Effect of Methotrexate on Thymidylate Synthetase in Cultured Parenchymal Cells Isolated from Regenerating Rat Liver

Robert J. Bonney and Frank Maley
Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201

SUMMARY

The effect of methotrexate (MTX) on thymidylate synthetase activity during liver regeneration was examined with parenchymal cells isolated 22 and 44 hr after partial hepatectomy and cultured as a monolayer. The synthetase activity in these cells decreased with a half-life of 18 to 24 hr, but if MTX (1.5 x 10^{-8} to 1.5 x 10^{-6} M) was present in the culture media, this decline could be delayed for at least 48 hr. In contrast, thymidine kinase activity decreased at a rate which was unaffected by MTX. Dihydrofolate reductase was inhibited at all concentrations of MTX used to block the decrease in synthetase activity. Folic acid at 10^{-4} M, although less effective than MTX, also delayed the decrease in synthetase activity. The addition of cycloheximide, puromycin, or actinomycin D to the culture media did not alter the response of the synthetase to MTX. The latter studies, coupled with those indicating that the rapid loss of synthetase activity in crude extracts could be prevented by MTX or, more effectively, by MTX plus deoxyuridine 5'-monophosphate, suggest that the primary effect of MTX on thymidylate synthetase in vivo is that of enzyme stabilization. Similar stabilizing effects were obtained in liver cell extracts with 10^{-8} M deoxyuridine 5'-monophosphate in combination with 10^{-4} M folate or 10^{-4} M dihydrofolate.

INTRODUCTION

dTMP synthetase and several other enzymes associated with DNA synthesis are induced in the rat liver following partial hepatectomy (7, 9, 18, 23, 26, 28). The nature of this inductive process is modified, however, by i.p. injection of MTX immediately following and again 24 hr after the operation. Thus it was shown by Labow et al. (23) that, of 5 enzymes measured in liver extracts from treated rats, only dTMP synthetase continued to increase at an accelerated rate beyond 48 hr. The kinetic profiles of the other 4 enzymes (thymidine kinase, thymidine kinase, deoxycytidine deaminase, and DNA polymerase), although similar, were inhibited at least 50% following MTX treatment. In addition, the injection of MTX into rats bearing minimal deviation hepatomas produced elevated dTMP synthetase levels in these tumors (27). The MTX effect is not limited to cells associated with liver; a similar increase in synthetase activity was observed in DON Chinese hamster cells (11) and in cells from a leukemia line (35).

Recently, procedures were reported for the isolation (1, 6, 37, 38) and cultivation (2, 5, 20) of homogeneous populations of adult rat liver parenchymal cells. A study was undertaken, therefore, to determine the effects of MTX on dTMP synthetase levels in parenchymal cells isolated from regenerating liver and maintained in primary culture. With this more direct approach, the complications produced by mixed cell populations and the influence of metabolites formed by other organs are avoided, and the mechanism by which the endogenous levels of dTMP synthetase are influenced by MTX can be examined.

MATERIALS AND METHODS

Materials. Ham's F12 medium was purchased as dry powder from Grand Island Biological Co., Grand Island, N. Y., and undialyzed fetal calf serum was from Microbiological Associates, Bethesda, Md. MTX was obtained from Lederle Laboratories Division, Pearl River, N. Y., and insulin was from Eli Lilly & Co., Indianapolis, Ind. Act D, puromycin, cycloheximide, folic acid, and dUMP were purchased from Sigma Chemical Co., St. Louis, Mo. [4,5-3H]Leucine, [6-14C]orotic acid, [methyl-3H]thymidine, and [5-3H]dUMP were obtained from Schwarz/Mann, Orangeburg, N. Y. The [5-3H]dUMP was purified by chromatography on a column of Dowex 1-formate (24) and lyophilized. FH2 was prepared from folic acid by the method of Blakley (3).

Animals. Albino Wistar rats, 120 to 130 g, were fed ad libitum a pelleted diet containing 26% protein and 7% fat (Charles River Formula 7RF). Partial hepatectomies (70%) were performed according to the method of Higgins and Anderson (15). Sham operations consisted of an abdominal incision and closure.

Cell Isolation and Culture Conditions. Parenchymal cells were isolated from unoperated rats, from rats 22 and 44 hr after partial hepatectomy, and from sham-operated animals, as previously described (6). This isolation technique permits the recovery of 20 to 50% of the available paren-
enchymal cells. For enzyme determinations, cell-free extracts were prepared from liver remnants or from freshly isolated cells in isotonic KCl containing 0.2 M mercaptoethanol, with the use of a Potter-Elvehjem tissue homogenizer. The homogenate was centrifuged at 105,000 × g for 1 hr at 4° in a Beckman Model L2-65B centrifuge. Alternatively, the isolated cells were suspended at a concentration of 0.6 × 10⁶ cells/ml in Ham’s F12 medium containing 15% fetal calf serum and 10 μinisulin per ml. Ten ml of the suspended cells were plated on Falcon plastic dishes (100 mm in diameter) as described in an earlier report (5). At specified times the cells were harvested as follows. The media and one 4-ml rinse with PBS were aspirated from the plates and discarded. One ml of KCl-mercaptoethanol was added to each plate, and the contents were removed with a rubber policeman. The cells from 2 plates were combined, homogenized with a tight-fitting glass Dounce homogenizer, and centrifuged at 105,000 × g for 1 hr. Enzyme activities were determined on the supernatant fraction.

**Enzyme Assays.** dTMP synthetase (EC 2.1.1.1b) was measured by the amount of tritium released from [5-³H]dUMP (34) and was determined to be linear with incubation time and with protein concentration. FH₂ reductase (EC 1.5.1.3) was assayed spectrophotometrically at pH 6.5 and pH 9.0 by the method of Mathews (29); thymidine kinase (EC 2.7.1.75) was assayed by the method described by Labow et al. (23).

**Determination of Macromolecular Synthesis.** Incorporation of radioactive leucine into material precipitated with 10% TCA was used as a measure of protein synthesis. At the time of initial plating, 10 μCi of [4,5-³H]leucine were added to each plate in the presence or absence of the various protein synthesis inhibitors. At specified times, the media and 2 rinses with PBS were aspirated, and the plates were placed on a block of dry ice and stored frozen. To determine the extent of incorporation, the cells were subsequently thawed, 4.0 ml of 10% TCA were added, and the contents of the plates were transferred to conical centrifuge tubes. After centrifugation at 3000 rpm for 10 min in a Sorvall RC III, the pellets were washed with 5% TCA until the radioactivity in the washes was less than twice background (3 to 4 washes). Each pellet was then dissolved in 1 N NaOH, and aliquots were removed for the determination of radioactivity and protein. The latter was determined by the method of Lowry et al. (25).

RNA synthesis was measured by the incorporation of [14C]orotic acid into material precipitated with 1 N NaOH. At the time of initial plating, 10 μCi of [14C]orotic acid were added to each plate. At specified times, the media and 2 rinses with PBS were aspirated, and the plates were placed on a block of dry ice and stored frozen. To determine the extent of incorporation, the cells were subsequently thawed, 4.0 ml of 10% TCA were added, and the contents of the plates were transferred to conical centrifuge tubes. After centrifugation at 3000 rpm for 10 min, the pellets were washed with 5% TCA until the radioactivity in the washes was less than twice background (3 to 4 washes). Each pellet was then dissolved in 1 N NaOH, and aliquots were removed for the determination of radioactivity and protein. The latter was determined by the method of Lowry et al. (25).

**RESULTS**

**Effect of MTX on dTMP Synthetase in Parenchymal Cells during Liver Regeneration.** The livers of rats given an injection of MTX immediately after a 70% partial hepatectomy and again after 24 hr possess a higher level of dTMP synthetase than those normally obtained at 48 and 72 hr after the operation (Chart 1). Similar results were reported by Labow et al. (23). To determine the cell type responsible for this increase, dTMP synthetase was measured in extracts prepared from purified parenchymal cells isolated 24, 48, and 72 hr after the partial hepatectomy of both untreated rats and rats given injections of MTX. The results presented in Chart 1 establish that the increase in activity in extracts from regenerating liver is associated with the parenchymal cell.

**Effects of MTX and Folic Acid on dTMP Synthetase in Cultured Parenchymal Cells.** Parenchymal cells isolated from liver 22 and 44 hr after partial hepatectomy were maintained in primary culture for 48 hr in the presence and absence of MTX. In the absence of MTX, dTMP synthetase...
produced a significant change in the dTMP synthetase activity.

In contrast to the MTX stimulation of thymidine kinase in Chang liver cells (12), no effect of MTX on kinase levels in parenchymal cells was observed. The kinase activity decreased rapidly in the cultures and could not be detected after 24 hr.

The primary intracellular target for MTX appears to be the enzyme FH₄ reductase (4). The activity of this enzyme in liver has been reported to be inhibited by MTX in some cases (23, 32) but not in others (8). In an attempt to resolve these conflicting results, the activity of dTMP synthetase and FH₄ reductase was determined in parenchymal cells maintained in culture for 24 hr in the presence of various concentrations of MTX. While the activity of dTMP synthetase was increased by MTX at concentrations be-

---

**Chart 1.** Effect of MTX on dTMP synthetase activity in regenerating liver and in parenchymal cells isolated during regeneration. MTX, when injected i.p. (0.5 mg/125-g rat) was administered just after the operation and again 24 hr later. Enzyme levels were determined in extracts prepared from the liver remnants or from isolated parenchymal cells. The values obtained for the liver remnants at 24 hr were the average obtained from 2 rats. All other points represent the mean ± S.D. of values obtained from 3 rats.

activity decreased, with a half-life of 18 to 24 hr. A similar decrease occurred even if the cells were isolated from rats that received MTX immediately following the operation (Chart 2A). On addition of 10⁻⁸ M MTX to the cultures, however, the activity of the enzyme was maintained at a constant level. Similar results were obtained whether the cells were isolated 22 or 44 hr after the partial hepatectomy, although the initial enzyme activities in the latter case were twice those in the former (Chart 2B). As indicated, the synthetase activity in cells from rats not given injections of MTX decreased rapidly in the control cultures, but on exposure of the cells to MTX following isolation, the activity decreased for 6 hr and then leveled off. These results are particularly striking in the case of cells from 44-hr regenerating liver (Chart 2B), and they suggest that parenchymal cells previously unexposed to MTX require a lag period before the MTX added to the cultures can influence synthetase activity.

Continuous exposure to MTX is essential if a constant synthetase level is to be maintained, since cells treated in culture with MTX for 24 hr and then incubated without MTX for another 24 hr decreased in enzyme activity to the same degree as did cells in control cultures.

Since folate also promotes an increase in dTMP synthetase in regenerating liver (23), the effect of this compound on synthetase levels in cultured parenchymal cells was tested. Folic acid (Table 1) prevented the decrease in dTMP synthetase activity observed with control cultures but, as in the case of the intact animal (23), the synthetase responded less effectively to folic acid than to MTX. Neither the addition of folic acid nor of MTX to parenchymal cells isolated from sham-operated animals and cultured for 24 hr

---

**Chart 2.** dTMP synthetase activity in parenchymal cells isolated during liver regeneration and maintained in primary culture with or without MTX. A, cells were isolated 22 hr after partial hepatectomy from animals given injections of MTX (●, ○) and cultured in the presence and absence of 10⁻⁸ M MTX or were isolated from animals that did not receive MTX (▲, △) and were cultured in the presence and absence of MTX; B, cells were isolated 44 hr after the operation from MTX-treated animals and cultured in the presence and absence of MTX or were isolated from untreated rats and cultured with and without MTX. The enzymatic assay was assayed in duplicate at the indicated times, with each point representing an average of the determinations from duplicate sets of plates. The variability of each point from the average was less than 10%.
Effect of MTX on Enzymes in Cultured Liver Cells

The cells were isolated from rats 22 hr after partial hepatectomy or 22 hr after a sham operation and were cultured as described in “Materials and Methods.” The zero-time specific activity was 0.680 for regenerating parenchymal cells and 0.040 for cells isolated after a sham operation. Each point is the average of duplicate determinations. A unit of activity represents the amount of enzyme necessary to convert 1 nmole of dUMP to dTMP in 30 min under the conditions of the assay.

### Table 1

Effect of folate and MTX on dTMP synthetase activity in parenchymal cells during 24 hr in primary culture

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Addition</th>
<th>Units/mg protein at 24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial hepatectomy</td>
<td>None</td>
<td>0.169</td>
</tr>
<tr>
<td></td>
<td>MTX, 10^{-6} M</td>
<td>0.500</td>
</tr>
<tr>
<td></td>
<td>Folic acid, 10^{-4} M</td>
<td>0.339</td>
</tr>
<tr>
<td>Sham operation</td>
<td>None</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>MTX, 10^{-6} M</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td>Folic acid, 10^{-4} M</td>
<td>0.034</td>
</tr>
</tbody>
</table>

MTX. As indicated in the legend to Table 4, the concentration in the media was found to be 1.00 to 1.03 \times 10^{-6} M, verifying the reliability of the assay method used. The drug was rapidly taken up by the cells over the first 2 hr with a maximum attained between 4 and 8 hr. By using a value of 2.48 μl of intracellular H2O per mg of protein the concentration of MTX in the cells was calculated to be 2.3 to 3.0 \times 10^{-6} M after 8 hr. Extracts prepared from control cells untreated with MTX did not inhibit the FH2 reductase used in the assay.

The total amount of MTX accumulated in the cells at 8 and 22 hr was found to exceed the FH2 reductase content of the cells by about 10-fold. Thus, it was determined (40) that the level of FH2 reductase in the cultured cells was 5.5 and 4.6 pmoles/mg protein at 8 and 22 hr, respectively.

Stabilization of dTMP Synthetase in Cell-free Extracts Prepared from Regenerating Liver Parenchymal Cells. The activity of dTMP synthetase in extracts prepared from regenerating liver parenchymal cells when incubated at 37° tween 1.5 \times 10^{-6} and 1.5 \times 10^{-6} M (Chart 3), that of FH2 reductase was almost completely inhibited under these conditions.

### Table 2

<table>
<thead>
<tr>
<th>Addition</th>
<th>Protein synthesis (µM)</th>
<th>Specific activity (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
<td>0.45</td>
</tr>
<tr>
<td>MTX, 10^{-6} M</td>
<td>97</td>
<td>1.02</td>
</tr>
<tr>
<td>MTX, 10^{-6} M + puromycin (3 µg/ml)</td>
<td>31</td>
<td>1.15</td>
</tr>
<tr>
<td>MTX, 10^{-6} M + cycloheximide (1 µg/ml)</td>
<td>4</td>
<td>1.05</td>
</tr>
</tbody>
</table>

MTX alone at 10^{-6} M had a minimal effect on the incorporation of [14C]orotic acid into RNA and decreased the incorporation in this experiment of [4,5-3H]leucine into protein by only 25% (Table 3). When 5 µg of Act D per ml were added to the cultures containing MTX, total RNA synthesis was decreased by 99%, while protein synthesis was unaffected. Despite this marked inhibition of RNA synthesis, MTX maintained a constant level of synthetase activity in the cultured cells for 24 hr.

MTX Levels in Cultured Cells. The above-described results indicate that MTX influences dTMP synthetase levels in the cultured cells independently of RNA or protein synthesis, an effect that could be attributed to the stabilization of the synthetase against metabolic turnover. This protection could be provided by MTX directly or could be due to certain compounds which are elevated by the action of MTX on cellular metabolism. Preliminary experiments indicated that the addition of MTX to crude cellular extracts at 10^{-6} M, the same level as that added to the cell cultures, did not stabilize dTMP synthetase against inactivation at 37°. Since MTX might be concentrated by the cells, as found for L1210 cells (40, 41), the level of MTX was determined (40) in the culture media and in cellular extracts at various times after the addition of 1.3 \times 10^{-6} M MTX.

The effects of protein synthesis inhibitors on the maintenance of dTMP synthetase in cultured cells by MTX

Cells were isolated from rat liver 24 hr after partial hepatectomy and were cultured for 24 hr. Enzyme activity, in addition to protein synthesis, was determined from cell-free extracts as described in “Materials and Methods.” The results for protein synthesis are the average of duplicate plates. The synthetase activities represent the average obtained from duplicate determinations in separate experiments (Experiments 1 and 2).
R. J. Bonney and F. Maley

**Table 3**

*dTMP synthetase activity in cultured cells in the presence of MTX and Act D*

Cells were isolated from rats 22 hr after partial hepatectomy and cultured for 24 hr. Enzyme activity, in addition to RNA and protein synthesis, was determined from cell-free extracts as described in “Materials and Methods.” The results for protein and RNA synthesis represent the average of determinations made from duplicate plates with a variability of less than 15%. The enzyme activity is the average of duplicate determinations.

<table>
<thead>
<tr>
<th>Addition</th>
<th>% of control with Specific activity RNA synthesis</th>
<th>Protein synthesis (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>MTX, 10⁻⁴ M</td>
<td>94</td>
<td>75</td>
</tr>
<tr>
<td>MTX, 10⁻⁴ M + Act D</td>
<td>1</td>
<td>75</td>
</tr>
<tr>
<td>(5 μg/ml)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 4**

*Intracellular accumulation of MTX by cultured parenchymal cells*

Cells isolated from rats 22 hr after partial hepatectomy were cultured for 22 hr in the presence of 1.3 × 10⁻⁴ M MTX. The media and cellular extracts were assayed for MTX content as described in “Materials and Methods.” The concentration in the media for Experiment 1 was found to be 1.02 to 1.03 × 10⁻⁴ M, and for Experiment 2, 1.00 to 1.03 × 10⁻⁴ M. The molarity of MTX was calculated using a value for intracellular H₂O of 2.48 μl/mg total protein. The results presented are from 2 separate experiments.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Total accumulation (pmoles/mg protein)</th>
<th>Intracellular concentration (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment 1</td>
<td>Experiment 2</td>
</tr>
<tr>
<td>0.5</td>
<td>35.6</td>
<td>40.6</td>
</tr>
<tr>
<td>2</td>
<td>43.2</td>
<td>48.5</td>
</tr>
<tr>
<td>4</td>
<td>57.2</td>
<td>74.0</td>
</tr>
<tr>
<td>8</td>
<td>56.0</td>
<td>74.0</td>
</tr>
<tr>
<td>22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

The results of these experiments suggest that the primary effect of MTX on dTMP synthetase in cultured parenchymal cells isolated from regenerating liver is that of enzyme stabilization. Several other reports have appeared on the

![Chart 4](chart4.png)

Chart 4. Effect of MTX and dUMP on the inactivation of dTMP synthetase in crude extracts. *A,* parenchymal cells were isolated 44 hr after partial hepatectomy, and equal portions of the cells were homogenized in buffer (0.25 M sucrose, 0.5 mM dithiothreitol, 0.1 M KCl, and 0.075 M Tris, pH 7.5) or buffer plus the various levels of MTX indicated in the chart. The high-speed supernatant fractions were incubated at 37°, and the enzyme was assayed at the indicated times. The initial specific activities were: no addition, 3.01; 10⁻⁴ M MTX, 3.00; 10⁻⁴ M MTX, 2.68; 5 × 10⁻⁴ M MTX, 2.31; 10⁻⁴ M MTX, 1.91. In a separate experiment, *B,* equal portions of the isolated cells were homogenized in buffer or buffer plus the indicated additions, and the high-speed supernatants were incubated at 37°. The synthetase was assayed at the indicated times. *Points,* average of duplicate assays with a variability of less than 10%.

![Chart 5](chart5.png)

Chart 5. Effect of dUMP, folic acid, and FH₄ on the inactivation of dTMP synthetase in crude extracts. Parenchymal cells were isolated 44 hr after partial hepatectomy, and equal portions of the cells were homogenized in buffer (see legend to Chart 4) or buffer plus the indicated additions. The high-speed supernatant fractions were incubated at 37°, and the enzyme assayed at the indicated times. *Points,* average of duplicate assays with a variability of less than 10%.
**Effect of MTX on Enzymes in Cultured Liver Cells**

regulation of enzyme turnover by specific ligands, e.g., FH₂ reductase turnover is decreased by MTX (16), tyrosine aminotransferase is decreased by L-tryptophan (10), and ornithine decarboxylase is decreased by several amino acids (17). In the case of dTMP synthetase in cultured liver cells, as indicated in this study, MTX alone or in combination with dUMP may bind to the enzyme and so protect it from metabolic turnover. A similar finding was reported by Roberts and Loehr (35) using a leukemia cell line, while McBurney and Whitmore (30) reported that MTX can inhibit dTMP synthetase directly in cultured Chinese hamster ovary cells, indicating that this compound can bind to the enzyme. Similar inhibitory effects of MTX on dTMP synthetase were obtained by us with the liver cells (see legend to Chart 4A).

Since the levels of MTX that were found to inhibit and to stabilize dTMP synthetase activity in extracts (Chart 4) were at least 10-fold higher than that added to the cell cultures, it was important to determine whether the cultured cells were capable of concentrating MTX in a manner similar to that found for L1210 cells in culture (40). As indicated in Table 4, liver cells accumulated MTX at a rapid rate, which finally reached a plateau at between 8 and 22 hr, to yield an intracellular concentration of MTX calculated to be 2.3 to 3.0 x 10⁻⁴ M. Chart 4 reveals that concentrations of MTX at 10⁻⁴ M or greater stabilize the enzyme in crude extracts. The calculated molarities are likely to be high, as MTX is in part bound to certain proteins such as FH₂ reductase. The latter effect may be offset somewhat by the finding that 10-fold more MTX accumulates intracellularly than is bound to FH₂ reductase, and by the fact that dUMP may enhance the stabilizing effect of MTX (Chart 4B). Although a direct extrapolation from crude extract to the cultured cells is not possible, the data suggest that dIMP synthetase is stabilized against turnover by a direct interaction with MTX alone or in combination with dUMP.

Similarly, 10⁻⁴ M folic acid, which only partially stabilized the enzyme in the cultured cells, also partially stabilized the enzyme in the crude extracts. The difference between folate and MTX stabilization could be due to a difference in affinity for dTMP synthetase. While MTX stabilization alone or in combination with dUMP may be the primary explanation for the maintenance of synthetase levels in culture, at present the possibility cannot be excluded that the alteration in the quantity and quality of intracellular folate derivatives by MTX may, in part, be responsible for the observed effects. Thus, since FH₂ reductase is completely inhibited at all concentrations of MTX associated with elevated synthetase levels, it is not inconceivable, under these conditions, that intracellular levels of FH₂ should increase and FH₄ and 5,10-methylene-FH₄ decrease, although evidence relating to these alterations has been contradictory (30, 33). If dUMP levels should rise simultaneously with FH₂, another potential means of stabilizing the synthetase would be provided (Chart 5). Evidence that FH₂ binds to the synthetase was obtained by demonstrating that FH₂ is a competitive inhibitor of 5,10-methylene-FH₄ (24) and also from circular dichroism studies (13). Recent findings by Kisliuk et al. (22) have shown that the polyglutamate derivatives of FH₂ inhibited the L. casei dTMP synthetase 10 to 100 times more effectively than did FH₂ alone. Since folate exists in rat liver primarily in the form of reduced polyglutamates (19, 39), the stabilizing effects of FH₂ and dUMP described in Chart 5 would be even more significant if the rat liver synthetase was affected by the dihydrofolypolyglutamates in a manner similar to that described for the L. casei enzyme.

The equilibrium level of a specific cellular protein is determined by its rate of synthesis and degradation, and any factor influencing either rate will shift the equilibrium accordingly. The maintenance of constant synthetase levels by MTX in the culture system suggests that while effective removal of the enzyme in question is prevented by MTX, little or no synthesis of the enzyme is occurring. In contrast, the synthetase levels in the regenerating livers of rats given injections of MTX (23) continue to increase considerably in specific activity at times when the synthetase levels in control rats are decreasing (Chart 1). Whether the increase represents a continuation of enzyme synthesis superimposed on an impaired decay rate is difficult to establish definitively without antibody to the synthetase.

Recent studies by Kawai and Hillcoat (21) suggest that dTMP synthetase and FH₂ reductase may influence each other’s activity in vitro mainly by their competition for MTX, FH₂, and dUMP. While competitive inhibition may explain some of the observed responses, the influence of these compounds on enzyme stability should be considered in the light of our studies. Also, the apparent stimulatory effect of MTX on dTMP synthetase activity that they reported was obtained in a case in which the ratio of FH₂ reductase to dTMP synthetase was extremely high. Of interest is their finding that MTX can enhance the incorporation of deoxyuridine into DNA, possibly as a consequence of the elevated levels of dTMP synthetase promoted by MTX. Our results tend to support this finding and suggest that any chemotherapeutic approach that promotes such an increase may require another agent to counteract the potential rebound effect that may attend the cessation of therapy or which might result from the development of resistance.

**ACKNOWLEDGMENTS**

We wish to thank Zeina Nimec for her technical assistance and Dr. John H. Galivan for performing the FH₂ reductase assays.

**REFERENCES**

R. J. Bonney and F. Maley


Effect of Methotrexate on Thymidylate Synthetase in Cultured Parenchymal Cells Isolated from Regenerating Rat Liver

Robert J. Bonney and Frank Maley


Updated version

Access the most recent version of this article at:

http://cancerres.aacrjournals.org/content/35/8/1950

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/35/8/1950. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.