Compartmental and semicompartmental approaches for measuring glucose carbon flux to fatty acids and other products in vivo

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Abstract We have attempted to estimate the flux of glucose carbon to total body fatty acids and to other metabolic end products in Bar Harbor 129/J mice fasted 5–8 hr. Tracer [U-^{14}C]glucose was injected intravenously, and the following data were obtained at various times up to 180 min: plasma glucose C specific activity, plasma glucose concentration, total body glycogen, and ^{14}C in total body fatty acid, total body lipid, unsaponifiable lipid, expired CO_2, and in hepatic and extrahepatic glycogen. The data were analyzed by three techniques, namely, multicompartmental, semicompartmental, and noncompartmental analyses. All three methods yielded comparable rates of glucose C conversion to total body fatty acids (2–3 μg of glucose C/min/20 g of body weight). Although the semicompartmental approach is extremely simple (it only requires analyses of plasma glucose specific activity as a function of time and ^{14}C-labeled fatty acid at one point in time), it gives an apparently valid approximation for the flux of glucose C to fatty acids. Other quantitative aspects of glucose metabolism in postabsorptive mice are also considered.

Supplementary key words multicompartmental analysis · [U-^{14}C]glucose · glycogen · carbon dioxide · noncompartmental analysis · mice

We have been interested in the metabolism of glucose in vivo and particularly in the quantification of transformation rates of glucose carbon to its metabolic products (1–3), including fatty acids (4). Several approaches, based on radioactive tracer techniques, have been used to study the conversion of a metabolic fuel to one or more of its products. Some of the experimental designs are quite simple from the standpoint of data analysis and mathematical computation, but they are somewhat complicated technically. For example, the conversion of glucose C to fatty acids has been studied (5) by a method in which [^{14}C]glucose had to be infused constantly throughout the experiment so that the specific activities of precursor and intermediate pools would remain constant during the experiment. The interpretation of such data is simpler than in technically easier experiments in which a single injection of a radioactive tracer is given and a multicompartmental analysis of complicated radioactivity curves is performed (6). Shipley et al. (7) have proposed a simple, noncompartmental method for analyzing data obtained from tracer experiments in which the single-injection technique has been used; however, their approach requires the isolation and measurement of all so-called end products[1] in order to calculate the conversion rate of a precursor to a single "end product."

We have tried to define the simplest experimental–mathematical approach for measuring the conversion rate of a precursor to a particular end product (4). It is a modification of the noncompartmental approach of Shipley et al. (7) and has been called a "semicompartmental" (4) approach since it combines features of both noncompartmental and multicompartmental analyses. Briefly, the semicompartmental analysis requires measurement of the specific activity–time curve of the precursor after a single intravenous injection of a radioactive tracer (as in the method of Shipley et al. [7]) and

Abbreviations: TLC, thin-layer chromatography; sp act, specific activity.

[1] The term "end products," as used here, includes pools that are slowly turning over, as defined by Shipley et al. (7). Of these, only expired CO_2 can be considered a true end product.

one measurement of the radioactivity in an "end product" at any point in time. From these data, by assuming a simple model, one may calculate the ratio at any time, t, of radioactivity in the measured end product $[q_i(t)]$ relative to that in all end products $[q_n(t)]$. The transfer rate of the precursor into the end product is then given by the following expression:

$$R_2 = R_1 \frac{q_i(t)}{q_n(t)}$$

where R_2 = μmoles/min of precursor converted to the particular end product of interest and R_1 = the irreversible disposal rate, μmoles/min, of the precursor (1, 7); the ratio $q_i(t)/q_n(t)$ has been defined above. Both R_1 and $q_n(t)$ are calculated from the specific activity–time curve of the precursor, and $q_i(t)$ is directly measured at just one point in time (4).

Although the semicompartmental approach is by far the simplest of the several isotopic methods that have been used in quantitative metabolic studies, its validity is yet to be tested by comparison with the more complicated, but theoretically more accurate, approach using multicompartmental analysis. In order to test the validity of the semicompartmental approach for approximating rates of fatty acid synthesis from glucose C, we have carried out a detailed study of the conversion of [U-^{14}C]glucose to its major end products, including total body fatty acids, in mice fasted for a short time. The data have been analyzed by three approaches, multicompartmental, noncompartmental, and semicompartmental analyses. The latter approach was found to give essentially the same values for lipogenic rates as the other, more complicated approaches and provided a valid approximation of rates of fatty acid synthesis from glucose C even in experiments of relatively short duration.

MATERIALS AND METHODS

Animals

Male mice (strain 129, Jackson Memorial Laboratories, Bar Harbor, Maine) weighing 17–24 g and housed 4–12/cage were fed Purina laboratory chow ad lib. On the experimental day, to eliminate intestinal glucose absorption, all animals were deprived of food, but not water, for 4–6 hr, starting about 9 AM.

Tracer injection

[U-^{14}C]Glucose (1 μCi; sp act, 5–42 mCi/mmole) was injected in 50 μl of water into a lateral caudal vein. Mice were restrained (8) and the exposed tails were immersed in 40–45°C water to dilate the veins immediately before the tracer was injected.

CO_2 collection

Prior to injection of tracer, each mouse was placed in a paired, sleeve-jointed, 1-oz, narrow-mouthed bottle (Braun no. 16058) from which the bottom had been removed. This simple arrangement was especially suited for preventing the animal from turning during breath collections. The tail remained exposed through the opening at one end. A rubber stopper (no. 1, single hole), fitted into the other end, served as a connector to the CO_2 traps. The gas-flow train consisted of two trapping vessels of measured efficiencies and a flowmeter joined in series using polyethylene tubing. Room air was swept by vacuum through the train via the rear opening of the mouse restraining chamber. Flow (0.5–1.0 l/min) was regulated with a vacuum filter flask fitted with stopcocks. Trapping vessels were 500-ml separatory funnels with standard taper joints and fitted with fritted gas inlet tubes (similar to Corning no. 98075) extending almost to the stopcock. Each vessel contained 50 ml of NaOH (either 1 N in water or 0.1 N in 50% ethanol–water). Flow was initiated just prior to injection of tracer. Respiratory CO_2 was collected continually for 30 min after injection of [U-^{14}C]glucose (four mice). At intervals (1, 5, 10, 15, and 30 min after injection), gas flow was interrupted momentarily by raising the dispersion tube of the first trap and draining 1 ml of trapping solution through the stopcock into graduated tubes. In order to maintain the initial 50-ml volume of the first trap, fresh alkali, equivalent in volume to that removed, was aspirated into the trap through the stopcock without interrupting the gas flow. Single 0.50-ml aliquots were assayed for radioactivity.

Analysis

Plasma glucose specific activity was determined after separation of glucose by TLC as described previously (8, 9). Plasma glucose concentrations were estimated enzymatically (9) from filtrates of deproteinized plasma or after separation of glucose by TLC. Glucose recoveries after TLC averaged 85% and were corrected to 100% recovery. Total body glucose was determined by the indirect method of Baker and Huebotter (4). Total body [^{14}C]glucose was calculated as a function of time from the product of glucose specific activity (t) × total body glucose. Total body ^{14}C-labeled lipid was measured in 15 mice (6 at 15, 6 at 30, and 3 at 60 min after injection). Aliquots (10 ml) of alcoholic (70%) homogenates (9) of the mice were heated at 80°C under N_2 to remove alcohol and then they were lyophilized. Residues were extracted and washed once according to Folch, Lees, and Sloane Stanley (10); aliquots (3–5 ml) of the total body lipid extract were then dried (60–80°C) in the counting vials for assay in a liquid scintillation counter. Lipid glyceride [^{14}C]glycerol was calculated by

assuming that total ^{14}C-labeled lipid minus total lipid ^{14}C-labeled fatty acid equals total lipid glyceride [^{14}C]-glycerol. A separate experiment was carried out to measure lipid ^{14}C-labeled fatty acid (4), unsaponifiable ^{14}C-labeled lipid (4), total body glycogen (labeled and unlabeled), and total body ^{14}C at various times after injection of [U-^{14}C]glucose. All analyses were initiated from an ethanolic alkaline digest as described previously (4). Glycogen was precipitated with additional alcohol from aliquots of the ethanolic alkaline digests and assayed enzymatically (11) and for ^{14}C after repeated alcoholic precipitation. Total body ^{14}C in the alcoholic digests was assayed directly after dispersion of particulate materials by ultrasonic vibration[2] for 2 min at 0°C. Liver glycogen ^{14}C was estimated (following purification as above) in three experiments. In experiment 1, the mice were anesthetized with subcutaneously injected pentobarbital, 2.5 mg/20 g body wt; the livers were removed rapidly and frozen in liquid nitrogen. In experiments 2 and 3, unanesthetized mice were stunned and immersed immediately in liquid nitrogen (expt. 2) or their livers were removed immediately and frozen in liquid nitrogen (expt. 3); in experiment 2, frozen livers were dissected from the frozen carcasses.

Radioactivity

All radioactivity measurements were performed in a Packard Tri-Carb liquid scintillation counter. Vials contained ^{14}C-labeled material in various forms: dissolved in ethanolic or aqueous extracts; dried, as in the case of lipid extracts; dissolved in alkali ($Na^{14}CO_3$ and total body residual ^{14}C). To the dried samples, 0.1–0.5 ml of 70% ethanol was added. Vials for assay of $^{14}CO_2$ and total lipid ^{14}C were filled (ca. 19 ml) with scintillator gel (9) and shaken occasionally during gelation. All other ^{14}C samples were dissolved and counted in a different, improved scintillator solution.[3] Appropriate ^{14}C standards were simultaneously assayed in each case, and quench corrections were applied when necessary.

Multicompartmental analysis, model, and assumptions

The SAAM 23 program of Berman, Shahn, and Weiss (12) was used to find a set of fractional rate constants which would give a "best" least squares fit for our data assuming the model shown in Fig. 2. An IBM 360 digital computer was used for the analysis. The following assumptions were made or are implicit in our model.

Body glucose (compartment 1) was treated as a single well-mixed compartment which was replaced primarily by gluconeogenesis in liver and kidney via a small pool of glucose-6-phosphate (not shown). The carbon from which the glucose was derived was considered, for purposes of simplification, to be derived from a heterogeneous mixture of compounds (compartment 2) all of which were formed partly from glucose, partly from relatively nonradioactive sources (I_2), and partly from extrahepatic tissue glycogen. Although chemically heterogeneous, compartment 2 was treated as a single well-mixed pool. Many intermediate steps between the major pools shown in the model were assumed to be so small and to turn over so rapidly that, for mathematical purposes, they could be ignored (6). Supporting arguments for many of these assumptions have been presented previously, e.g., homogeneity of body glucose pool (3, 7), recycling of glucose C (1–3, 13), and rapidity of turnover of intermediates (14, 15). Although a small amount of glucose C was also derived from glycerol by a relatively direct pathway,[4] the contribution of glycerol C to gluconeogenesis was assumed to be negligible. Our unpublished [^{14}C]glycerol experiments also showed that intermediates in gluconeogenesis turned over so rapidly that they could be ignored for mathematical purposes, e.g., [^{14}C]glycerol did not have to pass through compartment 2 before it was converted to glucose. Formation of glucose from liver glycogen and glucose derived from intestinal absorption were assumed to be negligible because of the dietary state of the animals (4). Additional evidence to support the assumption of negligible hepatic glycogenolysis to glucose is given in the text. Compartments 1, 2, 3, 5, 6, and 7 (Fig. 1) were assumed to be in the steady state with respect to total carbon content. Glyceride glycerol (compartment 8) and extrahepatic glycogen (compartment 3) were assumed to be formed from glucose by way of rapidly turning over pools which could be ignored for mathematical analysis; however, all other so-called end products (7) were assumed to form from glucose only after passage through the intermediates of compartment 2. All other intermediates in the formation of "end products" were excluded for purposes of simplification (see above), with the exception of the body bicarbonate–CO_2 compartments. Rate constants describing the turnover of the latter (compartments 5, 6, and 7) were taken from a previously published study using the same strain of mice (16). The fractional turnover rates of total body glyceride glycerol, lipid fatty acids, unsaponifiable lipids (such as cholesterol), protein, and nucleic acid were considered to be negligible during the period of the experiment (7); furthermore, negligible ^{14}C was assumed to recycle to glucose from any "end product" including the only true, major end product, expired CO_2 (compartment 4). No assumptions were

[2] Branson Sonifer, model LS75, using the micro tip and tuned at 3 amp (Branson Instruments, Stamford, Conn.).

[3] 8 ml of a 3:5 (v/v) mixture of Triton X-100 (Rohm and Haas) and scintillator solution (10 g of diphenyloxazole/l of toluene).

[4] Baker, N., and R. J. Huebotter. Unpublished observations.

necessary regarding the location of unlabeled inflows into the system. For example, calculation of all fractional rate constants shown in Fig. 1 are independent of location and magnitude of unlabeled inflows. Although one such inflow is shown (I_2), it is presented only to indicate that some such inflow is necessary in order to maintain steady state conditions for compartments 1, 2, and 3. At least one additional unlabeled inflow, I_5, would also be necessary (2), in order to account for the total CO_2 expired during the experiment. Assumptions regarding nonradioactive inflows would be required to calculate total flux of carbon in and out of the system; however, these assumptions are not necessary in order to calculate the flow of glucose carbon to its "end products" (7, 17). Only the latter flux calculations were made.

Semicompartmental analysis

The semicompartmental approach for estimating glucose C flux to fatty acids has been presented in an earlier study (4) and has been summarized briefly in the introduction, above, and in the legend to Table 2. Our approach assumes a two-compartment model in which all end products, including fatty acids, are formed from a common pool of intermediates:

→ glucose ⇄ intermediate pools → end products

The semicompartmental approach is essentially the same as the noncompartmental approach proposed by Shipley et al. (7), except that the total activity in the end products must be measured in the noncompartmental approach, whereas in the semicompartmental approach this value is calculated from a compartmental analysis of the glucose specific activity–time curve. Other assumptions and limitations of the semicompartmental approach have been discussed previously (4).

RESULTS

Steady state of total body glucose and glycogen

The total body glucose pool was in a low, near-steady state during the study and liver glycogen was essentially depleted (see below). The steady state of the glucose pool was evidenced by the constancy of the plasma glucose concentration during the first hour of the experiment (Table 1). Although the plasma glucose concentration was studied for only 1 hr, we have shown that the plasma glucose level in our mice remains relatively constant during an 8-hr fast, which is a period sufficient to deplete the liver glycogen completely (4). Additional evidence of steady state conditions was afforded by the observed levels of total body glycogen, which were low and constant (Table 1). Such low values indicate that the liver glycogen levels had been largely depleted during the 5-hr fasting period preceding the study. For example, in Table 1, in the last group of mice shown (t = 180 min after [^{14}C]glucose administration), the animals had been fasted for 8 hr; such animals are known to have negligible stores of liver glycogen (4). Therefore, the observed value, 30 mg total body glycogen/20 g body wt, must represent *extrahepatic* tissue glycogen. The values in mice fasted for shorter periods of time did not differ significantly from this value and were considerably less than the levels (80 mg/20 g body wt) which one would find in fed mice of this strain, which are known to have 50 mg of liver glycogen/20 g body wt (4). Since liver glycogen was depleted, no appreciable liver glycogen could have been in the process of net mobilization during the isotopic studies.

Plasma [^{14}C]glucose and total body [^{14}C]glucose

Plasma glucose specific activity as a function of time is

TABLE 1. Plasma glucose and total body glycogen content in 5-hr-fasted mice, and plasma glucose specific activity as a function of time after intravenous injection of [U-^{14}C]glucose

Time after [U-^{14}C]-Glucose Injection	Plasma Glucose		Total Body Glycogen
	Specific Activity	Concentration	
min	*% injected ^{14}C/mg glucose × body wt/20 g*	*mg/ml*	*mg/20 g body wt*
0		1.16 ± 0.06[a]	
1	13.0 ± 0.64	1.07 ± 0.18[b]	
5	7.2 ± 0.32	1.39 ± 0.07[b]	
15	3.6 ± 0.39	1.31 ± 0.15[b]	26.2 ± 3.1
30	2.1 ± 0.17	1.06 ± 0.17[b]	33.4 ± 1.6
60	1.0 ± 0.11	1.26 ± 0.12[a]	38.4 ± 5.3
120			39.2 ± 2.4
180			29.8 ± 2.8
Grand mean		1.21 ± 0.05	33.4 ± 0.52

Values are means ± SEM for four animals/group.
[a] Determined by analysis of deproteinized plasma.
[b] Calculated from glucose recovered after TLC.

shown in Table 1. These data could be described by the following function:

$$\text{sp act} = 10.8\ e^{-0.23t} + 4.4\ e^{-0.024t}$$

Since plasma glucose specific activity does not differ appreciably from total body glucose specific activity between 1 and 60 min after intravenous injection of tracer [^{14}C]glucose in mice (3), the total body [^{14}C]-glucose could be calculated from the plasma glucose specific activity data (see legend to Fig. 1).

The disappearance of [^{14}C]glucose from the total body glucose pool is shown in Fig. 1. Thus, over 90% of the injected [^{14}C]glucose left the body glucose pool in 60 min; however, about 50% of the glucose disappearance occurred during the first 5 min of the study. The fractional rate of disappearance was much slower between t = 30 and 60 min ($t_{1/2}$, 30 min). Similar kinetic phenomena, reported in previous studies in both mice (3) and rats (1, 2), have been interpreted as evidence that much of the [^{14}C]glucose was converted to products that recycled carbon back to glucose (1–3, 13).

Incorporation of [^{14}C]glucose into "end products"

Fig. 1 presents a summary of all of the observed ^{14}C data as well as values calculated by the SAAM program from the set of fractional rate constants shown in Fig. 2. The major metabolic product was CO_2 (compartment 4). Two-thirds of the injected ^{14}C was converted to CO_2 in 3 hr. However, only 2% of the dose was excreted as $^{14}CO_2$ at 5 min, when half of the injected [^{14}C]glucose had left the body pool. This indicates that glucose C had to pass through a large intermediate pool before it was oxidized to CO_2. It should also be noted that $^{14}CO_2$ was directly measured for only 30 min. $^{14}CO_2$ excretion at 60–180 min was estimated from the difference between the injected dose and the ^{14}C left in a total body alkaline digest. That is, we assumed that any ^{14}C not found in the total body had been excreted as $^{14}CO_2$. The latter indirect method gave values at 30 min which were identical with those obtained by direct $^{14}CO_2$ measurement. The validity of this approach was also established by comparison of $^{14}CO_2$ excretion predicted from the model on the basis of $^{14}CO_2$ data between 5 and 30 min with indirectly measured $^{14}CO_2$ between 60 and 180 min. The calculated and observed values agreed within experimental error.

Although almost half of the injected [^{14}C]glucose was converted to CO_2 in 1 hr, only about 1% of the dose was converted to total body lipid fatty acids (compartment 9) at that time. The values for ^{14}C-labeled fatty acid re-

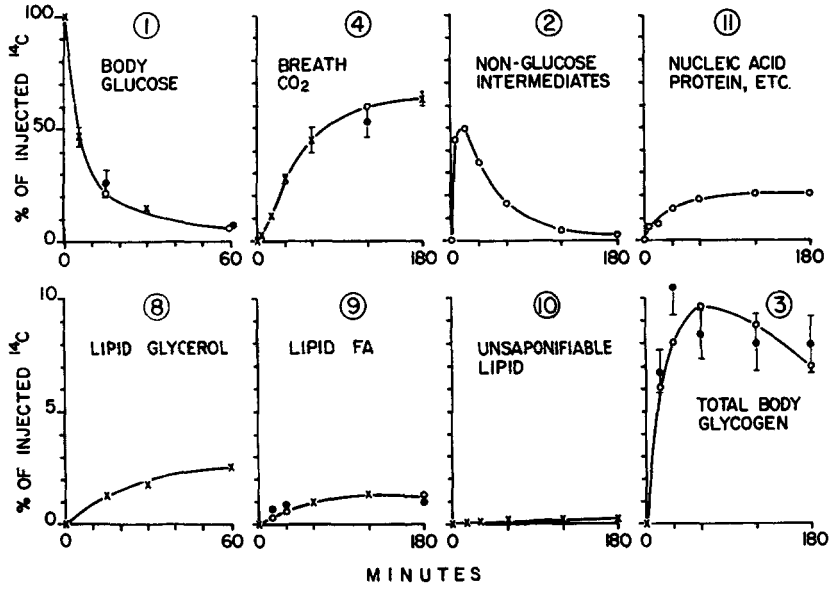

FIG. 1. Observed and calculated values for [^{14}C]glucose and its metabolic products after intravenous injection of tracer [U-^{14}C]glucose into briefly fasted mice. Calculated values were derived by digital computer (SAAM) using the model and fractional rate constants shown in Fig. 2. Observed values (± SD), ●; calculated values, O; equal values for observed and calculated values, ×. Values for the observed body [^{14}C]-glucose (t) were derived from plasma glucose specific activity ± SD (Table 2) multiplied by the mean body glucose pool size (6.7 mg/20 g body wt) as determined by computer extrapolation to zero time using a two-compartment model (4). Data for compartments 1 and 4 are based upon serial determinations in two separate groups of four mice each; for compartments 3, 9, and 10, n = 4 at each time point; for compartment 8, n = 6, 6, and 3 at 15, 30, and 60 min, respectively. Where no SD for data are shown, the deviations were smaller than the symbols. Encircled numbers correspond to the compartments shown in the model of Fig. 2.

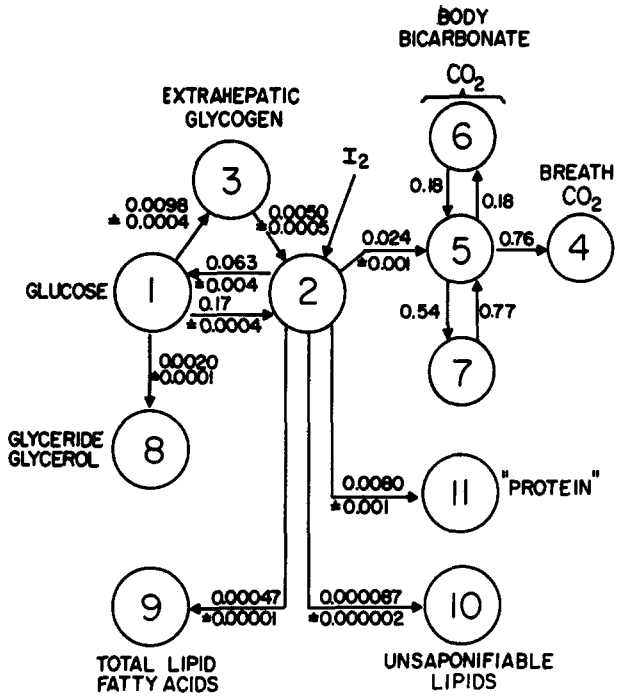

FIG. 2. Model of glucose metabolism in 5-hr-fasted mice. Fractional rate constants (min^{-1}) ± SD were derived by the SAAM program (12) from a least square fit to all of the data shown in Fig. 1. A comparison between observed and calculated values, using these rate constants, is also given in Fig. 1. All rate constants were allowed to vary except those related to the turnover of body bicarbonate (k_{56}, k_{65}, k_{57}, k_{75}, and k_{45}); the latter were taken from a previously published study (16). A rationale for the model's formulation as well as the assumptions made are given in the text. Note that only one nonradioactive inflow of carbon into the system is shown, entering compartment 2 (I_2). Actually, a small inflow probably enters compartment 1 due to formation of glucose from liver glycogen and from glycerol. In addition, a large input would have to enter compartment 5 due to CO_2 formation from relatively unlabeled substances which do not contribute carbon to compartments 1 or 2 (2). The calculation of fractional rate constants is independent of the number, location, and size of nonradioactive inflows into the system. However, I_2 is indicated to emphasize that some inflow of carbon is necessary to maintain the system in a steady state.

mained essentially constant between 60 and 180 min, as one would expect from published values for the turnover, 0.00016%/min, of carcass lipid fatty acids in mice (18). Thus, for practical purposes, lipid fatty acids behave as an "end product" during the experiment. Of course, it is likely that at least as many micromoles of fatty acids were mobilized as were synthesized in these briefly fasted animals. Lipid [^{14}C]glycerol (compartment 12) was also a minor product of glucose metabolism; however, more [^{14}C]glucose was converted to the glycerol moiety than to the fatty acid portion of the lipid molecules. Total lipid glycerol contained 2.5% of the injected ^{14}C at 1 hr. Unsaponifiable lipids (compartment 10) in the whole mouse contained about 15–20% as much ^{14}C at any time as did lipid fatty acids; only 0.2% of the dose was found at t = 60–180 min in this cholesterol-containing fraction.

A major product of glucose metabolism was glycogen (compartment 3). About 10% of the injected dose was recovered as ^{14}C-labeled glycogen from the whole animal at 30 min. Three separate studies showed that only 0.3% of the injected dose was in liver glycogen: expt. 1, 0.27 ± 0.10% (n = 6); expt. 2, 0.35 ± 0.17% (n = 6); expt. 3, 0.23 ± 0.06% (n = 6). Thus we conclude that essentially all of the newly synthesized glycogen was in extrahepatic tissues.

Only about 80% of the ^{14}C could be accounted for in compartments 1–10 between t = 60 and 80 min. The remainder of the ^{14}C must have been converted to other products (compartment 11), which were assumed to include protein and nucleic acids (7).

Fractional rate constants describing [^{14}C]glucose conversion to major "end products"

The data summarized in Fig. 1 were used to calculate fractional rate constants of glucose C conversion to various products. The model and the rate constants that gave the best fit for all of the data are shown in Fig. 2. The fit for most of the data was excellent, as may be seen by comparing the observed and calculated values for radioactivity in each compartment (Fig. 1).

Flux of glucose C to major "end products"

The fluxes of glucose C conversion to various products, expressed as μg of glucose C/min/20 g body wt, were calculated (2) from the fractional rate constants shown in Fig. 2 and the indirectly measured (4) body glucose pool size (3.1 mg of glucose C/20 g). The values are summarized in Fig. 3. The rate of glucose C conversion to fatty acids is shown to be only 3 μg of C/min/20 g, a rate only one-fiftieth the rate of glucose C oxidation to CO_2, and only one-tenth the rate of conversion to extra·

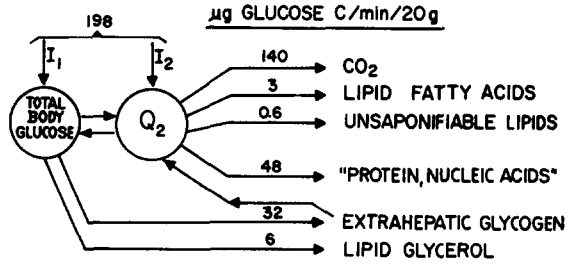

FIG. 3. Flux of glucose C to major "end products" as calculated from the fractional rate constants shown in Fig. 2. Two carbon inflows, I_1 and I_2, are shown; however, the values shown for all fluxes of glucose C are independent of the site of entry of carbon (glucose + non-glucose) into the system (see text). Units are μg of glucose C/min/20 g of body wt. The compartment designated Q_2 is ill defined (see text), but represents a composite of intermediates in the transformation of glucose C to its metabolic products.

TABLE 2. Comparison of compartmental, semicompartmental, and noncompartmental estimates of the rates of glucose C conversion to total body fatty acids in briefly fasted mice

Method of Calculation	Time after Injection	Rate of Glucose C Conversion to Fatty Acids
	min	*μg glucose C/min/20 g*
Compartmental	0–180	3.0
Semicompartmental[a]	30	3.2
	60	2.2
	180	1.9
Noncompartmental[b]	60	2.2
	180	1.9

[a] $R_2 = R_1 \frac{q_{fa}(t)}{q_n(t)}$; R_1 and $q_n(t)$ are calculated (4) from the plasma glucose specific actively curve between $t = 0$ and $t = 30$ or 60 min, where

$$R_1 = \frac{\text{total body glucose C } (\mu g/20 \text{ g body wt})}{A_1/g_1 + A_2/g_2} \quad \text{and}$$

$$q_n(t) = 100\left(1 + \frac{g_2 e^{-g_1 t} - g_1 e^{-g_2 t}}{g_1 - g_2}\right)$$

A_1 and A_2 are the normalized intercepts ($A_1 + A_2 = 1.0$) and g_1 and g_2 are the slopes, respectively, of the two exponential components of the plasma glucose specific activity–time curve, which is defined by the following equation:

$$\text{sp act } (t) = A_1 e^{-g_1 t} + A_2 e^{-g_2 t}$$

[b] $R_2 = R_1 \frac{q_{fa}(\alpha)}{\text{end product } {}^{14}\text{C } (\alpha)}$; R_1 is calculated as in the case of the semicompartmental approach; end product ^{14}C (α) is estimated from measured values at late times (7), as is $q_{fa}(\alpha)$.

hepatic glycogen. This rate of conversion of glucose C to fatty acids agrees well with our previously published values using the simpler semicompartmental approach in which fatty acid ^{14}C was measured at only one point in time, and no other data were obtained except the specific activity of plasma glucose (4). Application of the latter approach to the present data also yields essentially the same value, 2–3 μg of C/min/20 g at any time between $t = 30$ and 180 min, as shown in Table 2. We have also calculated the flux of glucose C into fatty acids by the noncompartmental method of Shipley et al. (7); the results (Table 2) agree exactly with those obtained by the semicompartmental approach.

DISCUSSION

The extremely slow rate at which glucose C is converted to fatty acids in postabsorptive mice and rats has been noted previously (4, 7, 19, 20). In the present study, 3 μg of glucose C/min/20 g was converted to fatty acids and sterols, including cholesterol. This rate may be compared with the conversion rates of glucose C to CO_2, extrahepatic glycogen, and "protein, etc.": 140, 32, and 48 μg of C/min/20 g body wt, respectively. The rate of fatty acid synthesis from glucose C was obtained using mice in which liver glycogen stores had probably been depleted by a minimum fasting period of 5 hr (4). However, we have reported that the rate of fatty acid synthesis from glucose C was 5 μg of C/min/20 g, i.e., just 67% higher than the present values, when similar studies were carried out using 2-hr-fasted mice in which liver glycogen levels were high (4).

The results of our study serve to validate the simple semicompartmental approach which was proposed and used to quantify lipogenic rates in our earlier experiments (4). Rates of glucose C conversion to fatty acids based upon three separate approaches, (*1*) multicompartmental, (*2*) noncompartmental, and (*3*) semicompartmental analyses, gave the following values: 3.0, 2.2, and 2.2 μg of C/min/20 g, respectively, calculated at $t = 60$ min in the case of the last two methods. Thus, the values obtained by all three approaches are similar. At 30 min, the time arbitrarily chosen for calculation in our earlier study (4), the transfer rate of glucose C to fatty acids obtained by our semicompartmental approach was exactly equal to the value derived by multicompartmental analysis of all of the data. On the other hand, theoretically, the semicompartmental analysis should yield a constant value at any time of analysis (4). This clearly was not the case; values ranged from 3.2 (at 30 min) to 1.9 μg of glucose C converted to fatty acids/20 g (at 180 min). The discrepancy was even greater when rates at 15 min were compared with those at 180 min. We conclude that our model, upon which the semicompartmental analysis was based, is not strictly correct. This was also evident from the observation that in our multicompartmental analysis a significantly better fit for the ^{14}C-labeled fatty acid data could be obtained if fatty acids were assumed to be formed directly from compartment 1 rather than (see Fig. 2) after passage of glucose C through compartment 2. (The latter multicompartmental analysis is not shown because calculations of flux of glucose C to fatty acids were unaffected by the change in model; moreover, presentation of all possible models and their analyses is not practical.) Nevertheless, despite the shortcomings associated with oversimplified and admittedly incorrect models, it appears that all of the approaches that we used yield comparable results between $t = 30$ and 180 min. Moreover, although any one of the three approaches may be used to study the effect of diet, hormones, etc. upon the rates of fatty acid synthesis from glucose C in vivo, the semicompartmental approach is by far the simplest.

A number of quantitative aspects of glucose metabolism in postabsorptive mice were derived from our multi-

compartmental analysis. First of all, glycogen was clearly a major product of glucose metabolism; yet, almost none of the newly formed [^{14}C]glycogen was found in the liver.[5] Second, the major, true end product of glucose metabolism, CO_2, accounted for 18% of the total CO_2 expired each min (140 μg of C/min/20 g from glucose C vs. 760 μg of C/min/20 g reported previously for total rate of CO_2 formation in the same strain of mice [16]). Third, $^{14}CO_2$ appeared in the breath only after passage of [^{14}C]glucose through a large intermediary pool having at least 2.5 times more carbon than was present in the entire body glucose pool. The nature of the intermediate carbon is unknown but presumably includes amino acids, tricarboxylic acid cycle intermediates, lactate, and pyruvate. Fourth, more than twice as much carbon from this pool was recycled back to glucose, presumably by way of the liver and kidneys, as was oxidized to CO_2. Thus, relative rates of recycling and oxidation to CO_2 of glucose C were comparable to those reported earlier for postabsorptive and fasted rats (2). In the case of rats, the high degree of recycling of glucose C has now been confirmed by several investigators who have used different experimental approaches (13, 21–24).

Our modification of the noncompartmental approach proposed by Shipley et al. (7) should serve as a rapid and useful tool for physiological studies of lipogenesis and its control in individual animals. Nevertheless, caution should be exerted in extending this approach to other "end products," to other conditions, and to studies of lipogenesis in specific organs. In each case, one would have to establish, at least for practical purposes, that no appreciable turnover of the ^{14}C-labeled product occurs during the experiment; alternatively, one may simply ignore the turnover of the labeled product, as we have done here, in which case all rates could be presented as minimal values. We have used the semicompartmental method for measuring lipogenesis from glucose C as a base line for evaluating the effects of dietary substances upon rates of fatty acid synthesis. One such study is the subject of another report (25).

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[5] In an earlier study of [U-^{14}C]glucose metabolism in mice (3), most of the labeled metabolites were found in the 70% ethanol-soluble fraction. These were probably derived to a large extent from extrahepatic [^{14}C]glycogen as a result of postmortem glycogenolysis. In those studies the mice were decapitated and violent muscular contractions occurred before the tissues were chilled, whereas in the present studies the mice were stunned and immediately immersed in liquid nitrogen.

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