JOURNAL OF NUCLEAR MEDICINE 5:746-762, 1964

Development of a Single Tracer Injection Method for C^{14} Glucose Kinetic Studies in Humans

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In 1954 N. Baker *et al* (1) proposed a model for the kinetics of glucose metabolism in humans after the injection of a single tracer dose of glucose, the sites of experimental measurement being the blood and the expired breath. Some data was presented to support the plausibility of their model and to set forth temporary values of the various parameters for normal individuals. Subsequently W. Shreeve *et al* (2) modified these normal values and extended the results to include persons with diabetes mellitus. B. Tolbert *et al* (3) in 1956 improved the experimental method of Baker *et al* so that continuous monitoring of the expired CO_2 and $C^{14}O_2$ became possible. M. Pollycove (4) then applied Tolbert's instrumentation and Baker's theory to study the effects of insulin, tolbutamide, and phenethylbiguanide on normal individuals.

Concomitant with this study of the effects of hypoglycemic agents on normal subjects an investigation of glucose kinetics was initiated in this laboratory on patients with diabetes mellitus and on patients with acromegaly. Most of these patients subsequently received heavy particle irradiation to their pituitary gland for the treatment of their acromegaly or their diabetic retinopathy. Several of these patients had a repeat glucose kinetic study performed some time after such therapy. It is the purpose of this paper to elucidate and to amplify the theory of Baker *et al*. A subsequent communication will present the results of these studies.

THEORY

The theory is that of Baker *et al* (1). Essentially, a steady state two compartmental model with unidirectional flow is assumed (Fig. 1). If the glucose pool (or compartment) is defined operationally to be that glucose of the body which dilutes the injected C^{14} glucose (5) and if the bicarbonate pool is defined similarly, then the assumptions are (1,6): (A-1) The tracer dose of glucose C^{14} injected intravenously mixes with a homogenous nonlabeled glucose pool without affecting glucose metabolism. (A-2) The amount of glucose in the glucose pool remains constant. That is, labeled glucose molecules in the glucose pool are replaced in time by nonlabeled glucose molecules at a rate which maintains a constant concentration of glucose in the glucose pool.

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(A-3) The rate at which glucose or CO_2 carbon leaves either the glucose or bicarbonate pool (and hence enters either pool) is proportional to the amount of carbon present in that pool.
(A-4) Some glucose is oxidized "rapidly" and irreversibly to CO_2 by some pathway(s) which will be called "the immediate oxidative pathway."
(A-5) The newly formed CO_2 mixes rapidly with the body bicarbonate pool and this CO_2 is produced at a rate which is constant and equal to that of its loss via the lungs.
(A-6) The "volumes" of these two pools remain constant.
(A-7) The theoretical CO_2 specific activity curve (which will be derived shortly) reaches its maximum point at the same time as does the experimental CO_2 specific activity curve for expired breath.

These assumptions have been incorporated into Fig. 1. Assumption (A-2) implies that there is no feedback of labeled glucose into the glucose pool during the time interval of the experiment once it has left this pool. It will be seen that under the experimental conditions of this study a single exponential decay curve for glucose pool specific activity is obtained in the time period under observation, provided a short period of time is allowed for mixing, thus supporting the plausibility of assumption (A-2).

In assumption (A-4), "rapidly" means rapid in relation to other pathways whereby C^{14} originally in glucose appears as C^{14} in CO_2. The pathways which bring about this rapid oxidation of glucose to CO_2 are combined under one title and called "the immediate oxidative pathway." Whether this "pathway" is the hexose-monophosphate shunt or the Embden-Meyerhof-Tricarboxylic acid pathway or a summation of these or other pathways is not of importance in the theory.

The work of Wrenshall and Hetenyi suggests that assumption (A-6) is a fair approximation to reality (7). Assumption (A-7) is partially justified by Baker *et al* (1).

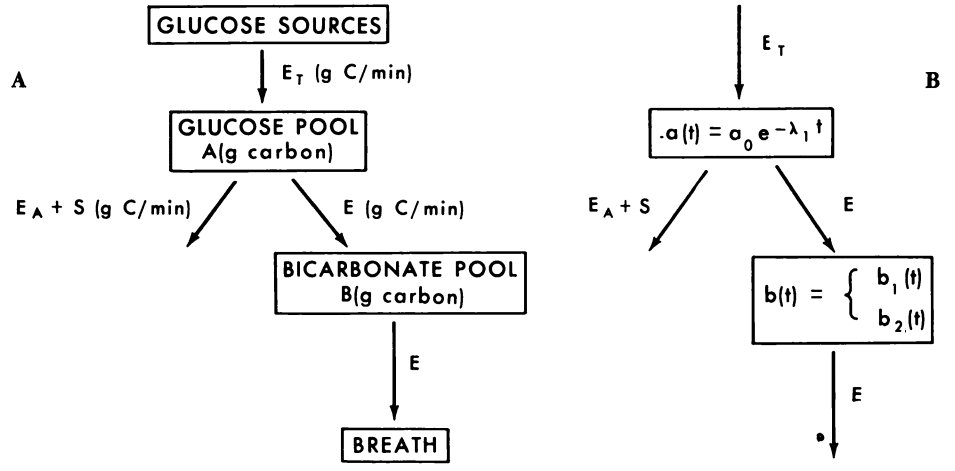

Fig. 1. The Baker *et al* model for glucose C^{14} kinetics. (A) represents the various pools and their interrelationships. (B) represents the equations for the specific activity in the corresponding pools obained in the theory of Baker *et al*.

Certain definitions are now needed to proceed with the mathematical expression of the model. These definitions differ slightly from Baker's in that here μc per gram of carbon (either in glucose or CO_2) is used for expressing specific activity whereas Baker *et al* (1) use per cent of injected activity per milligram of either glucose carbon or CO_2 carbon. Let

(D-1) $\alpha(t) = \mu c$ of C^{14} in the glucose pool at any time $t \geqq 0$. Then $\alpha(0) = \alpha_0$ is taken to be the dose of C^{14} injected as C^{14} glucose. (D-2) $\beta(t) = \mu c$ of C^{14} in the bicarbonate pool at any time $t \geqq 0$. (D-3) $a(t) = \mu c$ per gram of C in the glucose pool at any time $t \geqq 0$. This will be used to denote both the theoretical and experimental specific activities in the glucose pool since the theory is thought to closely approximate the experimental results. $a(0) = a_0$,

(D-4) $b(t) = \mu c$ per gram of C in the bicarbonate pool at any time $t \geqq 0$ under the assumption that all of the CO_2 in the bicarbonate pool is derived from glucose. This will be referred to as the "theoretical specific activity" in the bicarbonate pool as distinct from the experimental specific activity, $E^{SA}_{CO_2}$, in that pool. The reason for this distinction will become apparent later.

(D-5) A = grams of glucose carbon in the glucose pool.

(D-6) B = grams of CO_2 carbon in the bicarbonate pool.

(D-7) E = grams of CO_2 carbon excreted per minute in the breath.

(D-8) λ_1 = fraction of the glucose pool which turns over each minute.

(D-9) λ_2 = fraction of the bicarbonate pool which turns over each minute.

(D-10) t denotes time in minutes measured from the time of injection of C^{14} glucose.

(D-11) t_{max} = time at which CO_2 specific activity reaches its maximum value. (This will sometimes be denoted t_m.)

(D-12) $t_{\frac{1}{2}}$ = half-life of the specific activity in the glucose pool.

(D-13) $E_A + S$ = grams of glucose carbon removed from the glucose pool per minute by paths other than the immediate oxidative route.

(D-14) E_T = rate at which carbon enters and leaves the glucose pool in grams per minute.

As immediate consequences of the foregoing definitions we have:

(E-1) $$a(t) = \frac{\alpha(t)}{A}, \text{ by (D-1), (D-3), and (D-5).}$$

(E-2) $$b(t) = \frac{\beta(t)}{B}, \text{ by (D-2), (D-4), and (D-6).}$$

(E-3) $$\lambda_1 = \frac{E_T}{A}, \text{ by (D-5), (D-8), (D-1), (D-4).}$$

(E-4) $$\lambda_2 = \frac{E}{B}, \text{ by (D-6), (D-7), (D-9.)}$$

(E-5) $$E_T = E + E_A + S \text{ by (D-7), (D-13), (D-14).}$$

Clearly all of these are non-negative quantities.

The equation for the specific activity in the glucose pool is derived as follows: by assumption (A-3), at any time t, the rate of disappearance of radioactivity from the glucose pool is proportional to the radioactivity present in that pool. Thus

$$\text{(E-6)} \qquad \frac{d\alpha(t)}{dt} = -\lambda_1 \alpha(t),\ \lambda_1 \geq 0,\ \alpha(t) \geq 0,$$

where λ_1 denotes the constant of proportionality and the minus sign indicates that the radioactivity decreases with time.

Hence, $\frac{d\,\alpha(t)}{\alpha(t)} = -\lambda_1\, dt$

so that integration over the interval [0, t] yields $[\text{in}\ \alpha(t)]_0^t = -\lambda_1 t$.

Thus, $\text{in}\ \frac{\alpha(t)}{\alpha(0)} = -\lambda_1 t$, and

$$\text{(E-7)}\quad \alpha(t) = \alpha(0)e^{-\lambda_1 t}.$$

By (E-1) it follows that

$$\text{(E-8)}\quad a(t) = \frac{\alpha(t)}{A} = a0e^{-\lambda_1 t}.$$

This is expected to represent the actual specific activity in the glucose pool at any time $t \geqq \tau$, where τ is the "mixing time" and where assumptions (A-1), (A-2), (A-3), and (A-6) hold.

The analysis for the bicarbonate pool differs from that for the glucose pool in that the objective of the analysis is not to establish what actually happens in the bicarbonate pool but rather what would happen if all the CO_2 of this pool were to come from glucose. Then the experimentally obtained CO_2 specific activity can be compared with this theoretical CO_2 specific activity to obtain the fraction of CO_2 derived from glucose. That is, if the situation for the bicarbonate pool were as depicted in Fig. 2 where k is the fraction of CO_2 derived from glucose, then the specific activity in the bicarbonate pool would actually be

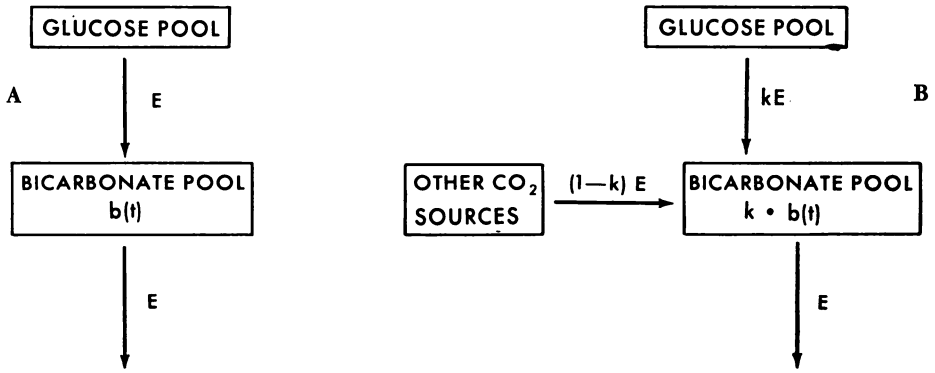

Fig. 2. (A) Theoretical Situation: All of the CO_2 is assumed to come from glucose. (B) Actual Situation: Only a fraction k of the CO_2 comes from glucose.

kb(t), rather than b(t). Thus it is clear that if we want to determine k we need only take the ratio $\frac{kb(t)}{b(t)}$ *i.e.*, the ratio of the experimental specific activity over the theoretical specific activity. (This will be clarified later.) This, in essence, is the purpose for the following analysis. Hence, to derive the theoretical expression for b(t) the following additional assumption is made:
(A-8) All of the CO_2 in the bicarbonate pool comes from glucose.

By assumptions (A-3) and (A-8) the rate at which radioactivity enters the bicarbonate pool from the glucose pool is proportional to the amount of radioactivity present in the glucose pool. Thus, $\frac{E}{A}\alpha$ μc of C^{14} enters the bicarbonate pool from the glucose pool each minute. Likewise, $\frac{E}{B}\beta$ μc of C^{14} leaves the bicarbonate pool each minute via the breath. Thus the total rate of change in the amount of radioactivity in the bicarbonate pool is

$$\text{(E-9)} \qquad \frac{d\beta(t)}{dt} = \frac{E}{A}\alpha(t) - \frac{E}{B}\beta(t).$$

Let $k_1 = \frac{E}{A}$ and $\lambda_2 = \frac{E}{B}$ (see (E-4)) so that

$$\text{(E-10)} \qquad \frac{d\beta}{dt} = k_1\alpha - \lambda_2\beta.$$

Equation (E-10) can be solved by the method of variation of parameters as follows: consider the homogeneous equation $\frac{d\beta}{dt} + \lambda_2\beta = 0$, and assume $\beta(t) = Ce^{-\lambda_2 t}$ Allowing C to vary with t, this assumed solution may be differentiated using the rule for differentiation of products of functions

$$\text{(E-11)} \qquad \frac{d\beta}{dt} = \frac{dC}{dt}e^{-\lambda_2 t} - \lambda_2 Ce^{-\lambda t_2}$$

$$= \frac{dC}{dt}e^{-\lambda_2 t} - \lambda_2\beta.$$

Comparing this with equation (E-10) shows that

$$\frac{dC}{dt}e^{-\lambda_2 t} = k_1\alpha.$$

That is, $dC = k_1\alpha e^{\lambda_2 t}\, dt$

so that by integrating over the interval [0, t] yields

$$C(t) - C(0) = k_1\int_0^t \alpha e^{\lambda_2\tau}d\tau.$$

Substituting $\alpha(\tau) = \alpha_0 e^{-\lambda_1\tau}$ gives

$$\text{(E-12)}\quad C(t) = k_1 \int_0^t (\alpha_0 e^{-\lambda_1 \tau} \cdot {}^{e\lambda_2 \tau})\, d\tau + C(0)$$

$$= \alpha_0 k_1 \int_0^t e^{(\lambda_2 - \lambda_1)\tau}\, d\tau + C(0).$$

Hence, $\beta(t) = \left(\alpha_0 k_1 \int_0^t e^{(\lambda_2 - \lambda_1)\tau}\, d\tau + C(0)\right) \cdot e^{-\lambda_2 t}$

Since at $t = 0$ the bicarbonate pool contains no C^{14}, it all being in the glucose pool, $\beta(0) = 0$. Thus

$$0 = C(0)$$

so that

$$\text{(E-13)}\quad \beta(t) = \left(\alpha_0 k_1 \int^t e^{(\lambda_2 - \lambda_1)\tau}\, d\tau\right) \cdot e^{-\lambda_2 t}.$$

Two cases must be considered, namely $\lambda_1 = \lambda_2$ and $\lambda_1 \neq \lambda_2$.

If $\lambda_1 = \lambda_2$ then

$$\beta(t) = \alpha_0 k_1 t e^{-\lambda_2 t}.$$

If $\lambda_1 \neq \lambda_2$ then

$$\beta(t) = \left(\alpha_0 k_1 \left[\frac{e^{(\lambda_2 - \lambda_1)\tau}}{\lambda_2 - \lambda_1}\right]_0^t\right) \cdot e^{-\lambda_2 t}$$

$$= \left(\alpha_0 k_1 \left(\frac{e^{(\lambda_2 - \lambda_1)\tau} - 1}{\lambda_2 - \lambda_1}\right)\right) \cdot e^{-\lambda_2 t}$$

$$= \frac{\alpha_0 k_1}{\lambda_2 - \lambda_1} (e^{-\lambda_1 t} - e^{-\lambda_2 t}).$$

Hence

$$\text{(E-14)}\qquad \beta(t) = \begin{cases} \alpha_0 k_1 t e^{-\lambda_2 t} & \text{, if } \lambda_1 = \lambda_2 \\ \dfrac{\alpha_0 k_1}{\lambda_2 - \lambda_1} (e^{-\lambda_1 t} - e^{-\lambda_2 t}) & \text{, if } \lambda_1 \neq \lambda_2 \end{cases}$$

By the definition of k_1 and consequences (E-1), (E-2), and (E-4) it follows that, for the case $\lambda_1 = \lambda_2$, $b(t)$ is given by

$$b(t) = \frac{\beta(t)}{B} = \frac{\alpha_0}{A} \cdot \frac{E}{B} \cdot t e^{-\lambda_2 t} = a_0 \lambda_2 t e^{-\lambda_2 t}$$

and in the case $\lambda_1 \neq \lambda_2$

$$b(t) = \frac{\beta(t)}{B} = \frac{\alpha_0 k_1}{B(\lambda_2 - \lambda_1)} (e^{-\lambda_1 t} - e^{-\lambda_2 t})$$

$$= \frac{a_0 \cdot A \cdot E/A}{B(\lambda_2 - \lambda_1)} (e^{-\lambda t} - e^{-\lambda_2 t})$$

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

so that the complete solution for b(t) is

$$\text{(E-15)} \qquad b(t) = \begin{cases} a_0 \lambda_2 t e^{-\lambda_2 t} & \text{, for } \lambda_1 = \lambda_2 \quad \text{(C-1)} \\ a_0 \dfrac{\lambda_2}{\lambda_2 - \lambda_1} (e^{-\lambda_1 t} - e^{-\lambda_2 t}) & \text{, for } \lambda_1 \neq \lambda_2 \quad \text{(C-2)} \end{cases}$$

To simplify the notation and to show the dependence of b on λ_1 and λ_2, write equation (E-15) as follows;

$$\text{(E-16)} \qquad b(\lambda_1, \lambda_2, t) = \begin{cases} b_1(\lambda_2, t) & \text{, for } \lambda_1 = \lambda_2 \\ b_2(\lambda_1, \lambda_2, t) & \text{, for } \lambda_1 \neq \lambda_2 \end{cases}$$

By L'Hôpital's rule, for each fixed λ_2 and t

$$\lim_{\lambda_1 \to \lambda_2} b_2(\lambda_1, \lambda_2, t) = b_1(\lambda_2, t)$$

so that b(t) is continuous at $\lambda_1 = \lambda_2$.

A test to determine whether $\lambda_1 = \lambda_2$ or $\lambda_1 \neq \lambda_2$, i.e., whether case (C-1) or (C-2) holds, can be made using assumption (A-7). By this assumption, the time, t_{max}, required for b(t) to reach its maximum value can be obtained from the experimental data for the bicarbonate pool (see (D-11)). Likewise λ_1 can be obtained directly from the experimental data for the glucose pool. Hence the following lemma can be used to determine whether or not $\lambda_1 = \lambda_2$.

(E-17) *Lemma:* $\lambda_1 = \lambda_2$ if and only if $t_{max} = \dfrac{1}{\lambda_1}$.

Proof: t_{max} is the time t such that $b'(t) = 0$. Letting primes denote derivatives with respect to t, (E-15) yields:

$$\text{(E-18)} \qquad b'(t) = \begin{cases} b_1'(t) = a_0 \lambda_2 e^{-\lambda_2 t}(1 - \lambda_2 t) & \text{, for } \lambda_1 = \lambda_2 \\ b_2'(t) = a_0 \dfrac{\lambda_2}{\lambda_2 - \lambda_1} (\lambda_2 e^{-\lambda_2 t} - \lambda_1 e^{-\lambda_1 t}) & \text{, for } \lambda_1 \neq \lambda_2 \end{cases}$$

Suppose $\lambda_1 = \lambda_2$. Then to find t_{max} the equation $0 = b_1'(t) = a_0 \lambda_2 e^{-\lambda_2 t}(1 - \lambda_2 t)$ must be solved for t. This yields $t_{max} = \dfrac{1}{\lambda_2} = \dfrac{1}{\lambda_1}$.

Suppose $\lambda_1 \neq \lambda_2$. It must be shown that in this case $t_{max} \neq \dfrac{1}{\lambda_1}$. Since $\lambda_1 \neq \lambda_2$, t_{max} is the solution of

$$0 = b_2'(t) = a_0 \frac{\lambda_2}{\lambda_2 - \lambda_1} (\lambda_2 e^{-\lambda_2 t} - \lambda_1 e^{-\lambda_1 t}).$$

Thus, $t_{max} = \dfrac{1}{\lambda_2 - \lambda_1}$ in $\dfrac{\lambda_2}{\lambda_1}$. Now it must be demonstrated that $\dfrac{1}{\lambda_1}$ cannot equal

$\frac{1}{\lambda_2 - \lambda_1}$ in $\frac{\lambda_2}{\lambda_1}$. To show this, one may argue by contradiction, i.e., show that $\frac{1}{\lambda_1} = \frac{1}{\lambda_2 - \lambda_1}$ in $\frac{\lambda_2}{\lambda_1}$ implies that $\lambda_1 = \lambda_2$ and thus obtain a contradiction to the supposition that $\lambda_1 \neq \lambda_2$.

If $\frac{1}{\lambda_1} = \frac{1}{\lambda_2 - \lambda_1}$ in $\frac{\lambda_2}{\lambda_1}$, then $\frac{\lambda_2 - \lambda_1}{\lambda_1} =$ in $\frac{\lambda_2}{\lambda_1}$, or, $\frac{\lambda_2}{\lambda_1} - 1 =$ in $\frac{\lambda_2}{\lambda_1}$

Let $x = \frac{\lambda_2}{\lambda_1}$. Then this equation is equivalent to $x -$ in $x = 1$. But $x = 1$ is the unique solution to this equation, because:

(1) $x = 1$ clearly is a solution ($1 -$ in $1 = 1 - 0 = 1$)
(2) all values of $x -$ in x are greater than 1 for values of $x \neq 1$.

To see this let $\varphi(x) = x -$ in x. Then $0 = \varphi'(x) = 1 - \frac{1}{x}$ implies $x = 1$. So φ has an extremum at $x = 1$. To see that this extremum is a minimum we note that $\varphi''(x) = \frac{1}{x^2} > 0$. Hence $\frac{\lambda_2}{\lambda_1} = 1$ so that $\lambda_2 = \lambda_1$ contradicting the supposition that $\lambda_2 \neq \lambda_1$.

This result is important in the calculation of λ_2 from the data and will be expanded later.

Equation (E-18) represents the specific activity of the bicarbonate pool under the proviso that all of the CO_2 in the bicarbonate pool originates from glucose. To further clarify the use of this expression for b(t), suppose that only kE grams of glucose carbon enter the bicarbonate pool each minute, k being the fraction of CO_2 carbon which originates from glucose, $0 < k < 1$. Then, by theory, the specific activity in the bicarbonate pool should be

$$p(t) = kb(t) \tag{E-19}$$

since, in the derivation of b(t), E appeared in the numerator. k here need not be constant. The following example will show that the assumption of "steady stateness" for the glucose and bicarbonate pools does not imply that k is constant. Consider the situation in Fig. 3 where E_T, E, E_A and S are positive constants. Steady-stateness of the glucose and bicarbonate pools implies that

$$E_T = kE + \tau(E_A + S). \tag{E-20}$$

Solving for τ,

$$\tau = \frac{E_T - kE}{E_A + S}$$

From this it can be seen that if k depends on time, then for each $t \geqq 0$, τ can be chosen to be

$$\tau(t) = \frac{E_T - k(t)E}{E_A + S}$$

Thus, at any time t, the inflow rate E_T and the outflow rate $k(t)E + \tau(t)(E_A + S)$ for the glucose pool are equal (by (E-20)) and constant as are the inflow rate $k(t)E + (1 - k(t))E$ and the outflow rate E for the bicarbonate pool. Hence, steady state conditions hold for both the glucose and the bicarbonate pools but k is not constant. It should be noted, too, that the nonglucose precursors of CO_2 need not be in a steady state; this, however, has no bearing on the present analysis.

The supposition that only kE grams of glucose carbon enter the bicarbonate pool per minute is not in reality a "supposition." It is well known that the carbon of CO_2 in the bicarbonate pool can originate from nonglucose precursors (8).

Notice should be made, too, that during the experimental procedure, the specific activity of CO_2 is not measured in the bicarbonate pool but in the breath. Heuristically one would expect that in time both of these would have nearly the same specific activity since homogeneous mixing seems plausible and since $C^{14}O_2$ and $C^{12}O_2$ behave quantitatively approximately the same in physiologic processes. Nonetheless, there is a lag period before these two sites of CO_2 develop nearly the same specific activity (9).

With these words of caution, the gap between the experimentally observed specific activity in the breath, denoted $E^{SA}_{CO_2}$, and the modified theoretical specific activity of the bicarbonate pool, p, is hurdled. That is, p is taken to be equal to $E^{SA}_{CO_2}$ so that for each $t \geqq 0$

$$k(t) = \frac{p(t)}{b(t)} = \frac{E^{SA}_{CO_2}(t)}{b(t)} \tag{E-21}$$

In calculating the data, k is determined at t_{max} thus allowing time for mixing and for diminution in the error due to lag.

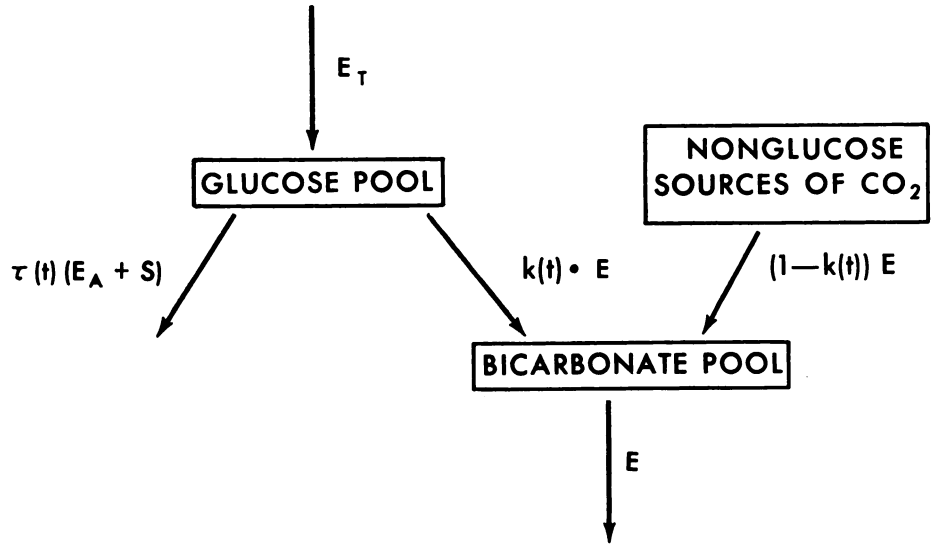

Fig. 3. An example to show k need not be constant.

Calculation of b(t) requires knowledge of a_0, λ_1, and λ_2. The experimental data for the glucose pool readily provides a_0 and λ_1. a_0 is the intercept on the specific activity axis of the straight line fit to the points obtained by plotting experimental glucose pool specific activity versus time, for $t \geqq 15$, on semilogarithmic paper. λ_1 is the slope of this line. To determine λ_2, the following results may be used.

Lemma 1: λ_2 must satisfy the equation $\lambda_1 e^{-\lambda_1 t_m} = \lambda e^{-\lambda t_m}$.

Proof: If $\lambda_1 = \lambda_2$ then clearly $\lambda_1 e^{-\lambda_1 t_m} = \lambda_2 e^{-\lambda_2 t_m}$. If $\lambda_1 \neq \lambda_2$, then b_2 holds in (E-16), and since at t_m, $b_2'(t_m) = 0$, the lemma follows from (E-18) and the fact that $a_0 \neq 0$ and $\lambda_2 \neq 0$.

Corollary 1: λ_2 must satisfy $Ce^{\lambda t_m} - \lambda = 0$ where $C = \lambda_1 e^{-\lambda t_m}$

Proof: Immediate from lemma 1.

Definition 1: Let φ be the function defined on $[0, \infty]$ by $\varphi(\lambda) = Ce^{\lambda t_m} - \lambda$ where $C = \lambda_1 e^{-\lambda_1 t_m}$. (See Fig. 4(a).)

Lemma 2: If $\varphi(\lambda_0)$ is the minimum value of φ, that is, $\varphi(\lambda) \geqq \varphi(\lambda_0)$, for all $\lambda \epsilon [0, \infty)$, then, $\varphi(\lambda_0) = 0$ implies $\lambda_2 = \lambda_1$, and, $\varphi(\lambda_0) < 0$ implies that there exists a unique $\lambda_2 \neq \lambda_1$ such that $\varphi(\lambda_2) = 0$.

Proof: The properties of φ which make this lemma possible are:

(P-1) $\lim_{\lambda \to 0} \varphi(\lambda) = C > 0$. This is clear since φ is a continuous function of λ.

(P-2) $\lim_{\lambda \to \infty} \varphi(\lambda) = \infty > 0$.

(P-3) φ has a unique minimum. To see this, the rules of differential calculus for finding extrema require that

$$0 = \varphi'(\lambda) = t_m Ce^{\lambda t_m} - 1 \tag{E-22}$$

be satisfied by any λ for which $\varphi(\lambda)$ is an extremal. (E-22) has the unique solution λ_0, given by

$$\lambda_0 = \frac{1}{t_m} \text{ in } \frac{1}{Ct_m}$$

the uniqueness being a result of the constancy of t_m and C. That $\varphi(\lambda_0)$ is a minimum follows from

$$\varphi''(\lambda_0) = t_m^2 Ce^{\lambda_0 t_m} > 0 .$$

These properties of φ are incorporated into Fig. 4(b).

From these properties it follows that φ can have at most two zeros, and the condition which must hold for φ to have two zeros is $\varphi(\lambda_0) < 0$. Since λ_1 is always one of these zeros (as is clear from the nature of C), it follows that when $\varphi(\lambda_0) < 0$, there exists a unique $\lambda_2 \neq \lambda_1$, such that $\varphi(\lambda_2) = 0$. When $\varphi(\lambda_0) = 0$, there is only one solution, this being $\lambda_1 = \lambda_0$.

Lemma 3: $\varphi(\lambda_0) = \dfrac{1 - \lambda_1 t_m + \text{in } \lambda_1 t_m}{t_m}$

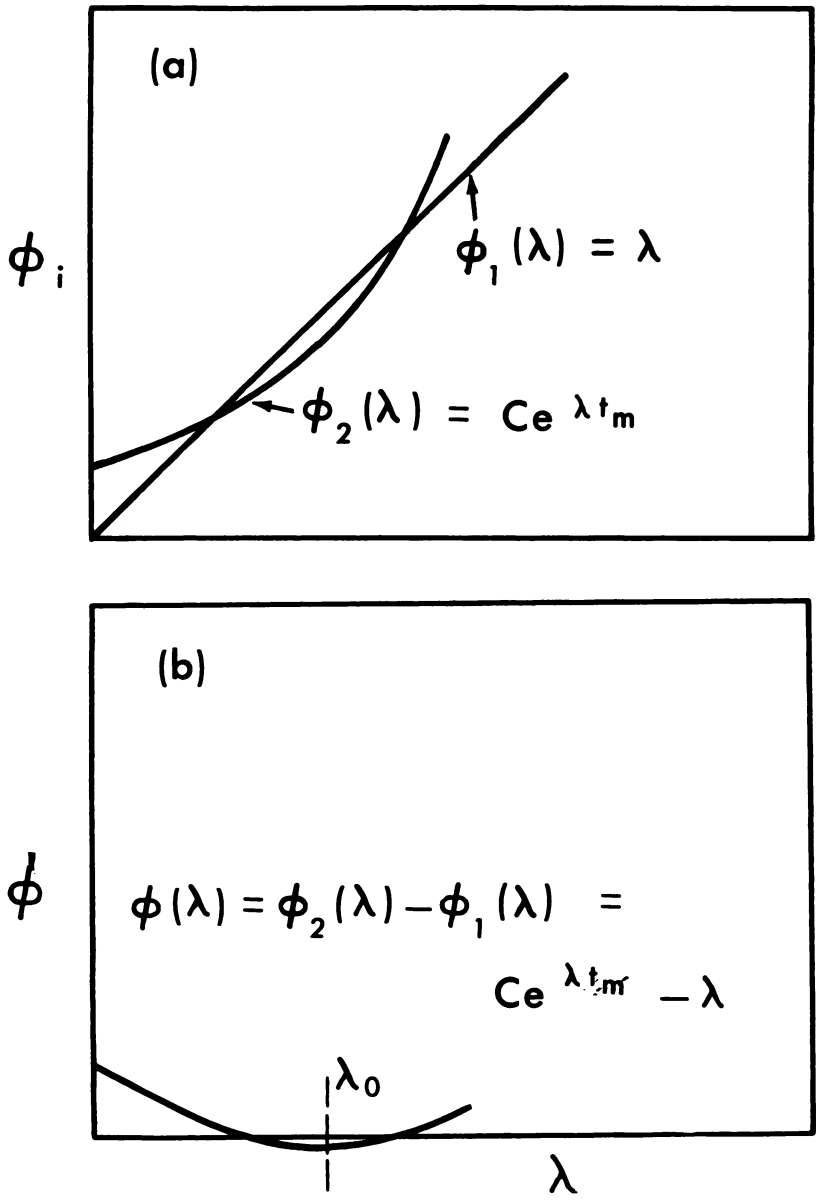

Fig. 4. (a) Graphs of $\phi_1(\lambda) = \lambda$ and $\phi_2(\lambda) = Ce^{\lambda t_m}$.

(b) Graph of $\phi(\lambda) = Ce^{\lambda t_m} - \lambda$ illustrating the case $\phi(\lambda_o) < 0$ where $(\lambda_o, \phi(\lambda_o))$ is the minimum point of ϕ. In this case there are two points where ϕ takes on the value 0.

Proof: $\varphi(\lambda_0) = Ce^{\lambda_0 t_m} - \lambda_0$ (by definition 1)

$= Ce^{\left(\frac{1}{t_m} \text{ in } \frac{1}{Ct_m}\right) \cdot t_m} - \frac{1}{t_m} \text{ in } \frac{1}{Ct_m}$ (by (E-25))

$= \frac{1 + \text{in } Ct_m}{t_m}$

$= \frac{1 + \text{in } \left(\left(\frac{ie^{-\lambda_1 t_m}}{t_m}\right) \cdot t_m\right)}{t_m}$ (by definition 1)

$= \frac{1 - \lambda_1 t_m + \text{in } \lambda_1 t_m}{t_m}$ Q.E.D.

Lemma 4: (a) If $\lambda_1 t_m = 1$, then $\varphi(\lambda_0) = 0$
(b) If $\lambda_1 t_m \neq 1$, then $\varphi(\lambda_0) < 0$

Proof: (a) follows immediately from lemmas 3 and 2.

This result was expressed earlier as part of (E-17).

(b) follows from noticing that the function

$$\Psi^*(x) = 1 - x + \text{in } x, \; x \geqq 0$$

has a unique extremum, it being a maximum, at $x = 1$. To see this, observe that $0 = \Psi^{*\prime}(x) = -1 + \frac{1}{x}$ has a unique solution, it being $x = 1$, and that $\Psi^*(1) = 0$. Thus, since $\Psi^{*\prime\prime}(1) = -\frac{1}{x^2}\Big|_{x=1} < 0$, it follows that for all $x \neq 1, \Psi^*(x) < 0$. Since $\varphi(x) = \frac{1}{t_m}\Psi^*(x)$, the lemma follows.

To determine λ_2, then, one should first calculate $\lambda_1 t_m$. If $\lambda_1 t_m = 1$ then $\lambda_2 = \lambda_1$ by (E-17) and also by lemma 2. If $\lambda_1 t_m \neq 1$ then, by lemma 4, $\varphi(\lambda_0) < 0$, and, by lemma 2, the existence of a unique $\lambda_2 \neq \lambda_1$, is assured. Since in this case $\lambda_2 \neq \lambda_1$, and since λ_1 is known, the unknown λ_2 can be calculated from $\lambda_1 e^{-\lambda t_m} = \lambda e^{-\lambda t_m}$ by successive approximations carried out by substituting various values of λ in this equation until $\lambda e^{-\lambda t_m}$ approximates $\lambda_1 e^{-\lambda_1 t_m}$ as closely as desired.

METHOD

The experimental technique and instrumentation are described in detail in the articles of Tolbert *et al* (3) and Pollycove (4). For purposes of measuring the glucose pool specific activity, a polyethylene catheter is inserted into a forearm vein and serial blood samples of 5 to 10 ml withdrawn in heparinized syringes starting before and continuing after a single rapid intravenous injection of approximately 10-15 μc of universally labeled C^{14} glucose[1] in the opposite arm. These samples are collected at 15 minute intervals from the first hour and at 60 minute intervals thereafter until a maximum point is discernible on the CO_2

[1]Nuclear-Chicago Corporation.

specific activity curve produced by the recorder. This usually requires 3-5 hours. The blood samples are analyzed for glucose and glucose radioactivity using a glucose oxidase method of glucose determination[2] (on protein free filtrate obtained by the method of Somogyi (10)) and the method of Searle and Chaikoff (11) respectively.

The breath is analyzed for CO_2 and CO_2 radioactivity every 12 seconds using an infrared CO_2 gas analyzer and a 21 liter ionization chamber having a vibrating reed electrometer, a 95 per cent theoretical efficiency, and a calibration constant of 1.40 μc of C^{14} per 10^{-11} amperes.

The patient's head is enclosed in a plastic helmet and the expired air passed through these instruments. A recorder produces tracings of the radioactivity and the CO_2 content of the expired breath as well as the ratio between these two quantities. During the course of the procedure, the patients sit in a reclining chair and are permitted to drink black coffee, to converse, and to remove the helmet for a few minutes.

CALCULATIONS

The theory presented earlier was synthesized to allow the calculation of certain quantities from data obtained through the application of the foregoing method. An orderly approach to these calculations is as follows:

(Q-1) Recall that $\alpha(0) = \alpha_0$ is the injected dose of C^{14} glucose in μc. (See (D-1).)

(Q-2) Plot a(t) versus time on semilogarithmic paper (see (D-3) Extrapolate to t = 0 and obtain a_0. This is μc/gm C in the glucose pool. In order to get μc/gm glucose divide a_0 by 2.5 (because, for each gm of carbon in glucose there are 2.5 gm of glucose) and define $A_0 = \frac{a_0}{2.5}$: this then is μc/gm glucose in the glucose pool. In Fig. 5, $a(t)$ has been plotted instead of $a(t)$, a(t) being $a(t)$ adjusted to an initial injected dose of 10 μc, i.e., $a(t) = \frac{10}{\alpha_0} a(t)$. (Q-3) $t_{\frac{1}{2}}$ is readily obtained from plot (Q-2) in that it is the time required for a_0 to drop to $\frac{1}{2}a_0$.

(Q-4) The glucose pool in grams, denoted by P_G, is obtained from $P_G = \frac{\alpha_0}{A_0}$. Let W represent body weight in kilograms. Then the glucose pool in grams per kilogram, denoted P_G^W, is $P_G^W = \frac{\alpha_0}{A_0 W}$. Note that A (see (D-5)) is given by $A = \frac{P_G}{2.5}$

(Q-5) λ_1, the fractional turnover rate for the glucose pool (see (D-8)), is the slope of the line plotted in (Q-2). It can also be obtained from $t_{\frac{1}{2}}$ by using $\lambda_1 = \frac{0.693}{t_{\frac{1}{2}}}$ which is obtained from $a(t) = a_0 e^{-\lambda_1 t}$ by taking logarithms and substituting $\frac{1}{2}a_0$ for a(t) and $t_{\frac{1}{2}}$ for t.

(Q-6) To obtain the glucose turnover rate in gm/kg/h, denoted T_G, use

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[2]Glucostat, Worthington Biochemical Corp., Trechold, N.J.

$$T_G = \lambda_1 \cdot P_G^W \cdot 60$$

(Q-7) The "glucose volume" in liters, denoted V_G, and in liters per kg of body weight, denoted V^w, and in per cent body weight, denoted V_G^W may be obtained as follows. Since V_G is defined to be the volume, in liters, which contains the P_G grams of glucose, $\frac{P_G}{V_G} = B_G \cdot 10^{-2}$ where B_G is the fasting blood glucose and the factor 10^{-2} enters in the conversion of mg/100 ml to gm/liter. Thus, $V_G = \frac{P_G}{B_G} \cdot 10^2$

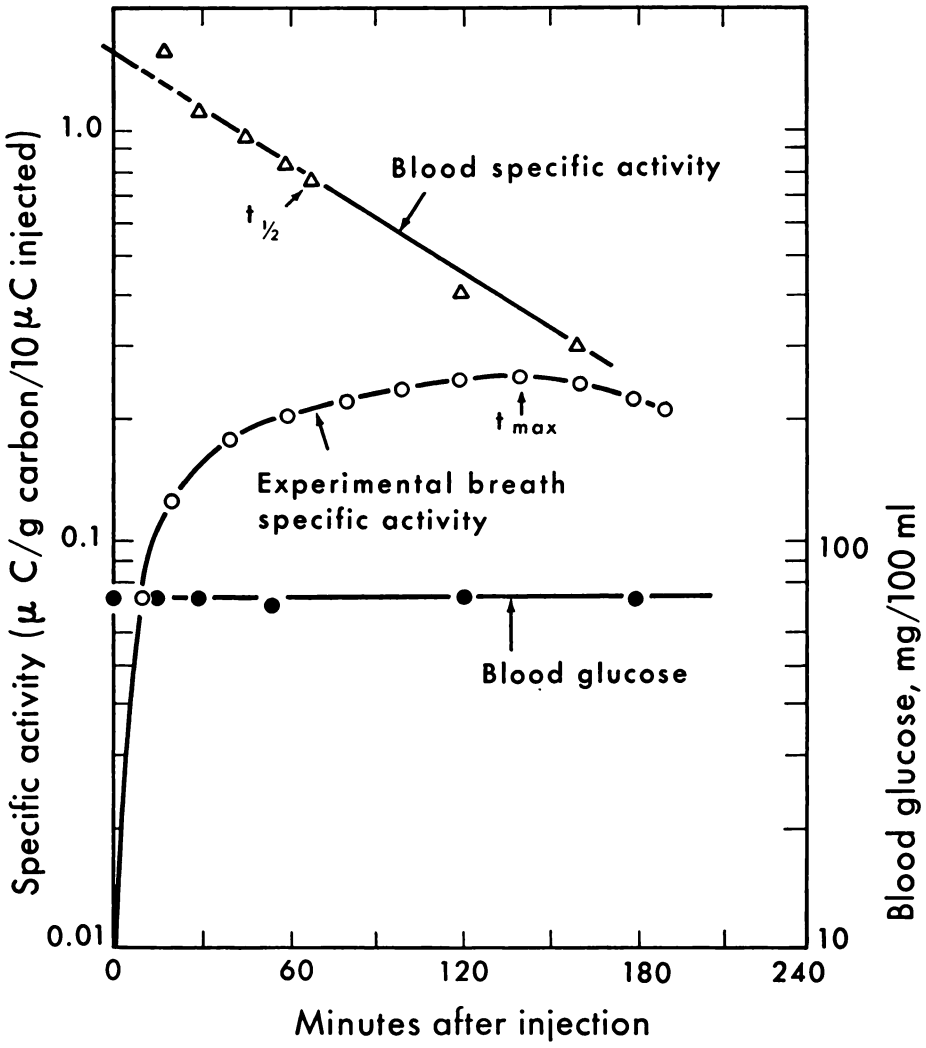

Fig. 5. Example of graphs of the data for $a(t)$, the blood glucose specific activity in μc/gmC adjusted to an injected dose of $10\mu c$, for $E^{SA}_{CO_2}(t)$ the experimental specific activity of the expired breath in μc/gmC, and for $B_S(t)$, the blood sugar in mg %.

liters and $V^w = \frac{P_G}{B_G} \cdot \frac{10^2}{W}$ liters/kg. If it is assumed that one liter is equivalent to one kilogram, then V^w is the fraction of body weight devoted to glucose and $V^w \cdot 100$ per cent is the per cent of body weight comprising the glucose pool.

Thus $V_G^W = \frac{P_G \cdot 10^4}{B_G \cdot W}$ per cent body weight.

(Q-8) λ_2 is obtained as described earlier.

(Q-9) The mean mg of CO_2 expired per minute, denoted $\left\langle \frac{d[CO_2]}{dt} \right\rangle$ was obtained by integrating the CO_2 experimental curve with the assistance of a mechanical integrator. This "mean" is an average over time. Note that this quantity is almost E (see (D-7)), the difference being essentially in units. E is in terms of gm C/min and $\left\langle \frac{d[CO_2]}{dt} \right\rangle$ is in terms of mg CO_2/min.

(Q-10) t_{max} (see (D-11)) is read off the experimental CO_2 specific activity curve t_{max} being the time at which this curve reaches its maximum value.

(Q-11) The experimental CO_2 specific activity, denoted $E_{CO_2}^{SA}$, is obtained at t_{max} directly by reading it off the experimental CO_2 specific activity curve at this point.

(Q-12) Since λ_1, λ_2, and t_{max} are known, from (E-15),

$$b(t_{max}) = \begin{cases} b_1(t_{max}) \text{ , for } \lambda_1 = \lambda_2 \\ b_2(t_{max}) \text{ , for } \lambda_1 \neq \lambda_2 \end{cases}$$

can be calculated.

(Q-13) The $\%CO_2$ derived from glucose, denoted $\%CO_2^G$ is obtained by (E-21)

$$\%CO_2^G(t_{max}) = \frac{E_{CO_2}^{SA}(t_{max})}{b(t_{max})} \cdot 100$$

(Q-14) The mg of glucose oxidized to CO_2 per minute, denoted G_{CO_2}, is

$$G_{CO_2}(t) = \frac{\%CO_2^G(t)}{100} \cdot \left\langle \frac{d[CO_2]}{dt} \right\rangle \cdot \frac{180}{6{\cdot}44}$$

because:
(fraction of CO_2 derived from glucose) . (mg CO_2/min) = (mg CO_2 derived from glucose/min) and one mg of CO_2 is equivalent to $\frac{180}{6{\cdot}44}$ mg of glucose as can be seen from

$$\underset{180 \text{ mg}}{C_6H_{12}O_6} \rightarrow \underset{6.44 \text{ mg}}{6CO_2} + 6H_2O$$

Since the fraction of CO_2 derived from glucose is $\frac{\%CO_2^G(t)}{100}$, the desired equation follows.

In terms of gm/kg/h, denoted $G^{W}_{CO_2}$, this is

$$G^{W}_{CO_2}(t) = \frac{G_{CO_2}(t)}{W} \cdot 60 \cdot 10^{-3}$$

Notice that G_{CO_2} is again almost E, the difference once more being essentially in the units.

(Q-15) The CO_2 turnover in gm/h per kg of body weight, denoted by T_{CO_2}, is

$$T_{CO_2} = \frac{\left\langle \frac{d[CO]}{dt} \right\rangle}{W} \cdot 60 \cdot 10^{-3} \qquad \text{gm/kg/h}$$

(Q-16) The CO_2 pool size in gm/kg, denoted by P_{CO_2} is

$$P_{CO_2} = \frac{T_{CO_2}}{\lambda_2} \cdot \frac{1}{60}$$

because:

(fraction of pool which turns over/min) · (pool size) = (total turnover/min)

implying that

$$\lambda_2 \cdot (\text{pool size}) = T_{CO_2} \cdot \frac{1}{60}$$

that is

$$(\text{pool size}) = \frac{T_{CO_2}}{\lambda_2} \cdot \frac{1}{60}$$

These then, are the various parameters measured or calculated.

DISCUSSION

Three methods of glucose kinetic studies using universally labeled C^{14} glucose are presently in vogue. One method, originating with Baker *et al* was described in this article. Another method, due to Steel *et al* (5,9) consists of simultaneously injecting a priming dose of C^{14} glucose and starting a continuous IV infusion of this tracer. The objective here is to maintain a constant blood glucose specific activity in order to simplify the determination of the asymptotic value of this specific activity. But even if this specific activity is not maintained at a constant value, fairly good results can be obtained by an approximation method outlined by Steele *et al.* This method also allows a determination of the fraction of CO_2 derived from glucose but again an asymptote is needed, this time of the ratio of the blood glucose specific activity to the specific activity of the expired breath.

The third method is that of Wrenshall and Hetenyi (7). It consists of giving successive intravenous injections of C^{14} glucose and determining the glucose volume after each injection by means of an isotope dilution equation. This method was originated to study the changes induced in glucose volume by modifications in the physiological state of the organism under study. Also, rates of glucose appearance and disappearance from the blood can be calculated. However, this method has not been used to answer questions regarding conversion of glucose to CO_2.

Each of these methods has its limitations, which have been discussed by their own authors. Baker's method has the following in its favor:

(1) It is relatively simple to perform.
(2) The equations involved are simple and fit the data sufficiently well to give consistent results.
(3) It provides a means for analyzing data regarding conversion of glucose to CO_2.
(4) All three methods give quite comparable results in the dog.

SUMMARY

The model for the study of glucose kinetics originated by Baker *et al* has been expanded and refined both in its theory and in its realization. The present paper is concerned with the refinements in theory. A subsequent report will present the data obtained when this method was applied to the study of normal individuals as well as patients with acromegaly or diabetes mellitus.

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