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A new allele sash (W^{sh}) at the *W*-locus and a spontaneous recessive lethal in mice

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SUMMARY

A mutation to an apparently new allele at the *W*-locus of the mouse arose spontaneously in a cross between two inbred strains. Heterozygotes have a broad white sash, leading to the name and symbol sash, W^{sh}. Homozygotes are black-eyed whites which are viable, fertile and not anaemic, although the gene does cause mild haematopoietic defects. The original mutant animal also carried a spontaneous recessive lethal mutation on chromosome 5, mapping at 2 cM distal to the *W*-locus.

1. INTRODUCTION

The *W* locus of the mouse has a high spontaneous mutation rate (Schlager & Dickie, 1971) with the result that a large number of mutant alleles are known, which have a varied constellation of effects on pigment formation, haematopoiesis and germ-cell development (Russell, 1979; Geissler, McFarland & Russell, 1981). The most obvious manifestation of the haematopoietic defect is macrocytic anaemia, but in addition animals of some *W*-genotypes show undue radiosensitivity, lack of colony-forming-unit-spleen (CFUs) cells, and lack of mast cells (Russell, 1979). The effects on pigment formation lead to varying degrees of white-spotting, sometimes accompanied by dilution of the remaining pigment in the coat, and the failure of germ-cell development results in sterility of both males and females of certain genotypes. Closely linked to the *W*-locus on chromosome 5 are three other gene loci affecting pigment formation and leading to white spotting, namely the loci of patch, *Ph*, rump-white, *Rw* and recessive spotting, *rs*. Thus, these loci form part of a gene complex or gene cluster, but the details of the organisation and function of this cluster are not yet understood (Geissler *et al.* 1981).

The present paper describes a new allele at the *W*-locus, which arose spontaneously and is apparently different from all previously described alleles. Its properties are of interest for further understanding of the genetics of the gene cluster.

The paper also describes a spontaneous recessive lethal mutation, found in the same animal as the original *W*-mutant. The lethal is closely linked to *W* but is apparently not functionally related to it. The finding of this lethal is of interest since there have been few reports of spontaneous mutations to early acting

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recessive lethals in the mouse. Most known early lethals in the mouse have some visible effect in the heterozygote (McLaren, 1976), and of course the well-known lethals of the *t*-complex exist as polymorphisms rather than new mutations (Bennett, 1975; Sherman & Wudl, 1977).

2. ORIGIN AND GENETICS

A single female with a broad white belt or sash was born among the offspring of a cross between two inbred strains, C3H/HeH × 101/H. When crossed to a normal male from the same F_1 stock she produced 39 white-sashed offspring like herself (Plate 1) and 32 normal offspring. This suggested that the character was

Table 1. *Segregation of* W^{sh} *in various types of crosses*

Cross	Genotype of parents	Offspring		
		$W^{sh}W^{sh}$	$W^{sh}+$	$++$
1	$W^{sh}l/++ \times ++/++$	—	196	186
2	$W^{sh}l/++ \times W^{sh}l/++$	9	542	253
3	$W^{sh}l/W^{sh}+ \times ++/++$	—	285	—
4	$W^{sh}+/++ \times W^{sh}l/++$	33	72	36
5	$W^{sh}+/+l \times W^{sh}+/+l$	94	209	4
6	$W^{sh}+/++ \times W^{sh}+/++$	109	215	95
7	$W^{sh}+/W^{sh}+ \times ++/++$	—	588	—
8	$W^{sh}+/W^{sh}+ \times W^{sh}+/++$	87	110	—
9	$W^{sh}+/W^{sh}+ \times W^{sh}+/W^{sh}+$	67	—	—

inherited as an autosomal dominant and this point was confirmed when outcrosses of sashed offspring of the original female to unrelated normal animals gave a 1:1 ratio of sashed to normal offspring (Table 1, cross 1). Intercrosses of sashed offspring at first did not produce any new phenotype that could be regarded as a possible homozygote and it was thus suspected that the homozygote was lethal.

(i) *Allelism with* W

The gene was next tested for allelism with the three closely linked dominant spotting loci on chromosome 5, each of which has at least one lethal allele, namely patch (*Ph*), rump-white (*Rw*) and the *W*-locus, using the viable allele, W^v. In each of the outcrosses to these three genes a new phenotype appeared, presumed to represent the double heterozygote. In the case of *Ph* and *Rw* the interaction with sash was little more than additive, and much pigment remained, particularly on the head (Plate 2). With W^v, however, there was a markedly above additive interaction, so that the putative double heterozygotes were black-eyed whites (b.e.w.), in some cases having small patches of pigment on the ears. This interaction suggested that the sash gene might be allelic with *W*, and it was given the name and symbol sash, W^{sh}.

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The allelism was confirmed by breeding tests of the putative heterozygotes (Table 2). All the types of double heterozygotes proved to be fertile, and W^{sh} showed close linkage with the three genes *Ph*, *Rw* and W^{v}. No crossovers were obtained but the data were not sufficiently extensive to exclude the possibility of occasional rare crossovers.

Table 2. *Offspring of crosses of* W^{sh} *to chromosome 5 spotting genes*

Cross		Offspring			
		MW^{sh}	$M+$	$+W^{sh}$	$++$
$Ph/+$	$\times W^{sh}l/++$	55	31	44	50
$Rw/+$	$\times W^{sh}l/++$	44	35	33	42
$W^{v}/+$	$\times W^{sh}l/++$	58	48	30	32
$W^{19H}/+$	$\times W^{sh}+/W^{sh}+$	37	—	71	—
$Ph+/+W^{sh}$	$\times ++$	—	105	188	—
$Rw+/+W^{sh}$	$\times ++$	—	123	164	—
$W^{v}+/+W^{sh}$	$\times ++$	—	61	53	—
$W^{19H}+/+W^{sh}$	$\times ++$	—	23	72	—

The genetics of W^{sh} as a *W*-allele was then studied further. Although early data suggested that intercrosses of $W^{sh}/+ \times W^{sh}/+$ yielded only two types of offspring and that the W^{sh}/W^{sh} type was lethal, later results (Table 1, cross 2) showed that occasional black-eyed white animals (b.e.w.) occurred in these crosses. These b.e.w. differed only slightly from the $W^{sh}+/W^{v}+$ animals in that they were more likely to have small patches of pigment on or near the pinnae, and also in some cases had small pigmented patches at the base of the tail (Plate 1). When these b.e.w. were outcrossed all of their offspring (a total of 285) had a typical white sash characteristic of $W^{sh}/+$ (Table 1, cross 3). Some of these offspring were crossed to $W^{sh}/+$ from other matings. Three behaved like the original sash heterozygotes and gave only phenotypically $W^{sh}/+$ and wild-type offspring; the remaining 5 produced a proportion of b.e.w. offspring as well as $W^{sh}/+$ and wild-type in the ratio of 1:2:1 (33 b.e.w.:72 $W^{sh}/+$:36 $++$) (Table 1, cross 4).

The interpretation was that the b.e.w. represented the W^{sh}/W^{sh} homozygote, and that the lethality encountered at first was not due to the W^{sh} gene itself but to a closely linked recessive lethal.

(ii) *Genetics of the lethality*

In order to test this point, attempts were made to map the position of the lethal (symbol *l*) relative to the *W*-locus. The data from the intercrosses, of 9/804 b.e.w., indicated a recombination of *l* with *W* of $1{\cdot}69 \pm 0{\cdot}57\%$. In order to determine whether *l* was proximal or distal to *W* putative $W^{sh}l/++$ heterozygotes were crossed with *go bf/go bf*, where *go* (angora) and *bf* (buff) are two linked markers located 8 cM and 24 cM respectively distal to the *W*-locus. Therefore, if *l* was indeed

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a linked gene 1·7 cM from *W*, the order of loci must be either *l-W-go-bf* or *W-l-go-bf*. Heterozygotes $W^{sh}l++/++go\ bf$ were crossed together. The few b.e.w. animals obtained, which putatively had arisen by crossing-over between W^{sh} and *l*, were tested for the presence of *go* and *bf*. In addition, a sample of animals which had arisen by crossing-over between W^{sh} and *go* were tested to find whether *l* had also exchanged with W^{sh}. A total of 6 b.e.w. were obtained among 460 offspring, (giving a W^{sh}-*l* R.F. of $1{\cdot}98 \pm 0{\cdot}81$ %). One died before testing, 4 proved to carry *go* and *bf*, and of these one was in fact homozygous for *bf* (genotypes $W^{sh}+go\ bf/W^{sh}l++$ and $W^{sh}+go\ bf/W^{sh}l+bf$). This latter animal must have arisen through crossovers occurring in both parents. These results show that loss of the lethal was indeed in most cases accompanied by recombination. The remaining b.e.w. proved not to carry *go* or *bf* (genotype $W^{sh}+++/W^{sh}l++$). Thus, if the order of loci is as shown, i.e. *W-l-go-bf*, the last animal could be explained as the result of a double crossover in the regions *W-l* and *l-go*, while the remaining 4 animals arose through single crossovers in the region *W-l*. Conversely, if the order were *l-W-go-bf* then 4 double crossovers and one single would be required. Hence, the order *W-l-go-bf* is more probable.

This was confirmed by the results of the tests of *W-go* crossovers for the presence of *l*. The genotypes, and the numbers of crossovers required, are shown in Table 3. Again, it is clear that loss of the lethal and crossing-over tend to occur together. The gene order *W-l-go-bf* requires fewer double and triple crossovers than the alternative order *l-W-go-bf*. Hence, the order *W-l-go-bf* is considered to be established.

The question next arose whether W^{sh} and *l* were in any way functionally related. In other words, did *l* form part of the complex locus or gene cluster which includes *W*, *Ph*, *Rw* and the recessive spotting gene *rs*? Three items of evidence bear on this point. First, no phenotypic difference was detected between the effects of $W^{sh}l$ and $W^{sh}+$ i.e. $W^{sh}l/++$ was indistinguishable from $W^{sh}+/++$, and $W^{sh}l/W^{sh}+$ and $W^{sh}+/W^{sh}+$ were indistinguishable. Thus, there is no evidence that *l* affected the expression of W^{sh}. Second, the time of death of homozygous *l*/*l* embryos was different from that of foetuses homozygous for *W*-alleles. Evidence on this came from dissection of pregnant $W^{sh}l/++$ females mated to $W^{sh}l/++$ males (Table 4). In these crosses 34·1 % of implanted embryos were dead at the small mole stage, well above normal levels. No foetuses were found suffering from anaemia, which is typical of *W*/*W* homozygotes, and none with cleft-face, typical of *Ph*/*Ph* homozygotes. The third item of evidence was the demonstration that the *l* gene retained its lethality when separated from W^{sh}. Animals from the $W^{sh}l++/++go\ bf$ linkage crosses were tested for the presence of the lethal by crossing to $W^{sh}l/++$, in order to find animals carrying the gamete $+l++$ (and therefore zygotically $+l++/++go\ bf$). These animals were then crossed to animals of genotype $W^{sh}+go\ bf/++go\ bf$ to give offspring of genotype $W^{sh}+go\ bf/+l++$. It was expected that when these were intercrossed the results would be complementary to those obtained from the original $W^{sh}l/++$ intercrosses, in that the wild-type homozygote would be the rare class. The results (Table 1, cross 5) agreed well with this expectation, the number of wild-type animals being 4/307, giving a

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recombination of 1·97 ± 0·99 %. This is fully consistent with the 9/804 of W^{sh}/W^{sh} homozygotes (giving an R.F. of 1·7 %) found previously in the $W^{sh}l/++$ intercrosses. Thus, the *l* gene exerted its lethal action when separated from W^{sh}.

Table 3. *Genetic tests of recombinant animals from* W^{sh}l++/++go bf *intercross*

Phenotype	Genotype found	No. animals	Crossovers required if gene order: W^{sh}-*l*-*go*-*bf*	Crossovers required if gene order: *l*-W^{sh}-*go*-*bf*
W^{sh}/W^{sh}	$\frac{W^{sh}+\ go\ bf}{W^{sh}l\ +\ +}$	3	1	2
	$\frac{W^{sh}+\ go\ bf}{W^{sh}l\ +\ bf}$	1	1*	2*
	$\frac{W^{sh}+\ +\ +}{W^{sh}l\ +\ +}$	1	2	1
W^{sh} *go* +	$\frac{W^{sh}l\ go+}{+\ +\ go\ bf}$	2	2	2
	$\frac{W^{sh}+\ go\ +}{+\ +\ go\ bf}$	1	2	3
W^{sh} *go bf*	$\frac{W^{sh}l\ go\ bf}{+\ +\ go\ bf}$	5	1	1
	$\frac{W^{sh}+\ go\ bf}{+\ +\ go\ bf}$	1	1	2
+ + +	$\frac{+\ +\ +\ +}{+\ +\ go\ bf}$	3	1	2
	$\frac{+\ l\ +\ +}{+\ +\ go\ bf}$	4	1	2
	Total crossovers			
	Single		17	9
	Double		4	11
	Triple		0	1

* Also one crossover in other parent.

Table 4. *Results of dissections of* W^{sh}l/++ *or* Ph/+ *females pregnant by* W^{sh}l/++ *males*

Female genotype	No. ♀♀	Corpora lutea	Implants	Live Embryos	Small moles	Large moles	Dead embryo
$W^{sh}l/++$	9	96	85	55	29	1	—
Ph/++	3	38	29	28	—	—	1

(iii) *Comparison of* W^{sh} *with other* W*-alleles*

In comparing W^{sh} with other *W*-alleles one needs to consider effects on pigmentation, blood formation and fertility.

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In the heterozygote $W^{sh}/+$ the pattern of distribution of the white spotting is distinctive. The white sash is present in almost all heterozygotes. Among several hundred studied, only one or two have lacked the sash, but have been shown by genetic test to carry the gene. The width of the sash is variable (Plate 1), but in any individual is similar on dorsum and ventrum. In some cases a white head blaze is also present. The tail typically has a white tip but is not wholly white. In the pigmented areas of the coat there was no dilution of coat colour. Geissler *et al.* (1981) have described a number of different spotting patterns in heterozygotes for different *W*-alleles, but none showed the distinctive white sash.

Table 5. *Haematological values of animals of various* W^{sh} *genotypes*

Genotype	No. of animals	Haematocrit (%) mean ± S.E.M.	Mean cell volume (μm^3) (range)
$W^{sh}/+$	29	46·95 ± 0·57	46–59
	10	46·70 ± 0·98	
W^{sh}/W^{sh}	10	41·80 ± 1·05	43–55
	10	44·05 ± 0·47	
W^{sh}/W^{v}	10	41·00 ± 0·49	51–63
	10	41·15 ± 0·99	
W^{sh}/W^{19H}	10	45·20 ± 0·98	42–54
$W^{sh}+/+Ph$	17	44·24 ± 1·31	46–55
	6	46·83 ± 0·79	
$W^{sh}+/+Rw$	3	45·0 ± 0·0	
$++/+Ph$	19	46·32 ± 0·55	
$++/++$	26	46·27 ± 0·37	

The pigmentation pattern of W^{sh}/W^{sh} homozygotes, which are white except for small patches on or near the pinnae, and occasionally at the base of the tail, (Plate 1) is similar to that found in some other *W*-alleles, particularly W^{39} (Geissler *et al.* 1981).

No deleterious effects on fertility were seen in either heterozygotes or homozygotes for W^{sh}. Numerous homozygotes of each sex were mated (Table 1, crosses 7–9) and produced many litters, of normal litter-size. In addition, double heterozygotes with W^{v} (W^{v}/W^{sh}), as already mentioned, were normally fertile (Table 2) although W^{v}/W^{v} homozygotes are sterile. Crosses were also made between W^{sh} and a newly discovered radiation-induced *W*-allele, W^{19H}. This allele resembled the allele *W* in its effect on pigment in the heterozygote (i.e. head blaze and belly spot but no dilution of coat colour), and was lethal when homozygous. Again, W^{19H}/W^{sh} double heterozygotes were fertile (Table 2), but there was a deficiency of $W^{19H}/+$ offspring.

To test for effects on haematopoiesis the packed cell volumes (PCV) of animals of various genotypes were measured (Table 5). In the double heterozygotes with W^{v} the PCV was slightly reduced, in both of two samples of 10 mice. In one of

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these groups the mean cell volume was measured and was slightly increased, indicating that the animals did indeed have a mild macrocytic anaemia. In one group of 10 W^{sh}/W^{sh} mice the PCV was again slightly low, but this was not repeated in a second group, and the mean cell volume was within the normal range. None of the other genotypes showed any difference from normal.

3. DISCUSSION

Geissler *et al.* (1981) have recently described a series of new *W*-alleles. From this it is clear that numerous different alleles exist. The range of effect on pigmentation in the heterozygote varies from almost no effect to almost total lack of pigment. The effects on blood formation and germ cell development vary also; some alleles cause severe anaemia and are lethal when homozygous while others have milder effects, and W^{44} has almost no effect. Similarly, most alleles cause severe impairment of fertility, whereas a few, such as W^{41} and W^{f} (Guenet *et al.* 1979), permit normal fertility. Sash, W^{sh}, appears to represent a new allele not only on the grounds of its distinctive coat pattern in the heterozygote, but also in that it has only minimal effects on both blood formation and germ-cell development, and all previously described alleles have had marked effects on one or other of these characteristics. The question may be asked whether it is correct to class W^{sh} as a *W*-allele. The linkage data indicate that it belongs to the gene cluster on chromosome 5 composed of *W*, *Ph*, *Rw* and *rs*, but are not sufficiently extensive to indicate whether rare crossovers occur. Should W^{sh} perhaps be assigned to one of the other known loci in this cluster, or even to a new locus?

The first point in the evidence for considering it as a *W*-allele is its interaction with the typical *W*-allele W^{v} in coat pigmentation. A second point is the slight interaction with W^{v} in reducing the PCV value, though it should be noted that $W^{v}/+$ heterozygotes do have a slight anaemia, even without the presence of other *W*-alleles. Further evidence of effects on haematopoietic cells has been obtained by Loutit who showed that $W^{sh}W^{sh}$ animals lack mast cells from both skin and intestine (Loutit, Peters & Stevens, 1981), and that CFUs are strongly reduced in $W^{19H}W^{sh}$ (much more than in $W^{19H}+$) and mildly reduced in $W^{sh}W^{sh}$ (Loutit, personal communication). However, the W^{sh} gene did not affect radiosensitivity, either alone in $W^{sh}+$ and $W^{sh}W^{sh}$ animals, or in compound with the alleles W^{e}, W^{v} or W^{19H} (Loutit, Corp & Adams, 1982). In sum, the evidence suggests that W^{sh} does have a mild effect on haematopoietic cells, and thus can validly be regarded as a *W*-allele.

The simultaneous discovery of W^{sh} and the lethal gene *l* is very interesting and unusual. One may speculate as to whether a single mutational event gave rise to both genes. In work with strong mutagens there have been cases in which mutations at two points on the same chromosome were apparently induced by the same event. An example is provided by the X-linked gene bare-patches, *Bpa*, in the mouse, which was apparently induced by the same event as an X-chromosome inversion with breakpoints both at some distance from the *Bpa* locus (Evans &

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Phillips, 1975). However, in this case there is no means of knowing whether the *l* mutation had arisen in the first sash female found, or whether it had been inherited from a previous generation. Recessive lethal genes which arise spontaneously in inbred strains are expected to persist for a few generations before being eliminated by natural selection (Haldane, 1936). Hence, it is possible that the lethal was being carried in the C3H/HeH or 101/H strains used to set up the cross in which the original carrier of W^{sh} was found.

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EXPLANATION OF PLATES

PLATE 1

Examples of dorsal pigmentation patterns of $W^{sh}/+$ (top) and W^{sh}/W^{sh} animals (bottom). Note pigmentation on or near ears in W^{sh}/W^{sh}.

PLATE 2

Typical pigmentation patterns of $W^{sh}+/+Ph$ (A), $W^{sh}+/+Rw$ (B), and W^{sh}/W^{v} (C). Note unpigmented ears of W^{sh}/W^{v}.

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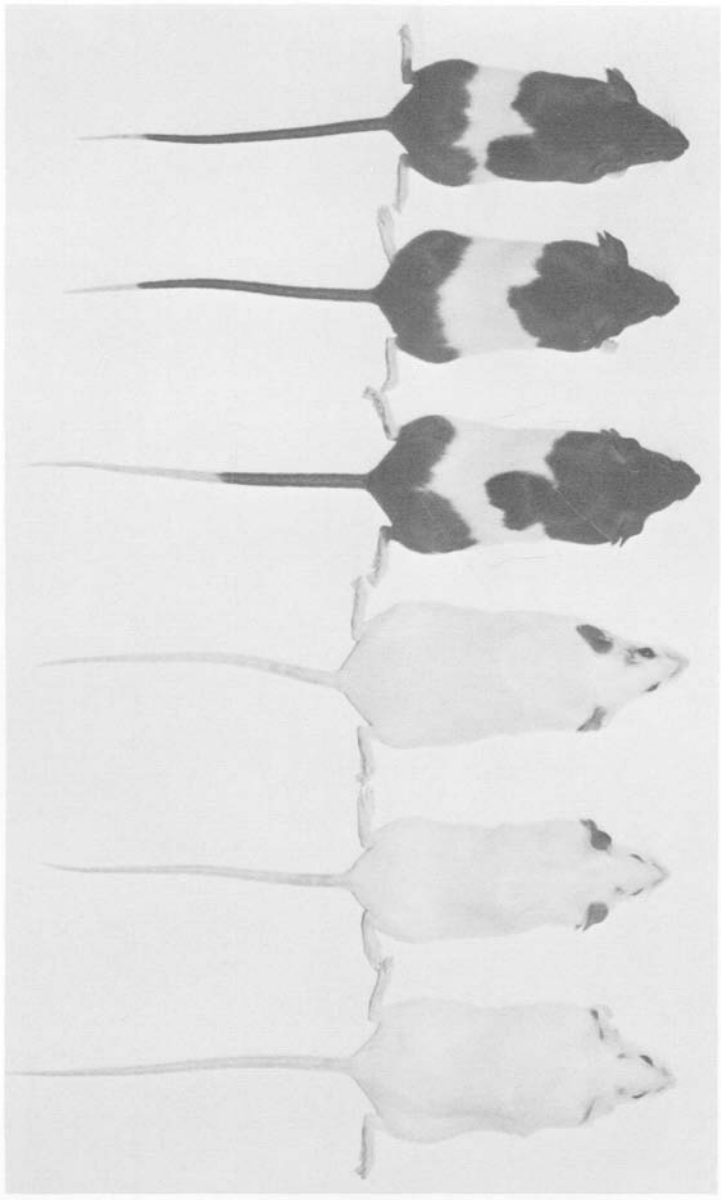

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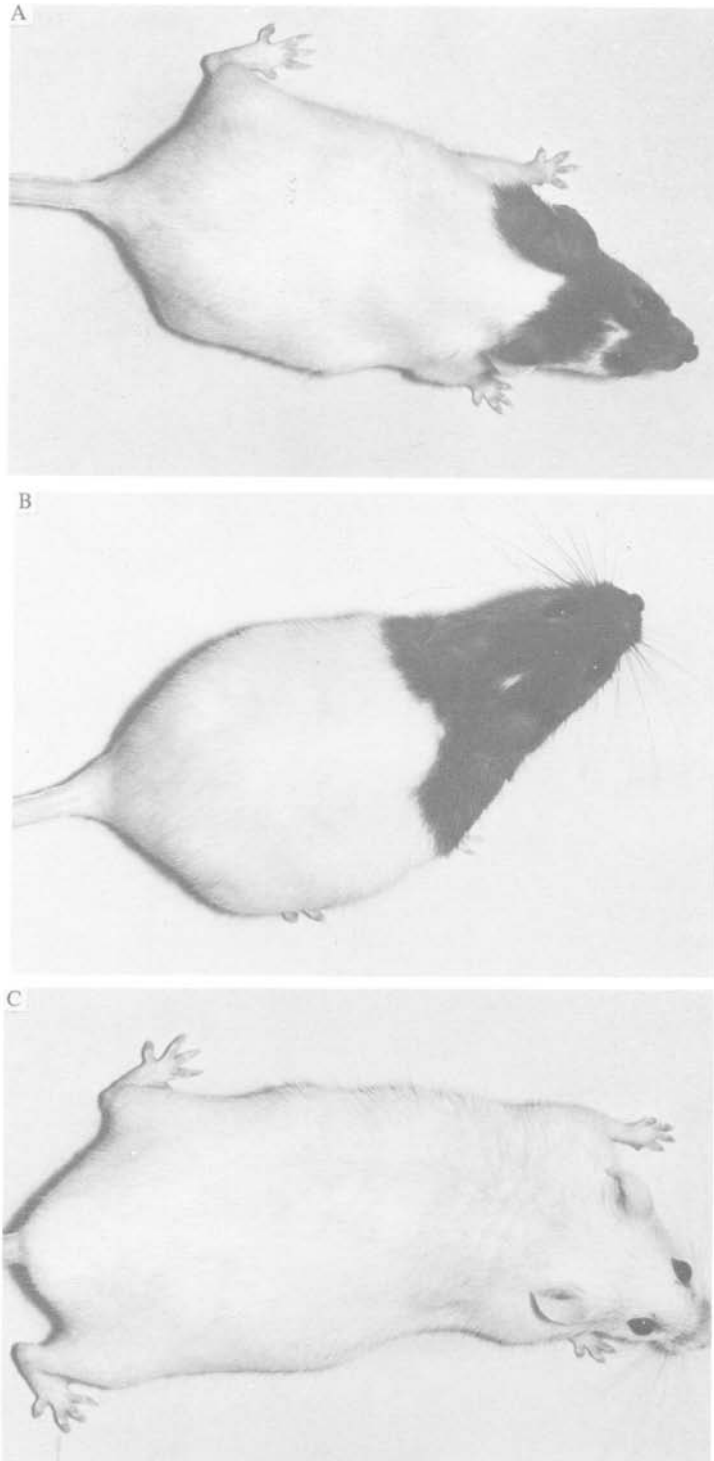

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