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# The Retention of Metabolic Radioactive Carbonate

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There is urgent need for a procedure for calculating the combustion rate of a $^{14}C$ metabolite from the amount of [$^{14}C$]carbon dioxide expired. In order to carry out such a calculation one must know the amount of $^{14}C$ retained in the bicarbonate, carbonate and carbamino compound pools of the body as a function of the amount of [$^{14}C$]carbon dioxide which appears in the respiratory carbon dioxide.

When [$^{14}C$]carbon dioxide is produced from a metabolite only in the circulating blood, or (to a good approximation) when [$^{14}C$]carbon dioxide is produced in a few localities and is presented to most of the body as blood [$^{14}C$]bicarbonate, it is possible to calculate the combustion rate directly from a preliminary experiment in which the retention of an instantaneous dose of [$^{14}C$]bicarbonate, administered intravenously, is established as a function of elapsed time. This is the method used by Kornberg (1953) and Kornberg, Davies & Wood (1954) to calculate the rate of conversion of [$^{14}C$]-urea into [$^{14}C$]carbon dioxide by the gastrointestinal bacteria of the intact cat.

This paper considers the possibility of deriving, from the equation describing the retention of an intravenous dose of [$^{14}C$]bicarbonate, an equation approximately describing the retention of a hypothetical dose of [$^{14}C$]bicarbonate introduced in the 'soft tissue compartment' of the real animal, the latter equation being the one which is suitable for calculating the combustion rate of a metabolite which is burned generally throughout the soft tissues of the body. The procedure followed is to establish the desired equation for an 'ideal' cat, and then to use the observations of Kornberg (1953) to find out to what extent the ideal situation is present in the real cat.

## THEORY

*The properties of the ideal cat.* Fig. 1 represents the situation as we might wish it to be in the cat. Since there are three terms in the exponential equation which represents the retention of an intravenous dose of [$^{14}C$]bicarbonate (Kornberg, 1953) and since we know that there is a central compartment which represents the circulating blood, we would like the other two terms of the three-term equation to reflect the presence of two discrete peripheral compartments. One of the two peripheral compartments we expect to represent the solid carbonate of bone with its slow rate of turnover and the other we expect to represent the

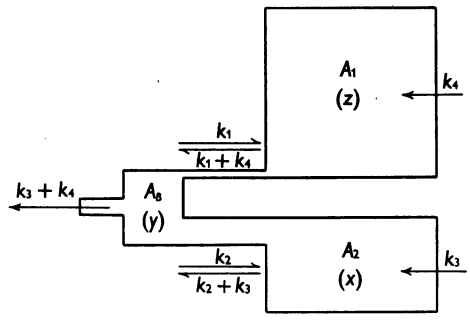


Fig. 1. Kinetic model for retention of $^{14}C$ introduced into the total acid-volatile $CO_2$ of the cat. $A_B$ (roughly the blood compartment) contains $A_B$ mg. of carbonate carbon and the fraction ($y$) at any time ($t$) of the original $^{14}C$ activity which was introduced at $t=0$. It loses $(k_3+k_4)$ mg. C/min. as expired $CO_2$ and its rate of $^{14}C$ loss to the expired air is $\frac{k_3+k_4}{A_B}(y)$/min. Its rates of $^{14}C$ loss to $A_1$ and $A_2$ are $\frac{k_1}{A_B}(y)$ and $\frac{k_2}{A_B}(y)$ respectively. $A_1$ (roughly the bone compartment) contains $A_1$ mg. carbonate carbon and the fraction ($z$) of the original $^{14}C$ dose. It gains $k_4$ mg. $^{12}CO_2$ C/min. by metabolic $CO_2$ production within $A_1$, and loses $^{14}C$ to $A_B$ at the rate of $\frac{k_1+k_4}{A_1}(z)$/min. $A_2$ (roughly the soft tissue compartment) is analogous to $A_1$.

bicarbonate of the soft tissues with its more rapid turnover. We would like every part of each of these two compartments to exchange carbonate carbon with the blood at a rate characteristic of the whole compartment in which it is located. Since we know that this is not what really happens we know that whatever relationship we derive for the ideal cat will be only an approximation to the truth.

As with the 'ideal' cat so with the 'ideal' metabolite; the metabolite we are calculating for is one which is burned in every small unit of the 'soft tissue' compartment at a rate characteristic for soft tissue and directly proportional to the volume of the unit, and in every small unit of the 'bone' compartment at a rate characteristic for bone and again directly proportional to the volume of the unit. Circulating glucose may represent such a metabolite to a very rough degree of approximation and is used as a concrete example in this discussion.

With regard to other assumptions which are implicit in the model of Fig. 1, no provision is made for carbon dioxide fixation because these reactions are negligible in quantity (Buchanan, 1951; Greenberg & Winnick, 1949; Skipper, 1952). Also no provision is made for the movement of carbon from the alveolar and tidal air into the gas exchange tissue of the lungs and thence into the blood bicarbonate. Kornberg and others (Kornberg, Davies & Wood, 1952; Wood, Lifson & Lorber, 1945) have observed that the blood bicarbonate and the respiratory carbon dioxide arrive at the same $^{14}C$ content (within 5%) very soon after [$^{14}C$]-bicarbonate administration, so that the alveolar air and tidal air carbon dioxide may for our purposes be considered as part of the blood carbon dioxide pool.

*The correspondence between the ideal cat and the real cat.* The equation of Kornberg for the fraction (designated as $1-P$) of the $^{14}C$ of an intravenous dose of [$^{14}C$]bicarbonate retained in the cat at time $t$ is

$$1-P=0{\cdot}284e^{-0{\cdot}243t}+0{\cdot}415e^{-0{\cdot}0234t}+0{\cdot}302e^{-0{\cdot}00119t}. \quad (1)$$

By means of the operations shown in the mathematical section it is established that the size of the blood compartment, $A_B$, is fixed by the equation at 75·9 mg. carbon when 6 mg. $CO_2$/min. are respired (2 kg. cat), and that the rates, $k_2$ and $k_1$, at which bicarbonate enters $A_2$ and $A_1$, respectively, from $A_B$ are 7·96 and 2·35 mg. C/min. It is also shown that the $CO_2$ carbon production ($k_4$ and $k_3$) in compartments $A_1$ and $A_2$ and the sizes of these compartments are interrelated in such a way that in fixing one of these values all of them are established. Inasmuch as $k_3+k_4$ must equal the rate of $CO_2$ expiration, or 6 mg. C/min., the values of $k_3$, $k_4$, $A_1$ and $A_2$ are confined within certain limits. Specifically, as $k_4$ varies from 0 to 6 mg. C/min., $A_1$ rises from 1397 to 4968 mg. C, $k_3$ falls from 6 to 0 mg. C/min., and $A_2$ falls from 273·8 to 156·2 mg. C. Furthermore, it is discovered that the identical $^{14}C$ retention equation arises regardless of how the $^{12}CO_2$ production actually happens to be distributed between the $A_1$ and the $A_2$ compartments. The sizes of these two compartments remain unfixed (although knowledge of carbon dioxide production in either would establish the size of both), but it is not necessary to know either their individual sizes or their individual rates of carbon dioxide production to utilize the retention equation for the calculation of metabolite combustion rates.

The slow compartment, $A_1$, is predominantly bone carbonate and the fast compartment, $A_2$, the bicarbonate of the soft tissues. However, the total amount of bone carbonate for the 1·69 kg. cat is given by Kornberg (1953) as 2000 mg. C, so that if $CO_2$ production in $A_1$ is close to zero as it probably is (Kornberg gives 0·001 mg. C/min. for the total $CO_2$ production by bone in the 1·69 kg. cat), then only $1397/(2000\times 2{\cdot}00/1{\cdot}69)$ or 1397/2360 of the total bone carbonate (2 kg. cat) is included in the equations. Indeed this is to be expected inasmuch as the expression of Kornberg for $^{14}CO_2$ retention covers only the first 5 hr. subsequent to the administration of [$^{14}C$]bicarbonate. Buchanan & Nakao (1952) have shown that bone has slow and fast components for carbonate turnover, and the equation of Kornberg obviously does not include the slower components. On the other hand, the amount of [$^{14}C$] left in this compartment 240 min. after intravenous [$^{14}C$]bicarbonate injection as calculated by eqn. 12 is about 95% of the total $^{14}C$ left in the cat, whereas Kornberg measured only about 30% of the residual $^{14}C$ in the bone carbonate at that point in time. We must be prepared for the possibility that not all of the slow compartment, $A_1$, is to be found in bone.

If $CO_2$ production in the bone compartment is close to zero the amount of total $CO_2$ carbon in the fast compartment is close to 270 mg. This is somewhat more than the 170 mg. or so which might be expected on the basis of the tissue analysis of Kornberg, and we must then be prepared for the possibility that some portion of the bone carbonate turns over rapidly enough to be included in the fast compartment. However, it should be noted that Wallace & Hastings's (1942) muscle $CO_2$ analysis gave 12 m-moles/kg. cat muscle as against Kornberg's 8·3 m-moles/kg.

Finally, the size of the 'blood' compartment, $A_B$, is fixed at about 76 mg. C, whereas Kornberg reported that the initial specific activity of blood bicarbonate after intravenous [$^{14}C$]bicarbonate was such that the $^{14}C$ could be considered as confined to the total $CO_2$ of the blood which for the 2 kg. cat would be about 45 mg. C. The selection of the exact 3-term exponential to represent the $^{14}CO_2$ retention

data is a fairly arbitrary procedure, and the equation of Kornberg was, of course, chosen without reference to the implications demonstrated here.

To clear up the discrepancy in the size of the 'blood' compartment and also to test the influence of arbitrary changes in the $^{14}CO_2$ retention equation, another exponential expression was chosen for which $A_B = 45$ mg. carbon at a $CO_2$ excretion rate of 6 mg. C/min.

$$1-P = 0{\cdot}34e^{-0{\cdot}371t} + 0{\cdot}31e^{-0{\cdot}0207t} + 0{\cdot}35e^{-0{\cdot}00185t}. \quad (2)$$

This expression for retained $^{14}C$ agrees within 6 % with Kornberg's retention equation between $t = 20$ min. and $t = 300$ min. The error at $t = 300$ (−6 %) was introduced in an effort to decrease the fraction of the retained $^{14}C$ present in the slow compartment at $t = 240$ by making the slopes of the last two exponentials as nearly alike as was thought to be reasonably consistent with the data. This effort was unsuccessful in that the fraction was reduced from 95 % by only a few per cent. Metabolic radiocarbonate retention calculated from this equation is compared below (see Table 1) with that calculated from Kornberg's original expression.

*Calculations of metabolite combustion rates.* As shown in the mathematical section, the introduction of [$^{14}C$]carbonate into each individual compartment of the system leads to a discrete set of equations for the retention of $^{14}C$ by the cat. The sum of the retentions in each of the individual compartments $(x+y+z)$ is the retention of $^{14}C$ by the whole cat, and is identical with Kornberg's expression designated as $(1-P)$ only when the [$^{14}C$]carbonate is introduced into the 'blood' compartment.

For the situation in which a circulating metabolite such as blood glucose is being converted into $CO_2$ at a uniform rate, is being kept at constant concentration by the secretion of liver [$^{12}C$]glucose, and is being kept at constant $^{14}C$ content by the constant infusion of a small amount of [$^{14}C$]glucose of high specific activity the following expression (as noted by Kornberg for [$^{14}C$]urea degradation) gives the fraction of the total $^{14}CO_2$ produced during $t$ min. which is retained by the cat at the end of the $t$ min.:

$$F = \frac{1}{t}\int_0^t (x+y+z)\,dt + c.$$

If one uses the expression $(x+y+z)_{A_B}$ (applying to injection of [$^{14}C$]carbonate into the 'blood' compartment) as Kornberg did, correctly, for the urease experiments the expression for $F$ becomes (from eqn. 13):

$$F_{(A_B)} = \frac{1}{t}[1{\cdot}169(1-e^{-0{\cdot}243t}) + 17{\cdot}74(1-e^{-0{\cdot}0234t}) + 253{\cdot}8(1-e^{-0{\cdot}00119t})].$$

However, the expression for $F$ when a metabolite is being burned in the 'soft tissue' compartment is (from eqn. 14):

$$F_{(A_2)} = \frac{1}{t}[-0{\cdot}310(1-e^{-0{\cdot}243t}) + 32{\cdot}8(1-e^{-0{\cdot}0234t}) + 258{\cdot}8(1-e^{-0{\cdot}00119t})].$$

Table 1. *Comparison of different methods of calculating metabolite combustion rate*

| $t$ (min.)* | $^{14}CO_2$ retained by the cat as a fraction of the total $^{14}CO_2$ which has been produced: Arbitrarily altered equation $F_{(A_2)}$† | Original equation $F_{(A_2)}$† | Original equation $F_{(A_B)}$† | Metabolite‡ combustion calculated by $F_{(A_B)}$ where $F_{(A_2)}$ applies. Calculated rate as percentage of true rate |
|---|---|---|---|---|
| 10 | 0·960 | 0·965 | 0·781 | 16·0 |
| 20 | 0·905 | 0·908 | 0·694 | 30·1 |
| 30 | 0·853 | 0·851 | 0·642 | 41·6 |
| 60 | 0·732 | 0·705 | 0·534 | 63·3 |
| 120 | 0·578 | 0·541 | 0·430 | 80·5 |
| 240 | 0·431 | 0·403 | 0·342 | 90·7 |

* Time in minutes which has elapsed since $^{14}CO_2$ began to be produced at a constant rate by combustion of a labelled metabolite.

† $F_{(A_2)}$ and $F_{(A_B)}$ are symbols for the exponential expressions (see text) representing $^{14}CO_2$ fractional retention by the cat when the $^{14}CO_2$ is produced at constant rate in the 'soft tissue' compartment ($A_2$) and the 'blood' compartment ($A_B$) respectively.

‡ This is a situation in which $^{14}CO_2$ arising in the soft tissues is erroneously treated as if arising in the blood. Suppose the $^{14}C$ content of a circulating labelled metabolite is $z$ $\mu$c/mg. C and it is burned in the soft tissues at the rate of 2 mg. C/min. or $2z$ $\mu$c/min. As shown by the value of $F_{(A_2)}$ at the 10 min. time interval, 96·5 % of the $20z$ $\mu$c of $^{14}CO_2$ produced are still present in the cat and only 3·5 % of $20z$ $\mu$c, or $0{\cdot}7z$ $\mu$c has been expired as $^{14}CO_2$. If we used $F_{(A_B)}$ for calculation we would suppose that the observed $0{\cdot}7z$ $\mu$c $^{14}C$ expired was (100–78·1) % or 21·9 % of the total $^{14}CO$ produced and would calculate total $^{14}CO_2$ production as being $0{\cdot}7z/0{\cdot}219$, or $3{\cdot}20z$ $\mu$c. This $3{\cdot}20z$ $\mu$c represents the production of 3·20 mg. $CO_2$ carbon from the circulating tagged metabolite during the 10 min. interval, or 0·32 mg. C/min. This is 16 % of the true combustion rate: 2 mg. C/min.

Table 1 shows how large the errors are which would be incurred at various times after the beginning of the experiment if $F_{(A_B)}$ were used for the calculation of [$^{14}$C]glucose combustion in the soft tissues, where $F_{(A_2)}$ properly applies. Table 1 also compares, at several values of $t$, the $F_{(A_2)}$ derived (from eqn. 15) for the alternative expression (eqn. 2) with the $F_{(A_2)}$ for Kornberg's own expression as given above. Since the figures are not much different it appears that any set of three exponentials which fits the retention data for intravenously injected [$^{14}$C]bicarbonate can be used for the calculation of metabolite combustion rates.

The $^{14}CO_2$ from many $^{14}$C metabolites which have been administered to animals appears rapidly in the respired air so there is reason to believe that not much of it arises in the 'slow' compartment, $A_1$. To establish this point in a quantitative way for any given metabolite it is necessary to kill an animal while it is in the process of metabolizing the $^{14}$C metabolite and to determine its total acid-volatile $^{14}CO_2$ for comparison with the total $^{14}CO_2$ respired up to the time of death. An additional amount retained in the animal over and above that predicted from the 'soft tissue' or $F_{(A_2)}$ equation above would allow a calculation for the $^{14}CO_2$ arising in the 'slow' compartment and so subject to the 'bone' equation.

On the other hand, some metabolites may produce $CO_2$ mostly in a single organ (e.g. liver) and for these the 'blood' compartment equation would be more nearly applicable, as was the case in the urease experiments of Kornberg. Here the ratio of $^{14}CO_2$ expired to the total acid-volatile $^{14}CO_2$ in the body of the animal at death would establish the fact that the $CO_2$ from this metabolite was acting as if produced in the 'blood' compartment, $A_B$.

## MATHEMATICAL

The differential equations which define the movement of $^{14}CO_2$ at any location in the model of Fig. 1 are as follow:

$$\left.\begin{aligned} (a)\quad \frac{dy}{dt} &= -K_1 y + K_2 x + K_3 z, \\ (b)\quad \frac{dx}{dt} &= -K_2 x + K_4 y, \\ (c)\quad \frac{dz}{dt} &= -K_3 z + K_5 y, \end{aligned}\right\} \qquad (3)$$

where $y$, $x$ and $z$ are $^{14}CO_2$ (in fractions of the original dose) in compartments $A_B$, $A_2$ and $A_1$ respectively at time $t$, and where

$$\begin{aligned} K_1 &= (k_1+k_2+k_3+k_4)/A_B, \\ K_2 &= (k_2+k_3)/A_2, \\ K_3 &= (k_1+k_4)/A_1, \\ K_4 &= k_2/A_B, \\ K_5 &= k_1/A_B. \end{aligned}$$

The several $K$'s represent those portions of the total $CO_2$ of the various compartments which are moved in the appropriate directions per min. in the steady state.

The characteristic equation (Morris & Brown, 1942) through which this set of differential equations can be solved is as follows:

$$m^3 + (K_1+K_2+K_3)\,m^2 + (K_1K_2 + K_1K_3 + K_2K_3 - K_3K_5 - K_2K_4)\,m + K_1K_2K_3 - K_2K_3K_4 - K_2K_3K_5 = 0. \qquad (4)$$

The roots of this equation ($m_1$, $m_2$, $m_3$) are the coefficients of $t$ in the following expressions for $y$, $x$ and $z$:

$$\left.\begin{aligned} y &= C_1 e^{m_1 t} + C_2 e^{m_2 t} + C_3 e^{m_3 t}, \\ x &= \left(\frac{K_4}{m_1+K_2}\right) C_1 e^{m_1 t} + \left(\frac{K_4}{m_2+K_2}\right) C_2 e^{m_2 t} + \left(\frac{K_4}{m_3+K_2}\right) C_3 e^{m_3 t}, \\ z &= \left(\frac{K_5}{m_1+K_3}\right) C_1 e^{m_1 t} + \left(\frac{K_5}{m_2+K_3}\right) C_2 e^{m_2 t} + \left(\frac{K_5}{m_3+K_3}\right) C_3 e^{m_3 t}, \end{aligned}\right\} \qquad (5)$$

where $C_1$, $C_2$ and $C_3$ are arbitrary constants which can be solved for at $t=0$ (the time $^{14}$C is introduced) for each case in which numerical values for $y$, $x$ and $z$ are known.

For the expression of Kornberg (eqn. 1) the values of $m_1$, $m_2$ and $m_3$ are $-0{\cdot}243$, $-0{\cdot}0234$, and $-0{\cdot}00119$, and the rate of loss of $^{14}CO_2$ from the whole system can be obtained by differentiating eqn. 1:

$$\frac{d(1-P)}{dt} = -0{\cdot}069012\,e^{-0{\cdot}243t} - 0{\cdot}009711\,e^{-0{\cdot}0243t} - 0{\cdot}00035938\,e^{-0{\cdot}00119t}.$$

This expression refers to the fraction of the original dose of $^{14}CO_2$ (introduced in compartment $A_B$) lost per min. from the whole system, and this loss occurs from $y$ (the $^{14}CO_2$ of the $A_B$ compartment) at the rate of $-6/A_B$ ($y$) per min. where 6 mg. $CO_2$ carbon/min. are expired from the $A_B$ mg. of $CO_2$ carbon present in the blood compartment. Thus

$$-\frac{6}{A_B}(y) = \frac{d(1-P)}{dt} \qquad (6)$$

and at $t=0$, where $y=1$,

$$A_B = 75{\cdot}8703.$$

(The value 6 mg. C/min. for respired $CO_2$ carbon is introduced for clarity and to demonstrate the calculated compartment sizes for the 2 kg. cat; actually the value N (mg. C/min.) if substituted in place of 6 cancels out later to give the same values for all the $K$'s as the value 6. Thus the relationships being derived here apply equally to large cats and small cats.)

From this value for $A_B$ the expression for $y$ (eqn. 6) is seen to be

$$y = 0{\cdot}87266\,e^{-0{\cdot}243t} + 0{\cdot}122796\,e^{-0{\cdot}0234t} + 0{\cdot}00454438\,e^{-0{\cdot}00119t}, \quad (7)$$

and by differentiating (7)

$$dy/dt = -0{\cdot}212056\,e^{-0{\cdot}243t} - 0{\cdot}00287343\,e^{-0{\cdot}0234t} - 0{\cdot}00000540781\,e^{-0{\cdot}00119t}.$$

The expression $dy/dt$ is the total loss of $^{14}CO_2$ from $A_B$, both to expired air and to compartments $A_2$ and $A_1$, or

$$-\frac{k_1+k_2+6}{A_B}(y)$$

and at $t=0$, where $y=1$:

$$-\frac{k_1+k_2+6}{75{\cdot}8703} = -0{\cdot}214935; \quad k_1+k_2 = 10{\cdot}3072. \quad (8)$$

Now since $k_3+k_4=6$ ($CO_2$ produced must equal $CO_2$ expired) and $K_1=(k_1+k_2+k_3+k_4)/A_B$

$$K_1 = 0{\cdot}214935, \quad (9)$$

eqn. 7 gives the values of the $C$'s, so that eqn. 5 for $x$ becomes

$$x = \left(\frac{K_4}{-0{\cdot}243+K_2}\right) 0{\cdot}87266\,e^{-0{\cdot}243t} + \left(\frac{K_4}{-0{\cdot}0234+K_2}\right) 0{\cdot}122796\,e^{-0{\cdot}0234t} + \left(\frac{K_4}{0{\cdot}00119+K_2}\right) 0{\cdot}00454438\,e^{-0{\cdot}00119t}.$$

But $K_4 = k_2/A_B = k_2/75{\cdot}8703$, so

$$x = k_2\left[\frac{0{\cdot}011502}{K_2-0{\cdot}243}\,e^{-0{\cdot}243t} + \frac{0{\cdot}0016185}{K_2-0{\cdot}0234}\,e^{-0{\cdot}0234t} + \frac{0{\cdot}0000598966}{K_2-0{\cdot}00119}\,e^{-0{\cdot}00119t}\right],$$

and since $x=0$ when $t=0$,

$$\frac{0{\cdot}011502}{K_2-0{\cdot}243} + \frac{0{\cdot}0016185}{K_2-0{\cdot}0234} + \frac{0{\cdot}0000598966}{K_2-0{\cdot}00119} = 0.$$

Similarly, for $z$,

$$\frac{0{\cdot}011502}{K_3-0{\cdot}243} + \frac{0{\cdot}0016185}{K_3-0{\cdot}0234} + \frac{0{\cdot}0000598966}{K_3-0{\cdot}00119} = 0.$$

Solving for $K_2$ and $K_3$ respectively:

$$K_2 = \frac{k_2+k_3}{A_2} = 0{\cdot}050979 \text{ or } 0{\cdot}0016801,$$

$$K_3 = \frac{k_1+k_4}{A_1} = 0{\cdot}0016801 \text{ or } 0{\cdot}050979.$$

At this point one must decide whether $A_1$ or $A_2$ is to be the compartment which turns over $CO_2$ at the slower rate. In Fig. 1 compartment $A_1$ has been designated as the slow compartment, so the values for $K_2$ and $K_3$ are

$$\left.\begin{aligned}\frac{k_2+k_3}{A_2} = K_2 = 0{\cdot}050979,\\ \frac{k_1+k_4}{A_1} = K_3 = 0{\cdot}0016801.\end{aligned}\right\} \quad (10)$$

Now in eqn. 4 the coefficient for the $m$ term is $(K_1K_2+K_1K_3+K_2K_3-K_3K_5-K_2K_4)$ and in the expansion of the roots of the characteristic equation for the Kornberg equation,

$$(m+0{\cdot}243)\,(m+0{\cdot}0234)\,(m+0{\cdot}00119) = 0,$$

the coefficient of the $m$ term is 0·006003216. Equating these coefficients, and substituting (from eqns. 9 and 10) the known values for $K_1$, $K_2$ and $K_3$ we have

$$K_5 = -30{\cdot}3428K_4 + 3{\cdot}21452.$$

Using the facts that $K_5 = k_1/A_B = k_1/75{\cdot}8703$ and $K_4 = k_2/A_B = k_2/75{\cdot}8703$ we observe that

$$k_1 = -30{\cdot}3428k_2 + 243{\cdot}887.$$

It is also known (from eqn. 8) that

$$k_1+k_2 = 10{\cdot}3072.$$

Solving these two equations for $k_1$ and $k_2$ we obtain

$$\left.\begin{aligned}k_1 = 2{\cdot}3468;\ k_1/A_B = K_5 = 0{\cdot}0309317,\\ k_2 = 7{\cdot}96039;\ k_2/A_B = K_4 = 0{\cdot}103921.\end{aligned}\right\} \quad (11)$$

It is to be noted that we have determined the values for $K_1$, $K_2$, $K_3$, $K_4$ and $K_5$ (eqns. 9–11) without having had to fix the exact values of $k_3$, $k_4$, $A_1$ and $A_2$. It is known that $(k_3+k_4)$ is 6, and that neither $k_3$ nor $k_4$ can be less than zero so (from eqns. 10 and 11) it is apparent that $k_3$, $k_4$, $A_1$ and $A_2$ are interrelated and are confined to certain regions as follows:

$$0 < k_4 < 6, \quad 1397 < A_1 < 4968,$$
$$6 > k_3 > 0, \quad 273{\cdot}8 > A_2 > 156{\cdot}2,$$

where $k_3+k_4 = 6$ mg. C/min.

The same procedures described above when applied to the alternative expression for $^{14}CO_2$ retention (eqn. 2) gives the following results:

$K_1 = 0{\cdot}352330$, $K_4 = 0{\cdot}157477$,
$K_2 = 0{\cdot}0384455$, $K_5 = 0{\cdot}0616493$,
$K_3 = 0{\cdot}00277430$,
$A_B = 45{\cdot}0435$,
$k_1 = 2{\cdot}7769$,
$k_2 = 7{\cdot}0933$,
$0 < k_4 < 6$, $1001 < A_1 < 3164$,
$6 > k_3 > 0$, $340{\cdot}6 > A_2 > 184{\cdot}5$,
$m_1 = -0{\cdot}371$,
$m_2 = -0{\cdot}0207$,
$m_3 = -0{\cdot}00185$,

where $k_2+k_4 = 6$ mg. C/min.

Combining the constants in eqns. 5 for the case in which (at $t=0$) $y=1$, $x=0$ and $z=0$ (for injection of $^{14}CO_2$ into the $A_B$ compartment) we have for the Kornberg expression

($^{14}CO_2$ starts in 'blood' or $A_B$),

$$\left.\begin{aligned} y &= 0{\cdot}872640\,e^{-0{\cdot}243t} + 0{\cdot}122816\,e^{-0{\cdot}0234t} \\ &\quad + 0{\cdot}00454375\,e^{-0{\cdot}00119t}, \\ x &= -0{\cdot}476814\,e^{-0{\cdot}243t} + 0{\cdot}467239\,e^{-0{\cdot}0234t} \\ &\quad + 0{\cdot}00957509\,e^{-0{\cdot}00119t}, \\ z &= -0{\cdot}111852\,e^{-0{\cdot}243t} - 0{\cdot}174905\,e^{-0{\cdot}0234t} \\ &\quad + 0{\cdot}286757\,e^{-0{\cdot}00119t}. \end{aligned}\right\} \quad (12)$$

The sum of the $^{14}CO_2$ in the three compartments is the total $^{14}CO_2$ retained by the cat and will be designated, for the case in which $^{14}CO_2$ starts in $A_B$, as $(x+y+z)_{A_B}$. Thus

$$(x+y+z)_{A_B} = 0{\cdot}284\,e^{-0{\cdot}243t} + 0{\cdot}415\,e^{-0{\cdot}0234t} + 0{\cdot}301\,e^{-0{\cdot}00119t}, \quad (13)$$

which is the original Kornberg expression.

For injection of $^{14}CO_2$ into the $A_2$ compartment $y=0$, $x=1$ and $z=0$ at $t=0$. Also for injection of $^{14}CO_2$ into the $A_1$ compartment $y=0$, $x=0$ and $z=1$ at $t=0$. Solving eqns. 5 for $C_1$, $C_2$ and $C_3$ under each of these sets of conditions and combining constants in each case:

($^{14}CO_2$ starts in 'soft tissue' or $A_2$),

$$\left.\begin{aligned} y &= -0{\cdot}231658\,e^{-0{\cdot}243t} + 0{\cdot}227006\,e^{-0{\cdot}0234t} + 0{\cdot}00465203\,e^{-0{\cdot}00119t}, \\ x &= 0{\cdot}126579\,e^{-0{\cdot}243t} + 0{\cdot}863617\,e^{-0{\cdot}0234t} + 0{\cdot}00980327\,e^{-0{\cdot}00119t}, \\ z &= 0{\cdot}0296932\,e^{-0{\cdot}243t} - 0{\cdot}323284\,e^{-0{\cdot}0234t} + 0{\cdot}293591\,e^{-0{\cdot}00119t}, \\ (x+y+z)_{A_2} &= -0{\cdot}0754\,e^{-0{\cdot}243t} + 0{\cdot}767\,e^{-0{\cdot}0234t} + 0{\cdot}308\,e^{-0{\cdot}00119t}; \end{aligned}\right\} \quad (14)$$

($^{14}CO_2$ starts in 'bone' or $A_1$),

$$\begin{aligned} y &= -0{\cdot}00609195\,e^{-0{\cdot}243t} - 0{\cdot}00952598\,e^{-0{\cdot}0234t} + 0{\cdot}0156179\,e^{-0{\cdot}00119t}, \\ x &= 0{\cdot}00332867\,e^{-0{\cdot}243t} - 0{\cdot}0362404\,e^{-0{\cdot}0234t} + 0{\cdot}0329117\,e^{-0{\cdot}00119t}, \\ z &= 0{\cdot}000780848\,e^{-0{\cdot}243t} + 0{\cdot}0135661\,e^{-0{\cdot}0234t} + 0{\cdot}985650\,e^{-0{\cdot}00119t}, \\ (x+y+z)_{A_1} &= -0{\cdot}00198\,e^{-0{\cdot}243t} - 0{\cdot}0322\,e^{-0{\cdot}0234t} + 1{\cdot}034\,e^{-0{\cdot}00119t}. \end{aligned}$$

For the alternative expression (eqn. 2) the corresponding solutions are:

$$\left.\begin{aligned} (x+y+z)_{A_B} &= 0{\cdot}340\,e^{-0{\cdot}371t} + 0{\cdot}310\,e^{-0{\cdot}0207t} + 0{\cdot}350\,e^{-0{\cdot}00185t}, \\ (x+y+z)_{A_2} &= 0{\cdot}0393\,e^{-0{\cdot}371t} + 0{\cdot}672\,e^{-0{\cdot}0207t} + 0{\cdot}368\,e^{-0{\cdot}00185t}, \\ (x+y+z)_{A_1} &= -0{\cdot}00256\,e^{-0{\cdot}371t} - 0{\cdot}0480\,e^{-0{\cdot}0207t} + 1{\cdot}051\,e^{-0{\cdot}00185t}. \end{aligned}\right\} \quad (15)$$

The same general procedure can be applied to a $^{14}CO_2$ retention equation with $n$ exponential terms, representing $(n-1)$ discernible peripheral compartments. For the three-peripheral compartment case where there are seven $K$'s a cubic equation leads to the evaluation of three $K$'s in place of the two $K$'s fixed by the quadratic equation leading to eqns. 10 above; three more $K$'s are determined in a manner analogous to the procedure leading to the evaluation of two $K$'s in eqns. 11 above, except that the coefficients of both the $m^2$ and the $m$ terms of the quartic equation produced by the expansion of the known roots $m_1$, $m_2$, $m_3$ and $m_4$ must be used. The $m^2$ and $m$ terms furnish two linear equations in three unknown $K$'s and a third linear equation in the same three $K$'s is furnished by

$$(K_4+K_5+K_7+K_1)=N,$$

where $-N$ is the value of $dy/dt$ at $t=0$, $y=1$, and $K_1$ is calculated from $d(1-P)/dt$ at $t=0$, $y=1$, and is the seventh of the seven $K$'s which are to be determined for the $^{14}CO_2$ retention equation.

## SUMMARY

1. A method is given and discussed for calculating the distribution of [$^{14}$C]bicarbonate in an idealized '3-compartment' animal.

2. The procedure has been demonstrated in detail, using the data of Kornberg (1953) and Kornberg *et al.* (1954), and shows a fair correspondence between the real cat and the model.

3. It is shown that different equations are required to express the retention of $^{14}CO_2$ formed uniformly throughout the soft tissues and the retention of $^{14}CO_2$ arising, or injected into, the blood. The parameters of the 'soft tissue' equation are derivable from those of the 'blood' equation.

4. It is shown how measurements of the ratio of $^{14}CO_2$ expired to the total $^{14}CO_2$ of the body can give information as to whether $^{14}CO_2$ is produced throughout the soft tissues or only in a small portion of them. In the latter case, the retention is more nearly described by the 'blood' equation.

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# Indirect Analysis of Corticosteroids

## 1. THE DETERMINATION OF 17-HYDROXYCORTICOSTEROIDS

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For the present purpose it is convenient to classify the known 17-hydroxycorticosteroids, according to the mode of substitution at the triad $C_{(17)}$–$C_{(20)}$–$C_{(21)}$, into the following four structural types: (*A*) 21-deoxy-17:20-ketols,* (*B*) 21-hydroxy-17:20-ketols (dihydroxyacetones), (*C*) 21-deoxy-17:20-glycols, and (*D*) 21-hydroxy-17:20-glycols (glycerols) (see Table 1). The last two groups (*C* and *D*) are degraded by periodate to 17-oxosteroids (cf. Reichstein & Shoppee, 1943); their indirect determination by measurement of the formed 17-oxosteroids as chromogens with *m*-dinitrobenzene and alkali (Zimmermann, 1935, 1936) was described by Talbot & Eitingon (1944) and by Fieser, Fields & Lieberman (1944). More recently Brooks & Norymberski (1952, 1953) have shown that bismuthate not only effects the above oxidations but also converts dihydroxyacetones (*B*) into 17-ketones and, on this basis, have developed an analytical procedure for the determination of 17-ketogenic steroids (types *B*, *C* and *D*).

The present report concerns a further development of the indirect analysis of 17-hydroxycorticosteroids by their conversion into 17-oxosteroids. The principal feature of this development consists in submitting an analytical sample to a sequence of reactions, the last of which is the oxidative fission of glycols, the presence and derivation of the latter being determined by the preceding operations. In particular, the following two analytical schemes have been evolved.

* Strictly applied the prefix 'deoxy' signifies the removal of an oxygen function already denoted in the name to which the prefix is added. Here, the term is used to indicate the absence of oxygen from a position at which it often occurs in closely related compounds; this provides a simple and unambiguous description for groups of compounds which will be frequently mentioned in the text.

### *Determination of all 17-hydroxycorticosteroids* (17-OHCS)

The analytical sample is reduced under conditions ensuring the quantitative conversion of ketones into alcohols, and is subsequently treated with sodium bismuthate: in the reductive step the 17:20-ketols (*A* and *B*) are converted into the corresponding 17:20-glycols (*C* and *D*), the latter, whether thus formed or originally present, are degraded to 17-ketones in the oxidative step. The total effect of this reaction sequence is the conversion of all 17-hydroxycorticosteroids (*A*, *B*, *C* and *D*) into 17-oxosteroids, whilst all other ketones originally present, including 17-oxosteroids, are reduced to alcohols. (Since the term '17-hydroxycorticosteroids' is currently employed to denote chromogens in the reaction of Porter & Silber (1950), i.e. dihydroxyacetone derivatives (*B*), it is suggested that, whenever confusion of terms is likely to occur, the group of compounds determined by the present method should be referred to as 'total 17-hydroxycorticosteroids'.)

### *Selective determination of* 21-*deoxy*-17:20-*ketols*

The analytical sample is treated with sodium bismuthate whereby 21-deoxy-17:20-ketols (*A*) remain unchanged while all other 17-hydroxycorticosteroids (*B*, *C* and *D*) are converted into 17-ketones. Subsequently the mixture is submitted to the reaction sequence described in the foregoing method, with the result that all 17-oxosteroids present in the final mixture of products are derived exclusively from 21-deoxy-17:20-ketols (*A*).

Each of the proposed schemes involves, at some stage, the reduction of ketones to alcohols. Sodium borohydride, which was first introduced by Chaikin & Brown (1949) for the selective reduction of