GENETIC AND PHYSIOLOGICAL CONTROL OF ESTERASES IN EXPERIMENTAL SMALL ANIMALS I. INHERITANCE OF SERUM ESTERASE IN MICE

GENETIC AND PHYSIOLOGICAL CONTROL OF ESTERASES IN EXPERIMENTAL SMALL ANIMALS
I. INHERITANCE OF SERUM ESTERASE IN MICE

By

Yoshio OKI, Mituru TAKEDA, Masaharu MANDA and Shusaku NISHIDA

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

(*Received June 30, 1966*)

Introduction

Variations among species, strains or individuals in electrophoretic mobilities and patterns of related esterases have been reported to occur in animal sera (1, 2, 3, 4, 5). Some of these enzymes having different electrophoretic patterns among breeds, strains or individuals have been demonstrated to be genetically controlled by autosomal allelic genes (6, 7, 8, 9). The various activity-levels of the pig serum arylesterase also have been demonstrated to be genetically determined by multiple allelic genes (10).

The differences among breeds or individuals on catalitic activities of esterases and multiple forms of cholinesterase in equine plasma have recently been reported (11). In our previous report (8,11) esterase zone D, a type of aliesterase, separated by starch-gel electrophoresis was found only in the draft horse breeds, Percheron and Belgian, but not in the light horse breeds, Thoroughbred and Standardbred, etc. The plasma of the Percheron-Thoroughbred crosses contained a weakly staining esterase zone D. It was suggested that the activity-levels of this enzyme might be genetically controlled.

In this connection, the present paper deals with the inheritance of activity-levels of serum esterase, a type of aliesterase, in inbred strains of mice.

Materials and Methods

Serum samples were obtained from mice of C_3H strain, DD/Sd strain, their crossbreds and AA strain in our laboratory.

The serum esterases were separated by vertical starch-gel electrophoresis (12) using half of the concentration of the discontinuous buffer of Polik (13) for preparing the gel. The esterases were characterized histochemically and spectrophotometrically using α-naphthyl acetate, β-carbonaphthoxycholine iodide

and benzoylcholine as substrates and using eserine and TEPP as inhibitors, according to the criteria of esterase classification in the previous report (11).

Results and Discussion

1. Difference and inheritance of activity-levels of serum esterase between two inbred strains of mice.

The serum esterases of C_3H strain, DD/Sd strain and their crossbreds were separated by starch-gel electrophoresis. Figure 1 shows the "zymograms" of the esterase zones detected using α-naphthyl acetate as the substrate. A highly active esterase zone D was present in all cases of the sera of the adult male and female mice of C_3H strain (Fig. 1 b), whereas this zone D was absent in those of the adult male and female of DD/Sd strain (Fig. 1 a). To exmaine the mode of the inheritance on the variation of the esterase activities, a number of crosses was carried out between the two strains of mice. The sera of F1 progeny contained

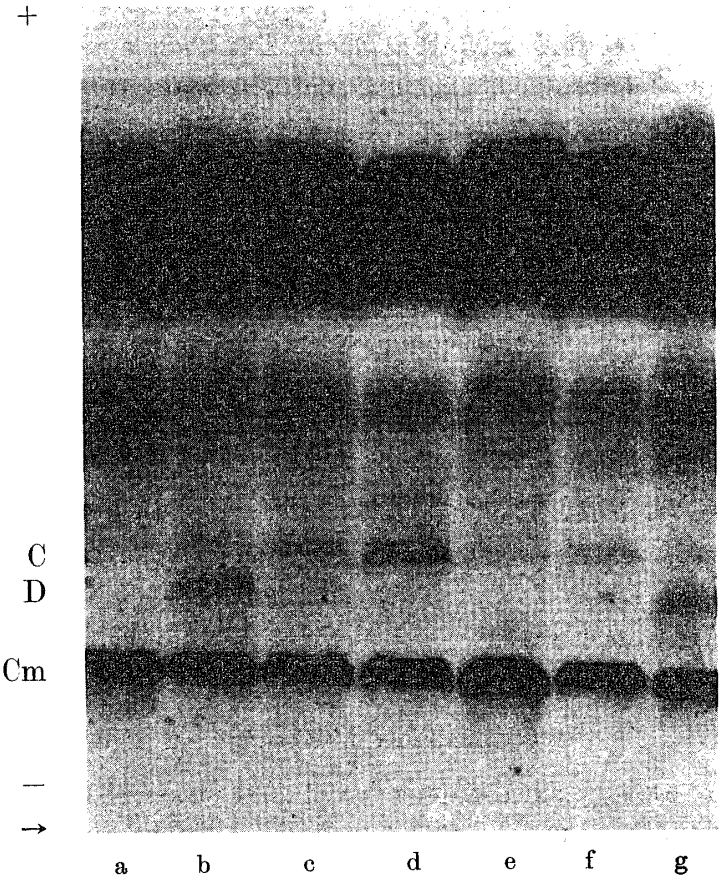

Fig. 1. Genetic variations of activity-levels of serum esterase zone D in two mouse strains and the crossbreds as revealed by starch-gel electrophoresis.

(a) DD/Sd strain, zone D is absent; (b) C_3H strain, highly active zone D; (c) and (d) (DD/Sd$\times C_3H$) F_1, low active zone D; (e), (f) and (g) $F_1\times F_1$, Zones D are absent, low active and highly active, respectively.

the esterase, although the activity was low in all cases of the adult mice (Fig. 1c and d). On the basis of the results, the genotypes of the mouse strains indicating the phenotypes of the highly active pattern and the negative pattern may be assumed to be homozygous EsD/EsD and Esd/Esd, respectively. Then, the genotype of F1 progeny indicating the phenotype of the low active pattern may be heterozygous EsD/Esd (Table 1). A number of the F1 progeny was intercrossed to determin whether the esterase activity segregates in accordance with the Mendelian law. In the expected ratio, 1: 2: 1, the three phenotypes, negative, low active and highly active patterns, were obtained in the adult mouse sera of the F_2 progeny (Fig. 1 e, f, g and Table 1). The phenotypes of the F_2 progeny were

Table 1. Types and inheritance of activity-levels of serum esterase in C_3H strain, DD/Sd strain and their crossbreds.

Strains and Matings \ Genotypes and Phenotypes	EsD/EsD ⫲～⫲	EsD/Esd +～⫩	Esd/Esd −
C_3H	22	0	0
DD/Sd	0	0	24
(DD/Sd×C_3H) F_1	0	17	0
$F_1 \times F_1$	12	23	11

Notes: ⫲～⫲ High active level. +～⫩ Low active level.
− Negative level.

independent of sex. It was considered that the three phenotypes corresponded to the three kinds of genotypes, Esd/Esd, EsD/Esd and EsD/EsD, respectively. Since the activity of the esterase of C_3H sera decreased during pregnancy, the sera of pregnant mice were not used for the analyse of the inheritance of the esterase levels in all cases.

The esterase zone D hydrolyzed α-naphthyl acetate, but not β-carbonaphthoxycholine iodide and benzoylcholine. Its activity was not inhibited by eserine, 10^{-5}M but was inhibited by TEPP, 10^{-5}M. The esterase is thus not a cholinesterase. It is considered that the enzyme is a type of aliesterase. Esterase zones C and Cm, which had respectively slightly greater mobility or smaller mobility than zone D, hydrolyzed β-carbonaphthoxycholine iodide and benzoylcholine, and their acitivities were inhibited by eserine, 5×10^{-5}M, and TEPP, 10^{-5}M. These esterase zones are thus different enzymes from the esterase zone D and considered to be isozymes of pseudocholinesterase.

2. Types and inheritance of activity-levels of serum esterase within an inbred strain of mice.

In the esterase patterns of sera of AA strain of mice, the activity—levels of the

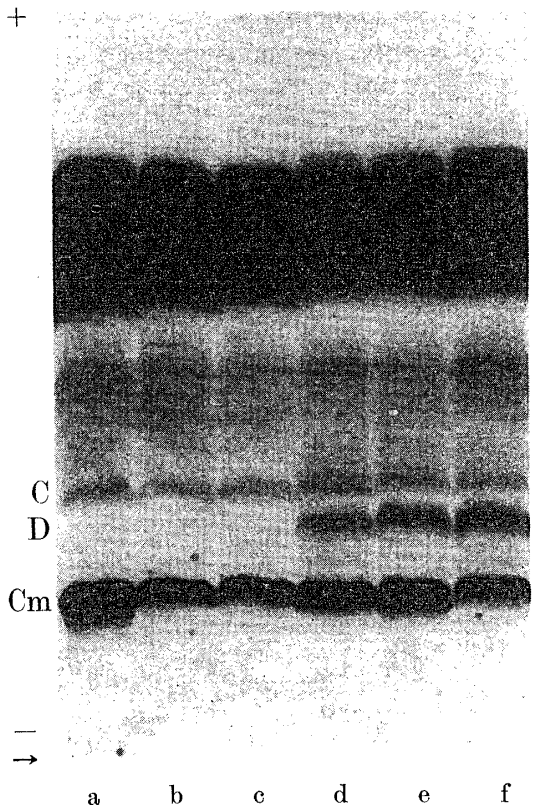

Fig. 2. Genetic variations of activity-levels of serum esterase zone D between groups of AA strain.

(a)-(c) A group of AA strain showing negative levels of esterase zone D; (d)-(f) A group of AA strain showing high active levels of esterase zone D.

esterase zone D exhibited considerable individual variation and were divided into three groups, corresponding to the high, negative and intermediate low activities, respectively. To certify the genetic control of the activity-levels of the esterase, a number of cross tests, including back-crosses, was performed with mice of the various phenotypes. In all matings where both parents were of negative level of the esterase, the progeny was always of the negative pattern (Fig. 2 a, b, c). In crosses between parents which both had highly active level of the esterase, all the progeny was always of the same active level as those of the parents (Fig. 2 d, e, f). On the basis of the results, it was considered that these parents were homozygous for the gene EsD and the gene Esd, respectively (Table 2). When female mice of the negative phenotype were crossed with the male mice of highly active phenotype, all the F1 progeny had intermediate low active level which may correspond to heterozygous EsD/Esd (Fig. 3 and Table 2). When both parents had the same low activity, the three phenotypes were obtained in the progeny according to Mendelian inheritance (Table 2). It was considered that the genotype of both parents was EsD/Esd and the three phenotypes of the progeny corresponded to

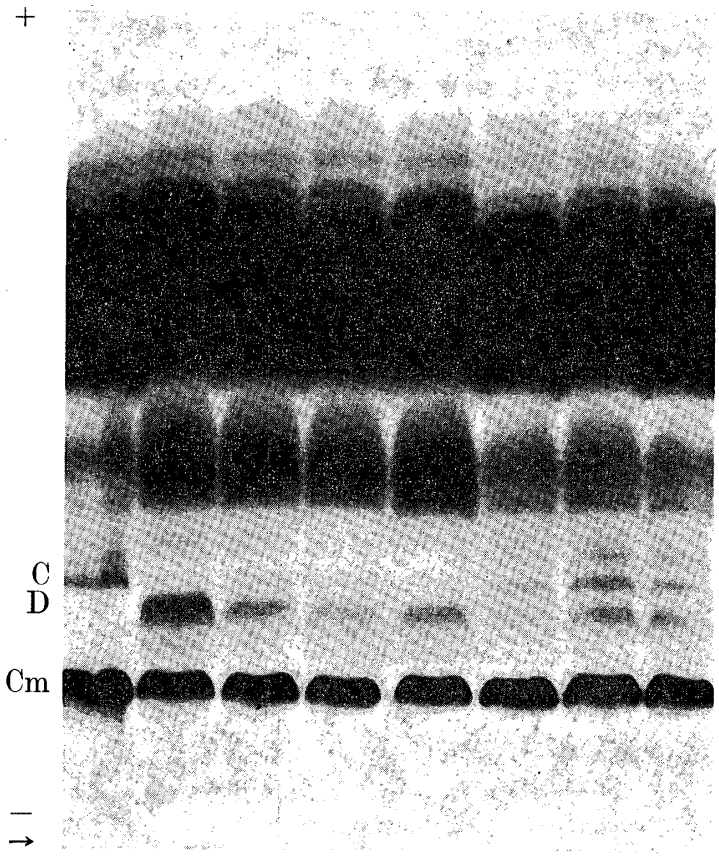

Fig. 3. Zymograms of parents and F_1 sera of strain AA showing the different activity-levels of esterase zone D.
(a) Female mouse of parent that zone D is absent;
(b) Male mouse of parent showing highly active zone D;
(c)-(e) Male mice of the F_1 progeny showing low active zone D;
(f)-(h) Female mice of the F_1 progeny showing low active zone D

Table 2. Types and inheritance of activity-levels of serum esterase in AA strain.

Matings \ Genotypes and Phenotypes	EsD/EsD ###～###	EsD/Esd +～##	Esd/Esd –
♀–×♂–	0	0	29
♀###×♂###	14	0	0
♀–×♂###	0	6	0
♀##×♂##	7	7	5
♀##×♂###	4	4	0

Notes: ###～### High active level.
+～## Low active level.
– Negative level.

three kinds of genotypes, EsD/EsD, EsD/Esd and Esd/Esd. Furthermore, when female mice of the low active phenotype were crossed with male mice of the high active phenotype, only the two phenotypes of the low and high active patterns which corresponded to genotypes, EsD/Esd and EsD/EsD, were obtained in the progeny. These results support the hypothesis that the biosynthesis and the activity of the serum esterase zone D is genetically determined by the autosomal allelic genes.

Summary

Serum esterases of mice were separated by starch-gel electrophoresis and the esterase types were exmained histochemically and genetically.

A highly active esterase zone D, a type of aliesteraes, was present in all sera of the inbred strain C_3H of mice, whereas this zone D was absent in all sera of the inbred strain DD/Sd of mice. In the sera of strain AA of mice, The activity-levels of the zone D were divided into three groups, corresponding to high, negative and intermediate low activities.

On the basis of the results of a number of cross-tests, it was thought that the three phenotypes corresponded to the three genotypes, EsD/EsD, Esd/Esd and EsD/Esd, respectively and that the activity-levels of the esterase zone D were genetically determined by autosomal allelic genes.

References

1) Augustinsson, K. -B. (1959). Acta Chem. Scand., **13**, 571.
2) Markert, C.L. and F. Møller, (1959). Proc. Natl. Acad. Sci., **45**, 753.
3) Markert, C.L. and R.L. Hunter, (1959). J. Histochem. Cytochem., **7**, 42.
4) Augustinsson, K. -B. and B. Olsson, (1959). Biochem. J. **71**, 477.
5) Lawrence, S.H., P.J. Melnick, and H.E. Weimer, (1960). Proc. Soc. Exptl. Biol. Med., **105**, 572.
6) Popp, R.A. and D.M. Popp, (1962). J. Heredity, **53**, 111.
7) Harris, H., D.A., Hopkinson, E.B. Robson, and M.W. Whittaker, (1963). Ann. Hum. Genet., **26**, 359.
8) Oki, Y., W.T. Oliver, and H.S. Funnell, (1964). Nature, **203**, 605.
9) Oki, Y. and M. Takeda, (1965). Jap. J. Vet. Sci., **27**, 475.
10) Augustinsson, K. -B. and B. Olsson, (1960) Nature, **187**, 924.
11) Oki, Y., W.T. Oliver, and H.S. Funnell, (1965). Canad. J. Physiol. Pharmacol., **43**, 147.
12) Smithies, O. (1959). Biochem. J., **71**, 585.
13) Poulik, M.D. (1959). J. Immunol., **82**, 502.