Effect of Steroid on Oyster—I. Activation of Respiration in Gonad by Estradiol-17 β *

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(Received June 24, 1968)

The studies on the hormones and reproduction in fishes have been reported and reviewed by several authors¹⁾. As HOAR said in his recent review¹⁾, it is clear that the gonadal hormones are universal in broad outline among the vertebrates; those in fishes are also steroid in nature and those synthesized by the testis are androgenic and those synthesized by the ovary are estrogenic. In the invertebrates, on the other hand, there have been few reports concerning the presence of sex control mechanisms by the steroid hormones, but material with estrogenic activity demonstrable by bioassay in rodents has been found in tissues of diverse groups of marine invertebrate²⁾. Recently, BOTTICELLI *et al.*³⁾ and LISK⁴⁾ have identified progesterone, estradiol-17 β , and some other estrogens in the ovaries of marine invertebrates including a marine bivalve, pecten. Estrogens, however, are well known also in the plant world¹⁾, and therefore there is a possibility that those in the marine invertebrates may be taken in through their mouth together with foods. Accordingly, the mere presence of a gonadal steroid is no evidence for the biosynthesis of the steroid sex hormone and its hormonal function. Very recently, we⁵⁻⁷⁾ demonstrated histochemically the presence of the Δ^5 -3 β - and 17 β -hydroxysteroid dehydrogenase activities in the tissues of maturing oysters and the seasonal change of the latter enzyme activity. It has been further proved that the glucose-6-phosphate dehydrogenase system participating in production of reduced nicotinamide-adenine dinucleotide phosphate which is concerned with a number of steps in steroidogenesis is closely related to the sexual maturation of oyster⁸⁾. These results led us to the conclusion that the biosyntheses of sex steroids such as estradiol-17 β or testosterone might exist in the marine bivalve. No informations, however, are available concerning the physiological effect of steroid on oyster. The present paper deals with the investigation on the effects of estradiol-17 β and testosterone on the tissue respiration of oysters during the sexual maturation.

Materials and Methods

The experiments were carried out late in July, 1966. The Japanese common

* An outline of this article was presented to the Ann. Meetg. of Japan. Soc. Sci. Fish., Tokyo, April 3, 1967.

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oysters, *Crassostrea gigas*, cultured for two or three years in Onagawa Bay, Miyagi Pref., by the raft-culture method were used as the materials. The genital canals and gonoducts appeared clear in most individuals, indicating that sexual maturation had proceeded considerably. It was found afterwards that spawning occurred from the beginning to the middle of September⁸. The fresh tissues of gonad, digestive diverticula, gill and pallial margin were sampled and those except gonad were minced with ophthalmic scissors or a safety razor.

Oxygen consumption was measured at 27°C by Warburg manometers, with single-arm vessels of about 17 ml in capacity. The gas phase was air. The shaking rate was about 115 cycles per minute with an amplitude of 35 mm. The main chamber of vessel contained 100 to 150 mg (in wet weight) of ovary, about 150 mg of spermary, 70 to 100 mg of digestive diverticula, about 100 mg of gill or about 200 mg of pallial margin in 1.8 ml of modified Herbst's artificial sea water buffered at pH 8.2 by the method reported previously⁹; 0.3 g of Tris (hydroxymethyl) aminomethane and 0.13 ml of 35% HCl were added to 100 ml of 90% artificial sea water. The final concentration of this solution was equal to about 34‰ in salinity. To absorb carbon dioxide, 0.3 ml of 17% KOH and a filter paper were placed in the center-well. The experimental solution (0.1 ml) in the side arm was the aqueous suspension for injection of estradiol-3-benzoate (0.1 mg) or testosterone propionate (0.05 mg) manufactured by Teikoku Hormone Mfg. Co., Tokyo. It was tipped in the main chamber 20 minutes after the start of measurement. The results showed the increase in the rate of respiration 30 minutes after tipping, because the rate was irregular just after tipping and became constant on and after 30 minutes. Control vessels containing no steroids were also used in each experiment.

Results

The effect of estradiol-17 β on the respiration in the tissues of female oysters is shown in Table 1. A clear increase of about 32% (average) in the respiratory rate was observed in ovary. An increase was also found in digestive diverticula, though it was lower. No definite effect was obtained in gill and pallial margin.

The effect of estradiol-17 β on the respiration in the tissues of male oysters is shown in Table 2. This steroid caused a marked increase of about 77% (average) in spermary respiration. No effect was found in digestive diverticula, except that a considerable decrease was observed in one oyster. No effect was seen also in gill and pallial margin.

The effect of testosterone on the respiration in the tissues of oysters is represented in Table 3. There was no tissue showing an increase in respiration by this steroid. A slightly decreasing tendency was exhibited in spermary and female digestive diverticula.

Table 1. Effect of estradiol-17 β^{*1} on the respiration in the tissues of female oysters during sexual maturation.

Tissue	Experimental No.	Rate of respiration* ² before tipping	Rate of respiration* ² 30 minutes after tipping	Increase in the rate of respiration, %
Ovary	1	22.8	30.6	34.2
	2	18.0	27.6	53.3
	3	25.6	32.0	25.0
	4	20.3	29.2	43.8
	5	20.0	29.0	45.0
	6	26.8	29.5	10.1
	7	26.2	30.1	14.9
	Average			32.3
Digestive diverticula	1	51.6	57.6	11.6
	2	34.3	42.9	25.1
	3	57.1	57.1	0
	4	40.3	52.3	29.8
	5	50.4	56.0	11.1
	6	48.1	52.2	8.5
	Average			14.4
Gill	1	38.4	35.4	-7.8
	2	28.2	30.6	8.5
	3	40.6	41.7	2.7
	Average			1.1
Pallial margin	1	25.8	24.0	-7.0
	2	21.2	22.0	3.8
	3	20.7	20.1	-2.9
	4	22.9	23.3	1.7
	Average			-1.1

*1. 0.1 mg of crystalline estradiol-3-benzoate in aqueous suspension.

*2. μ l. O₂/100 mg fresh wt./hr. measured at 27°C.**Table 2.** Effect of estradiol-17 β^{*1} on the respiration in the tissues of male oysters during sexual maturation.

Tissue	Experimental No.	Rate of respiration* ² before tipping	Rate of respiration* ² 30 minutes after tipping	Increase in the rate of respiration, %
Spermary	1	110.4	235.2	113.0
	2	122.4	187.2	52.9
	3	120.0	233.2	94.3
	4	114.4	174.0	52.1
	5	140.4	220.0	56.7
	6	104.8	200.0	90.8
	Average			76.6
Digestive diverticula	1	55.9	46.0	-17.7
	2	52.4	57.5	9.7
	3	39.0	37.7	-3.3
	4	43.8	45.0	2.7
	Average			-2.2
Gill	1	45.6	46.2	1.3
	2	37.7	39.3	4.2
	Average			2.8
Pallial margin	1	21.6	20.8	-3.7
	2	24.1	22.0	-8.7
	Average			-6.2

*1. 0.1 mg of crystalline estradiol-3-benzoate in aqueous suspension.

*2. μ l. O₂/100 mg fresh wt./hr. measured at 27°C.

Table 3. Effect of testosterone*¹ on the respiration in the tissues of oysters during sexual maturation.

Tissue	Experimental No.	Rate of respiration* ² before tipping	Rate of respiration* ² 30 minutes after tipping	Increase in the rate of respiration, %
Spermary	1	201.3	180.4	-10.4
	2	121.2	114.8	-5.3
	3	186.3	161.8	-13.2
	Average			-9.6
Ovary	1	29.4	28.8	-2.0
	2	31.0	32.4	4.5
	3	25.5	26.4	3.5
	Average			2.0
Digestive diverticula of the male	1	50.4	46.8	-7.1
	2	36.0	37.2	3.3
	3	40.8	44.4	8.8
	Average			1.7
Digestive diverticula of the female	1	55.8	57.0	2.2
	2	58.2	53.4	-8.2
	3	45.6	40.8	-10.5
	Average			-5.5

*1. 0.05 mg of crystals of pure testosterone propionate in aqueous suspension.

*2. μ l. O₂/100 mg fresh wt./hr. measured at 27°C.

Discussion

Observations on the changes of metabolisms in the target organs, *viz.*, uteri or vaginas after administration of estrogen have been often reported in mice or rats. They suggest a very rapid contact between estrogen and target organ¹⁰. According to KERLY¹¹, the respiration and glycolysis in rat uteri were accelerated during 24 hours after the subcutaneous administration of estrogen. With oyster ovary also, estradiol-17 β caused an increase in respiration as shown in Table 1. Besides, in some female oysters, such an increase was observed in the digestive diverticula which were known to show the activity of 17 β -hydroxysteroid dehydrogenase^{6,7}. No effects, however, were found in the gill and pallial margin which did not show this enzyme activity⁸. These results indicate that the oyster also has a clear tissue specificity with the physiological effect of the steroid.

Recently, SCOTT *et al.*¹² reported the formation of 4-androstenedione from testosterone added to ram, bull, dog and fowl semen. SEAMARK and WHITE¹³ observed that spermatozoa of several larger mammals cause the interconversion of estrone and estradiol-17 β , testosterone and 4-androstenedione, and progesterone and 20 β -hydroxyprogesterone. According to HATHAWAY¹⁴, similar steroid metabolisms in spermatozoa exist also in rainbow trout, sea urchin and oyster. The conversion of estradiol-17 β to estrone by sperm preparations is more active in oyster, *Crassostrea virginica*, than in sea urchins, *Arbacia punctulata* and *Echinarachnius parma*. When estradiol-17 β and testosterone are compared as substrates, sperm of *C. virginica*

converts about six times as much estrogen as androgen¹⁴⁾. However, the physiological function of such metabolisms in spermatozoa remains to be investigated. On the other hand, the respiration in the spermaries of considerably mature oysters, *Crassostrea gigas*, was markedly activated by estradiol-17 β (Table 2). The result of the present study together with that of HATHAWAY's one¹⁴⁾ strongly suggests that the metabolism of steroids by oyster sperm may be related to reproduction.

Testosterone, unlike estradiol-17 β , did not activate the respiration of oyster gonads and caused a slight decrease in it with spermary (Table 3). This may be due to unsuitable dosage and/or improper experimental time. Further investigations will be required in this connection.

Summary

1. The effects of estradiol-17 β and testosterone on the tissue respiration were studied, using the Japanese common oysters, *Crassostrea gigas*, during the sexual maturation.

2. Estradiol-3-benzoate caused an increase in ovary respiration. Moreover, it caused a marked increase in spermary respiration.

3. Testosterone propionate, however, did not activate the respiration of gonads and caused a slight decrease in it with spermary.

Acknowledgments

The author is indebted to Professor emeritus Takeo IMAI, Professor Chikayoshi MATSUDAIRA and Assoc. Professor Ryuhei SATO of Tohoku University for their interest in the work and their valuable advice.

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