MICROSCOPIC STUDIES OF CATTLE
HAIR PIGMENTATION

by

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INTRODUCTION

Considerable information has been recorded regarding color inheritance in cattle, especially among the more common breeds. Ibsen (1933) discussed the genes concerned in color inheritance in cattle, and gave each of the genes an appropriate symbol. Bogart and Ibsen (1937) studied the pigmentation in cattle skin and hair and advanced certain theories to correlate the apparent effects of the color genes on pigmentation.

It was thought that recent advances in technique justified a re-examination of the results they obtained, and that additional histological study of the pigment within the hair was essential to a more complete understanding of the effects of the color genes.

MATERIAL AND METHODS

Hair samples were selected from cattle carrying various color genes as postulated by Ibsen (1933). The animals were necessarily of various breeds and colors, and were chosen as representative of the particular phenotype being considered. The hair samples were taken from the same definite areas of the respective animals to assure more accurate comparisons.

The various kinds of hairs were studied microscopically both in whole mounts and as cross sections. To prepare the whole mounts, some hairs from each sample were washed thoroughly in xylol or carbon tetrachloride, then mounted directly in balsam.

The hair sample that was to be sectioned was washed and dried the same as for whole mounts, and then sectioned according to the method devised by Hardy (1935). The equipment patented by the latter consists of a small hand microtome which has a narrow slot into which the hairs can be placed. The hairs are then compressed tightly and a razor blade used to trim them even with the microtome plate. A calibrated plunger forces the hairs through the slot until they project the desired amount. The projecting ends of the hair are then coated with celloidin, and when dry the entire patch is cut off even with the microtome plate. The patch is then removed from the microtome and mounted directly in balsam, or in some instances it is affixed to the slide with a thin layer of balsam. Using this method, some sections as thin as four or five microns can be obtained.

Celloidin in alcohol-ether proved very satisfactory as the coating material for the projecting fibers.

Some cross sections were stained, using picric acid or picro-carmine. This procedure is explained in the discussion of white hair.

RESULTS

The distribution of pigment in cattle hairs was clearly distinguishable in cross sections. Both granular and diffuse pigments were observed in pigmented hairs. The granular pigment was black or red, and was present in both the cortex and medulla of the hair. The diffuse pigment ranges in color from black to very light red and was found in both the cortex and medulla.

The variations in color of pigment as well as pigment granule arrangement are described below.

Black Hairs

Whole mounts of Angus hair appeared black under the microscope. There was, however, some bluish diffuse pigment evident at the extreme base of the hair. When these hairs were cross-sectioned the granules could be observed in well defined clumps each about five microns or larger, Fig. 1 (Figures 1-6 inclusive are found in Plate I). These clumps were arranged in a series of three to five concentric circles from the medulla to the cuticula. When studied under the microscope these clumps appeared very clear and distinct, and the granules also were well defined. The interstices were filled with diffuse, or non-granular pigment.

Hair taken from young animals had a dull red diffuse pigment with black and dark red granules in the clumps. In older animals occasional hairs were found that had only black granules and black diffuse pigment. No case was found of hair with red granules and black diffuse pigment.

A commercial preparation, Clorox, was used to bleach intense black hairs. The hairs were left for variable periods of time in a bleach solution containing three drops of Clorox in 50 cc. of 50 per cent alcohol. It was found that within a few hours the diffuse black or dark red pigment became bright red, and after two weeks all but some of the very large black granules were red. These bleached granules were still arranged in clumps as before bleaching, Fig. 2. As the bleaching was continued for a period of several weeks, all of the pigment was removed and the hair appeared white. However, as long as the granules were even faintly visible they still appeared in the same pattern as before bleaching.

These observations tend to substantiate the interpretation of Baker and Andrews (1944) that the black pigment is changed to red by bleaching, rather than the theory of Bogart and Ibsen (1937) that the bleaching removed the black pigment and allowed the red to show.

Black hair of Holsteins was found to be very similar to Angus, Fig. 3. In younger animals red diffuse pigment was present and some hairs were found with only red or with red and black granules. However, there was no apparent difference in granule size or arrangement between the red and the black hairs. There were fewer red granules present in hairs from older animals. Most of the granules present were very intensely black, and some cases of black diffuse pigment were observed. The hairs from one old Holstein bull when sectioned at five microns showed such intense black granules that they were not separately distinguishable in the clumps.

Whole mounts of pigmented hairs from a black and white crossbred showed that some red hairs were present with the black. Cross sections showed that the pigment granules were present in irregularly shaped clusters, Fig. 4. There was no apparent difference in granule size or arrangement between the

EXPLANATION OF PLATE I

Fig. 1. Cross section (15 microns) of black Angus hairs (x 400), showing granules present in clumps.

Fig. 2. Cross section (15 microns) of black hairs from an Angus (x 400), showing clumps arranged in concentric circles.

Fig. 3. Cross section (20 microns) of black Holstein hairs (x 400), showing the resemblance to the pigment distribution in the Angus.

Fig. 4. Cross section (15 microns) of black and white hairs from a black roan (x 400). Although the pigment is clumped it is not present in clearly defined areas as in the Angus or Holstein (Figs. 1, 2 and 3).

Fig. 5. Cross section (10 microns) of black Negro hairs (x 400), showing the scattered distribution of the pigment granules.

Fig. 6. Cross section (15 microns) of hairs from a dark red Shorthorn (x 400). This example represents an extreme case of clumping, usually the granules were more generally distributed.

PLATE I

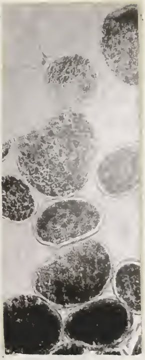

Fig. 1

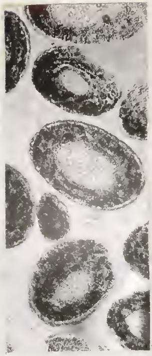

Fig. 2

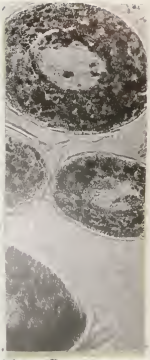

Fig. 3

Fig. 4

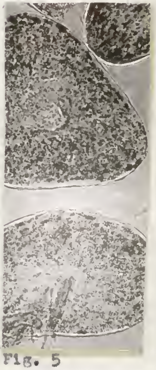

Fig. 5

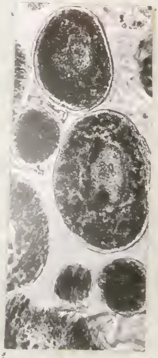

Fig. 6

red and the black hairs. Single granules ranged in size from less than one micron to as large as four or five microns.

The medulla, when present, was very small in both black and red hairs. Therefore the granule clusters and the diffuse pigment were distributed rather uniformly throughout the hair.

A study of Negro hair indicated that there was no red pigment of any type present. When the hair was cross-sectioned the granules appeared uniformly scattered, and therefore no pattern or grouping was evident, Fig. 5. There was seldom any indication of a medulla, although occasionally a large, intensely black granule was present in the center of the hair. This is shown in a microphotograph of Negro hairs in a paper published by Pipkin and Pipkin (1944).

When Negro hair was studied as a whole mount, the pigment appeared to be in streaks. The streaks were due to rows of closely aligned granules. Thus, although the granules had no pattern when viewed in the cross section, still some of the granules present were part of a long row of tightly arranged granules.

Hausman (1927) states that human hair of the same color has similar pigment characters, regardless of racial source of the hair.

Red Hairs

Two extreme types of hair were obtained from dark red Shorthorns. Whole mounts of one kind were dark red with a medulla, and the other was very small in diameter, light red and non-medullated. These differences were also apparent in cross sections, Fig. 6. The color of the hair varied with the color of the pigment, and the pigment granules were generally uniformly scattered with occasional clumping. Some of the granules were three or four microns in diameter, but not all hair had these large granules. There was a great deal of red diffuse pigment present in both the medulla and cortex of the hair.

The hair color in red-roan Shorthorns (some white hairs among the red) ranged from a very dark red to pure white. In the pigmented hair there were some extremely large clumps (10 microns) and granules as large as five microns, Fig. 7 (Figures 7-12 inclusive are found in Plate II). The different shades of red were very evident in the cross sections. The darker hairs had more diffuse pigment than the light colored hairs, and this pigment was a darker shade of red. Both granules and diffuse pigment were present in the medulla.

In a "salt-and-pepper" roan Shorthorn (many white hair distributed among the red) the hair also varied from dark red to white. The darker red hair had more pigment granules and the granules were a darker shade. There was also more pigment material present in the medulla of the darker hairs. The pigment granules were very scattered with practically no clumping, and there was no apparent consistent difference in the arrangement of granules in the light as compared with the dark hair, Fig. 8.

In a white Shorthorn that was examined the only pigmented hairs present were in the ears. These hairs were a very bright

EXPLANATION OF PLATE II

Fig. 7. Cross section (15 microns) of hairs from a red roan Shorthorn (x 400), showing the scattered arrangement of the granules and clumps.

Fig. 8. Cross section (10 microns) of hairs from a roan Shorthorn (x 400). There is very little clumping evident, and the granules are relatively small.

Fig. 9. Cross section (15 microns) of red hairs from the ear of a white Shorthorn (x 400), showing the non-uniform distribution of the pigment granules.

Fig. 10. Cross section (10 microns) of red hairs from a Herford (x 400), showing the scattered arrangement of the granules. Compare with Figs. 6, 7 and 8.

Fig. 11. Cross section (12 microns) of red hair from a Guernsey (x 400), showing the uniform distribution of red granules in the cortex. Some granules are also evident in the medulla.

Fig. 12. Cross section (17 microns) of red hair from a Jersey (x 400), showing the granule distribution in both the medulla and the cortex.

PLATE II

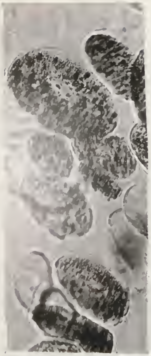
Fig. 7

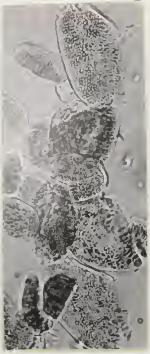
Fig. 8

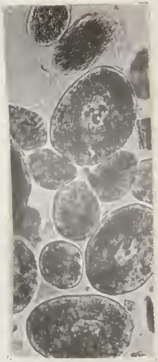
Fig. 9


Fig. 10

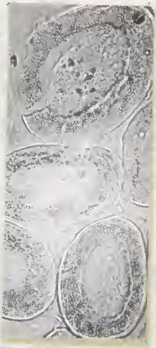
Fig. 11


Fig. 12

red, and in cross section they showed many bright red, loosely scattered pigment granules, Fig. 9. The hairs had a dark medulla which contained many closely packed, large red granules.

There was only red pigment evident in Herford hair. The granules and granule clumps were scattered uniformly, and the color of the red granules varied to produce the various shades of red in the Herford, Fig. 10. The range of color was not as extreme as in the case of the Shorthorn. Some of the hairs were medullated while some were non-medullated.

In the case of the Guernsey, the cross sections showed very light red diffuse pigment and red scattered granules, Fig. 11. Most granules were very small but an occasional hair did have relatively large granules and some clumping was also evident. Granules were present in both the cortex and medulla. Relatively more granules usually were present in the medulla than in the cortex, although they were present to a considerable extent in both.

The variation in the shade of the Guernsey was due to the difference in the intensity of the granular and diffuse pigment present in the hair. The granule size and arrangement appeared to be the same for both light and dark Guernseys.

In red Jersey hair as contrasted with "blackish" reds, there was a very light red diffuse pigment present, and the granules were very small and scattered, Figs. 12 and 13 (Figures 13-18 inclusive are found in Plate III). The hair was oval in shape, and there was great variation in size of medulla.

The difference in the color of Jerseys was due to the color of the pigment, and in the case of very light red (cream) there appeared to be fewer pigment granules present.

There was considerable variation in the granule arrangement of pigmented hairs from the Ayrshire. Figure 14 shows a group of hairs that have been sectioned. Examples can be seen of cross sections that have granules grouped around the medulla, some that have granules uniformly distributed, and some in which the granules are in clumps. The amount of pigment in the medulla also varies a great deal. Diffuse pigment was visually present to some extent in the medulla, and granules were often quite numerous, Fig. 15.

The shade of red in granules or diffuse pigment ranged from very light to dark red in the same animal. Some hairs had light red diffuse pigment and relatively dark red granules. These variations in shade of red are probably due to the action of intensity and dilution genes as discussed by Ibsen (1933). More precise information regarding the effect of these genes on granule size, shape and color will be necessary in order to understand more completely the actions of the major color genes. Investigations of this type have already been reported by Russell and Russell (1943) and Russell (1946) in the house mouse.

These observations of red cattle hairs are in contrast with the findings of Bogart and Ibsen (1937) who reported some black granules in red hair examined as a whole mount. Harman and Case (1941) could find no black granules in red hair of the guinea pig, and Jones (1947) reports that he could not find black pig-

EXPLANATION OF PLATE III

Fig. 13. Cross section (12 microns) of red hairs from a Jersey (x 400), showing granule arrangement. Note the oval-shaped hair.

Fig. 14. Cross section (12 microns) of red hairs from an Ayrshire (x 225), showing the variation in shade of red and granule arrangement present.

Fig. 15. Cross section (18 microns) of hairs from a blackish Ayrshire (x 400). These cross sections contain considerable dark pigment and are responsible for the "mahogany" color in the whole mounts. See Fig. 22.

Fig. 16. Whole mount (x 225) of white hair from an Ayrshire cow. The black-appearing medulla is very striking.

Fig. 17. Whole mount (x 225) of white hair from an Ayrshire bull showing an intermittent medulla and black cortical areas.

Fig. 18. Whole mount (x 225) of white hairs from a male Ayrshire. Compare with Figs. 16 and 17.

PLATE III

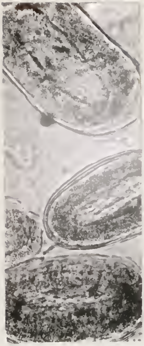

Fig. 13

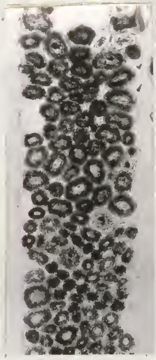

Fig. 14

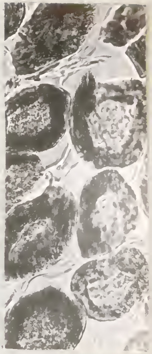

Fig. 15

Fig. 16

Fig. 17

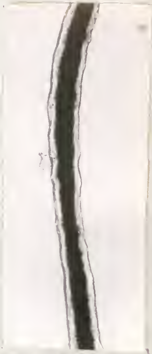

Fig. 18

ment in the red hair of Shorthorns. He also states that he could obtain results comparable to those of Bogart and Ibsen by certain methods of examination, but he considered these methods faulty.

White Hairs

When observed macroscopically various types of white hair appear to be very similar, but when studied microscopically characteristic differences are seen. Perhaps the most striking feature is the black-appearing medulla present in the white hair. This medulla varies from a wide, complete medulla, Fig. 16, to an intermittent medulla, Fig. 17, or in some cases a complete absence of medulla. The amount of black-appearing material present in the cortex and medulla of white hair from various genotypes has been described by Bogart and Ibsen (1937). Actually there seems to be a great deal of variation, even in a single animal, so it is difficult to establish a definite conclusion based on genotypic differences. For example, Fig. 17 is a typical hair from an Ayrshire bull, Fig. 16 is a typical hair from an Ayrshire cow, and Fig. 18 is a typical hair from their male offspring.

White hairs from the udders of Holstein, Jersey, Guernsey and Ayrshire cows were studied and compared. Although there are definite differences, especially in size and form, still they are no consistent breed differences. All seemed to be the result of individual variation.

There has been some difference of opinion in regard to the probable composition of the black-appearing medulla seen in white hair. Bogart and Ibsen (1937) consider that in cattle it is due to black pigment, while Harman and Case (1941) state that the white hair of guinea pigs may contain colorless granules, but no red, black or chocolate granules. Hausman (1921) states that the medulla appears dark in white hair because of light dispersion.

Various types of technique were utilized in an attempt to clarify this problem. For example, if white hairs similar to that in Fig. 16 were cross-sectioned, mounted in balsam and covered, the black-appearing medullary material apparently disappeared, Fig. 19 (Figures 19-22 inclusive are found in Plate IV). However, if a corresponding patch were affixed to a slide, but not mounted in balsam, the black-appearing medulla was again evident, Fig. 20. Figures 19 and 20 are adjacent sections cut by the Hardy device, which were treated the same except for the method of mounting. The cross sections in Fig. 20 were prepared by removing from the Hardy device the celloidin patch containing the cross-sectioned hairs and placing it, celloidin side down, on a slide that had a thin, sticky layer of balsam spread on it. Thus, the cross sections of the hairs were not covered or enclosed with any material whatsoever.

A slide containing cross sections similar to Fig. 19 was placed in xylol and the cover slip was removed. After the slide

EXPLANATION OF PLATE IV

Fig. 19. Cross section (x 225) of white hairs from an Ayrshire cow, showing the absence of the black-appearing medullary material. These cross sections are from hairs similar to the whole mount in Fig. 16.

Fig. 20. Cross section (x 225) of white hairs from an Ayrshire cow, showing the black-appearing medullary material. The sections in Figs. 17 and 20 are identical and differ only in method of mounting.

Fig. 21. Cross section (x 225) of white hairs from an Ayrshire bull, showing the cortical spots. These sections are from hairs similar to the whole mount in Fig. 17.

Fig. 22. Cross section (12 microns) of hairs from an Ayrshire bull (x 400), showing extreme blackish. Some of these hairs had black granules and black diffuse pigment.

PLATE IV

Fig. 19

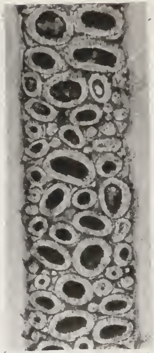

Fig. 20

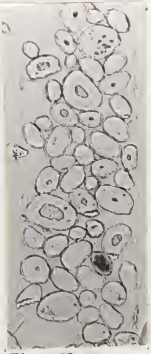

Fig. 21

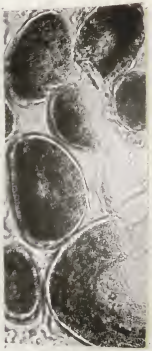

Fig. 22

was thoroughly dry the medullary material appeared dark as in Fig. 20. This could be explained by assuming that the medulla contained a translucent material which when in a normal, flaky form dispersed light and appeared opaque, but when cleared by a substance such as balsam the material no longer diffused the light and consequently appeared more transparent.

Staining white hair was attempted as an aid to microscopic study. After using many of the common stains it was found that picric acid and picro-carmine were most consistently satisfactory. If picric acid were used, the hair was placed in a saturated aqueous solution for 24 to 48 hours, then washed in 70 per cent alcohol for about six hours. After sectioning and mounting, the hair retained a yellow tint which made it considerably easier to study microscopically.

Staining with picro-carmine aided in studying the medulla. The stain was prepared according to Guyer (1936) and was used only on hair that was sectioned. The celloidin patch with the cross-sectioned hairs was affixed to a slide with balsam, allowed to dry, and then treated. The slide was first flooded with 50 per cent alcohol for three minutes, followed by picro-carmine for 15 minutes. Excess stain was washed off with 70 per cent alcohol, and the cross sections were then cleared for 10 minutes with beechwood creosote. They were then mounted in balsam and covered.

This type of staining served to show more clearly the medullary material present in white hair, and also gave the white hair some color so that it could be more easily seen under the microscope. The medullary material stained a darker shade of red than the cortex, and this differentiation remained whether the cross sections were mounted in balsam or left free.

When white hairs were bleached by a weak Clorox solution, some of the hairs showed a change in a few hours. The medulla was no longer distinguishable and the hairs became practically transparent. This change was readily discernable macroscopically as well as microscopically. The black-appearing material did not bleach to red as happens with true black pigment, but instead it became colorless without any intermediate shades or colors. The bleaching also tended to make the hairs soft and easily flattened.

If the bleached white hairs were cross-sectioned they appeared flat and misshapen due to the pressure exerted in wedging them into the Hardy device. If non-bleached and bleached white hairs were sectioned together and then stained with picro-carmine, the bleached sections stained the same as the non-bleached sections. Thus, although the bleached hairs were flattened and the medulla consequently made very much smaller than normal, the bleaching apparently had not affected the staining ability of the medullary material.

Some white hair from an Ayrshire bull had black-appearing spots in the cortex, Fig. 17. These dark-appearing areas were also visible in the cross sections of the same hair, Fig. 21, and apparently were distributed at random.

White hairs that were obtained from above the black skin spots of Holsteins had some red diffuse pigment present at the extreme proximal region. Bogart and Ibsen (1937) also report similar results from an Ayrshire. Microscopic examinations of white hairs from Angus, Herfords and roan Shorthorns did not disclose any form of pigmentation whatsoever. Jones (1947) states that he has unpublished data showing that microscopically the white hairs of white Shorthorns all show some small amount of red pigment.

Blackish Hairs

Blackish hairs were found in some Jerseys and Ayrshires. The hairs were distinguishable by having black pigment granules in an otherwise red hair. The red and black granules were present in clumps or scattered, and the color of the hair was directly influenced by the number of black granules present.

In young animals the black granules were relatively scarce but were more numerous in older animals. Bogart and Ibsen (1937) point out that there is also a sexual dimorphism in adult blackish animals that results in a greater amount of black pigment being in the hair of the male. Some blackish hair from an older Ayrshire bull were found to have only black granules and black diffuse pigment, Fig. 22. In younger animals apparently there was always some red pigment present. There was, however, no difference in granule arrangement of red pigment in hairs that showed blackish and in hairs that had no black granules. Both the red and black granules could be found in the same clumps, however, the black granules were more numerous near the tip of the hair than at the base.

When blackish hair was bleached the black granules became red within 96 hours. The granule arrangement appeared the same as in non-bleached hair.

Hair Form

The study of cross-sectioned cattle hairs showed striking differences in hair shape. An attempt was made to correlate hair shape with breed, type or color, but the exceptions in all cases were too numerous. Even in a single individual the hair varied from perfectly round to oval shaped, and the relative size of the medulla was also found to be extremely variable. It is interesting to note, however, that hairs from the crests of five dairy bulls were sectioned and were found to be without medullas in all cases. Females of corresponding breeds had medullated hair in that region.

A very brief examination of hair scales verified the Herford type as described by Fanova (1934). Other breed types were not studied extensively at this time mostly because hair scales were very difficult to see when ordinary mounting methods were used. In order to see them at all it is necessary to place a whole hair on a slide that has previously been coated with a very thin, sticky layer of balsam. Thus, when the hair is placed on the slide the entire upper surface is uncovered, and by carefully regulating the light it is possible to see the scales.

DISCUSSION

In the pigmented hairs studied both granular and diffuse pigment was present. The results of this study substantiate the theory of Ibsen (1933) regarding the action of certain color genes in cattle. However, the relation between red and black pigment is not entirely clear. Bleaching black hair artificially or by action of the sun apparently turns black pigment to red, yet the black in older animals appears to replace the red, and in blackish animals the black granules are more numerous in older animals. Pipkin and Pipkin (1944) were able to darken albino Negro hair by using the reducing agent, $SnCl_2$. Further chemical experimentation will probably be required to satisfactorily explain all of these results.

Chemical analysis will also aid in determining the exact role of the medullary material present in white hair.

SUMMARY

1. A microscopic study was made of whole mounts and cross sections of various kinds of cattle hairs.

2. Black hair of the Angus and Holstein breeds had red and black pigment granules present in well defined clumps arranged

circularly around the medulla. Usually there was red diffuse pigment present although examples of black diffuse pigment were found.

3. No black pigment was found in red hairs that were not blackish (baba). Since this differs with the findings of Bogart and Ibsen (1937), a modification of their concept is necessary. They stated that red hairs (ee babs) show a small amount of black pigment. It now seems certain that black pigment is never present.

4. These observations of black and red hair can be explained by the B, E and Bs genes postulated by Bogart and Ibsen (1937), with the exception noted above in regard to red hair.

5. The variation in shade of red hairs is due to the different intensities of the pigment they contain. Light red hair as in "cream" Jerseys apparently contains relatively more diffuse pigment and fewer pigment granules than dark red hair.

6. Black hair was bleached in a weak solution of Clorox. The black pigment changed to red and eventually became colorless.

7. If cross sections of white hair are mounted in balsam the medullary area appears transparent, but if the cross sections are affixed to a slide and not covered the black-appearing medullary material is evident. This can be explained by differences in light dispersion.

8. Bleaching white hairs did not cause the black-appearing material in the medulla to turn red, thus demonstrating that it was not true black pigment. The chemical nature of this material

is not understood.

ACKNOWLEDGMENTS

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