A PRELIMINARY REPORT ON THE POISONOUS EFFECT OF THE TOXOPNEUSTES TOXIN UPON THE HEART OF OYSTER

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A PRELIMINARY REPORT ON THE POISONOUS EFFECT OF THE *TOXOPNEUSTES* TOXIN UPON THE HEART OF OYSTER

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(With 11 Text-figures)

Most abalone-fishing divers have an intensive fear that the trumpet sea-urchin, *Toxopneustes pileolus* (LAMARCK), is poisonous and it is very dangerous to come in contact with their bodies. Nevertheless, no reports have been found, till to-day, concerning the biological research for so-called "*Toxopneustes* toxin", so far as we could know.

In the case of *Toxopneustes pileolus*, two kinds of modified pedicellariae, peculiar to this species, can be observed besides the ordinary pedicellariae, common in sea-urchins (Fig. 1). We will call the one "trumpet pedicellaria", and the other "giant pedicellaria". The body surface of this species, when living, is covered by numerous trumpet pedicellariae, each of which spreading out among numerous spines and opening fully its trumpet-like tip with three fine hooks. A few giant pedicellariae of three-lobed large tip with similar hooks are mingled here and there among these trumpet ones. The white mucous fluid is ejaculated through these fine hooks, if the fully opened tips of these pedicellariae are stimulated by a sudden contact to close themselves vigorously. The vigorous closing reaction is exceedingly remarkable in the response of giant pedicellariae. Such a mucous fluid, from the giant and trumpet pedicellariae, is supposed to contain the above-mentioned toxic substance of this species or "*Toxopneustes* toxin".

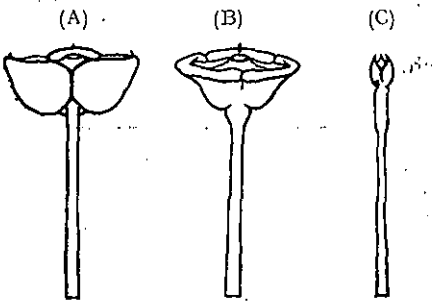

Fig. 1. Schematic representations, illustrating three kinds of pedicellariae of *Toxopneustes pileolus*. × 5.
(A) giant pedicellaria, (B) trumpet pedicellaria, (C) ordinary pedicellaria.

In this paper, therefore, the writers will report the results of preliminary experiments, which were undertaken to investigate some physiological effects of so-called *Toxopnesutes* toxin upon the rythmic pulsation of the isolated heart from the shoe oyster, *Ostrea nippona* SEKI.

MATERIAL AND METHODS

Toxopneustes pileolus and *Ostrea nippona*, used for this study, were collected from the rocky bottom a little off the coast of Mugi-machi, Tokushima Prefecture.

Through dissection, the oyster heart was carefully prepared, and then was suspended in the normal sea-water in a vessel of about 200 cc capacity. The pulsatory activities of the oyster heart were recorded by the kymographic method, experiments being made with or without the white mucous fluid containing the supposed *Toxopneustes* toxin. The following three different methods were employed to pour the mucous fluid into the oyster hearts.

(*I*) *Direct Ejaculation from a Giant Pedicellaria*

A fresh giant pedicellaria was separated from body surface of the sea-urchin by forceps and was kept in contact with the ventricle of oyster heart, prepared in the kymographic apparatus. If the separated pedicellaria was vigorous and undamaged, the hooks of this pedicellaria bit the heart wall actively, with a positive closing reaction of the tip caused by the contact stimulus. Simultaneously, the white mucous fluid was ejaculated from the giant pedicellaria directly into the ventricle of oyster heart.

(*II*) *Injection with Mucous Fluid of Trumpet Pedicellariae*

Many trumpet pedicellariae were separated from body surface of the sea-urchin by forceps and were treated with 0.5 mol KCl solution for several hours. During this treatment, the white mucous fluid was plentifully yielded and deposited from these pedicellariae, probably due to some unknown stimulation by K-ion there. The deposited mucous fluid was pipetted quickly and bathed in the normal sea-water repeatedly. In such a manner, the mucous fluid could be collected indirectly from the treated pedicellariae and used for the injection into the oyster heart.

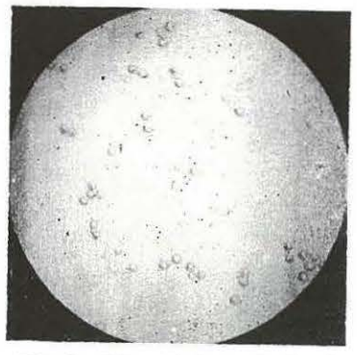

Fig. 2. Microphotograph showing "corpuscles" of mucous fluid, from trumpet pedicellariae. × 200.

(*III*) *Injection with "Corpuscles" and "Matrix" separated from Mucous Fluid.*

By the microscopic observation, we could find the numerous particular corpuscles of fine dumb-bell shape and the surrounding matrix of milky viscosity in the white mucous fluid, derived indirectly from trumpet pedicellariae (Fig. 2). These components of the mucous fluid, the corpuscles and the matrix, however, were separated into two different fractions by high centrifugal force. The one was the centrifugal fraction or corpuscles, and the other the centripetal fraction or matrix. Each fraction, therefore, could be independently injected into the oyster heart. Thus, the respective effect of "corpuscles" and of "matrix" upon the oyster heart was, also, investigated experimentally.

For the injection into the ventricle of oyster heart, a fine glass capillary or a minute syringe was utilized in the cases of *Experiments II* and *III*. As the control, the normal sea-water was injected into the ventricle of oyster heart in the same manner.

EXPERIMENTAL RESULTS

Experiment I. The pulsatory activities of oyster hearts, bitten by a giant pedicellaria, are all subjected to the inhibitory effects, but are somewhat different

Fig. 3. Pulsation of oyster heart No. 1, showing inhibitory effect caused by a small quantity of mucous fluid in *Exp. 1*.
Note: Time, marked per minute. Temperature, about 20°C.

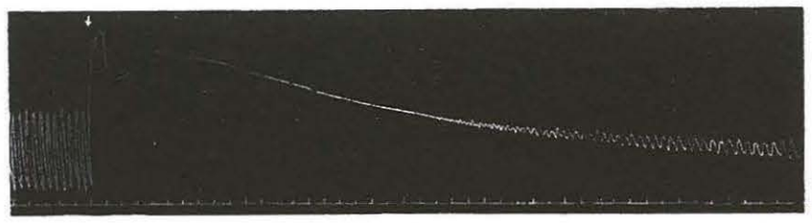

Fig. 4. Pulsation of oyster heart No. 2, showing inhibitory effect caused by a large quantity of mucous fluid in *Exp. 1*.
Time, marked per minute. Temperature, about 20°C.

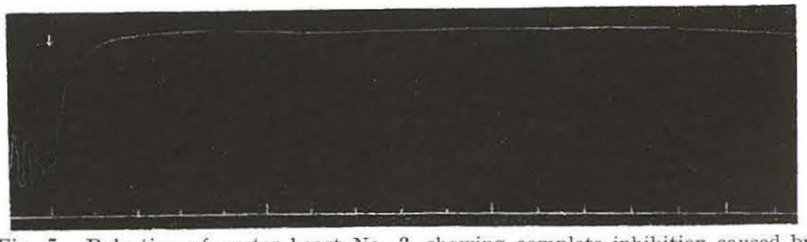

Fig. 5. Pulsation of oyster heart No. 3, showing complete inhibition caused by a larger quantity of mucous fluid in *Exp. 1*.
Time, marked per minute. Temperature, about 29°C.

from one another in aspect, as are ssen from Figs. 3, 4 and 5 respectively. In the case indicated by Fig. 3, the decayed gaint pedicellaria is empolyed for suppiying a little quantity of mucous fluid to the ventricle of oyster heart No. 1. Only a little inhibitory effect is recorded in this case. For several minutes immediately after ejaculation, the heart shows a little increasing tonus with faded amplitude, and soon recovers gradually to the original normal pulsation. In the case indicated by Fig. 4, the vigorous giant pedicellaria is employed for supplying much quantity of mucous fluid to the ventricle of oyster heart No. 2. A distinct inhibitory effect is produced on the pulsatory activities of the heart in this case. For several minutes immediately after ejaculation, the heart makes a sudden shortening with almost abolished pulsation. About twenty minutes later, the pulsatory power of heart begins to show a sign of recovering without the onset of initial shortening. About sixty minutes later or more, however, the pulsation cannot be recovered completely, although it shows a slightly dereasing tonus with gradually recovering amplitude. In the case indicated by Fig. 5, the most vigorous giant pedicellaria at higher temperature was employed for supplying a larger quantity of the mucous fluid to the ventricle of oyster heart No. 3. In this case, the pulsatory activities of oyster heart are affected with almost complete inhibition. The heart reacts into a clear-cut contracture at once, and abolishes its pulsation without any more recovering.

Experiment II. By the injection of white mucous fluid, derived indirectly from trumpet pedicellariae, the pulsatory activities of oyster hearts are, also, subjected to the inhibitions similar to those in *Exp. I.* These inhibitory effects are shown in Figs. 6, 7, 8 and 9 respectively. In the case indicated by Fig. 6, a little quantity of mucous fluid was injected by a fine glass capillary into the ventricle of oyster heart No. 4. For ten minutes immediately after the injection, the heart shows a slightly reduced pulsation with a little increased frequency and reduced amplitude, and soon begins to recover to the original pulsation. About twenty minutes later, the pulsatory power of oyster heart reaches the almost complete recovering. In the case indicated by Fig. 7, a small quantity of mucous fluid was injected through a fine glass capillary into the ventricle of oyster heart No. 5. For a few minutes immediately after the injection, the heart shortens rapidly and then shows an abnormally increased tonus with a gradual recovering to the normal pulsation. Thirty minutes later or more, however, the pulsatory activities are not yet completely recovered and remain to continue a slightly decreasing tonus with much reduced amplitude. In the case indicated by Fig. 8, much quantity of mucous fluid was injected by a minute syringe into the ventricle of oyster heart No. 6. A distinct inhibition appears on the pulsation of heart in this case. For a few minutes immediately after the injection, the heart reacts suddenly with the first increasing and then decreasing tonus. Several minutes later, the heart begins to show again the rapidly increasing tonus with increased frequency and much reduced amplitude. About eighteen minutes later, moreover,

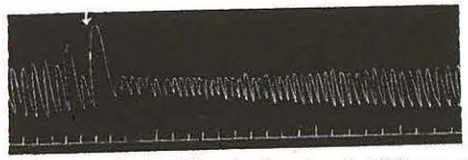

Fig. 6. Pulsation of oyster heart No. 4, showing inhibitory effect caused by a small quantity of mucous fluid in *Exp. II.*
Time, marked per minute. Temperature, about 20°C.

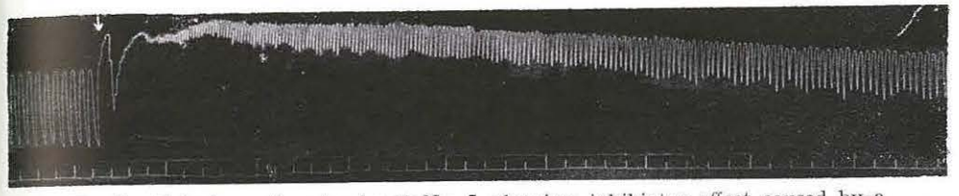

Fig. 7. Pulsation of oyster heart No. 5, showing inhibiotry effect caused by a small quantity of mucous fluid in *Exp. II.*
Time, marked per minute. Temperature, about 20°C.

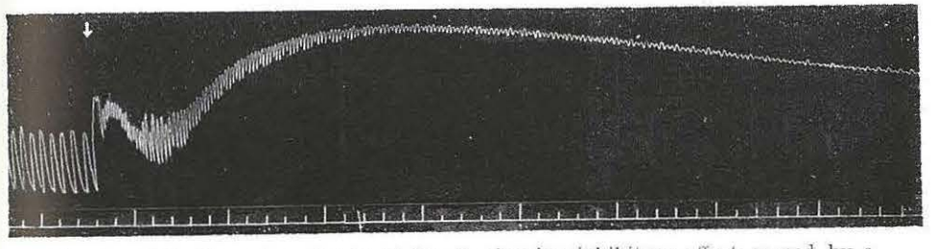

Fig. 8. Pulsation of oyster heart No. 6, showing inhibitory effect caused by a large quantity of mucous fluid in *Exp. II.*
Time, marked per minute. Temperature, about 20°C.

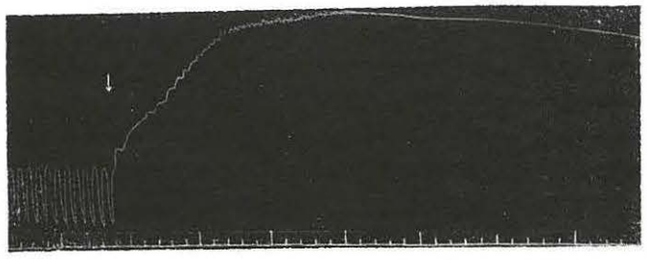

Fig. 9. Pulsation of oyster heart No. 7, showing complete inhibition caused by a larger quantity of mucous fluid in *Exp. II.*
Time, marked per minute. Temperature, about 20°C.

the tonus reaches the maximum and then decreases gradually with diminishing amplitude. About ninety minutes later, the heart abolishes the pulsation almost completely without any more recovering, tending to diastolic cessation. In the case indicated by Fig. 9, a larger quantity of the mucous fluid was injected by a minute syringe into the ventricle of oyster heart No. 7. The complete inhibition of the pulsation occurs at once in this case. As soon as the mucous fluid is injected into the ventricle, he heart shows a rapidly increased tonus, and diminished amplitude. About fifteen minutes later, the tonus reaches the maximum and its pulsation completely stops with gradual decrease of tonus in diastole.

Experiment III. Interesting results are obtained from the oyster hearts, injected with either fraction of the centrifuged mucous fluid. In the case indicated by Fig. 10, either centrifugal or centripetal fraction, densely suspended in sea-water, is injected alternately into the ventricle of oyster heart No. 8. In this case,

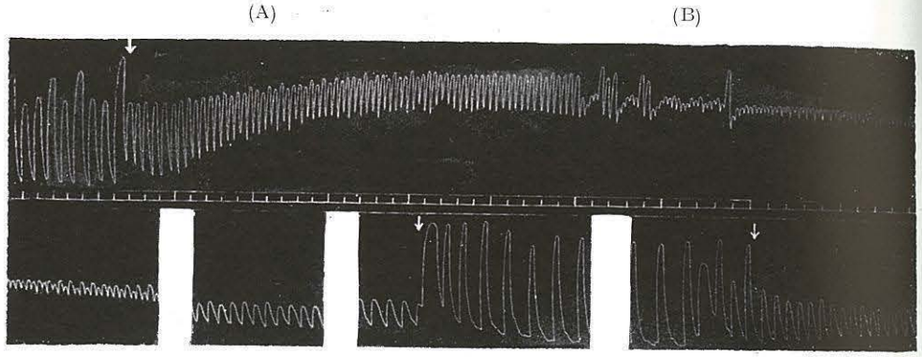

Fig. 10. Pulsation of oyster heart No. 8, showing effect of corpuscles and of matrix, separated from white mucous fluid.
Note : First injection, with corpuscles. Second injection, with matrix. Third injection, with corpuscles. Time, marked per minute. Temperature, about 20°C.

different effects occur on the pulsatory activities of oyster heart. For about twenty minutes immediately after the first injection with corpuscles, the heart shows the slightly increasing tonus with a little increased frequency and much reduced amplitude (Fig. 10 A). About thirty minutes later, the tonus reaches the maximum, when the previous feature of pulsation is interrupted repeatedly by abnormally diminished pulsations which go on thereafter (Fig. 10 B). About fifty minutes later, moreover, the heart decreases the tonus gradually (Fig. 10 C), and soon becomes stable in the diminished pulsation (Fig. 10 D). After that, the centripetal fraction or matrix was injected for the second time into the same ventricle. As soon as the second injection was given, the abnormally diminished

pulsation of oyster heart makes a sudden change and shows a greater amplitude than the original one, but is slowed down remarkably in its frequency (Fig. 10 E). Thirty minutes later, the corpuscle fraction was injected again into the same ventricle. The similar inhibitory effect of the corpuscles appears again and the heart suddenly diminished the pulsation with increased frequency and much diminished amplitude (Fig. .10 F).

GENERAL CONSIDERATIONS

In all experiments stated above, the oyster hearts may have been subjected to the possible inhibitory effect of the mechanical stimulus caused by the hooks of giant pedicellaria and by the glass capillary or needle of the syringe, in addition to the chemical stimulus of white mucous fluid under investigation. The inhibitory effect of the mechanical stimulus is here open to question. As the control to

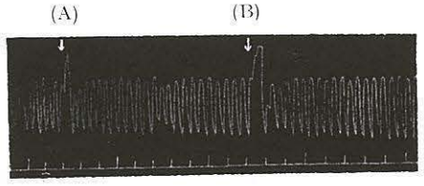

Fig. 11. Pulsation of oyster heart No. 9, showing two abnormal contractions caused by mechanical stimuli of injections.
Time, marked per minute. Temperature, about 20°C.

make clear this question, a small quantity of normal sea-water was injected repeatedly by a minute syringe into the ventricle of oyster heart No. 9.

At the first injection, the oyster heart reacts instantly with an abnormally increased contraction following the mechanical stimulation of injection, as shown in Fig. 11 A. The pulsatory power of oyster heart, however, recovers completely to the normal pulsation within a few minutes. At the second injection, a similar abnormal contraction also occurs on the pulsation of oyster heart, which shows the prompt recovering to the original rhythm (Fig. 11 B). As the results of this experiment, it is found that the mechanical stimulus just investigated causes only a temporarily increased contraction in the pulsation of oyster heart. Accordingly, the various inhibitions of the pulsatory activities of oyster heart mentioned above may safely be considered to be due to some toxic substances contained in the white mucous fluid.

On the other hand, it is doubtful that the inhibitory effect of poisonous corpuscles is quite different from that of poisonous matrix. A certain remarkable correlation like synergism or antagonism seems to be suggested in both poisonous effects between these different components of white mucous fluid. In order to

make clear this point, the research will be continued further from both physiological and chemical points of view. In conclusion, it has been confirmed by the present experiments that some remarkable poisonous effects upon the oyster heart are caused by the white mucous fluid secreted from the giant and trumpet pedicellariae of *Toxopneustes pileolus*.

SUMMARY

(1) In the present paper, the writers have reported the results of the experiments, which were undertaken to investigate the so-called poisonous effect of *Toxopneustes pileolus* upon the heart pulsation of *Ostrea nippona*.

(2) In different experiments, the prepared hearts of oysters for the kymographic apparatus have been injected directly or indirectly with the white mucous fluid, which was obtained from the "giant" and "trumpet" pedicellariae peculiar to this sea-urchin (Fig. 1).

(3) The conspicuous inhibitions of the pulsations of oyster hearts, shown in Figs. 3—9, may be assumed to be chiefly due to the poisonous effect of the white mucous fluid. The mechanical stimulus, due to the experimental procedure, affects the oyster heart causing only a temporary disturbance of pulsation as shown in Fig. 11.

(4) The white mucous fluid is composed of both poisonous corpuscles (Fig. 2) and poisonous matrix, which can be separated by high centrifugal force into two different fractions. The inhibitory effect of poisonous corpuscles is quite different from that of poisonous matrix, as shown in Fig. 10.

(5) In conclusion, the presence of so-called *Toxopneustes* toxin, complicatedly poisonous to the pulsatory activities of oyster heart, was confirmed experimentally in the white mucous fluid, secreted from the tips of giant and trumpet pedicellariae of *Toxopneustes pileolus*.