STUDIES ON THE PHOSPHOLIPIDS OF AQUATIC ANIMALS

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CONTENTS

1. INTRODUCTION

By the efforts of many investigators such as Tsujimoto, Toyama, Tsuchiya, Ueno, Hilditch, Lovern and others, the characteristics of fatty oils contained in aquatic animal bodies have been clarified to be different from those of fatty oils contained in land animal bodies. Many investigators[1)-4)] have studied on the conjugated lipids of land animal bodies. Nevertheless, only a few studies have been carried out on those of aquatic animal bodies.

In this paper the chemical properties and biochemical characteristics of the conjugated lipids of aquatic animal bodies are discussed.

Lecithin: Lecithin is one of the phospholipids, widely distributed in nature; they have been investigated from many years ago. The work of Gobley[1)] was particularly outstanding, since it demonstrated for the first time the presence of a phospholipids in egg yolk. This substance was later christened "lecithin" after the Greek word for egg-yolk, *lecithos*. In 1920, almost pure lecithin samples were separated from the egg or brain, and the hydrolysates of these lecithin samples were investigated by McLean[2)] and Levene and Rolf[3)]. They concluded that the lecithin was composed of two molecules of fatty acid and one molecule of glycerophosphoric acid and choline.

Two varieties of lecithin occur, depending upon whether the phosphate is attached to the α- or β-carbon of glycerol. These are referred to as α- and β-lecithins, respectively. Methods for the separation of the α- and β-forms of lecithin were worked out by Suzuki et al.[4)], but later it became clear that it is difficult to separate these two types of lecithin by their procedure, because the migration of phosphoric acid attached to the carbon of glycerol, occurred during alkaline hydrolysis[5)]. That ordinary lecithin is L-α-lecithin is indicated by the fact that most natural samples are optically active. It has become clear that the unsaturated fatty acid is attached to the α-carbon and the saturated fatty acid is attached to the β-carbon of glycerol[6)].

The fatty acids which have been reported in various lecithin preparations include the saturated fatty acids, myristic, palmitic and stearic and the unsaturated acids, oleic, linoleic, linolenic, arachidonic and clupanodonic. Furthermore, the fatty acid symmetrical lecithins, such as dipalmitoleyl-L-α-lecithin and dipalmitoyl-L-α-lecithin, have been obtained from natural sources[7)-10)]. In company with the progress of chemical studies on lecithin, the methods for its preparation have been discussed.

The most widely used method has been that of Levene and Rolf[3)], as modified

by Pangborn[11)-13)], wherein this lipid is isolated through a cadmium chloride complex. Sinclair[14)] has described a procedure for the isolation of egg lecithin involving the use of low temperature solvent fractionation.

Using adsorbent column chromatography, e. g., magnesium oxide[15)], aluminum oxide[16)17)] and cellulose[18)], many workers could separate lecithin. Besides these methods, counter current was used for the separation of the lecithin[19)20)]. Recently chromatography on silicic acid-celite has been used for the separation of the conjugated lipids containing lecithin etc. By the last mentioned method it is difficult to isolate pure lecithin from other phospholipids, e. g., acetal lipids.

To discuss the physiological activity of phospholipids in the living aquatic animal body is not the aim of this paper, but to make clear the properties of the lecithin samples obtained from certain parts, e. g., from the brain, egg, heart and liver.

Finally, changes in the lecithin, cephalin and other lipids during the development of the egg of salmon and crab and the distribution of conjugated lipids in the organs of aquatic animals are discussed.

Cephalin: Cephalin is one of the phospholipids, soluble in ether and slightly soluble in ethanol, widely distributed in nature; it has been studied since having been extracted from the brain in 1844 by Thudichum[20)], and christened "cephalin" after the Greek word for brain, *kephale*.

MacArther[21)], and Christensen and Hastings[22)], had already suggested the presence of amino acid other than ethanolamine in the cephalin. But until Folch's demonstrations[23)] of the multiple nature of cephalin, it was assumed that only two types thereof existed, which were identical because the phosphorylethanolamine was combined with the α- or β-carbon of glycerol[24)25)].

By dissolving the cephalin from the ox brain in chloroform and gradually precipitating the material with increasing concentrations of ethanol, Folch[26)27)] obtained the inositolphospholipid, phosphatidylserine rich and the phosphatidylethanolamine rich fractions.

Recently, a choline-like substance other than amino base[28)-32)], glutamic acid[33)34)] and threonine[35)-37)] etc. have been found in the hydrolysate of cephalin; the presence has been assumed of the new conjugated lipid combined with these substances. So it became clear that cephalin is not a single substance but a mixture. Several procedures have been employed for the preparation of cephalin from natural sources, one of which is the solvent fractionation method and another is the column chromatographic method using silicic acid-celite as an adsorbent[36) 38)-40)].

Phosphatidylethanolamine: Phosphatidylethanolamine is the substance formerly called cephalin; it consists of one molecule of glycerophosphoric acid and eth-

anolamine, and two molecules of fatty acids.

The fatty acids which have been found in various phosphatidylethanolamine preparations included the saturated fatty acids, palmitic, stearic and arachidic acid and the unsaturated fatty acids, palmitoleic, oleic, C_{20} unsaturated and C_{22} unsaturated fatty acids. L-α-phosphatidylethanolamine exists in nature.

Phosphatidylserine: Phosphatidylserine is a substance contained in cephalin; it consists of one molecule of glycerophosphoric acid and serine and two molecules of fatty acids. The fact that cephalin might contain an amino acid residue was first suggested by McArther[21] and again suggested by Christensen and Hasting[22] later.

Proof that ox brain cephalin may contain an amino acid fraction has recently been brought forward by Folch and Schneider[23]. Since their study, the existence of serine in the cephalin, widely distributed in nature, e. g., in the brain[41], liver[42], lungs[43], groundnuts[44], soybeans[45] and egg yolk[46], has been found by many investigators. The present writer has likewise observed the presence of serine containing phospholipid in the cephalin obtained from the viscera of aquatic animals.

It is suggested that the hydroxyl radical of serine is attached to glycerophosphoric acid, because it can be analyzed by the ninhydrin-carbon dioxide method, cannot be oxidized with periodate[47] and can synthesize DNP-derivatives when use is made of 2,4-dinitrofluorobenzene[48].

As a rule, phosphatidylserine shows low iodine number. The fatty acid which has been found in various phosphatidylserine preparations includes the saturated fatty acids, palmitic, stearic, arachidic and behenic acid and the unsaturated fatty acids, C_{16}, C_{18}, C_{20} and C_{22} unsaturated fatty acid.

Besides serine, some kinds of amino acids and amino bases have been found in the cephalin fraction obtained from natural sources mainly from bacteria[49] and plants[50].

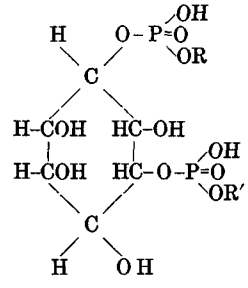
Three unknown bases were found in the ox brain cephalin by Levine et al.[32], a choline-like unkown amino base was detected in the muscle phospholipids of haddock and cod by Lovern et al.[28]-[31], glutamic acid was detected from the setashijimi phospholipid by Hori[34], and from the rat liver phospholipid by Pilgeram[33]. Writers[37] has obtained threonine from the cephalin obtained from tunny muscle; Blass[35] et al., detected this amino acid in the phospholipid obtained from the blood cells of humans and sheep; Rhodes et al.[36], reported the presence of threonine and some kinds of amino acid in the egg yolk phospholipids.

Inositolphospholipids: There are inositol-containing phospholipids in the cephalin fraction besides phosphtidylserine and phosphatidylethanolamine.

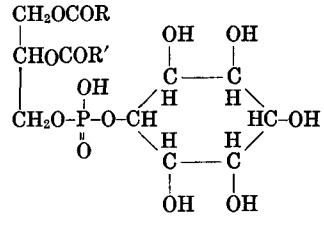
Since Anderson[51] obtained inositol from the hydrolysate of phospholipid pre-

pared from tubercle bacilli and Klenk et al.[52] obtained it from the soybean cephalin, many investigators have obtained inositol from phospholipids prepared from: soybean[52)-60)], groundnut[61)], corn[57)60)], linseed[57)60)62)], wheat germ[63)], cottonseed[60)], sunflower seed[60)], some kinds of bacteria[66)-68)]; from heart of oxen[69)70)], dogs[71)] and humans[72)], liver of the albino rat[72)], guinea pig[73)] and dog[71)], kidney, intestine, skeletal muscle, blood plasma and pancreas of dogs[71)] and humans[71)]; from brain of dog[71)] and ox[71)] and hen egg[45)]. There are several kinds of inositolphospholipids in nature; one of them is diphosphoinositide which was obtained from the ox brain cephalin by Folch[74)]. It has the structure shown in structure I. Another such phospholipid is inositolmonophospholipid which exhibits several structures. Structure II was obtained from soybean by Okuhara et al.[58)59)], and from heart of ox and wheat germ by Faure[69)]; structure III was obtained from groundnut by Malkin et al.[61)], structure IV was obtained from tubercle bacilli by Vilkas and Lederer[68)]; structure V was obtained from several kinds of plant seeds by Carter et al.[60)]

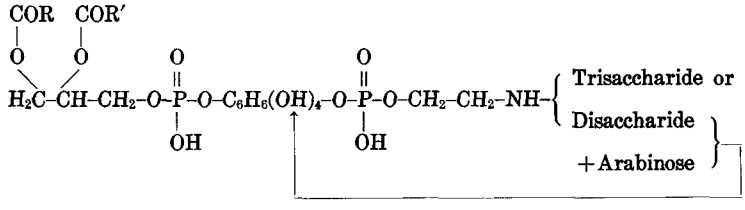
The present writer has also observed the presence of inositolphospholipid in tne cephalin of the brain of sperm whale[75)], rorqual[76)] and pollack[77)], likewise in the pollack heart[78)], the shell free chlamys[172)], the pecten liver[172)], and the eggs of pollack[79)] and crab[80)].



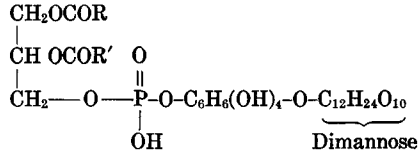
Structure I



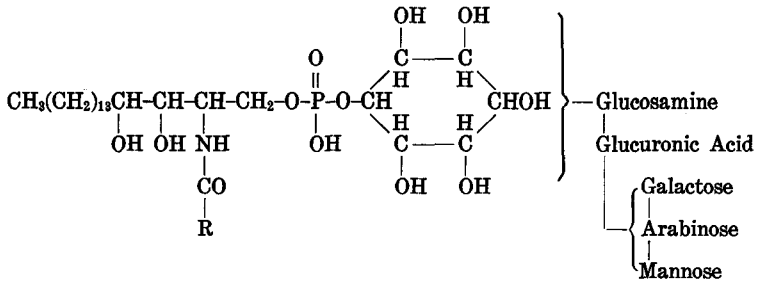
Structure II



Structure III



Structure IV



Structure V

Inositol was obtained as a hydrolysate from the inositol phospholipid prepared from the sperm whale brain[75], while inositol, inositoldiphosphate, glycerol and fatty acids were obtained and inorganic phosphate was detected in the hydrolysate of the inositolphospholipid obtained from the rorqual brain[81].

With the progress of studies on the chemistry of cephalin, it has become clear that almost all cephalin consists of phosphatidylserine and phosphtidylethanolamine.

Phosphatidylserine is believed to the mother substance of phosphatidylethanolamine; it is considered that some physiological significance exists between these two substances but the significance has not yet been clarified.

Ohno[82] suggested that by decarboxylation phosphatidylethanolamine might be obtained from phosphatidylserine contained in the living body but this hypothesis has not yet been proved true.

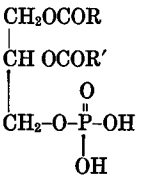
The writer found that only serine was present in the cephlin obtained from unfertilized pollack egg[79] and unfertilized crab egg[80] but only ethanolamine was present in fertilized crab egg as amino base. From this fact it is assumed that phosphatidylserine is decarboxylated into phosphatidylethanolamine during the course of fertilization and development.

But no marked differences were observed between the cephalin obtained from salmon egg before fertilization and 24 hours after fertilization.

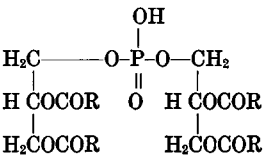
Phosphatidic Acids: Phosphatidic acids have a typical glycerophospholipid structure excepting the absence of a nitrogenous base. In a living body the phosphatidic acids are usually combined with calcium or magnesium. When those alkali metals were removed from phosphatidic acid, the phosphatidic acid became acidic.

As the phospbatidic acids are acidic, they form their salts easily with alkali metal, such as calcium, magnesium, barium and sodium etc.

Use being made of the solubilities of their salts in several kinds of solvents, phosphtidic acids are prepared and purified. Chibnall and Channon[83] first isolated this type of phospholipid from cabbage leaves and also from spinach; its structure



Structure VI



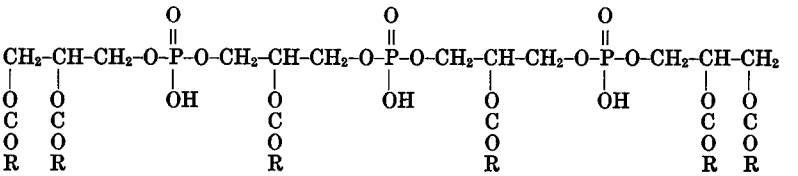
Structure VII

showed diglyceride phosphoric acid (Structre VI). Diphosphatidic acid obtained only by synthesis[84] but not separated from the natural substances shows structure VII.

Glyceroinositophosphatidic acid (Structure II) was obtained from the wheat germ and the ox heart by Faure[65)69)].

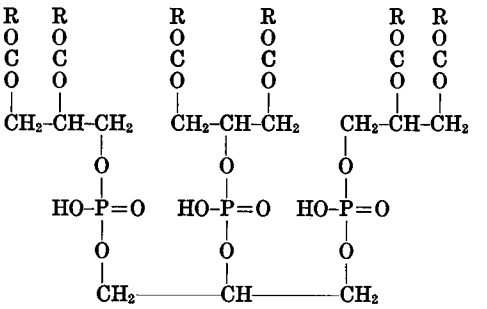
Cardiolipin is a phospholipid present in the ox heart which is now believed to be a glycerophosphatidic acid polyester. Pangborn[85)-87)] coined the name for a substance prepared from the ox heart which is responsible for the reaction with the sera of syphilitics. This substance was also obtained by Faure et al.[88)89)]

Later, in 1947, Pangborn[90)] reported observations on the chemical nature of the hydrolysis products of cardiolipin as follows: the hydrolysis products obtained were linoleic and oleic acid and polyglycerophosphoric acid. On the basis of these observations, Pangborn proposed the following structure (Structure VIII) for cardiolipin.



R: shows fatty acid. This fatty acid is made up of 5 molecules linoleic acid and 1 molecule oleic acid.

Structure VIII



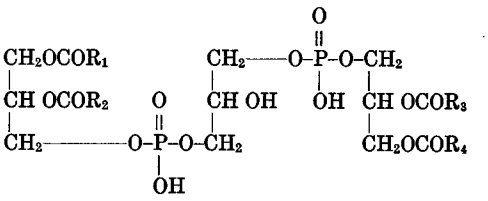
Structure IX

But Malaton[91)] proposed a structure, shown as structure IX, in the observa-

tion obtained from the result of experiments on monomolecular membrane.

Recent reports from Mcfarlane and Gray[92)93)] would tend to cast some doubt on the validity of the structures as proposed by Pangborn[90)] and Malaton[91)]. They obtained cardiolipin from the ox heart muscle by silicic acid chromatography, and also by Pangborn's and Faure and Morelec-Coulon's methods.

From the analytical results of these preparations, they proposed the following structure (Structure X).



Structure X

Cardiolipin shows the highest activity in the sera of syphilitics; other phosphatidic acids show no activity or only a weak activity in them. Hara[94)] obtained cardiolipin-like substances, which show activity in the sera of syphilitics, from the diphtheroid bacillus, dark colored muscle of tuna, sheep testicles, and human placenta.

Uroma et al.[95)96)] and Rein et al.[97)] obtained the cardiolipin-like substances called "sitolipin" from the wheat germ.

Furthermore, Faure et al.[89)] obtained a phosphatidic acid showing weak activity in the sera of syphilitics from the carrot, pea and groundnut. However, this substance is considered to be a mixture of monophosphatidic acid and cardiolipin-like substances.

Many phosphatidic acids, including cardiolipin, obtained from land plants and animals have shown some activity in the sera of syphilitics.

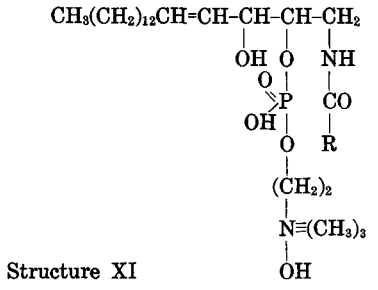
In this paper are reported the chemical properties of the phosphatidic acids obtained from the heart muscle of rorqual[98)] and pollack[78)], and from crab eggs[80)].

Also there are herein reported the activities of sera of syphilitics of the phosphatidic acids obtained from the heart muscle of rorqual[98)] and crab eggs[80)].

Sphingomyelin: Sphingomyelin is found together with other phospholipids and cerebrosides in the highest concentration in the brain and nervous tissue; it is also found in a relatively high concentration in the kidney, liver and spleen, particularly in the spleen which is being attacked by Niemann-Pick disease and more or less in blood and muscle.

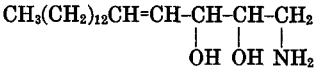
It is easily distinguished from other phospholipids because it contains two nitrogen atoms to each phosphorus atom in the molecule. It was first separated from

the human brain by Thudichum[99] in 1884. This substance later was christened "sphingomyelin" after the Greek word *sphingein*, which means to bind tight. Later Thudichum[99], Rosenheim and Tebb[100] and Levene[101] proved that they are composed of one molecule of fatty acid and phosphoric acid and two molecules of nitrogenous bases, one of which is choline and the other sphingosine. For sphingomyelin Levene[101] proposed a structure (Structure XI).



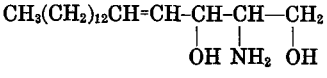
Structure XI

Klenk and Diebold[102] supported the above structure (Structure XI) for sphingomyelin on the basis of their experiments on sphingosine (Structure XII).



Structure XII

But this structure (Structure XII) was modified to structure XIII by Ohno[103] and by Carter et al.[104] independently. They studied the oxidation products obtained from sphingosine and their derivatives by oxidation with lead tetraacetate or periodic acid.



Structure XIII

Recently, Klenk et al.[105] recognized their mistake in the structure of sphingosine (Structure XII) which had been proposed, and corrected it to structure XIII on basis of the result of their study of oxidation product after ozonolysis of sphingosine.

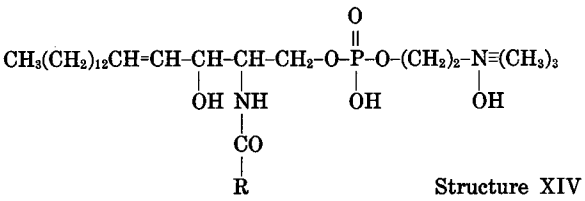
The stereochemistry of sphingosine was firmly established as D-Erythro-l, 3-dihydroxy, 2-amino, 4-trans, octadecene by the efforts of Carter et al.[106][107] Kiss et al.[108], Ohno[109], Mislow[110], and Marinetti et al.[111]

The identification of lignoceryl sphingosine as a product of partial hydrolysis offers considerable confirmation of the fact that the fatty acid and sphingosine are connected by a NH-CO linkage through the carboxyl and amino group, respectively. Furthermore, this is proved on the basis of the fact that lignoceryl sphingosine did not make its salt with mineral acids such as sulfuric acid and hydro-

chloric acid and no nitrogen was found in lignoceryl sphingosine after having been treated with nitrous acid.

In addition, phosphorylcholine and sphingosine phosphate have been isolated as hydrolysis products, so it is evident that phosphorylcholine must be esterified to one of the hydroxyl groups of sphingosine. The attaching position of phosphorylcholine was shown by Fujino[112] and Marinetti et al.[113][114] independently.

They proposed a modified structure of Levene et al.[101] and offered a structure (Structure XIV) for sphingomyelin.



Structure XIV

The differences found in fatty acid composition of sphingomyelin appear to be due to the use of different kinds of sources.

Sphingomyelins from lung[115] or spleen[117] yield equal proportions of palmitic and lignoceric acids while brain sphingomyelin yield stearic and nervonic acids, also small amounts of palmitic, arachidic, behenic, and hexacosenoic acid in addition to lignoceric acid.

Sphingomyelin is a white crystalline substance which is not hygroscopic and resistant to air and light. It is only slightly soluble in cold pyridine and ethanol, and can be crystallized from these solvents when cooled. Sphingomyelin is almost completely insoluble in cold acetone and cold ether, but it is easily soluble in warm ether, hot ethanol, chloroform, warm pyridine and glacial acetic acid.

These characteristics for solubilities of sphingomyelin have been used for the preparation of sphingomyelin[101], but by this solvent fractionation method it is impossible to obtain pure sphingomyelin, in which are contained no hydrolecithin and cerebrosides. To remove substances of these two sorts, cold saponification[116] or alumina chromatographic procedure[117] after cold saponification are used in the purification process. Recently, silicic acid chromatographic procedures have been employed for the separation of pure sphingomyelin.

Thus, previous workers succeeded in efforts to clarify the purification method, chemical structure and component fatty acids of sphingomyelin samples obtained from the land animals, but the properties of sphingomyelin samples of the aquatic animals have not yet been ascertained.

Therefore, the properties of sphingomyelin obtained from the sperm whale brain were studied as described in this paper.

Finally, the distribution of conjugated lipids in the organs of aquatic animals, e. g., herring, tuna, scomber and mollusca etc. have been discussed in this paper.

2. BRAIN PHOSPHOLIPIDS OF AQUATIC ANIMALS

Many investigators have studied the human and other mammalian brain lecithin but no one has ever reported that of aquatic animals.

In the present chapter is described the brain lecithin, cephalin and sphingomyelin of aquatic animals.

Materials

a) The sperm whales (*Physeter catodon*) used as the sources of material were caught off the coast of Onagawa, Miyagi Prefecture in June, 1954. Materials employed for the experiment were only the very fresh $2^1/_2$ brains (11.47 kg) treated immediately after the animals had been killed. Refrigerated brains were brought to the laboratory after having been washed with sea water carefully.

b) The rorquals (*Balaenoptera borealis*) used as sources of material were caught off the same coast as above in June, 1955. Three brains (8.37 kg) of rorqual used in this experiment were treated in the same way as described above for sperm whales.

c) The pollacks (*Theragra chalcogramma*) used as the sources of materials were chosen from the next two groups of pollack.

Material 1. One thousand and three hundred bodies of pollack used as the sources of material were obtained in Hakodate at the beginning of February, 1954. One thousand one hundred and sixty grams of brains were hashed in a meat grinder after having been washed carefully with physiological saline solution.

Material 2. Two thousand four hundred and nine grams of pollack brains (3110 brains) were obtained in Yoichi, Hokkaido in the middle of January, 1955. The brains were treated in same manner as described for material 1.

Method of Analysis

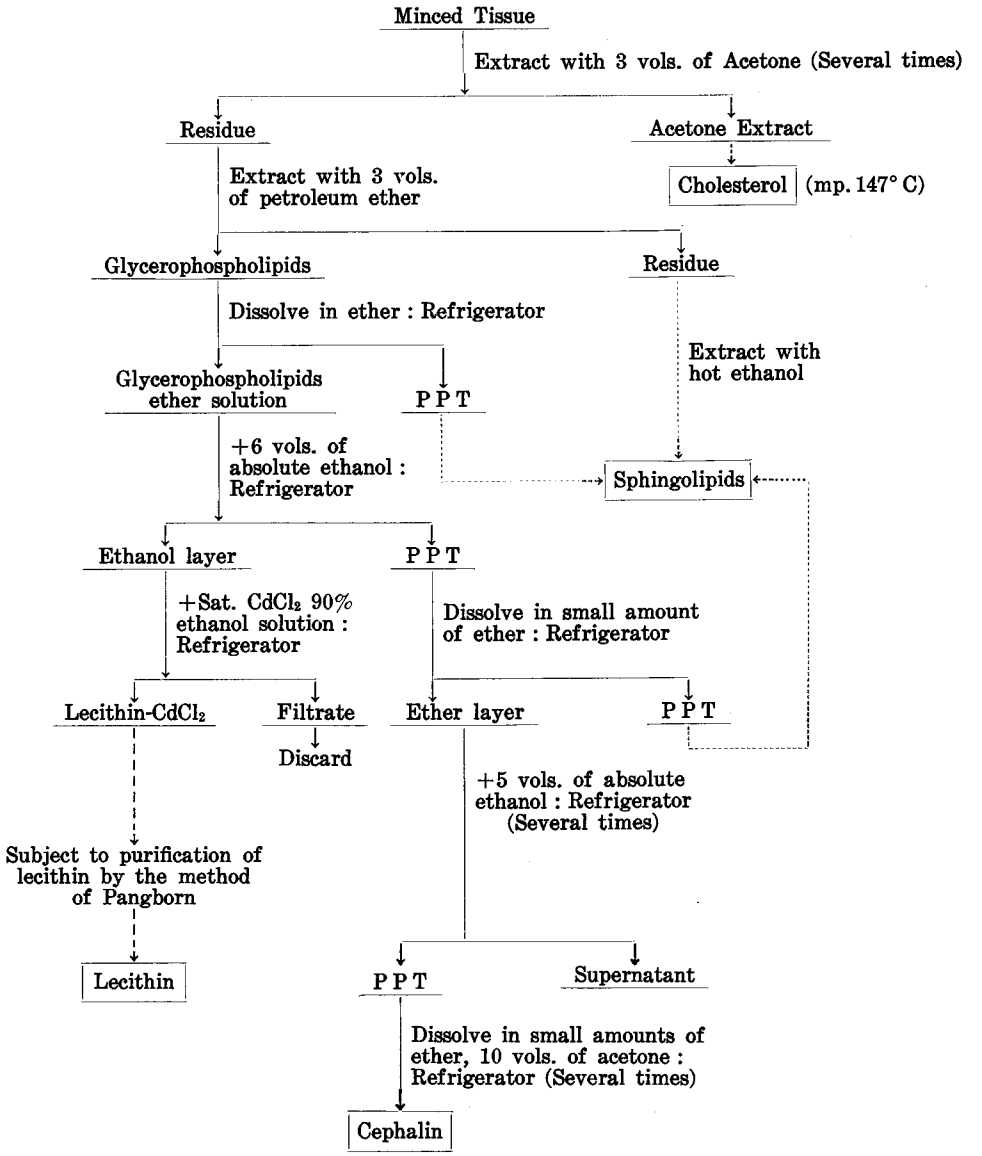
Phosphorous was determined by one of the three methods, i. e., Berenblum and Chain's[118], Fiske and Subbarow's[119] or Lieb's method[120]; nitrogen was determined by the micro Kjeldahl procedure; amino nitrogen by the Van Slyke manometric technique on acid hydrolysate; choline either by the Levine and Chargaff's[121] or by Beattie's method[122]; serine and ethanolamine either by Levine and Chagaff's[121] or by Nojima and Utsugi's method[123]; glycerol by Blix's[124]; iodine number by Wijs'

technique and bromine was estimated by a modified procedure of Stepanow[125]. Inositol was detected by Scherer's reaction.

Preparation of Lecithin and Cephalin

The procedures used in this study have been fully described in the writer's previous papers[75)-77),126)127)] so only a brief outline is herein shown as Fig. 1.

Fig. 1. Extraction and Purification of the Conjugated Lipids from Brain



Results

The properties of lecithin, cephalin and sphingomyelin prepared from the brain of sperm whale, rorqual and pollack are shown in Table 1.

Table 1. Properties of Lecithin, Cephalin and Sphingomyelin from Brain of Sperm Whale, Rorqual and Pollack

Material from		Sperm Whale 11470g (2.5 Brains)			Rorqual 8730g (3 Brains)		Pollack	
							2409g (3110 Brains)	1160g (1300 Brains)
Conjugated Lipid		Lecithin	Cephalin	Sphingomyelin	Lecithin	Cephalin	Lecithin	Cephalin
Yield	(g)	21.5	127	24.5 (Crude)*	24	170	26	28
P	%	3.61	4.04	3.56 (Pure)**	3.63	3.14	3.69	4.06
N	%	1.68	1.58	3.26	1.68	1.83	1.63	1.86
Choline	%	14.40	—	14.98	14.38	—	13.47	—
Ethanolamine	%	—	2.78	—	—	4.55	—	1.83
Serine	%	—	1.38	—	—	4.72	—	1.75
Glycerol	%	—	—	—	11.66	—	—	—
Amino N	%	—	1.32	—	—	1.81	—	1.74
N/P molar ratio		1.03	0.84	1.99	1.02	1.17	1.00	1.00
Glycerol/P molar ratio		—	—	—	0.99	—	—	—
Choline/P molar ratio		1.01	—	1.02	1.00	—	0.93	—
Ethanolamine/P molar ratio		—	0.34	—	—	0.73	—	0.23
Serine/P molar ratio		—	0.10	—	—	0.44	—	0.13
Iodine no.		71.5	90.9	38.8	70.73	85.4	92.6	95.8
Scherer's Reaction		−	+	−	−	+	−	+
Ash	%	—	10.91	—	—	9.48	—	10.03
Cholesterol	(g)	185 (mp. 147°C)			170 (mp. 147°C)		25 (mp. 147°C)	15 (mp. 147°C)

Notes: * Crude sphingomyelin was obtained from the sperm whale brain by the method of Levene et al.[101]

** Pure sphingomyelin was prepared from the substance by the alumina absorption technique.

(I) Brain Lecithin of Aquatic Animals

1) Hydrolysis of Brain Lecithin

A) Brain Lecithin of Sperm Whale[126]

a) Glycerophosphoric acid: Glycerophosphoric acid, as barium salt, was obtained from the hydrolysate of brain lecithin of sperm whale. The results of analysis were as follows:

$C_3H_7O_6PBa$ Calcd. : P 10.10%, Ba 44.69%
Found. : P 10.12%, Ba 44.67%

b) Choline: Choline, as platinum salt (mp. 234–234.5° C, decomp.) was also obtained from the above hydrolysate.

$(C_5H_{14}ONCl)_2\ PtCl_4$ Calcd. : Pt 31.68%
Found. : Pt 31.60%

B) Brain Lecithin of Pollack[127]

Barium glycerophosphate was also obtained from the hydrolysate of brain lecithin of pollack.

$C_3H_7O_6PBa$ Calcd. : P 10.10%, Ba 44.69%
Found. : P 10.19%, Ba 44.71%

2) Fatty Acid Composition of Brain Lecithin

The fatty acid composition of brain lecithin was estimated by the fractional distillation method or the spectrophotometric method[128)129)]. The results are shown in Table 2.

Table 2. Fatty Acid Composition of Brain Lecithin.

Fatty Acid from		Sperm Whale Brain Lecithin	Rorqual Brain Lecithin	Pollack Brain Lecithin
Neut. no.		199.5	196.6	201.2
Iodine no.		79.1	91.1	108.2
Myristic				4.3
Palmitic	Sat. acid %	16.6	49.0	19.5
Stearic		15.0		8.9
Arachidic		6.9		trace
Behenic+Lignoceric acid	%	9.4		
Tetradecenoic acid	%			1.6
Zoomaric acid	%			10.0
Oleic acid	%	23.7	33.4	32.9
Eicosenoic acid	%	2.5		
Linoleic acid	%		3.0	0.4
Linolenic acid	%		3.5	
Arachidonic acid	%	25.2	4.3	6.9
Clupanodonic acid	%		2.4	14.6
Docosahexaenoic acid	%		4.4	

(II) Brain Cephalin of Aquatic Animals

3) Fatty Acid Composition of Brain Cephalin

The fatty acid composition of brain cephalin is shown in Table 3.

4) Fractionation of Brain Cephalin

As shown in the previous papers[75)-77)] by dissolving a cephalin complex from

Table 3. Fatty Acid Composition of Brain Cephalin

Fatty Acid from		Sperm Whale Brain Cephalin	Rorqual Brain Cephalin	Pollack Brain Cephalin
Neut. no.		205.3	194.3	197.1
Iodine no.		105.7	98.8	121.8
Saturated fatty acid	%	33.0	46.7	27.1
Monoethylenic (as oleic) acid	%	40.0	33.2	44.9
Dienoic (as linoleic) acid	%	5.2	3.3	5.0
Trienoic (as linolenic) acid	%	7.9	3.6	5.1
Tetraenoic (as arachidonic) acid	%	1.3	5.1	5.5
Pentaenoic (as clupanodonic) acid	%	9.5	2.7	4.1
Hexaenoic (as docosahexaenoic) acid	%	2.7	5.4	3.3

brain in chloroform and gradually precipitating with increasing concentrations of ethanol, then were obtained inositolphospholipids, phosphatidylserine, and phosphatidylethanolamine rich portions. The results of this fractionation are shown in Table. 4.

5) Hydrolysis of Brain Cephalin

A) Brain Cephalin of Sperm Whale[75]

a) Glycerophosphoric acid: Glycerophosphoric acid was obtained as barium salt from the hydrolysate of Fraction 3 of this cephalin.

The results of analysis were as follows:

$C_3H_7O_6PBa$ Calcd. : P 10.10%, Ba 44.69%
Found. : P 10.16%, Ba 44.66%

b) Serine: Serine was obtained from the hydrolysate of Fraction 3 prepared from the cephalin and identified by the paper chromatographic method.

Table 4. Properties of Fractions Isolated from Brain Cephalin by the Chloroform-Ethanol Method

(I) Sperm Whale Brain Cephalin

Component		Fract. 1	Fract. 2	Fract. 3	Fract. 4	Fract. 5	Fract. 6
P	%	4.26	3.66	3.61	3.59	3.86	5.09
N	%	1.43	1.49	1.65	1.56	1.56	2.96
N/P molar ratio		0.76	0.96	1.01	0.98	0.84	1.29
NH_2-N	%	0.83	1.29	1.55	1.39	1.36	—
Serine	%	0.25	2.28	4.00	2.05	2.06	—
Ethanolamine	%	1.49	1.71	2.52	3.57	4.82	—
Inositol (Scherer's React.)		+	+	±	—	—	?
Ash	%	13.87	11.62	11.51	8.62	6.98	4.96
Iodine no.		73.6	89.3	101.1	149.9	133.1	205.3
Yield	%	42.6	17.6	5.6	3.6	25.0	5.6

(II) Rorqual Brain Cephalin

Component		Fract. 1	Fract. 2	Fract. 3	Fract. 4	Fract. 5	Fract. 6
P	%	3.40	3.05	3.20	3.68	3.47	4.86
N	%	1.76	2.04	2.05	1.35	1.93	1.59
N/P molar ratio		1.14	1.48	1.42	0.82	1.23	0.73
NH_2-N	%	1.59	1.92	2.07	1.46	2.08	1.65
Serine	%	5.83	9.33	9.38	1.18	0.89	0.81
Ethanolamine	%	1.96	2.32	2.62	5.29	8.10	6.11
Inositol (Scherer's React.)		++	+	+	±	−	?
Ash	%	12.14	11.09	10.77	5.87	6.59	4.51
Iodine no.		65.9	68.4	76.4	92.2	99.9	124.5
Yield	%	38.2	14.6	8.2	8.2	27.3	3.6

(III) Pollack Brain Cephalin

Component		Fract. 1	Fract. 2	Fract. 3	Fract. 4	Fract. 5	Fract. 6
P	%	4.29	3.56	3.57	3.37	3.07	−
N	%	2.12	2.01	1.44	1.73	1.39	−
N/P molar ratio		1.10	1.26	0.89	1.10	0.97	−
NH_2-N	%	2.07	1.80	1.32	1.59	1.19	−
Serine	%	0.42	−	2.10	−	0.26	−
Ethanolamine	%	?	−	0.07	−	1.92	−
Inositol (Scherer's React.)		++	+	+	±	−	?
Iodine no.		68.4	73.3	99.6	101.5	99.3	105.3
Yield	%	33	11	16	5	20	15

c) Ethanolamine: Ethanolamine was obtained as picrate from the hydrolysate of Fraction 5 prepared from the cephalin; mp. 158.5–159.0° C, mixed mp. with authentic samples 158.5 °C. The results of analysis were as follows:

$C_8H_{10}N_4O_8$ Calcd. : N 19.31%
Found. : N 19.42%

d) Inositol: Inositol was obtained from the hydrolysate of Fraction 1 obtained from the cephalin; mp. 225° C, mixed mp. with authentic sample 225° C. The analytical results were as follows:

$C_6H_{12}O_6$ Calcd. : C 39.98%, H 6.67%
Found. : C 39.87%, H 6.73%

B) Brain Cephalin of Rorqual

Further purification of inositolphospholipid, phosphatidylserine and phosphatidylethanolamine and studies on the hydrolysis products from the corresponding phospholipids were carried out.

i) Inositolphospholipid[81]: Fraction 1 prepared from rorqual brain cephalin was purified by chloroform-methanol method repeatedly, next precipitated with

methanol and finally with acetone. The precipitate was purified by suspending in 1 N-HCl and then by dialysis against distilled water. The undialysable material was precipitated with acetone. The results of analysis were as follows:

Table 5. Properties of Inositolphospholipid

Component		Fraction 1	Before Dialysis	After Dialysis
P	%	3.40	5.16	6.13
N	%	1.76	0.15	0.23
Glycerol	%	—	6.73	9.16
Ash	%	12.14	20.76	—
P/Glycerol molar ratio		—	2.27	1.99
Yield	%	100.0	15.7	11.6

6) Hydrolysis of Inositolphospholipid

a) Glycerol, Inositoldiphosphate and Phosphoric acid: Glycerol, as tribenzoate and inositoldiphosphate were isolated and phosphoric acid was precipitated as ammonium phosphomolybdate from the acid hydrolysate of this inositolphospholipid.

The results of analysis were as follows:

$C_{24}H_{22}O_6$ Calcd. : C 71.26%, H 4.98%
Found. : C 71.61%, H 4.60%

$C_6H_{14}O_{12}P_2$ Calcd. : P 18.22%, C 21.18%, H 4.15%,
Neut. equivalent 171.04
Found. : P 18.91%, C 21.13%, H 4.20%,
Neut. equivalent 171.06

b) Inositol: In addition to the above substances, inositol was also obtained from the complete acid hydrolysate; mp. 225° C, mixed mp. with authentic sample 225° C. The results of analysis were as follows:

$C_6H_{12}O_6$ Calcd. : C 39.98%, H 6.67%
Found. : C 39.97%, H 6.47%

c) Fatty Acid: The neutral no. of the mixed fatty acid obtained from this inositolphospholipid showed 195.9; the iodine no. of it showed 52.3; the composition of fatty acid of this inositolphospholipid was as follows: hexaenoic (as docosahexaenoic) acid 4.3 per cent, pentaenoic (as clupanodonic) acid 3.5 per cent, tetraenoic (as arachidonic) acid 1.7 per cent, trienoic (as linolenic) acid 1.3 per cent, dienoic (as linoleic) acid 1.8 per cent, monoethylenic (as oleic) acid 29.2 per cent, and saturated acid 58.2 per cent.

ii) Phosphatidylserine[130]: Phosphatidylserine was prepared from Fractions 2 and 3 of the rorqual brain cephalin[76] by chloroform-ethanol method of fractionation as described precisely in the previous paper[130], yield 33.5 per cent. The results of analysis were as follows: N 1.75%, P 3.24%, Serine 11.45%, Glycerol 10.35%, Ash 11.74%, Iodine no. 76.0, N : P 1.19 : 1.00, Serine : N 0.86 : 1.00, Glycerol : P 1.07 : 1.00.

7) Hydrolysis of Phosphatidylserine

a) Serine: Serine was isolated from the acid hydrolysate of this phosphatidylserine; mp. 226.5–227.0°C (decomp.) analytical results were as follows:

$C_3H_7NO_3$	Calcd.	: N 13.32%
	Found.	: N 13.08% (Calcd. as ash free; N 13.16%)
	Ash	0.6%

b) Fatty Acid: The neutral no. of the mixed fatty acid prepared from the phosphatidylserine showed 196.5 and the iodine no. of it showed 78.4; it consisted of hexaenoic (as docosahexaenoic) acid 6.6 per cent, pentaenoic (as clupanodonic) acid 2.3 per cent, tetraenoic (as arachidonic) acid 1.5 per cent, trienoic (as linolenic) acid 1.1 per cent, dienoic (as linoleic) acid 3.6 per cent and monoethylenic (as oleic) acid 54.4 per cent and saturated acid 31.5 per cent.

iii) Phosphatidylethanolamine[130]: Phosphatidylethanolamine was prepared from Fractions 4 and 5 of rorqual brain cephalin[76] by chloroform-ethanol method of fractionation as described in the previous paper[76], yield 35.6 per cent. The results of analysis were as follows: N 1.69%, P 3.14%, Ethanolamine 7.76%, Ash 6.49%, Iodine no. 98.1, N : P 0.99 : 1.00, Ethanolamine : N 0.98 : 1.00.

8) Hydrolysis of Phosphatidylethanolamine

a) Glycerophosphoric Acid and Ethanolamine: Glycerophosphoric acid, as barium salt, and ethanolamine, as picrate, were isolated from the barium hydroxide hydrolysate of the phosphatidylethanolamine. The analytical results were as follows:

$C_3H_7O_6PBa$	Calcd.	: P 10.10%, Ba 44.69%
	Found.	: P 10.06%, Ba 44.60%
$C_8H_{10}N_4O_8$	Calcd.	: N 19.31%
	Found.	: N 19.42%

b) Fatty Acid: The neutral no. of the mixed fatty acid prepared from the phosphatidylethanolamine showed 159.3 and the iodine no. of it showed 106.6; it was made up of hexaenoic (as docosahexaenoic) acid 9.2 per cent, pentaenoic (as clupanodonic) acid 3.2 per cent, tetraenoic (as arachidonic) acid 4.6 per cent, trienoic (as linolenic) acid 0.1 per cent, dienoic (as lineoleic) acid 3.4 per cent, monoethylenic (as oleic) acid 62.6 per cent and saturated acid 16.9 per cent.

(III) Sphingomyelin of Sperm Whale Brain

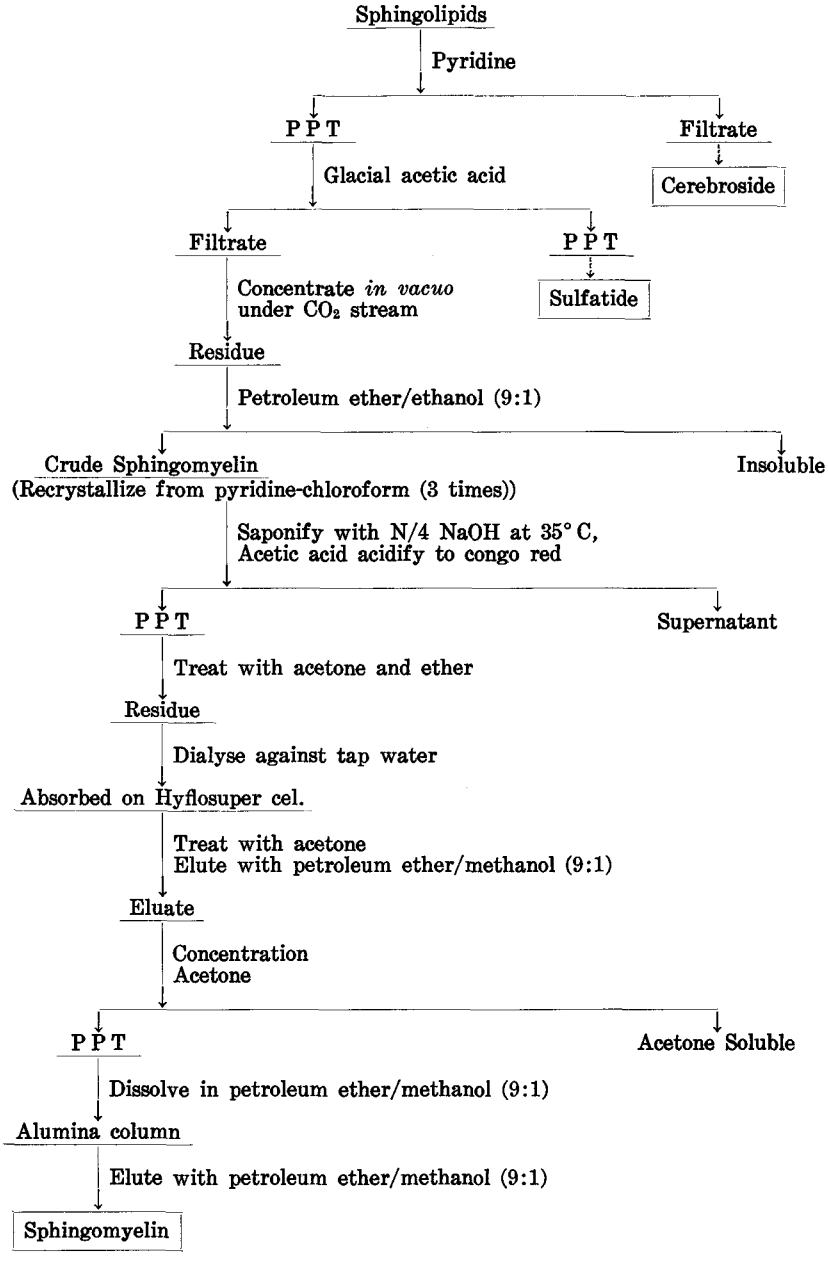
Preparation of Sphingomyelin

Syhingomyelin was prepared from the sphingolipids obtained from sperm whale brain as shown in Fig. 2.

Results

The properties of crude sphingomyelin recrystallized from pyridine and chloro-

Fig. 2. Preparation of the Sphingomyelin



form and pure sphingomyelin obtained from sperm whale brain are shown in Table 6.

9) Hydrolysis of Sphingomyelin

Table 6. Properties of Sphingomyelin

Component		Calcd. as $C_{47}H_{97}N_2PO_4$	Crude sphingomyelin (pyridine-chloroform)	Pure Sphingomyelin
P	%	3.72	3.21	3.56
N	%	3.36	3.52	3.26
Choline	%	15.44	13.01	14.98
Iodine no.		—	29.1	38.8
Sugar		—	(slightly positive)	—

a) Sphingosine and Fatty Acid: Sphingosine sulfate and fatty acid methylester (Sapon. no. 155.4, Iodine no. 22.1) were obtained from the hydrolysate of pyridine chloroform purified sphingomyelin. The analytical results were as follows:

$(C_{18}H_{37}NO_2)_2 \cdot H_2SO_4$ Calcd. : N 4.05%
Found. : N 4.13%

Stearic acid (Calcd.: Neut. no. 197.3[132], Found.: Neut. no. 197.5, mp. 69.1° C), lignoceric acid (Calcd.: Neut. no. 152.2[132], Found.: Neut. no. 152.7, mp. 85.0–85.2° C) and nervonic acid (Calcd.: Neut. no. 153.4[131], Iodine no.[131] 69.4, Found.: Neut. no. 153.8, Iodine no. 63.3) were detected as the component fatty acids of this sphingomyelin; the percentages were stearic acid 20 per cent, lignoceric acid 49 per cent and nervonic acid 31 per cent.

Discussion

In the present paper are described the brain lecithins of some aquatic animals, namely, sperm whale, rorqual and a pollack.

The presence of palmitic, stearic, arachidonic, linoleic and oleic acid in the brain lecithin was found by many investigators. Klenk et al.[133] reported that the fatty acid of the human brain lecithin consisted C_{16} saturated fatty acid 30 per cent, C_{18} saturated fatty acid 10 per cent, C_{20} saturated fatty acid 1.5 per cent, C_{16} unsaturated fatty acid 3 per cent, C_{18} unsaturated fatty acid 46 per cent, C_{20} unsaturated fatty acid 8.5 per cent, C_{22} unsaturated fatty acid 0.4–4.3 per cent.

The present investigation proved that the brain lecithin of sperm whale contained palmitic, stearic, oleic, and eicosatetraenoic acids (probably arachidonic acid) and several other kinds of fatty acid, such as C_{20}, C_{22} and C_{24} saturated and C_{20} monoethylenic acid; fatty acid composition of the brain lecithin of sperm whale was palmitic 16.6 per cent, stearic 15.0 per cent, arachidic 6.9 per cent, C_{22} and C_{24} saturated 9.4 per cent, oleic 23.7 per cent, eicosenoic 2.5 per cent and higher unsaturated fatty acid (mainly eicosatetraenoic acid) 25.2 per cent.

The fatty acid of rorqual brain lecithin was composed of hexaenoic (as docosahexaenoic) acid 4.4 per cent, pentaenoic (as clupanodonic) acid 2.4 per cent, tetraenoic

(as arachidonic) acid 4.3 per cent, trienoic (as linolenic) acid 3.5 per cent, dienoic (as linoleic) acid 3.0 per cent, monoethylenic (as oleic) acid 33.4 per cent and saturated fatty acid 49.0 per cent.

Though it is difficult to compare in detail the fatty acid composition of these two lecithins owing to the difference in analytical methods the component fatty acid of rorqual brain lecithin more richly contained C_{18} and C_{20} unsaturated fatty acid and more scantily contained C_{22} unsaturated acid than sperm whale brain lecithin.

The fatty acid composition of the brain lecithin of a pollack showed myristic 4.3 per cent, palmitic 19.5 per cent, stearic 8.9 per cent, physeteric 1.6 per cent, zoomaric 10.0 per cent, oleic 32.9 per cent, linoleic 0.4 per cent, arachidonic 6.9 per cent, clupanodonic acid 14.6 per cent and traces of arachidic acid. It was interesting to have detected the presence of myristic acid in the component fatty acid of the brain lecithin of a pollack, which could not be detected in that of brain lecithins of humans and the other mammals.

On the basis of these facts, it was concluded that the composition of fatty acid of the brain lecithin show a characteristic composition according to the species of animals and that the composition of fatty acid of the brain lecithin of the fish is very different from that of the whale.

It became apparent that the brain cephalins of sperm whale, rorqual and a pollack consist of inositolphospholipid, phosphatidylserine, and phosphatidylethanolamine respectively.

Levene et al.[134] showed that the fatty acid of the cephalin consisted of one mole. of stearic acid and one mole. of oleic or arachidonic acid. But Klenk[135] found that the component fatty acid of human cephalin was stearic, palmitic, unsaturated C_{18}, C_{20} and C_{22} acids.

In the present study, it was found that fatty acid composition of cephalin differs substantially greatly among the different species of animals as shown in Table 3.

Brain cephalin component fatty acid of the aquatic animals contained richly higher unsaturated fatty acid and a small amount of saturated fatty acid when compared with the brain cephalin component fatty acid of land animals, especially in the case of a pollack the saturated fatty acid content was very small whilst oleic acid content was comparatively large.

As described already, these cephalin fractions were made up of inositolphospholipid, phosphatidylserine and phosphatidylethanolamine, treated with chloroform-ethanol fractionation by Folch's method[27]. Though the results obtained by the writer were not so complete as Folch's[27], he has succeeded in fractionating the

material into inositolphospholipid, phosphatidylserine and phosphatidylethanolamine rich fractions.

On the basis of his results using his chloroform-ethanol method Folch stated that the fractionations of cephalin consisted of combined amino bases of cephalin but this experiment seems from the result of analysis of each fraction to have been affected not only by the combined amino bases but also by combined fatty acid.

Diphosphoinositide described by Folch[136] in Fraction 1 obtained from the brain cephalin of sperm whale, being its presence was first suggested by higher phosphorous content in Fraction I than in other Fractions and in having obtained inositol.

As shown in the result of analysis described above, serine was concentrated into Fraction 3 while ethanolamine was concentrated into Fraction 5 and glycerophosphoric acid, serine and ethanolamine were obtained from each corresponding phospholipid.

From the above observation it was clear that inositolphospholipid, phosphatidylserine and phosphatidylethanolamine existed in these samples of brain cephalin.

The content of ash was highest in Fraction 1; it decreased gradually towards Fraction 6 as shown in Table 4, (I). The above tendencies were observed in the samples of brain cephalin of rorqual and pollack.

In general it was recognized that the acetone soluble fraction (Fraction 6) showed high iodine no. though the fraction contained only traces of serine and relatively high content of ethanolamine.

It was accordingly considered that the velocity of decomposition of phosphatidylethanolamine was faster than that of phosphatidylserine and especially in the case of phosphatidylethanolamine connected with higher unsaturated fatty acids.

Purified phosphtidylserine and phosphatidylethanolamine were obtained from the brain cephalin of rorqual by the chloroform-ethanol method. From hydrolysate of each corresponding phospholipid, serine and ethanolamine were obtained respectively.

Comparing the component fatty acid of phosphatidylethanolamine with those of phosphatidylserine it was found that the content of the higher unsaturated fatty acids, docosahexaenoic, clupanodonic and arachidonic, were rich in the former.

The results obtained from this study did not agree with those of Klenk[137], who studied the component fatty acids of phosphatidylserine and phosphatidylethanolamine obtained from ox brain.

For the above-stated reason it was concluded that the fatty acid of phosphatidylserine and phospatidylethanolamine showed respectively a characteristic composition when prepared from the brain of the three species of aquatic animals.

Two types of inositol phospholipids have been obtained from the animal tissues: the one was glycerophosphoinsitide obtained by Faure[69] from the ox heart, it consisted of each one mole. of glycerol and inositolmonophosphate and two moles. of fatty acids; the other diphosphoinositide mainly inositoldiphosphate which was separated from the ox brain by Folch[136].

The inositolphospholipid obtained from the rorqual brain cephalin in this study, although containing a little nitrogenous base, did show the existence of glycerol, inositol, phosphoric acid and fatty acid from the complete hydrolysis and inositoldiphosphate (assumed to be inositolmetadiphosphate described by Folch[136], because of its neutral equivalent) from the half hydrolysate; the molar ratio between glycerol and phosphoric acid showed nearly 1 : 2.

So this inositolphospholipid can be explained as follows: glycerol connected with inositoldiphosphate and two moles. of fatty acid; their connecting positions of fatty acid were two hydroxyl groups of glycerol and the molar ratio of component fatty acid was 4 : 6 between saturated fatty acid and unsaturated fatty acid; this unsaturated fatty acid was mainly composed of monoethylenic acid.

From the above observations it could not be concluded that this inositol phospholipid was pure owing to the existence of nitrogen, but it consisted of glycerol, inositoldiphosphate and fatty acid showing molecular ratio 1 : 1 : 2. It was reasonable to presume the presence of trace nitrogenous base from the presence of glycerophospholipids in the inositol phospholipid.

Marked differences were not observed in the composition of the fatty acid between pollack brain cephalin and that of whales, compared with the case of these lecithins.

Sphingomyelin was obtained by the method of Levene et al.[101], but by use of this method the writer could not succeed to obtain sugar-free sphingomyelin. That substance was obtained from the next treatment; sphingomyelin was prepared by the method of Levene et al.[101] and later chromatographed on alumina after having been saponified at room temperature with mild alkaline solution. As the hydrolysis product of this sphingomyelin, sphingosine-sulfate and fatty acid methylester were obtained.

The presence of stearic, palmitic, C_{24} saturated and C_{24} monoethylenic acids in the sphingomyelin obtained from several kinds of sources have been reported, but in the case of brain sphingomyelin of sperm whale, the presence of C_{24} saturated and monoethylenic and stearic acid were observed. The ratio of these fatty acids was 20 per cent stearic, 49 per cent lignoceric and 31 per cent nervonic acid.

3. EGG PHOSPHOLIPIDS OF AQUATIC ANIMALS

Up to the present, many studies, have been carried out on the lecithin of hen eggs, but there have been only a few studies dealing with the fish egg lecithin; e. g., Masuda et al.[138] reported the content of phospholipids in several kinds of fish egg, Yokoyama and Suzuki[4] reported the herring egg lecithin and Anno[139] studied the salmon egg lecithin.

In this chapter are reported the properties of phospholipids, e. g., cephalin, lecithin, phosphatidic acid and sphingomyelin obtained from the egg of aquatic animals such as shark, pollack, crab, salmon and carp.

Mateaials

a) The sharks, (*Squalus sucklii*) used for the materials were caught off the coast of Esan, Hokkaido on January 10, 1954. One thousand six hundred and thirty grams of egg yolk (sixty eggs) were obtained as soon as possible after the fish had been killed and kept in ice during transport.

b) Four thousand eight hundred and ninety grams of pollack eggs (*Theragra chalcogramma*) were obtained at Yoichi, Hokkaido in the middle of January, 1955.

c) The crabs (*Paralithodes camtschatica*) used for the materials were caught off the coast of the Southern Kurile Islands at the end of March, 1955. One thousand and fifty grams of unfertilized eggs were obtained from the inside of crust and one thousand and ninety-six grams of fertilized eggs were obtained from the outside of crust of the crab.

d) The salmon (*Oncorhynchus keta*) used for the materials were caught in the Yūrappu River, Yakumo, Hokkaido at the end of November, 1955. There were obtained two thousand nine hundred and sixty grams (about eleven thousand four hundred eggs) of unfertilized and two thousand three hundred and sixty grams (about seven thousand nine hundred eggs) of fertilized egg (24 hours had been passed after fertilization).

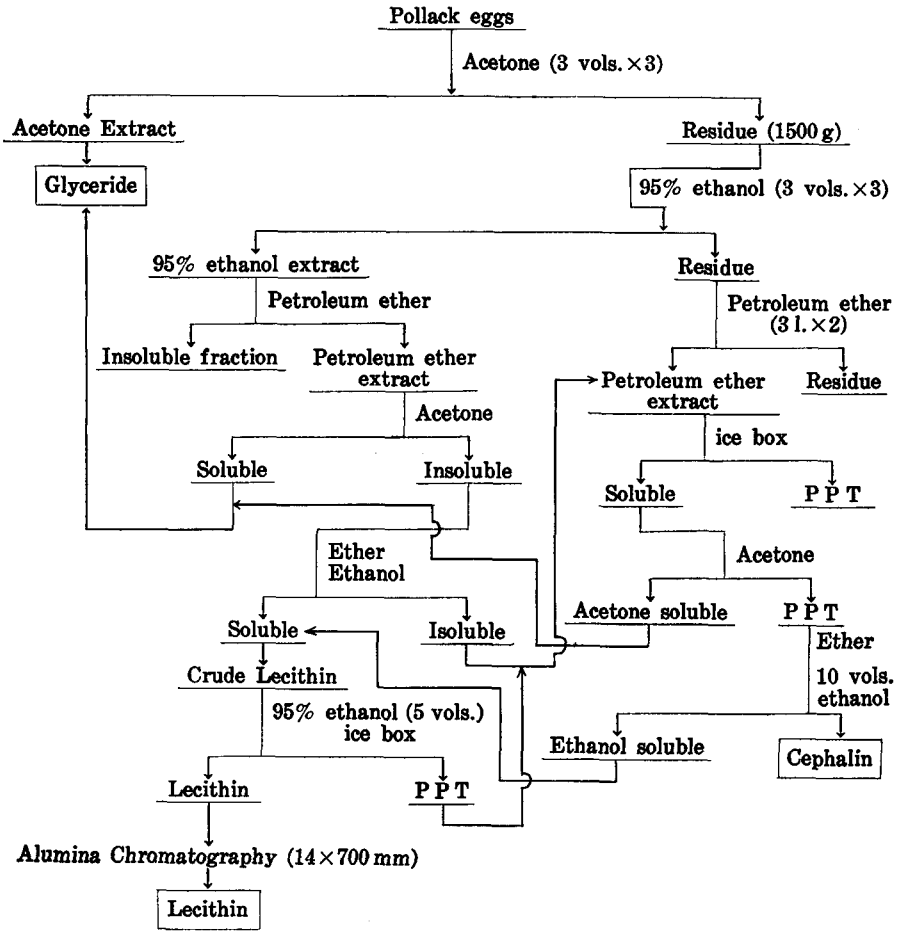
e) The carps (*Cyprinus carpio*) used for the materials were caught at the Nanae Fish-Culture Pond, Hokkaido, at the end of November, 1958. One thousand five hundred grams of eggs were obtained from five fishes.

Methods of Analysis

Phosphorous was determined by one of the three methods, i. e., Berenblum and Chain's[118], Fiske and Subbarow's[119] or Lieb's method[120], nitrogen was determined by the micro Kjeldahl procedure; choline was determined by one of the

three methods, i.e., Levine and Chargaff's[121], Beattie's[122] or Glick's method[140], serine and ethanolamine either by Levine and Chargaff's[121] or by Nojima and Utsugi's method[123], glycerol by Blix's[124], aldehyde by Wittenberg, Korey and Swenson's method[141], sphingosine by McKibbin and Taylor's method[142], galactose by Radin, Lavin and Brown's[143]; iodine number by Wijs' technique and bromine was estimated by a modified procedure of Stepanow[125]. Inositol was detected by Scherer's reaction.

Fig. 3. Preparation of Phospholipids of Pollack Eggs



Results

(I) Phospholipid of Shark Eggs

A) Shark Egg Lecithin[144]

Preparation of Lecithin from Shak Egg: From 1630 g of egg yolk, by the method of Pangborn[11)-18)], 43.6 g of lecithin was obtained as a waxy substance.

The results of analysis of shark egg lecithin were as follows: P 3.89%, N 1.79%, Choline 14.67%, N : P 1.01 : 1.00, Choline : N 0.95 : 1.00, Iodine no. 99.6.

1) Hydrolysis of the Lecithin

a) Glycerophosphoric Acid: Bariumglycerophosphate was obtained from the hydrolysate of the lecithin. The results of analysis were as follows:

$C_3H_7O_6PBa$ Calcd. : P 10.10%, Ba 44.69%
Found. : P 10.09%, Ba 44.64%

b) Fatty Acid: The neutral number of this acid showed 203.1, the iodine

Table 7. Composition of the Lecithin Before and After Passage Over Aluminium Oxide

Component \ Fraction		Crude Phospholipid	Fract. 1	Fract. 2	Fract. 3	Fract. 4	Fract. 5	Fract. 6
Elute	(ml.)	—	300	300	300	300	300	300
Yield	g	50.0	3.1	8.6	11.3	7.8	0.6	0.4
	%	100	6.2	17.2	22.6	15.6	1.2	0.8
P	%	3.69	3.73	3.69	3.79	3.76	3.72	3.65
N	%	2.05	1.71	1.67	1.79	1.79	1.73	1.64
Choline	%	10.12	14.21	13.91	14.20	13.89	9.23	8.51
N/P molar ratio		1.23	1.01	1.00	1.01	1.06	1.05	0.99
Choline/N molar ratio		0.70	0.98	0.96	0.96	0.95	0.63	0.59
Iodine no.		77.3	99.7	106.3	94.5	95.2	90.7	90.5

Table 8. Composition of the Component Fatty Acid of Lecithin and Glyceride of Pollack Egg (%)

Fatty Acid	Lecithin	Glyceride	Fatty Acid	Lecithin	Glyceride
Myristic	3	4	Oleic	23	18
Palmitic	16	25	Eicosenoic	—	2
Stearic	13	5	Linolenic + Octadecatetraenoic	12	12
Arachidic	—	2	Eicosatetraenoic	9	10
Zoomaric	10	12	Docosapentaenoic	14*	8
			Tetracosahexaenoic	—	2

* This fraction contains small quantities of docosahexaenoic acid.

number was 153.8 and the components of this acid were as follows: myristic small quantities, palmitic 34 per cent, stearic 10 per cent, oleic 20 per cent, linolenic 10 per cent, eicosatetraenoic (or arachidonic) acid 13 per cent and clupanodonic acid 13 per cent.

(II) Phospholipids of Pollack Eggs[145),79)]

Preparation of the Phospholipids of Pollack Eggs: The manner of obtaining glyceride, lecithin and cephalin fractions from pollack eggs is shown schemmatically in Fig. 3.

A) Pollack Egg Lecithin[145)]

Preparation of Pollack Egg Lecithin: The lecithin was purified with alumina adsorption column chromatography from the crude lecithin (Fig. 3). The results obtained are shown in Table 7.

As it may be considered that Fraction 1 to Fraction 4 were almost pure lecithin from the results given in Table 7, these fractions were combined and used in the following.

2) Hydrolysis of the Lecithin

a) Glycerophosphoric Acid: Barium glycerophosphate was obtained from the hydrolysate; results of its analysis were as follows:

$C_3H_7O_6PBa$ Calcd. : P 10.10%, Ba 44.69%
Found. : P 10.09%, Ba 44.75%

b) Fatty Acid: The composition of the fatty acids (Neutr. no. 200.2, 201.2, Iodine no. 160.4, 167.0) obtained from the lecithin and the glyceride are shown in Table 8.

B) Pollack Egg Cephalin[79)]

Five and twenty-nine one hundredths grams of cephalin was obtained from 4890 g. of egg and analytical results of this substance were as follows: P 3.69%, N 1.93%, Glycerol 10.76%, P : N : Glycerol 100 : 1.16 : 0.98, Serine 12.97%, N : Serine 1 : 0.90, Inositol+.

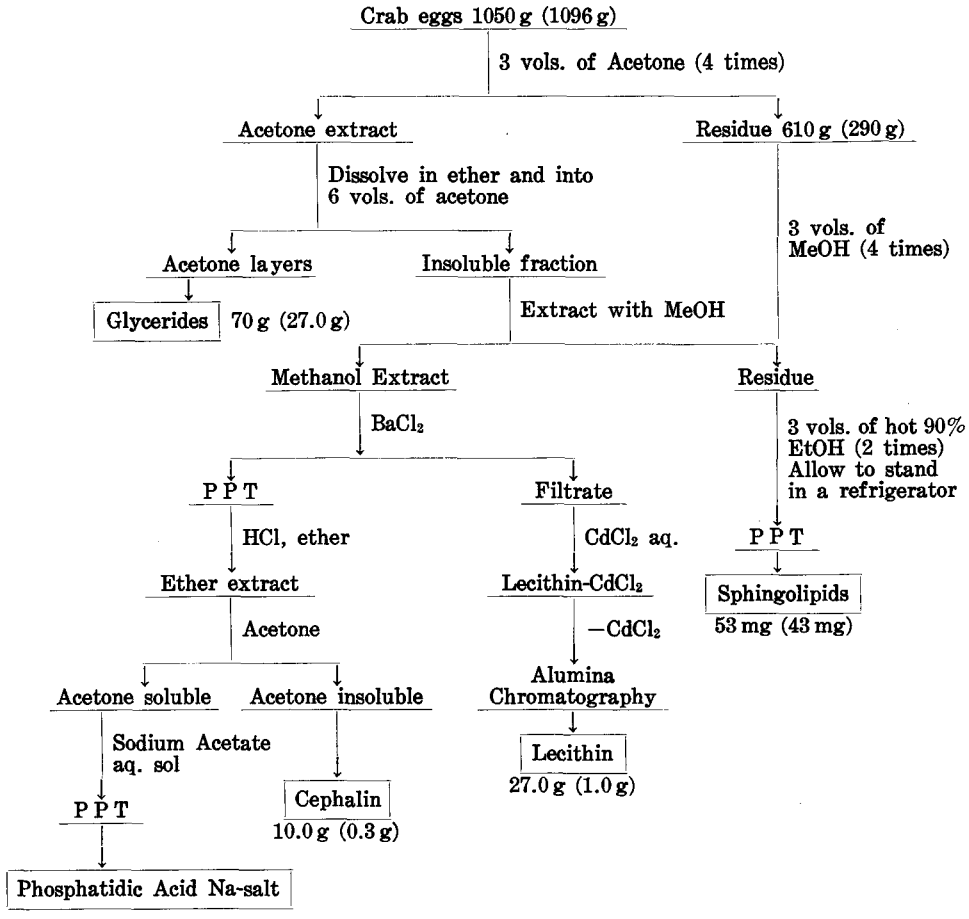
a) Fatty Acid: The composition of fatty acids (Neutr. no. 193.6, Iodine no. 159.6) of the cephalin was as follows: saturated acid 20.4 per cent, monoethylenic (as oleic) acid 43.0 per cent, dienoic (as linoleic) acid 6.0 per cent, trienoic (as linolenic) acid 5.0 per cent, tetraenoic (as arachidonic) acid 12.0 per cent, pentaenoic (as clupanodonic) acid 8.5 per cent and hexaenoic (as docosahexaenoic) acid 5.1 per cent.

(III) Phospholipids of Crab Eggs

Preparation of Phospholipids: The procedures used in this study have already been fully described in the writer's previous papers[146),80)] therefore only a brief outline is herein given in Fig. 4.

A) Crab Egg Lecithin[146)]

Fig. 4. Extraction and Fractionation of the Lipids from Crab Eggs



Note: Numbers are the results by weight of the experiments carried out on unfertilized eggs. Numbers in parenthese are the results from use of fertilized eggs.

Crab egg lecithin samples were obtained from the unfertilized and the fertilized eggs respectively. The analytical results are shown in Table 9.

a) Fatty Acid: The composition of the fatty acids of the lecithin obtained from unfertilized and fertilized crab eggs is shown in Table 10.

B) Crab Egg Cephalin[80]

Crab egg cephalin samples were obtained from unfertilized and fertilized eggs respectively. The results of analysis of these cephalin samples are shown in Table 11.

a) Fatty Acid: The fatty acid composition of these cephalin samples ob-

Table 9. Properties of Lecithin of Crab Eggs

Component \ Lecithin from		Unfertilized	Fertilized
Yield	g	27.0	1.0
	g/kg	25.7	0.9
Iodine no.		108.7	72.3
P	%	3.57	3.62
N	%	1.68	1.64
Choline	%	13.85	13.85
Glycerol	%	11.01	10.95
N/P molar ratio		1.02	1.03
Choline/P molar ratio		0.99	0.95
Glycerol/P molar ratio		1.04	1.02

Table 10. Composition of the Component Fatty Acid of Crab Egg Lecithin

	Fatty Acid		Unfertilized	Fertilized
	Neutr. no.		196.9	199.1
	Iodine no.		153.2	106.8
Saturated	Myristic acid	%	1	37.7
	Palmitic acid	%	9	
	Stearic acid	%	10	
Unsaturated	Monoethylenic (as oleic) acid	%	49	37.8
	Dienoic (as linoleic) acid	%	—	2.1
	Trienoic (as linolenic) acid	%	10	8.8
	Tetraenoic (as arachidonic) acid	%	13	9.8
	Pentaenoic (as clupanodonic) acid	%	8	2.4
	Hexaenoic (as docosahexaenoic) acid	%	—	0.4

tained from unfertilized and fertilized crab eggs is shown in Table 12.

C) Crab Egg Phosphatidic Acid[80]

Phosphatidic acid was obtained from both unfertilized and fertilized crab eggs; the results of analysis are shown in Table 13.

i) Paper Chromatography of Phosphatidic Acid: Each phosphatidic acid obtained from the unfertilized and the fertilized crab eggs was examined by paper chromatography as follows. Tōyō No. 50 paper was used with the solvent system n-propanol-acetic acid-water (8 : 1 : 1, v/v) with ascending-front chromatography (at room temperature). Detection was carried out for phosphatidic ester[147], choline[32], amino base[148] and unsaturated bonds[149], with the results shown in Table 14.

ii) Serological studies of the crab egg phosphatidic acid: Serological assay

Table 11. Properties of Cephalin of Crab Egg

Component \ Cephalin from		Unfertilized Crab Egg	Fertilized Crab Egg
P	%	3.31	3.22
N	%	1.60	1.64
Serine	%	9.52	—
Ethanolamine	%	—	6.05
Inositol (Scherer's Reaction)		+(Weakness)	+(Weakness)
Glycerol	%	9.89	9.79
N/P molar ratio		1.07	1.13
Glycerol/P molar ratio		1.01	1.02
Serine/P molar ratio		0.85	—
Ethanolamine/P molar ratio		—	0.95
Iodine no.		76.8	66.5
Yield	g	1.0	0.39
	g/kg	0.91	0.27

Table 12. Fatty Acid Composition of Crab Egg Cephalin

Fatty Acid			Unfertilized	Fertilized
Neutr. no.			203.6	205.1
Iodine no.			112.5	101.1
Saturated fatty acid		%	43.5	34.6
Unsaturated	Monoethylenic (as oleic) acid	%	29.5	45.2
	Dienoic (as linoleic) acid	%	2.5	3.7
	Trienoic (as linolenic) acid	%	3.3	5.2
	Tetraenoic (as arachidonic) acid	%	9.5	8.5
	Pentaenoic (as clupanodonic) acid	%	6.5	2.3
	Hexaenoic (as docosahexaenoic) acid	%	3.4	0.5

Table 13. Properties of Phosphatidic Acid of Crab Egg

Phosphatidic acid from		Unfertilized	Fertilized
Yield	mg	60	30
	mg/kg	57	27
P	%	3.87	3.66
Iodine	no.	84.5	71.5

with the crab egg phosphatidic acid, was carried out according to Wassermann's and the slide test was found to be active as shown in Tables 15 and 16.

Table 14. Paper Chromatography of Phosphatidic Acid of Crab Eggs (R_f value)

Phosphatidic Acid from	Unfertilized Egg	Fertilized Egg
Phosphoric acid ester	0.90	0.90
Unsaturated bond	0.90	0.90
Choline	−	−
Amino base	−	−

Table 15. Result of Tests of Serological Reactions to Syphilis Sera with Antigen Containing Cardiolipin or Phosphatidic Acid of Crab Egg (Unfertilized)

Serum No.	Cardiolipin		Crab Egg Phosphatidic Acid	
	Wassermann	Slide Test	Wassermann	Slide Test
1	###	##	##	+
2	###	##	+	+
3	###	##	+	−
4	###	##	+	−
5	###	###	+	−
6	##	##	−	−
7	##	##	−	+
8	+	##	−	+
9	−	−	−	−
10	−	−	−	−
11	−	−	−	−
12	−	−	−	−
13	−	−	−	−
14	−	−	−	−
15	−	−	−	−
16	−	−	−	−

IV) Conjugated Lipids of Salmon Eggs

Preparation of Conjugated Lipids from Salmon Eggs: The procedures used in this study have already been fully described in the writer's previous papers[150), 151)] only a brief scheme is herein shown in Fig. 5.

A) Salmon Egg Lecithin[151)]

Lecithin samples were obtained from the unfertilized and the fertilized Salmon eggs respectively. The results of analysis are shown in Table 17.

a) Fatty Acid: The fatty acid composition of the lecithin obtained from the unfertilized and the fertilized salmon eggs is shown in Table 18.

B) Salmon Egg Cephalin[151)]

Cephalin samples were obtained from the unfertilized and the fertilized salmon

Table 16. Result of Tests of Serological Reactions to Syphilis Sera with Antigen Containing Cardiolipin or Phosphatidic Acid of Crab Egg (Fertilized)

Serum No.	Cardiolipin		Phosphatidic Acid	
	Wassermann	Slide Test	Wassermann	Slide Test
1	±±±	±±	+	+
2	±±±	±±±	+	+
3	±±±	±±	+	+
4	±±±	±±±	±±	+
5	±±±	±±	−	−
6	±±±	±±	±±	+
7	±±	±±	−	+
8	+	±±	−	−
9	−	−	−	−
10	−	−	−	−
11	−	−	−	−
12	−	−	−	−
13	−	−	−	−
14	−	−	−	−
15	−	−	−	−
16	−	−	−	−

eggs respectively. The results of analysis of these cephalins are shown in Table 19.

C) Salmon Egg Sphingolipids[151]

Sphingolipids were obtained from the unfertilized and the fertilized salmon eggs respectively. Results of analysis of these sphingolipids are shown in Table 20.

V) Egg Lecithin and Glyceride of Carp

Preparation of Lecithin and Glyceride: Only a brief summary of this study is given here, since a detailed description is available in the writer's previous papers[152),153)].

1) Carp Egg Lecithin[153)]

The analytical results were as follows: P 3.90%, N 1.87%, Choline 14.82%, Glycerol 11.41%, Iodine no. 117.2, N : P 1.06 : 1.00, Choline : P 0.97 : 1.00, Glycerol : P 0.98 : 1.00.

Fatty Acid: The fatty acid composition of lecithin and glyceride of the carp egg are shown in Table 21.

Fig. 5. Extraction and Fractionation of the Conjugated Lipids from Salmon Eggs

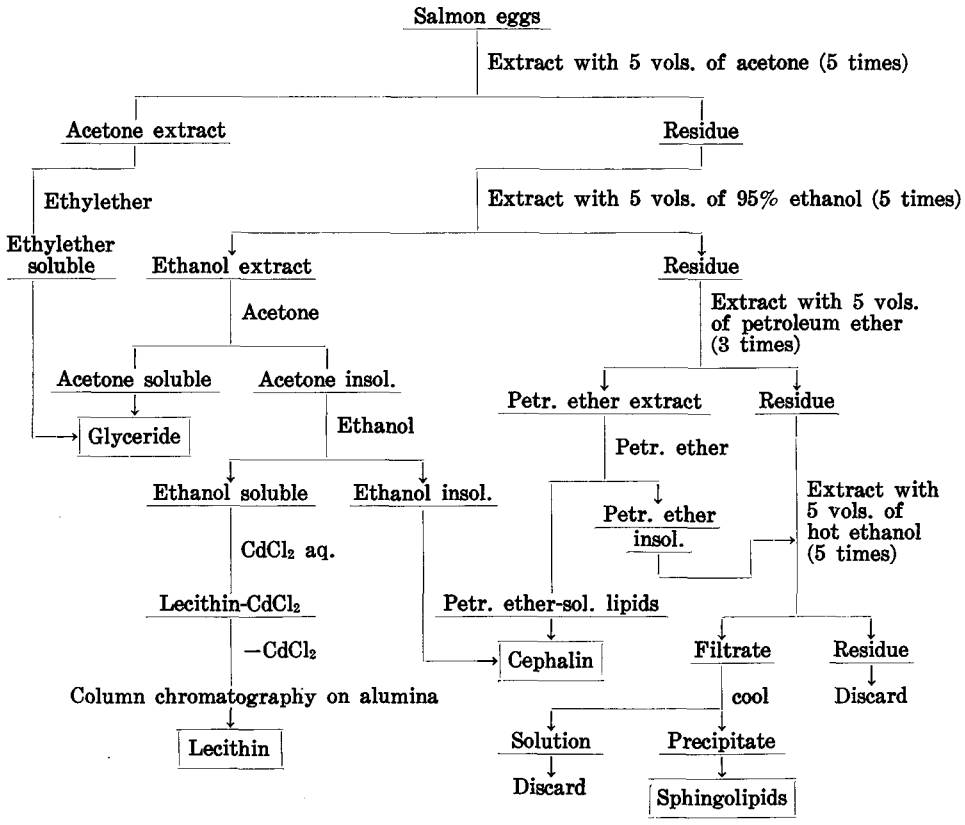


Table 17. Properties of Lecithin from Salmon Eggs

Component \ Lecithin		Unfertilized Egg	Fertilized Egg
Yield	g	38.5	25.3
	mg/individual egg	3.4	3.2
P	%	3.81	3.79
N	%	1.78	1.71
Choline	%	14.30	14.35
Glycerol	%	11.30	10.96
Aldehyde	%	0.02	—
Iodine no.		96.5	97.3
N/P molar ratio		1.03	1.00
Choline/P molar ratio		0.98	0.97
Glycerol/P molar ratio		1.00	0.97

Table 18. Composition of the Fatty Acid of Lecithin from Salmon Eggs (%)

Fatty Acid of Lecithin from		Unfertilized	Fertilized
	Neutr. no.	199.1	200.0
	Iodine no.	148.2	147.5
Methyl-ester	Sapon. no.	189.2	189.7
	Iodine no.	135.2	136.3
Saturated	C_{14}	3.5	3.1
	C_{16}	21.6	22.3
	C_{18}	8.7	9.8
	Total	33.8	35.2
Unsaturated	C_{16}	5.9(2.3)*	4.7(2.4)*
	C_{18}	35.6(3.2)	36.9(3.2)
	C_{20}	12.2(8.6)	12.3(8.5)
	C_{22}	12.5(10.1)	10.9(10.0)
	Total	66.2	64.8

* The numbers in parentheses indicate as measure of the unsaturation, the number of atoms of hydrogen required to saturate the fatty acid.

Table 19. Properties of Cephalin from Salmon Eggs

Component \ Cephalin from		Unfertilized Egg	Fertilized Egg	Phosphatidyl-serine*	Phosphatidyl-ethanolamine**
Yield	g	0.65	0.55	—	—
	mg/individual egg	0.057	0.069	—	—
P	%	4.01	4.02	4.06	4.31
N	%	1.90	1.90	1.84	1.95
Serine	%	4.75	4.96	13.79	0
Ethanolamine	%	4.53	4.26	0	8.51
Glycerol	%	13.19	12.30	12.09	12.83
Inositol		+	+	—	—
Aldehyde		trace	trace	—	—
Iodine no.		68.2	72.5	33.31	35.35
N/P molar ratio		1.05	1.05	1.0	1.0
Serine/P molar ratio		0.35	0.36	1.0	—
Ethanolamine/P molar ratio		0.57	0.53	—	1.0
Glycerol/P molar ratio		1.11	1.03	1.0	1.0

* Calculated for palmitoyloleoylglcerylphosphorylserine (M. W. 762.0)

** Calculated for palmitoyloleoylglcerylphosphorylethanolamine (M. W. 718.0)

Table 20. Properties of Shingolipid Fractions from Salmon Eggs

Component \ Sphingolipids from	Unfertilized Egg	Fertilized Egg	Sphingomyelin*	Cerebroside*
Yield { g	0.93	0.76	—	—
Yield { mg/individual egg	0.081	0.096	—	—
P %	2.00	2.11	4.30	0
N %	2.75	2.69	3.89	1.72
Sphingosine %	32.65	30.24	41.54	36.87
Choline %	7.83	8.60	16.81	0
Galactose	8.22	7.38	0	22.18
N/P molar ratio	3.04	2.82	2.00	—
Sphingosine/N molar ratio	0.56	0.53	0.50	1.00
Choline/N molar ratio	0.33	0.37	0.50	—
Galactose/N molar ratio	0.23	0.21	—	1.00

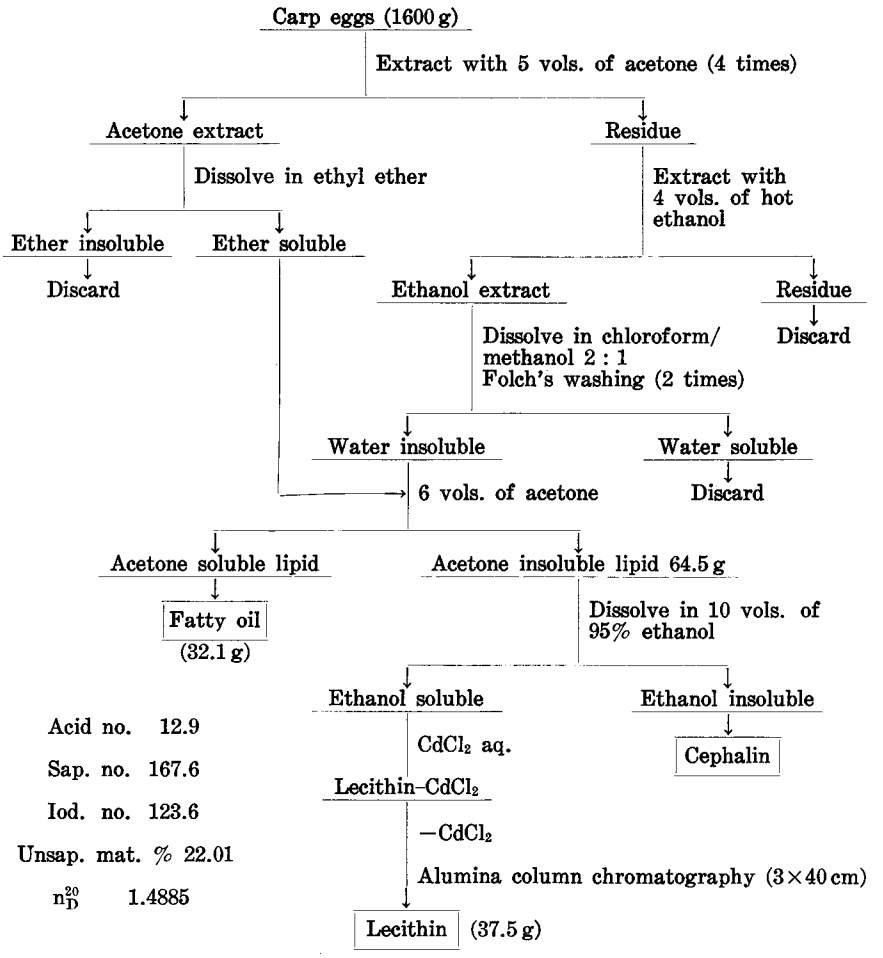
* Calculated for palmitoylsphingosylphosphorylcholine (M. W. 721.0)
** Calculated for lignocerylgalactosylsphingosine (M. W. 812.2)

Table 21. Fatty Acid Composition of Lecithin and Fatty Oil of Carp Egg (%)

		Lecithin	Fatty Oil
	Neutr. no.	201.3	202.3
	Iodine no.	172.2	150.7
Methyl-ester	Sapon. no.	190.5	191.5
Methyl-ester	Iodine no.	162.8	136.7
Saturated	C_{14}	2.6	2.2
Saturated	C_{16}	17.7	17.8
Saturated	C_{18}	8.1	8.1
Saturated	C_{20}	0.5	0.2
Saturated	Total	29.2	28.3
Unsaturated	C_{16}	7.1 (2.2)*	5.2 (2.2)*
Unsaturated	C_{18}	29.1 (3.2)	40.0 (3.7)
Unsaturated	C_{20}	24.2 (8.0)	25.0 (7.0)
Unsaturated	C_{22}	10.4 (9.8)	1.5 (8.0)
Unsaturated	Total	70.8	71.7

* The numbers in parentheses indicate as a measure of the unsaturation, the number of atoms of hydrogen required to saturate the fatty acid.

Fig. 6. Extraction and Fractionation of the Lipids from Carp Eggs



Discussion

In this paper are reported the properties of lecithin samples obtained from shark (*Squalus sucklii*), pollack (*Theragra chalcogramma*), crab (*Paralithodes camtschatica*), salmon (*Oncorhynchus keta*) and carp (*Cyprinus carpio*) and comparison between the fatty acid composition of the lecithin and that of glyceride obtained from the fish egg. The existence of palmitic, stearic, oleic, linolenic, arachidonic, and clupanodonic acids and traces of myristic acid were found in the component fatty acids of shark egg lecithin. The amounts of component fatty acids of lecithin are described in section A) and that of the glyceride are shown in Table 22.

Table 22. Composition of the Component Fatty Acid of Glycerides and Lecithin Obtained from Egg Yolk (%)

	Fatty acid	Shark egg		Hen egg[154]	
		Glyceride	Lecithin	Glyceride	Lecithin
Saturated	Myristic	3	Small quant.	0.7	—
	Palmitic	10	34	25.2	31.8
	Stearic	7	10	7.5	4.1
	Arachidic	4	—	—	—
Unsaturated	Zoomaric	13	—	3.3	—
	Oleic	21	20	52.4	42.6
	Eicosenoic	10	—	—	—
	Docosenoic	5	—	—	—
	Linolenic	5	10	8.6	8.2
	Eicosatetraenoic or Arachidonic	10	13	—	—
	Clupanodonic	10	13	2.3	13.3
	Tetracosapentaenoic	Small quant.	—	—	—

The data shown in Table 22 are those of Riemenschneider[154], who studied the lipids of hen eggs and found that the composition of fatty acid of lecithin did not accord with that of glyceride and that the component fatty acid of lecithin was composed of specific fatty acid. It might be concluded that in shark egg lecithin the content of unsaturated fatty acid is comparatively larger than that of the glyceride.

The egg lecithin of pollack was purified by the alumina column chromatographic method. The use of alumina column chromatography for the purification of egg yolk lecithin was first reported by Hanahan[16] and Faure[17].

No significant differences were observed in the properties of lecithin between those prepared by the method of Hanahan[16] and those by Faure's[17] nor by that of Pangborn[13], so it was accepted by the present writer that there are no remarkable differences between the egg lecithin of pollack prepared by the method of Hanahan and that prepared by the other two methods.

Myristic, palmitic, stearic, palmitoleic, oleic, linolenic, octadecatetraenoic, eicosatetraenoic, docosapentaenoic and docosahexaenoic acids were detected in the pollack egg lecithin. The amount of the component fatty acid of the lecithin has been described in Table 8, and the results of comparison with the component fatty acid of the glyceride are shown in Table 8. From the results tabulated in Table 8, it is apparent that the differences between the amount of saturated and monoethylenic acids of the lecithin and that of the glyceride are scarcely observable; that docosa-

pentaenoic acid is contained more in the lecithin than in the glyceride; that no arachidic, eicosenoic and tetracosahexaenoic acids, contained in glyceride are contained in the lecithin.

In the study on shark eggs and pollack eggs, unfertilized eggs were used for the material, but in salmon eggs both unfertilized and fertilized eggs (24 hours after fertilization) were employed. In order to study the change of lipids during the 24 hours after fertilization the glyceride, lecithin and cephalin were prepared from salmon eggs. The results obtained were as follows: in lecithins no decided changes were observed between the properties of lecithin from these two sources, whereas small amounts of acetal lipid were found only in the unfertilized eggs.

Concerning the changes of component fatty acid of these two lecithins during 24 hours after fertilization, it was recognized as exhibited in Table 18, that the contents of unsaturated C_{22} and C_{16} fatty acids of fertilized eggs were slightly decreased in comparison with the case of unfertilized eggs, while the contents of saturated and C_{18} unsaturated fatty acids were slightly increased. From these observations, it can be concluded that some kinds of specific highly unsaturated fatty acids of egg lipids were consumed slowly during the developmental stage, but no rapid changes occurred during this stage.

In comparison with the composition of fatty acids of lecithin and that of glyceride, the contents of saturated C_{16} and C_{18} fatty acids of the lecithins is larger, whereas their contents of unsaturated C_{16} and C_{20} fatty acids are relatively smaller than those of glyceride; no marked differences in the contents of unsaturated C_{18} and C_{22} acids between these two lipids were observed. As noted above, in comparison of the properties of lipids between the unfertilized and the fertilized salmon eggs, no marked differences were observed; it may be concluded that no marked changes occurred in the lipid composition of salmon eggs before and after fertilization, but that gradual changes occurred in lipid during the developmental period.

Futhermore, crab eggs were used to compare the properties of lipids between before and after fertilization. The purity of lecithin, prepared by the same method from these two conditions of crab eggs were the same, but the yield of lecithin obtained from the fertilized eggs was 96.3 per cent smaller than that from the unfertilized eggs.

The changes in combined fatty acid of lecithin were considered to be caused by the decrease of iodine number. This tendency was almost in agreement with the case of the fatty acid composition of the lipids obtained from unfertilized and fertilized salmon eggs. Therefore it was concluded that some kinds of highly unsaturated fatty acids were consumed during the period of egg development.

Table 23. Properties of Glyceride from Crab Eggs

Glyceride from		Unfertilized Egg	Fertilized Egg
Yield	g	70.0	27.0
	g/kg	66.7	24.6
Acid no.		31.8	62.6
Sap. no.		179.3	177.2
Iod. no.		190.5	181.4
n_D^{20}		1.4868	1.4860

Table 24. Composition of Component Fatty Acids of Crab Egg Glycerides (%)

Fatty Acid	Saturated	Unsaturated (Double Bond Number)			
		Mono	Tri	Tetra	Penta
C_{14}	2, (1)	—	—	—	—
C_{16}	10, (10)	5, (7)	1, (1)	—	—
C_{18}	3, (3)	17, (17)	—	11, (13)	—
C_{20}	1, (1)	11, (13)	—	—	16, (15)
C_{22}	—	4, (3)	—	—	13, *(13)**
C_{24}	—	—	—	—	6, **(3)**
Total	16, (15)	37, (40)	47, (45)		

Note: The numers in parenthese indicate the percentages of fatty acid obtained from the fertilized egg glycerides.
* It includes hexaenoic acid
** Mainly hexaenoic acid

No marked increase of the sphingolipids in the fertilized eggs was observed as compared with the amounts in the unfertilized egg. Tsuji[155] studied the changes of conjugated lipids during the development of hen egg and concluded that the sphingolipids were synthesized from the lecithin during this stage. In the present studies marked decrease of lipids of crab eggs was observed in lecithin, whereas no marked increase occurred in sphingolipids during this period; these results do not always agree with those of Tsuji.

As previously stated, changes in the composition of fatty acid of egg lecithin were observed during the developmental period, but no changes in the fatty acid composition of glyceride obtained during this period were observed. In this chapter are described the changes of the fatty acid composition of glyceride of egg obtained during this period.

As the details were reported elsewhere[156),157)], only the properties and the fatty acid composition of the glyceride obtained from unfertilized and fertilized crab eggs are shown in Tables 23 and 24. The total amount of 41.4 per cent

Table 25. Properties of Unsaponifiable Material Obtained from Crab Eggs

	Unsaponifiable Material	Unfertilized	Fertilized
Solid Portion	Yield g	2.2	1.2
	Liebermann-Burchard Reaction	+	+
	m. p. (°C)	136.0—137.5	137.0—138.0
	5,7 dehydrosterol %*	2.20	0.46
Liquid Portion	Yield g	1.7	0.8
	Liebermann-Burchard Reaction	+	+
	Iodine no.	123.7	113.5
	Acetyl no.	221.5	219.5

* This fraction was obtained from acetone-soluble fraction.

glyceride of fertilized eggs show decrease as compared with that of unfertilized eggs.

This fact is not very different from the case of eggs of other animals[158]. Acid number of glyceride of fertilized eggs increased as many as double when compared to that of unfertilized eggs. The reason for this phenomenon was not clear, for whether the fatty acid was freed from the glyceride or phospholipids, or from both lipids was unkown. The decrease of iodine number of the lipids obtained from the fertilized eggs was clearly due to the changes of component fatty acids. Actually no hexaenoic acid was found in glyceride obtained from unfertilized eggs. However, no remarkable changes were observed in the relationship between monoethylenic acids and saturated acids.

The amount of unsaponifiable matter in the lipids of fertilized eggs was decreased to 53.4 per cent of that of unfertilized eggs; this decrease is smaller than in the case of the glyceride. The decrease of unsaponifiable matter did not agree with that of glyceride.

As shown in Table 25, marked decrease of $\Delta^{5,7}$ sterol in the fertilized eggs was observed as compared to that in the unfertilized eggs; this finding is in good agreement with the results of Glover[159], who used material from the salmon egg.

No remarkable changes were observed in the acetyl values of alcohol fraction of unsaponifiable matter obtained from sorts of eggs, but there was observed the decrease of iodine number of the alcohol obtained from fertilized eggs as compared with that of unfertilized eggs. These results indicate that disappearance of highly unsaturated fraction of alcohol occurred together with that of fatty acids of these lipids.

Above have been discussed the differences between the component fatty acids of lecithin and those of glyceride of marine fish eggs. Furthermore carp eggs were used to study the differences between the component fatty acids of lecithin

and those of glyceride of fresh water fish eggs. No marked differences were observed between the composition of component fatty acids of the carp egg lecithin and those of the marine animal egg; glyceride obtained from carp eggs contained only 1.5 per cent of C_{22} unsaturated acid, which material is contained abundantly in the glyceride obtained from various marine animal eggs such as shark, pollack, salmon, and crab.

Lovern[160] reported that the content of C_{22} higher unsaturated fatty acids in marine fish oil, crustacea and plankton oil was a bit over ten per cent, however, these fatty acids were scarcely contained in fresh water fish oil which resembles green sea weed oil. The content of C_{22} unsaturated fatty acid of the carp egg glyceride was only 1.5 per cent of the total component fatty acids; it showed remarkable fresh-water-fish type fatty acid composition resembling that of carp body oil, whereas the component fatty acid composition of a salmon egg showed marine-fish type fatty acid composition.

So it was concluded that the fatty acid composition of egg glyceride might be affected by fat deposited in the body, but that of lecithin might not be affected by it.

Nottbohm[161] reported the quantity of cephalin in hen eggs and Masuda et al.[138], that of cephalin in fish eggs. Nishimoto[24] and Suzuki et al.[25] reported the properties of cephalin obtained from hen eggs. Carter et al.[63], and Dils et al.[46], found the presence of phosphatidylethanolamine and inositolphospholipid in hen eggs. Rhodes et al.[36], found the presence of serine and threonine in the hydrolysate of hen egg cephalin. In addition to the above a few investigations have been made on the properties of egg cephalin of aquatic animals. The purpose of presently reported study was to investigate the properties of cephalin obtained from the eggs of several kinds of aquatic animals, such as shark, pollack, salmon and crab.

Serine and very small amounts of inositol were found to exist but no ethanolamine was found in the hydrolysate of pollack egg cephalin. That fact suggests that the pollack egg cephalin comprised a small amount of inositolphospholipids besides phosphatidylserine but that phosphatidylethanolamine did not exist; however, the hen egg cephalin was composed of very small amount of phosphatidylserine and inositolphospholipids beside greater parts of phosphatidylethanolamine.

It was difficult to understand the reason for the very fact described just above whether due to the properties of fish egg cephalin themselves or to the properties of cephalin obtained from immature fish eggs; this pollack egg cephalin was mainly comprised of phosphatidylserine which was decarboxylated to phosphatidylethanolamine in company with the attainment of the stages of maturity, fertilization and development.

To confirm this point, two kinds of cephalin, which were obtained before and after fertilization of salmon eggs, were used for the material of the experiment; the properties of these cephalin samples were exactly compared with each other.

The yield of conjugated lipids per one egg is shown in Tables 17 and 19 in this chapter. The yield of cephalin obtained from salmon eggs after fertilization did not decrease very much as compared to that obtained from pre-fertilization salmon eggs though lecithin decreased markedly; these two sorts of cephalin consisted of phosphatidylethanolamine, phosphatidylserine and inositolphospholipid.

It might be concluded that phosphatidylserine existed in the immature eggs mainly, and it was decarboxylated into phosphatidylethanolamine as the eggs matured. But in the case of salmon egg cephalin, marked changes were not observed between the egg cephalin samples obtained from the unfertilized eggs and the fertilized eggs at 24 hours after fertilization.

To make clear the changes of properties of cephalin during the development of eggs, unfertilized and fertilized crab eggs obtained from the same body were used as materials.

As it was impossible to analyse the cephalin with absolute accuracy, two cephalin samples were prepared in the same way from either the unfertilized or the fertilized crab eggs and comparison was made of the properties of these cephalins. The yield of cephalin from fertilized eggs decreased to the amount of 31.0 per cent of that from unfertilized eggs and the decrease of iodine number was also noted. The amount of higher unsaturated fatty acid, such as hexaenoic and pentaenoic acid, was lower in the fertilized eggs than that in the unfertilized eggs. These facts showed that consumption of cephalin and selective consumption of higher unsaturated fatty acid of cephalin occurred during the developmental stage of crab eggs.

Two kinds of nitrogenous base were discovered in the cephalin, one of which was serine contained in the unfertilized crab egg cephalin while the other was ethanolamine contained in the fertilized crab egg cephalin. Traces of inositol were found in both sorts of cephalins. So it could be concluded that the cephalin of unfertilized crab egg is a mixture of a small amount of inositolphospholipids besides phosphatidylserine and decarboxylated into phosphatidylethanolamine in the course of maturation, fertilization and development. Notwithstanding the effort of Nishimoto[24], Suzuki et al.[25] and Carter et al.[63], only phosphatidylethanolamine was obtained and no phosphatidylserine was detected in the cephalin samples prepared from commercial hen eggs. Furthermore, in spite of the effort of Rhodes et al.[36], only small amounts of serine and threonine were detected in the hen egg cephalin, but the writer could detect a large amount of phosphatidylserine in the

cephalin prepared from unfertilized pollack eggs.

These matters could be explained by the supposition that phosphatidylserine contained in the cephalin obtained of immature eggs converted into phosphatidylethanolamine in the course of maturation and fertilization.

Though phosphatidylserine has previously been believed to be the mother substance of phosptidylethanolamine, these changes were not proved in the living body.

Nord[162] has suggested that ethanolamine originates in putrefactive anaerobes, by decarboxylation of serine. Later the presence of serine-decarboxylase was suggested in the living body, but it has not been proved actually. Therefore it remains unproved whether or not phosphatidylserine changes into phosphatidylethanolamine in the living body.

The iodine number and the amount of unsaturated fatty acid of brain phosphatidylserine was found to be lower than those of phosphatidylethanolamine, and vice versa in the egg cephalin. This fact shows that unsaturated fatty acids of cephalin were consumed and that decarboxylation of phosphatidylserine into phosphatidylethanolamine occurred at the same time.

The results of Scherer's reaction on the hydrolysate of the cephalin obtained from fertilized eggs was weaker than on that obtained from unfertilized eggs. From this fact it was considered by the present writer that the content of inositolphospholipid of cephalin obtained from the fertilized eggs was smaller than that obtained from unfertilized eggs.

No studies have been carried out on the presence of phosphatidic acid in fish eggs, but in the present study phosphatidic acids were prepared from the unfertilized and the fertilized eggs of crab. The content of phatidic acid as well as of the other phospholipids decreased during the developmental stage. Namely, in the case of crab eggs, the amount of phosphatidic acid obtained from fertilized eggs decreased to 47.4 per cent as compared to that from unfertilized eggs, and also a decrease of iodine number was observed. These facts prove that the consumption of phosphatidic acid and unsaturated fatty acid conjugated to phosphatidic acid occurred during the developmental stage.

The structures of these two phosphatidic acids obtained from the unfertilized and the fertilized eggs of crab did not show marked differences between them; the results of paper chromatographic analysis and a serological test did not show any differences. But it was uncertain that these phosphatidic acids samples were pure substances, though only one spot was detected by paper chromatography, when judged from the content of phosphorous and the results of the serological test of the phosphatidic acid.

Sphingolipids obtained from salmon eggs mainly comprised sphingomyelin and cerebrosides.

4. HEART PHOSPHOLIPIDS OF AQUATIC ANIMALS

Many studies have been carried out on the heart phospholipids of land animals since Pangborn[85)-87)] found cardiolipin in the ox heart, but no studies have been carried out on the heart phospholipids of aquatic animals.

In this chapter studies on the properties of heart phospholipids of aquatic animals such as rorqual and pollack, particularly the properties of phosphatidic acid are reported.

Materials

a) The rorquals (*Balaenoptera bolealis*), used as material were caught off the coast of Onagawa, Miyagi Prefecture in June, 1954.

Material employed for the experiments was only the very fresh heart treated immediately after the animal had been killed.

Refrigerated hearts (five thousand seven hundred grams) was brought to the laboratory after having been washed with sea water carefully.

b) The pollacks (*Theragra chalcogramma*), used as material were caught on the coast of Yoichi, Hokkaido at the end of December, 1957.

Materials employed for the experiments were only very fresh hearts treated immediately after the fish had been killed.

Nine thousand two hundred grams of refrigerated hearts (10975 bodies) were brought to the laboratory after having been washed throughly with physiological saline solution.

Method of Analysis

Phosphorous was determined by Lieb's method[120)], nitrogen by the micro-Kjeldahl procedure, choline, serine and ethanolamine by Levine and Chargaff's[121)], glycerol by Blix's method[124)] and iodine number was determined by Wijs' technique. Inositol was detected by use of Scherer's reaction.

Preparation of Phospholipids

The procedures, used in this study have already been fully described in previous papers[98),163)] and only a brief scheme is herein presented in Fig. 7.

Results

(I) Phospholipids of the Rorqual Heart

A) Rorqual Heart Lecithin[163]: Two and five-tenths grams of lecithin was obtained from 5700 grams of fresh heart tissues as outlines in Fig. 7. The results of analysis of the lecithin were as follows: N 1.76%, P 4.13%, Choline 14.57%, N : P 0.95 : 1.00, Choline : N 0.96 : 1.00, Iodine no. 91.6.

1) Hydrolysis of Lecithin

a) Glycerophosphoric Acid: This acid was obtained as barium glycerophosphate from the hydrolysate of the lecithin. The results of analysis were as follows:

$C_3H_7O_6PBa$ Calcd. : P 10.10%, Ba 44.69%
Found. : P 10.17%, Ba 44.96%

b) Fatty Acid: The neutral number of the mixed fatty acids obtained from the lecithin showed 198.4 and the iodine number showed 132.5; the components were hexaenoic (as docosahexaenoic) acid 2.5 per cent, pentaenoic (as clupanodonic) acid 6.2 per cent, tetraenoic (as arachidonic) acid 7.6 per cent, trienoic (as linolenic) acid 6.1 per cent, dienoic (as linoleic) acid 4.1 per cent, monoethylenic (as oleic) acid 52.9 per cent, and saturated acid 20.6 per cent.

B) Rorqual Heart Cephalin[163]: Three and one-tenth grams of cephalin was obtained from 5700 grams of rorqual heart by the method outlines in Fig. 7. The results of analysis were as follows: P 2.66%, N 1.37%, Serine 2.52%, Ethanolamine 4.90%, N : P 1.13 : 1.00, Inositol : Slightly positive, Iodine no. 122.3.

a) Fatty Acid: The neutral number of the components fatty acids of the cephalin showed 197.7 and the iodine number showed 201.6; the components were hexaenoic (as docosahexaenoic) acid 3.1 per cent, pentaenoic (as clupanodonic) acid 8.2 per cent, tetraenoic (as arachidonic) acid 10.6 per cent, trienoic (as linolenic) acid 16.5 per cent, dienoic (as linoleic) acid 0.8 per cent, monoethylenic (as oleic) acid 35.0 per cent and saturated acid 36.4%.

C) Rorqual Heart Phosphatidic Acid[98]: Seven hundred milligrams of phosphatidic acid was obtained from five thousand seven hundred grams of rorqual heart by the method described above in Fig. 8. The analytical results were as follows: P 3.96%, N 0.07%, Iodine no. 112.1.

2) Hydrolysis of Phosphtidic Acid

a) Glycerophosphoric Acid: Ba-glycerophosphates were obtained from the phosphatidic acid by the method shown in Fig. 9.

As the materials were scanty, Fractions 1 and 2 could not be further studied. The results of analysis of Fraction 3 are shown in Table 26.

Fig. 7. Extraction and Purification of the Phospholipids from Rorqual Heart

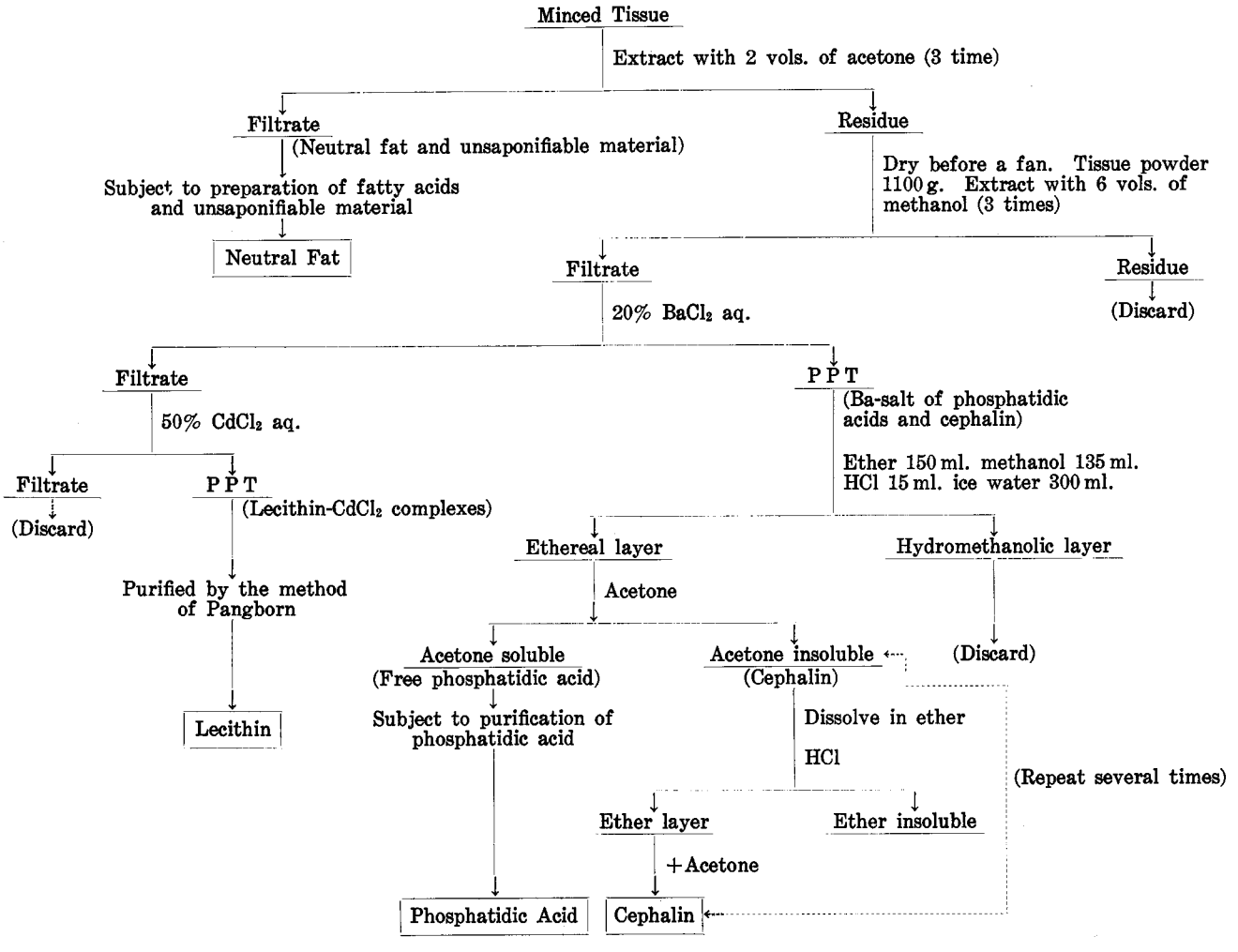
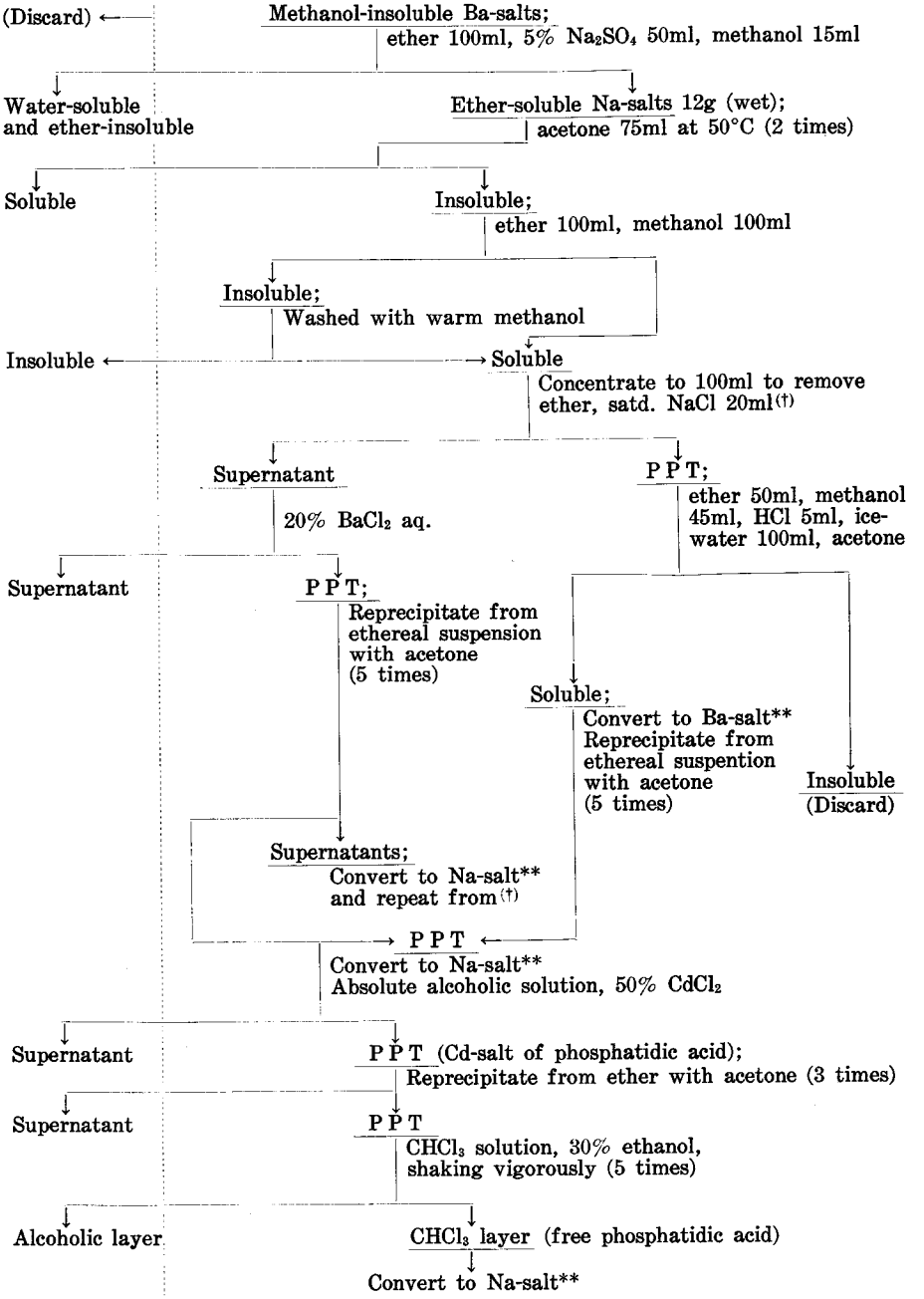


Fig. 8. Purification of Phosphatidic Acid of Rorqual Heart

(Rorqual-heart tissue is extracted first with acetone and then with methanol. Methanol-insoluble Ba-salts obtained by addition of $BaCl_2$ to the methanolic solution are converted to free lipids, which are then fractionated into acetone-soluble and-insoluble fractions. Then Ba-salt which is precipitated by addition of $BaCl_2$ to the methanolic solution of the acetone-soluble fraction is subjected to the purification presented below.)*



* See Fig. 7. ** In the same manner as that above outlined in this figure.

Fig. 9. Hydrolysis of Phosphatidic Acid

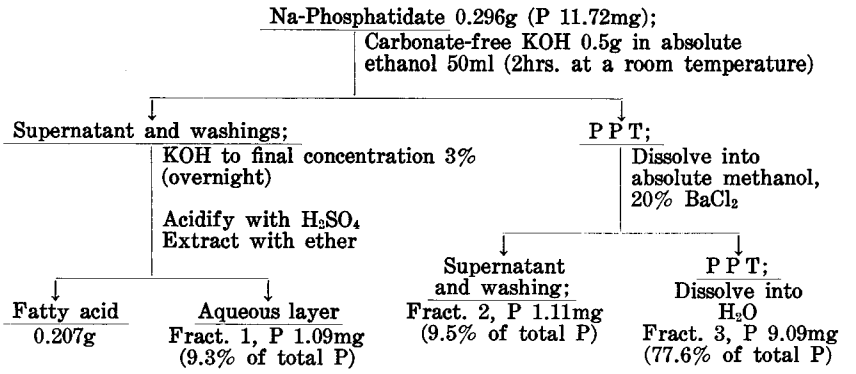


Table 26. Analysis of Ba-Salt of Fraction 3

Component		Found	Calculated as*		
			(A)	(B)	(C)
Ba	%	26.99	25.66	27.21	28.06
P	%	12.36	11.57	12.28	12.66
Glycerol	%	40.02	51.58	48.63	47.03

* Values calculated in per cent for the following formulas.
(A) $C_9H_{20}O_{13}P_2Ba$ (Glyceryl-di-glycerophosphate)
(B) $C_{12}H_{26}O_{18}P_3Ba_{3/2}$ (Glyceryl-tri-glycerophosphate)
(C) $C_{15}H_{32}O_{23}P_4Ba_2$ (Glyceryl-tetra-glycerophosphate)

b) Fatty Acid: The fatty acids (Neutr. no. 197.2, Iodine no. 163.5) obtained from the phosphatidic acid were composed of hexaenoic (as docosahexaenoic) acid 1.3 per cent, pentaenoic (as clupanodonic) acid 6.2 per cent, tetraenoic (as arachidonic) acid 12.1 per cent, trienoic (as linolenic) acid 23.6 per cent, dienoic (as linoleic) acid 9.0 per cent, monoethylenic (as oleic) acid 13.8 per cent and saturated acid 34.0 per cent.

i) Serological Studies of the Rorqual Heart Phosphatidic Acid: Serological assay was carried out with phosphatidic acid according to Wassermann's[164], Kahn's[164], and slide test[164] respectively, and the result was active as shown in Table 28.

The original antigen solutions were prepared after Wassermann's, Kahn's and slide test; their composition is shown in Table 27.

The results of serological assay shown in Table 28.

(II) Phospholipids of the Pollack Heart[78]

A) Pollack Heart Lecithin[78]: Three and five tenths grams of lecithin was obtained from 9200 grams of pollack heart. The results of analysis were as follows: P 3.94%, N 1.73%, Choline 14.53%, Glycerol 11.20%, Glycerol : P 0.96 : 1.00, Io-

Table 27. Composition of the Original Antigen Solution (% in weight)*

	Antigens for the Reaction of		
	Wassermann	Kahn	Slide test
Cardiolipin or phosphatidic acid of rorqual heart	0.01	0.1	0.03
Lecithin	0.04	1.0	0.30
Cholesterol	0.20	0.025	0.90

* Diluted with absolute alcohol.

Table 28. Result of Tests of Serological Reaction to Syphilis Sera with the Antigens Containing Cardiolipin or Phosphatidic Acid of Rorqual Heart

Serum No.	Cardiolipin			Phosphatidic acid of rorqual heart		
	Wassermann	Kahn	Slide test	Wassermann	Kahn	Slide test
1	###	###	+	###	###	###
2	##	−	+	##	+	+
3	###	+	##	###	+	###
4	###	−	##	###	+	##
5	−	−	−	+	+	##
6	−	−	−	+	+	+
7	##	−	−	##	+	+
8	−	−	−	±	−	+
9	−	−	−	−	+	+
10	−	−	−	−	−	−
11	−	−	−	−	±	−
12	−	−	−	−	±	+
13	−	−	−	−	−	−
14	−	−	−	−	−	−
15	−	−	−	−	−	−

dine no. 109.4.

a) Fatty Acid: From the component fatty acids (Neutr. no. 199.2 and Iodine no. 130.8) of the lecithin palmitic, stearic, arachidic, behenic, zoomaric, oleic, eicosenoic, linoleic and linolenic acid were detected by paper chromatography[165),166)]; the fatty acid consisted of saturated acid 35.3 per cent, monoethylenic (as oleic) acid 30.4 per cent, dienoic (as linoleic) acid 4.6 per cent, trienoic (as linolenic) acid 1.2 per cent, tetraenoic (arachidonic) acid 5.7 per cent, pentaenoic (as clupanodonic) acid 9.6 per cent and hexaenoic (as docosahexaenoic) acid 13.0 per cent.

B) Pollack Heart Cephalin[78)]: The yield of cephalin from 9200 grams of pollack heart was 2.5 grams. The obtained results of analysis were as follows: P 3.99%,

N 1.55%, Glycerol 10.85%, Serine 2.10%, Ethanolamine 5.03%, N : P 0.90 : 1.00, Glycerol : P 0.92 : 1.00, Iodine no. 102.7, Inositol+.

a) Fatty Acid: The fatty acid (Neutr. no. 198.5, Iodine no. 108.8) of this cephalin consisted of saturated acid 14.4 per cent, monoethylenic (as oleic) acid 50.3 per cent, dienoic (as linoleic) acid 8.6 per cent, trienoic (as linolenic) acid 4.4 per cent, tetraenoic (as arachidonic) acid 2.3 per cent, pentaenoic (as clupanodonic) acid 10.0 per cent and hexaenoic (as docosahexaenoic) acid 10.0 per cent.

C) Pollack Heart Phosphatidic Acid[78]: Two hundred and sixty-five milligrams of phosphtidic acid was obtained as a sodium salt from the pollack heart by the same procedure as outlines described in Figs. 7 and 8. Results of analysis thereof are shown in Table 29.

i) Paper Chromatographic Identification of Phosphtidic Acid: The phosphatidic acid obtained from the pollack heart was examined by paper chromatography as follows. Tōyō No. 50 paper was used with the solvent system n-propanol-acetic acid-water (8 : 1 : 1, v/v) with ascending front chromatography (at 25° C).

The R_f values detected by several reagents are shown in Table 30.

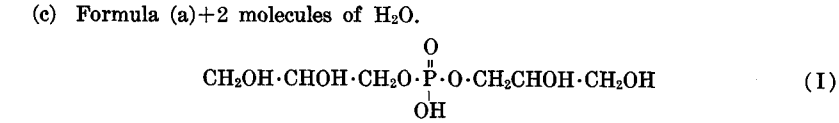
3) Hydrolysis of the Phosphatidic Acid

a) Glycerophosphoric Acid: Ba-glycerophosphate was obtained from the hydrolysate of phosphatidic acid as outlined in Fig. 9; results of analysis of Fraction 3 are shown in Table 31.

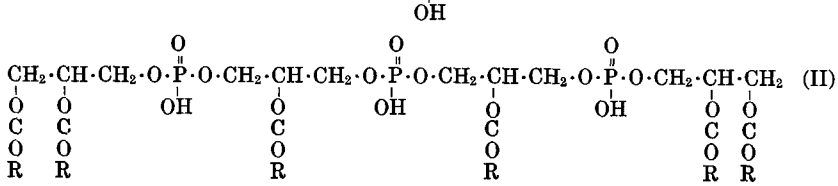
Table 29. Properties of Na-Phosphatidic Acid of Pollack Heart

Component		Found	Calculated as		
			(a)	(b)	(c)
P	%	4.21	4.23	4.20	4.17
Glycerol	%	16.51	16.78	16.64	16.58
Iodide	no.	93.2	127.2	126.5	125.1

Note: Values calculated in per cent by the following formulas.
(a) $C_{120}H_{208}O_{24}P_3Na_3$ with eleven double bonds (five linoleyl radicals and one oleyl) Mol. Wt. 2195 (Formula II).
(b) Formula (a)+1 molecule of H_2O.
(c) Formula (a)+2 molecules of H_2O.



(I)



(II)

Table 30. Paper Chromatography of Phosphatidic Acid from Pollack Heart

	R_f		R_f
Phosphoric acid (acid ester)	0.89–0.90	Amino base	—
Unsaturated bond	0.89–0.90	Choline	—
Fatty substance	0.89–0.90	Ammonia	—

Note: Phosphoric ester[138] was detected by spraying with an acid molybdate solution.
Unsaturated bond[140] was detected by spraying with a potassium permanganate solution.
Amino bases[139] were detected by spraying with a ninhydrin reagent.
Choline[32] was detected with a phosphomolybdic reagent.
Ammonia was detected by spraying with Nesseler's reagent.
Fatty substances[157] were detected by spraying with a Nile blue reagent.

Table 31. Analysis of Ba-Salt of Fraction 3

Component		Found	Calculated as*		
			(A)	(B)	(C)
Ba	%	27.01	25.66	27.21	28.06
P	%	12.40	11.57	12.28	11.66
Glycerol	%	47.83	51.58	48.63	47.02

* Values calculated in per cent for the following formulas.
(A) $C_9H_{20}O_{13}P_2Ba$ (Glyceryl-di-glycerophosphate)
(B) $C_{12}H_{26}O_{18}P_3Ba_{3/2}$ (Glyceryl-tri-glycerophosphate)
(C) $C_{15}H_{32}O_{23}P_4Ba_2$ (Glyceryl-tetra-glycerophosphate)

b) Fatty Acid: The fatty acid (Neutr. no. 196.7, Iodine no. 104.7) of this phosphatidic acid consisted of saturated acid 12.8 per cent, monoethylenic (as oleic) acid 81.5 per cent, dienoic (as linoleic) acid 1.9 per cent, trienoic (as linolenic) acid 0.5 per cent, tetraenoic (as arachidonic) acid 0.6 per cent, pentaenoic (as clupanodonic) acid 1.3 per cent and hexaenoic (as docosahexaenoic) acid 1.4 per cent.

Discussion

These studies have been undertaken in order to compare the properties of the phospholipids, such as the lecithin, cephalin and the phosphatidic acid, obtained from the heart of whale and fish. As materials rorqual and pollack hearts were used. Serological assays were carried out on the phosphatidic acid obtained from rorqual heart and paper chromatographic studies on that from pollack heart.

The yield of lecithin from the rorqual heart muscle was much smaller than that from the ox heart, which had reported by Pangborn[87]; in pollack heart lecithin, the yield was much smaller. Great differences were observed between the component fatty acids of these lecithins and those of ox heart lecithin. When com-

parison was made between the component fatty acids of the rorqual heart lecithin and those of the pollack heart many differences were observed, namely the heart lecithin of pollack contained more unsaturated fatty acid than that of rorqual, and clear differences were recognized between the component fatty acids of their lecithins and that of the glycerides[163] obtained from the same materials as well as from egg lipids. (See Table 32).

The phospholipids were extracted from the acetone treated rorqual heart muscle with methanol and were precipitated with barium chloride. Barium salts of the cephalin and phosphatidic acid were broken down with hydrochloric acid and then treated with acetone to separate into the cephalin and phosphatidic acid fractions. The cephalin fraction was purified with ether and ethanol. The heart cephalin of rorqual thus obtained showed high iodine number and high N : P molar ratio; an unknown aminobase other than serine and ethanolamine was contained in its hydrolysate. The existance of inositolphospholipid was doubtful in this cephalin, because Scherer's reaction in the hydrolysate of cephalin was very weak, whereas the existence of phosphatidylserine and phosphatidylethanolamine in this cephalin was clear. The heart cephalin of pollack was prepared as well as that of rorqual. The yield of the latter was 0.53 grams per kilogram and that of the former was 0.5 grams per kilogram. The pollack cephalin showed high iodine number, but low N : P molar ratio and glycerol : phosphorous molar ratio; it was positive in Scherer's reaction and the content of serine and ethanolamine was greater than that of rorqual heart cephalin. From results noted above it might be con-

Table 32. Composition of the Component Fatty Acids of Lecthin and Glyceride Obtained from Heart

Fatty Acid from		Ox Heart Lecithin	Rorqual Heart Lecithin	Pollack Heart Lecithin	Rorqual Heart Glyceride	Pollack Heart Glyceride
Saturated fatty acid	%	35	20.6	35.3	16	21.3
Monoethylenic (as oleic) acid	%		52.9	30.4	36*	
Dienoic (as linoleic) acid	%	45	4.1	4.6	6	
Trienoic (as linolenic) acid	%		6.1	1.2		78.7
Tetraenoic (as arachidonic) acid	%		7.6	5.7	16	
Pentaenoic (as clupanodonic) acid	%	15	6.2	9.6	16	
Hexaenoic (as docosahexaenoic) acid	%		2.5	13.2	—	

* This includes oleic, zoomaric, and eicosenoic acid.

cluded that this pollack cephalin consisted of phosphatidylserine, phosphatidylethanolamine and inositolphospholipid. The existence of an unknown amino base in the hydrolysate of rorqual heart cephalin suggested the presence of an unknown amino base containing phospholipid in the same cephalin.

Since Wassermann developed the serological diagnosis of syphilis and used as an antigen the salt solution extracts from the liver of the congenital syphilis fetus in 1926, it was found that the effective component was a kind of phospholipid. It was also found to be widely distributed in the normal viscera of the animal by the effort of many investigators. The ethanol extracts of the heart have been used for a long time as an antigen in the serological diagnosis of syphilis.

Pangborn[85)-87)] isolated the available compound from the ox heart and demonstrated that it was a kind of phosphatidic acid; she named it cardiolipin. Later this substance was isolated from the ox heart by Faure[88)] using a more simple method. Further a glycerophosphoinositide was obtained from the ox heart by Faure et al.[69)], but it did not have the activity of antigen for Wassermann's reaction.

In the present experiments a cardiolipin-like phosphatidic acid was obtained from the heart of rorqual and pollack by the simplified method of Pangborn[87)].

Since barium-glycerophosphates mainly obtained from the hydrolysate of these two phosphatidic acids were considered as barium-glyceryltriglycerophosphate, it might be concluded that these substances had a cardiolipin-like structure. The activity as antigen of the phosphatidic acid obtained from the rorqual heart shows as strong as that of commercial cardiolipin. In some cases the negative result was obtained by the writer in the Wassermann's test by use of the commercial cardiolipin, whereas slightly positive result was obtained by the rorqual heart phosphatidic acid. This fact was considered to be due to the contaminants contained in the rorqual heart phosphatidic acid or lecithin.

It had been reported that tetraacyl-bis-(L-α-glyceryl)-phosphoric acid, one of the phosphatidic acids synthesized by Baer et al.[84)], was active in Wassermann's test as antigen other than cardiolipin, but Tonks[168)] showed that this substance had only weaker activities than that of cardiolipin.

Faure et al.[88)] observed that the phosphatidic acid obtained from the carrot and other vegetables showed a weak activity as an antigen for serological diagnosis of syphilis being due to the small amount of contaminants mixed in the phosphatidic acid.

From the above observation, it was considered that the structure of these two phosphatidic acids obtained from rorqual heart and pollack heart to have a cardiolipin like structure.

As shown on pages 48 and 51 above, components of fatty acid differ greatly

from cardiolipin obtained by Pangborn, who found them to be comprised of oleic radical, 1 and linoleic radical, 5. This composition was due to the conjugation by alkaline and moreover to the spectrophotometry employed. However, the writer's analysis proved that their composition is more complicated than that stated by Pangborn, showing similar tendency with the results obtained by Macfarlane[92),93)] which showed palmitic acid 0.5 per cent, palmitoleic acid 5.2 per cent, stearic acid 0.8 per cent, oleic acid 11.0 per cent, linoleic acid 72.0 per cent, linolenic acid 8.0 per cent and C_{20} polyethylenic acid 1.5 per cent.

5. PHOSPHOLIPIDS OF MOLLUSCA

Only a few studies have been carried out on the mollusca phospholipids: Kitabayashi[169)] studied the squid (*Ommastrephes sloani pacificus*) muscle phospholipids, while Hori et al.[170,171)] studied the phospholipids of setashijimi (*Corbicula sendai*), and karasugai (*Anodonta woodiana*).

In the present chapter, the properties of lecithin and cephalin of mollusca particularly their fatty acid composition were studied.

Materials

1. Chlamys (*Chlamys nipponensis*) used as the materials were collected at the coast of Kamiiso, Hokkaido in March, 1957.

Four thousand nine hundred and twenty grams (250 bodies) of shell free materials were used as the sample.

2. Pecten (*Pecten yessoensis*) used as the materials were collected at the coast of Tokoro, Hokkaido in July, 1957.

Three thousand and five grams (202 livers) of pecten livers were used as the sample.

3. Octopus (*Octopus dofleini*) used as the material were caught off the coast of Kushiro, Hokkaido in December, 1955.

Seven thousand three hundred grams of octopus livers were used as the sample.

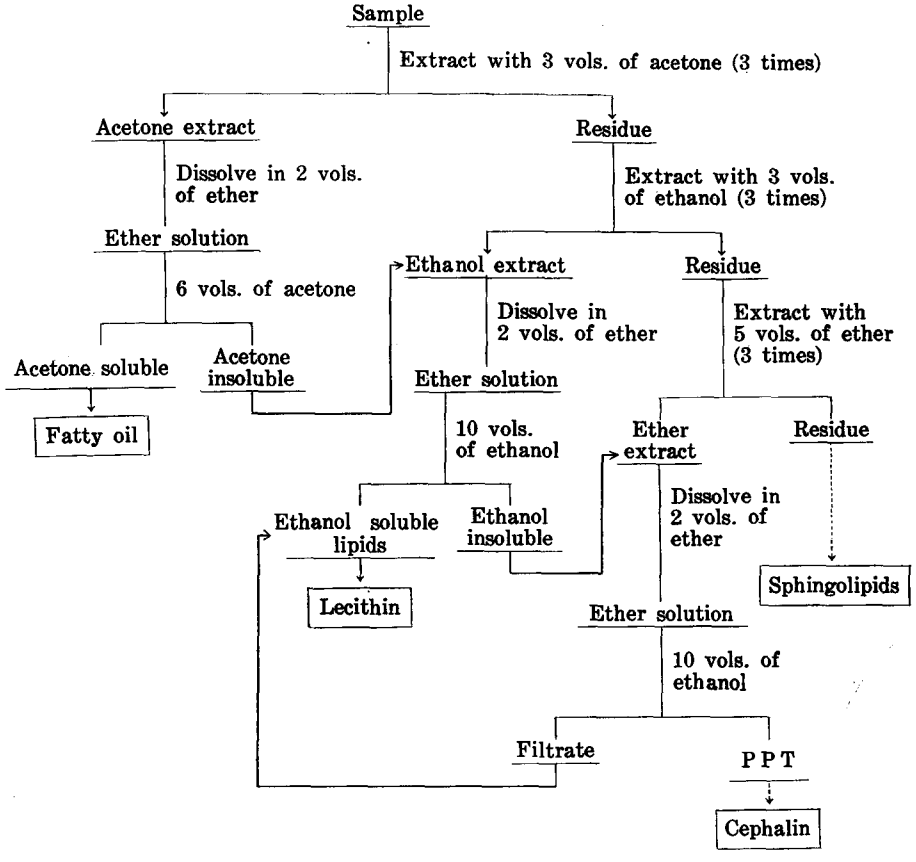
Method of Analysis

Phosphorous was determined by Fiske-Subbarow's method[119)], nitrogen by the micro-Kjeldahl procedure, choline by Beattie's[122)] ethanolamine and serine by Nojima and Utsugi's[123)], glycerol by Blix's[124)] and iodine number by Wijs' technique. Inositol was detected by Scherer's reaction.

Preparation of Lecithin and Cephalin

As the details of the isolation method of lecithin and cephalin from the viscera of mollusca have been described in the writer's previous paper[172], only a brief outline is herein given in Fig. 10.

Fig. 10. Extraction and Fractionation of Conjugated Lipids



Results

The properties of the lecithin and cephalin prepared from the shell-free chlamys, pecten liver and octopus liver are shown in Tables 33 and 34.

Fatty Acid Composition of Mollusca Lecithin and Cephalin: Lecithin and cephalin of mollusca were hydrolysed with 2N-KOH by refluxing on a water-bath for 1 hr. respectively. From these hydrolysates fatty acids were obtained by the

Table 33. Properties of Lecithin of Mollusca

Component \ Lecithin from	Chlamys (Shell free)	Pecten (Liver)	Octopus (Liver)
Yield g/kg	0.8	1.8	0.15
P %	3.99	4.03	3.56
N %	1.85	1.80	1.75
Choline %	14.62	13.24	15.00
Glycerol %	11.25	11.61	11.66
N/P molar ratio	1.02	0.99	1.09
Choline/P molar ratio	0.94	0.94	1.08
Glycerol/P molar ratio	0.95	0.94	1.01
Iodine no.	96.3	109.2	57.1

Table 34. Properties of Cephalin of Mollusca

Component \ Cephalin from	Chlamys (Shell free)	Pecten (Liver)	Octopus (Liver)
Yield g/kg	0.7	0.3	0.07
P %	4.05	4.02	3.30
N %	1.72	1.62	1.71
Ethanolamine %	5.65	5.82	6.20
Serine %	3.18	2.36	1.34
Glycerol %	11.34	10.32	10.04
Inositol	+	+	−
N/P molar ratio	0.94	0.86	1.14
Ethanolamine/P molar ratio	0.71	0.73	0.96
Serine/P molar ratio	0.23	0.17	0.11
Glycerol/P molar ratio	0.95	0.86	1.02
Iodine no.	75.4	60.8	−

usual manner and analysed by the method of Herb and Riemenschneider[128] and then calculated using the equation of Hammond and Lundberg[129]. The results are shown in Table 35.

Discussion

In this study, lecithin and cephalin, were obtained from the liver of octopus and pecten, and from the shell-free chlamys. The properties of these samples of lecithin and cephalin are shown in Tables 33 and 34. The composition of the component fatty acid of these lecithin and cephalin samples is shown in Table 35.

The yield of phospholipids obtained from the viscera of these mollusca was smaller than that of fatty oil obtained from these organs.

Table 35. Fatty Acid Composition of Lecithin and Cephalin of Mollusca

Fatty acid from		Chlamys (Shell free)		Pecten (Liver)		Octopus (Liver)
		Lecithin	Cephalin	Lecithin	Cephalin	Lecithin
Neutr. no.		197.0	198.2	199.3	198.8	198.2
Iodine no.		110.6	80.2	110.4	64.5	105.3
Saturated acid	%	12.1	55.3	6.7	58.6	20.8
Monoethylenic (as oleic) acid	%	69.9	16.0	80.7	19.5	61.6
Dienoic (as linoleic) acid	%	1.4	4.5	2.0	3.7	7.0
Trienoic (as linolenic) acid	%	2.8	3.1	1.8	2.3	2.6
Tetraenoic (as arachidonic) acid	%	4.1	3.9	3.4	3.6	3.4
Pentaenoic (as clupanodonic) acid	%	7.0	10.8	3.2	8.5	3.2
Hexaenoic (as docosahexaenoic) acid	%	2.7	6.4	2.2	3.8	1.4

Iodine number of the lecithin and the cephalin samples was markedly lower than that of the fatty oil. Large amounts of monoethylenic acid were contained in the fatty acid of these lecithin samples; particularly, pecten liver lecithin contained about eighty per cent.

On the other hand, the composition of cephalin obtained from mollusca liver amounted to about the same volume of saturated and unsaturated fatty acid.

In the case of mollusca phospholipids, some differences were observed between the component fatty acids of lecithin and those of cephalin obtained from the same organs.

Cephalin from shell-fish consisted of phosphatidylserine, phosphatidylethanolamine and inositolphospholipid, but the presence of inositolphospholipid in the liver cephalin of octopus was not assured.

6. DISTRIBUTION OF LIPIDS IN TISSUES OF AQUATIC ANIMALS

Javillar et al.[173], Bähr et al.[174], Kaucher et al.[175] and Matsumoto[176] have reported the distribution of the conjugated lipids in several fishes. A fully satisfactory analytical method for the conjugated lipids in the various tissues has not been discovered, in spite of many analytical methods having been used by several workers[174, 177-181].

Table 36. Comparison of

	Solvent used	Procedure	Solvent (ml/100g fresh tissue)×times
Fresh tissue	Petroleum ether	Shaking at room temperature	600×5
	Hot ethanol	Boiling for 30 mins.	600×7
	Ethanol/ethyl ether (3:1)	Shaking at room temperature	600×5
	Acetone	〃	600×3
Acetone powder	Ethyl ether	Soxhlet apparatus	48hrs
	Ethanol/benzene (32:68)	〃	〃
	Chloroform/methanol (2:1)	Shaking at room temperature	200×4

* Ordinary muscle of herring was used.

In the previous chapters of this paper there have been described in detail the properties of conjugated lipids from the brain, egg, heart and liver of several aquatic animals.

In this chapter is described the distribution of conjugated lipids in various tissues of aquatic animals together with the analytical method.

Extraction of Lipids

In order to compare the extractive power of solvents, the ordinary muscle of male herring was extracted with several kinds of solvents directly, or extracted with several kinds of solvents after having been extracted with acetone.

As shown in Table 36, the results of chloroform-methanol extraction of tissues after having been extracted with acetone was best in percentage, therefore this procedure was used in the presently described experiments.

Table 37. Calculation Formula for Conjugated Lipids

Lecithin (Mol.)=Choline (Mol.)−Sphingomyelin (Mol.), Phosphatidylethanolamine (Mol.) =Ethanolamine (Mol.)−Aldehyde (Mol.), Phosphatidylserine (Mol.)=Serine (Mol.), Acetal lipid (Mol.) = Aldehyde (Mol.), Sphingomyelin (Mol.) = Sphingosine (Mol.) − Hexose (Mol.), Cerebroside (Mol.)=Hexose (Mol.),

The following structures and molecular weights were taken for the calculation for the sake of convenience:

Palmitoyloleoylglycerylphosphorylcholine (M. W. 778) for lecithin, palmitoyloleoylglycerylphosphorylethanolamine (M. W. 718) for phosphatidylethanolamine, palmitoyloleoylglycerylphosphorylserine (M. W. 762) for phosphatidylserine, stearal plasmalogen (M. W. 466) for acetal lipid, N-palmitoylsphingosylphosphorylcholine (M. W. 721) for sphingomyelin, and N-lignoceryl-O-galactosylsphingosine (M. W. 812) for cerebroside.

Extracting Procedure*

The last extract, g/100g of fresh tissue	Total extract before washing, g/100g of fresh tissue	Total extract after washing, g/100g of fresh tissue**	Lipid-phosphorus, m Mol/100g of fresh tissue**	Shihgosine-nitrogen, m Mol/100g of fresh tissue**	Iodine no.
0.0112	3.3584	3.0584	0.310	0	124.2
0.0205	7.0916	3.8860	1.382	0.319	124.0
0.0164	4.3914	3.6400	1.295	0.206	128.7
—	4.1954	3.0750	0.480	0.072	134.7
—	0.6010	0.3400(3.4150)	0.185(0.665)	0.043(0.115)	123.6
—	1.1765	0.7430(3.8180)	0.886(1.366)	0.246(0.318)	118.9
—	1.2841	0.8294(3.9044)	0.900(1.380)	0.254(0.326)	116.5

** Numbers in parentheses show the values also containing those of aceton-etracts.

The results obtained in these experiments and analysis are shown in Tables 38–45.

Materials

Herring (*Clupea pallasii*), tuna (*Thynus orientalis*), mackerel (*Scomber japonicus*), octopus (*Octopus dofleini*), squid (*Ommastrephes sloani pacificus*), chlamys (*Chlamys nipponensis*), clam (*Mactra sachalinensis*), pecten (*Pecten yessoensis*) and neptune (*Neptunea* (*Barbitonia*) *arthritica*) were used for the materials; they were obtained mainly Hakodate.

Analytical Method

Phosphorous was determined by Fiske and Subbarow's meteod[119]; choline either by Glick's[140] or by Delsal's[182]; serine and ethanolamine by Nojima and Utsugi's method[123]; aldehyde by Wittenberg's[141], sphingosine by McKibbin and Taylor's[142] and hexose by the method of Radin et al.[143]; sterol was estimated by the digitonide method and iodine number by Wijs' technique.

Calculations

Lecithin, phosphatidylethanolamine, phosphatidylserine, acetal lipid, sphingomyelin and cerebrosides were calculated according to Table 37, respectively.

Results

The results are shown in Tables 38 to 45.

Table 38. Lipid Content of the Body Tissues of Herring (I)

Tissue		Ordinary muscle		Dark-colored
		♂	♀	♂
Total Lipid	g	3.9044	3.7370	19.6052
Lecithin	m mol.	0.657	0.625	1.779
Phosphatidylethanolamine	m mol.	0.319	0.291	0.956
Phosphatidylserine	m mol.	0.193	0.174	0.472
Acetal Lipid	m mol.	0.030	0.027	0.084
Sphingomyelin	m mol.	0.095	0.087	0.159
Cerebroside	m mol.	0.231	0.198	0.503
Cholesterol	m mol.	0.216	0.208	0.687
Total Phospholipids	m mol.	1.294	1.204	3.450
Total Sphingolipids	m mol.	0.326	0.258	0.662
Total Conjugated Lipids	m mol.	1.525	1.402	3.953
Iodine no.		129.6	127.0	137.9

Table 39. Lipid Content of the Body Tissues of Herring (II)

Tissue		Ordinary muscle		Dark-colored
		♂	♀	♂
Lecithin	mg	511.2	486.3	1384.2
Phosphatidylethanolamine	mg	229.0	208.9	686.4
Phosphatidylserine	mg	147.1	132.6	359.7
Acetal Lipid	mg	14.0	12.6	39.1
Sphingomyelin	mg	68.5	62.7	114.6
Cerebroside	mg	187.6	160.8	408.6
Cholesterol	mg	83.5	80.4	265.6
Total Phospholipids	mg	969.8	903.1	2584.0
Total Conjugated Lipids	mg	1157.4	1063.9	2992.6
Neutral Fat	mg	2663.5	2592.7	16347.0
Phospholipid/Cholesterol		11.6	11.2	9.7

Discussion

As shown in Table 36, the best result was obtained by the extraction with chloroform-methanol (2 : 1) mixture after the material had been extracted with acetone.

As shown in Table 38, the total lipid content in dark-colored muscle is the largest among various tissues of herring, whereas the conjugated lipids are contained in the highest quantity in liver.

The amount of each conjugated lipid in dark-colored muscle was higher than

(Indicated by Values in Mol. per 100g of Fresh Tissue)

muscle	Reproductive organ		Liver		Viscera	
♀	♂	♀	♂	♀	♂	♀
19.0524	5.7873	6.9515	7.2503	6.9515	5.1956	4.9515
1.886	0.763	1.308	0.981	0.896	0.463	0.417
0.870	0.568	0.254	1.294	1.126	0.340	0.272
0.435	0.187	0.034	0.262	0.222	0.207	0.195
0.075	0.043	0.029	0.074	0.066	0.036	0.022
0.168	0.170	0.085	1.280	1.295	0.952	0.863
0.607	0.385	0.195	0.665	0.679	0.346	0.355
0.630	0.109	0.266	0.842	0.845	0.671	0.611
3.434	1.731	1.710	3.891	3.595	1.998	1.769
0.775	0.555	0.280	1.945	1.974	1.298	1.218
4.4041	2.116	1.905	4.556	4.274	2.344	2.124
136.3	133.8	160.4	167.8	167.3	173.8	175.5

(Indicated by Values in mg per 100g of Fresh Tissue)

muscle	Reproductive organ		Liver		Viscera	
♀	♂	♀	♂	♀	♂	♀
1467.5	593.7	1017.7	763.3	689.4	360.3	324.5
624.7	407.8	182.4	929.6	808.5	244.1	195.3
331.5	142.5	25.9	199.6	169.2	157.7	148.6
34.9	20.0	13.5	34.5	30.7	16.8	10.2
121.1	122.6	61.3	922.9	933.7	686.4	622.2
493.0	312.2	158.4	540.1	551.5	281.0	288.3
243.6	42.1	102.8	325.6	326.7	259.4	236.2
2579.7	1286.6	1300.8	2849.4	2631.5	1465.3	1300.8
3072.7	1599.3	1459.2	3389.5	3183.0	1746.3	1589.1
15736.1	4145.9	5031.8	3535.2	3441.8	3189.9	3126.2
10.5	30.5	12.7	8.7	8.0	5.6	5.5

that in ordinary muscle. The amount of each conjugated lipid in viscera was smaller than that in other tissues except in ordinary muscle.

In the case of tuna the total lipid in ordinary muscle was higher in the anterior and middle portion of body than in the posterior, while the outer portion had a higher content than that in the inner portions. This trend agrees very well with the results obtained from yellowtail (*Seriola quinqueradiat*) by Tsuyuki et al.[183] The amount of each conjugated lipid in dark-colored muscle was much higher than that in ordinary muscle. The iodine number of lipid from the dark-colored muscle was also higher. Total lipid of the dark-colored and ordinary mus-

Table 40. Lipid Content of the Body Tissues of Tuna (I)

Tissue		Ordinary muscle		Dark-colored muscle
		Dorsal	Ventral	
Total Lipid	g	3.794	13.900	5.064
Lecithin	m mol.	0.213	0.824	0.889
Phosphatidylethanolamine	m mol.	0.184	0.700	0.340
Phosphatidylserine	m mol.	0.122	0.255	0.315
Acetal Lipid	m mol.	0.033	0.096	0.049
Sphingomyelin	m mol.	0.292	0.212	0.773
Cerebroside	m mol.	0.209	0.479	0.471
Cholesterol	m mol.	0.184	0.333	0.737
Total Phospholipids	m mol.	0.844	1.087	1.366
Total Sphingolipids	m mol.	0.501	0.691	1.244
Total Conjugated Lipids	m mol.	1.053	1.566	2.837
Iodine no.		108.7	147.0	163.6

Table 41. Lipid Content of the Body Tissues of Tuna (II)

Tissue		Ordinary muscle		Dark-colored muscle
		Dorsal	Ventral	
Total Lipid	g	3.794	13.900	5.064
Lecithin	mg	165.7	641.1	691.7
Phosphatidylethanolamine	mg	132.1	502.6	244.1
Phosphatidylserine	mg	93.0	194.3	240.0
Acetal Lipid	mg	15.4	44.7	22.8
Sphingomyelin	mg	210.5	152.9	557.4
Cerebroside	mg	169.8	389.0	382.6
Cholesterol	mg	74.1	128.8	385.1
Total Phospholipids	mg	616.7	1937.9	1756.0
Neutral Fat	mg	3007.5	11573.1	2925.4
Phospholipid/Cholesterol		8.6	8.5	6.2

cle were approximately the same. The parts of ordinary muscle which were high in total lipid content were also high in content of conjugated lipids, but the proportions were not always the same. The lipids in the inner portion, which is low in the total lipid content, had a total conjugated lipid/total lipid ratio higher than that of the outer portions. This fact is also evidenced in the dark-colored muscle. Namely, the inner dark-colored muscle contains a higer proportion of conjugated lipid in the total lipid as compared with the outer dark-colored muscle. The total phospholipid/cholesterol ratio was higher in the outer portions of the ordinary muscle than in the inner portion, followed in order by the outer portion of the dark-

(Indicated by Values in Mol. per 100g of Fresh Tissue)

Liver	Heart	Spleen	Pyloric appendages	Kidney	Stomach	Intestine
7.052	4.652	3.540	7.631	3.828	2.798	2.335
0.716	0.889	0.216	0.467	0.624	0.302	0.177
0.762	0.340	0.207	0.417	0.310	0.155	0.098
0.455	0.198	0.085	0.098	0.054	0.020	0.024
0.085	0.047	0.049	0.038	0.024	0.034	0.069
0.779	0.695	0.672	0.685	0.500	0.581	0.489
0.407	0.211	0.924	0.326	1.036	0.198	0.205
0.775	0.510	—	—	—	0.433	—
2.797	2.169	1.229	1.705	1.512	1.092	0.857
1.544	0.906	1.596	1.011	1.536	0.779	0.694
3.204	2.380	2.253	2.031	2.548	1.290	1.162
163.6	140.5	143.7	154.7	161.2	135.5	139.1

(Indicated by Values in mg per 100g of Fresh Tissue)

Liver	Heart	Spleen	Pyloric appendages	Kidney	Stomack	Intestine
7.052	4.652	3.540	7.631	3.828	2.998	2.335
557.1	691.7	168.1	363.4	485.5	235.0	137.7
547.1	244.1	148.6	299.4	222.6	111.3	70.4
346.7	150.9	64.8	74.7	41.1	15.2	18.3
59.6	21.9	22.8	17.7	11.2	15.8	32.1
561.7	501.1	484.5	493.9	260.5	418.9	352.6
330.6	171.4	750.5	264.8	841.5	160.8	166.5
299.6	197.2	—	—	—	167.4	—
2052.2	1609.7	888.8	1249.1	1120.9	561.2	473.4
4669.2	2870.9	1900.7	6117.1	2707.1	2076.0	1696.1
6.8	8.2	—	—	—	3.4	—

colored muscle, heart, liver, inner dark-colored muscle, and stomach. This fact suggests that muscle activity was most high in the outer ordinary muscle, whilst the inner ordinary muscle showed a relatively low muscle activity and also the outer dark-colored muscle showed rather high muscle activity. The phospholipid/cholesterol ratio values for heart agreed with the value (8) obtained by Bloor[184] for heart of cold-blooded animals, and the value for stomach agreed with the values (between 3 and 4) obtained by Bloor[184] and Kaucher et al.[175] for stomach of terrestrial animals.

The values for inner dark-colored muscle lies between those of ordinary mus-

Table 42. Lipid Content of the Body Tissues of Mackerel (I)
(Indicated by Values in Mol. per 100g of Fresh Tissue)

Tissue		Ordinary muscle		Dark-colored muscle		Liver	
Sex		♂	♀	♂	♀	♂	♀
Total Lipid	g	1.996	1.814	10.138	10.543	5.781	4.967
Lipid-P	m mol.	1.083	0.888	5.063	4.631	3.396	2.839
Lecithin	m mol.	0.195	0.308	0.142	0.995	0.533	0.478
Phosphatidylethanolamine	m mol.	0.359	0.255	2.008	1.818	1.968	0.896
Phosphatidylserine	m mol.	0.126	0.099	0.820	0.439	0.484	0.231
Sphingomyelin	m mol.	0.402	0.220	2.093	1.379	0.411	1.234
Cerebroside	m mol.	0.030	0.072	0.338	0.757	0.525	1.529
Total Conjugated Lipids	m mol.	1.112	0.960	5.401	5.388	3.921	4.368

Table 43. Lipid Content of the Body Tissues of Mackerel (II)
(Indicated by Values in mg per 100g of Fresh Tissue)

Tissue		Ordinary muscle		Dark-colored muscle		Liver	
Sex		♂	♀	♂	♀	♂	♀
Total Lipid	g	1.996	1.814	10.138	10.543	5.781	4.967
Lecithin	mg	151.7	239.6	110.5	774.1	414.7	371.9
Phosphatidylethanolamine	mg	257.8	183.1	1441.7	1308.5	1413.0	643.3
Phosphatidylserine	mg	96.0	75.4	624.5	334.5	368.8	176.0
Sphingomyelin	mg	289.8	158.6	150.9	994.3	296.3	889.7
Cerebroside	mg	24.4	58.5	274.5	614.6	426.3	1241.5
Total Phospholipids	mg	795.1	656.7	2327.6	2410.4	2491.8	2080.9
Total Conjugated Lipids	mg	819.7	715.2	2602.1	4026.6	2918.1	3322.4
Neutral Fat	mg	1176.3	1098.8	7535.9	6516.4	2862.9	1644.6

cle, one of the skeletal muscles (mammal 17, fowl 14 and cold-blooded animals 18), and those of the stomach, which is constructed of a smooth muscle; but slightly lower than that for heart and rather close to that for liver. In addition, the composition of the conjugated lipid and the distribution of cholesterol were similar to those of the liver. These facts suggest that the inner dark-colored muscle has a function more similar to that of liver, rather than that of skeletal muscle.

The amount of neutral and conjugated lipids in dark-colored muscle was higher than that in liver and ordinay muscle of mackerel. This tendency did not agree with the case of herring and tuna.

The distribution of each conjugated lipid in mollusca is shown in Tables 44 and 45. The content of each lipid in liver was much higher than the amount in muscle of shell-fish and squid.

Table 40′. Lipid Content of the Body Tissues of Tuna (1)
(Indicated by Values in Mol. per 100 g. of Fresh Tissue)

Tissue**		Total lipid, g	Iodine no. (Wijs)	Lecithin, m. Mol.	Phosphatidylethanolamine, m. Mol.	Phosphatidylserine, m. Mol.	Acetal lipid m. Mol.	Sphingomyelin, m, Mol.	Cerebroside, m. Mol.	Cholesterol, m. Mol
Dorsal	A I	3.444	137.9	0.225	0.169	0.134	0.028	0.338	0.216	—
	A E	8.456	143.2	0.417	0.262	0.165	0.075	0.328	0.316	—
	M I	3.794	108.7	0.213	0.184	0.122	0.033	0.292	0.209	0.184
	M E	7.590	109.5	0.427	0.245	0.137	0.050	0.335	0.271	0.187
	P I	1.952	103.5	0.158	0.135	0.076	0.023	0.219	0.128	—
	P E	4.112	103.8	0.423	0.175	0.107	0.054	0.320	0.247	—
Ventral	A I	12.508	153.9	0.761	0.540	0.242	0.092	0.291	0.332	—
	A E	12.962	165.8	0.823	0.368	0.333	0.125	0.346	0.307	—
	M I	13.900	147.0	0.824	0.700	0.255	0.096	0.212	0.479	0.333
	M E	7.754	148.1	0.568	0.280	0.196	0.053	0.106	0.233	0.137
	P I	1.584	120.5	0.357	0.095	0.095	0.035	0.085	0.164	—
	P E	2.872	141.0	0.449	0.120	0.126	0.035	0.121	0.258	—
Dark-colored	A I	4.085	153.7	0.716	0.301	0.540	0.084	0.534	0.299	—
	A E	11.683	166.6	1.216	0.747	0.643	0.047	0.934	0.579	—
	M I	5.064	163.6	0.889	0.340	0.315	0.049	0.773	0.471	0.737
	M E	6.712	164.5	0.771	0.389	0.293	0.038	0.534	0.513	0.449
	P I	7.078	156.6	0.624	0.489	0.390	0.068	0.688	0.492	—
	P E	5.636	160.5	0.466	0.243	0.265	0.069	0.402	0.354	—

** Abbreviations are used as follows: A, anterior; M, middle; P, posterior; I, internal; E, external.

Table 41′. Lipid Content of the Body Tissues of Tuna (2)
(Indicated by Values in mg per 100 g. of Fresh Tissue)

Tissue**		Lecithin, mg.	Phosphatidylethanolamine, mg.	Phosphatidylserine mg.	Acetal lipid, mg.	Sphingomyelin, mg.	Cerebroside, mg.	Cholesterol, mg.	Total phospholipids, mg.	Total phospholipids/cholesterol
Dorsal	A I	175.1	121.3	102.1	13.0	243.7	175.4	—	655.2	—
	A E	324.5	188.1	125.7	34.9	236.5	256.7	—	909.7	—
	M I	165.7	132.1	93.0	15.4	210.5	169.8	71.4	616.7	8.6
	M E	332.2	175.9	104.4	23.3	241.5	220.1	72.6	877.3	12.1
	P I	122.9	96.9	57.9	10.7	157.9	104.0	—	446.3	—
	P E	329.1	125.6	81.5	25.2	230.7	202.2	—	792.1	—
Ventral	A I	592.1	387.7	184.4	42.9	209.8	270.0	—	1416.9	—
	A E	640.4	264.2	253.7	58.2	249.5	249.4	—	1466.0	—
	M I	641.1	502.6	194.3	44.7	152.9	389.0	128.8	1937.9	8.5
	M E	442.0	201.0	149.4	24.7	76.4	189.2	53.0	893.5	16.9
	P I	277.8	75.4	72.4	16.3	61.3	133.2	—	503.2	—
	P E	349.4	86.2	96.0	16.3	87.2	209.6	—	636.1	—
Dark-colored	A I	557.1	216.1	411.5	39.1	385.0	242.9	—	1608.8	—
	A E	946.2	536.3	490.0	21.9	673.4	470.3	—	2667.8	—
	M I	691.7	244.1	240.0	22.8	557.4	382.6	285.1	1756.0	6.2
	M E	599.9	279.3	223.3	17.7	385.0	416.7	137.6	1465.2	10.6
	P I	485.5	351.1	297.2	31.7	496.1	399.6	—	1661.6	—
	P E	362.6	174.5	201.9	32.1	289.9	287.5	—	1061.0	—

** Abbreviations are used as follows: A, anterior; M, middle; P, posterior; I, internal; E, external.

7. CONCLUSION

In this paper are reported studies on the properties of lecithin, cephalin, phosphatidic acid and sphingomyelin samples obtained from the viscera of aquatic animals.

Lecithin samples were obtained from the brains of sperm whale, rorqual and pollack; from the eggs of shark, salmon, pollack, carp and crab; from the hearts of rorqual and pollack; from the livers of octopus, pecten and shell-free chlamys. The properties of these lecithins, particularly their fatty acid composition were studied. The fatty acid composition of the lecithin indicated a considerable difference between species.

Differences were observed in the fatty acid composition of the lecithin and glyceride obtained from the same organs.

Lecithin and glyceride, particularly the higher unsaturated fatty acids of these materials decrease markedly during the development of the egg of aquatic animals, but no marked changes in the fatty acid composition of these substances were observed at the beginning of the developmental period.

So it might be concluded that the changes of the component fatty acid of lecithin occurred gradually and the higher unsaturated fatty acids of lecithin were consumed selectively during the developmental period.

Cephalin samples were obtained from the brains of sperm whale, rorqual and pollack; from the eggs of pollack, crab, and salmon; from the hearts of rorqual and pollack; from the livers of octopus, pecten, and shell-free chlamys. The properties of these cephalin samples were studied. They were found to be mixtures of inositolphospholipid, phosphatidylserine and phosphatidylethanolamine except in the case of egg cephalin. No phosphatidylethanolamine was present in the egg cephalin obtained from the pollack egg and the unfertilized crab egg. Existence of inositolphospholipids in the liver cephalin of octopus was doubtful. The fatty acid composition of cephalin showed a considerable difference among the species of animal which supplied the materials. The content of higher unsaturated fatty acid in these cephalin samples was larger than that of the land animals as in the case of the lecithin. No relations between the component fatty acid of cephalin and that of lecithin were observed as has already been described by Klenk et al.[137] An unknown amino base was detected by the paper chromatographic analysis in the hydrolysate of rorqual heart cephalin. Unknown amino bases and amino acids other than serine and ethanolamine were detected in the several cephalin samples. The writer[87),185),186)] found the presence of a threonine containing phospholipid in the

Table 44. Lipid Content of the Body Tissues of Mollusca (I)

Tissue		Pecten	Neptune	
		Liver	Muscle	Liver
Total Lipid	g	16.937	1.159	5.470
Lecithin	m mol.	0.335	0.125	0.589
Phosphatidylethanolamine	m mol.	0.215	0.066	0.670
Phosphtidylserine	m mol.	0.240	0.022	0.199
Acetal Lipid	m mol.	0.024	0.011	0.052
Sphingomyelin	m mol.	0.276	0.078	0.485
Cerebroside	m mol.	0.580	0.301	0.589
Cholesterol	m mol.	0.691	0.060	0.480
Total Phospholipids	m mol.	1.090	0.302	1.995
Total Sphingolipids	m mol.	0.856	0.379	1.074
Total Conjugated Lipids	m mol.	1.670	0.603	2.584
Iodine no.		175.6	116.9	141.0

Table 45. Lipid Content of the Body Tissues of Mollusca (II)

Tissue		Pecten	Neptune	
		Liver	Muscle	Liver
Lecithin	mg	260.7	97.3	458.3
Phospatidylethonolamine	mg	154.4	47.4	481.1
Phosphatidylserine	mg	182.9	16.8	151.6
Acetal Lipid	mg	11.2	5.1	24.2
Sphingomyelin	mg	199.0	56.2	349.7
Cerebroside	mg	471.1	244.5	478.4
Total Phospholipids	mg	808.2	222.8	1464.9
Total Conjugated lipids	mg	1271.3	467.3	1943.3
Neutral Fat	mg	15657.7	691.7	3526.7

cephalin obtained from tuna muscle and it was interesting to have found the presence of new phospholipid in the cephalins. It was also observed that phosphatidylserine was the main component of the cephalin obtained from the unfertilized egg and that it was decarboxylated into phosphtidylethanolamine during the maturation, fertilization and development of eggs. Furthermore, decrease in cephalin, particularly the consumption of higher unsaturated fatty acid such as hexaenoic and pentaenoic acid occurred.

Inositolphospholipid obtained from the brain cephalin of rorqual was considered as a new phospholipid, because it consists of 1 molecule of glycerol, 1 molecule of inositoldiphosphate and 2 molecules of fatty acid; the fatty acid consists of 60 per cent saturated fatty acid and 40 per cent unsaturated fatty acid.

(Indicated by Values in Mol. per 100g of Fresh Tissue)

Chlamys	Clam	Squid			Octopus	
		Muscle	Liver ♂	Liver ♀	Liver ♂	Liver ♀
1.596	1.446	1.681	55.448	53.835	14.420	13.334
0.195	0.279	0.999	1.026	1.494	0.294	0.295
0.114	0.022	0.160	0.232	0.187	0.058	0.065
0.099	0.127	0.109	0.116	0.096	0.029	0.048
0.145	0.160	0.085	0.050	0.089	0.004	0.074
0.185	0.179	0.142	0.296	0.180	0.096	0.075
0.021	0.025	0.023	0.142	0.077	0.031	0.086
—	—	—	—	—	—	—
0.738	0.767	1.485	1.720	2.046	0.471	0.557
0.206	0.204	0.165	0.438	0.257	0.127	0.161
0.759	0.792	1.508	1.862	2.123	0.502	0.643
152.1	128.8	168.3	152.3	168.1	159.7	171.2

(Indicated by Values in mg per 100g of Fresh Tissue)

Chlamys	Clam	Squid			Octopus	
		Muscle	Liver ♂	Liver ♀	Liver ♂	Liver ♀
151.4	216.8	777.0	797.9	1162.7	228.9	229.5
81.6	15.5	114.8	166.0	134.2	41.4	46.2
75.0	96.4	82.9	83.4	69.4	21.7	36.1
67.3	74.6	20.8	23.3	41.4	1.7	34.4
133.1	128.8	102.2	213.0	130.0	69.0	54.2
17.0	20.3	18.6	115.5	62.3	24.8	69.6
508.4	532.1	1097.7	1283.6	1537.7	362.7	400.4
525.4	552.4	1115.3	1399.1	1600.0	387.5	470.0
1070.6	893.6	575.7	54049.3	52235.0	14032.5	12764.2

Both phosphatidylserine and phosphatidylethanolamine isolated from the brain cephalin of rorqual contained a large amount of monoethylenic acid (a bit over 80 per cent of the total fatty acid); comparing the fatty acid component of the two phospholipids it was observed that higher unsaturated fatty acid contained more phosphatidylethanolamine than that in phosphatidylserine.

Phosphatidic acid samples were separated from the heart of rorqual and pollack, and from the crab egg; the properties of these phosphatidic acid were studied. The results obtained from chemical analysis, serological assay and paper chromatographic analysis of these heart phosphatidic acids showed that these substances had similar structure to that of cardiolipin, although the component fatty acids were slightly different.

Though both phosphatidic acid obtained from unfertilized and fertilized egg showed similar R_f value from the paper chromatographic analysis, the singleness of these phosphatidic acids was doubtful, because the serological assays of these materials gave different results. The decrease of the phosphatidic acid during the development of crab egg was observed as in the case of the other phospholipids, but no marked changes of the properties of phosphatidic acid were considered to occur during this period.

Sphingomyelin was obtained from the sperm whale brain; the properties of this material were studied. The fatty acid composition of this material was 20 per cent stearic acid, 49 per cent lignoceric acid and 31 per cent nervonic acid.

The distribution of the conjugated lipid in the organs of aquatic animals was studied. Generally, the contents of conjugated lipids in the stock organs, such as liver, were higher than those of the motile organs such as muscles.

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