ERYTHROCYTE ESTERASE FORMS CONTROLLED BY MULTIPLE ALLELES IN THE DEER MOUSE[1,2]

SHERMAN RANDERSON[3]

Graduate Program in Genetics, Mammalian Genetics Center, Department of Zoology, The University of Michigan, Ann Arbor

Received June 25, 1965

STUDIES on the inheritance of nonspecific esterase forms of the house mouse (*Mus musculus*) have recently been reported from the serum (POPP and POPP 1962; PETRAS 1963), kidney (RUDDLE and RODERICK 1965), and erythrocytes (PELZER 1965). A brief report (SHAW 1964) indicated inheritable esterase differences in kidney homogenates of the deer mouse, *Peromyscus maniculatus*, and two rare genetically determined variants of an acetylesterase have been described from human red blood cells (TASHIAN and SHAW 1962; TASHIAN 1965). This paper presents the results of a study of the inheritance of certain erythrocyte esterases in the woodland deer mouse, *Peromyscus maniculatus gracilis* (Le Conte). A preliminary report has been published (RANDERSON 1964).

MATERIALS AND METHODS

Mice were obtained from three sources: (1) A feral population sample trapped by D. I. RASMUSSEN in the Huron Mountains area of Northern Michigan, June, 1961; (2) A laboratory population descended from mice trapped in Alger County, Michigan, by W. FRANK BLAIR in 1940 and VAN T. HARRIS in 1947; (3) Offspring from the above two sources.

Blood samples were obtained from the mice after they had reached the age of 24 days. Although previous reports (SHREFFLER 1960; POPP and POPP 1962; PETRAS 1963) indicate that changes occur in the proteins of *Mus musculus* during maturation, no obvious changes in esterase patterns of the mice used in this study were observed after 24 days. The blood was obtained by introducing a 1.5–2.0 mm capillary tube into the suborbital canthal sinus. Sodium citrate (4%) was used to prevent clotting. The samples were washed three times with approximately five volumes of saline and centrifuged for several minutes. The cells were then lysed and the stroma removed by adding two volumes of toluene followed by vigorous shaking, centrifugation for 20 to 30 minutes; the shaking and centrifugation were repeated once. Lysates thus obtained were stored at 3 to 5°C until used. Occasionally samples which had been frozen for some time at —15°C were used.

Starch gels were prepared according to the method of SMITHIES (1959) using 0.03 M borate buffer solution, pH 8.5, and a bridge buffer consisting of 0.3 M borate and 0.05 M NaCl, pH 8.0 (TASHIAN and SHAW 1962). From 0.01 to 0.25 ml lysate was placed in the gel slots. Vertical electrophoresis was carried out for 15 to 17 hours at 3 to 5°C with a voltage gradient of 6 volts per cm.

After electrophoresis, the gels were sliced in half and the halves were incubated at room temperature for 3 to 6 hours in a solution of 350 mg Blue RR salt, 40 ml Tris buffer (0.4 M,

[1] Part of a thesis to be submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Rackham Graduate School of the University of Michigan.

[2] This work was supported in part by Public Health Service grants 5-RO1-GM-7107 and HD-01259-05.

[3] Present address: Biology Department, Stout State University, Menomonie, Wisconsin.

pH 7.1), 400 ml distilled H_2O and 8 ml of a 1% substrate solution (MARKERT and HUNTER 1959). The substrates used were alpha-naphthyl acetate and alpha-naphthyl butyrate.

RESULTS

Up to 13 sites (bands) of erythrocyte esterase activity have been demonstrated using alpha-naphthyl acetate as substrate (Figures 1 and 2). These have been numbered I to XIII beginning with the fastest anodally migrating band. Using alpha-naphthyl butyrate as substrate, only Bands I, II, III, V, and VI could be demonstrated (Figure 3). Variant forms (presence or absence of a band) were found to occur at Bands I, II, III, and V. This paper is concerned only with the inheritance of Bands III and V. The presence of Bands I and II was usually difficult to determine due to extremely weak staining with both alpha-naphthyl acetate and alpha-naphthyl butyrate as substrate. Almost all lysates were classi-

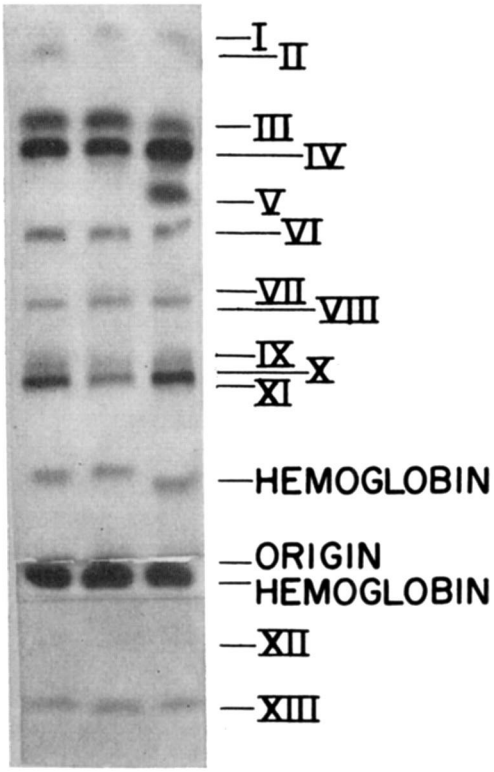

FIGURE 1.—Zymogram of erythrocyte esterases of the deer mouse using alpha-naphthyl acetate as substrate. The areas of esterase activity have been numbered I to XIII beginning with the fastest anodally migrating band. The variation in electrophoretic mobility exhibited by the anodally migrating hemoglobin band has not yet been investigated.

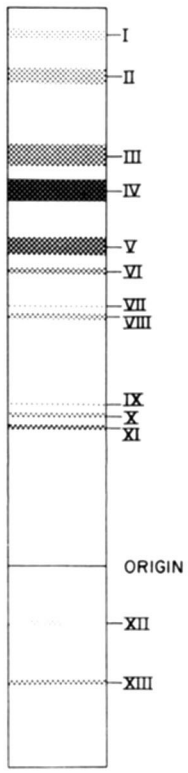

FIGURE 2.—Composite diagram of the nonspecific esterases from erythrocytes of the deer mouse.

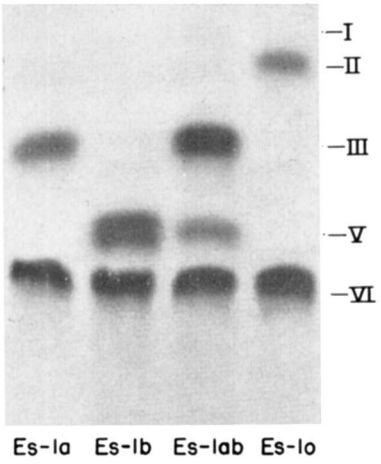

FIGURE 3.—Zymogram of erythrocyte esterases of the deer mouse using alpha-naphthyl butyrate as substrate. Only the five bands shown have been demonstrated with this substrate. Es-1a, Es-1b, Es-1ab, and Es-1o represent the four esterase phenotypes discussed in the text.

fied using alpha-naphthyl butyrate as substrate since Bands III and V were more readily demonstrated under this condition.

Four phenotypes were observed in regard to Bands III and V (Figure 3). These are designated Es-1a (presence of Band III, absence of Band V), Es-1b (absence of Band III, presence of Band V), Es-1ab (presence of both Bands III and V), and Es-1o (absence of both Bands III and V). The zymogram pattern of Es-1ab could be duplicated by mixing equal parts of Es-1a and Es-1b type lysates. Mixtures of Es-1a and Es-1o lysates produced the Es-1a pattern; mixtures of Es-1b and Es-1o lysates produced the Es-1b pattern.

Breeding tests were performed to determine the mode of inheritance of these four phenotypes (Table 1). The data in this table represent the classification by phenotype of 855 offspring from all of the ten possible matings by phenotype. Sex linkage can be excluded on the observation that matings of Es-1ab males with Es-1o females produced approximately ¼ Es-1a males, ¼ Es-1b males, ¼ Es-1a females, and ¼ Es-1b females. Data from reciprocal crosses (with respect to sex) have therefore been pooled in Table 1. Chi-square values in Table 1 have been computed on the basis of two genetic hypotheses: (1) The four erythrocyte esterase phenotypes are controlled by two unlinked autosomal loci, one locus determining Band III, the other locus determining Band V. (2) The inheritance of the four phenotypes is determined by one autosomal locus with three alleles, one allele producing Band III, another allele producing Band V, and a third allele producing no detectable activity. The fact that four of the χ^2 values computed on the two locus hypothesis are significant at the 0.01 or 0.001 level of significance excludes the first hypothesis. The data, however, are consistent with the hypothesis that the inheritance of the four erythrocyte esterase phenotypes is governed by one autosomal locus involving three alleles. It is proposed that the locus be designated *Es-1*, with alleles $Es\text{-}1^a$ (producing Band III), $Es\text{-}1^b$ (producing Band

TABLE 1

Summary of mating-offspring erythrocyte esterase phenotypes in the deer mouse

Parental phenotypes	Parental genotypes	Total offspring of various phenotypes Es-1a	Es-1b	Es-1ab	Es-1o	Two locus hypothesis χ^2	P	One locus hypothesis χ^2	P
Es-1a × Es-1a	*Es-1^a* .... × *Es-1^a*	69	0	0	0	...	...	...	..
	Es-1^{a}Es-1^o × *Es-1^a*	11	0	0	0	...	...	...	..
	Es-1^{a}Es-1^o × *Es-1^{a}Es-1^o*	13	0	0	3	0.08	0.8	0.08	0.8
Es-1b × Es-1b	*Es-1^b* .... × *Es-1^b*	0	12	0	0	...	...	...	..
	Es-1^{b}Es-1^b × *Es-1^b*	0	15	0	0	...	...	...	..
	Es-1^{b}Es-1^o × *Es-1^b*	0	5	0	0	...	...	...	..
	Es-1^{b}Es-1^o × *Es-1^{b}Es-1^o*	0	18	0	6	0.00	1.0	0.00	1.0
Es-1o × Es-1o	*Es-1^{o}Es-1^o* × *Es-1^{o}Es-1^o*	0	0	0	12	...	...	...	..
Es-1ab × Es-1ab	*Es-1^{a}Es-1^b* × *Es-1^{a}Es-1^b*	21	15	26	0	14.68	0.01	2.78	0.3
Es-1a × Es-1o	*Es-1^{a}Es-1^o* × *Es-1^{o}Es-1^o*	1	0	0	3	0.25	0.6	0.25	0.6
Es-1b × Es-1o	*Es-1^{b}Es-1^o* × *Es-1^{o}Es-1^o*	0	4	0	6	0.10	0.7	0.10	0.7
Es-1a × Es-1b	*Es-1^{a}Es-1^a* × *Es-1^{b}Es-1^b*	0	0	13	0	...	...	...	..
	Es-1^a .... × *Es-1^{b}Es-1^o*	13	0	19	0	...	...	...	..
	Es-1^a .... × *Es-1^b*	0	1*	12	0	...	...	...	..
	Es-1^{a}Es-1^o × *Es-1^{b}Es-1^o*	34	29	17	24	6.07	0.1	6.07	0.1
Es-1ab × Es-1o	*Es-1^{a}Es-1^b* × *Es-1^{o}Es-1^o*	36	38	0	0	75.02	0.001	0.01	0.9
Es-1ab × Es-1a	*Es-1^{a}Es-1^b* × *Es-1^a*	87	0	83	0	...	...	...	..
	Es-1^{a}Es-1^b × *Es-1^{a}Es-1^o*	38	21	27	0	22.41	0.001	2.00	0.4
Es-1ab × Es-1b	*Es-1^{a}Es-1^b* × *Es-1^b*	0	7	3	0	...	...	...	..
	Es-1^{a}Es-1^b × *Es-1^{b}Es-1^b*	0	5	3	0	0.13	0.7	0.13	0.7
	Es-1^{a}Es-1^b × *Es-1^{b}Es-1^o*	30	45	30	0	37.86	0.001	2.15	0.4

* Presumably this mouse was placed in this sibship by mistake.

V), and *Es-1*^{o} (producing no detectable activity). Alleles *Es-1*^{a} and *Es-1*^{b} are codominant, while *Es-1*^{o} is recessive to these two codominant alleles.

In the house mouse (PELZER 1965), loci controlling the inheritance of certain erythrocyte esterases have been designated *Ee-1* and *Ee-2*, where *Ee* indicates *erythrocyte esterase*. Since preliminary evidence (to be presented elsewhere) indicates that the inherited esterase forms (Bands III and V) described herein for Peromyscus are also present in tissues other than blood, a less tissue-specific designation seems more appropriate. Hence in the designation of *Es-1* as the locus controlling the inheritance of the four esterase phenotypes, *Es* indicates *esterase*, while the number 1 indicates the first such locus demonstrated for the esterases of the woodland deer mouse. Other esterase loci could be designated *Es-2*, *Es-3*, etc., while other alleles shown to belong to the *Es-1* locus could be designated *Es-1*^{c}, *Es-1*^{d}, etc.

DISCUSSION

It has not yet been possible to distinguish electrophoretically between lysates from homozygotes (*Es-1*^{a} *Es-1*^{a}, *Es-1*^{b} *Es-1*^{b}) and lysates from their respective heterozygotes (*Es-1*^{a} *Es-1*^{o}, *Es-1*^{b} *Es-1*^{o}). While differences in staining intensity do appear to exist, such differences do not seem to be correlated with the genotype of the mice involved. No quantitative studies measuring staining intensity were carried out. In Table 1, the genotypes of Es-1a and Es-1b parents were ascertained by inspection of their parents, or, in a few instances (when the number of offspring was sufficiently large) through inspection of their offspring. When the genotypes of Es-1a and Es-1b parents could not be determined, they were classified as *Es-1*^{a} . . . . and *Es-1*^{b} . . . .

The fact that no hybrid enzymes were observed in lysates from *Es-1*^{a} *Es-1*^{b} heterozygotes, as observed in heterozygous maize tissue (SCHWARTZ 1960, 1964), suggests that the esterases produced by alleles *Es-1*^{a} and *Es-1*^{b} are composed of a single polypeptide or aggregates of polypeptides produced by a single allele.

The different rates of electrophoretic mobility expressed by Bands III and V indicate a change in the structure of the enzyme. Hence it seems reasonable to assume that the *Es-1* locus is a structural rather than a regulatory locus. The possibility that variant Bands I or II represent the product of allele *Es-1*^{o} can be excluded on the grounds that lysates from heterozygous (*Es-1*^{a} *Es-1*^{b}) mice displaying esterase activity at all four sites (I, II, III, and V) were observed. If either Band I or Band II represented the product of *Es-1*^{o}, neither would be expected to be present in *Es-1*^{a} *Es-1*^{b} lysates. The hypothesis that *Es-1*^{o} is a closely linked regulatory gene rather than a defective structural allele of *Es-1*^{a} and *Es-1*^{b} cannot be excluded.

An hypothesis that *Es-1*^{o} is not an allele of *Es-1*^{a} and *Es-1*^{b}, but rather a dominant or recessive suppressor gene (either linked or not linked to the esterase locus *Es-1*) blocking the action of *Es-1*^{a} and *Es-1*^{b}, can be excluded by an examination of the phenotypes of the offspring of matings *Es-1*^{a} *Es-1*^{b} × *Es-1*^{o} *Es-1*^{o}. Es-1ab, Es-1o, or both such offspring would be expected from these matings (such offspring would also eliminate the three allele hypothesis) if such a suppressor locus were involved, but none were observed out of a total of 74 (Table 1).

The *Es-1*^{o} allele described herein for Peromyscus appears to be analogous to the *Ee-2*^{0} allele described by PELZER (1965). This *Ee-2*^{0} allele is responsible for a lack of esterase activity at the Ee-2a site in zymograms of erythrocyte esterases of the house mouse. RUDDLE and RODERICK (1965) described two alleles at separate loci which are responsible for a lack of esterase activity in house mouse kidney extracts. A "silent" gene has also been postulated as being responsible for a lack of cholinesterase activity in human serum (SIMPSON and KALOW 1964).

Lysates of erythrocytes from the house mouse and the deer mouse were subjected to electrophoresis side by side in the same starch gel by both PELZER (unpublished data) and this author in an attempt to correlate the various zones of esterase activity in each species with respect to electrophoretic mobility. Under the present conditions the deer mouse has two cathodally migrating forms, while the house mouse has no cathodally migrating forms. Of the 11 anodally migrating forms of each species, only two appear to have the same electrophoretic mobility: Band VI of the deer mouse appears to correspond to zone Ee-7 of the house mouse; Band X of the deer mouse appears to correspond to zone Ee-9 of the house mouse.

All the deer mice involved in the present study appeared to be healthy. Preliminary evidence suggests, however, that some type of selection involving the *Es-1* locus may be occurring. The direction of selection seems to be related to the background genotype ("wild" *vs.* "laboratory") of the mice. Preliminary evidence also indicates that the *Es-1* locus is rather closely linked to a locus responsible for another red cell phenotype in Peromyscus, i.e., the blood group locus *Pm* (RASMUSSEN 1961). This aspect, as well as the population distribution of alleles, will be considered in detail elsewhere.

I am especially indebted to DR. MORRIS FOSTER, Department of Zoology, for his encouragement and advice during the course of this study and in the preparation of the manuscript. I am also grateful to DRS. E. BARTO (Department of Zoology), C. SHAW (Hawthorne Center, Northville, Michigan), D. STRACHAN (Department of Anatomy), and R. TASHIAN and C. PELZER (Department of Human Genetics) for their advice and suggestions regarding the preparation of the manuscript.

SUMMARY

Vertical starch gel electrophoresis coupled with histochemical staining procedures has demonstrated up to 13 sites of esterase activity (2 cathodal, 11 anodal) in erythrocytes from the woodland deer mouse, *Peromyscus maniculatus gracilis*. With respect to Bands III and V of the anodally migrating forms, four phenotypes have been observed and have been designated Es-1a (presence of Band III, absence of Band V), Es-1b (absence of Band III, presence of Band V), Es-1ab (presence of both Bands III and V), and Es-1o (absence of both Bands III and V). Classification by phenotype of 855 offspring from all of the ten possible matings by phenotype indicates that the inheritance of the four esterase phenotypes is governed by one autosomal locus, designated *Es-1*, with alleles *Es-1*^{a}, *Es-1*^{b}, and *Es-1*^{o}.

LITERATURE CITED

MARKERT, C. L., and R. L. HUNTER, 1959 The distribution of esterases in mouse tissue. J. Histochem. Cytochem. **7**: 42–49.

PELZER, C. F., 1965 Genetic control of erythrocytic esterase forms in *Mus musculus*. Genetics **52:** 819–828.

PETRAS, M. L., 1963 Genetic control of a serum esterase component in *Mus musculus*. Proc. Natl. Acad. Sci. U. S. **50:** 113–116.

POPP, R. A., and D. M. POPP, 1962 Inheritance of serum esterases having different electrophoretic patterns. J. Heredity **53:** 111–114.

RANDERSON, S., 1964 The inheritance of an erythrocytic esterase component in the deer mouse. (Abstr.) Genetics **50:** 277.

RASMUSSEN, D. I., 1961 Erythrocytic antigenic differences between individuals of the deer mouse, *Peromyscus maniculatus*. Genet. Res. **2:** 449–455.

RUDDLE, F. H., and T. H. RODERICK, 1965 The genetic control of three kidney esterases in C57BL/6J and RF/J mice. Genetics **51:** 445–454.

SCHWARTZ, D., 1960 Genetic studies of mutant enzymes in maize: synthesis of hybrid enzymes by heterozygotes. Proc. Natl. Acad. Sci. U. S. **46:** 1210–1215. —— 1964 A second hybrid enzyme in maize. Proc. Natl. Acad. Sci. U. S. **51:** 602–605.

SHAW, C. R., 1964 The use of genetic variation in the analysis of isozyme structure. Brookhaven Symp. Biol. **17:** 117–130.

SHREFFLER, D. C., 1960 Genetic control of serum transferrin type in mice. Proc. Natl. Acad. Sci. U. S. **46:** 1378–1384.

SIMPSON, N. E., and W. KALOW, 1964 The "silent" gene for serum cholinesterase. Am. J. Human Genet. **16:** 180–188.

SMITHIES, O., 1959 An improved procedure for starch-gel electrophoresis: further variations in the serum proteins of normal individuals. Biochem. J. **71:** 585–587.

TASHIAN, R. E., 1965 Genetic variation and evolution of the carboxylic esterases and carbonic anhydrases of primate erythrocytes. Am. J. Human Genet. **17:** 257–272.

TASHIAN, R. E., and M. W. SHAW, 1962 Inheritance of an erythrocyte acetylesterase variant in man. Am. J. Human Genet. **14:** 292–300.