PRODUCTION OF LIPID PREPARATIONS.

I. ISOLATION OF NATURAL PHOSPHATIDYLCHOLINES

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The phospholipid compositions (qualitative and quantitative) are given of a wide range of natural sources — organs (brain, heart, liver, kidneys, spleen, lungs, pancreas) of two vertebrate animals (ox and pig) and 27 species of marine invertebrates of five types (coelenterates, arthropods, molluscs, echinoderms, and tunicates). Methods of isolating phosphatidylcholines from six sources — porcine brain and kidneys, a marine ascidian, a mussel, a starfish, and soybean phosphatides — are described and the compositions of the fatty acids of these phosphatidylcholines are given.

Phospholipids (PLs) both natural and synthetic of various structures, are widely used in the investigation of biological membranes [1]. Natural PLs are used as medicinal preparations [1-3]. The importance of phosphatidylcholine (PC) for the organism makes it necessary to develop simple and reliable methods for its isolation from various natural sources.

In the majority of methods described in the literature [4-8] for isolating PC, hens' egg yolks were used as a rule, or, very rarely, other sources (in one of the methods [4], rat liver). We have attempted to employ a wide range of sources for its isolation. With this aim, we have analyzed the PL compositions (qualitative and quantitative) and have determined the amount of total PLs in the organs of vertebrates (ox and pig) and 27 species of marine invertebrates. The results of the analysis are given in Table 1.

Of plant materials, we considered commercial soybean phosphatides, the PL composition of which we had determined previously [9]. All the sources considered, as a whole, proved to be suitable for the isolation of PC since in all cases this PL was the main one, but the calculation of the amount of raw material necessary for the isolation of 1 g of PC permitted the most suitable of them for preparative work to be determined (Table 2). In the experimental part we have given a description of the methods of isolating PC from 6 sources — ox kidneys and brain, marine invertegrates (*D. nipon*, *C. grayanus*, and *H. aurantium*), and commercial soybean phosphatides. The methods described are based on that of Singleton et al. [8] for the isolation of PC from hens' eggs. In all cases alumina was used to prepare the columns. The purity of the PC was not less than 99%. However, in the preparation of PC from porcine kidneys and soybean phosphatides, in order to achieve this purity it was necessary to use an additional silica gel column, as described in the experimental part.

Table 3 gives the compositions of the fatty acids of the PCs from the sources used in this work.

The amount of phosphorus in the PC preparations was 3.9% (in the PC from kidney, brain, soybean phosphatides, and *H. aurantium*), 3.8% (*C. grayanus*), and 3.6% (*D. nipon*) which are close to the theoretical figures (4.1, 4.0, 4.06, 3.99, 3.87, and 3.7%, respectively). The phosphorus:ester ratio was also close to theoretical (1:2). The PC preparations were readily soluble in chloroform, mixtures of chloroform and methanol, benzene, and propanol. The PC was stored at −4-20°C in dark bottles in chloroform or benzene. In the course of a month, the PCs showed no appreciable changes on micro-TLC.

EXPERIMENTAL

Bovine and porcine brain and kidneys were comminuted and extracted three times with acetone in a homogenizer using volume ratios of tissue to solvent of 1:5. The acetone ex-

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tract was centrifuged, the acetone was discarded, and the tissue residue was extracted by Folch's method [10]. The other tissues, without preliminary acetone treatment, were extracted by Folch's method [10] directly. The viscera were removed from the marine invertebrates, which were caught in Vityaz' Bay, Sea of Japan, and were extracted by the same method [10]. The lipid extract from commercial soybean phosphatides was prepared as described in [9]. Micro-TLC and the detection of the lipids were also performed as in [9]. The quantitative analysis of the PLs and the determination of phosphorus in the PC preparations were done by the method of [11]. The fatty acid methyl esters were analyzed as described in [12]. The determination of choline, glycerol, and ester groups in the PC preparations was carried out in accordance with Kates' handbook [13].

Isolation of PC from Porcine Kidneys. The total kidney lipids (12 g, obtained from 400 g of tissue) and 50 ml of chloroform were charged onto a column (3 × 27 cm) filled with 180 g of alumina (L 40/250 μ, alkaline, Czechoslovakia) in chloroform. The lipids were eluted successively with 0.9 liters of chloroform and 2 liters of chloroform–methanol (95:5). The last eluate was collected in 50-ml fractions, the course of separation being monitored by micro-TLC [9]. The fractions containing PCs were combined and, without evaporation, were passed through a column (3.8 × 19 cm) filled with 120 g of silica gel 40/100 μ (Czechoslovakia) in chloroform. The column was washed successively with chloroform–methanol mixtures in ratios of 60:40 (0.5 liters), 50:50 (0.9 liters), and 40:60 (1 liter). The eluates were discarded. Subsequent elution was carried out with chloroform–methanol (30:70) (1.5 liters). The eluate was collected in 50-ml fractions and was analyzed by micro-TLC. The fractions containing only PC were combined and evaporated. The yield of chromatographically pure TC was 1.4 g. The recovery of PC after the two chromatographic columns was of the order of 70% of the initial amount of PC in the lipid extract of the kidneys (Table 1 and 2).

Isolation of PC from Porcine Brain. The total lipids (7 g, obtained from 70 g of brain) in 50 ml of chloroform were transferred to a column (2.2 × 30 cm) filled with 120 g of alumina (L 40/250 μ, alkaline, Czechoslovakia) in chloroform. Elution was carried out successively with 0.7 liters of chloroform and 0.4 liters of chloroform–methanol (90:10). The last eluate was collected in 30-ml fractions, the course of separation being monitored by micro-TLC. The chromatographically pure PC fractions were combined and evaporated. The yield of PC was 0.8 g. The recovery of PC from the column was 75.5% of its initial amount in the lipid extract (Tables 1 and 2).

Isolation of PC from the Marine Ascidian *Halocynthia aurantium*. The total lipids of the ascidian (2.6 g, obtained from 150 g of tissues) in 50 ml of chloroform were transferred to a column (1.5 × 20 cm) filled with 40 g of alumina (L 40/250 μ, alkaline, Czechoslovakia) in chloroform. The column was washed successively with 0.8 liter of chloroform and 0.8 liter of chloroform–methanol (90:10). The last eluate was collected in 50-ml fractions and was analyzed by micro-TLC. The fractions containing PC were combined and evaporated. The yield of PC was 0.38-0.4 g. Its purity was of the order of 99% (with spingomyelin as a possible impurity). The recovery of PC from the column was about 80% of the initial amount of PC in the extract (Tables 1 and 2).

Isolation of PC from the Starfish *Distolasterias nipon*. The starfish lipids (5 g, obtained from 350 g of starfish viscera) in 200 ml of chloroform were deposited on a column (2 × 23 cm) filled with 80 g of alumina (L 40/250 μ, neutral, Czechoslovakia) in chloroform. The lipids were eluted successively with 0.7 liters of chloroform and 0.6 liters of chloroform–methanol (90:10), the last eluate being collected in 50-ml fractions which were analyzed by micro-TLC. The fractions containing PC were evaporated. The yield of chromatographically pure PC was 0.5 g. The recovery of PC from the column was of the order of 98% of its initial amount in the extract (Tables 1 and 2).

Isolation of PC from the Mussel *Crenomythilus grayanua*. The mussel lipids (2.4 g, obtained from 70 g of soft mussel tissues) in 100 ml of chloroform were transferred to a column (2 × 19 cm) with 45 g of alumina (L 40/250 μ, neutral, Czechoslovia) in chloroform. The column was washed successively with 0.9 liters of chloroform and with mixtures of chloroform and methanol in ratios of 90:10 (0.5 liters), 85:15 (0.2 liters), and 70:30 (2 liters), only the last eluate being collected, in 50-ml fractions which were analyzed by micro-TLC. The yield of PC was 0.3 g and its purity 99% (very small amounts of pigment were possibly present as impurities). The recovery of PC from the column was about 60% of its initial amount in the extract (Tables 1 and 2).

TABLE 1. Phospholipid Compositions of Various Natural Sources

Object of investigation	% TLs on the dry weight	%PLs on the dry weight	% of PLs on the TLs	% on the total PLs													
				PC	PCP	LPC	PE	PEP	LPE	SM	CAEP	DPG	PS	PI	PA	X	O
Ox*																	
Brain	8 4	4.7	56,3	30.1		1.5	29.7		2,2	14,9		6,4	12.2	1,7	Tr.		1,3
Kidneys	3.0	2 5	83.6	30,4		0,2	34,5		—	14,4		6,3	6,8	6 6	Tr.		0.8
Heart	3,1	2,3	61,4	41 9		1,5	27,9		—	7,2		9,2	3.5	7,3	0,8		0,7
Liver	6,5	3.6	54,9	48,6		3,0	27,9		1,1	5,5		2,5	2,6	6,5	—	1,0	0.3
Pig*																	
Brain	10,7	4,8	46,2	32,8		—	43.9		—	8,5		0,7	11.9	1,7	—		0,5
Kidneys	2,5	2.0	85,4	27,4		1,3	22,4		1,4	19.7		9,1	9.2	8,3	—	0,9	0,3
Heart	2,7	1.8	66.0	34,2		2.3	35.2		1,0	6 4		12,7	1.7	4,7	—	1 3	0,5
Liver	3 4	1,5	41.1	49,3		2,1	26.3		0,7	5 3		1.9	3,2	5,9	—	4,8	0 3
Spleen	3.4	1.2	35 6	27,4		3,4	18,3		4,2	19.7		1 8	10.7	5,1	Tr.	8,3	0,6
Lungs	1,9	0.8	40.2	42.9		—	21,7		—	14,9		1,2	9,3	3,6	1,1	4,9	0.4
Pancreas	4,3	1 0	22,5	40,3		0.5	23,9		1,1	12.4		1,2	3.4	5.9	—	9.6	1,7
Marine invertebrates																	
Coelenterates																	
Metridium senile	1,7	0,4	25,8	25,4	4,2	3,9	5,6	14.1	—	—	24.0	4,1	8.7	4,1	—	5,0	0,9
Arthropods																	
Hemigrapsus sanguineus	0,6	0,2	22,7	41.1	2.9	6.7	12.4	11.4	—	7,8	—	5,2	3,8	2,8	1,8	4,1	—
Pandalus latirostris	2,0	0,9	41,2	43,2	3.3	3,7	20.0	7,4		3,7	—	3.6	3,6	3,7	1,6	5.4	0,8
Pagurus brachiomastus	1,7	0,8	36,0	53,9	3 1	3,6	10.7	8 5	—	5.7	—	3,4	3,8	4,3	1,7	—	1.3
P. ochotensis	2,5	0,6	23,2	35 0	6,6	2,2	11 7	17.2	—	7.1	—	5,3	6.0	5.4	2,5	—	1.0
P. middendorffii	3,1	0,7	21.7	32.0	5 2	3,4	9,9	3.4	—	9,9	—	4,4	6,4	9.0	3.0	3,0	0,4
Paralitodes camtschatica	4,3	0,5	13.0	32,2	7,7	6,1	9,5	8,9	—	6,2	—	8.2	5,8	7,9	3,9	3,2	—

TABLE 1 (Continued)

Object of investigation	% TLs on the dry weight	% PLs on the dry weight	% of PLs on the TLs	% on the total PLs													
				PC	PCP	LPC	PE	PEP	LPE	SM	CAEP	DPG	PS	PI	PA	X	O
Molluscs																	
Crenomythilus grayanus	4,0	3,2	81,2	24,7	Tr.	3,6	9,8	18 6	—	—	17,5	3,2	9,8**	6 0	2,1	3,3	1,1
Modiolus difficilis	2,3	0,7	30,3	29,1	3,8	3,0	20,4	11,6	—	—	6,5	6,1	8,5**	5,1	—	3,8	2,1
Mytilus edulis	1 0	0,5	51,5	21,7	6,2	7,1	10,7	14,9	—	—	15,9	3,8	10,7**	5,9	1,6	—	1,5
Patinopecten yessoensis	3,8	0,7	17,9	29,2	3,5	2,2	8,3	16,9	—	—	13,3	3,0	10,8**	10,7	—	—	2,1
Swiftopecten swifti	3,0	0 3	10,9	49,2	5,0	2,1	18,3	5 3	—	—	5 8	3,4	3,3	2,1	2,8	2,0	0,7
Octopus sp.	3,0	0,4	13,7	31,9	4 2	3,2	9,4	15,7	—	0,3	10,0	3,7	4,4	6,8	—	3,8	0,6
Spisula sachalinensis	0,9	0,5	52,6	28,0	2,3	2,0	7,0	16,8	—	—	13 3	4,5	13 0	6,0	—	4,1	3,0
Acmea pallida	1,4	0,4	26,6	27,7	3,0	2,6	11,0	14,9	—	—	14,3	3,0	6,3	5,4	3,7	8,1	—
Echinoderms																	
Distolasterias nipon	1,4	0.4	29,5	32,2	5.3	6,8	3,4	20,6	—	10,0***	—	2.4	10,2	6.3	1,1	—	1,7
Patiria pectinifera	1,4	0,4	26,6	43,5	6,0	2,9	2,7	17,9	—	—	—	4,1	14,8	5,3	—	—	2,8
Aphelasterias japonica	0,9	0.4	43,3	36,1	4.1	3,8	3,6	18,4	—	8,2***	—	3,8	14,8	4,2	—	2,8	—
Asterias amurensis	6,5	1,6	24,0	52,0	5,9	3,8	4,3	18,4	—	—	—	4,5	6 0	3,0	—	—	2,1
Cucumaria fraudatrix	1,3	0,2	17,6	43,5	4,3	—	5,0	14,6	—	—	—	3,2	18,9	8,4	—	1,0	1,1
C. japonica	6,3	1,4	22,2	53.3	5 4	—	5,7	11,6	—	—	—	4,1	9,7	8 2	—	—	2,0
Stichopus japonicus	2,0	0,3	14 5	53,9	4,7	1,0	7,9	18,3	—	—	—	3,0	6,0	2,5	—	—	2,7
Strongylocentrotus nudus	11,7	2,8	24,0	51,4	3,3	0,8	11 3	20,2	—	—	—	4,3	5,3	2,5	—	—	0,9
St. intermedius	12,5	1,2	9,2	53,9	3,2	1,6	7,8	17,9	—	—	—	3,0	6,1	4,6	—	—	1,9
Tunicates																	
Halocynthia roretzi	3,8	0,8	22,9	33,3	4,0	5,5	7,7	16 0	—	16,6***	—	3,1	5,9	1,8	—	5,0	1,1
H. aurantium	1,8	0,9	49,7	33,7	3,7	4,7	9,3	9,3	—	10,5***	—	6,9	6,3	5,6	—	9,2	0,8
Stiela clava	2,1	0,6	30,6	26,8	9,0	4,8	7,6	12,3	—	16,8***	—	8,0	4,8	5,9	—	4,0	—

*Amount of the plasmalogen forms are not determined.

**Together with the PSP.

***Together with the SMH. PLs — phospholipids; TLs — total lipids; PC — phosphatidylcholine; PCP — plasmalogen form of PC; LPC — lyso-PC; PE — phosphatidylethanolamine; PEP — plasmalogen form of PE; LPE — lyso-PE; PS — phosphatidylserine; PSP — plasmalogen form of PS; SM — spingomyelin; SMH — sphingomyelin with a hydroxy acid; CAEP — ceramide aminoethylphosphonate; PI — phosphatidylinositide; PA — phosphatidic acid; DPG — diphosphatidylglycerol; X — unidentified PLs; O — start zone.

TABLE 2. Amounts of the PLs by Weight* in Natural Sources

Source	Amount of PLs (mg) per 1 g of tissue/amount of tissue (g) necessary for the isolation of 1 g of PLs	
	PC	PE
Ox		
Brain	14/71	14/71
Kidneys	7,6/132	8,6/116
Heart	9,5/105	6,4/155
Liver	17,5/58	10/100
Pig		
Brain	15,7/64	20/2,50
Kidneys	5,4/200	4,4/220
Heart	6,1/164	6,3/160
Liver	7,4/135	4,0/250
Spleen	3,2/310	2,2/500
Lungs	3,4/300	1,8/600
Pancreas	3,9/250	2,3/440
Marine invertebrates**		
M. senile f.	1,3/800	0,8/1250
H. sanguineus	0,6/1700	0,3/3300
P. latirostris	4,2/240	2,5/400
P. brachiomastus	4,6/220	1,5/670
P. pubescens	2,5/400	1,7/600
P. middendorffii	2,6/380	1,6/630
P. camtschatica	2,0/500	0,9/1100
A. pallida	1,2/840	1,0/1000
C. grayanus	7,9/130	9,1/110
M. difficilis	2,3/430	2,2/450
M. edulis	1,4/720	1,3/780
S. swifti	1,8/550	0,8/1250
P. yessoensis	2,3/440	1,7/600
Octopus sp.	1,4/720	1,0/1000
S. sachalinensis	1,5/700	1,2/840
D. nipon	1,5/700	1,0/1000
P. pectiniifera	2,0/500	0,8/1250
A. japonica	1,6/630	0,9/1140
A. amurensis	9,3/110	3,6/300
C. fraudatrix	1,0/1000	0,4/2600
C. japonica	8,2/125	2,4/420
St. japonicus	1,8/560	0,8/1250
St. nudus	15,4/65	7,8/130
St. intermedius	6,9/150	3,1/330
H. roretzi	3,0/340	1,9/530
H. aurantium	3,4/300	1,7/600
S. clava	2,1/480	1,2/840
Soybean phosphatides***	100/10	82/12,3

*The amounts of the main components of the PLs — PC and PE — are given (without taking into account the losses occurring in column chromatography).
**The amounts of PC and PE were calculated with the inclusion of their plasmalogen forms.
***The amounts of PC and PE (including N-acyl-PE) were calculated by using results obtained previously [9].

Isolation of PC from Commercial Soybean Phosphatides. The lipids obtained from 20 g of the phosphatides (10.3 g) in 60 ml of chloroform were deposited on a column (2.5 × 29 cm) filled with 140 g of alumina (L 40/250 μ, neutral, Czechoslovakia) in chloroform. The column was washed successively with 0.4 liters of chloroform and 0.7 liters of chloroform–methanol (90:10). The last eluate was collected in 35-ml fractions and these were analyzed by micro-TLC. The fractions containing PC contaminated with an unidentified PL were combined and, without evaporation, were passed through a column (2.5 × 10.5 cm) filled with

TABLE 3. Composition of the Fatty Acids of the PCs from Various Sources

Fatty acid	Pig		H. aurantium	C. grayanus	D. nipon	Soybean phosphatides
	brain	kidneys				
	% on the total fatty acids					
14:0	0,3		3,1	1,7		
15:0			1,1	0,4		
16:0	35,8	32.8	9 7	20,9	2,2	37,9
16:1	2,1		3,1	4,5		
17:0			2,6			
18:0	21,1	15.4	3,9	3,0	4 5	2,6
18:1	36,4	30,2	6,9	6,8	3,0	4,9
18:2	0,8	16,3	5 0	2,6		48,5
18:3ω3		0,7	1,1	5,2		6,1
18:4ω3			5,0	3,8		
20:0	0,7					
20:1			1,1		8,5	
20:2			2,7		7,2	
20:4ω6	2,8	4,5	1,6	5,8	10,5	
20:4ω3			3,6			
20:5ω3			32,6	19,4	50,0	
22:2				3,0		
22:5ω3			1,7	2,1		
22:6ω3			11,2	20,6	14,1	
Others		1.1	4.0	0,2		
$\Sigma_{saturated}$	57,9	48,2	20 4	26.0	6 7	40,5
$\Sigma_{monoenoic}$	38,5	30,2	11,1	11,3	11,5	4,9
$\Sigma_{polyenoic}$	3,6	20,5	64,5	62,5	81,8	54,6
Molecular weight of the PC	768	765	777	802	830	764

40 g of silica gel 40/100 μ (Czechoslovakia) in chloroform. The lipids were eluted successively with mixtures of chloroform and methanol in ratios of 90:10 (0.6 liters) and 40:60 (1.6 liters). The last eluate was collected in 20-ml fractions and these were analyzed by micro-TLC. The yield of chromatographically pure PC was 1.7 g. The recovery after two columns was of the order of 85% of the initial amount of PC in the extract (Table 2).

SUMMARY

1. The qualitative and quantitative PL compositions have been determined of a wide range of natural sources — the organs of two vertebrates (ox and pig) and of 27 species of marine invertebrates.

2. The amount of raw material necessary for the isolation of 1 g of PC has been determined.

3. Simple and effective methods have been described for isolating PC from six sources — vertebrate kidneys and brain, the tissues of three species of marine invertebrate, and commercial soybean phosphatides.

4. The compositions of the fatty acids of the PCs from these sources have been established.

LITERATURE CITED

1. R. P. Evstigneeva, E. N. Zvonkova, G. A. Serebrenikova, and V. I. Shvets, Lipids Chemistry [in Russian], Moscow (1983), p. 14.
2. J. L. Wood and R. G. Allison, Fed. Proc., 41, 3015 (1982).
3. R. Downs and A. Van Baak, Your Health Magazine, Reprinted from Best Ways, Carson City, Nevada (1982), p. 44.
4. L. D. Bergel'son, E. V. Dyatlovitskaya, Yu. G. Molotkovskii, S. G. Batrakov, L. I. Barsukov, and N. V. Prokazova, Preparative Biochemistry of the Lipids [in Russian], Moscow (1981), p. 105.
5. N. S. Radin, J. Lipid Res., 19, 922 (1978).
6. B. Lundberg, Acta. Chem. Scand., 27, 3545 (1973).
7. M. Marsh and R. T. Holzbach, Clin. Chem. Acta, 43, 87 (1973).
8. W. S. Singleton, M. S. Gray, M. L. Brown, and J. L. White, J. Am. Oil. Chem. Soc., 42, 53 (1965).

9. E. Ya. Kostetskii and N. I. Gerasimenko, Khim. Prir. Soedin., 35 (1984).
10. J. Folch, M. Lees, and G. M. Sloane-Stanley, J. Biol. Chem., 226, 497 (1957).
11. V. E. Vaskovsky [Vaskovskii], E. Ya. Kostetsky [Kostelskii], and I. M. Vasendin, J. Chromatogr., 114, 129 (1975).
12. E. Ya. Kostetskii, N. I. Gerasimenko, N. V. Naumenko, and V. E. Vas'kovskii, Bioorg. Khim., 7, 1069 (1981).
13. M. Kates, Techniques of Lipidology, Elsevier, New York (1972).

PRODUCTION OF LIPID PREPARATIONS.

II. ISOLATION OF NATURAL PHOSPHATIDYLETHANOLAMINES

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A description is given of the isolation of natural phosphatidylethanolamines from three sources — porcine kidneys, the marine ascidian *Halocynthia aurantium* and soybean phosphatides. The methods of isolating the phosphatidylethanolamines include the preparative fractionation of lipid extracts on alumina with the production of a fraction enriched with phosphatidylethanolamines and its subsequent further purification of a column with the mixed sorbent DEAE-cellulose—silica gel. The compositions of the fatty acids of the phosphatidylethanolamines from the sources used have been determined.

Phosphatidylethanolamines (PE), just like phosphatidylcholines are widely used in the investigation of biological membranes and lipid metabolism. PEs (together with phosphatidylserine) are potential anticoagulants in the blood-clotting system and, moreover, a number of membrane-bound enzymes exhibit an absolute specificity in relation to them [1].

The isolation of phosphatidylethanolamines in the chromatographically pure form is difficult, as a rule, because of their low stability resulting from their high degree of unsaturation [1-7]. Moreover, when they are isolated on silica gel columns, PEs proved to be contaminated with acidic phospholipids (phosphatidylserine, phosphatidylinositol), most frequently, phosphatidylserine [8-10]. This pattern is observed in the isolation of PEs from tissues in which the amount of acidic phospholipids is high. Consequently, egg yolk is more frequently used for the isolation of PEs [1, 11, 12] and, sometimes, bacteria [1] which contain none of these phospholipids or only trace amounts of them (bacteria).

However, our use as a sorbent for column chromatography of DEAE-cellulose mixed with silica gel and, as a preparative stage, alumina for obtaining fractions enriched with PEs has enabled us to obtain them also from sources in which the amount of acidic phospholipids is high — porcine kidneys (total amount of acidic phospholipids 17.5%; amount of PEs 22.4%), commercial soybean phosphatides (total amount of acidic phospholipids 20.8%; PEs together with N-acyl-PEs 29.5%); and the marine ascidian *Halocynthia aurantium* (sum of the acidic phospholipids 11.9%; PEs 18.6%).*

The use of alumina as a preparative step permitted the PE fraction to be obtained in approximately 1 h (since the elution of the lipids was carried out with the aid of vacuum, as described in the Experimental part) and with very small amounts of acidic phospholipids as impurities. The use of a silica gel column for the following purification of the PE fraction did not permit chromatographically pure PEs to be obtained because of the presence of impurities. However, a column with a mixed sorbent prepared as described in the Experimental part enabled these defects to be eliminated and a product of higher purity to be ob-

*The amounts of the individual classes of phospholipids are given in Table 1 of the preceding paper.

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