INHERITED MACROCYTIC ANAEMIAS IN THE HOUSE MOUSE

II. DOMINANCE RELATIONSHIPS

By HANS GRÜNEBERG

Department of Biometry, University College, London
(at Pathology Laboratory, Mount Vernon Hospital, Northwood, Middlesex)

Introduction

There exist two allelomorphs of 'dominant spotting' in the mouse, the lethal allel W and the viable allel W^V (formerly designated as W^1 and W^2 respectively, see Grüneberg, 1939). Both types of homozygotes and the compound (W/W, W^V/W^V and W/W^V) suffer from a marked macrocytic anaemia, and all three genotypes have white fur with black eyes ('black-eyed whites'*). W/+ and $W^V/+$ heterozygotes in unselected stocks show a variable amount of white spotting (variegation). $W^V/+$ heterozygotes in addition exhibit a general dilution of fur colour, as already noted by the discoverers of this gene, Little & Cloudman (1937); W/+ heterozygotes do not show this effect.

Dunn (1937) has investigated the dominance relations of W as regards variegation. Strains can be selected in which the W/+ heterozygotes are nearly completely white ('all-whites'), while in other selected strains the heterozygotes show no white spotting at all. In the former case, W behaves very nearly as a dominant gene, so far as spotting is concerned, in the latter case as a recessive. The strains, in which the W/+ heterozygotes are 'all-whites', carry a set of specific modifiers (m(W) complex) which, by themselves, have little or no effect on variegation; the strains with 'spotless' W/+ heterozygotes carry the normal allelomorphs of this complex. Whether W behaves as a dominant, incompletely dominant, or recessive gene thus depends on its genetic background of specific modifiers. No data about the dominance relations of spotting in $W^V/+$ heterozygotes have so far been published, but it seems safe to predict an analogous behaviour in that case. The 'pleiotropic' action of the W and W^V genes is set out below. It is relevant to inquire whether

* The 'black-eyed whites' of the fancy are of the constitution W/+; s/s; none of our animals carried the gene s for recessive spotting.

Genotype	Blood	Fur	
		Colour	Spotting
W/W	Severe anaemia	White	—
W/W^{v}	Medium anaemia	White	—
W^{v}/W^{v}	Mild anaemia	White	—
W/+	?	Normal	Variegation } depending
W^{v}/+	?	Diluted	„ } on *m*(W)

this 'pleiotropism' is genuine or spurious (Grüneberg, 1938). It might be suggested that the white fur colour of the first three genotypes is a physiological consequence of the anaemia; the anoxaemia, if already present during the critical interval of pigment determination, might interfere with that mechanism; and if this were so, a similar mechanism might be responsible for the dilute fur colour of W^{v}/+ heterozygotes, or the variegation found in both W/+ and W^{v}/+, or for both phenomena. Moreover, if this were true, some insight might be obtained into the mechanism of action of the genes of the *m*(W) complex. Supposing the variegation of heterozygotes were due to a hitherto undiscovered (presumably mild) anaemia, the *m*(W) genes might control the degree of semi-dominance of W and W^{v} respectively; in that case, 'all-whites' should be more markedly anaemic than heterozygotes with little or no spotting. On the other hand, if the *m*(W) genes control the relative stability of the pigment mechanism, the same degree of anaemia might lead to widely different results in the variegation produced.

A frontal attack on this problem would have to consist of answers to the following two questions. Does an anaemia exist during the critical time in development, and if so, is it sufficient to explain the effect? The latter could only be demonstrated if a comparable artificially produced anoxaemia led to spotting in genetically self animals. Unfortunately, this direct mode of attack is not practicable at present. We therefore have to be content with an indirect and greatly inferior method. An embryonic anaemia, if it exists, may persist into post-embryonic life, when heterozygotes can be distinguished phenotypically from their homozygous normal litter mates. Clearly an objection can be raised whatever the outcome of this inquiry. If no anaemia is found in the heterozygotes, the existence of one at the critical embryonic period is not thereby excluded; on the other hand, if an anaemia is discovered, it is not thereby proved that it was already present at the critical moment, nor, if it was, whether it was sufficient to produce the effect to be explained. Both these objections will detract from the conclusiveness of the interpretation.

As discussed in detail elsewhere (Grüneberg, 1942), it is still doubtful whether W and W^{v} in heterozygous condition have an effect on the

blood. Hagedoorn (1935), on very slender evidence, asserted that W/+ heterozygotes are mildly anaemic. Grüneberg (1939) failed to observe signs of semi-dominance of W^V, but his experiments were not designed to detect such a difference unless very obvious. The evidence in either case was slight and, as will be shown below, misleading.

MATERIAL AND METHODS

(1) A strain of W mice was obtained from a dealer, in which the W/+ heterozygotes were nearly completely white; in but few individuals was as much as 10% of the dorsal surface pigmented. The +/+ animals were solidly coloured black non-agoutis; feet and belly never showed any white, but a white tail-tip, and a very small blaze on the forehead (usually only a few hairs) were regularly present; this very slight indication of spotting in +/+ is presumably unconnected with the *m*(W) complex and due to a minor spotting gene in its own right. The W/W homozygotes were, of course, lethal anaemics.

(2) W/+ 'all-whites' of the strain mentioned above were outcrossed to the black agouti self pure line Strong *CBA*. The W/+ heterozygotes thus produced in F_1 were very uniform as to spotting; they showed a large blaze on the forehead, which in extreme cases covered nearly 10% of the dorsal surface, but no dorsal variegation elsewhere; all animals had a large white belly spot, which often covered more than one-half of the ventral surface; and all showed some white on feet and tail. Taken as a whole, these outcross W/+ heterozygotes were pigmented with a little white, while the heterozygotes mentioned under (1) were white with a little interspersed pigmentation. The +/+ mice in this outcross showed no sign of spotting.

(3) The W^V/+ heterozygotes used were later derivatives of a cross of a black silver W^V/+ male to Strong *CBA* females described previously (Grüneberg, 1939). They all showed a noticeable dilution of the fur colour, both on agouti and non-agouti background. Little & Cloudman (1937) ascribed this dilution to an interaction of W^V/+ with the gene for silver, either in homozygous or in heterozygous condition. I have never seen a W^V/+ mouse without the fur colour dilution amongst several hundred F_2, F_3 and F_4 animals, and am thus inclined to regard this dilution as a direct effect of W^V unconnected with the silver gene. Whatever explanation may be true, the diluting effect is peculiar to W^V and absent in W. The amount of variegation in my W^V/+ heterozygotes was very variable. In some cases there was no spotting at all, except a little white on feet and tail tip. On the other extreme of the scale

were animals with large belly spots, with a good deal of dorsal variegation in the region of the 'belt' and shoulders, and with a large blaze on the forehead. The +/+ homozygotes were entirely free from spotting.

Haematological measurements were carried out on 13–14 days old individuals from each of the three stocks mentioned. Equal numbers of heterozygotes and +/+, so far as possible of the same sex, were used from each litter sampled. Two further series of measurements were carried out with 9–10 days old animals and with adult individuals of stock (3), both sets including a few W^v/W^v homozygotes. Haemoglobin (Hb) estimations, red cell counts, and estimations of the volume of packed cells (haematocrit, Ht) were made in each case with the technique described recently (Grüneberg, 1941); the only difference was that in the majority of cases, Hb and Ht were determined from independent samples. From the three estimates, the mean Hb content (M.Hb.C.) of the cells in $\gamma\gamma$, the mean cell volume (M.C.V.) in μ^3, and the mean corpuscular Hb concentration (M.C.Hb.C.) in per cent. were calculated. This report is based on the blood pictures of 141 mice.

Results

The data on the blood pictures of W/+ heterozygotes and their +/+ litter mates (13–14 days old) are summarized in Table 1. Exp. A refers to the strain with 'all-white' heterozygotes, Exp. B to the outcross to the *CBA* strain. It is obvious that in neither case is there the slightest

Table 1

	n	Genotype	Weight g.	Hb g./100 c.c.	Erythrocytes per cu.mm.	Ht %	M.Hb.C. $\gamma\gamma$	M.C.V. μ^3	M.C.Hb.C. %
A	10 (4)	+/+	6·62	9·55	5,656,500	33·75	16·9	59·7	28·4
	10 (4)	W/+	6·42	9·70	5,763,500	34·50	16·9	59·9	28·2
B	10 (3)	+/+	5·07	10·90	6,081,000	37·05	18·0	61·1	29·5
	10 (3)	W/+	5·24	11·34	6,467,500	38·65	17·5	59·8	29·3

Note. '10 (4)' stands for '10 mice from 4 litters'.

suspicion of anaemia in the heterozygotes. On the contrary, in both experiments the W/+ heterozygotes are doing a little better than their +/+ litter mates in each of the three original measurements (Hb, cell count, and Ht), though this difference is scarcely significant. The decisive criterion is a comparison of the mean cell volumes. It has been shown previously (1939) that the pathological feature of the **W** anaemias is an increased cell volume, as in pernicious anaemia in man; it might thus be expected that, even though the cell count and Hb are not reduced, the mean cell volume would be increased. There is not the

slightest sign of such an effect. We conclude that at the age of 13–14 days, there is no indication of incomplete dominance of **W** as regards the blood, neither in 'all-whites', nor in heterozygotes with a low degree of variegation.

Both genotypes in Exp. B have significantly higher cell counts and Hb and Ht values than those in Exp. A. The reason for this difference is not clear. An environmental difference is unlikely, as both sets of observations were made simultaneously. The figures suggest that there might be some relation between blood pictures and mean weight; however, in neither set of data is there a correlation between body weight and blood count. Possibly differences in the genetic background may be responsible.

One would expect that what is true for the severe **W** anaemia should a fortiori be true for the mild $\mathbf{W^v}$ anaemia. However, this is by no means the case. The following Tables 2–4 present strong evidence that $\mathbf{W^v}$ is

Table 2

	n	Genotype	Weight g.	Hb g./100 c.c.	Erythrocytes per cu.mm.	Ht %	M.Hb.C. $\gamma\gamma$	M.C.V. μ^3	M.C.Hb.C. %
A	19 (10)	+/+	5·40	10·46	5,717,000	37·66	18·4	66·3	27·8
	21 (10)	$\mathbf{W^v}$/+	5·26	9·93	4,827,000	35·43	20·7	73·9	28·1
	6 (4)	$\mathbf{W^v/W^v}$	—	7·64	2,938,000	28·58	26·1	97·7	26·7
B	12 (7)	+/+	6·96	9·86	6,432,000	35·71	15·3	55·5	27·6
	12 (7)	$\mathbf{W^v}$/+	6·88	10·07	5,746,000	36·88	17·3	64·9	26·8
C	12 (6)	+/+	—	14·43	10,266,000	55·13	14·1	51·8	27·2
	12 (6)	$\mathbf{W^v}$/+	—	13·64	9,249,000	49·92	14·8	54·0	27·3
	7 (6)	$\mathbf{W^v/W^v}$	—	11·57	6,291,000	42·36	18·4	67·3	27·3

The animals in group A are 9–10, those in group B 13–14 days old; the six litters in group C were 80, 151, 153, 182, 238–9, and 256 days old respectively. The weights in group A are based on 10 individuals from four litters only.

Table 3. *Values of* $\mathbf{W^v}$/+ *heterozygotes divided by those of their* +/+ *sibs*

Group	Hb g./100 c.c.	Erythrocytes per cu.mm.	Ht %	M.Hb.C. $\gamma\gamma$	M.C.V. μ^3	M.C.Hb.C. %
A	0·95	0·84	0·94	1·12	1·12	1·01
B	1·02	0·89	1·03	1·13	1·17	0·97
C	0·95	0·90	0·94	1·05	1·04	1·00

semi-dominant in its effects on the blood, both in young and in fully adult animals. Turning first to Table 2, it will be seen that in each of the three age groups, the cell count of the $\mathbf{W^v}$/+ heterozygotes is markedly below that of their +/+ litter mates. On the other hand, Hb and Ht values are much less reduced; in the 13–14-days-old group (B of Table 2), they are indeed slightly higher than in the homozygous normals. As seen in Table 3, increases or decreases in Hb are accom-

panied by equally large increases or decreases of the Ht values. Hence the mean corpuscular Hb concentration of the heterozygotes does not differ from that of homozygous normals. This was to be expected, as that parameter is not affected in W/W and W^v/W^v homozygotes. As the cell count is much more reduced than the Hb and Ht values, the cells of the $W^v/+$ heterozygotes are on an average larger (macrocytic) than those of their +/+ sibs. The increased cell volume more or less compensates for the decreased cell number, and hence the Hb values are little, if at all, below normal. Taking the Hb value as a clinical criterion for an 'anaemia', we may say that $W^v/+$ heterozygotes are macrocytic, without being appreciably anaemic. As shown previously (1939) and again in Table 2, the W^v/W^v homozygotes are both macrocytic and anaemic; the increased cell size here does not compensate for reduced cell number any more, so that their Hb values are definitely below normal.

It remains to test the significance of the differences reported above. As the absolute values in the three age groups differ, three separate tests are necessary. Within each age group, litter mates show a correlation of their values; thus, whenever more than one pair of animals has been examined from a given litter, average values of two or more pairs have been compared; hence the number of degrees of freedom (D.F.) in each age group is one less than the number of litters sampled, that is 9, 6, and 6 respectively; in the latter case two pairs of males have been treated separately from two pairs of females examined on the subsequent day.

Table 4. *Student's t-test applied to the differences between* $W^v/+$ *and* +/+

Group	D.F.		Hb	Erythrocytes	Ht	M.Hb.C.	M.C.V.
A	9	*t*	3·543	5·882	2·446	5·111	5·187
		P	0·0064	0·0002	0·0462	0·0003	0·0003
B	6	*t*	1·090	3·687	1·556	4·417	7·922
		P	0·3176	0·0102	0·1711	0·0023	0·00011
C	6	*t*	4·477	5·520	4·367	2·197	1·458
		P	0·0043	0·0016	0·0046	0·0353	0·0978

In Table 4 are given the *t*-values testing the significance of the differences between $W^v/+$ and +/+; we have disregarded the mean corpuscular Hb concentration, as the differences between the means are obviously insignificant; it has also been deemed unnecessary to test the differences between W^v/W^v and $W^v/+$ or +/+, as these differences are significant beyond any reasonable doubt. The *P* values in the first three columns of Table 4 refer to both tails of the distribution; hence they give the probability of obtaining so bad or worse a fit between two sets of data,

regardless of whether the differences lie in the expected direction or not. In the case of the mean cell volume and the mean Hb content the P values refer to one tail of the distribution only, as the deviations are in the expected direction. This is not legitimate in the first three columns, as an expected increase in the size of the cells may or may not be associated with a reduction of the number of cells and/or Hb or Ht.

It will be seen that in young animals (A and B) both mean cell volume and mean Hb content of the cells of $W^v/+$ heterozygotes are increased, the differences being highly significant. The tendency clearly persists into adult life, though in the case of the mean cell volume it does not reach the conventional level of significance. The cell count is significantly decreased throughout, and the same applies to Hb and Ht in the first and last group; the slight increase in these values in group B is clearly not significant; it is probably accidental and spuriously inflates the increase in the mean cell volume and Hb content of the cells in that group. It is obvious that while the estimations of Hb, cell count, and Ht are independent of each other, those of the cellular characteristics depend on two of these basic estimates in each case.

DISCUSSION

It has been shown that, contrary to Hagedoorn's (1935) assertion, W has no measurable effect on the blood of heterozygotes; and that, contrary to Grüneberg's (1939) statement, W^v has such an effect. We have established these facts in the hope that they might throw some light on the question whether the manifold effects of W and W^v represent a case of genuine or spurious pleiotropism. It has already been pointed out in the introduction that the relatively inefficient approach, which we were forced to adopt, is open to two criticisms. The absence of semi-dominance in W at the age of a fortnight does not prove its absence during the critical period in embryonic life; and the presence of semi-dominance in W^v from the 9th day onwards does not prove that it was already present at the critical moment, nor, if it was, whether it would have been sufficient to produce the effect which we want to explain. These objections, though doubtless valid, are not helpful in evaluating our data. It seems sound at present not to go beyond the observed facts, and to disregard the 'might-have-beens', until their relevance has been demonstrated by observation. We shall therefore ask the following question: Supposing the findings in post-embryonic life to be representative of the conditions obtaining during the critical period in develop-

ment, what light do they shed on the causal relationship of the various pleiotropic effects of the genes **W** and $\mathbf{W^v}$?

(1) As **W**/+ heterozygotes, whether nearly white or nearly completely pigmented, have normal blood pictures, there is no foundation in observed facts for the assumption that variegation of the fur is physiologically conditioned by an anaemia. It is logical to extend this conclusion to the variegation of $\mathbf{W^v}$/+ heterozygotes.

(2) $\mathbf{W^v}$/+ heterozygotes differ from **W**/+ heterozygotes by a general dilution of their fur colour (as distinct from variegation), and by the abnormalities of their blood pictures described in this paper. It seems reasonable to suggest a causal relationship between these phenomena, it being clearly understood that the data at hand do not by any means prove it.

(3) Assuming that dilution of fur colour and blood changes in $\mathbf{W^v}$/+ heterozygotes are causally connected, it is difficult to see how the blood changes could be directly responsible for the changes in fur colour. The general and direct effects of an anaemia are due to the reduced oxygen carrying capacity of the blood (anoxaemia). These direct and unspecific effects of an anaemia are thus inversely proportional to the Hb content of the blood. However, the semi-dominance of $\mathbf{W^v}$ in heterozygotes does not lead to an appreciable reduction of the Hb content of the blood, and the distributions overlap broadly or completely those of +/+ mice. Under these circumstances the blood changes cannot very well be the direct cause for the dilution of the fur pigments by dint of an anoxaemia, unless we make the assumption, for which there is no foundation in observed facts, that at the critical period a much severer anaemia obtains. Even then, the relationship would be doubtful. Flexed-tailed mice (f/f), though they often have some ventral spotting, show no dilution of fur colour, although their anaemia is severe and probably starts before that of **W**/**W** anaemics.

The result is indeed meagre, so far as the pleiotropic mechanism of **W** and $\mathbf{W^v}$ is concerned. There is no evidence for causal relationship between blood changes and variegation in either case; a relationship between blood changes and fur colour dilution in $\mathbf{W^v}$, though suggested by the data, is not of a kind readily understandable. But, as pointed out on previous occasions, failure to demonstrate the spurious nature of pleiotropism constitutes no proof that it is genuine. There is at present no criterion how genuine pleiotropism can be recognized.

SUMMARY

The gene **W** in the mouse has no measurable effect on the blood in heterozygotes, regardless of the amount of variegation present. Mice heterozygous for $\mathbf{W^v}$ have macrocytic red blood corpuscles without being appreciably anaemic. It is suggested that this effect may somehow be causally connected with the general dilution of fur colour found in these heterozygotes, though the nature of this relation remains obscure. There is no evidence that the variegation of $\mathbf{W}/+$ or $\mathbf{W^v}/+$ is caused by an anaemia.

REFERENCES

DUNN, L. C. (1937). Studies in spotting patterns. II. Genetic analysis of variegated spotting in the house mouse. *Genetics*, **22**, 43–64.

GRÜNEBERG, H. (1938). An analysis of the 'pleiotropic' effects of a new lethal mutation in the rat (*Mus norvegicus*). *Proc. roy. Soc.* B, **125**, 123–44.

—— (1939). Inherited macrocytic anaemias in the house mouse. *Genetics*, **24**, 777–810.

—— (1941). The anaemia of flexed-tailed mice (*Mus musculus* L.). I. Static and dynamic haematology. *J. Genet.* **43**, 45.

—— (1942). *The Genetics of the Mouse*. Cambridge University Press (in the Press).

HAGEDOORN, A. L. (1935). Ueber Dominanz bei der Hausmaus. *Z. indukt. Abstamm. u. VererbLehre*, **70**, 509–12.

LITTLE, C. C. & CLOUDMAN, A. M. (1937). The occurrence of a dominant spotting mutation in the house mouse. *Proc. nat. Acad. Sci., Wash.*, **23**, 535–7.