Changes of Deoxyribonucleoside Triphosphate Pools Induced by Hydroxyurea and Their Relation to DNA Synthesis*

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Hydroxyurea inactivates ribonucleotide reductase from mammalian cells and thereby depletes them of the deoxyribonucleoside triphosphates required for DNA replication. In cultures of exponentially growing 3T6 cells, with 60–70% of the cells in S-phase, 3 mM hydroxyurea rapidly stopped ribonucleotide reduction and DNA synthesis (incorporation of labeled thymidine). The pool of deoxyadenosine triphosphate (dATP) decreased in size primarily, but also the pools of the triphosphates of deoxyguanosine and deoxythymidine (dCTP) were depleted. Paradoxically, the pool of thymidine triphosphate increased. After addition of hydroxyurea this pool was fed by a net influx and phosphorylation of deoxyuridine from the medium and by deamination of intracellular dCTP. An influx of deoxythymidine triphosphate increased. As possible explanations for this discrepancy, we discuss compartmentation of pools and/or vulnerability of newly formed DNA strands to nuclease action and pyrophosphorylation.

Hydroxyurea inhibits the growth of rapidly proliferating cells by blocking DNA synthesis (1–3). It is generally thought that this effect is secondary to a depletion of deoxyribonucleotides caused by inactivation of the enzyme ribonucleotide reductase (4–6). The active enzyme isolated from mammalian cells (7) or Escherichia coli (8) contains a tyrosyl free radical as part of its primary structure, and hydroxyurea inhibits the enzyme by scavenging this radical. A different form of ribonucleotide reductase, present in some procaryotes and lower eucaryotes, in which cobalamin replaces the function of the tyrosyl side.

Ribonucleotide reductases catalyze the reduction of all four common ribonucleotides. The deoxyribonucleoside triphosphates (dNTPs) required for DNA replication are thus produced by this enzyme. Somewhat surprisingly addition of hydroxyurea to mammalian cells in culture usually leads to depletion of only purine dNTPs (12–14). In secondary mouse embryo cells the dGTP pool was depleted first (12), while in 3T6 cells the dATP pool decreased primarily (15). In contrast, the dTTP pool increased in these and most other cell lines. We found recently that hydroxyurea in 3T6 cells stimulated the uptake of pyrimidine deoxyribonucleosides from the medium (16). Conditioned medium contains relatively large amounts of deoxyuridine (17) and phosphorylation of this nucleoside contributed to the rise of the dTTP pool. In the same cells the dCTP pool was rapidly halved after addition of hydroxyurea and then remained at a plateau value (16). Phosphorylation of deoxythymidine might have contributed to the maintenance of the size of the dCTP pool.

How are the changes in pool size linked to the inhibition of DNA synthesis? How rapidly does hydroxyurea inhibit ribonucleotide reduction and DNA synthesis? Are the kinetics of pool changes related to the inhibition of DNA synthesis? In this article we describe experiments with rapidly growing 3T6 cells that address these questions by analyzing short term effects of hydroxyurea on ribonucleotide reduction, dNTP pools, and DNA synthesis.2

EXPERIMENTAL PROCEDURES

Materials—Labeled dNTPs for pool determinations, [5-3H]cytidine (10–30 Ci/mmol) and [methyl-3H]thymidine (25 Ci/mmol) were from Amersham Corp., hydroxyurea from Sigma. A fresh solution of the drug was prepared for each experiment.

Growth and Incubation of Cells—All experiments were made with 3T6 cells derived from one original clone (18). Portions (1–2 × 10⁶ cells) were kept frozen in ampicils at liquid nitrogen temperature.

Cells were grown in Eagle's modification of Dulbecco's medium containing 10% horse serum at 37 °C in a 7.5% CO₂ atmosphere. The cells were maintained for at most six passages (one passage each third day) before use for an experiment. For each experiment the same amount of cells (usually 80,000) was distributed in 5 ml of medium to each of 20–50 Petri dishes (5 cm). Great care was taken to distribute equal amounts of cells to each dish and to keep the dishes at the same level in the incubator. After the first passage the cells had grown to a density of 0.5–1.0 million/dish (after 44–54 h) their number should vary by less that 10% and between 60 and 70% of them should be in S-phase. The medium was now reduced from 5 to 2 ml and, after additional incubation for 90 min at 37 °C, the experiment was started by addition of either hydroxyurea or labeled nucleoside.

Additions (0.01 or 0.02 ml volumes) were made rapidly from an automatic pipette without removing the dishes from the incubator. When indicated, the old medium was suctioned off and replaced with nonlabeled prewarmed medium. When conditioned medium was used for this purpose, it was obtained from parallel cultures not incubated with labeled nucleosides.

Analyses of DNA and dNTPs—To terminate incubations the medium was poured off rapidly and immediately replaced by 3–5 ml of ice-cold Tris saline to stop metabolism. Each dish was then washed rapidly twice with cold Tris saline and placed in a vertical position on ice for 1 min before removing the last traces of liquid by suction. Cold 60% methanol (1.5 ml) was then added to each dish to extract nucleotides. After at least 30 min at 0 °C the methanolic solution was carefully suctioned off, the cell layer was washed with 1 ml of 60%
methanol, and the combined methanolic extracts were used to determine dNTP pools. The cell layer remaining on the dish was dissolved overnight at room temperature in 2 ml of 0.3 M NaOH. If necessary the temperature was raised to 37 °C during the last hour. Isotope incorporation into DNA was determined as described (18).

The size and specific activity of dNTP pools were determined by the DNA polymerase assay (19-21). To this purpose the dry residues of the evaporated methanolic extracts were dissolved in 0.2 ml of 10 mM Tris-HCl, pH 7.4, and dNTP pools were measured. In experiments involving labeled cytidine, each residue was instead dissolved in 0.4 ml of 0.5 M HClO₄, centrifuged, and extracted with Freon/octylamine before the assay. This treatment served to remove radioactive compounds interfering with the determination of the specific activity of dCTP.

Assay of CDP Reduction after Addition of Hydroxyurea—In the experiment depicted in Fig. 1, we wished to detect and measure a very small incorporation of isotope from labeled cytidine into deoxyctydine phosphates. The specific activity of the dCTP pool was not expected to provide a sensitive enough assay for this purpose. Instead, we determined the total radioactivity incorporated into deoxycytidine phosphates (15). The evaporated methanolic extracts were dissolved in 1 ml of 1 M HClO₄, the solution was heated in a boiling water bath for 10 min, and neutralized with 4 M KOH. After removal of the precipitated KClO₄, dCMP was separated from CMP on a Dowex 50 column (22), and the total radioactivity present in dCMP was determined.

Measurements of Thymidylate Synthase Activity—In experiments using cytidine labeled with tritium in the 5-position of the pyrimidine ring, the amount of isotope transferred to water measured the synthesis of dTMP from dCMP via dUMP (23, 24). Such determinations were made by treating a portion of the medium with charcoal and measuring the radioactivity of the supernatant solution after removal of the charcoal by centrifugation (18).

All analytical values in the figures and tables are normalized to 10⁶ cells.

RESULTS

Complete Inhibition of Ribonucleotide Reductase by Hydroxyurea—Addition of 3 mM hydroxyurea to the medium of rapidly growing 3T6 cells resulted in a rapid and complete loss of the ability of the cells to incorporate labeled cytidine into the dCTP pool (Fig. 1). This inhibition was not due to a decreased phosphorylation of cytidine. The drug actually increased incorporation of isotope into cytidine phosphates (Fig. 1A), an effect probably caused by expansion of the CTP pool. In contrast, in the hydroxyurea-treated cells no isotope was recovered in deoxycytidine phosphates as late as 60 min after addition of labeled cytidine. This result clearly suggests a complete block of the reduction of CDP to dCDP by hydroxyurea.

Effects of Hydroxyurea on the Turnover of Pyrimidine dNTP Pools—Earlier experiments with 3T6 cells had shown that 3 mM hydroxyurea rapidly depleted the dATP and dGTP pools, decreased the dCTP pool to about 50%, but increased the size of the dTTP pool considerably (16). Part of the increase in dTTP was caused by an increased uptake and phosphorylation of deoxyuridine from the medium. We now demonstrate two additional effects that contribute to the maintenance of pyrimidine dNTP pools after addition of hydroxyurea: (i) synthesis of dTTP from dCTP and (ii) formation of dCTP from deoxycytidine in the medium.

dTTP Synthesis from dCTP Continues in the Presence of Hydroxyurea—In 3T6 cells most dTTP is formed from dCTP via intermediate deamination of dCMP to dUMP (17). This reaction sequence was monitored in experiments in which the dCTP pool was prelabeled with tritium in the 5-position of the pyrimidine ring.

Isotope was introduced from the medium as [5-³H]cytidine as shown schematically in Fig. 2. Inside the cells the nucleoside is rapidly phosphorylated to cytidine phosphates and pyrimidine deoxyribonucleotides are formed by reduction of CDP. Tritium is retained in deoxycytidine phosphates but not in thymidine phosphates, since isotope is lost from the pyrimidine ring and recovered as ²H₂O in the medium during formation of dTMP. During steady state conditions, when both the deoxycytidine phosphate pools and dUMP are at isotope equilibrium, the appearance of ²H₂O measures the synthesis of dTMP from dCTP while the appearance of isotope in DNA reflects incorporation of dCTP during DNA replication. These parameters can be expressed in absolute terms (pmol/min) if the specific activity of the dCTP pool is known. Tables I and II record results from two such experiments. In both cases the dCTP pool of cells was prelabeled from [5-³H] cytidine before addition of hydroxyurea. The drug then stopped further entry of isotope into the pool via reduction of CDP and it became possible to study the transfer of isotope from dCTP into other cellular compartments.

Table I records data from the first experiment involving the 10-min time period following addition of hydroxyurea. The size of the dCTP pool was almost halved, but its specific activity remained unchanged.

<table>
<thead>
<tr>
<th>Min after hydroxyurea</th>
<th>dCTP</th>
<th>dTTP</th>
<th>²H₂O DNA</th>
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<td>cpm</td>
<td>cpm x 10⁻³</td>
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<td>pmol/10⁶ cells</td>
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<td>151</td>
<td>660</td>
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<tr>
<td>Difference</td>
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<td>+55</td>
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Fig. 2. Scheme for the transformation of [5-³H]cytidine to various deoxynucleotides and DNA. Labeled end products are shown in boxes. Thymidine phosphates are not labeled since the isotope is lost during methylation of dUMP.

Fig. 1. Inhibition of ribonucleotide reduction by hydroxyurea. 3T6 cells were labeled from 0.3 PM [5-³H]cytidine 10 min after addition of 3 mM hydroxyurea. 30, 60, and 120 min thereafter the total radioactivity (•) of the nucleotide pool (A) and of deoxycytidine phosphates (B) was determined as described under "Experimental Procedures." Parallel cultures, not treated with hydroxyurea, served as controls (○).
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The relative contributions of the two pathways were now estimated in an experiment in which the accumulation of dTTP after addition of hydroxyurea was measured in two sets of parallel cultures, one lacking deoxyuridine in the medium, the other containing the nucleoside at 0.3 μM concentration. As shown by the results depicted in Fig. 3, deoxyuridine approximately doubled the initial accumulation of dTTP. The remaining increase then probably originated from deamination of dCTP. The presence of deoxyuridine in the medium had no effect on the decrease in the size of the dCTP pool (Fig. 3) nor did this small amount of nucleoside increase the size of the dTTP pool in the absence of hydroxyurea (data not shown). The concentration of deoxyuridine used in this experiment mirrored the amount of the nucleoside present in conditioned medium of 3T6 cells used under the growth conditions used.

The dCTP Pool Is Replenished from Deoxyxycytidine after Addition of Hydroxyurea—The kinetics of the disappearance of dCTP after addition of hydroxyurea varied, depending on the nature of the medium. When the drug was added directly to cells that had conditioned their medium during 2 days, the pool decreased during the first 10 min to approximately 50% of its original size but then remained fairly constant (Fig. 4). However, when cells had been transferred to fresh medium containing dialyzed horse serum immediately before addition of the drug, the depletion of the dCTP pool continued after 10 min. These results suggest that deoxyxycytidine present in conditioned medium but absent from fresh medium was used by the cells to replenish the dCTP pool during the time period following the first rapid decline of the pool.

Recycling of deoxyxycytidine was more directly demonstrated in the experiment depicted in Fig. 5. There the dCTP pool of cells was prelabeled for 60 min from [5-3H]cytidine. The cultures were then divided into two sets, one remaining in the labeled medium, while the other set was changed to nonlabeled conditioned medium. Hydroxyurea was added to both sets and the changes in the size of the dCTP pool (Fig. 5A) and its specific activity (Fig. 5B) were determined. The net loss of dCTP was identical in both sets, but the specific
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**FIG. 4. Effect of medium conditioning on the depletion of the dCTP pool by hydroxyurea (HU).** The drug was added to two separate sets of 3T6 cells. In one set (▲) the cells remained in the original growth medium (conditioned for 2 days), while in the second set (●) the cells had been shifted to fresh medium containing dialyzed horse serum 90 min before addition of hydroxyurea. The size of the dCTP pools was determined at various time intervals after addition of hydroxyurea. 100% corresponds to 100 pmol of dCTP in the cells kept in conditioned medium and 180 pmol in the second set.

**FIG. 5. Reutilization of deoxycytidine of the medium for synthesis of dCTP.** Two parallel sets of 3T6 cells were labeled from [5-3H]cytidine for 60 min before addition of 3 mM hydroxyurea (HU). 10 min thereafter the cells from one set (open symbols) were shifted to conditioned nonlabeled medium, while the cells of the second set (closed symbols) remained in the original labeled medium. A shows the size and B the specific activity of the dCTP pools at various times after addition of hydroxyurea.

activity decayed at least twice as fast in the cells kept in cold conditioned medium. Since ribotide reduction was blocked completely in both sets, the data suggest that the cells remaining in labeled medium recycled labeled deoxycytidine for dCTP synthesis while in the cold medium dCTP was formed from nonlabeled deoxycytidine.

**Correlation between Pool Changes and Inhibition of DNA Synthesis**—The experiments discussed so far illustrate some of the immediate effects of hydroxyurea on ribonucleotide reductase and the metabolism of pyrimidine dNTPs but do not explain the rapid inhibition of DNA synthesis by the drug. In the following experiment we correlated the effects of hydroxyurea on DNA synthesis with changes in purine and pyrimidine pool sizes following inhibition of the reductase.

DNA synthesis was measured by incorporation of isotope from prelabeled [3H]dTTP into DNA. A 10-min incubation period with [3H]thymidine sufficed to achieve isotope equilibrium between the nucleoside and dTTP. At this time hydroxyurea, at 0.1, 0.5, or 3 mM final concentration, was added to three sets of cultures and the continued incorporation of isotope into DNA was followed at 5-min intervals during the ensuing 15 min (Fig. 6). Since hydroxyurea during this time period only minimally influenced the specific activity of dTTP, incorporation of radioactivity mirrored faithfully the inhibition of DNA synthesis.

At all concentrations of the drug, DNA synthesis was rapidly inhibited. With 0.1 mM hydroxyurea, DNA synthesis was essentially normal during the first 5-min period, strongly inhibited between 5 and 10 min, and negligible thereafter. At the two higher concentrations of the drug, DNA synthesis was inhibited already during the first 5-min period and stopped completely thereafter.

Measurements of pool sizes from the same experiment are shown in Fig. 7. Only the pools from the experiment with 0.1 (Fig. 7A) and 3 mM hydroxyurea (Fig. 7B) were analyzed. At

**FIG. 6. Inhibition of DNA synthesis by increasing concentrations of hydroxyurea (HU).** Each set of cultures was incubated with 0.3 μM [3H]thymidine for 10 min before addition of 0.1, 0.5, or 3 mM hydroxyurea. The continued incorporation of isotope into DNA was measured at the indicated time intervals. The broken lines represent parallel control cultures.

**FIG. 7. Effects of 0.1 mM (A) and 3 mM (B) hydroxyurea (HU) on the size of dNTP pools.** The data are from the same experiment as the results shown in Fig. 6. Values are given as percentages of the following initial values (pmol/10⁶ cells): dATP (▲), 36 (A) and 37 (B); dGTP (●), 22.5 (A) and 25 (B); dCTP (●), 61 (A) and 71 (B); dTTP (○), 108 (A) and 119 (B).
both concentrations of the drug the pools of dATP, dCTP, and dGTP decreased in size while the dTTP pool increased. At 0.1 mM hydroxyurea, dATP was affected more than dCTP and dGTP, but such a difference was not apparent at the higher concentration of the drug. At both concentrations the pool changes were quite moderate, compared to the pronounced inhibition of DNA synthesis. As an example, Fig. 7 shows that 10 min after addition of 0.1 mM hydroxyurea the size of the dATP had decreased by 40%. The drop in the size of the dCTP and dGTP was still smaller. At this point the incorporation of thymidine into DNA ceased completely (Fig. 6). We shall return to this under the "Discussion."

**DISCUSSION**

The inhibition of ribonucleotide reductase by hydroxyurea and the molecular mechanism for this effect have been known for some time. Nevertheless doubts were sometimes expressed whether the inhibition of the reductase fully explains the effect of the drug on DNA synthesis and cell growth. We believe that this is the case. First, cells containing a cobalamin-dependent reductase (9-11), an enzyme that lacks the tyrosyl radical and therefore is not inhibited by hydroxyurea, are insensitive to growth inhibition by the drug. Second, in E. coli (25) and mammalian cells (26, 27), the presence of a mutated reductase confers an altered sensitivity for hydroxyurea to the cells.

The complete inhibition of ribonucleotide reductase leads to the cessation of the de novo synthesis of dNTPs and, consequently, first slows down and then rapidly stops DNA synthesis. The four dNTP pools vary in size and, after a block of de novo synthesis, the smallest pool(s) will limit DNA synthesis. In early experiments with secondary mouse embryo cells, the dCTP pool was by far the smallest, and the inhibition of DNA synthesis correlated nicely with the disappearance of this pool (12).

In the present investigation with 3T6 cells, the situation appeared to be more complicated. The pyrimidine pools are between 3- and 5-fold larger in size than the purine dNTPs in cells engaged in DNA synthesis, with dATP and dGTP being about equal. Addition of 3 mM hydroxyurea to 3T6 cells depleted both purine pools to about the same extent, but at lower concentrations of the drug the dATP pool was hit primarily (cf. also Ref. 15). However, as shown by a comparison of the results of Figs. 6 and 7, a moderate decrease in pool size was accompanied by a large effect on DNA synthesis. After 10 min, when DNA synthesis had apparently stopped completely, the size of the dATP pool had dropped only by 40%. We do not fully understand the reason for this discrepancy but can think of several possible explanations.

Maintenance of DNA synthesis might depend on critical pool sizes. A lowering of the dNTP concentration would first lead to a decreased rate of fork movement, and this might make the newly synthesized strands vulnerable to nuclease action. The decreased rate might also favor pyrophosphorolysis and recycling of deoxynucleotides, resulting in an underestimation of the rate of their polymerization. In line with this explanation, several authors have reported that any residual "DNA synthesis" after addition of hydroxyurea measured by incorporation of isotope from labeled thymidine occurred only into short DNA fragments (28-30).

A second possible explanation invokes compartmentation of purine dNTPs. Earlier work from this laboratory demonstrated the existence in 3T6 cells of more than one dCTP pool (31). Also dATP might be sequestered in separate metabolic compartments. Thus, if 3T6 cells contain a substantial dATP pool, not involved in DNA synthesis, our measurements would not reflect the amount of nucleotide available at the replication fork. One known compartmentation is caused by the heterogeneity of the cell population. Only 60-70% of our cells were in S-phase and pool measurements gave average values for dATP of the total population. In general, dNTP pools are larger in S-phase cells than in resting cells (32). However, this difference is less pronounced for dATP, and in the cell cultures used for our experiments as much as one-third of the dATP might have been present in resting cells. If the pool in resting cells was not affected by hydroxyurea, then the decrease in the size of the S-phase pools would have been larger than was apparent from the data of Fig. 7. This kind of compartmentation might well contribute to an overestimation of the amount of dATP present at replication forks in hydroxyurea-inhibited cells, but probably does not suffice to explain the discrepancy between the effects of the drug on DNA synthesis and the size of the dATP pool. A combination of several of the discussed effects might be at play.

We now turn to the effects of hydroxyurea on the metabolism of pyrimidine dNTPs. It has often been observed in different kinds of cells that hydroxyurea increases the size of the dTTP pool (12-15). Also, the dCTP pool may increase or remain relatively unchanged. A block of an enzyme responsible for the production of dNTPs is, however, not expected to lead to their accumulation. This paradox has sometimes been taken to support the erroneous belief that separate enzymes reduce pyrimidine and purine ribonucleotides (33).

In 3T6 cells the size of the dTTP pool more than doubled while the dCTP pool decreased to half its original size during the first hour after addition of hydroxyurea (16). This occurred during a time period when de novo synthesis of dNTPs was stopped completely. We now find that DNA synthesis, as measured by incorporation of radioactive dTTP, stopped 5 min after addition of the drug, calculated from data of Table I; B,

![Fig. 8. Rates of metabolic transformations of pyrimidine dNTPs after addition of hydroxyurea. A, values during the first 10 min after addition of the drug, calculated from data of Table I; B, values for the period between 10 and 70 min, calculated from data of Table II. In both cases the numbers next to the arrows give the interconversion as pmol/10^6 cells during the whole time interval.](image-url)
min after addition of hydroxyurea. However, deamination of dCMP continued and contributed to the accumulation of dTTP and the depletion of the dCTP pool. In addition, both pyrimidine dNTP pools were replenished from deoxynucleosides present in the medium. Together, these effects explain the unexpected changes found in the pool sizes of pyrimidine dNTPs.

In Fig. 8, we use the results compiled in Tables I and II to quantify the rates of the metabolic transformations of pyrimidine deoxyribonucleotides during the first 10 min (Fig. 8A) and during the period between 10 and 70 min (Fig. 8B) after addition of hydroxyurea. The numbers next to the arrows in the figure express the interconversions of the different pools as picomole per million cells during the whole time periods. For the calculations we assumed that isotope equilibration between the mono- and triphosphate pools of each deoxynucleoside was rapid enough to result in identical specific activities.

During the first 10 min after hydroxyurea (Fig. 8A), the loss of isotope from the dCTP pool can be translated to a loss of 71 pmol of dCTP, 25 of which were incorporated in DNA while 40 were used for dTTP synthesis. The remaining 6 pmol were probably excreted into the medium as deoxyctydine. This value agrees well with earlier results (16), demonstrating that 0.6 pmol/min of deoxyctydine was excreted after addition of hydroxyurea. This excretion was more than balanced by an influx of 10 pmol of deoxyctydine from the medium. We arrive at this value by comparing the loss of isotopic dCTP (71 pmol) and the net decrease in pool size (67 pmol) which shows that 4 additional pmol were introduced into the dCTP pool during the 10 min after hydroxyurea. This calculation involves a difference between two large values and cannot be taken as strictly accurate. It only serves to illustrate that synthesis of dCTP from the medium was small compared to that of dTTP.

The dTTP pool increased in size by 55 pmol. In addition, 32.5 pmol of dTTP were incorporated into DNA (the thymine/cytosine ratio of mouse DNA is 1.3:1). Thus, a total of 87 pmol of dTTP had to be synthesized during the 10-min period. Only 40 pmol were formed from dCTP. The remaining 47 pmol then were presumably derived from deoxyuridine and thymidine of the medium. This value represents the net influx of deoxynucleosides from the medium. We had demonstrated earlier (16) an excretion of 0.7 pmol/min of thymidine, which means that the total influx should have been 54 pmol.

In a similar way we arrived at the numbers shown in Fig. 8B for the period between 10 and 70 min after addition of hydroxyurea. The dCTP pool decreased in size by 20 pmol, but the deamination of dCMP amounted to as much as 43 pmol. The difference between these two values suggests a net influx of 23 pmol of deoxyctydine from the medium. Analysis of the dTTP pools showed a net increase of 60 pmol of which only 43 pmol were derived from dCMP. The difference (17 pmol) is then accounted for by a net influx of deoxyuridine and thymidine from the medium. For both dCTP and dTTP the total influx of deoxynucleosides from the medium is larger than the net influx because of the simultaneous excretion of deoxynucleosides from the cells.

One can schematically visualize the effect of hydroxyurea on dNTP metabolism as occurring in two phases: during the first 10 min the cessation of ribonucleotide reduction lowers the pool sizes of some dNTPs and, in particular, of dATP to a level that becomes critical for DNA synthesis. The movement of the replication fork slows down and soon becomes minimal. During the second phase, dNTPs are no longer consumed for DNA synthesis. Pool changes now arise from interconversion of dCTP to dTTP and from the activity of substrate cycles between deoxynucleosides and their phosphates, resulting in an influx of pyrimidine deoxynucleosides from the medium.

REFERENCES

2. Young, C. W., and Hodas, S. (1964) Science 146, 1172-1174