Genet. Res., Camb. (1982), **39**, *pp.* 31–39
With 2 plates
Printed in Great Britain

Allelic variation at several different genetic loci determines the major urinary protein phenotype of inbred mouse strains

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(*Received 3 June 1981 and in revised form 28 July 1981*)

SUMMARY

We have examined the major urinary protein (MUP) phenotype of three inbred mouse strains by one-dimensional isoelectric focusing in acrylamide gels. Each strain gave a distinct pattern of major and minor bands. In the three strains together, seven major and about seven minor bands were observed. F1 phenotypes were intermediate. F2 phenotypes can be explained by recombination between allelic variants at four or more different genetic loci. We propose that variation in MUP phenotype is due in fact, to allelic variation at approximately seven structural gene loci, some of which are linked on chromosome 4. The remainder may or may not be linked to these.

1. INTRODUCTION

The urine of laboratory mice contains substantial amounts of protein. Most of this is accounted for by a family of structurally related proteins, the major urinary proteins or MUPs. These are synthesized in the liver, secreted, and finally excreted. Male mice excrete 5–20 times as much MUP per days as do females, and MUP synthesis in females can be induced with testosterone (Rumke & Thung, 1964).

Finlayson and others (Finlayson & Baumann, 1958; Finlayson, Potter & Runner 1963; Hudson, Finlayson & Potter, 1967; Finlayson, Hudson & Armstrong, 1968) resolved the MUPs into three components, MUP 1, MUP 2 and MUP 3, by agarose and acrylamide electrophoresis. Two different patterns of MUP excretion were observed in inbred mouse strains. Essentially, strains in the C57–C58 group (but not C57BR) showed one pattern (MUP 2, 3) and all other strains examined showed another (MUP 1, 3) (Hudson *et al.* 1967). By means of crosses between C57BL and other strains, Hudson *et al.* (1967) were able to identify a genetic locus responsible for variation in the pattern of MUP excretion. Co-dominant alleles at this locus, the *Mup-a* locus, determined the presence or absence of components MUP 1 and MUP 2. It was therefore proposed that *Mup-a* is a structural gene locus, and that the alternative alleles, $Mup\text{-}a^1$ and $Mup\text{-}a^2$, specify the structures of MUP 1 and MUP 2. The *Mup-a* locus mapped to linkage group VIII (chromosome 4) (Hudson *et al.* 1967; Finlayson *et al.* 1969).

Szoka & Paigen (1978, 1979) showed that MUP 1 and MUP 2 are not simple allelic alternatives. *Mup-a*^{1} homozygotes contained traces of MUP 2 and likewise *Mup-a*^{2} homozygotes contained traces of MUP1. Based on this and other evidence Szoka & Paigen proposed that the *Mup-a* locus is regulatory rather than structural.

Recently it was shown that the mouse genome contains 15-20 MUP structural genes (Hastie, Held & Toole, 1979). It had earlier been shown that inbred strains of mice contain up to four different MUPs, rather than the three studied by Finlayson *et al.* and Szoka & Paigen (Hoffman, 1970). We therefore re-examined the MUPs of three inbred strains by acrylamide gel electrophoresis and isoelectric focusing. Our results show that there are at least seven major species of MUP, and in addition several minor species. Evidence from crosses between inbred strains show that their different MUP phenotypes are determined by allelic variation at 4 loci at least.

2. MATERIALS AND METHODS

Inbred mouse strains. The strains C57BL, BALB/c and JU were those maintained in the Department of Genetics (formerly Institute of Animal Genetics), University of Edinburgh. Strain JU was inbred from a stock of mixed origin (see Falconer, 1973).

Analysis of urine. Urine samples were taken at 8–12 weeks of age. Urine was collected by bladder massage and dialysed against 10 mM tris-acetate buffer, pH 5·4. Vertical acrylamide slab gels (10 % acrylamide, 0·25 % bis-acrylamide) measured 200 mm long × 160 mm × 2 mm. Each well received 10 μl of a 1 mg/ml solution of MUP in 10 mM tris-acetate, pH 5·4, 15 % sucrose. The gel buffer and the electrode vessel buffer were 10 mM tris-acetate, pH 5·4. Gels were run for 4 h at 20 V/cm, fixed for 30 min in 10 % TCA, stained with Coomassie Brilliant Blue R 250 in methanol/acetic acid/water (4/1/5) and destained with the same solvent.

Horizontal acrylamide IEF gels measured 170 mm from anode to cathode × 250 mm × 1 mm. Carbon rod electrodes were used, together with paper wicks soaked in 1 N-H_3PO_4 (anode) and 1 N-NaOH (cathode). Gels contained 4·85 % acrylamide, 0·15 % bis-acrylamide, 0·053 volume of Pharmacia ampholines, pH 4–6·5, and 0·013 % ammonium persulphate. Protein (10 μg) was applied at each station and the gels were run at 1·5 Va overnight, and then at 10 Va for 30 min. The gels were treated with 10 % TCA, 5 % sulphosalicyclic acid for 1 h, and with methanol/acetic acid/water (3/1/6) for 30 min, and were then stained and destained as described above. Each destained gel was placed on the plasticized side of a sheet of Benchkote (B.D.H.) and covered, in turn, with a piece of Whatman 3 MM paper soaked in methanol/acetic acid/water, a 2 in thick layer of paper towels, a glass plate and a 2 kg weight. After 24 h all but the 3 MM paper were removed. After a further 24 h the paper, with the dried gel firmly attached to it, could be peeled away from the sheet of Benchkote.

3. RESULTS

Greater resolution of the MUPs than that observed by previous authors was obtained by increasing the time of acrylamide gel electrophoresis at pH 5·5 (Fig. 1). BALB/c urine showed three major bands and one minor band. JU showed two major and two or three minor bands. C57BL showed four major and four minor bands. Fig. 1 shows an attempt to arrange the major bands in the groupings most likely to correspond to MUP 1, MUP 2 and MUP 3.

Still greater complexity was observed by isoelectric focusing (IEF). The number of major bands in each phenotype remained the same, but the number of minor bands increased to nine (BALB/c), seven or eight (C57BL) and nine (JU). The major bands have been labelled A, B, C, (DE) and F. Band (DE) quite frequently splits into two components, D and E, in C57BL samples and band D, but not E is found in BALB/c. Also, band A occasionally splits into two components (A1 and A2) in some heterozygotes. Thus there are probably at least seven major MUP components. A single minor band is labelled X.

The bands that focus between pH 4 and 4·3 appear to form a separate sub-set. To test whether these are also MUPs, the MUPs that focus between pH 4·4 and 4·7 were isolated from BALB/c and C57BL by preparative IEF, and antisera were prepared in rabbits. A mixture of these antisera precipitated the more acid bands which, by this criterion, are MUPs (data not shown).

By comparing the relative intensities of the different major bands and, more important, by comparing the three genotypes, it is possible to match the major IEF bands with the major electrophoretic bands. The IEF gel shows quite clearly that, at least in most cases, where a major band is missing from the urine of one or other inbred strain, a minor band with the same pI is found in its place. Several explanations of this are possible. For example, the minor band might be present in all three strains, but be obscured by the major band, when present. This explanation, however, invokes a series of coincidences that taken overall seem unlikely. Alternatively, major and minor bands with the same pI may represent different levels of expression of the same structural gene. In this case, the different patterns of expression would be due to *cis*-acting or *trans*-acting controlling elements.

(i) *F1 offspring of crosses between inbred strains*

Fig. 2 shows a BALB/c sample (track 1), a C57BL sample (track 8), and the urine of 6 male offspring of a BABL/c × C57BL cross. Inspection of bands B, C, (DE) and F suggests co-dominant inheritance in each case. A similar result was observed when the male offspring of a JU × C57BL cross were examined (Fig. 3).

(ii) *F2 offspring*

Fig. 4 shows urine samples from 12 males of the F2 generation of a cross between JU and C57BL. It is quite clear that novel patterns have arisen. Notice in

particular track 1, which resembles BALB/c, and tracks 3, 4 and 11, which show only two major components (C and F). Altogether these 12 F2 offspring show seven different patterns. This is quite clearly at variance with the hypothesis of Szoka & Paigen (1978) which suggests that the difference between the C57BL pattern and a BALB/c type pattern is due to alleles of a regulatory gene at the Mup-a locus. If so, we should observe only three patterns – the two parental patterns and the F1 pattern. Of the 12 F2 offspring shown here, none shows a C57BL pattern, one a JU pattern (track 5) and one an F1-type pattern (track 6).

Table 1. *Locus designations of the determinants proposed to control expression of the MUPs.*

MUP	Locus	MUP	Locus
A1	*Mup-2*	D	*Mup-6*
A2	*Mup-3*	E	*Mup-7*
B	*Mup-4*	F	*Mup-8*
C	*Mup-5*	X	*Mup-9*

Rather, the results suggest that a number of separate genes are segregating, each determining the presence or absence, or the intensity, of a single MUP. In the first instance, we have adopted the extreme form of this hypothesis, that the expression of each individual MUP is determined by alleles at a separate locus.

In what follows we use the symbols *Mup-2*, *Mup-3* etc. to denote the loci of the hypothetical genetic determinants of the different MUPs. (*Mup-1*, a synonym of *Mup-a*, has been used by Potter *et al.* (1973).) An allele that determines high-level expression is denoted by the superscript *a*, and one that determines low-level expression by the superscript *b*. Thus we have *Mup-2*^{a} and *Mup-2*^{b} or 2^a and 2^b. A list of these assignments is given in Table 1. The attribution of two loci to MUP-A is justified below.

To explore the hypothesis, we have examined a series of F2 progeny. We are now attempting to develop an objective method of scoring MUP phenotype. The data presently available, however, are based on subjective visual scoring, and are to some extent unreliable. For this reason we will draw only such conclusions as are unaffected by a moderate number of scoring errors.

In Table 2, we list the phenotypes of the male F2 progeny of crosses JU × C57BL and BALB/c × C57BL (54 and 42 progeny, respectively). Here, we use the symbol '+' (e.g. A+) to denote the presence of a band of major intensity, and the symbol '−' (e.g. A−) to denote either its absence or the presence in its place of a minor band. When it is possible to distinguish an intermediate phenotype, the symbol '+/−' (e.g. A+/−) is used. Using this notation, the MUP phenotypes of the three inbred strains are written as follows: BALB/c A+B+C−(DE)+F−; C57BL, A+B−C+(DE)+F+; JU, A+B+C−(DE)−F−.

Band A. A band designated A is found in all three parental strains. However, in the F2 of the cross JU × C57BL, three mice showed near absence of band A (see Fig. 3). We conclude that the genes responsible for the presence of band A in the

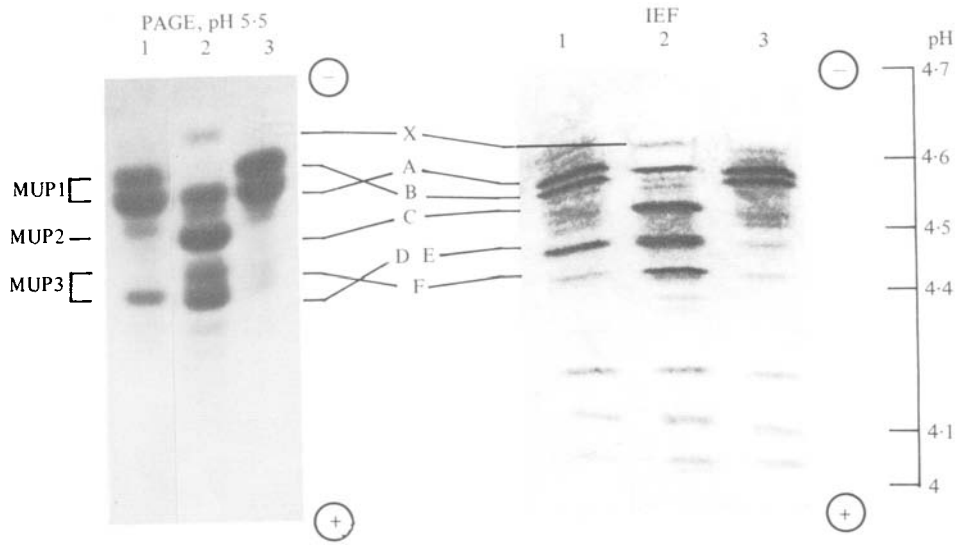

Fig. 1. Resolution of MUPs from male urine by polyacrylamide gel electrophoresis and isoelectric focusing. 1, BALB/c; 2, C57BL; 3, JU.

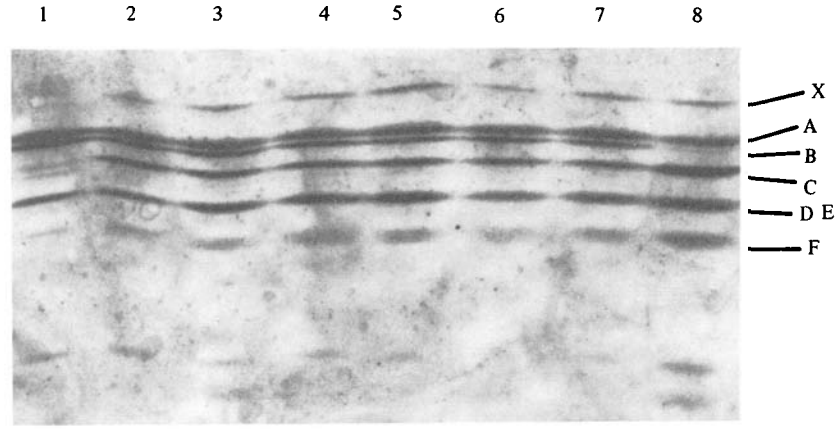

Fig. 2. IEF of MUPs from offspring of the cross BALB/C × C57BL. Lane 1, BALB/c; lane 8, C57BL; lanes 2–7, individual F1 males.

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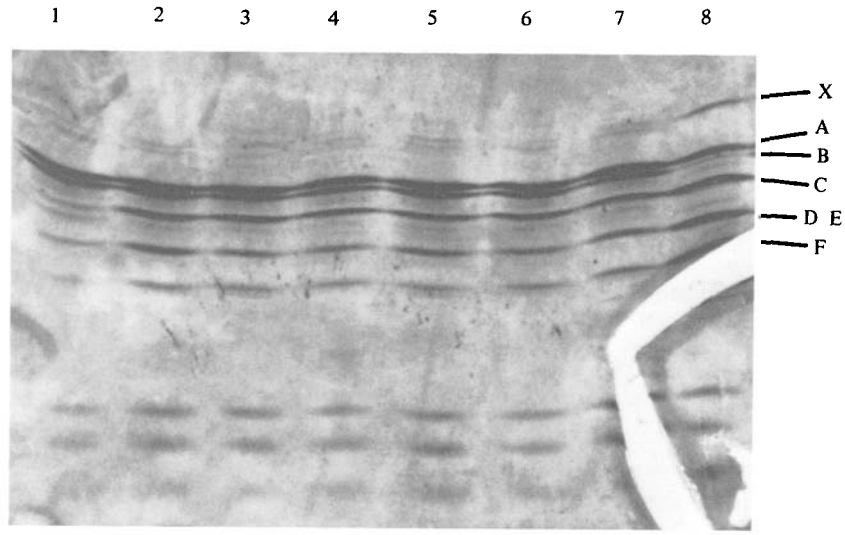

Fig. 3. IEF of MUPs from offspring of the cross JU × C57BL. Lane 1, JU; lane 8, C57BL; lanes 2–7, individual F1 males.

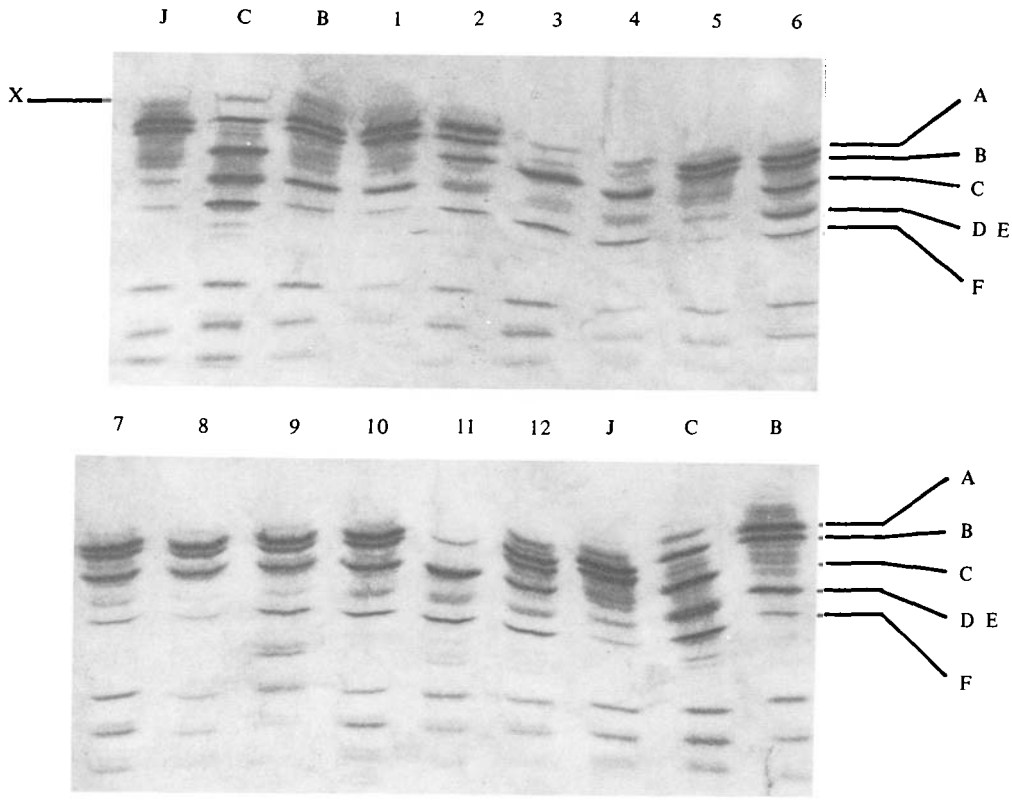

Fig. 4. IEF of MUPs from F2 progeny of the cross JU × C57BL. J, Ju; C, C57BL; B BALB/c; 1–12, individual F2 males.

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two parental strains are different. If they segregate independently we expect a 15:1 ratio of A+:A−. $\chi^2 = 0{\cdot}046$, which is consistent with this view. However, the possibility of loose linkage is not excluded. We call the loci *Mup-2* (JU) and *Mup-3* (C57BL). All three A− mice were also B−, suggesting linkage between A_1 and *B*. The argument assumes that such genotypes as $2^a2^a3^b3^b$, $2^a2^b3^a3^b$, $2^a2^a3^a3^b$ and $2^a2^b3^b3^b$ cannot be distinguished, all being scored A+.

Table 2. *MUP phenotypes of F2 progeny*

F2 of the cross

JU × C57BL

Mup-$2^a3^b4^a5^b(6, 7)^b8^b$ × *Mup-*$2^b3^a4^b5^a(6, 7)^a8^a$

A	B	C	DE	F			
				+	+/−	−	
			+	3	3	0	6
+	+	+	+/−	1	15	1	17
			−	0	4	3	7
				4	22	4	30
			+	0	1	0	1
+	+	−	+/−	0	1	2	3
			−	0	0	4	4
				0	2	6	8
			+	8	1	0	9
+	−	+	+/−	2	2	0	4
			−	0	0	0	0
				10	3	0	13
			+	0	0	0	0
−	−	+	+/−	0	3	0	3
			−	0	0	0	0
				0	3	0	3
							54

F2 of the cross

BALB/c × C57BL

Mup-$3^a4^a5^b6^a7^b8^b9^b$ × *Mup-*$3^a4^b5^a6^a7^a8^a9^a$

X	A	B	C	DE	F			
					+	+/−	−	
				+	4	16	1	21
+	+	+	+	+/−	0	1	0	1
					4	17	1	22
				+	1	0	1	2
+	+	+	−	+/−	0	0	0	0
					1	0	1	2
				+	0	0	3	3
−	+	+	−	+/−	0	1	2	3
					0	1	5	6
				+	3	7	2	12
+	+	−	+	+/−	0	0	0	0
					3	7	2	12
								42

Bands B and C. No B−C− progeny are found in either cross. If we make the assumption that genotypes 4^a4^a and 4^a4^b are indistinguishable, we expect a 3:1 ratio of B+ to B−. Similarly, if 5^a5^a and 5^a5^b are indistinguishable, we again expect a 3:1 ratio. The χ^2 values for band B are 0·62 (JU × ×C57B) and 0·29 (BALB/c × C57BL) and for band C, 2·25 (JU × C57BL) and 0·794 (BALB/c × C57BL). Thus the data are not inconsistent with the hypothesis. The absence of B−C− progeny shows that *Mup-4* and *Mup-5* are linked or allelic. Independent segregation would be expected to give about 3 B−C− F2 progeny in each cross.

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Band X. In the cross BALB/c × C57BL a minor band with a higher pI than band A could be scored (see Fig. 2). Based on the assumption that the parental genotypes are $4^a5^b9^b$ and $4^b5^a9^a$ we can work out the numbers of F2 phenotypes expected on the basis of complete linkage and independent segregation (Table 3). Only two putative recombinants were observed. Since these may have resulted from mis-scoring, we can conclude only that *Mup-9* is closely linked to *Mup-4* and *Mup-5*.

Table 3. *Segregation of Mup-4, Mup-5 and Mup-9. Cross BALB/c × C57BL*

Phenotype			Expectation		
X	B	C	Unlinked	Linked	Observed
+	+	+	15·8	21	22
+	+	−	7·9	.	2
+	−	+	7·9	10·5	12
−	+	+	5·3	.	0
−	+	−	2·6	10·5	6
−	−	+	2·6	.	0

Table 4. *Segregation of Mup-4, Mup-5 and Mup-8*

Phenotype			JU × C57BL			JU × BALB/c		
			Expectation			Expectation		
B	C	F	Unlinked	Linked	Observed	Unlinked	Linked	Observed
+	+	+	6·8	.	4	5·3	.	4
+	+	+/−	13·5	27	22	10·5	21	17
+	+	−	6·8	.	4	5·3	.	1
+	−	+	3·4	.	0	2·6	.	1
+	−	+/−	6·8	.	2	5·3	.	1
+	−	−	3·4	13·5	6	2·6	10·5	6
−	+	+	3·4	13·5	10	2·6	10·5	3
−	+	+/−	6·8	.	6	5·3	.	7
−	+	−	3·4	.	0	2·6	.	2

Band F. In both crosses it was possible to distinguish three F phenotypes, +, +/−, and −. We write the parental genotypes of both crosses $4^a5^b8^b$ (BALB/c and JU) and $4^b5^a8^a$. (C57BL). Taking the F+/− phenotype to represent the F1 genotype 8^a8^b (see Fig. 3) the numbers of F2 progeny expected from each cross on the basis of complete linkage and independent segregation are shown in Table 4. Clearly, the data do not support the hypothesis of complete linkage. For the hypothesis of independent segregation $\chi^2 = 32{\cdot}8$ and $P < 0{\cdot}01\,\%$ (JU × C57BL) and $\chi^2 = 21{\cdot}3$ and $P < 0{\cdot}5\,\%$ (BALB/c × C57BL). Thus the data strongly suggest that *Mup-9* is loosely linked to *Mup-4* and *Mup-5*, rather than segregating independently.

Bands D and E. These pose an extra problem, because they usually coincide in position. On those occasions when they resolve or partially resolve, it can be seen

that band D (higher pI) but not band E, is present in BALB/c. A useful analysis is possible only in the cross JU × C57BL, because both bands are absent in strain JU. Two possible situations can be envisaged: *Mup-6* and *Mup-7* may either be closely linked, or they may recombine with each other. If they are closely linked, they may either recombine with *Mup-4* and *Mup-5*, or may be closely linked to *Mup-4* and *Mup-5*. Expectations based on complete linkage of all four loci and independent segregation of *Mup-6* and *Mup-7* from *Mup-4* and *Mup-5* are shown

Table 5. *Segregation of Mup-4, Mup-5, Mup-6 and Mup-7. Cross JU × C57BL*

Phenotype			Expectation		
B	C	(DE)	Unlinked	Linked	Observed
+	+	+	6·8	.	6
+	+	+/−	13·5	27	17
+	+	−	6·8	.	7
+	−	+	3·4	.	1
+	−	+/−	6·8	.	3
+	−	−	3·4	13·5	4
−	+	+	3·4	13·5	9
−	+	+/−	6·8	.	7
−	+	−	3·4	.	0

in Table 5. Clearly, the hypothesis of complete linkage is unacceptable. Based on the hypothesis of independent segregation, $\chi^2 = 17{\cdot}56$ and $P = 2{\cdot}5\%$. Thus, if *Mup-6* and *Mup-7* are linked together, it is likely that they are loosely linked to *Mup-4* and *Mup-5*, but independent segregation of *Mup-6* and *Mup-7* from *Mup-4* and *Mup-5* cannot be ruled out at this stage.

If, on the other hand, *Mup-6* and *Mup-7* recombine with each other, they will generate a range of phenotypes intermediate between (DE)+ and (DE)−, which we are unable as yet to score. However, we can firmly conclude that whichever arrangement of *Mup-6* and *Mup-7* proves to be correct, some element (*6* or *7* or both) recombines with *Mup-4* and *Mup-5*.

4. DISCUSSION

This preliminary analysis is based on the extreme assumption that variation in the expression of each MUP is due to the existence of alleles at a single locus. The phenotypes of the F1 progeny suggest codominant expression. Among the F2 progeny, three and possibly four of these hypothetical loci (*Mup-4*, *Mup-5*, either *Mup-2* or *Mup-3*, and possibly *Mup-9*) did not recombine with each other, and must therefore be reasonably closely linked. On the other hand, we cannot exclude the possibility that the expression of the structural genes corresponding to bands X, B, C and A_1 or A_2 is determined by alleles at a single controlling locus of the sort proposed by Szoka & Paigen (1979). This explanation would carry the corollary

that these structural genes do not themselves vary significantly between the inbred strains.

However, the F2 phenotypes are consistent with recombination between some of the proposed loci. Loci *Mup-2* and *Mup-3* recombined to produce an A− phenotype. The *Mup-8* locus recombines with *Mup-4* and *Mup-5*. Either *Mup-6* and *Mup-7* recombine, or both together recombine with *Mup-4* and *Mup-5*. It is possible to explain these results by postulating the existence of a number of *trans*-acting controlling elements. However, the simplest hypothesis is that we are observing the recombination of alleles at structural gene loci. The fact that major bands are usually replaced by minor bands with the same pI in 'negative' strains suggests that the differences between alleles may be due to differences in *cis*-acting controlling elements (e.g. promoters). We presently favour the interpretation that variation of bands X, B and C is also due to allelic differences of the same sort between structural genes.

Hudson *et al.* (1967) and Finlayson *et al.* (1969) established linkage between a MUP component and the *brown* (*b*) locus on chromosome 4. It seems very likely that these authors were following the segregation of bands B and C. Thus, it is likely that the *Mup-4-5-9* complex is located on chromosome 4. Our data suggest that the *Mup-8* locus is loosely linked to *Mup-4* and *Mup-5*. Work with mouse × hamster cell hybrids has shown that many and perhaps all of the MUP structural genes are located on chromosome 4 (K. Bennett, P. Lalley and N. D. Hastie, in preparation). Our data are consistent with the possibility that *Mup-6*, *Mup-7* and one or even both of *Mup-2* and *Mup-3* are also linked to *Mup-4* and *Mup-5*.

We are grateful to Avril Duncan and Anne Walker for their assistance, and to Drs Bennet, Lalley and Hastie for access to their unpublished experiments. Mouse house facilities were made available by Drs W. G. Hill and A. Robertson. Invaluable help and encouragement was given by J. Isaacson and Dr C. Roberts. This work was supported by the Cancer Research Campaign and the Medical Research Council.

REFERENCES

FALCONER, D. S. (1973). Replicated selection for body weight in mice. *Genetical Research* **22**, 291–321.

FINLAYSON, J. S. & BAUMANN, C. A. (1958). Mouse proteinuria. *American Journal of Physiology* **192**, 69–72.

FINLAYSON, J. S., POTTER, M. & RUNNER, C. C. (1963). Electrophoretic variation and sex dimorphism of the major urinary protein complex in inbred mice: a genetic marker. *Journal of the National Cancer Institute* **31**, 91–107.

FINLAYSON, J. S., HUDSON, D. M. & ARMSTRONG, B. L. (1969). Location of the *Mup-a* locus on mouse linkage group VIII. *Genetical Research* **14**, 329–331.

HASTIE, N. D., HELD, W. A. & TOOLE, J. J. (1979). Multiple genes coding for the androgen-regulated major urinary proteins of the mouse. *Cell* **17**, 449–457.

HOFFMAN, H. A. (1970). Starch-gel electrophoresis of murine major urinary protein. *Proceedings of the Society for Experimental Biology and Medicine* **135**, 81–83.

HUDSON, D. M., FINLAYSON, J. S. & POTTER, M. (1967). Linkage of one component of the major urinary protein complex of mice to the brown coat colour locus. *Genetical Research* **10**, 195–198.

Potter, M., Finlayson, J. S., Bailey, D. W., Mushinski, E. B., Reamer, B. L. & Walters, J. L. (1973). Major urinary protein and immunoglobulin allotypes of recombinant inbred mouse strains. *Genetical Research* **22**, 325–328.

Rümke, Ph. & Thung, P. J. (1964). Immunological studies on the sex-dependent prealbumin in mouse urine and on its occurrence in the serum. *Acta Endocrinologia* **47**, 156–164.

Szoka, P. R. & Paigen, K. (1978). Regulation of mouse major urinary protein by the *Mup-a* gene. *Genetics* **90**, 597–612.

Szoka, P. R. & Paigen, K. (1979). Genetic regulation of MUP production in recombinant inbred mice. *Genetics* **93**, 173–181.