

GENETIC ASPECTS OF PIGMENT PRODUCTION
IN THE GUINEA PIG

by

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INTRODUCTION AND REVIEW OF LITERATURE

The purpose of the present study has been to correlate the processes of pigment formation as observed in fetal and adult guinea pigs with the theory of color inheritance as presented by Bogart and Ibsen (1937). The effects of some of the color-determining factors of the guinea pig and the time and manner of their action have been described.

The cytological origin of pigment has been studied by a number of workers, including Schultz (1912) and Ludford (1925 a,b), both of whom described pigment granules as arising from nuclear material; Renyi (1924) who found that pigment in the eye of the chick was formed from mitochondria; and Hooker (1915) who described a series of nucleocytoplasmic reactions which might result in pigment formation. Maximow and Bloom (1934) summarized the status of knowledge of pigment and its production in the skin and hair of man, from a histological point of view.

The foundations of the present knowledge of color inheritance in the guinea pig were laid by Castle and Wright (1916); Wright (1917, 1925, 1927), and others. Wright (1917) advanced an enzyme-theory of pigment production. In 1927 he presented this theory again in modified form and reviewed the literature on pigment production.

Kroning (1930) described the "dopa" reaction in the skin of rabbits and guinea pigs. Gortner (1911) studied the chemical nature of the melanins. Edwards and Duntley (1939) found a diffuse pigment in the living human skin, in close association with granular pigment. Gremmel (1939) found that the coat color of horses was due to different arrangements and concentrations of one kind of granular pigment. He found no diffuse pigment in horse hairs.

Esskuchen (1927, 1930) described the development of pigment in cattle fetuses. He found two types of pigment cells in the skin; he observed that dark color appeared earlier than red, and that the skin of the extremities showed pigmentation first.

The relation of pigmentation to color inheritance in pigeons was investigated by Hawkins (1931).

Hunt and Wright (1918) made a number of microscopic observations on the hair of guinea pigs. They described black and "yellowish" granules and a yellowish diffuse pigment in sectioned hairs.

Bogart and Ibsen examined the hair and in some cases the skin of various domestic animals, including cattle and guinea pigs, in an attempt to determine the effects of some color genes on the pigmentation of the hair and skin. They found both granular and diffuse types of pigment in

the hair of guinea pigs. They distinguished between black and chocolate granules and found that variations in the shade of the diffuse pigment, as well as in the amount and kind of granular pigment, were responsible for the different shades of red hairs. These results formed the immediate basis of the present study.

MATERIAL AND METHODS

Guinea pigs of known genetic composition with regard to the factors studied, were bred under observation and fetuses were obtained which ranged from 43 to 57 days, copulation age. A few newborn animals were also studied. Blocks of skin were taken from the nose, ears, back, and belly of each fetus and each adult animal killed. These tissues were studied microscopically as fresh smears and as fixed and sectioned material. A variety of fixatives was used, including 8 per cent neutral formalin and 95 per cent alcohol, neither of which altered the color of the pigment. Most of these tissues were sectioned and mounted without staining. Bouin's fluid was used as a fixative for tissues which were stained with hematoxylin and eosin and studied histologically.

Whole mounts were made of hair samples from animals of various ages. A series of weighed hair samples treated

with a 15 per cent solution of sodium hydroxide was examined.

By these methods the effects of the following color-determining genetic factors were studied:

The extension series: E, e^P, and e.

Black and chocolate: B and b.

The "albino" series: C, c^d, c^r, and c^a.

A few observations were made on the effects of the "pink-eyed dilution" factor p. Material was not available for a study of the factor c^k in the "albino" series.

The appearance of the living animal at various ages was checked with the microscopic observations. Color in animals is influenced by non-genetic as well as by genetic factors; therefore, the food, light, and temperature in the colony were kept as constant and as favorable as possible, and the animals used for breeding were not subjected to any experimental treatment.

In the discussion which follows, the age of embryos is given in days calculated after the date of copulation; other specimens are described either as newborn (less than one week old), or as adult (more than four weeks old). The sex of the specimen is indicated in some instances, although no study was made of the relation of sex to coloring.

OBSERVATIONS

Types of Pigment

Two types of pigment, granular and diffuse, were observed. The granular pigment was of three kinds, red, black, and chocolate. These granules were found in red, black, and chocolate hairs respectively. Black and chocolate granules only were found in the skin. No red granules were seen there. Colorless granules were found in the white hairs of c^r and c^a animals. These granules occupied a position corresponding to that of the colored granules in pigmented hairs. In every instance, the color of the hair was the same as that of the granules which it contained.

The diffuse pigment ranged in shade from a pale yellow to a deep red-brown. It was seen only in the cortex of the fully formed hair, and it was always found in association with red, black, or chocolate pigment granules.

Development of Pigment

The earliest stages of pigment formation were observed in fetuses of 43 days copulation age. Cells containing pigment granules were seen in the anlage of the hair when it was only a slight condensation of ectodermal cells

EXPLANATION OF PLATE I

Fig. 1. Transverse section (x 148) through the skin of the back of a self intense chocolate fetus, aged 45 days. Fixed in Bouin's fluid: stained with hematoxylin and eosin. The general histological relations of the developing skin and hair are shown.

Fig. 2. The base of the central follicle in Fig. 1 (x 466). The cone of pigment cells which develops from the bulb of the hair is shown. Note the absence of pigment in the papilla of the hair.

PLATE I

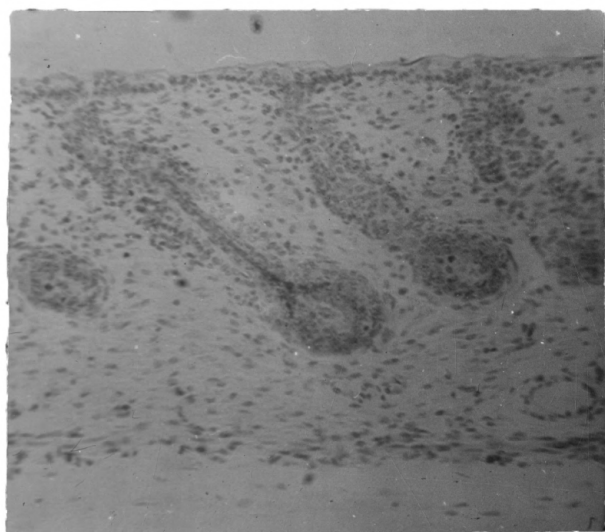


Fig. 1

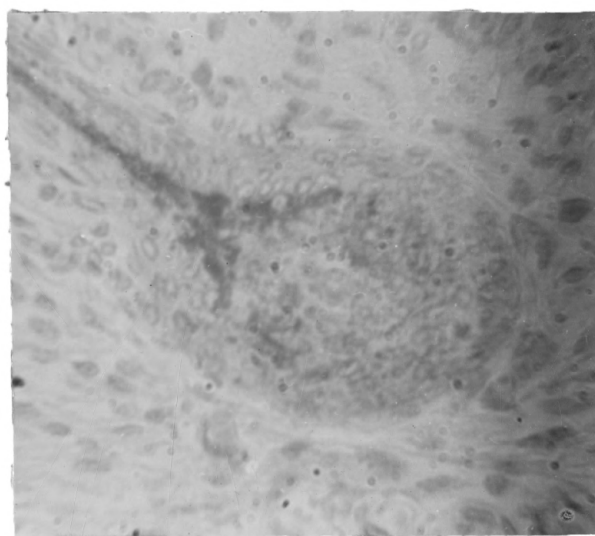


Fig. 2

projecting into the dermis. At the same time similar pigment cells were observed in the deep layer of the developing epidermis.

These pigment cells were larger than the ordinary cells of the dermis and epidermis, and usually were irregularly star-shaped, with long pseudopodia-like processes which extended far out between the surrounding cells. Mitotic figures were often seen in them. Cells of this type were found both in the epidermis and in the hair, but none occurred in the dermis, nor in the dermal hair papilla.

At a later stage of development (45 and 46 days) the branched pigment cells in the base of the hair follicle were arranged in a dense conical cap over the hair papilla (Figs. 1, 2). They were pushed upward by the division and growth of the cells next to the surface of the papilla, and they became differentiated into two types: the interlacing polygonal cells of the medulla and the spindle-shaped cells of the cortex of the hair. Branched pigment cells also occurred in the external root sheath of the follicle. They were especially conspicuous in black and chocolate animals (Fig. 3).

The definitive hair was composed of a large medulla

EXPLANATION OF PLATE II

- Fig. 3. Transverse section (x 156) through the tip of the ear of a self intense chocolate animal. Formalin-fixed, unstained. The distribution of pigment in the hair (seen in longitudinal section) and in the skin are typical. Note the thin layer of pigment in the wall of the hair follicle.
- Fig. 4. Transverse section (x443) through the skin of the ear of a self intense black animal. The stratum corneum is pulled away from the stratum granulosum. Note the absence of pigment in the dermis; the dense pigment in the stratum germinativum of the epidermis; and the "caps" of pigment over the peripheral ends of some of the nuclei. Pigment is more abundant in the basal layers than it is in the peripheral layers of the epidermis.

PLATE II

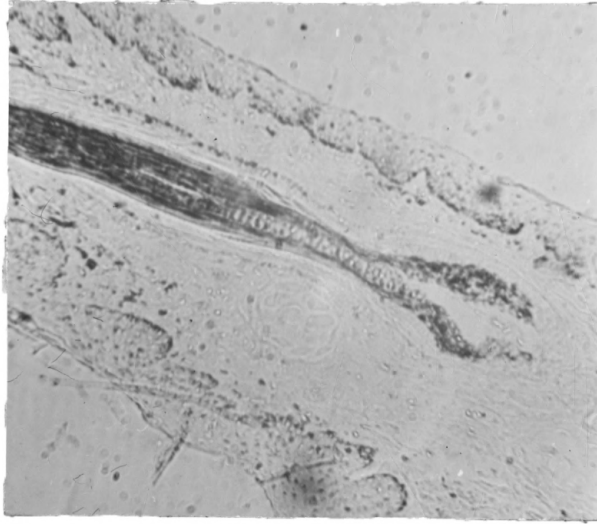


Fig. 3

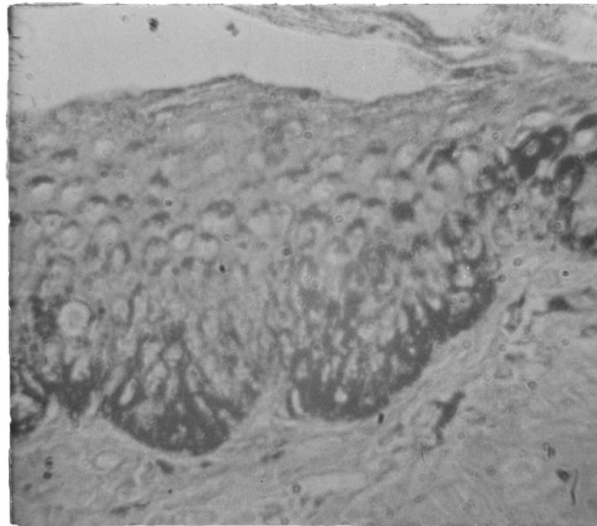


Fig. 4

made up of star-shaped cells between which lay anastomosing air spaces; a rather thin cortex made up of flattened spindle-shaped cells; and a cuticle of thin overlapping scales (Fig. 3). The medulla was narrow and discontinuous toward the distal end of the hair, and was absent at the tip.

The epidermis of the body was thin, and in pigmented animals branched pigment cells and scattered pigment granules were present in its deep layer. The epidermis of the nose and the ears became greatly thickened, and contained, in addition to the branched type of pigment cells, several layers of small rounded cells, in which granular pigment was found. (Figure 4.)

Pigment granules were seen in the cytoplasm of both types of pigment cells. They were either scattered or gathered into caplike masses over the peripheral surface of the nucleus, in the small rounded cells of the skin of the nose and ear. The flat cells of the stratum corneum contained fewer granules than the cells in the deep layer. In the branched type of pigment cell the granules were scattered through the cytoplasm or clumped in one or more rounded masses which were often so compact that the individual granules could not be distinguished. Either masses of granules or scattered granules or both

could be found in one cell. Pigment granules were also present between the cells of the hair bulb. Preliminary cytological observations indicated that the pigment did not originate directly from the nucleus, but was formed in the cytoplasm of the cell.

In their earliest stages of development no difference could be recognized between black, chocolate, and red granules. The time and place of development were the same for all three kinds of granules.

Study of the distribution of pigment granules in the hair has been complicated by the presence of refractive surfaces in the medulla which appear dark by transmitted light, and often look like pigment. The following methods were therefore employed in an effort to avoid confusion of pigment with "air spaces":

1. Comparison of cleared, uncleared, and partially cleared hairs
2. Examination of these hairs with reflected as well as transmitted light
3. Examination of sectioned hairs
4. Extraction of pigment with a 15 per cent solution of sodium hydroxide.

The results show that while pigment is present in the medulla of many hairs, air spaces are always present, and may look dark by transmitted light. It is possible that

in some cases where pigment has been described in "albino" hairs, the "air spaces" made observation difficult.

Description of Phenotypes

Intense Black (C- E- B- P-). The granular pigment of black animals was dark sepia in color. Rows and clumps of the granules were abundant in the cortex of black hairs, and "black" granules were densely packed in the shrunken cells of the medulla. A faint yellowish diffuse color was visible in the cortex of the hair. It was somewhat darker at the tip of the hair, and was almost absent at the base. "Black" granules were found in the pigment cells of the epidermis and of the wall of the follicle.

Intense Chocolate (C- E- bb P-). Chocolate granules were more translucent and were of a lighter brown color than the "black" granules. The amount and distribution of granules in the hair and the skin of chocolate animals was the same as it was in black animals. The pigment cells were the same in both types. The diffuse pigment in the cortex of a chocolate hair was more intensely red and more conspicuous than it was in a black hair. The granular pigment in the skin was of the same color as that in the hair.

There appeared to be no constant significant difference between black and chocolate with regard to the size, number, or distribution of the granules, or the time of their appearance. In the earlier stages of development, when only a few granules were present in the skin and hair, it was difficult to distinguish between black and chocolate granules. The difference in color was apparent only when a number of granules were observed together. The chocolate pigment appeared to be more susceptible than black to the effects of dilution factors such as c^r and p .

Deep Cherry Red (C- ee B- P-) (C- ee bb P-). All deep cherry red hairs which were studied contained translucent bright red-orange granules, abundant in the medulla and rather sparsely scattered in the cortex. A brilliant orange-red diffuse pigment was also present in the cortex. It was brighter near the tip of the hair than at the base. No black or chocolate granules were seen in any red hair, whether in a spot on an e^p animal or on a self red. The red granules were larger than black and chocolate granules, and they were more irregular in shape. Their color was entirely different from that of black or chocolate; no intergrades were found.

Red granules from different animals were sometimes different in shade; this variation seemed to be due in

part to differences in the age of animals, and in part to the influence of the factors B and b.

Sections of the skin of a spotted red-and-chocolate fetus were particularly valuable for comparing red and chocolate pigments. It was possible to observe in one field of the microscope follicles which were developing red hairs and follicles which were developing chocolate hairs. The differences could be observed easily and could not be ascribed to variations in the method of handling or preparation, since both were in the same tissue and were viewed under the same conditions of light and magnification. The red pigment in a spot on such a specimen appeared the same as that in a self red individual.

From observation of fresh whole mounts of the skin of black and red fetuses (litter mates) it was determined that the skin pigment is the same in both B- red and black animals. The branched pigment cells of the skin contained black granular pigment in both fetuses, although the hairs of the red fetus contained red granules and those of the black fetus contained black granules.

The skin of the nose and ear of B- red animals contained black granular pigment, although there was a little less present in red than in black animals. Chocolate granules were present in the same locations in bb red animals.

Newborn deep cherry red animals were a much deeper red and were more brownish than adults of the same genetic composition. This agrees with the observation that in the earlier stages of their development black, chocolate, and red granules look alike. However, microscopical and chemical observations showed that hairs from these young red animals behaved like reds with very concentrated pigment, rather than like chocolates or blacks.

Light Red ($c^d c^d ee bb smsm P-$) white at base. Hairs from animals of this composition had a few pale red granules in the cortex and the medulla. There was a small amount of yellowish diffuse pigment present in the cortex. It could be seen only at the tip of the hair. No chocolate granules were seen in the hair.

c^r Black and White ($c^r c^r eP- B- P-$); c^r Chocolate and White ($c^r c^r eP- bb P-$). Some of the c^r black animals were heterozygous for B; hence they produced both c^r black and c^r chocolate fetuses, which could not be distinguished from each other at an early age. When the difference could be recognized, as was possible in older fetuses, it was the same as the difference between C- black and C- chocolate, which has already been described. For these reasons, c^r black and c^r chocolate animals have been described together.

To gross observation, c^r black and c^r chocolate hairs appeared lighter in shade, especially near the base, than C- black and C- chocolate hairs. Microscopical examination showed that c^r hairs had fewer pigment granules both in the medulla and cortex, than C- hairs. The granules in c^r hairs were typically chocolate or black, as the case might be.

Red diffuse pigment was found in the cortex of c^r chocolate and c^r black hairs. It was less conspicuous in black than in chocolate hairs.

The white hairs of the c^r spotted animals contained neither red nor dark¹ granules nor diffuse pigment; however, colorless granules were present in them. The difference between white and dark hairs was especially evident in sections of the skin of these e^p (spotted) animals, which showed chocolate, white, and mixed hairs in the same field of the microscope. In the skin, scattered dark pigment granules were often present in regions where the hair was white as well as where it was pigmented.

¹

The term "dark" will be used as it was by Wright (1917) to include both black and chocolate granules, hairs, or animals, as distinguished from red or white.

c^r White (c^rc^r ee bb P-)(c^rc^r ee B- B-); "Albino"
White (c^ac^a ee bb P-). No difference was observed among
 the above three types of white hair. (E- bb and E-B-
 albinos were not available for study.) Neither red nor
 dark pigment granules, nor diffuse pigment, were found
 in any of the white hairs which were studied. Colorless
 granules were seen in both c^r and c^a white hairs. They
 occupied a position in the cell and in the tissue
 comparable to that of the pigment granules in pigmented
 hairs.

Treatment of Hairs with Sodium Hydroxide

Weighed hair samples were treated with 15 per cent
 sodium hydroxide solution and observed after 15 minutes
 and again after several days. The alkali destroyed the
 structure of the hair, but did not disintegrate the pigment
 granules, which, with the debris of the hair, were left
 floating on the liquid. The solutions assumed varying
 shades of yellow and red. The results of the treatment
 are outlined in Table 1. They corroborate the results of
 the microscopic observations in the following respects:

Black and chocolate hairs, both C- and c^r, contain red diffuse pigment.

Red hairs contain more diffuse pigment than dark hairs, and do not behave as if they contained dark pigment granules.

Chocolate hairs contain more diffuse pigment than black hairs.

There is no consistent difference among the types of white hair studied. None of them give evidence of containing red or dark pigment granules.

Hairs of newborn deep cherry red animals contain more red granular pigment and more diffuse pigment than do the hairs of adult animals of the same genetic composition.

In addition, the results of the treatment with sodium hydroxide suggest that c^r and c^a white hairs contain diffuse yellowish pigment. Possibly it is present in such a small amount that it escapes microscopic observation. It may be associated with the "colorless" granules in the same way that the more conspicuous diffuse pigment is associated with red and with dark granules.

Table 1. Results of treatment with 15 per cent sodium hydroxide solution

Color of hair sample	Color of solid	Color of liquid	
		After 15 minutes	After 5 days
c ^a bb ee white "albino"	white	clear pale yellow	pale yellow
c ^r bb ee white*	white	clear pale yellow	pale yellow
c ^r B- ee white	white	clear pale yellow	pale yellow
c ^d bb ee light red	pale yellow	clear pale yellow	pale yellow
C- bb ee deep cherry red	pale orange	deep orange red	deep orange red
C- B- ee deep cherry red	pale orange	deep orange red	deep orange red
C- B- ee deep cherry red (newborn)	pale orange	deep purplish red	deep purplish red
pp dilute chocolate	almost colorless	pale yellow-brown	medium red-brown
pp dilute black	light brown	very pale yellow-brown	light red-brown
c ^r chocolate	medium brown	pale yellow	medium red-brown
c ^r black	dark brown	very pale yellow	light red-brown
C- intense chocolate	medium brown	pale yellow	very dark red-brown
C- intense black	dark brown	very pale yellow	dark red-brown

* All animals were female except this one.

All animals were adult except one newborn, which is indicated.

The colors in this table were purposely described in general terms. The quantities of pigment concerned were only approximate, and fine gradations of color would have had little significance.

DISCUSSION

Development of Pigment

There has been some question as to the germ layer origin of the pigment and pigment cells of the mammalian skin and hair. Maximow and Bloom (1934) stated that the origin of the melanoblasts of both the skin and the hair (of man) was not definitely known. In those tissues of the guinea pig which were used in this study, the pigment was confined to the epidermis and its derivatives. No pigment cells were seen in the dermis or in the dermal hair papilla at any stage of development after the 43 day fetus, which was the youngest specimen in which they were seen.

The question has been raised by Maximow and Bloom (1934) and others as to whether the pigment cells of the skin and hair manufacture their own pigment or receive it from some other source. Maximow and Bloom (1934) state that the branched pigment cells of the basal layers of the skin (in man) are "dopa" positive and that the rounded pigment cells of the upper layers are "dopa" negative. Kroning (1930) showed that, in the guinea pig and the rabbit, the dopa reaction is stronger in the basal layers of the skin and in the hair papilla than it is in the peripheral layers. According to observations made in this

study, the pigment appeared first in the cytoplasm of the branched pigment cells in the hair and in the deep layer of the skin. This, like the above observations, may be evidence in favor of the theory that these cells elaborate the pigment which they contain.

The observation that the rounded cells of the skin are dopa negative need not be accepted as positive evidence that they do not produce the pigment which they contain. It is possible that they produce pigment early after their formation in the basal layers of the skin, and that they soon cease to manufacture it. Another explanation may be that the rounded pigment cells obtain enzyme from the branched melanoblasts, but manufacture the pigment within themselves.

Opinion is divided as to the cytological origin of pigment. Renyi (1924) working with the retina of the chick, concluded that pigment granules were metamorphosed mitochondria; Schultz (1912), basing his observations on pathological tissues of man, decided that pigment was formed from a chromidial network derived from the nucleus. In this he is supported by the observations of Ludford (1925 a, b) and others. Hooker (1915) found that pigment was formed in the cytoplasm immediately surrounding the nucleus in pigment cells of the skin of the frog.

Cytological observations on the guinea pig showed that most of the pigment was produced in cells although some pigment granules often appeared between the cells of the skin and hair. It was not determined what part of the cell was responsible for the manufacture of the pigment. The granules apparently did not come directly from the nucleus. The polar arrangement of the pigment and its frequent concentration in rounded masses at the side of the nucleus suggested possible activity of the Golgi apparatus in the formation of pigment in these cells.

Esskuchen (1927, 1930), working with cattle fetuses, found that the time at which the pigmentation appeared in the fetus varied with the color. He observed the first dark color in black embryos at 11.5 weeks and at 3.5 months he was able to distinguish the entire pattern. Reddish brown color was not observed until 7 months of gestation. In guinea pigs, no difference was found in the time of formation of red and of dark granules. The appearance of pigment in the hair was closely coincident with the development of the follicle. Pigment in the skin appeared at about the same time as in the hair. The dark pigment was more conspicuous than the red pigment to gross examination of the fetus. It seems improbable, however, that a difference in shade alone could account for the

great difference in time of appearance as observed by Esskuchen. Bogart and Ibsen (1937), found that the red color of cattle hairs was due solely to the reddish diffuse pigment. In guinea pigs, this red diffuse pigment appears much later than the granular pigment. The same may be true of cattle.

Esskuchen (1927) also stated that in cattle the extremities were the first to show pigmented areas. The same was found to be true of the guinea pig. In some types of guinea pigs, such as c^r whites and some "albinos", the extremities are the only places where pigment ever appears. In red animals, the color of the skin on the extremities shows the genetic composition of the animal more clearly than does the color of the hair.

The color of the eye is often more indicative of the genetic composition of a guinea pig than the color of the hair. This difference may be explained partly on the basis of the much earlier development of pigment in the eye (the iris shows pigmentation as early as 19 days), and in part by the fact that some of the eye color is derived from the choroid coat of the eye and some from the pigmented epithelium of the retina.

Action of Genetic Factors

The following observations agree with those of Bogart and Ibsen (1937):

1. Pigment in the hair of guinea pigs occurs in two forms: granular and diffuse.
2. The granular pigment is black in black hairs and chocolate in chocolate hairs.
3. Diffuse pigment occurs in several different shades of red.

The observations given below place a somewhat modified interpretation upon the conclusions of Bogart and Ibsen (1937):

4. Red hairs contain red granular pigment, but no black or chocolate pigment.
5. White hairs from all animals studied contain colorless granules, but no red, black, or chocolate granules.
6. Diffuse pigment is seen only in the cortex of the fully formed hair, and it is associated with some type of granular pigment. The intensity of the diffuse pigment is related to the type of granular pigment with which it is associated.
7. Red diffuse pigment occurs in c^R dark hairs, as well as in \underline{C} and \underline{c}^d hairs. None is found in white hairs.

Bogart and Ibsen (1937) described the diffuse red pigment in guinea pig hairs as if it alone accounted for the red color of red hairs. They thought that in dark hairs the black or chocolate granules were "extended", so

that they obscured the red diffuse pigment; and that in red hairs, the black or chocolate granules were present, but were not extended, thus permitting the red diffuse pigment to show through. They thought that a complete suppression of red diffuse pigment and the restriction of black or chocolate granular pigment to the medulla accounted for the colorless appearance of white hairs.

Neither black nor chocolate granules were found in ee (red and white) hairs. Red hairs contained red granules and white hairs contained colorless granules. The red granules are probably comparable to the "yellowish" granules which Hunt and Wright (1918) described in red and yellow guinea pig hairs. Hawkins (1931) described red granules as accounting for the red coloring in the feathers of pigeons. The colorless granules may not be homologous with the pigment granules of colored hairs.

Action of factors in the extension series. On the basis of the above observations, it seems that the factors E and e should be described as determining the production of a definite type of granular pigment (light or dark), rather than a variation in the amounts of diffuse and granular pigment. The light pigment produced in ee animals would then include the various shades of red granules, and possibly colorless granules. The dark pigment produced by

E- animals would include black and chocolate granules. Red or colorless granules are not seen in E- hairs; nor are black or chocolate granules found in ee hairs. The distribution of pigment in the hairs from the dark and light areas of e^p animals is the same as that in the hairs from E- and ee animals, respectively.

Action of factors in the "albino" series. The factors in the "albino" series will be discussed in terms of their relation to the factors E and e.

In C- animals, the factor E determines the presence of dark granules in the hair. The factor e determines the presence of red granules in comparable positions. The action in the skin is probably quantitative rather than qualitative, for no red granules were seen in the skin of any type of guinea pig studied.

Too little work was done with c^d animals to give a basis for describing the effects of that factor.

In c^r animals, the factor E produces dark granules in the hair, but e does not produce red granules. Instead, it may produce the colorless granules which we observed. Dark granular pigment appears in the skin of c^r white animals, especially in the extremities.

The factor c^a is epistatic to both E and e.

Neither red nor dark granules were seen in the hair of c^ac^a animals of our stock; but colorless granules were seen there as they were in c^r animals. Here again, the action is not the same in the skin as it is in the hair. "Albino" guinea pigs usually have dark pigment in the skin of the extremities. Because of the presence of this pigment, c^ac^a guinea pigs are not considered true albinos.

The relation between diffuse and granular pigment.

When the actions of the factors in the "extension" and the "albino" series are interpreted as above, it becomes evident that the diffuse red color need not be considered as one of the pigments which is primarily determined by the genetic composition of the animal. The granular pigments seem to be the direct expression of the action of the color-determining factors. The color of the hair in each case corresponds to the color of the granules in it. The red diffuse pigment appears to be formed in the cortex of the hair as a consequence of the presence there of some type of granular pigment. It behaves as if it were the product of a chemical or physical reaction between the granules and the cornified substance of the cortex of the hair.

Gortner (1911) in his description of two kinds of melanin, says:

Those melanins which are soluble in dilute acids are of a protein nature (melanoproteins). It appears probable that these melanoproteins are not present as granules, but that they are "dissolved" in the keratin structure.

The melanins which are insoluble in dilute acids are of an unknown constitution and are probably the "pigment granules" which may be seen in the hair and tissues.

Edwards and Duntley (1939), in studies of the living human skin, described a modified diffuse form of melanin which they called "melanoid". It resembled melanin closely in its spectrophotographic properties, and it occurred only in cells derived from melanin-bearing cells --that is, the cells of the stratum corneum. The amount of melanoid in any skin was related to the amount of melanin found there. Because of these relations, the authors regarded "melanoid" as a degradation product of melanin. It was present as a dissolved pigment, and reinforced the melanin, particularly in giving a purer yellow color than melanin alone.

The relations described by Edwards and Duntley (1939) for the human skin are similar to those found in the hair of the guinea pig. These authors did not study the hair of their subjects.

In c^r animals, white hairs take the place of red hairs

in C- animals. Bogart and Ibsen (1937) assumed that these hairs were white because they lacked red diffuse pigment. They expected that c^r black and chocolate hairs would also lack diffuse red pigment. The presence of red diffuse pigment in c^r black and chocolate hairs at first seemed contradictory to the description of the c^r factor (no red present). Its presence may be explained if one assumes that it is red granular pigment, rather than red diffuse pigment, which is determined by the C- factor in the presence of ee, and which will not appear in c^ree hairs. In the absence of these red granules no red diffuse pigment is formed; hence c^ree hairs are white. In c^rE- hairs the diffuse pigment is formed as it is in C-E- hairs, in association with the black or chocolate granules which are present in the hair. The close association between granular and diffuse pigment is parallel to the description of the "albino" series as given by Bogart and Ibsen (1937), in which there is no separate factor for the red diffuse pigment.

The absence of all pigment from the hairs of "albino" (c^ac^a) animals can be explained on this same basis. In such animals neither red nor dark granules are present in the hair; hence no diffuse pigment is produced, and the hair is colorless.

The differences between black and chocolate pigment granules have already been described (page 9). Part of the difference between the types of granules seems to lie in their relation to the diffuse type of pigment. Red granules are bright and translucent and are associated with a large amount of diffuse pigment. Chocolate granules are darker and denser and less of the diffuse pigment is found with them. Black granules are darker than chocolate granules, and are associated with an even smaller amount of diffuse pigment.

When deep cherry red guinea pigs are examined, it is evident that the B- red animals are of a deeper color than the bb reds, and that this difference is due as much to the shade of the hair as it is to the color of the skin of the ears, nose, and other exposed parts. The differences in the color of the skin may be explained on the basis of the presence of black pigment in the skin of the B- red animals, and chocolate pigment in the skin of bb red individuals. It was thought that the different shades of the red hairs might be due to the presence of black and chocolate granules in B- and bb hairs. Since in both types of hair, red granules only are present, some other explanation must be found for the difference in shade between the two types of red hair. It has already been mentioned

that there is some variation in the shade of the red granules. They are somewhat lighter in bb red hairs than they are in B- red hairs. The difference was seen only in material from adult animals. This leads to the conclusion that the factors B and b may have the same kind of effect on red granules as they do on dark granules. They may produce "light" and "dark" red granules in ee hairs just as they produce chocolate and black granules in E hairs.

CONCLUSIONS

1. The earliest stages of pigment formation in the hair and skin of the guinea pig were seen in fetuses of 43 days. At this stage no difference was apparent among black, chocolate, and red pigment granules.
2. In older fetuses and in adult animals, black, chocolate, and red granules were present in both the medulla and cortex of black, chocolate, and red hairs, respectively. Colorless granules, which may or may not have been homologous to the true pigment granules, were seen in white hairs. In every case, the color of the hair corresponded to the color of the granules which it contained.
3. Diffuse reddish pigment was seen only in the cortex of the fully formed hair in association with black,

chocolate, or red granular pigment. Hence it was present in C- red and dark hairs, and in c^r dark hairs, but it was absent in c^r and c^a white hairs.

4. It is possible to explain the actions of the color determining factors in terms of their relation to granular pigment alone. The diffuse pigment may be considered as the product of the granular pigment. The following outline presents a possible explanation of the action on the hair of the color-determining factors which were studied:

The factor E may determine the presence of dark granules.

The factors B and b determine whether these granules are black or chocolate.

The factor e in the homozygous condition may determine the presence of red granules.

The factors B and b may have a slight effect on the shade of the red granules.

In the presence of the factor C, both E and e may act as described above.

In the presence of the factor c^r, in the homozygous condition, E may act as described, but e may produce only colorless granules, or none at all.

Where the factor c^a is present in the homozygous condition, E does not produce dark granules and e does not produce red granules. Unless other factors are present, the hairs of c^ree and c^a animals are white.

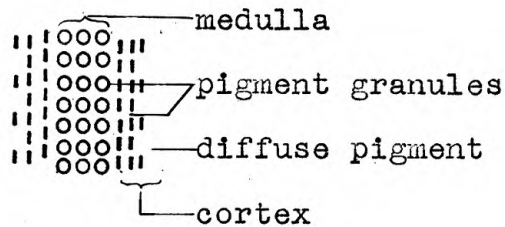
The action of the factors in the light and dark areas of e^p (spotted) animals corresponds to that in e and E animals respectively.

In the skin, the factors E and e, C, c^r, and c^a may affect the amount rather than the kind of pigment present. Where there is a small amount, it is found chiefly in the extremities. The factors B and b may control the color of the pigment in the skin, and act in the presence of either E or e.

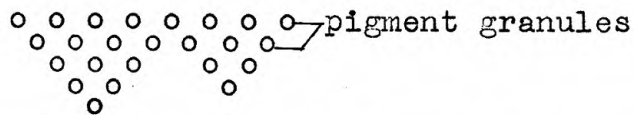
5. Treatment with a 15 per cent sodium hydroxide solution indicated the presence of a dilute form of red diffuse pigment in white hairs. This had not been observed in the microscopic examinations. With this exception the results of the two methods of observation agreed.
6. Observations on the histological and cytological origin of pigment in the guinea pig indicated that the pigment cells were ectodermal in origin and that pigment granules were not formed directly from the nucleus of the cell.

EXPLANATION OF PLATE III

This chart presents the phenotypic and genotypic relations of pigment in the guinea pigs which we have studied. Each of the twelve rectangles represents a genotype: rectangle 6, for example, represents an animal of the composition c^r-E-bb or the dark spots on an animal of the composition $c^r-eP-bb$. For each genotype, the distribution and relative amounts and shades of diffuse and granular pigment are given. The diagrams are to be interpreted as follows:



Longitudinal section of hair



Transverse section of epidermis from nose or ear

For the sake of completeness, we have included in the chart genotypes 9 and 10. Animals of this composition were not included in the material which we were able to study in detail.

PLATE III

	E- ← e ^p → ee		
	B-	bb	
C-	1	2	3
c ^r	5	6	7
c ^a	9	10	11

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