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In many experimental situations involving compartmental models, a more realistic model is obtained if the rate constants are regarded as random variables. We consider the problem of estimating the means and variances of the rate constants in a stochastic compartmental model when

a) a time series of observations of an individual system cannot be made (because of destructive sampling), and

b) exact expressions for the moments of the stochastic model cannot be found (as is the case with most multicompartment models).

Several estimation procedures will be described and compared using simulated experimental data from three different two-compartment pharmacokinetic models. While all the procedures seem to give reasonable estimates of the means, good estimates of the variances are obtained only if the covariance structure of the stochastic model is taken into account. In addition, the results suggest that the difference between the mean of the stochastic model and the deterministic model evaluated at the means of the rate constants is not as important in the parameter estimation problem as has been suggested by previous authors.

Parameter Estimation in Stochastic Multicompartment Models with Destructive Sampling

by

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I. INTRODUCTION

Compartment models are widely used for modeling many types of physical and biological systems. In such models, the system under consideration is viewed as a collection of homogeneous compartments, and some material of interest is assumed to move between the compartments (as well as into and out of the system) according to specified rate laws. The usual assumption is that the kinetics are linear, i.e., the rate at which the material leaves a compartment is proportional to the amount of material present in that compartment. With this assumption, the model can be represented mathematically by a system of linear, first-order differential equations, the solution of which provides a description of the behavior of the system over time as a function of the rate constants and the initial state vector.

An important statistical problem which arises in connection with compartmental models is estimation of the system parameters (i.e., the rate constants and initial state vector) from observations of the system. Because the equations describing the system are usually complicated functions of the parameters, the inverse problem generally involves some form of nonlinear parameter estimation. For the types of equations arising in compartmental analysis, graphical and numerical estimation methods are readily available (see, for example, Jacquez (1972) or Gibaldi and Perrier (1975)).

In many modeling problems, however, the deterministic formulation of the compartmental model is not adequate. Factors such as environmental effects, interindividual variation, and cyclic fluctuations of the rate coefficients may make it unrealistic to consider the rate constants and/or initial state vector as fixed parameters. Incorporation of uncertainties regarding the parameters into the model leads to a stochastic compartmental model, in which the parameters of the classical deterministic system are treated as random variables. The inverse problem in this model is the estimation of the means and variances (and possibly other distributional parameters) of the random variables.

Mention should perhaps be made of another type of stochastic compartment model, in which movement between the compartments occurs in discrete units according to a probabilistic transfer mechanism. Models of this sort, in which the lifetime of a unit in any compartment is a random variable, have been reviewed by Purdue (1979). Matis and Tolley (1979) and Matis and Wehrly (1979a, 1979b) have developed a unified model incorporating both types of stochasticity. We will not consider such models here, but will instead limit ourselves to the case in which the transfer mechanism is deterministic but the rate constants are time-invariant random variables.

Under this model, a system is deterministic for any particular realization of the rate constants. One possible approach to the estimation problem, therefore, is to estimate the constants for each system in a sample, and then use these individual estimates to estimate the population parameters. Often, however, situations arise in which estimation of the parameters of each individual in a sample is impossible. Estimation in a deterministic system usually requires a time series of observations, and such a time series may not be available. Many pharmacokinetics studies, for example, involve destructive sampling, since the small laboratory animals used must be sacrificed to obtain a tissue sample large enough for chemical analysis.

The usual approach to such a situation, where each animal (system) can be observed only once, is to observe several animals at each of several time points and fit the deterministic model to the sample means. In general, however, the behavior of the population mean over time is not the same as the behavior of the deterministic model evaluated at the means of the rate constants, so the wrong model is being used. Furthermore, even when the difference between the true model and the deterministic model is small, the deterministic model provides no estimate of population variances. Thus, the need for a more sophisticated method of parameter estimation for the stochastic model is evident.

This thesis will compare several methods of estimating the population means and variances of the rate constants in a stochastic multicomparment model when only one observation can be made on any one realization of the model. The next chapter reviews the results of the deterministic compartmental model and states the assumptions to be used in the following chapters. Chapter III discusses the difference between the deterministic model evaluated at the means of the rate constants and the stochastic model. In Chapers IV and V, the estimation methods are developed and their performances compared using simulated experimental data from three different two-compartment models. Finally, in Chapter VI, some concluding remarks are made and areas for further research are suggested.

II. PRELIMINARIES

2.1 The Deterministic Model

We briefly summarize results for the general p-compartment model. For a more detailed development, see Jacquez (1972).

Let $X_i(t)$ denote the amount of material in the i-th compartment at time t and $\theta_{ij} \geq 0$ the proportionality constant for flow from compartment i to compartment j $(i,j=1,2,\ldots,p,\ i \neq j)$. Define the state vector $\underset{\sim}{X}(t) = (X_1(t),\ldots,X_p(t))'$. Then the linear compartmental model can be written as

$$\frac{d\underset{\sim}{X}(t)}{dt} = \underset{\sim}{A}\underset{\sim}{X}(t) + \underset{\sim}{B}(t) \tag{1}$$

where $\underset{\sim}{A}$ is a pxp matrix and $\underset{\sim}{B}(t)$ is a p-vector of inputs. We will assume that there is no input to the system after time t=0, i.e., $\underset{\sim}{B}(t)=0$. The entries of the matrix $\underset{\sim}{A}$ are

$$a_{ij} = \theta_{ji},\ i \neq j$$

$$a_{ii} = -\sum_{\substack{j=1 \\ j \neq i}}^{p} \theta_{ij} .$$

In most compartmental systems of interest, the eigenvalues of $\underset{\sim}{A}$ will be non-positive and distinct (Soong and Dowdee, 1974); the solution of Equation 1 can then be written as a sum of exponentials,

$$X_i(t) = \sum_{j=1}^{p} c_{ij} \exp(\lambda_j t) \qquad (2),$$

where $\{\lambda_j\}$ are the eigenvalues of $\underset{\sim}{A}$ and both the λ_j and the coefficients c_{ij} are functions of the θ_{ij} (the c_{ij} are also linear functions of the initial state vector $\underset{\sim}{X}(0)$).

2.2 Assumptions and Definitions

The parameter estimation problem with which we are concerned arises from the following situation:

i) There is a population of compartmental systems available for observation, each system having the same (known) form.

ii) In any system, the r rate constants θ_{ij} are each a realization of a nonnegative random variable with finite mean μ_{ij} and variance σ_{ij}^2. The distributions of the θ_{ij} do not vary over time.

iii) Each system can be observed only once.

To simplify notation, we assume some ordering on the θ_{ij}, so we can write $\theta_k = \theta_{ij}$ for k=1,...,r. We can now define the r-vectors $\underset{\sim}{\theta} = (\theta_1,\ldots,\theta_r)'$ and $\underset{\sim}{\mu} = (\mu_1,\ldots,\mu_r)'$ and the rxr matrix $\underset{\sim}{\Sigma} = \mathrm{diag}(\sigma_k^2)$.

The following additional assumptions are made primarily for mathematical convenience. Although there is naturally some loss of generality, the resulting situation is certainly not unrealistic.

iv) The rate constants are mutually independent.

v) Only the i-th compartment is observed. (This assumption is made to avoid the complication of multiple observations corresponding to the same parameter vector. It appears to limit consideration to relatively small systems, since for larger systems observations from a single compartment may not provide enough information for estimation of the parameters. Such questions of identifiability have been discussed by Cobelli, Lepschy, and Romanin Jacur (1979) for deterministic systems, but the stochastic situation apparently has not been investigated.)

vi) The initial state vector $\underset{\sim}{X}(0)$ is known with probability one.

vii) The measurement error made in observing $X_i(t)$ is negligibly small, so the only source of variability in the data that need be considered is the variation in the rate constants.

We assume a sampling scheme whereby at m time points t_j, $n_j>1$ systems are observed. We will let x_{jk} denote the k-th observation of $X_i(t_j)$, with the i subscript indicating the compartment number being suppressed since only one compartment is observed.

2.3 An Example

As an example, consider the following typical protocol for a pharmocokinetics experiment. A particular drug is assumed to distribute in the body according to the two-compartment model diagrammed in Figure 1b. At time t=0 a known dose of the drug is injected into Compartment 1 (representing the blood, say) of each of a group of small laboratory animals. At various time points after injection, several animals are sacrificed and the amount of the drug in Compartment 2 (representing some other tissue of interest) is accurately determined. From the data thus collected, estimates of the means and variances of the two rate constants are desired.

The equations for the system are

$$\frac{dX_1(t)}{dt} = -\theta_1 X_1 + \theta_2 X_2$$

$$\frac{dX_2(t)}{dt} = \theta_1 X_1 - \theta_2 X_2$$

or in matrix form,

$$\frac{d\underset{\sim}{X}(t)}{dt} = \begin{bmatrix} -\theta_1 & \theta_2 \\ \theta_1 & -\theta_2 \end{bmatrix} \begin{bmatrix} X_1(t) \\ X_2(t) \end{bmatrix}$$

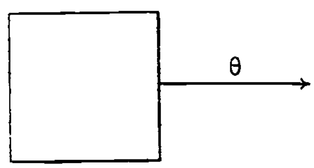
The general solution of the system can be found to be

$$X_1(t) = (\theta_2(X_1(0)+X_2(0))+(X_1(0)\theta_1-X_2(0)\theta_2)\exp(-\ell t))/\ell$$

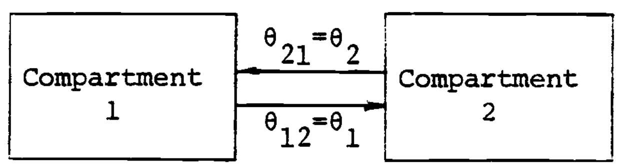
$$X_2(t) = (\theta_1(X_1(0)+X_2(0))+(X_2(0)\theta_2-X_1(0)\theta_1)\exp(-\ell t))/\ell$$

where $\ell = \theta_1+\theta_2$. We will assume a unit input at time t=0, so $\underset{\sim}{X}(0) = (1\ 0)'$. The relevant equation then becomes

$$X_2(t) = \frac{\theta_1}{\theta_1+\theta_2} (1-\exp(-(\theta_1 + \theta_2)t)) \qquad (3).$$



a. One-compartment model.



b. Two-compartment model.

Figure 1. Simple compartment models.

III. COMPARISON OF THE MEAN STOCHASTIC MODEL AND THE DETERMINISTIC MODEL

As noted in the Introduction, the mean of a stochastic compartmental model follows a different time course from the deterministic model evaluated at the means of the rate constants (see Jacquez, 1972, p.119). This difference has been pointed out by many authors, but no one appears to have attempted an analytical evaluation of the difference even for the one-compartment model. Such an evaluation is not possible for most multicompartment models, since a closed-form expression for the mean of the stochastic model usually cannot be found. For the two-compartment model of the previous chapter, however, a particular distributional assumption allows a closed-form expression for the mean to be obtained, and an analytical evaluation of the difference in mean functions is therefore possible.

Assume, then, that in Figure 1b the rate constants θ_1 and θ_2 have independent gamma distributions with shape parameters r_1 and r_2, respectively, and common scale parameter w, i.e.,

$$f(\theta_i) = \frac{1}{w^{r_i}\,\Gamma(r_i)}\,\theta_i^{r_i-1}\,\exp(-\theta_i/w),\quad 0<\theta_i<\infty.$$

Then it is easily shown that

$$L = \theta_1 + \theta_2 \sim \text{Gamma}\ (r_1+r_2, w)$$

$$U = \frac{\theta_1}{\theta_1+\theta_2} \sim \text{Beta}(r_1, r_2)$$

and that L and U are independent (Rao, 1973, p.164). It follows from Equation 3 that

$$g(t) = E\{X_2(t)\} = \frac{r_1}{r_1+r_2}\,(1-(1+wt)^{-(r_1+r_2)}) \qquad (4).$$

If we reparametrize in terms of

$$\mu_1 = r_1 w \qquad \mu_2 = r_2 w \qquad \sigma_1^2 = r_1 w^2$$

we have

$$g(t) = \frac{\mu_1}{\mu_1+\mu_2}\,(1-(1+\frac{\sigma_1^2}{\mu_1}\,t)^{-\mu_1(\mu_1+\mu_2)/\sigma_1^2})$$

and for μ_1 and μ_2 fixed,

$$\lim_{\sigma_1^2 \to 0} g(t) = \frac{\mu_1}{\mu_1+\mu_2}\,(1-\exp(-(\mu_1+\mu_2)t)) = \ell(t)$$

which is the deterministic model evaluated at the means of the rate constants. Thus the difference between the two functions decreases as the variances of the rate constants decrease. This is illustrated for a particular case by Figure 2. As a consequence of this asymptotic approach to the deterministic model, the true mean function is relatively insensitive to changes in σ_1^2 when σ_1^2 is close to zero, which affects the ability to obtain least squares estimates of $\underset{\sim}{\Sigma}$ using the true mean (as will be seen later).

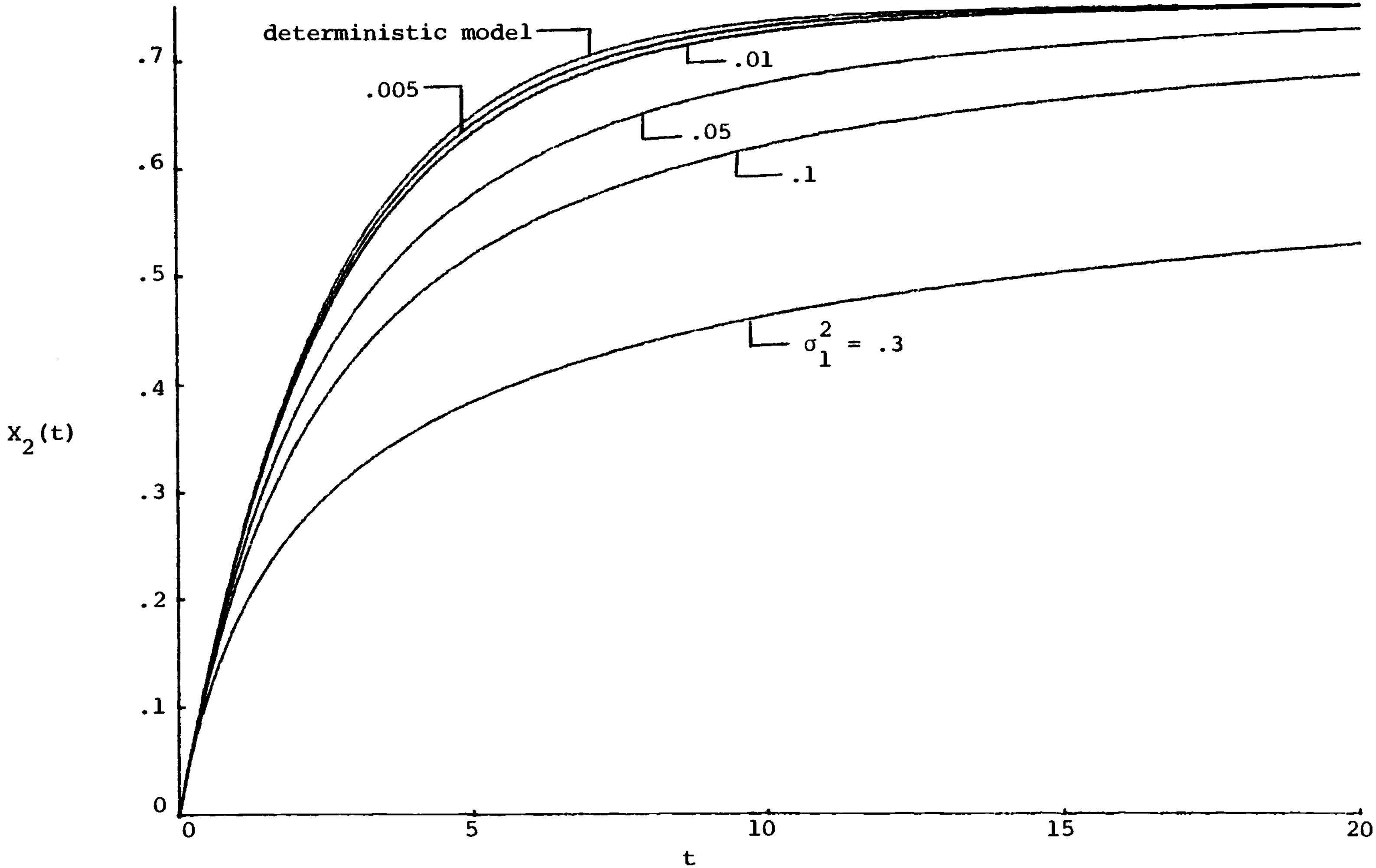


Figure 2. True mean functions for the stochastic two-compartment model of Chapter III with $\mu_1 = .3$, $\mu_2 = .1$, and various values of σ_1^2.

For a more quantitative assessment of the difference, we reparametrize by letting

$$u = \frac{r_1}{r_1+r_2} \qquad r = r_1+r_2 \qquad s = wt$$

Then

$$g(t) = \bar{g}(wt) = \bar{g}(s) = u(1-(1+s)^{-r}) \qquad \text{and}$$

$$\ell(t) = \bar{\ell}(wt) = \bar{\ell}(s) = u(1-e^{-rs}).$$

The difference between the two functions is

$$d(s) = \bar{\ell}(s)-\bar{g}(s) = u\{(1+s)^{-r}-e^{-rs}\}.$$

It can be shown (see Appendix 1) that d(s) has a maximum when

$$r = \frac{\ln(1+s)}{s-\ln(1+s)} \qquad (5).$$

For any particular value of r, let s* be the value of s which satisfies Equation 5. Then

$$d_{max} = \frac{us^*}{(1+s^*)^{r+1}}$$

One way of assessing the relative magnitude of d_{max} is to compare it to the variability of X_2 at time t* = s*/w. Figure 3 shows graphs of $\sqrt{Var(X_2(t^*))}/d_{max}$ against the coefficient of variation of θ_1 for various values of the ratio u. Numerical output from the program used to generate Figure 3 shows that the standard deviation of X_2 at t* is greater than d_{max} whenever C.V.(θ_1) is less than 165%,

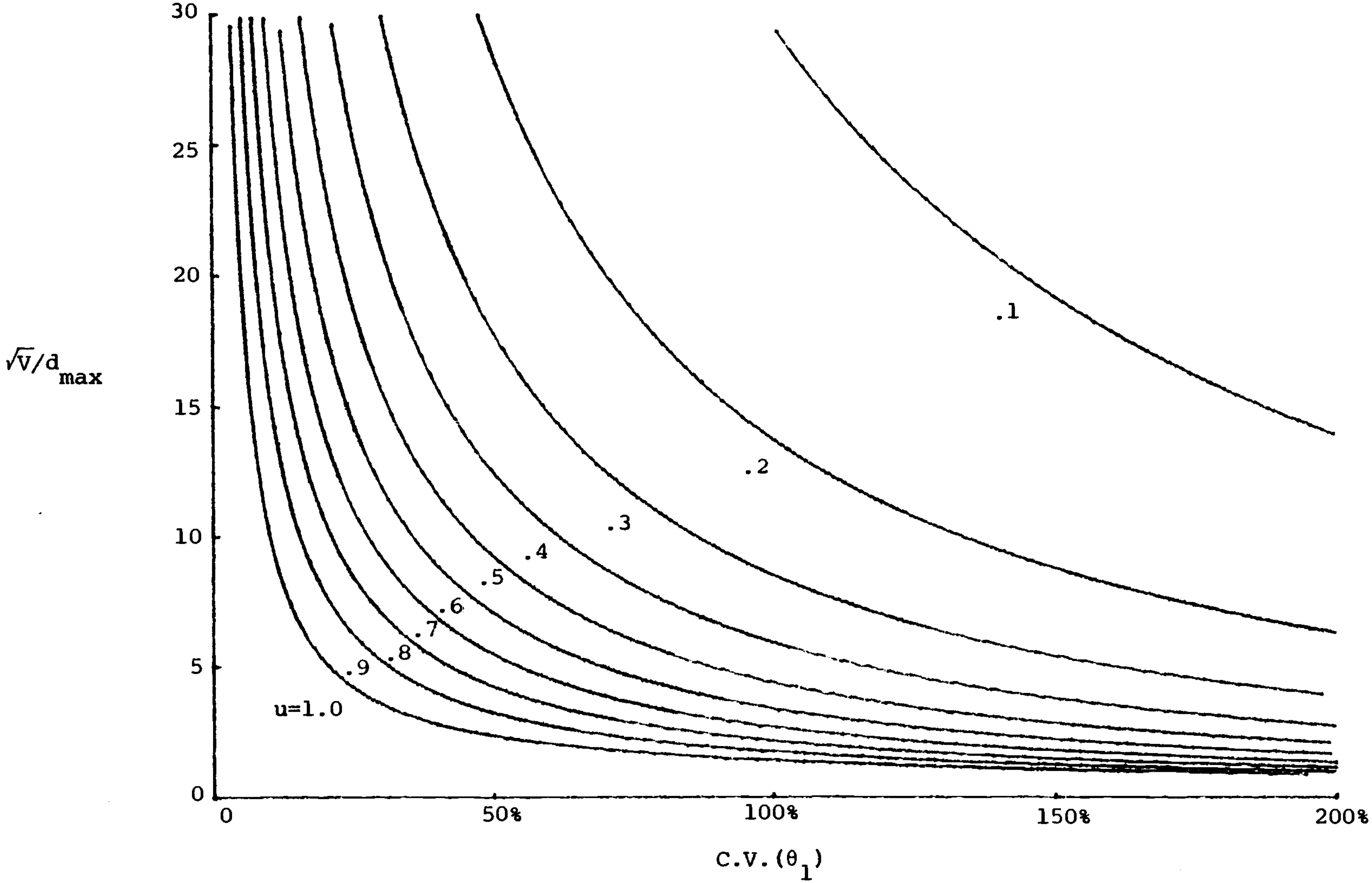


Figure 3. Graphs of $\sqrt{Var(X_2(t^*))}/d_{max}$ vs the coefficient of variation of θ_1 for different values of the ratio u.

and is at least 2.8 times greater when C.V.(θ_1) is less than 40%. The implication is that in most realistic situations the difference between the two functions is quite small in relation to the variability inherent in the data.

These results of course apply only to the rather specialized two-compartment model under consideration, and the extent to which they might generally be true is unknown. However, Tsokos and Tsokos (1976) obtained similar results in their investigation of a stochastic three-compartment system with normally distributed parameters. They do not discuss the matter directly, but their graphs clearly show the standard deviation of $X_i(t)$ to be at least two or three times as great as the difference between the deterministic and stochastic models.

It is also worth noting that the case u = 1.0 in Figure 3 is equivalent to the one-compartment model (Figure 1a) with $\theta \sim \text{Gamma}(r,w)$.

IV. METHODS OF PARAMETER ESTIMATION

4.1 One-Compartment Model

Parameter estimation in the one-compartment model (Figure 1a) is relatively straightforward since exact closed-form solutions for the moments of the stochastic model can be obtained. The equation for the deterministic system is

$$X(t) = X(0)\exp(-\theta t) \qquad (6).$$

If we assume that the probability distribution of θ has moment-generating function $M(t)$, then

$$E\{X(t)\} = X(0)M(-t)$$

Expressions for higher moments of $X(t)$ can be obtained in a similar manner. Matis and Wehrly (1979a) discuss the use of nonlinear least squares for parameter estimation in this and other stochastic one-compartment models.

Other estimation methods for the one-compartment model should also be noted. Lindstrom and Birkes (1979) discuss estimation using approximate likelihood functions based on Gram-Charlier expansions of the density of X (assuming θ is gamma-distributed and an additive normal error term). Their methods are very complicated even for the simple one-compartment model, and become much more so when extended to multicompartment models. Another obvious procedure (when $X(0)$ is known) is to solve Equation 6 to get an estimate of θ for each observation X(t), and use these estimates to estimate the population mean and variance.

4.2 Multicompartment Models

For most stochastic multicompartment models, closed-form expressions for the mean and variance are not available. It is necessary, therefore, to develop some sort of approximate model. We will describe below several models which have appeared in the literature, and will also develop from them two new models which have not previously been suggested. We note in passing that all of the approximate models could be applied to the one-compartment case if one does not wish to assume a particular distributional form for the rate constant.

Model 1: Assume that the eigenvalues λ_j in the sum of exponentials, Equation 2, are independent from each other and from the coefficients c_{ij}. Define

$$m_{ij} = E(c_{ij})$$

$$\eta_{ij}^2 = \mathrm{Var}(c_{ij})$$

$$\gamma_{ijk} = E(c_{ij}c_{ik})$$

$$M_j(t) = \text{moment-generating function of } \lambda_j.$$

Then we can write

$$E\{X_i(t)\} = \sum_{j=1}^{p} m_{ij} M_j(t) \tag{7}$$

$$\mathrm{Var}\{X_i(t)\} = E\left\{\sum_{j=1}^{p} c_{ij}\exp(\lambda_j t)\right\}^2 - \left(\sum_{j=1}^{p} m_{ij} M_j(t)\right)^2$$

$$= \sum_{j=1}^{p} \{(\eta_{ij}^2 + m_{ij}^2) M_j(2t) - m_{ij}^2 M_j(t)^2\}$$

$$+ \sum_{\substack{j=1 \\ j\neq k}}^{p} \sum_{k=1}^{p} (\gamma_{ijk} - m_{ij} m_{ik}) M_j(t) M_k(t) \tag{8}$$

Using Equation 7 as a regression function, we can estimate the m_{ij} and the distributional parameters of the λ_j. We then use these estimates in Equation 8 to estimate the η_{ij}^2 and γ_{ijk} from a least squares fit of the sample variances. Since the c_{ij} and λ_j are functions of the θ_{ij}, the estimates from this two-step procedure can be used to estimate $\underset{\sim}{\mu}$ and $\underset{\sim}{\Sigma}$ (see Campello and Cobelli, 1978).

As an example, consider the two-compartment model of Equation 3. We have

$$c_{21} = -c_{22} = c = \frac{\theta_1}{\theta_1+\theta_2}, \quad \lambda_1 = 0, \text{ and } \quad \lambda_2 = -(\theta_1+\theta_2).$$

Define $m = E(c)$ and $\eta^2 = \mathrm{Var}(c) = \mathrm{Var}(-c)$.

If we assume that $-\lambda_2$ has a Gamma(r,w) distribution, then

$$E(X_2(t)) = m(1-(1+wt)^{-r}),$$

which is just a reparametrization of Equation 4, and

$$\mathrm{Var}(X_2(t)) = \eta^2\{1-2(1+wt)^{-r}+(1+2wt)^{-r}\}+m^2\{(1+2wt)^{-r}-(1+wt)^{-2r}\}.$$

Using these equations, we obtain least squares estimates of the parameters m, r, w, and η^2. We then use these estimates to estimate the population means and variances from the relations

$$\mu_1 = E(\theta_1) = E(-c\lambda_2) = E(c)E(-\lambda_2) = mrw$$

$$\mu_2 = E(\theta_2) = E(-(1-c)\lambda_2) = (1-m)rw$$

$$E(\theta_1^2) = E(c^2)E(\lambda_2^2) = (\eta^2+m^2)r(r+1)w^2$$

$$E(\theta_2^2) = (\eta^2+1-2m+m^2)r(r+1)w^2$$

$$\mathrm{Var}(\theta_1) = rw^2(m^2+(r+1)\eta^2)$$

$$\mathrm{Var}(\theta_2) = rw^2((1-m)^2+(r+1)\eta^2)$$

This approach was first suggested by Soong and Dowdee (1974) and was extended by Chuang and Lloyd (1974) and Campello and Cobelli (1978). Its justification is the special two-compartment model of Chapter III, for which Equations 7 and 8 represent the true mean and variance. For other multicompartment models the assumption of independence of the c_{ij} and λ_j does not hold, but Soong and Dowdee suggest that the procedure is robust for estimation in the general stochastic multicompartment model.

Model 2: We expand the deterministic equation for $X_i(t)$ in a Taylor series about $\underset{\sim}{\mu}$ to get

$$X_i(t) = f(\underset{\sim}{\theta};t)$$

$$= f(\underset{\sim}{\mu};t) + \underset{\sim}{D}(\underset{\sim}{\mu};t)'(\underset{\sim}{\theta}-\underset{\sim}{\mu}) + 1/2(\underset{\sim}{\theta}-\underset{\sim}{\mu})'\underset{\sim}{G}(\underset{\sim}{\mu};t)(\underset{\sim}{\theta}-\underset{\sim}{\mu}) + R(t)$$

where

$$\underset{\sim}{D}(\underset{\sim}{\mu};t) = \left[\left.\frac{\partial f(\underset{\sim}{\theta};t)}{\partial \theta_1}\right|_{\underset{\sim}{\mu}}, \ldots, \left.\frac{\partial f(\underset{\sim}{\theta};t)}{\partial \theta_r}\right|_{\underset{\sim}{\mu}} \right]'$$

$$\underset{\sim}{G}(\underset{\sim}{\mu};t) = \left[\left.\frac{\partial^2 f(\underset{\sim}{\theta};t)}{\partial \theta_i \partial \theta_j}\right|_{\underset{\sim}{\mu}} \right]$$

and the remainder R(t) contains third and higher order partial derivatives. If we ignore the higher-order terms, we obtain the approximate expectation

$$E\{X_i(t)\} = f(\underset{\sim}{\mu};t) + 1/2\ E\{(\underset{\sim}{\theta}-\underset{\sim}{\mu})'G(\underset{\sim}{\mu};t)(\underset{\sim}{\theta}-\underset{\sim}{\mu})\}$$

$$= f(\underset{\sim}{\mu};t) + 1/2 \sum_{i=1}^{r} \left.\frac{\partial^2 f(\underset{\sim}{\theta};t)}{\partial \theta_i^2}\right|_{\underset{\sim}{\mu}} \sigma_i^2 \qquad (9).$$

From a nonlinear least squares fit of Equation 9 to the sample means, we obtain estimates of $\underset{\sim}{\mu}$ and $\underset{\sim}{\Sigma}$.

Birkes and Lindstrom (1977) developed this method for estimation from data consisting of a time series of $\underline{q}$ observations on each of n animals. However, the estimation procedure is not altered if the n animals observed at t_i happen to be different from the n animals observed at t_j, so the method extends directly to the present situation. In fact, it could be argued that the method is more properly applied here, since whatever additional information is contained in a time series of q observations on one animal (compared with observation of q different animals) is not utilized.

It might be supposed that by keeping more terms in the series expansion one would obtain an even better approximation to the true mean. That this is not necessarily the case is demonstrated by Figure 4, which shows three approximating functions for the two-compartment model of Chapter III where the true mean is known. It can be seen that the function described by Equation 9 lies closer to the true mean than does the function obtained by adding terms containing the third central moments. As more terms are added to the series, the resulting function more closely approximates the true mean for small values of time, but diverges greatly from the true mean as time increases. Such behavior is to be expected, since the radius of convergence of the series expansion depends on t as well as on the system parameters, and even within the region of convergence the convergence is oscillatory. However, the fact that $E\{R(t)\}$ may not be negligible for large values of t does not invalidate the use of Equation 9 as an approximating function. For the model used in Figure 4 (and in all other cases examined) the approximation seems quite adequate for all values of time.

Model 3: We retain only the first two terms in the series expansion for $X_i(t)$,

Figure 4 (page 23). True mean function and approximations for a two-compartment model.

$\theta_1 \sim \text{Gamma}(7.5,.04)$ $\quad$ $\theta_2 \sim \text{Gamma}(2.5,.04)$

$(\mu_1=.3,\ \sigma_1^2=.012)$ $\quad$ $(\mu_2=.1,\ \sigma_2^2=.004)$

1. Deterministic model evaluated at the means of the rate constants:

$$E(X_2(t)) = f(\underset{\sim}{\mu};t)$$

2. $$E(X_2(t)) = f(\underset{\sim}{\mu};t) + \frac{1}{2}\sum_{i=1}^{2} \frac{\partial^2 f(\underset{\sim}{\mu};t)}{\partial\theta_i^2}\sigma_i^2$$

3. $$E(X_2(t)) = f(\underset{\sim}{\mu};t) + \frac{1}{2}\sum_{i=1}^{2} \frac{\partial^2 f(\underset{\sim}{\mu};t)}{\partial\theta_i^2}\sigma_i^2 + \frac{1}{6}\sum_{i=1}^{2} \frac{\partial^3 f(\underset{\sim}{\mu};t)}{\partial\theta_i^3} E\{(\theta_i-\mu_i)^3\}$$

4. True mean function:

$$E(X_2(t)) = \frac{r_1}{r_1+r_2}\left(1-(1+wt)^{-(r_1+r_2)}\right)$$

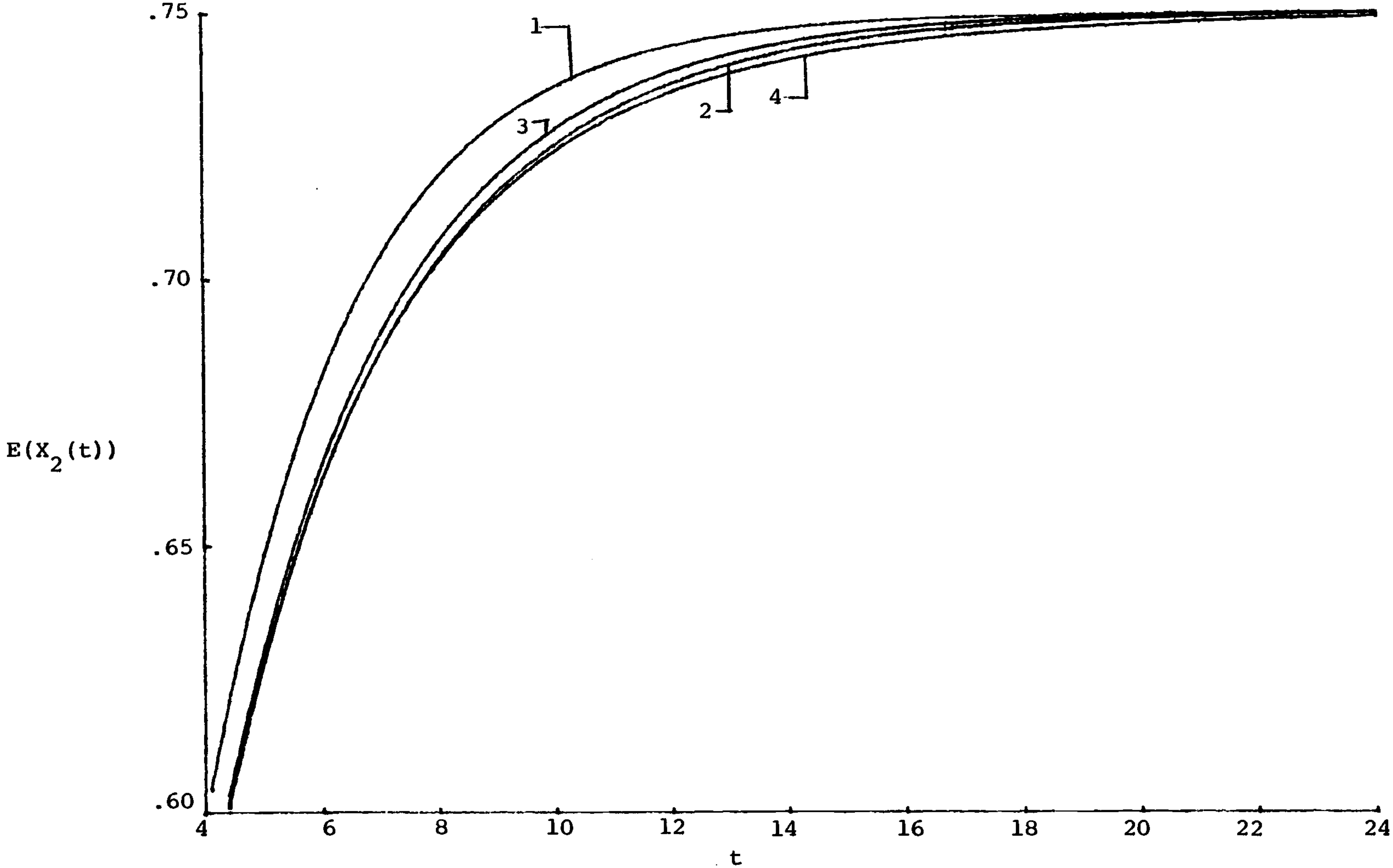


Figure 4.

$$X_i(t) = f(\mu;t) + D(\mu;t)'(\theta-\mu)$$

$$= f(\mu;t) + \varepsilon(t)$$

and treat $\varepsilon(t) = D(\mu;t)'(\theta-\mu)$ as an additive error term with mean zero and variance $V(\mu,\Sigma;t) = D(\mu;t)'\Sigma\, D(\mu;t)$

$$= \sum_{i=1}^{p} \left(\left.\frac{\partial f(\theta;t)}{\partial\theta_i}\right|_{\mu}\right)^2 \sigma_i^2 .$$

We note that the expression for the variance involves the unknown parameters. An obvious estimation procedure would therefore be to minimize the weighted least squares objective function

$$Q(\mu,\Sigma) = \sum_{j=1}^{m} \frac{\sum_{k=1}^{n_j} (x_{jk}-f(\mu;t_j))^2}{V(\mu,\Sigma;t_j)}$$

This sort of procedure fails in the present situation because $Q(\mu,\Sigma)$ can be made arbitrarily small by choosing the elements of Σ to be arbitrarily large. We will investigate three alternative estimation procedures in connection with Model 3.

a. (simple least squares): Define $\hat{\mu}$ to be the value of μ which minimizes

$$Q_1(\mu,\Sigma) = \sum_{j=1}^{m} \sum_{k=1}^{n_j} (x_{jk}-f(\mu;t_j))^2 .$$

Then define $\hat{\Sigma}$ such that

$$R(\underset{\sim}{\Sigma};\underset{\sim}{\beta}) = \sum_{j=1}^{m} (s_j^2 - V(\underset{\sim}{\beta},\underset{\sim}{\Sigma};t_j))^2$$

is minimized, where s_j^2 is the sample variance at time t_j. This is the same procedure that was used with Model 1. Note that the estimate of $\underset{\sim}{\mu}$ is obtained from fitting the deterministic model to the sample means.

b. (weighted least squares): Using an initial estimate $(\underset{\sim}{\beta}_0,\hat{\underset{\sim}{\Sigma}}_0)$, obtain an estimate $\underset{\sim}{\beta}_1$ by minimizing

$$Q(\underset{\sim}{\mu};\underset{\sim}{\beta}_0,\hat{\underset{\sim}{\Sigma}}_0) = \sum_{j=1}^{n_j} \frac{\sum_{k=1}^{n_j} (x_{jk} - f(\underset{\sim}{\mu};t_j))^2}{V(\underset{\sim}{\beta}_0,\hat{\underset{\sim}{\Sigma}}_0;t_j)} .$$

Next, obtain an estimate $\hat{\underset{\sim}{\Sigma}}_1$ of $\underset{\sim}{\Sigma}$ by minimizing $R(\underset{\sim}{\Sigma};\underset{\sim}{\beta}_1)$. Use $Q(\underset{\sim}{\mu};\underset{\sim}{\beta}_1,\hat{\underset{\sim}{\Sigma}}_1)$ to obtain an estimate $\underset{\sim}{\beta}_2$, and proceed iteratively.

c. (maximum likelihood): Assume that the distribution of $\varepsilon(t)$ is normal. Then $X_i(t)$ has a Normal $(f(\underset{\sim}{\mu};t),V(\underset{\sim}{\mu},\underset{\sim}{\Sigma};t))$ distribution and a maximum likelihood estimator can be found by minimizing

$$L(\underset{\sim}{\mu},\underset{\sim}{\Sigma}) = \sum_{j=1}^{m} \{n_j \ln(V(\underset{\sim}{\mu},\underset{\sim}{\Sigma};t_j)) + \frac{\sum_{k=1}^{n_j}(x_{jk} - f(\underset{\sim}{\mu};t_j))^2}{V(\underset{\sim}{\mu},\underset{\sim}{\Sigma};t_j)}\}.$$

The first two methods described above are similar to methods developed for the linear model by Jobson and Fuller (1980). The likelihood formulation was developed by Sheiner and co-workers (Sheiner, Rosenberg, and Melmon, 1972; Sheiner, Rosenberg, and

Marathe, 1977) as part of a general scheme for the estimation of population parameters from clinical data. The assumption of normality is not crucial for this method; one can look at $L(\mu,\Sigma)$ as an "extended" least squares objective function, with the $\ln V(\mu,\Sigma;t)$ term acting as a penalty function to prevent the variance from increasing without bound (Sheiner and Beal, 1980).

We see that Model 3 uses $f(\mu;t)$ as its mean and thus ignores the difference between the deterministic model evaluated at μ and the true mean. It seems logical to suppose that a better model would result if the difference were taken into account. Thus we are led to

Model 4: As in Model 3, except that the Taylor series approximation to the mean of the stochastic model (Equation 9) is used as the mean in place of $f(\mu;t)$. Because fitting this approximation is fairly time-consuming on the computer, only the likelihood estimation procedure will be used with this model.

We note that Model 2 approximates the true mean of $X_i(t)$, but ignores the variance. A least squares fit of this model involves the sample means but not the sample variances, as can be seen from the decomposition

$$\sum_{k=1}^{n_j} (x_{jk}-h(\mu,\Sigma;t_j))^2 = \sum_{k=1}^{n_j} (x_{jk}-\bar{x}_j)^2 + \sum_{k=1}^{n_j} (\bar{x}_j-h(\mu,\Sigma;t_j))^2$$

$$= (n_j-1)s_j^2 + n_j(\bar{x}_j-h(\mu,\Sigma;t_j))^2 \quad ,$$

where $\bar{x}_j$ is the sample mean at time t_j and $h(\mu,\Sigma;t)$ is the right-hand side of Equation 9, and the fact that the term involving s_j^2 is constant with respect to the minimization. A possible extension of Model 2 would involve incorporation of an approximate variance into the estimation procedure as was done with Model 3. However, the variance expression associated with Model 2 involves the third and fourth moments of the rate constants, and such an extension would thus double the number of parameters to be estimated.

Model 3, on the other hand, approximates the variance but ignores the difference between the mean functions. Estimation with this model (and with Models 1 and 4) uses both the sample means and sample variances. This is obvious for the least squares procedures; for the likelihood methods it follows from a decomposition similar to the one above (with $f(\underset{\sim}{\mu};t_j)$ in place of $h(\underset{\sim}{\mu},\underset{\sim}{\Sigma};t_j)$) and noting that the s_j^2 term is not constant with respect to minimization over $(\underset{\sim}{\mu},\underset{\sim}{\Sigma})$ when weighted by a variance term involving $\underset{\sim}{\mu}$ and $\underset{\sim}{\Sigma}$.

Models 1 and 4 approximate both the true mean and the variance of the stochastic model. It might therefore be expected that these models would perform better than the other two. We now proceed to investigate the performance of the four models using simulated data.

V. COMPARISON OF ESTIMATION METHODS

5.1 Generation of Simulated Experiments

To compare the estimation procedures discussed in the previous chapter, data from experiments of the type described in Section 2.3 were simulated. For each experimental animal, random values of θ_1 and θ_2 were obtained from independent gamma distributions using the IMSL gamma deviate generation routine GGAMR (IMSL, 1980). These values were then used in Equation 3 to produce an observation of $X_2(t)$ from a two-compartment animal. Each experiment consisted of five animals at each of eight time points between zero and fifteen.

One hundred experiments were generated for each of three different sets of parameter values:

Data Set I: $r_1 = 90$ $r_2 = 30$ $w_1 = w_2 = 1/300$

Data Set II: $r_1 = 7.5$ $r_2 = 2.5$ $w_1 = w_2 = 1/25$

Data Set III: $r_1 = r_2 = 9$ $w_1 = 1/30$ $w_2 = 1/90$

Representative experiments from each of the data sets are plotted in Figures 5 through 7. In the first two sets, the eigenvalue $\lambda = \theta_1+\theta_2$ and coefficient $u = \theta_1/(\theta_1+\theta_2)$ are independent, as noted in Chapter III (the sample correlation coefficient for Data Set II was $r = -0.00378$, $n = 4000$, $p>.1$). An exact expression for the mean is therefore given by Equation 4. For Data Set III λ and u are not

Figures 5 through 7. Representative experiments from three simulated data sets.

——————————— = deterministic model

— — — — = true mean function

-------------- = empirical mean function

Figure 5 (page 30). Data Set I

$\mu_1 = .3 \quad \mu_2 = .1 \quad \sigma_1^2 = .001 \quad \sigma_2^2 = .000333$

(The deterministic model and true mean are superimposed.)

Figure 6 (page 31). Data Set II

$\mu_1 = .3 \quad \mu_2 = .1 \quad \sigma_1^2 = .012 \quad \sigma_2^2 = .004$

Figure 7 (page 32). Data Set III

$\mu_1 = .3 \quad \mu_2 = .1 \quad \sigma_1^2 = .010 \quad \sigma_2^2 = .00111$

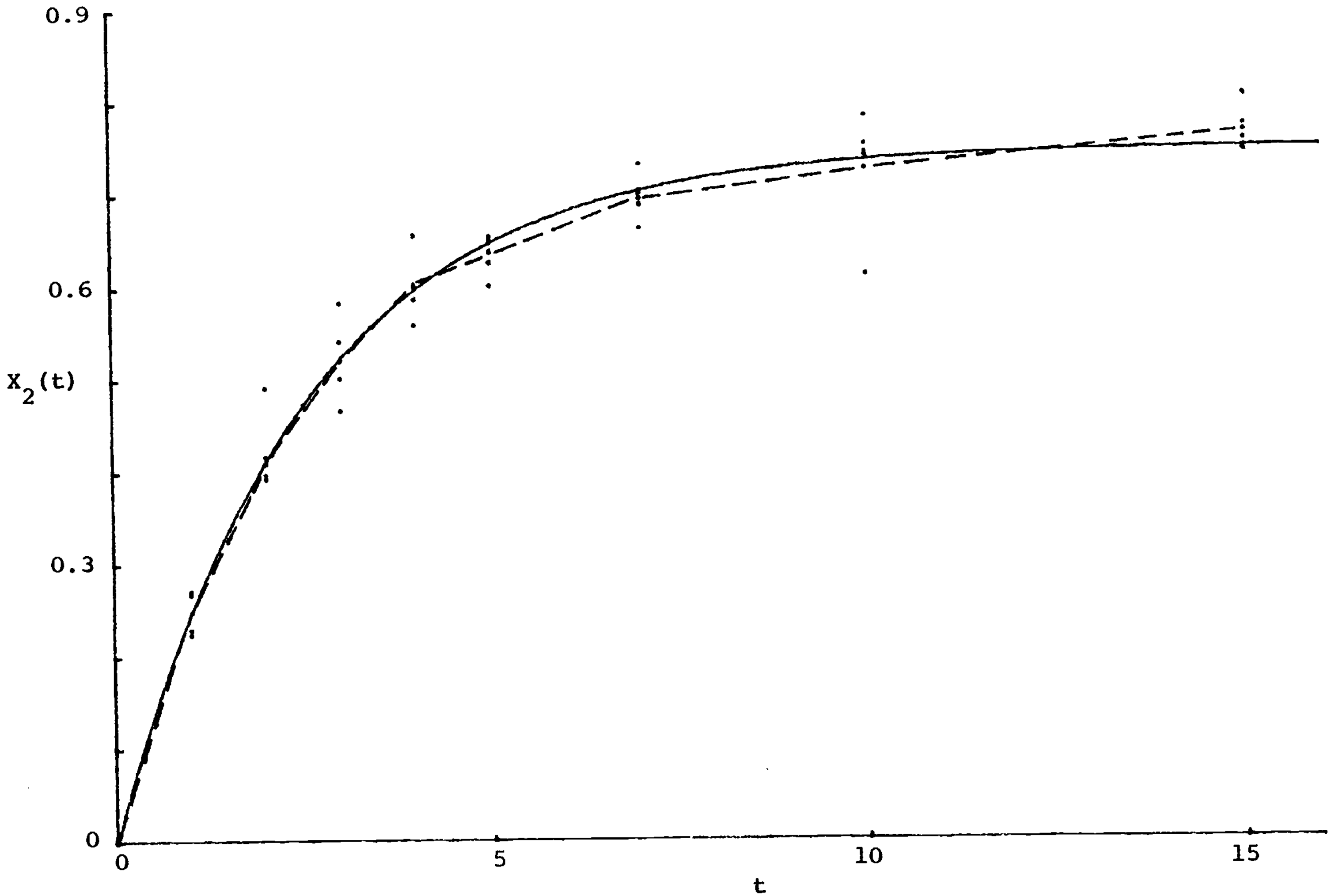


Figure 5.

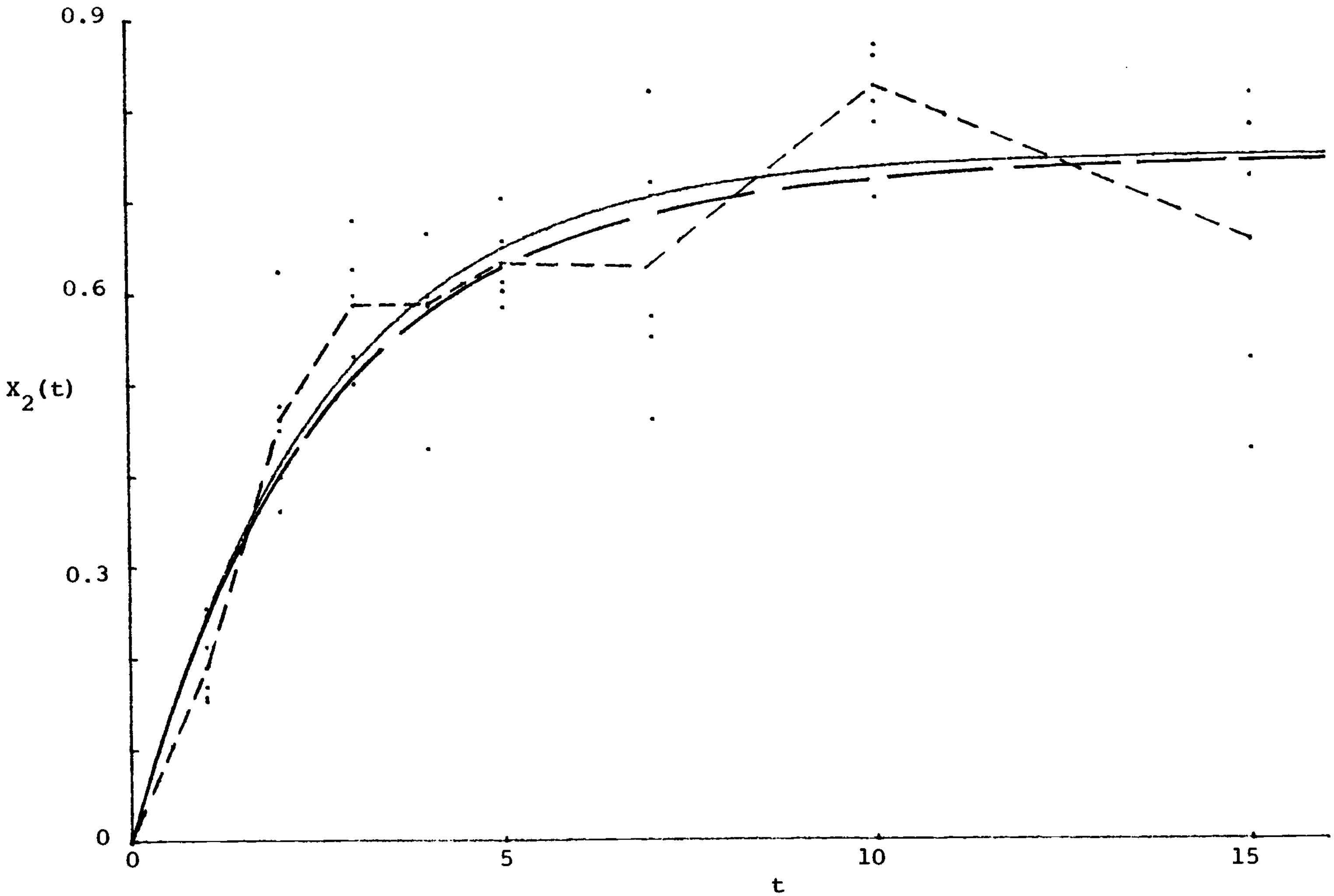


Figure 6.

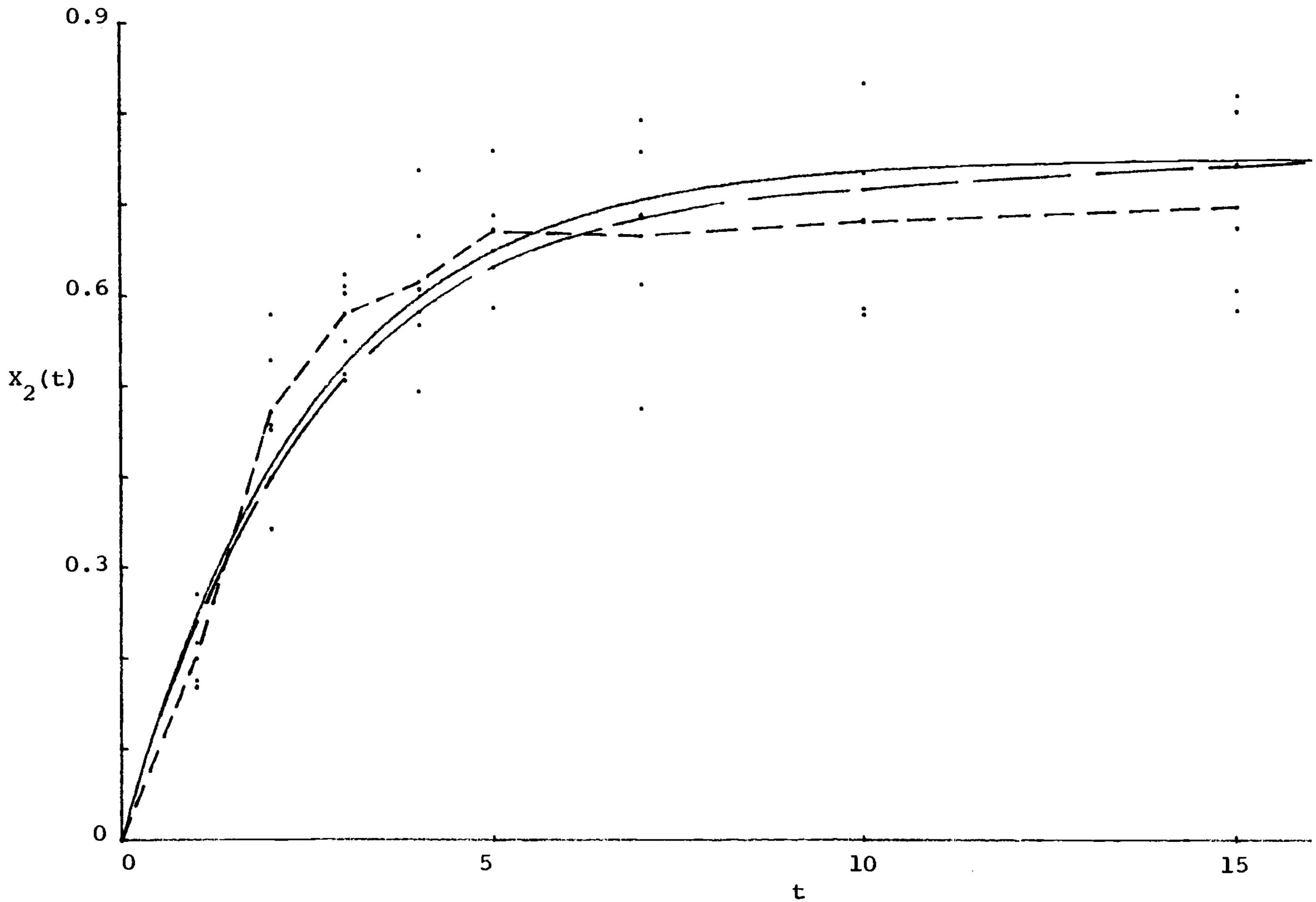


Figure 7.

independent ($r = .436$, $p<.001$), so an exact solution is not available; the true mean function shown in Figure 7 is an estimate obtained by averaging the 500 simulated observations at each time point.

The means of the rate constants ($\mu_1 = .3$, $\mu_2 = .1$) are the same in all three data sets. In Data Set I the variances are small enough that the difference between the stochastic mean and the deterministic model is negligible. In Data Set II, we attempted to make the difference as large as possible while keeping the variance of the observations at a level which seems reasonable for biological systems. In Data Set III both rate constants have the same coefficient of variation (C.V. = 33.3%), which again is near the upper limit of what we consider likely to occur in a physiological system.

The means and variances of the rate constants were estimated for each experiment by the six methods described in Chapter IV. For Data Sets I and II a seventh estimation method is available: We obtain estimates of the three parameters in the true mean function, Equation 4, by least squares and use these estimates to calculate estimates of $\underset{\sim}{\mu}$ and $\underset{\sim}{\Sigma}$. This method is not generally applicable and is included only for comparison. In theory, the estimates of $\underset{\sim}{\mu}$ from the exact solution and from Model 1 should be the same; numerical differences occur because different parametrizations of Equation 4 were used in the two procedures.

All function minimizations used the Nelder-Mead simplex method (Nelder and Mead, 1965), with the true parameter vector used as a starting point. All parameters were constrained to be nonnegative.

Several other points regarding the computations should also be noted:

1. Initial runs using Model 1 with the more variable data sets (II and III) gave several estimates of $m = E\{\theta_1/(\theta_1+\theta_2)\}$ which were greater than one. The constraint $m \leq 1$ was therefore included in the program for this model.

2. The iterative procedure of Model 3b was continued until all parameter estimates differed from their previous estimates by less than 10^{-5}. Usually three or four iterations were required; the maximum number was ten.

3. The least squares fitting of the exact solution and Model 1 to the sample means was weighted by the sample variances to correspond with previous applications of these procedures (Soong and Dowdee, 1974; Campello and Cobelli, 1978).

Listings of all computer programs appear in Appendix 2.

5.2 Results and Discussion

The means and root mean squared error of the estimates of the individual parameters are presented in Tables 1 through 3. Several points emerge from examination of these tables.

Fitting the exact solution resulted in zero estimates of $\utilde{\Sigma}$ in nearly half of the experiments. This reflects the insensitivity of the true mean function to changes in σ_1^2 for small values of σ_1^2, which in turn results from the asymptotic approach of the true mean to the deterministic model as discussed in Chapter III. Similar results are obtained from Model 2, which gives even more zero estimates of $\utilde{\Sigma}$.

TABLE 1

MEANS AND ROOT MEAN SQUARED ERROR OF
PARAMETER ESTIMATES: DATA SET I

Estimation Method	μ_1	μ_2	$\sigma_1^2 \times 10^3$	$\sigma_2^2 \times 10^3$
True value	.3	.1	1.0	.3333
Exact solution	.3067 (.01642)	.09938 (.009786)	15.29 (22.77) (48)[a]	4.425 (6.141) (48)
Model 1	.3067 (.01642)	.09938 (.009785)	5.881 (12.37)	.6690 (.8154)
Model 2	.3091 (.02003)	.1072 (.03849)	19.27 (27.42)	34.67 (81.98) (23)
Model 3a	.3005 (.009486)	.1019 (.008652)	.9873 (.4108)	.3375 (.2141) (94)
Model 3b	.3002 (.008911)	.1017 (.008885)	.9852 (.4104)	.3371 (.2161) (94)
Model 3c	.3004 (.008800)	.1020 (.008784)	.9406 (.3255)	.3180 (.2071) (95)
Model 4	.3013 (.008933)	.1018 (.008846)	.9436 (.3272)	.3209 (.2087) (95)

[a]Variance estimates which were equal to zero were excluded from the computations. The number of non-zero estimates, if less than 100, is indicated in parentheses below the mean and RMSE.

TABLE 2

MEANS AND ROOT MEAN SQUARED ERROR OF PARAMETER ESTIMATES: DATA SET II

Estimation Method	μ_1	μ_2	$\sigma_1^2 \mathrm{x} 10^3$	$\sigma_2^2 \mathrm{x} 10^3$
True value	.3	.1	12.0	4.0
Exact solution	.3243 (.07093)	.07550 (.04783)	120.6 (168.1) (59)[a]	15.79 (23.17) (46)
Model 1	.3243 (.07095)	.07542 (.04781)	67.05 (121.5)	3.144 (2.872)
Model 2	.3776 (.1615)	.1171 (.06530)	225.9 (413.1) (60)	31.62 (148.3) (27)
Model 3a	.2896 (.03367)	.1024 (.02871)	10.33 (4.829)	4.455 (3.657) (92)
Model 3b	.2934 (.03344)	.1052 (.03250)	10.62 (4.780) (98)	5.008 (5.642 (92)
Model 3c	.2926 (.03261)	.1041 (.03090)	10.26 (4.038)	4.334 (3.640) (90)
Model 4	.3029 (.03317)	.1039 (.03679)	10.65 (4.203)	4.487 (4.596) (94)

[a]Variance estimates which were equal to zero were excluded from the computations. The number of non-zero estimates, if less than 100, is indicated in parentheses below the mean and RMSE.

TABLE 3

MEANS AND ROOT MEAN SQUARED ERROR OF PARAMETER ESTIMATES: DATA SET III

Estimation Method	μ_1	μ_2	$\sigma_1^2 \times 10^3$	$\sigma_2^2 \times 10^3$
True value	.3	.1	10.0	1.11
Model 1	.3149 (.06025)	.08385 (.04279)	46.38 (105.9)	2.349 (3.052)
Model 2	.3320 (.08269)	.1208 (.06562)	99.65 (171.5) (53)[a]	102.9 (145.5) (29)
Model 3a	.2890 (.03093)	.1033 (.02442)	8.565 (3.402)	1.884 (1.846) (77)
Model 3b	.2892 (.02932)	.1032 (.02406)	8.619 (3.449)	1.948 (1.835) (74)
Model 3c	.2892 (.03050)	.1030 (.02492)	8.293 (3.523)	1.775 (2.222) (77)
Model 4	.2965 (.02889)	.09842 (.02745)	8.583 (3.588)	1.826 (2.492) (83)

[a]Variance estimates which were equal to zero were excluded from the computations. The number of non-zero estimates, if less than 100, is indicated in parentheses below the mean and RMSE.

Even when the zero estimates of variances are not considered, these two models give the worst estimates of both $\underset{\sim}{\mu}$ and $\underset{\sim}{\Sigma}$. Thus both of the methods which ignore the covariance structure of the stochastic model perform poorly relative to the other methods. It appears that there is too much variability in the data to distinguish between the stochastic and deterministic models on the basis of the difference in their means.

It is also apparent that Model 1 performs poorly in comparison to Models 3 and 4; Model 1 also does better with Data Set III than with Data Set II. These results were unexpected, since for Data Sets I and II Model 1 uses the true mean and variance functions while the other models use approximations. Again the difficulty in obtaining good least squares estimates from functions such as Equation 4 may be a factor. However, it is surprising that Model 3, which uses the deterministic model as the mean, should do better than a model which uses the true mean of the stochastic model.

From Figures 6 and 7 it can be seen that even for the more variable data sets the difference in means is still fairly small. Soong and Dowdee (1974) generated data using a χ_1^2 distribution (r=.5, w=2) for both rate constants. While such a distributional assumption seems quite unrealistic from a biological point of view, it does give a situation where the difference in mean functions is very large. Using one experiment with ten animals at eight time points (it is not clear whether destructive sampling was assumed), they obtained much more accurate results with the true mean function than with the deterministic model. When we simulated ten experiments with destructive sampling using five animals per time point and the same parameter values, we got the results shown in Table 4:

TABLE 4

ESTIMATES FROM TEN EXPERIMENTS WITH $\theta_i \sim$ GAMMA(.5,2)

	True Value	Exact Solution		Model 3c	
		Mean	Range	Mean	Range
μ_1	1.0	3.07	.325-17.9	1.04	.389-1.86
μ_2	1.0	8.41	0 -73.7	1.99	0 -5.40
σ_1^2	2.0	83.2	0 -827.	.802	.088-2.01
σ_2^2	2.0	1.01	0 -10.1	8.56	0 -33.7

Clearly neither estimation method is able to produce good estimates from such data. Again the variability of the data is so much greater than the difference between the mean functions that the difference is essentially undetectable with the small sample sizes commonly used in pharmacokinetics experiments.

Of the three estimation methods associated with Model 3, none seems to emerge as being preferable to the others insofar as the accuracy of the estimates is concerned. The least squares procedures would seem desirable since they involve no assumptions beyond those made in deriving the model; however, the assumption of normality may be necessary in situations requiring statistical inference. Model 4 does about as well as Model 3 with Data Set I, which might be expected since the difference between the true mean and deterministic model is very small. Even when the difference is larger, however, as in Data Sets II and III, Model 4 does not seem to be much of an improvement over Model 3.

Only Model 1 was able to give positive estimates of all four parameters in every experiment. Models 3 and 4 also did reasonably well, even though zero estimates of σ_2^2 were obtained in a few experiments even with the least variable data. Examination of several of the experiments which gave such aberrant results revealed no peculiarities (such as extreme outliers) which might account for them.

To provide an overall comparison of the methods, the relative Euclidean distance of each estimated parameter vector from its true value,

$$\text{r.d.} = \left\{\left(\frac{\hat{\mu}_1-\mu_1}{\mu_1}\right)^2+\left(\frac{\hat{\mu}_2-\mu_2}{\mu_2}\right)^2+\left(\frac{\hat{\sigma}_1^2-\sigma_1^2}{\sigma_1^2}\right)^2+\left(\frac{\hat{\sigma}_2^2-\sigma_2^2}{\sigma_2^2}\right)^2\right\}^{1/2},$$

was calculated for each experiment. The average relative distances are shown in Table 5. The distances from Models 3a, 3b, 3c, and 4 were tested for differences by regarding each experiment as a block in a randomized block design with four treatments. Using a distribution-free test based on Friedman rank sums (Hollander and Wolfe, 1973, pp.138-146), the following results were obtained:

Data Set I: $S = 7.97$, $p \simeq .046$

Data Set II: $S = .492$, $p > .25$

Data Set III: $S = .816$, $p > .25$

It should be noted that the relative distance is more closely related to the mean squared error of the estimates than to their means. Thus the estimates for Model 3c from Data Set I are on the average "closer" to the true values than those from Model 3b, using the relative distance: however, Table 1 shows that the mean estimates of all four parameters are closer to the true values with Model 3b than with Model 3c.

TABLE 5

MEAN RELATIVE DISTANCE OF PARAMETER
ESTIMATES FROM TRUE VALUES

Estimation Method	Data Set I	Data Set II	Data Set III
Exact solution	5.8510	5.4661	-
Model 1	9.9185	6.6301	5.2176
Model 2	29.5545	16.2059	29.0914
Model 3a	.6968	.8887	1.2098
Model 3b	.6996	1.0046	1.2031
Model 3c	.6353	.8744	1.2404
Model 4	.6375	.9669	1.2963

We should also point out that while Models 3 and 4 seem to perform equally well in estimating the parameters in the three sets of experiments examined, they are quite different in terms of the amount of computer time they require. Model 4 took nearly three minutes to estimate the parameters in 100 experiments, roughly six times as long as Model 3a, twice as long as Models 3b and 3c, and 1.5 times as long as Model 1.

VI. CONCLUSION

Two main conclusions emerge from the results presented here:

1. While the difference between the mean of the stochastic model and the deterministic model evaluated at the means of the rate constants may sometimes be considerable, it is relatively unimportant insofar as parameter estimation is concerned.
2. Of much greater importance, particularly for estimating the variances of the rate constants, is the incorporation of an expression for the variance of the stochastic model into the estimation procedure.

The first of these conclusions contradicts the statements of some previous authors (for example, Soong and Dowdee (1974) and Matis and Wehrly (1979a)), but it is strongly supported by several aspects of the present study: The poor performance of estimation methods based on an ordinary least squares fit of the true mean or an approximation; the excellent results from Model 3, which uses the deterministic model as the mean; and the lack of improvement of Model 4, which uses an approximation to the true mean, over Model 3. There may well be situations in which the difference in mean functions is more important, but for the experimental situation considered here, all indications are that the difference is negligible in relation to the variability of the data, and is of minor importance in the estimation problem.

In contrast, consideration of the variance of the model seems to be crucial for obtaining good estimates. Typically the variance of the observations is incorporated into an estimation scheme only through weighting by the sample variances. Our results indicate that this alone is not adequate. A comparison of the results obtained from fitting the exact solution for the mean by variance-weighted least squares with those from the two-stage estimation procedure of Model 1 shows that much better variance estimates are obtained when a variance expression containing the parameters is included in the estimation scheme. The poor performance of an ordinary least squares fit of an approximate mean function (Model 2) is further evidence for the importance of the variance of the observations.

These conclusions suggest several worthwhile areas for further research. Investigation of parameter estimation methods for non-linear models whose covariance structure involves the unknown parameters is clearly desirable. More complicated covariance structures than the one used here should also be considered. For example, the assumption that the rate constants are independent is quite unrealistic; a better model would have covariance terms included with the elements of $\underset{\sim}{\Sigma}$ to be estimated. Another extension of the present work would be to include some sort of measurement error in the formulation of the problem. It is straightforward enough to replace $V(\underset{\sim}{\mu},\underset{\sim}{\Sigma};t)$ by $V(\underset{\sim}{\mu},\underset{\sim}{\Sigma};t)+\sigma^2$ in Model 3, for example, but whether reliable estimates of σ^2 could be obtained with the methods used here remains to be investigated.

Additional areas for future research include relaxation of some of the other assumptions made in Chapter II. For example, in many situations it is more natural to model the initial state vector as a random variable rather than as a known constant; also, the possibility that the distributions of the rate constants vary over time should be considered. Finally, the selection of an optimal sampling scheme, both with regard to the choice of a set of time points and to the number of observations at each time point, is another area which merits investigation.

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APPENDICES

APPENDIX 1

We have $d(s) = u\{(1+s)^{-r}-e^{-rs}\}$

and $d'(s) = ur\{e^{-rs}-(1+s)^{-(r+1)}\}$

$$= 0 \text{ when } e^{-rs} = (1+s)^{-(r+1)} \leftrightarrow r = \frac{\ln(1+s)}{s-\ln(1+s)}$$

To show that this stationary point represents a maximum of d(s), we will show that d"(s) is negative when d'(s) = 0.

$$d''(s) = ur\{(r+1)(1+s)^{-(r+2)}-re^{-rs}\}$$

$$= ur\{(r+1)(1+s)^{-(r+2)}-r(1+s)^{-(r+1)}\} \quad \text{when } d'(s) = 0$$

$$= ur(1+s)^{-(r+2)}(1-rs)$$

So the sign of d"(s) at the stationary point is the sign of

$$1-rs = 1- \frac{s\ln(1+s)}{s-\ln(1+s)} .$$

Now $1<1+\ln(1+x)$ for all $x \rightarrow s<s+\int_0^s \ln(1+x)dx$ for all $s\varepsilon(0,\infty)$.

Integrating by parts, we get

$$s < s+s\ln(1+s)-\int_0^s\frac{x}{1+x}dx = s+s\ln(1+s)-\int_0^s (1-\frac{1}{1+x})dx$$

or $$s < s+s\ln(1+s)-s+\ln(1+s) = (1+s)\ln(1+s)$$

$$\rightarrow \frac{s-\ln(1+s)}{s\ln(1+s)} = \frac{1}{rs} < 1$$

$$\rightarrow 1 < rs$$

$\rightarrow d''(s) < 0'$ when $d'(s) = 0$.

APPENDIX 2

This appendix contains listings of the following Fortran IV programs:

1. Data generation program
2. Function minimization program (for Models 2, 3c, and 4)
3. Model 1
4. Model 3a
5. Model 3b
6. Nelder-Mead simplex subroutine

1.
```
      PROGRAM DATA1(TAPE1,TAPE2,TAPE3,OUTPUT)
      DIMENSION T(8),X(8,5),W(100),V(100)
      DOUBLE PRECISION DSEED1,DSEED2
      DSEED1=7545315
      DSEED2=7544196
      READ(1,10)K,N,M
   10 FORMAT(3I5)
      READ(1,20)A1,B1,A2,B2
   20 FORMAT(4F10.7)
      READ(1,30)T
   30 FORMAT(8F5.2)
      WRITE(2,20)A1,B1,A2,B2
      DO 50 I=1,K
      DO 40 J=1,M
      DO 70 L=1,N
      CALL GGAMR(DSEED1,A1,1,W,THETA1)
      CALL GGAMR(DSEED2,A2,1,V,THETA2)
      THETA1=B1*THETA1
      THETA2=B2*THETA2
      Z=THETA1+THETA2
      X(J,L)=THETA1*(1.-EXP(-Z*T(J)))/Z
   70 CONTINUE
      WRITE(2,80)T(J),(X(J,L),L=1,N)
   80 FORMAT(6(F8.4,2X))
   40 CONTINUE
   50 CONTINUE
      END
```

```
 2.   PROGRAM RFUNMIN(TAPE1,TAPE2,TAPE3)
      COMMON /SIMP/ N,ICOUNT,REQMIN,YNEWLO,YSEC
      COMMON /SIMP/ START(20),STEP(20),XMIN(20),XSEC(20)
      COMMON /DATA/ YBAR(10),T(10),SS(10),SAMPSIG(10)
      DIMENSION X(10),YDATA(10,50)
C
      READ (1,1) N,NSETS,ICOUNT,REQMIN
    1 FORMAT(3I4,F14.6)
      READ (1,2) (START(I),I=1,N)
    2 FORMAT (5F10.6)
      READ (1,2) (STEP(I),I=1,N)
C
      JCOUNT=ICOUNT
      DO 10 K=1,NSETS
C
      DO 3 I=1,8
      READ (2,4) T(I),(YDATA(I,J),J=1,5)
    4 FORMAT (6F10.6)
C
      SUMYBAR=SS(I)=0.0
      DO 71 J=1,5
      SS(I)=SS(I)+YDATA(I,J)*YDATA(I,J)
   71 SUMYBAR=SUMYBAR+YDATA(I,J)
      YBAR(I)=SUMYBAR/5.
      SS(I)=SS(I)-SUMYBAR*SUMYBAR/5.
      SAMPSIG(I)=SS(I)/4.
    3 CONTINUE
C
      CALL NELMIN
C
      DO 60 I=1,N
   60 X(I)=XMIN(I)*XMIN(I)
C
      WRITE(3,700)ICOUNT,(X(I),I=1,N)
  700 FORMAT(I6,3X,6(1X,F15.9))
      ICOUNT=JCOUNT
   10 CONTINUE
      END
      FUNCTION FX(X)
      COMMON /SIMP/ N,ICOUNT,REQMIN,YNEWLO,YSEC
      COMMON /DATA/ YBAR(10),T(10),SS(10),SAMPSIG(10)
      DIMENSION X(10),Z(10),DZ(10),Y(10),SIG(10)
      DO 10 I=1,N
   10 Y(I)=X(I)*X(I)
      SUM=0.0
C    LINE 46...INSERT FUNCTION CODE
   20 CONTINUE
      FX=SUM
   40 RETURN
      END
```

```
C
C   TAYLOR SERIES, MODEL 2
C
      A=Y(1)+Y(2)
      DO 20 I=1,8
      V=A*T(I)
      F=Y(1)*(1.-EXP(-V))/A
      B=(-2.*Y(2)+EXP(-V)*(-Y(1)*V*V+2.*Y(2)*V+2.*Y(2)))/A**3
      C=Y(1)*(2.+EXP(-V)*(-2.-2.*V-V*V))/A**3
      Z(I)=F+.5*(B*Y(3)+C*Y(4))
      DZ(I)=YBAR(I)-Z(I)
      SUM=SUM+DZ(I)*DZ(I)*5.


C
C    LIKELIHOOD METHOD, MODEL 3C
C
      A=Y(1)+Y(2)
      DO 20 I=1,8
      W=EXP(-A*T(I))
      Z(I)=Y(1)*(1.-W)/A
      DZ(I)=YBAR(I)-Z(I)
      F=SS(I)/5.+DZ(I)*DZ(I)
      C=(Y(2)+W*(Y(1)*A*T(I)-Y(2)))/(A*A)
      D=Y(1)*(-1.+W*(1.+A*T(I)))/(A*A)
      SIG(I)=Y(3)*C*C+Y(4)*D*D
      IF(SIG(I).GT.0.)GO TO 30
      FX=1.0E20
      GO TO 40
   30 SUM=SUM+ALOG(SIG(I))+F/SIG(I)


C
C    LIKELIHOOD METHOD, MODEL 4
C
      A=Y(1)+Y(2)
      DO 20 I=1,8
      V=A*T(I)
      F=Y(1)*(1.-EXP(-V))/A
      B=(-2.*Y(2)+EXP(-V)*(-Y(1)*V*V+2.*Y(2)*V+2.*Y(2)))/A**3
      C=Y(1)*(2.+EXP(-V)*(-2.-2.*V-V*V))/A**3
      Z(I)=F+.5*(B*Y(3)+C*Y(4))
      DZ(I)=YBAR(I)-Z(I)
      F=SS(I)/5.+DZ(I)*DZ(I)
      C=(Y(2)+EXP(-V)*(Y(1)*A*T(I)-Y(2)))/(A*A)
      D=Y(1)*(-1.+EXP(-V)*(1.+A*T(I)))/(A*A)
      SIG(I)=Y(3)*C*C+Y(4)*D*D
      IF(SIG(I).GT.0.)GO TO 30
      FX=1.0E20
      GO TO 40
   30 SUM=SUM+ALOG(SIG(I))+F/SIG(I)
```

```
C
C    EXACT SOLUTION
C
      A=Y(1)+Y(2)
      U=Y(1)/A
      S=Y(3)/Y(1)
      DO 20 I=1,8
      Z(I)=U*(1.-(1.+S*T(I))**(-A/S))
      DZ(I)=YBAR(I)-Z(I)
      SUM=SUM+DZ(I)*DZ(I)*5.0/SAMPSIG(I)
```

3.

```
      PROGRAM MODEL1(TAPE1,TAPE2,TAPE3,OUTPUT,TAPE4=OUTPUT)
      COMMON /SIMP/ N,ICOUNT,REQMIN,YNEWLO,YSEC
      COMMON /SIMP/ START(20),STEP(20),XMIN(20),XSEC(20)
      COMMON /DATA1/ T(10),YBAR(10),SIG(10),IFN,XMU,R,XNU
      DIMENSION YDATA(100,50)
C
      READ(1,1)N,NSETS,ICOUNT,REQMIN
    1 FORMAT(3I4,F16.6)
      READ(1,2) (START(I),I=1,N)
      READ(1,2) (STEP(I),I=1,N)
    2 FORMAT(5F10.6)
C
      JCOUNT=ICOUNT
C
      DO 100 K=1,NSETS
C
      DO 5 I=1,8
      READ(2,4) T(I),(YDATA(I,J),J=1,5)
    4 FORMAT(6F10.6)
      SUMY=SUMSQR=0.0
      DO 5 J=1,5
      SUMY=SUMY+YDATA(I,J)
      SUMSQR=SUMSQR+YDATA(I,J)*YDATA(I,J)
      YBAR(I)=SUMY/5.
      SS=SUMSQR-SUMY*SUMY/5.
      SIG(I)=SS/4.
    5 CONTINUE
C
      IFN=1
      N=3
      CALL NELMIN
C
      XMU=XMIN(1)*XMIN(1)
      R=XMIN(2)*XMIN(2)
      XNU=XMIN(3)*XMIN(3)
```

```
      IFN=2
      N=1
      T1=STEP(1)
      T2=START(1)
      STEP(1)=STEP(4)
      START(1)=START(4)
      CALL NELMIN
C
      STEP(1)=T1
      START(1)=T2
      ETA=XMIN(1)*XMIN(1)
      X1=XMU*R/XNU
      X2=R*(1.-XMU)/XNU
      X3=R*(ETA+XMU*XMU+R*ETA)/(XNU*XNU)
      X4=R*(1.-2.*XMU+ETA+XMU*XMU+R*ETA)/(XNU*XNU)
      WRITE(3,10)XMU,R,XNU,ETA,X1,X2,X3,X4
   10 FORMAT(1X,F8.4,2E10.4,F10.6,4F12.9)
      ICOUNT=JCOUNT
  100 CONTINUE
      END
      FUNCTION FX(X)
      COMMON /SIMP/ N,ICOUNT,REQMIN,YNEWLO,YSEC
      COMMON /DATA1/ T(10),YBAR(10),SIG(10),IFN,XMU,R,XNU
      DIMENSION X(4),Y(4),Z(10),DZ(10)
      DO 10 I=1,N
   10 Y(I)=X(I)*X(I)
C
      SUM=0.0
      IF(IFN.EQ.2)GO TO 20
      IF(Y(1).LE.1.0)GO TO 25
      FX=1.0E24
      RETURN
   25 DO 30 I=1,8
      Z(I)=Y(1)*(1.-(1.+T(I)/Y(3))**(-Y(2)))
      DZ(I)=YBAR(I)-Z(I)
      SUM=SUM+DZ(I)*DZ(I)/SIG(I)
   30 CONTINUE
      FX=SUM
      RETURN
C
   20 DO 40 I=1,8
      A=(XNU/(XNU+T(I)))**R
      B=(XNU/(XNU+2.*T(I)))**R
      C=XMU*XMU*(B-A*A)
      Z(I)=Y(1)*(1.-2.*A+B)+C
      DZ(I)=SIG(I)-Z(I)
      SUM=SUM+DZ(I)*DZ(I)
   40 CONTINUE
      FX=SUM
      RETURN
      END
```

```
 4.   PROGRAM MOD3A(TAPE1,TAPE2,TAPE3,OUTPUT)
      COMMON /SIMP/ N,ICOUNT,REQMIN,YNEWLO,YSEC
      COMMON /SIMP/ START(20),STEP(20),XMIN(20),XSEC(20)
      COMMON /DATA1/ T(8),YBAR(8),X1(2),SAMPSIG(8),LIND
      DIMENSION SS(8),X2(2),YDATA(8,5)
C
      READ(1,1)NSETS,ICOUNT,REQMIN
    1 FORMAT(4X,2I4,F16.6)
      READ(1,2)(START(I),I=1,4)
      READ(1,2)(STEP(I),I=1,4)
    2 FORMAT(4F10.6)
      TEMP1=START(1)
      TEMP2=START(2)
      N=2
C
      JCOUNT=ICOUNT
      DO 10 K=1,NSETS
C
      DO 3 I=1,8
      READ(2,4)T(I),(YDATA(I,J),J=1,5)
    4 FORMAT(6F10.6)
C
      SUMYBAR=SS(I)=0.0
      DO 71 J=1,5
      SUMYBAR=SUMYBAR+YDATA(I,J)
   71 SS(I)=SS(I)+YDATA(I,J)*YDATA(I,J)
      YBAR(I)=SUMYBAR/5.
      SS(I)=SS(I)-SUMYBAR*SUMYBAR/5.
      SAMPSIG(I)=SS(I)/4.
    3 CONTINUE
C
      LIND=1
      CALL NELMIN
C
      ICOUNT=JCOUNT
      DO 5 I=1,2
    5 X1(I)=XMIN(I)*XMIN(I)
C
      START(1)=START(3)
      START(2)=START(4)
      LIND=2
      CALL NELMIN
C
      ICOUNT=JCOUNT
      DO 6 I=1,2
    6 X2(I)=XMIN(I)*XMIN(I)
C
      WRITE(3,7)X1,X2
    7 FORMAT(3X,4F15.9)
```

```
      START(1)=TEMP1
      START(2)=TEMP2
   10 CONTINUE
      END
      FUNCTION FX(X)
      COMMON/ SIMP/ N,ICOUNT,REQMIN,YNEWLO,YSEC
      COMMON/ DATA1/ T(8),YBAR(8),X1(2),SAMPSIG(8),LIND
      DIMENSION X(10),Y(4),Z(8),DZ(8),Z1(8),Z2(8),SIG(8)
C
      DO 10 I=1,N
   10 Y(I)=X(I)*X(I)
      SUM=0.0
C
      IF(LIND.EQ.2)GO TO 50
C
      A=Y(1)+Y(2)
      DO 12 I=1,8
      Z(I)=Y(1)*(1.-EXP(-A*T(I)))/A
      DZ(I)=YBAR(I)-Z(I)
      SUM=SUM+5.*DZ(I)*DZ(I)
   12 CONTINUE
      FX=SUM
      RETURN
C
   50 A=X1(1)+X1(2)
      DO 22 I=1,8
      Z1(I)=(X1(2)+EXP(-A*T(I))*(X1(1)*A*T(I)-X1(2)))/(A*A)
      Z2(I)=X1(1)*(-1.+EXP(-A*T(I))*(1.+A*T(I)))/(A*A)
      SIG(I)=Y(1)*Z1(I)*Z1(I)+Y(2)*Z2(I)*Z2(I)
      DZ(I)=SAMPSIG(I)-SIG(I)
      SUM=SUM+DZ(I)*DZ(I)
   22 CONTINUE
      FX=SUM
      RETURN
      END
```

```
 5.   PROGRAM MOD3B(TAPE1,TAPE2,TAPE3,OUTPUT)
      COMMON /SIMP/ N,ICOUNT,REQMIN,YNEWLO,YSEC
      COMMON /SIMP/ START(20),STEP(20),XMIN(20),XSEC(20)
      COMMON /DATA1/ T(8),YBAR(8),SS(8),SAMPSIG(8),SIG(8),X1(4),IND
      DIMENSION YDATA(8,5),TEMP(4),Y(4)
C
      N=2
      READ(1,1)NSETS,ICOUNT,REQMIN
    1 FORMAT(4X,2I4,F16.6)
      READ(1,2)(START(I),I=1,4)
      READ(1,2)(STEP(I),I=1,4)
    2 FORMAT(4F10.6)
      DO 5 I=1,4
    5 TEMP(I)=START(I)
C
      JCOUNT=ICOUNT
      DO 10 K=1,NSETS
C
      DO 3 I=1,8
      READ(2,4)T(I),(YDATA(I,J),J=1,5)
    4 FORMAT(6F10.6)
C
      SUMYBAR=SS(I)=0.0
      DO 71 J=1,5
      SUMYBAR=SUMYBAR+YDATA(I,J)
   71 SS(I)=SS(I)+YDATA(I,J)*YDATA(I,J)
      YBAR(I)=SUMYBAR/5.
      SS(I)=SS(I)-SUMYBAR*SUMYBAR/5.
      SAMPSIG(I)=SS(I)/4.
    3 CONTINUE
C
      LCOUNT=0
   30 DO 6 I=1,4
    6 Y(I)=START(I)*START(I)
      A=Y(1)+Y(2)
      DO 7 J=1,8
      B=(Y(2)+EXP(-A*T(J))*(Y(1)*A*T(J)-Y(2)))/(A*A)
      C=Y(1)*(-1.+EXP(-A*T(J))*(1.+A*T(J)))/(A*A)
      SIG(J)=B*B*Y(3)+C*C*Y(4)
    7 CONTINUE
C
      IND=1
      CALL NELMIN
      ICOUNT=JCOUNT
      DO 8 I=1,2
      START(I)=START(I+2)
    8 X1(I)=XMIN(I)*XMIN(I)
C
      IND=2
      CALL NELMIN
```

```
      LCOUNT=LCOUNT+1
      IF(LCOUNT.EQ.10)GO TO 35
      ICOUNT=JCOUNT
      DO 9 I=1,2
    9 X1(I+2)=XMIN(I)*XMIN(I)
C
      DO 11 I=1,4
      IF(ABS(Y(I)-X1(I)).GT..00001)GO TO 25
   11 CONTINUE
      GO TO 35
C
   25 DO 26 I=1,4
   26 START(I)=SQRT(X1(I))
      GO TO 30
C
   35 DO 36 I=1,4
   36 START(I)=TEMP(I)
      WRITE(3,15)LCOUNT,X1
   15 FORMAT(I6,3X,4(1X,F15.9))
C
   10 CONTINUE
      END
      FUNCTION FX(X)
      COMMON /SIMP/ N,ICOUNT,REQMIN,YNEWLO,YSEC
      COMMON /DATA1/ T(8),YBAR(8),SS(8),SAMPSIG(8),SIG(8),X1(4),IND
      DIMENSION X(2),Y(2),Z(8),DZ(8)
C
      DO 10 I=1,2
   10 Y(I)=X(I)*X(I)
      SUM=0.0
C
      IF(IND.EQ.2)GO TO 50
C
      A=Y(1)+Y(2)
      DO 12 J=1,8
      Z(J)=Y(1)*(1.-EXP(-A*T(J)))/A
      DZ(J)=YBAR(J)-Z(J)
      SUM=SUM+(SS(J)+5.*DZ(J)*DZ(J))/SIG(J)
   12 CONTINUE
      FX=SUM
      RETURN
C
   50 A=X1(1)+X1(2)
      DO 14 J=1,8
      B=(X1(2)+EXP(-A*T(J))*(X1(1)*A*T(J)-X1(2)))/(A*A)
      C=X1(1)*(-1.+EXP(-A*T(J))*(1.+A*T(J)))/(A*A)
      SIG(J)=B*B*Y(1)+C*C*Y(2)
      DZ(J)=SAMPSIG(J)-SIG(J)
      SUM=SUM+DZ(J)*DZ(J)
   14 CONTINUE
      FX=SUM
      RETURN
      END
```

```
      SUBROUTINE NELMIN
      COMMON /SIMP/ N,ICOUNT,REQMIN,YNEWLO,YSEC
      COMMON /SIMP/ START(20),STEP(20),XMIN(20),XSEC(20)
      DIMENSION P(20,21),PSTAR(20),P2STAR(20),PBAR(20),Y(21)
      KCOUNT=ICOUNT
      ICOUNT=0
      RCOEFF=1.
      ECOEFF=2.
      CCOEFF=.5
      IF(REQMIN.LE.0.)ICOUNT=ICOUNT-1
      IF(N.LE.0.OR.N.GT.20)ICOUNT=ICOUNT-10
      IF(ICOUNT.LT.0)RETURN
      DABIT=2.04607E-35
      BIGNUM=1.0E38
      KONVGE=5
      DN=XN=FLOAT(N)
      NN=N+1
      DO 100 I=1,N
      XMIN(I)=XSEC(I)=0.
  100 P(I,NN)=START(I)
      YNEWLO=YSEC=0.
      Y(NN)=FX(START)
      ICOUNT=ICOUNT+1
      DO 2 J=1,N
      CHEK=START(J)
      START(J)=CHEK+STEP(J)
      DO 3 I=1,N
    3 P(I,J)=START(I)
      Y(J)=FX(START)
      ICOUNT=ICOUNT+1
    2 START(J)=CHEK
 1000 YLO=YNEWLO=Y(1)
      ILO=IHI=1
      DO 5 I=2,NN
      IF(Y(I).GE.YLO)GO TO 4
      YLO=Y(I)
      ILO=I
    4 IF(Y(I).LE.YNEWLO)GO TO 5
      YNEWLO=Y(I)
      IHI=I
    5 CONTINUE
      CHEK=(YNEWLO+DABIT)/(YLO+DABIT)-1.
      IF(ABS(CHEK).LT.REQMIN)GO TO 900
      KONVGE=KONVGE-1
      IF (KONVGE.NE.0) GO TO 2020
      KONVGE=5
      DO 2015 I=1,N
      COORD2=COORD1=P(I,1)
      DO 2010 J=2,NN
      IF (P(I,J).GE.COORD1) GO TO 2005
```

```
      COORD1=P(I,J)
 2005 IF(P(I,J).LE.COORD2) GO TO 2010
      COORD2=P(I,J)
 2010 CONTINUE
      CHEK=(COORD2+DABIT)/(COORD1+DABIT)-1.
      IF (ABS(CHEK).GT.REQMIN) GO TO 2020
 2015 CONTINUE
      GO TO 900
 2020 IF (ICOUNT.GE.KCOUNT) GO TO 900
      DO 7 I=1,N
      Z=0.
      DO 6 J=1,NN
    6 Z=Z+P(I,J)
      Z=Z-P(I,IHI)
      PBAR(I)=Z/DN
    7 PSTAR(I)=(1.+RCOEFF)*PBAR(I)-RCOEFF*P(I,IHI)
      YSTAR=FX(PSTAR)
      ICOUNT=ICOUNT+1
      IF (YSTAR.GE.YLO) GO TO 12
      IF (ICOUNT.GE.KCOUNT) GO TO 19
      DO 9 I=1,N
    9 P2STAR(I)=ECOEFF*PSTAR(I)+(1.-ECOEFF)*PBAR(I)
      Y2STAR=FX(P2STAR)
      ICOUNT=ICOUNT+1
      IF (Y2STAR.GE.YSTAR) GO TO 19
   10 DO 11 I=1,N
   11 P(I,IHI)=P2STAR(I)
      Y(IHI)=Y2STAR
      GO TO 1000
   12 L=0
      DO 13 I=1,NN
   13 IF (Y(I).GT.YSTAR) L=L+1
      IF(L.GT.1)GO TO 19
      IF(L.EQ.0)GO TO 15
      DO 14 I=1,N
   14 P(I,IHI)=PSTAR(I)
      Y(IHI)=YSTAR
   15 IF(ICOUNT.GE.KCOUNT) GO TO 900
      DO 16 I=1,N
   16 P2STAR(I)=CCOEFF*P(I,IHI)+(1.-CCOEFF)*PBAR(I)
      Y2STAR=FX(P2STAR)
      ICOUNT=ICOUNT+1
      IF(Y2STAR.LT.Y(IHI)) GO TO 10
      DO 18 J=1,NN
      DO 17 I=1,N
      P(I,J)=(P(I,J)+P(I,ILO))*0.5
   17 XMIN(I)=P(I,J)
      Y(J)=FX(XMIN)
   18 CONTINUE
      ICOUNT=ICOUNT+NN
      IF(ICOUNT.LT.KCOUNT)GO TO 1000
      GO TO 900
```

```
 19 DO 20 I=1,N
 20 P(I,IHI)=PSTAR(I)
    Y(IHI)=YSTAR
    GO TO 1000
900 DO 23 J=1,NN
    DO 22 I=1,N
 22 XMIN(I)=P(I,J)
    Y(J)=FX(XMIN)
 23 CONTINUE
    YNEWLO=BIGNUM
    DO 24 J=1,NN
    IF(Y(J).GE.YNEWLO) GO TO 24
    YNEWLO=Y(J)
    IBEST=J
 24 CONTINUE
    YSEC=Y(IBEST)=BIGNUM
    DO 25 J=1,NN
    IF (Y(J).GE.YSEC)GO TO 25
    YSEC=Y(J)
    ISEC=J
 25 CONTINUE
    DO 26 I=1,N
    XMIN(I)=P(I,IBEST)
    XSEC(I)=P(I,ISEC)
 26 CONTINUE
    RETURN
    END
```