LIII. THE ACTION OF PURE PHOSPHATIDES ON THE PERFUSED HEART OF THE FROG.

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THE study of the pharmacological properties of the phosphatides has until recently been hindered by lack of chemical criteria of purity, the result of our ignorance of the chemistry of these compounds. The work of Rosenheim, MacLean, Levene and others, chiefly in the last decade, has however brought us much nearer to an exact knowledge of their chemical constitution, and rendered it possible to use for biological experiments phosphatide preparations of a high degree of purity [see MacLean, 1918]. The object of the present work was to prepare samples of lecithin and kephalin of known purity and to test the influence of these substances on the perfused heart of the frog.

In considering criteria of purity it is necessary to survey briefly the present state of our knowledge of the chemistry of these compounds. Lecithin has been shown to have a molecular weight¹ of the order of 800 [Levene, 1921; Levene and Rolf, 1923]. It is a diglyceride, containing approximately equal proportions of saturated and unsaturated fatty acids. Of saturated acids only palmitic and stearic have been identified with certainty [Koch, 1902; Ulpiani, 1901; Cousin, 1906; Levene, 1921], but there are indications of several unsaturated acids [Levene, 1921; Levene and Rolf, 1923], of which oleic, linoleic and arachidonic have been confirmed. It appears then that a preparation of "pure" lecithin is a mixture of a series of lecithins with molecular weights ranging between 760 (palmityl-linoly) and 810 (stearyl-arachidonyl), having the generic formula

There are possibilities of space isomerism, which need not be considered in this connection. A kephalin apparently differs from a lecithin in containing the base colamine in the place of choline [MacLean, 1915].

A lecithin preparation may therefore have a nitrogen content anywhere between 1.70 and 1.85 % and still be quite pure. Neither the nitrogen content nor the phosphorus content is any test of purity, though if the present theory

¹ Some as yet unpublished work by the writer has shown that kephalin has an equivalent weight of the same order of magnitude. The full titration curves of both phosphatides will be published shortly.

is correct the atomic ratio of these two elements must always be unity, if the preparation is pure. Any mixture of lecithin and kephalin may satisfy this criterion, but estimation of the amino-nitrogen content affords an easy control on the proportion of kephalin present.

For the purposes of this work a lecithin sample was considered pure when its nitrogen-phosphorus ratio was unity and it contained no amino-nitrogen, and in addition gave negative tests for cholesterol, galactose and mineral impurities. A kephalin sample was tested in the same manner, and had to contain no non-amino-nitrogen.

PREPARATION AND PURIFICATION OF LECITHIN AND KEPHALIN.

Preparations were made of lecithin and kephalin from egg-yolk and from ox-brain. The egg-yolks had been separated from the whites and frozen without preservatives and in the absence of air; the ox-brains were always fresh. The method of treatment was the same in each case. It was adapted from the methods used at various times by Rosenheim, MacLean and Levene. The tissue was minced and dried by extraction with acetone; it was then exhaustively extracted with dry acetone, absolute alcohol and sodium-dried ether in succession. These and all subsequent operations were performed in an atmosphere of nitrogen. The fat-free residue was rejected and the extracts treated as follows.

Combined acetone extracts. These were maintained at 0° till no more solids crystallised out. The deposit was repeatedly extracted with small quantities of warm dry alcohol, each extract being precipitated with alcoholic CdCl_2 . These precipitates were mixed and subsequently purified.

Alcoholic extracts. These were precipitated at once with CdCl_2 ; all precipitates were mixed and subsequently purified.

Ether extracts. These were concentrated to half their bulk and crude kephalin precipitated by means of acetone.

Purification and decomposition of lecithin- CdCl_2 . The use of toluene, or of ethyl or methyl acetate, was considered undesirable in view of the relatively powerful pharmacological activities of these substances. The only purification adopted was "crystallisation" from moist ether. The compound freezes out from moist ether in granular lumps when a certain degree of purity is attained. It was then decomposed by Bergell's method [1900]. This is admittedly the weakest part of the technique. It involves heating an aqueous-alcoholic solution of the compound to 70° while a saturated solution of ammonium carbonate is added until the mixture is alkaline, when it is filtered hot. These conditions are very favourable to destruction, but by working in an atmosphere of nitrogen, and freezing the filtrate instantly, the risk was minimised as far as possible. The solution was kept at - 10° for an hour and the liquor decanted; this mother-liquor was added to the crude lecithin- CdCl_2 extracts. It is not wise to add acetone to the alcoholic filtrate as lecithin is very soluble in acetone-alcohol mixtures.

Purification of the free phosphatides. Lecithin. This was repeatedly precipitated with acetone from ether solution; it was then emulsified with water and coagulated with acetone, this being repeated several times. It was then dissolved in ether and reprecipitated with acetone to remove water. This also was repeated a few times. Finally the pure lecithin was extracted with dry acetone for several days at 0° to remove the last traces of ether and water. At this stage the lecithin had the appearance of a waxy, finely granular solid, quite white. It was dried in nitrogen over H_2SO_4 after as much acetone as possible had been pressed out. The product has been kept sealed up in nitrogen at low pressure for 2 years without any apparent deterioration.

Kephalin. This was obtained from the ether extract of the brain powder by repeated solution in ether and gradual reprecipitation with alcohol. The product was then repeatedly emulsified with water and coagulated with alcohol (not with acetone: alcohol is more effective). After several more precipitations from ether solution the product was extracted with dry acetone at 0° and finally dried in the same manner as the lecithin. P_2O_5 was tried as a desiccating agent, but it appeared to induce polymerisation of the traces of acetone present, causing the phosphatide to darken.

Analytical data tabulated.

Preparation	% P	% N	$\frac{\text{amino-N}}{\text{total N}} \times 100$	N/P ratio
Lecithin (egg)	4.01	1.85	12	100 : 98
Lecithin (brain)	3.92	1.87	5	100 : 95
Kephalin (brain)	3.88	1.82	86	100 : 96
Pure lecithin (theoretical)	3.8-4.1	1.7-1.85	0	100 : 100
Pure kephalin (theoretical)	4.1-4.4	1.85-2.00	100	100 : 100

THE PHARMACOLOGICAL ACTION OF THE PHOSPHATIDES.

The results of injection and ingestion of "lipoids" have in most cases in the past been vitiated by the doubtful purity of the preparations used. In the present work results are submitted which indicate that one at least of the supposed properties of the phosphatides is non-existent, and that such effects as have been observed by previous workers have probably been due to water-soluble impurities in the preparations used.

Clark [1914] showed that crude lecithin (alcoholic extract of egg-yolk: Kahlbaum) had a strong stimulating action on the hypodynamic heart of the frog, and that a similar action was produced by alcoholic extracts of serum and by sodium oleate and various other soaps. Lecithin of this nature also produced a slight stimulant action on the isolated gut of the rabbit and on the isolated uterus of the guinea-pig [Clark, 1920]. Van Leeuwen and Gyorgyi [1923, 1], working with pure samples of lecithin and kephalin prepared by Levene showed that pure lecithin had no action on the hypodynamic heart of the frog, but that kephalin had a strong stimulant action, and that an ultrafiltrate of kephalin had only a slight stimulant action. They showed however [1923, 2] that the ultrafiltrate from kephalin produced as great an action as the original kephalin in sensitising the isolated gut of the rabbit to pilocarpin,

and that an action of similar intensity was produced by sodium oleate. The writers mention, moreover, that the kephalin they were using had undergone a certain amount of decomposition.

Pharmacological tests. My samples of lecithin and kephalin were tested upon the hypodynamic heart of the frog. All samples examined had been prepared recently and kept in nitrogen at 0°. The results of these tests are shown in the table and in Figs. 1 and 2.

Fig. 1. Hypodynamic heart of frog (perfused 18 hours).
A. Perfusion with pure kephalin (0.002 %).
A 1. After 10 minutes' perfusion.
B. Perfusion with crude lecithin (egg) (0.002 %).
B 1. After 10 minutes' perfusion.
C. Perfusion with pure lecithin (egg) (0.004 %).
C 1. After 10 minutes' perfusion.
D. Perfusion with pure lecithin (brain) (0.004 %).
D 1. After 10 minutes' perfusion.

Fig. 2. Hypodynamic heart of frog (perfused 6 hours).
A. Perfused with water-soluble impurities extracted from lecithin (concentration less than 0.01 %).
A 1. After 10 minutes' perfusion.
B. Perfused with pure lecithin (brain) (0.01 %).
B 1. After 10 minutes' perfusion.

Pharmacological tests.

Concentration	Effect				
	Pure lecithin (brain)	Pure lecithin (egg)	Crude lecithin (egg)	Crude lecithin (brain)	Pure kephalin (brain)
1 : 25,000	Slight depression	Slight depression	400 % stim.	—	10 % stim.
1 : 50,000	—	—	400 % stim.	300 % stim.	Slight stim.
1 : 100,000	Doubtful effect	No action	400 % stim.	300 % stim.	Doubtful effect
1 : 200,000	No action	—	50 % stim.	—	—
1 : 500,000	—	—	No action	—	No action
1 : 1,000,000	—	—	—	—	—

At concentrations at which the crude lipin extracts of egg-yolk and ox-brain had powerful effects (10 parts per million) the pure phosphatides were without action. There would seem to be an impurity, perhaps a decomposition product (associated more particularly with kephalin), which is a strong cardiac stimulant. In this connection the activity of crude "lecithin" was compared with that of the water-soluble impurities extracted from it. Definite indications were obtained that the active principle was extracted by water (Fig. 2). Preliminary experiments showed that the water-soluble impurities also produced a stimulant effect on the guinea-pig's uterus but had no certain action on the isolated gut and uterus of the rat. The active principle or principles found cannot include choline, since this substance has a depressant action on the frog's heart. Sodium oleate may in part account for the action of the crude lipin extracts.

SUMMARY.

Samples of the phosphatides lecithin and kephalin have been prepared in a high degree of purity, particularly with respect to water-soluble impurities. In this state of purity neither phosphatide was found to be a cardiac stimulant, a result contrary to the experience of other workers. It is suggested that the activity of the specimens used by these workers was due to a water-soluble impurity, possibly of the nature of an organic base.

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