120. *Phosphatides of the Human Brain.*

IV. *The Separation of α - and β -Kephalins.*

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(Comm. by U. SUZUKI, M.I.A., Nov. 12, 1932.)

*Crude Kephalins*¹⁾: Two lots of crude kephalins were used separately in the first part of the experiments. The first lot was recovered from Cd-Cl₂ complex which was obtained as an ethereal solution during the purification of crude lecithins (lot 1). As the second lot (lot 2), the fraction of the mixture of lecithins and kephalins which is with difficulty soluble in alcohol, has been taken.

Purification: Both the lots were purified in a similar way as outlined as follows:

(1) The traces of lecithins were removed by treating Cd-Cl₂-kephalin with ether.

(2) The so-called white matters (cerebrosides, sphingomyelins etc.) were removed by repeating the precipitation of them by letting stand, over night, the ethereal solution of the kephalins in an ice box.²⁾

(3) The oxidative decomposition products were removed as insoluble precipitates by treating the preparation with glacial acetic acid.³⁾

(4) To make free from inorganic salts, the emulsion of the kephalin preparation in water was treated with 10% HCl.⁴⁾

The above procedures were repeated until no further treatment became necessary.

Properties of Kephalins: The purified kephalin mixtures were light brown coloured powders. The combined yields of the preparations extended to 0.34% of fresh brain. They were almost insoluble in dry ether but soluble in ether of 1% water content. In methyl alcohol, hot or cold, they were insoluble. It dissolved a little in absolute alcohol but insoluble in 92% alcohol. While hot, alcohol was a good solvent. The analyses given are:

1) c.f. Y. Yokoyama and B. Suzuki: Proc. 8 (1932), 18.

2) Levene & Rolf: J. Biol. Chem. 74 (1927), 713.

3) Levene and Rolf: Ibid. 68 (1926), 285.

4) Fränkel and Neubauer: Biochem. Z. 21 (1902), 321.

	M.P. (C)	I-value	P(%)	N(%)	NH ₂ -N(%)	N : P(%)	NH ₂ -N/Total N
Lot 1.....	166-168	53.08	3.60	1.58	1.57	1.00/1.02	0.993/1.00
Lot 2.....	167-169	59.04	3.59	1.58	1.59	1.00/1.02	1.00/1.00

Kephalin-lead acetate: It is a well known fact that kephalins form a definite compound with lead acetate¹⁾ in the presence of ammonia. When an alcoholic solution of basic lead acetate was added to an ethereal solution of kephalins, double compounds of the solutes were formed but they had no definite composition. It was found, however, that these double compounds are suitable for the separation intended. A part of the compounds (α -kephalins) was easily soluble, the other part was less soluble in ether while both the parts were insoluble in alcohol and soluble in benzene, toluene and chloroform. For the actual separation we used the mixture of ether and alcohol (1:2) or benzene and alcohol (1:2). To show how the separation ran: 3 g of lot 2 were dissolved in 30 cc. of ether and to the solution, an alcoholic solution of basic lead acetate (3 g in 30 cc.) was added. The precipitates were washed several times with the ether-alcohol mixture and dried in a vacuum desiccator. The dried material was dissolved in benzene, the insoluble inorganic substances being, thus, removed. The benzene solution was concentrated, the kephalin-lead acetate was recovered by adding methyl alcohol and washed thoroughly with the latter solvent. The solid compound was suspended in ether, a small quantity of 1% HCl was added and the suspension was well shaken, whereupon the free kephalins went to the ethereal layer. The ethereal solution was washed with water and the kephalins were recovered by adding acetone to the solution. To remove the last traces of lead, the process of (4) of the purification was adopted.

The portion which was easily soluble in ether alcohol mixture was combined with the washings. To the combined solution, water was added and the whole was shaken. The kephalin-lead acetate passed to the ethereal layer and it was precipitated by adding acetone to the solution. The recovering of the free kephalin from the double compound was carried out just in the same way with the above case.

Two kinds of kephalin preparation, obtained as above referred to, were hydrolysed by boiling with 10% baryta water for 6 hours and the quantities of α - and β -glycerophosphoric acid have been estimated following Karrer and Salomon's method.²⁾ Lot 1 was, also, similarly treated and analysed.

1) Maclean: Lecithin etc. (1918), p. 51.

2) Helv. chim. act. **9** (1926), 3.

Solubility	Lot 1		Lot 2	
	Easily	With difficulty	Easily	With difficulty
β -glycerophosphoric acid	2.22	91.49	7.27	80.97
α - " "	97.78	8.51	92.73	9.03

Thus we knew that kephalins of α - and β -series may be separated from each other by repeatedly treating kephalin-lead acetate compound with ether-alcohol or benzene-alcohol mixture.

The separation of α - and β -series: Thus far we had studied lot 1 and lot 2 separately but now that we found a way, applicable to both the lots, of separating α - and β -series, it had become unnecessary. So both the lots were combined and lead-acetate double compounds were formed and we treated the compounds with the mixed solvents along the line of the method we described in the preceding paragraph. When benzene was used, it was found very convenient to dissolve the lead acetate compound in the solvent and to precipitate by adding alcohol. Thus we obtained finally 2 fractions which contained either α - or β -series exclusively. From the with difficulty soluble fractions, β -glycerophosphoric acid has been recovered to the extent of 99.43% while the fraction, which was easily soluble, had shown only a slight sign of turbidity by adding the Karrer and Salomon's reagent. To show the yields; we obtained 36 g of a mixture of pure kephalins from 13 kg of human brain; from that quantity we were able to separate 23.7 g of β - and 5.2 g of α -kephalins, namely, the amount of β -kephalin overstrides that of α -form in the human brain.

The glycerophosphoric acid of α -kephalins: From hydrolysates the glycerophosphoric acid has been recovered as Ba-salt in the yield of ca. 96% of the theory. No turbidity has been observed by adding a solution of $\text{Ba}(\text{NO}_3)_2$ to an aqueous solution of the Ba-glycerophosphate. This is an ample proof that the glycerophosphoric acid is α -variety. The analyses had shown that the glycerophosphate contains P:9.83% (calc. 10.09) and Ba:44.67% (calc. 44.68).

The base of the α -kephalins: From the filtrate and washings of Ba-glycerophosphate, aminoethyl alcohol was isolated as the picrate. The picrate melted at 158–159°C and contained 19.21% (theory: 19.31) of N. The yield of the picrate was about 90% of the calculated amount and no other base had been found.

The glycerophosphoric acid of the β -kephalins: The isolation of glycerophosphoric acid was performed in the same way with the case of α -kephalins with the yield of ca. 95% of the calculated amount. 4.202 g of the Ba-salt was dissolved into 30 cc. of water and to this

solution, an aqueous solution (2.8 g in 25 cc.) of Ba-nitrate was added. After 15 hours, the precipitates were gathered, washed with cold water, alcohol and ether, dried and weighed. The yield extended to 99.52% of the amount used for the precipitation and we know, therefore, that the kephalins which form less soluble lead compounds, consist exclusively of the β -form of glycerophosphoric acid.

The base of the β -kephalins: Aminoethyl alcohol was isolated in the form of picrate as was the case with α -kephalins. The yield was ca. 87% of the theoretical amount and the picrate melted at 158.5–159° C and contained 19.37% of N. No other base had been found.