Review Article

Theme: Pharmacokinetics, Biopharmaceutics and Bioequivalence: History and Perspectives

Guest Editors: Marilyn Martinez and Lawrence Yu

Compartmental Analysis and its Manifold Applications to Pharmacokinetics

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Abstract. In this paper, I show how the concept of compartment evolved from the simple dilution of a substance in a physiological volume to its distribution in a network of interconnected spaces. The differential equations describing the fate of a substance in a living being can be solved, qualitatively and quantitatively, with the help of a number of mathematical techniques. A number of parameters of pharmacokinetic interest can be computed from the experimental data; often, the data available are not sufficient to determine some parameters, but it is possible to determine their range.

KEY WORDS: exit time; flow graphs; permanence time; residence time; tracer kinetics; transfer function; turnover time.

THE STUDY OF NATURE

Scientists like Plinius, Leonardo da Vinci, Linnaeus, Newton, and Buffon were equally interested in animals, plants, minerals, volcanoes, earthquakes, and so forth. The study of science was divided into two branches, natural history and natural philosophy. The distinction between those two branches was a distinction of methods, not of substance.

A few generations ago, that distinction was abandoned and science was divided between biological sciences and physical sciences. This distinction is strongly emphasized in our modern universities. However, the present tendency of Science is to minimize all artificial separations and look for a common ground on all aspects of scientific investigations.

Again, we observe that new findings in one branch of science can help investigations in another branch of science. Probably a couple of centuries ago, our methods of investigation were not powerful enough to show substantial differences between the physical and the biological world. However, today, our methods of investigation have shown those differences to be irrelevant.

PHARMACOKINETICS

Pharmacokinetics is a typical example of this tendency of modern science to ignore all artificial divisions and to use methods borrowed from different disciplines. Pharmacokinetics,

in its current form, is the study of absorption, distribution, metabolism, and elimination of drugs. However, since the times of Avicenna, (and probably centuries before him), it was known that drugs are absorbed, distributed, metabolized, and eliminated. For this reason, we can say that pharmacokinetics has always been a part of pharmacology, though it was not until more sophisticated methods of investigation became available that pharmacokinetics was considered to be a new discipline.

The study of pharmacokinetics has three objectives: prediction, description, and prescription. Predictive pharmacokinetics consists in identifying both the parameters that are relevant to the prediction of kinetic properties of the drugs and identifying methods for their determination. Descriptive pharmacokinetics consists of measuring the relevant parameters of the drug and/or the formulation. Prescriptive pharmacokinetics consists of using known concepts and previously determined parameters for achieving an appropriate clinical goal.

In all of its applications, pharmacokinetics utilizes the concept of “compartment,” even though this term may not always be explicit. Although a complete description of all applications of compartmental analysis to pharmacokinetic problems is impossible within the limited space of a single manuscript, I shall try to explore from a “bird’s eye” view a number of examples illustrating the past, present, and possibly future uses of the concept of compartment.

MY PERSONAL EXPERIENCE

When I was a freshman at the University of Milan many, many years ago, from time to time, some medical students came to ask us, students of physics and of mathematics, for help. Their physiology professor had the audacity, never heard of before, of embellishing his lectures with derivatives and integrals. We did our best to help them and felt a sense of superiority for our ability to manipulate the formulas that filled their lecture notes. Our sense of superiority of course

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was totally unjustified; if they did not understand those formulas, we did not understand their applications.

Years later, after graduating and after the disruption of war, I went to work in an Italian hospital that was experimenting, for the first time in that country, with ^{131}I , a radioactive isotope of iodine. My duty was simple enough, preparing the doses to be administered to the patients, measuring the subsequent radioactivity of different organs, showing the results to the physician in charge, and leaving to him their interpretation.

But I had not forgotten my earlier interactions with the medical students, and I wanted to know more. A graph showing radioactivity in the thyroid as a function of time could be easily plotted, but what was its meaning? The graph had an initial slope, a maximum value, an area, and so forth, but how could those geometrical properties be transformed to physical or physiological or pharmacological properties? I started looking into the existing literature.

PHARMACOKINETIC INVARIANTS

Widmark and Tandberg (1) studied the elimination of several narcotics from the blood and found that in their final phase, they eliminated in an exponential manner. However, his most important contribution was the definition of what we now call the *volume of distribution*. Let us assume that a dose, D , of a drug is given to a subject as a bolus intravenous dose at time $t=0$, the $c(t)$ is its plasma concentration at time t , and that $c^*(0)$ is the extrapolated value of drug's concentration after its mixing in the blood but before its elimination (see Fig. 1). In that case, the ratio

$$V = \frac{D}{c^*(0)} \quad (1)$$

is the volume of the plasma.

While this observation may seem obvious, the experimental values of D and $c(t)$ may result in volume estimates that differ from the expected volume of the plasma. There may be several reasons for this outcome. For example, the drug may be bound to some tissue before its distribution inside the plasma. Nevertheless, V , even if it is not the

Fig. 1. Extrapolation of $c(t)$ for $t \rightarrow 0$

physiological volume of the plasma, is an important pharmacokinetic quantity. Under appropriate conditions, V represents the quantity that when multiplied by the measured concentration gives the dose present in the plasma. We can say that it is a *pharmacokinetic invariant*.

A few years later, Gehlen (2) showed that the interval of time needed by a particular drug to reach its maximum concentration in the blood, what we call now $t_{\max}$, does not change when adjusting the dose within certain limits. This is another pharmacokinetic invariant. Although I will consider more invariants later in this manuscript, for now, I will describe the three papers that I found to be most influential in my efforts to understand the world of compartment models.

PHYSIOLOGICAL COMPARTMENTS

Behnke *et al.* (3) studied the phenomenon of nitrogen absorption by elimination from various tissues via the lung and circulation in dogs and man. They had a subject breathe pure oxygen, after which the amount of nitrogen eliminated from the breath was measured. The result of a typical experiment is shown in Fig. 2.

Behnke *et al.* called $Y(t)$ the cumulative amount of nitrogen eliminated up to time t and A the total amount of nitrogen contained in the body at time $t=0$, the instant the subject started breathing pure oxygen. They observed that the curve of Fig. 2 could be fitted by a function of the form:

$$Y(t) = A(1 - e^{-kt}).$$

To give a physical meaning to the constants A and k in the above function, we first observe that

$$\lim_{t \rightarrow \infty} Y(t) = A,$$

i.e., A is the total amount of nitrogen present in the body at time $t=0$ that is eliminated by breathing. Making a slight modification to the notation used by Behnke *et al.* results in the form

$$X(t) = A - Y(t),$$

where $X(t)$ is the amount of nitrogen still present in the body at time t . Accordingly, it follows that

$$X(t) = Ae^{-kt}.$$

Differentiating the last function leads to the expression

$$\frac{dX}{dt} = -kAe^{-kt}.$$

Upon substituting $X(t)$ for Ae^{-kt} from the last equation, we obtain the expression

$$\frac{dX}{dt} = -kX(t). \quad (2)$$

This equation shows that the rate of elimination of nitrogen from the body is proportional to the amount that remains; in other words, that elimination is a first-order

Fig. 2. Elimination of nitrogen from human subjects breathing pure oxygen (from A. Rescigno, *Foundations of Pharmacokinetics*, Kluwer, New York, 2003)

process. In modern terminology, we should say that Eq. 2 shows that the nitrogen eliminated by breathing behaves as a *compartment*. On closer examination, Behnke *et al.* found that by using longer observation times, the washout curves in their experiments could be better described as the superposition of two exponentials and a constant term. In other words, that the nitrogen present in the fat and water phases of the body was associated with distinctly different elimination rate constants.

Using the work of Behnke *et al.* as a backdrop, we can interpret those results using our modern notation. Call $x_1(t)$, $x_2(t)$, and $x_3(t)$ the amount of nitrogen present in the body fat, in the aqueous body spaces, and in the environment, respectively, at time t . Two different hypotheses are possible: the nitrogen is transferred from the lipoid phase to the aqueous phase, and from this one to the environment, or it is eliminated from both phases directly to the environment.

The first case can be described by three differential equations:

$$\begin{cases} \frac{dx_1}{dt} = -k_1 x_1(t) \\ \frac{dx_2}{dt} = +k_1 x_1(t) - k_2 x_2(t) \\ \frac{dx_3}{dt} = +k_2 x_2(t) \end{cases} \quad (3)$$

where k_1 is the fractional rate of transfer from the lipoid phase to the aqueous phase and k_2 the fractional rate of transfer from the aqueous phase to the environment.

The second case can be described by the corresponding set of differential equations:

$$\begin{cases} \frac{dx_1}{dt} = -k_3 x_1(t) \\ \frac{dx_2}{dt} = -k_4 x_2(t) \\ \frac{dx_3}{dt} = +k_3 x_1(t) + k_4 x_2(t) \end{cases} \quad (4)$$

where k_3 is the fractional rate of transfer from the lipoid phase to the environment and k_4 the fractional rate of transfer from the aqueous phase to the environment.

In both cases, the solution takes on the form

$$x_3(t) = A - B \cdot e^{-\beta t} - C \cdot e^{-\gamma t} \quad (5)$$

where the constants A , B , and C depend upon the initial conditions of $x_1(t)$, $x_2(t)$, and $x_3(t)$, and β and γ depend upon the rate constants.

At this point, two important observations must be made. First, Eqs. 3 and 4 imply the hypothesis that the transfer from one phase to another is strictly first order. Second, solution 5, in and of itself, does not adequately distinguish between the two elimination models. I shall elaborate about those two points in the next sections.

PHARMACOLOGICAL COMPARTMENTS

In 1937, Teorell (4,5) published a systematic study of the kinetics of drugs in a manner that differed from that of Behnke *et al.* in that while the compartments considered by Behnke were defined as specific substances in a specific part of the body, Teorell extended the idea of compartment to include the transformation of a drug substance to another chemical form (inactivation) without changing its spatial localization.

He considered the following exchanges between compartments:

- Resorption: passage of drug between the subcutaneous depot and the blood,
- Elimination: passage of drug from blood to urine,
- Tissue take-up: the exchange between blood and tissue,
- Tissue inactivation: change in the molecular form of the drug within tissue.

If $x(t)$ is the amount of drug in the depot, $y(t)$ is the amount of drug within the blood, $z(t)$ is the amount of drug within the tissue, call V_1 , V_2 , and V_3 the volumes of the respective compartments; then, the rate of resorption can be described by the expression

$$k_1 \left(\frac{x(t)}{V_1} - \frac{y(t)}{V_2} \right).$$

However, since $V_1 \ll V_2$, the rate of resorption can be reduced to

$$\text{rate of resorption} = \frac{k_1}{V_1} x(t).$$

Teorell described the other rates as:

$$\text{rate of elimination} = \frac{k_2}{V_2} y(t),$$

$$\text{rate of tissue take-up} = k_3 \left(\frac{y(t)}{V_2} - \frac{z(t)}{V_3} \right),$$

$$\text{rate of inactivation} = \frac{k_4}{V_3} z(t).$$

It should be noted that since each of the above rates contains a rate constant (k_1, k_2, k_3, k_4) and a specific amount of drug within the corresponding compartment at time t , the rates themselves will differ as functions of time.

In conclusion, he provided the following three differential equations to describe the movement of the drugs in the body following a depot administration:

$$\begin{cases} \frac{dx}{dt} = -\frac{k_1}{V_1} x(t) \\ \frac{dy}{dt} = +\frac{k_1}{V_1} x(t) - \frac{k_2}{V_2} y(t) - \left(\frac{k_3}{V_2} y(t) - \frac{k_3}{V_3} z(t) \right) \\ \frac{dz}{dt} = +\frac{k_3}{V_2} y(t) - \frac{k_3}{V_3} z(t) - \frac{k_4}{V_3} z(t) \end{cases}$$

Apart from this wider interpretation of the concept of compartment, the analysis of Teorell leads to a set of linear first-order differential equations, as did the work of Behne.

TRACER KINETICS

One year later, another fundamental paper (6) was published by three scientists, Artom, a physiologist, Sarzana, a histologist, and Segré, a physicist, all from the University of Palermo, Italy. This team had the necessary competence for doing innovative work. Unfortunately, soon after the publication of that paper, new racial laws compelled one of them to leave the country and put an end to the collaboration.

They administered inorganic phosphate labeled with the radioactive isotope ^{32}P to rats and measured the radioactivity present in inorganic phosphate of blood, in the lipid of liver and in the skeleton, at several times after administration. The results of their tracer study were described by the following notation:

N_s, N_f, N_ω are the number of atoms of ^{31}P (stable phosphorus) of the form of interest in blood, liver, skeleton, respectively.
 n_s, n_f, n_ω are the analogous number of atoms of ^{32}P .
 f/N_s is the probability per unit time for the fixation of a given atom of inorganic P by the liver.
 ω/N_s is the analogous probability for fixation by bone.

These notations have the implicit assumptions that the rate of phosphorus fixation, in any form and by any tissue, is constant and that the organism does not distinguish between ^{31}P and ^{32}P .

With the additional assumptions that the total amount of phosphorus in the tissues remains constant during the experiment and that the quantity of labeled P is sufficiently small

not to modify the metabolism of that subject, they wrote the following set of differential equations:

$$\begin{cases} \frac{dn_s}{dt} = -(f + \omega) \frac{n_s}{N_s} + f \frac{n_f}{N_f} + \omega \frac{n_\omega}{N_\omega} \\ \frac{dn_f}{dt} = f \frac{n_s}{N_s} - f \frac{n_f}{N_f} \\ \frac{dn_\omega}{dt} = +\omega \frac{n_s}{N_s} - \omega \frac{n_\omega}{N_\omega} \end{cases} \quad (6)$$

where f/N_f and ω/N_ω are the turnover rates of phosphorus in liver and bone, respectively.

Through integration, the authors reduced those three simultaneous equations to a sum of three exponential functions. Although I shall not show the detailed solution of those differential equations in this manuscript, the important point is that Eqs. 3, 4, and 6 are all linear first-order differential equations with constant coefficients. Accordingly, they all lead to the same general kind of solution, i.e., sums of exponential functions.

The fundamental difference among these three sets of equations is that while 3 and 4 assumed that the transfers between compartments were all first order, Eq. 6 allowed for the intercompartmental transfer to be of any order. The only critical assumption being made in Eq. 6 was that all compartments involved were at steady state. The difference between these critical assumptions is the reason why tracer kinetics had such an important role in the development of pharmacokinetic concepts.

A SINGLE COMPARTMENT

In its simplest form, a compartment is a constant volume V containing a variable quantity $x(t)$ of a substance; it is fully described by an initial value $x(0)$ and by the differential equation

$$\frac{dx}{dt} = -Kx(t) + r(t) \quad (7)$$

where K is the fraction of $x(t)$ leaving the compartment per unit time and $r(t)$ is the substance rate of entry into that compartment.

Equation 7 is just a conservation equation (i.e., mass balance); it states that at any time, the rate of entry minus the rate of exit is equal to the variation of the quantity present. Another fundamental hypothesis is declared by that equation, i.e., that the rate of exit from the compartment is proportional to the amount $x(t)$ present.

The quantity measured by $x(t)$ may have any dimension: mass, radioactivity, number of particles, and so forth; the only requirement is that it be conserved (i.e., mass balance).

Sometimes, the variable $x(t)$ is substituted by its concentration

$$c(t) = \frac{x(t)}{V}$$

so that Eq. 7 can be written in the form

$$\frac{dc}{dt} = -Kc(t) + \frac{r(t)}{V}.$$

It is important to note that when the variable in the differential equation is converted to concentration, we no longer have a conservation equation (concentration is not conserved!). Furthermore, $r(t)/V$ is then a quantity of unusual dimensions. For these reasons, a more meaningful expression is of the form

$$\frac{dx}{dt} = -KVc(t) + r(t). \quad (8)$$

In this equation, KV is the *clearance* (the volume of matrix totally cleared of the substance per unit time), an extensive quantity, and $c(t)$ is concentration, an intensive quantity. This fact is common to many fundamental equations studied in physics where the product of an intensive quantity by an extensive one is a conserved quantity; for example, density by volume is equal to mass, velocity by mass is momentum, and so forth. For a definition of extensive and intensive quantities, see Chapter 3 of Rescigno (7).

Using basic calculus, we find that the solution of Eq. 7 is

$$x(t) = e^{-Kt} \left(x(0) + \int_0^t e^{K\tau} r(\tau) d\tau \right),$$

but this formula is useful only when $r(t)$ has a very simple form.

A simple thought experiment can clarify the meaning of the constant K . If we administer the substance to the compartment at a constant rate, after a sufficiently long time, a steady state is reached with $dx/dt=0$ and $x(\infty) = x_{ss}$; at this point, we can write

$$\frac{\text{amount of substance present}}{\text{rate of elimination of substance}} = \frac{x_{ss}}{K x_{ss}} = \frac{1}{K},$$

but the ratio between the amount of substance present and its rate of elimination is the time (duration) for eliminating an amount of substance equal to the amount present; this time does not depend on $r(t)$. For this reason, $1/K$ is called the *turnover time* of the compartment.

Another parameter of great pharmacokinetic importance can be estimated from Eq. 7. Observe that

$x(t)$	amount of substance present at time t
$Kx(t)$	rate at which the substance leave the compartment
$Kx(t)dt$	amount of substance that left the compartment in the time interval from t to $t + dt$
t	time of this event

Therefore, the ratio

$$\frac{\int_0^\infty t Kx(t) dt}{\int_0^\infty Kx(t) dt} = \frac{\int_0^\infty t x(t) dt}{\int_0^\infty x(t) dt}$$

is the average time when the substance leaves the compartment. This parameter is called the *exit time* from the compartment, symbol Ω .

Two observations are needed at this point. First, the above identity is valid inasmuch as K is constant; this is one of the fundamental hypotheses we make on compartments. The second observation is that Ω is not an interval of time (duration), but a clock time; in other words, it is the average time “when” the substance leaves the compartment; therefore, it includes the time the substance spent in all precursors of that compartment. I shall return to this point in the next section when making a synopsis of all time constants.

LINEAR ALGEBRA

Other interesting parameters can be found by considering a set of compartments connected among them. The most general form of the equations of a system of n compartments is

$$\begin{cases} \frac{dx_1}{dt} = -K_1x_1(t) + k_{21}x_2(t) + \dots + k_{n1}x_n(t) \\ \frac{dx_2}{dt} = +k_{12}x_1(t) - K_2x_2(t) + \dots + k_{n2}x_n(t) \\ \dots \\ \frac{dx_n}{dt} = +k_{1n}x_1(t) + k_{2n}x_2(t) + \dots - K_nx_n(t) \end{cases} \quad (9)$$

where k_{ij} is the fractional rate of transfer from compartment i to compartment j and K_i is the fractional rate of exit from compartment i , with the initial conditions

$$x_i(0) = x_{i0}$$

for all values of i from 1 to n .

A better understanding of those equations is obtained using the methods of linear algebra. Equations 9 can be written

$$\left(\frac{dx_1}{dt} \frac{dx_2}{dt} \dots \frac{dx_n}{dt} \right) = - \begin{pmatrix} x_1(t) & x_2(t) & x_3(t) & \dots & x_n(t) \end{pmatrix} \cdot \begin{pmatrix} K_1 & -k_{12} & \dots & -k_{1n} \\ -k_{21} & K_2 & \dots & -k_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ -k_{n1} & -k_{n2} & \dots & K_n \end{pmatrix}$$

or with a more concise notation

$$\frac{d\mathbf{X}}{dt} = -\mathbf{X}(t) \times \mathbf{K}. \quad (10)$$

Equation 10 must be completed with the initial condition

$$\mathbf{x}(0) = \begin{pmatrix} x_1(0) & x_2(0) & x_3(0) & \dots & x_n(0) \end{pmatrix}.$$

The integral of Eq. 10 is

$$\mathbf{X}(t) = - \int_0^t \mathbf{X}(t) dt \times \mathbf{K} + \mathbf{X}(0). \quad (11)$$

If $\mathbf{K}$ is not singular, we call $\mathbf{T}$ its inverse

$$\mathbf{T} = \mathbf{K}^{-1}.$$

Multiplying each side of 11 on the right by $\mathbf{T}$, we obtain

$$\mathbf{X}(t) \times \mathbf{T} = - \int_0^t \mathbf{X}(t) dt + \mathbf{X}(0) \times \mathbf{T}.$$

We now estimate the limit for $t \rightarrow \infty$; if the system of compartments is open (8), no substance is left in it after a sufficiently long time; therefore,

$$\int_0^\infty \mathbf{X}(t) dt = \mathbf{X}(0) \times \mathbf{T}. \quad (12)$$

The physical interpretation of this identity is very important. Call t_{ij} the element of row i and column j of matrix $\mathbf{T}$. To investigate the meaning of that element, think of an experiment where all compartments are initially empty except compartment i ; identity 12 in this case is reduced to

$$t_{ij} = \int_0^\infty x_j(t) dt / x_i(0)$$

where i and j can take any value from 1 to n ; but $x_j(t)x_i(0)$ is the fraction of particles present in compartment j at time t that were fed from compartment i , while dt is the length of time spent by those particles in the interval from t to $t + dt$. The above fraction is the time spent in compartment j by a particle that entered compartment i at time 0. Therefore, if $i \neq j$, we call this quantity *residence time* from i to j ; alternatively, if $i = j$, we call it *permanence time* in i .

The permanence time in a compartment includes the time spent by a substance that entered it in its first passage and in all successive ones, if any; the residence time from one compartment to another includes only the time spent in this last compartment by the fraction of particles introduced into the first that actually reaches the second.

The parameters I have shown so far can be summarized as follows:

$$\text{Turnover time} \leq \text{Permanence time} \leq \text{Exit time},$$

$$\text{Residence time} \leq \text{Permanence time}.$$

Their relative ratios are:

$$\frac{\text{Permanence time}}{\text{Turnover time}} = \text{Turnover number} \geq 1,$$

$$\frac{\text{Residence time}}{\text{Permanence time}} = \text{Yield} \leq 1.$$

The Permanence time and the Turnover time of a compartment are equal when there is no recirculation in that compartment; the Residence time from one compartment to another and the Permanence time in the second compartment are equal when the whole substance that leaves the first compartment reaches the second one.

All the above parameters are measures of the time spent by a substance inside a compartment in different circumstances and in one or several passages. Conversely, the exit time is the time elapsed from the beginning of the observations to the exit of the

substance from the sampled compartment, and includes the time spent in all its precursors.

THE TRANSFER FUNCTION

A better and more complete understanding of the behavior of a set of compartments requires a description of the fate of the substances moving from one compartment to another. As before, basic concepts are borrowed from another discipline: in this case, system theory (9).

System theory deals with the analysis of sets of interacting parts and their reciprocal relationships, irrespective of their particular components; one of the main tools of system analysis is the *transfer function*.

The pharmacokinetic properties of a particular drug in a particular organism may be described by a particular transfer function; in other words, if the organism had an input, i.e., the drug to be administered, and an output, i.e., the drug in the target organ, the relationship between those two functions is its pharmacokinetic transfer function. All *kinetic* properties of a system, and only its kinetic properties, with the exclusion of its *dynamic* properties, can be reduced to the study of a particular transfer function. The whole information and only the information available from a pharmacokinetic experiment must be in its transfer function.

For a transfer function to exist, it is necessary that a system be linear and time-invariant. Both of these conditions are satisfied by the differential equations of the types shown so far (i.e., linear with coefficients that are constant over time).

I shall now rephrase the properties of a set of compartments using the terminology of system theory. Consider a particle in a living system and suppose that that particle can be recognized in two different states of the system where by state we mean a particular location or a particular chemical form or both. If one state is the precursor of the other (not necessarily the immediate precursor), then we can study the relationship among event $\mathbf{A}$ (the particle is in the precursor state), event $\mathbf{B}$ (transition from precursor to successor state), and event $\mathbf{C}$ (presence of the particle in the successor state).

For any t and τ such that $0 \leq \tau \leq t$, call $f(\tau)$ the probability of $\mathbf{A}$ at time τ and $h(t)$ the probability of $\mathbf{C}$ at time t . Suppose now that $\mathbf{B}$ depends only on the interval of time separating $\mathbf{A}$ and $\mathbf{C}$, so that we can call now $g(t - \tau)d\tau$ the conditional probability that a particle is in $\mathbf{C}$ at time t if it left $\mathbf{A}$ in the interval $\tau, \tau + d\tau$.

The product $f(\tau) g(t - \tau)d\tau$ therefore is the absolute probability that a particle leaves $\mathbf{A}$ in the interval of time between τ and $\tau + d\tau$ and is still in $\mathbf{C}$ at time t .

The integral of the above product must be the probability of a particle being in state $\mathbf{C}$ at time t irrespective of when it left $\mathbf{A}$, i.e.,

$$\int_0^t f(\tau)g(t - \tau)d\tau = h(t). \quad (13)$$

This is the well known *convolution integral* representing the relationship among the variables of a *linear, time-invariant* system.

Think of an experiment where a very large number of identical particles is used, then the numbers of particles

present in the precursor and in the successor states are good estimates of functions $f(t)$ and $h(t)$, respectively. Function $g(t)$ represents the probability that a particle that left $\mathbb{A}$ at time 0 will still be in $\mathbb{C}$ at time t ; therefore, in a hypothetical experiment where all identical particles left the precursor near time zero, the number of particles found in the successor will be given by $g(t)$.

The main properties of the convolution integral were described by Rescigno (7) on pages 181–185. The importance of the convolution integral in pharmacokinetics is that if the system under observation is linear and time-invariant, for any precursor defined by $f(t)$ and successor defined by $h(t)$, there exists a function $g(t)$ called *transfer function* from $f(t)$ to $h(t)$; that transfer function contains *in nuce* all properties of the compartments that connect the precursor to the successor. The next sections show the connection between the transfer function and the experimental data.

THE OPERATIONAL CALCULUS

The convolution integral introduced in the previous section may seem an abstract concept at first, but it easily becomes a very practical tool using *operational calculus*. An introduction to operational calculus may be found in several books (7,10,11); the [Electronic supplementary material](#) contains a simpler approach to it and is sufficient to understand the rest of this paper.

With the operational notation, convolution 13 becomes a multiplication,

$$\{f\} \times \{g\} = \{h\}$$

and those functions are treated as if they were real numbers.

The simplest one-compartment system with a bolus input and no recirculation, described by the differential equation

$$\frac{dx}{dt} = -Kx(t)$$

using identity 3 in the [Electronic supplementary material](#), becomes

$$s\{x\} - x(0) = -K\{x\}$$

or simply

$$x(0) \frac{1}{s+K} = \{x\}. \quad (14)$$

Here, $x(0)$ is the input, $1/(s+K)$ the transfer function, and $\{x\}$ the output.

A system of two compartments irreversibly connected, with a single bolus input, described by equations

$$\begin{cases} \frac{dx_1}{dt} = -K_1 x_1(t) \\ \frac{dx_2}{dt} = +k_{12} x_1(t) - K_2 x_2(t) \end{cases}$$

becomes

$$\begin{aligned} s\{x_1\} - x_1(0) &= -K_1\{x_1\} \\ s\{x_2\} &= k_{12}\{x_1\} - K_2\{x_2\} \end{aligned}$$

thence

$$\begin{cases} x_1(0) \frac{1}{s+K_1} = \{x_1\} \\ \{x_1\} \frac{k_{12}}{s+K_2} = \{x_2\} \end{cases}. \quad (15)$$

By eliminating $\{x_1\}$, we get

$$x_1(0) = \frac{k_{12}}{(s+K_1)(s+K_2)} \{x_2\}.$$

The fraction at the right-hand side is the transfer function from $x_1(0)$ to $\{x_2\}$.

If more compartments are involved and if reversible connections are present, the construction of the transfer function with this procedure may become very complicated. A more synthetic approach is needed.

SIGNAL FLOW GRAPHS AND FEEDBACK THEORY

Differential equations like 9 and integral equations like 13 can be solved the hard way, as we learn in a calculus class. We can even obtain solutions through the use of computers. In fact, there are several software packages that can solve problems containing a very large number of compartments. What those packages do not provide, however, is insight into the hidden parameters of potential biological significance. That view comes only from a synthetic approach. To this end, once again, such help is offered from a different discipline, in this case by control system engineering.

In 1953, Mason (12) described a method, called signal-flow graph, whereby a set of first-order linear differential equations can be transformed, with a few short steps, to the transfer function of a system of compartments. In short, a *flow graph* is a set of *nodes* connected by *arms*; each node corresponds to a variable or to an initial condition; each arm connects two nodes in a specific orientation and has the value of the transfer function between the two nodes it connects.

For example, to the one-compartment system of 14 corresponds the flow graph of Fig. 3 and to the two-compartment system of 15 corresponds the flow graph of Fig. 4. To the general set of differential Eq. 9 corresponds a flow graph whose nodes are $x_{10}, x_{20}, \dots, x_{n0}$, and $\{x_1\}, \{x_2\}, \dots, \{x_n\}$, and whose arms have the general form

Fig. 3. Flow graph of a one-compartment system

Fig. 4. Flow graph of a two-compartment system

if connecting node x_{i0} to node $\{x_i\}$, and

$$\frac{k_{ij}}{s + K_j}$$

if connecting node $\{x_i\}$ to node $\{x_j\}$. The number of possible nodes is equal to the number of initial conditions plus the number of variables. The number of possible arms is equal to the number of initial conditions plus the number of transfers between compartments. Of course, many initial conditions may be zero and not all compartments are connected with all other compartments. Nevertheless, the number of arms may be large and the flow graph may appear complicated. Therefore, there are some simple rules whereby the graph may be simplified and many properties of the system it represents can be obtained.

Thanks to the way that the flow graph is defined, a general rule can be stated: "Each node is equal to the sum of the products of all arms entering it times their nodes of departure." From this general rule, a number of special rules can be used to further simplify the graph and to determine its transfer function between any two nodes, as detailed in the literature (13):

Call *path* an ordered succession of arms such that the node where an arm ends, except the last one, is the node where the next arm begins. The value of a path is the product of the arms by which it is formed.

Choose an initial node p and a terminal node q .

Look for the existence of closed paths, i.e., for paths that start and end at the same node. Choose the minimum number of *essential nodes*, i.e., the minimum number of nodes that, if suppressed, interrupt all closed paths.

By following this procedure, an *essential graph* can be developed consisting of the initial node p , the terminal node q , a number of essential nodes, plus an arm from the initial node to the terminal node, an arm from the initial node to each essential node, an arm from each essential node to the terminal node, an arm from each essential node to each other essential node, and a *self-arm* from each essential node to itself. The terminal node may be an essential node. Each arm in an essential graph is equal to the sum of the paths it is formed by.

An essential node represents the recirculation of the substance from one compartment back to it; if the value of the essential node is φ , the transfer function of the first passage through it is φ , of the second passage φ^2 , of the third passage φ^3 , and so forth. From the well-known identity

$$1 + \varphi + \varphi^2 + \varphi^3 + \dots = \frac{1}{1 - \varphi},$$

we can conclude that an essential node can cancel by dividing the arms that enter it by the value of 1 minus the value of the self-arm.

Many interesting applications of graphs can be found in the literature (14).

AN ELEMENTARY EXAMPLE

Remember Oscar Wilde saying: "Like all people who try to exhaust a subject, he exhausted his listeners" (15). Instead of exhausting you with all the details of flow graphs, I will demonstrate with a simple example from the current literature what information we can get using operational calculus and flow graph methods.

Matthews (16) studied the fate of proteins injected into the bloodstream of animals. She started with the hypotheses that the injected protein is initially distributed into the intravascular compartment, that this central compartment is reversibly connected with a number of extravascular compartments, and finally that the protein is excreted via urine and feces, only from the intravascular compartment. From a typical experiment, she found, in the central compartment,

$$\frac{c(t)}{c(0)} = 0.33e^{0.080t} + 0.53e^{1.00t} + 0.14e^{14.4t};$$

we know that $c(0) = 1$; therefore, in operational notation, we can write

$$\{c\} = \frac{0.33}{s + 0.080} + \frac{0.53}{s + 1.00} + \frac{0.14}{s + 14.4}$$

and by expanding,

$$\{c\} = \frac{s^2 + 12.9076s + 5.37376}{s^3 + 15.48s^2 + 15.632s + 1.152}. \quad (16)$$

Using identity 3 in the [Electronic supplementary materials](#), we get

$$\begin{aligned} \{c'\} &= \frac{s^3 + 12.9076s^2 + 5.37376s}{s^3 + 15.48s^2 + 15.632s + 1.152} \\ &= \frac{(12.9076 - 15.48)s^2 + \dots}{s^3 + \dots}. \end{aligned}$$

Then using 5 in the [Electronic supplementary materials](#),

$$K_1 = \lim_{t \rightarrow 0} \frac{-dc/dt}{c(t)} = 15.48 - 12.9076 \approx 2.57.$$

The turnover time in the central compartment is 2.57 h.

Using identity 2 in the [Electronic supplementary materials](#),

$$\left\{ \int_0^t c(t) dt \right\} = \frac{s^2 + 12.9076s + 5.37376}{s(s^3 + 15.48s^2 + 15.632s + 1.152)},$$

then using 6 in the [Electronic supplementary materials](#),

$$\begin{aligned} \int_0^\infty c(t) dt &= \lim_{s \rightarrow 0} \frac{s(s^2 + 12.9076s + 5.37376)}{s(s^3 + 15.48s^2 + 15.632s + 1.152)}, \\ &= \frac{5.37376}{1.152} \approx 4.66; \end{aligned}$$

the permanence time in the central compartment is 4.66 h; the ratio $4.66/2.57=1.81$ is the turnover number of the central compartment.

Using identities 4 and 2 in the [Electronic supplementary materials](#), we can compute

$$\begin{aligned} \frac{\int_0^\infty tc(t)dt}{\int_0^\infty c(t)dt} &= \lim_{s \rightarrow 0} \frac{-\frac{d}{ds}\{c\}}{\{c\}} = \\ &= \lim_{s \rightarrow 0} \left(\frac{3s^3 + 30.96s + 15.632}{s^3 + 15.48s^2 + 15.632s + 1.152} - \frac{2s + 12.9076}{s^2 + 12.9076s + 5.37376} \right) \\ &= 13.5694 - 2.40197 = 11.167 \end{aligned}$$

The exit time from the central compartment is 11.17 h.

The transfer function in 16 has a denominator of degree 3; therefore, it shows that there are three compartments involved in the transfer of the substance. The flow graph has an arm of value $1/(s + K_1)$ from the initial value $c(0)$ to the central compartment $\{c\}$, followed by a self-arm of value $\varphi(s)/[(s + K_1)(s + K_2)(s + K_3)]$, with $\varphi(s)$ of degree less than 3, as shown in Fig. 5. As shown in the previous section, by eliminating the self-arm, the resulting transfer function becomes

$$\begin{aligned} \frac{1}{s + K_1} / \left(1 - \frac{\varphi(s)}{(s + K_1)(s + K_2)(s + K_3)} \right) \\ = \frac{(s + K_2)(s + K_3)}{(s + K_1)(s + K_2)(s + K_3) - \varphi(s)}. \end{aligned}$$

Comparing this expression with the numerical values given above, we find that

$$\begin{cases} K_2 + K_3 = 12.9076 \\ K_2 K_3 = 5.37376 \\ K_1 + K_2 + K_3 = 15.48 \end{cases}$$

thence

$$K_1 \approx 2.57, K_2 \approx 0.43, K_3 \approx 12.48.$$

Furthermore,

$$(s + K_1)(s + K_2)(s + K_3) - \varphi(s) = s^3 + 15.48s^2 + 15.632s + 1.152$$

Fig. 5. Flow graph of a three-compartment system with recycling

thence

$$\varphi(s) = 22.914s + 12.659. \quad (17)$$

The self-arm has value

$$\frac{22.914s + 12.659}{(s + 2.57)(s + 12.48)(s + 0.43)}.$$

This transfer function represents the transfer from the central compartment $\{c\}$ back to it, i.e., the recirculation through the peripheral compartments.

With the restrictive hypotheses made by the author, this self-arm has the form

$$\frac{k_{12}K_3}{(s + K_2)(s + K_1)} + \frac{k_{13}K_2}{(s + K_3)(s + K_1)},$$

and its solution is

$$k_{12} \approx 0.53, k_{13} \approx 1.82.$$

THE GENERAL SOLUTION

A general n -compartment system is determined by n^2 parameters, i.e., n turnover rates and $n(n - 1)$ transfer rates. The transfer function of such a system contains n coefficients in the numerator and n in the denominator. When $n > 2$, the solution of such system is undetermined, and it contains $n(n - 2)$ degrees of freedom. In the example shown in the previous section, the author made four assumptions to cause the problem to be fully determined, i.e., $K_2 = k_{21}$, $K_3 = k_{31}$, $k_{23} = 0$, and $k_{32} = 0$. Those assumptions may be justified by physiological considerations, but this may not always be the case. If the problem is not fully determined, it may be useful to compute a range for the parameters that have some degrees of freedom left.

The case of two degrees of freedom was studied by Berman and Schoenfeld (17); later, Rescigno (18) studied the general case using the methods of system theory, as proposed by Willems (19).

Using the terminology of system theory, the compartmental equations are written in the form

$$\frac{d\mathbf{X}}{dt} = -\mathbf{X}(t)\mathbf{K} + \mathbf{R}(t) \quad (18)$$

where $\mathbf{R}(t)$ is the row vector of the feeding functions $r_1(t)$, $r_2(t)$, ..., $r_n(t)$.

By integration, we get

$$\mathbf{X}(t) = \mathbf{R}(t) \otimes \exp(-t\mathbf{K}) \quad (19)$$

where the symbol $\otimes$ means “convolution.”

In a typical pharmacokinetic experiment, only a limited number of variables are controlled and a limited number are observed. Call $\mathbf{F}(t)$ the vector of the p input variables and $\mathbf{G}(t)$ the vector of the q output variables.

With the product $\mathbf{F}(t) \cdot \mathbf{A} = \mathbf{R}(t)$, we define a matrix $\mathbf{A}$ representing on row i and column j the weight of the input variable i on the state variable j . With the product $\mathbf{X}(t) \cdot \mathbf{B} = \mathbf{G}(t)$,

we define a matrix $\mathbf{B}$ representing on row i and column j the weight of the state variable i on the output variable j .

We multiply on the right both sides of expression 19 by $\mathbf{B}$ to get

$$\mathbf{G}(t) = \mathbf{F}(t)\mathbf{A} \otimes \exp(-t\mathbf{K})\mathbf{B}.$$

This expression shows that $\mathbf{A} \otimes \exp(-t\mathbf{K})\mathbf{B}$ is the transfer function from $\mathbf{F}(t)$ to $\mathbf{G}(t)$, and it is a generalization of expression 13.

We can write the identities

$$\begin{aligned} \mathbf{A} \otimes \exp(-t\mathbf{K})\mathbf{B} &= \mathbf{A}\mathbf{S}^{-1}\mathbf{S} \otimes \exp(-t\mathbf{K})\mathbf{S}^{-1}\mathbf{S}\mathbf{B} \\ &= (\mathbf{A}\mathbf{S}^{-1}) \otimes \exp(-t\mathbf{S}\mathbf{K}\mathbf{S}^{-1})(\mathbf{S}\mathbf{B}). \end{aligned}$$

In other words, the transfer function does not change with the transformation

$$(\mathbf{A}, \mathbf{B}, \mathbf{K}) \rightarrow (\mathbf{A}\mathbf{S}^{-1}, \mathbf{S}\mathbf{B}, \mathbf{S}\mathbf{K}\mathbf{S}^{-1}).$$

If this transformation exists, it defines a whole class of models that satisfy Eq. 18, but with different matrices $\mathbf{K}$. For this transformation to exist and to be physically realizable, it is necessary that

- $\mathbf{S}$ be invertible,
- $\mathbf{A}\mathbf{S}^{-1} = \mathbf{A}$,
- $\mathbf{S}\mathbf{B} = \mathbf{B}$,
- $\mathbf{S}\mathbf{K}\mathbf{S}^{-1}$ be physically realizable.

Observe that the second condition also implies $\mathbf{A}\mathbf{S} = \mathbf{A}$ and that the third condition also implies $\mathbf{B} = \mathbf{S}^{-1}\mathbf{B}$. The fourth condition requires some explanations. When we wrote Eqs. 7 and 18, we meant them to describe a system where matter is conserved; without the above restrictions on the elements of $\mathbf{K}$, the integrals of those equations would be mathematically correct, but could possibly contain negative values of some of the variables. This last condition is satisfied if all non-diagonal elements of $\mathbf{S}$ are non-positive and the sum of the elements of each row is non-negative.

Matrices $\mathbf{A}$ and $\mathbf{B}$ are determined by the experimental conditions. Matrix $\mathbf{S}$ must contain a number of arbitrary parameters equal to the degrees of freedom of the problem and be consistent with the first three above conditions. Matrix $\mathbf{K}$ can be filled with a number of arbitrary elements equal to the degrees of freedom of the system; all other elements must be computed to satisfy Eq. 18, without regards to the physical realizability of the resulting system. At this point, the product $\mathbf{S}\mathbf{K}\mathbf{S}^{-1}$ contains all solutions of Eq. 18; the fourth condition determines the range of the arbitrary parameters in matrix $\mathbf{S}$ that correspond to physically realizable solutions.

BEYOND COMPARTMENTS

Returning to Eq. 7, I said that compartmental theory is based upon the assumption of mass balance and that K is constant. However, any discussion of compartmental analysis would not be complete without considering what would happen if the rate of exit is not $Kx(t)$, with K constant. To this end, we can consider two cases: the substance measured by quantity $x(t)$ is uniformly distributed inside its volume or it

is not. In the first case, we can still say that we are dealing with a compartment, though a nonlinear one. Equation 7 must be rewritten with the exit term proportional to a nonlinear expression of $x(t)$. There is no general solution for nonlinear differential equation; each case must be solved in its own way, as shown in Chapter 12 by Rescigno (7).

In the second case, the substance is not uniformly distributed inside its volume; to the extensive quantity $x(t)$, we must substitute the intensive quantity $c(t)$ concentration. However, in this case, the concentration is a function of both time and location inside its volume of distribution. Equation 7 must be substituted by a partial differential equation of diffusion, where $c(t)$ appears in derivatives with respect to time and with respect to space. In the very special case of linear diffusion in one dimension only, the solution is relatively simple (20); for all other cases, there are no general solutions; see for instance Chapter 13 of Rescigno (7).

In the majority of cases, pharmacokinetic compartmental analysis is based on an assumption that upon the transfer of substance to a compartment, it is instantaneously distributed in a homogenous manner throughout that compartment. Although this assumption allows for some generalized description of the kinetic properties of that substance, the physiological validity of such an assumption is clearly flawed. Therefore, it is important to consider the degree to which deviations from that assumption will influence the validity of the conclusions derived from a compartmental analysis. The answer to that question clearly depends upon the objective for using the compartmental approach in the evaluation of a dataset.

A special problem arises when we collect data from different individuals and try to determine a pharmacokinetic parameter valid for that set or population. In its simplest form, the problem consists in administering a drug to m different subjects 1, 2, ..., m and measuring its concentration at n different times $t_1, t_2, \dots, t_n$ for all subjects. The results are represented by the rectangular matrix

$$\mathbf{C} = \begin{bmatrix} c_{1,1} & c_{1,2} & \cdots & c_{1,n} \\ c_{2,1} & c_{2,2} & \cdots & c_{2,n} \\ \vdots & \vdots & \ddots & \vdots \\ c_{m,1} & c_{m,2} & \cdots & c_{m,n} \end{bmatrix},$$

where $c_{i,j}$ is the concentration of the drug in patient i at time t_j . The substance of the problem does not change if some elements of matrix $\mathbf{C}$ are missing.

The above statement of the problem is somewhat ambiguous. For clarity, I shall rephrase the problem into two alternative ways:

- Find the function $c = c(t)$ that best represents the averages $\langle c, 1 \rangle, \langle c, 2 \rangle, \langle c, 3 \rangle, \dots, \langle c, n \rangle$ of each column.
- Find the average values of the parameters of the functions $c_1 = c_1(t), c_2 = c_2(t), \dots, c_m = c_m(t)$ that best represent the sets of experimental data in $\mathbf{C}$.

Both the above statements are acceptable, but they correspond to different problems, i.e., they satisfy different requirements. With the first statement, one tries to solve the

problem by concisely describing the data as they appear in matrix **C**. With the second statement, one tries to describe not the data themselves, but the individuals that generated them. More precisely, the second statement postulates a model for function $c = c(t)$; that model is embodied by some parameters; from the values of the parameters computed from the sample, one can estimate the corresponding parameters of the population.

The solutions of the two problems are quite different, as can be seen from the following example. Suppose the drug is administered to all patients as a unit bolus, it is totally and immediately absorbed, and it is eliminated by a simple first-order process. Suppose also that no experimental errors are present. The experimental results can be represented by equation

$$\frac{c_i(t)}{c_i(0)} = e^{-K_i t}, i = 1, 2, \dots, n \quad (20)$$

where K_i is the turnover rate of subject i . If we fit this equation with all data from patient i , we can calculate the parameter K_i ; then from $K_1, K_2, \dots, K_m$, we estimate the average turnover rate of the population from which our sample is coming from.

Suppose now that the turnover rate of the population is normally distributed with mean K and variance σ^2 . For any value of the sampling time, t_j , we can expand function 20 as a Taylor series around the value K

$$\begin{aligned} \frac{c_i(t_j)}{c_i(0)} &= e^{-K_i t_j} \\ &= e^{-K t_j} - t_j e^{-K t_j} (K_i - K) + \frac{1}{2} t_j^2 e^{-2K t_j} (K_i - K)^2 + \dots \end{aligned}$$

where the dots represent higher powers of $(K_i - K)$.

We now take the averages of the $c_i(t_j)/c_i(0)$ for each value of j

$$\begin{aligned} \left\langle \frac{c_i(t_j)}{c_i(0)} \right\rangle &= e^{-K t_j} - t_j e^{-K t_j} \langle K_i - K \rangle + \frac{1}{2} t_j^2 e^{-2K t_j} \langle (K_i - K)^2 \rangle + \dots \\ &= e^{-K t_j} + \frac{1}{2} t_j^2 e^{-2K t_j} \sigma^2 + \dots \end{aligned}$$

This identity shows that if Eq. 20 holds, then $\left\langle \frac{c_i(t)}{c_i(0)} \right\rangle$ is not in general equal to $e^{-(K_i)t}$; in other words, the turnover time computed from the average of functions $\frac{c_i(t)}{c_i(0)}$ does not coincide with the average of parameters K_i .

From the same experimental data contained in matrix **C** and the same Eq. 20, we can compute other pharmacokinetic parameters, for instance the volume of distribution $1/c(0)$, the turnover time $1/K$, and the clearance $K/c(0)$. However, we know that in general, $\langle 1/K \rangle$ is different from $1/\langle K \rangle$, that $\langle K/c(0) \rangle$ is different from $\langle K \rangle / \langle c(0) \rangle$, and so forth. It follows that to each parametrization corresponds a different “average” population.

To muse on a simple example, imagine having two cubes with sides 1 and 10 cm, respectively; we can compute their average side, their average surface area, their average

volume; the resulting “average cube” is a monster defying all laws of geometry!

	Side (cm)	Surface area (cm ²)	Volume (cm ³)
First cube	1	6	1
Second cube	10	600	1,000
“Average cube”	5.5	303	500.5

The moral is that if we observe a population of individuals, we can estimate their average drug concentration, their average turnover rate, their average clearance, and so forth, but all those averages do not converge to a platonic “mister average.”

CONCLUSION

The concept of compartment has come a long way from the first observations of less than a century ago to its modern use in pharmacokinetics. I have tried to show how, if certain specific hypotheses are valid, some parameters of pharmacokinetic interest can be determined from the available experimental data and how, in some cases, we can determine a range of some other parameters.

In recent years, it has become fashionable to oppose *physiological models* to *compartmental models* (21), as though a compartmental model had no physiological meaning. The truth is that any model must be based on some hypotheses (22). The models described by Widmark and Tandberg, by Gehlen, by Behnke and Thomson, in short by all pharmacokineticists I have cited and by a cohort of those I have omitted, are based on the hypotheses embodied in Eq. 7. Those hypotheses, once more, are that the substance under observation is uniformly distributed in a particular volume and that it moves to a different volume or a different state with a constant rate. In short, the hypotheses are that the system under observation can be *compartmentalized*.

As shown in “A SINGLE COMPARTMENT”, when function $r(t)$ has a particularly simple form, the integral of Eq. 7 is an exponential function or a sum of exponential functions. But it must be clear that any sufficiently regular function $f(t)$ can be approximated by a sum of exponential functions; therefore, the presence of exponential functions, *per se*, is not sufficient to prove the presence of compartments.

On the other hand, I have shown how to compute meaningful pharmacokinetic parameters from expressions like $\int_0^\infty c(t)dt$ and $\int_0^\infty tc(t)dt / \int_0^\infty c(t)dt$; but it has also been proven (23) that without those same compartmental hypotheses, the quantities computed from the so-called AUC and AUMC lose any valid meaning.

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