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Studies *in vivo* and *in vitro* on the Methylation of Phosphatidyl-*N*,*N*-dimethylethanolamine to Phosphatidylcholine in Rat Liver

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Summary: The transformation of specifically labelled phosphatidyl-*N*,*N*-dimethylethanolamine was investigated *in vivo* in the rat and in experiments *in vitro* with subcellular fractions of rat liver.
Shortly after the intravenous administration of the substrate, two thirds of the radioactivity recovered was found in the lecithin fraction. The isotope ratio in lecithin demonstrates that: 1) the entire phosphatidyl-*N*,*N*-dimethylethanolamine molecule enters the cell, and 2) the quaternization of the dimethylamino group takes place readily.
Experiments *in vitro* showed that this reaction is catalyzed by a methyltransferase bound to the endoplasmic reticulum of the liver cell which uses *S*-adenosylmethionine as a methyl group donor. The reaction velocity is influenced by the structure of the substrate. Substrates with a higher degree of unsaturation are methylated more rapidly. The phosphatidyl residue appears to determine the substrate specificity, since ceramide phosphoryl-*N*,*N*-dimethylethanolamine is not methylated. Parallel experiments *in vivo* using phosphatidylethanolamine with the same fatty acid composition indicate that the transformation of phosphatidylethanolamine to phosphatidylcholine is very active.

Untersuchungen in vivo und in vitro zur Methylierung von Phosphatidyl-N,N-dimethyläthanolamin zu Phosphatidylcholin in der Rattenleber

Zusammenfassung: Die Umwandlung von spezifisch markiertem Phosphatidyl-*N*,*N*-dimethyläthanolamin im Ganztier und im Experiment *in vitro* wurde in der Ratte bzw. mit subzellulären Fraktionen der Rattenleber untersucht. Nach intravenöser Injektion wurden zwei Drittel der wiedergewonnenen Radioaktivität in der Lecithinfraktion nach kurzer Zeit wiedergefunden. Das Isotopenverhältnis im Lecithin beweist, daß 1. das Substrat Phosphatidyl-*N*,*N*-dimethyläthanolamin als Gesamtmolekül in die Zelle aufgenommen wurde, 2. eine sehr rasche Quaternisierung der Dimethylaminogruppe erfolgte.
Versuche *in vitro* zeigten, daß diese Reaktion an ein Enzym des endoplasmatischen Reticulums der Leberzelle gebunden ist und *S*-Adenosylmethionin als Methyl-Donator verwendet. Die Reaktionsgeschwindigkeit wird durch die Struktur des Substrats beeinflußt. Höher ungesättigte Substrate werden bevorzugt methyliert. Der Phosphatidylrest bestimmt die Substratspezifizität, da Ceramidphosphoryl-*N*,*N*-dimethyläthanolamin nicht methyliert wird. Parallele Experimente *in vivo* mit Phosphatidyläthanolamin von der gleichen Fettsäurebesetzung zeigten, daß die Methylierung von Phosphatidyläthanolamin zu Phosphatidylcholin sehr wirksam erfolgt.

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Enzymes:

Cholinephosphotransferase, CDPcholine:1,2-diglyceride cholinephosphotransferase (EC 2.7.8.2)
Phospholipase A, phosphatide acyl-hydrolase (EC 3.1.1.4).

Several pathways have been elaborated for the biosynthesis of phosphatidylcholine. The biosynthesis *via* diglycerides and CDPcholine catalyzed by the cholinephosphotransferase first studied by Kennedy and Weiss[1] seems to be of major importance in animal tissues. The successive methylation of phosphatidylethanolamine by *S*-adenosylmethionine, as found by Bremer *et al.*[2] in the liver, is the sole biosynthetic pathway of phosphatidylcholine in many microorganisms[3,4]. Studies *in vivo*[5] have indicated that the methylating system is of considerable quantitative importance in rat liver, although it seems to play a minor role in extra-hepatic tissues. An additional pathway to phosphatidylcholine is a calcium-stimulated base exchange utilizing free choline[6]. The significance of this reaction is unclear.

The stepwise methylation of phosphatidylethanolamine to phosphatidylcholine is assumed to be catalyzed by a single enzyme[7]: *S*-adenosylmethionine-phosphatidylethanolamine methyl transferase. A mutant strain of *Neurospora crassa* was first shown to accumulate the two products of the reaction: phosphatidyl-*N*-methylethanolamine and phosphatidyl-*N,N*-dimethylethanolamine[8]. The latter was also shown to be an active component of the surfactant of the pulmonary parenchyma in mammals[9]. Recently Di Augustine[10] studied the rate of incorporation of [^{32}P]- and [^{14}C]palmitate in rat lungs. They are rapidly incorporated into lung phosphatidyl-*N,N*-dimethylethanolamine. Phosphatidyl-*N,N*-dimethylethanolamine appears to be an important biosynthetic end product. The methyl transfer to this product in the biosynthesis of phosphatidylcholine in the lung is negligible.

Most results so far were obtained with labelled choline or methionine. In this paper we wish to report the metabolic fate of several species of phosphatidyl-*N,N*-dimethylethanolamine in the rat *in vivo* and *in vitro*. Comparisons are made with phosphatidylethanolamine, phosphatidylcholine and ceramide 1-phosphoryl-*N,N*-dimethylethanolamine.

Results

1. Studies in vivo

1-[9,10,12,13-$^{3}H_4$]linoleoyl-2-[1-^{14}C]linoleoyl-*sn*-glycero-3-phosphoryl-*N,N*-dimethylethanolamine, spec. radioactivity 9.02×10^6 dpm ^{3}H and 0.235×10^6 dpm ^{14}C/μmol (^{3}H/^{14}C ratio 38.4:1) was administered intravenously to young rats (150 to 200 g) at 0.5 mg/g body weight. The degradation of the substrate was followed by measuring the respiratory $^{14}CO_2$ as described before[11]. After 6 h, the total respiratory $^{14}CO_2$ amounted to 16.5% of the administered radioactivity. The rate of release of $^{14}CO_2$ slowly decreased after this time period. After 24 h, about 60% of the administered ^{14}C radioactivity was recovered as CO_2. A comparable result was obtained with dilinoleoylphosphatidylcholine administered under the same experimental conditions[12].

After 6 h and 12 h, the animals were sacrificed, liver, lungs, kidneys, spleen, brain, heart, muscle and intestine removed and the lipids extracted. The results are given in Table 1.

The distribution of radioactivity in all organs, except liver, was assayed by thin-layer chromatography. Labelled lecithin was detected in all organs, particularly in lungs, spleen, kidneys and intestine. After 6 h, about one half of the recovered radioactivity was found in the neutral lipids and one half in the phospholipids, mainly in the phosphatidyl-*N,N*-dimethylethanolamine and phosphatidylcholine fractions. The ratio of ^{14}C radioactivity of phosphatidylcholine to phosphatidyl-*N,N*-dimethylethanolamine in the above organs ranged from 1:3 to 1:5. After 24 h, phosphatidyl-*N,N*-dimethylethanolamine had completely dis-

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Table 1. Distribution of radioactivity in organs of the rat after intravenous administration of 1-[9,10,12,13-$^3H_4$]linoleoyl-2-[1-^{14}C]linoleoylglycero-3-phosphoryl-*N,N*-dimethylethanolamine.

Radioactivity expressed as ^{14}C dpm.

Amounts injected: 6 h experiment: 100 μmol substrate, 58.75×10^6 dpm ^{3}H, 1.53×10^6 dpm ^{14}C; 24 h experiment: 100 μmol substrate, 54.3×10^6 dpm ^{3}H, 1.43×10^6 dpm ^{14}C, $^3H/^{14}C = 38.2$.

Organs	After 6 h		After 24 h	
	$10^{-3} \times {}^{14}C$ [dpm]	$^3H/^{14}C$	$10^{-3} \times {}^{14}C$ [dpm]	$^3H/^{14}C$
Liver	399	32:1	72.6	34:1
Lungs	17.6	28:1	5	17:1
Spleen	45.8	30:1	3.5	23:1
Kidneys	8.6	22:1	10	28:1
Intestine	38.7	33:1	21	20:1
Heart	1.9	30:1	1	41:1
Muscle	25.1	58:1	97	18:1
Blood	3.3	39:1	3	136:1
Brain	1.3	22:1	1	41:1
Total	541		214	

appeared and the radioactivity was found mostly in the phosphatidylcholine, the neutral lipid fraction, and to a small extent, in the phosphatidylethanolamine fraction (< 10%).

The radioactivity of the lipid extract from liver amounted to 26% of the administered radioactivity after 6 h and was reduced to 5% after 24 h. The liver lipids were separated by silicic acid chromatography into 1) neutral lipids, 2) ceramide, phosphatidic acids and cardiolipin, 3) phosphatidylethanolamine and phosphatidyl-*N,N*-dimethylethanolamine, 4) phosphatidylcholine, 5) lysophosphatidylcholine and sphingomyelin. Table 2 summarizes the distribution of radioactivity in the liver lipids.

The radioactivity in the neutral lipid fraction was found to reside almost exclusively in the triglycerides. In thin-layer chromatography the solvent system chloroform/methanol/conc. NH_4OH 65:25:4 separates the two bases phosphatidylethanolamine and its dimethyl analogue distinctly. No radioactivity could be found in the phosphatidylethanolamine in the 6 h experiment. The $^3H/^{14}C$ ratio in phosphatidyl-*N,N*-dimethylethanolamine and phosphatidylcholine was in both cases close to that of the original material. Phosphatidyl-*N,N*-dimethylethanolamine is extensively used in the biosynthesis of phosphatidylcholine. Two thirds of the radioactivity found in the liver lipids after 6 h resided in the phosphatidylcholine fraction. The phosphatidylcholine fraction was also subjected to phospholipase A hydrolysis. The free fatty acids and lysophosphatidylcholine were separated by silicic acid chromatography and esterified and transesterified respectively. Radio gas chromatographic analyses showed that the radioactivity resided mainly in the 18:2 fatty acid. Furthermore, 80% of the ^{14}C labelled fatty acid was located in the 2 position and 84% of the ^{3}H labelled acid had remained in the 1 position of the glycerol phosphate brackbone.

Phosphatidyl-*N,N*-dimethylethanolamine is rapidly metabolized. After 24 h the radioactivity is distributed in all lipid fractions although more than

Table 2. Distribution of radioactivity in liver lipids after intravenous administration of 1-[9,10,12,13-$^3H_4$]linoleoyl-2-[1-^{14}C]linoleoyl glycero-3-phosphoryl-*N,N*-dimethylethanolamine to the rat.

Amount injected: see Table 1.

	After 6 h			After 24 h		
	$10^{-3} \times {}^{14}C$ [dpm]	[%]	$^3H/^{14}C$	$10^{-3} \times {}^{14}C$ [dpm]	[%]	$^3H/^{14}C$
Triglycerides	42.0	10.6	–	23.4	32.2	–
Phosphatidic acids, Cardiolipin	38.0	9.5	–	–	–	–
Phosphatidylethanolamine	–	–	–	8.9	12.3	–
Phosphatidyl-*N,N*-dimethylethanolamine	78.5	19.7	32	8.2	11.3	40
Phosphatidylcholine	240.5	60.2	35	32.1	44.2	35
Total	399.0	100.0		72.6	100.0	

Table 3. Distribution of radioactivity in the liver lipids 6 h after intravenous administration of 1-[9,10,12,13-$^3H_4$]-linoleoyl-2-[1-^{14}C]linoleoylglycero-3-phosphoryl-*N,N*-dimethylethanolamine (4 μmol, 31.7×10^6 dpm ^3H; 0.83×10^6 dpm ^{14}C) and of 1,2-[9,10,12,13-$^3H_4$]*dilinoleoylglycero-3-phosphorylethanolamine* (4 μmol, 65×10^6 dpm ^3H) to the rat.

	Experiment with phosphatidyl-*N,N*-dimethylethanolamine $10^{-3} \times {}^{14}C$ [dpm]	%	Experiment with phosphatidylethanolamine $10^{-3} \times {}^{3}H$ [dpm]	%
Triglycerides	275	15.0	766	20.0
Phosphatidic acids, Cardiolipin	55	3.0	90	2.4
Phosphatidylethanolamine	–	–	*1260*	*32.9*
Phosphatidyl-*N,N*-dimethylethanolamine	242	13.2	–	–
Phosphatidylcholine	1192	65.0	1702	44.4
Lysophosphatidylcholine	63	3.8	10	0.3
Total	*1827*	*100*	*3828*	*100*

40% of the radioactivity was still located in the phosphorylcholine fraction.

Parallel experiments were also conducted with labelled phosphatidylethanolamine. 1-[9,10,12,13-$^3H_4$]linoleoyl-2-[1-^{14}C]linoleoylphosphatidyl-*N,N*-dimethylethanolamine and 1,2-[9,10,12,13-$^3H_4$]-dilinoleoylphosphatidylethanolamine were administered intravenously to rats. After 6 h the livers were removed and processed in the usual manner. A significantly larger percentage of radioactivity was recovered in phosphatidylcholine in the experiment with phosphatidyl-*N,N*-dimethylethanolamine. In this experiment, the ratio of radioactivities of the two phosphatidyl-*N,N*-dimethylethanolamine/phosphatidylcholine fractions was 1:5, while in the second experiment, the ratio of the radioactivity of phosphatidylethanolamine to that of phosphatidylcholine was only 1:1.3. Neither a demethylation of phosphatidyl-*N,N*-dimethylethanolamine nor a partial methylation of phosphatidylethanolamine could be observed. The results are summarized in Table 3.

The presence of this powerful quaternization system prompted us to extend our studies to the choline methyl group of the sphingomyelin. [4,5-$^3H_2$]-Dihydroceramide-*N,N*-dimethylphosphorylethanolamine (3 μmol, spec. radioactivity 37.6×10^6 dpm ^3H/μmol) was administered intravenously to rats. After 6 h, 30.2×10^6 dpm ^3H (26.8%) was recovered from the liver lipids. Radio thin-layer chromatography showed that this radioactivity was located exclusively in the original substrate: degradation or methylation products were not observed to any appreciable extent. The total liver lipids were subjected to alkaline hydrolysis. More than 90% of the total radioactivity was recovered and found to reside exclusively in the original substrate. This was established by thin-layer chromatography with authentic material.

2. Studies in vitro

The aforementioned studies were extended to experiments *in vitro*. The microsomal fraction was used, since Bremer and Greenberg[13] had shown that the methylating system of phosphatidylethanolamine is an enzyme of the endoplasmic reticulum. Labelled phosphatidyl-*N,N*-dimethylethanolamine was solubilized in Triton X-100 and incubated with *S*-adenosylmethionine and the rat or bovine liver $100000 \times g$ sediment. The lipid extracts were analyzed by thin-layer chromatography together with authentic materials. Fig. 1 summarizes the results of such an experiment.

The rate of methylation is strongly dependent on the concentration of *S*-adenosylmethionine, as shown in Fig. 2.

The concentration of native phosphatidylethanolamine is relatively high; ca. 1 μmol for 5 mg microsomal protein[5]. This could react competi-

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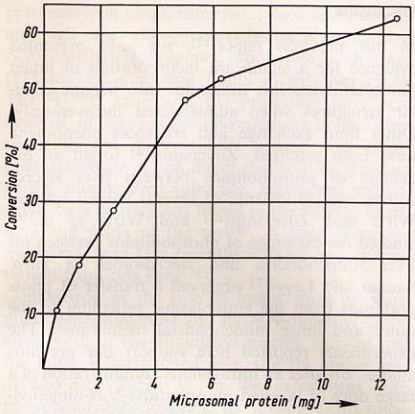

Fig. 1. Conversion of phosphatidyl-*N,N*-dimethylethanolamine to phosphatidylcholine by rat liver microsomes.

Each incubation mixture contained in a volume of 1.0 m*l*: 0.1 μmol 1-[9,10,12,13-$^3H_4$]linoleoyl-2-[1-^{14}C]-linoleoylphosphatidyl-*N,N*-dimethylethanolamine; 1.0 μmol *S*-adenosylmethionine; 100 μmol Tris buffer pH 8.4 and rat liver microsomal protein as given. The incubation lasted for 1 h at 37°C.

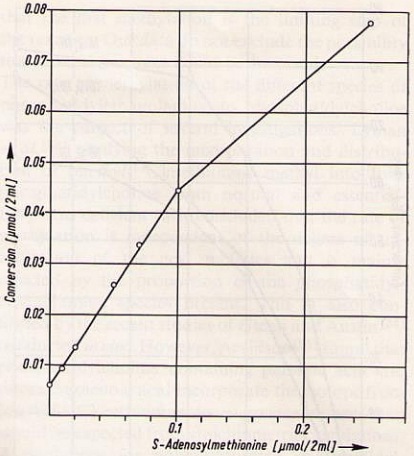

Fig. 2. Dependence of the degree of methylation of phosphatidyl-*N,N*-dimethylethanolamine upon *S*-adenosylmethionine concentration.

The complete incubation mixture contained in a total volume of 2.0 m*l*: 5 mg liver microsomal protein; 0.1 μmol 1-[9,10,12,13-$^3H_4$]linoleoyl-2-[1-^{14}C]linoleoyl-phosphatidyl-*N,N*-dimethylethanolamine; 200 μmol of Tris buffer pH 8.4 and *S*-adenosylmethionine as given. The incubation was carried out at 37°C for 1 h.

tively with the methylating system. In order to verify this effect, an incubation was performed with labelled dilinoleoylphosphatidyl-*N,N*-dimethylethanolamine and increasing amounts of unlabelled dilinoleoylphosphatidylethanolamine. The results are given in Table 4.

As shown in Table 4, concentrations of phosphatidylethanolamine up to 5mM did not significantly affect the rate of conversion of phosphatidyl-*N,N*-dimethylethanolamine (0.1mM) to phosphatidylcholine. Parallel incubations were conducted with 1,2-[9,10,12,13-$^3H_4$]dilinoleoylphosphatidylethanolamine as substrate. The conversion of phosphatidylethanolamine to phosphatidylcholine amounted to only about 1%.

The dependence of the rate of methylation upon the degree of unsaturation of the acyl moieties was studied using three species of labelled phosphatidyl-*N,N*-dimethylethanolamines: 18:2,18:2-; 18:0.18:2- and 18:0,18:1-.

In order to minimize the effect of a possible difference in solubility, care was taken to dissolve

Table 4. Conversion of labelled phosphatidyl-*N,N*-dimethylethanolamine to phosphatidylcholine in the presence of unlabelled phosphatidylethanolamine.

The incubation mixture contained in a total volume of 1.0 m*l*: 5.0 mg liver microsomal protein; 0.1 μmol 1-[9,10,12,13-$^3H_4$]linoleoyl-2-[1-^{14}C]linoleoylphosphatidyl-*N,N*-dimethylethanolamine; 1.0 μmol *S*-adenosylmethionine; 100 μmol Tris buffer pH 8.4 and a concentration of dilinoleoylphosphatidylethanolamine as given. The mixture was incubated for 1 h at 37°C.

Concentration of added phosphatidylethanolamine [mmol/*l*]	% Conversion
0	42.5
0.3	43.0
0.5	44.9
1.0	44.2
5.0	46.0

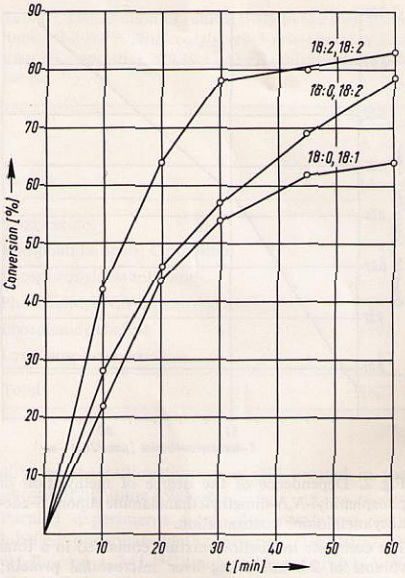

Fig. 3. Dependence of the methylation rate upon the degree of unsaturation of the acyl moieties.

Each incubation mixture contained in a volume of 4.0 ml: 0.5 μmol of substrate: 1-[9,10,12,13-$^3H_4$]-linoleoyl-2-[1-^{14}C]linoleoyl- or 1-[9,10,12,13-$^3H_4$]-stearoyl-2-linoleoyl- or 1-[9,10,12,13-$^3H_4$]stearoyl-2-oleoylphosphatidyl-*N,N*-dimethylethanolamine; 5.0 μmol *S*-adenosylmethionine; 400 μmol Tris buffer pH 8.4 and 10 mg rat liver microsomal protein. The tubes were incubated at 37°C. After the given time intervals 0.5 ml portions were transferred to 2 ml chloroform/methanol 2:1, the lipid mixtures extracted and chracterized by thin-layer chromatography in a system comprising chloroform/methanol/conc. NH_4OH, 65:25:4.

these compounds in Triton X-100. The results are summarized in Fig. 3.

As shown in Fig. 3, a preferential methylation rate was observed with substrates with a higher degree of unsaturation in the acyl moieties. The initial rate of methylation was higher for the 18:2,18:2 species than for the 18:0,18:2 species, the methylation rate of which was, in turn, slightly higher than that of the 18:0,18:1 species.

Discussion

In our previous paper[12], we have presented evidence for a significant incorporation of intact phosphatidylcholine molecules into hepatic cellular structures when administered intravenously. Other lipid exchange and transport phenomena have been reported. Zilversmit[14] found an exchange of phospholipids between liver microsomes and cytoplasma of several animal species. Wirtz and Zilversmit[15] and Wirtz *et al.*[16] studied the exchange of phospholipids between rat liver mitochondria and microsomes *in vitro*. Sauner and Levy[17] observed a transfer of phospholipids from the endoplasmic reticulum to the outer and inner mitochondrial membranes. The experiments reported here support our previous studies. 6 h after an intravenous administration of a large dose of labelled phosphatidyl-*N,N*-dimethylethanolamine to rats, 26% of the radioactivity was recovered in the liver (see Table 1). Almost 90% of this radioactivity was located in the phosphatidyl-*N,N*-dimethylethanolamine and phosphatidylcholine fractions. The $^3H/^{14}C$ ratio of these phospholipids was close to that of the substrate. That the radioactivity in the phospholipids was mainly due to a synthesis *de novo* from the free fatty acid pool could be discounted. Positional analysis of the fatty acids by phospholipase A hydrolysis revealed that, compared with the administered material, 80% of the radioactive label was retained in its original position.

The stepwise *N*-methylation of phosphatidylethanolamine is one of the pathways for the synthesis of phosphorylcholine *de novo*[5]. A rapid conversion of phosphatidyl-*N,N*-dimethylethanolamine to phosphatidylcholine was observed in our experiments. 6 h after an intravenous administration of 100 μmol of dilinoleoylphosphatidyl-*N,N*-dimethylethanolamine to the rat, 3 μmol of the substrate was recovered intact whereas 17 μmol was found as the corresponding phosphatidylcholine in the liver (see Table 2). Phosphatidylcholine was also detected in the other organs,

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particularly in lungs, intestine, spleen and kidneys, although the phosphatidylcholine/phosphatidyl-*N,N*-dimethylethanolamine ratios were much lower (1:3 to 1:5) than that found in the liver (5:1). The $^{3}H/^{14}C$ ratio and the positional fatty acid analysis of the pooled phosphatidylcholine after phospholipase A hydrolysis proved that phosphatidylcholine is the direct methylation product of the administered substrate. Significant methyltransferase activity was found in the kidney, heart, lung and testis of the rat[18]. A lipid *N*-methyl transferase was also recently isolated and characterized from dog lung[19].

Comparison of the rate of methylation of phosphatidylethanolamine and its *N,N*-dimethyl derivative, both of the dilinoleoyl species, showed that the latter is methylated at a slightly higher rate *in vivo* (see Table 3). In the experiment with phosphatidylethanolamine as substrate, no intermediate methylation product was found.

In contrast to the wide occurrence of phosphatidylethanolamine, its sphingomyelin counterpart, ceramide phosphorylethanolamine is present only in a few organisms. It was first found in the lipids of the housefly[20], of rumen protozoa[21] and later in several species of insects and microorganisms[22]. Its *N,N*-dimethyl derivative has never been observed in nature. It would be interesting to see whether a similar methylation reaction could take place. Labelled D-*erythro*-dihydroceramide-1-phosphoryl-*N,N*-dimethylethanolamine was administered intravenously to rats and the liver lipids were analyzed 6 h later. The substrate was incorporated intact into the liver but no sphingomyelin or demethylated product was found.

The methylation reaction was also tested *in vitro* (see Figures 1 and 2). Phosphatidyl-*N,N*-dimethylethanolamine is much more rapidly methylated to phosphatidylcholine than phosphatidylethanolamine of the same acyl species. The methylation reaction is not affected by large concentrations of added phosphatidylethanolamine (see Table 4). This confirms the conclusion reached earlier[18] that the first methylation is the limiting step of the reaction. Our data do not exclude the possibility that several enzymes could be involved.

The rate of methylation of the different species of phosphatidylethanolamine to phosphatidylcholine was the subject of several investigations. Lyman *et al.*[23], studying the incorporation and distribution of [*methyl*-^{14}C]methionine methyl into liver phosphatidylcholine from normal and essential-fatty-acid deficient rats, concluded that the rate of methylation is independent of the degree of unsaturation of the acyl moieties and is mainly affected by the proportion of the phosphatidylethanolamine species present. This is also confirmed by the recent studies of Glenn and Austin[24] conducted *in vivo*. However, Arvidson[25] found that phosphatidylcholine containing palmitic acid and docosahexaenoic acid incorporate the isotope from [*methyl*-^{14}C]methionine to a greater extent than would be expected from stoichiometric calculations. A preference for polyunsaturated phosphatidylethanolamine was shown by Fex[26], a possibility also suggested recently by Lyman *et al.*[27] and Holub and Kuksis[28]. The comparative rate of the last step of the methylation reaction was studied in rat liver microsomal fraction with three phosphatidyl-*N,N*-dimethylethanolamine species: 18:0,18:1-; 18:0,18:2- and 18:2,18:2-. As shown in Fig. 3, the initial rate of methylation increases with the increasing degree of unsaturation: 18:2,18:2- > 18:0,18:2- > 18:0,18:1-. Thus, at least for the last reaction step, the biosynthesis of phosphatidylcholine by the methylation of phosphatidylethanolamine is dependent upon the degree of unsaturation of the acyl moieties. In the intact animal, however, the concentration of the different phosphatidylethanolamine species might be the controlling factor of the pathway leading from phosphatidylethanolamine to lecithin *via* the methyltransferase reaction.

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Experimental

1-[9,10,12,13-$^3H_4$]linoleoyl-2-[1-^{14}C]linoleoyl-*sn*-3-glycerophosphatidyl-*N*,*N*-dimethylethanolamine, spec. radioactivity 9.02×10^6 dpm ^3H; 0.235×10^6 dpm $^{14}C/\mu$mol, and 1-[1-^{14}C]stearoyl-2-[9,10,12,13-$^3H_4$]-linoleoyl-*sn*-3-glycerophosphatidyl-*N*,*N*-dimethylethanolamine, spec. radioactivity 10.0×10^6 dpm ^3H; 0.58×10^6 dpm $^{14}C/\mu$mol, were obtained by partial demethylation of the corresponding phosphatidylcholine with sodium thiophenolate as described earlier [29]. The phosphatidylcholines were synthesized according to de Haas and van Deenen[30]. 1-[9,10,12,13-$^3H_4$]-Stearoyl-2-oleoyl-*sn*-3-glycerophosphatidyl-*N*,*N*-dimethylethanolamine, spec. radioactivity 16×10^6 dpm/μmol was prepared by the acylation of the corresponding lyso compound with oleoyl chloride. The labelled lyso compound was obtained by reduction of 1-linoleoyl-lysophosphatidyl-*N*,*N*-dimethylethanolamine in a tritium atmosphere with palladium on charcoal as a catalyst.

1,2-[9,10,12,13-$^3H_4$]Dilinoleoyl-*sn*-3-glycerophosphatidylethanolamine, spec. radioactivity 1.5×10^6 dpm/μmol was synthesized by coupling the corresponding diacyliodoglycerol with the silver salt of *N*-β,β,β-trichlorethoxycarbonyl-2-aminoethyl phosphoric acid using a modification of the method of Pfeiffer *et al.*[31]. Bovine brain sphingomyelin was partially demethylated to ceramide phosphoryl-*N*,*N*-dimethylethanolamine as described earlier[29]. This was, in turn, reduced in a tritium atmosphere with palladium on charcoal as a catalyst to form the corresponding 4,5-[$^3H_2$]dihydro derivative, spec. radioactivity 37.6×10^6 dpm/μmol. All products were purified by silicic acid chromatography and characterized by thin-layer chromatography, IR, NMR and mass spectra.

For studies *in vivo*, the labelled substrates were solubilized in saline solutions and injected into the tail veins of young male Wistar rats. The animals were kept in metabolic cages as described earlier[11]. Radioactive $^{14}CO_2$ was measured discontinously. The animals were killed by exsanguination, the blood was collected, and the organs immediately removed. Lipid extracts were carried out according to Folch *et al.*[32]. The lipid mixtures were separated by silicic acid chromatography as described before[11]. Phosphatidylethanolamine and phosphatidyl-*N*,*N*-dimethylethanolamine were separated by thin-layer chromatography with the solvent system chloroform/methanol/conc. NH_4OH *65:25:4. The pure phospholipid fractions were dis*solved in ether and subjected to phospholipase A (*Crotalus terr. terr.*, Boehringer Mannheim) hydrolysis overnight. The resulting fatty acids and lysophosphatides were separated by silicic acid chromatography and transesterified with 5% HCl in methanol. The fatty acid methyl esters were analyzed by gas-liquid chromatography, stationary phase 15% ethylene glycol succinate polyester on kieselguhr, column temperature 175°C, column length 200 cm. The radioactivity was monitored discontinously.

For experiments *in vitro*, the substrates were dissolved in Triton X-100 and sufficient buffer was added to obtain a clear solution. Rat or bovine liver $100000 \times g$ *sediment was used. A typical incubation included 5 mg* microsomal protein, 1 μmol *S*-adenosyl methionine (Boehringer Mannheim) and 0.1 μmol substrate, made up with 0.1M Tris buffer pH 8.4 to 1.0 ml. The mixture was incubated at 37°C for 1 h. The reaction was stopped with 2 ml chloroform/methanol 2:1, the lipids extracted and analyzed by thin-layer chromatography together with authentic substances. Microsomal protein was determined by the biuret method. The lipid phosphorus was analyzed by a modification of the method of Fiske and Subbarow[33]. Radiochromatograms were scanned with a Berthold Scanner Model LB 2722 or the Packard Scanner Model 7201. Liquid scintillation counting was performed with the Packard Model 3214 *or 3380.*

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