraction only (Table 5). The low activity of the mitochondrial enzyme probably reflects the low oxidative capabilities and small mitochondrial content of the white muscle tissue in the eel (Hulbert and Moon, 1978).

Again the question could be raised as to the effect that freezing may have on the tissue enzyme activities, so

TABLE 5

Specific activities (μmoles NADH/min/mg protein) of Asp AT from the tissue subcellular fractions of pooled tissue homogenates of fed and starved American eels. The Standard Assay was employed at pH 7.0 and 20°C.

TISSUE	FRACTION	FED	STARVED
LIVER	Cytosol	11.0	10.0
	Mitochondrial	36.5	312.2
RED MUSCLE	Cytosol	17.1	21.5
	Mitochondrial	52.1	37.7
WHITE MUSCLE	Cytosol	4.5	24.4
	Mitochondrial	7.7	9.7

the experiment was repeated using fresh tissue homogenates of five fed and five starved eels. At pH 6.5 the specific activities in these tissue fractions were in the same ratio as predicted by Table 5, but of lower specific activities since freezing tends to activate the enzyme in both subcellular fractions (see Table 4).

The substrate saturation curves for Asp ATs isolated from fed and starved tissue cell fractions again provided evidence that the total homogenate preparations masked changes in affinity and velocity. Also, the reciprocal substrate inhibition which had been referred to in Table 1 became dramatic and potentially of considerable importance when examined in the tissue fractionated enzymes. The representative graphs (Figs. 6, 7, 8, 9, 10 and 11) were plotted as % activity to make comparisons easier since large differences in specific activities were observed; summary Table 6 provides maximum velocity values.

The saturation curve for Asp in fed liver cytoplasm is dramatically inhibited at concentrations of Asp above 13 mM (Fig. 6) and α-KG above 4 mM (Fig. 7). This enzyme from the starved eel liver was less sensitive to Asp but more sensitive to α-KG (above 1 mM produces a sharp inhibition). While substrate inhibition was evident for the liver mitochondrial Asp AT, the emphasis changes; here the starved preparation is more sensitive to Asp than the fed, and little inhibition by α-KG was noted. It is apparent that starvation alters the role that substrate inhibition may play in reg-

Fig. 6.

Fed and food-deprived liver Asp AT activities as a function of Asp concentrations. Enzymes were isolated from fed and food-deprived Cornwall eel liver cytosol and mitochondrial fractions according to the Materials and Methods. Activities were assayed according to the Standard Assay at 1.83 mM α-KG as Asp was varied. Fed liver fractions were assayed at pH 8.0, food-deprived cytosol and mitochondrial AT at pH 7.0 and 6.0, respectively.

Symbols:

○ Fed liver Asp AT

△ Food-deprived liver Asp AT

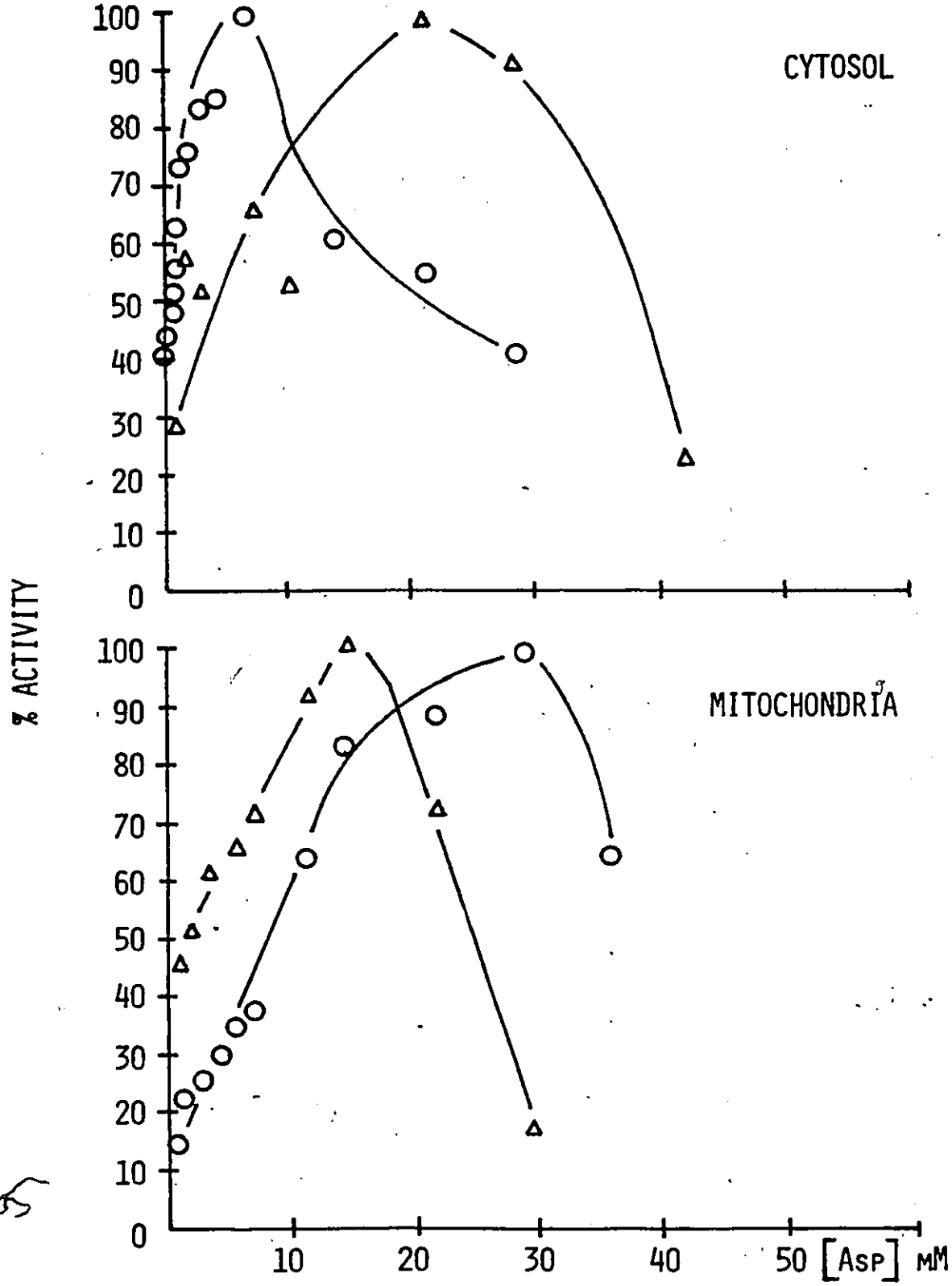
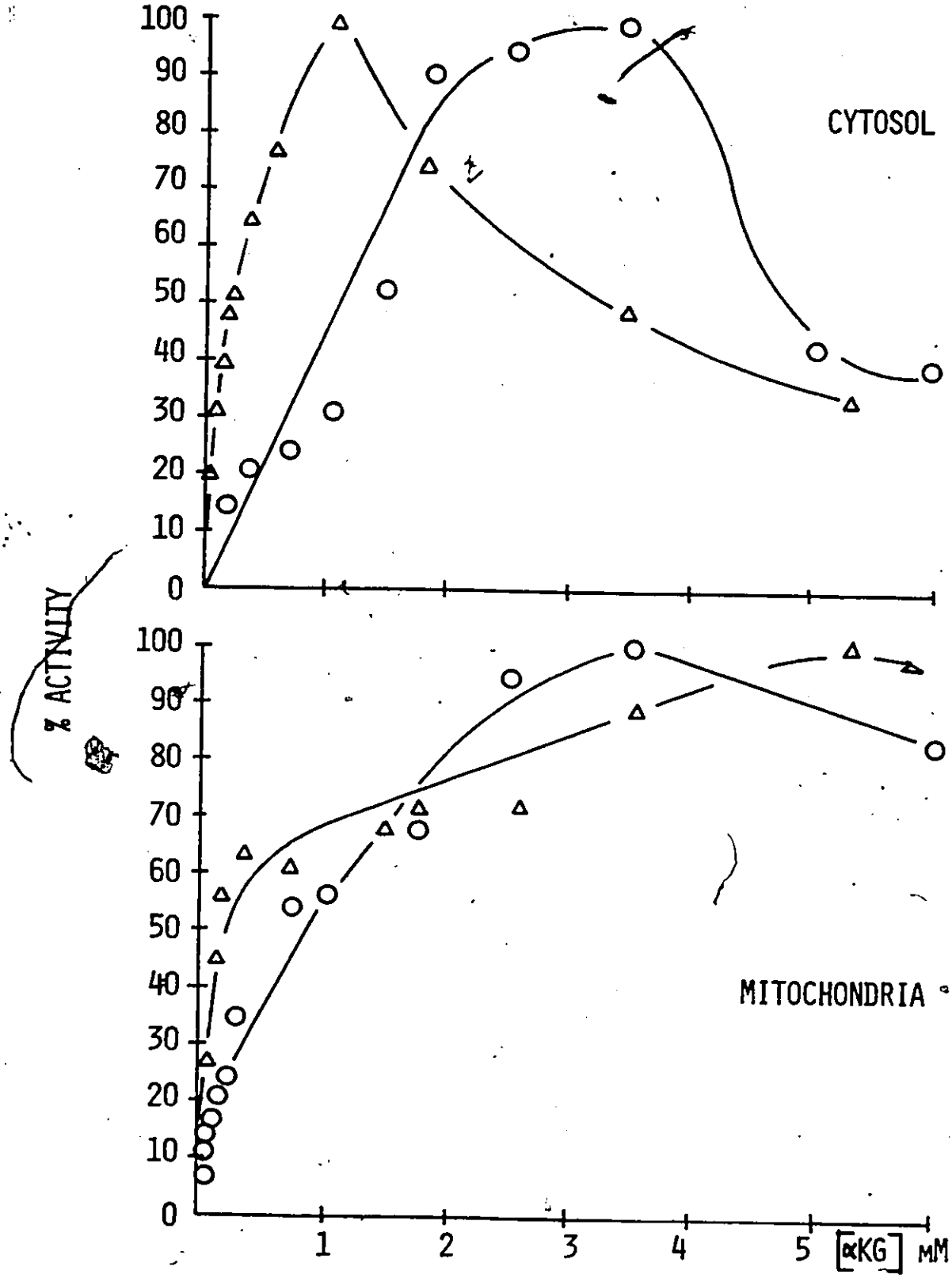

CYTOSOL
MITOCHONDRIA
% ACTIVITY
100
90
80
70
60
50
40
30
20
10
0
10
20
30
40
50
[Asp] mM

Fig. 7.

Fed and food-deprived liver Asp AT activities as a function of α-KG concentrations. Enzymes were isolated and assayed according to Fig. 6 at 7.35 mM Asp as α-KG was varied.

Symbols: ○ Fed liver Asp AT

△ Food-deprived liver Asp AT


CYTOSOL
MITOCHONDRIA
% ACTIVITY
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
[αKG] mM

ulating Asp AT from the cytosol and the mitochondria. Certainly α-KG is more important in terms of the cytosol enzyme while the effect of Asp is more complex. These kinetic differences predict mechanistic differences in the mitochondrial and cytosol enzymes.

Asp AT from fed red muscle cytosol demonstrated a sharp decrease in activity above 10 mM Asp (Fig. 8) and a decrease above 4 mM α-KG (Fig. 9). Ultimately the inhibition was about 60% at 28 mM asp and 45% at 7 mM α-KG. With starvation, again a pronounced inhibition occurred but only at concentration of Asp in excess of 25 mM (Fig. 8) and activity was approx. 75% decreased at 40 mM Asp. The α-KG profile was virtually unaltered by starvation in the cytosol with the same pronounced inhibition at high concentrations (Fig. 9). Mitochondrial Asp AT from the fed red muscle was inhibited above 30 mM Asp with 40% inhibition at 40 mM Asp. α-KG strongly inhibited the mitochondrial enzyme above 0.25 mM for the fed group. At 3.5 mM α-KG the enzyme was over 80% inhibited which was considerably different from the liver enzyme (Fig. 7). With starvation, both inhibitory responses are equally pronounced but occur at higher concentration; Asp inhibition occurs above 14 mM and reached 80% inhibition by 30 mM. The marked α-KG inhibition occurs at 1.7 mM α-KG and drops to approx. 30% of maximal activity by 7.0 mM. Thus with starvation, the red muscle enzyme demonstrates a reduced sensitivity to both Asp and α-KG in the mitochondrial fraction. Cytosol Asp AT control by α-KG remains unaltered, but

Fig. 8.

Fed and food-deprived red muscle Asp AT activities as a function of Asp concentrations. Enzymes were isolated and assayed according to Fig. 6. Red muscle cytosol enzymes were assayed at pH 7.5, fed and food-deprived mitochondrial enzymes at 8.0 and 6.0, respectively.

Symbols: ○ Fed red muscle Asp AT

△ Food-deprived red muscle Asp AT

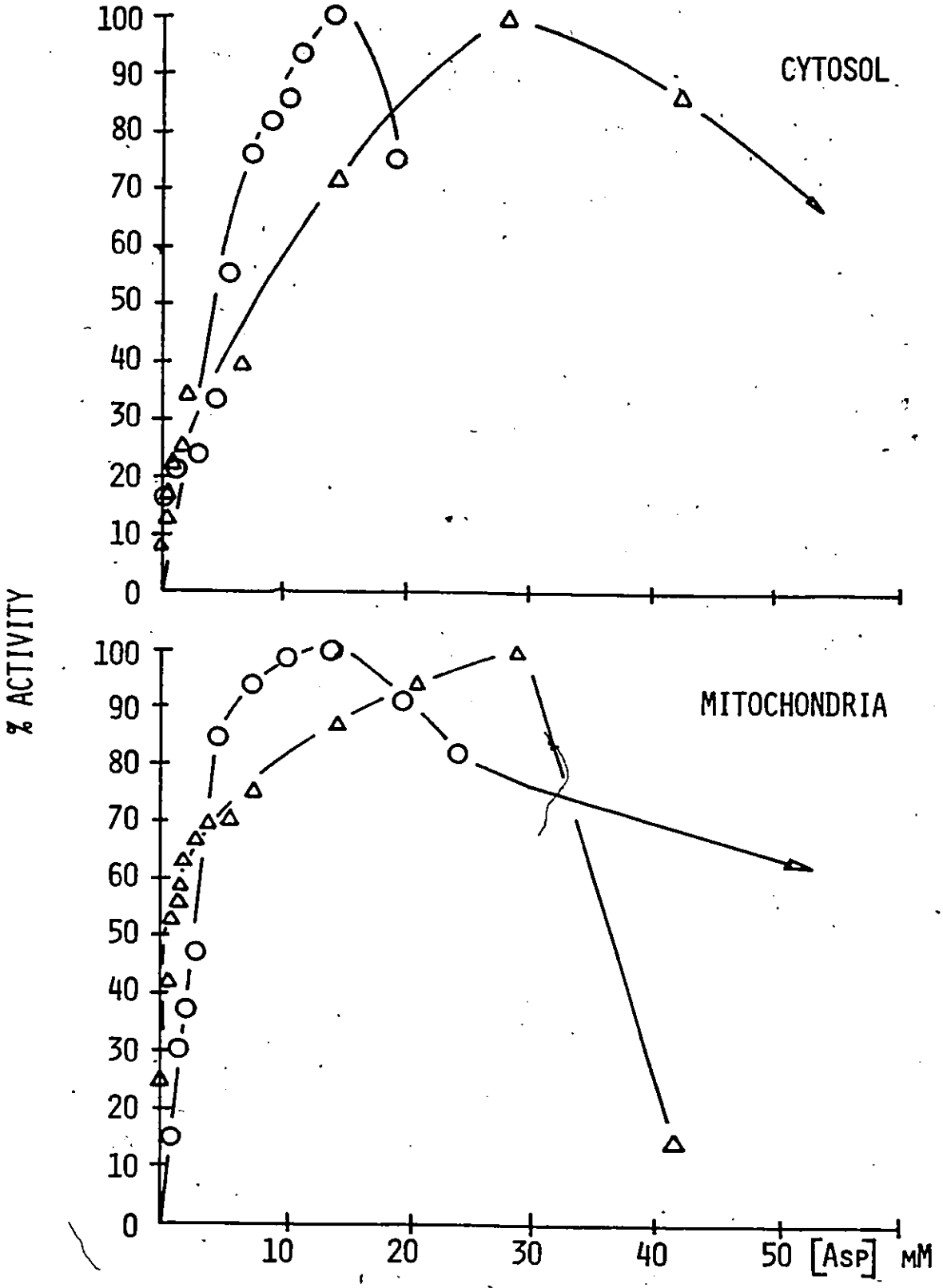
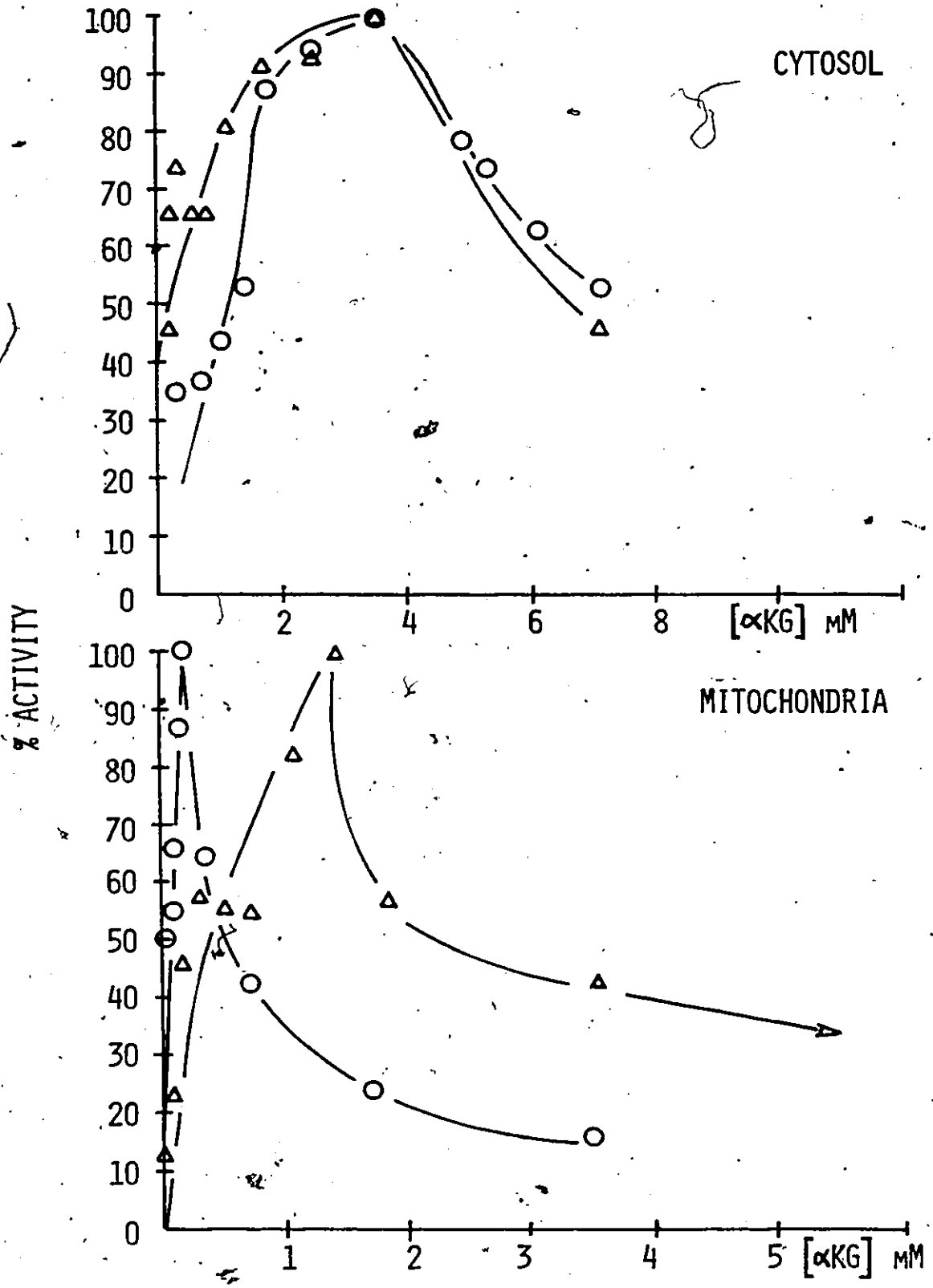

CYTOSOL
MITOCHONDRIA
% ACTIVITY
100
90
80
70
60
50
40
30
20
10
0
10
20
30
40
50
[Asp] mM

Fig. 9.

Fed and food-deprived red muscle Asp AT activities as a function of α-KG concentrations. Enzymes were isolated and assayed as in Fig. 8 at 7.35 mM Asp as α-KG was varied.

Symbols: ○ Fed red muscle Asp AT

△ Food-deprived red muscle Asp AT


CYTOSOL
MITOCHONDRIA
% ACTIVITY
100
90
80
70
60
50
40
30
20
10
0
2
4
6
8
[αKG] mM
1
2
3
4
5
[αKG] mM

Asp inhibits only at much higher concentrations with starvation. Unlike the liver enzymes, red muscle cytosol and mitochondrial Asp ATs in the fed and starved state appear to be tightly controlled by substrate inhibition at concentrations within the physiologic range.

Eel white muscle Asp AT presented yet another distinctive inhibitory pattern. In the fed state, the cytosol and mitochondrial Asp AT showed marked inhibition (up to 65%) by Asp concentrations above 30 mM in the cytosol and 20 mM in the mitochondria (Fig. 10). With starvation, this inhibition was reduced so that only a 20% decrease was seen at 70 mM Asp in the cytosol and a similar level of inhibition as noted in the fed state was not achieved until 70 mM Asp in the starved muscle mitochondria. This trend to reduce substrate inhibition is also observed for α-KG (Fig. 11). In the cytosol fraction, Asp AT in the fed fish was appreciably inhibited above 0.75 mM α-KG and reached 50% inhibition by 3.5 mM. With starvation, some inhibition was noted after 2.0 mM α-KG, but 10.0 mM α-KG had to be exceeded before substantial inhibition was noted. For mitochondrial Asp AT, the fed white muscle enzyme shows inhibition above 1.0 mM α-KG and approached 90% inhibition by 7.0 mM α-KG. Starvation again reduced the extent of inhibition, and the enzyme was only 50% inhibited by 5 mM α-KG (compared to almost 75% inhibition at this concentration in the fed state). It would appear that starvation results in a decreased sensitivity to high concentrations of substrate resulting in a reduction in the importance of this

Fig. 10

Fed and food-deprived white muscle Asp AT activities as a function of Asp concentrations. Enzymes were isolated and assayed according to Fig. 6. Fed white muscle enzymes and food-deprived cytosol activities were assayed at pH 8.0, while the food-deprived mitochondrial enzyme was assayed at pH 7.5.

Symbols: ○ Fed white muscle Asp AT

△ Food-deprived white muscle Asp AT

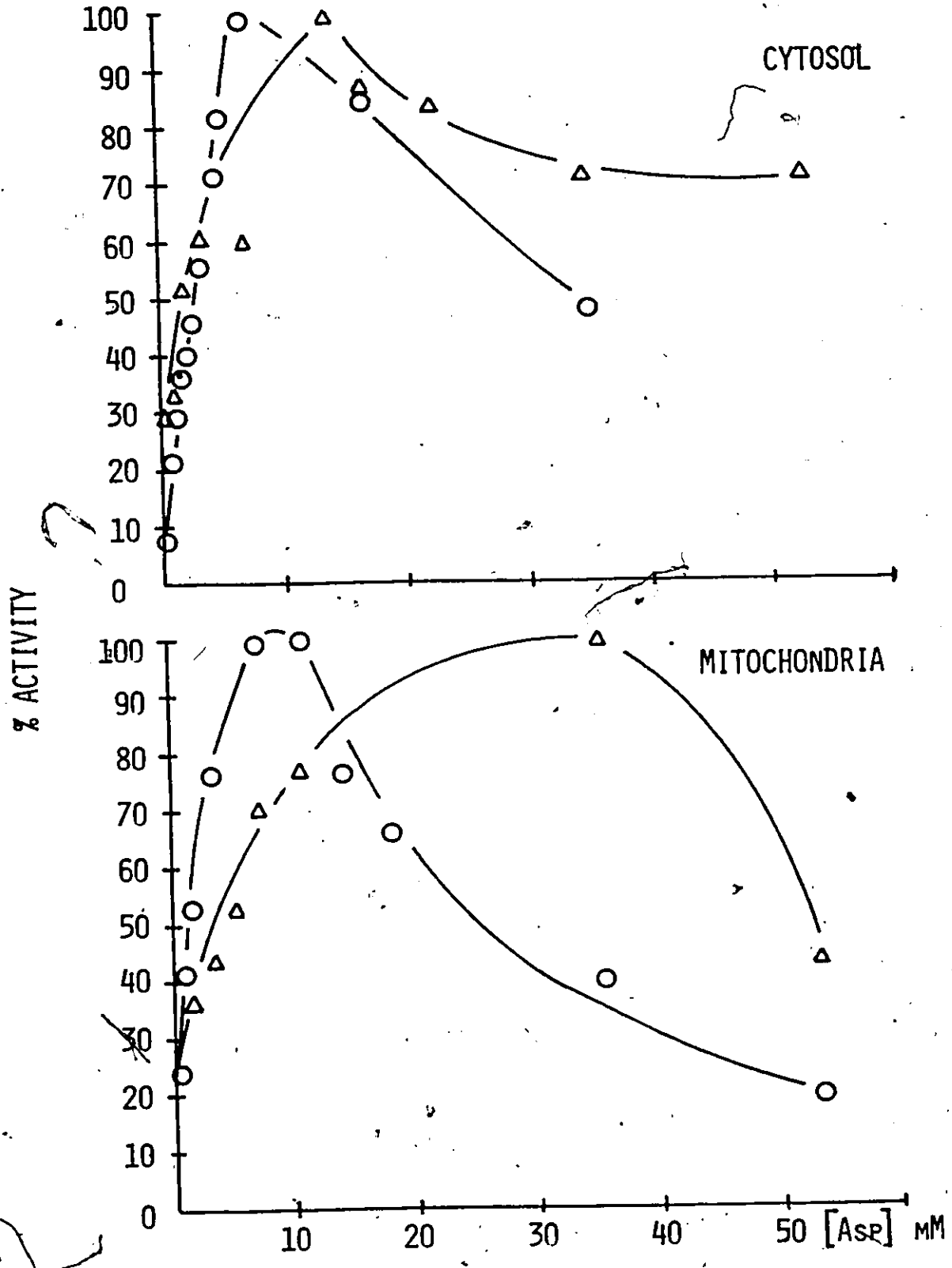
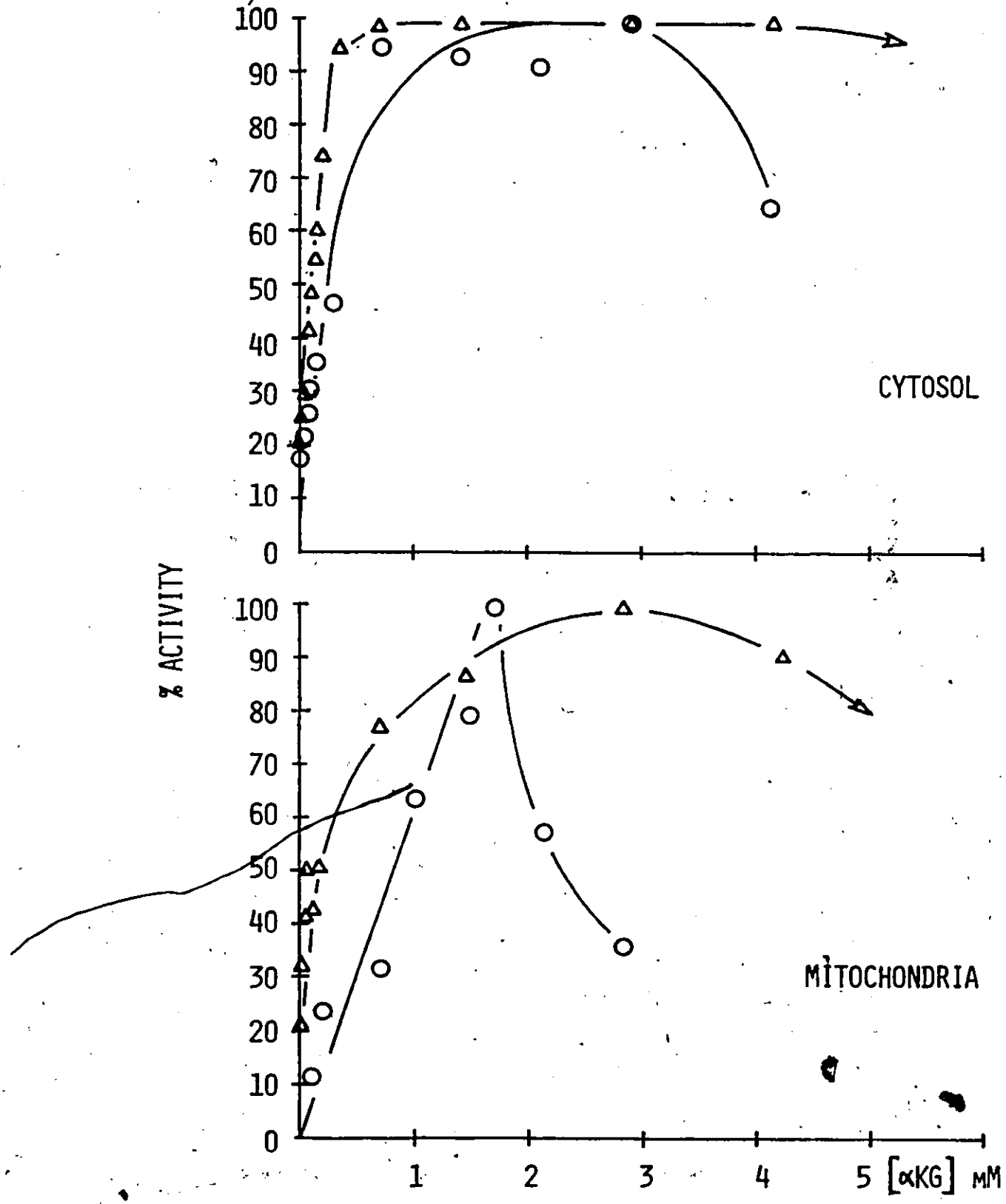

CYTOSOL
MITOCHONDRIA
% ACTIVITY
100
90
80
70
60
50
40
30
20
10
0
10
20
30
40
50
[Asp] mM

Fig. 11

Fed and food-deprived white muscle Asp AT activities as a function of α-KG concentrations. Enzymes were isolated and assayed according to Fig. 10 at 7.35 mM Asp as α-KG was varied.

Symbols: ○ Fed white muscle Asp AT

△ Food-deprived white muscle Asp AT


% ACTIVITY
100
90
80
70
60
50
40
30
20
10
0
CYTOSOL
MITOCHONDRIA
1
2
3
4
5
[αKG] mM

type of enzyme regulation in white muscle.

Previously (Table 1), it was found that the kinetic constants (Km and V_{max}) for tissue Asp ATs were dependent on the reciprocal substrate concentrations. The kinetic constants were estimated from saturation curves carried out at optimal concentrations of the reciprocal substrate. Also, since the curves were run at optimal pH, the values represent the maximal specific activities attainable for the particular enzyme. It is apparent that starvation alters both Km and V_{max} for the tissue fraction Asp ATs, but not necessarily in the same direction (Table 6). In general, Km is reduced and V_{max} is increased with some exceptions.

Liver Asp AT demonstrates an 8-fold increase in mitochondrial activity and a 2-fold decrease in cytosol activity. At the same time, $Km_{(\alpha\text{-KG})}$ decreases in the cytosol but not the mitochondria, and $Km_{(Asp)}$ does the reverse (Table 6). This increase in activity, coupled with the decrease in $Km_{(Asp)}$ or increased enzyme-substrate affinity, would enhance the ability of the liver mitochondrial enzyme to utilize Asp, as seen on Table 7. Changes in α-KG are similar in both fractions and of less magnitude to those of Asp (Table 7).

The red muscle enzymes were more variable than those in the liver, especially with respect to Km; activities were only marginally affected by starvation (Table 6). The $Km_{(Asp)}$ increased 4-fold in the cytosol but decreased by 2-fold in the mitochondria with starvation. The $Km_{(\alpha\text{-KG})}$ dropped by 5-fold in the cytosol with starvation and yet increases 3.5-

TABLE 6

Summary of Km and maximal velocity (V_{max}- as μmoles NADH/min/mg protein) values at the pH optima for Asp AT isolated from tissue fractions of fed and starved American eels at 20°C. Values were estimated from double-reciprocal plots (Lineweaver-Burke) of the corresponding saturation curves.

TISSUE	FRACTION	STATE	$Km_{(Asp)}$	$Km_{(\alpha\text{-KG})}$	V_{max}
LIVER	CYTO	FED	6.7	1.60	20.0
	CYTO	STARVED	6.7	0.15	10.5
	MITO	FED	3.8	0.43	48.1
	MITO	STARVED	0.7	0.40	349.1
RED	CYTO	FED	0.5	1.60	17.6
MUSCLE	CYTO	STARVED	1.9	0.33	39.5
	MITO	FED	4.3	0.08	62.4
	MITO	STARVED	2.1	0.29	56.6
WHITE	CYTO	FED	0.8	0.40	7.0
MUSCLE	CYTO	STARVED	0.8	0.18	41.4
	MITO	FED	7.6	0.19	9.7
	MITO	STARVED	0.4	0.66	12.0

TABLE 7

Summary of $Vmax/Km_{(S)}$ ratios for the data of Table 6. These values represent an estimate of the activity of the enzyme at very low (less than Km-levels) substrate concentrations (see Fersht, 1977).

$Vmax/Km_{(S)}$

		FED		STARVED	
		ASP	α-KG	ASP	α-KG
LIVER	CYTO	3.0	12.5	1.6	70.0
	MITO	12.7	111.9	498.7	872.8
RED MUSCLE	CYTO	35.2	10.0	20.8	119.7
	MITO	14.5	780.0	27.0	195.2
WHITE MUSCLE	CYTO	8.8	12.5	51.8	230.0
	MITO	1.3	51.1	30.0	18.2

fold in the mitochondria under the same conditions. These results suggest that the ability of red muscle Asp AT to utilize Asp changes little during starvation, but α-KG changes are increased in the cytosol but decreased in the mitochondria (Table 7).

Starvation doesn't affect the apparent $Km_{(Asp)}$ for white muscle cytosol Asp AT, but caused an approx. 20-fold increase in the mitochondrial enzyme affinity. The $Km_{(\alpha\text{-KG})}$ was reduced by half in the cytosol and tripled in the mitochondria by starvation. Maximal specific activities were increased by 6-fold in the cytosol and essentially unchanged in the mitochondria (Table 6). These changes are reflected in major alterations in activities at low substrate concentrations, especially in the cytoplasm (Table 7).

While the maximal values of tissue Asp ATs reported in Table 6 are slightly different from the Standard Assay at pH 7.0 (see Table 5), they should be considered as representing the maximum potential for Asp AT in any given tissue or fraction. Whether they represent a more physiologic measure of activity cannot be proven by these data since cellular pH is unknown, but they can be used to compare with studies of homologous enzymes from other species done under optimal conditions.

3. Changes in the Type of Tissue Asp AT

Numerous authors have found multiple electrophoretic forms or isozymes of cytosol and mitochondrial Asp AT (see Introduction). Eel tissue Asp ATs exist as multiple forms

as seen in Fig. 12. In the fed state, liver cytosol Asp AT consists of five darkly stained and four lightly stained bands on polyacrylamide electrophoresis (PAGE). The darker bands had been previously observed in a preliminary study using Fast Violet B dye as the staining agent; this was a less sensitive system and was subsequently replaced (see Materials and Methods). The red muscle and white muscle cytosol both demonstrated two heavily staining bands and four lightly staining bands with higher relative mobilities. The triplet seen in the fed mitochondrial fractions was consistent in all three tissues. These are presumed to be mitochondrial in origin from previous studies (see authors mentioned in Introduction) and from the electrophoresis of individual tissue fractions. It would appear that while the cytosol forms do not share the same isozymic banding pattern, the mitochondrial isozymes are electrophoretically identical..

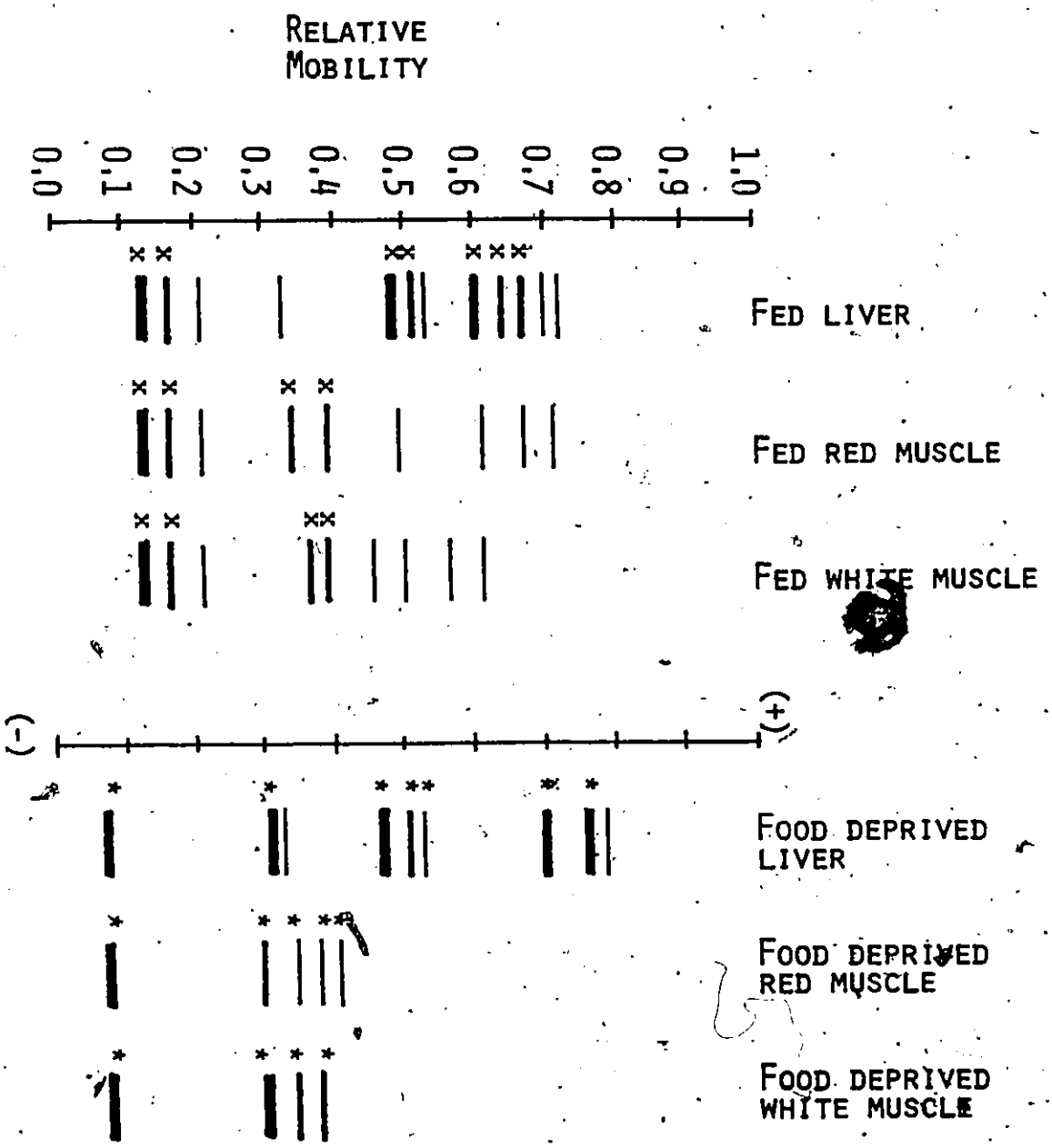
Starvation results in an overall reduction in band number as well as an alteration in mobility and staining intensity. All of these bands, with one exception, possessed sufficient activity to be distinguished with the less sensitive Fast Violet B stain and were previously found in the Quebec eels (see Fig. 12). Food deprivation reorganized the liver cytosol isozymes into three distinct groupings, differing in mobility from those of the fed state. Red muscle and white muscle cytosol Asp AT are reduced in band number compared to the fed state, especially the high mobility isozymes. The starved mitochondrial enzyme is the most

Fig. 12. Polyacrylamide electrophoresis.

Samples containing approximately 50 mg of protein were run and stained according to the Materials and Methods. Enzyme proteins were derived from frozen pooled tissue samples of the Cornwall eels homogenized in buffer less manisol and sucrose, cleared by centifugation and sucrose concentrated to the desired protein concentration.

Mobility of the bands is expressed as distance in cm. of migration of the band to distance in cm. of migration of the bromothymol blue marker. O represents the - end of the gel and 1 represents the + end of the gel.

Those bands with mobility <0.3 were mitochondrial and those >0.3 were cytosol (see Results). In addition, those bands marked with x were also seen in the 1976 survey of fed Cornwall eels and those with * in the 1974 survey of starved Quebec bronze eels.


RELATIVE MOBILITY
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
FED LIVER
FED RED MUSCLE
FED WHITE MUSCLE
(-)
(+)
FOOD DEPRIVED LIVER
FOOD DEPRIVED RED MUSCLE
FOOD DEPRIVED WHITE MUSCLE
MITOCHONDRIAL
CYTOSOL

radically altered in that the triplet fed banding pattern is replaced by a single intensely stained band equivalent to none of the fed bands, which was also found in the migrating Quebec eels. Freezing did not alter any electrophoretic pattern.

The PAGE technique had suggested that the enzyme type was altered with starvation, so isoelectricfocusing of the fed and starved liver homogenates was used to further characterize the nature of the changes. Unfortunately the resolution of Asp AT by this technique was insufficient to pick up the microheterogeneity seen with PAGE, but it did separate the mitochondrial and cytosol components. Fig. 13 provides evidence that starvation results in the virtual elimination of one activity band, presumed to be the cytosol component from both electrophoretic studies (Fig. 12) and activity measurements (Table 6). The isoelectric point for the mitochondrial and cytosol isozymes is approx. pH 5.5 and 8.0, respectively. Originally this technique was investigated as a possible purification method; however, the yield was very poor, and hence it was employed no further. If the technique could be refined to give better yields, it could be of great use in providing relatively pure enzymes from the two subcellular compartments for kinetics estimates. In this case it does provide a qualitative picture of what happens to liver Asp AT during starvation.

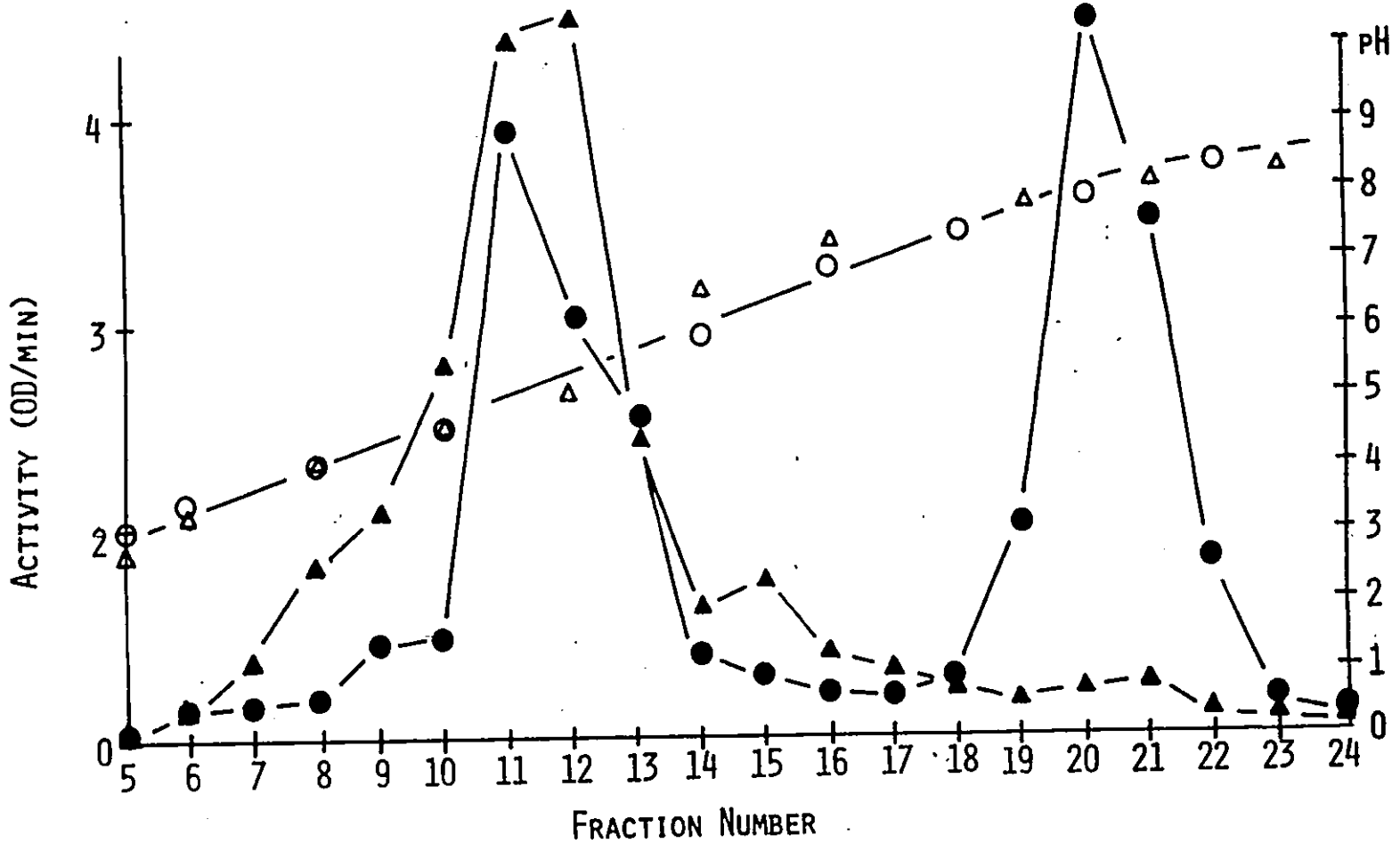
Since both PAGE and isoelectricfocusing indicated major changes in the enzymes occurred with starvation, gel sieving chromatography with Sephadex G-200 was used to further char-

Fig. 13.

Isoelectricfocusing of fed and food-deprived liver total homogenate Asp AT. Fed and food-deprived liver total homogenates were run concurrently in duplicate on a gel bed from pH 3 - 10 according to the Materials and Methods. Asp AT activities were assayed according to the Standard Assay.

Symbols:

- O pH of fed liver fraction
- △ pH of food-deprived liver fraction
- ● Asp AT activity in fed liver fraction
- ▲ Asp AT activity in food-deprived liver fraction


ACTIVITY (OD/MIN)
0
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
FRACTION NUMBER
pH
0
1
2
3
4
5
6
7
8
9

acterize any possible enzyme changes. All fed tissue Asp ATs except red muscle cytosol, have a molecular weight of approx. 19,000 daltons (Table 8). Red muscle cytosol Asp AT was much larger at 55,000 daltons. With starvation, the molecular weight determination becomes more variable, with values ranging between 15,500 and 26,500 daltons; interestingly, the red muscle cytosol Asp AT is approx. 50% of that seen in the fed state.

This molecular weight estimate of eel tissue Asp AT was very much lower than previously reported values (Jenkins, et al., 1959; Martinez-Carrion, 1967; Krista and Fonda, 1973; Owen and Hochachka, 1974). The consistently low molecular weights could result from the characteristics of molecular sieves to measure Stokes radii rather than true molecular weight (Owen and Hochachka, 1974). Also, it was necessary to verify that freezing damage did not alter the enzyme. When fresh starved liver cytosol Asp AT was run alone and in combination with substrate stabilizers, the estimate did not differ significantly from that of the frozen material. Therefore, it is apparent that starvation may alter the shape or size of the Asp AT enzyme, especially the red muscle cytosol form, but the exact mechanism for this change and the low molecular weight estimates attained can not at this time be explained.

TABLE 8

Estimation of molecular weights (±1500 daltons) of tissue Asp AT isolated from subcellular fractions of fed and 10 month starved American eels. The procedure is discussed in the Methods and Materials. Standard proteins were used as markers in the estimation: Ribonuclease A (13,700 daltons), K_{av} = 0.66; Chymotrypsinogen A (25,000 daltons), K_{av} = 0.47; Ovalbumen (45,000 daltons), K_{av} = 0.33; Aldolase (158,000 daltons), K_{av} = 0.10.

TISSUE	FRACTION	STATE	K_{av}	MOLECULAR WEIGHT (daltons)
LIVER	CYTOSOL	FED	0.56	19,000
		STARVED	0.50	24,000
	MITOCHONDRIA	FED	0.56	19,000
		STARVED	0.59	17,000
RED MUSCLE	CYTOSOL	FED	0.28	55,000
		STARVED	0.47	26,500
	MITOCHONDRIA	FED	0.56	19,000
		STARVED	0.63	15,500
WHITE MUSCLE	CYTOSOL	FED	0.56	19,000
		STARVED	0.50	24,000
	MITOCHONDRIA	FED	0.56	19,000
		STARVED	0.53	21,500

IV

DISCUSSION

DISCUSSION

The data presented in this thesis imply an alteration in the characteristics of the enzyme Asp AT has occurred with food deprivation in the American eel. No clear cut pattern is observed, however, possibly as a result of the number of tissues studied, their divergent metabolic roles, and the compartmentalization of Asp AT. In order to attempt an explanation of these results, the role of Asp AT as defined in animals will be reviewed, followed by an examination of each eel tissue enzyme studied in light of these possible functions, and finally a comparison will be made between eel tissue Asp ATs and the homologous enzymes from other organisms where it has been examined.

It is always difficult and even dangerous to model a tissue metabolism on the alterations in a single enzyme. Furthermore, the strategies utilized by fish during food deprivation may be quite different from those of mammals due to their major differences in metabolic rate, diet selection, and evolutionary history. However, it is one aim of comparative physiology-biochemistry to define possible species differences and attempt an explanation for them (Hochachka, 1976). Keeping in mind the shortcomings inherent in such an approach, only an approximation of the truth will be possible.

THE METABOLIC ROLE OF Asp AT

Although there is some disagreement about the precise mechanistic details, Asp AT has at least three major func-

tions in cells of mammals and other organisms. The first involves its critical role in the malate-aspartate shuttle to transfer reducing equivalents from the cytosol to the mitochondrial matrix. The second is an anaplerotic role, or the replenishment of carbon into a common metabolic pool which has been subject to constant drain by anabolic and/or catabolic processes (see Lehninger, 1975). A third is in transdeamination reactions to eliminate waste ammonia.

Mitochondrial membranes are impermeable to NADH generated by glycolysis in the cytosol (see Lehninger, 1975). In animal cells, two shuttles exist to transfer cytosol NADH across the mitochondrial barrier into the matrix space where it can be oxidized for energy production (La Noue et al., 1973; Williamson et al., 1973). These are termed the malate-aspartate shuttle and the α-glycerol phosphate (α-GP) cycle. The latter shuttle (α-GP cycle) is restricted to highly aerobic tissues such as insect flight muscles (Sacktor, 1976), the mantle muscle of fast swimming squid (Hochachka, 1976) and skeletal muscle of tuna (Guppy and Hochachka, 1978); although it can be demonstrated in rat heart (Safer et al., 1971), the α-GP cycle is considered to be of relatively minor importance in mammalian tissues (Safer and Williamson, 1972).

The malate-aspartate shuttle consists of the following components (see Hochachka, Storey et al., 1979): (a) cytosol and mitochondrial forms of both Asp AT and malate dehydrogenase (MDH); and, (b) at least two exchange mechanisms (malate in for α-KG out; glutamate in for Asp out).

This shuttle is diagramed in Fig. 14. As shown, the major role of this cycle is the transfer of reducing equivalents (NADH) into the mitochondrion and since it is a cycle, no carbon is lost to any other metabolic sequence.

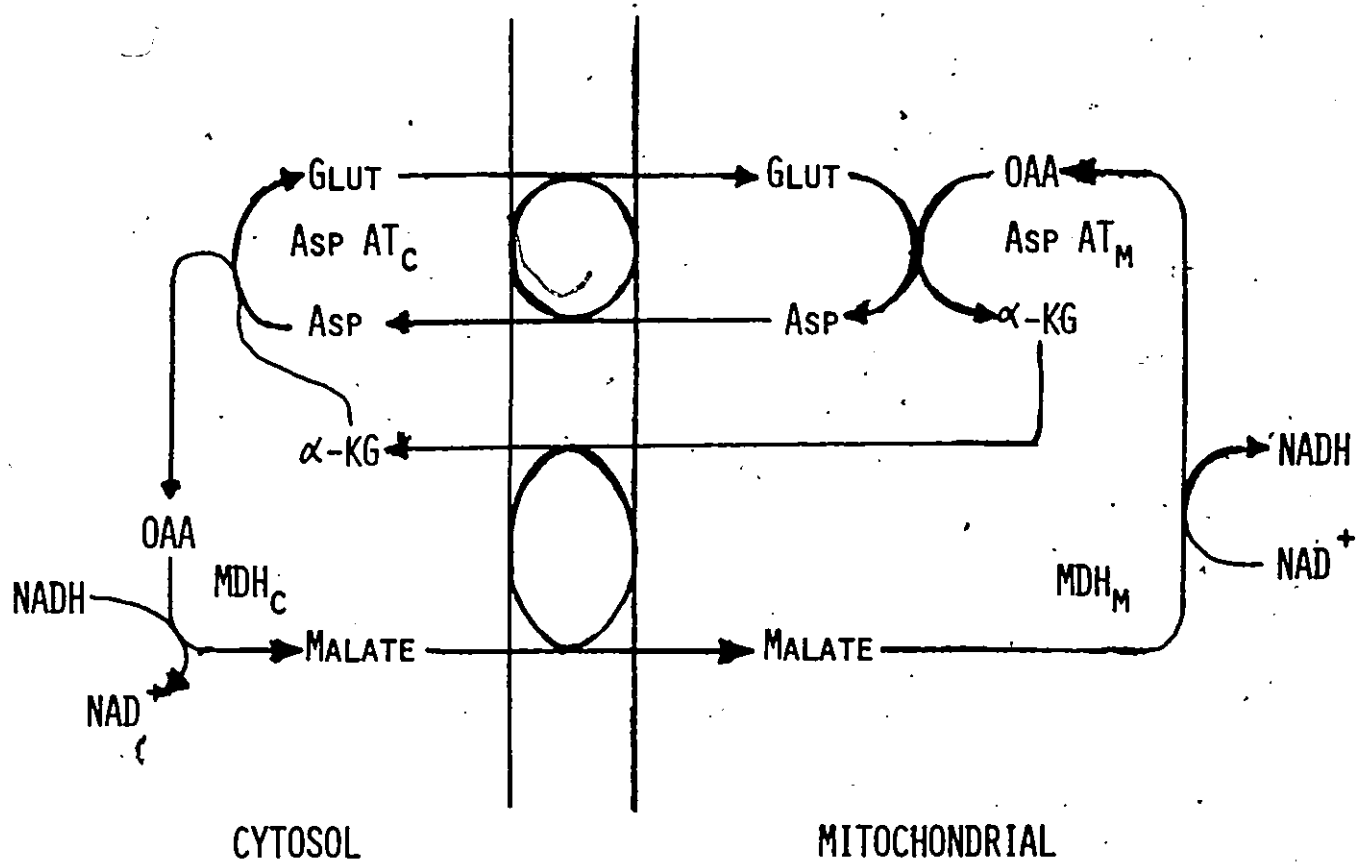
If the malate-aspartate shuttle is disconnected at either its cytosol or mitochondrial portions, it can become a translocator of carbon (and reducing equivalents) or it can act in an anaplerotic role by replenishing Krebs cycle carbons.

The best example of its translocatory role is during gluconeogenesis (see Krebs et al., 1976). In mammalian liver a cytosol and/or mitochondrial Asp AT is intermediate in urea synthesis, and links ureogenesis with gluconeogenesis (Krebs et al., 1976). Glucose production from lactate requires activities of mitochondrial Asp AT to translocate oxaloacetate carbon from the mitochondria to the cytosol where cytosol phosphoenol pyruvate carboxykinase (PEP CK) initiates glucose synthesis. When PEP CK is strictly cytosolic as in pigeons, Asp AT is not so involved (Söling and Kleineke, 1976). With alanine or pyruvate as glucose precusors, the bulk of the evidence is against Asp AT playing an important role (see Krebs et al., 1976).

The role of Asp AT in supplementing Krebs cycle intermediates is well documented (Williamson et al., 1973). It is agreed that the rate at which the Krebs cycle "spins" is a function of the availability of oxaloacetate (OAA). Normal levels of OAA have been reported to be less than 5 μmoles/g

Fig. 14

The malate-aspartate shuttle as modified from Hochachka, Storey et al. (1979). C, cytosol; M, mitochondrial.


GLUT
GLUT
OAA
ASP AT$_C$
ASP AT$_M$
ASP
ASP
α-KG
α-KG
NADH
OAA
NADH
MDH$_C$
NAD$^+$
MDH$_M$
MALATE
MALATE
NAD
CYTOSOL
MITOCHONDRIAL

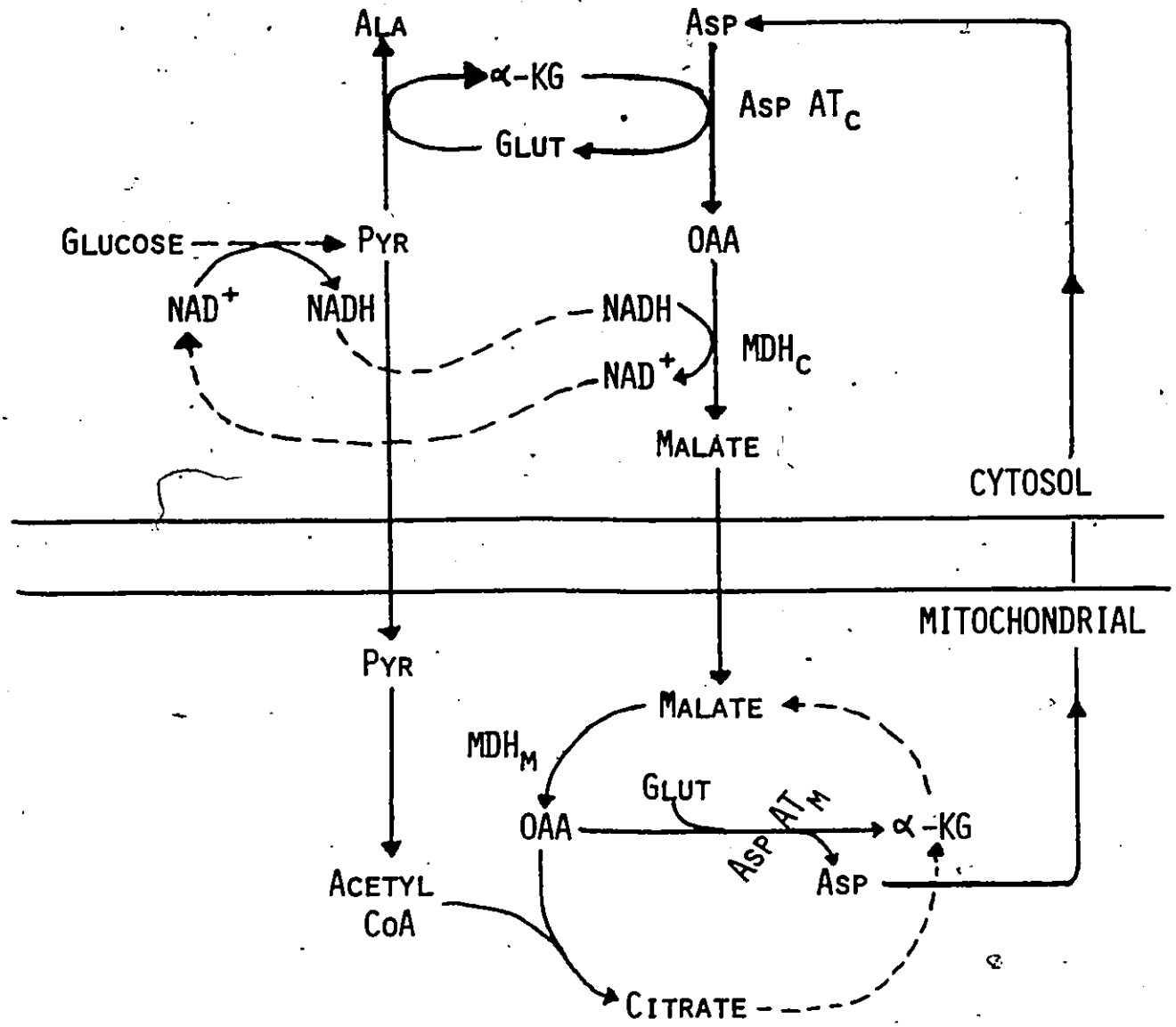
fresh tissue weight (Williamson et al., 1967; Safer and Williamson, 1973; Driedzic, 1975). To increase the "spinning rate" of the Krebs cycle as acetyl CoA becomes available (e.g., during the transition from low to high carbohydrate or fatty acid utilization), OAA levels must rise.

The best documented example of this was reported by Safer and Williamson (1973) for the rat heart burning glucose. They found a perfect stoichiometry between the augmentation of OAA plus other Krebs cycle intermediates and aspartate depletion. These results were explained by coupling cytosol Asp AT to alanine aminotransferae, and activating mitochondrial Asp AT (see Fig. 15). In this example, Asp not only augments OAA thus increasing the "spinning rate" of the Krebs cycle, but it is ultimately responsible for maintaining cytosol redox balance.

There is some evidence that a cytosol Asp AT is involved in the obligatory link between fatty acid oxidation and amino acids (Buse et al., 1972). Amino acids, in particular the branched-chain amino acids (leucine, isoleucine, valine), are transaminated with the resulting α-keto acids converted to activated CoA derivatives for incorporation into Krebs cycle intermediates. In this mechanism, glutamate and α-ketoglutarate tumble between Asp AT and the branched-chain transaminase. The ultimate result of this process is an enhancement of OAA levels to accept the acetyl CoA units from the β-oxidation of fatty acids. A recent review by Snell (1980), however, indicated that alanine aminotrans-

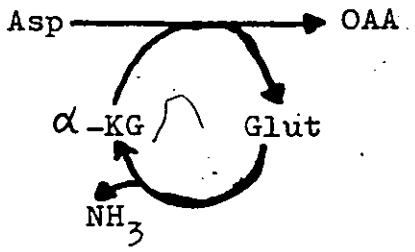
Fig. 15.

The augmentation of Krebs cycle intermediates by uncoupling the malate-aspartate shuttle. Modified from Safer and Williamson (1973); rat heart oxidizing glucose. Pyr, pyruvate; C, cytosol; M, mitochondrial.


ALA
ASP
α-KG
GLUT
ASP AT$_C$
GLUCOSE
PYR
OAA
NAD$^+$
NADH
NADH
NAD$^+$
MDH$_C$
MALATE
CYTOSOL
MITOCHONDRIAL
PYR
MALATE
MDH$_M$
GLUT
OAA
ASP AT$_M$
α-KG
ASP
ACETYL CoA
CITRATE

ferase may be more important than Asp AT in this process.

A final role for Asp AT, especially in liver tissue, is trans-deamination. In essence, instead of glutamate and α-ketoglutarate tumbling between Asp AT and another amino-transferase (i.e. for alanine or branched-chain amino acids), glutamate dehydrogenase (GDH) is coupled to Asp AT in the following manner (Cohen and Sallach, 1961; Lehninger, 1975):



(ammonia)

This mechanism has been found to be important to eliminate waste nitrogen as ammonia in mammalian liver and muscle (Miller et al., 1955; Owen and Hochachka, 1974; Krebs et al., 1976) and fish tissues (McBean et al., 1966; Janicki and Lingis, 1970; Creach and Serfaty, 1974).

It is obvious that the role of Asp AT is very complex, but equally obvious that it is a key enzyme in the metabolism of carbohydrates, fats and amino acids in probably all animal tissues. Although these mechanisms have evolved principally from mammalian studies, the metabolic machinery available in fish tissues, or other animal tissues for that matter, is thought to be similar and certain analogies would be expected between these systems (see Hochachka, 1976).

THE ROLE OF EEL TISSUE Asp ATs

The malate-aspartate shuttle has been demonstrated in subcellular preparations of fish hearts (Hochachka, Storey et al., 1979) and gills (Hochachka, Schneider et al., 1979), where it is important in the translocation of reducing equivalents. Alanine and aspartate aminotransferases have been demonstrated in a variety of tissues in many fish species (see Introduction), and there is much evidence to indicate a high degree of protein-amino acid metabolism in fish species (see Introduction). Therefore, each of the functions of Asp AT alluded to in the last section are possible in a given fish species.

1. Asp AT in Eel Liver

With food deprivation, there was a non-significant reduction in extractable (soluble) liver protein (Table 3) which was not reflected in a similar change in the hepatosomatic index (HSI) (Table 3). Other similar studies (Moon, 1980) have reported that eight months of food deprivation in this animal is associated with a slight decline in % water content (indicative of protein depletion; Love, 1970) and a drop in HSI. Where reported, changes in liver component indices are consistently less than that seen in skeletal muscle tissues (Love, 1970; Johnston and Goldspink, 1973; Moon and Johnston, 1980). Although the results in Table 3 do not concur precisely with these general trends (major size differences; see Results) they do suggest a relative protein sparing has occurred in the liver. This

sparing reflects the important role played by fish liver in gluconeogenesis (see e.g., Love, 1970; Cowey and Sargent, 1979; Knox et al., 1980; Moon and Johnston, 1980; 1981).

Maximal specific activities of total Asp AT rise slightly in liver (Table 3), but are enhanced by approx. 10-fold in the mitochondrial fraction (Tables 5, 6). These changes accompanied by an increased enzyme affinity especially for aspartate (Table 6; Figs. 6, 7), result in activity increases at low substrate concentrations of up to 40-fold (Table 7). Similar activity increases for liver Asp AT have been reported in carp (Creach and Serfaty, 1974), European eel (Larsson and Lewander, 1973) and rat (Murthy et al., 1980) with food deprivation. Unfortunately, these studies did not differentiate between cellular compartments. A recent study with food-deprived plaice found no change in mitochondrial activities, but significant increases in cytosol Asp AT activities (Moon and Johnston, 1981).

As noted in the last section, lactate gluconeogenesis depends upon a mitochondrial Asp AT to translocate OAA carbons out of the mitochondria to participate with a cytosol PEP CK for glucose synthesis (Krebs et al., 1976). Recent studies (Moon, 1980) have found increases in American eel liver cytosol relative to mitochondrial PEP CK activities after eight months of food deprivation, suggesting a system much like the rat system shown by Krebs et al. (1976) is operable in the eel liver. Also, Renaud and Moon (1980b) found a much higher rate of lactate incorporation into glu-

cose than alanine after eight months of food deprivation in eels, again supporting this hypothesis. Interestingly, plaice liver PEP CK is localized primarily to the mitochondrial fraction, and Asp AT activities do not change in this fraction with food deprivation (Moon and Johnston, 1980; 1981).

In addition to the previous functions, an enhancement of trans-deamination is also apparent. Table 4 demonstrated an increase in the already high activity levels of mitochondrial glutamate dehydrogenase with food deprivation. Thus, ammonia removal could be augmented by the combined increases in mitochondrial Asp AT and GDH activities. Certainly this pathway is thought to function in the liver of fish species (McBean et al., 1966), including eels (Janicki and Lingis, 1970), and it could be important when large quantities of amino acids are being oxidized (see Lehninger, 1975).

The kinetic changes seen in liver cytosol Asp AT (Tables 3, 7; Figs. 6, 7) are minor. This might be predicted from the fact that fish liver tissue has a low utilization of exogenous glucose (Love, 1970; Cowey et al., 1977b; Renaud and Moon, 1980a) and possibly little need for an active malate-aspartate shuttle. However, studies by Hayashi and Ooshiro (1979) with isolated Japanese eel hepatocytes found an enhanced specific activity of ^{14}C-aspartate arising from ^{14}C-pyruvate in the presence of the branched-chain amino acid leucine. These data suggest a cytosol Asp AT could be important for branched-chain transaminase activities as suggested by Buse et al. (1972). Furthermore, Moon (1980)

recently reported that liver contains higher activities of leucine aminotransferase than either red or white skeletal muscle of American eels, so this pathway could be important.

Since Asp AT is essentially at equilibrium in the cell (see Lehninger, 1975), substrate and product control over activities could be significant. With the exception of cytosol Asp AT, inhibitory concentrations of aspartate (Fig. 6), α-ketoglutarate (Fig. 7), and oxaloacetate and glutamate (Fig. 4) exceed their reported in vivo levels (see Williamson et al., 1967; Love, 1970). Therefore, substrate availability, not inhibition at high concentration, could control liver Asp ATs (Lehninger, 1975). Similarily, pH can be eliminated since pH curves are flat over the physiological range (Fig. 5).

It is apparent, therefore, that food deprivation results in a major increase in liver Asp AT especially that localized to the mitochondrial compartment. This major increase is probably correlated with an enhanced gluconeogenesis and ammonia metabolism, a pattern not unlike that observed in mammals.

2. Asp AT in Eel Red Muscle

As was noted for the eel liver with food deprivation, red muscle also demonstrated a reduced extractable protein level (Table 3). This result is very difficult to explain except that these values represent soluble (not total) protein which would consist primarily of enzymes; contractile proteins would not be included in this estimate. Moon

(1980) has found a general decline in eel red muscle glycolytic enzyme activities after eight months of food deprivation in eels but little change in water content; fat content tends to increase slightly. If these protein estimates represent primarily enzymes, the small change in total Asp AT specific activity compared to the significant decline in soluble protein, would suggest that Asp AT protein is spared during deprivation.

Specific activities of Asp AT in the cytosol and mitochondria are only moderately effected (Tables 5, 6), but the major changes in Km-values especially for α-KG (Table 6) result in major changes at very low levels of substrate (Table 7). Activities at low α-KG concentrations increase by 10-fold for the cytosol enzyme and decline by approx. 3-fold for the mitochondrial enzyme (Table 7). The interpretation of this result is difficult since α-KG levels are not known in this tissue, much less in the separate cell compartments. However, these results plus the strong substrate inhibition noted for both Asp and α-KG (but more so for the latter) in Figs. 8 and 9, indicate that substrates and α-KG could play a key role in controlling the activity of red muscle Asp AT; if a general pattern emerges, it is that food deprivation reduces this reliance on substrate control. As in the case of liver Asp AT, pH curves are quite flat (Fig. 5), so H^+-ions play a minor role in controlling activities of red muscle Asp ATs.

Generally, therefore, activity levels of red muscle Asp

ATs are not effected to the extent seen for liver. Since the metabolism of red muscle is quite diversified, the maintenance of this critical enzyme may be more significant than a substantial change. Certainly the literature indicates red muscle can oxidize carbohydrates, fats and proteins and that this muscle tissue is the prime locomotory muscle for the characteristic slow swimming movements of fish (Love, 1970; Bilinski, 1974; Driedzic and Hochachka, 1978; Cowey and Sargent, 1979; Johnston, 1980b; Johnston and Moon, 1980a, b). There is some indication that red muscle may re-cycle white muscle metabolites, especially lactate, back to glucose (see Braekkan, 1947; Wittenberger, 1973; Hulbert and Moon, 1978b; Knox et al., 1980), but there is much controversy regarding this matter (Driedzic and Hochachka, 1976; 1978; Moon and Johnston, 1980). Moon (1980) has found that next to liver, red muscle maintains the highest activity levels of the branched-chain aminotransferase, moderate alanine aminotransferase activities, but low GDH activities. Coupled transamination could be an important role for Asp AT in this tissue, but trans-deamination may be minor. Therefore, the maintenance of Asp AT activities during starvation allows eel red muscle maximum flexibility for substrate utilization to power aerobic swimming. Since fuels during food deprivation will be either fat or protein-amino acids, transaminase coupling and carbon translocation will both be important functions for red muscle Asp ATs.

Thus, the strategy of red muscle is the retention of

its unique ability to metabolize carbohydrates, fats and amino acids at all times. This strategy is necessitated by the aerobic nature of the muscle and its role as the principle locomotory muscle of fish. One component of this strategy is the maintenance of tissue Asp AT specific activities.

3. Asp AT in Eel White Muscle

Fish white muscle, as a result of its very low blood flow (see Johnston, 1980b), has a strictly anaerobic metabolism (see Love, 1970; Bilinski, 1974; Driedzic, 1975; Driedzic and Hochachka, 1976; 1978). It produces the major locomotory power of the infrequent burst swimming activity of most fish. Some fish, however, including the eel, will recruit white muscle at speeds well below burst as demonstrated by electromyographic recordings (Bone et al., 1978; Johnston, 1980b). Glycogen levels in this tissue are minimal, and it has been proposed that exogenous glucose is used to supply white muscle glycolysis (Bilinski, 1974; Driedzic and Hochachka, 1978). Such a postulate seems untenable considering the low blood flow to the muscle (Johnston, 1980b) and the low activity levels of hexokinase required to introduce glucose into the glycolytic pathway (Knox et al., 1980; Moon and Johnston, 1980).

Furthermore, Hulbert and Moon (1978) found few mitochondria and no intracellular triglyceride depositions in American eel white muscle. Fat utilization by fish white muscle is very low (Bilinski, 1974) and fat metabolizing

enzymes are in low activity levels (Johnston and Moon, 1980a). Therefore, the precise fuel for white skeletal muscle is still an enigma, but since fish are neutrally buoyant the energy requirement of this tissue will be small even though up to 90% of their body weight can be white muscle (Johnston, 1980b).

It has been suggested that fish white muscle serves as a protein store, especially by Creach and colleagues (e.g., Creach and Serfaty, 1973). The Introduction referred to many studies where starvation was associated with white muscle atrophy, giving some support for this hypothesis.

The American eels used in this study did not show a decline in extractable protein (Table 3), but just as was mentioned before, this estimate does not include structural or contractile protein which is subject to depletion, even in these animals (Moon, 1980). Activities of total (Table 3), and cytosol and mitochondrial (Tables 5, 6) Asp ATs tend to rise, supporting similar deprivation studies done with carp (Creach and Serfaty, 1973; Wittenberger and Giurgea, 1973) and plaice (Moon and Johnston, 1981). The largest increase was noted for the cytosol enzyme (Tables 5, 6). If activities at low substrate concentrations are examined (Table 7), a similar pattern is noted except for the mitochondrial enzyme with α-KG; a major increase in the $Km_{(\alpha\text{-KG})}$ for this enzyme (Table 6) markedly reduces the Vmax/Km-ratio (Table 7). Food deprivation reduces the sensitivity of the white muscle enzymes to Asp and α-KG inhibition

(Figs. 10, 11), but since levels required for inhibition are well above that normally seen in vivo, this change is probably of minor physiological consequence.

Unlike the liver or red muscle enzymes, white muscle cytosol Asp AT from the food deprived eel demonstrates a sharp pH optima at 8.0 (Fig. 5). It is possible that H^+-ions could, therefore, play some role in regulating this particular enzyme; without cellular pH measurement, however, it is impossible to predict its importance.

The major change noted with food deprivation is an enhanced white muscle cytosol Asp AT (Table 6). How would this fit into the proposed metabolic schemes which employ Asp AT?

The most plausible role for the enhanced cytosol Asp AT activity is to act in the interconversion of amino acids generated by white muscle proteolysis. Unfortunately there are few, if any, good estimates of amino acid changes in fish tissues with food deprivation. What are available suggest that no one (or few) specific amino acid changes but many show small changes (see Love, 1970). Only in the spiny dogfish is alanine found to predominate, but only during the first few weeks of an extended deprivation (Leech et al., 1979). This is quite unlike mammals where only a few critical amino acids change (see reviews by Felig, 1975; Snell, 1980).

Driedzic (1975; Driedzic and Hochachka, 1976) has proposed that glutamate and α-KG tumble between Asp AT and

branched-chain aminotransferase. The aspartate generated is directed into the purine nucleotide cycle which is very active in skeletal muscle (Lowenstein, 1972) to regenerate OAA for further transamination. The branched-chain α-keto acids produced can be released to the plasma for utilization by other tissues (e.g., liver; Snell, 1980), or oxidized within the white muscle Krebs cycle (Lehninger, 1975). Although Driedzic has circumstantial evidence for such a scheme in carp, white muscle of eel (Moon, 1980) and plaice (Moon and Johnston, 1981) are found to have very low activity levels of the branched-chain aminotransferase. Since there is good evidence for a functioning purine nucleotide cycle in fish muscle (Driedzic and Hochachka, 1976), the involvement of Asp AT may be more general, serving in the coupled transamination of a variety of amino acids.

Thus, the augmentation of white muscle cytosol Asp AT may be related to amino acid interconversions, and the maintenance of the mitochondrial enzyme may suggest that the malate-aspartate shuttle is operable in this tissue even though numbers of mitochondria are small. With the increased availability of amino acids during food deprivation and the implied use of carbohydrates as an energy fuel, these hypotheses appear plausible.

To summarize, even though the precise details are open to interpretation, it is possible to identify a physiological function for the changes seen in eel tissue Asp ATs after food deprivation. The increased liver mitochondrial acti-

vity is undoubtedly associated with an enhanced gluconeogenesis and ammonia metabolism; the maintenance of red muscle Asp ATs probably reflects the requirement for this tissue to retain flexibility of substrate choice; and the increase in the white muscle cytosol enzyme is probably related to an enhanced amino acid interconversion resulting from the increased availability of amino acids. These changes were associated with alterations in the physical properties of the Asp ATs as discussed in the following section.

THE COMPARISON OF EEL TISSUE Asp ATs WITH THE HOMOLOGOUS ENZYMES OF OTHER SPECIES

Since the literature concerning Asp AT is large, it was felt a comparison of the kinetic and physical properties of the enzymes studied in this thesis with other species would be appropriate.

1. Kinetic Properties

Total specific activities of liver Asp AT range from 100 - 350 μmoles/min/mg protein in fish species (Bell, 1968; Larsson and Lewander, 1973; Wittenberger and Giurgea, 1973; Cornish *et al*., 1978; Schlisio and Nicolai, 1978; Wilson, 1978; D'Appolonia and Anderson, 1980) and from 45 - 100 μmoles/min/gm tissue in rat, pig and birds (Sarkar, 1977; Cornish *et al*., 1978; Murthy *et al*., 1980). Sarkar (1977) reported mitochondrial and cytosol activities range from 5 - 10 and 35 - 60 μmoles/min/g, respectively, in the rat, pig and chicken.

Muscle values are more difficult to find, and few studies have separated red and white fibers. Wittenberger and Giurgea (1973) found carp red muscle values 6-fold higher than white muscle (50 vs. 8 μmoles/min/g). Where "skeletal muscle" has been examined (Sarkar, 1977; Cornish et al., 1978), cytosol and mitochondrial activities range from 25 - 35 and approx. 5 μmoles/min/g, respectively, in birds and mammals. Filosofova-Lyzlova (1972) found much lower values in the muscle of lamprey, pike, tench and frogs (0.8 and 0.05 - 2.4 μmoles/min/mg protein for cytosol and mitochondria, respectively).

The problem with comparing these values with those of the American eel (Tables 3, 5) is finding a basis for comparison. It was noted that whole tissue extracts do not necessarily reflect what is happening in the cell as vast differences can exist in the cellular compartmentation of Asp AT, and perturbations such as nutritional state can alter this distribution. In addition, problems such as the assay conditions, and the before mentioned separation of muscle types into red and white, can greatly alter activities. Thus, beyond the fact that the values on Tables 3 and 5 are within the range of reported values, a more detailed comparison would be unwise.

Two more interesting species comparisons can be made: (a) the mitochondrial to cytosol ratio of Asp ATs; and (b) the ratio of Asp AT to alanine aminotransferase (Ala AT).

Sarkar (1977) reported mitochondrial:cytosol Asp AT

ratios of 1:5 to 1:8 for rat, pig and chicken liver and muscle. Depending upon the state of the eel, and the tissue examined, values on Table 5 range between 1:1 and 30:1, except for starved white muscle where activities are 1:2. As was demonstrated by Table 4, some cross contamination between mitochondrial and cytosol Asp ATs is expected due to enzyme preparation (assuming differences between marker enzymes are real); however, it is apparent that the eel has much higher mitochondrial values than observed in mammals. Baumber and Doonan (1976) have reported ratios of 5:1 for rat liver, or just the opposite reported by Sarkar (1977), indicating much unexpected variation is possible. This ratio would be expected to follow closely the functional role of Asp AT in a particular tissue, as was indicated in the last section.

Since Ala AT and Asp AT are generally thought to be coupled (see the last two sections), the activity ratio of these two enzymes should also indicate something of their function. In general, mammalian liver Ala AT and Asp AT activities are similar, but Asp AT is at least 10-fold higher in avian livers (Sarkar, 1977; Cornish et al., 1978). In skeletal muscles, activities of Asp AT can be 10- to 100-fold higher than Ala AT (Sarkar, 1977; Cornish et al., 1978). Fish tissues follow the mammalian, not avian pattern (Wittenberger and Giurgea, 1973; Cornish et al., 1978; Moon and Johnston, 1981), and as was indicated in the Result section, Ala AT was very unstable and in low activities in

eel tissues (see Moon, 1980). Therefore, Ala AT may play a role in fish species, especially in the liver, but its extreme lability will make the elucidation of its role difficult.

By comparison, the estimate of Km-values may provide better species comparisons since it is considerably less effected by the problems mentioned above for specific activities. Estimated $Km_{(s)}$-values for eel tissue Asp ATs (Table 6) fall within the range reported for the homologous enzymes of other species; values differ little depending upon the tissue examined. Apparent $Km_{(Asp)}$-values range from 2.0 - 4.0 and 0.5 - 4.0 mM in cytosol and mitochondria, respectively, of birds and mammals (Henson and Cleland, 1964; Wada and Morino, 1964; Owen and Hochachka, 1974; Sarkar, 1974) and fish (Filosofova-Lyzlova, 1972; Cornish et al., 1978). Apparent $Km_{(\alpha\text{-KG})}$-values range from 0.15 - 4.3 and 0.5 - 4.5 mM in cytosol and mitochondria, respectively, of birds and mammals (Wada and Morino, 1964; Owen and Hochachka, 1974; Sarkar, 1974); values for fish tend to be slightly less (Filosofova-Lyzlova, 1972; Cornish et al., 1978). Owen and Hochachka (1974) have reported Km-values for OAA and glut of 0.10 and 9.4 mM, respectively, for mitochondrial Asp AT and 0.06 and 3.2 mM, respectively, for cytosol Asp AT isolated from the dolphin; no such data are available for fish species to the author's knowledge.

Changes in activities of tissue Asp ATs with food deprivation have been noted before in this thesis for mammals as

have been seen for the eel. Unfortunately, to the author's knowledge, no studies have reported changes in Km-values with food deprivation. As was previously emphasized, coupling specific activity and affinity changes can result in major changes in activity at low substrate concentrations (Table 7); since this enzyme is thought to operate at equilibrium (Lehninger, 1975), these major activity changes at low substrate concentrations are potentially of great importance and deserve to be examined in other species.

2. Modulators of Tissue Asp ATs

Eel tissue Asp ATs were found to be relatively insensitive to pH changes, although the starved white muscle mitochondrial enzyme did demonstrate a sharp optimum at pH 8.0 (Fig. 5). Since pH titrates exposed surface charges on protein molecules which could possibly alter the structure, and therefore, the activity of the molecule, eel tissue Asp ATs must compensate surface charges leading to no net change in charge at any pH (Hochachka and Somero, 1973; Lehninger, 1975: Fersht, 1977).

Owen and Hochachka (1974) studied pH as a modulation of dolphin muscle Asp ATs. Optimum activities of the cytosol and mitochondrial enzymes occurred at pH 7.3 - 8.3 and 6.3 - 7.3, respectively. This rather broad pH insensitivity of the enzymes was associated with a significant change in Km-values; Km-values for both amino acids (Asp and Glut) increased whereas for both α-keto acids, Km-values decreased as pH decreased below 7.3. They suggested that pH could play a

role in the modulation of enzyme activities, but without knowledge of intracellular pH, such postulates are unwise.

It is unlikely, therefore, that H^+-ion concentrations play a role in modulating eel tissue Asp ATs, a finding consistent with the lack of information in the literature concerning this factor. The only other report of the effect of pH on a fish Asp AT is that of Bell (1968) who found that the salmon liver enzyme functioned "best at a pH>7.5".

Temperature is a major perturbant of enzyme structure and function, and through complex and not fully understood mechanisms, enzymes of ectotherms have adapted to both a low and variable temperature regimen (see Hochachka and Somero, 1973; Prosser, 1973). Eel total tissue Asp ATs are equally sensitive to temperature changes, with an Arrhenius value (Ea) of 20.8 kcal/mole, regardless of the tissue enzyme examined (Fig. 2). Since the affinity constant, or Km, is not modified, Asp AT activities will be greatly reduced at low temperatures. The reduction in activity of tissue Asp ATs is consistent with the general metabolic decline expected of the eel during its low temperature, winter fast (e.g., Sinha and Jones, 1975). The only other temperature study reported for a tissue Asp AT to the author's knowledge is found in the work of Bell (1968); here enzyme activity rose linearly between 10 and 30°C, leveling off at 35-40°C.

The absolute requirement of Asp ATs for pyridoxal-5'-phosphate (P-5'-P) is well documented in mammals (e.g., Jenkins et al., 1959; Owen and Hochachka, 1974; Sarkar,

1977) and fish (Bell, 1968; Filosofova-Lyzlova, 1972; Schlisio and Nicolai, 1978). Eel liver Asp AT has a tightly-bound, nondialyzable P-5'-P fraction, but activities can be enhanced by adding up to 5 μM P-5'-P before the enzyme is saturated (Fig. 3A). The role of this cofactor is to form a Schiff's base linking the enzyme at its active site and the substrate (amino acid), an obligatory step in the enzyme mechanism (Lehninger, 1975).

Low concentrations of Mg^{++}-ions were also found to activate the liver enzyme (Fig. 3B). This observation has not been reported before, although Umbarger (1969) has reported a Mg^{++}-deinhibition of Asp AT from bacterial sources. Also, Patwardham (1960) reported that legume Asp AT had an iron requirement. What is responsible for this ion effect is unknown; the possibility of an osmotic effect (Moon et al., 1977) seems unlikely when the activating concentrations are so low.

It must be concluded that no unique modulator of eel tissue Asp ATs was found and that the eel enzymes are similar in this respect to the mammalian enzymes. With the exception of dolphin muscle Asp AT which might be controlled by specific metabolites (Owen and Hochachka, 1974), the evidence points to the availability of substrates as the limiting step in this enzyme reaction. Since the equilibrium constant is essentially 1.0, substrate availability will not only determine the rate of the reaction, but also the direction of enzyme catalysis (Lehninger, 1975).

3. Physical Properties

As was noted in the Introduction, the isozymal nature of tissue Asp ATs is well documented in mammals. This enzyme has also been reported to occur as polymorphs in herring (Odense et al., 1966), puppy fish (Turner, 1973) and Atlantic (European and American) eels (Pantelouris, 1976). In fact, Pantelouris (1976) reported four major zones of activity in eel homogenates, and a clear distinction between mitochondrial and cytosol forms. Figs. 12 and 13 establishes a similar pattern for the American eel tissues used in this study. Food deprivation tends to reduce the total number of activity bands, and the number of less dominant forms, resulting in fewer more darkly stained bands. This may be an economic measure on the part of the eel tissues, producing fewer forms of an enzyme better adapted to the prevailing physiological state of the animal; Hochachka and Somero (1973) have made a similar argument with respect to temperature. One thing is apparent; the changes in specific activities and affinity constants noted for the tissue Asp ATs with food deprivation (Table 6), are associated with a remodelling or refinement of the enzyme molecule as detected by electrophoretic mobilities.

Mammalian Asp ATs are dimeric proteins consisting of two identical subunits giving a total molecular weight of 80,000 to 120,000 daltons; the mitochondrial and cytosol forms are of similar molecular weights with approx. 50% sequence homology (see Krista and Fonda, 1973; Kagamiyana

et al., 1977). The eel Asp ATs molecular weight values reported here were all well below these mammalian estimates, and with only one exception (fed cytosol) were at least one-quarter of these values (Table 8). Since the marker enzyme proteins eluted from the column at their predicted K_{av}-values, the experiment itself ran properly. It should also be noted that freezing did not change these values, so the values on Table 8 are real estimates of eel Asp AT molecular weights. These very low values could be explained in at least two ways, but no data are available to make any conclusive decision.

Eel Asp AT could be a small protein of molecular weight 20,000 daltons, but this is unlikely considering other two-substrate enzymes are much larger (Lehninger, 1975). Instead, the native enzyme could be a dimer or even tetramer of 50,000 or 100,000 daltons which falls apart during gel filtration, but retains assayable activities. With the 55,000 dalton value for red muscle cytosol Asp AT, this hypothesis is attractive.

Another possibility could be the shape of the enzyme. Enzymes are typically globular proteins folded into a complex three dimensional array; fibrous proteins (e.g. keratin and collagen) assume a more elongate shape (Lehninger, 1975). Between the extremes of the true fibrous and globular structures, many shapes are possible. Gel filtration with Sephadex is sensitive to globular shapes, not fibrous or extended protein sheets. The low molecular weight found for eel Asp ATs may result from an extended sheet structure.

This hypothesis awaits validation.

Therefore, as with mammalian Asp ATs, eel tissue Asp ATs consist of electrophoretically distinct mitochondrial and cytosol forms, each with internal heterogeneity. With food deprivation, major changes occur in the electrophoretic banding pattern which may explain the concurrent changes in the kinetic properties of the enzymes. There is something very interesting intrinsic to the shape of the eel enzymes as suggested by molecular weight estimates, but further experiments are needed to elucidate this behaviour.

CONCLUSION

The major finding of this investigation was that with food deprivation eel tissue Asp ATs undergo substantial modification in kinetic properties. These changes apparently arise from an altered enzyme as evidenced primarily by the electrophoretic heterogeneity and the molecular weight determinations.

The importance of these changes are partially obscured by the problems of animal selection bias (see Results) and the lack of literature available for direct comparisons. Where data are available, the lack of distinction between tissue fractions and muscle types (red and/or white) made comparisons of little value.

It was found that with food deprivation in the eel liver, extractable protein decreased while an almost 10-fold increase in the mitochondrial Asp AT activity occurred with minimal changes in cytosol activities. The maintenance of mitochondrial activities even with decrease in soluble protein, suggests an important role for this enzyme during food deprivation. In red muscle, there was a modest decrease in extractable protein and activities of Asp AT were marginally altered. White muscle increases the amount of extractable protein and its cytosolic Asp AT increased activity almost 6-fold. While total tissue extracts alluded to these trends, none represented the real changes which had occurred.

In all tissue fractions, $Km_{(s)}$ was altered with food deprivation and it was suggested that both activity and

direction of catalysis would be linked to substrate availability. Substrate inhibition did occur, but inhibitory levels in most all instances exceeded the reported physiologic substrate concentrations. If a trend existed, food deprivation reduced the reliance on substrate concentration control of tissue Asp ATs.

From the indications of these results and based on the available literature, augmented mitochondrial Asp AT in the liver could fuel Krebs cycle activity. This is especially important for the reported enhancement of eel gluconeogenesis. Also, these increases in Asp AT could be important in the increased ammonia excretion which occurs during protein mobilization and the enhanced metabolism of amino acids. Red muscle Asp ATs were slightly activated and this could reflect the importance of maintaining the flexibility of substrate choice for this tissue which is the primary locomotor system of fish. White muscle augmentation of cytosol Asp AT could reflect an enhanced involvement of the enzyme in amino acid interconversions in a tissue thought to act as a store for protein mobilization. This role is apposed to an enhanced metabolic activity of this tissue.

Therefore, this study presents evidence to support the hypothesis that nutrient deprivation has augmented the activities of tissue Asp AT in at least liver and white muscle tissue fractions and, that in all tissues studied, the importance of amino acids during food deprivation was maintained or enhanced. Furthermore, it was found that the changes in

Asp AT were greatest in those tissues which were postulated to undergo the greatest reorganization in metabolic strategy.

In closing, the evidence in this thesis does answer some questions about amino acid metabolism in the American eel. However, even more questions arise; the mystery of the eel continues !

V

REFERENCES

REFERENCES

Aster, P.L. and T.W. Moon. 1979. Fatty acid synthesis in the American eel: Tissue slices, carbon sources and reducing equivalents. XIth International Congress Biochemistry. Toronto, July 8-13.

Amador, E. and W.E. Wacker. 1962. Serum glutamic-oxaloacetic transaminase activity. A new modification and an analytical assessment of current assay techniques. Clin. Chem. 8, 343-350.

Baumber, M.E. and S. Doonan. 1976. A quantitative study of the cellular distribution of aspartate aminotransferase isozymes in rat liver. Int. J. Biochem. 7, 119-124.

Beamish, R.W.H. 1964. Influence of starvation and routine oxygen comsumption. Trans. Am. Fish Soc. 93, 103-107.

Bell, G.R. 1968. Distribution of transaminases (Aminotransferases) in the tissues of Pacific salmon (Oncorhynchus), with emphases on the properties and diagnostic use of Glutamate-Oxaloacetic transaminase. J. Fish. Res. Bd. Can. 25, 1247-1268.

Bertin, L. 1956. Eels: A biological study. Cleaner-Hume. London.

Bertland, L.H. and N.O. Kaplan. 1968. Chicken heart soluble aspartate aminotransferase. Purification and properties. Biochem. 7, 134-142.

Bertland, L.H. and N.O. Kaplan. 1970. Conformations of the multiple forms of chicken heart aspartate aminotransferase. Biochem. 9, 2653-2665.

Bilinski, E. 1974. Biochemical aspects of fish swimming in Perspectives in Marine Biology, Edited by D.C. Malins and J.R. Sargent. Vol. 1, p. 239-288. Academic Press, New York.

Black, E.C., Bosomworth, N.J. and G.E. Docherty. 1966. Combined effect of starvation and severe exercise on glycogen metabolism of rainbow trout (Salmo gairdneri). J. Fish. Res. Bd. Can. 23, 1461-1463.

Bone, Q. 1966. On the function of the two types of myotomal muscle fibres in Elasmobranch fish. J. Mar. Biol. Ass. U.K. 46, 321-348.

Bone, Q., J. Kiceniuk and D.R. Jones. 1978. On the role of different fibre types in fish myotomes at intermediate swimming speed. Fish. Bull. 76, 691-699.

Borgman, A.I. and T.W. Moon. 1976. Enzymes of the normothermic and hibernating bat (Myotis lucifugus): Temperature as a modulator of pyruvate kinase. J. Comp. Physiol. 107, 185-199.

Boström, S.L. and R.G. Johansson. 1972. Enzyme activity patterns in red and white muscle of the eel (Anguilla anguilla) at different developmental stages. Comp. Biochem. Physiol. 42B, 533-542.

Braekkan, O.R. 1959. A comparative study of vitamins in the trunk muscles of fishes. Rep. Technol. Res. Norway Fish Ind. 3, 1-42.

Buse, M.G., J.F. Biggers, K.H. Friderici and J.F. Buse. 1972. Oxidation of branched chain amino acids by isolated hearts and diaphragms of the rat. The effect of fatty acids, glucose, and pyruvate respiration. J. Biol. Chem. 247, 8085-8096.

Butler, D.G. 1968. Hormonal control of gluconeogenesis in the north American eel (Anguilla rostrata). Gen. Comp. Endocr. 10, 85-91.

Butler, D.G., W.C. Clarke, E.M. Donaldson and R.W. Langford. 1969a. Surgical adenalectomy of a teleost fish (Anguilla rostrata LeSueur): effect on plasma cortisol and tissue electrolyte and carbohydrate conditions. Gen. Comp. Endocr. 12, 503-514.

Butler, D.G., E.M. Donaldson and W.C. Clarke. 1969b. Physiological evidence for a pituitary-adrenocortical feedback mechanism in the eel (Anguilla rostrata). Gen. Comp. Endocr. 12, 173-176.

Cannon, P.R., L.E. Frazier and R.H. Hughes. 1956. The influence of cortisone upon tissue metabolism. Archs. Path. 61, 271-279.

Chang, V.M. and D.R. Idler. 1960. Biochemical studies on sockeye salmon during spawning migrations. XII. Liver glycogen. Can. J. Biochem. Physiol. 38, 553-558.

Chen, S.H., R.P. Donahue and C.R. Scott. 1973. The genetics of glutamic-pyruvic transaminase in mice: Inheritance, electrophoretic phenotypes, and postnal changes. Biochem. Genet. 10, 23-28.

Chester-Jones, I., D.K.O. Chan, I.W. Henderson and J.N. Ball. 1969. In: Fish Physiology, Edited by W.S. Hoar and D.J. Randall. Vol. II, p.321-376. Academic Press, New York.

Cohen, P.P. and Sallach. 1961. Nitrogen metabolism of amino acids. In: Metabolic Pathways, Edited by D.M. Greenberg. Vol. II, p. 1-78. Academic Press, New York.

Cornell, N.W., P. Lund and H.A. Krebs. 1974. The effect of lysine on gluconeogenesis from lactate in rat hepatocytes. Biochem. J. 142, 327-337.

Cornish, E.C., C.M. Cussen, F.J.R. Hird and P.E.E. Todd. 1978. Comparative aspects of aminotransferases in the rat, pigeon and rainbow trout. Comp. Biochem. Physiol. 61B, 375-378.

Cowey, C.B. 1975. Aspects of protein utilization by fish. Proc. Nutr. Soc. 34, 47-63.

Cowey, C.B., J.W. Andron and D.A. Brown. 1975. Studies on the nutrition of marine flat fish. The metabolism of glucose by plaice (Pleuronectes platessa) and the effect of dietary energy source in protein utilization in the plaice. Br. J. Nutr. 33, 219-231.

Cowey, C.B., M. de la Higuera and J.W. Adron. 1977a. The effect of dietary composition and insulin on gluconeogenesis in rainbow trout (Salmo gairdneri). Br. J. Nutr. 38, 385-395.

Cowey, C.B., D. Knox, M.J. Walton and J.W. Adron. 1977b. The regulation of gluconeogenesis by diet and insulin in rainbow trout (Salmo gairdneri). Br. J. Nutr. 38, 463-470.

Cowey, C.B. and J.R. Sargent. 1979. Nutrition. In: Fish Physiology, Edited by W.S. Hoar, D.J. Randall and J.R. Brett. Vol. 8, p. 1-69. Academic Press, New York.

Creach, Y. and J.C. Murat. 1974. Le jeûne et la realimentation chez la carpe (Cyprinus carpo L.), VII. Metabolism du glucose-1-^{14}C et du glucose-6-^{14}C. Arch. Sci. Physiol. 28, 157-172.

Creach, Y. and A. Serfaty. 1974. Le jeûne et la realimentation chez la carpe. J. Physiol. (Paris) 68, 245-260.

D'Appelliona, S. and P.D. Anderson. 1980. Optimal assay conditions for serum and liver glutamate oxaloacetate transaminase and sorbital dehydrogenase for the rainbow trout (Salmo gairdneri). Can. J. Fish. Aquat. Sci. 37, 163-169.

Dave, G., M. Johansson, A. Larsson, K. Lewander and U. Lidman. 1975. Metabolic and hematological effects of starvation in the European eel (Anguilla anguilla L.). Carbohydrate, lipid, protein and inorganic ion metabolism. Comp. Biochem. Physiol. 52A, 423-430.

Davidson, R.R., J.A. Cortner, M.C. Rattazzi, F.H. Ruddle and H.A. Lubs. 1970. Genetic polymorphism of human mitochondrial glutamic oxaloacetic transaminase. Science 169, 391-392.

Davis, D.J. 1964. Disc electrophoresis. II-Method and application to human serum proteins. Ann. N.Y. Acad. Sci., USA 121, 404-427.

Davis, E.J. and J. Bremer. 1973. Studies with isolated surviving rat hearts. Interdependence of free amino acids and citric-acid-cycle intermediates. Eur. J. Biochem. 38, 86-97.

Davis, E.J., R.C. Lin and D.L.S. Chao. 1972. Energy metabolism and the regulation of metabolic processes. In: Mitochondria, Edited by M.A. Mehlman and R.W. Hanson, p. 211-238. Academic Press, New York.

Decker, L.E. and E.M. Rau. 1963. Multiple forms of glutamic-oxaloacetic transaminase in tissue. Proc. Soc. Exp. Biol. Med. 112, 144-149.

DeLorenzo, R.J. and F.H. Ruddle. 1970. Glutamate oxalate transaminase (GOT) genetics in Mus musculus: Linkage, polymorphism and phenotypes of the GOT-2 and GOT-1 loci. Biochem. Gen. 4, 259-273.

DeRosa, G. and R.W. Swick. 1975. Metabolic implications of the distribution of the aspartate aminotransferase isozymes. J. Biol. Chem. 250, 7961-7967.

Dixon, M. and E.C. Webb. 1964. Enzymes, 2nd edition, p. 950. Academic Press, New York.

Driedzic, W.R. 1975. Energy metabolism in carp white muscle. Ph.D. Thesis, Univ. of British Columbia, Vancouver.

Driedzic, W.R. and P.W. Hochachka. 1975. The unanswered question of high anaerobic capabilities of carp muscle. Can. J. Zool. 53, 706-712.

Driedzic, W.R. and P.W. Hochachka. 1976. Control of energy metabolism in carp white muscle. Am. J. Physiol. 230, 579-582.

Driedzic, W.R. and P.W. Hochachka. 1978. Metabolism in fish during exercise. In: Fish Physiology, Edited by W.S. Hoar and D.J. Randall. Vol. 7, p. 503-543. Academic Press, New York.

Drummond, G.I. 1971. Microenvironment and enzyme function: Control of energy metabolism during muscle work. Am. Zool. 11, 83-97.

Felig, P. 1975. Amino acid metabolism in man. Ann. Rev. Biochem. 44, 933-962.

Fersht, A. 1977. Enzyme Structure and Mechanism. Universities Press, Belfast, U.K.

Filosofova-Lyzlova, E.M. 1972. Isozymes of aspartate and alanine aminotransferase of somatic muscles of some lower vertebrates. Biokhimiya 37, 498-506.

Fontaine, M. and J. Hatey. 1953. Contibution a l'etude du metabolisme glucidique du saumon (Salmo salar L.) a diverses etapes de son developpement et de ses migrations. Physiol. Comp. Oecol. 3, 37-52.

Fontaine, M. and M. Olivereau. 1963. Nutrition et sexualite chez les poissons. C.N.E.R.N.A. 11A, 125-148.

Fritz, I.B., D.G. Davis, R.H. Holtrop and H. Dundee. 1958. Fatty acid oxidation by skeletal muscle during rest and activity. Am. J. Physiol. 194, 379-386.

Green, C.H. 1919a. Biochemical changes in the muscle tissues of king salmon during the fast of spawning migration. J. Biol. Chem. 39, 435-456.

Green, C.H. 1919b. Changes in nitrogenous extractives in the muscle tissue of the king salmon during the fast of spawning migration. J. Biol. Chem. 39, 157-477.

Goodridge, A.G. 1972. Regulation of the activity of acetyl Coenzyme A carboxylase by palmitoyl Coenzyme A and citrate. J. Biol. Chem. 247, 6946-6952.

Guppy, M. and P.W. Hochachka. 1978. Skipjack tuna white muscle: A blueprint for the integration of aerobic and anaerobic carbohydrate metabolism. In: Physiological Ecology of Tuna, Edited by A. Dizon and G. Sharp, p. 175-181. Academic Press, New York.

Guirard, B.M. and D.D. Snell. 1964. Vitamin B_6 function in transamination and decarboxylation reactions. In: Comprehensive Biochemistry, Edited by M. Florkin and E.H. Stotze. Vol. 15, p. 138-199. Elsevier Publishing Co., New York.

Guynn, R.W., D. Veloso and R.L. Veech. 1972. The concentration of malonyl-coenzyme A and the control of fatty acid synthesis in vivo. J. Biol. Chem. 247, 7325-7331.

Harper, A.E., N.J. Benevenga and R.M. Wohlhueter. 1970. Effects of injestion of disproportionate amounts of amino acids. Physio. Rev. 50, 428-458.

Hayashi, S. and Z. Ooshiro. 1979. Gluconeogenesis in isolated liver cells of the eel (Anguilla japonica). J. Comp. Physiol. 132, 343-350.

Henson, C.P. and W.W. Cleland. 1964. Kinetic studies of glutamate oxaloacetic transaminase isozymes. Biochem. 3, 338-343.

Herbst, R.M. and L.L. Engel. 1934. A reaction between α-ketonic acids and α-amino acids. J. Biol. Chem. 107, 505-512.

Hochachka, P.W. 1969. Intermediary metabolism. In: Fish Physiology, Edited by W.S. Hoar and D.J. Randall. Vol. 1, p. 351-390. Academic Press, New York.

Hochachka, P.W. and G.N. Somero. 1973. Strategies of Biochemical Adaptation. Saunders, Toronto.

Hochachka, P.W. 1976. Design of metabolic and enzymic machinery to fit lifestyle and environment. Biochem. Soc. Symp. 41, 3-34.

Hochachka, P.W., K.B. Storey, C.J. French and D.E. Schneider. 1979. Hydrogen shuttles in air verses water breathing fishes. Comp. Biochem. Physiol. 63B. 45-56.

Hochachka, P.W., D.E. Schneider and K.B. Storey. 1979. Hydrogen shuttles in gills of water verses air breathing osteoglossids. Comp. Biochem. Physiol. 63B, 57-61.

Hogeboom, G.H., W.C. Schneider and G.E. Palade. 1948. Cytochemical studies of mammalian tissues. I. Isolation of intact mitochondria from rat liver; some biochemical properties of mitochondria and submicroscopic particulate material. J. Biol. Chem. 172, 619-636.

Holmberg, B., S. Jenson, A. Larsson, K. Lewander and M. Olson. 1972. Metabolic effects of technical pentachlorophenol (PCP) on the eel (Anguilla anguilla L.). Comp. Biochem. Physiol. 43B, 171-183.

Hulbert, W.C. 1976. The functional role of red and white muscle in the eel, Anguilla rostrata: An ultrastructural and enzyme study. Ph.D. Thesis, University of Ottawa, Canada.

Hulbert, W.C. and T.W. Moon. 1978a. A histochemical, light electron mucroscope examination of eel (Anguilla rostrata L.) red and white muscle. J. Fish Biol. 13, 527-533.

Hulbert, W.C. and T.W. Moon. 1978b. The potential for lactate utilization by red and white muscle of the eel (Anguilla rostrata L.). Can. J. Zool. 56, 128-135.

Inabe, D., C.H. Ogino, C.H. Takamatsu, T. Ueda and K. Kurokawa. 1963. Digestability of dietary components in fishes. II. Digestability of dietary protein and starch in rainbow trout. Bull. Jap. Soc. Scient. Fish 29, 242-244.

Ince, B.W. and A. Thorpe. 1976. The effects of starvation and force-feeding in the metabolism of the northern pike (Esox lucius L.). J. Fish Biol. 8, 79-88.

Inui, Y. and Y. Oshima. 1966. Effect of starvation on metabolism and chemical composition of eels (Anguilla japonica). Bull. Jap. Soc. Scient. Fish, 32, 492-501.

Jacobs, R.A., W.S. Sly and P.W. Majerus. 1973. The regulation of fatty acid biosynthesis in human skin fibroblasts. J. Biol. Chem. 248, 1268-1276.

Janicki, R. and J. Lingis. 1970. Mechanism of ammonia production from aspartate in teleost liver. Comp. Biochem. Physiol. 37, 101-105.

Jedryczkowski, W. and Z. Fischer. 1973. Preliminary report on the metabolism of the silver eel (Anguilla anguilla L.). Pol. Arch. Hydrobiol. 20, 507-516.

Jenkins, W.T., D.A. Yphantis and I.W. Sizer. 1959. Glutamic aspartic transaminase I. Assay, purification and general properties. J. Biol. Chem. 234, 51-57.

John, R. and R. Jones. 1974. The nature of multiple forms of cytoplasmic aspartate aminotransfarase from pig and sheep heart. Biochem. J. 141, 401-416.

Johnson, W.A. and J.L. Connelly. 1972. Studies on the mutual influences of substrates on bovine α-keto acid metabolism. Biochem. 11, 2416-2421.

Johnston, I.A. 1980a. Quantative studies of muscle breakdown in the marine flat-fish (Pleuronectes platessa). Cell. Tis. Res. in press.

Johnston, I.A. 1980b. Structure and function of fish muscles. In: Vertebrate Locomotion, Edited by M.H. Day. Academic Press, London. Zool. Soc. Symp. 45, in press.

Johnston, I.A. and G. Goldspink. 1973. Some effects of prolonged starvation on the metabolism of red and white myotomal muscle of the plaice (Pleuronectes platessa). Mar. Biol. 19, 348-353.

Johnston, I.A. and T.W. Moon. 1980a. Endurance exercise training in the fast and slow swimming muscles of teleost fish (Pollachius virens). J. Comp. Physiol. 135, 147-156.

Johnston, I.A. and T.W. Moon. 1980b. Exercise training in brook trout skeletal muscle. J. Exp. Biol. in press.

Jonas, R.E. and R.A. MacLeod. 1959. Biochemical studies in sockeye salmon during spawning migration. X. Glucose, total protein, non-protein nitrogen and amino acid nitrogen in plasma. J. Fish Res. Bd. Can. 17, 125-126.

Kagamiyama, H., R. Sakakibara, H. Wada, S. Tanase and Y. Morino. 1977. The complete amino acid sequence of mitochondrial aspartate aminotransferase from pig heart. J. Biochem., Tokyo 82, 291-294.

Kamra, S.K. 1966. Effect of starvation and refeeding on some liver and blood constituents of Atlantic cod (Gladus morhua L.). J. Fish Res. Bd. Can. 23, 975-982.

Karmen, A. 1955. A note on the spectrophotometric assay of glutamate-oxaloactetic transaminase in human blood serum. J. Clin. Invest. 34, 131-133.

Knox, D., M.J. Walton and C.B. Cowey. 1980. Distribution of enzymes of glycolysis and gluconeogenisis in fish tissues. Mar. Biol. 56, 7-10.

Krista, M.L. and M.L. Fonda. 1973. Beef Brain cytoplasmic aspartate aminotransferase; purification, kinetics and physical properties. Biochem. Biophys. Acta. 309, 83-96.

Krebs, H.A. 1964. In: Mammalian Protein Metabolism, Edited by N.H. Monro. Vol. 1, p.125. Academic Press, New York.

Krebs, H.A., T. Gascoyne and B.M. Notton. 1967. Generation of extramitochondrial reducing power in gluconeogenesis. Biochem. J. 102, 275-282.

Krebs, H.A. and R.L. Veech. 1969. The Energy Level and Metabolic Control in Mitochondria, Edited by S. Papa, J.M. Tager, E. Quagliariello and E.C. Slater, p. 329-382. Adriatice Editrice, Bari.

Krebs, H.A. 1972. Some effects of the regulation of fuel supply in omnivorous animals. Adv. Enz. Reg. 10, 397-420.

Krebs, H.A., P. Lund and M. Stubbs. 1976. Interrelationships between gluconeogenesis and urea synthesis. In: Gluconeogenesis: Its regulation in Mammalian Species, Edited by R.W. Hanson and M.H. Mehlman, p. 269-291. Academic Press, New York.

LaNoue, K.F., E.I. Walajtys and J.R. Williamson. 1973. Regulation of glutamate metabolism and the interactions with the citric acid cycle in rat heart mitochondria. J. Biol. Chem. 248, 7171-7183.

Larsson, A. 1973. Metabolic effects of epinephrin and norepinephrin in the eel (Anguilla anguilla L.). Gen. Comp. Endocr. 20, 155-167.

Larsson, A. and K. Lewander. 1973. Metabolic effects of starvation in the eel (Anguilla anguilla L.). Comp. Biochem. Physiol. 44A, 367-374.

Layne, F.C. 1957. Spectrophotometric and turbidimetric methods of measuring proteins. In: Methods in Enzymology, Edited by S.P. Colowich and N.O. Kaplan. Vol. 3, p. 451-454. Academic Press, New York.

Lee, D.J. and G.B. Putnam. 1973. The response of rainbow trout to varying protein/energy ratios in a test diet. J. Nutr. 103, 916-922.

Leech, A.R., L. Goldstein, C.J. Chan and J.M. Goldstein. 1979. Alanine biosynthesis during starvation in skeletal muscle of the spiny dog-fish (Squalus acanthias). J. Exp. Zool. 207, 73-80.

Lehninger, A.H. 1975. Biochemistry, the molecular basis of cell structure and function, 2nd Ed. Worth Publishers Inc., New York.

Lewander, K., G. Dave, M. Johansson, A. Larsson and U. Lidman. 1974. Metabolic and hematological studies on the yellow and silver phases of the European eel (Anguilla anguilla L.). I. Carbohydrate, lipid, protein and inorganic ion metabolism. Comp. Biochem. Physiol. 47B, 571-581.

Lindsay, D.B. 1980. Amino acids as energy sources. Proc. Nutr. Soc. 29, 53-59.

Love, R.M. 1970. The Chemical Biology of Fishes. Academic Press, New York.

Lovern, J.A. 1939. Captive eels. Some observations on their behavior. Trout Mag. 94, 56-57.

Lowenstein, J.M. 1972. Ammonium production in muscle and other tissues: the purine nucleotide cycle. Physiol. Rev. 52, 382-414.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265-275.

Lundquist, F. and N. Tygstrup. 1973. Regulation of Hepatic Metabolism. Academic Press, New York.

Magee, S.C. and A.T. Phillips. 1971. Molecular properties of the multiple aspartate aminotransferases purified from rat brain. Biochem. 10, 3397-3405.

Martinez-Carrion, M., C. Turano, E. Chiancone, F. Bossa, A. Giartosio, F. Riva and P. Fasella. 1965. Isolation and characterization of multiple forms of glutamate-aspartate aminotransferase from pig heart. J. Biol. Chem 242, 2397-2409.

Martinez-Carrion, M., F. Riva, C. Turano and P. Fasella. 1967. Multiple forms of supernatant glutamate-aspartate transaminase from pig heart. Biochim. Biophys. Res. Comm. 20, 206-222.

Martinez-Carrion, M. and D. Tiemeier. 1967. Mitochondrial glutamate-aspartate transaminase. I. Structural comparison with the supernatant isozyme. Biochem. 6, 1715-1717.

McBean, R.L., M.J. Neppel and L. Goldstein. 1966. Glutamate dehydrogenase and ammonia production in the eel (Anguilla rostrata). Comp. Biochem. Physiol. 18, 909-920.

McGee, R. and A.A. Spector. 1975. The mechanism of short term control by exogeneous free fatty acids. J. Biol. Chem. 250, 5419-5425.

Miller, L.L., W.T. Burke and D.E. Haft. 1955. Interrelationships in amino acid and carbohydrate metabolism. Studies on the nitrogen sparing action of carbohydrate with the isolated perfused rat liver. Fed. Proc. 14, 707-716.

Moon, T.W., T. Mustafa, W.C. Hulbert, R.B. Podesta and D.R. Mettrich. 1977. The phosphoenol-pyruvate branch-point in adult Hymenolepis diminuta (Cestoda): A study of pyruvate kinase and phosphoenol-pyruvate carboxykinase. J. Exp. Zool. 200, 335-336.

Moon, T.W. 1980. Changes in tissue enzymes during food deprivation in the North American eel (Anguilla rostrata). Can. Soc. Zool. Jasper, Alberta. May 7-11.

Moon, T.W. and W.C. Hulbert. 1980. Characteristics of pyruvate kinases isolated from tissues of the American eel, (Anguilla rostrata LeSueur). Comp. Biochem. Physiol. 65B, 283-289.

Moon, T.W. and I.A. Johnston. 1980. Starvation and the activities of glycolytic and gluconeogenic enzymes in skeletal muscles and liver of plaice (Pleuronectes platessa). J. Comp. Physiol. 136, 31-38.

Moon, T.W. and I.A. Johnston. 1981. Amino acid transport and interconversions in tissues of fed and food deprived plaice (Pleuronectes platessa). Submitted.

Moon, T.W. and G. Ouellet. 1979. The oxidation of tricarboxcylic acid cycle intermediates with particular reference to isocitrate by intact mitochondria isolated from the liver of the American eel (Anguilla rostrata LeSueur). Archs. Biochem. Biophys. 193, 438-452.

Murthy, P.M., C.S. Shen and P. Mistry. 1980. Effect of starvation on the activities of enzymes related to gluconeogenesis in rat kidney. Nutr. Rep. Int. 21, 869-877.

Neely, J.R., R.M. Denton, P.J. England and P.J. Randle. 1972. The effects of increased heart work on the tricarboxylate cycle and its interactions with glycolysis in the perfused rat heart. Biochem. J. 128, 147-159.

Nyman, L. 1972. Some effects of temperature on eel (Anguilla) behaviour. Inst. Freshwater Res. 52, 90-102.

Odense, P.H., T.M. Allen and T.C. Leung. 1966. Multiple forms of lactate dehydrogenase and aspartate aminotransferase in herring. Can. J. Biochem. 44, 1319-1326.

Owen, T.G. and P.W. Hochachka. 1974. Purification and properties of Dolphin muscle aspartate and alanine transaminases and their possible roles in the energy metabolism of diving mammals. Biochem. J. 143, 541-553.

Palmer, T.N. and B.E. Ryman. 1972. Studies on oral glucose intolerance in fish. J. Fish Biol. 4, 311-319.

Pantelouris, E.M. 1976. Aspartate aminotransferase variation in the Atlantic eel. J. Exp. Mar. Biol. Ecol. 22, 123-130.

Pantelouris, E.M., A. Arnason and R. Bumphis. 1976. New observations on esterase variation in the Atlantic eel. J. Exp. Mar. Biol. 22, 113-121.

Pardridge, W.M. and L.S. Jefferson. 1975. Liver uptake of amino acids and carbohydrates during a single circulatory passage. Am. J. Physiol. 223, 1155-1161.

Patterson S. and G. Goldspink. 1973. The fine structure of red and white myotomal muscle in a marine teleost, the coal fish (Gladus virens). Z. Zellforsch. 146, 375-384.

Patterson, S., I.A. Johnston and G. Goldspink. 1974. The effects of starvation on the chemical composition of red and white muscle of the plaice (Pleuronectes platessa). Experientia 30, 892-893.

Patwardham, M.V. 1960. Glutamic - aspartic transaminase of Dolichos lablab: Participation of iron as a cofactor. Biochem. J. 75, 401-408.

Prosser, C.L. 1973. Comparative Animal Physiology, W.B. Saunders Co., Philadelphia.

Radola, B.J. 1969. The use of isoelectric focusing in the separation of proteins. Biochim. Biophys. Acta. 184, 335-338.

Renaud, J.M. and T.W. Moon. 1980a. Characterization of gluconeogenesis in hepatocytes isolated from the American eel (Anguilla rostrata LeSueur). J. Comp. Physiol. 135, 115-125.

Renaud, J.M. and T.W. Moon. 1980b. Starvation and metabolism of hepatocytes isolated from the American eel (Anguilla rostrata LeSueur). J. Comp. Physiol. 135, 127-137.

Ruderman, N.B., M.N. Goodman, M. Berger and S. Hagg. 1977. Effect of starvation on muscle glucose metabolism: studies with the isolated perfused rat hindquarters. Fed. Proc. 36, 171-176.

Sacktor, B. 1976. Biochemical adaptations for flight in the insect. Biochem. Soc. Symp. 41, 111-131.

Safer, B., C.M. Smith and J.R. Williamson. 1971. Control of the transport of reducing equivalents across the mitochondrial membrane. J. Mol. Cell. Cardiol. 2, 111-124.

Safer, B. and J.R. Williamson. 1973. Mitochondrial-cytosolic interactions in perfused rat heart. J. Biol. Chem. 248, 2570-2579.

Saier, M.H., Jr. and J.R. Williamson. 1967. Alanine aminotransferase. I. Purification and properties. J. Biol. Chem. 242, 91-100.

Sarkar, N.K. 1974. Alanine and aspartate amino transferase activities in serum and various subcellular fractions from the livers of different species. Int. J. Biochem. 5, 375-381.

Sarkar, N.K. 1977. Implications of alanine and aspartate aminotransferases, glutamate dehydrogenase in glucose production in rats, chicken and pigs. Int. J. Biochem. 8, 427-432.

Savina, M.W. and A.B. Wotzcak. 1977. Enzymes of gluconeogenesis and the synthesis of glycogen in various organs of the lamprey (Lampetra fluviailis). Comp. Biochem. Physiol. 57B, 185-190.

Schlisio, W. and B. Nicloai. 1978. Kinetic investigations on the behaviour of free amino acids in the plasma of two aminotransferases in the liver of rainbow trout (Salmo gairdineri Richardson) after feeding on a synthetic composition containing pure amino acids. Comp. Biochem. Physiol. 55B, 373-379.

Shrawder, E.J. and M. Martinez-Carrion. 1973. Simultaneous isolation and characterization of chicken supernatant and mitochondrial isoenzymes of aspartate aminotransaminase. J. Biol. Chem. 248, 2140-2146.

Singh, R.P. and T. Nose. 1967. Digestability of carbohydrates in young rainbow trout. Bull. Freshwat. Fish Res. Lab. 17, 21-25.

Sinha, V.R.P. and J.W. Jones. 1975. The European Freshwater Eel. University Press, Liverpool.

Slater, E.C. 1960. In: Structure and Function of Muscle, Edited by G.H. Bourne. Vol. II, p. 111, Academic Press, New York.

Söling, H.D. and J. Kleineke. 1976. Species dependent regulation of hepatic gluconeogenesis in higher animals. In: Gluconeogenesis: Its Regulation in Mammalian Species, Edited by R.W. Hanson and M.A. Mehlman. p. 369-462. Academic Press, New York.

Snell, K. 1980. Muscle alanine synthesis and hepatic gluconeogenesis. Biochem. Soc. Transact. 8, 205-213.

Stasko, A.B. and A.A. Romel. 1974. Swimming depth of adult American eels in saltwater bay as determined by ultrasonic tracking. J. Fish Board. Can. 31, 1148-1155.

Stimpson, J.H. 1965. Comparative aspects of the control of glycogen utilization in vertebrate liver. Comp. Biochem. Physiol. 15, 187-197.

Storer, J.H. 1967. Starvation and the effects of cortisol in the goldfish (Carassius auratus). Comp. Biochem. Physiol. 20, 939-948.

Swallow, R.L. and W.R. Flemming. 1969. The effect of starvation, feeding, glucose and ACTH on the liver glycogen levels of Tilapia mossambica. Comp. Biochem. Physiol. 28, 95-106.

Tarr, H.L.A. 1972. In: Fish Nutrition, Edited by J.E. Halver, p. 255-326. Academic Press, New York.

Tesch, F.W. 1977. The Eel, Biology and Management of Anguillid Eels. Chapman and Hill, London.

Turner, B.J. 1973. Genetic variation of mitochondrial aspartate aminotransferase in the teleost Cyprinodon nevadensis. Comp. Biochem. Physiol. 44B, 89-92.

Tyler, D.D. and J. Gonze. 1967. The isolation of mitochondrial and cytosolic aspartate aminotransferase. Meth. Enzymol. 10, 75-77.

Umbarger, H.E. 1969. Regulation of amino acid metabolism. Ann. Rev. Biochem. 38, 323-370.

Vladykov, V.D. 1955. L'anguille. Dept. des pêcheries, Quebec. Vol. 6.

Vladykov, V.D. 1964. Quest for the true breeding areas of the American eel (A. rostrata LeSueur). J. Fish Res. Bd. Can. 21, 1523-1530.

Wada, H. and Y. Morimo. 1964. Comparative studies on glutamate-oxalacetic transaminases from the mitochondrial and soluble fractions of mammalian tissues. Vit. Horm. 22, 411-444.

Walker, M.G. 1971. The effects of starvation and exercise on the skeletal muscle fibres of the cod (Gladus morhua L.) and coalfish (Gladus virens L.). J. Cons. Int. Explor. Mer. 33, 421-427.

Weinbach, E.C. 1961. A procedure for isolating stable mitochondria from rat liver and kidney. Anal. Biochem. 2, 335-343.

Williamson, D.H. 1978. Recent developments in ketone body metabolism. Biochem. Soc. Transact. 7, 1313-1321.

Williamson, D.H., O. Lopes-Vierira and B. Walker. 1967. Concentrations of free amino acids in the livers of rats subjected to various metabolic stresses. Biochem. J. 104, 497-502.

Williamson, J.R., B. Safer, I.F. LaNoue, C.M. Smith and E. Walajtys. 1973. Mitochondrial-cytosolic interactions in cardiac tissue: Role of the malate-aspartate cycle in the removal of NADH from the cytosol. Soc. Exp. Biol. Symp. 27, 241-281.

Wilson, R.P. 1973. Nitrogen metabolism in channel catfish (Ictalarus punctatus). I. Tissue distribution of aspartate and alanine amino transferases and glutamate dehydrogenase. Comp. Biochem. Physiol. 46B, 617-642.

Wittenberger, C. 1973. Metabolic interaction between isolated white and red carp muscles. Rev. Roum. Biol. 18, 71-76.

Wittenberger, C. and I.V. Diaciuc. 1965. Effort metabolism of lateral muscles in carp. J. Fish Res. Bd. Can. 22, 1397-1406.

Wittenberger, C. and R. Giurgea. 1973. Transaminase activities in muscles and liver of the carp. Rev. Roum. Biol. Zool. 18, 441-444.

Wittenberger, C., D. Coprean and L. Morar. 1975. Studies on the carbohydrate metabolism of the lateral muscle in carp (influences of phloridzin, insulin and adrenaline). J. Comp. Physiol. 101, 161-172.

Wodtke, E. 1974. Effect of acclimation temperature on the oxidative metabolism in the eel (Anguilla anguilla L.). J. Comp. Physiol. 91, 309-332.

Wroblewski, F. and J.S. LaDue. 1956. Serum glutamic-pyruvic transaminase in cardiac and hepatic disease. Proc. Soc. Exp. Med. 91, 569-571.

Yan-Hwa, L. and J.E. Churchich. 1975. Mitochondrial aspartate aminotransferase-independent function in the catalytic binding sites. J. Biol. Chem. 250, 5604-5608.

ADDENDUM

Due to the size differential between the fed and food deprived groups (Table 3) potentially biasing the results, circumstantial proofs are offered that size would not singularly be responsible for the experimental differences.

The location and direction of the migration of the fish would indicate that they were all in approximately the same life cycle stage at the time of capture. In the lab, these animals were sized and large animals from both groups were intended to be used; however, after the ten month holding period, only large food deprived and small fed animals remained. This was not a result of animals dying, but arose from factors outside of the author's control. Size differential in itself, however, may not reflect absolute maturational differences as Vladykov has found considerable overlap in size between groups differing in maturity (personal communication).

Also, certain enzyme data suggest that the results didn't arise from differing size: (a) the order of magnitude of Asp AT activities in Cornwall eels (approx. 600 g) and Quebec City eels (approx. 1 kg) were the same; (b) some tissue subcellular fractions maintained the same activities with food deprivation (Table 5); (c) Table 4 was derived from two groups of fresh eels all approx. 500 g, and again the relative activities are of the same magnitude; (d) based upon this size differential, an approx. 130% increase in metabolic activity would be predicted in the smaller group,

yet changes noted in tissue activities were either not this large or in great excess of this value; and, (e) since food deprivation is a common feature of the eel's life history, such adaptive changes in this enzyme might be expected.

Since the physiology of the eel does not allow for a physiologic fed control group during low temperature or migration food-deprivation, the only alteration in the methodology would have been to duplicate these experiments at the onset of the experiment and then to have fed and food-deprived the animals. After 10 months this would have established a pre-experimental control and then the relative changes from this group might have better represented how the perturbations of food deprivation affected the specific activities of tissue Asp ATs.

Therefore, the selection of eels of differing size was unfortunate but this choice probably did not contribute significantly to these results.