Effect of Methotrexate and 5-Fluorodeoxyuridine on Ribonucleotide Reductase Activity in Mammalian Cells

Howard L. Elford, Ernest L. Bonner, Bonnie H. Kerr, Stephan D. Hanna, and Mark Smulson

Department of Biochemistry and MCV/VCU Cancer Center, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298 [H. L. E.]; Department of Physiology and Pharmacology, Duke University Medical School, Durham, North Carolina 27710 [H. L. E., E. L. B., B. H. K., S. D. H.]; and Department of Biochemistry, Georgetown University, Schools of Medicine and Dentistry, Washington, D. C. 20007 [M. S.]

SUMMARY

A number of studies in bacteria have indicated that deoxythymidine 5'-triphosphate may be a repressor or corepressor of ribonucleotide reductase. For determination of whether a similar regulating mechanism exists in mammalian cells, HeLa cells and partially hepatectomized rats were treated with either methotrexate, 5-fluorouracil, or 5-fluorodeoxyuridine in order to block thymidylate synthesis and consequently lower the intracellular pools of deoxythymidine 5'-triphosphate. In HeLa cells there was a significant (360 to 400%) increase in reductase activity in both the methotrexate and 5-fluorodeoxyuridine-treated cells. The administration of methotrexate to partially hepatectomized rats resulted in a 2.7-fold enhancement of the hepatectomy-induced increase in reductase activity, and the 5-fluorouracil treatment yielded a 60% increment in the increase of ribonucleotide reductase activity after partial hepatectomy.

Cycloheximide prevented the increase in reductase activity after the exposure of HeLa cells to methotrexate and 5-fluorodeoxyuridine, indicating that the stimulation of ribonucleotide reductase activity was the result of enhancement of de novo enzyme synthesis rather than of enzyme activation. The data support the thesis that deoxythymidine 5'-triphosphate or a thymidylate metabolite may be involved in the regulation of ribonucleotide reductase levels in mammalian cells.

INTRODUCTION

Deoxyribonucleotide synthesis must occur prior to DNA synthesis in cellular replication because the pool size of deoxyribonucleotides in mammalian cells is inadequate to support DNA synthesis for more than 1 to 5 min (41, 46). The reduction of ribonucleotides to deoxyribonucleotides is an important and possibly a rate-controlling reaction in the sequence of events that leads to DNA synthesis and cellular replication. The specific activity of the enzyme, ribonucleotide reductase, that catalyzes this reductive step is closely correlated with cellular proliferation, as evidenced by the fact that specific activity of the enzyme increases with tumor growth rate (14), upon liver regeneration (22), on fertilization of sea urchin eggs (34), and during the DNA-synthetic portion of the cell cycle (32, 45). The dramatic changes in enzyme activity are due mainly to de novo enzyme synthesis and degradation rather than to enzyme activation (12, 22, 32, 45).

A number of previous studies with Escherichia coli (4, 33), Lactobacillus leichmannii (3, 18), and yeast (25) have indicated that a phosphorylated derivative of TdR, presumably dTTP, may serve as a repressor or corepressor of ribonucleotide reductase synthesis. For determination of whether dTTP is a repressor or corepressor of ribonucleotide reductase synthesis in mammalian cells, experiments designed to lower intracellular dTTP concentrations by inhibiting dTMP synthesis were undertaken. HeLa cells and animals with regenerating liver were subjected to MTX, 5FU, or SFUdR treatment in order to prevent dTMP synthesis and therefore reduce the pool size of dTTP.

The data presented in this paper indicate that inhibition of dTMP formation by treatment with MTX and 5FUdR, 2 different chemotherapeutic agents that ultimately affect thymidylate synthesis, results in an increase in ribonucleotide reductase levels in the cell. These studies are the first experiments to provide direct evidence in mammalian cells that a decrease in thymidylate formation results in an increase in ribonucleotide reductase activity and to support the proposal that dTTP or dTTP metabolite might be a repressor or corepressor of ribonucleotide reductase synthesis in mammalian cells.

MATERIALS AND METHODS

Materials. [3H]CDP was purchased from Nuclear Dynamics, Inc., El Monte, Calif. Radioactive [5-3H]dUMP was obtained from Amersham/Searle Corp., Arlington Heights, Ill., and [methyl-3H]TdR was supplied by New England

The abbreviations used are TdR, thymidine; MTX, methotrexate; 5FU, 5-fluorouracil; SFUdR, 5-fluorodeoxyuridine; CXM, cycloheximide.
various drugs for 16.5 hr, the cells were collected by treatments. The media already contain all other essential alleviate any impairment in purine, 1-carbon metabolism, described previously (43). In addition, during the experi spinner flasks with 10% fetal calf serum and antibiotics as were obtained from Calbiochem. San Diego, Calif., and Sigma Chemical Co., St. Louis, Mo., respectively.

HeLa Cell Culture and Fractionation. HeLa cells were maintained in Eagle's media for suspension culture in spinner flasks with 10% fetal calf serum and antibiotics as described previously (43). In addition, during the experiments described, the media were supplemented with adenosine (50 µM), glycine (0.5 mM), and serine (0.5 mM) to alleviate any impairment in purine, 1-carbon metabolism, or protein synthesis caused by the MTX, 5FU, or 5FUdR treatments. The media already contain all other essential amino acids, including methionine. After incubation with various drugs for 16.5 hr, the cells were collected by centrifugation and washed with 0.9% NaCl solution. The washed cells were lysed by hypotonic shock and disrupted in a Dounce homogenizer, as described in detail previously (42). Nuclei were removed by centrifugation at 900 × g for 10 min. The supernatant was centrifuged for 30 min at 16,000 × g, and the postmitochondrial cytosol was stored at −20° prior to enzyme assays.

Partial Hepatectomy. Partial hepatectomy was performed on 175- to 225-g male Sprague-Dawley rats (Holtzman Co., Madison, Wis.) according to the procedure developed by Higges and Anderson (19). The animals were killed by decapitation 36 hr after the operation.

Tissue Fractionation. The partially regenerated liver was dissected from the animal, washed with cold 0.9% NaCl solution, minced into small pieces, and washed again with the NaCl solution. The washed pieces of regenerative liver were suspended in 4 volumes by weight of buffer (0.25 M sucrose; 25 mM potassium phosphate, pH 7.4; 25 mM KCl, 5 mM MgCl₂, and 1 mM dithiothreitol) and homogenized in a Potter-Elvehjem homogenizer. The crude homogenate was centrifuged at 600 × g for 10 min to remove nuclei and cellular debris. This supernatant was further centrifuged twice at 10,000 × g for 10 min to remove mitochondria. The postmitochondrial supernatant was centrifuged at 78,000 × g for 2 hr to remove microsomes, and a soluble or cytosol fraction was obtained. Ribonucleotide reductase is located in this cytosol fraction.

Enzyme Assays. Ribonucleotide reductase was assayed by monitoring the conversion of CDP to dCDP with the use of a slightly modified assay procedure originally developed by Reichard et al. (36) as previously described (14). The incubation mixture (0.34 ml) contained 5 µCi [³H]CDP (specific activity, 12 Ci/m mole). One unit of activity is defined as the amount of enzyme that catalyzes the conversion of 1000 cpm of CDP to dCDP in 40 min at 30°. One unit of thymidylate synthetase was determined by the measurement of the release of tritium from [5-³H]dUMP by the method of Kämmen (21), with the use of charcoal to absorb the unreactive substrate as detailed in an earlier paper (14), except that the reaction was conducted for 18 min at 37°. The thymidylate synthetase assay mixture (0.10 ml) contained 0.34 µCi of [5-³H]dUMP (specific activity, 34 mCi/mmole). TdR kinase activity was measured by the method of Breitman (7) as outlined in the same earlier report (14).

The kinase assay mixture (0.10 ml) contained 0.10 µCi of [³H]TdR (specific activity, 4.54 mCi/mmole). Protein concentration of the different extracts was determined by the method of Lowry et al. (26), with the use of crystalline bovine serum albumin as the standard.

RESULTS

Effect of MTX and 5FUdR on Ribonucleotide Reductase Level in HeLa Cells. The effect of MTX or 5FUdR treatment of HeLa cells on the level of activity of ribonucleotide reductase is illustrated in Table 1. Treatment of HeLa cells with MTX results in a very pronounced effect on ribonucleotide reductase specific activity. An increase greater than 4-fold is observed. This increase in reductase activity is comparable to the effect that MTX has on thymidylate synthetase activity (Table 1). The greater than 4-fold increase in thymidylate synthetase activity also attests to the effectiveness of the MTX treatment because it has been shown previously that MTX treatment results in a higher level of thymidylate synthetase activity in mammalian cells (9, 10, 23, 38, 39). This elevation in synthetase activity has been attributed to enzyme stabilization by interaction with MTX or unreactive dUMP (6, 39), as well as to enzyme stabilization by increased enzyme synthesis (9, 10).

A marked increase in ribonucleotide reductase activity is also observed after incubation of HeLa cells with 5FUdR (Table 1). There is an enhancement of approximately 3.5-fold in reductase activity over that in the untreated cells. This increase is comparable to the increase observed with MTX. 5FUdR is reported to decrease dTTP pools by preventing thymydylate formation by directly inhibiting thymidylate synthetase. The thymidylate synthetase specific activity, on the other hand, was reduced approximately 90%. This effect is accounted for by the interaction between the enzyme with 5-fluorodeoxyuridine 5'-phosphate rather than by a reduction in the level of enzyme in the cell.

<table>
<thead>
<tr>
<th>Ribonucleotide reductase</th>
<th>Thymidylate synthetase</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specific activity</strong></td>
<td><strong>Specific activity</strong></td>
</tr>
<tr>
<td>(units/mg protein)</td>
<td>(units/mg protein)</td>
</tr>
<tr>
<td><strong>% change</strong></td>
<td><strong>% change</strong></td>
</tr>
<tr>
<td>Control</td>
<td>142.1</td>
</tr>
<tr>
<td>MTX</td>
<td>586.5</td>
</tr>
<tr>
<td>5FUdR</td>
<td>514.1</td>
</tr>
</tbody>
</table>

Table 1

Effect of MTX and 5FUdR on ribonucleotide reductase in HeLa cells

Three cultures of 100 ml each of HeLa cells (3 × 10⁴/ml) were grown in Eagle's media supplemented with adenosine (50 µM), glycine (0.5 mM), and serine (0.5 mM). One flask of cells received MTX (0.5 µM, final concentration); the second flask contained 5FUdR (1 µM, final concentration); the third flask, control, received no drug. The 3 flasks were incubated for 16.5 hr at 37°. Cells were washed and the cell extract material was prepared as described in "Materials and Methods." Assays are also described therein. One unit of ribonucleotide reductase activity is indicated by the conversion of 1000 cpm of CDP to dCDP in 40 min at 30°. One unit of thymidylate synthetase activity is defined as the release of 1000 cpm of the 5'-tritiated atom in 20 min at 37°.
In addition, TdR kinase activity in HeLa cells is increased significantly by incubation of HeLa cells with both MTX and 5FUdR (Table 2), an observation previously noted in cultured mammalian cells (9, 11, 39). An increase of over 3-fold is seen after exposure to MTX, and an increase of better than 4-fold is seen with the 5FUdR treatment, suggesting an induction of the salvage pathway enzyme by the 2 drugs.

The use of MTX for synchronization of HeLa cells has been well documented by other experiments from one of our laboratories (40). Cells will enter S phase only after administration of exogenous TdR, presumably due to depletion of dTTP pools caused by MTX inhibition of dTMP synthesis. Evidence to support the fact that dTTP pools were significantly lowered by MTX is provided by the following facts: (a) when the HeLa cells are released from the MTX block by the addition of TdR, they enter into S phase, as evidenced by uptake of [3H]TdR and increase in DNA content as they traverse through S phase; and (b) cells preferentially incorporated exogenous [3H]TdR in contrast to controls. The higher levels of TdR kinase noted in Table 2 could also contribute to the increase in exogenous TdR incorporation.

**Effect of MTX and 5FU on Ribonucleotide Reductase Activity in Regenerating Liver.** The following experiments were performed to confirm in the intact animal the results obtained with HeLa cells that indicate a regulatory role for dTTP in controlling ribonucleotide reductase synthesis. Little or no ribonucleotide reductase activity is discernible in normal adult rat liver under the assay conditions used. Activity increased at least 10-fold by 36 hr after partial hepatectomy (22). MTX treatment led to a significant, almost 3-fold enhancement of the partial hepatectomy-induced increase in reductase activity (Table 3). In comparison, the 5FU treatment resulted in a 60% increase in reductase activity. MTX stimulation of the regeneration-related increase in thymidylate synthetase activity was observed (Table 3). This 5-fold increase over the untreated 36-hr regenerating liver was comparable in magnitude to the results observed in the studies of Labow et al. (23) with 48-hr regenerating liver.

In contrast to the large inhibition of thymidylate synthetase activity seen in HeLa cells by the 5FUdR compound, treatment of a partially hepatectomized animal with 5FU had only a small effect on thymidylate synthetase activity as compared to the untreated 36-hr regenerating liver activity, perhaps suggesting that the 5FU dosage or the scheduling of administration of the drug for the partially hepatectomized rat may not have been optimum to obtain effective thymidylate synthesis inhibition.

The administration of MTX to animals after partial hepatectomy resulted in a decrease of the stimulation in TdR kinase activity as compared to the stimulation of the untreated hepatectomized animal (Table 4). Although these results are in contrast to the findings with HeLa cells, they are in agreement with data previously obtained after MTX treatment in regenerating liver (23), where a decrease of approximately 40% was seen with 48-hr regenerating liver after MTX treatment. Administration of 5FU also resulted in a decrease in TdR kinase activity.

**Inhibition of Protein Synthesis and Its Effects on the MTX- and 5FUdR-stimulated Increases in Ribonucleotide Reductase Activity.** Previous work by our laboratory (12) as well as by others (22, 25, 32, 45) has shown that the large increase in reductase activity concomitant with increased rates of cellular replication or with the entrance of cells into S phase of the cell cycle are due to de novo enzyme synthesis rather than to enzyme activation. For determination of whether the elevation in reductase activity that was seen after MTX or 5FUdR exposure in HeLa cell

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**Table 2**

<table>
<thead>
<tr>
<th>Cell treatment</th>
<th>Specific activity (units/mg protein)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>MTX</td>
<td>25.2</td>
<td>+332</td>
</tr>
<tr>
<td>5FUdR</td>
<td>33.1</td>
<td>+435</td>
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</table>

**Table 3**

<table>
<thead>
<tr>
<th>Source of enzyme</th>
<th>Treatment</th>
<th>Ribonucleotide reductase relative activity</th>
<th>Thymidylate synthetase relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rat liver</td>
<td>None</td>
<td>Not detected (12)*</td>
<td>&lt;0.1 ± 0.12*</td>
</tr>
<tr>
<td>Regenerating liver</td>
<td>None</td>
<td>1.0 ± 0.10* (34)</td>
<td>1.0 ± 0.08* (32)</td>
</tr>
<tr>
<td>Regenerating liver</td>
<td>MTX</td>
<td>2.7 ± 0.35* (39)</td>
<td>5.2 ± 0.85* (20)</td>
</tr>
<tr>
<td>Regenerating liver</td>
<td>5FU</td>
<td>1.6 ± 0.20* (22)</td>
<td>1.15 ± 0.25 (15)</td>
</tr>
</tbody>
</table>

* Normal rat liver ribonucleotide reductase activity cannot be detected but can be calculated to be less than 0.6 unit/mg of protein.

* Numbers in parentheses, number of separate rat livers used to determine enzyme activity.

* Mean ± S.E.
cultures was in fact due to an enhancement of enzyme synthesis and was not the result of the removal of a negative allosteric inhibitor, the effect of a protein synthesis inhibitor, CXM, on MTX- or 5FUdR-stimulated reductase activity was determined. The data presented in Table 5 support the proposal of enhanced enzyme synthesis. Incubation of HeLa cells with MTX or 5FUdR for 16 hr resulted in a 2.5-fold increase in reductase activity for the MTX-treated cells and a 3.4-fold increase in the 5FUdR-treated cells over the untreated cells. When CXM was included with the MTX or 5FUdR, the level of reductase activity was reduced to a value lower than that of the untreated cells, a value approximately one-third of that of the cells exposed to only MTX or 5FUdR. The addition of CXM alone resulted in a decrease of about 50% in the level of ribonucleotide reductase activity compared to that in untreated cells. Protein synthesis was inhibited approximately 80% by CXM, as indicated by the reduced level of amino acid incorporation into protein. Cell viability was essentially 100%, as determined by trypan blue staining.

**Possible Production of a Stimulating Substance or Removal of Inhibitory Material by the MTX or 5FUdR Treatment.** For determination that the stimulation of reductase activity by MTX and 5FUdR was not the result of alteration in concentration of some metabolite that directly affects the activity of the enzyme in the *in vitro* assay, mixing experiments were undertaken. When the MTX-treated HeLa cell extract and untreated cell extract were assayed together, the combination values were very close to the values that one would calculate based on the data obtained from the individual extracts (Table 6). The value obtained from the control and the 5FUdR extract mixing experiment was almost identical with that predicted on individual determinations. Therefore, the mixing experiments gave no indication of a major inhibitory substance in the control extract that was removed by the MTX or 5FUdR treatment. Dialysis or Dowex 1-CI- chromatography of the untreated, MTX-treated, or 5FUdR-treated extracts did not have a significant effect on the comparative levels of activities subsequently determined. The results would also tend to negate the idea that MTX or 5FUdR treatment led to the production of a stimulating substance.

**Table 4**

Effect of MTX and 5FU on TdR kinase activity in regenerating liver

The MTX and 5FU schedule and dosages are as outlined in Table 3. The assay is described under "Materials and Methods," and in Table 2. Average specific activity for 36-hr regenerating liver is 1.8 units/mg of protein.

<table>
<thead>
<tr>
<th>Source of enzyme</th>
<th>Treatment</th>
<th>Regenerating liver (36-hr) relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rat liver</td>
<td>None</td>
<td>0.10 ± 0.15*(5)*</td>
</tr>
<tr>
<td>Regenerating liver</td>
<td>None</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>Regenerating liver</td>
<td>MTX</td>
<td>0.6 ± 0.35</td>
</tr>
<tr>
<td>Regenerating liver</td>
<td>5FU</td>
<td>0.7 ± 0.4</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

* Numbers in parentheses, number of separate rat livers used to determine enzyme activity.

**Table 5**

Effect of CXM on MTX and 5FUdR stimulation of ribonucleotide reductase activity in HeLa cells

Cell cultures were incubated as in Table 1 with either no drug (control), MTX (0.23 μg/ml), MTX (0.23 μg/ml), and CXM (10 μM, final concentration); 5FUdR (1 μM), 5FUdR (1 μM), and CXM (10 μM); or CXM (10 μM) for 16 hr. Cells were approximately 100% viable after incubation as determined by trypan blue staining. Cell extracts were prepared from washed cells from the cultures as described in "Materials and Methods." Reductase activity was measured as previously outlined. The ribonucleotide reductase specific activity for the control cells in the MTX and MTX plus CXM experiment was 46.7 units/mg. The ribonucleotide reductase specific activity for the control cells in the 5FUdR and 5FUdR plus CXM experiment was 30.9 units/mg.

<table>
<thead>
<tr>
<th>Cell treatment</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.0</td>
</tr>
<tr>
<td>MTX</td>
<td>2.5</td>
</tr>
<tr>
<td>MTX + CXM</td>
<td>0.8</td>
</tr>
<tr>
<td>CXM</td>
<td>0.5</td>
</tr>
<tr>
<td>5FUdR</td>
<td>3.4</td>
</tr>
<tr>
<td>5FUdR + CXM</td>
<td>1.2</td>
</tr>
</tbody>
</table>

**DISCUSSION**

It has been known for over a decade that TdR, when added to the medium of mammalian tissue culture cells, inhibits DNA synthesis and cell replication (27). Its inhibitory action is manifest through a phosphorylated derivative because a mutant cell line lacking a TdR kinase is unaffected by the inclusion of TdR in the media (17, 29, 30). The inhibition of cellular replication by TdR has been utilized as a means to obtain partially synchronized cells (48).

The inhibitory action of TdR can be completely prevented by the supplementation of deoxycytidine to TdR-inhibited cells (5, 17, 28-30, 47). The biochemical explanation has been that TdR is phosphorylated to dTTP, which is a negative allosteric effector on the conversion of CDP to dCDP and of UDP to dUDP in mammalian cells (27, 31, 37). Therefore, the supplementation of deoxycytidine would prevent this inhibition both by supplying dCMP and, through the action of dCMP deaminase, to leading to the production of dUMP.

In this paper, we provide some evidence for an additional explanation on how dTTP or thymidylate metabolites could affect DNA synthesis other than through the allosteric inhibition of ribonucleotide reduction by dTTP. We propose that dTTP or thymidylate metabolites may affect DNA synthesis and cellular replication by decreasing the level of ribonucleotide reductase.
The significant increase in the level of ribonucleotide reductase activity after exposure of HeLa cells and partially hepatectomized rats to 2 antimitabolites of thymidylate synthesis supports the thesis that dTTP or a substrate dependent on thymidylate formation may act as a repressor or corepressor in mammalian cells. This hypothesis is further supported by the fact that this enhancement of reductase activity is dependent on continued protein synthesis. CXM, a protein synthesis inhibitor, prevented the enhancement of ribonucleotide reductase activity when it was added along with MTX or 5FUdR in HeLa cells. It is unlikely that MTX or 5FUdR treatment causes the production of a stimulatory substance, because when an untreated and a MTX- or 5FUdR-treated extract are assayed in combination (Table 6) the mixture does not result in a synergistic situation, but rather in values predicted by the level of activity in each extract measured separately. The possibility that MTX or 5FUdR exerts its effect by the removal or the lowering of the concentration of an inhibitory substance is more difficult to assess. However, mixing experiments gave no indication of the presence of an inhibitory substance.

There has accumulated from bacterial studies a considerable amount of evidence that dTTP serves as a repressor or corepressor of ribonucleotide reductase synthesis. Direct evidence for involvement of dTTP as a repressor of ribonucleotide reductase was provided by the findings of Biswas et al. (4), who found that thymine starvation of E. coli strain MT15 - the treatment of E. coli B with 5FUdR results in a 10-fold increase in reductase activity in cellular extracts which was lowered when thymine was added to the starved cells. This increase in enzyme levels apparently represents de novo protein synthesis because the increase in enzyme activity following thymine withdrawal was prevented if chloramphenicol was added concomitantly with thymine removal. This experiment is comparable to our results with CXM (Table 5). However, Cannon and Breitman (8) found that, when E. coli MT15 was grown in the presence of TdR, no appreciable difference in reductase activity was observed between the TdR-supplemented and unsupplemented cell extracts.

In L. leichmannii, a bacterium requiring cyanocobalamin or deoxyribosyl compounds for growth, it has been shown that the inclusion in the media of high concentrations of deoxyribonucleoside compounds or cyanocobalamin caused a lowering of reductase activity (18). In addition, in strain 7830, depleted in folic acid, thymine removal from the media caused a large increase in reductase activity (3).

The addition of 5FU to a synchronized culture of Saccharomyces cerevisiae enhanced the increase of ribonucleotide reductase activity during S phase (25). The increase and prolongation of reductase activity by 5FU could be blocked by CXM.

There are numerous studies showing that MTX, 5FUdR, or 5FU treatment leads to a decrease in the intracellular pools of dTTP in mammalian cells (1, 16, 20, 35, 44). It has been shown that in L929 cells (1), 5178Y lymphoma cells (44), human lymphocytic cell subline CCRF-CEM (16), and phytohemagglutinin-stimulated human lymphocytes (20, 35) MTX treatment leads to a rapid decrease in the pool size of dTTP. 5FU exposure also causes a large decrease in the dTTP pool in lymphoma cells (44). In contrast, Baumunk and Friedman (2) reported that they did not observe any change in the pool size of dTTP following the exposure of HeLa cells to MTX and 5FUdR; however, Tattersall and Harrap (44), who found a significant decrease in dTTP pool size after MTX or 5FU treatment, attribute the findings of Baumunk and Friedman to their washing procedure.

The effect of MTX and 5FU on the pool size of dATP, dGTP, and dCTP has been variable (1, 16, 20, 44). The pool sizes of these deoxyribonucleotides do not lend themselves to an obvious pattern which can be explained by the allosteric regulation of the conversion of ribonucleotides to deoxyribonucleotides.

The previous explanations for the inhibition of DNA synthesis and cellular replication by TdR, i.e., that it exerts its effect via dTTP as a negative allosteric effector on the reduction of CDP to dCDP, should be reexamined, inasmuch as enzyme repression may also be a contributing factor to the overall reduction in deoxyxypuridine synthesis after high TdR exposure. The data reported in this paper support a pivotal role of dTTP in the genetic expression of ribonucleotide reductase in the mammalian cell.

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16. Harrap, R. S., depleted in folic acid, thymine removal from the media caused a large increase in reductase activity (3).

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Effect of Methotrexate and 5-Fluorodeoxyuridine on Ribonucleotide Reductase Activity in Mammalian Cells

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