THE NATURE OF COAT COLOR DIFFERENCES IN MINK AND FOXES*[1]

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INTRODUCTION

COAT colors in laboratory mammals such as the mouse, rat, guinea pig and rabbit have long been used in genetic studies involving the physiology and transmission of genes. Horses, cattle, swine, sheep, goats and other domestic animals also present an array of different coat colors, but with the exception of the use of a particular color or color pattern as a breed marker, differences in pigmentation probably have little or no economic value in these forms. The situation is just the reverse with regard to the fur bearing animals raised in captivity; the value of the pelt is considerably influenced by color phase. Although color has always been one of the most important factors in determining pelt value, the fur trade became color phase conscious to a marked degree with the appearance of the first platinum silver fox pelts on the New York market in 1940. Since that time, mutant colors in mink and foxes have been in great demand.

The studies reported here deal with some quantitative and qualitative differences in pigmentation of the pelage of the silver, platinum and pearl color phases of the red fox (*Vulpes vulpes* L. = *V. fulva* species), and the dark, platinum and pastel color phases of the mink (*Mustela vison* Peale and Beauvois). The mutant color phases mentioned result from single gene substitutions, and so far as is known, all of the individuals contributing fur samples differed from their respective standard ranch-bred color phases only at the mutant locus indicated.

The main objective of the study was to isolate some of the links in the chain of events which intervene between the gene and its end point. This information should have value in studies of gene physiology. Three methods of approach to this problem have been considered: (1) histological aspects of guard hair pigmentation, (2) quantity of melanin pigment production as determined by weight, and (3) measurement of pigment granule length.

Coat color in mammals is generally attributed to melanins, organic compounds containing nitrogen, usually dark in color, and characterized by chemical inactivity. Observations such as the persistence of color in mutant spots or areas, and in transplantation experiments indicating the local pro-

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duction of pigment, and the resulting belief that the chain of reactions between gene and character is relatively short for mammalian coat color genes have led to many genetic analyses of the pigments produced in different genotypes of several mammals. WRIGHT (1942) has used the coat colors of the guinea pig as an example of some of the kinds of relations found between genes and characters.

The literature dealing with one or another phase of pigmentation is extensive and a number of investigators have given considerable attention to reviews of the literature. Since E. S. RUSSELL (1946) has given a rather complete selective review of the literature concerned with mammalian pigmentation, no attempt will be made at duplication here.

DESCRIPTION OF COLOR PHASES

Foxes

Most of the foxes raised in capitivity for their pelts are color phases of the American wild red fox (*Vulpes vulpes*, L.). A detailed description of the wild red fox and the black mutant that is the standard fox of the ranching industry has been dealt with elsewhere by COLE and SHACKELFORD (1943) and will not be repeated here except to state that in the black fox the red of the guard hairs and the albescence of the "white" areas of the wild red fox have been replaced by black. Two phenotypically similar mutations to black occurred in the wild red foxes of North America prior to the rise of the ranch breeding industry (ASHBROOK 1937); both are almost completely recessive to their respective non-mutant alleles, the one occurring in the Eastern United States and Canada designated as the Eastern Standard black, and the one occurring in Western Canada and Alaska as the Alaskan black.

The use of the term "black" was soon abandoned by the fur trade in favor of "silver" because of the exclusiveness, among fur bearers, of the type of silvering characteristic of the red fox (figs. 1a and 7 Dc). In most mammals, silvering refers to a sprinkling of white guard hairs throughout an otherwise colored pelage. The silvering of the red fox is dependent upon the portion of guard hairs with pigment-free subterminal bars scattered over the body (fig. 7Dc). Silvering appears to be inherited independently of color phase. KELLOGG (1941) interpreted degree of silvering to be dependent on several factors. Hereafter, the term "silver" will be used to designate the black color phase of the red fox.

Silver foxes long had such a hold on the trade that when a mutant color did appear, it was usually disposed of summarily as indicating lack of purity in the standard stock. In 1938 TUFF described a new type being raised in Norway which he referred to as "the platinum character in the silver fox." The general color of the platinum is lighter than that of the silver due not only to the grayish tone of the colored area but also to the smaller pigmented area. One of the most characteristic things about platinum is the considerable amount of white spotting that always accompanies it. MOHR and TUFF (1939) have well described it as follows: "White snout, a blaze along the nose and forehead

joining with a white color around the neck. The breast and a broad stripe on the belly are white and the same is true of the legs and the distal part of the tail. The size of the markings is somewhat variable, particularly on the legs, and the white collar may be lacking in dark individuals." It might be added that the amount of white on the face and the other parts of the body may in some cases be so small that the classification by phenotype might be doubtful as an index of genotype if it were not for the other characteristics of the platinum.

MOHR and TUFF (1939) presented breeding records clearly indicating "that the platinum character is due to an ordinary dominant gene," and suggested that it might be lethal in the homozygous condition. COLE and SHACKELFORD (1943) have discussed the occurrence and breeding histories of three separate mutations to platinum in the United States and Canada, which are presumably the same as the Norwegian platinum. Their data fully confirm the conclusions of MOHR and TUFF. White pups which are dead at birth or die shortly after, are occasionally found and are taken to represent individuals of the homozygous dominant class which have survived longer than is usual or by chance have been found. JOHANSSON (1947) has analyzed data from Swedish fox farms, and also concludes that the platinum character is due to an autosomal dominant gene which is lethal in the homozygous condition. Based on the results of limited experimental matings of platinum×platinum, however, he does not believe that homozygosity for the dominant mutant gene necessarily produces a white pelage.

A mutant coat color in the fox which is presumably homologous with maltese in the cat and the dilute character in other mammals is called pearl platinum, or simply pearl, by fox breeders. Pearl differs from the standard silver only in the general dilution of the black which gives the characteristic "blue" of this color phase; the pelage is a bluish gray throughout, and the eyes, nose, lips and claws are distinctly lighter than in the silver fox. BOWNESS (1944), who appears to be the first to have collected the breeding histories from several ranches on which pearl foxes were originally bred, says that LA DUE recalls having seen pearls in Minnesota as early as 1924. Previous to the popularization of the Norwegian platinum and the resulting general search for mutant color phases among silver foxes, however, pearls had occurred on several ranches in the United States and Canada and the character was understood among ranchers to be the result of homozygosity for a simple recessive gene. DEAKIN (1942) reported two different mutations in Canada which produce the pearl coat color. The coat colors resulting from these different mutations are so similar in appearance that they are phenotypically indistinguishable.

Mink

Domestic mink have been derived from the geographical races of the American mink (*Mustela vison*, Peale and Beauvois). The standard color of ranch-bred mink is similar to that of the wild mink; the pelage is usually dark brown, but may vary from light brown to near black. A stripe down the back, the tail and feet are darker than other parts of the body. The eyes, nose and claws are dark brown, and there are often white spots on the chin, throat, breast and

belly, although some individuals are free of all white markings. A number of color variations have arisen from ranch-bred mink; SHACKELFORD (1941), SMITH *et al.* (1941) and CASTLE and MOORE (1946) have described most of them and discussed the type of inheritance involved in each case.

Platinum[2] was the first mutant color phase to command the attention of mink breeders. This color phase had been observed in the wild, however, and the first platinum garment is reported to have been made from the pelts of animals taken by trappers over a period of several years. Platinum appears to have occurred first in captivity on the ranch of Mr. WILLIAM WHITTINGHAM of Arpin, Wisconsin, in 1929, but shortly thereafter made its appearance on several other ranches in the United States and in Canada. The pelage of the platinum mink is a light bluish gray in color. The claws, nose and lips are also lighter than in the dark mink. In other respects it is similar to the dark mink; the eyes are dark brown, ventral white spots are usually evident, and a stripe down the back, the tail and feet are darker than other parts of the body. The platinum character is due to homozygosity for an autosomal recessive, and is presumably homologous with pearl in the fox and the dilute character in other mammals.

Pastel, blond, and chocolate are a few of the names that have been applied to a light brown color phase in mink. This is similar in appearance to the "chocolate" mutation in other mammals.[3] The eyes, nose and claws are distinctly lighter than in the dark ranch-bred or average wild mink. It is often very difficult to distinguish pastel pelts from those of the lighter mink caught in the wild, which indicates that this color phase may have appeared in the wild and not been noticed. Since ranch-bred mink have been selected toward the dark brown to near black end of their range in coloring, no difficulty is experienced in distinguishing the pastel from dark mink under ranch conditions. The pastel character results from homozygosity for a simple recessive gene, and like most of the recessive color phases in both foxes and mink has occurred on several ranches in the United States and Canada. SHACKELFORD and COLE (1947) have reported an abnormal behavior pattern termed "screw neck" which is sometimes associated with this color phase.

HISTOLOGICAL ASPECTS OF PIGMENTATION

Several investigators have used the histological approach as an aid in determining the physical basis for color differences in hair. DURHAM (1904) noticed that in dilute mice the color of the pigment granule was unchanged from that of black animals, but that the pigment appeared to be clumped and reduced in quantity. HUNT and WRIGHT (1918) noted the clumping of pigment in dilute guinea pigs. GREMMEL (1939), in his study of the pigmentation of

[2] The name platinum was presumably given this color phase as a result of the popularity of the platinum silver color phase in the fox. The use of the same name to designate color phases in both species should not be taken as suggesting a similarity in expression of the mutant genes involved. The pelt of the platinum color phase is designated "Silverblu" by the MUTATION MINK BREEDERS ASSOCIATION.

[3] A brown mutant color phase called "Burgundy" is known in the red fox.

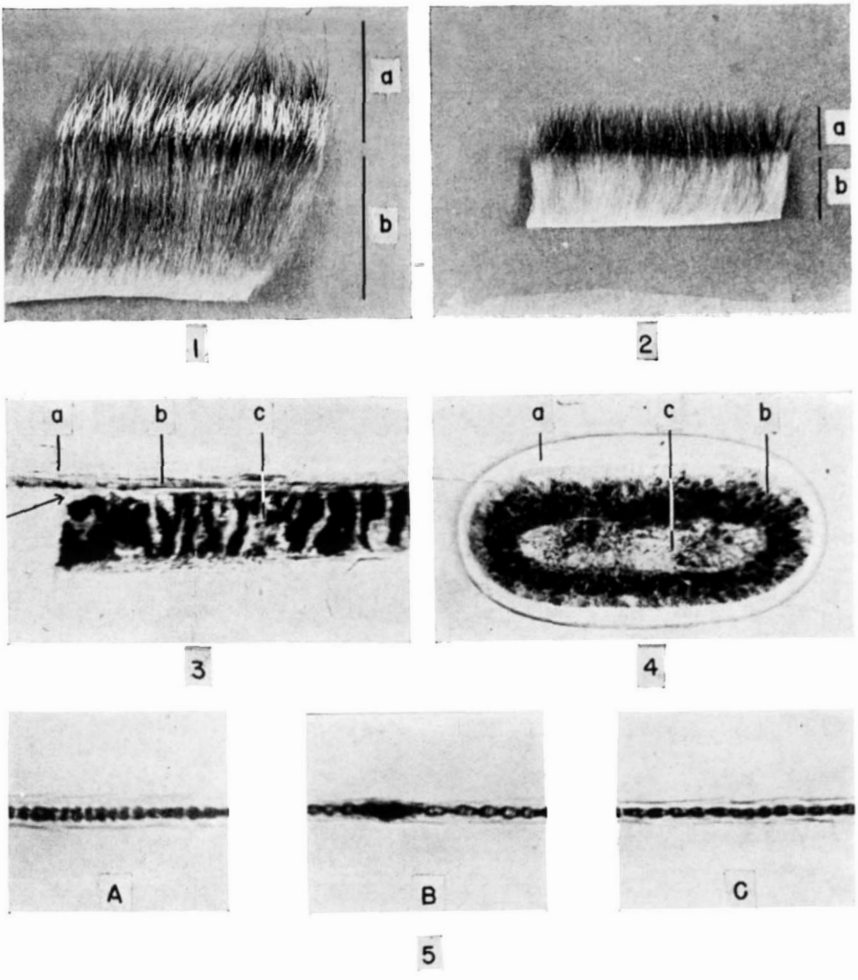


FIGURE 1.—A small section cut from the rump area of the pelt of a silver fox showing the underfur (b) and the portion of the guard hair (a) that projects beyond the underfur.

FIGURE 2.—A small section cut from the rump area of the pelt of a silver sable mink. The silver sable color phase of the mink is particularily well adapted for comparison of the guard hair (a) and the underfur (b) since the major effect of this dominant mutant gene is a general diminution of pigment production in the underfur with no effect on the guard hair except for the small number of white ones scattered over the entire body.

FIGURE 3.—Oblique section of a guard hair of the pearl fox. Arrow indicates separation of cortex (b) and medulla (c); pigment granules in the cortex oriented with long axis parallel to long axis of hair. ×370

FIGURE 4.—Cross section of a guard hair of a dark ranch-bred mink showing the cuticle (a), cortex (b), and medulla (c). ×370

FIGURE 5.—Portions from whole mounts of single hairs from the underfur of the three color phases of the mink; (A) dark, (B) platinum, and (C) pastel. ×400

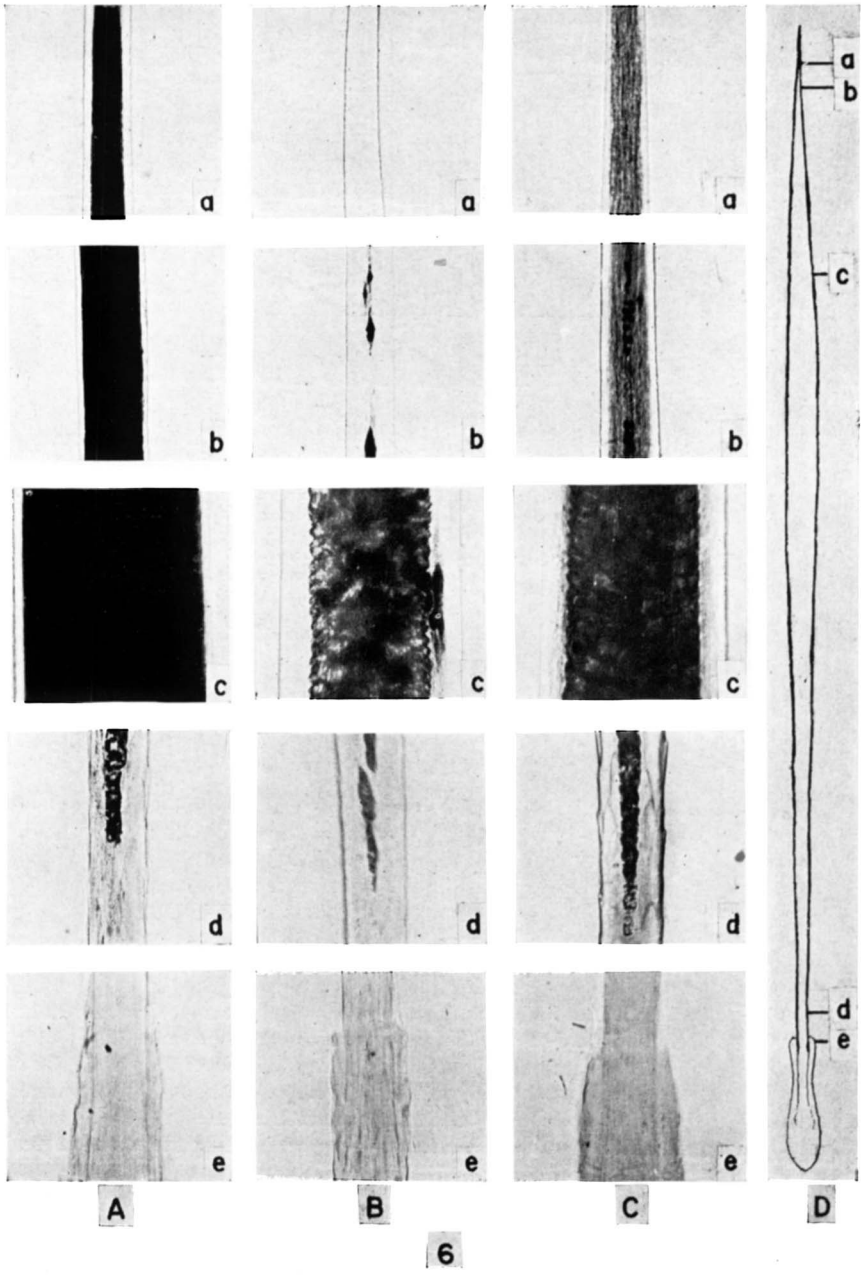


FIGURE 6.—Photon.icrographs of whole mounts of single guard hairs from three color phases of the mink; dark (A), platinum (B), and pastel (C). The approximate level at which the five portions of each hair were taken is indicated in the diagrammatic illustration (D). ×300

horse hair, found that "crowding of pigment to one side is characteristic of all dun horses." The histological technique has been used to distinguish differences in coat color by DANNEEL (1936) in the rabbit, HARMON and CASE (1941) in the guinea pig and E. S. RUSSELL (1946) in the house mouse.

The pelage of fur bearing animals consists of two rather distinct types of hairs which for purposes of classification can be designated arbitrarily as the underfur and the guard hair. The hairs composing the underfur are smaller, shorter, and more numerous (figs. 1b and 2b) than the guard hairs. These latter project beyond the underfur for one fourth to one third of their total length, the overhanging part being known collectively as the "veiling" or "covering" (figs. 1a and 2a).

Three areas are readily distinguished in the definitive hair: the medulla (fig. 4c), a central portion composed of loosely arranged cells with many intercommunicating spaces; the cortex (fig. 4b), a cylinder immediately surrounding the medulla and composed of keratinized cells; the outermost layer or cuticle (fig. 4a), composed of scales arranged in shingle fashion (figs. 5 and 6Cd) the overhanging edges toward the tip of the hair.

The young hair emerging from an active follicle is without a medulla for a short distance (fig. 6Ba and Ca), but as growth continues and the diameter increases the medulla begins to appear irregularly (figs 6Bb and Cb), becomes more continuous, and finally occupies the greater portion of the hair in the region of its greatest diameter (figs. 6Bc and Cc). As the diameter of the hair begins to regress, the medulla also begins to occupy less space until it becomes interrupted and finally disappears altogether a short distance from the root (figs. 6Ad, Bd and Cd). This series of events results in the hair having a cuticle and cortex that are continuous from tip to root, but with the distal tip and the base free of the medulla. The hairs of the underfur differ slightly from the above description. Their medulla is usually discontinuous throughout its length, the air spaces alternating in regular fashion with areas similar in appearance to the cortex (fig. 5).

Pigment granules in the cortex of the hair are oriented with their long axis parallel to the long axis of the hair, but in the medulla the granules appear to be arranged at random (fig. 3). This arrangement is characteristic of all color phases of mink and foxes, but is difficult to observe in the guard hairs of heavily pigmented color phases.

The tip of the guard hair is most diagnostic of color phase (figs. 6Aa, Ba, and Ca); consequently these observations have been limited to the distal one fourth of the guard hair. The type of pigment granule distribution in the silver color phase has been taken as the "wild type" for comparison with mutant colors in the fox since the pigment in the wild red (both red and black pigment) is arranged in a manner similar to that in the silver, and the type distribution found in the dark ranch mink as "wild type" for comparison with the mutant color phases in the mink.

Whole mounts and cross sections of representative guard hairs have been chosen for histological study because of the difficulties inherent in the study

of the underfur. The small diameter and the relative fragility of the underfur makes sectioning difficult, and the differences in pigmentation characteristic of the various color phases are less readily distinguished than in the guard hairs (Compare figs. 5 and 6).

Color of Pigment Granules

Color of the individual pigment granule is very difficult to determine. When seen in the hair (either in whole mounts or cross sections) under the microscope, the variation of a single granule from the darkest genotypes ranges from almost black to a light amber color, depending upon the amount of light admitted. Extracted pigment, either dry or in alcohol, appears dark brown in the silver, platinum and pearl color phases of the fox; the same is true of the dark and platinum mink, but the pigment from the pastel mink is distinctly lighter in color than in any of the other mink or fox color phases.

An attempt has been made to assign a color grade from RIDGEWAY'S *Color Standards and Color Nomenclature* to the pigment granules from each of the color phases considered. Color of the pigment granules does not appear to be the major cause of the differences in mutant coat color as compared to the standard coat color with the exception of the pastel mink.

Extracted granules from each of the color phases were placed on a glass slide in a thin layer, covered with a xylol solution of isobutyl methacrylate, and observed under oil immersion (objective 95×, ocular 20×). The maximum amount of light was admitted, a daylight filter being used on the lamp. Under these conditions, the granules from the silver, platinum and pearl color phases of the fox and the dark and silverblu color phases of the mink came nearest matching Sudan Brown. Granules from the pastel mink were somewhat lighter, being nearer Ochraceous-Tawny.

Materials and Methods

The number of different samples from which individual hairs have been taken for microscopical observation are shown in table 1. Most of these samples were plucked from the rump of living animals, although a few were taken from other parts of the body such as the shoulder, head and tail. All of the samples used for the photomicrographs, however, were collected from the rumps of live animals with a prime pelt (November, 1946). Guard hairs of silver, platinum and pearl foxes, and dark, platinum and pastel mink were cleaned by washing in an ether bath (described in the following section), and allowed to dry at room temperature. The hairs that were to be observed as whole mounts were placed on a glass slide and covered with a thin layer of methacrylate solution instead of being mounted in the usual manner. The low power (objective 10×, ocular 10×) of the microscope was found adequate in examining the whole mounts: all photomicrographs were taken with the high power (objective 44×, ocular 10×).

Hair is very difficult to section without tearing, and as pigment arrangement was the object of this study, a method to obtain undamaged cross sections was

of prime importance. HARDY'S (1935) method proved excellent for studying tufts of hair, but cross sections prepared in this way were usually crowded so much that one impinged upon the other, either distorting the individual section or interfering with illumination. This method is also inadequate when it is desired to cut cross sections as nearly the same thickness as possible. Further, it is important that comparable regions of hairs of comparable lengths be obtained, and this method allows little control over this operation. Since isolated and undamaged cross sections were necessary, other methods of sectioning have been investigated.

The following method, although somewhat time consuming, was found to

TABLE 1

Number of samples from which hairs have been taken for histological observations.

SPECIES	COLOR PHASE			NUMBER OF ANIMALS SAMPLED
Fox	Silver			70
	Pearl			29
	Platinum	Werth	15	43
		Cody	10	
		Norwegian	12	
		La Forrest	6	
Mink	Dark			35
	Platinum			20
	Pastel			24

give the best results. Guard hairs taken from the same samples as those used for the whole mounts and cleaned in the same way, were dipped individually into a xylol solution of isobutyl methacrylate polymer (E. I. DU PONT DE NEMOURS & CO., INC), and allowed to dry at room temperature. Drying was usually completed in one to two hours. Applications of the methacrylate solution were made until a layer of the dry material about 1 mm thick surrounded each hair. Three hairs prepared in this manner were placed together and dipped into the solution and allowed to dry overnight.

These groups of three hairs, encased in a coat of the methacrylate, were mounted in paraffin (melting point 50°–54°C) in the usual manner, and sectioned at ten micra. The paraffin ribbons were fixed on glass slides, allowed to dry overnight, and the paraffin and methacrylate removed with xylol. A thin layer of the methacrylate solution was applied to the slide in place of an ordinary cover glass.

The methacrylate coat served two purposes; it held the hair firmly and allowed it to be sectioned with a minimum of distortion, and the thickness of the methacrylate coat between each individual in the group of three hairs

allowed the proper spacing so that one section did not press against the other and either distort it or interfere with illumination. Sections cut much thinner than ten micra were apt to tear, and if much thicker, the more densely pigmented ones were too opaque to allow the arrangement of pigment to be studied in detail.

Results

The cross sections revealed a notable difference in shape of the guard hairs of the mink and foxes. The fox guard hair is approximately round in cross section throughout its length. Cross sections of mink guard hairs are oval in shape except at the tip and base which are round; the ventral side of the oval cross section is less curved than the dorsal side (fig. 4).

Mink

Dark

The guard hair of the dark mink is so heavily pigmented that the internal structure is completely obscured in views of whole mounts (fig. 6A), except that the cuticle can be seen to be pigment free. Cross sections reveal that the dark brown pigment granules are arranged singly or closely packed in groups in the cortex and medulla (fig. 8A). The non-medullated tip is more densely pigmented than any other part of the guard hair.

Platinum

The platinum (fig. 6B) shows the greatest contrast of any of the color phases when compared with the wild type (dark) distribution. The pigment granules are dark brown in color like those of the dark mink, but in longitudinal views of a guard hair the pigment gives the impression of irregular transverse bands alternating with light areas relatively free of pigment. Cross sections (fig. 8B) show that the pigment is mainly clumped in the cortex and medulla, and that most of the pigment is in the medulla, resulting in the cuticle appearing considerably thicker than in the dark mink. The non-medullated tip is free of pigment.

Pastel

Enough light passes through a whole mount of the guard hair of the pastel to reveal the cortex and medulla (fig. 6C). In cross section (fig. 8C), the pigment granules do not appear to be so closely packed as in the dark mink, and are distinctly smaller and lighter in color than in either the dark or platinum; except for the fact that the outer margin of the cortex is sometimes low in pigment, the distribution is similar to the wild type (dark).

Foxes

Silver

The denseness of the pigmentation in the silver fox obscures the internal structure of the hair as in the dark mink. The transparent pigment-free cuticle appears to be about one-third as thick as the cuticle of the mink guard hair

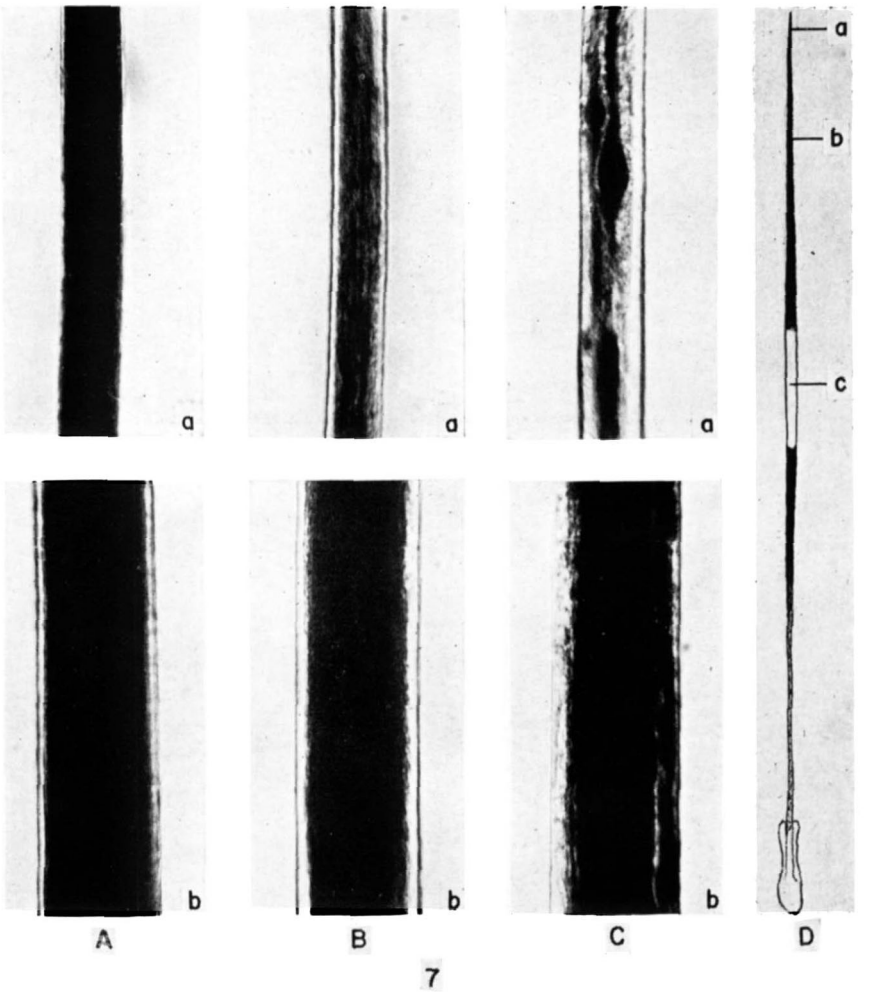
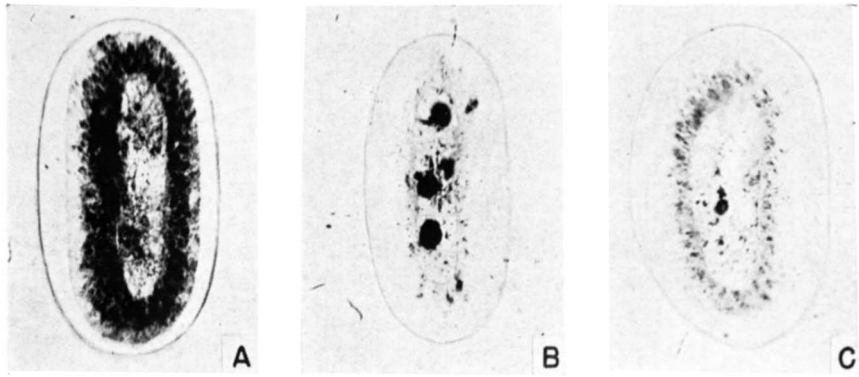


FIGURE 7.—Photomicrographs of whole mounts of single guard hairs from three color phases of the red fox; silver (A), platinum(B), and pearl (C). The approximate level at which the two portions of each hair were taken is indicated by the diagrammatic illustration (D). The "silver" bar characteristic of all color phases of the red fox is shown (c). ×200

(compare figs. 9A and 8A). A cross section (fig. 9A) reveals the numerous pigment granules oriented at random in the medulla, and a cortex packed from the inner to the outer margin with single granules and groups of granules. The most densely pigmented part of the guard hair is the non-medullated tip.

Platinum

No pigment can be seen at the extreme tip of the platinum guard hair, but pigment gradually appears (fig. 7Ba) below this area. The general sparseness of pigment allows the internal structure of the hair to be readily seen in light and medium colored individuals. In the darker platinums, only the extreme



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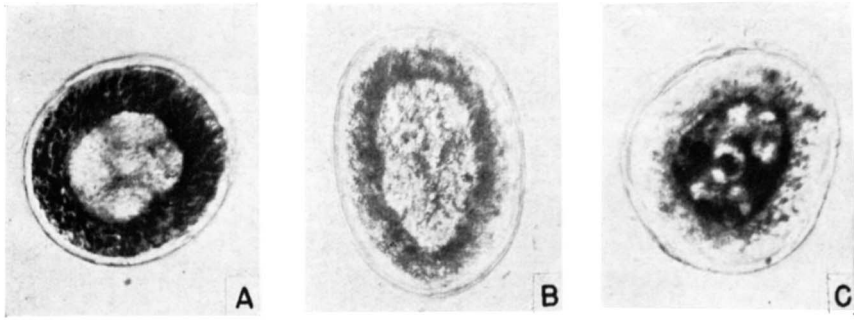

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FIGURE 8.—Photomicrographs of a cross section of a guard hair of the dark (A), platinum (B), and pastel (C) color phases of the mink taken at the level indicated in the diagrammatic illustration. ×300

FIGURE 9.—Photomicrographs of a cross section of a guard hair of the silver (A), platinum (B), and pearl (C) color phases of the red fox taken at the level indicated in the illustration. ×300

tip of the guard hair is diagnostic since the platinum and silver guard hair may be similar in appearance below the area where the medulla has become continuous, or in the vicinity of a "silver" bar. The translucency of the tip of the platinum guard hair is easily seen with the unaided eye; this characteristic has been especially useful in distinguishing some of the lightest "white-marked" silver phases (COLE and SHACKELFORD) from the true platinum color phase.

Granules in the platinum guard hair are distributed in the same manner as in the silver fox, except that there are smaller numbers of granules per unit area, as can be seen in a cross section (fig. 9B). The pigment in the cortex first appears in the area adjacent to the medulla and gradually approaches the outer margin of the cortex as the diameter of the hair increases. The cuticle appears to be thicker than in the silver, but this is the result of a tendency

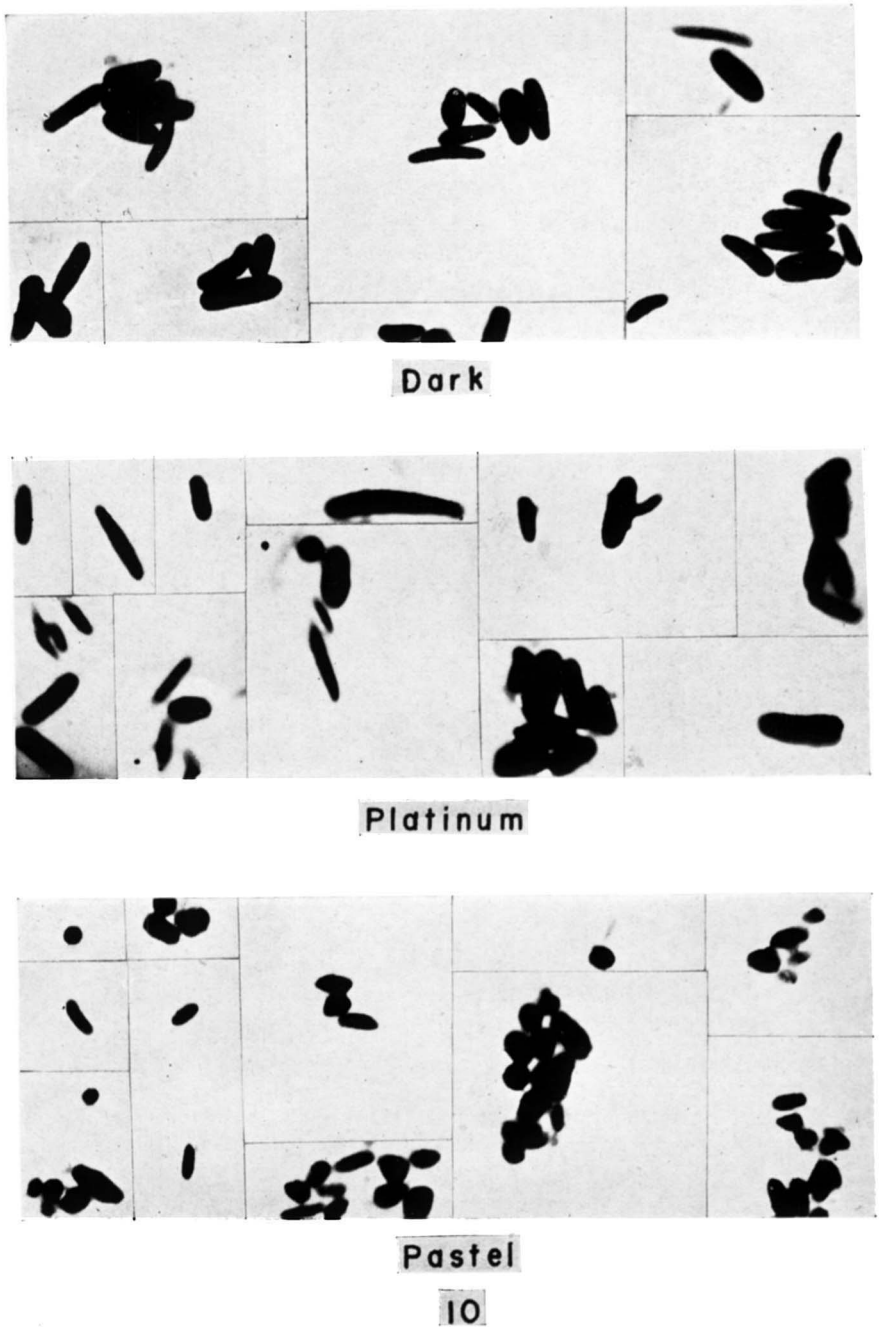


FIGURE 10.—Pigment granules extracted from the hair of three color phases of the mink. ×9,130

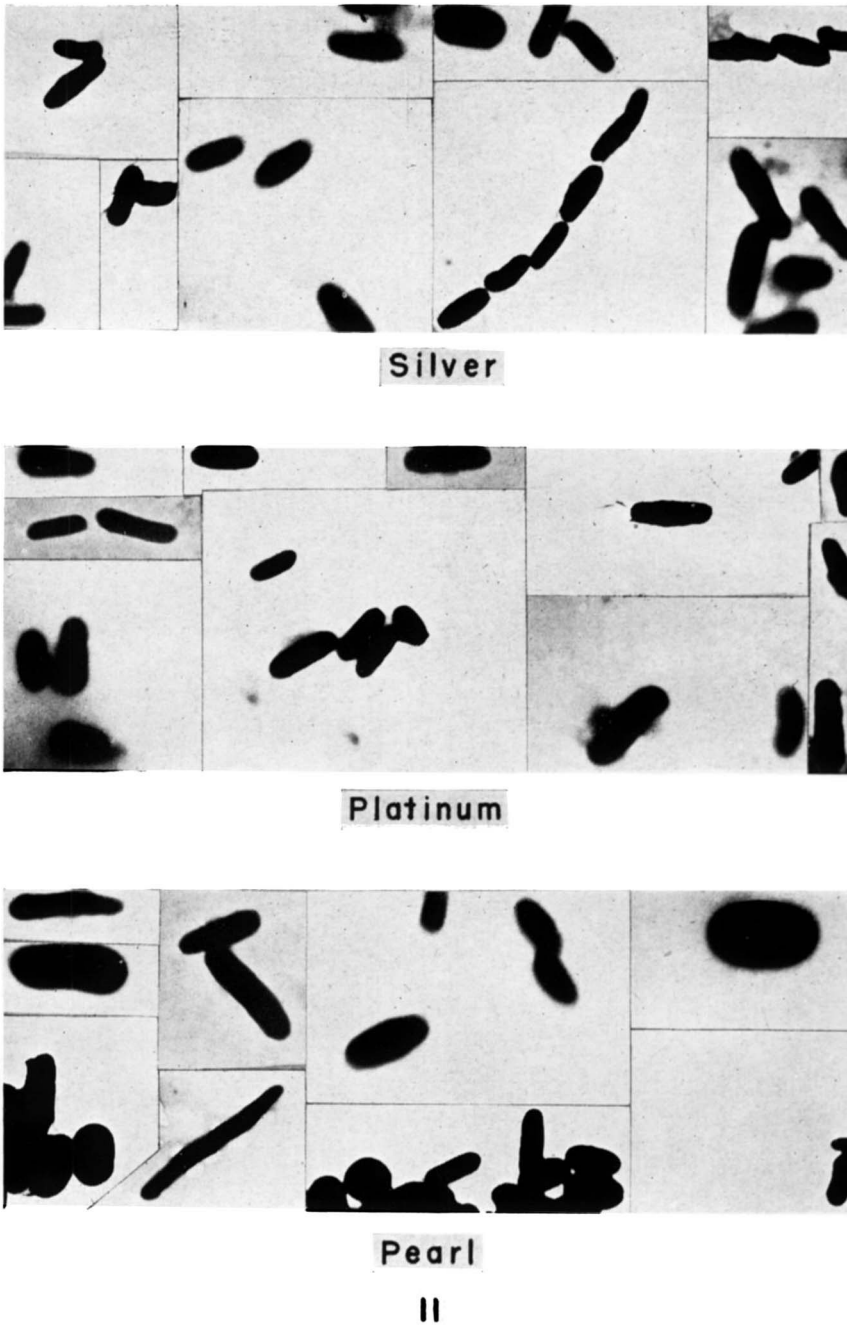


FIGURE 11.—Pigment granules extracted from the hair of three color phases of the red fox. ×9,130

for the small amount of pigment in the cortex to be arranged around the medulla. In the photomicrographs the granules appear lighter in color than in the silver as a result of more light passing through the hair section.

Pearl

The pigment clumps characteristic of this color phase appear as a series of irregular transverse bands in whole mounts (fig. 7C), making the pigment distribution in the pearl guard hair the easiest of all the color phases to distinguish from the "wild type" distribution. Clumping is most readily observed near the tip, but can be seen the entire length of the hair. As in the platinum, the cuticle appears to be thicker than in the silver guard hair.

The majority of the granules in this color phase are combined into relatively large aggregates, although a number of individual granules and small groups of granules are occasionally found (fig. 9C). Most of the clumps are in the medulla, but the majority of the pigment in the cortex is clumped and tends to occupy the area of the cortex contiguous to the medulla.

QUANTITATIVE MEASUREMENT OF MELANINS

The chemical inactivity of the melanins makes extraction difficult. EINSELE (1937) has considered the possible methods of separating melanins from the hair protein (keratin) and concluded that acid hydrolysis is the most suitable. He developed a technique which gave reliable results in the analysis of pigment production in mice of various genetic constitutions. EINSELE's method has been used in these studies.

This method depends upon the differential reaction of melanin and keratin to acid treatment; keratin is hydrolyzed by sufficiently long treatment, but melanin appears to be unaffected. E. S. RUSSELL (1939) has used this technique successfully to obtain pigment for her studies on quantitative differences in pigmentation in the sepia series of guinea pigs. She has called attention to the distinction made by GORTNER (1912) between acid-soluble and acid-insoluble melanins, and in this connection has commented as follows: "It should be noted that EINSELE's statement concerning the insolubility of mouse melanin depends on the fact that the color obtained in the acid hydrolysate is always pale yellow, showing no correlation with the amount of melanin in the hair treated. By the same reasoning the same conclusion holds for guinea pig melanins." The same results were obtained in the extraction of fox and mink melanins, the acid hydrolysate always being pale yellow regardless of the color of the hair treated.

Materials and Methods

Fur samples were obtained from living animals during the first week of May (1946) before the onset of the spring moult. A method thought to represent the different color phases adequately was the selection of two individuals that were approximately midway between the darkest and lightest in coat color characteristic of the range in their respective color phases. Fur samples from the standard silver, platinum and pearl color phases of foxes were collected

on a commercial fox farm; the samples of dark, platinum and pastel mink were obtained from animals in the University experimental herd.[4]

Although foxes are large enough to produce adequate samples in relatively limited areas of the body, the area chosen was the rump since there is greater uniformity of silvering between the various color phases on this area. The hair from an area approximately 10 cm×12 cm was removed for each sample. The small size of the mink and the shortness of the fur made it necessary to use a larger area; the fur on the back from the shoulders to the tail and well down the sides was removed for each sample. The fur samples were cut as close to the skin as possible by the use of ordinary scissors. The density and finess of the underfur, especially in the mink, made the use of electric clippers practically impossible.

Fat and dirt must be removed from the fur to circumvent two sources of error in determining the total hair weight attributable to melanin. Probably the greatest error results from the loss of granules that become entrapped in the soap ring formed inside the flask during hydrolysis, due to the action of acid on fat. Another source of error results from the increased weight of the hair due to impurities. EINSELE found that in mice dirt and fat could account for as much as 12 percent of the total weight of a hair sample.

Coarse dirt and some of the fat were removed by washing each fur sample in a 0.1 percent solution of sodium lauryl sulphate (sold commercially as "Dreft") for one hour as suggested by RUSSELL (1939). The solution was decanted and the fur rinsed three times with hot distilled water. After the last rinsing, the sample was divided into eight portions and each portion spread in a layer approximately .5 cm deep in the trays that are described below. The trays containing the wet fur were placed in a drying oven kept at 95°–105°C where they remained for four hours, then transferred to a desiccator until needed for further treatment.

EINSELE (1937) found that washing with water removed only part of the fat, and that further cleansing was necessary. He devised a bath which allowed the hair to be washed continuously with clean ether. The principle used by EINSELE has been applied in making the fat extractor for these studies.

A large glass vacuum desiccator (Scheibler) with the hole in the top was found satisfactory. The condensing coil was made of glass tubing. The hole fitted with a countersunk rubber stopper served as an entrance and exit for the coil. To insure sealing the stopper in the hole and the coil in the stopper, melted paraffin was poured over the stopper until it was flush with the surface of the collar. This procedure firmly attached the condensing coil to the top, and reduced the chance of breakage at each time the bath was opened. The top was sealed to the bowl of the desiccator by a thin coating of vaseline, and to prevent the escape of vapor due to pressure increases, two small thumbscrew type clamps were used to hold the top securely in place.

The trays for holding fur samples in the ether bath were made of copper screening (19 mesh). A rack to support the trays was fashioned of copper wire

[4] Cooperative experiments with the WISCONSIN CONSERVATION DEPARTMENT'S EXPERIMENTAL GAME AND FUR FARM, Poynette.

(No. 12), and had a capacity of four 10 cm×10 cm trays. The four posts of the rack extended 1.5 cm above the top tray, and served as a rest for the piece of copper screening which acted as a baffle in dispersing the drops of ether as they rained from the condensing coil.

The apparatus was set in a water bath on a tripod, and a 250 watt light bulb used as a source of heat. This arrangement kept the ether boiling, and the water running through the coil at tap temperature condensed it with sufficient speed to produce a continuous shower on the trays. Approximately 5 cm of ether was kept in the bottom of the desiccator; this had to be replenished each time the top was removed due to escape of vapor.

Preliminary tests indicated that the fur samples ceased to lose weight after two days in the ether bath, but to insure complete cleaning a four day treatment period was used. Each sample started at the bottom position on the rack, advanced upward by one position each day until at the end of the fourth day it was removed, the ether allowed to evaporate at room temperature, and the sample placed in the drying oven for one hour. Samples were stored in a desiccator until weighed; after three hours the fur samples ceased to lose weight, so a minimum of four hours in the desiccator was allowed before hydrolysis was begun.

Each portion of the sample from an individual, weighing 500–2000 mg in the fox and 500–1500 mg in the mink, was transferred to a 500 cc pyrex Florence flask fitted with a reflux condenser, 300 cc of 6N hydrochloric acid added, and the mixture boiled for two hours. The keratin appeared to be completely hydrolyzed after one hour, but boiling was continued for another hour to make sure all melanin granules had been liberated. After cooling, the acid solution was centrifuged at 3000 r.p.m. for 10 minutes and the liquid decanted. The residue was washed four times with distilled water and as the solution no longer gave a chloride test, the pigment was considered free of the keratin. In some of the lighter pigmented color phases, considerable difficulty was experienced in getting the melanin granules to settle out from the acid solution, even when centrifugation was continued for an indefinite time. This was overcome by diluting the solution with distilled water.

Following the fourth washing, the water was decanted and the pigment washed from the centrifuging tube into weighing bottles with 95 percent ethyl alcohol. The weighing bottles were placed in the drying oven and allowed to remain for one hour after the pigment appeared to be completely dry, then placed in a desiccator to remain until a constant weight had been attained (completed in all cases by four hours). These weights were recorded, and the percent of total weight of the hair sample represented by the melanin pigment was calculated. The final figure tabulated in table 2 is the mean of the eight portions of each sample.

Results and Discussion

Histological studies in several animals have indicated an apparent difference in the quantities of hair pigment in some of the lighter colored genotypes as compared to the darker. As early as 1904 Miss DURHAM made this observation

in her studies of dilute mice. The same observation was made in the histological studies of the guard hair of mink and foxes, but in certain color phases such as platinum in the mink and pearl in the fox the density of the individual pigment clumps could not be determined by visual inspection, so extraction was necessary to determine the relative quantities of pigment produced by the different color phases.

TABLE 2

The percent of melanin pigment in the fur of some color phases of mink and foxes.

SPECIES	COLOR PHASE		ANIMAL NUMBER	MEAN (%)	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY (%)
Mink	Dark		1	4.2	.1	2
			2	4.3	.1	2
	Platinum		1	1.2	.1	8
			2	1.4	.1	7
	Pastel		1	2.1	.1	5
			2	2.4	.1	4
Fox	Silver		1	5.0	.3	6
			2	5.0	.3	6
	Platinum	Werth	1	1.9	.1	5
			2	1.8	.1	6
		LaForest	1	2.1	.2	10
			2	1.9	.1	5
	Pearl		1	3.9	.1	3
			2	3.8	.3	8

The following generalizations can be made insofar as the two individuals of each color phase can be considered as representative of their respective color phases: in mink the approximate portion of the hair weight attributable to melanin pigment is 4.3 percent in the dark, 1.3 percent in the platinum and 2.3 percent in the pastel; for foxes, 5 percent in the silver, 1.9 percent in the platinum and 3.9 percent in the pearl. The pastel and dark mink bear the same relation to each other in quantity of pigment produced as reported by DUNN and EINSELE (1938) for brown and black mice; that is, pastel produces about one-half as much pigment as does the dark mink.

EINSELE (1937) found that as a result of long boiling, melanoids were formed during hydrolysis by the action of acid on keratin, and that these substances adhered to the melanin granules. By the use of albino hair he was able to determine that the quantity of melanoids formed and their ability to adhere

to the melanin granules were independent of the quantity of the granules in the hair, and thus relatively greater errors were introduced in determining the quantity of pigment produced in the lighter than in the darker genotypes. He arrived at a correction of this error in his work with mice as follows: "If such hairs are hydrolyzed together with heavily pigmented hairs, then the combined residues may be easily centrifuged out. Thus, if one uses as adsorbing agents the granules of a weighed quantity of pigmented hair of known pigment content, then from the differences between the weight of the total residue and the calculated weight of the granules used as adsorbing agents the percentage content of such lightly pigmented hairs can be easily calculated."

This correction was not applied in determining the pigment content of the fur of the various color phases of mink and foxes because pigment free fur was not available. No overlaps were observed between the different genotypes as to quantity of pigment produced, so the uncorrected determinations were considered adequate to indicate the relative differences in pigment production. DUNN and EINSELE (1938) found uncorrected weights satisfactory in their comparisons of the pigment producing abilities in the different genotypes of mice.

MEASUREMENTS OF PIGMENT GRANULE LENGTH

Some differences of pigment granule size and shape in the pastel and dark mink are apparent from cross sections of the guard hair; in the pastel most of the granules are considerably smaller, and appear to be more spherical than rod-shaped as are most of the granules in the dark mink. This observation suggested that some of the differences in fur color in the different genotypes of mink and foxes were related to granule size, shape, or a combination of these qualities.

Using preparations of melanin granules isolated by acid hydrolysis, EINSELE (1937) found that in mice different genotypes have characteristic granule size distributions. RUSSELL (1946) was able to measure the medullary pigment granules in various genotypes of mice from cross sections of the hair, stating that "The complete outline of individual granules is clearly visible in cross sections of even the most crowded types."

An approximation of the range in size of pigment granules characteristic of each genotype was desired, and this could not be obtained with cross sections because of the orientation of the granules in the cortex (fig. 3b) and the crowded condition in both cortex and medulla (fig. 4). Further, the clumping characteristic of two color phases (platinum in mink and pearl in the fox) possibly prohibit representative measurements of the individual granules in cross sections. Hence, in these studies the melanin granules extracted by acid hydrolysis from mink and fox fur were used.

RUSSELL'S (1946) observation that in dilute mice there "are large sharply defined clumps which may quite possibly be composed of the regular granules of the genotype concerned cohering to each other" applies to the present observations in mink and foxes. These clumps, however, appear to be completely broken up into their component granules by the acid treatment.

There has been considerable controversy since the first investigations of the underlying factors in hair color differences as to whether granular pigments were the only pigments involved. Several of the earlier investigators thought that diffuse pigments were present in addition to granular pigments in certain colors of hair, and at least one declared that the color of blond and red hair in humans was due solely to diffuse pigments. EINSELE (1937) has considered this question at length. He found that in the mouse "With the appearance of the least pigmentation granular pigments are always found . . . " and concluded that "Since we have been able to measure the pigment granules from hairs of all grades of pigmentation and to correlate their sizes with the visible hair colors it is unnecessary to assume the existence of diffuse pigments." No evidences of diffuse pigments were observed in mink and fox hairs, although it should be remembered that the colors here considered are comparable to the sepia series in other mammals, while the yellow series is the one involved in this controversy.

The minuteness of melanin pigment granules makes an approximation of their size and shape difficult with the ordinary light microscope, although both EINSELE (1937) and RUSSELL (1946) have accomplished this feat with mouse pigment. The great magnifying power of the electron microscope, however, makes it invaluable in a study of this kind.

Methods and Materials

An RCA model EMU-1 electromagnetic electron microscope has been used in these studies. Slides for use with the microscope must be prepared in a special manner. One way of making slides is as follows: the small screen disc (200 mesh) which acts as a support for a thin film upon which the material to be examined rests, is placed on an ordinary glass slide in the bottom of a dish of water; a film is prepared by placing a drop of parlodian on the surface of the water where it immediately spreads into a thin layer approximately .01 micra in thickness; the glass slide is raised toward the surface until the film comes to rest on the screen disc, then the slide and its contents are carefully removed and placed in a desiccator so that all moisture will be removed. The slide prepared in this special manner is placed in the microscope, and the objects to be observed are seen as silhouettes on a fluorescent screen.

Pigment granules obtained from the quantitative measurements were stored in 95 percent alcohol for use in the qualitative studies. The melanin extracted from the eight fur samples of each of the individuals representing their respective color phases was put into a single flask, and as a consequence a pigment sample taken from any one flask would contain granules produced by both animals, presumably in equal numbers. To make sure that the granules were thoroughly mixed at the time a sample was taken, the flask was shaken vigorously.

Great difficulty was experienced in getting individual granules free of their neighbors since extracted melanin granules cling together in large clumps. For use with the light microscope where the orthodox method of preparing slides can be used, a little "wet" pigment directly from the centrifuge tube diluted

with water makes a satisfactory preparation. Slides for use with the electron microscope can not be prepared in this manner, however, because the granules clump together on the parlodian film as the water evaporates.

The following method of preparing slides for use with the electron microscope was found satisfactory. About 2 cc of the stored granules were poured into a watch glass and the alcohol allowed to evaporate. As much of the resulting dry pigment as would cling to the end of a small glass rod was stirred with two or three drops of parlodian on a glass slide, the mixture taken into a pipette and dropped on the surface of the water. Spreading of the parlodian to form the film brought about two desired results: many granules were either isolated singly or in small groups in such a manner that their size and shape could be observed, and the granules appeared to be oriented with their long axis parallel to the surface of the film.

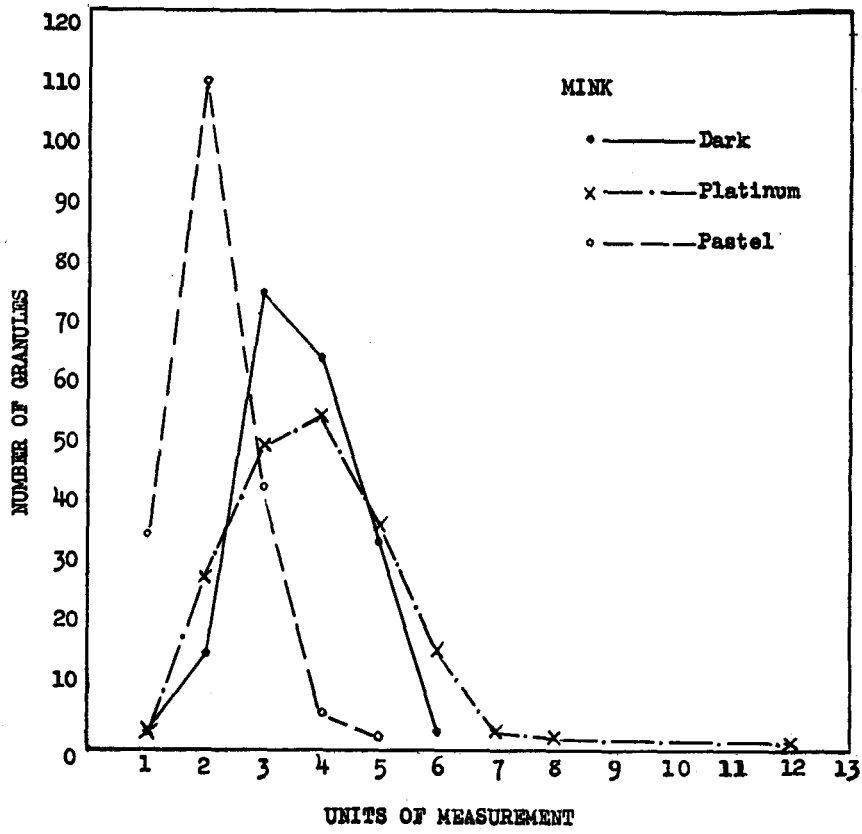
All of the unencumbered granules in a square chosen at random were observed and their lengths approximated. This process was continued until 200 granules from each of the color phases had been measured. The unit of measurement was taken as one-tenth of a side of one of the four squares of equal size on the fluorescent screen. At a magnification of 10,140 diameters, this unit was equivalent to .19 micra.

Results and Discussion

Several qualitative differences in the granules of the different genotypes can be seen from the photomicrographs shown in figures 10 and 11. In both species most of the granules are rod-shaped, the pastel mink being the only one in which appreciable numbers of round, or near round, granules occur. Further differences noted in the mink are that granules of the pastel are notably smaller in comparison with the other two genotypes, and that very large and long granules are occasionally seen in the platinum, which in other respects appears similar to those of the dark mink. In the three genotypes of the fox, such differences as exist are less noticeable than in the mink, the exception being the notably greater range in size of the granules in the pearl fox as compared with either silver or platinum.

Length is a component of size that can be directly approximated and therefore has been used as a measure of quantitative differences between the different genotypes. The frequency distribution of granule length in each of the different genotypes is shown in figure 12. Granules smaller than one unit (.19 micra) were included in the first class, but in only one genotype, pastel in mink, could this grouping have resulted in appreciable errors since there are relatively few granules in this class. Granules approximating .19 micra in greatest diameter or smaller are for all practical purposes round in shape.

The dark genotype in mink and the silver genotype in the fox have been taken as the standard with which to compare the mutant genotypes of their respective species. Two features of the granule size distributions have been tested against their respective standards; the difference in the means of the two distributions ("t" test) and a comparison of the class frequencies (χ^2 test). The results of these calculations are tabulated in tables 3 and 4.



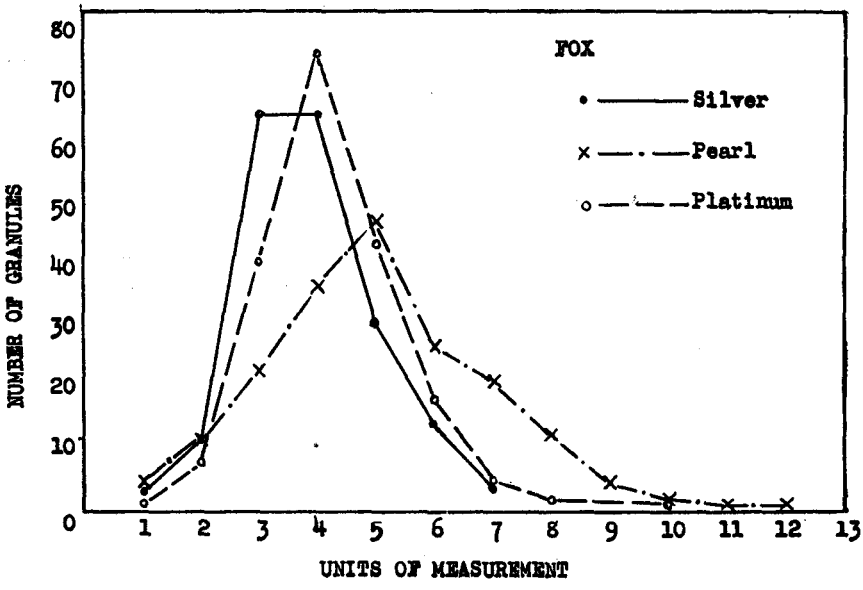


FIGURE 12.—Frequency distributions of the length of random samples of 200 pigment granules extracted from the hair of each of the color phases of the mink and fox indicated. 1 unit = .19 micra.

Foxes

Granules of the silver fox range from less than .19 micra to 1.33 micra in length with a mean length of .74 micra. In the platinum the range is from less than .19 micra to 1.90 micra with a mean length of .82 micra. The means of the two distributions differ significantly ($P < .01$), and the class frequencies are on the border line of significance ($P \cong .04$).

TABLE 3

Lengths of melanin pigment granules extracted from several color phases of the fox and mink.

SPECIES	COLOR PHASE	NUMBER OF GRANULES	MEAN (MICRA)	STANDARD DEVIATION
Fox	Silver	200	.74	.21
	Platinum	200	.82	.23
	Pearl	200	.97	.36
Mink	Dark	200	.68	.38
	Platinum	200	.74	.32
	Pastel	200	.40	.15

TABLE 4

Comparisons of the distributions of melanin pigment granule lengths of mutant color phases of mink and foxes to their respective "standards."

SPECIES	COLOR PHASES COMPARED	MEAN DIFFERENCES		CLASS FREQUENCY DIFFERENCES	
		"t" VALUE	PROB.	χ^2 VALUE	PROB.
Fox	Silver—Pearl	7.93	$< .01$	69.30	$< .01$
	Silver—Platinum	3.00	$< .01$	11.94	$\cong .04$
Mink	Dark—Platinum	2.06	$\cong .04$	15.37	$< .01$
	Dark—Pastel	9.60	$< .01$	191.07	$< .01$

The range in granule length in the pearl genotype is from less than .19 micra to 2.28 micra with a mean length of .97 micra. The means of the two distributions differ significantly ($P < .01$) as well as the class frequencies ($P < .01$).

Mink

The granules of the dark mink range in length from less than .19 micra to 1.14 micra with a mean length of .68 micra, and in the platinum genotype up to 2.28 micra with a mean length of .74 micra. It is questionable whether the differences between the means of these distributions are distinct enough ($P \cong .04$) to consider granule length a distinguishing feature. The size distribution of the granules of the platinum, however, differs significantly from that of the dark ($P < .01$).

Granules of the pastel mink range in length from less than .19 micra to .95 micra with a mean length of .40 micra. The pastel and the dark granule length distributions overlap but the means of the two distributions differ significantly ($P < .01$) as do the class frequencies of these distributions. The relation of the dark and pastel mink in regard to size of pigment granule approximates the results of DUNN and EINSELE's (1938) investigations with black and brown mice, that is, "The chief effect of the mutant change from B to b is thus to decrease the size of the units in which melanin is deposited."

Some of these observations are strikingly similar to RUSSELL's (1946) observations on pigment granule size in mice. She found the narrowest range in chocolate mice, this being similar to the pastel mink; the widest range was in dilute mice which is comparable to the pearl and platinum mutations. The granule lengths in her dilute mice, which are presumably comparable to platinum in the mink and pearl in the fox, tended to be skewed toward large size. This is also true in both pearl and platinum (fig. 12).

SUMMARY

An attempt has been made to characterize some of the quantitative and qualitative factors of pigmentation involved in coat color differences in several mutants of the red fox (*Vulpes vulpes* L. = *V. fulvus* species) and the mink (*Mustela vison* Peale and Beauvois). Three methods of approach have been followed: (1) histological aspects of guard hair pigmentation, (2) quantity of melanin pigment produced as determined by weight, and (3) measurements of pigment granule lengths. In the fox, the ranch-bred silver color phase has been taken as the standard to which the platinum and pearl mutants have been compared; for the mink, the standard for comparison of the platinum and pastel mutant color phases has been the ranch-bred dark.

Mink. The major effects of the platinum mutation on coat color appear to be the clumping of the pigment granules and the reduction in the quantity of melanin pigment to approximately one-third as compared to the dark mink. In both color phases the granules are dark brown in color. The granules of the platinum are more variable in length and shape than in the dark, and the frequency distribution curve of granule length is skewed toward larger size.

Pigment granules of the pastel as compared to the dark are notably smaller in size and lighter in color; greater numbers are round in shape, and the variation in length is less. The distribution of the pigment in the hair is similar to that of the dark mink.

Foxes. The major difference in the coat color of the platinum mutant as compared to the silver appears to result from a reduction in the number of pigment granules per unit area in the hair; the platinum has about 40 percent as much pigment by weight as the silver. The pigment granules are dark brown in both color phases and are the same shape. The mean length of granule in the platinum is significantly greater than in the silver, and the difference in frequency distribution between the two color phases is on the border line of statistical significance.

The pearl mutation appears to produce most of its effect on coat color by a

clumping, and reduction in quantity of the melanin pigment to approximately 80 percent of that characteristic of the silver. The granules of the pearl and silver are the same in color, but in the former the granule length and shape is more variable, and the frequency distribution curve of granule length is skewed toward the larger size.

These investigations of the nature of coat color differences in the various color phases of mink and foxes closely parallel the observations of earlier investigators working with the mouse in so far as the qualities considered are comparable and the probable homology of the different mutants of the three species obtain. The granular clumping characteristic of the dilute mutation in mice is also characteristic of the platinum mink and pearl fox; the dilute mouse, the platinum mink and the pearl fox have a greater variability in granule length than their respective standards of comparison, and the frequency distribution curves of granule length are skewed toward the larger size in all cases; in fact, the frequency distribution curve of granule length of the platinum mink and the pearl fox appear more similar to each other than either one does to the other color phases within its own species. The pastel mutation in the mink and the brown mutation in the mouse appear to be determined by a similar pattern in granule size. These color phases have the least variability in granule length of any of those investigated in their respective species. The pastel mink produces about one-half as much pigment by weight as the dark, which is comparable to the pigment producing abilities of brown mice.

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