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Nonlinear Models for Repeated Measurement Data

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David M. Giltinan

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Nonlinear Models for Repeated Measurement Data

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Preface

Analysis of repeated measurement data is a recurrent challenge to statisticians engaged in biological and biomedical applications. For example, data from clinical trials are often longitudinal in nature, with repeated measures of response taken over time. In pharmacokinetic studies, serial measurements of drug concentrations are taken from each participant. By definition, growth studies involve repeated measurements over time. In other applications, measurements on experimental units may be repeated across some other dimension, e.g. spatially rather than temporally.

Methods for *linear* modeling of repeated measurement data are well developed and well documented in the statistical literature; recent accounts include those by Crowder and Hand (1990), Lindsey (1993), and Diggle, Liang and Zeger (1994). In many biological applications, such as pharmacokinetic analysis and studies of growth and decay, however, *nonlinear* modeling is required for meaningful analysis. For this type of modeling, statistical approaches are less well understood, and discussion of appropriate methodology is scattered across a wide literature. Recent years have seen more attention to nonlinear repeated measurement data in the statistical literature; however, the economy of style imposed by many journals means that the material is sometimes presented in a manner that does not make it readily accessible to practicing statisticians. The result is that, although nonlinear modeling of repeated measurement data represents an area of some practical importance, it is one that still appears to engender a good deal of confusion among data analysts.

Our purpose in writing this monograph is to provide a clear delineation of currently available modeling approaches and inferential methods for nonlinear repeated measures. The goal is to make the material accessible to a wide audience. The book is targeted mainly to practicing biostatisticians in industry and academia, and to

graduate students in statistics or biostatistics. We have attempted to keep the exposition at an intermediate level, however, so that the majority of the material should also be accessible to pharmacokineticists and to researchers in the clinical and biological sciences.

The model framework that forms the basis for the inferential methods discussed in the book is that of the hierarchical nonlinear model for continuous response data. This may be viewed as an extension of standard nonlinear modeling techniques to accommodate multiple levels of variability (within and among individuals). Alternatively, it may be regarded as a generalization of the hierarchical linear model framework to include models that are nonlinear in parameters of interest. Hierarchical nonlinear modeling may thus be expected to inherit the computational difficulties intrinsic to both nonlinear regression and to hierarchical linear models. We have certainly found this to be true in practice; computational issues can be formidable at times, so that inference within this framework is not an enterprise to be undertaken lightly. We have included several case studies in later chapters; these represent 'real-life' data sets. We have tried to report analyses in sufficient detail to give the reader a realistic sense, not only of the potential scope and utility of the methods discussed, but also of the potential difficulties. Because computational aspects play a key role in the implementation of all the techniques described in this book, we have tried to include some discussion of available software in each of the relevant chapters. Most of the data sets considered in this book will be available on Statlib, together with code to implement model fits discussed in the text using various software packages.

Several friends and colleagues helped us while writing this book. We are grateful to Sharon Baughman, Doug Bates, Eric Chi, Art DeVault, Jim Frane, Tim Gregoire, Karen Higgins, Debbi Kotlovker, Cynthia Ladd, Nishit Modi, James Reimann, Alan Schumitzky, Anastasios Tsiatis, Jon Wakefield, and Fong Wang-Clow for comments on earlier drafts of the manuscript. Thanks go to researchers at Genentech, Inc., and elsewhere for permission to use their data in the book. Special thanks are due to Alan Hopkins and to Genentech for granting the second author a leave of absence to complete the manuscript and for encouragement throughout the writing process. Moral support was provided by Peter Compton, Carol Deasy, Ellen Gilkerson, Debbi Kotlovker, James Reimann, and Georgia Thompson. Finally, words cannot adequately express our debt of gratitude to Butch Tsiatis, without whose keen statistical insight, ongoing moral and culinary support, and unfailing

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Boston and San Francisco Marie Davidian
March 1995 David M. Giltinan

CHAPTER 1

Introduction

Data consisting of repeated measurements taken on each of a number of individuals arise commonly in biological and biomedical applications. For example, in longitudinal clinical studies, measurements are taken on each of a number of subjects over time. Similarly, participants in pharmacokinetic experiments undergo serial blood sampling following administration of a test agent. Pharmacodynamic studies may involve repeated measurement of physiological effect in the same subject in response to differing doses of a drug. By definition, studies of growth and decay involve repeated measurements taken on sample units, which could be human or animal subjects, plants, or cultures.

Modeling data of this kind usually involves characterization of the relationship between the measured response, y, and the repeated measurement factor, or covariate, x. In many applications, the proposed systematic relationship between y and x is nonlinear in unknown parameters of interest. In some cases, the relevant nonlinear model may be derived on physical or mechanistic grounds. In other contexts, a nonlinear relationship may be used simply to provide an empirical description of the data.

The presence of repeated observations on an individual requires particular care in characterizing the random variation in the data. It is important to recognize two sources of variability explicitly: random variation among measurements *within* a given individual and random variation *among* individuals. Inferential procedures accommodate these different variance components within the framework of an appropriate hierarchical statistical model. When the postulated relationship between y and x is linear in the unknown parameters, the relevant framework is that of the classical linear mixed effects model. An alternative is provided by Bayesian inferential methods for a suitable hierarchical linear model. There is an extensive literature on hierarchical linear models; Searle, Casella,

and McCulloch (1992) provide a comprehensive overview.

Methods for repeated measurement data where the relationship between y and x is *nonlinear* in the unknown parameters are less well developed. Treatment of existing techniques is scattered through a wide literature. The purpose of this monograph is to provide a unified presentation of methods and issues for nonlinear repeated measurement data. We begin by considering several examples to motivate our subsequent development.

1.1 Motivating examples

1.1.1 Pharmacokinetics of cefamandole

Figure 1.1 shows data from a pilot study to investigate the pharmacokinetics of cefamandole, a cephalosporin antibiotic (Aziz *et al.*, 1978). In this experiment, a dose of 15 mg/kg body weight of cefamandole was administered by ten-minute intravenous infusion to six healthy male volunteers. Blood samples were collected from each subject at each of 14 time points post-dose. Drug concentrations in plasma were determined for each sample by high-performance liquid chromatography (HPLC). The resulting plasma concentration-time profiles for each subject are plotted in the figure.

In characterizing the pharmacokinetics of a drug, it is common to represent the body as a system of compartments and to assume that the rates of transfer between compartments follow first-order or linear kinetics. Solution of the resulting differential equations shows that the relationship between drug concentration and time may be described by a sum of exponential terms. For instance, the biexponential equation

$$C(t) = \beta_1 \exp(-\beta_2 t) + \beta_3 \exp(-\beta_4 t), \quad \beta_1, \ldots, \beta_4 > 0, \qquad (1.1)$$

where $C(t)$ is drug plasma concentration and t is time post-dose, follows from the assumption of a two-compartment model to describe kinetics following intravenous injection (Gibaldi and Perrier, 1982).

The data in Figure 1.1 exhibit similarly shaped profiles for each subject, with possibly different parameter values for different subjects. Variation within each subject about the model in equation (1.1) arises mainly due to the HPLC assay. It is commonly recognized that intra-subject variation of this kind tends to increase with plasma concentration level (Beal and Sheiner, 1988).

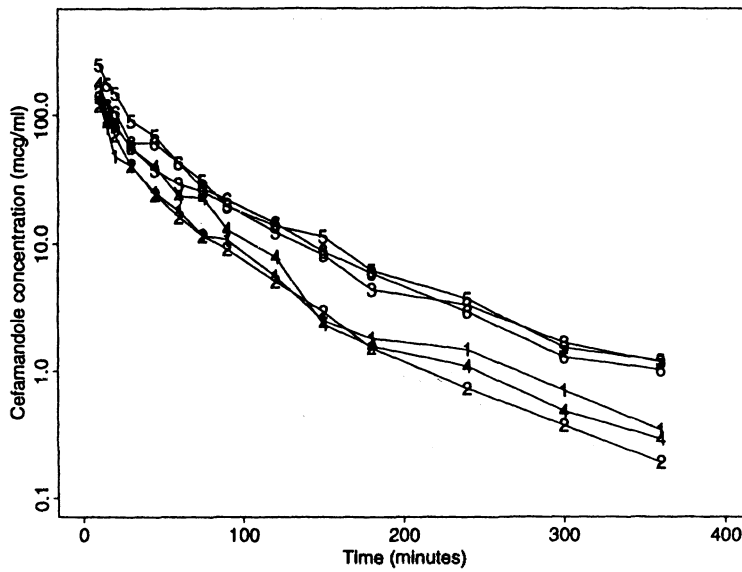

Figure 1.1. *Plasma concentration-time profiles for six subjects, cefamandole data.*

In pilot volunteer studies like this one, primary objectives are to establish an appropriate kinetic model, to obtain preliminary information on values of the model parameters, and to assess the nature of intra-subject variation. Typically, results from the analysis of a pilot study are used as a basis for subsequent investigation of kinetics in a larger, more heterogeneous patient population.

1.1.2 Population pharmacokinetics of quinidine

Data from a clinical study of the pharmacokinetics of the antiarrhythmic agent quinidine reported by Verme *et al.* (1992) consist of quinidine concentration (mg/L) measurements for 136 hospitalized patients (135 men, 1 woman) treated for either atrial fibrillation or ventricular arrhythmias with oral quinidine therapy. A total of 361 quinidine concentration measurements ranging from one to 11 observations per patient were obtained by enzyme immunoassay during the course of routine clinical treatment.

Measurements were taken within a range of 0.08 hours to 70.5

Table 1.1. *Partial data for two subjects, pharmacokinetic study of quinidine. Units for measurements are given in the text.*

time	*conc.*	*dose*	*SS*[1]	*age*	*wt.*	*creat.*[2]	*glyco.*[3]
			Subject 2				
0.0	–	166	–	58	85	> 50	82
6.0	–	166	–	58	85	> 50	82
12.0	–	166	–	58	85	> 50	82
18.0	–	166	–	58	85	> 50	82
25.0	1.2	–	–	58	85	> 50	82
height = 69, race = Latin, nonsmoker, ethanol abuse, moderate congestive heart failure							
			Subject 10				
0.0	–	201	8	73	79	< 50	254
2.2	3.9	–	8	73	79	< 50	254
288.0	–	201	8	73	79	< 50	176
290.0	5.4	–	8	73	79	< 50	176
504.0	–	201	8	73	79	< 50	150
506.0	2.8	–	8	73	79	< 50	150
816.0	–	201	8	73	79	< 50	127
816.2	–	201	8	73	79	< 50	127
817.0	3.1	–	8	73	79	< 50	127
1241.0	–	201	8	73	79	< 50	98
1249.0	–	201	8	73	79	< 50	98
7897.0	–	201	8	74	82	> 50	158
7897.8	1.6	–	8	74	82	> 50	158
height = 69, race = Caucasian, nonsmoker, no ethanol abuse or congestive heart failure							

[1] if numeric, subject has achieved steady state with given dosing interval; if blank, subject has not achieved steady state

[2] creatinine clearance

[3] α_1-acid glycoprotein concentration

hours after dose. Table 1.1 shows partial data records for two patients selected from the total of 136. Demographic and physiological covariate information was collected for each patient over an observation period ranging from 0.13 hours to 8095.0 hours. The following variables were available for the majority of patients: weight, height, and age, as well as information on race (Latin, Caucasian, Black), smoking status (yes, no), ethanol abuse (yes, no, previously), and status with respect to congestive heart failure

(severe, moderate, mild or none). Weight, age, smoking, and cardiac status were recorded periodically during the study. Creatinine clearance (ml/min), a measure of renal function, and α_1-acid glycoprotein concentration (mg/dL), the level of a circulating molecule that binds quinidine, were also measured periodically on all patients, although information on creatinine clearance was recorded in a categorical rather than continuous manner. Baseline albumin concentration (g/dL) measurements were available for some, but not all, patients. Oral quinidine may be administered in two different forms; in this study, it was given as quinidine sulfate to 53 patients, as quinidine gluconate to 57 patients, and in both forms to 26 patients. Doses were adjusted for differences in salt content between the two forms by conversion of both forms to milligrams of quinidine base.

One possible characterization of quinidine disposition is to use a one-compartment open model with first-order absorption (Verme *et al.*, 1992). Let $C(t)$ be the concentration of quinidine and let $C_a(t)$ be the apparent concentration of quinidine in the absorption depot at time t. Written in recursive form, the model is:

For the non-steady state at a dosage time $t = t_\ell$

$$\begin{aligned} C_a(t_\ell) &= C_a(t_{\ell-1})\exp\{-k_a(t_\ell - t_{\ell-1})\} + FD_\ell/V \\ C(t_\ell) &= C(t_{\ell-1})\exp\{-k_e(t_\ell - t_{\ell-1})\} + C_a(t_{\ell-1})\frac{k_a}{k_a - k_e} \\ &\quad \times \Big[\exp\{-k_e(t_\ell - t_{\ell-1})\} - \exp\{-k_a(t_\ell - t_{\ell-1})\}\Big]. \end{aligned} \tag{1.2}$$

For the steady state at a dosage time, $t = t_\ell$

$$\begin{aligned} C_a(t_\ell) &= (FD_\ell/V)(1 - \exp\{-k_a\tau_{ss}\})^{-1} \\ C(t_\ell) &= (FD_\ell/V)\frac{k_a}{k_a - k_e} \\ &\quad \times \Big[(1 - \exp\{-k_e\tau_{ss}\})^{-1} - (1 - \exp\{-k_a\tau_{ss}\})^{-1}\Big]. \end{aligned} \tag{1.3}$$

Between dosage times, $t_\ell < t < t_{\ell+1}$

$$\begin{aligned} C(t) = C(t_\ell)\exp\{-k_e(t - t_\ell)\} + C_a(t_\ell)\frac{k_a}{k_a - k_e} \\ \times\Big[\exp\{-k_e(t - t_\ell)\} - \exp\{-k_a(t - t_\ell)\}\Big]. \end{aligned} \tag{1.4}$$

In (1.2)–(1.4), t_ℓ, $\ell = 0, 1, \ldots$, are the times at which doses D_ℓ

are administered, $C_a(t_0) = FD_0/V$, $C(t_0) = 0$, F is the fraction of dose available, k_a is the absorption rate constant, $k_e = Cl/V$ is the elimination rate constant, Cl is the clearance, V is the apparent volume of distribution, $Cl, V, k_a > 0$, and τ_{ss} is the steady-state dosing interval.

The data in this example share certain characteristics with the cefamandole data: a common nonlinear model form for all subjects, values of the pharmacokinetic parameters that may differ from subject to subject, and probable heterogeneity of assay variation within subject. There are obvious differences as well. In contrast to the experimental setting, where essentially complete concentration-time profiles are collected for each subject, the quinidine data, collected in a clinical setting, are sparse, with relatively few observations per subject. This difference between data collected in a controlled experimental setting and routine clinical data is fairly typical. Clinical data may be available for a much greater number of patients, but data on any one individual patient tend to be sparse.

A second major difference between the quinidine and cefamandole data sets is the availability of demographic and physiological information that may help to explain inter-subject differences in the disposition of quinidine. This difference between experimental and clinical data is also usually the case. Early Phase I data are gathered frequently from a small number of healthy volunteers in a carefully controlled setting. Routine clinical data typically come from a much more extensive and heterogeneous patient population. This allows broader inferences to be drawn, although the task is complicated by the relative paucity of information at the individual subject level.

The major question of interest in the quinidine and similar clinical studies is identification of the demographical and physiological factors affecting drug disposition in a broad patient population. A thorough understanding of this issue may afford important clinical benefit: the dosage regimen for a given patient may be individualized based on relevant physiological and demographic information for the patient if a model is available relating drug disposition to measured patient covariates. Thus, more accurate titration of dosage may be feasible, avoiding possible suboptimal therapeutic benefit resulting from underdosing and minimizing potential toxicity associated with overdosing. Accurate dosing is of particular importance in drugs with a low therapeutic index, where the window of desirable serum concentrations is relatively narrow. For all

drugs, however, it is clearly beneficial to maximize understanding of factors affecting drug disposition.

Given a model which accurately predicts a subject's pharmacokinetic parameters as a function of physiological and demographic characteristics, there will still remain a random, or unexplained, component of variation among individuals. Quantifying this inter-subject variation in pharmacokinetic parameters is a secondary objective in population analysis of data such as those from the quinidine study. Achieving the analysis goals is complicated by several issues: (i) the sparsity of information on individual subjects; (ii) the generally nonlinear dependence of response on the relevant pharmacokinetic parameters; and (iii) inter-subject variability. Methods that allow valid inference in the face of these difficulties form the core of this book.

1.1.3 Growth analysis for soybean plants

Figure 1.2 shows data from an experiment to compare growth patterns of two genotypes of soybean: Plant Introduction #416937 (P), an experimental strain, and Forrest (F), a commercial variety.

In this study, data were collected in each of three consecutive years (1988–1990). At the beginning of the growing season in each year, 16 plots were planted with seeds, eight plots with each genotype. To assess growth, each plot was sampled eight to ten times at approximate weekly intervals. At each sampling time, six plants were randomly selected from each plot, leaves from these plants were weighed, and average leaf weight per plant was calculated for the plot. Different plots in different sites were used in different years.

Inspection of Figure 1.2 indicates that the usual logistic function

$$y = \frac{\beta_1}{1 + \beta_2 \exp(\beta_3 x)}, \quad \beta_1, \beta_2 > 0, \ \beta_3 < 0, \tag{1.5}$$

provides a reasonable representation of average leaf weight y and time x. It is evident from the figure that considerable variation in the parameters β_1, β_2 and β_3 that characterize the growth pattern exists among plots for a given genotype. For this kind of growth data, it is reasonable to expect serial correlation among measurements within the same plot. In addition, intra-plot variability may be expected to increase with the average level of response. Thus, these data have several features in common with the previous examples: (i) a nonlinear dependence of response on parameters of

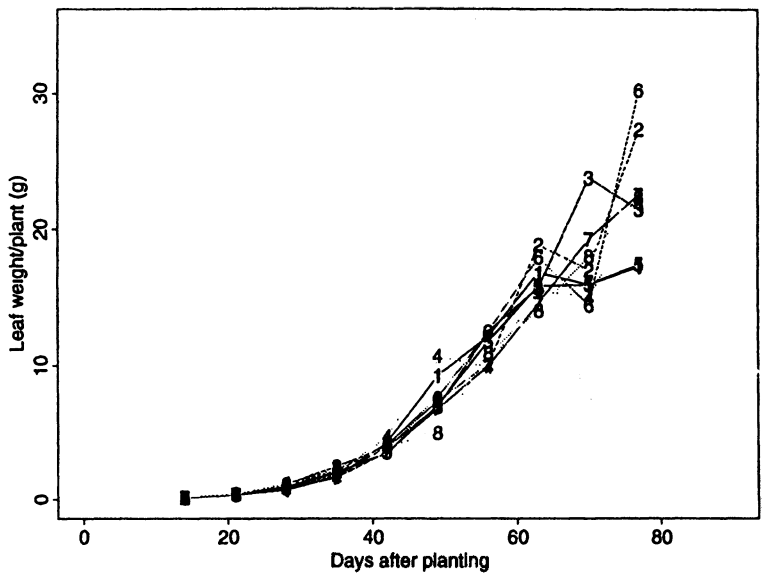

Figure 1.2. *Average leaf weight-time profiles for 8 plots planted with Plant Introduction #416937, 1988.*

interest; (ii) similarly shaped profiles for each plot; and (iii) heterogeneous within-plot variability, which may also include serial correlation. As with the cefamandole example, sufficient data are available for each plot to allow fitting of model parameters based on data from that plot only.

Variation in growth characteristics may depend on several factors. The primary objective of the experiment was comparison of growth characteristics (initial leaf weight, limiting leaf weight, and growth rate) for the two genotypes. Weather patterns differed considerably over the three years: 1988 was unusually dry, 1989 was wet,, and conditions in 1990 were normal. Comparison of growth across weather patterns was also of interest.

1.1.4 Bioassay for relaxin by RIA

Determination of the concentration of a particular protein in an unknown sample frequently relies on immunoassay or bioassay techniques. Bioassay methods are generally based on a relevant measure

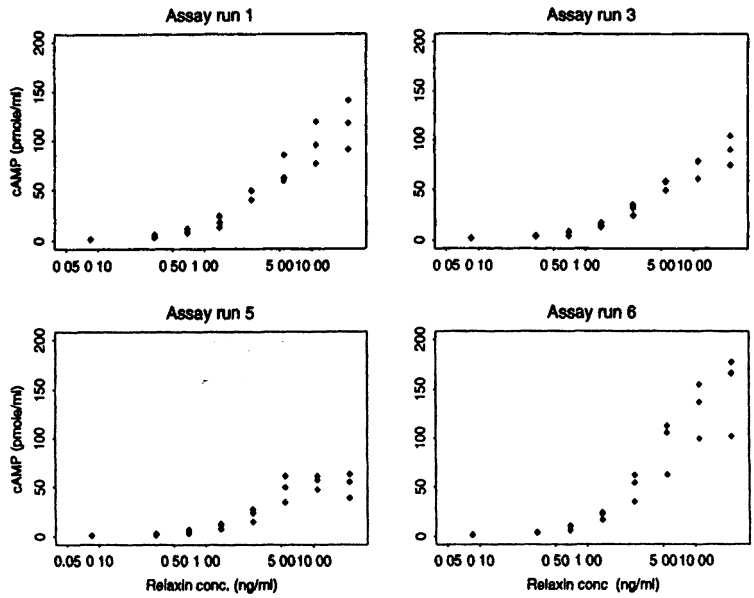

Figure 1.3. *Assay response (cAMP)-concentration data for four runs of the relaxin bioassay.*

of bioactivity of the protein in question and involve measurement of activity at several known (standard) concentrations of the protein (analyte). The resulting concentration-response curve is used to determine the protein concentration in unknown samples by inverse regression (calibration).

Figure 1.3 shows concentration-response data obtained for standard concentrations in four runs of a bioassay for the therapeutic protein relaxin (Fei *et al.*, 1990). For this assay, bioactivity of relaxin is measured by increased generation and release of intracellular adenosine-3′, 5′-cyclic monophosphate (cAMP) by normal human uterine endometrial cells in the presence of relaxin. (cAMP is an enzyme that plays a key role in regulating glycogen metabolism in the cell.) For each a total of nine runs, triplicate cAMP measurements were determined by radioimmunoassay for each of seven known relaxin concentrations. A single measurement at zero standard was also available for each run; by convention, the response at zero concentration has been plotted at two dilutions below the

lowest standard in Figure 1.3. A standard choice of dose-response model in describing this kind of assay data is the four-parameter logistic function

$$y = \beta_1 + \frac{\beta_2 - \beta_1}{1 + \exp\{\beta_4(\log x - \beta_3)\}}, \quad \beta_1, \beta_2 > 0. \qquad (1.6)$$

The response y in (1.6) is cAMP level (pmoles/ml), and x represents the known relaxin concentration (ng/ml). The parameters in (1.6) have the following interpretation: β_1 and β_2 represent response at 'infinite' and zero concentration, respectively; β_3 is the $\log EC_{50}$ value, that is, the logarithm of the concentration that gives a response midway between β_1 and β_2; and β_4 is a slope parameter governing the steepness of the concentration-response curve. It is evident from Figure 1.3 that values of the parameters may vary considerably from run to run. It is also clear from the plots that within-assay variability depends strongly on response level.

These data share several features with the previous examples: (i) nonlinear dependence of y on regression parameters; (ii) similarly shaped concentration-response profiles for each run, with possibly different parameter values from run to run; and (iii) within-run variability that increases with the level of the response.

What inferential questions are of interest for assay data of this kind? The primary focus is typically on calibration, or inverse regression; that is, estimation of the concentration of analyte in an unknown sample based on the observed response for that sample. Ancillary questions pertain to assay precision and performance. For example, with what precision are unknown samples calibrated? How may one form accurate confidence intervals about estimated concentrations? What is the 'acceptable range' of the assay, where calibrated estimates are sufficiently precise? What is the lowest limit of reliable assay measurement? Can one exploit the similarity among assay runs to improve calibration? These issues will be discussed in detail in Chapter 10. For now, we remark that the inferential challenge is the same as that in the previous examples, to address the questions of interest within a framework that correctly accommodates both the inter- and intra-assay variation.

1.2 Model specification

In the examples of the previous section, several common features of the data may be identified:

(i) Repeated response measurements taken on a number of different individuals (subjects, plots, or assay runs).

(ii) Nonlinear dependence of the response y on a set of unknown parameters, β, for each individual.

(iii) Response profiles that are similarly shaped across individuals, but that may have different values of the parameter vector β for different individuals.

(iv) A pattern of within-individual variability that is not necessarily homogeneous. Possible deviations from constant variation, or homoscedasticity, include a dependence of the variability on mean response, serial correlation among measurements within an individual, or both.

(v) Inter-individual variability between regression parameters that may be considered to be random, to be systematically related to individual-specific characteristics, or a combination of both.

To incorporate these features in an inferential setting, a useful strategy is to build a *hierarchical*, or staged, model. Full details are presented in Chapter 4; here, we sketch an outline of the approach, using a two-stage model.

The first stage specifies the mean and covariance structure for a given individual. For the ith of m individuals, assume that the $(n_i \times 1)$ response vector $\boldsymbol{y}_i$, satisfies

$$\mathrm{E}(\boldsymbol{y}_i|\boldsymbol{\beta}_i) = \boldsymbol{f}_i(\boldsymbol{\beta}_i) = \begin{bmatrix} f(\boldsymbol{x}_{i1}, \boldsymbol{\beta}_i) \\ \vdots \\ f(\boldsymbol{x}_{in_i}, \boldsymbol{\beta}_i) \end{bmatrix},$$

$$\mathrm{Cov}(\boldsymbol{y}_i|\boldsymbol{\beta}_i) = \boldsymbol{R}_i. \tag{1.7}$$

In (1.7), the function f characterizes the systematic dependence of the response on the repeated measurement conditions for the ith individual, summarized in the covariate vectors $\boldsymbol{x}_{i1}, \ldots, \boldsymbol{x}_{in_i}$. The regression function f depends in a nonlinear fashion on a regression parameter $\boldsymbol{\beta}_i$ specific to the ith individual and has the same basic functional form for all individuals; different individual response patterns are accommodated through the possibility of different $\boldsymbol{\beta}_i$ values for different individuals as well as through the individual-specific covariate vectors $\boldsymbol{x}_{ij}$. The matrix $\boldsymbol{R}_i$ is a covariance matrix summarizing the pattern of random variability associated with the data for the ith individual. Along with an assumption about the distribution of $\boldsymbol{y}_i$, the first stage model (1.7) thus describes both

systematic and random features of the data at the *intra-individual* level.

Inter-individual variation is characterized in a second stage, consisting of a *model* for variation in the regression parameters β_i. This variation can be modeled using a distributional assumption for the β_i at various levels of complexity. For instance, one might specify a parametric 'regression' model for the β_i, e.g.,

$$\beta_i = A_i\beta + b_i. \tag{1.8}$$

In (1.8), β_i is assumed to depend linearly and systematically on a vector of parameters β and on individual-specific information, such as physiological and demographic characteristics in the quinidine study or genotype and weather condition in the soybean growth study, summarized in a design matrix A_i. The 'error' b_i corresponds to the random component of inter-individual variation, which might be taken to have mean zero and covariance matrix D. One could add the further restriction that the distribution of the β_i belongs to a particular parametric family, for example, the multivariate normal distribution. In the example, this would amount to the assumption that

$$\beta_i \sim \mathcal{N}(A_i\beta, D).$$

We shall refer to the case where the variation in the inter-individual random parameters is specified by a parametric model like (1.8) and the random component is assumed to belong to a particular distributional family as the *fully parametric* model specification. At the other end of the spectrum, one might make no assumptions at all about the form or distribution of the β_i. We refer to this as the *nonparametric* model specification. An intermediate possibility is to specify a parametric model for the β_i as in (1.8), but to avoid the assumption of a particular distributional family for the random component. We refer to this kind of model specification as being *semiparametric*. Finally, the kind of two-stage model that we consider may be arrived at naturally from a Bayesian perspective. In the Bayesian view, individual-specific regression parameters β_i are considered to arise from a distribution whose mean and covariance are drawn from an appropriate prior distribution.

Each of the four kinds of model specifications for the random parameters – parametric, nonparametric, semiparametric, or Bayesian – leads to different inferential approaches. One other factor is a major determinant of the inferential technique to be applied: the relative amount of information that is available per individual. We

have seen in the context of pharmacokinetics that two quite distinct scenarios are possible: (i) sparse information on each of a large number of individuals and (ii) rich information on each of a small number of individuals. Intermediate scenarios are also a possibility, of course. Not surprisingly, the sampling design plays a role in determining what analysis methods may be employed; certain methods that can be used when sampling on an individual basis is relatively dense are not applicable to the sparse data case.

We shall use the term 'individual' throughout this book to refer to the experimental unit over which repeated measurements are available. In the repeated measurement literature, use of the term 'subject' is common in this context. Other terms include 'block' and 'stratum;' the term 'cluster' has also been proposed (Lindstrom and Bates, 1990). We avoid the latter term due to its more common usage elsewhere in the statistical literature, and use 'individual' in preference to 'subject' because of its greater generality.

1.3 Outline of this book

The goal of this book is to provide a unified presentation of modeling strategies and inferential procedures for the types of continuous repeated measurement data exemplified by the data sets described in section 1.1. This kind of data has recently received considerable attention, but discussion of inferential methods is scattered across a wide variety of sources, both in the statistical and subject-matter literature. We present methods suitable for *continuous* data of this type; techniques for binary or discrete data are not discussed. By providing a clear delineation of models and methods, we hope to make the relevant techniques more accessible both to statisticians and investigators faced with the challenge of analyzing nonlinear repeated measurement data.

For the most part, we have tried to keep exposition at an intermediate level, with emphasis throughout on applications. Familiarity with regression analysis at the level of a text such as Draper and Smith (1981) and with statistical inference at a first-year graduate level should provide adequate background for most of the material in this book. Chapters 7 and 8 are at a somewhat higher mathematical level than the remainder of the book, but can be omitted without significant loss of continuity.

Hierarchical *nonlinear* modeling provides the central framework for everything else in this book. A review of nonlinear regression methods is given in Chapter 2, which, in the context of repeated

measurement data, is relevant to data from a single individual only. Many of the techniques applicable to hierarchical nonlinear models are direct extensions of methods for individual data. Chapter 3 provides a comprehensive review of hierarchical *linear* models; a good understanding of this material is helpful in making the transition to the nonlinear case. Chapter 4 is central to the rest of the book; here, we lay out the various approaches to hierarchical nonlinear modeling in some detail. As mentioned above, these may be categorized as (i) fully parametric, (ii) semi- and nonparametric, and (iii) Bayesian. Each of these perspectives leads to different inferential strategies, discussed in Chapters 5–8. In Chapters 5 and 6, inferential procedures for the fully parametric (normal theory) hierarchical nonlinear model are presented. Chapter 5 discusses *two-stage* methods, which are applicable only in the case where sufficient data are available for each individual to allow estimation of individual-specific regression parameters based on data for that individual only. Methods presented in Chapter 6 are based on some type of *linearization* of the model and may also be applied to the 'sparse data' case. Chapter 7 is devoted to semiparametric and nonparametric inference. Bayesian methods are described in Chapter 8. Each of Chapters 9 and 10 treats a particular area of application in detail. Chapter 9 discusses population pharmacokinetic and pharmacodynamic modeling. The analysis of assay data, with particular reference to immunoassays and bioassays, is covered in Chapter 10. Case studies in the areas of crop science, forestry, and seismology, illustrating the general applicability of the methods, are presented in Chapter 11. The book concludes with a discussion of open issues and general comments (Chapter 12).

Schematically, the organization of the material may be represented as follows:

Chapter 1	Introduction
Chapters 2 and 3	Background material
Chapter 4	Model specification
Chapters 5–8	Inferential methods
Chapters 9–11	Applications and case studies
Chapter 12	Conclusion

It is inevitable that different readers will approach this book with different backgrounds and prior exposure to this material. Depending on background and the primary focus of the reader's interest, different reading strategies are possible. For instance, a researcher in pharmacokinetics, whose primary interest is in population pharmacokinetic and pharmacodynamic modeling, might

choose to include the following sections on a first reading: Chapter 1, Chapter 2, Chapter 3 (excluding sections 3.2.3, 3.3.3, 3.4, and 3.6), sections 4.1, 4.2, and 4.3.1, Chapters 5 and 6, and Chapter 9. This would provide comprehensive coverage of the fully parametric approach to population modeling. Other approaches, such as the nonparametric or Bayesian paradigms in Chapters 7 and 8, could be covered on subsequent readings.

Similarly, the above sequence might provide a useful first approach to statisticians unfamiliar with this material. For these readers, it would also be useful to include Chapters 10 and 11 to achieve a better understanding of the scope of the methods. Readers with interests in applications in biometry not directly related to pharmacokinetics or pharmacodynamics might cover the following chapters: Chapters 1–3 (excluding sections 3.2.3 and 3.3.3), Chapters 4–6, and Chapter 11, possibly including Chapter 10, depending on interest.

Computational aspects play an important role in the practical implementation of all of the techniques described in this book. For this reason, we have generally included a section in each of the relevant chapters that discusses software implementation. Some appreciation of potential computational difficulties is necessary if the methods discussed in this book are to be implemented sensibly.

CHAPTER 2

Nonlinear regression models for individual data

2.1 Introduction

Individual repeated measurement data often exhibit a relationship between response and measurement factors that is best characterized by a model nonlinear in its parameters. In some settings, an appropriate nonlinear model may be derived on the basis of theoretical considerations. In other situations, a nonlinear relationship may be employed to provide an empirical description of the data. In this chapter, as a prelude to discussion of the hierarchical nonlinear model for data from several individuals, we review techniques for nonlinear modeling and inference for data from a single individual only.

To fix ideas, consider the data in Table 2.1, taken from a study reported by Kwan *et al.* (1976) of the pharmacokinetics of indomethacin following bolus intravenous injection of the same dose in six human volunteers. For each subject, plasma concentrations of indomethacin were measured at 11 time points ranging from 15 minutes to 8 hours post-injection. In this chapter, we focus on the data for the fifth subject only for purposes of illustration; the concentration-time profile for this individual is shown in Figure 2.1. The usual approach to derivation of a suitable model for pharmacokinetic data of this kind is predicated upon the assumption of a compartment model for the human body, as described in section 1.1.1. This approach suggests that, except for random intra-individual variation, the biexponential function

$$y = \beta_1 \exp(-\beta_2 x) + \beta_3 \exp(-\beta_4 x), \quad \beta_1, \ldots, \beta_4 > 0,$$

is a reasonable representation of plasma concentration y as a function of time x.

Table 2.1. *Plasma concentrations (μg/ml) following intravenous injection of indomethacin for six human subjects.*

	Subject					
time (hrs)	1	2	3	4	5	6
0.25	1.50	2.03	2.72[1]	1.85	2.05	2.31
0.50	0.94	1.63	1.49	1.39	1.04	1.44
0.75	0.78	0.71	1.16	1.02	0.81	1.03
1.00	0.48	0.70	0.80	0.89	0.39	0.84
1.25	0.37	0.64	0.80	0.59	0.30	0.64
2.00	0.19	0.36	0.39	0.40	0.23	0.42
3.00	0.12	0.32	0.22	0.16	0.13	0.24
4.00	0.11	0.20	0.12	0.11	0.11	0.17
5.00	0.08	0.25	0.11	0.10	0.08	0.13
6.00	0.07	0.12	0.08	0.07	0.10	0.10
8.00	0.05	0.08	0.08	0.07	0.06	0.09

[1] outlier; not included in later analyses

Individual data that follow a nonlinear model often exhibit response variation that changes systematically with the level of the response. Heterogeneous variation occurs in nearly all fields of application, including those that are the focus of this book. For example, assay data typically exhibit intra-run variance that is an increasing function of the response level. Variation in pharmacokinetic data and data from growth studies is also widely acknowledged to be related systematically to mean response.

Another complication for repeated measurement data may arise from the tendency for observations on a given individual to be related. When measurement is repeated over time, serial correlation may be evident; in other contexts, correlation patterns may be due to factors such as adjacent positioning of samples on an assay microtiter plate, similarity in genetic composition of litter-mates, or spatial orientation of field samples.

Because of the frequency with which these features arise in practice, the overview of nonlinear regression modeling and inference given in this chapter includes detailed discussion of generalizations of the classical ordinary least squares approach to allow for heterogeneous response variance, often called heteroscedasticity, and for correlation. In section 2.2, the nonlinear regression model framework is introduced, and we discuss inference, including methods for

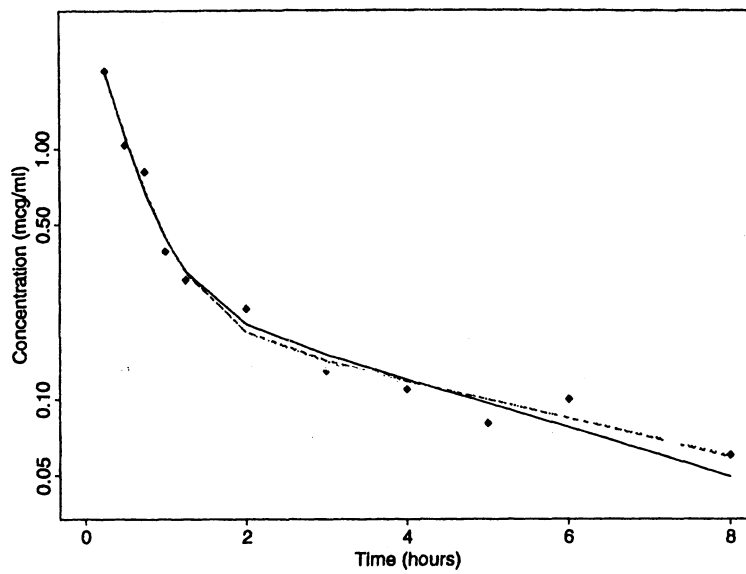

Figure 2.1. *Plasma concentration-time profile, indomethacin data, subject 5. Fits of model (2.43) are superimposed; see section 2.5. The solid line is the OLS fit ($\theta = 0$), the dotted line is the GLS fit with $\theta = 1$, and the dashed line is the GLS fit with θ estimated by PL ($\theta = 0.82$).*

handling heterogeneity of variance and correlation, in section 2.3. Notes on computational methods are given in section 2.4, and two examples are considered in section 2.5. In section 2.6, we comment on related approaches. Section 2.7 contains a brief discussion, and the chapter concludes with bibliographic notes in section 2.8.

2.2 Model specification

The classical nonlinear regression model described in this section is a direct extension of the linear case. We begin our discussion of this model in section 2.2.1 with a statement of the basic statistical model and notation. In section 2.2.2, we set out the classical assumptions and describe how they may be violated in practice. We describe a number of generalizations in section 2.2.3 to account for departures from the assumptions. For simplicity, the index i for individual is suppressed throughout this chapter.

2.2.1 Basic nonlinear regression model

The basic model for a response variable y has two main components: the nonlinear function characterizing mean response and a specification for intra-individual response variance. Let y_j denote the response taken at the jth covariate value x_j, $j = 1,\ldots,n$. The x_j are most often viewed as fixed quantities, and, in the case where they are random, the model and assumptions below are understood to be conditional on their values. In the repeated measurement context, the response vector $y = [y_1,\ldots,y_n]'$ summarizes the information available for a single individual taken at values $X = (x_1',\ldots,x_n')'$ of the repeated measurement factor(s).

The model for the jth observation is usually written as

$$y_j = f(x_j,\beta) + e_j. \tag{2.1}$$

In (2.1), the regression function f depends on β ($p\times 1$), the vector of regression parameters, in a nonlinear fashion. For the indomethacin data,

$$f(x,\beta) = \beta_1 \exp(-\beta_2 x) + \beta_3 \exp(-\beta_4 x), \tag{2.2}$$

with $\beta = [\beta_1,\ldots,\beta_4]'$. The random errors e_j reflect uncertainty in the measured response. For repeated measurement data, the vector $e = [e_1,\ldots,e_n]'$ thus summarizes the uncertainty for all observations on a given individual.

2.2.2 Classical assumptions

The classical nonlinear regression framework specifies that data arise according to (2.1) together with the following assumptions:

(i) The errors e_j have mean zero.

(ii) The errors e_j are uncorrelated.

(iii) The errors e_j have common variance σ^2, $\mathrm{Var}(e_j) = \sigma^2$, and are identically distributed for all x_j.

(iv) The errors e_j are normally distributed.

The first assumption ensures that the model f for mean response is correctly specified. This assumption is rarely called into question, as it is usually the case that the form of the covariate-response relationship is fairly well understood, especially for nonlinear relationships, where the model may result directly from theoretical considerations.

The remaining three assumptions are fairly restrictive and may not hold in some applications. Assumption (iv) is a reflection of

the emphasis of much of statistical methodology on the historical Gaussian paradigm and forms the basis for the standard approach to inference. In applications for which nonlinear models are appropriate, however, this assumption may be particularly unrealistic. For instance, for given x_j, the distribution of biological data may be heavily skewed or subject to a higher intensity of outlying observations than would be expected under normality.

If the assumption of normality (iv) does hold, along with that of uncorrelated errors (ii), then the errors are independently distributed. Thus, the assumption of independence is usually made directly, and under the full set of assumptions, the y_j are independently normally distributed with

$$\mathrm{E}(y_j) = f(x_j, \beta), \qquad \mathrm{Var}(y_j) = \sigma^2. \tag{2.3}$$

For repeated measurement data, however, even the less stringent specification of uncorrelated errors may be unrealistic; for example, measurements taken over time on a given individual may be serially related.

Assumption (iii), that of constant intra-individual response variance, is violated frequently in practice. For example, growth data often exhibit constant coefficient of variation rather than constant variance; that is, variance proportional to the square of the mean response. In this case, a more appropriate assumption than (2.3) would be

$$\mathrm{E}(y_j) = f(x_j, \beta), \qquad \mathrm{Var}(y_j) = \sigma^2\{f(x_j, \beta)\}^2, \tag{2.4}$$

where the scale parameter σ is the coefficient of variation. A general discussion of extensions of the classical model to accommodate heterogeneity of variance is given in the next section.

2.2.3 Generalizations of the classical framework

The classical nonlinear regression framework may be generalized to accommodate departures from the assumptions in a variety of ways. The following exposition is by no means exhaustive. We adopt the perspective taken in Chapters 2 and 3 of Carroll and Ruppert (1988) and Chapter 6 of Seber and Wild (1989); specifically, that of modeling systematic response variance and correlation patterns explicitly. We focus on this strategy because the hierarchical nonlinear model discussed in the remainder of the book adapts readily to account for these features. Other approaches are noted in section 2.7.