Hydroxyurea-induced Inhibition of Deoxyribonucleotide Synthesis: Studies in Intact Cells

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SUMMARY

Hydroxyurea-induced inhibition of thymidine incorporation by monolayers of HeLa cells was partially prevented and reversed by addition of deoxyadenosine, deoxyguanosine, and deoxycytidine to the culture medium. All 3 deoxyribonucleosides were required for optimal effect. Hydroxyurea inhibited incorporation of thymidine into DNA of embryos of the sand-dollar species Echinarachnius parma. The onset of inhibition was delayed for 3-4 synthesis (S) periods when the drug was added at fertilization, but was evident with the next replication cycle following drug addition between the 5th and 6th S period. Hydroxyurea did not alter rates of leucine incorporation into protein regardless of the time of drug exposure. Echinoderm ova possess significant quantities of preformed deoxyribonucleotides; previous studies with 5-fluorodeoxyuridine have suggested that these stores can supply the embryo with the thymidylate required for DNA replication over 6 S periods following fertilization.

These observations, i.e., partial reversal of drug-induced inhibition by exogenous deoxyribonucleosides in HeLa cells, and the insensitivity of sand-dollar embryos to hydroxyurea during initial S periods, are compatible with the postulate that this compound inhibits reduction of ribonucleotides to deoxyribonucleotides.

INTRODUCTION

Hydroxyurea interferes with synthesis of DNA in intact mammals (23, 28), mammalian cells in vitro (1, 4, 27, 29), bacteria (5, 20, 21), and echinoderm embryos (18). A considerable body of experimental evidence suggests that this drug inhibits DNA synthesis by decreasing the conversion of ribonucleotides to deoxyribonucleotides. Frenkel et al. (3) found formation of dCMP from CMP to be impaired in cell-free extracts of bone marrow from mice and patients treated with hydroxyurea.

In previously reported studies from our laboratory, the drug inhibited incorporation of CMP and GMP into DNA in cell-free extracts from HeLa monolayers, but did not alter rates of phosphorylation of TdR and deoxythymidylate nor impair the DNA polymerase reaction (29). In cultures of rabbit-kidney cells, Adams et al. (1) observed that hydroxyurea inhibited conversion of labeled cytidine into acid-soluble deoxynucleotides to a greater extent than could be achieved simply by inhibition of DNA synthesis. In a pyrimidine-deficient hamster cell line, Mohler (17) obtained partial protection against cytocidal and growth-inhibiting effects of hydroxyurea by addition of pyrimidine deoxyribonucleoside to the culture medium. However, the pyrimidine deoxyribonucleoside did not protect the cells from high concentrations of hydroxyurea nor alter the toxicity of that drug to a HeLa-cell line at any concentration. Purine deoxyribonucleosides had no apparent activity in his test system.

This communication supplies further evidence that hydroxyurea produces deoxyribonucleotide deprivation in intact cells. Two approaches were used: (a) further attempts were made to prevent or reverse the inhibitory effects of hydroxyurea upon HeLa cells by means of exogenous deoxyribonucleosides; (b) the effect of the drug on DNA synthesis was studied in an echinoderm embryo which is believed to contain pools of deoxyribonucleotides that serve the needs of the embryo until it reaches the early blastula stage. These organisms incorporate labeled TdR but not cytidine or uridine into DNA within 20-30 min following fertilization (7, 8, 24) even though they contain a preformed store of deoxyribonucleotides (25). Experiments with 5-fluoro-2'-deoxyuridine, which blocks TdR synthesis by inhibiting thymidylate synthetase, suggest that these deoxyribonucleotide stores can sustain the embryo through a number of replication and division cycles, before synthesis of TdR, de novo, becomes imperative (10, 19). By establishing the time course of hydroxyurea-induced inhibition of DNA formation in these embryos, we hoped to separate the effect of that drug upon deoxyribonucleotide formation from its other possible cytotoxic actions.

MATERIALS AND METHODS

Materials

HeLa cells were obtained from Microbiological Associates and maintained in monolayer culture by weekly subdivision. Eagle's minimal essential medium, supplemented with calf serum (to 10%), penicillin and streptomycin was used for maintenance of the cells. Sexually-mature sand dollars, Echinarchnus parma, were obtained during the months of July and August from the...
coastal waters about Mount Desert Island, Maine. They were kept in aquaria with running seawater until used.

**Chemicals**

3H- or 14C-labeled TdR, uridine, leucine, cytidine, CdR, and GdR were obtained from New England Nuclear Corp., Nuclear-Chicago, or Schwarz BioResearch, Inc. Nonradioactive ribonucleosides and deoxyribonucleosides were obtained from Calbiochem. Hydroxyurea was generously supplied by the Squibb Institute for Medical Research, New Brunswick, New Jersey.

**Isotope Incorporation Studies**

**HeLa Cells.** Drug effects upon uptake of tritium-labeled TdR, uridine, and leucine into nucleic acids and protein of HeLa cells were measured by a sequential isotope technic described in detail elsewhere (30). Monolayers of the HeLa cells growing on glass cover slips were first incubated in common in media containing a 14C precursor (TdR-2-14C, 0.025 µc/ml, 0.83 µM; uridine-2-14C, 0.016 µc/ml, 0.53 µM; or L-leucine-1-14C, 0.4 µc/ml, 16 µM) for 15 or 30 min at 37°C. After a rinse with warm medium, monolayers were placed in nonradioactive medium for 10-30 min. The monolayers were then removed in groups to medium containing the same chemical precursor previously used, now labeled with tritium (TdR-[methyl-3H], 0.42 µc/ml, 0.07 µM or 0.2 µM; uridine-5-3H, 0.8 µc/ml, 0.1 µM; or L-leucine-4,5-3H, 0.8 µc/ml, 0.2 µM). Drugs were present in the tritium-containing medium at zero time unless otherwise noted in the text; drug and reversing deoxyribonucleosides were dissolved in medium. After incubation at 37°C for 30-240 min, the monolayers were removed, rinsed with chilled saline, fixed with cold 5% TCA, extracted with ethanol (15 min) and ether (10 min) at room temperature, and air dried. In studies with leucine, nucleic acids were removed by exposing cells on the cover slips to 5% TCA, at 95°C, for 15 min prior to lipid extraction. The cover slips were fragmented and placed in (glass) liquid scintillation counting jars; the cells were then solubilized with hydroxide of Hyamine (Packard Instrument Co.), 1 M in methanol, and heat (65°C for 1 hr or 2 hr then 37°C overnight). Scintillation solution was added and tritium and 14C content in each sample was determined by the method of Kabara et al. (9) in a dual-channel liquid scintillation counter (Packard Instrument Corp., La Grange, III). The 3H/14C ratio served to quantitate precursor incorporation. Under these experimental conditions the labeled TdR, uridine, and leucine are incorporated only into DNA, RNA, and protein, respectively. The 14C content of a monolayer is related to the metabolic activity of its cells prior to exposure to drugs. Similarly, 3H content is a function of metabolic activity in the presence of drugs. Monolayers of cells exposed to identical concentrations of labeled precursors for an identical period of time will contain 3H and 14C in a relatively constant ratio unless the pattern of cellular metabolism alters significantly during the period of study. Significant departure from this constant ratio in association with exposure to a drug is interpreted as an effect of the agent. Since

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2 Eagle's medium lacking leucine, supplemented with calf serum to 1%, was used in experiments with labeled leucine.

4 Since the medium utilized in these studies was supplemented with nondialyzed calf serum, the presence of minute quantities of exogenous CdR in all experiments cannot be excluded.

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**RESULTS**

**Protective Effects of Mixtures of Deoxyribonucleosides.** A combination of 0.1 mM AdR, 0.1 mM GdR, and 1.0 µM CdR gave partial but significant protection against the inhibitory effects of hydroxyurea upon uptake of TdR into DNA of HeLa cells (Chart 1). To obtain a significant protective effect, addition of both AdR and GdR was required (Table 1); in the presence of AdR and GdR the addition of CdR afforded a slight, but consistent, increase in protection. Guanosine and adenosine could
CHART 1. Inhibition of DNA synthesis in HeLa cells by hydroxyurea. Protective effect of deoxyribonucleosides. Incorporation periods were 30 min; drug, thymidine-\(^{3}H\), and mixed deoxyribonucleosides were present at 0 time. Each point represents the mean value of at least 2 experiments upon triplicate samples. % control incorporation = 
\[
\frac{\text{\( ^{3}H/^{14}C \) experimental}}{\text{\( ^{3}H/^{14}C \) control}} \times 100
\]

Coefficient of variation within control groups, \( \leq 10\% \). AdR, deoxyadenosine; GdR, deoxyguanosine; CdR, deoxycytidine.

### TABLE 1

<table>
<thead>
<tr>
<th>Hydroxyurea (1.3 mM)</th>
<th>Nucleosides</th>
<th>% control uptake ( \times 100 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>AdR</td>
<td>GdR</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

\( ^{3}H/^{14}C \) experimental, AdR, GdR, CdR, AR, GR.

* 60-min incubations. Drug, nucleosides, and isotope present at onset of incubation.

Nucleoside concentrations: deoxyadenosine (AdR), deoxyguanosine (GdR), adenosine (AR), and guanosine (GR) 0.1 mM each; deoxycytidine (CdR), 1.0 \( \mu M \).

Each value represents the average of at least 2 experiments upon triplicate samples.

\[
\text{% control uptake} = \frac{\text{\( ^{3}H/^{14}C \) experimental}}{\text{\( ^{3}H/^{14}C \) control}} \times 100
\]

not substitute for GdR and AdR (Table 1). The concentrations of AdR and GdR used were quite critical; with 0.01 mM AdR and GdR no protective effect was seen, while 1 mM AdR itself caused inhibition of uridine, leucine, and TdR uptake (Table 2). The inhibition of TdR uptake induced by 1 mM AdR was not prevented by GdR and CdR. Addition of the mixed deoxyribonucleosides produced partial reversal when these compounds were added some time after the hydroxyurea effect had become manifest (Chart 2). A requirement of both GdR and AdR for significant reversal was again evident. A variety of concentrations of AdR, GdR, and CdR were tested in an unsuccessful effort to achieve complete reversal of the inhibitory effect of hydroxyurea upon DNA synthesis. The complexity of the reversal experiment is underlined by the fact that the exogenous deoxyribonucleosines are rapidly degraded, and the purine moiety enters the ribonucleotide pool. When HeLa monolayers were exposed to tritiated AdR or GdR, 80 to 90\% of acid-insoluble radioactivity was found in RNA (Table 3).

**Characteristics of the Embryos of E. parma.** In Chart 3 are illustrated the early developmental stages of the *E. parma* embryo. The times of appearance of cytotoxic effect induced by a number of chemical agents are indicated. Initial synthesis of DNA begins approximately 20 min following fertilization and is completed by 55 min (24). The 2nd synthesis or S period is initiated at 80 min, prior to the 1st cleavage, which occurs at 90 min. Thereafter, the cells divide about once every hr for approximately 10–12 hr; presumably cellular DNA is replicated at approximately 60-min intervals also. Cycloheximide inhibits synthesis of protein in these embryos (C. W. Young, unpublished observation); TdR incorporation into DNA and cleavage is also prevented (12). Dinitrophenol, by uncoupling oxidative phosphorylation, has a similar effect (12). Actinomycin D and 8-aza-
Effects of Hydroxyurea upon Development and Synthesis of DNA and Protein by Sand-dollar Embryos. In the presence of hydroxyurea, embryonic development is morphologically normal through the initial 3 cleavages. The 4th division, at 4.5 hr is completed successfully in some but not all experiments; after the 5th cleavage, the treated embryos are uniformly abnormal. Fig. 1 contains photos of normal and hydroxyurea-treated embryos shortly after the 4th cell cleavage. In the hydroxyurea-treated embryo this cleavage has not occurred although the blastula cavity is developing. If the morphologic evidence of defective cell division is related to a failure in the replication of DNA, incorporation of TdR into DNA should not be altered by hydroxyurea during the initial 3 S periods, but should be inhibited during the 4th or the 5th period of the DNA synthesis. Radioautographic observations (18) had suggested that incorporation of TdR was partially inhibited by hydroxyurea but the extent and time of onset of inhibition was unclear. Because of the small quantities of DNA formed in the first few S periods, samples were taken at intervals which were calculated to include S1 plus S2 plus S3 plus S6 plus S7, and S8. Incorporation of TdR followed the expected logarithmic curve between 2 hr and 6 hr in control samples (Chart 4); however, between 6 and 8 hr only a single S period occurred. In the initial 2 hr, radioactivity per unit material was less than that later achieved; this is in agreement with the observations of Hinegardner on TdR uptake into sea urchin embryos (7) and is consistent with a progressive decrease in the preformed pool of nonradioactive precursors of DNA thymine. As expected, the presence of (1.32 mM) hydroxyurea inhibited incorporation of TdR into DNA by 84% over the 8-hr incubation period; however, uptake of TdR in the initial 2 hr was not altered. Inhibitory effects became manifest between 4th and 5th S periods in some experiments and between 3rd and 4th S periods in others (Chart 4). This delay in onset of cytotoxicity following exposure of hydroxyurea, i.e., 4.5-5.5 hr by morphologic criteria, 3-4 hr with respect to DNA synthesis, is related to changing metabolic characteristics of the embryo. When embryos are first exposed to hydroxyurea 5 hr following fertilization, morphologic abnormalities can be detected within 2.5 hr. Since TdR uptake is inhibited by 75% in this period (Chart 5), the drug probably disturbs the 1st period of DNA synthesis following its addition.

In mammalian and bacterial cells hydroxyurea inhibits synthesis of DNA but does not alter rates of formation of RNA or protein (29). Similar inhibitory specificity probably obtains in the sand-dollar embryo. Hydroxyurea (1.32 mM) did not significantly inhibit incorporation of leucine into protein whether present between 0 and 5 hr following fertilization or added after 5 hr (Table 4).

Analysis of preliminary studies of cytidine incorporation into RNA and DNA discloses that cytidine-3H is rapidly incorporated

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**TABLE 2**

Effects of Purine Deoxyribonucleosides upon Incorporation of Thymidine-CH₃-³H, Uridine-5-³H, and Leucine-4-⁵H into HeLa cells

<table>
<thead>
<tr>
<th>Deoxyguanosine</th>
<th>Concentration (mM)</th>
<th>Thymidine</th>
<th>Uridine</th>
<th>Leucine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>83</td>
<td>99</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>50</td>
<td>56</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>26</td>
<td>12</td>
<td>44</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Deoxyadenosine</th>
<th>Concentration (mM)</th>
<th>Thymidine</th>
<th>Uridine</th>
<th>Leucine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>35</td>
<td>115</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>23</td>
<td>77</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>10</td>
<td>22</td>
<td>67</td>
<td></td>
</tr>
</tbody>
</table>

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* 30-min incubations. Nucleosides and labeled precursor present at onset of incubation.

* Each value represents the average of 2 experiments upon triplicate samples.

\[
\text{\% control incorporation} = \frac{\text{³H/¹⁴C experimental}}{\text{³H/¹⁴C control}} \times 100
\]
TABLE 3
Incorporation Specificity of TdR, GdR, and AdR in HeLa Monolayers

<table>
<thead>
<tr>
<th>Deoxyribonucleoside(s)</th>
<th>Total acid-insoluble radioactivity (% ± S.D.)</th>
<th>(^\text{H})</th>
<th>(^\text{H}^\text{C})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RNA</td>
<td>DNA</td>
<td>RNA</td>
</tr>
<tr>
<td>GdR-^3H</td>
<td>94.6 ± 1.0</td>
<td>5.4 ± 1.0</td>
<td>nd⁴</td>
</tr>
<tr>
<td>GdR-^3H and TdR-^14C</td>
<td>98 ± 1.5</td>
<td>7 ± 1.5</td>
<td>91 ± 0.3</td>
</tr>
<tr>
<td>AdR-^3H and TdR-^14C</td>
<td>88.7 ± 0.4</td>
<td>11.3 ± 0.4</td>
<td>nd⁴</td>
</tr>
</tbody>
</table>

* TdR, thymidine-2-^14C; GdR, deoxyguanosine-^3H; AdR, deoxyadenosine-^3H.

** RNA and DNA were extracted by a procedure modified from that of Schmidt, Thannhauser, and Schneider (26).

γ Purity of GdR-^3H (Schwartz BioResearch) and AdR-^3H (New England Nuclear Corp.) was checked by paper chromatography. Less than 1% of "GdR-^3H" radioactivity migrated with guanine or guanosine; 1% of "AdR-^3H" radioactivity migrated as adenine and 2% as adenosine.

δ Incorporation period was 2 hr.

† Radioactivity not detectable.

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**DISCUSSION**

If hydroxyurea inhibits synthesis of DNA by preventing reduction of ribonucleotides to deoxyribonucleotides, the drug should not alter rates of precursor incorporation into DNA when adequate concentrations of deoxyribonucleoside triphosphates are present. The failure of hydroxyurea to inhibit incorporation of deoxycytidylylate-^14C into DNA in a cell-free system containing deoxyadenylate, deoxyguanylate, dCMP, denatured DNA primer, and a 105,000 x g supernatant extract of HeLa cells is consistent with this expectation (29). The protection and reversal studies in intact HeLa cells described herein offer support for the postulated inhibitory mechanism. The fact that optimal effects were obtained in the presence of all 4 deoxyribonucleosides and that ribonucleosides were ineffective, indicates that the reversal phenomenon requires a high degree of molecular specificity and is not occasioned by a nonspecific reaction between...
Inhibition of Deoxyribonucleotide Synthesis by Hydroxyurea

Hydroxyurea and exogenous nucleosides. The latter possibility is made untenable by the observation that the mixed deoxyribonucleosides protected against 1.3 and 13 mM hydroxyurea with equal effectiveness. It is disconcerting, however, that incorporation of TdR did not reach normal levels in the presence of hydroxyurea and the four (exogenously supplied) deoxyribonucleosides. The partial nature of the reversal suggests that adequate concentrations of deoxyribonucleoside triphosphates cannot be achieved by addition of exogenous deoxyribonucleosides, or else that inhibition of multiple reactions is involved in the acute effect of hydroxyurea upon DNA synthesis.

The reversal experiment seems straightforward but actually is quite complex. Analysis of proliferating and nonproliferating tissues performed in many laboratories have detected small but measurable quantities of pyrimidine deoxyribonucleosides and deoxyribonucleotides but, with the exception of 2 reports (16, 22), purine deoxyribonucleosides or deoxyribonucleotides have been unmeasurable (2, 15). Klenow (13) and co-workers have demonstrated the presence of dATP only when Ehrlich ascites tumor cells were incubated with exogenous AdR or AdR plus GdR. These cells rapidly degraded AdR and dATP to hypoxanthine, deoxyinosine, and inosine; thus cellular dATP became undetectable when exogenous AdR was removed or AR was added (13). The HeLa cell line utilized in the present studies rapidly incorporates radioactivity from GdR-3H and AdR-3H into nucleic acids. However, 90% of the radioactivity in each instance was found in RNA with only 10% present in DNA (Table 3). Clearly, in mammalian cells at least, the purine deoxyribonucleoside pools are small because of rapid degradation. In the event of drug-induced arrest of all ribonucleotide reduction, the purine deoxyribonucleotide pools would be exhausted before those of the pyrimidines. This is consistent with our finding that the addition of AdR and GdR promoted incorporation of TdR-3H into DNA to a considerable degree in the apparent absence of exogenous CDR. On the other hand, Mohler (17)

TABLE 4

Incorporation of Leucine-14C into Protein of Embryos of Echinarchniius parma

<table>
<thead>
<tr>
<th>Incorporation period</th>
<th>Drug*</th>
<th>cpm/1000 embryos X 10^-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilization to 5 hr</td>
<td>None</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td>Hydroxyurea, 1.3 mM</td>
<td>18.9</td>
</tr>
<tr>
<td>5 to 8 hr postfertilization</td>
<td>None</td>
<td>23.3</td>
</tr>
<tr>
<td></td>
<td>Hydroxyurea, 1.3 mM</td>
<td>21.0</td>
</tr>
</tbody>
</table>

* Hydroxyurea and leucine-14C added simultaneously.
observed that exogenous TdR, deoxyuridine, and CdR partly reversed the growth-inhibiting effects of hydroxyurea in studies on V79H, a hamster cell line which had a partial nutritional requirement for exogenous cytidine, uridine, and TdR. In HeLa-S3, which does not require exogenous pyrimidine nucleosides (and was approximately 4 as sensitive to hydroxyurea as V79H), TdR did not reverse drug-induced inhibition of growth. It seems reasonable that in the pyrimidine-deficient V79H the pyrimidine deoxynucleotide pool derived from the de novo pathway was small enough to be a limiting factor on the rate of DNA synthesis. Thus the cellular content of purine and pyrimidine deoxyribonucleotides at the time hydroxyurea is added may determine whether TdR will reverse the drug-induced growth inhibition or be incorporated only to a negligible degree.

Further, the result obtained in the reversal experiment will be dependent, in part, upon the relative rates of phosphorylation and of degradation of the added deoxyribonucleosides. Cellular cleavage of exogenous purine deoxyribonucleosides produces free purines which are then utilized by the salvage pathway for purines. This may enlarge the ribonucleotide pool, and possibly impair phosphorylation of deoxynucleotides since the respective purine ribonucleoside monophosphates and deoxyribonucleoside monophosphates may employ the same kinases for phosphorylation to the diphosphate level (15). As the concentration of deoxyribonucleosides in the medium is increased they themselves, or their metabolites, inhibit the synthesis of nucleic acids and of protein. Thus when very large concentrations of deoxyribonucleosides are used in an attempt to reverse the effects of hydroxyurea, the net result may be to substitute one inhibitor for another.

The complexities of the reversal experiment with exogenous deoxyribonucleosides led us to seek a biologic system where significant intracellular pools of preformed deoxyribonucleotides are presumed to exist. Ova of amphibian and echinoderm species contain quantities of acid-soluble nucleotides, both riboside and deoxyriboside (6, 14, 25). Direct analytic studies have not been carried out to ascertain whether these acid-soluble deoxyriboside materials serve as precursors for DNA synthesis; however, these embryos are immune to cytotoxic effects of inhibitors of purine synthesis and thymidylate synthesis for some hours after fertilization. Possibly, it is the existence and utilization of preformed TdR which permits embryonic development in the presence of 5-fluorodeoxyuridine to reach an 8-cell (Paracentrotus lividus) or 64-cell stage (E. parma) (10, 19). Autoradiographic observations on sea-urchin embryos (8) and our preliminary studies in the sand dollar indicate that cytidine is not incorporated into DNA during the initial 2 replication cycles following fertilization. The fact that 5-bromodeoxyuridine is extremely toxic to the embryo in the early hours following fertilization while 5-bromouridine is nontoxic (D. A. Karnofsky, unpublished observation) further supports the concept that reduction of ribonucleotides does not occur before the 3rd or 4th S period. Either the enzymes which catalyze ribonucleotide reduction are absent initially or they are under physiologic "feedback" suppression by free deoxyribonucleotides until the latter are removed by incorporation into DNA.

Since E. parma embryos are injured by hydroxyurea, they seem eminently suitable for use in studying the relationship of cytotoxicity to effects of this drug on synthesis of DNA. If hydroxyurea prevents replication of DNA in intact cells by effects upon deoxyribonucleotide phosphorylation or polymerization or upon the DNA template, drug cytotoxicity should be apparent without delay. If, on the other hand, hydroxyurea acts by effects on ribonucleotide reduction, TdR incorporation into DNA of the embryo should be normal for at least several synthesis periods if deoxyribonucleotide stores are present and can be utilized for DNA. The time of onset of inhibition will be dependent upon the size of the precursor stores. In the 1st instance morphologic evidence of cytotoxicity may be evident from the time of the 1st cleavage, under the 2nd circumstance morphologic abnormalities should be seen shortly after the development of the biochemical lesion. The experimentally-observed effects of hydroxyurea correlate quite closely with those predicted for an agent which inhibits reduction of ribonucleotides.

Because exogenous deoxyribonucleosides did not reverse completely the drug-induced inhibition of TdR incorporation into HeLa cells, the possibility that hydroxyurea may interfere at multiple points in the synthesis of DNA remains unresolved. However, these studies offer further support, in 2 animal cell varieties, to the concept that hydroxyurea inhibits synthesis of DNA, at least in part, because it inhibits the formation of deoxyribonucleotides from ribonucleotides.

ACKNOWLEDGMENTS

We wish to thank Joan Walls for valuable assistance in the studies on sand-dollar embryos.

REFERENCES

Fig. 1. Embryo of *Echinorhynchus parma* 5 hr following fertilization showing normal development.

Fig. 2. Embryo of *E. parma* 5 hr following fertilization showing abnormal development in the presence of 1.3 mm hydroxyurea. Cytoceleavage at 4.5 hr has not occurred although the blastula cavity is developing.
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