LECITHIN FORMATION BY METHYLATION OF INTACT PHOSPHATIDYL DIMETHYLETHANOLAMINE

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DME** , a likely intermediate in the biosynthesis of choline, is readily incorporated into tissue phospholipids in vivo (Artom and Crowder, 1949) and in vitro (Crowder and Artom, 1952). After incubation of C^{14} labeled DME with rat liver slices, the isotope is present in the DME as well as in the choline moiety of the phospholipids. The latter finding may be explained by assuming: (a) that labeled DME is first methylated to choline, and this is subsequently incorporated into a phospholipid; or, (b) that labeled phosphatidyl DME is first synthesized and then converted to lecithin by methylation of its DME moiety. Suggestive evidence for the latter interpretation was supplied by experiments in which, after an initial incubation of liver slices with labeled DME, further incorporation of the isotope was inhibited by addition of non-labeled DME, or non-labeled choline (Artom, 1960). More direct evidence in this regard has been obtained now by incubating rat liver homogenates with labeled phosphatidyl DME in the presence, or in the absence, of AdMe.

Labeled phosphatidyl DME was obtained by the action of rat liver homogenates, or microsomes, on DME-2,3-C^{14}. The lipids extracted from the incubation products were chromatographed on a silicic acid-celite column and phosphatidyl DME was eluted with a chloroform-methanol (4:1) mixture, 5 ml. fractions being

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** Abbreviations used: DME for dimethylethanolamine, AdMe for adenosylmethionine.

collected. This phospholipid is eluted much more slowly than phosphatidyl ethanolamine (Artom, 1960). Accordingly, the material in fractions 17-40 only was used for the preparation of the labeled substrate. After hydrolysis of such material, 98% of the radioactivity was recovered in the DME; neither ethanolamine or choline was present in amounts detectable analytically. The material was emulsified in a 0.5% solution of albumin in Ringer and distributed in the incubation flasks.

After incubation, the proteins and lipids were precipitated with colloidal Fe_2O_3 and $MgSO_4$, and the precipitate was washed and extracted with cold ethanol-ether. An aliquot of the lipid extract was plated and counted. The remaining extract, after addition of carrier DME and choline, was hydrolyzed with 6N HCl in methanol. The solvent was removed, the residue was dissolved in water, and after filtration, DME was steam-distilled at pH 10 (Artom, 1957). Under the conditions of our determinations, 90-95% of the DME is recovered in the distillate. From the distillation residue, choline was precipitated as the Reineckate and its radioactivity was determined.

The results of a typical experiment are shown in Table I. The C^{14} contents of the unhydrolyzed lipids were essentially the same in the incubated flasks as in the non-incubated control, suggesting that little or no splitting of phosphatidyl DME occurred during the incubation. Likewise, after hydrolysis of the lipids, practically no isotopic choline was present in the flasks incubated without AdMe. However, when AdMe had been added, half or more of the radioactivity was recovered in the Reineckate isolated from the distillation residue. The presence of isotopic choline in the hydrolyzed lipids of flasks 4-6 (but not of flasks 1-3) was also demonstrated by using paper chromatograms developed with 5% NH_4OH in ethanol. With this solvent, DME migrates much further than choline. No isotope dilution effect was observed when unlabeled DME was added to the incubation flasks, a finding which seems to exclude the possibility that DME might have been first split off, methylated to choline, and finally incorporated into lecithin.

Similar experiments have been carried out also with pigeon liver homo-

Table I

Flask No.	Incubation time (hours)	Non-isotopic additions	C^{14} in lipids		
			Before hydrolysis	After hydrolysis	
				Choline	DME
1	0	None	1,030	0	1,130
2	2.5	None	1,160	38	1,040
3	"	DME	1,070	0	1,020
4	"	AdMe	1,020	500	530
5	"	AdMe	1,070	644	552
6	"	AdMe + DME	910	572	424

Values are expressed as c.p.m. per flask. The figures for the radioactivity in the distillate and in the residue have been corrected for the incomplete yield of DME, as determined on the non-incubated flask. Each flask contained in 5 ml. of Krebs-Ringer-phosphate (pH 7.4), 5 mg. of reduced glutathione, 3 mg. each of penicillin G and dihydrostreptomycin, 0.4 g. of homogenized rat liver, and approx. 1000 c.p.m. of isotopic phosphatidyl DME. Non-isotopic additions included 7 μM of AdMe, and 25 μM of DME. Flasks were incubated in a Warburg bath at 37° in air.

genate. This preparation, unlike rat liver homogenate, or its fractions, exhibits methylating activity, even without added AdMe, or methionine. Thus, after incubation of labeled phosphatidyl DME with pigeon liver homogenate in the absence, or in the presence, of added L-methionine (50 μM), the counts recovered as choline averaged 20%, or, respectively, 32% of the counts in the total lipids.

There seems, therefore, to be little doubt that, under the conditions of our experiments, phosphatidyl DME was converted directly to lecithin by methylation of the DME moiety of the intact phospholipid. This conclusion is in line with the results of Bremer and Greenberg (1960) who have shown that, in rat liver microsomes incubated with AdMe-methyl-C^{14}, labeled phosphatidyl

choline is formed, presumably by methylation of a water-insoluble acceptor present in the microsomes. However, in their experiments, no significant effect was observed by the addition of natural, or synthetic cephalin.

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