Experimental validation of measurements of glucose turnover in nonsteady state

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Radziuk, J., K. H. Norwich, AND M. Vranic. Experimental validation of measurements of glucose turnover in non-steady state. Am. J. Physiol. 234(1): E84-E93, 1978 or Am. J. Physiol.: Endocrinol. Metab. Gastrointest. Physiol. 3(1): E84-E93, 1978. - The aim of the present experiments is to validate, in conscious dogs, the tracer infusion methods of measuring nonsteady state turnover rates. This was done in nine experiments performed in four normal dogs by infusing isotopically labeled glucose (2-3H, 6-3H, 1-14C) and monitoring the concentrations of both the labeled and unlabeled substances. The validation is based on the observation that a high exogenous infusion of glucose will suppress endogenous glucose production and become the sole source of glucose in the body. By infusing glucose at a high, time-varying rate, calculating its rate of appearance, \( R_a \) and comparing it to the infused rate, the method can be verified. The calculations were based on: a) a single-compartment model with a modified volume of distribution; b) a two-compartment model; and c) a generalized dispersion model. The absolute values of the areas of the deviations of the calculated from the infused curves were found to be, respectively, 9.5, 8.4, and 7.8% of the total area under the infused curve. It was concluded that the tracer infusion method can reliably measure \( R_a \) of glucose when it is changing rapidly, and the system is out of steady state.

tracer methods; compartment models; dispersion models; glucose kinetics; glucose clearance; glucose production; insulin

This study was undertaken in order to validate the measurement of time-varying turnover rates in the glucose system. This system is an example of nonlinear metabolic systems in which the measurement of these rates is necessary to elucidate the control mechanisms regulating the concentrations of metabolites because this control takes place at the sites of their production and of their removal. Impaired tolerance for a substance or the resetting of its chronic level results from dysfunction of these control mechanisms. An early indication of this dysfunction could therefore be observed in the adaptive responses of the rates of production and removal to various metabolic challenges because changes in these rates are not necessarily reflected by the metabolite concentrations. For example, prolonged fasting (9), exercise (39), chronic administration of steroids (23), or growth hormone (1) cause a marked change in glucose turnover rates with only slight changes in its concentration. Arginine infusions do not induce any change in glucose concentration either in normal or in depancreatized insulin infused dogs, but in the former glucose turnover increases due to increased secretion of insulin and glucagon (5), whereas in the latter it does not change because the supply of insulin and glucagon was unaltered (6, 37).

Nonsteady glucose turnover has been measured most frequently by infusing labeled glucose at a constant rate; calculations were based on a modified single compartment model (36), but their validity for the glucose system has not yet been ascertained. Validation can be accomplished by comparing a known infusion rate of a substance (if it is not produced endogenously or if its endogenous production has been suppressed), to rates calculated by using nonsteady-state equations and the measured concentrations of labeled and unlabeled substance. Using the polysaccharide, inulin, as a test substance, we have previously validated nonsteady turnover measurements based on a one-compartment, a two-compartment, and a more general dispersion model (25, 29). Glucose differs fundamentally from inulin in that it strongly influences its own clearance through feedback control based on insulin. Its behavior is thus nonlinear in contrast to the linear behavior of inulin.

It was necessary therefore to determine the applicability of turnover calculations to the glucose system. Because glucose is produced endogenously, our approach to this validation was based on the observation that infusion of exogenous glucose, in normal dogs, suppresses endogenous glucose production (14, 19, 35). If we therefore infuse glucose at a sufficiently high rate, we may compare the calculated rate of production to the known rate of infusion. Although this experiment raises the levels of glucose appearance to the higher limits of those encountered physiologically, it will validate the calculations in general if the system remains nonlinear because the same feedback loops are operative as at lower rates of glucose production. As tracer we have used constant infusions of [6-3H]glucose, [2-3H]glucose, or [1-14C]glucose and have measured plasma concentrations of labeled glucose prior to and during the period of exogenous glucose infusion.

Our objectives were: a) to compare the magnitude of the errors in nonsteady turnover measurement based on the modified single-compartment, two-compartment, and dispersion models; b) to determine which radioactive label of glucose can be used under the conditions of
validations of nonsteady-state glucose turnover measurements

this experiment; and c) to initiate the development of a
general method for nonsteady turnover measurements
that may be applicable to systems other than the

glucose system.

MATERIALS AND METHODS

All experiments were performed using adult mongrel
dogs (12-15 kg). On the day before experimentation,
three cannulas (medical grade vinyl tubing, Becton-
Dickinson) were introduced into the two jugular veins
for the infusions of glucose and tracer and one into the

inferior vena cava via a saphenous vein for blood
sampling. This procedure was carried out under general
anesthesia.

Prior to experimentation, each dog was trained for
several days to stand quietly in a harness. Each validation
experiment was performed over a period of 2 days.
On the 1st day, a tracer injection was given, and the
plasma concentration of labeled glucose was determined
at frequent intervals for a period of 4 h. On the 2nd
day, an unprimed constant tracer infusion was given
for 80-100 min with a Harvard infuser. This was continued
while unlabeled D-glucose (AnalaR, British Drug
Houses) was infused with a second Harvard infuser at a
constant rate (4-5 times mean endogenous rate of

glucose production of normal fasting dogs) for at least
60 min before variations in this infusion were intro-
duced by manually changing the infusion rate on a
Sage 240-2 pump. Thirteen experiments were performed
in four dogs. In all four dogs the tracer, [6-3H]glucose
(Amersham/Searle) was used. In the last two dogs, the
same pair of experiments was repeated 3 days later
with an injection and infusion of mixed [1-14C]glucose
(New England Nuclear) and [2-3H]glucose (Amersham/
Searle). For a summary of the designs see Table 1.

Blood samples were collected in heparinized tubes,
and the blood was quantitatively replaced with saline.
Plasma glucose levels were determined by the glucose
oxidase method with the glucose analyzer (Beckman
Instruments) and serum immunoreactive insulin (IRI)
by the method of Hales and Randle (11) with the
Amersham/Searle kit. Plasma samples were prepared
for counting by deproteinization (32) and were passed
through an ion exchange resin (Bio-Rad Ag-2 x 8) to
remove acid components. Two milliliters of the effluent
solution were then evaporated at about 80°C, and the
residue was redissolved in 0.5 cc water. Finally 10 cc
in 1/2 of [1-14C]glucose in double-label experiments. t I refers to injec-
tions; the other values are for infusions.

vascular, interstitial, and intracellular fluids in a phys-
ically complex fashion, and the glucose system is there-
fore diagrammatically depicted in the uppermost dia-
gram as a nonuniform distribution of concentrations
which occurs as the net result of the flows and diffusion
taking place throughout the body. The next three dia-
grams are empirical representations of this system for
the purpose of measuring nonsteady turnover rates of

Table 1. Design of experiments

<table>
<thead>
<tr>
<th>Expt</th>
<th>Label</th>
<th>Amount Injected</th>
<th>Time Unlabeled</th>
<th>Infusion Rate</th>
<th>Time Variations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>6-3H</td>
<td>40 I</td>
<td>100</td>
<td>131</td>
<td>None</td>
</tr>
<tr>
<td>2A</td>
<td>6-3H</td>
<td>50 I</td>
<td>158</td>
<td>158</td>
<td>t = 230 min sinusoidal increase to t = 255 and decrease to t = 280</td>
</tr>
<tr>
<td>3A</td>
<td>6-3H</td>
<td>50 I</td>
<td>146</td>
<td>146</td>
<td>Same as in 3D</td>
</tr>
<tr>
<td>3B</td>
<td>6-3H</td>
<td>50 I</td>
<td>146</td>
<td>146</td>
<td>Increase to t = 160-280 sinusoidal increase, decrease and increase</td>
</tr>
<tr>
<td>4A</td>
<td>6-3H</td>
<td>50 I</td>
<td>221</td>
<td>221</td>
<td>Same as in 4D</td>
</tr>
<tr>
<td>4B</td>
<td>6-3H</td>
<td>50 I</td>
<td>221</td>
<td>221</td>
<td>Increase to 134 mg/min followed by a gaussian curve</td>
</tr>
<tr>
<td>5C</td>
<td>1-14C</td>
<td>0.301 100</td>
<td>146</td>
<td>146</td>
<td>Increase to 134 mg/min followed by a gaussian curve</td>
</tr>
<tr>
<td>5D</td>
<td>1-14C</td>
<td>0.302 100</td>
<td>146</td>
<td>146</td>
<td>Increase to 134 mg/min followed by a gaussian curve</td>
</tr>
</tbody>
</table>

* Each number (1-4) represents a different dog. A and C designate injection experiments and the injected tracer dose is given, except in dog 2; B and D designate infusion experiments: the constant infusion rates of tracer and glucose are shown; the varying rates of glucose infusion are described. T and C refer to [2-3H]glucose and [1-14C]glucose in double-label experiments. t I refers to injec-
tions; the other values are for infusions.

One-compartment model (Fig. 1B). Two assumptions
were made: 1) Glucose is uniformly distributed through-
out a smaller effective volume, pV. The pool fraction,
p, is a constant used empirically to account for the
nonuniformity of glucose distribution (34). 2) Our calculations with this model are performed with pool fractions of 0.5-0.75 because these have previously been found to be most appropriate (9, 25, 29, 34). The formula used (34) is

\[ R_a = \frac{R_a^*}{a} - \frac{pVc_a}{a} \frac{da}{dt} \]  

where \( a \) is the specific activity of glucose (\( C^*/C \)).

Two-compartment model (Fig. 1C). In order to eliminate the uncertainty introduced through the pool fraction a two-compartment model was also tested. One model which would allow all parameters to vary with time would be too complex because only one time-varying parameter can be uniquely calculated from the tracer data. The following assumptions are therefore made: 1) the first compartment contains the vascular space and hence this is where the concentrations of labeled and unlabeled glucose are determined and where glucose and tracer appear. 2) Because it is the disappearance of glucose that is most highly influenced by changing glucose concentrations, \( k_{12} \) and \( k_{21} \) are assumed constant, whereas the evaluation of \( k_{10}(t) \) and \( k_{02}(t) \) is performed in either of two ways: a) disappearance takes place only in the first compartment (\( k_{10} = 0 \), as suggested by Katz et al. (22) for glucose and previously used in the case of inulin (25, 27) or b) disappearance occurs from both compartments, but it is assumed that the fractional disappearance rates from both are equal (\( k_{01} = k_{02} \)).

The use of this method requires the preliminary estimation of the parameters: \( V_1, k_{12}, \text{and } k_{21} \). These are obtained by measuring concentrations of labeled glucose either after a tracer injection done previously or during a constant tracer infusion but prior to the commencement of the nonsteady state experiment (see Appendix 1). The equations describing these models are:

\[ \frac{dC_1}{dt} = -(k_{01} + k_{21})C_1 + k_{12}C_2 + \frac{R_a}{V_1} \]  

\[ \frac{dC_2}{dt} = k_{21}C_1 - (k_{12} + k_{02})C_2 \]

for glucose and analogous equations for the tracer. These are solved numerically for \( R_a(t) \) for both a) \( k_{02} = 0 \) and b) \( k_{01} = k_{02} \) above.

Dispersion model (impulse response method) (Fig. 1D). This model assumes less structural knowledge of the system. It could be any set of compartments or a region in which dispersion is governed by diffusion and convection. The assumptions made in using this model are: 1) the region which is accessible to sampling is well-mixed, and it is in this region that the inputs of glucose and tracer occur (the sampling pool (22)). 2) The second region does not assume a uniform distribution of glucose. Glucose and tracer disappear throughout this region. 3) In an out-of-steady-state experiment, the increment in the fractional disappearance rates is the same throughout the entire system, although the basal rates can be different at every spatial point. This is the most general system in which the rates of appearance can, at present, be calculated given the data obtained in these experiments. The equations used in this model are more complex and their solution utilizes deconvolution techniques (27). They are listed in Appendix 2 and their derivation is described elsewhere (28).

Errors and data smoothing. Because the quantities calculated, \( R_a \) and fractional disappearance rate, are proportional to the derivatives of the measured variables (equations 1-3), their sensitivity to measurement errors is increased. It is essential, therefore, to smooth the data. This is done by fitting the data points simultaneously to a series of 1st to 3rd degree polynomials joined together in such a way as to ensure continuity of function value and slope. The lowest degree possible is chosen in each subset of the data described in (25, 26, 29). The programs for the compartment calculations were based on the formula given in Appendices 1 and 2. The calculations for the impulse-response method are described in (27) and (28) and were implemented in Fortran IV on the IBM System 370, but a smaller computer would be equally applicable.

RESULTS

Validation of \( R_a \) calculations. In most experiments, immediately following the onset of the glucose infusion,
a brief peak was observed in the calculated values of the rate of appearance of glucose, after which the calculated rates matched the rates of infusion. The height of the initial peak above the rate of infusion (39, 23, 28, 38, 30, 28 mg/min in six experiments using two-compartment calculations) corresponded well with the preinfusion, steady-state rate of production of glucose (30.6 ± 2.7 SE mg/min).

In order to demonstrate how our calculations were made, we shall now examine one experiment in detail. The upper panel of Fig. 2 shows the result of an injection experiment (experiment 2A, see Table 1). The decay curve of labeled glucose is fitted by a double exponential function from which the parameters for the two-compartment model are obtained (Appendix 1). Alternatively they can be obtained from the integrated double exponential function fitted to the concentrations of labeled glucose (lower panel, Fig. 2) during the constant infusion of tracer but prior to the infusion of unlabeled glucose.

Figure 3 illustrates the procedure followed in performing and analyzing the infusion experiment. Tracer is infused from time 0, and the glucose infusion is initiated after 80 min at about 5 times the endogenous glucose production rate. At $t = 230$ min, the rate of glucose infusion is changed to mimic a physiological variation in glucose entry into the system. Glucose and tracer concentrations are monitored and smoothed and the calculations performed by means of the one-compartment model with two pool fractions: 0.5 and 0.75.

Figure 4 displays the results obtained using the two types of two-compartment model discussed and by the impulse response method (IR). In this experiment the tracer infusion method yielded satisfactory results irrespective of the model used. In contrast to these results the lower panel of Fig. 4 also illustrates the $R_a$ calculation (by deconvolution) based on the assumption that the fractional disappearance rate is constant and equal to that during the steady-state period (before glucose infusion begins). The difference between the two calculated curves is accounted for by a changing fractional disappearance which can only be calculated from the tracer concentrations.

The rates of appearance, calculated using the different models discussed for all experiments not shown in the figures with [6-3H]glucose as well as [1-14C]glucose corrected for recycling are given at selected times in Table 2 and compared to the infusion rates. In order to compare all the methods, an absolute percentage error (Table 3) between the actual and calculated curves is obtained in each case for any values occurring after the
following steady-state $R_a$ values were obtained in four experiments: 42.3 ± 9, 31.6 ± 2.9, and 34.8 ± 2.6 (SE) mg/min when $[2-^3H]$glucose, $[6-^3H]$glucose, and $[1-^{14}C]$glucose, respectively, were used as tracers. $[6-^3H]$Glucose and $[1-^{14}C]$glucose (corrected for recycling) measured essentially the same $R_a$, whereas $[2-^3H]$glucose gave a 34% higher result, as reported also by others (15, 18). It is shown in Table 4 however that during the period of glucose infusion, the error of $R_a$ measurements is of equal magnitude irrespective of the type of tracer used. An example of $R_a$ calculation with $[2-^3H]$glucose as tracer is shown in Fig. 6. This is also an example where the pool fraction method yielded an underestimate of the out-of-steady-state changes.

**DISCUSSION**

**Validation of $R_a$ calculations.** In order to verify the accuracy of nonsteady measurements of glucose appearance, the calculated $R_a$ had to be compared to a known rate. This can be accomplished by a sufficiently high exogenous rate of infusion of glucose that suppresses endogenous production (35). It was suggested that this inhibition in production as well as increased uptake of glucose is brought about by changes in secretion in hormones (10, 17, 20) and by the changes in glucose concentration in plasma (2, 4, 12, 23). Although the initial peak in glucose appearance when the glucose infusion was initiated could be the result of a smoothing artefact based on a sampling frequency insufficient to follow the step change accurately, the occurrence of all peak heights in a range (23-39 mg/min) comparable to the $R_a$ of glucose (31 ± 3 mg/min) in steady state suggests the gradual suppression of endogenous glucose production that occurs with the hyperglycaemia and hyperinsulinemia produced (14, 19, 35). It should be noted that, even if the suppression of endogenous glucose production is incomplete, the error that results can be neglected when exogenous glucose is infused at rates 5-10 times the initial endogenous $R_a$. For example, if we have achieved 80% suppression of endogenous $R_a$, the error in the total calculation will be less than 4%.

In order to achieve this suppression of endogenous production we have, of necessity, infused glucose at a high basal rate before making changes. The question arises whether a validation at this higher "operating point" carries over to the system when it is operating at a different level of glucose appearance. To answer this question, we must know whether the system is still nonlinear at this point and whether the nonlinearity is similar to that encountered at lower $R_a$'s. If we consider Table 5, we see that the fractional disappearance rates rise and fall throughout the experiments, and these changes follow those in the plasma insulin levels. These vary throughout the whole experiment and not just initially. Therefore, the nonlinearity of the system is present throughout the entire experiment. In addition, the calculated $R_a$'s give good results (e.g., Fig. 3–6) even in the face of a step change in $R_a$, a time when the system is extremely nonlinear.

Three approaches to calculating turnover rates were
VALIDATION OF NONSTEADY STATE GLUCOSE TURNOVER MEASUREMENTS

TABLE 2. $R_a$ calculations in with [6,3H] and [1,14C]glucose as tracers

<table>
<thead>
<tr>
<th>Exp</th>
<th>$\text{R}_a$ calculations</th>
<th>Basal*</th>
<th>$R_a$, mg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal*</td>
<td>$\text{R}_a$, mg/min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\text{Infusion rate}$</td>
<td>$\text{Single compartment, p = 0.75}$</td>
<td>Two-compartment $k_{eq} = 0$</td>
</tr>
<tr>
<td>1B</td>
<td>Time</td>
<td>103</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>2B</td>
<td>110</td>
<td>165</td>
</tr>
<tr>
<td></td>
<td>2C</td>
<td>100</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>3B</td>
<td>110</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>3C</td>
<td>110</td>
<td>145</td>
</tr>
<tr>
<td></td>
<td>4B</td>
<td>221</td>
<td>221</td>
</tr>
<tr>
<td></td>
<td>4C</td>
<td>110</td>
<td>145</td>
</tr>
</tbody>
</table>

$R_a$ calculations are given in milligrams per minute. C refers to [1,14C]glucose in double-label experiments. * Basal values of $R_a$ are averages of values obtained during the initial infusion of tracer before glucose is infused.

TABLE 3. Percentage error in $R_a$ calculations

<table>
<thead>
<tr>
<th>Exp</th>
<th>$\text{Percentage Error}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One-compartment model</td>
</tr>
<tr>
<td></td>
<td>$p = 0.5$</td>
</tr>
<tr>
<td>1B</td>
<td>7.5</td>
</tr>
<tr>
<td>2B</td>
<td>9.7</td>
</tr>
<tr>
<td>2C</td>
<td>7.0</td>
</tr>
<tr>
<td>3B</td>
<td>12.3</td>
</tr>
<tr>
<td>4B</td>
<td>11.0</td>
</tr>
<tr>
<td>3D(T)</td>
<td>13.7</td>
</tr>
<tr>
<td>3D(C)</td>
<td>14.2</td>
</tr>
<tr>
<td>4D(T)</td>
<td>8.5</td>
</tr>
<tr>
<td>4D(C)</td>
<td>11.0</td>
</tr>
</tbody>
</table>

Mean 10.5 9.6 9.4 8.6 8.1 7.8

Percentage error was calculated by expressing the absolute value of the differences in area between the actual and calculated curves as a percentage of the area under the infused curve. $T$ and $C$ in the same experiment number refer to the calculations using [6,3H]glucose and [1,14C]glucose, respectively, as tracer. Tested in these experiments: 1) the modified single-compartment method (34), 2) the two-compartment method; and 3) the impulse-response method, based on a more general dispersion model.

The purpose of these models is not to describe in detail the behavior of a substance in the body but to provide an empirically successful basis for the calculation of nonsteady-state turnover, namely, the rate of nonsteady-state turnover, that is, the modified single-compartment model yielded satisfactory results with a pool fraction of 0.65-0.75, as suggested earlier (8). The need for correcting the volume of distribution by an appropriate pool fraction is essential because, as shown in Fig. 5, the results were not satisfactory when the volume of distribution was not modified. In circumstances in which very rapid changes prevail, higher order models will be more consistent (e.g., Fig. 6). A time-varying pool fraction
findings indicate that the turnover calculations are taken to be a double exponential. Interestingly, our similar to those incurred in using the two-compartment rate. This method of calculation has also been tested in other systems (28). The varying parameters that would be applicable to any number of compartments or a dispersion system (28). The varying parameter is still restricted to the fractional disappearance of a, which changed after a metabolic challenge, has been suggested (19). As shown by this data higher order models (two-compartment, etc.) are sufficiently accurate in the face of the most rapid changes. All the methods used to model the glucose system will reproduce, within a 10% error, the time-varying infusion rates used to test the calculations.

Simultaneously, an attempt has been made to generalize the method of $R_e$ measurement inherent in one- and two-compartment models to a more general model that would be applicable to any number of compartments or a dispersion system (28). The varying parameter is still restricted to the fractional disappearance rate. This method of calculation has also been tested in these experiments, and the errors were found to be similar to those incurred in using the two-compartment model, a model to which it is equivalent when $k(t)$ is taken to be a double exponential. Interestingly, our findings indicate that the turnover calculations are quite insensitive to the model used, a fact which may account for the success with which the methods have been used, although previously untested (1, 5, 6, 9, 23, 39).

This success depends to a great extent on the effectiveness with which the tracer linearizes the glucose system. In other words, the fractional disappearance rate, $k_{ol}$, which in the glucose system depends on the glucose concentration, when used in the tracer system still is a function of glucose concentration. Therefore, in the tracer system, the $k_{ol}$, is a time-varying parameter, and the equations for the tracer system are, in effect, linearized. This is equivalent to the statement that we have everywhere in the system: $R^*_d = aR_e$ where $R_d$ and $R^*_d$ are the rates of disappearance of glucose and tracer, respectively, and $a$ is the specific activity.

Calculations of the $R_e$ have not been validated here directly but can be assumed valid because their determination is based on the same hypotheses as that of

## Table 4. Parameters for two-compartment calculations

<table>
<thead>
<tr>
<th>Expt</th>
<th>2-Exponential Fit Parameters*</th>
<th>Vol in 1-Compt Model, ml†</th>
<th>Vol of 1st Compartment (2-Compt model), ml</th>
<th>$R_e \times 10^{-6}$, min/pmol</th>
<th>$k_0, k_1, k_{ol}, k_{ol}X 10$</th>
<th>$k_0, k_1, k_{ol}, k_{ol}X 10$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1C</td>
<td>2.49 2.55 2.27 11.90</td>
<td>3150</td>
<td>1597</td>
<td>1.56</td>
<td>0.016 0.109 0.119 0.096</td>
<td>0.121 0.224</td>
</tr>
<tr>
<td>2B</td>
<td>1.07 3.01 0.85 7.95</td>
<td>4000</td>
<td>2721</td>
<td>1.67</td>
<td>0.029 0.097 0.079 0.014</td>
<td>0.065 0.104</td>
</tr>
<tr>
<td>3C</td>
<td>0.65 7.83 0.78 4.09</td>
<td>3275</td>
<td>2045</td>
<td>1.70</td>
<td>0.046 0.097 0.041 0.040</td>
<td>0.033 0.088</td>
</tr>
<tr>
<td>3B</td>
<td>4.20 2.83 1.26 6.10</td>
<td>3125</td>
<td>1580</td>
<td>1.71</td>
<td>0.072 0.049 0.061 0.064</td>
<td>0.054 0.141</td>
</tr>
<tr>
<td>3D(C)</td>
<td>1.81 1.12 1.14 7.16</td>
<td>3125</td>
<td>1518</td>
<td>0.662</td>
<td>0.066 0.041 0.072 0.056</td>
<td>0.048 0.170</td>
</tr>
<tr>
<td>4B</td>
<td>5.18 3.44 2.43 9.30</td>
<td>3175</td>
<td>1387</td>
<td>1.68</td>
<td>0.141 0.092 0.098 0.128</td>
<td>0.103 0.221</td>
</tr>
<tr>
<td>4D(C)</td>
<td>1.42 1.42 5.18 11.10</td>
<td>3150</td>
<td>1564</td>
<td>0.672</td>
<td>0.235 0.253 0.111 0.243</td>
<td>0.264 0.218</td>
</tr>
</tbody>
</table>

Compart, compartment. * The two-exponential fit is of the response to the initial tracer infusion and is $a_1 (1 - e^{k_1t}) + a_2 (1 - e^{k_2t})$. † Volume for one-compartment model is 25% of body weight. ‡ $k_1$, $k_2$, and $k_{ol}$ (0) have units of min$^{-1}$.

## Table 5. Serum IRI levels and fractional disappearance rates of glucose during glucose infusions

<table>
<thead>
<tr>
<th>Expt</th>
<th>Time</th>
<th>Insulin</th>
<th>$k_{ol}$</th>
<th>Insulin</th>
<th>$k_{ol}$</th>
<th>Insulin</th>
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</thead>
<tbody>
<tr>
<td>1C</td>
<td>98</td>
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The first value of immunoactive insulin (IRI) is prior to glucose infusion. * IRI units, μU/ml. † Fractional disappearance rates. units: min$^{-1}$, calculated from 2-compartment model with $k_{ol} = k_{ol}$. 

that would account for an effective volume of distribution, which changed after a metabolic challenge, has been suggested (19). As shown by this data higher order models (two-compartment, etc.) are sufficiently accurate in the face of the most rapid changes. All the methods used to model the glucose system will reproduce, within a 10% error, the time-varying infusion rates used to test the calculations.

Simultaneously, an attempt has been made to generalize the method of $R_e$ measurement inherent in one- and two-compartment models to a more general model that would be applicable to any number of compartments or a dispersion system (28). The varying parameter is still restricted to the fractional disappearance rate. This method of calculation has also been tested in these experiments, and the errors were found to be similar to those incurred in using the two-compartment model, a model to which it is equivalent when $k(t)$ is taken to be a double exponential. Interestingly, our findings indicate that the turnover calculations are quite insensitive to the model used, a fact which may account for the success with which the methods have been used, although previously untested (1, 5, 6, 9, 23, 39).

This success depends to a great extent on the effectiveness with which the tracer linearizes the glucose system. In other words, the fractional disappearance rate, $k_{ol}$, which in the glucose system depends on the glucose concentration, when used in the tracer system still is a function of glucose concentration. Therefore, in the tracer system, the $k_{ol}$, is a time-varying parameter, and the equations for the tracer system are, in effect, linearized. This is equivalent to the statement that we have everywhere in the system: $R^*_d = aR_e$ where $R_d$ and $R^*_d$ are the rates of disappearance of glucose and tracer, respectively, and $a$ is the specific activity.

Calculations of the $R_e$ have not been validated here directly but can be assumed valid because their determination is based on the same hypotheses as that of...
the hypothesis that \[2^{-3}H\]glucose measures the rate of dephosphorylation of glucose-6-phosphatase rather than the net production of glucose (21). This was not the case during the period of high glucose infusion, presumably due to the hyperglycemic and hyperinsulinemic conditions which cause a large flux of glucose and therefore of tracer into the hexose monophosphate pool, and subsequently into glycogen or the glycolytic pathways (e.g., 2, 3, 12, 13, 33), so that formerly labeled glucose does not reenter the circulation. Factors that increase the flux of glucose into storage of glycolysis would minimize tracer-dependent errors in calculated rates of appearance. When glycogenolysis and gluconeogenesis predominate or when in an experiment uptake and production of glucose prevail alternately, the choice of an appropriate label becomes a more critical factor.

In conclusion, our findings suggest that tracer infusion techniques can be used in measuring endogenous 

\[R_a\]. Comparisons of tracer-determined \(R_a\) with the \(R_d\) found with a glucose clamp technique (16, 31) would provide a further test. Clearance or fractional disappearance rates (Table 5) are more model-dependent quantities but can provide valuable information for modeling purposes (16, 29, 31, 38).

The experimental procedure for all the methods presented is identical: a preliminary unprimed infusion of tracer was found to give results as satisfactory as a primed infusion used previously (29) and obviates the necessity of an additional tracer injection experiment (29). The tracer does not reach steady state prior to the experiment, but this is inconsequential in a self-consistent method in which the tracer will later again be driven out of steady state.

In steady state, \([2^{-3}H]\)glucose measured a higher \(R_a\) than \([1^{-14}C]\)glucose or \([6^{-3}H]\)glucose. This corroborates
rates of glucose production not only in steady state but also when rapid changes in plasma glucose concentration or in turnover occur. A hierarchy of models of increasing complexity have been tested and their applicability to turnover measurement verified. All these models gave approximately the same results although the higher order models were more consistent when the turnover rate changed rapidly with time. These results can also be extrapolated to the restricted nonlinear systems described by assumptions 1-4 in MODELS and CALCULATIONS.

Under conditions of high hepatic uptake and low production of glucose, all the tracers employed, [1-14C]glucose corrected for recycling, [6-3H]glucose, and [2-3H]glucose were equivalent.

An initial step was taken towards a more general approach (dispersion model) to the measurement of turnover rates and was found to be valid when applied to the glucose system.

APPENDIX 1

One- and Two-Compartment Calculations

One-compartment. Equation 1 gives the formula by which $R_d$ is calculated. For glucose $V$ is taken at 25% of the body weight and $p$ is safely taken as 0.65. $R_d^*$ is the rate of infusion of tracer that is constant and known. At any time, values of $C$ and $C^*$ (and, therefore, $a$) are known or can be interpolated. $\frac{da}{dt}$ is determined from a smoothed curve of specific activities or from values of $C(t)$ and $C^*(t)$. $R_d$ can also be calculated in the one-compartment model using the formula

$$R_d = R_d^* - pV \frac{dc}{dt}$$

Two-compartment. Equations 2 and 3 describe the general two-compartment system. We shall consider the two cases described: i) $k_{12} = 0$ and ii) $k_{12} > 0$ with $k_{21}$ and $k_{21}$ time and/or concentration dependent.

i) $k_{12} = 0$. This case has been previously treated in detail (8, 10). The solution, neglecting initial conditions for simplicity, is reproduced here:

$$R_d(t) = R_d^* - \frac{V_1}{a_1} C_1(t) - V_2 k_{12} k_{12} \int_0^t e^{-k_2(t)} \left( C_1^*(\tau) - C_1(\tau) \right) d\tau$$

where $a_1(t) = C_1^*(t)/C_1(t)$ is the specific activity in compartment 1. The constant parameters $V_1, k_{12},$ and $k_{21}$ are obtained from the step-response curve

$$A \left(1-e^{-k_1} \right) + B \left(1-e^{-k_2} \right)$$

by standard techniques (25).

ii) $k_{12} > 0$. This alternative does not lend itself to a simple analytical solution. Instead equations 2 and 3 and their tracer analogues are made discrete as follows:

$$C_1(t+1) = (1-k_{12})C_1(t) - k_{10}(t)C_1(t) + k_{12}C_2(t) + \frac{R_d(t)}{V_1}$$

and

$$C_2(t+1) = k_{12}C_1(t) + (1-k_{12})C_2(t) - k_{10}(t)C_1(t)$$

These are solved for $k_{10}(t)$ and $R_d(t)$:

$$k_{10}(t) = \frac{1}{C_1^*(t)} \left[ (1-k_{12})C_1(t) - C_1^*(t+1) + k_{12}C_2^*(t) + \frac{R_d^*(t)}{V_1} \right]$$

and

$$R_d(t) = V_1 (C_1(t+1) + (k_{10}(t) + k_{21} - 1)C_1(t) - k_{12}C_2(t))$$

APPENDIX 2

Dispersion Model (Impulse Response Method) and a Simple Example

The formulas used in the dispersion model described are as follows

$$C_t(t)Q(t) = C_0(t) - R_d \int_0^t h(t)dt + \int_0^t h(t-\tau)R_d(\tau)Q(\tau)d\tau$$

for unlabeled glucose, and

$$C^*_t(t)Q(t) = \int_0^t h(t-\tau)R_d(\tau)Q(\tau)d\tau$$

for the tracer, where $C_t(t)$ and $C^*_t(t)$ are the sampled concentrations of glucose and tracer, respectively, $h(t)$ is the response of tracer concentration to a unit injection of tracer (that is, the concentrations of tracer in plasma at various times after injection divided by the amount of tracer injected). $R_d$ is defined as $C_0(t)/C_t(t)$ where $C_0(t)$ is the glucose concentration at time 0. (The initial tracer concentration is assumed to be zero.) $Q(t)$ is defined by exp (Integral $\Delta(t)dr$) where $\Delta(t)$ is the increment in the fractional disappearance rate, which is assumed the same at every point in the system. The second integral in equation A2-1 and the integral in equation A2-2 are convolution integrals. To find $R_d(t)$ we first solve equation A2-2 for $Q(t)$ because all other quantities are known, and then equation A2-1 for $R_d(t)$ by using numerical deconvolution (27). These equations are derived for both the model consisting of an arbitrary set of compartments and for a diffusion-convection reaction system (38).

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REFERENCES

VALIDATION OF NONSTEADY-STATE GLUCOSE TURNOVER MEASUREMENTS


