ethanol or dimethylaminoethanol to form products that are not the result of methylation.

The radioactive products are co-precipitated with carrier choline reineckate, but more careful recrystallization procedures reveal the loss of radioactivity with continued recrystallizations. The tetrahydrofolic acid effect probably resulted from the hydroxymethyltetrahydrofolic acid dehydrogenase present. In a similar way the effect of diphosphopyridine nucleotide could be explained by the presence of the formaldehyde dehydrogenase which uses DPN in oxidizing formaldehyde to formic acid.

With aminoethanol and formaldehyde as substrates, paper chromatography indicated the formation of N-formylaminoethanol which had an R_f value similar to that of choline. This probably is not the product of an enzymatic reaction.

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BROMINOLYSIS OF PHOSPHATIDES IN ORGANIC SOLVENTS[1]

Sir:

In a previous communication[2] the oxidation of lysolecithin by permanganate was reported. A search for another oxidant led to the finding that bromine in buffered aqueous solution was also capable of oxidizing lysolecithin.[2,3] In organic solvents, however, a novel reaction takes place. When lysolecithin (mixture of alpha and beta isomers) is treated with bromine in either chloroform or methanol, brominolysis occurs at room temperature to yield choline and lysophosphatidic acids (alpha and beta isomers). Oxidation does not occur to any appreciable extent.

Lecithin[4] and phosphatidylethanolamine[4] also undergo brominolysis but at a much slower rate than does lysolecithin. With these diester phosphatides the reaction requires 24–48 hours or longer (in contrast to 3–8 hours for lysolecithin) and there are produced phosphatidic acids and either choline or ethanolamine, respectively. Smaller amounts of phosphorylcholine and phosphorylethanolamine also may be formed.

In order to facilitate this study P^{32}-lysolecithin was used and was prepared biosynthetically from rat liver as described previously.[2] The time course of the reaction in chloroform is given in Fig. 1. At the time intervals indicated, appropriate aliquots of the reaction mixture were removed for paper chromatographic analysis and the radioactivity in the lysophosphatidic acid and in the lysolecithin was determined. Chromatography was carried out on silicic acid impregnated paper as described previously.[5] The R_f values of choline, lysolecithin and lysophosphatidic acid were 0.02, 0.21 and 0.64, respectively. The lysophosphatidic acid spot consisted of two overlapping components. Free choline also was identified by paper chromatography in other solvent systems.[6]

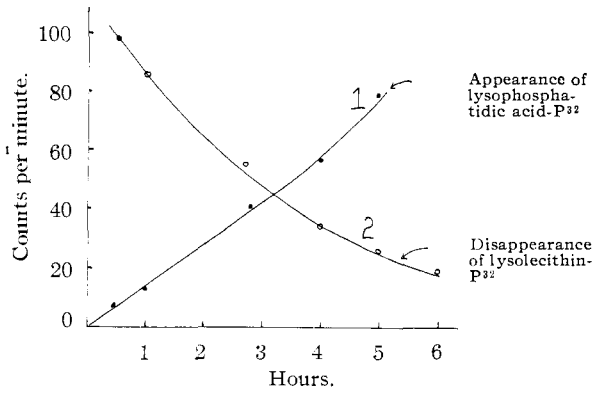

Fig. 1.—Brominolysis of P^{32}-lysolecithin in chloroform: five milligrams of P^{32}-lysolecithin was dissolved in 1.0 ml. of chloroform (Mallinckrodt, "Analytical Reagent"). To the solution was added 5 microliters of pure liquid bromine. The solution was mixed, stoppered and let stand at room temperature. At the time intervals indicated 10-microliter aliquots were removed for paper chromatographic analysis.[5] The lysolecithin and lysophosphatidic spots were cut from the chromatograms and the radioactivity in each was determined. Curve 1 shows the rate of appearance of the radioactive lysophosphatidic acid; curve 2 the rate of disappearance of the starting lysolecithin.

Brominolysis of lysolecithin in methanol occurs readily but the lysophosphatidic acids which are formed are hydrolyzed rapidly during the reaction. The maximum yield of lysophosphatidic acids in methanol is about 35% and is attained after 2-3 hours. The yield of lysophosphatidic acids in chloroform is 80–95% and is attained after 6–8 hours.

The reaction in chloroform was carried out on a larger scale using myristoyl-lysolecithin which was prepared from a synthetic dimyristoyllecithin[4] by snake venom hydrolysis.[2] The choline bromide precipitates out of solution in chloroform (but not in methanol) as the reaction proceeds and can be isolated easily. The lysophosphatidic acids were purified by column chromatography on silicic acid[5] and by converison to the barium salt. The free lysophosphatidic acids were found to contain 13.05% bromine.[7]

The brominolysis reaction allows for the preparation of lysophosphatidic acids and phosphatidic acids and complements the diazometholysis reaction[8] as a tool for establishing the stereochemical configuration of the phosphatides.

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(2) G. V. Marinetti, J. Erbland and E. Stotz, *Biochim. et Biophys. Acta*, **33**, 403 (1959).

(3) G. V. Marinetti and K. Temple, *Fed. Proceedings*, **18**, 281 (1959).

(4) Synthetic samples from Dr. E. Baer, University of Toronto, Toronto, Canada.

(5) G. V. Marinetti, J. Erbland and J. Kochen, *Fed. Proc.*, **16**, 837 (1957).

(6) G. V. Marinetti, D. Scaramuzzino and E. Stotz, *J. Biol. Chem.*, **224**, 819 (1957).

(7) The analysis for bromine was done by the Schwartzkopf Microanalytical Laboratory, Woodside, N. Y. The theoretical content for bromine varies depending on the nature of the product. The analysis indicates that a mixture of the brominated and non-brominated forms is present and could explain the finding that two components are seen on paper chromatograms.

(8) E. Baer and J. Maurukas, *J. Biol. Chem.*, **212**, 39 (1955).

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