EVIDENCE FOR THE IN VIVO INCORPORATION OF 2-AMINO-2-METHYLPROPANOL INTO RAT LIVER PHOSPHOLIPID*

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Wells (1956) first suggested 2-amino-2-methylpropanol (2A2MP)[1] as an inhibitor of choline synthesis. More recently he has reported a decreased rate of phosphatidyl choline formation from methionine and dimethylethanolamine in the presence of this compound (Wells and Remy, 1959; 1960). We have shown that ethanolamine and its N-methyl derivatives prevented fatty livers in weanling male rats being fed a choline deficient diet containing 2A2MP (Longmore and Mulford, 1959). The increased incidence of hemorrhagic kidneys produced by the 2A2MP has been found to be relieved by the N-methyl derivatives of ethanolamine (Outland et al, 1959). Ethanolamine itself led to an increase in the incidence and severity of damaged kidneys (Mulford et al, 1959). In studies on the effect of 2A2MP on phospholipid distribution in the liver of rats, using silicic acid paper chromatography, we observed that livers of animals on 2A2MP contained a new phospholipid component not present in the livers from control rats not receiving the 2A2MP.

These observations have led to a study of the possible incorporation of 2A2MP into phospholipid. Such incorporation might help explain the increase in liver fat and increased incidence of hemorrhagic kidneys in the rat. Stekol, during the discussion of a paper by Wells (1957), mentioned that 2A2MP occurred in tissue phospholipid and that it was apparently methylated by methionine. In the present communication evidence is given

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1 The abbreviation used is: 2A2MP, 2-amino-2-methylpropanol.

which indicates that 2A2MP is incorporated as such into the liver phospholipid of rats.

In all experiments male rats 17-18 days of age of the Sprague-Dawley strain were used. They were fed ad libitum the basal low choline diet, Diet A, used previously (Outland et al, 1959). Two series of experiments were carried out. In one series Diet A containing 10 mg of 2A2MP per gram of food was fed to the rats for five days. At the end of this time the animals were sacrificed by decapitation. In another series of experiments the animals were fed Diet A without 2A2MP for five days. At the end of this time 0.18 mg 2A2MP-1-C^{14} [2] (3.53 uc) was injected intraperitoneally into each rat. Three hours after injection, the animals were sacrificed by decapitation.

Immediately on sacrificing the animals the livers were removed for phospholipid extraction by a procedure similar to that of Hanahan, Dittmer and Worashina (1957). All phospholipids were stored at -18°C under acetone until studied.

Two dimensional silicic acid paper chromatography of the phospholipid fractions was carried out using solvents A and B of Marinetti and Stotz (1956) and the individual phospholipids were detected by Rhodamine-6-G development (Marinetti and Stotz, 1955), as well as by autoradiography where indicated. The chromatograms of the phospholipid fractions obtained from livers of animals receiving 2A2MP always showed a spot that did not appear in the chromatograms of the phospholipid fractions obtained from livers of control animals not receiving 2A2MP. The Rf of this spot was always slightly greater in both dimensions than that of phosphatidyl ethanolamine which was present in both liver phospholipid fractions. The autoradiograms of the phospholipid fraction of livers of animals injected with 2A2MP-1-C^{14} showed that all of the radioactivity was located in the same position relative to phosphatidyl ethanolamine as was the new spot on the chromato-

[2] The chemical synthesis of 2A2MP-1-C^{14} was carried out by esterification of α-aminoisobutyric acid-1-C^{14} with methyl alcohol followed by reduction with $LiAlH_4$.

grams of liver phospholipids from rats fed 2A2MP. Due to the small amount of 2A2MP-1-C^{14} injected, no spot was obtained with Rhodamine-6-G in the area containing the radioactivity.

The phospholipid fractions were hydrolyzed in 6N HCl in a sealed glass tube at 100°C for 6 hours. The hydrolysates were extracted repeatedly with petroleum ether to remove fatty acids and then concentrated in vacuo at room temperature. The products of hydrolysis were separated by one dimensional paper chromatography using the three solvent systems shown in Table 1. Solvents A and B were those used by Levine and Chargaff (1951) and Munier and Macheboeuf (1951), respectively. Solvent C was developed in our laboratory. Non-radioactive 2A2MP was detected with 0.1 percent ninhydrin in n-butanol containing 5 percent lutidine.

Table 1
Composition of Solvents

Solvent					
A ascending		B ascending		C descending	
	volume percent		volume percent		volume percent
n-Butanol	66.7	n-Butanol	61.7	n-Butanol	50.0
Diethylene glycol	16.7	2-Chloroethanol	12.3	Phenol	44.8
H_2O	16.6	NH_4OH(20 percent)	6.2	H_2O	5.2
		H_2O	19.8	(acetic acid atmosphere)	

The hydrolysates of the liver phospholipid fractions obtained from animals fed 2A2MP showed the presence of 2A2MP with each solvent system (Table 2). A major radioactive spot in the hydrolysate of liver phospholipid from animals injected with 2A2MP-1-C^{14} had the Rf value of 2A2MP. A second radioactive spot was also observed, but it has not yet been identified. It did not appear to be a methylated form of 2A2MP.

Table 2

Rf Values for the Components of the Experimental Phospholipid Hydrolysates, Known 2A2MP and Related Known Amino Alcohols

Material Used	Rf		
	Solvent A	Solvent B	Solvent C
2A2MP	0.67	0.74	0.37
2A2MP-1-C^{14}	0.63	0.78	0.34
Liver phospholipid hydrolysate (2A2MP fed rats)	0.66	0.74	0.37
Liver phospholipid hydrolysate (2A2MP-1-C^{14} injected rats)			
Major component	0.64	0.80	0.32
Minor component	0.43	0.01	0.17
Other related known amino alcohols			
Ethanolamine	0.47	0.52	0.10
Monomethylethanolamine	0.70	0.64	0.32
Dimethylethanolamine	0.49	0.50	0.45
Choline	0.46	0.23	0.57

The components of the hydrolysates were also separated by gas phase chromatography at 175°C using a 20 foot column of 25 percent by weight carbowax 2000 on alkaline washed firebrick. Under identical conditions known 2A2MP and a major component of the phospholipid hydrolysate of livers of animals receiving 2A2MP in their diets were eluted at identical times. No such component was eluted from the hydrolysate of the liver phospholipids of rats receiving no 2A2MP.

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