Hydrolecithin (dipalmityl lecithin) has recently been isolated by Thannhauser, Benotti, and Boncoddo from beef lung (1). The separation of this ether-insoluble phospholipide from sphingomyelin was achieved by a sequence of fractional extractions under control of an analytical method which is based on the observation that sphingomyelin is resistant to sodium or potassium hydroxide at 37° in contrast to hydrolecithin which is saponified under these conditions (2). Finally, an ether-insoluble substance was obtained which was completely saponifiable by sodium hydroxide at 37° and identified as dipalmityl lecithin.

The present paper deals with the application of this procedure to the lipides of brain and spleen. It will be demonstrated that hydrolecithin is present not only in the sphingomyelin fraction of lung, but also in brain and spleen, and that the hydrolecithin isolated from brain and spleen is identical with dipalmityl lecithin. The presence of large amounts of cerebrosides in brain necessitated the modification of the preparative procedure applied for the isolation of hydrolecithin from lung.

*Preparation of Hydrolecithin from Brain*

Batches not exceeding 25 pounds of beef brain were used for each single preparation, since the abundance of cholesterol and cerebrosides in this organ caused great practical difficulties which are not encountered in other material such as lung and spleen. These difficulties increase when the isolation is undertaken on a larger scale.

The fresh beef brains are minced, washed twice with acetone, filtered, and dried in a vacuum dryer at 60°. The material is then ground to a powder and extracted with hot acetone for 3 days in a large continuous extractor (3) in order to remove the bulk of the fat, cholesterol, and a part of the cerebrosides and monoaminophosphatides. The acetone extract is discarded despite the fact that it contains small amounts of hydrolecithin and sphingomyelin. The powder is continuously extracted with ether for 3 days. The crude ether extract is placed in the refrigerator and filtered

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on the next day with the aid of Hyflo filter aid (Johns-Manville). The filtered lipide mixture is reextracted with ether in a Soxhlet extractor for several days in order to complete the removal of the unsaturated monoaminophosphatides, cholesterol, fat, and small amounts of ceramides.

The residue (143 gm.) in the thimble is then taken up in about 1000 cc. of petroleum ether-methanol (9:1). The emulsion formed due to the presence of the Filter-Cel and cerebrosides is broken up by the addition of 1000 cc. of methanol. After standing overnight the precipitate, which consists essentially of cerebrosides and Filter-Cel, is filtered off. The filtrate is concentrated to a small volume of a thin syrupy consistency and precipitated with a large excess of acetone (1 to 2 liters). The suspension is placed in the refrigerator overnight. The precipitate (52 gm.) consists mainly of sphingomyelin, hydrolecithin, and still appreciable amounts of cerebrosides. This lipide mixture contains 2.4 per cent P of which 37 per cent is saponifiable P. (Saponifiable P represents saturated monoaminophosphatides (4).)

The material is treated with 10 volumes of glacial acetic acid (520 cc. for 52 gm.), which extracts the bulk of the phosphatides. On slight warming the major portion of the precipitate dissolves. The suspension is allowed to stand overnight at room temperature and is then filtered. The insoluble residue is extracted again with 10 volumes of acetic acid in order to extract the retained phosphatides. The glacial acetic acid filtrates are pooled and concentrated to a very small volume (viscous consistency) and precipitated with an excess of acetone (1000 to 2000 cc.). The suspension is allowed to stand overnight in the refrigerator and is then filtered. The precipitate (Substance A, 33 gm.) contains essentially sphingomyelin, small amounts of hydrolecithin and cerebrosides, and traces of amino acids.

The filtrate is concentrated to dryness under reduced pressure and dried over sodium hydroxide in an evacuated desiccator in order to remove the acetic acid as completely as possible. The residual substance is then suspended in an excess of acetone and is left overnight in the refrigerator. The precipitate is filtered (3.5 gm.). The analysis of this substance shows that 90 per cent of its total P is saponifiable P (hydrolecithin). This substance is recrystallized from a mixture of acetone-glacial acetic acid (40:1) (1 gm. of substance in 20 cc. of solution). The amount of hydrolecithin present in the ether-insoluble phospholipide fraction is approximately 25 per cent of its sphingomyelin content. Yield of hydrolecithin, 4 gm. from 25 pounds of fresh beef brains.

Physical Properties of Hydrolecithin from Brain—The substance is a white crystalline powder soluble in alcohol and glacial acetic acid, very slightly soluble in acetone, and insoluble in ether. The substance melts between 238-240°. (The mixed melting point with hydrolecithin from lung gave no depression.) The iodine number is 5.65.
Analysis—Calculated, N 1.86, P 4.1; found, N 1.81, P 4.15

The specific rotation was +6.25° (4 per cent solution of hydrolecithin in a mixture of chloroform–methanol, 1:1).

Products of Alkaline Hydrolysis of Hydrolecithin. Barium Salts of Fatty Acids—2.5 gm. of the substance were refluxed for 4 hours with saturated aqueous Ba(OH)₂. After cooling, the barium soaps were filtered and shaken with H₂O to remove soluble impurities. After filtering and drying, the crude barium salt of the fatty acid weighed 2.07 gm.

Identification of Fatty Acid by Vacuum Distillation of Methyl Ester—2 gm. of barium soaps were refluxed in a 5 per cent methanolic solution of H₂SO₄ for 4 hours on a water bath. The BaSO₄ formed was removed by filtration, and the methyl esters were extracted from the acid methanolic solution with petroleum ether. The dry weight was 1.25 gm.

The methyl esters were distilled at a pressure of 0.0015 to 0.002 mm., the substance distilling at a fairly constant temperature (94–96°), indicating the presence of only one methyl ester. The free acid was recrystallized at −15° to a constant melting point, 61° (palmitic acid, 62°); iodine number 0; mol. wt. 257 (palmitic acid, 256).

Choline—Choline, total P, and glycerophosphate were determined in the filtrate (150 cc.) of the alkaline hydrolysis. Choline was determined by the reineckate method in 1 cc. of the filtrate. Found, 345 mg. of choline; calculated for 2.5 gm. of dipalmitolecithin, 403 mg. A 1 cc. aliquot was analyzed for total P; found, 88.65 mg. of total P; calculated for 2.5 gm. of dipalmitolecithin, 103.0 mg. Both findings represent an 86 per cent recovery.

Barium Glycerophosphate—The remainder of the filtrate (145 cc.) was used for the isolation of glycerophosphate. After neutralization with acetic acid, basic lead acetate was added in slight excess. The precipitate was filtered and carefully washed with water. The lead salt was decomposed with hydrogen sulfide. The filtrate from the lead sulfide was concentrated to a small volume. A hot saturated solution of barium hydroxide was added until pH 9 was obtained. The barium salt was precipitated by the addition of 3 volumes of alcohol.

The barium salt was analyzed for glycerol (method of Blix) and for P (method of Fiske and Subbarow).

C₃H₅O₇PБa. Calculated. Glycerol 30, P 10

Found. 28.8, 9.71

The physical properties of the substance as well as the analysis of the split-products obtained by its alkaline hydrolysis show that hydrolecithin (dipalmityl lecithin) from brain is identical with dipalmityl lecithin isolated from lung.
Isolation of Hydrolecithin from Spleen

The method employed for the isolation of hydrolecithin from the ether-insoluble phosphatide mixture is essentially the same as previously reported for its isolation from lung (1).

Physical Properties of Hydrolecithin—Hydrolecithin isolated from spleen is a white crystalline substance soluble in alcohol and glacial acetic acid, very slightly soluble in acetone, and insoluble in ether. The substance melts between 238-240°. A mixed melting point with hydrolecithin obtained from lung and brain showed no depression of the melting point; iodine number 3.81.

Analysis—Calculated, N 1.86, P 4.1; found, N 1.91, P 3.98
Specific rotation, +6.25° (4 per cent solution of hydrolecithin in a mixture of chloroform-methanol, 1:1).

The amount of hydrolecithin present in spleen is approximately 25 to 40 per cent of its sphingomyelin content. Hydrolecithins (dipalmityl lecithin) isolated from spleen, brain, and lung are identical in their physical properties.

Discussion

In the paper on hydrolecithin from lung it has already been pointed out that this substance (dipalmityl lecithin) is not identical with hydrolecithin prepared by hydrogenation with colloidal palladium from unsaturated lecithins of egg yolk (4-6) and of brain (7). It is also not identical with the synthetic distearyl lecithin of Grün and Limpacher (8, 9). It seems to be identical with the hydrolecithin isolated by Lesuk and Anderson (10) from Cysticercus larvae.

The same statements are pertinent for hydrolecithin (dipalmityl lecithin) from brain and spleen described in this paper, since hydrolecithin from these organs is identical with hydrolecithin from lung.

The identity of the hydrolecithins isolated from different organs (brain, spleen, lung) contrasts with the non-identity of the sphingomyelins isolated from these organs, since the fatty acids of brain sphingomyelin are very different from those of lung and spleen sphingomyelin.

Summary

A method of isolation of crystalline hydrolecithin from beef brain is described.

The isolated saturated lecithin is dipalmityl lecithin and is identical with lung hydrolecithin.

Hydrolecithin was also isolated from spleen according to the procedure
applied for its isolation from lung. It is identical with dipalmityl lecithin prepared from brain and lung.

The yield of hydrolecithin in brain is approximately 4 gm. from 25 pounds of fresh beef brain, corresponding to 25 to 40 per cent of the sphingomyelin yield.

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ISOLATION AND IDENTIFICATION OF HYDROLECITHIN (DIPALMITYL LECITHIN) FROM BRAIN AND SPLEEN
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