GENETIC AND PHYSIOLOGICAL CONTROL OF ESTERASES IN EXPERIMENTAL SMALL ANIMALS II. INHERITANCE OF LIVER ESTERASE IN MICE

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II. INHERITANCE OF LIVER ESTERASE IN MICE

By

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Introduction

Relationships between genes and proteins have been defined through chemical genetic studies of variant proteins. Electrophoretic and immunochemical studies on the molecular types of enzymes having similar substrate specificities have revealed differences not only among species (1), individuals of the same species (2) or different organs of the same individuals (3), but also even within the single tissue of an individual (4, 5). The starch-gel electrophoresis (6) combined with histochemical methods for the location and identification of esterase (7) made it possible to ascertain more precisely the presence of esterase. Using this technique, Markert and Hunter (8) separated and partly characterized ten distinct bands of mouse liver esterases which compose a family of various enzymes with overlapping substrate specificities. Although several investigations have been undertaken concerning the inheritance of serum proteins and enzymes of mice (9, 10), little information is available on the genetic studies of the esterases of mouse tissues.

In this investigation, starch-gel electrophoresis was used to compare the qualitative differences of the liver esterases between two inbred strains of mice in order to demonstrate the genetic control of the enzymes.

Materials and Methods

Liver samples were obtained from adult mice of C_3H strain, DD/Sd strain and the progeny from first and second generation crosses between the two strains. The samples weighed by torsion balance and homogenized with a known volume of deionized water. One part of liver in three parts of water (1 : 3) was most adequate for the examination of the esterases. These homogneates were centrifuged at 12,

000 r.p.m. for 40 minutes at 2°C and the supernatant fluid was used for analysis. The liver esterases were separated by the vertical starch-gel electrophoresis (11) using the discontinuous buffer (12) and stained histochemically with α-naphthyl acetate and naphthanil diazo blue B in 0.2M phosphate buffer, PH 6.8.

Results and Discussion

The liver esterases of C_3H strain, DD/Sd strain and their crossbreds, F1 and F_2, were separated by starch-gel electrophoresis for comparing the electrophoretic mobilities and patterns of the enzymes. More than 10 distinct zones of esterase activity were observed on the starch-gels. The zymograms of the mouse liver samples are shown in Fig. 1. Although there were some differences of activity-levels

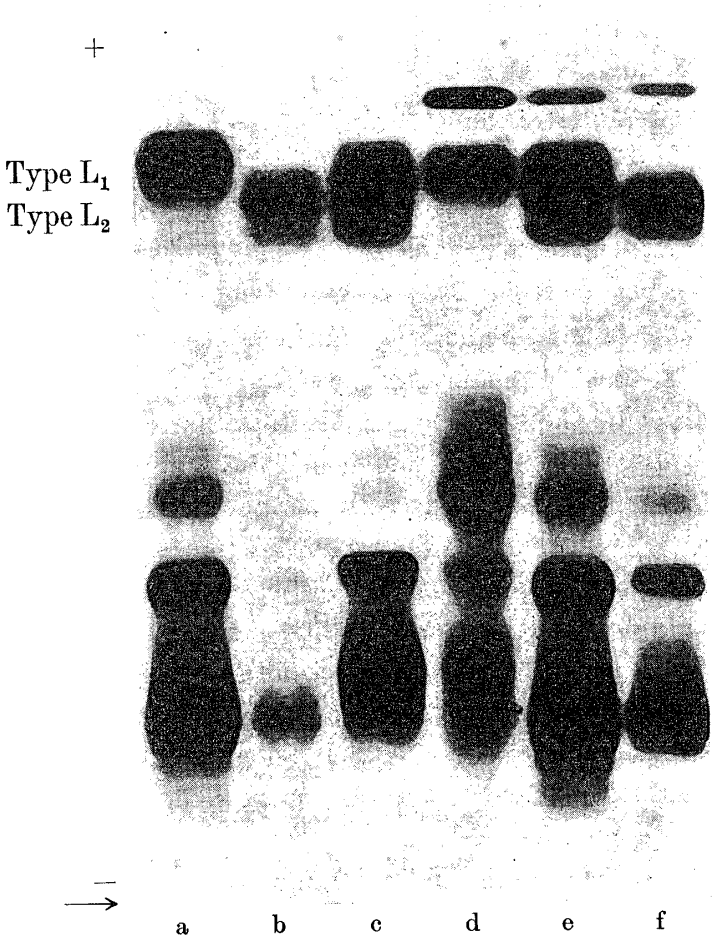

Fig. 1. Genetic variations of esterase types in C_3H, DD/Sd, F_1 and F_2 liver samples as revealed by starch-gel electrophoresis.

(a) C_3H esterase of Type L_1; (b) DD/Sd esterase of Type L_2; (c) (DD/Sd$\times C_3H$) F_1 esterase of Type $L_{1\cdot2}$ containing both the parental esterase components; (d), (e) and (f) F_2 esterases: the three esterase phenotypes segregated in the F_2 progeny, corresponding to the genotypes, EsL_1/EsL_1, EsL_1/EsL_2 and EsL_2/EsL_2, respectively.

between the esterase patterns of the two strains, this study was concerned only with the differences of mobility of the esterase which had secondly greater mobility in all the esterase zones. The mobility of the esterase zones of C_3H strain were slightly greater than that of DD/Sd strain (Fig. 1 a and b). This more rapidly migrating esterase in C_3H strain is designated Type LI, and the slower esterase in DD/Sd strain, Type L_2. Liver tissues from FI progeny of crosses between the C_3H strain and the DD/Sd strain contained both the parental Types L_1 and L_2 (Fig. 1 c). This phenotype will be referred to as Type $L_{1\cdot2}$. On the basis of the results, the genotypes of mice indicating the three phenotypes may be assumed to be homozygous EsL_1/EsL_1, EsL_2/EsL_2 and heterozygous EsL_1/EsL_2, respectively, as shown in Table 1. A number of the F_1 progeny was intercrossed

Table 1. Distribution of lvier esterase types in C_3H, DD/Sd, FI and F_2 mice.

Phenotypes and Genotypes / Strains and Matings		Type L_1 EsL_1/EsL_1	Type $L_{1\cdot2}$ EsL_1/EsL_2	Type L_2 EsL_2/EsL_2
C_3H		10	0	0
DD/Sd		0	0	10
(DD/Sd × C_3H) F_1		0	10	0
$F_1 \times F_1$	Obs	11	28	14
	(Exp)	(13.25)	(26.5)	(13.25)

to determin whether the esterase types segregates in accordance with Mendelian law. In the expected ratio, the three phenotypes were obtained in the mouse liver tissues of the F_2 progeny (Fig. 1 d, e, f and Table 1). It was considered that the three phenotypes of F_2 progeny corresponded to the three kinds of genotypes, EsL_1/EsL_1 EsL_1/EsL_2 and EsL_2/EsL_2, respectively. These results indicated that the liver esterase are genetically controlled by the allelic genes of a single locus, designated EsL.

Popp and popp (10) reported that the serum esterases having different electrophoretic patterns among the several inbred strains of mice were genetically controlled by allelomorphs of a single locus. However, the differences of such esterase patterns were not found between the sera of the C_3H strain and the DD/Sd strain exmained in the present study, and also the genetically controlled liver esterases, Types L_1 and L_2, were not found in the mouse sera. These serum and liver esterases thus were not linked genetically. Markert and Hunter (8) separated 10 distinct bands of esterase activity in mouse liver by starch-gel electrophoresis and examined the liver of mice carring the various genes, W, W^v,

Ay, cc, pp and obob etc., to find a genetically controlled difference in esterase activity. However, the zymograms from mice of the varied genotypes were alike and even the obese (obob) mice with exaggerated problems of fat metabolism exhibited the same pattern of liver esterase activity. The liver esterases, Types L_1 and L_2, in the present paper were also independent of coat color, obese mice and sex, although the activity of an esterase in other tissues is probably linked with obesity.

Summary

Zymograms of liver esterases from 2 inbred strains of mice were compared by starch-gel electrophoresis combined with histochemical staining method. The liver esterase of the C_3H strain had slightly greater mobility than that of the homologous esterase of the DD/Sd strain. Liver tissues of F_1 progeny from crosses between C_3H strain and DD/Sd strain always contained both the esterase components of the parental phenotypes. This analysis indicated that the enzymes were codominantly inherited in F_1 progeny. Tests of F_2 progeny indicated segregation of the parental and the F_1 esterase phenotypes with the frequencies expected from genetic control by a pair of alleles. It was concluded that the three esterase phenotypes were genetically controlled by the alleles designated as EsLI in the C_3H strain and EsL_2 in the DD/Sd strain.

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