

INHERITANCE OF SERUM ESTERASES HAVING DIFFERENT ELECTROPHORETIC PATTERNS

Among Inbred Strains of Mice

RAYMOND A. POPP AND DIANA M. POPP*

THE molecular structures of different enzymes capable of catalyzing similar chemical reactions may differ not only among species or strains but also within a tissue of an individual animal¹. Markert and Hunter² have shown that mouse tissues contain several electrophoretically separable esterases that possess overlapping substrate specificities. Some of these esterases are, nevertheless, sufficiently distinguishable in substrate specificity and enzymatic inhibition by eserine to suggest that their molecular structures differ and that their synthesis is controlled by separate genetic loci. However, a second possibility remains that the multiple forms may represent either polymeric states of a single enzyme—the degree of polymerization and manner of folding may alter the ionic charge of the molecule—or complexes of an enzyme with tissue proteins.

Demonstration that at least some of the multiple forms of mouse esterases are genetically distinct from one another would support the hypothesis that the molecular structure of each of the multiple forms is governed by a separate genetic locus. Differences among strains of mice in electrophoretic mobility and pattern of related serum esterases have recently been reported³, and data on the inheritance of these serum esterase differences are presented in this paper.

Materials and Methods

The inbred strains of mice used in this investigation were obtained from several laboratories, as indicated in Table I.

Blood from severed jugular veins was collected in 3-ml. centrifuge tubes. The

clots were allowed to retract for three hours at room temperature, and clear serum was obtained by centrifugation at $600 \times g$ for 10 minutes. Starch-gel electrophoresis, combined with a histochemical method⁴ and a modified biochemical assay for esterases⁵, was used to analyze serum esterases of inbred, F_2 , and BC_1 mice.

Electrophoresis was performed by placing 0.02 ml. of serum on a small piece (6×22 mm.) of Whatman No. 1 chromatography paper and inserting the wetted paper into a starch-gel block (potato starch obtained from Connaught Laboratories, Toronto), which was prepared with 0.03 M borate buffer at pH 8.5. The duration of electrophoresis was four hours, with a voltage drop of 7.5 volts/cm. The starch-gel blocks were removed from the plastic trays, sliced horizontally, and the distribution of esterases was revealed by incubating the starch-gel strips at room temperature in a substrate and dye solution containing 100 mg. Fast Blue RR (diazonium salt obtained from Dajac Laboratories, Philadelphia), 2 ml. 1 percent alpha-naphthyl acetate, 4 ml. 0.2 M phosphate buffer pH 6.8, and 100 ml. distilled H_2O .

For the modified Gomori procedure, 50 μ l. of serum were added to 1 ml. of distilled H_2O , and 10 μ l. of this mixture were withdrawn and pipetted into 1 ml. of distilled H_2O . Five ml. of a freshly prepared substrate solution, which contained 1 ml. of 0.046 M alpha-naphthyl acetate, 20 ml. of 0.2 M phosphate buffer pH 6.8, and 80 ml. of distilled H_2O , were added to the double-diluted serum sample. The reaction mixture was incubated in a water bath at 37° C. for 30

*Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.

minutes. One ml. of a freshly prepared color developer, which contained 100 mg. Fast Red ITR salt (obtained from General Dyestuff Company, Philadelphia), 7 ml. distilled H₂O, and 15 ml. 5 percent sodium lauryl sulfate, was added to the reaction mixture after the 30-minute incubation period. The color of the reaction was allowed to develop for 10 minutes and the optical density at 540 m μ was recorded.

Serum protein determinations⁸ were carried out by adding 5 ml. of biuret reagent to a mixture of 4 ml. H₂O and 1 ml. of the first serum dilution (50 μ l. of serum added to 1 ml. of distilled water). The biuret reaction was incubated for 30 minutes at 30° C., and the optical density at 555 m μ was recorded. The optical densities of the esterase and biuret reactions were used to calculate the specific activity of esterase for each serum sample; specific activity is expressed in mg. alpha-naphthol released/mg. of serum protein during 30 minutes of incubation.

Results

Serum samples of 21 strains and three partially inbred stocks of mice were examined by starch-gel electrophoresis and biochemical photometric techniques (Table I). Although zymograms of mouse serum reveal 10 or more electrophoretically separable esterase frac-

tions⁴, this study is concerned only with the region of the starch gel indicated in Figure 9. In that region of the zymogram, C57BL and C57L sera show a single band of esterase activity, whereas sera from the other strains studied reveal two bands of esterase activity that are difficult to resolve. Moreover, the

TABLE I. Esterase types in various strains of mice as revealed by starch-gel electrophoresis and biochemical assay

Strain*	Esterase type†	Strain	Esterase type
A/R1	II	FLEX/Rc‡	II
AKR/U _p	II	FU/R1	II
BALB/cJ	II	HR/R1	II
CASH/R1‡	II	NB/R1	II
CBA/J	II	RF/U _p	II
CFCW/R1	II	RUS/R1	II
CFW/R1	II	SEA/R1	II
C ₅ H/AnfCum	II	SEC/R1	II
C ₅ H-B/St	II	WC/Re	II
C57BL/Cum	I	11G/R1‡	II
C57L/R1	I	101/Cum	II
DBA/R1	II	129/R1	II

*Sources of mice: R1—W. Russell, and U_p—A. Upton, Biology Division, Oak Ridge National Laboratory; J—Jackson, and Re—E. Russell, Jackson Memorial Laboratory, Bar Harbor, Maine; Cum—Cumberland View Farms, Clinton, Tennessee; and St—L. Strong, Roswell Park, Springville, New York.

†Types I and II refer to patterns with one and two bands of esterase activity, respectively, with electrophoretic mobility similar to that of serum albumin. A minimum of eight mice was used to analyze the esterase type of each strain listed.

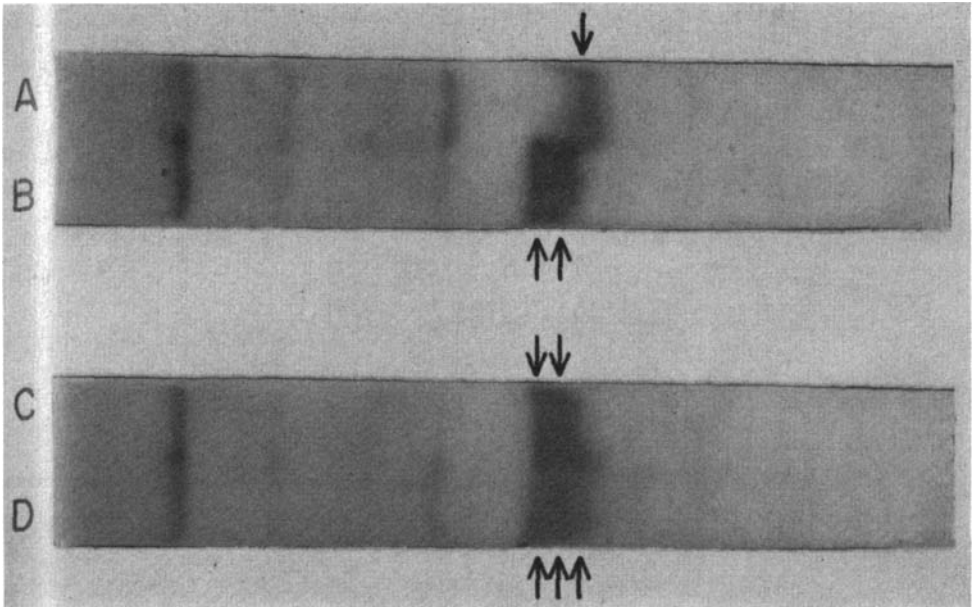
‡Partially inbred stocks.

TABLE II. F₂ and BC₁ progeny classified for serum esterase traits

Matings	Genotype of progeny					
	E ¹ /E ¹		E ¹ /E ²		E ² /E ²	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
(C57BL × 101)F ₁ × (C57BL × 101)F ₁	16	14.75	30	29.5	13	14.75
(C57BL × 101)F ₁ × C57BL*†	53	41.5	30	41.5
(C57BL × 101)F ₁ × 101*	31	33.5	36	33.5
(C57BL × BALB/c)F ₁ × (C57BL × BALB/c)F ₁	24	21.75	42	43.5	21	21.75
(C57BL × BALB/c)F ₁ × C57BL*	31	28	25	28
(C57BL × BALB/c)F ₁ × BALB/c*	15	19.5	24	19.5
(C57BL × SEC)F ₁ × (C57BL × SEC)F ₁	8	11	24	22	12	11
(C57BL × SEC)F ₁ × C57BL*	30	30	30	30
(C57BL × SEC)F ₁ × SEC*	37	36	35	36
Totals						
E ¹ /E ¹ × E ¹ /E ¹	48	47.5	96	95	46	47.5
E ¹ /E ¹ × E ¹ /E ²	114	99.5	85	99.5
E ¹ /E ¹ × E ² /E ²	83	89	95	89

*Results of reciprocal crosses have been combined.

†For unknown reasons this group deviates (P = 0.05) from an expected 1:1 ratio.



ESTERASE BANDS

Figure 9

Zymograms of C57BL, 101, and (C57BL \times 101) F_1 sera, showing the different electrophoretic mobilities and patterns of such serum esterases. A—C57BL; B and C—101; and D—(C57BL \times 101) F_1 sera. Serum was applied at the lateral edges of the filter paper insertion, allowed to diffuse toward the center and overlap, such that the two samples were mixed in the center but unmixed at both edges. Note that both the faster migrating esterase of Type I and slower migrating esterases of Type II are found in serum of F_1 progeny.

electrophoretic mobility of the single-banded C57BL esterase is slightly greater than that of the double-banded esterase of the other strains studied. For the purposes of this report, the single-banded pattern will be referred to as Type I and the double-banded pattern as Type II. On the basis of the data, the genotypes of mice showing Type I and Type II characteristics may be presumed to be Es^1/Es^1 and Es^2/Es^2 , respectively. The electrophoretic pattern of F_1 progeny, genotype Es^1/Es^2 , revealed all three esterase bands (Figure 9).

These electrophoretic patterns were correlated with differences in total esterase activity as judged by the photometric assay. A relatively lower specific activity (85 ± 12) was obtained for sera of C57BL and C57L mice than for sera of the other strains; the specific activity of sera of Type II mice was 187 ± 19 and the activity of sera of F_1 progeny was 140 ± 17 .

Strain C57BL mice, showing a single band of esterase activity appearing near the albumin fraction on starch gel and a lower specific activity for serum esterase, were mated with strain BALB/c, 101, and SEC mice, which possess a double band of slightly slower mobility on starch gel and a higher specific activity. F_1 progeny of these strains were intercrossed and backcrossed to mice of their respective parental types to determine the mode of inheritance of the different esterase patterns. Results of such studies are presented in Table II. The data indicate that the esterase patterns are controlled by different alleles at a single locus. Genotypes of the F_2 and BC_1 progeny of the crosses shown in Table II also indicated that the locus controlling the esterase patterns is not sex-linked nor is it linked with *c*, *Hb*, *Sol*, *a*, *b*, *d*, or *se*; these data are not included, however, in view of the negative evidence obtained.

Discussion

The electrophoretic and biochemical analyses reveal two types of serum esterase patterns among inbred strains of mice. The results of genetic tests are consistent with the idea that the factors determining the variant esterase patterns segregate as simple Mendelian autosomal alleles. Progeny of $F_1 \times C57BL$ matings could be classified either by the electrophoretic or biochemical method. The electrophoretic method was more subjective, however, for distinguishing Es^s/Es^s and Es^s/Es^l progeny of F_1 intercross matings or of F_1 backcrosses to the Es^s/Es^s parent. For this reason, the biochemical photometric method seemed preferable. The biochemical assay was performed on the serum of each animal, using sera of parental and F_1 mice for standard values; however, the specific activities in about 8 percent of the cases lay outside the standard deviation of the mean of the three esterase genotypes. This occurred most frequently in serum samples showing evidence of slight hemolysis. Such sera were re-examined by the electrophoretic method. Owing to subjective interpretation of electrophoretic patterns, the possibility remains, however, that 2 to 3 percent of the total progeny may have been misclassified.

During the preliminary phases of this experiment it was noted that age affected the esterase level. As previously reported by Markert and Hunter⁴, the esterase activity increased from weaning until maturity. Furthermore, females usually had a slightly higher esterase level than males of the same age. For these reasons, analyses were consistently made on serum of eight- to nine-week-old mice, at which time the enzyme level had reached nearly 100 percent of that found in four- to six-month-old mice. With the electrophoretic method, however, the two esterase patterns are distinguishable in weanling mice.

The role of esterases in biochemical processes is not well understood. The large difference noted in specific activity between Es^s/Es^s and Es^s/Es^l mice suggests that the enzyme level may be much in excess of essential requirements in Es^s/Es^s mice. Knowledge of whether the variant forms of these esterases have the same specificity on a large variety of carboxylic acid esters may further help to elucidate the role of esterases in metabolic processes.

The demonstration that the presence of one of the major components of esterase activity is clearly under genetic control and the earlier finding that another esterase (band G) is inhibited by eserine⁵ suggest that at least some of the bands of esterase must have different molecular structures and, therefore, that their synthesis may be controlled by separate genetic loci. Genetic variants of other serum esterases or physicochemical differences among such esterases must be demonstrated, however, before a direct gene-enzyme relation can be established for each electrophoretically distinguishable esterase. Studies in *Zea mays*⁶ and

in *Tetrahymena pyriformis*⁷ also reveal genetically determined differences in esterases.

It is not known whether differences noted in mouse serum are manifested in tissue homogenates. Markert and Hunter⁴ did not observe differences in the esterase activity of livers of mice carrying gene differences that affect coat color, hair structure, and hemoglobin synthesis; strains of mice carrying such genes were not mentioned.

The codominant inheritance of an esterase variation in the mouse seems similar to the genetic control of other enzyme and protein variations in mice and other mammals. Attempts have not been made to fractionate the serum esterases, but, if this could be done, the enzyme might be a suitable protein for studying gene-controlled molecular alterations. Indeed, chemical differences in human hemoglobin have been shown to be correlated with genetic variations⁸.

Summary

Sera of 21 strains and three partially inbred stocks of mice were examined by starch-gel electrophoresis and a colorimetric method for differences in zymogram patterns and levels of serum esterases. A single band of esterase activity that is electrophoretically similar to serum albumin was found in C57BL and C57L mice; two bands of slightly slower mobility were found in the other strains. This analysis indicated that these enzymes are codominantly inherited in F_1 progeny. Moreover, in the C57BL and C57L strains the level of esterase activity is lower than in sera of other strains of mice. Genetic tests indicated that such differences are controlled by allelomorphs of a single locus, designated *Es*.

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