A DISCRETE REPRESENTATION OF DNA DISTRIBUTION AND OBSERVABILITY FOR IDENTIFICATION OF CANCER CELL CYCLE KINETICS[1]

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ABSTRACT

Methods are developed for analyzing and interpreting DNA distributions of cell populations experimentally obtained by flow microfluorometric techniques. The first method derived can be used to compute the mean DNA synthesis rate and a transformation matrix relating a cell age distribution to its corresponding DNA distribution when these two distributions are known. This transformation matrix is used to compute the cell DNA distribution from a cell age distribution. The second part deals with computing the initial cell age distribution when several measurements of cell DNA distributions are available. This problem is shown to be equivalent to the observability problem for the discrete-time system representing the cell population. The observability condition in system theory and a computing method for determining the initial cell age distribution are discussed. An example using DNA distributions of Chinese hamster cells for the methods is presented.

INTRODUCTION

Cell cycle and proliferation kinetic studies have played an important role in elucidating the dynamics of tumor cell populations [1-9]. The radioautographic techniques [2] have been extensively used in experimental study of the cell cycle kinetics. Recently the introduction of flow microfluorometry (FMF) [10-12] makes it possible to determine rapidly the DNA content per cell and to generate the DNA distribution for a large number of cells.

The FMF technique has been reported to be increasingly employed in studying the cell cycle kinetics [4,13-18]. Quantitive methods for computing the fractions of cells in G1-, S-, and (G_2+M)-phases from FMF data were reported [16-19]. Kim et al. [20,21] developed the mathematical representation of the relationship between cell age distribution and its cell DNA distribution with knowledge of the mean DNA synthesis rate. Usually the mean DNA synthesis rate of a cell population is not readily available. In this paper analytical methods will be derived for computing the mean DNA synthesis rate and the cell age distribution from FMF data.

ANALTICAL METHODS

Mean DNA Synthesis Rate and Transformation Matrix

The problem is to compute the mean DNA synthesis rate of a cell population from an experimentally determined cell DNA distribution when its corresponding cell age distribution is known or can be estimated. When solved, it should also give the transformation matrix relating cell age distribution to its corresponding cell DNA distribution.

The experimental cell DNA vector $\tilde{z}(k)$ for experimental cell DNA distribution is defined,

$$\tilde{z}(k) = \text{transpose of } [\tilde{z}_1(k),\ldots,\tilde{z}_i(k),\ldots,\tilde{z}_e(k)] \quad (1)$$

where $\tilde{z}_i(k)$ is the number of cells at DNA content state i at time k observed experimentally.

Elements of $\tilde{z}(k)$ form the cell distribution according to the DNA content. Similarly, the cell age vectors $x(k)$ for proliferating cells and $q(k)$ for nonproliferating cells are defined.

$$\tilde{x}(k) = \text{transpose of } [x_1(k),\ldots,x_i(k),\ldots,x_n(k)] \quad (2)$$

$$q(k) = \text{transpose of } [q_1(k),\ldots,q_m(k)]$$

where $x_i(k)$ is the number of cells at age compartment i at time k.

In order to derive an algorithm for computing the mean DNA synthesis rate of a cell population from experimental cell DNA distributions and cell age distributions, the following assumptions are made.

1. The mean DNA synthesis rate is a function of the cell age within the cell cycle. A curve for the change in the DNA content of a single cell between successive age compartments within the S phase is assumed. Thus the DNA content of the cell must increase monotonically as it advances through the S phase of the cell cycle.
2. It is assumed that the disperison due to instrumental noise and nonuniform staining is described by the Gaussian distribution.

The probability density function for the dispersion is given by

$$P_j(d_i) = \frac{1}{(2\pi)^{1/2}\sigma_j} \exp[-1/2(\frac{d_i-\bar{d}_j}{\sigma_j})^2] \quad (3)$$

where $\bar{d}_j$ is the true mean DNA content before the dispersion due to the artifacts and d_i is the DNA content of the ith DNA content compartment.

The relation between the true cell DNA content distribution and experimentally observed cell DNA content distribution is,

$$\tilde{z}(k) = Gz(k) \quad (4)$$

where $z(k)$ is the r-dimensional true cell DNA content vector at time k. The element of row i and column j of matrix G is given by Eq. 3. The coefficient of variations is assumed to be the same for all DNA compartments.

3. Cells in a particular cell age compartment do not necessarily contain an identical DNA content. The DNA synthesis rate within a cell age compartment is assumed to be constant. This approximation can be improved by increasing the total number of cell age compartments. The relation between the true cell DNA distribution (without the dispersion due to instrumental and staining artifacts) and the cell age distribution at time k can be given.

$$z(k) = R_p x(t) + R_m q(t) \quad (5)$$

where R_p is the rxn transformation matrix and R_m is the rxm transformation matrix.

When a true DNA distribution and its corresponding age distribution are known, the mean DNA synthesis rate and the transformation matrices R_p and R_m in Eq. 5 and G in Eq. 4 can be determined. The cell age distribution is usually not experimentally observable and thus needs to be estimated, e.g., the asymptotic cell age

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distribution for an exponentially growing cell population is well known. If the cell population has noncycling cells, the asymptotic cell age distribution may be computed by using certain cell cycle kinetic models [20-24].

The first step is to compute the true cell DNA distribution by using Eqs. 3 and 4. If some parameters in the probability density function $p_j(d_i)$ is not known, this can be estimated by using methods of nonlinear least squares fit, such as the Levinberg-Marguardt method developed for computer programs [25].

Once the true DNA distribution is determined, this and the cell age distribution can be used to compute the mean DNA synthesis rate and the elements of R_p and Rm as follows. As cells progress through the S phase, changes in the DNA content are determined by the DNA synthesis rate. Since the DNA synthesis rate within a cell age compartment is assumed to be constant for simplicity, the curve for the DNA content vs the cell age near age compartment j and the DNA compartment i can be represented as in Fig. 1. The flow diagram of an algorithm for determining the DNA synthesis rate and transformation matrix R is shown in Fig. 2. This algorithm is developed by computing the fraction of cells in the jth cell age compartment with different DNA contents corresponding to DNA compartments (i-1), i, (i+1) which is a function of the DNA synthesis rate. This algorithm also computes the elements of transformation matrix R_p. Note that elements of the first and last row of R_p are eithr 1 or 0 and these can be dtermined by inspection because the elements of the first row are the coefficients for x_1 in the G_1 phase and those of the last row for x_i in the G_2+M phases. Similarly, R_m can be determined by inspection.

A Method for Determining Cell Age Distribution

It is useful to have knowledge of the cell age distribution of a cell population at any given time, since the cell age distribution describes the cell cycle kinetic state. An analytical method will be derived to determine the cell age distribution from experimental DNA distribution by using the cell DNA-cell age relationship developed (Eqs. 3,4,&5) and the dynamics of the cell cycle kinetics represented by [20,22].

$$\begin{aligned} \tilde{\underline{x}}(k+1) &= \Phi_{pp}\tilde{\underline{x}}(k) + \Phi_{pm}\underline{q}(k) \\ \underline{q}(k+1) &= \Phi_{mp}\underline{x}(k) + \Phi_{mm}\underline{q}(k) \end{aligned} \tag{6}$$

where Φ's are the local state transition matrices expressing the transformation from $\tilde{\underline{x}}$ and $\underline{q}$ at time k to $\underline{x}$ and $\underline{q}$ at time k+1.

Now the problem is defined as follows. Given experimental distributions at time 0,...,S described by $\tilde{\underline{z}}(k)$, $\tilde{\underline{z}}(k),\ldots,\tilde{\underline{z}}(S)$, one is required to determine the corresponding cell age distributions. The first step is to compute the true DNA distribution from experimental DNA distribution by using Eqs. 3 & 4, as described in the previous section. The second step is to use the computed DNA vectors $\underline{z}(0),\ldots,\underline{z}(S)$ and Eqs. 5 & 6 to determine the corresponding cell age vectors.

For convenience the notation for Eqs. 5 & 6 is simplified,

$$\underline{z}(k) = R\underline{x}(k) \tag{7}$$

$$\underline{x}(k+1) = \Phi\underline{x}(k) \tag{8}$$

where

$$\underline{x}(k) = \begin{bmatrix} \tilde{\underline{x}}(k) \\ \underline{q}(k) \end{bmatrix} \quad \Phi = \begin{bmatrix} \Phi_{pp} & \Phi_{pm} \\ \Phi_{mp} & \Phi_{mm} \end{bmatrix} \quad R = [R_p \; R_m]$$

Here x(k) = a (n+m) dimensional vector, Φ = the (n+m)x(n+m) local state transition matrix, and R = the rx(n+m) transofrmation matrix.

In this problem it is desired to determine at least $\underline{x}(0)$ from $\underline{z}(0),\ldots,\underline{z}(S)$, and, if possible, also $\underline{x}(1),\ldots,\underline{x}(S)$. Consider Eq. 7 with k=0

$$\underline{z}(0) = R\underline{x}(0) \tag{9}$$

Since the dimension γ for z is not necessarily the same as the dimension (n+m) for x, we premultiply both sides of Eq. 9 by the transpose of the transformation matrix R.

$$R^T\underline{z}(0) = R^TR\underline{x}(0) \tag{10}$$

Then (R^TR) is (n+m)x(n+m) matrix and if (R^TR) is invertible, then $\underline{x}(0)$ is obtained,

$$\underline{x}(0) = (R^TR)^{-1}R^T\underline{z}(0) \tag{11}$$

$\underline{x}(0)$ is the least square solution of Eq. 9 [26]. The rank of the rectangular matrix R and the square matrix (R^TR) is (n+m) if (R^TR) is invertible. If the rank of R is less than (n+m) the additional $\underline{z}$'s are included by using Eqs. 7 & 8.

$$\begin{bmatrix} \underline{z}(0) \\ \underline{z}(1) \\ \vdots \\ \underline{z}(k-1) \end{bmatrix} = \Theta_{(k)}\underline{x}(0) \tag{12}$$

where

$$\Theta_{(k)} = \begin{bmatrix} R \\ R\Phi \\ \vdots \\ R\Phi^{k-1} \end{bmatrix}$$

The minimum value of k for which the rank of $\Theta_{(k)}$ is (n+m) is the number of $\underline{z}$'s required to determine x(0). If this k exists, the system described by Eqs. 7 & 8 is defined to be observable and this k cannot exceed (n+m) [27].

Then $\underline{x}(0)$ can be obtained

$$\underline{x}(0) = (\Theta^T_{(k)}\Theta_{(k)})^{-1}\Theta^T_{(k)}\begin{bmatrix} \underline{z}(0) \\ \underline{z}(1) \\ \vdots \\ \underline{z}(k+1) \end{bmatrix} \tag{13}$$

In system theory $\Theta_{(k)}$ is called the observability matrix and the minimum k is the observability index.

EXPERIMENTAL DATA FOR ANALYSIS

FMF data for Chinese hamster cells (CHO Cells) will be analyzed by the derived methods. Puck et al. reported the cell generation time T of CHO cells to be 12.4 hrs. [28], while Kramer et al. [13] said the generation time T to be 16.5 hrs. and also T_{G1} for the G1 phase and Ts for the S phase are functions of cell concentrations and culture conditions for FMF experiments [19].

The CHO cell population grows exponentially and

thus they are cycling cells.

DNA Synthesis Rate

The cell DNA distribution of exponentially growing CHO cells in Fig. 3 [13] is used to compute the mean cell DNA synthesis rate and the transformation matrix for cell age and cell DNA distributions. The mean cell cycle time of this CHO cell populaiton is 16.5 hrs. Since the CHO cells comsidered here are exponentially growing, the asymptotic cell age distribution is [20,22]

$$\underline{x}^T = \frac{N}{\sum_{i=1}^{n} 2^{-i/n}}[2^{-1/n},\ldots,2^{-i/n},\ldots 2^{-1}]$$

Here N is the total cell population and n is the total number of the proliferating cell age compartments. The total number of the age compartments is chosen to be 19. The total number of the DNA content compartments is 80 which is the same as the maximum fraction number in the experimental cell DNA distribution. Using the nonlinear least square fit of the experimental cell DNA content distribution in Fig. 3 of the step 1, one can determine the cell DNA distribution with instrumental artifacts. For this DNA data, the cells in the G_1, S, and G_2+M phases constitute 53.6%, 38.9%, and 7.5% of the total population, respectively. The same results were also obtained by Dean and Jett [19]. The fraction numbers corresponding to the DNA contents of cells in the G_1 and G_2 phases (normalized DNA value of 1 and 2) are 36 and 72 respectivley. The true cell DNA distribution of cells in the S phase (after eliminating the dispersion due to instrumental and staining artifacts) is shown in fig. 4. This distribution excludes cells in the G_1 and G_2+M phases.

From the given fraction of cells in the G_1, S, and G_2+M phases and the given cell age distribution for exponentially growing cell population, one can compute the phase durations of the G_1, S, and G_2+M phases to be 7.6 hrs. 7.2 hrs., and 1.7 hrs. respectively. The cell age distribution of cells in the S phase is shown in fig. 4a. From the algorithm the cell DNA constant and the DNA synthesis rate are computed and shown in fig. 5. It is noted that the DNA synthesis rate is maximum slightly before the midpoint of the S phase.

An exponentially growing cell population of L5178Y was studied for the DNA synthesis rate The experimental cell DNA content distribution [19] was converted to the distribution without dispersion due to instrumental and staining noise which gave the fractions of cells in the G_1, S, G_2+M phases to be 20.1%, 64.3%, and 15.5%. The cell cycle time used is 12 hrs. The cell DNA distribution for only the S phase and the computed DNA synthesis rate is shown in Fig. 6.

Cell Age Distribution

Again, FMF data for CHO cells are used in this example of computing the corresponding cell age distributions from cell DNA distributions. The experimental DNA distributions of CHO cells [13] were recorded at various times (2.5, 3.5, 7.5, 8.5, 12.8, 13 hrs.) after release from thymidine block which is known to synchronize the cells at the G1-S interphase.

It is desired to compute the corresponding cell age distributions at as many time points as it can be computed. The true cell DNA distributions were computed by using Eq. 4. The optimum value of the coefficient of variations is found to be 0.69. Also the transformation matrices R_D and R_m for R in Eq. 7 computed in the previous example are used.

The rank of the transformation matrix R in Eq. 7 is found to be less than 19 (= the dimension of $\underline{x}(k)$), thus (R^TR) in Eqs. 10 & 11 is not invertible. This means that one cannot determine $\underline{x}(0)$ only from $\underline{z}(0)$ and additional $\underline{z}$'s at time k>0 are required. Also the local state transition matrix Φ in Eq. 8 is required. Since all the CHO cells are cycling cells, Φ can be shown to be [20,22]

$$\Phi = \begin{bmatrix} \beta & 0 & \ldots & 2\alpha & 2(1-\alpha-\beta) \\ (1-\alpha-\beta) & \beta & \ldots & 0 & 2\alpha \\ \alpha & (1-\alpha-\beta) & \ldots & 0 & 0 \\ 0 & \alpha & \ldots & 0 & 0 \\ \cdot & \cdot & \ldots & \cdot & \cdot \\ \cdot & \cdot & \ldots & \cdot & \cdot \\ \cdot & \cdot & \ldots & \cdot & \cdot \\ 0 & 0 & \ldots & \beta & 0 \\ 0 & 0 & \ldots & (1-\alpha-\beta) & \beta \end{bmatrix}$$

where α is the probability that cells in an age compartment advance two compartments after a unit time, and β is the probability that cells in an age compartment do not advance to the next compartment after a unit time. α and β are assumed to be the same since the probability distribution of the cell cycle time can be assumed to be Gaussian. Then only single parameter needs to be determined for Φ. α must be between 0 and 0.5. Since α is not known a priori, various values of α from 0.05 to 0.5 are used. For these values, the rank of the observability matrix $\Theta_{(2)}$ in Eq. 13 is computed to be 19. Thus Eq. 14 for $\underline{x}(0)$ exists i.e., the following equation can be solved for $\underline{x}(0)$.

$$\begin{bmatrix} \underline{z}(0) \\ \underline{z}(1) \end{bmatrix} = \begin{bmatrix} R \\ R\Phi \end{bmatrix} \underline{x}(0) \qquad (15)$$

Therefore, for this system only $\underline{z}(0)$ and $\underline{z}(1)$ are required to solve for $\underline{x}(0)$. Since at time 0 the cells are released from thymidyne block, all the cells are assumed to be at the last age compartment for the G1 phase. Among various α's the best fit is given by α=0.15. The computed cell age distributions at time 2.5, 7.5, and 12 hrs. are shown in Fig. 7. Each cell age distribution is determined from two cell DNA distributions at the corresponding time and one hour later. Note that the cells are still reasonably synchronized at 2.5 hrs. after the release from thymidine block but become more unsynchronized from 7.5 hrs. to 12 hrs. The computed true DNA distributions (without instrumental artifacts) are shown in Fig. 8. The cell DNA distributions computed by using the cell age distributions in Fig. 7 and Eqs. 7 & 8 are plotted in Fig. 9 with the experimental data superimposed.

DISCUSSION

The first algorithm demonstrated to be capable of determining the mean DNA synthesis rate of a cell population from a FMF cell DNA distribution and its corresponding cell age distribution in addition to calculating the fraction of cells in the G_1, S, and G_2+M phases. It also computes the matrix which transforms a cell age distribution at time k into the corresponding cell DNA distribution at time k. The cell age distribution of a cell population cannot be usually obtained by direct experimental measurements. Therefore, the cell age distribution is needed to be estimated for the algorithm.

For an exponentially growing population the

asymptotic cell age distribution is known. For a cell population comprising cycling and non-cycling cells, one can use the growth fraction and other cell cycle kinetic information to compute the asymptotic cell age distribution [20].

In the algorithm the cell DNA and age distributions are represented by the cell DNA content and age vectors which are discrete representations of the distributions. The accuracy can be improved by increasing the number of compartments representing the discrete DNA contents and cell age. The transformation matrices R_p and R_m in Eq. 5 determines the cell DNA content distribution from a cell age distribution. This relation is true as long as the mean DNA synthesis rate remains unchanged. If the DNA synthesis rate is changed, the new transformation matrices corresponding to the new synthesis will have to be computed.

If there exist variations in the actual DNA content of cells at a particular cell age compartment, these variations need to be represented in transformation matrices R_p and R_m. Then the mean DNA synthesis rate and transformation matrices G, R_p, and R_m can be more conveniently determined simultaneously by using methods of nonlinear least square fit [25].

In the second part of this paper it is shown that FMF DNA data can be used to compute the cell age distribution at a particular time considered. If the rank of the transformation matrix R is (n+m), (R^TR) in Eq. 10 is invertible. Thus Eq. 10 is solvable for $\underline{x}(0)$. That is, in order to compute the cell age distribution at time j, only the corresponding cell DNA distribution at time j is required. Let us examine $R=[R_p\ R_m]$ in Eqs. 5 & 7. R_p for the cycling cell age vector $\underline{x}(t)$ has the form,

$$R_p = \begin{array}{c} \begin{matrix} n_{G_1} & & n_s & & n_{(G_2+M)} \end{matrix} \\ r \begin{bmatrix} 1\ldots1 & & & & \\ & & & & \\ 0 & & & & 0 \\ & & RS & & \\ & & & & 1\ldots1 \end{bmatrix} \end{array}$$

where n_{G_1} = the number of age compartments in G_1
n_s = the number of age compartments in S
$n_{(G_2+M)}$ = the number of age compartments in G_2+M
R_s = the (rxn_s) matrix determined by the algorithm with the DNA synthesis rate.

The first n_{G1} columns of R_p has only one linearly independent column and the last $n_{(G_2+M)}$ column also has one. Therefore, the maximum number of linearly independent columns of R_p is possibly (n_s+2) or in R the maximum is (n_s+2+m). If n_{G_1} and n_{G_2} are not one, the rank of R is less than (n+m). Then one needs additional DNA distributions at time j+1, j+2,...,j+k-1, until the rank of the observability matrix $\Theta_{(k)}$ in Eq. 13 becomes (n+m). In Eq. 14 we need the state transition matrix Φ to compute the cell age distribution. It is computationally desirable to solve Eq. 12 by using a nonnegative least square method [29] than to solve Eq. 14 since the cell age vector x(j) is nonnegative.

If FMF DNA distributions are not measured consecutively, e.g., at time 0,5,10, then the observability matrix is

$$\Theta_{(3)} = \begin{bmatrix} R \\ R\Phi^5 \\ R\Phi^{10} \end{bmatrix}$$

If the rank of $\Theta_{(3)}$ is (n+m), then the following equation is to be solved for $\underline{x}(0)$.

$$\begin{bmatrix} \underline{z}(0) \\ \underline{z}(5) \\ \underline{z}(10) \end{bmatrix} = \Theta_{(3)}\underline{x}(0)$$

It is found that if the interval between one measurement and the next is large, the next measurement should be timed accurately so that the interval becomes integer multiples of the unit time. Otherwise inaccuracy results in the computation.

In the CHO cell population simulated, the state transition matrix Φ was not known but was determined in the process of computing the cell age distributions and the parameter α which gave the best fit. The parameter α can be regarded as the rate of unsynchronization.

Recently there has been a rapid increase in FMF study of antitumor agents [14,15,17,18,30,31] The knowldege of cell age distributions computed by the method can be potentially very useful in the study of the effects of antitumor agents to tumor populations. The cell age distributions before and after the application of drug can be used in conjunction with the state equation Eq. 6 to study analytically the sensitivity of antitumor agents as a function of the position in the cell cycle to a tumor population.

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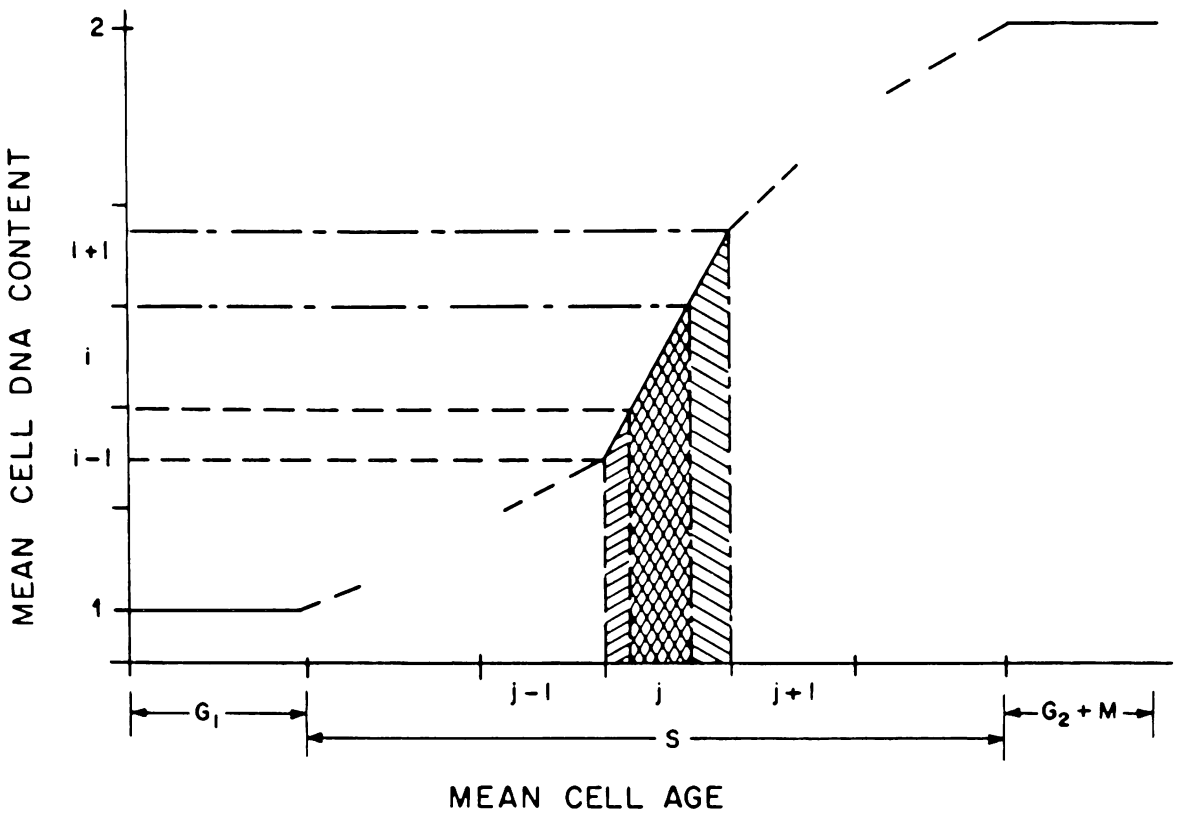

Figure 1. The mean DNA content vs. the mean cell age of a cell population. The DNA contents for G_1 and G_{2+M} are 1 and 2 respectively. Certain fractions of cells in the jth age compartment are assigned to the (i-1),i, and (i+1) DNA compartments. The fractions are determined as shown in the figure. Here the DNA synthesis rate is assumed to be constant within any age compartment but varys one age compartment to another.

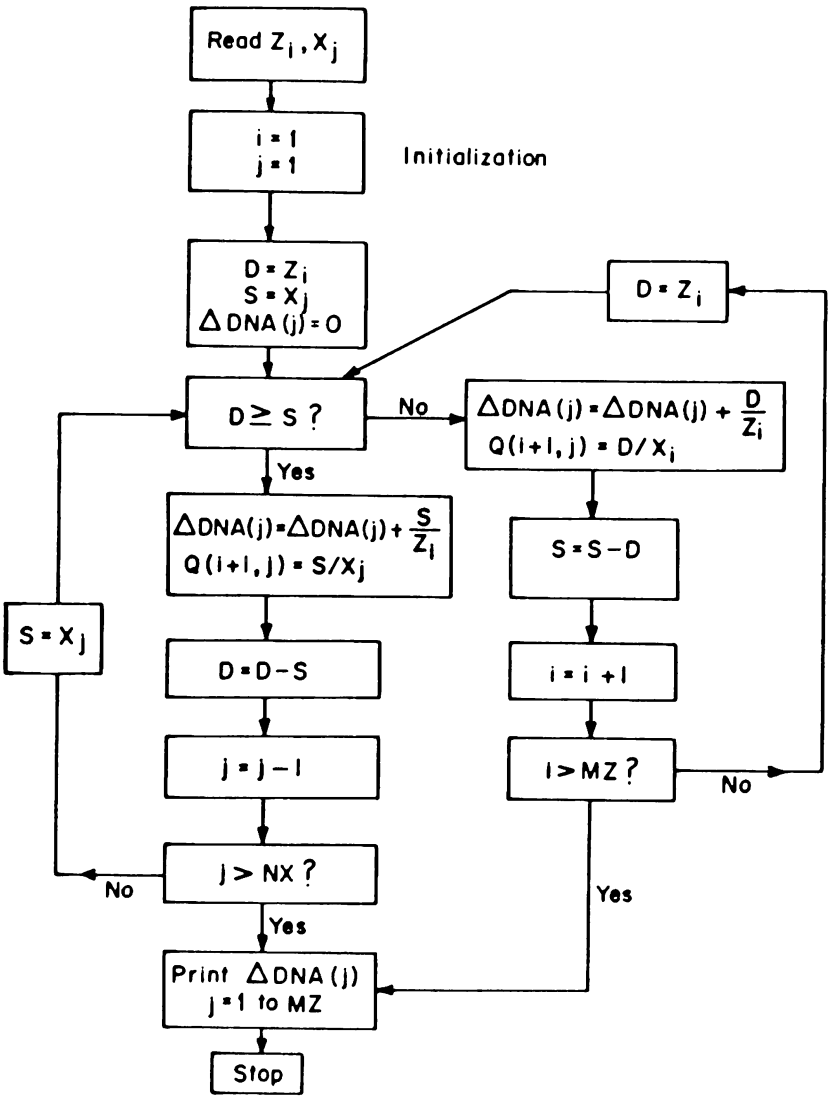

Figure 2. Flow diagram for computing the mean DNA synthesis rate from the true cell DNA content distribution and the cell age distribution. Subscript i indicates the ith DNA content compartment. Since only cells in the S phase are considered, i=1 is the DNA content interval $(1,1+\Delta\rho)$, i.e., the first DNA compartment for cells in the G_1 phase is excluded. Subscript j indicates the jth cell age compartment in the S ohase. Z_i is the number of cells at the ith DNA content compartment. x_j is the number of cells at the jth cell age compartment in the S. phase. ΔDNA(j) is the amount of DNA snthesized during the jth cell age interval i.e., the synthesis rate. NX is the total number of the cell age compartments in the S phase. MZ is the total number of the cell DNA content compartments between 1 (for G_1) and 2 (for G_2+M). Q(i,j) represents the fraction of cells in the jth S phase age compartment assigned to the ith DNA compartment

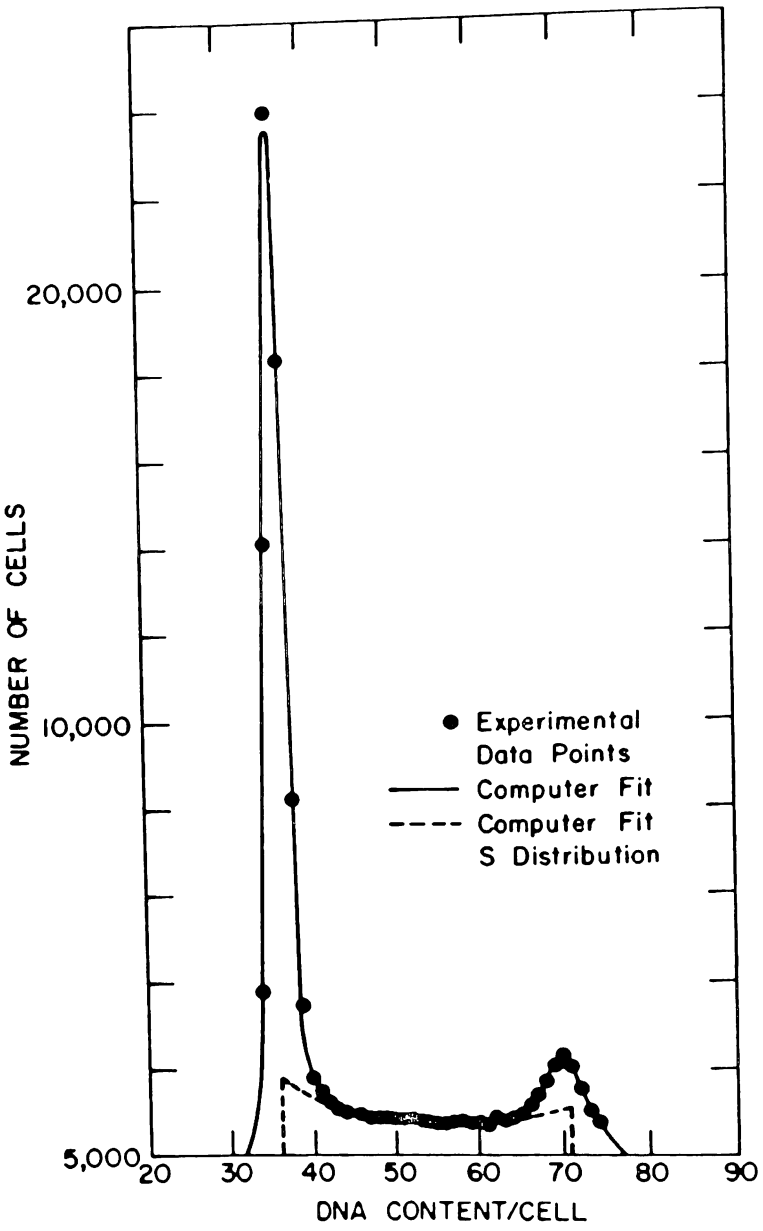

Figure 3. The cell DNA distribution of CHO cells (from Ref. 13)

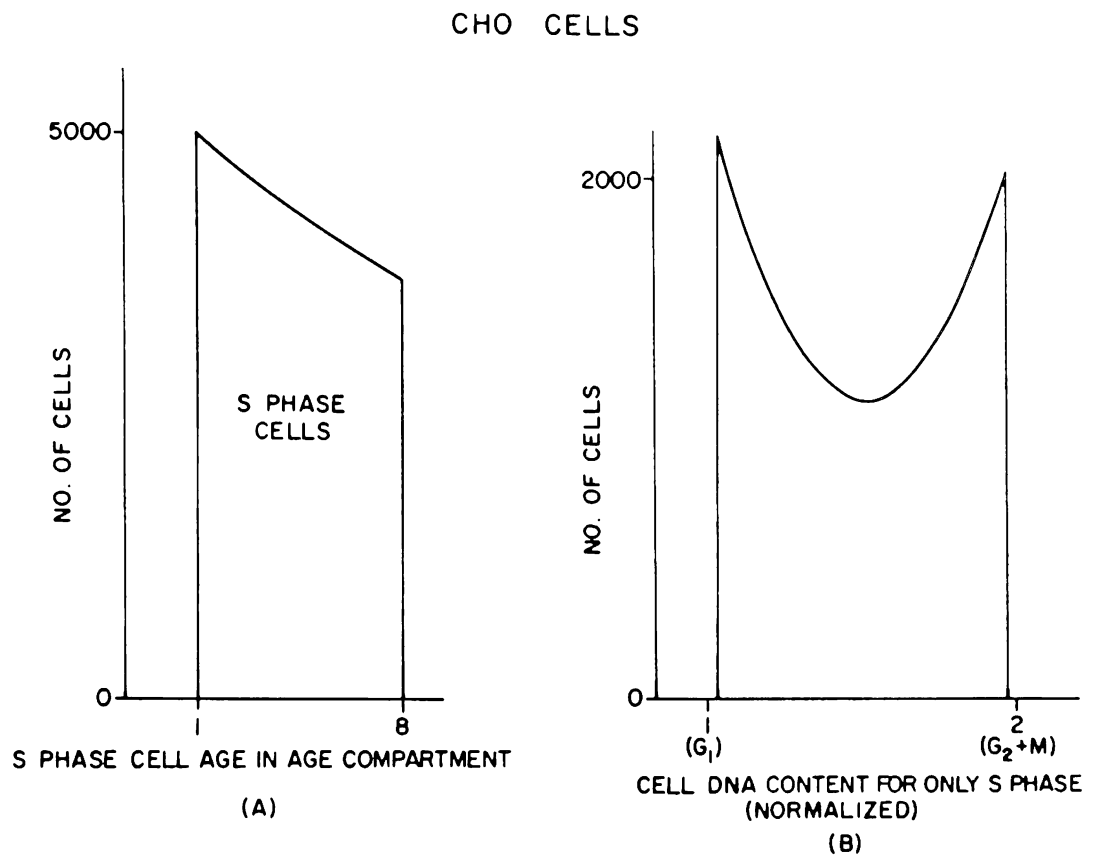

Figure 4 (A) The cell age distribution of S phase cells of an experimentally growing cell population. This age distribution is used for the CHO cells.

(B) The cell DNA content distribution of the CHO cells in the S phase after eliminating the dispersion due to instrumental noise and staining artifact.

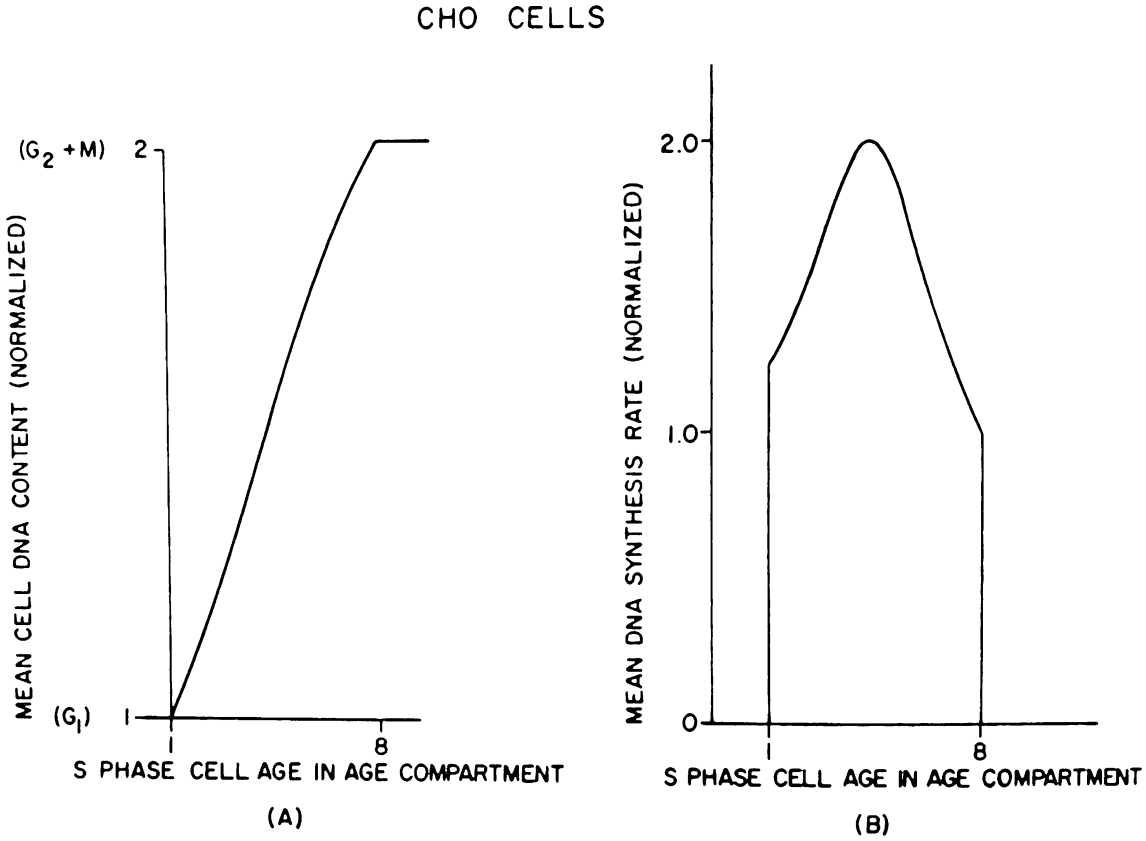

Figure 5 (A) The mean cell DNA content vs. the cell age computed by using the method derived.

(B) The computed mean DNA synthesis rate of the CHO cells as a function of the cell age.

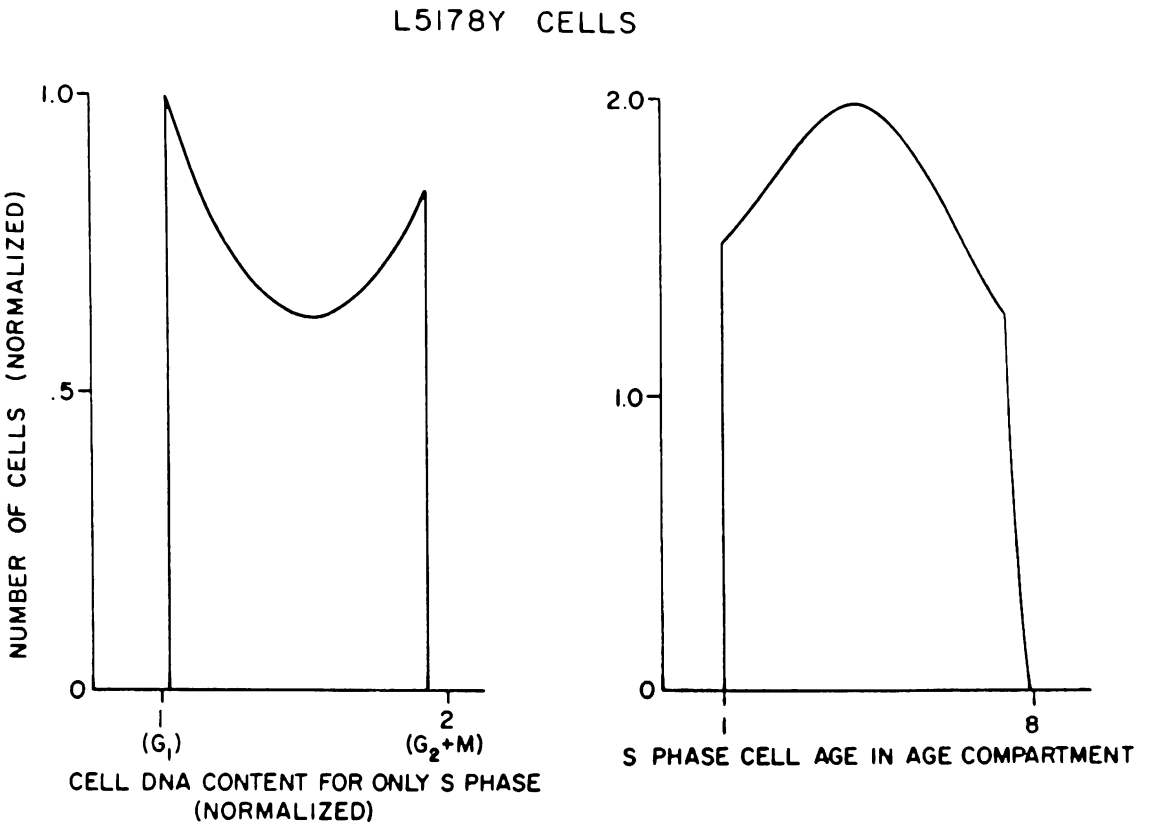

Figure 6 (A) The cell DNA distribution of L5178Y cells in the S phase after eliminating the dispersion due to instrumental noise and staining artifacts. The experimental DNA distribution from which this was computed is from Ref. 19.

(B) The computed mean DNA synthesis rate of the L5178Y cells as a function of the cell age.

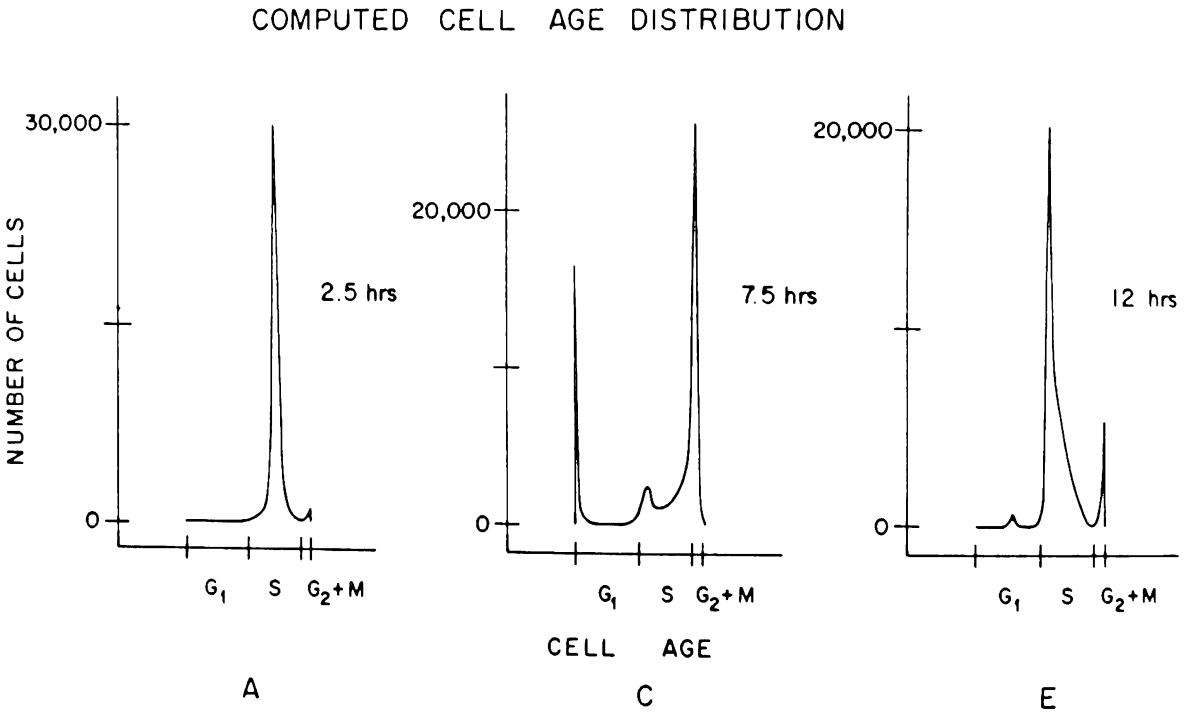

Figure 7 The computed cell age distribution of CHO cells at time 2.5, 7.5, and 12 hours after release from thymidine block.

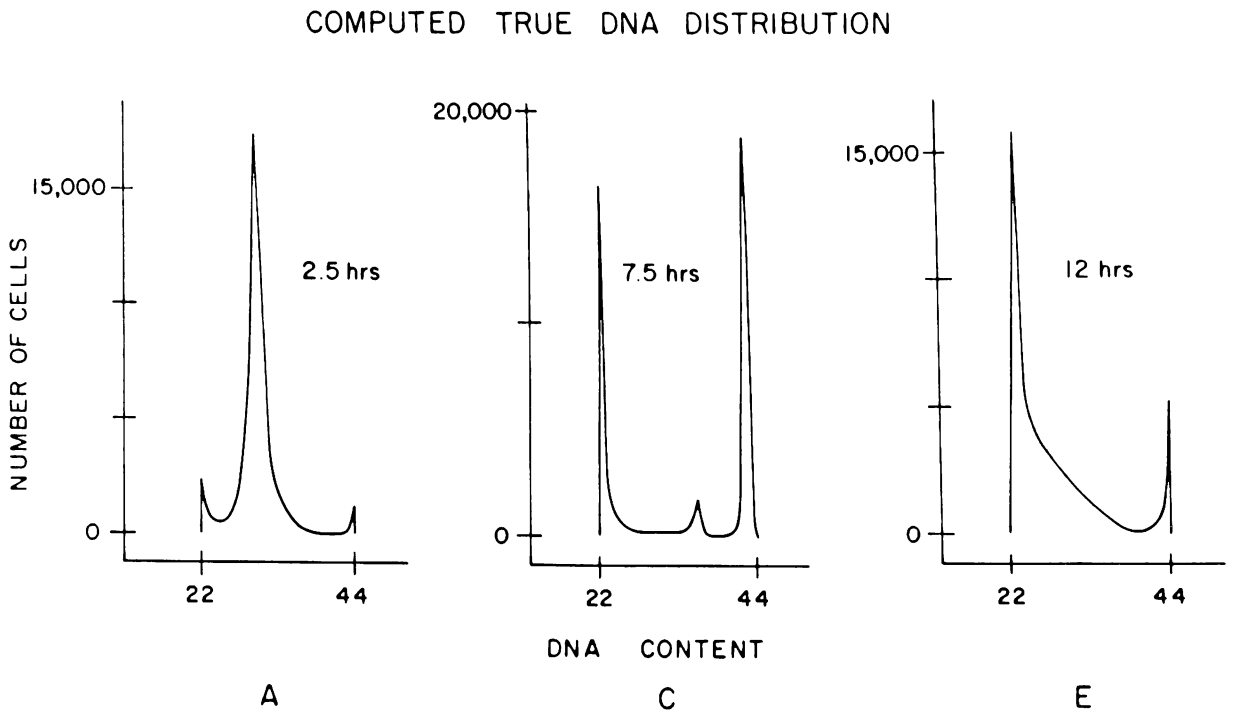

Figure 8 The computed true cell DNA distributions (without instrumental and staining artifacts) of CHO cells at time 2,5., 7,5., and 12 hours after release from thyimidine block.

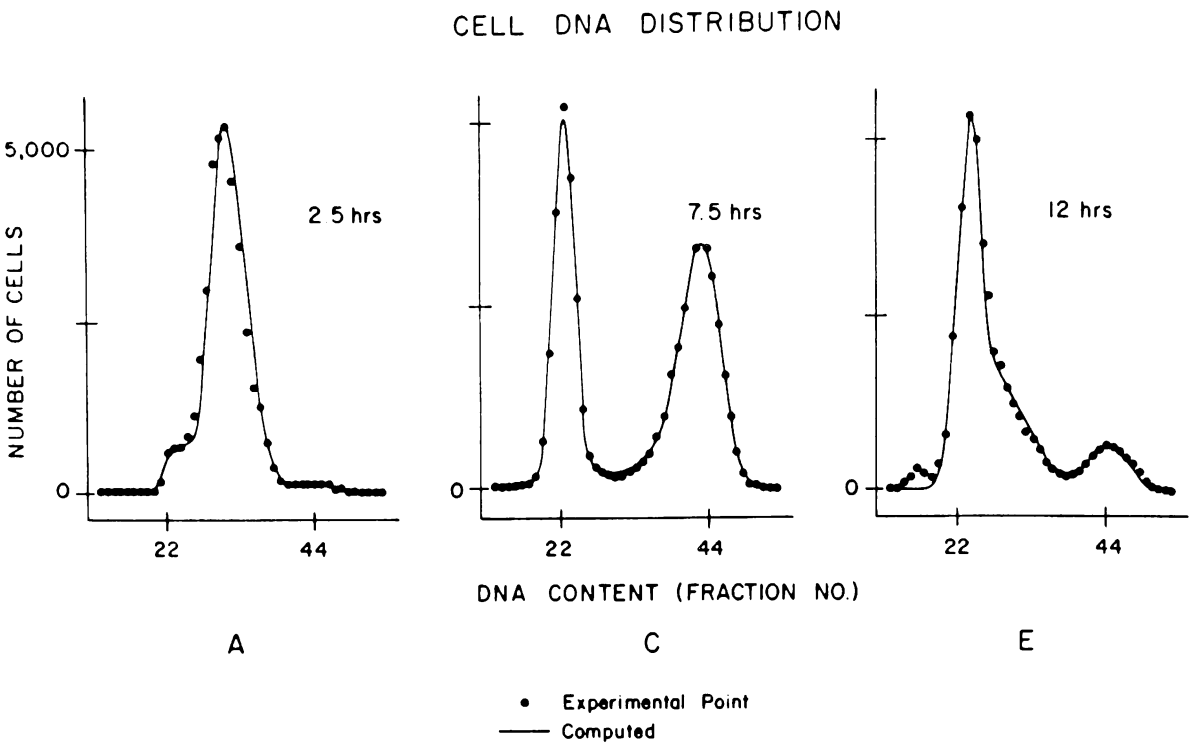

Figure 9 The computed cell DNA distributions of CHO cells at time 2.5, 7.5, and 12 hours after release from thymidine block. The experimental data are from Ref. 13.