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SHORT PAPERS

Linkage of one component of the major urinary protein complex of mice to the brown coat color locus

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1. INTRODUCTION

It has been known for some time that normal mice have a relatively high concentration of protein in the urine. Subsequent to the original observation of Parfentjev (1932, 1933), it has been shown that although other proteins are present in mouse urine, there is a major urinary protein (MUP) complex which has an average molecular weight of 17,800 (Finlayson & Baumann, 1958), migrates electrophoretically as a prealbumin (Thung, 1956; Finlayson & Baumann, 1958), and can be separated by moving boundary electrophoresis at pH 5·5 into three closely related peaks (Finlayson & Baumann, 1958).

More recently Finlayson *et al.* (1963) examined the urine of nineteen inbred strains of mice and two hybrids. Using agar-gel electrophoresis at pH 5·5 they detected three MUP components of which the most cathodal was designated component 1 and the most anodal, component 3. All of the inbred strains and hybrids tested exhibited component 3. For the various inbred strains, either component 1 or component 2 was present, but not both. On this basis several MUP phenotypes were designated (Finlayson *et al.*, 1963). When two parental strains of similar MUP type were mated, the MUP components of the respective F_1 hybrids were like those of the parent of the same sex. When two unlike parental strains were mated, the F_1 hybrids of both sexes had a different MUP type from either parent, all three components being present. Thus the function of components 1 and 2 as a genetic variant was suggested.

In the present study an experiment was designed to determine whether or not the MUP phenotype is linked with dilution and transferrin (linkage group II) as suggested by Roberts (cit. Finlayson *et al.*, 1963).

2. MATERIALS AND METHODS

The mice used in this study were the F_2 generation of a cross between the DBA/2N and C57BL/6N inbred strains of mice. The mice (1 to 5 months old) were maintained in plastic cages and fed pellets containing not less than 17% crude protein, 11% crude fat, and not more than 2% crude fiber. Tap water was available *ad libitum*. Urine was collected by holding the mouse over a test tube and gently massaging the bladder.

Samples (0·5 to 2 ml.) were collected from individual mice over a 5-day period during which the urines were stored at 5°C. They were then dialyzed against distilled water at 5°C. for 72 hours, quick-freeze dried, the protein redissolved in distilled water and the solutions diluted to 1·5% protein prior to electrophoresis.

Agar-gel electrophoresis was carried out on 3·25 × 4-in. glass plates with tris(hydroxy-

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methyl)aminomethane (Tris) acetate buffer at pH 5·5 and ionic strength 0·05. Details of this method have been previously described by Potter & Kuff (1961); however, certain modifications were made in the application of the samples. A small strip of Whatman No. 1 filter paper was saturated with the 1·5% protein solution and then inserted into a slit in the agar gel. Electrophoresis was carried out at room temperature for 3·5 hours with a constant voltage of 40 volts between the two wicks and a current of 32 milliamperes.

Acrylamide-gel electrophoresis was carried out by a method adapted from Sogami & Foster (1962). The gels were made by polymerizing 5% acrylamide and 0·2% N,N′-methylenebisacrylamide with fresh 10% ammonium persulfate (1 ml./100 ml.) and N,N,N′,N′-tetramethylethylenediamine (0·1 ml./100 ml.). After setting for 1 hour, the gels were dialyzed against distilled water at 5°C. for 24 hours, equilibrated with Tris acetate buffer of pH 5·5 and ionic strength 0·01, and stored at 5°C.

3. RESULTS

The MUP types of the two parental strains, DBA/2N and C57BL/6N, were 1,3 and 2,3, respectively. Members of the F_1 generation had a MUP type of 1,2,3. The F_2 generation displayed all three variations of MUP type, 1,3, 1,2,3 and 2,3, in a ratio of 1:2:1, thus suggesting that a pair of codominant alleles controls the production of components 1 and 2 of MUP (Plate I).

The coat color genotype of the DBA/2N is *aabbdd*, its phenotype being seen in the mouse's coat as dilute, brown, nonagouti. The C57BL/6N's genotype is *aaBBDD*, and phenotypically it is nondilute, black, nonagouti. As was expected, the ratios of black mice to brown and of nondilute mice to dilute in the F_2 generation were 3:1 (Table 1).

Since this experiment was designed to determine whether codominant alleles controlling the production of MUP components 1 and 2 were linked with dilution (linkage group II), the data were statistically analyzed (Green, 1963) to test this linkage hypothesis (see Table 1). For MUP components 1 and 2 to be linked with dilution, we would have to accept a recombination of $44 \pm 6 \cdot 1\%$ (standard error), which is not significantly different from 50%. The data thus do not give evidence for such a linkage.

On the other hand, the statistical tests for linkage of MUP components 1 and 2 with black and brown coat color yielded a recombination of $4 \cdot 1 \pm 2 \cdot 0\%$. One can thus conclude

Table 1. *Coat and major urinary protein (MUP) phenotypes in* F_2

Coat	MUP components present	Expected % if no linkage	Expected % if complete linkage	Observed distribution ($n = 99$)
	1,3	19	0	2
Black	1,2,3	37	50	42
	2,3	19	25	28
	1,3	6	25	25
Brown	1,2,3	13	0	2
	2,3	6	0	0
	1,3	19	0	18
Nondilute	1,2,3	37	50	33
	2,3	19	25	21
	1,3	6	25	9
Dilute	1,2,3	13	0	11
	2,3	6	0	7

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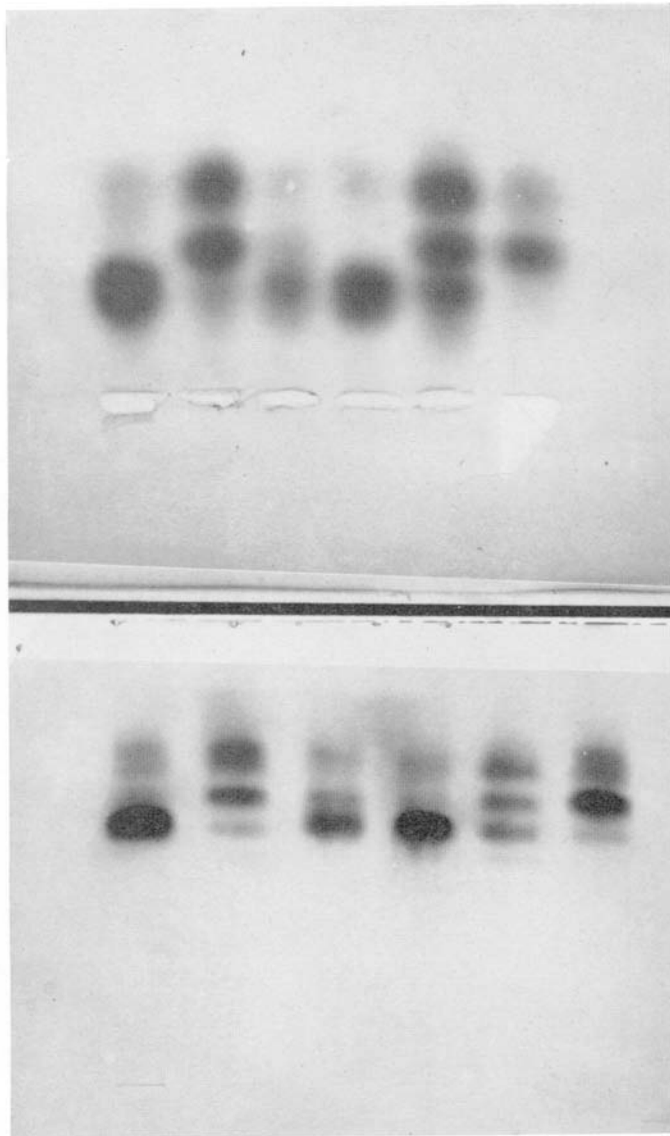

Plate 1. Electrophoresis of MUP at pH 5·5. Upper = agar-gel; lower = acrylamide-gel. Anode is at top. From left: samples from inbred mice with MUP types 1, 3 and 2, 3 respectively; sample from F_1; three samples from individuals of F_2 illustrating the three MUP types observed. All samples shown were from male mice.

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with a high degree of confidence that the codominant alleles controlling MUP components 1 and 2 are linked to those controlling black and brown coat color, which are on linkage group VIII.

4. DISCUSSION

Two methods of electrophoresis were used in this study. Assignments of MUP phenotypes were based on the results of agar-gel electrophoresis; however, in 'doubtful' cases or those in which smaller amounts of protein were available, acrylamide-gel, because of its superior resolving capacity, was used for confirmation. It was found that acrylamide-gel not only gave better resolution, but also split component 3 into two separate bands. This separation of component 3 may be due to the sieving properties of the acrylamide gel in view of the fact that on acrylamide-gel electrophoresis at pH 3·6 component 3 separates into four distinct bands (Finlayson *et al.*, 1965). The apparent confusion which might arise due to the heterogeneity of component 3 is not our concern in the present study since all mice exhibited component 3 and the electrophoresis of the protein variant under consideration, components 1 and 2, was clear and consistent.

The present data indicate that we have identified, by electrophoretic variations, two alleles of one of the components of the major urinary protein complex of mice. Therefore a genetic locus, which we propose here to designate *Mup-a*, has two alleles, $Mup\text{-}a^1$ and $Mup\text{-}a^2$, the superscripts corresponding to components 1 and 2, respectively. We do not know at present whether the *Mup-a* locus contains the structural gene controlling amino acid sequence or an interacting gene (e.g., controlling an enzyme which adds a carbohydrate moiety). Based on this system, the following inbred strains can be classified with respect to the *Mup-a* locus.

$Mup\text{-}a^1$	$Mup\text{-}a^2$
BALB/cAnN	C58
BL	C57BL/10-H-2^d new
C57BR/cd	C57BL/10-H-2^d old
C3H/Lwf	C57BL/Ka
DBA/2Lwf	C57BL/6N
DBA/2N	C57BL/10Sc
E−B+	C57L
F	Poly-2
I	
N	
NBL/N	
NH	
PBR	
SM	
129	

SUMMARY

Two codominant alleles, $Mup\text{-}a^1$ and $Mup\text{-}a^2$, controlling electrophoretic variation of one of the components of the major urinary protein (MUP) complex of the mouse *Mus musculus* have been found to be linked to the black–brown coat color alleles (linkage group VIII).

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