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著者	KOMEDA Kajuro, MANDA Masaharu, NISHIDA Shusaku
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Genetic and Physiological Control of Esterases in Experimental Small Animals

VII. Genetic Control of Esterase Zymograms in Mammary Gland and Uterus of Mice

Kajuro KOMEDA, Masaharu MANDA and Shusaku NISHIDA

Department of Animal Husbandry, Faculty of Agriculture,
Tohoku University, Sendai, Japan.

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Summary

The mammary gland and uterus esterase from 5 inbred strains of mice (CFW, C57BL/6, NC, SS, DSD) were studied by starch-gel electrophoresis combined with histochemical staining methods. The mammary gland and uterus of the C57BL/6 strain (Es-7A) had an esterase band (E_2) of greater electrophoretic mobility than the corresponding esterase, E_3, in similar tissues from the other four strains (Es-7B).

The mammary gland and uterus of F_1 progeny from crosses between C57BL/6 and NC strains always contained both esterase bands of the parent (Es-7AB). This analysis indicated that these enzymes were co-dominantly inherited in F_1 progeny. The activity of the greater mobility band was always lower than that of a slower band. On the basis of the genetic analysis, it is concluded that three phenotypes are genetically controlled by alleles (Es-7^A, Es-7^B) of a single autosomal locus.

These esterase bands were a type of aliesterase from substrate specificity and inhibition studies.

The starch-gel electrophoresis (1) combined with histochemical staining methods (2) are very suitable for detection of various enzymes. Recently, using this technique, a number of studies dealing with the genetical variations of various enzymes has been reported in many animals (3).

Although several investigators have reported on the inheritance of serum and erythrocyte esterase zymograms of mice (4–6), that of esterase zymograms of mice tissues has not yet been studied sufficiently (7–11). Moreover, the physiological role of these electrophoretically distinguishable esterases of mouse tissues are scarcely known.

Several investigators have been interested in a correlation between esterase activity and steroid biosynthesis in steroid-producing endocrine tissues (12, 13). It has been reported that the esterase activity was initiated and controlled by the

sex hormone in the reproductive organs (14, 15). Hence we study the genetic and physiological control of the esterase zymograms in the reproduction organs and mammary glands of mice.

The present report deals with variations among strains, genetic control and classification of the esterase zymograms in the mammary gland and uterus of mice.

Materials and Methods

Animals: Eight to fifteen week-old male and female mice of CFW inbred strain, eight to thirteen week-old female mice of four inbred strains (NC, C57BL/6, SS, DSD, 8–9 individuals of each inbred strain) and mice crossbred between C57BL/6 and NC were used in these studies.

Preparation of the enzyme: All animals were killed by cervical decapitation. The tissues were minced with scissors and washed in saline (0.85%) several times. The kidney and liver, mammary gland (abdominal) and uterus, and fatty tissue were homogenized with glass homogenizer in 8 volumes, 4 volumes, and 1 volume of distilled water, respectively. The homogenates were centrifuged at 15,000 rpm for 50 minutes at 0°C, and the supernatant was used for analysis. All samples were stored at –20°C until required for electrophoresis.

Electrophoresis: Electrophoresis was carried out in a vertical starch-gel system (1) using a modification of the buffer system of Ashton (16). The bridge buffer contained lithium hydroxide (1.2 g) and boric acid (11.8 g per litre) at pH 8.2. The gel buffer was mixed with nine volumes of the solution containing citric acid (1.2 g) and tris (4.8 g per litre), to one volume of bridge buffer. Hydrolyzed potato starch was employed at a concentration of 13%. Electrophoresis was performed with 9.7 V/cm for 8–9 hours at 15±2°C.

Staining for esterase: Esterases were stained histochemically by incubation of the gel (37°C) in a mixture containing α-naphthyl acetate with naphthanil diazo blue B in 0.2 M phosphate buffer at pH 6.8. Inhibition studies were carried out for 60 minutes in 0.2 M phosphate buffer (pH 6.8) with the concentrations used in Table 3.

Results

Variation among strains: Mammary gland and uterus esterase from 5 inbred strains were separated by starch-gel electrophoresis for comparing the electrophoretic mobility and patterns of the enzymes.

As shown in Fig. 1 and Fig. 2, a total of 10–12 esterase bands were detected with α-naphthyl acetate as a substrate on the starch-gel. This study was concerned only with the variation of mobility and activity-levels of the esterase bands which migrated near the anode end. The mammary gland and uterus

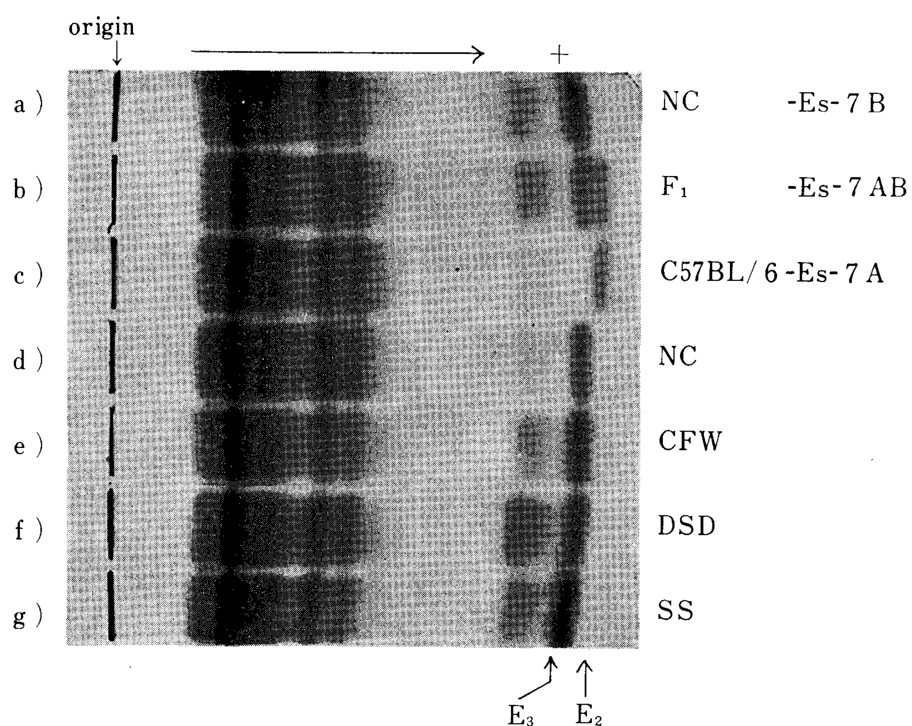

Fig. 1. Genetic variation of mammary gland esterase zymograms in mice.
Note: Esterase zymograms stained with α-naphthyl acetate as substrate.

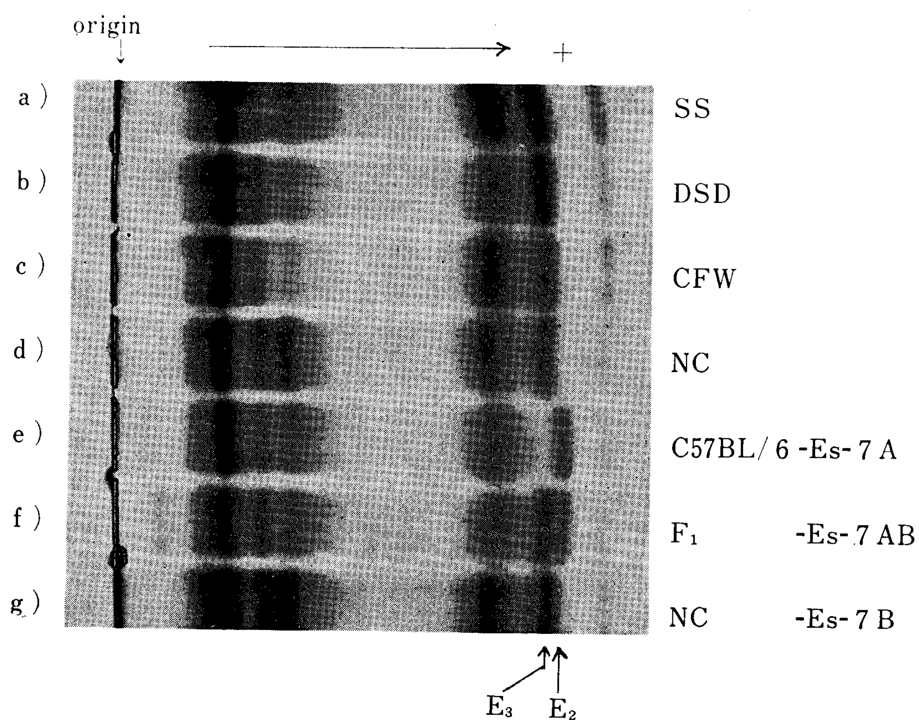

Fig. 2. Genetic variation of uterus esterase zymograms in mice.
Note: Esterase zymograms stained with α-naphthyl acetate as substrate.

TABLE 1. *Percentage Activity of E_2 and E_3 Esterase Bands of the Mammary Gland and Uterus in Mice*

	Strains	No.	Percentage activity of esterase bands		
			E_2	E_2 plus E_3	E_3
Mammary gland	C57BL/6	6	5. 25±0. 61%		
	F_1	8		6. 73±0. 84	
	NC	6			7. 19±0. 79
Uterus	C57BL/6	6	5. 35±1. 64		
	F_1	8		7. 23±0. 77	
	NC	6			8. 12±0. 94

Note: Density was measured by Densitometer Ozumor-82 type.

of C57BL/6 strain had an esterase band (labeled E_2) of greater electrophoretic mobility than the corresponding esterase, E_3, in similar tissues from the other four strains.

As shown in Table 1, the percentage activity of E_2 band was always lower than that of E_3. In the E_3 band, the variation of esterase activity-levels among the strains was not clear.

Genetic analysis: To examine the mode of genetic control of the variation among strains, reciprocal crosses were made between C57BL/6 and NC strains. As shown in Fig. 1, b and Fig. 2, f, all F_1 progeny always contained both E_2 and E_3 bands and the activity of E_2 band was always lower than that of E_3. The percentage activity of these bands in F_1 progeny were slightly higher than those of the intermediate in both phenotypes (Table 1).

The E_2, E_3 and double banded pattern will be referred to as Es-7A, Es-7B and Es-7AB respectively, and the genotype of Es-7A, Es-7B and Es-7AB of phenotype may be estimated as $Es\text{-}7^A/Es\text{-}7^A$, $Es\text{-}7^B/Es\text{-}7^B$ and $Es\text{-}7^A/Es\text{-}7^B$ respectively.

TABLE 2. *Distribution of Esterase Types in Mammary Gland and Uterus in Progeny from Various Matings*

Mating		Phenotypes of progeny					
		Es–7A		Es–7AB		Es–7B	
Strains	Types	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
NC×NC	Es-7B×Es-7B	0		0		8	
C57BL/6×C57BL/6	Es-7A×Es-7A	9		0		0	
C57BL/6×NC	Es-7A×Es-7B	0		14		0	
NC×57BL/6	Es-7B×Es-7A	0		8		0	
F_1*×F_1*	Es-7AB×Es-7AB	23	25. 75	51	51. 5	29	25. 75
C57BL/6×F_1*	Es-7A×Es-7AB	13	11. 5	10	11. 5	0	
F_1*×NC	Es-7AB×Es-7B	0		7	8	9	8

*F_1 (C57BL/6×NC)
Note: The phenotype of male was estimated from that of female.

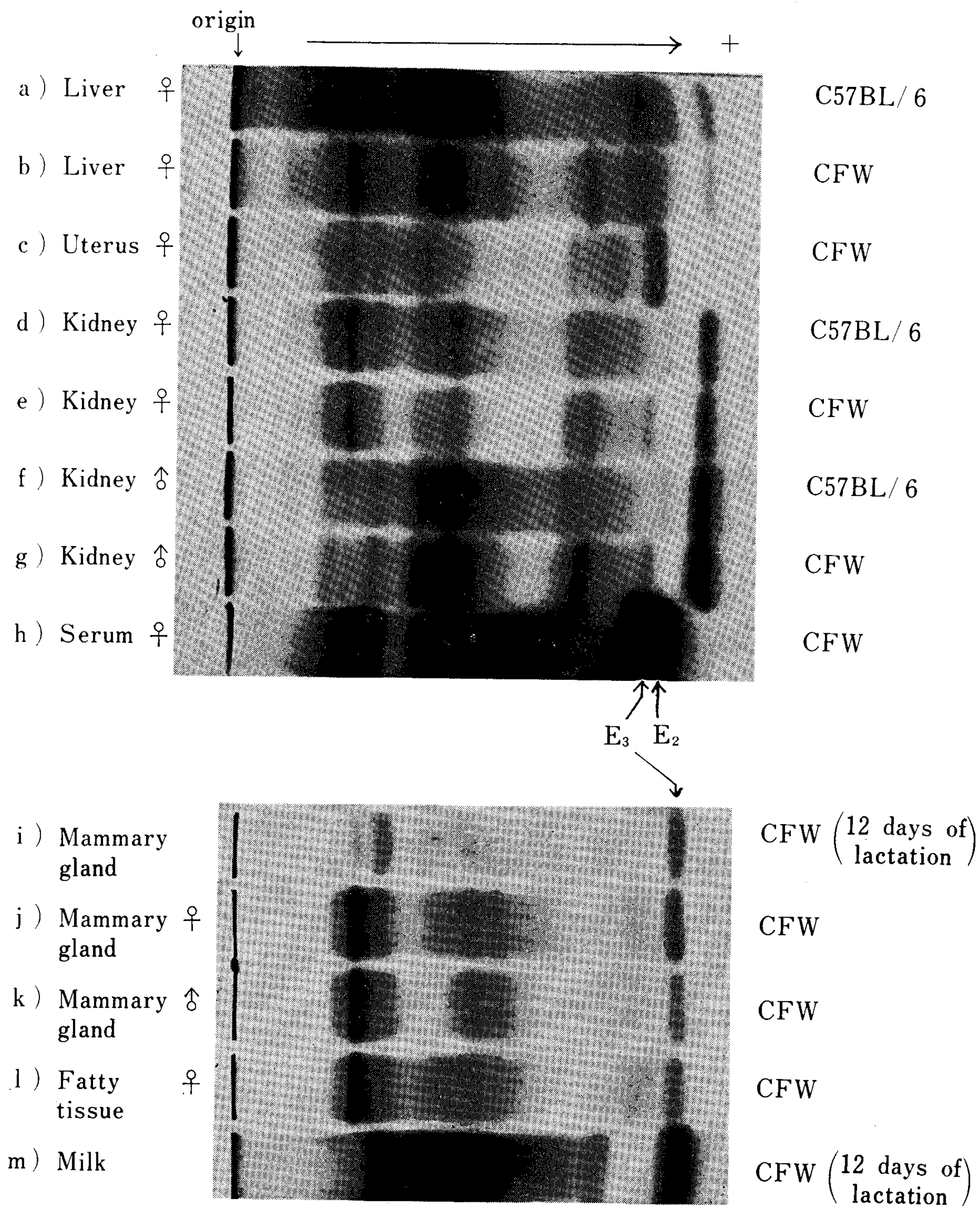

FIG. 3. Esterase zymograms of tissues and milk of mice.
Note: Esterase zymograms stained with α-naphthyl acetate as substrate.

The results of several crosses were summarized in Table 2. F_2 crosses yield Es-7A, Es-7AB and Es-7B phenotypes in close agreement with the expected ratio 1:2:1. Backcrosses yield Es-7A and Es-7AB or Es-7B and Es-7AB phenotypes in close agreement with the expected ratio 1:1. Sex-linkage was not detected. The esterase type of the mammary gland was identical to that of the uterus in the same individual mouse.

Tissue specificity: Several tissues esterase from 2 inbred strains were separated by starch-gel electrophoresis for comparing the tissue specificity and

patterns of E_2 and E_3 bands (Fig. 3). Although esterase zymograms of female mammary gland was identical to that of male mammary gland and female fatty tissue, variation of activity-levels among these tissues were detected only in E_3 band. The activity of E_3 band in female mammary gland was always higher than that of male mammary gland and fatty tissue. E_3 band was prominant in the mammary gland during lactation and in the milk. The esterase band (diffuse band) of serum was detected at the same mobility as E_3. On the other hand, the activity of E_3 band in the kidney and liver were always lower than that of E_3 in the uterus.

Substrate specificity and inhibition studies: Substrate specificity and inhibition studies carried out on E_2 and E_3 bands of both tissues (Table 3). E_2 and E_3 bands hydrolyzed α-naphthyl butyrate, β-naphthyl butyrate, and α-naphthyl

TABLE 3. *Characteristic Reactions of E_2 and E_3 Esterase Bands of Mammary Gland and Uterus in Mice with Various Substrates and Inhibitors*

	Esterase bands	
	E_2	E_3
Substrates		
α-Naphthyl acetate	++	+++
β-Naphthyl butyrate	++	+++
α-Naphthyl butyrate	++	+++
Indoxyl acetate	—	—
β-Carbonaphthoxycholine iodide	—	—
α-Naphthyl laurate	—	—
Inhibitors		
DFP (10^{-3}M. 10^{-4}M. 10^{-5}M)	—	—
Eserine (10^{-3}M. 10^{-4}M)	++	+++
EDTA (10^{-1}M.10^{-2}M)	++	+++
Tricresyl phosphate (10^{-2} M. 10^{-3}M)	—	—

Note: + indicate the presence, – the absence, of a staining reaction as a result of esterase activity.

acetate, but not indoxyl acetate or β-carbonaphthoxycholine iodine. E_2 and E_3 bands were highly sensitive to both DFP and tricresyl phosphate, but insensitive to both eserine and EDTA. There was not case in which the characteristic reactions with all substrates and inhibitors differed in the E_2 and E_3 bands. The E_3 region of serum was insensitive to eserine, DFP and tricresyl phosphate.

Discussion

A very early development of the mammary duct system of female mice occurs during growth, whereas the mammary ducts of the male remain in a rudimentary state (17). Making a comparison between esterase zymograms of female and male mammary glands, it seems probably that the sex difference of E_3 band in this

study reveals the difference of mammary gland development.

As α-naphthyl acetate employed in this studies had a wide substrate specificity, we had to study the characteristic reactions with various substrates and inhibitors on E_2 and E_3 bands. Three types of esterase have been described in mammalian tissues; arylesterase, aliesterase and cholinesterase (18, 19). The E_2 and E_3 bands detected here are probably of the aliesterase type, because these bands were insensitive to both eserine and EDTA, but highly sensitive to both DFP and tricresyl phosphate. In addition, these bands did not hydrolyze β-carbonaphthoxy-choline iodide.

The genetic control of isozymes which has been reported until now are classified into three types: unigenic, allelic and non-allelic isozymes (20). The mode of genetic control of variation among strains detected here seems to belong to the allelic isozyme type, because F_1 progeny always contained two bands and not hybrid enzyme. Moreover, in F_2 progeny the segregated ratio of esterase phenotypes was in close agreement with the expected 1:2:1 and in backcross progeny with the expected 1:1. Because of these factors and of the characteristic reactions to the substrates and inhibitors as previously mentioned, it is thought that the E_2 and E_3 bands in both tissues are allelic isozymes under the control of a pair of co-dominant alleles at a single autosomal locus (Es-7^A and Es-7^B).

Concerning the inheritance of eserine resistance esterase which migrated into the albumin region, three loci designating Es-1 (4), Es-4 (7) and Ee-1 (6) have been described. Popp (21) has reported a linkage of oligosyndactly (Os) with locus Es-1. Ruddle et al. (9, 22, 23) has reported that designating Es-4 as identical to Es-1 with a linkage data, phenotype of IV-20 and IV-30 (Es-4) in kidney esterase representing contamination from the serum, and nearly all of the organ extracts show at least a trace of these bands.

Tissue source of the enzyme and method of starch-gel electrophoresis which we employed in this study differed considerably with those which other investigators employed. It is difficult to compare their results with ours, although, the remarkable similarity between their results and ours are relative to electrophoretic mobility, insensitivity to eserine, and characteristic variation in C57BL/6 strain. However, difference between their results and ours are in the following respects: First, the activity of E_3 band in uterus were higher than that of E_3 in kidney and liver. E_2 and E_3 bands of the mammary gland and uterus were highly sensitive to both DFP and tricresyl phosphate, but E_3 esterase region of serum was insensitive to both DFP and tricresyl phosphate. E_2 and E_3 bands of mammary gland and uterus might differ with E_2 and E_3 region of serum in characteristics of esterase. Second, in the Es-7A and Es-7AB phenotypes, the activity of E_2 band was always lower than that of E_1. It is of interest that the difference of activity-levels between E_2 and E_3 bands may be genetically or physiologically controlled. In these respects, locus Es-7 described by us might differ with the three loci reported,

however this hypothesis requires additional demonstration, for example, a linkage of oligosyndactly (Os) with locus Es-7.

E_2 and E_3 bands in both tissues are distinguished from the other tissues in the following respects.* The activity of these bands in adults is higher than those in the immature. With various stages of sexual cycle, pregnancy and lactation, remarkable variations of activity-levels of these bands has been detected. It is of interest that the activity of these bands are easily influenced by physiological change. We are studying these problems from the developmental and physiological points of view.

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* Komeda, Manda and Nishida, unpublished observations