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DEPARTMENT OF RADIOLOGY AND RADIATION BIOLOGY

REPORT ON RESEARCH PARTICIPATION by Dr. Larrie Stone

MASTER

INTRODUCTION

Dr. Stone as he collaborated in experiments this summer learned techniques for synchronizing mammalian cells and obtained results supporting the hypothesis that mammalian cells are killed by chromosomal damage. These experiments which are discussed in detail below involved sensitization of synchronized Chinese Hamster cells with BUdR and the study of recovery of radiation damage in synchronized cells treated with cyclohexamide or potassium cyanide. The cysteamine experiments which were proposed were not conducted because subsequent to our proposal, reports on cysteamine effects on chromosomal damage in synchronized cells appeared in literature.

Sensitization of Synchronous CHO Cells with BUdR Pretreatment

The cells growing logarithmically were pretreated for 18 hours with 4 μ g/ml BUdR prior to selecting mitotic cells without Colcemid pretreatment. The CHO cells had a generation time of about 14 hours; therefore, essentially one strand of the DNA incorporated BUdR (about 10% replacement of T). Both the control TdR treated and BUdR treated cells were irradiated either at 1 hour after plating when they were in G₁ or at 11 hours after plating when they were in late S phase (as shown by pulse labeling replicate samples with tritiated thymidine). Cells were then analyzed as before for both chromosomal aberrations, and for lethality by the colony-plating technique. Results (Figs. 1 & 2) which were similar for chromosomal aberrations and lethality indicated that BUdR sensitized both the G₁ and late S phase cells by reducing the shoulders and slopes of the curves. The dose reduction factors for BUdR treated cells, based on chromosomal aberrations and lethality, respectively, were 1.7 - 2.6 and 2.0 - 2.6 for cells irradiated in G₁ and 2.1 - 2.3 and 2.6 - 4.3 for cells irradiated in late S phase.

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Recovery of Sublethal Damage and Restitution of Chromosomal Damage in CHO Cells Synchronized in Mitosis .

To study the recovery kinetics of cells irradiated with doses fractionated over short time intervals (5-120 minutes), Chinese Hamster Ovary cells (CHO) were synchronized by shaking loose mitotic cells without a Colcemid pretreatment. These cells were irradiated at 37°C with either a single dose of 600 rads or with two split doses of 300 rads each during the time interval between 8 and 11 hours after the cells had been plated and when over 90% of the cells were in DNA synthesis.

Results in Fig. 3 indicate that there was a significant amount of recovery of sublethal damage in 5-10 minutes with essentially complete recovery in 1-2 hours. Almost identical data were obtained for Don cells synchronized with Colcemid and irradiated at 37°C (data not shown). The frequency of chromosomal exchanges as a function of the fractionation time interval (Fig. 4) shows recovery kinetics very similar to that for the survival data, again indicating a significant amount of recovery in 5-10 minutes.

Effect of Cyclohexamide on Recovery of Sublethal Damage and Restitution of Chromosomal Damage

CHO cells were synchronized as before and irradiated with single or fractionated doses either with or without cyclohexamide (5µg/ml) added to the medium for about 2 1/2 hours during the irradiation period. As was expected from the data of Elkind et al (1967) and Kim et al (1966), the recovery of sublethal damage was not inhibited (data not shown), and the restitution of chromosomal damage also occurred during the 1 - 2 hour interval (Fig. 5). Thus, neither the recovery of sublethal damage nor the restitution of chromosomal damage were affected by a 90% inhibition of protein synthesis (Fig. 6). The restitution of chromosomal damage in mammalian cells during cyclohexamide treatment appears to differ from the studies of Wolff(see Elkind and Whitmore 1967_b) which implicate the necessity of protein synthesis for restitution of chromosomal damage in Vicia Faba.

1. M.M. Elkind, W.B. Moses, and H. Sutton-Gilbert, Radiation response of mammalian cells grown in culture. VI Protein, DNA, and RNA inhibition during the repair of x-ray damage. Radiation Res. 31, 156-173 (1967)
2. J.H. Kim, M.L. Eidinoff, and J.S. Laughlin, Recovery from sublethal x-ray damage of synchronized HeLa cells during inhibition of protein synthesis. Int. J. Rad. Biol. 11, 509-511 (1966).
3. M.M. Elkind and G.F. Whitmore, Chromosome damage and other cytological effects. In Radiobiology of Cultured Mammalian Cells, pp. 441-445, Gordon and Breach Science Publishers, New York, 1967_b.

Effect of Potassium Cyanide on Recovery of Sublethal Damage and Restitution of Chromosomal Damage .

CHO cells were synchronized as before and irradiated with single or fractionated doses either with or without KCN (0.001 M) added to the medium for about 2 1/2 hours during the radiation period. As shown in Fig. 7, neither the cloning efficiency nor the recovery of sublethal damage was inhibited by KCN treatment, although ³H leucine uptake was depressed by about 80%(data not shown). However, the progression of the cells through division was delayed considerably by the KCN treatment which resulted in a very low mitotic index, thus making an analysis of chromosomal damage impractical and unreliable.

Summary

A positive correlation was observed between X-ray induction of chromosomal aberrations and cell lethality in populations of synchronized cells (selected in mitosis) irradiated in different phases of the cell cycle. Cells irradiated in G₁ had less damage than those irradiated near the end of DNA synthesis. Furthermore, similar degrees of radiosensitization were observed for chromosomal aberrations and cell lethality in synchronized cells sensitized prior to synchrony by a pretreatment with BUdR. In addition, the recovery kinetics represented by both the frequency of chromosomal exchanges and cell survival following fractionated doses indicated a significant amount of recovery during a 10 minute period, and both types of recovery occurred during a 90% inhibition of protein synthesis with cyclohexamide.

Work to be Completed

In a BUdR experiment carried out this summer the cells were labeled with tritiated BUdR in such a way that one of the sister chromatids contained labeled parental DNA and the other sister chromatid contained newly synthesized unlabeled DNA. The analysis relating the location of the radiation induced aberrations with the location of these labeled and unlabeled regions remains to be completed. This analysis should show if BUdR has any sensitizing effect on the unlabeled sister chromatid structure.

Experiments are also being carried out on the recovery of sublethal and potentially lethal damage in cells treated at lower temperatures, and in these experiments the kinetics of recovery as measured by cell killing and chromosomal aberrations will be compared.

Dr. Stone, who is now at Dana College in Nebraska, will be assisting in these experiments during the year by carrying out the analysis of chromosomal damage. We hope that he will be able to actively participate in our experiments during the summer of 1970.

Figure 1 - Aberration frequency as a function of radiation dose is indicated for CHO cells synchronized in mitosis without Colcemid pretreatment but following an 18 hour pretreatment with either BUdR or TdR. The cells were irradiated at either 1 hour after plating when they were in G₁ or at 11 hours after plating when they were in late S phase.

Figure 2 - Survival is indicated for CHO cells synchronized in mitosis without Colcemid pretreatment but following an 18 hour pretreatment with either BUdR or TdR. As for Fig. 1 the cells were irradiated either in G₁ or late S phase.

Figure 3 - Fractional survival values are indicated for CHO cells irradiated with either single or split doses at different time intervals after plating cells synchronized in mitosis without Colcemid.

Figure 4 - Frequency of chromosomal exchanges is indicated for CHO cells irradiated with either single or split doses at different time intervals after plating cells synchronized in mitosis without Colcemid.

Figure 5 - Frequency of chromosomal exchanges is indicated for CHO cells irradiated with either single or split doses at different time intervals after plating cells synchronized in mitosis without Colcemid. Cyclohexamide (CHM of 5µg/ml) was added to the media between 8.5 and 11.5 hours as indicated.

Figure 6 - The suppression of protein synthesis in the cells described in Fig. 5 is indicated by the depression in uptake of tritiated leucine in cells treated with cyclohexamide.

Figure 7 - Survival per cent is indicated for CHO cells irradiated with either single or split doses at different time intervals after plating cells synchronized in mitosis. KCN (0.001M) was added to the media between 7.5 and 10.75 hours as indicated.

EXP. 74 B

Fig 1

Fig 2

EXP. 107

CHO CELLS

PE 76

Fig 3'

Fig 4

Fig 5

EXP. 120

Fig 6

Fig 7