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THE METABOLISM OF VARIOUSLY C¹⁴-LABELED GLUCOSE IN MAN AND AN ESTIMATION OF THE EXTENT OF GLUCOSE METABOLISM BY THE HEXOSE MONOPHOSPHATE PATHWAY

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Studies of glucose metabolism utilizing radioactive glucose have been reported in several animal species (1-3) including normal and diabetic man (4-6). In these studies the tracer employed has been glucose with the C¹⁴ uniformly distributed throughout the six carbon atoms. From the reactions of glucose catabolism in the Embden-Meyerhof pathway, Krebs' tricarboxylic acid cycle and the hexose monophosphate pathway, it is apparent that the individual carbon atoms of glucose may have different rates of oxidation to carbon dioxide as well as different metabolic fates in the body. Previous studies (7, 8) of ribose metabolism have shown that the hexose monophosphate pathway exists in man.

The studies reported here have been performed with glucose labeled with C¹⁴ located in specific carbon atoms, in order to ascertain differences in metabolism of individual parts of the sugar. From the results obtained an attempt is made to quantify in man the extent of glucose metabolism via the hexose monophosphate pathway.

METHODS

Three normal male and one female volunteer, aged 18 to 21 years, served as subjects for these experiments. All were maintained on a diet containing 300 g of carbohydrate and were fasted overnight prior to and throughout the performance of the study. An intravenous glucose tolerance test performed according to Amatuzio, Stutzman, Vanderbilt and Nesbitt (9) revealed normal glucose tolerance in all of the subjects.

Glucose-1-C¹⁴, specific activity (SA) 2.67 μC per mg, and glucose-2-C¹⁴, SA 5.56 μC per mg, were purchased from the Volk Radiochemical Co.; glucose-U-C¹⁴, SA 2.06 μC per mg, from Nuclear Chicago; and glucose-6-C¹⁴, SA 2.8 μC per mg, from Dr. H. Isbell of the National Bureau of Standards. These sugars were converted to gluconate and degraded by periodate to isolate C-1 and C-6. Virtually all of the label of glucose-1-C¹⁴ and glucose-6-C¹⁴ was in C-1 and C-6, respectively. No activity was

found in the C-1 or C-6 of glucose-2-C¹⁴. C-1 and C-6 of glucose-U-C¹⁴ each contained approximately 20 per cent of the total radioactivity. The glucose-1-C¹⁴ and glucose-6-C¹⁴ were chromatographically pure. To be absolutely certain that the glucose-1-C¹⁴ contained no gluconate which might be more rapidly metabolized than glucose, a known amount of the sugar was oxidized in the presence of potassium gluconate with periodate, and the CO₂ formed was trapped and counted as stated below. No radioactivity was liberated in this procedure. Since CO₂ is released from C-1 of gluconate and not glucose, the results of the procedure indicate that the starting glucose-1-C¹⁴ was free of gluconate-1-C¹⁴. Sodium bicarbonate C¹⁴, 11.9 μC per mg, was obtained from Nuclear Chicago. All C¹⁴ solutions were prepared by the radiopharmacy of the National Institutes of Health and found sterile and pyrogen-free prior to use.

The labeled glucose, dissolved in normal saline, approximately 1 μC per ml, was injected rapidly into an antecubital vein. Subjects C.K. and C.B., both male, each received 9.8 μC and 9.3 μC of C-1- and C-6-labeled glucose, respectively. Subject R.S., a male and A.H., a female, each received 5 μC of C-1, C-2, C-6 and uniformly labeled glucose. Expired CO₂ was collected in Douglas bags for 5- or 10-minute periods at various intervals for 6 hours, and blood was drawn for determination of the specific activity of glucose and for isolation of serum lipid. Urine was collected for 24 hours. Studies were performed at approximately monthly intervals. After such time-intervals no C¹⁴ derived from the previous study was detectable in expired air or blood glucose. In Subject C.B. the excretion of C¹⁴O₂ in expired air was determined after the intravenous injection of 0.64 μC NaHC¹⁴O₃ dissolved in 1 ml of normal saline.

The determination of CO₂ content and counting of samples were performed by the method of Fredrickson and Ono (10) with a liquid scintillation spectrometer (Packard Co., La Grange, Ill.) counting at 53 per cent efficiency. Blood glucose was isolated as potassium gluconate by the method of Blair and Segal (11). Gluconate was degraded by oxidation with periodate (12) to obtain C-1 as CO₂ and C-6 as formaldehyde which was converted to the dimedon derivative. CO₂ derived from C-1 was trapped in Ba(OH)₂. The resulting BaCO₃ was then acidified and the liberated CO₂ diffused into Hyamine base (13) and counted as described above. The

formaldehyde dimedon representing C-6 was counted directly in a toluene phosphor. For determination of total specific activity, the isolated gluconate was oxidized to CO_2 by a wet combustion (14). The CO_2 was collected as BaCO_3 which was processed for counting as described above. Urine was assayed directly for C^{14} as described (15). Blood glucose was determined by a glucose oxidase method employing the glucostat reagent.¹ Simultaneous determination of blood and plasma glucose revealed plasma glucose concentration to be 10 per cent higher than whole blood. Simultaneous determination of specific activity of blood and plasma glucose on several samples revealed no significant difference, and therefore whole blood glucose was routinely isolated.

Serum lipids were extracted in the following manner. Serum was lyophilized, the residue redissolved in a minimal amount of water and injected into 24 vol of chloroform-methanol (2:1). After shaking, this was allowed to stand 1 hour at room temperature; 5 ml of water per 1 ml of original water-serum solution was added and this was permitted to stand overnight. An aliquot

¹ Worthington Biochemical Corp., Freehold, N. J.

of the chloroform layer was evaporated to dryness under nitrogen; the residue was dissolved in toluene phosphor and counted.

In the study of glucose oxidation by blood, 2 ml of heparinized whole blood immediately after venipuncture was transferred to a modified Warburg vessel containing $0.3 \mu\text{c}$ of glucose-1- C^{14} in $20 \mu\text{l}$ of water. The vessel was gassed for 30 seconds with a 95 per cent O_2 , 5 per cent CO_2 mixture, sealed, and incubated for various times in a Dubnoff shaker at 37°C . At the end of the incubation, Hyamine was added to the center well and the reaction was stopped by addition of 0.3 ml of $6 \text{ N H}_2\text{SO}_4$ to the blood, thereby liberating CO_2 . The C^{14}O_2 was diffused into the Hyamine by shaking the flasks for 45 minutes. The vessels were then opened and the Hyamine placed in phosphor for scintillation counting.

RESULTS

C¹⁴-carbon dioxide excretion. The specific activity curves of expired CO_2 after administration of glucose-1 and 6- C^{14} to Subjects C.K. and C.B.

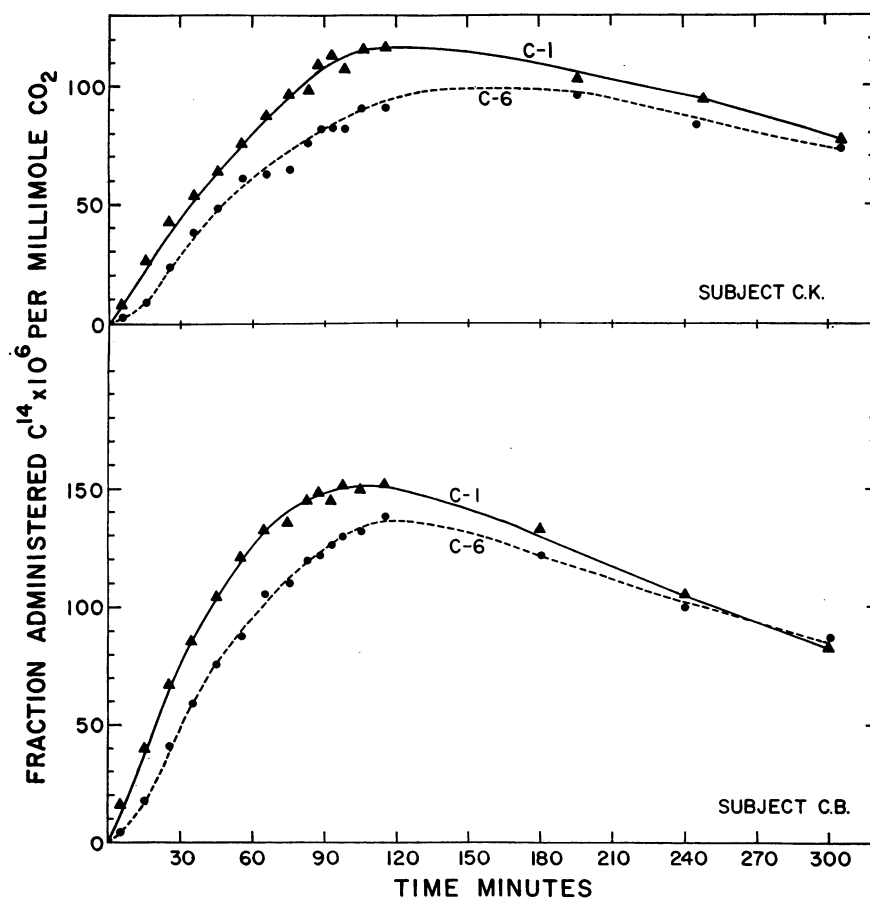


FIG. 1. SPECIFIC ACTIVITY OF EXPIRED CO_2 IN SUBJECTS C.K. AND C.B. AFTER ADMINISTRATION OF GLUCOSE-1- C^{14} AND GLUCOSE-6- C^{14} .

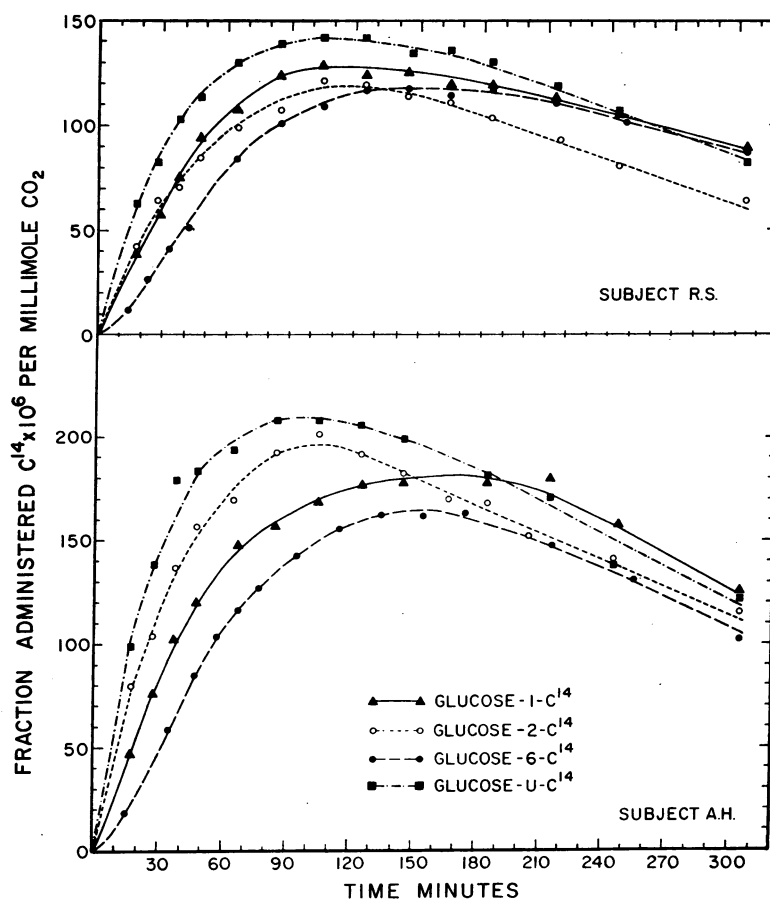


FIG. 2. SPECIFIC ACTIVITY OF EXPIRED CO₂ IN SUBJECTS R.S. AND A.H. AFTER ADMINISTRATION OF VARIOUS C¹⁴-LABELED GLUCOSE.

are shown in Figure 1. In these studies the expired air was continuously collected for 5- or 10-minute intervals up to 120 minutes, with subsequent collection of single samples. The points are plotted in the midpoint of each collection period. It is seen that after the administration of glucose-6-C¹⁴ there is a lag period of several minutes before a linear increase in specific activity occurs. After glucose-1-C¹⁴ injection, the CO₂ specific activity immediately increases without a lag phase. The peak specific activity after the C-1-labeled sugar is injected is earlier in time than that after C-6 in three of the four subjects. Since the C¹⁴O₂ specific activity curve after glucose-6-C¹⁴ is displaced by an initial lag, it is quite evident that ratios of specific activity of C¹⁴O₂ derived from the labeled sugars at any given time cannot be used to estimate differences in the metabolic fates of glucose-1-C¹⁴ and glucose-6-C¹⁴.

Figure 2 shows the specific activity curves of expired CO₂ in Subjects R.S. and A.H. after injections of 5 μg of C-1, C-2, C-6 and uniformly labeled glucose. In these subjects sample collections were made at 10-minute intervals for 4 hours in order to obtain more accurate delineation of the curves after 2 hours than was obtained in the subjects shown in Figure 1. It is clear that uniformly labeled glucose gives rise to faster and more extensive labeling of CO₂ than does any of the singly labeled sugars. The differences between the C-1 and C-6 excretion curves are very similar to those of studies shown in Figure 1. Of unusual interest are the curves of specific activity of CO₂ derived from glucose-2-C¹⁴. In both cases the curve parallels that derived from uniformly labeled glucose and has an earlier peak activity than that obtained from C-1- and C-6-labeled glucose.

TABLE I
CO₂ excretion after C¹⁴-glucose injection

Subject	Position C ¹⁴ adm. glucose	Blood* glucose	CO ₂ †	Administered C ¹⁴ in expired CO ₂ at				
				60 min	120 min	180 min	240 min	300 min
C.K. 72 kg	C-1	69	13.2	3.5	11.9	20.8	28.7	35.6
	C-6	70	14.2	2.5	9.2	17.5	25.3	32.1
C.B. 71 kg	C-1	67	13.1	5.7	17.1	28.6	38.2	45.7
	C-6	59	12.7	3.7	13.0	23.3	31.8	38.9
R.S. 81 kg	C-U	73	11.2	5.6	14.9	24.0	32.0	38.5
	C-1	77	11.0	3.9	12.0	20.3	27.8	34.3
	C-2	72	11.5	4.1	11.9	19.7	26.3	31.4
	C-6	77	10.6	2.3	8.9	16.2	23.4	29.5
A.H. 60 kg	C-U	76	7.9	5.8	14.7	23.4	30.9	36.9
	C-1	73	7.6	3.9	11.3	19.5	27.3	33.9
	C-2	71	7.3	5.2	14.2	22.8	30.1	36.0
	C-6	68	7.6	2.6	8.9	16.3	23.1	28.7

* Average of from 4 to 10 samples over a 3.5 hour period.

† Average of 14 to 18 samples.

Table I shows the rate of CO₂ output in millimoles per minute in each of the above studies. This value is remarkably constant in a given individual. Table I also reveals the cumulative C¹⁴O₂ expired at hourly intervals up to 5 hours, ex-

pressed in percentage of the dose of C¹⁴. Quite consistently the yield of C¹⁴O₂ derived from C-1-labeled glucose is greater than that from the C-6-labeled sugar. When all four labeled sugars are compared in a given subject, the oxidation of glucose-U-C¹⁴ appears to give the greatest yield of C¹⁴O₂.

Of course, differences of C¹⁴O₂ excretion might occur if the turnover of the glucose pool happened to differ on the days that the individual experiments were performed. Data obtained on the turnover rates of blood glucose in Subjects C.K. and C.B. after injection of glucose-1-C¹⁴ and glucose-6-C¹⁴ indicate that these rates were identical in a given subject during the study of both sugars (see below). Therefore the differences in the C¹⁴O₂ excretion patterns are due to differences in metabolic patterns after glucose has been delivered to the tissues.

C¹⁴-carbon dioxide excretion after NaHC¹⁴O₃ injection. It became apparent during the analysis of the data on excretion of C¹⁴O₂ derived from glucose that a knowledge of the bicarbonate system in man would be necessary. Baker and co-workers (4) have reported on some of the characteristics of this system after NaHC¹⁴O₃ injection into two subjects. Figure 3 shows the specific activity curve of expired C¹⁴O₂ after C¹⁴-bicarbonate injection into Subject C.B. who had also received labeled glucose. Because the realiza-

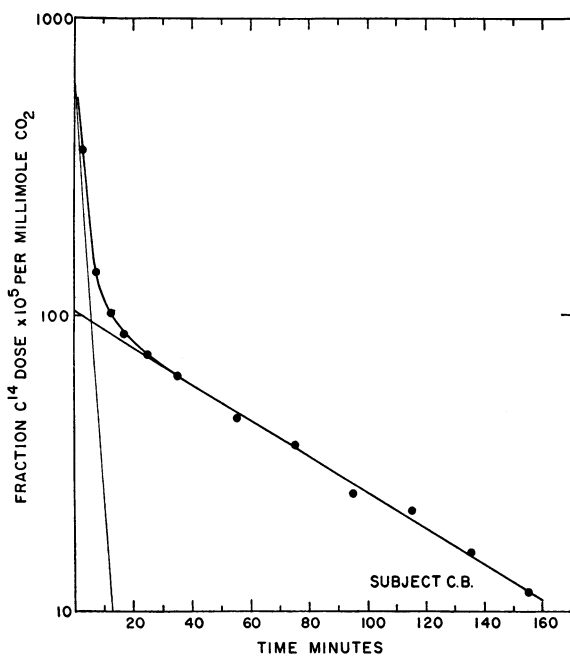


FIG. 3. SPECIFIC ACTIVITY OF CO₂ IN EXPIRED AIR RESULTING FROM A SINGLE BICARBONATE INJECTION. The data may be applied to a sum of two exponentials

$$B(t) = \sum_j B_j e^{-\beta_j t} = 0.00605 e^{-0.32t} + 0.0102 e^{-0.0132t}$$

tion of the importance of the bicarbonate system came relatively late in the course of these experiments, only Subject C.B. was available for study. In the investigations now in progress on endocrine influences on glucose metabolism, the bicarbonate system is studied in each patient. The curve presented here is similar to those of Baker and co-workers (4) and to other normal subjects studied by us. Examination indicates that for the purposes of this study small variations negligibly influence the results of the analysis. A study of the data in Figure 3, necessary for the interpretation of the present glucose oxidation data, is presented in the section on Analysis.

The oxidation of C¹⁴-glucose by whole blood. Since it is known that both red and white blood cells oxidize glucose-1-C¹⁴ to C¹⁴O₂ much more rapidly than glucose-6-C¹⁴ (16, 17), the possibility

TABLE II
*Glucose-1-C¹⁴ oxidation by whole blood **

Time of incubation	CO ₂ fraction of substrate C ¹⁴
<i>min</i>	
15	2.55 × 10 ⁻³
34	5.27 × 10 ⁻³
45	6.55 × 10 ⁻³

* Flask contained 2 ml whole blood, 0.3 μc glucose-1-C¹⁴.

arose that part or all of the difference in the curves of expired CO₂ shown above could be explained by this fact. That is, C¹⁴O₂ produced from glucose-1-C¹⁴ by the blood elements would be in a unique position, via rapid transit through the lung, to be excreted immediately in expired air. Therefore an experiment was performed *in vitro* to study glucose-1-C¹⁴ oxidation by whole blood.

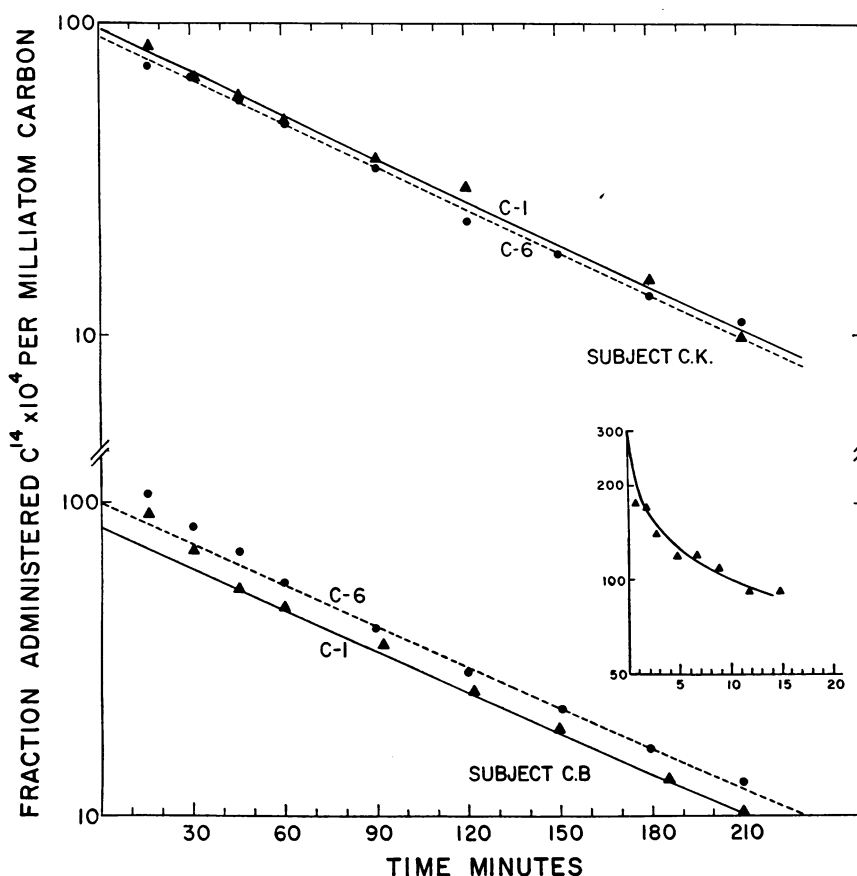


FIG. 4. SPECIFIC ACTIVITY OF C-1 AND C-6 OF BLOOD GLUCOSE AFTER ADMINISTRATION OF GLUCOSE-1-C¹⁴ AND GLUCOSE-6-C¹⁴, RESPECTIVELY. The inset is the specific activity of blood glucose after injection of glucose-1-C¹⁴ to Subject A.K. normalized to 15-minute value of Subject C.B.

TABLE III
Randomization of C¹⁴ in blood glucose after administration of specifically C¹⁴-labeled glucose

Subject	C ¹⁴ position	Time after injection hrs	Specific activity (SA)			Randomization*
			Total glucose dpm/mmole	Glucose C-1 dpm/mA.C†	Glucose C-6 dpm/mA.C	
C.K.	C-1	1	103,600	103,400	1,313	0
		2	63,400	64,750	2,124	0
		3	35,350	33,000	1,628	6.7
	C-6	1	91,600	0	97,200	0
		2	53,600	810	47,800	11.0
		3	30,500	780	27,710	9.2
C.B.	C-1	1	105,200	99,400	803	5.5
		2	54,900	53,800	2,015	2.0
		3	32,500	28,400	1,592	12.8
	C-6	1	117,200	766	114,800	2
		2	58,500	1,093	59,400	0
		3	38,090	5,460	34,000	10.6
R.S.	C-2	0.5	33,280	0	0	0
		1	21,690	0	0	0
		2	12,980	0	0	0
A.H.	C-2	0.5	49,300	1,271	1,362	5.6
		1.5	22,500	1,675	1,715	16.8

* For Subjects C.K. and C.B. calculated from:

$$\frac{\text{glucose SA} - \text{SA of carbon at original site of label}}{\text{glucose SA}}$$

for Subjects R.S. and A.H. who received glucose-2-C¹⁴ the calculation used is:

$$\frac{\text{SA of C-1} + \text{SA of C-6}}{\text{glucose SA} - (\text{SA of C-1} + \text{SA of C-6})}$$

† Disintegrations per minute per milliatom of carbon.

The results are shown in Table II. Under the conditions of this experiment, C¹⁴O₂ liberation was linear for about 30 minutes, and the turnover rate of glucose by the cells was calculated as 0.000163 per minute. This corresponds to 0.008 of the total glucose disappearance rate (from subsequent calculations).

C¹⁴ in blood glucose. The changes in the specific activity of blood glucose with time in Subjects C.K. and C.B. are shown in Figure 4. These data indicate that the behavior of C¹⁴ in blood glucose after injection of either C-1- or C-6-labeled glucose was nearly identical in each subject. In C.B. it appears that there is an initial phase of 16 to 60 minutes before the final portion of the curve becomes linear. This has been observed by us previously (11) and by others (18). In order to delineate the nature of the curve prior to 15 minutes, glucose-1-C¹⁴ was injected intravenously into a male subject, A.K., who weighed the same

as C.B., and blood was obtained at short intervals up to 15 minutes. In this experiment blood filtrates (7) were counted directly on the assumption that at these early times the C¹⁴ in blood was in glucose exclusively. The results of two such studies revealed curves with marked oscillations in the C¹⁴ activity. We felt that this was due to mixing phenomena exaggerated by the venostasis during the process of blood withdrawal from an indwelling needle. Therefore, the study was performed on blood samples obtained from the brachial artery. The curve thus obtained with the values normalized so that the 15-minute value equals that of C.B. is shown in the inset in Figure 4. These data in composite with those of C.B. and C.K. provide the basis for the further analysis of the glucose system shown later in this paper.

Randomization of C¹⁴ in blood glucose. The question often arises whether a specifically labeled substrate administered to an animal remains

specifically labeled over a period of time or whether resynthesis of the compound from labeled fragments causes randomization of radioactivity. The administration of glucose labeled in a single carbon provides an opportunity to study the randomization of C¹⁴ in blood glucose in man. Table III demonstrates the results of these experiments, in which the specific activity of blood glucose was determined as well as the specific activity of the 1 and 6 carbons of glucose. There appeared to be some labeling of C-1 when glucose-6-C¹⁴ was administered, and conversely of C-6 when glucose-1-C¹⁴ was given. In most cases the randomization is small. Up to 3 hours after injection of the C¹⁴-glucose, peripheral tissues were being presented with glucose principally labeled in the position where C¹⁴ originally resided. The per cent randomization as shown in Table III is quite variable. Much of this probably reflects methodological variations in the many steps involved in the isolation and degradations. In the studies in Subjects C.K. and C.B., essentially all of the C¹⁴ is in C-1 and C-6, which may be explained by hepatic synthesis of glucose from labeled pyruvate resulting from the metabolism of glucose by muscle. Since randomization is not extensive, the data indicate that glucose put out by the liver during the interval of study is either essentially unlabeled or labeled principally in the same position as the injected glucose.

The rapid incorporation of C¹⁴ into expired CO₂ after injection of glucose-2-C¹⁴ suggested that possibly this was being converted to glucose-1, 3-C¹⁴ via the oxidative pathway. The degradation studies of blood glucose indicate that this is not the case. It is possible, however, that randomization does occur intracellularly without being reflected in the pattern of labeling of blood glucose.

Incorporation of C¹⁴ from glucose into serum lipids. C¹⁴ was determined in total serum lipids in two studies with glucose-1- and 6-C¹⁴ 3 hours after injection of the label. The results are shown in Table IV. In both experiments more radioactivity appeared in lipids after administration of glucose-6-C¹⁴ than of glucose-1-C¹⁴. Although the amount of incorporation in the two studies varied, the ratio of activity derived from C-1- and C-6-labeled glucose is similar.

With preferential loss of C-1 of glucose via the hexose monophosphate pathway, a decrease in

TABLE IV
Radioactivity in serum lipids 3 hours after C¹⁴-glucose administration

Subject	C ¹⁴ position in glucose	Lipid C ¹⁴ fraction dose per 100 ml serum	Ratio C-1/C-6
C.K.	C-1	8.0 × 10 ⁻⁶	0.63
	C-6	12.7 × 10 ⁻⁶	
C.B.	C-1	19.7 × 10 ⁻⁶	0.57
	C-6	34.8 × 10 ⁻⁶	

radioactivity in the triose moieties derived via the Embden-Meyerhof pathway would be expected. This would be reflected in a decreased availability of labeled triose for lipid synthesis. A ratio comparing C¹⁴ in lipids from glucose-1-C¹⁴ and glucose-6-C¹⁴ would be less than 1; in this case it is about 0.6. Some authors (19) have felt that this ratio indicates the extent of operation of the two pathways, but Katz and Wood (20), in a recent theoretical treatment of this subject, demonstrate that the ratio of radioactivity in lipid cannot be used in this fashion and show that a ratio of 0.6 may be compatible with the metabolism of about 15 per cent of glucose via the shunt pathway.

Urinary excretion of C¹⁴. A normal subject may excrete glucose in urine (21). It is not surprising, therefore, that C¹⁴ should appear in the urine after labeled glucose injection; 1 to 2 per cent of the C¹⁴ administered appeared in the urine within 24 hours, except in the case of glucose-2-C¹⁴ where 4 per cent of the dose was excreted via this route. This C¹⁴ may represent compounds other than glucose.

ANALYSIS

The purpose of the analysis is to investigate the nature of the exchangeable glucose system and to derive a value for the magnitude of the hexose monophosphate pathway of glucose metabolism. It is apparent from the data that the C¹⁴ of glucose-1-C¹⁴ appears as CO₂ sooner than the C¹⁴ of glucose-6-C¹⁴. This implies the operation of a more direct pathway for C-1 of glucose to be oxidized to CO₂. Such a direct pathway has occasionally been referred to as a shunt. To account for the difference in the appearance of C-1 and C-6 of glucose in CO₂ in terms of a direct pathway for C-1, and to determine the magnitude of this pathway, it is necessary to examine the kinetics of glucose as well as the kinetics of bicarbonate in the plasma. From this examination a model is proposed, from which has been derived a value, *k*, which indicates the fraction of glucose being metabolized whose

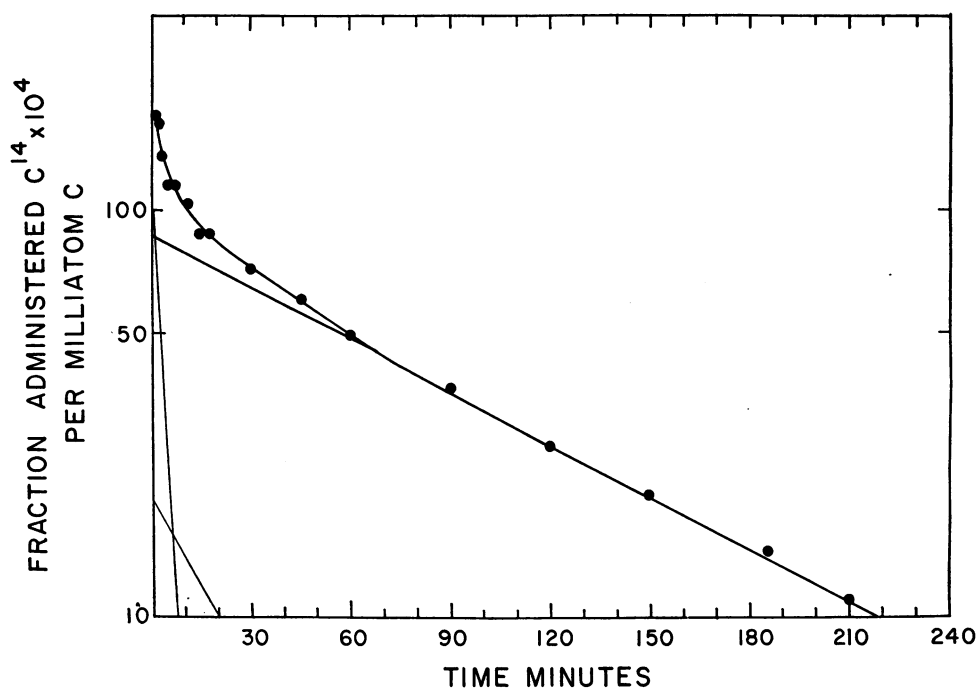


FIG. 5. COMPOSITE CURVE OF BLOOD GLUCOSE DATA BASED ON AVERAGE VALUES OF THE POINTS FOR SUBJECTS C.K. AND C.B. IN FIGURE 4. The early time data were normalized to the averaged 15-minute point. $x_1 = 0.01018e^{-0.326t} + 0.00194e^{-0.094t} + 0.00876e^{-0.01t}$.

fate is the direct oxidation of C-1 to CO_2 . This fraction, k , has been interpreted as the extent of glucose metabolism via the hexose monophosphate pathway.

Exchangeable glucose pools. A kinetic analysis of exchangeable glucose pools has been carried out with the SA data of plasma glucose, and the results are presented here. The analysis shows that a unique solution of the glucose system is not possible from our data. Several representative models that seem reasonable and that are discussed in the literature (22) have been assumed and values for their parameters calculated. This was done in order to examine what effect the choice of a model may have on the calculation of the relative size (k) of the direct oxidative pathway. For the purpose of estimating k , the analysis of the glucose system has suggested that this value may be ascertained independently of a knowledge of how the glucose pools are interconnected. This analysis of the glucose system is presented to demonstrate the reasoning employed to reach the above conclusion.

The plasma² glucose data for the two patients (Figure 4) are quite similar and were combined into a single curve for purposes of analysis. The special study dealing with the first 15 minutes was normalized to the combined curve, and a single composite curve for plasma glucose was obtained (Figure 5). These data were ap-

² Although blood glucose was isolated, its SA is the same as that of plasma glucose.

proximated analytically to a sum of three exponentials of the form:

$$x_1 = a_{11}e^{-\alpha_1 t} + a_{12}e^{-\alpha_2 t} + a_{13}e^{-\alpha_3 t} \quad [1]$$

where x_1 is the specific activity of glucose in the plasma, in units fraction of dose per milliatom of carbon. The values for a_{11} and α_1 are given in the legend of Figure 5. The three exponentials in Equation 1 mean that at least three exchangeable compartments can be resolved by the data. Since only one of these compartments was measured, a unique solution of all turnover rates and compartment sizes of the system is not possible (23). Some of the parameters of the model, however, may be solved for, and under special assumptions complete solutions may be obtained.

The size of the glucose pool for the sampled compartment (S_1) may be obtained from the reciprocal of x_1 at time $t = 0$.

$$S_1 = \frac{1}{[x_1]_0} = \frac{1}{a_{11} + a_{12} + a_{13}} = 47.9 \text{ milliatoms carbon}^3 \quad [2]$$

The volume of distribution (V_1) in liters of S_1 may be determined by using the average steady state concentra-

³ Since this refers to the quantity of C-1 or C-6 of glucose it is also a measure of the total molecules of glucose.

tion of nonlabeled glucose in the plasma (4.07 mmoles per L)⁴:

$$V_1 = \frac{47.9 \text{ mmoles glucose}}{4.07 \text{ mmoles/L}} = 11.8 \text{ L.}$$

When it is assumed that, after the initial mixing phases the specific activities of glucose in all three compartments are the same, and that glucose may leave the exchangeable system from all compartments with equal probability, then the "intercept method" may be used to estimate the size of the exchangeable glucose pool:

$$\text{exchangeable pool size} = \frac{1}{a_{13}}$$

The pool size so obtained for the composite curve of the five studies is shown in Table V and is in good agreement with values reported by others using the same method of computation (6).

For the assumed conditions, the rate of loss of glucose from the system may also be calculated as the pool size \times the smallest exponential constant:

$$\text{rate of loss of glucose} = \frac{\alpha_3}{a_{13}} \quad [3]$$

The calculated values are given in Table V.

In order to examine the nature of the exchangeable glucose system, several special models were assumed and solutions (23, 24) for the values of their parameters obtained⁵ (Table V). The sampled compartment, defined Compartment 1, corresponds roughly to the glucose content of the estimated extracellular space of the subjects. The next largest, Compartment 2, is nearly equal in size to Compartment 1, and Compartment 3 is small compared with 1 or 2. It was assumed for the solution of the pool sizes that the site of entry of new glucose is Compartment 1.

It is suggested in the literature (25, 26) that very little glucose exists in the intracellular space. It is therefore assumed here that Compartment 2 does not reflect intracellular glucose. It could represent a pool of glucose fragments in equilibrium with extracellular glucose, but this is not supported by our experiment in which the courses of carbon labels in the 1, 2 or 6 positions were studied and shown to remain predominantly in their original positions for a considerable time after injection. Lack of randomization of C¹⁴ in blood glucose demonstrates that blood glucose is not in equilibrium with a compartment of carbon consisting of carbon fragments, as has been proposed for the rat by Baker, Shipley, Clarke and

⁴ This value is the average of the blood glucose levels of C.K. and C.B., Table I, corrected to account for the fact that blood glucose equals $0.9 \times$ plasma glucose and converted from milligrams glucose to millimoles glucose.

⁵ In principle, it is possible to obtain the range of variation of all the parameters for all possible models (23). The calculations for this, however, become quite extensive and impractical without the aid of high-speed computers and special programs and have not been performed.

Incefy (22). The three-compartment glucose system seen in man appears to represent a spatial distribution of glucose or glucose metabolites which maintains the glucose carbon skeleton.

The only other glucose pool of any magnitude in the body free to exchange is in the form of liver glycogen and glucose intermediates on the synthetic pathway to glycogen. It seems a reasonable assumption that Compartment 2 reflects predominantly liver glycogen. Since the incorporation and release of glucose by glycogen occur at the outer tiers of the macromolecule (27), Compartment 2 could reflect only the exchangeable part of liver glycogen.

The size of Compartment 3 is about 20 per cent of Compartment 1, and its rate of exchange with either 1 or 2 is slow. It could be part of the extracellular space or some other slowly exchanging pool, and no attempt will be made to identify it.

The glucose entering the hexose monophosphate pathway must originate from one of the three compartments. Compartment 2 is excluded, because it is assumed to be predominantly a liver glycogen pool. Compartment 3 is also excluded, because its time of equilibration with the system is much too slow to account for the rapid rate of release of CO₂. Consequently, it is assumed that glucose for the direct oxidative pathway is in effect supplied by Compartment 1. Since Compartment 1 was sampled, it is not necessary for the purposes of the shunt determination, as will be seen later, to specify how the three exchangeable glucose compartments are interconnected.

The fraction of Compartment 1 which leaves the exchangeable glucose system, λ_{01} , will be required for subsequent calculations and may be determined as follows. The loss of labeled glucose from Compartment 1 due to metabolism in a time interval dt is $\lambda_{01}q_1 dt$. The total loss must eventually equal the amount injected:

$$\int_0^{\infty} \lambda_{01}q_1 dt = 1 \quad [4]$$

and

$$\lambda_{01} = \frac{1}{\int_0^{\infty} q_1 dt} \quad [5]$$

The amount of labeled glucose in Compartment 1, q_1 , may be obtained by normalization of Equation 1 to unit quantity at 0 time, and may be written as:

$$\begin{aligned} q_1 &= A_{11}e^{-\alpha_1 t} + A_{12}e^{-\alpha_2 t} + A_{13}e^{-\alpha_3 t} \\ &= 0.488 e^{-0.326t} + 0.093 e^{-0.034t} + 0.419 e^{-0.01t} \end{aligned} \quad [6]$$

Substituting [6] into [5]:

$$\lambda_{01} = 1 / \left(\frac{A_{11}}{\alpha_1} + \frac{A_{12}}{\alpha_2} + \frac{A_{13}}{\alpha_3} \right) = 0.0217/\text{min} \quad [7]$$

Knowledge of λ_{01} permits a calculation of the relative magnitude of the oxidation of glucose by the blood cells:

$$\frac{\text{blood cell oxidation}}{\text{total metabolism}} = \frac{0.000163/\text{min}}{0.0217/\text{min}} = 0.008 \quad [8]$$

TABLE V
Solutions of several possible glucose models

Model	S_1^\dagger Milliatoms Carbon	V_1^\S L	$\frac{S_2}{S_1}$	$\frac{S_3}{S_1}$	$\frac{S_1 + S_2 + S_3}{S_1}$	$S_1 + S_2 + S_3$ Milliatoms Carbon	Rate of Glucose Metabolism <u>Milliatom</u> <u>Min.</u>
	47.9	11.8	.794	.215	2.09	100.09	1.04
	47.9	11.8	.918	.215	2.13	102.01	1.03
	47.9	11.8	.861	.147	2.08	99.61	1.04
	47.9	11.8	.990	.169	2.16	103.44	1.09
	47.9	11.8	.830	.178	2.08	99.61	1.04
Intercept method	47.9	11.8				114.94	1.15

* Site of initial injection.

† Turnover rate fraction per minute.

‡ S_1, S_2, S_3 are sizes of compartments 1, 2 and 3 respectively.

§ V_1 is the plasma equivalent space of distribution of compartment 1 in liters.

Bicarbonate system. A study was made of the bicarbonate system by injecting C^{14} -labeled bicarbonate into the plasma and measuring its SA as a function of time (Figure 3).

It is suggested in the literature (28, 29) that when

CO_2 is liberated from glucose intracellularly, the CO_2 diffuses into plasma to be converted to bicarbonate in the red cells, and then equilibrates with plasma and other bicarbonate spaces. It has also been shown (30, 31) that the SA of expired CO_2 equals that of plasma bicarbonate.

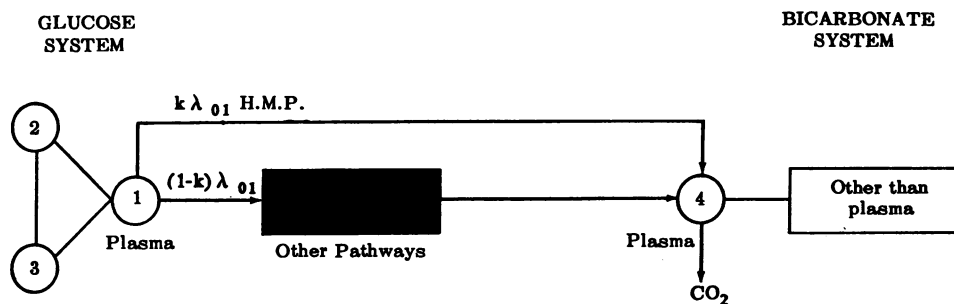


FIG. 6. THE MODEL USED FOR THE ANALYSIS OF THE METABOLISM OF GLUCOSE-1-C¹⁴ AND GLUCOSE-6-C¹⁴. The rate of loss of glucose from the glucose system is λ_{01} ; k is the fraction of glucose leaving the system whose C-1 is oxidized directly to CO₂; $(1-k)$ is the fraction of glucose metabolized by all other pathways and whose carbon may eventually be metabolized to CO₂. These other pathways are depicted by a "black box" which represents indeterminate transition states and intermediates as well as losses from the system.

It is therefore assumed that both the sites of entry and of loss of bicarbonate are in the plasma. This assumption makes the determination of the number of compartments in the bicarbonate system, their manner of interconnection, and relative sizes unnecessary for the calculation of the glucose shunt. If $B(t)$ is the response to a unit injection at time $t=0$, the response to an injection at time θ is $B(t-\theta)$. Any input rate function $F(t)$ may be considered as made up of a series of infinitesimal injections of magnitude $F(\theta) d\theta$. The response at any time t to one such injection at time θ is $B(t-\theta)F(\theta) d\theta$. The total response $G(t)$ to an input rate $F(t)$ may be expressed as the summation of the responses due to all the preceding injections, and, in the limit may be written as

$$G(t) = \int_0^t F(\theta)B(t-\theta) d\theta \quad [9]$$

Equation 9 assumes that no material is present in the system initially.

Determination of the direct oxidative pathway for glucose. Because of the difference in the C¹⁴O₂ patterns of C-1- and C-6-labeled glucose, it is assumed that in addition to their common pathways of metabolism (Embden-Meyerhof as well as other pathways) another pathway exists that differentiates between C-1 and C-6. Present information about glucose metabolism suggests that this alternate pathway is the so-called hexose monophosphate pathway. Figure 6 shows a model in which a fraction $(1-k)$ of the glucose C-1 is metabolized by way of the Embden-Meyerhof as well as nontriose pathways represented by a "black box,"⁶ and fraction k by way of an alternate path. Both fractions eventually release C-1 into the plasma bicarbonate from which the sampled CO₂ is derived. All of the C-6 of labeled glucose goes by way of the so-called black box representing both oxidative and synthetic pathways and has a fate identical with fraction $1-k$ of C-1 of glucose. Actually, a fraction of the C-6-labeled glucose must go by way of the alternate path-

⁶ *Black box* is a term used to represent transitions, intermediates, and losses which are undetermined.

way also. However, no labeled CO₂ is released, since only the C-1 carbon is liberated; the C-6 carbon stays with the remaining pentose. It is assumed here that the pentose returns rapidly to be metabolized.

The analysis that follows is an attempt to estimate k , the fraction of glucose that is metabolized via the pathway directly oxidizing the first carbon. Let $C_1(t)$ be the experimentally determined SA of plasma bicarbonate as a function of time from a single injection of unit amount of C-1-labeled glucose, and let $G(t)$ be the part contributed by the shunt pathway alone. Let $C_6(t)$ be the SA of plasma bicarbonate due to a single injection of unit quantity of C-6-labeled glucose. It follows from the model in Figure 6 that

$$C_1(t) = (1-k)C_6(t) + G(t) \quad [10]$$

$G(t)$ may be calculated by using Equation 9. $F(\theta)$, the rate of bicarbonate entry by way of the direct oxidative pathway or shunt at time θ is (Figure 6):

$$F(\theta) = k\lambda_{01}q_1(\theta)$$

where $q_1(\theta)$ is the amount of labeled glucose in the plasma at time θ . Substituting this in Equation 9, we get

$$G(t) = \int_0^t [k\lambda_{01}q_1(\theta)][B(t-\theta)] d\theta \quad [11]$$

TABLE VI
Value for hexose monophosphate pathway in man

Subject	k^*	SD of k
C.B.	0.090	±0.01
C.K.	0.070	±0.01
R.S.	0.081	±0.01
A.H.	0.120	±0.01
Mean	0.090	
Fraction of glucose-1-C ¹⁴ oxidation accounted for by blood cells	0.008	
Net shunt	0.082	

* Fraction of glucose metabolized by way of HMP.

TABLE VII

Estimation of the extent of glucose metabolism by the hexose monophosphate pathway

Tissue	Per cent shunt	Reference
Rat liver slice	65-72	(41, 43)
Rat liver slice	6-39	(44)
Rat liver <i>in vivo</i>	29-38	(45)
Rat liver perfused	56	(46)
Rat lactating mammary gland slice	40-60	(42)
Rat postlactation mammary gland slice	0	(42)
Rat diaphragm	0	(41)
Intact rat	0-9	(38)
Intact rat	0	(47)
Intact rat	15	(48)
Human red cells	11	(49)
Human leukocytes	<10	(50)

For $q_1(\theta) = \sum_i A_i e^{-\alpha_i \theta}$

and

$$B(t - \theta) = \sum_j B_j e^{-\beta_j (t - \theta)}$$

Equation 11 may be integrated⁷

$$G(t) = k\lambda_{01} \sum_j B_j e^{-\beta_j t} \sum_i \frac{A_i}{\alpha_i - \beta_j} [1 - e^{-(\alpha_i - \beta_j)t}] \quad [12]$$

Setting

$$g(t) = \frac{G(t)}{k} \quad [12a]$$

and substituting into Equation 10, we get

$$[g(t) - C_0(t)]k = [C_1(t) - C_0(t)] \quad [13]$$

in which k is the only unknown.

Equation 13 holds for each observation at times t_i ($i = 1, 2, \dots, m$). A least squares solution (32) for k to include all m data points, yields

$$k = \frac{\sum_{i=1}^m [g(t_i) - C_0(t_i)][C_1(t_i) - C_0(t_i)][\omega_i]}{\sum_{i=1}^m [g(t_i) - C_0(t_i)]^2 [\omega_i]} \quad [14]$$

The statistical weight for the datum at time t_i is ω_i .

⁷ This follows from repeated applications of the relation that

$$\int_0^t A_i e^{\alpha_i \theta} d\theta = \frac{A_i}{\alpha_i} [1 - e^{\alpha_i t}]$$

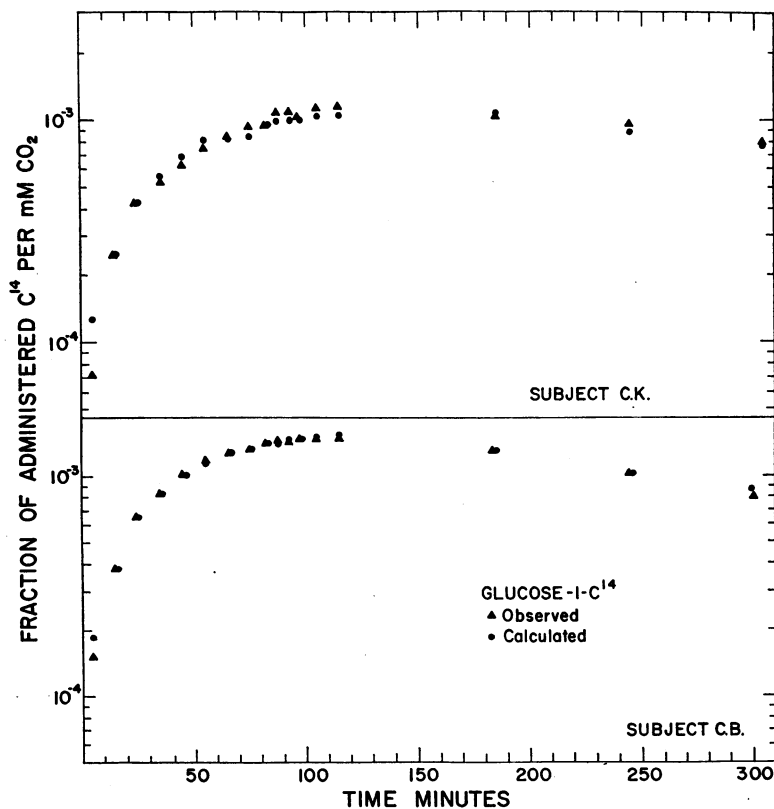


FIG. 7. A COMPARISON OF THE OBSERVED SPECIFIC ACTIVITY OF EXPIRED CO_2 AFTER GLUCOSE-1- C^{14} ADMINISTRATION WITH THAT CALCULATED IN THE DETERMINATION OF k , THE FRACTION OF GLUCOSE METABOLIZED VIA THE HEXOSE MONOPHOSPHATE PATHWAY.

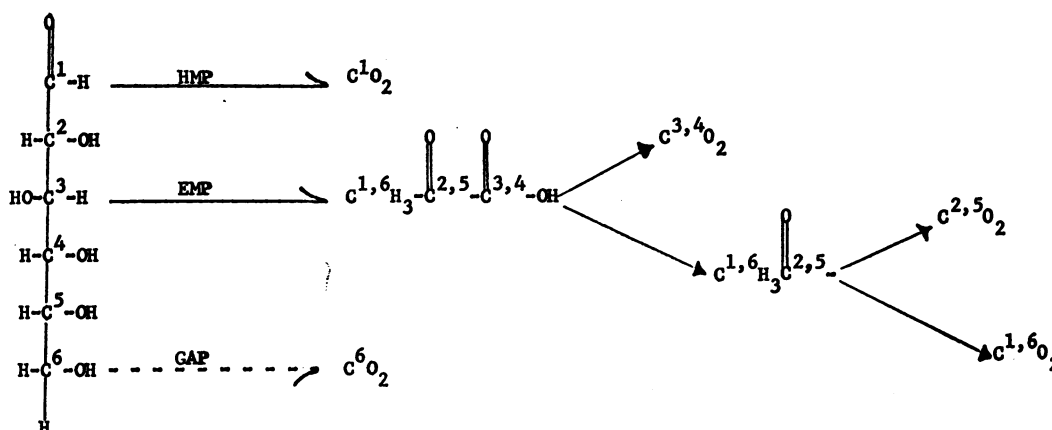


FIG. 8. SCHEMATIC DIAGRAM SHOWING THE CONVERSION OF INDIVIDUAL CARBON ATOMS OF GLUCOSE TO CO₂ BY THE CURRENTLY KNOWN PATHWAYS OF GLUCOSE METABOLISM IN MAMMALIAN TISSUE. Pathways indicated are: hexose monophosphate, HMP; Embden-Meyerhof, EMP; glucuronic acid, GAP; and tricarboxylic acid, TCA. The GAP arrow is broken to indicate a complex series of reactions compared with HMP pathway.

Using Equations 12 and 12a, $g(t)$ was calculated. $C_1(t)$ and $C_6(t)$ are the experimental values of C¹⁴O₂ derived from C-1- and C-6-labeled glucose, respectively. In this solution it is assumed that $g(t)$ and $C_6(t)$ are known exactly and that only $C_1(t)$ contributes to the variations.

The values of k and their standard errors were calculated for each study using Equation 14.⁸ These are given in Table VI. Figure 7 shows comparison of the observed values of the specific activity of C¹⁴O₂ after administration of glucose-1-C¹⁴ to those calculated from the value of C¹⁴O₂ specific activity after glucose-6-C¹⁴ injection.

The value of k as calculated above represents the fraction of glucose metabolized by direct oxidation of C-1 to CO₂ and includes the metabolism due to the blood cells. The mean value of k for the four studies shown in Table VI is 0.09. The fraction due to blood cells is 0.008 from previous calculations. Hence, the fraction due to this pathway other than blood cells is 0.082. It should be pointed out here that the fraction $1-k$ represents all other pathways for glucose metabolism, including the Embden-Meyerhof and synthetic routes.

DISCUSSION

Current concepts of glucose metabolism have recently been reviewed by Horecker and Hiatt (34). Two principal pathways of glucose catabolism are now known in mammalian tissue, the

Embden-Meyerhof (EM) and the hexose monophosphate (HMP). A third, the glucuronic acid pathway (GA) may be involved in glucose oxidation (35). Since other pathways of glucose catabolism exist in bacterial systems (36), it is possible that as yet undescribed pathways may exist in mammalian tissue. In addition, besides catabolism, glucose may enter into many synthetic reactions.

Based on the present state of knowledge, the metabolic fate of the individual carbon atoms of glucose appearing as CO₂ is depicted schematically in Figure 8. In the HMP pathway, described in detail by Horecker and Mehler (37), C-1 of C¹⁴-glucose gives rise to C¹⁴O₂ directly in two enzymatic steps. The remaining five carbon atoms may recycle to produce hexose such that the original C-2 of glucose appears in the 1 and 3 positions. Operation of the EM pathway results in conversion of one glucose to two pyruvate molecules from which acetate enters the tricarboxylic acid (TCA) cycle. In the GA pathway the sixth carbon of glucose may be released as CO₂ prior to oxidation of the other carbons but only after glucose is converted to uridine diphosphoglucose and subsequently to glucuronic acid. The GA pathway may be one not primarily involved in glucose oxidation to CO₂. It should be pointed out that not all of the glucose carbons in the EM path enter the TCA cycle nor does that carbon entering the cycle necessarily exit as CO₂.

⁸ A general computer program using an IBM 704 and a special program using an IBM 650 were used for the calculation involved in Equations 12 and 14, respectively. The features of the general computer program will be described separately (33).

Figure 8 shows that in the pyruvate resulting from operation of the EM pathway the original 1 and 6 carbons of glucose appear as the methyl group, the 2 and 5 carbons as the carbonyl, and the 3 and 4 carbons as the carboxyl group. Pyruvate first undergoes decarboxylation whereby the third and fourth carbons of glucose are liberated as CO_2 . Thus it would be expected that by this pathway glucose labeled with C^{14} in C-3 or C-4 would give rise to C^{14}O_2 earlier than glucose labeled in other carbons. Acetate, the remaining C-2 fragment, now enters the tricarboxylic acid cycle. If one follows the reactions of the TCA cycle it is evident that, after the entrance of acetate into the scheme, the second turn of the cycle will release the acetate carboxyl group as CO_2 (representing glucose C-2 and C-5) while a third turn of the cycle is required to oxidize the methyl carbon (glucose C-1 and C-6). The lag time observed here for C^{14}O_2 production from glucose-6- C^{14} may be due to the time required to label all of these intermediary compounds.

Because the carbons of glucose have relatively different metabolic fates and give rise to differing C^{14}O_2 excretion patterns, it may be possible to obtain more information concerning glucose metabolism in various pathological states by employing singly labeled glucose instead of uniformly labeled glucose, which is usually employed. It is possible that in some conditions the activity of the HMP pathway may be altered. Under such circumstances one would encounter C^{14}O_2 excretion patterns differing from normal when studied with singly labeled glucose even though the excretion pattern might not be significantly different from normal when studied with glucose-U- C^{14} . Using singly labeled glucose we have observed altered C^{14}O_2 excretion patterns in hyperthyroid patients. These data will be the subject of a future communication.

The present data demonstrate that the rate and extent of oxidation of various carbon atoms of glucose to CO_2 vary in man. Of the labeled carbons studied, the C^{14} of C-6 gives the least labeling of CO_2 . Glucose C-1 is oxidized to CO_2 more readily than is C-6. Since C-1 and C-6 are equivalent in the EM and other pathways, the greater C-1 oxidation may be explained by the operation of the HMP pathway. The oxidation

of glucose uniformly labeled gives rise to the greatest amount of C^{14} in CO_2 . This may be explained on the basis of rapid labeling of CO_2 from the 3 and 4 carbons via pyruvate decarboxylation plus the rapid oxidation of C-2 to CO_2 . Glucose-2- C^{14} oxidation to CO_2 was equivalent to or greater than glucose-1 and much greater than glucose-6- C^{14} .

The latter result requires some interpretation. It is possible that the fraction of glucose-2- C^{14} oxidized via the HMP pathway may, on rapid recycling, give rise to glucose-1,3- C^{14} (37) which, when oxidized, gives rise to C^{14}O_2 faster than glucose-6- C^{14} . However, this does not explain the equivalent or greater C^{14}O_2 production from glucose-2- C^{14} than from glucose-1- C^{14} . An explanation may be in the differential oxidation of the methyl and carboxyl carbon of acetate in the TCA cycle. Experimental observations of Bloom, Stetten and Stetten (38) suggest that our findings may reflect events in the TCA cycle. These investigators studied the oxidation of lactate labeled in single carbon atoms by the rat and observed about 50 per cent more C^{14}O_2 from lactate-2- C^{14} , compared with lactate-3- C^{14} .

Various studies have been performed in an attempt to ascertain the extent of glucose oxidation via the hexose monophosphate pathway in animal tissues. These are summarized in Table VII. A critique of the methods used in these experiments may be found in papers by Korkes (39), Wood (40) and Katz and Wood (20). Many of these values are based on interpretation of ratios of the C^{14} yield in CO_2 from glucose-1- C^{14} and glucose-6- C^{14} obtained at a given time-interval from the start of the experiment. Our data reveal that these ratios change with time and that one cannot estimate the extent of the shunt pathway by this method. This prompted us to apply a kinetic type of analysis to the data. The results suggest that about 8 per cent of glucose being metabolized in man occurs via the hexose monophosphate oxidative pathway. This amount does not differ markedly from some of the estimates in Table VII. It must be kept in mind that our results are for the whole body. No implication is made that this is the extent of the shunt in all the tissues. It is possible that some tissues have a much larger glucose catabolism via the

shunt and that this is counterbalanced by others like muscle which have little or no shunt pathway (41).

Some investigators (42) believe that the ratio of C¹⁴ in lipids after glucose-1-C¹⁴ and glucose-6-C¹⁴ injection is a reliable estimation of the HMP path. The ratio of about 0.6 obtained here would be interpreted as indicating that 40 per cent of glucose is metabolized via the shunt. Katz and Wood (20) have performed a very extensive analysis of the calculation of the shunt and show on very good grounds that the ratio derived from labeling of lipid after administration of specifically labeled sugars is not a true estimate of the shunt. They show on theoretical grounds that a 10 per cent shunt could give a ratio of approximately 0.7.

The results of the present study suggest that 8 per cent ($k \times 100$) of the initial glucose entering metabolic pathways is handled by the oxidative pathway. To our knowledge this is the first attempt, by a kinetic analysis, to calculate the hexose monophosphate pathway in the intact subject. Katz and Wood (20) have recently published an excellent analysis of the estimation of this pathway in tissue slices where a steady state of glucose metabolism has been achieved. This type of analysis cannot be employed in the single injection type of experiment. The value k derived here corresponds to the pentose cycle term used by Katz and Wood.

It should be noted that these experiments have been made in the fasting state, and it cannot be inferred that the estimated k value is of the same magnitude during periods of hyperglycemia or of hyperinsulinism. As a matter of fact, in fat tissue high levels of glucose and insulin appear to accentuate the hexose monophosphate pathway (51, 52).

Several aspects of the approach to the data analysis may be worth mentioning. Originally, only expired C¹⁴O₂ and labeled plasma glucose data were collected after C¹⁴-glucose injection, and the model proposed for the HMP pathway was a single glucose pool feeding into a single bicarbonate pool. A very good fit of the calculated and experimental C¹⁴O₂ data, similar to that shown in Figure 7, was obtained for this model. The uncertainty in the model parameter values (turnover rates, pool size, and so forth) was so large

that no estimate of k could be made. This indicated the need for more information on the bicarbonate system, which resulted in a separate bicarbonate study and a two-compartment representation for it. Efforts to fit the data with the more complex model resulted in inconsistencies in the fit. To eliminate this, a modification of the model was introduced for which more detailed knowledge of the early glucose curve was necessary. This resulted in a separate experiment. Thus, non-uniqueness of the solutions on the one hand and inconsistencies in the fit of the data on the other were the guiding criteria in evolving the model proposed.

SUMMARY

Various C¹⁴-labeled glucoses have been administered intravenously to normal human subjects, and the patterns of C¹⁴O₂ production, C¹⁴ disappearance and randomization of label in blood glucose, and incorporation of radioactivity into serum lipids were determined. The C¹⁴O₂ excretion curves obtained after injection of glucose-1-C¹⁴, glucose-2-C¹⁴, glucose-6-C¹⁴, and glucose-U-C¹⁴ differed considerably. Little randomization of C¹⁴ in blood glucose was observed. Greater incorporation of C¹⁴ into serum lipids occurred from glucose-6-C¹⁴ than from glucose-1-C¹⁴. The difference in C¹⁴O₂ patterns and lipid incorporation data in studies with 1- and 6-labeled glucose suggests the operation of the hexose monophosphate pathway of glucose metabolism in man. On the basis of the observed C¹⁴O₂ excretion data and a kinetic analysis of an assumed model system of glucose metabolism, it has been calculated that about 8 per cent of glucose is metabolized by the hexose monophosphate pathway in man.

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