APPENDIX

Models and Theory for Urea Metabolism

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1. Theories have been developed to try to interpret the effects of finite time of equilibration between plasma and body water, and the mechanism of renal function, on the variations of activity and specific activity of urea in plasma and urine after initial injection. 2. The magnitudes of errors likely to arise through approximations made in estimating the pool of body urea etc. have been derived. 3. Experimental results do not fit exactly with extreme models postulated, but are usually intermediate.

To interpret the observed time-dependence of total or specific activity in compartments of a communicating system investigated with isotopically labelled compounds, it is often necessary to postulate a model, because not all compartments may be accessible.

The basic model used in the present study (Scheme 1) envisages an overall steady state, i.e. total mass (M_i) of urea in any compartment i is independent of time (except, of course, in this instance for $i = 4$), but concentration is not necessarily uniform throughout a compartment. As usual, K_{ij} denotes the (constant) fraction of urea passing from compartment i into compartment j per unit time, and redistribution is regarded as instantaneous. If isotopically labelled urea is initially injected into the plasma only, the fraction X_i subsequently present in compartment i can be

calculated as a function of time, either from first principles, or with the help of the general solution of Rescigno (1956) to systems of equations:

$$\frac{dX_i}{dt} = \sum_{j=1}^n K_{ji}X_j - \sum_{j=1}^n K_{ij}X_i \quad (i = 1, 2 \dots n) \quad (1)$$

The phenomenon called 'excess excretion' may involve either of two processes, one due to the finite (although large) values of K_{13} and K_{31} and automatically allowed for in the solutions. The other, possibly due to the finite time for the dose to mix with plasma, would be a random rapid escape into the kidneys of a fraction E of the dose, equivalent to initial conditions $X_1 = (1 - E)$, $X_2 = E$. Although discussion has been mainly confined to these two possibilities the following treatment is general, allowing either to be selected as a special case.

If b_1 [k in the notation of the main paper (Regoeczi, Irons, Koj & McFarlane, 1965)] and b_2 are respectively the smaller and larger roots of the equation:

$$s^2 - s(K_{12} + K_{13} + K_{15} + K_{31}) + K_{31}(K_{12} + K_{15}) = 0 \quad (2)$$

$$b_1 + b_2 = K_{12} + K_{13} + K_{15} + K_{31} \quad (3)$$

$$b_1 b_2 = K_{31}(K_{12} + K_{15}) \quad (4)$$

and

$$\begin{aligned} X_1 &= (1 - E)[(K_{31} - b_1)e^{-b_1 t} \\ &\quad + (b_2 - K_{31})e^{-b_2 t}]/(b_2 - b_1) \\ &= (1 - E)(c_1 e^{-b_1 t} + c_2 e^{-b_2 t}) \end{aligned} \quad (5)$$

where

$$c_1 = (K_{31} - b_1)/(b_2 - b_1) \quad (6)$$

and

$$X_3 = (1 - E)(K_{13}e^{-b_1 t} - K_{13}e^{-b_2 t})/(b_2 - b_1) \quad (7)$$

Scheme 1. Interconnections of compartments that refer to urea: (1) in plasma; (2) in kidneys; (3) in body water; (4) excreted; (5) catabolized. Dashed lines represent possibility of return from kidneys or bladder. The dotted line separates system into two parts, the left-hand side being independent of the mechanism of kidney function (see the text).

The solutions for X_1 and X_3 of Scheme 1 are independent of what occurs to the right of the dotted line (assuming there is no return along the dashed routes). We are at liberty, therefore, to use these solutions in conjunction with alternative schemes for the kidneys.

For the renal mixing pool system illustrated:

$$X_2 = K_{12}(1-E)[c_1(e^{-b_1t} - e^{-K_{24}t})/(K_{24} - b_1) + c_2(e^{-b_2t} - e^{-K_{24}t})/(K_{24} - b_2)] + Ee^{-K_{24}t} \quad (8)$$

and

$$X_4 = K_{12}K_{24}(1-E)[c_1(e^{-K_{24}t}/K_{24} - e^{-b_1t}/b_1)/(K_{24} - b_1) + c_2(e^{-K_{24}t}/K_{24} - e^{-b_2t}/b_2)/(K_{24} - b_2)] + K_{12}(1-E)/(K_{12} + K_{15}) + E(1 - e^{-K_{24}t}) \quad (9)$$

The urine being excreted at any instant contains urea at the specific activity of the renal pool, X_2/M_2 .

An alternative (frontal) possibility visualizes the action of the kidneys as equivalent to a purely mechanical delay for a time t_d during which no isotope appears in the urine, but:

$$X_2 = K_{12}(1-E)[c_1(1 - e^{-b_1t})/b_1 + c_2(1 - e^{-b_2t})/b_2] + E \quad (10)$$

Subsequently

$$X_2 = K_{12}(1-E)[c_1e^{-b_1t}(e^{b_1t_d} - 1)/b_1 + c_2e^{-b_2t}(e^{b_2t_d} - 1)/b_2] \quad (11)$$

and

$$X_4 = K_{12}(1-E)[1/(K_{12} + K_{15}) - c_1e^{-b_1(t-t_d)}/b_1 - c_2e^{-b_2(t-t_d)}/b_2] + E \quad (12)$$

In this system the urine being excreted at any instant of time t ($> t_d$) has specific activity:

$$(1-E)[c_1e^{-b_1(t-t_d)} + c_2e^{-b_2(t-t_d)}]/M_1$$

corresponding to the plasma curve displaced by t_d to the right (Fig. 1).

For a given excretion rate and mass of urea in the kidneys, both mixing and frontal systems correspond to nearly the same value for t_d . Thus on a frontal basis:

$$t_d = M_2/M_2K_{24} = 1/K_{24}$$

whereas from eqns. (5) and (8) at long times:

$$(1-E)c_1e^{-b_1t}/M_1 = K_{12}c_1(1-E)e^{-b_1(t+t_d)}/M_2(K_{24} - b_1)$$

so that

$$e^{-b_1t_d} = M_2(K_{24} - b_1)/M_1K_{12} = (1 - b_1/K_{24}) \quad (13)$$

Fig. 1. Theoretical specific activity curves for urea in plasma (A) and in urine (B) leaving kidneys, based on primary (mixing) model (with $E=0$) and on typical experimental values for parameters as follows: $b_1=0.214$, $1/c_1=19$; $K_{12}=2.3$; $K_{15}=1.7$; $K_{24}=2.19$; $M_2K_{24}=24.1$ mg./hr. The ordinate unit is X_1/M_1 at $t=0$. The broken line represents the urine curve for a frontal delay mechanism.

In a typical situation b_1t_d is small enough for the exponential to be expanded as $(1 - b_1t_d)$. To this degree of approximation, therefore, t_d is still equal to $1/K_{24}$.

The experimentally measurable quantities are plasma urea (M_1) and its specific activity (X_1/M_1), the excretion rate ($M_1K_{12} = M_2K_{24}$), the excreted urea (M_4) and its specific activity (X_4/M_4), and the specific activity of urea excreted during a comparatively short time-interval. Theoretically, all the unknowns in any of the equations can be found by use of measurements made at several different times. In practice, because it is often very difficult to make these measurements with the necessary accuracy at times when there are, for instance, significant contributions from exponential terms other than those involving b_1 , extrapolation procedures are often used. From logarithmic plots of specific activity against time for either plasma or urea in the process of being excreted, b_1 can be obtained (Fig. 1). The horizontal distance (t_d) between the linear parts of these curves and the intercept $c_1(1-E)$ on the ordinate axis produced by extrapolation of the linear part of the plasma curve are also derived as a routine.

Another useful procedure, introduced by Walser & Bodenlos (1959), involved plotting X_4 against $1 - e^{-b_1(t-t_d)}$ and extrapolating to $t=t_d$. The significance of this is seen as follows. At sufficiently long times, eqn. (9) becomes:

$$X_4 = E + K_{12}(1-E)/(K_{12} + K_{15}) - K_{12}K_{24}(1-E)c_1e^{-b_1t}/b_1(K_{24} - b_1)$$

and the intercept the value of this at t_d . With eqn. (13) this reduces to:

$$(X_4)_{t_d} = E + K_{12}(1-E) [1/(K_{12} + K_{15}) - c_1/b_1]$$

which is also obtained with the alternative model by using eqn. (12). Eqns. (4) and (6) then show that:

$$(X_4)_{t_d} = E + K_{12}(1-E) (b_2 - K_{31})/b_2(b_2 - b_1)$$

which becomes E if we assume that K_{13} (and hence b_2) is practically infinite. If K_{31} and K_{13} are only moderately large, but E is zero, the extrapolation gives:

$$(X_4)_{t_d} = K_{12}(b_2 - K_{31})/b_2(b_2 - b_1) \simeq K_{12}/b_2$$

Extrapolation of X_4 as $t \rightarrow \infty$ gives:

$$E + K_{12}(1-E)/(K_{12} + K_{15})$$

in the first case and:

$$K_{12}/(K_{12} + K_{15})$$

in the second. Then:

$(X_4)_{t \rightarrow \infty} - (X_4)_{t_d} = K_{12}(1-E) c_1/b_1$ and $K_{12} c_1/b_1$ respectively. From eqns. (8) and (9) it is easily shown that summation of X_2 and X_4 at fairly long times, and extrapolation against $(1 - e^{-bt})$ leads at $t = 0$ to the same intercept:

$$E + K_{12}(1-E) (b_2 - K_{31})/b_2(b_2 - b_1)$$

Naturally, the same result also follows on a frontal basis. The error in assessing the contribution of X_2 is small whichever kidney mechanism is assumed, because at long times the values of X_2 given by eqns. (11) and (8) are in the ratio:

$$\begin{aligned} & (e^{b_1/K_{24}} - 1) (K_{24}/b_1 - 1) \\ &= (b_1/K_{24} + b_1^2/2K_{24}^2 \dots) (K_{24}/b_1 - 1) \end{aligned}$$

This becomes $(1 - b_1/2K_{24})$, neglecting higher-order terms as $b_1 \ll K_{24}$. The ratio is therefore close to unity.

The amount of urea in the body water can be deduced from the relation $M_1 K_{13} = M_3 K_{31}$ if K_{13}/K_{31} can be calculated. From eqns. (3), (4) and (6):

$$(K_{13}/K_{31}) = [(1 - c_1)/c_1] (1 - b_1/K_{31})^2$$

so that

$$(K_{13}/K_{31}) = (1 - c_1)/c_1$$

with a fractional error of $-2b_1/K_{31}$. This error is -0.033 in the example shown in Fig. 1, but zero if $K_{13} \rightarrow \infty$. If K_{13} and K_{31} are finite, the specific activities in body water and plasma compartments differ, the ratio $(M_1 X_3/M_3 X_1)$ being given at long times by eqns. (5) and (7) as:

$$M_1 K_{13}/M_3 (K_{31} - b_1) = K_{31}/(K_{31} - b_1)$$

which is 1.017 in the example shown in Fig. 1. There is a comparable error in assuming that the true synthesis rate:

$$M_1 (K_{12} + K_{15}) = b_1 (M_1 + M_3)$$

The experimental findings are not consistent with either of the extremes of kidney function postulated. Thus a kidney cannot conform to an ideal mixing pool because it contains regions of differing urea specific activity [Table 2 of the main paper (Regoecki *et al.* 1965)], nor can it exert a purely frontal delay, because (a) activity is found in the urine long before t_d and (b) the urine specific activity curve possesses a maximum. It must be concluded that the actual situation is equivalent to something between the extremes.

The origin of 'excess excretion', and allowance for it, poses problems. The shape of the plasma curve at short times implies that K_{13} is finite. However, in some cases where sufficient parameters were measured in a given experiment, an inconsistency arose which could be resolved only by assuming that both E and K_{13} were finite. It must therefore be concluded that neither the model with $K_{13} \rightarrow \infty$ nor the one with $E = 0$, but K_{13} finite, is adequate to explain all observations. Hence both types of contribution to 'excess excretion' should be taken into account if possible.

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