A QUANTITATIVE HISTOLOGICAL STUDY OF THE PIGMENT FOUND IN THE COAT-COLOR MUTANTS OF THE HOUSE MOUSE. I. VARIABLE ATTRIBUTES OF THE PIGMENT GRANULES[1]

ELIZABETH S. RUSSELL

Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

Received January 14, 1946

AN ANALYSIS of the genetics and physiology of coat color in mammals is very useful as a step toward understanding the processes which go on between original gene action and final character expression. Many studies have been made of genes which affect the whole organism, such as developmental lethals and size factors, including cases in which the effects of genes could be traced back to critical events in certain organs at definite stages in development. In these cases, particularly in mammals, it is difficult enough to discover what tissues are involved in the primary gene action without determining what happens to the metabolism of the cells involved. In contrast, the chain of reactions between gene and character must be relatively short for mammalian coat color genes. The persistence of color in mutant spots and in transplantation experiments indicates that the pigment is determined locally, and the processes concerned continue throughout life, as hair is regularly being shed and replaced. These advantages have been realized by a number of workers, and various types of physiological genetic analyses have been made of the pigments produced in different genotypes of several mammals. Some have been based on observation and grading of colors with the naked eye (LITTLE 1913; WRIGHT 1916, 1917–18, 1925, 1927; DUNN 1936; HADJIDIMITROFF 1933; and HALDANE 1927). Others have based their analyses on chemical isolation of the pigment (DUNN and EINSELE 1938; BAKER and ANDREWS 1944; DANIEL 1938; HEIDENTHAL 1940; E. S. RUSSELL 1939; WRIGHT 1942). SCHILLING (1936) has made a quantitative histological analysis of pigment in the guinea pig, but unfortunately has grouped several genotypes together in some cases, dealing only with grades of color. HARMON and CASE (1941) also have studied guinea pig hair pigment histologically. DANNEEL (1936) has done a genetically accurate histological analysis of rabbit hair pigment, but has not put his results on a quantitative basis. The same is true of LÜHRING'S (1928) analysis of squirrel pigment. HUNT and WRIGHT (1918) give a brief histological study of guinea pig hair pigment. WERNEKE (1916) has done an excellent histological analysis of pigment in the mouse. He describes the granules found in various genotypes, records their distribution between cortex and medulla, and their variation along the hair axis. In general, his conclusions

[1] This work has been aided by grants from THE COMMONWEALTH FUND, THE ANNA FULLER FUND, THE INTERNATIONAL CANCER RESEARCH FOUNDATION, THE JANE COFFIN CHILDS MEMORIAL FUND, and THE NATIONAL ADVISORY CANCER COUNCIL.

as to the effects produced by various genic substitutions still stand, although they, like those of DANNEEL, are not strictly quantitative.

Of the above mentioned authors, only HADJIDIMITROFF and WERNEKE have made any considerable use of one great advantage of hair pigmentation in physiological analysis, one which we feel will contribute greatly to our knowledge of gene action. From tip to base there is laid down an accurate account of the processes going on in a hair bulb throughout its active life. Any variation in pigment-producing activity with time will show up as a difference in amount or type of pigment deposited at different levels of the hair shaft. It is obvious that a histological study of the undisturbed pigment within the hair will provide a perfect place for analysis of this time factor in pigment-gene action.

There are a number of different allelic series of genes in the mouse which act within the hair follicle to affect the number, type, and distribution of pigment granules deposited in the hair as its cells are produced (REED and SANDER 1937; REED 1938a, 1938b; REED and ALLEY 1939). This local gene action may be studied in several ways. While it is occurring, a study may be made of the chemical substances present in the follicles in various genotypes. This has been done for the giunea pig by W. L. RUSSELL, who studied the concentration in a wide variety of genotypes, of an enzyme, dopa-oxidase, which is known to catalyze the change of a tyrosinelike amino acid, l-3,4,-dioxyphenylalanin, to the final hair pigment, melanin. A number of German workers have also studied the enzyme content of the Himalayan rabbit skin during the pigmentation process by a technique called under-cooling (ENGELSMEIER 1935; DANNEEL and SCHAUMANN 1938). LUBNOW (1939) has studied differences in the histological and cytological structure of the matrix-cells of the hair follicle of different genotypes and at different stages in the pigmentation process. Another possible approach is to study the end-product of the pigmentation process which has been deposited in the keratinized hair cells. Spectrophotometric analyses of this hair pigment have been made by DANIEL (1938) for the mouse and by BAKER and ANDREWS (1944) and GINSBURG (1944) for the guinea pig. An analysis of the chemical structure of the pigment would be ideal, but seems impossible at present, especially on the micro-scale necessary to these studies.

However, all of the pigment in mouse hair is granular, and a good deal can be ascertained as to the nature of the various gene actions from counts, measurements, and color determinations of the pigment granules in the cells of the various portions of the hair at successive levels along the hair shaft.

This present paper gives the results of these determinations on various combinations of several major allelic series of coat-color genes in the mouse. Included are the albino series (C, c^{ch}, c^{e}, and c^{a}), which alters the intensity of all colors; the agouti series (A^{v}, A^{w}, and a), which determines the distribution of yellow and sepia along the hair axis; black-brown (B and b), in which the recessive allele changes non-agouti pigment from some shade of black-sepia to a shade of brown; pink-eyed dilution (P and p), in which the recessive allele practically eliminates pigment in the eye and greatly reduces sepia pigment in

the hair; and dilution (*D* and *d*), which reduces the intensity and dulls the shade of all colors. Since this study involves a number of different methods and a wide variety of pigment attributes, it seems most satisfactory to devote the first paper of this series to a description of methods and a simple presentation of the differences found in the various genotypes, saving detailed interpretation of the nature of the changes involved for future publication. The author is very grateful to DR. W. L. RUSSELL for producing the invaluable mouse color stocks used in this investigation. She also wishes to thank both DR. W. L. RUSSELL and MISS LIANE BRAUCH for their many helpful suggestions.

GENERAL METHODS

In order to have a full record of the sequence of events during the pigmentation process in hair growth, it is essential to study whole mounts of representative hairs which have completed their growth. The hair cells are produced from the rapidly dividing matrix cells of the hair bulb (LUBNOW 1939) and very soon become keratinized and pushed above the skin surface. The hair shaft which is thus produced consists of three parts: the outermost coating, or cuticle, a very thin sheath of overlapping scales devoid of pigment; the cortex, a hollow cylinder inside the cuticle, made up of long narrow cells whose longitudinal axes are parallel with that of the hair; and the medulla, or core of the hair, a ladder-like arrangement with horizontal plates (septa) of disk-shaped cells alternating with air-spaces. Four types of hairs have been described in the mouse (DRY 1926) distinguished chiefly by the number of medullary cells per septum. In "lead" hairs there is one cell per septum throughout the length of the hair (the cortex is also especially heavy); these form two per cent of the first pelage (DRY 1926). In "awl" hairs the septa of the middle two-thirds of the hair contain three or possibly four medullary cells. "Auchene" hairs are very similar, except that there is a constriction in the mid-region where only one medullary cell is found in each septum. "Awls" and "auchenes" together constitute 14–15 percent of the first pelage (DRY 1926). The "zig-zag" hairs alternate blades with one medullary cell per septum with short curved constrictions (usually three in number) where only tiny remnants of medullary material remain. These "zig-zag" hairs constitute 83 per cent of the first pelage (DRY 1926). Thus zig-zags and awls or auchenes are the most representative hair types. In all hairs, outgrowth continues until the full length of 0.6–0.7 cm is reached (DRY 1926), after which the hair bulb itself shrinks and becomes keratinized. Fully grown hairs with keratinized bulbs, known as club hairs, can easily be plucked out. They are completely dead, but contain, from tip to base, a consecutive account of what has gone on in a hair bulb during its entire active life.

Club hairs of the first pelage were used in all these studies since their growth has been less influenced by environmental influences than that of later coats. At four or five weeks the first pelage hairs of the crown of the rump have reached the club stage (DRY 1926). Hair samples were plucked from this region

of six animals of each genotype, no more than two of which came from the same litter. The samples were stored in envelopes in darkness until used.

To make whole-mount slides, a small group of hairs were attached to a slide by means of pure egg-white (no glycerin), and after drying were put into each of the following solutions for at least three days: absolute alcohol, alcohol-xylol, and xylol. After this they were imbedded in balsam and covered. This produced preparations free of air-bubbles in the medullary spaces, and giving sharp definition of the unstained pigment granules in the transparent colorless cell matrix. Although glycerin has been used by many workers to remove air from the medullary spaces, its use was avoided here following the discovery by RALPH KELLOGG (unpublished) that glycerin sometimes reacts with the yellow pigment, leading to gradual breakdown of the yellow granules, leaving cells filled with a diffuse yellow coloration. This action has already led to considerable confusion in the literature, apparently accounting for many reports of diffuse non-granular yellow pigment (DANNEEL 1936; LÜHRING 1928; SCHILLING 1936 (slight)).

Individual hairs from each whole mount, usually one zig-zag and one awl or auchene, were studied at a succession of levels from the tip to the base. Distances along the shaft were estimated from the width of the microscopic field (approximately 85μ at 1800×), and generally were indicated simply by using one microscope field as a unit of length. At the very tip of the hair there is a short region with a solid rod of cortex. In zig-zag and awl-auchene hairs the length of this solid tip was found to average four fields (340μ). In order to get a clear measure of the amount of cortical pigment in each genotype, the first level studied in each hair was the most basal field with this solid cortex (arbitrarily called in every case the fourth field). The other levels studied were the 10th, 20th, 30th, 40th, 50th, and 60th fields. At each of the 10th–60th field levels, the number of pigment granules in one medullary cell was recorded. Selection of one medullary cell as a unit for measuring quantity of pigment deposited in the medulla seemed acceptable, since in 11 samples from seven genotypes there were no significant differences in the number of septa per microscopic field. The average number of cells per field (at 900×) was 39 ± 1 for awls (two to three cells per septum in the region counted), 29 ± 1 for auchenes (two cells per septum in the region counted), and 18 ± 1 in zig-zags (one cell per septum). To obtain a comparable measure of cortical pigment along the hair axis, it seemed desirable to determine the number of pigment granules in a volume of cortex approximately equal to that of a single medullary cell. The volume of a cylinder 15μ long from the solid cortex at the tip of the hair was found to be approximately equal to that of the average medullary cell (table 1). In lower regions, the thickness of the cortical wall was found to be 1–2μ, the cross-sectional area approximately twice that of the solid cortical tip, so the volume of the upper half of a 15μ long section in lower regions is sufficiently close to that of a 15μ section of solid cylindrical tip to allow some degree of comparison. Thus at all levels (4th and 10th to 60th at 10-field intervals) the number of pigment granules in a section of cortex 15μ long

(either solid cylinder or upper surface of hollow cylinder) was recorded in addition to the medullary cell counts for the 10th to 60th fields.

These whole mounts were ideal for determining number and variation in number of pigment granules per unit area in cortex and medulla for the various levels of the hair shaft. No significant differences in either cortical or medullary counts were found which might be related to differences among the three hair

TABLE 1

Determination of the length of solid cortical cylinder having volume equal to that of average medullary cell (considered here as a cylindrical disk).

SAMPLE	TYPE HAIR	DIAM. CORTEX IN μ (4TH FIELD)	DIAM. MED. CELL IN μ	HT. MED. CELL IN μ
158	zig-zag	5	6	4
			8	6
	awl	8	10	7
			11	6
139	zig-zag	6	10	6
			12	5
	awl	8	10	8
			10	6
122	zig-zag	6	14	6
	zig-zag	7	12	7
	awl	7	10	8
	awl	7	12	5
116	zig-zag	5	9	5
	zig-zag	5	11	6
	awl	8	13	7
	awl	7	10	6
108	zig-zag	6	12	8
	zig-zag	5	9	6
	awl	8	12	7
	awl	7	8	6
mean (diam.)		6.56	10.45	6.25
mean (rad.)		3.28	5.23	

Mean vol. medullary cell $= \pi r^2 h = \pi(5.23)^2\, 6.25 = 170.94\pi$.
Mean x-section area cortex $= \pi r^2 = \pi(3.28)^2 = 10.76\pi$.

Height cortical cylinder to give vol. equal to med. cell $= \frac{170.94\pi}{10.76\pi}$
$= 15.88\mu$

types studied. All of the counts for this paper were made in as short a time as possible in order to avoid possible personal differences in estimation of number of granules in the most crowded types.

Although observations were made of the size, shape, and color of the granules in the whole mounts, no attempt was made to arrive at quantitative indices for these attributes, because the thickness of the cells and the large num-

ber of granules in the more crowded types made detailed observations of individual granules difficult. However, two distinct types of differences in arrangement of granules within the medullary cell were noted. In some genotypes the granules filled the entire cell, while in others they tended to be located more distally, leaving a clear space at the proximal side of each cell. Also, in some types the granules were clumped together, while in others they were arranged more or less evenly. In each genotype the condition with regard to these two attributes, distal arrangement and tendency to clumping, was recorded.

To study the detailed structure of the individual granules, the hair mass remaining from each sample following the making of whole mounts was used for cross sections. These were prepared by the HARDY method (1935) for sectioning fibers. The essential equipment for this method is a small hand microtome, the size of a microscope slide, approximately, which contains a narrow slot into which hairs can be packed as in a vise, and held so tightly that sections cut from the projecting ends must be true cross sections. To operate the microtome, the hair-mass is moved up in the slot by means of a calibrated screw, the projecting hairs are coated with a quick-drying substance, which is allowed to dry, and sections are cut off with a razor blade. Accurate sections as thin as 3–4μ can be obtained by this method. Some tests were made of quick-driers, and collodion in alcohol-ether and methyl-crylate in ether were both found very satisfactory, the methyl-crylate slightly better for thin sections. The sections were affixed to slides by heating slightly, and were then covered with balsam and a cover slip.

The complete outline of individual granules is clearly visible in the cross sections of even the most crowded types. The greatest diameter of 100 granules from each of five samples of each genotype was determined. This diameter was estimated (at 1800×) to the nearest .25μ, using an ocular micrometer, with scale divisions indicating at this magnification almost exactly 1μ (48 ocular units = 50μ). The granules measured were taken from at least three plates of sections from each slide.

The shape of granules in each genotype was also recorded, being listed as round, short oval, long oval, or "irregular shred" (either elongated or roundish). In certain genotypes there were a few scattered pigment bodies very much larger than the others, forming a discrete group well separated from the rest of the size distribution curve; these were called clumps.

The color of the individual granules of each genotype was also determined from the cross sections. To evaluate this, the granules, as seen at 1800×, were matched with the color plates in Ridgway's Color Standards (1912). Since very slight differences in the intensity or color of the light transmitted through the microscope may alter the appearance of the granules, the same lamp, kept in a constant position, was used for all these observations, with the same daylight blue filter and the same setting of the iris diaphragm. The color plates were also always observed with a constant artificial illumination. Perfect matching was difficult, because the granules were seen by transmitted light, the color plates by reflected light. For this reason in many cases two

alternative color names, closely related in Ridgway's classification, were assigned to the same genotype, since it was impossible to say which was the better match. The color plates used fell into three color series, yellow, black-fuscous, and brown. Within the second and third series there were intensity differences in the color plates, varying with genotype, probably differences in concentration of pigments otherwise very similar or identical.

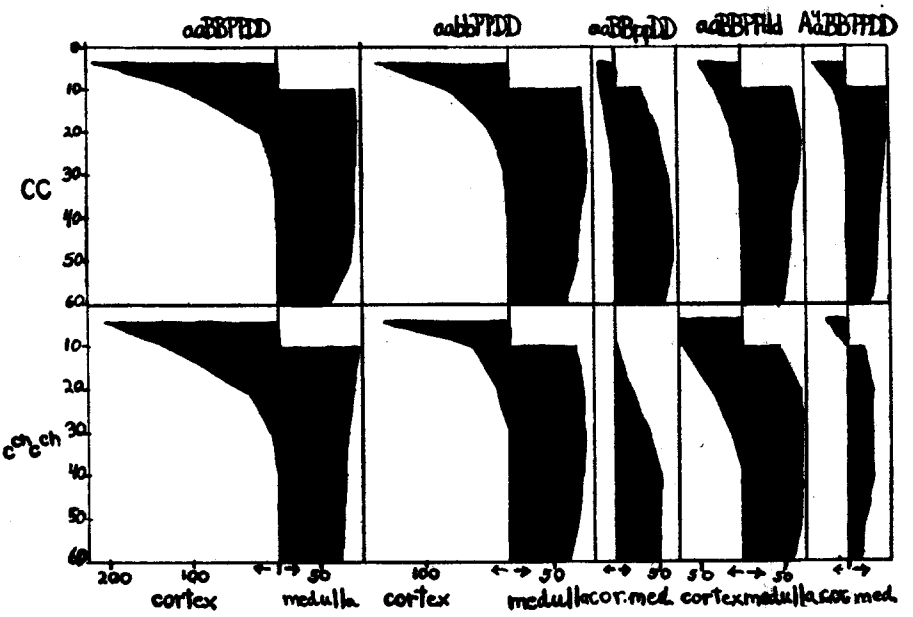

FIGURE 1.—The effect of certain single genic substitutions on cortical and medullary granule number. The figures to the left of the axis represent the mean number of pigment granules per unit volume of cortex at a succession of levels, from the 4th to the 60th field down from the tip of the hair. The figures to the right of the axis represent the mean number of granules per medullary cell at a succession of levels, from the 10th to the 60th field down from the tip of the hair.

The number of granules in a unit area ($25\mu^2$) of a cross section of medullary cell was determined for each genotype tested. Ten unit areas were counted for each of five samples of each genotype. Although these gave no measure of variation along the hair axis, they gave a value for the number of medullary granules in the mid-region of the hair of each genotype, useful for comparison with the more complete counts from whole mounts.

Thus we have observed seven attributes of the pigment granules in each of the 36 genotypes studied: First, attributes determined primarily from whole mounts: (1) Number of medullary granules per medullary cell, counted at 10-field intervals along the hair axis confirmed for the mid-region by independent counts from sections. (1a) (Corollary) Tendency toward a lag in the number per medullary cell produced near tip of hair. (2) Number of cortical granules per unit volume equal to that of average medullary cells. (3)

Tendency to distal arrangement of granules within medullary cells. Second, attributes determined primarily from cross sections: (4) Size of granules (greatest diameter). (5) Shape of granules. (6) Clumping of granules (also checked from whole mounts). (7) Color as matched against Ridgway's Color Standards. (7a) Intensity of color.

TABLE 2

The mean number of granules at successive levels in the non-agouti genotypes tested. (Cort. = granules/unit cortical volume; med. = granules/medullary cell; M = mean; E = standard error; field = width field at 1800×, approx. 85μ).

GENOTYPE	REGION	FIELD											
		4TH		10TH		20TH		30TH		40TH		50TH		60TH	
		M	E	M	E	M	E	M	E	M	E	M	E	M	E
aaCCBBPPDD	cort.	219	±7	113	±12	21	±5	4	±3	2	±2	0		0	
	med.			93	±14	95	±11	86	±2	90	±6	87	±2	61	±3
aac^{ch}c^{ch}PPBBDD	cort.	203	±8	128	±9	35	±4	8	±3	0		0		0	
	med.			98	±2	93	±3	85	±3	80	±3	77	±5	73	±2
aac^{e}c^{e}BBPPDD	cort.	20	±4	2	±2	0		0		0		0		0	
	med.			19	±2	38	±3	42	±3	38	±2	38	±2	33	±3
aac^{a}c^{a}BBPPDD	cort.	0		0		0		0		0		0		0	
	med.			0		0		0		0		0		0	
aaCCbbPPDD	cort.	160	±8	73	±3	21	±10	4	±3	0		0		0	
	med.			82	±3	90	±4	95	±4	85	±4	82	±8	70	±8
aac^{ch}c^{ch}bbPPDD	cort.	147	±5	44	±8	14	±5	1	±1	0		0		0	
	med.			79	±3	83	±3	88	±3	87	±4	81	±5	71	±5
aac^{e}c^{e}bbPPDD	cort.	31	±3	2	±2	1	±1	0		0		0		0	
	med.			20	±2	34	±2	50	±4	52	±5	39	±5	28	±4
aaCCBBppDD	cort.	18	±3	15	±1	4	±2	0		0		0		0	
	med.			32	±2	51	±3	62	±4	71	±5	69	±5	59	±5
aac^{ch}c^{ch}BBppDD	cort.	0		0		0		0		0		0		0	
	med.			1	±1	19	±4	40	±3	55	±4	51	±4	52	±4
aac^{e}c^{e}BBppDD	cort.	0		0		0		0		0		0		0	
	med.			0		0		0		0		0		0	
aaCCBBPPdd	cort.	49	±4	31	±3	12	±3	4	±2	0		0		0	
	med.			59	±5	73	±5	70	±5	58	±5	57	±6	52	±9
aac^{ch}c^{ch}BBPPdd	cort.	72	±15	71	±10	35	±11	11	±4	0		0		0	
	med.			47	±6	71	±3	72	±3	73	±5	70	±3	58	±8
aaCCbbppDD	cort.	17	±3	11	±3	1	±1	0		0		0		0	
	med.			30	±2	43	±2	47	±3	60	±4	59	±3	51	±4
aaCCbbPPdd	cort.	41	±5	29	±5	13	±4	5	±2	0		0		0	
	med.			40	±4	47	±4	53	±4	52	±7	49	±5	51	±7
aaCCBBppdd	cort.	0		0		2	±2	0		0		0		0	
	med.			9	±3	34	±3	48	±3	50	±4	59	±4	49	±4
aaCCbbppdd	cort.	0		0		0		0		0		0		0	
	med.			7	±3	32	±4	43	±3	47	±4	53	±5	42	±1

RESULTS

As stated in the description of methods, the number of both cortical and medullary granules in a unit volume was counted at successive levels in whole mounts of 12 hairs (six samples) of each of the 36 genotypes tested. The results for the non-agouti types are given in table 2, for yellows in table 3. The mean number of cortical granules per unit volume varies from 219 ± 7 for the 4th field of *aaCCBBPPDD* hairs down to zero for albinos and certain pink-eyed types. The mean number of granules per medullary cell varies from 90–100

TABLE 3

The mean number of granules at successive levels in the yellow genotypes tested. (Cort. = granules/unit cortical volume; med. = granules/medullary cell; M = mean; E = standard error; field = width field at 1800×, approximately 85μ.)

GENOTYPE	REGION	FIELD											
		4TH		10TH		20TH		30TH		40TH		50TH		60TH	
		M	E	M	E	M	E	M	E	M	E	M	E	M	E
$A^{v}aCCBBPPDD$	cort.	39	±7	12	±4	3	±2	0		0		0		0	
	med.			47	±2	47	±2	42	±2	40	±3	35	±3	29	±3
$A^{v}ac^{ch}c^{ch}BBPPDD$	cort.	25	±7	0		0		0		0		0		0	
	med.			22	±2	30	±1	30	±3	29	±3	20	±2	18	±2
$A^{v}ac^{e}c^{e}BBPPDD$	cort.			0		5	±3	0		0		0		0	
	med.					0		0		0		0		0	
$A^{v}aCCbbPPDD$	cort.	35	±3	19	±4	2	±1	0		0		0		0	
	med.			43	±3	43	±2	40	±2	34	±2	33	±2	31	±2
$A^{v}ac^{ch}c^{ch}bbPPDD$	cort.	23	±4	0		0		0		0		0		0	
	med.			18	±1	24	±2	24	±2	24	±2	22	±1	16	±1
$A^{v}ac^{e}c^{e}bbPPDD$	cort.	0		0		0		0		0		0		0	
	med.			0		1	±1	0		0		0		0	
$A^{v}aCCBBppDD$	cort.	14	±4	5	±3	0		0		0		0		0	
	med.			41	±2	45	±3	41	±3	35	±3	38	±2	34	±3
$A^{v}ac^{ch}c^{ch}BBppDD$	cort.	0		0		0		0		0		0		0	
	med.			13	±2	23	±4	20	±3	19	±3	22	±3	17	±2
$A^{v}aCCBBPPdd$	cort.	8	±3	2	±1	1	±1	0		0		0		0	
	med.			33	±4	41	±4	38	±3	41	±3	32	±3	24	±2
$A^{v}ac^{ch}c^{ch}BBPPdd$	cort.	5	±3	0		0		0		0		0		0	
	med.			11	±3	15	±3	16	±3	16	±3	14	±3	9	±2
$A^{v}aCCbbppDD$	cort.	15	±2	3	±2	0		0		0		0		0	
	med.			44	±2	37	±2	38	±2	38	±4	36	±3	24	±3
$A^{v}aCCbbPPdd$	cort.	5	±2	0		0		0		0		0		0	
	med.			29	±2	33	±3	32	±3	28	±5	27	±3	31	±6
$A^{v}aCCBBppdd$	cort.	0		0		0		0		0		0		0	
	med.			31	±4	44	±3	42	±4	38	±3	32	±2	36	±4
$A^{v}aCCbbppdd$	cort.	0		2	±2	0		0		0		0		0	
	med.			29	±2	39	±3	39	±4	42	±3	36	±3	42	±6

in the most intense non-agouti types down to zero in all albinos and certain extreme dilute types. The number of granules per unit volume varies along the length of the hair in different ways under different conditions. In general, heavy cortical pigment is found only near the tip, and only in certain very intensely colored types. It drops out irregularly in the mid-region of the hair. In certain other lightly colored types, no cortical pigment is produced. This

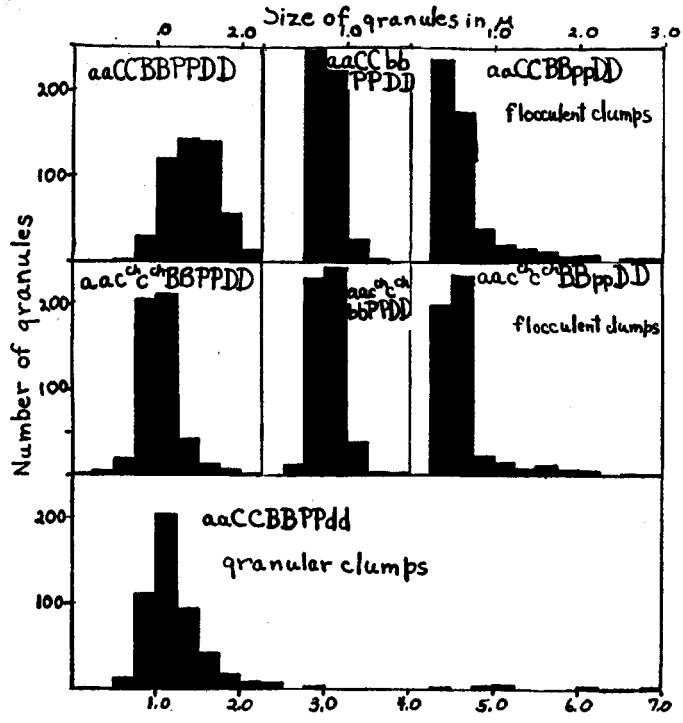

FIGURE 2.—Distribution of granule sizes in seven representative non-agouti genotypes. The total for each genotype is 500 granules; their greatest diameters are measured to the nearest 0.25μ interval.

is usually associated with a hesitation in the commencement of pigmentation in the medulla, likewise found only in lightly pigmented types (in intensely colored types the medullary pigment is evenly distributed along the length of the hair). We have called this tendency to smaller numbers of medullary pigment granules near the tip of the hair "pigmentation lag." Figure 1 shows graphically the amount and type of variation in granule counts found along the axis of ten different genotypes.

One further set of granule counts from whole mounts should be recorded here. Slides were made of six samples from each of six different light-bellied agouti genotypes. The means of the counts of these slides at successive levels are given in table 4. In each case, number (and also size, shape, and color of

granules, not listed here) of the granules in the agouti corresponds better with the non-agouti partner at the tip, then between fields six or seven and 15 to 18, with the yellow partner, then throughout the remainder of the hair length, with the non-agouti again.

TABLE 4

The mean number of granules at successive levels in six agouti genotypes. (Cort.=granules/unit cortical volume; med.=granules/medullary cell; M=mean; E=standard error; field=width at 1800×). The double vertical lines mark off the measurements corresponding with yellow.

GENOTYPE	REGION	FIELD											
		4TH		10TH		20TH		30TH		40TH		50TH		60TH	
		M	E	M	E	M	E	M	E	M	E	M	E	M	E
$A^wA^wCCBBPPDD$	cort.	201	±6	11	±6	26	±3	23	±4	0		0		0	
	med.			43	±3	99	±1	88	±3	95	±3	95	±3	93	±1
$A^wA^vc^cc^cBBPPDD$	cort.	50	±6	0		0		0		0		0		0	
	med.			3	±2	34	±4	39	±2	37	±3	44	±3	32	±2
$A^wA^wCCbbPPDD$	cort.	150	±10	12	±3	18	±9	0		0		0		0	
	med.			45	±2	85	±5	89	±5	82	±5	82	±4	86	±7
$A^wA^vc^cc^cbbPPDD$	cort.	38	±4	0		0		0		0		0		0	
	med.			2	±1	32	±5	51	±4	58	±4	62	±4	52	±4
$A^wA^wCCBBppDD$	cort.	49	±7	8	±2	3	±2	0		0		0		0	
	med.			52	±2	65	±4	78	±3	80	±4	88	±5	77	±3
$A^wA^vc^{ch}c^{ch}BBppDD$	cort.	0		0		0		0		0		0		0	
	med.			19	±2	32	±4	41	±4	56	±4	49	±4	38	±4

Description of individual medullary granules found in the various genotypes, as determined from cross sections, are given in table 5. The table includes for each genotype the matching with Ridgway's Color Standards, remarks on the granule shape, and the mean and standard error of the measurements of the greatest diameter of 500 granules from the cross sections of five different samples. The colors fall into three series, the aniline-yellow (or raw-sienna) series of the *A^va* genotypes, the black-fuscous series of the *aaBB* genotypes, and the carob-mummy brown series of the *aabb* genotypes. There are three grades of intensity in the black-fuscous series, grading down from intense black to fuscous black (or deep blackish-brown 3), and finally to fuscous (or dusky drab). There are two grades of intensity in the brown series, grading down from carob-brown (or chestnut-brown) to mummy brown (or raw umber). There is only one grade of intensity in the aniline-yellow series. The shape varies from long ovals for *aaCCBBPPDD* through shorter ovals, spheres, to irregular shred-like shapes which may be either elongated or roundish. The mean greatest diameter of medullary granules varies from $1.44 \pm .013\mu$ for *aaCCBBPPDD* down to $0.61 \pm .013\mu$ for *aaCCbbppDD*. Figure 2 shows graphically the distribution of the diameter measurements in seven representative

non-agouti genotypes, and demonstrates that there is considerable variation both in means and in type of distribution. The narrowest range of granule sizes is in full color brown, *aaCCbbPPDD*, where it is only 1.0μ, from 0.5 to 1.5μ. The widest range in one genotype is found in dilute black (*aaCCBBPPdd*) where it is 6.5μ, from 0.5μ up to 7.0μ. Genotypes with clumping such as will be described in the next section tend to have skewed distributions of granule size with a few very large pigment bodies. No distributions for yellow genotypes are given graphically here, since they show little variation among the genotypes tested.

TABLE 5

Description of individual granules, taken from cross sections, in all of the genotypes tested. The measurements are means of the greatest diameters of 500 granules from five samples of each type, graded by 0.25μ intervals. The colors are evaluated by matching with Ridgway's color charts. The shape is the usual one for each genotype, recorded for each sample at the time of measuring.

GENOTYPE	COLOR	SHAPE	DIAMETER
aaCCBBPPDD	intense black	long oval	1.44 ± .013
aac^{ch}c^{ch}BBPPDD	fuscous black	oval	1.05 ± .010
aac^{e}c^{e}BBPPDD	fuscous	round	0.94 ± .014
aaCCbbPPDD	carob-brown	round	0.77 ± .007
aac^{ch}c^{ch}bbPPDD	carob-brown	round	0.79 ± .008
aac^{e}c^{e}bbPPDD	carob-brown	round	0.77 ± .012
aaCCBBppDD	fuscous	long shreds	0.64 ± .018
aac^{ch}c^{ch}BBppDD	fuscous	long shreds	0.62 ± .015
aaCCBBPPdd	fuscous black	oval	1.23 ± .011
aac^{ch}c^{ch}BBPPdd	fuscous black	oval	1.12 ± .025
aaCCbbppDD	mummy-brown	short shreds	0.61 ± .013
aaCCbbPPdd	carob-brown	round	0.98 ± .020
aaCCBBppdd	fuscous	long shreds	0.73 ± .022
aaCCbbppdd	mummy brown	short shreds	0.67 ± .023
A^{v}aCCBBPPDD	aniline yellow	round	0.83 ± .013
A^{v}ac^{ch}c^{ch}BBPPDD	aniline yellow	round	0.77 ± .009
A^{v}aCCbbPPDD	aniline yellow	round	0.82 ± .010
A^{v}ac^{ch}c^{ch}bbPPDD	aniline yellow	round	0.76 ± .010
A^{v}aCCBBppDD	aniline yellow	round	0.80 ± .011
A^{v}ac^{ch}c^{ch}BBppDD	aniline yellow	round	0.66 ± .010
A^{v}aCCBBPPdd	aniline yellow	round	0.79 ± .012
A^{v}ac^{ch}c^{ch}BBPPdd	aniline yellow	round	0.77 ± .017
A^{v}aCCbbppDD	aniline yellow	round	0.79 ± .011
A^{v}aCCbbPPdd	aniline yellow	round	0.82 ± .015
A^{v}aCCBBppdd	aniline yellow	round	0.81 ± .013
A^{v}aCCbbppdd	aniline yellow	round	0.82 ± .017

Table 6 records the observations from both whole mounts and sections on the two remaining granule attributes, tendency to distal arrangement and tendency to clumping. Three grades of distal arrangement are distinguished: "none," indicating that the entire cell is evenly filled with pigment granules; "slight," indicating a small proximal space clear of pigment granules; and

TABLE 6

Description of granule arrangement within the medullary cells of the genotypes tested.

GENOTYPES	DISTAL ARRANGEMENT	CLUMPING
aaCCBBPPDD	none	none
aac^{ch}c^{ch}BBPPDD	none	none
aac^{e}c^{e}BBPPDD	cap	none
aaCCbbPPDD	none	none
aac^{ch}c^{ch}bbPPDD	none	none
aac^{e}c^{e}bbPPDD	cap	none
aaCCBBppDD	slight	flocculent
aac^{ch}c^{ch}BBppDD	cap	flocculent
aaCCBBPPdd	slight	granular
aac^{ch}c^{ch}BBPPdd	slight	granular
aaCCbbppDD	cap	flocculent
aaCCbbPPdd	slight	granular
aaCCBBppdd	cap	flocculent and granular
aaCCbbppdd	cap	flocculent and granular
A^{v}aCCBBPPDD	cap	none
A^{v}ac^{ch}c^{ch}BBPPDD	cap	none
A^{v}aCCbbPPDD	cap	none
A^{v}ac^{ch}c^{ch}bbPPDD	cap	none
A^{v}aCCBBppDD	cap	none
A^{v}ac^{ch}c^{ch}BBppDD	cap	none
A^{v}aCCBBPPdd	cap	granular
A^{v}ac^{ch}c^{ch}BBPPdd	cap	granular
A^{v}aCCbbppDD	cap	none
A^{v}aCCBBppdd	cap	granular
A^{v}aCCbbppdd	cap	granular

"cap," indicating that the greater part of the cell is clear with the pigment confined to a narrow distal cap. In the "slight" and "cap" types there is in addition to the proximal clear space another localization of the pigment granules in certain regions of the hair. In the middle sections of zig-zags, between the first and third bending constrictions, the medullary cells usually contain two distinct rows of granules, above and below a clear space which seems to be a rigid bar across the middle of the cell.

Two qualitatively different states are recorded in the "clumping" column of table 6; "flocculent" type and "granular" type. In the first there are loose flocculent masses, often as big as 3μ in diameter, which cannot easily be determined to be composed of clumped granules. In the granular type there are large sharply defined clumps which may quite possibly be composed of the regular granules of the genotype concerned cohering to each other. Points of deeper color, resembling individual granules, are often seen within the medullary cells but occasionally form separate entities attached to the inner wall of the cortical cylinder. This type of clump is often very large and conspicious,

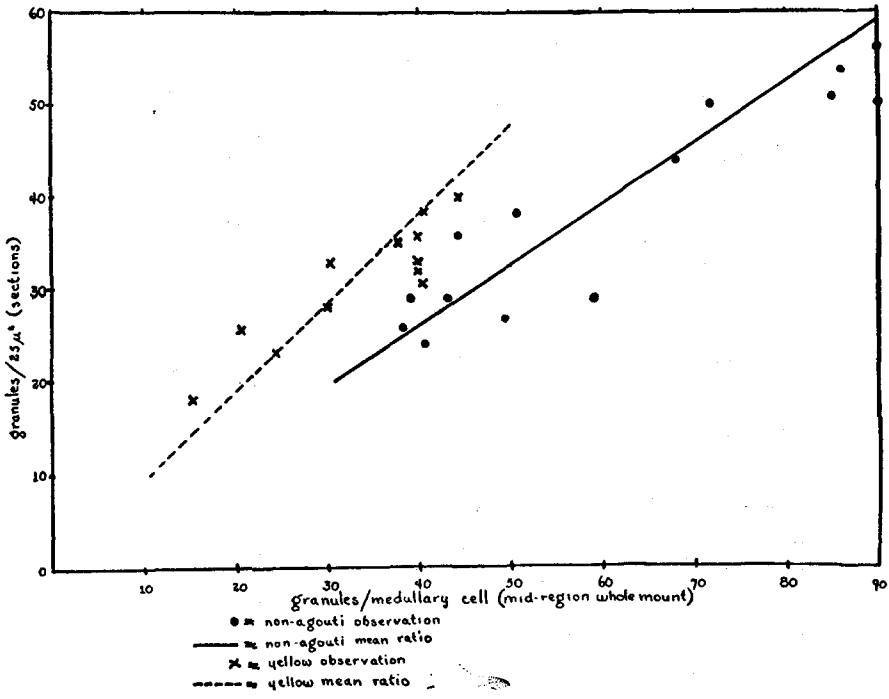

FIGURE 3.—Ratio of granule counts from cross-sections to counts from the mid-region of whole-mounts.

up to 7μ in diameter, and may be seen at magnifications considerably lower than those used in this study. Both types of clumps are very few in number relative to the smaller granules, as can be seen from the distributions in figure 2. In the types which have granular clumping many of the granules which are not actually clumped are somewhat irregularly arranged, giving the hair a spotty appearance at low magnifications. This irregularity and the large size of granular clumps make it possible to distinguish definitely between certain flocculently-clumped genotypes which also have granular clumping, and others which are similar except that they have no granular clumps.

Although the difficulties of making granule counts from cross sections, where the exact location on the hair axis cannot be controlled, have been mentioned, estimates have been made for all the genotypes tested and have led to results worth reporting. These cross-section counts are especially welcome, because they give independent confirmation of certain whole-mount findings such as essential identity of granule number in certain different genotypes (*aaCCBBPPDD*, *aac^{ch}c^{ch}BBPPDD*, *aaCCbbPPDD*, and *aac^{ch}c^{ch}bbPPDD*, for example). Table 7 and figure 3 give the mean of ten counts of the number of granules in a $25\mu^2$ section of a cross section of a medullary cell in each genotype tested, and compare these values with those obtained from the whole-mount counts. Since it seems that all of the sections must be taken from levels corresponding to the region of whole hairs lying between the 15th and 45th fields, the value used in comparing the whole mounts with the sections was the mean of the number of granules per medullary cell, combining counts from the 20th, 30th, and 40th fields. The non-agouti types show a fairly constant ratio of section to whole-mount value, the mean ratio being $0.65 \pm .02$, and only one value, 0.82 for *aaCCBBppdd*, differing from the mean of means by more than twice the standard error of the difference. The yellow types also show a fairly constant ratio of section to whole-mount count, although the mean ratio, $0.95 \pm .04$, is very different from that found for non-agoutis. This difference must be due at least in part to the extreme tendency for the granules of yellow to be concentrated in the distal portion of the cell, so that a fully pigmented area of $25\mu^2$ will contain most of the granules from an entire cell. In the yellows, means of three genotypes, *A^{v}ac^{ch}c^{ch}BBppDD*, *A^{v}ac^{ch}c^{ch}BBPPdd*, and *A^{v}aCCBBppdd*, differ from the mean of means by more than twice the standard error of the difference. Considering the amount of variation in pigment distribution along the hair axis and within the cells, the observed variations in ratio are not surprising, and the results of cross-section counts are sufficiently accurate to help in interpretation of results from the more accurate whole-mounts, particularly in cases where the whole-mounts are very crowded.

The results given in this paper have shown clearly that mouse coat color differences cannot arise merely from differences in diffraction from different sized pigment granules, as has been suggested as a possibility (DUNN and EINSELE 1938; DANIEL 1938). Table 8 shows that variations in many factors may lead to color changes. In certain cases there are differences in granule size which account for the entire difference in appearance between two genotypes, such as the black *aaCCBBPPDD* (mean greater diameter $1.44 \pm .013\mu$, granules/medullary cell, mid-region, 90 ± 2) and the dark sepia *aac^{ch}c^{ch}BBPPDD* (mean greater diameter $1.05 \pm .010\mu$, granules/medullary cell, mid-region 86 ± 2). The difference in appearance is not nearly as great here, however, as that found in other cases where granule size is identical. For instance, the mean size of granules in *A^{v}ac^{ch}c^{ch}BBPPDD* is $0.77 \pm .009\mu$, and the mean size in *aaCCbbPPDD* is $0.77 \pm .007\mu$; yet one is cream, the other dark brown. The aniline yellow color of the granules in *A^{v}ac^{ch}c^{ch}BBPPDD* and the carob-brown color of granules in *aaCCbbPPDD* appear to be much more pertinent

to the difference in general appearance than do the granule sizes. In this case, however, there is quite a difference between the types in number of granules, which undoubtedly contributes to the difference in appearance. Let us com-

TABLE 7

Medullary granule counts from unit areas ($25\mu^2$) of cross sections of genotypes tested, and their comparison with whole-mount counts from the corresponding mid-region (20th, 30th, 40th fields).

GENOTYPE	SECTIONS GRANULES/$25\mu^2$ M E	WHOLE-MOUNT GRANULES/MED. CELL M E	RATIO SECTIONS/WH. M. M E
aaCCBBPPDD	50±2	90±2	.56±.03
aac^{ch}c^{ch}BBPPDD	54±2	86±2	.63±.03
aac^{e}c^{e}BBPPDD	29±1	39±2	.74±.05
aaCCbbPPDD	56±1	90±2	.62±.02
aac^{ch}c^{ch}bbPPDD	52±1	85±2	.61±.02
aac^{e}c^{e}bbPPDD	29±1	43±2	.67±.04
aaCCBBppDD	29±1	59±2	.49±.02
aac^{ch}c^{ch}BBppDD	26±1	38±3	.68±.06
aaCCBBPPdd	44±1	68±3	.65±.03
aac^{ch}c^{ch}BBPPdd	50±1	72±2	.69±.02
aaCCbbppDD	27±1	49±2	.55±.03
aaCCbbPPdd	38±1	51±3	.75±.05
aaCCBBppdd	36±1	44±2	.82±.04*
aaCCbbppdd	24±1	41±2	.59±.04
mean of non-agouti ratios			.65±.02
A^{v}aCCBBPPDD	40±1	44±2	.91±.05
A^{v}ac^{ch}c^{ch}BBPPDD	28±1	30±1	.93±.05
A^{v}aCCbbPPDD	36±1	40±2	.90±.05
A^{v}ac^{ch}c^{ch}bbPPDD	23±1	24±1	.96±.06
A^{v}aCCBBppDD	38±1	41±2	.93±.05
A^{v}ac^{ch}c^{ch}BBppDD	26±1	21±2	1.24±.10*
A^{v}aCCBBPPdd	32±1	40±2	.80±.04
A^{v}ac^{ch}c^{ch}BBPPdd	18±1	16±2	1.13±.02*
A^{v}aCCbbppDD	35±1	38±2	.92±.05
A^{v}aCCbbPPdd	33±1	31±2	1.06±.08
A^{v}aCCBBppdd	31±1	41±2	.76±.04*
A^{v}aCCbbppdd	33±1	40±2	.83±.05
mean of all yellow ratios			.95±.04

* Differs from the mean of means for non-agoutis or yellows by more than twice the standard error of the difference.

pare $A^{v}aCCBBPPDD$ (mean greater diameter of $0.83 \pm .013\mu$, granules/medullary cell, mid-region 44 ± 2) with $aac^{e}c^{e}bbPPDD$ (mean greater diameter $0.77 \pm .008\mu$, granules/medullary cell, mid-region, 43 ± 2). Here both number and size of granules are very close in the two types, yet one appears yellow, the other light brown. The important fact here seems to be that the individual granules in the yellow type are aniline yellow, while those in the extreme dilute brown are carob-brown. We can also pick out cases where a difference in appearance is due entirely to a difference in number of granules. Let us compare two types which look just alike, *aaCCbbPPDD* (mean greater diameter

TABLE 8

Analysis of differences in granule attributes responsible for observed coat-color differences, demonstrating that these cannot be due merely to changes in granule size, but to different factors under different conditions.

		SIZE DOES IT		
aaCCBBPPDD	size	$1.44 \pm .013\mu$	gr./med. cell	90 ± 2
$aac^{ch}c^{ch}BBPPDD$	size	$1.05 \pm .010\mu$	gr./med. cell	86 ± 2
		COLOR DOES IT (AIDED BY NUMBER)		
$A^{v}ac^{ch}c^{ch}BBPPDD$	size	$0.77 \pm .009\mu$	color yellow (looks cream)	28 ± 1
aaCCbbPPDD	size	$0.77 \pm .007\mu$	color carob brown (brown)	56 ± 1
		COLOR ALONE DOES IT		
$A^{v}aCCBBPPDD$	size	$0.83 \pm .013\mu$	gr./med. cell	44 ± 2 (yellow)
$aac^{e}c^{e}bbPPDD$	size	$0.77 \pm .008\mu$	gr./med. cell	43 ± 2 (brown)
		NUMBER ALONE DOES IT		
aaCCbbPPDD	size	$0.77 \pm .007\mu$	gr./med. cell	90 ± 2 (dark)
$aac^{ch}c^{ch}bbPPDD$	size	$0.79 \pm .008\mu$	gr./med. cell	85 ± 2 (dark)
$aac^{e}c^{e}bbPPDD$	size	$0.77 \pm .012\mu$	gr./med. cell	43 ± 2 (light)

$0.77 \pm .007\mu$, granules/medullary cell, mid-region, 90 ± 2) and $aac^{ch}c^{ch}bbPPDD$ (mean greater diameter $0.79 \pm .008\mu$, granules/medullary cell, mid-region, 85 ± 2), with a third which differs from them only in granule number, $aac^{e}c^{e}bbPPDD$ (mean greater diameter, $0.77 \pm .012$, granules/medullary cell, mid-region, 43 ± 2). The first two of these types are dark brown, the third light brown. The pigment granules in all three are not only identical in size, they are all carob-brown. Thus only the difference in granule number can account for the change in appearance with this extreme-dilute substitution. The other granule attributes which we have studied have all undoubtedly contributed to the general coat color, although we cannot pick out any case where one of these is the sole cause of a difference in appearance; granule shape is important (particularly in pink-eyes), also arrangement of granules (especially the clumping of dilutes), and pigmentation lag (particularly in pink-eyes).

So we have an overall picture of a large number of different variable pigmentation attributes contributing to the differences in appearance of these coat-color mutants of the house mouse. In later papers we will discuss the interrelations of these attributes and attempt to determine the factors responsible for their observed variations.

SUMMARY

A quantitative histological analysis has been made of the pigment granules in representative mouse hairs from the first coat of animals carrying various combinations of the following allelic series of genes: albino series (*C*, c^{ch}, c^{e}, and c^{a}), agouti series (A^{w}, A^{y}, and *a*), black-brown, (*B*, *b*), pink-eye dilution (*P*, *p*), and dilution (*D*, *d*). The materials for these studies were whole-mounts, prepared with alcohol, alcohol-xylol, and xylol, and mounted in balsam, and cross sections, prepared by the Hardy method, using methyl-crylate as a coating substance, and mounted in balsam.

The granule attributes found to vary among the various genotypes were: (1) The number of granules per medullary cell, counted at 10-field intervals along the axis of whole-mount hairs. The mean for genotypes varied from 98 ± 2 for the 10th field of *aac*^{ch}*c*^{ch}*BBPPDD* down to zero in all albinos and certain extreme dilute types. In some types there is a tendency to variation in the number of granules per medullary cell at different points along the hair axis. (2) The number of cortical granules per unit volume equal to that of an average medullary cell. The cortical granules are generally found only near the tip of the hair, and the mean number at the 4th field varied from 219 ± 7 for *aaCCBBPPDD* down to zero for all albinos, unpigmented extreme dilutes, and certain pink-eyed types. (3) The tendency to distal arrangement of granules within medullary cells. In the most intensely colored types the granules fill the entire cell, but in intermediate types there is a small clear space at the proximal side of the cell, and in lightly colored types this clear space extends so far that all the pigment is located in a small distal cap. (4) Size of granules in medullary cells. The greatest diameter of 500 granules from each genotype showed that the mean size varied from $1.44 \pm .013\mu$ for *aaCCBBPPDD* down to $0.61 \pm .013\mu$ for *aaCCbbppDD*. The range of particle size also varies with genotype. The narrowest range, in *aaCCbbPPDD*, is 1μ from 0.5–1.5μ greater diameter; the widest, in *aaCCBBPPDD*, is 6.5μ, from 0.50μ–7.00μ greater diameter. In all types with a very wide range of granule sizes, the distributions are very skew, with a few particles (clumps) near the upper limit. (5) Shape of granules in medullary cells. Four basic granule shapes are found characterizing the various genotypes: long oval, oval, round, and irregular shred-shaped. (6) The clumping of granules. Two types of clumps are found in certain of the genotypes: loose flocculent masses (up to 3μ) and sharply defined granular clumps (up to 7μ diameter). (7) The color of pigment granules. These fall into three color series; (I) the yellow series, with only one grade of intensity, all granules matching with the aniline yellow or raw-sienna swatches in Ridgway's Color Standards; (II) the black fuscous series, with three grades of intensity, of which pure black is the darkest, fuscous black (or blackish-brown-3) is intermediate, and fuscous (or dusky drab) is lightest; and (III) the brown series, with two grades of intensity, of which carob-brown (or chestnut-brown) is the darker, and mummy-brown (or raw umber) the lighter.

Differences in all of these variable granule attributes have been shown to

make important contributions to the final coat color differences among the 36 genotypes observed in this paper.

LITERATURE CITED

BAKER, M. R., and A. C. ANDREWS, 1944 The melanins. I. Studies of the hair pigments of the guinea pig. Genetics **29**: 104–112.

DANIEL, J., 1938 Studies of multiple allelomorphic series in the house mouse. III. A spectrophotometric study of mouse melanin. J. Genet. **36**: 139–143.

DANNEEL, R., 1936 Die Färbung unserer Kaninchenrassen und ihre histogenetischen Grundlagen. Z.i.A.V. **71**: 231–264.

DANNEEL, R. and K. SCHAUMANN, 1938 Zur Physiologie der Kälteschwärzung beim Russenkaninchen. Biol. Zbl. **58**: 242–260.

DRY, F. W., 1926 The coat of the mouse. J. Genet. **16**: 287–340.

1928 The agouti coloration of the mouse (*Mus musculus*) and the rat (*Mus norvegicus*). J. Genet. **20**: 131–145.

DUNN, L. C., 1936 Studies of multiple allelomorphic series in the house mouse. I. Description of agouti and albino series of allelomorphs. J. Genet. **33**: 443–453.

DUNN, L. C., and W. EINSELE, 1938 Studies of multiple allelomorphic series in the house mouse. IV. Quantitative comparisons of melanins from members of the albino series. J. Genet. **36**: 145–152.

ENGELSMEIER, W., 1935 Nachweis der alternativen Modifikabilität der Haarfarbung beim Russenkaninchen. Z.i.A.V. **68**: 361–416.

GINSBURG, B., 1944 The effects of the major genes controlling coat color in the guinea pig on the dopa oxidase activity of skin extracts. Genetics **29**: 176–198.

HADJIDIMITROFF, P., 1933 Die Pigmentverteilung im Kaninchenhaar. Z. Zücht. **27**.

HALDANE, J. B. S., 1927 The comparative genetics of colour in rodents and carnivora. Biol. Rev. **11**: 199–212.

HARDY, J. I., 1935 A practical method of making thin cross-sections of fibers. Circ. U.S. Dept. Agric. **378**: 1–10.

HARMON, M. and A. A. CASE, 1941 Genetic aspects of pigment production in the guinea pig. Genetics **26**: 474–487.

HEIDENTHAL, G., 1940 A colorimetric study of genic effect on guinea-pig coat color. Genetics **25**: 197–214.

HUNT, H. R., and S. WRIGHT, 1918 Pigmentation in guinea pig hair. J. Hered. **9**: 178–181.

KELLOGG, R., 1941 The preparation of mouse hairs for gross microscopical examination, with special reference to yellow pigmentation. Unpubl. Summer Student Report, Jackson Memorial Laboratory.

LITTLE, C. C., 1913 Experimental studies of the inheritance of color in mice. Carnegie Inst. Washington Publ. **179**: 11–102.

LÜBNOW, E., 1939 Die Wirkung auf die Pigmentbildung im Kaninchenkaar. Z.i.A.V. **77**: 516–532.

LÜHRING, R., 1928 Das Haarkleid von *Sciurus vulgaris L.* und die Verteilung seiner Farbavrianten in Deutschland. Z. Morph. Okol. Tiere **11**.

REED, S. C., 1938a Determination of hair pigments. II. Transplantation results in mice, rats and guinea pigs. J. Exp. Zool. **79**: 347–354.

1938b Determination of hair pigments. III. Proof that expression of the black-and-tan gene is dependent upon tissue organization. J. Exp. Zool. **79**: 337–346.

REED, S. C. and ARMINE ALLEY, 1939 The production of pigment in grafts of mouse skin grown on the chorio-allantois of White Leghorn chicks. Anat. Rec. **73**: 257–267.

REED, S. C., and G. SANDER, 1937 Time of determination of hair pigments in the mouse. Growth **1**: 194–200.

RIDGWAY, R., 1912 Color standards and color nomenclature. Distributed by A. Hoen and Co.

RUSSELL, E. S., 1939 A Quantitative study of genic effects on guinea pig coat colors. Genetics **24**: 332–355.

RUSSELL, W. L., 1939 Investigation of the physiological genetics of hair and skin color in the guinea pig by means of the dopa reaction. Genetics **24**: 645–667.

SCHILLING, L., 1939 Untersuchung zur Variabilität der Meerschweinchen-haare und ihre Pigmente. Z.i.A.V. **76**:533–569.

WERNEKE, F., 1916 Die Pigmentierung der Farbenrassen von *Mus musculus* und ihre Beziehung zur Vererbung. Arch. EntwMech. **42**: 72–106.

WRIGHT, S., 1916 An intensive study of the inheritance of color and other coat characters in guinea pigs with especial reference to graded variation. Carnegie Instu. Washington Publ. **241**: Part II, 59–121.

1917–1918 Color Inheritance in Mammals, I–XI. J. Hered. **8** and **9**.

1925 The factors of the albino series of guinea pigs and their effects on black and yellow. pigmentation. Genetics **10**: 223–260.

1927 The effects in combination of the major color factors of the guinea pig. Genetics **12**: 530–569.

1942 The physiological genetics of coat color of the guinea pig. Biol. Symposia **6**: 337–355.