# EFFECT OF HYDROXYUREA ON REGENERATING RAT LIVER *

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Received April 5, 1965

Hydroxyurea has been shown to be a potent inhibitor of DNA synthesis in HeLa cells (17), ascites tumor cells (15), and bacteria (14). The suggestion has been made that its mode of action is at the level of conversion of ribonucleotides to deoxyribonucleotides since inhibition is seen in formation of dCMP when CMP is added to bone marrow preparations from rats previously treated with hydroxyurea (5). No effects have been observed on synthesis of RNA or protein (17,14).

Although there is evidence indicating that DNA and nuclear histone are synthesized during the same period of the cell cycle (1,2,6,9,13), the question of the possible interdependence of these two processes has not been resolved. Doses of radiation sufficient to inhibit DNA synthesis do not produce immediate inhibition of histone synthesis (12,16).

In order to investigate the interdependence of the synthesis of DNA and histone, we studied the effect of hydroxyurea on the incorporation of isotope into DNA, RNA, and several protein fractions of regenerating rat liver.

MATERIALS AND METHODS: Male Holtzman rats, weighing 300 to 350 grams

* This research was supported in part by Public Health Service Research Grant No. CA-01747-13.

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were used. Partial hepatectomies were performed by the method of Higgins and Anderson (7) as modified by Brues, Drury, and Brues (3). All partial hepatectomies were performed at 8 AM. Twenty-four hours later, the experimental animals received 100 mg of hydroxyurea in 1 ml of physiological saline by intraperitoneal injection; control animals received 1 ml of physiological saline. Thirty minutes later, the isotope was administered in a similar manner: 500 μc of carrier free $^{32}P$ as sodium phosphate (Iso-Serve Inc., Cambridge, Mass.) or 25 μc of glycine-1-$^{14}C$ (6.75 mc/mmole, Tracerlab, Waltham, Mass.).

One hour after injection of isotope the animals were sacrificed and their livers homogenized in ice cold 0.25 M sucrose.

DNA, RNA, and acid-soluble nucleotides were isolated and their specific activities determined as previously described (10).

A portion of the initial homogenate was centrifuged at 105,000 x g for sixty minutes. Protein was precipitated from the supernatant fluid with trichloroacetic acid. After extraction with hot trichloroacetic acid, ethanol, hot ethanol-ether, and ether, the protein was dissolved in dilute NaOH. This protein is hereafter referred to as cytoplasmic protein.

Nuclei were isolated by a modification of the method of Chauveau, Moulé, and Rouiller (4). The isolated nuclei were washed in 0.25 M sucrose and the acid-soluble nuclear proteins were extracted with cold 0.25 N $H_2SO_4$. Histone was purified by the method of Laurence, Simson, and Butler (8). The final product of this purification was dissolved in 0.05 N HCl and is hereafter referred to as histone although it is recognized that small amounts of other basic proteins are present.

The nuclear sediment from which the acid-soluble nuclear protein had been extracted was treated with buffered 10% NaCl at 100° to

solubilize the nucleic acids (10) and was then extracted with hot 5% trichloroacetic acid, ethanol, hot ethanol-ether, and ether. That portion of the residue which dissolved in dilute NaOH is referred to as acid-insoluble nuclear protein.

Specific activities of the protein samples were determined as previously described (9).

RESULTS: Table I shows the specific activity of RNA and DNA relative to that of the acid-soluble nucleotides. Incorporation of isotope into DNA is markedly reduced with respect to the control; incorporation of $^{32}P$ into RNA is not measurably affected.

TABLE I Effect of Hydroxyurea on Isotope Incorporation into DNA and RNA.

(Specific activity, c/m/μg P, shown as per cent of specific activity of acid-soluble nucleotide phosphorus)

| Control Animals | | Hydroxyurea Treated Animals | |
|---|---|---|---|
| DNAP* | RNAP* | DNAP* | RNAP* |
| 1.38 | 2.23 | 0.26 | 2.88 |
| 1.47 | 3.36 | 0.41 | 2.60 |
| 1.75 | 2.64 | 0.48 | 2.71 |
| 1.81 | 2.61 | 0.51 | 2.91 |
| 2.08 | 2.54 | 0.55 | 2.35 |
| 2.34 | 2.10 | 0.73 | 2.36 |
| 2.58 | 2.75 | 0.75 | 2.41 |
| 3.16 | 2.64 | | |
| 2.07 ± 0.21 (Mean ± S.E.) | 2.61 ± 0.13 | 0.53 ± 0.06 | 2.61 ± 0.04 |

*DNAP and RNAP denote DNA phosphorus and RNA phosphorus

Table II shows the specific activity of the three protein fractions. There is no significant difference between the hydroxyurea treated animals and the control group with respect to incorporation of glycine into the cytoplasmic protein and acid-insoluble nuclear protein. However, in the case of histone, the hydroxyurea treated animals show a mean value which is only 61% of that of the control

group. The difference of these means is significant at a level of $P = 0.02$.

TABLE II Effect of Hydroxyurea on Isotope Incorporation into Several Protein Fractions

(Specific activity expressed as c/m/mg protein)

| Control Animals | | |
|---|---|---|
| Histone | Cytoplasmic protein | Acid-insoluble nuclear protein |
| 2940 | 3450 | 6180 |
| 3220 | 3080 | 5310 |
| 3220 | 3620 | 5620 |
| 4160 | 4680 | 8000 |
| 4380 | 3900 | 5610 |
| 4860 | 4210 | 5740 |
| 3800 ± 310 (Mean ± S.E.) | 3820 ± 240 | 6080 ± 390 |
| **Hydroxyurea Treated Animals** | | |
| 1290 | 3170 | 5070 |
| 1640 | 3360 | 6090 |
| 1890 | 3340 | 5430 |
| 2080 | 3090 | 5040 |
| 3100 | 4210 | 7110 |
| 3880 | 3880 | 8000 |
| 2310 ± 410 | 3510 ± 170 | 6120 ± 500 |

DISCUSSION: The finding that hydroxyurea inhibits the incorporation of $^{32}P$ into DNA with no effect on RNA is in accord with the results of Young and Hodas (17) with HeLa cells and Yarbro, Kennedy and Barnum (15) with ascites tumor cells. The magnitude of inhibition of DNA synthesis in two fractions of isolated nuclei prepared as described by Niehaus and Barnum (10) was found to be comparable to that for total DNA (11) and the incorporation of the isotope into the RNA of these fractions did not appear to be affected by hydroxyurea (11).

The finding that hydroxyurea does not inhibit the incorporation of isotope into cytoplasmic protein or into nuclear acid-insoluble

protein is in accord with the work of Young and Hodas (17) who found no inhibition of protein synthesis in HeLa cell cultures, and with the work of Rosenkranz and Levy (14) who found no inhibition of protein synthesis in bacteria in the presence of concentrations of hydroxyurea which markedly inhibited DNA synthesis.

The specific inhibition of isotope incorporation into nuclear histone, concomitant with an inhibition of DNA synthesis, has not previously been reported.

Various workers have reported that histone and RNA are synthesized during the same period of the cell cycle (1,2,6,9,13) and it has been suggested that nucleoprotein synthesis is a unitary process (6) involving the simultaneous duplication of DNA and histone molecules.

Our findings are consistent with such an hypothesis, suggesting that synthesis of at least a portion of nuclear histone in regenerating rat liver is dependent on DNA synthesis.

SUMMARY: Hydroxyurea has been shown to be a potent inhibitor of DNA synthesis in regenerating rat liver with no measurable effect on RNA synthesis. No detectable difference was noted in incorporation of $^{14}$C-glycine into cytoplasmic protein or into acid-insoluble nuclear protein, but the incorporation into nuclear histone was only 61% of the control value, suggesting that hydroxyurea may inhibit the synthesis of histone in addition to, or as a consequence of, its effect on DNA.

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