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Glucose-2-*t* as a Tracer for Glucose Metabolism*

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ABSTRACT: Glucose labeled both in position 2 with tritium and uniformly with ^{14}C (glucose-2-*t*-U-^{14}C) was injected into rats. The specific activity of blood glucose and formation of labeled water were followed. The half-life of the tritium-labeled glucose was found to be 22 min and that of ^{14}C-labeled glucose, 34 min. Turnover of glucose calculated from tritium data was 3% of the glucose pool/min, as compared to 2%/min from ^{14}C data. The $t/^{14}C$ ratios of liver glycogen isolated 80–120 min after injection were one-third to one-half of the mean $t/^{14}C$ ratio during the experiment. Muscle glycogen contained very little tritium. Most of the tritium of metabolized glucose appeared promptly in water, and less than 5% was incorporated into glycogen and other compounds. Tritium from position 2 is liberated mainly in the isomerization of hexose 6-phosphates. It is concluded that glucose-^{14}C does not provide a correct estimation of glucose turnover, unless corrections for ^{14}C recycling are provided for. The loss of isotope from glucose-2-*t* is irreversible, and it provides a valid estimation of glucose utilization and production *in vivo*.

Glucose labeled with ^{14}C has been used extensively to study glucose metabolism *in vivo*. In the interpretation of tracer kinetics observed after injection of glucose-^{14}C, it has been frequently assumed that glucose newly formed during the experimental period contains no label. This assumption is, on theoretical grounds, questionable. Baker *et al.* (1959) concluded, from analysis of tracer kinetics of blood glucose specific activity, that up to one-half of the ^{14}C of the catabolized glucose is recycled. von Holt *et al.* (1963) and Reichard *et al.* (1963) provided direct evidence in rats and humans injected with glucose-^{14}C for the recycling of label through resynthesis from lactate. Dunn *et al.* (1965, 1966) recently investigated this problem with glucose doubly labeled with ^{14}C and tritium in position 6. They found in the rat the half-life of glucose-6-*t* to be considerably less than that of glucose-6-^{14}C, indicating extensive recycling of the ^{14}C into glucose.

For study of catabolism, an ideally labeled compound is one where the tracer is lost completely and irreversibly at an early stage of metabolism. In the case of glucose-2-*t* the tritium would be lost as water at the hexose-6-P stage. Glucose-2-*t* was synthesized and in the present paper experiments on its metabolism in rats are presented.

Theory

A consideration of hydrogen transfer from position 2 of glucose will be useful in the interpretation of our results. Rose and O'Connell (1961) have established that the isomerization of glucose-6-P to fructose-6-P involves a hydride shift between C-2 of glucose-6-P and C-1 of fructose-6-P, but that the hydrogen exchanges extensively with the protons of the medium. In a single transfer, part of the tritium from position 2 of glucose-6-P would appear in position 1 of fructose-6-P and part in water.

The metabolism of glucose *in vivo* is presented in the model of Figure 1. The scheme distinguishes be-

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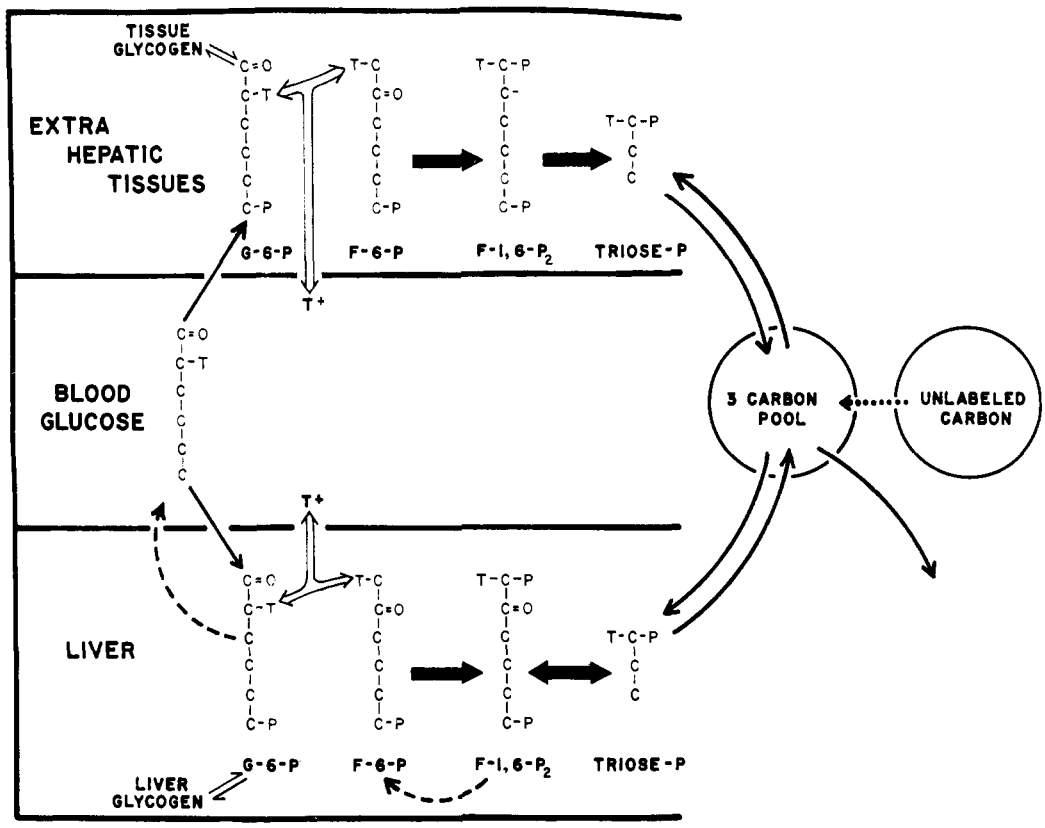

FIGURE 1: Model of metabolism of glucose-U-^{14}C-2-*t*. Double lines, isomerization of hexose 6-phosphates; broken lines, phosphatase reactions; single lines, other reactions; dotted lines, inflow of unlabeled compounds. All carbon atoms are labeled with ^{14}C. In each transfer (in both directions), catalyzed by hexose-6-P isomerase, part of the tritium from position 2 of glucose-6-P and position 1 of fructose-6-P exchanges with protons. G-6-P, glucose 6-phosphate; F-6-P, fructose 6-phosphate; F-1,6-P_2, fructose 1,6-diphosphate.

tween metabolism in liver, where the formation of glucose greatly exceeds its utilization, and extrahepatic tissue, where glucose is solely utilized (neglecting kidneys). At steady state, glucose is released by hepatic glucose-6-P phosphatase at a rate equal to that of the phosphorylation of glucose throughout the body. The glucose in the postabsorptive state is provided from liver glycogen and by resynthesis from three-carbon precursors. When glucose-^{14}C is injected, labeled lactate formed mainly in extrahepatic tissues will be reincorporated into glucose. If glucose from the circulation is utilized by liver, it would also label hepatic hexose-6-P and some of the label will be returned to circulating glucose. Thus, there are two possible sources for the recycling of ^{14}C into resynthesized glucose.

Glucose-6-P and fructose-6-P are reversibly isomerized in the body cells. In each isomerization (in either direction) one-half of the hydrogen in position 2 of aldose and position 1 of ketose will be exchanged with protons. After a single isomerization cycle of glucose to fructose and back, the tritium content at position 2 of glucose will be reduced to 25%. The activity of hexose-6-P isomerase is in general much higher than the rates of other reactions of the hexose phosphates, and the phosphate esters will be turned over several times before leaving the pool. The loss of tritium from the two esters will be different, and retention of *t* in glucose-6-P will be higher than in fructose-6-P. The retention will depend on the relative rates of hexose-6-P isomerase and the inflow and outflow from the hexose phosphate pools. In the limit, for very high isomerization rates, all tritium would be lost from position 2 of glucose-6-P and postion 1 of fructose-6-P.

Definitions. There seems to be some ambiguity in the terminology used in the literature for glucose utilization and turnover. *Glucose production* is designated here as the rate of output of glucose into the blood in the postabsorptive state. It equals the rate of glucose-6-P hydrolysis by phosphatase in liver and kidneys. *Glucose utilization* is the total rate of glucose phosphorylation in all tissues.[1] In the steady state, assumed to prevail in the postabsorptive animal, utilization equals production. Turnover time equals total body glucose divided by the rate of utilization (or production). *Fractional turnover rate* is the reciprocal of turnover time.

[1] Contribution of nonphosphorylating pathways is considered to be negligible. An exception is the synthesis of the glucose moiety of lactose in the lactating animal.

Methods

Glucose-6-P-2-*t* was prepared by incubating fructose-6-P with hexose-6-P isomerase in T_2O. The method in principle is that of Rose and O'Connell (1961) but was adapted to a millimolar scale. Glucose-6-P-2-*t* was hydrolyzed with acid phosphatase. Glucose-2-*t* was mixed with glucose-U-^{14}C to give a $t/^{14}$C ratio of counts of approximately 10:1, and the sugar was chromatographed on paper. The radioactive area was located by radioautography and eluted. The triazole derivative (Hann and Hudson, 1944) prepared from this sugar contained negligible amounts of tritium, indicating that essentially all the isotope was in position 2.

Rats were deprived of food in the early morning 4–5 hr before isotope injection. Procedures for injecting the rats were similar to those described by Depocas and Masuroni (1960). Indwelling cannulae were implanted in the aorta and vena cava of rats according to Popovic and Popovic (1960), permitting injection and blood sampling without anesthesia. About 5 mg of glucose, containing 200–400 μc of tritium and 10–20 μc of ^{14}C, were injected. At the end of the experiment (80–240 min) rats were anesthetized with nembutal and livers and muscle tissue were removed. Liver glycogen was extracted by homogenization in a high speed blender with 5% trichloroacetic acid, and muscle glycogen with boiling 30% KOH. The glycogens were precipitated with alcohol and hydrolyzed to glucose. The $t/^{14}$C ratio of glycogen was not affected by the different extraction procedures.

A 0.1-ml aliquot of plasma was diluted and deproteinized according to Somogyi (1945) in a final volume of 3 ml. A 2-ml aliquot was lyophilized in the apparatus described by Moss (1964) and the distillate collected for T_2O assay. The glucose in the residue was isolated by paper chromatography and eluted. The eluate was assayed for radioactivity and glucose was determined with glucose oxidase. Assay of tritium and ^{14}C was done according to Wu (1964), in a Packard liquid scintillation counter (Model 317 and 4000). Up to 1.5 ml of an aqueous sample may be assayed with 15 ml of Wu's solvent.

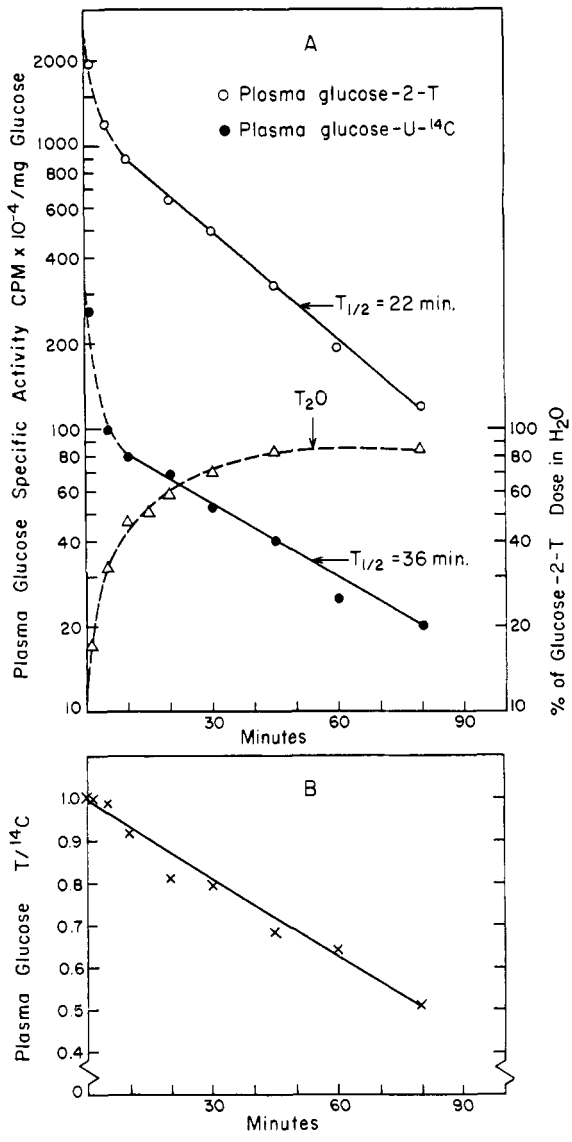

FIGURE 2: Specific activity of plasma glucose after injection of glucose-2-*t*-U-^{14}C (A) and (B) $t/^{14}$C ratio of plasma glucose. (A) Solid lines, specific activities; broken line, total tritium activity in body water (calculated on the basis of distribution in a water space of 75% of body weight).

Results

The semilogarithmic plot of the specific activity of plasma glucose after injection of glucose-U-^{14}C-2-*t* is illustrated in Figure 2. Curves for both isotopes show an initial rapid decline, followed after about 10 min by a linear decay curve. Our data are not sufficient to characterize precisely the rapid component. The isotope decay in this period probably is due mainly to mixing of blood glucose in the body pool and to metabolism. The initial slope of this curve indicates a half-life of approximately 5 min which is similar for both labels.

The slopes of the second component differ for the two types of glucose. The half-life for ^{14}C was 36 min and for tritium, 22 min. In Table I, the half-lives obtained in five experiments are presented. In four of these, the half-lives for the decay of glucose-U-^{14}C ranged from 32 to 36 min, and for glucose-2-*t* from 21 to 25 min. In one experiment a slower decay for ^{14}C- and tritium-labeled sugars (47 and 30 min, respectively) was observed. The $t/^{14}$C ratio of circulating glucose decreased to about half of the initial value 80 min after injection. This is illustrated at the bottom of Figure 2.

In Table I, the turnover rates of glucose, calculated from the decay of glucose-U-^{14}C and -2-*t*, were compared. From the tritium-labeled glucose, fractional turnover rates of about 3%/hr of the glucose pool are

TABLE I: Half-Lives and Turnover Rates of Glucose-2-*t* and Glucose-U-^{14}C in the Rat.

Expt No.	Rat Wt (g)	Half-Time (min)		Fractional Turnover Rate (% of glucose pool/min)	
		t	^{14}C	*t*	^{14}C
1	278	22	35	3.1	2.0
2	292	22	36	3.1	1.9
3	317	25	32	2.8	2.2
4	241	23	33	3.0	2.1
5[a]	271	30	47	2.3	1.5
Av (expt 1–4)	281	23	34	3.0	2.0

[a] Not included in average.

obtained, as compared to 2%/hr from glucose-U-^{14}C.

Appreciable activity was apparent in water 1–2 min after injection. Maximal activity in water was attained in all experiments after 80–100 min, and remained thereafter essentially constant. The concentration of T_2O in plasma depends on the amount of body water and the rate of distribution of water in this pool. To evaluate these parameters, five male rats, weighing from 300 to 400 g, were injected under ether anesthesia with T_2O in the tail vein, and blood was collected from the orbital sinus. The concentration 5 min after injection was 1.5 times the equilibrium values and that at 10 min 1.1–1.2 times equilibrium values. The amount of exchangeable body water ranged from 72 to 79% of body weight.

The fraction of the injected dose in water was calculated, assuming distribution in 75% of body space, and the results are presented in Figure 2. The specific activity of T_2O in plasma or blood will depend on the site of liberation of the T_2O and the rate of equilibration in the water pool. If, as is likely, T_2O is formed in a compartment (such as muscle) equilibrating rapidly with blood water, the initial specific activity of T_2O will be high and will decline with distribution in body water. Thus, in the initial period after injection (0–10 min) the assumption of prompt equilibration of T_2O (as used to calculate, Figure 2) will lead to a large overestimate of glucose breakdown. At 20 min after injection, 60% of the tritium dose was estimated to be in T_2O (Figure 2). This corresponds reasonably with a half-life of 22 min for glucose-2-*t*. In the period of from 20 to 80 min the appearance of *t* in water indicates a half-life of about 20 min. At 80 min, 85% of the dose was calculated to be in T_2O (if 70% water space is assumed, 80%). It is likely that at that time most of the residual activity is in glucose. The fraction of injected tritium estimated to be in liver glycogen at the end of the perfusion ranged from 0.5 to 1% in four experiments and was about 2% in one experiment. Muscle glycogen contained little tritium, and incorporation into this fraction was estimated to be at most 0.5%. Significant incorporation of tritium in other compounds is unlikely.

In Table II, the $t/^{14}$C ratios in liver and muscle glycogen isolated at the end of the experiments are compared with the calculated mean[2] $t/^{14}$C ratio of blood glucose. The $t/^{14}$C ratios in liver glycogen are one-quarter to one-half of the mean plasma glucose values. Most of the $t/^{14}$C ratios in leg muscle and heart glycogen ranged from 10 to 20% of the mean glucose ratio. The $t/^{14}$C ratios in glycogen indicate the extent of tritium labilization relative to carbon in glucose-6-P. From the extensive tritium loss in muscle glycogen it is apparent that the reversible isomerization of the hexose phosphates is much more rapid than the reaction *via* phosphofructokinase. The $t/^{14}$C ratio of fructose-6-P will always be less than that in glucose-6-P. The results indicate that most of the tritium of glucose-2-*t* metabolized in extrahepatic tissues is liberated as water at the hexose phosphate level.

The higher retention of tritium in liver glycogen, and thus hepatic glucose-6-P, reflects the large outflow from this pool *via* phosphatase. This rate must equal that of total glucose utilization in the body (neglecting the contribution to synthesis by kidney). In liver the rate of outflow from glucose-6-P greatly exceeds that from fructose-6-P into fructose 1,6-P_2. Under these conditions the tritium in glucose-6-P would be retained to a greater extent than in extrahepatic tissues. The specific activity of glucose-6-P would differ markedly from that of fructose-6-P, with the $t/^{14}$C ratio in fructose-6-P being less than that in glucose-6-P. The $t/^{14}$C ratio in fructose-6-P cannot be determined from our data.

Discussion

Dunn *et al.* (1965, 1967) reported a half-life of 41 min for glucose-6-^{14}C and 32 min for glucose-6-*t*. Comparison of these results with ours is of interest.

[2] If the specific activities of a compound labeled with two tracers is designated by $f_1(t)$ and $f_2(t)$, the mean ratio of the specific activities in an interval t_1 to t_2 is

$$\frac{\int_{t_1}^{t_2} f_1(t)}{\int_{t_1}^{t_2} f_2(t)}$$

With blood glucose the specific activities are described by a sum of two exponentials of the form $A_{0e}^{-\alpha t} + B_{0e}^{-\beta t}$. When *t* is 5 or more times $1/\alpha$, the integrals from 0 to *t* in the numerator and denominator are of the form $A_0/\alpha + (B_0 - y(t))/\beta$. A_0 and B_0 are the two intercepts at 0 time, α and β the two slopes of the exponential curves, and $y(t)$ the value of the specific activity at time *t*. Note that while the $t/^{14}$C ratio approaches 0 at high values of tritium, the mean ratio is finite and approaches a constant value.

TABLE II: $t/^{14}C$ Ratios in Plasma Glucose and Glycogen after Injection of Glucose-2-*t*-U-^{14}C.[a]

Expt No.	Duration of Expt (min)	$t/^{14}C$ Ratio in Glucose		$t/^{14}C$ in Glycogen from		
		Final	Mean[2]	Liver	Heart	Leg Muscle
1	120	0.31	0.80	0.16	<0.05	0.08
2	240	<0.1	0.71	0.27	0.06	0.15
3	80	0.55	0.81	0.42	0.15	0.13
4	80	0.51	0.81	0.32	<0.05	0.05
5	80	0.49	0.82	0.36	<0.05	0.06

[a] $t/^{14}C$ of injected glucose is taken as 1.0.

The decay curves of blood specific activity of glucose-6-^{14}C and -U-^{14}C are very similar when measured in the same animal (unpublished observations). The longer half-life for glucose-^{14}C reported by Dunn may be due to his use of older rats (390 g *vs.* 280 g in the present work). The turnover of glucose-2-*t* is about 1.5 times, and that of glucose-6-*t*, 1.3 times that of ^{14}C-labeled glucose.

With glucose-6-*t*, Dunn and co-workers (1967) observed complete retention of tritium in muscle glycogen and partial labilization in liver glycogen. Very little tritium from position 2 is retained in muscle glycogen, and losses are also extensive in liver glycogen. As discussed by Dunn (1967) *et al.* the loss of tritium from position 6 occurs mainly in the metabolism of pyruvate, including resynthesis to glucose. Tritium loss from position 2 is predominantly at the hexose-6-P stage. The use of glucose-6-*t* (see Dunn *et al.*, 1967) obviates to a large extent the need for corrections with glucose-^{14}C due to recycling of the label in the Cori cycle, the resynthesis of glucose from lactate. It does not correct for the recycling into circulating glucose of ^{14}C from labeled glycogen. Glucose-2-*t* corrects for both the Cori cycle as well as recycling into circulating glucose of isotope from labeled glycogen. (Some tritium is retained in liver glycogen (Table II), but most of this will be lost in the conversion of glycogen to glucose-6-P, due to the effects of reversible isomerase.)

The turnover of glucose-2-*t* was 1.5 times that of glucose-^{14}C, indicating that one-third of the ^{14}C derived from glucose is recycled. This agrees well with the estimates of Baker *et al.* (1959, 1961), who calculated that from one-third to one-half of the ^{14}C from glucose is recycled. Their analysis is based on a three-compartment model consisting of two glucose pools and one pool of glucose precursors. Such calculations presuppose numerous assumptions, and also require precise determination of slopes and intercepts of the glucose curve at an early period, when decay is very rapid.

Our data with glucose-2-*t* are as yet limited, but they indicate that glucose-2-*t* is an important tool for the study of glucose metabolism *in vivo*. This type of labeling appears to provide the most reliable measure of glucose turnover. Glucose-2-*t* is not yet commercially available, but it can be synthesized cheaply with high specific activities.

Of special value is the use of glucose doubly labeled with tritium and carbon-14. Parallel use of several types of labeling should be of great value in the elucidation of the sources for glucose synthesis such as glycogen, lactate, or other precursors. Such studies are in progress.

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