THE RELATION OF THE DISTRIBUTION OF BLACK AND RED SKIN AND HAIR PIGMENTS TO COLOR INHERITANCE IN CATTLE

by

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INTRODUCTION

Considerable work has been done on the inheritance of color patterns in cattle. The same is true of color, but on the other hand little or nothing has been accomplished in regard to the cytological basis for color. This applies especially to the mahogany, or blackish (Bs, "black-spotting") found in Ayrshires and Jerseys (Ibsen, 1933). Red animals and those carrying blackish (Bs) each vary considerably. For that reason there seems to be no clear cut difference between a dark red and a "minus" blackish animal. Because of the overlapping of red and blackish it was decided to study microscopically the pigments in the hairs and in skins from the noses of animals showing these colors in order to determine if possible what causes the differences and also the resemblances. In addition, it was decided to study the pigments in the hair and skin of animals carrying some of the other color genes.

LITERATURE

Bachrach (1930) in giving his technic for preparing hair slides for identification purposes, stated that one usually needs to bleach pigmented hairs with peroxide

(H_2O_2) before the internal structure can be seen.

Hausman (1927) states, "Color, in infra-hominid hairs, is produced: (1) by a diffuse yellowish, or reddish yellow, acid-soluble melano-protein, which uniformly stains the keratized protoplasm of the cortical cells, and sometimes the cells of the medullary column; (2) by granules or large masses of varying shades of brown, of an acid-soluble melanin substance, occurring in or among the cells of the cortex, or of the medulla, or of both; (3) rarely by a deposit, from skin glands, of a yellowish homogeneous pigment on the surface of the hair-shaft; and (4) by a combination of all of these, most often of the first two." No chemical results or other data were given in support of his statements regarding the chemical nature of these pigments.

Hunt and Wright (1918) in a microscopic study of guinea pig hairs found both diffuse and granular pigments. Granules were observed in sepias, blacks, yellows, and creams but not in reds. The diffuse pigment was observed in the cortex of red, yellow, and cream hairs. A general fading of the diffuse pigment near the surface of the hair was observed.

Ibsen (1933) postulated that in cattle inheritance red (R) is always present in the homozygous condition.

Black (B) and black-spotting, or "blackish" (Bs) were assumed to be epistatic to red, and B epistatic to Bs. The allelomorphs of B and Bs, according to his interpretation, are not red but merely the absence of these dominant genes. The double recessive, bb bs bs, usually allows the red (R), which is always present, to express itself.

A special pair of sex-limited modifiers, M, much black, and L, little black, was postulated, which affected the amount of black shown by Bs animals. He pointed out that, although a dominant dilution gene (D) and a recessive dilution gene (d) were known, these did not account for all the variations in shade found in cattle, especially reds. He quoted Wentworth as stating that the blackish hairs sometimes found in animals that are otherwise red have this appearance because the red granules are very closely packed.

Cole, Van Lone, and Johansson (1934) found pigment in both the medulla and the cortex of hairs from the "black" area in albinotic Holsteins, but no pigment whatever in hairs from the "white" area.

MATERIALS AND METHODS

Black (B) hairs from Holsteins and Angus, blackish (Bs) ones from Jerseys and Ayrshires, red (bb bs bs) from

Shorthorns, Herefords, and Guernseys, white from the white-spotted (*ss*) areas of Holsteins, Ayrshires, and Jerseys, white from white (*NN*) Shorthorns, and white from Herefords (s^H) have all been studied microscopically both from whole mounts and from sections. For sectioning, the hairs were dehydrated with alcohol and imbedded in celloidin. The celloidin block was cleared for twenty-four hours in a dehydrating oil mixture (Guyer, 1930) and then imbedded in 70° C paraffin (tinged with asphalt, and containing one per cent crude rubber). The hair sections were 5 microns in thickness and both transverse and longitudinal sections were obtained. The paraffin was dissolved with xylol and a thin cover was used. For whole mounts, the hairs were dehydrated with alcohol, passed through xylol to xylol plus cresote (50:50) and mounted in balsam on the slide. No stain of any kind was used.

Hairs of as many shades of reds as it was possible to secure from the Kansas State College herds were studied from whole mounts. Hairs from albino cattle at the Universities of Wisconsin and Minnesota were studied from whole mounts.

Black, blackish, red, and white hairs were bleached with 17 volume Notoxide (about 16 per cent H_2O_2) which was made alkaline by adding concentrated NH_4OH until the mixture was about one per cent NH_4OH. The usual bleaching time was about 48 hours.

Black skin from the noses of Holsteins, Angus, and black roan crossbreds, red and speckled skin from clean and "smutty" noses of Shorthorns, apparently unpigmented nose skin from the white (ss) areas on the noses of Holsteins, and white skin from the nose of Herefords (S^H) were studied in 8 microns transverse sections. One block of each skin type was fixed in 8 per cent formalin and another of each was fixed in 95 per cent alcohol. Orange G was used on some slides to stain for cell outline in order that one could determine the location of the pigments within the cells, but the colors of the pigments were determined entirely from unstained sections.

OBSERVATIONS

Black pigment is found in the hairs of all cattle in the form of irregular, opaque clumps. The latter differ greatly in amount and location, and consequently have a pronounced affect on hair color. In black (B) hairs the black clumps are closely packed throughout (Fig. 1). In blackish (Bs) hairs there are fewer clumps, and for that reason the red, which is present, shows to some extent both macro- and microscopically. The black clumps are more concentrated in the center or medullary region of Bs hairs (Fig. 2). In red hairs, RR bb bs bs, the black clumps are thinly scattered in the cortex and somewhat more closely packed in the medulla (Fig. 3). The red shows readily since the black clumps are very thinly scattered throughout.

White hairs from recessive white spots (s), Herefords (s^H), white Shorthorns (NN) and albinos all show black clumps in the medulla (Figs. 5 and 6). The hairs from the albino cattle, as previously stated, came from two sources. Dr. L. J. Cole, University of Wisconsin, furnished hair from a Holstein albino bull, and Dr. W. E. Peterson, University of Minnesota, sent samples from two cows in the University Dairy Department's herd of albino cattle. The hairs from the Minnesota cows have black pigment in the medulla only (Fig. 9), while those from the "black" area of the Wisconsin bull have black rather thinly scattered in the cortex as well as the medulla (Fig. 7). The "white" hairs from this bull have a large amount of black pigment in the medulla and a much smaller amount in the cortex (Fig. 8). Considerable variation in the amount of black pigment occurs in both areas. A few hairs are even entirely devoid of black pigment.

All black pigment examined thus far seems of uniform intensity. No hairs from dilute blacks, duns, have been investigated, however.

As previously stated, black hairs (B) have black pigment so closely packed that little can be seen regarding the internal structure. By bleaching with H_2O_2, making the black clumps transparent, red (R) is found to be present, the hairs appearing red even macroscopically (Figs. 1 and 4). Also, blackish (Bs) hairs show red without bleaching the black, since there is less black and less clumping. These hairs, therefore, are red in appearance after being bleached. As would be expected, red hairs show red pigment. The latter is found in a homogeneous, translucent condition, diffused through the hair. The bleaching of red hairs causes the black pigment which they contain to become colorless. This makes them a lighter shade than before, indicating that the black influences the shade of red. The same shade of red results from bleaching B, Bs, and bb bs bs hairs unless the red itself is in a dilute form. Where the red pigment is dilute the hair is yellowish in appearance.

Red pigment seems more stable than black. Bleaching, as stated before, causes the black pigment to become transparent but affects the red only slightly. With continued bleaching, however, the red pigment also becomes colorless.

If gray and fawn Jerseys are examined, it will be found that in both cases the hairs are blackish at the base, but that the hairs from the grays are a very dilute red at the tip while the fawns are a more intense shade. Upon bleaching the black at the base of these hairs, it will be noted that the red is the same shade throughout the hair length but is much lighter in the grays than in the fawns. This furnishes proof for the statement previously made that at least two shades of red are present in cattle.

None of the different kinds of white hairs previously mentioned have red pigment. All cattle, however, except possibly albinos, have pigmented areas in certain parts of the body where the hairs contain red pigment even though it may not show macroscopically. When white hairs are bleached, the black clumps in the medulla are made transparent and the hairs thus become completely colorless, and as a result have a translucent appearance (Fig. 10 and 11). This furnishes proof against the statement sometimes made that air spaces and not pigment granules produce the black in the medullae of white hairs. It also shows that bleaching does not change the black into red.

There is a faint indication of the diffuse red pigment in the hairs from the "black" area in the Wisconsin albino bull. Neither of the other two albinos show this tendency. The numbers concerned are too small to justify any definite conclusions, but there seems at least a possibility that the expression of the albino condition is sex-limited to the extent that some red shows in the mature males and none in the mature females.

It has been pointed out above that when intense red hairs of different shades are bleached, they become similar in shade. As stated, this is due to the bleaching of the black granules. One would therefore expect that the variations in the shade of red are due to the quantity and the distribution of the black clumps, and this proves to be the case. Light red Herefords and Guernseys show a small amount of black pigment in the cortex of the hairs. On the other hand, the black clumps are very noticeable in the medulla (Fig. 12). In the medium red hairs of Shorthorns, Herefords, and Guernseys there are comparatively large numbers of black clumps in the cortex (Fig. 13). Dark red hairs from Shorthorns and Herefords show still larger numbers of black clumps scattered throughout the whole hair (Fig. 14). What seems rather surprising is that dark red and blackish (Bs) hairs differ less in regard to the black clumps than do dark and light reds (Figs. 12, 13, 14, and 15). Blackish like reds, also vary in the degree to which the character is expressed, and this is brought about by means of the black clumps. The less blackish may have even fewer clumps in the cortex than the very dark reds. With the increase in number of black clumps in the cortex there is a corresponding increase in the blackness of the hair. Very black Bs animals have so much black in the hairs that little of the internal structure can be seen. They closely resemble animals carrying B. But, in no case is the black so closely packed that the red is completely obscured from view in whole mounts.

Variations in the size and distribution of the black clumps occur. The darker reds and blackish hairs have larger clumps than the lighter and less blackish hairs (Figs. 16 and 13). In some red animals the black is not found near the outer surface of the cortex. This sort of hair is much more dilute in appearance than one which has the black clumps nearer the surface. The amount of black in the medulla seems to have little effect upon the shade of red. Evidently the light is reflected largely from the surface of the hair.

The nose skin of cattle shows the black pigment generally less clumped than is the case in the hairs. In black (B) animals, Holsteins and Angus, the black pigment is found in all the epithelial cells (Fig. 17). It is concentrated in the cytoplasm near the nucleus, forming a half-moon clump in that part of the cell nearest the surface of the skin (Fig. 18). In most cases the black pigment appears to be formed in the stratum germinativum and is heaviest in the crypts of epithelium extending into the underlying connective tissue. In black roan (Angus x Shorthorn crossbred) the black pigment varies greatly in its distribution. Some regions are the same as in the Angus noses while other areas have much less pigment. This condition tends to give the nose a lighter appearance, corresponding to the interspersion of white hairs with black to produce a "blue-gray." Red pigment shows clearly in the regions of less black in the nose skin of the black roan. There is some indication of red in the pure black noses, but the black tends to mask its appearance.

Blackish (Bs) noses from Jerseys show the same half-moon type of clumping of the black pigment as is found in black (B) noses. There is less pigment present, however, and therefore the clumps are smaller. Considerable variation is found in different Bs animals. Dark Jerseys show darker noses and a corresponding greater amount of black pigment than lighter ones (Figs. 19 and 20). In all cases one can see the red since the black does not mask it in Bs animals.

Red (RR bb bs bs) noses from red and roan Shorthorns show both black and red pigment. There is only a small amount of black. It is not possible to see the effects of the roan in this type of nose because there is so little black present. Black pigment is found in the crypts and in the cornified cells. Red pigment apparently is present in larger quantities and can be seen distinctly especially in the crypts of the epithelium. It extends to the cornified area in most cases and sometimes is present in the cornified area. The red pigment takes on a somewhat granular appearance in the nose skins. The granules are definitely smaller than the black granules and there is no clumping (Fig. 21). In the red noses the black is granular, showing much less clumping than in Bs or B nose skins.

Smutty nose Shorthorns show a similar condition to the black roan. There are some areas which are about as black as is found in B noses and other areas which have pigmentation similar to the red Shorthorn nose (Fig. 22). This alternating variation in degree of pigmentation gives the nose a speckled or "smutty" appearance.

White, or pink, nose skin found in the ss white of Holsteins and the S^H white of Herefords differs markedly from B, Bs and red nose skins. As previously stated, the black and red pigments in the last three types of noses seem to be formed in the stratum germinativum. In the white noses no pigments are observed in that region. The black pigment is found only as fine, thinly scattered granules in the cornified cells (Fig. 23). A very small amount of red pigment seems to be present in some of the white skin spots. This is so minute as to give only a reddish tinge in certain areas. The skin of the one Hereford nose examined is thin, having only a thin layer of epithelium. This probably permits the blood in the underlying tissue to show through.

DISCUSSION

The observations herein reported have to some extent received support from the work of others. Hunt and Wright (1918) found both a diffuse and a granular pigment in the hairs of guinea pigs, but they made no statement regarding the color of the pigments. Hausman (1927), working with hairs of various mammals, found the diffuse pigment to be yellowish or reddish and the granular to be of varying shades of brown. It seems likely that the diffuse pigment as given by these workers and the red pigment in cattle hairs are analogous. Also, the granular pigment described by these workers and the black clumps in cattle hairs correspond, except that it is always black in cattle while other mammals may have chocolate as well as black granules.

There is reason for believing that B, Bs and Ps in cattle (Ibsen, 1933) are modifying factors affecting in one way or another the extension of a "fundamental" gene for black pigment. Thus B in cattle would correspond to E in guinea pigs and bb bs bs to ee in these animals. Bs and Ps, however, do not seem to have counterparts in other mammals.

SUMMARY

1. Black pigment is found in the hairs of all cattle, including albinos.

2. In black (B) animals the black pigment is closely packed and thus masks the red which is present in the hairs.

3. The shade of red (bb bs be) and blackish (Bs) hairs is affected by the amount and distribution of the black pigment. Darker shades of red and blackish hairs are brought about, (1) by a larger amount of the black pigment, (2) by an increase in the size of the black clumps, and (3) by having the black nearer the hair surface.

4. Red (R) is present in the homozygous condition in cattle, being diffused through the hair in the form of a homogeneous, translucent pigment. Black (B) and blackish (Bs) are epistatic to red because the large amount of black tends to mask the red macroscopically.

5. No red is found in white hairs, but no animal, except possibly an albino, is entirely white.

6. There are at least two intensities of red pigment in cattle.

7. There is more variation in the shade of red than in the shade of black cattle. This is probably explained by the fact that both the black and the red pigment have an influence in the case of red animals and only the black in the case of blacks.

8. The pigments in the skin from noses are similar to those found in hairs. There is generally less clumping of the black, however, and the red has a somewhat granular appearance.

9. The darker noses of black (B) and blackish (Bs) animals are due not only to a greater amount of pigment but also to the production in each cell of half-moon clumps in that part of the cytoplasm nearest the skin surface.

10. There seems to be justification for the conclusion that B, Bs, and Ps are due to different modifiers acting on the "fundamental" gene for black which is present in the homozygous condition in all cattle.

ACKNOWLEDGMENT

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The hair samples were secured from the animals in the Dairy and Animal Husbandry Departments, Kansas State College, and from the Universities of Wisconsin and Minnesota. The nose skins were secured from John Morrell & Company, Topeka, Kansas.

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Plate I

Explanations of Figures[1]

Fig. 1. Black (B) hair from a Holstein female (Q. Isabelle). Whole mount x 450. Showing the black closely packed throughout.

Fig. 2. Blackish (Bs) hair from an Ayrshire female (Petunia). Whole mount x 450. Showing many black clumps in the cortex and much black in the medulla. The red in this hair can also be seen.

Fig. 3. Red (bb bs bs) hair from a Hereford male (Hazford Tone 58th). Longitudinal section x 650, cut 5 microns in thickness. Showing both black and red pigments.

Fig. 4. Black (B) hair from a Holstein female (Q. Isabelle). Whole mount x 450. Showing how bleaching with hydrogen peroxide makes the black clumps color less and allows the red to show. The black has not been completely removed.

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1. All figures in all plates are photomicrographs.

Plate I

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Plate II

Fig. 5. White (ss) hair from an Ayrshire female (B. Ms. Cleopatre). Transverse section x 2500. Cut 5 microns in thickness. Showing the black clumps in the medulla only.

Fig. 6. White (ss) hair from an Ayrshire female (B. Ms. Cleopatre). Longitudinal section x 650. Cut 5 microns in thickness. Showing black clumps in the medulla only.

Fig. 7. "White" hair from the "black" area of the Wisconsin albino Holstein bull. Whole mount x 450. Showing black clumps in the cortex and apparently solid black in the medulla.

Fig. 8. White hair from the "white" area of the Wisconsin albino Holstein bull. Whole mount x 450. Showing considerable black in the medulla and much less in the cortex.

Plate II

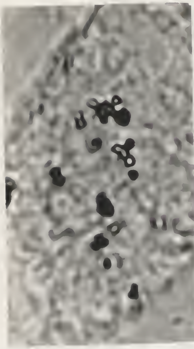

Fig. 5

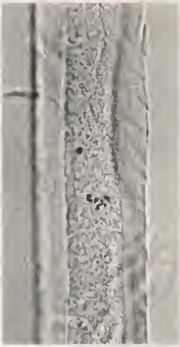

Fig. 6

Fig. 7

Fig. 8

Plate III

Fig. 9. White hair from a Minnesota albino cow. Whole mount x 450. Showing black in the medulla only.

Fig. 10. White (ss) hair from a Holstein female (Q. Isabelle). Whole mount x 450. Showing black in the medulla before bleaching with hydrogen peroxide.

Fig. 11. White (ss) hair from a Holstein female (Q. Isabelle). Whole mount x 450. Showing how bleaching with hydrogen peroxide removes the black and leaves the hair completely colorless.

Fig. 12. Red (bb bs bs) hair from a light red Guernsey female (P. K. Elsie). Whole mount x 450. Showing a small number of black clumps in the cortex.

Plate III

Fig. 9

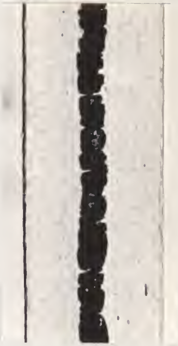

Fig. 10

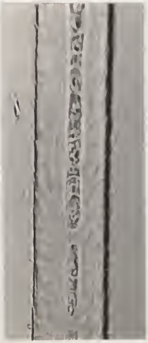

Fig. 11

Fig. 12

Plate IV

Fig. 13. Medium intense red (bb bs bs) hair from a Hereford male (Mathew's Anxiety 16th). Whole mount x 450. Showing considerable black pigment.

Fig. 14. Dark red (bb bs bs) hair from a Shorthorn female (06). Whole mount x 450. Showing many black clumps scattered throughout the hair.

Fig. 15. Blackish (bb Bs) hair from an Ayrshire female (B. Ms. Cleopatre). Partial longitudinal section x 650. Cut 5 microns in thickness. Showing black clumps in the medulla and in the cortex.

Fig. 16. Light red (bb bs bs) hair from a Hereford male (Mathew's Anxiety 17th). Longitudinal section x 650. Cut 5 microns in thickness. Showing small black clumps. Little black is present near the hair surface.

Plate IV

Fig. 13

Fig. 14

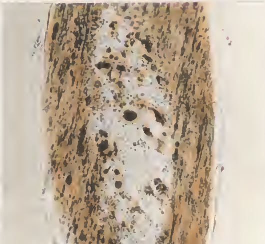

Fig. 15

Fig. 16

Plate V

Fig. 17. Nose skin of black (B) animal. Transverse section x 120. Cut 10 microns in thickness. Showing much black pigment in the crypts of epithelium and a clumping of the black throughout the epithelium.

Fig. 18. Black nose skin from a black roan (B Nn) animal. Transverse section x 600. Cut 10 microns in thickness. Stained with orange G. Showing in each cell a half-moon clumping of the black in the portion of the cytoplasm nearest the skin surface.

Plate V

Fig. 17

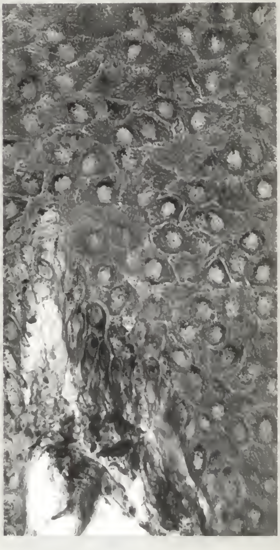

Fig. 18

Plate VI

Fig. 19. Dark blackish (Bs) nose skin from a dark Jersey. Transeverse section x 120. Cut 10 microns in thickness. Stained with Orange G. Showing less black but the same type of clumping as in B nose skin.

Fig. 20. Lighter blackish (Bs) nose skin from a Jersey. Transverse section x 120. Cut 10 microns in thickness. Stained with Orange G. Showing less black than in a darker Bs nose.

Plate VI

Fig. 19

Fig. 20

Plate VII

Fig. 21. Red (bb bs bs) nose skin from a red Shorthorn. Transverse section x 600. Cut 10 microns in thickness. Showing both black and red in the crypts of epithelium.

Fig. 22. Smutty nose from a red Shorthorn. Transverse section x 120. Cut 10 microns in thickness. Showing much black in some areas and little in others. The black, when present, extends to the skin surface.

Fig. 23. White nose skin from a white (NN) Shorthorn. Transverse section x 600. Cut 10 microns in thickness. Showing black granules in the cornified epithelial cells. There is a decrease in the number of black granules near the skin surface.

Plate VII

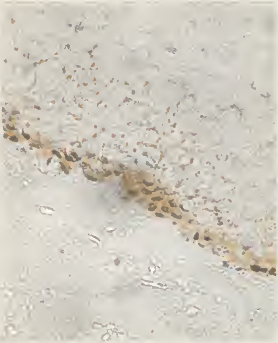

Fig. 21

Fig. 22

Fig. 23