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The liver/erythrocyte pyruvate kinase gene complex [*Pk-1*] in the mouse: regulatory gene mutations

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Summary

Nine enzyme activity variants and one charge variant of liver/erythrocyte pyruvate kinase have been found amongst laboratory and wild mice. Four of the enzyme activity variants were previously reported to be caused by allelic differences in the structural gene, *Pk-1s*. Analysis of two putative regulatory gene mutations is now reported, both of which map at, or close to, the structural gene on chromosome 3. One of these mutations, in the inbred strain SWR, is tissue specific, affecting enzyme concentration in the liver but not the erythrocyte the other, which arose in a mutation experiment, doubles the enzyme concentration in both tissues. The organization and the nomenclature in the [*Pk-1*] gene complex are discussed and are compared with the organization of other comprehensively analysed gene complexes in the mouse.

Introduction

The structural gene *Pk-1*, for liver/erythrocyte pyruvate kinase (Moore & Bulfield, 1981), codes for a different isoenzyme in each tissue (Imamura *et al.* 1972 Harada *et al.* 1978 Saheki *et al.* 1978, Marie *et al.* 1981, Moore & Bulfield, 1981, Simon *et al.* 1982); cDNA sequences for the gene have been cloned (Noguchi *et al.* 1983; Simon *et al.* 1983; Inoue *et al.* 1986; Lone *et al.* 1986). *Pk-1* has recently been mapped to mouse chromosome 3 (Sola *et al.* 1988).

Nine inherited enzyme activity variants and one electrophoretic allele of *Pk-1* have been found in the mouse (Bulfield *et al.* 1978; Moore & Bulfield, 1981 Bonhomme *et al.* 1984; Bulfield *et al.* 1984 Charles & Pretsch, 1984, 1987). Four of these activity variants are caused by allelic differences in the structural gene, *Pk-1s;* one of them (in the inbred strain C57BL) is an unusual tissue specific structural gene mutation (Fitton & Bulfield, 1989). In this paper we analyse two putative regulatory gene mutations. One of these mutations, in the inbred strain SWR, maps close to the structural gene and is tissue specific, affecting enzyme concentration in the liver but not the erythrocyte. The other mutation which arose in an ENU mutation experiment (Charles & Pretsch, 1984, 1987), also maps close to the structural gene and doubles the measurable activity of the enzyme in both tissues.

2. Materials and methods

(i) *Animals*

The inbred strains C57BL/6, SWR and C3H/He were obtained from B&K Universal Ltd, Grimston, Hull, U.K. The *Pk-1s*^{b} allele producing the W phenotype was recovered from feral mice (Moore & Bulfield, 1981). Animals from the C57L inbred strain and SWXL RI strains were imported from the Jackson Laboratory, Bar Harbor MA, USA. The MUN phenotype originated from an ENU mutation experiment on (101/E1 × C3H/E1)F_1 mice and is caused by a codominant mutation (Charles & Pretsch, 1984, 1987).

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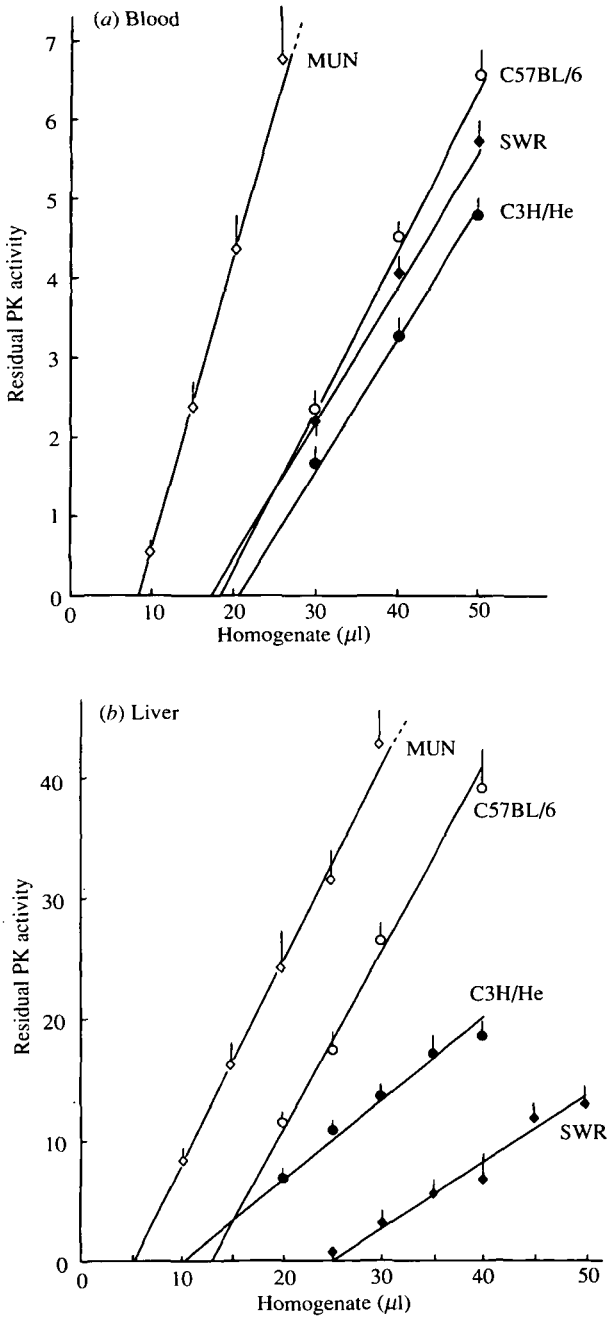

Fig. 1. Immunoprecipitation of pyruvate kinase in (*a*) blood and (*b*) liver homogenates of animals of four different phenotypes. Each point represents mean ± S.E. of 10 animals (each animal assayed three times).

(ii) *Determination of enzyme activity and concentration*

These measurements were performed as previously described (Bulfield & Moore, 1974 Moore & Bulfield, 1981; Fitton & Bulfield, 1989). Enzyme activity units were expressed as μmol min^{-1} (ml blood)$^{-1}$ or μmol min^{-1} (g wet weight liver)$^{-1}$ at 30 °C.

(iii) *Maximum likelihood analysis of segregation data*

Elston (1984) presented a maximum likelihood method of analysing a quantitative trait measured on two inbred parental strains, their F_1, and their backcross generations, with a view to choosing among a set of simple genetic hypothesis to account for the observed variability in the trait. The data are first transformed by a power transformation, if possible, to achieve approximate normality and homoscedasticity of the parental and F_1 generations. Likelihoods are then calculated from all the data, maximizing over unknown parameters, for a series of genetic hypotheses – one locus, polygenic, mixed major locus/polygenic, two unlinked loci, and two linked loci. In each case it is assumed that the variability about any genotypic mean is normally distributed with a common variance. A likelihood is also calculated for a general model that subsumes each specific hypothesis as a special case. For example, the general model contains a parameter, λ, such that $\lambda = 1$ corresponds to the mixed major gene/polygenic hypothesis, $\lambda = \frac{1}{2}$ corresponds to two unlinked loci and $0 < \lambda < \frac{1}{2}$ corresponds to two linked loci. Then significant departure from any specific hypothesis is judged by comparing the natural logarithm of the likelihood under that hypothesis with the natural logarithm of the likelihood under the general model twice the difference between these two quantities is, in large samples, distributed as chi-square if the hypothesis is true. One can also choose the 'best' hypothesis using Akaike's (1977) AIC information criterion. In this method one selects the hypothesis that gives rise to the largest likelihood as being the best, except that allowance is made for the fact that under different hypothesis the likelihood is maximized over different numbers of parameters. Elston (1984) listed 37 specific hypothesis, each being defined by a particular set of constraints on the parameters of the general model. These hypothesis were all examined, both for significant departure from the general model and for comparison with each other via the AIC information criterion.

3. Results

(i) *The activity of liver and blood pyruvate kinase in four phenotypes*

The activity of PK in blood and liver homogenates was determined for four phenotypes (Table 1). In relation to the C57BL/6 inbred strain, SWR animals have low PK activity in the liver but similar activity in the blood. The MUN phenotype has over twofold greater PK activity than C3H/He animals in both liver and blood; the mutation causing the MUN phenotype arose in a mixed C3H/101 background (see also Charles & Pretsch, 1984. 1987). Therefore, on the basis of enzyme activity SWR and C3H/He animals seem to have a similar phenotype in relation to C57BL/6 mice; that is a tissue-specific reduction in liver PK activity. The C57BL/6:C3H/He phenotype difference is caused by a tissue-specific mutation in the

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Table 1. *Activity of pyruvate kinase*[a] *in the blood and liver of four phenotypes of mice*[b]

	Liver				Blood			
			Percentage of				Percentage of	
Phenotype	Activity	*n*	B6	C3H	Activity	*n*	B6	C3H
C57BL/6	82·0 ± 2·80	28	100	192	7·58 ± 0·17	18	100	104
SWR	35·3 ± 1·45	14	43	82	8·57 ± 0·33	17	113	114
C3H/He	42·8 ± 2·40	19	52	100	7·52 ± 0·20	14	99	100
MUN	101 ± 4·40	22	123	236	16·9 ± 0·32	41	223	225

[a] Mean ± S.E. expressed as units (g liver)$^{-1}$ or units (ml blood)$^{-1}$ at 30 °C.
[b] Data on C57BL/6 and C3H/He taken from Fitton & Bulfield (1989).

Table 2. *Equivalence points and slopes of liver and blood PK of four phenotypes of mice determined by immunotitration*[a, b]

	Liver		Blood	
Phenotype	Equivalence point	Slope	Equivalence point	Slope
C57BL/6	13·5 ± 1·3	19·8 ± 1·0	18·5 ± 1·0	20·1 ± 1·3
W	10·1 ± 1·1	6·6 ± 1·2	17·2 ± 1·5	8·6 ± 0·8
SWR	25·3 ± 0·5	6·7 ± 0·1	17·6 ± 0·8	18·9 ± 0·5
C3H/He	10·7 ± 1·7	8·37 ± 0·8	21·0 ± 1·5	17·4 ± 1·8
MUN	5·10 ± 0·7	22·1 ± 0·8	8·50 ± 0·5	38·5 ± 2·1

[a] Mean ± S.E. of ten animals, each animal assayed three times, for each phenotype.
[b] Data on C57BL/6, W and C3H/He taken from Fitton & Bulfield (1989).

structural gene, *Pk-1s* (Fitton & Bulfield, 1989). The nature of the SWR and the MUN phenotypes are analysed in this paper.

(ii) *The concentration of liver and blood pyruvate kinase in four phenotypes*

The concentration of PK enzyme molecules in the four phenotypes was determined by immunotitration. From these data it was possible to calculate the equivalence points and slopes of the plots by linear regression analysis (Table 2, Fig. 1). The equivalence points are inversely proportional to the concentration of enzyme and the slopes proportional to the measurable activity, i.e. the product of the enzyme concentration × enzyme specific activity (Fitton & Bulfield, 1989).

From these analyses, it can be seen (Table 2, Fig. 1) that the PKs from SWR and C57BL/6 differ in equivalence points in the liver although they are similar in the blood (as would be expected from the enzyme activity measurements, Table 1). This indicates that the activity difference in liver PK between C57BL/6 and SWR animals is caused by a difference in enzyme concentration rather than a difference in enzyme specific activity. This implies that the C57BL/6:SWR phenotypic difference is probably caused by a regulatory gene mutation. The difference in liver PK activity between C57BL/6 and C3H/He animals, on the other hand, is caused by an alteration in specific activity of the enzyme as the equivalence points and hence enzyme concentration, are the same (Table 2; see also Fitton & Bulfield, 1989).

C3H/He and MUN animals differ over twofold in equivalence points for both liver and blood PK (Table 2, Fig. 1). This indicates that the twofold PK activity differences (Charles & Pretch, 1984 Table 1) between these two phenotypes in both tissues is due to a difference in enzyme concentration, implying that it is probably caused by a regulatory gene mutation.

(iii) *The SWR phenotype*

The immunotitration results suggest that the C57BL/6:SWR phenotypic difference is caused by a mutation in a regulatory gene(s). This is confirmed by kinetic determinations (Table 3) which show no difference in the affinity of liver PK for PEP or ADP between the C57BL and SWR phenotypes.

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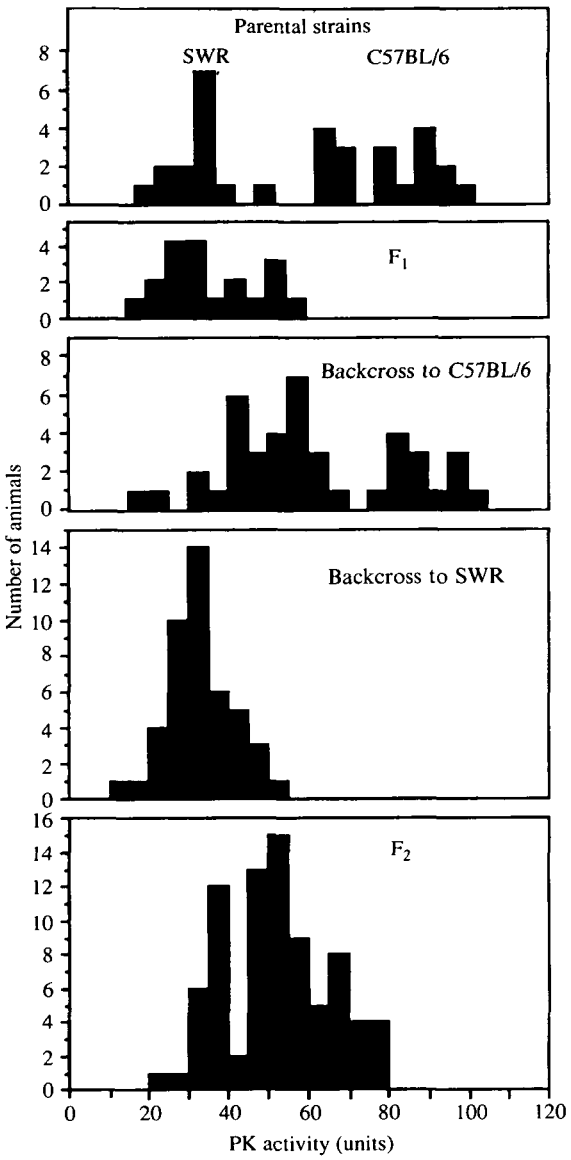

Fig. 2. Segregation analysis of liver PK activity among backcrosses and F_2s between animals of the SWR and C57BL/6 inbred strains.

Table 3. *Kinetic analysis of liver PK in the C57BL and SWR phenotypes*[a]

Phenotype	V_{max}	K_m PEP	K_m ADP
C57BL	60·5 ± 4·1	48·3 ± 0·55	327 ± 22
SWR	36·8 ± 13·5	48·7 ± 10·3	308 ± 9·5
Ratio SWR/C57BL	0·61	1·01	0·94

[a] V_{max} and K_m were measured by linear regression analysis.

A formal segregation analysis of liver PK activities between C57BL and SWR was performed (Fig. 2, Table 4). It can be seen that the F_1 values are displaced towards the SWR parental distribution. The backcross to C57BL suggest a bimodality as would be caused by monogenic segregation but the backcross to SWR does not. The F_2 values (Fig. 2) suggest a 1:2:1

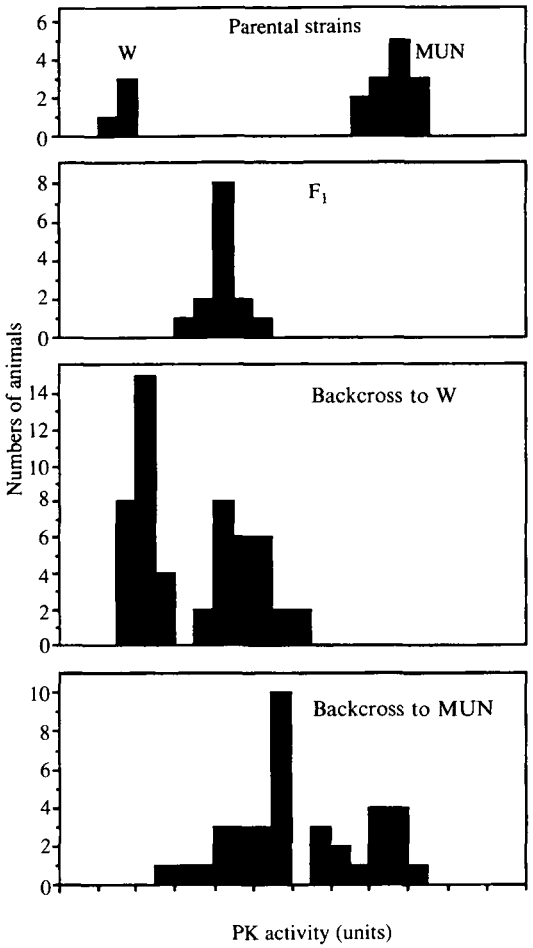

Fig. 3. Segregation analysis of blood PK activity among backcrosses between animals of the W and MUN phenotypes.

Table 4. *Liver PK activity*[a] *in C57BL/6, SWR, F_1 and backcross mice*[b]

Phenotype	Liver PK activity	*n*
C57BL/6	82·0 ± 2·8	28
SWR	35·3 ± 1·45	14
F_1	40·3 ± 2·5	20
Backcross to SWR	35·7 ± 2·3	45
Backcross to C57BL/6		
(i) Low group	52·9 ± 2·1	28
(ii) High group	92·1 ± 1·8	14

[a] Mean ± S.E. expressed as units (g liver)$^{-1}$ at 30 °C.
[b] Data on C57BL/6 and SWR taken from Table 1.

monogenic segregation but it is not clear-cut with significant overlap. A simple inspection of this data does not, therefore, allow conclusions over the existence of monogenic segregation in these crosses.

To try and resolve this problem, the distribution of PK activity levels was determined amongst the SWXL recombinant inbred strains and these data and the segregation data subjected to maximum likelihood analysis.

The progenitor strains of the SWXL RI strains are

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Table 5. *Liver PK activity*[a] *in C57BL/6, C57L, SWR and SWXL strain mice*

Strain	Liver PK activity	PK phenotype[b]	Coat colour phenotype
C57BL/6	82·0 ± 2·8	B	Black
C57L	56·5 ± 2·5	L	Leaden
SWR/J	35·3 ± 1·5	S	Albino
SWXL 4	38·0 ± 0·9	S	Leaden
SWXL 12	52·8 ± 1·9	L	Albino
SWXL 14	61·3 ± 2·3	L	Albino
SWXL 15	35·6 ± 1·9	S	Albino
SWXL 16	35·0 ± 1·0	S	Albino
SWXL 17	61·0 ± 3·0	L	Leaden

[a] Mean ± S.E. expressed as units (g liver)$^{-1}$ at 30 °C.
[b] Phenotypes: B, C57BL/6; L, C57L; S, SWR.

Table 6. *Maximum likelihood parameter estimates for segregation of a major gene between C57BL and SWR with polygenic effects*

Parameters	Estimates (natural log scale)
C57BL	4·38
F_1 mean	3·89
SWR mean	3·55
C57BL × F_1 backcross means	4·46, 4·11
SWR × F_1 backcross means	3·84, 3·84
Parental and F_1 standard deviation	0·19
Backcross standard deviation	0·21

the C57L and SWR inbred strains. C57L animals have a high liver-specific PK activity phenotype they do not, however, have activity levels as high as C57BL/6 mice (Table 5) and other genes with minor effect may cause this difference although they have the same general phenotype. Despite this difference between C57BL/6 and C57L mice, the C57L/SWR phenotypic differences segregates clearly amongst the six SWXL RI strains in a 1:1 ratio (Table 5). This indicates that the phenotypic difference between C57L and SWR is caused by a single major gene.

The segregation data was subject to maximum likelihood analysis (Elston, 1984). The initial analysis of the liver PK activities in C57BL/6, SWR mice and their F_1 hybrids indicate that these three classes are not significantly heteroscedastic ($\chi^2 = 2{\cdot}81$, 2 D.F., $P > 0{\cdot}2$) and that the best power transformation to obtain normality and homoscedasticity is $x = y^{0{\cdot}039}$. An analagous analysis of the RI strains also indicated lack of significant heteroscedasticity ($\chi^2 = 6{\cdot}70$, 6 D.F. $P > 0{\cdot}5$) and the power transformation was estimated to be $x = y^{0{\cdot}25}$.

Based on the results of this initial analysis, the data on C57BL/6, SWR, F_1 and their backcross offspring were analyzed after undergoing a logarithmic transformation, which corresponds to a power of zero. The hypothesis that is most compatible with the data, based on Akaike's (1977) AIC criterion, is one in which two linked loci are segregating. However, examination of the parameter estimates for that hypothesis show $\lambda = 0{\cdot}89$, i.e. a 'recombination fraction' of 0·89. Since this is biologically unrealistic, we note that the next best hypothesis is one in which both a major gene and polygenes are segregating, i.e. the so-called 'mixed model, and that the χ^2 for that hypothesis is not significant ($\chi^2 = 7{\cdot}59$, 3 D.F. $P > 0{\cdot}05$). All other hypothesis – single gene, polygenic or two unlinked loci – are rejected with $P < 0{\cdot}01$.

There is significant departure from the general mixed major gene/polygenic hypothesis ($\chi^2 = 7{\cdot}55$, 2 D.F., $P \sim 0{\cdot}02$), in which seven distinct means are estimated: means for C57BL/6, SWR and their F_1, two corresponding means for the C57BL/6 × F_1, backcross (but displaced because of polygenic effects), and two corresponding means for the SWR × F_1 backcross (also displaced). However, two of the means are estimated to be almost identical, and when a symmetrical relationship [symmetry B of Elston (1984)] is imposed on the means, the estimates remain virtually unchanged. Thus the χ^2 for fit of the model increases only slightly (to 7·59) but, because of the additional degree of freedom gained, is no longer significant. The parameters can be estimated for this hypothesis (Table 6). It should be noted that whereas the C57BL/6 × F_1 backcross segregates into two groups (with means 4·46 and 4·11 – displaced from 4·38 and 3·89 by polygenic effects), there is no such segregation in the SWR × F_1 backcross. The similarity of the single backcross mean (3·84) to the F_1 mean (3·89) is peculiar, and we are at a loss to explain it.

The analyses were repeated after using the transformation $x = y^{0{\cdot}039}$ and essentially the same results were obtained. The same mixed model is the only one to fit the data, though the fit is slightly better ($\chi^2 = 7{\cdot}38$, 3 D.F.). The estimates show the same peculiarity – the two SWR × F_1 backcross means are identical, and almost equal to the F_1 mean.

In conclusion, the analysis of the phenotypic difference between the C57L and the SWR strains using the SWXL RI strains indicates monogenic segregation. The analysis of the C57BL/SWR segregation data also indicate segregation of a major gene with polygenic effects. The immunotitration and kinetic data suggest this is a tissue-specific regulatory gene. The relationship of this gene to the gene complex [*Pk-1*] is discussed later.

(iv) *The MUN phenotype*

The MUN phenotype arose in an ENU mutation experiment and had previously been shown to be caused by the segregation of a single gene in a

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C3H/101 hybrid background (Charles & Pretsch, 1984, 1987). Homozygotes had 2·4-fold the activity of the progenitor strain and the gene is inherited additively with heterozygotes having 1·6-fold wild-type levels. The effect on PK activity is specific to adult liver and erythrocytes and PK activity in other tissues was not affected and therefore the phenotype is controlled by the [*Pk-1*] gene complex. The mutation does not effect heat stability, electrophoretic mobility or K_m PEP and it was therefore suggested that it was within a regulatory gene (Charles & Pretsch, 1984).

The immunotitration results (Table 2) confirm this conclusion demonstrating that the MUN phenotype has over twofold the number of PK molecules of C3H/He animals in both liver and blood. The mutation causing the MUN phenotype arose in a C3H/101 hybrid stock which has the same phenotype as purebred C3H/He mice (Charles & Pretsch, 1984). What now remains is to determine the relationship of the gene causing the MUN phenotype to the structural gene, *Pk-1s*.

Therefore animals of the MUN phenotype were mated to animals of the W phenotype which are homozygous for the reduced PK activity allele of the structural gene, *Pk-1s*^{b} (Moore & Bulfield, 1984; Fitton & Bulfield, 1989). The segregation data for PK activity in blood of W, MUN, F_1 and backcross animals are displayed in Fig. 3. The data show clear additive monogenic segregation; no recombinant activity classes are present. This segregation of the low activity *Pk-1s* structural gene mutation and the hyperactivity regulatory gene mutation causing the MUN phenotype, indicates that both the structural gene and regulatory gene are part of the same gene complex, [*Pk-1*]; there is also segregation in thermal stability as an indicator of the structural gene mutation (data not shown).

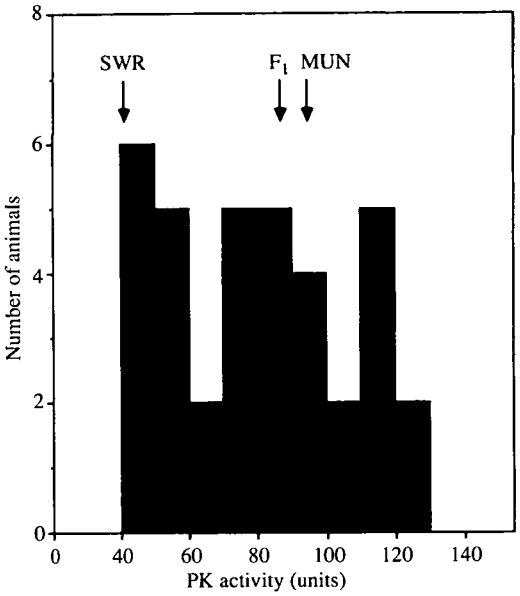

Fig. 4. Segregation of liver PK activity among F_2s of a cross between the SWR inbred strain and the MUN phenotype. Arrows mark the mean PK activity of SWR, F_1 and MUN animals.

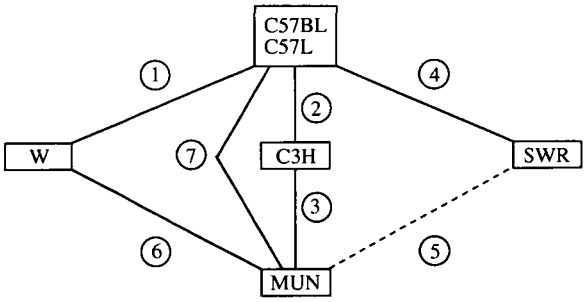

Fig. 5. Summary of segregation analyses between PK phenotypes. Lines between phenotypes indicate that monogenic segregation has been demonstrated as follows: 1, Moore & Bulfield (1981) 2, Fitton & Bulfield (1989) 3, Charles & Pretsch (1984) 4–7, this paper. The segregation of the MUN and SWR phenotypes remains to be confirmed (Fig. 4).

(v) *Further segregation analysis and relationships between the phenotypes*

It has now been possible to determine the relationship of the regulatory gene causing the MUN phenotype to the structural gene *Pk-1s*; they are closely linked. A similar segregation analysis is not possible between the *Pk-1s*^{b} structural gene mutation and the regulatory gene mutation causing the SWR phenotype as they both reduce PK activity to similar level (Table 1; Fitton & Bulfield, 1989). Liver PK levels do however differ enough between the SWR and MUN phenotypes (Table 1) to permit a segregation analysis. This was performed but the backcross data did not show monogenic segregation (data not shown) although there was some indication of a 1:2:1 segregation in the F_2 (Fig. 4). This analysis therefore remains inconclusive.

Taking all the segregation data together we get the relationships in Fig. 5 for monogenic segregation between phenotypes. In all these cases, except for the polygenic interference with the C57BL/6 – SWR segregation, there are no examples of recombinant phenotypes. This implies that all the genes involved are either alleles of the *Pk-1s* structural gene or alleles of genes closely linked to it within the [*Pk-1*] gene complex. For nomenclature purposes we have considered mutations affecting enzyme concentration as regulatory gene mutations closely linked to *Pk-1s*. This definition is based on phenotype and does not presume to imply anything about their action at the DNA level, this will have to await molecular analysis.

(vi) *Nomenclature of alleles and genes with the* [Pk-1] *gene complex*

It is now possible to assign alleles and genes within the [*Pk-1*] gene complex on chromosome 3 (Table 7).

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Table 7. *Haplotypes with the* [Pk-1] *gene complex*[a]

Strain	Other strains	[*Pk-1*] haplotype		
		Alleles at *Pk-1s*	Alleles at *Pk-1r*	Alleles, at *Pk-1c*
C3H	SM, A, 129, 101 C58, FS/t, NMR	*a*	*a*	*a*
W/PKD/PKL	—	*b, c, d*	*a*	*a*
C57BL	C57L, DBA/1, HTI	*1*	*a*	*a*
Mus spretus	—	*s*	*a*	*a*
MUN	—	*a*	*a*	*a–m*
SWR	NZB, NZW	*a*	*b*	*a*

[a] Information from this paper and Bulfield *et al.* (1978); Moore, (1981); Bonhomme *et al.* (1984); Charles & Pretsch (1984); Bulfield *et al.* (1984); Fitton & Bulfield (1989).

The most common phenotype amongst inbred mice (Bulfield *et al.*, 1978) and wild-caught European *M. m. domesticus* is that of C3H; this is therefore given the *a* allelic symbols for both the structural gene, *Pk-1s* and the regulatory gene, *Pk-1r*. The W, PKD and PKL phenotypes are all caused by mutations of the structural gene, *Pk-1s* amongst geographically different trapping of wild mice the alleles, *b*, *c* and *d* may be identical but this is unlikely. C57BL and C57L mice contain the tissue specific structural gene mutation $Pk\text{-}1s^{1}$ (Fitton & Bulfield, 1989) but are identical at the *Pk-1r* regulatory gene. The MUN phenotype is caused by a mutation in a gene regulating enzyme concentration; it is not known whether this is in the *Pk-1r* gene and therefore, as convention dictates, this gene is named *Pk-1c*, the allele in C3H/101 being *a* and the mutant allele causing the MUN phenotype *a-m* (*a-mutation*). Without molecular evidence it is not possible to tell if *Pk-1r* and *Pk-1c* are the same regulatory element or not.

4. Discussion

The basic unit of gene function in mammals appears to be a gene complex of a structural gene with closely associated regulatory gene(s) or elements (Paigen, 1979). The genetical analysis of these complexes has been difficult in mammals compared with *Drosophilia* or micro-organisms. What is apparent, however, from the genetical analysis of the phenotypic variation currently available to mammalian geneticists, is that there is a wide variety of different phenotypes caused by putative regulatory gene mutations. These include mutations in the response to hormone induction (Paigen, 1979; Pfister *et al.* 1982; Martin *et al.* 1984; Middleton *et al.* 1987) intracellular location (Lusis *et al.* 1977) and developmental profile (Paigen, 1979 Pfister *et al* 1982) as well as systemic and tissue-specific regulatory gene mutations (Paigen, 1979).

The liver pyruvate kinase gene complex, [*Pk-1*] appears to be no less complicated. In a previous paper we analysed four structural gene mutations found amongst inbred and wild-caught mice. Three of these mutations behaved in a predictable fashion affecting the specific activity of the enzyme in both the liver and erythrocyte. The fourth mutation (in the inbred strain C57BL) affected the specific activity of PK in the liver only (Fitton & Bulfield, 1989). As the liver and the erythrocyte isoenzymes are encoded by different mRNAs presumably by a splicing mechanism (Imamura *et al.* 1978; Harada *et al.* 1978; Saheki *et al.* 1978; Marie *et al.* 1981 Simon *et al.* 1982) it was hypothesized that the mutation in C57BL mice could be in a shuffled exon. This type of tissue specific structural gene mutation has never been reported before in mammals. In this paper, we show that the [*Pk-1*] gene complex is no less complicated as far as putative regulatory gene mutations affecting enzyme concentrations are concerned.

The difference between the SWR and C57BL phenotypes is caused by segregation of a major gene affecting tissue-specific liver enzyme concentration (although complicated by polygenic variation); this is confirmed by the segregation of these phenotypes amongst the SWXL RI strains. This tissue specific regulatory gene mutation can be contrasted to the ENU-induced mutation occurring in C3H/101 hybrid mice (Charles & Pretsch, 1984) which affects enzyme concentration in *both* liver and erythrocyte. The [*Pk-1*] gene complex therefore has given us two novel types of mutations to add to our portfolio of mammalian genetic variation: the $Pk\text{-}1s^{1}$, tissue specific structural gene mutation found in C57BL strain mice and, the $Pk\text{-}1r^{b}$, tissue specific regulatory gene mutation found in SWR strain mice.

In the four genetic systems in mice now analysed in some detail, a wide variety of types of gene mutations have been found (Table 8). Such a variety from only four systems suggests that the regulation of mammalian genes will be complicated and complex. As molecular evidence becomes available on the regulation of individual genes this complexity has begun to

Table 8. *Regulatory genes within gene complexes in mice*[a]

Gene complex					
[*Gus*][b]		[*Bgl*]	[*Hdc*][c]	[*Pk-1*]	
Gus-s	Structural gene	*Bgl-e* Structural gene	*Hdc-s* Structural gene	*Pk-1s* Structural gene	
Gus-r	Androgen regulator	*Bgl-s* Systematic regulator	*Hdc-c* Systemic regulator	*Pk-1r* Tissue-specific regulator	
Gus-t	Temporal regulator	*Bgl-t* Temporal regulator	*Hdc-e* Oestrogen regulator	*Pk-1c* Systemic regulator	
Gus-u	Systemic regulator		*Hdc-a* Androgen regulator		

[a] From: Paigen (1979); Pfister *et al.* (1982); Martin *et al.* (1984); Middleton *et al.* (1987); Fitton & Bulfield (1989); this paper.

[b] There is one unlinked gene affecting the regulation of the complex: *Eg* (Paigen, 1979).

[c] There is one unlinked gene affecting this complex, *hti* (R. J. Middleton, S. A. M. Martin & G. Bulfield, unpublished observations).

be defined in terms of DNA sequence; the metallotheionein II gene for example has at least nine 5′ regulatory sequence motifs controlling at least five regulatory functions (Lee *et al.* 1987). The mutations available in these four gene complexes (Table 8), especially those in the regulatory genes, will prove of great value in relating DNA sequence to gene function.

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