THE ROTATING FOURIER'S RING

AN ALTERNATIVE MODEL FOR CELL KINETIC STUDIES

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ABSTRACT A simple method of simulating cell kinetics experiments, suitable for use on small generally available computers and capable of any degree of accuracy without increase of store required, is described. No assumption is necessary as to cycle-time distribution; the computer program, after being adjusted to generate percentage labeled mitosis curves, will then, on being supplied with different data, generate the cycle-time distribution curve.

INTRODUCTION

In theoretical studies of cell kinetics systems there are two main approaches. One may either simulate directly the behavior of a cohort by a compartment-to-compartment transfer process, which starts with a cohort distributed in the prescribed manner about the cycle and imagines the cells to be transferred individually from one state to the next as the supposed conditions of the system determine; or one can consider that, by reason of the spread of cycle times in the cohort, the original distribution of the cohort will be lost as the faster cycling cells "diffuse" forward away from the cohort and the slower cycling ones diffuse backward.

Of these two methods, the former has been most widely used (see, for example, Barrett, 1966; Bronk et al., 1968; and Bronk, 1969) the latter having received but little attention (Merkle et al., 1965; Stuart, 1966).

Merkle et al. (1965) and Stuart (1966) showed that the behavior of a cohort of cells could be described by a differential equation formally identical with the diffusion equation, with translation, of classical physics. In applying the equation to various problems in cell kinetics, however, these authors converted the differential equation into a difference equation, thereby effectively reverting to the compartment-to-compartment transfer procedure, overlooking the fact that, provided that the original shape of the cohort can be written as a Fourier series, which is usually so, and certainly in the case of uniform and logarithmic phase distributions, explicit analytical solutions of the differential equations exist. In this paper, I show how these explicit solutions may be used to simulate various types of cell kinetic data.

The method uses the solutions of the differential equation relevant to the conduction of heat in a closed loop of wire; the problem is known, in physics, as Fourier's ring.

FOURIER'S RING

The theory of Fourier's ring considers a circular closed loop of a thermal conducting material, usually described as a wire. (By suitably specifying the conditions, the loop can be bent into any shape; it will, however, be convenient and less confusing, to restrict attention to the circular loop.) (Carslaw and Jaeger, 1947)

A distribution of temperature is supposed to have been established within the material of the loop at a given instant, for example $t = 0$, and it is required to calculate the distribution of temperature at all subsequent times. Since the theory is standard physical theory, it will not be discussed in detail here. The reader interested in more general or more detailed solutions should consult the reference above.

A case of particular interest is that in which there is initially a uniform temperature T between two points of the loop and zero temperature elsewhere on it. (Figs. 1 and 2). If, as the heat is conducted around the loop, the whole loop is allowed to rotate, the similarity between Fourier's ring and cell kinetics systems is immediately obvious (Fig. 3). In order to simulate the conditions of, say, a percentage labeled mitosis experiment, it is necessary only to place a "window," corresponding to mitosis, at some suitable position in the coordinate system and observe the integral of the temperature distribution curve between the limits of the window at a succession of suitable times (Fig. 4).

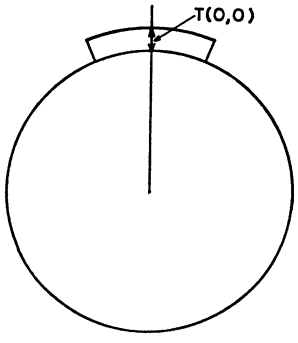

FIGURE 1

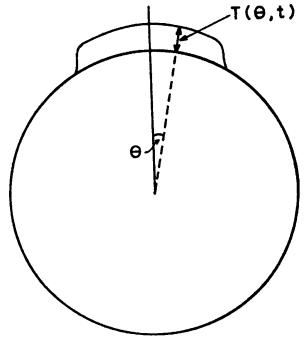

FIGURE 2

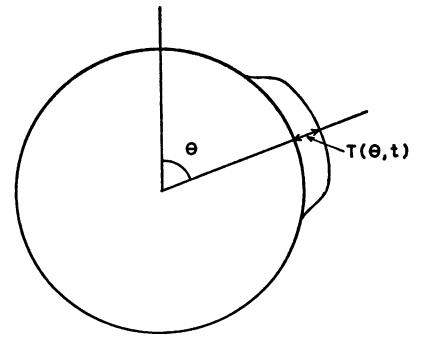

FIGURE 3

FIGURE 1 Representation of the initial state of a Fourier's ring with uniform temperature between two points and zero temperature elsewhere.

FIGURE 2 Representation of Fourier's ring showing the state of the system some time after the initial conditions have been established.

FIGURE 3 Representation of a Fourier's ring as in Fig. 2. but which has been rotated as the conduction of heat was taking place.

If the initial distribution of temperature is given by the Fourier series:

$$f(\theta) = \sum_{n=0}^{\infty} a_n \cos n\theta + \sum_{n=1}^{\infty} b_n \sin n\theta, \quad (1)$$

then the distribution at any subsequent time is given by

$$f(\theta, t) = \sum_{n=0}^{\infty} a_n \exp(-kn^2\pi^2 t/l^2) \cos n\theta + \sum_{n=1}^{\infty} b_n \exp(-kn^2\pi^2 t/l^2) \sin n\theta, \quad (2)$$

where k is the conduction constant and l is half the length of the loop.

This is readily converted to the notation of cell kinetics by remembering that half the length of the loop corresponds to half the cycle time and that the phase angle θ represents the position of a cell in the cycle as measured from some arbitrary position. It is found convenient to use the phase of the midpoint of the cohort as the origin.

The discussion has not so far taken into account the cycling of the system. The differential equation used by Stuart (1966) includes a term, the translation term, which takes this into consideration. It is, however, mathematically much simpler to solve the equation for the static case and impose the rotation on the results afterwards. This is the procedure followed here.

The system described here is an extremely simple one. It considers cohorts initially distributed uniformly with respect to phase, the cells having a cycle-time distribution with standard deviation σ.

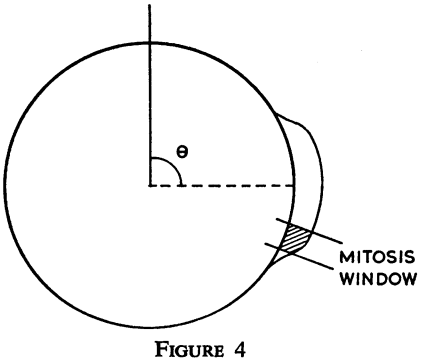

FIGURE 4

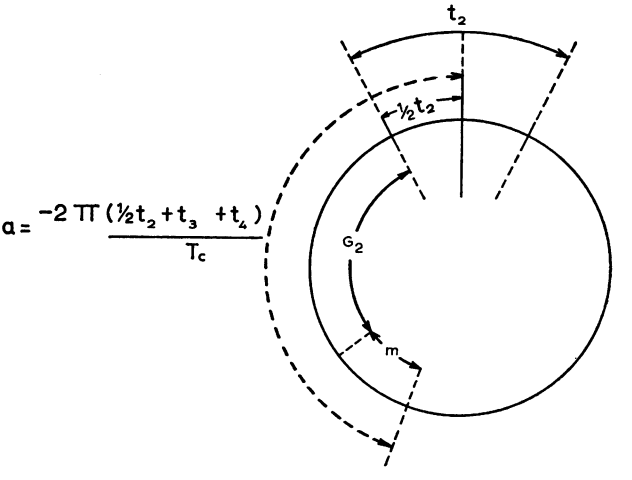

FIGURE 5

FIGURE 4 Scheme showing how, by the insertion of a window at some point in the cycle, the rotating Fourier's ring can be converted into a system analogous to a percentage-labeled mitosis system.

It deals with percentage labeled mitoses (which it is able to do without considering doubling at mitosis because the labeling index is only affected by the number of new individuals produced per cycle through the change in form of the waiting time in S [Bronk, 1969]), and synchronized cohorts, from which cycle-time distributions can be obtained.

Simple though it is, the system is remarkable for its success in reproducing experimental percentage labeled mitosis (PLM) curves and the cycle-time distributions derived therefrom. Aspects of the subject such as logarithmic phase distributions, doubling, cell loss, and individual and independent variances for the separate states, which have been dealt with by other techniques, can be dealt with by the Fourier ring treatment and versions of the computer program which do this are in preparation. As an illustration of the technique I consider first the reproduction of PLM curves.

PLM Curves

Using t_1, t_2, t_3, t_4, and T_c to represent the mean duration of the first growth stage, the synthesis stage, the second growth stage, mitosis, and the whole cycle, respectively (the European usage is $t(g_1)$, $t(s)$, $t(g_2)$, $t(m)$, and T_c), we evaluate the expression

$$\int_{a(t)}^{a(t)+t_4} f(\theta, t), \mathrm{d}\theta. \tag{3}$$

The quantity $a(t)$ represents the position of one boundary of the mitosis window at time t. In a PLM experiment, a will have the initial value given by

$$a(0) = -\frac{2\pi(\frac{1}{2} t_2 + t_3 + t_4)}{T_c}, \tag{4}$$

as illustrated in Fig. 5. If, as time passes and the distribution of the cohort decays, the value of a is altered to represent the cycling of the cohort in such a way that the position of the mitosis window relative to the cohort is always that which it would occupy at the time corresponding to the then state of the distribution, then a series of successive values of the integral would represent a PLM curve.

If, on the other hand, the initial cohort is very narrow (i.e., effectively synchronized) and is just leaving mitosis, then, provided, as is usually the case, that by the time it reaches the next mitosis the width of the cohort is much greater than the width of the mitosis window, the successive values of the integral give a representation of the cycle-time distribution.

A computer program which evaluates the integral at a suitable series of times has been written (copies of the program and instructions for its use are available on request). Some of the results obtained are shown and discussed briefly in Figs. 6–16.

A Comparison of the Fourier Ring and Other Treatments

Although the main justification of the Fourier ring treatment is its ability to reproduce, within the limits of experimental error the results of actual experiments, it is to be expected that a relation will be found between the method and other treatments which also yield satisfactory simulation of experimental results. This is indeed the case.

Bronk (1969) derives the approximate result:

$$M^*(t)/[M(t) + M^*(t)] = M^*/(M + M^*)\,|_\infty + ae^{-\lambda t}\cos(\omega_0 t - \delta), \qquad (5)$$

for the shape of a PLM curve. The right-hand side represents a constant term plus an exponentially decaying cosine term. (For a detailed explanation of the notation, the reader is referred to the original work.) For t large but not too large, the Fourier ring expression for a PLM curve also reduces to a constant term plus an exponentially decaying sinusoidal term, after a little manipulation. If the two expressions are to be equivalent, as Fig. 6 strongly suggests that they are, then the exponents in the exponential decay terms must be the same. Taking the value of λ from Bronk's paper (his equation 23), we find

$$\frac{2\pi^2\sigma^2}{T_c^3} = \frac{4k\pi^2}{T_c^2}, \qquad (6)$$

whence

$$k = \frac{\sigma^2}{2T_c}. \qquad (7)$$

This relationship is found to hold extremely well. In most cases where it has been possible to use it, the value of k so calculated has given a good fit to the experimental curves without further adjustment. (An exception will be discussed later.) Indeed, using the figures taken from Bronk et al. (1968), the method reproduced immediately the points shown in Fig. 6. Apart from the slight difference at the first peak, which is eliminated by using the Fourier series for a logarithmic distribution, the agreement is exact throughout.

Another relationship which, as its meaning is not as yet clear, will be described only briefly, appears in Hirsch and Engleberg (1966). If the exponent in their equation 28 is arbitrarily equated to zero, the expression

$$\frac{1}{s} = \frac{\sigma^2}{2T_c}, \qquad (8)$$

appears. s has the dimensions of $1/k$.

Finally, although the explicit assumption of a Fourier series expansion of the initial distribution of the cohort and the corresponding series expansion of its

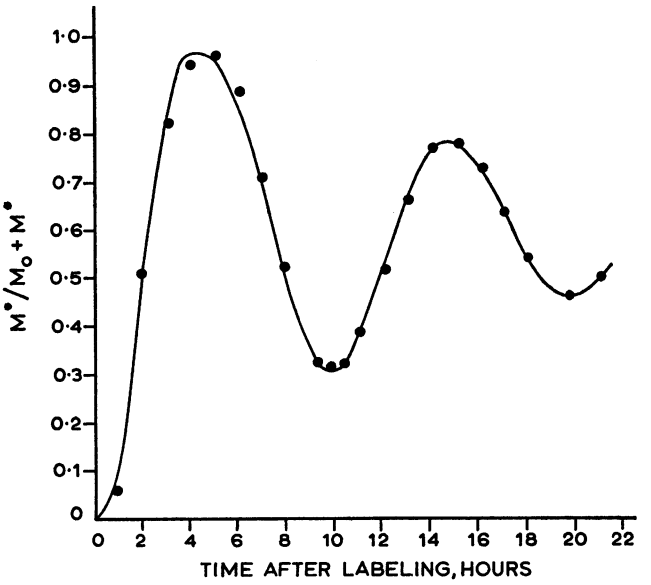

FIGURE 6 The curve is the continuous smooth curve in Fig. 11 of Bronk et al. (1968). The dots are the points computed by the program described here, using the values of the parameters used by these authors and calculating the value of k from the value of σ quoted by them. The slight disagreement, which is real, on the first peak, disappears if the Fourier series for a logarithmic distribution is used instead of that for a uniform distribution.

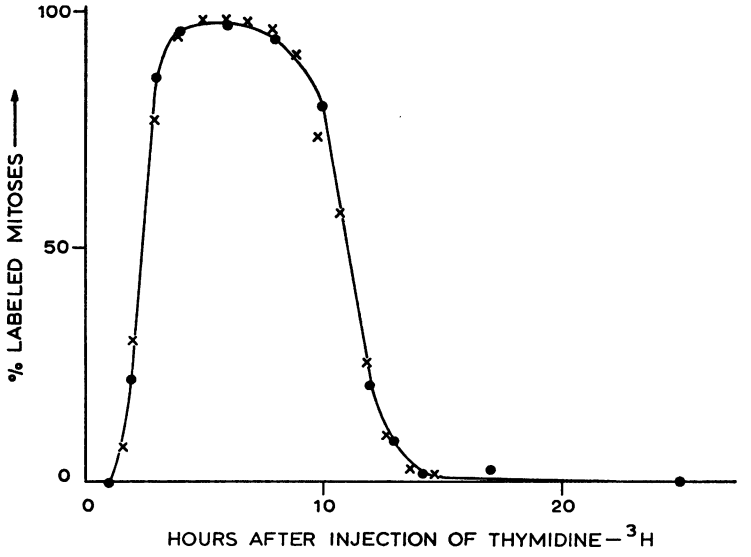

FIGURE 7 The curve is from Brown and Berry (1968). The crosses are the points computed in the present work. The slight disagreement can be made to improve by using a logarithmic distribution.

subsequent behavior do not appear to have been made before, there are cases in the literature in which Fourier series have been tacitly introduced by virtue of some mathematical assumption. Thus Hirsch and Engleberg (1966) assume a form for the Laplace transform of the growth rate (their equation 9) which is tantamount to the assumption of a Fourier series representation.

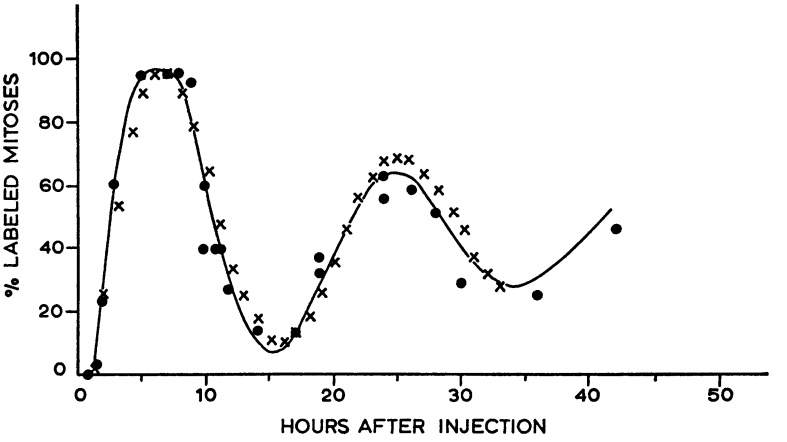

FIGURE 8 From Steel et al. (1966), (their Fig. 6., upper diagram). The line and points are from the original, the crosses are the computed points.

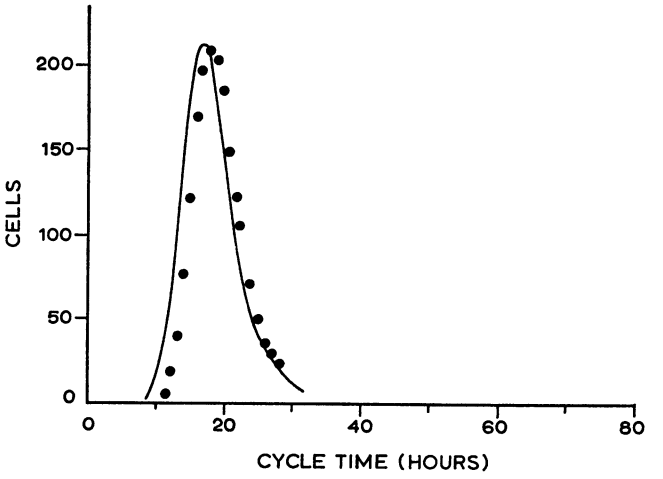

FIGURE 9 The cycle-time distribution curve for the tumor whose PLM curve is shown in Fig. 8. The points computed are shown superimposed on the curve published by Steel et al. If the mean cycle time used in the present work is reduced by 0.5 hr, which does not noticeably affect the fit of the PLM curve, the correspondence of the two cycle-time distributions is exact.

Despite the general success of the method, even in the very simple form presented here, there are results in which the simulated curve is less than completely satisfactory. These seem to be characterized by a group of points in the G_1 region, (i.e., the trough after the first peak of the PLM curve) which are higher than any curve which fits the rest of the points can be made to reach. Examples are Fig. 3 of Denekamp (1970) and Fig. 7 of Steel et al. (1966). It is not yet clear whether these points should be ignored or whether one has to admit that in these cases the best fit is not, in fact, a very good fit.

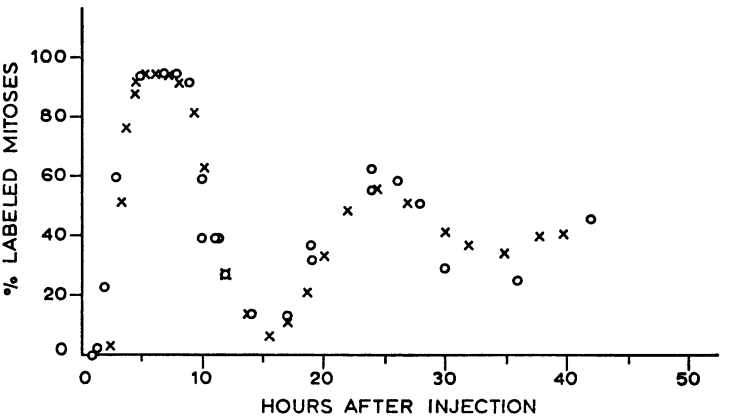

FIGURE 10 The points of the curve shown in Fig. 8, but with points superimposed upon it computed by supposing that (*a*) only 95% of the cycling cells in S were labeled and that (*b*) the decay parameter k was a linear function of t, i.e. that $k = k't$, where k' is constant.

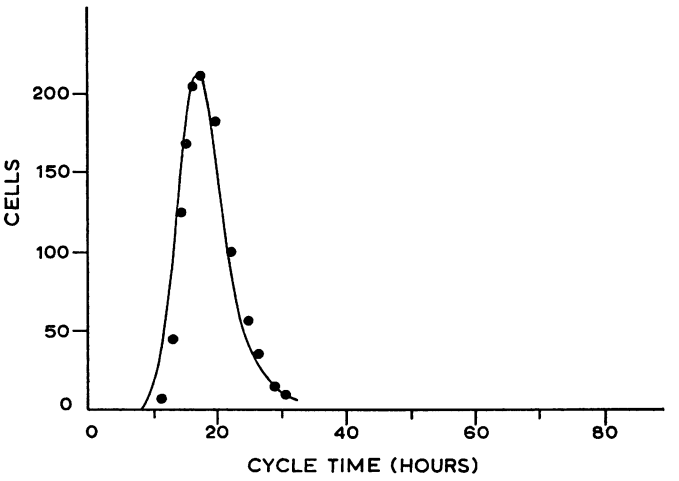

FIGURE 11 The cycle-time distribution for the tumor of Fig. 8 computed using a time-dependent decay parameter. The computed points agree well with the line representing the original results.

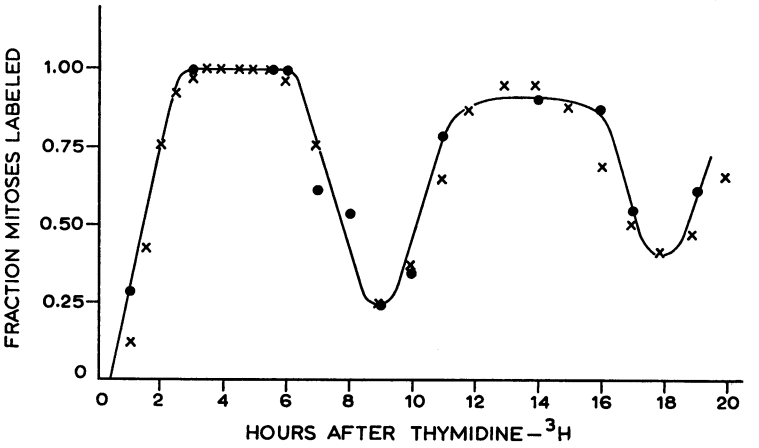

FIGURE 12 The line and solid points are taken from Wimber (1963). The crosses are the computed points. It is suggested that the computed points are a more realistic fit than the line drawn by Wimber as one does not expect the successive peaks of a PLM curve to broaden above the 50% mark.

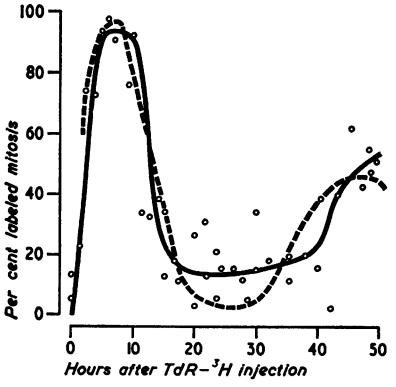

FIGURE 13

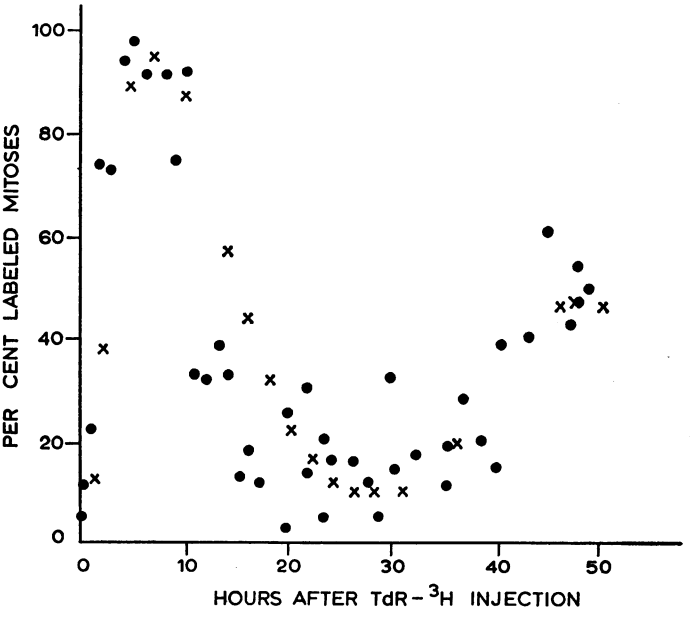

FIGURE 14

FIGURE 13 The PLM curve of the SSBl tumor (from Denekamp, 1970). The circles are the experimental points, the continuous curve is the best fit by eye and the broken curve is the best fit obtained from the Barrett Monte Carlo program. (Reprinted from *Cancer Research*, 30:396.

FIGURE 14 A spurious best fit to the Denekamp SSBl tumor PLM points. This result is invalid as it was obtained by increasing k until the heights of the first peak and the first trough were in the correct proportion and then increasing the scaling factor to obtain the correct absolute values. It illustrates the manner in which convincing but spurious results can be obtained if one does not understand the process being used. The scaling factor is determined by the relative durations of the cycle time and of mitosis and, given the value of these quantities, is not available for adjustment in this manner.

The difficulty is not one arising from the simplicity of the present system, as Barrett's treatment gives no better results. Indeed, the results derived by Barrett using a logarithmic initial phase distribution and independent phase transit time variances are practically identical, in the G_1 region of the PLM curves, with those derived using the present very simple form of the Fourier ring method.

An alternative approach which gives acceptable results in the case of the two PLM curves exhibited by Steel et al. (1966) is to use a Fourier ring system in which the decay parameter k is not a constant but is a linear function of t, i.e.,

$$k = k't, \tag{9}$$

where k' is constant. If, in Fig. 6 (upper diagram) of Steel et al. (1966) (Fig. 8, this paper), the plateau of the first peak is taken to be a true plateau (i.e., only 95% of the cycling cells in S took up the label) and the scaling factor in the computer program adjusted accordingly, then a good fit to the curve can be obtained using the Fourier ring technique modified as above (Fig. 10). As the corresponding cycle-time distribution curve agrees as well with that given by Steel et al. as that produced by the original Fourier ring method (see Figs. 9 and 11, for instance), it is not possible, on the evidence so far submitted, to judge between the two approaches.

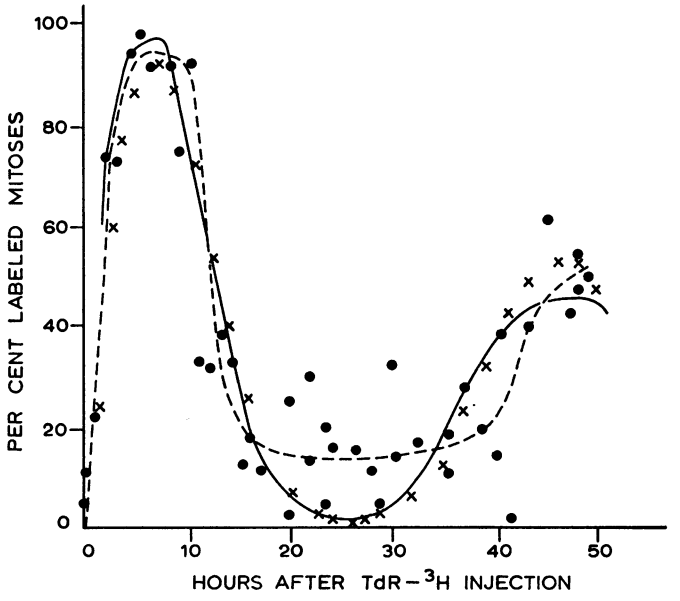

FIGURE 15 The figure of Fig. 9, redrawn, the roles of the continuous and broken lines being reversed and the best-fit Fourier ring computed points (shown as crosses) being superimposed thereon. There is general agreement between the Barrett best fit (the continuous line) and the Fourier ring points except in the values of the two maxima shown. Neither treatment, however, gives satisfactory values in the G_1 region.

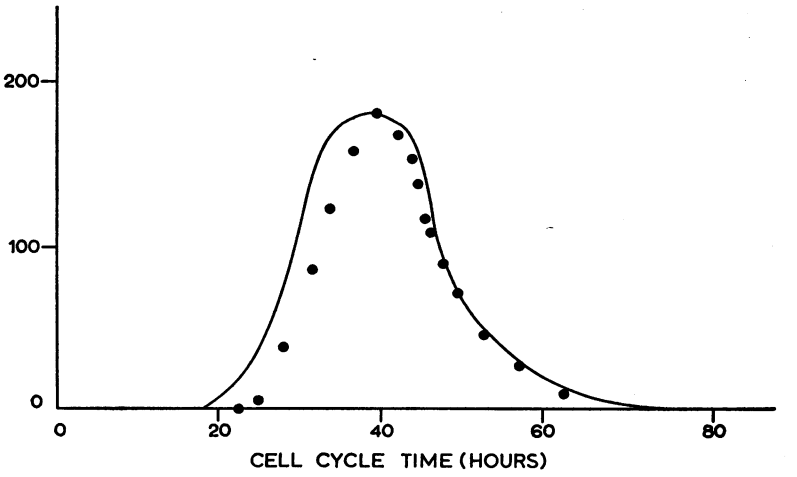

FIGURE 16 The cycle-time distribution curve of the SSBl tumor (Denekamp, 1970) with the Fourier ring computed points superimposed.

A similar treatment, except that 100% labeling is now assumed, gives a good fit to the BICR A2 curve of Steel et al. (their Fig. 6, lower diagram; Fig. 17, this paper), provided that a mean cycle time for 40 hr is assumed, instead of the value of 60 hr, used by Steel et al. Thus measurement of the cycle time by an alternative method may provide an indication of which is the correct approach.

The modified method is less successful in the Denekamp SSB1 tumor (Denekamp, 1970), in that one cannot obtain acceptable values for the first peak and the first trough without so damping the oscillation that the second peak does not appear at all. (Figs. 13–16).

DISCUSSION

The method of analysis of cell kinetic data presented here is a simple, easy method of simulating experimental PLM curves and deriving from them values of the various phase-transit times and the cycle-time distribution. It is suitable for use on small computers and the program runs very quickly, the program used throughout this work occupied under 4500 words of store (48 bits each) and generated the results in under 15 seconds.

Despite its extreme simplicity, it is as successful in generating the results of PLM experiments and the associated cycle-time distribution curves as the more elaborate method of Barrett (1966), which uses a logarithmic phase distribution of the original cohort and independent variances of the phase transit time distributions instead of a uniform initial phase distribution and a single variance (of the cycle-time distribution). This suggests that, with experimental techniques in their present state, the more refined methods, however correct in principle they may be, are unnecessarily complicated. In particular, the extraordinary agreement between the results obtained

by Bronk et al. and those obtained from the present method (Fig. 6) disposes once and for all of the idea that the results of a PLM experiment are materially affected by whether one starts with a uniform or logarithmic phase distribution of the original cohort.

The theoretical reason for this apparent independence of the results of the choice between uniform and logarithmic phase distribution may be appreciated by considering the respective Fourier series. In general the series which represents a broad shape containing obtuse angles gives a fair representation of the function after only a few terms, whereas a narrow shape or an acute angle requires many terms to represent the shape reasonably well. The logarithmic distribution may be regarded as a rectangular shape on which is placed an exponential curve. It may thus be regarded as being represented by the sum of two Fourier series, one of which represents well the rectangular shape after only a few terms and the other of which requires many terms to represent the "peaky" exponential part of the shape. In the case of the series representing the decaying distribution, each term is multiplied by an exponential term involving a power of $-n^2t$, so that shortly after the decay of the original distribution has begun, all the terms in higher values of n have been reduced to insignificance leaving only the terms for the rectangular distribution and a small fraction of the terms relevant to the exponential part.

This rapid disappearance of the higher order terms is useful also in the cycle-time distribution calculation, for which a very narrow (i.e., effectively synchronized) cohort just out of mitosis is observed passing through the next mitosis. For a cohort of the width used in this program (0.1 hr) thousands of terms would be required for an accurate representation of the cohort and the program, although still remaining simple, would become extremely long running on account of the number of terms involved, but for the fact that within a short time after the beginning of the experiment all the higher order terms are reduced to insignificance by the $-n^2t$ dependent exponential. A satisfactory result is thus obtained in both logarithmic phase distribution PLM studies and cycle-time distribution simulations by taking only the first ten terms of the relevant Fourier series.

The treatment of those cases for which the simple Fourier ring technique is inadequate raises a number of interesting problems. Apart from the Denekamp SSBl tumor (Denekamp, 1970), which can be satisfactorily accommodated only by ignoring the points in the G_1 region, satisfactory results can be obtained using either the modified Fourier ring technique (with $k = k't$) or the Barrett model with a disproportionately large variance of t_1. The difficulty with the Barrett method is that there is no apparent reason why the variance in t_1 should be disproportionately large. The chemical processes taking place in the cell are, largely speaking, of the same general nature throughout the cell-cycle and there seems to be no obvious reason to suppose that, in terms of the amount of randomness introduced, there is any difference between G_1 and the other states of the cell. The difficulty with the modified Fourier ring technique is that, although the expression derived by replacing

k by $k't$ is still a solution of the diffusion equation and although the effect intuitively suggests an interaction whereby the cells furthest away from the mean phase of the cohort drag with them those not so far away, it has not been possible, as yet, to devise an interaction mechanism which leads to the required differential equation. The question therefore remains, for the time being, an open one (see Note Added in Proof). Some evidence will be presented in a later paper indicating that an alternative approach to the subject, not involving an appeal to Fourier analysis, also suggests that a standard deviation, varying linearly with the square root of the time, of an initially synchronized cohort leads to the same results for the cycle-time distribution as other methods. Such evidence also supports the contention that the hypothesis of independent variances is, in fact, unnecessary.

A test of the relative merits of the two systems (the Barrett and the modified Fourier ring techniques, as applied to the two tumors discussed here) is possible if evidence other than that derived from a PLM curve fitting is available as to the cycle time of the BICR A2 tumor discussed by Steel et al. (1966). The modified Fourier ring method requires T_c = (about) 40 hr and fails if T_c = 60 hr in that it yields a plateau of several hours duration at PLM = 0% unless one so increases k' that the height of the first peak is reduced to less than 50%. This is, of course, somewhat similar to the circumstances of the Denekamp tumor described earlier. A further difficulty in comparing the two is that Barrett shows smooth lines rather than computed points and one is therefore unaware of the extent to which his judgement has affected his choice of the best line through the points which his program has computed.

Finally, the remarkable agreement between the results obtained by Bronk et al. and those obtained from the Fourier ring treatment (remembering that even the slight difference which exists between them when a uniform phase distribution is used in the Fourier ring analysis, disappears if a logarithmic phase distribution is used)

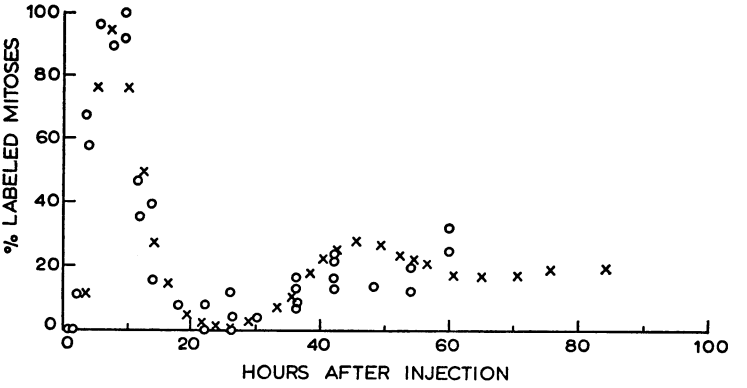

FIGURE 17 The results computed using the modified Fourier ring technique (crosses) superimposed on the experimental results for the BICR A2 tumor (Steel et al., 1966) (open circles).

suggests that despite the apparent differences in their approach, the two treatments are in fact identical. One is reminded, in this, of wave mechanics and matrix mechanics, which seem quite different but which have been shown, apart from relativistic electrodynamics, to yield identical results.

A TECHNICAL NOTE

With the exception of the BICR Ml tumor (for which a cycle-time of 18 hr appears to give the best fit) and the BICR A2 tumor (which can be fitted by the modified Fourier ring method if a cycle time of 40 hr is used) all the simulations described in this paper used the data given by the authors whose results were used.

Note Added in Proof. I have since been informed that the difference between systems obeying the chemical diffusion equation (k constant) and the Knudsen diffusion equation ($k = k't$) is that in the former case the elements (in this case the cells) are interacting with each other, whereas in the latter they are not. In other words, the interaction tends to hold the cohort together rather than cause it to separate, as suggested in the text.

I am indebted to the various authors whose results I have used for permission to reproduce their diagrams, and also to the International Atomic Energy Authority for permission to use Fig. 7.

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REFERENCES

BARRETT, J. G. 1966. A mathematical model of the mitotic cycle and its application to the interpretation of percentage labelled mitosis data. *J. Natl. Cancer Inst.* **37**:443.

BRONK, B. V. 1969. On radioactive labelling of proliferating cells; the graph of labelled mitosis. *J. Theor. Biol.* **22**:468.

BRONK, B. V., G. J. DIENES, and A. PASKIN. 1968. The stochastic theory of cell proliferation. *Biophys. J.* **8**:1353.

BROWN, J. M., and R. J. BERRY. 1968. Effects of X-irradiation on cell proliferation in normal epithelium and in tumours of the hamster cheek pouch. International Atomic Energy Agency Symposium on the effects of radiation on cellular proliferation and differentiation. SM-103/2, Monaco.

CARSLAW, H. S., and J. C. JAEGER. 1947. Conduction of Heat in Solids. The Clarendon Press, Oxford.

DENEKAMP, J. 1970. The cellular proliferation kinetics of animal tumours. *Cancer Res.* **30**:393.

HIRSCH, H. R., and J. ENGLEBERG. 1966. Decay of cell synchronisation; solutions of the cell growth equation. *Bull. Math. Biophys.* **28**:391.

MERKLE, T. C., R. N. STUART, and J. W. GOFFMAN. 1965. The calculation of treatment schedules for cancer chemotherapy University of California Lawrence Radiation Laboratory Report UCRL 14505, Pt. I and II.

STEEL, G. G., K. ADAMS, and J. C. BARRETT. 1966. Analysis of the cell population kinetics of transplanted tumours of widely-differing growth rates. *Br. J. Cancer.* **20**:784.

STUART, R. N. 1966. Kinetics of cell growth. University of California Lawrence Radiation Laboratory Report 70039.

WIMBER, D. E. 1963. *In* Cell Proliferation. L. F. Lamerton, and R. J. Fry, editors. Blackwell Scientific Publications Ltd., Oxford. 1–17.