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Arginine, Glutamate, and Proline as Substrates for Oxidation and for Glycogenesis in Cephalopod Tissues[1]

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ABSTRACT: In addition to the usual metabolic roles for arginine and proline in cephalopod metabolism (the first serving in anaerobic metabolism and the second in augmenting the Krebs cycle pool of intermediates), we found that arginine and proline were vigorously oxidized and that their catabolism appeared to proceed through two common intermediates, glutamate and ornithine. In addition, we found that glutamate and proline were both capable of supplying precursors for the gluconeogenic pathway. On a unit mass basis, highest rates of ^{14}C-glutamate and ^{14}C-proline incorporation into glycogen occurred in the kidney, but when overall organ and tissue mass were considered, muscle, kidney, and gill displayed comparable rates of glycogen formation from these amino acids. The possibility was considered that these interactions between arginine, proline, and glycogen metabolism may be utilized during replenishment of all three substrate stores during recovery from exhaustive exercise.

THE SWIMMING ABILITIES of different cephalopods vary greatly, but their energy metabolism shares three common features. First, working muscles contain little fat and little enzymic capacity for utilizing it (Ballantyne, Hochachka, and Mommsen 1981). Second, glycogen levels and glycolytic enzyme activities are substantial, pointing to carbohydrate as a major energy source for performance (Ballantyne et al. 1981, Gäde 1980). Third, free amino acids are present at high levels (over 200 μeq/g muscle; Hochachka, French, and Meredith 1978), and must also be considered as a possible source of energy. Of these, proline and arginine are particularly abundant and metabolically active. Proline is thought to be utilized during oxidative metabolism either directly as an energy source or as a means for augmenting the Krebs cycle (Storey and Storey 1978). Arginine, on the other hand, is thought to be involved in anaerobic metabolism, being stored as arginine phosphate under energy-rich conditions and being "dumped" into octopine during activated anaerobic metabolism, during burst swimming, for example (Gäde 1980).

So much attention has been devoted to these obvious functions of arginine and proline in cephalopod metabolism that other potentially important roles have generally been overlooked. We were led to consider such alternate metabolic functions for these two amino acids because preliminary experiments in this study revealed that arginine could be oxidized at surprisingly high rates by cephalopod tissues (see below; also see Hochachka et al. 1982) and because arginine and proline are potentially interconvertible through the common intermediates, glutamate and ornithine. This interconversion, known from mammalian and bacterial studies, is based on nutritional, tracer, and enzymatic evidence and can be schematically summarized as follows (Adams and Frank 1980):

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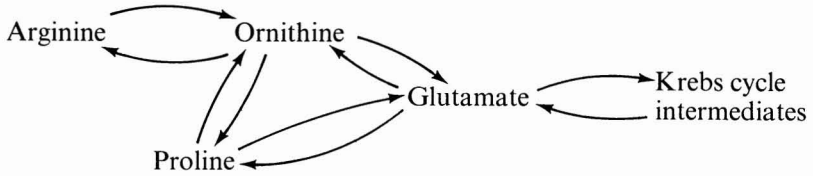


In parallel but independent studies, we confirmed that similar interconversions occur in *Sepia* studied in vivo (Hochachka et al. 1982) and in squid tissues studied in vitro (Mommsen and Hochachka 1981). In this study, we report that (1) arginine, proline, and glutamate can be vigorously oxidized in several cephalopod tissues, and (2) two of these (proline and glutamate), and probably all three, are capable of serving as glycogen precursors.

MATERIALS AND METHODS

Experimental Animals

All live experimental animals were captured in Tanon Strait by local fishermen, mostly working out of Bindoy, Negros Orientale, Republic of the Philippines. The species utilized in this study were *Octopus macropus*, *Sepioteuthis lessonia*, *Nototodarus sloani*, and *Nautilus pompilius*. Most of the results reported are for *Octopus macropus*.

In Vivo Experiments

Attempts to measure in vivo rates of ^{14}C-glutamate incorporation into tissue glycogen were made on four octopus specimens. Each animal was anesthetized in a seawater–ethanol bath and the vena cava was catheterized as described previously for *Sepia* (Hochachka et al. 1982). Following recovery (about 4 hr), a total of 1 ml of millipore filtered seawater containing 5 μmole/ml carrier glutamate and 10 μCi [U-^{14}C]glutamate was injected rapidly into the vena cava followed by $\frac{1}{2}$ ml of seawater to wash out the line. Two hours after injection, the animal was killed by decerebration; tissue samples were rapidly dissected out, weighed, cut into small cubes, and then extracted in boiling 30% KOH (1 : 3 wt/vol). Saturated $NaSO_4$ was added (0.5 ml/4 ml extract), and glycogen was precipitated with ethanol addition. The ethanol/KOH mixture was centrifuged in a Sorvall RC2B centrifuge (at about 2000 × *g*) to collect the precipitated glycogen. The resulting supernatant was decanted, and the glycogen precipitate was dried. It was then redissolved in water and twice reprecipitated with ethanol before finally being taken up in water and hydrolyzed in 0.7 M HCl by boiling for 3 hr. Following hydrolysis, the solution was neutralized and the amount of glucose released was assayed by coupling the hexokinase and glucose-6-phosphate dehydrogenase reactions (Bergmeyer 1974).

Tissue Slice Experiments

For in vitro experiments, tissues were isolated from anesthetized individuals held at ice temperatures. Each tissue sample was weighed, and tissue slices about 1 mm thick were prepared on ice blocks with a hand-held razor. Weighed samples (0.1–0.2 g) were placed in 25-ml Erlenmeyer flasks outfitted with aklali CO_2 traps (Kontes Scientific, Vineland, New Jersey) and containing 10 ml of filtered seawater plus 2 μCi of one of the following: [U-^{14}C]arginine, [U-^{14}C]proline, [U-^{14}C]glutamate, [U-^{14}C]glucose, or [U-^{14}C] taurine. Unless otherwise specified, the concentration of each substrate was 0.1 mM, approximately equivalent to physiological blood levels (Hochachka et al. 1978). In each case the experiment was initiated by addition of tissue slices, and the flasks were incubated with shaking at 22°C in a metabolic chamber for 60 min. The reaction was terminated by the addition of 1 ml of 1.4 M perchloric acid (PCA); $^{14}CO_2$ being driven off was collected in CO_2 traps containing filter paper wicks and 0.2 ml 30% KOH. After an additional 60 min with shaking, the CO_2 traps were transferred to scintillation vials containing 20 ml aquasol; radioactivity was determined in a scintillation

counter at about 85% efficiency. Initial specific activity in the arginine tissue slice experiments was 0.3 μCi/μmole substrate; CO_2 production rates were expressed in nmole/hr/g wet wt of tissue, assuming no change in specific activities through the experiment. Direct measurements indicated about a 10% uptake of the added labeled metabolite through the time course of these tests. The same assumptions were made in the proline and glutamate experiments.

The ^{14}C-glycogen formed in the tissue slices was determined by isolating and purifying glycogen as above. After two cycles of ethanol precipitation and water resolubilization, the glycogen was dissolved in water and aliquots (usually 0.2 ml) were added to 20 ml aquasol for counting. Other aliquots of glycogen were hydrolyzed in 0.7 M HCl as described above, and the amount of glucose released was quantitatively assessed. In tissue slice experiments measuring ^{14}C-glutamate or ^{14}C-proline conversion to glycogen, it was important to simultaneously assess ^{14}C incorporation into tissue glucose. The ^{14}C-glucose activity was determined using methods of French, Mommsen, and Hochachka (1981) and was found to be less than 2% of that found in glycogen. Therefore, for practical purposes, the rates of incorporation into glycogen were considered as a measure of flux in the gluconeogenic pathway under specified experimental conditions.

Enzyme Activities

Glutamate dehydrogenase was assayed at 25°C following the decrease in absorbance at 340 nm due to NADH oxidation. Assay conditions were: 0.2 mM NADH, 5 mM 2-ketoglutarate, 200 mM NH_4Cl, 2 mM ADP, 100 mM Tris–HCl, pH 8.0, total volume 1 ml. Assay conditions for fructose bisphosphatase were: 0.2 mM NADP, excess glucose-6-phosphate dehydrogenase and hexose isomerase, 2 mM fructose-1,6-bisphosphate, 100 mM Tris–HCl, pH 8.0. Tissues were prepared for enzyme assay by homogenizing weighed cubes of tissues in Tris–HCl buffer. Following homogenization, the preparation was centrifuged at about 5000 × g to remove cellular debris. The clear supernatant solution obtained was used directly as a source of enzyme activity. Background nonspecific NADP reduction or NADH oxidation was assessed by omitting the substrate for each enzyme assay. Enzyme activities were expressed in terms of micromoles of substrate converted to product per minute per gram wet weight of tissue, as in other systems (see, for example, Alp, Newsholme, and Zammit 1976).

Biochemicals

All organic and inorganic reagents were of analytical quality. Coupling enzymes, sub-

TABLE 1

OXIDATION RATES BY OCTOPUS TISSUE SLICES OF ARGININE COMPARED TO OTHER SUBSTRATES

SUBSTRATE	TISSUE	CONCENTRATION (mM)	CO_2 PRODUCTION (nmole/g/hr)	CALCULATED TOTAL TISSUE* INCORPORATION INTO CO_2 (nmole/hr)
Glucose	Gill	0.1	31.0	145.7
	Kidney	0.1	19.2	38.4
	Liver	0.1	20.9	480.7
Arginine	Gill	0.06	5.4	25.4
	Kidney	0.06	125.9	251.8
	Liver	0.06	20.6	473.8
Proline	Gill	0.1	96.8	454.9
	Kidney	0.1	94.0	188.0
	Liver	0.1	20.8	478.0
Taurine	Gill	0.1	0.3	1.4
	Kidney	0.1	2.4	4.8
	Liver	0.1	0.4	9.2

*Assuming a standard 500-g animal (see Table 2).

strates, and coenzymes were purchased from Sigma Chemical Co., St. Louis, Missouri. Aquasol and ^{14}C-labeled metabolites were purchased from New England Nuclear, Boston, Massachusetts.

TABLE 2

TISSUE AND ORGAN WEIGHTS FOR A STANDARD 500-g *Octopus macropus*

TISSUE/ORGAN	WEIGHT (g)
Mantle muscle and skin	61.0
Skinned mantle	30.0
Mantle skin	31.0
Liver	23.0
Gills	4.7
Branchial hearts	2.0
Kidneys	2.0
Systemic heart	0.55
Head and arms with skin	385.0

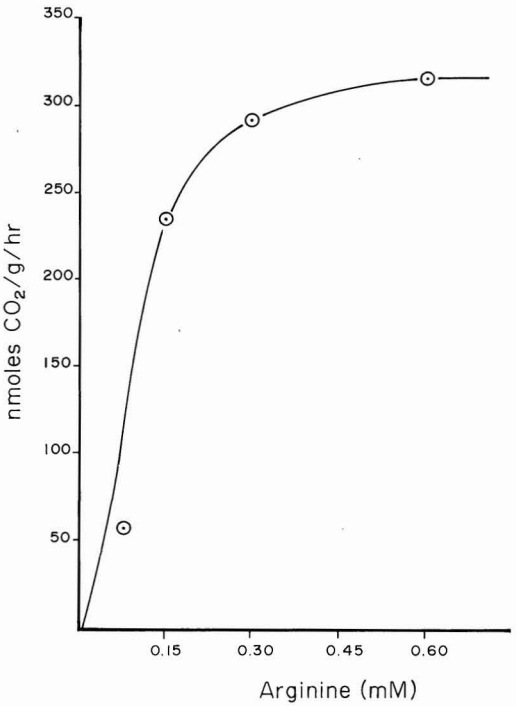


FIGURE 1. Effect of arginine concentration on [U-^{14}C]arginine oxidation by kidney slices of *Octopus macropus*. Specific activity held constant at 0.3 μCi/μmole arginine.

RESULTS

Arginine Oxidation

As mentioned above, so much attention has been directed to the role of arginine in phosphagen and octopine metabolism in cephalopods that we were surprised to find it was also oxidizable at relatively high rates. Oxidation rates for gill, kidney, and liver slices from *Octopus* are shown in Table 1 for arginine and three other substrates. Gill, kidney, and liver are all capable of oxidizing arginine, proline, and glucose. On a total tissue mass basis, liver capacity to oxidize arginine is greater than that of the kidney (Tables 1 and 2), but on a gram wet weight basis, the kidney displays highest arginine oxidation rates (4 and 20 times higher than in the liver and gill, respectively). Parallel studies of *Symplectoteuthis* (Mommsen et al., this issue), *Alloteuthis* and *Sepia* (Hochachka et al. 1982), and *Illex* (Mommsen and Hochachka 1981) indicate that other tissues (muscle, heart, brain, fin) are also able to oxidize arginine. The process of arginine oxidation is concentration-dependent; kidney slices show a half-saturation value of about 0.1 mM (Figure 1).

TABLE 3

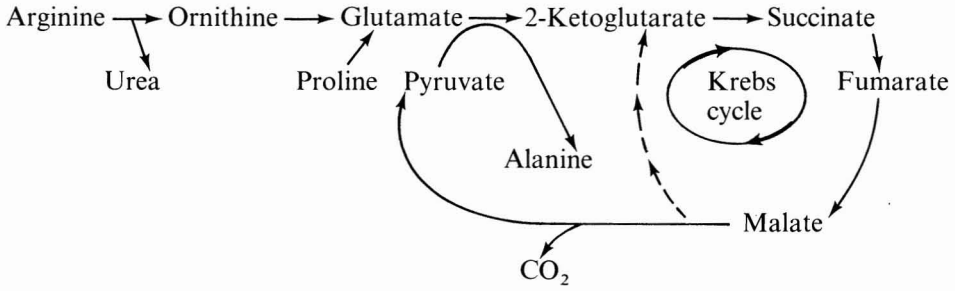
^{14}C–ARGININE OXIDATION BY OCTOPUS KIDNEY SLICES IN THE PRESENCE AND ABSENCE OF METABOLITES AND INHIBITORS

CONDITIONS	CO_2 PRODUCTION (nmole/g/hr)*
Control (0.1 mM arginine)	379.8 (4) [312–446]†
+1 mM AOA	141.0 (1)
+4 mM AOA	56.1 (1)
+2.5 mM pyruvate	419.0 (1)
+2.5 mM malate	251.8 (1)
+2.5 mM pyruvate + 2.5 mM malate	173.7 (2) [115–232]†
+10 mM proline	251.0 (1)
+10 mM glutamate	117.7 (2) [93–141]†
+0.5 mM CN^-	58.2 (2) [50.9–65.5]†

*The number of experiments is given in parentheses.
† Range of values.

Probable Pathway of Arginine Oxidation

During the *Alpha Helix* expedition, time did not allow a complete proof of the arginine oxidation pathway in any of the cephalopods local to the Philippines. However, a number of experimental observations bear directly on this problem (Table 3): (1) Aminooxyacetate (AOA), a transaminase inhibitor, strongly inhibits oxidation. (2) Pyruvate alone has no effect, but pyruvate in combination with malate is inhibitory. (3) High levels of proline or glutamate also lead to a reduction in $^{14}CO_2$ release from arginine. (4) Cyanide is extremely inhibitory, a result implying that aerobic metabolic processes are utilized in arginine oxidation, not just arginase coupled with urease. These results are consistent with the overall pathway subsequently shown by Mommsen and Hochachka (1981) for other cephalopods:



For this reason, and because glutamate is a known glucogenic amino acid, we turned our attention to the partitioning of glutamate between oxidation and glycogen formation.

Glutamate Incorporation into CO_2 and into Glycogen

As anticipated, most octopus tissues are capable of oxidizing glutamate, and ^{14}C from glutamate appears in CO_2 at high rates (Table 4). Kidney and gill slices oxidize glutamate at the highest rates, on a gram wet weight basis, while mantle muscle and branchial heart oxidation rates are the lowest. However, when calculated on the basis of a standard 500-g octopus (Table 2), the total tissue oxidation rates are similar for mantle, kidney, gill, and liver (Table 4).

On a wet weight basis, the kidney displays by far the highest capacity for glutamate incorporation into glycogen—about 20 times higher than mantle muscle and branchial heart, about 3 times higher than gill, and nearly two orders of magnitude higher than liver (Table 5). When standardized for amount of tissue present, however, three tissues (mantle muscle, kidney, and gill) display quantitatively highest potentials for glycogen formation from glutamate.

In vivo measurements of the incorporation of blood ^{14}C-glutamate into tissue glycogen indicate a similar tissue distribution of glyconeogenic capacities (Table 6). However, in vitro gill tissue displays a higher capacity for glutamate incorporation into glycogen than observed in vivo, possibly because of low glutamate levels in cephalopod blood (French, unpublished data, Hochachka et al. 1978) compared to the 5 mM levels utilized in the tissue slice experiments (Table 5).

Proline Incorporation into CO_2 and into Glycogen

Analogous experiments with ^{14}C-proline also indicate high rates of both oxidation and glycogen incorporation (Tables 7, 8). Although absolute rates of $^{14}CO_2$ release from proline and glutamate differ, so do their pool sizes, with proline levels typically higher than glutamate levels. Since only exogenous concentrations and ^{14}C activities are known

TABLE 4

^{14}C–GLUTAMATE OXIDATION IN OCTOPUS TISSUES

TISSUE	CO_2 PRODUCTION (nmole/g/hr)†	CALCULATED TOTAL TISSUE PRODUCTION* (nmole/hr)†
Mantle	155.2 [129.2–181.2]	4,656 [3,876–5,436]
Kidney	1,315.5 [1,288.0–1,342.9]	2,631 [2,576–2,686]
Branchial heart	122.9 [111.4–134.4]	246 [223–269]
Systemic heart	321.8 [297.9–345.6]	177 [164–190]
Gill	1,113.3 [1,076.3–1,150.2]	5,233 [5,059–5,406]
Liver	192.9 [169.2–216.5]	4,436 [3,892–4,980]

NOTE: Glutamate at 5 mM concentrations. Other conditions as given in the text.
*Assuming a standard 500-g animal (see Table 2).
†Values are averages for duplicate experiments; ranges of values are shown in brackets.

TABLE 5

^{14}C–GLUTAMATE INCORPORATION INTO GLYCOGEN BY ISOLATED OCTOPUS TISSUES ASSAYED AT 5 mM GLUTAMATE

TISSUE	^{14}C–GLUTAMATE INCORPORATION INTO GLYCOGEN (nmole/g/hr)†	(nmole/μmole glucosyl glycogen)†	CALCULATED TOTAL TISSUE INCORPORATION* (nmole/hr)†
Mantle	11.1 [10.8–114]	0.85 [0.8–0.9]	333 [324–342]
Kidney	230.5 [198.4–262.5]	8.6 [6.4–10.8]	461 [397–525]
Branchial heart	16.2 [13.4–18.9]	2.1 [1.6–2.5]	32.5 [27–38]
Systemic heart	34.7 [34.1–35.7]	1.95 [1.7–2.2]	18.5 [18–19]
Gill	79.1 [70.0–88.2]	10.1 [8.6–11.6]	372 [329–415]
Liver	2.7 [2.2–3.2]	0.75 [0.6–0.9]	62 [51–74]

*Assuming a standard 500-g animal (see Table 2).
†Values are averages for duplicate experiments; ranges of values are shown in brackets.

(tissue levels of intermediates were not determined in these studies), and because no correction has been made for $^{14}CO_2$ released from simple cycling through the Krebs cycle (with no net oxidation), the absolute $^{14}CO_2$ values from proline and glutamate are not directly comparable. Nevertheless, an indication of substrate preferences can be obtained by comparing single-substrate data for different tissues. Such a comparison indicates that mantle muscle is the main site of proline oxidation while gill and liver preferentially oxidize glutamate. Thus, the mantle oxidizes proline 3 times faster than does kidney, while glutamate is oxidized about 1.5 times faster by the mantle than by the kidney. On the other hand, mantle muscle oxidizes proline over 6 times faster than do gill and liver tissues, but all three oxidize glutamate at essentially similar rates (Table 9). These quali-

TABLE 6

IN VIVO ^{14}C–GLUTAMATE INCORPORATION INTO GLYCOGEN IN *Octopus macropus*

TISSUE	^{14}C–GLYCOGEN (DPM/g tissue)	^{14}C–GLYCOGEN (DPM/μmole glucosyl units)	CALCULATED TOTAL INCORPORATION* (DPM/2 hr)
Mantle	7,725	471.0	231,750
Kidney	190,875	4,140.5	381,750
Branchial heart	12,050	1,434.5	24,100
Gill	17,050	2,583.3	80,135
Systemic heart	24,375	892.9	13,406
Liver	1,850	560.6	42,550

NOTE: This experiment was initiated on four animals, three of which died before the tests were completed (probably because they pulled out the vena cava catheters). Radioactivity expressed as disintegrations per minute (DPM).
*Assuming a standard 500-g animal (Table 2).

TABLE 7

^{14}C–PROLINE OXIDATION IN OCTOPUS TISSUES

TISSUE	CO_2 PRODUCTION (nmole/g/hr)†	CALCULATED TOTAL TISSUE PRODUCTION* (nmole/hr)†
Mantle	33.1 [30.6–35.6]	993 [918–1,068]
Kidney	168.2 [143.6–192.8]	336 [287–385]
Branchial heart	11.8 [10.9–12.6]	235 [22–25]
Systemic heart	46.6 [38.0–55.1]	25.5 [21–30]
Gill	33.0 [31.5–34.4]	155 [148–162]
Liver	5.7 [5.2–6.1]	130 [120–140]

*Assuming a standard 500-g animal (see Table 2).
†Values are averages for duplicate experiments; ranges of values are shown in brackets.

tative patterns in oxidation capacities are consistent with different metabolic roles of both tissues and substrates; proline in muscle is probably an important substrate in energy metabolism, while glutamate in kidney, gill, and liver is probably an important source of waste nitrogen (NH_4^+).

Proline incorporation into glycogen occurs at highest rates in the kidney—approximately 5–10 times higher on a unit mass basis than in all other tissues assayed (Table 8). However, calculated total tissue capacity for this process indicates that the muscle and kidney can potentially synthesize glycogen at similar rates, while the gill is the next most capable (Tables 8, 9).

Enzyme Levels

Preliminary studies of the tissue distribution of fructose-1,6-bisphosphatase in *Octopus macropus*, as well as in several other cephalopods (Table 10), are completely consistent with the ^{14}C metabolite data presented above. Glutamate dehydrogenase, used as a mitochondrial marker enzyme, also shows a tissue distribution pattern (Table 10) consistent with tissue oxidative capacities.

DISCUSSION

Although the small sample sizes limit the quantitative implications of the data pre-

TABLE 8

^{14}C–PROLINE INCORPORATION INTO GLYCOGEN IN OCTOPUS TISSUES

TISSUE	^{14}C–PROLINE INCORPORATION INTO GLYCOGEN (nmole/g/hr)†	(nmole/μmole glucosyl glycogen)†	CALCULATED TOTAL TISSUE INCORPORATION* (nmole/hr)†
Mantle	7.5 [6.0–9.0]	0.8 [0.7–0.9]	225 [180–270]
Kidney	85.5 [76.5–94.5]	101.8 [82.4–121.2]	171 [153–189]
Branchial heart	7.3 [5.5–9.0]	5.0 [3.9–6.0]	14.5 [11–18]
Systemic heart	6.8 [6.0–7.5]	2.1 [1.7–2.5]	3.7 [3.3–4.1]
Gill	14.8 [13.5–16.0]	5.4 [4.5–6.2]	69.4 [63.5–75.2]
Liver	0	0	0

*Assuming a standard 500-g animal (see Table 2).
†Values are averages for duplicate experiments; ranges of values are shown in brackets.

TABLE 9

RELATIVE HOURLY RATES OF $^{14}CO_2$ OR ^{14}C–GLYCOGEN PRODUCTION FROM PROLINE AND GLUTAMATE BY TOTAL MANTLE (30 g) COMPARED TO OTHER TISSUES*

	SUBSTRATE SOURCE	
	PROLINE	GLUTAMATE
Ratio of CO_2 produced		
Mantle/Kidney	3.0	1.7
Mantle/Gill	6.4	0.9
Mantle/Liver	7.6	1.1
Mantle/Branchial heart	42.3	18.9
Mantle/Systemic heart	38.9	26.3
Ratio of glycogen formed		
Mantle/Kidney	1.3	0.7
Mantle/Gill	3.2	0.9
Mantle/Liver	Not measurable	5.4
Mantle/Branchial heart	15.5	10.2
Mantle/Systemic heart	60.8	18.0

*Assuming a standard 500-g animal (see Table 2).

sented, a common problem in isotope studies even with better-developed animal models (Katz et al. 1981), the experimental series are all internally consistent and help clarify the overall metabolic organization of these animals. The picture that is emerging from these and other parallel studies on cephalopods emphasizes potentially important connections and interactions among glucose, arginine, and proline metabolism. These occur during work as well as during recovery. The closest link is between glucose and arginine metabolism; during anaerobic work, the pathways of glucose and arginine phosphate mobilization are linked at the level of octopine dehydrogenase which serves as the terminal glycolytic dehydrogenase in cephalopods (Fields, Baldwin, and Hochachka 1976; Gäde 1980; Hochachka, Hartline, and Fields 1976). During aerobic work, glucose metabolism may be similarly but more loosely coupled to that of proline; glucose is converted to pyruvate which is oxidized in the Krebs cycle, and concomitantly proline augments the cycle intermediates, particularly oxaloacetate (Mommsen and Hochachka 1981). Because of this coupling, variable energetic demands on the muscle may result in depletion of glycogen plus arginine or of glycogen plus proline. Processes must therefore be provided for the replenishment of glycogen, proline, and arginine stores during recovery from exhaustive exercise.

Interestingly, as in their catabolism, the repletion of all three substrates may be related. That is why this study takes on especial significance; even if it does not complete the description of potential replenishment mechanisms, it clearly raises the possibility of close

TABLE 10

ACTIVITY OF GLUTAMATE DEHYDROGENASE (GDH) AND FRUCTOSE-1, 6-BISPHOSPHATASE (FBPASE) IN CEPHALOPOD TISSUES

SPECIES/TISSUE	GDH (μmole/min/g wet wt tissue)*	FBPase (μmole/min/g wet wt tissue)*
Octopus macropus		
Liver	2.3 (2) [2.0–2.6]	0.2 (1)
Kidney	62.7 (3) [17.3–112]	0.6 (1)
Gill	2.4 (1)	0.4 (1)
Mantle	2.1 (1)	1.1 (1)
Sepioteuthis lessonia		
Liver	14.9 (3) [6.2–22.7]	0.16 (3) [0.10–0.24]
Gill	4.1 (3) [2.4–7.5]	0.13 (3) [0.08–0.16]
Mantle	11.8 (3) [10.1–14.1]	0.33 (3) [0.31–0.38]
Nautilus		
Liver	—	0.37 (3) [0.24–0.47]
Gill	0.36 (2) [0.34–0.38]	0.39 (2) [0.35–0.43]
Kidney	0.8 (2) [0.8–0.83]	0.92 (2) [0.89–0.95]
Pericardial gland	0.76 (1)	0.70 (1)

*Number of enzyme preparations assayed is given in parentheses; ranges of values are given in brackets.

interactions, not only in catabolism, but also in the metabolism of proline, arginine, and glucose during recovery. The interactions between proline and arginine are based on their potential interconversions via the common intermediates, glutamate and ornithine (Adams and Frank 1980, Mommsen and Hochachka 1981). In principle, the depletion of one could be buffered by the other. Another important interaction may occur between the metabolism of these two amino acids and glycogenesis, since glutamate, proline, and probably arginine all can be used directly as gluconeogenic precursors. Thus, a great deal of versatility seems to be incorporated into the metabolic organization of these organisms.

As balanced a summary as we can currently generate is presented schematically in Figure 2. Such metabolic maps, as summaries of current understanding, are useful mainly in the questions they raise and the experiments they suggest. Three obvious problems for future work are suggested by Figure 2. First, we need to assess the partitioning of proline between deposition in alanine and complete oxidation in the Krebs cycle. Current estimates of the activity of malic enzyme imply that it is capable of pacing the Krebs cycle (Mommsen and Hochachka 1981), but this should be analyzed in greater detail. Second, a quantitative analysis is needed of the exchange of metabolites such as alanine and octopine between working muscles and blood. At this writing, literally no reliable information exists on this matter. In principle, the kinds of recovery processes suggested could occur in muscle, where actual replenishment of glycogen, proline, and phosphagen is ultimately required. This is a probable fate of octopine during recovery (Vollmer, Hochachka, and Mommsen 1981), but a simple reversal of the proline → alanine conversion is considered unlikely because of irreversible steps in the process (e.g., 2-ketoglutarate dehydrogenase). Thus, as in insects (Weeda et al. 1980), the replenishment of proline may require intertissue metabolic cooperation (Figure 2). Finally, it is important to assess the kinetics of replenishment of glycogen, proline, and arginine. As shown in Figure 2, the replenishment of these three substrate sources should display similar time constraints. However, the interactions between the various metabolic processes involved (Figure 2*B*) need not be stoichiometric and may be modified depending upon metabolite levels, energy status, and so forth. Thus, their kinetic properties need to be evaluated under different metabolic conditions, such as at rest, during work, and during recovery.

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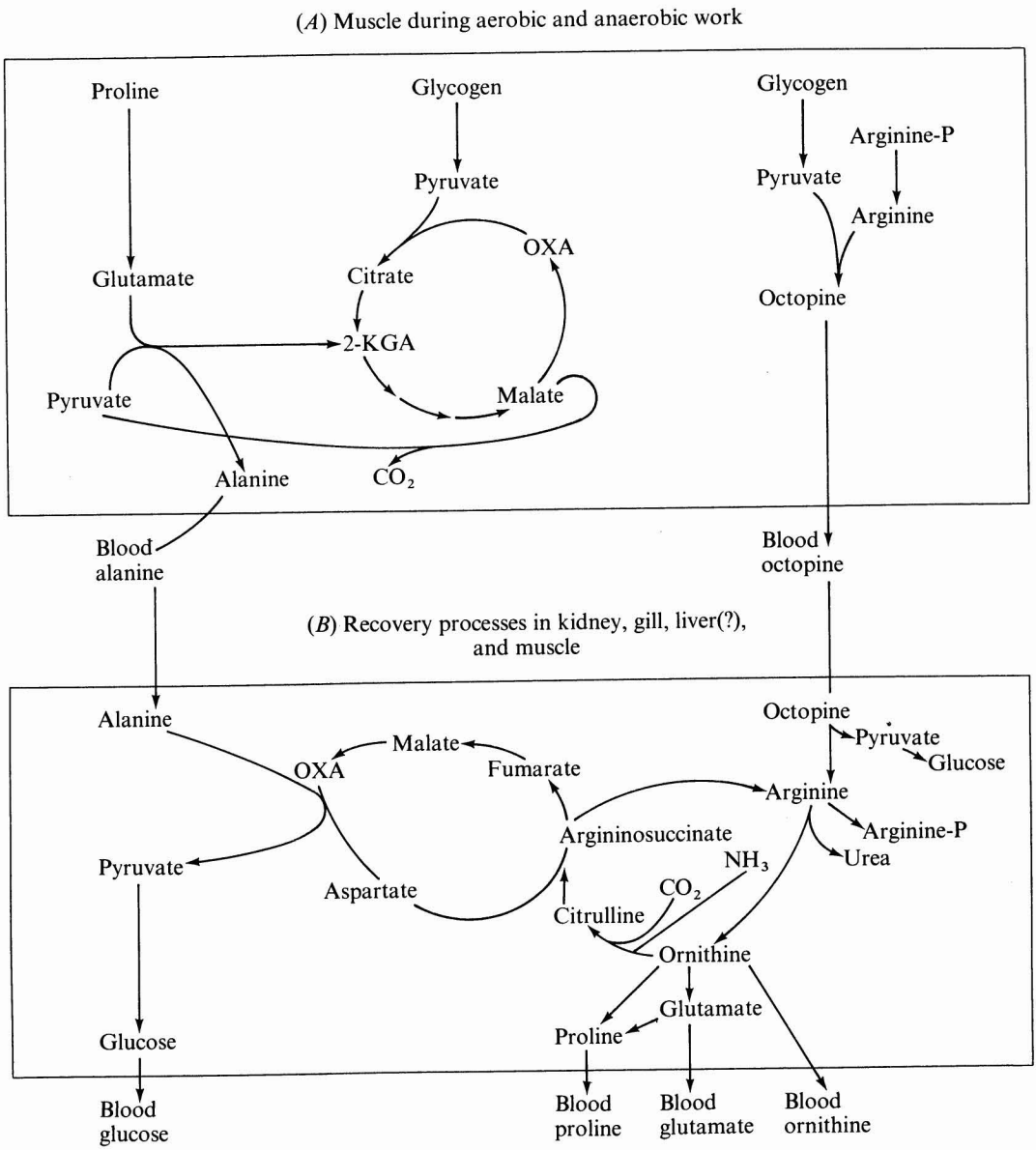

FIGURE 2. Summary of probable metabolic events in muscle: *A*, during exhaustive aerobic plus anaerobic work; *B*, during recovery. All recovery processes may not occur in any one tissue. See Hochachka et al. (1982) and Mommsen and Hochachka (1981) for comparable data for other species (*Sepia* and *Illex*).

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