PRESENCE OF Δ^5-3β-HYDROXYSTEROID DEHYDROGENASE ACTIVITY IN THE TISSUES OF MATURING OYSTERS

PRESENCE OF Δ^5-3β-HYDROXYSTEROID DEHYDROGENASE ACTIVITY IN THE TISSUES OF MATURING OYSTERS

By

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Introduction

It has been recently known that the Δ^5-3β-hydroxysteroids such as pregnenolone or dehydroepiandrosterone are oxidized to Δ^4-3-ketosteroids during the process of the biosynthesis of steroid hormones (1, 2). The enzyme responsible for this oxidation is called Δ^5-3β-hydroxysteroid dehydrogenase, and it replaced the older name of steroid-3β-ol-dehydrogenase. The presence of this enzyme has been demonstrated both by biochemical procedures (1, 3) and by histochemical techniques (4, 5) in mammalian tissues. The enzymic reaction seems to be limited to the organs such as the adrenal, ovary, and testis, all of which have been known as the sites of active steroid hormone production. During the histochemical studies of the enzyme systems in the oyster, the present authors found that Δ^5-3β-hydroxysteroid dehydrogenase activities in the tissues of maturing oysters. Their method employed dehydroepiandrosterone as a substrate and diphosphopyridine nucleotide as a cofactor.

There are no reports, as far as the present authors know, on the presence of steroid hormones in the oyster. No informations are available concerning the steroid sex hormones in the invertebrate tissues (6). The present study reports on the unexpected occurrence of Δ^5-3β-hydroxysteroid dehydrogenase activity histochemically demonstrated in the oyster tissues with the evaluation of the techniques employed.

Materials and Methods

The Japanese oyster, *Crassostrea gigas*, which had been cultured for two years in Matsushima Bay by the raft-culture method, was used as the materials. They were kept in the artificially warmed natural sea water at a temperature

between 24 and 27°C for three to six weeks from October to November in 1964, two months after their natural spawning. Under this condition, they began to maturate partially again. All oysters appeared to be healthy when observed grossly and by their mantle reaction on the mechanical stimulation. The mean shell size of the materials was 11.8 cm in length, 8.2 cm in width and 3.5 cm in depth.

The tissues adjacent to the adductor muscle and the visceral ganglion, and those containing the digestive diverticula, intestines, glycogen-bearing connective tissues and gonads of 11 oysters of both sexes were used. In a few individuals, the mantle and gill tissues were also examined.

The blocks of fresh tissues were placed in a cryostat (temperature, −20°C) for four to five hours. The blocks were then sectioned at 11 to 13 μ. Approximately 70 μ sections were prepared in a few cases. The sections mounted on cover glasses were stored in the cryostat and used within two hours.

Before incubation, the frozen sections were dried at room temperature for several minutes and rinsed in a cold (4°C) acetone bath, for five minutes in the case of thin sections or 10 minutes in the case of thick ones to remove the endogenous lipids. They were then immersed in 0.1M phosphate buffer, pH 7.1, at room temperature for five to 10 minutes. The sections were incubated in the medium containing dehydroepiandrosterone at 37°C for 30 to 120 minutes.

The medium employed was a slight modification of that used by Levy *et al.* (5). It cotained dehydroepiandrosterone (DHA: final concn. 5 mM), Nitro-Blue Tetrazolium (Nitro-BT: 0.16mM), diphosphopyridine nucleotide (DPN: 0.68mM) and phosphate buffer, pH 7.1 (0.057M). Propylene glycol and nicotinamide used in the earlier works of the same authors (5) were omitted, according to the report of Rubin *et al.* (7). The control medium lacked DHA. To test the DPN-dependency of this enzymic reaction, another control medium which contained only DHA and Nitro-BT was used.

After incubation, the sections were fixed for five minutes in 10 per cent neutral formalin and counterstained with Kernechtrot solution (8) for five minutes after washing in distilled water. They were then dehydrated through a series of ethanol and xylene, and finally mounted on slide glasses with Canada balsam. The control sections were treated in the same manner.

The fresh tissues of the adrenal gland, testis, liver and kidney of the albino rats were also studied to confirm the reliability of the above techniques employed by the present authors.

Observation

The presence of Δ^5-3β-hydroxysteroid dehydrogenase activities in the oyster and rat tissues are presented in Table 1, and illustrated in Figs. 1-10.

In four oysters, a very strong reaction was observed in an elongated tissue

Table 1. Histochemical demonstration of Δ^5-3β-hydroxysteroid dehydrogenase activity in the oyster and rat tissues.

Animal	Organ and Tissue		Incubation time in minutes			
			30	60	120	120*
Oyster	Elongated epitheloid tissue adjacent to adductor muscle and visceral ganglion		−～±	+～++	+++	−
	Connective tissue around the above-mentioned tissue		−	−	−～+	−
	Visceral ganglion		−	−	−	−
	Adductor muscle		−	−	−	−
	Intestine		−	−	±	±
	Digestive diverticulum	Duct	−	−	±～+	±
		Tubule	−	−	±	±
	Glycogen-bearing connective tissue		−	−	−	−
	Gill		−	−	−	−
	Mantle		−	−	−	−
	Egg		−	−	−	−
	Sperm		−	−	−	−
	Interstitial cell		−	+	+++	−
	Large type of amoebocyte		−	−	−	−
Rat	Adrenal gland	Zona glomerulosa	+	+～++	+++	−
		Zona fasciculata & reticularis	+++	+++～+++	+++	−
		Medulla	−	−	−	−
	Liver		−	−	−	−
	Kidney		−	−	−	−
	Testis	Spermatogenic cells	−	−	−	−
		Interstitial cells (Leidig)	−	±～+	+	−

* without dehydroepiandrosterone (Control experiment)

located adjacent to the adductor muscle and the visceral ganglion. The reaction was extremely intense after 120 minutes of incubation (Figs. 1, 3, 5, 8). In a few cases, the reaction was remarkable after 30 minutes of incubation. In the sections stained by hematoxylin and eosin, the elongated epitheloid tissues sometimes appeared to be tubular or sheet-like (Fig. 10). The Δ^5-3β-hydroxysteroid dehydrogenase reaction was found in the cytoplasm surrounding the dark rounded nuclei of the epitheloid cells. The distribution and histological characteristics of this tissue will be reported at another opportunity. Weak enzymic activity was also detectable in the connective tissue surrounding the epitheloid tissues after 120 minutes of incubation. As seen in Figs. 2, 4, 9, no positive reaction

was found in the epitheloid and the encircling connective tissues even after 120 minutes of incubation in the control medium.

The visceral ganglion and adductor muscle showed no enzymic activity after 120 minutes of incubation. A strong reaction was observed in the adductor muscle when the treatment with acetone and buffer was omitted. This reaction may be regarded as that of other dehydrogenases working on the endogenous substrates.

The glycogen-bearing connective tissue surrounding the intestines and digestive diverticula showed no formazan deposition. A slight deposition of the formazan was found in the epithelia of the digestive diverticulum and intestine. However, the deposition was also found in the control sections, indicating that it results from other dehydrogenases working on the remaining endogenous substrates. No activity of the Δ^5-3β-hydroxysteroid dehydrogenase was found in the gill, mantle, gonads of any of the individuals. In two individuals, a strong enzymic reaction was found in the interstitial cells, probably a smaller type of the amoebocytes, after 120 minutes of incubation (Figs. 6, 7). The reaction was negative in the control experiment. No reaction was observed in the larger type of amoebocytes.

In the rat, a strong Δ^5-3β-hydroxysteroid dehydrogenase activity was demonstrated in the zona glomerulosa, zona fasciculata and reticularis of the adrenal gland within 30 minutes of incubation. The interstitial cells (Leydig) of the testis also showed positive reaction after two hours of incubation. The tails of the spermatozoa were weakly reactive, but also positive in the control sections. The enzymic reaction was negative in the liver and kidney after the longest time of incubation. The above result showed a very good agreement with those of the previous reports on the same organs (4, 5).

Discussion

The histochemical demonstration of the Δ^5-3β-hydroxysteroid dehydrogenase has been proved to be highly specific and reliable. In mammals, the results obtained on the sites of the enzymic activity showed a very good agreement with that obtained biochemically. Namely, the presence of Δ^5-3β-hydroxysteroids and Δ^4-3-ketosteroids was found in the tissues in which the activity of this enzyme was demonstrable histochemically (1, 2, 3, 7).

In the present study, a strong histochemical reaction of the Δ^5-3β-hydroxysteroid dehydrogenase was observed in the elongated epitheloid tissues adjacent to the visceral ganglion and the adductor muscle, and in the smaller type of amoebocytes in the connective tissue. The reaction was negative when the incubation media did not contain DHA or DPN. The validity of the technic was also confirmed by the experiment done with the rat tissues, since the reaction of this enzyme was limited to the tissues which were already reported as the sites of such activity (4, 5, 7).

The deposition of formazan did not occur in the adductor muscles and in the epithelia of the intestine and digestive diverticulum, when the fresh frozen sections was treated with cold acetone before incubation. This indicated that the reduction of Nitro-BT during the incubation employed in the present study was not due to the reaction of other dehydrogenases working on the endogenous substrates.

The interpretation of the presence of the Δ^5-3β-hydroxysteroid dehydrogenase activity in the oyster tissues demands further investigations on this phenomenon, since no information was available on the presence of the steroid hormones in the oyster tissues. Assuming that the histochemical reaction of the Δ^5-3β-hydroxysteroid dehydrogenase actually indicated the active biosynthesis of the hormones in the oyster, further investigations should be done with the animals in the spring and early summer seasons when they are rapidly maturing. A report in this concern will be given at another opportunity.

Summary

The results in this investigation are summarized as follows:

1. Δ^5-3β-Hydroxysteroid dehydrogenase activity was demonstrated histochemically in the oyster, *Crassostrea gigas*, cultured in Matsushima Bay and collected in October and November of 1964.
2. The activity was found in the elongated epitheloid tissues adjacent to the visceral ganglion and the adductor muscle, and in a smaller type of amoebocytes in the glycogen-bearing tissues.
3. The reliability of the techniques employed in this study was confirmed by the control experiments, and by that on the rat tissues.

Acknowledgement

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Plate 1.

Explanation of the Figures

All microphotographs were taken with the 11-13μ fresh frozen sections fixed in 10 per cent neutral formalin after incubation. The incubation medium contained DHA, DPN, and Nitro-BT in 0.1M phosphate buffer, pH 7.1. Control sections were incubated in the medium containing only DPN and Nitro-BT.

Fig. 1. Δ^5-3β-Hydroxysteroid dehydrogenase reaction in the elongated epitheloid tissues (E) adjacent to the visceral ganglion (G) and adductor muscle (M). Kernechtrot nuclear stain. Two hours incubation. ×125

Fig. 2. Control section from the same oyster tissue. Only the nuclei were stained by Kernechtrot with no deposits of formazan. ×125

Fig. 3. Δ^5-3β-Hydroxysteroid dehydrogenase reaction in the epitheloid tissues of oyster. The reaction is in the cytoplasm surrounding the rounded nuclei of the epitheloid cells. ×500

Fig. 4. Control sections from the same oyster tissue. No reaction is seen. Kernechtrot nuclear stain. ×500

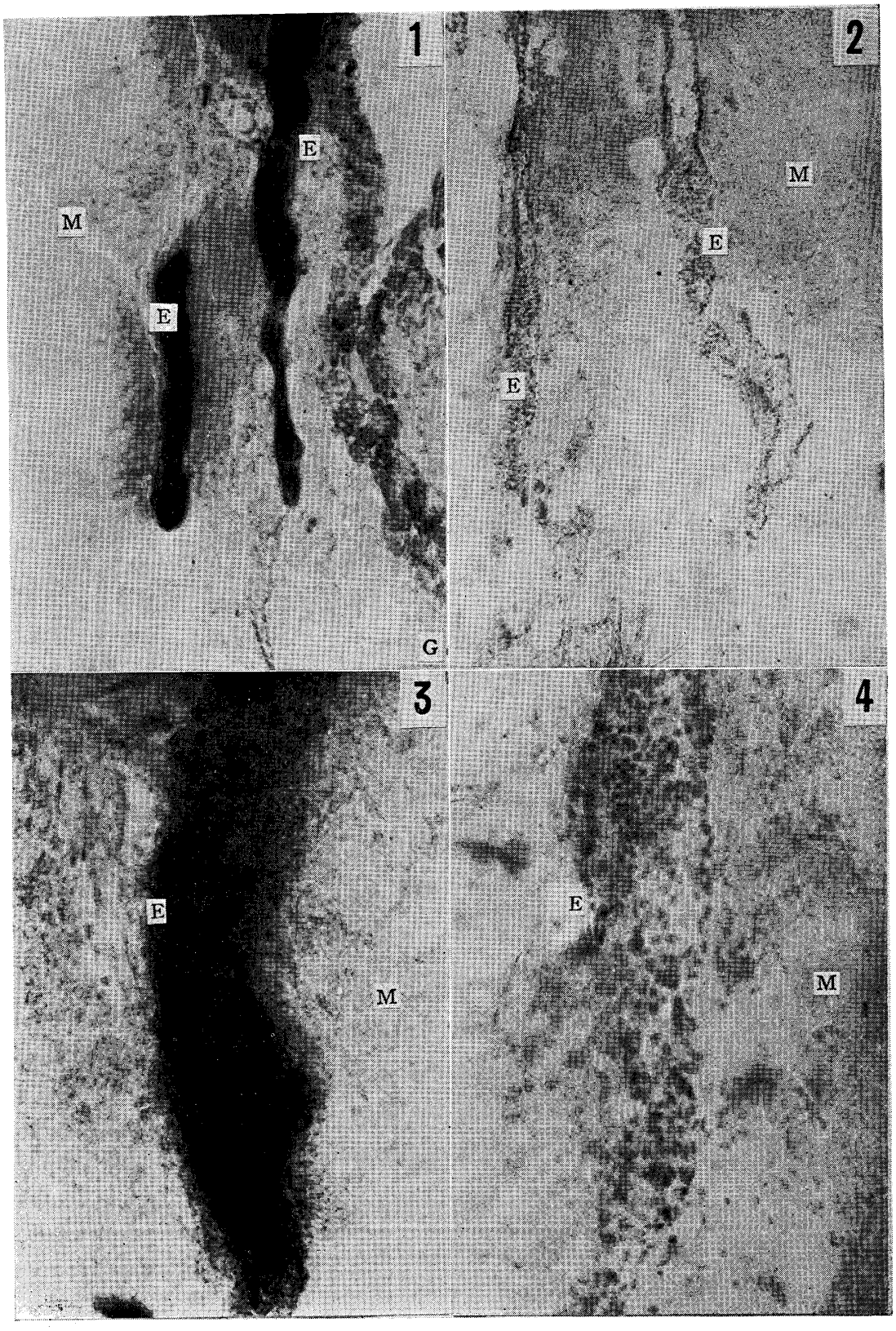
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Plate 2

Explanation of the Figures

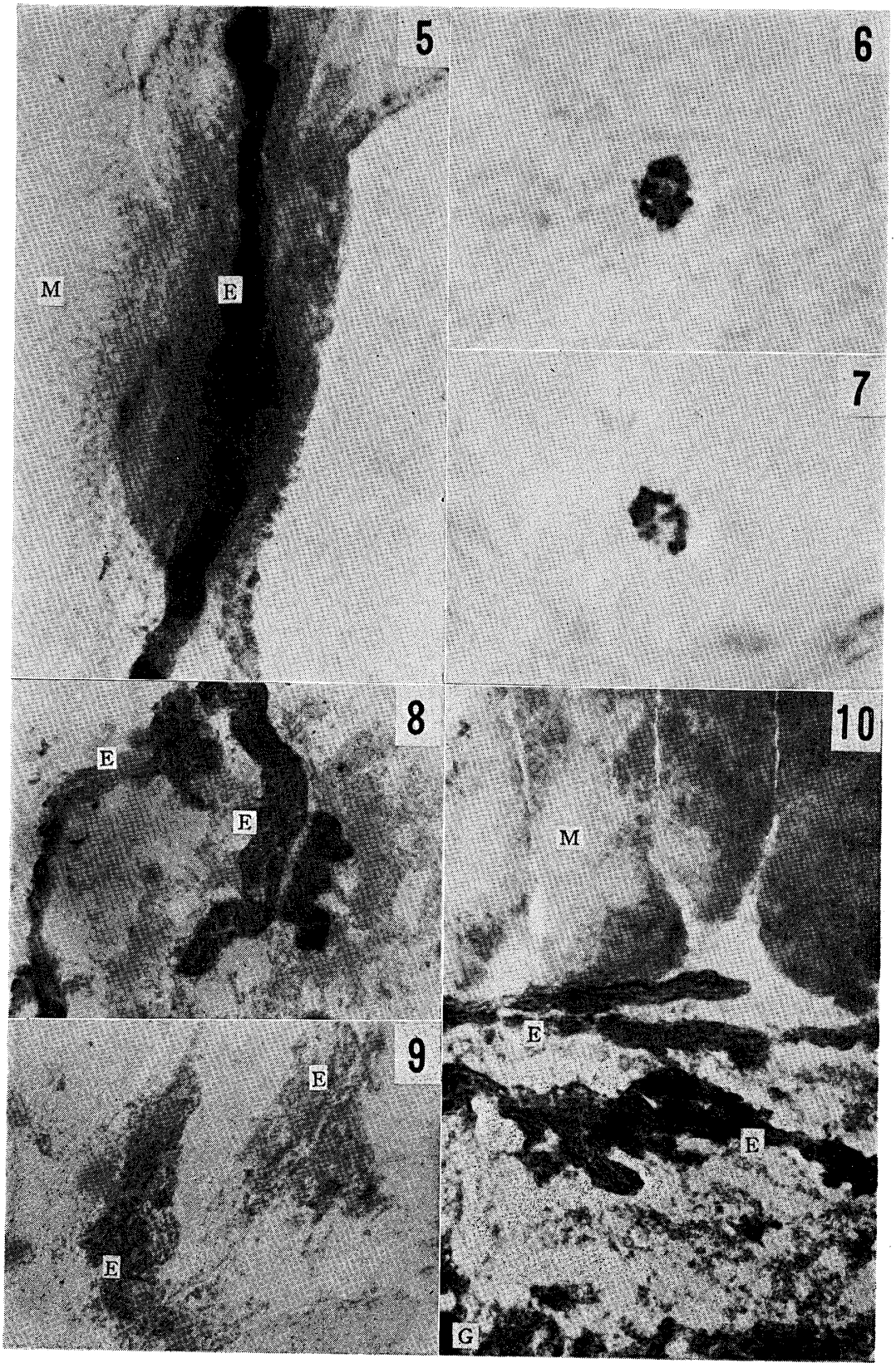
Fig. 5. Δ^5-3β-Hydroxysteroid dehydrogenase reaction in the epitheloid tissues of oyster (same individual as that of Figs. 1–2). ×125

Figs. 6 and 7. Δ^5-3β-Hydroxysteroid dehydrogenase reaction in the smaller type of amoebocytes in the glycogen-bearing connective tissue. The uniform granular depositions of formazan are noted. ×1600

Fig. 8. Δ^5-3β-Hydroxysteroid dehydrogenase reaction in the elongated epitheloid tissues of the oyster. The reaction is found exclusively in the epitheloid cells. ×90

Fig. 9. Control sections from the same tissues as in Fig. 8. No reaction is observed. The nuclei are stained by Kernechtrot. ×90

Fig. 10. The sections from the same tissues as in Figs. 8–9. Hematoxylin-eosin stain. The epitheloid tissues appear tubular in part. ×50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