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Suppression of Hematopoiesis by Ethanol*

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Anemia is a frequent finding in alcoholic patients for many reasons. One of the major causes is folate deficiency with associated macrocytosis and megaloblastic erythropoiesis (2). Whether the nutritional folate deficiency (presumed to be mainly from inadequate ingestion of folate-containing foods) in these patients is the sole cause of the macrocytosis and megaloblastic anemia, or whether other factors, among them alcohol, contribute to the anemia, has been a subject for much speculation. The concept that macrocytic anemia of liver disease may be the result of interference with blood formation has been entertained for at least 3 decades (3, 4). The frequent association of leukopenia and thrombopenia and the finding of "spontaneous" reticulocytosis in this anemia (upon admission to the hospital or soon thereafter) has been long noted (3–6), suggesting, among other possibilities, that cessation of alcohol ingestion removed a hematopoietic suppressant (6). Recent observations indicate that the minimal daily oral requirement for folic acid is in the range of 50 μg (7, 8), an amount exceeded many times by routine hospital diets and diets previously employed in studies of folate-deficient patients. Thus, the question of whether the hematologic improvement in alcoholics with megaloblastic anemia given no specific therapy is due to ingestion of folate (from the hospital diet or just before hospitalization), to removal of a possible hematosuppressant (alcohol), or to other factors has remained unresolved. An unexplored possibility to explain the occurrence of spontaneous reticulocytosis after cessation of alcohol ingestion is that chronic alcohol ingestion may result in malabsorption of dietary folate (as of other nutrients) (9) and that cessation of alcohol ingestion is followed by resumption of normal folate absorption.

The present study was designed to determine whether or not alcohol, in amounts commonly consumed by "heavy drinkers," could suppress the hematopoietic response of anemic folate-deficient patients to folic acid therapy. The results of these investigations form the basis of this report.

Methods

The patients were admitted to the medical wards of the Boston City Hospital and transferred to the Thorndike Metabolic Ward for study. All had normal serum electrolytes and blood urea nitrogen determinations. They were maintained on a diet containing approximately 5 μg of total folate per day, as determined by *Lactobacillus casei* assay (10), and given multivitamins [1] (exclusive of folic acid and vitamin B_{12}) and potassium chloride [2] supplementation.

Complete blood counts were performed by standard methods (11), and reticulocyte counts were performed daily by the dry method using cresyl blue. Bone marrow

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A preliminary report of a part of this study has been published in abstract form (1).

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[1] Berocca C, Roche Laboratories, Nutley, N. J. (Thiamine-HCl, 10 mg; riboflavin, 10 mg; niacinamide, 80 mg; pyridoxine-HCl, 20 mg; D-panthenol, 20 mg; *d*-biotin, 0.2 mg; ascorbic acid, 100 mg.) One ampule daily, by mouth, from day 17 to day 61, in Case 1.

Dayamin capsules, kindly supplied by Abbott Laboratories, N. Chicago, Ill. (Vitamin D, 10 μg; thiamine-HCl, 5 mg; calcium pantothenate, 5 mg; ascorbic acid, 100 mg.) One capsule daily, by mouth, beginning on day 61, in Case 1, and during entire course of Cases 2 and 3.

[2] Potassium chloride tablets, U.S.P. enteric-coated (Enseals), Eli Lilly, Indianapolis, Ind., 3 g, by mouth, daily.

examinations were performed on sternal and iliac crest aspirates.

Serum vitamin B_{12} and folate levels were assayed microbiologically with *Euglena gracilis* (12) and *L. casei* (13), respectively. Serum iron content and iron-binding capacity were determined by the method of Zak, Landers, and Williams (14). Formiminoglutamic acid excretion was measured in a 12-hour urine collection, after an oral dose of 20 g of L-histidine, by the urinary electrophoresis method of Zalusky and Herbert (15, 16). Gastric juice was obtained with augmented histamine stimulation (17) and was assayed for intrinsic factor by the *in vitro* guinea pig intestinal mucosa homogenate (GPIMH) technique of Sullivan, Herbert, and Castle (18). Urinary excretion of radioactivity was measured by a modification (19) of the Schilling (20) test, using a 2-μg oral dose of Co^{60}-labeled vitamin B_{12}.

Case Reports

Case 1. M.T., a 61-year-old waitress, was admitted to the Boston City Hospital in May 1962 because of polyuria and ankle edema. For 22 years she had lived alone and consumed unknown quantities of wine and whiskey daily. Her diet had been almost totally devoid of meat and fresh fruits or vegetables.

The patient was a 50-kg, 160-cm, pale, thin female with dyspnea after walking 20 yards, who appeared to be chronically ill. There was moderate papillary atrophy of the tongue. A tender liver edge was palpable 3 cm below the right costal margin, and a firm spleen tip was felt 2 cm below the left costal margin. Slight pedal and pretibial edema was present. The patient was disoriented to time and place and was inconsistent in historical details of her illness. She confabulated spontaneously.

The erythrocyte count was 1,200,000 per mm^3, the hemoglobin 5.6 g per 100 ml, and the hematocrit 19.1%. The erythrocyte mean corpuscular volume was 159 μ^3, and the mean corpuscular hemoglobin concentration was 29%. The reticulocyte count was 4.8% and the leukocyte count 8,300 per mm^3. A platelet count on the seventh hospital day was 117,000 per mm^3. A peripheral blood smear showed marked macroovalocytosis, poikilocytosis, anisocytosis, and marked hypersegmentation of the neutrophils. A sternal marrow aspirate revealed intense megaloblastic hyperplasia (Figure 1). Marrow hemosiderin was moderately increased. Several liver function stud-

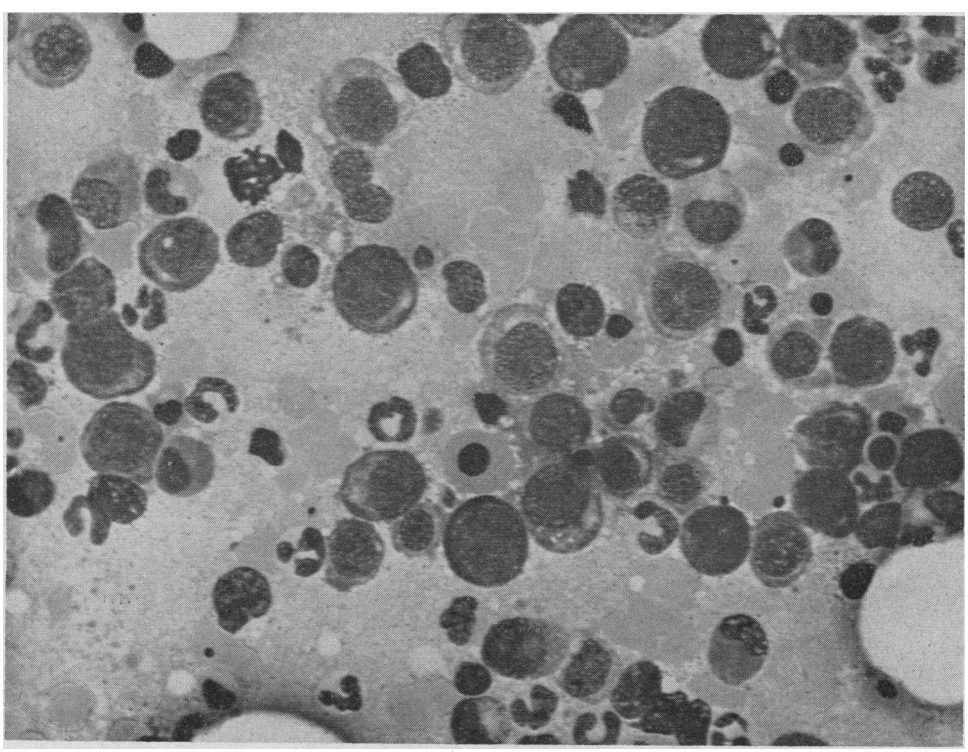

FIG. 1. BONE MARROW SMEAR ON ADMISSION (CASE 1). Erythropoiesis is intensely megaloblastic. The majority of the erythrocyte precursors are primitive megaloblasts. × 1,000.

TABLE I

Liver function studies before and after alcohol ingestion

Patient	Bromsulphalein retention after 45 min	Prothrombin time (patient/control)	Thymol tubidity	Cephalin flocculation	Serum				
					Albumin	Globulin	Bilirubin	Transaminase (SGOT)‡	Alkaline phosphatase
	%	*seconds*	*U*	*0 to 4+*	*g/100 ml*	*g/100 ml*	*mg/100 ml*	*U*	*U*
M. T.*	13.0	19.2/13.2	1.5	2+	3.0	3.1	4.0	26.0	1.7
M. T.†	6.0	16.2/14.0	1.4	3+	3.7	3.6	0.5	39..	2.0
E. F.*	11.4	18.4/13.7	1.6	2+					2.1
J. B.*	28.0	16.4/14.0			2.4	3.6	0.6	37.0	2.9
Normal	<5	12-14	0-2.3	0-1+	46-57% of total protein	43-54% of total protein	<1.2	8-40	0.8-2.8

* Before alcohol ingestion.
† After 22-day period of alcohol ingestion.
‡ Serum glutamate-oxaloacetate transaminase.

ies indicated mild liver dysfunction (Table I). An upper gastrointestinal series and a small bowel series were within normal limits. The serum folate was less than 1 nanogram per ml (normal, 7 to 16 nanograms per ml) (13), serum B_{12} was 331 picograms per ml (normal, 200 to 900 picograms per ml) (12), and the serum iron was 201 μg per 100 ml (normal, 60 to 180 μg per 100 ml) (14). Urinary excretion of formiminoglutamic acid was not measured because of incontinence. *In vitro* assay of gastric juice showed the presence of normal amounts of intrinsic factor (18).

Upon admission the patient was placed on a Dexin [3] and water diet of 2,000 calories daily for 5 days, then changed to a 2,200 calorie folate-free liquid formula (8) containing 300 g folate-free meal,[4] 30 g corn oil, 200 g sucrose, and 45 g gelatin powder for an additional 16 days. She was then transferred to the Thorndike Ward and, for the duration of the studies, placed on the diet containing 5 μg of total folate daily (10).

During the first 11 hospital days the reticulocyte count fell from the initial 4.8% to 1.4% (Figure 2). On day 12, therapy with 25 μg of folic (pteroylmonoglutamic) acid [5] (PGA) by mouth was instituted for a 14-day period without a significant increase in reticulocytes, but with continuation of the fall in erythrocyte count, hemoglobin, and hematocrit. (The hemoglobin and hematocrit determinations are not charted but paralleled the erythrocyte counts.) On the twenty-sixth hospital day a transfusion of 2 U of packed erythrocytes raised the hematocrit from 11.7% to 20.5% but did not raise the serum folate or B_{12} levels and was not followed by a rise in reticulocytes. The dose of folic acid was increased to 50 μg by mouth daily on day 26 and to 75 μg by mouth daily on day 43. An increase in reticulocyte count began on day 50 and reached a value of 6.8% on the fifty-third hospital day, when ingestion of 12 ounces of whiskey [6] and 32 ounces of wine [7] was instituted. There followed a fall in reticulocytes to 2.1% on day 59. Whiskey and wine were given until day 63, with continued reticulocytopenia. Five days after cessation of whiskey and wine the reticulocyte count rose to 3.4% and reached a peak of 21.5% 4 days later (seventy-second hospital day). The resumption of 15 ounces of whiskey daily on day 71 was followed by a fall in the reticulocyte count to 1.7% by day 77. This cycle of reticulocytopenia during ethanol ingestion and reticulocytosis after ethanol withdrawal was observed a total of four times with peak reticulocyte counts of 6.8, 21.7, 18.0, and 26.3%, respectively, and a fifth reticulocyte peak of 12% occurred after the final period of ethanol administration. For the fourth period, U.S.P.

[3] Purchased as Dexin (ingredients: 75% dextrins, 24% maltose, 0.25% mineral ash, and 0.75% water) from Burroughs Wellcome, Tuckahoe, N. Y.

[4] Folic-deficient diet, Nutritional Biochemicals Corp., Cleveland, Ohio.

[5] Tablets of folic acid, containing 25, 50, and 100 μg pteroylmonoglutamic acid each were specially prepared and supplied by Dr. Eugene H. Swanzey of Lederle Laboratories, American Cyanamid Co., Pearl River, N. Y.

[6] Imperial blended whiskey, 86 proof (alcohol 43% by volume), Hiram Walker & Sons, Peoria, Ill.

[7] Guild extra fine California muscatel, Guild Wine Co., Lodi, Calif. (alcohol 20% by volume).

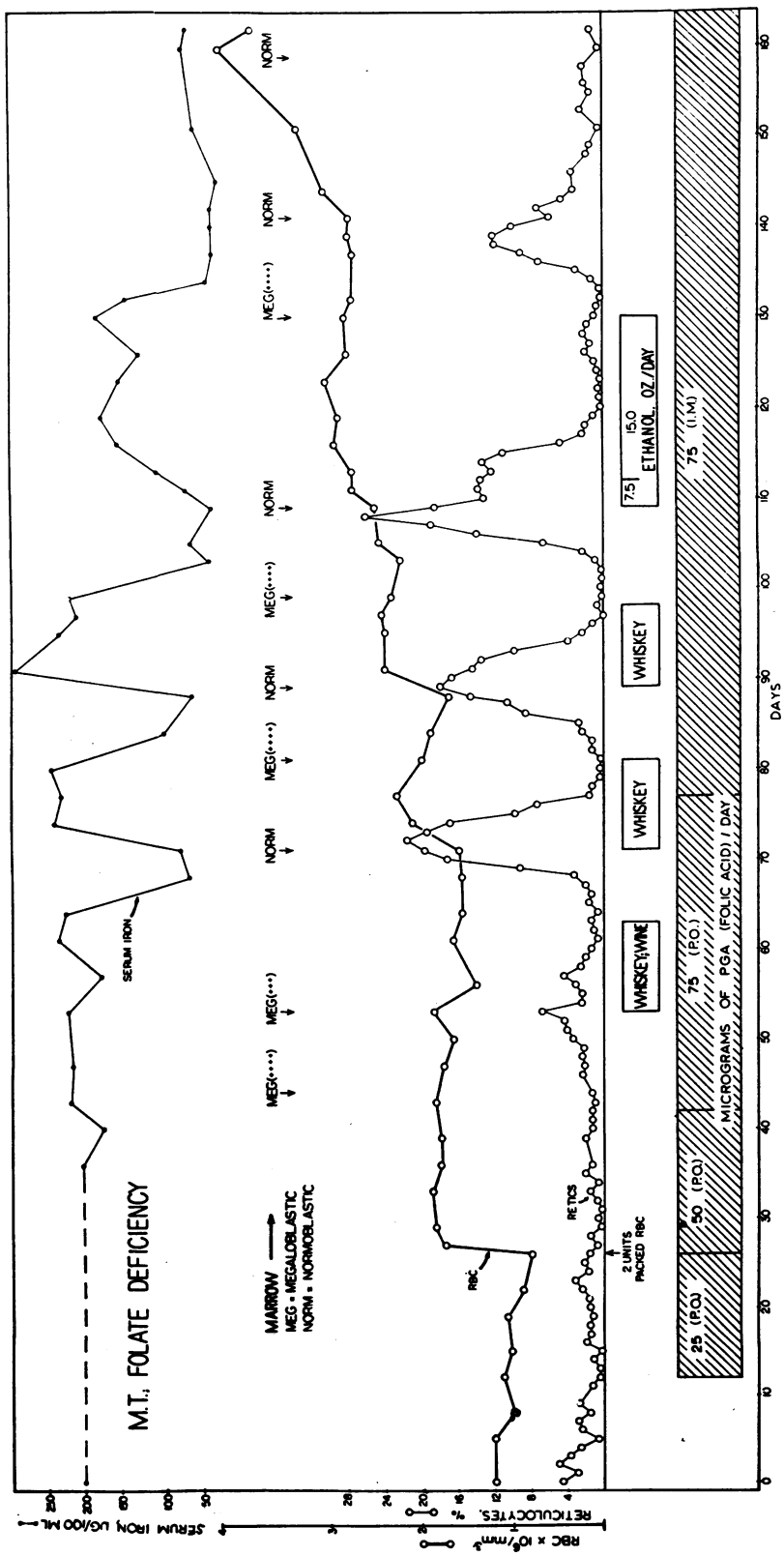

FIG. 2. RESPONSE TO 75 µG OF FOLIC ACID DAILY. Serial periods of suppression of reticulocytosis with wine, whiskey, and U.S.P. ethanol. Note changes in marrow morphology after each period of alcohol administration.

ethanol, 43% by volume, was administered. With 7.5 ounces there was only partial suppression of the reticulocyte count, but after the dose was increased to 15 ounces (equal to the amount of ethanol in the whiskey) the reticulocyte count fell to less than 1%. After the second period of hematosuppression folic acid was given intramuscularly instead of orally. During the entire period of therapy with 75 μg folic acid (PGA) daily there was gradual rise in the erythrocyte count, and hemoglobin and hematocrit returned to normal levels, although macrocytosis did not disappear until after day 214. Hypersegmentation of the neutrophils was present up to day 282. During the first two periods of alcohol administration, in addition to the fall in reticulocyte counts, there were also decreases in the erythrocyte count and hemoglobin and hematocrit levels. During the final two periods of alcohol administration the rate of improvement of these indexes was much slower than during the adjacent periods of abstinence.

Bone marrow smears, examined at the beginning and end of the periods of alcohol administration, in each instance showed marked conversion from normoblastic (Figure 3) to megaloblastic (Figure 4) morphology after 10 days of alcohol administration, with reversion toward normoblastic after cessation of alcohol. Bone marrow aspirates up to 4 days after cessation of alcohol remained predominantly megaloblastic, but by the end of 10 days erythropoiesis had become predominantly normoblastic.

At a later period, when the patient was no longer anemic, had a normoblastic marrow and slight macrocytosis (mean corpuscular volume, 100 μ^3), and a serum folate of 4.1 nanograms per ml, 15 ounces of whiskey was given daily while the patient continued to receive 75 μg PGA orally. The sternal marrow was megaloblastic after 5 days of alcohol ingestion. The erythrocyte count was 4.5 million per mm^3, hemoglobin 13.2 per 100 ml, and hematocrit 45%. In spite of sequential changes in the dose of folic acid every 6 to 10 days to 150 μg then 300 μg orally, and finally 150 μg intramuscularly, respectively, the bone marrow remained megaloblastic during this 35-day period. The serum folate levels rose with each change in folic acid therapy to average levels of 4.3, 5.7, 6.4, and 9.3 nanograms per ml, respectively. Whiskey and folic acid were then discontinued. The bone marrow 48 hours later showed partial conversion to normoblastic erythropoiesis, and 8 days later only rare intermediate megaloblasts were found. The serum folate, 6 days after cessation of whiskey and folic acid therapy, was 5.3 nanograms per ml.

Vacuoles (21) were occasionally found (in the nucleus or cytoplasm of the erythroid and myeloid precursors, or both, with equal frequency) after alcohol administration. Usually only 1 or 2 vacuoles were present per cell.

The initial serum iron values were high but characteristically fell to low levels at the beginning of each period of reticulocytosis (Figure 2). Within 48 to 72 hours after beginning alcohol ingestion the serum iron levels rose again to high values.

The "megaloblastic" changes in the large myeloid cells in the bone marrow showed slight improvement during periods of alcohol withdrawal. Because of slight hypochromia, as indicated by a fall in the mean corpuscular hemoglobin concentration (presumably due to the repeated venesections) to 29%, 300 mg ferrous sulfate orally four times daily was begun on day 89.

Studies for possible malabsorption were negative,[8] including xylose excretion, a 5-day stool fat analysis, small bowel X rays, and small bowel peroral biopsies (22) during the seventh and eighth hospital months, when the hematologic indexes were normal. There was no loss or blunting of villi or changes in jejunal epithelium before, during, or after alcohol administration (23). Large clusters of hemosiderin were localized in the submucosa of the villous tips, possibly related to the ingestion of ferrous sulfate. No iron was demonstrable in the columnar epithelial cells (24).

In the sixth month of study the Cr^{51}-labeled erythrocyte half-life (25), during a period of alcohol ingestion (when the serum folate was 4.5 nanograms per ml and the hematocrit was 47%), was 27 days (normal, 22 to 35 days); the $t_{\frac{1}{2}}$ clearance rate of Fe^{59} from the plasma (26) was 55

[8] The tests for malabsorption and the small bowel biopsies were kindly done by Drs. Sidney Winawer and Norman Zamcheck of the Gastrointestinal Research Laboratory, Boston City Hospital, Mallory Institute of Pathology.

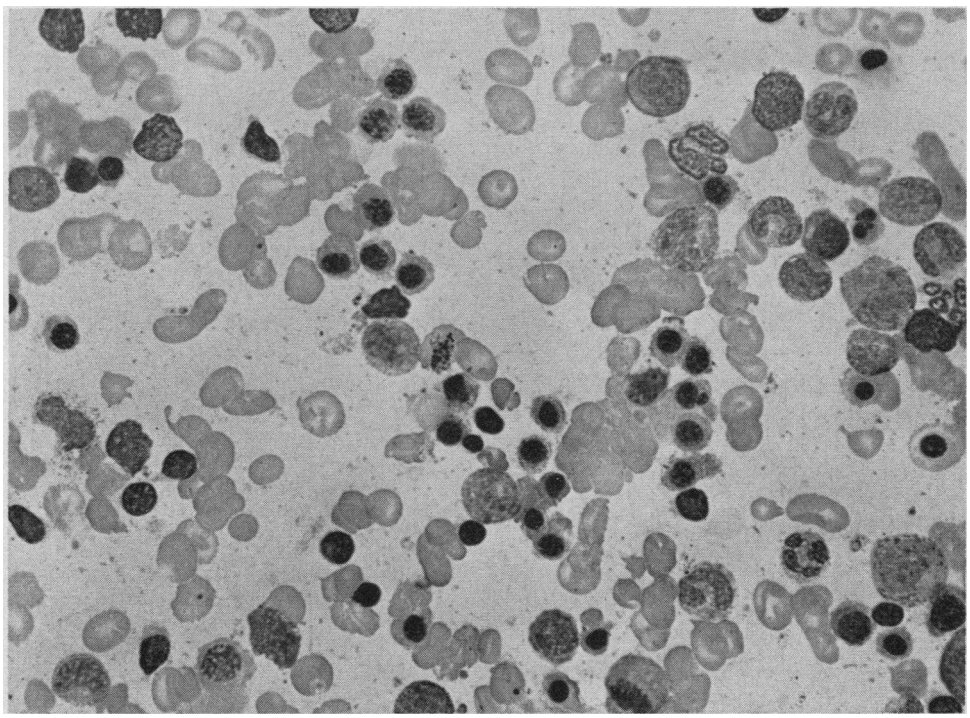

FIG. 3. BONE MARROW ON DAY 71. Erythropoiesis is essentially normoblastic, with acidophilic (late) normoblasts predominating. × 1,000.

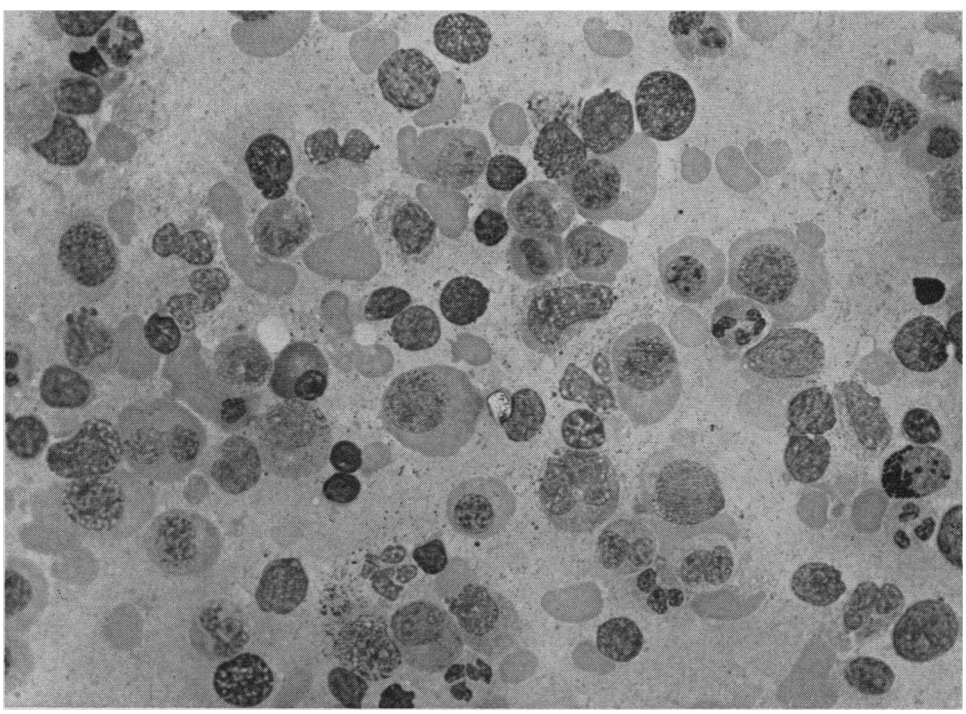

FIG. 4. BONE MARROW ON DAY 81. During ingestion of 15 ounces of 86 proof whiskey daily for 10 days, there has been marked reversion to megaloblastic erythropoiesis. The predominant erythrocyte precursor is now the orthochromatic megaloblast. × 1,000.

minutes (normal, 42 to 156 minutes); and the plasma iron turnover (27) was 36.2 mg (normal, 21 to 37 mg per day).

Percutaneous needle biopsy of the liver [9] in the seventh month showed minimal portal fibrosis and moderate accumulations of hemosiderin in the periportal areas of the liver lobules.[10] During the previous 4½-month period, the patient had received orally a total of 141 g of ferrous sulfate, which was discontinued at this point. In subsequent liver biopsies during the following 6 months the iron content appeared to remain approximately the same. No increase in stainable iron was found by microscopic examination of biopsies taken after 9, 22, and 35 consecutive days of whiskey ingestion.

Iron absorption studies, done when the hemogram was normal and 4 months after cessation of oral iron therapy, were normal during and after alcohol ingestion [11] (28).

There was a gradual rise in serum folate levels, while the patient received 75 μg folic acid daily, to the range of 3.0 to 5.6 nanograms per ml by the end of the third month of folic acid therapy. Serum B_{12} levels remained in the lower range of normal during her hospitalization.

The hepatomegaly and splenomegaly cleared by the second hospital month.

Case 2. E. F., a 38-year-old female, was admitted to the Boston City Hospital in June 1963 because of epistaxis and hematemesis. The patient had consumed an estimated ten whiskey highballs daily for 6 years. She rarely ate meat, fruits, or fresh vegetables.

She had been hospitalized for 2 months on the Thorndike Metabolic Ward 15 months previously because of megaloblastic anemia due to folate deficiency and had a reticulocyte response from 0.7% to 6.0% while being given 12.5 μg of folic acid intramuscularly daily on a folate-free diet (29). On the routine hospital diet she had had a second reticulocyte response (peak, 18.2%) with a rise in erythrocyte count and leukocyte count but had left the hospital against advice before complete hematologic remission. During the 13-month interval preceding her second admission she resumed ingestion of six to ten highballs daily and ate poorly.

She was a thin, 47-kg, 155-cm female with normal vital signs. On each ankle eight to ten petechiae were present, and a tourniquet test was positive. There was moderate papillary atrophy of the tongue. The liver and spleen were not enlarged.

A severe anemia was noted, with a hemoglobin of 3.4 g per 100 ml and hematocrit of 7%. Many oval macrocytes and hypersegmented polymorphonuclear leukocytes were present in the peripheral blood smear. The bone marrow was megaloblastic and contained heavy deposits of hemosiderin. Occasional vacuoles were present in the cytoplasm of megaloblasts and myelocytes. She was given 2 U of whole blood and a Dexin-and-water diet. On the second hospital day she was given the diet of foods containing 5 μg folate daily with multivitamin and potassium supplementation. After transfusion the erythrocyte count was 2,300,000 per mm^3; hemoglobin, 6.5 g per 100 ml; hematocrit, 21%; mean corpuscular volume, 91 μ^3; mean corpuscular hemoglobin concentration, 31%; reticulocyte count, 0.3%; leukocyte count, 3,400 per mm^3; platelet count, 8,000 per mm^3. The serum folate level was less than 1 nanogram per ml (indicating that the 2 U of whole blood transfused 36 hours previously did not significantly raise the serum folate level). Serum B_{12} was 176 picograms per ml, and serum iron was 55 μg per 100 ml with 38% saturation of iron-binding capacity. Urine formiminoglutamic acid excretion after a 20-g dose of L-histidine was 199 mg in 12 hours.

After a 10-day control period, she was given 75 μg of folic acid im and 15 ounces of 86 proof whiskey [12] daily for 10 days with no reticulocyte response (Figure 5). Seventy-two hours after whiskey had been discontinued (twenty-fourth hospital day) a rise in reticulocytes began, reaching a peak of 22.5% on the twenty-seventh hospital day, when ingestion of whiskey was resumed. The reticulocyte count fell progressively to 0.5% by the thirty-fifth hospital day. After change of therapy to 500 μg of DL-folinic acid [13] (only the

[9] We are grateful to Drs. Charles Lieber and Don Jones for performing several of the liver biopsies.

[10] We are grateful to Dr. Richard MacDonald for processing and interpreting the liver biopsies.

[11] This study was performed by Dr. Mortimer Greenberg.

[12] Imperial blended whiskey, 86 proof.

[13] Leucovorin, purchased from Lederle Laboratories, Pearl River, N. Y.

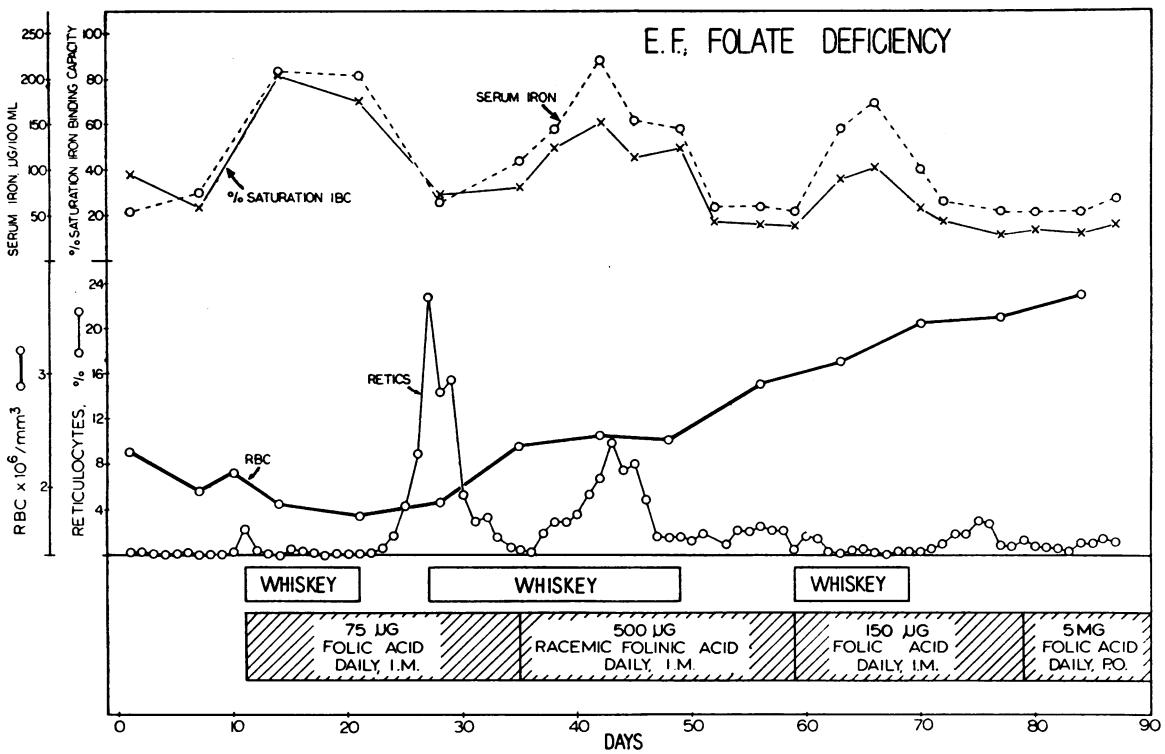

FIG. 5. FAILURE OF WHISKEY TO SUPPRESS THE RESPONSE TO 500 μG DL-FOLINIC ACID. Note the rises in serum iron and percentage of saturation of iron-binding protein during each period of whiskey ingestion. IBC = iron-binding capacity.

L-form is active in man) daily by im injection, a second reticulocytosis to 9.5% was observed, in spite of continued ingestion of whiskey. After a partial decline in the reticulocyte count, whiskey was discontinued. A low-grade reticulocytosis persisted until whiskey was resumed 10 days later. During the third period of ingestion of whiskey the reticulocyte count remained less than 1%, in spite of therapy with 150 μg folic acid intramuscularly. A slight rise in reticulocyte count to 2.8% occurred after cessation of alcohol, and no further rise followed the administration of 5 mg of folic acid daily.

Associated with periods of alcohol administration, the serum iron and the percentage of saturation of serum iron-binding capacity increased significantly within 48 to 72 hours, remained high until 24 to 48 hours after whiskey was discontinued, then fell. The initial total iron-binding capacity of the serum was low (145 μg per 100 ml) but increased to high levels during the patient's hospital course. While receiving therapy with 75 μg folic acid, 500 μg DL-folinic acid, 150 μg folic acid, and 5 mg folic acid, the averages of the serum folate determinations were 3.7, 9.1, 9.5, and 63 nanograms per ml, respectively. The serum vitamin B_{12} levels ranged from 93 to 248 picograms per ml (average, 163 picograms per ml) throughout her hospitalization. The platelet count increased from 8,000 per mm^3 on the second hospital day to 305,000 per mm^3 by the seventy-eighth hospital day, and the leukocyte count increased from 3,400 per mm^3 to 15,400 per mm^3 during the same period. Similar to Case 1, the megaloblastic bone marrow changed to normoblastic when alcohol was discontinued and reverted to megaloblastic when alcohol was resumed.

The patient was discharged on the eighty-seventh hospital day, with a hematocrit of 35%, on therapy with 5 mg pteroylglutamic acid daily by mouth.

Case 3. J. B., a 57-year-old electrician, was admitted to the Boston City Hospital in March 1964 because of weakness and shortness of breath. The patient had lived alone for more than 30 years and had consumed beer (two to four quarts), wine (one to two pints), and whiskey (three to

four highballs) daily during this time. He often went without food for several days. He had been hospitalized at Boston City Hospital for megaloblastic anemia secondary to folic acid deficiency in 1953 (30) and 1963. On each occasion he also had scurvy and had responded promptly to therapy with folic acid and ascorbic acid.

The patient was a 70-kg, 178-cm, pale man with multiple superficial ecchymoses, follicular keratin plugging, and perifollicular hemorrhages on the extremities. His teeth were carious, and the adjacent gums were swollen and dark red. His tongue was smooth, with papillary atrophy. The liver and spleen were not palpable.

His hematocrit was 28%, and leukocyte count was 4,450 per mm^3. The peripheral smear contained many oval macrocytes and hypersegmented neutrophils. The serum folate was less than 1 nanogram per ml; serum B_{12}, 116 picograms per ml; and serum iron, 144 μg per ml. Gastric

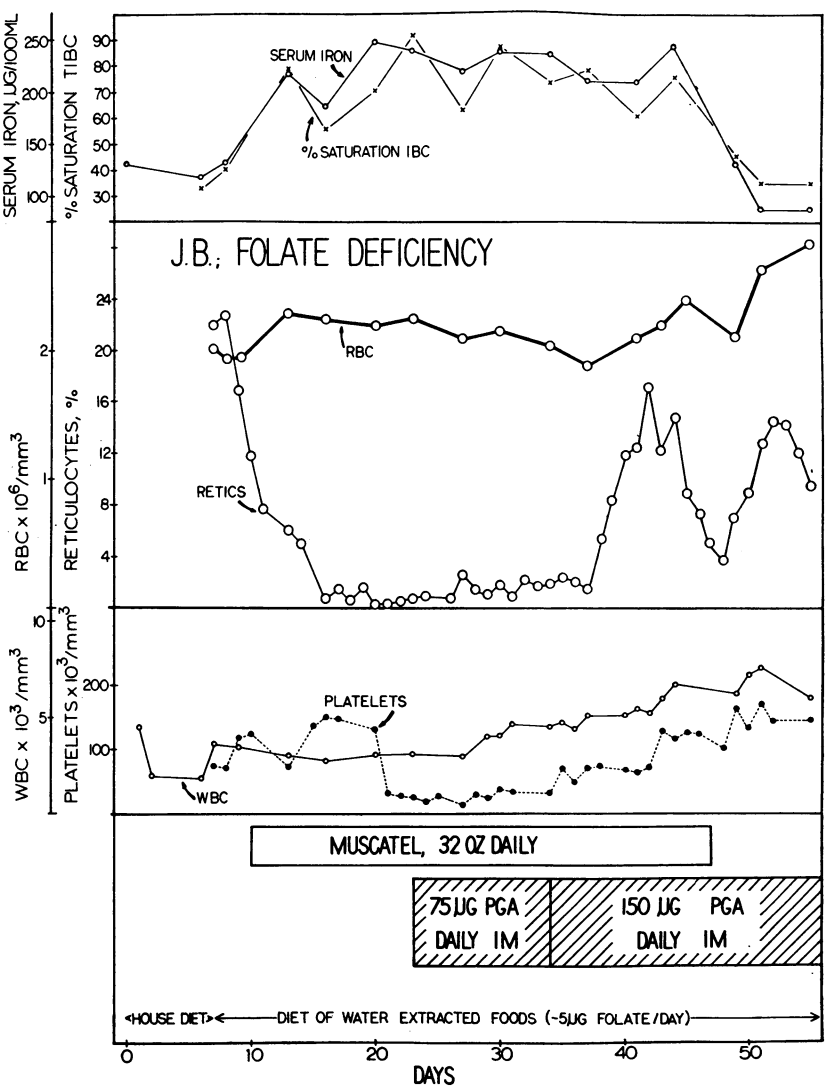

FIG. 6. FAILURE OF MUSCATEL WINE TO SUPPRESS THE RESPONSE TO 150 μG FOLIC ACID. Note the failure to respond to 75 μg while receiving muscatel, including the fall in platelet count to thrombopenic levels. With 150 μg folic acid there is a marked reticulocytosis and rise in platelet and leukocyte counts to normal levels in spite of continued ingestion of 32 ounces of muscatel daily. TIBC = total iron-binding capacity.

juice obtained with augmented histamine stimulation (17) had a pH of 1.3 and contained normal amounts of intrinsic factor on *in vitro* assay (18). Liver function studies are shown in Table I.

The patient was placed on a hospital diet plus ascorbic acid, 300 mg per day, and multivitamins.[14] Ecchymoses and perifollicular hemorrhages cleared rapidly.

On the seventh hospital day, when the patient's reticulocyte count was 22%, a bone marrow aspirate was obtained and found to be megaloblastic; the patient was transferred to the Thorndike Ward. At this time he had an erythrocyte count of 2.0 million per mm^3; hemoglobin of 8.2 g per 100 ml; hematocrit of 27%; mean corpuscular volume, 133 μ^3; mean corpuscular hemoglobin concentration, 31%; leukocyte count, 3,600 per mm^3; and platelet count of 76,000 per mm^3 (Figure 6).

On a diet of foods containing less than 5 μg folate daily the reticulocyte count fell to 11.8% by the tenth hospital day, when muscatel wine,[15] 32 ounces daily, was instituted. By day 16 the reticulocyte count was 0.7%. On day 23, folic acid, 75 μg daily im, was begun, with no significant rise in reticulocyte, leukocyte, or platelet counts. After increasing the dose of folic acid to 150 μg im daily on day 34, a brisk rise in reticulocytes began on day 38, reaching a peak of 18.4% on day 42 with an accompanying rise in erythrocyte count. The platelet count rose from 26,000 per mm^3 on day 34 to 65,000 per mm^3 on day 35, and to 140,000 per mm^3 on day 43. The leukocyte count rose from 3,000 per mm^3 on day 23 to 6,000 per mm^3 on day 45. After cessation of muscatel ingestion on day 47 a second reticulocytosis became evident on day 49, with a peak of 14.5% on day 53 and an accompanying rise in erythrocyte count. A bone marrow aspirate on day 48 showed the continued presence of striking megaloblastic changes, but 5 days later, the marrow smear exhibited predominantly normoblastic morphology. Ingestion of muscatel was resumed on day 56. The patient's hemogram continued to improve with 150 μg folic acid daily, so that on day 69 the erythrocyte count was 3.6×10^6 per mm^3; hemoglobin, 12.5 g per 100 ml; hematocrit, 40%; leukocyte count, 6,600 per mm^3; platelet count, 192,000 per mm^3; and reticulocyte count, 5.6% (not charted), although the marrow morphology had again become megaloblastic with alcohol ingestion. The serum iron and percentage of saturation of iron-binding protein both increased to high values with alcohol ingestion and remained high (including the period of the reticulocyte response to 150 μg folic acid), but promptly fell to normal values after cessation of muscatel (Figure 6). The Cr^{51}-labeled autologous platelet survival[16] during the second period of muscatel ingestion was 4 days, and when repeated after cessation of muscatel it was 7 days.

Discussion

Shortly after the appearance of a preliminary report of the present study (1), Beard, Barlow, and Tuttle reported reductions in hematocrit, hemoglobin, and leukocytes in normal dogs fed ethanol while ingesting an "adequate diet" (32). McFarland and Libre (33) noted a leukopenic response to severe bacterial infections in ten alcoholics and found a suboptimal leukocyte response to injected endotoxin. They concluded that their patients had a decreased marrow granulocyte reserve of unknown etiology. Because of the frequent finding of folate deficiency in alcoholism (2), the unknown factor might have been folate deficiency.

Before the development of both a palatable diet almost completely devoid of folate and of the *L. casei* assay of serum folate as a measure of folate deficiency, studies of folate-deficient patients presented difficulties of methodology and interpretation. Most commonly these difficulties were due to *1*) the use of diets containing substantial amounts of folate; *2*) use of *Streptococcus faecalis* as the microbiologic assay organism for evaluation of folate content of foods, which is inadequate[17]

[14] Engran baby drops, Squibb & Sons, New York, N. Y., 2 ml daily. Each 0.6 ml contains vitamin A, 5,000 U.S.P. U; vitamin D, 1,000 U.S.P. U; ascorbic acid, 70 mg; thiamine, 1.2 mg; riboflavin, 2.0 mg; niacinamide, 12.0 mg; pyridoxine, 2.0 mg; vitamin B_{12}, 6.0 μg; *d*-panthenol, 5.0 mg.

[15] Guild extra fine California muscatel (alcohol 20% by volume).

[16] Kindly done by Dr. Richard Aster, utilizing a recently reported method (31).

[17] *S. faecalis* does not measure N^5 methyltetrahydrofolate, which is a major folate form in man, but does measure pteroic acid, which has no folate activity in man (34).

and thus may be misleading; *3*) poor patient acceptance of synthetic folate-free diets; and *4*) the frequent observation of spontaneous reticulocytosis (5, 6) with hematologic improvement in patients with megaloblastic anemia.

With the 5 μg-folate diet (10), it has been demonstrated that man's minimal daily requirement for folate (as PGA) is in the range of 50 μg (7). On this diet, neither of the first two patients developed a spontaneous reticulocytosis during initial 10-day control periods although alcohol ingestion had stopped. In E. F., the increase in reticulocytes to 18.2% after institution of a routine hospital diet (on a previous admission for folate deficiency and anemia) suggests that the amounts of folate-active substances absorbed from such a diet are sufficient to cause a significant hematologic response. The reticulocyte count of 22% in Case 3, 1 week after admission (at the beginning of hematologic investigation), is probably a response to absorption of folate from the routine hospital diet and cessation of alcohol ingestion and possibly also other factors. Unfortunately, no earlier reticulocyte counts were done.

In the first two patients the administration of 75 μg of folic acid daily was accompanied by a brisk reticulocyte response beginning 3 to 5 days after cessation of alcohol ingestion, with a rise in erythrocyte count, hemoglobin, and hematocrit to normal levels after final cessation of ethanol, whereas there was no response to the administration of 75 μg folic acid during alcohol ingestion in any of the three cases.

The brisk hematologic responses in the second and third patients, resulting from the administration of 500 μg DL-folinic acid and 150 μg folic acid, respectively, in spite of continued ingestion of alcohol indicate that the block in hematopoiesis could be overcome by larger doses of folate. With these doses of folate, however, the marrow remained megaloblastic until alcohol was discontinued, whereupon marrow morphology became normoblastic within 4 to 10 days. In the third patient, there was a second reticulocyte response after cessation of alcohol. These observations suggest that the suppressive effects of alcohol were not completely eliminated by these amounts of folate and demonstrate that brisk hematologic response may occur despite failure of the bone marrow morphology to convert to normal. There was no evidence to suggest that folinic acid was more effective than folic acid in overcoming the suppression of hematopoiesis.

The fact that the serum folate level did not rise above the deficiency range for 50 days after institution of 75 μg folic acid daily in Case 1 is consistent with previous observations suggesting that the minimal daily requirement for folic acid is in the range of 50 μg. The period of 45 days between initial hematologic response and rise of serum folate also suggests that, with minimal doses of folic acid, serum folate levels rise as a late event, presumably after tissue folate stores are somewhat replenished.

The striking conversion of marrow morphology in each patient from normoblastic to megaloblastic within 4 to 10 days after the start of alcohol administration suggests that impairment of folate utilization may be caused by this agent. The marrow maturation time (from stem cell to mature erythrocyte) has been calculated as approximately 7 days (35). The 4- to 10-day interval required for definite changes of marrow morphology to occur in these studies is consistent with the hypothesis that alcohol may exert a significant effect at the stem cell level, where nucleoprotein synthesis is already impaired by folate deficiency (36). However, the prompt falls in reticulocyte count that occurred after beginning alcohol ingestion suggest that alcohol also affects erythroid cells beyond the stem cell level. Whether this is a direct effect of alcohol on hematopoietic cells or secondary to deranged folate metabolism (in the marrow or the liver) cannot be defined at this juncture. Dihydrofolic reductase activity was normal in percutaneous needle biopsies of the liver,[18] obtained from the first patient before and after a 5-day period of daily ingestion of 15 ounces of whiskey. Liver biopsies before and after 10-day periods of alcohol ingestion revealed slight accumulation of fat in the liver cells (38) but no other changes suggesting liver cell damage. The possibility of malabsorption of folic acid during alcohol ingestion accounting for suppression of hematopoiesis was excluded by intramuscular injection of folic acid after the second period of alcohol ingestion.

[18] We are indebted to Dr. J. R. Bertino for performing these determinations by a method previously described (37).

It is not yet certain that the hematosuppressive effect of alcohol acts solely via interference with folate metabolism. Patients with vitamin B_{12} deficiency have shown partial hematosuppression by ethanol (39). This, of course, is compatible with alcohol interfering with folate metabolism that is already impaired due to vitamin B_{12} deficiency (34). Observations on the effect of alcohol on erythropoiesis in other hematologic disorders are in progress to ascertain the degree of specificity of the observations reported herein (40).

The present study suggests that anemia, leukopenia, and thrombopenia may all occur in the alcoholic patient as a result of inadequate folate intake and ingestion of alcohol. The sharp rise in reticulocytes (6), leukocytes, and platelets (41) previously noted in patients with alcoholic cirrhosis simply on abstaining from alcohol and ingesting a normal diet is thus explainable, as previously suggested (41), by both of these factors. The platelet survival study during alcohol ingestion in the third patient suggests that alcohol may also shorten platelet survival in folate deficiency; the mechanism of this phenomenon is obscure at present.

The changes in serum iron concentration and percentage of saturation of iron-binding protein with ethanol are of considerable interest. Serum iron levels characteristically fell to much lower levels with hematologic responses to folic acid during abstinence from alcohol. On each occasion of ethanol administration, however, the serum iron rose to high levels and remained high until 24 to 72 hours after alcohol had been discontinued. The percentage of saturation of the serum iron-binding protein, followed in the second and third patients, was initially in the normal range but rose to high levels within 72 hours after beginning ethanol administration and fell to normal or low values within 48 hours after cessation of alcohol. In the second and third patients, the reticulocytosis occurring during alcohol ingestion was not accompanied by a fall in serum iron or percentage of saturation of iron-binding protein until after cessation of alcohol.

This dissociation of the changes in serum iron and saturation of iron-binding capacity from the reticulocyte response suggests that there may be an effect of alcohol on iron metabolism separate from the changes secondary to increased or decreased hematopoiesis. The finding of deposits of hemosiderin in the liver and in the submucosa of the villi of the small bowel in the first patient probably represents changes secondary to the prolonged anemia with possibly increased iron absorption and ineffective utilization over a long period. The interference of alcohol with iron metabolism may be of significance in the pathogenesis of some syndromes of iron overload in alcoholic subjects. MacDonald has recently shown that the iron content of some wines is considerable (42). The syndrome of iron overload in alcoholics probably results from many factors, including increased iron ingestion (in food and wine) and possibly increased iron absorption (43) and a block in normal iron utilization related to deficiencies or deranged metabolism of either folate (44), vitamin B_{12} (45), or pyridoxine (46). In such patients, the additional effect of alcohol on iron metabolism here demonstrated may lead to "pile-up" of iron in the serum and body tissues. The diagnosis of idiopathic hemochromatosis should probably not be made in alcoholic patients until it has been shown that the excessive iron stores in the liver are not due to deficiencies of these factors and cannot be reduced by cessation of alcohol and long-term therapy with folic acid, vitamin B_{12}, and pyridoxine (47).

The effects of alcohol on hematopoiesis are similar in several ways to those of chloramphenicol. Saidi, Wallerstein, and Aggeler (48) reported that chloramphenicol caused suppression of the hematologic response to large doses of vitamin B_{12} and to large doses of iron in patients with pernicious anemia and iron-deficiency anemia, respectively, in addition to producing vacuoles in the primitive marrow cells (especially the myeloid cells). By contrast, in our studies, the suppressive effects of alcohol were partially overcome by larger doses of folate. Secondly, the vacuoles reported in cases of chloramphenicol toxicity appear to be quite similar to those reported in acute alcoholism (21) and seen occasionally in the marrow of our three patients during alcohol ingestion. Thirdly, conversion of marrow morphology from normoblastic to megaloblastic during chloramphenicol ingestion in a patient with borderline folate stores, and reversion to normoblastic morphology after withdrawal of chloramphenicol, was reported by Gussoff, Lee, and Lichtman (49). The

fourth similarity is the elevation of serum iron levels and increase in the saturation of serum iron-binding protein during chloramphenicol therapy (50) or alcohol ingestion, with return of these findings to normal after withdrawal of the offending agent. These abnormalities may be the sequelae of marrow suppression from various causes, however, and do not imply that the site or mode of action of these agents is the same.

The low-normal serum B_{12} levels in the three folate-deficient patients may be due to repeated episodes of liver cell damage in the past, with reduction in the storage capacity for vitamin B_{12} by the liver and release of vitamin B_{12} from the liver into the plasma and excretion in the urine. These phenomena, combined with poor dietary intake of B_{12}, may lead eventually to vitamin B_{12} deficiency. We have observed such a sequence of events culminating in overt vitamin B_{12} deficiency in prior long-term observations of an alcoholic outpatient (40).

A B_{12} absorption study performed on Case 1 on a subsequent hospitalization showed a normal uptake by plasma (51) and accumulation in the liver (52) of an administered dose of 0.1 μg of Co^{57}-vitamin B_{12}, ruling against the possibility that the low serum B_{12} levels were the result of impaired vitamin B_{12} absorption.

Summary

Alcohol, in amounts readily consumed by "heavy drinkers," suppresses the hematopoietic response of anemic, folate-deficient patients to doses of folic acid in the range of the minimal daily adult folate requirement. This suppression can be overcome, either with larger doses of folic acid or by cessation of alcohol. The observation of "spontaneous" hematologic improvement seen in anemic alcoholics after hospitalization is, therefore, due to both ingestion of folate-containing foods and to cessation of ingestion of alcohol. Suppression of erythropoiesis, leukopoiesis and thrombopoiesis, and conversion of the bone marrow from normoblastic to megaloblastic within 10 days was observed with either commercially available alcoholic beverages or pure U.S.P. ethanol.

The serum iron concentration and saturation of iron-binding protein were increased during alcohol ingestion and fell upon alcohol withdrawal. This could be dissociated from the reticulocyte responses, suggesting an effect of alcohol on iron metabolism separate from the observed effects on hematopoietic activity. The effects of alcohol may be of significance in the evolution of syndromes of iron overload including hemochromatosis in alcoholic subjects.

The mechanism of the hematosuppressive effect of alcohol is presently unknown but may be partly via an effect on folate metabolism.

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