Plasma Homocysteine in Children with Acute Lymphoblastic Leukemia: Changes during a Chemotherapeutic Regimen Including Methotrexate

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ABSTRACT

Plasma homocysteine was determined in 12 children with acute lymphoblastic leukemia. The patients were investigated prior to chemotherapy (stage I), during seven weeks of induction chemotherapy (stage II), and thereafter during intermittent high-dose methotrexate (HD-MTX) therapy (stage III). The patients were followed for a period of three to 15 months, and the study included a total of 80 HD-MTX courses.

Before start of chemotherapy (stage I), the average plasma homocysteine level in the children with leukemia was 13.18 ± 6.23 (SD) μmol/liter, which is significantly (P < 0.001) higher than the level in control children (6.52 ± 1.21 μmol/liter). The plasma homocysteine level in the patients was positively correlated with the peripheral white blood cell count (P < 0.01) and negatively correlated with serum folate (P < 0.02). The serum folate was normal or subnormal in these patients.

During induction therapy with cytotoxic drugs such as vincristine, cisplatin, and intrathecal MTX (stage II), there was a drastic change in plasma homocysteine as a function of time. A reciprocal alteration in serum folate was observed, suggesting fluctuating intracellular folate status at this stage of therapy. At the end of stage II (about seven weeks), there was a significant (P < 0.01) reduction in total homocysteine (to 7.08 ± 3.84 μmol/liter).

HD-MTX (8 g/m²) therapy with 5-formyltetrahydrofolate “rescue” (stage III) was usually begun about seven weeks after start of chemotherapy, and the patients were followed for two to eight courses separated by three to eight weeks. Plasma homocysteine showed a transient increase (26–64%) following each MTX infusion. After three MTX infusions, basal plasma homocysteine was reduced to 5.56 ± 1.2 μmol/liter. During most MTX infusions, there was a variable reduction (17–56%) in plasma methionine followed by a rebound increase.

It is concluded that plasma homocysteine in children with acute lymphoblastic leukemia is elevated prior to therapy, probably because of occasional folate deficiency and increased burden of proliferating cells. During induction therapy, monitoring plasma homocysteine and serum folate both suggest a labile folate homeostasis, usually a deficiency state. HD-MTX induced a temporary intracellular folate depletion before 5-formyl-tetrahydrofolate was administered, as judged by a transient homocysteinaemia. The methionine depletion may interfere with the antileukemic effect of MTX.

INTRODUCTION

Plasma Hcy reflects the balance between the intracellular formation and utilization of this sulfur compound. Intracellular Hcy is a product of S-adenosylmethionine-dependent transmethylation reactions, and it is either degraded or remethylated to methionine. In most tissues the conversion to methionine is catalyzed by the enzyme methionine synthase, which requires 5-methyl-THF as methyl donor and methylcobalamin as cofactor. This is the biochemical basis for the observations that plasma Hcy shows a pronounced increase in folate (2) and cobalamin deficiencies (3). Plasma Hcy is also elevated in hyperproliferative states such as psoriasis and some malignant diseases, and during treatment with drugs interfering with cobalamin or folate metabolism or function, including the classic antifolate drug MTX (4). The plasma Hcy level merits attention because it may be a useful indicator during diagnosis and follow-up of these deficiency states, and it is a measure of dynamic effects of some drugs (4).

MTX is an antifolate drug that inhibits the regeneration of tetrahydrofolate from dihydrofolate (5) and thereby decreases the cellular content of reduced folates. It is used for the treatment of acute leukemia and several solid tumors (6), but also in the management of some nonmalignant diseases, such as psoriasis and rheumatoid arthritis (7, 8). Quite different MTX regimens are used in these diseases. In the nonmalignant states, MTX doses are 7–25 mg weekly (7, 8), whereas cancers are treated with doses from 100 mg to 33.6 g/m². HD-MTX (≥1 g/m²) with 5-formyl-THF “rescue” is currently used in the management of acute lymphoblastic leukemia in children (6).

The results obtained with cultured cells exposed to MTX (9) prompted us to investigate the effect of MTX on plasma Hcy. We have previously investigated the effect of 2 different MTX regimens given to patients with psoriasis (10) and solid tumors (11), respectively. Prior to treatment, the psoriasis patients had moderately elevated plasma Hcy assigned to the large burden of proliferating germinative cells or deteriorated folate status (10), whereas the plasma level was normal in most patients with solid tumor (11). The psoriasis patients receiving low-dose MTX (25 mg weekly) showed a maximal plasma Hcy increase 24–72 h after dosing, and the level reached pretreatment values within 1 week. This suggests that plasma Hcy is a sensitive measure of antifolate effect (10). MTX in doses of 1–3.8 g given to adult patients with solid tumors induced a rapid, marked increase in plasma Hcy, which was reversed upon administration of 5-formyl-THF “rescue” (11). These findings demonstrate the responsiveness of the plasma homocysteine level to MTX exposure and altered folate status.

Plasma Hcy seems to reflect the intracellular antifolate effect of MTX, which has been explained by efficient cellular depletion of 5-methyl-THF (12, 13) and thereby inhibition of Hcy remethylation (4). However, in vitro studies suggest that cellular Hcy egress and thereby plasma Hcy level is modulated by other factors that may rapidly change prior to and during chemotherapy of cancer. These factors include the burden of rapidly proliferating malignant cells and the cytotoxic effect of chemotherapy on cancer cells and normal tissues.

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: Hcy, homocysteine; 5-formyl-THF, 5-formyl-tetrahydrofolate; HD-MTX, high-dose methotrexate; 5-methyl-THF, 5-methyl-tetrahydrofolate; MTX, methotrexate; ALL, acute lymphoblastic leukemia; WBC, white blood cells.

4 H. Refsum, B. Christensen, R. Djuherus, and P. M. Ueland. The interaction between methotrexate, “rescue” agents and cell proliferation as modulators of homocysteine export from cells in culture, submitted for publication.
In the present work, we extended previous investigations on Hcy and MTX (10, 11, 14) by evaluating the modulation of plasma Hcy by the cancer state itself and the cytoreductive therapy including MTX in children with ALL. These patients were chosen because they have a large burden of rapidly proliferating cells, and preliminary observations (15) indicated an elevation of plasma Hcy in patients with ALL. We followed the patients prior to treatment, during induction and maintenance therapy with intermittent HD-MTX.

MATERIALS AND METHODS

Chemicals. MTX and 5-formyl-THF were obtained from Nycomed, Oslo, Norway. L-Methionine, L-homocysteine, 2-mercaptoethanol, and o-phthaldialdehyde were purchased from Sigma Chemical Co., St. Louis, MO. Sodium borohydride was from Fluka Chemie, AG, Switzerland, and monobromobimane was from Calbiochem-Behring Diagnostics, La Jolla, CA.

Patients and Controls. Twelve consecutive patients with ALL admitted to the Department of Pediatrics were enrolled in the study. Standard diagnostic procedures included bone marrow aspiration and immunocytochemical phenotyping, and all 12 patients had leukemia of the early B-cell lineage. Their age, sex, weight, serum cobalamin, and folate cytochemical phenotyping, and all 12 patients had leukemia of the early B-cell lineage. Their age, sex, weight, serum cobalamin, and folate values prior to treatment are given in Table 1.

Control subjects were 10 children (6 males and 4 females) admitted to the orthopedic department for elective surgery. Their mean age was 6½± 3½ years.

Protocol. All patients received the same chemotherapeutic regimen. This regimen is given in detail elsewhere (16). We divided the observation period of this study into 3 stages, referred to as stages I, II, and III in chronological order.

Stage I is the time of admission to the hospital immediately before chemotherapy was initiated.

Stage II is the first 7–8 weeks of induction therapy. The patients received weekly i.v. doses of vincristine (2 mg/m², maximum 2 mg) and intrathcal MTX (6–12 mg) for 4 weeks followed by i.v. administration of asparaginase (1000 IU/kg) for 10 days. Prednisone (60 mg/m²) was administered daily for 5 weeks.

Stage III is the period of HD-MTX therapy with 5-formyl-THF "rescue." This could be separated into consolidation and maintenance therapy. The first period (consolidation) usually started 7 weeks after start of chemotherapy (stage II), and then weekly until start of the HD-MTX courses (stage III).

During each HD-MTX course, blood samples were drawn before and 24, 36, 48, and 72 h after infusion.

All patients, except P. B. (followed during 2 infusions), were followed during 3 to 8 MTX courses, corresponding to 3–15 months.

Blood samples were collected into EDTA vacutainers, immediately placed on ice, and plasma prepared by centrifugation within 15 min. A portion of plasma was deproteinized by adding perchloric acid, which was neutralized and removed, as described (11, 17). Plasma was stored at −80°C until analysis.

Analytical Methods. Total Hcy in plasma was determined using a fully automated assay recently developed in our laboratory. The procedure involves reduction of protein-bound Hcy with NADH, followed by derivatization of reduced Hcy with monobromobimane and finally quantitation of the Hcy-monobromobimane adduct with fluorescence detection (18).

Plasma methionine was determined in deproteinized plasma with an assay based on derivatization with o-phthaldialdehyde and fluorescence detection (19). Serum cobalamin and serum folate were determined with radioassay kits from Diagnostic Product Corp., Los Angeles, CA.

MTX and 7-hydroxy-MTX were determined in plasma deproteinized by mixing samples with an equal volume of 100% acetonitrite. Samples of 25 µl were injected into a 10-cm 3-µm ODS Hypersil column equilibrated with 10 mmol/liter ammonium phosphate buffer, pH 5.5, containing 5% methanol. The column was eluted at ambient temperature with a methanol gradient in ammonium phosphate buffer. Methanol increased from 5 to 22.5% over 5 min. The flow rate was 2 ml/min. The absorbance of the effluent was recorded at 290 nm using a variable absorbance detector, model Spectroflow 773 from Kratos An-

<table>
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<th>Patient</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Wt (kg)</th>
<th>Hb (g/100 ml)</th>
<th>WBC* (10⁹ cells/liter)</th>
<th>Serum cobalamin* (nmol/liter)</th>
<th>Serum folate* (nmol/liter)</th>
<th>Plasma methionine (µmol/liter)</th>
<th>Plasma homocysteine (µmol/liter)</th>
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<td>H. S.</td>
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<td>3½/12</td>
<td>18</td>
<td>2.7</td>
<td>2.7</td>
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<td>4.8</td>
<td>3.0</td>
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<td>7.2</td>
<td>33.2</td>
<td>417</td>
<td>12.0</td>
<td>22.67</td>
<td>13.18</td>
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* Normal ranges for the age ¾–2 years, 10.5–13.5 g/100 ml; 2–6 years, 11.5–13.5 g/100 ml; 6–12 years, 11.5–15.5 g/100 ml; >12 years (males), 13–16 g/100 ml.

** Normal range for children, 4–10·10⁹ cells/liter.

† Normal range for children, >4·1 nmol/liter.

‡ Normal range for children, ≥4·1 nmol/liter.

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analytical Instrument. The retention times of MTX and 7-hydroxy-MTX were 4.8 min and 5.2 min, respectively.

Statistics. Comparison of paired samples was performed using the Wilcoxon signed-rank test. Unpaired samples were evaluated by means of the Mann-Whitney U test. Correlation between plasma Hey and other blood parameters was tested for using the Spearman rank correlation coefficient. Significant levels were always expressed as 2-tailed.

RESULTS

Plasma Hey and Other Blood Parameters before Start of Chemotherapy (Stage I). The pretreatment values for total plasma Hey, plasma methionine, serum folate, serum cobalamin, and some hematological parameters are listed in Table 1.

The mean plasma Hey in the patients is 13.18 ± 6.23 (SD) µmol/liter, which is significantly (P < 0.002) higher than in 10 healthy children (6.52 ± 1.21 µmol/liter). The plasma Hey for the patients showed a greater spread than the values for the controls. Five of 12 patients had pretreatment values higher than 3 SD above the mean value of control subjects.

Serum folate was in the lower normal range in 5 of 12 patients, and normal in 7 patients [mean, 12.0 ± 5.8 (SD) nmol/liter]. Serum cobalamin [mean, 417 ± 148 (SD) pmol/liter] and plasma methionine (mean, 22.67 ± 7.32 µmol/liter) were normal in all patients (Table 1).

Hemoglobin concentration was low [mean, 7.2 ± 2.0 (SD) g/100 ml, and WBC varied from 1.5 to 13.9-10⁹ cells/liter, except in patient P. B. with WBC of 327-10⁹ cells/liter at the time of admission (Table 1).

Plasma Hey level was not related to serum cobalamin, plasma methionine, or hemoglobin concentration. There was a positive correlation (P < 0.01) between plasma Hey and WBC, and a negative correlation (P < 0.02) between plasma Hey and serum folate (Fig. 1).

Variations in Plasma Hcy and Serum Folate during Induction Therapy (Stage II). During this period of 7–8 weeks, the patient received vincristine, asparaginase, and intrathecal MTX, as described in "Materials and Methods."

Both plasma Hey and serum folate changed markedly during the induction therapy and the response differed from one patient to another. Two different response patterns could be distinguished.

In 7 patients (K. B., E. A., K. H., H. S., K. R., L. T. S., and S. M.) we observed a transient increase in plasma Hey during this period. Notably, their pretreatment Hey (<10.2 µmol/liter) was less than 3 SD above mean value of controls. It increased significantly after start of therapy, remained elevated for 2 to 4 weeks (except in patient K. B.), and then decreased. Pretreatment serum folate was high (>10 nmol/liter) in most patients, and the changes in serum folate were essentially opposite that observed for plasma Hey (Fig. 2).

A different response was observed in the remaining 5 patients (S. B., H. K., P. B., L. S., and S. H.). In these patients, we observed a decrease in plasma Hey during induction. They had pretreatment levels (>15.5 µmol/liter) higher than 3 SD above mean value of controls. Again, serum folate showed a reciprocal profile, characterized by a low pretreatment level (<7.5 nmol/liter in most patients), which increased and then decreased towards the end of the period (Fig. 2).

The initial decrease in plasma Hey was particularly pronounced for patients P. B. and L. S. The plasma level before chemotherapy was 24.4 µmol/liter for patient P. B. and it decreased to 8.6 µmol/liter within 6 days of chemotherapy. In this period, his WBC was reduced from 327-10⁹ to 1.6-10⁹ cells/liter, and then dropped to 9.7 µmol/liter at day 5. His WBC decreased from 13.9-10⁹ cells/liter to 1.6-10⁹ cells/liter at day 5 (Fig. 3).

At the end of induction therapy, plasma Hey was reduced in all patients (mean 7.08 ± 3.83 µmol/liter; Fig. 4), except patients S. M. and L. T. S., and was not significantly different from that of control subjects.

Acute Response during a HD-MTX Course. Typical profiles for the alteration in plasma Hey and plasma methionine during a HD-MTX course are shown in Fig. 5. Plasma Hey increased during the 24-h infusion period and continued to do so until the administration of the 5-formyl-THF “rescue” at time 36 h
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Fig. 3. Changes in plasma Hcy and WBC during the first few days of induction therapy in two patients with high pretreatment levels.

Fig. 4. Reduction in plasma Hcy following the induction therapy and after 3 HD-MTX courses.

Fig. 5. Plasma profiles for Hcy and methionine during 24 h of MTX and the following 5-formyl-THF "rescue" therapy. Plasma curves for 4 courses in 3 patients are shown. The duration of the MTX infusion and the time of "rescue" therapy are indicated by the horizontal arrows (abscissae).

The mean increase in plasma Hcy 36 h after start of infusion during the first, second, and third course was 59, 37, and 40%, respectively, and no attenuation in response could be demonstrated during the following courses (Table 2).

The basal plasma Hcy tended to decrease during HD-MTX therapy, from 7.08 ± 3.83 μmol/liter at the end of induction therapy to 5.56 ± 1.12 μmol/liter after 3 HD-MTX regimens. Notably, in this period plasma Hcy levels showed a reduced spread, and all patients had plasma levels within the normal range after the third HD-MTX (Fig. 4). No further reduction was noted (Table 2).

The change in plasma methionine varied markedly between patients and HD-MTX courses (Table 2; Fig. 5). During the first 4 courses, the mean reduction in plasma methionine at 24 h after start of infusion was 36-56%. During the last 4 treatments, the decline was less pronounced (17-29%) (Table 2).

The percentage change in plasma methionine 24 h after start of MTX infusion showed a slight but significant correlation to the plasma levels of MTX (n = 51; P < 0.05) and 7-hydroxy-MTX (n = 51; P < 0.05) (Fig. 7).

The rebound increase in plasma methionine usually developed after 48 h in the first HD-MTX course. In the later courses, a somewhat different response pattern was observed. The increase developed earlier and was more pronounced (Table 2).

DISCUSSION

During the course of the chemotherapy of ALL, plasma Hcy is conceivably modulated by several factors other than the effect from MTX itself. Such factors are unstable folate homeostasis (20), a large burden of rapidly growing leukemic cells prior to therapy (21), and extensive cell kill and reduction in tumor burden during induction therapy. In this article, we evaluated
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For de novo synthesis of nucleic acid precursors (22). It has been reported that leukemic cells isolated from patients have a higher folate content than do nonleukemic cells at a similar stage of development, and the ratio between leukocyte folate and serum folate is markedly increased (23). Thus, there seems to be an increased folate requirement in patients with leukemia and solid tumors, which is reflected by reduced serum folate (20) and decreased folate catabolism and excretion (24) in such patients.

Plasma Hcy is probably the most convenient test among the few (25, 26) established indicators of folate function in vivo. Observations in humans (2, 27) and rats (28) suggest that plasma Hcy may reveal functional folate deficiency in subjects without overt deficiency and with serum folate within the normal range.

We found that plasma Hcy was increased in 5 of 12 patients with ALL (Table 1; Fig. 4). Since serum cobalamin was within the normal range (Table 1), the finding suggests folate deficiency in these patients. This is supported by the observation that plasma Hcy was negatively correlated to serum folate (Fig. 1).

At the time of admission, plasma Hcy was also related to WBC (Fig. 1). WBC at this time represents circulating leukemic cells, is an important prognostic factor in children with ALL (21), and may reflect total burden of malignant cells. Thus, proliferating leukemic cells may contribute significantly to plasma Hcy. Notably, cellular Hcy egress is positively related to the specific growth rate (29).

Plasma Hcy and Serum Folate during Induction Therapy (Stage II). During the induction therapy there is an extensive kill of leukemic cells, and all patients obtained remission within 5 weeks of treatment. During this period, we observed pronounced changes in plasma Hcy and serum folate (Fig. 2) and marked decline in both plasma Hcy and WBC was observed during the first few days in 2 patients (L. S., P. B.) with particularly high pretreatment values (Fig. 3). Fig. 4 shows that plasma Hcy is markedly reduced at the end of induction therapy in 10 of 12 patients, and the mean level is not different from that of controls.

In most patients (S. B., K. H., P. B., L. S., L. S.) with high pretreatment Hcy levels, Hcy decreased, whereas in those with low pretreatment Hcy, there was a transient increase followed by a decline (Fig. 2). We were not able to relate this difference in response to any clinical parameter, except that the former patients were older (9½ ± 1½ years) than the latter group (3½ ± 1½ years). Notably, essentially reciprocal changes in folate were observed, indicating that plasma Hcy is related to the alteration in plasma Hcy in 12 patients with ALL prior to therapy, during induction therapy, and then during 2–8 courses of HD-MTX.

Homocysteine and Folate in Leukemic Patients (Stage I). There is compelling evidence both from experimental and clinical studies of altered folate homeostasis in patients with leukemia and some forms of cancer. A high-affinity transport system for reduced folates seems to be overexpressed in leukemic and other malignant cells in culture, and this may be related to a possible increased requirement of reduced folates in response to any clinical parameter, except that the former patients were older (9½ ± 1½ years) than the latter group (3½ ± 1½ years). Notably, essentially reciprocal changes in folate were observed, indicating that plasma Hcy is related to

FIG. 6. Changes in plasma Hcy and serum folate during induction therapy (stage II) and consolidation and maintenance (stage III) in 2 patients. A, data for patient H. S. B. data for patient S. B.

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Table 2: Changes in plasma Hcy and plasma methionine during and after a 24-h infusion of 8 g/m² of MTX

The plasma levels of homocysteine and methionine prior to MTX infusion (0 h) are given as mean ± SD. The pretreatment value for each patient represents 100%, and the values after start of infusion (≥24 h) are given as mean ± SD.

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<th>Course no.</th>
<th>n</th>
<th>0 h (μmol/liter)</th>
<th>24 h (%)</th>
<th>36 h (%)</th>
<th>48 h (%)</th>
<th>72 h (%)</th>
<th>0 h (μmol/liter)</th>
<th>24 h (%)</th>
<th>36 h (%)</th>
<th>48 h (%)</th>
<th>72 h (%)</th>
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<tr>
<td>1</td>
<td>12</td>
<td>7.08 ± 3.84</td>
<td>130 ± 56</td>
<td>159 ± 45</td>
<td>116 ± 52</td>
<td>89 ± 37</td>
<td>18.99 ± 3.29</td>
<td>64 ± 39</td>
<td>88 ± 38</td>
<td>102 ± 61</td>
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<td>112 ± 34</td>
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<td>154 ± 59</td>
<td>147 ± 77</td>
<td>133 ± 73</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>6.21 ± 1.10</td>
<td>108 ± 27</td>
<td>129 ± 30</td>
<td>118 ± 32</td>
<td>109 ± 40</td>
<td>23.03 ± 6.40</td>
<td>83 ± 51</td>
<td>156 ± 16</td>
<td>205 ± 41</td>
<td>237 ± 157</td>
</tr>
</tbody>
</table>

All 80 5.79 ± 2.14 128 ± 44 143 ± 36 123 ± 36 110 ± 35 22.26 ± 2.42 63 ± 37 120 ± 55 151 ± 69 151 ± 86
METHOTREXATE AND HOMOCYSTEINE

Fig. 7. Correlation between the change in plasma methionine and the MTX and 7-hydroxy-MTX concentration in plasma 24 h after start of MTX infusion.

Thereafter it declined towards pretreatment levels (Fig. 5; Table 2).

The increased plasma Hcy probably reflects the ability of MTX to reduce cellular contents of reduced folates, including 5-methyl-THF. This process develops rapidly (within hours) at high MTX concentration and is reversed upon supplementing 5-formyl-THF (Fig. 5). In psoriasis patients receiving low doses of MTX, the antifolate effects measured as plasma Hcy developed slowly and reached a maximum within 2–3 days. It was not interrupted by supplementing antidote (10).

The antifolate effect and the resulting stimulation of cellular Hcy export probably precedes the cytotoxic effect of MTX. Cytotoxicity, cell death, and inhibition of cell growth lead to inhibition of Hcy export. This dual effect on Hcy export has been demonstrated in a cell culture system, and may explain an apparent inconsistency between the constant acute response to MTX described here (Fig. 5; Table 2) and the gradual attenuation in response and reduction in basal Hcy observed in patients with lymphomas and sarcoma receiving 1–13.6 g MTX (11). The patients with ALL received a cyto-reductive regimen (induction therapy), which normalized plasma Hcy (Figs. 2 and 4) prior to the HD-MTX courses, whereas in the patients with solid tumors, MTX was part of the primary cyto-reductive therapy leading to loss of cancer cells exporting Hcy. The HD-MTX courses only slightly decreased the plasma Hcy beyond the reduction obtained during the induction therapy (Fig. 4; Table 2).

Plasma Methionine. Broxson et al. (14) recently reported on the dramatic decline in plasma methionine during the HD-MTX infusion in patients with ALL. Notably, this decline developed rapidly within hours after start of infusion, and plasma methionine began to increase when plasma Hcy peaked (14). These data agree with the results of the present study. We observed that plasma methionine reduction preceded the homocysteinemia in most HD-MTX courses, but the methionine response, in contrast to the increase in plasma Hcy, was occasionally lacking, as demonstrated in Fig. 5 for patient S. B. An additional characteristic of the methionine response was that the hypomethioninemia was a dominating feature during the first infusions, whereas the rebound increase prevailed during the succeeding HD-MTX courses (Table 2).

In a previous study on patients with solid tumors receiving a 2–4-h infusion of 1 to 13.6 g/m² MTX, we did not observe hypomethioninemia except in one patient receiving the highest MTX dose (11). As suggested by Broxson et al. (14), lack of hypomethioninemia may be due to the short infusion time and the low doses used. They found that the methionine response was more pronounced at a dose of 8–33.6 g/m² than at a dose of 3 g/m², suggesting that this effect, in contrast to the homocysteinemia, is dose-dependent.

There are some indications that the hypomethioninemia is induced by MTX. The effect seems to be dose-dependent (14) and coincides with the peak plasma concentrations of MTX (33). We found a slight but significant correlation between reduction in plasma methionine and the plasma levels of MTX and its major metabolite (Fig. 7). Furthermore, the methionine effect is not produced by several other cytostatic agents (14).

Finally, high concentration of MTX (1000 μM) did not interfere with the determination of methionine in plasma samples.

The mechanism behind the methionine effect is not readily apparent. Nitrous oxide is an established inactivator of methi-

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**METHOTREXATE AND HOMOCYSTEINE**


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