SERUM TRANSFERRIN POLYMORPHISM IN THE DEER MOUSE[1]

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KNOWLEDGE of the inheritance of various protein differences in the deer mouse *Peromyscus maniculatus* has increased rapidly (RANDERSON 1965; SHAW 1964, 1965; SHAW and BARTO 1963, 1965; WELSER, WINKELMANN, CUTLER and BARTO 1965). Aside from indicating the wealth of individual variation within the species, these studies of protein polymorphisms within the deer mouse have been utilized in the analysis of subunit structure of enzymes (SHAW 1964, 1965; SHAW and BARTO 1963). Similar genetic markers may also serve in the analysis of the subunit structure of natural Mendelian populations (RASMUSSEN 1964). This study concerns the identification by starch gel electrophoresis and analysis of the inheritance of serum transferrin variants occurring within populations of the deer mouse.

MATERIALS AND METHODS

The deer mouse stock used for study was derived from approximately 200 wild trapped mice from several localities in Northern Arizona.

Blood was obtained by inserting a 1.5–2.0 mm capillary tube into the suborbital canthal sinus, and bleeding into 6 × 50 mm culture tubes. Samples were allowed to clot at room temperature for one hour. Following centrifugation and separation from erythrocytes, sera were immediately fractionated by horizontal starch gel electrophoresis.

The physical system employed and the procedures described in detail by KRISTJANSSON (1963) were followed in the preparation of gels and buffers. Gels were prepared at a concentration of 12% hydrolyzed starch (Connaught Medical Laboratories). Proteins were separated in a discontinuous system of buffers with a Tris-citrate gel buffer (pH 7.5) and sodium borate electrode chamber buffer (pH 8.7). A voltage drop of 10.0 v/cm was applied until the borate boundary had migrated 8.0 cm from the origin (about 2 hours).

Transferrin identification was made utilizing the Canalco Series 800 Iron Staining Kit (Canal Industrial Corporation, Bethesda) developed by L. ORNSTEIN (no date). A small amount of Ferric citrate was added to each serum sample prior to electrophoresis to intensify transferrin detection. A light-green color developed in areas of siderophilin activity, these areas were marked and the gel was then counterstained for 10 minutes in a saturated solution of Amidoblack in water, methanol and glacial acetic acid (5:5:1). Washing gels overnight in the solvent solution cleared the nonprotein areas of the gels.

RESULTS

Within individuals of the species *Peromyscus maniculatus*, the described electrophoretic procedure revealed serum transferrins of three mobilities (Figure 1). The three are designated transferrin a, transferrin b, and transferrin c. Trans-

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ferrin a has been observed in only one wild trapped mouse, a male, which to date has been bred in the laboratory with only limited success (Table 1). Although this component is described in this paper, definite designation of its genetic basis is precluded because of its rareness in our stocks. The other two transferrin components appear as unitary genetic characteristics inherited as a two-allele, one locus system. The locus responsible for the transferrin variants is designated by the symbol *Trf*, making the allele symbols Trf^b and Trf^c for the two alleles, and Trf-b, Trf-bc, and Trf-c for the three common phenotypes. This nomenclature follows that recommended by COHEN and SHREFFLER (1961) for the transferrin locus in the house mouse, *Mus musculus*. This similarity of symbols, however, is not meant to imply any protein homology between the two mouse species other than their identity as transferrins. The single wild trapped mouse exhibiting both the transferrin a and transferrin b component is designated with the phenotype Trf-ab and some of his offspring exhibit a phenotype designated Trf-ac (Figure 1), although definite evidence of the allelic relationship of the genetic unit determining the production of transferrin a is lacking. The common phenotypes observed occur in either sex and no ontogenetic changes have been observed from the time of weaning (about 3 weeks of age) to subsequent analysis at 6 to 12 months of age.

The proposed genetic basis for the phenotypes is based on the data presented in Table 1. In the tests of allelism of the Trf^b and Trf^c genes no sibship segregated for more than three phenotypes and no transferrin components were observed in offspring that were not present in their parents. The data are consistent with the

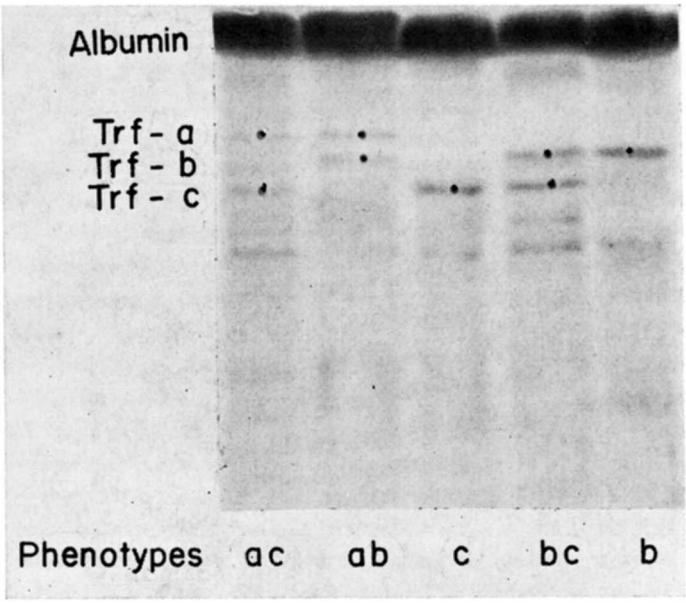

FIGURE 1.—Electrophoretic patterns of serum proteins in *Peromyscus maniculatus*. Gel stained in Amidoblack. Transferrin components revealed by the described technique are marked with a dot. Designation of individual phenotypes are given.

hypothesis that the two transferrin components, Trf-b and Trf-c, are controlled by two codominant alleles at a single locus. The very limited progeny data related to the rare Trf-a component is also consistent with a single locus model.

On another hypothesis namely that the two transferrins are determined by dominant genes at separate loci, a mouse of phenotype Trf-bc should be identifiable as a double heterozygote, having the genotype X^b/x, Z^c/z, if it satisifies either of the following criteria: (1) it has one parent of phenotype Trf-b and one of Trf-c, or (2) its offspring include mice exhibiting three phenotypes Trf-b, Trf-bc, Trf-c. Assuming that the offspring tested are representatives of the frequency of random genetic unions, 1/16 of the offspring from the matings between two such double heterozygotes are expected to exhibit a fourth phenotype of the genotype x/x, z/z. The probability of not observing individuals lacking both transferrin components, therefore, becomes $(15/16)^n$, where n represents the total offspring observed in Trf-bc by Trf-bc matings ascertained by the above criteria as double heterozygotes. A total of 47 offspring from such matings were observed, thereby giving a probability of less than 0.05 with the two-locus model for the observed offspring of this one mating type.

The observed phenotypic frequencies from several wild trapped populations exhibit a close agreement to the zygotic frequencies expected in a two-allele, one-locus panmictic population. For example of 85 wild trapped mice from the North Kaibab Plateau, Arizona, 58 exhibited the Trf-b phenotype, 23 the Trf-bc phenotype, and 4 the Trf-c phenotype. The estimated frequency of the Trf^b allele is 0.818 and the value for agreement of the phenotype frequencies to the expected zygotic frequencies of a one-locus system is chi-square = 0.65 ($df = 1$, $P > 0.3$).

The transferrin polymorphism appears to be a widespread geographical attribute of the species since laboratory stocks derived from mice trapped in Alger County, Michigan in 1940 and 1947 as well as mice from Contra Costa and Alameda counties, California trapped in 1963, exhibited both the Trf-b and Trf-c proteins.

Other species: During the course of field collecting, a limited number of mice from additional species of the genus Peromyscus have been collected at various localities in Arizona. Transferrin polymorphism was observed in each of species

TABLE 1

Summary of parent-offspring transferrin phenotypes in Peromyscus maniculatus

Phenotype of mating	Number of litters	Total number of offspring	Total offspring of various phenotypes					Chi-square*
			Trf-b	Trf-bc	Trf-c	Trf-ab	Trf-ac	
1. Trf-b × Trf-b	15	66	66	..	..	..	..	.....
2. Trf-c × Trf-c	1	4	..	..	4	..	..	.....
3. Trf-b × Trf-c	5	22	..	22	..	..	..	.....
4. Trf-b × Trf-bc	40	155	78	77	..	..	..	0.01 ($df = 1$ $P > 0.9$)
5. Trf-c × Trf-bc	3	12	..	7	5	..	..	.....
6. Trf-bc × Trf-bc	11	47	14	24	9	..	..	1.08 ($df = 2$ $P > 0.5$)
7. Trf-ab × Trf-bc	2	9	2	2	..	1	4	.....

* Chi-square tests are based on hypothesis of one-locus mode of inheritance.

collected. A sample of 23 individuals of the brush mouse, *Peromyscus boylii*, exhibits a polymorphism of two transferrin variants similar in electrophoretic mobility to the Trf-b and Trf-c components in *P. maniculatus* (Figure 2). Samples of three canyon mice, *P. crinitus*, and 13 cactus mice, *P. eremicus* both exhibit polymorphism for two transferrin components. Although the mobilities of the components are similar for these latter species, their transferrins have a markedly slower migration than the transferrins of *P. maniculatus* and *P. boylii* (Figure 2).

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SUMMARY

Serum transferrin polymorphism within natural populations of *Peromyscus maniculatus* was demonstrated utilizing horizontal gel electrophoresis. Electrophoresis has separated three transferrin fractions in populations of this deer mouse. The proteins are designated Trf-a, Trf-b, and Trf-c. The Trf-a apparently occurs as a rare variant in the populations studied. The other two transferrins occur in populations from Arizona, California, and Michigan. The two transferrin components (Trf-b and Trf-c) are inherited as if simply related to allelic factors designated Trf^b and Trf^c. The rareness of the *Trf-a* component precludes definitive allelic assignment at the present time. Serum transferrin polymorphisms were also observed in samples from Arizona localities of the species *Peromyscus crinitus*, *P. eremicus*, and *P. boylii*.

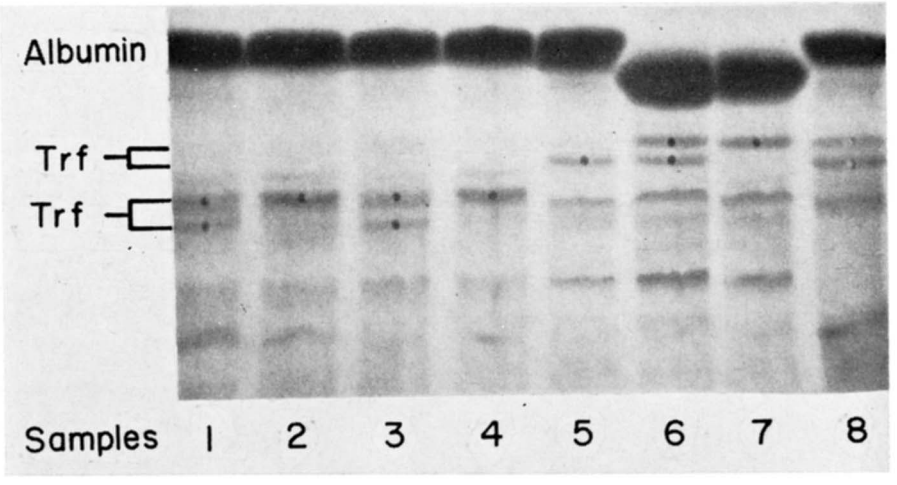

Figure 2.—Electrophoretic patterns of serum proteins in selected individuals of the genus Peromyscus. Transferrin components revealed by the described technique are marked with a dot. Samples 1 and 2, *P. crinitus*; samples 3 and 4, *P. eremicus*; sample 5, *P. truei*; samples 6 and 7, *P. boylii*; sample 8, *P. maniculatus* exhibiting Trf-bc phenotype.

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