microorganisms, and it is bound in greater quantities than cyanocobalamin, as evidenced by all 3 methods. The explanation is presumably that this substance can utilize other possibilities of binding. The fact that the binding capacity of hydroxocobalamin exceeds that of cyanocobalamin only upon addition of large quantities may be taken to support the assumption that hydroxocobalamin is primarily bound to the same proteins as cyanocobalamin, and that, when these bindings have been exhausted, hydroxocobalamin has further binding possibilities.

In other words, it is possible that hydroxocobalamin is bound partially to other protein groups than those which can be blocked by cyanocobalamin.

Summary. Using the same methods as in previous studies on the measurement of the vit. B_{12} binding capacity in serum, the author has now investigated the binding capacity for cyano- and hydroxocobalamin in CSF by two microbiological methods and a dialysis technique. The findings were as follows: 1. There is no difference between the binding pattern in the CSF and serum for cyanocobalamin. Its binding capacity was approximately 1/10 of that found in serum. 2. Upon addition of more than 10-15 times the normal vit. B_{12} content of the CSF, or 200-300 pg per ml and more, the binding capacity was found to be higher for hydroxocobalamin than for cyanocobalamin. 3. As the binding pattern for large quantities of hydroxocobalamin differs from that for the corresponding quantities of cyanocobalamin, it is concluded that the increased binding capacity for hydroxocobalamin may be due to a partial binding of this substance to proteins different from those to which cyanocobalamin is bound.

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Reduction of Glutamic Pyruvic Transaminase in Pyridoxine Deficiency in Liver Disease.* (30687)

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Serum glutamic pyruvic transaminase (GPT) activity increases markedly in patients with virus- or drug-induced hepatic necrosis(1,2,3). In contrast, there may be little or no increase of GPT with necrosis in liver disease of the alcoholic, even in the presence of jaundice, fever,

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and leukocytosis(4,5,6). Decrease in hepatic production and release of GPT may in part depend on pyridoxine deficiency since 30% of alcoholics with liver disease have low circulating B_6(7). Availability of a protozoological method for direct assay of vit. B_6 in tissues and biological fluids permits examination of the relationship between vit. B_6 and GPT(8). The present report shows a lowered serum and liver GPT activity in vit. B_6 deficiency in experimental liver injury and liver disease of malnourished alcoholics.

Materials and methods. Animal studies. One hundred and twenty weanling male Sprague-Dawley rats were fed for 10 days a diet supplemented with B-complex vitamins. Half the animals were then put on a vit. B_6-deficient diet for 6 weeks; litter-mate controls were maintained on the vitamin-supplemented diet. After 6 weeks on the vit. B_6-deficient diet, animals were given pyridoxine 1 mg/100 g intramuscularly daily for 2 to 21 days. Centrilobular necrosis was induced in B_6-deficient and -repleted rats 24 hours before killing by administering a single oral dose of 0.2 ml/100 g of CCl_4. At 48-hour intervals after initiating the vit. B_6-deficient diet, representative animals were anesthetized with ether; blood (3 to 6 ml) was obtained from the aorta and the liver was removed promptly. A segment of liver was saved for histological examination and the remainder homogenized. Homogenates 1 g/100 ml were prepared in ice-cold, pH 7.4, 0.1 M phosphate buffer. Vitamin B_6 was assayed by a protozoological method(8); GPT activity was spectrophotometrically(9) determined in serum and in the liver homogenate. Tissue enzyme activity and vitamin levels were standardized by dry weight and N content(10).

Human studies. Serum GPT and B_6 were determined in 11 healthy subjects and 35 malnourished alcoholics showing characteristic clinical features and histologic evidence of liver disease (12 fatty liver, 12 inactive cirrhosis, 11 cirrhosis with hyaline necrosis). Serum GPT and B_6 determinations were repeated in 24 patients given orally 100 mg pyridoxine daily for 10 days. GPT activity and vit. B_6 levels were also determined in percutaneous liver biopsy specimens from 5 healthy subjects and 15 alcoholics with liver disease (4 fatty liver, 5 inactive cirrhosis, 6 cirrhosis with hyaline necrosis). Tissue enzyme activity and vitamin levels were determined before and after pyridoxine therapy in 2 subjects with normal liver and 5 with hyaline necrosis. Percutaneous biopsy specimens were obtained with the Vim Silverman needle and processed as described for rat liver.

Results and discussion. Animal studies. The B_6 content of normal rat serum and liver was 340 $\pm$ 110 μg per ml and 32 $\pm$ 10 μg per mg, respectively. GPT activity in normal rat serum and liver was 41 $\pm$ 16 units per ml and 308 $\pm$ 60 units per mg, respectively. Rats maintained on the B_6-deficient diet had reduced tissue and circulating B_6 and GPT (Fig. 1). Coarsening of hair, acrodynia, and hind leg paralysis preceded reduction of serum and liver B_6; these signs appeared in some rats during the second week of pyridoxine deprivation. Six weeks after B_6 depletion, serum B_6 had decreased to 2%, liver B_6 to 32%, serum GPT to 20%, and liver GPT to 37% of control values. Administration of pyridoxine caused an immediate increase in serum B_6 with a less rapid rise in liver B_6 and GPT. Serum GPT activity, following CCl_4-induced liver cell necrosis, was 90% greater in pyridoxine repleted than in depleted animals (Fig. 2).

Human studies. Healthy subjects had a serum B_6 of 35 to 100 μg/ml, liver B_6 of 5 to 20 μg/mg, serum GPT of 10 to 45 units/ml, and liver GPT of 140 to 170 units/mg (Fig. 3). Patients with fatty liver and inactive cirrhosis had variable serum B_6 and GPT; those with hyaline necrosis had low serum B_6 without significant elevations of serum GPT. Reduced liver B_6 and GPT was noted in each biopsy specimen from patients with liver disease, with either dry weight or protein serving as reference base.

Pyridoxine administration restored normal serum and tissue B_6 levels. It did not alter serum GPT in patients with normal serum B_6; however, serum GPT increased in 9 of 15 patients with a low serum B_6. Liver GPT activity remained unchanged after administration of pyridoxine to subjects having normal tissue B_6. B_6 treatment caused clinical

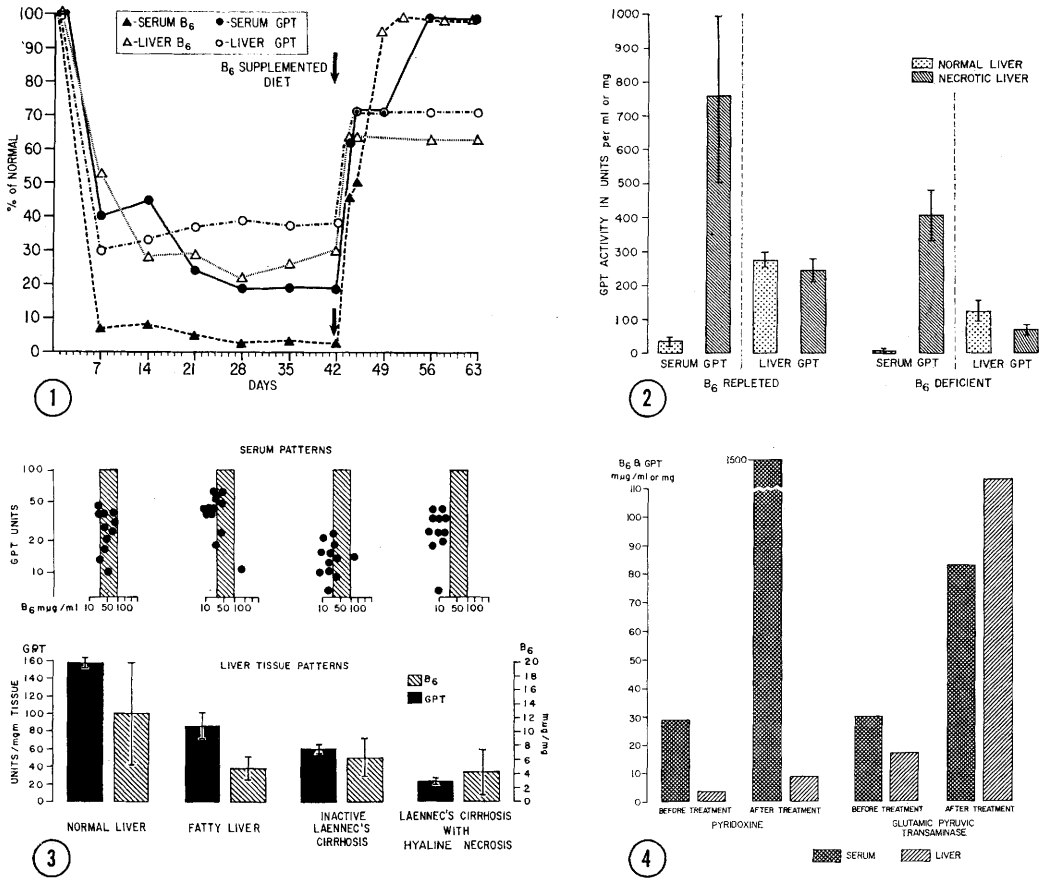

FIG. 1. Serum and liver glutamic pyruvic transaminase activity and B_6 levels in depleted rats.

FIG. 2. Serum and liver glutamic pyruvic transaminase activity in CCl_4-induced hepatic necrosis as affected by B_6 nutrition.

FIG. 3. Influence of B_6 status on serum and liver glutamic pyruvic transaminase activity in normal subjects and alcoholic patients with liver disease.

FIG. 4. Effect of pyridoxine (100 mg, oral daily) for 10 days on (a) serum and liver tissue glutamic pyruvic transaminase activity and (b) B_6, in a malnourished alcoholic with cirrhosis and hyaline necrosis.

improvement and increased tissue activity of GPT in 3 pyridoxine-depleted patients with cirrhosis and hyaline necrosis (Fig. 4). Pyridoxine repletion did not alter the disease picture or GPT activity in 2 pyridoxine-depleted patients with more severe degrees of cirrhosis and hyaline necrosis; they became worse and died.

These data show that low serum GPT activity in malnourished alcoholics with hepatic necrosis may be due to B_6 deficiency. Vitamin B_6 deficiency in such patients should be expected and should be treated promptly since it contributes to diminished protein and nucleic acid synthesis in liver disease(7). Reduced GPT activity may also result from deficient apoenzyme synthesis, or reduction in number of functional liver cells. When hepatic GPT is low less enzyme is released from necrotic liver cells.

Summary and conclusions. Hepatic glutamic pyruvic transaminase activity is decreased in diet induced B_6 deficiency and so prevents or diminishes increases in serum glutamic pyruvic transaminase following liver cell necrosis. Pyridoxine treatment restores normal tissue and serum glutamic pyruvic transaminase activity in uncomplicated vitamin B_6 deficiency; in contrast, low enzyme activity may persist in severe liver injury,

despite pyridoxine administration.

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Dietary Induction of Liver Glucose-6-Phosphate Dehydrogenase in the Rat.* (30688)

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It has recently been reported that the enzyme glucose-6-phosphate dehydrogenase (G-6-P DH) decreases in the liver during starvation and increases to a peak activity several times the level of non-starved controls 72 hours after refeeding a high glucose diet‡ (1,2). The present experiment was designed to determine which dietary ingredient is serving as the inducer of this enzyme.

Materials and methods. 48 Sprague-Dawley rats weighing 365 ± 25 (S.D.) g were used in this experiment. These animals had been previously fed Purina Rat Chow. At zero time, 36 animals were started on total starvation plus water *ad libitum* while 4 animals were started on the control diet of the following composition: casein 20, methionine 0.5, glucose§ 69.78, corn oil 5.0, salt mix 4164(2) 4.0, vitamin premix in casein(2) 0.5, α-tocopheryl succinate 0.02, vitamins A and D 0.10 (to provide 1500 I.U. vit A and 100 I.U. vit D/100 g diet). After seven days of starvation, 12 starved animals were sacrificed and the remaining 24 were divided into 3 groups of 8 rats each, which were refed *ad libitum* the following three diets: Group 1 control diet as above, Group 2 the same diet with starch replacing glucose and Group 3 a diet in which all carbohydrate was omitted and replaced by fat (hydrogenated vegetable oil‖) at a level to give a diet of essentially the same calorie/protein ratio as in the carbohydrate containing diets of Groups 1 and 2. The composition of this diet is as follows: casein 31.92, methionine 0.82, hydrogenated vegetable oil‖ 59.54, salt mix 6.55, vitamin premix in casein 0.82, α-tocopheryl succinate 0.04, vitamins A and D 0.15, choline chloride 0.16.

The food intakes of these animals were such as to give approximately 75 cals per day for Groups 1 and 2 and approximately 90

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§ Cerelose, Corn Products Co., New York.

‖ Crisco.