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REPAIR OF STRAND BREAKS IN SUPERHELICAL DNA OF ATAXIA TELANGIECTASIA LYMPHOBLASTOID CELLS

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SUMMARY

A number of different assay methods have been used to study repair of strand breaks in DNA after exposure of cells to ionizing radiation. Use of these methods indicates that fibroblasts from patients with ataxia telangiectasia (AT), a multiform genetic disease exhibiting high sensitivity to ionizing radiation, have a normal ability to repair strand breaks in DNA. All of these methods determine the extent of breakage of DNA and the resealing of these breaks but do not provide information on restoration of DNA configuration in the nucleus.

In this report we have used a sensitive technique to investigate restoration of the 3-dimensional structure of DNA in AT lymphoblastoid cells after exposure to ionizing radiation. This technique provides a means of lysing cells using a high concentration of salt and a non-ionic detergent, giving rise to structures called nucleoids which contain nuclear RNA and DNA, are depleted in protein, and sediment in a manner characteristic of supercoiled DNA. We have shown that the degree of supercoiling is the same in control and AT lymphoblastoid cells using sedimentation in the presence of ethidium bromide. The extent of breakage after exposure of cells to γ-radiation, and the rate of repair of these breaks are similar in both cell types. Rate of repair of strand breaks is dose dependent and the restructured rapidly sedimenting complex behaves similarly, on sucrose gradients containing ethidium bromide, to that extracted from unirradiated cells.

INTRODUCTION

Chromosomal instability and increased sensitivity to ionizing radiation have been demonstrated in cells from patients with ataxia telangiectasia (German, 1972; Taylor *et al.* 1975). This syndrome is included in a group of autosomal recessive syndromes, all of which have a characteristically high risk of developing cancer (Arlett & Lehmann, 1978). The molecular defect in ataxia telangiectasia (AT) has not been described but a deficit in the repair of γ-radiation damage has been reported using fibroblasts from some AT patients (Paterson *et al.* 1976) and lymphocytes (Lavin & Kidson, 1978), at high radiation doses.

Ionizing radiation gives rise to base modification and single-strand breaks in DNA at low to moderate doses (Ward, 1975). Repair of single-strand breaks is achieved in approximately 90 min postirradiation by a form of excision repair known as short-patch repair which involves the insertion of a small number of nucleotides into DNA (Ormerod, 1976). A number of reports indicate that AT cells have a normal level of proficiency in rejoining single-strand breaks in DNA (Vincent, Sheridan & Huang, 1975; Taylor *et al.* 1975; Paterson *et al.* 1976). However the radiation doses employed

in these experiments were high and the technique used, alkaline sucrose gradient analysis, has limitations in the resolution of high molecular weight species. Sheridan & Huang (1977) using alkaline-induced strand separation and S1 nuclease, a more sensitive technique, also demonstrated that AT cells did not have a reduced ability for rejoining single-strand breaks in DNA. This assay detects approximately 1 break/10^9 daltons of single-stranded DNA after a dose of 400 rads (4 J kg^{-1}) (Sheridan & Huang, 1977). We have studied the repair of breaks in DNA in AT cells using a sensitive method in which DNA appears to be intact, supercoiled, and circular in character (Cook, Brazell & Jost, 1976). These structures, resembling nuclei (nucleoids), containing superhelical DNA, nuclear RNA, and depleted in protein, are obtained by lysing cells in non-ionic detergent and high salt (Cook *et al.* 1976). Ionizing radiation introduces single-strand breaks into DNA, supercoiling is lost, and a decrease in sedimentation on sucrose gradients occurs. In this study we have compared degree of supercoiling, extent of breakage, and repair of DNA in nucleoids from control and AT cells. The AT homozygote cells are those previously used in this laboratory, all show increased sensitivity to ionizing radiation (Chen, Lavin, Kidson & Moss, 1978).

MATERIALS AND METHODS

Cell culture

Epstein Barr virus-transformed lymphoblastoid cells were used in this study. Cells were diploid at the commencement of this study and had a doubling time of approximately 24 h. The ataxia telangiectasia cells used were AT1ABR, AT2ABR, AT4ABR, AT5ABR, AT6ABR and GM1535 (Camden, Human Genetic Cell Repository, New Jersey). This nomenclature has been used for cell lines established in this laboratory (Houldsworth & Lavin, 1980). The control cells employed in the study were C3ABR, C4ABR, C5ABR and C8ABR. Cells were grown in RPMI 1640 medium (Gibco) supplemented with streptomycin (60 μg/ml), penicillin (100 IU/ml) and 10 % foetal calf serum, in an atmosphere of 5 % CO_2 in air at 37 °C.

Irradiation

Cells were irradiated in medium in a Gamma cell 220 (Atomic Energy of Canada Ltd) at a dose rate of 4·2–3·8 krad/min (42–38 J kg^{-1}/min). Zero time irradiations were carried out using cells suspended in ice-cold phosphate-buffered saline. Lead shielding was employed for low doses of radiation to reduce the effective output to 10 %.

Sucrose gradient analysis

Sedimentation of nucleoids was carried out on sucrose gradients using a modification of the method of Cook & Brazell (1975). Sucrose gradients (10–30 % sucrose; 13·4 ml; pH 8·0) contained NaCl (1·95 M), Tris (0·01 M) and EDTA (0·001 M). Ethidium bromide was added to the sucrose solutions in some experiments. Cells ($2-3 \times 10^5$) in 100 μl of phosphate-buffered saline were added to 3 vol. of lysis solution (2 M NaCl, 0·01 M EDTA, 0·5 % Triton X-100; pH 8·0). This mixture was in turn layered on top of a sucrose gradient using a wide bore pipette. Lysis was allowed to proceed for 10 min and the gradients were centrifuged at 37000 rev/min for 20–30 min at 12 °C in a Beckman L2-65B centrifuge, using an SW41 rotor. The position of nucleoids in the gradient was determined by pumping the gradient through an absorbance monitor at 254 nm wavelength. In each centrifugation run, either triplicate or duplicate sets of both unirradiated and irradiated cells were employed. Variability in sedimentation rates was reduced when centrifugation was carried out at 12 °C. Occasionally a double peak was observed on gradients.

RESULTS

DNA integrity and the degree of supercoiling determine nucleoid sedimentation (Cook & Brazell, 1976). Since ionizing radiation alters the integrity of DNA by introducing breaks into the phosphodiester backbone it is important to establish that the degree of supercoiling is the same in unirradiated AT and control cell lines. The results in Figs. 1 and 2 describe the sedimentation respectively of control and AT

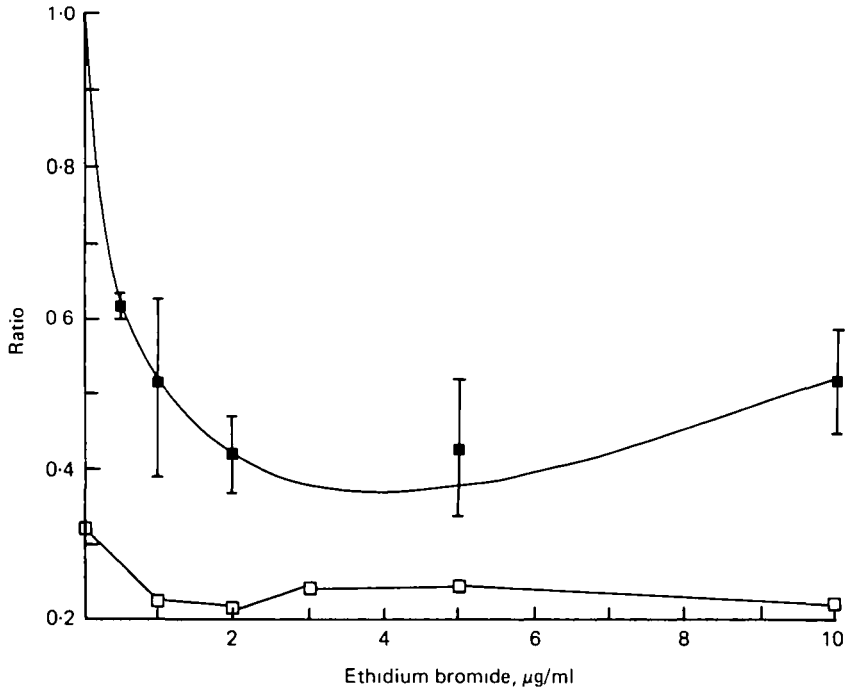

Fig. 1. Effect of ethidium bromide concentration on sedimentation of nucleoids from control lymphoblastoid cells. The upper curve (■) describes the sedimentation of nucleoids from unirradiated cells and the lower curve (□) describes that for nucleoids exposed to 2 J kg^{-1} of γ-rays. Distance sedimented by nucleoids in both cases is expressed as a ratio relative to the value obtained for unirradiated nucleoids sedimenting in the absence of ethidium bromide. Centrifugation conditions are outlined under Materials and methods. Two control cell lines, C3ABR and C5ABR, were employed in these experiments. Error bars give the standard error of the mean (S.E.M.).

nucleoids in the presence of the intercalating dye ethidium bromide (EB). A characteristic drop in sedimentation occurs for control cells at lower concentrations of EB followed by a rise at high concentrations (Fig. 1). A minimum in the sedimentation rate was obtained at about 3 μg/ml of EB for these cells. The results in Fig. 2 show that AT cells respond in a similar fashion to increasing concentration of EB, also reaching minimum sedimentation value at about 3 μg/ml. These data suggest that a similar degree of supercoiling occurs in both cell types. Furthermore treatment of cells with ionizing radiation (2 J kg^{-1}) relaxed the superhelical DNA to the same

extent in both types. This is evident from the results in Figs. 1 and 2 which show little further change in sedimentation of irradiated nucleoids with increasing concentration of EB.

The effect of increasing radiation dose on nucleoid sedimentation in the absence of EB is presented in Fig. 3. The rate of decrease in sedimentation and the extent of this decrease is similar for control and AT cells. The most marked effect for both cell types is seen at low radiation doses.

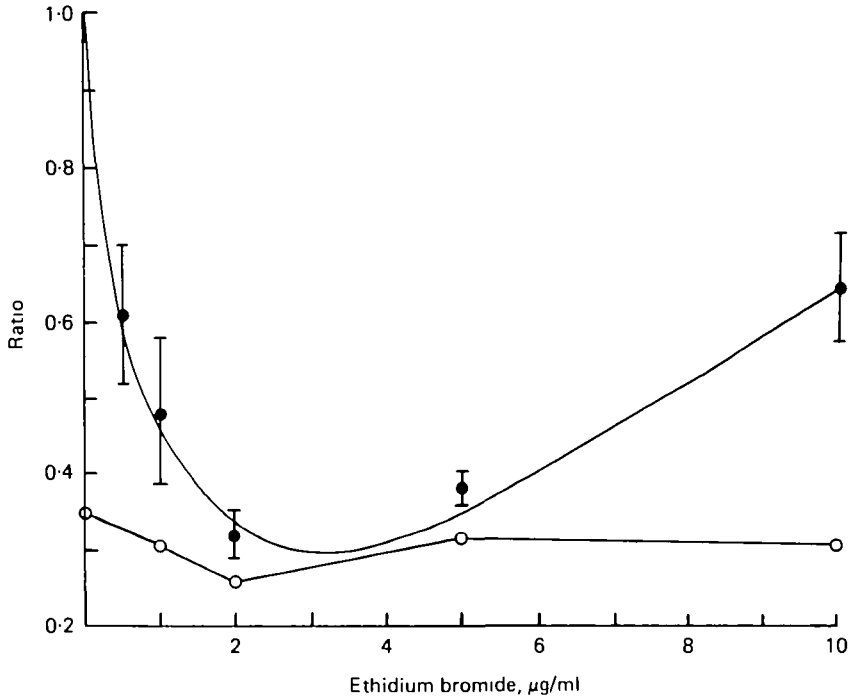

Fig. 2. Effect of ethidium bromide concentration on nucleoid sedimentation using AT lymphoblastoid cell lines. Upper curve (●) unirradiated cells, lower curve (○) irradiated cells (2 J kg^{-1}). Results are expressed as in legend to Fig. 1. Two AT cell lines were used, AT2ABR and AT6ABR. Error bars represent S.E.M.

A dose of 2 J kg^{-1} was selected to compare the time course of repair of radiation damage in the 2 cell types. The greatest effect on nucleoid sedimentation was seen immediately after irradiation which corresponds with the time at which most radiation-induced breaks in DNA are evident (Fig. 4). A rapid increase in sedimentation rate occurs with incubation time reaching a value of about 85% of that observed for unirradiated cells after 1 h incubation. After 3 h the rate of sedimentation is restored to that of unirradiated cells (Fig. 4). No significant difference is obtained when control and AT cells are compared. These results indicate that post-irradiation incubation of control and AT cells restores the same degree of supercoiling as that observed in unirradiated cells. In order to check that these rapidly sedimenting structures were indeed negatively supercoiled after incubation, we irradiated cells (2 J kg^{-1}) and allowed

them to carry out DNA repair for 2·5 h, followed by sedimentation in gradients containing ethidium bromide. A characteristic biphasic sedimentation pattern was again obtained for both cell types (Fig. 5).

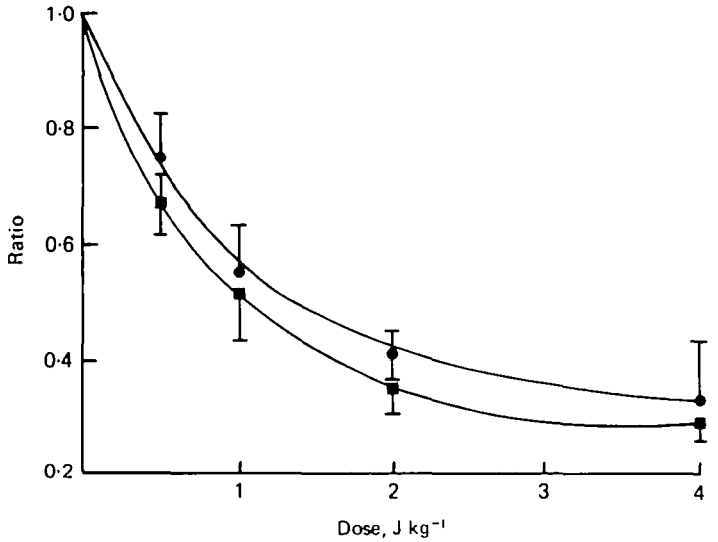

Fig. 3. Effect of radiation dose on sedimentation of nucleoids from control (■) and AT (●) cells. Distance sedimented by irradiated nucleoids is expressed as a ratio relative to unirradiated nucleoids sedimented in the same centrifugation run. Sedimentation is carried out in the absence of ethidium bromide. Three control cell lines C3ABR, C5ABR and C8ABR, and 4 AT cell lines AT1ABR, AT2ABR, AT4ABR and AT5ABR were used. Error bars represent S.E.M.

All of the AT cell lines used in this study have been shown to be more sensitive to ionizing radiation than control cells (Chen *et al.* 1978). Although no difference in the rate of DNA repair was evident at 2 J kg^{-1} in this study, it was possible that this dose was insufficient to discriminate between AT and control cells. Accordingly the time course of repair was determined at considerably higher doses (Fig. 6). Again both cell types showed a similar rate of repair, after radiation doses of 10 and 20 J kg^{-1}. After a dose of 10 J kg^{-1} the sedimentation rate for AT cells and controls increased to approximately 70% of the level of unirradiated cells in 2 h. Restoration of the supercoiled structure was slower after a dose of 20 J kg^{-1}, reaching unirradiated levels 16 h post-irradiation. When the dose was increased to 50 J kg^{-1} (results not shown) a slow increase in sedimentation rate was observed for both cell types reaching approximately 70% of the unirradiated levels after 16 h post-irradiation incubation.

DISCUSSION

The results obtained in this study not only confirm previous findings on the efficiency of repair of DNA strand breaks in AT cells, but also demonstrate that resealing of these breaks leads to the restoration of a DNA configuration similar to that obtained

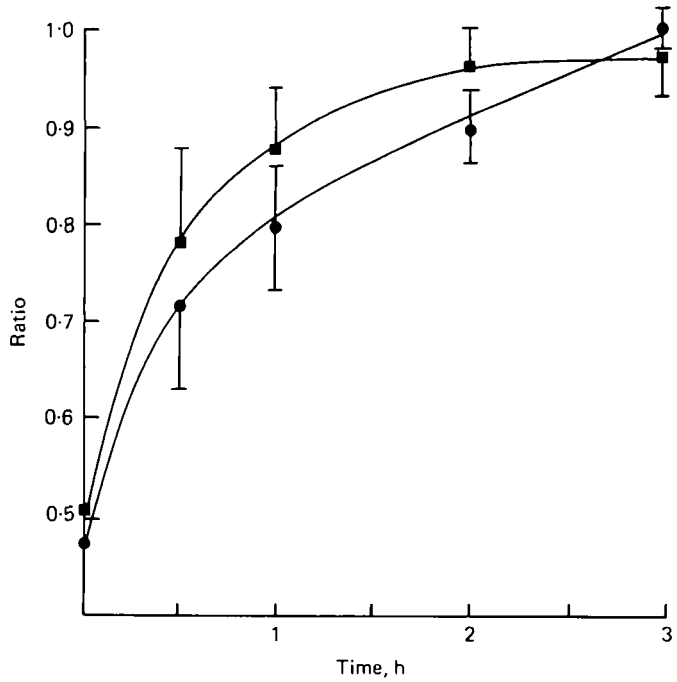

Fig. 4. Time course of repair of γ-radiation (2 J kg^{-1}) damage to DNA from control (■) and AT (●) cells. Zero time irradiation was carried out on ice and other incubations were carried out at 37 °C prior to sedimentation. In this case also sedimentation of nucleoids from irradiated cells, incubated for various times, were expressed as a ratio relative to unirradiated nucleoids. Four control cell lines, C3ABR, C4ABR, C5ABR and C8ABR, and 5 AT cell lines, AT1ABR, AT2ABR, AT4ABR, AT6ABR and GM1535 were used in experiments. Error bars represent S.E.M.

in unirradiated cells. Cook & Brazell (1975) suggest that variety of supercoiling may exist within the cell nucleus and that lethal effects of irradiation may be due to the incomplete restoration of the original superhelical properties. This study, which has determined the sedimentation characteristics of nucleoids from irradiated cells with incubation time (Fig. 4), and the sedimentation behaviour of repaired nucleoids in response to increasing concentration of EB (Fig. 5), would appear to rule out the introduction of an incorrect configuration into DNA as being responsible for cell death, at least at the gross level examined in these experiments.

The time course of repair of single-strand breaks, and the recovery of S1 nuclease resistance have been shown to be similar in both AT and control cells (Paterson *et al.* 1976; Sheridan & Huang, 1977). In both cases virtually all of the strand breaks are repaired in 90 min post-irradiation. In the more sensitive assay using S1 nuclease half of the alkali-labile sites are repaired in both control and AT cells 10–15 min after a radiation dose of 4 J kg^{-1} (Sheridan & Huang, 1977, 1979). It is evident from the results in Fig. 4 that the sedimentation value for irradiated cells (2 J kg^{-1}) has reached approximately half of that in untreated cells after 30 min post-irradiation incubation. This is in keeping with the kinetics of repair of single-strand breaks in control and

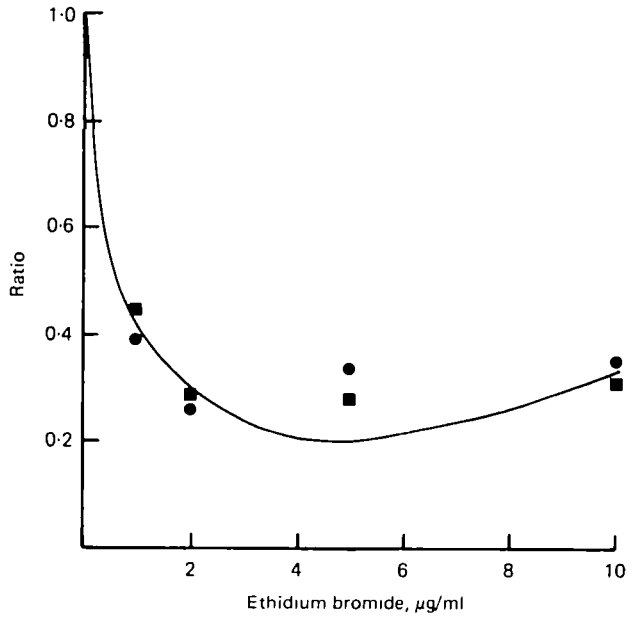

Fig. 5. Effect of ethidium bromide concentration on the sedimentation of nucleoids from irradiated control (■) and AT (●) cells. In these experiments cells were irradiated (2 J kg^{-1}) and allowed to carry out repair for 2·5 h, which restores the sedimentation value to that of unirradiated cells (Fig. 4). Sedimentation was then carried out in the presence of ethidium bromide. Sedimentation is expressed as outlined in Figs. 1 and 2. Two control cell lines C3ABR and C5ABR, and 2 AT cell lines, AT1ABR and AT4ABR were used; 2 experiments in each case.

AT cells described by others (Sheridan & Huang, 1979), and supports the contention that loss of supercoiling, and subsequent restoration with incubation, is indeed due to the appearance and loss respectively of single-strand breaks in DNA.

Our results also demonstrate a dose dependence in the restoration of superhelical structure (repair of single-strand breaks). At a dose of 2 J kg^{-1}, 85% of strand breaks have been repaired after 1 h incubation, while this level of recovery is achieved after 2–5 h in cells irradiated with 10 J kg^{-1}. The repair of damage due to γ-irradiation at 10 J kg^{-1} is of the same order as that observed in white blood cells by others using a similar dose of radiation (Cook & Brazell, 1976). This dose dependence of repair is further demonstrated at 20 J kg^{-1} (Fig. 5), and at 50 J kg^{-1} where a gradual increase in sedimentation up to 70% of the unirradiated value is observed 16 h post-irradiation (results not shown). The sensitivity of this method can be appreciated when comparison is made with data obtained using alkaline sucrose gradients to measure repair of single-strand breaks. At doses as high as 500 J kg^{-1}, it has been reported that 80–90% of single-strand breaks are repaired 90 min after irradiation (Dugle, Gillespie & Chapman, 1976; Paterson *et al.* 1976). Other results using lower doses (100–200 J kg^{-1}) provide values varying from 50–100% repair of breaks 1 h after irradiation (Kleijer, Lohman, Mulder & Bootsma, 1970; Taylor *et al.* 1975; Vincent *et al.* 1975). These results would seem to indicate that no dose dependence for repair of single-strand

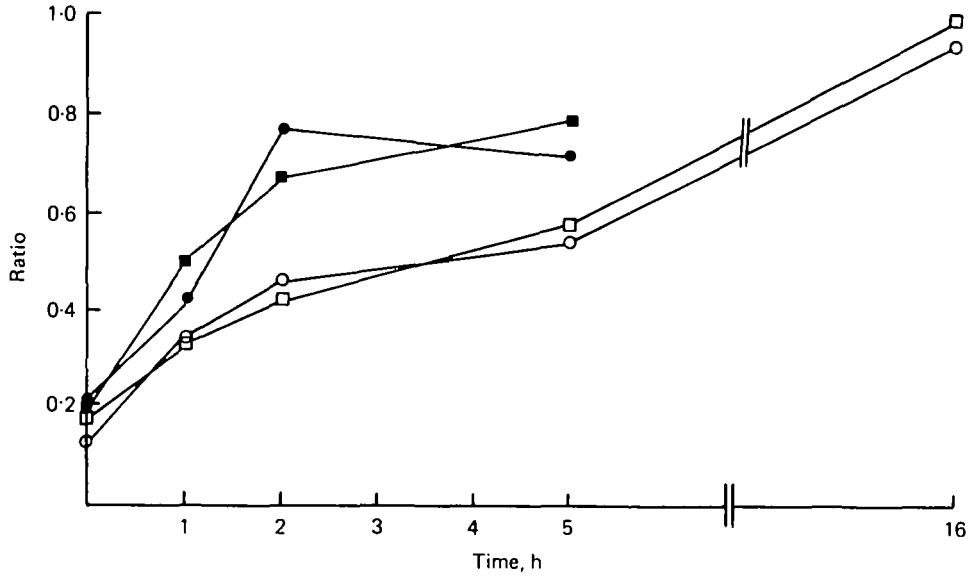

Fig. 6. Time course of repair of γ-radiation damage in control and AT cells. Two doses of radiation were used for both cell types: control 10 J kg^{-1} (■), control 20 J kg^{-1} (□); AT 10 J kg^{-1} (●), AT 20 J kg^{-1} (○). Conditions; the same as those described in the legend to Fig. 4. Repeat experiments were carried out for C4ABR, C5ABR, AT1ABR and AT4ABR.

breaks occurs, over the range 0–500 J kg^{-1}. More extensive results, using a dose range of 220–550 J kg^{-1}, and a repair period of 3·5 h, show a dose dependence for the presence of residual single-strand breaks after incubation (Dugle *et al.* 1976). It is obvious from the results using alkaline sucrose gradients that the apparent lack of a dose-dependent repair of single-strand breaks in DNA is due to lack of sensitivity of this method even at high radiation doses.

Conflicting data exists on the repair of double-strand breaks in mammalian cells (Dugle *et al.* 1976; Lehmann & Stevens, 1977). It has been estimated that the breakage efficiency of ionizing radiation is 0·1–0·2 double-strand breaks/10^9 daltons of DNA per 10 J kg^{-1} (Lehmann & Stevens, 1977; Hutchinson, 1978). The highest dose used in these studies (50 J kg^{-1}) would give rise to approximately 0·5–1 double-strand breaks/10^9 daltons based on these estimates, which is the same molecular weight as that estimated for nucleoids released in unirradiated cells (Cook & Brazell, 1975). Failure to repair a double-strand break would have a continued and marked effect on nucleoid sedimentation, and since the sedimentation value is restored to 70% of that in unirradiated cells 16 h after exposure of cells to 50 J kg^{-1}, it would strongly support the findings that double-strand breaks are indeed repaired in mammalian cells (Lehmann & Stevens, 1977). Cytogenetic studies, which demonstrate increased levels of radiation-induced aberrations in AT cells compared to controls, seem to indicate that a small proportion of DNA strand breaks remain unrepaired in these cells and thus contributes to the radiation sensitivity (Taylor, 1978). The method employed here would not detect failure to repair a small proportion of double-strand breaks in DNA.

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