Lipids in shellfish

by Akira Hayashi

Original title: Kairui no shishitsu

From: Yukagaku (Oil Chemistry), 20(10) : 726-736, 1971

Translated by the Translation Bureau (GN)
Foreign Languages Division
Department of the Secretary of State of Canada

Department of the Environment
Fisheries Research Board of Canada
Halifax Laboratory
Halifax, N. S.
1972

28 pages typescript
Lipids in shellfish

Kairui no shishitsu

Yukagaku

Oil Chemistry

Japan Oil Chemists' Society

DATE OF PUBLICATION
DATE DE PUBLICATION

PAGE NUMBERS IN ORIGINAL
NUMÉROS DES PAGES DANS
L'ORIGINAL

1971  20  10

726-736

28

APR 18 1972

REQUESTING DEPARTMENT
MINISTÈRE-CENT

Environment

TRANSLATION BUREAU NO.
NOTRE Dossier N° 0565-M

BRANCH OR DIVISION
DIRECTION OU DIVISION

Fisheries Research Board

TRANSLATOR (INITIALS)
TRADUCTEUR (INITIALES) G.N.

PERSON REQUESTING
DEMANDÉ PAR

Dr. R.G. Ackman

DATE OF REQUEST
DATE DE LA DEMANDE

Feb. 10, 1972

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement
LIPIIDS IN SHELLFISH

Akira Hayashi

Department of Chemistry, Faculty of Science and Technology, Kinki University
(321 Kowakae, Higashiosaka-shi, Osaka)

1. INTRODUCTION

From ancient times, the shellfish has been a creature very close to man, and the meat of shellfish has been widely used as a food rich in nutritional value. While it may be for such reasons, the number of studies concerning their lipids are many. The direction of these studies, however, has been "an attempt to chemically analyze the lipids themselves, without seeking the biological activity of the lipids in the shellfish or doing follow-up research on the metabolism of lipids" as Hori stated nine years ago. Since then, considerable research on metabolism and biosynthesis may be said to have occurred in certain areas involving the lipids in shellfish, but even in the field of lipid biochemistry which has undergone such remarkable development, what is still lacking is virtually the same as nine years ago, and we can only hope for greater developments in this field of research in the future. For all this, great strides have been made in the study of compound lipids in shellfish over the past ten years, and particularly because of research in our country, new sphingo-type compound lipids have been announced one after another, thereby contributing greatly to research in this field.
The study of lipids in shellfish was restricted to acetone-soluble lipids until about ten years ago, involving research on the properties of shellfish oil and sterols in the main. Even during this period, it is a well-known fact that the research performed by the scientists in our country played a very big role. In recent years, with advances in various types of chromatography, new light has been thrown upon the research done in the past to enable a second look. Studies in this field which were once felt to be irritatingly slow-going have now been accelerated by the direct identification of various components and the possibility of analysis using various types of equipment. This point is the same in the field of compound lipids which has undergone great development in the past ten years as well. In this paper, therefore, we have dealt mainly with research subsequent to 1960, and for studies prior to this, the readers are referred to other texts 2), 3).

At this point, we should like to touch briefly on the biological classification of shellfish. Belonging to the Mollusca order, the shellfish are grouped by such generic names as Gastropoda (snails), Pelecypoda (bivalves), Scaphopoda (Dentalium) and Polyplacophora (Hizaragai genus). They are further subdivided but the classification of shellfish is extremely complicated, and for the sake of expediency we simply divided them into snails, bivalves and Hizaragai. Shellfish live in salt water, brackish water, fresh water and on land, and they differ in their feeding habits and ecology. There are known to be about 120,000 species in the world, of which there are approximately 8,000 species in our country.

2. ACETONE-SOLUBLE LIPIDS (NEUTRAL LIPIDS)

Shellfish oil has a colouration ranging from dark rust red to dark brown, and at normal temperatures some are liquid but many are semi-solid containing solids. Its content in relation to shellfish meat is in the order of 1-5%, rarely exceeding 10%, and there is more in the viscera than in such muscle tissues as the adductor muscle. In addition to a special odour, it is characterized by a high acid value (30-100), iodine value (120-190) and unsaponifiable content (20-40%). The fact that
it contains many highly unsaturated acids is seen to be characteristic of marine animal oils but it differs from fish oil in smell. Even after 1960, there have been a number of studies 4)-7) carried out on these points.

2.1 Fatty acid composition and fatty acid distribution

Since entering the sixties, studies on the composition of fatty acids have been carried out 6)-10) using gas chromatography (GC). However, because the qualitative analysis of highly unsaturated acids by GC has its limits, of equal importance are the results based on the method 11) of finding the polyunsaturated acid content by measuring the ultraviolet absorption spectrum of alkali isomerized fatty acids as used in the fifties 12)-15). In the future, GC-MS would probably be useful on this point. The fatty acid composition of several types of shellfish oil based on GC has been presented in Table 1.

In general, palmitic acid is the most dominant saturated acid, comprising more than 50% of the saturated acids. The unsaturated acids comprise 50-70%, characterized by the predominance of pentanoic and hexanoic acid. From the acetone-soluble lipids of the snail *Helix pomatia*, Thiele et al. 16) obtained triglyceride (TG) and diglyceride (DG) by silica gel chromatography, and examining their constituent fatty acids by GC, they discovered that both TG and DG were 50% comprised of palmitic acid, stearic acid and oleic acid and that in the free fatty acid (0.8%) was contained 20% C₂₀:₄ acid. No branched fatty acid has yet been detected from shellfish.

There are also a number of studies on the positional distribution of the fatty acids in TG. Brockerhoff et al. 17) found a predominance of palmitic acid in position α and a predominance of such unsaturated acids as C₂₀:₅ in position β of TG in the adductor muscle of scallops, while detecting some random distribution in the shellfish oil as well. Next, they examined the characteristic three-dimensional fatty acid distribution with respect to TG in the scallop (liver, pancreas) and the periwinkle, reporting 18) that saturated acids (palmitic acid, stearic acid) are predominant in position 1, polyenic acids are predominant in position 2, and that while
Table 1. Fatty acid composition of shellfish oil

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Bivalve</th>
<th>Snail</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oyster*</td>
<td>Corbicularia*</td>
</tr>
<tr>
<td>C_{16:0}</td>
<td>14.5</td>
<td>20.3</td>
</tr>
<tr>
<td>C_{16:1}</td>
<td>7.0</td>
<td>10.7</td>
</tr>
<tr>
<td>C_{18:0}</td>
<td>3.2</td>
<td>5.1</td>
</tr>
<tr>
<td>C_{18:1}</td>
<td>10.9</td>
<td>8.3</td>
</tr>
<tr>
<td>C_{18:2}</td>
<td>2.1</td>
<td>1.9</td>
</tr>
<tr>
<td>C_{18:4}</td>
<td>4.2</td>
<td>3.4</td>
</tr>
<tr>
<td>C_{20:1}</td>
<td>5.1</td>
<td>9.0</td>
</tr>
<tr>
<td>C_{20:4}</td>
<td>3.9</td>
<td>4.8</td>
</tr>
<tr>
<td>C_{20:5}</td>
<td>22.8</td>
<td>8.6</td>
</tr>
<tr>
<td>C_{22:2}</td>
<td>3.4</td>
<td>2.9</td>
</tr>
<tr>
<td>C_{22:4}</td>
<td>1.1</td>
<td>3.0</td>
</tr>
<tr>
<td>C_{22:5}</td>
<td>-</td>
<td>3.9</td>
</tr>
<tr>
<td>C_{22:6}</td>
<td>10.7</td>
<td>10.3</td>
</tr>
</tbody>
</table>

* Includes viscera. The rest were rid of viscera and only flesh retained.

position 3 is virtually the same as position 1, acids of longer chain are more predominant. Based on the findings of Brockerhoff et al., Litchfield\(^{19}\) proposed a formula to estimate the positional distribution of C\(_{22:5}\) and C\(_{22:6}\) acids within the TG of marine animals. From the fact of the difference in the coefficient of each formula for positions 1, 2 and 3, the acylation of positions 1, 2 and 3 in the biosynthesis of TG in shellfish was deemed to occur independently, thus supporting the biosynthetic pathway put forth by Lands et al.\(^{20}\)

2.2 Seasonal variation and metabolism

With regard to the seasonal variation in shellfish oil content, there is the study involving the true oyster done by Masumoto et al.\(^{21}\) in the 1930s. It was
reported to increase to a maximum in the genetic enlargement period (March-June), decreasing sharply in the reproductive period (August-September) and thereafter gradually rising again, and there was a certain connection with glycogen. This was something quite ahead of the times for similar occurrences were subsequently verified in studies\(^22\)-\(^29\) on a variety of shellfish including the pearl-oyster and sea mussel. There is also the report\(^24\) that the content is high in winter and low in summer in fresh-water Nagatanishi, Tatebochi and Hydriopsis schlegeli while the fresh-water bred Corbicula sandai revealed the same variation as the true oyster.

The snail *Pila globosa* is a curious shellfish which estivates, and during the period of estivation the fat content decreases and the utilization rate is said\(^30\) to be \(\frac{1}{4}\) of glycogen while the unsaturated fatty acids of the liver and pancreas increase during the estivation period and this is deemed to be due to the antioxidation function of ascorbic acid\(^31\).

With regard to lipids in the organs of living bodies, there is the report\(^32\) that when spermatozoa of *Hydriopsis schlegeli* are incubated under favourable conditions the total lipid quantity increases by 1.5 times but the compound lipids decrease, while there is another report\(^33\) on the lipids in the neuron of the seahare.

No research has yet been seen on metabolism using radioisotopes. From the fact that the fat content increases when snails (*Pila viridis*) are given filter paper alone, Meenakshi\(^30\) ascertained that the synthesis of fat occurs from carbohydrates, thus verifying the correctness of the thinking of Masu moto et al.\(^21\) who deduced from the difference in seasonal variation between the glycogen level and fat content that the glycogen gets converted into fat.

3. **STEROLS**

3.1 **Composition and content**

With regard to the sterols in shellfish, studies have been going on from olden times, and the achievements of the scholars of our country in particular have contributed greatly in this field. This fact is also made clear in the general
papers\textsuperscript{34)-36)} on the sterols in shellfish.

The sterol content in shellfish meat, while it would vary with the shellfish, is 15-175 mg\textsuperscript{31),37)} per 1.00 g of shellfish meat, and a seasonal variation can be seen. Reports that this variation is connected with the reproductive activity of shellfish have been made on oysters\textsuperscript{38)}, sea mussels\textsuperscript{39)} and scallops\textsuperscript{40)}, indicating that the sterol level is low prior to the release of genetic matter, that it increases rapidly after release and thereafter levels off. There are other reports\textsuperscript{37),41)}, however, which say that it is low in autumn and high in spring, and in certain species of shellfish this is thought to be due to different reproductive methods and periods.

Idler et al.\textsuperscript{40)} examined the seasonal variation in respect of individual component sterols, discovering that while 22-dehydrocholesterol and brushkasterol are virtually stable, cholesterol and 24-methylene cholesterol decrease in June and in February. This is felt to be something related to the metabolism of each sterol, and as research of this type gets carried out more widely in the future, some very interesting results can be anticipated.

There have been many studies on the composition of sterols contained in shellfish. While they have been known to contain many types of sterols, in some cases the same sterol has been given different names or those known to be mixtures are included, so these have been consolidated as much as possible and presented in Table 2.

As shown in Table 2, the general trend of recent results of analysis by GC reveals that such salt-water and brackish-water bivalves as the scallop, oyster, clam, short-necked clam and razor-clam contain 25-41\% cholesterol, 13-26\% 24-methylene cholesterol, 14-22\% brushkasterol, 5-13\% 22-dehydrocholesterol, 4-6\% \(C_{26}\)-sterol and 8-15\% \(C_{29}\)-sterol. Of interesting contrast is the fact that fresh-water bivalves (fresh-water mussel) and one brackish-water bivalve, Corbicula japonica, are 60-70\% cholesterol and salt-water snails are 70-100\% cholesterol, while such primeval gastropods as the wreath shell, Nordotis discus and Lunella coronata are virtually all cholesterol.
Table 2. Sterols in shellfish

<table>
<thead>
<tr>
<th>Sterol</th>
<th>Structural characteristic - mp (polarity)</th>
<th>Snails</th>
<th>Bivalves</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(C\textsubscript{26}-sterol)</strong></td>
<td></td>
<td>Boshubora(1.1-1.6\textsuperscript{78}), whelk(2.1\textsuperscript{78}), ivory-shell(1.9\textsuperscript{78})</td>
<td>Oyster(5.64\textsuperscript{2}, 2-5\textsuperscript{48}), clam(4.8\textsuperscript{42}), scallop(4.44\textsuperscript{2}, 6.44\textsuperscript{44}), Corbicula japonica(3.9\textsuperscript{78}), short-necked clam(4.5\textsuperscript{78}), razor-clam(4.0\textsuperscript{78})</td>
<td></td>
</tr>
<tr>
<td><strong>(C\textsubscript{27}-sterol)</strong></td>
<td>Cholesterol (\Delta^5)</td>
<td>Periwinkle\textsuperscript{15}(15), 45), Nassa obsolaeta\textsuperscript{46}, Nerita peleronta\textsuperscript{46}, Hapetanishi\textsuperscript{3}, Helix pomatia\textsuperscript{41}, Nina(71.1\textsuperscript{48}), wreath shell(100.0\textsuperscript{13}), Nordotis discus(86.1, 91.8\textsuperscript{78}), Lunella coronata(81.2\textsuperscript{78}), Hapetanishi(70.1\textsuperscript{78}), Kawanai(76.0\textsuperscript{78}), Boshubora(59.3, 69.1\textsuperscript{78}), whelk(70.6, 74.1\textsuperscript{78}), ivory-shell(78.8\textsuperscript{78}), Naganishi(70.9\textsuperscript{78})</td>
<td>Atrina pectinata japonica\textsuperscript{15}, oyster(41.4\textsuperscript{42}, 33.9-46.5\textsuperscript{48}), clam(36.7\textsuperscript{42}), scallop(25.7\textsuperscript{42}, 26.8\textsuperscript{40}\textsuperscript{44}), short-necked clam(34.6\textsuperscript{78}, 36.2\textsuperscript{78}), mussel(38.1\textsuperscript{48}), fresh-water mussel(71.0\textsuperscript{78}), Corbicula japonica(60.2\textsuperscript{78}), razor-clam(26.3\textsuperscript{78})</td>
<td>Hizarae(9.5\textsuperscript{48})</td>
</tr>
<tr>
<td>Cholestanol</td>
<td>142(+24)</td>
<td>Scallop(small amount)\textsuperscript{44}, mussel(31.0\textsuperscript{48}), oyster(10.7-19.8\textsuperscript{48})</td>
<td>Hizarae(6.2\textsuperscript{48})</td>
<td></td>
</tr>
<tr>
<td>(\Delta^7)-cholestanol (Latosterol)</td>
<td>124(+42)</td>
<td></td>
<td>Hizarae(48,49)</td>
<td></td>
</tr>
<tr>
<td>7-dehydrocholesterol</td>
<td>(\Delta^5,7)</td>
<td>Buccium undatum(27.5\textsuperscript{51}), ivory-shell(35\textsuperscript{51}), oyster(10.8\textsuperscript{78}), Helix pomatia\textsuperscript{41}, Nordotis discus(3.5\textsuperscript{78}), Hapetanishi(8.0\textsuperscript{78}), Boshubora(9.6\textsuperscript{78})</td>
<td>Modiolus demissus(35-50\textsuperscript{52}), Corbicula japonica(1.0\textsuperscript{78})</td>
<td></td>
</tr>
<tr>
<td>22-dehydrocholesterol</td>
<td>(\Delta^5,22)</td>
<td>Nordotis discus(0.8\textsuperscript{78}), Lunella coronata(3.6\textsuperscript{78}), Hapetanishi(6.1\textsuperscript{78}), Kawanai(3.2\textsuperscript{78}), Boshubora(3.0\textsuperscript{78}), whelk(4.7\textsuperscript{78}), ivory-shell(6.8\textsuperscript{78}), Naganishi(1.9\textsuperscript{78})</td>
<td>Scallops(40.58)(13.44\textsuperscript{2}, 14.14\textsuperscript{44}), oyster(2.94\textsuperscript{2}, 4.6-5.2\textsuperscript{48}), clam(7.9\textsuperscript{42}), short-necked clam(6.7\textsuperscript{47}, 10.4\textsuperscript{78}), mussel(4.2\textsuperscript{48}), fresh-water mussel(3.0\textsuperscript{78}), Corbicula japonica(4.8\textsuperscript{78}), razor-clam(13.9\textsuperscript{78})</td>
<td></td>
</tr>
<tr>
<td>(\Delta^5,7,22)-cholestatrienal</td>
<td>(\Delta^5,7,22)</td>
<td></td>
<td>Sea mussel\textsuperscript{54}, Hototogisu\textsuperscript{55}</td>
<td></td>
</tr>
<tr>
<td><strong>(C\textsubscript{28}-sterol)</strong></td>
<td>Brushkasterol (Shakosterol) (\Delta^5,22)</td>
<td>Viviparas japonicus\textsuperscript{56}, Lunella coronata(4.4\textsuperscript{78}), Hapetanishi(4.3\textsuperscript{78}), Kawanai(10.7\textsuperscript{78}), Boshubora(22.6\textsuperscript{78}), whelk(13.3\textsuperscript{78}), ivory-shell(2.1\textsuperscript{78}), Naganishi(2.9\textsuperscript{78})</td>
<td>Corbicula(57), 61\textsuperscript{1}(7.1\textsuperscript{78}), oyster 58\textsuperscript{1}(16.0\textsuperscript{42}, 6.5\textsuperscript{48}), Modiolus demissus\textsuperscript{59}, clam(14.1\textsuperscript{42}, 14.1\textsuperscript{42}, scallop(14.14\textsuperscript{2}, 16.34\textsuperscript{44}), fresh-water mussel(15.5\textsuperscript{78}), short-necked clam(15.7\textsuperscript{47}, 20.1\textsuperscript{78}), razor-clam(22.6\textsuperscript{78})</td>
<td></td>
</tr>
<tr>
<td>Substances</td>
<td>Formula</td>
<td>Content (%)</td>
<td>Species</td>
<td></td>
</tr>
<tr>
<td>----------------------------------</td>
<td>---------</td>
<td>-------------------</td>
<td>--------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>24-methylene cholesterol</td>
<td>$d^5,24(28)$</td>
<td>144(-42)</td>
<td>Nordotis discus($1.0)<em>{78}$, Lunella coronata($1.6)</em>{78}$, Himetanishi($6.4)<em>{78}$, Kawanina($5.5)</em>{78}$, Boshubora($4.7)<em>{78}$, whelk($4.6)</em>{78}$, ivory-shell($1.8)<em>{78}$, Naganishi($10.6)</em>{78}$, Nina($21.5)_{48}$</td>
<td></td>
</tr>
<tr>
<td>Ergosterol</td>
<td>$d^5,7,22$</td>
<td>243-C3H5, 165(-130)</td>
<td>Oyster($25,942$), $13,2548$, $3668$, clam($53,682$), $29,242$), scallop($40$), $19,542$, $26,044$), Kariyama($49$), short-necked clam($21.5$), $4.7$, $19,678$), fresh-water mussel($7.1)<em>{78}$, Corbicula japonica($6.2)</em>{78}$, razor-clam($20.7)<em>{78}$, mussel($39.5)</em>{48}$)</td>
<td></td>
</tr>
<tr>
<td>(C29-sterol) $\beta$-sitosterol</td>
<td>$d^5$</td>
<td>137(-37)</td>
<td>Hametanishi($5$), Nina($9.4)<em>{48}$, Ubagai($12$), mussel($6), 67$), scallop($4.1)</em>{48}$, oyster($4.8-6.9)_{48}$</td>
<td></td>
</tr>
<tr>
<td>Clionasterol</td>
<td>$d^5$</td>
<td>243-C2H5, 148(-43)</td>
<td>Mud-snail($56$), periwinkle($45$), Ubagai($12$), scallop($49$), clam($60$), mussel($67$), Atrina pectinata japonica($15$), $65$), corbicula($61$)</td>
<td></td>
</tr>
<tr>
<td>7-dehydro-clionasterol</td>
<td>$A^5,7$</td>
<td>243-C2H5, 139(-96)</td>
<td>Periwinkle($45$)</td>
<td>Sea mussel($66$)</td>
</tr>
<tr>
<td>Polyphellasterol</td>
<td>$d^5,22$</td>
<td>243-C2H5, 156(-50)</td>
<td>Mud-snail($56$), Clam($60$), corbicula($61$), mussel($67$)</td>
<td></td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>$d^5,22$</td>
<td>243-C2H5, 170(-51)</td>
<td>Nina($21.6)<em>{48}$, Scallop($2.2)</em>{48}$, oyster($11.5-17.2$), mussel($7.2)_{48}$</td>
<td></td>
</tr>
<tr>
<td>Isofucosterol</td>
<td>$d^5,24(28)$</td>
<td>126(-42)</td>
<td>Short-necked clam($14.1)_{47}$</td>
<td></td>
</tr>
<tr>
<td>7-dehydro-stigmasterol</td>
<td>$A^5,7,22$</td>
<td>243-C2H5, 154(-114)</td>
<td>Corbicula($68$), $69$), $72$), fresh-water mussel($70$), short-necked clam($71$)</td>
<td></td>
</tr>
<tr>
<td>C29-sterol</td>
<td>$A^5,24(28)$</td>
<td>154(-114)</td>
<td>Nordotis discus($3.3)<em>{78}$, Lunella coronata($1.1)</em>{78}$, Himetanishi($2.8)<em>{78}$, Kawanina($2.1)</em>{78}$, Boshubora($1.3)<em>{78}$, whelk($2.3)</em>{78}$, Naganishi($6.6)<em>{78}$, Oyster($8.2)</em>{42}$, clam($11.8$), mussel($4.4)<em>{42}$, scallop($3.0$, $5.542$, $4.044$), short-necked clam($5.8)</em>{78}$, razor-clam($8.9)_{78}$</td>
<td></td>
</tr>
</tbody>
</table>

* Figures in ( ) denote level of content (%) in sterol.

While $d^5,7$-sterol has been included in Table 2 as well, there are many reports$^6,12,15,49,52,55,74-76$ wherein $d^5,7$-sterol (provitamin D) has been quantitatively analyzed by the method$^74$ using an ultraviolet extinction coefficient, and these have been consolidated in Table 3. There are reports$^52,55$ that say the sea mussel and Hototogisu which belong to the sea mussel family of bivalves contain 30% or more. While differences can be seen in both Table 2 and Table 3 depending on...
the measurer, these are believed to have been caused by differences in habitat, feeding, season and method of measurement.

With regard to C_{26}-sterol, Idler et al.\textsuperscript{77} recently used GC and fractionated from the sterol of scallops a peak of 0.6-0.7 retentivity time in terms of cholesterol. By MS, NMR and IR, its structure was determined to be 22-trans-24-Norcholesta-5, 22-dien-3\beta-ol as shown in Fig. 1. Hayashi\textsuperscript{43} has also confirmed by GC-MS that the C_{26}-sterol of oysters is of the dien-type. Peaks of even smaller retentivity time than this sterol (R cholesterol = 0.5) can ordinarily be seen in the sterols of salt-water bivalves but these have not been clarified as yet.

C_{29}-sterol is made up of 2-3 components, and in addition to the fact that it sometimes contains \(\beta\)-sitosterol and stigmasterol and sometimes not, there are believed

![Fig. 1. 22-trans-24-Norcholesta-5, 22-dien-3\beta-ol](image)

\textbf{Table 3. \(\Delta^5,7\)-sterol content}

<table>
<thead>
<tr>
<th><strong>Snails</strong></th>
<th><strong>5% or more</strong></th>
<th><strong>1-5%</strong></th>
<th><strong>0-1%</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Madaragaiushii(11.0)\textsuperscript{15}, Chicoreus asinus(10.0)\textsuperscript{49}, Hominerite japonica(5.3)\textsuperscript{49}, Thais bronni(14.1)\textsuperscript{49}, Thais clavigera problematica(17.5)\textsuperscript{49}, Himetanishi(19.3)\textsuperscript{47}, Onaiinumaiai(9.4)\textsuperscript{44}, Vivarasa japonicus(7.6)\textsuperscript{74}, 5.9\textsuperscript{75}, Cinango paludina chinensis mallenta(5.9-7.2)\textsuperscript{75}, Neverta didyma(7.9)\textsuperscript{75}</td>
<td>Monodonta labio(49), Lunella coronata(49), Chlorostoma arcrostroma lishkeii(49), Nateira(49), Tristichothroctus uncinus(49), Thais bronni(47), Thais clavigera problematica(47), Neverta didyma(47), Kawanishi(75), Cinango paludina chinensis mallenta(75), ivory-shell\textsuperscript{47}</td>
<td>Ivory-shell (meat)\textsuperscript{74}, Callana toreuma(49), 74\textsuperscript{74}, Ueshintonsume\textsuperscript{74}) (wreath shell)\textsuperscript{49}</td>
<td></td>
</tr>
<tr>
<td><strong>Bivalves</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sea mussel(35-50)\textsuperscript{52}, soft clam(12.6)\textsuperscript{15}, short-necked clam(9.9)\textsuperscript{76}, cockle(6.2)\textsuperscript{76}, fresh-water mussel(7.2-11.8)\textsuperscript{75}, 15.5\textsuperscript{76}, Atrinapectinata japonica(15.8)\textsuperscript{15}, 6.0\textsuperscript{76}, Hishiketalaya(14.2)\textsuperscript{74}, 15.3\textsuperscript{76}, Ubagai(5.6)\textsuperscript{12}, Kotumai(7.7)\textsuperscript{74}, Mototogri(50)\textsuperscript{55}, Karingeai(9.8)\textsuperscript{49}, mussel(6.7)\textsuperscript{6}, Corbicula sandai(9.3)\textsuperscript{76}, Numanai(13.0)\textsuperscript{75}, Dobucei(11.3)\textsuperscript{75}, Corbicula japonica(8.0)\textsuperscript{75}, common fresh-water clam(9.4)\textsuperscript{75}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kemushihizaragai(12.5)\textsuperscript{49}</td>
<td>Hizaragai\textsuperscript{49}, Nishikihizaragai\textsuperscript{49}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Denotes viscera only.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
to be unknown components as well. Hoda \(^47\) recently discovered the presence of isofucosterol in the sterol of the short-necked clam. This is the first time in shellfish.

Apart from this, the gorgosterol \(^78\) found in the coelenterate coral was established \(^79\) to have a cyclopropane ring in the side chain, but this has not been found in shellfish.

There was also a report \(^64\) on the sterol of tetrane, but since then nothing has been verified.

In the foregoing paragraphs on the sterols in shellfish, we have also alluded to research done before 1960. Mention was made of these because we felt that many of them needed to be re-examined using new analytical equipment recently developed. The pioneers would also be gratified if these could contribute in any way to the development of subsequent studies.

3.2 Biosynthesis

There is a report by Idler \(^80\) that as the lipid content in \textit{Itavagai} decreases the sterol content increases. Tamura et al. \(^81\) observed the effects of feeding oysters with a diet containing no sterol and a diet containing \(\beta\)-sitosterol \((C_{29})\). In the former case, while the growth of the oyster was not inhibited, because of a reduction in the sterol level part of the endogenous sterol was surmised to be of the exogenous type. Also, the cholesterol level increased and became stable while 24-methylene cholesterol also stabilized so it was surmised that oysters are capable of cholesterol synthesis and also capable of converting cholesterol to 24-methylene cholesterol. When fed with a diet containing \(C_{29}\)-sterol, the \(C_{29}\)-sterol within the body first decreased and then increased. This was believed to be because \(C_{29}\)-sterol is indispensable to oysters. With regard to research on these points, there is a whole series of studies by Fagerlund and Idler which show by radioisotopes that the sea mussel and clam absorb the radioactivity of \(2^{-14}C\)-acetate into digitonin precipitates \(^62\), and that the clam \((1)\) converts squalene into digitonin precipitates.
(mainly 45-sterol\textsuperscript{82}), (2) converts cholesterol into 24-methylene cholesterol\textsuperscript{83}, and (3) is capable of inducing an unsaturated bond in the side chain\textsuperscript{84}. In addition, Voogt\textsuperscript{85} has reported the fact that slugs absorb radioactivity from 1\textsuperscript{-14}C-acetate into fatty acid and unsaponifiable matter. In the snail Helix pomatia, however, Addink\textsuperscript{86} has reported that the radioactivity from 1\textsuperscript{-14}C-acetate was absorbed into cholesterol but not as much as fatty acid and not absorbed into squalene at all. Voogt\textsuperscript{87} also says that the synthesis of sterol from acetate can be seen only in grass-eating snails (Unonshii, Monodonta labio) while Zandee\textsuperscript{88},\textsuperscript{89} too has obtained results supporting this, reporting that carnivorous shellfish (ivory-shell) do not synthesize 3\beta-sterol from acetate. Salaque\textsuperscript{90} has found in oysters that there is no 2\textsuperscript{-14}C-mevalonic acid or 14C-methyl-L-methionine.

From the foregoing results, further detailed studies on the biosynthesis of sterols in shellfish are hoped for, and we believe that the connection with feeding habits must also be thoroughly investigated.

There is a report\textsuperscript{91} that a sterol compound obtained from the clam had the effect of lowering the blood cholesterol in baby chicks.

4. STEROIDS

With regard to steroids in shellfish meat, there are reports of the presence of \(\beta\)-olsterol\textsuperscript{92} in the ovary and spermary of seahares and of estrogen and progesterone\textsuperscript{93} in scallops. From the fact that in the liver and pancreas of the sea mussel, when TG increases the steroids decrease and that the steroid content is high during the reproductive period, Monnier et al.\textsuperscript{29} have surmised that it is essential to the production of genetic matter, while Chapat et al.\textsuperscript{94} have also obtained results to support this.

As far as the biosynthesis of steroids is concerned, Hathaway\textsuperscript{95} has found in the spermatozoa of oysters that estradiol 17\(\beta\) gets converted to estrone and a small quantity of testosterone becomes androstane-dione. Mori et al.\textsuperscript{96} also found in the digestive tract of oysters that the activity of 17\(\beta\)-exysteroid dehydrogenase increases
together with the maturation of genetic matter and decreases after the release of genetic matter. Gottfried et al. have discerned from the ovaries of the slug that 11-ketotestosterone, testosterone and 17α-oxyprogesterone are synthesized from endogenous steroids. When the seminal receptacle (containing spermatozoa) was used, the formation of estrone and estradiol 17β was detected but no intermediate metabolite of estrone synthesis such as progesterone, pregnenolone, testosterone and androstane-dione could be detected. Idler et al. discovered the fact that the genetic tissue of the scallop converts 17α-oxyprogesterone into androstenedione but not into 11β-oxyandrostanedione or testosterone, reporting that androstenedione and testosterone etc., cannot be labelled from pregnenolone and that similar phenomena could be seen in the liver and pancreatic tissues as well.

The reason that we can detect only a portion of the biosynthetic pathway which can be seen in higher animals is because, as Gottfried says, the turnover rate of the precursors is so rapid, or the intermediate precursors are created elsewhere and are selectively concentrated, both of which are interesting topics for the future.

5. HYDROCARBONS, HIGH-GRADE ALCOHOLS, BILE ACIDS

With regard to hydrocarbons, there is a report of the discovery of heptacosane in the snail Nassa obsoleta. As for high-grade alcohols, there is the report by Toyama et al. that chimyl alcohol and batyl alcohol were detected in Hizaragai and corbicula while unsaturated selachyl alcohol and something which looks like a liquid hydrocarbon were found in corbicula. There being the possibility of secondary products, future research on this is awaited. There has been no report of bile acids being found in shellfish.

6. GLYCEROPHOSPHOLIPIDS

Tremendous advance has been made in the study of phospholipids (PL) in shellfish over the past ten years. The PL content in shellfish in relation to the total lipids is generally 25-50%, extending in some cases as high as 70% as in the abalone.
6.1 Distribution and composition

The main PL in both bivalves)100,102-112) and snails)33,100,101,108,109,111,113-116) are ethanolamine, serine and choline phospholipid (designated as PE, PS and PC respectively). Inositol phospholipid (PI) and cardiolipin (CL) also exist in small quantities)101,106,108,111,114,116). In addition, Liang et al.114) have detected diglyceride derivatives of 2-aminoethyl phosphonate (2-AEP) from Radix auricularis japonicus. Of noteworthy interest is the discovery of a glycerol-form of lipid (phospholipid) containing a C-P bond which generally exists as a sphingo-form in shellfish. Table 4 shows the phospholipid composition in the short-necked clam)110) and abalone)101).

![Table 4. Phospholipid composition (%) of shellfish](image)

As for PL containing an ether bond, Rapport et al.104,105) have reported the presence of a large quantity of plasmalogen (alkenyl ether PL) in the PL of mussels, soft clams and scallops (13% of total lipids), it being particularly prevalent in the cephalin fraction but scarce in PC. This fact can also be seen in other shellfish (short-necked clam)110), abalone)101), oyster)108), Ishigai)111), etc.), and in the case of the abalone it extends to 23% of the total PL. Thompson et al.109) found that aside from plasmalogen there is a large quantity of glyceryl ether-form (alkanyl ether-form) PL.
Table 5. Proportion of glyceryl ether-form PL in various PL

<table>
<thead>
<tr>
<th>PL</th>
<th>Slug 113)</th>
<th>Radix auricularis japonicus 114)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphatidic acid + CL</td>
<td>22 mol%</td>
<td>- 15.4(3.4)</td>
</tr>
<tr>
<td>PE</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>PL + PS</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>49</td>
<td>44.5(2.5)</td>
</tr>
</tbody>
</table>

Figures in ( ) denote proportion of plasmalogen-form

In slugs, it comprised 11% of the total PL, in *Thais bronni* 7% and in the short-necked clam 3%. In addition, the glyceryl ether-form, as shown in Table 5, is contained in virtually all PL, it being particularly prevalent in PC and also abundant in the visceral PL of slugs.

In general, the component fatty acids of diacyl-form PL in shellfish are mostly unsaturated acids, there being some highly unsaturated acids (C_{20:4}, C_{20:5}, C_{22:5}, C_{22:6}) as well. In abalone 101) 64% is unsaturated acid, the breakdown of which was oleic acid 17%, tetranoic acid 20%, pentanoic acid and hexanoic acid 14% each. As a saturated acid, palmitic acid is predominant. In PL containing an ether bond, the main component of the ether bond side chain (position α) is 16 and 18 carbon atoms, while the fatty acid in position β is 75% unsaturated acid (C_{18:1}, C_{18:2}) in slugs 109), 113).

With regard to the positional distribution of fatty acids of diacyl-form PL, there is a study on PC of scallop muscles by Brockerhoff et al. 17) who found position α to be 69% palmitic acid and position β to be 30% C_{20:5} and 21% C_{22:6}.

6.2 Biosynthesis

With regard to the metabolism of diacyl-form PL, there is the study by Shieh 112) who used 14C-methyl choline, 14C-ethanol amine and 3-14C-serine and looked at their absorption into the PL of scallops.

On the PL biosynthesis of ether bonds, a detailed study has been carried out on slugs by Thompson et al. 113), 117)-119) from absorption experiments 113) using...
6-$^{14}$C-glucose, it was believed that the glycerin portion of ether PL was formed from glucose not unlike the case of the diacyl-form, with $\alpha$-glycerophosphonate being an important medium. In addition, when the absorption of $^{14}$C-palmitic acid and $^3$H-chimyl alcohol was traced over a 1-16 hr period, equivalent absorptions into glyceryl ether-form and diacyl-form PL were discerned. Absorption into neutral lipids was far more than PL and considerable absorption was also seen into diacyl glyceryl ether, but this radioactivity decreased sharply with time while that of ether-form PL gradually increased. However, the absorption into plasmalogen was low, being 10-20% of glyceryl ether PL. But when observations over a long period (3 days) were made, the relative radioactivity of plasmalogen reached approximately half that of the glyceryl ether-form. Also, $^3$H-chimyl alcohol was absorbed into ether-form PL intact, while the absorption into plasmalogen was far greater than $^{14}$C-palmitic acid.

From these findings, it was felt that diacyl-form PL is not the precursor of plasmalogen but that plasmalogen is formed by the desaturation reaction (this reaction is extremely slow) of glyceryl ether PL created from diacyl glyceryl ether. In addition, when the absorption of chimyl alcohol whose side chain was labelled with $^{14}$C and glycerin portion with $^3$H, was watched over a 7-day period, 84% of the absorbed radioactivity was found in PL, of which 65% had been absorbed into choline PL and 22% into ethanol amine PL. The value of $^{14}$C/$^3$H in both neutral lipids and PL did not differ much from that of the chimyl alcohol given, but the $^{14}$C radioactivity of plasmalogen was 33% of glyceryl ether PL and 20 times the ester bond fatty acids. From this fact, it became clear that the ether bond of glyceryl ether PL is retained intact during desaturation and turns into plasmalogen. Fig. 2 depicts the biosynthetic pathway of plasmalogen.

There are also a number of studies on the PL in various organs of shellfish. Higashi has conducted research on the function of PL in the spermatozoa of *Hydrorlipsis schlegeli*, clarifying the fact that the palmitic acid and stearic acid formed by the decomposition of PE which is the principal compound lipid in spermatozoa,
Glycerophosphonate

Triglyceride

Diacyl glyceryl ether

\[ \text{Glycerophosphonate} \quad \rightarrow \quad \text{Diacyl glyceryl ether} \quad \rightarrow \quad \text{H}_2\text{C}-\text{O}-\text{CH}_2-\text{CH}_2-\text{R} \quad \longrightarrow \quad \text{H}_2\text{C}-\text{O}-\text{CH}_2-\text{CH}_2-\text{R} \]

\[ \rightarrow \quad \text{HC}-\text{O}-\text{COR}' \quad \longrightarrow \quad \text{HC}-\text{O}-\text{COR}' \]

\[ \text{H}_2\text{C}-\text{O}-\text{P}-\text{N} \text{ base} \quad \rightarrow \quad \text{H}_2\text{C}-\text{O}-\text{P}-\text{N} \text{ base} \]

Glycerol ether PL  Plasmalogen

Fig. 2. Biosynthetic pathway of plasmalogen

are the main energy sources for respiration. With regard to the nervous system, there is the report\(^{33}\) that the PL in the neurons of the seahare has more PS than PE with some PI being seen, and both PE and PS are virtually all ether bonds without any sphingomyelin at all. Another report\(^{111}\) says that the PL in the ganglia of Ishigai and the snail Helix pomatia is mostly PC, followed by PE, PI and CL, the content being 1/2-1/3 that of vertebrates and again with no sphingomyelin in evidence.

7. SPHINGOPHOSPHOLIPIDS

The sphingophospholipids (SPL) of shellfish are extremely unique in comparison to those of higher animals, and there exist several types of C-P bonded SPL (sphingophosphonolipid) and sphingoethanolamine (SE) not found in higher animals while sphingomyelin (SM) common in higher animals is present in limited quantities in only several species of shellfish. Consequently, SPL is the most unique aspect of lipids in shellfish.

7.1 Sphingophosphonolipids

With regard to lipids with C-P bonds, fairly detailed papers have been written\(^{122)-126}\) so that here we shall confine our remarks to where shellfish are concerned. Research on sphingophosphonolipids in shellfish began in 1964 when Hori et al.\(^{127),128}\) discovered ceramide 2-aminoethyl phosphonate (CAEP) from the fresh-water bivalve Corbicula sandai and determined its structure. Thereafter, a method of isolating\(^{129),130}\) this lipid was established, and a thorough structural analysis was done using phospholipase C\(^{131),132}\). Their presence was also detected in abalone by Koning\(^{101),133}\), in the true oyster by Hayashi et al.\(^{134}\) and in the short-necked clam...
by Hoda 135), and at present they are known to exist widely in various species of bivalves and snails as shown in Table 6. The sphingophosphonolipid content makes

Table 6. Distribution of sphingophospholipids in shellfish

<table>
<thead>
<tr>
<th>Family</th>
<th>CAEP</th>
<th>CMAEP</th>
<th>SE</th>
<th>SM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primeval Gastropoda</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monodonta labio</td>
<td>+148),149)</td>
<td>+139)</td>
<td>+148),149)</td>
<td>+147)</td>
</tr>
<tr>
<td>Bateira</td>
<td>+149)</td>
<td>+139)</td>
<td>+149)</td>
<td></td>
</tr>
<tr>
<td>Ashiyaga</td>
<td>+139)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorostoma aryzyrostoma lishkei</td>
<td>+148),149)</td>
<td></td>
<td>+148),149)</td>
<td></td>
</tr>
<tr>
<td>Halioitob midae</td>
<td>+101)</td>
<td></td>
<td>+101)</td>
<td></td>
</tr>
<tr>
<td>Halioitob garneri</td>
<td>+147)</td>
<td></td>
<td>-147)</td>
<td></td>
</tr>
<tr>
<td>Bokkogasa</td>
<td>+147)</td>
<td></td>
<td>-147)</td>
<td></td>
</tr>
<tr>
<td>Wreath shell</td>
<td>+148)</td>
<td>+138)</td>
<td>-148)</td>
<td></td>
</tr>
<tr>
<td>Mesogastropoda</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Himetanishi</td>
<td>+149)</td>
<td></td>
<td>+149)</td>
<td></td>
</tr>
<tr>
<td>Nagetanishi</td>
<td>+144)</td>
<td>+144)</td>
<td>+147)</td>
<td></td>
</tr>
<tr>
<td>Kawanina</td>
<td>+147)</td>
<td>+139)</td>
<td>+146)</td>
<td>-147)</td>
</tr>
<tr>
<td>Batillaria multiformis</td>
<td>+147)</td>
<td></td>
<td>+146)</td>
<td>-147)</td>
</tr>
<tr>
<td>Neogastropoda</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thais bronni</td>
<td>+148),149)</td>
<td></td>
<td>+148),149)</td>
<td>+147)</td>
</tr>
<tr>
<td>Hemifusus ternatanus</td>
<td>+147)</td>
<td></td>
<td></td>
<td>-147)</td>
</tr>
<tr>
<td>Pulmonata</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radix auricularis japonicus</td>
<td>+114)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bivalves</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corbicula sandai</td>
<td>+128)</td>
<td>+139)</td>
<td>-148),149)</td>
<td>-147)</td>
</tr>
<tr>
<td>Corbicula japonica</td>
<td>+128)</td>
<td></td>
<td>-149)</td>
<td></td>
</tr>
<tr>
<td>Common fresh-water clam</td>
<td>+149)</td>
<td></td>
<td>-149)</td>
<td></td>
</tr>
<tr>
<td>Fresh-water mussel</td>
<td>+128)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Numagani</td>
<td>+148)</td>
<td></td>
<td>-148)</td>
<td>-147)</td>
</tr>
<tr>
<td>Hydriopsis schlegeli</td>
<td>+136)</td>
<td></td>
<td>-148)</td>
<td></td>
</tr>
<tr>
<td>Ishigai</td>
<td>+128)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tateboashi</td>
<td>+128)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearl-oyster</td>
<td>+148),149)</td>
<td></td>
<td>+148),149)</td>
<td>+147)</td>
</tr>
<tr>
<td>True oyster</td>
<td>+134)</td>
<td></td>
<td>-149)</td>
<td></td>
</tr>
<tr>
<td>Short-necked clam</td>
<td>+135)</td>
<td></td>
<td></td>
<td>110)</td>
</tr>
</tbody>
</table>
up 53-75\% \textsuperscript{228}) of the lipids insoluble in acetone, ether and pyridine, and constitutes the main ingredient of SPL. The main constituent fatty acid of CAEP, except for the spermatozoa of Hydriomena schlegeli (96\% stearic acid)\textsuperscript{136}, is palmitic acid. In addition, CAEP containing oxyacid which may be deemed to be its metabolic medium, has been isolated from the pearl-oyster\textsuperscript{137}).

In 1969, Hayashi et al.\textsuperscript{138}) isolated a N-methyl derivative of CAEP from the wreath shell and determined it to be ceramide 2-N-methyl aminoseryl phosphonate (CMAEP). Hori et al.\textsuperscript{138}) also discerned its presence in Monodonta labio, investigating its structure in even more detail and verifying its existence in several species of shellfish (Table 6). While the fatty acid of CMAEP is also predominantly palmitic acid, some cases have been encountered where oxyacid is the main constituent fatty acid\textsuperscript{115,140}).

Furthermore, N-acylates of CAEP and CMAEP have recently been discovered by Hori et al.\textsuperscript{141,142}) The former has been isolated from the pearl-oyster, the latter from Monodonta labio and both from Corbicula sandai. Hereafter, the discovery of even newer phosphonolipids is anticipated\textsuperscript{143}). As the data in Table 6 include findings based on thin layer chromatography alone, the interrelationship between sphingophosphonolipids and the species of shellfish warrants careful examination in future. Fig. 3 shows the structural formula of sphingophosphonolipid and SE contained in shellfish.

\[
\text{Ceramide}^* \quad \text{CH}_3(\text{CH}_2)_{12}\text{CH-CH-CH-CH}_2-\text{O-P-CH}_2\text{CH}_2\text{NH}_2
\]

\[
\text{Ceramide}^* \quad \text{CH}_3(\text{CH}_2)_{12}\text{CH-CH-CH-CH}_2-\text{O-P-CH}_2\text{CH}_2\text{NH}_2
\]

\[
\text{Ceramide}^* \quad \text{CH}_3(\text{CH}_2)_{12}\text{CH-CH-CH-CH}_2-\text{O-P-CH}_2\text{CH}_2\text{NH}_2
\]

\[
\text{Ceramide}^* \quad \text{CH}_3(\text{CH}_2)_{12}\text{CH-CH-CH-CH}_2-\text{O-P-CH}_2\text{CH}_2\text{NH}_2
\]

\[
\text{Ceramide}^* \quad \text{CH}_3(\text{CH}_2)_{12}\text{CH-CH-CH-CH}_2-\text{O-P-CH}_2\text{CH}_2\text{NH}_2
\]

Fig. 3. Structural formula of SPL (* long chain base deemed as $C_{18}$-sphingosine)
7.2 Sphingoethanolamine and sphingomyelin

SE was discovered from Nagatanishi by Hori et al. \(^{144}\) in 1965, and detailed studies \(^{131,132,145,146}\) were carried out on its isolation method and chemical structure. Its distribution at the moment is confined to snails and one bivalve, the short-necked clam, and there seems to be a relationship between it and the species of shellfish (Table 6).

SM, which had been the only SPL contained in animals until SE was discovered, has been found to date in no more than several species of shellfish as shown in Table 6. In shellfish, it may be that phosphonolipids play the role of substitute for SM \(^{125,126}\). As mentioned earlier, there are reports \(^{33,111}\) that SM cannot be seen in the nervous system of Ishigai and such. However, these reports have not touched upon phosphonolipids.

7.3 Biosynthesis

With regard to the biosynthesis of SPL in shellfish, research has progressed in connection with phosphonolipids. When Liang and Rosenberg \(^{150}\) injected \(^{32}\)P-phosphoric acid into slugs, they found as shown in Fig. 4 that while free aminoethyl phosphonate (AEP) is initially labelled, its relative radioactivity decreases after peaking at the 5-hr point, and from the fact that the activity of the other fractions increases, they surmised that free AEP is first synthesized and then combines with the lipid components. This is diametrically opposite to the biosynthetic process \(^{151}\) in protozoans. Research was also done on the precursor of the carbon framework of AEP, and as with protozoans, phosphoenal pyruvic acid was deemed to have the highest possibility.

Injecting \(^{32}\)P-phosphoric acid into
Table 7. Absorption of $^{32}$P-phosphoric acid into AEP lipids in various organs of *Hydriopsis schlelei*.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Relative radioactivity (cpm/µmol AEP lipid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>1907</td>
</tr>
<tr>
<td>Adductor muscle</td>
<td>245</td>
</tr>
<tr>
<td>Mantle membrane</td>
<td>175</td>
</tr>
<tr>
<td>Branchiae</td>
<td>327</td>
</tr>
<tr>
<td>Others</td>
<td>104</td>
</tr>
</tbody>
</table>

Next, using liver homogenate and examining the absorption of $^{32}$P-phosphoric acid in vitro, AEP was found to be synthesized in 4 hrs at $37^\circ C$.

Recently, when liver homogenate was divided by centrifugal separation into the mitochondrial fraction, microsomal fraction and supernatant fraction (50000xg, 60 min) and the absorption from $^{32}$P-phosphoric acid into the C-P compounds was examined, it was found to be largest in the supernatant fraction, with the absorption of radioactivity being seen only in free AEP just as in the findings of Liang et al.\textsuperscript{150} and there was virtually no absorption into the lipid-bonded or protein-bonded forms.

From the foregoing results, it is certain in shellfish that free AEP is first synthesized and then it combines with the lipid components. There have been no reports on the biosynthesis of SE and SM, nor is the role of SPL in vivo clear.

8. GLYCOLIPIDS

As far as glycolipids in shellfish are concerned, their first mention occurred in 1959 when Nakazawa\textsuperscript{154} reported a new lipid form from oysters known as O-(14-methyl-4-pentadecanoyl) choline N-O-(l-fucopyranosyl-1,4-D-glucopyranosyl-1,4-D-glucopyranosyl)-lactyl taurate. Thereafter, from studies on *Corbicula sandai*, true oyster, wreath shell and *Hydriopsis schleleli* by Hori et al.\textsuperscript{155}-\textsuperscript{157} as well as Hayashi et al.\textsuperscript{158},\textsuperscript{159}, it has been made clear in every case that the main component is a gulobosidic form of mucolipid with an extremely complicated saccharic section.

Such structurally simple glycolipids as ceramide monohexoside and dihexoside have also been obtained from *Corbicula sandai*\textsuperscript{160} (glucosyl ceramide, galactosyl
ceramide, galactosyl mannosyl ceramide), Hydriopsis schlegeli spermatozoa\textsuperscript{156}) (galactosyl ceramide) and Hesokikubogai\textsuperscript{116}) (galactosyl ceramide), with the majority being mucolipids.

The constituent fatty acids, in every case are mainly palmitic acid and stearic acid, except that in the case of Chlorostoma argyrostoma lishkei\textsuperscript{116}), oxypalmitic acid is the constituent component in place of stearic acid.

The presence of two types of mucolipids\textsuperscript{155}) has been reported in fresh-water bred Corbicula sandai, but these are virtually identical in fatty acid and long chain base and differ only in the sugar component. That is, they contain glucose, mannose, xylose, glucosamine and mannose-6-phosphoric acid\textsuperscript{161}) as common sugar components in addition to which one contains 3-O-methyl fucose\textsuperscript{162}) while the other contains fucose and 4-O-methyl galactose\textsuperscript{163}). Neither contains sialic acid. The fact that the saccharic section thus consists of several sugars makes it very different from the glycolipids of higher animals. Also, this saccharic section, compared to that of glycolipids in higher animals, is exceedingly unstable towards acid so that its structural determination has been delayed, but Hori et al. have hypothesized\textsuperscript{157}) the following structural formula based on analytical results of oligosaccharide obtained by weak oxyhydrolysis. The fatty acids of this mucolipid are mainly such saturated acids as C\textsubscript{16} and C\textsubscript{18}, and there is no unsaturated acid like C\textsubscript{24} as can be seen in the glycolipids of higher animals.

\[
\text{Ceramide-glucose-mannose-mannose-6-phosphoric acid-glucosamine-xylose}
\]

\[
\begin{align*}
\text{3-O-methyl fucose} \\
\text{Fucose} \\
\text{Ceramide-glucose-mannose-mannose-6-phosphoric acid-glucosamine-xylose}
\end{align*}
\]

\[
\begin{align*}
\text{4-O-methyl galactose}
\end{align*}
\]

The long chain base is sphingosine.

A mucolipid\textsuperscript{156}) quite similar to that of corbicula has been obtained from the
spermatozoa of Hydriopsis schlegeli as well. The constituent sugars are glucose, fucose, xylose, mannose and glucosamine, with the long chain base being sphingosine.

Also, from the adductor muscle, viscera, branchiae and mantle membrane of the salt-water bivalve, the oyster, a gulobosidic-form of mucolipid likewise similar to corbicula has been obtained as the main ingredient. The constituent sugars are glucose, galactose, fucose, O-alkyl fucose, galactosamine and glucosamine. While it is still unclear as to the position and type of alkyl base of this O-alkyl fucose, Fujiwara et al. recently discovered 4-O-methyl fucose from glycolipid-like matter in the oyster, an interesting comparison with the fact that the glycolipid of the corbicula contains 3-O-methyl fucose. The long chain base of this mucolipid has a special characteristic in that, in contrast to the long chain base of corbicula being sphingosine normally seen in glycolipids of higher animals, there is very little sphingosine in the oyster and sphingadienine is the main ingredient with a fair quantity of C₁₆-sphingosine also being seen. While a diene-form of long chain base also exists in SM of human blood-plasma, in this case the position of the double bond is different, being 4 and 14. In the oyster it is 4 and 8, and apart from the oyster this sphinga-4,8-dienin also exists in the glycolipids of the wreath shell as well as in the sphingophosphonolipids of Hizaragai and Cellana toreuma.

A mucolipid has likewise been obtained as the main ingredient from a salt-water snail, the wreath shell, as well. The fatty acids are predominantly palmitic acid and oxypalmitic acid, and the long chain base is sphingosine and sphinga-4,8-dienin while the constituent sugars are glucose, galactose, fucose and glucosamine.

In this way, the glycolipids of shellfish are common in every case with a gulobosidic-form of mucolipid being obtained, while their special characteristics are the fact that many unusual sugars not seen in the glycolipids of higher animals are contained in the saccharic section and the fact that a diene-form is contained in many of the long chain bases in salt-water shellfish.

The glycolipid content in terms of the total lipid in shellfish is in the
order of 1-2%. There has been no report on the presence of glycosyl glyceride, nor has any report been seen on the metabolism of glycolipids in shellfish.

(Accepted June 1, 1971)

BIBLIOGRAPHY

1) Hori, Shishitsu seikagaku kenkyu (Lipid biochemical research), 10 (1962).
4) Itasaka, Hori, Higashi, Seikagaku (Biochemistry), 33, 206 (1961).
7) Hamada, Ueno, Yukagaku (Oil chemistry), 17, 39 (1968).
12) Toyama, Takagi, Nikka (Japan Chem.), 75, 1238 (1954).
14) Takagi, Toyama, Nikka (Japan Chem.), 78, 93 (1957).
22) Tanaka, Hatano, Nikka (Japan Chem.), 73, 870 (1952).
34) W. Bergmann, Progr. in Chem. Fats and other Lipids, 1, 18 (1952).
43) Hayashi, unpublished, partially reported at the 1966 Annual Oil Chemists Research Symposium.
47) Hoda, Yukagaku (Oil Chemistry), 19, 1014 (1970).
49) Kita, Toyama, Nikka (Japan Chem.), 81, 485 (1960).
57) Matsumoto, Toyama, Nikka (Japan Chem.), 65, 258 (1944).
58) Matsumoto, Toyama, Nikka (Japan Chem.), 65, 310 (1944).
65) Takagi, Maeda, Toyama, 
67) Toyama, Tanaka, Nikka (Japan Chem.), 77, 636 (1956).
69) Hori, Nikka (Japan Chem.), 75, 1144 (1954).
72) Tamura, Kuniuma, Matsumoto, Nikka (Japan Chem.), 77, 987 (1956).
73) Hoda, Yukagaku (Oil Chemistry), 20, 479 (1971).
74) Toyama, Takagi, Nikka (Japan Chem.), 75, 1241 (1954).
76) Toyama, Takagi, Nikka (Japan Chem.), 76, 240 (1955).
107) Itasaka, Hori, Higashi, Seikagaku (Biochemistry), 33, 206 (1961).
110) Hoda, Yukagaku (Oil Chemistry), 16, 596 (1967).
115) Hayashi, Matsuura, Matsuura, Yukagaku (Oil Chemistry), 18, 118 (1969).
122) Horiguchi, Tampakushitsu, kakusan, koso (Proteins, nucleic acids, enzymes), 12, 317 (1967); Kagaku to seibutsu (Chemistry and life), 6, 425 (1968).
125) Hori, Yukagaku (Oil Chemistry), 20, 2 (1971).
135) Hoda, Yukagaku (Oil Chemistry), 18, 239 (1969).
160) T. Hori, O. Itasaka, M. Kamimura, J. Biochem., 64, 125 (1968).
169) Hayashi, Matsuura, unpublished.