50. *Phosphatides of the Human Brain.*

Part I. The Separation of α - and β -Series of Lecithins.

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In a previous paper,¹⁾ the authors had presented an account of their experiment concerning the procedures of separating α - and β -series of soy bean lecithins. The separation had been carried out with good results by treating lecithin- CdCl_2 complex with acetone and it was found that the so-called soy bean lecithin is a mixture of, at least, 9 simple lecithins. Having been encouraged by the experiment, the authors turned, now, to the study of the lecithins of the human brain.

To see whether or not the same process is applicable to lecithin mixtures of animal origin in the same way with those of vegetable origin, as we have no definite proof with regard to the fact that the lecithins of two origins are categorically the same or different, the authors came to consider it necessary to test the process, preliminarily, with some animal lecithin which can be readily obtained.

As the material for preparing lecithin, salted and dried herring roe was selected. The roe was powdered finely, extracted several times with warm alcohol. By adding acetone to the combined and concentrated alcohol extracts, the phosphatides were precipitated. Lecithins and kephalins were separated by treating with alcohol. Crude lecithins had been purified to a certain degree by repeating the method of dissolving into alcohol and precipitating with acetone. CdCl_2 - complex was obtained and the complex was washed thoroughly with ether. From 3 Kg of the material, some 82 g of the complex were yielded. To see the purity, free lecithins were prepared from a part of the double compound following Levene and Rolf's²⁾ method and analysed. N: 1.76%, P: 4.01%, N:P=1:1.02, Amino-N (after Van Slyke): 0.00%.

The remaining complex had been separated into two fractions which are less and more soluble into acetone. From more soluble

1) Proc. **8** (1930), 341.

2) J. Biol. Chem. **72** (1927), 587.

part, the compound had been recovered by adding ether to the acetone solution and it was purified by repeating the same process. The less soluble fraction was washed thoroughly with acetone. These procedures were guided by analysis and repeated until the analysis showed that either one of the glycerophosphoric acids are solely contained. From the more soluble fraction β -Ba-glycerophosphate was obtained in an almost theoretical yield by adding $\text{Ba}(\text{NO}_3)_2$ solution but by treating Ba-glycerophosphate of the less soluble fraction, similarly, no turbidity at all has been observed.¹⁾

After the lecithins were hydrolysed with HCl the fatty acids were identified as follows:

From α -lecithins (CdCl_2 -complex are with difficulty soluble in acetone):—

Palmitic acid (m.p.: 61°C , C: 75.01, H: 12.60% (theory, C: 74.94, H: 12.59%)), dibromostearic acid (Br: 36.17% (36.15)), tetrabromostearic acid (m.p.: 114°C , Br: 52.93 (53.32)) and hexabromostearic acid (m.p.: 178°C , Br: 63.38% (63.43)).

From β -lecithins (CdCl_2 -complex are more soluble in acetone):—

Palmitic acid (m.p.: 61°C , C: 75.10, H: 12.49%), dibromostearic acid (Br: 36.10%), tetrabromostearic acid (m.p.: 113°C , Br: 53.73%) and hexabromostearic acid (m.p.: 178°C , Br: 62.90%).

The separation of α - and β -lecithins is successful, therefore, with regard to the animal lecithins likewise those of vegetable origin.

Human brains (10 brains, about 13 Kg) were made as free as possible from blood vessels and meninges, soaked in a sufficient quantity of acetone and macerated to fine powder. The dried powder were extracted several times with ether. Ether was driven off from the combined ether extracts and the residue was treated with alcohol, repeating the extraction 5 or 6 times. The alcoholic extracts were combined and concentrated when acetone was added. The extraction with alcohol and the precipitation by acetone were repeated and to the final alcoholic extract, an alcoholic solution of CdCl_2 was added, whereupon lecithin- CdCl_2 complex precipitated (584 g) and the following separation and analysis were carried out in the same way with the above case.

The free lecithins;— N: 1.85%, P: 4.06%, N:P=1.01:1, amino-N: 0.00%.

Choline was identified as the chloroplatinate (m.p.: 232°C (decom-

1) Helv. Chim. Acta **3** (1927), 9.

posing), Pt: 31.26% (theory 31.68). As fatty acids, dibromostearic acid (Br: 36.05%), tetrabromostearic acid (m.p.: 114° C, Br: 53.27%), octabromoarachidic acid (m.p.: 205° C (decomposing), Br: 67.90% (theory 67.76) and palmitic acid (m.p.: 61° C, C: 74.78, H: 12.83%) were obtained in the following proportions. The % were calculated, taking the combined amount of the fatty acids actually obtained as 100.

	Yield (g)	%
Oleic acid	3.39	85.4
Linolic acid	0.05	1.3
Arachidonic acid	0.13	3.3
Palmitic acid	0.40	10.0

α - and β -series were separated by the process already referred to, and from each series, the following fatty acids were found.

α -lecithins (CdCl₂-complex of which are soluble with difficulty in acetone):—

Oleic acid (as dibromostearic acid, Br: 36.15%), arachidonic acid (as octabromarachidic acid, m.p.: 205° C (decomposing), Br: 67.40%) and palmitic acid (m.p.: 61° C, C: 74.94, H: 12.59%) but no trace of linolic acid.

β -lecithins (CdCl₂-complex of which are easily soluble in acetone):—

Oleic acid (as the dibromide, Br: 36.06%), linolic acid (as tetrabromides, m.p.: 114° C, Br: 52.81%), arachidonic acid (as octabromides, m.p.: 205° (decomposing), Br: 67.20%) and palmitic acid (m.p.: 61° C, C: 74.65, H: 12.82%).

Solubility or non solubility of the complexes of both the series are relative properties, accordingly the loss of the material during the separation and purification was considerable.

The separation of each member of lecithins of the mixtures of α - and β -series, are under way in our laboratory.

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