COLORIMETRIC DETERMINATION OF THE AMOUNTS OF MELANIN IN THE HAIR OF DIVERSE GENOTYPES OF THE GUINEA PIG.[1]

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THE melanin pigments of mammals fall into two major categories, the darker pigments or eumelanins and the orange yellow pigments or phaeomelanins. These presumably differ qualitatively although the exact chemistry of the melanins is not yet known.

The hairs of wild cavies and of the so-called agouti variety of the guinea pig are predominantly eumelanic but have a phaeomelanic subterminal band. Replacement of *E*- by *ee* in the genotype of the agouti guinea pig (*EABCFP*) causes the whole hair to be yellow (at birth) without changing the intensity in the subterminal region. Replacement of *A*- by *aa* (with *E* present) causes the whole hair to be eumelanic without change of intensity of the regions which are eumelanic in agoutis. The combinations with *eeaa* are yellows (or whites), not distinguishable in the guinea pig at birth from those with *ee*A-. In adult animals, a small amount of eumelanin (sootiness) often develops near the tips of the hairs in yellows and this is inhibited in the characteristic subterminal band in the presence of *A*. There is a second allele of *E* (namely e^p), the presence of which (e^pe^p, e^pe) results in a tortoiseshell pattern of eumelanic and phaeomelanic areas similar in intensity to colors of corresponding varieties with *E* and *ee* respectively, except that some hairs may have mixtures of both kinds of pigment in reduced amounts.

Two major kinds of eumelanin, must be distinguished namely sepia and brown, depending in the guinea pig on *B*- and *bb* respectively (in association with genes which permit eumelanin to develop at all). A second pair of alleles distinguishes relatively intense (*P*-) and pale (*pp*) varieties of both sepia and brown. The intensities of all combinations of these genes are affected by a series of five alleles C, c^k, c^d, c^r, c^a. In albinos (c^ac^a) no pigment is present at birth, though some may develop later under the influence of low temperature. Intensities are highest in most cases with *C*, and intermediate, but in diverse orders, in the remaining combinations of the *C*-series.

The intensities of phaeomelanin are not affected appreciably by *B*, *b* or *P*, *p* but are affected by *F*, *f* (which have no apparent effect on eumelanin in the presence of *P*). The *C*-series effects the yellow as well as the dark pigments. The highest intensities are found with *C*. There is no yellow in c^ac^a, c^rc^a and c^rc^r (in this case even on exposure to low temperatures). The order of effect of the *C* series differs from any found in the eumelanic series. In the combination *ffpp*, replacement of *ee* by *E* reduces the intensity of phaeomelanin or wholly

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eliminates it, without replacing it by eumelanin, except perhaps in minute traces. The *ff*-yellows tend to become paler after birth to a much greater extent than the *F*-yellows.

Summing up, we may distinguish the following seven major series of uniformly pigmented varieties of the guinea pig, within each of which intensity depends on the *C*-series (WRIGHT 1927).

	Uniform eumelanic series	
Dark sepias	$E\text{-}aa\ B\text{-}P\text{-}\ (C, c^k, c^d, c^r, c^a)$	No effect of *F*,
Pale sepias	$E\text{-}aa\ B\text{-}ppF\text{-}\ (C, c^k, c^d, c^r, c^a)$	
Dark browns	$E\text{-}aa\ bbP\text{-}\ (C, c^k, c^d, c^r, c^a)$	No effect of *F*, *f*
Pale browns	$E\text{-}aa\ bbppF\text{-}\ (C, c^k, c^d, c^r, c^a)$	
	Uniform phaeomelanic series	
Persistent yellows	$ee\ F\text{-}\ (C, c^k, c^d, c^r, c^a)$	No effect of *A*, *a*; *B*, *b*; *P*, *p*.
Fading yellows	$ee\ ff\ (C, c^k, c^d, c^r, c^a)$	No effect of *A*, *a*; *B*, *b*; *P*, *p*
Anomalous yellows	*E-aaC-ffpp* (no pigment without *C*)	No effect of *B*, *b*.

Orange yellow solutions can be obtained in dilute alkali from the phaeomelanic varieties. These can be compared without difficulty with a single standard solution, making possible determination on a single scale.

The eumelanic series yield tea colored solutions in dilute alkali which cannot be compared colorimetrically with the yellow standard but which can be compared fairly well with a single standard from black hair. There are, however, slight but characteristic differences in quality in certain cases. These are not due to mingled granules of eumelanin and phaeomelanin. Mixtures of the two kinds of granules can indeed be seen under the microscope in some hairs of tortoiseshells and in the transition regions in agouti hairs. In the uniform eumelanics (*E-aa*), however, only one quality of granule can be recognized in each case. If there is a mixture it must be within the granules themselves. The appearance of pale yellow in *EaaCffpp* in which eumelanin is suppressed, in contrast with the uniformly eumelanic character of all granules in other genotypes with *Eaa*, suggests that some phaeomelanin may be mixed with the latter and be responsible, through variations in its proportions, for slight differences in quality. The qualitative differences among combinations of the *C*-series seem to fit this interpretation but those between sepias and browns, especially pale sepias and pale browns, do not and indicate a real qualitative difference rather than mixture, in this case.

It should be added that three additional series of uniform colors may be distinguished on the basis of imperfect dominance of *F*. There are also other minor variations in intensity that have a genetic basis but for which genes have not yet been isolated. The attempt has been made to avoid these by selection for intensity within each series of colors but they have been an important source of variability in the present study.

Finally it may be noted that several pairs of alleles are known (*S*, *s*; *Si*, *si*;

Gr, *gr*) in which the mutant allele causes loss of pigment (white) or in some cases dilution, in areas of the coat or in individual hairs, without affecting the intensity of the color in other areas as determined by the rest of the genotype. We shall not be concerned with effects of these genes in this paper.

For many years, it has been part of the routine in our guinea pig colony to grade the colors of all animals at birth by comparison with standard series of pelts (WRIGHT 1927). Sepias and browns have been graded by the same scale, ranging from grade 1, barely distinguishable from white, to 21 intense black. Yellows have been graded by a different scale ranging from grade 1, again barely distinguishable from white, to 13 the most intense red. In a relatively small number of cases, solutions of pigment have been made from weighed samples of hair for colorimetric comparisons with standard solutions. Two papers have been published from this laboratory giving such determinations for a considerable number of genotypes (E. S. RUSSELL 1939, HEIDENTHAL 1940). These dealt largely with the various kinds of yellows and with dark sepias. The primary purpose of this paper is to present data on pale sepias and dark and pale browns of diverse genotypes as well as additional dark sepias. A secondary purpose is to establish the relation between the colorimetric values and the grades assigned the same animals as a means of transforming the averages based on grades into estimates of colorimetric value. The results of this transformation will, however, be deferred to a later paper.

The grades are used here to provide a means by which colorimetric values based on different standard solutions may be reduced to comparability, and to determine by internal evidence a correction for the gradual fading of the standard during the rather long period (19 months) in which the present data were obtained.

GENOTYPES

There is so much overlapping in intensity that genotypes can not be assigned on the basis of coat color alone. Eye color gives some help since this is black in the presence of *BP*, and brown in the presence of *bbP*, except for slight reduction in c^rc^rBP (dark red) and c^rc^rbbP (brown red), greater reduction in c^rc^aBP (light red) and c^rc^abbP (light brown red), and complete absence of pigment in c^ac^aBP and c^ac^abbP (both pink). With *pp*, eye color is always pink, though small amounts of pigment are present in most cases. A gene *sm* which GREGORY (1928) has described as reducing eye pigmentation has not been present in this stock.

The primary basis for assignment of genotype in cases of overlap is pedigree. Except for tests of doubtful cases, matings have been largely restricted to animals whose genotypes were considered certain in the significant respects, from color and ancestry, and matings have usually been made in such a way as to avoid segregation of genotypes likely to be confused. The genotype assigned each mated animal is consistent with its own appearance, with the genotypes of its parents and through these with those of all of its ancestors to the beginning of the records. It is consistent with the colors of its offspring and with the genotypes assigned all mated offspring and through these with the colors and genotypes assigned to all descendants to the end of the period studied. Where alternative interpretations have been possible this has been indicated in the records. The greatest difficulty has been caused by a few cases in which animals considered homozygous in some respect on the basis of fairly extensive evidence have turned out to have been heterozygous by the appearance of recessive segregants two or more generations later. No unexpected color has appeared in this series in which mutation seemed, after careful study, to be the most probable explanation.

EXTRACTION OF PIGMENT

The method of extracting eumelanin from hair samples was essentially that described by HEIDENTHAL (1940) except that filtration was substituted for centrifugation. It involved the following steps. (1) The hair was washed in 0.1 percent sodium lauryl sulphate (Dreft), (2) placed in nichrome baskets and rinsed four or five times with hot distilled water, (3) dried in an oven at 90°–100°C, (4) placed in apparatus devised by RUSSELL (1939, p. 342) and exposed there to dripping distilled ether for five days, (5) dried. (6) Two samples of the dried hair from each animal were weighed (samples of about 100 mgm from the darker colors, of about 200 mgm for the paler colors and white). (7) These were boiled in a glass reflux condenser for $1\frac{1}{2}$ to 2 hours with 50 cc of 6N HCl to dissolve the keratin. (8) The pigment was collected by filtration through an asbestos mat in a Gooch crucible. (9) The mat with pigment was washed with distilled water until the filtrate was free of Cl as indicated by the absence of reaction with $AgNO_3$. (10) The mat with pigment was put in a reflux flask. (11) The sides of the crucible were washed with KOH and the washings were added to the flask. (12) Amounts of 0.2N KOH, varying from 15 cc to 75 cc according to amount of pigment were put in the flask and boiled three hours. (13) the contents were passed through a Gooch crucible (which collected the asbestos) and a funnel with filter paper into a volumetric flask. The asbestos was squeezed with a glass rod and washed with KOH into the flask until the desired volume was reached.

COLORIMETRY

A large quantity of a standard solution was prepared from black hair. The solutions from hair samples were compared with this in a Klett Biometer, adjusting the concentrations so that the depths in the cups were approximately the same. Each of the two samples from an animal was read against standard ten times, five times in the left cup and five times in the right cup. The positions of the cups were never changed. Sample A was also read against sample B five times in each cup and sample B was read against sample A five times in each cup.

The amount of error in the colorimetry can be compared with that involved in taking a sample of hair and preparing a solution from it. The average reading (R_1) of A against B is affected by possible real differences in intensity, by the errors of preparation of the two samples and by the colorimetric error of the average of ten readings. The reciprocal (R_2) of the average reading of B

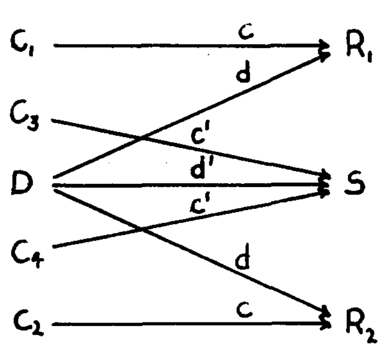
TABLE 1

Statistical analysis of colorimetric tests of the darker colors (dark sepia, dark brown) and of the paler colors (pale sepias, pale browns, whites). R_1 is the average of ten readings of sample A against sample B. R_2 is the reciprocal of the average of ten readings of sample B against sample A. S is the ratio of the average of ten readings of sample A against standard to the average of ten readings of sample B against standard. The table shows the mean and standard deviation of each of these ratios, the correlations among them and all of the standard errors.

	DARK EUMELANINS (76 CASES)		PALE EUMELANINS (75 CASES)	
RATIO	$\bar{R}$ $SE_{\bar{R}}$	σ SE_σ	$\bar{R}$ $SE_{\bar{R}}$	σ SE_σ
R_1	.9991 ± .0053	.0460 ± .0037	.9951 ± .0076	.0656 ± .0054
R_2	.9935 ± .0054	.0471 ± .0038	.9931 ± .0075	.0651 ± .0053
S	.9993 ± .0059	.0512 ± .0042	.9923 ± .0088	.0758 ± .0062
RATIOS	r SE_r		r SE_r	
R_1R_2	.977 ± .005		.992 ± .002	
R_1S	.895 ± .023		.935 ± .015	
R_2S	.883 ± .025		.931 ± .016	

against A is affected by the same differences and errors of preparation but by the independent colorimetric error of the average of ten different readings. The ratio (S) of the ten readings of A against standard to those of B against standard is again affected by the same differences and errors of preparation but in this case by the average errors of two independent sets of readings.

It seems desirable to consider separately the tests from the relatively intense dark eyed sepias and browns and those from the relatively pale pink-eyed sepias, pink-eyed browns, and albinos. Table 1 shows statistics relating to each of the three ratios referred to above and to the correlations among them. It may be seen that the means of the ratios are all close to 1. The variances in the



case of the paler samples are about twice as great as in the case of the darker ones, indicating greater reliability of the ratios in the latter case. Ratios R_1 and R_2 show a coefficient of variability of 4.7 percent for the darker colors, 6.5 percent for the paler ones. As might be expected the coefficients of variability of S are somewhat greater (5.1 percent for the darker colors, 7.6 percent for the paler ones) since these involve two opportunities for colorimetric error instead of one and the colorimetry involves matching of extracts from different genotypes with a single standard instead of mere matching of two samples from the same animal. The correlations are all high.

The factors underlying the deviations in the ratios are indicated in the accompanying figure. D represents real differences plus errors of preparation between A and B, while C_1, C_2, C_3 and C_4 are mean colorimetric errors of 10 readings in matching A against B, B against A, A against standard and B against standard respectively.

It is assumed that the colorimetric errors are all independent of each other and of the differences between samples from the same animal. The variances of R_1 and R_2 can each be analyzed into two independent components.

$$\sigma^2_R = \sigma^2_{R(D)} + \sigma^2_{R(C)}.$$

Here $\sigma^2_{R(D)} = d^2\sigma^2_R$, and $\sigma^2_{R(C)} = c^2\sigma^2_R$ where d^2 and c^2 measure the degrees of determination of R by D and C respectively. The value of d^2 can be estimated from the equation $r_{12} = d^2$.

Samples from dark eyed animals

$d^2 = .9767$	$\sigma^2_{R(D)} = .002117$	$\sigma_{R(D)} = .0460$
$c^2 = .0233$	$\sigma^2_{R(C)} = .000050$	$\sigma_{R(C)} = .0071$
1.0000	$\sigma^2_R = .002167$	$\sigma_R = .0465$

Samples from pink eyed animals

$d^2 = .9923$	$\sigma^2_{R(D)} = .004234$	$\sigma_{R(D)} = .0651$
$c^2 = .0077$	$\sigma^2_{R(C)} = .000033$	$\sigma_{R(C)} = .0057$
1.0000	$\sigma^2_R = .004267$	$\sigma_R = .0653$

This analysis indicates that the colorimetric error of the average of ten comparisons of two preparations from the same animal is measured by a coefficient of variability of only 0.7 percent (dark eyed) or 0.6 percent (pink eyed). This source of error is negligible in comparison with that due to errors of sampling and preparation.

In the case of the ratio of the comparisons of A and B with standard we have

$$\sigma^2_S = \sigma^2_{S(D)} + 2\sigma^2_{S(C)}.$$

The variance due to differences between A and B should remain the same ($\sigma^2_{S(D)} = \sigma^2_{R(D)}$) giving the equation $\sigma^2_{S(C)} = \frac{1}{2}(\sigma^2_S - \sigma^2_{R(D)})$. This yields $\sigma^2_{S(C)} = .000253$, $\sigma_{S(C)} = .0159$ in the case of dark eyed animals and $\sigma^2_{S(C)} = .000740$, $\sigma_{S(C)} = .0272$ in the case of the pink eyed ones.

The colorimetric error in the average of ten comparisons with standard is thus considerably greater than in matching two preparations from the same animal (CV = 1.6 percent instead of 0.7 percent for the dark eyed, 2.7 percent instead of 0.6 percent for the pink eyed). Nevertheless it is less than the errors of sampling and preparation of one sample ($.707\ \sigma_{R(D)}$), 3.3 percent for dark colors, 4.6 percent for the pale ones.

The correlation between ratio R_1 (or R_2) and S should be given by $dd' = \sigma^2_{R(D)}/\sigma_R\sigma_S$. This yields .888 for the dark eyed animals which is unexpectedly close to the observed values .895 and .883. The estimated value .855 for the pink eyed animals does not however agree well with the observed values .935 and .931, but does not disagree sufficiently to invalidate the principal conclusions. The total standard error of sampling, preparation and colorimetry of the average of two samples is $.50\sigma_S$ or 2.6 percent for the dark color, 3.8 percent for the pale ones.

RELATIONS BETWEEN COLORIMETRIC VALUE AND GRADE

It was shown in previous studies (RUSSELL 1939, HEIDENTHAL 1940) that the coefficients of variability of the colorimetric values for the various grades are more nearly constant than are the standard deviations. This suggests that

TABLE 2

Regressions of log M (where M is the geometric mean of colorimetric values) and of log (M+6) on grade, and analysis of the variance about the regression lines. Four series of colors are considered separately. Statistical data are also given for white. n is the number of individuals, k the number of grades, $\bar{x}$ *the mean grade,* y_0 *the estimated value of y at grade O,* $SE_{\bar{y}}(=\sigma_{y.x(total)}/\sqrt{n})$ *the standard error of* $\bar{y}$*, b the regression coefficient,* $SE_b(=\sigma_{y.x(total)}/\sigma_x\sqrt{n-2})$ *the standard error of the regression coefficient. The variance about the regression line is analyzed in the usual way. F is the ratio of the "between" estimate ((k−2) degrees of freedom) to the "within" estimate ((n−k) degrees of freedom) and Prob. is the probability that accidents of sampling might give as much variability of log M or of log (M+6) about the regression line as observed (from Fisher & Yates tables 1938).*

COLOR	y	n	k	x	y_0	$SE_{\bar{y}}$	b	SE_b	ESTIMATES OF $\sigma^2_{y\cdot x}$ "WITHIN"	"BETWEEN"	"TOTAL"	F	PROB.
D Sep	log M	29	7	17.276	.7857	.0176	.0611	.0051	.00949	.00694	.00902	.73	>.20
P Sep	"	42	11	6.167	.5087	.0123	.0906	.0039	.00445	.01307	.00639	2.94	.01–.05
D Br	"	47	8	14.021	.7596	.0120	.0610	.0062	.00597	.01170	.00673	1.96	.05–.20
P Br	"	21	7	6.095	.3941	.0220	.1042	.0102	.00796	.01650	.01021	2.07	.05–.20
W	"	12	1	0	.4660	.0186	—	—	.00414	—	—	—	—
D Sep	log (M+6)	29	7	17.276	.9159	.0163	.0559	.0047	.00803	.00644	.00773	.80	>.20
P Sep	"	42	11	6.167	.9108	.0078	.0582	.0025	.00175	.00520	.00258	2.97	.01–.05
D Br	"	47	8	14.021	.9417	.0104	.0524	.0054	.00458	.00806	.00504	1.76	.05–.20
P Br	"	21	7	6.095	.8592	.0153	.0620	.0071	.00408	.00722	.00491	1.77	.05–.20
W	"	12	1	0	.9504	.0060	—	—	.00044	—	—	—	—

the logarithms are distributed more normally than the actual values and that logarithms of colorimetric values are preferable in statistical studies.

It was also shown in the previous studies that there is a roughly linear relation between the logarithms of the colorimetric values and the grades, as expected if the successive grades, chosen to give barely perceptible steps, differ by a constant percentage.

Table 2 (top 5 rows) shows an analysis of the present data from this standpoint. The dark sepias, pale sepias, dark browns and pale browns and whites are considered separately.

The most significant deviation from linearity is found in the case of pale sepias (probability between .01 and .05). The slopes are virtually the same in the cases of dark sepia (b = .0611) and dark brown (b = .0610) but are much greater in the cases of pale sepia (b = .0906) and pale brown (b = .1042). Thus it is obvious that the relation of the logarithms of colorimetric value to grades can not be treated as linear over the entire range of either sepias or browns. Since the colorimetric values in the region of overlap of the dark and pale colors (grade 11 in the case of sepia, grade 9 in the case of brown) are rather similar it appears possible that satisfactory linear relations might be obtained by using a different function of the colorimetric values. By using log (M+a) for log M, where M is the geometric mean of the colorimetric value V, and a is a positive constant the slope is reduced more at low grades than at high ones. It was found by experiment that the function log (M+6) makes the slopes more nearly alike than with any other integral value of a. The lower five rows in table 2 give the statistical analysis using this function. The variance within grades was estimated by multiplying the original sum of squares at each grade by $[M/(M+6)]^2$ since $\delta \log (V+6) = \delta V/(V+6)$ and $\delta \log V = \delta V/V$, approximately, with the consequence that $\sigma^2_{\log (V+6)} = [\bar{V}/(\bar{V}+6)]^2 \sigma^2_{\log V}$. This transformation was tested in four cases (10 blacks of grade 21 ($\bar{M} = 115.64$), 10 browns of grade 16 ($\bar{M} = 49.35$), 10 browns of grade 15 ($\bar{M} = 44.09$), and 12 whites ($\bar{M} = 2.22$) by finding log (V+6) for each individual and calculating $\sigma^2_{\log (V+6)}$ without approximation. The ratios of the estimates to the actual values were .992, 983, .999 and 1.066 respectively, indicating that the transformation is sufficiently accurate, except possibly for the palest colors.

In order to test for possible changes in the standard solution, log M_c was estimated for each grade in each series of colors on the basis of the linear regression of log (M+6) on grade, and the deviation (d) of the logarithm of each colorimetric value (log V) was found. The correlation between this deviation and the time (t) in months from the beginning of the experiment, between this deviation and the grade (x) assigned the animal in question and between time and grade, and other statistics necessary for calculating the partial regression of deviation on month for a given grade are given in table 3.

TABLE 3

Statistical properties of the distribution of colorimetric determination with respect to time (t) (months since beginning of the experiment), grade (x), deviation (d) of individual determinations from log M_c where M_c is derived from the linear regression of log (M+6) on grade. The regression of deviation on time for a given grade is estimated from the correlations r_{dt}, r_{tx}, r_{dx} and the standard deviations. The grand average (weighting by the inverse squares of the standard errors) indicates fading of the standard at rate 2.24 percent per month.

COLOR	n	TIME (MONTHS)		GRADE		DEVIATION log V − log M_c		CORRELATIONS			REGRESSION		
		t	σ_t	$\bar{x}$	σ_x	$\bar{d}$	σ_d	r_{dt}	r_{tx}	r_{dx}	$b_{dt\cdot x}$	SE_b	b/SE
D Sep	29	2.62	2.78	17.28	3.50	+.0002	.094	.494	−.084	−.007	+.0169	±.0058	2.9
P Sep	42	7.26	3.05	6.17	3.22	−.0003	.077	.236	−.575	−.016	+.0085	±.0039	2.2
D Br	47	5.26	5.77	14.02	1.94	+.0003	.082	.568	−.212	+.016	+.0085	±.0018	4.7
P Br	21	7.38	4.95	6.10	2.21	+.0004	.111	.493	−.113	−.090	+.0110	±.0046	2.4
W	12	11.42	2.58	0	0	−.0004	.064	.708	—	—	+.0177	±.0056	3.2
Total	151										+.00985	±.00141	7.0

It may be seen that there is a significant positive partial regression in each series of colors. The grand average is a regression of +.00985±.00141 per month, which indicates a fading of standard at the rate of 2.24 percent per month or 8.7 percent in four months. This is somewhat greater than a rough estimate made by HEIDENTHAL (6.5 percent during the four months in which her data were obtained).

The greatest regression was in 12 whites (+.0177±.0056) in which the color measured is due wholly to melanoids resulting from the treatment. These were determined relatively late in the experiment (mean month 11.4). Nearly as great a regression was, however, found among 29 dark sepias (+.0169

TABLE 4

Means and standard deviations of logarithms of colorimetric values in three series. Those from present data (ZIB) are corrected for fading of standard, the means obtained by GH are adjusted to the same standard by subtracting .1054, the colorimetric values of ESR are reduced by 9 and log M is adjusted to the same standard as the others by subtracting .1074, as discussed in the text.

	DARK SEPIA (ZIB)			DARK SEPIA (GH)			DARK SEPIA (ESR)			DARK BROWN (ZIB)		
x	n	log M′	$\sigma_{\log V'}$	n	log M′	$\sigma_{\log V'}$	n	log M	$\sigma_{\log V}$	n	log M′	$\sigma_{\log V'}$
21	10	2.063	.070	18	2.048	.063	9	2.021	.044			
20				13	2.004	.059	5	1.911	.080			
19	5	1.881	.132	17	1.894	.068	6	1.927	.098			
18				10	1.841	.065	1	1.866	—			
17				9	1.763	.093	2	1.826	.073	3	1.721	.074
16	5	1.685	.030	4	1.786	.037	1	1.847	—	10	1.693	.076
15				7	1.677	.042	6	1.701	.100	10	1.644	.055
14	3	1.612	.057	6	1.608	.083	2	1.633	.000	5	1.543	.079
13	3	1.580	.120	2	1.574	.019	3	1.562	.052	7	1.520	.087
12	2	1.526	.092				1	1.484	—	7	1.379	.052
11	1	1.404								4	1.360	.016
10												
9										1	1.227	—

	PALE SEPIA (ZIB)			PALE SEPIA (GH)			PALE BROWN (ZIB)		
x	n	log M′	$\sigma_{\log V'}$	n	log M	$\sigma_{\log V}$	n	log M′	$\sigma_{\log V'}$
12				1	1.435	—			
11	3	1.366	.017	2	1.410	.051			
10	5	1.353	.064	1	1.486	—			
9	7	1.303	.061				1	1.265	—
8	2	1.154	.023				6	1.138	.105
7	4	1.037	.080				5	1.036	.035
6	2	.983	.054				3	1.057	.053
5	2	1.072	.061						
4	5	.802	.031				2	.582	.058
3	5	.680	.089				3	.629	.050
2	5	.537	.076				—		
1	2	.478	.003				1	.455	—
0	12	.347	.050						

$\pm .0058$) which, as a group, were determined early (mean month 2.6). There seems no reason to attribute any reality to the differences among the regressions or to assume anything other than a uniform rate of fading of the standard.

Every individual log V was accordingly corrected for fading of the standard by subtracting .00985 t where t is the number of months (taking account of fractions) since the beginning of the experiment. Table 4 shows the corrected means ($y = \log M' = \overline{\log V'}$ and the standard deviations of the corrected values ($\sigma_{\log V'}$) for each grade from the determinations of the junior author (ZIB), as well as for previous determinations, adjusted as described below, to the same standard.

It is desirable to combine these determinations with those obtained by RUSSELL (1939) and HEIDENTHAL (1940). The latter made colorimetric determinations of 86 dark sepias and 4 pale sepias but no browns. The former used a wholly different technique, titration of extracted pigment with $KMnO_4$. Only the dark sepias (36 in number) and dark browns (6 in number) will be considered as the results by this method for the paler colors were (as noted) too erratic to give more than the order of the amount of pigment.

HEIDENTHAL gives the mean colorimetric values ($\bar{V}$) and the standard deviations (σ_V) for the various grades (her table 4). To compare with the present data, the values of $\overline{\log V}$ and $\sigma_{\log V}$ should be obtained for each grade. It was deemed sufficiently accurate to use the transformation formulae based on the assumption of normal variability on the logarithmic scale

$$\overline{\log_{10} V} = \log_{10} \bar{V} - \tfrac{1}{2} \log_{10} \left[1 + (\sigma_V/\bar{V})^2\right]$$
$$\sigma^2_{\log V} = .4343 \log_{10} \left[1 + (\sigma_V/\bar{V})^2\right].$$

RUSSELL's data are less directly comparable both because of the difference in method referred to above and because 100 mgm of black pigment of known permanganate value were added to each sample following a suggestion of EINSELE (1937). It is estimated that the permanganate value for white (12 on the scale used) consisted of about 9 parts due to added black and 3 parts due to melanoids. Thus 9 has been subtracted from the permanganate numbers as published (RUSSELL's table 11). Estimates of $\overline{\log V}$ and $\sigma_{\log V}$ were then calculated as above.

As the standards used in these three series of experiments were not related, it is necessary to determine the relations among them from internal evidence. This might be done either from comparisons of the logarithm of the colorimetric values of grades or of the genotypes. As the ultimate object is to obtain combined estimates of genotypes, it seemed best to use the logarithms of the colorimetric values of the grades in adjusting for differences in the standards. This in turn could be done either by comparison of the regression lines or by averaging the differences at different grades. As no determinations were made of several of the grades of sepia in the present data, the best method here seems to be that of finding the difference between the two regression lines to be compared at that point (x) at which the standard error of the difference is minimum: Letting y_1 and y_2 be the estimated values of y ($= \log M'$) in the two bodies of data at the same value of x,

$$y_1 = \bar{y}_1 + b_1(x - \bar{x}_1) \qquad \sigma^2_{y_1} = \sigma^2_{\bar{y}_1} + (x - \bar{x}_1)^2\sigma^2_{b_1}$$

$$y_2 = \bar{y}_2 + b_2(x - \bar{x}_2) \qquad \sigma^2_{y_2} = \sigma^2_{\bar{y}_2} + (x - \bar{x}_2)^2\sigma^2_{b_2}.$$

We wish to find the value of x at which $\sigma^2_{(y_2-y_1)}$ is minimum.

$$\frac{d}{dx}\sigma^2_{(y_2-y_1)} = \frac{d}{dx}(\sigma^2_{y_2} + \sigma^2_{y_1}) = 2(x - \bar{x}_2)\sigma^2_{b_2} + 2(x - \bar{x}_1)\sigma^2_{b_1} = 0$$

$$x = (\bar{x}_1\sigma^2_{b_1} + \bar{x}_2\sigma^2_{b_2})/(\sigma^2_{b_1} + \sigma^2_{b_2}).$$

TABLE 5

Statistical analysis of three series of colorimetric determinations of dark sepia. Mean logarithms of colorimetric values (y) related to grade (x). Symbols as in table 1.

D SEP	y	n	k	$\bar{x}$	y_0	$SE_{\bar{y}}$	b	SE_b	ESTIMATES OF $\sigma^2_{y \cdot x}$ "WITH-IN"	"BE-TWEEN"	"TO-TAL"	F	PROB
ESR	log M	36	10	17.778	.9908	.0122	.0539	.0042	.00575	.00390	.00531	.68	>.20
GH	log M	86	·9	18.291	.8443	.0073	.0620	.0032	.00441	.00612	.00455	1.39	>.20
ZIB	log M′	29	7	17.276	.7411	.0156	.0619	.0045	.00731	.00593	.00706	.81	>.20

Table 5 gives the principal statistics for comparison of the results of RUSSELL (ESR) and HEIDENTHAL (GH) with those of the junior author of this paper (ZIB) in the case of dark sepias. The slopes are virtually identical in HEIDENTHAL's and the present data (.0620 *versus* .0619). Thus the difference between the regression lines is nearly the same at all grades. However, the grade at which the standard error of the difference is minimized comes out 17.607 by the above formula for x. At this grade the value of y from HEIDENTHAL's data is 1.9360 and in the present data is 1.8306. The difference .1054 has accordingly been subtracted from all values of log M from HEIDENTHAL's data to reduce them to the same standard as in the present data. The same adjustment has been applied to the four values for pale sepias in her data. These adjusted values (log M′) as well as the estimates of $\sigma_{\log v}$ are given in table 4.

Because of the difference in method it has not seemed desirable to combine RUSSELL's data with those of HEIDENTHAL and BRADDOCK in obtaining a formula for transforming grades into colorimetric values. It is, however, important to make comparisons as far as possible in order to use her figures for genotypes. It turns out that the grade at which the standard error of the difference is minimum is 17.845 (for dark sepias). The calculated ordinate at this grade is 1.9526 in RUSSELL's data and 1.8452 in the combined data of HEIDENTHAL and BRADDOCK giving .1074 as the quantity to be subtracted from RUSSELL's figures to bring them into comparability. The adjusted figures and the estimates of $\sigma_{\log v}$ are given in table 4.

The values of log (M′+6) from the present data (corrected for fading of standard) have been combined with HEIDENTHAL's (after adjustment to a common standard as above). These are shown for each grade in each series of colors in table 6. Table 7 gives an analysis of the relations between log (M′+6) and grade (x) in the various eumelanic series separately and on combining pale sepia with dark sepia and pale brown with dark brown. The slopes are all closely similar (.056 to .058) and in no case do they differ significantly.

TABLE 6

A function of the colorimetric values (data from GH and ZIB adjusted to a common standard and combined) which gives approximately linear relations to the color grades assigned at birth.

	DARK SEPIA			DARK BROWN			PALE SEPIA				PALE BROWN		
x	n	TOTAL log (M′+6)	σ	n	TOTAL log (M′+6)	σ	x	n	TOTAL log (M′+6)	σ	n	TOTAL log (M′+6)	σ
21	28	2.075	.062				12	1	1.522	—			
20	13	2.029	.055				11	5	1.480	.030			
19	22	1.923	.077				10	6	1.473	.063			
18	10	1.877	.060				9	7	1.417	.047	1	1.388	—
17	9	1.806	.084	3	1.768	.067	8	2	1.306	.016	6	1.296	.073
16	9	1.776	.055	10	1.743	.068	7	4	1.228	.052	5	1.227	.022
15	7	1.729	.037	10	1.700	.049	6	2	1.194	.033	3	1.241	.035
14	9	1.669	.062	5	1.612	.068	5	2	1.250	.040			
13	5	1.642	.074	7	1.592	.074	4	5	1.091	.016	2	.992	.023
12	2	1.597	.078	7	1.477	.042	3	5	1.033	.040	3	1.011	.021
11	1	1.496	—	4	1.466	.013	2	5	.975	.028			
10							1	2	.954	1.000	1	.947	—
9				1	1.359	—							
							0	12	.915	.014			

TABLE 7

Statistical analysis of combined colorimetric determinations of all eumelanic colors. Log (M′+6) where M′ is corrected geometric mean, related to grades. Symbols as in table 1.

COLOR	y	n	k	$\bar{x}$	y_o	$SE_{\bar{y}}$	b	SE_b	ESTIMATES OF $\sigma^2_{y \cdot x}$ "WITHIN"	"BETWEEN"	"TOTAL"	F	PROB
D Sep	log (M′+6)	115	11	18.035	.8765	.0061	.0564	.0023	.00426	.00518	.00434	1.22	>.20
P Sep	"	46	12	6.587	.8688	.0071	.0578	.0021	.00170	.00445	.00233	2.61	.01–.05
Total Sep	"	161	23	14.764	.8768	.0048	.05644	.0008	.00363	.00434	.00373	1.19	>.20
D Br	"	47	8	14.021	.8202	.0085	.0576	.0044	.00342	.00323	.00339	.94	>.20
P Br	"	21	7	6.095	.8365	.0121	.0580	.0056	.00232	.00525	.00309	2.26	.05–.20
Total Br	"	68	15	11.574	.8466	.0069	.05579	.0017	.00313	.00361	.00322	1.15	>.20
W	"	12	1	0	.9150	.0039	—	—	.00018	—	.00018	—	

The grade at which the standard error of the difference between the calculated values for log (M′+6) for dark sepia and pale sepia is minimum comes out 12.740. The calculated value for log (M′+6) for dark sepia is here 1.595 and for pale sepia 1.606, with a difference of .011 ± .020 which is not significant.

In view of this and of the close similarity of the slopes it is legitimate to fit a single line to all of the sepias. The analysis of variance in table 7 (line 3) shows that the deviations from linearity are not significant. The formula for this line is as follows

$$\log (M' + 6) = .8768 + .05644x.$$

This may thus serve as a means of transforming observed average grade into log (M′+6) and ultimately geometric mean (M′) for any genotype. Fig. 1

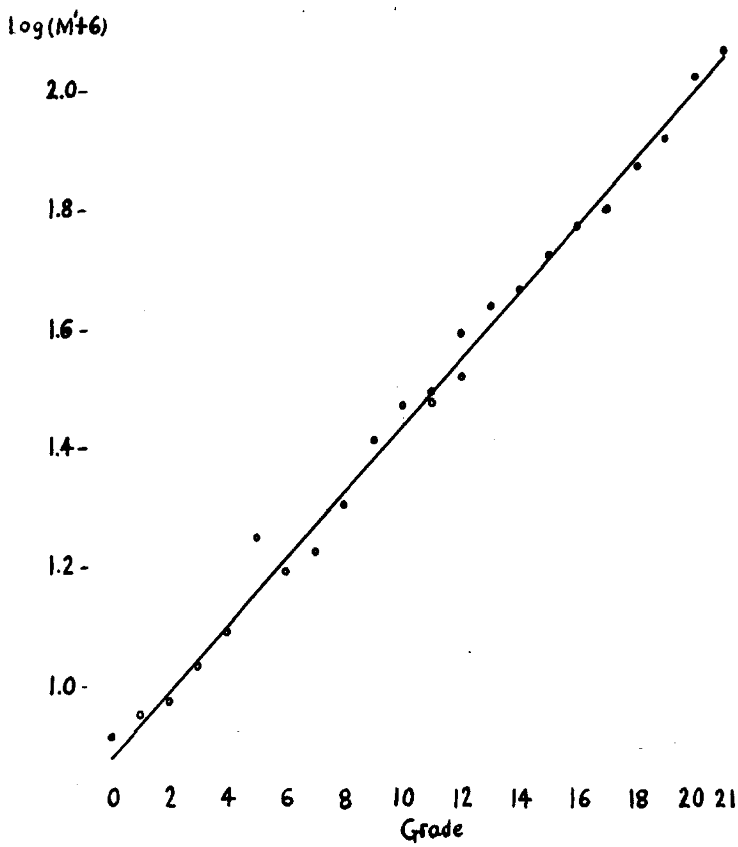


FIGURE 1.—Values of log (M′+6) plotted against visual grade of pale sepia (open circles) and dark sepia (solid circles). M′ is the geometric mean of the colorimetric value, after correction for fading of the standard. The formula of the regression line is log (M′+6) = .8768+.05644x.

shows the relations between the values of log (M′+6) for sepias (see table 6) and the fitted line.

Similarly in the case of the dark and pale browns, the grade at which the standard error of the difference of the regression lines is minimum comes out 9.130. The calculated values of log (M′+6) are here 1.346 for dark brown and 1.366 for pale brown with an insignificant difference .020 ± .028. The slopes are very similar again making it legitimate to calculate a single regression line

$$\log (M' + 6) = .8466 + .05579x.$$

The deviations from this line are shown in table 7 (line 6) to be insignificant. The relations between the values of log (M′+6) for browns from table 6 and the fitted line are shown in fig. 2.

In the case of the dark sepias and dark browns, the grade at which the standard error of the differences is minimum is 14.871. The calculated values

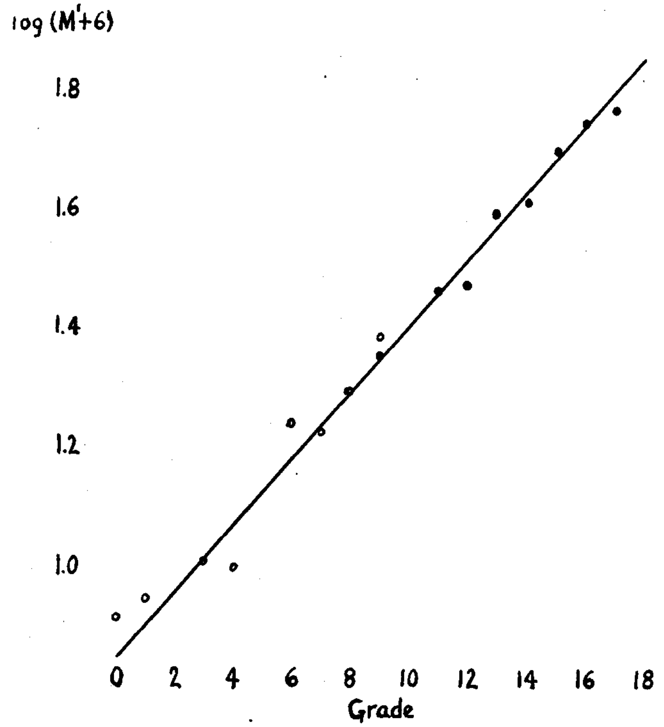


FIGURE 2.—Values of log (M′+6) plotted against visual grade of pale brown (open circles) and dark brown (solid circles). M′ is the geometric mean of the colorimetric value, after correction for fading of the standard. The formula of the regression line is log (M′+6) = .8466+.05579x.

of log (M′+6), 1.715 for dark sepias, 1.676 for dark browns, differ by .039 ±.0135, which is significant. The pale sepias and pale browns differ in the same sense although with less significance. The grade at which the standard error of the difference is minimum is 6.385. The calculated values of log (M′+6), 1.238 for pale sepia and 1.206 for pale brown, differ by .032±.015.

On comparing the regression lines for total sepias and total browns the slopes (.0564 and .0558 respectively) do not differ significantly (.0008±.0019) but the values of log (M′+6) for sepias are systematically high. The point at which the standard error of the difference is minimum is 12.196. The calculated values of log (M′+6) are 1.565 for sepia, 1.527 for brown, with a difference of .038±.008 which is highly significant.

There is no necessity that pelts which appear of the same intensity to the eye should yield solutions of the same colorimetric value. One might have such small granules that all of the pigment functions almost alike in the absorption of light while the other might have such large granules that light never reaches the interior. Thus E. S. RUSSELL (1946) has shown that in mice "dilute" blacks (genotype *dd*) actually have as much or more pigment than intense blacks, but have larger globules and so appear paler. While there are marked histologic differences between the pigment granules of pale and dark sepias and of pale and dark browns it appears that they are not sufficient to cause any appreciable error in estimating colorimetric value from superficial appearance. In the case of sepias and browns, on the other hand, it appears that there is a systematic error, leading to overestimation of the colorimetric value of

TABLE 8

Means and standard deviations of logarithms of colorimetric values of genotypes of eumelanic varieties in three series, adjusted to a common standard.

	ESR			GH			ZIB		
EBP	n	log M	$\sigma_{\log V}$	n	log M	$\sigma_{\log V}$	n	log M′	$\sigma_{\log V}$
C-	7	2.015	.063	16	2.042	.066	10	2.063	.070
c^kc^k	3	1.927	.100	1	1.892	—			
c^dc^d	2	1.879	0	10	1.887	.071			
c^kc^r	1	2.089	—	3	2.000	.083			
c^kc^a	1	1.852	—	7	1.839	.048	3	1.799	.023
c^dc^r	8	1.930	.102	8	1.948	.104	2	2.005	.136
c^dc^a	8	1.581	.129	10	1.637	.081	5	1.556	.087
c^rc^r	3	1.972	.076	8	2.026	.061			
c^rc^a	6	1.714	.100	17	1.729	.096	9	1.631	.103
EBpp									
C-				4	1.435	.045	21	1.266	.123
EbbP									
C-	5	1.748	.046				16	1.683	.075
c^kc^a	1	1.762	—				4	1.557	.073

browns if the transformation formula for sepias is used. This is probably not due to differences in granule size although there may well be a difference similar to that found by RUSSELL in mice. It may be due to the fact that the grades are based on the exposed distal portion of the hair while the colorimetric values are based on the whole hair. The lower colorimetric value of grades of brown as compared with sepia may thus indicate a relatively lower intensity in the proximal portion of the hair in the browns. It may also be noted that sepias respond to the lower temperatures to which the skin is exposed after birth by increased pigmentation while browns do not to an appreciable extent. Since the hair samples for colorimetric determination were taken two or three weeks after birth, it is not unlikely that the same transformation function would apply to the pigment that develops in the two series of colors before birth.

TABLE 9

Logarithms of geometric means of colorimetric values, of all series combined, for genotypes of eumelanic color varieties. The geometric means, adjusted to give 100 for genotype EBCP (intense black) are also given.

EBCP = 100

EBP	n	log M′ ± SE		$\sigma_{\log V}$	M″ ± SE	
C-	33	2.043	.017	.067	100.0	3.9
c^kc^k	4	1.918	.048	.083	74.5	8.5
c^dc^d	12	1.886	.028	.064	69.1	4.6
c^kc^r	4	2.022	.048	.081	95.2	10.8
c^kc^a	11	1.829	.029	.043	60.4	4.2
c^dc^r	18	1.946	.023	.102	79.7	4.3
c^dc^a	23	1.600	.020	.103	34.8	1.7
c^rc^r	11	2.012	.029	.066	93.0	6.4
c^rc^a	32	1.698	.017	.105	44.1	1.8
Av.				.086		
EBpp						
C-	25	1.293	.019	.130	16.1	0.8
c^kc^k	1	1.085	.097	—	9.2	2.5
c^kc^a	6	.825	.039	.163	4.1	0.6
c^dc^r	3	.796	.056	.022	3.7	0.7
c^dc^a	8	.630	.034	.149	1.9	0.3
c^rc^a	3	.520	.056	.035	1.0	0.4
Av.				.132		
EbbP-						
C-	21	1.698	.021	.074	44.1	2.2
c^kc^k	1	1.782	.097	—	53.9	12.4
c^kc^r	1	1.654	.097	—	39.7	9.3
c^kc^a	5	1.598	.043	.113	34.6	3.6
c^dc^a	10	1.375	.031	.047	19.9	1.5
c^rc^r	2	1.641	.068	.052	38.4	6.4
c^rc^a	13	1.509	.027	.128	27.8	1.8
Av.				.091		
Ebbpp						
C-	15	1.096	.025	.095	9.5	0.7
c^kc^a	2	.582	.068	.058	1.5	0.6
c^dc^a	3	.629	.056	.050	1.9	0.5
c^rc^a	1	.455	.097	—	0.6	0.6
Av.				.089		
White	12	.347	.014	.050	0.0	

GENOTYPES

Table 8 shows the estimated value of log M and of $\sigma_{\log v}$ for the eumelanic genotypes measured by RUSSELL and HEIDENTHAL in comparison with those of the junior author, after reduction to a common standard. For the most part there is good agreement where the numbers are at all adequate. Table 9 shows the averages and standard deviations of eumelanic genotypes on

TABLE 10

Means and standard deviation of logarithms of colorimetric values of yellows in two series and in combinations of these. The values obtained by GH are increased by .2558 to adjust to the same standard as those obtained by ESR. The last two columns give the values of the function log (M+10) and the corresponding standard deviation as a function which gives a more nearly linear relation to grade than does log M.

x	ESR n	ESR log M	ESR $\sigma_{\log}$	GH n	GH log M	GH $\sigma_{\log}$	TOTAL n	TOTAL log M	TOTAL $\sigma_{\log}$	TOTAL log (M+10)	TOTAL σ
12	1	2.424	—	3	2.521	.037	4	2.497	.057	2.511	.055
11	9	2.472	.085	6	2.430	.053	15	2.455	.081	2.470	.078
10	15	2.449	.091	13	2.413	.081	28	2.432	.087	2.448	.084
9	2	2.349	.034	6	2.317	.062	8	2.325	.056	2.345	.053
8	6	2.123	.145	5	2.181	.053	11	2.149	.112	2.179	.105
7	34	2.002	.113	27	2.041	.071	61	2.019	.098	2.059	.089
6	12	1.881	.094	10	1.983	.086	22	1.927	.097	1.976	.086
5	17	1.778	.129	7	1.858	.108	24	1.802	.126	1.865	.109
4	22	1.731	.098	13	1.616	.099	35	1.688	.112	1.769	.093
3	12	1.535	.124	7	1.474	.132	19	1.512	.127	1.629	.097
2	4	1.368	.093	2	1.375	.062	6	1.371	.078	1.525	.054
1	1	1.086	—	3	1.050	.100	4	1.059	.084	1.332	.045
0	3	1.068	.096	4	1.010	.038	7	1.035	.070	1.319	.037

combining all of the results. The standard deviation is based on the formula $\sigma^2_{total} = \Sigma[(n-1)\sigma^2 + n(y-\bar{y})^2]/(\Sigma n - 1)$ where y is the mean, σ the standard deviation and n the number of cases in a series of determinations and $\bar{y}$, σ_{total} and Σn the corresponding statistics of the total. The standard error of log M is based on a generalized standard deviation (.0965) obtained from all classes except the whites. The whites differ from the others in that the standard deviation is entirely experimental error while in the other cases there are undoubtedly real variations in amount of pigment in what was called the same grade. Geometric means (M″) are given on a scale in which the amount of pigment in genotype *EBCP* is 100 by the formula

$$M'' = .92515[M' - 2.22]$$

which involves subtraction of a term 2.22 from M′ to allow for melanoids (which give a spurious colorimetric value to white) and multiplication by the appropriate factor. The standard errors (generalized) were obtained by the formula

$$SE_{M''} = .92515 \times 2.3026M' \times .0965/\sqrt{n} = .2055M'/\sqrt{n}.$$

TABLE 11

Statistical analysis of colorimetric values of two series of determinations of yellows and their combinations. Symbols as in table 1.

YELLOW	y	GRADES	n	k	$\bar{x}$	y_0	$SE_{\bar{y}}$	b	SE_b	ESTIMATES OF $\sigma^2_{y \cdot x}$ "WITHIN"	"BETWEEN"	"TOTAL"	F	PROB
ESR	log M	1–12	135	12	6.333	1.1855	.0102	.1201	.0040	.0125	.0312	.0139	2.49	.001–0.01
GH	"	1–12	102	12	6.765	1.1310	.0110	.1277	.0041	.0090	.0427	.0124	4.73	< .001
Total	"	1–12	237	12	6.5190	1.1618	.0072	.1235	.0027	.0105	.0492	.0121	4.70	< .001
"	"	1– 8	182	8	5.3846	1.1301	.0084	.1296	.0047	.0117	.0441	.0127	3.78	.001–.01
Total	log (M+10)	1–12	237	12	6.5190	1.3115	.0060	.1092	.0023	.0080	.0222	.0086	2.77	.01–.05
"	"	1– 8	182	8	5.3846	1.3148	.0069	.1080	.0039	.0086	.0112	.0087	1.30	> .20

YELLOW GRADES

While no new data have been obtained for phaeomelanic grades and genotypes, it is desirable to put those obtained by RUSSELL and by HEIDENTHAL in the same form as used here for the eumelanic ones. Log M and $\sigma_{\log\ v}$ were estimated from the published data by the method described earlier. The results are shown in table 10 after addition of a constant term (.2558) to HEIDENTHAL'S figures to adjust them to the same standard as RUSSELL'S. This term was calculated in a different way from that used above. Regression of log M on grade is roughly linear as noted by the authors but the deviations from linearity (analyzed in table 11) are significant. These deviations are of

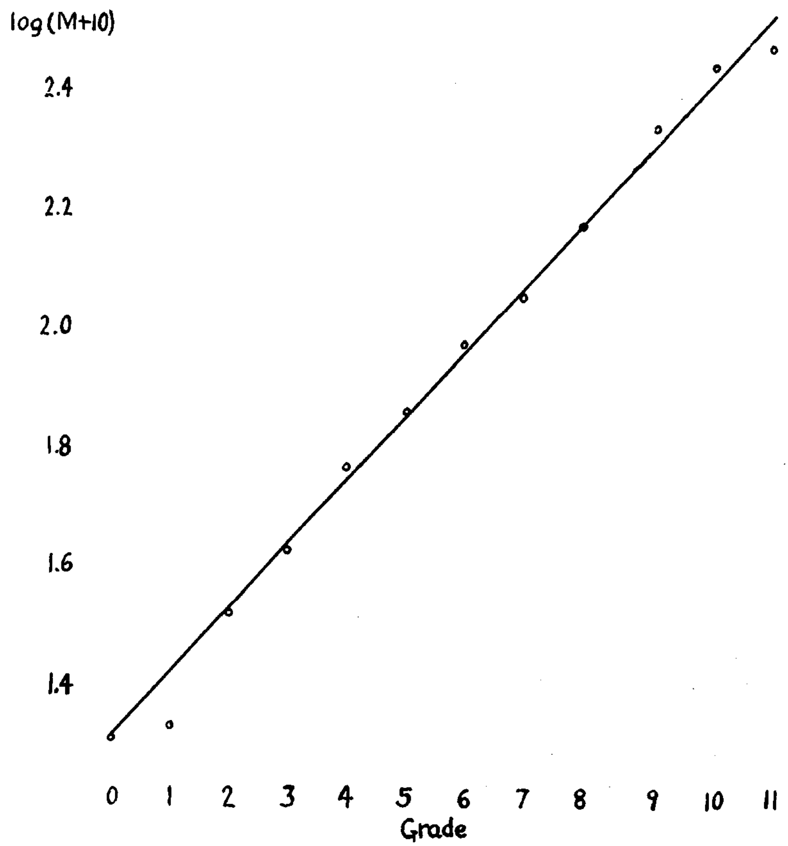


FIGURE 3.—The values of log (M+10) plotted against visual grade of yellow. M is the geometric mean of the colorimetric values estimated from the data of E. S. RUSSELL and G. HEIDENTHAL. Visual grades 9, 10 and 11 are raised to 9.25, 10.25 and 11.25 to allow for an apparent excessive gap between grades 8 and 9. The formula for the regression line, based on grades 1 to 8, is log (M+10) = 1.3148+.10801x.

such a nature as to indicate imperfection in the scale of grades which can not be wholly corrected by the type of transformation used in the case of the eumelanins. There is too great an increase in colorimetric value from grade 8 to grade 9 and too little beyond grade 10 in both series. Since there is considerable parallelism in the numbers tested at the various grades by RUSSELL

TABLE 12

Means and standard deviations of logarithms of colorimetric values of genotypes of phaeomelanic varieties in two series adjusted to a common standard and in the combination of these. Standard errors of the means (combined data) are based on a generalized standard deviation ($\sigma_{\log V}=.1168$) derived from all classes excluding white. The geometric means and their standard errors are given in the next to the last column on a scale on which that for eeCFF (intense red) is 100.

Yellow

	ESR			GH			Total					
eeFF	n	log M	$\sigma_{\log V}$	n	log M	$\sigma_{\log V}$	n	Log M	$\pm SE_{\log V}$	$\sigma_{\log V}$	M″	$\pm SE_{M''}$
C-	27	2.448	.089	28	2.408	.089	55	2.428	.016	.090	100.0	3.8
c^kc^k	10	1.970	.079	8	1.952	.059	18	1.962	.028	.070	31.5	2.3
c^kc^d	6	1.987	.150	1	2.042	—	7	1.995	.044	.139	34.3	3.9
c^dc^d	23	2.037	.144	21	2.078	.082	44	2.056	.018	.119	40.1	1.8
c^kc^r	13	1.773	.120	1	1.518	—	14	1.755	.031	.134	17.9	1.6
c^kc^a	4	1.687	.044	4	1.965	.104	8	1.826	.041	.153	21.8	2.5
c^dc^r	19	1.750	.140	10	1.796	.137	29	1.766	.022	.140	18.5	1.1
c^dc^a	18	1.664	.134	1	1.701	—	19	1.666	.027	.131	13.8	1.1
eeff												
C	7	1.943	.068	11	2.049	.093	18	2.008	.028	.098	35.4	2.5
c^kc^k	—			2	1.004	.083	2	1.004	.083	.083	0	0.8
c^dc^d	10	1.423	.146	10	1.408	.144	20	1.415	.026	.141	5.9	0.6
Effpp												
C-	2	1.669	.048	—			2	1.669	.083	.048	13.9	3.5
White	3	1.068	.096	4	1.010	.038	7	1.035	.044	.070	0	0.3

and HEIDENTHAL, it appears that the term for reducing to a common standard can be obtained better from the weighted average difference between determinations of the same grade than from the regression lines. The differences were weighted by the quantity $n_1n_2/(n_1+n_2)$ and gave .2558 as the average.

The grand average for log M and $\sigma_{\log v}$ for the total data are shown in table 10. The analysis of variance in table 11 brings out the high significance of the deviations from linearity in the relation of log M to grade whether all grades are considered or merely those from 1 to 8. On fitting a straight line to log (M+10) the deviations are still significant if all grades are considered but grades 1 to 8 are well fitted. The line

$$\log (M + 10) = 1.3148 + .10801x$$

can thus be used as a transformation function for the lower grades.

In order to relate the amount of pigment in genotypes other than *eeCF-* (virtually never exceeding grade 8) to that in intense yellows of genotype *eeCFF* (virtually never below grade 9) it seems necessary to make a special adjustment for the higher grades. First it may be noted that the colorimetric values of grades 11 and 12 differ so little from each other and from grade 10 that it appears that the superficial appearance (based on the tip of the hair) is seriously misleading in these cases. It has seemed best to combine grade 12 (rare in these series) with grade 11. The average value of log (M+10) of grades 9 to 12 was 2.4436. The average of these grades (combining 12 with 11) was 10.20 while the average grade at which log (M+10) should have the above value, as estimated from the regression line based on grades 1 to 8, was 10.45. Thus the excessive gap between grades 8 and 9 can be corrected by adding 0.25 to the average grade of the more intense genotypes before transforming. The relation between the values of log (M+10) for yellows (from table 10) and the fitted line are shown in fig. 3.

YELLOW GENOTYPES

Table 12 shows the values of log M and $\sigma_{\log v}$ for the various phaeomelanic genotypes, estimated from the figures published by RUSSELL and HEIDENTHAL. Generalized standard errors are based on $\sigma_{\log v} = .1168$ derived from estimates of $\sigma_{\log v}$ for all genotypes except those that are white. The geometric means corrected for melanoids in whites and reduced to a scale on which that for genotype *eeCFF* is 100, are derived from the formula .3892 (M − 10.84) and their generalized standard errors from the formula $.3892 \times 2.3026\ M \times .1168/\sqrt{n} = .1047\ M/\sqrt{n}$.

The comparison of these geometric means with those deduced by transformation of grades of much larger numbers of animals will be reserved for a later paper.

SUMMARY

The self colored varieties of the guinea pig may be classified in two primary categories—those with predominantly dark or eumelanic pigment and those with yellow or phaeomelanic pigment. There are four major series in the first category (1) dark sepias of genotype *EaaBP*; (2) pale sepias *EaaBppF*; (3) dark browns *EaabbP*; (4) pale browns *EaabbppF*. The intensities of all of these vary with the combination of the series of alleles C, c^k, c^d, c^r, c^a. Three major series may be distinguished among the yellows (5) the relatively persistent yellows of genotype *eeF-*, (6) the fading yellow of genotype *eeff* and (7) a class of anomalous yellows of genotype *Eaaffpp*. There are variations of intensity in all of these due to combinations of the *C* series. Minor divergences from (2), (4) and (5) are due to incomplete dominance of *F*.

The present paper presents a statistical analysis of colorimetric determinations of amount of pigment in the eumelanic varieties, especially dark and pale browns, and pale sepias, supplementing previous determinations, especially of dark sepias, and of the phaeomelanic series.

The relation of the colorimetric determinations to grades based on comparisons of animals at birth with standard pelts is analyzed statistically. These relations are used to correct for fading of the standard solution and to adjust colorimetric determinations made with different standards to a common basis.

Transformation formulae are derived by which average grades of genotypes,

based on large numbers of animals, may be transformed into the relative quantities of pigment characteristic of these genotypes.

The corrected geometric means of the colorimetric values of diverse genotypes are presented on scales on which intense black of *EaaBCP* is 100 (eumelanic genotypes) or on which intense yellow of *eeCFF* is 100 (phaeomelanic genotypes).

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