FEEDING AND DIGESTIVE PROCESSES OF *STICHOPUS JAPONICUS*

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I. Introduction

In the annual changes of the feeding activity Tokuhisa (1915) reported that *Stichopus japonicus* has some period under estivation when it ceases feeding after spawning; at that time the intestines are observed to be fill with sand or mud (which is abridged together as food below) excluding the above period, especially showing maximum feeding activity in February and March. Yamanouchi (1941, 1942, 1956) stated, in the daily rhythm of the feeding activity, that genera Holothurian and Stichopus are classified into the following three groups according to their feeding habits on the basis of anatomical observations on materials collected from the coral reef of Palao Island and in Mie Prefecture.

1) Group 1 takes food actively day and night.

2) Group 2 gives up feeding at night, and burrows into the ground,

3) In the 3rd group, the animal hides itself under the marine plants or the stones instead of burrowing into the sand or the mud while fasting.

Clark (1954) noted that holothurian and other bottom animals generally extract organic matter out of sand or mud which pass through their intestines. In the holothurians, Tokuhisa (1915) commented that they extract organic matter out of sand or mud taken together. Hunt (1925) stated that they digest the plankton and organic deposits contained in sand or mud. In the digestion of the intestine, several digestive enzymes were examined quantitatively on *Caudina chilensis* by Sawano (1927). So far as the present author is aware, however, there are few studies concerning the mechanism of the digestion of organic matter taken as food by those animals.

As to the feeding by holothurians, as noted above, there are many items of information along the lines of ecology and physiology, but no one has examined the whole process of digestion and absorption of the food taken from bottom materials.

Accordingly, it may be necessary for the understanding of their feeding habits to study from ecological view points the seasonal changes in the amounts of food taken by the holothurians, and the dynamic processes employed in the nutrition absorption by their intestines for transmission to their tissues and in the excretion of non-digested organic material.

The present study is designed to clarify systematically the above mentioned processes of *Stichopus japonicus*. Reported herein are the results obtained by comparison of the feeding amounts in December 1956 with those in July and October 1957, by examining the decrease of the nutritive matter, especially of nitrogen contents in their intestinal food caused by absorption, and by investigation nitrogen contents in digestive juice. At same time the variations of the components of their body fluids are predictively reported which their body fluids might intervene between their intestines and tissues during the absorption. This study was carried out at Usu Bay in Hokkaido.

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II. Process of digestion

It is convenient to keep animals under fasting condition in the examination of their digestive process, which indicates the removal of the food in their intestines. Therefore, in the present work animals were observed in December 1956, July and Octobr 1957 under the above condition; in December, July and October the diminution in the quantities of food in intestines was studied; in July the change in amounts of nitrogen content and non protein nitrogen (NPN) in intestinal juice was observed, and in December study was made of the decrease in nitrogen content by the digestion of the food taken, with the progress of time.

(*a*) *Time of digestion*

The material used consisting of 166 specimens were collected from the entrance of Usu Bay by a landing net. Animals were removed immediately to a box like a fish preserve and then the observation was started. Their excrements fell out of the box and the animals were kept so as not to be able to find their food. After that the animals are taken out at regular intervals to measure total body weight and total wet weight of the intestinal food. The feeding amounts were converted into the intestinal food weight per total body weight as this is the most convenient unit to show the relative food amounts, and observations were continued for 30 hours in December, 165 hours in July and 45 hours in October.

As shown in Fig. 1, no difference in the average feeding amounts was found between December and July, viz., 4.4 and 4.6% respectively. The time of digestion, the total hours used in the excretion of entire intestinal food required about 30 hours both in July and December. However, the feeding amounts in October decreased 2%, and in general the feeding amounts in October were clearly different from those in July and December. The intestines of animals used in October were smaller in size being more shrunken than those used in July and December. Moreover in a few specimens it was impossible to find them because of their degeneration.

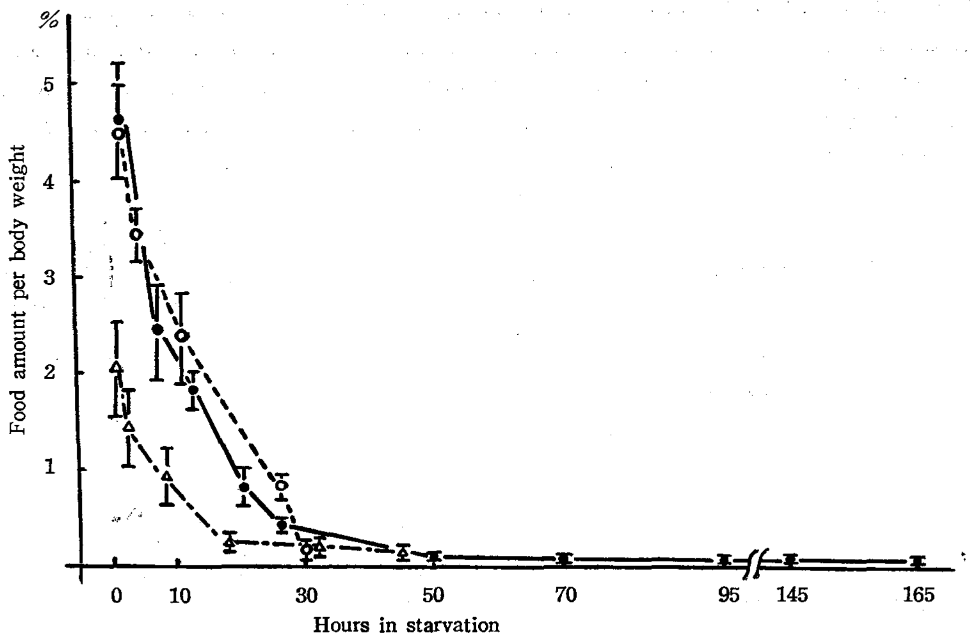

Fig. 1. Decreases in the intestinal food of ***Stichopus japonicus*** under starvation
The feeding amounts of the animal collected from field are shown at the starting point. Vertical lines indicate the standard error of the mean. The symbols indicatete as follow: ○-------○ Experiment in December, ●———● Experiment in July, △— - —△ Experiment in October.

It seems possible, therefore, that the feeding activity in October is sluggish in comparison with that of July and December. The time of digestion was also different from the results obtained in July and December decreasing to about 0.25% during 18 hours. This difference seems to be due to the length of their intestines becoming smaller by degeneration, and, in addition, to the influence of their intestinal contents. In the July observation, extending over 165 hours, most intestines of animals still held a little food, the average amounts never showing zero per cent in 165 hours throughout the examination period.

Tokuhisa (1915) reported that animals pass some periods under an abnormal condition,* estivation, during which their intestines degenerate owing to starvation after spawning, and that it is impossible to find their intestines during this period, which becomes shorter in northern regins, and in Hokkaido extending for two weeks in July. Kinosita (1939) stated, however, that he cannot perceive such condition as notes above in his own observation in Hokkaido from May to September and he is positive in denying it. Fig. 1 indicates that amounts of food decrease distinctly in October, and the intestines

*) This word denotes the same meaning with 'estivation'

become smaller in the process of degeneration or some other unrecognizable process. Therefore, it is clear that the animals are under estivated condition in early October.

Tokuhisa (1915) mentioned that the estivation begins in the water at over 16°C. No animals, however, were under such condition in the present observations in July, the water temperature showing about 17°–19°C in the process of becoming warmer. In October when the water temperature showed 16°–18°C which trended to come down, they were under estivated condition. Thus it may be suggested that the estivation is indirectly influenced by the water temperature which successively effects the physiological activities controlled by water temperature, as Yamanouchi (1942) noted.

(*b*) *Intestinal juice*

Those observations were made simultaneously with the above experiment. The intestinal juice was sufficiently extracted from the small intestines, but it was hardly possible to take it out from the large intestines. NPN in the intestinal juice was separeted with the addition of a 10% trichloroacetic acid. Total nitrogen contents and NPN are measured by Kjeldahl method and photo-electric colorimeter. In the experiment in December estimation was made of the percentage of NPN per total nitrogen contents, and progressive decrease in total nitrogen was examined in July.

Fig. 2 summarizing the results shows that percentage of NPN was 58% in July at the beginning of the present experiment; it gradually increased for 12 hours and thereafter declined more gradually. NPN was contained in the amount of 75% of the total nitrogen after 165 hours of experiment in July. The fluctuations show the identical tendencies, though the experimental terms were different, in December and July. The amounts of nitrogen in intestinal juice measured 71 mg per cent at the beginning of this experiment, but after 50 hours they showed 34 mg per cent. It clearly depends upon the extraction from the food through digestion that such amounts of nitrogen are contained in the intestinal juice from the beginning to the end of the 50 hours period (Fig 2). Therefore it is evidently due to the digestion that the percentage of NPN increases from beginning of the present observation to 12 hours in spite of decreasing the amounts of nitrogen intake. This observation clearly demonstrates that the digestion is active for 12 hours and not after then, as total nitrogen and NPN both decrease during the 12 to 50 hours period. After 50 hours the nitrogen and NPN keep almost at the same amount of about 35 mg per cent and 70 per cent respectively. Accordingly, it may be presumed that those nitrogen contents are not included in the foods but are body components.

From Fig. 2 it is understood that the average pH values intestinal juice, when food is seen in the small intestines, turn from 6.1 in the beginning of the experiment to 5.6 within 12 hours. After that no food is found in them, and at 50 hours the values increase to 5.9; afterwards they keep the same value.

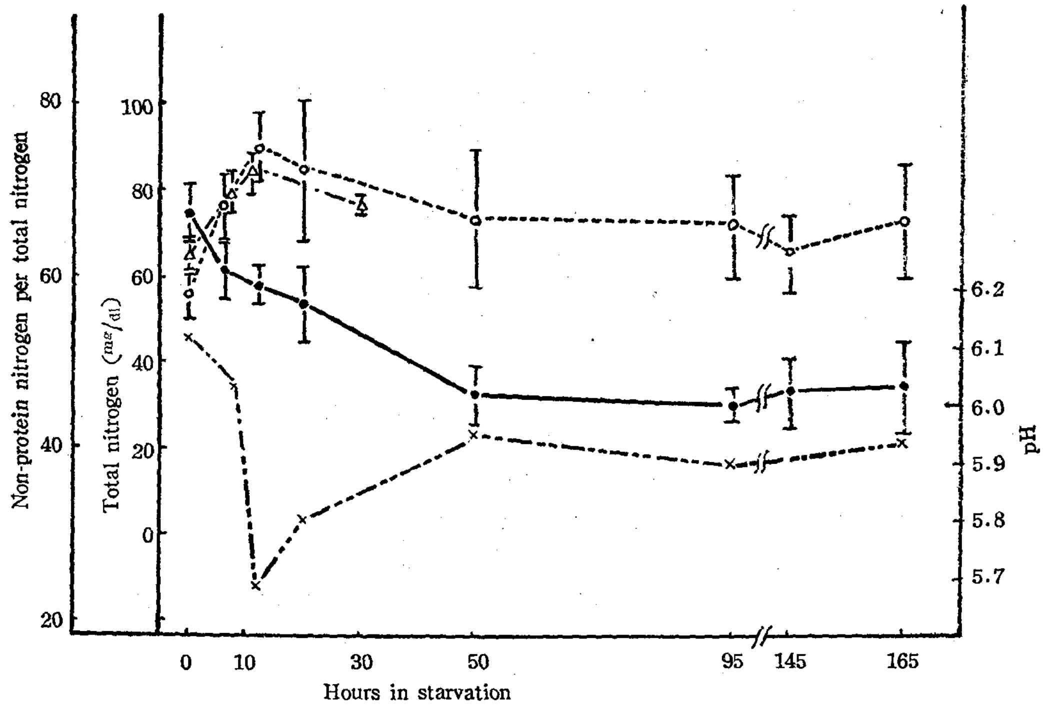

Fig. 2. Changes of components in the intestinal juice of *Stichopus japonicus* kept under starvation
Each bar indicates the standard error. The symbols indicate as follow:
●———● Total nitrogen, ○------○ Percentage of non protein nitrogen in Jnly,
△———△ Percentage of non protein nitrogen in December,
×------× Average pH value.

Sawano (1927) reported of *Coudina chilensis* that pH values of the intestinal juice are between pH 7.3 and 7.7 by colorimetric estimation. On the other hand, in eight holothurian species Yamanouchi (1941) reported that those values range from 5.5 to 6.7 when the creatures' intestines are filled with food and from 5.0 to 6.2 when empty. This wide difference between the values obtained by the present author and those by Sawano may be ascribed to the different species used as material.

Although the values indicate the results obtained by observations, it is assumed that change in the values may be due to the intensive activity of digestion which varys with amounts of the food in the intestines. Accordingly pH values may be conceivable as one of the effective means for the estimation of the degree of digestion.

(*c*) *Changes of nitrogen contents in the food during period from feeding to excretion*

Although the feeding habits of *Stichopus japonicus* have been observed by many researchers, they merely reported that the creature's nutrition depends upon organic materials and plankton. So the present author examined total nitrogen contents of food,

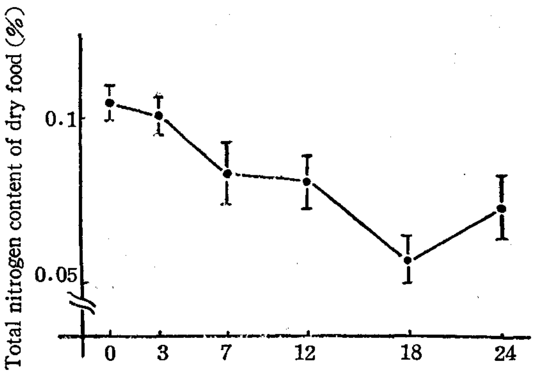

Fig. 3. Changes of nitrogen contents in food in process of digestion
Vertical lines represent the standard error.

strictly speaking, of the sand in gullet and one near anus in the course of digestion and absorption. The animals examined in December numbered 69 specimens. Their intestines are removed from body cavity after dissection. About 0.2 gr of samples in wet weight are taken out of the food at the part nearest the mouth in their intestines, as the food in gullet moves towards anus in entire shape. Total nitrogen contents in the food were measued by kjeldahl method after drying; the results of analysis are presented as percentage in dry materials.

As shown in Fig. 3, average total nitrogen included in food is 0.11% at the beginning of this observation; it decreases to 0.06% in 18 hours. It is evident that this diminution of nitrogen is due to absorption. Total nitrogen, however, shows 0.07% after 24 hours, and this increment may not be caused by digestion and absorption but may be ascribable to the minority of animals, because a few of them have not food in their intestines.

Total nitrogen measured, as already stated, at 0.11% in the food in their gullet when collected in the field, whereas bottom materials in collecting place included 0.03% in nitrogen. This difference is attributed to the fact that the animals take the sand in which much organic materials are included, being piled over the upper surface of bottom. A test performed in Mutsu Bay showed essentially similar outcome to the above description.

III. Process of feeding

The process of feeding of this animal was studied in specimens which were fed after starvation of 165 hours in July and after 45 hours in October, respectively. The very same animals were used both at the beginning of the observation (Figs. 4,5) and at the end of starvation (Figs. 1,2). In July and October the changes in quantity of the food in their intestines were examined; also, in July, the increases in total nitrogen and NPN in intestinal juice were observed.

(*a*) *Time of feeding*

The starved animals were put for feeding in the wirecages of 70 × 70 × 20 cm, settled so as to push into bottom sand, so that the cages were always one quarter full of sand. The animals were observed at regular intervals and feeding amounts were estimated by the same method as that stated above.

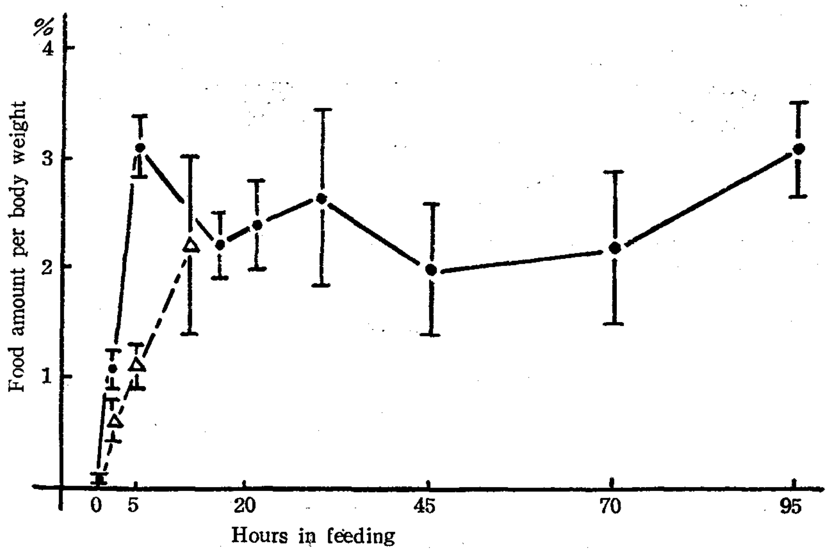

Fig. 4. Increases of the intestinal food of *Stichopus japonicus* upon feeding after starvation
Each symbol and vertical bar is the same as those in Fig. 1.

In July amounts of food taken increase by about 3% in 5 hours as shown in Fig. 4 and the intestines of all the animals were filled with food. However, no food was yet found near the anus in their intestines for 1.5 hours from the beginning of the observation. They were filled likewisely with food after 5 hours. On the other hand, in October the feeding amounts augment by 0.1% after 1.5 hours, 1.1% after 5 hours and 2.1% after 12 hours respectively from the beginning of the observation; after 5 hours a few animals have food in their intestines, while the intestines observed in 12 hours are replete with food. Therefore length of time of feeding differs to some extent between July and October. This difference may reasonable be attributed to the fact that feeding activity is normal in July but is not in October (Fig. 1), the latter owing to the degeneration of their intestines on account of having given up feeding.

In July the average feeding amounts show 4.6% when the animals are collected from the field, but they show about 3% when they are fed after starvation. In October the average shows about 2% both in feeding quantity of the collected animals and in those after starvation (Figs. 1,4). The diminution in July may be caused by artificial starvation, for in October feeding ability may not be influenced by the starvation for 45 hours, but is influenced by for 165 hours' starvation in July.

As understood above, the estivating animals are under an abnormal condition in which their intestines degenerate on account of giving up feeding for some unknown reason. The factors inducing estivation and artificial starvation employed in this study differ from each other. Long starvation makes the intestine capacity smaller, so the feeding amounts after starvation are smaller than those before it (Fig 3).

(*b*) *Intestinal juice*

The material and the technique used in this observation are exactly the same as described in (I).

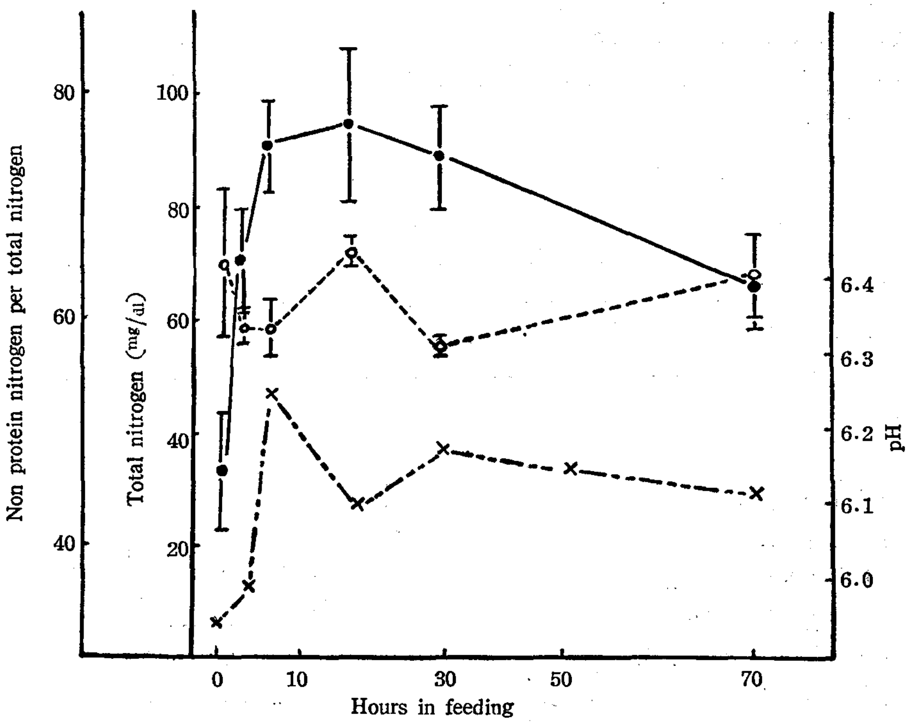

Fig. 5. Changes of components in the intestinal juice of *Stichopus japonicus* when fed after starvation
Each symbol and vertical bar is the same as those in Fig. 2.

As shown in Fig. 5, total nitrogen rose very rapidly to 90 mg per cent in 5 hours and attained to maximum of 94 mg per cent in 16 hours. It is clear that the nitrogen increased because it had been removed from the food by the digestion. The absolute nitrogen amounts removed out of the food, that is, the true nutritive element, are higher in quantities than those values shown in Fig. 5, for some nutrition is absorbed into the intestine.

The increasing nitrogen in the juice for 16 hour period indicates that the amount removed from the food is higher in qantity than that absorbed, and the latter gradually becomes higher between 16 and 70 hours. At 70 hours it comes down to the normal degree as observed in collecting time from field (Figs. 2,5).

The percentage of NPN attains to a maximum of 73% in 16 hours. It is easily known that this high percentage is due to the active digestion when much nitrogen is contained in intestinal juice. Decline of percentage from 16 hours to 30 hours does not occur in the least, but because of the dullness of digestion, or otherwise that percentage is influenced by the absorption of NPN.

Average pH value augments to 6.2 in 5 hours and holds a constant level of about 6.1 after 16 hours, being essentially similar to pH value in animals collected from the field (Figs. 2,5). There can be seen a considerable correspondence between the range of pH values treated in part (I) and (II) by the present author and that shown by Yamanouchi (1941).

IV. Sugar and non protein nitrogen in the body fluid

This study was made in July in conjunction with the above investigation examined and the animals used numbered 105. The fluid was allowed to stain and the clot, containing wandering blood cells, was removed by filtration. NPN in the coagulum-free filtration of body fluid was determined by Kjeldahl method, the proteins being first precipitated with trichloracetic acid. Reducing sugar in the filtration was determined by Somogi method.

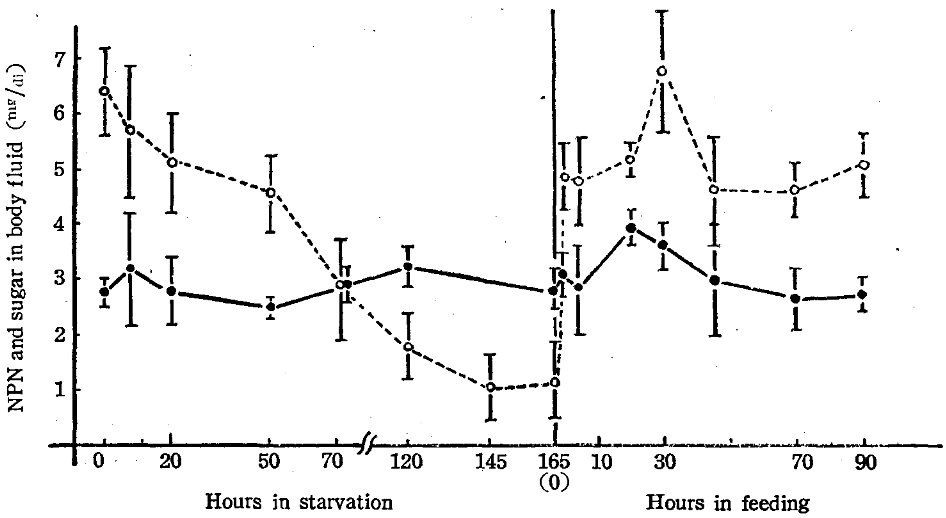

Fig. 6. Changes in the amounts of sugar and NPN in the body fluid of *Stichopus japonicus* in the processes of starvation and feeding
Vertical lines cover the range of the standard error of the mean. The symbols indicate follws: ○---------○ Sugar, ●———● Non protein nitrogen.

As shown in Fig. 6, sugar and NPN in the body fluid, when the animals are collected from the field, are usually present in the body fluid of *Stichopus japonicus* to the extent of about 6.4 mg per cent and 2.8 mg per cent respectively, while the average amounts of sugar are gradually decreased to 1.2 mg per cent after 165 hours of starvation. NPN, however, does not change markedly in either full or empty intestines. Sugar in the fluid rises very rapidly to the maximum of 6.8 mg per cent after 30 hours from the beginning of the feeding after starvation; after 45 hours it comes down to about the same level as observed in collecting time from the field. NPN does not increase conspicuously for the duration of the above experimental period, but it is observed to increase slightly in 16 hours.

Lasker and Giese (1954) reported that no seasonal variation of sugar and NPN are recognized in body fluid of sea urchins under normal condition, and that those constituents increase remarkably upon feeding with special food after starvation; similarly NPN and sugar show like tendency under starvation in *Stichopus japonicus*. Lasker and Giese interpret their results as meaning that nitrogen compound stored in sea urchins gonad must be liberated upon starvation to maintain the supply of soluble NPN and therefore the gonad is almost completely resorbed.

As noted above, a considerable correspondence is seen between the results of this study and those by Lasker and Giese, though increase in rate of NPN shows discrepancy between the two result. This difference may be ascribed to the fact that sea urchins are fed upon a high protein diet.

Although the nitrogen compounds in sea urchins are stored in their gonad, the animals examined in the present study are mostly in spent condition as shown by their having very small gonads. It may be shown, therefore, that NPN is kept at constant level under starvation owing to the liberation from body tissues, not from the gonads different to the case in the sea urchins.

In Fig. 6, constant amounts of NPN and decreased sugar suggest that nitrogen compounds stored in tissues are liberated to supply soluble NPN, but that no sugar is liberated for storage as kinetric energy in tissues.

V. Discussion

Yamanouchi (1941, 1956) mentioned that holothurians in Palao Island can be divided into three groups according to their feeding habit. If this categorization is applicable to Hokkaido animals then it is dangerous to deal with such a thing as satiation in feeding condition of animals collected from field. Yamanouchi (1942) further stated, having studied *Stichopus japonicus* in Mie Prefecture, that it takes food uninterruptedly all day long and its intestine is always filled with food. Accordingly the animals used in the study of the present author is not collected at any special time on the supposition that it is always under satiation. For all intestines of the animals used in each observation are seen to be filled with food.

Time of digestion in *Stichopus japonicus*, until their intestines become empty, is reported to be 12 hours by Isono (1925) and to be 24 hours by Yamanouchi (1929). From Fig. 1 it is clear that the time of digestion is over 30 hours in December and July, and that it is 25 hours under abnormal feeding conditions in October.

As for the time of feeding, Yamanouchi (1941) reported in holothurians of Palao that the intestine of *Holothuria atra* is filled in 4–5 hours, of *H. edulis* in 5 hours, of *H. scabra* in 2–2.5 hours, of *H. vitiensis* in 3–4 hours, of *H. bivittata* in 3–5 hours, *Stichopus rariegatus* in 2–3 hours. Consequently, the intestines of the above species are filled with the food in 2–5 hours. Further he continues that the intestin of *Caudina chilensis* from Asamushi is filled with the food in 2 hours. Though a wide difference can be seen between duration of digestion and of feeding, that difference may be ascribed to the feeding conditions under which the animal in question gives up feeding and takes food occasionally rather than to some internal factor, e.g., digestion.

As to the effect of temperature upon the time of digestion, many investigators have already pointed out that the digestion takes place slowly under comparatively lower temperatures in vertebrata. It is clearly demonstrated, however, that digestion in *Stichopus japonicus* takes place to the same degree in two different temperatures, under either 16°C–18°C in July or 2°C–5°C in December (Fig. 1). Therefore, the digestion is not effected in the least by the temperature within the range as shown above except for the abnormal feeding condition in October. It is reasonable to suggest that the effect of temperature upon digestion is comparatively weak in this animal.

As noted above, the animal must move widely on the bottom surface in order to obtain sufficiency of organic matter. If seasonal changes in movement are provoked, there must be seen some change in the amount of organic materials absorbed. That change may exert effect on the creature's growth.

In this study it is shown clearly that the animal spends some period under estivation, and yet that period cannot be determined because no examination of feeding amounts for successive long period has been made. The behavior of the animal under this condition may be evidently explained by the seasonal changes in its physiological activities, and at the same time the cause leading to the estivation may be found out. Accordingly, the metabolism of this animal must be a author subject for further reseach.

The sugar in body fluid rapidly exerts a influence upon the digestion and absorption of food in the intestines; this effect may be interpreted to be due to the following two causes. The body fluid of the animal contains blood corpuscles (Kawamoto 1927). The fluid and the blood have a chance to mix with each other (Yazaki 1930). In holothurians have a indistinct boundary between a blood-vessel and body cavity, thus the fluid which varies in amounts in a short time may intervene between blood-vessel and tissues during absorption of nutriment. On the other hand, if the nutriments remove directly to tissues from blood-vessels, the change in amounts of sugar content shown in Fig. 6 may indicate less amounts than those practically removed to their tissues, for the blood-vessels of this animal are open.

VI. Summary

This study was executed using *Stichopus japonicus* kept under starvation and under feeding after starvation in order to observe the feeding and digestive processes, and constituent of body fluid. The place was Usu Bay in Hokkaido, and the dates December 1956 and July and October 1957. The results obtained may be summarized as follows;

(1) The average feeding amounts in December and in July are 4.4 and 4.6% of the body weight respectively. Time of digestion in both periods is about 30 hours. Time of feeding varies from 1.5 to 5 hours; no difference can be seen between above two data obtained in December and those in July. The feeding amounts taken in October, differing from those in December and July, show 2.1%; some of the animals give up feeding owing to degeneration of intestines. Estivating animals to be found in Hokkaido.

(2) No digestion and absorption in their intestines is observed after 50 hours of starvation. Intestinal juice is always contained to the amounts of about 30 mg per cent of total nitrogen of body components.

(3) Total nitrogen of food lying near gullet and anus is varied from 0.11 to 0.06% respectively by digestion and absorption.

(4) The food in the gullet contains as much nitrogen as 3-5 times the amount of that contained in the bottom materials near which *Stichopus japonicus* lives.

(5) The average amount of sugar in body fluid comes to 6.4 per cent at the time of collection from field, and it decreases to 1.1 mg per cent after 165 hours of starvation. In the feeding after starvation it increases to 6.8 mg per cent. On the other hand, NPN shows constant level of about 3 mg per cent under both starvation and feeding.

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Appendices Full data showing variation of food amount, and of chemical components in intestinal juice and in body fluid

1. Experiment in July

Treatment	Hours	Number of individuals	Range of body weight gr	Feeding amounts Range %	Feeding amounts Average %	NPN/Total N in intestinal juice Range %	NPN/Total N in intestinal juice Average %	Total N in intestinal juice Range mg/dl	Total N in intestinal juice Average mg/dl	pH Range	pH Average	Sugar in body fluid Range mg/dl	Sugar in body fluid Average mg/dl	NPN in body fluid Range mg/dl	NPN in body fluid Average mg/dl
Starvation	0	9	111.1-215.8	2.9-7.0	4.6	50.7-62.8	57.6	0.51-0.91	0.65	6.1-6.2	6.1	5.0-9.2	6.4	2.5-2.8	2.7
	6	6	134.9-278.4	1.4-3.8	2.4	67.5-68.0	68.8	0.49-0.91	0.61	5.9-6.2	6.0	3.7-8.0	5.7	1.7-3.4	3.2
	12	5	135.1-186.1	1.2-2.2	1.8	67.6-80.2	75.1	0.48-0.58	0.54	5.6-5.8	5.6	3.7-6.7	4.8	——	——
	20	7	141.5-164.0	0.3-1.1	0.8	55.9-81.6	72.1	0.41-0.77	0.54	5.6-6.1	5.8	2.5-6.7	5.1	1.5-3.8	2.8
	26	5	100.5-190.6	0 -0.4	0.2	——	——	——	——	5.8-6.0	5.9	2.5-6.7	5.0	——	——
	50	5	71.4-206.2	0 -0.5	0.1	57.9-79.5	66.6	0.16-0.59	0.33	5.9-6.0	5.9	3.0-6.2	4.2	2.2-2.7	2.5
	70	5	107.6-219.4	0 -0.2	0.05	——	——	——	——	5.7-5.9	5.8	2.5-5.5	2.7	2.2-3.9	2.9
	95	5	72.2-127.6	0 -0.2	0.06	56.1-74.1	67.6	0.21-0.41	0.31	5.9-6.1	5.9	0 -5.5	2.3	——	——
	145	5	96.5-139.2	0 -0.5	0.1	——	——	——	——	5.7-6.1	5.9	0 -3.0	1.1	——	——
	165	5	62.4-113.1	0 -0.1	0.04	59.2-88.0	73.6	0.28.0.51	0.36	5.8-6.2	5.9	0 -2.5	1.2	2.5-3.5	2.8
Feeding	1.5	7	97.8-227.2	0.9-1.7	1.1	54.8-74.3	62.1	0.43-0.80	0.71	5.9-6.2	6.0	5.0- 6.2	4.9	2.2-3.7	3.2
	5	5	88.5-141.4	1.9-4.2	3.0	52.5-64.9	58.7	0.81-1.0	0.91	6.2-6.3	6.2	2.5- 6.7	4.8	2.5-4.8	2.9
	16	6	73.6-146.2	1.1-3.4	2.2	69.5-78.9	73.9	0.68-1.33	0.94	5.9-6.2	6.1	3.7- 6.2	5.2	3.5-4.1	3.9
	30	5	61.1-108.6	1.4-4.5	2.9	55.7-56.2	56.0	0.64-1.11	0.89	6.1-6.2	6.2	5.0-10.0	6.8	3.0-4.1	3.6
	45	4	109.3-161.8	1.7-2.1	2.0	——	——	——	——	6.1-6.2	6.2	3.7- 5.0	4.6	2.3-3.7	3.0
	70	5	103.5-186.5	0.5-3.5	2.1	68.6-79.3	75.9	0.55-0.74	0.65	6.0-6.2	6.1	3.0- 6.2	4.6	1.2-4.1	2.7
	95	5	71.0-129.4	2.4-4.9	3.6	——	——	——	——	——	——	3.7- 6.2	5.1	2.2-3.2	2.7

2. Experiment in October

Treatment	Hours	Number of individuals	Range of body weight gr	Feeding amounts Range %	Feeding amounts Average %
Starvation	0	11	61.5–201.5	0 –4.3	2.0
	2	9	49.1–260.5	0 –3.4	1.4
	8	5	32.5–100.2	0.6–1.9	0.8
	18	5	35.5– 88.4	0 –0.6	0.2
	32	4	39.0– 97.6	0 –0.3	0.1
	45	5	54.0– 87.6	0 –0.2	0.1
Feeding	1.5	6	64.6–115.7	0 –1.4	0.6
	5	5	47.6–185.0	0 –2.5	1.0
	12	5	62.7–153.5	0.6–3.4	2.1

3. Experiment in December

Treatment	Hours	Number of individuals	Range of body weight gr	Feeding amounts Range %	Feeding amounts Average %	NPN/Total N in intestinal juice Range %	NPN/Total N in intestinal juice Average %
Starvation	0	19	65.8–227.9	2.5–3.7	4.4	60.0–74.8	68.3
	3	22	35.6–164.0	1.3–5.1	3.4	59.8–79.3	68.8
	10	10	61.7–247.6	1.2–3.4	2.1	67.9–87.0	76.8
	26	12	56.4–128.9	0.2–1.5	0.8	———	———
	30	7	32.6– 52.6	0 –0.6	0.2	69.5–77.3	73.3