

entirely ruled out, the significant differences between the HA2 and HA4 structures in the present case are probably due to the sequence. This implies a subtle but well-marked influence of the distance of the A:T base pair from the end of the oligonucleotide helix on the DNA conformation. At 2.5 Å resolution, accurate details of the conformational changes in HA4 are not visible. Nevertheless, a substantial and significant deviation from other Z-DNA hexamer structures indicates an inherent plasticity in the DNA molecule which is apparent even under the close-packed conditions found in Z-DNA crystals.

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Damage and repair of DNA of human epidermoid carcinoma cells X-irradiated in the presence of chlorpromazine

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X-irradiation of human epidermoid carcinoma cells (Hep-2) in the presence of the phenothiazine drug chlorpromazine (CPZ) enhances X-ray-induced cytotoxicity and chromosomal aberrations. The cytotoxicity is partly repairable during holding of the cells in the liquid before plating. The extent of double-strand breaks (DSB) produced in the DNA during these treatments was assayed by pulse field gel electrophoresis. DSBs produced in the cell X-irradiated in the presence of CPZ is the sum of the DSBs produced by X-rays and CPZ separately. Repair of DSBs after X-irradiation in the presence of CPZ is less than that after X-irradiation without CPZ.

CHLORPROMAZINE (CPZ) is a substituted phenothiazine used clinically as an antiemetic antipsychotic agent¹ and recently it is being considered as an adjuvant to cancer chemotherapy for its ability to enhance cytotoxic effects of anticancer drugs² and radiation³. We have shown that

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CPZ increases cytotoxic and cytogenetic effects of X-rays on human cells and the cytotoxic effects may be repaired to some extent by holding the cells in the liquid medium before plating⁴. The mechanism of this CPZ-induced enhancement of cytotoxicity is not clear. It is well known that CPZ acts on the membrane. It has been shown that the intracellular concentration of some anti-cancer drugs increases in the presence of another CPZ-like drug trifluoperazine (TFP), making the cells more susceptible to the cytotoxic effect of the drugs⁵. CPZ also enhances genetic effects of radiation like chromosome aberration and sister chromatid exchange⁶. CPZ might directly affect the amount and nature of the lesions produced in DNA by X-irradiation or it might affect other cellular constituents which in turn increase the damages in the DNA.

In this work, cells in culture were treated with different combinations of CPZ and X-rays and the state of the DNA at each point was determined by pulse field electrophoresis, immediately or 4 h after the damage. Clonogenic survival of the cells after such a treatment was also measured. Human epidermoid carcinoma cell line Hep-2 was used in these experiments. Stationary phase cells were irradiated by X-rays from a Phillips-Muller MG 150 machine (run at 80 kV; dose rate 1 Gy/min) with or without the presence of CPZ. After irradiation the cells were washed and then either plated immediately or were kept in complete medium for 4 h before plating. As reported earlier⁴, liquid holding allowed the cells to repair part of the damage, so that a higher survival was seen for such cells, X-irradiated alone or X-irradiated in the presence of 5 µg/ml CPZ, the extent of repair being higher in the former case (data not shown).

To follow the state of the DNA during these treatments, the cells were first labelled with ¹⁴C-thymidine for 48 h. They were then washed and chased in a cold medium containing 50 µm thymidine for 1 h and then damaged. Agarose blocks were prepared immediately or 4 h after the damage. Blocks were digested with detergent, proteinase K and then incubated with RNase⁷. DNA thus obtained was almost intact. The blocks were electrophoresed in a pulsed field with hexagonal electrodes using Pharmacia LKB 2015 Pulsaphor electrophoresis unit applying 5 V/cm with a pulse time of 250 s for 72 h.

At this condition, all intact human DNA remained within the groove and only degraded DNA moved out into the gel. So,

$$\text{amount of degraded DNA} = \frac{\text{input count in the block before run} - \text{count in the groove after run}}{\text{total count}}$$

Therefore, the relative amount of degraded DNA or the

fraction of activity released

$$\text{FAR} = \frac{\text{amount of degraded DNA}}{\text{total count}}$$

So, each block was prepared in duplicate. One of them was preserved and the other was electrophoresed. After electrophoresis, these blocks were solubilized in 0.1 N HCl and counted to give FAR. Figure 1 shows the variation of FAR as the cells were X-irradiated in the presence or absence of 10 µg/ml CPZ and then the plugs were made, immediately following damage or 4 h later. FAR decreased, indicating repair of double-strand breaks (DSB) when the cells were held in liquid before assay. The rate of repair in the cells X-irradiated alone is, however, much more than in the cells X-irradiated in the presence of CPZ. The reactivation factor may be defined here as $\text{FAR}_{0\text{h}} - \text{FAR}_{4\text{h}}$. If this is plotted against $\text{FAR}_{0\text{h}}$, we get a much steeper curve for cells X-irradiated without CPZ (Figure 2). This indicates that the DSBs produced by X-rays CPZ combination are repaired less than those induced with X-rays alone.

Figure 3 shows the values of FAR when the cells were incubated with different concentrations of CPZ for 1 h, some of them being irradiated with 20 Gy X-rays halfway through the incubation. Blocks were made either immediately or 4 h after damage. We see that there was some breakdown of DNA caused by incubation of cells with CPZ alone, and this was repaired to a great extent during holding in liquid. This is in agreement with an earlier observation where Py 815 mastocytoma cells grown in the presence of CPZ showed a lot of DNA breakage, which resealed after removal of the drug⁸.

DSBs produced by X-rays + CPZ treatment were essentially the sum of DSBs produced by X-rays and

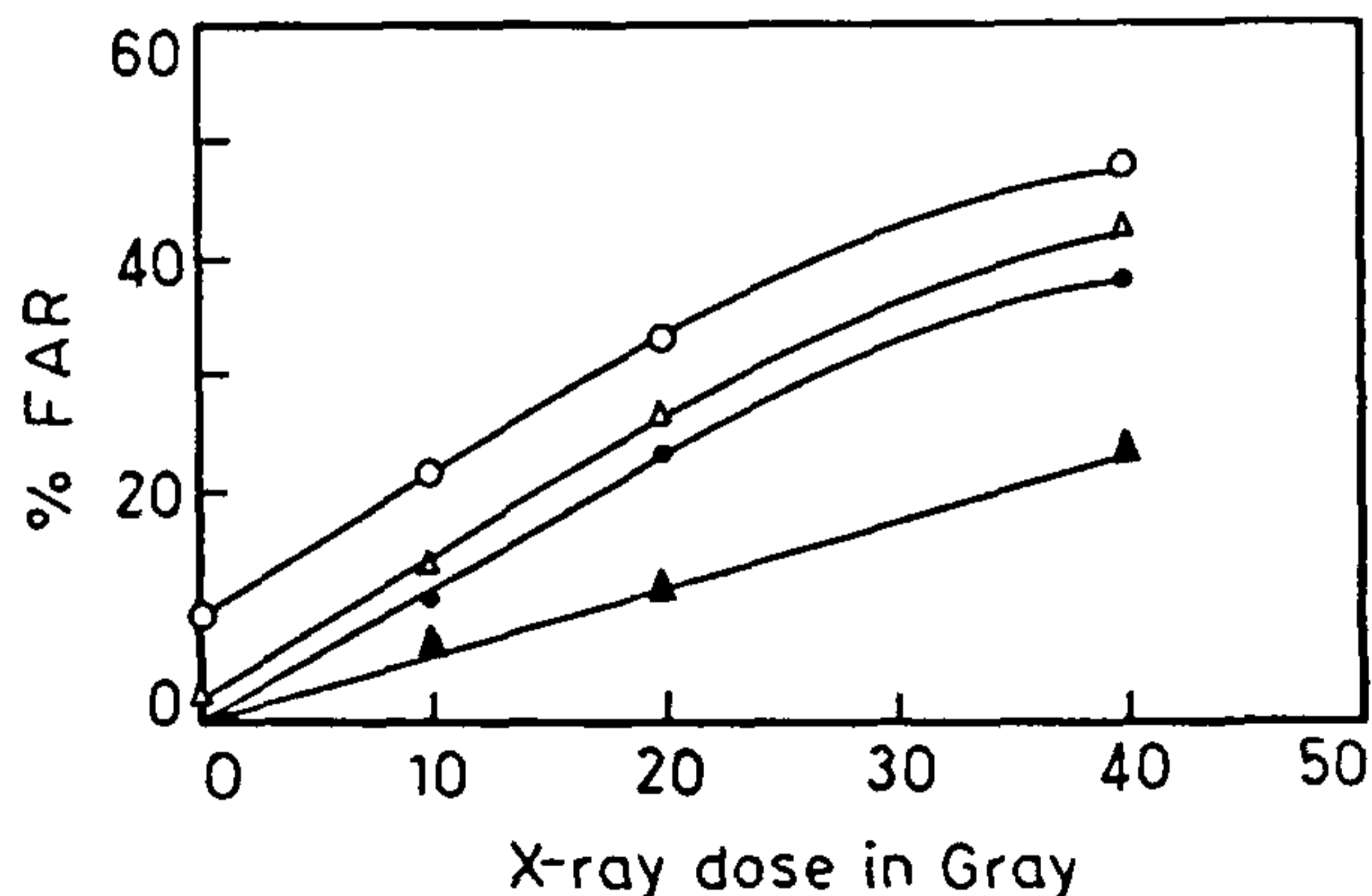


Figure 1. Degradation of DNA (as measured by FAR (%)) in cells X-irradiated in the presence of 0 or 10 µg/ml CPZ and blocks made immediately or 4 h after damage of cells. ● No CPZ, 0 h; ▲ no CPZ, 4 h; ○ 10 µg/ml CPZ, 0 h; △ 10 µg/ml CPZ, 4 h.

CPZ treatment separately (Figure 3). However, the repair of the damage produced by the combined treatment was much less than the repair of the damages produced by the agents individually.

A general model has been proposed to explain the action of intercalative and non-intercalative drugs, postulating their inhibitory effects on DNA nicking and closing enzymes⁹. It seems likely from our data that CPZ also inhibits some components of the cellular repair

system. Further, this impairment of repair makes CPZ more attractive as a possible adjuvant to radiotherapy.

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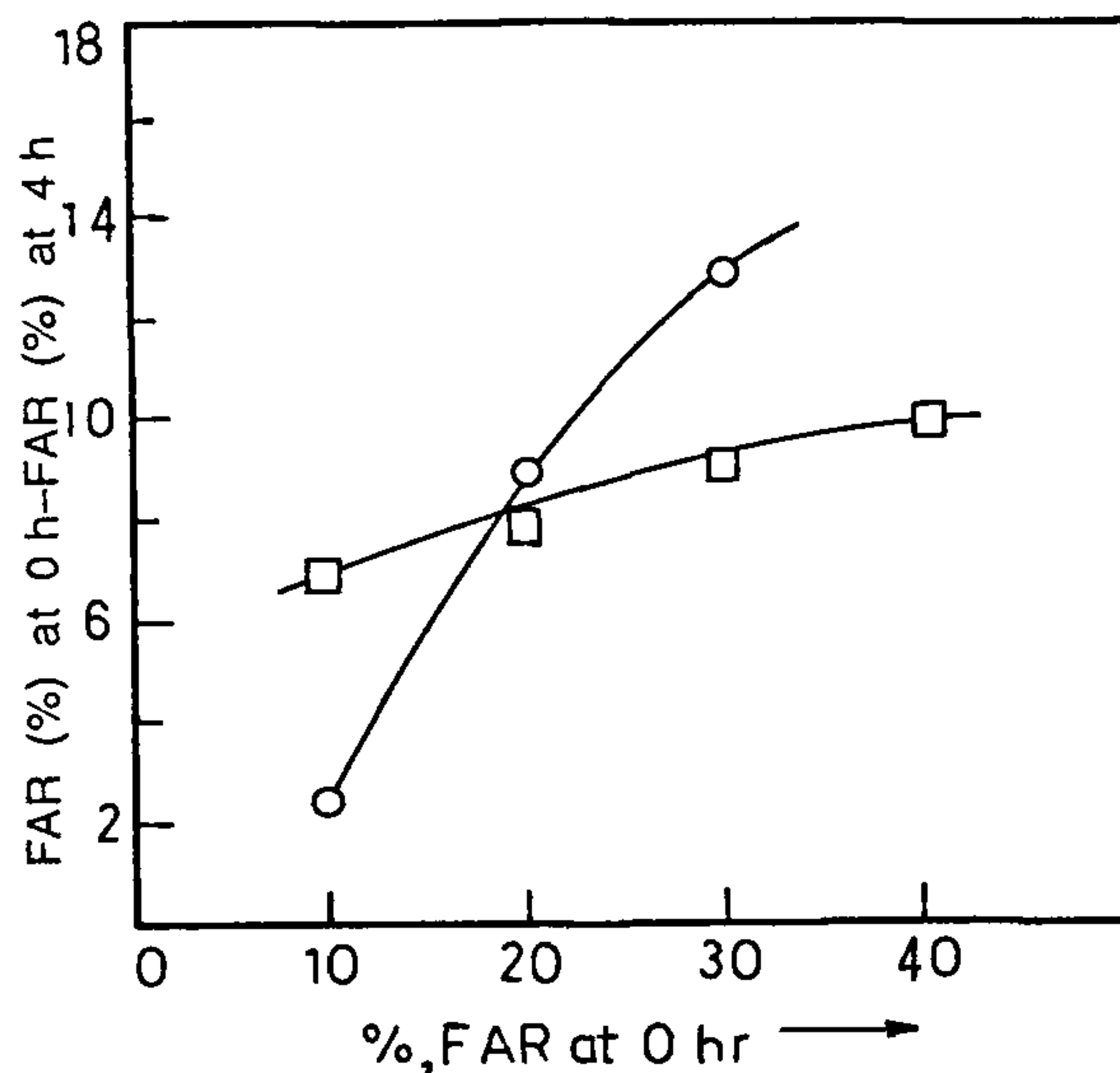


Figure 2. FAR (%) at 0 h - FAR (%) at 4 h against FAR (%) at 0 h. Data derived from Figure 1. ○ X-rays, □ X-rays + CPZ for 1 h.

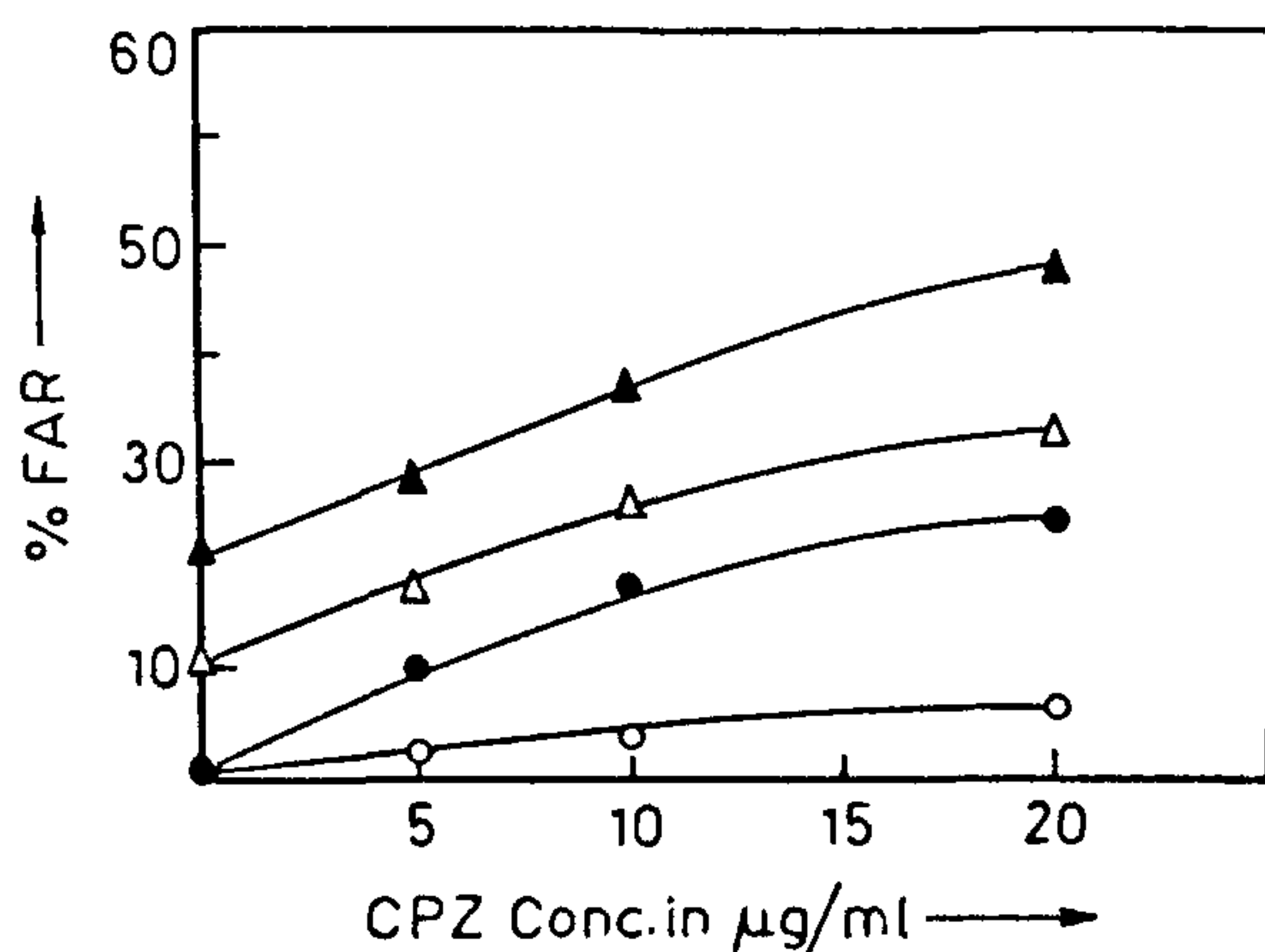


Figure 3. FAR (%) in cells incubated for 1 h with different doses of CPZ with or without X-irradiation (20 Gy). ● Blocks made immediately; ○ 4 h after incubation, ▲ cells X-irradiated during CPZ incubation and blocks made immediately; Δ 4 h after incubation.

Gibberellic-acid-induced changes in α -amylase activity of barley seeds and their reversal by chlorflurenol

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α -amylase activity was measured in barley seeds soaked in water, gibberellic acid (GA, 10^5 and 10^{-3} M) and chlorflurenol (CFL, 10^{-10} and 10^{-5} M) for 20 h. A high background activity was observed in controls. Seeds soaked in GA inhibited the enzyme activity, probably due to an endogenous superoptimal concentration. The higher concentration of CFL (10^{-5} M) reversed their inhibition. The action of CFL is attributed to interference in GA's action.

CHLORFLURENOL, a synthetic growth substance¹, inhibits seedling growth. In barley, this could be reversed by simultaneous application of GA, suggesting a role of GA in CFL-induced inhibition². However, the lack of effect of CFL on GA-induced α -amylase activity in embryoless half seeds of barley, a specific bioassay for gibberellin action, tempted Krelle and Libbert³ to question the mechanism of CFL action *vis-à-vis* GA mediation. Our findings (unpublished) also showed that CFL does not inhibit the induction of α -amylase activity by externally applied GA. Nevertheless, it was observed that