FUTILE CYCLES IN ISOLATED PERFUSED RAT LIVER AND IN ISOLATED RAT LIVER PARENCHYMAL CELLS

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SUMMARY: Isolated livers from fed rats were perfused with a medium containing glucose labeled uniformly with ^{14}C and specifically with ^{3}H. There was considerable formation of glucose from endogenous sources but simultaneously uptake of about half of the ^{14}C in glucose. After 2 hours the $^{3}H/^{14}C$ ratios in perfusate glucose decreased by 55-60% with (2-^{3}H, U-^{14}C), 40-50% with (5-^{3}H, U-^{14}C), 25-30% with (3-^{3}H or 4-^{3}H, U-^{14}C) and by 10-15% with (6-^{3}H, U-^{14}C) glucose. Qualitatively comparable patterns were obtained with rat hepatocytes. These results demonstrate recycling of carbon between glucose and pyruvate. Superimposed upon this there is an extensive futile cycle between glucose and glucose 6-P. There is also futile cycling between fructose 6-P and fructose 1,6 P_2 and to a small extent between phosphoenol pyruvate and pyruvate.

Introduction

Experiments with ^{14}C and ^{3}H labeled glucoses have demonstrated the presence of a futile cycle between glucose and glucose 6-P in rat hepatocytes (1, 2) and have indicated another cycle between fructose 6-P and fructose 1,6 P_2 in both rat hepatocytes (1, 2) and in the *in vivo* rat liver (3). These cycles cause the hydrolysis of ATP and energy dissipation. The occurrence of the fructose 6-P: fructose 1,6 P_2 futile cycle has been questioned by Hue and Hers (4). These authors showed that liver extracts, unsupplemented with ATP and Mg, detritiated (2-^{3}H) and (5-^{3}H) glucose 6-P but not (3-^{3}H) glucose 6-P. In the presence of ATP and Mg however there was loss of tritium from (3-^{3}H) glucose 6-P. These results indicate that tritium can be lost from position 5 of fructose 6-P without the formation of fructose 1,6 P_2 and its cleavage by aldolase. Hue and Hers (4) suggested that glyceraldehyde 3-P is labeled in position 2 by transaldolase exchange between the glyceraldehyde moiety of (5-^{3}H) fructose 6-P (carbons 4,

5 and 6) and glyceraldehyde 3-P. The tritium in triose-P is then exchanged with protons by the action of triose-P isomerase. The loss of ^{3}H by this sequence of reactions is an exchange without energy dissipation. The detritiation of (3-^{3}H) glucose 6-P in the supplemented liver extracts of Hue and Hers (4) cannot be attributed to this exchange reaction. This results indicates the occurrence of the fructose phosphate futile cycle in liver.

In the present paper the occurrence of the fructose 6-P: fructose 1,6 P_2 futile cycle in rat hepatic tissue has been reinvestigated by using (3, 4, 5 and 6-^{3}H, U-^{14}C) glucose.

Methods

Materials: Isotopes were purchased from the Radiochemical Centre, Amersham, England; Mono filament nylon mesh filters were from Tuta Laboratories (Australia) Pty. Limited, Lane Cove, N.S.W., Australia; Dacron filters were a kind gift from Pioneer Filters Inc. Beaverton, Oregon, U.S.A.

Perfusion of Rat Liver. Male rats of the Wistar strain, 140-190g, had free access to a standard diet of rat pellets and water or were starved for 48 hours. Rats were anesthetized with nembutal and the isolated liver perfused essentially by the method of Hems et al. (5). Livers of fed rats were perfused with 40 ml Krebs-Ringer bicarbonate buffer containing 25% washed bovine erythrocytes, 3% bovine serum albumin and 2.8 mM glucose. Livers of fasted rats were perfused with 40 ml whole fresh rat blood. Before use both media were filtered through 4 g of tightly packed Dacron wool (6). The perfusate, before entering and after leaving the liver, was filtered through nylon blood transfusion filters packed with 0.3 g Dacron wool. Samples (0.5 ml) of the perfusion medium were deproteinized with 2.5 ml 95% v/v ethanol and assayed for glucose. The supernatant was passed through three columns: Amberlite CG-120 (H^{+}), Dowex 1 (acetate) and Dowex 1 (borate). The borate column retained glucose which was eluted with 6M formic acid and assayed for ^{14}C and tritium.

Hepatocytes. The preparation of hepatocytes and methods of incubation were as previously described (1).

Results and Discussion

The labeled glucose was added to the perfusion medium 30 minutes prior to the attachment of the liver. Samples taken during this period served as a control for the metabolism of glucose by erythrocytes. There was glycolysis only with fresh rat blood but no significant changes in the perfusate glucose $^{3}H/^{14}C$ ratios. The metabolism of (5-^{3}H, U-^{14}C) glucose by livers from 48 hour fasted rats perfused with fresh rat blood is shown in Figure 1A. Upon the introduction of the liver there was rapid glucose synthesis with its concentration increasing from 3 to 7-11 mM within 40 minutes. The mean maximal

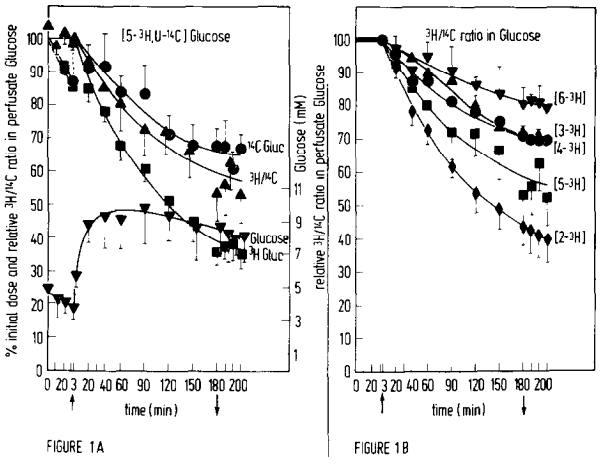

FIGURE 1A FIGURE 1B

Figure 1A. The Metabolism of (5-^{3}H, U-^{14}C) Glucose and Glucose Production in the Livers of Rats Starved for 48 Hours. Figure 1B. The ^{3}H/^{14}C Ratios in Perfusate Glucose During the Metabolism of (2, 3, 4, 5 or 6-^{3}H) Glucose in the Livers of Rats Starved for 48 Hours. Livers were perfused with 40 ml of fresh rat blood containing 20 μCi of (2, 3, 4, 5 or 6-^{3}H) glucose and 10 μCi of (U-^{14}C) glucose. The results with (2-^{3}H, U-^{14}C) and (5-^{3}H, U-^{14}C) glucose are the mean (±SD) for 3 animals. Those with (3-^{3}H, U-^{14}C) and (6-^{3}H, U-^{14}C) glucose are the mean (SD) for 2 animals while the (4-^{3}H, U-^{14}C) glucose perfusion was with 1 animal.

synthesis of glucose in these experiments was 240 μmoles. There was sufficient residual glycogen in the fasted livers to supply at least 50% of this glucose. Net glucose production from this source is unlikely however as the glycogen content appeared to remain constant or increase slightly during these perfusions. About one quarter of the glucose synthesized can be derived from lactate in the perfusion medium and the rest presumably arises from amino acids within the blood and from endogenous sources (5). Although there was synthesis of glucose one third to one half of the ^{14}C in perfusate glucose was utilized. In part this is due to glycolysis by erythrocytes. The nature of the labeled products was not determined in these perfusions. In hepatocytes from fasted rats ^{14}C from glucose was recovered mainly in CO_2, although lactate, amino acids and cellular glycogen and lipids were also labeled (1, 7). The apparent utilization of ^{14}C glucose in the presence of glucose synthesis indicates simultaneous catabolism and synthesis, and recycling of carbon between glucose and pyruvate.

The loss of tritium from glucose was more rapid than that of ^{14}C. The detritiation of (2-^{3}H) glucose was the most rapid and after 3 hours only 22 to 28% of the original activity was present in the circulating glucose. There was a large variability in the rates of glucose synthesis and in ^{14}C glucose catabolism; nevertheless the $^{3}H/^{14}C$ ratios which measure the relative retention of the two tracers in the same preparation were fairly reproducible. The results are shown in Figure 1B. The detritiation from position 2 is more rapid than that from position 5. The initial rate of tritium loss from position 4 appears to be more rapid than that from position 3, but after 2 hours there was no significant difference in these ratios. In the first hour the $^{3}H/^{14}C$ ratio with (6-^{3}H) glucose was not significantly different from that of (3-^{3}H) glucose.

Results with livers of fed rats are shown in Figure 2. The livers were perfused with a buffer containing albumin and bovine erythrocytes (see Methods). In Figure 2A the synthesis of glucose and the uptake of isotope from (3-^{3}H, U-^{14}C) glucose is shown. There was considerable variation between

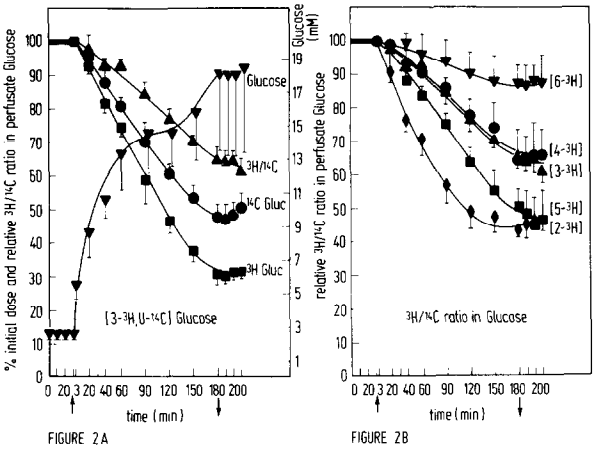

FIGURE 2A FIGURE 2B

Figure 2A. The Metabolism of (2-^{3}H, U-^{14}C) Glucose and Glucose Production in the Livers of Rats Fed Ad Libitum. Figure 2B. The $^{3}H/^{14}C$ Ratios in Perfusate Glucose During the Metabolism of (2, 3, 4, 5 or 6-^{3}H) Glucose in the Livers of Rats Fed Ad Libitum. Livers were perfused with 40 ml of Krebs Ringer bicarbonate buffer containing 25% washed bovine erythrocytes, 3% bovine serum albumin and 2.8 mM glucose labeled with 20 μCi (2, 3, 4, 5 or 6-^{3}H) glucose and 10 μCi (U-^{14}C) glucose. Each point is the mean (±SD) of 4 animals.

Table 1. The Utilization of Labeled Glucose by Hepatocytes from Fasted and Fed Rats.

(18 mg cell protein in experiment 1 and 30 mg in experiment 2) incubated in 2 ml Krebs-Henseleit bicarbonate buffer, 10 mM in glucose, doubly labeled uniformly with ^{14}C and specifically with tritium.

Diet	Incubation Period Hours	Glucose Uptake	Label in Glucose	Isotope Uptake**	Final $^{3}H/^{14}C$ Ratio in Glucose***
		μmoles/100 mg*/hr		% Dose	
Fasted	3	-4.0	U-^{14}C	8.4	-
			1-^{3}H	13	0.97
			2-^{3}H	27	0.78
			3-^{3}H	16	0.96
			4-^{3}H	17	0.95
			5-^{3}H	20	0.93
			6-^{3}H	14	0.97
Fed	2	-7.2	U-^{14}C	19	-
			1-^{3}H	30	0.93
			2-^{3}H	54	0.66
			3-^{3}H	37	0.80
			4-^{3}H	39	0.82
			5-^{3}H	45	0.77
			6-^{3}H	32	0.93

* Milligrams of cell protein

** The isotope uptake from ^{14}C glucose represents the ^{14}C recovered in CO_2, lactate, amino acids and glycogen. Water constituted between 90-95% of the utilized tritium activity from (2, 3, 4 and 5-^{3}H) glucose and from 80-90% from (1 and 6-^{3}H) glucose.

*** The initial ratio is 1.00.

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animals in the amount of glucose synthesized but the average rates of catabolism of ^{14}C glucose were quite similar. The synthesis of glucose was biphasic with rapid synthesis for the first 40 to 60 minutes. The glucose is presumably derived both from glycogen and from lactate plus amino acids. Although the

increase in concentration of glucose in the medium was considerably greater than that with livers from fasted rats, the uptake of ^{14}C was also greater. At the end of the 3 hour period, the tritium content of the medium glucose was much depleted, with (2-^{3}H) and (5-^{3}H) glucose containing only 18 to 22% of the initial activity. The $^{3}H/^{14}C$ ratios in glucose are shown in Figure 2B. The pattern was much the same in the experiments with fasted rats but in this case there is a much greater difference between the ratios obtained with (3-^{3}H, U-^{14}C) and (6-^{3}H, U-^{14}C) glucose.

In Table 1 results obtained with hepatocytes on the detritiation of glucose doubly labeled with ^{14}C and in all six positions with tritium are shown. In these experiments the concentration of glucose was 10 mM and only a small fraction of the activity was utilized. For this reason the decline in the $^{3}H/^{14}C$ ratios was rather small. Still the pattern is much the same as in perfused liver. The detritiation of glucose from position 2 is much greater than that of other positions, and the loss of tritium is in the order: 2> 5> 3 4> 1 ~6.

The occurrence of futile cycles in glucose metabolism and the methods proposed for the quantitation of recycling are discussed in a forthcoming review by Katz and Rognstad (8). There are difficulties in these estimates in ideal systems under steady state conditions. In the present experiments the rate of glucose synthesis was non-uniform and steady state approximations can not be applied. Only qualitative interpretations are possible. Some results however are brought out. 1) The pattern of detritiation in hepatocytes and in the perfused liver are similar. 2) There is recycling of carbon between glucose and pyruvate. 3) Superimposed upon this overall recycling of carbon there is a rapid futile cycle between glucose and glucose 6-P. 4) There is also a futile cycle between fructose 6-P and fructose 1,6 P_2. If the labeling of triose-P were solely through the aldolase cleavage of fructose 1,6 P_2 the detritiation from (3-^{3}H) and (5-^{3}H) glucose should be about equal. Since the loss from position 5 exceeds that from position 3, it is possible that the

excess tritium loss is via transaldolase exchange (4). 5) The utilization of (6-^{3}H) glucose is only slightly greater than that of (U-^{14}C) glucose.

The change in ^{3}H/^{14}C ratios in circulating glucose after intravenous injection has been investigated in vivo in several species. In rat (9) there was no significant difference between the ^{3}H/^{14}C ratios with (3, 5 or 6-^{3}H) glucose but the decay was slower than that with (2-^{3}H) glucose. In rabbits (10) the rate of detritiation of (2-^{3}H) and (5-^{3}H) glucose is nearly the same and more rapid than that of (6-^{3}H) glucose. The loss of tritium from (3-^{3}H) glucose in rabbits was intermediate between that of (5-^{3}H) and (6-^{3}H) glucose. In mice (4) the rate of detritiation decreases in the order (2-^{3}H) > (5-^{3}H) > (3-^{3}H) $\simeq$ (6-^{3}H) glucose (4). Conversely there was little difference in the rates of detritiation of (2, 3 or 6-^{3}H) glucose in sheep (11). In all species by far the greater fraction of the liberated ^{3}HOH is due to extrahepatic glucose catabolism. In rat it is estimated that only 10% of ^{3}HOH formed in the body from (2-^{3}H) glucose is derived by hepatic recycling (9). The fraction of ^{3}HOH formed in liver from the other position is much less. In mice (4) and rabbits (10) hepatic detritiation of (5-^{3}H) glucose, possibly catalyzed by transaldolase and triose-P isomerase, might make a significant contribution to body water. Our results suggest that (6-^{3}H) glucose is the tracer of choice to study the contribution of the Cori cycle to glucose synthesis. It should be pointed out however that all available methods including those employing the randomization of ^{14}C (12) will overestimate to some extent the cycling of carbon between the periphery and liver, since as shown here in perfused liver and in hepatocytes there is hepatic recycling of carbon.

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