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THE RÔLE OF CHEMICAL STIMULATION IN THE SPAWNING REACTIONS OF OSTREA VIRGINICA AND OSTREA GIGAS

By Paul S. Galtsoff

U. S. Bureau of Fisheries*

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It is a well-known fact that chemical stimulation plays an important rôle in the sexual phenomena of many invertebrates. In the majority of cases the complexity of the reaction, consisting of a series of sexual reflexes, precludes the possibility of an accurate physiological analysis. In several water animals, especially in those that possess no organs of copulation, the reaction occurs in a simpler form and, therefore, presents a better opportunity for an experimental study. In 1913 Lillie and Just demonstrated that the males of *Nereis limbata* placed in the water in which the female worm of the same species had been kept for some time immediately began to shed sperm. The presence of sperm in the water in turn incited the shedding of eggs by the female. The reaction, according to these authors, is almost mechanical in its regularity. Fox (1924) working on sea urchins found that extrusion of genital product by one individual causes spawning of all other ripe individuals in the neighborhood.

The present investigation was carried out with two species of oyster, *Ostrea virginica*, the common American species, and *Ostrea gigas*, the Japanese oyster grown in Puget Sound. Because of the character of the spawning reaction of the oyster, it was possible to carry out the work under controlled laboratory conditions and to measure both the strength of the stimulus and the effect produced by it. Oysters used in the experiments were kept in 30-liter glass tanks; the temperature of the water, its oxygen tension, pH, and salinity were kept constant. The movement of shell was recorded by means of a slow motion kymograph. Experiments with *O. virginica* were carried out during the summers of 1927–1929 at Woods Hole. All the experiments with *O. gigas* were made in October, 1929, at Hopkins Marine Station, Pacific Grove, Calif.

Spawning of the female oyster consists of a series of the following reactions: The eggs are discharged from the ovary and fill up the mantle

cavity; the edges of the mantle on the two opposite sides come together and close the cavity, leaving but a small opening; the adductor muscle undergoes a series of rhythmical contractions, causing the discharge of the eggs, which are expelled into the water through the opening between the edges of the mantle.

Churchill (1920), Nelson (1928), and Prytherch (1929) have shown that, in nature, oysters spawn when the temperature of the water has remained for some time above 20°C. Under laboratory conditions, only a few females could be induced to spawn by a rise of temperature. Out of over two hundred experiments with *O. virginica*, positive results were obtained only with two specimens that spawned after they were taken from

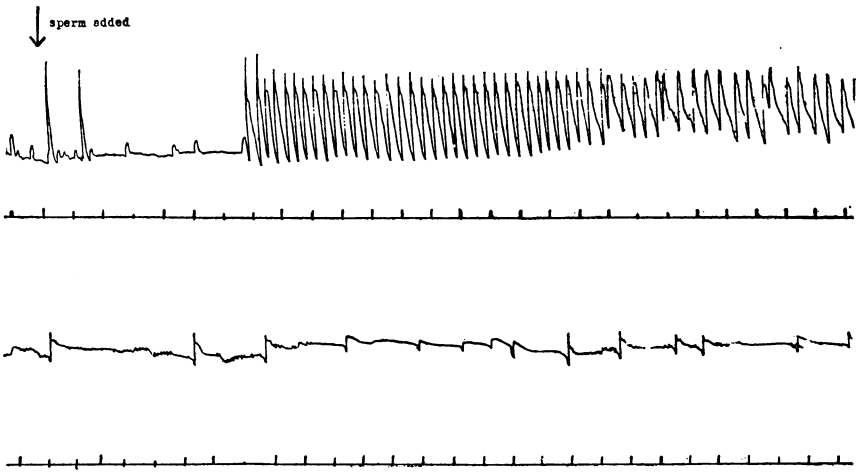

FIGURE 1

Kymograph record of the spawning reaction of *Ostrea virginica*. Lower line represents normal shell movement of the same specimen. Time intervals—one minute.

water of 19° and kept for several hours at a temperature of 27.5°. Spawning of *O. gigas* could be very easily induced by raising the temperature to 30°. In that case the response was immediate.

In both species spawning can be provoked by the addition of a small amount of sperm to the water; a typical case of the reaction is shown in figure 1. In several instances when the oyster was not ripe or after it had already spawned several times, the contractions of the adductor muscle were not accompanied by the discharge of eggs. This indicates that the adductor muscle is not stimulated by the presence of eggs in the mantle cavity or by their passage from the ovary to the cavity. No closing up of the mantle edges was observed when eggs were not discharged.

The latent period of the reaction in *Ostrea virginica* varied from 6 to 38 minutes and, as can be seen by examining table 1, was independent

of the concentration of sperm added. The coefficient of correlation computed from the data presented in table 1 is $r = \pm 0.093 \pm 0.106$, indicating that no significant correlation existed between the two variables. In *O. gigas* the latent period varied from $9^1/_2$ to 17 minutes.

TABLE 1

SHOWING NO SIGNIFICANT CORRELATION BETWEEN THE CC. OF SPERM ADDED AND THE DURATION OF THE LATENT PERIOD

LATENT PERIOD, IN MINUTES	CC. OF SPERM ADDED 0–1.9	2.0–3.9	4.0–5.9	8.0–10.0	TOTAL
35.0–40.0	1	1	1	1	4
30.0–34.9	1	1	1		3
25.0–29.9		1	1		2
20.0–24.9	1	2	2		5
15.0–19.9	2		1	1	4
10.0–14.9	7	3	2		12
5.0– 9.9	5	2	3		10
Totals	17	10	11	2	40

The duration of the reaction varied from $3^1/_2$ to 74 minutes in *Ostrea virginica* and from $13^1/_2$ to 64 in *O. gigas*. No correlation was found either between the concentration of sperm and the duration of the reaction, or between the latent period and the duration. Spawning reaction appears to follow the "All or none principle." It can be initiated by a minimum dose of sperm, which was found to be equal to 0.03 cc. of standard sperm suspension (1 gm. of gonad tissue in 100 cc. of sea water) added to 30 liters of water. This represents a concentration of approximately 150 spermatozoa in each cubic centimeter of water.

Temperature of 20° or above is prerequisite for the spawning of *Ostrea virginica;* if the water is colder than 20°, the sperm fails to provoke the reaction. For *O. gigas*, the temperature limit is 25°.

An analysis of the kymograph tracings shows that so far as the contractions of the adductor muscle are concerned, there is no difference whether the reaction has been initiated by the temperature or by the sperm. The inference can be made that the active principle of sperm suspension does not directly affect the neuro-muscular mechanism but either initiates a chain of reactions which terminate in the contractions of the adductor muscle or causes a release from the tissues of the organism of some substance which affects the neuro-muscular mechanism in a peculiar way. The action of sperm is entirely different from the action of adrenalin, which also causes rhythmical contractions of the adductor muscle. In the latter case, besides the difference in the character of the curve, the latent period lasts only a few seconds.

The spawning reaction is apparently specific, because no positive response was obtained when the sperm of *Mytilus* or *Mya* was used. Control

experiments with the same specimens of oysters gave positive reactions. Unfortunately, opportunity did not present itself to test the effect of sperm of *O. gigas* on *O. virginica* and vice versa.

The spawning reaction of the female is followed by the refractory period during which the organism is insusceptible to sperm. In *Ostrea virginica* this period lasts from 2 to 5 days.

In order to determine whether the active principle is located in the spermatozoa or is present in solution, sperm suspensions were filtered through collodion membranes. In all the experiments, the filtrate was inactive, while the residue contained the active principle. These experiments prove that the active principle is either insoluble in the sea water or is of a large molecular size and fails to pass through the membrane.

The active principle is labile; it is destroyed by heating for fifteen minutes at 55°C.

The spawning reaction of the male consists in shedding of sperm, which is discharged through the cloaca and is carried away with the outgoing stream of water. The adductor muscle is not involved in the reaction, and the shells of the spawning males remaining wide open all the time. In some of the males, shedding of sperm can be provoked by the rise of temperature to 22–23°. Nearly all of them spawn when the temperature is increased to 26–27°. The reaction can be induced also by the addition of egg suspension, the males responding almost immediately by the discharge of sperm. The latent period of the reaction varies from 5 to 12 seconds. The reaction is not followed by a refractory period and can be repeated a number of times until the male is spent.

The active principle of egg suspension is soluble in the sea water and withstands boiling for 10 minutes.

The difference in the latent periods of the spawning reactions of the female and male oysters is apparently due to the fact that the active principle of egg suspension, being soluble in the sea water, directly affects the receptors of the male; whereas the active principle of sperm suspension, because of its insolubility in water or on account of its failure to penetrate through the cell walls, must be first absorbed by the organism and affects the female through the circulatory system. Inasmuch as it has been shown by Yonge (1926–28) that absorption in the oyster takes place only through the digestive tract, one may assume that the sperm affects the female after it has reached its stomach and digestive gland. An indirect evidence corroborating this suggestion is found in the fact that particles suspended in water and caught by the gill epithelium reach the stomach in not less than 5 minutes. That the sperm may act upon the organism through the digestive tract has been demonstrated by Just (1914), who found that shedding of eggs by *Platynereis megalops* is induced by the sperm which had been introduced into its mouth by the male.

Male oysters are not only stimulated by egg suspensions but respond also to the presence of sperm in the water. In several instances ripe males attached to the kymograph were kept for two or three hours in water at 24° but failed to spawn. The discharge of sperm was, however, provoked by the addition of sperm. In that case, the latent period of the reaction, similar to the case of a spawning reaction of a female, varied from 9 to 21 minutes.

Mutual stimulation of the two sexes plays an important rôle in the propagation of oysters. The males respond more readily than the females to the increase in temperature and, under natural conditions, are apparently the first to spawn. The reaction once started by one of the males, stimulates the females and the males nearby, which in turn stimulate the others. In that way spawning spreads all over the oyster bank. The chemical stimulation is influenced in two ways by the temperature: the latter determines the lowest limit (20° for *Ostrea virginica*, 25° for *O. gigas*), below which the females fail to spawn, and supplies the initial stimulus for the spawning of the male.

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ON THE FREE ENERGY OF GLUCOSE AND OF TRIPALMITIN

BY HENRY BORSOOK AND HOWARD M. WINEGARDEN

KERCKHOFF BIOLOGICAL LABORATORIES, CALIFORNIA INSTITUTE OF TECHNOLOGY

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The theoretical maximum amount of work derivable from a chemical reaction is a quantity which we may designate as the reversible work, and which is equal to the decrease in the free energy of the system plus the change in the pressure–volume product. In the form of an equation this is: $W_R = -\Delta F + \Delta(PV)$.

Since the magnitude of the change in the pressure–volume product is rarely significant compared to the free energy change, for practical purposes we may consider the theoretical maximum amount of work derivable from the chemical reactions discussed here as a quantity equal to the decrease in