0031-3998/86/2004-0285$02.00/0
PEDIATRIC RESEARCH

Vol. 20, No. 4, 1986
Printed in U.S.A.

Cerebrospinal Fluid Biogenic Amine Metabolites in Children during Treatment for Acute Lymphocytic Leukemia

FAYE S. SILVERSTEIN, RAYMOND J. HUTCHINSON, AND MICHAEL V. JOHNSTON

Departments of Pediatrics [F.S.S., R.J.H., M.V.J.] and Neurology [F.S.S., M.V.J.], University of Michigan Medical School and The Center for Human Growth and Development [M.V.J.], Ann Arbor, Michigan 48104

ABSTRACT. To learn more about the impact of intrathecal methotrexate and cystosine arabinoside therapy on neuronal metabolism, we measured serial cerebrospinal fluid concentrations of homovanillic acid and 5-hydroxyindoleacetic acid, major metabolites of the neurotransmitters dopamine and serotonin, in children with acute lymphocytic leukemia. Multiple sequential cerebrospinal fluids were obtained from 30 children with acute lymphocytic leukemia evaluated prospectively from the time of diagnosis. We focused on the period of induction and intensification when children received weekly intrathecal chemotherapy. Paired cerebrospinal fluid specimens were also obtained at 3-month intervals from 60 children with acute lymphocytic leukemia in remission. Homovanillic acid and 5-hydroxyindoleacetic acid were measured using high performance liquid chromotography with electrochemical detection. We found that pretreatment metabolite values were no different from those in age-matched subjects in remission. In the first 5 wk of treatment, there were no significant changes in metabolite levels in patients treated exclusively with methotrexate. There was a transient decrease in homovanillic acid ($-28 \pm 10\%$, $p < 0.001$, Student's t test) and 5-hydroxyindoleacetic acid ($-28 \pm 12\%$, $p < 0.05$) in five of six patients after a single intrathecal dose of cytosine arabinoside. In the next 4 wk there was a gradual rise in levels of homovanillic acid ($p = 0.001$, by analysis of variance) and 5-hydroxyindoleacetic acid ($p = 0.029$, analysis of variance); this pattern did not correlate with administration of cranial irradiation. In children in remission, there were no significant changes in metabolite levels over a 3-month period. The data suggest that intensive therapy including methotrexate and cytosine arabinoside alters central neurotransmitter metabolism and/or transport in children with acute lymphocytic leukemia. (*Pediatr Res* 20: 285–291, 1986)

Abbreviations

HVA, homovanillic acid
5 HIAA, 5-hydroxyindoleacetic acid
DHFR, dihydrofolate reductase
DHPR, dihydropteridine reductase
ALL, acute lymphocytic leukemia
CSF, cerebrospinal fluid
MTX, methotrexate
Ara-C, cytosine arabinoside
I.T., intrathecal
CNS, central nervous system

Received April 26, 1985; accepted November 18, 1985.
Address correspondence and reprint requests to Michael V. Johnston, M.D., Neuroscience Laboratory Building, University of Michigan, 1103 East Huron, Ann Arbor, MI 48104.
This work was supported by The Children's Leukemia Foundation of Michigan, USPHS grant K07 NS 00603 (M.V.J.) and a fellowship from the Medical Research Council of Canada (F.S.S.).

Inclusion of CNS prophylaxis in treatment protocols for ALL has contributed greatly to improved disease free survival rates. Intrathecal cytotoxic drugs—most commonly MTX or Ara-C with cranial irradiation—are routinely administered to children with no signs of CNS disease. However, clinical, radiologic, and pathologic evidence indicates that this treatment may produce neurotoxic side effects. The clinical spectrum of posttreatment sequelae includes behavior disorders, learning problems, seizures, and dementia (1–10).

Acute, subacute, and chronic neurologic dysfunction have been attributed to I.T. methotrexate alone or in combination with cranial irradiation (3, 4, 6, 9–12). Transient headache, nausea, vomiting, meningismus, fever, and CSF pleocytosis occur within 12 h in 5–40% of patients and this acute toxic syndrome is thought to represent a chemical arachnoiditis (3). Acute encephalopathy also occurs in a fraction of patients treated systemically with high dose MTX, suggesting that the drug may have direct acute metabolic effects on the brain (2). Observations based on patients with toxic effects of acute intrathecal overdoses also support this proposition (13, 14). Months to years after treatment, a progressive encephalopathy evolves in a few patients, especially those exposed to high doses of MTX (4, 7, 11, 15–17). In these patients leukomalacia is seen on CAT scan of brain, and myelin basic protein, a myelin breakdown product, is usually elevated in spinal fluid during the active phase. Histopathologic examination of brain shows multifocal coagulative necrosis in white matter, as well as astrogliosis, demyelination, and dystrophic calcification (4, 16). However, neuronal elements are also disrupted and axonal swelling has been described as an early finding (12, 17). These effects are thought to be related directly to the cumulative dose of MTX administered (3, 4, 12).

Considerable effort has been directed toward understanding methotrexate's effect on white matter but metabolic effects on neurons have received less attention. To examine toxicity of MTX, we measured serial CSF concentrations of metabolites for two biogenic amine neurotransmitters, dopamine and serotonin, in children before and during induction of therapy for ALL. Both transmitters are produced in the brain by rate limiting hydroxylation of precursor amino acids, a step that is dependent on adequate levels of tetrahydrobiopterin cofactor. Previous studies suggested that MTX might reduce concentrations of the pteridine hydroxylation cofactor by inhibiting its primary synthetic enzyme, DHPR, and also by reducing DHFR activity (18–22). Reduction in cofactor would be expected to reduce endog-

enous levels of dopamine and serotonin and their CSF metabolites, HVA and 5-HIAA (23, 24).

Disruption in neurotransmitter synthesis might be associated with a variety of changes in appetite, behavior, attention, and level of consciousness (25). We hypothesized that if MTX is able to inhibit neurotransmitter synthesis at toxic levels, it might produce more subtle but detectable changes in neurotransmitter metabolites in a general population of newly diagnosed leukemic children.

METHODS

Subjects. One hundred sixty children age 1 to 21 yr, who attended the Pediatric Hematology–Oncology Clinic at The University of Michigan Medical Center between 1981 and 1984, were the subjects for this study (Table 1). The protocol was approved by The University of Michigan Institutional Review Board.

Multiple sequential CSF's were obtained from 30 children (group 1) evaluated prospectively from the time of diagnosis of ALL. Pretreatment specimens were available along with samples from 2 to 9 wk after initiation of therapy in 26 of the 30. Four or more samples were obtained from the same patient in 26 cases. CSF was saved from fluid withdrawn during clinically indicated lumbar punctures. Although all patients received lumbar punctures and intrathecal therapy at similar time intervals, occasional CSF samples were lost to study because the small amount of fluid obtained was consumed by other clinically indicated examinations. None of the patients had CNS disease. At diagnosis, none had elevation of CSF protein. One patient had 33% blasts in his initial CSF but had only 1 white blood cell/mm^3 and did not meet Children's Cancer Study Group criteria for CNS disease. No other patient had CSF pleocytosis or abnormal cytology. At most recent follow-up (mean duration of follow-up = 21 months) none of the 26 survivors had clinical evidence of treatment-induced encephalopathy. All were treated according to Children's Cancer Study Group protocols. During induction, systemic therapy included vincristine, prednisone, and L-asparaginase for all patients. In addition, a small number of patients received a single dose of cyclophosphamide or up to four doses of daunorubicin in induction. In the second or intensification phase, all patients were randomized to receive: 1) 6-mercaptopurine; or 2) thioguanine, Ara-C, L-asparaginase and oral methotrexate; or (3) Ara-C, cyclophosphamide, and 6-mercoptopurine.

All patients received seven doses of intrathecal drug during the induction and intensification phases of therapy: 24 of 30 received MTX (12 mg/M^2) alone, and six who were considered at high risk because of large peripheral leukemic cell burden received an intial dose of Ara-C (30–70 mg) followed by six doses of methotrexate (12 mg/M^2) (at lumbar puncture number 2, 4, 5, 6, 7, 8). Typically 2 wk elapsed between the first and second lumbar punctures, and the remainder were done at weekly intervals. Eighteen patients were begun on prophylactic cranial irradiation during the 4th or 5th wk of treatment.

Table 1. *Patient groups*

Group	N	Age range (yr)	Diagnosis	Interval from previous I.T. MTX at time of CSF sampling
1	30	2–16	ALL	1 wk*
2	60	3–20	ALL	>3 mo†
3	70	1–21	ALL, lymphoma, or solid tumor	≥3 mo†

* Except 2 wk between CSF 1 and CSF 2.

† Indicates patients who have completed treatment.

A second group of children who were in remission (group 2) were studied to determine variability in the CSF metabolites over several months. Two or more CSF specimens obtained at 3-month intervals were available from 60 children in this group, which included those who were no longer on treatment and others receiving maintenance I.T. MTX every three months. Multiple (up to 10) samples were available from some group 2 subjects.

A third group (group 3) was comprised of children in remission from leukemia, lymphoma or solid tumors from whom only a single CSF sample was obtained.

Analysis of CSF. CSF was frozen at −20° C within an hour after withdrawal and stored at −70° C until the time of analysis. CSF levels of HVA and 5-HIAA were measured using high performance liquid chromatography with electrochemical detection (26). Samples were thawed, spun in a microfuge (Beckman) for 1 min, and then 25 μl of the supernatant was injected directly into the chromatography system, which included a precolumn (Brownlee), a C-18, 5-μ, reverse phase column (Altex), and electrochemical detector with glassy carbon electrode set at 0.8 V *versus* an Ag/AgCl electrode (Bioanalytical Systems). The mobile phase was 0.1 M phosphate-citrate buffer, pH 3, with 0.0001 M EDTA, and 0.012% sodium octyl sulfonate. Sodium octyl sulfonate was used because we also examined CSF dopamine levels (results not reported). HVA and 5-HIAA peaks were identified by calculation of retention times and the peaks of CSF metabolites coeluted with those of added standards. Concentrations were calculated by comparison with peak heights of external standards (Sigma).

Statistics. Statistical analysis of the data was carried out using the Michigan Interactive Data Analysis System. Where appropriate, HVA and 5-HIAA data were examined independently using Student's *t* test and least squares linear regression analysis with significance of the correlation determined by analysis of variance.

RESULTS

Children with acute leukemia are often systemically ill at the time of diagnosis and it was of interest to determine if their initial pretreatment HVA and 5-HIAA values were abnormal. Concentrations of both metabolites normally change markedly with age, declining by approximately 50% from birth to age 3 yr and diminishing sharply afterward until adolescence (27, 28). Therefore, the pretreatment values in the patients (Figs. 1 and 2) were compared to values measured in age matched patients during remission (groups 2 and 3) and no significant difference was found (paired *t* tests). Furthermore, the leukemic patients' pretreatment values resembled closely our previous results from a large group of age-matched children who received lumbar punctures for a variety of reasons including surveillance of ALL in remission (28). Their values also are close to results from other normal children (27).

Inspection of the sequential data from group 1 patients during induction and intensification therapy (Figs. 1 and 2) suggested that HVA values followed a modestly downward trend over the first 5 wk, but rose over the next 4 wk. Linear regression analysis was performed separately on data from each period. Despite the trend downward, the linear correlation attempted on the early group of data was not statistically significant. In contrast, values from the 6th through the 9th wk did rise significantly (analysis of variance, $p = 0.001$). Data on 5-HIAA concentrations were very similar, remaining stable over the first 5 wk and then rising significantly from wk 6 through 9 (analysis of variance, $p = 0.029$). The data analyzed in Figures 1 and 2 included all data points gathered on the entire patient group, but independent analysis of individual data from 17 patients for whom it was possible to calculate early and late phase slopes yielded similar results. CNS prophylaxis in all these patients was started in similar fashion except that six of the 30 group 1 children received

a single, initial dose of Ara-C instead of MTX. Excluding these patients from the statistical analysis of the early HVA and 5-HIAA data did not alter the results.

When the metabolites were measured 2 wk after the first dose of I.T. drug (Figs. 3 *A* and *B*), in patients who had received MTX, there were no significant changes. In contrast, in five of six patients who had received an initial dose of Ara-C, HVA fell by 28 ± 10% ($p < 0.001$, Student's t test) and 5-HIAA declined by 28 ± 12% ($p < 0.05$). The pretreatment HVA levels in the patients who received Ara-C were unremarkable (mean difference from age-matched controls +3.3 ± 3.4 ng/ml) while in the postreatment group, HVA values were 21.7 ± 8.3 ng/ml lower than age-matched controls. Thus, it is unlikely that the reduction

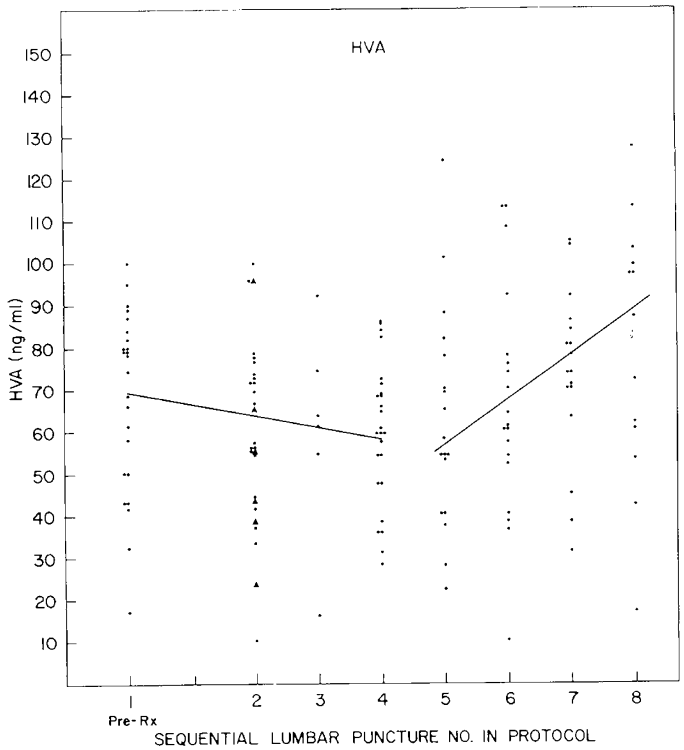

Fig. 1. The distribution of CSF HVA values in 30 children with ALL (group 1) during induction and consolidation phases of treatment (Rx). The pre-Rx lumbar puncture (L.P.) is designated as 1. The time interval between the first and second specimens is 2 wk while the interval between the seven subsequent L.P.s is 1 wk. *Filled triangles* represent values from patients who had an initial dose of Ara-C. The mean slope lines which are shown are calculated by least square linear regression. The attempted linear correlation for L.P.s 1–4 was not significant statistically but the correlation for the later group rose significantly as described in the text.

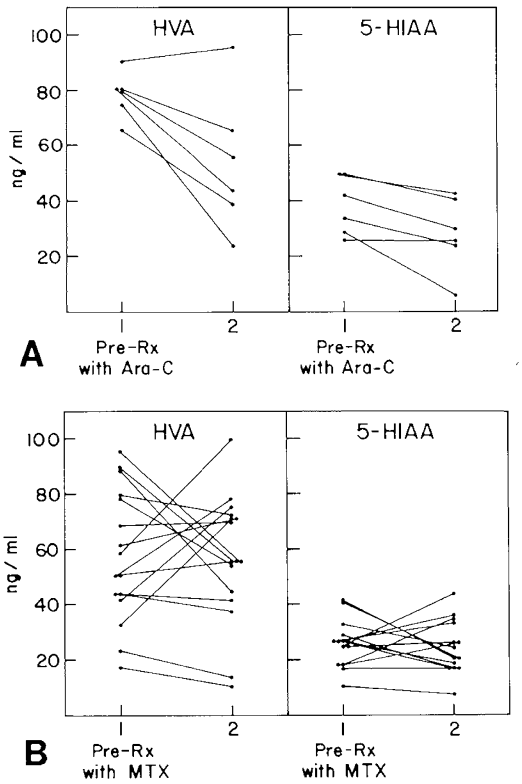

Fig. 3. *A*, compares HVA and 5-HIAA levels before treatment (pre-Rx) and 2 wk later in six children who received IT Ara-C at the first lumbar puncture. HVA declined by 28 ± 10%, $p < 0.001$, Student's t test, and 5-HIAA decreased by 28 ± 12%, $p < 0.05$, Student's t test. *B*, compares HVA and 5-HIAA levels pre-Rx and 2 wk later in 16 children who received IT MTX at the first lumbar puncture. Mean levels did not change.

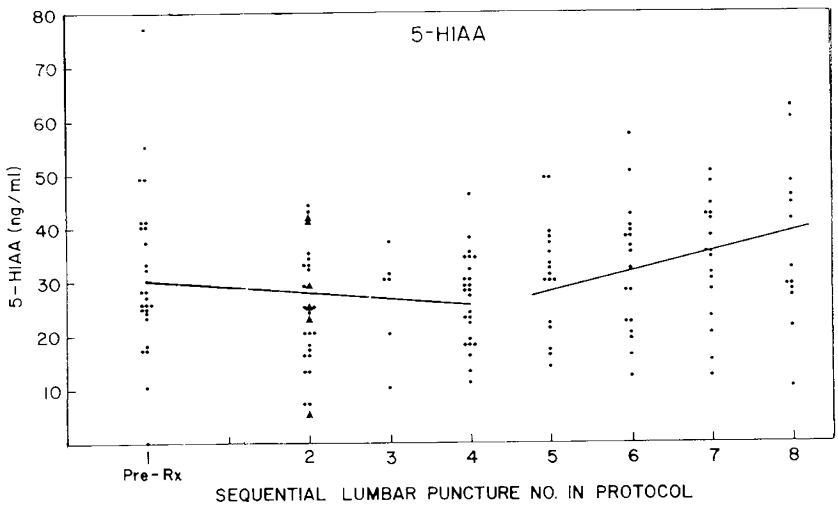

Fig. 2. The distribution of CSF 5-HIAA values in 30 children with ALL during induction and consolidation phases of treatment (Rx). There is a 2-wk interval between the first and second specimens while the seven subsequent L.P.s were done at weekly intervals. *Filled triangles* signify values from patients who had an initial dose of cytosine arabinoside. Mean slopes which are shown are calculated by least square linear regression. The values rose significantly for specimens 5–8 as described in text.

Table 2. *Selected illustrative cases**

I.D.	Age	Sex	Diagnosis	Timing of LP	HVA (ng/ml)	5-HIAA (ng/ml)	Comment
1	4	F	ALL, newly diagnosed	LP-1	61	17	She received 8 doses of IT MTX, and no radiation during induction; LP-9 was done 3 months after LP-8.
				LP-2	71	25	
				LP-4	66	28	
				LP-8	108	44	
				LP-9	60	19	
2	13	M	ALL, newly diagnosed	LP-1	74	28	He showed marked early depression of HVA and 5-HIAA; he developed somnolence syndrome but is clinically normal at 1 yr follow-up.
				LP-2	23	5	
				LP-4	36	11	
				LP-7	63	12	
3	6	M	ALL, in remission	5/82	80	31	In 8/82, he developed headaches, and decreased responsiveness 1 month after a dose of I.T. MTX; no cause was identified; he recovered completely.
				8/82	23	18	
				11/82	74	24	
				4/82	79	33	
4	12	F	ALL, in remission	11/81	79	44	She has clinical and CT scan evidence of leukoencephalopathy; HVA and 5-HIAA levels are normal.
				8/82	84	38	
5	21	M	ALL, in relapse	1/83	28	18	He relapsed with CNS leukemia; with +++ blasts, protein = 95 and glucose = 10 mg % in CSF, HVA and 5-HIAA were still normal.
6	2	M	Neuroblastoma	1/83	10	30	He had an LP done before spinal cord compression was diagnosed; the HVA/HIAA ratio is reversed which is typical of CSF block.

* For newly diagnosed patients, specimens are identified by sequential lumbar puncture (LP) number; for children in remission dates of specimens are given.

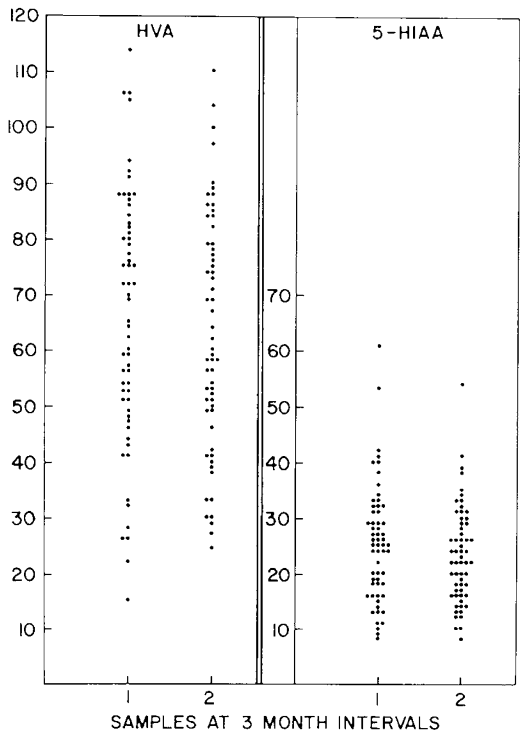

Fig. 4. Paired CSF HVA (*left side*) and 5-HIAA (*right side*) levels in 60 children with ALL in remission (group 2). Three months elapsed between the two lumbar punctures. There are no statistically significant differences between metabolite values in the two sets of CSFs.

could be related to any normalization of CSF dynamics by Ara-C therapy.

The results indicated that both HVA and 5-HIAA rose significantly only from wk 6–9 of treatment, and this prompted a search for variables that might be responsible for the effect. Eighteen patients received cranial irradiation beginning between wk 4 and 5, but elevated metabolite levels were also seen by wk 9 in patients who had not received this treatment. Type of systemic chemotherapy could not be related to the rise in metabolites. There was no relationship observed between elevation of CSF protein (in patients in both groups 1 and 2) and metabolite levels (*e.g.* patient 5, Table 2).

There are few data available about the stability of neurotransmitter metabolite levels in an individual over time. To examine this issue, we evaluated paired specimens obtained 3 months apart in children in remission (Fig. 4). Over this interval, values for HVA and 5-HIAA were remarkably stable. For example patient 1 (Table 2), who had marked elevations returned to HVA and 5-HIAA values in remission that were nearly identical to pretreatment values.

Very few of the patients examined in these studies experienced sharp declines in CSF metabolites; data from three children are included in Table 2. Patient 2 had a rapid and prominent fall in both HVA and 5-HIAA at two weeks after his first dose of I.T. Ara-C. Although he had no neurological signs at the time, 2 months later he developed a typical somnolence syndrome which resolved, leaving him normal at 1 yr follow-up. Patient #3 did have prominent somnolence, depression, and headache in association with very low HVA and 5-HIAA values 1 month after a maintenance dose of I.T. MTX. Both his metabolite values and mental status returned to normal at subsequent follow-up. We could not relate the occurrence of somnolence syndrome to any

pattern of neurotransmitter metabolite changes in other patients. The only patient in our series with documented chronic MTX leudoencephalopathy (patient 4, Table 2) had normal CSF metabolites, when studied 3 yr after she had received intensive I.T. MTX and cranial irradiation. Finally, the most dramatic reduction in CSF HVA was measured when a lumbar puncture was done in a patient in whom the spinal canal was blocked by neuroblastoma tumor (patient 6). Although neuroblastomas secrete HVA systemically (29), there is no evidence of significant blood to CSF transport of HVA. CSF HVA which is transferred primarily from the brain downward into the lumbar sac via CSF was markedly reduced. In contrast 5-HIAA, produced partially in the spinal cord, was preserved. Thus, the CSF block inverted the normal HVA/5-HIAA ratio.

DISCUSSION

These observations provide new information about CNS dopamine and serotonin metabolism in children with ALL undergoing treatment with systemic chemotherapy and intrathecal MTX or Ara-C. It is noteworthy that their pretreatment values were normal, since previous studies found early EEG abnormalities at this time (30, 31). Although the response to treatment has not been investigated before in a prospective fashion, an earlier report based on random CSF specimens from children already started on chemotherapy showed that monoamine metabolites were normal in most but were low in a few (32). The reductions in that report seemed to correlate with the administration of I.T. MTX. Based on that preliminary data, as well as the possibility that MTX might inhibit brain dihydrofolate and dihydropteridine reductases, we hypothesized that monoamine metabolites might decline in these patients.

Temporary disruption in neurotransmitter synthesis might be associated with a variety of changes in appetite, behavior, attention, and level of consciousness (25). This is supported by observations made in children with variant forms of phenylketonuria. In these patients, deficient pteridine cofactor production is associated with abnormalities in CSF neurotransmitter metabolites and neurologic deficts which respond in part to neurotransmitter replacement therapy (23, 33). Similar replacement therapy had been suggested to treat toxic MTX encephalopathy (13, 18). Therefore, the late elevation we observed in both HVA and 5-HIAA levels during wk 6–9 for the study group (group 1) was unexpected.

Based on the human data alone, it is impossible to decide whether the metabolites are elevated because of an increase in dopamine and serotonin turnover within central neurons or alternatively because their egress from CSF is impeded (25). All patients received vincristine, prednisone, and L-asparaginase during induction. These drugs have no known direct effects on CNS neurotransmitter metabolism. By inhibition of protein synthesis, L-asparaginase could limit neurotransmitter production—but this was not observed. One way to determine if turnover was increased would be to measure levels after the acid transport system which removes the metabolites was blocked with probenecid (32). However, this would be unsatisfactory since the drug frequently makes patients uncomfortable because of nausea and because probenecid may also inhibit removal of MTX from CSF (34, 35). Alternatively, animal studies may provide insight into the effects of MTX. Our experiments with effects of infusions of MTX into the lateral ventricles of rhesus monkeys and rats suggest that the elevation in monoamine metabolites is caused by delayed egress rather than enhanced turnover (36, 37). Constant infusion of MTX (0.05 mg/day for 5 days) into the lateral ventricle of monkeys produced a nearly 3-fold elevation in CSF concentrations of HVA compared with untreated controls and a smaller but significant rise in 5-HIAA (36). However, direct brain tissue (corpus striatum) measurements of metabolites after repeated intraventricular injection of MTX in rats did not produce any significant elevation, suggesting a more distal site of accumulation (37). While it remains possible that the CSF changes are related to some medication or combination of treatment other than intrathecal chemotherapy, the data from both humans and animals suggest that this component of therapy is capable of causing the changes we observed. Since daily CSF production is, normally, four to five times the total CSF volume, it is unlikely that the weekly or biweekly lumbar punctures would lead to altered metabolite concentrations unless CSF production was itself inhibited (for which there is no evidence).

Elevation in biogenic amine metabolites, independent of changes in neuronal metabolism, would not be expected to affect behavior or be neurotoxic. However, the observation has several other possible implications. MTX is eliminated from CSF by bulk flow and active transport, partially blockable by probenecid (34, 38–42). If the elevated metabolites reflect a cumulative toxic reduction in function of the organic acid pump, then transit of other molecules, such as MTX itself, might be slowed. This would be reflected in a lengthening of CSF MTX elimination kinetics with prolonged or intensive treatment. This effect has not been reported but might be expected based on our data. It is possible that chemotherapy may alter the egress of HVA and 5-HIAA through a subtle nonspecific inflammatory effect seen with MTX, or by a more specific biochemical action (15). Based on the absence of elevated CSF protein (data not shown) and absence of clinical symptoms of arachnoiditis in the patients, a specific biochemical effect seems more likely. If the changes noted reflect accumulation of metabolite in a probenecid-like fashion, possible depression in dopamine or serotonin turnover by a direct action would be partially masked unless allowance is made for the rising baseline.

We hypothesized that I.T. MTX might suppress biogenic amine metabolism if the drug reached a high enough concentration in lateral ventricular CSF bathing the caudate nuclei, regions of dense innervation by dopamine nerve terminals. Dopamine nerve terminals are the primary sites of tetrahydrobiopterin synthetic capacity and endogenous cofactor stores in the caudate and they are a major source of HVA in lumbar CSF (43, 44). Clinical and animal studies suggest that the extent of entry of MTX into the lateral ventricles after lumbar injection is variable (38, 40, 41). Two patients had marked reduction of CSF HVA during therapy. In both, there was at least one HVA value approximately 70% lower than values which preceded or followed them and both also had behavioral abnormalities noted by their attending physicians. In the case of the child in whom low HVA and 5-HIAA values occurred at the second and fourth lumbar puncture (patient 2, Table 2) during induction, a "somnolence syndrome" was delayed several weeks. In the other (patient 3), the low HVA and 5-HIAA values were obtained at the time he was sleepy, depressed, and complaining of headaches 1 month after a dose of I.T. MTX. It is conceivable that such symptoms could be related to the neurochemical abnormalities. Dopamine and serotonin pathways send diffuse projections to multiple brain regions including the basal ganglia, cortex, and hypothalamus and disruptions in their metabolism have been linked to alterations in wakefulness, memory, appetite, activity, and attention (25). Therefore the two cases lend some limited support to the proposition that biogenic amine abnormalities might be involved in certain temporary subacute encephalopathic signs and symptoms. The single patient with chronic severe leukoencephalopathy had normal metabolite levels, suggesting that chronic degeneration of these neurotransmitter pathways does not accompany the impressive destruction of myelinated areas. However, these findings do not exclude the possibility of preceding transient derangements of neurotransmitter metabolism.

It was unexpected that I.T. Ara-C administration was associated with a fall in biogenic amine metabolites 2 wk later. The biochemical explanation for this is not clear since this drug has no known effect on biogenic amine metabolism. However, both MTX and Ara-C have been shown to produce a similar spectrum

of acute and subacute neurotoxic effects as well as chronic leukoencephalopathy (1, 10). Although Ara-C is thought to act predominantly by inhibiting DNA polymerase, it also disrupts synthesis of RNA and protein (45, 46). Like other pyrimidine antineoplastic drugs it causes neurotoxic effects in patients (47). MTX, in addition to its prominent effects on DNA synthesis, also inhibits protein synthesis and inhibits synthesis of the amino acids glycine and methionine, necessary for methyltransferase reactions (46). Therefore, these two antimetabolites share a common action to disrupt protein synthesis in nondividing brain cells. Inhibition of protein synthesis needed for turnover and synthesis of essential myelin constituents, such as myelin basic protein, would be a rational mechanism by which they cause neurotoxic leukoencephalopathy. Reduction in biogenic amine synthesis might reflect a specific action of the drug on transmitter metabolism or a nonspecific toxic neuronal response to restricted protein synthesis.

Although neurotransmitter abnormalities may be involved in transient behavioral responses to CNS prophylaxis, their causal relationship to serious long-term leukoencephalopathy is tenuous. However, it is reasonable to consider whether transmitter metabolite levels could be used to assess the intensity of brain exposure to MTX. It is possible that transiently high ventricular concentrations might produce a prolonged effect on brain biochemical events through active polyglutamated storage forms of MTX (38). Data from rat cultured pineal glands suggest that high MTX concentrations will reduce concentrations of tetrahydrobiopterin in brain (24). MTX in concentrations that completely inhibited dihydrofolate reductase had no effect on levels of the cofactor but MTX concentrations in the range of 10^{-6} M reduced tetrahydrobiopterin by 30%. Although this recent information suggests that DHFR activity plays little role in generating cofactor, high brain concentrations of MTX in a range that may be reached with certain treatment protocols, might be expected to suppress pteridine reductase. This is consistent also with our data from monkeys in which lower doses of MTX raised monoamine metabolite levels, but high dose continuous infusions produced marked reductions in association with transient encephalopathy (36). Our human data do not make it clear if this phenomenon occurs in humans at clinically relevant doses. Further prospective longer term evaluation is warranted, especially in patients treated with high dose MTX who are at high risk for encephalopathy.

Acknowledgments. Statistical consultation was provided by Ms. S. Sereika and Dr. A. Shork, University of Michigan School of Public Health, Biostatistics Department. We are grateful for the cooperation of the Hematology-Oncology staff at Mott Children's Hospital.

REFERENCES

1. Allen JC 1978 The effects of cancer therapy on the nervous system. J Pediatr 93:903–909
2. Allen JC, Rosen G 1978 Transient cerebral dysfunction following chemotherapy for osteogenic sarcoma. Ann Neurol 3:441–444
3. Bleyer WA 1981 Neurologic sequelae of methotrexate and ionizing radiation: a new classification. Cancer Treat Rep 65:89–97
4. Crosley CJ, Rorke LB, Evans A, Nigro M 1978 Central nervous system lesions in childhood leukemia. Neurology 28:678–685
5. DeVivo DC, Malas D, Nelson JS, Land VJ 1977 Leukoencephalopathy in childhood leukemia. Neurology 27:609–613
6. Kay HEM, Knopton PJ, O'Sullivan JP, Wells DG, Harris RF, Innes EM, Stuart J, Schwartz FCM, Thompson EN 1972 Encephalopathy in acute leukemia associated with methotrexate therapy. Arch Dis Child 47:344–354
7. McIntosh S, Klatskin EH, O'Brien RT, Aspnes GT, Kammerer BL, Snead OC, Kalavsky SM, Pearson HA 1976 Chronic neurologic disturbance in childhood leukemia. Cancer 37:853–857
8. Pavlovsky S, Castano J, Leiguarda R, Fisman N, Chamoles N, Moreno R, Arizaga R 1983 Neuropsychological study in patients with ALL. Amer J Ped Hematol Oncol 5:79–86
9. Pizzo PA, Bleyer WA, Poplak DG, Leventhal BG 1976 Reversible dementia temporally associated with intraventricular therapy with methotrexate in a child with acute myelogenous leukemia. J Pediatr 88:131–133
10. Pizzo PA, Poplack DG, Bleyer WA 1979 Neurotoxicities of current leukemia therapy. Am J Pediatr Hematol Oncol 1:127–140
11. Peylan-Ramu M, Poplack DG, Pizzo PA 1978 Abnormal CT scans of the brain in asymptomatic children with acute lymphocytic leukemia after prophylactic treatment of the central nervous system with radiation and intrathecal chemotherapy. N Engl J Med 298:815–818
12. Shapiro WR, Allen JC, Horten BD 1980 Chronic methotrexate toxicity to the central nervous system. Sloan-Kettering Memorial Hospital Clin Bull 10:49–52
13. Ettinger LJ 1982 Pharmacokinetics and biochemical effects of a fatal intrathecal methotrexate overdose. Cancer 50:444–450
14. Poplack DG 1984 Massive intrathecal overdose: "Check the label twice!". N Engl J Med 311:400–401
15. Bleyer WA, Drake JC, Chabner BA 1973 Neurotoxicity and elevated cerebrospinal fluid methotrexate concentration in meningeal leukemia. N Engl J Med 289:770–773
16. Price RA, Jamieson PA 1975 The central nervous system in childhood leukemia II. Subacute leukoencephalopathy. Cancer 35:306–318
17. Rubinstein LJ, Herman MM, Long TF, Wilbur JR 1975 Disseminated necrotizing leukoencephalopathy: a complication of treated central nervous system leukemia and lymphoma. Cancer 35:291–305
18. Abelson HT 1978 Methotrexate and central nervous system toxicity. Cancer Treat Rep 62:1999–2001
19. Liang BT, Vaccaro KK, Perelle BA, Perlman RL 1981 Studies on dihydropteridine reductase activity in pheochromocytoma cells. J Neurochem 37:1164–1169
20. Milstein S, Kaufman S 1975 Studies on the phenylalanine hydroxylase system in liver slices. J Biol Chem 256:4777–4781
21. Nichol CA, Viveros OH, Duch DS, Abou-Donia MM, Smith GK 1983 Metabolism of pteridine cofactors in neurochemistry. In: Pfleiderer W (ed) Chemistry and Biology of Pteridines. Walter de Gruyter and Co, New York, pp 131–151
22. Pollock RJ, Kaufman S 1977 Dihydrofolate reductase is present in brain. J Neurochem 30:253–256
23. Brewster TG, Moskowitz MA, Kaufman S 1979 Dihydropteridine reductase deficiency associated with severe neurologic disease and mild hyperphenylalaninemia. Pediatrics 63:94–99
24. Culvenor AJ, Miller LP, Levine RA, Lavenburg W 1984 Effects of methotrexate on biopterin levels and synthesis in rat cultured pineal glands. J Neurochem 42:1707–1714
25. Johnston MV, Singer HS 1982 Brain neurotransmitters and neuromodulators in pediatrics. Pediatrics 70:57–68
26. Silverstein FS, Donn S, Buchanan K, Johnston MV 1984 Concentrations of homovanillic acid and 5-hydroxyindoleacetic acid in cerebrospinal fluid from human infants in the perinatal period. J Neurochem 43:1769–1772
27. Langlais PJ, Walsh FX, Bird ED, Levy HL 1985 Cerebrospinal fluid neurotransmitter metabolites in neurologically normal infants and children. Pediatrics 75:580–586
28. Silverstein FS, Johnston MV, Hutchinson RJ, Edwards NL 1985 Cerebrospinal fluid neurotransmitter abnormalities in the Lesch-Nyhan syndrome. Neurology 35:907–911
29. Brewster MA, Berry DH, Moriarty B 1977 Urinary 3-methoxy-4-hydroxy phenylacetic acid (homovanillic) and 3-methoxy-4-hydroxy mandelic (vanillyl-mandelic) acids: gas liquid chromatography methods and experience with 13 cases of neuroblastoma. Clin Chem 23:2247–2249
30. Ch'ien LT, Aur RJA, Stagner S, Cavallo K, Wood A, Goff J, Pitner S, Hustu O, Seifert MJ, Simone JV 1980 Long term neurological implication of somnolence syndrome in children with acute lymphocytic leukemia. Ann Neurol 8:273–277
31. Mahoney DH, Britt CW, Kellaway P, Fernbach DJ 1981 Childhood leukemia: implications of EEG findings at time of diagnosis. J Pediatr 98:437–440
32. Shaywitz BA, Cohen DJ, Bowers MB 1980 Cerebrospinal fluid monoamine metabolites in neurological disorders of childhood. In: Wood JH (ed) Neurobiology of Cerebrospinal Fluid. Plenum Publishing Corporation, New York, pp 219–236
33. Koslow SH, Butler IJ 1977 Biogenic amine synthesis defect in dihydropteridine reductase deficiency. Science 198:522–523
34. Howell SB, Olshen RA, Rice JA 1979 Effect of probenecid on cerebrospinal fluid methotrexate kinetics. Clin Pharmacol Ther 26:641–645
35. Ramu A, Fusner JE, Blaschke T, Glaubiger DL 1978 Probenecid inhibition of methotrexate-cerebrospinal fluid pharmacokinetics in dogs. Cancer Treat Rep 62:1465–1470
36. Johnston MV, Silverstein FS, Greenberg HS, Ensminger WD, Chandler WF, Phillips TW, Knutsen CA 1983 Intraventricular methotrexate alters cerebrospinal fluid homovanillic acid levels in Rhesus monkeys. Neurology 33(suppl 2):109
37. Silverstein FS, Eickholt M, Johnston MV 1983 Intraventricular methotrexate produces seizures and changes in biogenic amine metabolites in rats. Neurology 33(suppl 2):67
38. Jolivet J, Cowan KH, Curt GA, Clendeninn MJ, Chabner BA 1983 The pharmacology and clinical use of methotrexate. N Engl J Med 309:1094–1103
39. Schornagel JH, McVie JG 1983 The clinical pharmacology of methotrexate. Cancer Treat Rep 10:53–75
40. Bleyer WA 1977 Clinical pharmacology of intrathecal methotrexate. II. An improved dosage regimen derived from age-related pharmacokinetics. Cancer Treat Rep 61:1419–1425

41. Bleyer WA, Dedrick RL 1977 Clinical pharmacology of intrathecal methotrexate. I. Pharmacokinetics in nontoxic patients after lumbar injection. Cancer Treat Rep 61:703–708
42. Bode U, Magrath IT, Bleyer WA, Poplack DG, Glaubiger DL 1980 Active transport of methotrexate from cerebrospinal fluid in humans. Cancer Res 40:2184–2187
43. Levine RA, Lovenberg W, Curtius H-Ch, Niederwieser A 1983 In: Pfleiderer W (ed) Chemistry and Biology of Pteridines. Walter de Gruyter and Co, New York, pp 833–837
44. Levine RA, Miller LP, Lovenberg W 1981 Tetrahydrobiopterin in striatum: Localization in dopamine nerve terminals and role in catecholamine synthesis. Science 214:919–921
45. Kaplan RS, Wiernik PH 1982 Neurotoxicity of antineoplastic drugs. Semin Oncol 9:103–125
46. Gilman AG, Goodman LS, Gilman A 1980 The Pharmacological Basis of Therapeutics. MacMillian Publishing Co, New York, pp 1272–1274 Winkelman MD, Hines JD 1983 Cerebellar degeneration caused by high-dose cytosine arabinoside: a clinicopathological study. Ann Neurol 14:520–527