Measurement of Size and Turnover Rate of Body Glucose Pool by the Isotope Dilution Method

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ABSTRACT

Glucose uniformly labeled with C\textsuperscript{14} was administered intravenously in minute amounts to unanesthetized dogs in the postabsorptive state as an initial dose followed by a continuous infusion. The C\textsuperscript{14} content of the plasma glucose was determined at intervals. When the ratio of priming dose to infusion rate was suitable, the plasma glucose specific activity remained relatively constant during the 60-180 minute period of the infusion, whereas during the first 60 minutes it decreased in a manner indicating the presence of two compartments exchanging glucose with the plasma glucose compartment. For a typical experiment of this kind, the amounts of glucose in these compartments and the rates at which these bodies of glucose underwent mixing with the plasma glucose were calculated. It was then possible to determine the magnitude of the errors in body glucose pool size and inflow-outflow (i.e., turnover) rate which are made when measurements are carried out at various ratios of priming dose to infusion rate. These errors are incurred when the usual simplifying assumption is made that instantaneous mixing occurs throughout the body glucose pool. It was found that there is an extensive range of ratios of initial dose to infusion rate over which the errors are small enough (less than \( \pm 5\% \)) to be ignored; it is not necessary to carry out a preliminary experiment on each dog to establish a desirable ratio. Average values of body glucose pool size and glucose inflow-outflow rate obtained in 10 experiments with seven normal dogs are compared with values which have been reported by others. The physiological significance of these parameters measured by isotope dilution is discussed.

ISOTOPICALLY-TAGGED glucose has been used in the measurement of the size and turnover rate of the body glucose pool in the rat (1-3), the dog (4-7) and in man (8). Surprisingly, the method has not been critically considered with respect to the experimental conditions required for accurate measurements and the physiological meaning of the parameters of carbohydrate metabolism which are determined.

Searle et al. (7) have found that the commonly used method, which involves a single injection of C\textsuperscript{14} glucose, gives values for body glucose pool size which seem to be too high. They accept from such an experiment the rate of fall of plasma glucose C\textsuperscript{14} content (specific activity) as a measure of the ratio between pool size and inflow-outflow rate. They then perform a second experiment 3 weeks later on the same dog, using a ratio between priming dose and infusion rate of C\textsuperscript{14} glucose which is calculated from the results of the first experi-

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The second experiment is successful if a nearly constant plasma glucose specific activity is maintained beginning at the end of the 1st hour. Under these conditions the absolute values of pool size and inflow-outflow rate can be calculated from the value of this constant plasma glucose specific activity.

A method has been in use for some time in our laboratory which is similar to the second experiment of Searle et al. in that it employs an initial intravenous priming dose of C14 glucose followed by a continuous intravenous infusion. However, a treatment of the data has been developed which allows the determination of pool size and inflow-outflow rate from this experiment alone, thus eliminating the initial single injection procedure.

In the course of a long series of such experiments on normal and hypophysectomized dogs, it has become clear that the fall in plasma glucose specific activity which is due to the slow mixing of the priming dose with the body glucose pool is evident for about an hour. This mixing delay is responsible for errors in the calculations of glucose pool size and inflow-outflow rate from the observed changes in plasma glucose specific activity. These errors are incurred when the usual simplifying assumption is made that the body glucose is a well-mixed pool.

The present report evaluates these errors for the various procedures by which labeled glucose may be used for the measurement of glucose pool size and turnover rate. It defines the condition under which the simplifying assumption referred to above leads to calculated values for pool size and turnover rate which are approximately correct. A critical analysis of the physiological significance of these values as calculated from isotope data is presented.

**MATERIALS AND METHODS**

**Animals.** The experiments were carried out on unanesthetized adult male or female dogs which had been trained to lie quietly on a table under the conditions of the experiment. The animals were in the postabsorptive state (17–18 hr. fasted). The standard maintenance diet has been described (9). The same dog could be used again for a similar experiment after an interval of at least 3 weeks.

**Apparatus.** Respiratory CO₂ containing C14 was collected by means of the 'respiratory mask' and the CO₂ absorber shown in figure 1. The respiratory mask consisted of a transparent plastic cylinder, 18 inches long and 10 inches in diameter. One end plate of this cylinder, containing a flanged aperture, was removable and also split horizontally to allow it to be fitted around the dog's neck. Additional end plates with different size apertures were available to fit the various dogs. A sheet rubber (dentist's dam) collar, about 6 inches wide, was fitted around the dog's neck and cemented to the flange of the aperture, thus making an airtight seal between the neck and the mask. An additional opening was provided at each end of the cylinder to allow air to enter and be withdrawn.

Air was drawn through the respiratory mask at the rate of 30 l/min., thence through a flow meter (to allow continuous observation of the flow rate) and subsequently through the sintered glass spargers (C porosity) connected in parallel for dispersion of the air drawn from the respiratory mask. The lid of the CO₂ absorber pot was made of one-half inch plastic, grooved to fit the pot rim and sealed to the pot with a rubber gasket.

C14 Glucose. Uniformly tagged radioactive glucose of high specific activity was prepared. Isolated Canna leaves were exposed to an atmosphere of C14O₂ under illumination for 24 hours by the method of Gibbs et al. (10). The alcoholic extract of the leaves after preliminary purification was subjected to hydrolysis with HCl and the resulting mixture of glucose and fructose was separated chromatographically on a column of 'Magnesol' by the method of Noggle and Bolomey (11). The radiochemical purity of the isolated glucose was established by paper chromatography and radioautography. The preparation had a specific activity of 20 μc/mg glucose and was shown to contain less than 1% of its C14 in compounds other than glucose.

The uniform distribution of the C14 among the carbon atoms of the glucose was ascertained by degrading the glucose by the method of Wood et al. (12). A bac-
Material fermentation was employed to convert glucose to lactic acid which was subsequently degraded chemically in steps so that each lactic acid carbon was obtained as CO₂ which was assayed for C¹⁴.

**Experimental Procedure.** The dog was placed on the table with its head enclosed in the respiratory mask (fig. 1) and the air flow was started. An indwelling catheter was inserted into the bladder for continuous urine collection throughout the experiment. A priming dose of about 3/4 mg of glucose (containing about 15 μc of C¹⁴) in 10 ml saline was injected into the saphenous vein. Simultaneously, an intravenous infusion of C¹⁴ glucose in saline was started. The infusion solution was delivered at about 0.5 ml/min. by a constant infusion pump (Bowman Model no. 2) through a polyethylene tube inserted a distance of 3-4 inches into the cephalic vein (exposed under local anesthesia). The infusion solution contained enough C¹⁴ glucose so that 15 μc was delivered in about 110 minutes.

Samples of blood were drawn from the exposed femoral vein (or from the femoral artery), and collected in heparinized tubes for the determination of the concentration of plasma glucose and its specific activity. At least four samples were collected during the 60- to 180-minute period of the constant infusion.

At the end of the experiment the dog was transferred to a respiration chamber with a transparent plastic top and kept there until the respiratory C⁴O₂ reached a low level (4 days). During this period the chamber was aerated at the rate of over 100 l/min. by means of a blower which delivered the outflowing air into a chemical hood.

**Chemical Analyses.** Determinations of plasma glucose were made by the Hagedorn-Jensen method (13) on aliquots of Somogyi zinc-barium filtrates (14). Another 10-ml aliquot of each filtrate was mixed with an exactly measured (about 13 mg) quantity of C⁹ glucose, and the phenylosazone derivative was prepared, recrystallized and dried for combustion (6). Combustion of the phenylosazones to C⁴O₂ and counting of the C⁴O₂ in the gas phase were done by the method of Van Slyke et al. (15).

**RESULTS**

The specific activities (μc/gm C) observed for the isolated phenylosazones were corrected for dilution with carrier nonradioactive glucose and with phenylhydrazine carbon atoms, and were then expressed as μc of C¹⁴ per gram of original plasma glucose carbon. These values were plotted against time as shown in figure 2, which represents an experiment performed on a normal dog.

In a series of 10 normal dogs and 7 hypophysectomized dogs to which the method was applied, it was found that the observed plasma glucose specific activities at 20 minutes and 40 minutes were always higher than would be expected from the values which were observed subsequent to 60 minutes after the priming dose. Figure 2 shows this effect in an experiment in which a large number of observations were made during the first 60 minutes. The early phase of this experiment is typical of the way in which the priming dose of C¹⁴ glucose mixes with the glucose of the body pool.

Since the mixing of glucose added to the blood plasma is not instantaneous with respect to the whole of the body glucose pool, it is necessary to inquire whether or not there are conditions under which calculations can be simplified by a method which ignores the process of mixing.

By a mathematical treatment of the whole process, including the mixing phase, it is possible to evaluate the errors in the determination of glucose pool size and of inflow-outflow rate which result from the simplified method of
calculation which ignores the mixing process. It is shown that these errors are small when an approximate balance between priming dose and infusion rate is achieved.

In the simplified method the calculation of the glucose pool size and the inflow-outflow rate is carried out by means of equation 1.

$$\bar{S}A_t = \frac{F}{g} + \left( \frac{P}{C_0} - \frac{F}{g} \right) e^{-t/t_C}$$  \hspace{1cm} (1)

$\bar{S}A_t$ = plasma glucose specific activity in $\mu$C C$^{14}$/gram carbon at time $t$; $t$ = time in minutes after the priming dose was injected; $F$ = infusion rate in $\mu$C C$^{14}$/min. (glucose in 'weightless' amount); $g$ = inflow-outflow rate of glucose in grams carbon/minute (inflow equals outflow at constant blood sugar level); $P$ = priming dose in $\mu$C C$^{14}$ (glucose in 'weightless' amount); $C_0$ = body glucose pool size in grams of carbon.

This equation treats the body glucose as an instantaneously mixed pool in a steady state of turnover. In this idealized pool, the initial value of specific activity ($P/C_0$) is determined by the dilution of the priming dose ($P$) by the body glucose ($C_0$). The eventual value of plasma glucose specific activity approaches as an asymptote the value $F/g$, a value which is independent of the priming dose and depends only on the dilution of the infused C$^{14}$ ($F'$) by the C$^{12}$ glucose ($g'$) flowing into the pool from the liver. The derivation of equation 1 is given in APPENDIX I.

In using equation 1, the observed values of plasma glucose specific activity ($\bar{S}A_t$) between 60 and 180 minutes after the priming dose are employed. Earlier values are too much influenced by the mixing process and values subsequent to 180 minutes have shown a tendency to be too high because of recycling. Recycling refers to the release into the blood of labeled glucose resynthesized from C$^{14}$ fragments derived from the injected labeled glucose. This effect is shown by data presented in the succeeding paper (16).

The values of plasma glucose specific activity between 60 and 180 minutes are plotted against time on ordinary graph paper. A smooth curve drawn through the points is extrapolated back to zero time and forward to the asymptotic value expected to be approached after a long period of infusion. Using the zero time intercept as the trial value of $P/C_0$ and the asymptote as the trial value of $F/g$, values for $C_0$ and $g$ are obtained since $P$ and $F$ are known. The trial values are inserted in equation 1 and a few values of $\bar{S}A_t$ between 60 and 180 minutes are calculated to see whether or not they fall on the observed curve for plasma glucose specific activity. If not, a new value of $P/C_0$ or $F/g$ (or both) is selected and the above process repeated until satisfactory values of $g$ and $C_0$ are obtained. In our experiments a punched-card electronic computer was used to fit the observed points to equation 1 by the least squares method after the initial approximation had been made; however, with practice a satisfactory fit can be obtained by hand after only a few trials. If a straight horizontal line is the best fit to the points, the relationship $\bar{S}A_t = P/C_0 - F/g$ may be used to calculate $C_0$ and $g$.

In the case of the curve shown in figure 2, the values of $g$ and $C_0$ obtained by the least squares method were 0.0149 gm carbon/min. and 1.75 gm carbon, respectively. The curve described by equation 1 for these values of $g$ and $C_0$ is represented in figure 2 by the dotted line designated as $\bar{S}A_t$.

The more complicated calculation which takes into account the mixing process in the body glucose pool is described in APPENDIX II. Here the reasonable assumption is made that the mixing process consists of the exchange of glucose between a central plasma compartment and peripheral interstitial fluid compartments. It is then possible to derive from the shape of the plasma glucose specific activity curve (fig. 2) the number of peripheral compartments, the size of each compartment, and the rate of glucose flow in and out of each compartment. As a result of this information, it is possible to derive the specific activity vs. time curve for each of the compartments. In figure 2 the curve $Q$ represents the plasma glucose specific activity and is the curve fitted (by trial and error) to the observed values (open circles). The curve $I$ represents the calculated specific activity of the rapidly equilibrating part of the body glucose pool and the curve $L$ represents the specific activity of the slowly equilibrating part of the body glucose pool. The values of glucose inflow-outflow ($g$) and glucose pool size ($C_0$) calculated by this method (see APPENDIX
were .0153 gm carbon/min. and 1.67 gm carbon, respectively. Thus the inflow-outflow rate calculated from the simplified relationships (eq. 1) is about 3\% lower than that calculated from the more complex expression (APPENDIX II). The glucose pool size calculated from the simplified relationship is about 4\% higher than that calculated from the complex expression. It should be noted that this comparison is based on a real experiment in which errors in observation affect the differences in results by the two methods. In an idealized experiment in which the plasma glucose specific activity would follow exactly the curve Q of figure 2, the value of pool size derived from equation 1 would be about 4\% lower than that given by the more complex expression, and the value of inflow-outflow rate would be about 1\% higher.

APPENDIX II and the legend for figure 3 describe how the errors involved in the estimation of pool size and inflow-outflow rate by the use of equation 1 can be calculated for ratios of priming dose to constant infusion rate other than the ratio used in the experiment in figure 2. These errors are summarized in figure 3. It is seen that results accurate to within ±2.5\% for \( g \) and ±5\% for \( C_0 \) are obtained in the region in which from 50\% to 110\% of the ratio of infusion rate to priming dose shown in the experiment of figure 2 are employed. Satisfactory accuracy is afforded by the use of a standard ratio of priming dose to infusion rate which results in a relatively constant (and preferably slightly declining) plasma glucose specific activity between 60 and 180 minutes. For the experiment illustrated by figure 2, the closest approximation would have been attained if the infusion rate had been about 70\% of the rate actually used; under this condition the plasma glucose specific activity would have declined from 7.80 to 6.76 during the 60 to 180-minute interval.

Values of body glucose pool size and inflow-outflow rate determined by the use of equation 1 for a large number of normal and hypophysectomized dogs are discussed in a separate publication (16). Table I summarizes our results with normal dogs and compares these with values previously reported by other investigators.

Figures 4 and 5 give the specific activities of the various compartments of the body glucose pool as calculated for the two extreme ratios of priming dose to infusion rate considered in figure 3. In figure 4 the infusion rate is zero (the single injection experiment) and in figure 5 the priming dose is zero (constant infusion with no initial injection). The basic cause of the errors incurred by the use of equation 1 is seen to be the fact that the plasma glucose specific activity is not characteristic of the specific activity of the whole body glucose pool.

**DISCUSSION**

**Body Glucose Pool Size.** The material which is called the body glucose pool is believed to consist for the most part of the glucose dissolved in the blood and the interstitial fluids. In discussing the possibility that glucose in other locations may be included, it is necessary to deal with the fact that the method of isotope dilution is capable of falsely including as 'glu-
Table 1. Glucose Utilization and Glucose Space in Normal Dogs as Determined by Isotope Dilution Methods

<table>
<thead>
<tr>
<th>Method, Ref.</th>
<th>No. of Exp.</th>
<th>Dog Wt. (W, Mean Value and Range)</th>
<th>Glucose Utilization (Mean Value and Range)</th>
<th>Plasma Glucose Conc. (Mean Value and Range)</th>
<th>Glucose Space (Mean Value and Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single dose*</td>
<td>7 (4 dogs)</td>
<td>6.9 (5.6-8.6)</td>
<td>0.58 (0.4-0.8)</td>
<td>118 (105-140)</td>
<td>38 (27-63)</td>
</tr>
<tr>
<td>Two step†</td>
<td>3 (5 dogs)</td>
<td>6.8 (6.4-8.0)</td>
<td>0.53 (0.5-0.63)</td>
<td></td>
<td>31 (30-32)</td>
</tr>
<tr>
<td>Present‡</td>
<td>10 (7 dogs)</td>
<td>19.1 (13.8-24.1)</td>
<td>0.49 (0.42-0.67)</td>
<td></td>
<td>27 (22-31)</td>
</tr>
</tbody>
</table>

* Results obtained by analysis of the plasma glucose specific activity vs. time curve after a single injection of C14 glucose.
† Results obtained by the method in which the ratio of priming dose to infusion rate is determined in a preliminary experiment.
‡ Results obtained by the present method in which a standard ratio of priming dose to infusion rate is employed.

the carbon of other compounds which are in rapid reversible equilibrium with glucose.

The cells of the extrahepatic tissues contain minimal amounts of free glucose under ordinary circumstances and so could contain only a small fraction of the body glucose pool. With regard to the possibility that extrahepatic cells deliver glucose to the blood, Drury et al. (17) demonstrated that in the hepatectomized rabbit no C12 glucose is released from any extrahepatic tissue to dilute the C14 glucose of the circulating blood, except for a small amount from the kidneys. The kidneys release glucose only during severe hypoglycemia.

In the case of the liver there is no doubt that glucose can both enter and leave the hepatic cells. Furthermore, inside the hepatic cell the carbon of glucose enters and leaves the intermediary metabolites. Thus the liver is capable of transferring carbon atoms back and forth between extracellular glucose, the intermediary metabolites of the hepatic cells and the stable compounds (e.g., glycogen, protein and fat) of the hepatic cells. In the liver the boundaries of the body glucose pool as measured by isotope dilution are vague. Liver glycogen has a relatively slow 'turnover rate' (18-20), and we have observed that the injection of adrenaline at the end of a 5-hour infusion of C14 glucose results in a release of glucose that is about 90% C12 glucose and about 10% glucose labeled to the same degree as the plasma glucose. Thus the error in the calculation of glucose pool size attributable to liver glycogen is negligible.

The body glucose pool is most strictly defined as the glucose which dilutes the injected C14 glucose, but our working concept of this material is that it is mostly glucose in solution in the extracellular fluids. Body glucose pool size should thus be proportional to body weight and might reasonably be expected to vary in direct proportion with the prevailing plasma glucose concentration in the postabsorptive state. To compare results from one dog to the next, a glucose 'space' can be calculated which takes into account the variable plasma glucose concentration. The small differences in water content of plasma and interstitial fluid are neglected, and glucose space is calculated by finding what total volume of fluid of the same glucose concentration as plasma is required to contain the number of grams of glucose in the body pool. The volume so calculated is converted to kilograms, assuming a fluid specific gravity of 1000 and is expressed as percentage of body weight.

In the normal dog in the present experiments, the glucose space so calculated constitutes 27% of the body weight (16). If the glucose of the erythrocytes, in which the glucose concentration was found to be 30-40% of that in the plasma, is subtracted from the glucose pool, the corrected glucose space (25% of body weight) approximates the reported inulin space of 20-25% of body weight in the dog (21, 22). Since inulin is distributed only in the extracellular fluid, it would appear that the glucose pool may also be distributed mainly in the extracellular fluid. Such a distribution suggests that the glucose pool represents glucose in transit from the liver to tissue cells and, as such, constitutes a fundamental parameter of carbohydrate metabolism.

Relationship Between Plasma Glucose Specific Activity, the Glucose Pool Size and the Glucose Inflow and Outflow Rates. In the present method the size of the glucose pool is established by the dilution of the injected C14 as shown by the values of plasma glucose specific activity during the 60 to 180-minute period. On the other hand, the inflow to the extracellular fluid, it would appear that the glucose pool may also be distributed mainly in the extracellular fluid. Such a distribution suggests that the glucose pool represents glucose in transit from the liver to tissue cells and, as such, constitutes a fundamental parameter of carbohydrate metabolism.

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Taken together, both influences are described by equation 1.

After a nearly uniform C\textsuperscript{14} concentration has been established in the body glucose pool (after 60 minutes, see fig. 2), it is the inflow of C\textsuperscript{12} glucose from the liver which dilutes the C\textsuperscript{14} glucose of the constant infusion. An increase in outflow rate from the body glucose pool (e.g., after insulin) has no effect on the specific activity of the plasma glucose. However, if the C\textsuperscript{12} glucose inflow rate from the liver changes (e.g., in response to hypoglycemia), there is then a change in plasma glucose specific activity.

Glucose Utilization as Related to the Inflow-Outflow Rate. In the postabsorptive state, in the absence of a change in plasma glucose concentration, it is concluded that the rate of glucose inflow into the body pool equals the rate of outflow from the body pool. Both rates are given by the same number, the inflow-outflow rate (\(g\)). The disappearance of glucose from the body pool constitutes sugar uptake or utilization by the tissues since no glucose is excreted. The term ‘utilization’ is to be distinguished from combustion, which denotes conversion of sugar CO\textsubscript{2} and which represents the fate of only a part of the sugar which is ‘utilized.’

The inflow-outflow calculated from isotope data differs from ‘glucose output by the liver’ as measured by arterio-hepatovenous glucose concentration difference and the rate of blood-flow through the liver. The liver itself probably utilizes some plasma glucose, and the isotope method measures, along with inflow-outflow, that labeled glucose which the liver removes from the blood and replaces with C\textsuperscript{12} glucose. The magnitude of this process is as yet not known. Since no glucose concentration change results, this kind of ‘utilization’ and ‘liver output’ does not enter into the results obtained by the hepatic vein catheterization procedure. Of course, labeled glucose which goes from the blood into hepatic cells and is promptly returned to the blood is not measurable by either of the two methods.\(^4\)

Glucose utilization is a metabolic rate which should be more closely proportional to body surface area than to body weight. Another theoretically satisfactory method of comparing results from one dog to the next is to divide the glucose utilization rate by the 0.6 power of the body weight in each case (23); this method was used in preparing table 1 because the surface areas of the dogs used by the previous investigators were not reported. For normal dogs over the weight range included in the present study, the results of the two methods of comparison are nearly identical.

The ‘turnover time’ of the body glucose pool is the pool size divided by the glucose inflow

\(^4\) Whether the method of calculation handles uptake into a liver constituent as increased pool size or as utilization rate depends on whether, and how fast, the carbon of the liver constituent comes to isotopic equilibrium with the plasma glucose carbon. A turnover process which is rapid in comparison with the mixing time in the glucose pool would result in a positive error in pool size; a slow process such as glycogen turnover (at least in the inner branches of the glycogen tree) would be included with glucose utilization; an irreversible process such as the manufacture, partly from glucose, of a liver protein secretory product would be included with utilization.
outflow rate. The turnover time should become smaller as dog weight decreases, as indicated by the above discussion.

Comparison of Measurements With Those Previously Published. Table I includes values for normal dogs in the postabsorptive state as determined (1) by the single-injection method, (2) by the two-step method of Searle et al., and (3) by the present method. It is probable that the high values for glucose space, as measured by the single-injection method, arise not only from mixing delay but also from the phenomenon of recycling.

At the end of 5 hours in the single-injection experiment, the plasma glucose specific activity has fallen to about one-fifth of the 1-hour value (4). The amount of recycled $^{14}C$ which raises specific activity by 10% in the constant infusion experiment would raise it by 50% in the single-injection experiment.

The effect of recycling in the single-injection procedure is to cause the line fitted to the observed points to be rotated counterclockwise around the earliest observed point (1 hr.). Thus the intercept when the line is extrapolated back to zero time is too low, which makes the calculated glucose pool size too high. In the present series of dogs, it has been observed that recycling is more evident in some experiments than in others; this would explain the extreme variability of the glucose space measurements by the single-injection procedure.

When such a recycling error occurs in the single-injection experiment, the slope of the line is also in error, and in the two-step procedure of Searle et al. (7) this error is carried on into the design of the second experiment. For this reason, in a long series of determinations by the two-step method, it would be expected that many second experiments would be encountered in which plasma glucose specific activity, instead of remaining constant, would fall during the 1–5-hour period of constant infusion.

Significance of the Compartments of the Body Glucose Pool. The rapidly equilibrating 'compartment' (1 of fig. 2) may represent blood-flow-limited transcapillary exchange between plasma glucose and part of the interstitial fluid glucose. The slowly equilibrating compartment (L) is not understood but is perhaps related to the slow compartments for sucrose exchange which have recently been described (24). The existence of a compartment which comes to equilibrium with a half-time greater than that of the glucose turnover process, analogous to the very slow sucrose compartments described by Cotlove (24), cannot be unequivocally seen in the case of glucose because the 'recycling' process obscures the long-term course of the plasma glucose specific activity curve; there is, however, the possibility that some of the 'recycling' effect is caused by such a compartment.

Equation 1 is derived by integrating the expression for the rate of change of total $^{14}C$ in a well-mixed pool, in a steady state of glucose replacement, into which is flowing $^{14}C$ glucose in 'weightless' quantity. The symbol $x$ is used to denote total $^{14}C$ at time $t$ for the purpose of the derivation.

The rate of $^{14}C$ inflow (the infusion rate) is $F$ and the rate of $^{14}C$ outflow at any time, $t$, is the fraction of the pool replaced per minute $(g/C_0)$ times $x$. Thus:

$$\frac{dx}{dt} = F - \frac{g}{C_0} \cdot x$$

Integration, evaluation of the constant of integration at $t = 0$, where $x = P$ (the priming dose), and replacement of the expression $x/C_0$ by its equivalent, $SA_t$, gives equation 1.

APPENDIX I
When the glucose of the plasma is mixing with the glucose of the interstitial fluids and is being replaced without change in glucose concentration by an inflow of $^{14}C$ glucose, a unit dose of $^{14}C$ glucose introduced into the plasma at $t = 0$ would be retained as follows (x is the fraction of the unit dose present at time $t$),

$$x = A_1e^{-m_1t} + A_2e^{-m_2t} + \cdots + A_n e^{-m_nt} \quad (2)$$

where the number of exponential terms which must be used is determined by the shape of the observed retention curve (25). One exponential term is required for each compartment peripheral to the plasma and one more for the replacement rate of the pool as a whole. Thus the value of $m_1$ is glucose inflow rate divided by total glucose pool size, or $(g/C_o)$.

When a priming dose ($F\mu C$) of $^{14}C$ glucose is given at $t = 0$, the number of microcuries of $^{14}C$ left at time $t$ is then:

$$C_{14}^t = F(A_1e^{-m_1t} + A_2e^{-m_2t} + \cdots + A_n e^{-m_nt}) \quad (3)$$

and where a constant infusion ($F\mu C$/min.) of $^{14}C$ glucose is also maintained, the amount of $^{14}C$ due to the infusion, present at time $t$ (25) is:

$$C_{14}^t = F \left( \frac{A_1}{m_1} (1 - e^{-m_1t}) + \frac{A_2}{m_2} (1 - e^{-m_2t}) + \cdots + \frac{A_n}{m_n} (1 - e^{-m_nt}) \right) \quad (4)$$

Thus the total amount of $^{14}C$ in Q at time $t$ is $C_{14}^t + C_1^t$ or:

$$C_{14}^t = (P - \frac{F}{m_1}) A_1 e^{-m_1t} + \left( P - \frac{F}{m_2} \right) A_2 e^{-m_2t} + \cdots + \left( P - \frac{F}{m_n} \right) A_n e^{-m_nt} \quad (5)$$

$$\left| F \left( \frac{A_1}{m_1} \frac{A_2}{m_2} \cdots \frac{A_n}{m_n} \right) \right|$$

and the specific activity of the plasma glucose carbon is the above expression divided through, term by term, by the grams of glucose carbon in the plasma.

For the experiment of figure 2 it was found that three exponential terms in equation 5 were required to make the curve fit the observed values of plasma glucose specific activity between 0 and 180 minutes, so $m_2$ (equal to $1/gC_o$) became $m_3$. Notice that in selecting this portion of the curve to carry out the fitting, it was tacitly assumed that there is no compartment containing glucose which mixes with the plasma glucose by a process with a greater half-time than the turnover half-time of the whole glucose pool. This assumption is considered briefly in the discussion section of the text.

Values of $g$, $C_o$, $A_1$, $A_2$, $A_3$, $m_1$, and $m_2$ were then found (by trial and error) such that the observed values of specific activity at all values of time were predicted by equation 5. Equation 2 then became:

$$x = 0.804e^{-0.05t} + 0.959e^{-0.08t} + 0.101e^{-0.028t} \quad (6)$$

The value of $m_1$ was not accurately determined because no observations were made between 0 and 5 minutes; it is at least 1.5 because mixing was complete with this ‘fast’ compartment sometime prior to 5 minutes, when the first plasma sample was taken for determination of glucose specific activity.

By a process essentially the same as that described elsewhere in another connection (25), it was possible to deduce the complete characteristics of the three compartment system having a plasma glucose compartment (Q) of 0.197 gm C (estimated from blood volume, hematocrit and plasma glucose concentration), and having the plasma glucose $^{14}C$ content curve given by equation 5 above. The values for the constants of the system are given in the legend for figure 2.

Once the system had been determined, the specific activity curve of each of the individual compartments could be calculated. The equations corresponding in form to equation 6 (for $x$) and 7, which represent the fraction of a single time-zero dose ($P\mu C$ of $^{14}C$ glucose) which is present at time $t$ in compartments L and I, respectively, are as follows:

$$y = -0.577e^{-0.05t} - 0.486e^{-0.08t} + 0.544e^{-0.028t} \quad (7)$$

$$z = -0.751e^{-0.05t} + 0.399e^{-0.08t} + 0.361e^{-0.028t} \quad (8)$$

For each of the equations (6 through 8), the coefficients of the three exponential terms represent $A_1$, $A_2$ and $A_3$, respectively, for equation 5, and the coefficients of $t$ represent $m_1$, $m_2$ and $m_3$ for equation 5. Thus to find the total $^{14}C$ ($\mu C$) present in compartment L at any time (t) during a priming dose-constant infusion experiment, the coefficients in equation 7 are used in equation 5; the specific activity ($\mu C$/gm C) in L at any time (t) is the resulting equation divided through by 0.785, the size of L in grams glucose carbon. This is the way in which the compartment specific activity curves for figure 2 were obtained.

For the curves in figure 4 (single injection of $^{14}C$ glucose), the same procedure was followed except that $P$ (eq. 5) was set at zero instead of 0.138 $\mu C$/min. For the curves in figure 5 (constant infusion of $^{14}C$ glucose with no priming dose), the same procedure was followed except that $P$ (eq. 5) was set at zero instead of 15.0 $\mu C$.  

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6 Starting about 5 min. after an intravenous dose of $^{14}C$ glucose, the specific activity of the plasma glucose decreases as if equilibration were taking place with a single interstitial compartment. A similar finding has been reported by Baker et al. for the rat (3). Prior to this, equilibration is already nearly complete with a large amount of interstitial fluid glucose as is shown by the fact that plasma glucose specific activity at the end of the first 5 min. has about one-sixth the value calculated on the basis of dilution of the intravenous $^{14}C$ glucose dose by the glucose contained in the total plasma volume.
As an example of the calculation of plasma glucose specific activities at 60, 120 and 180 minutes in the preparation of figure 3, take the case in which the infusion rate is 0.8 F and the priming dose is 1.0 P. Equation 5 was used as just described after inserting the constants from equation 6. With $P = 15.0 \mu g$ and $F = 0.8 (0.138) \mu g - 0.110 \mu g$, setting $t$ equal to 60, 120 and 180 minutes in turn then gave the required values.

REFERENCES