MUSCULAR ACTION IN *HOLOTHURIA GRISEA*

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(3 Figs.)

Holothuria grisea is an exceedingly common animal on rocky shores of the south of Brazil. Its anatomical organisation is typical of the genus. Its body consists essentially of a highly muscular body wall of circular muscle to the inner surface of which are attached the five powerful radially arranged longitudinal muscles. Within the body wall is a large cœlomic space filled with fluid and containing the gut and other viscera. At the posterior end of the gut, is the powerful muscular cloaca into which open the large branched diverticula, the "respiratory trees". These lie freely in the cœlomic cavity.

The shape of the body and its movements depend on the contractions of the muscular body wall acting against the volume of fluid within the body cavity. This "action system" is characteristic of animals with a hydrostatic skeleton — the "hohlorganartige Tiere" of JORDAN (1914 ; 1929). In the present paper we shall give an account of the mechanics of muscular action of this system in this animal.

Volume of the body

Most of the fluid in the body cavity, against which the muscular system acts, consists of cœlomic fluid. But it also includes sea-water which has been pumped by the cloaca into the respiratory trees. The pumping movements have a considerable effect on the volume of the animal, as we shall see. But though the cloacal pumping mechanism has long been a subject of interest (BABÁK 1921) there appear to have been no quantitative observations.

References to earlier work on the respiratory movements of the cloaca in Holothurians, particularly rhythmical activity of the circular and radial muscles, may be found in BUDINGTON (1937).

If we examine a living *Holothuria*, we see that the cloaca opens rhythmically about 5 times a minute. This opening is an active process and, as we shall see later, has to be executed against a positive internal cœlomic pressure. The cause of this opening appears to be contraction of the radial muscles which attach the cloaca to the posterior end of the body. Their contraction causes the posterior end of the body to become narrower and more pointed as the cloaca opens and fills with water. The suction is strong enough to take in air if the cloaca is above water level. After the cloaca is filled, the anus closes and the powerful contraction of its circular musculature drives water into the respiratory trees. The rhythmic contraction thus begins with the radial muscle and follows with that of the circular muscles. About every tenth contraction of the cloaca, a considerable volume of sea water is forced out of the body.

These volume changes are clearly of great importance to the animal. Quite apart from their significance in respiration, they alter the volume of fluid against which the muscular system of the animal acts. It was therefore necessary to examine the phenomenon quantitatively as well as by direct observation. A simple apparatus was constructed to record the changes in volume of an intact *Holothuria* (Fig. 1). The animal was inserted into a large glass tube, 25 cms x 3 cms, immersed in a large dish of sea-water. A thin rubber diaphragm (made from a child's toy balloon) was stretched over one end of the tube. The posterior end of the animal was pushed through a circular hole in the diaphragm and allowed to project for a fewcentimetres into the surrounding sea-water. The rubber diaphragm prevented leakage of sea-water.

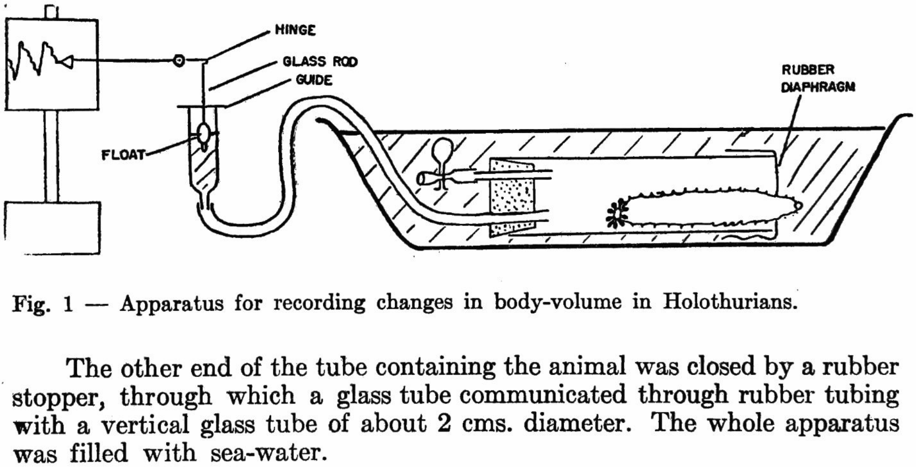

Fig. 1 — Apparatus for recording changes in body-volume in Holothurians.

The other end of the tube containing the animal was closed by a rubber stopper, through which a glass tube communicated through rubber tubing with a vertical glass tube of about 2 cms. diameter. The whole apparatus was filled with sea-water.

Changes of volume of the animal, due to cloacal pumping, drive water into the vertical glass tube. The level of the water in this tube was recorded kymographically by means of a float attached to the recording lever. At the end of an experiment the animal was removed and the rubber diaphragm closed with a cork. The apparatus was now calibrated by adding 5 and to 10 ml of sea water and noting the change in height of the recording lever. Finally the volume of the animal at the end of the experiment was determined by finding the volume of sea water which it could displace when placed in a glass vessel.

Fig. 2 shows a typical record (26° C) from an animal about 17 cms long, with a volume of 150 ml at the end of the experiment. The cyclical changes in volume are very clear. A succession of about ten cloacal contractions increases the internal volume by 10 to 15 ml. (i. e. about 10% of the whole volume of the animal). Each cloacal contraction drives in about 1 ml. Every few minutes the cloaca opens and the body wall contracts. The whole of the accumulated water is thus driven out, and the volume falls.

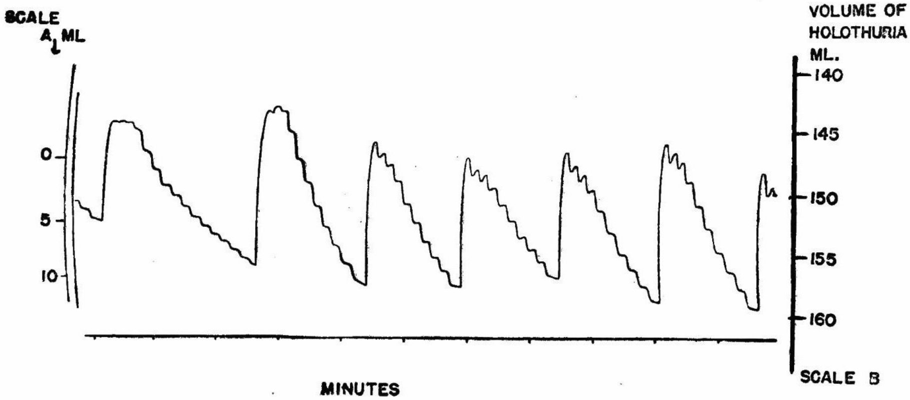

Fig. 2 — Volume changes in an intact *Holothuria grisea* recorded with the apparatus shown in figure 1. Time scale in minutes. Scale *A* shows calibration of apparatus by addition of 5 ml and 10 ml of sea water to float chamber. The final volume of the animal was found to be 150 ml, and scale *B* is constructed about this value using the calibration measurements of scale A.

The figures are of the same order as an approximate estimate by CROZIER (1920) of the volume of water involved in cloacal pumping in *Stichopus*.

Both the cloacal contractions and the periodic expulsion of water are more or less rhythmic processes. But the expulsion process is apt to vary considerably in frequency so that the average volume of the animal may rise or fall to new levels. Much of this semi-permanent change in volume is easily lost or re-gained and would therefore seem to be due to changes in the average volume of water in the "respiratory tree" system. But the animal can also change the volume of its cœlomic fluid so that it can more or less permanently assume a distended or a highly contracted condition. There is in fact a real variation in the volume of the cœlomic fluid itself.

The water movements involved in these changes in volume of the cœlomic fluid must be brought about through transport of water to and from the cloacal-respiratory tree system. Thus the importance of the "respiratory tree" mechanism in regulating the volume and mechanics of muscular action in these animals is at least equal to its importance in respiration.

The Coelomic pressure

We may now consider the cœlomic pressure against which the muscular system of the body wall must act. To investigate the action of the muscular system in the living animal, the pressure of the cœlomic fluid was measured. The apparatus employed was essentially similar to that described by BATHAM & PANTIN (1950) for the measurement of the cœlenlenteric pressure of Actinians. A canula, attached to the manometer apparatus by about 20 cms of thin flexible rubber tubing, was inserted into the body wall of the *Holothuria* and the pressure recorded at intervals of 15 secs. on a water manometer. The animal was placed in a large dish containing about 3 litres. It was free to move about in the dish. Fig. 3 shows a typical record from an animal 16 cms long and 2.5 to 3.5 cms in diameter.

The average pressure in seven experiments on various animals varied from 7 to 21 mm of water. The mean of the averages of these experiments was 16 mm of water pressure. The Figure shows that, as in the case of the Actinians, active contractions cause a rise of pressure. This occurs particularly when the animal elongates through contraction of the circular muscle. Throughout the experiment rhythmic contractions of the cloaca were taking place regularly at about ten in 90 secs. These were not accompanied by any obvious changes in cœlomic pressure. After every ninth or tenth contraction there was a powerful expulsion of water lasting several seconds as already described. This expulsion was commonly accompanied by a temporary fall in cœlomic pressure of about 5 mm..

The cœlomic pressure is greatly influenced by locomotor and other movements of the body. During normal bodily movements, the cœlomic pressure frequently approached, but did not exceed, 3 cms of water. Strong mechanical stimulation caused contraction of the body wall which produced pressures varying from 4.5 to 16 cms of water in different cases. There appears to be no simple maximal reflex muscular response in this *Holothuria* comparable to the maximal retraction of the Actinian.

The normal range of cœlomic pressures is far below the maximum pressure that can be set up by maximal muscular contraction. Since both circular and longitudinal muscles are highly sensitive to acetylcholine, it was found convenient to induce maximal contraction by injection of this substance into the cœlom. Thus after the experiment illustrated in figure 3, 2 ml of 1% acetylcholine chloride were injected. The pressure rose during several minutes and finally reached a steady value at 19 cms of water. The muscle system is thus normally working at tensions much less than the maximal tension.

The pressures set up by injection of acetylcholine into the living animal are in general agreement with the maximal isometric tensions that can be recorded in the isolated longitudinal muscle. Thus after the experiment shown in fig. 3, one of the five longitudinal muscles was removed. This muscle was then exposed to successive doses of 0.5 ml of 1% acetylcholine added to about 10 ml of sea water surrounding the muscle. A maximal tension of 25 gms. was recorded. The total maximal isometric tension of the five longitudinal muscles may thus be taken as about 125 gms. The diameter of the body of the normal animal at its widest was 3.5 cms. A tension of 125 gms. acting over the area of cross-section of the body would thus generate a pressure of $125/\pi \times 1.75^2 = 13$ gms per sq/cm. Bearing in mind the variability of the shape of the body and the possibility of some degree of damage on removal of the muscle from the body, this value is in fair agreement with the maximal pressure of 19 cm of water developed by the living animal.

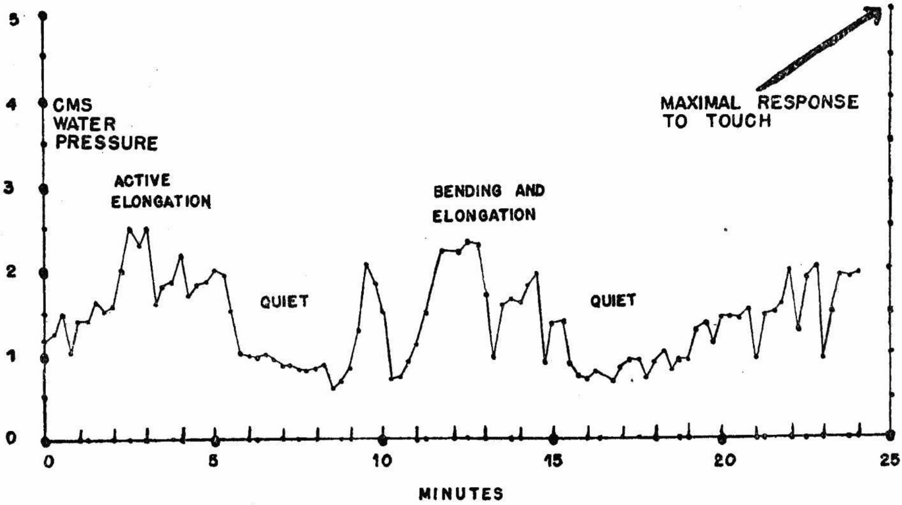

Fig. 3 — Graph of pressure changes in *Holothuria grisea*

Measurements of the isometric tension developed in the circular muscle layer were less satisfactory. Thus in the experiment quoted a transverse strip of the body wall with its circular muscle layer was at first insensitive to stimulation. Later it developed a tension of 6.5 gms in the presence of 0.4 ml of 1% acetylcholine in 5 ml of sea water. Such strips of tissue contract greatly when cut from the animal, and one can only approximately ascertain the original width occupied by the strip when *in situ* in the normal body wall. In the present case this width appeared to be 7 mm. The region of the body from which the strip was taken had a normal diameter of about 2.5 cms. To balance a tension of 6.5 gms in a circular strip of body wall 7 mm wide and 2.5 cms in diameter, would require a pressure of 7.5 gms per sq/cm. This is considerably below the maximal value of 19 cm of water pressure in the cœlom as measured in life. But some degree of damage and fatigue is inevitable in this preparation.

These experiments show that, as in the case of the actinian *Metridium* (Batham & Pantin 1950) and of *Arenicola* (Chapman & Newell 1947) the normal movements of the body involve tensions in the muscular wall which are well below 50% of the maximum isometric tension. That is the muscles are operating under conditions in which contraction can cause great shortening and consequent deformation of the shape of the body. It is however of interest to note that values for the average basal pressure and for the maximal isometric pressure in *H. grisea* (16 mm and 200 mm) are well above the corresponding values for the much less muscular *Metridium* (2.6 mm and 13 mm). On the other hand they are below the values found by Chapman & Newell for *Arenicola* (130 mm and 800-900 mm)

(Batham & Pantin 1950). Holothuria is a much heavier animal than *Metridium* and its normal habit of moving through crevices in rocks, necessitates more powerful muscular action. On the other hand, the burrowing *Arenicola* requires even greater muscular power to penetrate its environment of sand and mud.

This relation between the internal pressure and the environmental conditions seems to be of some general significance. Zuckerkandl (1950) records maximal pressures of 960 mm of water in *Sipunculus nudus* when burrowing in sand. These pressures are similar to those in *Arenicola.* But the most interesting comparison is between the *Holothuria grisea* which lives in rock crevices and the burrowing Holothurians. Yazaki (1930) records cœlomic pressures in the sand-burrowing *Caudina chilensis* of about 150 mm under normal conditions and 450-500 mm on maximal stimulation, whilst Prosser (1950) records figures from unpublished records of Zuckerkandl on *Thyone* sp. which are equivalent to about 24 mm of water pressure during rest and about 390 mm during activity. The maximal pressures in these burrowing species are about double the maximum pressure in the crevice-living *Holothuria grisea.*

On some occasions *Holothuria grisea* extruded the gut during the course of an experimental measurement of the cœlomic pressure. When extrusion took place, the pressure was high, usually 12-18 cms of water. But on one occasion, extrusion of the gut took place at a pressure as low as 8 cms of water. Moreover these animals often develop maximal tension without extrusion of the gut. From this it seems that extrusion can sometimes occur at only moderate internal pressures and that some other factor as well as pressure operates in the extrusion reaction.

We may note here one other point. Locomotion in *Holothuria* takes place as in other Echinoderms by means of the tube-feet of the water-vascular system. In most Echinoderms this system is in free communication with the low pressure of the surrounding sea-water by way of the madreporite on the surface of the body. If this had been the condition in *Holothuria grisea* the relatively high cœlomic pressure would inevitably have caused the collapse of the water vascular system. This would have prevented the tube-foot system from functioning. Accordingly, it is of interest to note that in these Holothurians the madreporite has become internal, so that the water pressure in the water-vascular system must be in equilibrium with that of the cœlomic fluid of the body cavity, and the water vascular system will not become collapsed.

It would be interesting to know the hydrostatic relations between the cœlomic fluid and the water-vascular system in the primitive and aberrant Echinothuroids such as *Asthenosoma.* These are Echinoids, but the body is flexible and muscular. Nevertheless they possess well developed external madreporites (Mortensen 1935). How do they prevent collapse of their water-vascular system? At present we know no answer to this question.

Summary

1) The mechanics of muscular action in *Holothuria grisea* are investigated. The longitudinal and circular muscles act against the fluid in the body cavity most of which is cœlomic.

2) The control of the volume of the body by the cloacal contractions is studied. An apparatus is described for recording changes in volume of the body. Each cloacal contraction drives about 1 ml of water into the body. About every 2 minutes, that is about every tenth contraction, there is an expulsion of acumulated sea-water amounting to about 10% of the body volume. The mechanical importance of this system is discussed.

3) The cœlomic pressure accompanying movement is measured. The average internal pressure is from 7 to 21 mm of water pressure. Maximal contractions reached 19 cms of water pressure. The values are in general agreement with the recorded tensions in the muscles of the body wall. The mechanics of the system are discussed and compared with that in *Metridium*, *Arenicola* and other animals. It is pointed out that the high cœlomic pressure provides the functional explanation of the fact that in *Holothuria* the madreporite is internal.

Resumo

Holothuria grisea é um dos animais comuns nas praias rochosas do sul do Brasil. A forma e a construção do corpo é tal que os seus movimentos dependem das construções de paredes musculares do corpo atuando contra o volume do fluído que se encontra dentro da cavidade do corpo. Êste sistema de ação é característico dos animais com um esqueleto hidrostático — os "hohlorganartige Tiere" de JORDAN (1914 ; 1929). No presente trabalho trataremos da mecânica de ação muscular dêste sistema nêste animal.

Volume do corpo do animal

A maior parte do fluído da cavidade do corpo, consiste de fluído celômico, que contém água do mar aspirada pela cloaca para a árvore respiratória. Os movimentos de sucção da cloaca têm efeito considerável sôbre o volume do animal.

Examinando a Holothuria viva verificamos que a cloaca se abre ritmicamente cêrca de 5 vêzes por minuto.

A causa dêste movimento da cloaca parece ser a contração dos músculos radiais que prendem a cloaca à extremidade posterior do corpo. Sua contração provoca o estreitamento da extremidade posterior do corpo que se afila quando a cloaca se abre e se enche de água. A sucção é suficientemente forte para absorver ar se a cloaca estiver acima do nível da água. Depois de cheia a cloaca o anus fecha-se e, pela poderosa contração da musculatura circular, a água é lançada na árvore respiratória.

Inicia-se, então, a contração rítmica pela musculatura radical e seguida da dos músculos circulares. Aproximadamente em cada décima contração da cloaca um volume considerável de água é expelido do corpo.

Estas alterações do volume são evidentemente de grande importância para o animal. À parte sua significação na respiração, êles alteram o volume do fluído contra o qual age o sistema muscular do animal. Era, portanto, necessário examinar o fenômeno quantitativamente tanto como pela observação direta. Construiu-se um aparelho simples para registrar as mudanças no volume de uma Holotúria intata (Fig. 1). O animal foi inserido num grande tubo de vidro, 25 cms x 3 cms, imerso em uma grande cuba de água do mar. Um delgado diafragma de borracha (feito de uma bola de borracha de brinquedo de criança) foi esticado sôbre uma das extremidades do tubo. Intrometeu-se a extremidade posterior do animal através de uma cavidade circular no diafragma de maneira a permitir projetar-se por alguns centímetros na água do mar circundante. O diafrágma de borracha impede o extravasamento da água do mar.

Fechou-se com uma rolha de borracha a outra extremidade do tubo que contém o animal, através da qual um tubo de vidro se comunicou por meio de um tubo de borracha com um tubo de vidro vertical de cêrca de 2 cms. de diâmetro. Encheu-se totalmente o aparelho com água do mar. As modificações do volume do animal, devido ao bombeamento cloacal alteram o nível da água no tubo vertical, alterações essas que se registraram no quimógrafo com o auxílio de uma alavanca presa ao flutuador. No fim da experiência removeu-se o animal e fechou-se o diafragma com uma rolha. Calibrou-se, então, o aparelho ajuntando-se 5 a 10 ml. de água do mar, anotando-se a mudança de altura da alavanca inscritora. Finalmente, determinou-se o volume do animal no término da experiência pelo volume de água do mar por êle deslocado quando disposto no vaso de vidro. A fig. 2 mostra o registro típico (26.°C) de um animal de ca de 17 cms. de comprimento com um volume de 150 ml no fim da experiência. Calculou-se a pressão do fluido celômico pelo aparelho semelhante ao descrito por Batham e Pantin (1950) para medida da pressão celentérica de Actínias. Uma cânula, ligada ao manômetro do aparelho por meio de um tubo de borracha flexível de ca de 20 cms. foi inserída na parede do corpo da Holothuria e a pressão registrada com intervalos de 15 segundos. Colocou-se o animal em uma parede cristalizada contendo 3 litros de água do mar, no qual êle podia mover-se livremente. A Fig. 3 mostra um registro típico de um animal de 6 cms de comprimento e 2,5 a 3,5 cms de diâmetro.

Conclusões

1) Os mecanismos da ação muscular de *Holothuri grisea* são investigados. Os músculos longitudinal e circular agem contra o fluido na cavidade do corpo, que em grande parte é celômico.

2) O contrôle do volume do corpo pelas contrações cloacais é estudado. Descreveu-se um aparelho para registro das alterações no volume do corpo. Cada contração cloacal conduz ca de 10% do volume do corpo. A importância mecânica dêste sistema é discutida.

3) Mediu-se a pressão celômica que acompanha o movimento. Em média a pressão interna é de 7 a 21 mm de pressão de água. As contrações máximas atingem 19 cms de pressão de água. Êstes valores estão, em geral, de acôrdo com a tensões registradas nos músculos da parede do corpo. Os mecanismos de sistema são discutidos e comparados com os de *Metridium*. *Arenicola* e outros animais. Indicou-se que a pressão celômica alta auxilía a explicação funcional do fato de na *Holothuria* ser interna a placa madrepórica.

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