Studies on the Lipids of Egg Yolk

Part II.† Nature of Sphingolipids

Sir:

Egg yolk is known to contain about 30% of lipids. We recently examined and reported the chemical composition of the lipids. In the course of the study, we noticed that the egg yolk lipids possessed a small amount of sphingolipids, which were once recorded in the literature but had not been indentified yet. The present paper describes the classification and the chemical constitution of sphingolipids in egg yolk.

![Thin-layer Chromatogram of Sphingolipid Fraction of Egg Yolk.](image)

Developed with chloroform-methanol-water (65:25:4) and detected by spraying with 50% sulfuric acid.

- a. Sphingolipid fraction obtained from egg yolk
- b. Standard ceramide
- c. Standard cerebroside
- d. Standard sphingomyelin

† I. Commun: See 1).


Fresh egg yolk of White Leghorn was lyophylyzed and extracted with chloroform-methanol (2:1) to obtain total lipids. Neutral lipids were removed as much as possible from the lipids by washing with cold acetone. The residue was treated with mild alkali to decompose ester-lipids leaving sphingolipid fraction. Thin-layer chromatography of the fraction revealed three spots, as shown in Fig. 1, corresponding respectively to ceramide, cerebroside and sphingomyelin, the last being superior to the former two.

The sphingolipid fraction was subjected to silicic acid column chromatography with chloroform and methanol, and each lipid eluted was purified by recrystallization in ether. The respective sphingolipid gave a single spot on the thin-layer chromatogram, and perfectly agreed with the authentic samples in the infrared spectrum.

In order to investigate the chemical composition, each sphingolipid was hydrolyzed with potassium hydroxide in methanol or with hydrochloric acid in methanol. The resultant hydrolysates, i.e., fatty acids, long chain bases and carbohydrates, were analyzed by thin-layer chromatography and by gas-liquid chromatography in the usual manner.

6) e.g., see A. Makita, In Methods of Biological Chemistry (in Japanese) VII, ed. by Editorial Board of Protein-Nucleic acid-Enzyme, Kyoritsu Shuppan Co., Ltd., Tokyo, 1967, pp. 106~128.
Ceramide was found to contain as the component acid sixteen kinds of fatty acid covering lignoceric, behenic, tricosanoic and so forth, among which lignoceric acid was predominant; and as the component base two kinds of long chain base, sphingosine and dihydrospingosine, the former being in larger amount. Thus, the representative ceramide in egg yolk was postulated to be N-lignoceryl-sphingosine. Cerebroside was found to contain fourteen fatty acids covering lignoceric, stearic, hydroxylignoceric, hydroxytricosanoic(?), hydroxybehenic and so forth, among which hydroxy fatty acids were dominating. It also contained two long chain bases, sphingosine and dihydrospingosine, the former being in the majority; and two hexoses, glucose and galactose, the amount of which was equal with each other. Therefore, cerebroside in egg yolk was considered to consist of two types, glucosyl ceramide and galactosyl ceramide, in the ratio of 1:1. Sphingomyelin was recognized to contain eleven fatty acids covering palmitic, lignoceric, stearic, behenic and so forth, among which palmitic acid was prevailing; and two long chain bases, sphingosine and dihydrospingosine, the former being in a greater amount. Accordingly, the typical sphingomyelin in egg yolk was assumed to be N-palmityl-sphingosyl-phosphorylcholine.

These data show that egg yolk used in the experiment comprised at least three kinds of sphingolipid, ceramide, cerebroside and sphingomyelin and that sphingolipids in the egg yolk had the different pattern of the component fatty acids and the similar pattern of the constituent long chain bases.

Details of the study will be published elsewhere in the near future.

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