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Effects of Estrogen on the Activities of Glucose-6-phosphate, Isocitrate, Succinate and Malate Dehydrogenases in the Tissues of the Japanese Oyster, *Crassostrea gigas*[*1]

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Summary

The *in vitro* and *in vivo* effects of estrogen on the activities of glucose-6-phosphate, isocitrate, succinate and malate dehydrogenases (G–6–PDH, IDH, SDH and MDH, respectively) were investigated histochemically in the tissues of the Japanese oyster, *Crassostrea gigas*. There were no essential differences in results between the *in vitro* and *in vivo* experiments during the present study, despite the fact that the kind of estrogen used was different, i.e., estrone in the former experiment and 17β-estradiol in the latter. Estrogen stimulated the activities of G–6–PDH in the epithelia of the duct of digestive diverticula, genital canal and intestine, IDH in the epithelia of intestine and nephridium and SDH in the epithelia of the duct of digestive diverticula and nephridium. However, this steroid inhibited the activities of G–6–PDH in the epithelium of nephridium and MDH in the epithelia of the duct of digestive diverticula, nephridium and intestine. These results indicate that the response of each tissue to estrogen differs with different enzymes examined. *In vivo* experiments, the effects of 17β-estradiol on enzymatic activity were generally observed from 8 to 24 hours after its injection and completely disappeared within 2 weeks after that. From the investigation on the effects of different doses of 17β-estradiol on G–6–PDH activity, it was found that the minimum dose of 17β-estradiol-3–benzoate required by the oyster to inhibit this enzyme activity in the epithelium of nephridium was 10 μg/oyster; this dose is equal to 0.9 μg/g of soft body wet weight.

It has been known since the early 1930's that some marine invertebrates such as a sea urchin, *Echinus miliaris*, and three molluscs, *Aplysia*, *Octopus* and *Eledone*, contain material with estrogenic activity demonstrable by bioassay in rodents (1).

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*1 Effect of Steroid on Oyster—VII.
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However, the chemical identity of substances responsible for this activity remained unknown until the early 1960's. Botticelli *et al.* (2, 3) identified the presence of 17β-estradiol and progesterone in the ovary of the starfish, *Pisaster ochraceous*, in 1960 and in the ovaries of the sea urchin, *Strongylocentrotus franciscanus*, and the pecten, *Pecten hericius*, in 1961. Lisk (4) demonstrated the presence of 17β-estradiol in the eggs of the American lobster, *Homarus americanus*, in 1960. These studies are regarded as the first chemical identity of steroid hormones in invertebrates.

Estrogens, however, are well known also in the plant world, and therefore there is a possibility that those in the marine invertebrates may have been taken in through their mouth 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. In *Crassostrea gigas*, the presence of the activities of Δ^5-3β- and 17β-hydroxysteroid dehydrogenases, which are considered to be responsible for the biosynthesis of steroid hormones, has been demonstrated histochemically in the limited tissues of maturing stage (5–7). Their study has also showed the possible presence in the oyster of 17β-estradiol dehydrogenase which is specific for 17β-estradiol (6). The results of these studies, together with those of the physiological effects of 17β-estradiol on the oyster (8–13), suggest that the biosynthesis of steroid hormones related to reproduction may exist and that at least 17β-estradiol may be functional in this marine bivalve.

Our previous studies revealed that 17β-estradiol has an accelerating effect on the glycogenolysis in female *C. gigas* (12, 13). The fact that such an effect is observed only in the female is of great interest in connection with the evidence that 17β-estradiol is capable of accelerating the sexual maturation in female *C. gigas* (11) and of inducing sex reversal from male to female (10), supporting the idea that this estrogen functions as a female gonadal steroid in the oyster. Hence, the purpose of this study is firstly to evaluate the physiological effects of estrogen on the oyster at the enzyme level, and secondly to refer to the metabolic causes and significance of glycogenolysis during sexual maturation and spawning.

In the present study, which is a continuation of the physiological analysis of the effects of steroid hormone on the Japanese oyster (8–13), we used histochemical methods to investigate the effects of estrogen on the activities of glucose-6-phosphate dehydrogenase (G–6–PDH) of the pentose phosphate pathway and isocitrate, succinate and malate dehydrogenases (IDH, SDH and MDH, respectively) of the TCA cycle, *in vitro* and *in vivo*.

Materials and Methods

The present study was performed from 1977 to 1979. Two-year-old Japanese oysters, *C. gigas*, under hanging culture in Mangoku-Ura, Miyagi Prefecture, were transplanted to nearby Onagawa Bay and used as the experimental materials.

The various experiments were carried out in June (during the process of sexual maturation), July and August (just before the spawning period in 1977 and 1978 and during the partial spawning period in 1979) and October (after the spawning period). The mean shell size$\pm$SD on 100 oysters measured in June, 1978, was 100.2$\pm$14.3 mm in height, 53.0$\pm$6.2 mm in length and 39.3$\pm$5.7 mm in width.

In the *in vitro* experiments, 7 ml of each incubation medium for histochemical visualization of G–6–PDH, IDH, SDH and MDH, respectively, were added to 1 mg of estrone (not 17β–estradiol*) dissolved in a small amount of acetone to form a thin film at the bottom of the incubation dish after evaporation of acetone. The medium for G–6–PDH contained barium salt of G–6–P (final conc., 5 mM), Nitro-Blue Tetrazolium (Nitro-BT: 0.16 mM), NADP (0.56 mM), potassium cyanide (KCN: 5 mM) and veronal buffer, pH 7.8 (57 mM). The incubation medium for IDH consisted of barium salt of DL-isocitrate (5 mM), Nitro-BT (0.16 mM), NADP (0.56 mM), KCN (5 mM) and phosphate buffer, pH 7.8 (57 mM). Sodium salt of succinate (5 mM), Nitro-BT (0.16 mM) and phosphate buffer, pH 7.8 (57 mM) constituted the solution for SDH. The MDH solution was composed of the sodium salt of L-malate (5 mM), Nitro-BT (0.16 mM), NAD (0.68 mM), KCN (5 mM) and phosphate buffer, pH 7.8 (57 mM). The albumen embedded blocks of nephridium, visceral ganglion, adductor muscle, intestine, digestive diverticula and gonad prepared in an acetone and isopentane bath at –80°C were sectioned at 12 μm in a cryostat (–20°C), treated with buffer for 5 minutes and incubated in each medium at 37°C for 60 minutes to detect enzymatic activities. The control media lacked either estrone, substrate or coenzyme. After incubation, the sections were rinsed in distilled water for several seconds, treated with ethyl ether/ethyl alcohol (1:1) for one minute and 70% ethyl alcohol for 10 seconds, then fixed for 15 minutes in 10% neutral formalin and counterstained with Kernechtrot solution 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 incubation media and histochemical procedures employed here were slightly modified from those used previously by Mori (15).

In the *in vivo* experiments, an injection of 17β-estradiol–3–benzoate (EB), 100 μg/oyster as 0.1 ml aqueous suspension, was made into the ovary which had been exposed by cutting off a part of the right valve by means of a small hand-saw (10). Injection of 100 μg/oyster was equivalent to 9 μg/g of soft body wet weight. The exposed ovary of the control oyster was injected with modified Herbst's artificial sea water, 0.1 ml/oyster. After injection, the oysters were suspended in a large-sized outdoor tank in running natural sea water, at ambient temperature, using wire-netting cages (50$\times$50$\times$12 cm deep). The albumen embedded blocks

* 17β-Estradiol is unable to be used in this kind of *in vitro* experiment, since it is one of the hydroxysteroids which may convert Nitro-BT into formazan using NAD or NADP as an intermediate hydrogen acceptor (14).

containing the same tissues as in the *in vitro* experiment described previously were prepared 0, 4, 8, 12 and 24 hours, and 2 weeks after injection, sectioned and incubated in the same media for visualization of G–6–PDH, IDH, SDH and MDH as used in the *in vitro* experiment. The incubation time was 60 minutes for G–6–PDH and 120 minutes for IDH, SDH and MDH at 37°C.

In the other *in vivo* experiment designed to determine the effects of different doses of EB on the activity of G–6–PDH, 0, 1, 10 and 100 μg/oyster of the steroid suspended in 0.1 ml of modified Herbst's artificial sea water were injected into the exposed ovaries and the albumen embedded blocks were prepared 12 hours after injection, sectioned and incubated in the medium for G–6–PDH. The sections after incubation were treated in the same way as in the *in vitro* experiment.

The degree of dehydrogenase activities as judged microscopically by the intensity of intracellular formazan granule deposition was rated from – or 0 (negative reaction) to ++++ or 4 (maximum reaction).

Results

In Vitro Experiments

a) *Effect of Estrone on G–6–PDH Activity*

In the G–6–P plus NADP without estrone system, a moderate or considerably intense enzyme reaction was found in the epithelia of nephridium and duct of digestive diverticula in August, 1977, just before the spawning period of oyster (Table 1). The G–6–PDH reaction in these tissues observed in October, after the spawning period, was weaker than that in June and August. These results were in close agreement with those of Mori (15). In the G–6–P plus NADP plus estrone system, the reaction was stronger especially in the epithelia of the duct of digestive diverticula and genital canal as compared with that in the above-mentioned

TABLE 1. *Effect of estrone on the activity of glucose-6-phosphate dehydrogenase in the tissues of female C. gigas (In Vitro Experiment). Incubation: 1 hr at 37°C.*

Organ and tissue	D-Glucose-6-phosphate*1									without substrate		
	plus NADP plus E*2			plus NADP without E			without NADP plus E			plus NADP plus E		
	I	II	III	I	II	III	I	II	III	I	II	III
Nephridium	++	++	–	+	++	–	–	–	–	–	–	–
Visceral ganglion	±	±	–	–	±	–	–	–	–	–	–	–
Adductor muscle	–	–	–	–	–	–	–	–	–	–	–	–
Intestine	±	+	±	±	±	–	–	–	–	–	–	–
Duct of D.D.*3	++	+++	+	+	++	±	–	–	–	–	–	–
Germ cell	–	–	–	–	–	–	–	–	–	–	–	–
Genital canal	++	++	+	+	+	±	–	–	–	–	–	–

*1 disodium salt. *2 estrone. *3 digestive diverticula.

I: June; II: August; III: October (1977)

system. No formazan deposition was detected in any of the tissues incubated in the control media lacking either NADP or G–6–P but containing estrone. These results indicate that the G–6–PDH in the epithelia of the duct of digestive diverticula and genital canal is activated by estrone.

b) *Effect of Estrone on IDH Activity*

In the isocitrate plus NADP without estrone system, only a trace or very weak reaction of IDH was observed in the epithelium of nephridium, the visceral ganglion and the epithelia of intestine and the duct of digestive diverticula before spawning (Table 2). After spawning, the epithelium of nephridium alone among them showed a trace reaction. In the isocitrate plus NADP plus estrone system, the IDH activity in the epithelia of nephridium and intestine was a little higher than in the previous system without estrone. No reaction was found in any of the tissues incubated in the control media lacking either NADP or isocitrate but containing estrone. These observations suggest that the IDH activity in the epithelia of nephridium and intestine is slightly stimulated by estrone.

TABLE 2. *Effect of estrone on the activity of isocitrate dehydrogenase in the tissues of female C. gigas (In Vitro Experiment). Incubation: 1 hr at 37°C.*

Organ and tissue	DL-Isocitrate*									without substrate		
	plus NADP plus E			plus NADP without E			without NADP plus E			plus NADP plus E		
	I	II	III	I	II	III	I	II	III	I	II	III
Nephridium	+	+	±	±	±	±	–	–	–	–	–	–
Visceral ganglion	±	+	±	±	±	–	–	–	–	–	–	–
Adductor muscle	–	–	–	–	–	–	–	–	–	–	–	–
Intestine	±	+	±	–	±	–	–	–	–	–	–	–
Duct of D.D.	±	+	±	±	±	–	–	–	–	–	–	–
Germ cell	–	–	–	–	–	–	–	–	–	–	–	–
Genital canal	–	±	–	–	–	–	–	–	–	–	–	–

* trisodium salt.
Abbreviations as in Table 1.

c) *Effect of Estrone on SDH Activity*

In the usual incubation medium for SDH (without estrone), a strong reaction was detected in the epithelia of nephridium and duct of digestive diverticula just before the spawning period (Table 3). However, the SDH activity in these tissues declined after spawning. This decline in activity coincides with that of G–6–PDH (Table 1). In the succinate plus estrone system, the SDH activity was higher in the epithelia of nephridium, intestine, duct of digestive diverticula and the germ cell as compared with that in the usual medium (Table 3). None of the tissues examined were positive in the control medium lacking succinate but containing estrone. This data indicates that the SDH activity in some tissues such as nephridium and intestine is enhanced by estrone.

TABLE 3. *Effect of estrone on the activity of succinate dehydrogenase in the tissues of female C. gigas (In Vitro Experiment). Incubation: 1 hr at 37°C.*

Organ and tissue	Succinate*						without substrate		
	plus E			without E			plus E		
	I	II	III	I	II	III	I	II	III
Nephridium	++	+++	++	+	+++	+	–	–	–
Visceral ganglion	±	+	+	±	±	±	–	–	–
Adductor muscle	+	+	±	±	+	±	–	–	–
Intestine	++	++	+	+	+	+	–	–	–
Duct of D.D.	+++	+++	+	++	+++	+	–	–	–
Germ cell	+++	+++	+	++	++	±	–	–	–
Genital canal	±	+	±	±	±	±	–	–	–

* sodium salt
Abbreviations as in Table 1.

d) *Effect of Estrone on MDH Activity*

The MDH activity as judged by the formazan deposition in the usual incubation medium for this enzyme was widely demonstrated in the oyster tissues (Table 4). This finding agrees with the SDH data (Table 3). A weaker reaction of MDH occurred especially in the epithelium of nephridium when estrone was added in the incubation medium (Table 4). All of the tissues investigated were negative in the control media lacking either NAD or malate but containing estrone. These results indicate that the MDH activity in the epithelium of nephridium is inhibited by estrone.

TABLE 4. *Effect of estrone on the activity of malate dehydrogenase in the tissues of female C. gigas (In Vitro Experiment). Incubation: 1 hr at 37°C.*

Organ and tissue	L-Malate*									without substrate		
	plus NAD plus E			plus NAD without E			without NAD plus E			plus NAD plus E		
	I	II	III	I	II	III	I	II	III	I	II	III
Nephridium	++	++	+	+++	+++	++	–	–	–	–	–	–
Visceral ganglion	±	±	±	+	+	±	–	–	–	–	–	–
Adductor muscle	+	+	+	+	+	+	–	–	–	–	–	–
Intestine	++	++	++	++	+++	++	–	–	–	–	–	–
Duct of D.D.	+	++	+	++	++	+	–	–	–	–	–	–
Germ cell	+	++	±	++	++	+	–	–	–	–	–	–
Genital canal	+	++	±	+	++	+	–	–	–	–	–	–

* sodium salt.
Abbreviations as in Table 1.

In Vivo Experiments

a) *Effect of 17β-Estradiol on G-6-PDH Activity*

In June, 1978, the G-6-PDH activity was enhanced slightly in the epithelia

of intestine, duct of digestive diverticula and genital canal by administration of EB, whereas it was a little inhibited in the epithelium of nephridium by this steroid. In July, as shown in Fig. 1, the activity was induced to a small extent in the former three tissues, but inhibited distinctly in the latter tissue by the steroid. Much the same results as in July were obtained in October (Fig. 2). No effect of the steroid was seen in the adductor muscle during the present study.

b) *Effect of 17β-Estradiol on IDH Activity*

In June, the IDH activity increased slightly only in the epithelium of intestine after injection of EB. In July, the steroid induced the activity considerably in the epithelia of not only intestine but also genital canal and nephridium (Fig. 3). In October, a distinct increase in activity was observed in the epithelia of the duct of digestive diverticula and nephridium (Fig. 4). It was noticed that such an enhancing tendency as in July was not obtained in the genital canal in October.

c) *Effect of 17β-Estradiol on SDH Activity*

In June, a very slight rise in SDH activity was found only in the epithelium of genital canal after administration of EB. In July, the enzyme activity in the epithelia of intestine, duct of digestive diverticula and nephridium was induced by the steroid to such a degree as shown in Fig. 5. In October, a high degree of increase in activity was demonstrated in the epithelium of nephridium (Fig. 6). The epithelium of the duct of digestive diverticula showed an appreciable enhancement of activity.

In the experimental oysters injected with EB in October, a remarkable infiltration by small- or medium-sized amoebocytes was present in the epithelia of the duct and tubule of digestive diverticula and the surrounding connective tissue 8 to 24 hours after injection. Some of these amoebocytes exhibited a moderate activity of SDH. However, such an infiltration by amoebocytes was not observed in any of the control animals.

d) *Effect of 17β-Estradiol on MDH Activity*

It was found that the MDH activity decreased after injection of EB in the epithelia of all the tissues investigated during the present study, although the seasonal change in the degree of decrease differed considerably from tissue to tissue (Figs. 7–9). The decline in activity was especially marked in the epithelium of intestine 12 hours after injection in October.

e) *Effects of Different Doses of 17β-Estradiol on G-6-PDH Activity*

In June, 1979, the G-6-PDH activity in the epithelium of nephridium was observed to decrease gradually as the amount of EB injected increased from 1 to 100 μg/oyster, although there was no appreciable difference in activity between 0 (control) and 1 μg/oyster (Fig. 10). In July (during the partial spawning period), no definite relationship could be found between the activity and the amount of

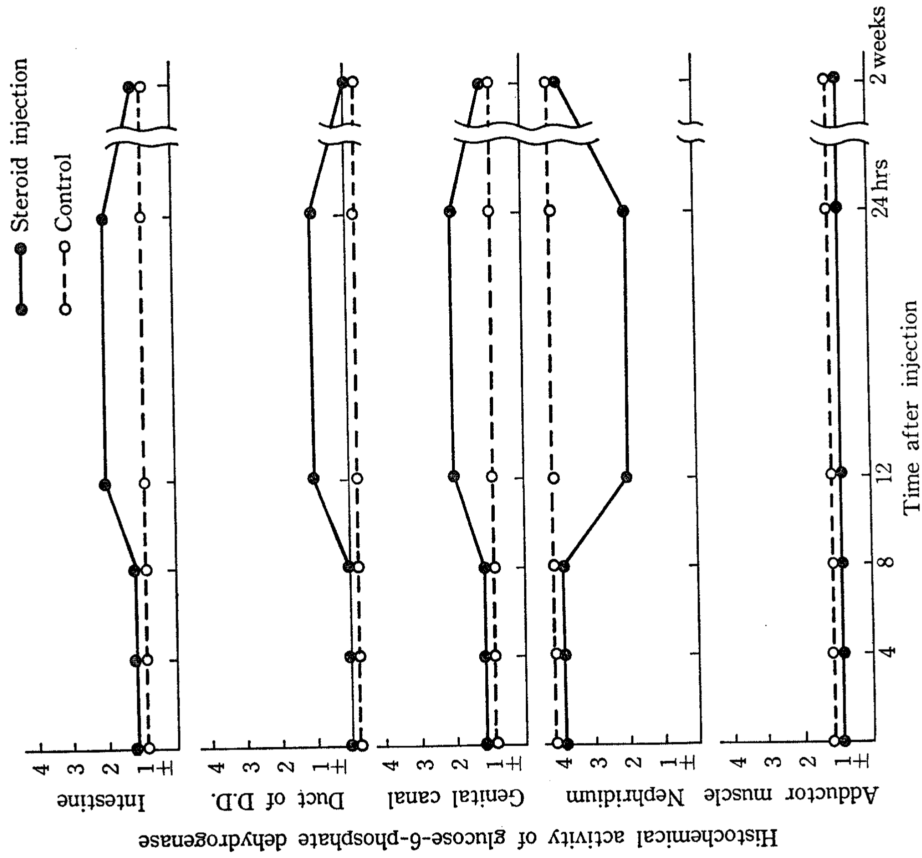

FIG. 2. Effect of 17β-estradiol-3-benzoate on the activity of glucose-6-phosphate dehydrogenase in the tissues of female *C. gigas* (*In vivo* Experiment). Injection: October 24, 1978

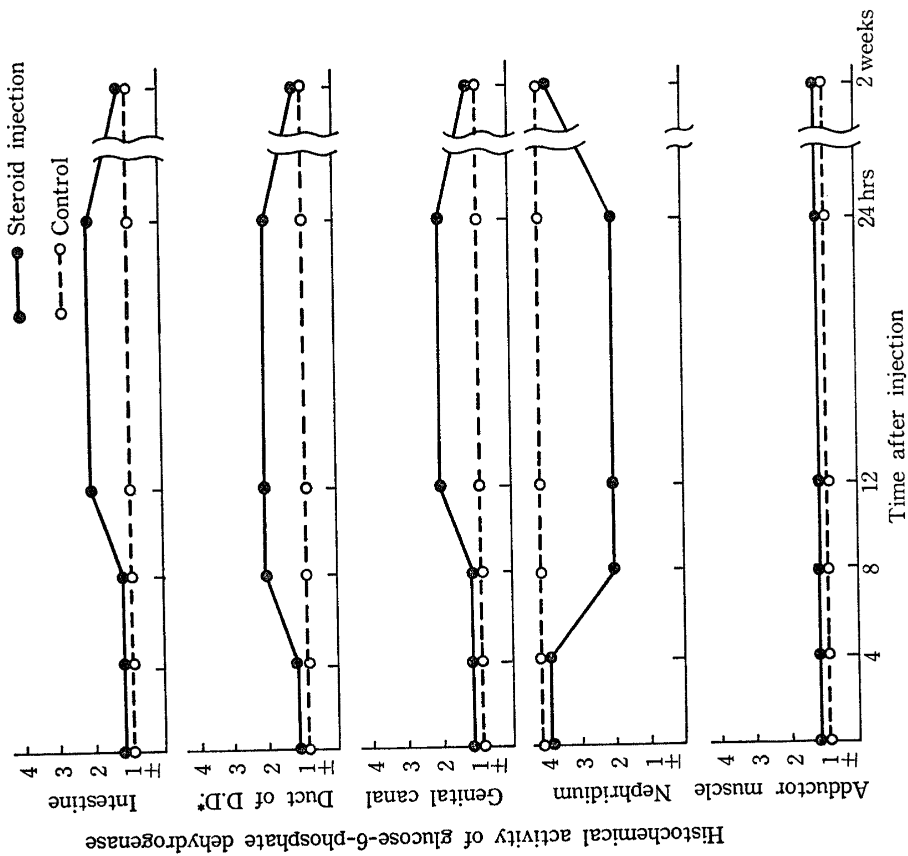

FIG. 1. Effect of 17β-estradiol-3-benzoate on the activity of glucose-6-phosphate dehydrogenase in the tissues of female *C. gigas* (*In vivo* Experiment). Injection: July 28, 1978
* digestive diverticula

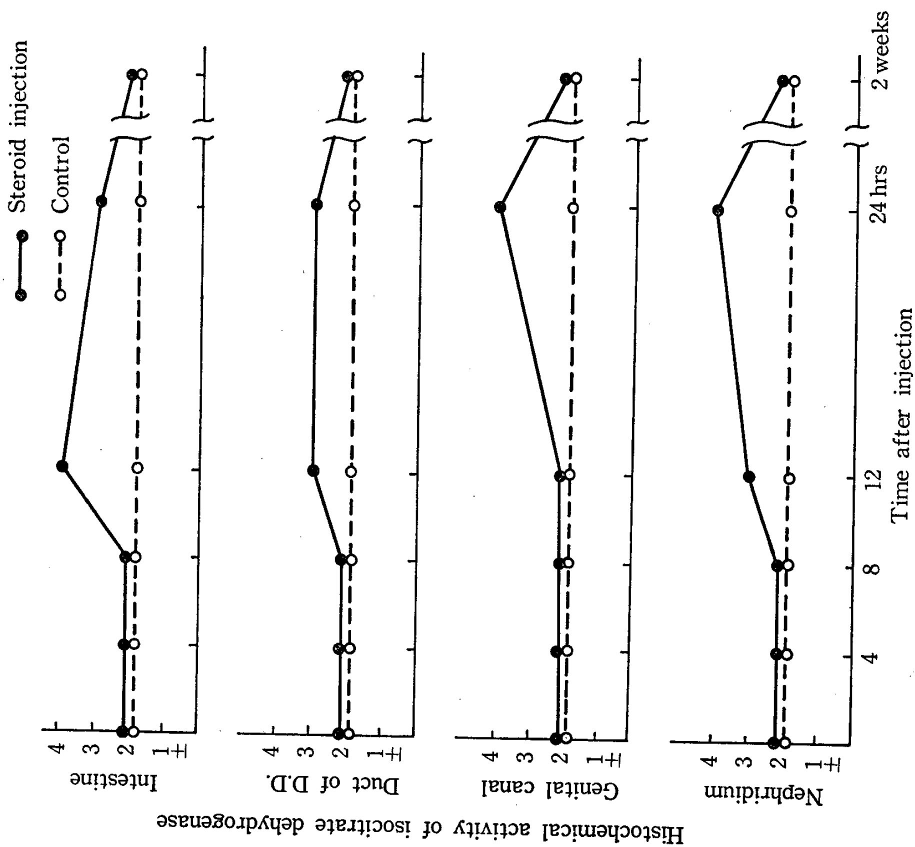

FIG. 3. Effect of 17β-estradiol-3-benzoate on the activity of isocitrate dehydrogenase in the tissues of female *C. gigas* (*In vivo* Experiment). Injection: July 28, 1978

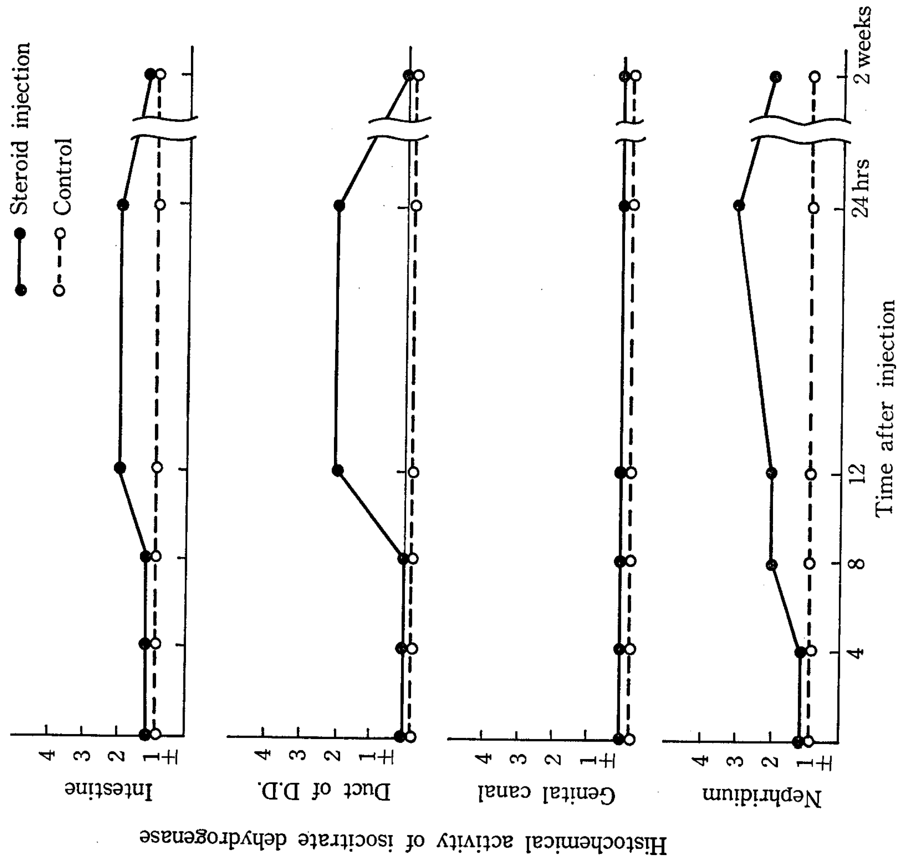

FIG. 4. Effect of 17β-estradiol-3-benzoate on the activity of isocitrate dehydrogenase in the tissues of female *C. gigas* (*In vivo* Experiment). Injection: October 24, 1978

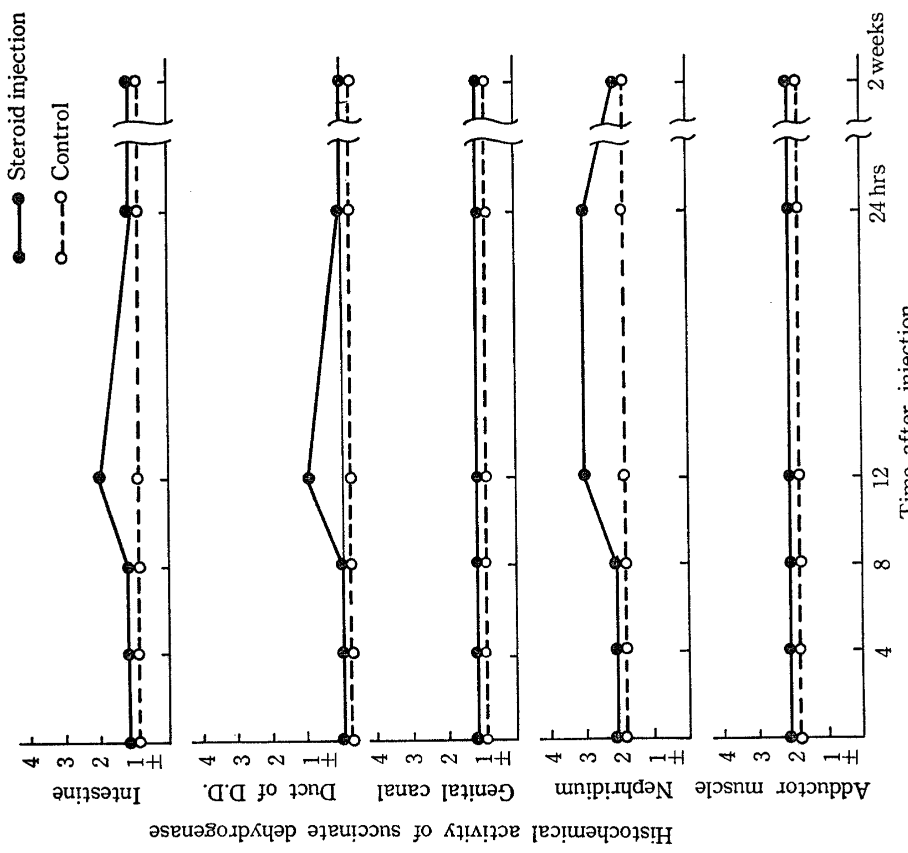

FIG. 5. Effect of 17β-estradiol-3-benzoate on the activity of succinate dehydrogenase in the tissues of female *C. gigas* (*In vivo* Experiment). Injection: July 28, 1978

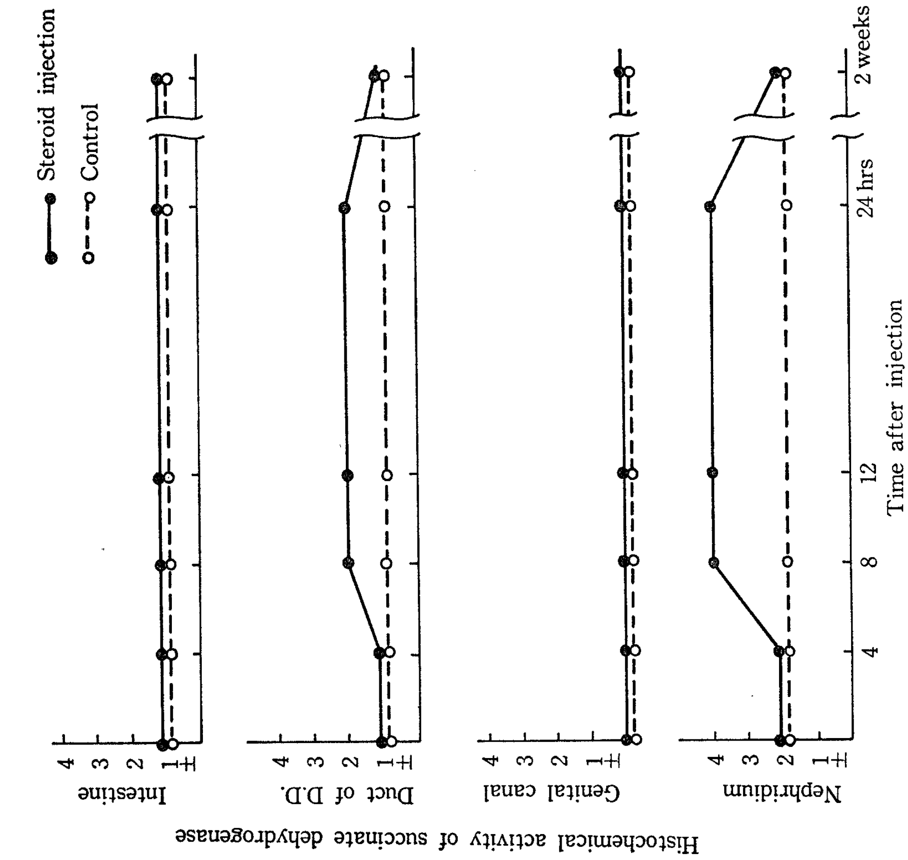

FIG. 6. Effect of 17β-estradiol-3-benzoate on the activity of succinate dehydrogenase in the tissues of female *C. gigas* (*In vivo* Experiment). Injection: October 24, 1978

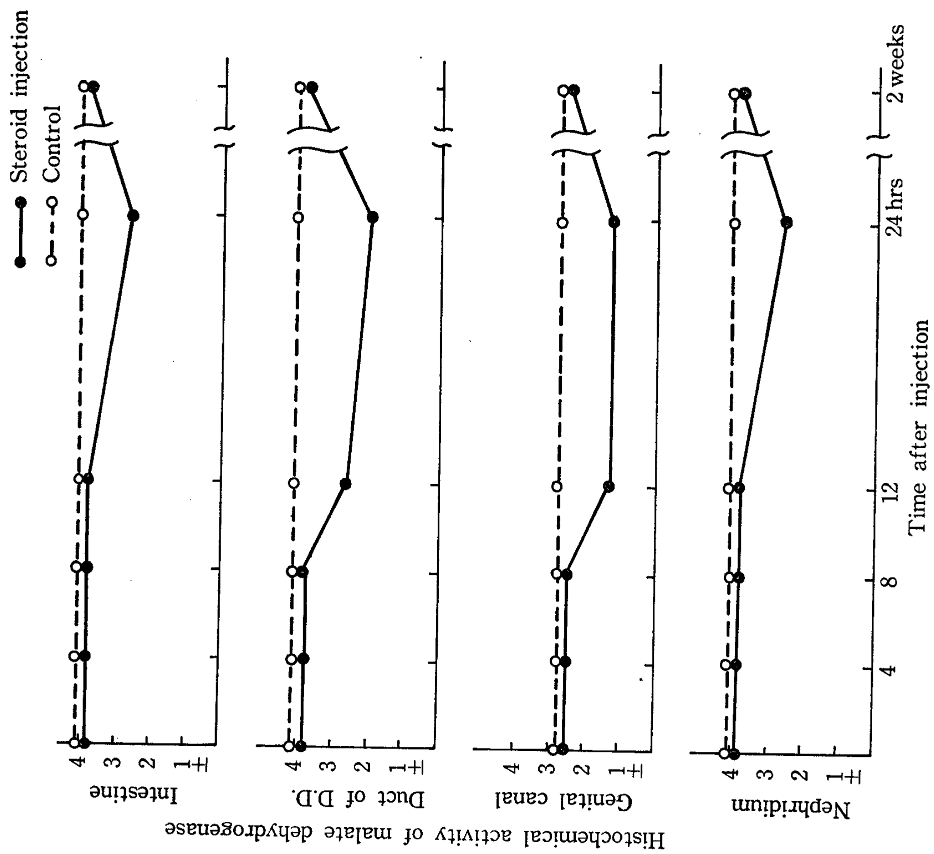

FIG. 7. Effect of 17β-estradiol-3-benzoate on the activity of malate dehydrogenase in the tissues of female *C. gigas* (*In vivo* Experiment). Injection: June 24, 1978

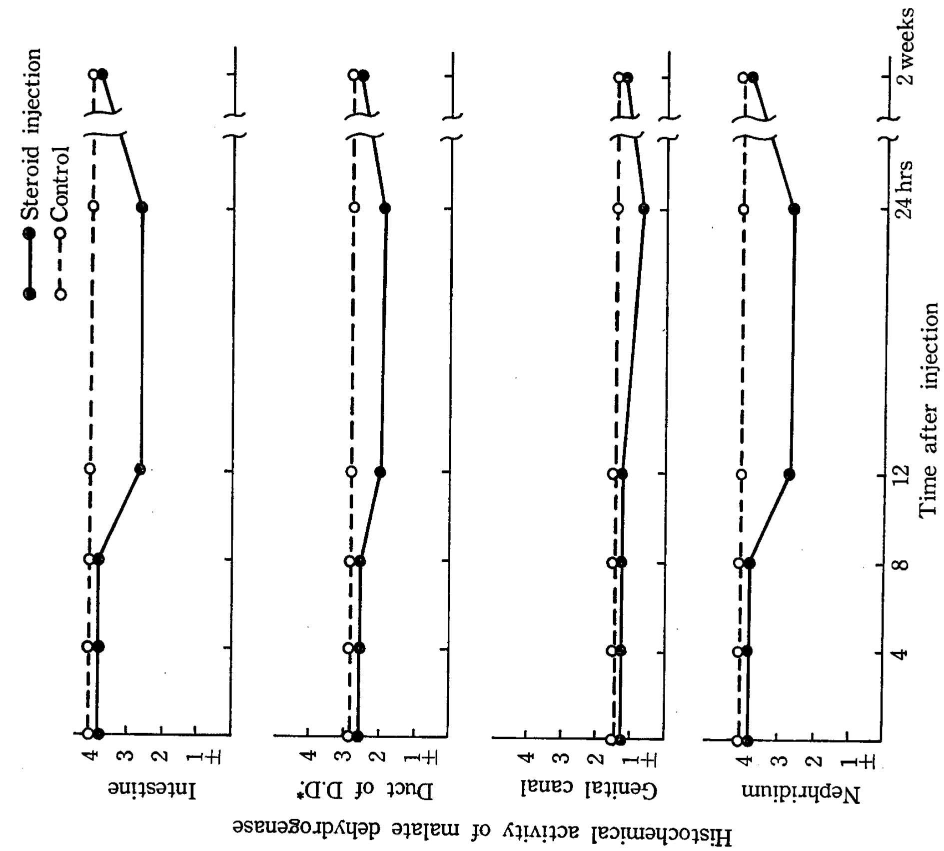

FIG. 8. Effect of 17β-estradiol-3-benzoate on the activity of malate dehydrogenase in the tissues of female *C. gigas* (*In vivo* Experiment). Injection: July 28, 1978
* digestive diverticula

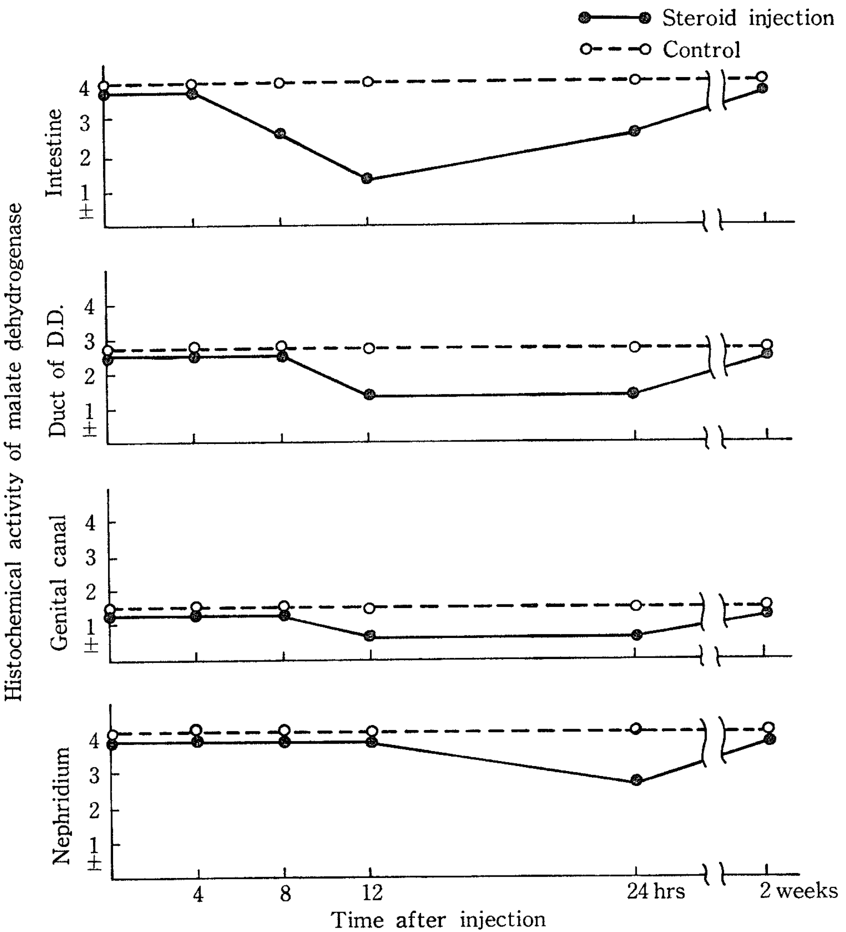

FIG. 9. Effect of 17β-estradiol-3-benzoate on the activity of malate dehydrogenase in the tissues of female *C. gigas* (*In vivo* Experiment). Injection: October 24, 1978

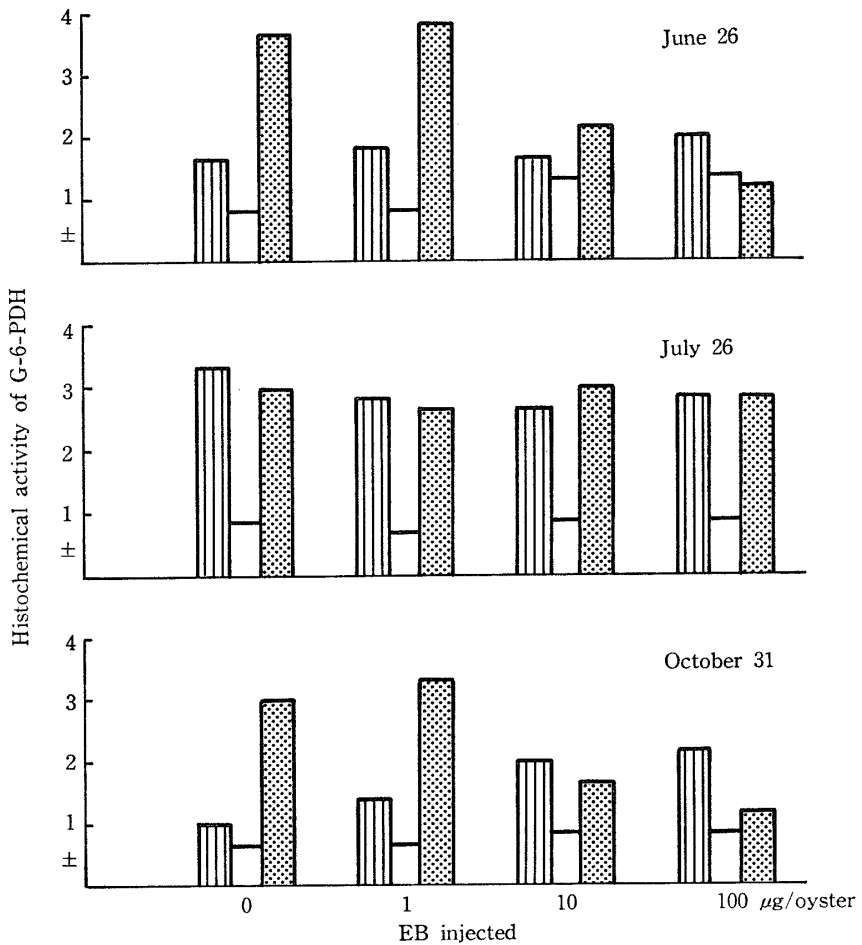

FIG. 10. Effects of 17β-estradiol-3-benzoate (EB) on the activity of glucose-6-phosphate dehydrogenase (G-6-PDH) in the tissues of female *C. gigas* (*In vivo* Experiment, 1979).
▥: Duct of D.D.; □: Tubule of D.D.; ▒: Nephridium

EB in any of the tissues examined. In October, the activity in the duct of digestive diverticula increased slightly with an increase in the amount of EB administered, whereas the activity in the nephridium showed about the same tendency as in June.

These results indicate that the response of the oyster to 17β-estradiol differs with different conditions of gonad development and with different tissues of the animal.

Discussion

There were no essential differences in results between the *in vitro* and *in vivo* experiments during the present study, despite the fact that the kind of estrogen used was different, i.e., estrone in the former experiment (Tables 1–4) and 17β-estradiol in the latter (Figs. 1–9). The response of each tissue to estrogen in both of the experiments was found to differ with the different enzymes examined. The effects of 17β-estradiol on enzymatic activity were generally observed from 8 to 24 hours after its injection and completely disappeared within 2 weeks after that. The duration of the effects and the peak of response remain unknown, since there is no detailed data between 24 hours and 2 weeks.

On the basis of the results described above, the authors have compiled a schematic diagram illustrating the effects of estrogen on the dehydrogenases of pentose phosphate cycle and TCA cycle in the oyster (Fig. 11).

This diagram indicates that the activities of G–6–PDH in the epithelia of the duct of digestive diverticula, genital canal and intestine, IDH in the epithelia of intestine and nephridium and SDH in the epithelia of the duct of digestive diverticula and nephridium are stimulated by estrogen.

G–6–PDH of the pentose phosphate pathway and NADP-dependent IDH of the TCA cycle have been well known to participate in production of NADPH which is concerned with a number of steps in steroidogenesis including the ring closure and hydroxylation. The production of NADPH also leads to fatty acid, nucleic acid and amino acid biosynthesis (15–17). As stated, it seems likely that the biosynthesis of steroid hormones related to reproduction exists in *C. gigas* (5–13, 18). It has been suggested that an activation of the G–6–PDH system by estrogen in the epithelia of the digestive diverticula and intestine leads to an increased synthesis of fats in these epithelia and eggs during the sexual maturation of the oyster (15, 19, 20). The progress of the reactions of these NADP-dependent dehydrogenases is presumed to be accompanied by the following transhydrogenation reaction: NADPH+NAD $\rightleftarrows$ NADH+NADP (21). The major fraction of the biologically useful energy (ATP) of the cell results from oxidative phosphorylation coupled with the oxidation of NADH by NADH-cytochrome c reductase and the cytochrome system. If the transhydrogenation reaction were under the hormonal control of estrogen, this would provide for the regulation of energy-yielding reactions and thus of the amount of energy available for synthetic processes.

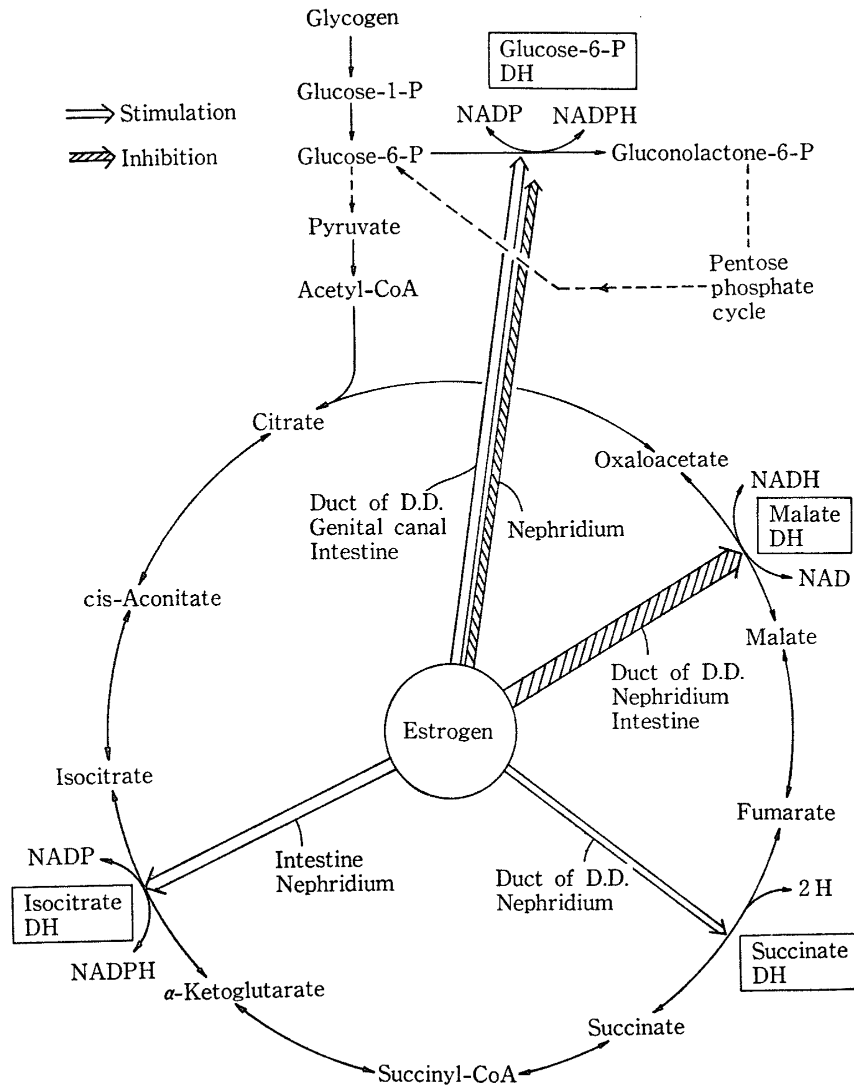

Fig. 11. Effect of estrogen on the dehydrogenases of pentose phosphate cycle and TCA cycle in the female oyster

The IDH and SDH systems are considered to be the rate-controlling steps for the whole TCA cycle. Hence, the stimulation of these enzyme systems by estrogen seems to lead to increased turnover rates of the TCA cycle, resulting in an increase in production of ATP.

In the female oyster, there is clear evidence in tissues of gonad and digestive diverticula that the addition of 17β-estradiol leads to a significant increase in oxygen consumption (8), supporting the above idea that estrogen causes an increase in production of energy-rich phosphate. If the supply of biologically useful energy in the cell were increased, the rates of synthesis of proteins, lipids and nucleic acids, all of which involve energy-requiring reactions, could be increased in female *C. gigas* during progressive development of the gonads.

Fig. 11 shows that the activities of G-6-PDH in the epithelium of nephridium

and MDH in the epithelia of the duct of digestive diverticula, nephridium and intestine are inhibited by estrogen. From the investigation on the effects of different doses of 17β-estradiol on G–6–PDH activity (Fig. 10), it was found that the minimum dose of EB required by the oyster to inhibit this enzyme activity in the epithelium of nephridium was 10 μg/oyster; this dose is equal to 0.9 μg/g of soft body wet weight. At present, however, there is no available data concerning the metabolic causes and physiological significance of the above inhibition.

The most important observations have been made on the relationship between the seasonal biochemical cycles and the annual reproductive cycle in bivalves (see Galtsoff (22), Giese (23) and Gabbott (24) for review). However, there is not yet enough evidence to explain the metabolic causes and physiological significance of the seasonal changes in biochemical composition such as glycogen. In view of this fact, Gabbott and Bayne (25) suggested that *M. edulis* shows a shift from reliance on glycogen as the main source of energy during the summer to a greater reliance on protein as the main source of energy during the winter. Waldock and Holland (26) have suggested that the large amount of lipid stored in the *M. edulis* eggs during the autumn and early winter (the stage of oogenesis and vitellogenesis) is derived from both the diet and glycogen reserves prestored in the mantle tissue during the summer. More recently, Gabbott and Head (17) have attempted to relate the changes in NADP-dependent dehydrogenase activities to the annual reproductive cycle, since gonad development involves considerable metabolic activity, including the production of NADPH for fatty acid and sterol synthesis. They have also described some of the properties of cytoplasmic NADP-dependent IDH from the digestive gland and mantle tissue of *M. edulis* (27). G–6–PDH from these tissues of this bivalve was investigated over two years for changes in specific activity and the apparent Michaelis constants for G–6–P and NADP (28).

NADPH is provided from the breakdown of glycogen *via* the pentose phosphate pathway, therefore glycogenolysis is necessarily activated in steroid biosynthesis (29). In the Japanese oyster, *Crassostrea gigas*, it has been well known that the glycogen content decreases markedly as sexual maturation proceeds (see Giese (23) and Mori (30) for review). In relation to this decrease, a large seasonal variation was observed in the respiratory quotient of the digestive diverticula and pallial margin, indicating the possible changes in the respiratory substrate of these tissues occurring during gonad development (20, 31). Mori *et al.* (7) and Mori (15) investigated the relationship between reproduction and steroid metabolism in the oyster. The results of their studies evidenced a reciprocal relationship between seasonal variations in glycogen content and those in activities of 17β-hydroxysteroid dehydrogenase and glucose–6–phosphate dehydrogenase. These results, together with those on the physiological effects of 17β-estradiol on the oyster (8–13), lead us to assume not only that the biosynthesis of functional sex steroids such as 17β-estradiol exists in the marine bivalve, but also that at least a part of glycogenolysis

during sexual maturation is related to the steroid biosynthesis. A hypothesis was previously proposed concerning the glycogenolysis, steroidogenesis and decline in physiological activity of the oysters during sexual maturation (15). It is reasonable to conclude that the process of glycogenolysis in sexually maturing oysters consists of at least two stages which are as follows: one stage related to the production of NADPH which is necessary for steroid, fatty acid, nucleic acid and amino acid biosynthesis and the other stage connected with the supply of the sources and energy which are required for the gonad development stimulated by estrogen. However, detailed physiological effects of estrogen on the spawning of oysters remain unknown, hence further investigations will be required on this aspect.

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