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Glucose and Proline Metabolism in *Nautilus*[1]

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ABSTRACT: The rates of incorporation of [U-^{14}C]proline and [U-^{14}C]glucose into CO_2 and glycogen were assessed in *Nautilus pompilius* under both in vitro and in vivo conditions. Both substrates exhibited tissue-specific rates of metabolism. However, overall higher rates of incorporation into CO_2 and glycogen were observed with glucose, both with tissue slices and in the intact, catheterized organism.

PROLINE IS AN ABUNDANT COMPONENT of the free amino acid pool in cephalopod tissues and is considered to contribute to aerobic metabolism (Mommsen and Hochachka 1981, Storey and Storey 1978). At the same time, high activities of glycolytic enzymes and ample supplies of glycogen (Ballantyne, Hochachka, and Mommsen 1981; Hochachka and Fields, this issue) also imply an important role for carbohydrate in cephalopod intermediary metabolism. However, there is little information on the relative contributions of proline and glucose to different metabolic processes. In preliminary experiments with squid and octopus, Hochachka and Fields (this issue and unpublished data) found higher rates of proline oxidation than of glucose oxidation. The data suggest higher proline oxidation rates in fast-swimming squid than in the more sluggish octopus. Since the whole-organism metabolic rate of *Nautilus* is lower than that of *Octopus macropus* (Johansen, personal communication), we were interested in comparing its relative rates of glucose and proline incorporation into CO_2 and glycogen. We found that in *Nautilus*, glucose is far more vigorously oxidized than is proline in all tissues analyzed. Not surprisingly, glucose is also incorporated into tissue glycogen at much higher rates than is proline.

MATERIALS AND METHODS

Experimental Animals

All experimental *Nautilus* were obtained from local fishermen working out of Bindoy in Tanon Strait, Negros Orientale, Republic of the Philippines, in October 1979.

In Vitro Experiments

Tissue slices were prepared as described by Hochachka and Fields (this issue), and, in general, all other manipulations were also essentially identical. An important difference, however, was in the incubation medium used. In the first study, *Nautilus* blood was used directly as an incubation medium. *Nautilus* blood contains a mixture of amino acids, glucose, and octopine (Hochachka, French, and Meredith 1978; Storey et al. 1979), presumably in addition to other unidentified metabolites. A total of 5 ml of blood was added to experimental flasks containing 2 μCi of either [U-^{14}C]proline or [U-^{14}C]glucose. Although the blood levels of proline and glucose are similar (about 0.5–1.0 μmole/ml; Hochachka et al. 1978, Storey et al. 1979), the tissue levels of proline can be assumed to be much higher, particularly in muscle. Since the

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tissue levels were not ascertained in these experiments, the exact specific activities of proline and glucose were not known. Thus, the rates of metabolism were only qualitatively comparable.

In a second set of experiments, the same procedure was used but the blood was first vacuum dialyzed.

In Vivo Experiments

In four separate *Nautilus* preparations, the vena cava was catheterized using procedures described elsewhere (Johansen, Redmond, and Bourne 1978). In two preparations, 10 μCi of [U-^{14}C]glucose were taken up in 3 ml of seawater and injected into the blood, followed by a 1-ml washout injection of seawater. In the other two preparations, 10 μCi of [U-^{14}C]proline were injected in similar fashion. Small-volume (0.1–0.2 ml) blood samples were drawn at 5, 10, 30, 60, 90, and 120 min following initiation of the experiment. The $^{14}CO_2$ was driven off with perchloric acid, collected in KOH, and counted in Aquasol. Similarly, tissue glycogen was extracted, purified, and counted, in both cases using procedures described by Hochachka and Fields (this issue). The $^{14}CO_2$ yields in the blood showed tremendous variation between individuals. For example, one *Nautilus* retained up to 3000 disintegrations per minute (DPM) per ml blood at about an hour after injection of ^{14}C-proline, while another showed between 100 and 200 DPM for the duration of the experiment, although in both cases the time for 50% removal of radioactive metabolites from blood was about 15 min. In the glucose experiments, the yields of $^{14}CO_2$ in the blood were not so variable but showed drastic differences in time course. Since, under these conditions, unknown and variable amounts of $^{14}CO_2$ were also lost across the gill epithelium, this variability in the in vivo experiment was not surprising. However, it did preclude direct comparison of the in vivo oxidation rates of proline and glucose using these procedures. At the completion of these experiments (2 hr after injecting labeled substrates), the animals were gradually chilled in ice-cold seawater for another 2 hr. The animals were quiescent through this manipulation and were in effect cold anesthetized when sacrificed. The tissues were quickly removed, glycogen was extracted, and ^{14}C-glucose incorporation was compared to ^{14}C-proline incorporation as described by Hochachka and Fields (this issue).

RESULTS

Proline and Glucose Oxidation Rates

Two interesting implications arise from assessing rates of CO_2 release from proline and glucose. First, as indicated in Tables 1 and 2, rates of oxidation of glucose by all tissues examined greatly exceed rates of proline oxidation. Although large differences in intracellular pool sizes of proline and carbohydrate may contribute to the different rates of ^{14}C-proline and ^{14}C-glucose incorporation into $^{14}CO_2$, the overall pattern stands in striking contrast to that observed for *Octopus macropus* kidney and gill (Hochachka and Field, this issue) and squid kidney, heart, and inner mantle (Mommsen and Hochachka 1981; Mommsen et al., this issue), where ^{14}C-proline oxidation rates exceed those of glucose.

Second, despite the general predominance of glucose metabolism in essentially all tissues examined, some clear-cut tissue specificities emerge. Heart oxidation rates of proline are higher than in any other tissue (particularly when dialyzed blood is utilized as the incubation medium); at the same time, glucose oxidation rates by the heart are relatively low, lower than for kidney, pericardial gland, or gill (Tables 1, 2). In other cephalopods examined, a similar relatively high capacity for proline metabolism has been observed (Mommsen et al., this issue).

Proline and Glucose Incorporation into Glycogen

As in our parallel studies of glutamate and proline incorporation into glycogen in *Octopus macropus*, our experiments with *Nautilus* establish the capacity for this process in essentially all tissues studied (Tables 1, 2).

TABLE 1

[U-^{14}C]PROLINE AND [U-^{14}C]GLUCOSE INCORPORATION INTO CO_2 AND GLYCOGEN IN *Nautilus* TISSUES

SUBSTRATE	TISSUE	$^{14}CO_2$ (DPM/g tissue)	^{14}C-GLYCOGEN (DPM/μmole glucose)	^{14}C-GLYCOGEN (DPM/g tissue)
^{14}C-Proline	Kidney	225	57	2,390
	Pericardial gland	182	75	1,166
	Gill	669	480	2,543
	Systemic heart	946	72	2,868
	Retractor muscle	263	25	621
	Liver	—	—	—
^{14}C-Glucose	Kidney	14,677	6,332	223,513
	Pericardial gland	10,456	5,696	73,484
	Gill	48,695	48,007	326,446
	Systemic heart	7,220	3,560	192,231
	Retractor muscle	2,503	483	12,037
	Liver	4,780	491	4,176

NOTE: Undialyzed blood was used as the incubation medium. Temperature, 22°C.

TABLE 2

[U-^{14}C]PROLINE AND [U-^{14}C]GLUCOSE INCORPORATION INTO CO_2 AND GLYCOGEN IN *Nautilus* TISSUES

SUBSTRATE	TISSUE	$^{14}CO_2$ (DPM/g tissue)	^{14}C-GLYCOGEN (DPM/μmole glucose)	^{14}C-GLYCOGEN (DPM/g tissue)
^{14}C-Proline	Kidney	284	32.9	2,723
	Pericardial gland	204	70.3	3,481
	Gill	808	270.3	2,731
	Systemic heart	2,162	27.1	2,515
	Retractor muscle	272	29	2,413
	Liver	—	34.6	1,313
^{14}C-Glucose	Kidney	15,003	4,826	459,946
	Pericardial gland	11,113	1,365	68,951
	Gill	29,376	17,920	206,081
	Systemic heart	8,259	3,182	320,129
	Retractor muscle	5,738	813	83,607
	Liver	2,979	1,463	48,584

NOTE: Dialyzed blood was used as the incubation medium. Temperature, 22°C.

However, the marked tissue specificity seen in *Octopus macropus* (Hochachka and Fields, this issue) is not so strikingly displayed. Also, the actual incorporation rates are low in comparison with *Octopus* (Tables 1, 2), whether the incorporation is expressed in terms of ^{14}C incorporated per micromole glucosyl glycogen or in terms of ^{14}C-glycogen formed per gram of tissue.

In contrast, very high rates of incorporation of ^{14}C-glucose into glycogen typifies most of the tissues analyzed. Lowest rates occur in liver slices; highest rates, in kidney, gill, and systemic heart (Tables 1, 2).

In Vivo Proline and Glucose Incorporation into Glycogen

Because of large percentage differences in the amounts of glycogen present in different individuals, pooling of data from different specimens seemed to cover up more information than it summarized; therefore, the incorporation rates are given for each *Nautilus*

TABLE 3

IN VIVO [U-^{14}C]PROLINE INCORPORATION INTO GLYCOGEN IN TWO *Nautilus* FEMALES

TISSUE	GLYCOGEN LEVEL (μmole glucose/g)	^{14}C-PROLINE INCORPORATION INTO GLYCOGEN/4 hr	
		(DPM/μmole glucose)	(DPM/g tissue)
Nautilus #1 (679 g total weight)			
Kidney	13.5	31.8	429.3
Pericardial gland	—	—	—
Gill	5.9	27.9	164.1
Systemic heart	23.0	22.3	512.9
Retractor muscle	8.6	45.7	391.2
Liver	3.0	17.0	50.7
Nautilus #2 (789 g total weight)			
Kidney	43.1	75.4	3,248
Pericardial gland	32.6	20.4	665
Gill	12.7	83	1,054
Systemic heart	61.9	28	1,733
Retractor muscle	89.1	45.7	476
Liver	48.1	8.2	394

NOTE: Tissue levels of glycogen varied extensively, so the data are not pooled.

TABLE 4

^{14}C-GLUCOSE INCORPORATION INTO GLYCOGEN IN TWO *Nautilus* FEMALES

TISSUE	GLYCOGEN LEVEL (μmole glucose/g)	^{14}C-GLUCOSE INCORPORATION INTO GLYCOGEN/4 hr	
		(DPM/μmole glucose)	(DPM/g tissue)
Nautilus #1 (678 g total weight)			
Kidney	26.3	881	23,176
Pericardial gland	4.0	13,593	54,099
Gill	1.7	120,241	199,600
Systemic heart	—	—	—
Retractor muscle	3.3	4,386	14,431
Liver	1.4	4,523	6,288
Nautilus #2 (667 g total weight)			
Kidney	28.7	494	14,187
Pericardial gland	30.2	2,274	68,684
Gill	0.7	352	254
Systemic heart	44.2	2,355	104,072
Retractor muscle	5.7	2,459	14,040
Liver	3.4	1,014	3,396

separately (Tables 3, 4). As expected from the in vitro experiments, in vivo incorporation rates of ^{14}C-glucose into tissue glycogen pools greatly exceed the rates of ^{14}C-proline incorporation. For proline, highest rates of incorporation appear in kidney, gill, and systemic heart (Table 3). In comparison, highest rates of glucose incorporation appear in pericardial gland, kidney, systemic heart, and retractor muscle; the results for the gill are equivocal since very high rates of ^{14}C-glucose incorporation into glycogen occurred in one individual, but very low rates in the other (Table 4). Although the basis for this discrepancy is

not understood, it may be worth emphasizing that the level of endogenous glycogen in the gill varied in the two cases.

CONCLUSIONS

Even if these experiments must be considered as preliminary, they are instructive in establishing a rather marked difference in the rates of utilization of proline and glucose both in oxidation and in replenishing glycogen depots in *Nautilus* tissues. In contrast to the situation in the octopus, glucose appears to be a particularly useful substrate for both these metabolic functions. In view of these results, it would be surprising if glucose were not the main carrier of carbohydrate carbon in blood and the primary immediate precursor of glycogen in most tissues of the *Nautilus* body. From the in vivo experiments, a high clearance rate for both substrates implies the existence of an effective circulation and mechanisms for the rapid clearance of metabolites such as glucose and amino acids. However, the kinetics of exchange between tissues and blood have not been clarified, and this remains a subject for further research into metabolic biochemistry not only of *Nautilus* but of other cephalopods.

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