# Genetic and Physiological Control of Esterases in Experimental Small Animals IX. Influence of Gonadal Hormones on the Kidney Esterase Isoenzymes of Mice

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# Genetic and Physiological Control of Esterases in Experimental Small Animals

## IX. Influence of Gonadal Hormones on the Kidney Esterase Isoenzymes of Mice

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### Summary

The kidney esterases in mice of CFW inbred strain were separated by starch-gel electrophoresis for comparing the zymograms of females with males. The activities of five bands ($E_1$, $E_8$, $E_{13}$, $E_{14}$, and $E_{15}$) were much greater in normal adult males than the corresponding bands in females and those of three bands ($E_{17}$, $E_{18}$ and $E_{19}$) were much greater in normal adult females than males. No sex difference was observed in immature mice. Several endocrine studies suggest that the activities of four bands ($E_1$, $E_8$, $E_{13}$ and $E_{14}$) are induced and those of three bands ($E_{17}$, $E_{18}$ and $E_{19}$) are reduced by testosterone propionate.

The $E_1$ and $E_{14}$ bands showed a much lower intensity in RR inbred strain than in the other four inbred strains (CFW, C57BL/6, SS, and DSD).

Hormonal control among the esterase isoenzymes was also discussed.

In recent years, it was reported that the activity of electrophoretically distinguisable esterases were dependent on sex-hormones in several tissues of various species (1–7). However, the physiological role of these esterases was unknown. One way to demonstrate the physiological role of the esterase polymorphisms is the establishment of regulatory mechanisms among isoenzymes for hormonal control.

Although sex differences and hormonal control of kidney esterases in mice have been reported (3, 4, 8, 9), the regulation mechanisms for isoenzymes has not been well demonstrated. The present report deals with the kidney esterase polymorphisms in mice with special reference to its sex difference, interstrain variation and the influence of gonadal hormones.

### Materials and Methods

Mice of five inbred strains (CFW, C57BL/6, SS, DSD, RR, a total of 67 individuals) were used in these studies. Castration was performed under anethesia

at 45 days of age in the CFW strain and at maturation in the other four strains.

Hormone administration, preparation of the emzyme, electrophoresis and detection of the enzyme were carried out as previously described (7, 8). Average protein concentration of the samples were 4.8 mg/ml (7).

## Results

*Sex difference of kidney esterases*: Kidney esterases in mice of CFW inbred strain were separated by starch-gel electrophoresis for comparing the patterns of females with males. As shown in Fig. 1, a total of fifteen to sixteen esterase bands in adult mice were detected with $\alpha$-naphthyl acetate as substrate and labled $E_1$ to $E_{24}$ (7). Distinct sex differences on eight bands of the enzyme were detected in adult mice (Fig. 1 c and d). In immature mice, however, no sex difference of the enzyme was detected (Fig. 1 a and b). The activities of $E_1$. $E_8$, $E_{13}$, $E_{14}$ and $E_{15}$ bands were much greater in adult males than in the corresponding bands in adult

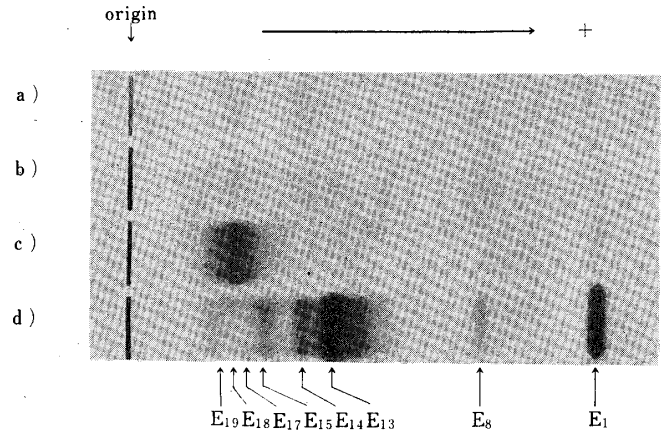


FIG. 1. Sex differences of kidney esterase zymograms in mice of the CFW inbred strain. Note: Esterase zymograms stained with $\alpha$-naphthyl acetate as substrate. a) immature female, b) immature male, c) adult female, d) adult male.

females. On the contrary, $E_{17}$, $E_{18}$, and $E_{19}$ bands showed much lower activity in adult males than females. $E_{13}$, $E_{15}$ and $E_{19}$ bands were lacking in immature mice. The $E_9$ band showed sex difference in the C57BL/6 inbred strain (Fig. 3 d and e). In our previous report (7), the sex difference of the liver esterases in mice was detected in only the $E_1$ band with $\alpha$-naphthyl acetate.

*Influence of gonadal hormones on the kidney esterases*: To determine whether or not the activities of the eight bands were influenced by gonadal hormones, several endocrine studies were carried out. Ovariectomy caused no changes in the zymograms, whereas castration caused distinct changes. $E_1$, $E_8$, $E_{13}$, $E_{14}$, $E_{17}$, $E_{18}$ and $E_{19}$ bands in castrated males showed nearly an equal intensity to those in adult females (Fig. 2 d and f). When testosterone propionate were injected into

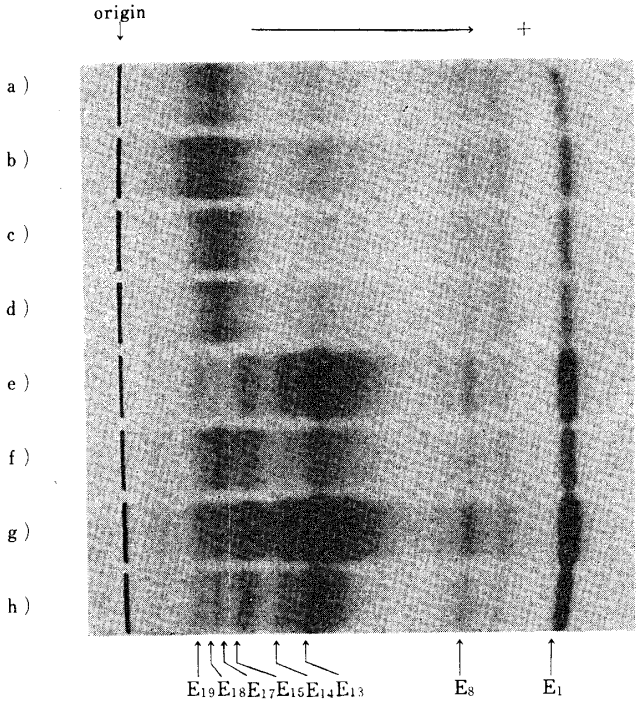


FIG. 2. Influence of gonadal hormones on the kidney esterase zymograms in mice of the CFW inbred strain.

Note: Esterase zymograms stained with α-naphthyl acetate as substrate. a) ovariectomized female given progesterone, b) ovariectomized female given estrone, c) ovariectomized female, d) normal adult female, e) normal adult male, f) castrated male, g) castrated male given testosterone propionate, h) normal female given testosterone propionate.

castrated males and adult females, these zymograms resembled closely those of adult males except for the following points. The activity of the $E_1$ band was always slightly lower in females given testosterone propionate than of the corresponding band in adult males. The $E_{18}$ band always indicated a slightly higher intensity in normal adult females given testosterone propionate than in adult females (Fig. 2 e and h). When estrone and progesterone were separately injected into ovariectomized females, the zymograms showed no changes (Fig. 2 a-d). Therefore, it seems probable that the activities of the $E_1$, $E_8$, $E_{13}$ and $E_{14}$ bands are induced and those of the $E_{17}$, $E_{18}$ and $E_{19}$ bands are reduced by testosterone propionate. The sex differences detected in these studies are independent of the influence of estrone and progesterone. The $E_{15}$ band showed no observable changes in castrated males and mice given gonadal hormones (Fig. 2). The $E_{15}$ band is independent of the influence of gonadal hormones.

The $E_{13}$ and $E_{14}$ bands which were found by us correspond to the male kidney esterase described by Show and Koen (3). The $E_{17}$, $E_{18}$ and $E_{19}$ bands probably correspond to the two bands between the male kidney esterase and origin, which were described by Show and Koen (3). The $E_{13}$ and $E_{14}$ bands correspond to band

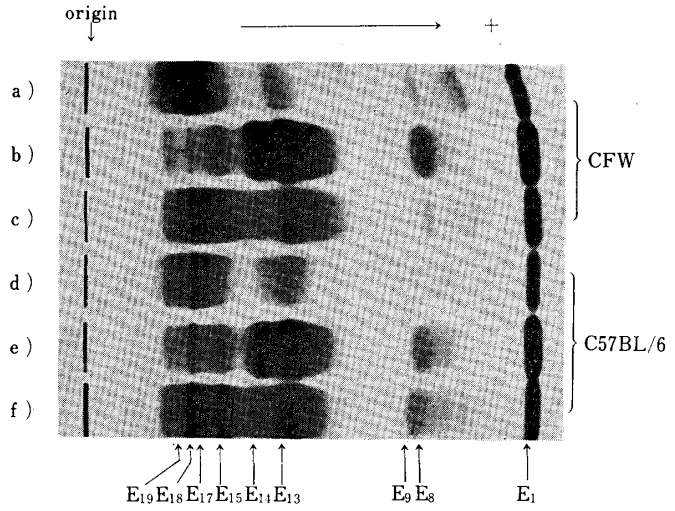


FIG. 3. Interstrain variations of kidney esterase zymograms in mice.
Note: Esterase zymograms stained with $\alpha$-naphthyl acetate as substrate. a) and d) adult female, b) and e) adult male, d) and f) castrated male.

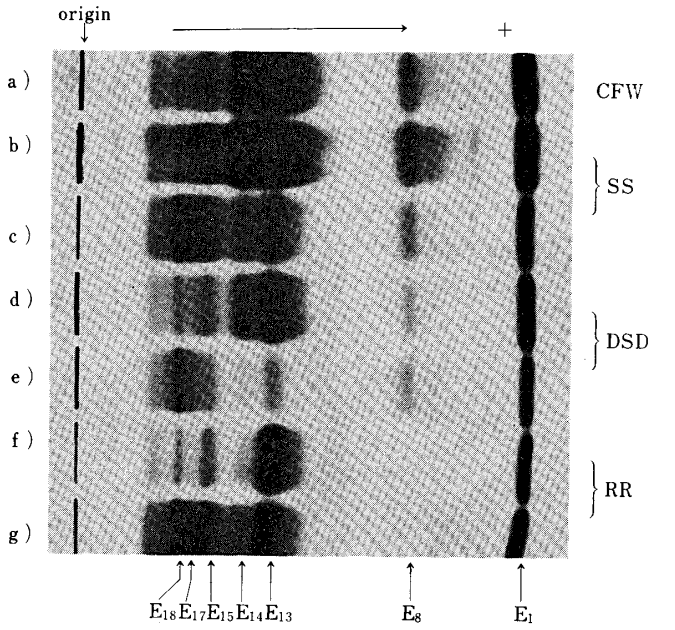


FIG. 4. Interstrain variations of kidney esterase zymograms in mice.
Note: Esterase zymograms stained with $\alpha$-naphthyl acetate as substrate. a), b), d) and f) normal adult male, c), e) and g) castrated male.

III-30 and III-20 of the kidney esterase which were described by Ruddle and Harrington (10), respectively.

*Interstrain variation on the esterases*: The $E_1$ and $E_8$ bands of adult males showed much greater intensity in the CFW and SS than C57BL/6, DSD and RR inbred strains. The activity of the $E_{14}$ band in adult male of RR inbred strain was much lower than the corresponding band in the other four inbred strains (Fig. 3

and Fig. 4). Castration produced distinct changes in the $E_1$, $E_8$, $E_{13}$, $E_{14}$, $E_{17}$ $E_{18}$ and $E_{19}$ bands of the five inbred strains except for the following respects. There were no observable changes in the $E_1$ band of RR and the $E_8$ band of DSD and RR inbred strains (Fig. 4 d-g). The $E_1$ band had no interstrain variation for castrated males of these strains and the $E_{17}$, $E_{18}$, and $E_{19}$ bands were the same (Fig. 3 and Fig. 4). The activities of $E_{13}$ and $E_{14}$ bands in castrated males differed in these inbred strains (Fig. 3 e and f, Fig. 4 c, e and g). These result might indicate that sensitivity to testosterone propionate differs in inbred strains of mice.

*Substrate specificity and inhibition studies*: To determine the characteristic of kidney and liver esterases, substrate specificity and inhibition studies were carried out. $E_1$, $E_{13}$, $E_{14}$, $E_{15}$, $E_{17}$, $E_{18}$ and $E_{19}$ bands of both tissues hydrolyzed with nearly an equal amount of $\alpha$-naphthyl acetate, $\alpha$-naphthyl butyrate and $\beta$-naphthyl butyrate. These bands were inhibited completely by DFP at concentrations of $10^{-4}$M and $10^{-5}$M, but were not inhibited by eserine, EDTA, and PCMB, at concentrations of $10^{-4}$M, $10^{-2}$M, and $10^{-3}$M, respectively. These enzymes are not cholinesterase nor arylesterase but a type of aliesterase (11). The $E_8$ band was not inhibited by any of the inhibitors previously mentioned.

## Discussion

Sex differences in esterase polymorphisms were reported on various tissues of several species (3, 4, 6, 7, 9, 10). These results showed that the activity of the enzyme were much greater in males than in females except for the liver of rat and the submaxillary gland of mouse. From the physiological aspect, it is of interest that there are two case of sex difference observed in these studies; one is much greater and another is much lower in males than females.

Several investigators (3, 4, 7, 9) reported on the sex differences and hormonal control of kidny esterases in mice, but they did not detected any sex differences in the eight bands of the enzyme as described in these studies. Such a difference in our reports may derive from the following: First, the resolution of starch-gel electrophoresis has been considerably improved by us. Second, the number of bands which show sex difference on the zymograms differ in various inbred strains.

The $E_{15}$ band is independent of the influence of gonadal hormones, but this band shows a distinct sex difference. The cause of this difference is probably complex, and includes such factors as difference in the influence of extra gonadal hormones, as well as in the tissue function.

In the adult males, a distinct interstrain variation was detected on the $E_1$ and $E_{14}$ bands, which were influenced by testosterone propionate. But no interstrain variation showed on the activity of the $E_1$ band in castrated males. We may conclude that the activity-levels of the esterase which were induced by testosterone propionate are genetically controlled. It is intended to ascertain whether or not the activities of the $E_1$ and $E_{14}$ bands are genetically controlled.

It was reported that the $E_1$ band of liver esterase was induced by estrone and progesterone in females, and testosterone propionate in males (7). However, the $E_1$ band of kidney esterase is induced by only testosterone propionate. The $E_{13}$, $E_{14}$, $E_{17}$, $E_{18}$ and $E_{19}$ bands of the liver esterses are independent of the influence of gonadal hormones (7). On the other hand, these kidney bands are influenced by testosterone propionate. These bands in both tissues, however, have the same properties for various substrate and inhibitors. Considering these results for the esterase isoenzymes, it would indicate that the mechanisms of gonadal hormone actions on esterase activity differ according to tissue. It is of interest that the difference in the mode of hormonal influence are apparent between the $E_1$, $E_{13}$ and $E_{14}$, and $E_{17}$, $E_{18}$ and $E_{19}$ bands.

Although the physiological role of the male kidney esterase has been unknown, Show and Koen (3) suggested that it is an adaptive enzyme that functions in the secretion of male steroid products. Ruddle and Harrington (10) reported that a sexual dimorphism in esterase activity may be related to the patterns of sexual behavior. In addition to sex difference, it may be that an interstrain variation in the $E_{14}$ band is an adequate explanation of the physiological role of the male kidney esterase of mice.

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