NONSTEADY-STATE THREE COMPARTMENT TRACER KINETICS

I. THEORY

TOBIAS L. SCHWARTZ *and* FRED M. SNELL

From the Department of Biophysics, State University of New York at Buffalo, New York 14214. Dr. Schwartz's present address is the Department of Neurology, Laboratory of Neurophysiology, College of Physicians and Surgeons, Columbia University, New York 10032.

ABSTRACT A set of differential equations is derived which describes the four unidirectional fluxes of a substance across the boundaries of the central compartment of a serially arranged three compartment system, and the amount of this substance present in the central compartment. An analytic solution is obtained which yields all of these quantities as functions of time. The analysis is associated with a defined set of repetitive experiments from which the necessary data are obtained and during which the two outer compartments must be subject to experimental control. The solution is applicable to both the initial steady state and a transient, time-dependent state created by making a step change in the initial conditions. It describes the fluxes and compartment size without assuming that constant kinetic coefficients relate the fluxes to compartmental quantities but is limited by the requirement that the response of the system be repeatable in time.

INTRODUCTION

The strengths and weaknesses of tracer compartmental analysis have been thoroughly discussed (see for instance Solomon, 1949, 1953, 1960, 1964; Robertson, 1957, 1962; Sheppard, 1962). Although there appears to be no theoretical obstacle to the examination of multicompartment, nonsteady-state systems (Lax and Wrenshall, 1953; Landahl, 1954; Hart, 1957), a major source of difficulty lies in the fact that such systems yield linear differential equations with nonconstant coefficients. Analytic solutions generally cannot be obtained, and numerical techniques must often be utilized (Sheppard, 1962). Consequently the literature is almost entirely confined to systems in a steady state.

It is well known that a study of the transient response of a system can be more revealing of underlying mechanisms than a simpler steady-state investigation. It is shown in this paper that an analytic solution may be obtained which yields the four unidirectional fluxes in a serially arranged three compartment system and the size of its middle compartment as functions of time. This analytic solution describes the

transient state following a step change in conditions from an initial steady state, and makes use of an experimental procedure designed to obtain the required tracer information. This experimental procedure and the associated analysis have been applied to the sodium-ion fluxes across the urinary bladder of the toad, *Bufo marinus*, and the results are reported in the following paper (Schwartz and Snell, 1968).

ANALYSIS

1. The System

The system to be analyzed is described in Fig. 1. It consists of three compartments in series. The two outer compartments, 1 and 3, represent accessible bathing solutions and the central compartment, 2, represents the tissue which, it is assumed, can

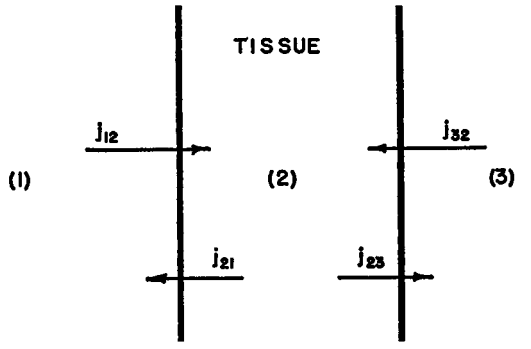

FIGURE 1 Three compartment model.

be approximated as a single homogeneous compartment. Let S_n denote the total amount of the substance under investigation present in compartment n, and j_{nm} denote its unidirectional flux from the nth to the mth compartment. The corresponding tracer quantities are denoted by superscripted asterisks. From the conservation of mass we then have

$$\frac{dS_2}{dt} = (j_{12} + j_{32}) - (j_{21} + j_{23}) \tag{1}$$

$$\frac{dS_2^*}{dt} = (j_{12}^* + j_{32}^*) - (j_{21}^* + j_{23}^*). \tag{2}$$

The specific activity of tracer in any compartment is defined by

$$a_m = \frac{S_m^*}{S_m} \tag{3}$$

giving

$$j_{mn}^* = a_m j_{mn}\,, \qquad m \gtrless n \tag{4}$$

if it is assumed that there is no discrimination between isotopes in the process.[1]

[1] Tracer amounts were measured as count rates in this work and the units of specific activity were consistent with this practice. A different method of specifying tracer amounts merely results in different units for specific activity.

Using equations 3 and 4, equations 1 and 2 can be combined to give

$$\frac{S_2}{a_2}\frac{da_2}{dt} = j_{12}\left(\frac{a_1}{a_2} - 1\right) + j_{32}\left(\frac{a_3}{a_2} - 1\right), \tag{5}$$

and a_2 can be eliminated to obtain

$$\frac{d}{dt}\ln j_{2n} = \frac{d}{dt}\ln j_{2n}^{*} - \frac{j_{12}}{S_2}\left(a_1\frac{j_{2n}}{j_{2n}^{*}} - 1\right) - \frac{j_{32}}{S_2}\left(a_3\frac{j_{2n}}{j_{2n}^{*}} - 1\right), n = 1, 3. \tag{6}$$

This expresses the species fluxes in terms of experimentally measurable time varying tracer fluxes. Equations 1 and 6 are insufficient to determine the four unidirectional fluxes and S_2. Further independent information is required, and a set of repetitive experiments must be devised for this purpose.

2. *Set of Experiments*

We wish to analyze the transient, time-dependent state following an imposed step change in conditions from an initial steady state. The existence of two kinds of steady states can be utilized to design the required experiments. Both types of steady state are defined by the time invariance of the concentrations or compartment sizes of the substance traced, and of its fluxes. In the first kind the tracer and the total test substance are both in a steady state, but in the second kind only the total test substance is in a steady state. The second kind is commonly approximated by the sudden addition of a trace amount of isotope to a system otherwise in a steady state.

A sudden step change in conditions from initial steady states of both the first and second kinds can be sequentially employed. Furthermore, the spatial symmetry of the system about compartment 2 makes it possible to repeat this procedure with tracer initially in either compartment 1 or 3. Four possible experiments thus result. The data to be analyzed consist of standards drawn from the "hot" incubating solutions, and the tracer activity in the appropriate outer compartment both during the initial steady state and during the ensuing transient state. The outer compartments may be monitored by serial sampling or any other method consistent with the required boundary conditions.

The step change may involve any of a variety of conditions which affect the fluxes whose time dependence is to be ascertained. A change in transmembrane voltage, transmembrane current, temperature, or the sudden application of activating or inhibiting drugs are all possibilities. In the study reported in the following paper (Schwartz and Snell, 1968) we have employed a sudden transition from an open-circuited to a short-circuited state—i.e. voltage clamping at zero potential difference between compartments 1 and 3.

A detailed description of the experimental protocol and its associated boundary conditions is in order. A set of four sequential runs are performed during which compartments 1 and 3 are constantly flushed with the proper flowing solution.

Run A. Compartment 3 contains high tracer activity. Compartment 1 is continuously flushed with "cold" solution. After a steady state of the first kind is reached, the monitoring of tracer activity in the effluent from compartment 1 is initiated. This point is denoted as $t = 0$. Following a suitable period of duration $t = \theta$, the step change in conditions is applied. Monitoring continues for the desired period. Conditions: $a_1(t) \backsimeq 0$, i.e., $a_1 \ll a_2$; $a_3(t) = a_3(0) =$ constant.

Run B. Compartment 3 initially contains high tracer activity and compartment 1 is continuously flushed with "cold" solution. After a steady state of the first kind is reached, compartment 3 is opened and also flushed continuously with "cold" solution. Following the washout of this compartment, the system is in a steady state of the second kind and monitoring of tracer activity in the effluent from compartment 1 is then initiated. This point is denoted as $t = 0$, and corresponds to the similarly denoted point in Run A. Following a period of duration $t = \theta$, the step change in conditions is applied. Monitoring continues for the desired period. Conditions: $a_1(t) \backsimeq 0$, i.e. $a_1 \ll a_2$; $a_3(t) \backsimeq 0$, i.e. $a_3 \ll a_2$.

Run C. This corresponds to Run A, with the procedures with respect to compartments 1 and 3, reversed. Conditions: $a_1(t) = a_1(0) =$ constant; $a_3(t) \backsimeq 0$, i.e. $a_3 \ll a_2$.

Run D. This corresponds to Run B, with the procedures with respect to compartments 1 and 3, reversed. Conditions: $a_1(t) \backsimeq 0$, i.e. $a_1 \ll a_2$; $a_3(t) \backsimeq 0$, i.e. $a_3 \ll a_2$.

The validity of an analysis based on such a set of experiments depends upon the response of the system being the same each time the change in conditions is reimposed. This implies that the changes in the initial conditions produce no irreversible effects on the processes involved so that the system returns to its initial state—except for the distribution of tracer—at the start of each run. In practice it is convenient to employ separately analyzable isotopes, one for Runs A and B, and another for Runs C and D.

3. *Analysis of the Transient Period*

Normalized variables based on experimentally measurable quantities are defined for the indicated runs as follows:

$$\xi_{21} = \frac{j^*_{21}}{a_3(0)} \qquad \text{during Run A} \tag{7a}$$

$$\eta_{21} = \frac{j^*_{21}}{a_3(0)} \qquad \text{during Run B} \tag{7b}$$

$$\xi_{23} = \frac{j^*_{23}}{a_1(0)} \qquad \text{during Run C} \tag{7c}$$

$$\eta_{23} = \frac{j^*_{23}}{a_1(0)} \qquad \text{during Run D.} \tag{7d}$$

In equations 7*b* and 7*d*, $a_3(0)$ and $a_1(0)$ refer to the initial specific activities used to incubate the tissue with tracer during the associated runs. In equations 7*a* and 7*c* they represent the actual activities in the pertinent compartments at $t = 0$ and throughout the run.

Employing these variables and the appropriate boundary conditions for each run we obtain from equation 6 the relations

$$\frac{d}{dt} \ln j_{21} = \frac{d}{dt} \ln \xi_{21} + \frac{j_{12} + j_{32}}{S_2} - \frac{j_{32} j_{21}}{S_2 \xi_{21}} \tag{8a}$$

$$\frac{d}{dt} \ln j_{21} = \frac{d}{dt} \ln \eta_{21} + \frac{j_{12} + j_{32}}{S_2} \tag{8b}$$

$$\frac{d}{dt} \ln j_{23} = \frac{d}{dt} \ln \xi_{23} + \frac{j_{12} + j_{32}}{S_2} - \frac{j_{23} j_{12}}{S_2 \xi_{23}} \tag{8c}$$

and

$$\frac{d}{dt} \ln j_{23} = \frac{d}{dt} \ln \eta_{23} + \frac{j_{12} + j_{32}}{S_2} \tag{8d}$$

which together with equation 1 comprise a set sufficient to describe the transient periods of Runs A–D. Proceeding, we define

$$\alpha_{2n} = \frac{j_{2n}}{S_2}, \qquad n = 1, 3 \tag{9}$$

which gives

$$\frac{d}{dt} \ln S_2 = \frac{j_{12} + j_{32}}{S_2} - (\alpha_{21} + \alpha_{23}) \tag{10}$$

with equation 1. Equations 8*a* and 8*b*, and similarly 8*c* and 8*d* can then be combined to yield

$$0 = \frac{d}{dt} \ln \left(\frac{\xi_{21}}{\eta_{21}} \right) - \frac{\alpha_{21} j_{32}}{\xi_{21}} \tag{11a}$$

and

$$0 = \frac{d}{dt} \ln \left(\frac{\xi_{23}}{\eta_{23}} \right) - \frac{\alpha_{23} j_{12}}{\xi_{23}}. \tag{11b}$$

Equations 8*b* and 8*d* with 10 yield

$$\frac{d}{dt} \ln \alpha_{21} = \frac{d}{dt} \ln \eta_{21} + (\alpha_{21} + \alpha_{23}) \tag{12a}$$

$$\frac{d}{dt} \ln \alpha_{23} = \frac{d}{dt} \ln \eta_{23} + (\alpha_{21} + \alpha_{23}). \tag{12b}$$

Since η_{21} and η_{23} are known from the data of Runs B and D respectively, equations 12 can be solved simultaneously for α_{21} and α_{23}. Equations 11 can then be solved for j_{32} and j_{12} since ξ_{21} and ξ_{23} are known from the data of Runs A and C, respectively. Finally equation 10 can then be solved for S_2.

Proceeding as outlined we subtract 12*a* from 12*b* to obtain

$$\frac{d}{dt} \ln \left(\frac{\alpha_{23}}{\alpha_{21}} \frac{\eta_{21}}{\eta_{23}} \right) = 0 \tag{13}$$

whose solution is

$$\alpha_{23} = K \left(\frac{\eta_{23}}{\eta_{21}} \right) \alpha_{21} \tag{14}$$

where K is the constant of integration. Equations 12*a* and 14 then give

$$\frac{\eta_{21}}{\alpha_{21}} \frac{d}{dt} \ln \left(\frac{\alpha_{21}}{\eta_{21}} \right) = \eta_{21} + K\eta_{23} \tag{15}$$

which can be rewritten as[2]

$$\frac{d}{dt} \left(\frac{\eta_{21}}{\alpha_{21}} \right) = - (\eta_{21} + K\eta_{23}) \tag{16}$$

from which the solution

$$p = \frac{\eta_{21}}{\alpha_{21}} = C - \int_{\theta}^{t} (\eta_{21} + K\eta_{23})\, d\tau \tag{17}$$

follows, and the varible p can be defined for convenience as shown. Here τ is a dummy variable, C is a constant of integration, and the lower limit of integration marks the time of application of the step change in conditions. It then follows that

$$\alpha_{21} = \frac{\eta_{21}}{p} \tag{18a}$$

and

$$\alpha_{23} = \frac{K\eta_{23}}{p} \tag{18b}$$

and j_{21} and j_{23} can be obtained from equations 9. When equations 17 and 18 are substituted into equations 11, we find that

$$j_{32} = p \frac{d}{dt} \left(\frac{\xi_{21}}{\eta_{21}} \right) \tag{19a}$$

and

$$j_{12} = \frac{p}{K} \frac{d}{dt} \left(\frac{\xi_{23}}{\eta_{23}} \right). \tag{19b}$$

---

[2] This follows from the fact that for any quantity μ

$$\frac{1}{\mu} \frac{d}{dt} \ln \mu = - \frac{d}{dt} \left(\frac{1}{\mu} \right).$$

Since all of the fluxes and the α's are now known, equation 10 can be solved for S_2. We can rewrite it as

$$\frac{d}{dt}\ln S_2 = \frac{p}{S_2}\frac{d}{dt}\left[\frac{1}{K}\left(\frac{\xi_{23}}{\eta_{23}}\right) + \left(\frac{\xi_{21}}{\eta_{21}}\right)\right] - \left(\frac{\eta_{21} + K\eta_{23}}{p}\right). \tag{20}$$

Upon differentiation, equation 17 gives

$$\frac{\eta_{21} + K\eta_{23}}{p} = -\frac{d}{dt}\ln p \tag{21}$$

which yields

$$\frac{d}{dt}S_2 - S_2\frac{d}{dt}\ln p = p\frac{d}{dt}\left[\frac{1}{K}\left(\frac{\xi_{23}}{\eta_{23}}\right) + \left(\frac{\xi_{21}}{\eta_{21}}\right)\right] \tag{22}$$

when substituted into equation 20. With the use of $1/p$ as an integrating factor (Ford, 1933), equation 22 becomes

$$\frac{d}{dt}\left\{\frac{S_2}{p} - \left[\frac{1}{K}\left(\frac{\xi_{23}}{\eta_{23}}\right) + \left(\frac{\xi_{21}}{\eta_{21}}\right)\right]\right\} = 0 \tag{23}$$

giving, upon integration,

$$S_2 = pg \tag{24}$$

where

$$g = F + \frac{1}{K}\left(\frac{\xi_{23}}{\eta_{23}}\right) + \left(\frac{\xi_{21}}{\eta_{21}}\right) \tag{25}$$

and F is a constant of integration.

To summarize then, the unidirectional fluxes are

$$j_{21} = \eta_{21}g \tag{26}$$

$$j_{23} = K\eta_{23}g \tag{27}$$

$$j_{32} = p\frac{d}{dt}\left(\frac{\xi_{21}}{\eta_{21}}\right) \tag{19a}$$

$$j_{12} = \frac{p}{K}\frac{d}{dt}\left(\frac{\xi_{23}}{\eta_{23}}\right) \tag{19b}$$

and the total amount of associated substance in compartment 2 is

$$S_2 = pg. \tag{24}$$

Each has been expressed in terms of experimentally measurable quantities.

The constants of integration, C, K, and F, can be evaluated from the initial conditions. Should there be a discontinuity in any of the variables at $t = \theta$, the instant of the step change in conditions, the values at $t = \theta+$ must be used. This question of continuity will be examined later. From equation 9 and 17

$$C = \frac{\eta_{21}(\theta)S_2(\theta)}{j_{21}(\theta)}, \tag{28}$$

and from equations 9 and 14

$$K = \frac{j_{23}(\theta)}{j_{21}(\theta)} \frac{\eta_{21}(\theta)}{\eta_{23}(\theta)}. \tag{29}$$

It will be demonstrated later that $F = 0$.

We have developed the solution for the nonsteady state from a particular set of experiments designed to gather the necessary independent information about the system. While it may be possible to design some other set of experiments for this purpose, the content of the solution would necessarily be the same since it must be unique.

The initial steady states must be examined to evaluate the integration constants.

4. Analysis of the Steady State

A. General Considerations. Prior to imposing the step change in conditions during Runs A through D, i.e. in the interval $0 \leqq t \leqq \theta$, the system is in a steady state of either the first or the second kind. Thus for all of these runs the conditions

$$\frac{d}{dt} S_2 = \frac{d}{dt} j_{21} = \frac{d}{dt} j_{23} = \frac{d}{dt} \xi_{23} = \frac{d}{dt} \xi_{21} = 0 \tag{30}$$

prevail throughout this period. However

$$\frac{d}{dt} \eta_{2n} \neq 0, \qquad n = 1, 3, \qquad 0 \leqq t \leqq \theta \tag{31}$$

since in Runs B and D we have a steady state of the second kind. For brevity, all quantities remaining constant during this initial period will hereafter be denoted by a parenthesized zero for t. Thus we define

$$m = \alpha_{21}(0) + \alpha_{23}(0) \tag{32}$$

and utilize equations 30 and 31 to write equations 10–12 in their steady-state forms:

$$0 = \frac{j_{12}(0) + j_{32}(0)}{S_{21}(0)} - m \tag{33a}$$

$$0 = \frac{d}{dt} \ln \eta_{21} + \frac{\alpha_{21}(0) j_{32}(0)}{\xi_{21}(0)} \tag{33b}$$

$$0 = \frac{d}{dt} \ln \eta_{23} + \frac{\alpha_{23}(0) j_{12}(0)}{\xi_{23}(0)} \tag{33c}$$

$$0 = \frac{d}{dt} \ln \eta_{21} + m \tag{33d}$$

$$0 = \frac{d}{dt} \ln \eta_{23} + m. \tag{33e}$$

Combining equations 33*b* with 33*d*, and 33*c* with 33*e*, we can write

$$m[\xi_{23}(0) - \xi_{21}(0)] = \alpha_{23}(0) j_{12}(0) - \alpha_{21}(0) j_{32}(0). \tag{34}$$

This gives

$$\xi_{23}(0) - \xi_{21}(0) = j_{23}(0) - j_{32}(0) \tag{35}$$

with the use of 33*a*. The steady-state net flux across compartment 2 is thus given by the difference between the measured normalized, unidirectional tracer fluxes of Runs A and C. One can demonstrate that an analogous expression is valid for a serially arranged four compartment system, and its applicability to a two compartment system is obvious. This suggests that equation 35 is independent of the assumed homogeneity of the central compartment. Other more general arguments toward this end have also been advanced (Schwartz, 1966), and Kedem and Essig (1965) have demonstrated the validity of equation 35 in a generalized, continuous, noncompartmentalized system by utilizing the techniques of nonequilibrium thermodynamics.

Equations 33*d* and 33*e* show that $\ln \eta_{21}$ and $\ln \eta_{23}$ are linear functions of time. This property can be utilized to check the validity of the assumption that the experimental system can be represented by a three compartment model. Unfortunately, however, these equations are not independent. The steady-state set, equations 33, is thus comprised of only four equations in the five unknowns—the four unidirectional fluxes and S_2—and the steady-state system is underdetermined. An additional independent experiment is needed. One which leads to an independent determination of either $S_2(0)$ or $S_2^*(0)$ would serve. The following two alternate experiments thus suggest themselves.

Run E. As in Runs C and D, compartment 1 is flushed with solution containing high tracer activity while compartment 3 is flushed with "cold" solution. When a steady state has been achieved, compartment 2—the tissue—is removed and analyzed for the value of $S_2^*(0)$.

Run E'. The tissue is analyzed for the value of $S_2(0)$ by some appropriate procedure.

B. Steady-State Calculations with $S_2^*(0)$ Known. From equations 3, 4, 7*c*, 9, 33*c*, and 33*e* it follows that

$$j_{12}(0) = m\left[\frac{S_2^*(0)}{a_1(0)}\right]. \tag{36}$$

It is seen from equations 33*d* or 33*e* that m can be evaluated from the slope of a semilogarithmic graph. Therefore $j_{12}(0)$ can be determined. Equations 33*c* and 33*e* yield

$$\alpha_{23}(0) = m\frac{\xi_{23}(0)}{j_{12}(0)} \tag{37}$$

so that $\alpha_{23}(0)$ can be calculated. Then

$$\alpha_{21}(0) = m - \alpha_{23}(0) \tag{38}$$

follows from the definition (32). With $\alpha_{21}(0)$ now known we can compute

$$j_{32}(0) = m\frac{\xi_{21}(0)}{\alpha_{21}(0)} \tag{39}$$

from equations 33*b* and 33*d*. From 33*a* we can calculate

$$S_2(0) = \frac{j_{12}(0) + j_{32}(0)}{m} \tag{40}$$

and, in turn, from the definitions (9)

$$j_{21}(0) = \alpha_{21}(0)S_2(0) \tag{41}$$

and

$$j_{23}(0) = \alpha_{23}(0)S_2(0). \tag{42}$$

Thus values for all the steady-state fluxes as well as for $S_2(0)$ can be determined.

C. Steady-State Calculations with $S_2(0)$ Known. Once again according to equations 33*d* or 33*e*, m can be evaluated from the slope of a semilogarithmic plot of available data. Then from 33*c*, 33*e*, and the definition of α_{23} in equation 9

$$j_{12}(0)j_{23}(0) = mS_2(0)\xi_{23}(0) \tag{43}$$

while from 33*a*

$$j_{23}(0) = mS_2(0) - j_{21}(0). \tag{44}$$

The net flux, known from the data and equation 35, can also be written as

$$j(0) = j_{12}(0) - j_{21}(0) \tag{45}$$

and equations 43, 44, and 45 can be combined to yield the quadratic expression

$$j_{12}^2(0) - j_{12}(0)[mS_2(0) + j(0)] + mS_2(0)\xi_{23}(0) = 0 \tag{46}$$

whose solution gives the two roots $j_{12}^{(1)}(0)$ and $j_{12}^{(2)}(0)$. Knowing $j_{12}(0)$ we can now calculate $j_{23}(0)$ from equation 43, $j_{21}(0)$ from equation 44, and $j_{32}(0)$ from equation 33*a*. Thus all four unidirectional steady-state fluxes are again determined.

Parenthetically we note that

$$j_{12}^{(1)}(0)j_{12}^{(2)}(0) = mS_2(0)\xi_{23}(0). \tag{47}$$

Comparison with equation 43 shows that

$$j_{12}^{(1)}(0) = j_{23}^{(2)}(0) \tag{48a}$$

and

$$j_{12}^{(2)}(0) = j_{23}^{(1)}(0), \tag{48b}$$

indicating that the two boundaries of compartment 2 cannot be distinguished from each other when the steady-state values are calculated in this manner, unless one has additional information as to which root provides the correct value for $j_{12}(0)$.

5. The Constants of Integration. The integration constants appearing in the solution describing the transient, time-dependent state (equations 28 and 29) can be evaluated from knowledge of the initial steady-state fluxes and $S_2(0)$ pro-, vided that there is no discontinuity in these values in going from $t = \theta-$ to $t = \theta+$ the point of application of the step change.

From equations 3, 4, 7*b*, and 28 one can deduce that

$$C = \frac{{}^{B}S_2^*(\theta)}{a_3(0)} \tag{49}$$

where the alphabetical superscript denotes the run to which the quantity refers. Since ${}^{B}S_2^*(\theta)$ is an amount of tracer in an inaccessible compartment it cannot change discontinuously. Therefore C must also be continuous. It is seen that C is the normalized amount of tracer present in compartment 2 at $t = \theta$ during Run B.

One may similarly ascertain from equation 29 that

$$K = \left[\frac{a_1(0)}{a_3(0)}\right]\left[\frac{{}^{B}a_2(\theta)}{{}^{D}a_2(\theta)}\right], \tag{50}$$

and since the a_2's are specific activities in an inaccessible compartment they too cannot change discontinuously. Thus K is also continuous at $t = \theta$. This constant corrects for any difference between the specific activities in compartment 2 during Runs B and D at $t = \theta$ due to causes other than a corresponding difference between the specific activities of the "hot" incubating solutions.

Only F of equation 25 remains for discussion. From equations 17, 24, and 25

$$F = \frac{S_2(\theta)}{C} - \left\{\frac{1}{K}\left[\frac{\xi_{23}(\theta)}{\eta_{23}(\theta)}\right] + \frac{\xi_{21}(\theta)}{\eta_{21}(\theta)}\right\}. \tag{51}$$

This together with equations 28 and 29 gives

$$F = \frac{j_{21}(\theta)}{\eta_{21}(\theta)}\left\{1 - \left[\frac{\xi_{23}(\theta)}{j_{23}(\theta)} + \frac{\xi_{21}(\theta)}{j_{21}(\theta)}\right]\right\}. \tag{52}$$

But it follows from equations 9 and the steady-state relations (33) that

$$\frac{\xi_{23}(\theta)}{{}_{23}(\theta)} + \frac{\xi_{21}(\theta)}{j_{21}(\theta)} = 1 \tag{53}$$

and F thus vanishes identically during the steady state. The continuity of this constant at $t = \theta$ follows from the fact that

$$F = \frac{S_2(\theta)}{C} - \left\{\frac{1}{K}\left[\frac{{}^{C}a_2(\theta)}{{}^{D}a_2(\theta)}\right] + \frac{{}^{A}a_2(\theta)}{{}^{B}a_2(\theta)}\right\} \tag{54}$$

which can be deduced from equations 4, 7, and 51.

SUMMARY AND CONCLUSIONS

Biological systems that can be approximated by a three compartment model are common. The two outer compartments often represent bathing media accessible to experimental control, while the central compartment represents the tissue. Until now the fluxes across the boundaries of the central compartment and the amount of tracer in that compartment could be thoroughly investigated by tracer techniques only during a steady state.

We have designed a set of four experiments which yield sufficient information to investigate a nonsteady state. It was necessary, for this purpose, to make a careful distinction between two types of steady states. The set of differential equations which describes the experiments was derived, and analytically solved for the desired fluxes and compartment size. The solution is valid for the time-dependent, transient state prevailing during the relaxation of the system to a final steady state after a step change has been made in certain of the conditions prevalent during an initial steady state. With the addition of one experiment it also describes the initial state. The assumption that constant kinetic coefficients relate the fluxes and compartmental quantities was not required. The associated integration constants were evaluated in terms of initial conditions.

The detailed investigation of time dependent states, which can be more revealing of transport mechanisms than steady-state experiments, is thus made easier. But success depends upon the system returning to the same state each time that the initial

conditions are reproduced, and then responding identically whenever the step change in conditions is reimposed. While this analysis is based on a given set of experiments, the content of a solution involving the same imposed changes but using some other experimental set would necessarily be the same for reasons of physical uniqueness.

Finally, we have have presented evidence that, for a system in a steady state of the first kind, the difference between the normalized unidirectional tracer fluxes $\xi_{23}(0)$ and $\xi_{21}(0)$, yields the net flux across the middle compartment even if that compartment is inhomogeneous.

Received for publication 9 January 1968.

REFERENCES

FORD, L. R. 1933. Differential Equations. McGraw Hill Book Co., Inc. New York.
HART, H. E. 1957. *Bull. Math. Biophys.* **19**:61.
KEDEM, O., and ESSIG, A. 1965. *J. Gen. Physiol.* **48**:1047.
LANDAHL, H. D. 1954. *Bull. Math. Biophys.* **16**:151.
LAX, L. C., and WRENSHALL, G. A. 1953. *Nucleonics.* **11**:18.
ROBERTSON, J. S. 1957. *Physiol. Rev.* **37**:133.
ROBERTSON, J. S. 1962. *In* Handbook of Physiology. Section 2: Circulation. American Physiological Society, Washington, D. C. 617.
SCHWARTZ, T. L. 1966. Doctoral Dissertation. State University of New York at Buffalo, Buffalo, N.Y.
SCHWARTZ, T. L., and SNELL, F. M. 1968. *Biophys. J.* **8**:818.
SHEPPARD, C. W. 1962. Basic Principles of the Tracer Method. John Wiley & Sons, Inc., New York.
SOLOMON, A. K. 1949. *J. Clin. Invest.* **28**:1297.
SOLOMON, A. K. 1953. *Advan. Biol. Med. Phys.* **3**:65.
SOLOMON, A. K. 1960. *In* Mineral Metabolism. C. L. Comar and F. Bronner, editors. Academic Press, Inc., New York. 119.
SOLOMON, A. K. 1964. *In* Transcellular Membrane Potentials and Ionic Fluxes. F. M. Snell and W. K. Noell, editors. Gordon & Breach Science Publishers, Inc., New York. 47.