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Characterization and mapping of a viable anaemic mutant in the mouse: a new allele, *mk*^{van}, at the *microcytic anaemia* locus

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Summary

A viable anaemic mouse mutant arose in the stock of a mouse fancier and has been characterized haematologically and genetically. Anaemic animals were less viable than normal animals (especially from 0 to 2 weeks of age) and had lower haemoglobin levels, percentage packed-cell volumes, higher red cell counts and lower mean cell volume than normal animals. Peripheral blood films showed a wide range of abnormal cells and extreme microcytosis. Linkage studies showed the mutant to be linked to the chromosome 15 markers *Na Ca* and *bt*; recombination with *Ca* was $1{\cdot}37 \pm 0{\cdot}68$% for females and $10{\cdot}5 \pm 7{\cdot}41$% for males. This position is similar to the *microcytic anaemia, mk*, mutant, and crosses between the viable anaemia mutation and *mk/mk* homozygotes showed the two to be allelic. Viable anaemia is therefore a second allele at the *mk* locus *mk*^{van}; new data give its position on chromosome 15.

1. Introduction

Mutations at seventeen different loci in the mouse cause anaemia (reviews: Russell, 1979; Green, 1981); only one of these mutants, however, the autosomal recessive *mk*, has persistent microcytic anaemia. It is of interest, therefore, that a mutant which was anaemic and viable and arose in a stock from a mouse fancier, also displayed microcytic anaemia. We report the haematological characterization of this new mutant and demonstrate that it is allelic with *mk* on chromosome 15.

2. Materials and Methods

(i) *Animals*

The *viable anaemia* mutant first arose in a stock of *himalayan* mice obtained from a mouse fancier (Wallace, 1979). In linkage experiments the following stocks were used: (*a*) *himalayan*, c^h, for chromosome 7. (*b*) *hammer-toe*, *Hm*, *twirler*, *Tw*, *Sombre*, E^{so}, *brown*, *b* and *short-ear*, *se*, for chromosomes 5, 18, 8, 4 and 9 respectively and (*c*) *naked*, *N*, *caracul*, *Ca*, and *belted*, *bt* for chromosome 15. The *microcytic anaemia*, *mk*, mutation was purchased, segregating in strain MKW, generation 26, from the Jackson Laboratory, Bar Harbor, MA 04609 USA.

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(ii) *Haematology*

The determination of various haematological parameters was performed on either a Coulter Model F_n or a Coulter Model S.

3. Results

(i) *Genetical analysis of the* viable anaemia *mutant*

The recessive mutation (initially called *viable anaemia, van*) arose in a stock of *himalayan* mice (Wallace, 1979) a few generations after the stock was obtained from a fancier. The latter's records showed that the c^h mutation was a *de novo* event, since the mutant traced to a mating homozygous for *chinchilla*, c^{ch} (Wallace, 1972). The fancier said she had never imported mice from the U.S.A., where the first *himalayan* mutant had been reported. It seems unlikely therefore that both *himalayan* and *viable anaemia* were descendants of existing alleles arising in the U.S.A. Unless there is new evidence from records in

Table 1. *Alleles transmitted by* NCa+/++van *heterozygotes backcrossed to* ++van/++van *homozygotes*

Recombination event	Locus *N Ca van*	Numbers of progeny: Heterozygous parent Female	Male	Totals	
None	*N Ca*+	161	11	172	301
	++ *van*	123	6	129	
N[a]	*N*+*van*	0	0	0	3
	+ *Ca* +	3	0	0	
van	*N Ca van*	3	0	3	5
	++ +	1	1	2	
Ca	*N*+ +	0	0	0	1
	+*Ca van*	0	1	1	
Grand total				310	

[a] In this event *N* and its normal allele are exchanged in relation to the parental arrangement. In the next event below, this is true for *van*. And so on.

Table 2. *Time of death of anaemic animals*

Period	Died in period	Culled in period	Total	Percentage of all deaths per period[a]
0–2 weeks	48	9	57	75
2–4 weeks	13	40	53	20·3
1–4 months	3	38	41	4·7
Over 4 months	0	31	31	0
Totals	64	118	182	–

[a] Deaths in a particular period as a percentage of total deaths.

Table 3. *Haematological parameters on viable anaemic animals (mean* ± S.E.)

Phenotype (*n*)	Haemoglobin (g/dl)	Red cell count ($\times 10^{12}$/l)	Percentage packed cell volume	Mean cell volume (fl)
Anaemic animals (5)	8·36 ± 1·17	11·2 ± 1·15	24·8 ± 2·89	24·8 ± 0·58
C57BL/10 animals (12)	14·6 ± 0·26	9·99 ± 0·17	44·1 ± 0·68	44·5 ± 0·39
Anaemics as a percentage of C57BL/10	57·3	112	56·2	55·7

the U.S.A., they must both be taken as *de novo* events. Anaemic mice were noticed, by their pale body colour, soon after birth in the first and third litters from two matings of related *himalayan* mice. These matings yielded a total of 13 anaemics out of 79 young, all seen once at 0–7 days old. This is significantly less than the 1/4 expected from heterozygous parents of a recessive condition. Since deaths could have occurred between birth and being seen, classification was then done at birth, and anaemics identified by a tail clip, until it

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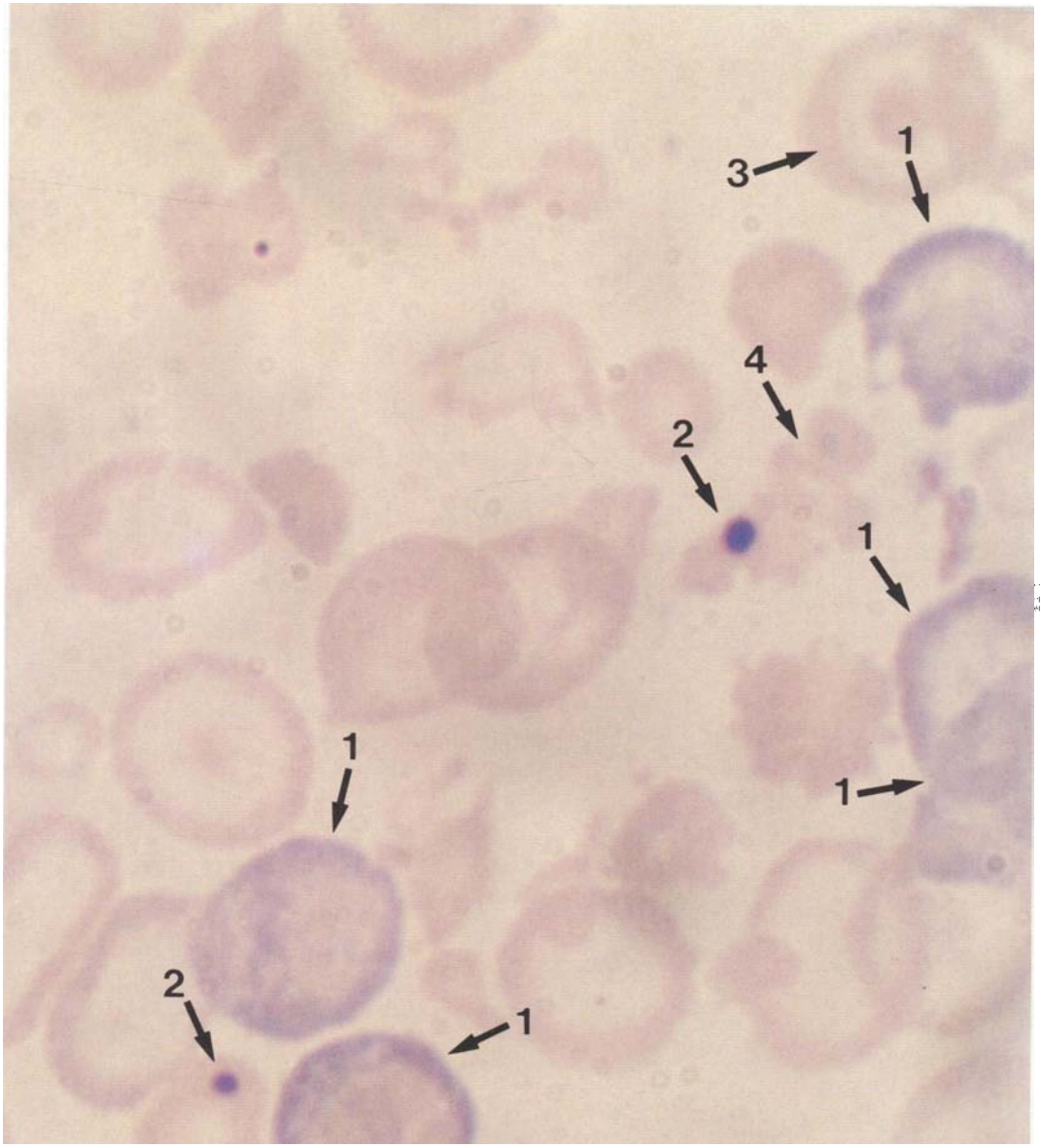

FIGURE 1. Peripheral blood film from a *viable anaemic* mouse. This shows the following features: extreme anisocytosis and poikilocytosis, increased polychromasia (1), hypochromasia, Howell-Jolly bodies (2), target cells (leptocytes) (3) and schistocytes (4).

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was established that the mutant is a fully penetrant recessive.

This is shown from matings between anaemics, which gave 36 young, all anaemic. It was clear then that anaemics which had grown hair were distinguishable from normals by the pale palmar surface of the feet and by the pale tail vein in pale-furred mice. The anaemic mutant was unsuccessfully tested for linkage to: *Hm*, *Tw*, E^{s^o}, *b*, *se* and c^h on chromosomes 5, 18, 8, 4, 9 and 7 respectively. In a test with the chromosome 15 markers *N*, *Ca* and *bt*, however, linkage was established (Wallace & Ferguson, 1980). When *N Ca*+/++*van* female heterozygotes were crossed to ++*van*/++*van* male homozygotes (Table 1) the complementary pair of phenotypes *N*++ and +*Ca van* was the one with the lowest frequency, 1/310. This then is the pair arising by double crossing-over and indicates the order *N–Ca–van*. The recombination between *van* and *Ca* can be estimated as $1{\cdot}37 \pm 0{\cdot}68$% for females and $10{\cdot}53 \pm 7{\cdot}41$% for males (Table 4). The recombination data between *van* and *Ca* are similar to those between *Ca* and another anaemic mutant *microcytic anaemia*, *mk* (McFarland & Russell, 1975), raising the question as to whether *van* and *mk* are allelic.

The identity between *van* and *mk* was studied in two ways. Homozygotes for both mutations were analysed haematologically (see below) and an allelism test was performed. Both *van/van* and *mk/mk* females are poor breeders, so crosses were made between an *N Ca/van* female and an *mk/mk* male (one mating) and +/*mk* females and *van/van* males (two matings). These matings together gave 13 anaemics and 20 non-anaemics. The seven non-anaemic offspring of the *N Ca/van* female were all *N Ca* and the 8 anaemic offspring all lacked *N* and *Ca*. Therefore *van* is allelic with *mk* and will now be called mk^{van}.

(ii) *Characterization of the* viable anaemia *mutant*

The viability of mk^{van}/mk^{van} animals in relation to age was studied. From the matings between homozygotes 12 of the 36 anaemic young (33%) died before weaning. The viability of anaemics in the linkage testing experiments was also examined. In the linkage test involving *Hm*, *Tw*, E^{s^o} and c^h, classification of all mutants was complete by 2 weeks of age, when there were 53 anaemic and 40 normal animals. This is close to the expected 1:1 for full viability, indicating that the anaemics have normal viability to 2 weeks in this stock. In the tests involving *se*, classification was complete at 18 days of age; 117 normals and 26 anaemics were produced, whereas 25% were expected for full viability. This is 10 less anaemics than expected, giving an increase in mortality of 28% relative to normal animals.

In the backcrosses involving *N* and *Ca* a more detailed survey of the viability of anaemic animals was carried out. The age of death or killing was noted weekly up to 8 weeks and monthly thereafter. Anaemic animals can be classified from births onwards, *caracul* from the whiskers at 3 days and *naked* from the fur at about 11 days; after classification unwanted mice were culled. Amongst these backcross animals at 2 weeks of age there were 177 normals and 133 anaemics (Table 1), a deficiency of 44 anaemics, indicating a mortality of 25% compared with normals. A detailed analysis of the age of death of anaemic animals (Table 2) shows greatest mortality during the 0- to 2-week period (26·4% of animals born and 75% of all deaths at all ages), diminishing to zero in animals over 4 months of age; the trend is significant (χ^2_3, testing heterogeneity, is 95·96, $P < 0{\cdot}001$). These data, divided according to sex (not shown), show a slight trend towards greater mortality of females than of males at 0–2 weeks of age, with a reversed trend thereafter, but these differences are not significant. The few anaemic females which bred, however, only produced a few litters and died earlier than unmated females.

Haematological parameters were measured on male and female anaemic mice and the data pooled and compared with normal animals from the C57BL/10 inbred strain (Table 3). Anaemic animals had a reduced amount of haemoglobin, packed cell volume and mean cell volume compared with C57BL/10 animals (the one heterozygous litter-mate of anaemic animals analysed in this series had the same haematological parameters as C57BL/10 animals). One anaemic female and one anaemic male had a very marked erythrocytosis, with RCC of 13·5 and 14·3 respectively, but the most consistent and unusual observation is the low mean cell volume (Table 3). For technical reasons, accurate reticulocyte counts were difficult to perform on the anaemic mice; the counts did, however, show extreme variability and were usually very high, often over 80%, but in some animals as low as 20%, although still higher than controls.

Peripheral blood films were examined from 8 female and 7 male anaemic animals (Plate 1). They were all abnormal and showed the following features: extreme anisocytosis, extreme poikilocytosis, markedly increased polychromasia, hypochromasia, numerous target cells, variable numbers of cells containing Howell-Jolly bodies, cell fragments (schistocytes) and bizarre-shaped cells. The films also showed extreme microcytosis, but this was partially obscured by the presence of cells with a large diameter but a very low MCV due to being unusually thin (leptocytes). Occasional coarsely crenated cells were seen on some films; true spherocytes were absent but there was microspherocytosis.

4. Discussion

Mutant alleles, each leading to some type of anaemia at some or all stages of development in mice, have already been identified at 17 loci (Russell, 1979). A

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Table 4. *Recombination values from present and published data*

Source	Segment	Female heterozygotes R.F.(%)	S.E.	Male heterozygotes R.F.(%)	S.E.
Wallace & Mallyon (1972)	*N-Ca*	0·4931	0·1555	2·2749	0·3280
	Ca-bt	3·7968	0·4244	11·1326	0·6924
McFarland & Russell (1975)	*Ca-mk*	0·7026	0·4017	2·6316	2·5668
Present data	*N-Ca*	1·0309	0·5922	5·2632	5·1253
	Ca-van	1·3746	0·6840	10·5263	7·4090

Table 5. *Comparison of haematological parameters in* mk/mk, mk^{van}/mk^{van} *and normal mice*[a]

Genotype	Haemoglobin (g/dl)		Red cell count ($\times 10^{12}$/l)		Percentage packed cell volume		Mean cell volume (μm^3)	(fl)
mk/mk	10·9	—	13·6	—	39·6	—	29	—
mk^{van}/mk^{van}	—	8·36	—	11·2	—	24·8	—	24·8
normal	17·5	14·6	10·4	9·99	49·3	44·1	47	44·5

[a] Data on *mk/mk* from Russell *et al.* (1970*b*), Table 1, sexes averaged; data on mk^{van}/mk^{van} from Table 3.

newly discovered viable anaemic mutant (Wallace, 1979) has first, therefore, to be analysed genetically and haematologically to see whether it is allelic with any of the existing mutations.

The new recessive mutation showed full penetrance. Linkage studies with a wide range of chromosome markers showed it to be linked to *N–Ca–bt* on chromosome 15 (Table 1), suggesting allelism with microcytic anaemia, *mk*. Recombination values for this section of chromosome 15 are displayed in Table 4 including the data on the linkage of *viable anaemia* presented in this paper. The values from a large balanced three-point cross for *N–Ca–bt* (Wallace & Mallyon, 1972) are taken as the baseline for comparison. They show that the *Ca-mk* values compatible with the *Ca-van* values and give the order *N-Ca-van*. Allelism between *van* and *mk* was established. The *van* allele is therefore renamed mk^{van}. The *N-Ca-van* data thus give a position for the *mk* locus inside the *Ca-bt* segment of chromosome 15 with the order *N-Ca-mk-bt*.

It is interesting that in the three-point cross of *N-Ca-bt* the recombination values for males greatly exceed those of the females; the data from all the other crosses in Table 4 confirm this. The *N-bt* segment of chromosome 15 is the only one to show such a big difference in recombination values between the sexes.

The microcytic anaemia (*mk/mk*) mutant in the mouse differs from all other haematological mutants in showing an increase in the production of erythrocytes of half-normal size, low packed cell volume and haemoglobin concentration. All these features of *mk/mk* mice are found in viable anaemic mk^{van}/mk^{van} animals; a comparison of the haematological parameters shows a remarkable similarity, especially as the two alleles are on different genetic backgrounds (Table 5, Russell *et al.* 1970*b*). The *mk/mk* mutants have relatively poor early postnatal viability, which varies in extent depending on genetic background (Russell *et al.* 1970*a*). Viable anaemic animals also show early postnatal mortality (Table 2); the absolute mortality of anaemics from matings between anaemics, and the relative mortality from the backcross involving *N* and *Ca*, indicate an overall mortality of mk^{van}/mk^{van} animals of 33% by weaning, with a tendency towards greater mortality in the third week. The trend towards higher mortality in the first 4 weeks is borne out by the more detailed analysis of the backcross data (Table 2). This analysis also shows relative longevity in those mice which survived 4 months, indicating that some mice are less severely affected than others; this suggests that selection from the less affected mice might improve viability. By contrast, in the backcross involving *Hm*, *Tw*, E^{so} and c^h there was no difference in mortality between anaemic and normal animals up to 2 weeks of age, indicating normal viability of mk^{van}/mk^{van} animals in this genetic background. Therefore, both in terms of early postnatal mortality and in response to genetic background *mk/mk* and mk^{van}/mk^{van} animals are very similar.

It is concluded that the viable anaemic mutation derived from the mouse fancy is a second mutant

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allele at the *mk* locus with both sets of homozygotes showing similar – perhaps identical – haematological parameters and viabilities. How similar these two alleles are cannot be determined until they are placed in the same genetic background.

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