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Choline Phosphotransferase and Phosphatidyl Ethanolamine Methyltransferase Activities in Spleen Microsomes of Mice Infected with Friend Virus[1] (38203)

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Increased phospholipid synthesis has been observed with a number of viral infections (1–4). Shope virus was first reported to stimulate phospholipid synthesis in rabbit papilloma (1). Phosphatidyl choline is the major phospholipid in the membranes of the cell, plasma membrane (5), mitochondria and microsomes (6). This lipid represents 48.5% of the total lipid-P of microsomes (6). Phosphatidyl choline biosynthesis in microsomes is known to occur by 2 major different pathways. The Kennedy (7) pathway involves choline phosphotransferase which catalyzes the following reaction: cytidine diphosphocholine + α,β-diglyceride to form phosphatidyl choline + CMP. The Greenberg (8) 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 spleen microsomes of mice during the development of tumors by Friend virus. Friend virus was discovered in 1957 as a murine leukemia infecting spleen tissue (9) and is a RNA virus (10).

Material and Methods. Sprague–Dawley, BALB/c male mice were divided into 2 groups and fed Purina Laboratory Chow *ad libitum*. Group I served as controls. The animals of Group II were infected with Friend virus by intraperitoneal injection of 0.2 ml of virus as a 33% cell-free extract of spleen obtained from 14 day old infected animals. The animals were killed by decapitation at 5, 10, 14 and 21 days following viral inoculation. The spleen was removed, rinsed with cold water, blotted, weighed, pooled and homogenized with ice-cold 0.25 *M* sucrose in a Potter–Elvehjem homogenizer with Teflon pestle. The microsomal fraction was isolated by differential centrifugation (11). 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 (12).

Choline phosphotransferase assay. The 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 (13). The materials used were cytidine diphosphate-1,2-^{14}C-choline (ICN Tracerlab Chemical and Isotope Division, Irvine, California) and Tween-20 (Sigma Chemical Co., St. Louis, Missouri). Diglycerides were prepared from egg lecithin by the method of Gurr *et al.* (14) and purified by the chromatography method of Barron and Hannahan (15). Each reaction mixture contained 50 μmoles Tris–HCl (pH 8.0), 2 μmoles 1,2-diglycerides emulsified in 0.1 ml of 1% Tween 20, 10 μmoles $MgCl_2$, 0.5 μmoles CDP-1,2-^{14}C-choline (specific activity, 4×10^5 cpm/μmole) and 10 mg microsomal protein. The

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final volume of the reaction mixture was 1.3 ml. The reaction time was 6 min.

Phosphatidyl ethanolamine methyltransferase assay. The assay of the enzyme phosphatidyl ethanolamine *S*-adenosyl methionine methyltransferase (EC 2.1.1.c) was done by the method of Rehbinder and Greenberg (14) and used L-distearoyl-α-glyceryl phosphoryl-*N*,*N*-dimethylethanolamine as substrate. The materials used were ^{14}C-methyl-*S*-adenosyl methionine (New England Nuclear Corp., Boston, Massachusetts); unlabeled *S*-adenosyl methionine (Calbiochem, Los Angeles, California) and L-distearoyl-α-glycerylphosphoryl-*N*,*N*-dimethylethanolamine (Schwarz-Mann, Orangeburg, New York). Each reaction mixture contained 1 μmole L-distearoyl-α-glycerylphosphoryl-*N*,*N*-dimethylethanoline emulsified in 1 ml of 0.2 *M* Tris–HCl (pH 8.6) containing 0.4% deoxycholate, 0.2 μmoles *S*-adenosyl-L-methionine-methyl-^{14}C (specific activity, 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.

Biosynthesis Phosphatidyl Choline Fractions. The specificity of the incorporation of 1,2-^{14}C-choline and 1,2-^{14}C-ethanolamine into the phosphatidyl choline fractions as a means of measuring these two biosynthetic pathways of lecithin synthesis has been determined (17). Phosphatidyl cholines of fractions 1 and 2 are chiefly incorporated from 1,2-^{14}C-ethanolamine and provide a lecithin rich in polyunsaturated fatty acids (17). The phosphatidyl cholines of fractions 3 and 4 are mainly synthesized by the 1,2-^{14}C-choline pathway (17). Choline-1,2-^{14}C (sp. act. 3.7 mCi/mmole) and ethanolamine-1,2-^{14}C (sp. act. 3.51 mCi/mmole) were purchased from Mallinckrodt Nuclear, St. Louis, MO.

Additional mice were inoculated intraperitoneally with Friend virus and killed after 14 days. One, 2 and 3 hrs before the animals were killed the mice were injected intraperitoneally with 3.33 μCi/100 g of body weight of the isotopic compounds. The spleens were removed and microsomes prepared by differential centrifugation (11) and lipids extracted by the method of Folch, Lees and Stanley (18). Phosphatidyl cholines were isolated from the lipid extract by thin-layer chromatography by the method of Parker and Peterson (19). Fractionation of the phosphatidyl choline fractions was carried out by thin-layer chromatography on silica gel H impregnated with silver nitrate by the method of Arvidson (20). The phosphatidyl choline fractions were extracted from the gel and the lipid phosphorus (21) and radioactivity were determined. The details of the methods were previously reported (22). All radioactivity measurements were made in a Packard Tri-Carb liquid scintillation counter. Specific activity is expressed as counts per min per microgram of lipid phosphorus.

Results and Discussion. There are a number of indications that the invasiveness of cancer cells may be related to the characteristics of the cell surface membranes (23–26). It is now known that phospholipids are essential components of all cell membranes including both plasma membrane and the highly specialized membranes of the mitochondria and endoplasmic reticulum. Phosphatidyl choline is the major phospholipid in these membranes and is synthesized in the microsomes. The enzymatic activity of choline phosphotransferase and phosphatidyl ethanolamine methyltransferase was shown to be linear with time and concentration of enzyme (27). Figure 1 gives the enzymatic activity of choline phosphotransferase and phosphatidyl ethanolamine methyltransferase of the microsomes of spleen from control and infected mice with Friend virus at 5, 10, 14 and 21 days. There is a marked stimulation of the choline phosphotransferase activity in the infected spleens as shown by the specific activities. The peak of enzymatic activity at 5 days following inoculation of the virus is similar to the peak of virus titer reported by Budillon *et al.* (28). The average weight of the control spleens was 81 mg. The spleens of the viral infected mice in 5 days had increased 5 times in weight and in 10 days had reached the maximum, an increase of 17 times in weight. The viral infection stimulates to a greater degree the phosphatidyl choline pathway involving the CDP-

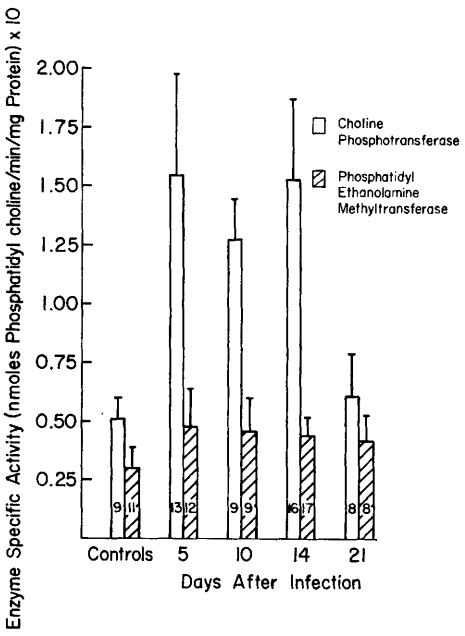

FIG. 1. Choline phosphotransferase and phosphatidyl ethanolamine methyltransferase specific activities (nmoles/min/mg protein) × 10 of spleen microsomes from control and Friend virus infected BALB/c male mice at various time intervals following inoculation of the virus. Reaction mixtures are given in *Material and Methods*. The vertical lines intersecting the tops of the bars indicate values for standard deviations of means. The number of animals are indicated in each bar.

choline:α,β-diglyceride. This pathway would provide phosphatidyl cholines which would be low in polyunsaturated fatty acids for the membranes of the endoplasmic reticulum, mitochondria and plasma membrane (17). Table I gives the total whole spleen microsomal activity of the 2 enzymes involved in phosphatidyl choline biosynthesis. It is apparent from the data that the viral infection greatly stimulates the choline phosphotransferase. The decrease in activity of the two enzymes involved in lecithin biosynthesis that was observed at 21 days after viral infection occurs when the animals begin to die from rupture of the spleen and metastasis of the tumor. It has been demonstrated that 1,2-^{14}C-choline has a specificity for incorporation into phosphatidyl choline fractions (17) and represents a measurement of the Kennedy pathway (7). It is

TABLE I. Total Enzymatic Activity of Choline Phosphotransferase and Phosphatidyl Ethanolamine Methyltransferase of Whole Spleen Microsomes of Mice Infected with Friend Virus.[a]

Time after infection, days	Choline phosphotransferase	Phosphatidyl ethanolamine methyl-transferase
	(nmoles phosphatidyl choline/min) × 10	
0	0.7 ± 0.1	0.4 ± 0.2
5	28.0 ± 4.2	10.0 ± 3.9
10	37.0 ± 10.0	13.1 ± 4.8
14	43.4 ± 11.7	13.2 ± 3.8
21	17.7 ± 6.3	12.8 ± 6.6

[a] Numbers preceded by ± are standard deviations.

apparent from the data in Table II that there is a greater incorporation of 1,2-^{14}C-choline into the total phospholipids, total lecithin and the phosphatidyl choline fractions in the spleens of the viral infected animals than controls. The specific activities of the phosphatidyl choline fractions are greater in the incorporation data from 1,2-^{14}C-choline than from 1,2-^{14}C-ethanolamine. This data would suggest that the CDP-choline:α,β-diglyceride pathway is more active in the viral infected animals. The incorporation data and the greatly stimulated choline phosphotransferase activity would support the idea that the biosynthesis of phosphatidyl choline by the CDP-choline:α,β-diglyceride reaction is stimulated by the Friend virus.

The existence of 2 pathways of phosphatidyl choline biosynthesis provides a source of different lecithin molecules for the normal function and integrity of the membranes of the cell. Friend virus greatly stimulates the CDP-choline:α,β-diglyceride reaction, the choline phosphotransferase. These phospholipid changes in the membranes of the cells that are due to the viral infection may help to alter the characteristics of the cell membrane and thus be part of the malignant process that is seen in the cell in the production of viral tumors.

Summary. Choline phosphotransferase and phosphatidyl ethanolamine methyltransferase enzymatic activities (nmoles phos-

TABLE II. Incorporation of 1,2-^{14}C-Choline and 1,2-^{14}C-Ethanolamine into Total Phospholipids, Total Phosphatidyl Choline and Phosphatidyl Choline Fractions in Normal and Friend Virus Infected Mouse Spleens.[a]

Compound injected	Time after injection of isotopic compounds, hr.	Specific activity (cpm/μgP)					
		Total phospholipids	Total lecithin	Phosphatidyl choline fractions			
				1	2	3	4
1,2-^{14}C-Choline	1	3.90 ± 0.93	9.15 ± 1.18	0.43 ± .24	3.48 ± .48	7.09 ± .27	7.66 ± 1.3
		(9.30 ± 5.6)	(17.30 ± 10.70)	(11.1 ± 3.20)	(12.5 ± 9.50)	(15.8 ± 9.6)	(6.9 ± 3.3)
	2	(4.46 ± .38)	9.83 ± 1.22	6.38 ± .59	5.99 ± 1.32	8.03 ± .83	0.5 ± .02
		(7.0 ± .90)	(15.40 ± 4.90)	(13.0 ± 3.10)	(13.9 ± 5.1)	(13.8 ± 5.2)	(3.6 ± 2.8)
	3	4.95 ± .58	10.99 ± 1.51	9.07 ± 1.5	7.89 ± .31	8.72 ± .44	0.6 ± .06
		(26.0 ± 19.5)	(57.5 ± 8.20)	(14.30 ± 1.30)	(6.2 ± 2.5)	(15.9 ± 2.4)	(13.9 ± 5.8)
1,2-^{14}C-Ethanolamine	1	1.80 ± .30	0.22 ± .03	1.38 ± 4.0	2.12 ± .89	.58 ± .03	.40 ± .07
		(3.12 ± .2)	(.58 ± .07)	(.53 ± .06)	(.46 ± .07)	(.44 ± .06)	(.45 ± .09)
	2	2.21 ± .82	.28 ± .02	.40 ± .01	.12 ± .01	.12 ± .05	.13 ± .01
		(7.54 ± 2.0)	(.90 ± .3)	(2.21 ± .21)	(.63 ± .24)	(.61 ± .29)	(.44 ± .04)
	3	2.86 ± .38	.50 ± .18	.40 ± .09	.22 ± .04	.12 ± .04	.10 ± .05
		(7.60 ± 3.4)	(3.84 ± 3.2)	(4.94 ± 2.20)	(4.99 ± 3.0)	(3.49 ± 3.3)	(1.1 ± .63)

[a] Numbers preceded by ± are standard deviations. Values in parentheses are the viral infected spleens.

phatidyl choline/min/mg protein) have been determined in spleen microsomes of Friend virus infected BALB/c male mice at 5, 10, 14 and 21 days following inoculation of the virus. There is marked stimulation of the choline phosphotransferase activity in the virus infected spleens. There is less stimulation of the phosphatidyl ethanolamine methyltransferase.

The incorporation of 1,2-^{14}C-choline and 1,2-^{14}C-ethanolamine into the total phospholipid-P, total lecithin-P, and the phosphatidyl choline-P fractions of spleen microsomes at 1, 2 and 3 hrs after intraperitoneal injection of the isotopic compounds in 14 day old viral infected mice was studied. There was a greater incorporation of 1,2-^{14}C-choline into the total phospholipid-P, total lecithin-P and phosphatidyl choline fractions in the microsomes of the viral infected spleens than in control mice. Friend virus greatly stimulates the choline phosphotransferase which catalyzes the reaction of CDP-choline-α,β-diglyceride to form phosphatidyl choline.

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