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Glucose Production Rates in Dogs Determined by Two Different Tracers and Tracer Methods

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INTRODUCTION

Published tracer methods designed to determine the rate of appearance of a plasma component (i.e. a substance contained in blood plasma), such as glucose are based either on *quantity dilution* or *rate dilution* or both. Tracer methods using quantity dilution determine the rate of decrease with time in the specific activity of a selected substance in the plasma of a living subject, following the rapid injection and intermixing of a trace amount of the same substance suitably labeled. One such tracer method, in which the rate of appearance of glucose is calculated using values extrapolated to the time of tracer injection, will be designated as tracer Method A (1). When used repeatedly this corresponds to the method of successive measured injections of tracer (2).

In tracer methods employing rate dilution, the tracer substance is infused at a constant measured rate into the blood stream of an animal (3). During this process the specific activity of the traced substance will approach an equilibrium level, providing that the substance is in a dynamic steady state in the plasma. The length of time required for this constant level to be closely approached can be greatly reduced by the intravenous injection of a certain priming dose of the tracer at the start of the tracer infusion (4). From a figure for the equilibrium level of specific activity and the measured rate of infusion of the labeled substance, a value for the rate at which the unlabeled substance is appearing in the subject's plasma can be calculated using the dilution principle. For the purposes of discussion, this basic procedure together with its elaboration into a series of successive primed increments in the rate of infusion of tracer (5) will be referred to as Method B.

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The objectives of this paper are: a. to compare the calculated rates of glucose appearance (endogenous production) as obtained by simultaneous and alternate determinations using tracer *Methods* A and B; b. to investigate the applicability of (6-^{3}H) glucose as compared with glucose uniformly labeled with ^{14}C, (^{14}C)glucose, as *tracers* for determining the endogenous rate of appearance of unlabeled glucose in the fasting conscious dog. In working toward both objectives, the design of the experiments in Group (ii) is such as to permit the resolution by statistical procedures of the effects of method from those of isotopic tracer on the calculated rate of glucose appearance.

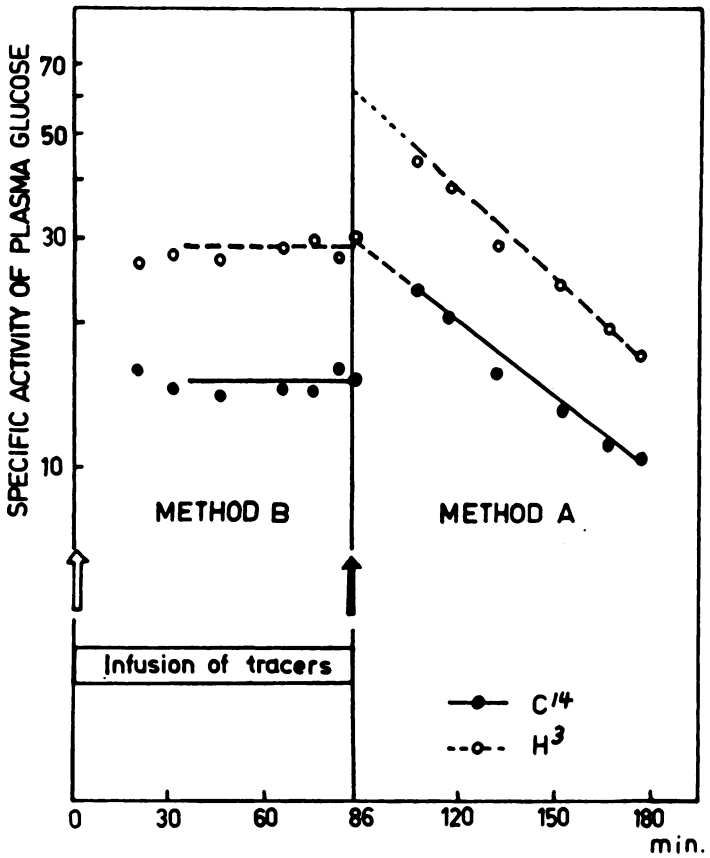

Fig. 1. Experiment No. 23 (in Table IIA) demonstrating the experimental design followed in Group (ii). The changes in the specific activities of 6-^{3}H glucose (circles) and ^{14}C glucose (dots) with respect to time are shown. Abscissa: time in minutes. Ordinates: specific activity of plasma glucose, dpm/μg glucose. White arrow indicates the simultaneous injection of a priming dose of ^{14}C glucose and 6-^{3}H glucose. The horizontal block indicates the infusion of these two tracers at a constant rate (Method B). The black arrow shows the second simultaneous injection of the tracers this time not followed by a tracer infusion (Method A). The rates of appearance of glucose are calculated at the time of the second tracer injection (black arrow), in this graph at 86 min in the abscissa. In the first period using Method B calculated rates of appearance of glucose (mg/kg min) were 3.28 and 3.08 with tritiated and ^{14}C-labeled glucose respectively. In the second period using Method A 3.56 and 3.02 were the corresponding calculated rates.

Tracer-method A was shown to give valid absolute rates of glucose appearance in the eviscerated dog (6). In previous tests of performance Method B did not provide adequate information concerning changes in the content of an inaccessible compartment of an experimental two-compartment hydrodynamic model system. However, this method did provide valid figures with small standard deviations for the rate of water inflow (appearance) into the accessible compartment in such a system in a dynamic steady state (5,7). The results of a few preliminary experiments of this series were published previously (8).

MATERIAL AND METHODS

Animals: All experiments were performed in conscious healthy fasting dogs trained either to stand or lie quietly. Technical details of the tracer injections and infusions and the procedures for withdrawing and processing blood samples have been described previously (9).

Determinations of the concentration and the specific activity of plasma glucose: Glucose in the plasma was determined enzymatically (10) after deproteinization (11). Paper chromatography and the elution of the chromatograms (12) were used for the separation of glucose. The chromatographic eluates were collected directly into counting vials. The latter then were placed in a vacuum oven set to 60° and the eluates evaporated. Distilled water, 0.15 ml, was next added and care was taken that the water cover the bottom of each vail. Following this, 10 ml of Bray's solution (13) was added from a burette. Two sets of quench standards, altogether 16 vials, were prepared, one set containing a measured amount of (^{14}C) glucose, the other a mixture of measured amounts of ^{3}H and ^{14}C-labeled glucose in water of a varying volume (0.10–0.20 ml).

Double-label counting with correction for quenching differences was carried out essentially as described by Hendler (14). A program of Fortran II for the calculation of the specific activities of both ^{14}C and ^{3}H and including the fittings of exponential functions has been set up for the IBM 7094 Digital Computer, and was used to analyze the experimental data. Specific activities were calculated as disintegrations per minute per microgram of glucose.

Calculations: The rate of appearance of glucose and the amount of glucose intermixing with the injected tracer, referred to by some authors as the "pool size" (15), was calculated using Method A (1). The formulas used for the calculations of the intermixing amount (N) and the rate of appearance (R_a) of glucose at the time of the injection of tracer (T) are (6):

$$N_T = \frac{n(S - (s - s')_T)}{(s - s')_T} \tag{1}$$

$$(R_a)_T = \frac{-N_T}{(s - s')_T} \cdot \left[\frac{ds}{dt}\right]_T \tag{2}$$

where n is the injected amount (mg) of tracer glucose, S its specific activity, s'_T the specific activity of plasma glucose at the time of tracer injection, and s_T the specific activity of plasma glucose extrapolated back to the time of tracer injec-

tion from values following the tracer injection. All s *vs* t curves used in the present study were single exponential functions.

Using Method B the rate of glucose appearance at the time of tracer injections and start of tracer infusion was calculated by the rate dilution equation:

$$(R_a) = \frac{r_a (S - s_\infty)}{s_\infty} \qquad (3)$$

where r_a is the rate of infusion of tracer glucose (mg/min), S its specific activity, and s_∞ the constant specific activity of plasma glucose after approaching a constant value ("plateau").

Experimental design: Thirteen experiments were carried out on nine dogs with a total of 45 tracer injection cycles. A given dog is referred to by the same number in all Tables, with an added a or b when used twice. The experiments are subdivided into groups (i), (ii) and (iii) as shown below.

In Group (i) four experiments were performed on two dogs. In the first experiment on each dog 6-^{3}H glucose was used as a tracer with Method A and ^{14}C glucose with Method B. In the second experiment the combination of tracers and methods was reversed. Each experiment consisted of two cycles of both methods, the injections of the second pair of tracers terminating the first cycles. In this design the rates of glucose appearance as calculated by Methods A and B do not coincide in time, the one calculated by Method A was referred to the time of injection of the tracer, whereas, the other, calculated by Method B, was referred to the later time at which the specific activity of glucose reached a plateau level in the plasma. Since these experiments did not permit the calculation of the variance due to a difference in methods and tracers nor the interaction between methods and tracers, the experiments described in group (ii) were carried out.

In Group (ii) four experiments were carried out on four dogs. In the first part of each experiment the rate of appearance of glucose was calculated by Method B with both tracers simultaneously. This entailed the simultaneous primed infusion of both tracers. This period was terminated after 86-105 minutes by a second simultaneous injection of a measured amount of both tracers. The rate of appearance of glucose could be calculated again at the time of tracer injection by both tracers using Method A.

Since the second injection of tracers coincides in time with the latest point on the "plateau" of the specific activity *vs* time plot of the first period, a direct comparison of the four rates can be made *at this one time,* and the variations due to tracers, methods and the interaction between these two factors could be calculated by an appropriately designed analysis of variance. Data from a typical experiment of this design are shown in Figure 1.

Group (iii) consists of five experiments performed on three dogs. In this group, only Method A was used with both types of labeled glucose. This was done in order to permit the comparison in the same subject of the calculated rates of appearance and intermixing amounts of glucose as obtained with ^{14}C gucose and 6-^{3}H glucose as tracers. In the first two experiments two injections of each tracer were made alternately at time intervals of 25-40 minutes. The

specific activity of plasma glucose was followed for 60-75 minutes when the next injection of the same tracer was given. In two other experiments the alternate injections of tracers were followed by glucose labeled with ^{14}C and ^{3}H one or two simultaneous injections. In one experiment only a single simultaneous injection of the two tracers was followed.

RESULTS

The results of the experiments of Group (i) are shown in Table I. The average rates of appearance calculated by the two methods were 2.90 mg/kg min with Method A and 2.83 mg/kg min with Method B. There is some tendency to obtain higher rates with 6-^{3}H glucose as tracer in Dog Number 11, but not in Dog Number 12. However, because of the balance in the experimental design, the overall averages of the rates as obtained by the two methods in the four experiments agree.

TABLE I

Dog No.	*Method A* *Label in tracer glucose used*	*Method B*	*Method A* *Ra (mg/kg min)*	*Method B*
11a	^{3}H	^{14}C	2.88	2.71
11b	^{14}C	^{3}H	1.95	2.29
12a	^{3}H	^{14}C	3.24	3.22
12b	^{14}C	^{3}H	3.54	3.09
Mean:			2.90	2.83

Rates of appearance of glucose (mg/kg min) determined by two different tracer methods and two forms of labeled glucose: 6-^{3}H glucose and ^{14}C glucose used as tracers in two dogs. Each value on the table is an average of two consecutive determinations.

Calculated rates of glucose production in the experiments of Group (ii) are shown in Table IIA, and the analysis of variance in Table IIB. The use of 6-^{3}H glucose as tracer leads to an average calculated rate of 3.73 mg/kg min with Method A and 3.70 mg/kg min with Method B. The respective rates obtained using ^{14}C glucose as tracer were 3.48 and 3.33 mg/kg minute. It appears that 6-^{3}H glucose as a tracer yields significantly higher rates of glucose production than those calculated with ^{14}C glucose as tracer irrespective of which method is used. This difference is not conspicuous in every experiment. Nevertheless, it is statistically significant for the group. The rates calculated by the two methods A and B led to essentially identical values, namely to 3.73 and 3.70 mg/kg min with 6-^{3}H glucose, and 3.48 and 3.33 with ^{14}C glucose as tracers, respectively. The small differences between methods when using the same tracer were not significant statistically. There was no interaction between the tracers and the methods used, indicating that each tracer was handled identically by the body irrespective of the method applied.

TABLE IIA

Rate of Appearance of Glucose (mg/kg min)

Dog No.	*Method A*		*Method B*	
	^{3}H	^{14}C	^{3}H	^{14}C
21	4.13	3.68	4.17	4.03
22	3.84	3.93	3.88	3.41
23	3.28	3.08	3.56	3.02
24	3.69	3.22	3.18	2.87
Mean:	3.73	3.48	3.70	3.33

Rates of appearance of glucose (mg/kg min) calculated simultaneously by two different tracer methods and two forms of labeled glucose: 6-^{3}H glucose and ^{14}C glucose used as tracers.

In Table III the rates of glucose appearance are compared in the same dog using Method A only, employing both alternate and simultaneous injections of the two forms of labeled glucose. The rates obtained by using 6-^{3}H glucose as tracer are on the average 25% higher than those where ^{14}C glucose was employed.

Table IV shows the "pool" (15), *i.e.* the amounts of intermixing glucose calculated by Method A in those experiments where the two forms of tracer glucose were injected simultaneously. There is a considerable variation but no consistent difference between the results calculated using the two tracers, the average ratio of the pools determined by the two tracers, 1.03 ± 0.047, simultaneously.

TABLE IIB

ANALYSIS OF VARIANCE OF THE DATA ON TABLE IIA

Source of variation	*d.f.*	*SS*	*MS*	*F*
Total	15	2.626	—	—
Between dogs	3	1.784	0.5945	13.05*
Between sets of determinations	3	0.4324	0.1441	—
Methods	1	0.0333	0.0333	n.s.
Tracers	1	0.3875	0.3875	8.51**
Methods vs. Tracers	1	0.0116	0.0116	n.s.
Error	9	0.4101	0.0456	—

*P < 0.01

**P < 0.025

d.f. = degrees of freedom; SS = sum of squares; MS = mean square; F = F-value of Snedecor; n.s. = not significant.

TABLE III

Dog No.	*Cycle Tracer*	*1* ^{14}C	*2* ^{3}H	*3* ^{14}C	*4* ^{3}H	*Simult. 1.* ^{14}C	^{3}H	*Simult. 2.* ^{14}C	^{3}H	*Average of all determinations* ^{14}C	^{3}H
31a		2.72	3.31	2.71	3.38	—	—	—	—	2.72	3.35
31b		3.10	4.30	2.89	3.74	—	—	—	—	3.00	4.02
32a		2.86	3.18	—	—	2.42	2.78	2.82	3.14	2.70	3.03
32b		2.24	2.98	2.57	—	1.90	2.98	—	—	2.24	2.98
33		—	—	—	—	4.29	4.99	—	—	4.29	4.99

Successive and simultaneous determinations of the rate of glucose appearance (mg/kg min) using (6-^{3}H) glucose and (^{14}C) glucose as tracers with Method A.

In the same eight experiments for which results are shown in Table IV, the exponential decay constants of the specific activity *vs* time curve of plasma glucose were higher using 6-^{3}H glucose as tracer than the corresponding value obtained with ^{14}C glucose. The average ratio of the two decay constants was 1.24, and 1.04–1.47 the range between.

TABLE IV

Dog No.	*Intermixing Amount Determined by* ^{14}C *glucose*	6-^{3}H *glucose*	Ratio $\frac{N(^{14}C)}{N(^{3}H)}$
12	4.06	3.12	1.30
22	4.54	3.67	1.24
23	3.15	3.67	0.86
24	3.71	4.03	0.92
32a	3.25	3.00	1.08
32a	3.18	3.13	1.02
32b	2.67	2.83	0.94
33	2.20	2.48	0.89
		Mean	1.03
		S.e.m.	0.047

Simultaneous determinations of the amounts of intermixing glucose (N) in grams using 6-^{3}H glucose and ^{14}C glucose as tracers with Method A.

DISCUSSION

As summarized in Table IIB, rates of glucose production calculated by Methods A and B do not differ significantly from each other. Such an agreement is regarded as circumstantial evidence for the validity of the calculated magnitude of the rate of glucose production obtained by tracer methods. In contrast rates calculated by using 6-^{3}H glucose as tracer tend to be higher than those calculated with ^{14}C glucose. The absence of any significant interaction between tracers and methods emphasizes even more the difference in the handling of 6-^{3}H glucose and ^{14}C glucose in the body.

These results do not lend support to the prediction that rates of appearance of glucose calculated by the method of a primed infusion of tracer (Method B) would be lower by about 12% than those obtained by Method A, that is by the single injection-extrapolation method in dogs (16). This prediction was made on the assumption that glucose in the mammalian body is contained in two compartments, the contents of the one intermixing rapidly, the other much more slowly with the injected tracer. More recently the experimental evidence for such a hypothesis has been critically reviewed (17,18). Also it was shown in hydrodynamic models that even in such a two-compartment system drastic changes made in the content, and rate of efflux from the second compartment, had little or no detectable influence on the concentration of tracer in the first compartment into which the tracer was being infused at a constant rate (5,7). Transient changes which occur in the *fast* compartment of the glucose pool (e.g. as a response to insulin) very likely would have little effect in a second compartment. The realization of this condition led de Bodo *et al* (19) to use, arbitrarily, one half of the total glucose "pool"-occupying about 13% of body space- in their calculation of rates of glucose transfer. This procedure, however useful, involves the additional supposition that glucose is taken up by all cells from the first *fast* compartment only.

The difference in rates calculated with the two kinds of tracers used in this study deserves special comment. Since no significant difference between the intermixing amounts of glucose was found when they were determined simultaneously by the two tracers using Method A, the difference in the rates is due to the consistently higher decay constant of the exponential specific activity *vs* time curve of 6-^{3}H glucose. Such a difference was indicated in the experiments by Dunn and Strahs (20) on rats, and attributed to an exchange of ^{1}H for ^{3}H on the 6th C-atom of glucose, possibly in the liver. The ratio of the exponential decay constants reported in this paper indicates a smaller difference between the rates of removal of ^{3}H and ^{14}C from the glucose pool than appears from the changes in the "isotope ratio" reported by Dunn and Strahs (20). This may be due to a difference in species. The data presented in Tables IIA and IIB demonstrate that such a difference in the handling of the two kinds of tracer glucose in the body leads to a difference in the magnitude of the calculated rates, also when a primed infusion (rate-dilution) method, Method B, is used. Since the calculated rates of appearance of glucose were found to check with known rates of glucose infusion in eviscerated dogs when ^{14}C glucose was used as a

tracer (6), it seems that rates calculated employing 6-^{3}H glucose as tracer are spuriously high.

It has been recognized (2) that larger intervals must exist between injections of tracer than between the intervening collection of samples for determining tracer concentration as a function of time, representing a limitation on tracer Method A. As a consequence it was noted that quick changes in glucose kinetics cannot be followed efficiently with this method. Table III demonstrates the possibility of using two forms of tracer glucose, so that the interval between tracer injections—and thus between successive determinations of the rate of appearance of glucose and the "pool"—can be reduced to much less than half. However, different forms of tracer glucose, labeled at different carbon atoms with ^{14}C, will have to be used, since 6-^{3}H glucose was found to yield spuriously high results.

SUMMARY

The rate of endogenous glucose production was calculated in fasting conscious dogs utilizing data obtained simultaneously with two different tracers, (6-^{3}H)glucose and glucose uniformly labeled with ^{14}C, and by two methods, A and B. Method A utilized successive measured injections of tracer (quantity dilution), while in Method B the rate was calculated by rate dilution employing the primed infusion of the tracer at a constant rate.

Statistical analysis of the results demonstrated that both tracer *methods* yield equal rates for glucose production.

However, 6-^{3}H glucose as a *tracer* yielded glucose production rates significantly higher than when ^{14}C glucose was used as tracer. Since with Method A the use of ^{14}C glucose had been shown to yield valid glucose production rates as eviscerated dogs, 6-^{3}H glucose is rejected as a tracer for glucose transfer rate studies in dogs.

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Due to unforeseen reasons, the "2nd International Conference on Methods of Preparing and Storing Labelled Compounds" Brussels June 6-11, 1966 has been postponed, and will take place at the following dates:

November 28—December 3, 1966