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Erythrocytic antigenic differences between individuals of the deer mouse, *Peromyscus maniculatus**

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All previously published detailed reports of erythrocytic antigenic variation within the genus *Peromyscus* have employed for absorption pooled cells from several individuals thereby hindering the detection of antigenic individuality. Gorer (1936), when investigating antigenic individuality in the house mouse, *Mus musculus*, avoided the problems of small blood volume and individual variation by using pooled cells from inbred (sibship mating) lines. Species specific erythrocytic antigenic differences between deer mice of the species *Peromyscus maniculatus* and *P. leucopus* were demonstrated by Moody (1941) using immune rabbit sera. Cotterman (1944) found that normal human group-B sera absorbed with pooled erythrocytes from individuals of various species within the genus *Peromyscus* possess agglutinins showing quantitatively different reactions against erythrocytes of various *Peromyscus* species. Moody (1948) demonstrated differential cellular antigenic components between populations of the species *Peromyscus maniculatus*. These cellular antigens were demonstrated with the use of immune rabbit sera produced by inoculation of pooled erythrocytes from mice of a given stock. The immune sera were absorbed with pooled blood obtained from several mice of various stocks.

The following study concerns individual erythrocytic antigenic variation in the deer mouse, *Peromyscus maniculatus*, demonstrated by hemagglutination tests using unabsorbed isoimmune mouse sera and immune rabbit sera absorbed with erythrocytes from individual deer mice. Two antigenic types have been detected which appear as unitary genetic characteristics inherited as a two-allele, one-locus system. These two antigens are designated antigen A and antigen B and are not to be considered antigenically related to any other AB antigen system including the familiar A-B antigen system of humans. The alleles at the locus responsible for these antigenic phenotypes are designated as Pm^A and Pm^B.

MATERIALS

Deer mice used for injection and absorption were obtained from laboratory stocks maintained and kindly provided by Dr Elizabeth Barto, Department of Zoology, the University of Michigan. The individuals, of the subspecies *Pero-*

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myscus maniculatus gracilis (LeConte), represent the fifteenth to twenty-fifth generation descendants of feral animals trapped in Alger County, Michigan, in 1940 by W. Frank Blair and in 1947 by Van T. Harris. The stock has not been purposely inbred, although inbreeding has resulted from the limited number of individuals available.

In addition, individuals of *P. m. bairdii* (laboratory stock, University of Michigan), *P. leucopus* (feral animals from Washtenaw County, Michigan) and *P. polionotus* (laboratory stock, University of Michigan) were used for immunization, although the results herein reported are primarily concerned with the antigenic characteristics of individuals of the subspecies *P. m. gracilis* exhibited by means of anti- *P. m. gracilis* rabbit sera.

METHODS

The preparation of erythrocytes and sera, as well as typing methods, followed standard serological procedures (cf. Dunsford and Bowley, 1955). Blood was obtained from the mice by clipping the tail or by inserting a 1·5–2·0 mm. capillary tube into the suborbital canthal sinus. Approximately 0·5 c.c. of blood could be collected, and rarely were there fatalities.

All agglutination reactions were performed by the standard saline agglutinin tube test. Macroscopic readings of reactions were observed before and after centrifugation. The agglutinating reactions characterizing the A and B antigens here reported were easily defined, stable agglutinated masses of cells. In anticipation of the genetic data presented later, no differences were noted as to strength of positive reaction between heterozygote and homozygote positives. All typing series performed after initial identification of the antigens included known positive and negative cell types.

Heteroimmunization

In heteroimmunizing a rabbit with mouse erythrocytes an individual mouse was used for the complete immunization schedule of a rabbit. The initial injection consisted of a mixture of 0·5 c.c. Freund's (complete) bacto-adjuvant and 0·15 to 0·2 c.c. of washed, packed mouse erythrocytes introduced subcutaneously in the shoulder region of the rabbit. On the twenty-fourth and again on the twenty-fifth day following the subcutaneous injection, the rabbit was injected intravenously with a 50% suspension of erythrocytes from the same donor mouse. A total of 0·15 to 0·2 c.c. of packed cells was injected on those subsequent days.

Unabsorbed rabbit serum samples were periodically titred by the doubling dilution technique. The immune anti-mouse titre of unabsorbed rabbit sera rose to a final plateau in the range of 1:2000 to 1:4000 approximately 21 days following the subcutaneous injection, at which time final bleeding of the rabbit was performed.

Absorption

Multiple absorption analysis of immune rabbit sera was performed by standard procedures which are briefly summarized as follows. A total volume of approxi-

mately 0·1 c.c. of immune rabbit serum was absorbed stepwise through aliquots of erythrocytes (usually three) containing a total volume of 0·2 to 0·3 c.c. of packed cells from an individual mouse. The final reagents, exhibiting no agglutination reaction toward the absorbing cells, were tested for agglutinins against cells of other mice used for absorption of the immune sera.

Analysis of immune sera from three rabbits immunized with *P. m. gracilis* cells absorbed with individual cell samples of blood from forty-nine different mice showed patterns of absorption indicated by agglutination tests as summarized in Table 1. Cells were classified into three groups on the basis of absorption and reaction characteristics. The two reactive agglutinins are designated as anti-A and anti-B. Attempts by absorption to fractionate the anti-A and anti-B reagents were unsuccessful, and these two reagents are therefore considered to represent specific antibodies within the sera.

Table 1. *Summary of absorption analysis of heteroimmune anti-*Peromyscus maniculatus gracilis *sera with* P. m. gracilis *erythrocytes*

	Immune rabbit sera								
	anti-group 1			anti-group 2			anti-group 3		
	Types of absorbing cells								
Tested erythrocytes	1	2	3	1	2	3	1	2	3
Group 1 (type AB)	−	+	+	−	−	+	−	+	−
Group 2 (type A)	−	−	+	−	−	+	−	−	−
Group 3 (type B)	−	+	−	−	−	−	−	+	−

\+ = agglutination.
− = non-reactivity.

Absorption of the anti-AB serum with type A cells and, subsequently, with type B cells rendered the serum non-reactive with the donor type AB cells. These results exclude the existence of an interaction antigen, in this particular anti-AB serum, of the sort noted with some heterozygote serotypes (Cohen, 1960). The titres of the anti-A and anti-B reagents when tested by doubling dilution technique against cells from the immunizing or donor mouse were 1/64 and 1/32, and typing reactions were performed using 1/12 and 1/8 dilutions, respectively. Those dilutions gave strong diagnostic reactions. Following the original identification of each of the antibodies in various immune sera, a single rabbit serum was chosen for preparation of each of the reagents. A limited number of previously tested absorbing bloods were repeatedly used for preparation of typing reagents.

Judging from the absorption methods, two unitary saline (complete) agglutinins were isolated from the immune sera and each exhibits a different pattern of reactions toward erythrocytes of individual mice. All *P. m. gracilis* tested possessed either or both of the antigenic types; no doubly non-reactive mouse was observed in the 344 individuals of the laboratory stock tested.

Isoimmunization

Two methods of isoimmunization within the *P. m. gracilis* stock were used in an attempt to obtain isoimmune sera.

A series of three intraperitoneal injections, each of a 25% suspension of 0·1 to 0·2 c.c. packed erythrocytes, was used in a group of ten donor-recipient pairs. The injections were administered at 3-day intervals, and 7 days following the last injection the recipient mouse was bled into an uncitrated tube. The unabsorbed sera of the recipient bloods were tested for agglutination against erythrocytes of the donor mice and in all cases were negative. Subsequent typing of mice with immune rabbit sera revealed that six of the donor-recipient pairs of this group differed antigenically such that an immune response might have been expected.

Subsequent to the exhibition of differences by immune rabbit sera, nine new donor-recipient pairs of mice were used for isoimmunization; of these, five pairs consisted of serotype A donors and serotype B recipients and four pairs consisted of serotype B donors and serotype A recipients. The initial dose consisted of a subcutaneous injection of an emulsion of 0·05 to 0·1 c.c. of washed, packed erythrocytes and 0·2 c.c. of Freund's (complete) bacto-adjuvant. Twenty-two days later, 0·5 c.c. of a 20% erythrocyte suspension was injected intravenously into the ventral base of the tail, and six days thereafter the recipient mouse was bled and the unabsorbed serum tested for hemagglutination with donor and control cells.

In a doubling dilution series, in which each of the nine isoimmune sera were tested against erythrocytes from the donor individual, agglutinating titres were as follows: isoimmune anti-A sera, 1/64, 1/16, 1/8, 1/1 and a dilution greater than 1/1; isoimmune anti-B sera, 1/2048, 1/256, 1/128, 1/1.

Reactions of the unabsorbed isoimmune sera paralleled those of the immune rabbit sera and indicated that the isoimmune sera represented the same agglutination system as defined by the heteroimmune sera.

The success of the second procedure appears to indicate that a prolonged exposure (e.g. subcutaneous adjuvant emulsion) followed by a large direct circulatory injection of the antigen was necessary for the elicitation of an observable isoimmune response.

GENETIC BASIS OF ANTIGENIC POLYMORPHISM

A summary of mating types and observed offspring in the laboratory stock of *P. m. gracilis* is given in Table 2. In these tests of allelism of the two antigenic factors no sibship segregated for more than three phenotypes. Moreover, the data are consistent with the simplest hypothesis that the two antigenic types are controlled by two co-dominant alleles at a single locus.

On another hypothesis, namely that the two agglutinogens are determined by dominant genes at two separate loci, a mouse of the AB blood type should be identifiable as a double heterozygote, having the genotype X^A/x, Z^B/z, if it satisfies either of the following criteria: (1) it has one type A and one type B parent, or (2)

Table 2. *Summary of mating-offspring antigenic phenotypes in* Peromyscus maniculatus gracilis*

Antigenic phenotype of mating	Number of sib-ships	Total number of off-spring	Total offspring of various phenotypes				χ^2
			A	AB	B	Non-reactive	
1. A × A	2	15	15	0	0	0	—
2. B × B	2	12	0	0	12	0	—
3. A × B	10	57	0	57	0	0	—
4. A × AB	7	50	26	24	0	0	0·08, d.f. = 1, p = 0·75–0·90
5. B × AB	13	61	0	27	34	0	0·81, d.f. = 1, p = 0·25–0·50
6. AB × AB	18	127	38	55	34	0	2·29, d.f. = 2, p = 0·25–0·50
7. B × unknown	1	13	0	0	13	0	—
8. Unknown	9	9	2	4	3	0	—
Total	62	344					

Data of mating types numbers 7 and 8 have been included primarily to indicate the absence of a non-reactive type, and to indicate the total number of individuals with serologically untyped parentage contributing to the colony.

* All χ^2 tests are based on hypothesis of two-allele, one-locus mode of inheritance.

its offspring include mice exhibiting the three blood types, A, AB, and B. Assuming that the typed offspring are representative of the frequency of random gametic unions, one-sixteenth of the offspring from matings between two such double-heterozygotes are expected to exhibit a fourth, non-reactive, phenotype of the genotype x/x, z/z. The probability of not observing individuals lacking both antigens, therefore, becomes $(15/16)^n$, where n represents the total offspring observed in AB by AB matings ascertained by the above criteria as double heterozygotes. A total of 112 offspring of such matings was observed, thereby giving a probability of less than 0·001 with a two-locus model.

The breeding data indicate that the two antigens are determined by two co-dominant alleles, Pm^A and Pm^B.

In addition to the summary data, no individual pedigree of any mating (in some cases through seven generations) necessitated use of a two-locus model to explain the observed breeding results.

OCCURRENCE OF THE A, B ANTIGENS IN OTHER FORMS

The two antigens have been noted in both feral and laboratory individuals of *P. m. gracilis* from Alger County, Michigan, and in feral and laboratory stock of *P. m. bairdii* from Washtenaw County, Michigan.

Only the A antigen has been observed in all forty-eight individuals of a laboratory stock of the beach mouse, *Peromyscus polionotus*. The eight ancestral mating pairs of these animals were collected in 1951 from Marion County, Florida, by Paul G. Pearson. The stock has undergone considerable inbreeding due to the small size of the colony.

The anti-A reagent (at the typing dilution of 1/12) and anti-B reagent (at 1/8) caused no agglutination of (1) erythrocytes of the house mouse (*Mus musculus*), representing various laboratory strains—STOLI/Lw, C3H, SEC/Re, C57 BL/6, ST/JAX, (2) with human erythrocytes possessing the human A, B, M or N antigens, and (3) with cells of eleven mice of the species *Peromyscus leocupus* collected by Michael Petras and the author in Washtenaw County, Michigan.

OTHER ANTIGENS

During this study other agglutinins were noted which appeared as inter-specific agglutinins. The absorption of anti-*P. maniculatus* rabbit serum (either anti-*P. m. gracilis* or anti-*P. m. bairdii*) with *P. leucopus* cells resulted in an anti-*P. maniculatus* reagent which reacted with cells of all twelve (eight *P. m. gracilis*, four *P. m. bairdii*) individuals of *P. maniculatus* tested; the converse was also true (with all eight individuals of *P. leucopus* tested.) These findings are similar to those noted by Moody (1941). Although anti-*P. m. gracilis* serum when absorbed with cells from individuals of *P. m. gracilis* resulted in reagents which could be used to type individuals of *P. m. gracilis* or *P. m. bairdii* for the A and B antigens, the absorption of anti-*P. m. gracilis* sera with cells from type AB individuals of *P. m. bairdii* resulted in an anti-*P. m. gracilis* reagent reacting with cells of all twenty-four *P. m. gracilis* tested. The absorption of anti-*P. m. bairdii* sera with cells from type AB individuals of *P. m. gracilis* produced an anti-*P. m. bairdii* reagent reacting with the cells of all seven individuals of *P. m. bairdii* tested. These population antigenic differences are similar to those reported by Moody (1948), although the differences here reported were observed using erythrocytes from individual mice for absorption.

Anti-*Peromyscus polionotus* sera could not be fractionated by multiple absorption analysis using the laboratory stock of *P. polionotus* previously mentioned.

The results indicate that absorption and immunization procedures using samples of erythrocytes from individuals of small mammals are feasible for the isolation and identification of antigenic characteristics dependent upon simple genetic systems. Such genetic characters should be a valuable aid in the study and analysis of various problems of polymorphisms in populations of small mammals.

SUMMARY

1. Antigenic polymorphism between individuals of the subspecies *Peromyscus maniculatus gracilis* is demonstrated.

2. Absorption analysis of heteroimmune rabbit sera using erythrocytes of individual mice revealed two unitary complete (saline) agglutinins, designated as anti-A and anti-B, reactive to erythrocytic antigens in this species.

Isoimmunization apparently demonstrated these same antigenic differences.

3. The two antigenic characters (A and B) defined by the agglutinins are inherited as if simply related to allelic factors designated Pm^A and Pm^B respectively.

4. These two antigens are found in representatives of both the subspecies *P. m. gracilis* and *P. m. bairdii*, and, in addition, the A antigen was observed in a

laboratory stock of *P. polionotus*. Tested individuals of the species *P. leucopus* and *Mus musculus*, however, possessed neither antigen, and samples of human blood also lacked both antigens.

5. Species-specific agglutinins distinguishing individuals of the species *P. leucopus* and *P. maniculatus*, and population-specific agglutinins which distinguished between individuals of the subspecies *P. m. bairdii* and *P. m. gracilis*, have been observed.

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