# THE RESPONSE OF HISTONE FRACTIONS TO X IRRADIATION IN CULTURED CHINESE HAMSTER CELLS*

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## SUMMARY

X irradiation of cultured Chinese hamster cells resulted in dissociation of small amounts of histones from chromatin. However, during the division delay period which followed irradiation, histones reassociated with the chromatin. Histone f3 accumulated in 50% excess over non-irradiated controls during this period. This excess f3 may contribute to death of x-irradiated cells. Part of the excess f3 deposited during the division delay period was synthesized 5 to 10 hours prior to its deposition in the chromatin and offers further evidence that there exists within the cell a non-chromatin pool of histones.

## INTRODUCTION

When Chinese hamster cells growing exponentially in culture are irradiated with biologically significant doses of x rays (200 to 1000 rads), cell division ceases for a period of time which is dependent on the x-ray dose (1). During this division delay period, the cells repair the damage caused by the ionizing radiation to the extent that cell division can again occur. Thus, the end of this repair period is marked by the resumption of exponential cell growth in the culture. This post-irradiation exponential growth will continue for one or more cell generations, but eventually a portion of the cells will die [the surviving fraction being dependent upon the dose (2)]. Therefore, radiation-induced lesions resulting in cell death may induce effects which require relatively long times for expression in the cell.

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Experiments have recently been carried out in this Laboratory to determine in what way the macromolecular structures of irradiated cells may have changed which might prevent their ultimate survival. This report describes some changes which occur in histone fractions of chromatin following x irradiation.

## METHODS

Chinese hamster cells (line CHO) were grown in suspension culture as previously described (3). To uniformly label their proteins, the cells were cultured for four generations in 6 liters of growth medium containing 50 μCi of reconstituted $^{14}C$-protein hydrolysate (Schwarz BioResearch, Inc.) per liter of growth medium (4). The cells were then resuspended in medium containing no $^{14}C$ and allowed to continue in exponential growth. Two hours after resuspension, the culture was divided into identical 1-liter cultures. Each culture (except the control) was irradiated with 900 rads of x rays as previously described (1). At various times after irradiation, a culture was sampled to determine its cell concentration using an electronic particle counter and its cellular mass of DNA and RNA using a modified Schmidt-Thannhauser procedure (3). The remaining $2 \times 10^8$ cells were then used to prepare histone fractions by the methods of Johns and of Phillips and Johns adapted to tissue culture as described by Gurley and Hardin (3). Aliquots of the histone preparations were taken to measure their radioactivity by liquid scintillation counting and their protein concentration by the method of Lowry *et al.* (3).

## RESULTS

After x irradiation of a culture growing with a generation time of 16.8 hours, exponential growth continued for 1 hour (Fig. 1). This occurs because those cells in the culture which are within 1 hour of cell division in their life cycle are insensitive to division delay by x irradiation (1,5). This continuing cell division accounts for the small decrease in the culture's

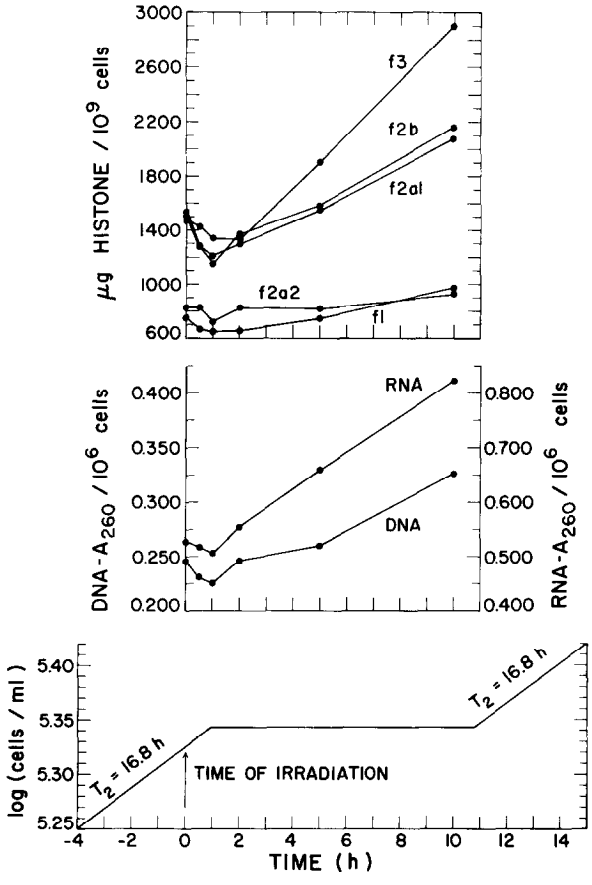


Fig. 1. Cellular content of DNA, RNA, and chromatin histone fractions following x irradiation of cultured Chinese hamster cells. DNA and RNA cellular masses are presented as a function of their absorbances at 260 mμ in 0.5 N $HClO_4$ and 0.2 N $HClO_4$, respectively, obtained by a modified Schmidt-Thannhauser procedure.

average DNA and RNA per cell observed in the first hour, as well as part of the decrease in histone per cell (Fig. 1).

From 1 to 10.8 hours after irradiation, cell division was delayed by the x-ray dose as predicted (1). Experiments to be reported elsewhere have shown that, during this division delay period, DNA synthesis continued at the same rate as during exponential growth and that RNA synthesis was only slightly retarded (6). Therefore, the average DNA and RNA per cell continued to increase during the division delay period (Fig. 1). The mass of histone per cell also increased during this time, the mass of f3 increasing to the great-

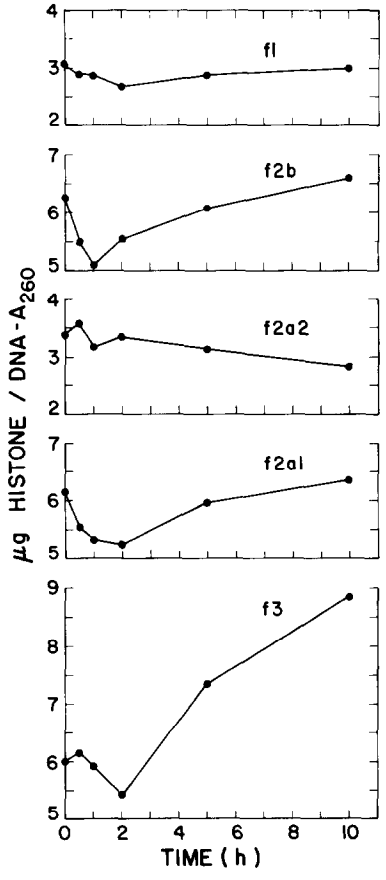


Fig. 2. Ratio of various histone fractions to DNA in chromatin following x irradiation of cultured Chinese hamster cells.

est extent (Fig. 1).

From ratios of histone-to-DNA (Fig. 2), it was found that small amounts of various histones had been lost from the chromatin during the first 2 hours after irradiation; however, by the end of the division delay period the ratio of histones f1-, f2b-, and f2a1-to-DNA had returned to the level of the unirradiated control. In contrast, the ratio of histone f3-to-DNA was 1.5 times greater at the end of this period than in the unirradiated culture. The ratio of f2a2 was slightly lower at the end of the division delay period. This small loss is most likely an artifact produced by the grossly enlarged f3 preparation which is known to be contaminated with small amounts of f2a2 (7). How-

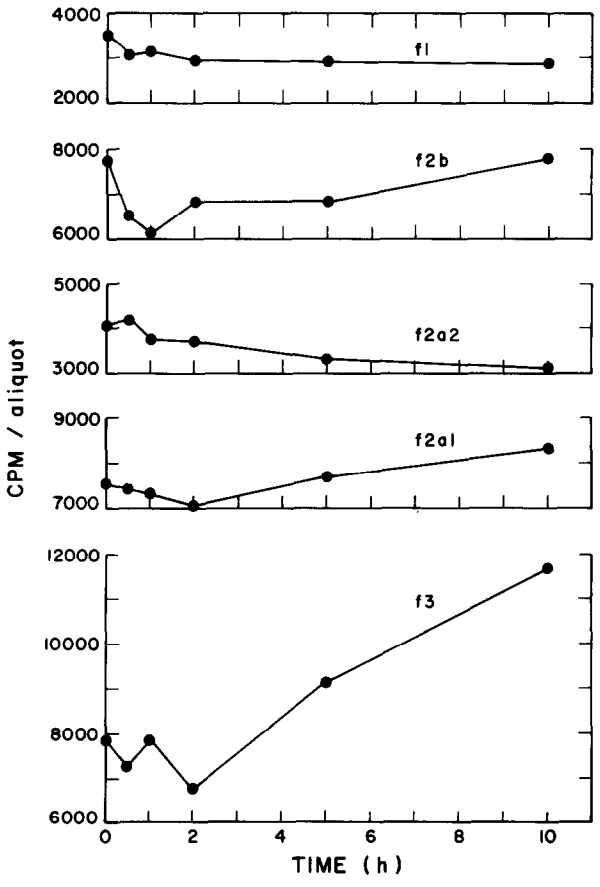


Fig. 3. Radioactivity of chromatin histone preparations following x irradiation of cultured Chinese hamster cells labeled with $^{14}$C-amino acids prior to irradiation.

ever, electrophoresis showed that the large increase in f3 was not due to this small f2a2 contamination.

The radioactivity of histone fractions is shown in Fig. 3. Of particular interest is the large increase in radioactivity of the f3 preparation after irradiation. If the excessive amount of f3 accumulating in chromatin after irradiation (Fig. 2) had been synthesized during the division delay period, this excess of f3 would not have been labeled and the radioactivity of the f3 preparation would have remained constant. The fact that the increase in amount of f3 was accompanied by an increase in radioactivity indicates that some of the excess f3 was synthesized during exponential growth of the cell 5 to

10 hours prior to deposition in the saline-insoluble chromatin.

Specific activities of histones (Fig. 4) decreased during the division delay period as a result of dilution of the prelabeled histones with non-labeled, newly synthesized histone (4). The lines in Fig. 4 represent the predicted decrease of specific activity if non-labeled, newly synthesized histones were deposited in the chromatin at just the rate to maintain a constant histone-to-DNA ratio while DNA replication was occurring. The specific activity of f3 decreased slower than the predicted rate as a result of pre-synthesized f3 entering the chromatin. Specific activities of f2b, f2a2, and

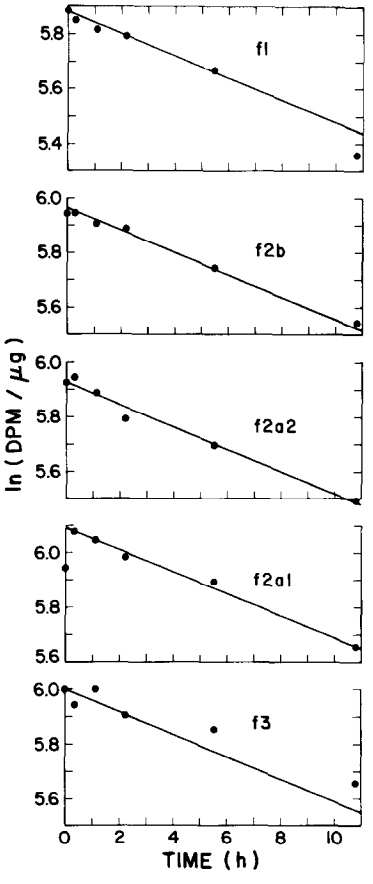


Fig. 4. Specific activities of various histone fractions following x irradiation of cultured Chinese hamster cells labeled with $^{14}$C-amino acids prior to irradiation. Data points show actual rate of decrease in specific activity. The line is a theoretically predicted decrease in specific activity defined in the text.

f2a1 decreased at the predicted rate, indicating that the chromatin neither picked up extra presynthesized histone nor lost histone due to turnover (4). Histone f1, which normally turns over in exponentially growing Chinese hamster cells (4), showed no sign of turnover in the first 5 hours after irradiation. However, between 5 and 10 hours the turnover of f1 began to occur again. Though this observation has been repeated, more data will be required to establish the timing of return of f1 turnover following irradiation and to determine whether there is a correlation between reduction of f1 turnover following irradiation and f1 phosphorylation (8).

## DISCUSSION

Dissociation of nucleoprotein from DNA early in the post-irradiation period has been previously described (9). Results in this report indicate that, in cultured Chinese hamster cells, dissociated histones reassociate with DNA during the division delay period. However, 50% more histone f3 was associated with the chromatin during this repair period than existed in the chromatin of unirradiated cells. The arginine-rich histones are known to suppress RNA synthesis (10), and recently some specific details of f3 suppression of RNA synthesis have been reported by Spelsberg *et al.* (11). Therefore, excess f3 in post-irradiated chromatin may contribute to the delayed effects of radiation damage by altering essential RNA synthesis.

Ord and Stocken (8) have shown that 1000 rads will cause oxidation of the f3-1 thiols *in vivo*. It is possible that excess f3 in post-irradiated cells may be involved in this oxidation process in some way.

Lehnert and Okada (12) observed an increase in ratio of whole histone-to-DNA in regenerating rat liver after irradiating with 800 rads. They attributed this increase to the fact that, in regenerating rat liver, DNA synthesis is reduced by irradiation while histone synthesis is not. Since this is not the case with exponentially growing Chinese hamster cultures (6) and since the liver histones were not fractionated, further comparisons of

the two systems are impossible at this time.

Previous experiments in this Laboratory have led us to conclude that cells have a non-chromatin pool of histones involved in processes such as histone turnover (4) and regulation of histone deposition in chromatin (13). This conclusion is in agreement with the observations made on various aspects of histone metabolism in other laboratories (14,15). The fact that part of the excess f3 in post-irradiated cells was synthesized 5 to 10 hours prior to deposition in the saline-insoluble chromatin is further evidence that there exists within the cell a non-chromatin pool of histones.

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