A QUANTITATIVE HISTOLOGICAL STUDY OF THE PIGMENT FOUND IN THE COAT COLOR MUTANTS OF THE HOUSE MOUSE. II. ESTIMATES OF THE TOTAL VOLUME OF PIGMENT[1]

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A DETAILED quantitative study of the histology of the pigment granules found in the coat color mutants of the house mouse has been undertaken as a step toward understanding the action of the genes affecting this pigmentation. The first paper of a series giving the results of this study (E. S. RUSSELL 1946) has described the variable attributes of these granules and their condition in each of the genotypes tested. The presentation was entirely analytical: the granules were counted and measured, and their shape, color and distribution recorded. No attempt was made to synthesize the results into a measure of the total amount of melanin produced in each genotype. A strictly analytical approach was maintained as a reaction against one of the chief limitations in earlier quantitative studies of mammalian pigment (DUNN and EINSELE 1938, E. S. RUSSELL, 1939) where the observed differences in weight or volume could not be analyzed further. This difficulty has made it impossible to distinguish qualitatively between any different types of pigment reduction which might result from different genic substitutions. Histological analysis certainly has made such qualitative distinctions possible and in some cases obvious.

Since the writing of the above-mentioned paper, however, it has become clear that some sort of estimate of the total amount of pigment found in each genotype is essential to interpretation of certain types of data. For example, measurements of enzyme content of various genotypes (W. L. RUSSELL 1939, BRAUCH and RUSSELL 1946a) need to be compared with the amounts of pigment present to determine the directness of the relationship between enzyme concentration and pigment production.

There are various possible ways of comparing the total amount of pigment present in various genotypes. Up to the present it has not been possible to obtain the limited comparisons possible with mean color grades determined for each genotype, as no visual series of color grades, such as used by WRIGHT (1915, 1917, 1927) for the guinea pig, has been made for the mouse. Weights of pigment have been determined for some of the sepia genotypes by DUNN and EINSELE (1938). Pigment weight determinations, however, are very difficult to make for the yellow genotypes, as the pigment is very light. A thorough

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study of the amount of pigment in extracts of the yellow genotypes of the mouse has been made by BRAUCH and W. L. RUSSELL (1946b) but no one has as yet been able to find a method which will extract the very insoluble sepia pigments. Thus the quantity of pigment in all mouse color genotypes has never been measured by a single method, and adequate material is not available for the comparisons which must be made for interpretation of gene action.

This second section of the quantitative histological study of mouse hair pigment is an attempt to find a series of comparable values, obtained by the same method for all genotypes, which are proportional to the volume of pigment deposited either in a complete hair or in some particular region thereof. Much of the data quoted is taken directly from the first paper of the series, where the reader may find definition of the terms used and description of the general methods of study. The destruction by fire of W. L. RUSSELL'S stocks of mice carrying the various genetic color combinations upon which all of these studies have been based has made doubly important the immediate publication of the best possible estimate of pigment content from data already available.

METHOD OF ESTIMATING MEAN GRANULE VOLUME

Obtaining an estimate of the total volume of pigment in a hair or portion of a hair from histological data involves finding a value (1) proportional to the total number of granules in that region and multiplying this figure by a value (2) proportional to the mean volume of these granules. While the value proportional to granule number can be determined very easily, the published data on granule size are not so easily adapted to the present need. The size of pigment granules is given in the first paper of this series as the mean greater diameter of 500 granules of each genotype, estimated to the nearest .25μ. There is a wide range of sizes in certain genotypes, and differences in length of single granules which make relatively small differences in the mean when only one diameter is considered become much more important in measures of volume. This is especially true where there are very large pigment bodies, with lengths as much as seven times the mean, and widths proportionately large. Also, there is variation in granule shape both within and between genotypes which makes it impossible to translate measures of length directly to measures of volume.

Therefore, it seemed necessary for estimates of mean granule volume to get a value proportional to the volume of each individual granule by multiplying its length by the square of its width (lw^2). Both diameters were estimated to the nearest .25μ, although this could not be done very accurately as the micrometer scale was divided in 1μ units. As this is a tedious procedure, only 100 granules of each genotype were measured. Accuracy was checked by comparing the mean of the newly determined lengths with the completely independent measurements of the mean greater diameters of 500 granules previously given in paper I (table 1).

Although the newly determined lengths agree fairly well with the more accurate older data, these measurements can not give a perfect picture of granule volume. Since all had to be made on cross-section slides which include

pieces from the 15th to the 45th fields (unit of hair length) only, any variation in granule size near the ends of the hair would not be included in the data (there often is reduction at the apex). Also, the presence of a few very large pigment bodies, called "granular clumps" (E. S. RUSSELL 1946) in certain

TABLE 1

A comparison of two types of determinations of the mean length, in μ, of granules in the coat color mutants of the mouse. The first column gives the mean for each type of the length of 100 granules estimated to the nearest .25μ. The second gives the mean length of 500 granules estimated to the nearest .25μ.

GENOTYPE	NEW (100 GR.) MEAN LENGTH	OLD (500 GR.) MEAN LENGTH S.E.
aaBBCCDDPP	1.41	1.44± .013
aaBBc^{ch}c^{ch}DDPP	1.07	1.05± .010
aaBBc^{e}c^{e}DDPP	0.86	0.94± .014
aabbCCDDPP	0.81	0.77± .007
aabbc^{ch}c^{ch}DDPP	0.80	0.79± .008
aabbc^{e}c^{e}DDPP	0.83	0.77± .012
aaBBCCDDpp	0.66	0.64± .018
aaBBc^{ch}c^{ch}DDpp	0.64	0.62± .015
aaBBCCddPP	1.33	1.23± .011
aaBBc^{ch}c^{ch}ddPP	1.10	1.12± .025
aabbCCDDpp	0.68	0.61± .013
aabbCCddPP	0.90	0.98± .020
aaBBCCddpp	0.69	0.73± .022
aabbCCddpp	0.51	0.67± .023
A^{v}aBBCCDDPP	0.81	0.83± .013
A^{v}ac^{ch}c^{ch}DDPP	0.73	0.77± .009
A^{v}abbCCDDPP	0.81	0.82± .010
A^{v}abbc^{ch}c^{ch}DDPP	0.75	0.76± .010
A^{c}aBBCCDDpp	0.80	0.80± .011
A^{c}aBBCCDDpp	0.80	0.80± .011
A^{v}aBBc^{ch}c^{ch}DDpp	0.71	0.66± .010
A^{v}aBBCCddPP	0.76	0.79± .012
A^{v}aBBc^{ch}c^{ch}ddPP	0.77	0.77± .017
A^{v}abbCCDDpp	0.72	0.79± .011
A^{v}abbCCddPP	0.82	0.82± .015
A^{v}aBBCCddpp	0.85	0.81± .013
A^{v}abbCCddpp	0.92	0.82± .017

genotypes may lead to inaccuracies in estimate of pigment volume. To minimize difficulty from this source, studies of the distribution, frequency and size of clumps were undertaken, using both the cross sections and whole mounts of hairs of each dilute genotype. Although all types were examined, the best measurements of *frequency* of clumps were made in the whole mounts of the dilute yellow genotypes (five in all), where the low total number of pigmented bodies make it possible to count relative numbers of granules and clumps more accurately than in the more heavily pigmented sepias. In these yellows, clump frequency was determined in both awl and zigzag hairs at 10-field in-

tervals. Great irregularity of distribution was found both within and between hairs. The estimates obtained by this method for the ratio of clumps to total pigment bodies varied from 0.8 percent to 1.5 percent, with a mean of 1.1 percent. The most accurate measurements of mean clump *volume* were made from the cross-section slides. The length and width of 100 clumps from each dilute genotype was determined to the nearest micron. Unfortunately, both the slides and the measurements of six of these ten types were lost by fire, but as the value proportional to the mean volume of the clumps of the four remaining

TABLE 2

Values proportional to the mean size of pigmented bodies in dilute genotypes, where both small granules and large clumps contribute appreciably to total pigment. The first column gives the means of length×squared width of 100 granules (volume under 5μ^3) of each dilute genotype. In the second column this value is multiplied by 98.9 percent, the mean proportion of granules among total pigmented bodies. The third column gives the means of length×squared width of 100 clumps (volume over 5μ^3) of each dilute genotype. In the fourth column this value is multiplied by 1.1 percent, the mean proportion of clumps among total pigmented bodies. The final column gives the value proportional to mean volume of all pigmented bodies, that is, column 2 plus column 4.

GENOTYPE	GRANULE lw²/n S.E.	×98.9%	CLUMP lw²/n	×1.1%	TOTAL
aaBBCCddPP	1.63± .13	1.61	58.2	.64	2.25
aaBBc^{ch}c^{ch}ddPP	0.91± .06	0.90	54.5	.60	1.51
aabbCCddPP	0.79± .04	0.78	40.5	.45	1.23
aaBBCCddpp	0.31± .07	0.31	(50)	.55	0.86
aabbCCddpp	0.16± .04	0.16	(50)	.55	0.71
A^{v}aBBCCddPP	0.51± .05	0.50	41.6	.46	0.96
A^{v}aBBc^{ch}c^{ch}ddPP	0.58± .08	0.57	(50)	.55	1.12
A^{v}abbCCddPP	0.62± .03	0.61	(50)	.55	1.16
A^{v}aBBCCddpp	0.72± .06	0.71	(50)	.55	1.26
A^{v}abbCCddpp	0.56± .05	0.55	(50)	.55	1.10

dilute types ranged only from 41.6 to 58.2 cubic microns, it seems safe to estimate the other values as 50 (see table 2). In all dilute genotypes, the volume value upon which estimates of total pigment are based is determined by combining the mean size and frequency of both clumps and granules (mean granule volume×98.9 percent plus mean clump volume×1.1 percent, table 2).

Thus a method has been developed by which it is possible to measure reasonably satisfactorily the mean volume of the pigment granules in all of the coat color mutants. The data have been recorded as the mean and standard error of a value proportional to the mean volume of individual granules (lw²/n) of each of the genotypes (table 3).

TOTAL VOLUME OF PIGMENT DEPOSITED IN THE HAIR OF THE VARIOUS GENOTYPES

The mean volume data can be used with measures of granule number to determine the total volume of pigment in each genotype. In some cases the

TABLE 3

Values proportional to the amount of pigment produced in full-grown hairs of each of the genotypes tested. The first column gives a value proportional to the mean volume of individual granules of each type (lw^2/n). The second column gives the sum of all counts of the cortical granules, and is thus proportional to the total number of cortical granules deposited. The third is the sum of all counts of medullary granules and is proportional to the total number of medullary granules deposited. Multiplying the sum of the second and third column figures by the mean granule volume value gives column 4, the estimate of a value proportional to the total volume of pigment in a full-grown hair. Multiplying the third column only by the mean volume value gives column 5, a value proportional to the total volume of pigment in the medulla.

GENOTYPE	lw^2/n S.E.	C	M	$(lw^2/n)(C+M)$	$(lw^2/n(M)$
aaBBCCDDPP	1.53± .05	359	512	1332	783
aaBBc^{ch}c^{ch}DDPP	0.79± .06	374	506	695	400
aaBBc^{e}c^{e}DDPP	0.64± .06	22	208	147	133
aaBBc^{a}c^{a}DDPP		0	0	0	0
aabbCCDDPP	0.63± .06	258	504	480	318
aabbc^{ch}c^{ch}DDPP	0.62± .03	206	489	431	303
aabbc^{e}c^{e}DDPP	0.62± .05	34	223	159	138
aaBBCCDDpp	0.38± .08	37	344	145	130
aaBBc^{ch}c^{ch}DDpp	0.37± .12	0	218	81	81
aaBBc^{e}c^{e}DDpp		0	0	0	0
aaBBCCddPP	2.25	96	369	1046	830
aaBBc^{ch}c^{ch}ddPP	1.51	189	391	876	590
aabbCCDDpp	0.33± .06	29	290	105	96
aabbCCddPP	1.23	88	292	467	359
aaBBCCddpp	0.86	2	249	216	214
aabbCCddpp	0.71	0	224	159	159
A^{y}aBBCCDDPP	0.63± .06	54	240	185	151
A^{y}aBBc^{ch}c^{ch}DDPP	0.55± .05	25	149	96	82
A^{y}abbCCDDPP	0.61± .10	56	224	171	137
A^{y}abbc^{ch}c^{ch}DDPP	0.51± .07	23	128	77	65
A^{yy}aBBCCDDpp	0.66± .08	19	234	167	154
A^{y}aBBc^{ch}c^{ch}DDpp	0.41± .04	0	114	47	47
A^{y}aBBCCddPP	0.96	11	209	211	201
A^{y}aBBc^{ch}c^{ch}ddPP	1.12	5	81	96	91
A^{y}abbCCDDpp	0.45± .03	18	217	106	98
A^{y}abbCCddPP	1.16	5	180	215	209
A^{y}aBBCCddpp	1.26	0	223	281	281
A^{y}abbCCddpp	1.10	0	227	250	250

desired value may be the total amount produced throughout a complete growth cycle of the hair follicle, that is, the amount deposited in an entire hair. In others it may be more helpful to know what is deposited during a certain stage of hair growth, or in a certain region of the hair, such as cortex or medulla. By making the proper combination, of the data given in the first paper of this series on the number of granules deposited at successive levels along the hair shaft in both cortex and medulla, with these present data on mean granule volume, it should be possible to obtain the value desired for any purpose. As examples, it may be useful to give the values proportional to the total amount

produced throughout the entire growth cycle (table 3, column 5), the total amount deposited in the medulla of the hair (table 3, column 6), and the amount produced by the hair follicle while the mid-region of the hair (20th to 40th fields) is growing (table 4).

DISCUSSION

Each of the three series of values given in the preceding section on the total volume of pigment in each genotype may be of service in some particular type

TABLE 4

Values proportional to the amount of pigment deposited in the mid-region of the hair of each of the genotypes tested. Left is the non-agouti series, right the yellow. The figures represent the product of the names of length×squared width (lw^2/n) of the granules, multiplied by M_2, the sum of the counts of number of granules per medullary cell at the mid region (mid), that is, the 20th, 30th and 40th fields.

BACKGROUND GENOTYPE	M_2 mid	NON-AGOUTI (lw^2/n) (M_2)	M_2 mid	YELLOW (lw^2/n) (M_2)
BBCCDDPP	271	415	129	81
BB$c^{ch}c^{ch}$DDPP	258	204	89	49
BB$c^{e}c^{e}$DDPP	118	76		
bbCCDDPP	270	170	117	71
bb$c^{ch}c^{ch}$DDPP	258	160	72	37
bb$c^{e}c^{e}$DDPP	136	84		
BBCCDDpp	184	70	121	80
BB$c^{ch}c^{ch}$DDpp	114	42	62	25
BBCCddPP	201	452	120	115
BB$c^{ch}c^{ch}$ddPP	216	326	47	53
bbCCDDpp	150	50	113	51
bbCCddPP	152	187	93	108
BBCCddpp	132	114	124	156
bbCCddpp	122	87	120	132

of investigation. The first series, giving the total volume of pigment in full-grown hairs, should be especially useful for comparison with extractions of pigment, as all of the pigment, both cortical and the medullary, would be present in the extract. There is one limitation to the use of this series, however, which also limits extract work: minor modifiers and/or environmental variations appear to influence cortical pigment more than medullary, which complicates evaluation of the effects of major genic substitutions.

Thus the second series, in which medulla only is considered, reducing the causes of variation, may sometimes be more useful for the type of evaluation mentioned above. However, this series also has limitations, especially in the highly pigmented types. It is clear from table 3 that as medullary pigment increases cortical pigment usually increases even more, so that in considering medulla only a considerable portion of the increase in activity is missed.

The third series is more useful when there is a time factor in the data to be correlated. It has already been shown in the first paper of this series that

many genotypes have different rates of pigment production at different stages in the hair growth cycle. The usual finding is a "pigmentation lag," or low rate of production, near the tip of lightly or moderately pigmented types. This variable factor must be taken into account in collecting data where the figure obtained represents the condition at a particular moment rather than over a long period of time as, for example, the enzyme concentration at a certain stage in a growing hair follicle. BRAUCH and RUSSELL (1946a) have been very careful to measure the dopa reaction at a well-selected uniform stage, from six day old animals, in which the mid-region of the hair of the first pelage is developing. The data in table 4 are probably the best histological material avail-

TABLE 5

A comparison of pigment volume in corresponding dilute and intense genotypes. The first column of figures is the ratio of the volume of cortical and medullary pigment in dilute to intense (dd/DD) against each background, the second the ratio of medullary volumes only, and the third of the medullary volume of the mid-region.

BACKGROUND	RATIO *dd/DD* TOTAL	RATIO *dd/DD* MEDULLA	RATIO *dd/DD* MID-MEDULLA
aaBBCCPP	0.79	1.06	1.09
aaBBc^{ch}c^{ch}PP	1.24	1.48	1.60
aabbCCPP	0.97	1.13	1.10
aaBBCCpp	1.48	1.65	1.63
aabbCCpp	1.51	1.66	1.74
A^{v}aBBCCPP	1.14	1.33	1.42
A^{v}aBBc^{ch}c^{ch}PP	1.00	1.11	1.08
A^{v}abbCCPP	1.26	1.53	1.53
A^{v}aBBCCpp	1.69	1.76	1.95
A^{v}abbCCpp	2.36	2.56	2.59

able for comparison with their dopa results. Other workers who have timed their experiments differently could easily prepare from tables 2 and 3 of the first paper a different series of granule numbers to suit their needs.

Thus by obtaining a value proportional to the mean volume of the pigment granules and combining it with already available data on granula number, it has been possible to estimate the pigment content of each genotype tested. Data have been given for the purposes of comparison with extracts of pigment, for measurements of effects of genic substitution, and for comparison of pigment deposition at a certain stage with enzyme concentration at the same stage.

As this paper is intended solely as an addition to the body of data on variable granule attributes, the discussion should be restricted largely to a simple presentation of the differences found among the various genotypes. Further interpretation will be limited to correlation of the volume data with the condition of other granule attributes:

1. The highest members of the black-fuscous color series of genotypes

(*aaBB*) have by far the greatest volume of pigment. The highest volume in any yellow genotype is only about one-fifth that in the highest black-fuscous type, the highest brown slightly more than one-third.

2. In general within a color series the visual impression of color intensity depends more upon the total volume of pigment than upon either granule size (or shape) or number taken alone. For example, *aaBBc^{e}c^{e}DDPP* and *aaBBCCDDpp* have granules of very different size ($0.64 \pm .06\mu$, $0.38 \pm .08\mu$, table 3) and shape, the first round, the second shred-shaped. They also differ in total granule number (C+M, 230 and 381, table 3). However, the total volume of pigment is more nearly the same for the two (147 and 145, table 3), and the general appearance corresponds with the pigment volume.

3. An outstanding exception to the parallelism of pigment volume and visual impression of color is found in the dilute (Maltese) genotypes. In all cases the dilute type appears much lighter than its non-dilute counterpart. Yet in 27 of the 30 comparisons which it is possible to make (table 5, three measurements of pigment volume for each of ten corresponding pairs) between dilute and intense genotypes, the dilute has a higher pigment content than the corresponding intense type. While these data are not as accurate as could be desired, they do confirm completely the extract findings on dilute and non-dilute pairs (BRAUCH and RUSSELL 1946b). The visual effect can be attributed to the concentration of a large amount of the pigment in dilutes into the large clumps where it can have very little effect on light absorption.

SUMMARY

As a continuation of quantitative histological analysis of the action of the major coat color genes of the house mouse, this paper presents the volume of pigment, estimated in three different ways, in each of the 26 pigmented genotypes listed. The materials used were the slides prepared for the first paper of this series, and much of the data has been taken from that paper (E. S. RUSSELL 1946). The chief additional information is an estimate of the mean granule volume in each of these genotypes, based upon new measurements of the length and width of individual granules and clumps. These estimates of granule volume have been combined first with counts proportional to the total number of pigment granules in both cortex and medulla to get a series of values proportional to the total volume of pigment in each genotype, especially useful for comparison with extract data and for certain cases of quantitative evaluation of the effects of genic substitution. They have also been combined with counts proportional to the number of pigment granules in the medulla only, to get a second series of values proportional to the volume of pigment in the medulla of each genotype, which is more satisfactory for many cases of measurement of the effect of genic substitution. Still a third series of values, useful for comparison with data on enzyme concentration at a given time, was obtained by combining the mean volume estimates with counts proportional to number of medullary granules deposited during that time. Suggestions are made for the compilation of other series of pigment volumes which might be useful in other timed experiments.

The correlation of total pigment volume with other granule attributes is discussed briefly. The highest members of the black-fuscous color series are found to have by far the greatest volume of pigment. In most cases within a color series the visual appearance corresponds closely with the volume of pigment. An outstanding exception is found in types with Maltese dilution, which, although they appear much lighter than the corresponding intense types, have as high or higher total volume of pigment.

Comparable values proportional to the total volume of pigment deposited in the hair have now been obtained for 26 pigmented mouse genotypes by a single method. The results are far from perfect, but it is hoped that they are sufficiently accurate to provide a basis for interpretation of gene action.

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