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THE EFFECTS OF HYDROXYUREA ON CULTURED SOMATIC CELLS OF THE CHINESE HAMSTER, **CRICETULUS GRISEUS**

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INTRODUCTION

Hydroxyurea has been under investigation as a carcinostatic agent, and, as a result, considerable interest has developed in its mode of action. Young and Hodas (1964), using HeLa cells, reported that hydroxyurea inhibits incorporation of thymidine into DNA and suggested that hydroxyurea interferes with ribonucleotide (diphosphate) reduction. Sinclair (1965) reported that hydroxyurea has a lethal effect on cultured lung cells of the Chinese-hamster that are actively synthesizing DNA; cells at other mitotic stages, when exposed to the compound, survive and appear to progress until just before the beginning of the next period of DNA synthesis. Furthermore, Sinclair stated that hydroxyurea has no serious effects on dividing cells at concentrations of 10^{-3}M or less after six hours of exposure.

This paper is concerned with the effects of hydroxyurea on the mitotic rates of mammalian cells surviving treatment with the compound for periods of 24 to 48 hours.

MATERIALS AND METHODS

Cultures of Chinese-hamster lung cells (Cell Repository Designation CCL 16) were grown at 37°C in culture bottles or T-flasks containing Puck's Medium N-16 supplemented with 15% fetal calf serum. Cells used in the tests were trypsinized (0.25% trypsin in Hank's Balanced Salt Solution), transferred to Leighton tubes with cover slips and allowed to proliferate in fresh medium for three to five days. Fresh medium containing hydroxyurea at concentrations of 0.65×10^{-3}M, 1.3×10^{-3}M, 2.6×10^{-3}M, and 4.29×10^{-3}M was added and the tubes incubated for 24 or 48 hours. At the end of the exposure period the medium containing hydroxyurea was replaced with fresh medium and the cultures allowed to grow for 24 hours. The cells were then incubated for about 12 hours in culture medium containing a 10^{-7}M solution of colchicine, then fixed in ethanol and glacial acetic acid (3:1), air dried and stained in aceto-orcein (Paul, 1960). Controls were run simultaneously for each experiment. Each concentration-exposure period test combination was replicated twice; control tests were replicated four times for each time period.

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To determine the effects of hydroxyurea, the slides were analyzed microscopically with the aid of an ocular grid. All cells within the grid and the cells in some stage of cell division were scored. Ten randomly chosen fields were counted on each replicate. A mitotic index, calculated according to the method of Hanks and Fawcett (1955) was determined for prophase, metaphase, and the total mitotic figures. The mitotic index is expressed as the percent of cells in division out of the total number of cells scored. The percentages for the two tests at each concentration were averaged.

RESULTS AND CONCLUSIONS

The results are presented in Table 1. The percent of nuclei undergoing division is slightly higher than the sum of the cells in prophase and metaphase since the total figures include the small number of anaphase figures which were not tabulated separately.

After 24 hours of exposure the effects of hydroxyurea were variable and there was no significant reduction in the recovery of mitotic activity except at a concentration of 4.29×10^{-3}M (figure 1). Sinclair (1965) has shown that at a concentration of 10^{-3}M hydroxyurea the cells which survive the drug and are arrested in a stage just prior to DNA synthesis will divide more rapidly than untreated cells when placed in fresh medium. The percent of cells in prophase following treatment for 24 hours at a concentration of 2.6×10^{-3}M may be the result of this. There were fewer cells present in the two replicates as shown in Table 1 but a higher percentage were in prophase.

The percent of cells in prophase following 24 hours of treatment indicate that mitotic inhibition is not permanent except at the highest concentration where only 0.31 percent of the cells were in prophase at the end of the recovery period.

For cells exposed to hydroxyurea for 48 hours there is a definite reduction in the percent of cells recovering mitotic activity (figure 2). Recovery is inversely proportional to concentration with no activity observed at a concentration of 4.29×10^{-3}M. following the 24 hour recovery. At this high concentration many cells appeared dead. The chromatin in the nuclei was clumped and deeply stained. Nuclei with this appearance were not seen in any of the other cultures. While this was not studied quantitatively, it does indicate that hydroxyurea at this concentration and exposure time has an adverse effect on cells.

Young and Hodas (1964) have suggested that hydroxyurea is metabolized to hydroxylamine, a compound which interferes with DNA synthesis. Sinclair (1965) has shown that cells not synthesizing DNA are not lethally damaged by exposure to 5×10^{-3}M or 10^{-3}M hydroxyurea for more than five hours. The data presented in figures 1 and 2 indicate that prolonged treatment reduces the ability of cells to recover mitotic activity. The failure of cells to recover from high concentrations and prolonged treatment indicates that under hese condi-

tions hydroxyurea effects cells that are not synthesizing DNA. This concentration is between 2.6 and 4.29 x 10^{-3}M for exposures of from 24 to 48 hours.

LITERATURE CITED

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Table 1. Cell line Don treated with Hydroxyurea

Exposure Period		24 hours					48 hours				
Concentration in M/liter x 10^{-3}		0	.65	1.3	2.6	4.29	0	.65	1.3	2.6	4.29
Number of nuclei counted	Rep. 1	789	1034	855	343	1078	628	612	595	308	356
	Rep. 2	861	1180	906	335	542	798	807	481	333	521
	Rep. 3	958					508				
	Rep. 4	1324					643				
Ave. % of counted nuclei in prophase		3.31	2.03	3.23	5.01	0.31	5.39	4.22	3.25	2.18	0.0
Ave. % of counted nuclei in metaphase		1.04	0.41	0.51	0.29	0.06	1.07	0.91	0.56	0.16	0.0
Ave. % of counted nuclei in division		4.45	2.44	3.80	5.31	0.37	6.56	5.14	3.81	2.49	0.0

Figure 1. The percent of prophase, metaphase and total division products observed after 24 hour exposure to varying concentrations of hydroxyurea.

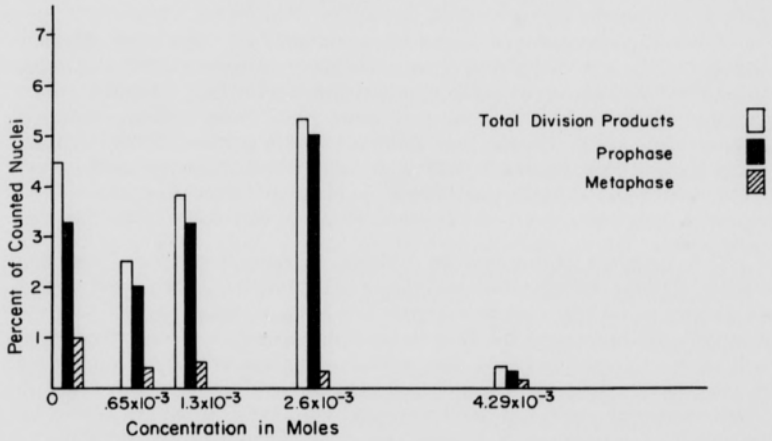

Figure 2. The percent of prophase, metaphase and total division products observed after 48 hour exposure to varying concentrations of hydroxyurea.