tions 1 and 2 were incubated with rat ovary slices, and the formation of tritiated water determined. During three hr at 37°, approximately 3.5, 10.0, and 15% of the total tritium present in progesterone-1,2-^{3}H, androstenedione-1,2-^{3}H, and testosterone-1,2-^{3}H respectively was converted to ^3H_2O by 100 mg of tissue. The addition of FSH had no effect upon the aromatization of any of the substrates tested, although one of the preparations tested contained a contaminant that significantly inhibited the aromatization of androstenedione.

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Radiation Responses of Embryonal and SV40 Transformed Hamster Cells in Culture* (33381)

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The mechanisms of virus–host cell interaction resulting in the transformation of normal cells to neoplastic cells has been examined in several systems. Elegant experiments with the SV40-3T3 mouse fibroblast system reported by Todaro and Green (1) demonstrated that active cell replication and DNA synthesis are required to fix the transforming event between virus and target cell. Stoker (2) observed that the frequency of transformation of polyoma virus was increased among BHK-21 hamster cells which have survived X-irradiation. Exposure of mouse fibroblast cells to radiomimetic thymidine analogues enhanced the efficiency of SV40 transformation fivefold (3). Borek and Sachs observed that 300 R of X-irradiation induced the *in vitro* transformation of hamster embryo cells when cultured on mouse cell feeder layers (4). Processes associated with cell replication were essential soon after irradiation to fix the transformed state (5). A hereditary and a physiological state of susceptibility to transformation appeared to exist in X-irradiated cells and mechanisms for repair of radiation damage ("error correction") directly controlled the development of the transformed state (6).

Recent studies in this laboratory demonstrated that exposure of primary hamster embryonic cells to 150 R of X-irradiation prior to infection produced a 15- to 50-fold increase in the number of transformed clones when compared with unirradiated controls (7). Radiation alone under these conditions did not produce transformed cells. Characterization of the radiation responses of normal embryonal cells in culture is fundamental to understanding the role of irradiation in sensitizing these cells to transformation by SV40 virus. The present report describes results obtained in the investigation of several parameters of radiation induced changes in normal hamster cells in culture. A parallel study was conducted on the radiation response of SV40 transformed isogenic hamster cells to define potential viral induced changes that may exist.

Materials and Methods. Preparation of embryonal cells. Hamster embryonic lung and kidney cells were prepared by a modification

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of a method suggested by Dr. C. P. Stanners, University of Toronto (personal communication). Syrian Golden hamsters (Lakeview Hamster Colony, Newfield, N. J.) were the source of all embryos. Pregnant hamsters in the fifteenth to sixteenth day of gestation were anesthetized and the embryos were removed aseptically to a petri dish containing Hanks' balanced salt solution (HBSS). Lung and kidneys were removed from the embryos, freed to connective tissue and rinsed twice with HBSS. The pooled organs were minced and suspended in 30 ml of 0.1% trypsin in saline citrate [1.0 g of trypsin (Difco Laboratories, Detroit, Michigan) 2.96 g of sodium citrate, 3.0 g of sodium chloride in 1000 ml of glass distilled water]. The suspension was stirred for 5 min at 25° and then the upper 10 ml of the suspension was discarded. Ten ml of fresh trypsin–saline–citrate solution was added and gentle stirring was continued for an additional 15 min at 25°. The upper 20 ml of this suspension was removed, 2 ml of fetal calf serum were added and the cells were held at 25° for later use. Twenty ml of trypsin were added to the remaining suspension of cells and stirring was continued for 15 min. Tissue fragments were removed from the suspended cells by passage through a stainless steel wire mesh and 3 ml of fetal calf serum were then added. These cells were pooled with the first lot and immediately centrifuged at 225*g* for 10 min, the cell pellet was collected in 20 ml of medium 199 supplemented with a double complement of vitamins, essential amino acids, and L-glutamine in HBSS plus 10% fetal calf serum and antibiotics (100 units of penicillin/ml, 100 μg of streptomycin/ml and 2.5 μg of amphotericin B/ml), henceforth referred to as special 199 medium. Viable cells were enumerated by the trypan blue dye exclusion test and usually standardized to 5×10^5 cells/ml for culture studies. Two ml of this suspension were added to each tube. Tube cultures were gassed in a 40–60% atmosphere of CO_2 immediately after seeding to rapidly lower the pH to 6.0–6.5 and the tube caps were then tightened to hold the pH. Failure to adjust the pH in this manner results in poor adherence of the cells to the tubes and a large portion of the cells soon die.

SV40 hamster tumor cells. The F5-1 line of SV40 hamster tumor was used. This line does not shed SV40 virus but recently a similar line has been reported to yield infectious virus following Sendai virus fusion with primary monkey kidney cells (personal communication, Dr. A. Girardi). The cells were routinely cultivated *in vitro* in medium 199 containing 10% calf serum plus antibiotics. Passage levels 90–95 were used for these studies.

X-irradiation. Irradiation was performed on cells in suspension in special medium 199 in 60-mm plastic dishes employing a General Electric Maxitron "300" X-ray machine equipped with 0.5 mm aluminum and 0.42 mm copper filters. The cells were exposed at a distance of 22 cm and the X-ray unit was set at 250 kVp, 20 mA delivering 540 R/min as determined by a Victoreen Condenser R meter placed under the culture dish lid.

Measurement of cell survival. Following irradiation, cells from replicate tube cultures were removed from the tube wall by brief trypsinization at the desired time intervals, diluted in HBSS and viable cells were enumerated by the trypan blue dye exclusion technique. Empirical observations suggested that the seeding level for normal embryo cells should be 2.5 times the number of tumor cells to compensate for plating efficiency differences between the two cell types.

Measurement of deoxyribonucleic acid (DNA) synthesis. Replicate tube cultures of embryonic and tumor cells were rinsed gently with 1 ml of growth medium, and 2 ml of fresh, special medium 199 containing 2.5 μCi/ml of thymidine-^{3}H (0.97 C/mmole, Mallinckrodt Co., St. Louis, Mo.) was added to each tube. The cells were incubated for 1 hr at 37°. The radioactive medium was then removed, the cell sheets were gently rinsed twice with fresh medium, and the cells were treated with 1.0 ml of 0.25% trypsin at 37° to detach them from the tubes. Labeled cells were collected by centrifugation at 2500*g* for 15 min and extracted for 30 min with 0.3 *N* perchloric acid at 4°. The acid precipitable material was collected by centrifugation and

resuspended in scintillation fluid and assayed for radioactivity in a Nuclear Chicago Liquid Scintillation System.

For autoradiographic studies, replicate Leighton tubes of embryonal and SV40 transformed cells were cultured in special 199 medium containing 2.5 μCi/ml of thymidine-^{3}H (0.97 Ci/mmole, Mallinckrodt Co., St. Louis, Mo.). The cells were incubated from 0.5 to 3 hr at 37°. The medium was then removed, and the cell sheets were rinsed twice with HBSS. The cells were fixed with aceto–ethanol (one part glacial acetic acid to 3 parts absolute ethanol) for 1.5 hr at room temperature. The cover slips were removed, air dried, and mounted in balsam on slides with the cell side up. The following day the slides were dipped in a liquid nuclear-track emulsion (Kodak NTB-3), air dried, and incubated with a desiccant in black slide boxes at 4° for 6 days. Then the slides were developed at 21° for 3.5 min with Kodak D-19 developer, and development was stopped with 28% acetic acid. The slides were acid fixed for 3 min, rinsed in water for 10 min, and air dried. The cells were stained with toluidine blue O stain, cleared with two changes of tertiary butyl alcohol and mounted in balsam. Grain counts were done under the oil immersion lens at 1000 diameters. Total DNA determinations were made using the diphenylamine method described by Burton (8).

Determination of number of chromosomes. Chromosomes were enumerated by the method described by Cooper and Black (9).

Morphologic observations. Cells for examination by microscopy were cultured in Leighton tubes on slides and were fixed at the desired time with absolute methanol and stained with May-Grünwald Giemsa stain.

Results. Preparation of embryonal cells. A variety of procedures for preparing primary cells for *in vitro* culture were evaluated. The method described in the previous section was found to be superior to methods which employed prolonged exposure to high trypsin concentrations. Cells prepared by this new method routinely produced plating efficiencies of 80–95% in 60-mm plastic culture dishes and 25–33% in glass culture tubes.

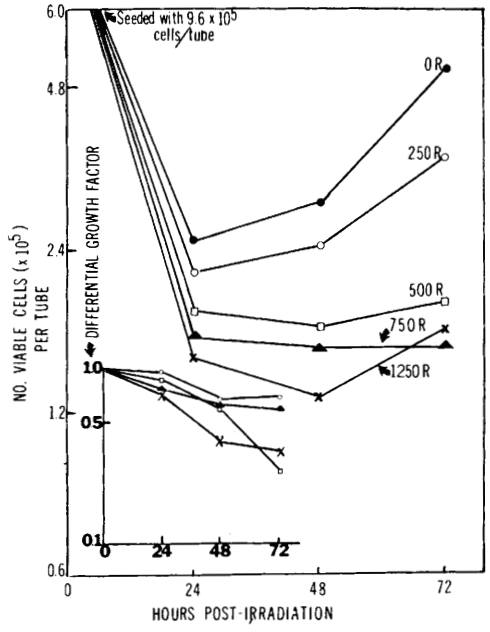


FIG. 1. Viability of X-irradiated embryo cells *in vitro*.

Several factors were found to contribute to these high plating efficiencies. The use of 0.1% trypsin solution for tissue dissociation and the brevity of the exposure time to the enzyme, greatly enhanced survival of the cells. Serum addition was most effective in preserving the viability of the cells after trypsinization. Adjustment of the pH to 6.0–6.5 with CO_2 was absolutely essential to obtain maximal attachment of the cells.

Radiation lethality studies. The survival of embryonic lung and kidney cells in tissue culture following exposure to increasing doses of X-irradiation was determined. Vital staining was employed as the method for distinguishing living from dead cells postirradiation. Parallel cultures for each X-ray dose were sampled at 24, 48, and 72 hr after exposure and the results are given in Fig. 1. These data were expressed both as the number of viable cells per tube and the differential growth factor. Differential growth factor was used to express radiation damage as a function of both decreased survivors and mitotic inhibition of growth. This expression was used in preference to fraction survival since the latter term was invalid when control

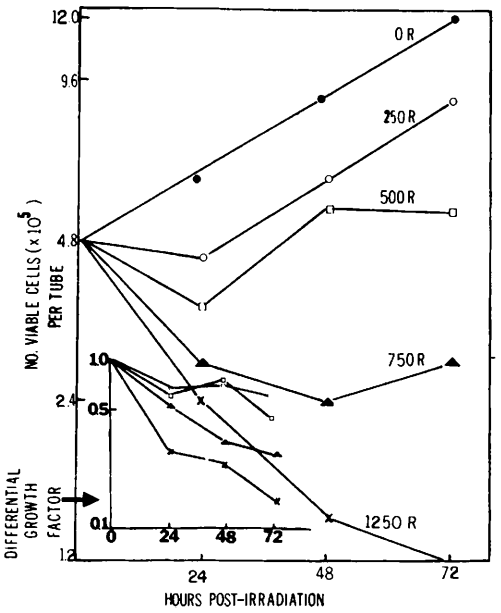


FIG. 2. Viability of X-irradiated tumor cells *in vitro*.

cell values (unirradiated cells) increased over the period of assay. Differential growth factor was obtained by dividing observed viable counts for irradiated cells by values obtained for unirradiated cells. A factor of 1 indicated growth equivalent to unirradiated cells. The growth differential plot, which is primarily an expression of surviving fraction at 24 hr, shows that 95% of the population survived the initial effects of X-ray exposures of 250 R. Exposures of 1250 R or less caused only 30% of the population to die in the first day. An examination of the viable cell plot reveals that after an initial decrease in cells resulting primarily from the combined effects of immediate radiation death and plating efficiency, a dose-related delay ensued for the irradiated cells during the next 24 hr. After 48 hr, cell populations receiving 250 R resumed growth albeit with a decreased generation time.

In a similar experiment, the radiation response of SV40 transformed hamster cells was evaluated and results are presented in Fig. 2. Unirradiated cells initiated growth immediately as shown in the viable cell plot in contrast to the 24-hr delay observed for normal cells. The SV40 tumor cells showed a greater sensitivity to X-irradiation than was observed for normal cells as demonstrated by viable cell count and differential growth, especially at the higher radiation levels.

The growth differential pattern of normal hamster cells was observed to vary with the length of time lapsed between exposure to any given dose of irradiation and the performance of the vital stain procedure (Fig. 3). Results obtained at 24 hr showed no difference between exposures of 500 R or higher, whereas striking differences became apparent between these doses when the vital staining procedure was withheld until 96 hr postirradiation. The response patterns tend toward the exponential and became less bimodal by 96 hr. Similar results, not presented, were obtained for SV40 transformed hamster cells.

X-irradiation effects on DNA synthesis. The *in vitro* incorporation of thymidine-^{3}H into normal and SV40 transformed hamster cell DNA was determined at regular intervals after irradiation (see Table I). The DNA synthesis in unirradiated embryonal hamster cells proceeded at a constant rate per cell over the 72-hr period examined. Exposure to 250 R of X-rays diminished the rate of DNA

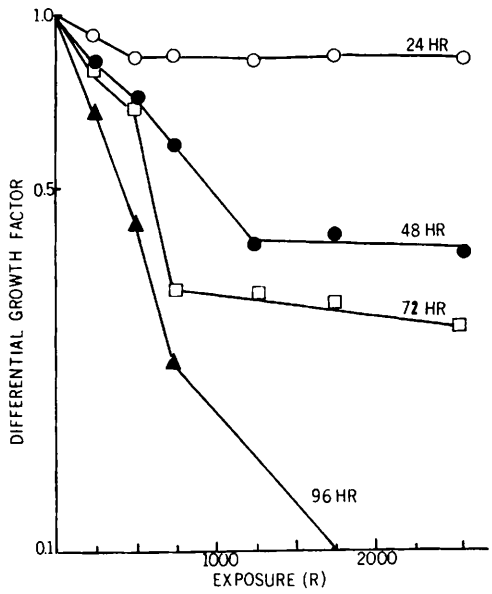


FIG. 3. Effects of X-irradiation on embryo cells determined by vital staining at intervals postirradiation.

TABLE I. DNA Synthesis in X-Irradiated Embryo and SV40 Transformed Hamster Cells *in Vitro*.

X-ray exposures (R)	Thymidine-^{3}H uptake per viable cell per hour (10^{-3}) (cpm)[a] at hour postirradiation when labeled					
	Embryo cells			Transformed cells		
	24	48	72	24	48	72
0	7.80 ± 0.93[b]	7.10 ± 0.85	7.90 ± 2.10	40.8 ± 6.1	22.4 ± 5.1	17.6 ± 3.1
250	3.10 ± 0.47	6.70 ± 0.47	6.70 ± 0.73	49.0 ± 5.4	30.4 ± 3.3	13.6 ± 1.9
500	3.30 ± 0.66	3.30 ± 1.1	4.30 ± 0.86	60.0 ± 9.0	31.1 ± 1.2	14.6 ± 1.8
750	2.20 ± 0.28	3.30 ± 0.16	4.50 ± 0.87	42.6 ± 6.7	42.5 ± 6.8	29.2 ± 6.7

[a] Values given are from two different experiments run in duplicate.

[b] Each value is written in the form A ± B, where A is the mean rate of DNA synthesis for both experiments and B is the range.

synthesis by one-half at 24 hr but DNA synthesis returned to the level observed for unirradiated cells by 48 hr. Suppression of DNA synthesis at 500 or 750 R in embryonal hamster cells was still observed 72 hr postirradiation. Recent results indicate that a 30% diminution in DNA synthesis rate can be detected as early as 1 hr postirradiation.

Unirradiated SV40 transformed cells were observed to uniformly maintain 5 times the rate of DNA synthesis occurring in normal hamster cells at 24 hr after inoculation into culture. It should be noted that the generation time of the tumor cell line was 12–15 hr and the normal hamster embryo cell usually divides every 23–25 hr after adapting to tissue culture conditions. Forty-eight hr after seeding, thymidine-^{3}H incorporation by unirradiated tumor cells diminished to almost one-half the rate observed at 24 hr and even more at 72 hr. Tumor cells formed confluent cell sheets by 24 hr and cell populations in the tubes were extremely crowded by 72 hr possibly accounting for the diminishing rate of DNA synthesis in unirradiated controls.

Unlike embryo cells, DNA synthesis in surviving tumor cells was not suppressed by the X-ray exposures employed. In fact, DNA synthesis rates per viable cell were routinely increased 24 hr postirradiation. It is important to recognize that the higher rates of synthesis observed for irradiated tumor cells at 24 hr correlate well with the initiation of active cell replication observed for X-irradiated cells as shown in Fig. 2. Of the X-ray exposures tested, 500 R produced the highest rates of DNA synthesis. The results were repeatedly obtained in several experiments and may correlate with growth release from mitotic delay.

The rates of DNA synthesis for embryo and SV40 transformed hamster cells as measured by autoradiography are shown in Table II. The difference in the rates of DNA synthesis per cell can be accounted for by the difference in the number of cells in S-stage for the two cell types. The X-irradiation induced a decrease in the number of cells in S-stage for both the embryo cells and SV40 transformed cells. In embryo cells, the decrease in the number of cells in S-stage agreed with the diminished rate of DNA synthesis as measured by thymidine-^{3}H incorporation.

TABLE II. Measurement of Rates of DNA Synthesis for Embryo and SV40 Transformed Hamster Cells by Autoradiography.

X-ray exposure (R)	Cells labeled (%)[a]	
	Embryo cells	Tumor cells
0	19.6[b] (18.2–23.3)	58.4 (54.6–60.0)
250	12.1 (10.2–14.0)	41.0 (37.8–43.4)

[a] Values obtained for 24-hr culture.

[b] Data are given as mean value (%) obtained from observing 2500 cells with more than 10 grains per nucleus and range is given in parentheses.

TABLE III. Nuclear Characteristics of Embryo and SV40 Transformed Hamster Cells.[a]

Cell type	DNA ($\mu\mu$g/cell)	No. of nuclei/cell[b]	Nuclear volume (μ^3)[b]	No. of chromosomes/nucleus[c]
Embryo cells	9.9 (5.9–12.0)	1	1.50 (1.1–2.0)	44
SV40 transformed cells	16.7 (13.1–19.8)	1.32	3.64 (2.0–5.2)	61.0 (56.7–65.3)

[a] Values obtained from 48-hr culture.
[b] Mean and range for 500 cells scored.
[c] Mean and 95% confidence interval from scoring 50 cells.

Nuclear characteristics of normal and tumor cells. The differences in rates of DNA synthesis between normal and tumor cells stimulated an investigation of the nuclear morphology of the two cell types. Results shown in Table III summarized a comparison of the two cell types. Tumor cell nuclei were uniformly more than twice as large as em-

TABLE IV. Effects of X-irradiation on Nuclear Size of Embryo and SV40 Transformed Hamster Cells *in Vitro*.

Cell type	X-ray exposure (R)	Nuclear dimensions[a] (μ); hour postirradiation		
		24	72	144
Hamster embryo cells	0	8.0 × 7.8 (6 × 3–10 × 10)	8.0 × 7.8 (6 × 3–10 × 10)	8.0 × 7.8 (6 × 3–10 × 10)
	500	9.0 × 7.8 (7 × 4–12 × 17)	13.6 × 11.2 (7 × 7–18 × 22)	36.0 × 15.0 (12 × 7–30 × 40) (10–15% PNGC)[b]
	1000	9.8 × 7.8 (7 × 4–12 × 10)	25.8 × 15.4 (10 × 7–32 × 22)	36.8 × 15.0 (7 × 10–50 × 26) (5–10% PNGC)
	2500	8.8 × 8.0 (7 × 4–10 × 12)	26.2 × 26.0 (4 × 12–50 × 41) (20–25% PNGC)	26.2 × 26.0 (10 × 20–52 × 30) (10–20% PNGC)
SV40 tumor cells	0	9.6 × 11.4 (7 × 7–14 × 10)	9.6 × 11.4 (7 × 7–14 × 10)	9.6 × 11.4 (7 × 7–14 × 10)
	500	9.5 × 11.8 (7 × 7–15 × 10)	8.0 × 13.4 (7 × 4–25 × 15) (20–25% PNGC)	20.0 × 20.0 (8 × 10–30 × 20) (70–80% PNGC)
	1000	9.4 × 11.0 (7 × 4–15 × 11)	8.0 × 13.4 (7 × 6–28 × 16) (60–70% PNGC)	(100% PNGC)[c]
	2500	9.0 × 11.0 (7 × 10–21 × 12)	8.0 × 13.4 (7 × 6–27 × 17) (70–80% PNGC)	(100% PNGC)[c]

[a] The cross-section of the nuclei were generally ellipsoidal and values are given in a multiplicative notation of the major and minor axis of 25 representative cells. The range is also given for these values.
[b] PNGC = polynuclear giant cell.
[c] All cells contained 5–20 micronuclei/cell and no measurement of the size could be performed.

TABLE V. The Effects of X-irradiation on Chromosome Number in Embryo and SV40 Hamster Cells *in Vitro*.

	X-ray exposure (R)	Chromosome count/cell[a]; hour postirradiation		
		24	48	72
SV40 transformed cells	0	61.0 (56–65)	61.0 (56–65)	61.0 (56–65)
	500	74.0 (66–97)	81.0 (60–103)	71.3 (61–84)
	1000	68.6 (44–89)	82.0 (60–131)	77.0 (72–105)
	2500	86.0 (74–102)	87.0 (65–85)	97.0 (80–133)
Embryonal cells	0–2500	44	44	44

[a] Duplicate slide preparations from several experiments were examined and more than 50 cells were examined per slide. Numbers represent mean values and ranges from all counts.

bryonal hamster cell nuclei and contained at least twice the complement of DNA. The number of chromosomes in tumor cell nuclei varied from 56 to 65. Fifteen to 20% of the tumor cells contained two nuclei per cell and 1–2% of the cells had more than two nuclei.

An evaluation of changes in nuclear size as a function of X-ray exposure and time postirradiation was performed. Results (Table IV) show that a general increase in nuclear size of surviving embryo cells was apparent at all radiation levels by 72 hr after exposure. The nuclei of embryo cells 144 hr postirradiation uniformly measured three to four times the size of unirradiated cell nuclei and the estimated volume increased by a factor of 9–12.

The SV40 transformed cell nuclei increased somewhat in size 72 hr postirradiation but not to the degree observed for surviving embryonic cells. A major contrast in nuclear and cytoplasmic size between embryonic and tumor cells treated with 2500 R of X-irradiation can be seen in Fig. 4. Giant cell formation was apparent in both cell types, but the majority of embryo cells uniformly contained one large nucleus whereas tumor cells always possessed several smaller nuclei. Occasionally irradiated enbryonal cell populations contained a few polynuclear giant cells.

The SV40 tumor cells, capable of mitosis (an inherent feature of the chromosome counting procedure to get mitotic figures) after irradiation showed a general increase in the average number of chromosomes per nucleus (Table V and VI). Embryo cells capable of mitosis maintained the normal chromosome number. The total DNA content for each cell type, shown in Table VI, correlated well with the radiation-induced ploidy changes. That is, the embryo cells showed no change of total DNA per viable cell, whereas the SV40 transformed cells showed a significant increase in the amount of DNA per viable cell.

Discussion. Radiation and its role in viral transformation. The mechanism of neoplastic transformation has been intensively studied and a definitive description of the events lead-

TABLE VI. Effect of X-irradiation on the Total DNA Content of Embryo and SV40 Transformed Hamster Cells.[a]

Cells	X-ray exposure (R):	Total DNA ($\mu\mu$g/cell)[b]			
		0	250	1250	2500
Embryo cells		9.9 (5.9–12.0)	13.1 (10.7–22.6)	9.0 (6.0–12.0)	10.1 (7.0–14.3)
SV40 transformed cells		16.7 (13.9–18.6)	13.4 (12.7–13.8)	47.7 (38.1–57.0)	52.9 (43.2–61.4)

[a] Values obtained 144 hr after seeding.

[b] Values are given in the form A $(x–y)$, where A is the mean of five determinations and $(x–y)$ is the range.

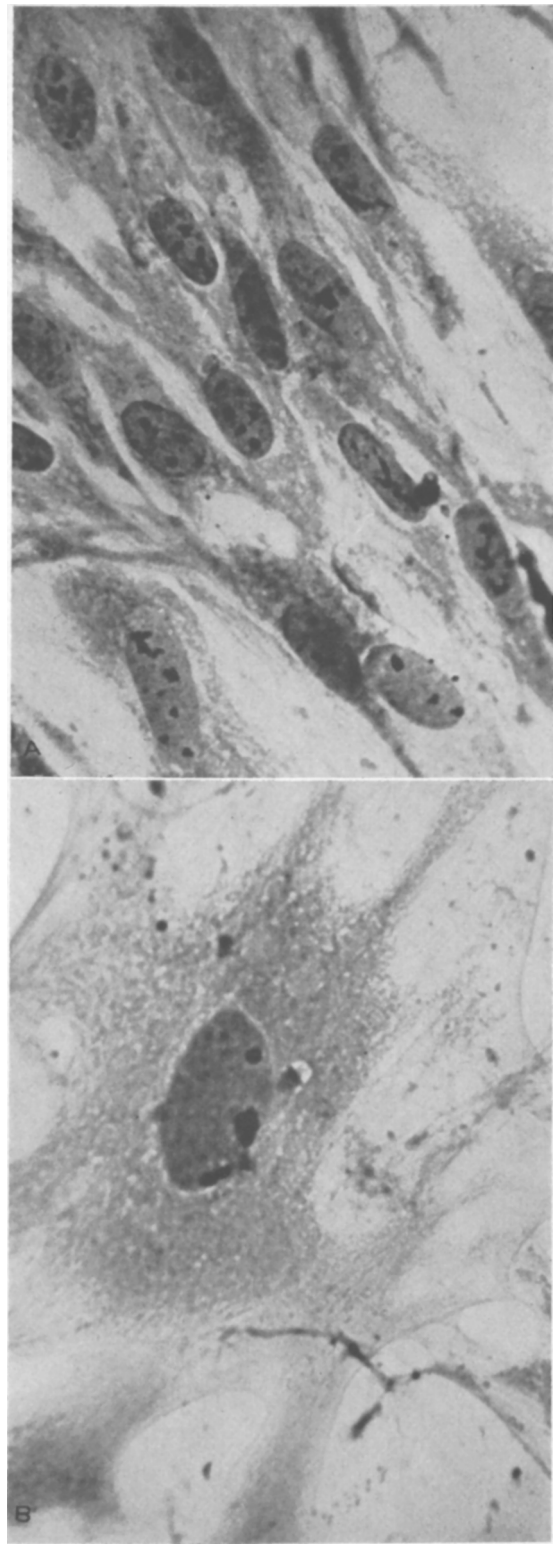
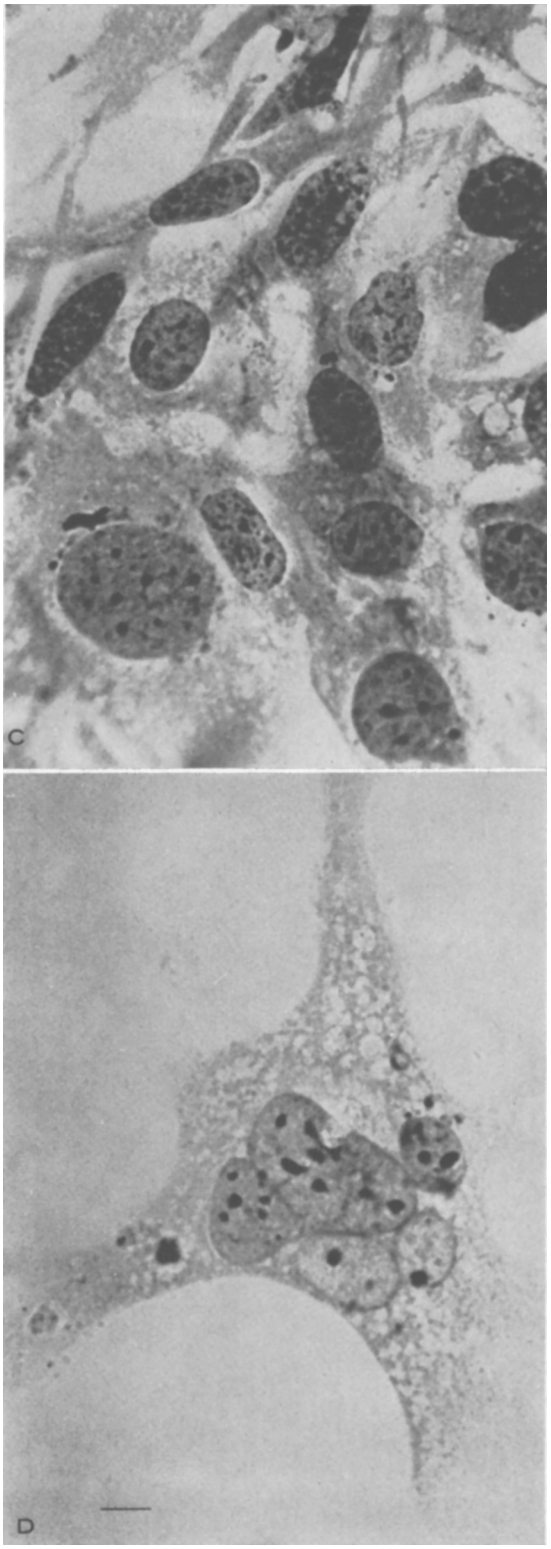


FIG. 4. Morphology of irradiated embryonal and SV 40 transformed hamster cells (scale 10 μ). (a) Embryo cells 144 hr in culture; (b) Embryo cell 144 hr after exposure to 2500 R of



X-irradiation; (c) SV40 Transformed cells 144 hr in culture; (d) SV40 Transformed cell 144 hr after exposure to 2500 R of X-irradiation.

ing to transformation has been most elusive. Recent investigations (1, 5, 6) have shown that radiation or virus-induced transformation occurred in cells during periods of active DNA synthesis and the transforming event appeared to be fixed early after radiation exposure or infection with the virus. Agents which damage DNA directly or during replication were found to potentiate SV40 virus transformation (2, 3, 7). Our studies were initiated to describe the early radiation responses of embryonic hamster cells which relate to cell survival, periods of DNA synthesis postirradiation, and other characteristics of embryonic and SV40 transformed cells in a program to define the role of X-irradiation in enchancing SV40 transformation.

Cell survival following irradiation has been effectively determined in a variety of ways. Puck and Marcus (10) determined cell survival by observing colony production at selected intervals after irradiation. Embryonic cells usually clone with low efficiency making them somewhat undesirable for cloning studies. Therefore, it was necessary to measure cell survival by some other technique. Assuming that the transforming event was fixed early after infection, we selected a method for determining cell survival that had the capability of demonstrating immediate radiation lethality and employed the vital staining technique which has been successfully used by Watanabe and Okada to describe the early stages of radiation-induced cell death in mouse leukemia cells (11).

X-Ray exposures of 250 R caused the early death (within 24 hr) of only 5% of the hamster embryo cell population. These dead cells seem to be ruled out as potential targets for viral transformation since they lack replicative potential or cellular integrity.

Periods of DNA synthesis after irradiation were measured by the uptake of thymidine-^{3}H into acid-precipitable material and by autoradiography. Results (Table I) indicated that a 60% depression in the rate of DNA synthesis per viable cell occurred after exposure to 250 R of X-rays. Autoradiographic studies (Table II) demonstrated a 40% decrease in the number of cells in DNA synthesis after this same exposure. Therefore, a large fraction of the observed decline in DNA synthesis resulted from a decrease in the number of cells actively synthesizing DNA rather than from a true 50–60% depression in the rate of DNA synthesis per viable cell postirradiation. However, the decrease in the number of cells synthesizing DNA postirradiation as detected by autoradiography cannot account for the total diminution in DNA synthesis. A minimum 20% depression in the rate of DNA synthesis per cell occurred since this differential cannot be eliminated from the total rate of depression by results obtained from autoradiography studies. These results suggest that the primary effects of irradiation were twofold. The rate of DNA synthesis per cell was depressed and the number of cells synthesizing DNA was decreased.

These findings indicate that there were at least two populations of surviving cells which were potential targets for SV40 transformation. One population would include those cells undergoing depressed DNA synthesis and the second group would be composed of cells trapped in some stage of the cell cycle and unable to initiate DNA synthesis following irradiation. The studies of Todaro and Green have suggested that active DNA synthesis is an essential requirement for the tranformation of 3T3 mouse fiboblasts by SV40 virus (1). If indeed active DNA synthesis is a requirement to fix transformation in irradiated cells, then neither of these cell populations are likely targets for viral transformation.

The data presented here suggest that the transforming event may be delayed until some time between 24 and 48 hr following irradiation. At this time, DNA synthesis returned to a normal rate in X-irradiated embryo cells and cell division was initiated. The observation has been made in our laboratory that the rate of transformation of hamster embryo cell in culture by SV40 was increased from an efficiency of 0.2–27% when cells were subjected to low doses of X-ray just prior to infection with the virus (7). When the addition of SV40 virus to irradiated hamster cells was delayed 10 hr, a decrease in

transforming efficiency to 15% was observed (7). The enhancing principle of irradiation therefore, must be a transient condition of the irradiated cell. Either nonreplicating cells conducting depressed DNA synthesis or cells trapped in some stage of the cell cycle immediately after irradiation may indeed be the prime target cells for transformation, and delay in the infection of these cells with virus reduces the frequency of transformation.

If the target cells are those trapped in some specific cell stage, this sensitive period would appear to be the G-2 stage. Supporting this conclusion are the observations of Howard (12) and Kelly (13) who showed that radiation-induced depression of DNA synthesis directly resulted from the phenomenon of mitotic delay. Therefore, the radiation-induced block occurred before mitosis, during the G-2 period. This interpretation is compatible with the observation of Bascilico and Marin (14), who have reported that synchronized cultures of the Syrian hamster cell line BHK-21 exhibited an increased susceptibility to polyoma virus transformation. Transformation was dependent on the proper timing of infection with the G-2 stage of the cell cycle.

Stoker (2) observed that polyoma virus transformation was enchanced almost twofold when BHK-21 hamster cells were pretreated with 500 R instead of 188 R of X-rays. Results presented here show that hamster cells exposed to 500–750 R generally failed to replicate and that these cells conducted DNA synthesis at a rate one-half that of irradiated cells. Thus, the observation of Stoker that 500 R was most effective in enhancing viral transformation further suggested that cells undergoing depressed DNA synthesis or trapped in G-2 stage were prime candidates for viral transformation. In future work it will be important to determine whether or not nonproliferating embryo cells which are capable of successful replication postirradiation are the "target" cells of viral transformation.

Radiation responses of embryo and tumor cells. The SV40 transformed hamster cells derived *in vivo* produced a dose response pattern for survivors similar to that observed for embryo cells. The differential growth plot, however, showed tumor cells to be more sensitive to X-irradiation. These data were best explained by the observation that unirradiated tumor cells, unlike embryo cells, replicated soon after inoculation into culture. After irradiation tumor cells would be expected to show mitotic death or mitotic inhibition more quickly than the slower growing embryonal cells.

Unirradiated tumor cells had a DNA synthesis rate five times that of embryo cells. This observation can be accounted for by the number of cells in S-stage (Table II), the amount of DNA present, the number of nuclei present, the number of chromosomes present, and the nuclear volume of the respective cell types (Table III). The rate of DNA synthesis for unirradiated embryo cells was constant throughout the 72 hr period of observation whereas tumor cells demonstrated a depressed rate of synthesis after the first 24 hr. Macieira-Coehlo (15) recently reported that SV40 transformed human fibroblast cells synthesized DNA at a rate inversely proportional to the cell population density. Human tumor cell concentrations were at the same level as the hamster tumor cells used in these studies when the rate of DNA synthesis began to diminish.

Irradiation does not appear to affect the rates of DNA synthesis in tumor and embryo cells in the same manner. Irradiation of embryo cells caused a suppression in the rate of DNA synthesis similar to that reported for a variety of other cell types. Tumor cells exposed to 750 R of X-rays maintained a higher rate of DNA synthesis than unirradiated controls suggesting that irradiation enhanced DNA synthesis. The observed increase may be an artifact resulting from the cell density phenomena. That is, radiation diminished the cell population as a function of dose and surviving cells were less restricted than cells growing in higher population densities, and therefore, maintained a higher rate of DNA synthesis.

This postirradiation increase of DNA synthesis can further be explained by the results obtained in chromosome studies. Tumor cells

surviving X-irradiation possessed an increased number of chromosomes and also an increased amount of DNA. Radiation-dependent chromosome changes similar to those reported here, have been observed in X-irradiated Chinese hamster tumor cells (16). It would be expected that increased chromosome counts or increased amounts of DNA relate directly to an increased rate of DNA synthesis.

The observed nuclear changes in tumor and embryo cells after irradiation are in agreement with the changes reported by Enders *et al.* (17). In our study, surviving tumor cells uniformly contained many small nuclei whereas surviving embryo cells usually possessed one extremely large nucleus. Surprisingly, giant embryo cells (Table VI) contained no more DNA per cell than unirradiated cells. The available data offered no explanation for the increase in nuclear size, however, the presence of this new material may result from aberrant histone or nucleolar synthesis. Results obtained with the May-Grünwald stain suggested that the large nuclei contained excess RNA. Studies are underway to determine the chemical composition of giant cell nuclei.

Polynuclear giant cells (PNGC) formed after irradiation of tumor cells contained more DNA than its unirradiated counterpart. It should be noted that the irradiated tumor cells uniformly developed into PNGC. The occurrence of this response in all surviving cells suggested the presence of a particular radiosensitive "target" in the genome of tumor cells which controls nuclear separation, or daughter cell formation, or both. An occasional PNGC was observed among irradiated embryo cells and the low percentage (15%) suggested that the "target" controlling nuclear response is either smaller or more radioresistant in embryo cells than a similar locus in tumor cells.

It seems unlikely that selective processes resulting from repeated subpassage of the tumor cells may have yielded a cell type which responded to irradiation by the production of PNGC. Early passage levels of the tumor line were not observed to show any morphological difference postirradiation from the results reported here. However, other SV40 transformed hamster cell lines in another laboratory (17) have also been observed to respond to X-irradiation by the formation of PNGC. The suggestion has been made and it was affirmed here, that the SV40 virus genome must in some way be responsible for the peculiar radiation responses of these tumor cells. An understanding of the mechanism by which SV40 converts normal cells to neoplastic cells may give some insight into the basis for the observed differences in the radiation response. These studies are the subject for future experimentation in this laboratory.

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