Carbohydrate Metabolism in Fish—II. Effect of Dietary Composition on Metabolism of Glucose-6-^{14}C in Carp

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In order to confirm that carbohydrate would not be a primary requirement for energy source in carp, from a view point of intermediate metabolism of glucose, incorporation of glucose-6-^{14}C into glycogen and blood glucose, its excretion to $^{14}CO_2$ and randomization of ^{14}C in glucose molecule in carp which were fed the various diets consisting of carbohydrate and protein were investigated.

Glucose-6-^{14}C was scarcely incorporated into glycogen in all groups but oxidized to $^{14}CO_2$. When more than 50% of protein was contained in diet, oxidation of glucose-6-^{14}C decreased remarkably, and at the same time blood glucose pool and the extent of randomization of ^{14}C in glucose were increased.

From these observations it was suggested that carp possesses the active and reversible Embden-Meyerhof pathway but that glycogen is not a principal storage depot of energy.

In the previous paper[1] it was suggested that metabolic correlations among carbohydrate, protein and lipid in carp are substantially different from those already established in mammals, and that carbohydrate is not a primary requirement for energy supply in carp.

Though there have been few detailed studies on individual enzymes involved in glucose metabolism in fish, some studies on the multienzyme systems of glucose metabolism have been reported,[2–7] and the Embden-Meyerhof pathway, the pentose cycle and the TCA cycle in fish appear to be nearly identical with those in mammals. However, the extent of contribution of glucose metabolism to total energy requirement and the detailed features in glucose metablism of fish are still now obscure.

In the present paper, the fate of glucose in carp was investigated with glucose-6-^{14}C as a tracer in order to obtain additional evidences on the energy metabolism of carp reported in the previous paper.[1]

Materials and Methods

Materials and the methods of breeding. Materials and the methods of breeding in these experiments are identical with those described in the previous paper.[1] After being fed diet C for 10 days at least, carp, *Cyprinus carpio*, weighing 15–25 g were satisfactorily fed the respective diets (HC, 90% carbohydrate; C, 50% carbohydrate; LC, 10% carbohydrate)[1] during a definite period in the morning and evening twice a day, and used for analyses.

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Assays of radioactivities. After being fed diets HC, C and LC respectively for 76 days, each group of carp was employed for radioactivity analyses. two hours after feeding, 0.2 ml of 1 μc: of glucose-6-^{14}C in 1 mg of carrier glucose dissolved in 0.75 % NaCl solution was intraperitoneally injected and one carp was placed in a stoppered vessel (2l flask) according to BROWN[4] with slight modifications. After carp was kept at 20°C for 6 hours in flask (fasted during this period), incorporation of radioactivity from glucose-6-^{14}C into glycogen, respiratory rates to $^{14}CO_2$ and the residual activities in blood glucose were measured. By introducing CO_2-free air into the flask containing carp for aeration, respirated $^{14}CO_2$ was recovered at hourly intervals by passing through an alkaline trapping solution (absolute ethanolamine) and the radioactivity of $^{14}CO_2$ was counted by liquid scintillation counter (Aloka, Model LSC–502). All other radioactivities of ^{14}C in glycogen and blood glucose were counted by gas-flow counter (Aloka, Model JDC–104).

Isolations of ^{14}C-glycogen. Isolation of ^{14}C-glycogen in hepatopancreas was made by digestion of 0.2–0.3 g of the tissue with strong alkali (60 % NaOH) heating.[8] Isolated ^{14}C-glycogen was completely purified in turn by 95 % ethanol and ether, and the radioactivity of ^{14}C-glycogen was measured by gas-flow counter with a correction curve for $BaCO_3$.[9] Determination of glycogen was made according to the method of MONTGOMERY.[10]

Hydrolysis of glycogen. Isolated glycogen was dissolved to a certain concentration (10–15 mg per 3 ml). To 3 ml of the solution, 1 ml of 3 N HCl was added and the mixture heated 2 hours in boiling water bath (capped to avoid evaporation). After neutralization with an equivalent volume of 0.75 N NaOH solution, liberated glucose was determined by glucose oxidase in order to confirm completeness of the hydrolysis.

Degradation of glucose—especially about isolation of carbon-6. By measuring randomizations of ^{14}C in glycogen glucose and blood glucose, degradations of glucose and its resynthesis from its smaller fragments could be estimated to some extent,[11,12] which might be regarded as an aspect of glucose utilization. Though both glucose-1-^{14}C and glucose-6-^{14}C which form lactate-3-^{14}C as a common intermediate product of their catabolism are available as the precursors of glucose resynthesis,[13] in this experiment glucose-6-^{14}C was used in order to avoid effect of decarboxylation in carbon-1 by the hexose-monophosphate shunt on $^{14}CO_2$ from the TCA cycle.[14]

The radioactivities of labeled carbon-6 of blood glucose and glycogen glucose were counted as formaldemethone according to DUNN *et al.*[15] which is precipitated from formaldehyde with dimedon. Formaldemethone was stoichiometrically obtained from glucose and the radioactivity was counted with a correction curve for $BaCO_3$.[9] The blood samples were deproteinized with Ba–Zn by the method of SOMOGYI-NELSON.[16] The resulting protein-free filtrate was applied on the mixed column of Amberlite IR–120(H^+) and IRA–400(OH^-) to deionize. Glucose in aliquot of the resulting eluate was determined by glucose oxidase and the remainder was used to isolate carbon-6 as formaldemethone

as described above.

Results and Discussion

Respiratory rate of glucose-6-^{14}C to $^{14}CO_2$. As shown in Fig. 1, the respiratory rate in group HC was overwhelmingly higher than those in the other two groups. During the experimental period, difference of respiratory rate of $^{14}CO_2$ between group C and LC was found slight. Total yield of $^{14}CO_2$ activity in 6 hours in group HC was about 31% and in both groups C and LC they were 10% respectively.

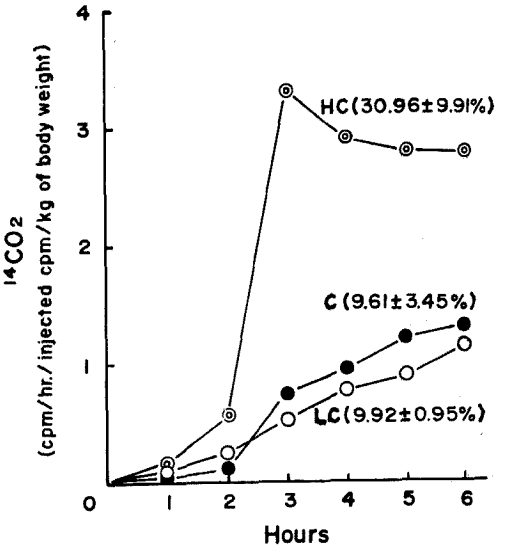

Fig. 1. Effect of diet composition on respiratory rate of glucose-6-^{14}C to $^{14}CO_2$ of carp bred for 76 days. Each curve represents the average of assays on carp. Values in parentheses are total yields in 6 hours.

Incorporation of glucose-6-^{14}C into glycogen. As shown in Table 1, the activities of ^{14}C incorporated into glycogen in 6 hours were found very slight. It is demonstrated here, as pointed out in the previous paper,[1] that the interconversion rate between glucose and glycogen is very low and little glucose is incorporated into glycogen. The least inflow of ^{14}C in group HC might be due to glucose being actively oxidized to $^{14}CO_2$ by adaptation of metabolism because of insufficient supply of protein in addition to an inferior capacity of the conversion from glucose to glycogen. Then, in group C furnished an appropriate quantity of protein, a little more glucose might be incorporated into glycogen. Less incorporation of ^{14}C into glycogen in group LC would be due to glucose being utilized to

Table 1. Effect of diet composition on incorporation of the injected glucose-6-^{14}C into glycogen and distribution of ^{14}C in glycogen glucose of carp bred for 76 days.

Diet	Glycogen content (%)	^{14}C incorporated (%)	Distribution of ^{14}C (%)	
			C-1 to 5	C-6
HC	10.56±1.546	0.29±0.29	33±11.5	67±11.5
C	9.14±0.976	1.11±1.03	37±12.6	63±12.6
LC	8.28±2.186	0.38±0.25	26±21.5	74±21.5

Each carp was given an intraperitoneal injection of 0.2 ml of 1 μc of glucose-6-^{14}C in 1 mg of carrier glucose dissolved in 0.75% NaCl solution. All carp were killed for assay 6 hours after the injection. Each value is the average±the standard deviation of assays on five fishes.

maintain anaerobic glycolysis (the Embden-Meyerhof pathway) rather than accumulated as glycogen which is expected to be active by the fact that oxidations of glucose-6-^{14}C to $^{14}CO_2$ by groups C and LC were held to the same extent.

Distribution of ^{14}C in glycogen glucose. As shown in Table 1, randomization of ^{14}C in glucose molecule of glycogen, showing little differences among the groups was still highest in group C. But distribution ratio of ^{14}C into carbon-1 to 5 was high, reflecting an active degradation of blood glucose to 3 carbon breakdown products and "resynthesis" of glucose from them.

Distribution of ^{14}C in blood glucose. As for randomization of ^{14}C into carbon-1 to 5 in blood glucose, group HC showed the lowest ratio while groups C and LC showed slightly higher, although little difference being found between the latter two (Table 2). More extent in degradation of blood glucose than of glycogen glucose may reveal that much more glucose is rapidly metabolized via blood glucose than is incorporated into glycogen. Since all these distribution ratios are very high, it is reasonably estimated that degradation and resynthesis of blood glucose molecule by the Embden-Meyerhof pathway and/or gluconeogenesis action is very active.

Table 2. Effect of diet composition on distribution of ^{14}C in blood glucose of carp bred for 76 days.

Diet	mg/100 ml*	cpm/mg glucose**	Distribution of ^{14}C (%)	
			C-1 to 5	C-6
HC	57±14	15196± 5615	55± 3.3	45± 3.3
C	90±15	43840±12188	65±11.2	35±11.2
LC	98±44	18346± 4041	66±10.6	34±10.6

* Determined by the method of SOMOGYI-NELSON.[16]
** Determined by glucose oxidase.

Injection of 1 mg of carrier glucose (although this quantity had been expected hardly to affect blood glucose level) gave high levels of blood glucose in groups C and LC after 6 hours (Table 2). Blood glucose level in group HC, however, showed normal level[1] after 6 hours, suggesting that oxidation mechanism of glucose in group HC was more operative. Therefore, the lower specific radioactivity in blood glucose as well as the lowest randomization of ^{14}C in group HC would be due to a reduced gluconeogenesis action and an increased oxidation to $^{14}CO_2$ (Fig. 1). These results would suggest that glucose tolerance capacity of hepatopancreas in group HC was increased whereas the capacities of groups C and LC were low or fell down. Augmented pool of blood glucose in groups C and LC would be resulted from the reduced capacity of glucose tolerance and the decreased utilization of glucose.

That little glucose-6-^{14}C is, as a whole, incorporated into glycogen and the extent

of randomization in blood glucose is astonishingly high would characterize glucose metabolism of carp in energy utilization. Thus, the reversible reaction of anaerobic glycolysis resulting in randomization of carbon atoms in blood glucose is assumed to be maintained active and this gluconeogenesis action seems to be inactivated slightly in group HC, and so the oxidative capacity of glucose in carp would be considered to be decided by inflowing quantity of glucose through the Embden-Meyerhof pathway to the TCA cycle. Then the inflow of glucose in groups C and LC which took more than 50% protein in diet might be to be limited to the same extent, probably resulting in an augmented pool of blood glucose.

When, as described above and pointed out in the previous paper[1], it is considered that carbohydrate is not a primary requirement but that the adequate quantity of carbohydrate must be taken, an active system consisting of hexose, hexose-phosphate and triose-phosphate mentioned here should be present in carp, which is supposed to make a certain contribution to carp's energy utilization from protein taken into body.

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