ON THE OCCURRENCE OF A MON-AMINO-DIPHOSPHATIDE LECITHIN-LIKE BODY IN EGG YOLK

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In making some investigations on the ethereal extract of egg yolk, I succeeded in separating a body of the general nature of a lecithin, but containing in its molecule two parts of phosphorus to one part of nitrogen.

This compound is of the same type as that separated from heart muscle by Erlandsen,¹ and called by him 'Cuorin,' though, as shown below, it differs in certain respects from this substance. A preliminary note giving the N and P content which characterise it as a mon-amino-diphosphatide was published some time ago,² but as this portion was prepared from a comparatively small number of eggs, no account of its properties or probable elementary composition was given.

During this winter, however, I have isolated the substance from a large quantity of egg yolk, and having used certain modifications in the preparation of different portions, will now proceed to give a short account of its isolation, composition and chief properties.

PREPARATION

The egg yolk of fresh eggs was separated from the white portion, and after being spread out in thin layers on a glass plate was dried by means of a current of air generated by a fan-like arrangement attached to a motor. When thoroughly dry the mass was broken up into small pieces, and finally passed through a coffee mill. In this way a very fine powder was obtained, which was carefully extracted five times with ether. The ethereal extracts were mixed together, evaporated to a fairly small volume, and treated with acetone. The precipitate was dried in vacuo over H_2SO_4 and divided into two parts. Portion A was treated by a combination of the methods used by Stern and Thierfelder³ for purifying lecithin, and by

1. *Zeitschrift f. physiol. Chemie.*, Bd. LI, S. 92.
2. *Ibid.*, Bd. LVII, S. 304.
3. *Ibid.*, Bd. LIII, S. 370.

Erlandsen⁴ for the isolation of cuorin; as will be seen, this combination is rather tedious in carrying out.

Portion B was treated by a much simpler method, which was found exceedingly easy to carry out, and capable of giving quite a pure substance.

TREATMENT OF PORTION A

This portion was again dissolved in ether and gave a markedly turbid solution. By means of the centrifuge a perfectly clear fluid was obtained, the residue in the centrifuge tubes being of a whitish colour, and with difficulty soluble in ether. The clear solution was again precipitated with acetone and dried. This process of purification was repeated five times, and by this means impurities such as cholesterin and fat were in great part got rid of. The final material gave a perfectly clear solution in ether, but only after standing for some time, being at first slightly turbid. The solution was now treated with about four times its volume of absolute alcohol, and left to stand under CO₂ in a closed vessel for twenty-four hours; an alcohol insoluble residue remained, which was separated by filtration. This substance was washed with cold alcohol, and the united filtrates evaporated in vacuo to a small bulk, precipitated with acetone, and dried as usual. This precipitate was now treated with absolute alcohol, when a small portion remained behind, which was added to the above alcohol insoluble part. Thus, the ethereal solution was divided into two parts—the part soluble in cold alcohol consisting of ‘lecithin.’

The alcohol insoluble part was now placed in an incubator and heated for a fairly long time with alcohol at 65° to 70° C. By this means part of the substance went into solution, but separated again on the alcohol cooling. This process was repeated three times, and finally boiling alcohol was used in an attempt to obtain an alcoholic solution that would remain quite clear after cooling. In every case, however, the alcoholic filtrate on cooling became slightly opalescent, though only faintly so. By this procedure the part insoluble in cold alcohol was divided into two parts, one of which (portion *a*) was soluble, the other (portion *b*) insoluble in hot alcohol.

Portion (*a*) was thoroughly washed with cold alcohol and treated as described later.

Portion (*b*) was dissolved in ether and left to stand over night under CO₂; next day a slight precipitate had settled out, the solution itself being

4. *Loc cit.*

almost clear. On centrifuging, the perfectly clear solution obtained was precipitated by acetone; on again dissolving in ether it gave practically a clear solution. This solution being again precipitated, the substance was dissolved in hot ethyl acetate, out of which it separated on cooling. It was then filtered, dried and analysed.

TREATMENT OF PORTION B

This portion was thoroughly extracted with cold absolute alcohol, whereby the greater part went into solution. This solution was evaporated to a small bulk and the syrupy residue dissolved in ether; the ethereal solution was then precipitated by acetone. The precipitate was dried, redissolved in ether and reprecipitated by acetone. By this means ordinary 'lecithin' was obtained in a pure condition, the ordinary contaminating substances present in the raw ethereal extract mass being relatively insoluble in cold alcohol.

Residue insoluble in cold alcohol was now treated with hot alcohol exactly as described under Portion A. By this means impurities such as cholesterin and fat went into solution. This treatment with hot alcohol was repeated four times, boiling alcohol being ultimately used. The residue was then further purified by dissolving in ether, precipitating with acetone, and finally dissolving in hot ethyl acetate, as described above; it was then dried and analysed.

The hot alcoholic solution on cooling deposited a voluminous precipitate of floccular masses. This was obviously composed in great part of fat and cholesterin, and was not examined.

This method of separating the raw material is exceedingly simple and quite efficient. It is much more quickly carried out than the method adopted under Portion A, and can be recommended as an easy means of separation of the mono- and di-phosphatides present in the ethereal extract of egg yolk, heart muscle, and probably in other substances.

In the following analyses Parts I and II were prepared as described under Portion A. Part III was prepared as mentioned under Portion B.

ANALYSIS

I

Nitrogen (Kjeldahl)

0.2596 gm. substance used 1.5 c.c. $\frac{n}{10}$ H_2SO_4 = 0.81 %
 0.3122 " " " 1.82 c.c. " = 0.82 %

Phosphorus (Neumann)

0.2411 gm. substance used 15.66 c.c. $\frac{n}{2}$ NaOH = 3.60 %
 0.1565 " " " 10.3 c.c. " = 3.64 %

Elementary Analysis

0.1625 gm. substance gave 0.3527 gm. CO_2 = 59.19% C
 and 0.1388 gm. H_2O = 9.56 % H

II

Nitrogen

0.3140 gm. substance used 1.78 c.c. $\frac{n}{10}$ H_2SO_4 = 0.794 %
 0.6952 „ „ „ 4 c.c. „ = 0.806 %

Phosphorus

0.4139 gm. substance used 26.55 c.c. $\frac{n}{2}$ NaOH = 3.55 %
 0.2729 „ „ „ 17.56 c.c. „ = 3.56 %

Elementary Analysis

0.1541 gm. substance gave 0.3329 gm. CO_2 = 58.92 % C
 and 0.1296 „ H_2O = 9.41 % H
 0.1701 „ „ „ 0.3686 „ CO_2 = 59.09 % C
 and 0.1413 „ H_2O = 9.29 % H
 0.1448 „ „ „ 0.3140 „ CO_2 = 59.14 % C
 and 0.1217 „ H_2O = 9.40 % H

Iodine Number

0.1691 gm. substance bound 0.1317 gm. Iodine = 76.8
 0.2254 „ „ „ 0.1759 „ „ = 77.1

III

Nitrogen

0.4836 gm. substance used 2.85 c.c. $\frac{n}{10}$ H_2SO_4 = 0.83 %

Phosphorus

0.2132 gm. substance used 13.82 c.c. $\frac{n}{2}$ NaOH = 3.59 %

Elementary Analysis

0.1128 gm. substance gave 0.2453 gm. CO_2 = 59.31 % C
 and 0.0963 „ H_2O = 9.55 % H

	Portion I		Portion II			Portion III	Average	Average of Cuorin (Erlandsen)
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.		
C	59.19	—	58.92	59.09	59.14	59.31	59.12	61.63
H	9.56	—	9.41	9.29	9.4	9.55	9.44	9.03
N	0.81	0.82	—	0.794	0.806	0.83	0.812	1.015
P	3.60	3.64	—	3.55	3.56	3.59	3.59	4.46
O	—	—	—	—	—	—	27.048	—

Relation N : P = 1 : 2.

Since the only substance of this nature hitherto isolated is the cuorin of Erlandsen, a comparison of the two substances is of some interest. A reference to the analyses shows that they are not identical. If, for instance, we consider the percentage of N and P in the two compounds, it is seen that the difference is relatively high. Again, the O content of my substance is higher than that of cuorin. At first it was thought that this difference in percentage composition might possibly be dependent on the occurrence of a certain amount of oxidation, prior to, or during, the preparation of the substance, and the extreme facility with which cuorin undergoes oxidation lent colour to this view. An examination of the

figures, however, shows that possible oxidation does not account for the difference; *a priori* the probability of any marked oxidation was unlikely, since all manipulations were so conducted so as to exclude as far as possible the presence of oxygen.

Since cuorin contains a relatively higher percentage of N and P than this substance, it is obvious that the addition of more O would lower the N and P percentage, and so tend to lessen the difference in composition.

For a specimen of oxidised cuorin, Erlandsen gives the following figures:—O 30.72, N 0.92, P 4.06 per cent.; while my substance gives O 27, N 0.812, P 3.59 per cent.

If we consider cuorin oxidised only to the extent of 27 per cent. (instead of 30 per cent. as given), this portion must contain something over 0.92 per cent. N and something over 4.06 per cent. P. Since my substance, oxidized to 27 per cent., contains only 0.812 per cent. N and 3.59 per cent. P, it is clear that possible oxidation is not the cause of the difference. It is probable that this is really accounted for by a difference in the fatty acids of the two substances.

Again, though the substances differ somewhat in their iodine figures, this difference is not great enough to satisfy the assumption that the one is but an oxidized form of the other.

Whether the somewhat prolonged treatment with hot alcohol necessary for the isolation of the substance in a pure condition has any action in causing some decomposition, and so a slight change in the ultimate formula of the phosphatide is perhaps worth consideration; if this is so, it is possible that the exact percentage composition may vary slightly, depending on the amount of hot alcohol treatment. Since, however, the different portions mentioned above yielded practically the same results, this is not very probable; in any case, it does not affect the fact that in egg yolk there is contained a well defined lecithin-like substance containing N : P in the proportion of 1 : 2.

PROPERTIES

This substance is obtained as a yellowish brown brittle substance, which, after being carefully dried, is easily ground to an exceedingly fine powder; it is much more brittle than cuorin, and though hygroscopic, is by no means markedly so in comparison with certain other phosphatides. In common with all lecithins it undergoes oxidation on exposure to air. It is insoluble in cold alcohol but somewhat soluble in boiling alcohol, and thus differs from cuorin, which is said to be insoluble in boiling

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alcohol; in chloroform, ether and petroleum ether it dissolves easily at the ordinary temperature. In ethyl acetate it dissolves on heating, to be thrown out on cooling. From its ethereal solution it is precipitated by acetone. With water it forms an emulsion. With platinum chloride it gives double salts, and by hydrolysis gives fatty acids and glycerophosphoric acid; no base of the nature of choline could be obtained. When heated to 90° to 100° C. it changes colour, but an exact melting point could not be determined. After hydrolysis with weak hydrochloric acid no reducing carbohydrate was obtained. All its general properties show that here we have a typical phosphatide substance.

WHITE SUBSTANCE SOLUBLE IN HOT ALCOHOL AND SEPARATING OUT ON COOLING

This substance (Portion *a*, page 163) was dissolved in hot alcohol, filtered hot, allowed to cool, and again filtered. By this means a voluminous precipitate of whitish snowflake-like material was obtained, which on drying shrunk to a very small volume. This process was repeated four times. By this means a fine whitish granular powder was obtained, which was completely soluble in ether, chloroform and benzene. On the addition of acetone to any of these solutions it was, after a little time, but not immediately, precipitated.

As some doubt exists as to the composition of this 'white substance,' Erlandsen having found N but no P in apparently similar material, while Thierfelder and Stern found both N and P, I made some further investigations with the following results.

In this particular preparation there was neither N nor P present. Melting point was easily observed being 62° to 63° C. When burned on platinum foil it left no inorganic residue. After boiling for some time with HCl no substances of a reducing nature were obtained. Saponification with Ba(OH)₂ failed to give any substances of basic nature precipitated by platinum chloride. From these observations it was apparent that this substance was probably of the nature of a fat. A combustion experiment showed this fat to be tripalmitin.

Analysis

0.1068 gm. substance gave 0.2964 gm. CO₂ = 75.70 % C
and 0.1179 „ H₂O = 12.35 % H

Calculated for Tripalmitin

C₅₁H₉₈O₆ = 75.86 % C
and 12.24 % H

I would here remark that no investigation of any of the filtrates used in purifying the above substance was made. This tripalmitin seems to be somewhat soluble in 'lecithin,' so it is doubtful if it is possible to obtain 'lecithin' absolutely free from fat.

SUMMARY

The ethereal extract of egg yolk contains as well as ordinary 'lecithin' with N : P as 1 : 1 another lecithin-like substance—a mono-amino-diphosphatide body—with N : P as 1 : 2.

This substance, as above obtained, differs somewhat in constitution and properties from the substance, cuorin, prepared from heart muscle, the difference being probably dependent on the presence of different fatty acids.

The other substance in the ethereal extract which dissolved in hot alcohol and separated out on cooling was found to be pure tripalmitin.