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Determination of Glucose Utilization in the Dog with [2-^{3}H], [6-^{3}H]-, and [U-^{14}C]Glucose[1] (39421)

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Glucose labeled with ^{14}C has been widely used as a tracer for the study of *in vivo* glucose metabolism in various species. The application of [U-^{14}C]glucose kinetics to glucose turnover studies is, however, complicated by the recycling of carbon which greatly limits the interpretation of the experimental data (1-6). An alternative approach, based on the use of [^{3}H]glucose, has been proposed in order to minimize this problem (4, 6-9). The use of tritiated glucose as a tracer of glucose metabolism has been investigated in various species (3, 4, 7-17). The loss of tritium generally exceeds the loss of ^{14}C from circulating glucose, suggesting less recycling of tritium than of ^{14}C (4, 7-9). Formation of water is the predominant early fate of tritium from metabolized glucose (7, 9) and the dilution of tritium from glucose in a large pool of body water minimizes the recycling of this tracer to the glucose pool.

[2-^{3}H]Glucose and [6-^{3}H]glucose have been used most frequently as ^{3}H-labeled tracers for estimating parameters of glucose metabolism (7-11, 13-16). Their use in combination with [U-^{14}C]glucose have allowed various investigators to estimate the degree of glucose-carbon recycling in intact animals (9, 18). Only a limited number of studies have been conducted which compare the loss of tritium from [2-^{3}H]glucose and [6-^{3}H]glucose in the same experiment. Such studies would allow one to estimate the rate of glucose utilization as well as to obtain some information on the extent of operation of "futile cycles."

The purpose of this study was to determine the rate of glucose utilization in the dog using [2-^{3}H]- and [6-^{3}H]glucose in combination with [U-^{14}C]glucose.

Materials and methods. Experimental animals and diet. One-year-old female beagles[2] were housed in individual pens with raised floors and were fed a maintenance level of a commercial diet.[3] The diet contained 36, 40, and 24% of metabolizable calories from protein, fat, and carbohydrate, respectively. Water was available *ad libitum*. Lights in the temperature-controlled rooms were on from 7:00 AM to 7:00 PM and off from 7:00 PM to 7:00 AM. At the time of the experiment, the dogs weighed approximately 8 to 10 kg.

Turnover study. Glucose turnover studies were performed on seven dogs fasted for 48 hr. One hour prior to the experiment, indwelling catheters were inserted in the jugular vein of unanesthetized dogs. Teflon catheter needles[4] were used to eliminate surgery during the implantation. Dogs were in their pens and allowed access to water during the experiment. Four dogs were injected with [2-^{3}H]glucose[5] (250 μCi) and [U-^{14}C]glucose[5] (20 μCi) mixture in 5.0 ml of 0.9% NaCl. Simultaneous administration of [6-^{3}H]glucose[5] (250 μCi) and [U-^{14}C]glucose (20 μCi) was performed in the other three dogs. Blood samples (5.0 ml) were withdrawn at timed intervals up to 5 hr. The initial samples were taken at 3-min intervals for the first 15 min, and at 5 min intervals for the next 15 min. Subsequent blood samples were taken at 15-min intervals up to 1 hr, and then at 30-min intervals thereafter. Blood samples were collected in

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[2] Laboratory Research Enterprises, Inc., Kalamazoo, Mich.

[3] Ken-L-Ration, The Quaker Oats Company, Chicago, Ill.

[4] Becton, Dickinson & Co., Rutherford, N.J.

[5] Amersham/Searle Corporation, Arlington Heights, Ill.

chilled tubes containing heparin and sodium fluoride. Dogs were handled gently and did not appear excited during sampling. The total volume of blood withdrawn per dog per day was approximately 90 ml. After 1 week dogs previously injected with [2-^{3}H]glucose were administered with [6-^{3}H]glucose and vice versa.

Glucose and glucose-specific radioactivity determinations. Plasma was promptly separated and deproteinized with barium hydroxide and zinc sulfate. Glucose was isolated by passing an aliquot of the deproteinized plasma through an ion exchange column (9, 17). Determination of glucose-specific radioactivity by paper chromatography verified that the column separated [U-^{14}C]glucose from its ^{14}C-labeled metabolites. Further, results from a preliminary experiment showed that recovery of labeled glucose after passage through the ion exchange column averaged 95%. The column eluate and washings were lyophilized and taken up in a small volume of water. Scintillation cocktail[6] was added and the radioactivity in the sample was quantitated by liquid scintillation spectrophotometry. Tritium counts were corrected for spillover of ^{14}C-counts. The counting efficiency was obtained with an external standard. Plasma glucose concentration was determined in other aliquots with glucose oxidase.[7] Tritium and ^{14}C-specific radioactivity of glucose was then calculated.

Calculations. Parameters of glucose metabolism estimated by single injection of labeled glucose were calculated using both graphical and exponential analysis. The linear plot of glucose specific radioactivity versus time was constructed. The glucose replacement rate, transit time, total body glucose mass, glucose-carbon recycling, and glucose mass of sampling pool were then calculated graphically according to Katz *et al.* (6, 9). The log of specific radioactivity versus time curve of glucose was also constructed. The curve obtained was fitted into a two-component exponential equation. Estimates of the coefficient and exponent of each component were obtained by a curve "peeling" process. The terminal exponential of the curve was fitted by least square procedure, and then, by subtraction of this line from the earlier part of the curve, a second curve was obtained. Glucose replacement rate and total body glucose mass in the dog were then calculated using the two-component exponential equation.

Statistical analyses. The data were treated statistically by Student's *t* test (19).

Results. Plasma glucose levels were relatively constant throughout the experimental period. The coefficient of variation of glucose concentration (14–19 samples) was less than 7%. Body weight and plasma glucose levels in dogs injected with [2-^{3}H]glucose were not significantly different from those in dogs administered [6-^{3}H]glucose (Table I).

Figure 1 presents a linear plot and Fig. 2 a semilogarithmic plot of plasma glucose radioactivity versus time after a single injection of [2-^{3}H]- or [6-^{3}H]glucose. These curves show an initial rapid decline followed after about 20 to 30 min by a curvilinear change up to 5 hr postinjection. From the areas under these curves (Fig. 1), parameters of glucose metabolism were calculated by the graphical method of Katz *et al.* (6, 9). In Fig. 2 the straight lines represent exponential functions that give the line of the best fit for the decay curves. These curves were represented by an exponential equation with two terms.

Estimates of glucose metabolism parameters in dogs fasted for 48 hr are presented in Table I. The glucose replacement rate using the graphical method of analysis averaged 4.6 mg/min/kg body weight for [2-^{3}H]glucose. This value is similar to values reported by others (13, 15). The glucose replacement rate calculated by graphical or by exponential analysis was higher when [2-^{3}H]glucose rather than [6-^{3}H]glucose was utilized as tracer. However, values obtained by exponential analysis were more variable and thus differences between [2-^{3}H]glucose and [6-^{3}H]glucose were statistically different only when the graphical analysis was utilized. Values for the rate of glucose utilization obtained by exponential analysis were also slightly higher than values obtained by graphical analysis (Table I).

Estimates of glucose-carbon recycling

[6] 3a70, Research Products International Corporation, Elk Grove Village, Ill.

[7] Glucostat T. M., Worthington Biochemical Corp., Freehold, N.J.

TABLE I. GLUCOSE METABOLISM IN FASTED DOGS ESTIMATED BY USING A SINGLE INJECTION OF MIXTURES OF [2-^{3}H]GLUCOSE OR [6-^{3}H]GLUCOSE AND [U-^{14}C]GLUCOSE.

	Tritiated tracer	
	[2-^{3}H]glucose	[6-^{3}H]glucose
Body weight (kg)	9.42 ± 0.28[a]	9.43 ± 0.30
Plasma glucose (mg/100 ml)	102 ± 2	97 ± 5
Glucose replacement rate		
[^{3}H]glucose (mg/min/kg)	4.6 ± 0.2	3.7 ± 0.2[b]
	(5.3 ± 0.9)	(3.9 ± 0.4)
[^{14}C]glucose (mg/min/kg)	3.2 ± 0.2	3.1 ± 0.1
Glucose-carbon recylcing (%)	31 ± 2	14 ± 2[b]
Glucose transit time (min) ^{3}H[c]	67 ± 3	76 ± 4
^{14}C[c]	91 ± 3	89 ± 5
Glucose body-mass (mg/kg) ^{3}H	308 ± 15	280 ± 18
^{3}H	(261 ± 15)	(225 ± 17)
^{14}C	284 ± 18	290 ± 21
Glucose mass of sampling pool (mg/kg) ^{3}H	210 ± 16	199 ± 15

[a] Results expressed as mean ± SE for seven dogs. Numbers in parenthesis indicate values calculated by exponential analysis.
[b] [2-^{3}H]Glucose versus [6-^{3}H]glucose, significantly different ($P < 0.05$).
[c] Indicates tracer utilized in the calculations.

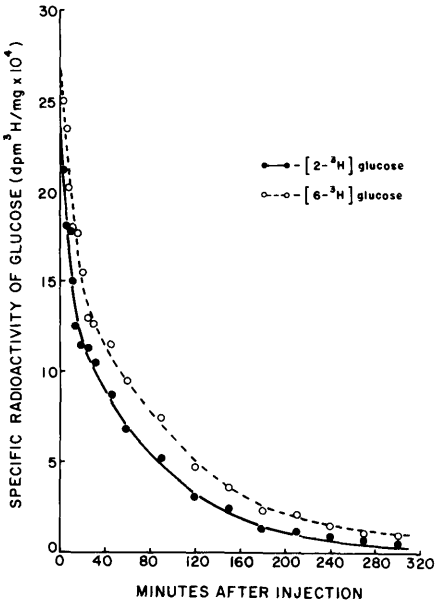

FIG. 1. A linear plot of glucose-specific radioactivity and time after single injection of [2-^{3}H]- or [6-^{3}H]glucose in a dog fasted for 48 hr.

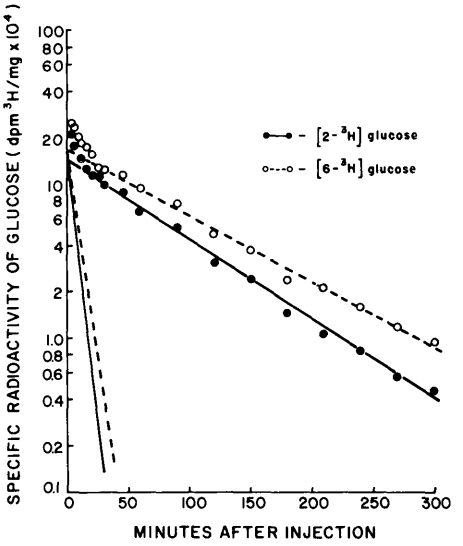

FIG. 2. A semilogarithmic plot of glucose-specific radioactivity and time after single injection of [2-^{3}H]- or [6-^{3}H]glucose in a dog fasted for 48 hr.

were obtained from the glucose replacement rates of [2-^{3}H]- or [6-^{3}H]glucose and [U-^{14}C]glucose. Glucose-carbon recycling estimated with [2-^{3}H]glucose in fasted dogs averaged 31% (Table I). This value compares favorably with a previous report (13). The degree of glucose-carbon recycling obtained with [2-^{3}H]glucose was significantly higher than when [6-^{3}H]glucose was utilized (Table I).

The time which a glucose molecule is present in the body from the time of synthesis to the time of its catabolism or loss (transit time) was also estimated using [2-^{3}H]-, [6-^{3}H]-, or [U-^{14}C]glucose as tracers. The glucose transit time obtained with [6-^{3}H]glucose was 76 min; this value was 12% longer than that obtained with [2-^{3}H]glucose. However, the difference in transit time between the two tracers was not statistically significant (Table I). Estimates of transit time obtained with ^{14}C as tracer averaged approximately 90 min.

The total body mass of glucose in fasted dogs determined by graphical analysis averaged 308 ± 15 and 280 ± 18 mg/kg body weight with [2-^{3}H]- and [6-^{3}H]glucose, respectively. These values were in close agreement with the estimates of body mass obtained with [^{14}C]glucose (Table I). Values obtained by exponential analysis were relatively lower but not significantly different from those obtained by graphical analysis. There were no significant differences in total body glucose mass obtained with [2-^{3}H]- or with [6-^{3}H]glucose. The glucose mass of the sampling or rapidly mixing pool was determined from the zero-time intercept of the glucose specific activity curves. Estimates of glucose mass of the sampling pool were also similar whether [2-^{3}H]glucose or [6-^{3}H]glucose was utilized as tracer.

Discussion. In the present study the *in vivo* metabolism of [2-^{3}H]glucose and [6-^{3}H]glucose was compared in dogs fasted for 48 hr. The rate of glucose utilization estimated with [6-^{3}H]glucose was about 20% lower than when [2-^{3}H]glucose was utilized as the tracer. This indicates that the loss of tritium from [2-^{3}H]glucose exceeds the loss of tritium from [6-^{3}H]glucose in the dog. The *in vivo* detritiation of [2-^{3}H]glucose has also been reported to be faster in rabbits (18), sheep (16), mice (20), and chickens (17) than the detritiation of [6-^{3}H]glucose. Recent reports suggest that this greater loss of tritium from [2-^{3}H]glucose than from [6-^{3}H]glucose is due to the operation of a "futile" cycle between glucose and glucose-6-phosphate (18, 21). It thus appears that significant recycling between glucose and glucose-6-phosphate is probably occurring in the dog and that our estimates of glucose utilization obtained with [2-^{3}H]glucose (Table I) represent the total rate of glucose utilization including glucose recycling at the hexokinase-glucose phosphatase level.

Simultaneous administration of tritiated and [U-^{14}C]glucose has been used to estimate glucose-carbon recycling, i.e., reappearance of ^{14}C-labeled three-carbon fragments in newly synthesized glucose. In the present study the extent of glucose-carbon recycling estimated with [6-^{3}H]- and [U-^{14}C]glucose was 55% lower than when [2-^{3}H]- and [U-^{14}C]glucose was utilized. Since the degree of glucose-carbon recycling is obtained from the difference between the [^{3}H]- and [^{14}C]glucose replacement rates, the higher estimates obtained with [2-^{3}H]glucose than with [6-^{3}H]glucose further suggest the presence of a "futile" cycle between glucose and glucose-6-phosphate in the dog. Altszuler *et al.* (22) have also recently reported that glucose turnover using [2-^{3}H]glucose was higher than when [6-^{14}C]glucose was used. Our estimates of the magnitude of this "futile" cycle depend in part on how fast glucose molecules subjected to this cycle in gluconeogenic organs of the dog equilibrate with the plasma pool relative to the rapidity with which three-carbon intermediates are formed. Estimates of the relative rates of these two processes were not obtained in the present study.

With graphical method of analysis, our estimate of the total glucose mass averaged 308 ± 15 and 280 ± 18 mg/kg with [2-^{3}H]glucose and [6-^{3}H]glucose, respectively. Our estimates compare with the reported values obtained with [2-^{3}H]glucose for rats (260 mg/kg) (9), rabbits (290 mg/kg) (9), or dogs (290 mg/kg) (23). Estimates of the glucose mass of the sampling or rapidly mixing pool are only rough approximations. As discussed by Katz *et al.* (9) due to the rapid change in slope of the initial curve (Fig. 1), these estimates are subject to considerable error.

Exponential analysis of the data has been extensively used in studies with labeled glucose. In the present study comparisons between the graphical and exponential methods of analysis were made. Theoretically, these two methods of analysis should provide similar results. Comparable results

were obtained for glucose replacement rate and total body glucose mass; however, the values obtained with the exponential analysis were more variable than with the graphical method. Possibly the data would have been better represented if we had considered models containing more than two glucose pools.

Summary. Parameters of *in vivo* glucose metabolism in adult female dogs fasted for 48 hr were estimated after a single injection of [2-^{3}H]- or [6-^{3}H]glucose in combination with [U-^{14}C]glucose. The glucose replacement rate estimated with [2-^{3}H]glucose averaged 4.6 mg/min/kg and was about 25% higher than the values obtained with [6-^{3}H]glucose. Estimates of glucose-carbon recycling were more than twice as high (31 vs 14%) when [2-^{3}H]glucose was utilized as tracer than when [6-^{3}H]glucose was utilized. These differences suggest the presence of a "futile" cycle between glucose and glucose-6-phosphate in the dog.

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