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Liver Microsomal Phosphatidyl Choline Biosynthesis in Choline Deficiency[1] (37940)

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Phosphatidyl choline biosynthesis in liver microsomes is known to occur by two major different pathways. The Kennedy (1) pathway involves choline phosphotransferase which catalyzes the following reaction: cytidine diphosphocholine + α,β-diglyceride to form phosphatidyl choline + CMP. The Greenberg (2) pathway involves phosphatidyl ethanolamine methyltransferase which catalyzes the following reaction: phosphatidyl ethanolamine + *S*-adenosyl methionine to form phosphatidyl choline. In this report the enzymatic activity of choline phosphotransferase and phosphatidyl ethanolamine methyltransferase has been determined in liver microsomes from choline-deficient. animals.

Material and Methods. Female Albino rats of Sprague–Dawley strain weighing 140–160 g were divided into three groups. Group I was fed Purina Laboratory chow. Group II served as controls and was fed purified 25% casein-5% fat diet (3) for 7 days. The animals of Group III were fed 5% casein-5% fat diet (4, 5) plus 1% guanidoacetic acid for 7 days to produce a choline deficiency. The fat was Crisco (Procter and Gamble Co., Cincinnati, OH). Wire-bottomed cages were used to house individual rats, and the rats were allowed free access to food and water. The fatty acid composition of the laboratory chow and fat used in the diet has been determined (5).

Female Sprague–Dawley rats (Sprague-Dawley, Inc., Madison, WI) and female Buffalo rats (Simonsen Lab. Inc., Gilroy, CA) were fed Purina laboratory chow. The female guinea pigs (Gopher State Caviary, St. Paul, MN) received Purina guinea pig chow.

Phosphatidyl choline biosynthesis. The materials used were ^{14}C-methyl-*S*-adenosyl methionine (New England Nuclear Corp., Boston, MA); unlabeled *S*-adenosyl methionine (Calbiochem, Los Angeles, CA); L-distearoyl-α-glycerylphosphoryl-*N*,*N*-dimethyl ethanolamine (Schwarz-Mann, Orangeburg, NY); cytidine diphosphate-1,2-^{14}C-choline (ICN Tracerlab Chemical and Isotopes Division, Irvine, CA); and Tween-20 (Sigma Chemical Co., St. Louis, MO). Diglycerides were prepared from egg lecithin by the method of Gurr *et al.* (6) and purified by the chromatography method of Barron and Hannahan (7).

At the end of the dietary regimen, the control and choline-deficient rats were killed by decapitation, livers removed, rinsed with cold water, blotted, weighed, and homogenized with ice-cold 0.25 *M* sucrose in a Potter–Elvehjem homogenizer with a Teflon pestle. The microsomal fractions was isolated by differential centrifugation (8). The nuclear and mitochondrial fractions were separated from the homogenate by centrifuging for 10 min at 14,500*g*. The supernatant solution was centrifuged at 78,450*g* for 45 min to sediment the microsomal pellet. Protein was determined by a modified Biuret method (9).

Choline phosphotransferase assay. The

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assay of the reaction catalyzed by the enzyme CDP-choline:1,2-diglyceride choline phosphotransferase (EC 2.7.8.2) was done by the method of Kennedy (10). Each reaction mixture contained 50 μmoles Tris–HCl (pH 8.0), 2 μmoles 1,2-diglyceride emulsified on 0.1 ml 1% Tween-20, 10 μmoles $MgCl_2$, 0.5 μmole CDP-1,2-^{14}C-choline (sp act 4×10^5 cpm/μmole), and 10 mg of microsomal protein. The final volume of the reaction mixture was 1.3 ml. The reaction time was 6 min.

Phosphatidyl ethanolamine methyltransferase assay. The assay of the reaction catalyzed by the enzyme phosphatidyl ethanolamine *S*-adenosylmethionine methyltransferase (EC 2.1.1.c) was done by the method of Rehbinder and Greenberg (11) and used L-distearoyl-α-glycerylphosphoryl-*N*,*N*-dimethylethanolamine as substrate. Each reaction mixture contained 1 μmole L-distearoyl-α-glycerylphosphoryl-*N*,*N*-dimethyl ethanolamine emulsified in 1 ml of 0.2 *M* Tris–HCl (pH 8.6) containing 0.4% deoxycholate, 0.2 μmole *S*-adenosyl-L-methionine-methyl^{14}C (sp act 2.3×10^5 cpm/μmole), and 6 mg of microsomal protein. The final volume of the reaction mixture was 1.7 ml. The reaction time was 10 min.

Results and Discussion. Figures 1 and 2 give the linearity of the assay of the choline phosphotransferase and phosphatidyl ethanolamine methyltransferase of liver microsomes. It is apparent from the data in Table I that in liver microsomes from animals fed a choline-deficient diet there is a significant decrease in the activity of the phosphatidyl ethanolamine methyltransferase when compared to controls fed a 25% casein diet. This substantiates the finding of an impairment in the conversion of phosphatidyl ethanolamine to phosphatidyl choline in choline deficiency which was noted with a five fold decrease in the incorporation of 1,2-^{14}C-ethanolamine into phosphatidyl choline fractions 1 and 2 (12). The biosynthesis of phosphatidyl choline fractions 1 and 2, which contain the highly polyunsaturated fatty acids (13), represents the Bremer–Greenberg pathway (2). The phosphatidyl ethanolamine methyltransferase activity in liver microsomes of the guinea pig is one-tenth of that seen in the Sprague–Dawley rats or is 9.6 times less when expressed per gram of liver (Tables I and II). It has been demonstrated that liver microsomal phosphatidyl choline fractions 1 and 2 of the guinea pig which are synthesized by the Bremer–Greenberg (2) represent only 5% of the total phosphatidyl choline-P and that 90% of the total phosphatidyl choline-P is found in phosphatidyl choline fractions 3 and 4 (14) which are synthesized by the CDP-choline pathway (1). The

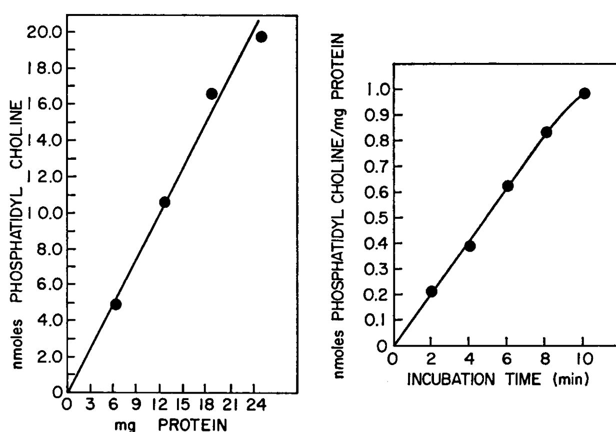

FIG. 1. Choline phosphotransferase assay. The reaction mixtures consisted of the following components in a total volume of 1.3 ml; 50 μmoles Tris–HCl (pH 8.0); 2.0 μmoles 1,2-diglyceride (egg) emulsified in 0.1 ml of 1% Tween, 20; 0.5 μmole CDP-1,2-^{14}C-choline (sp act 4.5×10^5 cpm/μmole); 10 μmoles $MgCl_2$; and 10 mg microsomal protein.

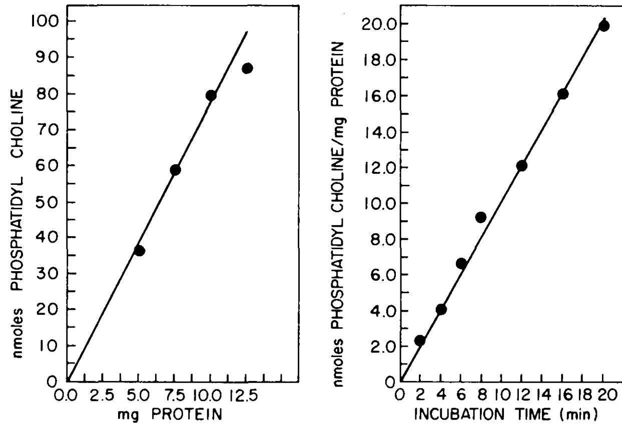

FIG. 2. Phosphatidyl ethanolamine methyltransferase assay. The reaction mixture consisted of the following components in a total volume of 1.7 ml; 1.0 μmole L-Distearoyl-α-glycerylphosphoryl-*N*,*N*-dimethyl ethanolamine emulsified in 1.0 ml of 0.2 *M* Tris–HCl (pH 8.6) containing 0.4% deoxycholate; 0.2 μmole *S*-adenosylmethionine-methyl-^{14}C (sp act 3.3×10^5 cpm/μmole), and 6 mg microsomal protein.

TABLE I. The Effect of Choline Deficiency on the Choline Phosphotransferase and Phosphatidyl Ethanolamine Methyltransferase Activity in Liver Microsomes of Female Rats[a]

		Enzyme activity (nmoles/min/mg protein) × 10	
Diets	Number of animals	Choline phosphotransferase	Phosphatidyl ethanolamine methyltransferase
Purina lab chow	4	1.77 ± 0.32	6.43 ± 1.98
25% casein-5% fat	5	0.46 ± 0.25**	5.17 ± 3.24
5% casein-5% fat + 1% guanidoacetic acid	5	0.32 ± 0.28	2.33 ± 1.67*

[a] Numbers preceded by ± are standard deviations. The test of significance was applied between mean values of control (25% casein-5% fat) and choline deficient (5% casein-5% fat + 1% guanidoacetic acid). Probability for chance occurrence of this difference was $*P < 0.02$. The test of significance was applied between the mean values of the animal fed the 25% casein-5% fat diet and the Purina Lab Chow. Probability for chance occurrence of this difference was: $**P < 0.01$.

TABLE II. Choline Phosphotransferase and Phosphatidyl Ethanolamine Methyltransferase Activity in Liver Microsomes of Different Female Species[a]

		Enzyme activity (nmoles/min/mg protein) × 10	
Species	Number of animals	Choline phosphotransferase	Phosphatidyl ethanolamine methyltransferase
Buffalo rats	4	1.85 ± 0.32	4.99 ± 0.80
Guinea pigs	2	1.14 ± 0.12	0.69 ± 0.27

[a] Numbers preceded by ± are standard deviations. Animals were fed Purina Laboratory Chow.

activities of phosphatidyl ethanolamine methyltransferase and choline phosphotransferase in liver microsomes from the Buffalo rat are similar to that seen in the Sprague–Dawley rat. However, the Buffalo rat has been shown to have increased potential to develop a fatty liver after ethanol ingestion (15). A genetic defect in the metabolism of hepatic one-carbon metabolism was postulated and a decrease formiminotransferase activity has been demonstrated in the Buffalo rat (16).

The choline phosphotransferase activity in the liver microsomes of the choline-deficient animals fed a 5% casein-5% fat diet which contains no choline is similar to that observed in animals fed a 25% casein-5% fat diet (Table I). The 25% casein diet is similar to Purina laboratory chow; however, it does not contain any choline. The choline phosphotransferase activity in the liver microsomes of the rats fed the 25% casein diet with no choline is reduced by 74% when compared to the animals fed the Purina laboratory chow containing 23% protein and 2.4 mg choline/g of diet (Table I). This observation may suggest that dietary choline might induce or regulate the choline phosphotransferase activity. Lysolecithin, a degradation product of phosphatidyl choline, stimulates the enzyme phosphorylcholine cytidyltransferase which catalyzes the formation of CDP-choline and thus may represent a positive feedback mechanism for the control of phosphatidyl choline biosynthesis (17). Choline oxidase, the enzyme involved in degradation of choline, is inhibited by fat when the liver cell becomes fatty (18). The liver cell must have a regulatory mechanism for cellular choline. When choline is needed for the formation of phosphatidyl choline, less is degraded.

Summary. In choline deficiency, there is a decrease in activity of the phosphatidyl ethanolamine methyltransferase of liver microsomes. There is an impairment in the conversion of phosphatidyl ethanolamine to phosphatidyl choline *via* the Bremer-Greenberg pathway. The phosphatidyl choline synthesized *via* the Kennedy pathway shows that the choline phosphotransferase activity is unaltered in animals fed a diet made choline deficient by feeding 5% casein-5% fat and 1% guanidoacetic acid when compared to controls fed a 25% casein-5% fat diet. However in animals fed Purina laboratory chow which contains 2.4 mg choline/g diet, the choline phosphotransferase activity is 3.8 times greater than the values from rats fed the 25% casein-5% fat diet with no dietary choline.

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