Linkage of Es-1 and Es-2 in the Mouse

RAYMOND A. POPP

IN a paper on the inheritance of esterases found in mouse erythrocytes[4], I reported that RFM/Un mice lack the fastest migrating anodal esterase. The serum esterase pattern of RFM/Un mice is similar to that reported by Petras for the feral mouse with the *Es-2*^{a} allele[1]. *Es-2*^{a}, the silent allele, expresses no esterase; the esterase identified as Es-2b is expressed in mice that have the *Es-2*^{b} allele. RFM/Un is the first and only inbred strain of laboratory mice known to carry the *Es-2*^{a} allele. Data reported here describe briefly the effect of *Es-2*^{a} on the esterase pattern of tissues from the RFM/Un mouse and show that *Es-2* is linked to *Es-1*.

Materials and Methods

The inbred strain of laboratory mice in which the *Es-2*^{a} allele was found is the RFM/Un, the foundation stock from which other closely related sublines have been derived in the past. It is important to distinguish the RFM/Un from its sublines because three of the sublines tested carry the *Es-2*^{b} allele.

Blood was obtained from the tail or jugular veins of the mice. Tissues were rinsed in saline before being homogenized in a Potter-Elvehjem homogenizer using an equal volume of distilled water. Serum or tissue extract was applied to Whatman no. 1 filter paper and then inserted into starch gels for electrophoresis. Methods for starch gel electrophoresis and localization of the esterases by a diazonium dye-coupling reaction have been described previously[5]. Alpha naphthyl acetate or α naphthyl butyrate was used as substrate.

RFM/Un mice (*Es-1*^{b} *Es-2*^{a}/*Es-1*^{b} *Es-2*^{a}) were mated to C57BL/Cum mice (*Es-1*^{a} *Es-2*^{b}/*Es-1*^{a} *Es-2*^{b}), and the F_1 progeny were intercrossed or backcrossed to parental strain mice. Serum from the F_2 and BC_1 mice was analyzed to classify the mice for their *Es-1* and *Es-2* genotypes. Differences in the electrophoretic patterns of Es-1a and Es-1b esterases[5] were used to classify the mice for their *Es-1* alleles. The basis for classifying the mice for their *Es-2* alleles will be discussed later in the text.

Two analyses were made on many sera, and sera from all mice that showed recombination of alleles at *Es-1* and *Es-2* were reanalyzed. This was done because, as indicated previously[3,5], a single analysis does not always permit definite distinction of Es-1a/Es-1b from Es-1b/Es-1b. Moreover, as will be discussed later, it is sometimes difficult to distinguish Es-2a/Es-2b from Es-2b/Es-2b in a single analysis of serum. In a few cases, tissue extracts were analyzed to confirm results obtained from the serum samples. The advantages of using tissue extracts for the classification of mice for alleles at *Es-2* will be discussed later in the text.

Strain RFM/Un mice were also mated to a subline of mice derived from the feral mouse of Petras. This subline was kindly provided by Dr. Robert L. Hunter, Stanford Medical School, Palo Alto, California. Serum from the F_1 hybrids was analyzed for its Es-2 type.

Results and Discussion

Sera from more than 20 strains of laboratory mice have been examined for the presence or absence of Es-2b. Most of these strains are listed in a previous paper[5]; additional strains examined were C57BR/RL, SWR/Re, RF/J, RF/Lo, and Y/He. The only strain that lacks Es-2b is RFM/Un. Zymograms of serum from RFM/Un, C57BL/Cum and F_1 hybrid mice are illustrated in Figure 1. The zymograms show that Es-2b is absent from RFM/Un serum, intermediate in quantity in the serum of F_1 hybrids, and present in larger quantities in the serum of C57BL/Cum mice. Extracts from the kidneys of the same mice are shown in Figure 2. Again one sees the same relationship between genotype and quantity of esterase in the Es-2b region. Although not shown, Es-2b has a higher affinity for α naphthyl butyrate than for α naphthyl acetate and it becomes inactivated after heating to 56°C for 30 minutes.

Serum from the F_1 progeny of RFM/Un and the subline of the feral mouse was examined for the presence or absence of Es-2b. The results showed that Es-2b was absent in such mice; therefore, the *Es-2*^{a} allele in RFM/Un mice is considered to be identical to that described by Petras[1].

Altogether, $156F_2$ and $153BC_1$ mice were classified for their *Es-1* and *Es-2* genotypes. The criteria used to classify the mice for their *Es-2* alleles were as follows: Mice homozygous for the *Es-2*^{a} allele lacked Es-2b, and the intensity of staining of Es-2b was used to distinguish *Es-2*^{a}/*Es-2*^{b} and

The author is affiliated with the Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830. Analyses on the progeny of Petras subline and strain RFM/Un mice were done in collaboration with Dr. R. C. Allen of this Division. This research was sponsored by the U. S. Atomic Energy Commission under contract with the Union Carbide Corporation.

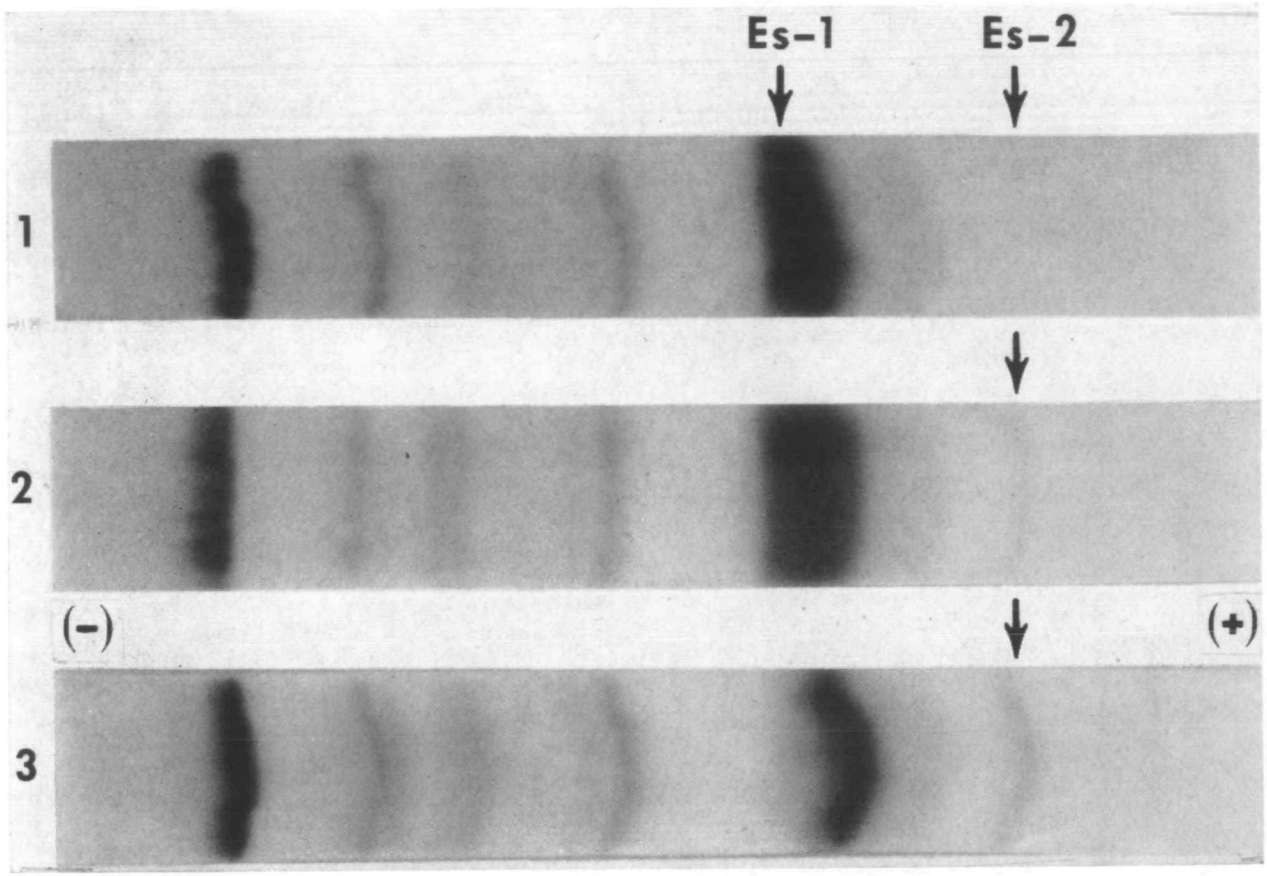

FIGURE 1—Zymograms showing the Es-1 and Es-2 phenotypes of mouse serum. Duration of electrophoresis was 4 hours; alpha-naphthyl butyrate was used as substrate. The strains of mice and their Es-1 and Es-2 phenotypes are as follows: 1—RFM/Un, Es-1b and Es-2a; 2—(C57BL × RFM)F_1, Es-1a+b and Es-2a+b; and 3—C57BL, Es-1a, and Es-2b. Both electrophoretic mobility and pattern were used to identify Es-1 phenotypes. Es-2a represents the absence of Es-2 esterase and the intensity of staining was used to distinguish phenotypes Es-2b and Es-2A +b.

Es-2^b/Es-2^b individuals, as shown in Figures 1 and 2. The differences in the staining intensity of Es-2b in homozygous and heterozygous individuals is more pronounced in tissue extracts than in serum samples (cf. Figures 1 and 2). Thus, tissue extracts were analyzed when the results of serum samples were ambiguous. The genotypes of the F_2 and BC_1 mice are given in Table I; the combined recombination frequency of alleles at *Es-1* and *Es-2* was calculated to be 0.1100 ± 0.0178.

The phenotypic expression of *Es-2^a* in strain RFM/Un mice is indistinguishable from that in the feral mouse. Both lack the fastest migrating esterase in the serum[1] (Figure 1), and tissues such as the liver, kidney, and duodenum lack Es-2b in the feral mouse[1] and in RFM/Un mice (Figure 2). Moreover, the serum from F_1 hybrids of RFM/Un mated to a subline of the feral mouse lacks Es-2b. It is therefore concluded that strain RFM/Un mice carry the *Es-2^a* allele.

Several sublines have been derived from strain RFM/Un mice, and serum from a few of these was obtained for study. Dr. A. C. Upton (Biology Division, Oak Ridge National Laboratory) has a closed, but random bred, colony of RF mice that are related to RFM/Un, but thay have been maintained separately since 1949. These RF random-bred mice have the Es-2b esterase. Strain RF/J originated from RFM/Un at generation 19 in 1954. Illustrations in a report of Ruddle and Roderick[6] on kidney esterases suggested that RF/J mice carry the *Es-2^b* allele. Indeed, serum samples from three RF/J mice received from Dr. G. D. Snell (The Jackson Laboratory, Bar Harbor, Maine) showed that RF/J mice do express Es-2b. It has been shown previously that strain RFM/Un and RF/J mice also differ at the *H-2* locus[2]. Strain RF mice were sent at generation 25 in 1957 to Dr. J. Spaulding (Los Alamos Scientific Laboratory, Los Alamos, New Mexico). Serum samples were taken from six RF/Lo mice and each showed the presence of Es-2b. Analyses of the *Es-2* genotype of RFM/Un mice were begun at generation 55. All RFM/Un mice tested to date lack Es-2b. Either the *Es-2* genotype of the RFM/Un mice had not become fixed when the RF/J and RF/Lo mice were sent to those laboratories or a mutation from *Es-2^b* to *Es-2^a* occurred and became fixed in the RFM/Un breeder line at Oak Ridge

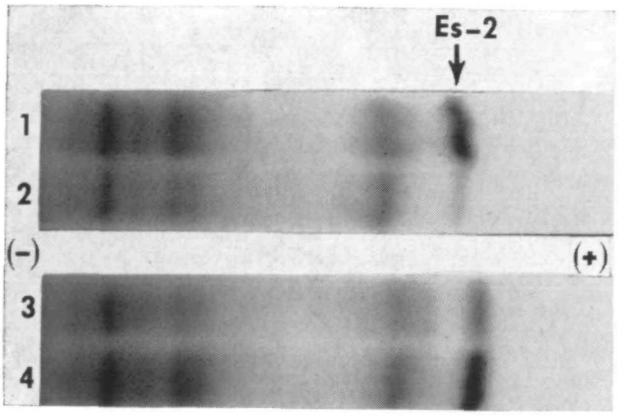

FIGURE 2—Zymograms showing the effect of Es-2 alleles on the esterase pattern of kidney extracts of mice. Alpha-naphthyl acetate was used as substrate. Two samples were electrophoresed in the same starch gel. The strains of mice used are as follows: 1 and 4 —C57BL; 2—RFM/Un; and 3—(C57BL × RFM)F_1. The differences in the intensity of staining in the Es-2 region were used to identify the Es-2 phenotypes. The esterase band in the Es-2 region of the RFM/Un sample is not Es-2b, because Es-2b is heat labile and a similar component is observed in heat-treated extracts of C57BL kidneys.

between generations 25 and 55. It is not possible to decide between these alternatives because serum from mice of the intervening generations is not available for testing.

Linkage data in Table I establish that *Es-2* and *Es-1* are linked, and *Es-1* has been shown to be linked with *Os* in linkage group XVIII[3]. Preliminary results suggest that the linear order of loci in linkage group XVIII is *Hk*, *Es-1*, *Os*, and *Es-2*.

Summary

The fastest migrating esterase in mouse serum and tissue extracts is controlled by alleles at *Es-2*. The $Es\text{-}2^a$ allele does not produce a recognizable esterase product, and $Es\text{-}2^b$ controls the synthesis of Es-2b. The silent allele, $Es\text{-}2^a$, in strain RFM/Un mice seems to be identical to the one previously found in a feral mouse. Strain RFM/Un is the only known inbred strain of laboratory mice that has the $Es\text{-}2^a$ allele; all other strains of laboratory mice examined have the $Es\text{-}2^b$ allele. Linkage studies show that *Es-2* is linked to *Es-1* in linkage group XVIII; a recombination frequency of 0.1100 ± 0.0178 was observed among the 309 mice analyzed.

Literature Cited

1. Petras, M. Genetic control of a serum esterase component in *Mus musculus*. *Proc. Natl. Acad. Sci. U. S.* 50.113–116. 1963.

2. Popp, D M and D. B. Amos. An H-2 analysis of strain RFM/Un mice. *Transplantation* 3.501–508. 1965.

3. Popp, R. A. Loci linkage of serum esterase patterns and oligosyndactyly. *J. Hered.* 56·107–108. 1965.

4. ————. Inheritance of an erythrocyte and kidney esterase in the mouse. *J. Hered.* 57:197–201. 1966.

5. ———— and D. M. Popp. Inheritance of serum esterases having different electrophoretic patterns. *J. Hered.* 53·111–114. 1962.

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

Table I. F_2 and BC_1 mice classified for their *Es-1* and *Es-2* genotypes

Matings	Offspring: Nonrecombinants*			Offspring: Recombinants*				Totals
	Es-1 / *Es-2* a/a b/b	a/b b/a	b/b a/a	a/a a/b	a/b a/a	a/b b/b	b/b a/b	
$\frac{Es\text{-}1^a\ Es\text{-}2^b}{Es\text{-}1^b\ Es\text{-}2^a} \times \frac{Es\text{-}1^a\ Es\text{-}2^b}{Es\text{-}1^b\ Es\text{-}2^a}$	35	67	37	5	5	3	4	156
$\frac{Es\text{-}1^a\ Es\text{-}2^b}{Es\text{-}1^a\ Es\text{-}2^b} \times \frac{Es\text{-}1^a\ Es\text{-}2^b}{Es\text{-}1^b\ Es\text{-}2^a}$	62	55		6		9		132
$\frac{Es\text{-}1^b\ Es\text{-}2^a}{Es\text{-}1^b\ Es\text{-}2^a} \times \frac{Es\text{-}1^a\ Es\text{-}2^b}{Es\text{-}1^b\ Es\text{-}2^a}$		8	11		1		1	21
	Recombination frequency 0.1100 ± 0.0178							

* In the first cross, half the cases of crossovers in both parents are indistinguishable from noncrossovers. Based on the observed recombination frequency, one or two such individuals might be included here. Thus, the true recombination frequency is perhaps slightly greater than the one given. Also, note that individuals of the other possible recombinant classes representing crossovers in both parents (*i.e.*, $Es\text{-}1^{a/a}\ Es\text{-}2^{a/a}$ and $Es\text{-}1^{b/b}\ Es\text{-}2^{b/b}$) were not observed among the mice examined.