A SIMPLE GRAPHIC METHOD OF COMPUTING THE PARAMETERS OF THE LIFE CYCLE OF CULTURED MAMMALIAN CELLS IN THE EXPONENTIAL GROWTH PHASE

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The relationship of all parameters of the life cycle of cultured mammalian cells in the exponential growth phase, namely the duration and fraction of cells in four stages, is expressed by 11 sets of equations (1–3). When computations are made of all parameters of life cycle, the calculations involving these equations are often time consuming unless a computer is used (3). The purpose of this note is to describe a simple graphic method of computing life cycle parameters by use of semilogarithmic, one cycle, graph paper.

In the exponentially growing cell population, a fraction of the cells at age t in the life cycle is proportional to $2\,e^{-at}$ (4) where $a = ln\,2/$(generation time) and an age t in the life cycle is zero at the beginning of the life cycle and equal to generation time at the end of the life cycle. Thus, the logarithm of the fraction is linearly related to age t. With these basic principles, computations by this method are as accurate as those involving the laborious calculations involving 11 equations (3). To illustrate the method of computation, we use as an example the mouse lymphoma cell line (L5178-Y) in the exponential growth phase at 37°C (3).

STEP 1 Before computation can be started, it is necessary to obtain at least four parameters of the life cycle using various methods of life cycle analysis (3). In L5178Y cells, the parameters easily obtained from experiments are (*a*) the population doubling time by counting cell numbers, (*b*) the mitotic index, (*c*) the per cent of S stage cells, pulse-labeled with tritiated thymidine, and (*d*) the duration of G_2 period by the method of Puck and Steffen (2). The fraction of dead cells

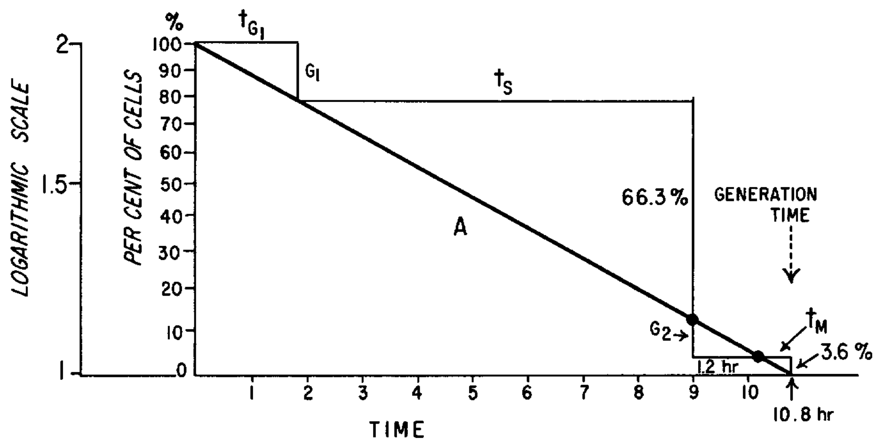

FIGURE 1 A computation of life cycle of mammalian cells in the exponential growth phase.

was estimated by eosin dye exclusion test (3) and by subtraction of maximum labeled cells from the total cell count (3). After introducing corrections for dead cells (3), the generation time was found to be 10.8 hr, the fraction of M stage cells was 3.6%; the fraction of S stage cells was 66.3%, and the duration of G2 period was 1.2 hr (3).

STEP 2 The complete life cycle can be computed with semilogarithmic, one cycle, graph paper. The time scale on the abscissa extends from 0 to 10.8 hr, the generation time. On the ordinate, only the portion from 1 to 2 is used. 0% was placed on the scale at 1, and 100% was placed on the scale at 2 Then, a straight line (*A*) was drawn from 100% to the 0 hr to 0% at 10.8 hr, the generation time (Fig. 1).

STEP 3 The next step is to build steps representing all parameters of the life cycle along the straight line (*A*). In the case of the L5178Y cell line, a vertical line of 3.6% was drawn at 10.8 hr (the generation time) to represent the fraction of cells in the M stage. Then, a horizontal line was drawn from this point to line *A*. The distance to the intercept on line *A* is equal to 0.6 hr which is the duration of M stage. From the intercept, the horizontal line was extended by 1.2 hr, the duration of G_2 period. Then, a vertical line was drawn to line *A*. The length of this line to the new intercept with line *A* is equal to 8%, the fraction of cells in G_2 stage. The vertical line is extended to 66.3% representing the fraction of S stage cells, and a new horizontal line was drawn from this point toward the zero time. The length of this line to the intercept with line *A* is equal to 7.2 hr, equal to the duration of S period. From the new intercept, a vertical line was drawn to 100%. This equals 22%, the fraction of cells in G_1 stage. Then, a new horizontal line was drawn to the 0 hr. The length of this line is 1.8 hr, the duration of G_1 stage.

The principle involved in the graphic method is the same as that in the more lengthy calculations of life cycle parameters using the equations. This method can be used not only to compute all the parameters of life cycle, but, also, to determine what parameters of life cycle must be experimentally estimated in order to complete the life cycle analysis.

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