Starch-Gel Electrophoresis of Murine Major Urinary Protein (34992)

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The occurrence of high concentrations of protein in the urine in the laboratory mouse, *Mus musculus*, appears to be a normal homeostatic mechanism and a metabolic curiosity (1–3). Urinary protein concentrations, both those determined in this laboratory and those reported by Thung (4), ranged from 0.14%–2.5% where age and sex of animal are the main factors which determine the protein concentration level (5, 6). The use of this protein complex, termed "major urinary protein" (MUP) by Finlayson *et al.* (7), as a genetic marker based on agar-gel electrophoresis (7) and the relationship of the protein complex to the endocrine system (7, 8) have been previously reported. This paper describes the electrophoretic phenotype of the major urinary protein on starch-gel electrophoresis and presents the classification of 35 inbred strains and substrains of mice.

Materials and Methods. Inbred strains of mice were obtained from the Laboratory Aids Branch, NIH (N), The Jackson Laboratory (J), and from the colonies of Drs. Andervont (An), Deringer (De), Heston (He), Hoffman (Hf), Law, (Lw), and Russell (Rl).

Urinary protein patterns were obtained from either undialyzed urine samples, urine dialyzed exhaustively against distilled water, or from urine passed through a Sephadex G-75 column (2.5 × 45 cm) with 0.02 M ammonium bicarbonate, pH 8.2, as the elution solvent. The female urinary protein patterns shown in Fig. 1 are from 3× concentrated urine samples.

Horizontal starch-gel electrophoresis was performed according to Smithies (9) with slight modification; a 13.2% hydrolyzed starch slurry was gelled by the method of Kristjansson (10) with Tris–EDTA–borate, pH 9.1 (0.09 *M* Tris, 0.0025 *M* EDTA, 0.009 *M* boric acid). The electrode chambers contained 0.3 *M* boric acid and 0.05 *M* sodium hydroxide. A voltage gradient of 15 V/cm was applied for 75 min at 4–5°. After electrophoresis the gel was sliced in half and stained with 1% amido schwartz 10B in 2% acetic acid.

Results and Discussion. The electrophoretic patterns of the major urinary protein complex are of two distinct types, a three-band pattern and a four-band pattern (Fig. 1). The three-band pattern, bands 1, 2, and 4 (the slowest, or most cathodal, is designated band 1 and the fastest, or most anodal band 4), and the four-band pattern, bands 1, 2, 3, and 4, are designated urinary protein types Mup-1a[1] and Mup-1b, respectively. In Fig. 1 strains DBA/2e and BALB/c demonstrate the protein type Mup-1a and strains C57BL/10 and P the Mup-1b protein type. In all strains classified as Mup-1a both males and females exhibited similar three-band protein patterns, while in strains classified as Mup-1b the male protein pattern and the female protein pattern were different (Fig. 1). Males exhibit all four bands and females show only bands 1, 3, and 4. Whether this is a true qualitative difference or a result of a lower protein concentration is not known at the present time.

The protein complex proved to be quite stable in the laboratory procedures employed in this study. Identical electrophoretic patterns were obtained from fresh undialyzed urine, urine exhaustively dialyzed against distilled water, urinary protein isolated by

[1] The nomenclature used in this report conforms with the recommendations of the Committee on Standardized Genetic Nomenclature for Mice (12). *Mup-1* indicates the genetic locus and *a* the allele.

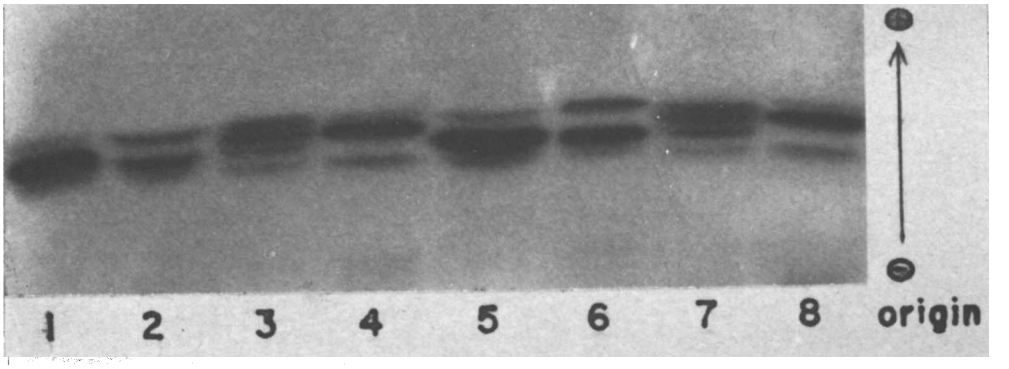

FIG. 1. Starch-gel electrophoresis of urinary protein types Mup-1a and Mup-1b. (1) DBA/2e ♂, Mup-1a; (2) DBA/2e ♀, Mup-1a; (3) C57BL/10 ♂, Mup-1b; (4) C57BL/10 ♀, Mup-1b; (5) BALB/c ♂, Mup-1a; (6) BALB/c ♀, Mup-1a; (7) P ♂, Mup-1b; (8) P ♀, Mup-1b.

column chromatography, and urine samples or protein solutions which have been frozen and thawed several times. The number of protein components, their relative concentrations, and their electrophoretic mobilities are not changed after the protein complex is eluted from a starch gel and electrophoresed a second time. This suggests that the multiplicity of the electrophoretic pattern is not a result of protein aggregation nor an artifact of the analytic procedures.

On agar-gel electrophoresis, Tris acetate at pH 5.5 (7, 11), electrophoretic zones for Mup-1a and three electrophoretic zones for Mup-1b are distinguishable; Mup-1a and Mup-1b would be designated 1, 3 and 1, 2, 3, respectively (7). When agar zone 3 is eluted and rerun on starch-gel electrophoresis at pH 9.1, agar zone 3 separates into starch bands 1 and 4. On acryamide-gel electrophoresis the Mup-1a and Mup-1b protein patterns are identical to the starch-gel patterns with the Tris–EDTA–borate, pH 9.1, buffer system and with the Tris acetate, pH 5.5, buffer system they are identical to the agar-gel patterns. It seems, therefore, that the apparent electrophoretic phenotype of the urinary protein complex depends on the pH and buffer system.

In Table I are listed the urinary protein types of 35 inbred strains and substrains. Strain classification was based on a minimum of 10 individual urine samples for each strain and sex. Related strains of the C57 and C58 family of strains are all Mup-1b except C57BR which probably represents gene segregation rather than a mutation from Mup-1b to Mup-1a. Strains DBA/2, BL, and BALB/c and strains descended from them or through crosses are all Mup-1a.

TABLE I. Urinary Protein Types in 35 Inbred Strains and Substrains.

Strain	Mup type	Strain	Mup type
A/He	1a	DBA/1J	1a
AKR/Lw	1a	DBA/2eDe	1a
AL/N	1a	DD/He	1a
BALB/cHf	1a	HR/De	1b
BL/De	1a	I/An	1a
BRSUNT/N	1a	LP/J	1a
CBAf/Lw	1a	NBL/N	1a
CE/J	1a	MA/J	1a
CFCW/R1	1a	NH/Lw	1a
C3Hf/De	1a	P/J	1b
C57BL/An	1b	RF/Lw	1a
C57BL/He	1b	RIII/An	1b
C57BL/6Hf	1b	ST/J	1a
C57BL/10N	1b	STR/N	1a
C57BR/edJ	1a	STR/IN	1a
C57L/He	1b	SWR/De	1a
C58/Lw	1b	YBR/He	1b
		129/J	1a

Qualitative variation, the presence or absence of a protein band, was not observed within an inbred strain except for the sex difference in Mup-1b type (Fig. 1). Quantitative differences in the relative concentration of the protein components was quite pronounced between inbred strains with different lines of origin. Component 4 in the Mup-1a

type and components 2 and 3 in the Mup-1b type exhibited concentration differences. Whether these differences are a reflection of different alleles at the Mup-1 locus or an interaction with a second locus remains to be determined. Only components 2 and 3 appear to be under the genetic control of the Mup-1 locus. Since the biological function of the urinary protein complex is not known (8), these conclusions are speculative at this time.

Summary. Two murine urinary protein electrophoretic phenotypes, Mup-1a and Mup-1b exhibit three protein components and four protein components, respectively. Thirty-five inbred strains and substrains of mice were classified for one of the two urinary protein types, and the classifications are listed.

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