Biochem. J. (1974) **142**, 161–170
Printed in Great Britain

Evaluation of Glucose Turnover, Body Mass and Recycling with Reversible and Irreversible Tracers

By JOSEPH KATZ,* H. ROSTAMI* and ARNOLD DUNN†
**Cedars–Sinai Research Medical Center, Los Angeles, Calif.* 90054, *U.S.A.*, *and*
†*Department of Biological Sciences, University of Southern California, Los Angeles, Calif.* 90006, *U.S.A.*

(*Received* 3 *July* 1973)

1. Methods are presented for the calculation of rates of synthesis or loss, mean transit time and total body pool of compounds from specific-radioactivity curves, without assuming a multicompartmental model and without fitting the data by exponential expressions. The methods apply to the steady state after either single injection or continuous infusion of a labelled compound. 2. The use of irreversible and reversible tracers and the effects of recycling of carbon on the estimations of the parameters of glucose metabolism are discussed. Methods for quantitatively determining recycling of glucose carbon by the use of glucose doubly labelled with ^{14}C and ^{3}H are presented.

Isotopic tracers for metabolic compounds may be divided into two classes, irreversible and reversible. An irreversible tracer is one in which the labelled catabolite is not reincorporated into the mother compound. Circulating proteins labelled with radioactive iodine, or compounds labelled with ^{3}H in which water is the sole labelled product, represent this class. In the second class a fraction of the labelled breakdown products is reincorporated. Most ^{14}C-labelled compounds belong to this class. The recycling of labelled carbon from glucose *in vivo* has long been realized, and methods of correcting for recycling have been proposed by von Holt *et al.* (1961) and Reichardt *et al.* (1963). An alternative approach, based on the use of irreversible labelling of glucose with ^{3}H, was introduced by us (Katz & Dunn, 1967). At that time we did not use a theoretically valid method to determine recycling quantitatively. Although recycling of glucose has been shown to occur in a number of species its effect on the interpretation of isotopic data has so far received inadequate attention. In the present paper we propose a procedure for the quantitative determination of recycling, based on the utilization of doubly labelled glucose, and we discuss some of the implications of recycling on estimations of parameters of glucose metabolism. The application of these methods to experiments with rats and rabbits is shown in the following paper (Katz *et al.*, 1974).

Most isotopic studies of glucose metabolism *in vivo* have hitherto been based on the assumption of a multicompartmental model, and have used mathematical analysis which required fitting of experimental curves to exponential equations. It has been pointed out by a number of workers that assumption of a multicompartmental model is not essential, and moreover is an unnecessary restraint on the system. It has also been pointed out (e.g. Zierler, 1962) that in theory all the essential parameters can be derived from the experimental curves without recourse to fitting by exponential expressions. The advantage of the non-compartmental approach to the estimation of glucose-replacement rate has been stressed by Shipley & Clark (1972). However, more general application of non-compartmental analysis, and especially of graphical methods for the evaluation of mean transit time and total body pool, has been very rare. We have applied these procedures to our data for rats and rabbits (Katz *et al.*, 1974). In the present paper we present a concise description of the calculations, and indicate their advantages over conventional methods.

(I) Non-compartmental Methods for the Estimation of Parameters of Glucose Metabolism in the Steady State

Model for body glucose

It is established that intravenously injected labelled glucose distributes itself very rapidly (taking less than 1 min in small animals) in a mass greater than that in the circulation, whereas equilibrium distribution within the total body glucose is considerably slower. Accordingly, in the model shown in Fig. 1 total body glucose is divided into a rapidly mixing pool in which the specific radioactivity is always equal to that in the blood, and the rest of the body glucose. There are no assumptions about the distribution of glucose within that mass. The system may be graphically depicted in several ways. A reasonably realistic representation of the distribution of body glucose is probably that shown in Fig. 1(*a*). A network of tubes emanates from a central well-mixed compartment. The tubes may branch, expand or constrict in any possible fashion, forming a labyrinth.

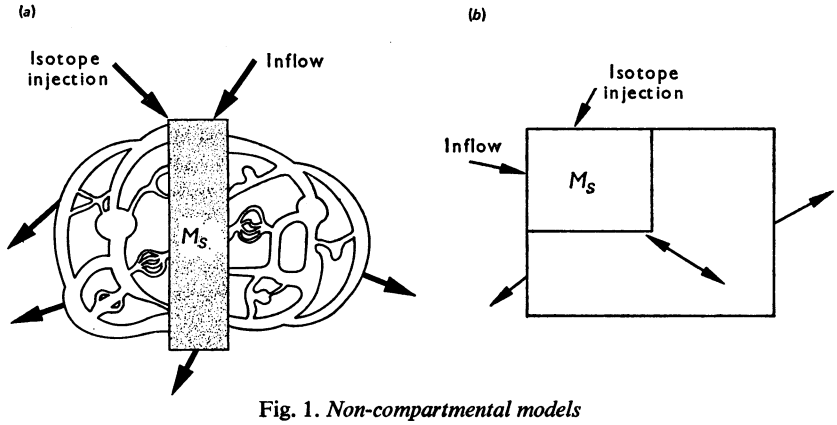

Fig. 1. *Non-compartmental models*

An alternative representation of the glucose system at steady state. Glucose in a rapidly mixing pool, M_s, is exchanging with glucose in the rest of the body. The entry into the system is solely into M_s, but catabolism or loss may occur anywhere. The body pool outside M_s is depicted in (*a*) as a labyrinth, but in (*b*) the nature of the body pool is not specified (see the text.) Note that after a single injection of radioisotope the specific radioactivity throughout the system is not uniform outside M_s but may vary from point to point and will be, when equilibrated, higher than the specific radioactivity in M_s.

Such a labyrinth was used by Zierler (1962) in the analysis of blood flow through an organ, except that in his system there was no central pool. Equivalent alternative treatments are possible (see Fig. 1*b*). Thus Nosslin (1964) in his analysis of plasma protein kinetics considered the extravascular space to consist of an infinite number of pools, whose connexion to each other and to the central rapidly mixing plasma pool is not specified. The most general stochastic approach is probably that of Bergner (1962, 1964, 1965). Although the mathematical methods used by Zierler (1962), Nosslin (1964), and Bergner (1962, 1964, 1965) are quite different they all lead to the same expressions.

In the model of Fig. 1 the sole site of inflow of newly synthesized glucose is the rapidly mixing pool. On the other hand there is no restriction on the site of catabolism or loss from the system, which may occur from the central pool or in one or many sites of the labyrinth. Special cases with a restricted site of catabolism will also be considered.

The central compartment includes the blood and is accessible for injection and sampling; it will be referred to as the sampling pool. The curve of specific radioactivity in this compartment will be referred to for convenience as the plasma curve, although the mass of the compound in the sampling pool frequently exceeds that in the plasma. It will be shown that nearly all the valid information may be obtained from the plasma curves, whether or not they are exponential.

Single and continuous infusion

The two basic methods for the administration of a labelled dose are the single injection (S.D.) and continuous infusion (I.R.). In the past there has been a prolonged controversy as to the validity of these two procedures for the study of glucose metabolism. It is easily shown that for unit dose/unit time, the continuous infusion curve represents the integral of that with the single injection. The ordinate of the continuous infusion curve at any time, t, represents the area from 0 to t under the single-injection curve. Obviously both methods, when properly used, yield identical results. Expressed as a sum of exponential terms, the specific radioactivity after a single injection may be represented as:

$$\text{Specific radioactivity} = A_1e^{-\alpha_1 t} + A_2e^{-\alpha_2 t} + \ldots = \Sigma A_t e^{-\alpha_t t}$$

and with continuous infusion as:

$$\text{Specific radioactivity} = B_1 + B_2 + \ldots - B_1e^{-\alpha_1 t} - B_2e^{-\alpha_2 t} = \Sigma[B_t(1-e^{-\alpha_t t})]$$

The exponents α are the same and the coefficients A and B are related as shown by eqn. (1):

$$B_t = \frac{A_t}{\alpha_t} \times \frac{\text{I.R.}}{\text{S.D.}} \qquad (1)$$

where I.R. and S.D. refer to Infused and Single Injection respectively. If, as is commonly the case, one of the exponents is much larger than the others, the transformation from a single injection to continuous infusion leads to a marked decrease in one coefficient. For instance, with a curve for a single injection, represented by two exponential terms with equal coefficients $A_1 = A_2$ and $\alpha_2 = 0.1\alpha_1$, the transformation would yield B_1 to be one-tenth of B_2, and the relative contribution of the first term would be

small. Hence in practice for the same system a curve for continuous infusion can often be adequately depicted by a smaller number of terms than that for a single injection.

Parameters of glucose metabolism

The parameters that can be determined from the plasma curve are as follows. (1) M_s, the mass of glucose in the rapidly mixing pool. (2) M, the total body mass of glucose which can be calculated only in a special case (as will be shown below) and in general only the limiting minimal and maximal masses $M_{min.}$ and $M_{max.}$ can be estimated. (3) R_0, the rate of glucose utilization or replacement (which are equal in the steady state). The rate of replacement or synthesis in the post-absorptive animal (no glucose from the gut) is defined best in biochemical terms as being equal to the rate of hydrolysis of glucose 6-phosphate by glucose 6-phosphatase. The utilization of glucose is practically equal to the phosphorylation of glucose, which in post-absorptive animals nearly all occurs in non-hepatic tissue. There is some utilization of glucose without prior phosphorylation, namely in the formation of fructose by the sorbitol pathway and incorporation into the glucose moiety of milk lactose. However, except during lactation the contribution of non-phosphorylating pathways is small. (4) R_{11}, the rate of exchange of glucose between the rapidly mixing pool and the rest of the body glucose. Another parameter is the mean transit time (or time of sojourn) $\bar{t}$. The true transit time can be evaluated only in some cases, and in general only a range corresponding to the experimental data, between the minimal and maximal transit times, can be obtained. The product of R_o and $\bar{t}$ is total mass of glucose.

Graphic calculations of these parameters use only the curve (either that for continuous infusion or that for single injection). The expressions are either taken directly or are modified from Zierler (1962) and Nosslin (1964). Formal proofs can be found in their papers as well as those of Bergner (1962, 1964) and the monographs of Rescigno & Segre (1966), Steele (1971) and Shipley & Clark (1972).

Calculations

The methods of calculating R_o, M_s, R_{11} and total body mass are summarized in Table 1. Expressions are presented for the calculation of these values from either experiments with single injections (expressions with suffixes *a* or *b*) or continuous-infusion experiments (suffixes *c* or *d*). Expressions with suffixes *a* and *c* use the experimental curves themselves. Analogous expressions utilizing the coefficients and exponents of the exponential equations are also given, with suffixes *b* and *d*. The values of R_0, M_s and R_{11} can in theory always be uniquely determined from the curves, and the calculation does not depend on the site of catabolism. On the other hand the calculation of the mean transit time and the total mass requires knowledge or assumption of the site of catabolism or outflow.

Rate of replacement. The rate R_0 is obtained in single-injection experiments by dividing the injected dose by the area from 0 to ∞ under the plasma curve, or by dividing the infused rate by the plateau specific radioactivity ($S.a._{(T)}$) in the plasma. In expressions 2(*a*) and 2(*c*) the area and the plateau value, as measured directly, are used, and in expressions 2(*b*) and 2(*d*) these are expressed in terms of exponents and coefficients. These expressions are well established and have been widely used.

Mass (M_s) and outflow (R_{11}) from the sampling pool. The mass of the sampling pool may be determined by single-injection experiments, by dividing the dose by the (extrapolated) specific radioactivity at zero time (expressions 3*a* and 3*b*), or by continuous-infusion methods by dividing the rate by the initial slope (expressions 3*c* and 3*d*). The outflow from the sampling pool, by catabolism and exchange with the periphery, can be obtained from the single injection curve by multiplying M_s by the initial slope (expressions 4*a* and 4*b*). The calculation from the continuous-infusion curve requires the evaluation of the second derivative of the specific-radioactivity curve at zero time. In practice the evaluation of the second derivative is subject to great error, which makes this method unpractical.

Total body mass. Total body mass is in general equal to the product of the replacement, R_0, and the mean time of transit (or sojourn), $\bar{t}$, of the compound. Transit or sojourn time is the period for which a molecule is present in the body, from the time of synthesis to the time of catabolism or loss (Perl & Chinard, 1969). The mean transit time, irrespective of the site of loss, after a single injection equals the area from 0 to ∞ under the curve for total body radioactivity [for proof see Bergner (1962) or Shipley & Clark (1972)]. Hence total body mass is obtained by dividing the area under the total body radioactivity curve by the area from 0 to ∞ under the plasma specific-radioactivity curve (expression 5*a*).

The determination of the total body radioactivity is frequently difficult but it is possible if the catabolic products are promptly eliminated from the body. Thus by using iodinated plasma proteins from which the liberated radioactive iodide is fairly promptly excreted, total body radioactivity can be determined from whole body counting or by collecting the urine. This method has been used by Rossing (1971). Attempts to estimate the total body radioactivity after a single injection of [^{3}H]glucose will be discussed in the following paper (Katz *et al.*, 1974). With continuous infusion the determination of total

Table 1. *Equations for parameters of glucose kinetics with irreversible tracers with inflow solely into sampling pool*

Symbols: α, exponent of the exponential terms; A and B, coefficients of exponential terms for single-injection and continuous-infusion curves respectively; α_n smallest (final) exponent; S.a., specific radioactivity (as function of time); $S.a._{(0)}$, intercept of single-injection curve at zero time; $S.a._{(T)}$, plateau specific-radioactivity curve of single-injection curve; $Slope_{(0)}$, slope of both types of curve at zero time; Area, area (0 to ∞) under single-injection curve or area between continuous-infusion curve and plateau ordinate.

				Single injection (S.D.)		Continuous infusion (I.R.)	
Parameter	Expression no.	Symbol	Site of loss	General expression (*a*)	Exponential expression (*b*)	General expression (*c*)	Exponential expression (*d*)
Replacement rate	2	R_0	Anywhere	$\frac{\text{S.D.}}{\text{Area}}$	$= \frac{\text{S.D.}}{\Sigma(A_i/\alpha_i)}$	$\frac{\text{I.R.}}{S.a._{(T)}}$	$= \frac{\text{I.R.}}{\Sigma B_i}$
Mass of sampling pool	3	M_s	Anywhere	$\frac{\text{S.D.}}{S.a._{(0)}}$	$= \frac{\text{S.D.}}{\Sigma A_i}$	$\frac{\text{I.R.}}{Slope_{(0)}}$	$= \frac{\text{I.R.}}{\Sigma[B_i\alpha_i]}$
Outflow from sampling pool	4	R_{11}	Anywhere	$\frac{Slope_{(0)}\times\text{S.D.}}{[S.a._{(0)}]^2}$	$= \frac{\Sigma(A_i\alpha_i)\times\text{S.D.}}{(\Sigma A_i)^2}$	$\frac{\text{First derivative of slope}_{(0)}\times\text{I.R.}}{[Slope_{(0)}]^2}$	$\frac{\Sigma[B_i\alpha_i^2]\times\text{I.R.}}{[\Sigma(B_i\alpha_i)^2]}$
Total mass	5	M	Anywhere	$\frac{\int_0^\infty(\text{Total body activity})dt}{\text{Area}}$	—	$\frac{\text{Final body activity}}{S.a._{(T)}}$	—
Total minimal mass	6	$M_{min.}$	Sampling pool	$\frac{\int_0^\infty(\text{S.a.}\times t)dt}{(\text{Area})^2}$	$= \frac{\Sigma A_i/\alpha_i^2\times\text{S.D.}}{[\Sigma(A_i/\alpha_i)]^2}$	$\frac{\text{Area}\times\text{I.R.}}{[S.a._{(T)}]^2}$	$= \frac{\Sigma B_i/\alpha_i\times\text{I.R.}}{[\Sigma B_i]^2}$
Total mass	7	M_u	Uniform rate everywhere	—	$\frac{1}{\alpha_n}\cdot R_0$	—	$\frac{1}{\alpha_n}\cdot R_0$
Maximal mass of total body pool	8	$M_{max.}$	Point of longest transit time*	Not determined	$\sum\left(\frac{1}{\alpha_i}\right)\times R_0$	Not determined	$\sum\left(\frac{1}{\alpha_i}\right)\times R_0$

* For a catenary system outflow from end pool furthest from sampling pool.

body mass is very simple in principle but difficult in practice. Equal specific radioactivity throughout the body is approached asymptotically and total body radioactivity of the labelled species ultimately becomes practically constant. When this situation is attained, dividing total body radioactivity by the final specific radioactivity yields body mass (expression 5*c*). Total radioactivity in the glucose is the difference between the radioactivity infused at time t, and the ^3H radioactivity in the body water at that time. The latter can be estimated, but since total body glucose radioactivity is a small difference between two similar values, its determination is subject to considerable error which makes the method unreliable in our hands. In the general case the mean transit time cannot be obtained solely from the plasma specific-radioactivity curves [see Shipley & Clark (1972) and Steele (1971)]. It may be obtained in a special case such as in Fig. 1, in which the tracer labels the inflow into the system, provided that there is a known sole site of outflow. With these restrictions it is possible to obtain from the plasma specific-radioactivity curve a minimal and maximal value for the mean transit time, thus defining the range of values for the total body mass which is consistent with the plasma specific-radioactivity curve. If the range is great the information is of little value. It will be shown, however, that at least under some conditions the computed range for total body mass of glucose is quite narrow in rabbits and rats.

Minimal mass. The minimal transit time and minimal mass are the transit time and mass if it is assumed that catabolism is solely from the sampling pool. The minimal transit time is obtained from the plasma curve, after a single injection, by the expression:

$$\frac{\int_0^\infty \text{S.a.}_{(t)}\, t\, dt}{\int_0^\infty \text{S.a.}_{(t)}\, dt}$$

Two graphical procedures for the evaluation of this integral for a single injection are available. In one procedure a curve is generated by multiplying the specific radioactivity by time (the values of the abscissa and ordinate for each point), and dividing each product by the area under the plasma curve. The area under the resulting curve is the mean transit time. An alternative and simpler method is to plot the cumulative area under the plasma curve, dividing each point by the total area under the plasma curve. The area between the curve which is so generated and its asymptote equals the mean transit time. The procedure is a transformation of the data from a single injection to those of continuous infusion. The use of both methods is illustrated in the following paper (Katz *et al.*, 1974). Multiplying the mean transit time by R_0 yields the mass.

Estimation of the minimal mass from continuous-infusion experiments requires simply the determination of the area bounded by the plasma specific-radioactivity curve and the plateau ordinate. This is readily done graphically. It is surprising that the method of expressions 6(*c*) or 6(*d*), although it was described years ago (Zierler, 1962), has not been used so far.

Maximal mass. The maximal mean transit time and maximal mass for any system would be obtained if the loss of material all occurred at a site or region removed as far as possible from the sampling pool ('far' here refers to the path requiring the longest time). If the system were made of n pools, this would correspond to a catenary system, with an outflow from the end compartment furthest from the sampling pool. The transit time through such a system may be shown to be the sum of the reciprocals of the exponents. By multiplying this transit time by replacement rate, the maximal mass, $M_{max.}$, which is consistent with the experimental data, is obtained (expression 8). We were not able to devise a graphical method for this expression, without fitting data to an exponential curve. As shown in the following paper (Katz *et al.*, 1974), at least in rats and rabbits the expression is of little practical interest. If the semilogarithmic plot of the plasma curve attains a terminal constant slope, total body mass may also be calculated if it is assumed that the fractional rate of catabolism is uniform throughout the system. This mass is designated M_u. Expression 7 is essentially that derived by Steele (1971). The terminal logarithmic slope α_n is obtained by plotting the experimental values for the last few hours on semilogarithmic paper. For continuous infusion, the difference between the asymptote and the experimental points is plotted in this way. Since α_n is much smaller than the other exponents, the difference between M_u and $M_{max.}$ is small in practice.

Other methods of measuring body mass

Total body mass has been frequently estimated by extrapolating the plasma specific-radioactivity curve after injection of [^{14}C]glucose to zero time. It is assumed that complete and uniform mixing occurs within minutes after injection, and that total body glucose may be assumed to be a single well-mixed pool. If this were the case, specific-radioactivity curves with [^3H]glucose should become rapidly linear. However (see Katz *et al.*, 1974), linearity is not attained before 15min in rats and before 30min in rabbits. It seems therefore that the assumption that body glucose is a single rapidly mixing pool is not satisfactory, at least in small animals. Also, as shown by Katz *et al.* (1974), extrapolation in

practice depends to a large extent on arbitrary choice by the investigator.

A combination of the methods of single injection and continuous infusion is primed infusion. With this frequently used method, a suitable priming dose gives a rapid increase in plasma specific radioactivity. The estimation of body mass with primed infusion is more difficult than with other methods of radioisotope administration. Steele *et al.* (1957) introduced an approximate method based on the injection of an 'ideal' priming dose, which requires prior knowledge of the plateau (see also Steele, 1971). This approach has obvious limitations and is not generally valid. In theory it is possible to calculate all the parameters of Table 1 (see above) from primed-infusion data, but this requires frequent sampling immediately after the administration of the dose and throughout the experiment. Unless the conditions require a very short experimental period, primed infusion appears to us to offer no theoretical advantages over continuous infusion.

Advantages of graphical analysis

It has been repeatedly pointed out that the choice of the number of terms to fit exponential equations to experimental curves is arbitrary, depending essentially on the degree of experimental error [see, e.g., Bergner (1964) or Shipley & Clark (1972)]. The construction of metabolic models with the number of pools equal to the exponential terms has little anatomical or physiological reality and all the significant information on the system can be obtained without recourse to such models. Non-compartmental analysis of indicator dilution and isotope kinetics has been treated with differing degrees of rigour by Bergner (1962, 1964), Zierler (1962), Nosslin (1964) and Rescigno & Segre (1966), and more extensively by Shipley & Clark (1972). A similar stochastic analysis has been used by engineers for the evaluation of continuous-flow systems, and Aris (1966) and Wingard *et al.* (1972) have pointed out the merit of this approach to the study of circulation and metabolism. Properly used the various ways of analysis of isotope kinetics should yield the same information. However, as may be seen from Table 1, the evaluation of the replacement rate and masses depends on the determination of areas. It appears illogical first to fit curves to assumed functions, a rather unstable operation, and then to integrate, when the areas can be measured in the first place simply and reliably.

In addition, the fitting of data by non-linear least-squares methods offers some difficulties (see, e.g., Atkins, 1969). With a single injection, the early numerically large values carry more weight than later smaller ones. Ottaway (1971) stressed the importance of applying suitable weighting factors, but as he points out there is no objective criterion for these corrections. The theoretical advantage of numerical integration of areas over fitting to exponential equations was discussed by Normand & Fortier (1970). It will be shown in the following paper (Katz *et al.*, 1974) that graphical integration with paper and pencil is not difficult. Moreover, the integrations can be performed readily by the use of Simpson's or the trapezoidal rule and the operations can be programmed for a desk calculator or a simple computer program.

The determination of the sampling pool M_s and of the rate of its exchange with the rest of the glucose, R_{11}, depends on the evaluation of slopes and intercepts only in the very first minutes of the experiments and requires very early sampling. Extrapolation from later points either manually or by computer may be quite misleading. Owing to limitations in our sampling technique our estimates of M_s and R_{11} are only rough approximations.

(II) Recycling of Glucose Carbon

Effects of recycling on the calculation of apparent mass and apparent replacement rate

The effects of recycling are best shown by examining an example. It is assumed that, as in the starved animal, there is no input of glucose from liver glycogen stores. It is also assumed that the system is in a steady state with respect to glucose and that glucose mass is maintained constant by synthesis from lactate. The metabolized glucose is all converted into pyruvate (or lactate), which mixes with endogenous unlabelled lactate. The lactate is in part oxidized to CO_2 and in part reconverted into glucose. The system is a gross oversimplification and the model shown in Fig. 2 is not intended to simulate a physiological state, but to serve as a numerical example to illustrate the effects of recycling on the calculation of the rate of glucose synthesis or total body mass. Qualitatively the conclusions would apply with more realistic and more complex models. The model, shown in Fig. 2, consists of glucose in two exchanging pools, with catabolism restricted to pool 1. Newly synthesized glucose enters pool 1, the injected and sampled compartment. The product (lactate) enters compartment 3, which also has an unlabelled endogenous inflow. The outflow from this compartment is divided between irreversible loss and resynthesis of glucose. The fraction of labelled carbon reincorporated into glucose equals the ratio of glucose synthesis to the total inflow into the lactate pool. It should be noted that in this model the tracer labels glucose but not the inflow into the system, which is lactate. Systems in which the inflow is uniformly labelled with tracer may be designated 'homogeneous', whereas systems such as the model

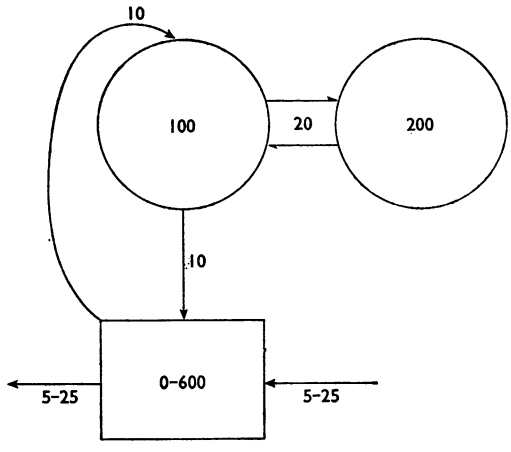

Fig. 2. *Model of glucose metabolism with inflow of carbon into the precursor pool*

The mass of glucose (300), the rate of synthesis (10) and the rate of exchange between glucose pools (20) remain constant, but inflow into the lactate pool (square) and its mass are varied as shown. Arbitrary units of mass and time are used. [^{14}C]Glucose is injected into pool 1. The specific-radioactivity curves for this pool are shown in Fig. 3.

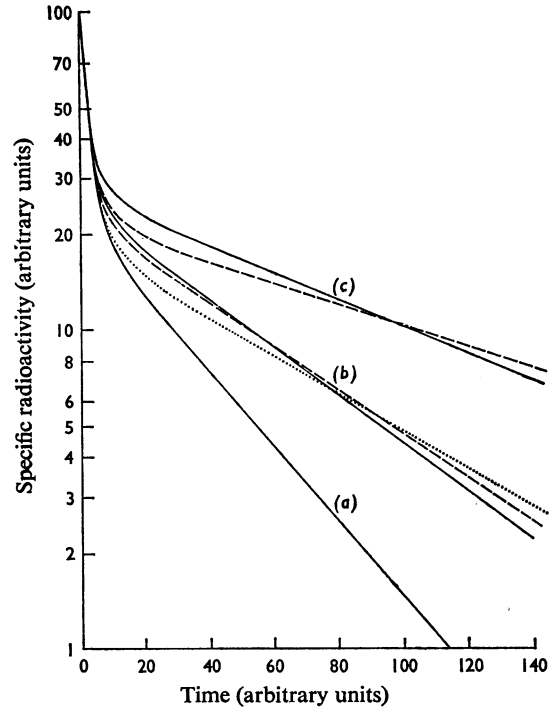

Fig. 3. *Specific-radioactivity curves for the model of Fig.* 2

Curve (*a*), no recycling; curves (*b*), with an inflow of 15 into the lactate pool or 40% recycling; curves (*c*), inflow of 5 into the lactate pool or 67% recycling. The mass of the lactate pool was either 50 (——), 200 (– – – –) or 600 (· · · ·). Note that with 40% recycling or less, the curves for a mass of 50 and 200 are very similar and in practice could not be distinguished.

of Fig. 2 in which the tracer is introduced in a site or compartment other than at the inflow may be designated 'non-homogeneous'. In the example the mass of glucose and its rate of synthesis are kept constant, but the mass of lactate and inflow into the system are varied. In Fig. 3 a family of curves with 0, 40 and 67% recycling, and with different masses in the lactate pool, are shown. It is apparent that when inflow into the system is higher than the rate of glucose synthesis, the shape of the curves is not sensitive to changes in the mass of the product pool. When recycling is less than 25%, the total mass of the system cannot be, because of experimental error, reliably determined from the glucose specific-radioactivity curves.

The apparent replacement rates and the apparent minimal and maximal masses were calculated from the plasma curve as if the system were homogeneous, and the calculated values obtained with this (erroneous) assumption are shown in Table 2. Inflow was varied from 5 to 25 units of mass, corresponding to from 28 to 67% recycling, and the mass in the product (lactate) pool from 50 to 600 units of mass. The total mass of glucose was 300 units of mass and the rate of synthesis 10 mass units per unit time. The surprising result is that the apparent total minimal mass did not differ much from the mass of glucose. For instance, with 28% recycling and a total mass three times that of glucose the apparent mass was only 15% higher than that of glucose. Thus an incorrect calculation using [^{14}C]glucose provides under some conditions a fair approximation to glucose mass. This is well illustrated in the experiments with rabbits and rats (Katz *et al.*, 1974). The reason for this is that recycling decreases replacement rate but increases mean transit time, so that the change in their product is relatively small. The apparent maximal mass would be, however, considerably greater than without recycling (404 mass units in this example), and this has also been observed (Katz *et al.*, 1974).

Irreversible loss

The apparent replacement rate, obtained by dividing the injected dose by the area from 0 to ∞ under the respective plasma curves, has been commonly

Table 2. *Apparent mass calculated for the model of Fig. 2*

The units are arbitrary. The mass of glucose in the model is 300 and the rate of synthesis 10. The apparent masses may be calculated, assuming a homogeneous system, by the equations of Table 1 but they were in fact obtained by the following short-cut:

$$M_{\text{min.}} = \left|\frac{dF_{i(s)}}{ds}\right|_{(s=0)} \Big/ \left|F_{1(0)}\right|^2$$

where $F_{(s)}$ is the Laplace transform of the specific radioactivity in the sampling pool. $M_{\text{max.}} = (B/C) \times (1/F_{1(0)})$, where B and C are the coefficients of first and zero degree respectively for the characteristic equation of the set of differential equations.

Carbon inflow ...	25			15		10		5	
Percentage recycling ...	28.5			40		50		66.7	
Apparent replacement ...	7.15			6.0		5.0		3.33	
Real mass									
Products	Total	$M_{\text{min.}}$	$M_{\text{max.}}$	$M_{\text{min.}}$	$M_{\text{max.}}$	$M_{\text{min.}}$	$M_{\text{max.}}$	$M_{\text{min.}}$	$M_{\text{max.}}$
50	350	304	386	308	380	312	375	322	367
100	400	308	402	316	400	325	400	345	400
150	450	312	414	324	425	337	425	367	422
300	600	324	457	348	480	375	500	432	533
600	900	348	540	396	600	450	650	564	733

designated the rate of irreversible disposal. Shipley & Clark (1972) have thoroughly discussed the distinction between irreversible disposal and production (or utilization) of the compounds. The irreversible disposal of glucose carbon may be defined as the mass of glucose that forms compounds not recycled back into glucose, or as the part of glucose synthesis derived from carbon not previously labelled from glucose. The definition has limitations, since if [^{14}C]glucose is applied in large amounts or infused for long periods practically all the body carbon will contain ^{14}C, and contribute ^{14}C to newly formed glucose. However, the turnover of this carbon is much slower than that of glucose and this secondary recycling is neglected, just as is the recycling of ^{3}H from labelled water. As shown by the example in Fig. 2 irreversible disposal cannot be represented by a definite enzyme rate or a metabolic path, but is a complex metabolic parameter. It is of considerable physiological interest, but does not indicate the rate of glucose production by liver and kidney, nor utilization of glucose by body tissues. These, however, are the most significant pieces of information about glucose metabolism, and to obtain them the use of doubly labelled glucose and evaluation of recycling are required.

Quantification of recycling

Consider a system in which glucose is synthesized from endogenous or exogenous carbon precursors, and catabolic products from glucose enter carbon compounds, with some of these being reincorporated into glucose. For simplicity assume the glucose to be present in one well-mixed pool. If glucose labelled with ^{3}H, forming water as sole labelled product, is injected the plasma specific-radioactivity curve will be represented by a single exponential term. If the glucose is labelled with ^{14}C there will be two isotopic inputs, one due to the initial injection and one due to the input via recycling. The second input function may be simple or complex depending on the nature of the pathways.

If the mass of glucose is designated M, its rate of synthesis and loss R_0 and the total radioactivities Q_H and Q_C for ^{3}H- and ^{14}C-labelled glucose respectively, the specific radioactivities are then $Q_H/M = q_H$ and $Q_C/M = q_C$. The specific radioactivity of the precursor of glucose is designated q_R. R_0 has been previously defined as being essentially equal to the rate of glucose phosphorylation or glucose 6-phosphate phosphatase. The direct precursor is glucose 6-phosphate, which may be formed from liver pyruvate, glycerol, glycogen etc.

The trivial differential equation in the absence of recycling would be $dQ/dt = -R_0 q$, but with recycling an additional input, $R_0 q_R$, under the conditions specified, occurs. Dividing throughout by M the differential equation for [^{14}C]glucose is:

$$\frac{dq_c}{dt} = \frac{-R_0}{M} \cdot q_c + \frac{R_0}{M} \cdot q_R \qquad (2)$$

This equation only holds if there is no direct unlabelled input into the initially labelled (glucose) pool. If there is a direct input, e.g. glucose from dietary carbohydrate in the gut, the rates of synthesis and of loss are not equal, and the system is not in a steady state.

If ^{14}C is used as the sole tracer, R_0 is unknown and eqn. (2) cannot be solved. If ^{3}H and ^{14}C are used

simultaneously, R_0 is known from the ^3H curve (see Table 1, and eqn. (2) can be solved for q_R. We define R_a as an apparent replacement rate, equal to the injected dose divided by the area from 0 to ∞ under the ^{14}C specific-radioactivity curve, and R_0 will be equal to the area from 0 to ∞ under the ^3H curve.

Eqn. (2) may be transformed into the following integral equation (see the Appendix):

$$\int_0^\infty q_c dt = \frac{1}{R_0} + \int_0^\infty q_R dt \quad (3)$$

which leads to:

$$R_a = \frac{1}{\int_0^\infty q_c dt} = \frac{R_0}{1 + R_0 \int_0^\infty q_R dt} \quad (4)$$

From the definition of R_0 it also follows that:

$$\int_0^\infty q_R dt = \int_0^\infty q_c dt - \int_0^\infty q_H dt \quad (5)$$

or simply the difference in the areas from 0 to ∞ of the normalized ^3H- and ^{14}C-labelled glucose specific-radioactivity curves. The expression is also valid if the glucose system is not a well-mixed pool and Q_H is multiexponential.

It is convenient to describe recycling by the ratio:

$$\frac{\int_0^\infty q_R dt}{\int_0^\infty q_C dt} = \frac{\int_0^\infty q_C dt - \int_0^\infty q_H dt}{\int_0^\infty q_C dt} \quad (6)$$

If the apparent replacement rate for glucose ^{14}C is R_a we obtain:

$$\text{Fraction recycled} = \frac{\int_0^\infty q_R dt}{\int_0^\infty q_C dt} = \frac{R_0 - R_a}{R_0} \quad (7)$$

Because of the limitations attached to eqn. (2), this definition only applies to homogeneous systems as defined above. For instance, although it might well apply to glucose metabolism in starved animals, it would not apply to animals after a carbohydrate-containing meal.

It is difficult to define a 'rate' of recycling. Eqn. (7) is an expression for the fraction of carbon of newly synthesized glucose that came from glucose. Thus when the percentage recycling is 40, 40% of the glucose carbon formed is recycled one or more times and 60% is 'virgin' carbon not previously in the form of glucose.

The input function q_R may be obtained by deconvoluting the difference between the ^{14}C and ^3H specific-radioactivity curves, with the ^3H curve serving as weighting curve. Such a curve represents the theoretical specific radioactivity of the immediate precursor of the recycled glucose. Such curves are presented by Katz *et al.* (1974).

Tracers for the determination of recycling

Glucose labelled with ^3H in positions 2, 3, 4 and 5 may serve as irreversible tracers. The metabolism of glucose labelled in these positions was studied by Katz & Rognstad (1966, 1969) and Katz & Wals (1971, 1972) in rat adipose tissue and mammary gland. Water is the major primary product from all these tracers and under some conditions may account for up to 99% of the utilization of ^3H from positions 2 and 5. ^3H from all positions may appear to some extent in glycogen and that from positions 2, 3 and 4 in lipids. However, little of the ^3H in muscle glycogen and lipids is likely to be reincorporated into glucose. Use of glucose labelled simultaneously with ^3H and ^{14}C provides maximal information on glucose metabolism with practically no more effort than the use of singly labelled glucose. It is also possible, by following the approach of von Holt *et al.* (1961) and Reichardt *et al.* (1963), to determine synthesis, body mass and recycling with glucose labelled in C-1 or C-6, but degradation of blood glucose to determine the randomization of label between both positions is required. By subtracting the ^{14}C on the 'top' from the 'bottom' carbon, (or vice versa) a specific-radioactivity curve that should be equivalent to that with an irreversible tracer is obtained. The procedure is much more laborious than the use of doubled-labelled glucose, but comparison of results by the two techniques should be of interest.

We acknowledge the support of grant N.I.H. no. 5R01AM-12604-05 to J. K. and grant no. 501AM07-215 to A. D.

References

Aris, R. (1966) in *Intracellular Transport* (Warner, K. B., ed.), vol. 5, pp. 167–169, Academic Press, New York

Atkins, G. L. (1969) *Multicompartmental Models for Biological Systems*, chapter 5, Methuen and Co., London

Bergner, P. E. E. (1962) *Acta Radiol. Suppl.* **210**, 1–10

Bergner, P. E. E. (1964) *J. Theor. Biol.* **6**, 137–158

Bergner, P. E. E. (1965) *Science* **150**, 1048–1050

Katz, J. & Dunn, A. (1967) *Biochemistry* **6**, 1–5

Katz, J. & Rognstad, R. (1966) *J. Biol. Chem.* **241**, 3600–3610

Katz, J. & Rognstad, R. (1969) *J. Biol. Chem.* **244**, 99–106

Katz, J. & Wals, P. (1971) *Arch. Biochem. Biophys.* **147**, 405–417

Katz, J. & Wals, P. (1972) *Biochem. J.* **128**, 879–899
Katz, J., Dunn, A., Chenoweth, M. & Golden, S. (1974) *Biochem. J.* **142**, 171–183
Normand, M. & Fortier, C. (1970) *Can. J. Physiol. Pharmacol.* **48**, 274–281
Nosslin, B. (1964) in *Appendix to Metabolism of Human Gamma Globulin* (Andersen, S. A., ed.), pp. 103–120, Blackwell Scientific Publications, Oxford
Ottaway, J. H. (1971) *Biochem. J.* **125**, 44P–45P
Perl, W. & Chinard, F. A. (1969) *Science* **160**, 260
Reichardt, G. A., Moury, N. F., Hochella, N. J., Patterson, A. & Weinhouse, S. (1963) *J. Biol. Chem.* **238**, 495–501
Rescigno, A. & Segre, G. (1966) *Drug and Tracer Kinetics* Blaisdell Publishing Co., Waltham, Mass.
Rossing, N. (1971) *Human Albumin Metabolism*, Munksgaard, Copenhagen
Shipley, R. A. & Clark, R. E. (1972) *Tracer Methods in Vivo*, Academic Press, New York
Steele, R. (1971) *Tracer Probes in Steady State Systems*, C. C. Thomas, Springfield, Ill.
Steele, R., Wall, J. S., de Bodo, R. L. & Altszuler, N. (1957) *Amer. J. Physiol.* **187**, 15–24
von Holt, C., Schmidt, H., Feldmann, H. & Hallmann, I. (1961) *Biochem. Z.* **334**, 524–533
Wingard, L. B., Chorbajian, L. & Galla, S. J. (1972) *J. Appl. Physiol.* **33**, 264–275
Zierler, K. (1962) in *Handbook of Physiology, Circulation* (Hamilton, W. F. & Dow, P., eds.), vol. 1, pp. 585–615, American Physiological Society, Washington, D.C.

APPENDIX

Solution of eqn. (2) of main paper:

$$\frac{dq_C}{dt} = \frac{-R_0}{M} \cdot q_C + \frac{R_0}{M} \cdot q_R$$

q_C and q_R are any functions of t. For convenience, replace $\frac{R_0}{M}$ by k. Then:

$$kq_R = \frac{dq_C}{dt} + kq_C = e^{-kt} \cdot \frac{d}{dt}(q_C e^{kt}) \qquad (A1)$$

Integrating from 0 to t

$$q_C e^{kt} - q_C(0) = k \int_0^t e^{kt'} \cdot q_R \, dt' \qquad (A2)$$

$q_C(0)$ = injected dose/M. If the dose is set to unity, $q_C(0) = 1/M$. Substituting this and dividing through by e^{kt} we obtain:

$$q_C = \frac{1}{M} e^{-kt} + k e^{-kt} \int_0^t e^{kt'} \cdot q_R \, dt' \qquad (A3)$$

Integrating from 0 to ∞:

$$\int_0^\infty q_C \, dt = \frac{1}{M} \int_0^\infty e^{-kt} \, dt + k \int_0^\infty e^{-kt} \, dt \int_0^t e^{kt'} \cdot q_R \, dt' \qquad (A4)$$

By using the fact that

$$\int_0^\infty e^{-kt} \, dt \int_0^t e^{kt'} f(t') \, dt' = \frac{1}{k} \int_0^\infty f(t) \, dt \qquad (A5)$$

for any function f which has a convergent integral, then:

$$\int_0^\infty q_C \, dt = \frac{1}{M} \int_0^\infty e^{-kt} \, dt + \int_0^\infty q_R \, dt$$

$$= -\frac{1}{Mk} \{e^{-kt}\}_0^\infty + \int_0^\infty q_R \, dt$$

$$= \frac{1}{Mk} + \int_0^\infty q_R \, dt$$

Substituting R_0/M for k leads to:

$$\int_0^\infty q_C \, dt = 1/R_0 + \int_0^\infty q_R \, dt \qquad (A6)$$