TIME LAPSE CINEMICROGRAPHIC STUDIES OF X-IRRADIATED HeLa S3 CELLS

I. Cell Progression and Cell Disintegration

CAMILLA HURWITZ *and* L. J. TOLMACH

From the Division of Radiation Biology, Mallinckrodt Institute of Radiology, School of Medicine, and the Committee on Molecular Biology, Washington University, St. Louis, Missouri 63110

ABSTRACT Time-lapse cinemicrographs of synchronous HeLa S3 cells irradiated with 220 kv X-rays at various stages of interphase provided data for constructing pedigrees, measuring the duration of both generation cycles and mitoses, and scoring events associated with cell disintegration for up to seven postirradiation generations. The onset of the first mitosis after doses of 500 rads was delayed as expected from previous studies of the age dependence of "mitotic delay." The interval between this first mitosis and the next was indistinguishable from that for unirradiated control cells, while the subsequent two generations were again prolonged, on the average, though not so severely as was the irradiated generation. The duration of mitosis was increased proportionally more than interphase. Cell disintegration took place by way of two morphologically distinct processes. In three-quarters of the cases the cells were rounded and apparently trapped in metaphase when they disintegrated; the remaining disintegrations occurred in spread, interphase cells. In cells disintegrating from the rounded configuration, the generation preceding disintegration was prolonged relative to that in cells which divided; in cells disintegrating from either configuration, the penultimate generation was also prolonged. The mitotic times were disproportionately increased in both of these generations. It is suggested that in this system X-ray damage is preferentially expressed as derangement of the mitotic process; such damage ultimately brings about permanent mitotic arrest in the majority of cells.

INTRODUCTION

The molecular events that give rise to the cellular manifestations of exposure to ionizing radiations are obscure. In particular, the mechanism(s) by which mammalian cells lose the capacity for sustained proliferation (Puck and Marcus, 1956) have yet to be elucidated. In the case of this most important consequence of radiation exposure, the conventional methodology—scoring of the fraction of cells that gives

rise to macroscopic colonies after many days of incubation—does not provide observational data concerning changes that occur during the first few days following irradiation. During this period, the cell or its descendants lose the capacity to divide and give rise to so called abortive colonies, giant cells are formed, and cell disintegration occurs.

In efforts to describe these aspects of cellular response to irradiation, a number of investigations have been concerned with the physiological and morphological characteristics of irradiated cells. Time-lapse cinemicrography consitutes a useful technique for such studies, as essentially continuous observations of irradiated cells can be recorded. These provide information that might offer clues to the subcellular changes caused by the radiation, changes that may be pertinent not only to loss of reproductive ability, but also to other aspects of the cellular radiation syndrome. The fate of the irradiated cell can be determined, and comparisons can be made between irradiated and control populations that permit quantitative assessment of: (*a*) distortions of the cell generation cycle, (*b*) cell disintegration, (*c*) induction of cell fusion and giant-cell formation, and (*d*) multipolar mitosis. These responses to irradiation can be examined as a function of cell age, radiation dose, and environmental conditions. This paper deals with (*a*) and (*b*); (*c*) and (*d*) will be the subject of another report.

The present study, in which the doses of radiation administered were such that very few of the cells were able to continue to proliferate indefinitely, has involved analysis of the histories of substantial numbers of irradiated cells for up to seven post-irradiation generations. The results not only permit refinement of previous descriptions of the behavior of X-irradiated HeLa S3 cells (Marin and Bender, 1966; Froese, 1966), but also reveal hitherto unreported aspects of the radiation response of these cells.

MATERIALS AND METHODS

Cultures of HeLa S3 cells with a generation time of about 18 hr were maintained by conventional techniques in medium N16HHF (Ham and Puck, 1962). Synchronous populations were obtained by mitotic selection (Terasima and Tolmach, 1963 *a*) of cells that had been grown for 2 days in medium N16FCF (Phillips and Tolmach, 1966) after trypsinization. The collected mitotic cells, in medium N16FCF, were introduced into two plastic Petri dishes and and were incubated under standard conditions (4.8% CO_2 in air saturated with water vapor, at 38°C) until irradiation.

Irradiation of one of the two dishes was carried out with 220 kv X-rays (constant potential; 15 ma; added filtration of 0.25 mm Cu + 1.0 mm Al, yielding a half-value layer of 1.0 mm Cu; dose rate 83 rads/min; 37°C; gas phase 4.8% CO_2 in air). The second Petri dish served as a sham-irradiated control. Doses of 500 rads were used for most of the experiments, yielding from about 1 to 20% colony-forming cells, depending on the age of the cells at the time of irradiation (Tolmach et al., 1965). A few experiments were performed with 1000 or 1500 rad doses.

Postirradiation incubation of the two dishes was carried out on the stages of two inverted microscopes housed in a single incubator maintained under standard conditions.

Filming commenced shortly after irradiation, and was carried out with 16 mm Kodak Plus-X reversal film, processed without reversal. Using ×10 phase contrast objectives, the light intensity could be maintained at a level sufficiently low to permit continuous illumination without detectable effects on the cells; the mean generation time of unirradiated cells was 18.4 hr in the three generations subsequent to plating (see Table II). Fields containing about 50 cells were selected, and exposures were made at regular intervals, about 5 min apart, for up to 7 days. Analyses of 10 films of irradiated cells are included in this report. Table I lists the X-ray dose for each film, the time after collection of mitotic cells at which irradiation took place, and the presumptive corresponding stage of the cell cycle.

Analysis of films, which was facilitated by use of a Tagarno 16 projector, permitted the construction of histories of individual cells. The times of occurrence of cell rounding, division, and disintegration were determined; these events are described below. Although an attempt was made to record the history of each cell present in the initial frame, and its descendants, this was often not possible: inadequacies of the optics precluded following many of the cells especially in the periphery of the field, some migrated out of the field, others were obscured by debris from cells that had already disintegrated, and control cells were inevitably lost from observation because of crowding after a few days of growth. It is assumed that no bias has been introduced into the data by the absence of complete pedigrees for each cell. Analysis of one of the films was carried out independently by two observers, with nearly complete agreement.

Construction of cell histories involved identification of a number of events:

1. On entering mitosis, a cell gradually became refractile and assumed a nearly circular outline, *R* (for round); this was retained until the cell divided or disintegrated (Fig. 1). The metaphase plate was often discernible in at least one frame during mitosis (e.g., frames 216, 220); its frequency of detection varied among the films (see Table V), presumably as a function of precision of focusing. Since the circular shape was assumed only gradually, and the state of the nucleus could not be ascertained in these films, the time at which a cell entered mitosis could not be determined precisely. Accordingly, *R* was assigned to the frame at which the cell reached an essentially constant area (frame 214 in Fig. 1). While this convention probably identified a point after the inception of mitosis, it is assumed that it was a common point early in the mitotic process.

TABLE I
AGE OF CELLS AT IRRADIATION AND X-RAY DOSE EMPLOYED, BY FILM NUMBER

Film No.	Time of irradiation after cell collection	Presumptive stage of cycle	Dose
	hr		*rads*
1	3.1	G1	500
2	4.8	G1	500
3	8.9	G1-S	500
4	12.1	S	500
5	15.8	S-G2	500
6	16.5	S-G2	500
7	4.0	G1	1000
8	10.0	S	1000
9	14.0	S	1000
10	3.7	G1	1500

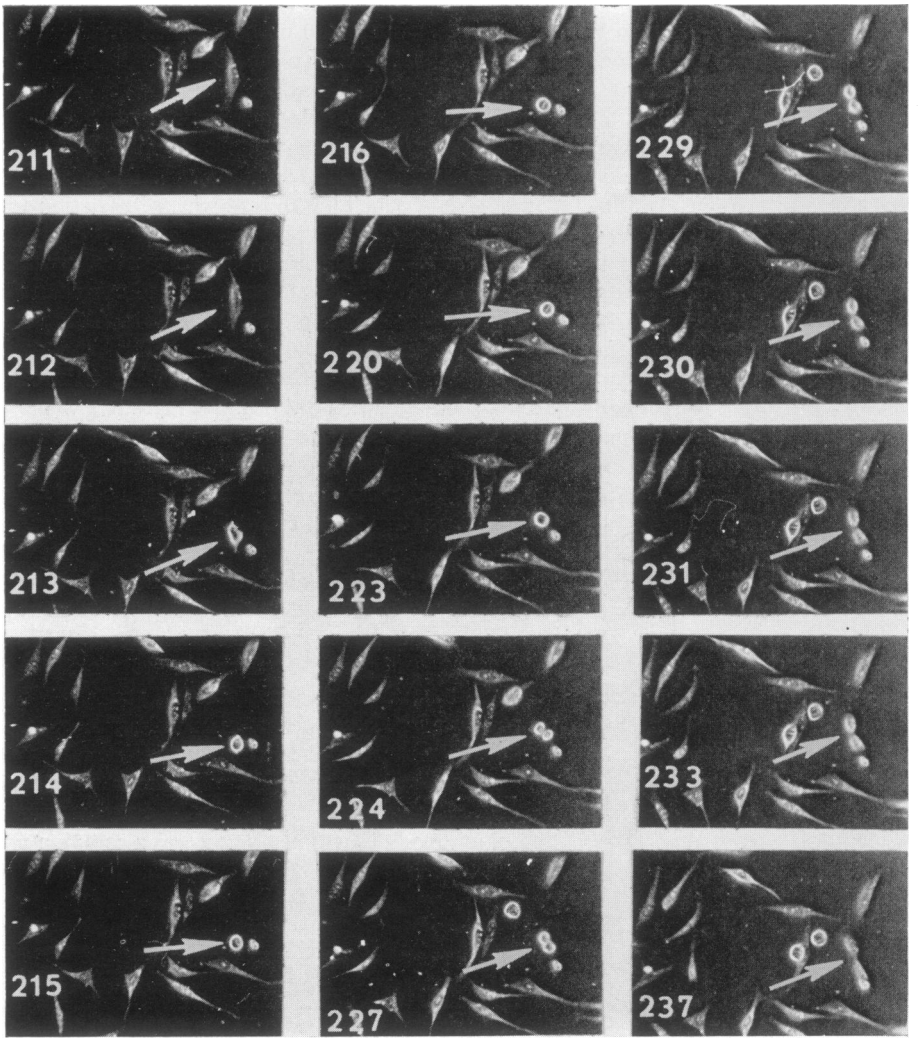


FIGURE 1 Film sequence illustrating the rounding of a cell (arrow) as it entered mitosis, *R*, and its subsequent division, *C*. Enlarged (×6.5) prints of the original film were cropped and numbered, and pertinent frames selected. Consecutive frame numbers represent exposures made 5 min apart. The sequences in Figs. 1–3 have been taken from the same film. See text for description of events. Two other cells can be seen to round in the later frames.

2. Cell division, *C* (for cytokinesis), was scored at the first frame in which either a dumbbell-shaped configuration or two separated cells could be seen (Fig. 1, frame 224). Division normally occurred within a 5 min interval and so could be scored quite precisely.

3. Disintegration of irradiated cells, *D*, could almost always be categorized as occurring according to one or another of a small number of fairly distinct patterns. It took place both in spread, interphase cells, D_s, and, more frequently, in cells which had apparently

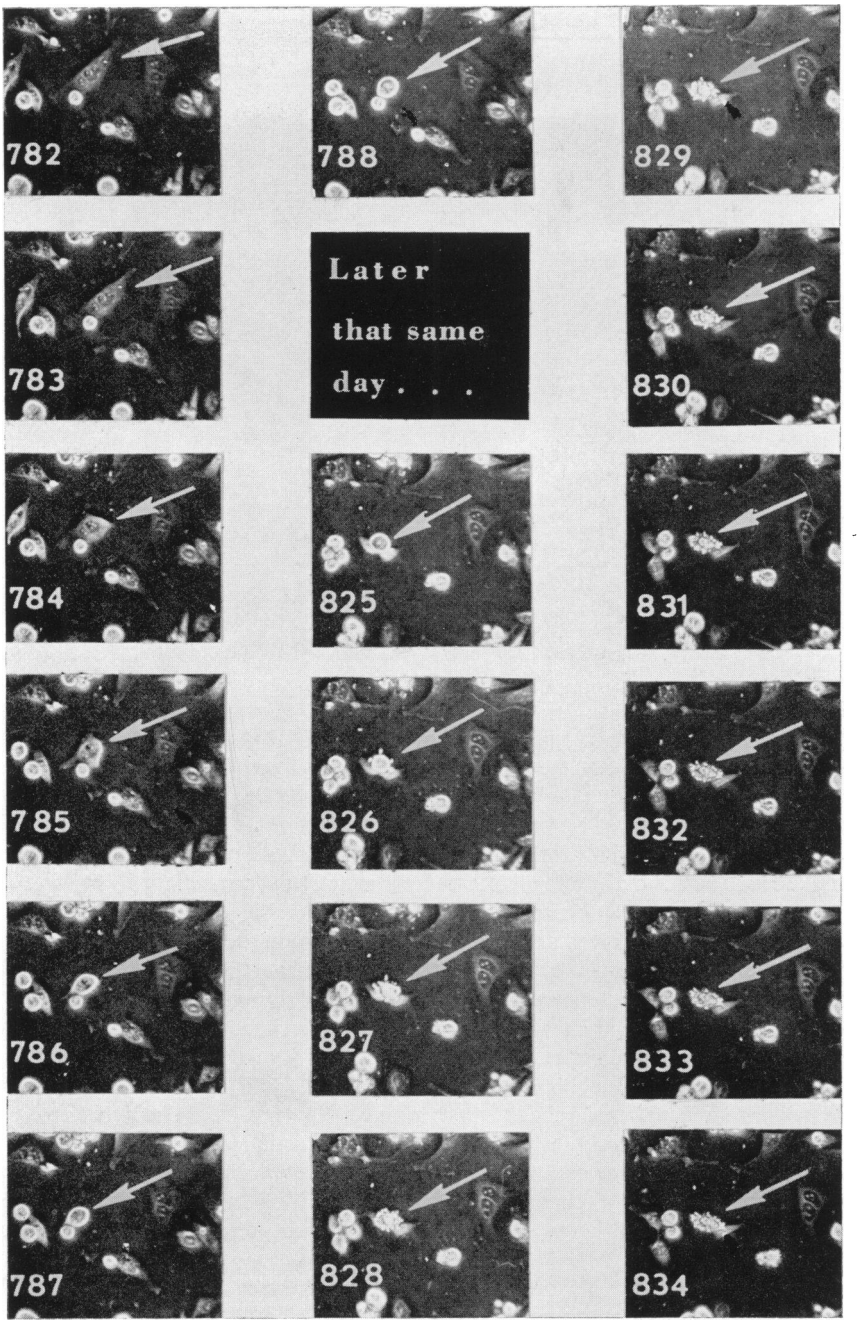


FIGURE 2 Film sequence illustrating rounding of a (small giant) cell (arrow) and its subsequent disintegration, D_r, with blebbing. See legend to Fig. 1 for details of preparation of figure, and text for description of events. From frame 825 on, two spread cells (daughters of the mitotic cell seen immediately below the cell under consideration in frames 782–788) lie beneath the disintegrating cell. Another rounded cell blebs in frame 834.

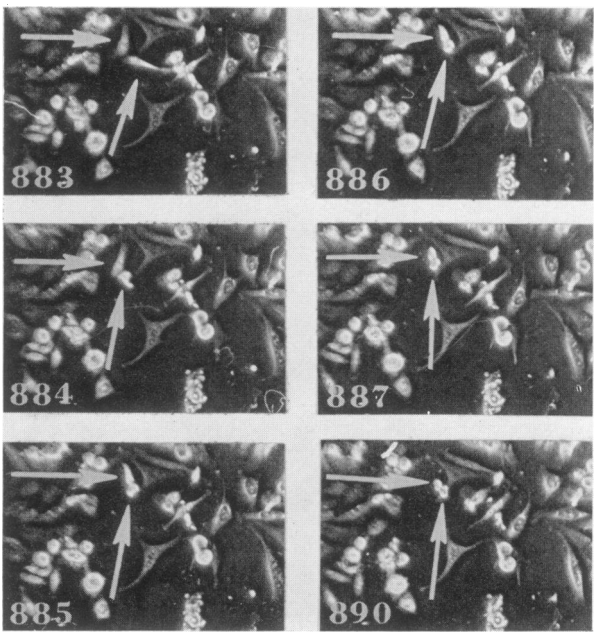


FIGURE 3 Film sequence illustrating disintegration of spread cells, D_s, without blebbing. The two cells (arrows), which disintegrated within 5 min of each other, were sisters. See legend to Fig. 1 for details of preparation of figure, and text for description of events. The cluster to the right of center at the lower margin contains the cell that disintegrated in Fig. 2, together with the two cells that lay beneath it.

rounded for mitosis, D_r. Disintegration of rounded cells, in turn, occurred either via a process involving the violent blebbing and collapse or fragmentation of the cell, or by collapse or disintegration into debris without blebbing. It may be noted that because cell movement (changes in position and/or shape) as well as the instantaneous form of a cell can be detected in time-lapse pictures, the criteria used to identify cell disintegration were in effect both morphological and physiological. Even in the case of disintegration of a rounded cell which did not move (except to drift in the field), the disintegration process often occupied several frames, so that its dynamic aspects could be detected.

Fig. 2 illustrates D_r with blebbing: frames 783 through 788 display the initial steps of what appears to be rounding of a (giant) cell entering mitosis; frames 789 through 824 (not shown) covered a period of 2.9 hr during which the cell remained rounded; finally, frames 825 *et seq.* show the initiation of blebbing and the disintegration of the cell. Frame 826, at which blebbing commenced, was selected for D_r. Evidence indicating that the rounding preceding cell disintegration is identical with that which accompanies mitosis and division is discussed in the Results, section 3 *a*.

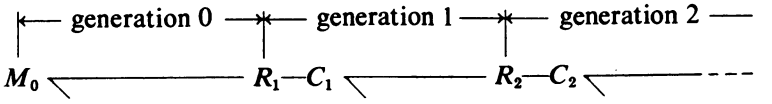
D_r without blebbing is not illustrated. It frequently took place gradually over a number of frames, in which case its occurrence was scored, but the time at which it occurred was not recorded.

Disintegration from the spread configuration, D_s, also occurred with or without blebbing; Fig. 3 illustrates the latter process for two cells. D_s always occurred over only a small number of frames; frames 884 and 885 in Fig. 3 were designated as the ones in which disintegration of the two cells, respectively, occurred.

The relatively few cases in which D_r could not be distinguished from D_s were placed in a separate category, D_u (for unclassified).

Cell fusion, which will be discussed in a subsequent report, was a common occurrence in the irradiated cultures. Fused cells were scored as one cell.

Notation. Data are presented according to the following notation regarding generation number, the rounding (R) of cells on entering mitosis, and cell division (C):



M_0 denotes the mitosis in which cells were engaged at the time of collection. Irradiation took place during generation 0. Generations were counted from R_i to R_{i+1} rather than from C_i to C_{i+1} in order to score cells which rounded but did not complete division (Results, section 3). Mean generation times were not significantly different when calculated from one interval or the other. As a matter of convenience, the intervals between rounding were often compared, though the conventional generation times (i.e. from one division to the next) were considered when appropriate.

The time interval between two events has been denoted by $X_i \rightarrow Y_j$, and the corresponding mean value for all cells undergoing this progression, by $\langle X_i \rightarrow Y_j \rangle_{av}$. When a cell failed to divide after rounding and entering mitosis, the previous generation time has been denoted $R_{i-1} \rightarrow R_i(D)$, in order to distinguish it from the generation time of cells which did undergo C_i.

Statistical treatment. Most of the data are presented with 95% confidence limits. Determination of these limits required two different types of calculations. The first pertains to the confidence limits that can be placed on the difference between the means of two normally distributed populations (Wilks, 1948). The data to which the calculation has been applied here, however, such as generation times or mitotic times, are not normally distributed, and the calculated confidence limits must therefore be inaccurate. The magnitude and direction of the error cannot easily be determined; we assume that the inaccuracy is not large enough to vitiate the conclusions drawn from the data. A χ^2 test was applied in many instances to test the similarity of the medians of the two distributions in question (Snedecor, 1956) in order to verify the conclusions drawn from consideration of the means and their confidence limits.

The second calculation concerns the confidence limits that can be placed on the parameter p, given by $p = x/n$, where x is a chance quantity (i.e. the number of times a given event occurs in n trials) distributed according to the binomial distribution (Wilks, 1948). The calculation is direct and can be applied to many of the data presented here (for example, the probability of dividing) without reservation.

RESULTS

1. *Cell Progression through the Generation Cycle*

Exposure of mammalian cells to X-rays is known to cause a dose-dependent delay in progression to the next cell division. In many cell lines the delay presumably occurs largely in the G2 portion of the generation cycle, and its duration is age dependent (Elkind and Whitmore, 1967). The subsequent rate of cell proliferation has not been well documented, however; in particular, it has not been clear whether the progres-

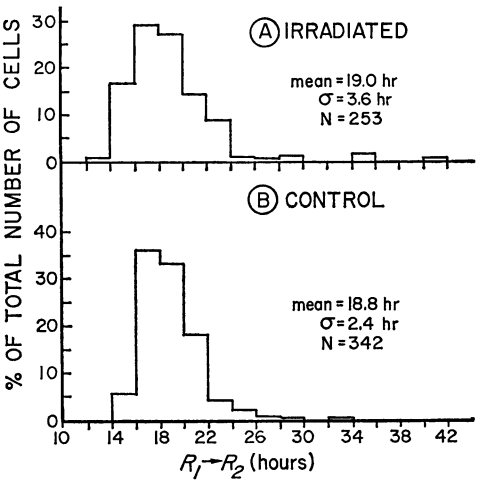


FIGURE 4 Frequency distribution of the duration of generation 1, measured as the interval $R_1 \rightarrow R_2$ (see Materials and Methods) in cells irradiated with 500 rads (A) and in unirradiated control cells (B). Data from six cultures irradiated at different times in the generation cycle (Table I) are combined. The means with their standard deviations, σ, are given, together with the sample sizes, N. Only cells which completed C_2 are included.

sion of cells is delayed in generations subsequent to the one in which they are irradiated.

Elkind et al. (1963) concluded that after the initial delay, growth of X-irradiated Chinese hamster V-79 cells (both those that ultimately gave rise to colonies and those that had lost the capacity for sustained proliferation) resumed at its normal rate, which suggested that cell progression was not perturbed in succeeding generations (though this rate, of course, was not maintained for many hours after moderate to large doses, as cessation of cell division and cell disintegration began to affect the net growth rate). Adams and Gregg (1966) reported that time-lapse cinemicrographs of X-irradiated U-12 fibroblasts showed delay of cell progression only in the irradiated generation, not in subsequent generations.

In contrast, Froese (1966) concluded from time-lapse measurements of the distribution of generation times of Chinese hamster cells in the first postirradiation generation, that a fraction of the cells suffered a delay in that generation; Marin and Bender (1966) reported that the mean duration of interphase in HeLa cells was longer than normal in the postirradiation generations; and Thompson and Suit (1967) found that the generation times were increased in the three generations following the one in which mouse L-P59 cells were irradiated.

The frequency distributions of $R_1 \rightarrow R_2$, $R_2 \rightarrow R_3$, and $R_3 \rightarrow R_4$ for all cells irradiated with 500 rads and for the corresponding control cells, are shown in Figs. 4, 5, and 6, respectively. Only cells which divided at C_{i+1} have been included. (The combined frequency distribution for irradiated cells in all generations except generation 0 is shown in Fig. 11 *B*.) Table II lists, by generation number, the mean generation times for each culture, together with the difference (with 95% confidence limits) in mean generation times between irradiated and control cells. (Values for generation 3 are given only for the combined data for all films because data for the

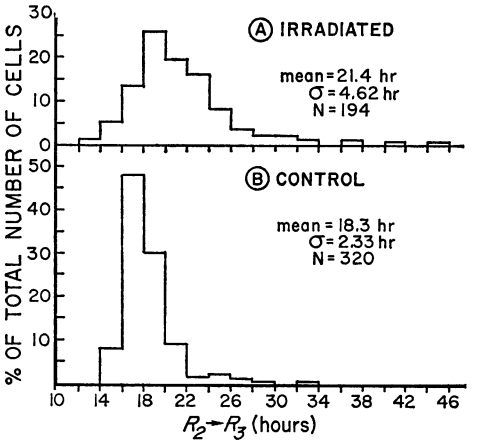


FIGURE 5 Same as Fig. 4, but for generation 2.

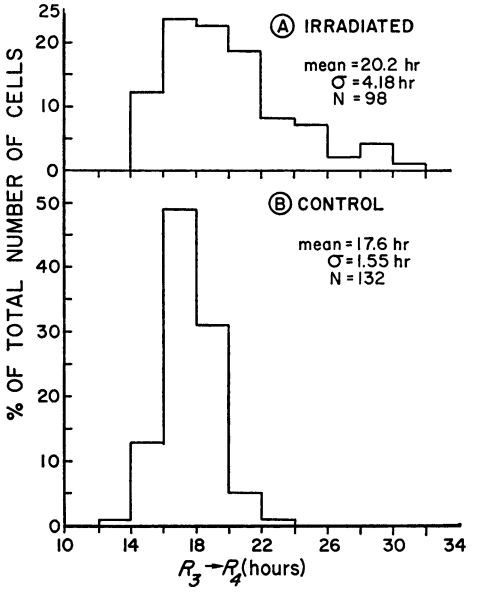


FIGURE 6 Same as Fig. 4, but for generation 3.

individual films were scanty.) It is recognized that since the ages at which the different cultures were irradiated were not randomly selected, any age dependence in the generation times might result in a distorted mean value for the combined data from the six films. Nevertheless, as the chosen irradiation times spanned most of interphase, significant trends should be detectable, and comparisons between generations should be valid.

It is apparent that the mean generation times in generation 0 for the unirradiated cultures were variable and generally longer than the corresponding values in later generations. These perturbations probably arose from manipulation of the cultures

TABLE II

MEAN GENERATION TIMES* FOR IRRADIATED (500 RADS) AND CONTROL CELL POPULATIONS‡ AND CORRESPONDING DELAYS§ IN PROGRESSION

Film No.	Time of irradiation (after collection)	Generation 0			Generation 1			Generation 2			Generation 3‖		
		$\langle M_0 \rightarrow R_1 \rangle_{av}$ Irradiated	Control	Delay	$\langle R_1 \rightarrow R_2 \rangle_{av}$ Irradiated	Control	Delay	$\langle R_2 \rightarrow R_3 \rangle_{av}$ Irradiated	Control	Delay	$\langle R_3 \rightarrow R_4 \rangle_{av}$ Irradiated	Control	Delay
	hr												
1	3.1	28.4 (75)	22.0 (74)	6.4	19.7 (76)	18.6 (115)	1.1 ± 0.9	23.2 (34)	¶	—	—	—	—
2	4.8	30.6 (33)	29.3 (18)	1.3	19.7 (38)	20.0 (30)	−0.3 ± 1.4	22.2 (24)	18.2 (41)	4.0 ± 1.6	—	—	—
3	8.9	34.8 (30)	23.6 (35)	11.2	18.9 (22)	20.1 (59)	−1.2 ± 1.2	20.3 (10)	19.3 (76)	1.0 ± 2.1	—	—	—
4	12.1	30.8 (25)	18.3 (20)	12.5	18.8 (27)	17.3 (35)	1.5 ± 1.6	21.7 (17)	17.2 (56)	4.5 ± 1.5	—	—	—
5	15.8	30.1 (25)	22.8 (9)	7.3	18.3 (34)	18.4 (17)	−0.1 ± 1.8	21.4 (42)	17.3 (29)	4.1 ± 1.0	—	—	—
6	16.5	30.5 (44)	21.3 (54)	9.2	18.1 (56)	18.5 (86)	−0.4 ± 0.7	20.2 (67)	18.5 (118)	1.7 ± 0.3	—	—	—
	All films	30.4 (232)	22.4 (210)	8.0 ± 1.4	19.0 (253)	18.8 (342)	0.2 ± 0.5	21.4 (194)	18.3 (320)	3.1 ± 0.6	20.2 (98)	17.6 (132)	2.6 ± 0.8

* Data are given in hours.

‡ The numbers in parentheses refer to the number of cells included in the calculation of the mean.

§ Delays ± 95% confidence limits.

‖ There were too few cells to provide a meaningful comparison between individual films.

¶ This film could not be analyzed.

at the time of irradiation. The reliability of the measured delays in cell progression caused by irradiation is accordingly in doubt (hence the 95% confidence limits for the difference between irradiated and control cells are omitted). Nevertheless, the 8 hr delay for all films combined agrees with that previously measured for random populations (Tolmach, 1961). Furthermore, both the magnitudes of the individual delays and their age dependence also are similar to those reported previously (Terasima and Tolmach, 1963 *b*; Froese, 1966), though test of the statistical significance of the differences in delay among the various cultures has not been made, again because of the abnormally long generation times of the control cells.

Table II reveals a complex pattern of delay in progression of irradiated cells through the postirradiation generations. The values for the increase of $\langle R_1 \rightarrow R_2 \rangle_{av}$ in each film (and for the sum of all films) are close to zero; i.e., the rate of progression in generation 1 was normal, irrespective of the age of the parent cells at irradiation. Progression through the subsequent two generations, however, was again delayed, though not so severely as in the irradiated generation. Because of the uncertainties concerning the accuracy of the calculation of 95% confidence limits on the means (see Materials and Methods), the medians for the combined data were analyzed as well. χ^2 values were calculated for the hypothesis that the median generation times for irradiated and control cells are the same. For each value of χ^2, the probability, P, that the null hypothesis is true was determined from the accumulative distribution of χ^2. Table III lists values of χ^2 and P for generations 0 through 3. It is clear that there is a large chance that the medians for generation 1 are the same, but virtually none that this is so for generations 0, 2 or 3. Thus, the analysis of median values supports the conclusion reached from consideration of the means. Fig. 7 (open circles) depicts the mean delays as a function of generation number.

The cause of this behavior of irradiated cells is obscure. It will be seen below that an increase in generation time was characteristically detectable in the generation preceding cell disintegration, and the delay in progression through generations 2 and 3 might be attributable to the approaching disintegration of sizable fractions of the population. Such an explanation suggests that the origin of the delay in the postirradiation generations is different from that in the irradiated generation. The different age dependence of delay for the irradiated and first postirradiation generations of

TABLE III
STATISTICAL TESTS OF DIFFERENCE IN MEDIAN GENERATION TIMES OF IRRADIATED (500 RADS) AND CONTROL CELLS

Generation	χ^2	P
0	183	~0
1	1.09	0.32
2	86.5	~0
3	16.1	~0

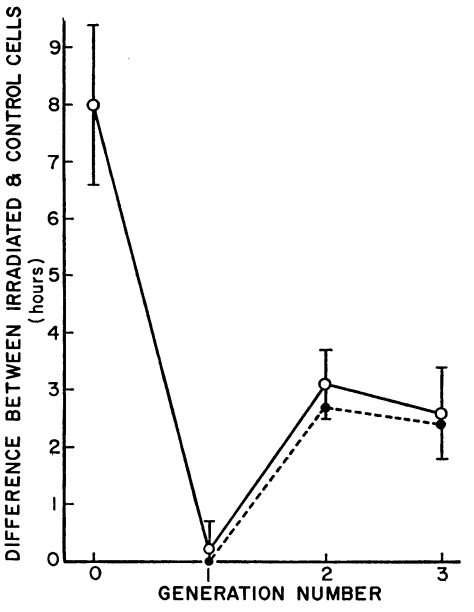


FIGURE 7 Mean delay in progression of irradiated cells (500 rads) through the irradiated and first three postirradiation generations (open circles). delay is given by the difference between $\langle R_i \rightarrow R_{i+1} \rangle_{av}$ for irradiated and control cells. The intervals $R_i \rightarrow R_{i+1}(D)$ are not included. The bars show the 95% confidence limits. The solid circles show the mean differences in interphase time, $\langle C_i \rightarrow R_{i+1} \rangle_{av}$, between irradiated and control cells for the first three postirradiation generations.

the Chinese hamster cells studied by Froese (1966) would be consistent with this suggestion. However, if delay in the postirradiation generations is related to approaching disintegration, one might have predicted an increasing delay from generation 1 through 3, as progressively larger fractions of cells disintegrated during this period (see Fig. 13). In fact, the delays in generation 2 and 3 were about the same (Fig. 7). Again, an age dependence for this delay, paralleling that for loss of colony-forming ability (Terasima and Tolmach, 1963 *b*), might have been expected. However, no statistically significant age dependence for prolongation of generation 2 is evident (Table II) (unaccountably, none was found for loss of the ability to divide, either; see section 3 *c*), although the increase in mitotic time does seem to be age dependent (section 2). Finally, a strong dose dependence of the pattern of generation time increase might be anticipated on the basis of this model, but the data are insufficient for the analysis.

Fig. 7 also shows (solid circles) the differences in $\langle C_i \rightarrow R_{i+1} \rangle_{av}$ $i = 1, 2, 3$ (mean interphase time for generations 1 through 3) between irradiated and control cells. It is seen that only a small absolute fraction of the increase in generation time took place in mitosis (difference between each pair of points); this fraction appears to be disproportionately large, however, in terms of the relative durations of mitosis and interphase (see section 2).

2. Cell Progression through Mitosis

The duration of mitosis, $R_i \rightarrow C_i$, in all generations is shown in Fig. 8 as separate frequency distributions for cells irradiated with 500 rads and for control cells. While

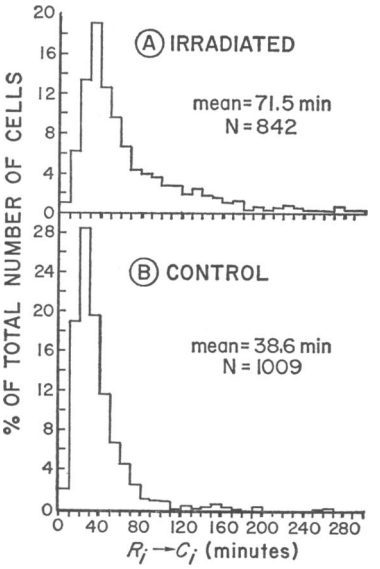


FIGURE 8 Frequency distributions of the duration of mitosis, measured as $R_i \rightarrow C_i$, in cells irradiated with 500 rads (A) and in unirradiated control cells (B), for all generations. The mean durations and sample sizes, N, are given.

both distributions are skewed toward long times, it is clear that the distribution for irradiated cells is considerably broader and has a larger modal value; the mean duration of mitosis was almost twice as long for irradiated cells as for the controls. A similar comparison by Marin and Bender (1966) revealed a larger number of prolonged mitoses in irradiated than in control cells, but no difference in modes was apparent, probably because of the paucity of data.

Because both generation times and mitotic times were increased in irradiated cells, it was of interest to determine whether a constant fraction of the generation time was spent in mitosis. The data for the increase of $\langle R_i \rightarrow R_{i+1} \rangle_{av}$ and $\langle C_i \rightarrow R_{i+1} \rangle_{av}$ shown in Fig. 7 indicate that it was not, the mitotic time (difference between these values) being disproportionately increased. Calculation of the ratio $\langle R_{i+1} \rightarrow C_{i+1} \rangle_{av} : \langle C_i \rightarrow C_{i+1} \rangle_{av}$ for 790 control cells and 607 irradiated cells confirmed this indication. The values (with standard deviations) of this ratio are 0.0351 ± 0.0252 and 0.0590 ± 0.0450 respectively, for the two populations; the difference (with 95% confidence limits) is 0.0239 ± 0.0019. That is, the fraction of the generation time spent in mitosis by irradiated cells was 69% greater than by control cells. This disproportionate sensitivity of mitotic time to irradiation might be related to cell disintegration (section 3).

Although the increase in generation time (in generations 1 and 2) was not found to depend on the age of the parent population at the time of irradiation (Table II; cf. Froese, 1966), the data for the increase in mitotic time do indicate an age dependence. $\langle R_i \rightarrow C_i \rangle_{av}$ was determined separately for each of the cultures irradiated with 500 rads, and for their respective controls. Fig. 9 *A* shows the mean values of the duration of mitosis for control and irradiated cells, as a function of the age of the

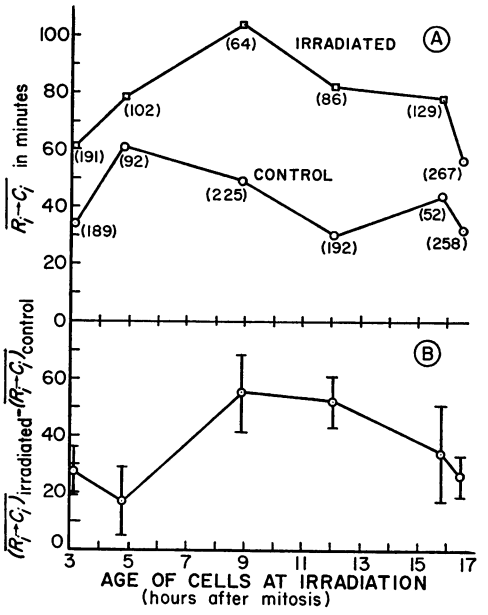


FIGURE 9 Age dependence of the increase in mitotic time resulting from irradiation. The mean duration of mitosis, $\langle R_i \rightarrow C_i \rangle_{av}$, shown (A) for all cells except those in generation 0 as a function of time after mitosis at which the collected cells were irradiated with 500 rads (squares). The mitotic times for the corresponding unirradiated control cultures are shown by the circles. The number of cells from which each value was calculated is given in parentheses. The increase in mitotic time, with 90% confidence intervals (bars), is given (B) as the difference between the means for the irradiated and control cells.

cells at irradiation; Fig. 9 *B* shows the differences between these values, with 95% confidence limits. In spite of appreciable variation (possibly reflecting slight differences in growth conditions) among the control cultures, the differences among the values for prolongation of mitosis appear to be statistically meaningful. Cells irradiated during S (9 and 12 hr) were affected more than those irradiated in G1 or probably G2. The data from each pair of films (irradiated and control) were subjected to χ^2 tests, assuming that the medians are the same. Only for the cells irradiated at 5 hr is χ^2 small enough (1.43) to yield a reasonable probability (0.24) that the medians are the same; all the remaining values of χ^2 (ranging from 12.1 to 87.1) indicate that the medians are almost certainly different, confirming the conclusion reached from consideration of the means. The increase in mitotic time as a function of the age of the cells at irradiation was also examined for each generation separately. While the data were insufficient to determine the shape of the age response curves with precision, each of the curves again showed a minimum for cells irradiated at 5 hr, followed by a maximum for those irradiated at 9 hr, and a decrease for those irradiated later in the cycle. This age dependence resembles that for prolongation of generation time in generation 0 (Table II), but the relation between the two phenomena is not apparent. It may be noted also that the lethal damage inflicted on S phase cells by the drugs vinblastine and vincristine is expressed only at mitosis (Madoc-Jones and Mauro, 1968). Possibly both X-rays and these drugs interfere with some sensitive mitosis-related process that takes place during S.

It will be seen below that cells which ceased dividing and underwent disintegration

TABLE IV
DISTRIBUTION OF CELLS AMONG THE VARIOUS OBSERVED PATERNS OF DISINTEGRATION

Pattern of disintegration*	Number of cells	Per cent of cells
D_r with blebbing	186	54
D_r without blebbing	60	18
D_s	77	23
D_u	18	5
Total	341	100

* The patterns are described in Materials and Methods.

spent a longer time in their last completed mitosis than did those irradiated cells in the same generation which divided at least once more. Although it was anticipated that the prolongation of mitosis would be found to increase with generation number because of the strong generation dependence for failure of division (see section 3 *c*), the data in Fig. 7 indicate no clear trend of this sort.

Examination of the dose dependence of mitotic time suggested that the prolongation of mitosis was greater after larger doses. Compared with the distribution of $R_i \rightarrow C_i$ for cells irradiated with 500 rads (Fig. 8 *A*), that for cells receiving 1000 rads was flatter and the median was 10 min greater, though the mean was only 1 min greater, probably because of the small sample size (only 74 cells were scored) and consequent truncation. (Since mitotic time increase showed an age dependence, $R_i \rightarrow C_i$ properly should not be compared for cells receiving 1000 rad doses and those receiving 500 rads, inasmuch as the ages of the two were not the same; Table I.)

3. Cell Disintegration

In this section we consider disintegration of individual irradiated cells or their descendants. As indicated in Materials and Methods, a number of different patterns by which cells change from intact entities to necrotic masses could be detected. While the present time-lapse pictures have yielded no information about changes in the cell membrane (as might be revealed, for example, by altered staining characteristics) or other subcellular structures, it has been possible to examine the occurrence of overt cell necrosis as a function of disintegration pattern and of various kinetic parameters. In addition, correlations have been sought between cell disintegration and deviations from normal cell progression. We have previously termed cell disintegration "metabolic death", and scored it by the loss of the cell from the monolayer on the culture dish (Tolmach, 1961).

Table IV shows the frequency with which the different patterns of disintegration were detected following an X-ray dose of 500 rads. A total of 341 disintegrations,

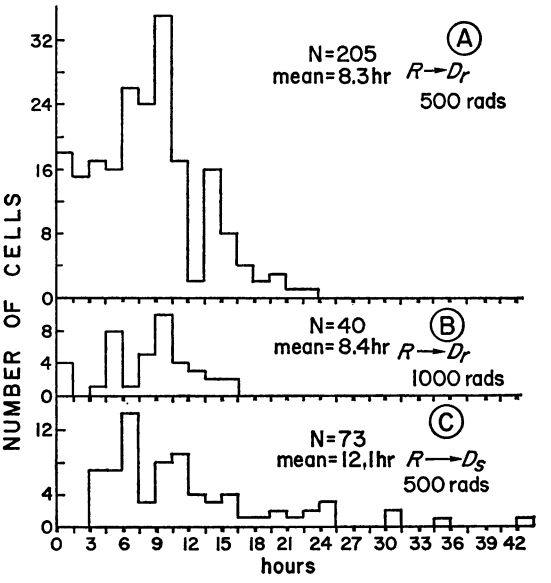


FIGURE 10 Frequency distribution of the interval between the final rounding of a cell and its disintegration, for cells disintegrating from a rounded configuration, D_r, after 500 rads (A) or 1000 rads (B), or from a spread configuration, D_s, after 500 rads (C). The number of cells in each distribution, N, is indicated, together with the mean interval. The 205 cells included in (A) represent all those which blebbed, but only 19 of the 60 which disintegrated without blebbing, because of difficulty in identifying the frame at which the latter occurred. A similar selection was necessary in (B).

occurring in all postirradiation generations, were observed; all but 18 could be assigned to one of the three patterns described. These patterns may correspond to the processes described earlier (from discontinuous observations) by which cells are lost from a monolayer (Tolmach and Marcus, 1960).

Only very occasional instances were recorded in these films of the sequence of events, observed by others (P. I. Marcus, personal communication), wherein a cell rounds as if for mitosis, but fails to divide or to disintegrate, and subsequently assumes an interphase configuration.

a. *Disintegration of Rounded Cells.* Disintegration of a rounded cell (D_r) occurred at any time within 24 hr after rounding, as shown by the histograms in Figs. 10 *A* and *B*, though only a very few cells were still intact after 16 hr. The mean duration of the rounded state was 8.3 hr. There appears to have been a preferred time for disintegration between 6 and 10.5 hr after rounding (the virtual hiatus in disintegration between 12 and 13.5 hr in Fig. 10 *A* is probably not significant). The duration of the rounded state would appear not to have any strong dose dependence: both the shape and the mean (8.4 hr) of the distribution shown in Fig. 10 *B* for cells receiving 1000 rad doses are similar to those for cells treated with 500 rads (Fig. 10 *A*).

Little dependence of the distribution on the generation in which disintegration occurred was found, though the mean duration dropped from 9.4 hr for generations 1 + 2, to 8.3 hr for generation 3, and 7.5 hr for generations 4 through 6. When the data of Figs. 10 *A* and *B* were plotted in the form of "survival" curves, i.e., the fraction of cells not yet disintegrated as a function of time after rounding, survival appeared to approach exponential decrease, with the same terminal slope.

If the rounded configuration preceding disintegration can be identified with a mitotic event, then the most common pattern of HeLa S3 cell disintegration, under the experimental conditions described, would appear to involve failure of the mitotic process. Two types of evidence are offered which indicate that such rounding does in fact constitute entrance into mitosis.

1. Frequency distributions for the generation times $R_{i-1} \rightarrow R_i$ and $R_{i-1} \rightarrow R_i$ (D) for all cells irradiated with 500 rads (the interval $M_0 \rightarrow R_1$ is omitted) are presented in Fig. 11. Similar distributions were obtained for each generation separately. It can be seen that the minimum durations of the two generation times were nearly the same, and that the mean (and mode) for the distribution of $R_{i-1} \rightarrow R_i$ (D), is only 2 hr longer than that of $R_{i-1} \rightarrow R_i$; i.e., rounding occurred at about the time expected for mitosis. (The difference between the distributions is discussed in section *c*, below.)

2. Films were examined for the presence of metaphase plates in rounded cells destined either to divide or to disintegrate. In each film, the per cent of disintegrating rounded cells in which metaphase plates were detectable was almost identical to the corresponding value for rounded cells which subsequently divided (Table V). The fact that this close correspondence held not only for the total cell population but for the six individual films as well, in which detectability varied from 43 to 90%, strongly

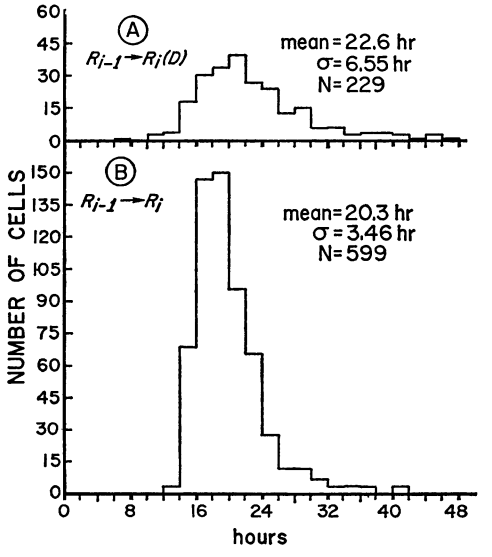


FIGURE 11 Frequency distribution (A) of the final generation times, $R_{i-1} \rightarrow R_i$ (D), of rounded cells prior to disintegration, and (B) of the corresponding intervals, $R_{i-1} \rightarrow R_i$, for cells which divided at C_i.

supports the conclusion that all cells observed to round up did so at the time of a mitotic event.

The foregoing analysis does not deal with the question of whether disintegrating rounded cells remained in mitosis until they disintegrated. Cells persisted for several hours in a rounded configuration, and they might have reverted to interphase without dividing or spreading. Accoringly, the films were examined for the peristence of metaphase plates after the normal mitotic time and up to the moment of disintegration. Fig. 12 shows the frequency distribution among 79 cells (the remainder of the 170 cells recorded in Table V could not be adequately examined) of the ratio ($R \rightarrow$ last observation of metaphase plate):($R \rightarrow D_r$). It is clear that for most of the cells the plate was detectable through the major part of the period between rounding and disintegration. We conclude that the majority of cells undergoing disintegration entered mitosis and became trapped in metaphase from which they did not emerge,

TABLE V

DETECTABILITY OF METAPHASE PLATES IN ROUNDED CELLS DESTINED EITHER TO DIVIDE OR DISINTEGRATE

Film No.	Dividing cells			Disintegrating cells		
	No. of cells	Metaphase plates		No of cells	Metaphase plates	
		No.	%		No.	%
1	66	30	45	32	16	50
2	183	93	51	56	24	43
3	102	81	79	40	33	82
4	85	75	88	30	27	90
5	126	88	70	52	36	69
6	266	233	88	39	34	87
Total	828	600	72.5	249	170	68.3

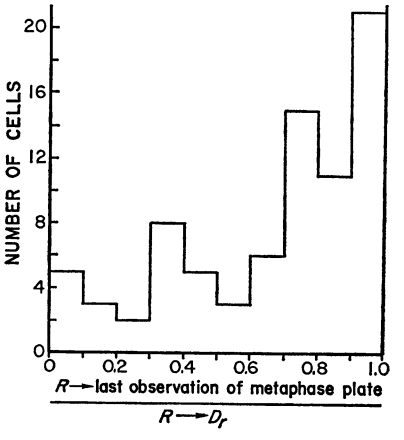


FIGURE 12 Frequency distribution of the period, measured from final rounding, during which a metaphase plate could be seen in cells undergoing disintegration from the rounded configuration, D_r, after irradiation with 500 rads, expressed as the fraction of the interval from rounding to disintegration, $R \rightarrow D_r$.

although we can not rule out the possibility that the metaphase configuration may have disappeared immediately before cell disintegration (cf. Watanabe and Okada, 1966).

The cause of the arrest in metaphase, the characteristics of the arrested cell, and the nature of the disintegrative processes are unknown; neither is it known whether disintegration without blebbing is a fundamentally different event from disintegration with blebbing, which occurred three times as often (Table IV).

b. *Disintegration of Spread Cells.* Analysis of the disintegration of spread cells, D_s, was hampered by a paucity of data, but it seems clear from both morphological and kinetic considerations that this process did not involve mitosis. Fig. 10 *C*, which shows the frequency distribution of $R \rightarrow D_s$ for cells irradiated with 500 rads, indicates that the shape, the mean, and the mode of the distribution are all irreconcilable with the corresponding parameters for the distribution of either normal generation times or of $R_{i-1} \rightarrow R_i$ (*D*) (Fig. 11).

Further indication that disintegration of rounded cells and of spread cells might be fundamentally different processes was gained by comparison of $R \rightarrow D$ for the two processes. (The latter cells, of course, did not remain rounded, but divided, attached, and spread before disintegrating.) Both the shape of the distribution of $R \rightarrow D_s$ and the mean, 12.1 hr, (Fig. 10 *C*) seem to differ significantly from those for $R \rightarrow D_r$ (Fig. 10 *A*).

D_s occurred with an over-all frequency only one-third that of D_s (Table IV), but its relative frequency increased threefold between generations 1 and 3 (Table VI *A*), apparently with statistical significance. Total cell disintegration exhibited a similar dependence on generation number (see Fig. 13) and occurred sooner at higher doses

TABLE VI

RELATIVE FREQUENCY OF DISINTEGRATION OF SPREAD CELLS AS A FUNCTION OF GENERATION AND OF X-RAY DOSE

A. Dependence on Generation (500 rads)

Pattern of disintegration*	Number of cells			
Interval	$M_0 \rightarrow C_2$	$C_2 \rightarrow C_3$	$C_3 \rightarrow C_4$	$C_4 \rightarrow R_8$
D_s	9	19	33	16
D_r	71	87	55	33
$D_s/(D_s + D_r)$	0.11	0.18	0.38	0.33
95% confidence interval	0.04–0.19	0.13–0.28	0.28–0.48	0.22–0.47

B. Dependence on Dose (All Generations)

Dose (rads)	500	1000	1500
D_s	77	10	6
D_r	246	42	22
$D_s/(D_s + D_r)$	0.24	0.19	0.21
95% confidence interval	0.20–0.29	0.10–0.34	0.07–0.38

* The patterns are described in Materials and Methods.

(see Fig. 14). It is therefore of interest that although the relative frequency of D_s was probably not dependent on dose in the range examined here (Table VI *B*), preliminary study of cultures irradiated with higher doses indicates that $D_s/(D_s + D_r)$ also increases markedly with dose, approaching 0.9 at 4000 rads. Perhaps D_s is the manifestation of more severe damage than is represented by D_r.

The cause and mechanism of interphase cell disintegration are as obscure as the nature of mitotic disintegration.

c. *Disintegration and Growth Kinetics.* Since, under the conditions of these experiments, a cell that ceased dividing disintegrated within at most one day, the rate of disintegration could be studied alternatively in terms of loss of divisional ability. The two responses have slightly different rates, but if they are measured as functions of postirradiation generation number rather than of time, identical curves result. It is convenient to measure divisional ability in terms of the probability, $p\,(i)$, that a cell will divide in generation i:

$$p(i) = \frac{\text{Number of cells dividing at end of generation } i}{\text{Number of cells entering generation } i},$$

generations being measured in the conventional manner from C_i to C_{i+1} (Whitmore and Till, 1964). $1-p(i)$ vs. i is thus a measure of the dependence of disintegration on generation number. Froese (1966) has reported that $p(i)$ for both Chinese hamster V79-1 and HeLa S3 cells was reduced for $i = 0$ after doses in the range 500–1000 rads, and was further reduced in Chinese hamster cells for $i = 1$ (data for HeLa were not reported). Thompson and Suit (1967) have reported that $p(i)$ for mouse L-P59 cells was reduced for $i = 0$ following a dose of 736 rads, further reduced for $i = 1$, and then remained essentially constant for $i = 2$ and 3. This behavior, however,

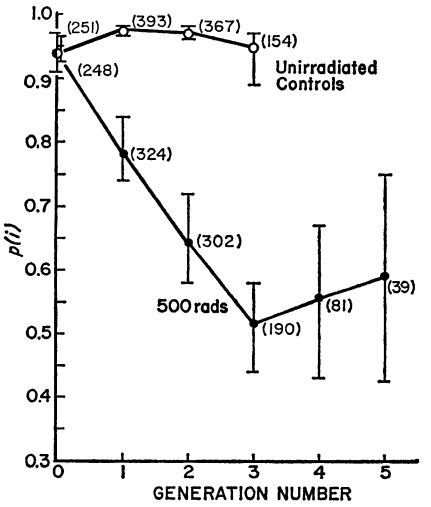
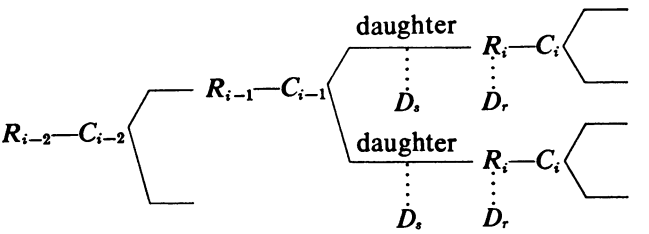


FIGURE 13 Probability of cell division, $p(i)$, as a function of generation number, for all cells irradiated with 500 rads (solid circles) and for unirradiated control cells (open circles). For each point, the number in parentheses gives the sample size, and the bars indicate the 95% confidence interval.

depended on the stage of the generation cycle at which the cells were irradiated (see below).

Fig. 13 (solid circles) shows the composite behavior during the irradiated and first five postirradiation generations of the cell populations irradiated with 500 rads. The same fraction of cells divided at the end of the irradiated generation as in unirradiated controls (which ranged between 0.936 and 0.975 in the first four generations; open circles). $p(i)$ then fell approximately linearly over the next three generations. It subsequently remained sensibly constant for two generations; the descendants of the few colony-forming survivors may have become detectable at this point. When the normalized cumulative "survival" of divisional ability was calculated from the foregoing data, that is, the ratio of the number of cells that divided to the total population that would have resulted had no cells distintegrated, it was found that 24% of this potential population divided at the end of generation 3, and 8% after generation 5.

As described above, cells which were destined to disintegrate in mitosis spent about 2 hr longer, on the average, in the preceding generation than did cells which divided (Fig. 11). In addition, the distribution for $R_{i-1} \rightarrow R_i$ (D) is flatter than that for $R_{i-1} \rightarrow R_i$. Apparently, the disintegration of a cell was preceded, on the average, by distorted growth kinetics. To examine the possibility that an indication of approaching disintegration may be manifested still earlier, the durations of the *previous* interphase and mitosis, and their sum, were determined; cells undergoing D_s were included. The generations in question can be identified from the following scheme:



The intervals $C_{i-2} \rightarrow R_{i-1}$, $R_{i-1} \rightarrow C_{i-1}$, and $C_{i-2} \rightarrow C_{i-1}$ for cells whose daughters subsequently divided (solid lines) were compared with the corresponding intervals for cells of which one or both daughters disintegrated (dotted lines).

Table VII *A* lists the respective means and standard deviations of the three intervals for the two groups of cells, as well as the differences in means, with 95% confidence limits. It is seen that all three intervals were longer in cells whose daughters were to undergo disintegration. The last successful division cycle, from C_{i-2} to C_{i-1}, was prolonged an average of 2.9 hr, which is as long as the average prolongation in the final generation cycle in those cells which underwent D_r (Fig. 11). Furthermore, about one-fourth of this delay occurred in mitosis; $\langle R_{i-1} \rightarrow C_{i-1} \rangle_{av}$ was

increased by 90%. Thus, indication of impending disintegration was exhibited at least a generation before it occurred. In fact, such evidence might have been manifested still earlier; when $\langle R_{i-2} \rightarrow C_{i-2} \rangle_{av}$ was compared in cells in which only one daughter disintegrated and those in which both daughters were lost, it was found (Table VII *B*) that this interval was 59% longer in the latter group. This observation suggests that mitotic prolongation may be quantitatively related to the amount of damage harbored by a particular cell.

The ability of irradiated mammalian cells to divide in the first few postirradiation generations has been shown previously to be quite dose sensitive (Engelberg, 1960; Tolmach, 1961; Elkind et al., 1963; Froese, 1966). The present data for HeLa S3 again show a dose dependence; $p(i)$ is given as a function of dose, for $i = 0$ and 1, in Fig. 14. It is seen that after a 500 rad dose, division occurred as often in the irradiated generation as in unirradiated cells (cf. Tolmach, 1961); only after larger doses did any significant amount of cell disintegration occur before one successful postirradiation division had taken place. The ability of a cell to divide twice after irradiation was apparently more dose sensitive than the ability to divide once.

It was expected, in view of the intimate relation between cell division and the formation of a colony, that X-ray inactivation of the former function would exhibit a strong age-dependence, as does the latter. Such was the case for L-P59 cells, as reported by Suit and Thompson (1967); $p(i)$, for $i = 0$ and 1, and probably $i = 2$ and 3 as well, became progressively smaller as cells were irradiated later in

TABLE VII

DELAY IN PROGRESSION OF CELLS APPROACHING DISINTEGRATION

*A. Comparison of Dividing with Nondividing Cells**

Interval‡	Mean ± σ (hr)		Difference ± 95% confidence limit
	Both daughters dividing (118 cells)	One or both daughters disintegrating (191 cells)	
$\langle C_{i-2} \rightarrow R_{i-1} \rangle_{av}$	17.3 ± 2.5	19.5 ± 4.2	2.2 ± 0.83
$\langle R_{i-1} \rightarrow C_{i-1} \rangle_{av}$	0.83 ± 0.62	1.58 ± 1.1	0.75 ± 0.22
$\langle C_{i-2} \rightarrow C_{i-1} \rangle_{av}$	18.2 ± 2.6	21.1 ± 4.6	2.9 ± 0.91

B. Comparison of Partially Dividing with Nondividing Cells.

Interval‡	One daughter dividing (75 cells)	Neither daughter dividing (71 cells)	Difference ± 95% confidence limit
$\langle R_{i-2} \rightarrow C_{i-2} \rangle_{av}$	1.18 ± 0.70	1.88 ± 1.36	0.70 ± 0.35

* Generation 0 not included.

‡ See text for definitions of intervals.

§ Generation 0 included, to obtain sufficient data for analysis; $\langle R_1 \rightarrow C_1 \rangle_{av}$ was no longer than the mean values of subsequent mitotic times.

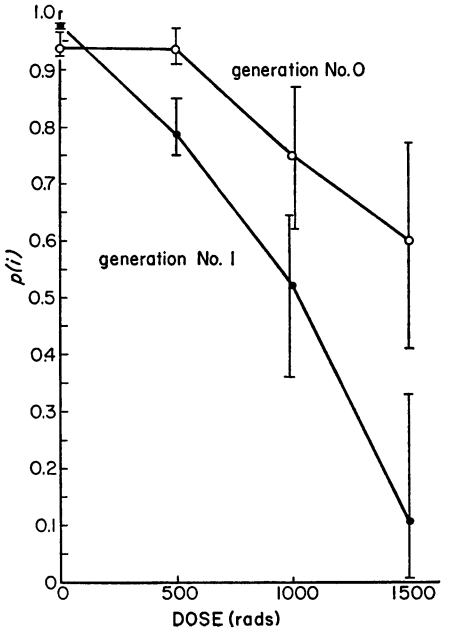


FIGURE 14 Probability of cell division, $p(i)$, as a function of dose, for the irradiated (open circles) and first post-irradiation (solid circles) generations. The bars show the 95% confidence intervals.

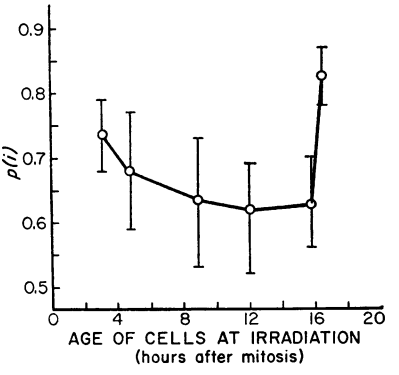


FIGURE 15 Probability of cell division in all generations, $p(i)$, as a function of age of the collected population at the time of irradiation with 500 rads. The bars indicate the 95% confidence intervals.

the cycle. (Whether this pattern followed that for survival of colony-forming ability is not clear; see Whitmore et al., 1965.) However, the present results for HeLa S3 do not indicate any strong age dependence for $p(i)$. Fig. 15 shows no statistically significant difference in $p(i)$ (all generations combined) for cells irradiated with 500 rads between 3.1 and 15.9 hr after collection; only cells irradiated at 16.5 hr showed a markedly changed response, and we do not regard this as reliable, as the population should have been at nearly the same age as the one irradiated at 15.9 hr.

DISCUSSION

The results of the foregoing analysis indicate that the mitotic process is particularly sensitive to X-rays. Cell disintegration, and hence loss of ability to form a

colony, appears to be intimately connected with a mitotic event in the majority of cases; 75% of the classified disintegrations occurred in rounded cells (Table IV) apparently trapped in mitosis (section 3 *a*). This generalization is supported by the further observations that, on the average: *1.* the mitotic time in irradiated cells was prolonged by 85% (Fig. 8), such prolongation being greater than proportional to the fraction of the generation time normally occupied by mitosis (the fraction was 69% greater in irradiated cells); *2.* the prolongation depended on the age at which the cells (or their antecedents) were irradiated (Fig. 9), as does loss of colony-forming ability (Terasima and Tolmach, 1963); *3.* the mitotic time of moribund cells in their last completed generation was 90% longer than that of irradiated cells of the same generation that divided at least once more (Table VII *A*), and the mitotic time in the preceding generation was 59% longer in cells with no dividing daughters than in cells with one daughter that divided (Table VII *B*); and *4.* mitotic time seemed to be dose dependent. While the concept of a preferential expression of radiation damage by the mitotic process is scarcely novel (see for example, Lea, 1956; Harrington, 1961; and Watanabe and Okada, 1967, for references to early work concerned with "mitotic death"), description of the relevant phenomena has been incomplete and quantitation has been lacking.

It cannot be predicted whether or not a similar strong correlation between mitotic derangement and cell destruction will be found in most other mammalian cell systems. Certainly there is evidence that other types of cells, e.g. lymphoid cells, express radiation damage in fundamentally different fashion (see for example Kelly, 1961; Watanabe and Okada, 1966), and it would not appear that the observations made here apply generally even to cells with properties more like those of HeLa S3. Thus, Thompson and Suit (personal communication) have found little evidence of correlation between mitosis and disintegration in mouse L cells.

The pattern of postirradiation generation time increases shown in Fig. 7 does not resemble any of those described hitherto (Froese, 1966; Thompson and Suit, 1967). Froese's (1966) results indicated that this response to irradiation is strain specific, and it is possible that the behavior observed with this system is unique. However, the pattern we have described would not appear to be spurious inasmuch as: *1.* each of the films, obtained with cells irradiated at different ages, showed the same fluctuations (Table II); *2.* statistical test indicates a high probability that there is a difference between the generation times for generations 1 and 2 (or 3); and *3.* the delays in progression in generation 0 conform qualitatively and quantitatively to the pattern previously described (Terasima and Tolmach, 1963 *b*) for HeLa S3 cells, in spite of the slowed progression of the unirradiated control cells.

Our suggestion that the delay in generations 2 and 3 may have a different genesis from that in generation 0 has little experimental basis. It would be of interest to determine whether delay occurs in G2; its localization at a different point in the cell cycle from that associated with "mitotic delay" in the irradiated generation would support the notion of different mechanisms. Furthermore, the absence of

an increased delay in progression through generation 3 over that found for generation 2, and the lack of any significant age dependence for the delay in generation 2 (Table II), appear not to support the suggestion that these delays are related to the imminence of disintegration. Nevertheless, explanation of the pattern of delay (Fig. 7) in terms of a single mechanism will be difficult, and we are reluctant, therefore, to dismiss the possibility of two distinct processes.

The increased average generation times of moribund cells in their penultimate (Table VII) and final cycles ($R_{i-1} \rightarrow R_i\,(D)$; Fig. 11), as compared with irradiated cells that undergo C_i, suggest that slowed cell progression heralds the approach of cell disintegration. Again, it would be of interest to determine which phase(s) of the generation cycle, in addition to mitosis, are prolonged, but as the increase in the duration of interphase was only about 2 hr (after 500 rads), the analysis might prove difficult, especially as pure populations of cells about to disintegrate may not be readily obtainable.

The present results are in accord with the concept of a progressive development of damage following a brief exposure to ionizing radiation (see Bacq and Alexander, 1961). Thus, the incidence of cell disintegration following a dose of 500 rads progressively increased during the first four generations from about 6% (the same as in the unirradiated controls) to about 50% by the third postirradiation generation (Fig. 13), in essential agreement with previous studies. Again, in accord with previous work, the rate of occurrence of this event was dependent on the dose of radiation administered (Fig. 14); however, there was no clear age dependence for disintegration (Fig. 15), though the data do not preclude such dependence.

The increasing incidence of cell disintegration during the postirradiation generations (Fig. 13) is inconsistent with the model for loss of colony-forming ability which proposes that irradiation decreases the probability of cell division, thereby leading, via stochastic considerations, to the observed survival curves for colony-forming ability (Whitmore and Till, 1964). As further test of the idea that loss of the ability to divide (i.e., disintegration) may occur randomly in the irradiated population, the clonal distribution of cell disintegration was examined. It was found, in concurrence with reports of Froese (1966) and of Thompson and Suit (1967), that disintegration of only one of two sister cells occurred less frequently than expected on the basis of a randomly distributed event.

The nature of the presumptive developing damage remains obscure. Mechanisms based on the idea, for example, of a progressive dilution of a necessary cell constituent whose synthesis has been terminated as the result of irradiation, or of a relatively slowly developing change in structure of nucleoprotein whereby it becomes progressively more "sticky," are equally possible (if unattractive). As indicated, we know virtually nothing about the mechanism(s) of cell disintegration, albeit the blebbing and collapse of irradiated cells in culture was described many years ago (for example, Pomerat et al., 1957). In the present study, disintegration was found most often to occur in cells which apparently had entered mitosis (Table

IV). Disintegration of these cells occurred at no strongly preferred time during the 16 hr following rounding, and the distribution of times from rounding to disintegration showed no detectable dose dependence (Fig. 10). It occurred more frequently in the earlier postirradiation generations than in later ones, but was the preferred mode in all generations (Table VI). Preliminary study indicates, however, that disintegration of spread interphase cells becomes more frequent at higher doses.

We have presented data suggesting that disintegration from the rounded configuration (section 3 *a*) may be a process fundamentally different from disintegration of spread interphase cells (section 3 *b*), but in fact there is no compelling evidence that the processes are different. Indeed, entrapment in mitosis and cell disintegration might be essentially independent events. That is, the two processes might represent alternative manifestations of radiation damage, or possibly mitotic arrest is a relatively direct consequence of irradiation, while disintegration of the arrested cell is a physiologically more remote sequela (Tolmach, 1961; though it would be hazardous to regard entrapment in mitosis as the only important manifestation of radiation-induced damage, as disintegration of interphase cells is a far from rare event). In any case, since HeLa S3 cells irradiated with doses up to 1500 rads most often disintegrate while apparently trapped in mitosis, further study of the arrested cells should yield information pertinent to the lethal action of X-rays.

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