days. The same technic of thoracotomy and thoracic inferior vena caval constriction, as employed in the second control group, was applied to this series. Positive endotracheal oxygen pressure was given throughout. No antibiotics were utilized in the postoperative period. Following operation, daily paracenteses were performed, and the formation of ascites was usually demonstrated in 3 to 4 days. This fluid was examined for total protein and A/G ratio every 7 days for a period of 2 months. No appreciable change in average total protein or A/G ratio was noted when compared with the fluid produced in the animals with thoracic inferior caval constriction alone. Total protein averaged 4.0 g per 100 cc, A/G ratio 1.4 and specific gravity 1.013 to 1.017. Table III outlines the results obtained in this group of animals.

Conclusion. 1. Acute hepatic artery ligation in the rabbit resulted in 100% mortality in this experiment. 2. Massive penicillin therapy following hepatic artery ligation in rabbits resulted in a 66% survival rate. 3. Ascites produced by thoracic caval constriction in the rabbit is not prevented or appreciably affected by previous hepatic artery ligation.

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Influence of Vitamin B_{12} and Folacin on the Synthesis of Choline and Methionine by the Rat.* (18882)

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It was reported(1,2) that vit. B_{12} exerted a sparing action on the choline requirement of rats and chicks. It was later noted(3) that both folacin and vit. B_{12} were involved in the methylation process. Schaefer *et al.*(4) reported that chicks required vit. B_{12} for the synthesis of choline from methylaminoethanol and methionine or betaine. Jukes *et al.*(5) noted that vit. B_{12} was essential for the methylation of homocystine for growth in chicks fed a methionine-deficient diet. Oginsky(6) observed that livers of vit. B_{12}-deficient rats exhibit a lower ability to form methionine from homocystine and either betaine or choline as compared with those from animals dosed with vit. B_{12}. The role of vit. B_{12} and folacin in the methylation of guanidoacetic acid was reported by Dinning *et al.*(7). Stekol and Weiss(8) concluded that rats are able to grow on a diet free of all the known labile methyl group donors but

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TABLE I. Basal Diet.

		Vitamins added to supply mg/kg diet	
Alcohol extracted peanut meal*	25.00	Thiamine	2
Oxidized Casein†	6.00	Riboflavin	4
Sucrose	39.09	Pyridoxine	2
Vitamin Mix‡	5.00	Calcium pantothenate	10
Minerals (undried)§	4.40	Niacin	20
Cod liver oil‖	1.00	Inositol	200
Lard	19.00	Alpha-tocopherol	25
L-cystine	.26	Alpha-tocopherol acetate	25
DL-tryptophan	.10	2-Methyl-1,4-naphthoquinone	5
DL-homocystine	.15		

* After treating the peanut meal as described by Engel, R. W., *J. Nutrition*, 1948, v36, 739, it was extracted with methanol in a continuous Soxhlet apparatus for 7 days. Choline analysis on a 50-g sample was negative. Protein analysis = 60%; methionine supplied in the diet from 25% peanut meal = 0.11%.

† Prepared as described by Toennies, G., *J. Biol. Chem.*, 1942, v145, 667, as modified by Hove, E. L., Copeland, D. H., and Salmon, W. D., *J. Nutrition*, 1949, v39, 397.

‡ Thiamine, riboflavin, pyridoxine, calcium pantothenate, niacin and inositol added to sucrose to furnish the amounts listed per kg of diet.

§ Salmon, W. D., *J. Nutrition*, 1947, v33, 155.

‖ The tocopherol and 2-methyl-1,4-naphthoquinone were added to the cod liver oil.

TABLE II. Methionine Requirement of Rats Fed the Basal Diet Minus Homocystine Supplemented with 30 μg Vit. B_{12} and 2 mg Folacin per kg of Diet.

Dietary supplement					
Choline, %	DL-methionine, %	No. of rats	Avg body wt gain, g	Incidence renal damage, %	Avg liver fat* (dry basis), %
.20	0	8	3	0	27
.20	.08	8	43	0	15
.20	.10	8	50	0	13
.20	.20	4	53	0	12
.20	.30	4	58	0	12
.20	0 †	8	58	0	13

* Total ether-extractable material.

† Methionine (0.18%) supplied by regular extracted casein replacing the oxidized casein.

which contained vit. B_{12}, folacin, and homocystine or homocysteine. The biological synthesis of labile methyl groups has been confirmed by Arnstein(9), du Vigneaud *et al.* (10), and Sakami(11). Sakami and Welch (12) reported that choline and methionine methyl groups are synthesized from formate. Arnstein(9) showed that methanol, formate, the β-carbon atom of L-serine and the α-carbon atom of glycine are precursors of choline methyl groups, but the incorporation of these precursors into the methyl group of choline appears to be slow as compared with their incorporation into the aminoethanol moiety.

In a continuation of studies on the interrelationship of vit. B_{12}, folacin, and choline, the effect of vit. B_{12} and/or folacin on the synthesis of both choline and methionine in the presence or absence of a dietary source of labile methyl was investigated. The *in vivo* synthesis of choline and methionine is implied by the favorable response observed in rats when the basal diet was supplemented with various precursors of these factors.

Experimental. Since a study of choline metabolism is directly related to the supply of dietary methionine, a diet deficient in both choline and methionine and supplemented with homocystine was employed. Composition of the basal diet is given in Table I. 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.

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10. du Vigneaud, V., Ressler, C., and Rachele, J. R., *Science*, 1950, v112, 267.

11. Sakami, W., *J. Biol. Chem.*, 1950, v187, 369.

12. Sakami, W., and Welch, A. D., *J. Biol. Chem.*, 1950, v187, 379.

TABLE III. Effect of Vit. B_{12} and Folacin on the Utilization of Choline or Betaine for Synthesis of Methionine from Homocystine.

Dietary supplement	Vit. B_{12}, μg/kg	Folacin, mg/kg	No. of rats	Avg body wt gain, g/2 wk	Incidence renal damage, %	Mortality, %
0	0	0	10	0	100	100
0	150	10	8	0	100	50
0.25% glycine, 0.20% DL-serine	150	10	8	−2	100	75
1% methanol	150	10	4	0	100	50
1% methanol, 0.05% aminoethanol	150	10	8	0	88	50
0.06% choline chloride	60	2	4	22	100	0
0.08% " "	60	2	8	35	0	0
" " "	60	0	8	28	50	0
" " "	0	2	12	17	50	0
" " "	0	0	8	14	75	0
0.10% " "	60	2	8	42	0	0
0.12% " "	60	2	8	49	0	0
0.16% " "	60	2	12	62	0	0
" " "	60	0	8	56	0	0
" " "	0	2	8	19	0	0
" " "	0	0	8	14	0	0
0.06% choline chloride, 0.06% betaine HCl	60	2	4	43	0	0
0.08% choline chloride, 0.04% betaine HCl	60	2	8	48	0	0
0.08% choline chloride, 0.08% betaine HCl	60	2	8	65	0	0

Feed and water were supplied *ad lib*. 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. In general, each treatment consisting of 4 rats per group was repeated 1 to 3 times.

The additional dietary methionine supplement required for maximum growth was approximately 0.10% when the basal diet minus homocystine, was supplemented with adequate choline, vit. B_{12}, and folacin (Table II). At the 0.30% level of DL-methionine, growth was equal to that obtained when alcohol-extracted casein was used in place of the oxidized casein. Dietary supplementation of 0.08% DL-methionine supported nearly maximum growth (43 g per 2 wks). When the diet was supplemented with 0.20% choline chloride, the kidneys were protected against hemorrhage even though the diet was deficient in methionine; however, liver fat was increased from 12% to 27% (dry basis) by the omission of the methionine supplement.

The requirement for dietary choline to supply choline *per se* and sufficient methyl for methionine synthesis from homocystine is indicated by the data in Table III. Supplementation of the homocystine basal diet with vit. B_{12} and folacin and either glycine and serine, or methanol and aminoethanol was ineffective in preventing renal hemorrhage and for the support of growth of the weanling rats. The effect of either vit. B_{12} or folacin, or both folacin and vit. B_{12} on the utilization of choline for the methylation of homocystine was studied at 2 levels of choline, 0.08% and 0.16%, which supported suboptimal to optimal growth, respectively. (Table III). When the diet was supplemented with 0.08% choline chloride, both folacin and vit. B_{12} were essential for protection against renal damage and for support of fair growth (35 g in 2 weeks). Increasing the choline chloride level to 0.16% without vitamin B_{12} and folacin protected against renal damage; however, average weight gain was only 14 g in 2 weeks. The addition of vit. B_{12} alone to the diet supported maximum growth (56 g in 2 wks), whereas the addition of folacin alone was without benefit.

As a methyl donor for the synthesis of methionine from homocystine, betaine was as effective as choline, mole per mole (Table III), in the presence of vit. B_{12} and folacin. When the diet containing 0.08% choline chloride was supplemented with 0.04% betaine

TABLE IV. Role of Vit. B_{12} and Folacin on Synthesis of Choline and Methionine from Betaine, Aminoethanol and Homocystine.

Dietary supplement						
Betaine HCl, %	Amino-ethanol, %	Vit. B_{12}, μg/kg	Folacin, mg/kg	No. of rats	Avg body wt gain, g/2 wk	Incidence renal damage, %
.32	0	0	0	4	1	100
.32	0	60	2	8	25	62
.32	.03	0	0	4	−1	100
.32	.03	60	0	8	0	100
.32	.03	0	2	4	0	100
.32	.03	60	2	8	40	0
.35	0	0	0	4	8	100
.35	0	60	2	8	32	25
.35	.04	0	0	8	6	100
.35	.04	60	0	4	31	50
.35	.04	0	2	4	3	100
.35	.04	60	2	8	42	0
.41	0	0	0	4	9	100
.41	0	60	2	4	34	25
.41	.05	0	0	4	8	100
.41	.05	60	0	4	32	0
.41	.05	0	2	4	15	50
.41	.05	60	2	4	45	0
.60	0	0	0	4	26	25
.60	0	60	0	4	39	0
.60	0	60	2	4	43	0
.60	.06	0	0	4	33	0
.60	.06	60	0	4	42	0
.60	.06	0	2	4	30	0
.60	.06	60	2	4	43	0

HCl. growth was equal to that obtained with 0.12% choline chloride. Likewise, 0.08% choline and 0.08% betaine were equally as effective as 0.16% choline.

By using varying levels of betaine to replace an equivalent amount of choline, it was noted that for the replacement of metabolic choline, *per se*, 3 moles of betaine were required to replace 1 mole of choline. When the basal diet was supplemented with vit. B_{12} and folacin, 0.10% to 0.12% choline chloride (Table III) was as effective as 0.32% to 0.41% betaine HCl plus aminoethanol (Table IV) for promotion of growth and protection against renal damage. The synthesis of choline and methionine from betaine, aminoethanol and homocystine is shown by the data in Table IV. Supplementing the basal diet with betaine or betaine and aminoethanol failed to support growth and prevent renal hemorrhage. The addition of vit. B_{12}, folacin and 0.32% or 0.41% betaine resulted in an incidence of renal damage of 62 or 25% respectively. The further addition of aminoethanol gave complete kidney protection and supported near maximum growth. Rats fed the basal diet supplemented with 0.32% betaine HCl and aminoethanol required both vit. B_{12} and folacin for normal development. By increasing the level of betaine to 0.41%, vit. B_{12} (without folacin) protected against renal damage and supported fair growth. As the betaine level was increased to 0.60%, aminoethanol was not required for the prevention of renal damage (Table IV). The addition of vit. B_{12} alone to the diet was as effective in promoting growth as were both vit. B_{12} and folacin.

Discussion. The earlier studies(3), in which it was reported that vit. B_{12} and folacin exerted a sparing action on the choline requirement of rats, can now be interpreted as being the result of the function of these vitamins in the biological synthesis of choline and methionine. By using a diet deficient in choline and methionine, the importance of vit. B_{12} and folacin in the synthesis of choline and methylation of homocystine was demonstrated.

Vit. B_{12} was as effective as a combination of vit. B_{12} and folacin for the support of maximum body weight gain when the diet con-

tained an ample methyl supply (0.16% choline). When the methyl supply was suboptimal (0.08% choline) both vit. B_{12} and folacin were essential. A possible explanation of these data and recent observations(8,12) is that folacin functions primarily in methyl synthesis and vit. B_{12} functions in either the transfer or utilization of labile methyl.

For the methylation of homocystine to methionine, betaine is at least as effective a methyl donor as choline. When animals are fed a diet deficient in choline, 3 moles of betaine are required to replace 1 mole of choline. This substantiates previous reports (13,14) that betaine replaces choline *per se* mainly by virtue of supplying one methyl group per mole of betaine.

Vit. B_{12} and folacin were essential for the synthesis of choline and methionine from aminoethanol, homocystine, and a limited supply of betaine. Under the experimental conditions employed, the amount of biological synthesis of labile methyl or of the utilization of methyl from methanol, or synthesis of the entire choline moiety, was insufficient to meet the entire choline requirement necessary for the prevention of renal hemorrhage in the *weanling rat.*

Summary. The role of vit. B_{12} and folacin in the biosynthesis of choline and methionine has been studied and the following conclusions are warranted: 1. In the presence of adequate methyls (from choline), vit. B_{12} alone was as effective for methionine synthesis as when combined with folacin. When fed alone, folacin was ineffective. 2. In the presence of a limited supply of methyl groups, both folacin and vit. B_{12} were essential for the normal development of weanling rats. 3. Vit. B_{12} and folacin were essential for the synthesis of choline and of methionine from aminoethanol, homocystine, and a limited supply of betaine. 4. As a methyl donor for the synthesis of methionine from homocystine, betaine was at least as effective as choline, mole per mole. 5. Under the experimental conditions employed, the rate of synthesis of the methyl group or utilization of methanol for the methylation of homocystine to methionine and aminoethanol to choline was not fast enough to support normal development of *weanling rats.*

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Changes in Acetylcholine Content of the Brain During Exposure to Cold. (18883)

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That acetylcholine (ACh) may play a role in the temperature regulatory mechanism of the mammalian organism and that its effect might be modified by changes in body temperature are suggested by the findings of several investigators. Thus, White(1) in 1929 reported that atropine (an anticholinergic drug) in moderate or excessive amounts produced a marked hyperthermia in infants and children. On the contrary, Burn and Dutta(2) showed that the body temperature of mice is lowered after atropine administration. Grosse-Brockhoff(3) reported therapeutic effects of atropine in hypothermia by diminish-

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