VALIDITY OF A TRACER-INJECTION METHOD FOR STUDYING GLUCOSE TURNOVER IN NORMAL DOGS

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In the measurement of glucose turnover one method—that of successive measured injections of tracer (SMIT) (*1*)—has been partially validated. Although an absolute validation of SMIT has been carried out in eviscerated dogs (*2,3*), it has not previously been done in normal animals because normal production of glucose makes matching of infused rate with tracer-determined rate impossible. What might be called a nonabsolute validation has also been reported (*4*) in which the injection method is compared to the equally unvalidated infusion method of Steele (*5*) under near dynamic steady-state conditions.

However, if labeled glucose will trace the entry of any unlabeled glucose into the intermixing region, it should also measure the entry of any differently labeled glucose into the same region. Therefore an absolute validation of any tracer for glucose can be made if the measured appearance rate is the rate for a type of glucose which is not confused with glucose produced by the liver, and yet is chemically indistinguishable from it. With this in mind, we have undertaken a validation by measuring the rate of appearance of 1-^{14}C-glucose by the SMIT method using injections of 6-^{14}C-glucose.

MATERIALS AND METHODS

We used nine normal unselected dogs in twelve experiments. Six of the dogs were used in the six three-stage experiments outlined below. Of these six dogs, four were studied with the glucose at near dynamic steady-state conditions, while the remaining two were studied with a linearly increasing and a linearly decreasing glucose appearance rate, respectively. The seventh dog was used in two single-stage experiments, one with a linearly increasing and one with a linearly decreasing glucose appearance rate. The eighth dog was used in three single-stage experiments with steady, linearly increasing and linearly decreasing glucose appearance rates. The ninth dog was used in a single-stage experiment with a near dynamic steady state. Changes in the appearance rate of a substance do not represent a departure from a dynamic steady state if they are paralleled by corresponding changes in disappearance rate. However, linear changes in the infusion rate of the substance will induce similar, almost linear, changes in the intermixing mass of the substance; hence they represent departures from dynamic steady states. The parameters of the experimental design are given in Table 1.

1-^{14}C-glucose was used as the analog of unlabeled endogenous glucose and was infused at a known rate while 6-^{14}C-glucose was injected and used as the tracer to calculate the tracee infusion rate by the same technique used to calculate the production rate

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TABLE 1. PLAN OF EXPERIMENTS

Dog No.	Sex	Initial weight (kg)	Experiment No.	State of experiment	No. of stages
1	F	14.8	1	DSS*	3
2	M	11.8	2	DSS*	3
3	M	13.6	3	DSS*	3
4	M	10.0	4	DSS*	3
5	M	10.5	5	increasing R_a	3
6	F	16.3	6	decreasing R_a	3
7	F	11.1	7a	increasing R_a	1
7	F	11.1	7b	decreasing R_a	1
8	M	15.2	8a	DSS*	1
8	M	15.4	8b	increasing R_a	1
8	M	16.1	8c	decreasing R_a	1
9	M	12.6	9	DSS*	1

* DSS = dynamic steady state.

of unlabeled glucose in the usual SMIT method. If the calculated infusion rate of the tracee, determined by the injected tracer, is the same (within error limits) as the known value, then the tracer technique being tested can be said to give valid appearance rates under those conditions.

To calculate the rate of entry of the tracee, it was necessary to define a new specific activity, the "comparative" specific activity (G), which at any given time is the ratio of tracer (6-^{14}C) label to tracee (1-^{14}C) label in plasma glucose. With this one modification the rate calculation for the method tested remains unchanged.

Thus the comparative specific activity has been defined as

$$G = \frac{\text{dpm tracer (6-}^{14}\text{C)/mg plasma glucose}}{\text{dpm tracee (1-}^{14}\text{C)/mg plasma glucose}}.$$

G is independent of the amount of unlabeled plasma glucose or of any changes in that amount which changing rates of production or disappearance of unlabeled glucose can produce; G and any variation in G is not a function of unlabeled glucose values.

The intermixing amount of the tracee is calculated by extrapolating a single exponential function, fitted to paired values of G and time, back to the time of injection of the tracer. The value of G at that time is G_0. If n′ is the intermixing amount of the tracee at the time of tracer injection, and if n is the amount of injected tracer, then $n' = n/G_0$.

In conventional tracer experiments this relationship is usually $n' + n = n/G_0$ because the injected tracer is also considered part of the intermixing mass, but in the double-tracer method the tracer is not part of the tracee, and the first formula applies.

Clearly, because n and G are independent of unlabeled, endogenously produced glucose, n′ is also independent of endogenously produced glucose. The rate of appearance (R_a) of the tracee is calculated as

$$R_a = n'k'$$

where $$k' = \frac{1}{G}\left(\frac{dG}{dt}\right),$$

and hence R_a is also independent of any influence from unlabeled endogenously produced glucose.

Since both labeled glucoses used in this study contained ^{14}C, but in different positions, it was necessary to show that 1-^{14}C glucose from the infusion would not be used or transformed in such a way that any appreciable amount of ^{14}C from this source would appear in the sixth carbon position and interfere with determinations of the injected tracer. Similarly it had to be shown that ^{14}C from the injected 6-^{14}C-glucose would not appear in significant quantities in the first carbon position and interfere with the determinations of the tracee levels.

To eliminate this possible source of error, experiments on the first six dogs were done in three stages. The first stage was merely a primed infusion of 1-^{14}C-glucose (tracee) with periodic blood sampling for 120 min. The level of 6-^{14}C-glucose which appeared over that period was expressed as a fraction of the infusion level of 1-^{14}C-glucose and was found to be too small to influence the results significantly. The second stage of the experiment, carried out a few days later, was a 6-^{14}C-glucose injection (the tracer) followed by 90 min of periodic blood sampling; accumulating levels of the tracee were also expressed as a fraction of the levels of the tracer. These too were found to be negligible.

Nonetheless, measurements based on these first two stages were applied as corrections to the third or combined stage which was performed a week after the second stage (Fig. 1). The corrections consisted of small, almost equal, subtractions from both 1-^{14}C and 6-^{14}C levels, affecting final calculated rates by less than 2%. A further small correction was added to the known infusion rate to account for the endogenous resynthesis of the tracee, as indicated by stage 1, but was not necessary for the tracer because data for the tracer were estimated from a function extrapolated to time of tracer injection.

The last six experiments were undertaken in Dogs 7, 8 and 9, and because the corrections deduced from the first two stages had been small and con-

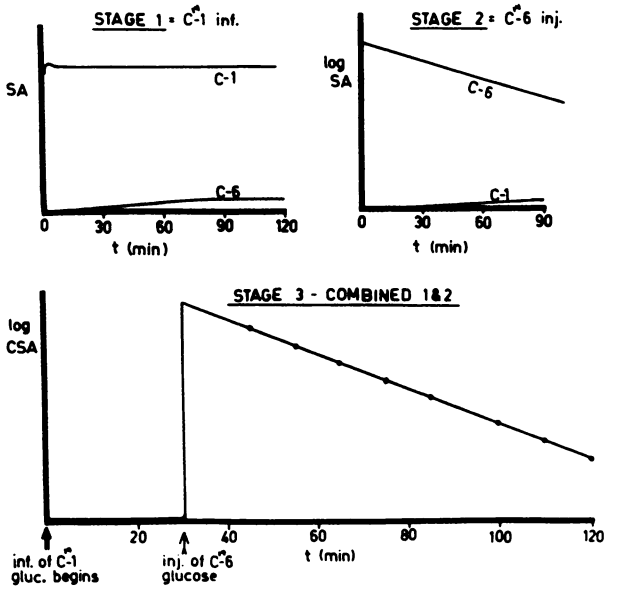

FIG. 1. Graphic representation of three-stage validation experiment: Stage 1, tracee infusion; Stage 2, tracer injection, and Stage 3, combined. Used in Experiments 1–6.

sistent in the previous six dogs, only the combined stage was done in Experiments 7a, 7b, 8a, 8b, 8c and 9.

In the 6 experiments with linearly increasing or decreasing glucose appearance rates (5–7b, 8b and 8c), the rate of infusion was adjusted incrementally with a Sage pump every 2 min during the 120-min infusion. The rate of change of the infusion was such that the infusion rate was either halved or doubled during the middle 100 min of the 120-min infusion.

In all experiments infusions were made through an in-dwelling catheter into a cephalic vein, and in all experiments except No. 3 which is discussed below tracer injection and blood sampling was done through a cannula in a saphenous vein.

Six to eight blood samples were drawn in the 90 min following each tracer injection. Fifteen or 20 min were allowed for intermixing before the first sample was taken. The separation of 6-C from 1-C glucose carbon was by Reichard's technique (*6*) and the activity was measured by liquid scintillation counting. Plasma glucose was determined by the glucose oxidase method (*7*). Estimates of the error of the calculated rates were made by the standard method of combining the errors of the component determinations.

RESULTS

A summary of the validity of the appearance rates determined is given in Table 2.

Experiments 1–4, 8a and 9 deal with near dynamic steady states with respect to the tracee. The second experiment has a ±8% indeterminancy in the ratio of calculated R_a to known R_a because of partial failure of an infusion pump. The estimates of error of other processes are not changed.

TABLE 2. COMPARISON OF DETERMINED-TO-KNOWN RATES

Experiment No.	State of Experiment	R_a tracer-det. / R_a known
1	DSS*	101%
2	DSS*	102–118%
3	DSS*	176%
4	DSS*	107%
5	increasing R_a	131%
6	decreasing R_a	119%
7a	increasing R_a	139%
7b	decreasing R_a	98%
8a	DSS*	109%
8b	increasing R_a	141%
8c	decreasing R_a	112%
9	DSS*	107%

* DDS = dynamic steady state.

Note: Estimated relative error from combined errors of component processes was ±12% (Exp. 1), ±10% (Exp. 2–7b) and ±7% (Exp. 8a–9).

The third experiment had a calculated R_a equal to 176% of the known R_a. This was attributed to the only difference in execution between this experiment and the others—the fact that both the tracer injection and blood sampling in this experiment were done through a jugular cannula so that both infusion and sampling were from the venous portion of the superior circulation; this did not allow time for the adequate intermixing that was possible when the samples were taken from the inferior venous circulation in the other experiments. The inevitable falsely high 1-^{14}C levels and the resultant falsely high intermixing amount produced the inflated value of the calculated R_a derived from the formula $R_a = n'k'$ because of the deceptively high value of n'.

Experiments 5, 6, 7a, 7b, 8b and 8c show that the SMIT method is less valid with fairly rapid departures from dynamic steady state than it is for situations near dynamic steady state. Experiments 5 and 6 suggest that in circumstances where R_a is increasing, the method will give a relatively higher value for R_a than when R_a is decreasing. This hypothesis is confirmed by the statistically significant difference between Experiments 7a and 7b and between Experiments 8b and 8c. These two pairs of experiments were each carried out in the same dog to obviate the effects of variation between subjects. By grouping Experiments 5, 7a and 8b and Experiments 6, 7b and 8c, one can see that in the case of increasing R_a the tracer-determined rate was higher by 31–41% than the known rates. In the case of decreasing R_a the tracer-determined values of 119%, 98% and 112% of the known rates are not significantly different from the results in the dynamic steady state (Experiments 1, 2, 4, 8a and 9).

The tracee infusion which comprised the first stage of the first six experiments showed that as soon as the 1-^{14}C-glucose tracee infusion began, 6-^{14}C-glucose appeared in the plasma. This amount of 6-^{14}C was found to be 0.4 ± 0.2% of the amount of 1-^{14}C, and was due to impurities in the manufacture of the 1-^{14}C-labeled glucose. However, during the 120 min of the infusion, this level rose in all six cases, so that at 120 min the amount of 6-^{14}C relative to 1-^{14}C was 2.4 ± 1.1%. This was attributed to the recycling of glucose carbons into new endogenous glucose production. There was evidence toward the end of the 120 min period that the level of 6-^{14}C-glucose was reaching a plateau.

Similarly, the second stage of the first six experiments, which was a 6-^{14}C-glucose injection, showed some shift of the label to other carbon positions. Immediately after injection the ratio of ^{14}C on the first five glucose carbons to ^{14}C on the sixth glucose carbon was 1.5 ± 0.6%. This rose over the 90-min sampling period to 2.3 ± 1.2% of the initial level of 6-^{14}C.

DISCUSSION

The SMIT method yields values close to correct for the calculated glucose appearance rate in normal dogs near a dynamic steady state. Results in Experiment 3 shown on Table 1, however, are a reminder that to achieve this, intermixing criteria must be satisfied. While it is clear that intermixing was quite unsatisfactory in this experiment, it is not likely to be perfect under any physiological situation. Thus even in experiments in which conditions for intermixing appeared satisfactory, there was still a tendency to give slightly overestimated values. It is important to remember that in this context not only the intermixing of the tracer, but also that of the tracee, will influence the results. There is evidence that injected tracer glucose is satisfactorily intermixed after 15–20 min.

In experiments not concerned with validation, the tracee is unlabeled, endogenously produced glucose. Since this glucose is produced continually, some of the more recently produced is more centrally distributed than the injected tracer, which after a suitable "intermixing" period reaches the final state of its distribution throughout the system. Such a situation would lead to an overestimation of the appearance rate. In this validation study Experiments 1, 2, 4, 8a and 9 yield glucose rates determined by SMIT of 101–110% of the true value with the mean at 107%. Problems of tracer intermixing within a system have been discussed in general terms by Steele (*8*), Bergner (*9*) and Forbath *et al* (*10*).

It appears that for all practical purposes the SMIT method is satisfactory in near dynamic steady states for glucose. It follows that the tracer infusion method described by Steele (*5*) must also be valid for glucose under comparable circumstances (*4*).

The experiments conducted under nonsteady-state conditions showed that the SMIT method yielded high values for R_a for glucose when R_a increased in a rapid linear fashion after the tracer was injected, but did not show a corresponding drop in measured R_a when compared to true R_a in the case of a rapid linear decrease in R_a.

In part this may be caused by the fact that in the increasing R_a and decreasing R_a cases, the infusions were mirror images of each other. Thus while the absolute rate of change of R_a per unit time was the same in both cases, at the time of the tracer injection the proportional rate of change in R_a was much higher in the experiments with R_a increasing than in those where R_a decreased.

A weak point in the SMIT technique is the arbitrary fitting of a single exponential function to the specific activity versus time curve (*11,12*). While it is true that a perfect extrapolation of such a curve to injection time will always give the correct appearance rate in theory the use of a single exponential function is theoretically justified only if the appearance rate and the intermixing amount ("pool") do not change with respect to time. Empirically, a single exponential function may describe a specific activity versus time curve satisfactorily in many other cases, but it will introduce a serious limitation into the interpretation of the data since its exponential constant (k) is not permitted to vary at any point along the curve. Thus k becomes fixed at a value not representative of time zero, but rather of a time midrange in the sampling period. This in turn affects the zero-time intercept of the curve so that a partial compensatory effect on the calculated appearance rate is felt. Thus when R_a varies, the SMIT technique probably measures an appearance rate that lies between time zero and the midpoint of the sampling period.

This in fact is demonstrated in the three experiments where R_a had been *increased* linearly. The three experiments where R_a has been decreased gave results commensurable with those of constant rates.

SUMMARY

A method is described for testing the validity of absolute appearance rates of a given substance determined *in vivo* in plasma by isotopic labels. In twelve experiments on unanesthetized dogs the method of successive measured injections of tracer (SMIT) was tested for glucose, six times in near dynamic steady-state conditions and three times each with linearly increasing and decreasing appearance rates. In these tests a known rate of appearance (i.v. infusion) of 1-^{14}C-glucose was compared with its calculated rate using a single injection method with 6-^{14}C-glucose as tracer. Data on recycling of ^{14}C into endogenously produced glucose are also presented, as well as some observations on intermixing. The absolute appearance rate determined by the SMIT method was found to be valid within 10% for glucose in normal dogs near dynamic steady states. With a rapid linear increase in the rate of appearance of 1-^{14}C-glucose the tracer-determined rate was 31–41% higher than the known value at time of injection, while with a similar linear decrease in the

appearance rate, the tracer-determined rate did not vary significantly from that determined in the near dynamic steady state cases.

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