Folate Deficiency in Chronic Liver Disease

By Frederick A. Klipstein and John Lindenbaum

SINCE THE INITIAL case reports in 1949,1,2 the occurrence of megaloblastic anemia as a complication of alcoholic cirrhosis has been reported with increasing frequency in recent years.3-14 Jarrold and Vilter reviewed the marrows of 30 patients with cirrhosis and found 3 to be megaloblastic.1 Krashnow et al. found the incidence of megaloblastic anemia in cirrhotics to be 7 per cent in a group of 96 patients who underwent bone marrow examination.5

Folic acid deficiency appears to be the underlying cause of the megaloblastic anemia associated with cirrhosis. Where serum assays have been performed, serum folate levels have been subnormal,6-13 and serum vitamin B12 levels have been normal or elevated.15-17 Hematologic responses to physiologic doses of 50 μg. of folic acid have been demonstrated in several of these patients;4,7,9 such responses are considered to be specific for folic acid deficiency.8,9,18 In other patients a seemingly “spontaneous” hematologic improvement, without added hematinic therapy, has been attributed to quantities of free folic acid present in the hospital diet approximating 50 to 100 μg. a day.4,8,9

All of the reported cases of megaloblastic anemia in cirrhosis have been in chronic alcoholics, usually with a history of a poor dietary intake and evidence of malnutrition and multiple vitamin deficiencies. No cases of megaloblastic anemia in nonalcoholic individuals with postnecrotic cirrhosis have been reported to date. There have been several reports of megaloblastic anemia associated with hemochromatosis, but all of these patients have been chronic alcoholics.19,20 Thus, Jarrold1 and Herbert8 have speculated that dietary deficiency of folic acid is the principal factor leading to megaloblastic anemia in cirrhotics. On the other hand, Cherrick and his associates have proposed that decreased hepatic avidity for folic acid, and a possible deficiency in enzymes necessary in the metabolism of folic acid may play a significant role.21

In a recent study reported from Boston, Herbert, Zalusky, and Davidson found subnormal serum folate levels in 80 per cent of a group of 70 poorly nourished alcoholic cirrhotics.7,12 Morphologic abnormalities characteristic of folate deficiency were observed in the peripheral blood smears and in bone marrow aspirates when obtained from 31 patients who had serum folate levels below 3.0 μg./ml. as well as from the majority of the 25 patients who had folate concentrations of 3.0 to 4.9 μg./ml. The nature of the patient material studied by this group precluded any conclusions regarding correlations be-
between the incidence of folate deficiency with either adequacy of dietary intake or severity of impairment of hepatic function.

In the present study, similar observations have been conducted on 55 patients with liver disease of varied etiology and severity. A consideration of such factors as alcoholism, adequacy of diet, severity of impairment of liver function, adequacy of hepatic blood flow, absorption of folic acid, and status of bone marrow activity in affecting these determinations is presented.

Methods and Materials

Forty subjects (Cases 1–40) were consecutive patients seen with liver disease. Fifteen patients (Cases 41–55), who presented with alcoholic cirrhosis and a megaloblastic anemia, have been studied during the past 3 years. Serum folate concentrations were assayed on all patients and folic acid clearances determined in Cases 1 through 40. The subjects may be divided into three groups: (1) Thirteen patients with nonalcoholic liver disease, including 10 patients with chronic liver disease, primarily postnecrotic cirrhosis, and 3 patients with acute infectious hepatitis. (2) Seven patients with alcoholic cirrhosis who had been abstaining from alcohol for periods of time greater than a year. (3) Thirty-five patients with alcoholic cirrhosis who continued to have an excessive consumption of alcoholic beverages up until the time of hospital admission.

Twenty-eight patients had the clinical diagnosis of liver disease supported by biopsy or autopsy material; in the remaining patients the diagnosis was based on characteristic abnormalities of the liver chemistries, physical findings, and, in some instances, evidence of portal hypertension or recurrent episodes of hepatic coma. In a few actively drinking alcoholics in whom a liver biopsy was not obtained, the differentiation between acute fatty liver and cirrhosis could not be made with certainty. However, all of these patients had hepatomegaly and abnormal liver chemistries. The severity of impairment of liver function was classified on the basis of liver chemistries as follows: Mild—patients with serum bilirubin concentrations of less than 1.5 mg. per cent, cephalin flocculations of 0 to 1+, and BSP retention of less than 20 per cent at 45 minutes; all of these patients had normal serum albumin concentrations; Moderate—patients with bilirubin concentrations of 1.5 to 3.0 mg. per cent, cephalin flocculations of 2+, and BSP retention of less than 40 per cent at 45 minutes; the majority of these patients had normal serum albumin concentrations; Severe—patients with bilirubin concentrations of greater than 3 mg. per cent, cephalin flocculations of 3+ or 4+, and BSP retention greater than 40 per cent at 45 minutes; all of these patients had subnormal serum albumin concentrations.

A history of dietary intake prior to hospitalization was obtained from each patient. Although such histories may be of dubious validity at times when obtained from alcoholic cirrhotics, additional information obtained from the patient’s family or objective evidence of severe malnutrition or concomitant vitamin deficiencies, such as scurvy or Wernicke’s encephalopathy, was usually also available to assist in categorizing the adequacy of dietary intake. Diets have been categorized as good, when they were equivalent to a normal dietary intake; fair, when they were substandard as to caloric or protein intake but still contained sufficient quantities of meat or vegetables to assure an adequate folate intake, and poor, when they were considered to be grossly inadequate in caloric and folate intake. Only actively imbuing alcoholic cirrhotics were in the poor category. The majority of these patients were admitted from the Bowery and had subsisted on large quantities of alcohol and small quantities of carbohydrate for at least several months prior to hospital admission. The folic acid studies were conducted within 48 hours following hospital admission in order to minimize the effect of hospital diet on nutritional status.

Bone marrow aspirations were performed in 37 subjects. As has been noted by other investigators, macrocytosis in patients with liver disease does not correlate with folate deficiency, and this morphologic index of folate deficiency did not prove useful in this study. Evidence for hypersplenism was considered to be the presence of leukopenia.
and/or thrombocytopenia associated with splenomegaly in patients in whom megaloblastic anemia, drug toxicity, or other recognized causes of leukopenia or thrombocytopenia had been ruled out.

Serum folate levels were assayed with *L. casei* using the method introduced by Baker, Herbert, and their colleagues.24 This assay has been shown to reflect primarily the concentration of 5-methyltetrahydrofolic acid, the folate coenzyme naturally present in the serum in measurable quantities.25 Folic acid clearance studies were performed by the technic described by Chanarin, Mollin, and Anderson,26 using an intravenous dose of 15 μg. folic acid per Kg. body weight; the serum folic acid activity of the 3- and 15-minute samples was assayed with *S. faecalis*, which measures folic acid and those reduced forms of folic acid which are not ordinarily found in significant quantities in the serum. The absorption of folic acid was studied by determination of the serum folic acid activity with *S. faecalis* assay in hourly serum samples following an oral dose of 40 mμg./Kg. body weight given 38 hours after completion of saturation with parenteral folic acid. Normal subjects have peak serum levels of greater than 40 mμg./ml. at 1 to 3 hours following an oral test dose.27,28 Serum vitamin B₁₂ levels were assayed with *Lactobacillus leichmannii*.29

**RESULTS**

Results are presented in table 1 and figure 1.

**Nonalcoholic Liver Disease**

Six of 10 patients with chronic liver disease were anemic (i.e., had hemoglobin concentrations of less than 12 Gm. per cent); 5 of these 6 patients had either gastrointestinal bleeding or an overt hemolytic anemia. The folic acid clearance was abnormally rapid and the serum folate concentration subnormal in 2 anemic patients and 1 nonanemic patient; the clearance was abnormally rapid in 2 additional anemic patients. Bone marrow aspirations were obtained in 3 patients and showed normoblastic erythropoiesis in every instance, including 2 of the patients who had abnormal folic acid determinations. Folic acid clearances and serum folate levels were normal in all 3 patients with biopsy-documented acute infectious hepatitis (Cases 11-13).

**Alcoholic Liver Disease**

(1) Three of the 7 patients who were abstaining from consumption of alcoholic beverages were anemic; 1 of these 3 patients had gastrointestinal bleeding. Bone marrow aspirations were normoblastic in all 3. Serum folate levels were normal in every patient in this group; 1 nonanemic patient had an abnormally rapid folic acid clearance.

(2) Thirteen of 20 consecutively studied, actively imbibing alcoholic cirrhotics were anemic; 8 of these patients had gastrointestinal bleeding. Nine patients, all of whom were anemic, had abnormally rapid folic acid clearances and 8 had subnormal serum folate concentrations (fig. 1). Bone marrow aspirations were obtained in 7 patients with abnormal folic acid determinations; erythropoiesis was normoblastic in 3, and megaloblastic changes were seen in 4; in 2, erythropoiesis was overtly megaloblastic, and in 2, megaloblastic changes were limited to the presence of giant metamyelocytes in the bone marrow and hypersegmentation of the neutrophils in the peripheral blood smear. Bone marrow aspirates were also obtained from 4 nonanemic patients.
who had normal folic acid studies; all 4 showed normoblastic erythropoiesis.

(3) Serum folate concentrations were subnormal in all 15 actively drinking alcoholic cirrhotics who have presented during the past 3 years with a megaloblastic anemia.

Serum Vitamin B₁₂ Concentrations

Thirty-five patients had serum vitamin B₁₂ levels that were within the normal range, and 19 had increased concentrations of 1000 μg./ml. or greater. The degree of impairment of liver function in the 19 patients with high serum B₁₂ levels was mild in 2, moderate in 4, and severe in 13.

Hematologic Results of Therapy

Dietary or vitamin therapy resulted in a hematologic response in all 19 patients who had a megaloblastic anemia. Five of these patients, all poorly nourished, actively drinking alcoholic cirrhotics (Cases 44, 45, 49, 53, 55),
Table 1.—Results of Vitamin Assays

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Hb</th>
<th>Bone Marrow†</th>
<th>Serum Vit. B12</th>
<th>Serum Folate</th>
<th>Folic Acid Clearance S. faecalis</th>
<th>Serum Levels 3 min.</th>
<th>15 min.</th>
<th>Dietary Intake</th>
<th>Impairment of Liver Function</th>
<th>Complications‡</th>
</tr>
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</table>
|          | Gm. % | µg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg./ml. | mgg/
had a reticulocytosis accompanied by a rise in hemoglobin concentration following introduction of a regular hospital diet without any vitamin therapy. Observations conducted in 1 of these patients (Case 53) indicate that such a hematologic response is associated with a gradual rise in serum folate concentration to normal. Treatment of the 14 other patients with megaloblastic anemia, with vitamin B₁₂ in 2 and folic acid in the remainder, resulted in a reticulocytosis in every instance; this was accompanied by a rise in hemoglobin concentration in all except 3 cases, all of whom had gastrointestinal bleeding of a significant degree. Eleven patients had rapid folic acid clearances, with subnormal serum folate levels in seven, without morphologic abnormalities. Treatment with folic acid did not result in a hematologic response in any of these patients.

Factors Related to Abnormal Folic Acid Determinations

(1) Folic acid absorption studies were normal in 8 patients, 4 of whom had normal serum folate levels (Cases 17, 23, 26, 31), 1, a subnormal serum folate level but normoblastic erythropoiesis (Case 25), and 3, subnormal serum folate concentrations with megaloblastic bone marrows (Cases 27, 39, 55). Two patients, both of whom had a megaloblastic anemia, had subnormal absorption of folic acid. Case 54 had a peak serum _S. faecalis_ folate level of 33 μg/mL; no other absorption studies were performed. Case 40 had a peak level of 16 μg/mL; she also had other evidence of jejunal malabsorption with subnormal xylose and fat absorption.

(2) The presence of ascites did not appear to influence the results of the folic acid clearance studies. Fourteen of the patients studied had ascites; abnormally rapid clearances were observed in only 7. Further, simultaneous _S. faecalis_ folate concentrations were determined of the serum and ascitic fluid following an intravenous test dose of 15 μg. folic acid/Kg. body weight in 3 patients. In no case was there a significant increase over baseline ascitic fluid levels after the intravenous injection of folic acid, with observations extending to 1 hour, even though simultaneous serum levels contained as much as 25 times the activity measured in the ascitic transudate. Thus under conditions of the clearance study, significant amounts of folic acid do not appear in the ascitic fluid. However, if doses much larger than those used in the performance of clearance studies are given, detectable increases in the ascitic fluid levels are obtained, as reported by Condit and Grob.³⁰

(3) Portacaval shunt. The folic acid clearance was studied in 6 patients (Cases 1, 2, 14, 17-19) who were felt to have patent portacaval shunts in order to determine whether the clearance of the vitamin from the plasma might be delayed as a result of impairment of blood flow through the liver. This did not appear to be the case since clearance values were normal in 3 patients and abnormally rapid in 3.

(4) Plasma volume. The interpretation of clearance tests in cirrhosis may be complicated by the presence of an expanded blood volume in many of these patients. Blood volume determinations were not carried out in the present study; however, a comparison of folic acid clearance values with 5-minute
BSP clearance values suggest that this factor did not play a significant role in those patients with abnormally rapid folic acid clearances. Five-minute BSP values of less than 80 per cent, suggesting increased plasma volume, were observed in 4 of 16 determinations; in these 4 subjects, the folic acid clearance was abnormally rapid in only 1, a patient who also had a subnormal serum folate level with a megaloblastic anemia.

(5) Adequacy of dietary intake. Thirty-one of 35 actively drinking alcoholic cirrhotics were considered to have a poor dietary intake. Of these 31 patients, 19 had subnormal serum folate levels with a megaloblastic anemia, 2 had subnormal serum folate levels with normoblastic bone marrows, and the remaining 10 had normal folic acid determinations. Abnormal folic acid determinations were also found in 5 patients with nonalcoholic liver disease, 1 patient with alcoholic liver disease who was no longer imbibing, and 3 actively imbibing alcoholic cirrhotics who were considered to be on an adequate diet. None of these patients had megaloblastic changes in the bone marrow.

(6) Recent alcoholism and type of alcoholic beverages. It has recently been reported that alcohol may have a suppressant effect on erythropoiesis, which, in one case at least, produced a megaloblastic arrest even in the presence of a normal serum folate concentration. It is difficult to evaluate this factor in the present series. All 19 patients in this study who had megaloblastic changes were actively drinking alcoholics. In addition, 3 of the 7 patients who were admitted in a state of alcoholic intoxication had a megaloblastic anemia. Folic acid clearances were abnormally rapid in 3 of 5 acutely intoxicated patients studied.

Herbert has reported that beer contains considerable amounts of folic acid, wine contains little, and whisky, none. In the present study, no correlation was found between tests of folic acid deficiency and type of beverage consumed, although the small number of patients in each beverage category precludes statistical significance. Nonetheless, it is of interest that rapid folic acid clearances were observed in 2 of 3 beer drinkers (Cases 25, 31, 38) and a fourth beer drinker (Case 44) had a megaloblastic bone marrow and subnormal serum folate. Further, the inclusion of beer among the preferred beverages imbibed by some of the patients who drank a variety of beverages did not appear to prevent the development of abnormal folic acid determinations.

(7) Degree of impairment of liver function. Eight of 19 patients with megaloblastic changes and subnormal serum folate levels had mild or moderate impairment, and eleven had severe impairment of liver function. Abnormally rapid folic acid clearances were observed in 6 of 21 patients with mild or moderate impairment and 9 of 19 patients with severe impairment.

(8) Bone marrow activity. In the 40 patients in whom folic acid clearance studies were performed, evidence of gastrointestinal bleeding was observed in 13 patients, hypersplenism in 15 patients (8 of whom had gastrointestinal bleeding in addition), and overt hemolytic anemia in 1 patient. A hyperactive bone marrow is commonly associated with these conditions and was observed in those patients who had marrow aspirations. One or both of these
complications were present in 14 of the 15 patients who had abnormally rapid folic acid clearances. Further, whereas fast clearances were observed in only 1 of 19 patients who had none of these complications, they occurred in 9 of 13 patients with gastrointestinal bleeding, 3 of 7 patients with hypersplenism alone, as well as in the patients with overt hemolytic and megaloblastic anemias.

**DISCUSSION**

The present study confirms the recent report from Boston\textsuperscript{12} that megaloblastic anemia due to folate deficiency is a frequent complication of actively drinking, poorly nourished alcoholic cirrhotics. In addition, abnormal folic acid determinations were observed in some patients with alcoholic as well as nonalcoholic liver disease in the absence of morphologic abnormalities of the peripheral blood or bone marrow. Some comments regarding the significance of these determinations seem appropriate.

Patients with folate deficiency states have been shown to excrete abnormal amounts of formimino glutamic acid (FIGLu) and urocanic acid in the urine after metabolical loading with histidine.\textsuperscript{30-37} These metabolites of histidine have been found repeatedly in the urine of the majority of patients with cirrhosis.\textsuperscript{13,14,38,39} However, large quantities of these substances frequently continue to be found in the urine of cirrhotics after therapy with large doses of folic acid.\textsuperscript{13,26} Their presence in the urine, therefore, has not been considered to be a reliable indication of folate deficiency in these patients and has been attributed to a possible deficiency in the enzyme FIGLu reductase.\textsuperscript{39} In addition, the sensitivity of the test in detecting mild states of folate deficiency has been questioned.\textsuperscript{37,4} Therefore these determinations have not been included in this study.

Abnormally rapid plasma clearances of folic acid, as measured with \textit{S. faecalis}, can occur in the presence of folate deficiency, in the presence of increased folate utilization, or when both folate deficiency and increased utilization are present. The clearance is rapid in patients with megaloblastic anemia secondary to either folate or vitamin B\textsubscript{12} deficiency.\textsuperscript{26} The rapid clearances sometimes observed in patients with inadequate nutritional intake,\textsuperscript{41} malabsorption,\textsuperscript{28,42} and associated with anticonvulsant drug therapy\textsuperscript{45} in the absence of megaloblastic changes of the bone marrow, have been interpreted to represent evidence of "subclinical" folate deficiency. When folate requirement is increased above normal, as in pregnancy,\textsuperscript{44,45} hyperthyroidism,\textsuperscript{46} chronic myelofibrosis,\textsuperscript{42} and conditions associated with hyperactivity of the bone marrow, such as hemolytic anemia\textsuperscript{47,48} and leukemia,\textsuperscript{49,50} the rapid clearance probably represents increased utilization when the bone marrow is normoblastic and a combination of increased utilization and deficiency when the bone marrow is megaloblastic. Occasionally, rapid clearances may be observed in polycythemia\textsuperscript{50} and uncomplicated iron deficiency anemia;\textsuperscript{26,50,51} in these circumstances, the fast clearance probably reflects solely increased utilization. The definitive interpretation of the folic acid clearance test awaits the correlation of clearance values with direct assay of tissue folate stores.
Serum folate levels are subnormal in those megaloblastic anemias associated with folate deficiency.\textsuperscript{6,52,53} Simultaneous assay of liver biopsy material in a few such overtly deficient patients has shown reduced tissue folate stores.\textsuperscript{50} Subnormal folate levels have also been observed in patients on anticonvulsant drug therapy,\textsuperscript{43} with malabsorption,\textsuperscript{28,54} pregnancy,\textsuperscript{55} hemolytic anemia,\textsuperscript{48} and in poorly nourished infants\textsuperscript{56} who did not have overt morphologic abnormalities. The most likely interpretation of these observations is that the subnormal serum folate values reflect an initial or "subclinical" decrease in folate stores which antedates deficiency of a degree sufficient to produce overt morphologic changes. Such a sequence has been described in experimental nutritional folate deficiency in human volunteers where subnormal serum folate levels preceded the appearance of peripheral blood or bone marrow changes.\textsuperscript{40,57} Whether serum folate levels can be depressed secondary to increased utilization in the absence of tissue depletion is unknown. The results of the serum folate determination in the present study correlated with the folic acid clearance test in 36 or 40 patients; in 4 patients, clearances were rapid in the presence of normal serum folate concentrations.

In the present study, all 19 patients who had a megaloblastic anemia had subnormal serum folate levels and all 4 of these patients studied had rapid folic acid clearances. These 19 patients were clearly folate deficient. In addition, 11 patients had rapid folic acid clearances, and 7 had subnormal serum folate levels with normal peripheral blood smears. Normoblastic erythropoiesis was observed in all 5 of these patients in whom bone marrow aspirations were obtained. Ten of these 11 patients had complications which tend to induce hyperactivity of the bone marrow. It is likely that the abnormal folic acid determinations in these patients reflect increased utilization of folate; whether they also indicate "subclinical" folate deficiency is unknown.

The results of this study indicate that such factors as the presence of ascites, a patent portacaval shunting procedure, malabsorption of folic acid (with 2 exceptions), and the type of alcoholic beverage imbibed do not play a significant role in the development of folate deficiency in chronic liver disease. The degree of impairment of liver function per se also appears to be of little if any significance. It has been postulated\textsuperscript{21} that impaired storage capacity or decreased hepatic avidity for folic acid might result in depletion of this vitamin in chronic liver disease. However, megaloblastic changes and subnormal serum folate levels were found to occur almost as frequently in patients who had mild or moderate impairment of hepatic function as in those with severe derangement (fig. 1). Nor did the severity of impairment of liver function appear to influence results of the folic acid clearance test. In normal subjects, the intravenous dose of folic acid used in this test disappears rapidly from the plasma and is retained in the body.\textsuperscript{26,58} Since the liver is considered to be the major site of body stores of folate derivatives,\textsuperscript{57} it has been postulated that hepatic uptake of the administered vitamin accounts for the major part of its clearance from the plasma,\textsuperscript{59} although the extent to which other organs, such as the bone marrow, may contribute to the initial rapid removal of the vitamin has not been investigated. It is apparent from the present study that marked impairment of liver function does not result in a delayed plasma...
clearance but is associated with normal, or even more rapid than normal, rates of folic acid clearance. Indeed, in 3 patients in terminal hepatic coma associated with marked abnormalities of hepatic function, the clearance rate was well within normal limits in 2 and abnormally rapid in the third.

Inadequate dietary intake of folate appears to be of major significance in the development of folate deficiency in liver disease. Restriction of dietary folate has been shown to result in subnormal serum folate levels and rapid folic acid clearances, with or without megaloblastic changes, in experimental subjects46,57 and in malnourished populations.41,56,60 Previous reports of megaloblastic anemia in cirrhosis have all been in poorly nourished alcoholics,1-14 and all 19 patients in the present study who had a megaloblastic anemia were actively imbibing alcoholic cirrhotics who were considered to have an inadequate dietary intake of folate. Further, megaloblastic anemia was not seen in any of the patients who were on an adequate diet. It is probable that nutritional deficiency was the sole cause of folate deficiency in the 9 patients on a poor diet who had no complications and was the major cause of folate deficiency in the 10 patients on a poor diet who had complications. However, in the 9 other patients who had abnormal folic acid determinations despite what was considered to be an adequate dietary intake of folate, factors other than dietary deficiency must be invoked.

Increased activity of the bone marrow is known to result in an increased demand for folate which, in some situations, may occasionally exceed supply, resulting in a secondary deficiency and megaloblastic anemia. This appears to have been the major cause of folate deficiency in the more than 50 instances of megaloblastic anemia complicating chronic hemolytic states which have been reported.48 In these patients, folic acid clearances have been abnormally rapid, serum folate levels subnormal, and a therapeutic requirement for at least four to eight times the usual physiologic dose of 50 µg. of folic acid has been demonstrated.48 In the present study, a correlation was noted between abnormal folic acid determinations and states which tend to induce hyperactivity of the marrow in cirrhosis—gastrointestinal bleeding, hypersplenism, and overt hemolysis. At least one of these factors tending to stimulate marrow activity was present in 20 of the 30 patients in this study who had abnormal folic acid determinations including 8 of the 9 patients with abnormal folic acid determinations whose dietary intake was considered to be adequate.

It seems likely that inadequate nutritional intake of folate is the major cause of, and a prerequisite for, the development of folate deficiency of sufficient degree to produce megaloblastic anemia in chronic liver disease. Dietary deficiency may well be the sole factor operative in the development of folate deficiency in alcoholics with uncomplicated mild liver disease. On the other hand, the additional factor of increased folate requirement secondary to hyperactivity of the bone marrow may contribute to the development of folate deficiency in both alcoholic and nonalcoholic cirrhosis. The abnormal folic acid determinations observed in adequately nourished patients with nonalcoholic and alcoholic liver disease, who did not have megaloblastic changes, probably reflect this factor; however, in the absence of the additional factor of
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nutritional deficiency, this increased requirement did not result in folate deficiency of sufficient severity to produce megaloblastic arrest in these patients. The severity of impairment of liver function is probably of significance in the development of folate deficiency only in that the complications of severe liver disease, such as bleeding varices and hypersplenism, result in hyperactivity of the bone marrow. It remains uncertain as to the degree which the compensated hemolytic state, usually present in nonanemic patients with cirrhosis, may contribute to increased marrow demands for folate.

Summary

Fifty-five patients with liver disease of varied etiology and severity have been studied. Serum folate concentrations were subnormal and folic acid clearances rapid, when studied, in 19 actively imbibing alcoholic cirrhotics who had a megaloblastic anemia. Eleven patients, from both the nonalcoholic and alcoholic groups, had rapid folic acid clearances, with subnormal serum folate levels in seven, in the absence of morphologic evidence of folate deficiency. Serum B₁₂ concentrations were uniformly normal or elevated.

Dietary deficiency appeared to be the major cause of folate deficiency; all 19 patients who had megaloblastic changes were considered to have an inadequate dietary intake. Increased requirement for folate due to hyperactivity of the bone marrow secondary to gastrointestinal bleeding, hypersplenism, or hemolysis appeared to contribute to the development of abnormal folic acid determinations in many patients in both the alcoholic and nonalcoholic groups. Two of 10 patients studied had malabsorption of folic acid. Such factors as the presence of ascites, an expanded plasma volume, a patent portacaval shunting procedure, the type of alcoholic beverage imbibed, and the severity of impairment of liver function did not appear to be of significance in the development of folate deficiency.

Summary in Interlingua

Esseva studiate 55 patientes con morbo hepatic de varie etiologias e varie grados de severitate. Le concentrationes seral de folato esseva subnormal e le clearance de acido folic esseva rapide in 19 activemente alcoholic cirrhoticos qui habeva un anemia megaloblastic. Dece-un patientes—ab le grupo non-alcoholic e ab le grupo alcoholic—habeva un accelerate clearance de acido folic (con subnormal nivellos seral de folato in septe), in le absentia de evidentia morphologic de carentia de folato. Le concentrationes seral de vitamina B₁₂ esseva uniformemente normal o elevate.

Carentia dietari pareva esser le causa major de carentia de folato. Esseva opinate que omne le 19 patientes con alterationes megaloblastic habeva haine un inadequate ingestion dietari. Un augmentate requerimento de folato in consequentia de hyperactivitate del medulla ossee secundari a sanguination gastrointestinal, hypersplenismo, o hemolyse pareva contribuer al disveloppamento de anormal valores pro acido folic in multe patientes in le grupo alcoholic e etiam in le grupo non-alcoholic. Duo de 10 patientes studiate habeva malabsorption de acido folic. Il pareva que le disveloppamento de
carentia de folato non dependeva significativamente de factores como le presentia de ascites, un expandite volumine de plasma, un patente shunting in le porta cave, le typo de alcohol usate, e le severitate del dysfunction hepatic.

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Folate Deficiency in Chronic Liver Disease

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