Radiation Increases the Solubility of L Cell DNA in a Detergent-Based Lysing System (35171)

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During the past 5 years ultracentrifuge methods have become widely used to study radiation induced damage to both bacterial and mammalian cell DNA. Many workers employ a modification of the method originally described by McGrath and Williams (1). For this, a small volume of a cell suspension is gently layered upon a layer of lysing solution which has, in turn, been layered upon an alkaline sucrose gradient. After gently mixing of the cells and lysing solution, the gradient is centrifuged. A most attractive feature of this method is the avoidance of much hydrodynamic shear to the DNA which would occur if extensive pipetting were used.

In our laboratory we have used a lysing solution, originally described by Humphrey *et al.* (2), for ultracentrifuge experiments. This solution contains the detergent TIPNS (triisopropylnaphthalene sulfonic acid) *p*-aminosalicylic acid, and *sec*-butyl alcohol. In some of our ultracentrifuge studies, applying the McGrath and Williams' technique, we have observed minute strands of material, not in solution, dispersed throughout the tube at the end of centrifugation. Since these observations suggested that DNA solubility could possibly be altered by the conditions of lysis, we performed a series of experiments in which larger volumes of cell suspension and lysing solutions were used, as compared with the ultracentrifuge studies. The results indicated that, indeed, changes in DNA solubility did occur as a function of radiation dose, and conditions of lysis.

Methods. The methods used for culturing and handling of the L-929 cells have already been described (3). The cells are maintained as monolayers. At 24 hr before irradiation, tritiated thymedine (Amersham, 21.9 Ci/mmole) was added to the medium, final concentration 0.2 μCi/ml. At the end of the labeling period, the cells were detached with dilute trypsin, washed twice with glucose-free Hanks' balanced salts solution (HBSS) and then suspended in HBSS at a concentration of 10^6 cells/ml. We should point out that this cell concentration is the same as used for ultracentrifuge experiments. The suspension was maintained at 37° and gently agitated with a magnetic stirrer. Three-ml samples (3×10^6 cells) were pipetted into test tubes and then irradiated with X-rays (250 kVp, 0.5 mm Cu HVL, 500 rads/min); doses from 250 to 10,000 rads were used. At 10 min after a given dose of radiation, 3 ml of lysing solution was added. This solution contained 2% TIPNS, 1% *p*-aminosalicylic acid, and 6% *sec*-butyl alcohol; the materials were dissolved in distilled water, and adjusted to pH 12.5 with NaOH. Since equal volumes of lysing solution and cell suspension are used for the ultracentrifuge experiments, the same cell concentration and the same ratio of cell suspension volume to lysing solution volume is maintained for the present studies.

Results and Discussion. In some instances paired samples were irradiated. At the time the initially described lysing solution was added to the first tube, 3 ml of a second lysing solution was added to the second tube. The second lysing solution was identical to the first type, except that it also contained 0.05 *M* EDTA (ethylenediaminetetraacetate). Immediately upon addition of the lysing solutions, the cell suspensions lost their opalescent character and became clear. Microscopic examinations showed all of the cells to

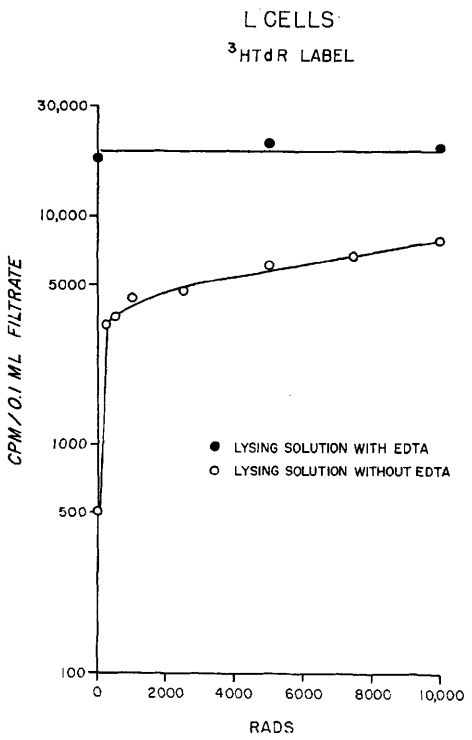

FIG. 1. Experimental results. The upper curve (●) shows the influence of the lysing solution with EDTA. No change of the count rate of the filtrate (see text) occurred as a function of dose. The lower curve (○) shows the effect of radiation on cells lysed with the solution without EDTA. The count rate of the filtrate increased rapidly as a function of dose.

be totally lysed; the degree of lysis provided by both solutions was equal.

The tubes were allowed to stand in the cold (2°) overnight. For the cells lysed with solution without EDTA, the controls and the low-dose samples (250–1000 rads) were found to contain clearly discernible mucoid-like precipitates. The higher dose samples on the other hand, showed smaller precipitates. The samples were filtered (Whatman No. 1 paper) to remove the precipitates and 0.1-ml portions of the filtrates were counted with a liquid scintillation counter. These results are shown by the lower curve of Fig. 1 which indicates the response to be somewhat nonlinear at the lower does. There is a monotonic increase in the amount of radioactivity in solution as a function of dose.

The cells lysed with the EDTA-containing solution did *not* show the pecipitate. Both gross and microscopic examinations were negative; no evidence of the mucoid-like precipitate could be found. As before, 0.1-ml portions were counted. These results are shown by the upper curve of Fig. 1, which indicates that much more radioactivity is in solution, as compared with cells lysed by the solution without EDTA. Also, there is no change in the counts in solution as a function of dose.

The precipitates produced by the lysing solution without EDTA were analyzed by a modified Schmidt–Thannhauser method (4). This analysis showed the precipitates to contain both deoxyribose and protein. Also, the DNA fraction contained virtually all of the radioactivity contained in the precipitate. Consequently, the precipitate probably represents a DNA–protein complex. For nonirradiated controls, approximately 95% of the labeled DNA is rendered insoluble when EDTA is omitted from the lysing solution.

The next point concerns the nature of the material in solution after treatment with the lysing solution without EDTA. A portion of the filtrate from a nonirradiated control sample was applied to a 5–23% alkaline sucrose gradient (5), and then centrifuged for 4 hr at 24,000 rpm. A SW 25.1 swinging bucket rotor was used. After centrifugation, 10-drop fractions were collected and the radioactivity was measured with a liquid scintillation counter. A parallel analysis was made of the filtrate from a nonirradiated control cell suspension which had been lysed with the EDTA solution. In both instances the materials in solution were the same. They both banded near the same location, which is characteristic of DNA (see Fig. 2).

The only difference between the two portions of the experiment concerns the presence or absence of EDTA in the lysing solution. When EDTA is used, all DNA from the lysed cells remains in solution. When EDTA is excluded, a considerable amount of the DNA does not remain in solution. A possible explanation for these findings is based upon the work of Kirby (6), who found that divalent metal ions are necessary to form a

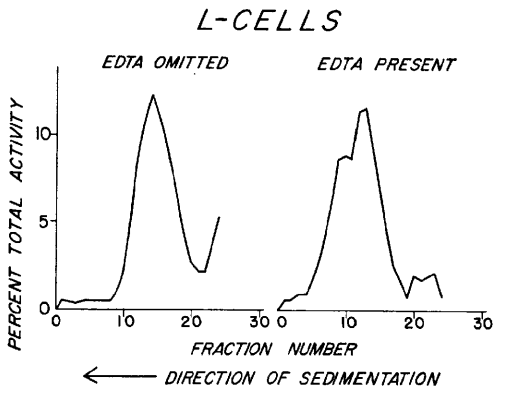

FIG. 2. Alkaline sucrose sedimentation analysis. For this experiment L cells were labeled with ^{3}HTdR and suspended in HBSS. A portion of the cell suspension was lysed with EDTA-free lysing solution while the other portion was lysed with solution which contained 0.05 *M* EDTA. Following overnight storage at 2°, the samples were filtered; 50-μl portions of the supernatants were layered upon alkaline sucrose gradients, and centrifuged as described. The results, expressed as percentage of total activity, applied to the gradient, are plotted as a function of the fraction number. As shown, the sedimentation patterns are similar for the two samples. Comparison with the sedimentation of T2 bacteriophage [mol wt 6.7 $\times$ 10^7 Daltons (9)] indicates the molecular weights of the L cell DNA to be 5.38 $\times$ 10^7 Daltons for cells lysed with the EDTA-free solution and 1.29 $\times$ 10^8 Daltons for cells lysed with EDTA-containing solution. The method described by Noll was used for estimation of the molecular weights of L cell DNA. At present, we have no explanation for the differences in DNA molecular weights.

DNA–protein–metal ion complex which is insoluble in an organic–aqueous system. Exclusion of the metal ions prevented the precipitate from appearing. Since EDTA binds divalent metal ions by chelation, the Kirby mechanism could explain the failure of the precipitate to appear when the cells were lysed with the solution which contained EDTA.

The *change* in DNA solubility in the lysing solution, when EDTA is omitted, as a function of radiation dose is more difficult to explain. Possibly radiation causes some change in the intracellular proteins, such that an insoluble DNA–metal ion–protein complex forms less efficiently than in the case of nonirradiated controls. A second, and perhaps less likely possibility concerns the influence of single- and double-strand DNA breaks upon DNA solubility. Certainly, we cannot exclude the possibility that DNA breaks could increase the solubility of DNA in the lysing solution. We should point out, however, that the 10-min period between irradiation and lysis was selected because this period of time is sufficient for this strain of L cells to rejoin single-strand DNA breaks (7, 8). Additional studies to determine the influence of both protein and DNA damage on DNA solubility are currently in process in our laboratory.

Summary. Treatment of L cells with a detergent-based lysing solution caused the formation of a precipitate which contained DNA and protein. Increasing doses of X-rays decreased the amount of DNA which precipitated; at the same time, the amount of DNA which remained in solution increased. Inclusion of EDTA in the lysing solution prevented the formation of the precipitate.

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