RADIATION-INDUCED BREAKS OF DNA IN CULTURED MAMMALIAN CELLS

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ABSTRACT Mouse leukemic cells (L5178Y) in suspension culture were irradiated and the extent of single-strand breaks and double-strand cuts of DNA was estimated by sucrose gradient centrifugation. The radiation produced 3.0 single-strand breaks per cell (G_1 stage) per rad and approximately 0.3 double-strand breaks per cell (G_1 stage) per rad.

INTRODUCTION

Using low energy electrons with limited power of penetration in protoplasm, Cole et al. (1963) demonstrated that the nucleus is more radiosensitive than the cytoplasm of cultured mammalian cells (mouse fibroblast, *L*). Furthermore, they showed that radiosensitization of cells by 5′-bromodeoxyuridine (BUdR)-labeling occurs only if the nucleus A is irradiated. Djorjevic and Szybalski (1960) and Erikson and Szybalski (1963) reported that substitution of DNA-thymine with halogenated pyrimidine, e.g. bromouracil or iodouracil, also sensitized mammalian cells (D98/AG) to the action of radiations. Suicide experiments by Ragni and Szybalski first showed that cells were killed more efficiently by decay of DNA-^{32}P than by other intracellular ^{32}P-containing molecules (1962). These observations suggest that the nucleus is the radiosensitive "site" of cultured mammalian cells and that DNA molecules are the radiosensitive "target" or, at least, a component of the target.

Radiation damage to DNA of lethally irradiated cells is not always obvious because of the lack of sensitive methods for detecting minor changes in these macromolecules. Recently, McGrath and Williams (1966, 1967) studied the DNA of irradiated bacteria by means of an alkaline sucrose gradient centrifugation technique. They observed that the single-strand breaks of DNA were repaired in a radioresistant strain but not in a radiosensitive strain. Kaplan (1966) then confirmed the existence of radiation-induced single-strand breaks in DNA and of repair mechanisms in another strain of bacteria. He also showed that no repair took place when "double-strand cuts" were produced in DNA. Lett et al. (1967) and Alexander

and Lett (1966) applied a similar method of study to cultured mammalian cells (L5178Y).

The present paper uses a modified version of the method of Lett et al. for calculation of breaks of DNA.

METHODS AND MATERIALS

Cell line

The mouse leukemic cells (L5178Y) used in these experiments had been maintained in this laboratory for 5 yr. All experiments were carried out with cultures in the exponential growth phase suspended in Fischer's medium supplemented with 10% horse serum (Grand Island Biological Co., Grand Island, N.Y.). The characteristics (Watanabe and Okada, 1967) of this cell line are: generation time, 10.8 hr; duration of G_1, 1.8 hr; duration of S, 7.3 hr; duration of G_2, 1.2 hr; and duration of M, 0.55 hr; the chromosome number is 40 ± 1.

Labeling of Cells with Radioactive Precursors

In early experiments, cells in the exponential growth phase were incubated for approximately one generation time (9–12 hr.) in a medium containing either thymidine-^{3}H (0.05–0.1 μc/ml) or thymidine-^{14}C (0.025–0.05 μc/ml) at a total thymidine concentration of 10^{-6} M. Since this was known to maintain a constant specific activity in the newly synthesized fraction of DNA only up to one generation (Burki and Okada, unpublished data), the total thymidine concentration was raised to 10^{-4} M and 10^{-5} M deoxycytidine was added to minimize the toxic effects of the thymidine; those conditions assured uniform labeling for four generations (Burki and Okada, unpublished data). It should be noted that the CsCl sedimentation pattern of the first generation labeled under both conditions was the same.

In some experiments, cells were incubated for 30–60 min with uridine-^{3}H (4 μc/ml, 5 mc/mM); in others, the cells were labeled with a mixture of amino acids-^{14}C (New England Nuclear Corp., Boston, Mass.) (0.25 μc/ml) for one generation time (approximately 10 hr). In all cases, the cells were sedimented and subjected to the sucrose gradient centrifugation procedure.

X-Irradiation

The cells were centrifuged, resuspended in 5 ml of fresh warm medium, and placed in a tissue culture vessel (15 ml). The vessel was placed 8 cm from the target of a million volt X-ray machine (General Electric Co.) and irradiated for up to 10 min at a dose rate of 76,000 rads/min. In other experiments, 5 ml of the cell suspensions were added to tissue culture tubes (15 ml) and placed in a water bath at 37°C. The water bath was taken to the X-ray unit and the cell suspension irradiated at the dose rate of 10,000 rads/min. The dose rate was estimated by means of a ferrous sulfate dosimeter and a Radcon.

Alkaline Sucrose Gradient Centrifugation

After irradiation, a volume of cell suspension containing 1.6×10^7 cells was removed and centrifuged. The cell pellet, so obtained, was then lysed by addition of 0.4 ml of 1% deoxycholate in 0.14 M sodium chloride and 0.14 M of sodium citrate. Using an 0.5 ml graduating pipette with a cutoff tip, 0.1 ml of the lysate was pipetted off gently and placed on top of the sucrose gradient in a nitrocellulose centrifuge tube. The sucrose gradient was composed of 4.8 ml of 5–20% linear sucrose gradient (pH 12), overlayered with 0.1 ml of 0.5 M NaCl.

Three tubes were sedimented simultaneously at 35,000 rpm for 2 hr in a Spinco L2 preparative centrifuge with SW39 rotor operating at a temperature of 4°C. After centrifugation, the bottom of each tube was punctured and, typically, 21 fractions containing 20 drops each were collected. In most of the cases, the last fraction contained less than 20 drops and, in some cases, e.g., the second set of fractionating apparatus, one or two additional fractions had to be collected. For the second apparatus, the total number of x fractions was always adjusted to 21 fractions by multiplying with $21/x$.

Neutral Sucrose Gradient Centrifugation

In studies involving neutral sucrose gradient centrifugation, the same procedure was used except for the neutral pH of the sucrose solution.

Assay of Radioactivities

From each fraction, 0.2 ml of the eluent was mixed with 1 ml of water and 10 ml of Bray's solution in a low background glass bottle and counted in a liquid scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.)

Standardization of Sedimentation Rate in Relation to the Size of Single-Strand and Double-Strand DNA

An aliquot of T_4 and T_7 phage suspension, both labeled with thymidine-^{14}C, was mixed with an equal volume of a solution containing 100 μg/ml of lysozyme and 0.1 M EDTA at pH 8. After incubating the mixture for 1 min at 65°C, 0.1% sodium dodecyl sulfate was added and incubated 10 min more at 65°C. 0.001 mm of lysed phage containing 0.1 μg DNA was added to the sucrose gradient. The sedimentation patterns of the phage DNA were determined in both alkaline and neutral sucrose gradient centrifugations. Table I summarizes these results. The distance from the top of the sucrose gradient expressed as the fraction number is assumed to follow the empirical equation of Burgi and Hershey (1963): (distance) = K (molecular weight)$^{\alpha}$. For single-strand DNA, α was 0.284 and K was 0.067; and for double-strand DNA, K value was estimated to be 0.016 by using $\alpha = 0.35$ (Burgi and Hershey, 1963).

TABLE I

CALIBRATION OF SUCROSE GRADIENT OF THE PRESENT EXPERIMENTS*

DNA	ph	Phage	$S_{1/2}$‡		Molecular weight§
					daltons
Single-stranded	12	T_7	8.0 6.2	7.1	1.3×10^7
Single-stranded	12	T_4	10.7 11.4	11.1	6.0×10^7
Double-stranded	7	T_4	9.0	9.0	1.2×10^8

* Assuming that (distance) = K (MW)$^\alpha$ (Burgi and Hershey), α and K were calculated from observed $S_{1/2}$ and molecular weight of single-stranded DNA. $\alpha = 0.284$ and $K = 0.067$.

‡ $S_{1/2}$ is expressed in "fraction number" from the top (where the whole solution was divided into 21 fractions). $S_{1/2}$ is the distance beyond which ½ of the mass of DNA sediments.

§ The molecular weight at pH 12 was assumed to be half that at pH 7. The molecular weight of T_7 phage and T_4 phage are respectively, 2.5×10^7 daltons and 1.2×10^8 daltons.

Theory Upon Which Analysis Is Based

The parameter of the degraded distribution related most directly to the number of breaks induced by irradiation is the *number-average* molecular weight, MW_n, defined by

$$MW_n = \frac{\text{Total mass of DNA per cell}}{\text{Number of pieces of DNA per cell}}.$$

But the change in the number of pieces of DNA per cell is equal to the number of radiation-induced breaks in the DNA of each cell. Rearranging the definition of $(MW)_n$,

$$\text{number of pieces of DNA per cell} = \frac{\text{total mass of DNA per cell}}{MW_n}$$

Thus, the number of radiation-induced breaks per cell is given by

$$\text{breaks per cell} = (\text{mass of DNA per cell}) \cdot [1/MW_n \text{ after irradiation} - 1/MW_n \text{ before irradiation}]$$

However, MW_n is difficult to measure directly. Other parameters of the distribution are more convenient. In particular, $MW_{1/2}$, defined as that molecular weight which is exceeded by one-half of the mass of molecules in the distribution, can be measured graphically from the sedimentation patterns of DNA (e.g., Fig. 1). What is needed is a relationship between MW_n and $MW_{1/2}$.

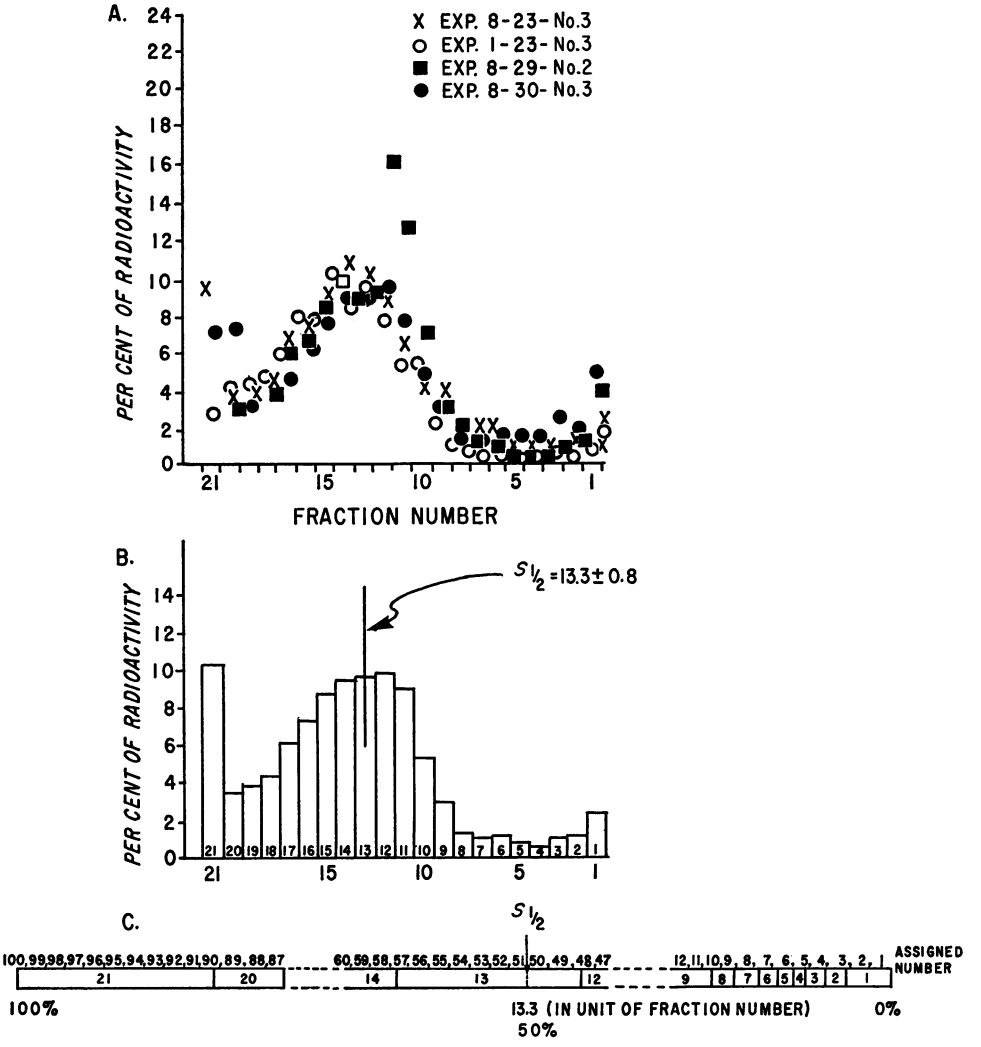

FIGURE 1 Sedimentation pattern of single-strand DNA from nonirradiated cells and method of obtaining $S_{1/2}$. $S_{1/2}$ is the distance (in fractions) beyond which one-half the mass of molecules sediments; $MW_{1/2}$ is defined by $S_{1/2} = k\ (MW_{1/2})^{\alpha}$; for single-strand DNA, $k = 0.067$ and $\alpha = 0.284$.

Using Poisson statistics, one can show that the distribution resulting from the random degradation of an infinitely long strand of a polymer is given by

$$N(p) = A \times p^2 \times e^{-MW \times p}$$

where $N(p)$ is the number of molecules having a molecular weight $(MW)_n$ after a density of degradation of p. A is a normalization constant.

Charlesby has shown that after a density of degradation equivalent to about five breaks per average molecule any distribution approaches the form of this distribution resulting from random degradation of an infinite polymer (Charlesby, 1954). This approximation applies to all of the irradiated samples in this paper.

For the distribution resulting from random degradation, one can calculate both MW_n and $MW_{1/2}$:

$$(MW)_n = \frac{\int_0^\infty N(p) \times MW \, d(MW)}{\int_0^\infty N(p) \, d(MW)}$$

$$\int_0^{MW_{1/2}} N(p) \times MW \, d(MW) = \tfrac{1}{2} \int_0^\infty N(p) \times MW \, d(MW)$$

By substituting the expression of $N(p)$ for the randomly degraded distribution, one obtains the following simple relationship:

$$(MW)_n = 0.60 \, MW_{1/2}$$

Replacing $(MW)_n$ in the expression for breaks per cell:

$$\text{Breaks per cell} = \frac{(\text{mass of DNA per cell})}{0.60} \left[\frac{1}{MW_{1/2}}_{\text{after}} - \frac{1}{MW_{1/2}}_{\text{before}} \right]$$

This is the equation used to calculate number of single-strand and double-strand breakages.

RESULTS

Nature of DNA Peak in Sucrose Gradient Centrifugation

Cells labeled with uridine-^{3}H were mixed with thymidine-^{14}C labeled cells and subjected to sucrose gradient centrifugation at pH 7 and 12. After alkaline sedimentation, most of the ^{14}C-activity was found in a peak in the middle of the fractions and some, essentially unsedimented, near the top. ^{3}H-activity was mostly found in the top fractions (Fig. 2 A). In the neutral sedimentation, ^{14}C- as well as ^{3}H-activity occurred in the middle of the fractions and both types of activities were also found in the top fractions (Fig. 2 B).

After mixing cells labeled with ^{14}C-amino acid with those labeled with thymidine-^{3}H, the gradient centrifugation patterns obtained at pH 12 and 7 are shown in Fig. 2 C and D. At pH 12 there is a fairly good separation of ^{14}C-activity from the peak of ^{3}H in the middle of the sedimentation pattern (Fig. 2 C) while such clear-cut separation was not seen at pH 7 (Fig. 2 D).

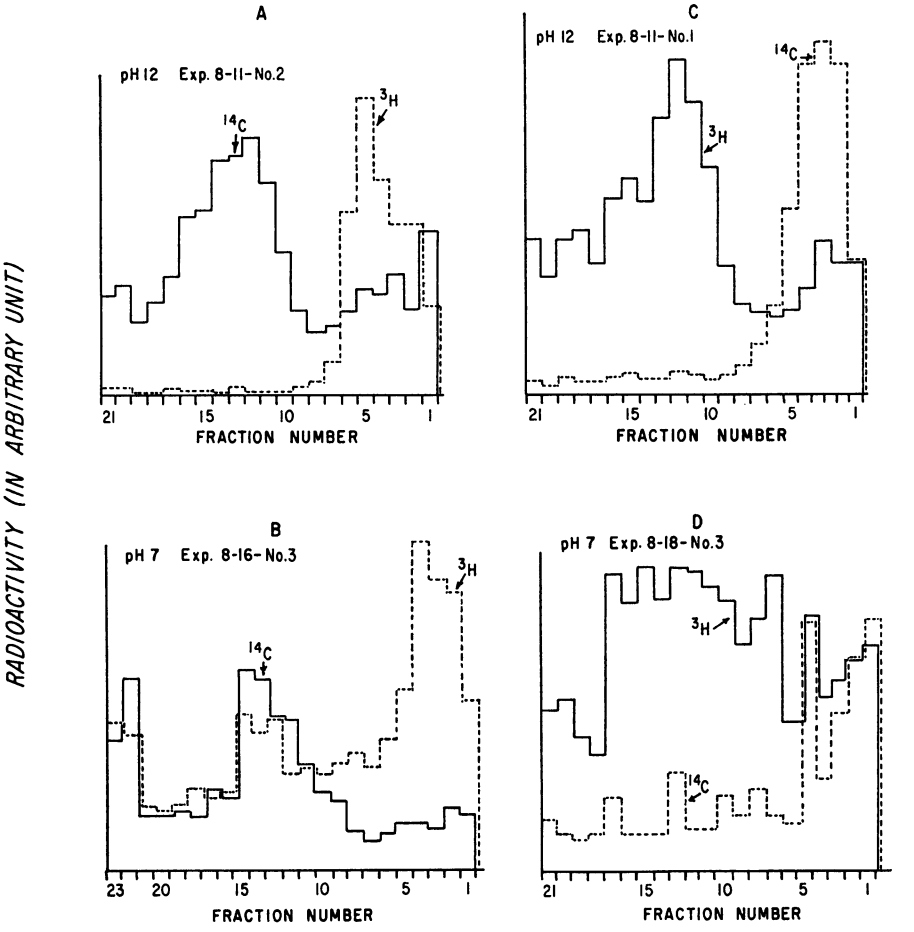

FIGURE 2 Sedimentation patterns of lysate labeled with thymidine, uridine, and amino acids. (*A*) Uridine-^{3}H-labeled and thymidine-^{14}C-labeled cells at alkaline pH. (*B*) Uridine-^{3}H-labeled and thymidine-^{14}C-labeled cells at neutral pH. (*C*) Amino acid mixture-^{14}C-labeled and thymidine-^{3}H-labeled cells at alkaline pH. (*D*) Amino acid mixture-^{14}C-labeled and thymidine-^{3}H-labeled cells at neutral pH.

In previous experiments using radioautography (Burki and Okada, 1968), most of the thymidine-^{3}H incorporated under the present labeling condition was found in the nucleus of the cells; 85% of ^{3}H-activity was in the DNA fraction isolated by a modified method of Schneider and Tannhauser (Burki and Okada, 1968); 99% of ^{3}H-activity in acid-insoluble fraction was digested by deoxyribonuclease. Thus, the radioactivity associated with radioactive thymidine is most likely to be incorporated into DNA. Fig. 2 suggests that at pH 12, the DNA peak is rather free from contamination with RNA and proteins, while a gross contamination is expected in the DNA peak of pH 7.

Effects of Concentration of DNA after Sucrose Gradient Centrifugation

In early runs, attempts were made to obtain a better resolution between irradiated and nonirradiated DNA by mixing irradiated cells labeled with thymidine-^{14}C with nonirradiated cells labeled with thymidine-^{3}H and subjecting them to sucrose gradient centrifugation. With the cell concentrations used (1–4 $\times$ 10^7 cells), an interaction occurred between the irradiated DNA and nonirradiated DNA. To avoid such interaction of DNA, most of the work was carried out without mixing different groups, e.g., irradiated and nonirradiated cells. It should be pointed out

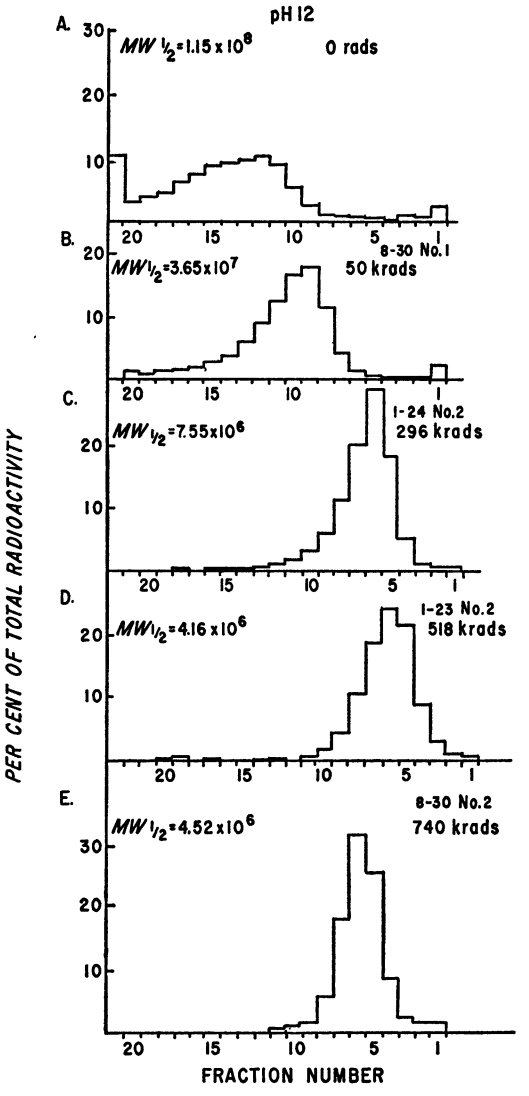

FIGURE 3 Sedimentation patterns of single-strand DNA from irradiated cells.

TABLE II

SINGLE-STRAND BREAKS

Radiation dose	$S_{1/2}$*	$(MW)_{1/2}$‡	Number of pieces of DNA cell§	Number of radiation-induced single-strand breaks/cell‖	Number of radiation-induced single-strand breaks/cell rad	Exp. no.
krads						
0	13.3 ± 0.8¶	1.15×10^8	7.54×10^4	0	—	—
50	9.62	3.65×10^7	2.38×10^5	1.63×10^5	3.26	8-30 1
50	9.32	3.20×10^7	2.71×10^5	1.97×10^5	3.94	1-24 3
200	7.94	1.82×10^7	4.76×10^5	4.01×10^5	2.00	8-29 1
296	6.51	9.10×10^6	9.53×10^5	8.78×10^5	2.96	1-23 1
296	5.67	7.55×10^6	1.15×10^6	1.07×10^6	3.61	1-24 2
518	5.30	4.16×10^6	2.08×10^6	2.00×10^6	3.86	1-23 2
740	5.11	3.80×10^6	2.28×10^6	2.20×10^6	2.98	8-30 2
740	5.37	4.52×10^6	1.92×10^6	1.84×10^6	2.49	1-25 3
				Average 3.1 ± .7		

* $S_{1/2}$ is the distance (in fractions) beyond which ½ of the mass of DNA sediments.

‡ $(MW)_{1/2}$ is calculated from $S_{1/2} = 0.067\ (MW_{1/2})^{0.284}$ or $MW_{1/2} = 1.3 \times 10^4\ (S_{1/2})^{3.52}$.

§ Number of pieces of DNA/cell $= \dfrac{\text{Total mass of DNA/cell}}{0.6 \times MW_{1/2}} = \dfrac{5.2 \times 10^{12} \text{ daltons}}{0.6 \times MW_{1/2}}$.

‖ Number of radiation-induced breaks/cell = Number of pieces/cell − Number of pieces at zero dose.

¶ The average of four independent runs.

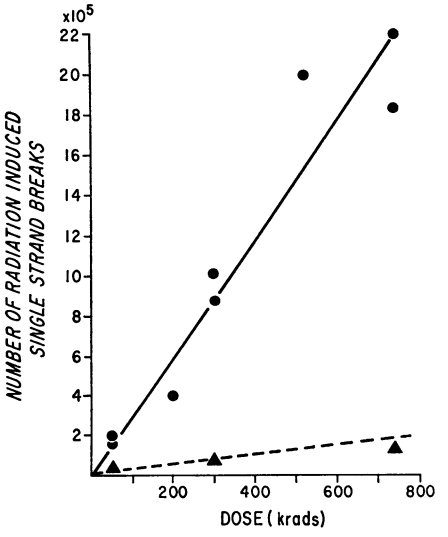

FIGURE 4 Relationship of number of breaks to radiation dose. Full line with solid circles represents single-strand breaks and the dotted line with triangles, double-strand cuts.

that, when small cell numbers were used (from 2×10^5 to 4×10^6 cells), the sedimentation profile was similar to that obtained using 10^7 cells. In most of the experiments, 4×10^6 cells were used.

Single-Strand Breaks

The sedimentation profile of nonirradiated and irradiated DNA at pH 12 is shown in Fig. 3 and is summarized in Table II. The quantitative relationship of the number of breaks and the radiation dose is shown in Fig. 4. The number of breaks increases linearly with the radiation dose (Fig. 4) and amounts to 3.1 ± 0.7 or approximately three breaks per cell per rad.

It should be pointed out that in all experiments the cells were labeled for one to one-and-a-half generations with radioactive thymidine and the radioactivity, therefore, reflects mainly newly replicated strands. Fig. 5 shows an example in which the cells were labeled for four generations with thymidine-^{14}C in the presence of 10^{-4} M thymidine and 10^{-5} M deoxycytidine followed by incubation for one generation with thymidine-^{3}H. All newly replicated strands were labeled with ^{3}H, while pre-

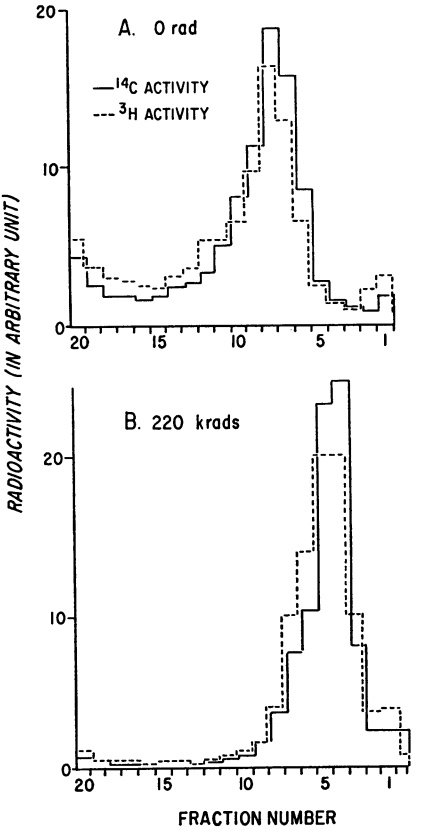

FIGURE 5 Single-strand breaks in the newly replicated (dotted line) and previously replicated strands (full line) of DNA.

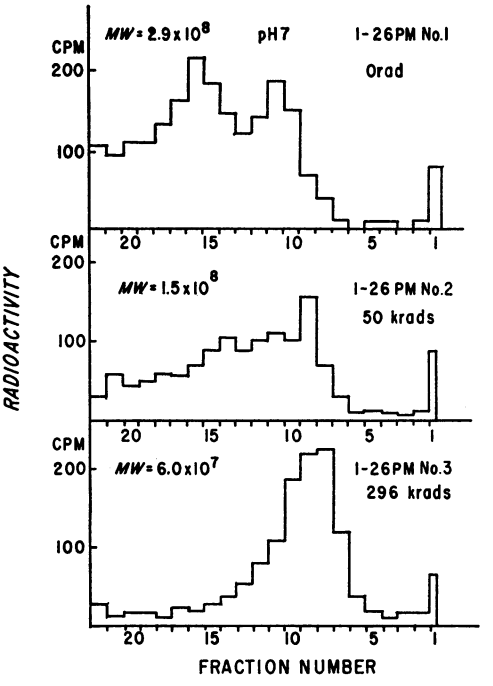

FIGURE 6 Sedimentation patterns of double-strand DNA from irradiated cells.

TABLE III

DOUBLE-STRAND CUTS

Radiation dose	$S_{1/2}$	$MW_{1/2}$*	Number of pieces of DNA/cell‡	Number of radiation-induced double-strand cuts/cell§	Number of radiation-induced double-strand cuts/cell/rad	Exp. No.
krads						
0	14.16	2.9×10^8	2.99×10^4	0	—	1-26 1
50	11.14	1.5×10^8	5.78×10^4	2.79×10^4	0.56	1-26 2
296	8.22	6.0×10^7	1.05×10^5	7.5×10^4	0.25	1-26 3
740	7.99	5.5×10^7	1.57×10^5	1.27×10^5	0.17	1-25 1
					Avrage 0.33 ± .17	

* $S_{1/2} = k\,(MW_{1/2})^{\alpha}$; $\alpha = 0.35$ was used (Burgi and Hershey, 1963); double-stranded DNA of T_4 phages (1.2×10^8 daltons) has an $S_{1/2}$ of 10.7; $10.7 = k\,(1.2 \times 10^8)^{0.35}$ yields $K = 0.016$; $MW_{1/2}$ was calculated from $S_{1/2}$ by using $S_{1/2} = 0.016\,(MW_{1/2})^{0.35}$.

‡ Number of pieces of DNA/cell $= \dfrac{\text{Mass of DNA/cell}}{0.6 \times MW_{1/2}} = \dfrac{5.2 \times 10^{12} \text{ daltons}}{0.6 \times MW_{1/2}}$. (The amount of DNA/cell is that of G_1 stage cell.)

§ Number of double-stranded cuts/cell = Number of pieces of DNA/cell − Number of pieces at zero dose.

viously replicated strand was labeled with ^{14}C. The results showed that both strands were degraded to the same extent in the sucrose gradient centrifugation, indicating that both strands of DNA are probably equally radiosensitive (Fig. 5).

Double-Strand Cuts

The sedimentation profile of nonirradiated and irradiated DNA in the neutral sucrose gradient centrifugation is shown in Fig. 6. Admittedly, the DNA peak is grossly contaminated with proteins and RNA, but irradiation still resulted in a decrease in the distribution of the size of DNA fragments. In order to obtain a rough idea of the number of double-strand cuts, an analysis similar to that described above was applied and the results are summarized in Table III and Fig. 6. The number of double-strand cuts is about 0.3 per cell per rad. As discussed previously, this number presumably indicates the order ofmagnitude of the double-strand cuts in the DNA of the irradiated cells.

DISCUSSION

The Method of Analysis and Sucrose Gradient Centrifugation

In determining random breaks in irradiated DNA, it was necessary to satisfy several conditions; in practice, however, some of these conditions could not be satisfied completely. To what extent the present experiments fulfilled these requirements is discussed in this section.

Condition A. The distribution of radioactivity in sedimentation patterns should reflect accurately the distribution of DNA in the cells. The conditions of labeling used were such that the specific activity (expressed in the radioactivity per newly synthesized fraction of DNA) is likely to be a constant (Burki and Okada, unpublished data). In other words, the radioactivity in DNA labeled for one generation represents that in the newly replicated strand of the period; it does not represent the other strand which had been replicated two generations before. The comparative studies of newly replicated strand and old strand showed that the radiosensitivities of both strands of DNA were the same (Fig. 5); this means that the result of the studies can be applied to all DNA within the cell.

Condition. B. The relationship of the sedimentation rate and the molecular weight of DNA must be clearly known. In the present experiments, labeled T_4 phage and T_7 phage were used to standardize the relationship of the sedimentation rate and the molecular weight. In alkaline sucrose gradient centrifugation, the molecular weight of DNA of T_2, Pl, γ, and T_7 phages was half of the molecular weight of native DNA (Abelson and Thomas, 1966). In T_5 phage, Abelson and Thomas, for example, reported that the size of DNA measured in alkaline pH is several times smaller than half of the native DNA. Although there is no concerete

evidence for possible interruption of single strandedness in T_4 phage, this could have introduced an error. Another possible source of error is the purity of DNA. If RNA and proteins were associated with DNA, the molecular weight based on DNA might be subject to correction, especially in the neutral pH sedimentation patterns. The concentration of DNA (12 μg/ml) used in this experiment is rather high so that the sedimentation rate itself may be subject to an error of a few per cent (Burgi and Hershey, 1963).

Condition C. The difference between the sedimentation patterns of irradiated and nonirradiated DNA must be due solely to the number of random breaks introduced by the ionizing radiation in the DNA. If the sedimentation pattern of nonirradiated DNA includes inherent breaks due to the extraction procedures, this assumption will be correct only in either one of the following situations: first, if the number of radiation-induced breaks is so much higher than the number of inherent breaks of nonirradiated DNA so that the latter can be ignored; and second, if the number of breaks in the nonirradiated DNA is that for inherent breaks while the number of breaks in the irradiated DNA is the sum of the number of radiation-induced breaks and inherent breaks.

Condition D. The radiation must randomly break DNA molecules in the cells.

Condition E. The cultured mammalian cells (Lett et al., 1967) possess the mechanism to rejoin single-strand breaks of DNA. When estimating the extent of breaks, the experiments should be carried out under the conditions where little or no rejoining process takes place. One of the conditions which inhibits the rejoining process was found to be low temperature (below 5°C) (Sawada and Okada, unpublished data). For example, when the cells were irradiated with 50 krads (the minimum dose used in the present experiment) at 37°C and 5°C and their alkaline sucrose gradient sedimentation profiles compared, the sedimentation profile of the samples irradiated at 37°C was superimposable on those at 5°C for a postirradiation period of at least 10 min, indicating that even if the rejoining process occurred at 37°C, it was too slow to interfere with the estimation of single-strand breaks (Sawada and Okada, unpublished data).

Single-Strand Breaks and Double-Strand Cuts

The number of single-strand breaks in cultured mammalian cells was found to be about three breaks per cell per rad while the number of double-strand cuts was somewhere around 0.3 cuts/cell per rad. Lett et al. (1967) obtaind the value of 70 ev per one single-strand break for the same leukemic cell line and this, by using 5.2×10^{12} daltons of DNA per cell, would give eight single-strand breaks per cell per rad, which is about twice the present value of 3.1 ± 0.7. Such a difference may be primarily attributed to the difference of media used during irradiation, saline (Lett et al., 1967)

vs. Fischer's media supplemented with 10% horse serum and antibiotics, and the sublines, D_o = 38 rads (Lett et al.) vs. 180 rads (Watanabe and Okada, 1966).

When the number of breaks or cuts is expressed in terms of the D_o dose, the value would be equivalent to the average number of breaks or cuts occurring for one cell to be killed. Since D_o dose of L5178Y cells in our suspension culture was 180 rads (Watanabe aAd Okada, 1966), 540 single-strand breaks and 60 double-strand cuts would be produced per D_o dose. In suicide experiments (Burki and Okada, 1968), the number of tritium atoms in DNA which had to decay in order to kill one L5178Y cells has been estimated to be about 170 in the unifilar labeled cells and 80 in the bifilar labeled cells. The number of chromosome bridges induced by irradiation in L5178Y cells is estimated to be 5×10^{-5} bridges per cell (rad)[2] (Doida, unpublished data), corresponding to 1.6 bridges per cell at D_o = 180 rads. Therefore, most of the breaks and cuts as well as damage to DNA by tritium decay would not result in killing or in chromosomal damage.

In order to account for cell killing from so much damage to DNA molecules, it would be necessary to introduce one of the following assumptions: (a) Most of the breaks and cuts have been repaired and the few remaining unrepaired cuts cause the biological effect, or improperly rejoined breaks or cuts (misrepairs) cause the biological effect; (*b*) most cuts and breaks occurring in the "inactive part" of DNA are not lethal, and only those in the "active part" of DNA are lethal; (*c*) breaks or cuts of DNA are not lethal in themselves but some of them have a certain probability of progressing to lethal damage (such as chromosome aberration). All of these assumptions remain to be explored.

Factors which would affect the extent of radiation-induced breaks have been studied. They are: (*a*) Radiosensitive and radioresistant strains. In mouse leukemic L5178Y cells, the extent of the radiation-induced single-strand breaks was similar in both sensitive and resistant strains (Alexander and Lett, 1966). In *Escherichia coli*, the number of breaks was also independent of strains (McGrath and Williams, 1966). (*b*) Oxygen. The number of single-strand breaks produced in the presence of oxygen was nearly twice that in the absence of oxygen (in the presence of nitrogen) in L5178Y cells (Lett et al. 1967). In *E. coli*, the oxygen enhancement ratio was somewhere between 3.5–4 (Achey and Whitefield, 1968). (*c*) Two strands of DNA. Parental strand (previously replicated strand) has the same radiosensitivity as the daughter strand (newly replicated strand) as is shown in the present paper. (*d*) Bromodeoxyuridine. In *E. coli*, incorporation of bromodeoxyuridine into DNA resulted in an increase of single-strand breaks (Kaplan, 1966). (*e*) Rejoining process. Single-strand breaks have been shown to rejoin in some radioresistant strains of *E. coli* (McGrath and Williams, 1966, and Kaplan, 1966) and in mouse L5178Y cells (Lett et al. 1967). Lack of rejoining of breaks was observed in a radiosensitive strain of *E. coli* (McGrath and Williams, 1966) and in double-strand cuts of DNA in radioresistant strain of *E. coli* (Kaplan, 1966).

TABLE IV

SINGLE-STRAND BREAKS AND DOUBLE-STRAND CUTS OF DNA FROM THE IRRADIATED ORGANISMS

Organism	Irradiation conditions	Nucleic acid content	D_{37} or D_o for killing	Number of breaks/rad/organism		Number of breaks/rad/10^{12} daltons DNA		Number of breaks/D_{37} or D_o organism		Reference
				Single	Double	Single	Double	Single	Double	
		daltons	*rad*							
Tobacco mosaic virus	Suspension (broth)* frozen state	2×10^6	200,000	5×10^{-6}	—	2.5	—	1	—	Englander et al., 1960
			200,000	2×10^{-6}	—	1	—	0.4	—	
Phage α	Suspension (buffer + histidine)*	3×10^7	27,300	7.4×10^{-5}	3.7×10^{-6}	2.4	0.12	2	0.1	Freifelder, 1966
Phage T7‡	Suspension (buffer)	2×10^7	20,000	5×10^{-4}	4×10^{-5}	25	2	12	1	Freifelder, 1965
	Suspension (buffer + histidine)*	2×10^7	84,000	$5\text{–}7 \times 10^{-5}$	5×10^{-6}	3	0.26	4–6	0.4	
	Suspension (buffer + histidine + cysteine)*	2×10^7	170,000	—	6×10^{-6}	—	0.3	—	1	
Phage λ	Bacterium O_2 (in vivo)*	—	—	—	—	1.4	—	—	—	Summers and Szybalski, 1967
	Bacterium N_2	—	—	—	—	0.5	—	—	—	
Phage ϕ 29	Suspension (diluted saline)	3×10^7	7,000	—	1.4×10^{-4}	—	4.7	—	1	Boyce and Tepper, 1968

E. coli B_{s-1}	Suspension (growth medium)*	2.8×10^9	3,000	6×10^{-4}	—	0.2	—	2	—	McGarth and Williams, 1966
		2.5×10^9	2,730	2.7×10^{-3}	—	1.1	—	7.4	—	Freifelder, 1968
E. coli B/r	Suspension (growth medium)*	2.8×10^9	15,000	6×10^{-4}	—	0.2	—	10	—	McGrath and Williams, 1966
L5178Y	Suspension (growth medium)*	5.2×10^{12}§	180	3.1	0.3	0.6	0.06	560	60	Present paper
L5178Y	Suspension air (saline) N_2	5.2×10^{12}§	65	7.7	—	1.5	—	290	—	Lett et al., 1967
				3.5	—	0.7	—	240	—	

* Presence of "protective agents."
‡ Phage B_s was said to have the similar radiosensitivity (Freifelder, 1966).
§ See Okada, 1968. (The amount of DNA/cell is that of G_1 stage cells.)

Comparison of Single-Strand Breaks and Double-Strand Cuts in Various Organisms

Table IV summarizes the limited data available on the quantitative estimation of the number of breaks and cuts in DNA of irradiated viruses and bacteria. In T_7 phage, the ratio of single-strand breaks to double-strand cuts is about 10:1 which is similar to that observed in L5178Y cells. When comparing the number of single-strand breaks per D_{37} dose or per D_o dose, the number ranged from 0.4 to 600. The high values observed in the mammalian cells might mean that they are killed by mechanisms quite different from those in viruses and bacteria. If the number of breaks or cuts are expressed per rad per unit (10^{12} daltons) DNA, the values ranged from 0.2 to 3 (with the exception of 25 of T_7 phage in buffer). This means that the radiosensitivity of DNA is of the same order of magnitude in all organisms, including viruses, bacteria, and cultured mammalian cells.

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