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Phospholipids of *Tenebrio molitor* larvae were separated on columns of DEAE-cellulose and silicic acid-Hyflo Super Cel. The major phosphatide components were identified by various analytical procedures.

Phosphatidyl choline was demonstrated to be the predominant phospholipid with a high percentage of phosphatidyl ethanolamine. A small amount of inositol-containing phosphatides were present. Phosphatidyl serine was undetectable but an unknown amino acid-containing phosphatide comprised one of the fractions. Evidence is also presented for the existence of a choline-containing sphingolipid.

The results show that the phospholipid pattern was similar to those of other insects.

PHOSPHOLIPIDS OF TENEBRIO MOLITOR LARVAE

by

FRANCIS XAVIER KAMIENSKI

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MASTER OF ARTS

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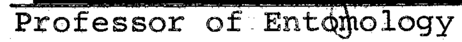
APPROVED:



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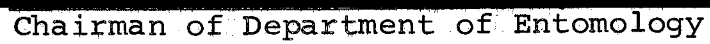
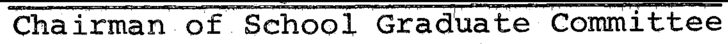
In Charge of Major



Chairman of Department of Entomology



Chairman of School Graduate Committee

Dean of Graduate School

Date thesis is presented May 14, 1964

Typed by Erma McClanathan

To the Holien Family

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LEGEND OF ABBREVIATIONS

PC	Phosphatidyl Choline
PEA	" Ethanolamine
PI	" Inositol
Sph	Sphingomyelin
EA	Ethanolamine
Chol.	Choline
Ser	Serine
N/P	Nitrogen/Phosphorous
E/P	Ester/Phosphorous
C:M	Chloroform:Methanol
Unk	Unknown
A.A.	Amino Acids

PHOSPHOLIPIDS OF TENEBRIO MOLITOR LARVAE

Although many facets of insect biochemistry particularly hemolymph composition have been investigated, the phospholipids of these animals have not been studied in any great detail. Such studies were hindered by lack of adequate methods for the isolation and characterization of phospholipids and the large amount of material needed for analysis. However, development of such analytical methods as column and thin-layer chromatography and infra-red spectroscopy helped overcome some of the difficulties, although no single method appears to resolve completely a complex mixture of phosphatides. The most frequently used separation is chromatography on silicic acid-Hyflo Super Cel columns. Rouser's (38) separation of phospholipids on DEAE-cellulose is one of the most recent chromatographic techniques and is receiving wide application. The chemistry, methods of analysis, and characterization of phospholipids have been reviewed by several persons. Hanahan's (23) book is probably the most comprehensive.

Insects show remarkable differences in their morphology and physiology as well as in their compositions

and biochemical properties. The content of the chief chemical constituents in the body of different insect species varies widely. According to Niemierko (34) probably the major differences concern the amount of lipids.

Reviews on insect fat have been made by Gilmour (21), and Hilditch (26). Timon (43) found the fat composition amounted to less than 1% of the total weight in certain Lepidoptera. The amount of fat in some insects is often surprisingly high, making up 69% of the dry weight of the male locusts (Fawzi, 1961).

It is difficult to generalize about the lipid composition in so diverse a group since the published information is fragmentary and almost all analyses are for whole insects and not for that extracted from isolated body tissue.

According to Niemierko (34), the content of phospholipids and other compound lipids in the insect body is usually low; but in the beetle larvae, *Pachymerus dactis*, tissue phosphatides comprise as much as 50% of the total lipid (Collin, 1933).

The amounts of different classes of insect phospholipids have been investigated, but quantitative data of individual phosphatides have seldom been reported.

Choline was generally present as the major base in the phosphatides, while the bases ethanolamine, serine, inositol, and sphingosine were present in lesser amounts (Beament *et al.*, 1963).

Patterson *et al.* (35) have studied the phosphatides in the central nervous system of the honey bee, *Apis mellifera*. This study, although mainly qualitative, showed that phosphatidyl ethanolamine was the predominant phosphatide, comprising 70% of the total phospholipid. They also showed that the central nervous system of the bees contained the same types and roughly the same proportions of lipids as are found in vertebrates. In contrast to the vertebrates, no cerbroside was detected in insect brain tissue. Among dietary lipid constituents which have the greatest effect on fecundity in *Doryphora*, Gaston (20) noted from different lipid fractions that the choline phospholipid was predominant and most important. Comparison between sexes demonstrated the female to contain double the amount of choline phospholipid as the male.

Wren and Mitchell (46) have qualitatively studied the phosphatides of adult *Drosophila melanogaster* and found phosphatidyl ethanolamine to be the predominant phospholipid in this organism. They were, however, unable

to detect any sphingomyelin, which is contrary to what has been found in other systems. In *Aedes* DDT-resistant and susceptible larval strains Fast *et al*. (11) observed phosphatidyl ethanolamine to be the major phospholipid, comprising 84% of the phospholipid fraction, while the phosphatidyl choline composition was 14%. In a very brief note Sridhara and Bhat (42) reported that phospholipids in pupal fat of *Bombyx mori* contain 62% phosphatidyl choline but only 8.1% phosphatidyl serine, 16% sphingomyelin and 3.6% phosphatidyl ethanolamine. Crone and Bridges (10) reported that the adult housefly contains 65% phosphatidyl ethanolamine as well as an ethanolamine sphingolipid. In contrast, Chojnacki and Korzybski (8) have shown phosphatidyl choline to comprise 47% of the total lipid and phosphatidyl ethanolamine 45% in the moth *Arctia caia*. Sphingomyelin was estimated as 10% of the phosphatides.

The most recent and comprehensive investigation of insect phospholipids is on the blowfly, *Phormia regina*, as presented by Bieber *et al*. (2). Quantitative data on the various phosphatide fractions is given. Phosphatidyl ethanolamine was shown to comprise 63% of the egg, larva, and adult stages; while phosphatidyl choline was the second main component representing 19% of the phospholipid.

An ethanolamine sphingolipid was also demonstrated (3). When the larvae were reared on an amino acid diet and choline was replaced by carnitine, the majority of the choline in the lecithin fraction was replaced by β-methyl choline (3). If the dietary choline is replaced by either dimethyl aminoethanol or 1-dimethyl aminoisopropyl alcohol, these compounds appeared as phospholipid components (4).

Fraenkel (16) has shown the mealworm, *Tenebrio molitor*, to require carnitine as a dietary constituent. Carnitine has been found in many sources (17) and although it is often present in large amounts, little is known of its biosynthesis and metabolism. Early biochemical investigations have been reviewed by Fraenkel (17, p. 74-118), and the biosynthesis and metabolic fate of carnitine has been studied by many groups. A stimulation of fatty acid oxidation was found by Fritz (18,19). Yue and Fritz (47) noted that most of the tritium-labeled carnitine injected into dogs was excreted as carnitine or deposited in the tissues. Of the latter, a small amount of activity was found in the lecithin fraction of phospholipids. Mehlman and Wolf (33) have found a small amount of bound carnitine in the phospholipids of hen's eggs after they had been injected with chain-labeled carnitine. Carnitine precursors have been studied in the rat by Lindstedt and

Lindstedt (29,30,31) as well as by Bremer (5,6,7). They reported γ-butyrobetaine was a carnitine precursor. Bieber *et al.* (3) results suggest that carnitine is decarboxylated to β-methyl choline by *Phormia* larvae.

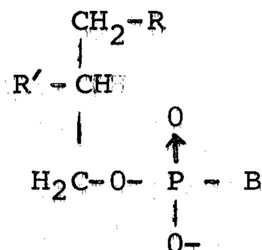
The undertaking of this particular research stems from previous studies in this laboratory where it was found that substitution of various dietary compounds were shown to have a marked effect on phospholipid composition of *Phormia regina*. When choline was replaced by carnitine, a β-methyl choline lecithin was observed.

Since *Tenebrio* requires in addition to choline a substantial amount of carnitine for maximal growth, it seems reasonable to postulate that when larvae are reared on a diet containing carnitine, a phospholipid in which choline is replaced by carnitine or β-methyl choline might be observed.

This thesis will describe the principal phospholipids of *Tenebrio molitor* larvae which were reared on a natural diet.

The structures of the principal phosphatides described in this thesis are shown below.

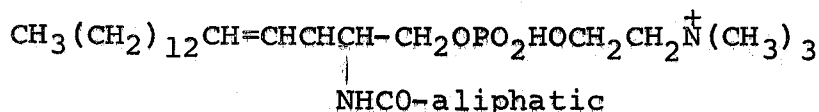
The general structure of the glycerol-containing phospholipids is



When the phospholipid is in the diester form R and R' are fatty acid residues (-O-CO-aliphatic); when the phosphatide is a plasmalogen at least one of the R groups contains a vinyl ether (-O-$\overset{H}{C}$=$\overset{H}{C}$-aliphatic); if it is in the lyso form at least one of the R groups is an -OH. The structure of B can vary greatly.

Name of Phospholipids	Structure of B
Phosphatidyl choline (lecithin)	$-O-CH_2-CH_2-\overset{+}{N}(CH_3)_3$
" ethanolamine	$-O-CH_2-CH_2-NH_2$
" serine	$-O-CH_2-CH\overset{+}{N}H_3-COO^-$
" carnitine	$(CH_3)_3-\overset{+}{N}-CH_2-CHOH-CH_2-COO^-$
" inositol	$C_6H_{12}O_6$
Phosphatidic acid	-OH

Sphingomyelin has the following structure:



MATERIALS

Tenebrio larvae were obtained from Carolina Biological Supply Company; 100% whole wheat flour from Fischer Flouring Mills Company; Brewer's Yeast-USP, phosphatidyl ethanolamine, serine, phosphatidyl serine and phosphatidyl inositol were obtained from Nutritional Biochemicals Company; cardiolipin from Sigma Chemical Company; myo-inositol from Fischer Scientific Company; ethanolamine from Eastman Organic Corporation; choline chloride from Merck and Company; chloroform and methanol were Baker analyzed reagents; sphingomyelin from Sylvana Chemical Company; lecithin from British Drug Houses Ltd.; 100 mesh silicic from Mallinckrodt Chemicals; Hyflo Super Cel from Johns-Manville Products; *Kloeckera apiculata* No. 9774 from the American Type Culture Collection; inositol assay media from Difco Laboratories; Baker analyzed hydrogen peroxide from J. T. Baker Chemical Company; DEAE-cellulose (Bio-Rad-Cellex-D) from Bio-Rad Laboratories; silica gel H (without calcium sulfate binder) from Brinkmann Instruments Incorporated.

METHODS

Commercially obtained *Tenebrio* larvae were reared on a natural diet consisting of 100% whole wheat flour and Brewer's yeast as described by Fraenkel (16). Twenty-five grams of larvae, approximately the same size and age (six months) were collected for each experiment. The larvae were killed in chloroform and homogenized immediately.

The entire tissue was extracted by three successive treatments in a Waring blendor for five minutes in the presence of chloroform:methanol (2:1). One-hundred-seventy mls. of this solvent was used for each homogenization.

The homogenized material was allowed to stand in the solvent for one hour after the second treatment and three hours after the third treatment. The organic phase was separated from a solid phase by centrifugation. No special precautions were taken to avoid oxidation. The total volume of the solvent was twenty times the weight of the wet tissue. The chloroform:methanol extracts were combined and washed with 0.2 volume of 0.79%/NaCl and then washed 0.2 volume of a 50:50 mixture of 0.79% NaCl and methanol.

The lipid isolation procedure was tested for

completeness in the following manner. The centrifuged residue was extracted a fourth time with chloroform: methanol (2:1) by homogenizing for five minutes in a Waring blendor and letting the mixture stand for twenty-four hours at room temperature. After filtering the suspension, the total amount of phosphorous in the extract was found to be 1.2 μmoles which was less than 1% of the phospholipid phosphorous obtained in the first three extractions. Therefore, extraction of phosphatides with this solvent pair appears to have been reasonably effective with three extraction operations, as routinely used.

Loss of lipid phosphorous during the washing procedures was also tested. The combined aqueous wash was extracted three times with chloroform:methanol (2:1) and the amount of lipid phosphorous in this wash was below the limits of detection.

The washed lipid extract was filtered and diluted to a known volume. Aliquots were taken and total phosphorous was determined by Bartlett's method (1). The extract was evaporated to dryness under vacuum and at less than 45°C. It was then redissolved in 4 mls. of chloroform. A small amount of chloroform-insoluble material was evident after the evaporation.

The redissolved lipid extract was applied to a 13 mm. diameter column containing 10 grams of silicic acid and 5 grams of Hyflo-Super Cel as described by Hanahan et al. (22). Prior to use the silicic acid had been dried at 110° overnight. The neutral lipids were eluted with 500 mls. of chloroform and the phospholipids with 500 mls. of methanol.

The methanol fraction was evaporated to dryness in vacuo at 45° C. The material was redissolved in 4 mls. of chloroform, applied to a DEAE-cellulose column and eluted according to the procedure of Rouser et al. (37). This column was 13 mm. in diameter and consisted of 12 grams of DEAE-cellulose which was washed after the method of Rouser. After the final chloroform wash, the DEAE-cellulose was poured into the column as a slurry in chloroform. The elution scheme is shown in Figure 1. Fractions of 400 drops were collected with an automatic drop counting fraction collector.

The positions of the phospholipid peaks were determined by phosphorous analysis. The tubes containing the individual peaks were combined and diluted to a known volume. An aliquot of each peak was taken to determine the recovery and total phosphorous. Recovery of total

lipid phosphorous from DEAE-cellulose ranged from 90-93%.

The pooled samples, which were shown by thin-layer chromatography to be mixtures of various phosphatides, were rechromatographed on silicic acid Hyflo-Super Cel columns and eluted with varying amounts of methanol in chloroform depending on the type of separation desired. Fractions of 400 drops were collected. The positions of the phospholipid peaks were determined by phosphorous analysis and the tubes containing the individual peaks were combined and diluted to a known volume. An aliquot of each peak was taken to determine the recovery and total phosphorous. Recovery of total lipid phosphorous from these columns was 98-99%.

Various analytical determinations were done on the material which comprised the different fractions. Nitrogen was determined by the method of Lang (27). The ester content was estimated by the method of Snyder and Stephens (41). Paper chromatography of the nitrogenous bases was done on 6 N HCl hydrolysates. The initial detection of spots was with iodine vapor. Ethanolamine and amino acids were detected with ninhydrin and choline with Dragendorf's reagent. Inositol was detected by the method of Feigel and Gentil (13) and determined quantitatively by microbiological assay with *Kleockera apiculata* (ATCC 9774).

The assay medium resembled closely that of Williams _et al_. (45).

Separation and detection of unhydrolyzed lipid fractions was performed according to the procedure of Skipski _et al_. (40) using purified phospholipids as reference compounds. The initial detection was with iodine vapors. Phosphatides were detected with the Zindzase reagent specific for phosphorous containing compounds. Phosphatidyl ethanolamine and amino acid-containing phospholipids were detected with a mixture of 0.2% ninhydrin reagent in butanol; phosphatidyl choline with Dragendorf's reagent; plasmalogens with Schiff's aldehyde reagent; and sphingomyelin with sodium fluorescein under ultra-violet light (Saito, 1960).

The following solvent systems were used for paper chromatography of 6 N HCl hydrolysates: 95% ethyl alcohol: 28% NH_4OH (95:5), phenol: 1-butanol: 98-100% formic acid: water (50:50:3:10) plus solid KCl with papers impregnated with 1% KCl, 1-butanol: acetic acid: water (4:1:5).

RESULTS

The results of the separation of different classes of phospholipids from Tenebrio larvae on DEAE-cellulose are shown in Figure 1. The total phosphorous in each fraction was determined by combining the eluates of each individual peak, diluting to a known volume, and then determining the total phosphorous content of the fraction. This was equivalent to the organic phosphorous in the fraction, since the inorganic phosphorous was negligible. Recovery of the phosphatides varied from 90-93%.

Figures 2, 3 and 4 show the results after rechromatography of the three major peaks in Figure 1 on silicic acid-Hyflo Super Cel columns. Each individual peak was diluted to a known volume and total phosphorous determined. Figure 5 shows the separation of the unhydrolyzed lipids from the various peaks obtained on DEAE-cellulose and silicic acid-Hyflo columns.

Analytical data on the various peaks is presented in Table I. Peak Ia(PEA) was shown to contain only phosphatidyl ethanolamine and was eluted as a yellow fraction with the solvent. A small amount of a non-phosphorous material was also present. The Schiff aldehyde reagent indicated the phospholipid to be a plasmalogen. The N/P

ratio was 1.0 and E/P ratio was 2.0. This fraction comprised 1.9% of the total lipid phosphorous.

Phosphatidyl choline was the only component present in peak Ib(PC) and accounted for 20.8% of the total phospholipid phosphorous. The N/P and E/P ratios were in good agreement with that expected for phosphatidyl choline. No plasmalogen was demonstrated.

The third fraction, peak Ic(Sph) had a N/P ratio of 2.0 and a E/P ratio of zero indicating a sphingolipid. Comparison of Rf values of this component with reference sphingomyelin together with its reaction with sodium fluorescein confirmed the presence of sphingomyelin. A positive Dragendorf test on 6 N HCl hydrolysates and unhydrolyzed lipid show choline as the only nitrogenous base. This material comprised 1.1% of the total lipid phosphorous and contained only one phosphatide.

Phosphatidyl ethanolamine was present as the only phosphatide constituent of Peak IIa (PEA), and comprised 33.3% of the total lipid phosphorous. Traces of free ninhydrin positive components were detected. A positive aldehyde test indicated the presence of plasmalogen. This fraction had a N/P ratio of unity and a E/P ratio of less than 2.0.

The second fraction, Peak IIb (PC) contained only phosphatidyl choline and comprised 22.1% of the total phospholipid phosphorous. The N/P and E/P ratios were slightly lower than the expected value. No plasmalogen was detected.

Peak IIc (Sphingomyelin) in Figure 3 contained choline as the only base and accounted for 5.7% of the total phospholipid phosphorous. A N/P ratio of 2.0 and an E/P ratio of zero indicated a sphingolipid. Thin-layer chromatography of unhydrolyzed lipid indicated the sphingolipid to be the only phosphatide present.

Peak III of Figure 1 contained a high E/P ratio. Thin-layer chromatography demonstrated the presence of phosphatidyl ethanolamine along with two other unidentified phosphatides. See Figure 6. A positive test for an aldehyde was observed. Since this peak represented only 5.3% of the total lipid phosphorous it was not investigated further.

Phosphatidyl ethanolamine was the only constituent in Peak IV and represent 0.6% of the total lipid phosphorous. It had a N/P ratio of 2.2 in accordance with the large amount of ninhydrin positive amino nitrogen. An anomolous E/P ratio of 10.2 was observed. This

fraction was not investigated further since it represented such a small percent of the phospholipid.

Peak V represented 4.7% of the total lipid phosphorous and had high N/P and E/P ratios. Paper chromatography of 6 N HCl hydrolysates indicated this fraction to contain two unidentified amino acids neither of which was serine or threonine. See Figure 7. Thin-layer chromatography of unhydrolyzed lipid failed to reveal any phosphatide components.

The smallest fraction, Peak VIa, contained a phosphatide component which had Rf values similar to cardiolipin. This peak was not investigated further since it represented only 0.7% of the total lipid phosphorous.

Peak VIb (PI) contained two phosphatide components one of which was an inositol phospholipid. Thin-layer chromatrography has shown the other component to have Rf values similar to cardiolipin. The N/P ratio was 4.1 and subsequent washing failed to lower this ratio significantly. The E/P ratio was 1.6. If this were all momophosphoinositide an E/P ratio of 2.0 would be expected. Microbiological assay shows a inositol/P ratio of 0.41. This is a minimum estimate of the inositol present, since it is known that phosphatidyl inositol is difficult to hydrolyze

completely under rather mild conditions. The microbiological method will not differentiate diphospho- and triphosphoinositide from monophosphoinositide. This peak represented 6.7% of the total lipid phosphorous.

Peaks Ia, IIa, and III contained plasmalogen. Since the aldehyde test is qualitative no estimate is available of what percent of the lipid is in the plasmalogen form.

Figure 1

Chromatography of phospholipids on a DEAE-cellulose column. 12 grams DEAE-cellulose; 13 mm. column diameter. Collected 400 drops per tube. Phosphorous was determined on each tube. PE,PEA=phosphatidyl ethanolamine; PC=phosphatidyl choline; Sph=sphingomyelin; UNK=unknown; PI=phosphatidyl inositol; P=phosphorous; $HCCl_3$= chloroform; M=methanol; HAc=acetic acid; AM=ammonium hydroxide.

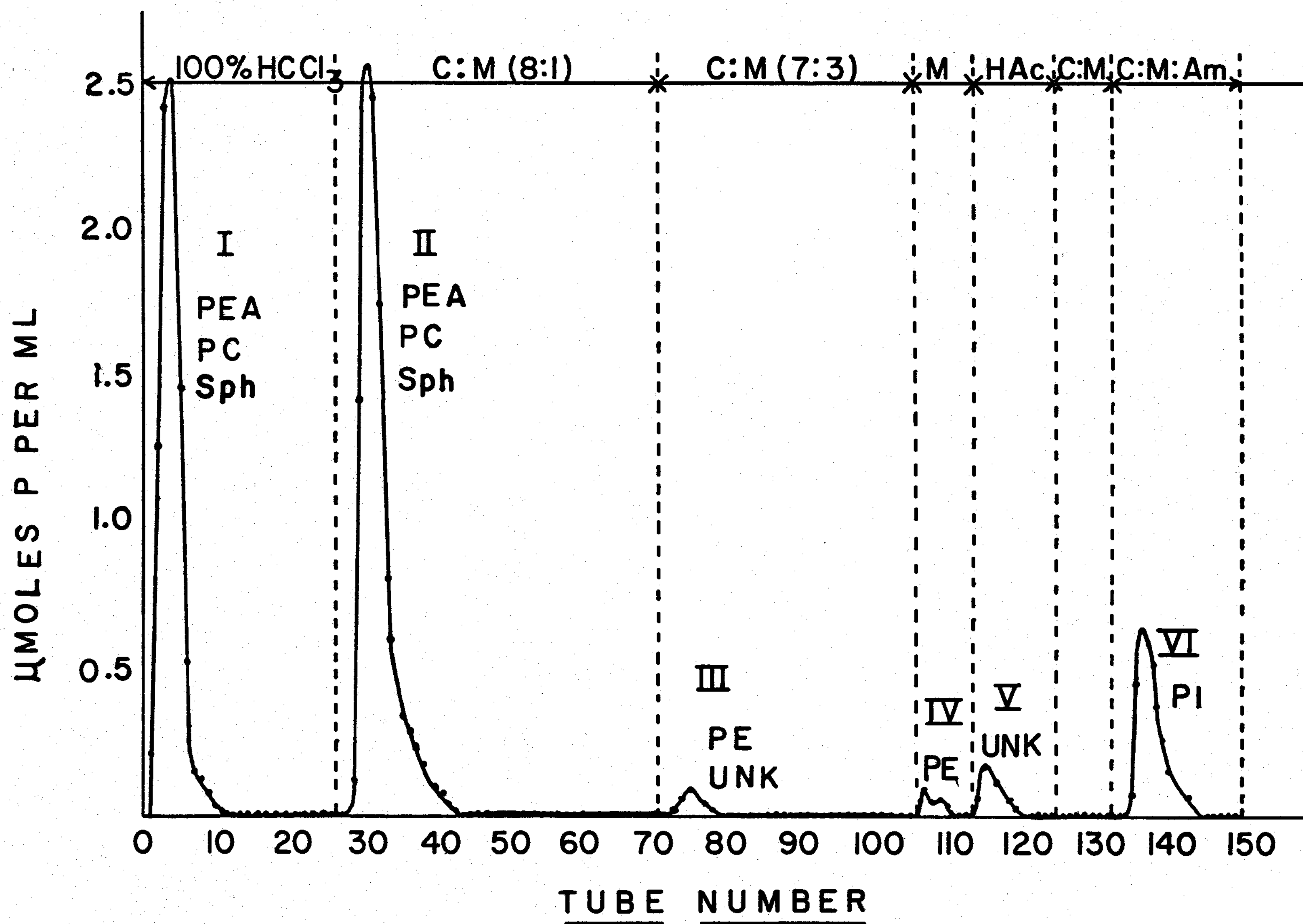

100% HCCl3
C:M (8:1)
C:M (7:3)
M
HAc
C:M
C:M:Am
2.5
2.0
1.5
1.0
0.5
I
PEA
PC
Sph
II
PEA
PC
Sph
III
PE
UNK
IV
PE
V
UNK
VI
PI
μMOLES P PER ML
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
TUBE NUMBER

Figure 2

Rechromatography of Peak I of Figure 1 on 8 grams silicic acid-4 grams of Hyflo Super Cel. Column diameter 13 mm. Collected 400 drops per tube. Phosphorous was determined on each tube. The neutral lipid was eluted from the column with chloroform prior to elution with C:M (4:1). $CHCl_3$, C = chloroform; M = methanol; PEA = phosphatidyl ethanolamine; PC = phosphatidyl choline; Sph = sphingomyelin.

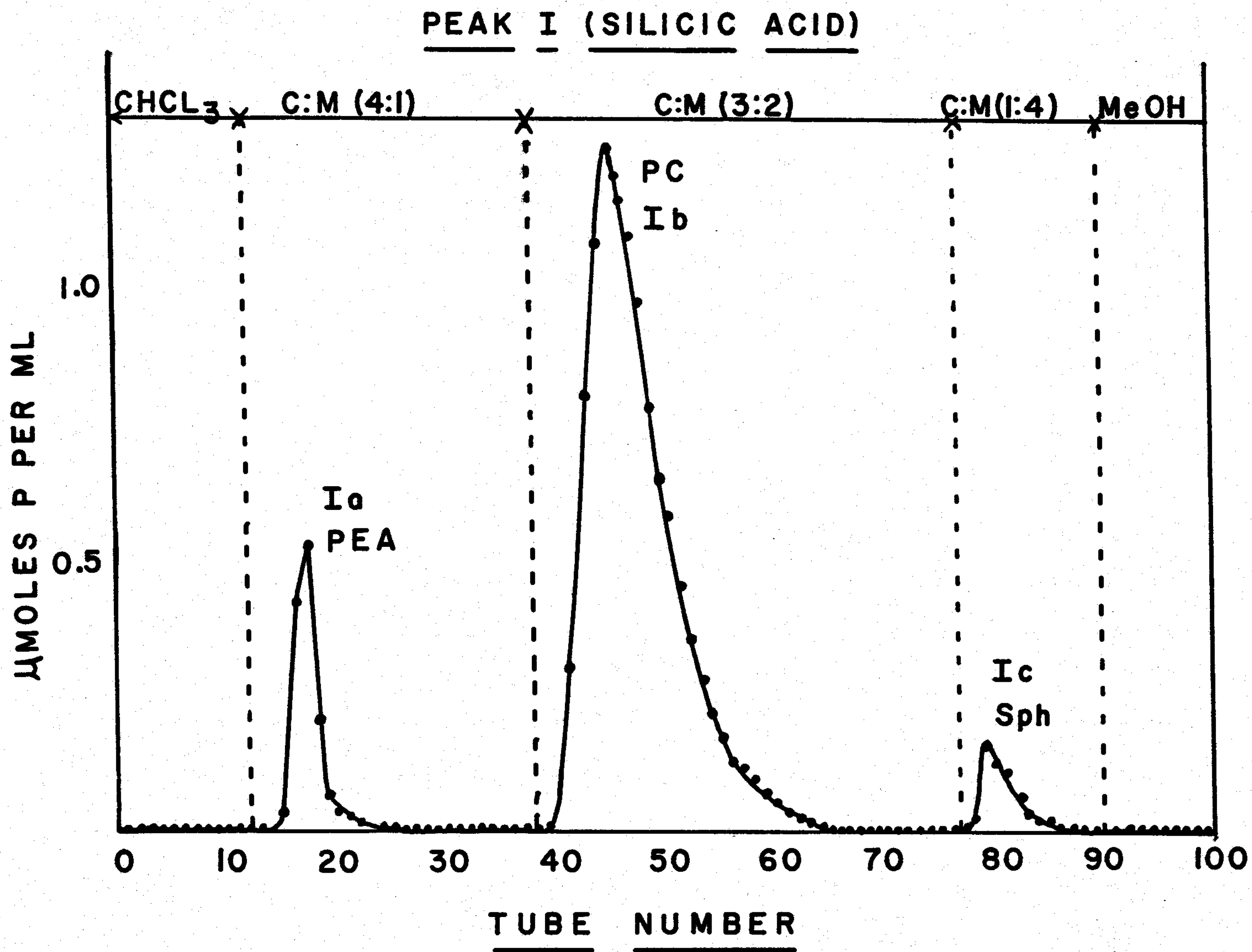

PEAK I (SILICIC ACID)
CHCL3
C:M (4:1)
C:M (3:2)
C:M(1:4)
MeOH
PC
Ib
Ia
PEA
Ic
Sph
μMOLES P PER ML
1.0
0.5
0
10
20
30
40
50
60
70
80
90
100
TUBE NUMBER

Figure 3

Rechromatography of Peak II of Figure 1 on 8 grams silicic acid; 4 grams Hyflo Super Cel; column diameter 13 mm. Collected 400 drops per tube and phosphorous determined on each tube. The neutral lipid was eluted from the column with chloroform prior to elution with C:M (4:1). $HCCl_3$,C = chloroform; M = methanol; PEA = phosphatidyl ethanolamine; PC = phosphatidyl choline.

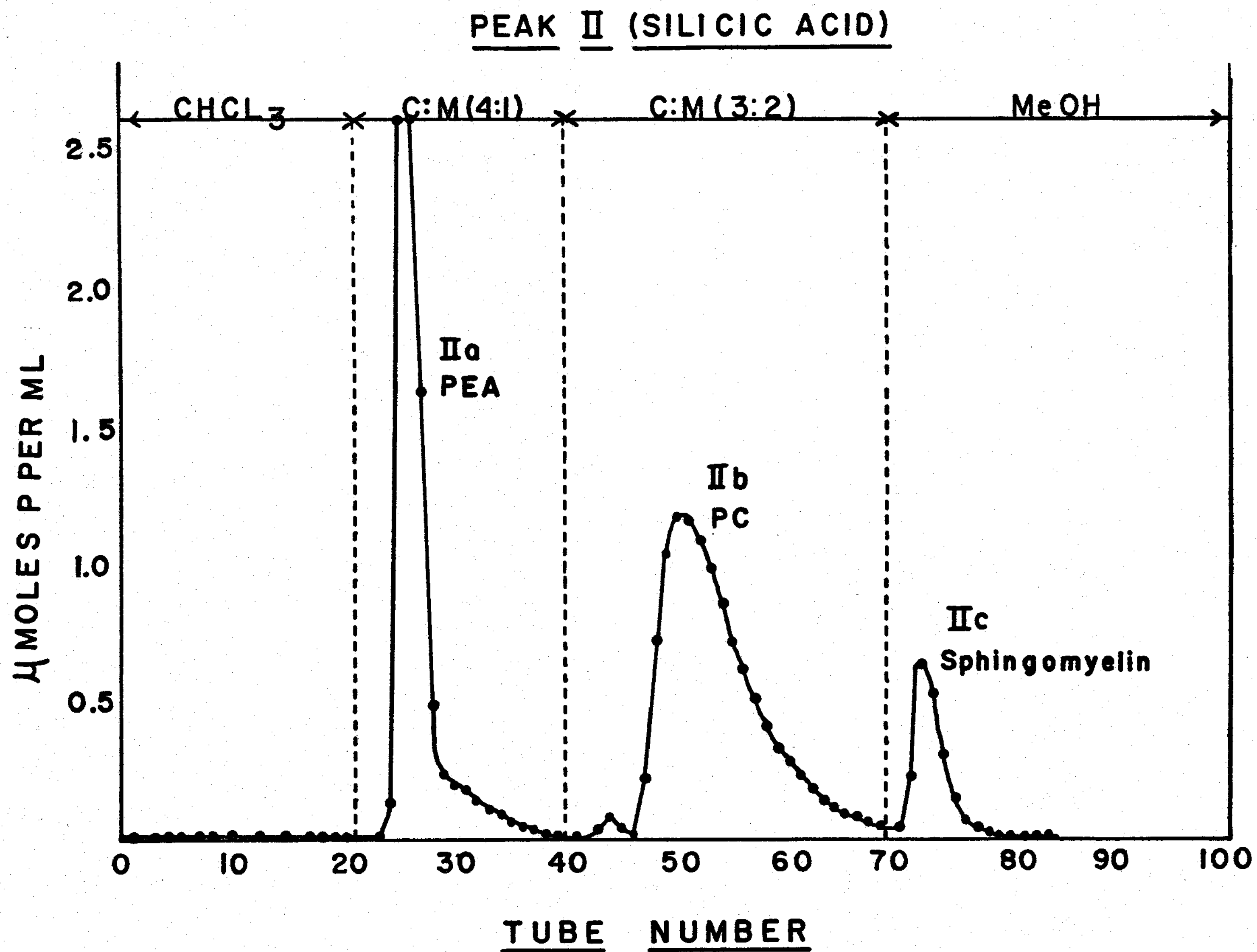

PEAK II (SILICIC ACID)
CHCL3
C:M(4:1)
C:M(3:2)
MeOH
IIa
PEA
IIb
PC
IIc
Sphingomyelin
μ MOLES P PER ML
2.5
2.0
1.5
1.0
0.5
0
10
20
30
40
50
60
70
80
90
100
TUBE NUMBER

Figure 4

Rechromatography of Peak VI of Figure 1 on 8 grams silicic acid; 4 grams Hyflo Super Cel; column diameter 13 mm. Collected 400 drops per tube and phosphorous determined on each tube. The neutral lipid was eluted from the column with chloroform prior to elution with C:M (7:1). C = chloroform; M = methanol; PI = phosphatidyl inositide. UNK = unknown phosphatide.

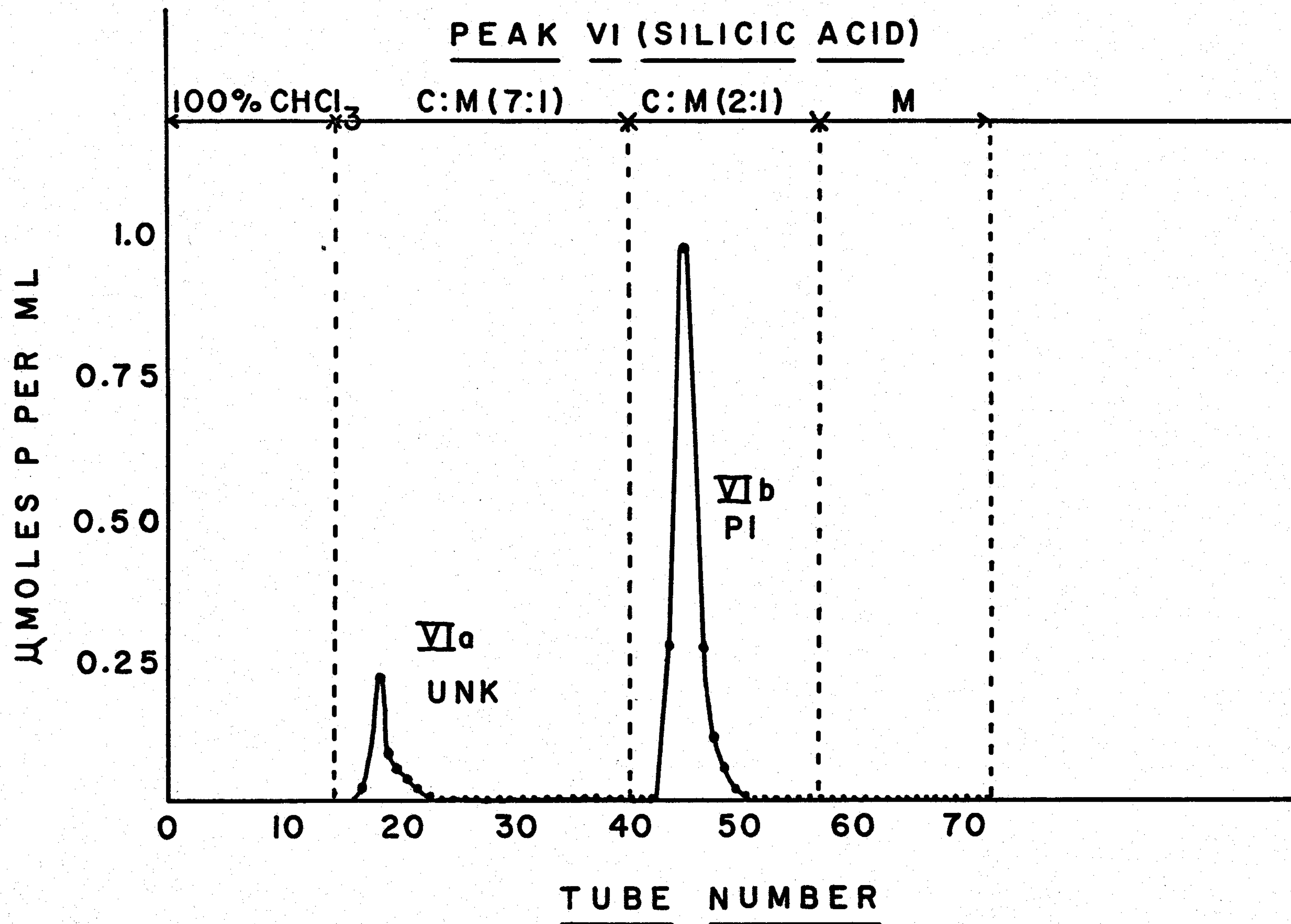

PEAK VI (SILICIC ACID)
100% CHCl3
C:M (7:1)
C:M (2:1)
M
μMOLES P PER ML
1.0
0.75
0.50
0.25
VIa
UNK
VIb
PI
0
10
20
30
40
50
60
70
TUBE NUMBER

Figure 5

A typical thin-layer chromatographic separation (on Silica Gel H without calcium sulfate binder) of the unhydrolyzed lipids from the various peaks obtained on DEAE-cellulose and silicic acid-Hyflo Super Cel columns. Solvent system: chloroform-methanol-glacial acetic acid-water (50:25:7:3). SF = solvent front; 0 = origin; Lys = lysolecithin; Ch = phosphatidyl choline; Sph = sphingomyelin; PI = phosphatidyl inositol; PS = phosphatidyl serine; Car = cardiolipin; EA = phosphatidyl ethanolamine.

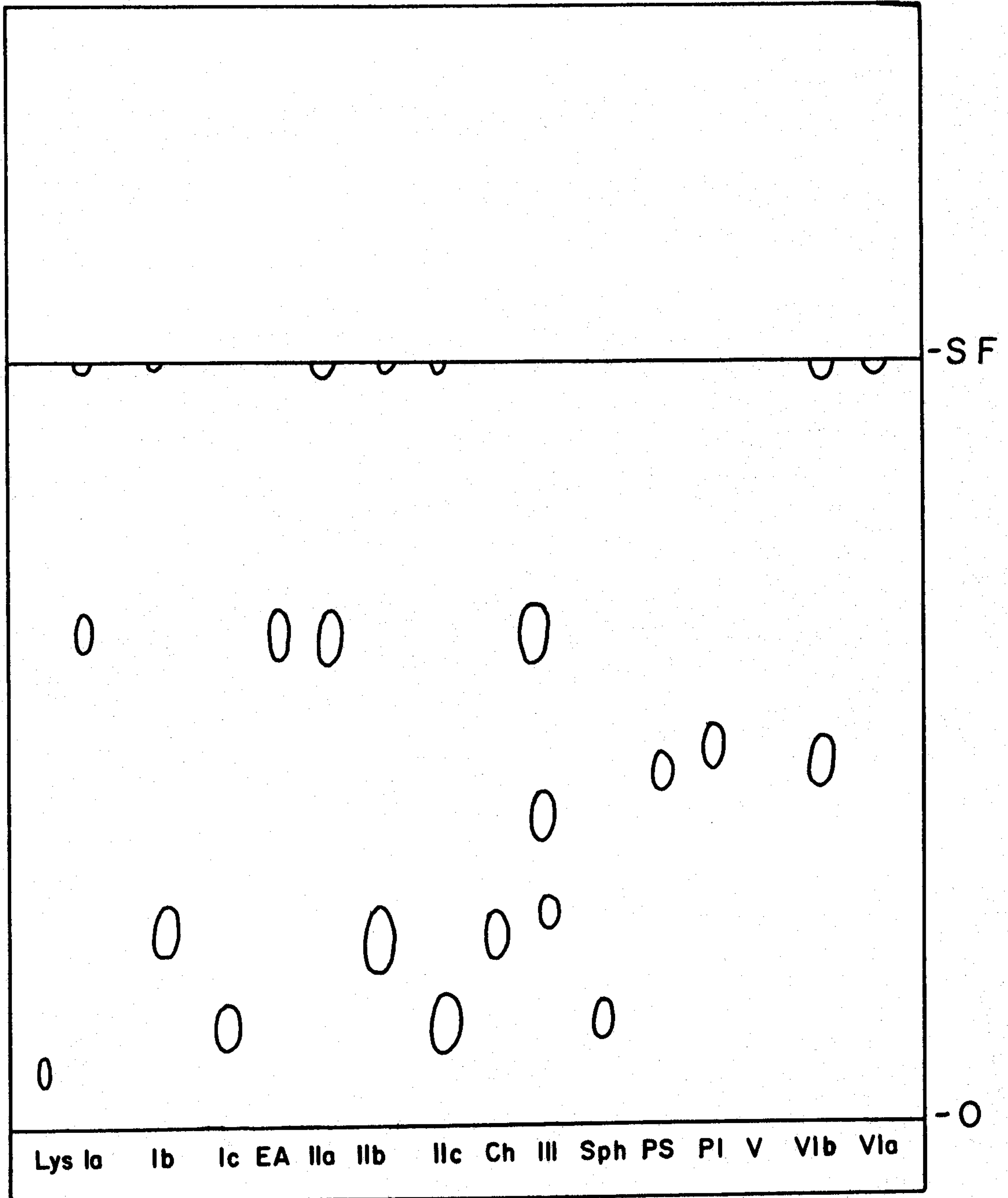

-SF
-O
Lys
Ia
Ib
Ic
EA
IIa
IIb
IIc
Ch
III
Sph
PS
PI
V
VIb
VIa

Figure 6

Paper chromatogram of 6 N HCl hydrolysates of Peak V on Whatman #1 paper. Developing solvent: butanol-acetic acid-water - 40:10:15 (v/v). EA = ethanolamine; Val = valine; Thr = threonine; Ser = serine.

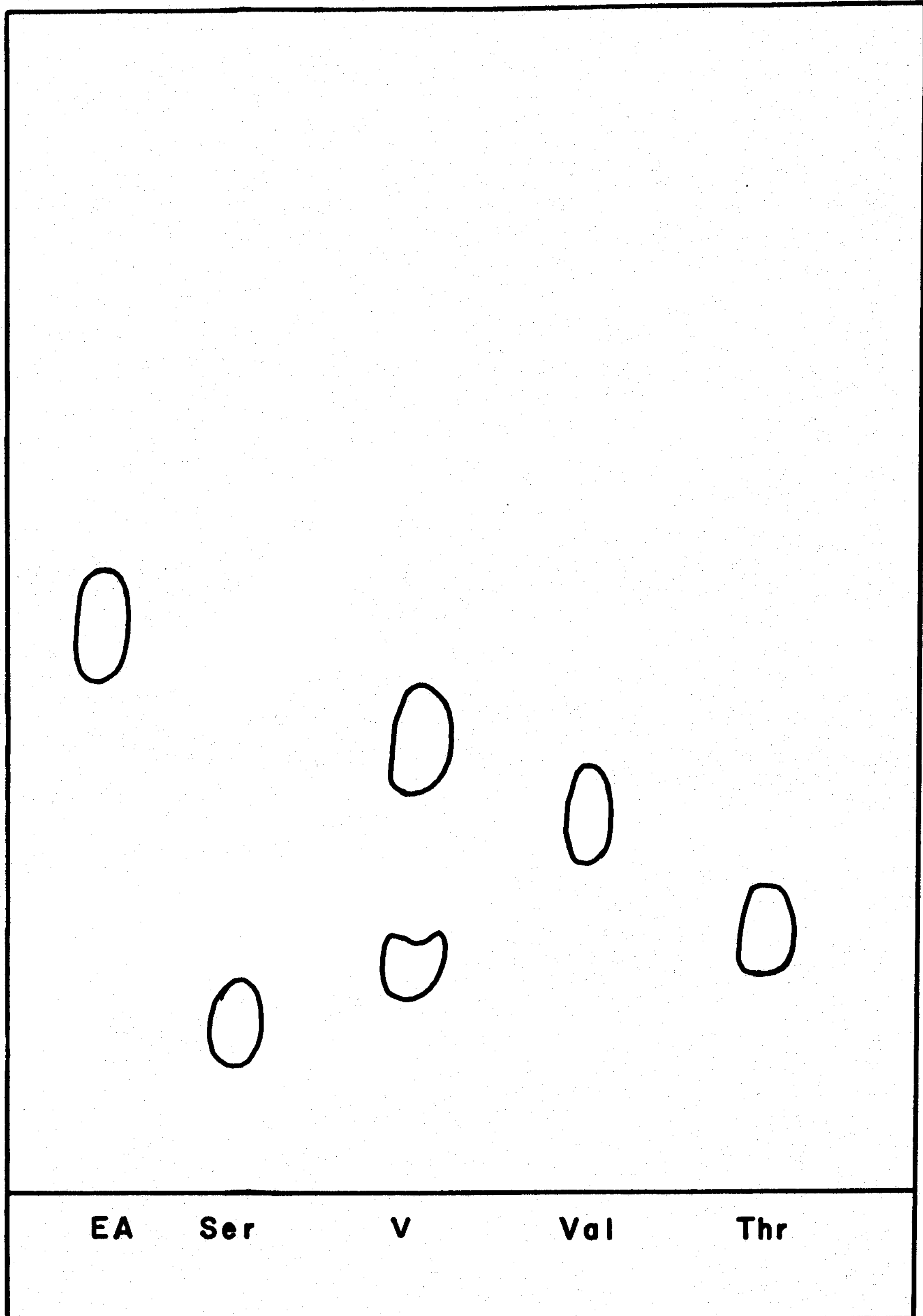

EA
Ser
V
Val
Thr

Figure 7

Thin-layer separation of the phosphatides comprising Peak III of Figure 1. Developing solvent: chloroform-methanol-glacial acetic acid-water 50:25:7:3 (v/v). SF = solvent front; 0 = origin; PE = phosphatidyl ethanolamine; PPC = phosphatidyl choline; PS = phosphatidyl serine.

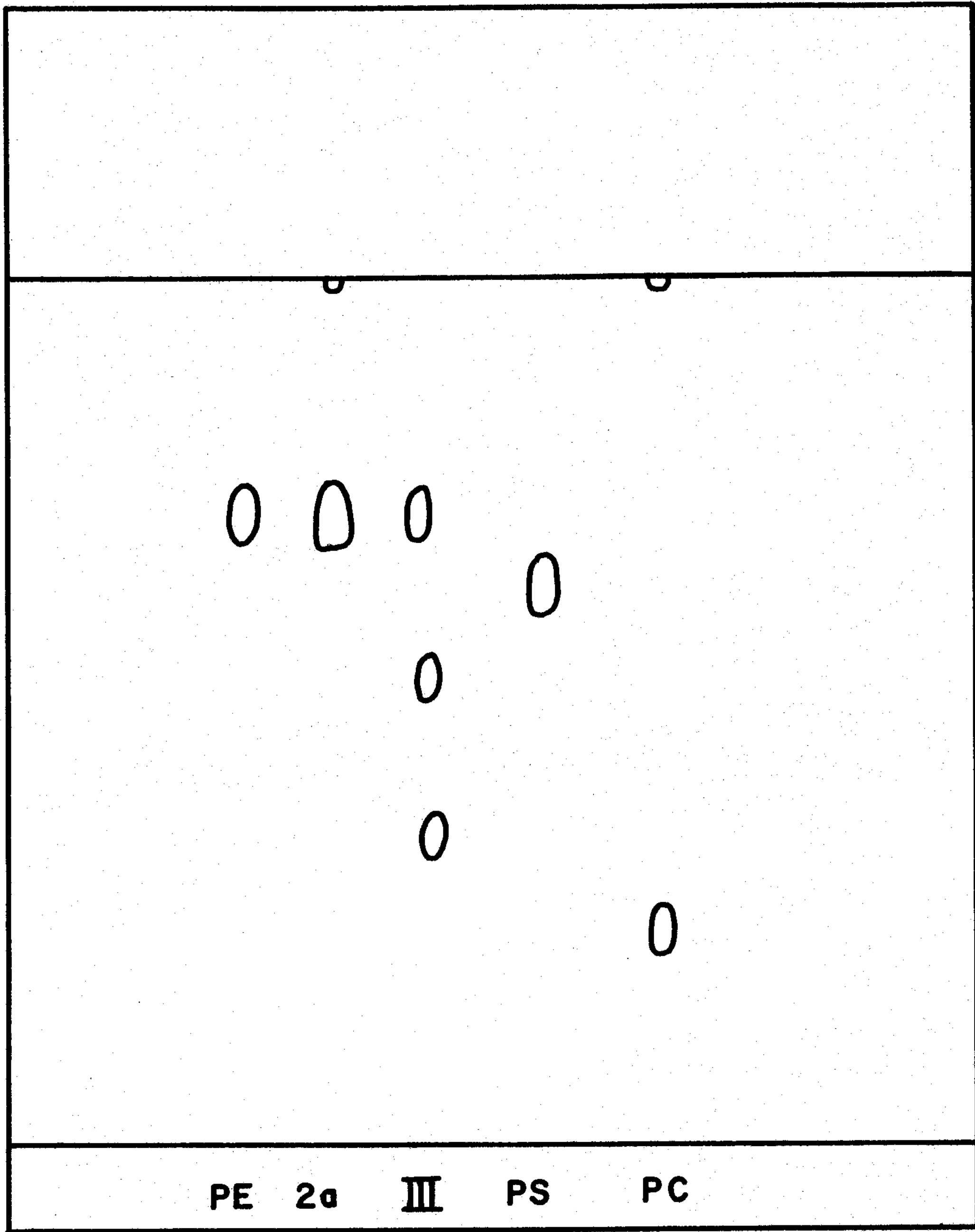

-S F
-O
PE
2a
III
PS
PC

Table I

Analysis of
Phospholipid Fractions in _Tenebrio Molitor_ Larvae

Fraction	μ moles P	N:P	E:P	% Total P	Base
Ia	6.2	1.0	2.1	1.9	EA
Ib	66.4	0.9	2.0	20.8	Choline
Ic	3.3	1.9	.0	1.1	Choline (Sph)
IIa	106.6	1.0	1.9	33.3	EA
IIb	70.7	0.9	1.9	22.1	Choline
IIc	18.5	2.0	.0	5.7	Choline (Sph)
III	17.0	1.1	4.9	5.3	EA + 2 unk
IV	2.1	2.2	10.2	0.6	EA
V	15.0	1.5	3.0	4.7	Unknown A.A.
VIa	2.4	-	-	0.7	Unknown (Cardiolipin?)
VIb	11.7	4.2	2.0	3.7	Inositol

Table II

Comparison of Phospholipids of Various Insects

Genus	Stage	%PEA	%PC	%PS	%PI	%Sph (base)	Reference
Phormia	Larva	63	19	3	4.3	2-3 (EA)	(2,3)
Tenebrio	Larva	41	43	-	3.7	6.8 (Chol.)	
Bombyx	Pupae	3.6	63	8.1	-	16.1 (?)	(42)
Musca	Adult	65	17	3.5	3.2	3.5 (EA)	(10)
Aedes	Larva	84	14	-	-	1.3 (?)	(11)
Apis (brain)	Adult	70	12	-	-	8.8 (?)	(35)
Arctia	Pupae	25	50	15	1.0	15 (?)	(8)

DISCUSSION

Phospholipids of _Tenebrio molitor_ have been separated, purified and identified. The data demonstrate phosphatidyl choline to be the predominant phospholipid, comprising 43% of the total phosphatides. Phosphatidyl ethanolamine was the second major component representing 41% of the total phospholipid. Although phosphatidyl serine was not detected, this component is present in small amounts in other insect systems (see Table II).

The original DEAE-cellulose fractions gave high N/P and E/P ratios, but purification of these fractions by re-chromatography on silicic acid-Hyflo columns lowered these ratios considerably.

Amino acids were present in varied amounts in phospholipids hydrolysates, but their status as constituents of phospholipid molecules was not investigated. The possibility exists that they are artifacts or contaminants, since the free amino acid concentration in insects is high (31, p. 63) and phospholipids have the property of rendering normally lipid insoluble compounds soluble in lipid solvents. Amino acids have also been detected in phospholipid extract by others (74, p. 328; 98, p. 283; 78, p. 381; 89, p. 74). Evidence has been presented by

Hendler (24,25) that amino acids and peptides are covalently linked to phospholipids. Vilkas et al. (44) has shown this structure $CH_3(CH_2)_nCO-(Val_2,Thr_2,Ala_1,Pro_1)OCH_3$ to exist in the phospholipid molecules. The significance of this information in insects cannot be evaluated with certainty at this time, but their involvement as intermediate carriers of amino acids entering the cell is not rejected.

A choline sphingolipid was detected in Tenebrio which is different from other insects thus far investigated containing ethanolamine as the base in addition to sphingosine.

The inositol fraction isolated from silicic acid appears to be very heterogenous. It contained a considerable amount of ninhydrin positive material in addition to a second phosphatide. No attempt to further purify this material was made.

Comparison of Figures 2 and 3 show the re-chromatographed fractions to be qualitatively identical. It is surprising to find phosphatidyl ethanolamine in the first two DEAE-cellulose fractions since Rouser (37) shows that this component should be eluted as a pure compound in the chloroform:methanol (7:3) solvent. The author does not

believe this to be caused by overloading since the loading factor was not exceeded.

Tenebrio larvae would seem to be an ideal source of material to study because they can be reared on a chemically defined diet and of the ease of identifying the stages of development. It should be mentioned that *Tenebrio* was reared on a natural diet and the results presented in this thesis would apply only to the later larval stage of development.

Failure to detect a phosphatidyl carnitine component is rather surprising since carnitine comprises 1-2% of the defined diet of *Tenebrio*. The close structural relationship between carnitine and choline, both of which are of importance in the nutrition of certain organisms, initiates the problem of whether these two substances are interchangeable. From dietary studies on other organisms it is obvious that choline can not replace carnitine in the nutrition of the few insects requiring carnitine, since the carnitine-deficient diet always contains large amounts of choline. This is shown to apply to *Tenebrio*. Bieber (3) has shown that carnitine can replace choline completely in the diet of *Phormia*, but the effect carnitine exerts on choline is unknown.

Considering the amount of carnitine required by the mealworm it might be expected to appear as part of the phospholipids or have some effect on the phospholipid profile. No major difference between the phospholipid pattern of *Tenebrio* and other insect systems was observed. However, the absence of phosphatidyl carnitine and the high percentage of phosphatidyl choline and phosphatidyl ethanolamine indicate the possibility that *Tenebrio* may be capable of converting carnitine into ethanolamine and choline via two alternate metabolic pathways. This possibility should not be excluded since Bieber (3) reported that *Phormia* is capable of decarboxylating carnitine to β-methyl choline. No β-methyl choline phospholipid was observed in *Tenebrio* phosphatides. Absence of phosphatidyl carnitine may be attributed to the fact that carnitine is difficult to detect chemically and could have easily gone undetected if present in trace amounts.

It is, of course, impossible to come to any conclusions concerning the function of carnitine in metabolism when one considers the dearth of information. On the basis of what little evidence is available, it would seem that carnitine is strongly implicated in the

utilization of fat in the animal body (Fraenkel, 1957).

A comparison between the results for the phospholipids of whole insects and those obtained by other authors for various vertebrate tissue show some differences which would distinguish insects from other animals. Insect tissues appear to have a lower plasmalogen content than vertebrates although it must be remembered that the comparison is being made between a whole insect and various tissues separated from vertebrates.

Presence of cardiolipin was suggested to be present in *Tenebrio* and according to Rouser (37) would be eluted with the inositide fraction. If this component were present it would be a definite departure from the vertebrates and other insect systems investigated.

SUMMARY

1. The phospholipids of *Tenebrio molitor* have been examined by chromatography on columns of DEAE-cellulose and silicic acid.
2. The major constituents consist of choline and ethanolamine phospholipids. Inositol phosphatides represented a relatively small portion of the total phospholipid.
3. A choline sphingolipid was detected by methods described in this thesis. This is in contrast to ethanolamine sphingolipids reported for other insects.
4. Only ethanolamine phosphatides were detected in the plasmalogen form.
5. No lysolecithin compounds were demonstrated in *Tenebrio*.
6. Cardiolipin may well comprise a small amount of the total phospholipid.
7. No phosphatidyl serine was detected.
8. The overall phospholipid pattern was qualitatively similar to other insects.

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