Genetic and Physiological Control of Esterases in Experimental Small Animals VI. Differences among Strains and Inheritance of Serum Cholinesterase Isozymes in Mice

Tohoku Journal of Agricultural Research
Vol. 20, N0. 2, June 1969
Printed in Japan

Genetic and Physiological Control of Esterases in Experimental Small Animals

VI. Differences among Strains and Inheritance of Serum Cholinesterase Isozymes in Mice

Masaharu MANDA, Yoshio OKI* and Shusaku NISHIDA

*Department of Animal Husbandry, Faculty of Agriculture, Tohoku University, Sendai, Japan, National Institute of Animal Health, Tokyo, Japan.**

(Received, June 5, 1969)

Summary

The sera of 12 mouse strains and some of their crossbreds were examined for differences among strains and inheritance in serum cholinestrase isozyme, zone C_3, which was detected by starch-gel electrophoresis combined with the histochemical staining method. Serum samples were obtained from mice of both sexes from 12 mouse strains, namely the CFW, CF♯1, C57BL/6, C3H/He, DDK, KK, NC, RR, SS, AA, DSD and dd strain.

Some esterase patterns in the sera of the mice showed considerably different activities. The activity-level of serum cholinesterase isozyme, zone C_3, in female mice was always higher than those in male mice with the exception of the KK strain and in some strains, such as the CF♯1 strain and DDK strain, exhibited considerable individual variation. The activity-level of zone C_3 in female mice of these strains were roughly divided into three groups, corresponding to high (CFW, CF ♯1, dd, SS, RR), intermediate (DDK, C57BL/6, NC, AA) and low activity (KK, C3H/He, DSD), respectively. Those in male mice were divided into two groups, corresponding to high (CFW, CF♯1, dd, KK, DDK, C57BL/6) and low activity (SS, RR, NC, AA, C3H/He, DSD), respectively.

In consequence of a number of cross-tests, it was suggested that the three esterase phenotypes in female mice were genetically controlled by allels designated as chE and che. Thus we conclude that the activity-level of serum cholinesterase isozyme, zone C_3, in mice may be controlled not only genetically but also physiologically by sex hormone.

In our previous paper (1), concerning physiological variation and hormonal regulation in activities of serum cholinesterase isozymes, a wide variation of activity-levels was found for them among strains or individuals of mice. It was suggested that such a wide variation of activity-levels of serum cholinesterase isozymes could be controlled not only physiologically by sex hormone but also genetically.

Yuhas and Angel (2, 3) have previously stated in their reports that the activity-levels of serum cholinesterase among the 23 strains of mice varied significantly, as a result of the chemical quantitative measurement. However, differences among strains of mice in these electrophoretic patterns (isozymes) have not yet been examined satisfactorily, and little is known about the genetic and hormonal mechanism controlling the same enzyme.

The present paper deals with differences of serum cholinesterase isozyme, zone C_3, among various strains of mice detected by starch-gel electrophoresis and with the mode of its inheritance.

Materials and Methods

Serum samples were obtained from mice of both sexes from 12 mouse strains, namely the CFW, CF#1, C57BL/6, C3H/He, DDK, KK, NC, RR, SS, AA and DSD inbred strain of the National Institute of Animal Health and the dd strain of the Medical Deparment of Tohoku University. Ages and numbers of each mouse strain used for this examination are shown in Table 1. The CFW, KK, DSD, dd strain and their crossbreds were used to examine the mode of the inheritance on the variation of the esterase patterns. These sera were obtained from adult mice under ordinary physiological conditions. Castrations of both sexes from the CFW, NC and DSD inbred strain were performed through mid-ventral incisions under ether anesthesia.

The serum esterases were separated by vertical starch-gel electrophoresis (4) using half of Polik (5) for preparing the gel and stained histochemically with α-naphthyl acetate and naphthanil diazo blue B in 0.2 M phosphate buffer, pH 6.8. The density of some esterase patterns was measured by a Densitometer Ozumor-82 Type.

TABLE 1. *Ages and Numbers of Mice Used in This Experiment*

Strain	Age of month	No. of mice
CFW	5～ 8	18
CF#1	5～ 7	16
C57BL/6	5～ 7	12
C3H/He	3～ 6	12
DDK	3～10	16
KK	3～ 7	12
NC	4～ 7	15
SS	4～ 7	11
AA	2～12	11
RR	5～ 7	12
*DSD	3～ 6	14
dd	2～ 4	12
Total		161

Note : * In 1963 from laboratory of animal breedig Tohoku University to National Institute of Animal Health in 36th generation of the DSD strain.

Results and Discussion

Difference of Activity-levels of Serum Cholinestrase Isozyme, Zone C_3, Among Strains of Mice.

The serum estrases of each mouse strain was separated by starch-gel electrophoresis, and detected using α-naphthyl acetate as the substrate. The activity-levels of estrase patterns were judged by the naked eye, and graded into six levels of ⫲, ⫲′, ⫳, ⫩, + and −, according to activity level. Table 2 shows the difference of the activity-levels of serum cholinestrase, zone C_3, among strains of both sexes of adult mice.

TABLE 2. *Difference of Activity-levels of Serum cholinesterase, Zone C_3, Among Strains of Mice*

Strain	CFW	CF#1	SS	RR	dd	DDK	C57B /6	NC	AA	C3H /He	KK	DSD
Age of month	5~8	5~7	4~7	5~7	2~4	3~10	5~7	4~7	2~12	3~6	3~7	3~6
No. of mice	16	12	11	11	12	15	12	12	11	11	12	14
Female	⫲ ⫲ ⫲ ⫲ ⫲ ⫲ ⫲ ⫲	⫲ ⫲′ ⫲′ ⫳ ⫳ ⫩	⫲′ ⫲′ ⫲′ ⫳ ⫳	⫲ ⫲′ ⫲′ ⫲′ ⫲′ ⫲′	⫲′ ⫲′ ⫲′ ⫲′ ⫲′ ⫲′	⫲′ ⫳ ⫳ ⫳ ⫳ ⫳ ⫳ ⫳	⫳ ⫳ ⫳ ⫳ ⫳ ⫳	⫳ ⫳ ⫳ ⫳ ⫳ ⫳	⫲′ ⫳ ⫳ ⫳ ⫳	⫳ ⫳ ⫳ ⫳ ⫳	⫩ ⫩ ⫩ ⫩ ⫩	⫩ ⫩ ⫩ ⫩ ⫩ ⫩ + +
Male	⫩ ⫩ ⫩ ⫩ ⫩ ⫩ ⫩	⫩ ⫩ ⫩ ⫩ ⫩ +	+ + + + + +	⫩ + + + +	⫩ ⫩ ⫩ ⫩ + +	⫩ ⫩ ⫩ ⫩ + +	⫩ ⫩ ⫩ ⫩ + +	⫩ ⫩ + + + +	⫩ + + + + +	+ + + + + +	⫩ ⫩ ⫩ ⫩ ⫩ ⫩ +	+ − − − − −

Notes : ⫲~⫲′ High active-level in female. ⫳Intermediate active level in female. ⫩~+ Low active level in female. ⫩High active level in male. +~− Low active level in male.

In female mice the activity-levels of zone C_3 were always higher than those in male mice with the exception of the KK strain and some strains, such as the CF# 1 strain and the DDK strain exhibited considerable individual variation. The activity-levels of zone C_3 in female mice were roughly divided into three groups, corresponding to high (CFW, CF#1, dd, SS, RR), intermediate (DDK, C57BL/6, NC, AA) and low activity (KK, C3H/He, DSD), respectively.

In male mice the activity-levels of zone C_3 was consistently lower with the exception of the KK strain, and it was rather difficult to divide into three groups as against those in female mice, since the activity was too low to be judged by the naked eye. Those in male mice were roughly divided into two groups, corres-

ponding to high (CFW, CF#1, dd, KK, DDK, C57BL/6) and low activity (SS, RR, NC, AA, C3H/He, DSD), respectively.

In females the lowest activity was found in the DSD strain and the highest in the CFW strain. In males the lowest activity was found in the DSD strain. Activity-levels of serum cholinestrase isozyme, zone C_3, in a typical mouse strain of each group were shown in Fig. 1.

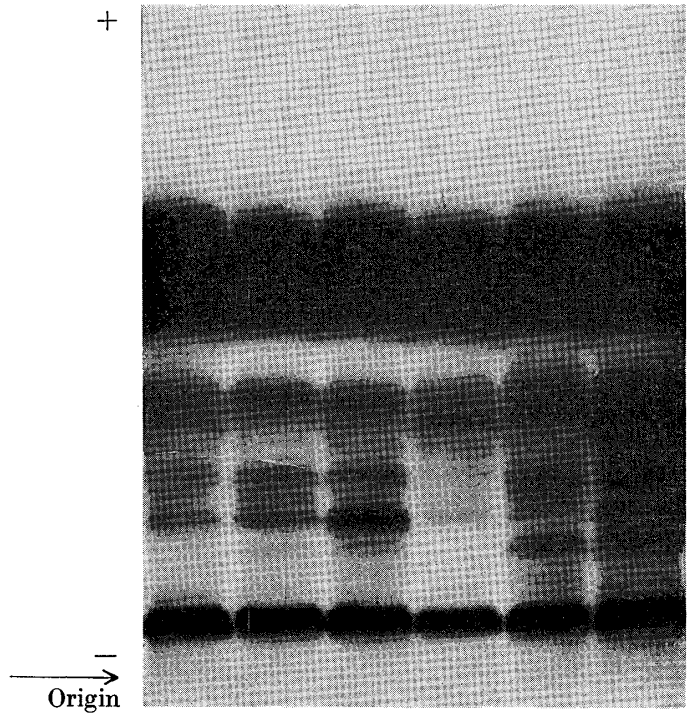

FIG. 1. Zymograms of strains in mice showing the different activity-levels of serum cholinesterase isozyme, zone C_3.
(a) Female of DSD strain showing low activity-level; (b) Female of NC strain showing intermediate activity-level; (c) Female of CFW strain showing high activity-level; (d) Male of DSD strain showing low activity-level; (e) Male of NC strain showing low activity-level; (f) Male of CFW strain showing high activity-level.

Percentage of activity of zone C_3 in a typical mouse strain of each group are shown in Table 3. Active values of zone C_3 for the CFW female strain in the high active group were twice as much as those for the DSD female strain in the low active group.

Some castrated mice and immature mice (10 days) were used to examine whether differences of the activity-levels of zone C_3 among strains may be due to differences in the sex hormone, since activity-levels of zone C_3 were regurated by sex hormone as reported in the previous paper (1). Castration of both sexes from the CFW, NC and DSD inbred strain resulted in marked differences of this zone among strains from normal adult mice as shown in Fig. 2. In immature mice the highest

TABLE 3. *Percentage Activity of Serum Cholinesterase Isozyme, Zone C_3, in the Sera of Mice.*

Sex	Strain	No. of mice	Percentage activity of zone C_3
Female	CFW	5	8.7±1.02
	C57BL/6	5	7.3±2.05
	DSD	5	4.3±1.60
Male	CFW	5	4.7±0.98
	C57BL/6	5	5.5±1.07
	DSD	5	3.6±2.40

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

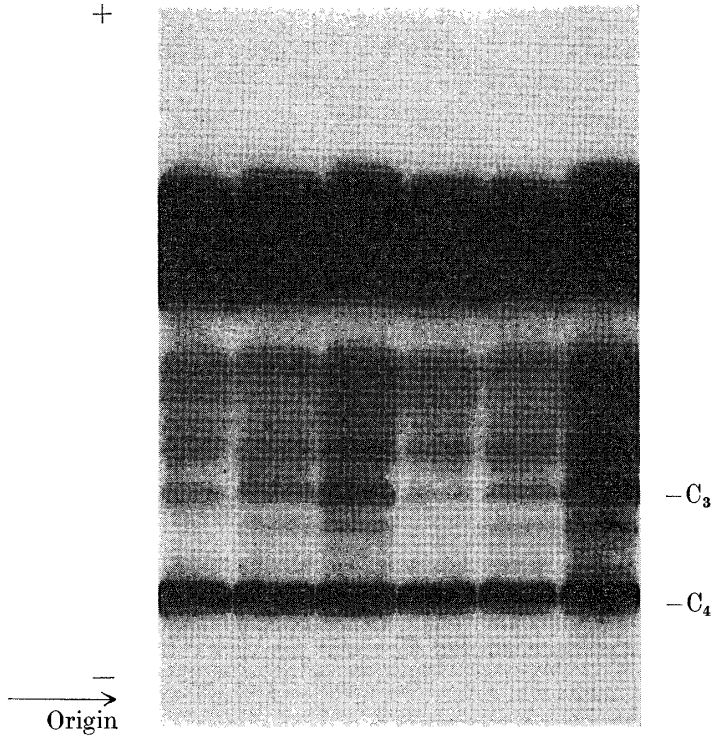

FIG. 2. Zymograms of strains in castrated mice showing the different activity-levels of serum cholinesterase isozyme, zone C_3.
(a) Castrated female of DSC strain; (b) Castrated female of NC strain; (c) Castrated female of CFW strain; (d) Castrated male of DSD strain; (e) Castrated male of NC strain; (f) castrated male of CFW strain.

activity was also found in the CFW strain and differences among the strains of this zone were exhibited as shown in Fig. 3. The results suggested that differences among strains of activity-levels of zone C_3 were independent of the sex hormone.

Reports dealing with multiple forms of various enzymes have increased rapidly since the introduction of starch-gel electrophoresis, and it has been shown recently that multiple forms of serum cholinestrase also occured in humans and the horse. Oki et al. (6) have reported that the five zones, C_1 to C_5, were classified

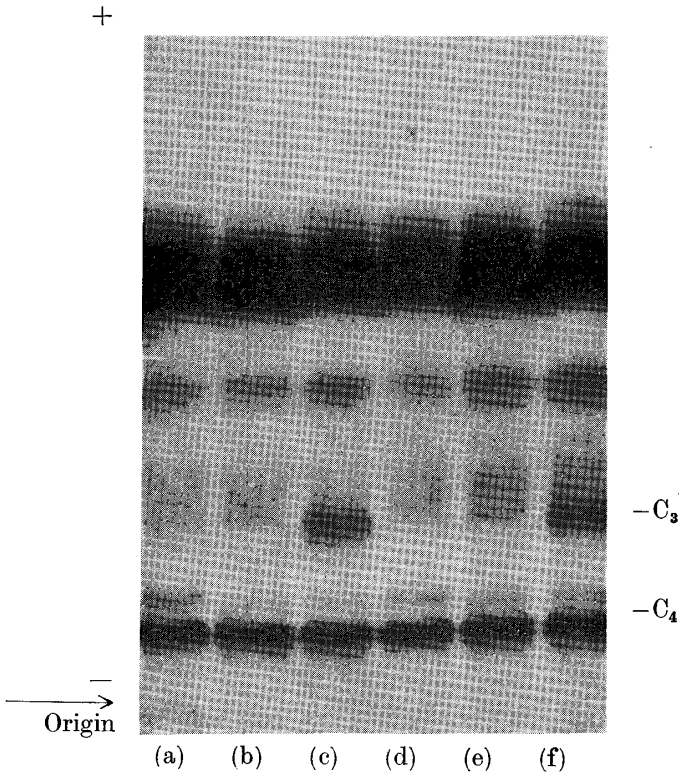

FIG. 3. Zymograms of strains in immature mice (ten days) showing the different activity-levels of serum cholinesterase isozyme, zone C_3.
(a) Immature female of DSD strain; (b) Immature female of NC strain; (c) Immature female of CFW strain; (d) Immature male of DSD strain; (e) Immature male of NC strain; (f) Immature male of CFW strain.

as cholinesterase in horse plasma and that one of these zones, C_4, always predominated; another zone, C_5, a variant form, exhibited differences among individuals. These finding were similar to those of human serum reported by Harris et al. (7). However, enough investigation to clarify has not been made further variations of the other zones of cholinesterase isozymes in animal.

The four zones, C_1 to C_4, were classified as cholinestrase of mouse serum and tissues as reported in the previous paper (1). In the present investigation, it was found that one of these zones (activity-levels of zone C_3) exhibited marked differences among the strains of mice. Activity-levels of the main cholinesterase isozyme, zone C_4, also appeared to exhibit slight differences among strains.

Inheritance of the Activity-levels of Serum Cholinestrase Isozyme, Zone C_3, in Female Mice.

To examine the mode of the inheritance on the variation of the cholinesterase activities in zone C_3, a number of crosses were carried out between two strains of mice (namely dd×DSD and CFW×KK). Adult female mice, which it is relatively easy to judge by the naked eye, were examined in this investigation.

The sera esterases from F1 progeny of crosses between the dd strain and the

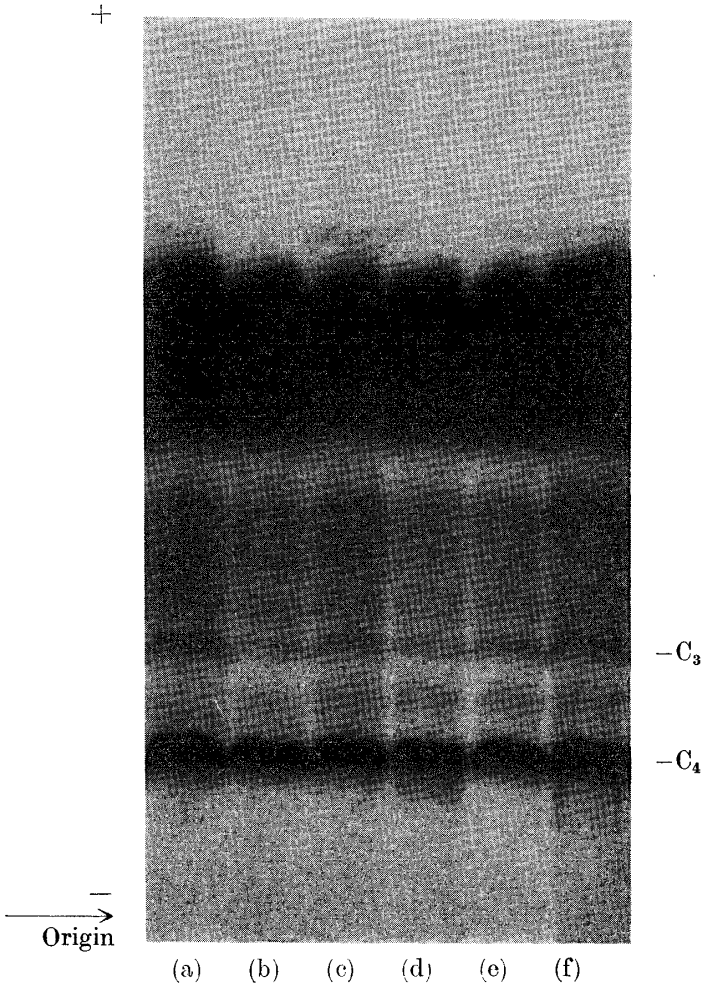

FIG. 4. Genetic variations of activity-levels of serum cholinesterase isozyme, zone C_3, in two strain, F_1 and F_2 in female mice as revealed by starch-gel electrophoresis.
(a) dd strain showing high active-level of zone C_3; (b) DSD strain showing low active-level of zone C_3; (c) F_1 progeny showing intermediate active-level of zone C_3; (d), (e) and (f) F_2 progeny: the three phenotypes segregated in the F_2 progeny, corresponding to low, intermediate and high activity-levels of zone C_3, respectively.

DSD strain contained intermediate activities between both parents, as shown in Fig. 4 and Table 4. A number of the F_1 progeny were intercrossed to determine whether the esterase activity segregates in accordance with the Mendelian law. In the expected ratio, 1:2:1, the three phenotypes, high, intermediate and low active patterns, were obtained in the adult mouse sera of the F_2 progeny (Fig. 4 and Table 4). Crosses between the CFW strain and the KK strain conducted gave the same result, as shown in Table 5. On the basis of the results, it was concluded that the three esterase phenotypes in females were genetically controlled by alleles designated as chE and che.

In extensive human family studies, Harris et al. (8) demonstrated electro-

TABLE 4. *Types and Inheritance of Activity-levels of Serum Cholinesterase, Zone C_3, in the dd Strain, DSD Strain and Their Crossbreds in Female Mice.*

Strains & Matings \ Genotypes & Phenotypes	ChE/ChE ⫲⫲⫲～⫲⫲⫲′	ChE/Che ⫲⫲	Che/Che ⫲～+
dd	39	0	0
DSD	0	0	55
(dd×DSD) F_1	0	23	0
$F_1 \times F_1$*	33	66	37

Note : ⫲⫲⫲～⫲⫲⫲′ High active level. ⫲⫲ Intermediate active level.
⫲～+ Low active level.
* x^2-test: $0.90>p>0.75$

TABLE 5. *Types and Irheritance of Activity-levels of Serum Cholinesterase, ZoneC_3, in the CFW Strain, KK Strain and Their Crossbreds in Female Mice.*

Strains & Matings \ Genotypes & Phenotypes	ChE/ChE ⫲⫲⫲～⫲⫲⫲′	ChE/Che ⫲⫲	Che/Che ⫲～+
CFW	14	0	0
KK	0	0	9
(CFW×KK) F_1	0	20	2
$F_1 \times F_1$*	13	24	10

Notes : ⫲⫲⫲～⫲⫲⫲′ High active level. ⫲⫲ Intermediate active level.
⫲～+ Low active level.
* x^2-test: $0.90>p>0.75$

phoretically that zone C_5, a variant form of serum cholinesterase, was genetically controlled. But little has been known about genetic control of the other zones in serum cholinesterase isozymes. In the present investigation, it was found as a result of a number of cross-tests that the three phenotypes of activity-level of zone C_3 in female mice were also genetically controlled. Activity-levels of zone C_3 also were regulated by sex hormone as reported in the previous paper (1). Thus we conclude that the activity-levels of serum cholinestrase isozyme, zone C_3, in mice may be controlled not only genetically but also physiologically by sex hormone. Such reports the genetic and hormonal mechanism controlling the same enzyme pattern have not yet been knwon.

Augustinsson et al. (9, 10) have reported that blood plasma arylesterase in pigs is genetically determined and the various phenotypic activity-levels are produced by a set of multiple alleles designated as a, A_1, A_2, A_3 and A_4. Activity-levels of cholinesterase isozyme, zone C_3, in mice sera extended over a wide range. Therefore futher studies will be needed to examine whether the above mentioned variety of isozymes are controllable by multiple alleles.

Acknowledgement

The authors express their appreciation to Dr. Takayoshi Ino in National Institute of Animal Health for advice and supply of mice, and Mr. Sakio Yoshikawa for technical assistance.

References

1) Oki, Y., Manda, M., Takeda, M. and Nishida, S., *Tohoku J. Agr. Res.*, **17**, 201 (1967)
2) Yuhas, J.M., Angel, G.R., Mahin, D.T., Farris, R.D., Woodward, K.T. and Storer, J.B. *Genetics, Austin, Tex.*, **57**, 613 (1967)
3) Angel, C.R., Mahin, D.T., Farris, R.D., Woodward, K.T., Yuhas, J.M. and Storer, J.B. *Science, wash.*, **156**, 529 (1967)
4) Smithies, O., *Biochem. J.*, **71**, 585 (1959)
5) Poulik, M.D., *J. Immunol.*, **82**, 502 (1959)
6) Oki, Y., Oliver, W.T. and Funnell, H.S., *Can. J. Physiol. Pharmacol.*, **43**, 147 (1965)
7) Harris, H., Hopkinson, D.A. and Roblon, E.B., *Nature*, **196**, 1296 (1962)
8) Harris, H., Hopkinson, D.A., Robson, E.B. and Whittaker, M., *Ann. Human Genet.*, **26**, 359 (1963)
9) Augustinsson, K. -B. and Olsson, B. *Nature*, **187**, 924 (1960)
10) Augustinsson, K. -B. and Olsson, B. *Hereditas*, **47**, 1 (1961)