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Computer assisted two-compartment pharmacokinetics for the individualization of drug dosing

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COMPUTER ASSISTED TWO-COMPARTMENT PHARMACOKINETICS FOR THE INDIVIDUALIZATION OF DRUG DOSING

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3/17/82

Date

COMPUTER ASSISTED TWO-COMPARTMENT PHARMACOKINETICS FOR THE INDIVIDUALIZATION OF DRUG DOSING

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INTRODUCTION

Owing to their unique abilities of rapid computation, and man's abilty to reduce complex problems and thoughts to exercises in binary arithmatic, digital computers have revolutionized many fields in the natural and social sciences. One such area is the study of pharmacokinetics. A brief history of the field will explain.

It is believed (65) that the originator of multicompartment analysis of "tracer" or (as it has now come to be referred to) pharmacokinetic data was Teorell (78) who in 1937 proposed that physiologically the body behaved as a two-compartmental open system, rather than as a single compartment. Riggs, in 1963, (66) was one of the first to give a rigorous mathematical treatment to many of the theories and observations that had been set forth concerning the existence of biophysiologic compartments and the kinetics of substance transfer between them (11,19,20,55,71,72,75,76,78).

As he stated, a common mathematical form for the general equation of compartmental analysis is: (see Appendix A for detailed derivation)

$$Cp = \sum_{i=1}^{n} C_{i} * e^{-\lambda_i * t} \qquad (\#1)$$

where Cp = drug concentration in plasma

C_i = C1, C2 . . . Cn = numerical coefficients

λ_i = 1, 2 . . . n = numerical exponents

e = the natural base e

t = time

* = multiplication symbol

Most often, this analysis is used to understand the behavior of a substance following bolus intravenous injection at time t=0 with subsequent frequent determinations of plasma concentration. (This route of administration is the most common as it allows one to assume that drug absorption is essentially instantaneous and complete, thus greatly simplifying calculations). If the body is treated as exhibiting the behavior of a single compartment a schematic view would be as shown in figure 1. The corresponding equation, derived from equation #1 would be:

$$Cp = C*e^{-\lambda*t} \qquad (\#2)$$

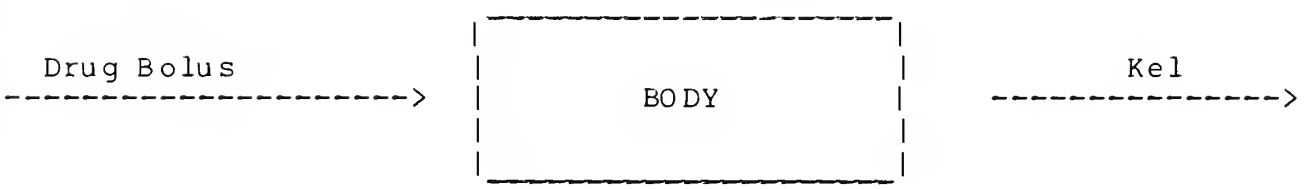
However, as Riegelman et al. (65) and others have discussed, it is physiologically and mathematically more accurate to use a two-compartment open model with elimination from the central compartment. Such a model is shown in figure 2 and is represented by the equation:

$$Cp = C_{1}*e^{-\lambda_1*t} + C_{2}*e^{-\lambda_2*t} \qquad (\#3)$$

This is frequently rewritten as

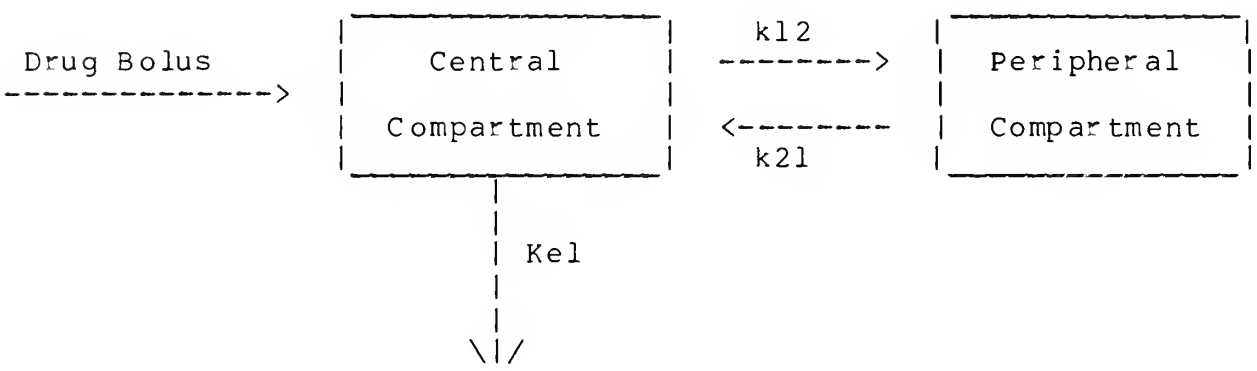
$$Cp = A*e^{-\alpha*t} + B*e^{-\beta*t} \qquad (\#4)$$

FIGURE 1



Kel = constant of elimination of drug from the single compartment

FIGURE 2



Kel = constant of elimination of the drug from the central compartment.

k12 = constant of transfer of the drug from the central to the peripheral compartment.

k21 = constant of transfer of the drug from the peripheral to the central compartment

for ease in referral to the various constants. (Whenever possible this notation will be used for the remainder of this paper).

Just as we derived figure 2 from the format of figure 1 by the insertion of another compartment, in an analogous fashion an infinite number of additional compartments with access to and from the central compartment described by constants k13/k31, k14/k41 etc. can be added. In practice, it is rarely necessary to refer to any model more complex than three compartments (11 - ref. 10) as even a large number of physiological constants can be combined, and an essentially equivalent, yet simpler, model derived.

Note here, that each compartment has a unique set of entrance and exit constants (referred to here as klN and kNl respectively). Each constant describes the rate of drug movement in one direction only, and contrary to our intuitive sense, is not necessarily equal to its partner. Indeed movement in the opposite direction may occur at a wholly different rate, presumably due to variations in transport mechanism, membrane permeability, protein binding etc.

In this system of modeling, compartment one, often referred to as the central compartment, represents that area of the body into which the drug is introduced, in the sense that it will be a medium of drug transport throughout the body. All excretion of the drug from other compartments must be mediated via the central compartment. This is usually felt to typify the plasma

(27,38,65,66) but could conceivably include extracellular fluid or some portion thereof, if this portion was always in a constant equilibrium with the plasma, with respect to the drug in question (i.e., if klN and kNl equaled infinity).

The second compartment, often called the tissue compartment, "is not clearly identified but presumably includes at least some 'intracellular fluid' " (66, p. 205). Its distinguishing characteristic is the existence of a finite kinetic constant k, which can be utilized to derive the rate at which a drug enters (or leaves) the tissue compartment from (or into) the central one. The relevant equation is:

$$dC/dT = kC \qquad (\#5)$$

This indicates that the instantaneous rate of change in concentration of a drug in a given compartment at a given time, is the product of the constant k, and the concentration C at that point in time.

If a third compartment is invoked it might be referred to as a "slowly accessible" tissue space, whereas the second one would be "rapidly accessible" (38). In the model we are considering, access to and from the third compartment is via the central one only. Finally, in this type of model it should be re-emphasized that all elimination from the body occurs via the central compartment. Thus a drug in the tissue compartment (e.g.,

adipose tissue) must first return to the central compartment before it is excreted by the appropriate means (whether in the urine, feces, sweat, etc.)

THEORY

The reason that a given drug is subjected to pharmacokinetic evaluation is that once the appropriate constants are derived, one can calculate the half-life, volume of distribution and other relevant values which can then be used to plan rational drug therapy with respect to dose, dosage interval, desired peak and trough blood levels (82, also see Appendix A). The last item can be particularly important when using agents where one needs to attain a certain peak level for efficacy yet also achieve a given trough to minimize toxicity. Clinically this becomes difficult when drugs have a low therapeutic index. The most frequent such situations encountered are probably with the aminoglycosides, and cardiac glycosides.

With a single-compartment model, there are only two parameters of interest k, and Vd (the volume of distribution of the drug). These may be considered to be roughly equivalent to, or derivable from, the terms in equation #2. Calculation of C is relatively simple. Equation #2 may be converted to equation #6 (below) by taking the natural logarithm of both sides:

$$\log Cp = -\lambda * t + C \qquad (\#6)$$

This equation is linear, and of the form $Y = mX + B$. When a plot of log Cp vs t is made, the slope is $-\lambda$ and the y-intercept is C. Then, using simple linear regression (21, p. 1-43) an exact solution for all constants can be found.

Unfortunately, with higher order models, the situation is not as straight-forward. For illustrative purposes, consider the two-compartment model. Here, there are four constants (see Figure 1) k12, k21, k10 and Vp. These can be converted to the four constants of equation #4 -- A, Alpha ,B and Beta -- using methods outlined in Appendix A. While this second set of constants is "equivalent" in the sense that each set is derivable from the other (as with the single-compartment model), there is no direct correlation between any members of each set, but rather a complex inter-relationship (64,80,82 p. 254-257).

Problems arise when one takes the logarithm of equation #4:

$$\log Cp = \log (A*e^{-\alpha*t} + B*e^{-\beta*t}) \qquad (\#7)$$

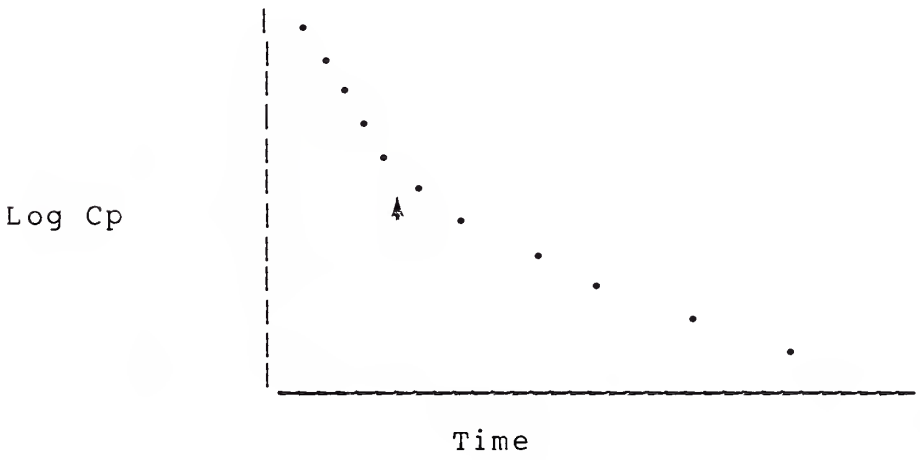
Equation #7 cannot be simplified further due to the limitations of logarithmic manipulations (see Appendix B). Thus it cannot be "linearized" as was done with equation #2 and no exact solution exists, only estimated ones (21, p. 263-304)

In the early days of pharmacokinetics most investigators

used one-compartment models in treating their data (even if they did not think of it this way at the time, compartmental theory being quite new). Thus, the problem of how best to estimate two-compartment parameters, was not a problem, or at least not a recognized one. However in the late 1940's and early 1950's, when scientists began to analyze their data in this fashion (19,55,66,75,76), a search began for good non-linear methods of estimation. Before the availability of digital computers or calculators, the commonest way to estimate the parameters was to try to manually/visually resolve the plot of log Cp vs time into its two component curves (9). Each curve could then be treated as a separate problem in one compartment kinetics and all parameters be obtained. This method is one of curve "stripping" or "peeling".

However curve stripping is a process based on an incorrect assumption. While it is true that the first term of the right side - $A*e^{-\alpha*t}$ - constitutes most of (and contributes primarily to) the initial so-called "alpha" phase of the curve (see Figure 3), with the term $B*e^{-\beta*t}$ being similarly associated with the "beta" phase, this is indeed just an association, and each term clearly contributes to values thoughout the entire curve (56,57,60). In fact, the closer the values of Alpha and Beta are to each other, the more difficult they will be to separate (either manually or by computer) (46). In addition, experimental error, or lack of data points at crucial times (i.e., around the

FIGURE 3



Typical plot of log Cp vs time for a drug introduced by bolus intravenous injection and exhibiting two-compartment pharmacokinetic behavior. The arrowhead separates the so-called "alpha" phase (left) from the terminal "beta" phase (right).

curve "crossover") can present great problems (60).

The reason crude methods such as visual resolution were employed was that more accurate solutions required non-linear regression analysis using complex iterative techniques involving innumerable detailed calculations (21) (see appendix B for reasons why iterative techniques must be used, and an appropriate example of these methods). With the advent of digital computers it suddenly became feasible to apply these iterative methods and obtain much improved solutions. Predictably, over the next few years many programs appeared in the literature and were applied to both new and existing pharmacokinetic data (3,6,9,13,24,25,35,36,40-42,44,45,52,57,59,60,62,69,88-90).

Some of these are capable of examining data for a two, three or more compartment fit and, using various criteria, of selecting the best fit (36,42,57,62,89). Others limit themselves to two-compartment evaluation (44,59,60,90).

As is a feature of iterative techniques in general, some starting value for each parameter in question is needed as an initial crude estimation which will then be refined by the program. As many workers in the field have noted, the speed with which the interation converges upon a solution, and even whether or not it will converge at all, is very dependent on the choice of good starting values, i.e., initial estimates (21,44,56,57,60,77); see also Appendix B for details.

Most of the programs mentioned obtain their starting values

via computerized curve peeling/stripping, i.e., a subroutine of the program which "separates" the curve into its "alpha" and "beta" phases (assuming a two-compartment model; with a three-compartment model the final phase might be the "gamma" phase). This, as mentioned above, is precisely what was done manually before computers became available. Once curve stripping has been done it is simple to apply one-compartment models to each phase, calculate the appropriate parameters, and use these as the initial estimates for non-linear regression analysis.

Many of these programs require a large computer system. Recognizing that not everyone has access one, several self-contained curve-stripping programs have been published (12,26,36,50,68), to be run on mini-computers or programmable calculators. These are intended to be run separately to provide good initial parameter values which can subsequently be used as starting points for non-linear regression, or can even themselves suffice as "final" values if only rough estimates are needed. Perhaps the most impressive achievement in this area is that of Muir (44) who devised a non-linear least squares regression analysis program for use on a TI-59 pocket programmable calculator.

When published reports of comparisons of the various programs (using the same sets of data) are examined, it is evident that in the vast majority of cases, the values obtained are within 25% of each other. For example, Muir's TI-59 program

(44) yields almost identical results as the program NONLIN (57) in the two-compartment data analysis used. Similarly Yeh and Kwan (90) compare results with the program COMPT (60) and three out of four parameters are within 25% of each other. Wijnan and Timmer (86) compared four different programs. In 15 out of 16 parameters calculated results varied by less than 20%. Various calculated values for the remaining parameter vary by over 100%, although it should be noted that this parameter was one to two orders of magnitude less than the others, and thus larger differences between programs were of less ultimate significance.

The lesson here is that while there may be inter-program differences in parameter estimation that is of great interest to the pharmacokineticist and/or computer programmer, these differences are rarely of such a magnitude as to be of practical interest to the clinician. Indeed one can show (see Appendix C) that over a given time period after drug administration (i.e., the period before the next scheduled dose), quite similar predicted drug levels may be calculated using remarkably different sets of parameters. Additionally when calculations are carried one step further parameters differing by as much as 30% do not yield significantly different drug doses or dosing schedules for most medications (see again Appendix C). It would appear that the "balance" of an entire set is more important than differences seen between individual items in given sets.

All of the programs mentioned above require some form of

advanced knowledge in order to be used. This ranges from a relatively minor pre-requisite of knowing how the data is to be entered and what other information is required, to writing a short FORTRAN IV computer program that "runs" the original program (42). In addition, the output of these programs are often in complex form and without any documentation, thus rendering them practically uninterpretable. For example, none of the programs have sub-routines included which will provide explanation or directions at run time (the actual time of program execution), or which will aid the user in processing the final parameters once they have been calculated. Even with the appropriate documentation found in the literature describing the programs it is obvious that they are not designed for use by the inexperienced.

Given this state of affairs, it would appear that many existing computer programs, while of great utility to pharmacologists studying the kinetics of a drug, are of limited use to clinicians.

With this in mind I wrote the computer program NOVICE, which is described below. It allows for most of the features of the previously mentioned programs. Specifically, it will treat data from IV bolus drug injection or peroral administration in a two-compartment fashion by performing curve-peeling to arrive at initial parameter estimates. NOVICE then will utilize a modified version of COMPT (60) (a non-linear regression program) to

perform non-linear least squares analysis using these initial estimates.

NOVICE has extensive documentation and instructions for the user and is designed to be accessible to the clinician with only a very limited pharmacokinetic or computer backround. It provides for substantial run-time interaction, allowing the user flexibility in deciding how to treat the data. Much of this flexibility has been extended to COMPT. NOVICE is also equipped with a library of appropriate parameters for commonly used drugs, these having been derived from population studies. (These drugs include: penicillin G, ampicillin, methicillin, oxacillin, erythromycin, cephalexin, cephradine, cefazolin, cephalothin, cefoxitin, theophylline, digoxin, propranolol, lidocaine, procainamide, quinidine, bretylium.) The user has access to these either for comparison with his/her own data, or for simulation of drug kinetics under varying conditions.

NOVICE was designed to be used in conjunction with an existing program, LEVEL3 (77) which will take the calculated parameters and, again with extensive run-time interaction, allow the user to design a schedule for drug administration. The user is able to choose desired serum levels, dose, dosing interval etc., and thus will be able to rapidly and directly apply the parameter values from NOVICE to the clinical setting.

NOVICE is a 2700 line program written in the language AlphaBASIC (2), a superset of BASIC marketed by Alpha Microsystems, Irvine, CA. It was written on an Alpha Microsystems model AM-1051 computer (using facilities kindly provided by Clinical Analytics, Inc., New Haven, CT) but may be used with all Alpha Microsystems and Olivetti S-6000 computers. A complete listing of the program is available from the author upon request. A flow chart is presented in Figures #4-8. A detailed description of the program is presented below.

NOVICE itself performs curve stripping. The values derived serve as initial estimates for non-linear regression analysis as performed by a modified version of the program COMPT (60). Source code for this program can be found in the original reference.

Testing of the program consisted of using data sets which had served as input for several other non-linear regression programs, results of which appeared in the literature (6,36,42,44,73,81,90). This data was used as input for NOVICE and the parameters obatined were compared with the previously published results.

NOVICE PROGRAM SEQUENCE

The user is first given the opportunity to view a detailed set of directions. He/she must then to choose between either utilizing existing pharmacokinetic data (herein referred to as

utilizing existing pharmacokinetic data (herein referred to as the "HAVE'DATA" mode) or running a simulated trial (the "EQUATION" mode).

The trial assumes IV bolus drug administration, and lets one introduce a fixed amount of random error (scatter) into a theoretical data set, generated by having the user type in known values of A, B, Alpha and Beta, or, if preferred, for k12, k21, kel and Vp. Known values for commonly used drugs are provided if desired. These values have been gathered from population studies in the literature (1,8,10,18,22,23,29,33,37,39,48,49,51,53,61,67,85). The scatter takes the form of a maximum error from the ideal that can be tolerated (e.g., 10%), which is then used to derive a standard deviation for the error distribution (which is normal with a mean of 0%). The standard deviation, at any sample point, is the product of the drug levels and the maximum deviation. This is similar to the procedure outlined by Cutler (15). The number of simulated drug levels is preset at 10. These are spaced logarithmically and are designed so as to cover a period of 2-3 half-lives of the drug in question. The original purpose of this simulation was to provide data sets to use in testing the curve peeling sub-routine of NOVICE. The introduction of random error to the data sets is necessary since a curve peeling algorithm may work perfectly with perfect data but give markedly inaccurate results when an element of scatter is introduced into one of the

variables. Since such error will always be present to a greater or lesser degree in any measurements make in a clinical setting, it is appropriate to introduce it in data simulation. It soon became obvious that simulation might be useful in others ways as well (see below) and it was integrated into the final version of the program.

In the HAVE'DATA mode the user analyzes a set of serum drug levels which have already been obtained. The data may be entered at run time, or optionally stored in a data file which is then specified by name at the program's execution. If data is supplied by the user, he/she will be asked for the drug name. If it is a commonly used drug, known parameter values from the literature will be made available later for comparison. With user-supplied data, the drug dosage is also required.

Additionally, with existing data, the program requests the route of administration. If the route is intravenous, then all the data entered is used in the program. If instead peroral administration is used, the program searchs for peak serum drug levels, and discards all levels prior to the peak. It then treats the data as if they referred to an IV bolus injection with the first level being the peak level and having a time=0 assigned to it. (The implications of this are discussed below).

In either mode, the user then has the opportunity to select what units of drug dosage and serum drug levels should used in data input and output.

be used in determining the best fit. The weighting factor, abbreviated as W is used in the equation describing the weighted sum of the squared residuals (SS) below:

$$SS = \sum_{i=1}^{n} (C'_i - C_i)^2 / W \qquad (\#8)$$

where C = measured serum drug level

C'= serum drug level calculated by regression (or curve peeling) derived pharmacokinetic parameters.

The user is given a choice of four weighting factors. The details and rationale of this system are discussed below.

After the weighting factor has been entered the program requests information for use in its non-linear regression phase. This consists of the convergence testing factor (CTF) and the maximum number of iterations to be used. The CTF is the value used to test whether the iterative technique has led to convergence for a minimum value for SS. The actual equation is (60):

$$(SS_{i+1} - SS_i) / SS_i <= CTF \qquad (\#9)$$

CTF is set by default to $1 * 10^{-6}$ (1E-6 in computerized notation), and the maximum number of iterations is set to 100.

notation), and the maximum number of iterations is set to 100. The user may change both of these if desired. If at any point in the iterative process the criterion of equation #9 is satisfied a "tentative" solution will be deemed to have been reached. (See Appendix D for details on how the "final" solution is actually reached). If the CTF is achieved, the number of iterations actually performed will be sub-maximal. If however, the solution converges so slowly that the CTF is never attained, after the maximum number of iterations, the program will cease cycling and use the last solution as its "tentative" one.

At this juncture, all the data and necessary criteria have been entered. It should be noted that all questions posed to the user, for which the programmer can suggest a probable response (e.g., the value for CTF) are handled by a special routine which asks the question and follows it with the expected response. The program then pauses and allows the user to either approve of this answer (by typing an carriage return) or to enter his/her own reply in its place.

The program now proceeds in its calculations. A flow chart is provided in Appendix E. Essentially, the program performs sequential curve stripping starting with the assumption that the "beta" phase is represented wholly by the last two data points and ending with the assumption that is it represented by all but the first two data points. All points not in the "beta" phase are automatically assigned to the "alpha" phase. If there are N

data points, this will yield N-3 possible combinations as theoretical models.

For each model a weighted SS is computed. This is done by calculating values for A, Alpha, B and Beta for each model via linear least squares. Then, using these values, the curve is reconstructed yielding "calculated" drug levels at the appropriate times. The calculated levels (C') are compared with the actual levels (C) and using the weighting factor selected by the user, SS's are determined. The model with the lowest SS is chosen within the limits of certain constraints. These are:

1. If the program is using data generated by random scatter about input parameters (i.e., the EQUATION mode) then no model is acceptable if any of its parameters vary by more than a factor of two from their corresponding input (or idealized) parameter.

2. Whether the EQUATION or HAVE'DATA mode is being used, all chosen parameters must be greater than zero.

If no model satisfies the criteria above an appropriate message is printed. In the HAVE'DATA mode this indicates that the probable reasons for failure to find a model were that either data entry was done incorrectly (e.g., not in chronological order) or that the model could not be fit to two-compartment

pharmacokinetics. In the EQUATION mode, it is explained that in all likelihood the error chosen was too large. The user is then allowed to reset the error and/or parameters as desired.

Once the model is chosen, the program outputs values for the number of points considered to constitute the "beta" phase and the parameters of that phase, B and Beta. It then displays the values calculated with the remaining points for A and Alpha. Standard errors for each phase are also given.

The user then has the option of viewing the following data for each model, or for just the model chosen: calculated values for A, Alpha, B and Beta, input values for A, Alpha, B and Beta (if applicable), a table comparing values for actual drug levels and calculated ones at each time, the weighted SS, and the correlation coefficient. If the program is being run in the EQUATION mode, the table will include a drug level assuming no error, and three weighted SS's will be given, comparing all combinations of "perfect" data (with no error), "given" data (with error), and "generated" or "calculated" data (levels calculated by the program using the best model, which was chosen to minimize the SS of given vs generated data). Following this, the parameters A, Alpha, B and Beta may be converted to k12, k21, kel, and Vp if desired.

Next, the program allows the user to compare the calculated parameter values with those derived from population studies, if the drug being used is one for which this data has been put in

the program's drug library.

The user may now reset the weighting factor. If he/she desires to do so, the program will then recycle through all pertinent routines using this new weight and will display the model chosen accordingly. New weighting factors may be selected until all have been chosen.

After each model calculation using a new weighting factor, the user may view a table of values for each parameter as calculated with the different weights used up to that point. This is intended to aid the user in the next task, which is selecting which of the weighting factors should be the one used in the remainder of the program. Once this weight is chosen, (if the HAVE'DATA mode is being utilized, and least squares analysis was requested) the program runs non-linear regression using an adapted version of the program COMPT (60).

Some of these changes were necessary as COMPT is written in one superset of BASIC (Leasco Time-Sharing modification of the Hewlett-Packard interpreter) (60), while the AlphaMicro AM-1051 uses a different superset, namely AlphaBASIC. Most changes in source code involved problems of syntax. (Source code is the text form of the program is written by the programmer in a "high level language," analagous to English. Source code must be translated by the computer into object code, the sequence of numbers, expressed as 0's and 1's, which directs the steps the computer must take to execute the program.) However, AlphaBASIC

does not have a system command capability for matrix manipulation which was present in the original language of COMPT. Thus, appropriate subroutines for matrix addition, matrix multiplication, matrix inversion and scalar multiplication were added using programs from Poole and Borchers (63) as a model. In addition, new routines for matrix equalization and matrix transposition were written by the author. Other changes made in COMPT included provisions for varying weighting factors and alterations in the format of data input and output. The mathematical and statistical portions of the program were left essentially intact.

After non-linear regression analysis has been finished, control of the program returns back to NOVICE, all major calculations having been completed at this point. The user now views the final data using the same format as had been applied previously (i.e., for the preliminary model derived from curve stripping); to this is added the number of iterations, convergence, standard deviations of the parameters, and appropriate half-lives. Program execution is now transferred to LEVEL3, which has been briefly described above. A demonstration of this can be found in Appendix F. If non-linear regression analysis was not done, transfer to LEVEL3 occurs whenever the user declines to reset the weight.

In addition, I have created a program entitled SINGLE, which is a version of NOVICE edited to perform one-compartment

pharmacokinetic calulations. I applied it to data sets subject to analysis by NOVICE to demonstrate the difference in parameters obtained when one assumes a one- vs two-compartment model.

RESULTS

Sample program executions which demonstate style and format can be found in Appendix F.

Table I below compares NOVICE with a curve-peeling program written by Foss (26), using tracer data cited and reproduced by that author. Here, NOVICE was able to duplicate the original results, when the proper weighting factor (so as to maximize the correlation coefficient) was used (i.e., unweighted). (A more detailed discussion of weights and correlation coefficients will be presented below).

Table II compares NOVICE and NOVICE/COMPT with results of Smith et al. (73) using their original data for pentobarbital (IV injection) and a non-linear regression program SAAM-23 (6). In none of these cases is there an exact duplication of results, however this would not really be expected. Although curve-peeling is a standardized procedure, non-linear regression is not (as evidenced by the large number of programs written to perform it). The scheme used in COMPT is but one of many feasible for the process. What is evident, is that in almost

TABLE I

	A	Alpha	B	Beta	SS	CC
Foss wt(5)	9.294	0.8596	1.134	0.3113		
NOVICE wt(1)	9.292	0.859	1.134	0.3113	0.01748	0.9998
NOVICE WT(2)	9.858	0.7468	0.1895	0.1646	0.04785	0.9985
NOVICE wt(3)	10.79	0.784	0.1222	0.1330	0.4584	0.9969
NOVICE wt(4)	10.79	0.7846	0.1222	0.1330	0.1148	0.9969
SINGLE			4.003	0.4275		0.5915

Weight codes are as follows:

1 = 1

2 = $1/C$

3 = $1/C^2$

4 = $1/(C + C')^2$

5 = $1/\sigma^2$

where C = the actual drug level
C' = the calculated drug level
σ = the variance (available only for multiple samples)

CC = the correlation coefficient

Units of A and B are mg/hr. Units of Alpha and Beta are 1/hr.

TABLE II

	k12	k21	kel	Vp	SS	CC	% kel diff	% Vp diff
Subject I								
Smith	1.524	0.599	0.043	34	0.004			
NOVICE wt(3 & 4)	1.454	0.574	0.042	34.6	0.0067	0.995	2.4	1.7
NOVICE/ COMPT	1.472	0.582	0.041	34.6	0.0063		4.8	1.7
Subject II								
Smith	0.860	0.573	0.041	42	0.017			
NOVICE wt(3 & 4)	0.598	0.479	0.039	41.1	0.0334	0.951	2.7	2.1
NOVICE/ COMPT	0.612	0.494	0.040	42.2	0.0313		2.7	0.5
Subject III								
Smith	1.405	0.508	0.036	39	0.003			
NOVICE wt(3 & 4)	1.338	0.504	0.036	42.8	0.0418	0.979	0.3	9.7
NOVICE/ COMPT	1.450	0.535	0.036	42.3	0.0096		0.5	8.5
Subject IV								
Smith	1.568	0.614	0.033	45	0.012			
NOVICE wt(3)	1.337	0.614	0.030	45.5	0.1293	0.975	7.9	1.1
NOVICE/ COMPT	1.408	0.646	0.030	45.2	0.125		7.9	0.5
NOVICE wt(4)	1.943	0.726	0.038	38.3	0.0295	0.977	13.9	14.9
NOVICE/ COMPT	1.701	0.650	0.033	40.4	0.0273		3.0	10.2

(Continued on next page)

Subject V	k12	k21	kel	Vp	SS	CC	% kel diff	% Vp diff
Smith	0.455	0.281	0.036	46	0.11			
NOVICE wt(3 & 4)	0.367	0.233	0.033	54.6	0.123	0.949	7.2	18.7
NOVICE/ COMPT	0.529	0.312	0.039	49.7	0.123		9.4	8.0

Weight codes are the same as in Table I

CC = Correlation Coefficient

Use of NOVICE/COMPT is always with the weight(s) listed immediately above for NOVICE alone. In the case of multiple weights, weight #4 was used to calculate the SS.

Units of k12, k21, and kel are 1/hr. Units of Vp are liters.

all cases COMPT improved NOVICE's initial results (with respect to duplicating Smith's SAAM-23 answers - whether or not this actually is an improvement is debatable). In only one instance (case IV) did the two primary data weighting schemes give different results. For comparison's sake, the percent difference between kel and Vp were used - kel since it is used to calculate the plasma half-life, possibly the most important parameter to clinicians, and Vp because it is a "global" parameter derived from all the others. Table III compares NOVICE with results from Muir's TI-59 program (44), and NONLIN (42), on data cited and reproduced by Muir. COMPT was not utilized on this data as drug dosage was not supplied, and interconversion from A, Alpha, B and Beta (the parameters used by Muir and NOVICE) to k12, k21, kel and Vp (the parameters used by COMPT) requires this piece of information. Two points bear mention. First, while it is obvious that parameters calculated by NOVICE differ by as much as a factor of about 70 from those of NONLIN, in no case does the best NOVICE model (as measured by the correlation coefficient) produce drug levels which on the average differ from the actual drug levels by more than 20%. (Indeed, in but one case the average difference is less than 10%). Thus, as was mentioned above, markedly different parameters can, as a group, yield quite similar (and accurate) blood levels. Secondly, the original table by Muir (44), not reproduced here, gives standard deviations for the parameters, which in some cases are as much as

TABLE III

	A	Alpha	B	Beta	Corr Coeff	Avg Level Diff
Subject I						
NONLIN	0.94627	0.03761	0.23748	0.00209		
TI-59	0.94638	0.03760	0.23729	0.00209		
NOVICE wt(3)	0.74885	1.9388	0.25221	0.14185	0.960	8.4
NOVICE wt(4)	0.84925	2.24801	0.30220	0.17189	0.987	5.9
Subject II						
NONLIN	10.5271	0.39822	0.61991	0.00685		
TI-59	10.5236	0.39817	0.61984	0.00685		
NOVICE wt(3)	0.57710	2.19310	0.40135	0.24004	0.879	20.9
NOVICE wt(4)	0.86887	4.3534	0.44044	0.25993	0.925	20.0
Subject III						
NONLIN	0.60006	0.02441	0.09298	- 0.00012		
TI-59	0.60065	0.02440	0.09265	- 0.00015		
NOVICE wt(3 & 4)	0.64224	2.52802	0.17082	0.10646	0.965	8.2
Subject IV						
NONLIN	1.23932	0.09393	0.64843	0.00560		
TI-59	1.23997	0.09422	0.64955	0.00561		
NOVICE wt(3 & 4)	1.16726	4.64235	0.58951	0.29353	0.990	4.8
Subject V						
NONLIN	1.71104	0.10826	0.44583	0.00254		
TI-59	1.71174	0.10852	0.44663	0.00259		
NOVICE wt(3)	1.23140	3.54244	0.33794	0.07995	0.936	9.5
NOVICE WT(4)	1.37386	4.20068	0.35905	0.09291	0.950	8.8

Units of A and B are mg/hr. Units of Alpha and Beta are 1/hr.

ten times greater than the parameter values themselves. Thus, even with NONLIN, which is "probably the most frequently used [program]" (44), confidence limits can be huge. In one case, the programs even arrived at a negative value for Beta.

Table IV compares NOVICE and NOVICE/COMPT with the results of a non-linear regression program written by Yeh and Kwan (90). The data used was originally published by Wagner et al. (81) and is reproduced by Yeh and Kwan. Here, all weighting factors produced extremely high correlation coefficients, although the closest average drug levels (by percentage) were not reproduced by the model with the best correlation coefficient. Further, COMPT did not improve the average drug levels, although it did reduce the SS. All two-compartment models proved superior to the one-compartment model (found by SINGLE) as measured by the correlation coefficient and the average drug level difference.

TABLE IV

	k12	k21	kel	Vp	CC	SS	Avg Level diff
Yeh & Kwan	0.136	0.247	0.545	7.83			
NOVICE wt(1)	0.867	1.601	0.695	6.25	0.9989		7.8
NOVICE/COMPT	0.835	1.541	0.669	6.67			
NOVICE wt(2)	0.867	1.601	0.695	6.25	0.9989		7.8
NOVICE/COMPT			failed to converge				
NOVICE wt(3)	0.152	0.329	0.547	7.78	0.988	0.037	5.1
NOVICE/COMPT	0.159	0.345	0.574	7.59		0.024	10.8
NOVICE wt(4)	0.153	0.329	0.547	7.78	0.988	0.009	5.1
NOVICE/COMPT	0.158	0.342	0.569	7.65		0.006	7.7
SINGLE					0.936		16.5

Units of k12, k21, and kel are 1/hr. Units of Vp are liters.

DISCUSSION

A number of authors over the preceding twenty years have attested to the fact that drug doses and/or dosing schedules must be altered in patients with renal disease (4,16,17,79,81). In particular, Dettli et al. (16) developed detailed procedures for adjustment of drug dosage based on a patients creatinine clearence, whereby clinicians could calculate how changes in the glomerlular filtration rate (GFR) would alter the normal kel. The normal kel was determined by studies on populations of varying sizes; requiring, of course, a separate study for each drug. The primary difficulty here is that this "normal" kel represents an averaged figure and may well be incorrect for any given patient under consideration. Another problem, is that no provision is made for patients who might require altered drug dosing on a basis other than, or in addition to, renal disease; for example the patient with cirrhosis or congestive heart failure receiving a drug metabolized partly or in whole via an hepatic route.

It is for these reasons that I developed NOVICE, wrote it to be used with a modified form of COMPT and LEVEL3, and made it accessible to individuals with little, if any, experience with computers and pharmacokinetics. It should prove particularly valuable when being used with a drug with a low therapeutic index where careful maintanance of appropriate blood levels is imperative.

NOVICE was programmed to allow the user to have the option

of two-compartment pharmacokinetic simulation. The subroutine EQUATION contains most of the source code for this. It is anticipated that simulation will be used primarily by pharmacologists; applications would include the following:

1. The prediction of serum blood levels with a given set of "estimated" parameters, thus enabling one to make educated decisions as to when serum blood levels should be determined in an actual pharmacokinetic experiment. Proper timing would insure that adequate numbers of blood samples are obtained from periods where there is a rapid change in drug levels (e.g., the "alpha" phase).

2. The determination of the maximum amount of error that can be introduced into a generated set of data, while still enabling one to rederive the parameters used to produce the data. Researchers developing a drug assay would then know in advance the degree of precision that was required.

3. The calculation (with the aid of LEVEL3) of a number of different dosing schedules based on the introduction of varying levels of error into a given data set. This would facilitate comparison of the effect of errors in drug assay on ultimate drug dosage.

NOVICE is designed so that non-linear least squares analysis in parameter estimation is not performed when the EQUATION mode is being used. This was done since non-linear regression, while a useful tool in data analysis, rarely produces changes greater than 20% in the parameter estimates -- and usually produces changes far less -- as was shown above. Since in this mode the data is generated from "theoretical" or "estimated" parameters (to which error is then introduced) there seems little need to further adjust the "preliminary" estimates of NOVICE's curve-stripping. Indeed the EQUATION mode was not designed for exact parameter derivation from any given set of starting values and error. Its real purpose lies in comparing the differences in parameters calculated from the same set of starting values, and varying levels of error.

There are, of course, limitations to the program(s):

1. To use them, at least four (and preferably more for increased accuracy) serum drug levels must be available following a single drug dose. This could be a problem if serum drug determinations are inconvenient to perform, or, if in an emergent situation a loading dose and them maintanance doses of a drug must be administered rapidly without waiting for the appropriate blood samples to be drawn.

2. In a patient with fluctuating levels of renal function, the results of a trial dose and subsequent parameter determination may not apply the next day, as GFR's can change rapidly. This, of course, is a limitation with all computerized kinetic programs.

3. Pharmacokineticists may not be interested in LEVEL3, but for clinicians it will be of primary concern, as it does the work of changing the data from kinetic parameters to a drug dosing schedule. However, this results in two problems. First, if peroral administration is the chosen route, there are no provisions for individualizing the fraction of a drug absorbed (Fabs) and the kinetic rate constant of absorption (Kabs). Instead, Fabs is assumed to be a bioavailability of 100% (although the user enter a different value), and Kabs - if not available from the literature - is set to have drug absorption at greater than 90% after one hour. These settings are obviously gross estimates but should prove adequate in most clinical situations.

4. While adding COMPT to NOVICE always results in a reduction of the SS, it can take quite some time to achieve convergence (and convergence is required for non-linear regression to be completed).

Pharmacokineticists will likely be willing to make this sacrifice, clinicians may not. This is particularly true when one realizes that COMPT does not always give better results for drug levels, as measured by the average percentage of level difference. This can be seen in tables II and IV. A reduction is COMPT time can be achieved by choosing a larger CTF, or reducing the maximum number of iterations. Alternatively, the option is offered to bypass non-linear regression altogether and pass directly to LEVEL3.

5. Use of LEVEL3 requires one to specify in advance a desired minimum and maximum serum drug level. For antibiotics this should be simple since minimum inhibitory concentrations are widely available, and the maximum level might be a factor of 8 to 16 times greater (a relationship perinent to serum bacteriocidal concentration). Even for other drugs this should not be a problem as most drugs for which an assay exists are also drugs for which optimum serum levels have been determined (e.g., digoxin, lithium, quinidine, theophylline, pentobarbital etc.).

6. The ability of both NOVICE and NOVICE/COMPT to accurately derive the kinetic constants Alpha and

> Beta is very much dependent on the magnitude of difference between them. Myhill et al. (33) found that with an error in data measurement of 2% they could resolve rate constants (with an accuracy of 10-15%) differing by a factor of two; with an error of 6% a ratio of four between costants was required; with an error of 10%, a ratio of six. While detailed studies such as these were not performed on this program, a similar trend was clearly noted.

The flow-chart for the sub-routine CORRECT'BETA (figure 8) deserves comment. In either mode of operation it is required that all parameters be positive for a model to be considered acceptable. The justifications for this are primarily ones of tradition and reason. Many of the pharmacokinetic computer programs cited here (NONLIN and Muir's TI-59 program are exceptions) incorporate this criterion. Beyond this though, it would seem that any model used in calculations should conform to reality which obviously requires that once a drug is administered and fully absorbed the amount of drug present in the body must decline rather than rise. The reader should be aware however, that there is another school of thought. Smolen (8, p. 365) for example, has proposed that "[C]ompartment models are not necessarily unique and realistic descriptions of the biokinetic behavior of a system.. It is suggested that . . . it may be

justifiable to abandon all pretenses of requiring compartment models to possess physical reality." (We often do abandon reality when we speak of the concept of a volume of distribution. For example, a drug with a long half-life, such as digitalis, commonly will have a volume of distribution far in excess of a patient's total body water.)

In addition, in the EQUATION mode it is required that any model chosen have parameters within a factor of two of those used to generate the data. This criterion is necessary, because when blood levels are drawn close together -- e.g., in the "alpha" phase -- errors in the opposite direction in the drug concentration tend to be magnified by a small t in linear slope determination, such that the derived values for A and Alpha will be very different from the original ones. This is particularly true if the number of points in the "alpha" phase is small. For example, if the original data were:

time = .1 hr level = 100 units
time = .2 hr level = 200 units

then the true slope, Δ level/Δtime would equal 12.5 units/hr. If an error of say 20% is applied in opposite directions the levels at .1 and .2 hours might be 120 and 64 units respectively yielding a slope of almost 20 units/hr. These effects are consistent with the results of Myhill et al. (46) in their experiments on the introduction of random error into data

generated by a bi-exponential equation.

The use of two as a cutoff factor is somewhat arbitrary, but may be justified as allowing enough parameter variation to tolerate errors of up to 20% (certainly within the limits of most laboratory assays (32)) but still rejecting models where parameters vary by greater than 100% from their original values. In the mode of existing data (HAVE'DATA) there is no analagous cutoff factor.

Other major points to be addressed are the accuracy of NOVICE (and its combination with COMPT) as compared with other programs in existence, how best to use this program, and how confident may one be that its parameters can be translated into desired drug levels and drug doses. To treat these fully, one must understand the meaning and use of weighting factors.

Ideally, in curve fitting, a value for an observation at any given time should be assigned a weight inversely proportional to its variance (54,57). Thus, "[i]f the reproducibility of the estimation of any variable at any sampling point is bad . . . the large variance at that point automatically ensures that the local normalized sum of squares will be small, i.e., that sample will not play a large role in determining the final fit." (54, p. 730)

Unfortunately, in the vast majority of cases, data used here will provide only single observations at each sampling point and thus no variance is available. A number of solutions have been devised for this problem, none totally sataisfactory. Some of

the more complex have involved division of each data point by the lowest data point in each equation (46), or attempting to calculate an estimated variance equal to the residual sum of squares divided by the residual degrees of freedom (the latter being the number of data points minus the number of parameters to be estimated) (57).

Ottoway (54), in a detailed discussion of data weighting felt that (based on extensive testing) the rather empiric factor of $1/(C + C')^2$ produced the best results. (Here C is the actual measured serum drug level, and C' is the value calcuated using the set of derived constants). When error was constant $1/(C + C')^2$ became functionally equivalent to $1/(C)^2$. With this in mind, the user of NOVICE/COMPT has been offered the option of weighting data by a factor of 1 (i.e., unweighted), $1/C$, $1/(C)^2$, or $1/(C + C')^2$. The latter two are strongly recommended, however the first two options might be more desireable if for some reason one wanted to give greater weight to numerically higher results (e.g., if an assay was inaccurate at low levels).

While the SS gives a rational basis for choosing the best model within a given weighting scheme, it offers no clue as to which best model between weighting schemes should be used, since interscheme variation in SS depends upon data values and not just goodness of fit. There is no such interscheme variability with the correlation coefficient; this parameter can

therefore be a valuable guide in choosing between schemes. Indeed in Table II, the model with the best correlation coefficient was always the model with the lowest average level difference. However in Table IV, that relationship did not hold in every case, which may attest to the fact that both the correlation coefficient and the average level difference are imperfect guides to goodness of fit. Other guidelines might be accuracy of the assay as suggested above, or desired serum blood levels i.e., very high or rather low. When inis doubt with such conflicting results, Ottaway's suggestion of $1/(C + C')2$ as a weighting factor would probably be the most sensible choice.

Addressing the issue of translation of calculated parameters into accurate dosage recommendations, NOVICE/COMPT has demonstrated its ability to derive parameters which can be used to predict durg levels consistently within 20% of their true values, and generally well within 10% (see Tables III and IV). Certainly this is within the range of acceptable error for all drugs used and comparable to the error inherent in most drug assays (69). However, the caveats above still apply (especially with regard to Kabs and Fabs, and changes in renal function).

The question remains of NOVICE/COMPT's performance vis a vis other similar programs. With some data and programs its results are impeccable (Table I), with others quite good (Tables II and IV) and with still others, poor (Table III), at least as regards parameter values. But, as was mentioned above, if prediction of

drug levels is the ultimate criterion, then even results such as those in Table III are adequate. In any case, to judge one program by another is a questionable process since it implicitly affirms the validity of the original program by holding it up as a "gold standard." There can be little basis for doing so. SS's can be compared, but only if the same weighting system is used. Even NONLIN reached convergence on a negative parameter value (Table III) and can produce huge (>100%) standard deviations. Indeed the authors of that program have stated "We have little interest in comparing the performance of different computer programs on one set of data. We have often said that given two computer programs A and B . . . we can find two sets of data, set I and set II, such that program A is 'better' with set II and program B is 'better' with set I." (43, p.445). Other problems with COMPT e.g., failure to converge with one set of data with a given weight (see Table IV), and dependence of accuracy on initial estimates are "common to all Gauss-Newton-based programs to a greater or lesser extent." (44, p. 13, 77)

Some pharmacokinetic programs are designed to fit a given set of data to a multi-compartment model with the ultimate number of compartments in the model to be determined by whatever number yields the "best fit" (in accordance with a pre-specified criterion of "goodness of fit"). However, it has been stated that most pharmacokinetic problems can be solved adequately with a two or three compartment model (70). For simplicity's sake,

and because NOVICE is designed for relative novices, three compartment models are not considered. The pharmacokinetic purist may find this unsavory 84, p. 448) but I believe the sacrifice in accuracy is well worth the gain in simplicity and ease of understanding. In further defense, it should be noted that using the same set of data, different programs can fit the set to models with different numbers of compartments. Nagashima et al. (47) fit IV Coumarin to a three-compartment model, while Yamaoka et al. (89), using the same data, arrived at a two-compartment one. A graphical analysis (89) of actual serum drug levels versus those predicted on the basis of either model indicate that the primary difference is in the terminal points (i.e., those at very low serum levels) when the three-compartment model exhibits a "gamma" phase non-existent in the two-compatment one. Similarly in simulated three-compartment data with 5% error (89) again the major predictive differences are at the terminal portion of the curve. (In stark contrast, a single-compartment model produces a curve with significant variations from the two- and three- compartment models at all drug levels (24)). To the clinician variations at the end of the curve should make little difference as this section of the curve represents extremely low serum drug concentrations and most patients will be maintained on much higher drug levels. These higher levels will fall in mid-range of the curve being used for analysis. Regardless of whether a two- or a three-compartment model is being used the

mid-range of the curve is the most accurate section since the early "alpha" phase may contain large errors due to problems of closely spaced samples (as discussed above) and the terminal portion of the curve is affected by the difficulty of performing an assay when the drug concentration is very low.

COMPT was chosen as the companion program to NOVICE simply because its source code was in the public domain, and available rapidly as it had been reprinted in the original publication describing the program (1). It was fortuitous that its facilities matched well with those planned for NOVICE. The only major difficulty was translating from one superset of BASIC to another and writing the necessary additional subroutines.

In summary, I have written a new computer program called NOVICE to perform two-compartment pharmacokinetic analysis in a manner both understandable and useful to the working clinician. It was designed for integration with COMPT, a non-linear least squares regression analysis program, and LEVEL3, a program to determine drug dosing using two-compartment parameters. NOVICE performs curve peeling in a highly interactive fashion, allowing the user to select various weighting factors, make appropriate interconversions, and view detailed statistical analyses. NOVICE may be used for simulation or with clinically derived data. Simulation will be of interest to the pharmacokineticist who

wants to predict in advance when to draw blood samples in an actual experiment. It will also prove useful to the clinical chemist who needs to know what degree of accuracy he/she must achieve in a drug assay. Using "real" data with the program, the clinician can determine any patient's two-compartment pharmacokinetic parameters for a given drug (with the aid of COMPT) and then using LEVEL3 translate these into an exact dosing schedule based on desired maximum and minimum blood levels. Studies show this combination of programs is able to perform pharmacokinetic calculations which predict drug levels accurate to within the confidence limits of the great majority of drug assays.

REFERENCES

1. Abramson, F.P., Two-Compartment Pharmacokinetic Models: Computer Simulations of Their Characteristics and Clinical Consequences., J. Pharm. Sci., 70:141-146 (1981).

2. "AlphaBASIC User's Manual", DWM-00100-01, Rev. 802, Alpha Microsystems, Irvine, California.

3. Atkins, G.L., A versatile digital computer program for nonlinear regression analysis, Biochim. Biophys. Acta., 252:405-420 (1971), ref. 9, as cited in Pedersen, 1977, vide infra.

4. Azarnoff, D.L., Application of Blood Level Data to Clinical Trials., Clin. Pharm. Ther., 16;183-188 (1969).

5. Benet, L.Z., and Galeazzi, R.L, Noncompartmental Determination of the Steady-State Volume of Distribution., J. Pharm. Sci., 68:1071-1074 (1979).

6. Berman, M., and Weiss, M.F., "Users Manual for SAAM", National Institutes of Health, Bethesda, MD, 1968, ref. 6, as cited in Pedersen, 1977, vide infra.

7. Bjornsson, T.D., and Shand, D.G., Estimation of Kinetic Parameters From a Two-Point Determination of the Drug Cumulation Factor., Clin. Pharmacol. Ther., 26:540-547 (1979).

8. Blouin, R.A., et al., Tobramycin Pharmacokinetics in Morbidly Obese Patients., Clin Pharmacol. Ther., 26:508-512 (1979).

9. Booth, G.W., and Peterson, T.L., "Nonlinear Estimation", IBM Share Program, No. 687 WLNL7, 1958, ref. 2, as cited in Pedersen, 1977, vide infra.

10. Borgstrom, L., et al., Pharmacokinetics of Propranolol, J. Pharmacokinet. Biopharm., 9:419-429 (1981).

11. Bronwell, G.L., Isotopes: Radioactive; Tracer Experiments, Theory., In "Medical Physics", edited by O. Glasser, Vol. 2 pp. 353-364, Year Book Publishers, Inc., Chicago, 1950, ref. 18, as cited in Riggs, 1963, vide infra.

12. Brown, R.D., and Manno, J.E., ESTRIP, a BASIC Computer Program for Obtaining Initial Polyexponential Parameter Estimates., J. Pharm. Sci., 67:1687-1691 (1978).

13. Chamber, J.M., Computer system for fitting models to data., Appl. Stat., 18:249-263 (1969), ref. 7, as cited in Pedersen, 1977, vide infra.

14. Chiou, W.L., New Calculation Method for Mean Apparent Drug Volume of Distribution and Application to Rational Dosage Regimens., J. Pharm. Sci., 68:1067-1069 (1979).

15. Cutler, D.J., Numerical Deconvolution by Least Squares: Use of Prescribed Input Functions., J. Pharmacokinet. Biopharm., 6:227-238 (1978).

16. Dettli, L., et al., Multiple Dose Kinetics and Drug Dosage in Patinets with Kidney Disease., Acta. Pharmacol. Toxicol., (Kbh.), 29(Suppl. 3):211-224 (1971).

17. Dettli, L.C., Drug Dosage in Patients with Renal Disease., Clin. Pharm. Ther., 16:274-280 (1969).

18. Dittert, L.W., et al., Pharmacokinetic Interpretation of Penicillin Levels in Serum and Urine After Intravenous Administration., "Proceedings of the IX Interscience Conference on Antimicrobial Agents and Chemotherapy.", edited by G.L. Holoby, pp. 42-48, American Society of Microbiology, Bethesda, MD, 1969.

19. Dominguez, R., Kinetic of Elimination, Absoprtion and Volume of Distribution in the Organism., In "Medical Physics", edited by O. Glasser, Vol. 2 pp. 476-489, Year Book Publishers, Inc., Chicago, 1950, ref. 33, as cited in

Riggs, 1963, vide infra.

20. Dominguez, R., Am. J. Physiol., 114:204 (1935), ref. 23, as cited in Riegelman et al., 1968a, vide infra.

21. Draper, N.R., and Smith, H., "Applied Regression Analysis.", John Wiley and Sons, Inc., New York, 1966.

22. Ehrnebo, M., Pharmacokinetics and Distribution Properties of Pentobarbital in Humans Following Oral and Intravenous Administration., J. Pharm. Sci., 6:1114-1118 (1974).

23. Ehrnebo, M., et al., Pharmacokinetics of Ampicillin and its Prodrugs Bacampicillin and Pivampicillin., J. Pharmacokinet. Biopharm., 7:429-451 (1979).

24. Eisenpress, H., et al., "Nonlinear Regression Equations and Systems, Estimation and Prediction", 7090 G21BM0035 IBM, Hawthorne, NY, ref. 12, as cited in Pedersen, 1977, vide infra.

25. Fletcher, R., "Report R6799", Atomic Energy Research Establishment, Harwell, U.K., 1971, ref. 8, as cited in Pedersen, 1977, vide infra.

26. Foss, S.D., A Method of Ibtaining Initial Estimates of the Parameters in Exponential Curve Fitting., Biometrics., 580-584 (Sept. 1969).

27. Gibaldi, M. et al., Relationship Between Drug Concentration in Plasma or Serum and Amount of Drug in the Body., J. Pharm. Sci., 58:193-197 (1969).

28. Gibaldi, M., and Perrier, D., Drug Elimination and Apparent Volume of Distribtuion in Multicompartment Systems., J. Pharm. Sci., 61:952-953 (1972).

29. Guentart, T.W., et al., Quninidine Pharmacokinetics in Man: Choice of a Dispositional Model and Absolute Bioavailability Studies., J. Pharmacokinet. Biopharm., 7:315-330 (1979).

30. Hartley, H.O., The Modified Gauss-Newton Method for the Fitting of Non-Linear Regression Fuctions by Least Squares., Technometrics, 3:269-280 (1961).

31. Hornbeck, R.W., "Numerical Methods", Quantum Publishers Inc., New York 1975.

32. Jatlow, P., Personal Communication., Dept. of Laboratory Medicine, Yale-New Haven Hospital, New Haven, CT 06510.

33. Jelliffe, R.W., A Mathematical Analysis of Digitalis Kinetics in Patients with Normal and Reduced Renal Function., Math. Biosci., 1:305-325 (1967).

34. Kaplan, S.A., Pharmacokinetic Profile of Sulfisoxazole following Intravenous, Intramuscular, and Oral Administration to Man., J. Pharm. Sci., 61:773-778 (1972).

35. Kuester, J.L. and Mize, J.H., "Optimization Technique", McGraw-Hill, New York, 1973, Chap 6., ref. 11 as cited in Pedersen, 1977, vide infra.

36. Leferink, J.G., and Maes, R.A.A., STRIPACT, an Interactive Curve Fit Programme for Pharmacokinetic Analyses., Arzneimittelforschung., 29:1894-1898 (1979).

37. Lesko, L.J., et al., Pharmacokinetics and Relative Bioavailability of Oral Theophylline Capsules., J. Pharm. Sci., 68:1392-1394 (1979).

38. Levy, G., et al., Multicompartment Pharmacokinetic Models and Pharmacologic Effects., J. Pharm. Sci., 58:422-424 (1969).

39. Lima, J.J., et al., Clinical Pharmacokinetics of Procainamide Infusions in relation to Acetylator Phenotype, J. Pharmacokinet. Biopharm., 7:69-85 (1979).

40. Marquardt, D.W., "Least Squares Estimation of Nonlinear

Parameters", IBM Share Library Program, Distribution No. 309401, 1966, ref. 3, as cited in Pedersen, 1977, vide infra.

41. Metter, D.A., "Program GAUSHAUS", Numerical Analysis Laboratory, University of Wisconsin, Madison, 1966, ref. 4, as cited in Pedersen, 1977, vide infra.

42. Metzler, C.M., "A User's Manual for NONLIN", The Upjohn Co., Technical Report 7292/69/7292/005, Kalamazoo, MI, 1969, ref. 1, as cited in Pedersen, 1977, vide infra.

43. Metzler, C.M., and Elfring, G.L., Curve Fitting and Modeling in Pharmacokinetics: A Response., J. Pharmacokinet. Biopharm., 6:443-446 (1978).

44. Muir, K.T., Non-Linear Least Squares Regression Analysis in Pharmacokinetics - Application of a Programmable Calculator., Comput. Biomed. Res., 13:307-316 (1980).

45. Murray, W., et al., Division of Numerical Analysis, National Physical Laboratory, Teddlington, Middlesex, U.K., ref. 10, as cited in Pedersen, 1977, vide infra.

46. Myhill, J., et al., Investigation of an Operator Method in the Analysis of Biological Tracer Data., Biophysical J., 5:89-107 (1965).

47. Nagashima, R., Comparative Pharmacokinetics of Coumarin Anticoagulants IV, J. Pharm. Sci., 57:1888-1895 (1968).

48. Narang, P.K. et al., Pharmacokinetics of Bretylium in Man After Intravenous Administration., J. Pharmacokinet. Biopharm., 8:363-372 (1980).

49. Neu, H.C. et al., Cefotaxime Kinetics After Intravenous and Intramuscular Injection of Single and Multiple Doses., Clin. Pharmacol. Ther., 27:677-685 (1980).

50. Nielsen-Kudsk, F., A Pharmacokinetic Program for TI-59., J. Pharmacol. Methods., 3:345-355 (1980).

51. Nightingale, C.H., et al., Pharmacokinetics and Clinical Use of Cephalosporin Antibiotics., J. Pharm. Sci., 64:1899-1927 (1975).

52. "Nonlinear Parameter Estimation and Programming", Catalog of Programs for IBM System 360 Models 25 and Above, IBM, White Plains, NY, ref. 15, as cited in Pedersen, 1977, vide infra.

53. Odar-Cederlof, I., and Borga, O., Kinetics of Diphenylhydantoin in Uremic Patients: Consequences of Decreased Plasma Protein Binding., Eur. J. Clin. Pharmacol., 7:31-37 (1974).

54. Ottaway, J.H., Normalization in the Fitting of Data by Iterative Methods., Biochem J., 134:729-736 (1973).

55. Pappenheimer, J.R., et al., Filtration, Diffusion, and Molecular Sieving Through Peripheral Capillary Membranes. A Contribution to the Pore Theory of Capillary Permeability., Amer. J. Physiol., 167:13-46 (1951), ref. 83, as cited in Riggs, 1963, vide infra.

56. Peck, C.C., and Barrett, B.B., Nonlinear Least-Squares Regression Programs for Microcomputer., J. Pharmacokinet. Biopharm. 7:537-541 (1979).

57. Pedersen, P.V., Curve Fitting and Modeling in Pharmacokinetics and Some Practical Experience with NONLIN and a New Program FUNFIT., J. Pharmacokinet. Biopharm., 5:513-531 (1977).

58. Pedersen, P.V., Curve Fitting and Modeling in Pharmacokinetics: Reply from the Author., J. Pharmacokinet. Biopharm., 6:447-449 (1978).

59. Pedersen, P.V., Model-Independent Method of Analyzing Input in Linear Pharmacokinetic Sustems Having Polyexponential Impulse Response II: Numerical Evaluation., J. Pharm. Sci., 69:305-312 (1980).

60. Pfeffer, M., COMPT, a Time-Sharing Program for Nonlinear Regression Analysis of Compartmental Models of Drug

Distribution., J. Pharmacokinet. Biopharm., 1:137-163 (1973).

61. Phillips T.A., et al., Pharmacokinetics of Oral and Intravenous Fluorouracil in Humans., J. Pharm. Sci., 69:1428-1431 (1980).

62. Poland, H., and Woloszczak, R., A Program Library for Computing the Parameters of Linear Compartment Models in Pharmacokinetcs., Int. J. Biomed. Comput., 11:115-128 (1980).

63. Poole, L. and Borchers, M., "Some Common Basic Programs", Adam Osborne and Associates Inc., Berkeley, CA 1977.

64. Riegelman, S. et al., Concept of a Vloume of Distribution and Possible Errors in Evaluation of This Parameter., J. Pharm. Sci., 57:128-133 (1968).

65. Riegelman, S. et al., Shortcomings in Pharmacokinetic Analysis by Conceiving the Body to Exhibit Properties of a Single Compartment., J. Pharm. Sci., 57: 117-127 (1968).

66. Riggs, D.S., "The Mathematical Approach to Physiologic Problems.", The Williams and Wilkins Company, Baltimore, 1963.

67. Schwartz, S.N., et al., A Controlled Investigation of the Pharmacokinetics of Gentamicin and Tobramycin in Obese Subjects., J. Infect. Dis., 138:499-505 (1978).

68. Sedman, A.J., and Wagner, J.G., CSTRIP, a Fortran IV Program for Obtaining Initial Polyexponential Parameter Estimates., J. Pharm. Sci., 65:1006-1010 (1976).

69. SHARE Program Library Agency, Triangle Universities Computation Center, P.O. Box 12076, Research Triangle Park, North Carolina 27709, ref. 14, as cited in Pedersen, 1977, op.cit.

70. Sharney, L., et al., Am. J. Med. Electronics., 4:249

(1965), ref. 10, as cited in Riegelman et al., 1968a., op.cit.

71. Shepard, C.W., and Householder, A.S., J. Alppl. Physiol., 22:519 (1951), ref. 24, as cited in Riegelman et al., 1968a, op.cit.'

72. Skinner, S.M., et al., Complete Solution of the Three Compartment Model in Steady State After Single Injection of Radioactive Tracer., Amer. J. Physiol., 196:238-244 (1959), ref. 97, as cited in Riggs, 1963, op.cit.

73. Smith, R.B., et al., Pharmacokinetics of Pentobarbital After Intravenous and Oral Administration., J. Pharmacokinet. Biopharm., 1:5-16 (1973).

74. Smolen, V.F., Quantitative Determination of Drug Bioavailability and Biokinetic Behavior from Pharmocological Data for Opthalmic and Oral Administrations for a Mydriatic Drug., J. Pharm. Sci., 60:354-365 (1971).

75. Solomon, A.K., Compartmental Methods of Kinetic Analysis., In "Mineral Metabolism", edited by C.L. Comar and F. Bronner, Vol. 1, Part A, Chap. 5, Academic Press, Inc., New York, 1960, ref. 102, as cited in Riggs, 1963.

76. Solomon, A.K., Equations for Tracer Experiments., J. Clin. Invest., 28:1297-1307 (1949), ref. 101, as cited in Riggs, 1963. op.cit.

77. Spiesel, S.Z., "Clinical Management Software", Clinical Analytics, Inc., New Haven, CT 06511.

78. Teorell, T., Arch. Intern. Pharmacodyn., 57:205 (1937), ref. 22, as cited in Riegelman et al., 1968a, op.cit.

79. Vesell, E.S., Relationship Between Drug Distribution and Therapeutic Effects in Man, Ann. Rev. Pharmacol., 14:249-270 (1974).

80. Wagner J.G., and Northam, J.I., Estimation of Volume of

Distribution and Half-Life of a Compound After Rapid Intravenous Injection, J. Pharm. Sci., 56:529-531 (1967).

81. Wagner, J.G. et al., Int. J. Clin. Pharmacol., 1:137 (1973), ref. 9, as cited in Yeh and Kwan, 1979, vide infra.

82. Wagner, J.G., "Biopharmaceutics and Relevant Pharmacokinetics.", Drug Intelligence Publications, Hamilton, IL, 1971.

83. Wagner, J.G., "Fundamental of Clinical Pharmacokinetics", Drug Intelligence Publications., Hamilton, IL, 1975/

84. Wagner, J.G., Linear Pharmacokinetic Equations Allowing Direct Calculation of Many Needed Pharmacokinetic Parameters from the Coefficients and Exponenets of Polyexponential Equations Which have been Fitted to the Data., J. Pharmacokinet. Biopharm., 4:443-467 (1976).

85. Welling, P.G., and Craig, W.A., Pharmacokinetics of Intravenous Erythromycin., J. Pharm. Sci., 67:1057-1059 (1978).

86. Wijnand, H.P., and Timmer, C.J., Curve-Fitting and Modeling in Pharmacokinetics: All Programs Are Equal, But Some Programs Are More Equal Than Others., J. Pharmacokinet. Biopharm., 7:681-684 (1979).

87. Wong, D., Fitting Concentration-Time Data to Biexponential Equations., J. Pharmacokinet. Biopharm., 7:97-100 (1979).

88. Wood, F.S., "Nonlinear Least Quares Curve-Fitting Program, 360D 13.6.007 IBM/360, 1971, ref. 13, as cited in Pedersen, 1977, op.cit.

89. Yamaoka, A., Application of Akaike's Information Criterion (AIC) in the Evaluation of Linear Pharmacokinetic Equations., J. Pharm. Sci., 6:165-175 (1978).

90. Yeh, K.C., and Kwan, K.C., Kinetic Parameter Estimation by

Numerical Algoriths and Multiple Linear Regression: Application to Pharmacokinetics., J. Pharm. Sci., 68: 1120-1124 (1979).

APPENDIX A

For the two-compartment open model which is the focus of this paper and was shown in figure 2, the appropriate differential equations (adapted from refs.35 and 10) are:

$$V_1 * dC_1/dt = -V_1 * k12 * (C_1 - C_2) - V_2 * kel * C_1 \qquad (\#A1)$$

$$V_2 * dC_2/dt = V_1 * k12 * (C_1 - C_2) \qquad (\#A2)$$

where V_1 = volume of compartment #1 (central compartment)

V_2 = volume of compartment #2 (peripheral compartment)

C_1 = concentration of the drug in V_1

C_2 = concentration of the drug in V_2

k12 = first order rate constant representing the fraction of the drug being instantaneously transferred from compartment #1 to #2

kel = first order rate constant representing the fraction of the drug being instantaneously eliminated from the system via compartment #1

and Vd_{ss} = the steady state volume of distribution = $V_1 + V_2$

Integration of the equations yields:

$$C_1 = A*e^{-\alpha*t} + B*e^{-\beta*t} \qquad (\#A3)$$

and

$$C_2 = A'*e^{-\alpha*t} + B'*e^{-\beta*t} \qquad (\#A4)$$

where Beta < Alpha, by convention.

Of interest to us is equation #A3 because its left-hand side, C_1, is an easily measured quantity, namely serum drug level. Unfortunately, equation #A4 is of much less value. C_2, a tissue drug level, is not so simply obtained.

To solve for each of the constants in terms of the others we obtain the well known relations:

$$A = \frac{(k12 + kel - \beta) * (C_1)}{(\alpha - \beta)} \quad t=0 \qquad (\#A5)$$

$$B = \frac{(k12 + kel - \alpha) * (C_1)}{(\beta - \alpha)} \quad t=0 \qquad (\#A6)$$

where C_1 at time t=0 equals A + B.

Rearranging yields:

$$k12 = (A*\alpha + B*\beta)/(A + B) \qquad (\#A7)$$

$$kel = (\alpha*\beta)(A + B)/(A*\beta + B*\alpha) \qquad (\#A8)$$

$$V_1 = D/(A+B) \qquad (\#A9)$$

$$V_2 = V_1 * (k12 * kel)/(\alpha*\beta) \qquad (\#A10)$$

$$= V_1 * (k12/k21)$$

Thus Vd(ss), the volume of distribution of the entire system at steady state, is:

$$Vd(ss) = V_1 + V_2 = V_1*(1 + k12/k21) \qquad (\#A11)$$

And,

$$\alpha = (b + sqr(b^2 - 4*c))/2 \qquad (\#A12)$$

$$\beta = (b - sqr(b^2 - 4*c))/2 \qquad (\#A13)$$

$$b = k12 + k21 + kel \qquad (\#A14)$$

$$c = k21 * kel \qquad (\#A15)$$

where sqr is the square root function.

Using these formulae, when A, Alpha, B and Beta are obtained (by whatever means), k12 is found with equation #A7, kel with equation #A8, and k21 is derived as follows:

Since,

$$C_1 = \frac{D}{V_1} * \frac{(k21-\alpha)}{(\beta-\alpha)} * e^{-\alpha*t} + \frac{D}{V_1} * \frac{(k21-\beta)}{(\alpha-\beta)} * e^{-\beta*t} \qquad (\#A16)$$

after a sufficiently long period of time has elapsed, the first term has a negligible contribution and a straight line through the graph of log C_1 vs t can be drawn. This results in a plot whose y-intercept is:

$$B = D*(k21-\beta)/V_1*(\alpha-\beta) \qquad (\#A17)$$

Similarly, the y-intercept of the other component of the

biexponential is:

$$A = D*(k21-\alpha)/V_1*(\beta-\alpha) \qquad (\#A18)$$

From equation #A9 $D/V_1 = A+B$ and thus equation #18A reduces to:

$$A = (A+B)*(k21-\alpha)/(\beta-\alpha) \qquad (\#A19)$$

We can now solve this for k21:

$$k21 = -\alpha + A*(\beta-\alpha)/(A+B) \qquad (\#A20)$$

APPENDIX B

The method of least squares is designed to fit a function to a given data set so as to minimize the error, where error is measured by the sum of the (weighted or unweighted) squares of the residuals from the difference between the actual data point and a function-derived "calculated" point (at any given value of the independent variable).

Thus, from Draper and Smith (21, p.9-11), if we measure values for a variable Y, and propose that they are a function of the variable X, then $Y' = b_0 + b_1 * X$ where Y' is the

"predicted" (or "calculated") value of Y for a given X. The residual then for any given point Y_i is:

$$Y_i - Y_i' = Y_i - b_0 - b_1 * X_i \qquad (\#B1)$$

and the sum of the squared residuals SS is:

$$SS = \sum_{i=1}^{n} (Y_i - b_0 - b_1 * X_i)^2 \qquad (\#B2)$$

To minimize this function one need only take the first derivative(s) and set it (them) equal to zero, as below:

$$SS/\ b_0 = -2 \sum_{i=1}^{n} (Y_i - b_0 - b_1 * X_i) = 0 \qquad (\#B3)$$

and

$$SS/\ b_1 = -2 \sum_{i=1}^{n} (X_i) * (Y_i - b_0 - b_1 * X_i) = 0 \qquad (\#B4)$$

These expand respectively into the so-called "normal" equations of the system:

$$b_0 * n + b_1 * X_i = Y_i \qquad (\#B5)$$

$$b_0 * X_i + b_1 * (X_i^2) = X_i * Y_i \qquad (\#B6)$$

which can them be solved as two equations in two unknowns for

b_0 and b_1, in terms solely of X_i and Y_i.

This basic approach, i.e., a solution through the normal equations, will work for all linear systems and can be adapted iteratively for some non-linear systems as well. With the particular non-linear system we are considering, an attempt (not shown here) to derive normal equations arrives at this equality:

$$(1/B)*(\Sigma Y*e^{-\alpha*t} - A*\Sigma e^{-\alpha*t}* e^{-\alpha*t}) = e^{-\alpha*t}*e^{-\beta*t} \quad (\#B7)$$

The next step is to solve this equation for Beta. Unfortunately no explicit solution can be found thus this method will fail.

The most commonly utilized alternative is the Gauss-Newton method or a modified version thereof. A non-linear estimation using this approach is complex; it will be more easily understood if we first apply the method to a linear solution. (The following, known as Gauss elimination, has been adapted from Hornbeck (31)).

Consider these three equations in three unknowns (b_1, b_2 and b_3):

$$X_{11}*b_1 + X_{12}*b_2 + X_{13}*b_3 = Y_1 \quad (\#B8)$$

$$X_{21}*b_1 + X_{22}*b_2 + X_{23}*b_3 = Y_2 \quad (\#B9)$$

$$X_{31}*b_1 + X_{32}*b_2 + X_{33}*b_3 = Y_3 \quad (\#B10)$$

These can be represented in matrix form as:

$$\begin{bmatrix} X_{11} & X_{12} & X_{13} \\ X_{21} & X_{22} & X_{23} \\ X_{31} & X_{32} & X_{33} \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \\ b_3 \end{bmatrix} = \begin{bmatrix} Y_1 \\ Y_2 \\ Y_3 \end{bmatrix} \qquad (\#B11)$$

When the top row of the matrix is divided by X_{11} we get:

$$\begin{bmatrix} 1 & X_{12}' & X_{13}' \\ X_{21} & X_{22} & X_{23} \\ X_{31} & X_{32} & X_{33} \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \\ b_3 \end{bmatrix} = \begin{bmatrix} Y_1' \\ Y_2 \\ Y_3 \end{bmatrix} \qquad (\#B12)$$

where a prime (') is used to show that the original element has been changed arithmetically. The first row is now multiplied by X_{21} and subtracted from the second row yielding:

$$\begin{bmatrix} 1 & X_{12}' & X_{13}' \\ 0 & X_{22}' & X_{23}' \\ X_{31} & X_{32} & X_{32} \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \\ b_3 \end{bmatrix} = \begin{bmatrix} Y_1' \\ Y_2' \\ Y_3 \end{bmatrix} \qquad (\#B13)$$

Repeating this sequence with the first and third rows gives:

$$\begin{bmatrix} 1 & X_{12}' & X_{13}' \\ 0 & X_{22}' & X_{23}' \\ 0 & X_{32}' & X_{33}' \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \\ b_3 \end{bmatrix} = \begin{bmatrix} Y_1' \\ Y_2' \\ Y_3' \end{bmatrix} \qquad (\#B14)$$

If the second row is divided by X_{22}', multiplied by X_{32}' and subtracted from the third row, we obtain:

$$\begin{vmatrix} 1 & X_{12}' & X_{13}' \\ 0 & 1 & X_{23}' \\ 0 & 0 & X_{33}' \end{vmatrix} \begin{vmatrix} b_1 \\ b_2 \\ b_3 \end{vmatrix} = \begin{vmatrix} Y_1' \\ Y_2' \\ Y_3' \end{vmatrix} \qquad (\#B15)$$

Elements already changed from their original values, and thus marked "prime" have not been double- or triple-primed even if they have subsequently been changed in value again.

Finally the third row is divided by X_{33}' yielding:

$$\begin{vmatrix} 1 & X_{12}' & X_{13}' \\ 0 & 1 & X_{23}' \\ 0 & 0 & 1 \end{vmatrix} \begin{vmatrix} b_1 \\ b_2 \\ b_3 \end{vmatrix} = \begin{vmatrix} Y_1' \\ Y_2' \\ Y_3' \end{vmatrix} \qquad (\#B16)$$

The final row is now equivalent to $b_3 = Y_3'$ and thus b_3 is solved for. Substituting into the second row:

$$b_2 + X_{23}' * b_3 = Y_2' \qquad (\#B17)$$

will then give b_2; and b_1 is similarly obtained.
(Actually, the final substitutions can be compacted into further matrix manipulation, giving as a final result:

$$\begin{vmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{vmatrix} \begin{vmatrix} b_1 \\ b_2 \\ b_3 \end{vmatrix} = \begin{vmatrix} Y_1'' \\ Y_2'' \\ Y_3'' \end{vmatrix} \qquad (\#B18)$$

double primes indicate that the values of Y_1 through Y_3 have been changed from equation #B15. This method - Gauss-Jordan

elimination - is the same as Gauss elimination in principle but derives the identity matrix at the end, thus dispensing with the need for substitutions.)

The process outlined above can be represented as:

$$X * B = Y \qquad (\#B19)$$

where capital letters stand for matrices. Obtaining the final solution, or solving for the b's is then a matter of deriving:

$$B = (X)^{-1} * Y \qquad (\#B20)$$

Here, $(X)^{-1}$ is the inverse matrix of X such that $X * (X)^{-1}$ yields the identity matrix.

If the system to be solved is (adapted from 4):

$$Y_i = b_1 * X_{1i} + b_2 * X_{2i} * b_3 * X_{3i} \qquad (\#B21)$$

then to minimize the sum of the squared residuals (SS) where

$$SS = \sum_{i=1}^{n} (Y_i - Y')^2 \qquad (\#B22)$$

we obtain the normal equations represented by:

$$\begin{bmatrix} X_{1i}^2 & X_{1i}*X_{2i} & X_{1i}*X_{3i} \\ X_{1i}*X_{2i} & X_{2i}^2 & X_{2i}*X_{3i} \\ X_{1i}*X_{3i} & X_{2i}*X_{3i} & X_{3i}^2 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \\ b_3 \end{bmatrix} = \begin{bmatrix} X_{1i}*Y_i \\ X_{2i}*Y_i \\ X_{3i}*Y_i \end{bmatrix} \quad (\#B23)$$

or:

$$(X' * X) * B = X' * Y \quad (\#B24)$$

where X' = the transverse matrix of X. Here:

$$X = \begin{bmatrix} X_{1i} & X_{2i} & X_{3i} \end{bmatrix} \quad (\#B25)$$

$$X' = \begin{bmatrix} X_{1i} \\ X_{2i} \\ X_{3i} \end{bmatrix} \quad (\#B26)$$

$$B = \begin{bmatrix} b_1 \\ b_2 \\ b_3 \end{bmatrix} \quad (\#B27)$$

and $$Y = [Y_i] \quad (\#B28)$$

So that $$B = (X' * X)^{-1} * (X' * Y) \quad (\#B29)$$

In the linear system specified by equation #B21 the normal equations are also linear, and thus equation #B29 is singlularly soluble. However, as Muir (44) has stated, "[T]he normal equations describing the least-squares fit to a nonlinear system are also nonlinear in the parameters and there exists no direct

solution for the minimum (and unique) sums of squares. Thus iterative techniques are invariably employed in any attempt to solve them."

The Gauss-Newton (or Taylor series) method "linearizes" the function via a first-order Taylor series approximation using initial parameter estimates, and then iteratively corrects these estimates. Linear normal equations can then be derived.

For example, with equation #4 in the text rewritten as:

$$Cp_i = Ae^{-\alpha * t_i} + Be^{-\beta * t_i} \qquad (\#B30)$$

to indicate that it is in fact a summation of observations, a first order Taylor series expansion is:

$$Cp_i = A_1 * e^{-\alpha_1 * t_i} + B_1 * e^{-\beta_1 * t_i} + \qquad (\#B31)$$

$$(A' - A_1)*(\partial Cp_i/\partial A_1) + (\alpha' - \alpha_1)*(\partial Cp_i/\partial \alpha_1) +$$

$$(B' - B_1)*(\partial Cp_i/\partial B_1) + (\beta' - \beta_1)*(\partial Cp_i/\partial \beta_1)$$

A prime denotes a "theoretical" true regression-obtained value for a variable; a numerical subscript, an approximation (i.e., A_1 is thus the first approximation of A').

Since the first calculated value is

$$C'p_i = A_1 * e^{-\alpha_1 * t_i} + B_1 * e^{-\beta_1 * t_i} \qquad (\#B32)$$

then:

$$Cp_i - C'p_i = (A' - A_1)*(\partial Cp_i/\partial A_1) + (\alpha' - \alpha_1)*(\partial Cp_i/\partial \alpha_1) + (B' - B_1)*(\partial Cp_i/\partial B_1) + (\beta' - \beta_1)*(\partial Cp_i/\partial \beta_1) \quad (\#B33)$$

This is of the form

$$Y_i = b_1*X_{1i} + b_2*X_{2i} + b_3*X_{3i} + b4*X4i \quad (\#B34)$$

and can be solved as outlined in equations #B22 - #B29.

For a variable V, the first iteration solves for $(V' - V_1)$. V_1 being the first approximation, is known, and thus $V_2 = (V' - V_1) + V_1$ is used as the second approximation, and the iteration is repeated. "Since the first-order Taylor series approximation of the fuction in the region of the initial estimates is not exact" (4), $V_2 = (V' - V_1) + V_1$ will <u>not</u> actually equal a true V' but will be an improved estimate.

This procedure is repeated until successive iterations satisfy the convergence testing factor.

Actually, the non-linear regression program used here, COMPT solves the model via equation #A16 using #A11, #A14 and #A15 for substitutions and thus obtains its results in terms of k12, k21, kel and Vp. A, <u>Alpha</u>, B and <u>Beta</u> are then derived from

equations #A12, #A13, #A17 and #A18. Wong et.al. (87) has demonstrated that this method yields equivalent results to solving directly for A, Alpha, B and Beta and then calculating the other set via methods discussed above.

Readers who desire more detailed information concering COMPT, including a flow-chart, program lising and description of how the partial derivatives are calculated are urged to read the original reference. However, an abbreviated explanation of the program can be found in Appendix D.

APPENDIX C

As an example of how similar drug levels and SS's can be obtained with one weighting system by quite different parameters, the EQUATION mode of the program was executed using data from the literature (18) for penicillin G with $1/(C' + C)2$ as a weighting factor, and a maximum deviation of 10%. With this input a trial run generated data whereby a "beta" phase of eight (of ten) points was chosen by the program as the best model with an SS = 0.004038. The next two closest models made use of "beta" phases of seven and four points with SS's of 0.004839 and

0.004234 respectively. Table C1 compares the two best models.

It is easily appreciated that the major difference in the parameter sets is between A's and Alpha's, since the calculated drug levels vary from each other most in the "alpha" phase and early "beta" phase (when these two coefficients exert their greatest numerical influence on the calculated levels).

Note, though, that while three of the four parameters in the two models vary by greater than 5%, none of the calculated drug levels do.

TABLE CI

	"Beta" Phase = 8	7	% difference	Input Values
SS	0.004038	0.004234	4.6	
A	0.1325	0.2071	36.0	0.146
Alpha	24.84	60.57	60.0	5.80
B	0.9018	0.8960	6.4	0.853
Beta	1.010	1.007	0.3	0.996

Time (hrs)	Calculated Level "Beta" Phase = 8	7	% difference	"Given" Level
0.008	1.004	1.008	1.4	1.004
0.016	0.9776	0.9623	1.6	0.9776
0.031	0.9348	0.8994	3.8	0.8976
0.063	0.8748	0.8460	3.3	0.9178
0.125	0.8008	0.7900	1.3	0.7415
0.250	0.7009	0.6965	0.6	0.6787
0.500	0.5444	0.5415	0.5	0.5664
1.000	0.3286	0.3272	0.4	0.3198
2.000	0.1197	0.1195	0.2	0.1143
4.000	0.0159	0.0160	0.3	0.0164

Note that in the "Given" levels the drug concentration is greater at 0.063 hrs than at 0.031. This is an artifact produced by the random error generator. Such error is found in real data too (see ref. #73, Table I, subject B.G.). It is taken into account in parameter calculation, but of course not reproduced in levels generated by those parameters.

APPENDIX D

While a full explanation of COMPT is beyond the scope of this paper, a few salient points are in order. First, as was mentioned above, the program has been modified for use with NOVICE. The changes introduced included the addition of new subroutines, (necessitated by the use of a slightly different programing language), changes in data input and output, and the provision for variation in the weighting scheme.

Second, the program's actual algorithm uses a modification of the Gauss-Newton method, proposed by Hartley (30). Briefly,

Hartley's procedures is used to correct the estimations when they are in the neighborhood of an SS minimum by approximating the function as a quadratic and then interpolating the quadratic function to locate the minimum. A detailed description can be found in the original paper (30).

Third, once a solution has been interatively converged upon so that the CTF is satisfied, a direct search of the parameter space (defined by the space limit vector - see below) is performed to insure that the solution was a true (i.e., global) minimum and not a local one. This direct search, a relatively time-consuming process, divides the parameter space into 100 equal intervals and varies each parameter individually thoughout while testing whether any of these new parameter combinations results in a reduction in the SS. If not, the solution previously converged upon is considered a global minimum and the search is terminated. If however a new combination with a reduced SS is found then it becomes the set of so-called "initial" estimates for a new set of iterations, (and the interation counter is reset at zero).

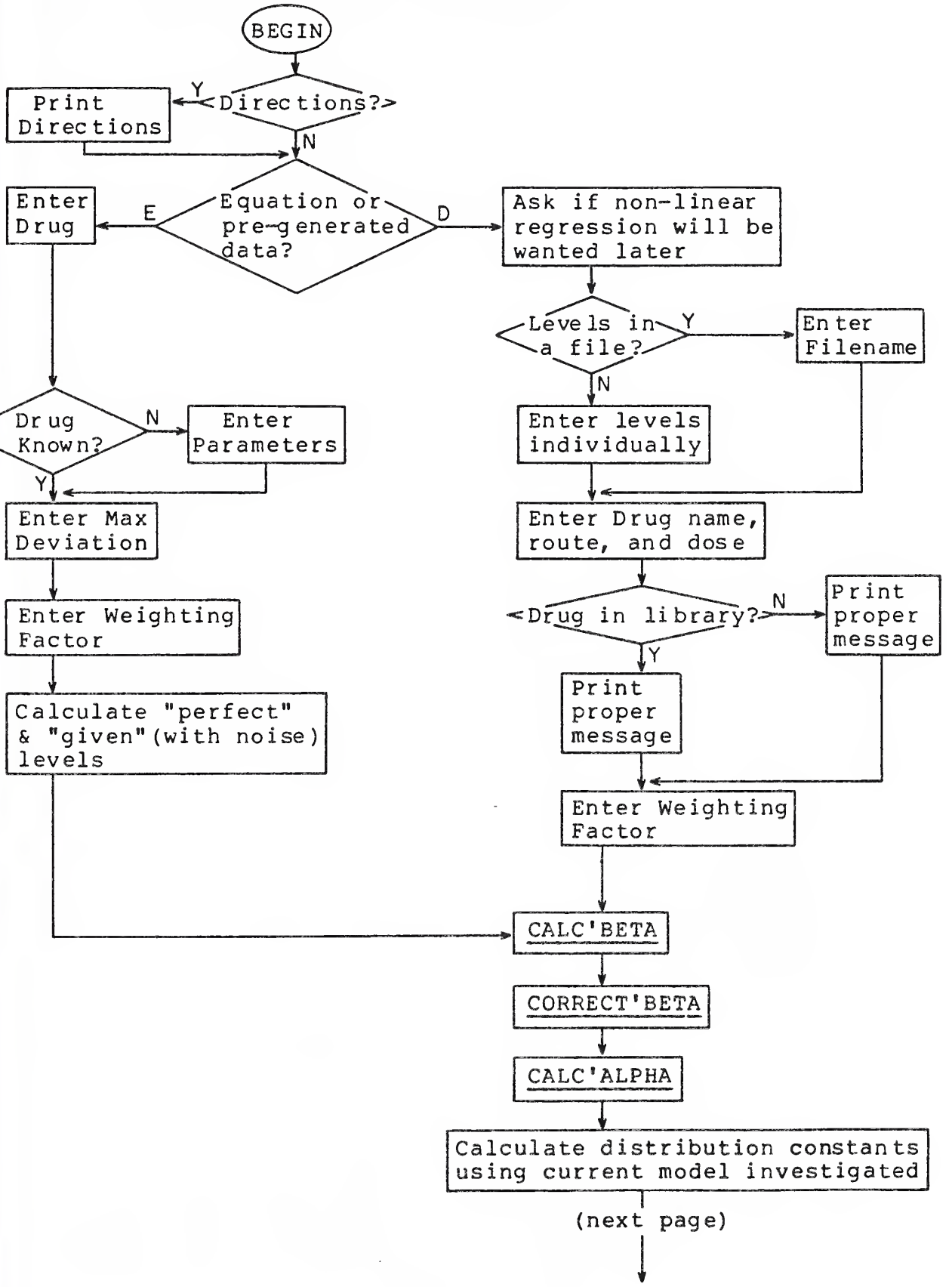
(The parameter space limit vector is a constraint imposed on the parameter values (i.e., k12, k21, ke, and Vp) to facilitate speedy solution of the model and help prevent divergence of solutions. In the case of COMPT any new estimates of the parameters must be within a factor of ten of the initital estimates. This factor was a somewhat arbitraty choice in the

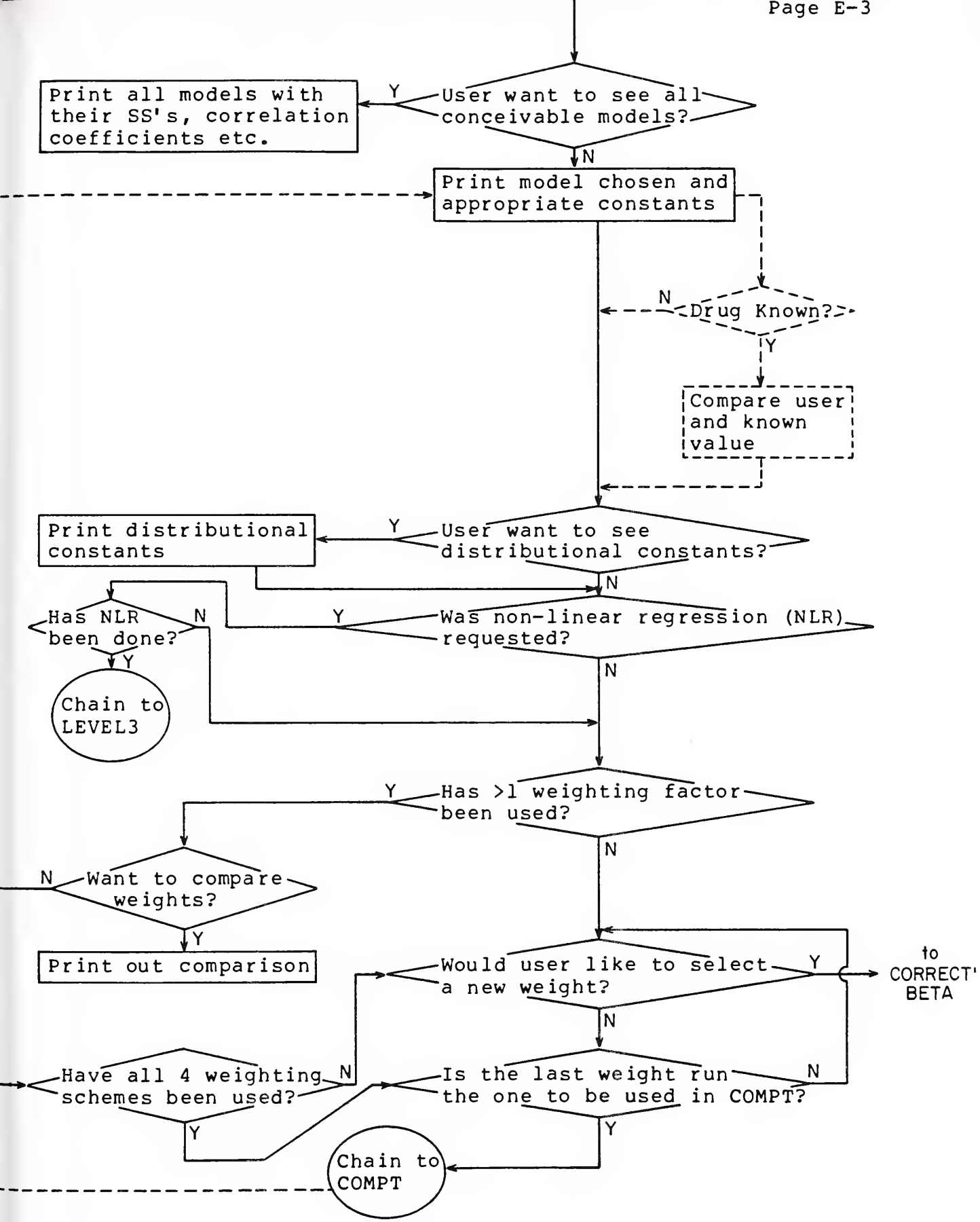
original program but testing showed that expanding the limit resulted in no reduction of SS, and required more computer time (via more interations) for convergence.)

APPENDIX E

Figure #4

NOVICE Flow Chart




User want to see all conceivable models?
Y
Print all models with their SS's, correlation coefficients etc.
N
Print model chosen and appropriate constants
N
Drug Known?
Y
Compare user and known value
User want to see distributional constants?
Y
Print distributional constants
N
Was non-linear regression (NLR) requested?
Y
Has NLR been done?
N
Y
Chain to LEVEL3
N
Has >1 weighting factor been used?
Y
N
Want to compare weights?
N
Y
Print out comparison
Would user like to select a new weight?
Y
to CORRECT' BETA
N
Is the last weight run the one to be used in COMPT?
N
Y
Have all 4 weighting schemes been used?
N
Y
Chain to COMPT

Here, double lines have been used to indicate the somewhat altered flow chart of NOVICE when it has been re-entered via COMPT as opposed to being run de-novo.

Note that a few branch points having to do with error trapping during data input have been omitted from this flow sheet, and can be found in figure #5. Flowsheets for individual subroutines (here capitalized and underlined) can be found in figures #6-8).

In addition, the user may reset the weighting factor even if non-linear regression was not requested. This is not shown in the flow chart because of space limitations.

Figure #5

Data Entry Flow Chart

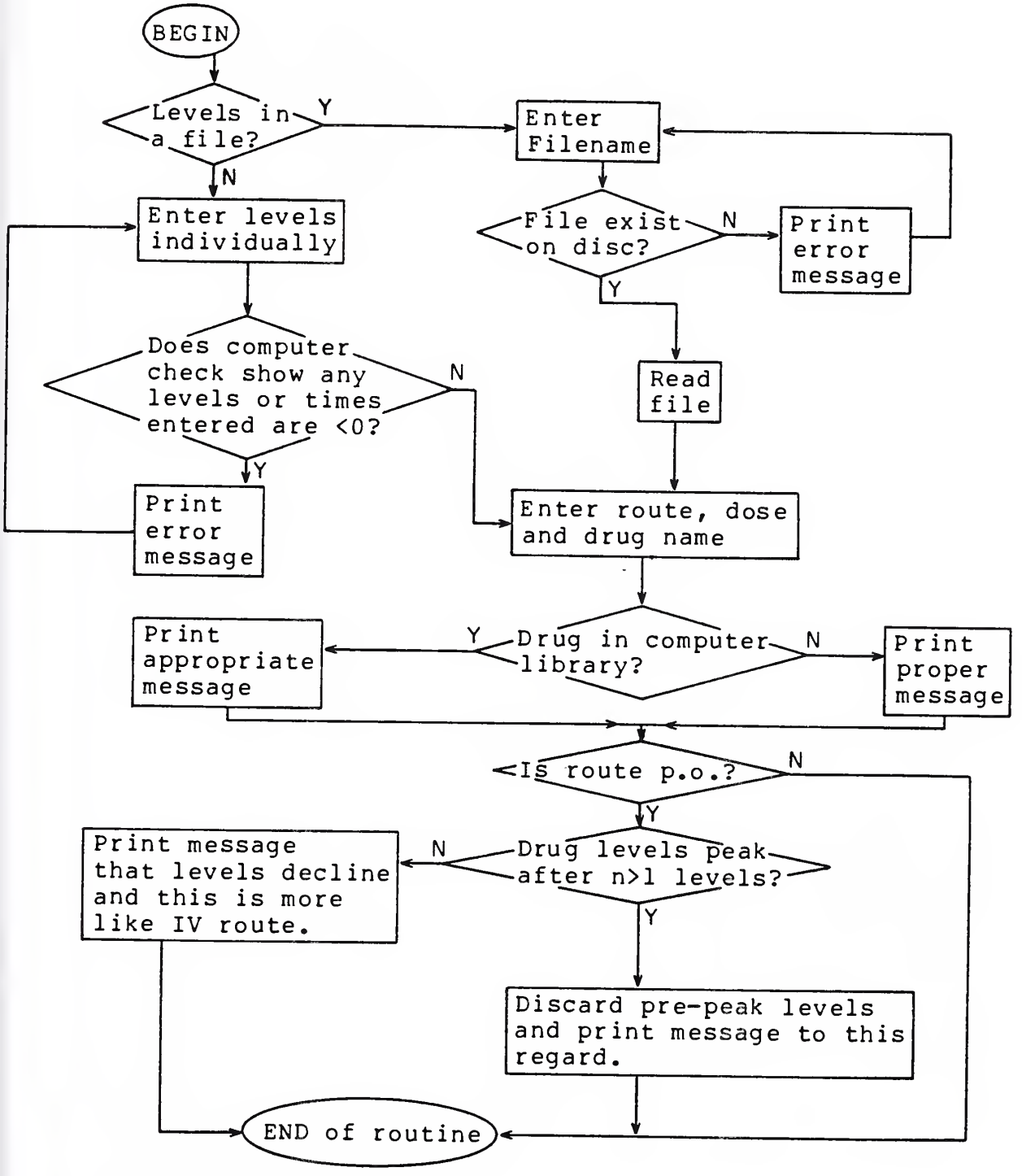


Figure #6

Flow Chart for CALC'BETA

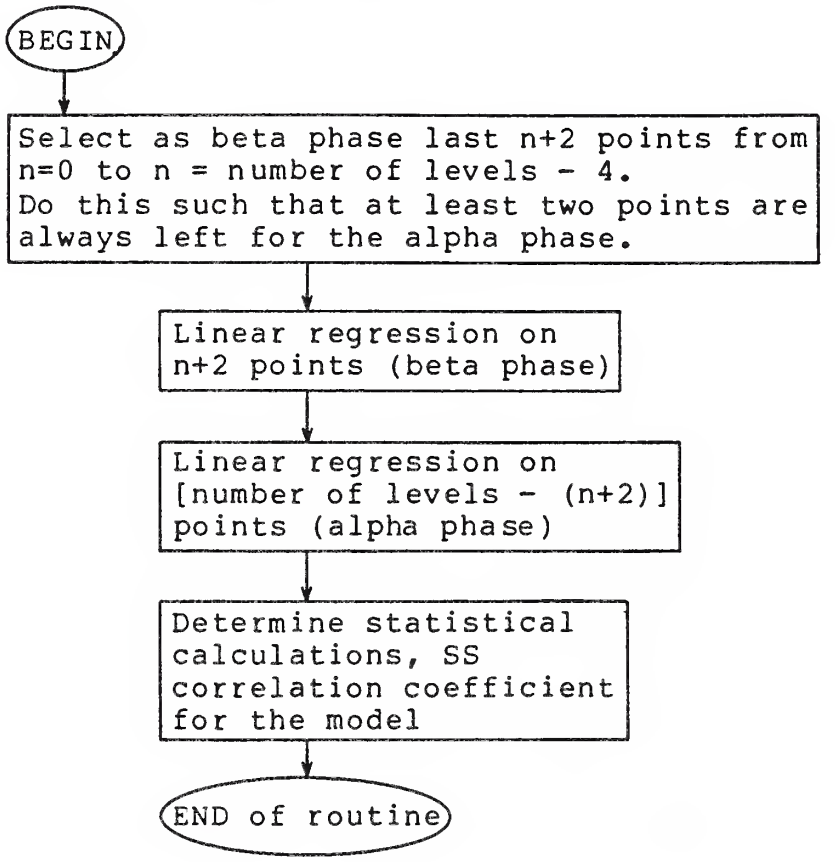


Figure #7

Flow Chart for CORRECT'BETA

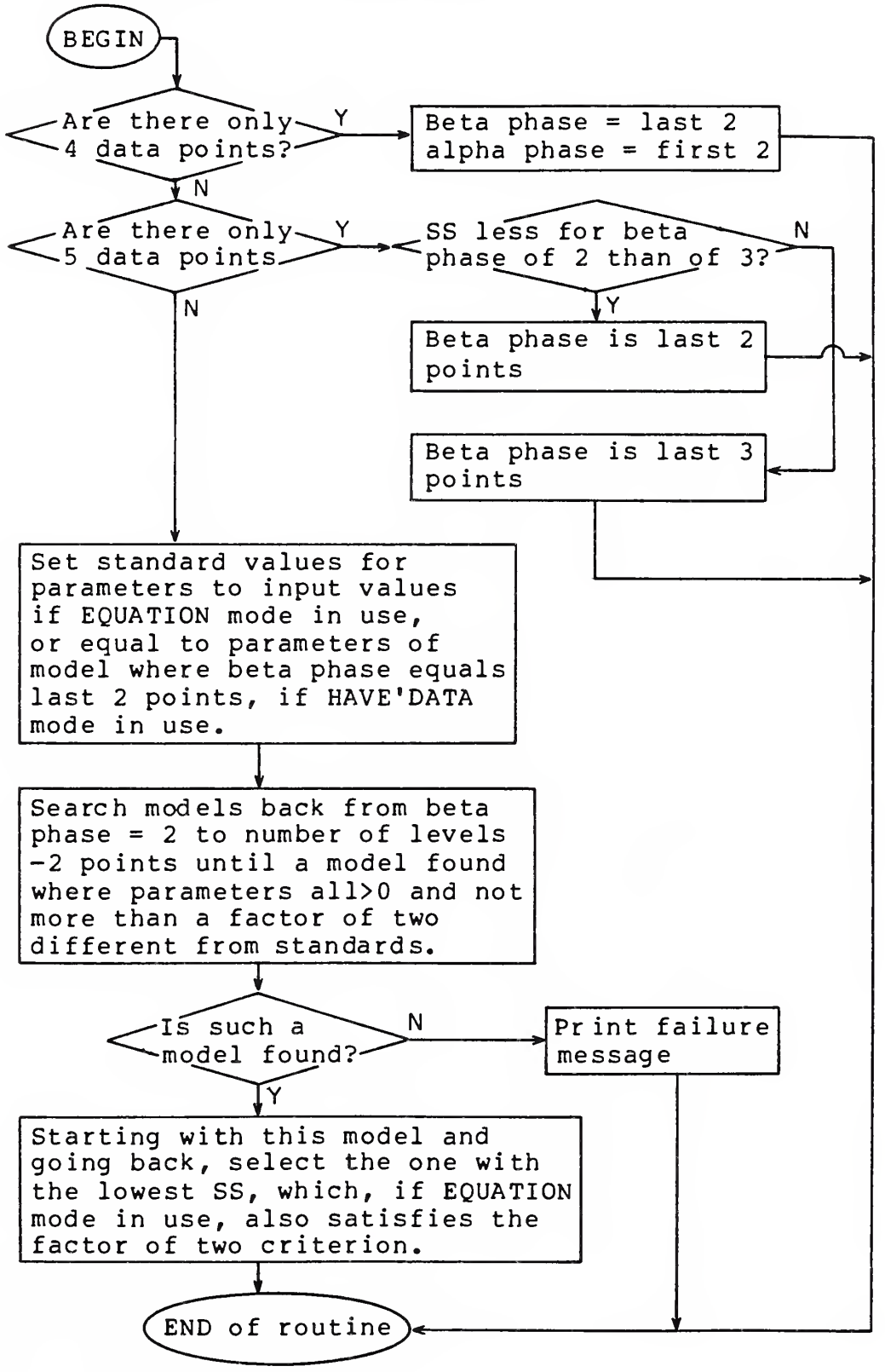
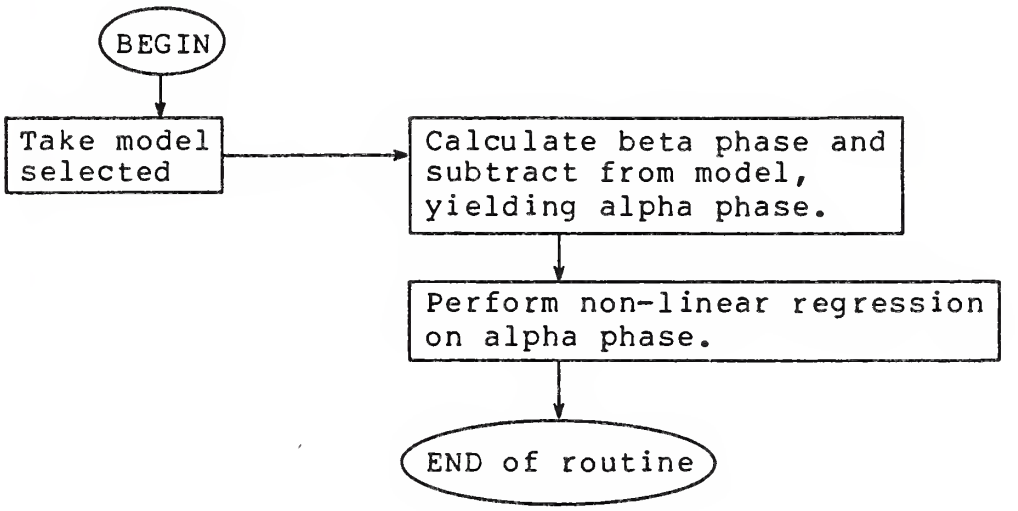


Figure #8

Flow Chart for CALC'ALPHA



Note, the final linear regression on the "alpha phase" should yield identical results as that performed in CALC'BETA when the model was being considered in that routine. It is recalculated now as previous values were not saved in order to conserve storage space in memory.

APPENDIX F

****
2 COMPARTMENT PARAMETER TESTING
****

you need directions? Y

ese are the detailed directions for this two compartment
armacokinetic parameter program. As you are probably aware,
st recommended drug dosages are calculated from pharmaco-
netic constants derived from pooled population data. While
most clinical situations this is probably adequate to enable
e clinician to feel assured that his/her patient is indeed
ceiving a reasonably correct dosage of a given drug to main-
in intended blood levels, there will be occasional exceptions
this. Most commonly these will occur in the patient with
tered renal function, although congestive heart failure and
patic insufficiency are other frequent causes. Additionally,
en administration of a drug with a very low toxic/therapeutic
tio is being considered, a need for this program may arise.

states of decreased renal function the clinician can use
rmulas that calculate altered dosage and dose intervals
compensate, however these formulas universally assume a one
mpartment pharmacokinetic model, a state which in reality
rely exists. For some drugs this will only result in minor
rors for dosing, however for other drugs errors in the range
50% will occur.

ess <RETURN> to continue:

particular if the drug is one that will be administered
ronically and which has a low therapeutic index -- e.g.
goxin or theophylline -- then it may well be worthwhile
derive an individual patient's own two compartment kinetic
rameters to enable you to individualize your dosing scheme
r that particular patient. This program is designed to
do that for intravenous medication. It can also be of
nsiderable use if the drug is to be given per-orally,
wever, it will not produce as accurate results. This
ll be explained below.

he program may be run in one of two modes, 'EQUATION' or 'DATA'. he latter is the mode of deriving the kinetic constants for a rug and is the one of interest for most clinicians. To use the rogram in this fashion you need to administer orally or by IV bolus he drug of interest, and subsequently draw a minimum of four, but referably more, serum blood levels. At least two of these blood evels should be drawn within the first 15 minutes after the drug as been given. The remainder can be after any reasonably length f time has past, (e.g. - 3 and 6 hours) and can be done at your onvenience. When you run the program you will be asked what drug ou are going to be using, and if this drug is known to the rogram library, it will make suggestions as to appropriate times. ress <RETURN> to continue:

hichever drug route you choose should be the one by which ou plan to administer the drug chronically, and the one for hich you are using this program in the first place.

However, please be aware, that calculations for IV administation ill be more accurate than those for PO, as in the latter case a population derived averaged constant of absorbtion will be used to calculate the rate by which the drug is absorbed from the GI tract. In the case of the IV route, this inaccuracy will not need to be introduced.

This program will first give initial data results using a 'curve-peeling' technique, and then use these initial estimates for the more sophisticated non-linear regression estimation. During the the latter there will be a series of revised estimates for the parameters printed on the screen as the program cycles through each iteration. The full process can take quite some time, so please be patient.

Press <RETURN> to continue:

The 'EQUATION' mode will be of interest to pharmacokineticists In this option mode you will be asked to specify the four parameters (A, Alpha, B and Beta) of 2 compartment pharmacokinetics. You will then be able to simulate the IV administration of a drug, during which 10 blood levels will be drawn for you at appropriate intervals. These so-called 'perfect' levels will correspond exactly to the parameters you've entered. You will be asked to specify a percentage of random error to be introduced, and the computer will do so and then use its curve peeling routines to try and rederive the initially entered parameters. From these it will arrive at 'calculated' levels for the drug at the various sampling times. Its accuracy in reproducing the parameters is a function of their relationship to each other, the amount of random error chosen, and of chance in that a given amount of error has different effects at various sampling times depending on the interval between them. Note that non-linear regression is not run in the 'EQUATION' mode as the initial estimates of curve peeling are sufficient for the purposes of the simulation.

Press <RETURN> to continue:

Most of the program is self explanatory, only a few points need be raised. Many questions asked will be followed by anticipated answers. If they are correct just hit a carriage return, if not, just type in the correct one which will erase the first one. With regard to data entry you may either type the data in line by line at the time you are running the program, or preferably -- for you -- you may create a file of any name, with the extension LST and enter the data into the file.

To do the latter type VUE DATA#.LST substituting a one or two digit number of your choice for the # sign. If you are totally unfamiliar with computers or data files let me suggest that discretion being the better part of valor you will find it much easier to just enter the data as the program runs.

Now a word concerning data weighting. In order to fit the best possible curve to the data the program must decide what numerical weight to assign to each data point.

Press <RETURN> to continue:

There are several options which it will present to you, one of equal weights (1), one of weighting by the inverse of the actual data point (1/C), one weighted by the inverse squared (1/C**2), and one of the inverse of the square of the sum of the actual point and the calculated point (1/(C + C')**2). Recommended choices are equal weights if the drug levels vary by an order of magnitude or less, and (1/(C + C')**2) if they vary by more than an order of magnitude. We STRONGLY urge that if you are unfamiliar with data weighting you use one of the latter two methods.

Press <RETURN> to continue:

Finally, a little explanatory note on non-linear regression. The last information you will be asked for is the convergence and number of iterations you desire. If you are unfamiliar with these terms they may be thought of as the criterion which you use to decide if a good model has been chosen, and the number of times that you want to cycle through the program, making adjustments in your model, until the criterion is filled. Default answers have been provided and I suggest you use them unless you know what you are doing. In any case the amount of time this phase of the program takes to run is inversely proportional to the size of the convergence, as is the accuracy of the model. The number of iterations has much less effect on the run-time and is best left alone.

```
Enter 1 for simulation, 2 for your own data: 2



Will you be wanting non-linear regression analysis? Y


Data is available for simulation and/or comparison
for the following drugs.
ANTIBIOTICS     CARDIO-ACTIVE  ANTI-EPILEPTIC        OTHER

Pen G           Digoxin        Pentobarbital         Theophylline
Ampicillin      Quinidine      Dilantin
Oxacillin       Procainamide
Dicloxacillin   Bretylium
Methicillin     Lidocaine
Erythromycin    Propranolol
Cephalexin
Cephradine
Cefazolin
Cephalothin
Cefoxitin
Cefotaxime
Gentamicin
Tobramycin

If you are using data of one of these drugs, I will compare your
parameters values with those stored in my memory once calculations
are complete.
Press <RETURN> to continue:


Is the data already in a file? Y


Filename?   YEHX8.LST

Was the drug given P.O. or I.V.?      IV

What is the name of the drug?    SPECTINOMYCIN



Is the data already in a file? Y


Filename?   YEHX8.LST

Was the drug given P.O. or I.V.?      IV

What is the name of the drug?    SPECTINOMYCIN




The drug you are studying is not in my library and therefore
I will be unable to help you with comparisons of the results
of your data and population studies.  As this comparison serves
as an important part of error detection, BEWARE.
```

The choice of units for dosage in grams (1), milligrams (2), micrograms (3), and 'units' (4).

What units would you like to use (code number 1 to 4) 2

Using the units that you have just selected,
what was the dose of the drug? 500

1 = mg/dl
2 = mcg/dl
3 = ng/dl
4 = mg/ml
5 = mcg/ml
6 = ng/ml
7 = units/ml (e.g. - for Penicillin)

What units do you use for serum levels
If you are using your own data, these units
MUST be the same as the input levels. 5

The units that you have chosen to use for both dose and serum levels will be retained throughout the entire program, but used only in dosage scheduling. In the other tables that appear, drug levels are in mcg/ml. Should you be using a dose in 'units', then in tables, levels are in units/ml.

Rate constants are in units of 1/hr for alpha, beta, k12, k21, and kel. They are in mg/hr for A and B Volumes are in units of liters.

Weighting factors are set by the user. For equal weights type 1, for 1/C type 2, for 1/C**2 type 3 for (1/(C + C')**2) type 4.

Here, C = the actual experimental or random generated drug level and C' is the calculated level.

Again, if you are not familiar with data weighting we URGE you to pick option 3 or 4 (the latter is especially recommended).

The desired weighting factor for error minimization is: 3

I now require additional information for the non-linear regression portion of the program, as was explained in the directions.

What is the convergence testing factor? .000001

What is the maximum number of iterations to be used? 1

My analysis yields 2 points as the correct number for the 'beta' phase. Using this:

Beta= 0.22366 and B = 11.67066

The standard error of Y= 0.00000

Using the remaining 6 values to calculate ALPHA and A, I obtain:

Alpha= .804431 and A = 52.6153

The standard error of Y = 610.981

Would you like to see the model chosen? Y

Calculated A = 52.615301
Calculated Alpha = 0.804431 Num'levels = 2
Calculated B = 11.670663 Weight = (1/C)**2
Calculated Beta = 0.223656

TIME(HRS)	GIVEN LEVEL	CALCULATED LEVEL
0.167	63.3000000	57.2558304
0.333	50.6000000	51.0734615
0.500	43.3000000	45.6269186
1.000	31.0000000	32.8687958
2.000	18.3000000	17.9906894
4.000	6.9000000	6.8775487
6.000	3.0500000	3.4716498
8.000	1.9500000	2.0343785

The weighted sum of the squared residuals = 0.037007
The correlation coefficient = 0.988024
Press <RETURN> to continue:

ould you like to see the preliminary distribution constants? Y

K12 = 0.152291
K21 = 0.329092
Kel = 0.546705
Vp = 7.778

ress <RETURN> to continue:

ould you like to reset the weight? N

he program will now shift into its non-linear regression phase.
ou are allowed only one weight in the interests of time and to
et this weight it is necessary that you re-select it unless it
as your previous choice.

our previous weight choice was 3

his corresponds to a weight factor of (1/C)**2

s this the scheme you want to use? Y

STARTING ESTIMATES FOR THE ITERATIONS

ITERATION	K(12)	K(21)	K(10)	V(1)
1	.152291	.329092	.546705	7777.75
2	.160857	.347603	.577456	7777.75
3	.160697	.347256	.576881	7539.28
4	.160516	.346867	.576234	7546.77
5	.160318	.346439	.575523	7555.23
6	.160114	.345997	.574788	7564.53
7	.159938	.345617	.574158	7574.17
8	.15987	.34547	.573913	7582.47
9	.159866	.345462	.573899	7585.69
10	.159864	.345457	.573892	7585.87
11	.159863	.345454	.573888	7585.98
12	.159862	.345453	.573886	7586.03
13	.159862	.345453	.573884	7586.06

The non-linear regression phase of the program has been completed.
You will now have an opportunity to view the finalized data
and the many parameters automatically calculated for you.

fter 13 iterations, convergence = 0.0000002
inal SS = 0.02443

ara- eter	Estimated values	Standard Deviation	95% Confidence Limits (By group on top) (Independently below)
K12	0.1599	0.0340	0.0278 TO 0.2920(S)
			0.0180 TO 0.3017 (U)
K21	0.3455	0.1281	-0.1520 TO 0.8429(S)
			-0.1888 TO 0.8797 (U)
Kel	0.5739	0.0205	0.4943 TO 0.6535(S)
			0.4884 TO 0.6594 (U)

Press <RETURN> to continue:

Volumes of Distribution
V'TOTAL= 2.161 V'CENTRAL= 7586.070 V'PERIPHERAL= 3510.535
V(STEADY STATE) = 11096.6058

Half Times
T(12)= 4.3350 T(21)= 2.0061 T(el)= 1.2076

Plasma Clearance Rate = 4353.5234

Calculated A = 53.944762
Calculated Alpha = 0.844423
Calculated B = 11.965519 Weight = (1/C)**2
Calculated Beta = 0.234775

TIME(HRS)	GIVEN LEVEL	CALCULATED LEVEL
0.167	63.30000	64.98073
0.333	50.60000	53.17761
0.500	43.30000	48.28153
1.000	31.00000	36.47147
2.000	18.30000	17.44721
4.000	6.90000	6.51920
6.000	3.05000	3.26533
8.000	1.95000	1.89193

The weighted sum of the squared residuals = 0.024431

Would you like to see the final distribution constants? Y

K12 = 0.159862
K21 = 0.345452
Kel = 0.573884
Vp = 7.586

Press <RETURN> to continue:

Spectinomycin blood levels are usually measured in units of mcg/ml

-- are these the units you are accustomed to using (Y or N)? Y

Spectinomycin doses are usually measured in milligrams

-- are these units satisfactory (Y or N)? Y

Spectinomycin formulated for IV usually has
a bioavailability of 100 %

-- does the preparation of spectinomycin which you are using
have a similar bioavailability (Y or N)? Y

Would you like a dose schedule based on desired blood levels (Y or N)? Y

Enter desired maximum serum level of spectinomycin in mcg/ml: 64

Enter desired minimum serum level of spectinomycin in mcg/ml: 1

Assuming that the patient does not handle spectinomycin in an unusual fashion, the following doses and dose interval are recommended to achieve an equilibrium serum minimum of 1 mcg/ml and an equilibrium serum maximum of 64 mcg/ml:

Estimated optimum loading dose = 478 milligrams

Estimated optimum maintenance dose = 478 milligrams

Estimated optimum dose interval = 10 hours and 45 minutes

Though this is the only combination which will achieve the desired serum maximum and minimum level, different -- and perhaps more convenient -- combinations of dose and interval can be selected to give a similar area-under-the curve (AUC).

Would you like another dose-interval combination (Y or N)? Y

Enter desired interval between maintenance doses

Example:

6	[hours]
4:30	[hours:minutes]
24	[hours]

Desired interval [hr:min]? 12

```
nter smallest practical division in which your preparation of
            spectinomycin can be dispensed -- often corresponds to 1/2 tablet

            Example:
                              2.5           [milligrams]

mallest division (in milligrams)? 250

djusted maintenance dose = 500 milligrams

nter date for 1st dose (press <RETURN> for today): F

nter smallest practical division in which your preparation of
            spectinomycin can be dispensed -- often corresponds to 1/2 tablet

            Example:
                              2.5           [milligrams]

mallest division (in milligrams)? 250

djusted maintenance dose = 500 milligrams

nter date for 1st dose (press <RETURN> for today): FEB 28, 1982

nter time for 1st spectinomycin dose

nter time

        Example:

                  6 AM                NOON
                  4:03 PM             MIDNIGHT

ime? 8 AM
```

ESTIMATED BLOOD LEVELS FOR SPECTINOMYCIN

ATE	TIME	DOSE COUNT	DOSE MILLIGRAMS	BLOOD LEVEL (MCG/ML) BEFORE DOSE	AFTER DOSE
eb 28	8:00 AM	1st	500	0	65.9
eb 28	8:00 PM	2nd	500	.717	66.6
ar 1	8:00 AM	3rd	500	.76	66.7
ar 1	8:00 PM	4th	500	.763	66.7
ar 2	8:00 AM	5th	500	.763	66.7
ar 2	8:00 PM	6th	500	.763	66.7
ar 3	8:00 AM	7th	500	.763	66.7
ar 3	8:00 PM	8th	500	.763	66.7
ar 4	8:00 AM	9th	500	.763	66.7
ar 4	8:00 PM	10th	500	.763	66.7

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