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Structural effects in drug distribution: whole animal pharmacokinetics

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Computer-generated data simulating the results of assays of homogenized whole animals as a function of time following rapid intravenous injection have been employed to calculate various pharmacokinetic constants in both one and two compartment open models. The results have been compared to those obtained with data representing analysis of blood samples at identical time intervals. Data for whole animal analysis are equally reliable in calculating the first-order constants for distribution and elimination when applied to the appropriate cases. However, the range of utility is smaller when the whole animal method is used. The whole animal approach does offer some unique advantages over the classical plasma data method and these are discussed. The range of utility and the method for calculating pharmacokinetic rate constants from whole animal data are clearly defined.

This report defines methodology for carrying out pharmacokinetic modeling using whole animal data rather than the plasma concentration data normally employed. Both the utility and the limitations of the method are compared to those of the plasma analysis method. The range of applicability of whole animal pharmacokinetic analysis is less than that of the plasma method but the methods work equally well in the region where both are operable. Not only can the whole animal method provide the desired pharmacokinetic information that is conventionally derived from the plasma level method, but it is particularly advantageous in situations that preclude the use of plasma techniques; namely, when available assay methods are not sufficiently sensitive for plasma analysis and when the study requires the use of small animals with limited blood volumes.

One familiar type of investigation that may benefit from the whole animal approach involves the separation of structure-activity relations from structure-pharmacokinetic relations within a series of closely related chemical compounds. The significance of such studies and the limited progress made to date have been reviewed recently by Notari (1973). He has emphasized that discussions of substituent effects upon drug-receptor interactions, though quite common, may often be inadequate. Typically a group of analogues is compared using some biological response as a measure of relative potency. Some assumptions are generally made regarding the interaction between the parent compound and the biological receptor and molecular modifications are employed to test these assumptions. In many cases conclusions are based upon dose-response curves and the administered dose is assumed to be responsible for the magnitude of the response. In recent years it has become recognized that the time course for drug at the receptor must also be considered. Thus, the onset, duration and intensity of the observed effect would appear to depend upon both the interaction between drug and receptor and the availability of drug to the

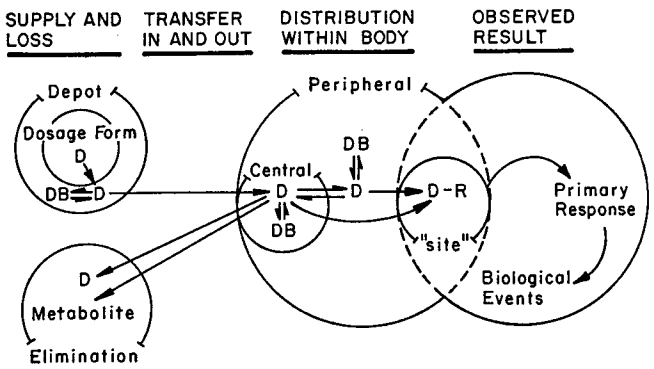

FIG. 1. Diagram of potential rate processes which can be influenced by molecular modification. D, free drug; R, receptor; DB, bound drug; the terms "central" and "peripheral" are defined in the text. (Taken from Notari, 1973, with permission of the copyright holder.)

receptor. The time course for drug at the site of action is influenced by many rate processes. Some of these are illustrated in Fig. 1. Riegelman, Loo & Rowland (1968) have discussed the physiological aspects of such models wherein the term "central" refers primarily to blood and "peripheral" represents all other drug-bearing tissues.

Pharmacokinetic methods for compartmental analysis are now commonplace and have been described in a number of textbooks (Notari, 1971; Wagner, 1971a; Swarbrick, 1970). A simple compartmental scheme to accommodate both one and two compartment models as discussed by Notari (1971) is shown in Fig. 2. Here, B is the central compartment (or blood), T is the peripheral compartment (or tissue) and C is the compartment for drug eliminated by excretion and metabolism. The object of such an approach is to account for the time course of drug within the body. One of the primary advantages is that the animal (or patient) is used as its own control. That is, the pharmacokinetic study is carried out on an intact animal. Generally, blood and/or urine are assayed for drug content and, where possible, metabolites. The compartmental scheme and the time course for drug in each compartment is then deduced using kinetic calculations. Often minimum therapeutic and maximum safe blood levels can be determined and a dosage regimen can be calculated using these parameters. However, it is well recognized that these values are operational parameters only and are not meant to imply that the blood itself is the site of action. The search for relations between pharmacological response and pharmacokinetic analysis has been the subject of several papers during recent years. In many cases a

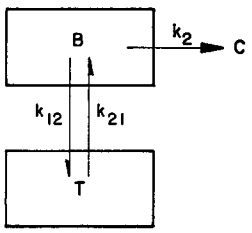

FIG. 2. Pharmacokinetic scheme to accommodate one or two compartment model. Rate constants are first order, B is central compartment, T is tissue compartments and C is the sum of elimination by all routes.

simple correlation of the time course for drug in the body and the intensity of pharmacological response has been successfully established (Levy, 1966). More complex analyses have determined the compartment(s) (if any) whose time course most closely resembles that of intensity of pharmacological response. Levy, Gibaldi & Jusko (1969) correlated performance scores with the fraction of dose of lysergic acid diethylamide in the slowly accessible compartment of a three compartment model. Reuning, Sams & Notari (1973) correlated left ventricular ejection time index values with digoxin tissue levels in a two compartment model. Many such examples could be sited. Wagner (1971b) has written an extensive review on the relations between drug concentration and pharmacologic response.

One complication associated with correlations of this type is the non-homogeneity of the peripheral (or tissue) compartment. The time profile for drug at the site of action might be quite different from that of either the blood or one of the tissue compartments. Ideally, the actual time course for drug at the receptor should be correlated with the time course for pharmacological response to provide a direct comparison of the effects of various drug analogues. It is difficult, if not impossible, to define and then locate the receptor involved. Many times it is possible to specify a target organ or anatomical site of action for a given drug. The most common approach to determining the concentration of drug in a specific organ or site is by death of the animal and analysis of the tissue homogenate. Generally, this is done after some chosen time interval and kinetic aspects are neglected. However, through the use of whole animal assay and pharmacokinetic modeling, it is possible to evaluate structure-activity and pharmacokinetic relationships simultaneously.

Such an approach requires a judicious selection of conditions. The pharmacological response should be conveniently measurable without trauma to the animal so that the pharmacokinetic parameters will not be influenced by abnormal physiological conditions. Thus, the response measurement should require no surgical procedure. The various analogues should have a common anatomical site of action and this site should be well-defined. Some method of monitoring the drug content in the organ or region containing the target site is essential. This may require death of the animal and removal of a specific organ or tissue. In this instance, the animal no longer can serve as its own control as it does in the classical pharmacokinetic approach. Because of biological variability it becomes necessary to analyse several animals for each datum point. Thus, it is economically desirable to use small animals such as mice. The drugs should be capable of being administered by rapid intravenous injection in solution to eliminate the effects of the region designated as the depot in Fig. 1. The fraction of the dose in the whole animal and the fraction of the dose in the organ or site of interest may then be determined as a function of time. Ideally, the analogues being compared should be eliminated only by metabolism and the assay should be specific for intact drug. If the drug is excreted intact the additional technique of removing the urine from the bladder or assaying its contents separately must be included to determine the content of drug actually within the animal. This eliminates the possibility of assaying intact drug within the bladder as part of the whole animal homogenate. By applying the techniques described in this paper the time profile for drug in various compartments can be compared to the observed time profile for pharmacologic response. The overall effect of chemical modification upon the distribution of drugs to the target organ and the resultant response at that organ can then be evaluated.

METHODS

Equations for whole animal pharmacokinetics. Fig. 2 represents either a one or two-compartment open model with rapid intravenous injection as discussed by Notari (1971). The total amount of unchanged drug in the body at any time would be the sum of the amount in the blood compartment (B) and the tissue compartment (T). An equation defining the time course for total drug in the body D_t, following a rapid intravenous dose, D, has been derived by Wagner (1971a) for a two compartment open model:

$$D_t = \frac{D}{\alpha - \beta}[(\alpha - k_{12} - k_{21})e^{-\alpha t} + (k_{12} + k_{21} - \beta)e^{-\beta t}] \quad .. \quad .. \quad (1)$$

where α and β are pseudo first-order rate constants made up of k_{12}, k_{21} and k_2. The fraction of the dose remaining in the animal, $f = D_t/D$, may be described by

$$f = \left(\frac{k_{12} + k_{21} - \alpha}{\beta - \alpha}\right) e^{-\alpha t} + \left(\frac{k_{12} + k_{21} - \beta}{\alpha - \beta}\right) e^{-\beta t} \quad .. \quad .. \quad (2)$$

which is obtained by dividing equation 1 by D and which can be simplified to

$$f = ae^{-\alpha t} + be^{-\beta t} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where

$$a = (k_{12} + k_{21} - \alpha)/(\beta - \alpha) \quad .. \quad .. \quad .. \quad .. \quad (4)$$

and

$$b = (k_{12} + k_{21} - \beta)/(\alpha - \beta) \quad .. \quad .. \quad .. \quad (5)$$

At time zero, equation 3 may be solved to give

$$a + b = 1 \quad .. \quad .. \quad .. \quad .. \quad .. \quad (6)$$

which can be combined with either equation 4 or 5 to derive the expression

$$(k_{12} + k_{21}) = b\alpha + a\beta \quad .. \quad .. \quad .. \quad .. \quad (7)$$

Substitution for $(k_{12} + k_{21})$ in the known relationship (Riegelman & others, 1968)

$$\alpha + \beta = k_{12} + k_{21} + k_2 \quad .. \quad .. \quad .. \quad (8)$$

provides

$$k_2 = a\alpha + b\beta \quad .. \quad .. \quad .. \quad .. \quad .. \quad (9)$$

which can be used to calculate the value for k_2 from the values for α and β and the intercept values (a and b) obtained by the method of feathering (Notari, 1971) or by nonlinear regression based on equation 3 (Metzler, 1969). The remaining constants may then be calculated using the known equations (Riegelman & others, 1968)

$$k_{21} = \alpha\beta/k_2 \quad .. \quad .. \quad .. \quad .. \quad .. \quad (10)$$

and

$$k_{12} = \alpha + \beta - k_2 - k_{21} \quad .. \quad .. \quad .. \quad (11)$$

Pre-equilibrium case

When distribution between the central (B) and peripheral (T) compartments is sufficiently rapid relative to elimination, or $[(k_{12} + k_{21})/k_2] \rightarrow \infty$ in Fig. 2, the system will approach the equilibrium value

$$K = k_{12}/k_{21} = f_T/f_B \quad .. \quad .. \quad .. \quad .. \quad (12)$$

where f_T is the fraction of D in T and f_B is the fraction of D_t in B. The case where pre-equilibrium occurs has been referred to as a one-compartment open model (Notari, 1973) since it can be described by a monoexponential equation (Notari, 1971). Thus, the rate of change of the fraction of dose, f, remaining in the whole animal, may be written

$$-df/dt = k_{obs}f = k_T f_B \quad .. \quad .. \quad .. \quad .. \quad (13)$$

and since

$$f = f_B + f_T \quad .. \quad .. \quad .. \quad .. \quad (14)$$

it can be shown using equations 12 and 14 that

$$f_B = f/(K + 1) \quad .. \quad .. \quad .. \quad .. \quad (15)$$

Thus, combining equations 13 and 15 yields

$$k_{obs} = k_2/(K + 1) \quad .. \quad .. \quad .. \quad .. \quad (16)$$

Substituting the ratio k_{12}/k_{21} for K shows that the observed rate constant, k_{obs}, is equal to β which is the elimination constant normally calculated from blood level data (Notari, 1971) since f_c is f_b/f,

$$k_{obs} = \left(\frac{k_{21}}{k_{12} + k_{21}}\right)k_2 = f_c k_2 = \beta \quad .. \quad .. \quad (17)$$

Rewriting equation 13 in terms of β yields

$$-df/dt = \beta f \quad .. \quad .. \quad .. \quad .. \quad .. \quad (18)$$

which can be integrated and rewritten in the logarithmic form as

$$\text{Ln } f = \text{Ln } 1 - \beta t \quad .. \quad .. \quad .. \quad .. \quad (19)$$

Therefore, a first-order plot of data for the fraction of the dose remaining in the whole animal would have a slope of minus β and intercept of Ln 1 if the drug behaves according to a one compartment model.

Testing validity of equations. Before application of these equations to whole animal studies, it is appropriate to determine how well they can be expected to work. This is conveniently done by using an analog computer to simulate the model in Fig. 2. A variety of one and two compartment systems was simulated by changing the values of the three first-order constants, k_{12}, k_{21} and k_2. In each case data were generated to simulate the fraction of dose remaining in the whole animal and the fraction of the dose remaining in the central compartment. This allowed calculation of the constants in the model (k_{12}, k_{21} and k_2) from whole animal data and from data

representing results obtained by sampling blood. Since the values originally employed to generate the analogue data were known, the % difference between the calculated value and the original value was determined for each estimate. The adequacy of using whole animal data is thus compared to the commonly employed methods which use blood sampling.

Data were compared in three ways. Nonlinear regression analysis was employed to estimate the rate constants and their standard deviations. In this case α and β in equation 3 were written in terms of k_{12}, k_{21} and k_2 and the biexponential equation for plasma data was written in a similar manner (Notari, 1971). The data were chosen to represent 8 points in the alpha phase and 8 points in the beta phase for each case studied. A constant value for f_T/f_B was used as the criterion for the beginning of the beta phase. In a second study the ability to use whole animal data to estimate the constants was compared to blood level data by feathering (Notari, 1971). In a third study the values for the apparent first-order rate constant, β, and the intercepts of the first-order plots used to calculate this constant were examined for a series of primarily pre-equilibrium cases using both whole animal data and blood data.

RESULTS

Table 1 summarizes the comparison of whole animal pharmacokinetic analysis to that of the blood level method using nonlinear regression analysis to estimate the parameters. The methods appear equally reliable within the range of values shown. These cases represent two-compartment models with both alpha and beta phases. However, in the cases where the value *b/a* exceeds 10 the alpha phase is barely detectable by inspection of whole animal data although the computer analysis gave satisfactory results. The results in Table 2 show that data associated with *b/a* ratios of 10 or more could not be adequately treated using feathering.

The analysis of data representing a one compartment model, where $(k_{12} + k_{21}) >> k_2$, provided identical values for β regardless of which type of data was employed–plasma or whole animal. The intercept values, *B* and *b*, represent the only difference between the methods. This difference is examined in the discussion section.

Table 1. *Comparison of whole animal data to blood level data in calculating the microconstants for a two compartment model. Data were generated by analog computer and analysed by nonlinear regression analysis.**

Analog values			Whole Animal			Blood		
k_{12}	k_{21}	k_2	k_{12}(±s.d.); %Δ	k_{21}(±s.d.); %Δ	k_2(±s.d.); %Δ	k_{12}(±s.d.); %Δ	k_{21}(±s.d.); %Δ	k_2(±s.d.); %Δ
5	5	1	†4·13(0·31); 17	4·99(0·19); 0·2	0·885(0·02); 11	4·74(0·01); 5·2	4·79(0·03); 4·2	0·956(0·03); 4·4
3	3	1	†2·93(0·18); 2·3	3·15(0·11); 5·0	0·936(0·01); 6·4	2·84(0·01); 5·3	2·87(0·03); 4·3	0·950(0·005); 5·0
2	2	1	1·97(0·04); 1·5	2·09(0·02); 4·5	0·946(0·005); 5·4	1·91(0·004); 4·5	1·97(0·01); 1·5	0·982(0·002); 1·8
1	1	1	0·957(0·08); 4·3	0·994(0·01); 0·6	0·944(0·002); 5·6	0·954(0·004); 4·6	0·987(0·01); 1·3	0·954(0·003); 4·6
1	1	2	0·969(0·05); 3·1	0·941(0·04); 5·9	1·92(0·02); 4·0	0·951(0·02); 4·9	1·01(0·06); 1·0	1·92(0·02); 4·0
1	1	5	1·01(0·03); 1·0	1·03(0·03); 3·0	4·79(0·02); 4·2	0·932(0·06); 6·8	1·13(0·18); 13	4·79(0·06); 4·2
3	1	1	2·69(0·09); 10	0·961(0·02); 3·9	0·909(0·01); 9·1	2·86(0·009); 4·7	0·993(0·01); 0·7	0·957(0·01); 4·3
5	1	1	4·82(0·13); 3·6	1·02(0·01); 2·0	0·921(0·01); 7·9	4·75(0·01); 5·0	0·994(0·01); 0·6	0·965(0·01); 3·5
5	3	1	†4·36(0·27); 13	3·02(0·09); 0·7	0·887(0·02); 11	4·68(0·01); 6·4	2·87(0·01); 4·3	0·983(0·003); 1·7
8	3	1	†7·72(0·72); 3·5	2·97(0·11); 1·0	0·942(0·04); 5·8	7·66(0·01); 4·3	2·92(0·01); 2·7	0·954(0·01); 4·6

* The program NONLIN was used (Metzler, 1969). † The ratios *b/a* were greater than 10. All of the *B/A* ratios were less than 1.

Table 2. *Comparison of whole animal data to blood level data in calculating the microconstants for a two compartment model. Data were generated by analog computer and analysed graphically using the method of feathering.**

Analog values			Whole Animal				Blood			
k_{12}	k_{21}	k_2	(b/a)	k_{12}; %Δ	k_{21}; %Δ	k_2; %Δ	(B/A)	k_{21}; %Δ	k_{21}; %Δ	k_2; %Δ
1	1	4	0·38	1·07; 7·0	0·99; 1·0	4·30; 7·5	0·06	1·00; 0	1·09; 9·0	3·88; 3·0
1	2	4	0·61	1·04; 4·0	2·06; 3·0	3·85; 3·8	0·16	0·95; 5·0	1·89; 5·5	3·94; 1·5
4	2	3	2·3	3·92; 2·0	1·98; 1·0	2·90; 3·3	0·20	3·76; 6·0	2·24; 12	2·91; 3·0
8	2	5	2·2	8·20; 2·5	1·93; 3·5	5·04; 0·8	0·11	7·66; 4·2	1·93; 3·5	4·78; 4·4
2	8	5	4·3	2·10; 5·0	7·86; 1·8	4·87; 2·5	1·3	2·01; 0·40	8·90; 11	4·77; 4·6
3	2	2	3·2	2·89; 3·7	1·98; 1·0	1·90; 5·0	0·31	3·18; 6·0	2·17; 8·5	2·03; 1·5
4	3	1	10·9	—†	—†	—†	0·57	3·91; 2·2	3·05; 1·7	0·99; 1·0
8	8	1	30·1	—†	—†	—†	0·88	8·13; 1·6	8·14; 1·7	1·02; 2·0

* The method is described by Notari (1971). † The α phase is difficult to analyse using the method of feathering whole animal data when $b/a \geqslant 10$. Table 1 has successful entries for similar ratios using nonlinear regression.

DISCUSSION

The applicability of whole animal data has been determined for evaluating the time course of those drugs which can adequately be described by either a one or two compartment model following rapid intravenous injection. Results of pharmacokinetic calculations on simulated whole animal data have been compared to those using simulated plasma data. The observed difference in the methods can be attributed to the difference in the coefficients of the analogous equations. The fraction within the central compartment, based on the dose, may be described by the equation

$$f_B = Ae^{\alpha - t} + Be^{-\beta t} \quad .. \quad .. \quad .. \quad .. \quad (20)$$

which differs from equation 3 (for whole animal data) only in the coefficients A and B. The values for the coefficients in the case of whole animal data (defined in equations 4 and 5) are related to those in equation 20 in the following manner.

$$a = A + k_{12}/(\beta - \alpha) \quad .. \quad .. \quad .. \quad .. \quad (21)$$

$$b = B + k_{12}/(\alpha - \beta) \quad .. \quad .. \quad .. \quad .. \quad (22)$$

Thus, the intercept for the alpha phase is either a or A and that for the beta phase is b or B, depending on which types of data are considered, f or f_B.

There are no standard criteria in the literature for deciding whether a one or two compartment model is most appropriate in a given case. It has been suggested that a one compartment model is sufficient when $\alpha >> \beta$. In practice, failure to observe a biphasic first-order plot has generally resulted in treating the data according to a one compartment model. There are several factors controlling the degree of success in analysing data for both the alpha and beta phase in order to calculate values for k_{12}, k_{21} and k_2. If the alpha phase is too rapid for adequate sampling, the value for α and its intercept cannot be estimated. However, the condition $\alpha >> \beta$ does not in itself preclude the calculation of k_{12}, k_{21} and k_2 provided that adequate sampling is possible.

A second limitation in calculating both α and β and their intercepts is the relative intensity of the alpha phase data compared to that of the beta phase. This is a function of the relative values of a and b in the whole animal case and A and B in the plasma case. For example, if the coefficients are equal in value then half

of the total change may be ascribed to each phase. Conversely, if either B or b is nearly equal to one then the alpha portion will represent a small fraction of the total change. The feasibility of evaluating a relatively small fraction of the total change will depend upon the analytical methodology available. However, it generally becomes difficult to accurately assess a change of much less than 10% of the total and still obtain reliable estimates of the microconstants.

As shown in equation 6, the total change possible is unity since the data are described on a fractional basis throughout this paper. The portion of the total change belonging to each phase is related to the relative magnitudes of the coefficients. The ratio of the coefficients will indicate the feasibility of evaluating both the alpha and beta phase (provided that the alpha phase is not too rapid to allow adequate sampling). If we accept the arbitrary, but reasonable, estimate of a 10% minimum, as discussed above, the acceptable limits for the coefficient ratios become: 0·1 $<$Ratio $<$10. Since the values for the coefficients themselves are dependent only on the *relative* values of the rate constants, the ratio, $k_{12} : k_{21} : k_2$, also defines the coefficient ratios, b/a or B/A. Using equations 4, 5, 8 and the known relationship (Notari, 1971)

$$\alpha = 0{\cdot}5\,(k_{12} + k_{21} + k_2 + C) \quad .. \quad .. \quad .. \quad .. \quad (23)$$

it can be shown that

$$\frac{b}{a} = \frac{k_{12} + k_{21} - k_2 + C}{k_2 - k_{12} - k_{21} + C} \quad .. \quad .. \quad .. \quad .. \quad (24)$$

where $C = \sqrt{(k_{12} + k_{21} + k_2)^2 - 4k_{21}k_2}$. A similar treatment applied to the plasma case yields

$$\frac{B}{A} = \frac{k_{21} - k_2 - k_{12} + C}{k_{12} + k_2 - k_{21} + C} \quad .. \quad .. \quad .. \quad .. \quad (25)$$

Thus the acceptable range of 0·1 to 10 for the ratios b/a or B/A is dependent on the ratio $k_{12} : k_{21} : k_2$. Assuming any one of the three constants may be larger or smaller than the remaining two, leads to the consideration of six limiting cases. These six cases, wherein the coefficient ratios approach the limits of 0·1 or 10, are examined in Tables 3–5 and the following discussion.

Optimum conditions for the analysis of both phases might be regarded as those cases where half the observable change is attributed to each phase. Thus b/a or B/A may be set equal to unity and equations 24 and 25 solved to give:

$$(k_{12} + k_{21})/k_2 = 1 \quad .. \quad .. \quad .. \quad .. \quad (26)$$

for *whole animal data* where $b/a = 1$ and

$$k_{21} = k_2 + k_{12} \quad .. \quad .. \quad .. \quad .. \quad (27)$$

for *plasma data* where $B/A = 1$. The cases described by equations 26 and 27 are shown to give coefficient ratios of one in Tables 3 and 4.

Table 3 illustrates that $b/a = A/B$ when k_{12} is varied while holding the values for k_{21} and k_2 equal and constant. The reason for this can be shown by substituting k_2 for k_{21} in equations 24 and 25 to yield

$$b/a = A/B = (C_1 + k_{12})/(C_1 - k_{12}) \quad .. \quad .. \quad (28)$$

where $C_1 = \sqrt{k^2_{12} + 4k_{12}k_2}$. Table 3 further illustrates that as k_{12} is increased both b/a and A/B ratios increase. As the pre-equilibrium case of $(k_{12} + k_{21}) >> k_2$ is approached, more of the total change is due to the *alpha* phase with plasma data and the *beta* phase with whole animal data. In both cases the coefficient ratio of 10 is exceeded when $(k_{12} + k_{21}) = 10\,k_2$ and the optimum ratio of 1 is approached as k_{12} approaches zero.

Table 3. *Effect of k_{12} on ratios of coefficients.**

$(k_{12} + k_{21})/k_2$	A/B	b/a	$(k_{12} + k_{21})/k_2$	A/B	b/a
1	1·00	1·00	8	8·88	8·88
1·05	1·25	1·25	10	10·91	10·91
1·5	2·00	2·00	12	12·92	12·92
2	2·62	2·62	14	14·93	14·93
3	3·73	3·73	16	16·94	16·94
4	4·79	4·79	18	18·95	18·95
5	5·82	5·82	20	20·95	20·95
6	6·85	6·85			

* $k_{21} = k_2$

Table 4. *Effect of k_{21} on ratios of coefficients.**

$(k_{12} + k_{21})/k_2$	B/A	b/a	$(k_{12} + k_{21})/k_2$	B/A	b/a
1·0	~0	10·00	6	3·52	26·96
1·1	0·03	1·10	8	5·39	50·98
1·2	0·06	1·22	10	7·31	82·98
1·3	0·10	1·39	12	9·26	123
2	0·38	2·62	14	11·22	171
3	1·00	5·83	16	14·18	258
4	1·77	10·90	18	16·16	326
5	2·62	17·94	20	17·15	362

* $k_{12} = k_2$

Table 4 illustrates the effect of k_{21} on the ratios of the coefficients. Both the B/A and b/a ratios increase as k_{21} increases. Thus, the fraction of the total change represented by the alpha phase becomes smaller as pre-equilibrium conditions are approached. If 10% of the total change is needed to assess the alpha phase, plasma data could be analysed up to ratios of $(k_{12} + k_{21})/k_2 = 12$ while whole animal data would be limited to the value $(k_{12} + k_{21})/k_2 \leqslant 4$. As k_{21} approaches zero, b/a approaches the optimum ratio of 1 whereas B/A reaches the lower limit of 0·1 at $(k_{12} + k_{21})/k_2$ equal to 3. Thus the overall range of workable rate constant values for whole animal data is somewhat less than with plasma data when $k_{12} = k_2$.

The pre-equilibrium condition has been defined as the case where $[(k_{12} + k_{21})/k_2] \to \infty$ in Fig. 2. The resulting kinetics are characteristic of a one compartment model or the case when the relationship $k_{12}/k_{21} = f_T/f_B$ is time independent and therefore $f_c = k_{21}/(k_{12} + k_{21})$ (Notari, 1971). A simple case to consider is that of $k_{12} = k_{21}$ with the equilibrium value of 0·5 for f_c. Since the coefficients are in terms of fractions the values for A and B would also be 0·5. Table 5 summarizes a comparison of the coefficients A, B, a and b for the case where $k_{12} = k_{21}$ and k_2 is varied. Pre-equilibrium might be said to begin at approximately $(k_{12} + k_{21})/k_2 = 10$ where the observed

Table 5. *Effect of k_2 on coefficients as the microconstant ratios* are changed from a two- to a one-compartment pharmacokinetic model.*

$(k_{12}+k_{21})/k_2$	A	B	B/A	a	b	b/a
0·10	0·998	0·002	0·002	0·948	0·052	0·051
0·18	0·992	0·008	0·008	0·902	0·098	0·11
0·25	0·99	0·01	0·01	0·86	0·14	0·16
0·50	0·95	0·05	0·05	0·72	0·28	0·38
0·75	0·90	0·10	0·11	0·60	0·40	0·67
1·0	0·85	0·15	0·17	0·50	0·50	1·00
2·0	0·72	0·28	0·38	0·28	0·72	2·62
4·0	0·62	0·38	0·61	0·14	0·86	6·34
6·0	0·58	0·42	0·72	0·09	0·91	10·2
10·0	0·55	0·45	0·82	0·05	0·95	18·1
14·0	0·53	0·47	0·87	0·04	0·96	26·1
18·0	0·53	0·47	0·89	0·03	0·97	34·1
24·0	0·52	0·48	0·92	0·02	0·98	46·0
32·0	0·52	0·48	0·94	0·02	0·98	62·0
60·0	0·51	0·49	0·97	0·01	0·99	118
100·0	0·502	0·498	0·98	0·005	0·995	198
200·0	0·501	0·499	0·99	0·002	0·998	398

* $k_{12} = k_{21}$

value for B is 10% below the equilibrium value or at some higher ratio such as $(k_{12} + k_{21})/k_2 = 14$ where a difference of 6% is observed. It is apparent that plasma level data might be evaluated at the largest ratio listed, $(k_{12} + k_{21})/k_2 = 200$, provided the alpha phase is not too rapid, since at the equilibrium ratio for B/A half of the total observable change would belong to each phase. Conversely, the ratio b/a indicates that whole animal data would reach the point where the alpha phase represents less than 10% of the total observable change at $(k_{12}+k_{21})/k_2 = 6$ where $b/a = 10{\cdot}2$. Plasma data reach the lower limit of acceptability, $B/A = 0{\cdot}1$, when $k_{12} : k_{21} : k_2$ becomes 3 : 3 : 8 whereas b/a does not reach this limit until the ratio for the rate constants becomes 1 : 1 : 11. Thus the overall ranges of values for the rate constants $(k_{12} : k_{21} : k_2)$ when $k_{12} = k_{21}$ are roughly 3 : 3 : 8 to 7 : 7 : 1 for *plasma* data and 1 : 1 : 11 to 3 : 3 : 1 for *whole animal* data.

There are some general conclusions which can be made in comparing the range of applicability for whole animal data to that of plasma data when either k_{21} or k_2 are altered so that a pre-equilibrium model is approached, $[(k_{12} + k_{21})/k_2] \rightarrow \infty$. Both b/a and B/A will increase but the rate of increase is much more rapid for whole animal data. This difference in rate of increase is illustrated in Fig. 3 where the most rapid increase in ratio is for b/a when k_{21} is increased with k_{12} held constant and equal to k_2. In the case of whole animal data the value for a decreases and b increases as α becomes large relative to β regardless of which of the three microconstants is increased in value. Conversely, when the increase in α is due to an increase in k_{12} then the value for A increases and B decreases. Thus, the whole animal data and plasma data behave in opposite ways with respect to k_{12}, but similarly with respect to k_{21} and k_2. These patterns are illustrated in Fig. 4.

It is obvious in pharmacokinetic modeling that some measurable change must be due to the alpha phase in order to allow evaluation of the slope, α, and intercept, a or A. The fraction of the total observable change which is required to be due to the alpha phase is dependent upon factors such as assay sensitivity. However, it can easily be concluded by examining Fig. 4 that the range of applicability of whole

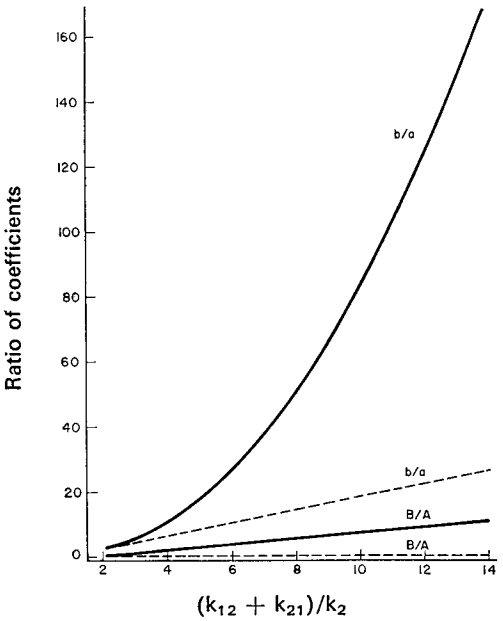

FIG. 3. Comparison of the rate of increase of coefficient ratios for whole animal data (b/a) to that for plasma level data (B/A) as a function of k_{21} (——) and k_2 (– – – –). In each case the values of the remaining constants are equal to each other.

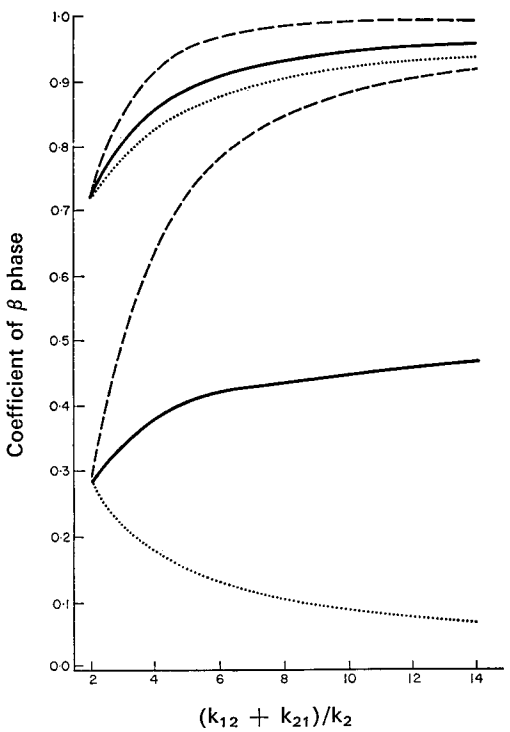

FIG. 4. Comparison of change in b (initial values 0·72) to change in B (initial values 0·28) as a function of k_{12} (. . . . .), k_{21} (— — —) and k_2 (——). In each case the values of the remaining constants are equal to each other.

animal data is generally less than that of plasma data regardless of what fraction is chosen. This figure shows that b approaches unity faster than B regardless of which microconstant is being altered.

When all three constants are equal in value the value of b is 0·72 whereas B is 0·28, indicating that the fraction attributable to the alpha phase, at $(k_{12} + k_{21})/k_2 = 2$, is smaller for whole animal data. However, within the range of applicability, the success achieved with whole animal data is in fact similar to that of plasma level data as can be seen in Tables 1 and 2.

Wagner (1971a) has advised against concluding that a one compartment model is operative based upon data representing total drug remaining in the body. Wagner employed two examples to illustrate this point. The values for k_{21}, k_{12} and k_2 (h^{-1}) were: 1·93, 0·993, 0·730 and 4·16, 2·94, 0·403. In both cases the alpha phase was markedly evident with plasma data but nearly absent when total drug in the body was examined. The ratios for the coefficients calculated from the above rate constants are: $B/A = 1{\cdot}16$; $b/a = 8{\cdot}52$ for the first example and $B/A = 1{\cdot}26$; $b/a = 40$ for the second. Based on the previous discussion regarding the range of whole animal applicability, one would predict that the first case could be analysed with reliable results but not the second case. The values given by Wagner were employed to generate data using an analog computer. The results were analysed by non-linear regression using methods identical to those outlined in reference to Table 1. As predicted, the whole animal method was applicable to the first case but showed rather large errors in the estimates for the second case. The results were as follows: (as calculated value, standard deviation and % difference for each constant in the order k_{21}, k_{12}, k_2 in h^{-1}) for case 1, whole animal data = 1·89 (0·045) 2·1%; 0·861 (0·035) 13%; 0·675 (0·0045) 7·5%; plasma data = 1·83 (0·017) 5·2%; 0·992 (0·047) 0·1%; 0·698 (0·0013) 4·4%; for case 2, whole animal data = 5·14 (0·60) 23%; 3·50 (0·92) 19%; 0·381 (0·0024) 5·5%; plasma data = 3·98 (0·023) 4·3%; 2·76 (0·01) 6·1%; 0·387 (0·0076) 4·0%. The second case is clearly out of the range of utility for whole animal analysis. Results for the first case, however, are reasonable even though the alpha phase was shown to be nearly absent in a semilog plot of total body content versus time (Wagner, 1971a).

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