

PIGMENTATION IN GUINEA-PIG HAIR

Microscopical as Well as Chemical Studies Reveal Many Differences Between the Black and Red Colors—Genetic Investigation Reveals but a Single Difference—the Problem is Suggested of Explaining the Many Observed Differences as Results of a Single Fundamental Physiological Difference

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IN RECENT years many interesting facts have been discovered concerning the inheritance of coat color in guinea-pigs. It seems to the writers that the time is now ripe to correlate these facts of heredity with the color, form, and chemical constitution of the pigments which impart the color to the different forms of guinea-pig hair. Progress in science is frequently made by attacking a problem from a new angle. Such a correlation as the one just mentioned should throw some light on the physiology of pigment formation in mammals.

In all, twenty-eight samples of guinea-pig hair have been studied so far. These were dehydrated in absolute alcohol, cleared in xylol, imbedded in paraffin, sectioned and mounted in the usual way, and studied, unstained, with the oil immersion.

The cortical, or peripheral, and the medullary, or central, regions of the guinea-pig's hair present characteristic differences. The medulla contains extensive communicating air spaces (see figures), and its substance stains readily with eosin. The cortical material lacks this reaction with eosin and air spaces are entirely absent. The cuticle forms a thin outer covering for the hair.

The color of the hair depends upon two forms of pigment—diffuse and granular. The former lends color to the hair in much the same way that substances in solution may color a solvent. The adjective "granular" sufficiently explains the character of the other kind of pigment.

The color and the distribution of these pigment substances in the medulla and cortex demonstrate some interesting facts in the physiology of pigment formation.

OBSERVATIONS

Black Hair.—The most noteworthy fact in the case of black hair is that both the medulla and the cortex have an abundance of black granular pigment. (Fig. 10.) The cortical granules are short cylindrical rods whose diameters are, roughly, half or two-thirds their length. The average length is about 0.8 of a micron. Their long axes lie lengthwise of the hair. The medullary granules vary in size and shape, the smallest ones being about 0.2 of a micron in diameter and the largest approximately $3\frac{1}{2}$ micra in their greatest dimension.

In the same samples of hair, the granules are often more closely aggregated in cross-sections of small diameter than in larger sections. The smaller cross-sections were probably cut nearer than the larger ones to the distal ends of the hair. Since the tips of the hairs are usually darker in color than the bases, one would expect to find the condition just described—more granules near the tips.

Sepia Hair.—In sepia hair the granules are black. They vary considerably in size and are abundant in both cortex and medulla. The cortical granules as in black hair are predominantly short bacillus-like rods, oriented with their long axes parallel to the long axis of the hair. The distribution and

the number of the granules observed in sepia hair seems to be practically the same as in black hair. However, since black hair is darker than sepia, it certainly must contain a slightly larger amount of black pigment than sepia hair.

Red Hair.—Red hair, like black and sepia, has a large amount of granular pigment in the medulla, but unlike them it has *very few* granules in the cortex. As will appear later, this absence of cortical granules is a significant fact.

The granules in red hair vary widely in size and shape. Nearly all are spherical, having a diameter, as accurately as could be computed, of 0.2 to 0.3 micron. It is difficult to see these small ones clearly except when an oil immersion objective is used. The largest granules, which are relatively few in number, are elongated. Some of them are as large as 6 micra x 2 micra, about eighteen times as long as the smallest particles. Between these two extremes is found a great variety of intermediate sizes.

The color of the pigment particles is distinctly yellowish. The difference in color between them and the pigment in black hair is very clearly seen when one compares sections of the two kinds of hair under the same conditions of illumination and magnification.

Diffuse yellowish pigment is abundant in the cortex of some hairs. Probably it is present in every hair but can be seen only in those sections where it is relatively concentrated. It is most dense around the medulla, fading away to invisibility near the surface of the hair.

Yellow Hair.—The number of granules in the cortex of yellow hair is extremely small. The medulla contains a greater quantity of granular pigment, but even this is considerably less than in the medulla of red hair. Comparing red with yellow guinea pigs, one finds that the yellow color is really a dilute red. The difference in the quantity of granular pigment observed in the hair sections may explain this color dilution.

The granules in the yellow hair vary markedly in size and color. There are

a few of the large yellow type such as are found in red hair. Of the smaller granules, a large part seem to be as *black* as the smaller ones in black hair, while the rest are yellowish. Probably these black granules are more highly oxidized melanin than the yellow ones. Yellow hair is sometimes slightly sooty in appearance. The black granules are undoubtedly responsible for this sootiness.

A slightly yellowish tint in the cortex of a few hairs suggests that diffuse yellow pigment may be present, but is not always visible on account of the thinness of the sections.

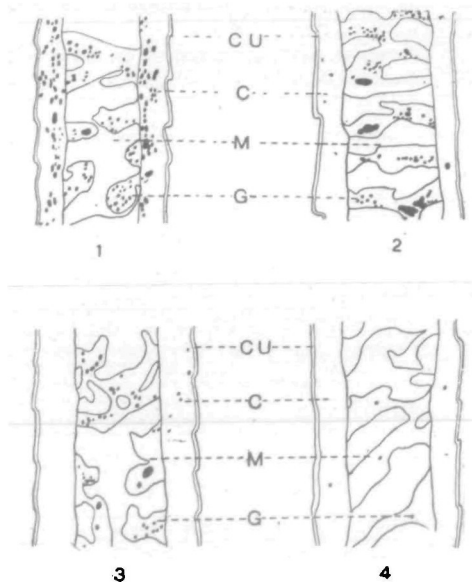
Cream Hair.—The medulla and cortex of cream hair contain a very few granules, apparently black, of approximately the size of the granules in red hair. The sections do not furnish evidence of diffuse pigment, but the dark color and the small number of the pigment granules can hardly account for the color of the hair, which is not black but cream. Therefore diffuse yellow pigment is probably present, but in such small quantities that in the sections it does not visibly alter the color of transmitted light.

DISCUSSION OF OBSERVATIONS

The preceding discussion has brought out a number of distinct ways in which red hair differs from black. First, there are differences in the character of the pigment. Black hair contains only granules and these are dark in color. Red hair contains both granules and diffuse pigment, and these are light colored. There is a difference in distribution; in black hair, granules are abundant in both cortex and medulla, while in red hair they are found almost exclusively in the medulla.

Next there is a difference in the effect produced when the same genetic factors are introduced into black and red stocks of guinea pigs. The case of most interest here is the effect of introducing grades of albinism. Complete albinism reduces both black and red to white. the dilution factor, an allelomorph of albinism, when introduced into black stock, produces sepia, a dark brown color, which grades into black. No

certain distinction could be observed under the microscope between samples of sepia and of black. When the same dilution factor is introduced into reds, the color is changed to yellow or cream, and the sections show a marked reduction in the number of granules as compared with red hair. With a still lower grade of imperfect albinism, red is wholly reduced to white, while black is still only very slightly affected. This greater susceptibility of red to the influence of dilution factors is the rule among mammals.



MAGNIFIED HAIRS

Longitudinal sections of guinea-pig hairs magnified 1550 diameters.

Fig. 1, Black hair; Fig. 2, red hair; Fig. 3, yellow hair; Fig. 4, cream hair; CU, cuticle; C, cortex; M, medulla; G, pigment granule. (Fig. 10.)

To these differences between black and red may be added certain ones observed by other workers. Miss Durham¹ found a distinct difference in solubility of the pigments. Dilute alkali dissolved red pigment easily but black hardly at all. Gortner² observed a pronounced chemical difference. Black

pigment from several sources contained considerable iron, while red pigment contained virtually none. Onslow³ discovered a difference in the enzyme content of the skins of black and yellow rabbits. He could extract a peroxidase from the skins of black rabbits but not from yellow. With this peroxidase he could produce dark pigment resembling melanin.

At first sight it seems necessary to suppose that black and red guinea pigs differ from each other by many physiological factors in order to account for these many differences. But there is probably only one primary physiological difference between black and red hair, because a single genetic factor is enough to effect the change. All the observed differences are, in some way, direct or indirect effects of this primary difference.

It is generally agreed that melanin pigment is produced by the oxidation, through the influence of oxidizing enzymes, of protein metabolic products such as tyrosin. The simplest explanation of the difference between black and red hair seems to be that a certain specific enzyme (enzyme II), which is present in black, is absent in red. This enzyme increases the oxidizing power of another, more fundamental, enzyme (enzyme I) whose presence is necessary for the production of *any* pigment. (Wright.)⁴ The more thorough oxidation of the chromogens in the case of blacks is probably responsible for the differences in color between black and red hairs, the more thoroughly granular nature, and the decreased solubility of black pigment.

The difference in iron content, noted by Gortner, seems at first to indicate a specific difference in the chromogens of blacks and reds, as well as in the enzymes producing them. But the following explanation is possible. The chromogens oxidized in the red hair are also oxidized in black, but in addition iron-containing chromogens are oxidized in the latter.

The presence of granular pigment in

¹ Durham, F. M., 1904. *Proc. Roy. Soc. London.*, 74:310-313.

² Gortner, R. A., 1911. *Biochem. Bull.*, 1:207-215. 1912. *Proc. Soc. Exp. Biol. and Med.*, 9:3-4.

³ Onslow, H., 1915. *Proc. Roy. Soc.*, B-99:36-58.

⁴ Wright, S., 1917. *JOUR. HEREDITY*, 8:224-235.

the cortex of black and sepia hair and its almost complete absence in red and yellow, shows that the constitution of the cortex imposes some obstacle in the way of cortical pigment production in red and yellow hair. The enzyme for black overcomes this obstacle; therefore pigment appears in the cortex of black and sepia hair. It seems likely that this obstacle is the slowing up of the oxidation of chromogens in the cortex. This inhibiting action of the cortex is

similar to the effect which the dilution factors have upon pigment production. The dilution factors reduce very little the quantity of pigment in black hair, but considerably diminish the amount in red. *Thus it seems plausible that there is some characteristic peculiarity of the cortex of guinea-pig hair which diminishes the quantity of cortical granular pigment in much the same way as the dilution factors reduce the total granular pigment content.*

Budding Incompatible Cottons.

An experiment has come to light in the field notes of the late R. M. Meade which should be of interest to plant breeders. Mr. Meade has been trying for several years to secure hybrids between American Upland cotton, *Gossypium hirsutum*, and two Asiatic species, *G. herbaceum* and *G. indicum*. From the standpoint of fertile seeds these attempts proved to be unsuccessful but in many instances the cross-pollinated fruits remained on the plants for several days longer than those not pollinated, indicating that initial growth had been stimulated by the application of foreign pollen.

That growth was started seemed to mean that fertilization might have taken place and that complete development was prevented by some form of chemical incompatibility that caused the shedding of the young bolls. Mr. Meade ingeniously planned to overcome this obstacle by budding one species upon the other with the idea that the sap of the stock would exert an influence upon the chemical composition of the floral organs of the budwood. No difficulties were encountered in getting buds of American Upland varieties to grow on Asiatic stock and vice versa and several successfully budded plants were secured. Unfortunately the plants were budded so late in the season that only one of the resulting branches produced flowers and this at a time when no flowers were open on the stock plant.

That the sap of the stock may alter the chemical composition of the budded branches was shown by an experiment in budding two distinct Upland varieties. The variety used for budwood was Willet's Red Leaf. This variety has dark red foliage and stems, which are very distinct in color from those of normal green varieties. The stock was a normal green variety called Trice. Several buds of the Willet's Red Leaf were inserted on the Trice stock; these buds developed rapidly, producing large well-formed leaves and branches. The first leaves on the young budded branches were red in color like the plant from which the bud came but the succeeding leaves became lighter and lighter in shade until at the end of the season they were only half as dark as those of the parental plant which were all dark red. This fact certainly seems to support Mr. Meade's hypothesis that the chemical composition of budded branches might be influenced through the stock, and leaves open the possibility of accomplishing the hybridization of American Upland and Asiatic species of cotton.

A more adequate investigation of this interesting phenomenon is highly desirable. It is hoped that this method of overcoming cross sterility, the testing of which was interrupted by Mr. Meade's untimely death, may be of interest to other investigators.