

# C<sup>14</sup> STUDIES IN CARBOHYDRATE METABOLISM

## I. THE OXIDATION OF GLUCOSE IN NORMAL HUMAN SUBJECTS

By NOME BAKER, WALTON W. SHREEVE, REGINALD A. SHIPLEY,  
GENEVIEVE E. INCEFY, AND MAX MILLER

(From the Radioisotope Unit, Veterans Administration Hospital, and the Departments  
of Biochemistry and Medicine, School of Medicine, Western Reserve University,  
and Lakeside Hospital, Cleveland, Ohio)

(Received for publication, March 22, 1954)

After a single injection of uniformly labeled glucose-C<sup>14</sup> into rats (1) and dogs (2), the specific activity of plasma glucose, highest immediately after the injection, declines exponentially. The decline is due to a progressive replacement of the tagged compound with relatively non-labeled glucose. The slope or rate constant of the decay curve denotes the rate of turnover of the glucose pool expressed as the fraction of its total glucose content replaced per unit of time. The curve of specific activity for expired CO<sub>2</sub> at first rises gradually, and then, after reaching a peak at approximately 1 hour, declines with a slope somewhat less than that of glucose. As pointed out by Stetten *et al.* (3), this curve is incompatible with the assumption of a one pool model which was made by earlier workers (1) who studied glucose oxidation rates. However, the contour of the CO<sub>2</sub> curve is compatible with that of a glucose end-product incorporating C<sup>14</sup> atoms which have traversed at least *two* sizable pools, each with a turnover time of appreciable magnitude. One of these pools is obviously glucose. Another one which is known to exist in the direct pathway of glucose degradation is the large reservoir of HCO<sub>3</sub>-CO<sub>2</sub>.

If it is assumed that the two pools mentioned above are the only sizable ones in the pathway of early glucose degradation, the rates of glucose oxidation may be estimated. The mathematical treatment of the kinetics of enrichment and dilution of tracer material passing through a two pool series is well known. An exponential formula, long in use to describe radioactive transformations (4), has been applied both in metabolic problems (5-13) and in studies of blood flow in which dye dilution was employed (14). For application of the formula in the present study the rate constants of turnover for the glucose and HCO<sub>3</sub>-CO<sub>2</sub> pools must be known. That of glucose may be approximated, while that of HCO<sub>3</sub>-CO<sub>2</sub> may be either independently measured or calculated. It is important to note that the classic formula which describes the concentration of test material in the second pool is ordinarily applied on the assumption that enrichment and dilution of this pool are accomplished without entrance of diluting

material except from the first pool. In the body such is not the case. The  $\text{HCO}_3\text{-CO}_2$  pool also may be diluted by  $\text{CO}_2$  derived from essentially unlabeled stores of protein and fat and from metabolites of glycogen not diverted to the glucose pool. The time-concentration curve of  $\text{C}^{14}$  in  $\text{CO}_2$  would thereby be symmetrically depressed below the curve expected if 100 per cent of the  $\text{CO}_2$  were derived from glucose.

In the present study the per cent  $\text{CO}_2$  derived from glucose is estimated by comparison of the height of the observed  $\text{CO}_2$  curve with that of a theoretical curve if glucose were the sole source of  $\text{CO}_2$ . Approximations have also been made of the rate of glucose combustion, the size of the glucose pool, and its volume of distribution in normal humans. In addition, specific activity-time curves of excreted  $\text{C}^{14}\text{O}_2$  following the intravenous injection into humans of fructose- $\text{C}^{14}$ , acetate-1- $\text{C}^{14}$ , and bicarbonate- $\text{C}^{14}$  are presented as evidence in support of theoretical considerations. Finally, the data of Feller *et al.* (2) have been recalculated by this method. The rates of glucose oxidation in dogs were found to compare more favorably with the results obtained by a different technique; namely, that of constant infusion of glucose- $\text{C}^{14}$  (15).

#### EXPERIMENTAL

Glucose- $\text{C}^{14}$  (approximately 1 mmole, specific activity, 90  $\mu\text{c.}$  per mmole) in a volume of 9.0 to 10 cc. of isotonic saline was injected intravenously into each of four subjects considered normal with respect to carbohydrate metabolism (Table I). Patients, at rest throughout the experimental period, were injected with tracer  $\text{C}^{14}$  in the morning; blood and breath samples were collected for a period of 6 to 8 hours thereafter.

At least four successive venous blood samples were drawn from 1 to 8 hours after the injection of the labeled glucose. Plasma was separated from blood cells, deproteinized according to Somogyi (16), and concentrated under vacuum to a volume of 2 cc. prior to the addition of phenylhydrazine hydrochloride and Na acetate (9.6 mg. and 8.2 mg., respectively, per mg. of glucose). Phenylglucosazones were then prepared at pH 5, washed with water and ether several times, redissolved, filtered, recrystallized from 30 per cent ethanol, isolated, suspended in 30 per cent ethanol in  $\text{H}_2\text{O}$ , and plated directly on tared, flat, aluminum disks for the determination of specific activity. The osazones obtained in this manner had melting points agreeing closely with those of authentic phenylglucosazone samples prepared from D-glucose. Further purification failed to alter either the melting point or the color of the crystals. Passage of the Somogyi filtrate over Duolite C-3 and A-4 cation and anion exchange resins resulted in osazones having specific activities practically identical with those found when this procedure was omitted. Approximately 2 mg. of osazone

were plated on the disks; weighings were accurate to  $\pm 0.05$  mg. An empirically determined correction factor was used to convert the counts per minute of osazone- $C^{14}$  on aluminum to counts per minute of an infinitely thin layer of  $BaC^{14}O_3$  on filter paper. Samples were counted under an ordinary shielded, thin, end window Geiger tube, having an over-all efficiency estimated at 8 per cent.

Breath samples, for assay of  $C^{14}O_2$ , were collected for exactly 1 minute in an apparatus such as that illustrated in Fig. 1. It consisted simply of an aviator's oxygen mask, equipped with inlet and outlet valves, connected

TABLE I  
*Information on Subjects Used in Glucose- $C^{14}$  Experiments*

Subject	Initial blood glucose	Age	Sex	Height	Weight	Diagnosis
	<i>mg. per cent</i>	<i>yrs.</i>		<i>cm.</i>	<i>kg.</i>	
N1	87	24	M.	175	72	Remote hemiplegia
N2	89	45	F.	157	65	Carcinoma of breast with metastases
N3	92	37	M.	179	90	Conversion reaction, chronic
N4	92	76	F.	162	65	Hiatus hernia

The subjects were fasted for 16 hours prior to the start of and during the experiment with the exception of Subject N1, who ate a meal approximately 4 hours after the experiment was begun. Water was allowed *ad libitum*. The preexperimental diet (about 3000 calories) consisted of approximately 59 per cent carbohydrate, 23 per cent fat, and 18 per cent protein. (Normal Subjects N5 to N9 and diabetic Subjects D1 and D2, injected with either  $C^{14}$ -labeled bicarbonate, acetate, or fructose, were fasted for 15 to 24 hours prior to the experiment. Subject D7 (Fig. 6) was in the fed state in the two experiments which he underwent.) Blood sugar was determined on whole blood according to Somogyi (16) at the time of  $C^{14}$  administration. Glucose tolerance tests, performed 1 to 3 days before the experiment, were normal.

to an evacuated rubber beach ball by means of an intermediate glass T-joint.

The breath samples were then drawn by vacuum through 40 cc. of 1 N  $CO_2$ -free NaOH in an absorption column fitted with a fritted glass gas disperser. The absorption efficiency was approximately 95 per cent. Aliquots were taken to determine the specific activity (per cent of the injected  $C^{14}$  per mg. of carbon) and the total amount of  $CO_2$  excreted in 1 minute. The latter was done by adding an excess of  $BaCl_2$  to the NaOH- $Na_2CO_3$  aliquot and titrating the resulting  $BaCO_3$  with standard HCl between the phenolphthalein and brom cresol green end-points.

Specific activity was determined by converting  $Na_2CO_3$  to  $BaCO_3$  with  $BaCl_2$ , filtering the precipitate on tared filter paper, weighing, and then

counting the radioactivity. The activity was corrected for mass absorption to that of an infinitely thin layer of  $\text{BaCO}_3$ .

Total  $\text{C}^{14}$  activity of the injected glucose, fructose, acetate, and bicarbonate was determined to an accuracy of  $\pm 5$  per cent by oxidation (generation with acid in the case of bicarbonate) to  $\text{CO}_2$  (17, 18) and subsequent assay of the radioactivity as described above for the  $\text{CO}_2$  breath samples.

The design of experiments in which evenly labeled fructose- $\text{C}^{14}$  (approximately 1 mmole, 90  $\mu\text{c}$ . per mmole), Na acetate-1- $\text{C}^{14}$  (approximately 1 mmole, 1 to  $2 \times 10^2$   $\mu\text{c}$ . per mmole), and  $\text{NaHC}^{14}\text{O}_3$  (approximately 0.02

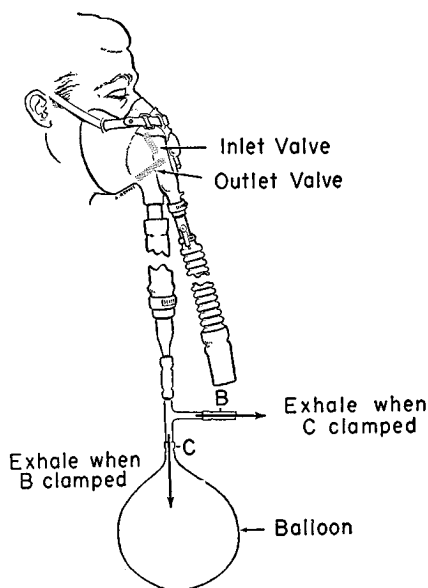


FIG. 1. Apparatus used to collect breath samples

mmole,  $1.5 \times 10^3$   $\mu\text{c}$ . per mmole) were employed was essentially identical with that of experiments with glucose- $\text{C}^{14}$  except that analyses were performed only on  $\text{CO}_2$ .

Uniformly labeled glucose- $\text{C}^{14}$  and fructose- $\text{C}^{14}$  were purchased from the Nuclear Instrument and Chemical Corporation, Chicago.  $\text{BaC}^{14}\text{O}_3$ , obtained from Oak Ridge, was used to synthesize acetate-1- $\text{C}^{14}$  according to Calvin *et al.* (19). Tracer  $\text{NaHC}^{14}\text{O}_3$  was prepared in a Baruch flask (18) from  $\text{BaC}^{14}\text{O}_3$  by generation of  $\text{CO}_2$  with acid, followed by diffusion of the gas into  $\text{NaOH}$ . The resulting  $\text{NaHCO}_3$ - $\text{NaOH}$  solution was transferred into a volumetric flask and partially titrated with dilute standard  $\text{HCl}$ , so that an excess of only 0.8  $\mu\text{mole}$  of  $\text{NaOH}$  per cc. remained. The molarity of the resulting solution was equivalent to that of isotonic saline

(0.15 M NaCl). Radioactive glucose, fructose, and acetate were dissolved in isotonic saline. All compounds were autoclaved in sealed containers before use.

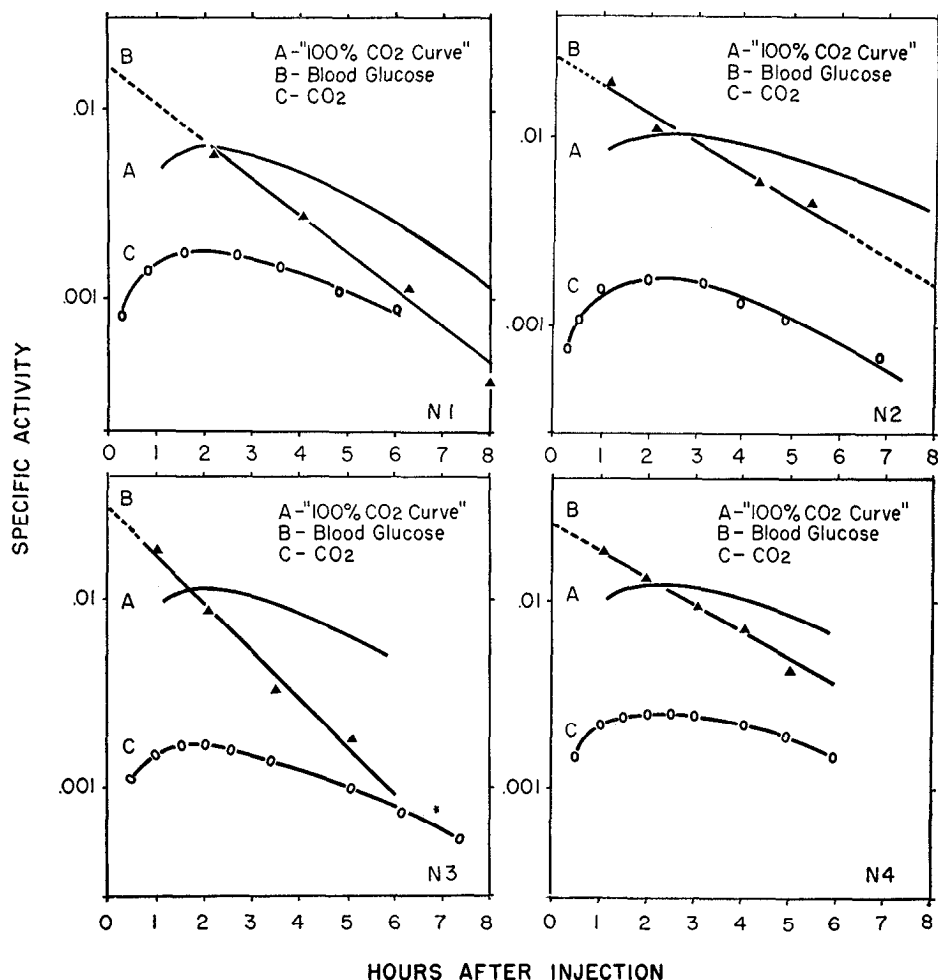


FIG. 2. Specific activity-time curves of blood glucose and respiratory CO<sub>2</sub> for four normal subjects plotted semilogarithmically; specific activity is expressed as the per cent of injected activity per mg. of either glucose or CO<sub>2</sub> carbon.

## RESULTS AND DISCUSSION

### *Rate of Glucose Oxidation*

The specific activity-time curves of glucose and CO<sub>2</sub> following the injection of a tracer dose of glucose-C<sup>14</sup> into four normal subjects are plotted

semilogarithmically in Fig. 2. Additional experimental data may be found in Table II. In each section of Fig. 2, Curve B represents the exponential decline in specific activity of plasma glucose, while Curve C depicts the rise and fall of the specific activity of expired CO<sub>2</sub>. Curve A, which is mathematically derived, represents the theoretical specific activity of CO<sub>2</sub> had the latter arisen exclusively from the oxidation of glucose via immediate oxidative pathways (see below). It will be termed the "100 per cent CO<sub>2</sub>" curve, and its method of derivation will be described in the following section. It should be pointed out again that the 100 per cent CO<sub>2</sub> curve is intended to serve as a standard of reference in such a manner that the proportion of CO<sub>2</sub> derived from glucose may be estimated at any time after 1 hour by a simple comparison of the CO<sub>2</sub> activities of the observed and derived curves at any given instant. Thus at time  $t$

$$(1) \quad \% \text{ CO}_2 \text{ derived from glucose} \\ = \frac{\text{specific activity CO}_2 \text{ carbon (observed)}}{\text{specific activity CO}_2 \text{ carbon (100\% curve)}} \times 100$$

*Derivation of 100 Per Cent CO<sub>2</sub> Curve*—It will be assumed for practical purposes (1) that following the intravenous injection of glucose-C<sup>14</sup> the tracer is rapidly mixed with a homogeneous<sup>1</sup> non-labeled glucose pool, and that the labeled molecules are then replaced by relatively non-radioactive glucose at a rate which maintains the total glucose concentration at a constant level, (2) that a portion of the glucose is converted to tissue CO<sub>2</sub> irreversibly by way of such small and rapidly replaced pools that virtually no time delay occurs en route (this process will be referred to as the "immediate oxidative pathway"), and (3) that the newly formed CO<sub>2</sub> mixes rapidly with the body bicarbonate pool and is produced at a rate which is constant and equal to that of its loss via the lungs.

A schematic representation of the metabolic system under consideration is portrayed in Model I, Fig. 3. The differential equation which would express the rate of change of activity in a CO<sub>2</sub> pool derived solely from glucose is as follows:

$$(2) \quad \frac{d\beta}{dt} = E \left( \frac{\alpha}{A} - \frac{\beta}{B} \right)$$

The symbols employed in this equation and referred to elsewhere in the text are explained in the legend of Fig. 3. In order to obtain an expression for the specific activity of the CO<sub>2</sub> carbon ( $\beta$ ) at any time ( $t$ ) in a theoretic

<sup>1</sup> While it is convenient, in analyzing data of this sort, to think of a sharply delineated and homogeneous pool, it is recognized that glucose, which is largely contained in interstitial fluid, is not necessarily of equal concentration in all tissues, nor are rates of removal necessarily identical in all organs.

cal system wherein all of the  $\text{CO}_2$  carbon is derived solely from glucose, equation (2) may be integrated, algebraic substitution made, and the following formula<sup>2</sup> obtained.

$$(3) \quad b = a_0 \frac{\lambda_2}{\lambda_2 - \lambda_1} (e^{-\lambda_1 t} - e^{-\lambda_2 t})$$

Similar equations have been used to study the rates of diverse metabolic processes, *e.g.* phospholipid turnover rate (5), the kinetics of xylose and galactose distribution and utilization (6, 7), and the rate of protein synthesis in humans (8). Many theoretical discussions regarding applications and limitations of such an approach to biological processes have also appeared (8-14). It is apparent that construction of the 100 per cent  $\text{CO}_2$  curve by means of expression (3) requires that the following values be known: (1) the specific activity of glucose carbon at zero time ( $a_0$ ); (2) the fractional rate of replacement of the glucose pool (decline in specific activity of glucose carbon as in Fig. 2, B) ( $\lambda_1$ ); and (3) the fraction of  $\text{CO}_2$  ( $\text{HCO}_3$ ) pool which turns over each minute ( $\lambda_2$ ). The latter was determined experimentally on two separate subjects as follows.

*Fractional Turnover Rate of Bicarbonate Pool* ( $\lambda_2$ )—15  $\mu\text{c.}$  of  $\text{NaHC}^{14}\text{O}_3$  (0.02 mmole) were injected intravenously and serial breath samples collected. The composite curve of specific activity of  $\text{CO}_2$  carbon in expired air is plotted in Fig. 4. The rapid decline in the early segment of the curve probably represents diffusion of the tracer from plasma and mixing with the bicarbonate of both interstitial and cellular fluids. The remaining segment, which approximates a straight line, is considered to represent replacement of the  $\text{C}^{14}$  with unlabeled carbon derived from oxidative processes. Rate constants for the terminal portion of the curves, after mixing was presumed to be "complete," were 0.0099 and 0.0094 per minute. Although it is assumed that the injected  $\text{C}^{14}\text{O}_2$  is removed from the bicarbonate pool primarily by way of the breath, there is no doubt that an additional undetermined portion may be diverted into various metabolic systems and subsequently reenter the pool. Such a phenomenon would attenuate the slope of the distal portion of the curve and lead to an underestimation of  $\lambda_2$ . Therefore, for the estimate to be precise, such recycling must be minimal.

A mathematical estimate of  $\lambda_2$  also may be obtained indirectly. By solving expression (3) for  $db/dt = 0$ , the following equation relating  $\lambda_1$  and  $\lambda_2$  at  $t_{\text{max.}}$  is obtained.

$$(4) \quad \lambda_1 e^{-\lambda_1 t_{\text{max.}}} = \lambda_2 e^{-\lambda_2 t_{\text{max.}}}$$

<sup>2</sup> The use of expressions (3) and (5) is limited to instances in which  $\lambda_1$  does not equal  $\lambda_2$ . Furthermore, as the value of  $\lambda_1$  approaches  $\lambda_2$ , the possible error in the calculation is increased.

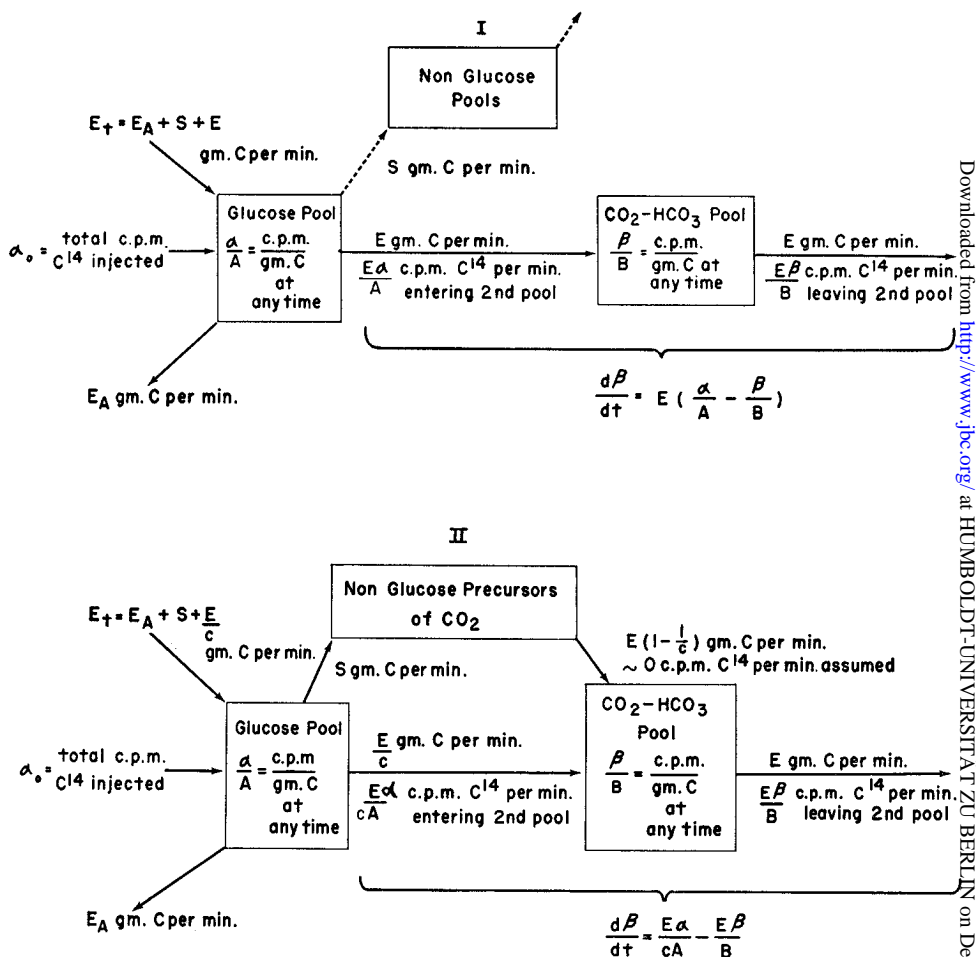


FIG. 3. Model (I) for the hypothetical 100 per cent  $\text{CO}_2$  curve in which  $\text{CO}_2$  is derived solely from glucose carbon. Assumed model (II) for the normal oxidation of glucose. Only a fraction  $(1/c)$  of the  $\text{CO}_2$  is derived from glucose carbon, while the remainder of the  $\text{CO}_2$   $(1 - 1/c)$  is derived from non-glucose precursors of  $\text{CO}_2$ . In Model I, at  $t = 0$ ,  $\alpha_0$  c.p.m. of glucose- $\text{C}^{14}$  are injected into the body glucose pool containing  $A$  gm. of glucose carbon. Three outlets from the glucose pool having rates  $E_A$ ,  $S$ , and  $E$  gm. of  $C$  per minute are shown; only the latter is explicitly defined.  $E$  gm. of  $C$  per minute is the rate at which glucose carbon enters the  $\text{CO}_2$  pool from the glucose pool by way of rapidly turning over intermediates (immediate oxidative pathway). Glucose carbon is replaced by non-labeled carbon at the rate  $E_t$  gm. of  $C$  per minute  $= E_A + S + E$ ; thus the glucose pool is maintained in a steady state. Therefore, the radioactivity of carbon in the glucose pool decays exponentially; at any time,  $t$ ,  $\alpha = \alpha_0 e^{-\lambda_1 t}$ , where  $\lambda_1 = E_t/A$ . The steady state will be maintained in the  $\text{CO}_2$  pool only if the rate at which the carbon leaves is equal to the rate at which it enters the  $\text{CO}_2$  pool. The activity at any time in the  $\text{CO}_2$  pool is determined by the



Since  $t_{\max.}$  is assumed to be similar for the observed and 100 per cent  $\text{CO}_2$  curves and  $\lambda_1$  is already known,  $\lambda_2$  may be calculated. When this was done in the four subjects (Table II), the derived value for  $\lambda_2$  varied from 0.70 to 1.0 per cent per minute and averaged 0.86 per cent per minute.

From the data obtained after  $\text{C}^{14}\text{O}_2$  injection, the mean size of the body bicarbonate pool was calculated to be 12 mmoles of  $\text{CO}_2$  per kilo of body weight. From the relationship  $\lambda_2 = E/B$ , the mean size of the body bicarbonate pool was also determined in the subjects who received glucose (Table II). A mean value of 12 mmoles ( $5.4 \times 10^2$  mg.) of  $\text{CO}_2$  per kilo of body weight was obtained. These figures are similar to the value of 15 mmoles of  $\text{CO}_2$  per kilo of body weight reported by Harper *et al.* in dogs (20).

*Validity of Model As Formulated in Expression (3)*—Use of the simple formula for a two pool system may be justified by several separate arguments and experimental observations. It has just been shown that  $\lambda_2$  as determined by indirect calculation is similar to that obtained experimentally on two separate subjects. This means that either of the values substituted in equation (4) will give a similar time for  $t_{\max.}$ . If an appreciable delay occurred in the intermediate pathways during the interval of experimental observation, the observed  $t_{\max.}$  would tend to lie farther to the right than that of  $t_{\max.}$  of the 100 per cent curve as calculated by equation (4), in which the experimentally determined  $\lambda_2$  was employed (Fig. 4). The effect of a delay within a hypothetical intermediate pool is illustrated in Fig. 5. The glucose decay curve is represented by Curve I which has a rate constant equal to 0.0070 per minute. Curve II is the calculated 100 per cent  $\text{CO}_2$  curve when  $\lambda_2$  equals 0.0090 per minute. Curve III is the 100 per cent  $\text{CO}_2$  curve which would be obtained if a third intermediate pool (not shown) with a rate constant of 0.010 per minute were interposed.

rate at which activity enters and leaves this pool; thus in Model I,  $d\beta/dt = E((\alpha/A) - (\beta/B))$ , whereas in Model II,  $d\beta/dt = (E\alpha/cA) - (E\beta/B)$ . Other definitions of symbols used in Fig. 3 and in the text are defined as follows:  $\alpha_0$ , dose in counts per minute of injected glucose- $\text{C}^{14}$ ;  $\alpha$ , counts per minute of  $\text{C}^{14}$  in glucose pool at any time;  $\beta$ , counts per minute of  $\text{C}^{14}$  in  $\text{CO}_2$  pool at any time;  $a_0$ , specific activity of glucose at 0 time ( $1/10A$ );  $a$ , specific activity of glucose at time  $t$  ( $\alpha/10\alpha_0A$ );  $b$ , specific activity of  $\text{CO}_2$  pool at time  $t$  ( $\beta/10\alpha_0B$ );  $A$ , gm. of glucose carbon in body pool;  $B$ , gm. of  $\text{CO}_2$  carbon in  $\text{CO}_2\text{-HCO}_3$  pool;  $E$ , gm. of  $\text{CO}_2$  carbon excreted per minute in breath;  $\lambda_1$ , fraction of glucose pool which turns over each minute ( $E_t/A$ );  $\lambda_2$ , fraction of  $\text{CO}_2\text{-HCO}_3$  pool which turns over each minute ( $E/B$ );  $t$ , time in minutes;  $1/c$ , fraction of  $\text{CO}_2$  carbon derived from glucose;  $t_{\max.}$ , time at which  $\text{CO}_2$  specific activity reaches a maximal value (minutes);  $t_{1/2}$ , half life of a given pool;  $E_A + S$ , gm. of glucose carbon removed from the glucose pool per minute by paths other than the immediate oxidative route;  $E_t$ , rate at which carbon enters and leaves the glucose pool in gm. per minute; specific activity, per cent of injected activity per mg. of either glucose or  $\text{CO}_2$  carbon.  $A$ , Model I, is not equal to  $A$ , Model II.

In such an instance the observed specific activity of  $\text{CO}_2$  after dilution by non-glucose sources of unlabeled  $\text{CO}_2$  ( $c = 5$ ) would approximate Curve IV. It will be observed that the  $t_{\max.}$  of Curves III and IV is shifted to the right and that the contour is altered from that of the two pool system.

Similar arguments may be applied to the question of reentry of labeled carbon from side pools. If a significant amount of  $\text{C}^{14}$  were diverted from the immediate oxidative paths of glucose into side channels, such as those

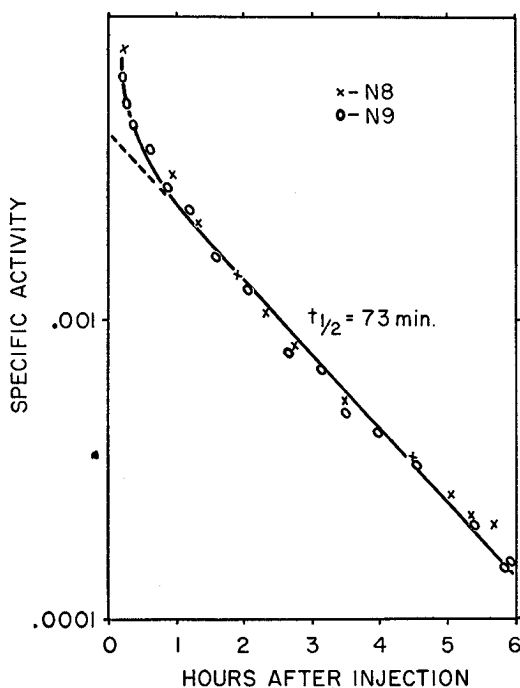


FIG. 4. Specific activity of respiratory  $\text{CO}_2$  following the injection of tracer amounts of bicarbonate- $\text{C}^{14}$  into two human subjects. Data plotted semilogarithmically. Units of specific activity as in Fig. 2  $\times 0.65$ .

in equilibrium with protein and fat, and subsequently reentered the direct pathway of carbohydrate oxidation, a contour distortion of the observed  $\text{CO}_2$  curve with respect to the reference curve would be anticipated. Recycling of  $\text{C}^{14}$  back into glucose also must be considered. Since a composite of the glucose curves in Fig. 2 approximates a straight line, one might conclude that such a phenomenon is insignificant (1). However, this argument cannot be pressed too strongly, for the data might also represent a relatively linear portion of a complex exponential function, in which case appreciable recycling need not be precluded. It should be emphasized

that diversion of  $C^{14}$  into side pools *without reentry* into the system glucose  $\rightarrow$   $CO_2$  would not affect the specific activity of the main stream of metabolites which feed into the  $CO_2$  pool, and hence expression (3) would still be valid.

Confirmatory evidence of absence of delay in intermediate pools was brought forth by the experiments in which either  $C^{14}$ -labeled acetate or fructose was injected and the activity of expired  $CO_2$  determined in the same manner as after an injection of glucose. After the administration of

TABLE II  
*Characteristics of Glucose and  $CO_2$  Pools*

These values were calculated from blood glucose and respiratory  $CO_2$  specific activity-time curves (Fig. 2). Determination of the  $CO_2$  pool size and turnover rate and of the glucose space required, in addition, measurements of the rate of  $CO_2$  excretion in the breath and of the blood glucose concentration.

Subject	Glucose						$CO_2$		
	Pool size, mg. glucose $\times 10^{-2}$ per kilo body weight	Volume of distribution, per cent body weight	Rate constant, per cent per min., $\lambda_1 \times 10^{2*}$	Glucose turnover rate, mg. glucose per hr. per kilo body weight ( $T$ )	Oxidation to $CO_2$ †		Pool size, mg. $CO_2 \times 10^{-2}$ per kilo body weight	Rate constant, per cent per min., $\lambda_2 \times 10^2$	Turnover rate, mg. $CO_2 \times 10^{-2}$ per hr. per kilo body weight
					Mg. glucose per hr. per kilo body weight ( $Q$ )	Per cent of glucose turnover diverted to $CO_2$			
N1	2.2	25	0.72	95	51	54	4.3	1.0	2.6
N2	1.5	17	0.58	52	39	75	8.1	0.70	3.4
N3	0.92	10	0.96	53	28	53	5.4	0.73	2.4
N4	1.5	17	0.53	48	34	71	3.7	1.0	2.2
Average.	1.5	17	0.69	62	38	63	5.4	0.86	2.7

\*  $\lambda_1 = 0.693/t_{1/2}$ .

† Via immediate oxidative pathway (see the text).

acetate (Fig. 6) a peak activity was attained within not more than 10 to 15 minutes. Such an early appearance of  $t_{max}$ , suggests a very rapid rate via the pathways between acetate and  $CO_2$ . A mathematical estimate of the rate constant which applies jointly to mixing, diffusion, and acetate removal may be made by application of expression (4). The value  $\lambda_1$  will now represent this unknown constant. When  $\lambda_2$  equals 0.0090 per minute and  $t_{max}$ , 10 or 15 minutes,  $\lambda_1$  may be calculated to equal 0.35 and 0.20 respectively. Such a rapid rate is within the probable range of the rate constant for diffusion and mixing alone and thus suggests that negligible delay is contributed by the metabolic transport of acetate carbon to the  $CO_2$  pool. It may be noted that approximately 15 minutes after the injection

of acetate- $C^{14}$ ,  $C^{14}O_2$  specific activity declines in a simple exponential manner and has a rate of decay similar to that obtained after the injection of bicarbonate- $C^{14}$ . Evidence for the rapid disposal of acetate has also been presented by Harper and coworkers (20) who injected acetate-1- $C^{14}$  into dogs and showed that practically all of it had disappeared from the blood by the time  $t_{max}$  of  $CO_2$  had been attained.

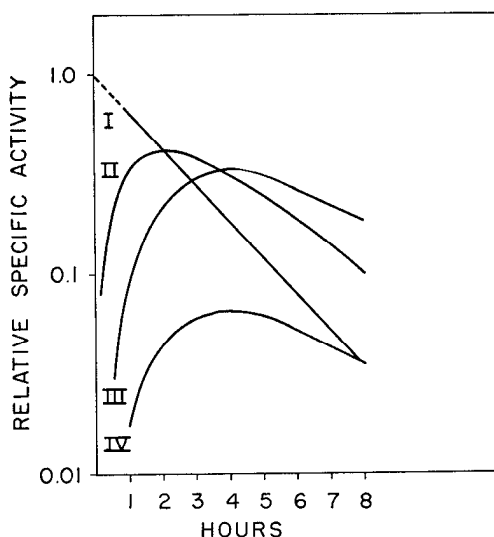


FIG. 5. The effect of an intermediate pool between glucose and  $CO_2$  on the characteristics of the  $CO_2$  specific activity-time curve. 100 per cent  $CO_2$  curves are drawn semilogarithmically. First pool,  $\lambda_1 = 0.0070$  per minute (Curve I); if there were a second pool having  $\lambda_2 = 0.0090$  per minute, Curve II would be obtained; if a third pool having  $\lambda = 0.010$  per minute were interspersed, Curve II would be shifted to Curve III. If only 20 per cent of the carbon of Curve III were derived from I, Curve IV would be obtained.

Curves of specific activity of expired carbon after the injection of fructose- $C^{14}$  into three subjects are reproduced in Fig. 7. From the two lower curves it is apparent that  $t_{max}$  was reached just as rapidly as after acetate injection. The top curve differs in that a maximum is observed at about 1 hour; yet the first sample collected at 10 minutes had already risen to 88 per cent of the activity reached at 1 hour. The variability in pattern of the three curves is probably due to a partial conversion of fructose to glucose. Thereafter both serve as precursors which contribute  $C^{14}$  to the  $CO_2$  pool. The early portions of the curve appear to be dominated by fructose degradation and the terminal portion by glucose. It has been shown by a determination of the  $C^{14}$  content of blood glucose after an injection of

fructose- $C^{14}$  that approximately half of the radioactivity appears promptly in glucose.<sup>3</sup>

*Estimate of Per Cent  $CO_2$  Derived from Glucose*—In Table III values are presented for the per cent  $CO_2$  derived from glucose as determined graphically by application of equation (1). The value for  $\lambda_2$  used in constructing the 100 per cent curve was derived from expression (4) in each instance. It was judged preferable to calculate this constant rather than to use the

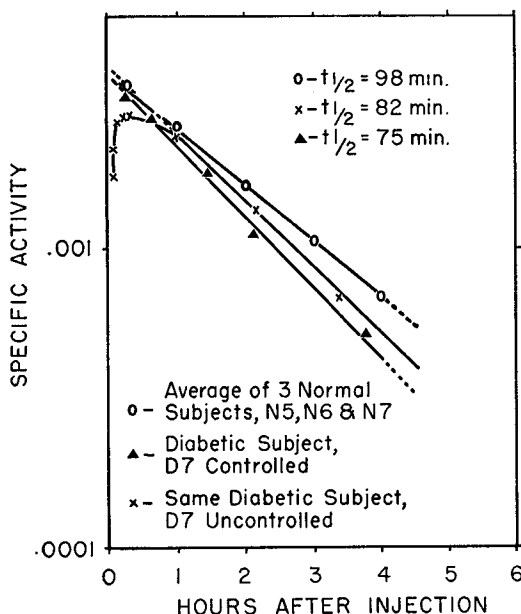


FIG. 6. Specific activity of respiratory  $CO_2$  following the injection of tracer amounts of acetate- $C^{14}$  into four human subjects. Data plotted semilogarithmically. Units of specific activity as in Fig. 2.

observed values obtained by injection of  $HC^{14}O_3$  into two other subjects. Justification for the computation has been discussed under the section devoted to the validity of the model used. It is apparent that little difference exists in the per cent  $CO_2$  derived from glucose whether an estimate is made at 1 hour or at the other points up to 6 hours. The grand mean for the four subjects was 21 per cent.

Because of the fact that the 100 per cent  $CO_2$  curve intersects, *i.e.* is equal to, the glucose curve at  $t_{max.}$ , it is permissible to compare the observed  $CO_2$  activity with that of glucose at a time equal to  $t_{max.}$ ;<sup>4</sup> however,

<sup>3</sup> Unpublished observations of Dr. Walton W. Shreeve.

<sup>4</sup> When  $\lambda_1$  and  $\lambda_2$  are similar or equal (see foot-note 2), the ratio at  $t_{max.}$ ,  $a/b \times$

beyond this point use of the glucose curve is not valid for such a comparison. It should be noted that Feller *et al.* (1, 2), who made a simple comparison of glucose and CO<sub>2</sub> activities to calculate the per cent CO<sub>2</sub> derived from glucose, chose this portion of the curve and hence obtained excessively high values.

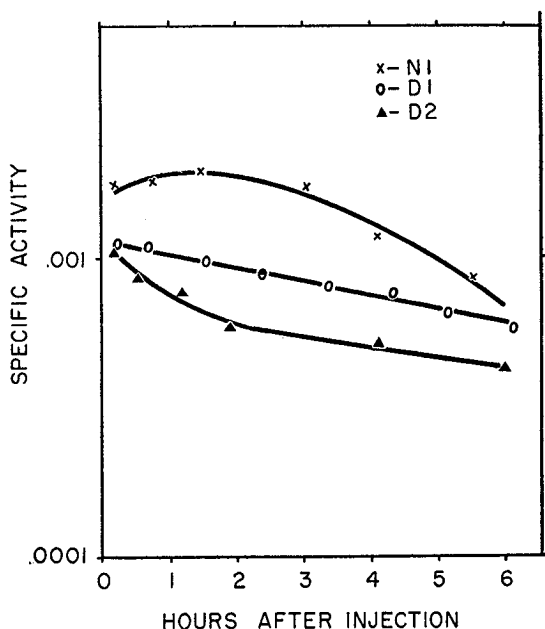


FIG. 7. Specific activity of respiratory CO<sub>2</sub> following the injection of tracer quantities of uniformly labeled fructose-C<sup>14</sup> into three human subjects. Data plotted semilogarithmically. Units of specific activity as in Fig. 2.

The fraction of CO<sub>2</sub> derived from glucose may be determined mathematically by combining equations (1) and (3). This dispenses with the graphical construction of curves. The formula is as follows:

$$(5) \quad \% \text{ CO}_2 \text{ from glucose} = \frac{100}{c} = \frac{100}{a_0} \frac{\lambda_2 - \lambda_1}{\lambda_2} \left( \frac{b}{e^{-\lambda_1 t} - e^{-\lambda_2 t}} \right)$$

A model in which only a fraction of the CO<sub>2</sub> (1/c) is derived from the oxidation of glucose is represented in Fig. 3, Model II. Equation (5) also is obtained by integration of the differential equation (expression (6)) which describes the rate of change of activity in the CO<sub>2</sub> pool of this model.

100, may still be calculated with an accuracy equal to that of the original observations.

$$(6) \quad \frac{d\beta}{dt} = \frac{E\alpha}{cA} - \frac{E\beta}{B}$$

*Reappraisal of Data of Feller et al.*—From the data of Feller *et al.* (2) a recalculation has been made of the per cent CO<sub>2</sub> derived from glucose in normal and diabetic dogs. If, by an optional method of computation described above, comparison of CO<sub>2</sub> activity with that of glucose is made at  $t_{\max.}$ , values of 28 and 16 per cent total CO<sub>2</sub> derived from glucose are obtained for normal and diabetic dogs respectively.

In calculating the per cent CO<sub>2</sub> carbon derived from glucose from the typical *constant infusion* experiments reported by Strisower and Searle (15),

TABLE III  
*Per Cent of Expired CO<sub>2</sub> Carbon Derived from Oxidation of Glucose\**

Subject	Ratio 1		Ratio 2		Ratio 3		Ratio 4		Mean (Ratios 1 to 4)
	<i>t</i>	Per cent	<i>t</i> †	per cent	<i>t</i>	Per cent	<i>t</i>	Per cent	Per cent
N1	60	26	120	25	240	30	360	34	29
N2	60	18	156	17	240	15	360	17	17
N3	60	16	120	17	240	15	360	17	16
N4	60	23	132	21	240	22	360	23	22
Average.....	60	21	132	20	240	21	360	23	21

\* As determined at various times by the ratio (observed CO<sub>2</sub> specific activity) × 100/(100 per cent CO<sub>2</sub> curve specific activity).

†  $t_{\max.}$

values of approximately 25 and 11 per cent for the same dog in the normal and diabetic states, respectively, were obtained. These figures agree quite favorably with those derived from the recalculated data of Feller *et al.*, suggesting that the two methods of study may yield comparable results.

*Theoretical Error Due to Difference in Specific Activity of Plasma and Interstitial Fluid*—It is pertinent to examine the common assumption that the specific activity of a compound in plasma is identical with that in extracellular fluid after diffusion and mixing between the two compartments are complete. Although Feller *et al.* (1) have shown in the rat that the specific activity of glucose in red cells reaches a level within 1 hour which approximates that of plasma, this does not necessarily mean that interstitial fluid in an intact subject would maintain an exact equality with plasma after this time. Since plasma undergoes dilution by endogenous, non-labeled glucose prior to the extrahepatic interstitial fluid, a slight gra-

dient between the two would be anticipated. Thus after a period of transient equilibrium the specific activity of interstitial fluid will lag in being diluted; *i.e.*, possess a higher activity than plasma. In the case of bicarbonate- $C^{14}$  formed intracellularly, the specific activity-time relationship between plasma and extraplasma spaces will be the reverse. The magnitude of the difference will depend upon the relative rates of capillary interchange and either plasma or extraplasma dilution. By using models such as those in Fig. 3 where plasma, interstitial, and cellular pools are treated as though they were a single, mixed pool, an error is introduced in the calculations of pool size and turnover rate constants. It is presumed that such errors are small. Studies designed to determine the magnitude of such errors are in progress.

#### *Glucose Space, Rate of Turnover, and Amount Oxidized*

When the decay curve for the specific activity of glucose is extrapolated to the ordinate, the value at zero time is an approximation of the degree of dilution of glucose- $C^{14}$  by that of the effective glucose pool. Thus the dose in total counts per minute divided by the counts per minute per gm. of glucose at zero time will approximate the size of the glucose pool in gm. The mean of the four subjects was 0.15 gm. of glucose per kilo (Table II). Division of the total weight of glucose by the blood glucose concentration yields a figure for the volume in which glucose is distributed. This averaged 0.17 liter per kilo or 17 per cent of body weight (Table II).

Dominguez *et al.* (21) have reported that after mannitol injection a simple extrapolation of this sort gives values for volume of distribution which are approximately 10 per cent too high. The rate constant for the disappearance of mannitol after mixing is similar to that for glucose- $C^{14}$ , and the rates of diffusion of the two compounds also are probably similar. The value herein obtained for glucose space therefore is not likely to be more than 10 per cent too high. Moreover it is of interest to note that the results compare favorably with the value of 16 per cent of body weight reported for inulin space in man (22-24). Wick *et al.* have shown in eviscerated-nephrectomized rabbits that glucose is distributed in a volume equal to that of thiocyanate space (25). Similar results have been obtained in normal dogs (26). Thus it would appear that the effective glucose pool approximates that of extracellular fluid. Searle *et al.* have attempted to explain the higher values of 35 to 65 per cent of body weight obtained in both rats (1) and dogs (2) using the single injection technique on the basis of uncertainties regarding the fate of the injected glucose- $C^{14}$  prior to its complete mixing with the body pool (26).

The rate of glucose turnover is proportional to  $\lambda_1$ , the slope of the declining activity of the specific activity curve for glucose. It may be seen



from Table II that  $\lambda_1$  averaged 0.69 per cent per minute (range 0.53 to 0.96 per cent per minute). The turnover rate ( $T$ ) of glucose (mg. per hour per kilo of body weight) may be calculated by the relationship

$$(7) \quad T = (\text{mg. glucose per kilo body weight}) (\lambda_1 \text{ as fraction per min.}) \times 60$$

The quantity of glucose which is oxidized to  $\text{CO}_2$  per hour (mg. per kilo of body weight,  $Q$ ) may be calculated according to equation (8):

$$(8) \quad Q = \left( \frac{\text{mg. CO}_2 \text{ excreted per hr. per kilo body weight}}{\left( \frac{\% \text{ CO}_2 \text{ from glucose}}{100} \right)} \right) \left( \frac{180 \text{ mg. glucose}}{6 \times 44 \text{ mg. CO}_2} \right)$$

Values for  $Q$  and  $T$  are included in Table II. The rate of  $\text{CO}_2$  expiration varied markedly in a given experiment (eight to twelve samples), presumably because of the large potential error inherent in taking a breath sample of 1 minute. However, it was calculated on the basis of an assumed R. Q. of 0.82 (sex, age, and mean weight being considered) that the subjects studied should have excreted 17 gm. of  $\text{CO}_2$  per hour. The average observed rate of  $\text{CO}_2$  excretion (19 gm. per hour) agreed favorably with this calculated value.

#### SUMMARY

1. Four normal human subjects were given a single injection of glucose- $\text{C}^{14}$ , following which serial samples of blood and respiratory  $\text{CO}_2$  were collected.

2. From the specific activity determinations of blood glucose- $\text{C}^{14}$  and respiratory  $\text{C}^{14}\text{O}_2$ , an attempt was made to approximate the size, volume of distribution, and fractional rate of turnover of the glucose pool. The mean pool size was 0.15 gm. of glucose per kilo of body weight distributed in a volume equivalent to 17 per cent of the body weight. An average of 0.69 per cent of the pool was turned over each minute.

3. An expression was derived for approximating the relative contribution of glucose to expired  $\text{CO}_2$  following a single injection of glucose- $\text{C}^{14}$ . The mean value determined at different times between 1 and 6 hours after the injection was 21 per cent. This represented an average of 38 mg. of glucose per hour per kilo of body weight oxidized to  $\text{CO}_2$ .

4. It was further calculated that the amount of glucose oxidized to  $\text{CO}_2$  accounted for approximately 60 per cent of the glucose removed from the body pool during a given time interval.

5. From the change in the specific activity of  $\text{C}^{14}\text{O}_2$  with time following the administration of trace amounts of  $\text{C}^{14}$ -labeled fructose, acetate, and bicarbonate, it seemed likely that the  $\text{C}^{14}$  which was transported from glucose to  $\text{CO}_2$  during the experiment had traversed pools having rapid turnover times.

We wish to acknowledge the assistance of Mr. Richard E. Clark, Mr. Jack Krohmer, Dr. Donald Buchanan, and Dr. Edward Strisower for their enlightening discussions and criticisms. Dr. James Craig was especially helpful in the management of tests performed on those patients who were studied at the Lakeside Hospital. The figures were drawn by Mr. Al Rendes.

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**C<sup>14</sup> STUDIES IN CARBOHYDRATE  
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*J. Biol. Chem.* 1954, 211:575-592.

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