

Plasma Homocysteine in Children with Acute Lymphoblastic Leukemia: Changes during a Chemotherapeutic Regimen Including Methotrexate¹

Helga Refsum,² Finn Wesenberg, and Per Magne Ueland

The Clinical Pharmacology Unit, Department of Pharmacology and Toxicology [H. R., P. M. U.], and Department of Pediatrics [F. W.], University of Bergen, N-5021 Haukeland Hospital, Norway

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) $\mu\text{mol/liter}$, which is significantly ($P < 0.001$) higher than the level in control children (6.52 ± 1.21 $\mu\text{mol/liter}$). 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, asparaginase, 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 $\mu\text{mol/liter}$).

HD-MTX (8 g/m^2) 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 total plasma homocysteine was reduced to 5.56 ± 1.12 $\mu\text{mol/liter}$. 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 homocysteinemia. The methionine depletion may interfere with the antileukemic effect of MTX.

INTRODUCTION

Plasma Hcy³ 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 (1). 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/m^2 . HD-MTX (>1 g/m^2) 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* studies⁴ 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.

⁴ H. Refsum, B. Christensen, R. Djurhuus, 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.

Received 9/10/90; accepted 11/6/90.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by grants from the Norwegian Cancer Society, from Astri og Birger Torsteds legat til bekjempelse av kreft, and the Norwegian Research Council for Science and the Humanities.

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.

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-homocystine, 2-mercaptoethanol, and *o*-phthalaldehyde 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 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 $6\frac{1}{2} \pm 3\frac{1}{12}$ 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 intrathecal 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 admission and lasted for about 6 weeks. During this interval, the patients received 3 HD-MTX courses separated by 3 weeks. At the end of consolidation, the patients were given 4 doses of cytarabine (each dose of 300 mg/m²) plus teniposide (165 mg/m²) over 10 days. The second part, the maintenance therapy, consisting of weekly, p.o. doses of MTX (20 mg/m²) plus 6-mercaptopurine, and 5 courses of HD-

MTX with 5-formyl-THF "rescue" separated by intervals of 2 months.

The patients were given a total of 8 HD-MTX courses, and the study included a total of 80 HD-MTX courses.

In all patients remission was obtained within 5 weeks of treatment.

High-Dose MTX Infusion, Hydration, Alkalinization, and 5-Formyl-THF "Rescue". The patients were given 8 g/m² of MTX as an infusion of 24 h. No chemotherapy was given concomitantly with HD-MTX. During this period the patients received a sodium bicarbonate solution i.v. (240 mmol/m²/24 h) to keep the urine pH above 7.0 and a high urinary output. Most patients also received intrathecal MTX (3–6 mg) immediately before the MTX infusion.

"Rescue" therapy with 5-formyl-THF was started 12 h after cessation of HD-MTX infusion. It started with a 1-h infusion of 75 mg/m² followed by p.o. or i.v. administration of doses of 15 mg/m² every third and later every 6 h. 5-Formyl-THF administration continued until plasma MTX was less than 0.1 μmol/liter.

Blood Sampling and Processing. Blood samples were collected at the time of diagnosis before chemotherapy, the first, second, and third day 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 NaBH₄ 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*-phthalaldehyde 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% acetonitrile. 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 1 Patient characteristics

Patient	Sex	Age (yr)	Wt (kg)	Hemoglobin ^a (g/100 ml)	WBC ^b (10 ⁹ cells/liter)	Serum cobalamin ^c (pmol/liter)	Serum folate ^d (nmol/liter)	Plasma methionine (μmol/liter)	Plasma homocysteine (μmol/liter)
H. S.	M	3 ³ / ₁₂	18	2.7	2.7	389	24.0	13.88	8.43
S. M.	M	2 ⁸ / ₁₂	20	9.1	4.6	302	14.3	39.31	8.47
L. T. S.	F	1 ⁸ / ₁₂	12	7.7	1.5	542	13.9	22.45	5.91
H. K.	F	9 ⁹ / ₁₂	55	6.0	12.8	354	7.2	ND	20.61
P. B.	M	13 ³ / ₁₂	70	8.2	327.0	787	5.9	30.69	24.45
K. B.	M	5 ⁴ / ₁₂	35	6.9	4.1	428	15.5	20.35	8.57
E. A.	F	2 ³ / ₁₂	14	7.0	10.9	363	17.9	18.30	8.94
K. R.	F	2 ³ / ₁₂	12	8.2	4.0	396	6.8	21.72	10.10
L. S.	M	2 ¹ / ₁₂	22	9.4	13.9	404	14.0	23.54	15.56
S. H.	F	8 ⁸ / ₁₂	35	9.9	5.1	321	6.5	13.38	20.12
S. B.	M	13 ³ / ₁₂	50	6.7	9.2	201	5.4	24.45	18.18
K. H.	F	4 ² / ₁₂	20	4.8	3.0	518	12.3	21.33	8.82
Mean		5 ¹ / ₁₂	30	7.2	33.2	417	12.0	22.67	13.18
SD		4 ³ / ₁₂	19	2.0	92.6	148	5.8	7.32	6.23

^a Normal ranges for the age $\frac{1}{2}$ –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.

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

^c Normal range for children, ≥ 103 pmol/liter.

^d Normal range for children, ≥ 4.1 nmol/liter.

alytical 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 Hcy and other blood parameters were tested for using the Spearman rank correlation coefficient. Significant levels were always expressed as 2-tailed.

RESULTS

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

The mean plasma Hcy in the patients is 13.18 ± 6.23 (SD) $\mu\text{mol/liter}$, which is significantly ($P < 0.002$) higher than in 10 healthy children (6.52 ± 1.21 $\mu\text{mol/liter}$). The plasma Hcy 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 $\mu\text{mol/liter}$) 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 $13.9 \cdot 10^9$ cells/liter, except in patient P. B. with WBC of $327 \cdot 10^9$ cells/liter at the time of admission (Table 1).

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

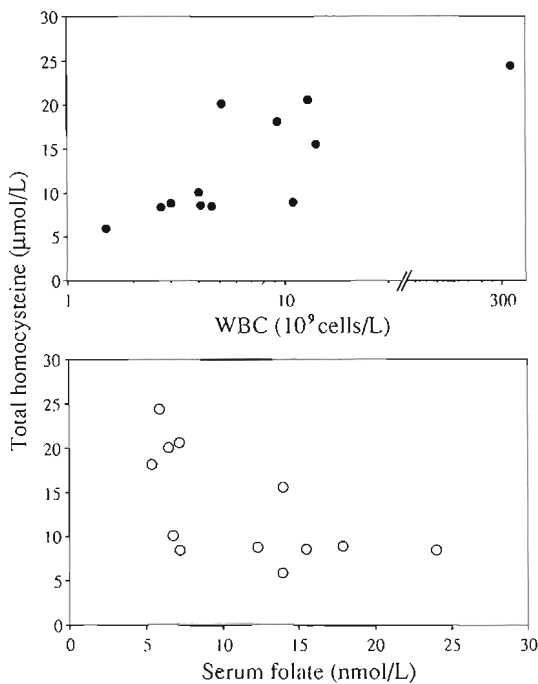


Fig. 1. Correlation between plasma Hcy and WBC and serum folate at the time of admission.

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 Hcy 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 Hcy during this period. Notably, their pretreatment Hcy (<10.2 $\mu\text{mol/liter}$) 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 Hcy (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 Hcy during induction. They had pretreatment levels (>15.5 $\mu\text{mol/liter}$) 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 Hcy was particularly pronounced for patients P. B. and L. S. The plasma level before chemotherapy was 24.4 $\mu\text{mol/liter}$ for patient P. B. and it decreased to 8.6 $\mu\text{mol/liter}$ within 6 days of chemotherapy. In this period, his WBC was reduced from $327 \cdot 10^9$ to $1.6 \cdot 10^9$ cells/liter. Similarly, patient L. S. had a plasma Hcy of 15.6 $\mu\text{mol/liter}$ prior to chemotherapy, it increased to 20.4 $\mu\text{mol/liter}$ 2 days after start of therapy, and then dropped to 9.7 $\mu\text{mol/liter}$ at day 5. His WBC decreased from $13.9 \cdot 10^9$ cells/liter to $1.6 \cdot 10^9$ cells/liter at day 5 (Fig. 3).

At the end of induction therapy, plasma Hcy was reduced in all patients (mean 7.08 ± 3.83 $\mu\text{mol/liter}$; 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 Hcy and plasma methionine during a HD-MTX course are shown in Fig. 5. Plasma Hcy 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

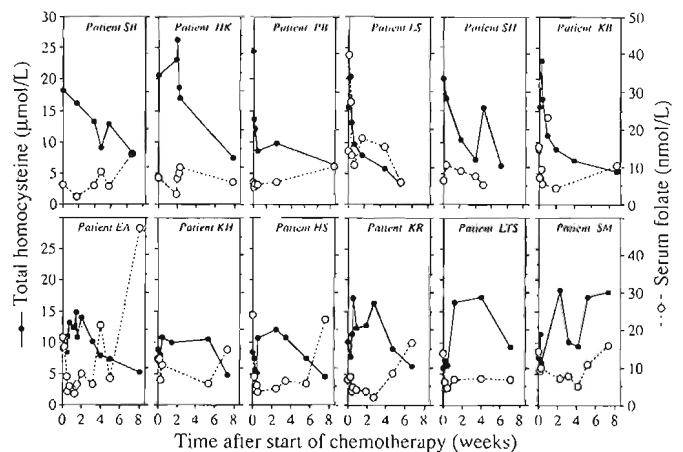


Fig. 2. Changes in plasma Hcy and serum folate during induction therapy (stage II).

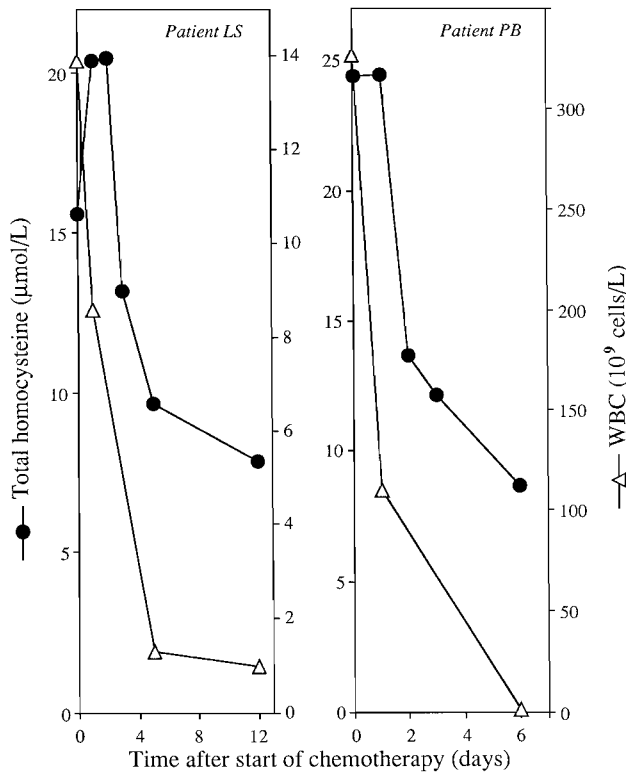


Fig. 3. Changes in plasma Hcy and WBC during the first few days of induction therapy in two patients with high pretreatment levels.

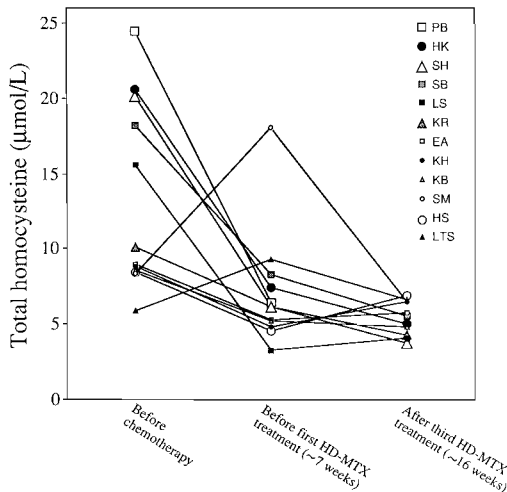


Fig. 4. Reduction in plasma Hcy following the induction therapy and after 3 HD-MTX courses.

after start of the infusion. From this time point, plasma Hcy decreased towards pretreatment levels.

Plasma methionine usually showed a biphasic change (Fig. 5). It decreased during the MTX infusion and often reached levels below 10 µmol/liter at time 24 h. Thereafter, patients usually showed a rebound increase to above pretreatment methionine levels.

Multiple Courses of HD-MTX and Long-Term Effects. The effect from 8 courses of HD-MTX on plasma Hcy in 2 patients (S. B. and H. S.) are shown in Fig. 6. Each course gave a transient increase in plasma Hcy, and no attenuation of the Hcy response was observed.

The effects during multiple courses in all patients are sum-

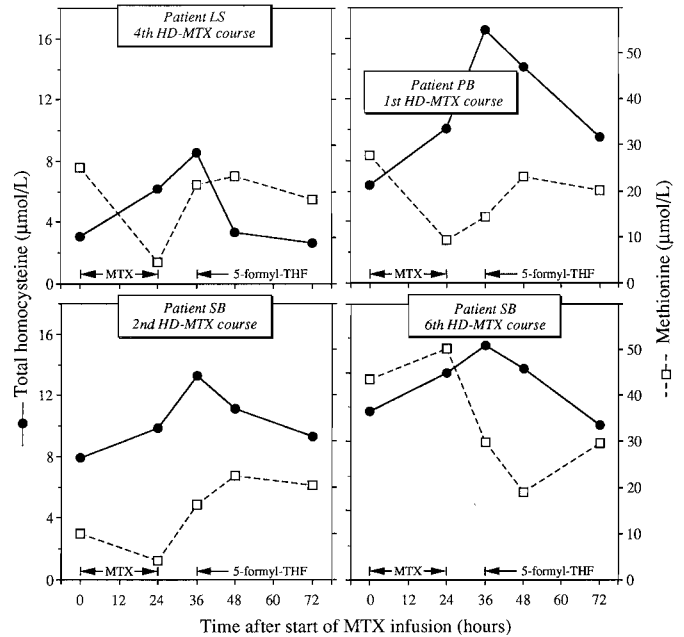


Fig. 5. Plasma profiles for Hcy and methionine during 24 h of MTX and the following 5-formyl-THF "rescue" therapy. Plasma curves for 4 courses in 3 patients are shown. The duration of the MTX infusion and the time of "rescue" therapy are indicated by the horizontal arrows (abscissae).

marized in Table 2, which also shows the variations in plasma methionine during and after the infusion.

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

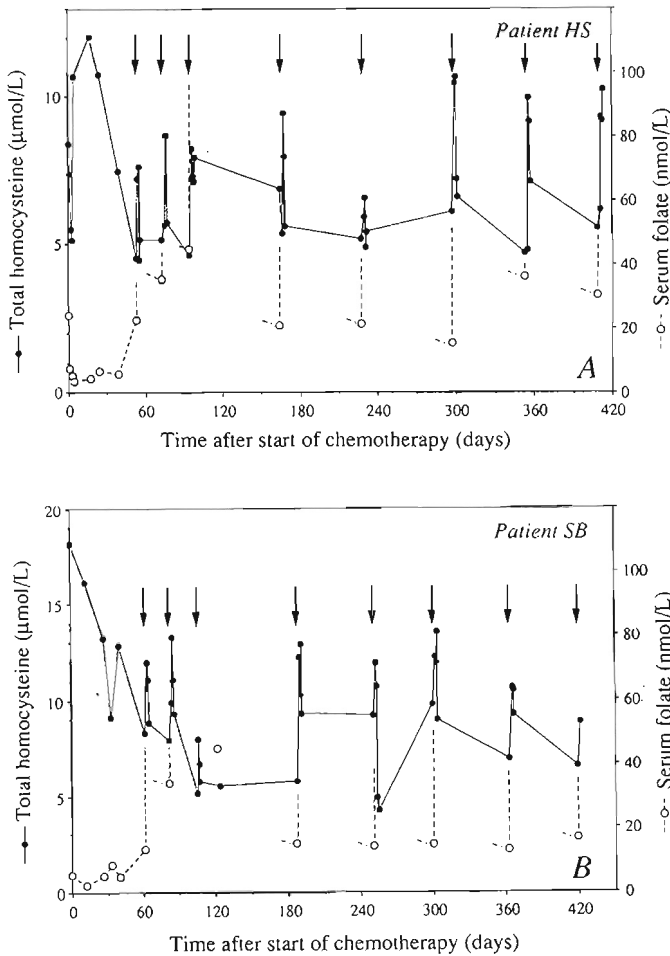


Fig. 6. Changes in plasma Hcy and serum folate during induction therapy (stage II) and consolidation and maintenance (stage III) in 2 patients. A, data for patient H. S. B, data for patient S. B.

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.

Folate and Hcy Homeostasis 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

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).⁴

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 ($9\frac{1}{2} \pm 1\frac{1}{2}$ years) than the latter group ($3\frac{3}{12} \pm 1\frac{1}{2}$ years). Notably, essentially reciprocal changes in folate were observed, indicating that plasma Hcy is related to

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 \pm SD. The pretreatment value for each patient represents 100%, and the values after start of infusion (≥ 24 h) are given as mean \pm SD.

Course no.	n	Time after start of MTX infusion									
		Plasma homocysteine					Plasma methionine				
		0 h (μ mol/liter)	24 h (%)	36 h (%)	48 h (%)	72 h (%)	0 h (μ mol/liter)	24 h (%)	36 h (%)	48 h (%)	72 h (%)
1	12	7.08 \pm 3.84	130 \pm 56	159 \pm 45	116 \pm 52	89 \pm 37	18.99 \pm 8.29	64 \pm 39	88 \pm 38	102 \pm 61	121 \pm 46
2	12	5.80 \pm 1.55	111 \pm 24	137 \pm 35	118 \pm 32	112 \pm 34	16.07 \pm 6.55	48 \pm 27	117 \pm 35	137 \pm 67	122 \pm 39
3	11	5.14 \pm 1.28	124 \pm 31	140 \pm 21	136 \pm 27	128 \pm 25	20.53 \pm 5.04	44 \pm 32	124 \pm 23	176 \pm 65	175 \pm 60
4	10	5.01 \pm 1.64	160 \pm 64	164 \pm 39	129 \pm 40	110 \pm 31	23.70 \pm 6.29	56 \pm 30	133 \pm 65	133 \pm 48	128 \pm 61
5	10	5.74 \pm 1.82	134 \pm 35	126 \pm 25	103 \pm 33	91 \pm 22	22.88 \pm 10.44	75 \pm 35	156 \pm 73	201 \pm 86	206 \pm 124
6	10	5.66 \pm 1.99	126 \pm 30	132 \pm 29	128 \pm 34	114 \pm 23	29.82 \pm 12.85	71 \pm 50	125 \pm 70	138 \pm 59	121 \pm 45
7	8	5.37 \pm 1.35	115 \pm 39	142 \pm 37	138 \pm 35	139 \pm 47	28.04 \pm 11.93	74 \pm 41	154 \pm 59	147 \pm 77	133 \pm 75
8	7	6.21 \pm 1.10	108 \pm 27	129 \pm 30	118 \pm 32	109 \pm 40	23.03 \pm 6.40	83 \pm 51	156 \pm 16	205 \pm 41	237 \pm 157
All	80	5.79 \pm 2.14	128 \pm 44	143 \pm 36	123 \pm 36	110 \pm 35	22.36 \pm 9.32	63 \pm 37	128 \pm 55	151 \pm 69	151 \pm 86

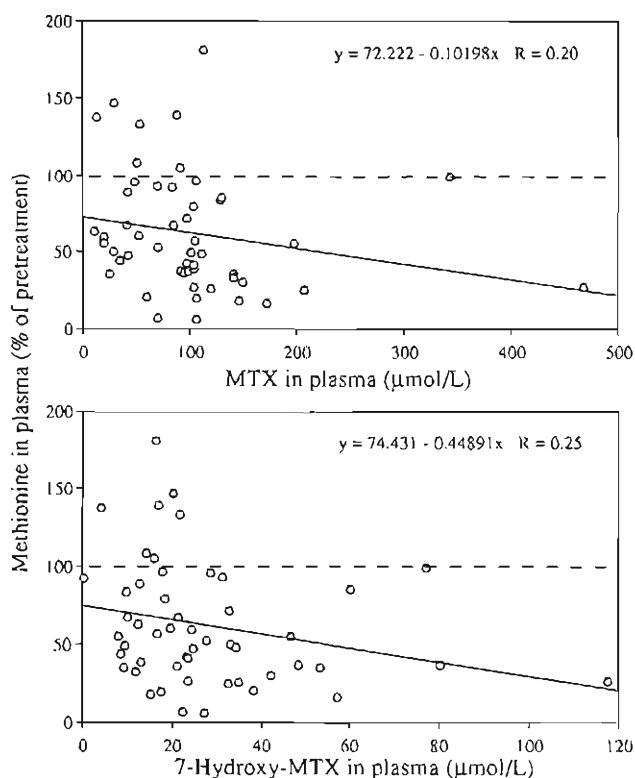


Fig. 7. Correlation between the change in plasma methionine and the MTX and 7-hydroxy-MTX concentration in plasma 24 h after start of MTX infusion.

folate status also under the unstable conditions occurring during induction therapy.

Monitoring both serum folate and plasma Hcy may provide complementary information on folate status. Serum folate may be artificially high due to cytolysis and leakage of intracellular folate, and occasional high values were observed in these serum samples. Furthermore, under conditions of rapid changes in folate homeostasis and availability, serum folate, in contrast to plasma Hcy, may not mirror the intracellular folate status (2).

Nitrous oxide, trimethoprim, and intrathecal MTX were administered during the induction therapy, and their effect on plasma Hcy should be considered. Duration of nitrous oxide exposure was probably too short to interfere with cobalamin function of methionine synthase,⁵ and there are only occasional and conflicting reports on the antifolate effect of trimethoprim in humans (30). According to Broxson *et al.* (14), intrathecal MTX does not affect plasma Hcy.

In conclusion, the fluctuation in plasma Hcy during induction therapy (Fig. 2) probably reflects both unstable folate homeostasis and cytotoxic action of the regimen. It is conceivable that the variable intracellular folate status during or at the end of induction therapy is an important determinant of the response to the oncoming HD-MTX courses. Results from experimental studies (31, 32) may support this possibility.

Acute and Long-Term Effects of HD-MTX. The acute effects of MTX on plasma Hcy in cancer patients have been described previously in 2 articles (11, 14). These are confirmed and extended by the findings in the present study. Plasma Hcy increased during and after MTX infusion until the administration of 5-formyl-THF "rescue," *i.e.*, at 36 h in the present work.

⁵ A. A. M. Ermens, H. Refsum, J. Ruprecht, L. J. M. Spijkers, A. B. Guttormsen, J. Lindemans, P. M. Ueland, and J. Abels. Monitoring cobalamin inactivation during nitrous oxide anesthesia by determination of homocysteine and folate in plasma and urine, *Clin. Pharmacol. Ther.*, accepted for publication.

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,⁴ 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 cytoreductive 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 cytoreductive 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.⁶

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

⁶ H. Refsum, unpublished observation.

onine synthase (34) and reduces plasma methionine (35). Supposing that the reduction in methionine by MTX is caused by inhibition of Hcy remethylation, we would expect the same relation between the methionine and Hcy response produced by either nitrous oxide or MTX. However, we observed that nitrous oxide induced a rapid increase in plasma Hcy within 90 min, and 2-4 hours later, a transient hypomethioninemia sometimes developed.⁵ MTX is not a direct inhibitor of methionine synthase (14). Mechanisms not involving inhibition of Hcy remethylation should therefore be considered, such as effect of MTX on the cellular transport (36), distribution, and renal excretion of methionine. The alkalization and hydration and the methionine balance prior to therapy may also contribute to the effect. The rebound increase in plasma methionine above normal (Table 2) and the occasional lack of plasma methionine reduction (Fig. 5) might be expected if these mechanisms are operating.

Methionine is involved in vital processes such as synthesis of the versatile methyl donor S-adenosylmethionine, is furnishing the aminopropyl moiety for the polyamine synthesis, and is utilized for protein synthesis (37). Therefore, if the low plasma methionine reflects low intracellular concentration, it may have major impact on cellular function and growth. In addition, methionine modulates MTX polyglutamation (38) and cytotoxicity (39, 40) *in vitro*, and low methionine may play a role in MTX hepatotoxicity (41). The causes and consequences of hypomethioninemia as a part of the HD-MTX regimen should be further investigated.

Summary and Conclusion. Plasma Hcy is elevated in 5 of 12 patients with ALL. This is probably related to intracellular folate deficiency, and export of Hcy from leukemic cells. During the cyto-reductive induction therapy, determinations of plasma Hcy and serum folate suggest large fluctuations in folate status, and when remission is obtained, plasma Hcy is normalized in most patients. The unstable folate homeostasis is a possible determinant of therapeutic effect of the oncoming HD-MTX regimens. The HD-MTX courses lead to a transient homocysteinemia that peaks when 5-formyl-THF "rescue" is administered, and probably reflects depletion of reduced folates. The Hcy response is preceded by a transient hypomethioninemia, which probably is induced by the MTX infusion by an unknown mechanism. Plasma Hcy is a potentially useful indicator of folate status prior to and during chemotherapy of ALL, whereas the low plasma methionine observed during MTX infusion may affect the therapeutic results. Determination of plasma Hcy and plasma methionine should be evaluated as prognostic factors and as useful parameters to monitor therapeutic effect from chemotherapy in patients with ALL.

ACKNOWLEDGMENTS

The technical assistance of Gry Kvalheim, Elfrid Blomdal, and Halvard Bergesen is highly appreciated. The authors are grateful to the staff of the pediatric ward for collecting blood samples.

REFERENCES

1. Finkelstein, J. D. Methionine metabolism in mammals. *J. Nutr. Biochem.*, *1*: 228-237, 1990.
2. Kang, S-S., Wong, P. W. K., and Norusis, M. Homocysteinemia due to folate deficiency. *Metabolism*, *36*: 458-462, 1987.
3. Allen, R. H., Stabler, S. P., Savage, D. G., and Lindenbaum, J. Diagnosis of cobalamin deficiency. I. Usefulness of serum methylmalonic acid and total homocysteine concentrations. *Am. J. Hematol.*, *34*: 90-98, 1990.
4. Ueland, P. M., and Refsum, H. Plasma homocysteine, a risk factor for

- vascular disease: plasma levels in health, disease, and drug therapy. *J. Lab. Clin. Med.*, *114*: 473-501, 1989.
5. Goldman, I. D., and Matherly, L. H. The cellular pharmacology of methotrexate. *Pharmacol. Ther.*, *28*: 77-102, 1985.
6. Ackland, S. P., and Schilsky, R. L. High-dose methotrexate: a critical reappraisal. *J. Clin. Oncol.*, *5*: 2017-2031, 1987.
7. Nyfors, A. Methotrexate therapy in psoriasis and psoriatic arthritis. *Rheumatology*, *9*: 60-87, 1986.
8. Wilke, W. S., and Mackenzie, A. H. Methotrexate therapy in rheumatoid arthritis. *Current status. Drugs*, *32*: 103-113, 1986.
9. Ueland, P. M., Refsum, H., Male, R., and Lillehaug, J. R. Disposition of endogenous homocysteine by mouse fibroblast C3H/10T1/2 Cl 8 and the chemically transformed C3H/10T1/2MCA Cl 16 cells following methotrexate exposure. *J. Natl. Cancer Inst.*, *77*: 283-289, 1986.
10. Refsum, H., Helland, S., and Ueland, P. M. Fasting plasma homocysteine as a sensitive parameter to antifolate effect. A study on psoriasis patients receiving low-dose methotrexate treatment. *Clin. Pharmacol. Ther.*, *46*: 510-520, 1989.
11. Refsum, H., Ueland, P. M., and Kvinnsland, S. Acute and long-term effects of high-dose methotrexate treatment on homocysteine in plasma and urine. *Cancer Res.*, *46*: 5385-5391, 1986.
12. Allegra, C. J., Fine, R. L., Drake, J. C., and Chabner, B. A. The effect of methotrexate on intracellular folate pools in human MCF-7 breast cancer cells. Evidence for direct inhibition of purine synthesis. *J. Biol. Chem.*, *261*: 6478-6485, 1986.
13. Baram, J., Allegra, C. J., Fine, R. L., and Chabner, B. A. Effect of methotrexate on intracellular folate pools in purified myeloid precursor cells from normal human bone marrow. *J. Clin. Invest.*, *79*: 692-697, 1987.
14. Broxson, E. H., Stork, L. C., Allen, R. H., Stabler, S. P., and Kolhouse, J. F. Changes in plasma methionine and total homocysteine levels in patients receiving methotrexate infusions. *Cancer Res.*, *49*: 5858-5862, 1989.
15. Kredich, N. M., Hershfield, M. S., Falletta, J. M., Kinney, T. R., Mitchell, B., and Koller, C. Effects of 2'-deoxycoformycin on homocysteine metabolism in acute lymphoblastic leukemia (Abstract). *Clin. Res.*, *29*: 541A, 1981.
16. Moe, P. J., Wesenberg, F., and Kolmannskog, S. Methotrexate infusions in poor prognosis lymphoblastic leukemia: II. High-dose methotrexate (HDM) in acute lymphoblastic leukemia in childhood: a pilot study from April 1981. *Med. Pediatr. Oncol.*, *14*: 189-190, 1986.
17. Refsum, H., Helland, S., and Ueland, P. M. Radioenzymic determination of homocysteine in plasma and urine. *Clin. Chem.*, *31*: 624-628, 1985.
18. Refsum, H., Ueland, P. M., and Svoldal, A. M. Fully automated fluorescence assay for determining total homocysteine in plasma. *Clin. Chem.*, *35*: 1921-1927, 1989.
19. Krishnamurti, C. R., Heindze, A. M., and Galzy, G. Application of reversed-phase high-performance liquid chromatography using pre-column derivatization with *o*-phthalaldehyde for the quantitative analysis of amino acids in adult and fetal sheep plasma, animal feeds and tissues. *J. Chromatogr.*, *315*: 321-331, 1984.
20. Magnus, E. M. Folate activity in serum and red cells of patients with cancer. *Cancer Res.*, *27*: 490-497, 1967.
21. Gustafsson, G., Garwicz, S., Hertz, H., Johansson, G., Jonmundsson, G., Moe, J. P., Salmi, T., Seip, M., Siimes, M. A., Yssing, M., and Åhrström, L. A population-based study of childhood acute lymphoblastic leukemia diagnosed from July 1981 to June 1985 in the five nordic countries. *Acta Paediatr. Scand.*, *76*: 781-788, 1987.
22. Sirotnak, F. M. Obligate genetic expression in tumor cells of a fetal membrane property mediating "folate" transport: biological significance and implications for improved therapy of human cancer. *Cancer Res.*, *45*: 3992-4000, 1985.
23. Hoffbrand, A. V., and Newscombe, B. F. A. Leucocyte folate in vitamin B12 and folate deficiency and in leukemia. *Br. J. Haematol.*, *13*: 954-966, 1967.
24. Saleh, A. M., Pheasant, A., Blair, J. A., Allan, R. N., and Walters, J. Folate metabolism in man: effect of malignant disease. *Br. J. Cancer*, *46*: 346-353, 1982.
25. Das, K. C., Manusselis, C., and Herbert, V. Simplifying lymphocyte culture and the deoxyuridine suppression test using whole blood (0.1 mL) instead of separated lymphocytes. *Clin. Chem.*, *26*: 72-77, 1980.
26. Morgan, S. L., Baggott, J. E., and Altz-Smith, M. Folate status of rheumatoid arthritis patients receiving long-term, low-dose methotrexate therapy. *Arthritis Rheum.*, *30*: 1348-1356, 1987.
27. Brattström, L. E., Israelsson, B., Jeppson, J.-O., and Hultberg, B. L. Folic acid—an innocuous means to reduce plasma homocysteine. *Scand. J. Clin. Lab. Invest.*, *48*: 215-221, 1988.
28. Lin, J.-Y., Kang, S-S., Zhou, J., and Wong, P. W. K. Homocysteinemia in rats induced by folic acid deficiency. *Life Sci.*, *33*: 319-325, 1989.
29. Iizasa, T., and Carson, D. A. Differential regulation of polyamine synthesis and transmethylation reactions in methylthioadenosine phosphorylase deficient mammalian cells. *Biochim. Biophys. Acta*, *844*: 280-287, 1985.
30. Lambie, D. G., and Johnson, R. H. Drugs and folate metabolism. *Drugs*, *30*: 145-155, 1985.
31. Bunni, M., Doig, M. T., Donato, H., Kesavan, V., and Priest, D. G. Role of methylenetetrahydrofolate depletion in methotrexate-mediated intracellular thymidylate synthesis inhibition in cultured L1210 cells. *Cancer Res.*, *48*: 3398-3404, 1988.
32. Jolivet, J., Faucher, F., and Pinard, M.-F. Influence of intracellular folates

METHOTREXATE AND HOMOCYSTEINE

- on methotrexate metabolism and cytotoxicity. *Biochem. Pharmacol.*, *36*: 3310-3312, 1987.
33. Borsi, J. D., and Moe, P. J. A comparative study on the pharmacokinetics of methotrexate in a dose range of 0.5 g to 33.6 g/m² in children with acute lymphoblastic leukemia. *Cancer (Phila.)*, *60*: 5-14, 1987.
 34. Banerjee, R. V., and Matthews, R. G. Cobalamin-dependent methionine synthase. *FASEB J.*, *4*: 1450-1459, 1990.
 35. Nunn, J. F. Clinical aspects of the interaction between nitrous oxide and vitamin B12. *Br. J. Anaesth.*, *59*: 3-13, 1987.
 36. Scanlon, K. J., Cashmore, A. R., Kashani-Sabet, M., Pallai, M., Dreyer, R. N., Moroson, B. A., and Saketos, M. Inhibition of methionine uptake by methotrexate in mouse leukemia L1210 cells. *Cancer Chemother. Pharmacol.*, *19*: 21-24, 1987.
 37. Stipanuk, M. H. Metabolism of sulfur-containing amino acids. *Annu. Rev. Nutr.*, *6*: 179-209, 1986.
 38. Rhee, M. S., Johnson, T. B., Priest, D. G., and Galivan, J. The effect of methionine on methotrexate metabolism in rat hepatocytes in monolayer culture. *Biochim. Biophys. Acta*, *1011*: 122-128, 1989.
 39. Chello, P. L., and Bertino, J. R. Effect of methionine deprivation on L5178Y murine leukemia cells in culture. Interference with the antineoplastic effect of methotrexate. *Biochem. Pharmacol.*, *25*: 889-892, 1976.
 40. Scanlon, K. J., Kashani-Sabet, M., Cashmore, A. R., Pallai, M., Moroson, B. A., and Saketos, M. The role of methionine in methotrexate-sensitive and methotrexate-resistant mouse leukemia L1210 cells. *Cancer Chemother. Pharmacol.*, *19*: 25-29, 1987.
 41. Barak, A. J., Tuma, D. J., and Beckenhauer, H. C. Methotrexate hepatotoxicity. *J. Am. Coll. Nutr.*, *3*: 93-96, 1984.