Calculation of the Albumin Catabolic

Rate in the Non-Steady State

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ABSTRACT Methods which are in current use for the calculation of the albumin breakdown rate apply only to the steady state animal. In this paper a simple but more general method based on analyses of I^{131}-albumin tracer data is presented. It utilizes easily measured plasma specific activity and excretory data and is equally applicable to the steady and non-steady states.

INTRODUCTION

In subsequent papers of this series we report I^{131}-albumin tracer studies in rabbits following extensive bowel resection (1, 2). These experiments present a particular problem in the calculation of the albumin breakdown rate, or efflux, because the plasma volume and total intravascular albumin of many animals decreased markedly during experimental periods. While various methods of estimating albumin breakdown have been described (3–8), none of them is entirely satisfactory for the non-steady state animal. We present here an extension of certain of these methods which has proved useful to us. It appears to be theoretically sound and equally applicable to the steady and most non-steady states. We base our derivation on the model developed by Reeve and Roberts (7) because we believe it provides a realistic description of albumin catabolism in the rabbit. Any system, however, which assumes breakdown in or closely associated with the vascular compartment may be used.

METHOD

The Albumin Model in the Steady State

Fig. 1 shows the deterministic model developed by Reeve and Roberts for the distribution and transfer of unlabeled albumin (Fig. 1, A) in an animal, and for the distribution, transfer, breakdown, and excretion of I^{131}-albumin and its breakdown products (Fig. 1, B) after injection into an animal. There are $\bar{x}$ and $\bar{y}$ grams of native albumin in the intravascular and extravascular com-

partments (Fig. 1, A), respectively. $\bar{k}_s$ grams per day are synthesized, while $\bar{k}_3$ grams per day leave the plasma and are catabolized in an unknown site. $\bar{k}_1$ grams per day pass out of the capillaries into the extravascular compartment, while $\bar{k}_2$ grams per day return to the vascular compartment, primarily via the lymphatics. When the animal is in the steady state, $\bar{x}$, $\bar{y}$, and the albumin fluxes, i.e., the $\bar{k}$'s, are constants. I^{131}-albumin follows this same schema of distribution and transfer after injection into the vascular compartment (Fig. 1, B). At any time, t, thereafter, the fraction of total administered radioactivity attached to albumin in the vascular compartment is x and in the
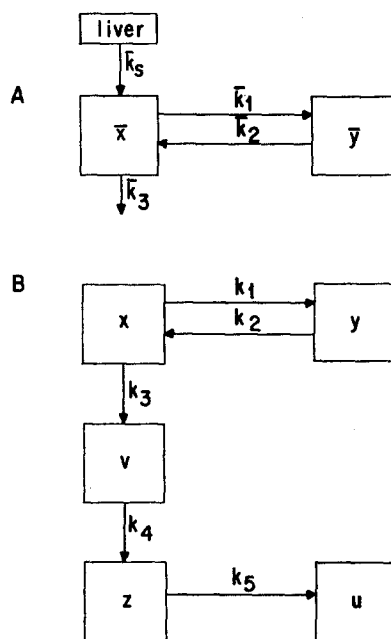

Figure 1. Model of distribution and transfer of unlabeled (A) and labeled (B) albumin in an animal. From Reeve and Roberts (7).

extravascular compartment is y. Since I^{131}-albumin is continually broken down with liberation of radioactive breakdown products, there are additional compartments: a breakdown compartment which at t contains a fraction, v, of the total activity; a breakdown products compartment which at t contains a fraction, z, of the total activity; and an excretion compartment in which radioactivity accumulates and which at t contains a fraction, u, of the total activity. The model can be described by the following set of differential equations:

$$\frac{dx}{dt} = k_2 y - (k_1 + k_3)x \tag{1}$$

$$\frac{dy}{dt} = k_1 x - k_2 y \tag{2}$$

$$\frac{dv}{dt} = k_3x - k_4v \qquad (3)$$

$$\frac{dz}{dt} = k_4v - k_5z \qquad (4)$$

$$\frac{du}{dt} = k_5z. \qquad (5)$$

In the steady state the tracer k's are constants.

These equations predict the form of the tracer functions, $x(t)$, $y(t)$, $v(t)$, $z(t)$, and $u(t)$, and provide solutions for the tracer k's. As confirmed by experiment (7), the plasma radioactivity in the rabbit in the steady state can be described by

$$x = C_1e^{-at} + C_2e^{-bt} \qquad (6)$$

and, since $b \gg a$, by

$$x = C_1e^{-at}. \qquad (7)$$

when $t \geq t_a$. Similarly, when the thyroid is blocked, the excretory data can be described, $t \geq t_a$, by

$$1 - u = C_3e^{-at} \qquad (8)$$

in which u represents the cumulative values of excreted (fecal and urinary) radioactivity. Numerical values for the intercepts and the slopes of these functions can be obtained by the methods of graphical analysis. These, in turn, provide a numerical solution for k_3 (5, 7),

$$k_3 = [C_1/a + C_2/b]^{-1}. \qquad (9)$$

Alternatively, k_3 can be calculated from equation 26 (8) or 27 (7) given below. Since k_3 represents the fraction of plasma I^{131}-albumin broken down each day, it also represents the fraction of intravascular native albumin broken down each day. The amount, therefore, of catabolized albumin in grams per day is $k_3\bar{x}$ or

$$\bar{k}_3 = k_3\bar{x}. \qquad (10)$$

Regardless of whether k_3 is obtained from equation 9, 26, or 27, calculation of the breakdown rate depends upon measurement of x and of $\bar{x}$.

Plasma Data in the Non-Steady State

Because measurements of the plasma concentration of radioactivity and of albumin can be performed simply and accurately, plasma data are conveniently expressed in terms of fractional specific activity, s, as follows:

Let

r = plasma radioactivity concentration in counts per minute per milliliter,

c = plasma albumin concentration in grams per milliliter,

V = plasma volume in milliliters,

and r_0, c_0, V_0, and $\bar{x}_0 = r$, c, V, and $\bar{x}$, respectively, at $t = 0$.

Then s is defined as the radioactivity per gram of albumin at any time t divided by the radioactivity per gram at $t = 0$. That is,

$$s = \frac{r}{c} \bigg/ \frac{r_0}{c_0}. \tag{11}$$

Multiplying the numerator by V/V and the denominator by V_0/V_0, we obtain

$$s = \frac{x}{\bar{x}} \bigg/ \frac{x_0}{\bar{x}_0}.$$

By definition, $x_0 = 1$, and therefore,

$$s = \frac{x}{\bar{x}} \bar{x}_0. \tag{12}$$

In steady state experiments, where $\bar{x}$ is constant, x is determined by measuring s. No such convenient means of estimating x is available when $\bar{x}$ is a variable, nor can $\bar{x}$ itself be readily measured. It is possible that x and $\bar{x}$ could be obtained by repeated plasma volume measurements since both are products of a concentration and V; however, this is not usually practicable. One of the requirements, therefore, of a non-steady state solution for the albumin efflux is that it not depend on measurement of x and $\bar{x}$.

Derivation of a Non-Steady State Solution for the Efflux

In this derivation, $\bar{x}$, $\bar{y}$, the albumin fluxes, and, consequently, k_1, k_2, and k_3 are time-dependent variables. The original notation is retained for convenience. k_4 and k_5 are treated as constants. k_4 cannot be measured directly, but measurements in highly unsteady states (2) indicated that k_5 remained constant.

For the first group of experiments reported in this series (1), the derivation was simplified by the nature of the data. Despite marked changes in V and $\bar{x}$, the excretory data could be described by equation 8 and the plasma data by

$$s = E_1 e^{-\alpha t} + E_2 e^{-\beta t} \tag{13}$$

and, when $t \geq t_a$, by

$$s = E_1 e^{-\alpha t}. \tag{14}$$

The similarity between equation 13 and equation 6 may be fortuitous, or perhaps reflects the exponential nature of $\bar{x}$ in these experiments. Proceeding from equations 3, 4, and 5, we obtain

$$k_3 x = u' + v' + z' \tag{15}$$

or in terms of u' and its derivatives

$$k_3 x = u' + u''/k_4 + u''/k_5 + u'''/k_4 k_5. \tag{16}$$

If the excretory data can be described by equation 8, $u'' = -au'$, $u''' = a^2 u'$, and

$$k_3 x = u' \cdot \frac{k_4 - a}{k_4} \cdot \frac{k_5 - a}{k_5}, \quad t \geq t_a. \tag{17}$$

Substituting from equation 12,

$$k_3 \bar{x} = \bar{x}_0 \frac{u'}{s} \cdot \frac{k_4 - a}{k_4} \cdot \frac{k_5 - a}{k_5}, \tag{18}$$

which we write, from equation 10, as

$$\bar{k}_3(t) = \bar{x}_0 \frac{u'}{s} \cdot \frac{k_4 - a}{k_4} \cdot \frac{k_5 - a}{k_5} \tag{19}$$

to emphasize that the albumin efflux, $\bar{k}_3$, may be a variable in the non-steady state.

From equations 8 and 14, we have

$$\bar{k}_3(t) = \frac{\bar{x}_0 FaC_3}{E_1} e^{(\alpha - a)t}, \quad \text{with} \quad F = \frac{k_4 - a}{k_4} \cdot \frac{k_5 - a}{k_5} \tag{20}$$

where a, α, C_3, and E_1 are constants which can be obtained by graphical analysis of the plasma and excretory data. The term F is a correction factor for the small amount of radioactivity retained as v and z when $t \geq t_a$. Reeve and coworkers (7, 9) obtained approximate values of 2.0 and 2.5 days^{-1} for k_4 and k_5, respectively, and we used these values in our calculations. Since k_4 and k_5 are much larger than a, errors in their estimation have little effect, and F itself alters $\bar{k}_3$ only slightly (4 to 8 per cent in our experiments).

Finally, to find the mean albumin catabolic rate, $\bar{\bar{k}}_3$, during an experimental period, $t_1 \leq t \leq t_2$, with $t_1 \geq t_a$, we use

$$\bar{\bar{k}}_3 = \int_{t_1}^{t_2} \frac{\bar{k}_3(t) \, dt}{t_2 - t_1} = \bar{x}_0 F \frac{\displaystyle\int_{t_1}^{t_2} \frac{u'}{s} \, dt}{t_2 - t_1} = \bar{x}_0 F \frac{aC_3}{E_1} \int_{t_1}^{t_2} \frac{e^{(\alpha - a)t} \, dt}{t_2 - t_1} \tag{21}$$

or, $\alpha \neq a$,

$$\bar{k}_3 = \bar{x}_0 F \frac{aC_3}{E_1} \frac{e^{(\alpha-a)t_2} - e^{(\alpha-a)t_1}}{(\alpha - a)(t_2 - t_1)}. \tag{22}$$

In acute experiments, such as those reported elsewhere in this series (2), it may not be possible to fit s and $1 - u$ to exponential functions, and additional measurements may be required. While the activity, v, in the unknown breakdown site cannot be measured, z, which is in general equivalent to the fraction of injected activity in the iodide space (9), can be estimated fairly readily. Then the efflux can be calculated as follows:

From equations 15 and 12, we have

$$k_3\bar{x} = \bar{x}_0 \left[\frac{u' + v' + z'}{s} \right] \tag{23}$$

and from equations 4, 5, and 10,

$$k_3(t) = \bar{x}_0 \left[\frac{1}{k_4} \frac{z''}{s} + \frac{k_4 + k_5}{k_4} \frac{z'}{s} + k_5 \frac{z}{s} \right]. \tag{24}$$

Then, as with equation 21,

$$\bar{k}_3 = \bar{x}_0 \left[\frac{\frac{1}{k_4} \int_{t_3}^{t_4} \frac{z''}{s} dt + \frac{k_4 + k_5}{k_4} \int_{t_3}^{t_4} \frac{z'}{s} dt + k_5 \int_{t_3}^{t_4} \frac{z}{s} dt}{t_4 - t_3} \right] \tag{25}$$

where $t_4 > t_3 \geq 0$. If empirical equations for z and for s can be obtained such that the integrations can be performed, the mean efflux $\bar{k}_3$ between t_3 and t_4 can be estimated. In practice, handling of z data in this way is difficult and compromises may have to be made (2).

DISCUSSION

Other investigators have used excretory and plasma data to calculate the albumin breakdown rate. One widely used equation is that of McFarlane (8),

$$k_3 = \frac{u_2 - u_1}{\xi(t_2 - t_1)} \tag{26}$$

in which the numerator represents the fraction of injected radioactivity excreted between t_1 and t_2 and ξ the mean value of x, when $t_1 \leq t \leq t_2$. This equation was modified by Reeve and Roberts in terms of their model.

With k_3 constant, equation 17 is integrated between t_1 and t_2 to give

$$k_3 \frac{\int_{t_1}^{t_2} x \, dt}{t_2 - t_1} = \frac{\int_{t_1}^{t_2} u' \, dt}{t_2 - t_1} \frac{k_4 - a}{k_4} \cdot \frac{k_5 - a}{k_5} .$$

Then

$$k_3 = \frac{u_2 - u_1}{\xi(t_2 - t_1)} \frac{k_4 - a}{k_4} \cdot \frac{k_5 - a}{k_5} . \tag{27}$$

A variation of equation 27, derived by Takeda and Reeve (10), can be obtained from equation 18. Substituting from equation 11,

$$k_3 \bar{x} = \bar{x}_0 \frac{u'}{r} \cdot \frac{r_0}{c_0} \cdot \frac{k_4 - a}{k_4} \cdot \frac{k_5 - a}{k_5}$$

and, since $\bar{x}_0 = V_0 \cdot c_0$ and $R = V_0 \cdot r_0$,

$$k_3 \bar{x} = R \frac{u'}{\frac{r}{c}} \cdot \frac{k_4 - a}{k_4} \frac{k_5 - a}{k_5}$$

where R is the total radioactivity in counts per minute injected at $t = 0$. Integrating and dividing by $t_2 - t_1$, as with equation 27,

$$k_3 \bar{x} \frac{\int_{t_1}^{t_2} \frac{r}{c} \, dt}{t_2 - t_1} = R \frac{\int_{t_1}^{t_2} u' \, dt}{t_2 - t_1} \cdot \frac{k_4 - a}{k_4} \cdot \frac{k_5 - a}{k_5}$$

or

$$k_3 \bar{x} = \frac{R(u_2 - u_1)}{\psi(t_2 - t_1)} \frac{k_4 - a}{k_4} \cdot \frac{k_5 - a}{k_5} \tag{28}$$

where $R(u_2 - u_1)$ is the total activity in counts per minute excreted between t_1 and t_2 and ψ is the mean specific activity in CPM/gm, when $t_1 \leq t \leq t_2$. Unlike equations 9, 26, and 27, equation 28 does not depend on measurement of x and $\bar{x}$. However, it treats not only k_3, but also the albumin efflux, $k_3 \bar{x}$, as constant. While these parameters may not vary in certain special circumstances, a general solution for $k_3 \bar{x}$ in the non-steady state requires that they be treated as functions of time.

Nearly all methods assume that breakdown of albumin takes place in or closely associated with the plasma compartment; there is good evidence to

support this assumption (11, 8, 12, 7). Additionally, all methods in which plasma and excretory data are used together require that k_4 and k_5 be treated as constants. Otherwise, the solutions cannot be carried beyond equation 15. In practice, small shifts in either k_4 or k_5 would not alter the efflux measurement significantly.

From the foregoing, it is clear that estimating the non-steady state albumin efflux from equation 21 combines several advantages. The estimate does not depend upon knowledge of x or $\bar{x}$, nor does it require any assumptions about the number of compartments or the distribution of albumin in the extravascular space. During the 3 to 5 day period before s and $1 - u$ become single component exponential functions, "biologic screening" (13) of the I[131]-albumin occurs, eliminating a possible source of error. On the other hand, if s and $1 - u$ are not exponential, it is possible to estimate the efflux from equation 25 or, if good excretory data are available, from a similar development of equation 16:

$$\bar{k}_3(t) = \bar{x}_0 \left[\frac{u'}{s} + \frac{k_4 + k_5}{k_4 k_5} \frac{u''}{s} + \frac{1}{k_4 k_5} \frac{u'''}{s} \right] \tag{29}$$

and

$$\bar{k}_3 = \bar{x}_0 \frac{\left[\int_{t_3}^{t_4} \frac{u'}{s} dt + \frac{k_4 + k_5}{k_4 k_5} \int_{t_3}^{t_4} \frac{u''}{s} dt + \frac{1}{k_4 k_5} \int_{t_3}^{t_4} \frac{u'''}{s} dt \right]}{t_4 - t_3} . \tag{30}$$

These equations provide general solutions for $\bar{k}_3$ and $\bar{\bar{k}}_3$ in terms of u and s. Finally, all equations of the method treat the product $k_3 \bar{x}$ as a single function and make no assumptions about either k_3 or $\bar{x}$.

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