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Liver Damage in Children with Acute Leukaemia and non-Hodgkin's Lymphoma on Oral Maintenance Chemotherapy

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Summary. *Eight of 36 children receiving maintenance chemotherapy for acute lymphoblastic leukaemia or non-Hodgkin's lymphoma had liver biopsies on the basis of clinical abnormalities and/or elevated serum enzyme levels. Six biopsies were abnormal, including one in a boy with spider naevi who showed micronodular cirrhosis; he appeared to retain methotrexate in the blood for a prolonged period and his SGOT level did not return to normal for 19 months after maintenance chemotherapy was discontinued. The five other abnormal biopsies showed minor changes in the portal tracts. The six children with abnormal liver histology showed a wide variation in their early handling of an oral methotrexate dose.*

There was a statistically significant rise in mean SGOT and alkaline phosphatase during treatment, but the wide scatter in values precluded their use as accurate indicators of liver damage in these children.

Introduction

In 1955 Colsky [3] drew attention to the possible toxic effect of methotrexate (MTX) on the liver in children with acute leukaemia. In contrast, Wetherley-Mein and Cottom [19] suggested that the liver fibrosis seen in such children was caused by infiltration of the portal tracts by leukaemic cells rather than by MTX. The incidence and aetiology of liver abnormalities in children receiving maintenance chemotherapy remain controversial, and as the life expectancy of these children improves, so the importance of liver damage increases. This report is a description of the serum enzyme and liver histology findings in children receiving maintenance chemotherapy for acute lymphoblastic leukaemia (ALL) or non-Hodgkin's lymphoma (NHL).

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Patients and Methods

Thirty-six children, 26 with acute lymphoblastic leukaemia and 10 with non-Hodgkin's lymphoma, had liver function tests at about 6-monthly intervals whilst receiving oral maintenance chemotherapy, a total of 126 samples being taken. There were 24 boys and 12 girls, the age range being 3–15 years. Selected children also had percutaneous liver biopsy.

Induction therapy was performed with vincristine, prednisolone, adriamycin, and L-asparaginase. Central nervous system prophylaxis, given in each case, consisted of 2400 rads cranial irradiation and five injections of intrathecal (IT) MTX, 10 mg/m^2, not exceeding 12 mg in any single injection. Oral maintenance chemotherapy consisted of MTX in a dose of 25–30 mg/m^2 PO weekly, 6-mercaptopurine 50–70 mg/m^2 PO daily, and in the case of NHL patients cyclophosphamide 200 mg/m^2 PO weekly. The intended duration of oral maintenance chemotherapy was 24 months for ALL and 36 months for NHL.

Where there was clinical evidence of liver dysfunction and marked elevation of serum glutamic oxaloacetic transaminase (SGOT) and alkaline phosphatase (AP), or where there was marked elevation of serum enzymes alone (particularly where the SGOT was markedly above 40 IU/litre; see Fig. 1) liver biopsy was performed after full discussion with the parents. Biopsy sections were stained with both haematoxylin and eosin and for reticulin, and the result contributed to a clinical decision to stop or continue maintenance chemotherapy.

Because slow MTX absorbers may be more susceptible to liver damage [5], six of the eight children who had liver biopsies also had a MTX absorption test after their normal weekly dose of the drug. Blood levels were measured at intervals up to 4 h after the dose of MTX by a radioimmunoassay developed at the University of Surrey, Guildford. Folinic acid was given at the end of the absorption test in those children who had previously stopped taking MTX.

Results

Serum Enzyme Levels

Mean SGOT and AP levels rose during maintenance treatment and fell when it was stopped. There was a statistically significant rise in mean SGOT from 40.2 IU before treatment to 84.4 IU in the second year of treat-

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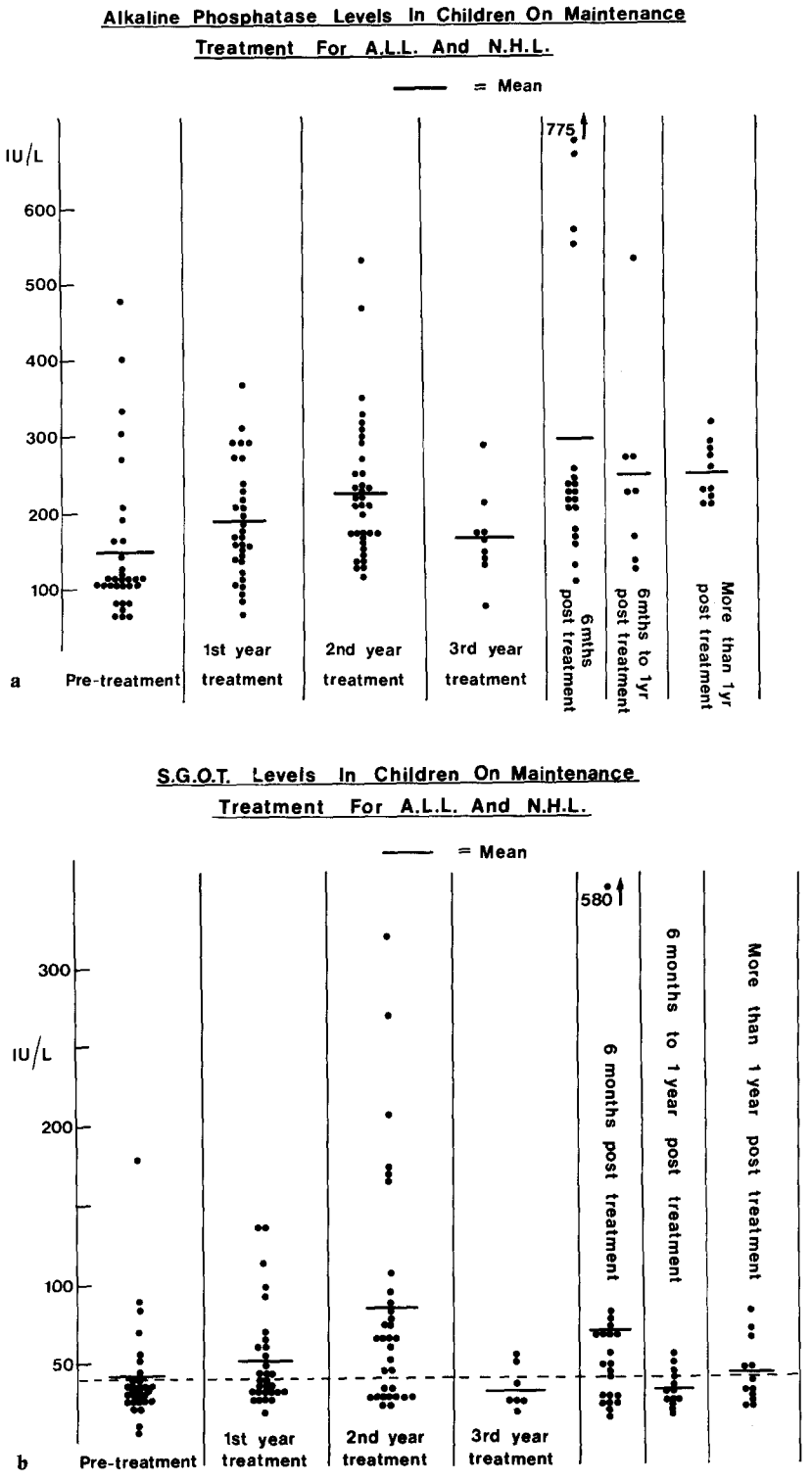

Fig. 1a and b. Alkaline phosphatase (**a**) and glutamic-oxaloacetic transaminase (**b**) levels in children in the current study

ment; the mean then fell to 55.5 IU in the first year after treatment (Table 1, Fig. 1). Similarly, mean AP levels rose significantly from 150.7 IU before treatment to 226.2 IU in the second year of treatment, but levels remained significantly above the pretreatment levels in the first year *after* treatment.

This rise in mean AP and SGOT was reflected in the *percentage* of patients who showed abnormal values for these enzymes before, during, and after maintenance treatment (Table 1).

The upper limit of normal for SGOT was taken to be 40 IU for all ages since this enzyme level is not known to

Table 1. Serum enzyme levels in relation to treatment

	Mean	Standard deviation	Number of samples	Significance of difference of mean from pre-treatment mean	Proportion of patients showing abnormal results
Serum glutamic oxaloacetic transaminase					
Pretreatment	40.2 IU	31.93	30	–	6/17 = 35.3%
First year of treatment	50.4 IU	32.40	30	$p = 0.1$	
Second year of treatment	84.4 IU	74.7	31	$p = > 0.001^a$ < 0.01	21/27 = 77.7%
First year posttreatment	55.5 IU	94.5	34	$p = 0.5$	7/15 = 46.7%
Alkaline phosphatase					
Pretreatment	150.7 IU	99.3	32	–	5/16 = 31.3%
First year of treatment	185.7 IU	74.2	31	$p = 0.1$	
Second year of treatment	226.2 IU	92.3	35	$p = > 0.001^a$ < 0.01	16/27 = 59.3%
First year posttreatment	278.2 IU	173.5	28	$p = < 0.001^a$	9/14 = 64.3%

[a] Statistically significant at 5% level according to Student's *t*-test

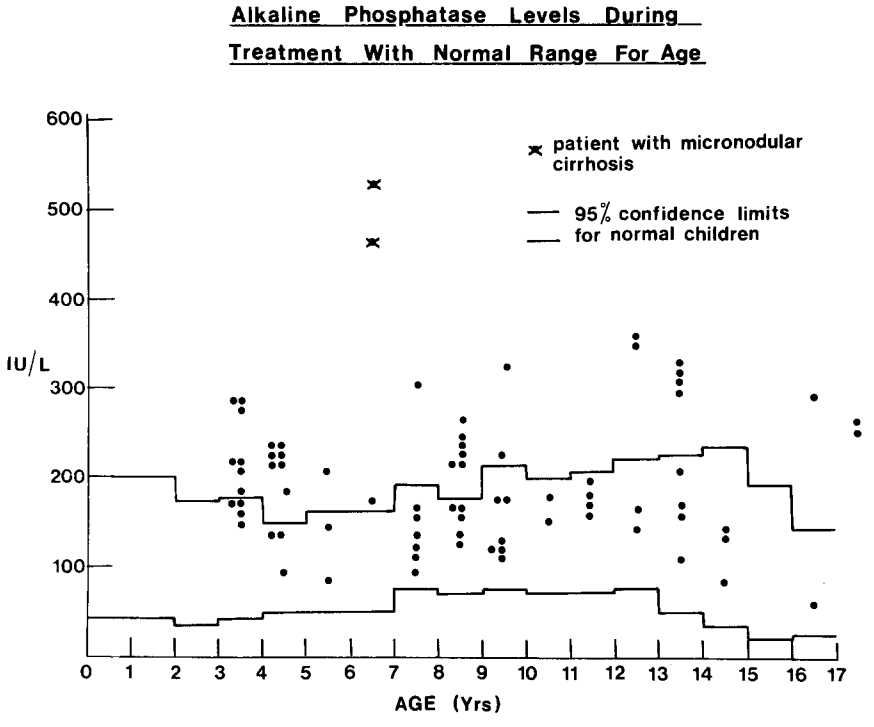

Fig. 2. Alkaline phosphatase results during treatment with normal ranges for age

vary within the ages of the children in this study [17]. With AP the normal range was considered to be within the 95% confidence limits from the studies of Round [15] and A. Stott [personal communication]. These limits are shown graphically in Fig. 2. Fifty-nine percent of the children showed elevated alkaline phosphatase levels during maintenance treatment, including the particularly high levels in the child with cirrhosis.

The wide scatter of results is emphasised by the large standard deviations at all times for both SGOT and AP.

Liver Histology

Eight children had a liver biopsy whilst receiving maintenance treatment (Table 2). There were abnormalities in six, with micronodular cirrhosis in one boy who showed numerous spider naevi on clinical examination (Fig. 3). The other five childrens' liver biopsies showed infiltration of the portal tracts with small lymphocytes or eosinophils, some increase in reticulin within the widened portal tracts, and mild fatty change in the hepatocytes. The histological

Table 2. Patients who had liver biopsies

Patient no.	Age	Disease	Weekly dose MTX	Duration of therapy to biopsy	Total dose MTX per m^2	Other drugs given for maintenance	Highest SGOT IU/l	1-h serum MTX	Histology
1	7	ALL	25 mg	17 months	2.33 g	6-mercaptopurine 60 mg daily	107	192 ng/ml	Micronodular cirrhosis
2	10	ALL	20 mg	24 months	1.87 g	Thioguanine 25 mg daily	320	1200 ng/ml	Slight increase in fibrous tissue in portal tracts. Degenerative change in some hepatocytes
3	5	ALL	25 mg	17 months	2.46 g	6-mercaptopurine 40 mg daily	206	390 ng/ml	Mild fine fatty change. Evidence of increased liver cell turnover. A few eosinophils present
4	13	ALL	20 mg	16 months	0.77 g	6-mercaptopurine 50 mg daily	77	40.5 ng/ml	Mild focal chronic inflammatory cell infiltrate
5	11	NHL	20 mg	24 months	1.51 g	6-mercaptopurine 50 mg daily; cyclophosphamide 200 mg weekly	85	–	Normal liver
6	4	ALL	20 mg	10 months	1.2 g	6-mercaptopurine 35 mg daily	133	141 ng/ml	Widened and increased connective tissue on portal tracts. Fine droplet fatty change
7	4	ALL	25 mg	19 months	2.25 g	6-mercaptopurine 35 mg daily	95	–	Cell plates expanded in peri-portal region. Increased number of binucleated hepatocytes. Fine fatty change
8	4	ALL	40 mg	10 months	1.25 g	6-mercaptopurine 50 mg daily	96	165 ng/ml	Mild centrilobular fatty change

changes in these five biopsies were considered mild and probably reversible.

After maintenance chemotherapy was stopped in one child whose liver biopsy had shown normal histology (patient 5), the SGOT returned to normal within 2 months, but in the boy with cirrhosis, SGOT levels remained above normal (65–78 IU/litre) for 19 months after discontinuation of treatment (Fig. 4). AP rose to a maximum of 775 IU/litre 2 months after his treatment was stopped, possibly due to renewed growth, but his alkaline phosphatase levels had been very high during treatment.

Methotrexate Absorption Studies

MTX blood levels after a normal weekly oral dose showed wide variation (Fig. 5), resembling those seen in leukaemic children not known to have liver damage [4]. In the boy with cirrhosis a low level of MTX (0.90 ng/ml) in the blood was detectable before the oral dose was given, indicating that he had retained MTX for 23 weeks since the last dose of MTX was given. When he was retested 7 months later no residual MTX was detected. Another child (patient 4) with mildly abnormal liver histology showed 1.1 ng MTX/ml before an oral dose of the drug, suggesting retention of MTX since his oral dose 2 weeks previously.

Discussion

There has been only one previous description of cirrhosis in association with treated ALL [12] (Table 3). We consider it likely that the liver damage we have reported is drug-induced by prolonged oral maintenance chemotherapy. The significant rise in mean SGOT and AP during treatment is consistent with this.

Study of the possible liver toxicity of MTX or other oral maintenance drugs in ALL has been overshadowed

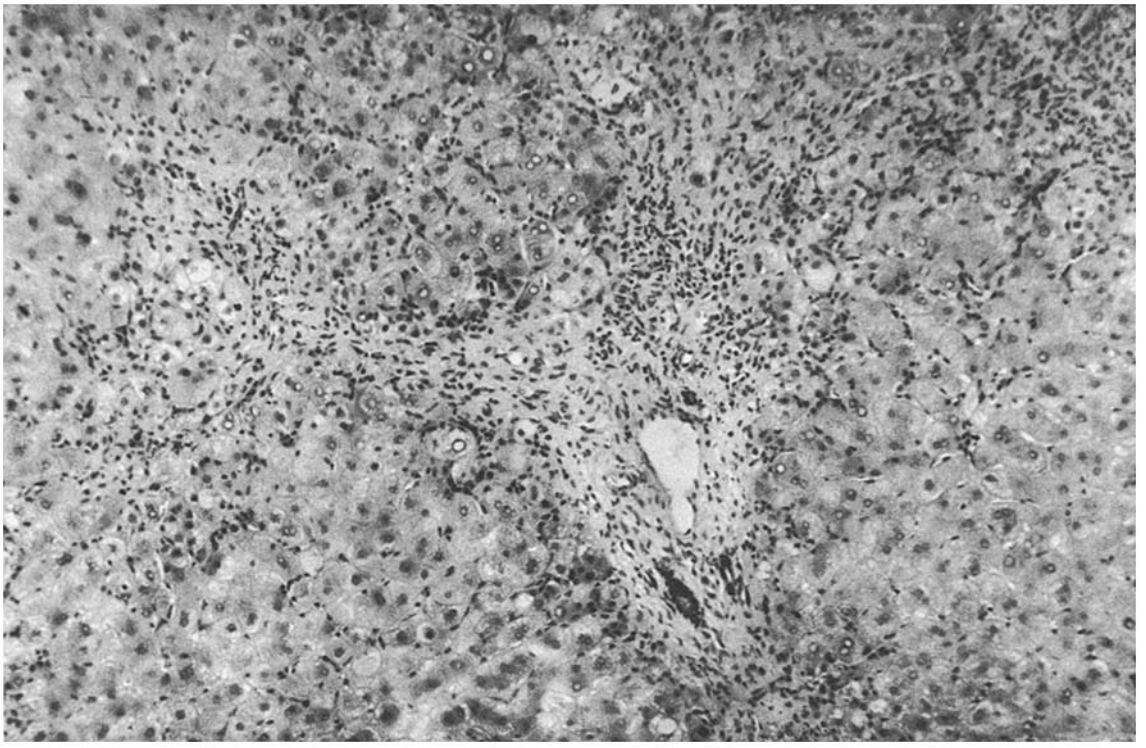

Fig. 3. Liver biopsy from patient 1, showing micronodular cirrhosis. Haematoxylin and eosin

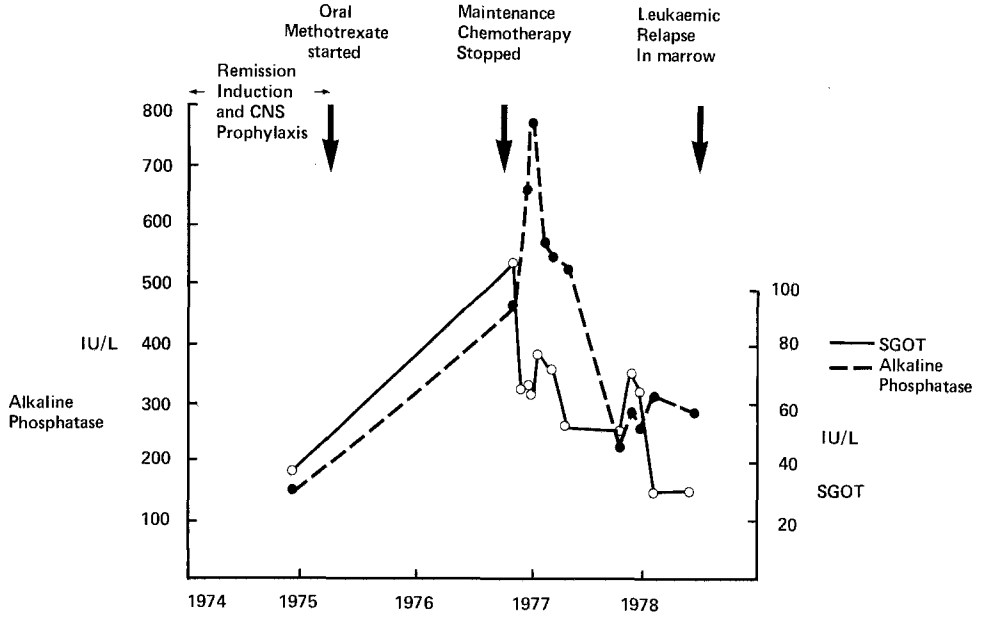

Fig. 4. Serial alkaline phosphatase and glutamic-oxaloacetic transaminase levels in patient 1 with micronodular cirrhosis

by the wealth of literature on the liver toxicity of MTX used for psoriasis. MTX does appear to produce liver damage in a minority of psoriatics, especially when given as a daily oral dose in cumulative total dose of more than 2–4 g over a period of more than 1 year. Cirrhosis appears in about 6% of patients, and the valuable studies of Nyfors [14] show a statistically significant increase in cirrhosis and liver fibrosis during a period of MTX therapy between liver biopsies. Such prospective studies virtually negate the suggestion that the liver damage seen is

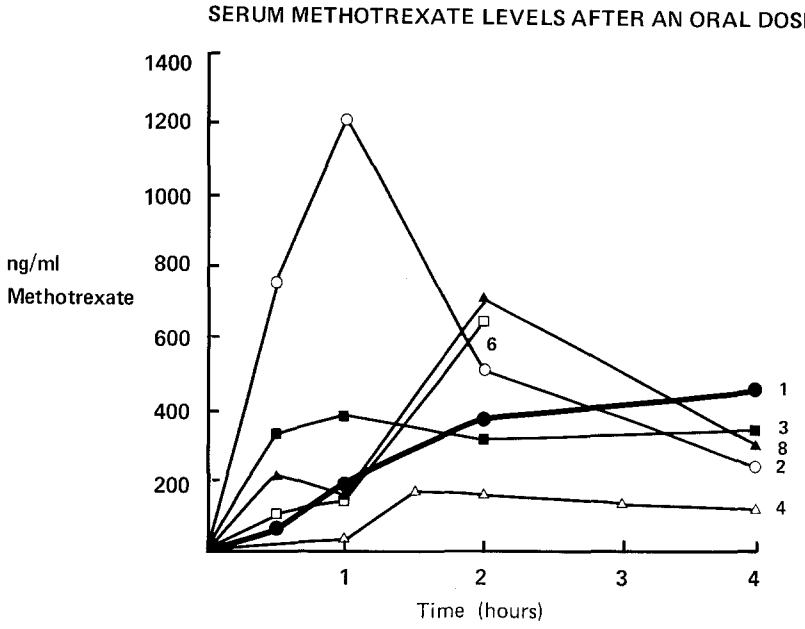

Fig. 5. Serum MTX levels after the usual weekly dose in six children who had liver biopsies. Reference numbers are as given in Table 2

Table 3. Liver toxicity associated with MTX therapy for acute leukaemia of childhood

No. of patients	Dose of MTX	Duration of MTX treatment	Other drugs given	Toxicity seen	Authors
7	1–10 mg MTX or aminopterin daily	9–12 months	Nil	5 of 7 children developed portal fibrosis	Colsky et al. [3]
21	7 had MTX or aminopterin. 14 no MTX	Maximum 49 days	Various	13 of 21 children showed increased collagen in portal tracts of liver	Wetherley-Mein and Cottom [19]
273	–	–	Corticosteroids, 6-mercaptopurine	31% of children showed hepatic fibrosis before MTX therapy. 80% showed fibrosis after therapy	Hutter et al. [8]
32	0.025 mg/kg/day	Less than 1 month	Prednisolone, 6-mercaptopurine	Acute hepatitis demonstrated by liver biopsy in 4 patients	Taft [18]
10	10–22 mg/m^2 IV for 5 days, repeated up to 4 times	40 days	Nil	Liver enzyme elevation in serum and portal inflammatory infiltrate on biopsy	Hersch et al. [7]
216	30 mg/m^2 twice weekly PO	Longer than 4 months	Children's cancer study group 903	80 children showed elevated serum liver enzymes. 5 developed cirrhosis. 9 had fibrosis	Nesbit et al. [12]
7	80 mg/m^2 2-weekly	Usually $1\frac{1}{2}$–$2\frac{1}{2}$ years	Cytosine arabinoside, cyclophosphamide	4 developed portal fibrosis	McIntosh [11]
1	1.25 mg daily (total dose 2.5 gm)	$5\frac{1}{2}$ years	Vincristine, prednisolone	Portal fibrosis, hepatoma	Ruymann et al. [16]

associated with psoriasis per se, although the possible interaction between alcohol and MTX is not excluded and may be important [13].

Similar MTX hepatotoxicity in leukaemic children cannot be assumed, as they are younger, the underlying disease is known to infiltrate the liver [19], the MTX dosage schedules differ, and other potentially hepatotoxic antileukaemic drugs are given concurrently.

Table 3 shows that the most commonly reported changes during ALL maintenance treatment are fibrosis, hepatitis, and elevation of serum enzymes, with cirrhosis reported by only one group [12]. Our survey suggests that this toxicity is not due to a difference in the immediate handling of MTX after an oral dose but possibly to its abnormal retention over a long period. This requires further investigation.

There is clear evidence that 6-mercaptopurine [2, 6, 10] and possibly cyclophosphamide [1] can produce liver cell necrosis or cholestasis when given alone, but they are not known to produce cirrhosis. They are probably cofactors in production of the liver damage in these children.

It is important to establish a histological diagnosis of cirrhosis in a child in remission of acute leukaemia who shows both clinical and biochemical evidence of liver damage, as this will lead to a change in drug treatment. By contrast, it is not clear that children who show biochemical but no *clinical* evidence of liver damage should have liver biopsies. Our study shows only mild structural changes in such childrens' liver biopsies, which are probably reversible. This is in accord with Lascari's comment [9] that "changes in liver function tests normally return to normal within weeks or months of stopping MTX or 6-mercaptopurine".

It is not possible to say whether further patients whom we did not biopsy have cirrhosis, and a reliable noninvasive test is needed to separate children who are going to develop irreversible liver damage from those who will not be severely affected.

Although this study has shown that the mean AP and SGOT rise during maintenance treatment, the wide scatter of readings at all times invalidates these as a test for irreversible liver damage.

A test of MTX retention such as has been described here could be of value in this respect, and could lead to a modification of drug dose or schedule in patients at particular risk. The results of the MTX retention test could be interpreted together with those of tests of liver damage, such as serum enzyme values, bromsulpthalein retention, and prothrombin time, to give an index of the risk of developing liver damage.

Should one stop all maintenance chemotherapy when biopsy indicates reversible damage, or should one substitute another less hepatotoxic drug for MTX? This problem could only be answered by a prospective controlled trial, and at present we consider the prognosis for children with ALL and particularly NHL does not permit treatment to be stopped early unless there is evidence of clear and worsening hepatotoxicity.

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