APPENDIX

An Analysis of Errors in Estimation of the Rate of Protein Synthesis by Constant Infusion of a Labelled Amino Acid

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Rates of protein synthesis in tissues can be calculated from the specific radioactivity of free and protein-bound amino acids at the end of a constant infusion of a labelled amino acid (Garlick, Millward & James (1973) *Biochem. J.* **136**, 935–945]. The simplifying assumptions used in these calculations have been criticized [Madsen, Everett, Sparrow & Fowkes (1977) *FEBS Lett.* **79**, 313–316]. A more detailed analysis using a programmable desk-top calculator is described, which shows that the errors introduced by the simplifying assumptions are small, particularly when the specific radioactivity of the free amino acid rises rapidly to a constant value.

Determination of rates of protein synthesis in tissues by constant intravenous infusion of a labelled amino acid, as described by Garlick *et al.* (1973), requires that measurements are made of the relative specific radioactivity of the free and protein-bound amino acid at the end of the infusion. In addition, an estimate of the rate of rise to a plateau value of the specific radioactivity of the free amino acid is required. Although compartmental analysis showed that this rise to the plateau value would be complex, it was suggested that the rise could be approximated to a single exponential, provided that the infusion time was long compared with the time taken for the intracellular amino acid to reach plateau. Three different approximations were suggested, depending on the amino acid infused and the tissue. It is now appropriate to reconsider the validity of these approximations for the following reasons. (i) The original calculations were made with experience of infusion of tyrosine, which reached plateau values very rapidly. In the accompanying studies proline was infused and in the muscle it rose to plateau values comparatively slowly. (ii) During the course of stretch-induced hypertrophy described in Laurent *et al.* (1978*b*) the marked changes in free proline pool sizes will alter the rate of rise to plateau, requiring that different approximations be used in the calculation at various times in the study. (iii) Madsen *et al.* (1977) attempted a compartmental analysis by using a computer program to obtain the rate of synthesis without approximation of the rate of rise to plateau of the precursor amino acid. They stated that the approximation suggested by Garlick *et al.* (1973) introduced an error of up to 34%. We believe these results to be incorrect because transfers of label between the compartments of the model analysed by Madsen *et al.* (1977) were described by inappropriate differential equations. However, the computer analysis approach can clearly by-pass the need for approximations, which were originally devised to simplify the analysis in the absence of computing facilities. Here we describe a comparison of the approximate method of Garlick *et al.* (1973) with a more complete compartmental analysis using a widely available programmable desk-top calculator.

Method 1 (*full analysis*)

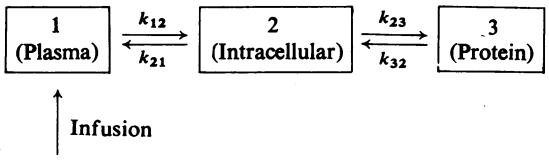
The model and notation of Madsen *et al.* (1977) was used (Scheme 1), and the equations describing the specific radioactivities in the three compartments (A_1, A_2 and A_3) in the steady state are:

$$A_1 = A_{1(\max.)}\,(1 - e^{-\lambda_p t}) \qquad (1)$$

$$dA_2/dt = k_{12}\frac{q_1}{q_2}A_1 - (k_{21} + k_{23})A_2 \qquad (2)$$

$$dA_3/dt = k_{23}\frac{q_2}{q_3}(A_2 - A_3) \qquad (3)$$

These equations differ in the following ways from those of Madsen *et al.* (1977). Eqn. (1) describes the observed specific radioactivity in the plasma as a single exponential with rate constant λ_p as observed in a number of studies (e.g. Waterlow & Stephen, 1967; James *et al.*, 1976; Laurent *et al.*, 1978*a*) rather than by its equivalent differential equation. Eqns. (2) and (3) include ratios of pool sizes (e.g. q_1/q_2) as described by Atkins (1972). The omission of these terms by Madsen *et al.* (1977) means that their analysis is only valid when the three pools are of equal size, which is never the case. In addition, breakdown of



Scheme 1. *Three-compartment model to represent amino acid incorporation into protein*

The model is used to predict the rate of rise to plateau of intracellular amino acid specific radioactivity during constant infusion of a labelled amino acid as described in the text. The three compartments have specific radioactivities A_1, A_2 and A_3 and contain amounts of amino acid q_1, q_2 and q_3. The rates of transfer of amino acid between compartments are described by the first-order rate constants k_{12}, k_{21}, k_{23} and k_{32}.

labelled protein has been allowed for in eqn. (3) (Zilversmit, 1960), but not in eqn. (2). This component is very small in tissues with slowly turning over proteins such as muscle, but has been included to improve the validity of the equations for tissues with more rapidly turning over protein, such as liver.

With suitable computing facilities these equations could have been solved to obtain the rate of protein synthesis from the experimental data. Instead they were first simplified by expressing all the k values as known multiples of the rate of protein synthesis. k_{23} is the rate of protein synthesis expressed as a fraction of the intracellular free amino acid pool transferred in unit time. However, we have generally expressed the rate of protein synthesis as a fraction of the protein pool and called this rate the fractional rate of protein synthesis, k_s. Hence $k_s = (q_2/q_3)k_{23}$, which in the steady state is equal to k_{32}, the fractional rate of protein breakdown.

Eqn. (3) then becomes:

$$dA_3/dt = k_s(A_2 - A_3) \qquad (4)$$

Eqn. (2) can be simplified by substitution from the following equations. In the steady state:

$$k_{21} = (q_1/q_2)k_{12}$$

and at plateau values:

$$(dA_2/dt) = 0 = k_{12}(q_1/q_2)A_{1(max.)} - (k_{21} + k_{23})A_{2(max.)}$$

where $A_{1(max.)}$ and $A_{2(max.)}$ are the plateau values of A_1 and A_2. Hence:

$$k_{21} = k_{23}A_{2(max.)}/(A_{1(max.)} - A_{2(max.)})$$

Substituting $A_{1(max.)}/(A_{1(max.)} - A_{2(max.)}) = P$ gives:

$$k_{21} = (P - 1)k_{23}.$$

The factor P, also used in Method 2, is the ratio of total rate of entry of amino acid into the intracellular pool to the rate of entry from protein breakdown alone. The final substitution is $R = q_3/q_2$ so that $k_{23} = Rk_s$. Eqn. (2) now becomes:

$$dA_2/dt = Rk_s[(P - 1)A_1 - PA_2] \qquad (5)$$

Eqns. (1), (4) and (5) were then solved simultaneously using a programmable desk-top calculator (Hewlett–Packard 97) to obtain a value of k_s for an experimental set of values for A_1, A_2, A_3, λ_p, P, R and t. Briefly, the program selects an estimate of k_s (k_s') by solving the approximate formulae described in Method 2. This estimate is then used to derive corresponding values of A_3 and A_2 from eqns. (1), (4) and (5) by calculation of the increments in A_3 (ΔA_3) and A_2 (ΔA_2) for a given increment in time (Δt) (usually taken to be 0.1 h). ΔA_3 and ΔA_2 were then summed from $t = 0$ to the end of the infusion. The value of A_3 at the end of the infusion was then compared with the experimentally derived value and if the two were not equal a better estimate of k_s (k_s'') was calculated from the expression:

$$k_s'' = k_s' \cdot \frac{A_{3\,(experimental)}}{A_{3\,(calculated)}}$$

This process was repeated until a value of k_s was found for which the calculated value of A_3 agreed with the experimental value.

Method 2 (with approximations)

This method was originally derived by analytical solution of eqn. (2) to obtain a two-exponential expression for A_2 (Garlick *et al.*, 1973). Rather than using this two-exponential expression, it was approximated to a single exponential, i.e. $A_2 = A_{2(max.)}(1 - e^{-\lambda_i t})$. This expression was substituted in eqn. (4), which was then solved to obtain a formula relating k_s to the experimental values of A_2 and A_3 at the end of the infusion:

$$\frac{A_3}{A_2} = \left(\frac{\lambda_i}{\lambda_i - k_s}\right)\left(\frac{1 - e^{-k_s t}}{1 - e^{-\lambda_i t}}\right) - \left(\frac{k_s}{\lambda_i - k_s}\right) \qquad (6)$$

This formula contains λ_i, but, provided the infusion time is long compared with the time taken to reach plateau, λ_i need only be known approximately and therefore three approximations were suggested, which took account of the fact that, regardless of the actual turnover rate of the intracellular pool (PRk_s), λ_i must always be either equal to or less than λ_p. Thus the choice of which approximation to use for λ_i for a given tissue and amino acid was made by applying the following criteria, which required that the rate of protein turnover in that particular tissue was known approximately:

(i) when $PRk_s > Rk_s > \lambda_p$, $\lambda_i = \lambda_p$

(ii) when $\lambda_p > PRk_s > Rk_s$, $\lambda_i = PRk_s$

(iii) when $PRk_s > \lambda_p > Rk_s$, $\lambda_i = Rk_s$

(P, R, λ_p and k_s were defined under 'Method 1').

This gave rise to three alternative formulae for calculating k_s by substituting one of the three expressions for λ_i in eqn. (6), depending on the tissue and the amino acid infused.

Results and Discussion

Table 1 shows estimates of the rate of protein synthesis in the anterior latissimus dorsi muscle of the fowl calculated by both methods from data in Laurent *et al.* (1978*b*). In the control muscle (day 0) the estimates of k_s made by the approximate method and by the more complete compartmental analysis are in very close agreement, as they are for muscles after 7, 28 and 58 days of hypertrophy. By contrast, after 1 and 3 days of hypertrophy the estimates by the approximate formula are about 10% lower than those from the full analysis. At each of these times the appropriate approximation to use for calculation by Method 2 is $\lambda_i = PRk_s$, whereas calculations at 0, 7, 28 and 58 days, where the two estimates of k_s are in good agreement, use the approximation $\lambda_i = Rk_s$. However, if the approximation $\lambda_i = Rk_s$ had been used for all muscles, the estimates of k_s after 1 and 3 days of hypertrophy would still be about 10% in error, but in the opposite direction. Whichever approximation is selected, the error in using the approximate formula is unlikely to amount to more than 10%.

The difficulty with the approximate method in the present case lies in the slow rise to plateau of [^{14}C]-proline specific radioactivity in the intracellular pool. During hypertrophy there are very large alterations in the size of the intracellular proline pool, which is a major determinant of the rate of rise to plateau. With amino acids that rise to plateau more quickly (e.g. tyrosine), the plateau is maintained for a high proportion of an infusion lasting 6h and therefore alterations in the rate of rise to plateau have a correspondingly smaller influence on the estimate of k_s obtained. For example, for 6h infusions with calculation by Method 2, a doubling of the value of λ_i from 15 to 30 days^{-1} (cf. proline) alters the estimate of k_s by about 13%, whereas a doubling from 50 to 100 days^{-1} (cf. tyrosine) alters the estimate of k_s by only 4%. With infusions of tyrosine in various tissues of rats, the difference between the two methods of calculation is not more than 2% (data of Garlick *et al.*, 1975). By contrast, Madsen *et al.* (1977) concluded that the difference between the methods of calculation for tyrosine in dog heart was as high as 34%. The reason for this difference is the very slow, almost linear, rise in specific radioactivity of intracellular tyrosine predicted by their equations (results kindly supplied by I. A. MacDonald and G. E. Lobley). Such a slow rise results from the incorrect formulation of their equations (as discussed above), and is unlikely to occur since in rabbit muscle (Nicholas *et al.*, 1977) and mouse brain (Garlick & Marshall, 1972) the plateau value for specific radioactivity of intracellular tyrosine is achieved within 1h of infusion. In rat muscle when k_s is 11.0% per day the measured value of λ_i is 40 days^{-1}, which is very close to the value predicted by Rk_s (44 days^{-1}) (D. J. Millward & P. C. Bates, unpublished work). Madsen *et al.* (1977) did not give enough information to enable recalculation by the methods described here, but, when we estimate k_s from similar results of Everett *et al.* (1977), the discrepancy between the

Table 1. *Estimates of the rate of protein synthesis (k_s, % per day) in the anterior latissimus dorsi muscle of the fowl by two methods*

Birds were infused with [^{14}C]proline and results calculated from data in Laurent *et al.* (1978*b*). k_s (full analysis) was calculated by Method 1, and Rk_s and PRk_s were calculated by using this value. k_s (approximate) was calculated as described in Method 2 by using the appropriate approximation for λ_i (i.e. $\lambda_i = Rk_s$ or $\lambda_i = PRk_s$) as marked with an asterisk (*). λ_i' is the numerical value of λ_i that, when used with the approximate formula (eqn. 6), would give the same result as the full analysis (Method 1). λ_i' is useful for calculating the rate of synthesis of single proteins or protein fractions [e.g. sarcoplasmic and myofibrillar fractions; see Laurent *et al.* (1978*a*)] by the approximate formula (eqn. 6) since the compartmental analysis as shown is not suitable for this purpose. λ_p was taken to be 40 days^{-1} in all cases.

Hypertrophy (days)	k_s (%/day)		Rk_s (days^{-1})	PRk_s (days^{-1})	λ_i (days^{-1})
	Full analysis	Approx.			
0	17.0	17.6	18.4*	51.2	22.2
1	32.4	29.2	7.1	19.9*	11.4
3	32.2	29.0	9.0	25.1*	13.8
7	31.5	31.9	22.7*	63.2	24.5
28	25.8	25.2	32.9*	91.8	28.0
58	17.9	17.9	26.1*	72.8	26.1

methods appears to be no more than 4%. [Everett *et al.* (1977) gave no value for R. We have assumed a value of $R = 400$.]

We may conclude therefore that the approximation described by Garlick *et al.* (1973) will not result in serious error in the estimate of synthesis rate provided that their original condition, that the infusion time is long compared with the time taken to reach plateau, is met. When the choice of amino acid for infusion is governed by consideration other than its rapid increase to plateau, as was the case with proline in the fowl, an error of around 10% might result from use of the approximate formulae. In such cases the more complete analysis (Method 1) would result in improved accuracy. It should be noted, however, that even the full analysis gives only an estimate of the rise to plateau. For maximum accuracy it is best either to measure the rise to plateau by serial biopsy as did Nicholas *et al.* (1977), if this is possible, or to lengthen the infusion time to make the rising part of the curve an insignificant part of the total infusion time.

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