

## ACTION OF X-RAYS ON MAMMALIAN CELLS\*· †

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### PLATES 26 AND 27

Study of the mechanisms of action of ionizing radiation in higher animals has been impeded by lack of a precise method for measurement of reproductive potential in single mammalian cells comparable to that available for microorganisms, such as the plating technique of the bacteriologist (1) or the plaque method of the virologist (2). While a number of quantitative and semiquantitative procedures for titrating animal cell reproductive capacity have been proposed (3), these generally depend on complex interactions between members of large populations, and their results are difficult to translate in terms of the fate of the individual cell. A variety of different criteria have been used as an index of cell "killing" by irradiation, and "cell lethal doses" have variously been defined over a range extending from the neighborhood of 100 r to more than 100,000 r (3-5). The extent to which the metabolic state of the individual animal cell can alter its radiation susceptibility is only poorly apprehended. The degree to which the lethal radiation dose to an entire animal depends on radiobiologic damage to reproductive processes within cells of specific types is uncertain. And the rates of mammalian cell mutation have been very rarely susceptible to experimental determination (6) because of lack of a reasonably rapid method for cultivation of large populations from the single cell mutants produced by irradiation, as is done in a routine manner with microorganisms, (see reference 7, for example).

With the development of simple, rapid methods for growing single mammalian cells into macroscopic colonies with a plating efficiency of 100 per cent (within the limits of sampling error) (8-9), it became possible to carry out studies on such cells in precisely the same way as has been done for bacteria. In this paper are presented experiments describing quantitatively the effects of high energy irradiation on the reproductive capacity of single HeLa cells, derived from a human cervical carcinoma (10).

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*Methods and Materials*

The plating procedures used to obtain colonial growth from single HeLa cells on Petri dishes have been described (9). Both the method employing a feeder layer and that involving more gentle trypsinization which makes the feeder layer unnecessary, were observed to yield similar results, so that after the first several experiments the use of feeders was discontinued. The clonal strain S3 was employed in most of these experiments, although a few survival curve determinations carried out on the wild type HeLa cell and on another mutant, S1, yielded essentially the same result.

Known aliquots of monodisperse HeLa cell suspensions were deposited on 60 mm. Petri dishes containing 4 to 5 cc. of standard growth medium (9), or a modification thereof containing 10 or 15 per cent porcine serum as the only serum component. (Use of porcine rather than human serum permits a larger number of colonies on each plate because of the greater compactness of the resulting growth (9).) In the standard procedure the plates were incubated in 5 per cent CO<sub>2</sub> in air at 37°C., for at least 5 hours and not more than 24 hours, during which time the cells attach to the glass. Inspection of the plates just before irradiation confirmed that more than 90 per cent of the cells are indeed single at this point and in interphase. The covered Petri plates were then exposed for measured time intervals to a Westinghouse x-ray tube operating at 230 KVP<sup>1</sup> and 15 ma., filtered with 1 mm. of aluminum and 0.5 mm. of Cu. Dose rates of 100 to 200 r/minute were employed. These were measured with a Victoreen r meter whose calibration had been confirmed by comparison with meters standardized by the National Bureau of Standards. The meter was placed in exactly the same position as the sample, and covered with a Petri dish lid to duplicate the conditions of cell exposure. Readings were corrected to standard temperature and pressure.

After irradiation the plates were incubated in the CO<sub>2</sub> chamber (9) for at least 9 days, and sometimes as long as 17 days, as will be described. In some experiments, fresh growth medium was substituted immediately after irradiation in case toxic materials should have been produced in the suspending fluid, but experience soon demonstrated that this measure had no appreciable influence on the experimental results for the dosages used in this study. It was also necessary to determine whether toxic products arising from the irradiated cells might inhibit growth of true survivors on the same plate. The following experiment showed this effect to be negligible within the dose ranges here employed: Plates containing thousands of cells were irradiated with 2000 to 4000r, a dose range sufficient to permit no survivors. Immediately afterward a known inoculum of normal cells was added, and the plates incubated. In every case the colony count obtained closely approximated the number of normal cells added to the heavily irradiated plate.

Unirradiated cells exhibit 100 per cent colony-forming efficiency under the conditions employed. Hence, it is reasonably certain that the experimental procedure subjects these cells to no major stress other than that of the irradiation. Throughout this paper the words, "survival," "viable," and "killing" are used in the sense which has become standard in microbiology; *i.e.*, referring only to the ability of the individual cell to multiply into a macroscopic colony. We shall designate  $D_0$  as the number of Roentgens needed to reduce a population to the fraction  $1/e = 37$  per cent, and shall use this as a measure of the slope of the survival curve in regions where it is a linearly logarithmic function of the dose.

<sup>1</sup> KVP, kilovolt peak.

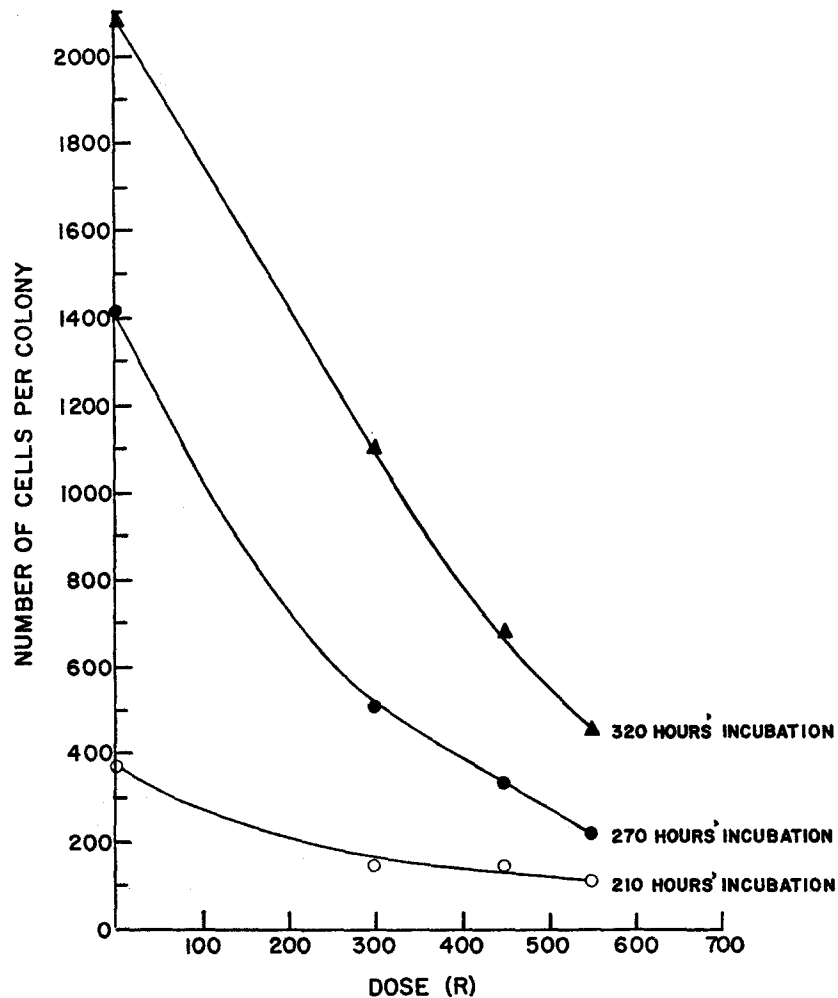
## EXPERIMENTAL RESULTS

*Reduction in Growth Rate among the Survivors, and the Formation of Abortive Colonies.—*

Irradiation of a plate seeded with isolated HeLa cells kills part of the population, the proportion depending on the dose. In addition, however, the survivors may grow more slowly. These two effects are demonstrated in the plates of Figs. 1 and 2, showing the colonies developing on two plates which have been treated identically except that one was irradiated with 300 r. On the latter fewer colonies have grown out and those which have developed are clearly smaller than the colonies of the unirradiated control. This effect of progressive slowing down of reproductive rate by increasing amounts of radiation is quantitatively shown in the data of Text-fig. 1, in which the average number of cells per colony has been determined for a control plate and for the survivors of a series of irradiations. The growth-delaying effect is also illustrated by the fact that the largest colony produced by the survivors of a dose of 550 r contained fewer cells than the average colony on the unirradiated plate.

The existence of this growth-delaying action adds some difficulty to the scoring of survivors, since an incubation period perfectly adequate to produce well developed colonies from all of the cells of a control plate may be insufficient to permit identification of some slow growing survivors. The situation is further complicated by the fact that cells lethally damaged by the irradiation do not at once cease all activity but may go on multiplying for as much as five or six generations before terminating their reproduction. In this respect, their behavior is exactly analogous to that of irradiated bacteria (11). Therefore, in scoring survivors, it becomes necessary to distinguish clearly between the abortive colonies and those of slow growing survivors. An unambiguous determination of the survival of the capacity to reproduce as a function of radiation dose can be obtained by increasing the time of incubation in the growth medium. Ultimately even the slowest growing surviving colonies reach diameters of 3 to 4 mm., while the abortive colonies attain a limiting size after which they remain constant or even degenerate. These processes are clearly illustrated in the plates of Figs. 3 and 4, in which the macroscopic appearance of colonies on plates irradiated with 0 and 300 r, respectively, is shown after 17 days of incubation, as contrasted with the 9 day old plates of Figs. 1 and 2. It is obvious that on long term incubation, many of the questionable survivors of Fig. 2 have failed to maintain their growth, and that the plate of Fig. 4 makes possible a count of the survival of the capacity to reproduce, which is as definitive as that in a bacterial population.

The abortive colonies are also usually distinguishable from slow growing but true survivors by the preponderance of giant cells in their constituency, as will be discussed later. Experience has shown that an accurate count of



TEXT-FIG. 1. Demonstration of growth-slowing action of x-irradiation on single HeLa cells. Plates containing single cells deposited in the standard manner were x-irradiated, incubated for the times shown, then fixed and stained. The cells per colony were counted by microscopic examination, for all the colonies on each plate, or if these were too large in number, for a random sample of 10 to 20 colonies. In order to avoid weighting the data through the effect of abortive colonies, only colonies with more than 50 cells were counted.

colony-forming survivors can be obtained after only 11 to 13, instead of 17, days of incubation if the criterion employed for survival is that at least 50 normal cells be present in the colony at the end of this period. The standard practice has been adopted of scoring all the colonies which, to the unaided eye, are obviously in active growth. Questionable colonies are then examined microscopically using 50 or more normal cells as the index of retention of

multiplication potential. With this system of scoring, the colony counts are definitive and remain constant with continued incubation.

A typical example will illustrate the results obtained: A series of plates, each seeded with 200 S3 cells, was irradiated with 300 r, and incubated for 9, 12, and 17 days, respectively. The plate stained after 9 days had a total of 117 aggregates visible to the unaided eye, ranging in diameter from 0.1 to 1.0 mm. Estimation of the actual number of these which would go on to further growth is difficult with the eye alone. This uncertainty was eliminated on further incubation, for the 12 day and 17 day plates unequivocally differentiated between cell aggregates which had remained less than 1 mm. in diameter, and those which now formed large colonies of 2 to 5 mm. The number of these latter was 31 and 35 on the plates incubated for 12 and 17 days respectively, values agreeing well within sampling uncertainty. However, by employing the criterion that each colony with 50 or more normal cells is to be counted as a survivor, the counts obtained on all 3 plates agreed, consisting in 35, 36, and 36, for the 9, 12, and 17 day incubations, respectively. It is impossible to avoid a certain degree of arbitrariness in the criterion which will indicate loss of reproductive function. We believe the operational definition employed in this study is biologically analogous to that which has proved useful in microorganisms, in addition to furnishing a readily reproducible end-point.

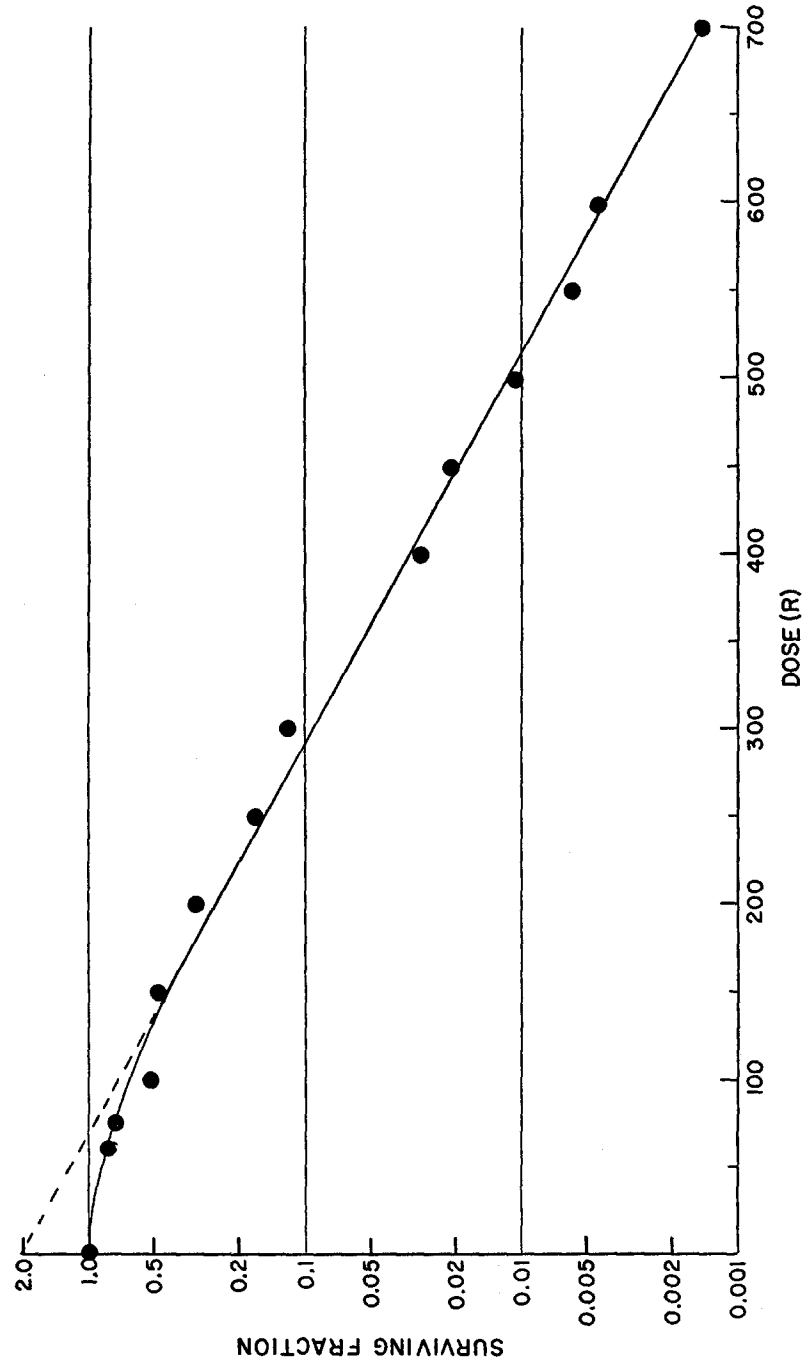
#### *Shape of the Survival Curve.—*

The survival curve for single cells of the S3 HeLa strain determined by this procedure is presented in Text-fig. 2. Survival determinations at each dose were carried out with duplicate or triplicate platings whose individual values almost invariably agreed within sampling uncertainty. All but one of the points shown on the curve are the means of values determined in a series of 2 to 10 such separate experiments carried out over a period of several months. At each dose the standard deviations of individual survival values obtained from such a series of independent experiments usually lay in the neighborhood of 10 to 25 per cent of the mean. As is to be expected, the reproducibility of individual points is less good at doses greater than 500 r, when inocula as high as  $10^4$ – $10^5$  cells are required on each plate to yield a countable number of survivors. Study of survival after doses of 800 r or more necessitates such tremendous cell crowding as to require changes in the experimental technique.

The existence of the shoulder in the survival curve of Text-Fig. 2 is unequivocal and constitutes evidence for a multiple hit killing mechanism. This curve furnishes strong contrast to the single hit curve obtained with haploid cells like *E. coli* (12). The straight portion of the HeLa survival curve has a slope corresponding to a value for  $D_0 = 96$  r; *i.e.*, in this linear region, an increase in dose of 96 roentgens further reduces the survivors by a factor of 37 per cent. (See Discussion.)

#### *Fate of the Cells Inactivated by Irradiation.—*

The cells which fail to form self-sustaining colonies suffer one of three fates. They may undergo a limited number of divisions, forming microcolonies with a cell number that almost never exceeds 50, even after 17 to 20 days of incuba-



TEXT-Fig. 2. Survival of reproductive capacity in HeLa cells as a function of x-ray dose. The points fit the equation  $S = [1 - (1 - e^{-D/66})^2]$  within the limits of uncertainty of the experimental procedure.

tion; they may remain as single cells; or they may disappear, leaving no trace on the plate which is fixed and stained. Concomitant with the first two processes which we shall lump together under the name "abortive colony formation" even when only a single cell remains, is the development of giant cells from the non-reproducing cells which continue to grow, reaching diameters as great as 0.8 mm.

At low doses almost every killed cell will still multiply several times, so the non-survivors form microcolonies of 20 to 40 individuals corresponding to 4 to 5 divisions. Practically all these microcolonies have several giants at the time of staining, and some consist only of giants. As the x-ray dose increases, the number of divisions decreases and the abortive colonies become smaller in cell number. At doses greater than 600 r the ability to divide even once is

TABLE I

Illustration that with increasing x-ray dose, there occurs a decrease in number of cells per abortive colony, and an increase in the proportion of giant cells in such non-surviving populations, until at 800 to 1000 r, a population consisting practically entirely of single giant cells is to be found on the plate.

Dose	Average cells/clone in abortive colonies	Proportion of giants in the cells of the abortive colonies
<i>r</i>		<i>per cent</i>
50	27.	6.7
200	23.	23.
400	13.	28.
600	1.7	100.
1000	1.0	100.

In unirradiated populations the frequency of giant cell occurrence is 0.19 per cent.

greatly reduced, and at doses equal to or greater than 800 r practically all the killed cells remain single. In addition, the per cent of giant cells in this population which fails to form self-sustaining colonies increases steadily with dose. These relationships are illustrated in Table I. Thus, exposure of a plate containing as many as  $10^4$  cells to a dose of 1000 r or more will, after incubation and staining, yield a population consisting entirely of single, giant cells (Figs. 5 to 7).

The irradiation needed to stop the biosynthetic activities involved in giant formation is enormously larger still. A dose as high as 10,000 r applied to a population of single cells may yield 5 to 20 per cent of the original inoculum as isolated, well developed giants.

*Properties of the Giants Resulting from Radiation.—*

These giant cells can be produced in very high yields, and in almost any desired degree of freedom from admixture with normal cells. For example, a

population of single cells irradiated with 600 r will have approximately 20 per cent of its population converted into abortive colonies. From Table I, it is seen that the average number of giants developing in each of these is 1.7, so that the number of giants recovered is 30 to 35 per cent of the initial cell population. If smaller x-ray doses are employed, one can readily obtain more giants than the initial number of normal cells employed, but these will be mixed with a larger number of the latter which have survived and multiplied. With doses greater than 1000 r, but less than 10,000 r, the giant yield falls to 10 to 15 per cent of the original inoculum, but extremely pure preparations can be obtained. By adjustment of these parameters, it becomes readily possible to prepare populations of giant cells of  $10^8$  or more, with any desired degree of purity.

Though study of these bizarre forms has only begun, the following facts may be mentioned here:—

The glass-attached, stretched giant may occupy an irregular area roughly circular but sometimes elliptical or elongated. They can readily be trypsinized, and assume a spherical shape with diameter of about  $50 \mu$ , indicating a cell volume of approximately  $6 \times 10^4 \mu^3$ , about 15 times as great as a normal HeLa cell. The cytoplasm is often less densely staining than in normal cells, but the nuclei stain intensely. The nucleus is always enlarged, and sometimes contains several huge nucleoli. Occasionally normal, discrete, double nuclei are visible but these are not particularly more frequent than in the normal cells. Nuclear fragmentation, and bizarre rosette-shaped nuclear configurations are often seen.

These cells metabolize at a high rate, changing the pH of standard growth medium at a rate roughly comparable to that of an equal mass of actively dividing cells. They can be maintained in the metabolically active state for long periods if the medium is regularly replaced as it becomes exhausted. Large numbers of these cells have been kept active at  $37^\circ\text{C}$ . in this fashion for 3 weeks.

The giants adhere tightly to glass as do normal cells. On removal by trypsinization they can be resuspended in growth medium and will then reattach to a new glass surface, with apparently the same configuration and properties as before. Reattachment to glass after dispersal by trypsinization is presumably an index of the ability to recrete a new outer protein layer.

The "feeder" layer of irradiated cells which we have described earlier (8-9), consists entirely of giants. These are able to promote colony formation of single cells which have been traumatized by trypsinization procedures which cause extensive leakage of the cell constituents and loss of ability to multiply in isolation.

The ability to form large, deeply staining, nuclear bodies is direct evidence of the retention by these cells of biosynthetic as well as catabolic activity. It



was of particular interest to determine whether the giant cells have indeed lost the power to multiply. Populations of giant cells observed during 20 days of incubation in complete growth medium which was regularly renewed every 3 to 4 days showed no increase in numbers, whereas normal cells multiply with a generation time of 20 hours under these conditions. A more critical test was performed by examination of large numbers of these cells for evidence of mitosis. Populations of at least  $10^5$  cells were examined for mitotic figures by microscopic scanning. While occasionally giant cells were found with nuclear configurations resembling those of early prophase, not a single case of late prophase, metaphase, or telephase was found. Mitotic figures corresponding to these latter stages occur in unirradiated cells in a frequency of 3 to 6 per cent under these conditions. It may be concluded that the ability to multiply in our standard conditions has been lost practically completely by the irradiation-produced giants.

Although they have lost the ability to multiply, these giant cells are highly susceptible to destruction by virus. Addition of 200 particles of Newcastle's disease virus to a plate containing  $10^6$  giants causes almost complete disintegration of the cell layer within 5 days. Addition of this virus to a mixed population of normal and radiation-produced giant HeLa cells selectively produces greater destruction of the latter cell type, possibly because of its larger surface. HeLa cells suspected of carrying a latent virus, were found to produce massive areas of destruction when plated on these giants. Further details of these processes will be presented in another publication.

#### DISCUSSION

The data here presented distinguish four x-radiosensitive processes in the isolated HeLa cell: (a) Loss of the ability of single cells to form a macroscopic colony. This reproductive function possesses a shoulder in the neighborhood of 75 r, and thereafter exhibits a radiosensitivity such that the surviving population is reduced by a factor of 37 per cent for each additional 96 r. Cells injured mortally by this process in the dose range up to 600 r retain the ability to multiply for 1 to 5 generations. After multiplication ceases, an appreciably large proportion of the cells continue to grow, forming giants. (b) Slowing of growth among the survivors. This effect is more difficult to quantitate, but is already distinct with doses of 100 to 200 r. (c) With doses greater than 600 r, damage to the reproductive apparatus itself appears, since cells so exposed rarely divide even once. However, these cells still form giants, so that a considerable part of their metabolic equipment is still functional. (d) With even greater doses, cells disappear so that the yield of giants drops. This process is much less radiosensitive than any of the others, an appreciable fraction of the original population still forming giants after exposure to 10,000 to 20,000 r.

The tremendous sensitivity of the HeLa cell to loss of reproductive potential

as revealed by these experiments demonstrates that the reproductive apparatus of warm-blooded animal cells is enormously more radiation-sensitive than that usually found in microorganisms. The  $D_0$  value of 96 r for this cell may be compared with its equivalent in round numbers for the following systems: about 4000 r for *E. coli* (12); 40,000 r for T2 bacteriophage (13) and Newcastle's disease virus (14); 5000 to 18,000 r for haploid, diploid, triploid, or tetraploid yeast (15 *a, b*); and more than 15,000 r for *Chilomonas paramecium* (16). Admittedly the HeLa cell, which derives from a carcinoma rather than from normal human tissue, may have an abnormal x-ray sensitivity. Similar determinations with human cells from normal tissues are now in progress.

The following questions arise: Does the seat of this killing action in the HeLa cell lie in the genetic or physiological parts of the cell? If genetic, does the significant locus involve a single gene, or a larger part of one or more chromosomes?

The following considerations constitute good evidence (though not absolute proof) that lethal effect occurs in the genetic apparatus, at least for doses up to 800 r:

(a) The ability of the killed cells still to reproduce for a limited number of times appears to indicate that the mitotic mechanism is essentially intact. This phenomenon of phenotypic lag is characteristic of lethal and other types of mutations in bacteria (11, 17). It is difficult to conceive of a damage not confined within the self-replicating part of the cell, which cannot be repaired by the cell, which delays its expression for 4 or 5 generations, and which then appears in every one of the progeny.

(b) The very minuteness of the value of 96 r indicates the existence of an exceedingly sensitive structure whose inactivation can cause irreparable damage. This amount of irradiation is equivalent to only  $10^{-12}$  calories per cell. Even with 100 per cent efficiency, this amount of energy could break only about  $2 \times 10^7$  atomic bonds per cell,<sup>2</sup> and since these will be randomly distributed, the number of such bonds that can be broken in any particular structure must be very small indeed. The sensitivity of genetic structures to such exceedingly minute changes is well established. The existence of non-genetic cell constituents able to destroy reproductive capacity as a result of so small an amount of energy absorption has never been demonstrated.

(c) The multiple unit form of the survival curve qualitatively agrees with that expected from hits localized in the genetic structure of a polyploid cell and quantitatively yields the hit number of 2 as the following analysis shows:—

If loss of a given function in an irradiated population occurs as a result of at least one ionizing event in any of  $m$  different kinds of units, and if there are  $n$  units of each

<sup>2</sup> A value of 96 r is equivalent to  $96 \times 2 \times 10^{-8}$  or  $2 \times 10^{-4}$  calories per gm. These cells have a mass of about  $4 \times 10^{-9}$  gm. (9), so that the maximum energy per cell is roughly  $8 \times 10^{-13}$  calories. Assuming an average only of 20,000 calories per gm. atom for atomic binding energies, this represents a maximum of  $\frac{8 \times 10^{-13}}{2 \times 10^4} = 4 \times 10^{-17}$  gm. atomic weights; or  $6 \times 10^{23} \times 4 \times 10^{-17} = 2 \times 10^7$  atoms.

kind present, all of which must be inactivated before the function is lost, the fraction of the population in which this function survives is given by  $S$  in the formula

$$S = [1 - (1 - e^{-D/D_0})^n]^m \quad (1)$$

in which  $D$  is the x-ray dose, and  $D_0$  is the 37 per cent lethal dose for an individual site. This equation was originally derived by Luria (18) and its applicability has been examined in detail by Atwood and Norman (19). We shall call  $n$  the hit number. In genetic processes  $n = 1$  for haploid, 2 for diploid, and 3 for triploid cells, etc.; we call  $m$  the unit number since it represents the number of different kinds of genetic structures whose inactivation in all replicates present can lead to loss of the function under consideration. For  $n = 1$ , the survival curve is a linear logarithmic function of the dose. For any value of  $n \geq 2$ , a plot of the log of the survivors against dose exhibits an initial shoulder, after which it descends linearly, as in Text-fig. 2. Formula 1 assumes all sites have an equal radiation sensitivity. As the dose increases, contributions to cell killing may be made by more radioresistant loci, and each time this happens, the survival curve will add an increment to its slope (19). By confining our attention to the first linear region of the survival curve, we consider only the  $m$  loci which are maximally sensitive to the lethal effects of radiation. The values of  $n$ ,  $m$ , and  $D_0$  for such loci may be determined as follows: Extrapolation of the linear portion of the curve to its intercept on the axis yields a value  $S^0 = n^m$ . The slope of the linear portion of the curve is  $-m/D_0$ .

Application of these considerations to the survival curve of Text-fig. 2 yields the following values:  $n = 2$ ;  $m = 1$ ; and  $D_0 = 96$  r. The equation

$$S = [1 - (1 - e^{-D/96})^2]$$

fits the experimental points well within experimental accuracy.<sup>3</sup>

These data make it most unlikely that the lethal action is due to a simple, single gene mutation. The value of 96 r is completely incompatible with that for any known single gene mutation. This figure leads to a sensitive volume calculated by the method of Lea (20) in the neighborhood of  $1 \mu^3$ , a value more than  $10^6$  times the size commonly accepted for a single gene (21). While the theoretical uncertainties of volume calculations of this kind have been pointed out many times (19, 22) these almost never introduce an error greater than a factor of 10 or at most 100. The enormous divergence in the present case appears sufficient to rule out the single gene mechanism.

The site in question could well be chromosomal with the lethal action, a reflection of chromosomal breakage without normal restitution.

The data can be quantitatively explained if one chromosome pair in the cell possesses a radiosensitivity appreciably greater than the others; or if the killing

<sup>3</sup> While the extrapolation of Text-fig. 2 actually yields a value of 2.1, we consider this to be identical to 2 within the experimental precision, although the possibility is real that a process with a higher value of  $n$  is participating to a very small extent in the observed killing process, yielding an average  $n = 2.1$  (19).

action is an expression of aberrations resulting from production of 2 or more chromosome breaks, producing mitotic bridges, translocations, and genic imbalance. Experiments testing these and other possibilities are in progress.

It is of interest that experimental behavior similar to that observed here was obtained by Lucke and Sarachek (15 *a*) who found a 1 to 1 correspondence between the values of  $n$  determined from survival curves and the true genetic ploidy for haploid, diploid, triploid, and tetraploid yeast cells respectively. They too observed a value of  $m$  equal to unity for all four types of cells. Zirkle and Tobias (15 *b*) have studied diploid yeast cells with survival curves indicative of high values of  $m$ .

The enormous radiosensitivity of the HeLa cell strongly suggests that the killing action studied here may contribute materially to the processes which determine the mean lethal dose for whole body irradiation of mammals. At 500 r, the 50 per cent human lethal dose, 99 per cent of the cells sharing the sensitivity of the HeLa would be killed. The ability of such cells still to multiply for several generations, or to persist as giants may contribute to the delay before the full development of symptoms, which so strongly characterizes the radiation syndrome. Studies are currently under way comparing the x-ray susceptibility of various clonal human cells obtained from normal tissues, and the effects thereon of changing the metabolic state of the cell population. Experiments describing mutation occurrence among the cell survivors of various doses of x-irradiation will be presented in a forthcoming report.

The parallelism between the giant cell formation here observed and the development of huge snake-like forms on irradiation of *E. coli* (23) is striking. Giant formation in mammalian cells has also been observed by Graham and associates (24) who have carefully studied the appearance of these forms in irradiated tumors, and have based an index of the tumor susceptibility on the per cent of giants appearing in smears after various x-ray doses. The present data are completely consistent with those of Graham and leave little doubt that the processes observed *in vitro* are close counterparts to those seen in the body. Experimental extension of the survival curve presented in Text-fig. 2 is now in progress. Such data would provide a means of estimating the radiation dosage needed to sterilize tumors of various sizes, provided all cells have a uniform radiation sensitivity. The need for study of these relationships with a variety of normal and malignant cells is obvious.

#### SUMMARY

The effects of x-irradiation have been quantitatively studied on single cells of a human cervical carcinoma (HeLa) under conditions such that 100 per cent of the unirradiated cells reproduce in isolation to form macroscopic colonies. This technique eliminates complexities due to interactions of members of large cell populations.

Survival of single cells (defined as the ability to form a macroscopic colony within 15 days) yields a typical 2 hit curve when plotted against x-ray dose. The initial shoulder extends to about 75 r, after which a linear logarithmic survival rate is obtained, in which the dose needed to reduce survivors to 37 per cent is 96 r. This radiation sensitivity is tens to hundreds of times greater than that of any microorganism for which the equivalent function has been studied.

Evidence, though not proof, is presented that the lethal effect is due to a radiation-induced genetic defect which, however, cannot be a simple single gene inactivation. The locus of the action could be chromosomal.

Beginning at doses of 100 r, or possibly earlier, growth-delaying effects of radiation are visible.

Cells in which the ability to reproduce has been destroyed by doses below 800 r, can still multiply several times. At higher doses even a single cell division is precluded.

A large proportion of the cells killed by radiation at any dose gives rise to one or more giant cells. These metabolize actively, grow to huge proportions but never reproduce under the experimental conditions employed. Methods of preparing large populations of giant cells are described. These giants are particularly susceptible to virus action.

Some of the irradiated cells disappear from the plate, presumably by disintegration. This action of radiation is by far the least efficient, since even after 10,000 r, 5 to 10 per cent of the original cell inoculum is recoverable as giants.

#### BIBLIOGRAPHY

1. Wilson, G. S., and Miles, A. A., Topley and Wilson's Principles of Bacteriology and Immunity, Baltimore, The Williams & Wilkins Co., 4th edition revised by G. S. Wilson and A. A. Miles, 1955, 99.
2. Luria, S. E., Animal Virology, New York, John Wiley and Sons, 1953, 39.
3. Reviewed by Stroud, A. N., and Bruez, A. M., *Texas Rep. Biol. and Med.*, 1954, **12**, 931.
4. Halberstaedter, L., Goldhaber, G., and Doljanski, L., *Cancer Research*, 1941, **2**, 28.
5. Reid, T. R., and Gifford, M. P., *J. Nat. Cancer Inst.*, 1952, **13**, 431.
6. Russel, W. L., *Cold Spring Harbor Symp. Quant. Biol.*, 1952, **16**, 327.
7. Demerec, M., and Latarjet, R., *Cold Spring Harbor Symp. Quant. Biol.*, 1946, **11**, 38.
8. Puck, Theodore T., and Marcus, Philip I., *Proc. Nat. Acad. Sc.*, 1955, **41**, 432.
9. Puck, T. T., Marcus, P. I., and Cieciora, S., *J. Exp. Med.*, 1956, **103**, 273.
10. Gey, G. O., Coffman, W. D., and Kubicek, M. T., *Cancer Research*, 1952, **12**, 264; Scherer, W. F., Syverton, J. T., and Gey, G. O., *J. Exp. Med.* 1953, **97**, 695.
11. Demerec, M., and Cahn, E., *J. Bact.*, 1952, **65**, 27.
12. Lea, D. E., *Actions of Radiations on Living Cells*, Cambridge, Cambridge University Press, 1947, 318.
13. Watson, J. D., *J. Bact.*, 1950, **60**, 701.

14. Puck, T. T., Marcus, P. I., and Cieciora, S., data to be published.
15. (a) Lucke, W. H., and Saracher, A., *Nature*, 1953, **171**, 1014. (b) Zirkle, R. E., and Tobias, C. A. *Arch. Biochem. and Biophysics*, 1953, **47**, 282.
16. Ducoff, H. S., *Radiation Research*, 1954, **1**, 130, abstract 15.
17. Newcombe, H. G., *Genetics*, 1948, **33**, 447; 1953, **38**, 134.
18. Luria, S. E., *Proc. Nat. Acad. Sc.*, 1947, **33**, 253.
19. Atwood, K. C., and Norman, A., *Proc. Nat. Acad. Sc.*, 1949, **35**, 696.
20. Lea, D. E., *Actions of Radiations on Living Cells*, Cambridge, Cambridge University Press, 1947, 83.
21. Muller, H. J., *Cold Spring Harbor Symp. Quant. Biol.*, 1941, **9**, 151.
22. Puck, T. T., in Nickson, J. J., *Symposium on Radiobiology*, New York, John Wiley and Sons, 1952, 385.
23. Lea, D. E., *Actions of Radiations on Living Cells*, Cambridge, Cambridge University Press, 1947, 300.
24. Graham, R. M., and Goldie, K. R., *Cancer*, 1955, **8**, 71; Graham, R. M., and Graham, J. B., *Cancer*, 1955, **8**, 59.

#### EXPLANATION OF PLATES

##### PLATE 26

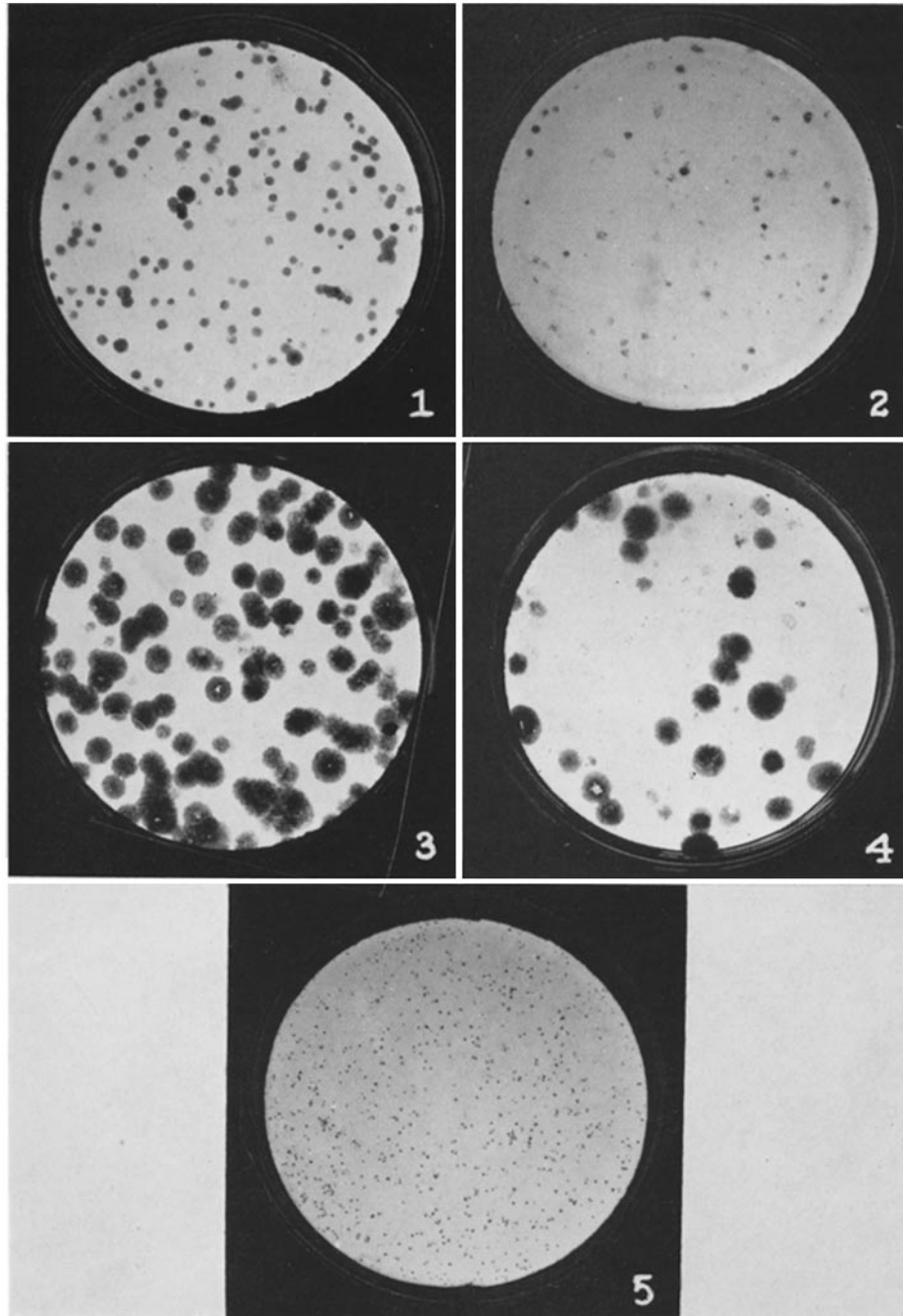
FIG. 1. Colonies developing on a plate seeded with 200 S3 HeLa cells and incubated 9 days in growth medium. Actual size.

FIG. 2. Colonies developing on a plate identical with that of Fig. 1 in every respect except that the plate was irradiated with 300 r before incubation. Actual size.

FIG. 3. Plate identical with that of Fig. 1 (*i.e.*, no irradiation), but which has incubated 17 days instead of 9 days. Actual size.

FIG. 4. Plate identical with that of Fig. 2 (*i.e.*, irradiated with 300 r), but which has incubated 17 days instead of 9 days. Actual size.

FIG. 5. Plate on which 5700 cells were seeded, and which was then irradiated with 900 r. After 12 days of incubation the plate was fixed with Bouin's solution and stained with hematoxylin. Only one actual colony developed on two such plates. Each of the visible spots shown is a single giant cell. Actual size.



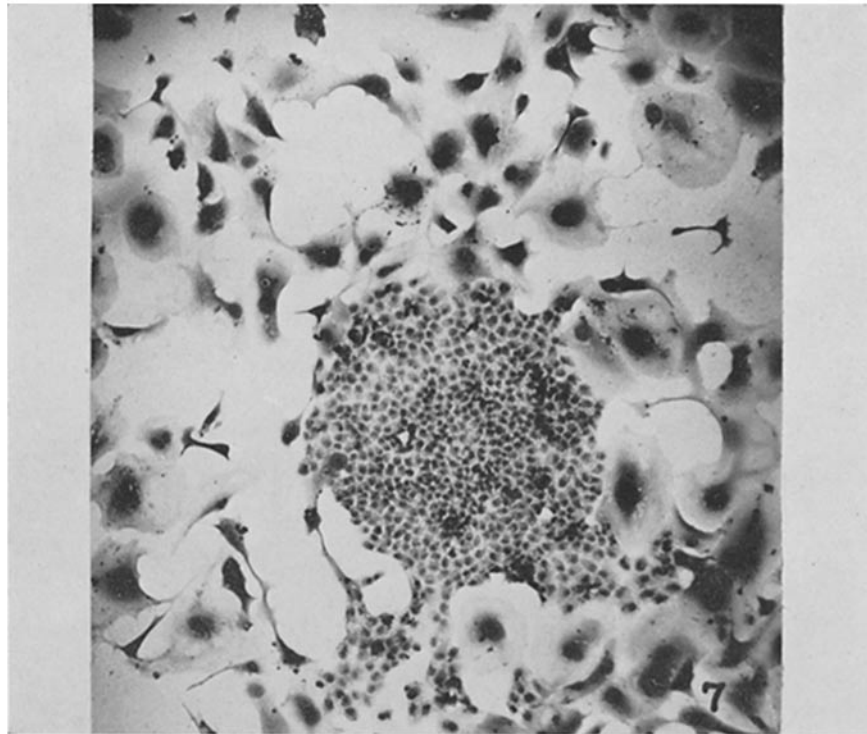
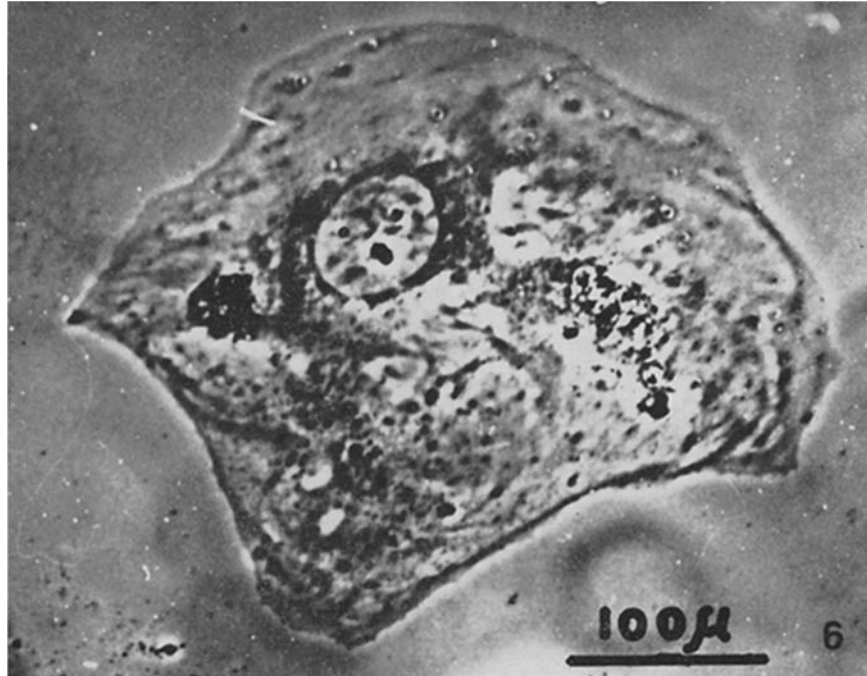
(Puck and Marcus: Action of x-rays on mammalian cells)

PLATE 27

FIG. 6. Single giant cell from plate like that in Fig. 5. (The scale is indicated.) Phase contrast photomicrograph.

FIG. 7. Photomicrograph of a normal colony which has grown up amongst the radiation-inactivated cells that have formed giants.  $\times 25$ . The photograph demonstrates that the giants achieve spread diameters that are 7 to 10 times that of the normal cell.





(Puck and Marcus: Action of x-rays on mammalian cells)