KINETICS OF CELL VOLUME CHANGES OF MURINE LYMPHOMA CELLS SUBJECTED TO DIFFERENT AGENTS IN VITRO

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ABSTRACT Cell volume distributions obtained with an electronic particle analyzer were used to study the changes in volume of individual cells in the absence of cell division. Cultures of murine lymphoma (strain L5178-Y) cells in suspension were used in these studies. During a division delay following ionizing radiation, individual cells increased exponentially in volume with equal rate constants; these rate constants were indistinguishable from that describing the increase in cell number of an unirradiated population. When an originally log phase population of cells was prevented from increasing in number by inhibitors of DNA synthesis, individual cells increased exponentially in volume for about one generation time with the same rate constant as observed after exposure to ionizing radiation; thereafter, only the cells defining the upper half of the volume distribution continued to increase in volume, and they apparently did so with a first order rate constant proportional to their amount of DNA exceeding that present in one diploid complement of chromosomes in G_1. Cells arrested in mitosis with colchicine increased in volume for approximately 4 hr after which they remained constant in volume for almost one generation time; eventually these cells again increased in size. Inhibitors of protein and RNA synthesis inhibited the cell volume growth of irradiated cells.

INTRODUCTION

The advent of an electronic particle-analyzer which obtains the particle volume distribution has greatly facilitated studies on growth and division of cells in suspension. Some theoretical aspects of such an analyzer have already been described (1). Recently it has been demonstrated by Bell and Anderson how the volume spectrum of a log phase population can be used to study the properties of cellular growth and division (2). In the present paper we describe some studies on originally log phase populations of cells which have been treated with agents which eventually prevent an increase in cell number. The kinetics of individual cell volume growth, under

conditions when the population is constant in number, is inferred from observed changes in median cell volume and shape of the volume spectrum with time. Results of such studies, using ionizing radiation and chemical agents to inhibit increases in population number are discussed and compared.

MATERIALS AND METHODS

Fisher's murine lymphoma L5178-Y cells, which remain essentially spherical throughout the cell cycle and which grow in suspension with a doubling time of 8-11 hr, were used in all the studies. The approximate time table for the cell cycle of these cells grown under similar conditions (3) is: M, 0.5 hr; G_1, 1.8 hr; S, 7.3 hr; and G_2, 1.2 hr. The culture medium consisted of S103 (4) supplemented with 1.76% (by weight) glutamine and 10% (v/v) horse serum. The cells were inoculated at a concentration of about 2×10^4 cells/ml; hence, no dilution was necessary (except for the controls) for purposes of counting and obtaining the volume distribution without coincidence loss and without distortion of the distribution in cellular volumes.

An electronic particle counter and analyzer (1, 5) in combination with a Coulter orifice, 100 μ in diameter and about 75 μ long, was used to obtain the cell volume spectra. Each spectrum was obtained in 100 channels of an available 400 and was defined by 5000 or more events.

In order to obtain the median cell volumes of populations of cells as expeditiously as possible and to facilitate the calculations needed for plotting representations of the volume distributions, the subtotal integral of each distribution was automatically performed by a TMC Resolver Integrator, Model 522, and then driven to an IBM typewriter by a TMC Typewriter Drive Unit, Model 510.

The lower limit for the integrations was set for the channel in which the minimum number of counts appeared. Although it was possible to represent a single volume distribution with about 80–90 points, for purposes of clarity, only some of them will be shown on the graphs in the present paper.

The source of ionizing radiation was either a 250 kv X-ray machine operating at 15 ma filament current (half-value layer [HVL] = 1.5 mm Cu) or γ-rays from a ^{60}Co source. The approximate dose rate from the X-ray machine was 80 rads/min and the dose rate from the ^{60}Co source was 10,000 rads/min. Cell suspensions were irradiated at concentrations between 1×10^5 and 5×10^5 cells/ml and then diluted into fresh medium at 37°C. The radiations were done at room temperature and an unirradiated control sample was carried along in all other procedures.

The following chemical agents were used in the present study: fluorodeoxyuridine (FUdR) and hydroxyurea (HU), inhibitors of DNA synthesis; cycloheximide, an inhibitor of protein synthesis; actinomycin D, an inhibitor of RNA synthesis; and colchicine, a mitotic inhibitor. The chemical solutions were made up with distilled water and filtered through a Nalgene filter unit (Nalgene Laboratory Wares, Rochester, N. Y.) with a 0.45 μ grid membrane before being added to the cell suspensions. These solutions were added in volumes too small to cause any detectable osmotic effects.

The cultures were kept in tightly stoppered Erlenmeyer flasks in a constant temperature water bath (37°C) between samplings, and sterile technique was employed when aliquots were taken. Before a measurement was made, the aliquot (about 5 ml) was withdrawn and expelled against the side of the sample bottle about eight times with a pipette in order to minimize cell clumping.

Each experiment was performed two or more times with similar results. In the present

paper, however, results of single sets of experiments are shown. In most cases the concentrations of chemical agents used were just sufficient to prevent an increase in cell number.

THEORY

In order to facilitate analyses of possible changes in shapes of cell volume spectra, a mathematical description for the volume distribution of a log phase population was sought. It was found that the volume spectrum for L5178-Y cells in log phase growth can be represented quite well by a log normal distribution. That is,

$$N(v) = \frac{C}{v} \text{EXP} - \left[\frac{(\log v - \mu)^2}{2\sigma^2}\right]$$

where, $N(v)$ is the number of cells per unit volume at v. C is a normalization constant such that the area under the distribution of log v is unity. μ is the mean log volume. σ^2 is the variance of log volume about μ.

In other words, the logarithms of the cellular volumes are described by a Gaussian relationship and when the percentage of cells with volumes equal to or less than v is plotted against v on logarithmic probability paper, a straight line results. During a period when the cells are not dividing, changes in the volume distribution curves may be interpreted in terms of changes in volume of individual cells more readily than if the spectrum were always partly defined by newly formed daughters. Furthermore, when division comes to a halt shortly after the cells have been treated, changes (if any) in the volume spectrum may occur initially because of changes in volume of cells in all stages of the cell cycle. Therefore, if different portions of the volume distribution can be associated with cells in specific stages of the cycle, then the kinetics of volume growth as a function of cycle stage under the particular conditions may be determined. In the following, we will consider originally log phase populations of cells for which cell number has become constant; the original spectrum, assumed to be log normally distributed, will refer to the one existing for such a population at the time division stopped (i.e. at $t = 0$). The first two sections are brief discussions which include a comparison between exponential and linear volume growth in terms of the cell volume spectra at different times, t, after division stopped.

Volume Equal to the Product of the Cell's Original Volume and a Time-Dependent Function

If the volume of every cell within a population is given by $v_t = v_0 f(t)$ [where v_t is the volume of a cell some time, t, after cell division was halted; v_0 represents the cell's volume at $t = 0$; $f(t)$ is any time-dependent function such that $f(0) = 1$], then the volume spectra for the population at times t will be log normal and the variances about the mean log volume will be equal to that of the original distribution; mean log volumes will be given by $\mu_t = \mu + \log f(t)$ (where μ represents the mean log volume at $t = 0$).

The consequence of the above assumed kinetics in terms of the representations of the volume distributions at various times on logarithmic probability paper is that each representation will be parallel to that of the original ($t = 0$) spectrum. The time-dependent function, $f(t)$, may be estimated by measuring, at different times, any population volume parameter such as mean or median cell volume. For the particular case where $f(t) = e^{kt}$ (with k a constant), cell volume growth is exponential and log median (or log mean, etc.) cell volume will increase linearly with time (slope = k).

Volume Equal to the Sum of the Cell's Original Volume and a Time-Dependent Function

If the volume of every cell within the population at time t is given by $v_t = v_0 + f(t)$, where $f(t)$ in this case must equal zero at $t = 0$, then the volume spectra for the population will not be log normal at times greater than $t = 0$; therefore, the representations on logarithmic probability paper of these spectra will not be parallel to that of the original volume distribution. With $f(t) = kt$ (with k a constant) each cell volume increases linearly and the slope of the growth curve of median (or mean) cell volume will be equal to k.

Age Dependence of Cell Volume Changes

If the pattern of volume change depends on cell age (unlike the examples considered so far), the nature of the age dependence can be deduced from observed changes in the volume distribution only when the relation between cell age and cell volume is known. For purposes of the present paper, it will be assumed that the older the cell the greater its volume; cell age is here defined as the elapsed time from the birth of a cell by the process of division.

High Sensitivity of Method Utilizing the Distribution Shape

Utilizing the shape of the volume distribution provides a more sensitive method to deduce growth rate laws than a method which employs only the mean volume. This can be illustrated by comparing linear versus exponential growth patterns of individual cells.

For example, over a period during which the mean volume doubles, the maximum percentage difference in mean volume between an exponential and linear mean volume growth curve is only 6%. The same is true for the median or mode volume.

However, the coefficient of variation, a measure of the shape of the distribution, will remain constant over the period during which the mean volume doubles for the case of exponential growth, whereas at the end of that period it will have been reduced by 50% for case of linear growth.

RESULTS

Evidence for Log Normality of the Volume Spectrum of L5178-Y Cells in Log Phase Growth

Fig. 1, curve *A*, shows a typical representation on logarithmic probability paper of an observed volume spectrum for L5178-Y cells in log phase growth. The straight line drawn through the points was fitted by eye. The linearity of the representation implies that the volume distribution is log normal. Curve *B* shows the representation of the same data on normal probability paper for comparison.

Kinetics of Cell Volume Changes for Cells Exposed to Ionizing Radiations

In Fig. 2 *a*, curves *A* and *B* show the log cell number and log median cell volume, respectively, of unirradiated L5178-Y cells as a function of time. Curves *C* and *D*, respectively, depict the same parameters plotted for a culture exposed to approximately 1000 R of γ-radiation. It will be observed that during the period when the number of cells in the irradiated population did not increase (i.e. during the radiation-induced division delay) the median volume increased with a rate constant indistinguishable from that describing the increase in cell number of the unirradiated control culture. Both parameters, cell number and median cell volume, are plotted in arbitrary units and all curves were made to coincide on the graph at the first observation point.

Fig. 2 *b* shows some representations of the volume distributions on logarithmic probability paper for the nonirradiated population (curve *A*) and irradiated population (curves *B* and *C*) obtained at different times during the same experiment. The

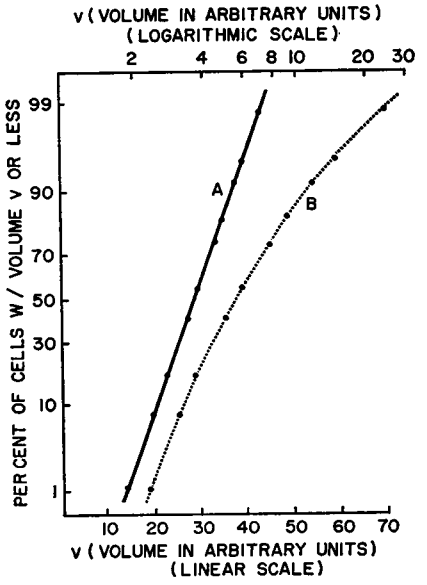

FIGURE 1 Typical representations of a volume spectrum for L5178-Y cells in log phase growth on *A*, logarithmic; and *B*, normal probability paper.

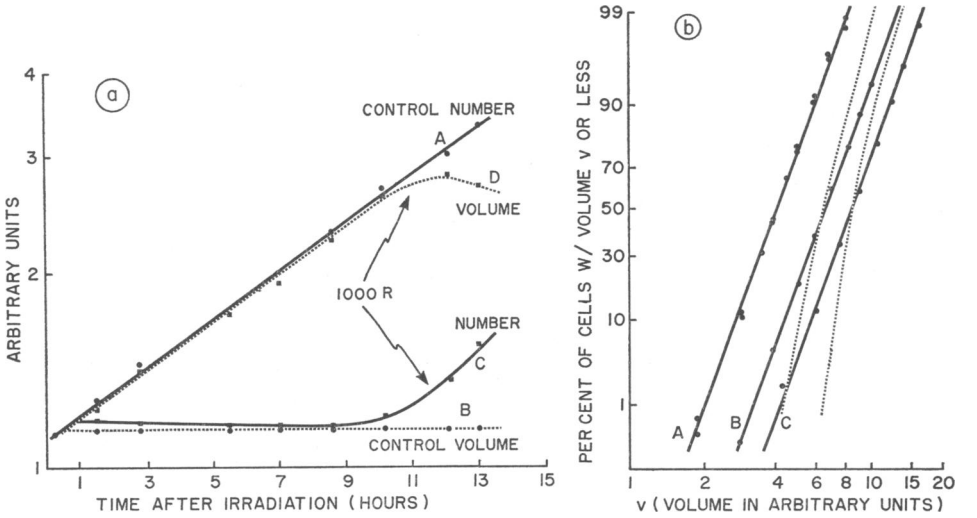

FIGURE 2 *a* Semilogarithmic plots against time of median cell volume (curves *B* and *D*) and cell number (curves *A* and *C*) for a population irradiated with 1000 R γ-rays (*C* and *D*) and for an untreated population (*A* and *B*). (*b*) Representations of the volume spectra obtained at different times on logarithmic probability paper; curve *A*, the control population at the beginning of the experiment and 10 hr later; curves *B* and *C*, the irradiated population 6 hr and 10 hr, respectively, after exposure. The dotted lines indicate the expected representations for *B* and *C* under the assumptions of linear cell volume growth (see text).

representation of the control population was essentially invariant with respect to both shape and median volume during the course of the experiment. The solid lines labeled *B* and *C* were constructed parallel to line *A* which was fitted to the data (indicated by the solid symbols) by eye. Curve *C* represents the volume distribution obtained at the end of the division delay period. The dotted lines indicate the theoretical representations for the irradiated population assuming the volume of each cell increased linearly from the time of onset of division delay to the time the distribution was obtained with a rate constant equal to that observed for the median volume.

Similar experiments were performed with different doses of ionizing radiations from 100 R to 1000 R of X-radiation and from 200 R to 6700 R of γ-radiation. The results with regard to the kinetics of median cell volume changes and representations of the volume distributions during the division delay were similar to those described above; only the duration of division delay depended on radiation dose. For example, after 6700 R the median cell volume did not begin to level off until after 1.9 doubling times for cell number in the untreated population.

Kinetics of Volume Changes during the Absence of Cell Division Induced by Inhibitors of DNA Synthesis

When a log phase population of cells was exposed to either FUdR (10^{-8} M) or HU (10^{-3} M), known inhibitors of DNA synthesis, cell division stopped approximately

2 hr later (i.e. after a time $\simeq G_2 + M$ for these cells). In Fig. 3 *a*, curve *C* shows the observed changes in median cell volume (log scale) as a function of time for cells exposed to FUdR or HU. The arrow indicates the time at which FUdR and HU were added to the cell suspensions; note that the median cell volume does not begin to increase until about 2 hr later (at which time cell division had stopped as was indicated by the absence of further increase in number of cells). Curve *A* shows the observed increase in cell number (log scale) of the untreated population as a function of time while curve *B* displays the changes in the median cell volume of that population. The median cell volume of the control population was observed to increase slightly during the course of the experiment (indicating a slightly unbalanced growth situation). When the slope of the steeply rising portion of curve C was reduced by that observed for curve *B*, the corrected slope was indistinguishable from that of curve *A* which describes the increase in cell number of the control popula-

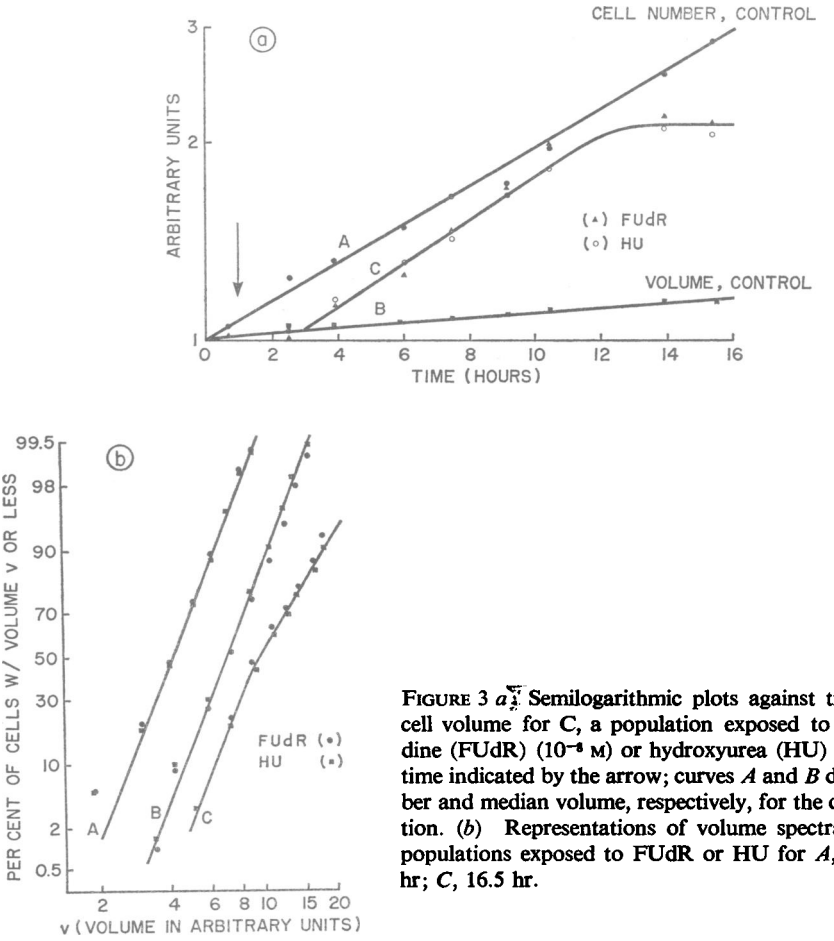

FIGURE 3 *a*, Semilogarithmic plots against time of median cell volume for C, a population exposed to fluorodeoxuridine (FUdR) (10^{-6} M) or hydroxyurea (HU) (10^{-3} M) at the time indicated by the arrow; curves *A* and *B* depict cell number and median volume, respectively, for the control population. (*b*) Representations of volume spectra obtained for populations exposed to FUdR or HU for *A*, 1.5 hr; *B*, 7.5 hr; *C*, 16.5 hr.

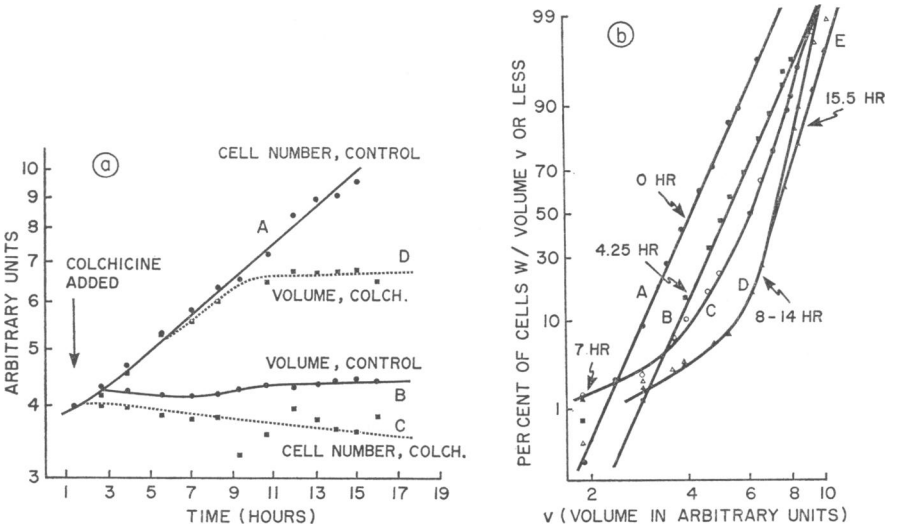

FIGURE 4 *a* Semilogarithmic plots against time of median cell volume (curves *B* and *D*) and cell number (curves *A* and *C*) for a population exposed to 10^{-4} M colchicine (*C* and *D*) and for an untreated population (*A* and *B*). (*b*) Representations of the volume spectra for the population exposed to colchicine for the indicated times.

tion.[1] After the population had been exposed to FUdR or HU for about one mean doubling time, the median cell volume was no longer observed to increase; it remained essentially constant during the remaining observation period of 6.5 hr (only 4 hr are indicated in Fig. 3 *a*).

Fig. 3 *b* depicts the representations of the volume distributions for the cells exposed to HU and FUdR observed at three different times: A, just before the population stopped increasing in number; B, about 6 hr; and C, 15 hr after division stopped (and about 6.5 hr after the median cell volume curve leveled off—see Fig. 3 *a*).

Representations of the volume distributions taken at other times during the steeply rising portion of curve *C* in Fig. 3 *a* were all essentially parallel to curves *A* and *B* of Fig. 3 *b*. The two distinct, approximately linear, portions of curve *C* in Fig. 3 *b* indicate that during the period when the median cell volume remained constant, the volume distribution was becoming bimodal. The upper portion of curve *C* was truncated because at the particular amplifier gain setting used for the observation, signals larger than for the highest channel (due to large cells which represented 5–10% of the population) were automatically made to appear in the upper five to seven channels of the analyzer.

[1] In another experiment in which HU was used to inhibit DNA synthesis, the median cell volume for the untreated population remained constant during the course of the experiment; the rate of increase of the medium volume of the treated population was then indistinguishable from the rate at which the cell number increased in the control.

Blockage of Mitosis with Colchicine

In Fig. 4 *a*, curve *C* displays the observed effect of colchicine (10^{-4} M) on cell number as a function of time; there is no detectable increase in number following the addition of colchicine. Curve *A* describes the increase in cell number for the control population. Curve *D* depicts the change with time of the median cell volume for the population of cells exposed to colchicine and curve *B* denotes the slight variation in median cell volume of the control population. The median cell volume of the population exposed to colchicine was seen to increase for about 7.8 hr (0.8 of the control doubling time) up to approximately 1.6 times that of the control population and then to remain essentially constant for 7.5 hr.

Fig. 4 *b* shows the graphical representations on logarithmic probability paper of some of the volume distributions obtained at various times during the course of the experiment. Curve *A* represents the volume distribution at the time just before colchicine was added to the cell suspension. Curves *B*, *C*, *D*, and *E* depict the progress of the volume spectrum in chronological order for the population of cells exposed to colchicine. It should be noted that until more than 4 hr in the presence of colchicine the representations of the volume spectra remained parallel to that of the original log phase population. Curve *D* represents the spectra obtained between 8 and 14 hr after the addition of colchicine; note that these times were all during the interval for which the median cell volume remained constant (see Fig. 4 *a*) and that the slope of the main portion of curve *D* is greater than the slope of curve *A*. The lowest portion of the curves obtained after curve *B* and which represent from 8 to 10% of the observed events indicate that a small percentage of cells had undergone division. Curve *E* displays the volume distribution obtained 15.5 hr after colchicine was added and the larger cells seem to have begun to increase again in size as indicated by the displacement of the upper portion of the spectrum with respect to curve *D*.

Similar results were obtained using concentrations of colchicine as low as 10^{-7} M. At concentrations below 10^{-7} M division was not completely halted.

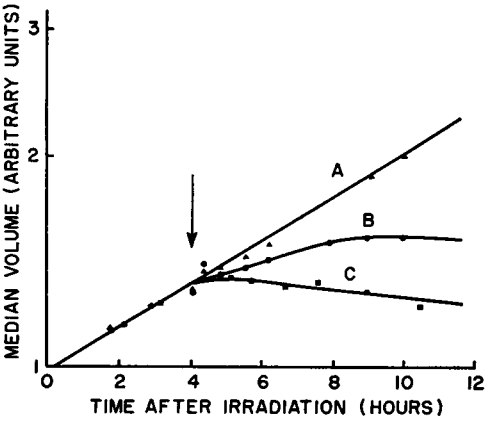

FIGURE 5 Semilogarithmic plots against time of median cell volume for populations exposed to 5000 R γ-radiation (curve *A*) and subsequently (see arrow) to 0.12 μg/ml actinomycin D (curve *B*) or 5 μg/ml cycloheximide (curve *C*).

Inhibitors of RNA and Protein Synthesis

Fig. 5 shows the effects of actinomycin D and cycloheximide on median cell volume of a previously irradiated (5000 R γ-rays) population. Curve *A* indicates the progression of the median volume for the irradiated population. Curves *B* and *C* depict the median cell volume for populations which were irradiated and subsequently exposed to actinomycin *D* (0.12 μg/ml) and cycloheximide (5 μg/ml), respectively, at the time indicated by the arrow. It will be noted that both agents were able to prevent the cell volume growth observed during a radiation-induced division delay; plots of the volume distribution (not shown) revealed that the volumes of all cells within the populations were affected by these agents in the same way as observed for the median volumes.

DISCUSSION

Ionizing Radiation

It has been known for a long time that after exposure to ionizing radiations many types of cells are prevented or delayed from dividing while they continue to increase in size for some time. The mode of individual cell volume growth for "living" bacterial (*Escherichia coli*) cells suffering a radiation-induced division delay was found to be exponential; furthermore, the first order rate constant was approximately the same as that characterizing the increase in cell number of the unirradiated populations (6). It was possible to distinguish exponential from linear volume growth because the duration of division delay was long compared to the interdivision time of the bacterium and cell volume could be measured over a period during which it increased tenfold. Also, volume changes could be determined by measuring the length of an individual cell at different times because the diameter of these rod-shaped organisms remained essentially constant; thus, a 30% increase in length reflected a 30% increase in bacterial volume.

In contrast, when many other types of cells (e.g. mammalian cells) are exposed to ionizing radiations, the duration of the induced division delay is often less than or about equal to the interdivision time of the cell. Therefore, the kinetics of cell volume changes following irradiation would be more difficult to determine for mammalian cells, especially if the volume is calculated from optical measurements of cell diameter; a 30% increase in cell volume corresponds to only about a 9% increase in diameter.

Some workers have studied the mean cell volume, as calculated from the concentration and the "hematocrit" (7), and the modal volume of populations of irradiated mammalian cells; the latter determined from volume distributions obtained with instruments based on the same principle as the one used in the present study (8). However, any method which employs only a population parameter is not only less sensitive for distinguishing between two possible growth models than one which considers the entire volume spectrum (as illustrated in the Theory section), but it

also cannot reveal whether the individual cells within the population are exhibiting the same kinetics as observed for that parameter.

Our studies on irradiated L5178-Y cells utilizing the cell volume distribution are compatible with the hypothesis that during a radiation-induced division delay nearly every cell within the population increases exponentially (not linearly) in volume with equal rate constants; these rate constants are independent of radiation dose and, furthermore, are indistinguishable from the constant describing the increase in cell number for an untreated population in log phase growth (see Figs. 2 *a* and 2 *b* and the Theory section).

Since the irradiated cells were originally in log phase growth and since division ceases shortly after irradiation, the volume distribution was initially defined mainly by cells which were in all parts of S, G_1, and G_2 phases of the cell cycle; therefore, the results indicate that the kinetics of cell volume growth after irradiation are the same during these cell cycle stages. If it is assumed that irradiated G_1 cells enter and proceed through the S phase of the cycle without any appreciable delay and that irradiated S cells continue to synthesize DNA (although possibly at a reduced rate) as has been shown for HeLa $S3$ cells (9), then our results suggest that *synthesis* of DNA does not in itself affect the rate of cell volume changes. Also, since for at least a period of time equal to G_1, or about 2 hr after exposure, the amount of cellular DNA presumably ranged from one to two diploid complements of that present in chromosomes in G_1, our results indicate that the amount of DNA is not the rate-limiting factor for volume changes. However, results from experiments in which inhibitors of DNA synthesis were used (discussed in the following section) indicated that the amount of cellular DNA can eventually become the rate-limiting factor for such changes.

Inhibitors of DNA Synthesis

Figs. 3 *a* and 3 *b* depict the results obtained when an originally log phase population of cells was exposed to inhibitors of DNA synthesis. The experiment illustrates a situation where a population parameter (median cell volume) reflects changes in volume of every cell over one interval of time but does not do so during another.

During the interval of time for which the median cell volume was rapidly increasing, each cell within the population apparently increased exponentially in volume with the same rate constant as observed during a radiation-induced division delay. One would expect from the time table for the cell cycle of these cells and from the fact that cell division did not stop until about 2 hr after the addition of the inhibitors that the population during that interval of time consisted of cells of which about 50% were trapped in all parts of the S phase (and therefore possessed from one to two diploid complements of DNA) while the remaining cells were in G_1 (and each of them presumably contained only one diploid complement of DNA). Therefore, the results obtained during the period when the median cell volume was increasing sup-

port the suggestions made in the immediately preceding section regarding the independence of the rate constant to the amount and to the synthesis of cellular DNA.

However, about one mean doubling time after the cells were first exposed to HU or FUdR, the median cell volume ceased to increase significantly. The representations of the volume distributions obtained thereafter showed that larger cells, represented by the upper half of the volume spectrum (presumably those trapped in the *S* phase of the cycle), continued to increase in size while the cells represented by the lower half of the spectrum (presumably all of which were blocked at the G_1–*S* boundary by this time) remained essentially constant in volume. Furthermore, the cells which continued to increase in volume did so with apparently unequal first order rate constants as indicated by the changing slope of the upper half of the representation of the volume distribution. (Curve *C* in Fig. 3 *b* represents the final volume distribution obtained during the experiment). The rate constant for the largest cells (those which probably had almost two diploid complements of DNA) was approximately equal to that observed for all cells during the period when the median volume was increasing rapidly.

The simplest interpretation which is compatible with all of our observations is that the amount of cellular DNA was not the rate-limiting factor for volume growth until after one generation time; thereafter, individual cells began to increase in volume with first order rate constants proportional to the amount of DNA exceeding that present in one diploid complement of chromosomes in G_1. That the observed increase in cell volume for cells treated with inhibitors of DNA synthesis did indeed reflect a corresponding increase in RNA and protein is supported by the work of other investigators (9, 10). These investigators found, however, that RNA and protein synthesis essentially stopped in HeLa cells after about one mean doubling time in the presence of inhibitors of DNA synthesis. Clearly, the relationship between volume growth and measured synthesis under these conditions (i.e. after treatment with inhibitors of DNA synthesis) need to be studied in further detail.

Arrest in Mitosis with Colchicine

According to current concepts, DNA acts as a template for RNA synthesis, and mRNA, which in most cells has a limited life time, in turn specifies protein synthesis. In the absence of mRNA, proteins cannot be synthesized. If, in the present experiments, cell volume changes reflect protein synthesis, an arrest of volume increase may have been caused by the decrease or absence of mRNA in the cell.

The results recorded in Fig. 5 would tend to support these assumptions. Firstly, in Fig. 5 curve *C* shows that cycloheximide prevents, almost immediately, a further increase in cell volumes, as is to be expected if the inhibitor of protein synthesis acts directly at the level of the ribosomes. The results of the experiment in which actinomycin was used to inhibit cell volume growth of previously irradiated cells (Fig. 5, curve *B*) can best be interpreted in terms of a short-lived mRNA. Thus, the 4 hr be-

tween the time the inhibitor was added and the time at which the median cell volume stopped increasing may be taken as a rough estimate of the average lifetime for mRNA if it is assumed that actinomycin immediately prevents synthesis of mRNA. Other workers have obtained similar results using other experimental approaches. For example, Tobey et al. reported a 3 hr lifetime for division-associated mRNA in hamster CHO cells (11) and Tobey and Campbell found that CHO cells treated with actinomycin incorporated leucine for 4.5 hr (12).

Colchicine in sufficient concentration has been shown to arrest cells in metaphase or in a quasi-metaphase, stages in which the chromosomes are highly condensed and believed to be unavailable as templates for (m) RNA synthesis (13–17). Thus, under the above assumptions any increase in the volume of cells arrested with colchicine could be due to the utilization for protein synthesis of mRNA which had been made previously in these cells, and the period during which the arrested cells continue to increase in volume would be an estimate of the average lifetime for mRNA in the mitotic cells. Observations of volume spectra at different times after an originally log phase population of cells has been exposed to colchicine might, therefore, also be used to obtain such an estimate.

For example, curve *B* in Fig. 4 *b* represents the volume distribution on logarithmic probability paper of the population which was exposed to colchicine (and which stopped increasing in number) 4.25 hr before the spectrum was obtained; the fact that the representation was still parallel to that of the original log phase population (curve *A*) indicates that every cell within the population increased in volume by the same percentage over the 4.25 hr period. The representations of the volume distributions obtained at times after the one designated by curve *B* in Fig. 4 *b* indicated by their curvature that the larger cells (presumably the arrested cells) were unable to increase further in volume (until about one generation time later) while the smaller cells continued to grow in volume. In view of the above discussion, the method utilizing the shapes of the volume distributions for cells exposed to colchicine yields a rough estimate for the average lifetime of mRNA in mitotic L5178-Y cells of about 4.25 hr which is in good agreement with the estimate obtained from the experiments in which actinomycin was used to block RNA synthesis; we feel the agreement supports the above hypothesis regarding the effect of colchicine on cell volume growth.

That the cells treated with colchicine were able to increase once more in size suggests that their chromosomes eventually decondensed to a state which once again supports mRNA synthesis. The eventual resorption of C mitoses in colchicine or colcemide is well documented (18, 19).

SUMMARY AND CONCLUDING REMARKS

The kinetics of individual cell volume changes for cultured L5178-Y cells during the absence of cell division was inferred from observed changes in median cell volume

and in shape of the volume spectra with time. The results of such studies, using chemical agents and ionizing radiations to stop division, have suggested the following: (*a*) The kinetics of cell volume growth for these cells is probably independent of all stages (G_1 , G_2 , S, M) of the cell cycle; the cell increases exponentially (not linearly) in volume with a first order rate constant which is indistinguishable from that which describes the increase of cell number for a log phase population in balanced growth. (*b*) The average lifetime for mRNA is about 4 hr under the specified conditions. (*c*) DNA synthesis, per se, does not contribute significantly to changes in cell volume. (*d*) The amount of DNA, ranging from one to two diploid complements, is not the rate-limiting factor for volume growth until after about one generation time, at which time each cell begins to increase in volume with a first order rate constant proportional to the amount of DNA exceeding one diploid complement.

Ionizing radiation of doses up to 6700 R apparently do not alter the kinetics of cell volume growth. Also, the observation that nearly every cell continued to increase in volume for more than one mean doubling time after exposure to sufficiently high doses of radiation implies that DNA replication did occur in irradiated cells as was argued in the discussion on ionizing radiations. If DNA synthesis, in these nondividing cells, proceeds only up to the premitotic amount (i.e. two times the G_1 amount) and if one generalizes the conclusions reached from the experiments in which inhibitors of DNA synthesis were used, then one might not expect irradiated cells to increase in volume for more than approximately two generation times. In fact, following 6700 R γ-irradiation the median cell volume stopped increasing after 1.9 mean doubling times for cell number in the control population.

The cessation and eventual resumption of cell volume growth of cells exposed to colchicine may be explicable on the basis that condensed chromosomes, unlike decondensed chromosomes, are unable to serve as templates for RNA synthesis. However, the mechanism is more obscure that regulates volume growth in cells rendered incapable of synthesizing DNA and presumably not having condensed chromosomes. Cytological and biochemical studies performed concomitantly with physical measurements of cell volume should aid in the further understanding of the observed kinetics of cell volume changes under different conditions.

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REFERENCES

1. GREGG, E. C., and K. D. STEIDLEY. 1965. *Biophys. J.* **5**:393.
2. BELL, G. I., and E. C. ANDERSON. 1967. *Biophys. J.* **7**:329.
3. WATANABE, I., and S. OKADA. 1967. *J. Cell Biol.* **32**:309.
4. SWIM, H. E., and R. F. PARKER. 1958. *J. Lab. Clin. Med.* **52**:309.

5. ADAMS, R. B., W. H. VOELKER, and E. C. GREGG. 1967. *Phys. Med. Biol.* **12**:79.
6. LEA, D. E. 1956. Actions of Radiations on Living Cells. University Press, Cambridge, 2nd edition.
7. KELLY, L. S., J. D. HIRSCH, G. BEACH, and N. L. PETRAKIS. 1957. *Proc. Soc. Exp. Biol. Med.* **94**:83.
8. ELKIND, M. M., A. HAN, and K. W. VOLTZ. 1963. *J. Nat. Cancer Inst.* **30**:705.
9. RUECKERT, R. R., and G. C. MUELLER. 1960. *Cancer Res.* **20**:1584.
10. COHEN, L. S., and G. P. STUDZINSKI. 1967. *J. Cell Physiol.* **69**:331.
11. TOBEY, R. A., E. C. ANDERSON, and D. F. PETERSON. 1966. *Proc. Nat. Acad. Sci. U. S. A.* **56**:1520.
12. TOBEY, R. A., and E. W. CAMPBELL. 1965. *Virology.* **27**:11.
13. HSU, T. C. 1962. *Exp. Cell Res.* **27**:332.
14. JOHNSON, J. C., and J. J. HOLLAND. 1965. *J. Cell Biol.* **27**:565.
15. TERASIMA, T., and L. J. TOLMACH. 1963. *Exp. Cell Res.* **30**:344.
16. PRESCOTT, D. M., and M. A. BENDER. 1961. *Exp. Cell Res.* **26**:260.
17. TAYLOR, J. HERBERT. 1960. *Ann. N. Y. Acad. Sci.* **90**:409.
18. PUCK, T. T., and J. STEFFEN. 1963. *Biophys. J.* **3**:379.
19. PUCK, T. T., P. SANDERS, and D. PETERSON. 1964. *Biophys. J.* **4**:441.