THE ISOLATION OF A METHYLCHOLINE CONTAINING PHOSPHOLIPID FROM PHORMIA REGINA LARVAE[1]

Loran L. Bieber, Victor J. Brookes, Vernon H. Cheldelin and R. W. Newburgh[2]

Science Research Institute
Oregon State University
Corvallis, Oregon

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Although carnitine, β-hydroxy-γ-butyrobetaine, occurs in many animal systems, its function and intermediary metabolism remains obscure (Fraenkel 1957).

The results of previous experiments in our laboratory indicated that on a chemically defined diet, carnitine and several related compounds, including γ-butyrobetaine, etc., replaced the choline requirement. (Hodgson, Cheldelin and Newburgh 1956, 1960). Since choline is a major constituent in Phormia regina phospholipids it seemed reasonable to postulate that when larvae are reared on a diet devoid of choline but containing carnitine, a phospholipid in which choline is replaced by carnitine might be observed. This communication reports the formation of a new "lecithin" when blowflies are reared in the presence of carnitine.

MATERIALS AND METHODS

The larvae were grown aseptically on an amino acid diet similar to the method described by McGinnis et al. (1956) except that the vitamin content was increased 2.5 fold and the agar concentration changed to 1.8 g per 100 ml liquid. In addition each 500 ml flask contained 0.4 g of Hammersten quality casein, 160 mg RNA and 3.4 g of amino acid mixture.

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Choline was replaced by carnitine in the appropriate samples. Microbiological assay of the carnitine diet using a choline requiring strain of Neurospora crassa indicated a negligible amount of choline.

The chloride of methylcholine (1-trimethylamino isopropyl alcohol) was synthesized from 1-amino isopropyl alcohol and an excess of methyl iodide by a method similar to the one that Mazzetti and Lemmon (1957) used for the synthesis of carnitine from 3-hydroxyl-4-aminobutyric acid. The compound gave a positive Dragendorf test. After recrystallization from ethanol-ether the chloride melted at 163-165°C. The melting point of the chloride of trimethylamino isopropyl alcohol has been reported to be 165°C.

The phospholipids were isolated and separated by a method similar to the one used by Bieber et al. (1961) except that smaller samples and columns were used (12 g silicic acid and 6 g Hyflo). The phospholipids were hydrolyzed overnight in 6 N HCl at 105°C and chromatographed by the ascending technique on washed Whatman #1 paper in one or more of the following solvents.

A. 95% ethyl alcohol :28% NH_4OH (95:5)
B. Phenol :1-butanol :98-100% formic acid :H_2O (50:50:3:10) + solid KCl
C. 1-butanol :methanol :benzene :H_2O (2:1:1:1)
D. Butanone :methyl cellosolve :20% HOAc (40:15:20)

DISCUSSION AND RESULTS

Sterile larvae which had been grown on a cholineless, carnitine-containing diet were used as a source of material. Tissue lipids were extracted with chloroform-methanol (2:1) and placed on a silicic acid-Hyflo column. The phospholipid profile appeared to be very similar to that reported earlier for larvae grown on a normal diet. However, a Dragendorf-detectable substance in an HCl hydrolyzate of the phospholipid corresponding to the lecithin peak did not coincide with choline in the chromatographic solvent used. The compound was less sensitive to Dragendorf reagent, the color was slower in developing, and it gave a slightly different color than choline. The color difference was more

pronounced when the nitrogen bases were detected with iodine vapors prior to spraying with Dragendorf's reagent. The compound was not methylaminoethanol, dimethylaminoethanol, carnitine or betaine.

The chloride of methylcholine was found to have the identical Rf as the unknown in the four different solvents used, as shown in Table I. The color of the Dragendorf spot was the same for both unknown and methylcholine.

TABLE I

Solvent[a]	Rf Unknown Hydrolyzed in 6N HCl	Rf Choline Chloride	Rf Methylcholine Chloride
A	0.62	0.49	0.62
B	0.72	0.59	0.72
C	0.41	0.36	0.41
D	0.62	0.56	0.62

(a) refers to solvents A, B, C, D given in Materials and Methods section.

The phospholipid in question travels slightly faster than the normal lecithin does under similar conditions on a silicic acid column. It represents approximately 20% of the total phospholipid obtained from elution of the column. The results represent at least four different batches of sterile larvae grown on a carnitine-containing diet. Each column gave the same general phospholipid profile and methylcholine was the principal nitrogenous component of the phospholipid eluted in the position expected for lecithin. In one of the columns a trace of choline was detected in this peak but the choline/phosphorus ratio determined by microbiological assay was less than 0.01. The same assay on another peak indicated the complete absence of choline. These results also demonstrate that methylcholine is unable to replace choline in the strain of Neurospora crassa (strain #34486) used for assay of choline. Nitrogen/phosphorus ratios of this fraction from two of the columns were 1.05 and 1.2, and the ester/phosphorus ratio was 2.0.

In order to determine whether the abnormal phospholipid was caused by the amino acid diet or carnitine, larvae were grown on an identical diet, except that choline had been restored, replacing carnitine. The phospholipid pattern was identical to the profile obtained from larvae grown in the presence of carnitine except for the "lecithin" peak. This fraction was examined and the nitrogen base was found to be choline and no methylcholine was detected.

From the results obtained it appears that carnitine is responsible for the presence of methylcholine as a phospholipid constituent in the larvae of Phormia regina grown on a diet where choline is replaced by carnitine. Further, while growing on such a diet a new "lecithin" is formed in which the nitrogen base appears to be methylcholine rather than choline.

A more detailed report of these investigations will be reported elsewhere.

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