THE CAUSE OF BLUE COLOR AS FOUND IN THE BLUEBIRD (SIALIA SIALIS) AND THE BLUE JAY (CYANOCITTA CRISTATA).*

BY CARL GOWER.

INTRODUCTION.

ALL the colors which one observes in bird feathers are due to one of two things: either to a pigment, or to some modification in the structure of the barb, producing what is known as structural color. The pigment colors are black, brown, red, yellow, and in a few cases green, while the blues, whites, some of the greens and metallic and iridescent colors are due to structure. These facts are accepted by biologists and have been worked out by them and by physicists. The work presented in this paper has been done to determine if possible something of the real nature of the structures causing the color found in the Blue Jay (*Cyanocitta cristata*) and the Bluebird (*Sialia sialis*).

The cause of blue color in feathers was first investigated by Fatio (1), who published a paper on this subject in 1866. He discovered that the blue color is localized in a distinct layer, but does not mention the fact that the color is restricted to the walls of the cells of the layer. He called this layer "email." He also said that there appears under the layer of prismatic cells a layer "de grandes cellules polygonales a noyau colors," but of the later workers, Gadow supposes this to be an optical illusion on the part of Fatio.

Following Fatio, Gadow (1) published a more intensive paper in 1882, including all types of coloration. His explanation of blue color is of particular interest. He attributes the blue to a series of fine lines running parallel to the long axis of the cones (color-producing cells), although taking into account other factors as a possible cause. One paragraph from his paper will give his conclusions better than I can summarize them.

"Let us throw only a furtive glance at some of the changes which the light falling upon and passing through a blue feather is likely to undergo. First, part of the rays will be simply reflected from the outer surface . . . ; secondly, the rest, before passing through this stratum, will be variously broken and reflected *before* reaching the coating . . . , since the stratum . . . is not homogeneous, but consists apparently of several irregular scales and secondary strata; thirdly, the coating, . . . , breaks the rays again and partly reflects them, and, if it is only 0.0006 mm. thick, as in *Pitta*, it is thin enough to allow the application of the theory

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of thin-plate colors; fourthly, the system of ridges; fifthly, some rays will reach the layer of brownish pigment. How much of them is absorbed, how much reflected as brownish light, and what the changes are of this brown light before it comes up again to the surface, we cannot tell. Again, the ray *a* will be under different conditions to the ray *c*. To follow and to calculate all these changes would be almost a superhuman task. We know only the result, namely blue color."

Thus we see that as far as coming to any definite conclusions regarding the cause of the blue color Gadow's work was inconclusive, but it has been of great value to later investigators on this problem in that it has given them some basis from which to work.

In 1902 Häecker and Meyer (2) published a paper in which they followed up Gadow's lead, but due probably to better facilities for research they reached entirely different conclusions as to the blue color. Their reasons as given by them are:

"1. The difference between the refractive indices of the cell substance and air without, involving the hypothesis that this difference is distinctly greater for blue than for red.

"2. The small size of the pores whose diameter is small in comparison with a wave-length of light."

In other words, they believe that the blue light is due to the scattering of light by minute pores in the walls of the blue-producing cells.

Mason's work (3) (4) published in 1923 is the only work that has been done in this country on this problem. He was aided in this research by Bancroft, Chamot and Merritt, all of Cornell University. As far as results are concerned, his work has added absolutely nothing to that of Häecker and Meyer, and it will not be necessary to say more than to quote his conclusions.

"1. Non-iridescent blues of feathers are due to the scattering of blue light by very fine pores in the walls of the outer layer of cells of the barbs of the feather. This is the blue described by Tyndall, which is commonly observed in turbid media.

"2. No blue pigments, and no other structural causes of blue color have been observed in non-iridescent blue feathers.

"3. Green feathers are essentially the same as blue feathers, except that the blue cells are overlaid by a transparent yellow layer."

With this work as a basis, I have chiefly attempted to discover, if possible, the nature of the structures which are responsible for the break-up of the white light. From the first, I have never been able to see any thing which resembled pores, and so I have been interested in checking Mason's work, to determine, if possible, the exact nature of the structures present.

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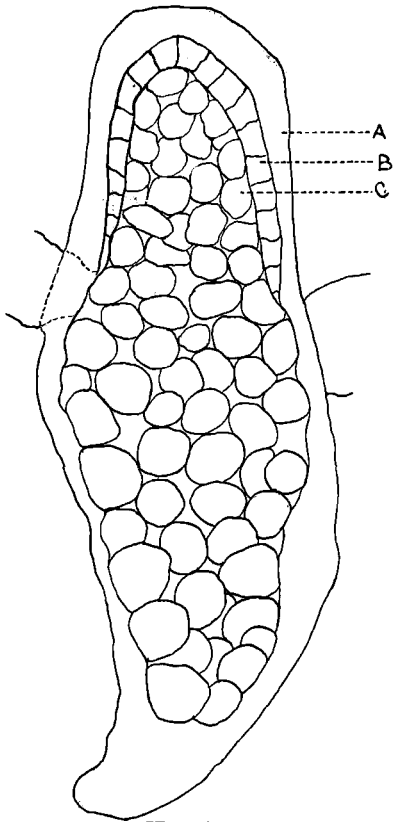

Fig 1

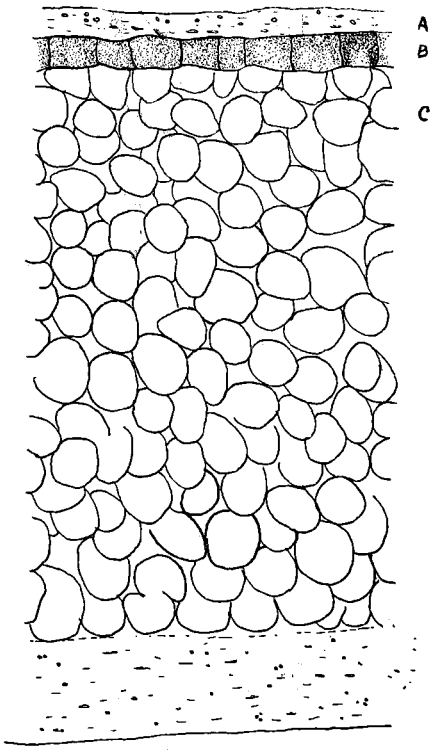

Fig 2

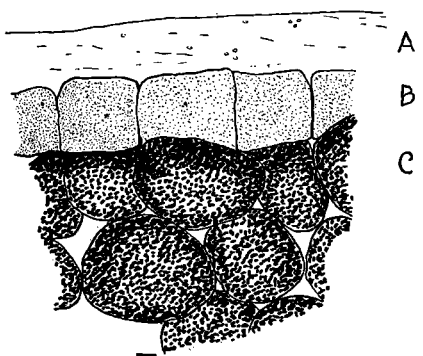

Fig 3

Fig. 1. A cross section of a Blue Jay barb showing shape of barb and size, position, and relation of various parts. A, sheath; B, blue producing cells; and C, pigment cells. (× 450.)

Fig. 2. A camera drawing through a sagittal section of a Blue Jay barb. A, dorsal portion of sheath; B, blue producing cells; and C, pigmented cells. (× 450.)

Fig. 3. Camera drawing of the blue producing cells with bodies and pigment cells with pigment. A, dorsal portion of sheath; B, blue producing cells; and C, pigment cells. (× 1000.)

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METHODS.

The problem here was one of acquiring a technique by which sections of a known thickness might be cut. This is extremely difficult due to the brittleness of the keratin of the sheath, and a great many different methods were used before the author was able to make sections suitable for study.

In preparation for sectioning the barbs are first cut close to the shaft and made into a roll by means of a fine thread, then treated as follows:

1. 24 hours in the following solution: Nitric acid (conc.) 20 parts; .5% Chromic acid 20 parts; Mecuric chloride in 20% alcohol, 20 parts; Picric acid (st. sol. in H_2O) 10 parts. Absolute alcohol 30 parts.
2. Wash in 50% Iodine alcohol for 12 hours, and then slowly dehydrated.
3. Cleared in Methyl salicilate.
4. Infiltrated and imbedded in 55° paraffin.
5. Sectioned 4–20 microns (best at 10).
6. Mounted unstained in balsom.

Staining was found to be quite superfluous, and was not used.

Bleaching was done by using a 3% solution of Hydrogen peroxide and also by using a 1% solution of sodium hydroxide. Feathers were left a month or longer in the latter mixture to complete the process.

Gross sections were best made by embedding in clear shellac, after which cuts were made free hand and mounted on slides with balsam. Such cuts were quite valuable in studying the relationship of different layers of the barb.

MORPHOLOGY OF THE BARB.

In order to clarify the process of blue production by the feather, it is necessary to describe in detail the structure of the barb. Gadow (1), Häecker and Meyer (2), and Mason (3) have each described the structure of the color-producing barbs, but their description seems to me to be inadequate. In each case, they have failed to make clear the exact location of the color-producing cells, and this gives the impression that they occur as a complete cylinder within the sheath. Their description also gives the impression that the barb would appear round in cross section, and this is not the case at all.

When examined with a magnification of 450, a cross section of a barb of a Blue Jay feather appears to be shaped more or less like an Indian Club, the dorsal portion of the barb representing the handle of the club, and the ventral portion being extended into a drawn out, pointed base, which is curved slightly to one side (Fig. 1). The three different regions of the feather are plainly visible, but some explanation of their relationship is necessary. The sheath is very variable in thickness, and from a number of measurements made with an ordinary ocular micrometer, I obtained a range of 7.2 to 21.6 microns in thickness. By far the thickest portions of the sheath are at the dorsal and ventral margins of the barb, while the lateral portions of the sheath are about one half as thick (Fig. 1). The blue-producing cells do not occur as a complete cylinder within the sheath, but are found on the dorsal side and extending about half way down the side of the barb. This location is further proven by the fact that only the dorsal side of the barb is blue, while the ventral side is black. The center of the barb is filled with large roundish cells containing large granules of melanin pigment. This pigment is so thick as to give the feather a black appearance wherever the blue-producing cells are absent.

The sheath is more or less transparent, having a slightly yellowish tinge in sections, and showing granulations and irregularities throughout. These irregularities appear as air bubbles, and stratification lines due probably to the uneveness in laying down. The keratin seems to be laid down in very uneven layers, and thus produces the lines referred to. In many places there are small foreign bodies which appear to have been imbedded in the keratin when it was laid down. These extremely small particles are in most cases smaller than the pigment granules. Gadow (1) has described the inner surface as being "waved" or scalloped to fit closely to the underlying cells, which he says are mound-shaped; but the author found these cells to resemble cuboidal epithelial cells in that they are almost cubical in shape, and hence very even in outline. The function of the sheath seems to be entirely protective. One thing would seem to indicate this, and that is that the sheath is thicker at the dorsal and ventral margins than on the side; the dorsal and ventral portions of the barb being more exposed to mechanical injury than any other part of the barb, would need better protection.

Underneath the sheath, on the dorsal portion of the barb, lies a layer of polygonal cells (appearing square in section) which under the lower powers (under 450) appear as nothing but slightly visible cell walls. The cells measure approximately 15 microns in diameter, while the height varies with the position. The cells in the center are the tallest, the height decreasing ventrally, giving the layer of cells a crescent appearance. Under the higher magnifications (1000–2000) these cells appear to have a cavity in the center,

roughly spherical in shape, and the walls appear tuberculated. In the walls of these cells are minute, almost ultra-microscopic bodies. Häecker and Meyer (2) and Mason (3) have referred to these as pores, and have given measurements of .3 micron for them in the *Malurus* feather. In the Blue Jay feather and Bluebird feather, from which this study has been made, these bodies were found to be immeasurable with ordinary Leitz micrometers. They have no regular arrangement, but seem to be evenly dispersed throughout the cell wall. I am considering them bodies, because of evidence which I shall present later. Gadow (1) has described the transparent cells as having laminae, but I was unable to find any evidence of these.

The cellular part of the barb is made up chiefly of the pigment cells, which, aside from the layer of transparent cells, occupy the entire cavity of the feather. These cells are roughly cuboidal, rounded at the corners, and have a spherical cavity in the center. They are approximately 19–20 microns in diameter, and there are often open spaces between cells, probably due to the withdrawal of the connective tissue when the cells around it die. The pigment cells lying next to the transparent cells, however, present a very smooth surface to those cells, and are more heavily pigmented than any of the other cells. The pigment granules are elliptical in shape, and measure about 1.6 by .8 microns. They appear brown in sections, but when very densely concentrated appear black. They are zöomelanin, which, according to Gadow, is probably the most common pigment found in animals.

EXPERIMENTAL WORK AND RESULTS.

In the preceding section, I have stated that the structures effecting the breaking up of white light, and thus giving rise to the blue color, are bodies. Häecker and Meyer, and Mason have each after a great deal of study concluded that the structures are pores in the cell walls of the color-producing cells, and this statement has never been questioned. Mason's experiments have been checked in this work, and the problem has also been investigated from another angle, that of microscopic examination, and the results obtained all indicate that the structures are solid bodies and not pores.

Mason based his conclusions chiefly upon the experimental work which he did involving the permeation of the feather by a liquid of refractive index of approximately 1.54. According to his experiments when a feather is allowed to become permeated by such a liquid, it loses its blue color and assumes a brownish-black color due to the underlying pigment. This, he explains, is due to the fact that the pores become filled with the liquid, and the difference in refractive index between the walls of the pore and the air in the lumen is eliminated, thus destroying the blue because the light is no longer broken up by the pores. In other words, the blue-producing pores,

when filled with a suitable liquid, no longer exist optically, but the entire layer of color-producing cells has become homogeneous and transparent to light. Mason further states that as soon as the liquid is allowed to evaporate the blue color is restored, the pores once more becoming functional. He does not state whether or not all the blue feathers mentioned in his paper were treated thus, but his conclusions seem to imply that all blue feathers are alike in this respect. He used a number of liquids for this work, and I have checked with enough of these to eliminate any error on that score. Most of those used have a refractive index of 1.52–1.54, or approximately that of the feather. I have checked Mason's work with methyl salicilate (refractive index of 1.534), with xylene (refractive index of 1.49), and with cedar oil (refractive index of 1.51). Barbs of Blue Jay and Bluebird feathers have been left in these liquids for as long as six weeks without any noticeable change in the blue color. This period is long enough to eliminate any question of whether the feather is permeated or not, but the fact that a feather may be completely bleached in 48 hours would prove that permeation of the feather does not take such a long period. According to Mason's hypothesis any liquid having the proper refractive index should have the same effect on the blue, and enough different liquids of the proper refractive index have been used in this investigation to offer a reasonable check on his work.

A careful microscopic study of the structures in question also bears out that they are bodies and not pores. They were studied with a Zeiss microscope, having a magnification of 1980 times, and they appear as small black bodies in the walls of the transparent cells. If they were pores, they would show a lumen as one focused up and down on them, but this is not the case. In focusing up and down at no time was there seen anything that resembled the appearance of a lumen. Other capable people have checked this point and their observation agrees with those set forth here. The fact is that in certain bacteria, where pores are present, the pores are plainly visible as such and show a distinct lumen. This makes it reasonable to suppose that if pores were present in the cells of the barb they would show the same characteristic.

Another thing which would tend to prove that the structures are bodies and not pores is the fact that when treated with a solution of .3% potassium hydroxide in hydrogen peroxide the bodies disappear. This solution bleaches very rapidly and barbs which have been treated with this solution for 48 hours show absolutely no trace of the bodies. When bleached in ordinary .3% hydrogen peroxide, however, the bodies are unchanged. This would indicate that they are of such a nature that they are soluble in a very weak solution of hydroxide. A solution this weak will not effect a softening of the barb itself in less than two months.

CONCLUSIONS.

1. The structural color of blue feathers (turbid blue) is due to the scattering of light by small irregular bodies which have been laid down in the transparent layer of cells of the barb, and also to the fact that the pigment cells, in acting as a dark background, allow only the blue light to show.

2. These transparent blue-producing cells are found only on the dorsal surface of the barb.

3. With magnification of 1900 or less the bodies are not measurable.

4. Neither in appearance nor by immersion in a suitable liquid do they resemble pores.

5. There are pores and granulations in the sheath of the barb which may aid to some extent in the breaking up of the light.

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