LIPOPROTEINS (LIPOSTICHAERINS) IN THE ROE OF BLENNY, STICHAEUS GRIGORJEWI HERZENSTEIN.

LIPOPROTEINS (LIPOSTICHAERINS) IN THE ROE OF BLENNY, *STICHAEUS GRIGORJEWI* HERZENSTEIN*.

By

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Introduction

In the earlier publications (1, 2), the authors reported on the occurrence of a toxic lipoprotein and at least two toxic lipids by intraperitoneal injection in the roe of blenny fish, *Stichaeus grigorjewi* Herzenstein.

Thus it is the purpose of the present investigation to clarify the correlation between toxic lipids and lipoproteins. Hitherto, only the parenteral toxicity was

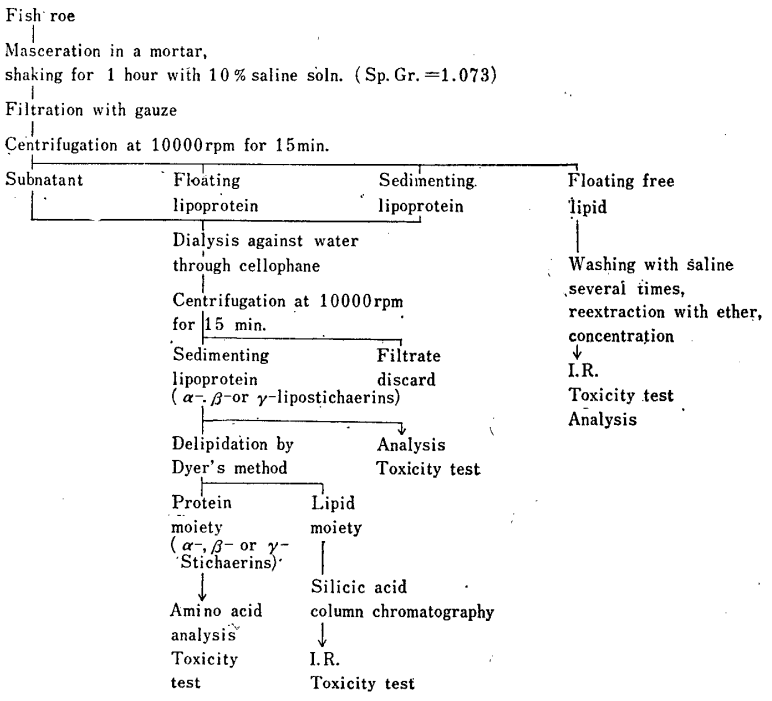


Figure 1. Flow sheet of fractionation of lipoproteins in blenny roe.

* Most of the present work was given as a lecture at the Annual Meeting of the Japanese Society of Scientific Fisheries held at Tokyo in April 3, 1965.

checked, however, needless to say, the oral toxicity should be examined since the actual food poisonings are caused exclusively by the oral route. Therefore, the feeding test of lipids and lipoproteins was performed to determine the oral poison in fish roe.

Experimental

1. Fractionation of lipoproteins in the roe utilizing their differences in specific gravity.

After many trials to fractionate the lipoproteins, the authors established tentatively the following fractionation method. The flow sheet of the method is given in Figure 1.

Wet fish roe was mascerated in a mortar to which an equal volume of 10% sodium chloride solution (specific gravity=1.073) was added, then shaken for 1 hour on a mechanical shaker. The suspension was freed from tissue residue by filtration through gauze and the filtrate was centrifuged for 15 min. at 10000 rpm. After centrifugation, it could be divided into four fractions, namely, a floating free lipid, a floating lipoprotein (low density lipoprotein, named α-lipostichaerin*), a lipoprotein contained in the subnatant (high density lipoprotein, β-lipostichaerin*), and a sedimenting lipoprotein (high density lipoprotein, γ-lipostichaerin*).

Each lipostichaerin fraction was dialyzed against water through a cellophane sack and the dialyzate was centrifuged for 15 min. at 10000 rpm. (α-, β- and γ-lipostichaerins.)

A floating free lipid fraction was collected, washed several times with saline solution to remove any traces of lipostichaerin, reextracted with ethyl ether and the extract was freed from ether by evaporation. (free lipid fraction).

These lipostichaerin and lipid fractions were the materials for subsequent analysis and toxicity tests.

2. Analysis of lipostichaerins and stichaerins.

Nitrogen was determined by micro Kjeldahl method, phosphorus by Allen's method. Isoelectric points determination of lipostichaerins was carried out as follows; A series of buffer solutions with different pH were prepared. The pH

* Proposal of the revision for naming the roe lipoproteins: At first, the authors tentatively named the toxic lipoprotein in the roe by intraperitoneal injection "Dinogunellin" from its scientific name "Dinogunellus". However, the scientific name of the fish was revised recently to "Stichaeus". Therefore, the authors wish to change the name of "Dinogunellin" in the previous report as "lipostichaerin". This new name is analogous to "Lipovitellin" in the hen egg yolk. Since three lipoproteins were fractionated, the authors distinguished them by placing the prefix "α". "β" and "γ" at the head of the name "Lipostichaerin" α- β- and γ-Lipostichaerins represent the lipoproteins with different specific gravities (different protein/lipid ratio) and α- β- and γ-Stichaerins represent the protein moieties of the corresponding lipostichaerins.

Table 1. Analysis of lipostichaerins and stichaerins.

Fraction	Yield	N%	P%	PI*
α-lipostichaerin	12.6–14.4%	3.56	0.75	3.8 5.8
β-lipostichaerin	34.5–38.6	11.37	1.25	4.7
γ-lipostichaerin	1.5– 2.1	11.43	0.69	4.6 5.2
α-stichaerin	—	14.89	0.72	—
β-stichaerin	—	13.42	1.14	—
γ-stichaerin	—	14.61	0.32	—
free lipid**	1.4	nil	0.07	—

* Isoelectric points. pI values of α- and γ-forms are not so sharp as that of β-form.

** Floating free lipid which separate on the surface during fractionation with 10% saline solution.

Table 2. Automatic analysis of amino acid contents of α-, β- and γ-stichaerins.

Amino acids	α-stichaerin μmols/mg	β-stichaerin 〃	γ-stichaerin 〃	α-vitellin* 〃	β-vitellin* 〃
Lys.	0.578	0.580	0.471	0.50	0.51
His.	0.153	0.150	0.150	0.19	0.15
NH_3	0.356	0.376	0.680	—	—
Arg.	0.314	0.312	0.268	0.47	0.52
$CySO_3H$	0.083	0.067	0.102	0.12	0.13
Asp.	0.500	0.479	0.567	0.60	0.67
Thr.	0.363	0.343	0.365	0.35	0.36
Ser.	0.512	0.565	0.428	0.54	0.51
Glu.	0.657	0.638	0.557	0.82	0.86
Pro.	0.317	0.257	0.444	0.43	0.44
Gly.	0.432	0.339	0.405	0.38	0.38
Ala.	0.626	0.706	0.403	0.63	0.61
Val.	0.386	0.327	0.389	0.56	0.61
Met.	0.008	—	—	0.19	0.21
Ileu.	0.285	0.302	0.226	0.45	0.49
Leu.	0.564	0.532	0.482	0.70	0.73
Tyr.	0.193	0.153	0.206	0.23	0.25
Phe.	0.245	0.241	0.233	0.27	0.25
Try.	—	—	—	0.13	0.13

* Data obtained by Cook et al.

ranges were from 3.19 to 12.15. The buffer solutions used were acetate buffer (pH 3.19–5.90), phosphate buffer (pH 5.90–8.04), and glycine buffer (pH 8.93–12.15). Half an ml. of each protein solution was added to 4.5ml of each buffer solution, mixed vigorously, left to stand and then centrifuged. After centrifugation, 1 ml of the supernatant was taken for protein analysis by modified Folin method. (3). The corresponding pH value to the minimum amount of protein in the supernatant aliquot (least optical density) was regarded as isoelectric point (pI) of the

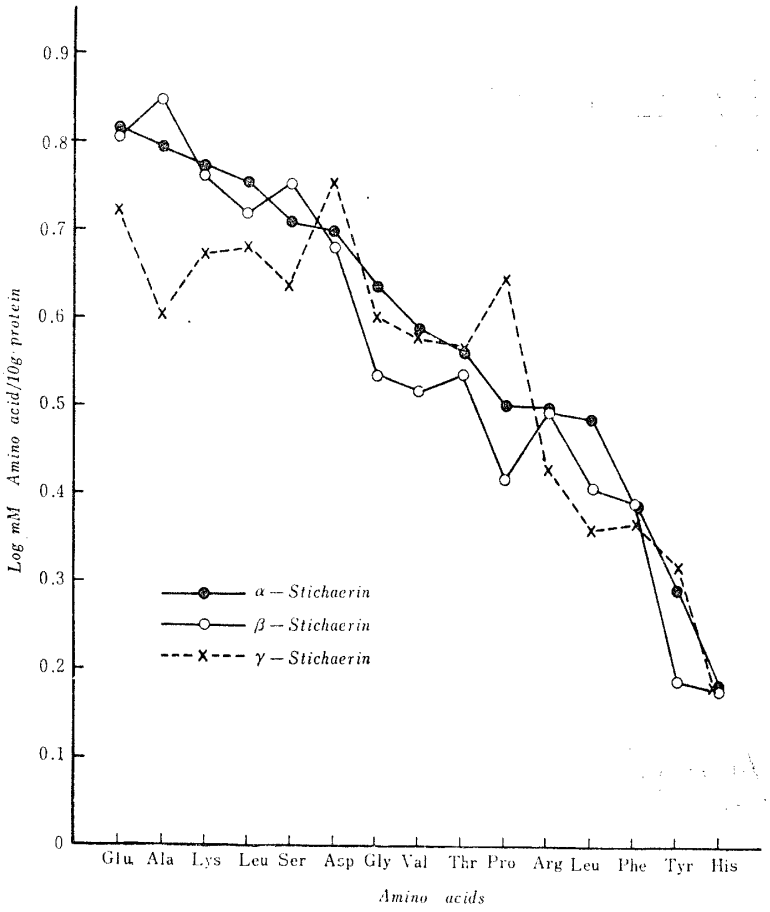


Figure 2. Content of15amino acids in Stichaerins

lipostichaerin tested. The data are summarized in Table 1.

Automatic analysis of amino acids of the hydrolyzates of α-, β- and γ-stichaerins was carried out by using KLA–2 Hidachi amino acid analyzer. The results obtained are shown in Table 2 and Figure 2, together with the results of α- and β-vitellins by Cook et al (4) form comparison.

Gas liquid chromatography of the fatty acid methyl esters prepared from the lipid moieties of lipostichaerins was carried out by using Shimadzu Type GC–2B hydrogen flame ionization detector. The column of ethylene glycol succinate polyester was used with nitrogen (45 ml/min.) as mobile phase. The results obtained was shown in Table 3.

3. Correlation between the toxicity and infrared absorption spectra of lipid fractions.

A. Lipid moieties of lipostichaerins and a free lipid.

The authors prepared α-, β- and γ-lipostichaerins from fish roe as described in the former section, delipidated by Dyer's method (5), fractionated each lipid moiety by repeated silicic acid column chromatography as reported previously (2).

They also prepared a free lipid fraction by the extraction of freeze dried fish roe

Table 3. Percentage fatty acid composition (as methyl esters) of lipostichaerins.

Fatty acid	From α-lipostichaerin	β-lipostichaerin	γ-lipostichaerin
14:0	1.6%	1.1%	1.2%
15:0	0.4	0.3	0.3
16:0	24.8	12.6	23.0
16:1	10.2	9.1	9.5
17:0	2.3	4.3	3.0
18:0	5.5	6.2	6.8
18:1	20.4	23.2	19.3
18.2	0.3	1.6	1.0
18:3 20:1	2.6	2.5	2.1
18:4	0.3	0.3	0.2
22:1	1.7	3.1	2.5
20:4	—	0.2	0.2
20:5	12.0	14.1	11.1
22:4	0.4	—	—
22:5	1.2	—	1.1
22:6	15.6	20.4	17.8

Shimdazu type GC–2B hydrogen flame ionization detector.
Ethylene glycol succinate polyseter column. Mobile phase nitrogen (45 ml/min.)

with ethyl ether alone. Alternatively, when lipostichaerins are prepared by fractionation with 10% saline solution, a free lipid fraction floats on the surface of emulsion. They collected this lipid, washed several times with saline solution to remove any traces of lipostichaerin. Sometimes the free lipid layer was further extracted with ethyl ether, and the extract was evaporated to remove solvent. (free lipid).

Table 4. Toxicity test of the "free lipid" fraction.

Sample	route of administration	body weight & sex of mice (m) or rats (r)	volume or weight given	survival or death
free lipid extracted with ether alone (Soxhlet)	intraperitoneal injection (i.p.)	(m) 20.3 g ♀ 21.1 ♀ 22.4 ♀	1 ml 〃 〃	survived 〃 〃
floating free lipid*	i.p.	(m) 20.4 ♂ 22.9 ♂ 21.9 ♂	1 ml 〃 〃	survived 〃 〃
floating free lipid*	oral administration	(r) 142 g ♂ 190 ♂	2 ml (647 mg)** 〃	survived 〃

* This was obtainedby fractionation with 10% salin and floatation. Liver oil like, pale yellowish, purified by repeated washing with saline solution.
** Emulsified with Tween 60. Forced feeding.

Table 5. Toxicity test of the lipid fractions obtained from the lipid moieties of lipostichaerins.

Sample	Source of sample	route of administration	body weight & sex of mice (m) or rats (r)	volume or weight given	Survival or death
Fract. 3*	lipid moiety of α-lipostichaerin	i.p.	(m)		
			23.0 g ♂	1 ml(40.6 mg)	died
		〃	19.2 ♂	〃	〃
		〃	21.0 ♂	〃	〃
Fract. 3*	lipid moiety of β-lipostichaerin	〃	22.2 ♀	1 ml(41.3 mg)	died
		〃	23.1 ♀	〃	〃
		〃	24.2 ♀	〃	〃
Fract. 3	lipid moiety of γ-lipostichaerin	〃	23.8 ♀	1 ml(48.0 mg)	survived
		〃	22.8 ♀	〃	〃
		〃	23.4 ♀	〃	〃
Fract. 3	lipid moiety of α-lipostichaerin	oral administration**	(r)		
			158 g ♂	2 ml(618 mg)***	survived
		〃	128 ♂	〃	〃
Fract. 3	lipid moeity of β-lipostichaerin	〃	106 ♂	2 ml(627 mg)	survived
		〃	108 ♂	〃	〃
Fract. 3	lipid moiety of γ-lipostichaerin	〃	100 ♂	2 ml(622 mg)	survived
		〃	156 ♂	〃	

* Fraction 3 obtained by silicic acid column chromatography. Experimental conditions were the same as reported in the previous report (2). Other fractions (Fractions 1, 2 and 4) were usually nontoxic by i.p.. Data on other fractions were omitted for economy of space.

** Forced feeding using polyethylene catheter.

*** Emulsified with Tween 60.

Table 6. Toxicity test of lipostichaerins.

Sample	route of administration	body weight & sex of mice(m) or rats (r)	volume or weight given	Survival or death
		(m)		
α-lipostichaerin	i,p,	19.2 g ♂	1 ml (45.4 mg)	died
	〃	18.7 ♂	〃	〃
	〃	21.2 ♂	〃	〃
β-lipostichaerin	〃	17.6 ♂	1 ml (53.3 mg)	died
	〃	16.4 ♂	1 ml (67 mg)	〃
	〃	15.3 ♂	〃	〃
γ-lipostichaerin	〃	20.3 ♂	1 ml (47.8 mg)	died
	〃	18.9 ♂	〃	〃
	〃	19.9 ♂	〃	〃
α-lipostichaerin	oral administration*	(r)		
		122 g ♂	5 g in 3 ml saline	survived
	〃	140 ♂	〃	〃
β-lipostichaerin	〃	148 ♂	〃	survived
	〃	124 ♂	〃	〃
γ-lipostichaerin	〃	132 ♂	〃	survived
	〃	136 ♂	〃	〃

* Forced feeding using polyethylene catheter.

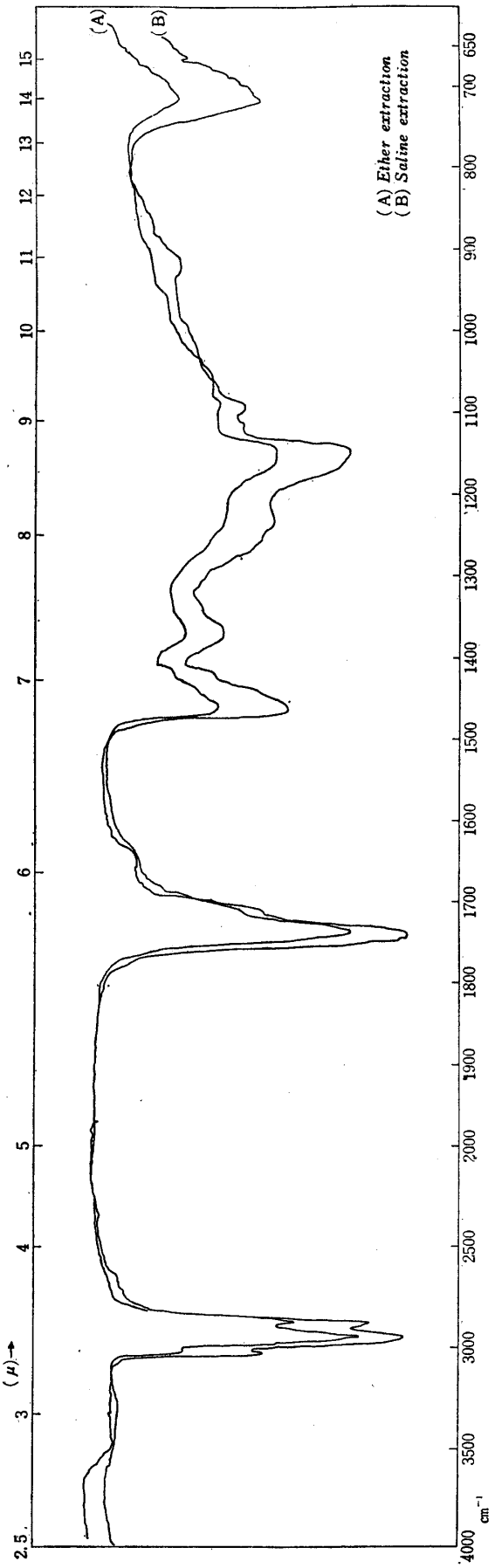


Figure 3. I.R. spectra of free lipid fractions

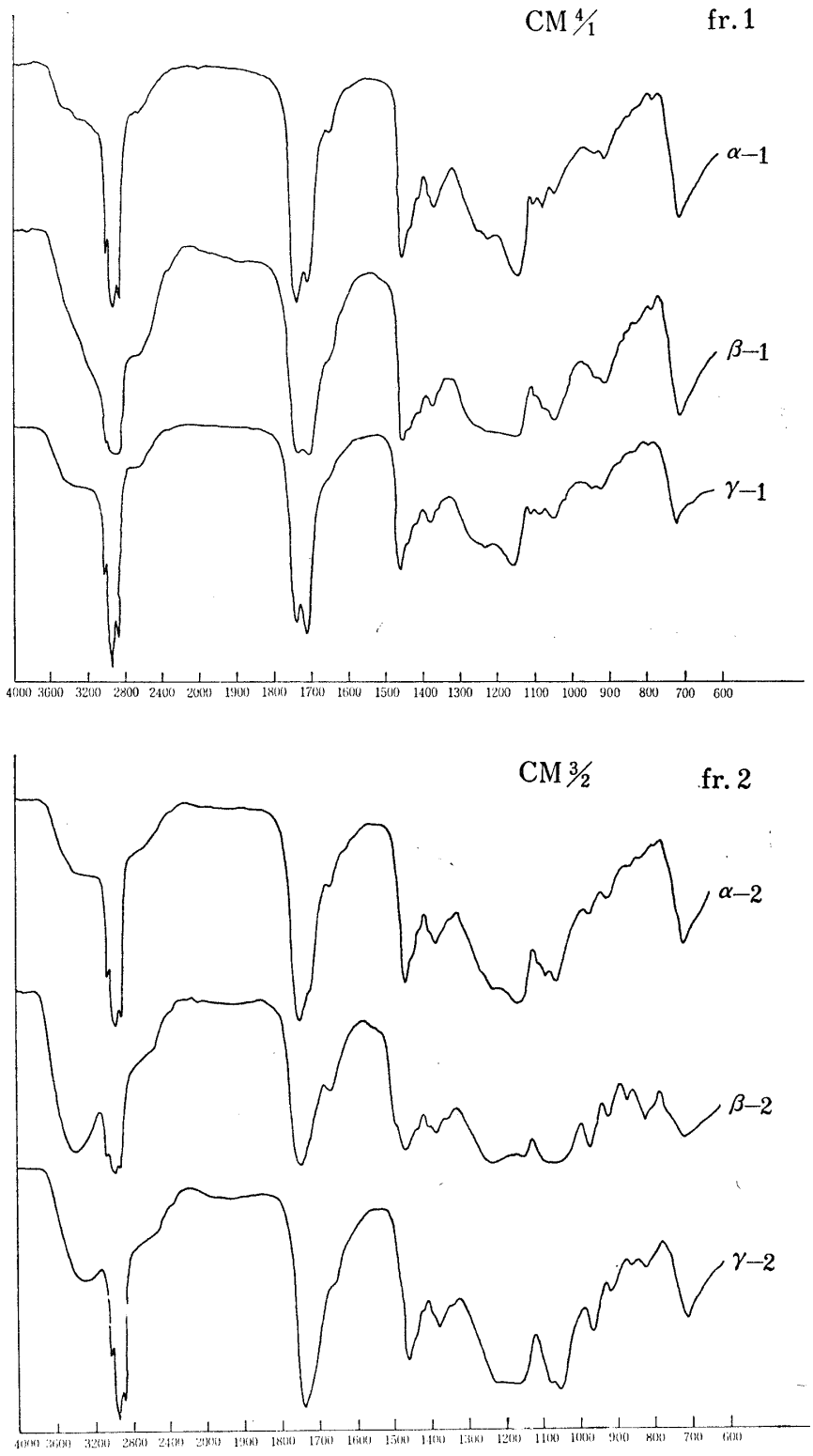
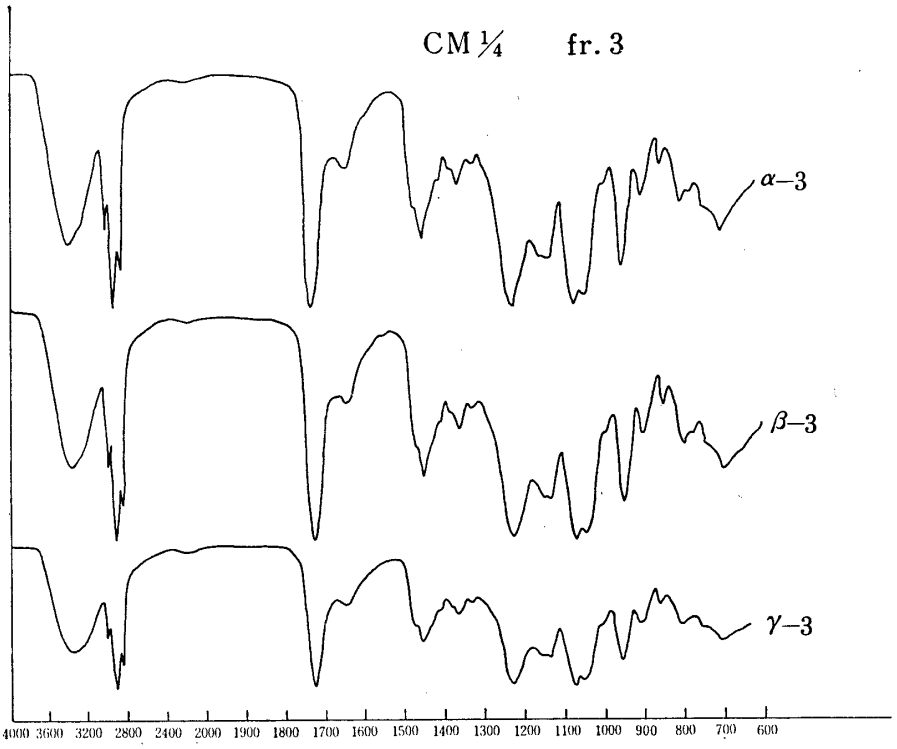


Figure 4. I.R. spectra of lipid moieties of Lipostichaerins



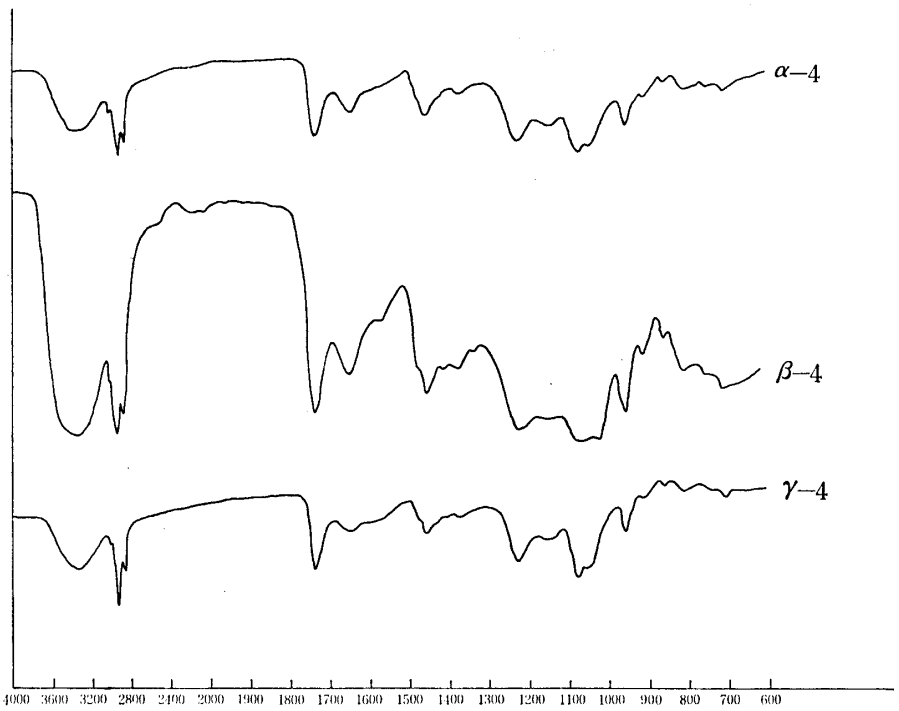


Figure 4. (Continued)

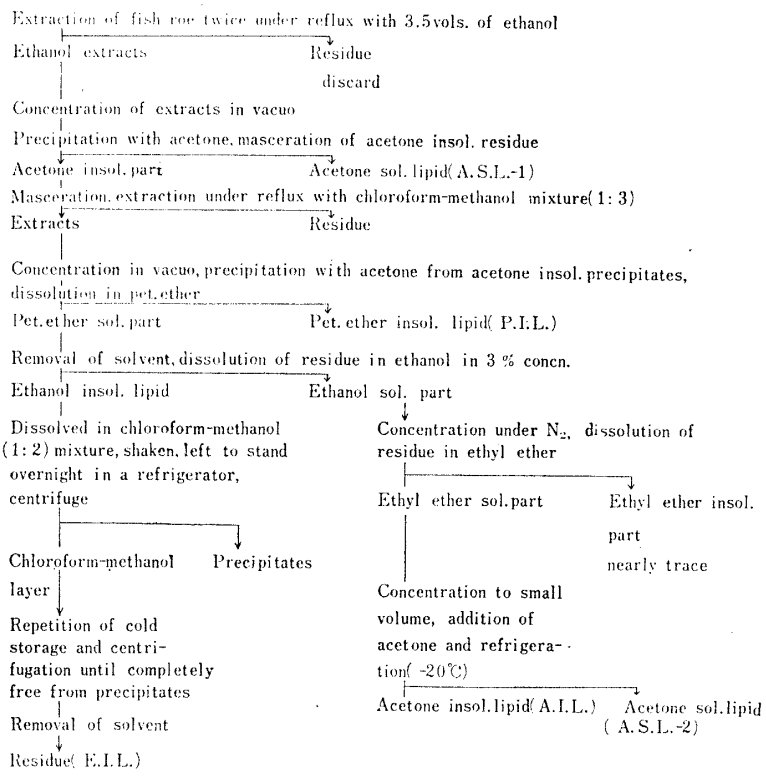


Figure 5. Flow sheet of the modified Thiele method.

Toxicity tests of all lipid fractions were examined by both parenteral and oral routes. (Tables 4–6)

Simultaneously with toxicity tests, the infrared absorption spectra of all lipid fractions were measured. Infrared spectra of the free lipid fractions which were prepared by direct ether extraction or by fractionation with saline and floatation, were compared in Figure 3.

Infrared spectra of all lipid fractions obtained by silicic acid column chromatography of the lipid moieties of lipostichaerins were illustrated in Figure 4. The experimental conditions were the same as in the previous report (2).

B. Lipid fractions fractionated by modified Thiele's method.

Lipid fractions fractionated by modified Thiele's method were also examined on their parenteral and oral toxicities and their infrared spectra. The flow sheet of the modified method is shown in Figure 5.

The results of toxicity tests are given in Table 7. Abbreviated symbols of each fractions are the same as those in Figure 5.

Simultaneously, the infrared spectra of each fractions are shown in Figure 6.

The results of toxicity tests so far examined are summarized as in Table 8.

C. Similarity of infrared spectra of the lipid moieties of lipostichaerin and A.I.L.

Acetone insoluble lipid (A.I.L.) ,the most predominant fraction in amount by Thiele method, was thought in early stage of the study to be a predominant poison in

Table 7. Toxicity test of lipid fractions fractionated by modified Thiele method.

Sample	route of administration	body weight & sex of mice (m) or rats (r)	volume or weight given	Survival or death
		(m)		
A.S.L.-1*	i.p.	17.4 g ♀	1 ml (42.0 mg)	survived
	〃	17.6 ♀	〃	〃
	〃	16.6 ♀	〃	〃
P.I.L.	〃	17.1 ♀	1 ml (43.7 mg)	survived
	〃	16.9 ♀	〃	〃
	〃	17.7 ♀	〃	〃
E.I.L.	〃	16.9 ♀	1 ml (41.5 mg)	died
	〃	17.3 ♀	〃	〃
	〃	16.7 ♀	〃	〃
A.I.L.	〃	17.5 ♀	1 ml (45.0 mg)	died
	〃	18.5 ♀	〃	〃
	〃	17.2 ♀	〃	〃
A.S.L.-2	〃	19.5 ♀	1 ml (41.7 mg)	survived
	〃	18.6 ♀	〃	〃
	〃	18.4 ♀	〃	〃
A.S.L.-1	oral administration	(r) 143 g ♂	2 ml (640 mg)**	survived
	〃	110 ♂	〃	〃
P.I.L.	〃	114 ♂	2 ml (610 mg)	survived
	〃	98 ♂	〃	〃
E.I.L.	〃	100 ♂	2 ml (636 mg)	died
	〃	112 ♂	〃	〃
	〃	146 ♂	2 ml (616 mg)	〃
	〃	187 ♂	〃	〃
A.I.L.	〃	90 ♂	2 ml (606 mg)	survived
	〃	130 ♂	〃	〃
A.S.L.-2	〃	150 ♂	2 ml (635 mg)	survived
	〃	174 ♂	〃	〃

* Abbreviated symbols of each fraction are the same as in Figure. 5.
** Foreced feeding using polyethylene catheter. Emulsified with Tween 60.

fish roe. Tne infrared spectrum of A.I.L. was compared with those of the lipid moieties of α- and β-lipostichaerins. These are shown in Figures 7 and 8, indicating the similarity (identity) of both spectra.

4. The oral poison. (Ethanol insoluble lipid fraction. E.I.L.)

The true poisonous principle in fish roe should be, of course, an oral poison. According to the results of screening the oral toxicities of all lipid and lipostichaerin fractions, it was found by the authors that only one oral poison is so-called "ethanol insoluble lipid fraction" fractionated by modified Thiele's method. (Table 8).

This oral poison is, at the present stage, not completely pure and should be purified further by conjunctive use of other techniques. However, the analytical data so far obtained are summarized in Table 9.

Choline was determined by Glick's method or by Kushner's method, Iodine vlue by Wijs method, aldehyde by Wittenberg method or by Schiff's modification

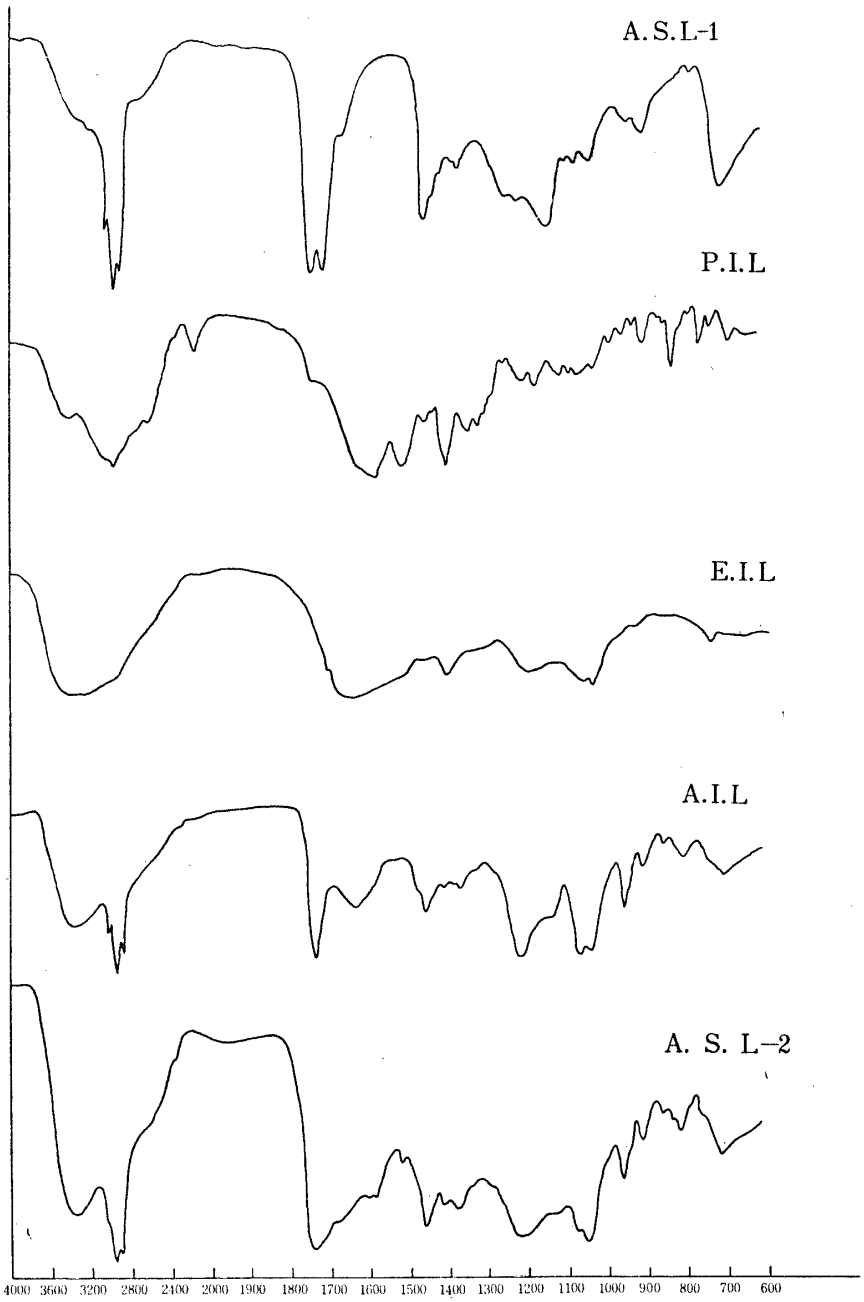


Figure 6. I.R. spectra of Lipid fractions by Thiele method.

method, and glycerol by Renkonen's method (6).

Automatic analysis of amino acids of the hydrolyzates of the oral poison was carried out as before. (Table 10). Gas liquid chromatography of the fatty acid methyl esters prepared from the oral poison was also conducted. (Table 11)

Discussions

Usually for the fractionation of lipoproteins, the preparative procedure which involves the floatation of the various lipoprotein classes in sequence by successively

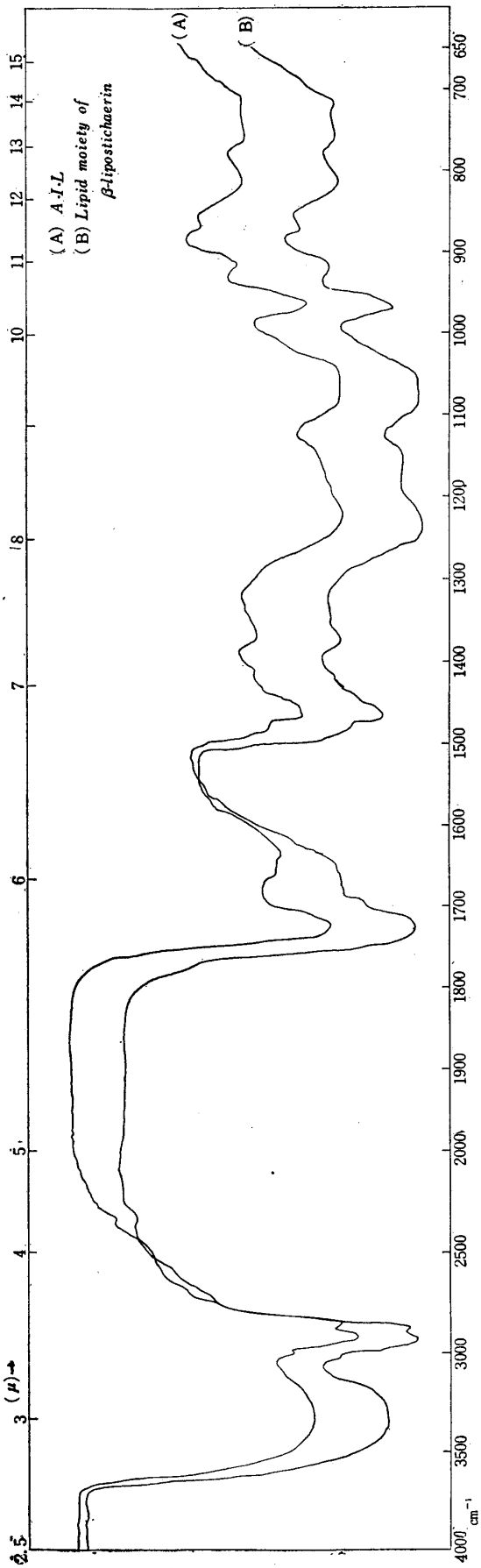


Figure 7. I.R. spectra of A.I.L. and lipid moiety of β-lipostichaerin.

Table 8. Summary of toxicity tests.

Fraction	Intraperitoneal injection into mice	Oral administration to rats
Fractionation with 10% saline solution		
α-lipostichaerin	+	−
β-lipostichaerin	+	−
γ-lipostichaerin	+	−
Lipid moiety of α-lipostichaerin*	+	−
Lipid moiety of β-lipostichaerin*	+	−
Lipid moiety of γ-lipostichaerin*	−	−
free lipid	−	−
Fractions by Thiele method		
A.S.L.-1	−	−
P.I.L.	−	−
E.I.L.	+	+
A.I.L.	+	−
A.S.L.-2	−	−

Remarks: + toxic, − nontoxic

* Fraction 3 or Fraction 3-3, obtained by silicic acid column chromatography.

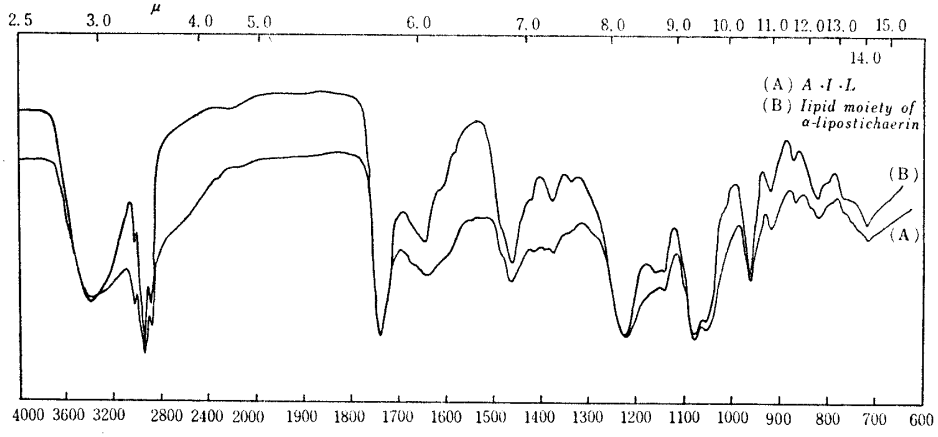


Figure 8. I.R. spectra of A.I.L and lipid moiety of α-lipostichaerin.

increasing the density of the medium, is often employed. Thus, the authors tried the Chargaff method (7), the Fevold methods (8), the Cook method (9) and the Hillyard method (10) to prepare the lipostichaerins from fish roe. However, the authors found that these procedures are not successful for the preparation of lipostichaerins, therefore, they established the fractionation method with 10% saline solution as schemed in Figure 1. By this method, three lipostichaerins (α-, β- and γ-lipostichaerins) and a free lipid fraction were obtained.

Isoelectric point determination of lipostichaerins showed that β-lipostichaerin has only one pI(4.7), while in case of α- and γ-forms more than one pIs exist.

Table 9. Nature of the oral poison (E.I.L.)

		Data given in the previous report
Parenteral toxicity (i.p).	+ (Mice)	+(Mice)
Oral toxicity	+ (rats)	
Infrared spectra	cf. Figure 6	
Nitrogen content (%)	5.75–6.05	7.48
Phosphorus content (%)	2.16–2.32	2.07
N/P	5.74–5.9	8.02
Choline content (%)	1.48–3.30%	trace
Iodine value	96	107
Glycerol content (%)	6.56	+
Aldehyde content (%)		8.34(W) 8.00(S)
Amino acids in the hydrolyzate	cf. Table 10 Lys,His,Arg,$CySO_3H$,Tau, Asp,Thr,Ser,Glu,Pro,Gly, Ala,Val,Ileu,Leu,Tyr,Phe.	
Fatty acids	cf. Table 11	

Table 10. Automatic analysis of amino acid content of the oral poison (E.I.L.)

Amino acids	μmols/ml	Amino acids	μmols/ml
Lys.	0.331	Glu.	0.656
His.	0.111	Pro.	0.334
NH_3	1.541	Gly.	0.733
Arg.	0.192	Ala.	0.527
$CySO_3H$	0.131	Val.	0.197
Tau.	1.080	Ileu.	0.096
Asp.	0.648	Leu.	0.142
Thr.	0.423	Tyr.	0.098
Ser.	0.771	Phe.	0.074

The experimental conditions are the same as in Table 2.

Table 11. Percentage fatty acid composition of lipid moieties of the oral poison (E.I.L.).

Fatty acids	%	Fatty acids	%
14:0	0.5	18:3	1.9
15:0	0.1	20:1	
16:0	11.9	18:4	0.1
16:1	4.9	22:1	6.4
17:0	1.9	20:4	0.3
18:0	12.0	20:5	20.9
18:1	14.7	22:5	1.5
18:2	1.4	22:6	20.8

The experimental conditions are the same as in Table 3.

Starch gel electrophoresis or gel filtration with Sephadex G–200 of β-lipostichaerins showed an homogeneous pattern. Therefore a purification procedure of β-lipostichaerin which involves the fractionation with 10% saline followed by gel filtration with Sephadex G–200 is promising, whereas in case of α- and γ-lipostichaerins gel filtration with Sephadex (G–200, G–75) was unsuccessful, indicating some heterogeneity in them. Although further work is necessary to obtain the completely pure lipostichaerins, the authors carried out preliminary chemical analysis and toxicity tests of lipostichaerins and their lipid moieties. (Table 1) These lipostichaerins were delipidated with chloroform-methanol by Dyer's method (5) to separate into lipid and protein classes (α-, β- and γ-stichaerins). The amino acid analysis of the hydrolyzates of α-, β- and γ-stichaerins proved that the amino acid compositions of these stichaerins are very similar to each other and unlike to vitellins from hen egg yolk (markedly different in Arg, Glu, Val, Met, Ileu and Leu. contents). (Table 2 and Figure 2)

Fatty acid composition of three lipostichaerins are also similar to each other (rich in 16: 0, 16: 1, 18: 1, 20: 5, 20: 6, fatty acids.)

Correlation between the parenteral and oral toxicities and the infrared spectra of lipid fractions were also examined.

In the first place, the authors regarded the free lipid as the lipid extractable with ethyl ether alone, or as the lipid floating spontaneously on the surface of emulsion during fractionation with 10% saline solution. (without using any organic solvents). As shown in Table 4 and figure 3, both types of "free lipid" were proved to be nontoxic when injected intraperitoneally into mice or administered orally to rats. The infrared spectra of both free lipids coincide with each other and differ from those of derived lipid fractions from the lipid moieties of lipostichaerins. It is a significant evidence that the toxic substance does not occur in free lipid state but occur in a complex lipid state in the roe.

Concerning the toxicity of the lipostichaerins, these have only an intraperitoneal toxicity but do not have an oral toxicity as shown in Table 6.

Next, the lipid moieties from each lipostichaerins were fractionated by silicic acid column chromatography using chloroform-methanol solvent in varying ratio, or the lipid fractions were prepared by modified Thiele's method. All lipid fractions obtained by these methods were screened by toxicity tests and the infrared spectra were measured and compared with each other simultaneously.

The results obtained by toxicity tests are summarized in Table 8. Since α-, β- and γ-lipostichaerins have parenteral toxicity but not have oral toxicity and oral poison should be responsible for human intoxication, one must distinguish the parenteral poison and oral poison. Although α-, β- and γ-lipostichaerins prepared by 10% saline method differ in their protein-lipid ratio, these appeared to be essentially similar entities, considering from their amino acids and fatty acid

compositions. (Tables 2 and 3) The infrared spectra of lipid fractions fractionated by silicic acid column chromatography (cf. Figure 4) are also similar in their characteristic bands. The intraperitoneal toxicity of these lipid fractions was found only in fraction 3 (or more polar fraction), however, none of them show oral toxicity.

Among the lipid fractions obtained by Thiele's method, the intraperitoneal toxicity were found in A.I.L. and E.I.L. fractions, and the oral toxicity was exclusively found in E.I.L. fraction. Therefore, E.I.L. (ethanol insoluble lipid fraction) is only one oral poison that the authors could discover.

A.I.L. fraction, the predominant parenteral (i.p.) poison in fish roe, was thought in early stage of study to be a principal poison in fish roe by Hokkaido workers. The infrared spectra of A.I.L. is very similar (or identical) with those of fraction 3 derived from the lipid moieties of lipostichaerins (Figures 7 and 8).

This is also a evidence that A.I.L. might be derived by delipidation of lipostichaerin with polar solvents.

The infrared spectrum of E.I.L. fraction is very peculiar and there is nothing to equal it among all spectra of lipid fractions so far obtained. Analysis of some properties of the oral poison (E.I.L.) was performed, although it was not completely pure yet. (Table 9) The exact molecular structure, molecular size of the oral poison are to be pursued further, however, the authors assumed from the data that it may be a lipoprotein or lipopeptide containing phosphorus, choline and glycerol. It also has fatty acid ester and fatty acid amide groups in its molecule. The authors named the oral poison (E.I.L.) tentatively as "δ-lipostichaerin" owing to its lipoprotein nature.

Summary

1. The authors obtained three lipoproteins (named α-, β- and γ-lipostichaerins) and a free lipid by the fractionation of fish roe with 10% saline solution.

α-, β- and γ-lipostichaerins have intraperitoneal toxicity (parenteral poison) but did not have oral toxicity. Lipid moieties of lipostichaerins have also intraperitoneal toxicity but did not have oral toxicity. Free lipid had neither parenteral nor oral toxicities.

2. Among the lipid fractions by Thiele's method, E.I.L. and A.I.L. fractions have intraperitoneal toxicity, and oral toxicity was found only in E.I.L.

A.I.L. fraction, the most predominant parenteral poison, has no oral toxicity.

E.I.L. fraction is the only oral poison so far discovered.

3. The authors assumed that the oral poison (E.I.L.) is a lipoprotein or lipopeptide containing phosphorus, glycerol and choline. The authors named the oral poison (E.I.L.) tentatively as "δ-lipostichaerin".

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