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## CHEMICAL STUDIES ON THE MEAT OF ABALONE

(*Haliotis discus hannai* INO)-II\*

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### V. DETECTION OF MUCOPROTEIN IN ABALONE MEAT

There is found a mucous substance on the surface of the abalone body. This may be due to a gland secretion similar to that in the snail. When a small quantity of the mucous substance collected by rubbing off from the body surface was tested by Biuret reaction, Xanthoprotein reaction, Millon reaction, Sakaguchi reaction and diazo reaction, all of them showed positive. That is to say, the presence of a protein substance was definitely and certainly proven. The presence of free sugars, amino sugars and uronic acid was likewise ascertained by Molish reaction and naphthoresorcinol reaction. Therefore, the mucous substance might be supposed to be a mucoprotein. Here the authors describe their examination of properties of the mucoprotein.

#### 1. Extraction of mucoprotein from abalone meat

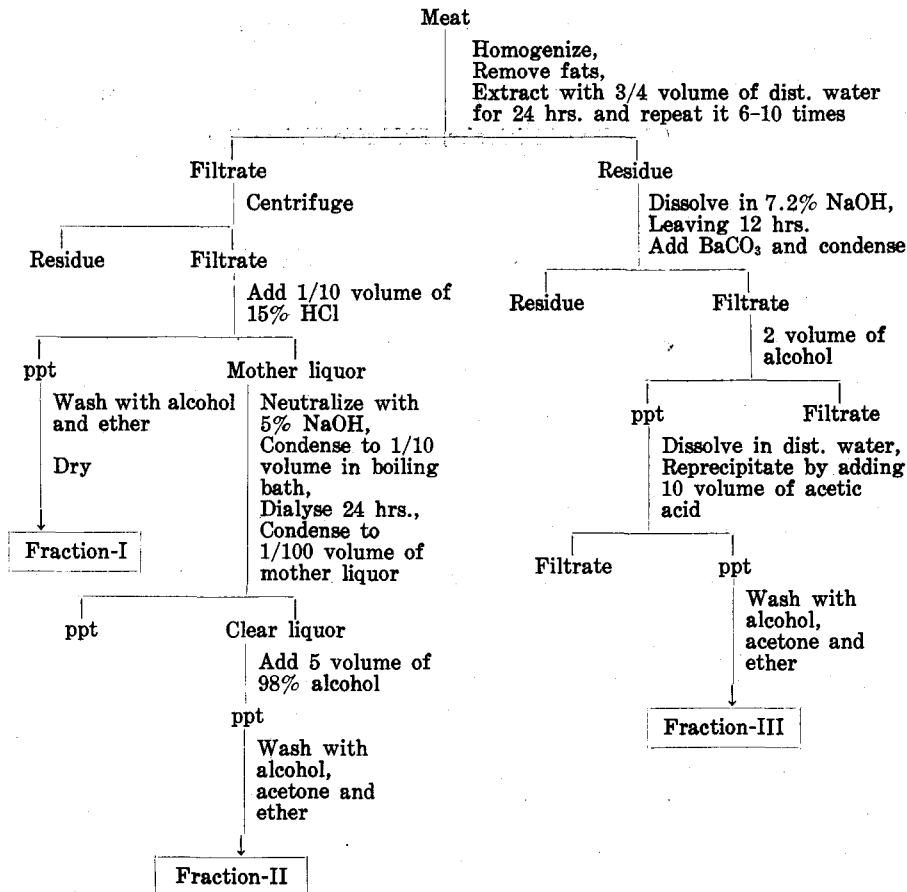
For the preparation of the mucoprotein from the meat of abalone, the meat was extracted with water, then the mucoprotein was fractionated after Scheme 1, following the methods employed by Blix<sup>1)</sup>, Meyer<sup>2)</sup> and Tanabe<sup>3)</sup>.

The eviscerated bodies of abalone were crushed and then defatted with ether. The prepared meat was extracted with three-fourth volume of water for 6 hours. Thus the extraction was repeated seven times at 24-hour intervals. The extracted solution collected was centrifuged. One-tenth volume of 15% HCl solution was added to the upper transparent liquor obtained after the centrifugation. The precipitate obtained here was called Fraction I. The precipitate was filtered, and the deposit was washed with water thoroughly until Cl<sup>-</sup> disappeared. The washed substance was treated with alcohol and ether and dried after Blix<sup>1)</sup>.

The filtrate obtained by removal of Fraction I was neutralized with 5% NaOH solution, and was evaporated in a dish to the volume of one-tenth on a water-bath. The condensed liquor was dialyzed in running water for 24 hours.

\* Continued from the previous paper I, published in this Bulletin, 12 (3), 212 (1961).

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Scheme 1. Preparation of mucoprotein from *H. discus hannai* meat

After dialysis, the liquor was evaporated again to the volume of one one-hundredth. After the condensation, the liquor was cooled and centrifuged to remove the deposit. Ninety-five % alcohol was added to the upper transparent liquor, then some precipitate was obtained. This precipitate was treated with alcohol and ether and then dried. This final material was called Fraction II.

The residue of water extraction of abalone meat was treated with 300 cc of 7.2% NaOH for 24 hours. BaCO<sub>3</sub> was added to the alkaline extracted solution obtained and it was condensed on a water-bath. The condensed solution was centrifuged to remove the deposit. Two times volume of 95% alcohol was added to the upper transparent brown liquor, then grey precipitate was obtained. The precipitate was dissolved with water. Ten volumes of glacial acetic acid was

added to the solution, then some precipitate was obtained. This precipitate was centrifuged. The precipitate was treated with alcohol, acetone and ether, and then it was dried on  $P_2O_5$  in a desiccator. This was called Fraction III. In the upper transparent liquor after the centrifugation, many colourless needle-shaped crystals were produced after standing over one night.

Thus Fractions I, II and III were obtained. The yield amount of Fraction I was the least (0.05% of original meat), the amounts of Fractions II and III were 0.17% and 0.31%, respectively.

Samples of each fraction were tested qualitatively by means of various reactions similarly to those used for the mucous substance on the surface of the abalone meat. In consideration of the results obtained, in each fraction there are certainly protein, amino-sugar, uronic acid similarly to the contents of sea cucumber meat<sup>4</sup>).

The substances in each fraction were not precipitated by mercuric chloride or by heating.

## 2. Detection of the kinds of carbohydrates by means of paper chromatography

The kinds of carbohydrates in the substances of each fraction were detected by means of paper chromatography after Hirase and Araki<sup>5</sup>), and Satake<sup>6</sup>).

### (1) Preparation of sample for chromatography

Writers hydrolyzed each 5 mg samples of the substances of each fraction to which was added about 3 cc of 1 N  $H_2SO_4$  in a closed glass tube on a water bath at 100°C for 5 hours. After hydrolysis,  $Ba(OH)_2$  was added to each solution to make pH value 5.8, and then preparate was centrifuged. The upper transparent liquor obtained was used for paper chromatography.

### (2) Developing solvents and revealing reagents

As developing solvents, a mixture of butanol, acetic acid and water (5:1:2) was used. The method of development was by one-dimensional chromatography by ascending solvent. As revealing reagents, a mixture of butanol containing acetylacetone (0.5 cc) and 80% alcohol containing 10% KOH (butanol : alcohol = 1 : 50) was used. The developed spots were sprayed with the mixture causing acetylation, and then heated at 105°C for 5 minutes. After the treatment, the spots were revealed with Ehrlich reagent.

The paper chromatograms revealed by Ehrlich reagent are shown in Fig 1.

As seen in Fig. 1, in Fraction I glucuronic acid, galactose, and N-acetylglucosamine were detected; in Fraction II glucuronic acid, galactose, fructose, N-acetylglucosamine, and fucose were detected; and in Fraction III glucuronic acid, galactose, fructose, and N-acetylglucosamine were detected.

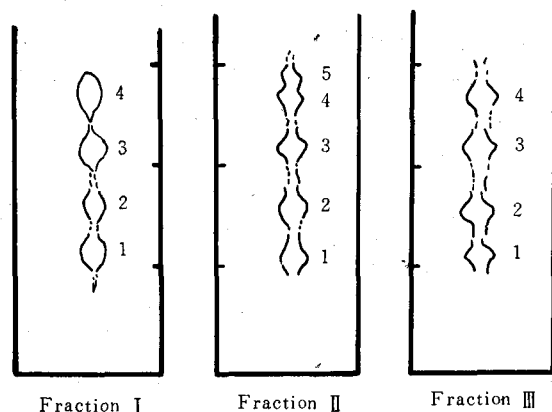


Fig. 1. Paper chromatograms revealed by Ehrlich reagent

1. Glucuronic acid
2. Galactose
3. Fructose
4. N-acetylglucosamine
5. Fucose

## (3) Quantitative estimation of mucoprotein in the meat of abalone

The total amount of nitrogen was estimated by the micro-Kjeldahl method. Acid-hydrolyzable sulfur was estimated gravimetrically as  $\text{BaSO}_4$  after hydrolysis with 1 N HCl solution. The amount of glucuronic acid was estimated by Tanabe's method<sup>7)</sup>. The estimation of reducing power was carried on after Fujita<sup>8)</sup>. The amount of galactose was estimated by Hagedorn-Jensen's method which was employed by Gale<sup>9)</sup>. The estimation of acetyl radical was done after Suzuki<sup>10)</sup>. The estimation of the amount of amino sugar was done by colorimetry which was employed by Masamune and Nagazumi<sup>11)</sup>.

Results obtained are shown in Table 1, in which the results obtained from sea cucumber meat<sup>4)</sup> are presented in company with those from abalone meat.

Table 1. Comparative results on mucoprotein prepared from abalone (*H. discus hannai*) meat with sea cucumber meat

Items	Fractions	Fraction I	Fraction II	Fraction III	Sea cucumber <sup>4)</sup>
Total-N (%)		11.51	39.8	16.50	13.03
Acid-hydrolyzable sulfate (%)		0.72	1.31	0.97	1.27
Glucuronic acid (%)		0.52	5.56	8.10	7.83
Reducing power		22.60	16.70	21.40	25.75
Galactose (%)		8.23	4.30	7.42	7.81
Acetyl (%)		1.71	2.85	3.62	4.83
Ash (%)		0.98	3.68	6.61	1.08

## (4) Nitrogen distribution of the protein moiety in the mucoprotein

The substances in each fraction from the prepared mucoprotein were hydrolyzed with 20% HCl solution. After hydrolysis, the nitrogen distribution was determined by Van Slyke's method. The results obtained are shown in Table 2.

Table 2. Nitrogen distribution in each fraction of mucoprotein isolated from abalone (*H. discus hannai*) meat

Items	Fractions	Fraction I	Fraction II	Fraction III
Total-N		100	100	100
Amide-N		1.87	2.36	5.67
Humine-N		7.30	8.50	3.70
Basic total-N		32.12	36.43	37.14
Basic amino-N		6.90	7.58	5.51
Mono amino total-N		52.10	51.04	55.60
Mono amino amino-N		28.70	30.28	37.06

As seen in Table 2, the ratios of the amount of basic amino acid nitrogen to the total amount of nitrogen in Fractions I, II and III were 32.1, 36.5 and 37.1%, respectively, while the ratios of the amount of mono amino acid nitrogen to the amount of the total nitrogen were 52.1, 51.0 and 55.6%, respectively. Comparing those values with the fractionated proteins, which are detailed in Article II, in previous paper I<sup>12)</sup> the nitrogen distribution of mucoprotein was observed to be almost the same as that of the raw abalone meat and hot water extractives. That is to say, the distribution of nitrogen of Fraction II was like that of abalone meat and the nitrogen distributions of Fractions I and II were like hot water extractives of abalone meat.

## (5) On the colourless needle-shaped crystals

As above mentioned, colourless needle-shaped crystals remained in the upper transparent liquor after addition of acetic acid to the water extraction of abalone meat and centrifugation.

The upper transparent has shown a strong flow birefringence. After the liquor was filtered, the filtrate showed no flow birefringence. The crystals on the filter paper were collected and dissolved into water. This aqueous solution showed flow birefringence. When the crystals were burned, they melted then carbonized. The crystals were ascertained to contain C, N and S by qualitative tests. The m.p. was 144°C. The crystals will be studied in future to confirm whether they are hyaluronic acid.

## VI. DECOMPOSITION OF THE ABALONE MEAT

The abalone is used for preparing canned food or dried food. Moreover, it is used for eating as raw meat ("Sushi" or "Sashimi"). Therefore, it is important to know the freshness of the meat and the limit of the freshness for using as raw material of canned food or dried food.

Here, the author describes his attempts to learn the velocity of the falling of freshness, and the products due to decomposition when the abalone meat was left and allowed to decompose.

### 1. Changes in the amount of volatile basic nitrogen when the abalone meat is left

#### (1) Experimental method

Fresh *Haliotis discus hannai* caught near Hakodate was used. From the abalone viscera mass was removed and the body was employed as samples, while the abalone without removal of visceral mass was also employed. Each two carcasses were left as they are at 35° ( $\pm 2^\circ\text{C}$ ), 20° ( $\pm 2^\circ\text{C}$ ) and 5°C ( $\pm 2^\circ\text{C}$ ) respectively, and the abalone meat was allowed to decompose aerobically. At certain definite intervals of leaving time, a sample of each abalone meat was taken off; on such portions the amount of volatile basic nitrogen (V.B.-N) was estimated by Weber and Wilson's method<sup>13)</sup>. The hardness of the abalone meat was estimated by Tauchi's hardness-meter<sup>14)</sup>. From the amounts of V.B.-N produced, limit of the leaving time at a certain temperature as raw material were determined.

Table 3. Changes in amount of V. B.-N, pH and hardness of abalone in shell at 35°C

Leaving time (hrs.)	V. B.-N(mg%)	pH	Hardness	Organoleptic test
0	2.01	6.2	80	Fresh smell
5	3.34	6.2	55	"
12	6.48	5.8	65	" , Softening of meat
16	13.35	6.0	59	Fishy smell, "
19	21.80	6.2	56	Slight putrefactive odor
21	24.25	6.4	58	Strong putrefactive odor
14	40.50	6.4	59	"
28	46.54	6.6	62	"
48	64.20	6.8	65	"

## (2) Results

At the respective leaving temperatures, the variations in amount of V.B.-N in the abalone meat, pH and hardness are as shown in Tables 3~7 and Fig. 2.

Table 4. Changes in amount of V. B.-N, pH and of abalone in shell at 20°C

Leaving time (hrs.)	V. B.-N (mg%)	pH	Hardness	Organoleptic test
0	1.33	6.2	64	Fresh smell
13	4.54	6.0	70	"
19	15.34	6.2	54	Slight putrefactive odor
22	24.60	6.4	50	Strong putrefactive odor
26	35.56	6.4	50	"
35	41.81	6.6	55	"
54	55.35	6.4	57	"

Table 5. Changes in amount of V. B.-N, pH and hardness in shell at 5°C

Leaving time (hrs.)	V. B.-N (mg%)	pH	Hardness	Organoleptic test
0	3.34	6.2	30	Fresh smell
144	4.68	6.2	53	"
168	6.02	6.4	60	"
192	9.72	6.4	60	"
216	16.54	6.4	58	Slight putrefactive odor
230	22.03	6.4	62	"
254	27.38	6.6	57	"
278	36.20	6.6	55	"
302	39.07	6.6	55	"

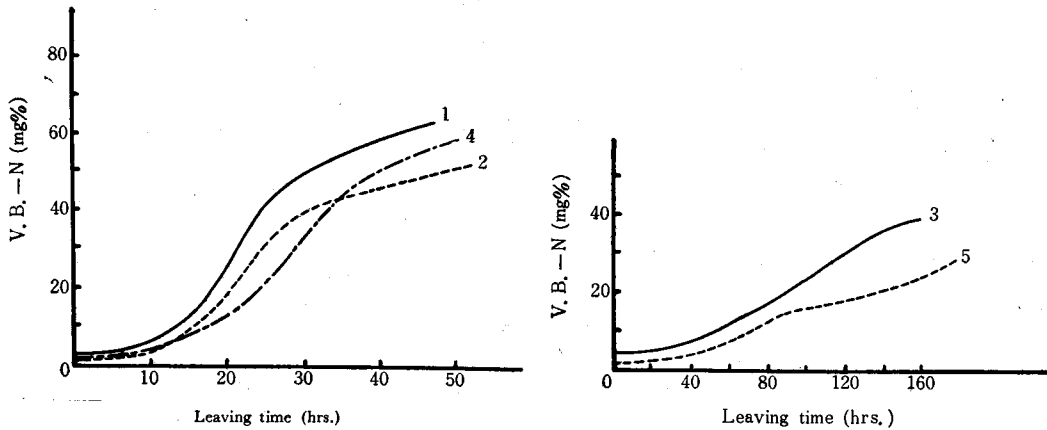
Table 6. Changes in amount of V. B.-N, pH and hardness of eviscerated abalone meat at 20°C

Leaving time (hrs.)	V. B.-N (mg%)	pH	Hardness	Organoleptic test
0	1.33	6.2	64	Fresh smell
13	5.74	5.8	45	"
16	6.02	6.0	43	" , Softening of meat
19	14.78	6.0	40	Slight putrefactive odor
22	17.35	6.2	36	Strong putrefactive odor
30	34.80	6.2	37	"
36	44.24	6.4	39	"
50	58.10	6.6	42	"



Table 7. Changes in amount of V. B.-N, pH and hardness of eviscerated abalone meat at 5°C

Leaving time (hrs.)	V. B.-N (mg%)	pH	Hardness	Organoleptic test
0	1.32	6.2	64	Fresh smell
24	4.68	6.2	60	"
48	6.96	6.1	65	"
72	12.68	6.0	72	"
96	15.35	6.2	62	"
120	18.21	6.2	63	Slight putrefactive odor
144	21.90	6.4	60	"
168	30.78	6.4	65	Strong putrefactive odor

Fig. 2. Changes in amount of volatile basic nitrogen during the leaving of *H. discus hannai*

1. Abalone in shell (35°C)
2. Abalone in shell (20°C)
3. Abalone in shell (5°C)
4. Eviscerated abalone (20°C)
5. Eviscerated abalone (5°C)

As seen in Tables 3~7 and Fig. 2, the pH value of the abalone meat decreased once at the stage in which the amount of V.B.-N increased to 6~8 mg%, then it increased. The hardness of the abalone meat increased also at the stage in which the pH value decreased, and thereafter decreased; the meat became soft. When the amount of V.B.-N increased above 15 mg%, the putrefactive odour was slightly noticeable. When it reached above 20 mg%, the putrefactive odour became strong. In the case of leaving abalone meat at 5°C ( $\pm 2^\circ$ ), when the amount of V.B.-N increased to 20 mg%, the abalone meat gave off slightly a

putrefactive odour.

The leaving time of abalone in shell at which the amount of V.B.-N reached to 20 mg% was about 140 hours. The reason why the abalone kept fresh for longer time at 5°C will be interpreted by Kinoshita and Nakagawa's studies<sup>15)</sup> in which they have clarified that abalone in shell is alive for a long time, if it is left at about 6°C.

## 2. Calculations of decomposition velocity coefficient, temperature coefficient and temperature constant

From data obtained as shown in Fig. 2, the decomposition velocity coefficient, ( $K$ ), temperature coefficient ( $Q_{10}$ ) and temperature constant ( $B$ ) were calculated in the same manner as in the case of Alaska pollack (*Theragra chalcogramma*) meat<sup>16)</sup>.

The results are shown in Tables 8 and 9.

Table 8. Decomposition velocity coefficient ( $K$ ) of abalone meat at various temperatures

Abalone in shell		Eviscerated meat after removed from the shell	
Storing temp. (°C)	$K = k \times 10^3$	Storing temp. (°C)	$K = k \times 10^3$
35	136	20	52
20	121	5	17
9	13	—	—

Table 9. Values of temperature coefficient, ( $Q_{10}$ ), and temperature constant ( $B$ ) of abalone meat

Abalone in shell			Eviscerated meat after removed from the shell		
Storing temp. (°C)	$Q_{10}$	$B$	Storing temp. (°C)	$Q_{10}$	$B$
5~20	4.79	24,000	5~20	4.82	24,000
20~35	1.10	1,390			

As seen in Table 8, except in the case of leaving temperature of 5°C, the higher the leaving temperature, the larger the value of " $K$ ". The value of " $K$ " of abalone meat in shell was larger than that of viscerated meat removed from the shell. This may be due to the fact that decomposition of the meat in shell was rapid owing to the digestive enzymatic action after death in the shell. At 5°C leaving temperature, the abalone in shell may perhaps be alive for a rather long time. In order to keep fresh, abalone must be stored at low tem-

perature at which it keeps alive.

The value of "K" of abalone at various temperatures is compared with those of other marine creatures obtained by Tanikawa<sup>4, 17-20</sup>) as shown in Table 10.

Table 10. Values of "K" in abalone meat compared with those of other marine creatures ( $K \times 10^3$ )

Species	5°C	15°C	20°C	25°C	35°C
Sea cucumber <sup>4)</sup>		17		28	29
Atka mackerel <sup>17)</sup>		22		36	61
Crab <sup>18)</sup>		22		74	91
Mackerel <sup>19)</sup>		30		99	114
Squid <sup>20)</sup>				103	151
Abalone in shell	13		121		136
Eviscerated abalone meat after removed from the shell	17		52		

As seen in Table 10, the raw abalone meat is easily decomposable at comparatively high temperature after death.

The values of " $Q_{10}$ " and " $B$ " of abalone meat at 20° or 25°C in comparison with those of other marine creatures are shown in Table 11.

Table 11. Values of " $Q_{10}$ " and " $B$ " of abalone meat and those of other fish meat

Species	Temp. range	$Q_{10}$	$B$
Atka mackerel <sup>17)</sup>	{ above 25°C	1.5	$8 \times 10^3$
	{ below 25°C	3.5	$21 \times 10^3$
Mackerel <sup>19)</sup>	{ above 25°C	1.2	$3 \times 10^3$
	{ below 25°C	3.6	$22 \times 10^3$
Abalone in shell	{ above 20°C	1.1	$1 \times 10^3$
	{ below 20°C	4.8	$24 \times 10^3$
Eviscerated abalone meat after removed from the shell	below 20°C	4.8	$24 \times 10^3$

As seen in Table 11, at comparatively high storing temperature raw abalone meat is decomposable, so it must be kept alive at about 5°C in order to keep it fresh.

### 3. Calculation of initial decomposition velocity coefficient

As it is important to know the initial decomposing coefficient, the authors have determined the relation between the storage time and amount of V.B.-N produced until that amount reaches to 20 mg% at which the putrefactive odour

begins to be noticeable and the pH value increases rapidly, as seen in Tables 3~7.

The coefficient of the velocity of incipient putrefaction, "P", was calculated in the same manner as in the case of Alaska pollack (*Theragra chalcogramma*) meat.<sup>16)</sup>

The values of "P" obtained are compared with these from other fish meat<sup>20,21)</sup> as shown in Table 12.

Table 12. Values of coefficient of the velocity of incipient putrefaction "P" of abalone meat compared with corresponding values of other fish meat

Temp. (°C)	Species	Autumn squid meat <sup>20)</sup>	Salmon <sup>21)</sup>	Abalone	
				in shell	Eviscerated abalone after removed from the shell
35		0.082	0.367	0.040	—
25		0.023	0.023	—	—
20		—	—	0.027	0.029
10		0.003	0.013	—	—
5		—	—	0.002	0.002

As seen in Table 12, at temperature lower than 20°C, raw abalone meat is less decomposable than other raw fish meat in the initial stage of storing.

#### 4. Decomposition products during the leaving of abalone meat

Various kinds of decomposed products will be produced in the decomposition of raw abalone meat. The authors have employed the amount of V.B.-N as an index of the degree of decomposition. Here are described the efforts to detect the kinds of decomposed products by means of paper chromatography at various stages of decomposition, at which the amounts of V.B.-N were estimated.

As sample use was made of raw abalone meat viscerated after removal from the shell. To detect the kinds of volatile basic nitrogen by paper chromatography, 30 g of the crushed raw abalone meat was steam-distilled in 5 cc of 10% NaOH solution. The distillate was collected in 5 cc of 1 N HCl solution. Then about 50 cc of the distillate was obtained; the distillate was evaporated in a dish on a water bath. The remainder substance was dissolved with 0.5 cc of water. This solution was employed as a sample for paper chromatography.

As a developing solvent, water was added to a mixture of benzyl alcohol and glacial acetic acid (3:1) until the mixture became turbid. The mixture was shaken before use. The method of development was by one-dimensional chromatography. As revealing agent 0.1% ninhydrin-butanol solution was used.

To detect histamine, 10 g of the crushed raw meat of abalone was made

acidic (below pH 3.0) by addition of acetic acid, and the meat was left for a short time after the addition of 50 cc of 95% alcohol; then the mixture was centrifuged. The upper transparent liquor was evaporated on a water-bath. The condensed substance was dissolved with a small quantity of water to make the volume to about 1 cc. This solution was used as a sample for paper chromatography.

As a developing solvent butanol solution saturated with 1 N ammonia solution was employed. As a revealing reagent a mixture of dilute hydrochloric acid solution containing 0.3% sulfanilic acid and 5% sodium nitrite solution (1:1) was used. This revealing reagent was sprayed on spots in the filter paper after which 1 N NaOH solution was sprayed.

To detect volatile organic acids, 30 g of the crushed raw abalone meat was steam-distilled with 50 cc of water containing 5 cc of conc. sulfuric acid. About 300 cc of distillate was extracted with ether for 18 hrs., then the ether was evaporated to make the solution about 1 cc. Thirty cc of absolute alcohol and 7.5 cc of conc.  $H_2SO_4$  were added to the condensed solution to esterify at  $140^\circ C$  for 2 hrs. The not completely esterified solution was neutralized with sodium carbonate. The completely esterified solution was distilled at  $70^\circ C$ , to make potassium hydroxamate with hydroxylamine HCl-salt. This solution was employed as a sample. As the developing solvent n-butanol solution saturated with water was used. As revealing reagent use was made of methanol solution saturated with  $FeCl_3$ .

Results obtained are shown in Table 13, and Fig. 3. As seen in Fig. 3,

Table 13. Organic acids and bases detected at various leaving time (hrs.)

Leaving time (hrs.)	V. B.-N (mg%)	Organic bases detected
13	5.74	Tyrosine, Histidine
16	6.02	Tyrosine, Histidine
19	14.78	Tyrosine, Histidine, Agmatine, Cadaverine, Putrescine, Formic acid
22	17.35	Tyrosine, Agmatine, Cadaverine, Putrescine, Formic acid
30	34.80	Tyrosine, Agmatine, Iso-amylamine, Formic acid, Acetic acid
36	44.24	Tyrosine, Histamine

when the amount of V.B.-N reached to 14.78 mg% after the abalone meat had been left for 19 hours, agmatine, cadaverine, putrescine, formic acid were detected. Further more, when the amount of V.B.-N reached to 17.35

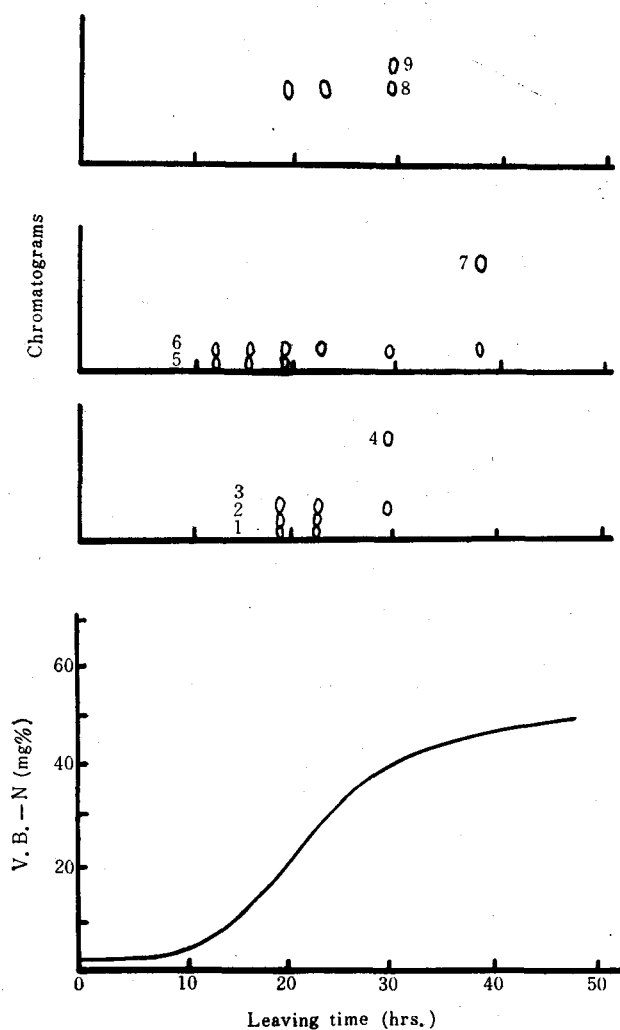


Fig. 3. Relation between the kinds of organic acids and bases detected and the leaving times of eviscerated abalone meat (20°C)

- |                  |                |                |
|------------------|----------------|----------------|
| 1. Putrescine    | 2. Cadaverine  | 3. Agmatine    |
| 4. Iso-amylamine | 5. Histidine   | 6. Tyrosine    |
| 7. Histamine     | 8. Formic acid | 9. Acetic acid |

mg% after the meat had been left for 22 hours, the same kinds of organic acids and bases were detected. In the same way, at 34.80 mg% of V.B.-N among the organic bases cadaverine and putrescine disappeared, they may have decomposed by bacterial action. After 36 hours' leaving, histamine was detected besides the organic bases above mentioned.

When the amount of V.B.-N reached to 15~20 mg%, the putrefactive odour began to smell slightly.

## VII. SUMMARY

From the results obtained by chemical studies of abalone meat, including those reported in the previous paper I<sup>12)</sup>, the authors wish to summarize their conclusions as follows:

(1) Seasonal changes of general chemical composition of abalone meat were studied. The amounts of protein, fat and glycogen were maximum just before the spawning season of July to August. On the contrary, the amounts of water content and ash were then minimum. Those changes were almost same as are found in other fish meats.

(2) Distribution of nitrogen in raw abalone meat and in hot water extractive was studied. From the results obtained, within the total content of nitrogen 57% was estimated to be mono amino acids, whilst 35% was diamino acids. The amount of diamino acids in raw abalone meat was larger than that of other fish meat. This may be concerned with the taste of the abalone meat.

(3) From the histological observation of raw abalone meat, it was noted to be different from that of fish meat. In fish meat, many muscle bundles are situated in parallel, and between the separate muscle bundles there is connective tissue. But the connective tissue of abalone is found only at the part of epithelial tissue, while the muscle fibres run horizontally and vertically in all directions. Those muscle fibres connect with epithelial tissue at their ends.

(4) The amount of water soluble protein of abalone meat was 22% of the total amount of protein. The solubilities of abalone meat with acid solution, alkaline solution and various salts solutions were observed. In general, the solubilities by acid solutions differ from those by hydrochloric and sulfuric acids, and the solubility by alkaline solution increased with the increase of the concentration.

(5) The isoelectric point of water soluble protein of the abalone meat was pH 4.8~5.2, and that of NaCl solution soluble protein was pH 5.2. The reason why the isoelectric point has some range is the fact that the protein does not have a single components, but consists of several components.

(6) It was observed that the phenomenon of the flow birefringence in the water-extractives of abalone meat does not occur in the first extraction, but several extractions later. The phenomenon does not appear after the filtration of the extractives and furthermore also the phenomenon disappears after the

heating of the extractive above 65°C. As histologically observed, the muscle fibres of the abalone meat are fine and arranged like net-work. So the appearance of the pattern of flow birefringence in the extraction of abalone meat is considered to be due to the mechanical deformation of muscle fibre, by which the fibre flakes will be dispersed in the extractive showing the pattern of flow birefringence.

(7) When abalone meat is soaked in water at various temperatures, the ratio of dehydrating of abalone meat decreased largely at higher temperatures like to that of sea cucumber meat. Accordingly it is assumed that the muscle fibre of abalone meat takes the form of a net-work construction like that of sea cucumber meat.

(8) The heat coagulation of the extractives of abalone meat with water or 0.5 N NaCl solution was observed. According to the results obtained, the heat coagulation of water-soluble protein was completed within two temperature ranges from 45° to 50°C and from 60° to 65°C. The main component of the coagulable protein in this case is considered to be myogen.

The protein which is coagulated at the lower temperature (40°~55°C) is actually myosin, while that at the higher temperature (60°~70°C) is considered to be myogen. The presence of two kinds of proteins was also observed in the heat coagulation curve of 0.5 N NaCl solution-soluble protein and in the dehydrating curve.

(9) The degrees of swelling of the abalone meat in the solution of salts are different according to the concentration of the solutions, similarly those in solutions having various pH values are different according to the pH value. Generally speaking, the degrees of swelling of the abalone meat are less than those of fish meats such as Alaska pollack or Atka mackerel. This is due to the histological characteristics of the abalone meat muscle which shows a net-work construction of muscle fibres. This net-work construction of the muscle tissue prevents penetration of water into muscle tissue resulting in difficulty of hydration.

(10) As to the hydration of the abalone meat, the water-content ( $g$ )—relative vapour pressure ( $p/p_0$ ) curve in the fresh raw meat was determined. According to the results obtained, the hydrating properties of abalone meat protein are not remarkably different from those of other marine creatures, even though the amount of the protein differs from that of others.

(11) Mucoprotein was fractionated, and the amount obtained was 0.54%. The prosthetic part of the carbohydrate to the mucoprotein was proven to be N-acetyl glucosamine, fructose, galactose, glucuronic acid and fucose by paper



chromatography. The nitrogen distribution was estimated to be the same as that of the raw meat of abalone.

(12) The decomposition of abalone meat will advance at comparatively high temperatures, but at about 5°C the decomposition velocity was small; especially it is noteworthy that abalone in shell is alive for a long time.

The putrefactive odour begins to be noticeable at 15~20 mg% of V.B.-N on the way to decomposition. Therefore, abalone must be preserved at low temperature before the processing if it is desired to have live material.

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