17130. Interrelationship of Vitamin B_{12} and Choline.* I. Effect on Hemorrhagic Kidney Syndrome in the Rat.

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Following the isolation of vitamin B_{12} by Rickes and co-workers,[1] several investigators[2-4] have reported data indicating the identity or close relation of this vitamin with the "animal protein factor." Studies by Bird, Rubin, and Groschke[5] have indicated that methionine can function as a partial substitute for the "animal protein factor" in soybean protein diets for chicks. In view of the relationship between methionine and choline, it seemed of interest to determine whether vitamin B_{12} might exert a sparing action on the choline requirement of the rat.

It is the purpose of this paper to report that the incidence and the severity of renal hemorrhage in weanling rats receiving a diet low in choline and methionine were significantly decreased by supplementing the diet with vitamin B_{12} in concentrate or crystalline form.

Experimental. Weanling rats of the AES (Alabama Experiment Station) strain weighing 40-50 g were placed in individual cages and uniformly grouped with respect to number, weight, sex and litter. Feed and water were supplied *ad libitum*. The basal diet contained extracted peanut meal 30,[6] sucrose 39.5, extracted casein 6,[7] salt mixture[7] (undried) 4.4, L-cystine 0.1, cod liver oil 1, lard 19. Vitamins were added, mg/kg of diet, as follows: thiamine 2, pyridoxine 2, riboflavin 4, calcium pantothenate 10, niacin 20, i-inositol 200, alpha-tocopherol 25, alpha-tocopherol acetate 25, and menadione 5. The total methionine and choline content of the diet was .3% and .007% respectively.

On this basal diet without added choline or methionine, a 100% incidence of fatal kidney hemorrhage occurs in weanling rats of the AES strain in 2 weeks or less. In the present experiments, various sub-protective levels of choline or methionine were added to the basal diet. The effect of adding vitamin B_{12} in concentrate or crystalline form to the diet for each of the treatments was then determined. In one experiment the effect of adding vitamin B_{12} concentrate to the basal diet supplemented with an adequate protective level of choline (.2%) was determined. Except in the latter experiment, all rats were necropsied after death or at the end of a 14-day experimental period, and the kidneys were carefully examined for gross pathological lesions.

The results on the effects of vitamin B_{12} with sub-protective levels of choline are shown in Table I. At levels of .04% and .06% of choline chloride without vitamin B_{12}, the

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TABLE I.
Choline-Sparing Action of Vitamin B_{12} for Protection Against Hemorrhagic Kidneys.

Dietary supplement: Choline Cl., %	Dietary supplement: Source of vitamin B_{12}, $\cong$ μg/kg diet	No. of rats	Mortality	Avg wt gain of survivors g/2 wks	Incidence of renal damage, %
.04	0	25	24	19	100
.05	0	4	0	36	75
.06	0	16	0	44	69
.20	0	4	0	57 (130)*	0
.02	Conc. No. 1† $\cong$ 30	4	50	50	75
.03	" " 1 $\cong$ 60	4	0	38	25
.03	" " 1 $\cong$ 30	4	25	39	50
.04	" " 1 $\cong$ 30	21	9.5	55	38
.05	" " 1 $\cong$ 30	4	0	45	25
.06	" " 1 $\cong$ 30	10	0	57	20‡
.20	" " 1 $\cong$ 30	4	0	58 (132)*	0

* Avg wt gain at 4 weeks.
† Merck & Co., Inc.—charcoal adsorbate No. 8R 5704.
‡ Very slight renal damage in 2 of the 10 rats.

TABLE II.
Choline-Sparing Action of Vitamin B_{12} for Protection Against Hemorrhagic Kidneys.

Dietary supplement: Choline Cl., %	Dietary supplement: Source of vitamin B_{12}, $\cong$ μg/kg diet	No. of rats	Mortality, %	Avg wt gain of survivors, g/2 wks	Incidence of renal damage %
.04	None	4	25	14	100
.04	Crystalline B_{12}, 30	4	25	43	50
.04	Conc. No. 1 $\cong$ 30	4	25	39	50

TABLE III.
Methionine-Sparing Action of Vitamin B_{12} Concentrate for Protection Against Hemorrhagic Kidneys.

Dietary supplement: DL-methionine, %	Dietary supplement: Source of vitamin B_{12}, $\cong$ μg/kg diet	No. of rats	Mortality, %	Avg wt gain of survivors, g/2 wks	Incidence of renal damage, %
.150	0	4	75	46	100
.192	0	8	25	27	100
.150	Conc. No. 1 $\cong$ 30	4	25	57	25
.192	" " 1 $\cong$ 30	8	0	60	12

incidence of kidney damage was 100% and 69%, respectively. The inclusion of a charcoal adsorbate to supply 30 μg of vitamin B_{12} per kg of diet to the above treatments reduced the incidence of kidney damage to 38% and 20%, respectively. It appeared that .03% of choline with vitamin B_{12} was as effective in preventing kidney damage as .06% of choline without the vitamin.

In general, the severity of renal damage in the rats receiving vitamin B_{12} was relatively mild in comparison with the damage in the control rats not receiving the vitamin. Only 2 of 21 rats receiving .04% of choline with vitamin B_{12} concentrate died; the average weight gain of the survivors for the 2-week period was 55 g. Of the 25 rats receiving .04% of choline chloride without vitamin B_{12}, 6 died and the average weight gain of the survivors for the 2-week period was only 19 g. The increased gain of the rats receiving the vitamin B_{12} preparations was in all probability a direct result of the protective action of the vitamin. It certainly emphasizes the protective effect of the vitamin because in the absence of such protection the increased gain

would have increased the incidence and the severity of the kidney lesions.

That vitamin B_{12} was the active principle in the concentrates used is indicated by the results shown in Table II. In this series crystalline vitamin B_{12} was compared with the charcoal adsorbate at the .04% level of choline chloride.

Vitamin B_{12} likewise increased the efficiency of methionine for the prevention of kidney damage (Table III). Supplementary DL-methionine was added to the basal diet at levels of .15% and .192%. The incidence of kidney damage was 100% at both levels. However, when the diet was further supplemented with vitamin B_{12}, the incidence of kidney damage decreased to 25% at the .15% level of methionine and to 12% at the .192% level.

The results when vitamin B_{12} was added to the diet with an adequate protective level of choline are shown in Table I. There was no effect on weight gains or appearance of the rats at the end of the second or fourth week. Rats in this laboratory have consistently made normal growth on this diet when adequate choline was added without either folic acid or vitamin B_{12}. When .2% choline chloride was added to the basal diet the need for supplementary vitamin B_{12} was eliminated. It thus appears that dietary choline has a significant sparing action on vitamin B_{12}.

Discussion. The results of these experiments show that vitamin B_{12} decreases the dietary choline or methionine required for protection against the hemorrhagic kidney syndrome in rats. It appears that, under the conditions of these experiments, about one-half of the choline required for protection against this syndrome can be replaced by 30 μg of vitamin B_{12} per kg of diet. Moreover, the results of one experiment indicate that the need for vitamin B_{12} is markedly decreased by the inclusion of adequate choline in the diet.

Thus, there is established the existence of an interrelationship between vitamin B_{12} and choline or methionine when the latter functions as a choline precursor. This interrelationship assumes added significance in view of the apparent importance of these factors in the maintenance of normal hemoglobin levels in humans as well as in experimental animals. Vitamin B_{12} has been shown by West[8] to be effective in the treatment of Addisonian pernicious anemia. Spies and his collaborators[9] have recently reported satisfactory hematologic responses to vitamin B_{12} therapy in persons suffering from pernicious anemia, nutritional macrocytic anemia, or tropical sprue. They also found the vitamin to be effective in relieving the subacute combined nervous system degeneration associated with pernicious anemia.

Engel[6] has reported anemia and edema in rats on low-protein diets that were deficient in choline. The addition of choline chloride prevented both the anemia and the edema. McKibbin, Thayer and Stare[10] have reported lowered hemoglobin values in dogs receiving diets deficient in choline. We have observed severe anemia in dogs on a choline-deficient diet similar to the diet used in the experiments herein reported.[11]

Choline has been used for the treatment of anemia in humans with variable results. Moosnick, Schleicher and Peterson[12] have reported successful treatment with choline of a case of pernicious anemia that was refractory to parenteral liver therapy. Davis and Brown[13] have reported hematologic responses to choline therapy in 4 case of pernicious anemia and one case of megaloblastic anemia of pregnancy; choline was ineffective, however, in one other case of pernicious anemia and 4 other cases of megaloblastic anemia.

The demonstration of a specific interrelationship of vitamin B_{12} and choline in protecting against the hemorrhagic kidney syn-

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drome in rats, may suggest that the use of choline as a supplementary aid in the treatment of various types of anemia should be further explored. Some of the clinical results referred to above indicate that it may be of particular value in cases complicated with liver damage. Further investigations on the nutritional implications of this interrelationship are underway in this laboratory.

Summary. 1. The incidence and severity of renal injury in weanling rats fed diets low in choline and methionine were markedly decreased by supplementing the diet with a vitamin B_{12} concentrate or crystalline vitamin B_{12}.

2. Under the conditions of these experiments, 30 μg of vitamin B_{12} per kg of diet could replace about one-half of the supplementary choline or methionine required for protection against kidney damage.

3. When sub-protective levels of choline were fed, the addition of vitamin B_{12} caused a significant increase in weight gain. However, when an adequate protective level of choline was fed, no increase in weight gain was obtained from the addition of the vitamin.

4. The results established the existence of an interrelationship between vitamin B_{12}, and choline or methionine.

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17131. Relation of Oxygen and Temperature in the Preservation of Tissues by Refrigeration.

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An inquiry has been made into conditions which may influence the viability of 1x1 cm areas of biopsied rabbit skin during refrigeration at 0° and 6-8°C. Since the availability of oxygen, as well as the nature of the storage medium, had an important influence on the preservation of viability at these two temperatures, these relationships may be of interest to those wishing to store or ship tissues for surgical and other purposes.

Tissues separated from the circulation rapidly become anoxemic and necrotic at room or body temperatures. This condition may be prevented in uterine or intestinal strips by oxygenation or by chilling.[1,2] The survival of ligated limbs[3] and of the cells in whole embryos[4,5] or organs[5,6] is optimal (among the widely spaced temperatures which have been studied) at 0°. At this temperature respiration is minimal, while oxygen solubility in water is twice that at 30°.

Though Lambert,[7] Carrel,[8] and Hetherington and Craig[5] found 0 to 7° favorable for preserving the small masses of crowded cells in embryonic tissue fragments, there is considerable evidence that the thin perimeter of migrating and dividing cells in established tissue cultures has maximal longevity around 30°[9,10] and are unable to re-establish growth after refrigeration for a few days.[9,11-13] Upon considering the fact that large tissues are

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