

Effect of Quinacrine on Survival and DNA Repair in X-irradiated Chinese Hamster Cells¹

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SUMMARY

Quinacrine (5 $\mu\text{g}/\text{ml}$) sensitizes cultured Chinese hamster cells (A1 subline) to killing by X-rays and inhibits the repair of single-strand breaks in their DNA. The relevance of the chemical inhibition of repair systems to the radiation treatment of cancer is discussed.

INTRODUCTION

Considerable attention has been given, of late, to the production of DNA chain breaks by X-irradiation, and the kinetics of their rejoining with the use of alkaline (29) or neutral sucrose gradient procedures (23). Several studies have indicated that there is a relationship between the capacity of cells to repair DNA single-strand breaks and their sensitivity to the lethal effects of X-rays (25, 29, 30, 41). This suggests that one way to enhance the effect of ionizing radiation on cells would be the chemical inhibition of cellular recovery (repair) systems. For that purpose, several substances have been used: actinomycin D (4, 11, 17, 21, 37, 38, 42), 5-fluorodeoxyuridine (10, 42), acriflavine (4, 24), ethidium bromide (4), proflavine (40, 42), quinacrine (12), chloroquine (13, 14), hydroxyurea (26, 31, 35, 37), dinitrophenol (9, 32, 37, 43), lucanthone (Miracil D) (5), and phleomycin (42).

In this study, we have investigated the inhibitory effect of quinacrine (Atabrin) on the rejoining of DNA single-strand breaks and its effect on the radiosensitivity of Chinese hamster cells.

Quinacrine is an aminoacridine that is used in malaria therapy. It appears to be selectively taken up by certain experimental tumors (1-3) and to show a limited carcinostatic activity (19, 20). Quinacrine has the ability to combine with nucleic acids *in vivo* as well as *in vitro* (27, 46). Recently, quinacrine (34) and quinacrine mustard (6, 7, 15) were used to stain selected chromosomal regions in plant, animal, and human cells as evidence for chemical differenti-

ation along the chromosomes. The model of interaction between DNA and quinacrine suggests that the planar triple-ring system of the drug becomes intercalated between adjacent base pairs of the double helix (27).

Fuks and Smith (12) have demonstrated that quinacrine is a potent radiation sensitizer that irreversibly inhibits the *recA* gene-controlled repair of DNA single-chain breaks in bacteria. It therefore seemed of value to test the effectiveness of quinacrine in this regard on mammalian cells with the view of assessing the potential usefulness of this drug as a radiation sensitizer in the treatment of cancer.

MATERIALS AND METHODS

Cells and Culture Techniques. HA1 cells, a subline of Chinese hamster ovary cells, were maintained in a monolayer in exponential growth in glass bottles (37°, humidified atmosphere of 5% CO₂-95% air) in MEM⁴ supplemented with 15% fetal calf serum (18). Under these conditions, the generation time was 15 to 16 hr. All experiments were carried out with the cells in exponential growth phase.

Determination of the Toxicity of Quinacrine. The cells were plated at appropriate dilutions in 4 ml of MEM in 60-x 15-mm plastic Petri dishes and were kept for 4 hr at 37° in a humidified atmosphere of 5% CO₂ and 95% air. Solutions of quinacrine dihydrochloride (Sigma Chemical Co., St. Louis, Mo.) at various concentrations (2.5 to 250 $\mu\text{g}/\text{ml}$) were prepared just before the start of an experiment by dissolving the drug in 0.15 M NaCl. One ml of these quinacrine solutions was added to each of the culture dishes (5 dishes/point were used), and the dishes were then incubated for from 5 to 240 min. The medium containing the drug was removed, and the dishes were washed twice with 0.68% NaCl solution and then were overlaid with 5 ml fresh prewarmed medium containing no drug. Cell survival was determined by counting the colonies after 10 days of incubation. Plating efficiencies were between 75 and 100%.

Sensitization of the Cells to X-rays by Quinacrine. Dishes of cells were prepared as in the toxicity experiments. The sensitizing effect of quinacrine was studied by: (a) exposing the cells to quinacrine, (final concentration, 5 $\mu\text{g}/\text{ml}$), at 37° for various intervals of time (30 to 240 min) before irradiation with a single dose of 700 rads. Immediately after irradiation, the drug was removed, and the cells were washed twice with MEM, overlaid with fresh prewarmed

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⁴ The abbreviation used is: MEM, Eagle's minimal essential medium.

medium, and incubated for 10 days at 37°; (b) exposing the cells to quinacrine (5 $\mu\text{g}/\text{ml}$) for 30 or 120 min at 37° and then irradiating with various doses of X-rays (100 to 1100 rads), followed by removal of the drug and incubation in fresh medium; (c) adding quinacrine (5 $\mu\text{g}/\text{ml}$) immediately after irradiation and exposing the cells to the drug for 30 min at 37° followed by removal of the drug and incubation in fresh medium. The cells were X-irradiated at a dose rate of 131 rads/min (85 kV, 9.6 ma, 1-mm beryllium filtration). Cell survival was determined by counting colonies after 10 to 12 days of incubation.

Sedimentation Analysis. The DNA of HAI cells was labeled by growing them in plastic Petri dishes for approximately 20 hr in medium containing thymidine- ^3H (6.7 Ci/mmmole), which was added in 2 batches, each of 0.5 $\mu\text{Ci}/\text{ml}$ at 10-hr intervals. The cells were then washed twice with phosphate-buffered saline (0.05 M phosphate buffer, pH 7.0, in 0.68% NaCl solution), overlaid with fresh nonradioactive medium, and incubated again for 30 min at 37° (to reduce the acid-soluble radioactive pools). The cells were then trypsinized (18), counted, and diluted with MEM to about 1×10^5 cells/ml, and then were held at 0° until used. The total radioactivity in the cells was usually about 0.25 to 0.5 cpm/cell as measured on filter discs by liquid scintillation counting (see below).

In studies in which quinacrine was added before X-irradiation, a 5-ml cell suspension containing the appropriate quinacrine concentration was incubated in a gyratory water bath at 37°. After the desired length of incubation, aliquots (0.25 ml) were irradiated at 0° in plastic vessels, after which 0.1 ml of the cell suspension was layered on the lytic layer of the gradient.

In experiments in which quinacrine was added after the irradiation, 4 aliquots of 0.25 ml of the trypsinized cell suspension were irradiated as described above, the 4 aliquots were transferred to 1 culture tube, the appropriate amount of quinacrine was added, and then incubated in a gyratory water bath. At the end of the incubation, 0.1 ml of the cell suspension was added to the gradients.

In some experiments in which the repair of X-ray-induced DNA breaks was followed, a slightly different method was used. After the customary labeling and rinses, the cells in complete MEM were exposed to the desired quinacrine concentration while kept in the incubator. This was followed by irradiation at 0°, removal of the drug-MEM solution, and addition of fresh MEM after 2 MEM washes. The cells were then placed in the incubator (37°) for the desired post-X-ray incubation time, after which the cells were trypsinized, counted, and added to the gradient as usual. In other experiments, the cells were treated first with X-rays, followed by drug treatment at 0° with or without additional incubation at 37° in drug-free medium before trypsinization.

Irradiations were carried out at 0° with 30 kilorads using a twin-tube beryllium window X-ray unit (50 kV, 50 ma, 48 ma, 0.3-mm aluminum filtration, 8.1 or 7.0 kilorads/min, depending upon the geometry of the sample) (28).

Sedimentation analysis was performed by means of a modification of the method of McGrath and Williams (29).

Linear 5 to 20% sucrose gradients were prepared in 5-ml nitrocellulose centrifuge tubes with a Buchler triple-gradient maker. The 5% sucrose solution contained 0.3 M NaOH, 0.25 M sodium acetate, 0.5 M NaClO_4 , and 1 mM EDTA. The 20% sucrose solution contained 0.1 N NaOH. Sucrose gradient tubes prepared at room temperature were held at 4° for 30 to 60 min before use. A 0.2-ml volume of the lysing solution (0.25 N NaOH, 0.05 M EDTA, and 0.20% sodium-deoxycholate) was gently layered on the top of each gradient, followed immediately by 0.1 ml of cell suspension containing 3 to 7×10^3 cells. The top 2 layers were gently mixed with a pin. The tubes were kept for 30 min at room temperature and then were centrifuged in a SW 50.1 swinging bucket rotor on a Beckman L2 65B ultracentrifuge, initially for 15 min at 1,000 rpm. The rotor was subsequently accelerated at 2,000 rpm/min until a speed of 30,000 rpm was attained and then was run for an additional 150 min at 20°.

After centrifugation, the bottoms of the tubes were pierced and 25 8-drop fractions were collected on Whatman No. 3MM paper discs. After drying, the discs were washed twice in 5% trichloroacetic acid, and once each in ethanol and acetone, and dried. The discs were placed in glass vials and counted in 5 ml of counting solution (4 g PPO and 0.1 g POPOP per liter of toluene). The gradient tubes were rinsed with 0.5 ml 0.5 N NaOH, and the radioactivity content of 0.1 ml was measured as described above.

Under these conditions, an average of 88 to 94.5% of the radioactivity of DNA has been routinely found at a reproducible position in the gradient, and 5.5 to 12% sedimented to the bottom of the tube. Protein and RNA were recovered in 99% yield near the top of the gradient. When compared with phage T2 marker DNA, the DNA of unirradiated Chinese hamster cells had an average molecular weight [the molecular weight corresponding to the position on the gradient beyond which one-half of the DNA sediments (39, 45)] of about 3.5×10^8 daltons.

RESULTS

Toxicity of Quinacrine. The toxicity of quinacrine is a function of both the concentration and the exposure time (Chart 1). Cell viability decreased by 30% after being exposed to the drug for 7 min at a concentration of 50 $\mu\text{g}/\text{ml}$, for 12 min at 25 $\mu\text{g}/\text{ml}$, and for 30 min at 5 $\mu\text{g}/\text{ml}$. These experiments suggest that quinacrine requires time to reach the cellular receptor sites (nuclear DNA?) and to express its toxic effect. The amount of time required depends upon the concentration of the drug in the medium. Gittelson and Walker (16) have shown, *in vitro*, that the number of molecules of proflavine bound per molecule of DNA is a function of the free proflavine concentration in the medium.

Sensitization of Cells to X-rays by Quinacrine. Chart 2 shows the survival of unirradiated cells treated with quinacrine (5 $\mu\text{g}/\text{ml}$) and of cells irradiated with a single dose of 700 rads given after various periods of treatment with quinacrine (5 $\mu\text{g}/\text{ml}$). The survival of the quinacrine-treated

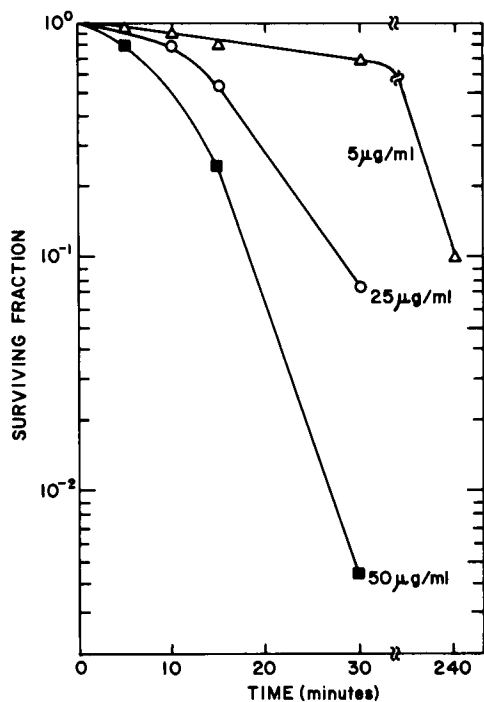


Chart 1. Survival curves for Chinese hamster HA1 cells exposed to different concentrations of quinacrine as a function of time at 37°.

irradiated cells decreased proportionally with the exposure time to the drug. After 240 min of exposure to quinacrine, there was a 10-fold sensitization of the cells to X-rays (after correction for toxicity of the drug to unirradiated cells).

The survival curves of X-irradiated cells without quinacrine treatment, with 30 min of exposure to the drug (5 $\mu\text{g/ml}$) immediately after irradiation, and with 30 min or 120 minutes of exposure to the drug before irradiation, are given in Chart 3. These curves show the net sensitization effect of quinacrine (after correction for toxicity of the drug to unirradiated cells). The sensitizing effect was higher when quinacrine was added before irradiation than when added after irradiation. Also, a longer time of exposure to the drug sensitized the cells to X-rays to a larger extent. The extrapolation numbers (\bar{n}) for the survival curves (Chart 3) were all the same (~ 2.5), but the D_0 values changed with increasing drug treatment times (no quinacrine, $D_0 = 200$ rads; quinacrine for 30 min after irradiation, $D_0 = 180$ rads; quinacrine for 30 min before irradiation, $D_0 = 160$ rads; and quinacrine for 120 min before irradiation, $D_0 = 135$ rads). The corresponding dose-reduction factors (ratio of D_0 values) are 1.11, 1.25, and 1.48, respectively.

Effect of Quinacrine on DNA Single-Strand Rejoining.

When quinacrine (5 $\mu\text{g/ml}$) was added 30 min before irradiation (and was present during irradiation), the rejoining of DNA single-strand breaks appeared to be completely prevented, after a dose of 30 kilorads, even when the cells (after removal of the quinacrine) were incubated in fresh medium at 37° for 30 min (Chart 4). The treatment of unirradiated cells with quinacrine under these conditions had essentially no effect on the sedimentation of the DNA.

When quinacrine was added immediately after irradiation,

the DNA from cells incubated for 30 min with the drug showed evidence of a moderate amount of repair. This amount of repair was not increased if the cells were incubated (after removal of the drug) for an additional 30 min in normal growth medium (Chart 5). If a higher concentration of quinacrine was used (25 $\mu\text{g/ml}$), the amount of repair was less (gradient data not shown).

To determine whether the small amount of repair observed when quinacrine was added after irradiation was due to the time required for the drug to reach the DNA, the cells were irradiated at 0° and then incubated with quinacrine for 30 min at 0°. Holding the cells at 0° in the absence of drug does not irreversibly inhibit the rejoining process (data not shown). After the quinacrine was removed, the cells were reincubated in fresh medium for 30 min at 37°. Chart 6 shows that, under these conditions, the rejoining of DNA single-strand breaks was prevented to approximately the same extent as it was when the quinacrine was added before irradiation (compare Chart 4).

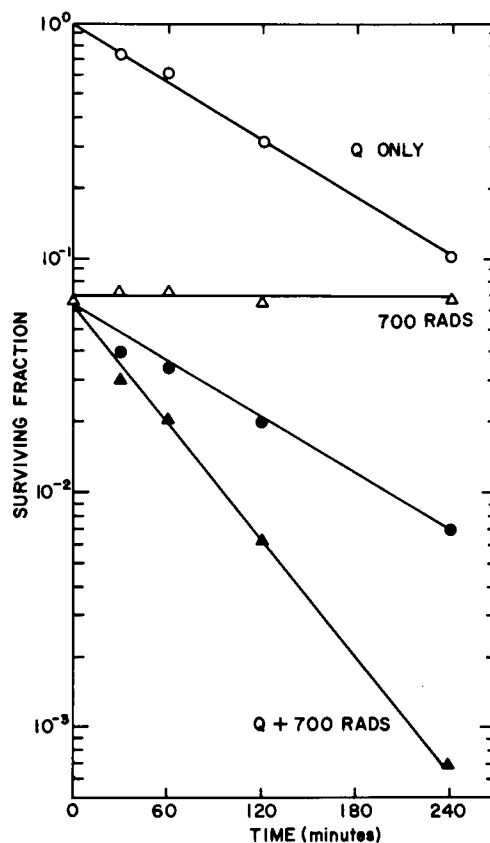


Chart 2. Survival curves for HA1 cells exposed to quinacrine (5 $\mu\text{g/ml}$) for various intervals at 37° and then irradiated with 700 rads. Immediately after irradiation, the medium containing the drug was removed, and the cells were washed and overlaid with drug-free medium; O, unirradiated controls treated with quinacrine (Q); Δ , survival of untreated cells after 700 rads; \blacktriangle , cells treated with quinacrine for various times and then irradiated with 700 rads; \bullet , irradiation-sensitizing effect of quinacrine corrected for the toxicity to the unirradiated cells.

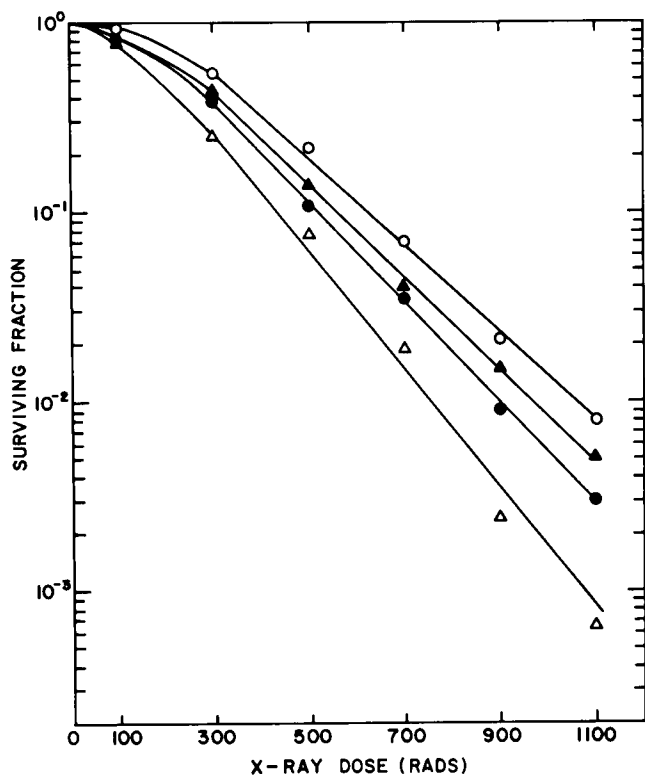


Chart 3. X-ray survival curves for untreated HA1 cells and for cells exposed to quinacrine (5 $\mu\text{g}/\text{ml}$) at 37° for either 30 or 120 min before irradiation or 30 min immediately after irradiation: O, untreated cells; ▲, treated immediately after irradiation for 30 min; ●, treated 30 min before irradiation; or Δ, treated 120 min before irradiation. In each case, the toxicity to the unirradiated controls treated similarly has been subtracted. The survival of unirradiated cells after exposure to quinacrine (5 $\mu\text{g}/\text{ml}$) for 30 min was 74%; after 120 min of exposure, it was 31%. The extrapolation number for all the curves is ~ 2.5 .

DISCUSSION

Quinacrine (5 $\mu\text{g}/\text{ml}$) sensitizes Chinese hamster cells to killing by X-rays (Chart 3). This effect appeared to be greater when the drug was added before than when added after irradiation, but the sensitization increased proportionally, in both cases, with exposure time to the drug. When cells were treated with the drug for 120 min before irradiation (our longest treatment time), the degree of sensitization expressed as the dose-reduction factor was 1.48. The extrapolation number (~ 2.5) was not affected by the drug treatment.⁵

This degree of sensitization may be compared with the effect of Lucanthone (having a planar triple-ring system, as

does quinacrine) on HeLa cells (5). The control cells (no drug) had a $D_0 = 180$ rads, $\bar{n} = 1.5$ and, at a Lucanthone concentration that was toxic to 50% of the unirradiated cells, the irradiated cells had a $D_0 = 140$ rads, $\bar{n} = 1.0$. The dose-reduction factor was 1.28. Chinese hamster cells treated with a nontoxic concentration (0.02 $\mu\text{g}/\text{ml}$) of actinomycin D (a drug that also intercalates with DNA) exhibited an X-ray dose-reduction factor of 1.14 (11).

The postirradiation treatment of *Escherichia coli* K-12 with quinacrine (75 $\mu\text{g}/\text{ml}$) yielded a dose-reduction factor of 1.46 (12). The D_0 for untreated cells was 3.8 krad ($\bar{n} = 2$) and that for treated cells was 2.6 krad ($\bar{n} = 1$).

The Chinese hamster cells were sensitized by quinacrine at 5 $\mu\text{g}/\text{ml}$ while 75 $\mu\text{g}/\text{ml}$ was required for a comparable reduction in D_0 in *E. coli* K-12 (12). The cellular penetration by the drug appears to be passive in the mammalian cells but active in *E. coli*. This is evidenced by the fact that, at 0°, quinacrine can exert its effect on mammalian cells (Chart 6) but not on *E. coli* (12).

With the use of sedimentation analysis to follow the effect of quinacrine on the rejoining of X-ray-induced DNA single-strand breaks, it was observed that the drug almost completely prevented the repair process when it was added before and was present during irradiation. Since the presence of the drug, at the concentration used, did not significantly modify the number of DNA single-strand breaks induced by the irradiation (Chart 4), this suggested that quinacrine exerted its sensitizing effect by inhibiting the repair process.

This hypothesis was substantiated by experiments in which the quinacrine was added after the irradiation.

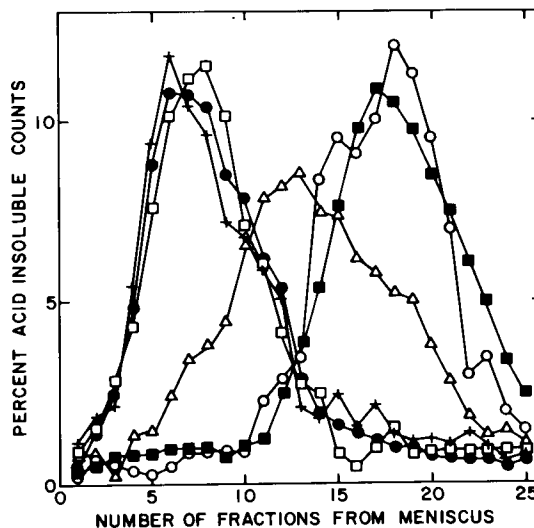


Chart 4. Alkaline sucrose gradient sedimentation profiles of DNA from HA1 cells exposed to quinacrine (5 $\mu\text{g}/\text{ml}$) 30 min before irradiation. The cells (treated or nontreated) were irradiated at 0° with 30 krad and then reincubated in fresh medium for 30 min at 37°: O, unirradiated; ■, unirradiated control treated with quinacrine; Δ, untreated cells, irradiated and incubated for 30 min in fresh medium at 37°; □, cells treated, irradiated, and reincubated in fresh medium for 30 min at 37°; ●, cells treated and irradiated but not reincubated; and +, untreated cells irradiated but not reincubated.

⁵ Since this paper was submitted, it has been determined that the sensitizing effect of quinacrine on the survival of X-irradiated cells is not observed if the drug treatment is immediately followed by trypsinization for purposes of cloning the cells (R. Roots, unpublished observations). Since trypsinization reduces the metabolic rate of cells for several hours (36), this may give the cells more time to excrete the quinacrine before metabolic events essential to repair are initiated.

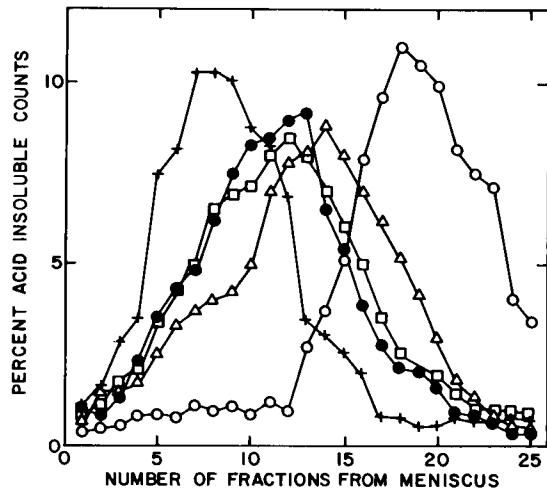


Chart 5. Alkaline sucrose gradient sedimentation profiles of DNA from HA1 cells exposed to quinacrine (5 $\mu\text{g}/\text{ml}$) immediately after irradiation. The cells were irradiated at 0° with 30 kilorads, the drug was added, and the cells were incubated for 30 min at 37°: O, unirradiated; Δ , irradiated, untreated, incubated for 30 min in fresh medium at 37°; \bullet , irradiated, treated for 30 min with quinacrine; \square , irradiated, treated for 30 min with the drug, and then reincubated for an additional 30 min at 37° in the absence of drug; and +, cells irradiated and not reincubated.

Quinacrine did exert an inhibitory effect on DNA strand rejoining when it was added immediately after irradiation, but the degree of inhibition was not as complete as it was when the drug was added before irradiation. This difference can probably be explained by the time required for quinacrine to reach and interact with the nuclear DNA.

When the cells were irradiated and held at 0° in the absence of drug, the repair of DNA single-strand breaks was reversibly inhibited (data not shown). When quinacrine was added to the cells at 0° and held under these conditions for 30 min (to allow time for the drug to penetrate the cell before repair was initiated), and then the temperature was raised to 37° (to permit the repair process to proceed), the inhibition of repair by quinacrine was as complete as if the drug had been added 30 min before the irradiation.

Although the precise mechanism by which quinacrine exerts its radiation-sensitizing effect is not well understood, it could prevent the rejoining of DNA single-strand breaks by some mechanism of steric hindrance and/or by a general alteration of the metabolism of the cell.⁶ Quinacrine (33, 44) and proflavine (22) prevent the action of DNA and RNA polymerase. In *Tetrahymena*, quinacrine (15 $\mu\text{g}/\text{ml}$) inhibits the synthesis of DNA (almost completely), RNA (70%), and protein (50%), and almost completely blocks the incorporation of labeled acetate into lipid components (8). Quinacrine also interferes with oxidative phosphorylation (47). Dinitrophenol, also an uncoupler of oxidative phosphorylation, prevents the rejoining of DNA single-strand breaks in mammalian (32) and bacterial cells (43).

⁶ In comparing the inhibitory effects of quinacrine on survival and on repair, it should be kept in mind that, because of the relative insensitivity of the gradient assay technique, much higher doses of radiation were used for the biochemical studies than were used for the survival studies.

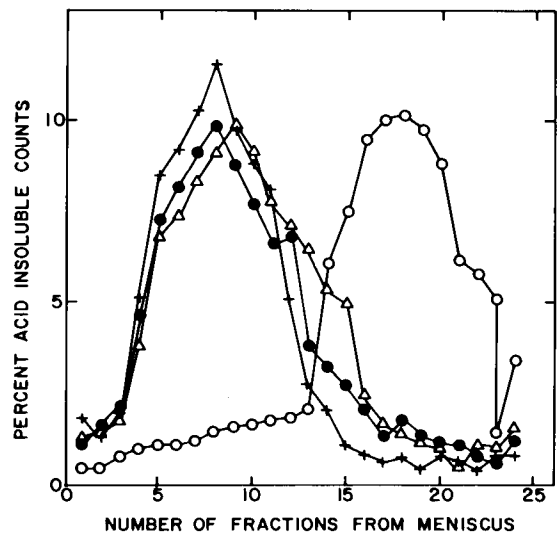


Chart 6. Alkaline sucrose gradient sedimentation profiles of DNA from HA1 cells exposed to quinacrine (5 $\mu\text{g}/\text{ml}$) for 30 min at 0° immediately after irradiation at 0° with 30 kilorads; O, unirradiated; \bullet , irradiated and treated with quinacrine for 30 min at 0°; Δ , irradiated, treated for 30 min at 0°, and reincubated in drug-free medium at 37° for an additional 30 min; and +, irradiated but not reincubated.

Because of the importance of repair systems in determining the radiation sensitivity of cells (26, 29, 41), drugs that are selective in their inhibition of these repair systems should prove to be beneficial adjuncts to the use of radiation in the treatment of cancer.⁷ Although repair inhibitors more effective than quinacrine may well be developed, this drug has the advantage of a long history of clinical use in the treatment of malaria, so that human tolerances are well known. However, any assessment of the value of the use of one drug in preference to another must take into account the toxicity of the drugs. Quinacrine appears to show radiation sensitization only at concentrations that show toxicity to unirradiated cells. Such toxicity may be tolerated in a therapeutic situation if the drug is tissue specific. Some tissue specificity has been observed for quinacrine (Refs. 1 to 3; Z. Fuks, unpublished observations).

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⁷ However, *in vivo* experiments performed so far with quinacrine on the response of the KHT sarcoma, the EMT6 carcinoma and normal mouse skin, have failed to uphold this promise (J. M. Brown and Z. Fuks, unpublished observations). These experiments have involved either single or multiple injections (i.p.) given at various times before and after either single or multiple fraction X-irradiations. In none of these circumstances has any reproducible potentiation of the radiation effect been observed. However, an increase in the skin reactions of irradiated mouse legs was observed when quinacrine was absorbed directly into the epidermis of the legs by topical application 0.5 and 24 hr prior to X-irradiation.

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