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An Overview of Models of Cell Proliferation

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Some different types of models of cell proliferation are discussed. The importance of basing the models on experimental data is emphasised, but warnings are given about some of the pitfalls in fitting models to data. The importance of investigating alternative models which might lead to similar experimental findings is stressed. The use of simulation to assess the ability of an analytical method to extract correct information from experimental data is advocated. In this instance, the modelling process takes place in advance of the data collection. Models described relate to cell proliferation in a transplantable tumour, the prostate of the castrate mouse stimulated with testosterone, and stratified squamous epithelium. Experimental techniques include measurement of tumour size, calculation of labelling and mitotic indices over time, and the fraction labelled mitoses method.

Keywords: Models, simulation, cell proliferation

INTRODUCTION

It is unusual to begin a mathematical paper with a resumé of its author's career, but this is not a typical mathematical paper. Perhaps it is not one at all, as it contains a great deal of what the reader may regard as subjectivity and personal bias, which the author prefers to think of as experience. I was initially trained as a mathematician, graduating in that subject and 'natural philosophy' as Edinburgh, at least in its Arts Faculty, still terms physics. Perhaps this nomenclature has inclined me to the belief that rational thought is at least as important as equations. I then did postgraduate work in computing science before obtaining a post as a medical statistician, the description I have applied to myself in a professional capacity in the 26 years since. Workers in these three branches of knowledge tend to regard models as rather different things, and it is perhaps not unnatural that my own views should have been coloured by the three diverse attitudes I have encountered.

The applied mathematicians I have met, whether or not working in biology, have subscribed to the view of modelling typical of the mathematical physicist. They believe in the essential simplicity of natural phenomena and in their own ability to represent them through differential equations. The solution of these equations in particular circumstances may be difficult because of the dimensionality of the problem, its boundary conditions, or its ill-conditioning; but they know that their models are, if not absolutely true, at least entirely adequate representations of the reality they wish to describe. They believe in

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inverse-square laws, solid bodies and homogeneous substances. Their belief in linearity has only recently been shaken. Sometimes they test their models by reference to experimental data.

Computer scientists are really games-players. They invent their own universes and create life in them. Their processes involve discrete entities living in geometrically simple habitats, functioning in accordance with basic laws. Once in a while their clockwork models have a passing resemblance to some aspects of reality.

Statisticians are principally distinguished by their love–hate relationship with variability. When they hate it most they designate it 'error' and ignore it. They call this fitting data. They claim, sometimes with justification, that they are motivated by data, but their models too often are little better than a complicated way of putting a curve through a set of points without providing any insight into the process whereby the data were generated. They are obsessed with estimating 'confidence limits' for the parameters of their equations.

THE FUNDAMENTAL QUESTIONS

There are two key questions we might consider before looking at examples of models of cell proliferation. The first is the more commonly asked; the second may be equally useful.

- What is a good model?
- Why, in any particular case, do we wish to build one?

In spite of the diversity of approach which I have just summarised, we might find among most model-builders a degree of agreement that a good model is a simplification, by just the right amount, of the system it seeks to describe. The skill in constructing a model is to decide which aspects of the system must be precisely modelled and which may be treated more impressionistically or even ignored. The following extract summarises a common view (Rényi, 1967).

> ... one can construct many mathematical models for the same practical situation, and one has to choose the most appropriate, that which fits the situation as closely as practical aims require (it can never fit completely). At the same time it must not be too complicated, but still must be mathematically feasible.

Nowadays the necessity for mathematical tractability is less important as the solution of the equations may usually be obtained satisfactorily by computer.

We can only come to a rational decision about the type of model we need if we are clear why we are building a model in the first place. There are basically two common reasons: for pragmatic purposes or for enlightenment. These have been called convenient and mechanistic models (Ripley, 1987). We shall later consider a third: for planning experiments.

EXAMPLE 1: TUMOUR GROWTH

Let us begin our discussion of actual models by considering one which is very clearly pragmatic. We wished to estimate the relative growth rate of a tumour — a transplantable mouse fibrosarcoma — at particular times during its growth phase (Gratton, Appleton and Alwiswasy, 1978). This was because independent estimates of cell birth rate were available, and it was proposed to compare these two measures of proliferation to try to estimate the rate at which cells were being lost from the tumour.

The data are summarised in Figure 1, which also shows the result of fitting a Gompertz equation and a logistic equation to the data. These data have been discussed previously (Appleton, 1995) but the units on the vertical axis there should read 'g', not 'mg'. It is not possible to distinguish with much certainty between the adequacy of these models by reference to the data, though the Gompertz does have a smaller residual standard deviation: 0.205 compared to 0.265, both of the fits being to the natural logarithms of the tumour weights. The Gompertz equation gives an estimate of 0.42 g/g/day as the growth rate on day 7, while the logistic equation gives 0.51 g/g/day. The precision of the estimates

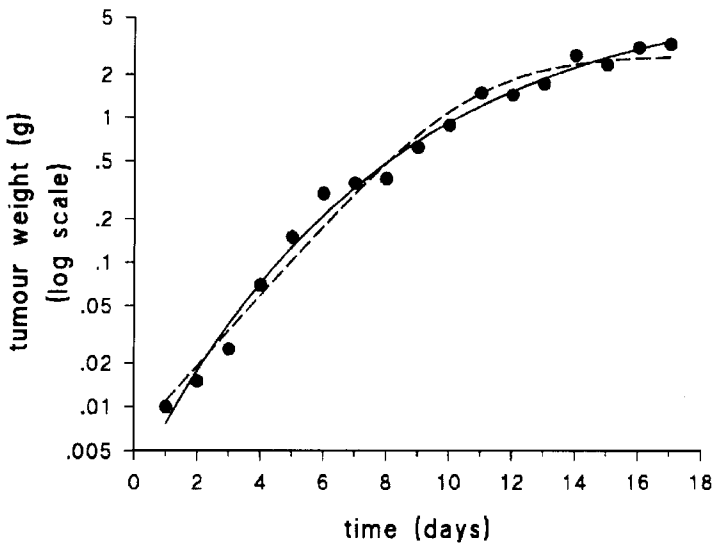

FIGURE 1 Tumour weight as a function of time for a transplantable fibrosarcoma in mice. The continuous line fits the equation $\log w = a - be^{-ct}$ and the dashed line $\log w = a - \log(1 + be^{-ct})$.

may be given in terms of 95% confidence intervals: Gompertz 0.39 to 0.44; logistic 0.45 to 0.56.

It is clear that the results are model-dependent, to an extent that the precision of the estimate of growth rate from either model is spurious. The same phenomenon is even clearer if we extrapolate the curves to try to determine the ultimate weight to which the tumours will grow: we obtain 95% confidence intervals of 4.3 g to 18.8 g (Gompertz) and 2.0 g to 3.7 g (logistic). Notice that the Gompertz equation now leads to the less precise estimate.

Which of the two models should we prefer? Since we are merely trying to estimate something and are paying no heed to mechanisms of proliferation, it is inappropriate to base our decision on biological considerations which are in any case sham. It is true that the Gompertz equation may be derived from a differential equation which treats the relative growth rate as a linear function of the logarithm of tumour size, whereas the logistic equation follows from assuming a linear relationship with the size itself. Neither of these models, however, is likely to be more than approximately correct in a few special cases. I suggest that when results are as dependent as they are here on the choice of model, neither can be regarded as satisfactory. It may be possible to improve the situation by taking a Bayesian approach to model specification (Draper, 1995), but this may be thought of as merely placing one's reliance on a different type of model.

Notice that we can only know of our difficulty if we apply more than one model to the data.

EXAMPLE 2: THE MOUSE PROSTATE

We now look at a model constructed for a quite different purpose: to try to explain the proliferative response of the prostate of the castrate mouse to injections of testosterone (Morley, Wright and Appleton, 1973). As part of this explanation we wished to estimate the durations of the phases of the cell cycle during the response, but more particularly to describe the possible mechanism behind the prostate's ability to regenerate from its small unstimulated size to that which it would normally be in the uncastrated state.

Data were available over time on the mitotic index and the DNA-labelling index for both the seminal vesicle and the coagulating gland. The labelling index data are illustrated in Figure 2. In these experiments the mice had been castrated 14 days previously. For the duration of the experiment

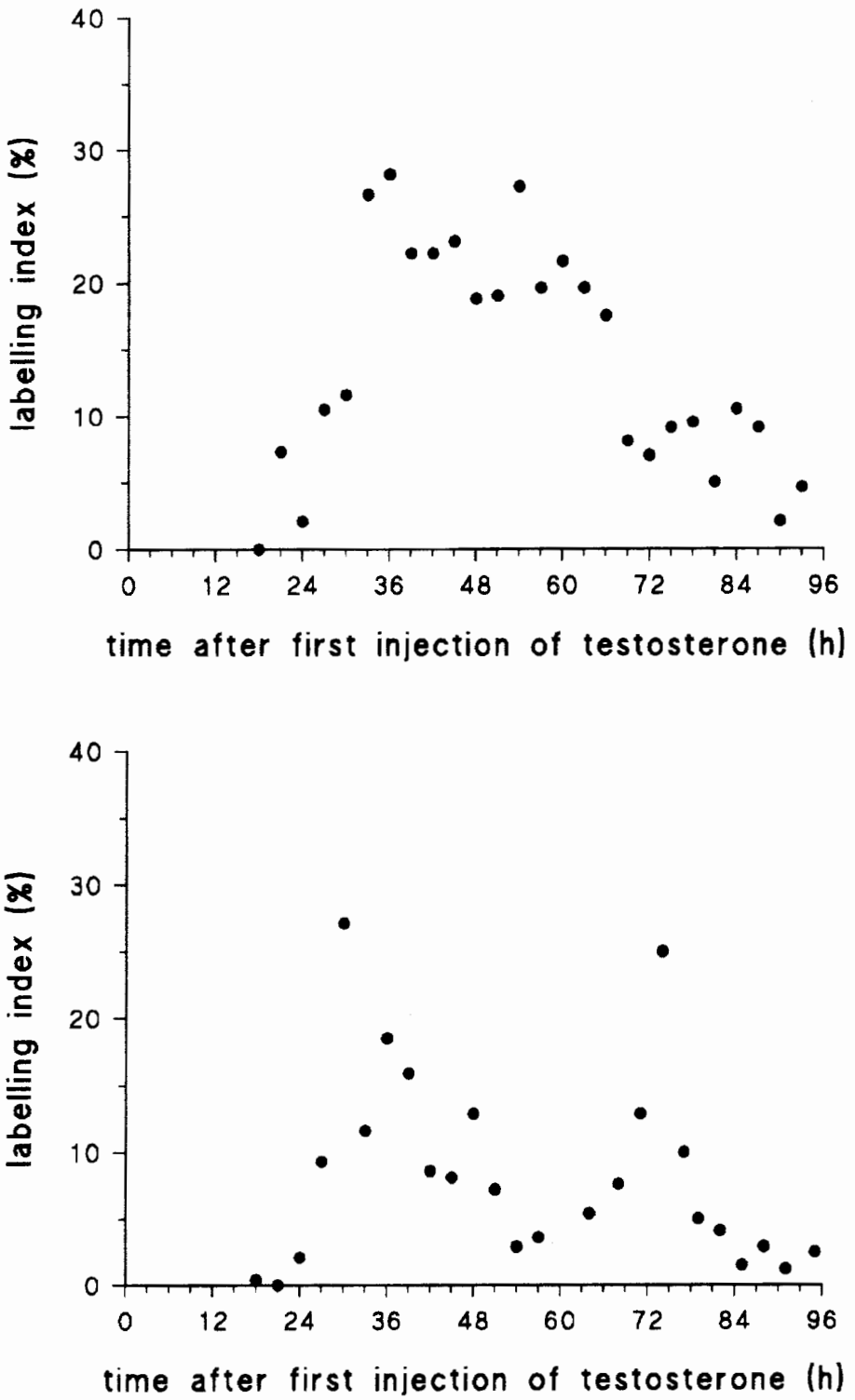

FIGURE 2 Labelling index as a function of time after testosterone injection in (top) the seminal vesicle and (bottom) the coagulating gland of the castrate mouse.

they received essentially continuous treatment of testosterone.

Had only the data on the seminal vesicle been available, it might have been tempting to suggest that the quiescent cells each underwent a single cell cycle beginning at times distributed in a somewhat skew fashion between 20 and 100 hours. Doubt is immediately cast on this hypothesis by what is seen in the coagulating gland where two distinct peaks in response are apparent. This leads to the more general model which is shown in Figure 3.

Although it is permissible to use different parameters for the seminal vesicle and coagulating gland, the labelling and mitotic data for each tissue must of course be described by the same parameters. We shall discuss the fitting of such data later.

We may translate the model from flow-diagram form into equations (Appleton, Morley and Wright, 1973). For example, if a proportion ρ of the N_0 cells initially in the G_0 state leave at times distributed as $f(t)$, we may calculate the numbers of cells in the different states as

$$G_0(t) = N_0\left\{1 - \rho\int_0^t f(\theta)\,\mathrm{d}\theta\right\},$$

$$D(t) = 2\sum_{r=1}^{k}\int_{\omega_r^{(m)}}^{t}\vec{\eta}_r(\theta)\,P(\theta-\omega_r^{(m)}) \times f(\theta-\omega_r^{(m)})\mathrm{d}\theta,$$

$$\text{and } N(t) = N_0 + \sum_{r=1}^{k}\int_{\omega_r^{(m)}}^{t}P(\theta-\omega_r^{(m)}) \times f(\theta-\omega_r^{(m)})\mathrm{d}\theta$$

$$\text{where } P(\theta) = \rho N_0 2^{r-1}\Pi_{j=0}^{r-1}\left\{1-\vec{\eta}_j(\theta+w_j^{(m)})\right\}.$$

The equations are easily solved numerically by moving the cells through the various components of the system in successive small time intervals. With a gamma distribution for the times of leaving G_0 it was possible to fit the data well with one proviso: the decycling probability could not be constant, but had to increase as time passed. By making it a decreasing function of the total number of cells in the system a satisfactory fit could be obtained. The fitted parameters for the seminal vesicle are shown in Table I; none is contradicted by our biological knowledge of comparable systems. Taking into account the variance of the times in G_0, there are ten parameters.

In the coagulating gland not all of the cells left G_0 and the cell-cycle phase durations were different.

It is possible to use the model to predict the outcome of other experiments, for example the shape over time of a continuous-labelling index and the total DNA content of the tissue (Alison *et al.*, 1974; Morley *et al.*, 1975). When it was shown that experimental data was in line with these predictions, for example that by 100 hours after stimulation the

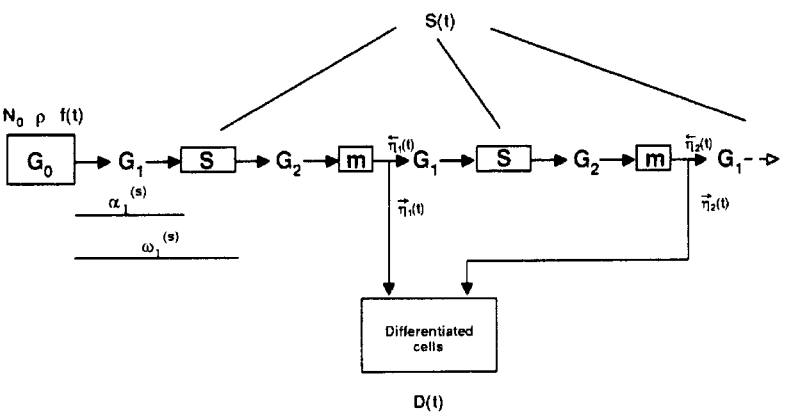

FIGURE 3 A model for the proliferative response to testosterone in the prostate of the castrate mouse. All cells begin in G_0; some (or all) move through one or more cell cycles after each of which they may recycle or differentiate. Beginnings and ends of phases in the cycle are denoted by α and ω respectively, with suitable suffices; $\overleftarrow{\eta}$ and $\vec{\eta}$ are the recycling and decycling probabilities.

TABLE I Cell cycle parameters for fitting the data of Figure 2 (top) using the model shown in Figure 3

Mean time in G_0	28 h
Duration of first G_1	22 h
Duration of G_1 otherwise	7 h
Duration of S	10 h
Duration of G_2	1.3 h
Duration of mitosis	0.7 h
Proportion of cells leaving G_0	100%
Decycling probability	$0.75 + 0.075 \times D(t)/D(0)$
Value of $N(t)$ by 108 hours	$2.41\ N(0)$

seminal vesicle contained 2.4 times as many cells as the unstimulated gland, we believed that the model was an adequate representation of what was happening and it was subsequently used to look at the proliferative response of the gland at different times after castration (Alison *et al.*, 1976).

As well as estimating the cell-cycle phases, this model was therefore capable of suggesting that the prostate's proliferative response was controlled by a negative-feedback mechanism. It was a great success. Has it any shortcomings?

Its main weakness is that it concentrates all the variability in response to the times of leaving G_0; all other times are assumed to be constant. Also, because the data were fitted subjectively, no estimates of the precision of the estimated parameters are available. The second of these points is compensated for by the knowledge which the user of the model gains through changing the parameters in various combinations and seeing what happens to the shape of the various responses. The first seems not to matter, as the variability in the relatively short cell-cycle phases is negligible compared to that at the beginning of the response. However, the shape of the single distribution postulated could be influencing our belief in the hypothesis of a negative feedback.

EXAMPLE 3: STRATIFIED SQUAMOUS EPITHELIUM

The third model we wish to describe was constructed to formalise a situation which is really rather obvious — though more so with the model to guide one. We were not in this instance concerned with fitting data; instead we wished to show that a particular assumption about a system was crucial, at a qualitative level, to what would be observed.

The system in question is stratified squamous epithelium such as exists in some skin. (Appleton, Wright and Dyson, 1977; Duffill *et al.*, 1977). Proliferation takes place at least in a basal layer, and possibly in higher layers. There is migration upwards through the layers. For the moment we may model the situation by considering there to be k layers in which all cells proliferate. We shall assume that the cell-cycle time, T, is constant for all cells in all layers. The fact that these assumptions are probably quite untrue does not for our present purposes matter; the purpose of this model is to make a theoretical point, not explain data.

We shall work in terms of the age distribution of cells and suppose that for layer k it is $y_k(x)$, that is the probability that a cell in that layer is between age x and $x + \delta x$ $(0 < x < T)$ is $y_k(x)\delta x$. Since the only easily distinguishable proliferating cells are mitoses we shall wish to have an expression for the mitotic index M_k: if the duration of mitosis is τ this is approximately $\tau y_k(T)$.

Suppose that at each mitosis in layer k one daughter cell remains in that layer. The other also remains with probability p_k and displaces another cell at random into layer $k + 1$; otherwise it moves to layer $k + 1$ itself displacing a cell from that layer at random into layer $k+2$. The appearance of a cell in any layer causes a random cell in that layer to migrate into the layer above.

The proportion of cells in layer k of age $x + \delta x$ is the same as the proportion of age x, less those cells of that age which have been pushed out by the horizontal mitoses in that layer during time δx, less those displaced by any mitosis in any of the lower $k - 1$ layers, plus those pushed up from the layer below by horizontal mitoses in that layer, plus those arriving because of any mitoses in the $k - 2$ layers below that. We may deduce that

$$y_k'(x) = -y_k(x)\left\{y_k(T)p_k + \sum_{i=1}^{k-1} y_i(T)\right\} + y_{k-1}(x)\left\{y_{k-1}(T)p_{k-1} + \sum_{i=1}^{k-2} y_i(T)\right\}$$

and if we write

$$C_k = y_k(T)p_k + \sum_{i=1}^{k-1} y_i(T)$$

we have

$$y_k'(x) + C_k y_k(x) = C_{k-1} y_{k-1}(x)$$

which has solution

$$y_k(x) = A_k \, e^{-C_k x} + C_{k-1} \, e^{-C_k x} \int_0^x y_{k-1}(x) \, e^{C_k x} dx \tag{1}$$

where A_k is a constant chosen to satisfy the boundary conditions $y_k(0) = (1 + p_k)y_k(T) + (1 + p_{k-1})y_{k-1}(T)$.

Particular Cases

For the basal (or only) layer the solution of equation (1), ignoring the subscript, is

$$y(x) = \frac{1+p}{p} \frac{\log(1+p)}{T} \exp\left\{-\frac{\log(1+p)}{T}x\right\}.$$

As $p \to 0$ $y(x) \to 1/T$. The mitotic index is

$$\frac{\tau}{T} \frac{\log(1+p)}{p}$$

which tends to τ/T as p tends to zero. Therefore, if all daughter cells stay in the basal layer, the mitotic index is only 69% of what it would be if one daughter cell always left the layer.

A more interesting question is how the mitotic index in the second layer is affected.

If $p_k = 1$ for both layers, then

$$M_1 = M_2 = \frac{\tau}{T} \log 2,$$

but if $p_k = 0$ for both layers then

$$y_2(x) = \frac{e^{1-x/T}}{(e-1)T}$$

whence

$$M_2 = \frac{1}{e-1} \frac{\tau}{T}$$

so that M_2 is only 58% of M_1.

The mode of migration is therefore crucial to what we see. If daughter cells remain in the layer which their parent cell occupied and all cells in the two bottom layers are proliferative, then the mitotic indices of the two layers will be the same, whereas if one daughter cell moves upwards, the mitotic index in the second layer will be substantially less than that in the basal layer. Such a finding, therefore, need not be evidence that there are fewer proliferative cells in the suprabasal layer than in the basal layer.

We said that this was a fairly obvious result, at least qualitatively. It is: if the second layer is being supplied with young cells its mitotic index cannot be as high as if cells are arriving at random with respect to age.

Notice that this model gives warning that an experiment based solely on counting mitotic cells is liable to give uninterpretable results about the proliferative fraction of cells in the suprabasal layer. Furthermore it suggests an experiment: it is necessary to observe the orientation of mitotic cells to see how frequent is each mode of migration.

EXAMPLE 4: THE USE OF SIMULATION

Before carrying out an experiment it can be helpful to use Monte-Carlo simulation to model a possible mechanism for the system under investigation with a view to finding out whether the analysis to be used on the data — another model which perhaps is known only to be wholly justified under certain restrictions — will be satisfactory (Appleton, 1985). This is a standard statistical methodology which is applied when we wish to know whether a proposed analytical technique is robust to departures from the assumptions under which it was derived. The advantage of this technique is that we know precisely the details of the system we are studying. In the context of cell proliferation modelling, CELLSIM may provide a suitable language for generating the simulated data (Donaghey, 1975).

Figure 4 shows a program which simulates a standard cell proliferation experiment in which the fraction labelled of all mitoses (FLM) is measured at

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CELL TYPES UNLB, LBLD;
STATES (1, 2) G1, S, G2, M;
FLOW (1, 2) G1-S (ALL), S-G2 (ALL), G2-M (ALL), M-G1 (ALL);
TIME IN STATES (1, 2) G1: TRAP (0, 2, 8, 24), S : TRAP (0, 2, 8, 24),
                      G2: TRAP (0, 1, 4, 12), M : 2.0;
NUMBER OF GROUPS (1, 2) = 200;
MAXIMUM NUMBER OF GROUPS = 800;
PROLIFERATION (1, 2) M : 2;
INOCULUM (1) 1000;
KILL (1) BETWEEN 100 AND 106 S : 0.8;
MOVE, AT TIME = 112, S(1) TO S(2);
REPORTS 112 HOURS;
CHANGE REPORTS, AT TIME = 1, 1 HOUR;
GRAPH 100*M(2) / (M (1) + M (2));
RANDOM NUMBER SEED = 328511;
SIMULATE 148 HOURS;
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FIGURE 4 A CELLSIM program which models an FLM experiment conducted after a cytotoxic insult.

various times after an administration of tritiated thymidine has labelled all cells in the DNA-synthetic phase at time zero (Appleton, 1983). The system modelled has suffered an insult 12 hours previously resulting in a loss of 80% of cells in S-phase at that time. As a result the cells are not cycling asynchronously whereas the analytical method assumes that they are (Gilbert, 1972). Figure 5 shows the data generated and fitted. Although the fit is not good, in the variable world of biology it might be accepted as adequate and the discrepancies near the end of the experimental period explained away in terms of artefacts. Table II however clearly shows that the method is sensitive to the assumption of asynchronous proliferation being violated. This approach should help to prevent erroneous conclusions being drawn from such an experiment; indeed, unless a more appropriate method of analysis can be found, it may save a pointless experiment from being performed.

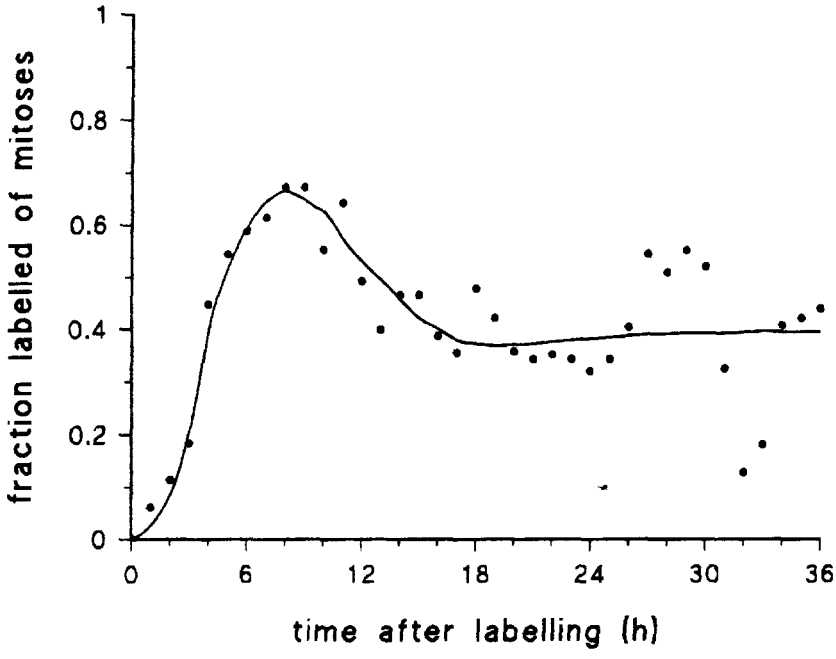

FIGURE 5 The data generated by the program in Figure 4, with a curve fitted by an analytical method assuming asynchronous proliferation.

TABLE II Mean phase durations estimated from data of Figure 5, compared with actual values used in defining the simulated system

Parameter	Actual value	Estimated value
Duration of $G_1 + \frac{1}{2}$ M (h)	10.2	3.1
Duration of S (h)	9.2	5.2
Duration of $G_2 + \frac{1}{2}$ M (h)	5.6	5.5
Cell cycle time (h)	25.0	13.8
CV of cell cycle time (%)	32	75

Fitting Curves to Data

It is worth pointing out that the method of least squares (or maximum likelihood) is often quite inappropriate for fitting cell-proliferation models. When we are studying a response over time to a stimulus it is often true that as well as a variation in the magnitude of response there is variation in its timing. Let us consider a very abstract model, which nevertheless can be thought of in the context of cells proliferating.

A process exists at a constant level η until time τ when it begins to increase at a rate β per unit time. For any individual η, τ and β are observations from a trivariate Normal distribution with variances σ_η^2, σ_τ^2, and σ_β^2 and with correlation of magnitude ρ between any pair of variables (the higher the value of η, the higher is β likely to be and the smaller τ). In addition the observation of the value at any time has Normally distributed error with variance σ^2. This is by no means a realistic model of a proliferative index in a tissue stimulated out of a steady state, but it is possible to think of it in these terms, with the correlation structure representing a tendency of individuals who start high to respond earlier and to a greater degree than individuals who start low.

Figure 6 shows the theoretical response for the following values of the parameters: $\eta = 5$, $\tau = 2$, $\beta = 3$, $\sigma_\eta = 1$, $\sigma_\tau = 2$, $\sigma_\beta = 0.1$, $\rho = 0.8$ and $\sigma = 0.5$. It also shows the mean and the deciles, calculated at 12-minute intervals from the start of the experiment to 4 hours, of 1000 simulations. It is unlikely that a single experiment would be capable of detecting the nonlinearity, the skewness of the distribution of residuals or its increasing variance.

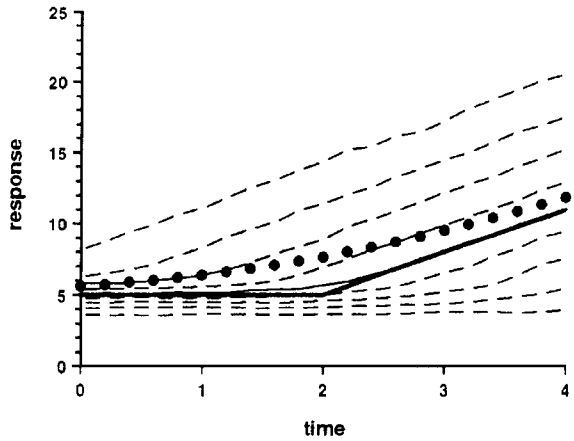

FIGURE 6 A simple model of a system changing with time as described in the text. The heavy line is the expected response, the points the mean observed at each time point. The deciles of the distribution of responses at each time point are shown as dashed lines, except that the median is shown as a continuous line.

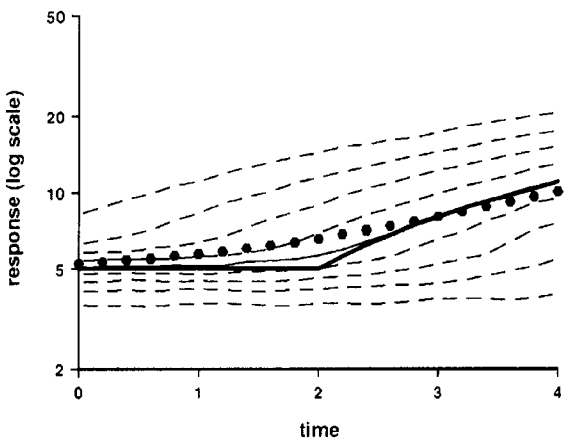

FIGURE 7 The same data as in Figure 6 except that the scale of the response axis is logarithmic, and geometric means rather than means are plotted for each time point.

A least-squares fit might well be used on a sample of such data.

The data-analyst would therefore be unlikely to detect that a change occurred around $\tau = 2$, and would probably considerably underestimate the rate of increase of the response. This sort of problem is well known to statisticians who study the growth of children, and is similar in nature to 'attenuation' in regression analysis (Sprent, 1969). The method of

exemplifying it here is of course through the use of simulation as described in Example 4.

Paradoxically perhaps, the situation could be considered worse if there were sufficient data to suggest that the fitted equation should not be a straight line and that the variance of the observations increased with time. Then the analyst might take logarithms of the response variable and be happy with the resultant relatively constant variance and straight-line fit. Figure 7 shows that this would be a not unreasonable attitude. Unfortunately the description of the underlying process, in suggesting an exponential increase in the response variable, would be quite wrong.

CONCLUDING OBSERVATIONS

We have tried to show how mathematical and statistical attitudes may be applied to the study of cell proliferation, and have strongly advocated the use of simulation. Because we regard the experimental method as the key to knowledge in biology we have pre-eminently had regard to data, but have indicated that the difficulty of fitting it with a mathematical model is sometimes underestimated. We have also suggested that the best time for the modelling process is not always in the analysis of experimental data but may be after the definition of the study protocol but before data is collected.

We believe that a great deal of thought must be given to reconciling data and models, and that this almost always means the construction of alternative models. We have not expanded on the balance between parsimony and closeness to reality, though this is always an aspect of modelling which is important. The modelling of variability as well as the expected response of a system should be carefully considered: not all models which are called 'stochastic' (as our Example 3 might be described) handle variability.

We have shown that a good fit to data does not mean that a model is correct, or even useful from a pragmatic point of view. A parameter may be estimated precisely (i.e. with a small standard error) and still be quite wrong.

Modelling is an extremely powerful tool. That implies that it is dangerous if not treated with care. A balanced view of its potential benefits and pitfalls is essential (Renshaw, 1991).

References

Alison, M. R., Appleton, D. R., Morley, A. R. and Wright, N. A. (1974) Cell population growth in the castrate mouse prostate complex: experimental verification of computer simulation. *Cell Tissue Kinetics*, **7**, 425-431.

Alison, M. R., Wright, N. A., Morley, A. R. and Appleton, D. R. (1976) Cell proliferation in the prostate complex of the castrate mouse. *J. Microscopy*, **106**, 221-237.

Appleton, D. R. (1983) The fraction labelled mitoses method: general considerations, in N. A. Wright and R. S. Camplejohn (eds) *Psoriasis: Cell Proliferation*, (Churchill Livingston, Edinburgh), pp. 24-33.

Appleton, D. R. (1985) Aspects of statistics in studies of cell proliferation. IV. Simulation. *Cell Tissue Kinetics*, **18**, 133-135.

Appleton, D. R. (1995) What do we mean by a statistical model? *Stat. Med.*, **14**, 185-197.

Appleton, D. R., Morley, A. R. and Wright, N. A. (1973) Cell proliferation in the castrate mouse seminal vesicle in response to testosterone propionate. II. Theoretical considerations. *Cell Tissue Kinetics*, **6**, 247-258.

Appleton, D. R., Wright, N. A. and Dyson, P. (1977) The age distribution of cells in stratified squamous epithelium. *J. Theoret. Biol.*, **65**, 769-779.

Donaghey, C. E. (1975) *CELLSIM II Users Manual*, University of Houston, Houston.

Draper, D. (1995) Assessment and propagation of model uncertainty. *J. Royal Stat. Soc. B*, **57**, 45-97.

Duffill, M. B., Appleton, D. R., Dyson, P., Shuster, S. and Wright, N. A. (1977) The measurement of the cell cycle time in squamous epithelium using the metaphase arrest technique with vincristine. *Br. J. Dermatol.*, **96**, 493-502.

Gilbert, C. W. (1972) The labelled mitosis curve and the estimation of the parameters of the cell cycle. *Cell Tissue Kinetics*, **5**, 63-63.

Gratton, R. J., Appleton, D. R. and Alwiswasy, M. K. (1978) The measurement of tumour growth rates, in A-J. Valleron and P. D. M. Macdonald (eds) *Biomathematics and Cell Kinetics*, (Elsevier/North-Holland, Amsterdam), pp. 325-332.

Morley, A. R., Appleton, D. R., Wright, N. A. and Alison, M. R. (1975) The proliferative response of the coagulating gland of the castrated mouse under continuous androgen stimulation: an experimental and computer simulation model. *J. Endocrinol.*, **65**, 353-361.

Morley, A. R., Wright, N. A. and Appleton, D. R. (1973) Cell proliferation in the castrate mouse seminal vesicle in response to testosterone propionate. I. Experimental observations. *Cell Tissue Kinetics*, **6**, 239-246.

Renshaw, E. (1991) *Modelling Biological Populations in Space and Time*, (Cambridge University Press, Cambridge).

Rényi, A. (1967) *Dialogues on Mathematics*, (Holden-Day, San Francisco).

Ripley, B. D. (1987) *Stochastic Simulation*, (Wiley, New York).

Sprent, P. (1969) *Models in Regression and Related Topics*, (Methuen, London).