JCI The Journal of Clinical Investigation

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J Clin Invest. 1971;50(3):627-641. https://doi.org/10.1172/JCI106533.

Research Article

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Effects of Thyroid Disease on Glucose Oxidative Metabolism in Man. A Compartmental Model Analysis

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ABSTRACT Glucose oxidation to CO_2 in man at the fasted, steady state has been investigated in normal, hypothyroid, and hyperthyroid patients by monitoring the specific activity of plasma glucose and expired CO_2 after intravenous injection of glucose-1-^{14}C, glucose-6-^{14}C, and sodium bicarbonate-^{14}C in tracer amounts. Making certain stoichiometric assumptions about the oxidation of the C-1 and C-6 carbons of glucose to CO_2, the data are incorporated into a multicompartmental model describing the kinetics of plasma glucose, plasma bicarbonate, and the conversion of glucose to CO_2 by the hexose monophosphate pathway and all other series and parallel pathways which oxidize glucose carbon to CO_2 (EMP-TCA). This formulation separates the distribution kinetics of glucose and bicarbonate from the kinetics of glucose oxidation to CO_2. It allows the calculation of a minimal fraction (ϕ_t) of glucose irreversibly oxidized to CO_2 which is based entirely on the duration of the experimental data. This calculation is independent of the extrapolative implications of the model beyond the experimental interval and of the particular model chosen to fit the data. All modeling and data fitting were performed on a digital computer with the SAAM program.

Based on a 300 min experiment the analysis suggests that in hypothyroidism there is a decrease in the rate of glucose metabolized irreversibly (ρ_G). There is also a decrease in the minimal fraction (ϕ_{300}) which is completely oxidized to CO_2 by way of the EMP-TCA. ρ_G and ϕ_{300} are 0.56 and 0.42 mmole/min respectively as compared to 0.89 and 0.50 mmole/min respectively in normals. However, the fraction of the C-1 of glucose metabolized irreversibly which undergoes oxidation to CO_2 by the hexose monophosphate pathway (Ψ) is not different from normal (0.07 and 0.07 respectively). The hyperthyroid studies suggest that ρ_G and ϕ_{300} are within the normal range (1.01 and 0.46 mmoles/min respectively as compared to 0.89 and 0.50 mmole/min respectively in normals). However, Ψ is decreased to less than half the normal value (0.03 as compared to 0.07 in normals).

An abstract of this work was submitted to Spring Meetings, American Society for Clinical Investigation, 1968.

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Received for publication 12 August 1970.

INTRODUCTION

Glucose oxidation to CO_2 in man studied by a single intravenous injection of universally labeled glucose-^{14}C has been the subject of several reports (1–4). Glucose-^{14}C labeled specifically in the C-1 and C-6 positions was also used (5) to determine the fraction of the C-1 of glucose which undergoes oxidation to CO_2 by the hexose monophosphate pathway (HMP). The effects of increased and decreased thyroid activity on these measures of glucose oxidation have been studied in a semiquantitative manner in the rat (6–8) and in man (9).

This report describes steady-state kinetic studies of glucose oxidation to CO_2 in patients with hyper- and hypothyroidism using glucose-1-^{14}C and glucose-6-^{14}C. A compartmental model similar to that proposed by Segal, Berman, and Blair (5) was formulated to calculate the fraction of C-1 of glucose and C-6 of glucose which is oxidized to CO_2 by the HMP and EMP-TCA respectively. The EMP-TCA as defined here, represents all pathways except C-1 oxidation of glucose-6-phosphate by the HMP through which glucose carbon may pass before being oxidized to CO_2. These pathways include transport of glucose carbon through noncarbohydrate pools such as lipid and protein. By assuming particular stoichiometries, the extent of glucose oxidation and CO_2 production via the HMP and EMP-TCA are estimated.

METHODS

Subjects

Four normal volunteer subjects, three males and one female, aged 18–21 yr, served as controls. Data collected on these subjects have previously been published (5). Four patients with hypothyroidism and two with hyperthyroidism were the subjects of the present experiments. These patients are briefly described as follows.

Hypothyroid patients. M. M. was a 57 yr old Caucasian female weighing 60 kg who had spontaneous hypothyroidism, which in the past had been treated with thyroxine for 4 yr. She had been without therapy for several months before the study. Her BMR was −19%, PBI 1.8 μg/100 ml, RAI uptake after 3 days of thyroid stimulating hormone (TSH) stimulation, 6.2% after 24 hr, and cholesterol 416 mg/100 ml. Intravenous glucose tolerance as estimated by the method of Amatuzio, Stulzman, Vanderbilt and Nesbitt (10) gave a half-time of 17.5 min.

E. M. was a 36 yr old Caucasian female weighing 99 kg who had undergone complete thyroidectomy for carcinoma about 3 yr before study. She had been placed on vitamin D, 50,000 U/day, and 3 grains of thyroid extract daily. At the time of the study no thyroid had been taken for three months, and she had had symptoms of hypothyroidism for 2 months. Her BMR was −18%, PBI 2.3 μg/100 ml, RAI excretion 94.5% in the urine 72 hr after the administration of the dose, and cholesterol 343 mg/100 ml.

L. S. was a 26 yr old Caucasian male weighing 94 kg who underwent complete thyroidectomy for thyroid carcinoma 3 yr before study. He had been on vitamin D, 150,000 U/day, and triiodothyronine, 125 μg/day. Thyroid medication had been removed for 2 months before the study. BMR was −9%, PBI 0.6 μg/100 ml, and the cholesterol 377 mg/100 ml.

G. R. was a 56 yr old Caucasian male weighing 63 kg who had onset of spontaneous hypothyroidism 4 yr before study. He had been taking ½ grain of thyroid extract per day. This therapy was stopped 1 month before study. His BMR was −30%, PBI 1.0 μg/100 ml, RAI uptake 2% after 24 hr both with and without TSH stimulation, and cholesterol 424 mg/100 ml.

Hyperthyroid patients. C. N. was a 26 yr old Negro female weighing 58 kg who had had symptoms of hyperthyroidism for about 12 months. She had been treated with propylthiouracil, but had been without this drug for 3 wk at the time of study. Her BMR was +29%, PBI 13 μg/100 ml, and cholesterol 167 mg/100 ml. Intravenous glucose tolerance test revealed a half-time of 25 min.

A. F. was a 27 yr old Negro female weighing 74 kg who had had symptoms of hyperthyroidism for about 1 yr. She had no treatment before the first of a series of three studies. At the time of her first study (A.F.1) her BMR was +72%, PBI 21 μg/100 ml, RAI uptake 43% at 24 hr, and the cholesterol 93 mg/100 ml. Intravenous glucose tolerance revealed a half-time of 5 min. After the first series of studies with labeled glucose, she was started on propylthiouracil, 300 mg/day, and was on this medication for about 10 wk, at which time she was again studied with radioactive glucose injection. At the time of her second study (A.F.2) her BMR was 0, PBI 6.2 μg/100 ml, and her weight 85 kg. Because of the nature of her CO_2 expiration curves and a continued abnormal intravenous glucose tolerance test with a half-time of 10 min, it was felt that she was not euthyroid with respect to glucose tolerance despite the lack of symptoms and normal BMR and PBI. 1 month after the second series of glucose experiments she underwent subtotal thyroidectomy because she was thought to be a poor risk for continued propylthiouracil medication. After subtotal thyroidectomy, the symptoms of hyperthyroidism returned and she was given a course of radioactive iodine therapy with subsequent good control of her symptoms. She was again studied for a third time (A.F.3) 1½ yr after the cessation of symptoms of hyperthyroidism. She was taking no medication and was thought to be euthyroid with a PBI of 6.2 μg/100 ml, BMR of −2%, cholesterol of 186 mg/100 ml, and a weight increase to 101 kg. At this time her glucose tolerance test revealed a half-time of 17 min.

Experimental design

Glucose-1-^{14}C, specific activity 2.67 μCi/mg, was obtained from Volk Radiochemical Co., Burbank, Calif. Glucose-6-^{14}C, specific activity 2.8 μCi/mg, was obtained from Dr. H. Isbell of the National Bureau of Standards. The sugars were converted to gluconate and degraded by periodate to isolate C-1 and C-6. Virtually all of the label in glucose-1-^{14}C and glucose-6-^{14}C was in the C-1 and C-6 carbons respectively. The glucose-1-^{14}C and glucose-6-^{14}C were chromatographically pure. Sodium bicarbonate-^{14}C, 11.9 μCi/mg, was obtained from Nuclear-Chicago Corp. All ^{14}C solutions were prepared by the radiopharmacy of the National Institutes of Health and found sterile and pyrogen free before use.

All subjects were maintained on a diet containing 300 g of carbohydrate and were fasted overnight before and throughout the performance of the study. Labeled glucose dissolved in isotonic saline, approximately 1 μCi/ml was injected rapidly into an antecubital vein. All subjects received 5 μCi of C-1– and C-6–labeled glucose except A. F. to whom these doses were given on three occasions. Expired CO_2 was collected in Douglas bags for 5- or 10-min periods at various intervals for 6 hr, and blood was drawn for determination of the specific activity of glucose. Studies were performed at approximately 1 wk intervals. At such time intervals no ^{14}C from previous studies was detectable in expired air or blood glucose. In each subject the excretion of $^{14}CO_2$ in expired air was determined after the intravenous injection of 0.64 μCi of sodium bicarbonate-^{14}C dissolved in 1 ml of isotonic saline.

The determination of CO_2 content and counting of samples were performed by the method of Fredrickson and Ono (11) using a liquid scintillation spectrometer counting at 53% efficiency. Blood glucose was isolated as potassium gluconate by the method of Blair and Segal (12). The gluconate was degraded by oxidation with periodate (13), to obtain C-1 as CO_2 and C-6 as formaldehyde which was converted to the dimedon derivative. CO_2 derived from C-1 was trapped in barium hydroxide. The resultant barium carbonate was acidified and the liberated CO_2 diffused in hyamine base and counted as described above. The formaldehyde dimedon representing C-6 was counted directly in the toluene phosphor. For determination of total specific activity, the isolated gluconate was counted by mounting on paper by the technique of Blair and Segal (14). Blood glucose was determined by a glucose oxidase method employing the Glucostat reagent.

Analysis

Fig. 1 shows the block diagram and compartmental model formulated from the kinetic data. Each subsystem was first considered and analyzed independently and then incorporated into the full model. After an overnight fast glucose is sup-

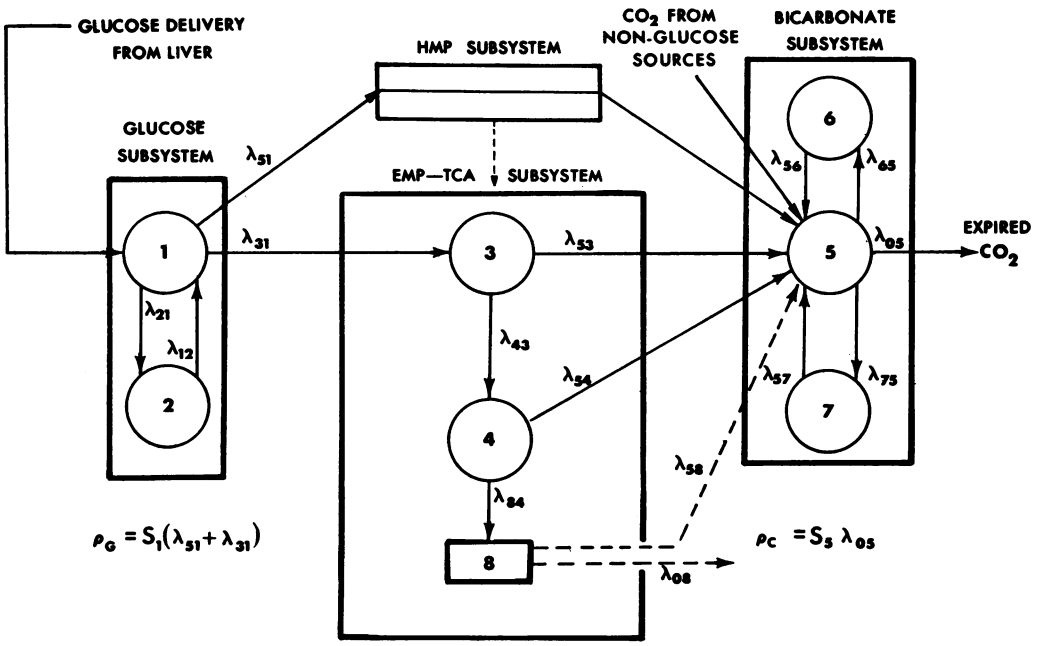

FIGURE 1 Compartmental model and block diagram describing the conversion of glucose-^{14}C to $^{14}CO_2$ by the HMP and EMP-TCA. The HMP is defined as that pathway which preferentially oxidizes the C-1 of glucose-6-phosphate to CO_2. The EMP-TCA represents all the series and parallel pathways, recycling, and nonrecycling (except C-1 oxidation of glucose-6-phosphate by the HMP) through which glucose carbon may pass before being oxidized to CO_2. Heavy lines enclose the glucose, bicarbonate, HMP, and EMP-TCA compartmental subsystems of model. The dotted lines in the figure represent pathways that cannot be resolved from available data. They should be considered, however, for long-range estimates, such as steady states. The λ_{ij} are rate constants of glucose-^{14}C transfer into compartment i from compartment j in units min^{-1}. The S_i are compartment sizes in mmoles. ρ_G is the rate of glucose metabolized irreversibly in mmoles per min. ρ_C is the rate of CO_2 excretion in expired air in mmoles per minute.

plied to the glucose subsystem by the liver. Two pathways of glucose oxidation to CO_2 are distinguished. In one, C-1 of glucose is irreversibly lost through the HMP and in the other C-1 and C-6 of glucose are lost through the EMP-TCA. Recycling of three carbon fragments back to plasma glucose via the Cori cycle is assumed to occur in the glucose subsystem. The bicarbonate subsystem is supplied CO_2 from the glucose as well as nonglucose sources. It is assumed that all metabolites are in a steady state.

By making certain stoichiometric assumptions, estimates of the pool sizes of glucose and bicarbonate, rates of glucose and bicarbonate loss from the plasma, and the extent of glucose oxidation to CO_2 by both the HMP and EMP-TCA can be calculated.

All modeling and data fitting were performed on a digital computer with the SAAM program (15–17).

Glucose subsystem

The specific activity (SA) data (fraction of administered dose of ^{14}C per mmole of glucose) of plasma glucose-1-^{14}C, $X_{G_1}(t)$, and glucose-6-^{14}C, $X_{G_6}(t)$, were fitted jointly to sums of two exponentials with the constraint that they be proportional to each other.

$$X_{G_1}(t) = a_{11}e^{-\alpha_1 t} + a_{12}e^{-\alpha_2 t} = \frac{1}{c}X_{G_6} \qquad (1)$$

The proportionality constant, c, slightly different from unity, was assumed to reflect variations of glucose pool size in the same patient from one experiment to the next.

In a previous publication (5) the plasma glucose SA data were approximated by three exponentials rather than two. Here it was decided to neglect the fastest component since in many of the studies it could not be resolved.[1]

A two compartment model (19) was chosen to explain the data. Since the general two compartment model is not resolvable by these data alone (20), a particular model (Fig. 1) with irreversible loss from the sampled compartment only was chosen (5).

The plasma equivalent space of distribution, V_G, was derived from the relation

$$V_G = \frac{(S_1 + S_2)}{[G]_p} \qquad (2)$$

where $[G]_p$ is the plasma glucose concentration, and $(S_1 + S_2)$ is the calculated pool size.

[1] In accordance with the Stewart-Hamilton principle (18) the rate of irreversible loss of a metabolite from a compartment injected with a tracer is inversely proportional to the area under the SA curve. The very fast component of the glucose SA curve neglected here contributes less than 5% to the area.

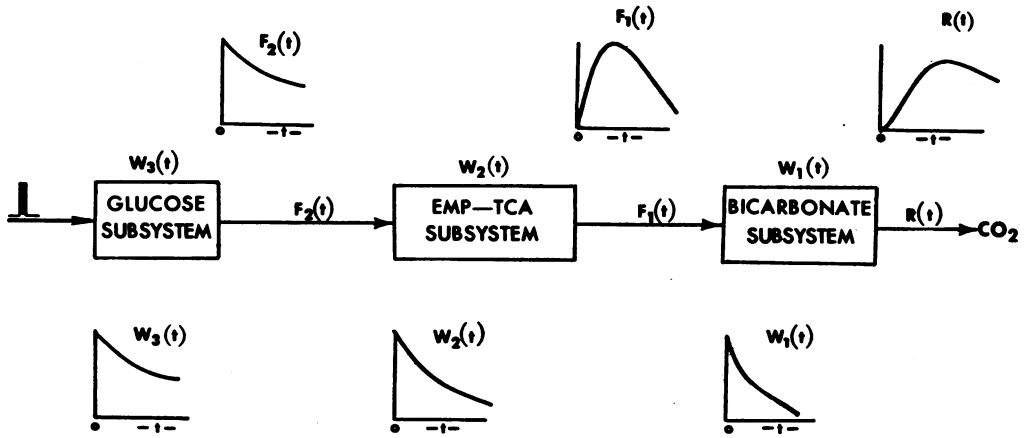

FIGURE 2 Input-output relations describing the conversion of unit intravenous injection of glucose-6-^{14}C to $^{14}CO_2$ by the EMP-TCA. $R_1(t)$ is the $^{14}CO_2$ SA response in expired air after a unit intravenous injection of glucose-6-^{14}C. $W_1(t)$ is the $^{14}CO_2$ SA response in expired air after a unit intravenous injection of bicarbonate-^{14}C. $F_1(t)$ is the calculated rate of $^{14}CO_2$ output of the EMP-TCA after a unit intravenous injection of glucose-6-^{14}C and is obtained by the deconvolution of $R_1(t)$ with $W_1(t)$. $F_2(t)$ is the calculated rate of glucose-6-^{14}C input to the EMP-TCA after a unit intravenous injection of glucose-6-^{14}C. $W_2(t)$ is the calculated $^{14}CO_2$ rate of output of the EMP-TCA after a "unit injection" of glucose-6-^{14}C into the EMP-TCA directly and is obtained by the deconvolution of $F_1(t)$ with $F_2(t)$. $W_3(t)$ is the total plasma glucose-6-^{14}C activity response to a unit intravenous injection of glucose-6-^{14}C.

Bicarbonate subsystem

The SA of expired CO_2 was measured after a unit intravenous injection of ^{14}C-labeled sodium bicarbonate tracer. It has been pointed out elsewhere (21, 22) that the CO_2 SA of expired air approximates that of plasma bicarbonate. Evidence that the plasma is also the site of entry of CO_2 into the bicarbonate pool has also been presented (23, 24).

Based on these assumptions a three compartment mammillary model (Fig. 1) was chosen to describe the kinetics of bicarbonate in the plasma. Since the bicarbonate subsystem serves only as a weighting function for the prediction of other inputs, the nature of the model chosen is immaterial (25).

Convolution principle

An "*input*" to a system is defined as a function describing the rate of material entry into the system via a particular pathway. The "*response*" of a system is any function generated within the system as a result of its input. We define the "*output*" of a system as the rate of loss of material from the system via a particular pathway. The output is a special response. A "*weighting function*" is defined as the response of a system to an input which is a unit impulse.

If the weighting function, w(t), of a linear system with time-invariant parameters is known, then the response, r(t), to any input function, f(t), can be calculated by the convolution integral (26):

$$r(t) = \int_0^t f(\theta)w(t - \theta)d\theta. \quad (3)$$

This may also be written as the convolution of f(t) with w(t)

$$r(t) = f(t)*w(t).$$

It can be shown that given w(t) and r(t), f(t) can be calculated by an inverse process, deconvolution (26). We shall write this formally as

$$f(t) = w(t)\bar{*}r(t)$$

to mean that "w(t) is deconvolved from r(t)."

Since convolution is commutative, that is

$$r(t) = f(t)*w(t) = w(t)*f(t),$$

it also follows that

$$w(t) = f(t)\bar{*}r(t).$$

EMP-TCA subsystem

The technique of deconvolution can be used to derive a weighting function for the EMP-TCA subsystem. Since the C-6 of the glucose[2] that traverses the HMP does not yield

[2] Randomization between the C-1 and C-6 carbons of glucose in plasma over the time interval of these experiments was negligible in normal subjects (5) and not significantly different from normal in the hypo- and hyperthyroid patients studied here. This suggests that hepatic randomization of glucose carbon from labeled three carbon fragments before intrahepatic oxidation to CO_2 or resynthesis to glucose is also small over this time interval since hepatic glucose mixes rapidly with plasma glucose (27). Peripheral tissue randomization of glucose carbon between the C-1 and C-6 positions is also assumed to be negligible over the time course of these experiments.

CO_2, the glucose-6-^{14}C to $^{14}CO_2$ conversion can be approximated by the model shown in Fig. 2. It consists of three subsystems characterized by $W_1(t)$, $W_2(t)$, and $W_3(t)$ which are the weighting functions for the bicarbonate, EMP-TCA, and glucose respectively. The functions $F_2(t)$, $F_1(t)$, and $R(t)$ are the inputs and outputs of the various subsystems that result from a unit glucose-6-^{14}C injection into plasma. $W_3(t)$ is the total amount of glucose-6-^{14}C in plasma after unit injection. $F_2(t)$ is the rate of ^{14}C-6 transport (fraction per minute) from the glucose into the EMP-TCA subsystem and is given by (Fig. 1):

$$F_2(t) = \lambda_{31}W_3(t) = \frac{\lambda_{31}(a_{11}e^{-\alpha_1 t} + a_{12}e^{-\alpha_2(t)})}{(a_{11} + a_{12})}. \quad (4)$$

$R(t)$ is the specific activity (fraction per mmole) of the bicarbonate subsystem as seen in the expired CO_2, after a unit injection of glucose-6-^{14}C. $W_1(t)$ is the activity of this subsystem for unit injection of bicarbonate-^{14}C. Both quantities were determined experimentally. Hence, by deconvolution,

$$F_1(t) = W_1(t)\bar{*}R(t).$$

$F_1(t)$ is the rate (fraction per minute) of ^{14}C-6 entry into the bicarbonate subsystem after unit glucose-6-^{14}C injection and is also the rate of output of ^{14}C-6 from the EMP-TCA subsystem. Since the rate of input into the EMP-TCA subsystem is $F_2(t)$ it follows that

$$W_2(t) = F_2(t)\bar{*}F_1(t).$$

Thus, $W_2(t)$—the output (fraction per minute) of the EMP-TCA subsystem due to an input of a hypothetical unit injection—can be determined. One compartmental model for the EMP-TCA subsystem that is compatible with the derived $W_2(t)$ is shown in Fig. 1.

The schematized curves in Fig. 2 represent the relative shapes of $R(t)$, $F_1(t)$, $F_2(t)$, $W_1(t)$, $W_2(t)$, and $W_3(t)$.

Since all the ^{14}C-6 of glucose leaves plasma by way of the EMP-TCA path, the integral

$$\int_0^\infty W_2(\theta)d\theta = \phi_\infty \quad (5)$$

is the fraction of ^{14}C-6 of glucose oxidized to $^{14}CO_2$. However, the data allow calculations of $W_2(t)$ only up to 300 min and integration to $t = \infty$ requires extrapolation. To avoid errors due to extrapolation, we define

$$\int_0^{300} W_2(\theta)d\theta \equiv \phi_{300} < \phi_\infty. \quad (6)$$

ϕ_{300} represents a minimal estimate (with respect to the duration of the data) of the fraction of C-6 of glucose oxidized to CO_2 by the EMP-TCA. This measure is independent of the model chosen to describe $W_2(t)$.[3]

The three compartment model (Fig. 1) used to describe the EMP-TCA is a minimal structure required by the data. The glucose-6-^{14}C entering the EMP-TCA and not accounted for in the $^{14}CO_2$ data is lumped into another compartment 8, with dotted arrows indicating potential recycling to $^{14}CO_2$. This compartment represents all intermediate pathways of glucose-6-^{14}C conversion to $^{14}CO_2$ with "transit times" too great to be observed in the data collected. These pathways as do those represented by compartments three and four may include incorporation of C-6 of glucose into amino acids, proteins, free fatty acids, triglycerides, nucleotides, etc. Eventually most of this glucose carbon leaves the body as CO_2 in expired air, although some may exit by other routes such as stool, urine, and desquamation. It is because the kinetics of these slower processes cannot be deduced from the data that the calculation of ϕ is qualified here (ϕ_{300}) with respect to the time interval of the data.

If it is assumed that the C-6 of glucose becomes the methyl carbon of triose phosphate and is the last carbon of the triose phosphate carbon skeleton to be oxidized to CO_2, and that complete equilibration of triose phosphates occurs before oxidation, ϕ_{300} also represents a minimal estimate of the fraction of glucose which is completely oxidized to CO_2 by the EMP-TCA. Estimates of the minimal rate of glucose oxidation to CO_2 by the EMP-TCA, $\rho_{G\text{-EMP(min)}}$, and the minimal rate of CO_2 production by the EMP-TCA, $\rho_{C\text{-EMP(min)}}$, may be given by

$$\rho_{G\text{-EMP(min)}} = \rho_G\phi_{300} \quad (7)$$

and

$$\rho_{C\text{-EMP(min)}} = 6\rho_G\phi_{300} \quad (8)$$

where $\rho_{G\text{-EMP(min)}}$ and $\rho_{C\text{-EMP(min)}}$ are expressed as mmoles per minute of glucose and CO_2 respectively. The minimal fraction of the total CO_2 excretion rate, ρ_C, coming from glucose oxidation via the EMP-TCA is then $\rho_{C\text{-EMP(min)}}/\rho_C$. Since the maximum possible rate of CO_2 production in mmoles per minute from the complete oxidation of glucose by all pathways oxidizing glucose to CO_2 equals $6\rho_G$, the maximum possible fraction of ρ_C derived from glucose carbon is therefore $6\rho_G/\rho_C$.[4]

Direct glucose oxidative pathway

The technique of calculating the magnitude of direct HMP oxidation of C-1 of glucose by using the expired CO_2 SA curves, $C_1(t)$ and $C_6(t)$, resulting from the injection of glucose-1-^{14}C and glucose-6-^{14}C respectively, has been presented elsewhere (5). It involves the fitting of the $C_1(t)$ SA curve to a linear combination of the $C_6(t)$ SA curve and a function, $g(t)$, obtained by the convolution of $F_2(t)$ with $W_1(t)$. The fraction of C-1 of glucose oxidized to CO_2 by the HMP, ψ, can be calculated from the equation

$$C_1(t) = (1 - \psi)C_6(t) + \psi g(t) \quad (9)$$

where

$$g(t) = \int_0^t F_2(\theta)W_1(t - \theta)d\theta. \quad (10)$$

ψ may also be expressed (Fig. 1) by the relation

$$\psi = \frac{\lambda_{51}}{\lambda_{51} + \lambda_{31}}. \quad (11)$$

Since the source of every ^{14}C-1 oxidized to $^{14}CO_2$ by the HMP is a molecule of glucose-1-^{14}C, ψ also represents the fraction of glucose molecules entering the HMP. The rate of

[3] The significance of ϕ_{300} becomes more clear when compared to other measures of glucose-6-^{14}C oxidation to $^{14}CO_2$ which can be obtained over a 300 min experiment. For example, a patient E. M. excreted 33% of the injected ^{14}C as $^{14}CO_2$ in 300 min. Since 16% of the injected ^{14}C remained in the glucose subsystem at this time, the minimal fractional conversion of glucose-6-^{14}C to $^{14}CO_2$ is $0.33/0.84 = 0.39$. However, another 4% of the injected ^{14}C is distributed in the bicarbonate subsystem as bicarbonate-^{14}C. Thus the minimal fractional conversion of glucose-6-^{14}C to $^{14}CO_2$ becomes $(0.33 + 0.04)/0.84 = 0.44 = \phi_{300}$.

[4] ρ_G and $6\rho_G$ would be overestimated if a slower component of the glucose SA curve, $X_{G_6}(t)$, existed beyond the time of the last datum. Consequently $6\rho_G/\rho_C$ could significantly overestimate the maximal fraction of CO_2 deriving from glucose.

glucose entry into the HMP, $\rho_{G\text{-}HMP}$, in mmoles of glucose per minute is therefore

$$\rho_{G\text{-}HMP} = \rho_G \psi. \quad (12)$$

Because of recycling of pentose (generated by the HMP) back to glucose-6-phosphate (G6P), the rate of G6P entry into the HMP is greater than $\rho_{G\text{-}HMP}$. Calculation of the rate of G6P entry into and rate of CO_2 production from the HMP cannot be made from our data without certain stoichiometric assumptions. If, as assumed by Katz and Wood (28, 29), two-thirds of the glucose carbon entering the HMP recycle back to G6P, one-sixth goes to CO_2, and the other sixth goes to triose phosphate irreversibly, it can be shown by the appropriate steady-state calculations that the rate of entry of G6P into the HMP in mmoles per minute is given by $\rho_G\psi/(1 - 0.67\psi)$. Since upon entering the HMP 1 mmole of G6P produces 1 mmole of CO_2, the rate of CO_2 production by the HMP, $\rho_{C\text{-}HMP}$, in mmoles per minute is also given by $\rho_G\psi/(1 - 0.67\psi)$. However because ψ is 0.07 or less in all studies reported here, $\rho_{C\text{-}HMP}$ is closely approximated by the relation

$$\rho_{C\text{-}HMP} \approx \rho_G \psi. \quad (13)$$

If it is assumed that all or almost all of the glucose carbon exists from the body as CO_2, the minimal fraction of the glucose-derived CO_2 produced via the HMP is

$$\frac{\rho_{C\text{-}HMP}}{6\rho_G} = \frac{\rho_G\psi}{6\rho_G} = \frac{\psi}{6}. \quad (14)$$

The maximal fraction of the CO_2 produced from glucose via the HMP (as calculated from data collected over a 300 min interval) is

$$\frac{\rho_{C\text{-}HMP}}{\rho_{C\text{-}EMP(min)} + \rho_{C\text{-}HMP}} = \frac{\psi}{6\phi_{300} + \psi} \approx \frac{\psi}{6\phi_{300}} \quad (15)$$

since $6\phi_{300} \gg \psi$.

It can be noted in Fig. 1 that no compartmental description of the HMP subsystem is formulated. This process(es) is too fast to be resolved by the early portion of the $C_1(t)$ SA data and is therefore assumed to be instantaneous.

Complete model

After all subsystems of the model were independently formulated they were connected together as in Fig. 1. All the parameters of the full model were then adjusted jointly by SAAM to fit all the data obtained on each patient until a least squares solution was obtained. The uncertainties of the parameter values were estimated from the least squares solution.

RESULTS

Glucose kinetics. Fig. 3 shows the plasma glucose SA curves of each patient after the intravenous injection of a unit amount of glucose-1-^{14}C and glucose-6-^{14}C. The glucose-1-^{14}C SA curve is usually slightly below or equal to the glucose-6-^{14}C SA curve.

The rate of glucose irreversibly metabolized (ρ_G), the sampled compartment size (S_1), the total pool size ($S_1 + S_2$), and the plasma equivalent space (V_G), of the total glucose pool were calculated from the glucose SA curves using the model presented. The values for each study are shown in Table I.

A composite glucose SA curve was used for the four normal subjects. Consequently only single values for S_1, V_G, $S_1 + S_2$, and ρ_G are given. The value of ρ_G in the euthyroid patients, 0.89 mmoles/min, was close to the average value for the two hyperthyroid patients. A significantly smaller average value for ρ_G, 0.56 mmoles/min, was calculated for the hypothyroid group. The decrease in ρ_G in patient A. F. following the control of her hyperthyroidism primarily reflects normalization of ρ_G to a 70 kg weight. She gained 27 kg between studies A.F.1 and A.F.3.

The physiological significance of compartment 2 is unclear. Calculated values for V_G substantially exceed average estimates of extracellular fluid volume in man (30), making it unlikely that compartment 2 is a sequestered portion of the extracellular fluid. A three carbon fragment pool either in peripheral tissues, extracellular fluid, liver, or in all of these is unlikely because of only minimal randomization between the C-1 and C-6 carbons of glucose over a 3 hr period. The magnitude of the glucose

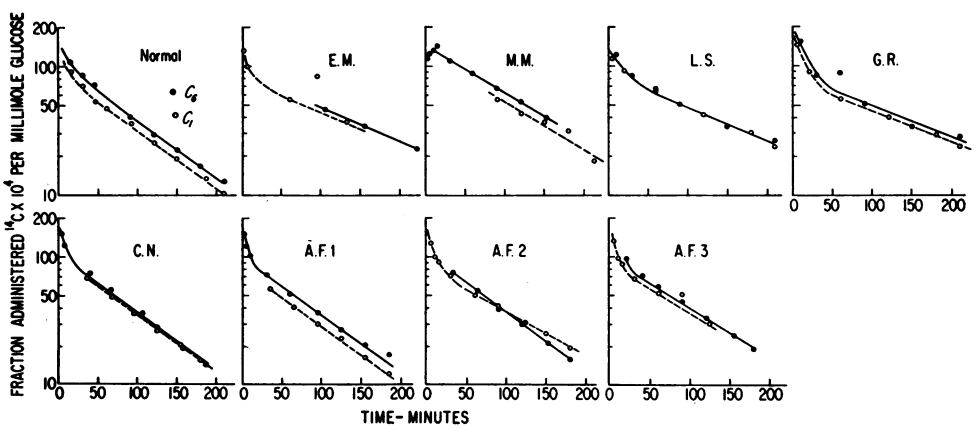

FIGURE 3 Specific activity of blood glucose in normal, hypo-, and hyperthyroid patients after single intravenous injection of glucose-6-^{14}C and glucose-1-^{14}C. "Normal" curve is average of four patients studied previously (5).

TABLE I

*Measures of Glucose and Bicarbonate Subsystems**

Subject	Condition	S_1	$(S_1 + S_2)$	V_G	ρ_G	S_5	$(S_5 + S_6 + S_7)$
		mmoles	*mmoles*	*liters*	*mmoles/min*	*mmoles*	*mmoles*
C. B.	Normal	73 ±5	92 ±3	23 ±1	0.89 ±0.06	205 ±17	810 ±20
C. K.	Normal	73 ±5	92 ±3	23 ±1	0.89 ±0.06	182 ±27	695 ±30
R. S.	Normal	73 ±5	92 ±3	23 ±1	0.89 ±0.06	147 ±9	590 ±11
A. H.	Normal	73 ±5	92 ±3	23 ±1	0.89 ±0.06	131 ±10	525 ±12
Average						166	655
E. M.	Hypothyroid	40 ±7	81 ±7	24 ±2	0.47 ±0.09	110 ±21	510 ±34
M. M.	Hypothyroid	‡	76 ±4	20 ±1	0.67 ±0.02	148 ±26	748 ±92
L. S.	Hypothyroid	56 ±4	83 ±2	22 ±1	0.44 ±0.05	121 ±10	490 ±23
G. R.	Hypothyroid	60 ±6	120 ±4	28 ±1	0.64 ±0.08	186 ±34	840 ±91
Average		52	90	24	0.56	141	647
C. N.	Hyperthyroid	79 ±4	100 ±4	24 ±1	1.11 ±0.05	235 ±11	682 ±16
A.F.1	Hyperthyroid	65 ±4	92 ±3	25 ±1	0.92 ±0.06	200 ±17	720 ±16
A.F.2	Treated hyperthyroid	43 ±3	80 ±7	22 ±2	0.79 ±0.06	75 ±10	638 ±22
A.F.3	Treated hyperthyroid	38 ±6	61 ±5	16 ±1	0.61 ±0.10	58 ±9	510 ±17

S_1, sampled glucose compartment size; V_G, plasma equivalent space of glucose pool; S_5, "sampled" bicarbonate compartment size; $(S_1 + S_2)$, glucose pool size; ρ_G, rate of irreversible glucose metabolism; $(S_5 + S_6 + S_7)$, bicarbonate pool size.

* Normalized to 70 kg weight; value ±SD.

‡ Early glucose data were of poor quality. Consequently only slower component could be resolved.

carbon flux into and out of compartment 2 also makes it unlikely that this is a pool of Cori cycle intermediates. An equilibrating intracellular glucose pool also seems unlikely (31, 32). Equilibration of the labeled glucose with the outer tiers of liver glycogen would be consistent with the magnitude and fractional turnover rate of and total glucose flow through compartment 2. However, this is inconsistent with observations on glycogen-^{14}C (33) in the fasting dog undergoing a constant infusion of glucose-^{14}C.

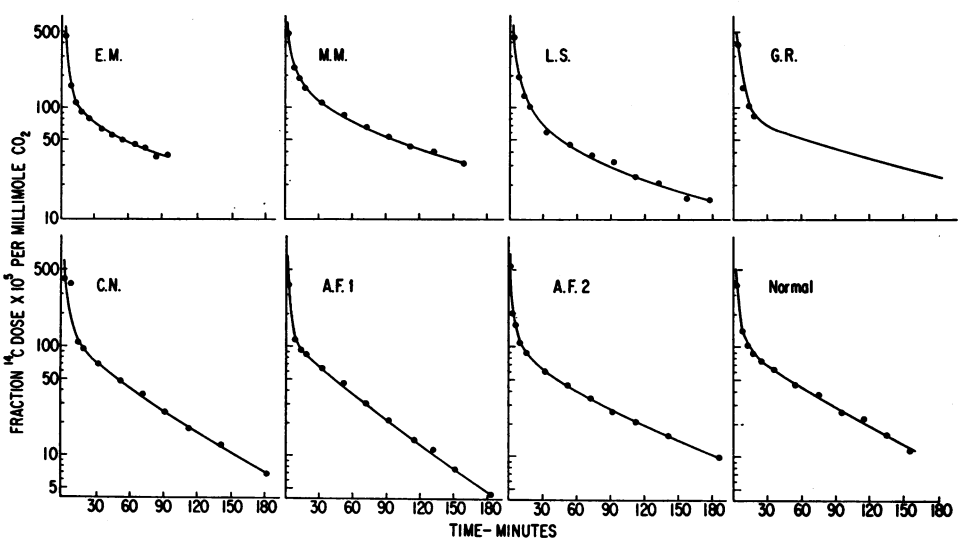

FIGURE 4 Specific activity of CO_2 in expired air in normal, hypo-, and hyperthyroid patients after single intravenous injection of bicarbonate-^{14}C. Because of hyperventilation during the middle of the bicarbonate study of G. R., only the first four data were considered reliable. The tail of the curve was fitted subject to the statistical constraint that the total bicarbonate pool size be 750 ±150 mmoles. "Normal" curve represents a single study.

TABLE II

Blood Glucose Concentration and CO_2 Excretion

Subject	Condition	Position ^{14}C administered glucose	Blood glucose*	CO_2 excretion‡
			mg/100 ml	*mmoles/min*
C. B.	Normal	C-1	67	13.0
		C-6	59	14.0
C. K.	Normal	C-1	69	12.8
		C-6	70	12.4
R. S.	Normal	C-1	77	9.5
		C-6	77	9.2
A. H.	Normal	C-1	73	8.9
		C-6	68	8.9
Average				11.1
E. M.	Hypothyroid	C-1	64	5.7
		C-6	63	5.7
M. M.	Hypothyroid	C-1	60	7.7
		C-6	70	7.0
L. S.	Hypothyroid	C-1	65	7.2
		C-6	67	6.6
G. R.	Hypothyroid	C-1	75	7.9
		C-6	78	7.4
Average				6.9
C. N.	Hyperthyroid	C-1	61	14.6
		C-6	75	16.6
A.F.1	Hyperthyroid	C-1	66	14.1
		C-6	68	14.3
Average				14.9
A.F.2	Treated hyperthyroid	C-1	68	8.4
		C-6	66	9.0
A.F.3	Treated hyperthyroid	C-1	70	6.7
		C-6	70	7.4
Average				7.9

* Average of 4–10 samples over a 3.5 hr period.

‡ Average of 14–18 samples over a 5.0 hr period; normalized to 70 kg weight

Bicarbonate-^{14}C kinetics. Fig. 4 shows the CO_2 SA data of each patient after intravenous administration of a unit amount of bicarbonate-^{14}C. Although not strikingly different, the CO_2 SA in expired air declines faster than normal in the hyperthyroid studies and slower than normal in the hypothyroid studies. The sampled compartment size (S_5) and total pool size ($S_5 + S_6 + S_7$) for the bicarbonate subsystem in each study are shown in Table I. A bicarbonate pool size of about 650 mmoles in all studies is similar to values obtained by others (1, 3, 34) in normal man.

Carbon dioxide excretion. The rate of CO_2 excretion in mmoles per minute was measured in each patient. These values are presented in Table II. The average values for the three groups show an increased rate of CO_2 excretion in hyperthyroidism and a decreased rate of CO_2 excretion in hypothyroidism as compared to the euthyroid patients.

The SA curves of expired CO_2 after a unit intravenous injection of glucose-1-^{14}C and glucose-6-^{14}C in each patient are shown in Fig. 5. Each study shows that the $C_1(t)$ SA curve rises faster but does not always have a greater maximum value than the $C_6(t)$ SA curve. The area between $C_1(t)$ and $C_6(t)$ SA curves in the hyperthyroid studies is smaller than those observed in the normal and hypothyroid studies suggesting that glucose oxidation to CO_2 by the HMP might be proportionately smaller than by the EMP-TCA in the hyperthyroid group.

Measures of EMP-TCA subsystem. The calculated values of ϕ_{300} for each patient are given in Table III. As mentioned earlier, ϕ_{300} is a minimal estimate of the fraction of C-6 of glucose in compartment 1 which is oxidized to CO_2 with respect to the time interval over which the experimental data were collected. The values of ϕ_{300} overlap in the normal, hyperthyroid, and treated hyperthyroid studies. However, the values of ϕ_{300} in the four hypothyroid studies are the lowest values in the entire series (except A.F.1). The average minimal estimate calculated for the rate of glucose oxidation to CO_2 via the EMP-TCA, $\rho_{G\text{-}EMP(min)}$, is decreased in hypothyroidism to about half the value for normal and hyperthyroid patients. This results from a combined decrease in ρ_G and ϕ_{300}. $\rho_{G\text{-}EMP(min)}$ in the hyperthyroid patients is quite close to the values calculated for the normal patients.

Table III also includes the values of $\rho_{C\text{-}EMP(min)}$, $\rho_{C\text{-}EMP(min)}/\rho_C$, and $6\rho_G/\rho_C$. The calculated values for $\rho_{C\text{-}EMP(min)}/\rho_C$ show overlap in all groups suggesting that the average rate of oxidation of other substrates to CO_2 is altered by abnormal thyroid activity to the same extent as in the rate of glucose oxidation to CO_2. The calculated values for $6\rho_G/\rho_C$ represent the largest possible fraction of expired CO_2 which might result from the oxidation of glucose. These values also overlap among groups and range from 0.39 to 0.59. Since eventually most of glucose carbon exits from the body via expired air, $6\rho_G/\rho_C$ is probably closer to the true fraction of CO_2 derived from glucose at the fasted steady state than is the minimum value, $\rho_{C\text{-}EMP(min)}/\rho_C$. Reservations with respect to the magnitude of $6\rho_G/\rho_C$ have been noted.[4]

EMP-TCA weighting function. The function $W_2(t)$ is an input-output description of the EMP-TCA subsystem decoupled from the distribution kinetics of glucose and bicarbonate. In most of the studies, $W_2(t)$ is a bi-exponential function, but in some a single exponential adequately fits the curve. The calculated values and standard deviations of $W_2(t)$ for all studies are shown in Fig. 6. $W_2(t)$ could not be resolved over the first 10

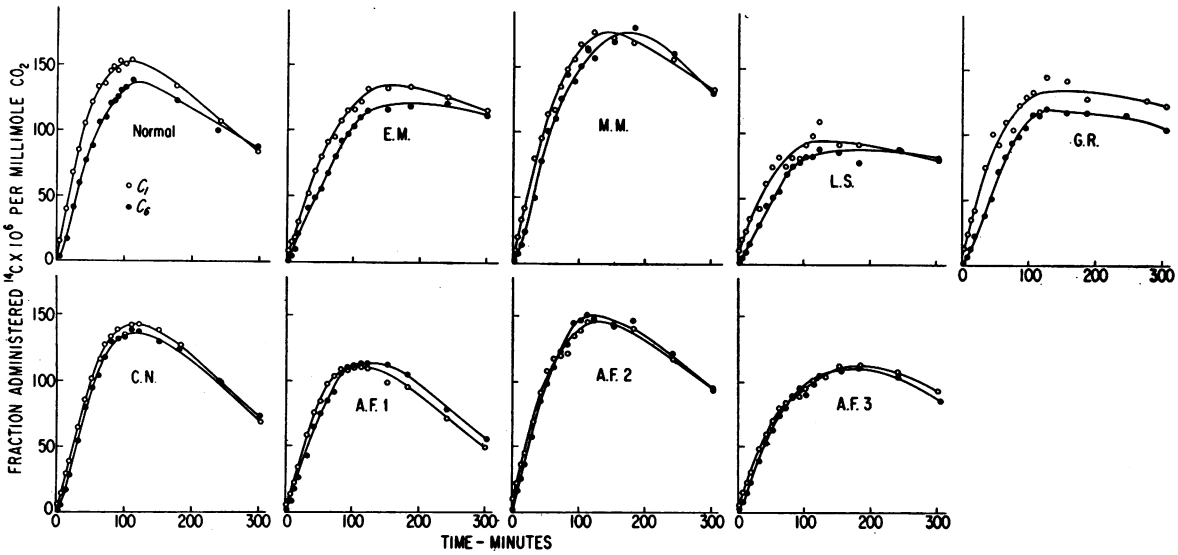

FIGURE 5 Specific activity of expired CO_2 in normal, hypo-, and hyperthyroid patients after single intravenous injection of glucose-6-^{14}C and glucose-1-^{14}C. "Normal" curve is average of four patients studied previously (15).

min and its uncertainty increases with time after 60 min.

The four classes of patients cannot be differentiated on the basis of the shapes of $W_2(t)$. However, the changes in $W_2(t)$ of one patient (A. F.) studied on three separate occasions (A.F.1, A.F.2, and A.F.3) are worth noting. During the first study (A.F.1) she was plainly hyperthyroid. In the second (A.F.2) she was questionably euthyroid, and in the third (A.F.3) she was euthyroid by all the usual criteria. As noted here $W_2(t)$ changed from a fast single exponential function to a somewhat slower bi-exponential function to finally an even slower bi-exponential function, suggesting an increased lag in the metabolic pathways oxidizing glucose to CO_2 as the hyperthyroidism is controlled. However, ϕ_{300} did not change significantly in these three studies.

No attempt is made here to identify any of the metabolic pathways of glucose conversion to CO_2 by the EMP-TCA with the compartmental system corresponding to $W_2(t)$. The most simple compartmental system possible was fitted to the calculated value of $W_2(t)$.

HMP subsystems. Calculated values for Ψ, the fraction of C-1 of glucose oxidized to CO_2 by the HMP, are shown in Table III. There is a great deal of overlap between the normal and hypothyroid patients. The values of Ψ for the two hyperthyroid patients are less than half those calculated for any of the normal or hypothyroid patients. It is worth noting that the value of Ψ in hyperthyroid patient A.F.1 did not change significantly when her hyperthyroidism was controlled, A.F.3.

Also shown in Table III are the values for $\rho_{C\text{-}HMP}$ and $\rho_{C\text{-}HMP}/6\rho_G$. The rate of CO_2 production from glucose by the HMP, $\rho_{C\text{-}HMP}$, is less than normal in both hypo- and hyperthyroidism. In the hypothyroid case this is because ρ_G is decreased with Ψ unchanged, while in the hyperthyroid case Ψ is decreased with ρ_G unchanged. The values of $\rho_{C\text{-}HMP}/6\rho_G$ in all studies show that a minimum of about 1% of the CO_2 coming from glucose results from oxidation by the HMP. The maximum possible fraction of glucose-derived CO_2 resulting from glucose oxidation by the HMP is therefore about 2% (equation 15).

Data fitting and model parameters. Fig. 7 is a representative example of the fit of the compartmental model in Fig. 1 to all the data obtained on a given patient, A.F.2. The glucose-1-^{14}C, glucose-6-^{14}C, bicarbonate-^{14}C, C-1-$^{14}CO_2$, and C-6-$^{14}CO_2$ SA data were fitted simultaneously (16).

The parameter values of the model in Fig. 1, averaged for the four normal studies, the four hypothyroid studies, and the two hyperthyroid studies, are presented in Table IV. Although some of the values have large uncertainties (per cent standard deviations as great as 50%), the measures derived from these parameters presented in Table I and III are much less uncertain due to cancellations resulting from high correlation coefficients.

DISCUSSION

The experimental data and theoretical analysis presented here suggest that hypothyroidism in man produces a decrease in the rate of irreversible glucose metabolism at the fasted steady state, ρ_G. The minimal fraction of the

TABLE III
Measures of Glucose

Subject	Condition	ϕ_{300}	$\rho_{G\text{-}EMP(min)}$	$\rho_{C\text{-}EMP(min)}$
			mmoles/min	*mmoles/min*
C. B.	Normal	0.57 ±0.02	0.51 ±0.04	3.1 ±0.22
C. K.	Normal	0.51 ±0.04	0.45 ±0.03	2.7 ±0.19
R. S.	Normal	0.47 ±0.02	0.42 ±0.03	2.5 ±0.18
A. H.	Normal	0.45 ±0.01	0.40 ±0.03	2.4 ±0.17
Average		0.50	0.45	2.7
E. M.	Hypothyroid	0.44 ±0.03	0.21 ±0.04	1.2 ±0.20
M. M.	Hypothyroid	0.44 ±0.03	0.28 ±0.01	1.6 ±0.07
L. S.	Hypothyroid	0.41 ±0.03	0.18 ±0.02	1.1 ±0.15
G. R.	Hypothyroid	0.41 ±0.03	0.27 ±0.03	1.6 ±0.19
Average		0.42	0.24	1.4
C. N.	Hyperthyroid	0.48 ±0.02	0.53 ±0.02	3.3 ±0.17
A.F.1	Hyperthyroid	0.43 ±0.02	0.40 ±0.03	2.4 ±0.17
A.F.2	Treated hyperthyroid	0.54 ±0.02	0.43 ±0.04	2.6 ±0.26
A.F.3	Treated hyperthyroid	0.47 ±0.03	0.29 ±0.04	1.8 ±0.27

ϕ_{300}, minimal estimate of fraction of irreversibly metabolized C-6 of glucose which is completely oxidized to CO_2 by EMP-TCA; $\rho_{G\text{-}EMP(min)}$, minimal estimate of rate of glucose oxidation to CO_2 by the EMP-TCA; $\rho_{C\text{-}EMP(min)}$, minimal estimate of rate of CO_2 production resulting from glucose oxidation by the EMP-TCA; $\rho_{C\text{-}EMP(min)}/\rho_C$, minimal estimate of fraction of CO_2 excretion resulting from glucose oxidation by EMP-TCA; $6\rho_G/\rho_C$, maximal estimate of fraction of CO_2 excretion resulting from glucose oxidation; ψ, fraction of irreversibly metabolized C-1 of glucose which is oxidized to CO_2 by the HMP; $\rho_{C\text{-}HMP}$, rate of CO_2 production resulting from glucose oxidation by the HMP; $\rho_{C\text{-}HMP}/6\rho_G$, minimal fraction of glucose-derived CO_2 resulting from glucose oxidation by the HMP.
* Normalized to 70 kg weight; value ±SD.

C-6 of glucose oxidized to CO_2 by the EMP-TCA, ϕ_{300}, is also reduced although the fraction of C-1 or glucose oxidized to CO_2 by the HMP, Ψ, is unchanged. In the hyperthyroid studies ρ_G and ϕ_{300} are within the normal range, while Ψ is reduced.

It should be pointed out that a ρ_G of 0.89 mmoles/min computed from our normal studies is about 25% higher than the average values calculated for normal subjects by other investigators (3, 4). The average rate of CO_2 excretion, ρ_C, in our normal subjects is also 25–30% higher than that measured by Manougian, Polycove, Linfoot, and Lawrence (3). If one were to conjecture that our normal subjects are located at the upper end of the probability spectrum with respect to ρ_G and ρ_C, it would be concluded that ρ_G and $\rho_{G\text{-}EMP(min)}$, the minimal estimate of glucose oxidation to CO_2 by the EMP-TCA, are increased in hyperthyroidism rather than unchanged. All other conclusions would remain unchanged.

An extensive analysis of the techniques used and problems involved in determining the magnitude of glucose oxidation by the HMP in tissue slices using C-1– and C-6–labeled glucose was published by Katz and Wood (28, 29). In one of these reports (29) the authors suggest that our calculation of Ψ assumes no recycling of pentose back to G6P. This results, we think, from a misinterpretation of our meaning of Ψ. As defined in the analysis section, Ψ is the fraction of C-1 of glucose entering tissues which is directly oxidized to CO_2 by the HMP. Since the ^{14}C-1 of glucose-1-^{14}C which enters the HMP and is directly oxidized to $^{14}CO_2$ does not recycle, the determination of Ψ is independent of the degree of recycling of pentose back to G6P. Not independent of the extent of recycling, however, are the rate of G6P entry into the HMP and the rate of CO_2 production from the HMP, $\rho_{C\text{-}HMP}$. These two rates are equal on a mole per mole basis and can be calculated by the relation $\rho_G\Psi/(1-0.67\Psi)$ if the stoichiometry discussed in Methods is assumed.

It is important to distinguish between the concepts of Ψ as defined here and PC defined by Katz and Wood (29) as "the fraction of the glucose that is metabolized to CO_2 and glyceraldehyde-3-P via the pentose cycle." Metabolism of a G6P molecule by the HMP is defined by Katz and Wood as the complete degradation of the molecule to $3CO_2$ and one molecule of triose phosphate. Consequently they would conclude from our data and stoichiometric assumptions that the net rate of G6P metabolism by the HMP is one-third of $\rho_G\Psi/(1-0.67\Psi)$

*Oxidation to CO_2**

$\frac{\rho_{C\text{-}EMP(min)}}{\rho_C}$	$\frac{6\rho_G}{\rho_C}$	Ψ	$\rho_{C\text{-}HMP}$	$\frac{\rho_{C\text{-}HMP}}{6\rho_G}$
			mmoles/min	
0.22 ±0.02	0.39 ±0.04	0.079 ±0.010	0.071 ±0.009	0.013 ±0.002
0.22 ±0.02	0.43 ±0.04	0.051 ±0.009	0.045 ±0.010	0.009 ±0.002
0.27 ±0.02	0.58 ±0.04	0.075 ±0.007	0.067 ±0.007	0.012 ±0.001
0.27 ±0.02	0.59 ±0.04	0.068 ±0.008	0.061 ±0.007	0.011 ±0.001
0.25	0.50	0.068	0.061	0.011
0.21 ±0.03	0.49 ±0.07	0.060 ±0.008	0.028 ±0.004	0.010 ±0.001
0.23 ±0.01	0.57 ±0.02	0.063 ±0.006	0.042 ±0.005	0.010 ±0.001
0.16 ±0.02	0.39 ±0.05	0.072 ±0.010	0.031 ±0.004	0.012 ±0.002
0.21 ±0.03	0.52 ±0.05	0.076 ±0.010	0.049 ±0.006	0.013 ±0.002
0.20	0.49	0.068	0.038	0.011
0.20 ±0.01	0.42 ±0.02	0.032 ±0.003	0.035 ±0.004	0.005 ±0.0005
0.17 ±0.01	0.39 ±0.02	0.023 ±0.004	0.021 ±0.004	0.004 ±0.0007
0.28 ±0.03	0.52 ±0.06	0.028 ±0.004	0.022 ±0.003	0.005 ±0.0007
0.25 ±0.04	0.53 ±0.06	0.031 ±0.006	0.017 ±0.003	0.005 ±0.001

which reduces to $\rho_G\Psi/(3-2\Psi)$. At the steady state the net amount of G6P lost through complete degradation to $3CO_2$ and triose phosphate is replaced molecule for molecule by glucose. Thus the net rate of glucose metabolism by the HMP would also be given by $\rho_G\Psi/(3-2\Psi)$ which is equal to the relation $\rho_G PC$ according to the above definition of PC. Consequently the relationship between PC and Ψ can be given directly by the equation $PC = \Psi/(3-2\Psi)$ which reduces to $PC \approx \Psi/3$ when Ψ is small.

Effects of hyper- and hypothyroidism on glucose oxidation by the EMP-TCA and HMP have been studied in the rat in vivo by several investigators using C-1- and C-6-labeled glucose. The experimental design and data analysis employed in these semiquantitative studies differ from our in several ways. Spiro and Ball (6) and Dow and Allen (7) did not perform their experiments at the basal steady state since enough carrier glucose was injected intraperitoneally to produce a rise in the blood glucose concentration. The experimental design of Necheles, Spratt, Ford, and Beutler (8), using glucose tracer without carrier, was more like ours. However, their analysis is based solely on the area under the CO_2 SA data which was collected for only 10 min. No bicarbonate weighting function or data on plasma glucose disappearance is presented. Consequently their calculation of "rate of oxidation" (and that of Spiro and Ball and of Dow and Allen also) represents a summation of processes which are separated in this report.

Gordon and Goldberg (9) studied the $^{14}CO_2$ expiration pattern in hypothyroid, hyperthyroid, and normal men and women after the injection of glucose-1-^{14}C and glucose-6-^{14}C. The patients were loaded with 50 g of oral glucose 30 min before the studies, which were terminated after 60 min of data collection. No data were obtained on the distribution and turnover kinetics of either the glucose or bicarbonate systems. These differences in experimental design and the semiquantitative data analysis employed by these investigators make comparison with our results somewhat difficult. They conclude that glucose oxidation to CO_2 in hypothyroidism is decreased "particularly in regard to pentose shunt activity." Our data suggest that $\rho_{G\text{-}EMP(min)}$ and $\rho_{G\text{-}HMP}$ are decreased proportionately. They also conclude that there is increased "shunt activity" in hyperthyroidism, whereas our data as measured by Ψ, $\rho_{G\text{-}HMP}$, and $\rho_{C\text{-}HMP}$ suggest the reverse.

No attempt is made in this report to ascribe the changes in any of the calculated measures of glucose

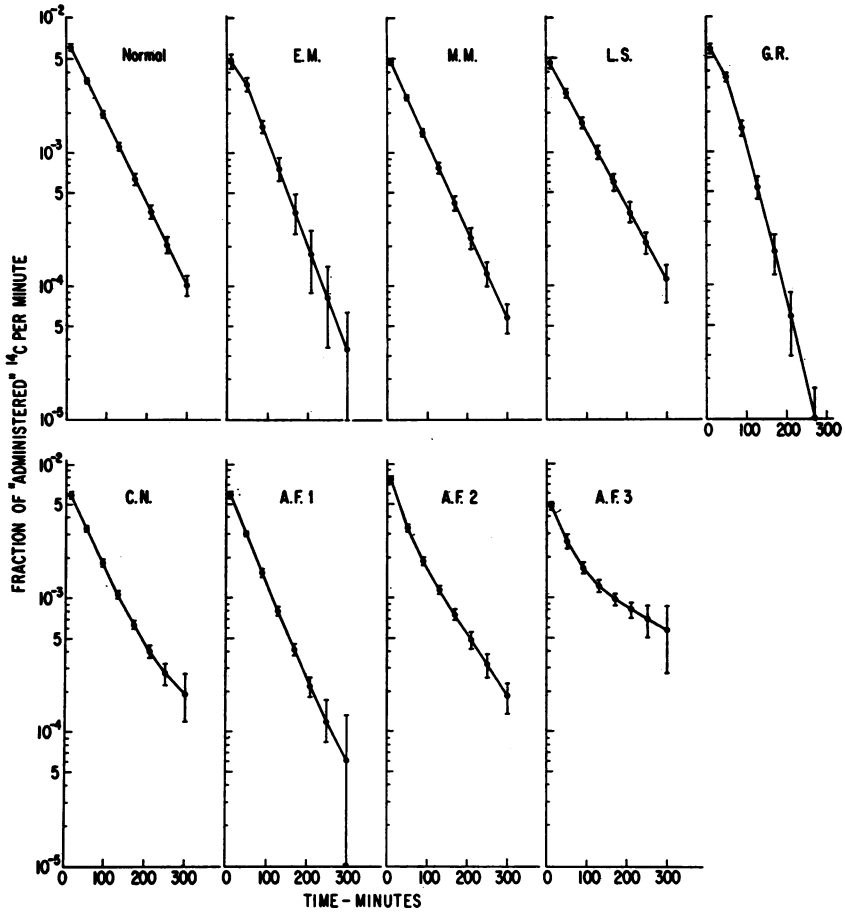

FIGURE 6 Calculated unit response of the EMP-TCA in normal, hypo-, and hyperthyroid patients. Response is expressed as the fraction of a hypothetical dose of glucose-6-^{14}C injected directly into the EMP-TCA which leaves the EMP-TCA as $^{14}CO_2$ per minute. This response is defined in text as $W_2(t)$. "Normal" curve is average of four patients studied previously (5).

oxidation to a particular tissue. It is worth pointing out, however, that the central nervous system which accounts for a large portion of the total glucose utilization in the fasted condition, exhibits very little oxidation by the HMP (35). This would suggest that certain other tissues exhibit greater HMP activity than would be implied by the average value for all the tissues (Ψ) calculated here.

The convolution-deconvolution methodology employed in this report has been well documented in the biological literature (5, 36–39). It is particularly useful in the in vivo analysis of a steady-state precursor-product system in which one or more intermediate pools separate precursor from product. The unit output response of a conversion mechanism CM(t) in this case $W_2(t)$, can be decoupled from the distribution and loss kinetics of both precursor and product. Determination of CM(t) may be quite important in understanding the effects of a steady-state perturbation. It is possible that a perturbation may produce no change in the fraction of precursor converted to product $\left(\int_0^\infty CM(\theta)d\theta\right)$ yet result in an obvious difference in the shape of CM(t). It is important to note that estimation of the steady-state fraction of precursor converted to product requires t (∞) extrapolation of CM(t). To avoid this extrapolative uncertainty, CM(t) may be integrated over the time interval in which the experimental data were obtained to yield $\int_0^t CM(\theta)d\theta$. The latter represents a minimal estimate of the steady-state fraction of precursor converted to product and is independent of the extrapolative implications of the particular compartmental or noncompartmental model chosen to fit the data.

Studies somewhat similar to ours have been performed in normal man. Baker, Shreeve, Shipley, Incefy, and

Miller (1) have formulated a two compartment model to describe blood glucose SA and expired CO_2 SA following a single injection of universally labeled glucose-^{14}C (glucose-U-^{14}C). Manougian (2) used Baker's model to analyze the same kind of data in a larger group of normal, diabetic, and acromegalic patients. Baker concluded that the fraction of CO_2 coming from glucose averaged 21% and the fraction of glucose oxidized to CO_2 averaged 60%. The same measures calculated by Manougian et al. (3) in normal patients were 32 and 47% respectively. Although these values are similar to ours, differences in experimental design and data analysis are worth mentioning. As noted by Segal et al. (5) the CO_2 SA excretion curves in the same patient resulting from glucose-6-^{14}C and glucose-U-^{14}C are markedly different. When glucose-U-^{14}C is used as precursor, the ^{14}C-3 and ^{14}C-4 are oxidized first (with the ^{14}C-1 which traverses the HMP) followed by the ^{14}C-2 and ^{14}C-5 and finally the ^{14}C-1 and ^{14}C-6. The SA of expired CO_2 rises to an earlier and greater maximum value than the $C_e(t)$ curves shown here. Thus a calculation of ϕ_t, a *minimal* estimate of the fraction of ^{14}C of glucose oxidized to $^{14}CO_2$ as calculated from data collected from time 0 to t would be greater using glucose-U-^{14}C than glucose-6-^{14}C. As the duration of the experiment is increased, however, ϕ_t for glucose-U-^{14}C and glucose-6-^{14}C would become closer and would probably merge at ϕ_∞. The closeness of our calculations of ϕ_{300} and $\rho_{G\text{-}EMP(min)}/\rho_C$ to those of Baker and Manougian and their colleagues (using glucose-U-^{14}C) lends support to our assumption that glucose-6-^{14}C may be used to estimate the oxidation of the total glucose molecule to CO_2 if data are collected over a 5 hr interval.

TABLE IV

Parameter Values of Model (Fig. 2) Averaged for Normal, Hypothyroid, and Hyperthyroid Patients

Parameter (min^{-1})*	Normal		Hypothyroid		Hyperthyroid	
λ_{21}	0.0042	±0.0008	0.030	±0.006	0.017	±0.007
λ_{12}	0.019	±0.004	0.036	±0.007	0.049	±0.014
λ_{31}	0.0113	±0.0012	0.0088	±0.0009	0.0137	±0.007
λ_{61}	0.00082	±0.00015	0.00064	±0.00010	0.00040	±0.00005
λ_{42}	0.0076	±0.0016	0.071	±0.010	0.0091	±0.0005
λ_{63}	0.0058	±0.0003	0.0051	±0.0003	0.0069	±0.0004
λ_{04}	0.0050	±0.0012	0.011	±0.001	0.00032	±0.00015
λ_{54}	0.0012	±0.0007	0.0066	±0.0003	0.00014	±0.00001
λ_{05}	0.068	±0.007	0.052	±0.004	0.067	±0.005
λ_{65}‡			0.18	±0.06		
λ_{56}‡			0.14	±0.04		
λ_{75}	0.19	±0.02	0.13	±0.01	0.17	±0.02
λ_{67}	0.063	±0.004	0.036	±0.004	0.069	±0.006

* λ_{08} and λ_{58} are not resolvable from data; value ±SD.

‡ λ_{65} and λ_{56} are not resolvable from bicarbonate weighting function data in normal and hyperthyroid patients.

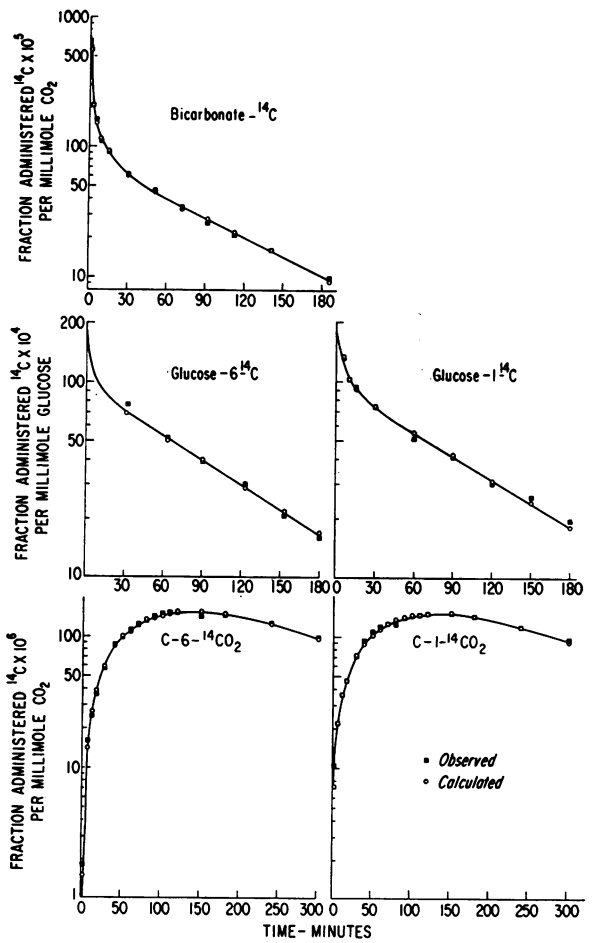

FIGURE 7 Representative example of fit of the compartmental model (Fig. 1) to all data obtained in a given study (A.F.2).

Several theoretical differences between the two techniques can also be pointed out. Baker et al. (1) and Manougian et al. (3) use the maximum value of the CO_2 SA curve rather than all the data in calculating the parameters of their model. They also do not give an estimate of the uncertainties of the calculated parameter values. Their two compartment model implies instantaneous conversion of glucose to CO_2 via the EMP-TCA, an assumption which is inconsistent with the data presented in this report. In a later model (40) however, developed by Baker et al. to describe glucose oxidation to CO_2 in the rat, a nonglucose intermediate pool is included. Another difference between the two techniques is our calculation of ϕ_t which is a minimal estimate of the steady-state fraction of glucose oxidized to CO_2 by the EMP-TCA. This calculation is independent of extrapolation and of the particular model chosen to fit the data. Employment of this "minimal fraction" concept may help to resolve discrepancies arising from different rates of glucose oxidation to CO_2 being calculated from different compartmental and noncompartmental models

which fit the data equally well but which extrapolate differently beyond the time of the last datum.

A more recent study (41) using Baker's two compartment model, analyzes the effects of moderate exercise as compared to rest on glucose oxidation to CO_2 in man. During exercise the rate of plasma glucose metabolism is only slightly increased, while the fraction of the injected glucose-^{14}C expired as $^{14}CO_2$ in 4.5 hr more than doubles. This suggests that the EMP-TCA is more affected by exercise than the glucose or bicarbonate subsystems. However, because a bicarbonate weighting function was not obtained and a deconvolution analysis not employed to calculate the CO_2 response of the EMP-TCA to a unit impulse, comparison with $W_2(t)$ of our studies is difficult.

The investigation presented here portrays a picture of glucose oxidation to CO_2 in normal, hypo-, and hyperthyroid man in the fasted steady state. It is noted that the conclusions of this study are based on a small number of patients in each group. Integration of all the studies into a simple consistent pattern, however, reinforces the individual studies as part of a larger population spectrum. The experimental procedure and data analysis employed form the basis for a more extensive investigation of glucose oxidative kinetics in thyroid disease and in other conditions using many more patients. Furthermore, this analysis suggests new experiments which might be performed. One would be the repeat of these studies after the rise of plasma glucose to new steady states resulting from constant rate glucose infusions of varying magnitudes. This would permit elucidation of possible nonlinear characteristics of the system by conventional linear analysis (42, 43). The transient characteristics of the system might then be profitably investigated especially if the system had already been intensively studied at different steady states. Separation of some of the pathways of the EMP-TCA might be accomplished with the use of differently labeled pyruvate and glucose labeled in positions other than C-1 and C-6. The use of glucose labeled with tritium in positions C-2 and C-6 would also be helpful in determining more precisely the extent of recycling of glucose carbon through liver glycogen and the Cori cycle (44, 45). Methods presented in this report may allow changes in glucose and bicarbonate kinetics to be separated from perhaps more subtle changes in the biochemical mechanism oxidizing glucose to CO_2.

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