Computer Simulation of the Non-Steady-State Radioactive Labelling of a System of Metabolite Pools with Constant Rates of Influx and Efflux

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The derivation is given of the general case of a differential equation describing the labelling of a single metabolite pool that is subjected to constant rates of influx of radioactive material when the pool size is itself changing at constant rate. A programme, written in ALGOL, is briefly described that solves a set of these differential equations, and that can be used to simulate the radioactive labelling of a system of metabolite pools. A copy of the programme, together with examples of input and output, has been deposited as Supplementary Publication no. SUP 50001 at the National Lending Library for Science and Technology, Boston Spa, Yorks. LS23 7BQ, U.K., from whom copies can be obtained on the terms indicated in *Biochem. J.* (1970), 116, 7.

In the preceding paper (Randle, England & Denton, 1970) a model was described for the radioactive labelling of the tricarboxylate-cycle metabolites in rat heart perfused with [$1\text{-}^{14}\text{C}$]acetate. The model was defined by a series of differential equations describing the flow of radioactivity between metabolites within the system; these equations were then solved by digital computer to yield values of the pool specific radioactivities at times after the start of the simulation. The differential equations required to describe the system shown in Randle *et al.* (1970) were unusual in that it was necessary to derive equations that allowed the values of the pool sizes to change at the same time as the specific radioactivities of the pools were also changing, i.e. a non-steady-state system with respect to both flow of metabolite and flow of label. In this paper the derivation of the general case of the differential equation describing the flow of label into a metabolite pool, with any number of constant influxes and effluxes when the pool size is changing linearly with time, is given. A brief description of a programme used to solve sets of such equations is also given.

THEORY

Given a metabolite pool of initial size P , let there be i influxes, at constant rates k_i , and specific radioactivities a_i , so that the total flux of label into the system is $\sum_1^i k_i a_i$. Similarly, let there be j effluxes at rates k_j , so that the total flux of label out of the pool is $\sum_1^j k_j \alpha$, where α is the specific radioactivity of the pool at time t . It should be noted that the k_i and k_j are not rate constants, but have the dimen-

sions of amount $\times$ time $^{-1}$. Some values of k_i and k_j may be identical, as when the metabolite pool is linked to another with which it exchanges label without any net flow of material.

If q is the quantity of label in the pool:

$$\frac{dq}{dt} = \sum_1^i k_i a_i - \sum_1^j k_j \alpha \quad (1)$$

If Q is the quantity of material in the pool at time t , then:

$$Q = P + \left(\sum_1^i k_i - \sum_1^j k_j \right) t \quad (2)$$

and

$$\frac{dQ}{dt} = \sum_1^i k_i - \sum_1^j k_j \quad (3)$$

At any time:

$$\alpha = q/Q \quad (4)$$

Differentiating eqn. (4) with respect to t , using the quotient rule, gives:

$$\frac{d\alpha}{dt} = \frac{1}{Q} \cdot \frac{dq}{dt} - \frac{q}{Q^2} \cdot \frac{dQ}{dt} \quad (5)$$

Substituting α for q/Q in eqn. (5):

$$\frac{d\alpha}{dt} = \frac{1}{Q} \cdot \frac{dq}{dt} - \frac{\alpha}{Q} \cdot \frac{dQ}{dt} \quad (6)$$

Further substitution of eqns. (1) and (3), and then eqn. (2), in eqn. (6) gives:

$$\begin{aligned} \frac{d\alpha}{dt} &= \left[\sum_1^i k_i a_i - \sum_1^j k_j \alpha - \alpha \left(\sum_1^i k_i - \sum_1^j k_j \right) \right] / Q \\ &= \sum_1^i k_i (a_i - \alpha) / \left[P + \left(\sum_1^i k_i - \sum_1^j k_j \right) t \right] \end{aligned} \quad (7)$$

Two simplified systems, which can be derived from the general eqn. (7), are:

(a) where there is a single influx (k_1), and a single efflux (k_2), then:

$$\frac{d\alpha}{dt} = k_1(a - \alpha) / [P + (k_1 - k_2)t]$$

where a is the specific radioactivity of the material flowing into the pool.

(b) where the pool is of constant size:

$$\frac{d\alpha}{dt} = \sum_1^i k_i(a_i - \alpha) / P$$

The above analysis assumes that the mixing within the pool is uniform and instantaneous. The above derivation is also applicable to systems involving tracers other than radioactive ones, when concentration or atom per cent replaces specific radioactivity, as long as the tracer does not perturb the system.

PROGRAMME

The simulation of the labelling of a series of interconnected metabolite pools requires the solution of the set of differential equations describing the system. A programme was written in the Elliott 503 dialect of ALGOL 60 to perform this function. The set of equations were written explicitly into the programme in the form of a procedure, which was then called by the integration routine to give values of the functions at each step in the solution. The equations were written in terms of the initial pool sizes, the flow constants and metabolite specific radioactivities. One equation was present for each metabolite pool, there being a total of n pools. The programme was written in dynamic form to accept any number of pools, but if $n > 12$ changes in the printing format are required. The initial conditions required to start the solution are values of the metabolite specific radioactivities at $t = 0$.

The method chosen for the numerical solution of the differential equations was essentially Process 6 as described by England (1969). This is an explicit Runge-Kutta type of method, of order four, giving a fifth-order error estimate with six function evaluations per step. The error estimate was used to automatically adjust the integration-step length in the following way. If $e_i^{(j)}$ is the error in the i th specific radioactivity at the j th integration step, the step was accepted if:

$$\max. \left| \frac{e_i^{(j)}}{a_i^{(j-1)} - a_i^{(j)}} \right| < \text{preset error limit} \\ (i = 1, \dots, n)$$

If any $e_i^{(j)}$ caused this inequality to become false, the step was rejected, the step length halved and the integration repeated. If the left-hand side of the inequality was less than (error limit/2) the step length was doubled for subsequent steps. Whenever a step was accepted the e_i were subtracted from the respective increments in the a_i to obtain higher accuracy in the estimates of the a_i . This routine, although requiring approximately twice as much computer time as standard fourth-order Runge-Kutta solution routine (see e.g. Fox & Mayers, 1968), was used in preference since the latter gave no error estimates, and the stability and accuracy of the results are therefore dependent on the step length chosen. The normal present error limit was 0.01. With this error limit, the errors on the fourth-order approximation were calculated precisely enough to give overall errors of less than 0.01% after 100 steps when the method was tested on equations that could be solved analytically. The accuracy of the solutions are directly related to the accuracy of the error estimates, and England (1969) has quoted values of error estimates obtained with this routine. After some 60 integration steps with a system of five differential equations, the cumulative error estimates agreed to within 4% of the true errors.

A copy of the programme listed above, together with examples of input and output, has been deposited as Supplementary Publication no. SUP 50001 at the National Lending Library for Science and Technology, Boston Spa, Yorks. LS23 7BQ, U.K., from whom copies can be obtained on the terms indicated in *Biochem. J.* (1970), **116**, 7. A limited number of listings of the programme are also available from the author.

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