Genetic and Physiological Control of Esterases in Experimental Small Animals VIII. Influence of Gonadal Hormones on the Liver Esterases of Mice

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Genetic and Physiological Control of Esterases in Experimental Small Animals VIII. Influence of Gonadal Hormones on the Liver Esterases of Mice

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Summary

The liver esterases of mice were separated by starch-gel electrophoresis for comparing the zymograms of females with males. The activities of the two bands (E_1 and E_{10}) were much greater in normal adult males than the corresponding bands in females. The E_{10} band was preferencially hydrolyzed indoxyl acetate.

The E_1 and E_{10} bands disappeared in ovariectomized mice. The E_1 band in castrated males diminished to nearly the same intensity as that in normal adult females. The E_{10} band showed no changes in castrated males.

Several endocrine studies suggest that the activity of the E_1 band are induced by estrone, progesterone and testosterone and that the activity of the E_{10} band are induced by estrone and progesterone.

Other factors such as the influence of extra gonadal hormones on the sex differences was also discussed.

A dependent relationship between the esterases and sex-hormones in the uterus and epididymis of mice was described (1, 2). In recent years, various investigators reported on the sex differences in the electrophoretically distinguishable esterases of liver (3), kidney (4–8), submaxillary gland (7), and plasma (1) in mice, rats and hamsters. In addition, it was reported that the activities of these enzymes were dependent on sex-hormones (1, 4, 5, 8).

One way to demonstrate the physiological role of the esterase polymorphisms is the establishment of regulatory mechanisms among isoenzymes on the hormonal control.

Although sex differences of liver esterases in rats were reported by Schwark and Ecobichon (3), hormonal control of liver esterase polymorphisms is not resolved. The present report deals with sex differences and with the influence of gonadal hormones on the liver esterase zymograms of mice.

Materials and Methods

Animals: Immature and adult male and female mice of CFW inbred strain were used in these studies. Male and female mice were castrated under ether anesthesia at 45 days of age.

Hormone administration: Estrone, progesterone and testosterone propionate were separately injected intraperitoneally into gonadectomized and normal female mice 0.1 μg, 150 μg, and 0.5 mg daily for 7 days from 60 days of age, respectively.

Preparation of the enzyme: The tissues were removed, minced with scissors,

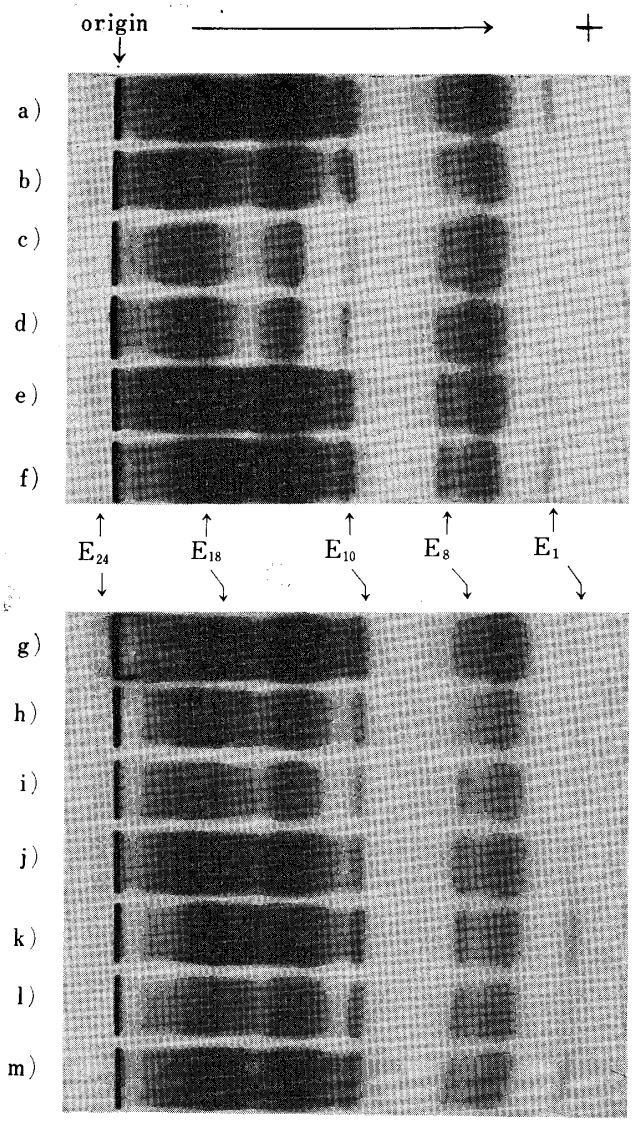

FIG. 1 Zymograms of liver esterases in mice.
Note: Esterase zymograms stained with α-naphthyl acetate as substrate. a) normal female given testosterone propionate, b) and i) ovariectomized female, c) immature female, d) immature male, e) and j) normal female, f) and k) normal male, g) ovariectomized female given progesterone, h) ovariectomized female given estrone, l) castrated male, m) castrated male given testrosterone propionate.

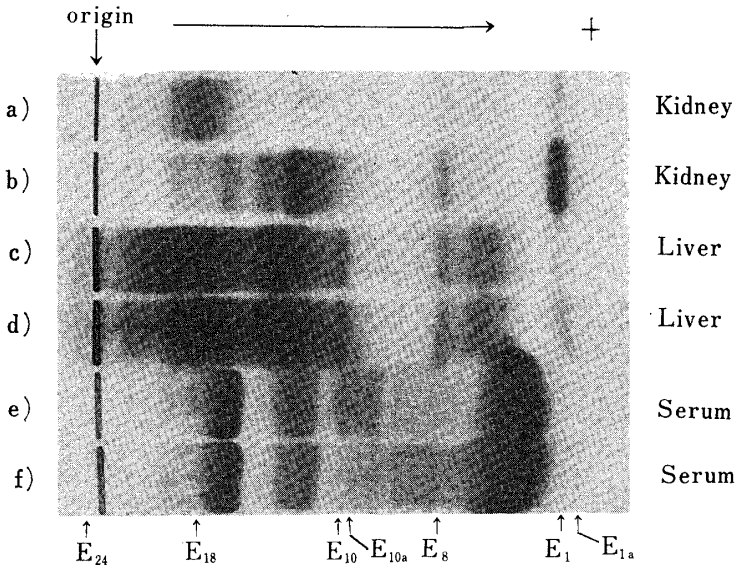

FIG. 2 Zymograms of tissue and serum esterases in mice.
Note: Esterase zymograms stained with α-naphthyl acetate as substrate.
a), c), and e) normal adult female, b), d), and f) normal adult male.

washed in saline (0.85%) several times and weighted after the animals were killed by cervical decapitation. The tissues were homogenized with glass homogenizer in 8 volumes of distilled water at 0°C. Protein determination were performed by means of a modification of the methods of Lowry et al (9). The average protein concentration of the samples (total 41) were 0.62 mg/ml.

Electrophoresis: Electrophoresis was carried out for 8–9 hours with 9.7 V/cm at 15±2°C in a vertical system using the modification of Ashton's buffer system (10). The esterases were stained by incubation of the gel (37°C) in 0.2 M phosphate buffer containing either α-naphthyl acetate, α-naphthyl butyrate, or β-naphthyl butyrate as substrate (10) and in 0.2 M trisaminomethane buffer, pH 7.0 containing indoxyl acetate (0.5% in 2-ethoxyethanol) with potasium ferrocyanide and potasium ferricyanide as oxidation catalyst(11).

Results and Discussion

The liver esterases of mice were separated by starch-gel electrophoresis for comparing the zymograms of females with males. As shown in Fig. 1 and Fig. 2, a total of 16–18 esterase bands (labeled E_1 to E_{24}) were detected with α-naphthyl acetate as a substrate.

Distinct sex differences of liver esterases were detected in normal adult mice. The activity of E_1 band was always greater in normal adult males than in females with α-naphthyl acetate (Fig. 1 e, f, j and k). However E_1 band absented in immature mice (Fig. 1 c and d).

Although no sex difference of E_{10} band in normal adult mice was detected with α-naphthyl and β-naphthyl butyrate and α-naphthyl acetate, a distinct sex difference was detected with the substrate of indoxyl acetate. E_{10} band showed much greater

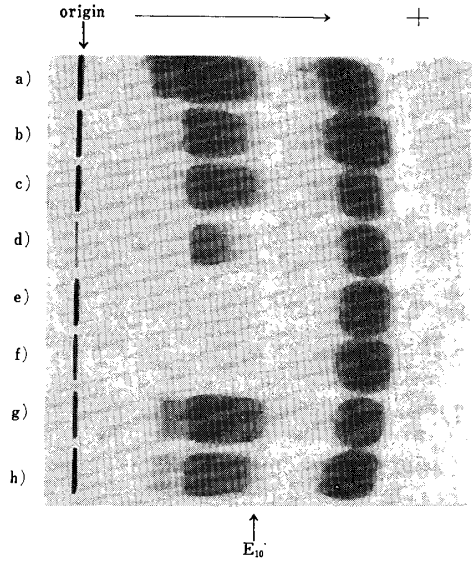

FIG. 3 Zymograms of liver esterases in mice.
Note: Esterase zymograms stained with indoxyl acetate as substrate. a) normal male, b) normal female, c) castrated male, d) ovariectomized female, e) immature male, f) immature female, g) castrated male given testosterone propionate, h) ovariectomized female given estrone.

intensity in normal adult males than the corresponding band in females, but E_{10} band absented in immature mice (Fig. 3 a, b, e and f). Schwark and Ecobichon (3) reported that the band 4 of female liver esterase stained with greater intensity than the corresponding band in male liver. Mice are the opposite of rats in respect to sex differences of liver esterases.

To determine whether the activities of E_1 and E_{10} bands were influenced by sex-hormones or not, several endocrine studies were carried out. E_1 band always disappeared in ovariectomized females and the activity of E_1 band in castrated males diminished to nearly the same as that in normal adult females (Fig. 1 i, j and l). E_{10} band always disappeared in ovariectomized females, but castration of males produced no perceptible changes in E_{10} band (Fig. 3 a-d).

When estrone and progesterone were injected separately into ovariectomized females, E_1 and E_{10} bands appeared again with nearly an equal intensity to that in normal females (Fig. 1 g, h, and j, Fig. 3 b and h). On the other hand, when testosterone propionate was injected into the castrated males and normal adult females, E_1 band appeared again with nearly an equal intensity to that in normal adult males (Fig. 1 k, l and m), and an injection of testosterone propionate produced no perceptible changes in E_{10} band (Fig. 3 a and g). Therefore, it is considered that the activities of E_1 and E_{10} bands are induced by estrone and progesterone in females and the activity of E_1 band by testosterone propionate in males. These results differed from those which the activities of kidney esterase isoenzymes are induced by testosterone in mice and are controlled by both testosterone and estradiol in rats (4, 5). It is of interest that difference in the mode of hormonal influence between E_1 and E_{10} bands are apparent in males.

The activities of E_1 and E_{10} bands were much greater in castrated males than the corresponding bands in ovariectomized females. The cause of this difference is probably complex and includes such factors as differences in the influence of extra gonadal hormones, as well as in the tissue function. It was reported that thyroid hormone affects the activities of various enzymes in liver (12–14). It may be that the activities of E_1 and E_{10} bands are induced by many hormones, for example, the thyroid hormone during development. It is intended that developmental changes and the influence of adrenal and thyroid hormones in E_1 and E_{10} bands be further examined.

A sex difference of the E_1 band was also found in kidney, but not of the E_{10} band. In serum, sex differences were found on E_{1a} and E_{10a} bands migration slightly faster than E_1 and E_{10} (Fig. 2). These observations show that the E_1 and E_{10} bands are not influenced by serum esterases. To demonstrate the character of the liver esterases, substrate specificity and inhibition studies were carried out. The E_1 band hydrolyzed β-naphthyl butyrate, α-naphthyl butyrate and α-naphthyl acetate almost equally. The E_1 band was completely inhibited by DFP at a concentration of 10^{-4}M to 10^{-6}M, but the E_{10} band was not inhibited. The E_1 and E_{10} bands were not inhibited by eserine, EDTA and PCMB at concentrations of 10^{-4}M, 10^{-2}M and 10^{-3}M, respectively. The E_1 band is a type of aliesterase, but the E_{10} band is not.

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