Estimation of Glucose Turnover and the Cori Cycle Using Glucose-6-*t*-^{14}C*

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ABSTRACT: Glucose-6-*t*-^{14}C was administered to rats and the specific activity and $t/^{14}$C ratio of plasma glucose and tissue glycogens were determined. The decay rates of the tritium and carbon-14 specific activities of plasma glucose both followed first-order kinetics but the decay rate of tritium was faster than that of carbon-14. The half-times of tritiated and ^{14}C glucose were 32 and 41 min, respectively. The fractional turnover rate as measured with tritium was 2.2% of the glucose pool/min in contrast to 1.7%/min for ^{14}C.

The plasma glucose $t/^{14}$C ratio decreased linearly to about 65% of its initial value in 100 min. The $t/^{14}$C ratio of muscle glycogen was the same as the average ratio of circulating glucose, while that of liver glycogen was lower than the ratio of plasma glucose at the time of sacrifice. The rate of tritium loss from plasma glucose approximated the rate of ^{14}C recycling as measured by incorporation of ^{14}C from C-6 into C-1 through C-3 of glucose. It is proposed that loss of tritium occurs in the liver during glucose resynthesis from lactate. The use of glucose-6-*t* in turnover studies corrects for recycling and measures the production of new glucose from three-carbon units. The simultaneous use of glucose-6-*t* and glucose-6-^{14}C provides an estimation of the Cori cycle.

Glucose labeled with carbon-14 has been widely used to study glucose turnover in intact animals. In these studies it was usually assumed that glucose formed during the period of an experiment contained insignificant amounts of recycled carbon-14, although Cori (1931) demonstrated that lactate derived from glucose catabolism in extrahepatic tissues does contribute to hepatic glucose synthesis. Baker *et al.* (1959, 1961) concluded from kinetic analysis of blood glucose specific activity that as much as one-third to one-half of degraded glucose is recycled. von Holt *et al.* (1961) and Reichard *et al.* (1963) directly demonstrated recycling of ^{14}C label following injection of glucose-^{14}C into rats and humans, respectively.

In earlier studies in this laboratory glucose-6-*t* was used to measure glucose turnover in rats (Dunn and Strahs, 1965). In these experiments significantly higher turnover rates were obtained with tritium than had previously been reported for glucose-^{14}C (Depocas and Masuroni, 1960). These differences suggested that the fate of tritium is different from that of carbon. Experiments reported in this paper demonstrate that there is an extensive loss of tritium over ^{14}C in plasma glucose, following administration of glucose-6-*t*-^{14}C to intact rats. This loss can be accounted for by the removal of tritium during glucose resynthesis from three-carbon units; *i.e.*, recycling. It is proposed that use of glucose-6-*t* in turnover studies corrects for recycling and gives an accurate measure of new glucose production. Combined with glucose-^{14}C, it provides a simple means of estimating the Cori cycle.

Materials and Methods

Preparation and Treatment of Animals. Experiments were performed on unanesthetized male Sprague–Dawley rats having indwelling polyethylene cannulae placed into the aorta and vena cava according to Popovic and Popovic (1960). The animals were maintained on Purina Chow before and after surgery and were used only if they returned to or exceeded their presurgical weights. Postabsorptive rats were obtained by removal of food 5 hr before the isotope was administered. Except when stated, all experiments were performed on postabsorptive animals. Fasting conditions were obtained by removing food 24 hr before the start of the experiment.

Experimental Procedure. At 9:00 AM a rat was placed into a metabolic chamber described by Depocas and Masuroni (1960). One hour later, a tracer amount of glucose tagged at position 6 with 150–300 μc of tritium and 15–30 μc of carbon-14 was administered intraarterially. In two experiments similar amounts of glucose-1-*t* and glucose-1-^{14}C were administered instead of the 6-labeled glucose. (Tritiated glucose and glucose-^{14}C were obtained from the New England Nuclear Corp. of Boston, Mass.) Serial samples of blood (0.25 ml) were taken at intervals between 15 and 130 min after isotope administration; plasma glucose concentration and glucose-^{14}C and tritium glucose specific activities were determined. At the end of the experiment, the rat was anesthetized with Nembutal and liver,

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heart, diaphragm, and leg muscle samples were taken for glycogen isolation.

Chemical Analysis and Determination of Radioactivity. The plasma glucose concentration was measured in an aliquot of zinc–barium filtrate using the glucose oxidase procedure modified by Meites and Bohman (1963). Plasma glucose was isolated for radioactive assay as the phenyl-*d*-glucosatriazole derivative using the method of Steele *et al.* (1957). In some experiments plasma glucose was isolated by paper chromatography according to Depocas (1959). The $t/^{14}C$ ratio of chromatographed glucose was identical with that of the triazole derivative, eliminating the possibility of tritium loss from carbons 1–3 during preparation of the triazole derivative. Glucose carbons 1–3 were isolated as 2-phenyl-4-formylosatriazole following periodate oxidation of the glucosatriazole derivative (Hann and Hudson, 1944). Proof of the identily of this aldehyde derivative was indicated by melting point determination and infrared spectroscopy. This aldehyde derivative has colorless crystals and is easily soluble in an ethanol–toluene phosphor system. No activity was found in the aldehyde derivative when it was made from glucose-6-^{14}C. Quantitative recovery of carbon-14 was obtained from glucose-1-^{14}C.

Tissue glycogens were extracted with boiling KOH and precipitated with ethanol (Good *et al.*, 1933). Following acid hydrolysis, glucose determination and triazole formation were performed as above.

Radioactive assay of tritium and carbon-14 was done with a Nuclear-Chicago liquid scintillation spectrometer, Model 720. The triazole was dissolved in a counting vial using 5 ml of borated alcohol (13.8 g of H_3BO_3 in 500 ml of absolute ethanol (Steele *et al.*, 1957)), followed by addition of 10 ml of phosphor solution (300 mg of PPO[1] and 5.0 g of dimethyl-POPOP dissolved in 1 l. of toluene). When paper chromatography was used to isolate glucose, an aliquot of eluate was placed in a counting vial and dried in an air stream. The glucose residue was dissolved in the same solvent system and assayed as above.

Determination of Recycling. The extent of glucose recycling was determined directly by measuring the incorporation of ^{14}C from C-6 to C-1–3. The per cent contribution of this recycling to the glucose pool was calculated according to von Holt *et al.* (1961). From this, F, the fraction of glucose-^{14}C which does not contain recycled isotope, is readily obtained using the expression $F = (^{14}C_T - 2(^{14}C_{1-3}))/^{14}C_T$, where $^{14}C_T$ equals the ^{14}C activity per mole of glucosatriazole (C_{1-6}) and $^{14}C_{1-3}$ is the activity per mole of the 2-phenyl-4-formylosatriazole derivative. The term $^{14}C_{1-3}$ is multiplied by a factor of 2, on the assumption that for each labeled three-carbon fragment incorporated into glucose carbons 1–3 another is incorporated into carbons 4–6.

Glucose recycling was also measured with doubly labeled glucose using $f = 1 - (t_{1/2}/t'_{1/2})$, where f is the fraction of glucose recycled and $t_{1/2}$ and $t'_{1/2}$ are the half-times of glucose-6-t and glucose-6-^{14}C, respectively. The derivation of this equation is given in the appendix.

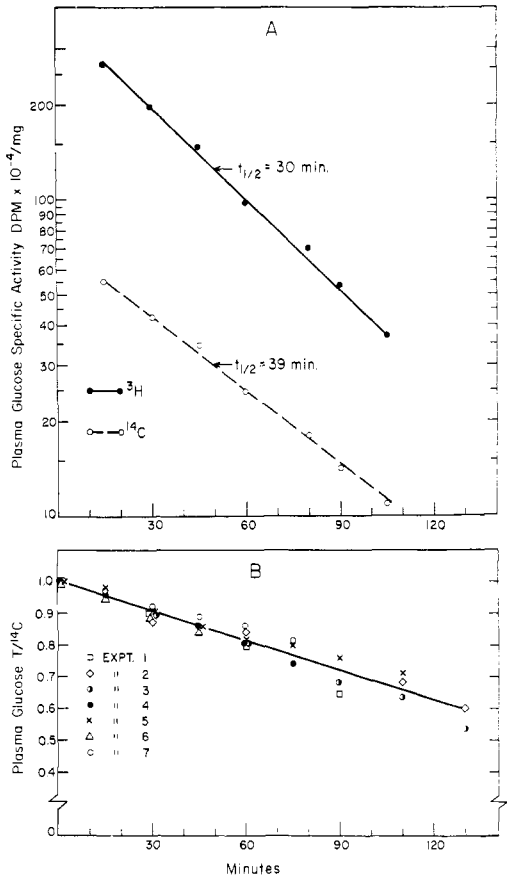

FIGURE 1: (A) Specific activity of plasma glucose following administration of glucose-6-t-^{14}C; (B) $t/^{14}C$ ratios of plasma glucose.

Results

Figure 1A shows the plasma glucose specific activities of a representative experiment following injection of glucose-6-t-^{14}C. The decay of both specific activities plotted logarithmically with respect to time is linear. However, the decay rate of tritium is faster than that of carbon-14; the half-time of glucose-^{14}C is 39 min, while that of tritiated glucose is 30 min. The excess loss of tritium over ^{14}C is reflected in a linear decrease in the $t/^{14}C$ ratio as illustrated in Figure 1B. Within 60 min about 15–20% more tritium is lost than carbon-14 and at 130 min the excess loss is about 40%.

Since the possibility existed that some tritium loss might occur as a result of metabolic activity of blood cells, whole rat blood was incubated *in vitro* at 37° with glucose-6-t-^{14}C. The plasma glucose concentration decreased with time indicating an active glucose metabolism. However, there was no decrease in the $t/^{14}C$

[1] Abbreviations used: PPO, 2,5-diphenyloxazole; POPOP, 1 4-bis[2-(5-phenyloxazolyl)]benzene.

TABLE I: Half-Lives and Turnover Rates of Plasma Glucose-6-*t* and Glucose-6-^{14}C in Rats and the Per Cent Contribution of Recycling to Glucose Production.

Expt No.	Rat Wt	Half-Life (min)		Turnover Rate (% of glucose pool/min)		Calcd Contribution of Recycling[a]
		t	^{14}C	*t*	^{14}C	
1	354	33	43	2.10	1.61	23
2	390	34	42	2.04	1.65	19
3	375	32	40	2.16	1.73	20
4	405	30	40	2.31	1.73	25
5	404	31	39	2.23	1.77	21
6	371	31	42	2.23	1.65	26
Av std dev	386	31.8 ± 1.3	41.0 ± 1.4	2.18 ± 0.008	1.69 ± 0.002	23

[a] The fractional contribution of recycling to glucose production was calculated from the half-times of ^{14}C and tritiated glucose using the equation, $f = 1 - (t_{1/2}/t'_{1/2})$ (t is time), derived in the appendix.

TABLE II: $t/^{14}$C Ratios in Plasma Glucose and Glycogens Following Administration of Glucose-6-*t*-^{14}C.[a]

Expt No.	Duration of Expt (min)	$t/^{14}$C of Plasma Glucose		$t/^{14}$C of Glycogens			
		Final Sample	At Midpoint	Leg Muscle	Diaphragm	Heart	Liver
2	150	0.60	0.77	...	0.74	...	0.32
4	75	0.74	0.88	...	0.86	...	0.60
5	120	0.71	0.87	0.81	0.85	0.87	0.59
6	75	0.82	0.91	0.96	0.97	0.93	0.74
7	110	0.71	0.80	0.81	0.75	0.75	0.40

[a] $t/^{14}$C ratio of administered glucose is 1.0.

ratio or in the tritium and ^{14}C specific activities, showing that an excess tritium loss does not result from the metabolism of blood cells.

The half-lives and the turnover rates of glucose-6-*t* and glucose-6-^{14}C are presented in Table I. The average tritiated glucose half-life is 31.8 ± 1.3 min, whereas the average half-life of ^{14}C is 41 ± 1.4 min. The average fractional turnover rate for tritiated glucose was 2.19%/min. In contrast, the average turnover rate for glucose-^{14}C was 1.69%/min.

Table II shows the $t/^{14}$C ratio of liver, heart, diaphragm, and leg muscle glycogen and compares them with the isotope ratio of the plasma glucose at the midpoint[2] and at the end of each experiment.

The $t/^{14}$C ratios of heart, diaphragm, and leg muscle glycogens are approximately equal to plasma glucose ratios at the midpoint. However, the $t/^{14}$C ratio of liver glycogen in every experiment is lower even than the final isotope ratio of plasma glucose. These results indicate that an excess loss of tritium over carbon-14 occurs at some step, or steps, leading to liver glycogen synthesis, but does not occur in skeletal muscle.

The low $t/^{14}$C ratio of liver glycogen suggested that tritium loss may occur in the liver during hexose resynthesis from three-carbon fragments. To confirm this possibility a direct measurement of glucose recycling was made in fasted rats to determine whether it correlates quantitatively with tritium loss. The results are presented in Table III. The randomization of ^{14}C from C_6 to C_{1-3} could be detected within 15 min, and in 2 hr as much as 35–46% of the ^{14}C activity of plasma glucose came from recycled isotope. The $t/^{14}$C ratio of glucose carbons 1–3, with the exception of the initial samples,[3] was less than 0.1, indicating virtually com-

[2] In experiments of relatively short duration the plasma glucose $t/^{14}$C ratio at the midpoint approximates the mean isotope ratio of plasma glucose throughout an experiment. If the duration of an experiment exceeds 150 min, a more accurate value of the mean isotope ratio can be calculated by integrating the tritium and glucose-^{14}C specific activities from time 0 to time *t* (Katz and Dunn, 1967).

[3] The $t/^{14}$C ratios detected in the first 15 min are subject to large errors because of the low ^{14}C activity in the initial samples.

TABLE III: Per Cent of Plasma Glucose-^{14}C Activity from Recycling. Comparison of $t/^{14}$C Ratio of Plasma Glucose (C_{1-6}) with the Fraction of Glucose-^{14}C Activity Containing No Recycled ^{14}C. The $t/^{14}$C Ratio of Recycled Fragments (C_{1-3}).

Expt No.	Min after Isotope Administration	^{14}C Spec. Act. (dpm/mmole × 10^{-2})		($^{14}C_T$ − 2 × $^{14}C_{1-3}$)/C_T	$t/^{14}$C of C_{1-3}	$t/^{14}$C of C_{1-6}
		Glucosatriazole C_{1-6}	Formylosatriazole C_{1-3} × 2			
8	0					1.0
	15	617	43	0.93		0.94
	30	458				0.90
	45	273	45	0.84		0.84
	60	257	56	0.78		0.77
	80	148	56			
	100	130	49	0.62		0.59
	120	107	46	0.55		0.50
9	0					1.0
	15	1328	42	0.97	<0.27	0.94
	30	1131				0.93
	45	996	131	0.87	<0.1	0.89
	60	705	107	0.85	<0.1	0.85
	80	571	145	0.75	<0.1	0.81
	100	570	149	0.74	<0.1	0.74
	120	374	135	0.64	<0.1	0.70
10	0					1.0
	15	1749				0.95
	30	1291	87	0.93	<0.24	0.90
	45	888	97	0.90	<0.1	0.88
	60	771	121	0.84	<0.1	0.82
	80	559	133	0.76	<0.1	0.74
	100				<0.1	
	120	387	135	0.65	<0.1	0.69
11	0					1.0
	30	879	62	0.92	<0.30	0.92
	45	616	70	0.88	<0.1	0.86
	60	521	80	0.84	<0.1	0.81
	80	387	95	0.76	<0.1	0.74
	100	294	76	0.74	<0.1	0.61
	120	222	87	0.60	<0.1	0.58

plete loss of tritium during recycling. The correlation between tritium loss and recycling is indicated by the closeness with which the $t/^{14}$C ratios of glucose (C_{1-6}) approximates $^{14}C_T - 2(^{14}C_{1-3})/^{14}C_T$, the fraction of total glucose-^{14}C activity which contains no recycled isotope.

It follows that if the plasma glucose-^{14}C specific activities are corrected for recycling according to von Holt *et al.* (1961), the half-times and turnover rates should be equal to those obtained with glucose-6-*t*. These two sets of values, presented in Table IV, are indeed essentially the same for each experiment. When the per cent contribution of recycling to total production is calculated from the corrected and uncorrected glucose-^{14}C half-times, the values are the same as those obtained using the tritium and uncorrected ^{14}C half-times. The contribution of recycling in postabsorptive rats was also calculated, and the results are presented in Table I. There are no marked differences between fasted and postabsorptive rats either in per cent recycling or in the turnover rates. However, the results of one experiment with fasted rats (expt 9) did not agree closely with the other three in this group.

If the reactions involved in recycling are responsible for the decrease in the $t/^{14}$C isotope ratio of glucose-6-*t*-^{14}C, a similar decrease should occur following administration of glucose-1-*t*-^{14}C, since carbons 1 and 6 of glucose both undergo the same reactions during glucose resynthesis. Figure 2 presents the results of two experiments in which glucose-1-*t*-^{14}C was administered. The results are similar to those presented in Figure 1B

TABLE IV: Half-Times and Turnover Rates of Plasma Glucose-6-*t*, Glucose-6-^{14}C, and Glucose-6-^{14}C Corrected for Recycling in Fasted Rats. The Per Cent Contribution of Recycling to Glucose Production.

Expt and Conditions	Half-Time (min)			% Contribution of Recycling to Glucose Production[b]		Turnover Rates (% of glucose pool/min)		
	^{14}C	Cor ^{14}C[c]	*t*	Cor ^{14}C[c]	*t*	^{14}C	Cor ^{14}C[c]	*t*
Postabsorptive 1–6 av[a]	41		32		23	1.69		2.18
Fasted								
8	40	30	30	25	25	1.73	2.31	2.31
9	64	41	43	36	33	1.12	1.69	1.61
10	38	31	32	18	16	1.82	2.24	2.16
11	47	35	34	25	27	1.47	1.98	2.04

[a] From Table I. [b] Recycling calculated as in Table I using ^{14}C along with tritium and corrected ^{14}C half-times. [c] $^{14}C_T - 2(^{14}C_{1-3})$.

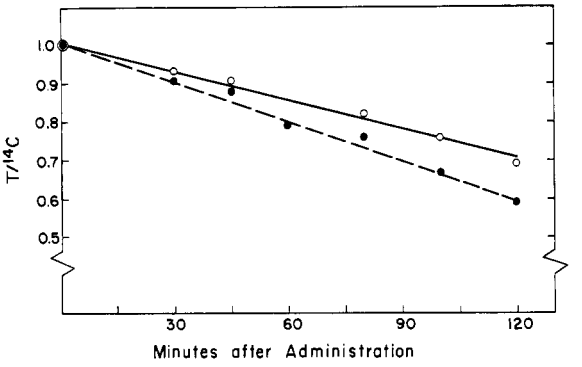

FIGURE 2: $t/^{14}$C ratio of plasma glucose following administration of glucose-1-$t/^{14}$C.

for glucose-6-*t*-^{14}C.

Discussion

Our results show that the turnover rate of plasma glucose-6-*t* is one-third faster than that of glucose-6-^{14}C. The $t/^{14}$C ratios of heart, diaphragm, and leg muscle glycogens are similar to that of circulating glucose, indicating that there is no loss of tritium from carbon 6 in extrahepatic tissues. However, the liver glycogen $t/^{14}$C ratio is always lower than that of plasma glucose, showing that loss of tritium occurs in this organ.

Foster and Bloom (1961), however, reported that no excess loss of tritium occurs *in vitro* when glucose-6-*t*-^{14}C or glucose-1-*t*-^{14}C is incorporated directly into glycogen in liver slices. We have confirmed these findings. The differences between the results obtained *in vivo* and *in vitro* can be explained if the contribution of the Cori cycle to hepatic hexose synthesis in the intact animal is considered. The lower $t/^{14}$C ratio of liver glycogen *in vivo* may well result from hexose synthesis from labeled extrahepatic lactate which enters the liver and in subsequent reactions loses tritium but retains ^{14}C.

During synthesis of glucose in the liver from lactate, pyruvate is carboxylated to oxalacetate *via* the pyruvic carboxylase reaction and oxalacetate is then decarboxylated to phosphoenolpyruvate *via* the phosphoenol carboxykinase reaction. The role of this dicarboxylic acid shuttle in the reversal of glycolysis is well established (Wood and Utter, 1965). One hydrogen from the methyl group of pyruvate is lost in the carboxylation step. Furthermore, if oxalacetate equilibrates with malate and fumarate, hydrogen originating in the methyl group of pyruvate would be exchanged with protons. Equilibration of the carboxylic acids is extensive, as evidenced by the randomization of C-2 of pyruvate incorporated into glucose (Hiatt *et al.*, 1958). Thus, tritium from C-6 of glucose would be extensively diluted relative to carbon in these reactions. The low $t/^{14}$C ratio (<0.1) of carbons 1–3 of resynthesized glucose demonstrated that essentially all tritium is lost in recycling.

The simultaneous use of glucose-6-*t* and glucose-^{14}C allows a simple calculation of the Cori cycle. Our experiments show 19–26% of glucose production comes *via* the Cori cycle in the postabsorptive rat. von Holt *et al.* (1961) investigated glucose resynthesis by measuring the randomization of label from C-6 to C-1 and reported 12% resynthesis in the fed rat. This value increased to 50% after fasting for 15 hr. In our experiments only one out of four fasted rats showed any appreciable increase over postabsorptive values. Ashmore (1961) concluded from comparable experiments in fasted rats that less than 10% of ^{14}C in blood glucose could be attributed to the Cori cycle. Reichard *et al.* (1963), measuring the incorporation of ^{14}C from carbon 1 into carbon 6 of glucose in postabsorptive human subjects, found that 12–20% of

glucose production is derived *via* the Cori cycle.

The use of glucose-6-*t* in turnover studies corrects for recycling of label *via* the Cori cycle and measures the production of new glucose. However, since glucose-6-*t* does not lose tritium label when incorporated into liver glycogen as intact hexose, it cannot measure the contribution of glycogenolysis to total glucose production. Katz and Dunn (1966) recently measured glucose turnover using glucose-2-*t*-U-^{14}C. The turnover rate of glucose-2-*t* is 1.5 times faster than that of glucose-^{14}C. The faster turnover rate of glucose-2-*t* relative to glucose-6-*t* is probably due to the almost complete removal of tritium from carbon 2 at the phosphoglucoisomerase step prior to incorporation into liver and muscle glycogen. Thus, glucose-2-*t* may be used to measure total glucose production (new glucose plus glycogenolysis) which is equal to the rate of glucose-6-P hydrolysis by liver phosphatase. The use of glucose, labeled in various positions with tritium, and combined with glucose-^{14}C, allows for simultaneous measurement of a variety of parameters of carbohydrate metabolism.

Appendix

Determination of Turnover Rates. Under steady-state conditions, the turnover of the glucose pool with respect to ^{14}C and tritium is depicted as follows

$$G \xrightarrow[\text{production}]{k} \boxed{\frac{G^{14}C}{G};\ \frac{G_t}{G}} \underset{\text{recycling}}{\overset{k}{\underset{k_f}{\rightleftarrows}}} \text{utilization} \quad \boxed{\frac{G^{14}C}{G};\ \frac{G_t}{G}}$$

where G is new glucose produced (unlabeled), G_t/G is glucose-6-*t* specific activity, $G^{14}C/G$ is the glucose-^{14}C specific activity, k is the first-order rate constant, and k_f is the rate constant for recycling. In the case of glucose-6-*t*, k_f is zero.

A. Glucose-6-t. The rate of dilution of specific activity is expressed as

$$\frac{dG_t}{dt} = -k\frac{G_t}{G} \tag{1}$$

which upon integration between the limits G_{t_0} at time 0 and G_{t_t} at time t gives

$$G_{t_t} = G_{t_0}\, e^{-k_t/G} \tag{2}$$

The fractional turnover rate can be obtained from the half-time, when $G_{t_t}/G = {}^1/_2((G_{t_0})/G)$

$$k = \frac{0.693}{t_{1/2}} \tag{3}$$

$k \times 100$ = the per cent of the glucose pool turned over per minute.

B. Glucose-6-^{14}C.

$$\frac{d^{14}C}{dt} = -k\frac{G^{14}C}{G} + k_f\frac{G^{14}C}{G} \tag{1a}$$

$$G^{14}C_t = G^{14}C_{t_0}e^{k-(1-f)/G} \tag{2a}$$

$$k(1-f) = \frac{0.693}{t'_{1/2}} \tag{3a}$$

Determination of Fraction of Glucose Recycled (f) Using Double Labeling. f is obtained from eq 3 and 3a above: $f = 1 - (t_{1/2}/t'_{1/2})$, where $t_{1/2}$ and $t'_{1/2}$ are the half-times of glucose-6-*t* and glucose-6-^{14}C, respectively.

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