ANALYSIS OF PLEIOTROPISM AT THE W-LOCUS IN THE MOUSE: RELATIONSHIP BETWEEN THE EFFECTS OF W AND W^v SUBSTITUTION ON HAIR PIGMENTATION AND ON ERYTHROCYTES[1]

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THE dominant-spotting genes of the mouse, *W*, W^v, and *w*, have been studied extensively by DEABERLE (1925, 1927), GOWEN and GAY (1932), LITTLE and CLOUDMAN (1937), GRÜNEBERG (1939, 1942a), and FEKETE, LITTLE and CLOUDMAN (1941). They are especially noteworthy in that the six possible genotypes differ in a regular manner in a number of apparently independent characteristics: size and number of erythrocytes, development and functioning of gonads, and intensity and location of hair pigment (table 1), as well as in such probably secondary characteristics as viability. Attempts to trace these separate effects back to a common embryological origin should give

TABLE 1

General description of genic effects at the W, W^v, w locus in the mouse.

GENOTYPE	ERYTHROCYTES		VIABILITY	GONAD DEVELOPMENT	PIGMENT	
	NUMBER	SIZE			INTENSITY	SPOTTING
ww	normal	normal	normal	normal	full	none
Ww	normal	normal	normal	normal	full	belly spot
WW	extreme reduction	macrocytic	90% die 0–7 days without postnatal development	deficient	white	?
W^vw	slight reduction	slightly macrocytic	normal	normal	diluted	belly spot
W^vW^v	considerable reduction	macrocytic	normal	deficient	white	?
WW^v	greater reduction	macrocytic	53% die 0–21 days, others fairly normal	deficient	white	?

valuable information on the paths of gene action, nature of causes of congenital macrocytic anemia, and nature of genic effects on gonad development and pigment intensity and location. Analysis is greatly aided by the existence of two dominant deleterious alleles, *W* and W^v, which make it possible to compare the effects of six rather than the usual three levels of gene "dosage."

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Even before embryological studies are undertaken, evidence as to the closeness of relation between two end-effects of the genes can be garnered from comparisons of the order and magnitude of effect of the six "dosage" levels upon the end-product in question. It is such evidence for pigment intensity and erythrocyte count which is presented in this paper. The blood picture will be presented more fully elsewhere, and only those counts and measurements pertinent to these studies included here. However, this is intended as a full presentation of all data on intensity of pigmentation available purely from observation of completed hairs.

The concept of pleiotropism, or multiple effects of single genic substitutions, is, of course, of great interest to the student of gene action. One aspect of the problem has been described recently by GRÜNEBERG (1938a, b, 1943a): the distinction between "genuine" pleiotropism, where the gene involved has more than one primary effect ("doing two things directly by means of different mechanisms"), and "spurious" pleiotropism, where there is "unity of gene action," the gene having a single primary action with two effects traceable to a common cause or with one effect subordinated to the other. He has presented these possibilities clearly in diagram form (1943a). As he correctly points out, "the existence of genuine pleiotropism has never been conclusively demonstrated, while the existence of spurious pleiotropism is beyond doubt." He himself has demonstrated clearly by extremely able analysis (1938b, 1943a) the great contribution studies of "pedigree of causes" can make to our knowledge of *paths* of gene action.

For the purpose of this paper, however, the question of distinction between "genuine" and "spurious" pleiotropism seems less pertinent than certain other considerations, and it seems advisable to present another classification of types of pleiotropism (figure 1). Of these possibilities, A2 and D correspond exactly with GRÜNEBERG'S classification of "genuine" pleiotropism. In all other classes there is a single primary gene action in the strictest sense that only one original gene product need be assumed to enter and act in the cytoplasm. The chief difference between the present chart and GRÜNEBERG'S earlier one (1943a) is the introduction of the possibility that "unity of gene action" could exist without "tissue or cell specificity." It is logically possible, as listed in C of figure 1, that the same original gene product could by its intrinsic nature be active in two types of cells, long separated in development and different in structure. The gene in question might either act on the same substrate which has been independently produced in two different localities, or, perhaps more probably, it might be general enough in its action to affect two substrates. Certain oxidative enzymes might be examples of the second possibility. Where two types as different in origin and function as erythrocytes and matrix cells of the hair-follicle are involved it seems this category of pleiotropism must be considered. In one sense there is unity of gene action, since the gene releases only one product. In another sense there is real pleiotropism, as this one gene product is "doing two things directly by means of different mechanisms."

Brief examination of table 1 shows that there is parallelism in the effect of genes of the *W*-series on erythrocytes and on pigmentation to the extent that

the three most anemic genotypes (WW, W^vW^v, and WW^v) are all completely lacking in pigment in the hair, while the three types with higher blood counts are all pigmented. It is futile, however, to study these three white extreme anemic genotypes further for correlation between the two characters, as there is no variability in pigmentation. The three pigmented genotypes are more promising. The normal homozygote, ww, has of course, full color intensity and

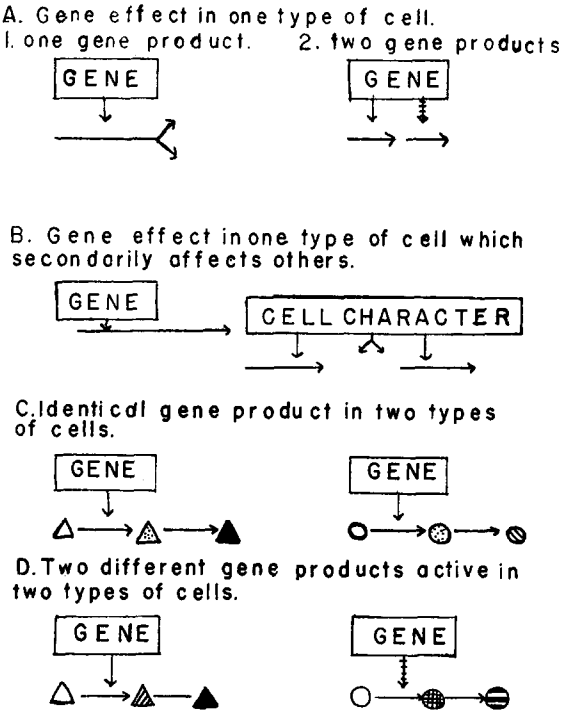

FIGURE 1.—Theoretically possible types of pleiotropism

normal blood picture. GRÜNEBERG, however, after having first described both heterozygotes as normal in blood picture (1939), changed his view and later (1942a) concluded that although the blood picture was completely normal in ww and Ww, the W^vw genotype had a lower number of erythrocytes, and a larger mean cell volume, indicating that W^v was partially dominant in its effect on blood, while W was completely recessive. *A priori* this seems improbable, since the effect of two doses of WW is more extreme than that of two W^vW^vs. However, preliminary experiments at that time (1943) by the author on heterogeneous material showed that in each of the five stocks tested, in litters segregating for W^vw and ww there were significantly fewer erythrocytes in the W^vw littermates, as previously indicated by GRÜNEBERG. As shown in table 1, these three pigmented genotypes also are distinguishable by their pigmentation. Both types of heterozygote always differ from the normal homozygote by the presence of a white belly spot. However, black or full-

color agouti animals of the *Ww* and *ww* genotypes are indistinguishable from the dorsal surface, and the pigment on the colored portion of the ventral surface is usually though not universally of full intensity in the *Ww* heterozygote. Black $W^v w$ heterozygotes show quite marked reduction in dorsal pigmentation, and invariably even greater reduction on the pigmented portion of the belly. Thus W^v appears partially dominant in a second type of effect where *W* is completely recessive. This apparent parallelism in dominance relations of the two alleles on pigment intensity and erythrocyte number suggested that careful quantitative evaluation of both characters might be helpful in analyzing the nature of the pleiotropism. These stimulating possibilities have led to two types of experiments. First, breeding experiments were undertaken to place both dominant genes on the same uniform genetic background so that differences observed with genic substitutions could definitely be attributed to those genes and not to modifiers which might differ between stocks. It was felt that this step was particularly essential for the comparisons of erythrocyte number and size. Second, in order to obtain a more thorough understanding of the W^v effect on pigmentation intensity, crosses were made leading to litters segregating for $W^v w$ and *ww* on five different colored backgrounds, involving three qualitatively different pigments (black-fuscous, brown, and yellow), and two other types of pigment reduction (pink-eyed pale sepia, C-series) (RUSSELL 1946). It was hoped that with the existing knowledge of the physiology of pigmentation, histological studies of these particular stocks would test the type of pigment reduction involved, and that accurate counts and measurements of the pigment granules in segregating littermates in each stock would lead to further conclusions as to the basic nature of the gene actions involved.

In the first type of experiment mentioned above, the two dominants *W* and W^v were introduced into the Jax C57 black strain. The animals used in the erythrocyte counts recorded here were from the second and third back-cross generations, so within each littermate comparison, 75 to 88 percent of the genes other than *W*-alleles should be identical. The exact nature of the residual heterozygosity may differ from litter to litter. Determinations are recorded on seven litters segregating for $W^v w$-*ww* (table 2) and on eight segregating for *Ww*-*ww* (table 3). Two erythrocyte counts (number per cu mm) and two haematocrit readings (proportion of cell volume to total blood volume) were made for each animal, using blood obtained from the tail (heated) of non-anaesthetized individuals between four and five weeks of age. The erythrocyte counts were made with a Levy haemacytometer and regulation red-counting pipettes, and the accuracy of sampling can be judged from the mean difference between the two samples taken from each animal, 0.43×10^6 for the 50 animals involved in this experiment. The haematocrit readings were made by diluting the blood with 1.3 percent sodium oxalate in Van Allen haematocrit tubes, and centrifuging in a clinical centrifuge at 2,700 rpm for one-half hour. As pointed out by PONDER (1944) the values obtained for proportional blood volume vary somewhat with the conditions of centrifugation and dilution oxalate in particular differing from other diluents. Thus the mean cell volumes obtained in this study may not be strictly comparable to those obtained by

other workers (GRÜNEBERG 1939, 1942a), but they should give satisfactory comparison within the current experiment. The personal accuracy may be judged from a mean difference of 1.2 percent between the two samples taken from each of the 50 animals used here. In as far as possible, two animals of each genotype were taken from each litter, and the values given in tables 2 and 3 are the means of the two samples from each individual.

Using means of litters as a basis, paired comparisons were made for each type of measurement of W^vw and *ww* segregants. In each, there was a signifi-

TABLE 2

Erythrocyte counts, haematocrit readings, and determinations of mean cell volume of 4–5 week old animals in litters segregating for W^vw and ww.

LITTER	rbc×10⁶	*ww* haem. %	M.C.V., mu³	rbc×10⁶	W^vw haem. %	M.C.V., mu³
1	8.55	40.8	47.7	7.32	43.3	59.2
1	8.72	41.3	47.4	6.48	39.0	60.2
2	10.68	44.8	42.0	7.87	39.0	49.6
2		47.0	44.0	9.08	42.5	46.8
3	9.26	41.5	44.8	6.41	38.8	60.5
3	7.89	39.8	50.4	8.85	36.5	41.2
4	9.39	36.0	38.3	6.95	31.5	45.3
4	8.87	36.5	41.1	8.08	—	—
5	9.30	45.3	48.7	7.68	43.8	57.0
6	7.63	40.5	53.1	7.05	36.0	51.7
7	7.93	38.5	48.5	6.67	37.0	55.5
7	7.24	40.0	55.2	6.72	41.0	61.0
mean	8.72	41.0	46.8	7.43	38.9	53.5

cant difference between the genotypes (erythrocyte counts, *ww* higher than W^vw, haematocrit reading, *ww* higher than W^vw, mean cell volume, W^vw (macrocytic) higher than *ww*, probability of chance deviation less than 0.01 in each case). The same three types of paired comparisons were made of *Ww* and *ww* segregating littermates, but in these no significant differences were found (probability of chance difference in erythrocyte number, 0.5, of chance difference in haematocrit percentage, 0.3, of chance difference in mean cell volume, 0.3). Thus the observation that W^v is partially dominant in its effect on blood has been confirmed, using material well on its way toward homogeneity. With similar material, *W* has been shown to have no dominance. One fact which has been added by this study is that in this material, at least, in spite of the definite macrocytosis of W^vw heterozygotes, there is still sufficient reduction in cell number to result in a significant decrease in total cell volume.

Thus the first type of experiment, although still not completely finished, has demonstrated rather clearly the difference in dominance relations of the two alleles in their effect on the flowing blood. Further studies on more isogenic material will be needed to measure the exact degree of W^v dominance.

Investigations on the second problem, the nature of the effects of W^v substitution upon a variety of genetically different color types, and the question of existence of an effect of W substitution in pigment intensity, have been carried out, using five different color backgrounds for the W^v substitutions, only one for W substitution. No attempt was made here to develop isogenic stocks, as in general the major color genes are extremely regular in their manifestation.

Samples of hair were taken from the rump of eight four to five week old animals of each of the following genotypes: *aaBBCCDDPPW^{v}w; aaBBc^{ch}c^{ch}DDPPW^{v}w; aabbCCDDPPW^{v}w; aaBBCCDDppW^{v}w; A^{v}aBBCCDDPPW^{v}w-A^{v}aBBCCDDPPww;* and *aaBBCCDDPPWw-aaBBCCDDPPww.*

TABLE 3

Erythrocyte counts, haematocrit readings, and determinations of mean cell volume of 4-5 week-old animals segregating for Ww and ww.

LITTER	rbc×10^6	*ww* Haem. %	M.C.V., mu^3	rbc×10^6	*Ww* haem. %	M.C.V. mu^3
1	9.93	41.0	41.3	7.89	40.3	51.0
2	8.67	40.3	46.5	8.84	47.5	48.3
2	9.75	43.3	46.4	10.39	44.5	42.9
3	8.21	38.0	46.3	7.74	41.5	53.6
4	7.76	46.0	59.3	10.36	44.8	43.2
4	8.15	40.5	49.9	9.58	40.5	42.3
5	7.85	35.5	45.3	8.30	39.0	47.0
5	7.95	42.0	52.8	8.65	38.8	44.8
6	8.05	40.8	50.6	10.64	51.0	47.9
6	7.85	37.0	47.2	8.75	42.0	48.0
7	8.08	41.0	50.6	8.47	39.0	46.1
7	9.55	49.3	52.1	8.86	46.5	54.1
8	7.50	40.3	53.7	8.02	37.8	47.1
mean	8.41	41.2	49.2	9.04	42.6	47.4

In the first four genotypes, the differences from the corresponding *ww* genotypes were so great that it was felt no error in interpretation would result from comparing the present values with the means of corresponding *ww* types taken from unrelated animals described in the general paper of RUSSELL (1946). In the case of the last four genotypes, bracketed together in pairs, the differences between *W^{v}w* and *ww* pairs of genotypes were not obvious, and for that reason samples were taken from litters segregating for these genes to reduce extraneous variability. Cross sections and whole mounts were made of the hair samples (full grown hairs of the first pelage) by the standard methods of RUSSELL (1946). Counts of granules in a unit volume of cortex and in a single medullary cell were made at a series of levels from tip to base of the hair (4th, 10th, 20th, 30th, 40th, and 50th microscopic fields, at 1800×). The mean values at each level for each genotype are given in table 4, combined, for the first four geno-

types, with the earlier published means of corresponding *ww* genotypes. In figure 2 histograms are given comparing the mean values for $W^v w$ and *ww* genotypic pairs by superimposing them on the same diagram. The values for *ww* are connected by a solid line, those for $W^v w$ by a broken line. It will be noticed that the medullary numbers in particular are always lower in the $W^v w$

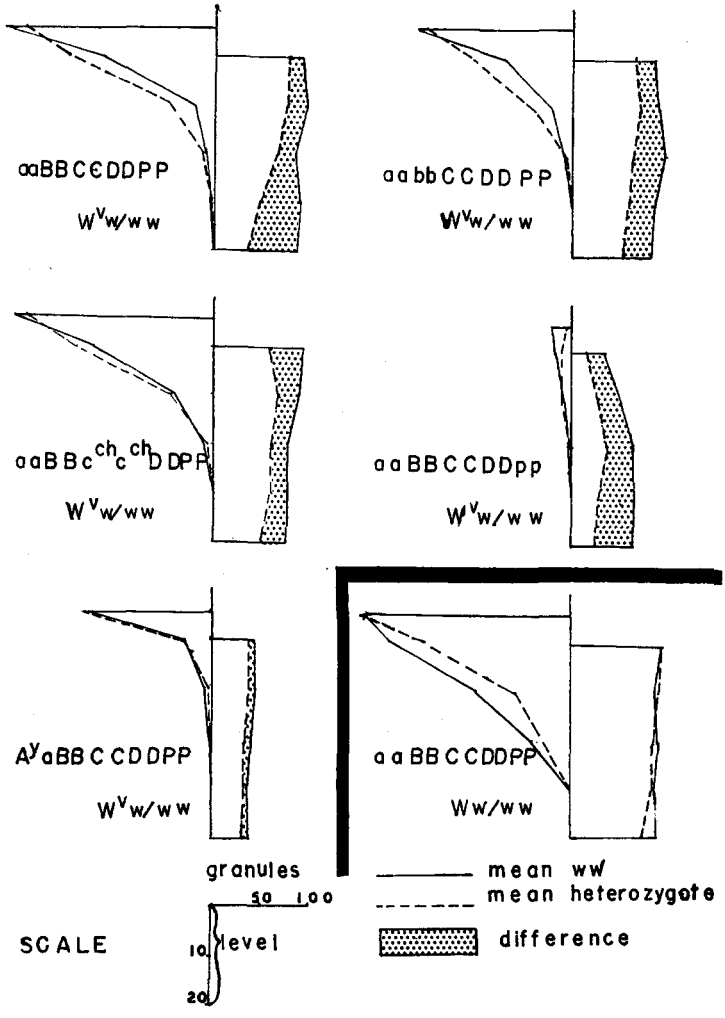

FIGURE 2.—Comparisons of numbers of pigment granules in the medulla and cortex in pairs of genotypes segregating for $W^v w/ww$ or Ww/ww. Each histogram compares number of granules at various levels in pairs of genotypes differing by the indicated substitution. Values to the left of vertical axis are for cortical number per unit volume, to the right, mean number of granules per medullary cell.

member of a pair, and the shaded area in each histogram represents the amount of reduction in medullary granule number with $W^v w$-*ww* substitution in that genetic background. It is clear that the degree of reduction, as suggested above is less in the yellow stock than in the first four $W^v w$-*ww* stocks. The sixth histo-

gram in figure 2, and the last column in table 4, show a littermate comparison of *aaBBCCDDPPWw-aaBBCCDDPPww*. Here no area has been shaded since casual inspection fails to show any reduction of pigment granule number. The slight degree of $W^{v}w$ reduction in the yellow background, and the apparent lack of reduction with *Ww-ww* substitution on a black background raise once more the question of the nature of these pigment effects. If the yellow type is significantly affected, the $W^{v}w$-*ww* pigment reduction is probably a general

TABLE 4

Number of pigment granules at successive levels in corresponding $W^{v}w$-*ww and Ww-ww genotypes (cort. = granules per unit cortical volume; med. = granules per medullary cells; M = mean, E = standard error; field = width field at 1800×, approximately 85μ).*

GENOTYPE	REGION	4TH		10TH		20TH		30TH		40TH		50TH	
		M	E	M	E	M	E	M	E	M	E	M	E
aaBBCCDDPPW^{v}*w*	cort.	195	± 4	153	±27	49	±16	5	±3	0		0	
	med.	—		81	± 5	77	± 4	65	±7	47	±7	38	±8
	cort.	219	± 7	113	±12	21	± 5	4	±3	2	±2	0	
	med.	—		93	±14	95	±11	86	±2	90	±6	87	±2
aaBBc^{ch}*c*^{ch}*DDPPW*^{v}*w*	cort.	189	±13	143	±19	41	±11	7	±4	0		0	
	med.	—		61	± 4	71	± 2	63	±3	67	±4	58	±4
aaBBc^{ch}*c*^{ch}*DDPPqw*	cort.	203	± 8	128	± 9	35	± 4	8	±3	0		0	
	med.	—		98	± 2	93	± 3	85	±3	80	±3	77	±5
aabbCCDDPPW^{v}*w*	cort.	149	± 6	112	± 9	40	±11	2	±1	0		0	
	med.	—		68	± 2	73	± 3	66	±3	62	±3	56	±4
	cort.	160	± 8	73	± 3	21	±10	4	±3	0		0	
	med.	—		82	± 3	90	± 4	95	±4	85	±4	82	±8
aaBBCCDDppW^{v}*w*	cort.	3	± 2	9	± 3	8	± 3	0		0		0	
	med.	—		14	± 3	30	± 4	34	±4	30	±6	27	±6
aaBBCCDDppww	cort.	18	± 3	15	± 1	4	± 2	0		0		0	
	med.	—		32	± 2	51	± 3	62	±4	71	±5	69	±5
A^{v}*aBBCCDDPPW*^{v}*w*	cort.	131	± 6	34	±12	2	± 2	1	±1	0		0	
(littermates)	med.	—		40	± 3	40	± 2	36	±3	41	±3	36	±3
A^{v}*aBBCCDDPPww*	cort.	111	±15	30	± 9	5	± 4	0		0		0	
	med.	—		47	± 4	46	± 3	44	±3	43	±3	44	±3
aaBBCCDDPPWw	cort.	220	±10	152	±10	60	±11	28	±6	0		0	
(littermates)	med.	—		98	± 2	94	± 2	84	±4	86	±5	75	±6
aaBBCCDDPPww	cort.	212	±10	186	±13	100	±16	35	±4	0		0	
	med.	—		97	± 2	90	± 2	94	±2	85	±3	92	±2

one, since all of the qualitatively different pigments here investigated are affected. If yellow is not significantly affected, the *W^{v}w-ww* reduction is limited to eumelanotic pigments. On the other hand, while there is no apparent regular reduction in granule number with *Ww-ww* substitution on a black background, there are points where such reduction can be found (30th and 50th field medullary granule counts) and it would be valuable to have some method of determining whether or not the over-all picture indicates significant reduction. However, statistical treatment of the data is complicated by the fact that the individual measurements which can be paired between littermates (number of granules per medullary cell at the 10th microscopic field of *W^{v}w-ww* segregants, for example) are not independent of each other, as the successive levels come from the same hair, and there may be associations with factors affecting that particular hair, or with hair type (zig-zag or awl), with particular levels of the hair, and with factors common to littermates, as well as the factors whose

TABLE 5

Analysis of variance in medullary granule number in litters segregating for A^{u}aBBCCDDPPW^{v}w-A^{u}aBBCCDDPPww.

SOURCE OF VARIATION	SUM OF SQUARES	DEGREES FREEDOM	MEAN SQUARE	F RATIO
genotype	3176	1	3176	19.05**
litters	2175	4	544	3.26*
error (a)	2334	14	167	—
levels	431	4	108	1.11
hairtypes	581	1	581	5.98*
level×hairtype	2969	4	742	7.63**
level×litter	2528	16	158	1.63
litter×hairtype	222	4	55	0.57
genotype×level	224	4	56	0.58
genotype×hairtype	3	1	3	0.02
error (b)	14202	146	97	—

* Significance at the 5% level.
** Indicates significance at the 1% level.

effects are being determined, those associated with the *W^{v}w-ww* genotypic difference. At the suggestion of EARL L. GREEN, and with a great deal of help from him which the author wishes to acknowledge here, a method for determining the contribution of each of such a complex of factors to the total variance of medullary granule numbers was developed. Contributing factors analyzed were the effects of difference in genotype (*W^{v}w-ww* or *Ww-ww*); of single factors varying among litters, levels of hair and hairtypes; and of interacting factors common to hairtype and level of hair, to litter and level of hair, to hairtype and litter, to genotype and hairtype, and to genotype and level. This method was applied only to the yellow *W^{v}w-ww* segregants, since it is only here that the reality of *W^{v}w* reduction is in doubt, and also, of course, to

the black *aaWw-ww* segregants. Results of these analyses of variance are given in tables 5 and 6. It is immediately clear from table 5 that the difference between W^vw and *ww* genotypes is the major source of variance in the W^vw-*ww* yellow stock. The difference between the two genotypes is highly significant (F is 19.05, much greater than $F_{0.01}$, 8.86). By the same test, the difference shown in table 6 between *aaWw* and *aaww* segregants is clearly not significant. (Before leaving these tables one further significant source of variation should be mentioned, that due to factors common to level and hairtype, which is also

TABLE 6

Analysis of variance in medullary granule number in litters segregating for aaBBCCDDPPWw-aaBBCCDDPPww.

SOURCE OF VARIATION	SUM OF SQUARES	DEGREES FREEDOM	MEAN SQUARE	F RATIO
genotype	604	1	604	2.62
litters	2967	3	989	4.3*
error (a)	2535	11	230	—
levels	3827	4	957	1.66
hairtype	926	1	926	1.60
level×hairtype	412	4	103	.18
level×litters	2676	12	223	.39
litters×hairtype	1601	3	534	.93
genotype×level	1506	4	264	.46
genotype×hairtype	843	1	843	1.46
error (b)	66401	115	577	—

* Significance at the 5% level.
** Indicates significance at the 1% level.

significant at the 1 percent level ($F = 7.63 > F_{0.01} = 3.44$.) This means that in these particular stocks the degree of difference between zig-zag and awl hairs depends upon the level of the hair.) These analyses of variance have confirmed statistically the suspected difference in dominance relations between the W^v and W alleles in their effect on pigment intensity, and have indicated that the W^v dominant effect is a general one, since in the doubtfully affected yellow stock, the differences between genotypes is by far the greatest source of variation.

The fact that granule number is affected by W^vw-*ww* substitution in three qualitatively different pigments (black-fuscous, brown, and yellow), and in differently arranged shred-granuled *pp* types has already shown quite a bit about the nature of the W^vw effect on pigment intensity, i.e., has shown its generality. Another deduction which might be drawn from a histological examination might come from a study of the effects of this gene substitution on granule size. In an earlier study of the interrelationships among variable granule attributes (RUSSELL 1949a) one of the four key pigmentation characteristics described was the degree of pigmentation, which referred to a "group

phenomenon in which a number of attributes vary together in response to one basic factor, the general degree of pigmentation." "Among the attributes conditioned by this key factor are medullary number itself . . . and size of black-fuscous granules. . . . These attributes vary with changes in general degree of pigmentation whether such changes are produced by genic substitution or by non-genetic effects such as position along the hair axis." Yellow granule size can be affected by changes in degree of pigmentation only in very extreme cases, and pink-eyed granules and brown granules have not been shown to be affected in any case as yet. Therefore, if the *W^{v}w-ww* effect on pigment intensity is to be classified into one of the key pigmentation characteristics, its effect on the mean size of various types of granules will help determine whether or not it is a change in the general degree of pigmentation. Table 7 compares the mean greater diameter of corresponding *W^{v}w*, *Ww* and *ww* types, based on littermate comparisons, and shows quite clearly that in this case *Ww-ww* substitution does not reduce the size of black-fuscous granules, while *W^{v}w-ww*

TABLE 7

The effect of W^{v}w-ww and Ww-ww substitution on granule size in various genetic backgrounds. Value given is the mean and standard error of the greater diameter of 100 granules of each genotype. An asterisk indicates a significant difference.

BACKGROUND GENOTYPE	*W^{v}w*		*Ww*		*ww*		*ww-W^{v}w*	*ww-Ww*
	M	E	M	E	M	E	diff. E	diff. E
aaBBCCDDPP	1.43	±.008	1.53	±.011	1.50	±.008	0.07 ±.012*	−.03 ±.014
aaBBc^{ch}c^{ch}DDPP	0.99	±.008	—		1.03	±.010	0.04 ±.013*	—
aabbCCDDPP	0.78	±.007	—		0.77	±.007	−0.01 ±.011	—
aaBBCCDDpp	0.55	±.031	—		0.58	±.015	−0.03 ±.020	—
A^{v}aBBCCDD	0.77	±.010	—		0.78	±.009	−0.01 ±.013	-

substitution significantly reduces the mean greater diameter of two black-fuscous genotypes (*aaBBCCDDPPW^{v}w-ww; aaBBc^{ch}c^{ch}DDPW^{v}w-ww*) and does not significantly affect brown, pink-eyed black, or yellow. This shows clearly that the effect of *W^{v}w-ww* substitution is very similar to other general changes in degree of pigmentation, since the effect on granule size is limited to the same types of granules.

DISCUSSION

An extensive parallelism of effect of *W^v* and *W* substitutions on two very different localities, (a) blood and (b) pigment-producing cells of the hair follicle, has been demonstrated. In both tissues, the double dominant types are severely affected; they, *WW*, *WW^v*, and *W^{v}W^v*, are black-eyed whites; they also have rather extreme macrocytic anemia, though its exact degree varies with the particular alleles present, those with *W* being the most anemic. One dose of *W*, however, has no effect either on erythrocyte count or hair pigment intensity. One dose of *W^v* has a slight but significant effect on both

tissues. The nature of the effect of $W^v w$-ww substitution on hair pigment intensity has been investigated sufficiently to say that it is a general reduction, affecting all the types of pigment which were studied. The basic question of interest in a study of these pleiotropic effects is the place and time of original gene action. Certainly the two types of tissues studied here, affected so similarly by the same series of genes, come from widely separated cell lineages. All mammalian haematopoietic tissues come directly or indirectly from the primitive mesenchyme (BLOOM 1938), and the main sites of haematopoiesis are successively the yolk sac, the liver, and the bone marrow. All hair pigment in the mouse is believed to come from melanophores which migrate out from the neural crest between the eighth and twelfth days of gestation (RAWLES 1947). It hardly seems possible that the gene action responsible for these widely separated effects can have taken place in a common ancestor of the mesenchyme and neural crest cells, since so many other descendants of the same cells appear to be unaffected. Rather, one effect must be dependent upon the other for its appearance (figure 1, B), or both must result from gene action independently in two types of cells (figure 1, C or D), or both tissues must be in an especially susceptible state to be affected by some gene action external to both at a particular embryonic stage (special double case of figure 1, B). The type of data given here cannot distinguish definitely among these possibilities. One possibility, of course, is that the anemia arises early enough in embryonic development to inhibit the melanophores so either their number or pigment-producing capacity is limited. (It should be noted that the extent of final *migration* of melanophores is restricted by one dose of either W or W^v, since both types always carry a belly spot. This effect is not parallel with the blood effect, however.) The concept of inhibition of development by an anemia has been used to explain the secondary effect of *ff* siderocyte anemia on the tail and on extent of pigmentation (GRÜNEBERG 1942b). In considering this possibility, however, it should be mentioned that in most cases where arrest of development at a particular time has been postulated as a manner of gene action, a skeletal effect has been involved, since some part of the axial skeleton is actively growing at almost any stage in embryonic development. The W series appears to have no effect at all on the skeleton. The third possibility, that some change external to both tissues acts similarly on the two at a critical time in development, should be considered in the same light: since no skeletal effect is involved, it is doubtful if such an outside change could be a general arrest in development; this does not exclude some other type of external change such as change in concentration of specific chemicals or alteration of the percent survival or rate of motion of wandering cells. The first step in investigating these possibilities is, of course, a determination of the time of first appearance of the anemia, and this is being carried out at present. A second possibility is that the anemia, which continues throughout life, may in some way diminish the supply to the hair follicle of some substance essential for pigment formation. (It should be remembered, however, that the actual haemoglobin level was thought by GRÜNEBERG to be the same in $W^v w$ and ww individuals due to the macrocytosis counteracting the reduction in numbers;

our data do indicate a slight but significant decrease in total cell volume.) Data somewhat pertinent to the first point and extremely important to the idea of an effect from a continuing anemia would be obtained by transferring skin between types differing in their blood pictures, as autonomy or nonautonomy of pigment deposition in transplants would determine their dependence upon the nature of the blood supply. It should also be mentioned that if such transplants should prove to be nonautonomous, it would be a definite exception to the general rule, which is that pigmentation is strictly locally determined. The only previous exceptions known to the author are certain cases where cells near the edge of skin transplants have appeared to develop nonautonomous pigment which must have been determined in some way by a very limited type of migration of pigment-forming cells between the marginal cells of host and donor tissues (REED and HENDERSON 1940).

The final alternative possibility that conditions in the haematopoietic tissues and the hair follicle both independently produce a situation in which the *W*-genes have similar or identical effects must be considered seriously. To prove local gene action is extremely difficult, to disprove it somewhat easier, and the author feels that the best procedure is to test out all possibilities of action from a distance. Possibly in favor of local action, however, is the observed exactness in relation between gene dosage and final character in both tissues. In his analysis of the nature of the action of the *ff* gene-pair (siderocyte anemia, tail flexure, belly spot) GRÜNEBERG (1942b) makes considerable use of the fact that the anemia is a much more regular manifestation of the gene than is tail flexure as evidence for his conclusion that the anemia is the primary result of gene action. It would be impossible to say in the case of the *W*-series which effect is most regular, as both blood and pigment effects occur in all cases in exact concordance with the genotype. Either the dependence of the degree of pigmentation upon the anemia is in very delicate adjustment, or precursors of both tissues must be identically affected by some externally-located effect of the gene during embryological development, or both phenomena must result from independent original gene action in the affected tissues.

Further mention might be made at this point of the nature of the $W^v w$-ww pigment reduction. It appears to be a general change in the degree or level of pigmentation, affecting all qualitatively different types of pigment which have been tested. Two other examples of such general change have been established (RUSSELL 1949b). The first is the change produced by *C*-series (albino) gene substitutions in which the gene action appears to result in a change in the concentration of the enzyme dopa-oxydase (RUSSELL, L. B., and W. L. RUSSELL 1948). The second is the non-genic change in pigmentation level along the axis of the hair in many genotypes with an intermediate amount of total pigmentation. Because of the close parallelism of effect of $W^v w$-ww substitution on blood and pigmentation this knowledge of the nature of the pigment effect may be of help in interpreting the nature of the basic gene action on blood as well as on hair pigment-producing cells.

Although the $W^v w$ pigment reduction is allied to the albino-series reduction,

it is not identical with it, since the two allelic series have somewhat different types of effects upon medullary granule number. An important point in the interpretation of the effect of $c^{ch}c^{ch}$-CC substitution (RUSSELL 1949b) on a black or brown background was that granule number is not affected, and any reduction in total pigment is due to a reduction in granule size. The assumption made was that all available sites of pigment deposition were already active at the chinchilla level, and addition of further enzyme could act only by increasing the size of particles at each site. The fact that the medullary granule numbers in full color blacks (total from the 10th to the 50th fields, 451), full color browns (total, 434), chinchilla blacks (total, 433), and chinchilla browns (total, 418) are almost identical, while the estimated total volume of medullary pigment varies widely, with full color blacks very high (783 units), full color browns low (318 units), chinchilla blacks intermediate (400 units) and chinchilla browns low (303 units) (RUSSELL 1948), strongly supports the concept that in all of these a maximum number of granules has been reached. If W^vw reduction were identical in nature to C-series reduction, its effect on full color blacks certainly should have been a reduction in granule size much greater than any effect on number. Actually, in this case number is much more effected than is size (figure 2, tables 4 and 7). In the two types where size is affected, full color and chinchilla black, the reduction is significant but very small. The reduction in granule number is much greater. A curious and probably significant fact is that in each of three backgrounds where *ww* genotypes have identical medullary granule numbers but differing total volumes of pigment, W^vw substitution has the same proportional effect on granule number (in full color black, 68 percent, in chinchilla black, 74 percent, and in full color brown, 75 percent), and of course very different proportional effect on pigment volume. Two other backgrounds, where the *ww* type was not near this maximum, had very different proportional reductions in granule number (full color yellow, 86 percent, and pink-eyed sepia, 47 percent). This suggests the possibility that the W^vw action on pigment intensity either removes or incapacitates some of the sites of pigment deposition, and the amount of reduction in any particular background depends upon the relation of the pigment producing potentialities of the rest of the genotype to the new restricted limit on sites of pigment deposition.

Thus this paper has summarized evidence on the nature of dominance relations of W and W^v in their effect upon erythrocytes and on hair pigment intensity, with the conclusion that although the W gene has no dominance in either tissue, W^v is dominant in both, a single dose producing a significant effect. Emphasis has been placed on the close parallelism of the effects of the entire W-series of genes upon the blood and pigment-producing tissues, especially in these peculiar dominance relationships. The fact has been stressed that in both cases the gene W with no effect in one dose has a severe effect in two doses (WW), while the gene W^v with some effect in one dose has a milder effect (compared to WW) in two doses (W^vW^v). An attempt has been made to analyze the nature of the W^v dominant effect on hair pigmentation, with the conclusion that it is a general change in all pigmentation, probably in-

volving restriction in sites of pigment deposition. Suggestions have been made as to the possible significance of these results to a further analysis of the pleiotropic effects of the *W*-series genes.

SUMMARY

1. Studies were made of the blood picture of month-old littermates in stocks approaching genetic uniformity. In litters segregating for $W^v w$ and *ww*, the $W^v w$ individuals had significantly lower erythrocyte counts and hematocrit readings, significantly higher mean cell volumes than their *ww* littermates. In litters segregating for *Ww* and *ww* there were no significant differences between the genotypes.

2. Studies were made of the number and size of hair pigment granules in five differently colored $W^v w$ genotypes. In full-color blacks, chinchilla blacks, full-color browns, pink-eyed sepia, and full-color yellow backgrounds, the $W^v w$ type has fewer pigment granules than its *ww* counterpart. In some types there is also reduction in granule size.

3. An analysis of variance of medullary granule numbers in litters segregating for $A^v aBBCCDDPPW^v w$-*ww* (the least reduced of the five stocks mentioned above) proved statistically that the difference between the $W^v w$ and *ww* genotypes is highly significant and is by far the greatest source of variability.

4. Studies were made of the number and size of pigment granules in litters segregating for *Ww* and *ww* in a full-color black stock (*aaBBCCDDPPWw-ww*). There is no reduction in granule size, and an analysis of variance of medullary granule number proved statistically that there is no significant difference between *Ww* and *ww* segregants.

5. The observed effects of $W^v w$ substitution upon granule number and size are compatible with the concept that this substitution causes a reduction in the general level of pigmentation.

6. In both blood and pigment-forming cells of the hair, *W* has no effect in one dose, a very severe effect in two doses, while W^v has a significant effect in one dose and a milder effect than *W* in two doses. This close correlation in the dominance relations of the two alleles indicates a very close connection between the *W*-gene action leading to blood changes and that leading to pigment-intensity changes.

7. The bearing of these findings upon further analyses of the pleiotropic effects of the *W*-series and determination of the nature of gene action is discussed.

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