TABLE II.
Changes in Total and Residual Chromogen in Urine.
(10 ml of normal urine diluted 1:10; 4 mg glucose added, and 3 ml borate buffer in 50 ml of water).

Contents of tube	Urine	Urine + Borate	Urine + Glucose	Urine + Glucose + Borate
Total Chromogen as μg Uric Acid	53.9	53.6	97.4	54.4
After the incubation with uricase, and borate buffer.				
Residual Chromogen as μg Uric Acid			8.6	8.6
Apparent "True Uric Acid"			88.8	45.8

roneously high values result. Addition of borate to both the aliquot treated with uricase, and to the total color determination eliminates the interference of glucose.

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Effect of Vitamin B_{12} on Experimental Hepatic Injury.* (17686)

PAUL GYÖRGY AND CATHARINE S. ROSE.

From the Department of Pediatrics and the Medical Clinic, School of Medicine, University of Pennsylvania, Philadelphia.

Observations on the effect of crude liver extract in experimental hepatic injury in rats have been previously reported(1-3). It has been shown that liver extract given by mouth is without influence on, or will even promote the development of acute diffuse hepatic necrosis. On the other hand, liver extract will not only prevent cirrhosis but has a definitely beneficial effect in the treatment of hepatic cirrhosis. Thus, the effect of liver extract may be comparable to that of choline, the latter also having a beneficial effect on cirrhosis, and no effect or a deleterious one on necrosis(1,4,5). The fact that the combination of liver extract with methionine or with protein proved to be superior in the treatment of experimental cirrhosis(3) to the effect of methionine or protein alone seemed to indicate that the beneficial effect of the liver extract might not have been due to its content of choline but perhaps to some other constituent, such as vitamin B_{12}. This supposition was based on the assumption that choline, methionine and protein (caesin) all act similarly as sources of "labile methyl" and it was difficult to believe that a simple increase in supply of the same factor over and above a sufficient supply could have improved the therapeutic results as distinctly as was seen on the addition of liver extract to methionine and protein.

In the course of their studies on the lipotropic effect of liver extract Hall and Drill(6) reached similar conclusions. They found liver extract a much more potent lipotropic agent

* This represents work carried out under the auspices of the Commission on Liver Disease of the Armed Forces Epidemiological Board, Office of the Surgeon General, U. S. Army, Washington, D.C.

1. György, P., and Goldblatt, H., *J. Exp. Med.*, 1942, v75, 355.

2. György, P., and Goldblatt, H., *J. Exp. Med.*, 1949, v90, 73.

3. György, P., *Med. Clin. N. Am.*, 1949, 1657.

4. Daft, F. S., Sebrell, W. H., and Lillie, R. D., PROC. SOC. EXP. BIOL. AND MED., 1942, v50, 1.

5. Himsworth, H. P., *The Liver and Its Diseases*, Ed. I, Oxford, England, Blackwell, Scient. Publ., 1947.

6. Hall, C. A., and Drill, V. A., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 3.

than could have been expected from its content of choline alone. A more direct proof of the sparing effect of vitamin B_{12} on the choline ("labile methyl") requirement of rats and chicks was first presented by Schaefer, Salmon and Strength(7). They were able to prevent "hemorrhagic kidney" in rats, and promote growth in chicks with suboptimal amounts of choline and methionine, provided B_{12} was added to the diet (3 γ/100 g). This interrelationship between vitamin B_{12} and methylating compounds has been confirmed on chicks by Gillis and Norris(8).

In the light of this new evidence it became desirable to study the effect of B_{12} as such in various forms of liver injury, in order to be able to distinguish between the effects of choline and B_{12} in liver extract.

Massive hepatic necrosis. Hepatic necrosis in rats fed a diet low in casein occurs but only very irregularly(1,9). A more severe, truly massive hemorrhagic necrosis of the liver can be produced in almost 100% of the experimental animals by using a diet with a proper preparation of yeast as sole source of protein(5,10-12). Yeast is free from B_{12}(13), whereas casein, if not especially purified, may contain traces of B_{12}. Thus the question arises whether a deficiency of B_{12} may play a part in the development of this especially severe fulminating massive hepatic necrosis when yeast rations are used. It may even be surmised that the irregular occurrences of hepatic necrosis in rats fed casein-containing rations are related to traces of B_{12} remaining in the purified casein. The often observed, slightly injurious effect of liver extract in this form of hepatic necrosis would then be due to the presence of choline.

This assumption, however, was not borne out by experiments in which the incidence of hepatic necrosis was compared in groups of rats receiving a "necrogenic" yeast ration with or without supplements of vitamin B_{12}. The composition of the yeast rations was as follows: A. Yeast,† 18; corn starch, 79; salt mixture (USP II), 3. B. Yeast corn starch, 59; lactose, 20; salt mixture (USP II), 3. Peanut oil, 0.5 ml and cod liver oil, 2 drops, were added for each 8 g of the dry mixture (ration A or B). All animals received daily 20 γ "Synkayvite"‡ and supplements of 20 γ thiamine, 25 γ riboflavin, 20 γ pyridoxine, and 100 γ calcium pantothenate, dissolved together in 1 ml water. Rats were of the Sprague Dawley strain, males, weanlings, with an average weight of 50 g. Twelve rats received ration A without supplement of B_{12}§, 6 rats received the same ration with vitamin B_{12}, 1 γ daily by mouth. Seven additional rats were fed ration B with vitamin B_{12}, 1 γ daily by mouth. Thus, altogether 13 rats received a necrogenic yeast diet with supplement of vitamin B_{12}, and 12 rats were used as controls without B_{12}. All rats in both groups died from typical massive hemorrhagic necrosis, involving preferentially the left lobe or left half of the liver. There was no statistically significant difference in length of survival of the animals in the two groups; rats fed without B_{12} died on the average after 34, those receiving supplement of B_{12} after 36, experimental days. Thus, vitamin B_{12} given by mouth was without any influence on the production of massive hepatic necrosis in rats receiving a "necrogenic" yeast diet.

Lipotropic effect of B_{12}. Experimental dietary hepatic cirrhosis is due to insufficient supply of lipotropic factors, and is accompanied, at least initially, by fat infiltration of the

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13. Hartman, A. M., Dryden, L. P., and Cary, C. A., *J. Am. Dietetic Assn.*, 1949, v25, 929.

† "D.C.L. Vit. B_1 yeast" from Distillers Co., Ltd., Glasgow C.4, Scotland.

‡ Water-soluble vitamin K preparation, Hoffmann-LaRoche, Nutley, N.J.

§ Kindly supplied by Chas. Pfizer and Co., Inc., Brooklyn, N.Y.

liver. Thus, it may be assumed that substances with lipotropic activity should have a beneficial effect on the production and perhaps even on the treatment of dietary hepatic cirrhosis. Direct evidence may only be obtained in long-term experiments in which the effect of the substance in question is studied on the prevention and treatment of hepatic cirrhosis. Such investigations on the effect of B_{12} in the treatment of dietary cirrhosis of the liver are in progress.‖ The present report includes experiments designed for the study of the lipotropic activity of B_{12}.

The same general procedures were followed as were previously reported(14,15). Young adult female rats of the Sprague Dawley strain, weighing 145-175 g, were used. As usual, control and treated animals were run simultaneously. When all of the animals of an experiment could not be run at one time, equal numbers of the various groups were started together. Two types of rations were used: a) Low protein-high fat (V_4), and b) Low protein-low fat (V_{11}). *V_4 (Exp. 31)*: Casein, G.B.I. Vitamin Test, 8; "Vream"¶, 40; Sucrose, 48; Salt mixture (USP II), 4. *V_{11} (Exp. 33:* Casein, 8; "Vream", 5; Sucrose, 83; Salt mixture, 4. Each animal received daily 20 γ thiamine, 25 γ riboflavin, 20 γ pyridoxine, 100 γ calcium pantothenate dissolved in 1 ml of water. Three drops of percomorph oil and 3 mg of α-tocopherol were given weekly. When methionine was given, the daily dose (50 mg dl-methionine)** was dissolved in 1 ml of water and mixed with the vitamin B supplement. B_{12} was administered similarly, in form of a solution contain-

TABLE I.
The Lipotropic Effect of B_{12}. Ten rats in each experiment.

Exp.	Diet	Treatment	Liver Wt, g	Liver Total fat, %	Rat weight Initial, g	Rat weight Change, %	Food intake, g/day
31	V_4 (High Fat)	Control	7.7 ± .40	27.8 ± 2.6	156	— 8.8 ± 3.3	6.1 ± .36
		B_{12}	6.7 ± .24	23.0 ± 2.2	156	— 8.7 ± 1.2	5.9 ± .19
		Methionine	6.5 ± .41	18.3 ± 1.4	152	— 7.0 ± 1.6	5.5 ± .30
		Meth. + B_{12}	5.9 ± .35	14.7 ± 1.6	152	—11.3 ± 1.8	5.0 ± .25
33	V_{11} (Low Fat)	Control	9.3 ± .44	35.0 ± 1.7	160	—12.7 ± 2.1	8.1 ± .36
		B_{12}	8.0 ± .56	20.5 ± 1.6	158	— 2.6 ± 1.4	9.4 ± .20
		Methionine	6.5 ± .48	14.6 ± 1.8	161	— 8.3 ± 2.7	8.1 ± .43
		Meth. + B_{12}	6.6 ± .27	10.4 ± 1.3	158	— 4.5 ± 2.9	8.6 ± .41

‖ Investigations are also carried out at the Army Hepatic and Metabolic Center, Valley Forge General Hospital, on the metabolic and therapeutic effect of B_{12} in man (normals and patients with subacute hepatitis and cirrhosis), in collaboration with Dr. V. M. Sborov and Lt. D. Sutherland.

14. György, P., Rose, C. S., and Shipley, R. A., *Arch. Biochem.*, 1947, v12, 125.

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¶ Vegetable shortening, obtained from Swift & Co., Chicago.

** Kindly furnished by Wyeth, Inc.

ing 0.5 γ of the crystalline compound.†† At the end of the 21 day experimental period, the animals were sacrificed and the liver analyzed for total lipide with the same technique used in the previous experiments.

The results obtained are summarized in Table I. In Exp. 31, using a low protein-high fat diet, administration of B_{12} was followed by a slight statistically insignificant reduction of the relative liver fat content. When combined with methionine, B_{12} had increased only slightly, without statistical significance the lipotropic effect of methionine alone. It should, however, be pointed out that the average weight of the liver, and in consequence, the absolute fat content of the liver in Exp. 31 were distinctly reduced in the groups of animals receiving supplements of B_{12} (with or without methionine) to their low protein-high fat ration.

The lipotropic effect of B_{12}, suggested in Exp. 31, became clearly evident in Exp. 33 when the low protein-low fat diet was used. It appeared to be especially marked in the group of animals receiving B_{12}, without methionine, as supplement to the basal ration. The slightly increased average food intake and better average weight curve in the groups of animals receiving B_{12} over those in the control groups should have, if anything, counteracted to some extent the lipotropic effect of B_{12}(16,17).

The lipotropic effect of a B_{12} concentrate was found recently also by Drill and McCormick(18). These authors used a lipotropic diet with moderately high protein (16%) and high fat (51%) content. The B_{12} concentrate was given by injection (0.2-1.0 γ, 3 times per week).

†† Kindly supplied by Merck & Co.

16. Treadwell, C. R., Tidwell, H. C., and Gart, J. H., *J. Biol. Chem.*, 1944, v156, 237.

17. Treadwell, C. R., *J. Biol. Chem.*, 1945, v160, 601.

18. Drill, V. A., and McCormick, H. M., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 388.

We were able to show that B_{12} may exert its lipotropic effect also when given by mouth. Further, the experiments here reported indicate that a low protein-high fat diet will hinder the demonstration of lipotropic activity by B_{12}, whereas a low protein-low fat diet, or, as in the experiments of Drill and McCormick, a moderately high protein-high fat diet, will not interfere with the lipotropic reaction following the administration of B_{12}. These observations may also be related to the findings of Bosshardt, Paul and Barnes(19) on the growth-promoting effect of B_{12} in mice receiving varying proportions of fat and protein in their diet. Special studies are required to answer the questions whether with increased dosage of B_{12} the lipotropic effect may become evident in rats fed a low protein and high fat diet, or whether the lipotropic effect as such may be potentiated by giving higher amounts of B_{12}. It should be pointed out that in our experiments methionine has proved to be a more effective lipotropic agent than vitamin B_{12}. Experiments are contemplated to show whether additional amounts of vitamin B_{12} will increase its lipotropic effect, or whether the sparing effect of vitamin B_{12} could be made more evident with reduced suboptimal amounts of the lipotropic compound (choline, methionine).

Summary. Vit. B_{12} (1 γ daily, by mouth) had no effect on the development of massive hepatic necrosis in rats fed experimental rations with yeast as sole source of protein.

Vit. B_{12} (0.5 γ daily by mouth) has shown significant lipotropic activity in rats fed a low protein-low fat ration. Under the experimental conditions chosen methionine was more effective than vitamin B_{12} in preventing fat deposition in the liver. A low protein-high fat diet seemed to interfere with the lipotropic effect of B_{12}.

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