STUDIES ON POST-MORTEM CHANGES IN THE CHEMICAL CONSTITUTION OF THE MEAT OF SEA CUCUMBER (*STICHOPUS JAPONICUS* SELENKA)*

I. Changes in the Amounts of Lactic Acid and Glycogen in the Meat of *Stichopus japonicus* during the Period of Rigor Mortis

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When *Stichopus japonicus* is processed into dried merchandise or canned food, it is very important that the raw material of *Stichopus japonicus* must be prepared before the freshness of the meat falls. However, owing to the conditions of fishing it may happen that the raw material of *Stichopus japonicus* can not be treated in fresh state. In this case, the post-mortem changes of *Stichopus japonicus* should be known.

There has been hitherto no published study on such a subject. As to the post-mortem changes of *Stichopus japonicus*, the authors have tried to investigate the changes in rigor mortis, autolysis and putrefaction of the so-called "meat."

The rigor mortis which hardens the muscle of animals is also found to occur in the body of *Stichopus japonicus*. Investigation has been made of the change in the amounts of lactic acid and glycogen in the meat of *Stichopus japonicus* during the period of rigor mortis.

Experimental

1. The change of the hardness of the meat of *Stichopus japonicus* after death

Tauchi[1] tried to judge the freshness of fish meat by his hardometer on the basis of the fact that the fish body gradually becomes soft after the rigor mortis. But his hardometer has a defect of yielding various data even on the same part of the fish body. Tanikawa & Konno[2] have previously tried to judge the freshness of squid meat using Konno's fatiguesmeter of muscles of sportsmen and they succeeded in determination of the freshness.

The present authors have also tried to judge the freshness of the meat of *Stichopus japonicus* by the same instrument in this experiment.

(1) Sample

Living bodies of *Stichopus japonicus* which were caught in the sea near Hakodate were eviscerated and left at room temperature (about 10°C) and the hardness of the bodies was estimated. In this case, the time at which the bodies were eviscerated was supposed to be the time of the death of *Stichopus japonicus*.

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* This "Studies on post-mortem changes in the chemical constitution of the meat of sea cucumber" is the second part of "Chemical studies on the meat of sea cucumber (*Stichopus japonicus SELENKA*)."

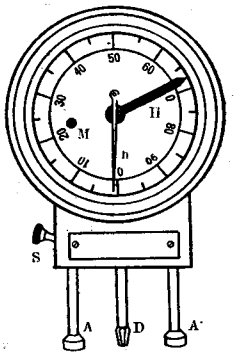

Fig. 1. Fatiguesmeter (Muscle hardness test meter)

(2) Experimental method

To judge the freshness of the meat of *Stichopus japonicus*, the fatiguesmeter of muscles of sportsmen as shown in Fig. 1 was used.

This fatiguesmeter resembles Tauchi's hardometer. In Fig. 1, this meter has 3 feet, A, A' and D, of which A and A' have the same action. D has a weaker spring than A and A'. The needle H is the main needle and the needle h is a sub-needle. M is notice board, connected with S. M and S move by the action of D. Scale is divided into 90, and is read to decimal order. This instrument is used for the determination of the fatigue of human muscles by estimation of the difference of hardness between the strained muscle and the relaxed state of the fatigued muscle by the setting of this meter on the muscle. The needles A and A' are pushed into the loosened muscle to a definite depth, that is to say, when the definite power is applied to the needle D, the switch S indicates "off", with a clicking noise. At the same time, the board changes to red at part M. The scale which is indicated by the needles, H and h, is read. After an estimation, when the switch S is turned back, the red board returns to the white board at M, and the needle H returns to zero. Next, the needles A and A' are pushed into the consciously strained muscle, and so on.

The difference of the values of hardness of strained and relaxed muscles indicates the elasticity of the muscle. The loosened meat of *Stichopus japonicus* was estimated by laying down the body of *Stichopus japonicus* on the table, taking care that the end part of the needles of the instrument should not face to the papillae on the surface. The strained condition of the meat was brought about by laying down the body on a triangular pillow and bending it.

Estimations were made five times and the average value was taken.

(3) Experimental results

The results obtained are shown in Fig. 2. As seen there, the body of *Stichopus japonicus* became hard from the 4th hour after the death until the 44th hour, that is to say, there is a period during which the meat of *Stichopus japonicus* is hard.

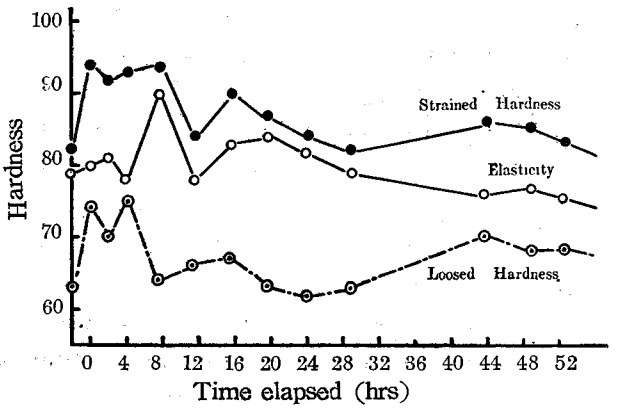

Fig. 2. Changes in the hardness of meat of *Stichopus japonicus* at room temperature (about 10°C)

This phenomenon that the meat becomes hard during such a period may be called "rigor mortis".

2. The post-mortem changes in the amounts of lactic acid, and glycogen in the meat of *Stichopus japonicus*

It is of interest to know the changes in the amounts of lactic acid and glycogen and values of pH in the meat of *Stichopus japonicus* during rigor mortis. The experiment was divided into two parts.

(1) Sample

For the first experiment living *Stichopus japonicus* which was caught in the sea near Hakodate in June was eviscerated and crushed and then left at the room temperarure of 10°C. During the leaving of the crushed meat the changes in amounts of lactic acid and glycogen were observed. For the second experiment, living *Stichopus japonicus* which was caught in July was treated in the same manner. To the material were added each 5 cc of toluene and chloroform. The material was left at room temperature of 15°-18°C; changes of the same items were estimated.

(2) Experimental method

Estimation of the amount of lactic acid: To the crushed meat was added 10 cc of 20% trichloracetic acid to remove the protein. After filtration, the amount of lactic acid in a definite volume of the filtrate was estimated by Friedmann's method.[3]

Estimation of the amount of glycogen: The crushed meat was previously treated by Simonovits' method[4] and the amount of glycogen was estimated by Hagedorn-Jensen's method.[5]

The estimation of the value of pH: The value of pH was estimated by the glass electrode meter.

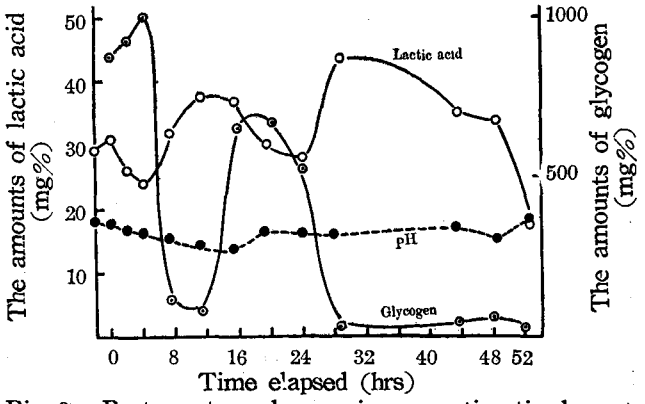

Fig. 3. Post-mortem changes in non-antiseptized meat of *Stichopus japonicus* at 10°C

(3) Experimental results

Experimental results obtained are shown in Fig. 3.

As seen in Fig. 3, the values of pH of the meat of *Stichopus japonicus* decreased once gradually with the lapse of time after the death, and increased again from the 16th hour after death. Thereafter the value became constant for some hours and increased again from the 50th hour.

The variation of the values of pH has corelated with the variations of the amount of lactic acid. The amount of lactic acid increased after the death. It indicated maximum at the 12th hour, decreased gradually to the minimum amount at the 24th hour, and then increased again until the 30th hour. Thereafter the amount decreased. The amount of glycogen content varied with the increasing and decreasing of the amounts of lactic acid inversely. The rigor mortis of the body of *Stichopus japonicus* continued from about the 4th hour until 44th according to the degree of hardness as previously noted.

In these experimental results, the variation of the amounts of lactic acid and glycogen, when graphed, showed some peaks and valleys.

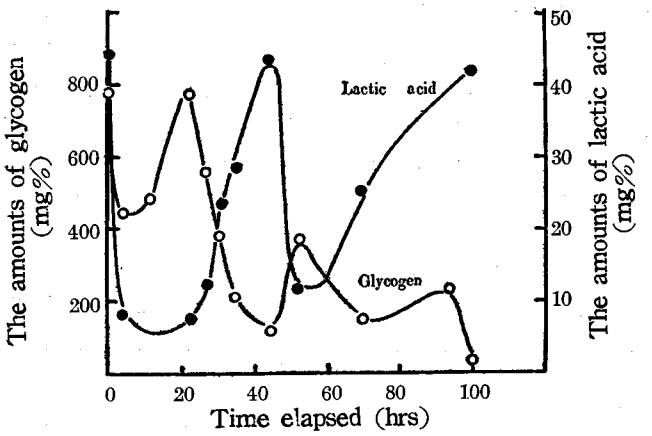

Fig. 4. Post-mortem changes in antiseptized meat of *Stichopus japonicus* at 15°–18°C

In the second experiment, the crushed meat was antiseptized by the addition of toluene and chloroform and the variation in the amounts of lactic acid and glycogen were observed to ascertain whether the results are similar to the first experimental results, or not. The second experimental results obtained are shown in Fig. 4.

As seen in Fig. 4, the amounts of lactic acid and glycogen showed some peaks and valleys of increase and decrease like the first experimental results. But in the second experimental results, as bacterial action in the crushed meat is inhibited by the antiseptants added, the number of mountains and valleys is smaller than in the first experimental results. Further, as the temperature of the first experiment is higher than the second, the position of the mountains in the latter experiment situated to right. In the first experiment, at the time when the increase of the amount of lactic acid stopped, the smell of ammonium began to be noticeable.

Some observations on the results obtained may be worth consideration. The increase of the amount of lactic acid in the meat of *Stichopus japonicus* during the period of rigor mortis is considered to be due to the decomposition of glycogen.

The amount of lactic acid in the meat of *Stichopus japonicus* did not increase slowly, but rapidly for a short time, then decreased and again increased after some interval. The same observation was made by Yamada[6] of variation in the increase of lactic acid in fish meat during the period of the post-mortem change. The phenomenon of decrease and increase of lactic acid during post-mortem change in fish meat is considered to be due to the formation of pyroracemic acid from lactic acid by the action

of lactic acid dehydrogenase, also to be due to the decrease of the amount of lactic acid by the intracellular respiraton remaining after the death of fish.

In the present experiment, the amount of pyroracemic acid and the amounts of various types of phosphoric acid were not estimated, so the variation in the amount of lactic acid can not be explained clearly by the above mentioned chemical changes.

The senior author has observed histologically the meat of *Stichopus japonicus* and ascertained that the edible part of *Stichopus japonicus*, the body wall, is really connective tissue consisting of a network of collagen fiber.[7] The fact that water soluble protein, NaCl solution-soluble protein and dil. NaOH solution-soluble protein are fractionated from the body wall, suggests that various kinds of soluble protein may be considered to be bound with a large amount of water (90% of the weight of the body) in that network of collagen fiber in the connective tissue. From the characteristics of the construction of the tissue, the glycolytic action of *Stichopus japonicus* meat is considered differ instinctively from that of fish meat. As to this point, further detailed investigation should be made.

Summary

(1) The variation of the hardness of the meat of *Stichopus japonicus* after death was examined, and the rigor mortis which is seen in fish meat was likewise observed in the "sea cucumber" body.

(2) The variation of the increase and the decrease of the amounts of lactic acid and glycogen was observed to be converse. Those variations are complex, but it is clear that they indicate the phenomenon of the glycolytic action.

Literature cited

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