STUDIES ON INHERITANCE IN PIGEONS

II. A MICROSCOPICAL AND CHEMICAL STUDY OF THE FEATHER PIGMENTS¹

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SIXTY-THREE FIGURES—SEVEN PLATES

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¹ Papers from the Department of Experimental Breeding of the Wisconsin Agricultural Experiment Station, No. 4. The paper by Cole ('14) is the first in the series of "Studies on inheritance in pigeons."

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INTRODUCTION

1. *Method of genetics*

The aim of most of the work now being done on the study of inheritance is to identify in the hereditary complex the discrete units, factors, genes, which apparently control the external appearance of the organisms.

The method commonly in use to accomplish this genetic analysis is hybridization or cross-breeding. Forms differing in one or more respects are mated, the crossbred offspring are mated together and with the parents, and, depending on how the characters are distributed, the gametic structures of the animals are formulated.

The most that can be done by this method of crossing and hybridization is to discover the relation of one character to another as they *appear* in heredity, and from this we may form a hypothesis concerning the relations of the factors to each other as they *act* in the ontogeny of the organisms. We cannot get beyond hypothesis until we are able to go behind form and color as we see them, to the mechanical and chemical nature of the processes and the factors which control them.

Pernitzsch ('13) is attempting such an analysis of the races of Axolotl which Haecker has been using in his genetic experiments. After speaking of the hybridization methods as a means of identifying the factors of heredity, he says (p. 149):

Eine völlige Sicherheit ist wohl auf einem anderen Wege erreichbar, indem man nelmlich die Unterschiede zweier Rassen, die durch mendelnde äussere Merkmale verschieden sind, möglichst genau morphologisch und physiologisch untersucht, und ihre Erstehung und allmähliche Divergenz im Verlauf der Ontogenese bis zum Ei zurückverfolgt.

Pernitzsch, in his study up to the present time, has established certain striking differences in form and behavior of pigment-cells in the integument of the three varieties of Axolotl. Certain other simple analyses of this nature have already been accomplished and have illuminated the field and prepared for suggestive interpretation of the facts. As examples: The compound nature of the coloring in agouti hairs and the ability of this form to give rise to the other colors; the rôle of oxydases and chromogens in producing animal pigments; the inhibiting action of certain oxyphenols on this reaction and its bearing on dominant whites. The facts presented in this paper are offered as a contribution to the method for the experimental study of heredity, described above. The form used is the tumbler pigeon and the characters subjected to analysis are the various self-colors found in this variety.

2. Granular nature of melanin pigments

The pigments concerned in the production of color in *Columba livia* are of the class known as melanins. Melanin pigments, as far as is known, are granular in nature; often the granules are of definite shape and of measurable size. Post ('94, p. 491) states that a particular shape is peculiar to each genus of animals. For example, the pigment granules in the dog are whetstone-shaped; in the guinea-pig, short and thick; in pigeons, rods of measurable size. Haecker ('90, p. 70,) speaking of feathers in general, says that the pigment granules are oval, spindle-shaped or circular in form. He specifies that the granules of the rust-

brown feathers of a certain variety of pigeons are spherical and he gives some good figures of these. Strong ('02, p. 155) held that for birds the granule shape is always rod-like and of a definite size for each species. He later ('03, p. 269) qualified this statement to account for spherical granules which he discovered in the iridescent neck feathers from the pigeon. At this time he took the position, since abandoned by him, that these so-called metallic colors were dependent directly on the spherical form of the granules.

3. Origin of pigment in feathers

Investigations on the processes of pigmentation of the developing feathers have been carried on by Post ('94) working on the dove, Rabl ('94) on the chick, and Strong ('02), on the common tern (*Sterna hirundo*). Strong was fortunate in the choice of his material for this study, since in the tern the pigment cells are comparatively simple and the process is direct.

At least one fact concerning pigmentation in birds is now well established, that is, that the entire process is confined to the epidermal layer of the feather. Post and Rabl early agreed that this is the case, and Strong in 1902 laid considerable emphasis on the matter. A large number of feather germs has been examined in the course of my own work, and among all the preparations not a single case suggesting melanin formation in the pulp has appeared.

In the feathers of the common tern, which contain black pigment, Strong ('02, p. 165) described the pigment as first appearing in certain of the intermediate cells near the pulp region before the differentiation of the ridges. The pigment corpuscles, at first exceedingly small, increase in size, become deeper in color and more numerous, until they finally form a complete ball. These dense black balls, two or three of which are seen in each ridge, are the pigment-cells. When the pigment-cells reach this stage they send out processes which finally arrive at the future barbule cells, and in some way distribute pigment to them.

Although this description in its essential features will answer for the pigmentation of pigeon feathers, it must be considerably modified in details to fit the process in certain of the colors, as will be shown later.

Text figure A illustrates diagrammatically the parts of a fully developed definitive feather, while figure B shows the general relationship of the parts in the feather germ. It is believed these figures will facilitate following the discussion and understanding the positions and relations of the drawings in the plates. For an account of the structure of feathers the reader is referred to Mascha ('05), while the general process of development and of pigment formation is described by Strong ('02).

4. Materials and methods

As mentioned above, the material for this work was taken entirely from tumbler pigeons. These birds as they are handled by the fancier exist in six different so-called self-colors, namely: red, yellow, black, dun, blue and silver. Another self 'color,' white, due to the absence of pigment, is not considered in this paper. These six colors will be described in detail, as they are treated individually. The particular individuals mentioned in this work are among those used in the breeding experiments of the Department of Experimental Breeding at the University of Wisconsin. They are of known parentage and genetic constitution and can be identified in other publications of this department (Cole '12, '14) since the birds are referred to by their permanent record numbers. Young birds of any color were to be had almost throughout the year, so that, all things considered, the material available was almost ideal for an investigation of this sort.

The methods used by Strong ('02) were found to be generally satisfactory and were essentially followed. Portions of whole feathers were cleared in chloroform and mounted in gum dammar. The most instructive preparations for a study of pigmentation in the adult feather were made by shearing the barbules from the barb with a fine scissors, cutting parallel

Fig. A Diagram of a portion of the vane of a cornified feather representing parts of two adjacent barbs with their two kinds of barbules, and showing the way in which the latter are interlocked by means of the minute hooks.

Fig. B Diagrammatic cross-section of a feather fundamen-
 B

with the barb and toward the shaft. In this way large numbers of barbules collect on the blades of the scissors and can be scraped on to a slide and mounted direct. This flattens out the barbules and exposes them to view throughout their whole extent. Sections of dry feathers were made by mounting in paraffin from ehloroform and cutting on a Minot microtome. In order to obtain ribbons so as to preserve the parts in their proper relation on the slide, it was necessary to cover the cutting surface of the block with a thin film of celloidin after each section was cut.

As fixing reagents for feather fundaments Kleinenberg's picro-sulphuric, and also the fluids of Bouin and Gilson, were found satisfactory. As a clearing agent xylol proved to be unsuitable, its action being to harden and to increase the brittleness of the feather germ. The use of chloroform obviated this difficulty. Best results were got when the change from chloroform to paraffin was made gradually, by saturating the clearing chloroform with paraffin before transferring to pure paraffin. For good results infiltration with paraffin was continued for at least three days. In sectioning, the series would ribbon off nicely near the umbilical end of the feather germ, but the sections showed an increasing tendency to fall out of the ribbon as the cornified part of the feather was approached. The final sections in the series invariably fell out of the ribbon, and it was necessary to pick them up carefully by a moistened needle, and remove them one by one to the slide.

In staining, for the most part, weak cytoplasmic stains were used, for it is easy to obscure the pigment granules, and to confuse them with other cell structures which stain heavily when any considerable coloration of the sections with basic stains is produced.

The invaluable suggestions and criticisms of Dr. L. J. Cole, given throughout the course of the work, have been an essential factor in accomplishing whatever results are here presented. The writer also wishes to express gratitude for valuable assistance given by Dr. R. M. Strong, at his laboratory in the University of Chicago, for a short period at the inception of the work.

EXPERIMENTAL FINDINGS

1. *Red color of pigeons*

a. Distribution of red pigment in the feather structures. The so-called 'red' of the domestic pigeon belongs to the series of browns rather than reds. Ridgway's ('12) chestnut (plate 2), or pecan-brown (plate 18), are nearest the typical red of pigeons. These colors may be bright or dull, light or dark, but the range of variability is small and reds of whatever kind are easily classified as such. The most extreme variance from the typical red is the color called, in our work, 'plum color,' or 'wine color,' to be considered later.

If the pigment masses in a preparation from a red feather are examined under a microscope by transmitted light they appear red-brown in color, very much as when examined by gross methods and reflected light. Apparently the individual granules in a red feather are never dense enough to exclude all the light, thereby appearing black by transmitted light. Further, it is impossible, in the small space within any feather structure, for the granules to be thickly and deeply enough packed to become opaque. When feathers have been cleared in chloroform and xylol even the thickest parts of the barbs are readily pervious to light and their red color unmistakable. Further evidence, if it were needed, for the existence of a pigment substance red in color, has been obtained by chemical methods, and will be brought out in the section devoted to that subject.

Typically, all the structural elements in the red feather contain pigment material. Figure 1 is a cross-section of a red feather vane, and shows this condition. If there is any reduction from the maximum pigmentation (i.e., light red) the first structures to suffer loss (the pigment-free areas at the cell boundaries are not considered here) are the haemules or hooks, followed by the hair-like terminal extension, first of the curved barbules and then of the hook barbules. In all kinds of red (also, as will be seen, in colored pigeon feathers generally) the hook barbules are more heavily pigmented than the curved. Figures 3 and 4 show the relative intensity of pigmentation in

these two kinds of barbules. Any reductions of pigment further than those in the haemules and terminal fibers, mentioned above, fall most heavily on the curved barbules; they can be almost devoid of pigment while at the same time the hook barbules show practically no reduction in pigmentation. This condition, which results in a grating of parallel pigmented and non-pigmented lines, is associated with the effect known as 'grizzled,' and will be dealt with more fully at another time.

The barb in typical reds contains some pigment (figs. 1 and 5). The cortex, in covert feathers, is uniformly pigmented from apex to ventral ridge. The cavities of the medullary cells are also copiously lined with pigment granules. In flight feathers, in which the barb is always thin and high the portions of the cortex beneath the barbules are more sparsely pigmented, causing these feathers to appear dull in color when viewed from the ventral aspect. Figure 45 is from a black feather, but shows this condition of distribution. The dull ashen color seen on the under side of the wing in typical red birds is due to this condition.

As pointed out, the region near the transverse walls of the barbule cells generally tends to be free of pigment. This serves to break up the pigment into separate masses, giving a segmented appearance to the barbule. The degree of this segmentation—that is, the breadth and clearness of this pigment-free area—has an influence on the color effect of the feather. Lightness and darkness in reds (shown later) depends chiefly on the number of granules per given area, but sparse pigmentation is generally associated with a broadening of the pigment-free band. The boundaries of this area are not cleancut, however. They are broken by invading rows of granules, and occasional granules may be found scattered within the area (fig. 4). When more granules are present the pigment encroaches on the pigment-free band, narrowing it and the red is darkened, or perhaps, more properly, enriched. Light and dark reds can be compared by examining figures 1 and 5 and also figures 6 and 7. Figures 1 and 7 are drawn from 5 B, a dark mahogany red; figure 5 is from 93 B a 'washed-out' pale red, and figure

6 from a light red feather of 894 B. The light or dark condition is clearly a function of the number of pigment granules present and involves no change in kind or form of granules.

b. Granule form. In red birds the granule form is without exception spherical. The granules are for the most part, though not always, uniform in size in each barbule. The typical size is about $0.3\ \mu$. In typical reds, or even better in dark reds, where the spherical granules are packed in tightly, they assume more or less the arrangement taken by round shot if placed on a plain surface and crowded together, presenting the appearance of rows running with the length of the barbule, the granules alternating in the rows (fig. 7). This linear arrangement is not due alone to being packed into the cell, however, as it is also seen in the light reds where the granules are thinly scattered (fig. 6). Haecker ('90, p. 70) also observed this latter condition in referring to the spherical granules of the rust-brown pigeon feather, "welche in perlschnurartigen, der langsrichtung des betreffenden Organs folgenden Reihen angeordnet sind."

c. Plum color. There appears in some of our red birds, especially in the region of the breast and belly, but often spread over the shoulders and back, a greatly modified condition of the typical red. In our work we have spoken of this as plum color or wine color. Ridgway's indian red ('12, plate 27) is a near representation of this color. The color also lacks the brightness and warmth characteristic of red; it is cold and possesses a bloom like that seen on an unwiped plum rather than the sheen seen normally on reds.

The granules of pigment making up this color are spherical in shape but remarkably large in size, often reaching 2.5 to $3\ \mu$ in diameter (figs. 22-23). The distribution in the barb is much the same as in normal red, with the exception that the ventral part of the cortex bears fewer granules (fig. 24). In the barbules segmentation is always marked, and the boundaries of the pigment masses always clean-cut and distinct. These broad clean-cut pigment-free areas, combined with heavy pigmentation, doubtless play a rôle in producing this color effect.

d. Development of red pigment. First signs of pigment formation in red pigeon feathers appear in the intermediate and inner sheath cells some time before the formation of the ridges (fig. 8). The pigment appears promiscuously throughout this region as minute granular clumps, in or between the cells; at a somewhat later stage, but before ridge formation, this promiscuous elaboration of pigment becomes more general; the clumps are denser and more numerous. Often just before ridge formation this pigmentation is so generalized that one gets the impression that practically all the cells in the field are taking part in the process.

The epidermal layer is now cut up into the ridges, each ridge enclosing its share of pigment material and active cells, which are scattered more or less throughout the tissue of the ridge (fig. 10). At this time certain of the cells begin to increase in size and activity, pigment accumulates in them, becomes closely packed and either sends out its own branches or becomes associated with other branches and pigment paths formerly existing. These are the so-called melanoblasts, melanophores or pigment cells of writers. They most frequently lie near the base of the ridge, but have been found in all situations within the inner sheath cells.

The fact that such specialized cells do occur in developing feathers, that they are large, that they are actively forming pigment and that they extend apparently continuous branches into the outer regions, has led some workers in this field to ascribe the whole duty of pigment formation to them; some unusual substance is supposed to be resident in these cells which endows them alone with the faculty of elaborating the pigment, and further to distribute it to other cells of the tissue, generally by means of pseudopod-like ramifications. As Strong ('02, p. 168) pointed out, in the case of the dove, the pigment cell processes are irregular in form. In the tumbler pigeon this condition is extremely marked. In fact, the processes are so tortuous that in working with a series of cross-sections it is very difficult, often impossible, to establish their continuity. Bending as they must so sharply upon themselves, they appear in the

sections as isolated, irregular, elongated pigment masses, occurring promiscuously throughout the ridge tissue.

Klee ('86, p. 139) believed that besides the pigment from the branched cells there was another source; he says that "Anderseits sah ich an den Zellen der Strahlen, etc., noch eine sozusagen freie Pigmentbildung." Strong ('02, p. 170) has stated that he cannot deny that there is a free formation of pigment in the barbule cells independent of that supplied by the pigment cells. Winkler ('10, p. 618) observed in regenerating tissue of *Salamandra* "dass schon sehr frühzeitig die oberflächlichste kernlose Zellenlage reich mit Pigmentkörnchen erfüllt ist," and that these or other cells later developed into branched pigment cells. Pigment development in tumbler pigeon feathers furnishes demonstrative evidence that this faculty is by no means confined to the specialized pigment cells. As has been shown in the earlier stages, the process is exceedingly generalized throughout the tissue, and I am unable to conclude that this activity on the part of the intermediate cells in general, ceases upon the development of more specialized cells. Any of the intermediate cells, it seems, may be stimulated to increased pigment-forming activity, whatever their location, and all stages of activity are seen, from the extremely specialized branching cells to those forming but a few granules. It is even possible that the entire pigmentation of the feather may take place without the activity of specialized pigment cells. The series represented by figures 11 to 15 represents such a condition. Abundant pigmentation is taking place in this feather, but scarcely ever is a well-developed pigment cell seen. Figure 16 also shows the feather structures becoming well pigmented, but specialized pigment cells are absent.

2. Yellow

a. Nature and distribution. Among self-colored birds the colors red and yellow are perfectly distinct. Even the lightest reds and darkest yellows are easily placed in their proper class. In appearance these colors bear the relation to each other of dilute and intense conditions of the same pigment material.

That yellow is a dilute condition can be demonstrated by even a hasty microscopic examination of the feathers, and the fact is further borne out to the degree of certainty by a comparative chemical study of the two kinds of feathers. Microscopically the pigment is seen to be far more sparsely distributed than in reds. Often under strong light the pigment in the barbules is almost imperceptible. In cross-sections of cornified feathers, especially, the attenuated condition of the pigment is well demonstrated (fig. 17). As regards the relative distribution to the various feather parts, there are some constant differences from the conditions seen in red. In the barbules the segmented condition of the pigment is seldom to be seen (fig. 18) though the hook barbules may show it in a weakly developed condition (fig. 19). It is not likely that this condition is a fundamental one correlated with yellow color, since yellows are perfectly capable of developing this condition if enough pigment is present, as shown often in the hook barbules and even occasionally in the curved barbules in dark yellows. Also, as a further result of sparse pigmentation, the nuclear areas are not so well marked out. In all preparations of yellow that I have examined the hooks or haemules are always (unlike red) pigment-free (fig. 19).

The barb in yellow feathers (figs. 17 and 20) contains far less pigment proportionately than the barb in reds (figs. 1 and 5). In the former it is almost entirely confined to the apex; the lateral sheets of cortex and the medullary cells are usually pigment-free. Occasionally, however, large dense masses of pigment, irregular in shape but distinct in outline, occur in the cortex substance.

b. Granule form. The crucial difference between yellow and red, however, has not yet been mentioned. It is concerned with the nature and form of the pigment granules. As shown above, the pigment in reds exists as distinct clean-cut spherical granules, typically $0.3\ \mu$ in diameter. No such spheres have ever been found in a yellow feather, in the course of my work. The pigment exists as irregular formless clumps, or agglomerations. These blotches of pigment appear finely granular in nature,

but are so fine, that discrete particles are beyond the limits of a 1.8 mm. oil immersion objective (figs. 18-20). This difference between reds and yellows is sharp and is constant. When minute patches of reddish pigment are present on a dun, black or white feather, it is frequently difficult or impossible by gross examination to classify the color as red or yellow; but microscopic study of such areas serves unfailingly to distinguish the colors by virtue of the morphological difference above cited.

c. Development. As regards the development of the pigment in the yellow feather fundament, the process is practically identical with that described under red, except that the end-product is, on the one hand, distinct spheres, on the other, irregular pigment masses. A study of pigment formation in yellows bears out the assumption that a large part of the pigment is elaborated by non-differentiated intermediate cells. Specialized pigment cells often are not present at all in the basal regions, while abundant and generalized pigment formation is going on in the barbule region. Figure 21 shows a small, and rather inactive pigment cell (*pg.cl.*) such as is often seen in these feathers.

3. Black

a. Nature of the pigment. Haecker ('90, p. 70) assumes that the 'rust-brown' pigment, such as is found in red pigeon feathers, "underlies all brown, gray, black and blue colors," that is, outside of the structural distribution of pigment these colors bear only a quantitative relation to one another. These relations are hypothetically possible if the pigment substances were always seen by transmitted light, since in this case any particle of substance impervious to the visible spectrum would appear black, although by reflected light this particle might give any of the spectrum colors. I have found that a microscopic examination of black feathers at once reveals striking differences from red or yellow and immediately places them, on the basis of the color of pigment, in a wholly different category from these colors. This work has indicated that the color of the pigment in a black bird is a true black and not an effect produced by the massing of dark brown color material. In a black feather

regions can always be found where the pigment granules are thinly scattered so that individual particles can be examined. The smallest granules, about $0.3\ \mu$, are not altogether impervious to light. The light which they transmit, however, shows no effect of differential absorption and is essentially white. This gives a color to the granules which might be described as 'attenuated black' or dun. In those young regions of the feather germ where the pigment is so sparsely scattered and finely divided as to be perceptible only as a faint nebula, its identification as black pigment is already possible. Finally the existence of a black pigment substance is demonstrated beyond further doubt by its isolation and purification by chemical methods.

b. Distribution. Black feathers are richly supplied with pigment. Its distribution in the different feather structures is in general similar to that in red. As in red, the hook barbules are almost invariably more heavily pigmented than the curved barbules, and the pigment-free areas at the cell boundaries, though generally well marked, are frequently very narrow. An expression of the richness of the pigment supply in blacks is the condition of the haemules, which are here more heavily laden with pigment than in any other cases examined. In reds this region carries but slight amounts.

c. Granule form. As regards granule form, blacks present varying conditions. If the barbules are heavily pigmented and the granules closely massed, as is generally the case with hook barbules, the granule form cannot be determined. In the lighter colored curved barbules where the pigment is thinly scattered, regions can always be found where individual granules can be distinguished and their size and shape determined with fair accuracy.

A study of feathers from certain black birds in our colony shows the pigment to exist as well-defined, uniform, spherical granules about $0.5\ \mu$ in diameter, packed in the cell in much the same way as the granules in a red bird (figs. 27-28). If in such a bird feathers from various regions of the body are examined, this condition of the granules will be found throughout.

In those structures too heavily pigmented to allow the observation of individual granules, the uniformly 'pebbled' appearance of the surface of the pigment mass yet serves easily to distinguish it as made up of spheres; such a bird is 1037 B (figs. 27-28). Feathers from other black birds may show an entirely different condition, for example, 7 A. Practically no spherical granules can be found in this bird. The pigment in all parts of the body exists in rod form about 0.8 to 1.5μ long, and 0.2μ in diameter (figs. 25-26). In general, the long axis of these rods is parallel to the long axis of the barbule, though in the region of the old cell nucleus they are often tilted as if they rested or were pressed against the walls of the nucleus, thus forming in optical section, a roughly circular cavity. Cross-sections of black feathers show in all cases spherical granules, a result to be expected, for cut across and viewed on their ends, even the rod forms appear circular in outline. These birds, 1037 B and 7 A, are similar in color, in intensity, and in sheen.

The type of blacks represented by 1037 B, in which a rod-like granule is practically never seen, is not common. Neither do birds showing as nearly an absolute lack of spheres as 7 A appear frequently. The commonest condition is for one kind of granule to predominate greatly and the blacks can generally be classed as 'predominantly spheres' or 'predominantly rods.' A few cases have been seen where the rods and spheres were about equal in number. Sixty-five black birds have been thoroughly studied in this respect, but up to the present time the granule shape has not been found correlated with either age, sex, or genetic constitution. Conditions here are very suggestive and a more detailed study of this matter will be made in the immediate future.

d. Development. Black pigment, at its first appearance in the feather fundament, is not unlike red in form and behavior. Black pigment is seen first between certain of the outer intermediate and inner sheath cells, as a fine granular haze characteristic in color for the black pigment of the feather. The corpuscles making up this nebula rapidly increase in size, appar-

ently by a 'running together' of the finer particles, and well defined particles are seen at an early stage. Figure 29 shows the position in which this pigment first appears, and also the promiscuous way in which it is scattered throughout the tissue. The spot marked *X* is shown under higher magnification as figure 30. It is hard to see how this pigment could have arisen in any other manner than an autochthonous activity of non-differentiated intermediate cells. Meanwhile, the cells or regions elaborating this pigment become more numerous till a condition of general but very sparse pigmentation is produced. By the time ridges have formed, certain of the cells have increased in size and become specialized for pigment production. These pigment cells generally develop among the inner intermediate cells, but frequently they are found nearer to the future barbule cells. At a very early stage they send out branches which at first seem to have no particular direction (fig. 31) but later only those directed toward the barbule cells remain active; the others either become spent or are withdrawn.

The well developed pigment cells of black feathers furnish better evidence than do those of red, that the pigment processes are continuous and actually function as paths for the translocation of the pigment granules from the pigment cell to the outer regions of the barbule. Every pigment cell is provided with one or more such rays, and their course is not so devious as those in red feathers. In thin sections their turnings and sudden bendings have not been so frequently cut across as in the case with the much twisted ray of the red feather and their continuity is more easily established.

Of data which suggest the nature or manner of the force which regulates the granule shape in black, I have none whatever. Frequently in the feather germs of birds which bear both rod- and sphere-shaped granules, one pigment cell will be elaborating rods, while another immediately adjacent cell may be producing predominantly spheres; figures 32 and 33 represent two such adjacent cells.

4. *Dun*

a. General. Dun color in pigeons is produced by the same pigment which produces black; the difference between the two colors is chiefly a quantitative one. In duns the pigment is distributed in different feather structures in the same manner proportionately as it is in blacks, but of course always less in absolute amount.

Self-duns have some specific limitations, however, in regard to granule shape and size. The pigment is more finely, also probably more uniformly, subdivided than in black. The measurement of particles so minute as these granules is at best difficult, and with the apparatus which was available for this work, inaccurate. The granules of dun are plainly smaller than those of black—as near $0.2\ \mu$ as could be determined—and their perfect spherical shape is undoubted (fig. 35).

This lack of rods in self-dun birds is very striking, but even here it is not absolute, for at least two dun birds, 265 A and 892 B, display this form of granule. In these cases the admixture of rods is exceedingly slight, however, and one may safely say that self-duns are as a rule characterized by granules spherical in shape and less than $0.25\ \mu$ in diameter.

b. Dun color in other birds. The conditions described above are in all cases associated with self-dun color. We may have dun colors appearing in birds which are very unlike self-duns in genetic constitution, that is, in blue or in black birds, but in these cases the dun color is made up in a different way; a similar color effect is produced but a different set of conditions prevails.

The body or wing covert feathers from a blue bird are not uniformly colored from tip to base (fig. 46). The distal portion shows the characteristic blue effect, which gradually merges into a typical dun color in the proximal two-thirds (or thereabouts) of the feather. The granule form of the pigment in this portion of the feather is always rod-like. The rods are always longer than in the black birds; they are confined to the dorsal portions of the barbule, and they are not found in the

haemules. Such conditions are shown in figures 43 and 44 and will be dealt with later, under blue.

The color in black birds may become so weak in certain regions that it may be described as dun. This condition is found most frequently in the side-of-body contour feathers, or under-wing coverts near the axilla. This effect is simply the result of a quantitative reduction in the number of pigment granules, those which are present being characteristic in size and shape for black birds. The black is 'diluted' without the aid of the *dilution factor*. These four kinds of duns—namely, self-dun, dun on the bases of blue feathers, dun color in pale black feathers and the dun on the under side of black flight feathers—are similar to the eye, yet only one of them is the *character* known as dun in our breeding work.

Dun also occurs in the wing bars of a silver bird. Study of a microscopic preparation of such a dun could not distinguish it from the dun of a self-colored bird.

c. Development. The process of development of pigment in duns is essentially the same as that which has already been described for blacks. Figure 36 shows a young ridge with three pigment cells. The processes are unusually straight and regular. It will be noticed that one is advancing along the outer side of each lateral plate. This should be compared with the position of the pigment processes in blues where they are confined mostly to the axial region of the ridge.

5. Blue

a. Nature of blue color in birds. Haecker ('90), Krukenberg ('84), Gadow ('82) and others, showed that blue as seen in bird feathers is a 'structure color.' Franz Samuely ('11) discusses several cases of animal pigment substances, blue in color, occurring in the tissues of certain coelenterates, and in the integument of certain fishes. No blue pigment substance has ever been discovered in the integument of higher vertebrates, though this color is both brilliant and common, especially in birds. A thoroughly satisfactory explanation of the exact optical properties and relations of the feather structures which

produce blue color has not yet been given. This is a problem demanding elaborate and refined physical technique, and biologists have had difficulty in solving the problem.

The color called 'blue' in domestic pigeons has very little claim to that name. It is not at all comparable to the blue of the bluebird, jay or indigo bird, but resembles more the so-called blue of the rabbit or maltese of the cat. In other words, the color belongs more properly among the grays than among the blues. The 'gull-gray' of Ridgway ('12, plate 53) is a fair representation of the blue of the domesticated pigeon. Typical spectrum blue, however, is found among tropical members of the pigeon family.

b. Distribution of the pigment. The pigment in these feathers is the same as that concerned in the production of black and dun. In distribution and granule shape, however, it presents striking differences from these colors. It is, in fact, this peculiar distribution of the pigment in the tissue of the feathers which produces the blue effect. In the barb the pigment is restricted entirely to the apex (figs. 37-39). The lateral sheets of cortex are altogether without pigment and the medullary cells, richly supplied with pigment in black and in duns, are entirely free from pigment in blue feathers. This is in marked contrast to the conditions seen in the true blue shown by the birds above mentioned, i.e., the jay or indigo bird (Haecker '90; Strong '02). Here the medullary cells are packed with pigment which is overlaid with pigment-free cells and tissue.

Striking as are these conditions in the barb they are not the chief factors concerned in the production of the 'blue.' In the barbule the pigment, instead of being spread throughout the cells in a fairly uniform layer, is massed closely together in the middle of each barbule cell. Figure 40 shows part of a barbule laid flat on its side. This highly exaggerated segmental arrangement of the pigment provides large areas for the reflection of light, modified only by the properties of the keratin and uninfluenced by underlying pigment substance. Light from such areas would be substantially white. Now if these areas are finely interspersed with regions which reflect no

light (that is, black) ideal conditions for the production of gray are presented. The manner in which this gray is modified to give the blue effect will now be explained.

When in position on the barb the conditions of light reflections from the barbules are not so simple. On the feather the barbules are arranged as a series of thin, curved, lamellae set more or less on edge. The pigment masses are so situated within these lamellae that when viewed from either edge (that is, from either dorsal or ventral aspect of the feather) the pigment must be viewed through a certain depth of colorless semi-transparent keratin substance (fig. 37). Moreover, this pigment-free keratin presents to the eye a series of convex surfaces which offer other optical possibilities for reflection and refraction of light. It is the combination of these conditions which produces the condition called blue in pigeon feathers.

That the blue effect is produced by a layer of pigment-free material intervening between the eye and the pigment mass is further demonstrated by the condition seen in the base of blue wing coverts, and often even in black flight or tail feathers. It will be recalled that the wing coverts from a blue pigeon are not blue throughout their entire extent. Only the distal part is blue, while the proximal unexposed part is dun color. These color relations of the different regions of the feather are in evidence only when it is viewed from the dorsal (outer) aspect. Viewed on their ventral aspect these feathers are blue from tip to base. A study of figures 43 and 44 reveals the cause of these differences in color, seen on the two surfaces of such a feather. The clumping of the pigment is largely broken down, i.e., the pigment is spread, in the bases (proximal region) of these wing coverts, but the pigment in the barbule has spread in only one direction, that is, toward the dorsal, recurved, margin. The ventral half of the barbule is still pigment-free, and from this aspect the pigment mass must still be viewed through colorless keratin, producing the blue color.

Frequently the flight feathers of a black bird, which are a good black when viewed dorsally, show a bluish cast on their ventral surface. This is again due to the presence of practi-

cally pigment-free keratin, but in this case the keratin is of the barb, not the barbule. The high thin form of the barb in flight feathers will be recalled (fig. 45) and when the pigment is confined to the dorsal portion of this structure a bluish cast is produced on the ventral surface of the feather. This is more marked when the surface is viewed at an angle other than ninety degrees, than it is when viewed exactly at right angles, simply because more of the pigment-free keratin intercepts the eye. This condition on the ventral surface of the black flights is exactly comparable to the dullness or ashen color seen on the ventral surface of red flight feathers.

c. Granule form. The combination of factors which produces blue in a bird also has an influence on granule shape. The pigment in the blue regions of a feather, which occurs massed in clumps, invariably exists in spherical granules (figs. 40-42). The granules exceed in size the largest ever found occurring in a black feather. When massed in characteristic fashion they have a diameter of about 0.8 to 1 μ and are remarkably uniform and constant in this respect (fig. 42). If the pigment masses are small and the degree of compactness slight the granules assume a larger size, sometimes reaching 2 μ in diameter (fig. 41). The alternating rows and string-of-pearl-like arrangement of the granules are well marked in blues.

As pointed out in the discussion of dun, the pigment in the bases of blue feathers (which region is generally dun in color), assumes striking differences in form from the pigment in the blue region, that is, it invariably shows rod-shaped granules, larger in dimension and more distinctly marked than the rod granules of a black (fig. 43).

The pigment in the black bar of a blue bird consists of spherical granules, in this respect resembling black birds of the type of 1037 B (fig. 27).

d. Development. The study of the origin of the pigment in blue feathers is attended with certain difficulties and uncertainties. There are invariably on the common blue pigeon two wing bars of black color involving the secondaries and secondary coverts. These bars are made up, as shown in

figure 47, by a series of black spots on the exposed half of the vane. They do not always occur at the same level and they are not the same width in different birds. This condition excludes these feathers as material for the study of origin of the pigment in the blue because one can not be certain whether the sections are taken from the blue, or from the black bar portion of the feather. The 'wing bow'—that is, the region of the tertiary and lesser wing coverts—shows the so-called blue in its purest condition. It has been pointed out, however, that these feathers are not blue throughout their whole extent but blend into a dun in the proximal unexposed portion. Here, also the granules are rod-shaped, not spherical. These long slender rods always arise as such, while the spheres in the blue portion of the feather arise as spheres. In a study of the manner of origin of these two kinds of granules the stage of development of the feather germ and the level from which the series is cut must be carefully determined. The linear dimension of that part of the formative region where the origin of pigment is taking place is at best very small (about 1 mm.) and entirely different results are obtained, depending on whether the series is taken from a level which is in the distal blue region or in the proximal dun region or in an intermediate position. The stage of developmental advancement of the specimen studied will in turn determine from which of these levels the series must be taken. If the feather germ is old enough for the distal portion, which is blue (about two-thirds of the feather), to be even partially cornified, the series will necessarily be taken from the proximal dun part because the cornified region is too hard to section. No spherical granules will be seen at this level. A pigment cell from such a region is shown in figure 48; from the nuclear membrane to the end of the pigment process the constancy of this rod form of the granules is distinct and striking. In the mature feather this region will show a dun color.

On the other hand, if the feather germ selected is in a considerably earlier stage of development, the series will be taken from a level which is destined to be blue. Figure 49 represents such a region fairly well, but is near the border of the dun region.

Here the pigment is almost entirely in the form of spherical granules of a size characteristic for blue. In a feather slightly older than this, but younger than that mentioned in the preceding paragraph, sections through the growing portion strike an intermediate region where both rods and spheres are present (fig. 50). Moreover, in a series of sections from such an intermediate region those sections farther from the base show fewer and fewer rods (figure 49 is just within this region), while passing in the other direction the proportion of rods becomes greater and greater.

Without very thorough study these conditions might easily give rise to the opinion that in these birds the pigment arises as rods and is probably translocated as such, but as development proceeds the rods are metamorphosed into spheres. Strong ('03, p. 269), studying the spherical granules which he found in certain iridescent neck feathers of the pigeon, concluded that "in the ontogeny of these barbules they receive rod-shaped granules of melanin from typical pigment-cells or chromatophores During the differentiation of the barbule cells however these granules are metamorphosed from the rod-like shape to a spherical form." The possibility of explaining the origin of spherical granules in a red pigeon in this manner is quite out of the question, for at no stage in their development is anything like a rod-shaped pigment granule present in them. The facts presented in regard to the development of pigment in blacks and particularly blues are, in the mind of the writer, ample proof that in other colors of pigeons also pigment granules arise directly in the form which they will permanently hold and that the apparent metamorphosis of rods into spheres is to be explained by the peculiar relation of these forms described above.

Apparently, in the color under discussion there is less free pigment formation in the barbule cells than is the case with black or red feathers. Also, pigment translocation does not begin so early in blue as in black or red. Another condition which characterizes blue is the more straight, direct and orderly manner in which the processes of the pigment cells proceed

outward. Moreover, they are generally confined to the axial region of the ridge, whereas the paths followed by the pigment processes in other colors may extend in any direction and between any cells of the ridge. Clearly some force is at work which has a controlling influence in the transportation of the pigment. As a result of this influence pigment granules enter the barbule only on the inner (future dorsal) side, while in the other colors considered they may find their way into the barbule cells from all directions (figs. 31 and 11). Figure 51 is from a blue feather germ at a stage when the barbules are just beginning to differentiate. Only a part of the hook barbules are shown. The pigment here is scattered somewhat promiscuously throughout the barbules, but the amount of it on the outer (future ventral) side of the nucleus is always small. Figure 52 is a similar region at a later stage; the pigment has withdrawn entirely from the outer (future ventral) side of the barbule cells, but still occupies all of the inner side. In figure 53 we have a similar region at a still later stage. Here the pigment has finally become clumped in the manner characteristic for blue.

This series of figures is typical for all cases of developing blue; though the pigment is never spread throughout the barbule cells, as in the case of black, the extent of spreading in the early stages is considerably greater than in the completed feather; there is in fact a 'clumping process' which goes on in the feather, changing the conditions of distribution from what would produce a black (viewed from the dorsal surface alone, but blue on the ventral surface of the feather) to a completed blue.

Certain preparations from blue feather germs which I have among my slides present a striking variation in development. At the very earliest appearance of the pigment, conditions are the same as those commonly seen, but as pigmentation proceeds the granules assume an extraordinary size, sometimes three or four times that of ordinary blue. Figure 54 is drawn from a young region of a feather germ before ridge formation. It shows two centers of pigmentation in close proximity, in one of which the normal blue granule size is seen, while the un-

usually large granules are present in the other. Figure 55 is a pigment cell forming these granules shown in its position in the ridge. Five feather germs showing these conditions have been studied. The point of interest is that they have in every case been taken from the blue rump of an otherwise black bird. In this connection it should be mentioned that the rump color of a bird is apparently controlled by a factor different from that which produces blue on other regions of the body (Cole '14).

6. *Silver*

a. General. The condition known among pigeon fanciers as silver is a very pale smoky gray or pearl. The amount of pigment present in these feathers is exceedingly small, in fact it is with difficulty that any pigment granules at all can be discovered in some specimens. Microscopic examination of these feathers shows them to possess the distribution of pigment essential to the production of blue, that is, in the silver portions of the feather whatever pigment is present is clumped in the middle of the barbule cells (figs. 57-58). As in blue, the curved barbules generally show this clumping in the middle of the barbule cells to a less degree than do the hook barbules.

b. Granule shape. A study of the granule shape in silvers is made exceedingly unsatisfactory by the extreme roughness of the barbule surface (fig. 61). This roughness has been noticed in other birds by several writers and has been described variously as due to striae, ridges, knobs, pits, blisters or 'ghost granules,' on the barbule surface. They are scattered with great geometric disorder over the surface, and unquestionably make the feather a poorer reflecting surface than it otherwise would be. These irregularities, though apparently most abundant on silvers, are also evident on blues and often even on duns. As mentioned above, they are so marked on silvers that the exact boundaries of the granules are obscured and a knowledge of their size rendered uncertain (fig. 62). A study of the developing feathers, however, escapes this difficulty and furnishes evidence that in silver (fig. 59) the granule shape is similar to that of dun (fig. 35), but in size the granules are somewhat

smaller and their incomplete opacity in the microscopic field speaks for a lesser degree of density.

Silvers like blues commonly have wing bars and in the same position and made up in the same way. They are, however, dun in color instead of black and the conditions microscopically are typical of dun feathers. As concerns the development of silver, the process is the same as seen in blue and nothing further need be added.

7. *Chemical studies*

a. Methods. The obstacles encountered in a chemical study of animal pigments are of various and numerous kinds and the chemical work done on the pigments of the feather in this investigation was of a relatively superficial and general nature.

In general the feathers were treated much after the manner Gortner ('10 b) describes for sheep's wool. The weighed feathers are put into a beaker and a quantity of 2.5 per cent NaOH poured over them (20 cc. liquid for each gram of feathers). The feathers are then digested at boiling temperature till almost completely disintegrated—a period of about 25 minutes for black and 12 minutes for red feathers. The mass is then thrown on a porcelain filter lined with white silk cloth, and filtered by suction. Filtering is very slow and difficult on account of the gelatinous remains of the feather structures, particularly the shafts. The filtrate is now made slightly acid with HCl, which process throws down a very light flocculent precipitate. The precipitate is allowed to settle, is separated and washed several times by decantation, boiled in 95 per cent alcohol for a few minutes, which removes considerable of the keratin products, and is then dried in a weighed dish.

No claim is made for even approximate purity nor for exact identity of the material so isolated with the pigment as it exists in the feather structure. The work, however, has furnished some suggestive data, which the writer hopes will be considered only in that light.

b. Red pigment. When red feathers suspended in a 2.5 per cent NaOH solution are heated, almost at once the liquid becomes

colored. After a few minutes' digestion the solution has taken on the chestnut brown characteristic of the feathers and if the remaining feather mass be freed of all liquid by pressure it is seen to have lost most of its color. Thus the pigment goes easily and completely into solution, and after filtration the solution is a clear, transparent, red-brown. The precipitate thrown down upon acidification contains practically all the pigment, but the supernatant fluid nevertheless retains a brownish tinge. The pigment from yellow feathers behaves in exactly the same way.

c. Black pigment. Black feathers, in the first stages of digestion, impart no color whatever to the NaOH solution. Not until the barbules have completely broken down and the pigment granules themselves have been liberated, does the digestion mixture become black in color. The black granules are extremely resistant and even after long digestion microscopic examination of a drop of the mixture reveals many of the granules intact in their original size and shape. The filtrate, as it was obtained, contains considerable pigment yet in granular form, but also much in a dissolved condition. Acidification throws down practically all the pigment material and the supernatant liquid is practically clear.

The red pigment dries slowly, remaining for some time as a pasty mass which becomes darker as it dries. When completely dried and powdered it is a very dark brown, but distinctly different from black pigment in the same condition. The black pigment dries without difficulty and without apparent change.

d. Quantitative relations. An attempt was made to determine the quantitative relations between red and yellow color, and between black and dun. The figures presented in table 1 do not represent absolute amounts of pigment in the feathers. As the pigment was weighed it contained keratin degradation products and salts left in by incomplete washing. The feather masses were all treated by the same methods, however, and the figures do approximate the truth as regards the relative amounts of pigment existing in the different kinds of feathers.

TABLE 1

Showing the relation of pigment content in dilute and intense birds

COLOR OF FEATHERS	WT. FEATHERS USED; GRAMS	WT. PIGMENT SECURED; GRAMS	PER CENT OF PIGMENT	RATIO OF INTENSE TO DILUTE
Red.....	4.415	0.2	4.530	
	10.270	0.385	3.747	
			av. 4.138	
Yellow.....	4.435	0.055	1.26	3.28:1
Black.....	7.80	0.26	3.333	
Dun.....	7.55	0.09	1.191	
	3.80	0.04	1.053	2.9 : 1
			av. 1.122	

The data in table 1, crude as they are, give us an idea of the magnitude of the influence of the factor for intensity, and furnish a numerical expression for its value. Acting in the skin of either a red or a black bird it appears to increase the amount of pigment laid down by about three times over what it would have been if this factor were lacking. Sufficient material for a study of the quantitative relations of pigment in blue and silver feathers was not available at the time, and these determinations were not made.

Much time and labor were spent in the course of this work in an attempt to demonstrate, in the soft growing regions of the feather tissue where pigmentation is actively going on, the presence of any oxydases or enzymes which might be concerned in the process. Young birds, fifteen to twenty days old, were killed, remeges and rectrices pulled out, the soft unpigmented portion cut off and placed in a mortar. This tissue was triturated with sand and extracts were made with distilled water, normal saline solution, chloroform water, and glycerine. Varying quantities of these extracts were added to tubes containing solutions of different amino acids, chiefly tyrosin, the reaction carefully controlled, H_2O_2 added to certain of the tubes and the tubes incubated.

The presence of tyrosinase, an oxidase which is a true enzyme, in the body wall of various forms, has been repeatedly demonstrated by several workers, particularly Gortner ('10 and '11) for insects; and Durham ('04) has reported the occurrence of tyrosinase in the skins of fetal rabbits.

In the writer's own work, as outlined above, many cases of pigmentation *in vitro* were obtained. The colors produced, however, did not correspond with the colors of the birds from which the extract was taken and their behavior with solvents and reagents was entirely different from that of the pigments prepared from adult feathers. Despite the best efforts evidence of bacterial action was often detected, which action alone might account for the appearance of color; and moreover repeated attempts often failed to reproduce cases of pigmentation once secured. Plainly this phase of the work warrants no conclusions as to the biochemical nature of the process concerned in pigmentation.

Summary of facts

The following points have been brought out in the preceding pages:

1. There is a red-brown pigment substance which produces the colors red and yellow in tumbler pigeons.

2. In red birds this pigment always exists as spherical granules, which are in 'typical red' about $0.3\ \mu$ in diameter, but in 'plum color' they are $2.0\ \mu$ or more in diameter.

3. In reds abundant pigmentation takes place in the intermediate cells of the epidermis, independently of specialized pigment cells.

4. In yellows the pigment is so finely divided that its granule form can not be determined.

5. There is a black pigment substance which under different conditions produces the colors, black, dun, blue and silver.

6. In black birds this pigment may exist as spheres $0.5\ \mu$ in diameter, or as rods $1.0\ \mu$ long. Some blacks show entirely one category of granule form, and some entirely the other. In most blacks, however, both kinds are to be seen, although there is commonly a marked predominance of one or the other type.

7. In blacks the pigment cells are better developed than in reds and elaborate a greater proportion of the pigment.

8. Pigment granules in dun birds are invariably spherical in form and about 0.3μ in diameter.

9. Dun color may be seen in birds which are not dun genetically, but differences in granule form and distribution show that this is not the character dun which behaves as a unit in inheritance.

10. 'Blue' of pigeons is produced by the black pigment clumped and distributed in such a way as to produce the blue effect.

11. Granules in blues are spherical, 0.8μ to 1.0μ in diameter.

12. The development of the pigment in blues is not so promiscuous and 'disorderly' as in blacks. It is apparently controlled by an influence lacking in blacks.

13. After the pigment arrives in the barbules it undergoes a 'clumping' process which gives it its characteristic distribution.

14. The feathers from blue rumps on black birds show granules much larger in size than those in other blue feathers.

15. Pigment in silver feathers is distributed in the manner characteristic for blue.

16. In silvers the barbule surface appears greatly pitted and roughened.

17. The red and the black pigments show pronounced differences in their behavior towards reagents and solvents.

18. The intensity factor has about the same quantitative value in blacks and reds, and results in the formation, in intense birds, of about three times the amount of pigment present in the dilute birds.

DISCUSSION

1. The Mendelian factors concerned

It is interesting now to consider the bearing which the facts presented have on our interpretation of color inheritance in pigeons and on the pertinent questions of genetics generally. Cole ('14) in his breeding work with pigeons, has postulated certain genetic factors which are concerned in the formation of the six self colors (excluding white) of tumbler pigeons recognized

by fanciers. The ultimate recessive of these six colors is that known as yellow. A reddish pigment is seen in birds of this constitution and to account for its existence we may conveniently assume a factor *R* for red pigment. This red factor is apparently a fundamental one and no birds have been found which do not carry it. Strictly speaking, this factor is not entitled to a name until it has been seen to drop out of the hereditary complex and has later been re-introduced in an unchanged condition. But it is desirable to have something to which we may refer when occasion demands, and remembering the possible compound nature of the factor—in fact, that *R* might stand for ‘residuuum’ just as well as for red—utility will be served by using the term.

There is a factor *B* for black pigment, dominant or epistatic to *R*, which added to a yellow produces black pigment and, when not acted on by other modifying factors, results in a silver bird. Another factor—*S* for ‘spreading’—added to a silver bird produces the condition known as dun. Moreover, these three conditions can be modified by the action of an intensifying factor *I*, which changes the above colors into red, blue, and black, respectively. Thus the six self colors and their genetic relationships are accounted for.

2. Influence of the several factors

a. Influence of factor B. When the factor *B* is introduced into a red or a yellow bird changes are brought about in the processes that go on within the feather fundamēt, which result in black pigment being laid down instead of red. The results of Bertrand ('08), von Furth and Schneider ('01) and many others, have shown that oxidases, acting on certain amino acids as chromogens, are able to produce a whole series of color changes. Riddle ('09, p. 329) in discussing the factor hypothesis for color, assumes that “in an animal that produces melanic color there exists all the machinery necessary to produce a series or scale of these colors,” and that the different colors seen in animals represent simply degrees of stages in a single process.

The black pigment in pigeon feathers by virtue of its color and its resistance to stains can be detected and studied at a very early stage in development. Lower down in the feather fundament, in the exceedingly finely divided stage when the pigment first becomes visible under a magnification of 1200 diameters, the fine haze is easily seen to be distinctly black in color. The possibility cannot be denied that at a time when the quantity of pigment was so small and its subdivision so fine as to be altogether invisible, the process of pigment formation might already have passed through a red stage. However, in higher and more developed regions of the feather where the rate of pigmentation is tremendously increased new pigment masses are forming. The process here is so rapid that even in their earliest stages pigment masses are sufficiently dense to be distinctly seen, and there is no indication of a red tinge in these masses at any stage.

Another fact which speaks for the complete developmental independence of these two pigments is their behavior physiologically. As pointed out (page 469) 'free' pigment formation is much more abundant and generalized in reds than in blacks. Oftentimes large, specialized pigment cells are rare in the former, while practically all the pigment comes from these cells in black feathers. This is a difference which might be overlooked in a hasty study, but examination of a large number of series substantiates the conclusion with compelling force. There is little ground for the assumption that merely an activation of the pigment-forming mechanism to a further stage should thus change the place of origin and manner of distribution of the pigment. When the factor *B* is added to the constitution of a yellow bird (*Rbi*) there is also a striking morphological change in the pigment, the irregular, formless masses of yellow being substituted in the resulting duns or silvers for spherical granules $0.3\ \mu$ in diameter.

We can thus study the properties of factor *B* by observing its influence in several distinct and independent ways on the pigmentation processes of the feather. The changes which it produces in the color, the mechanics of formation and distribution,

the physical form, and the chemical properties of the feather pigment all speak for an individuality and a value for factor *B* coördinate with *R*, *I* or *S*.

b. Factors concerned in the production of blue. The nature of the blue color, and the processes concerned in its production have already been discussed (p. 471). Though we are hardly justified in the statement that blues pass through a black stage, yet it is clear that black is a less differentiated condition of pigmentation than is blue, and if translocation of pigment should be arrested in a blue feather at the proper stage the resulting color would be more nearly black than blue (p. 477, fig. 51). This study furnishes strong evidence that some controlling force (we might call it a factor *C*, for 'clumping' is at work from the very beginning of pigmentation, which carries development beyond this non-differentiated point. It guides the pigment branches in their course, assembles the pigment into clumps, regulates the size of granules, and results in a blue feather. The assumption of a positive factor *C* for clumping in blue birds lead us, however, into logical difficulties, for blue is recessive to black, in which clumping does not exist. The pigment in blacks in fact, is spread uniformly throughout the barbule and this property (i.e., the spread condition of the pigment) was chosen by Cole ('14, p. 325) as a vehicle for a symbol representing a positive character present in the dominant blacks, and the factor *S* for 'spreading' is used by him. But although the factor *S* for spreading satisfactorily indicates the conditions in the adult feather, and although it makes good workable formulae and accurately expresses genetic relationships as revealed by experimental breeding, yet it is not compatible with developmental processes as they are unmistakably revealed by a microscopic study. On the assumption of a spreading factor, when a black ♂ (*BIS*) is mated to a blue ♀ (*BIs*) there is introduced into the 'blue' egg an element *S*, the action of which in ontogeny is to disperse the clumps, spread the pigment uniformly throughout the barbule cells, reduce the size of the granules—in fact, to produce a black bird. But plainly, black birds are not the result of such a series of changes. *S*

does not disperse the clumps, for they are never allowed to assemble in its presence; it is not S which is accountable for the spread condition of the pigment in blacks, for this condition exists, in certain stages of development, in birds which lack S . S , then, instead of being a factor which initiates developmental processes in ontogeny is rather a factor which prevents them from taking place; under the influence of this factor the processes which would take place in the primitive blue are stopped at a state short of completion, i.e., at a stage when conditions of pigmentation are such as to cause a black bird. It seems wise to the writer to retain the symbol S in the genetic formulae for pigeons, but with this modification, that we consider it a factor for 'stopping' rather than for 'spreading.' In the absence of the inhibiting influence of S (i.e., s) ontogeny runs its primitive course and produces a clumped condition: a blue bird. With this understanding of the factor S the logical relation between the positive and negative characters are maintained and yet a nice adjustment of hypothesis to fact is secured.

It must be recalled (Cole '14, p. 326) that the existence of factor S can be seen only in birds which carry B , for in birds lacking B (red or yellow) no conditions of coloration have been found to be correlated with the presence or absence of S . S may or may not be present in birds lacking B , but the alternative must be decided by a breeding test. In other words, S appears to act only on B and not upon R .

c. The influence of factor I . The most obvious and without doubt the essential influence of factor I is to stimulate the pigmentation process so that the amount of pigment laid down in intense birds is about three times the amount found in dilute birds. In this respect, I probably has a constant and uniform effect, whether acting on a yellow, silver or dun bird.

But besides producing this quantitative change, the factor for intensity acts in another totally unrelated way by regulating the form of the particles in which the pigment exists. However, I produces by no means a simple and constant change on granule shape when acting on dilute birds of different constitution, i.e., yellow, silver or dun. The irregular ill-defined

pigment masses of yellow feathers are substituted in reds by clean-cut, optically perfect, spherical granules. These two conditions are beautifully differentiated; there is never the faintest tendency for spherical granules of red to appear in a bird lacking the *I* factor, and vice versa. In dun birds *B* alone, without the aid of *I*, insures the existence of the pigment in spherical granules, again $0.3\ \mu$ in diameter. In other words, the secondary function of *I* as judged by its action on yellows has already been fulfilled by *B*. *I* has a further influence in a dun (*RbIS*) bird, however, and the size of the spherical granules is increased from 0.3 to $0.5\ \mu$; also an entirely new form, rod-shaped granules may appear. Of course one could with equal justice argue that these latter changes are produced by *B* alone when acting on a red (*RbI*) bird, and strictly speaking we should say that *I* acting with *R* alone has a different effect than when acting with both *R* and *I*. When *I* is added to silver (*RBis*) the $0.3\ \mu$ spheres are again increased, in this case from 0.8 to $1.0\ \mu$ in diameter; and moreover rods, longer and more slender, appear in certain regions.

TABLE 2

Showing the changes in granule condition brought about by the factor for intensity, I, when added to birds of dilute constitution

DILUTE			
GENETIC FORMULA	COLOR	GRANULE CONDITION	
		Shape	Size
<i>RbIS</i> (or <i>s</i>).....	yellow	undeterminable	undeterminable
<i>RBis</i>	silver	spheres	$0.3\ \mu$
<i>RBiS</i>	dun	spheres	$0.3\ \mu$
INTENSE			
GENETIC FORMULA	COLOR	GRANULE CONDITION	
		Shape	Size
<i>RbIS</i> (or <i>s</i>).....	red	spheres	$0.3\ \mu$
<i>RBI s</i>	blue	spheres and rods	$0.8\ \mu$ - $1.0\ \mu$
<i>RBIS</i>	black	predominatingly spheres or rods	$0.5\ \mu$

Table 2 summarizes the changes in granule condition when *I* is added to birds of dilute constitution.

This method of studying the properties of a factor by observing its effects when acting in combination with other factors, is the best method available to genetists, but it is a superficial one. As an analogy, let us assume that we are to make a study into the chemical properties of a given substance and that the investigation must be prosecuted by the use of methods similar to those above. A series of beakers is obtained containing substances and mixtures of substances which happen to be at hand; the true chemical natures of these substances and mixtures are themselves unknown. The substance under investigation is introduced into the various beakers and the resulting changes described on the basis of color, granular or flocculent nature of the precipitate, the shape and size of crystals, etc. Clearly, with this information alone, we are a long way from understanding the absolute nature of the substance in question, but we have at least amassed a certain amount of definite data, such as it is, concerning it: we have done the best we can under the circumstances. It is largely to such empirical and superficial methods that the modern genetist is limited in his attack on the nature and properties of factors.

3. Granule shape in blacks

Excluding blacks, the wide and inconstant differences in granule form above discussed are associated with the presence or dropping out of what is otherwise a single unit factor, *I* (intensity). The case of the granule form in blacks must be developed further. Unlike other groups, the granule form in these birds bears apparently no simple constant relation to their color, black. To be sure, blacks of whatever sort are characterized by certain features which distinguish them from duns or blues, but within the color there are distinct types, and the distribution of these types is apparently without regard to genetic constitution, as it has yet been worked out, neither does it bear any relation to age or sex. That the granule shape may be here controlled by a separate factor which has this specific effect,

would be the easiest explanation. But it must be assumed further than the factor is powerless to exert any influence unless acting in a bird of black constitution, for as mentioned above, all the other colors are invariably characterized by their own peculiar conditions of granule form. This, of course, makes possible numerous genotypes of other colors, which carry the factor for granule shape as a cryptomere. A research to test this hypothesis is now in progress.

4. Analysis of characters

This study of granule shape amounts, in effect, to a partial and preliminary analysis of certain unit characters of pigeons. We are now able to speak of them in terms of their anatomical and morphological nature, rather than on the basis of general optical effect. We have been wont to describe these color characters by a single term, and have been assuming them to be as simple as our description. However, the facts brought out by this study are highly suggestive that underneath what we can see macroscopically and what we name, a whole series of changes is being enacted; there is an elaborate interaction of factors moulding the size and form of the pigment granules, creating, in effect, new characters as distinctly different from each other as blue color is from black, as seen by the naked eye. It is improbable that these various conditions of granule form are in any way optically concerned in producing the colors with which they are associated. The base of a blue feather contains long slender rods of pigment but is just as good dun as the feathers of a self-colored dun bird; and a lightly pigmented side-of-body contour feather near the axilla, from a black bird, can not be distinguished by color alone from a true dun, though the spherical granules are one-third larger and the pigment may even exist entirely as rods (p. 470). Likewise blacks (p. 468) identical in appearance in every way may contain granules of entirely different categories.

It is natural that geneticists should first have been entirely occupied with the obvious characters. When we limit ourselves to such characters, however, we are working the surface of the

field only; failure to see it with the naked eye is no assurance that a microscopic mechanism is not at work, and it is easily possible that previously baffling ratios may be explained by the revelations of a more minute analysis.

5. Concluding summary

A "Summary of facts" containing a brief statement of the experimental findings has already been given (p. 482). The following summary is concerned with the "Discussion" (pp. 483-491) only.

1. The six fundamental self colors of tumbler pigeons have been accounted for by the interaction of four genetic factors: *R*, red; *B*, black; *I*, intensity; *S*, spreading.

2. Evidence as to the nature of the factor *B* has been secured from its effect on the feather pigment with respect to (a) color, (b) manner of formation and distribution, (c) physical form, and (d) chemical properties. All of these seem to indicate a different mechanism from that which produces red pigment, rather than simply a later stage of the same process.

3. If uninfluenced by other factors, the final result of the pigmentation process in a bird carrying *B*, is the clumping of the pigment into the middle of the barbule cells. The factor *S* when present stops this clumping process and results in a 'spread' condition of the pigment. *S* may properly be considered as an inhibitive or 'stopping factor.'

4. As regards the increment of pigment substance factor *I* probably has a constant effect when acting on dilute birds of different constitutions, namely, to increase by about three times the amount of pigment produced. As regards its influence on granule shape, on the other hand, it reacts in a different manner with each combination of factors.

5. The facts concerning the granule shape in blacks suggest the possible existence of a factor not yet determined which is specifically concerned with granule shape.

6. Genetic research which is confined only to obvious characters is often superficial, and in such cases microscopic research is necessary to distinguish the independently heritable characters involved.

POSTSCRIPT

Certain features in a paper by Spöttel which has just come before the writer, deserve comment. In an analysis of "Felsentauben blau" Spöttel describes the large black spherical granules of the distol, and the smaller or even rod shaped granules of the proximal parts of the feather.

As to the relations between these two kinds of granules however Spöttel's descriptions do not coincide with those of the present writer. Spöttel speaks of a "Verschmelzung" of several spherical granules which thus give rise to long oval, or rod-shaped granules, and pictures (M_2) a series of pigment masses which represent various stages in this fusion. A study of the developing structures furnishes unmistakable evidence that the form of the granules is determined very early in their existence, at a period in fact, long before they have attained anything like their ultimate size. The mere circumstance of what position the granules eventually come to occupy would seem unable to change their fundamental nature. It can not be denied that before cornification, while the granules are yet in a plastic condition, if they are subject to crowding, their original spherical nature will be masked—as is shown by Spöttel (fig. O_2C). It is to be noted that these granules do not fuse together in a homogeneous mass, however, and it is far less likely that linearly arranged spheres would melt together to form true rods. Pictures such as shown in figure M_2c , and d would be produced, of course, if the center granule was displaced from the linear arrangement but remained in the plane of the observer, allowing the end granules slightly to approximate one another.

Spöttel apparently has not restricted himself to feathers from birds of the Wild Rock pigeon color. He speaks (pp. 415-417) of rusty brown, reddish grey, feathers on the breast and the head regions of his birds. In these feathers, especially at the tips, he finds pigment granules varying from chocolate color to golden yellow. Granules of this color always have a spherical form. They become less numerous towards the proximal regions, being replaced by small dark or black granules, frequently rod

like in form. These are essentially the conditions which obtain in the juvenal plumage of black birds. As far as the writer can discern, Spöttel does not specify the breeding of the birds from which he obtained the material, but such conditions as he describes, and pictures in the plate have never been observed in the blues of the rock pigeon type which have been raised in our colonies.

SPÖTTEL, WALTER 1914 Ueber die Farben der Vogelfedern. II. Die Färbung der *Columba livia* nebst Beobachtungen über die mechanischen Blauverhältnisse der Vogel feder. Zool. Jahrb. Abteilung für Ontogenie der Thiere, Bd. 38, Heft 3, pp. 357-426. 70 Text Fig. Taf. 22.

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ABBREVIATIONS

<i>a.</i> , apex of barb	<i>lt.pl.</i> , lateral plate
<i>b.</i> , barb	<i>med.cl.</i> , medullary cells
<i>b.m.</i> , basal membrane	<i>n.</i> , nuclear region of barbule cell
<i>c.bbbl.</i> , curved barbule	<i>p.</i> , pulp region (dermis)
<i>c.b.</i> , cell boundary	<i>pg.</i> , pigment mass
<i>cy.cl.</i> , cylinder cell layer	<i>pg.cl.</i> , pigment cell
<i>d.</i> , down	<i>pt.c.</i> , point at which barbule curves
<i>e.</i> , epidermal region	<i>rec.m.</i> , recurved margin
<i>h.</i> , hooks or haemules	<i>sh.</i> , feather sheath
<i>h.bbbl.</i> , hook barbule	<i>st.</i> , shaft
<i>int.cl.</i> , intermediate cells	<i>t.f.</i> , terminal fiber
<i>in.s.cl.</i> , inner sheath cells	<i>v.</i> , vane
<i>lt.sh.</i> , lateral sheet	<i>v.r.</i> , ventral ridge of barb

PLATE 1

EXPLANATION OF FIGURES

1 Cross-section of red feather showing 2 barbs with barbules; shows well how haemules interlock with clefts in curved barbules; pigmentation general in all parts; (slightly diagrammatic). $\times 100$.

2 Cross-section of a curved barbule, showing grooved nature of barbule, recurved margin and cleft; pigmented throughout with spherical granules. $\times 1100$.

3 Hook barbule from red bird (1144 *C*); pigmented more heavily than curved barbule from same bird. Note the narrow pigment-free areas at cell boundaries. $\times 280$.

4 Curved barbules from 1144 *C*; more lightly pigmented; compare with figure 3. $\times 280$.

5 Cross-section from barb from weakly pigmented red bird (93 *B*); ventral ridge is partly broken off. $\times 480$.

6 Portion of curved barbule from light red bird (894 *B*) showing thinly scattered spherical granules. $\times 1600$.

7 Portion of pigment mass from dark red feathers (5 *B*); granules same size and arrangement as in light red, but more closely massed. $\times 1600$.

8 Cross-section of red feather fundament before ridge formation, showing first traces of forming pigment scattered promiscuously throughout intermediate cell region. $\times 480$.

9 Diagram showing from what part of the feather (marked *x*) figure 8 was drawn.

10 Drawing from down region of a feather fundament just after ridge formation and before arrangement of lateral plates, showing generalized pigmentation (intermediate cell region). Parts of three ridges are shown. $\times 480$.

PLATE 2

EXPLANATION OF FIGURES

11 to 15 These figures are from a single feather in similar regions but at successive levels and show the several steps in feather differentiation.

11 Ridges are formed and the lateral plates well defined, two pigment cells are present. $\times 430$.

12 The cells of the lateral plates have elongated and are developing into barbules. Specialized pigment cells are not active. $\times 430$.

13 Barbules well developed; barb becoming defined. The pigmentation process is not concerned with pigment cells. $\times 430$.

14 Barb and barbules well defined. $\times 430$.

15 Withdrawal of barb, showing some 'residual' pigment remaining in the epidermal tissue not built into the feather. The cornified portion is here breaking away from the central supporting structure. $\times 430$.

16 Single ridge from cross-section of a feather fundament, showing copious pigmentation of feathered parts without the aid of a pigment cell. $\times 430$.

PLATE 3

EXPLANATION OF FIGURES

17 Cross-section of barb and barbules from yellow feather (76 *B*). $\times 480$

18 Portion of curved barbule from a yellow feather (19 *B*) showing the nature of feather masses, the lack of segmentation and sparse pigmentation. $\times 480$.

19 Portion of hooked barbule from a yellow feather (19 *B*), showing heavier pigmentation but also lack of segmentation. The hooks (haemules) in yellows are pigment-free. $\times 480$.

20 Feather ridge from cornified portion of fundament from yellow bird (2 *A*) showing the nature of pigment masses, also the cellular nature of the barb cortex just before complete cornification. The high attitude of the barb shown here is characteristic of flight feathers. $\times 480$.

21 Feather ridge from yellow feather fundament. A small and comparatively inactive pigment cell characteristic for yellow is shown here. Lateral plates are well formed, but the barbs are without definition. $\times 280$.

22 Cross-section of curved barbule from plum-colored feather (894 *B*) showing the inordinately large spherical granules typical of this color. $\times 1100$.

23 Portion of barbule from plum-colored feather laid flat, showing arrangement of large spherical granules. $\times 1600$.

24 Cross-section of barb or plum-colored feather (894 *B*), showing many granules within the medullary cells. $\times 280$.

PLATE 4

EXPLANATION OF FIGURES

- 25 Portion of barbule from black bird with rod-shaped granules. $\times 430$.
- 26 Area in same barbule under higher magnification (fig. 25) showing the well defined rod granules. $\times 1600$.
- 27 Portion of barbule from black bird (1037 *B*) with the 'pebbled' effect due to spheres. $\times 430$.
- 28 Area in same barbule, highly magnified (fig. 27) showing spherical granules. $\times 1600$.
- 29 A region from feather fundament before ridge formation, showing the first appearance of black pigment. $\times 430$.
- 30 Area marked *x* in figure 29 under higher magnification, showing appearance of pigment in 'free pigmentation.' $\times 1600$.
- 31 Ridge from black feather fundament from bird 1147 *E* (poorly fixed) showing copious pigmentation and promiscuous direction of branches of pigment cells. $\times 430$.
- 32 Young pigment cell from black bird forming predominantly spheres. $\times 1600$.
- 33 Pigment cell adjoining figure 32 in same ridge but forming predominantly rods. $\times 1600$.
- 34 Portion of barbule from dun feather (959 *B*) showing sparse pigmentation and very little segmentation. $\times 430$.
- 35 Area in same barbule (fig. 34) under higher magnification showing entirely spherical granules. $\times 1600$.
- 36 Ridge from dun feather fundament, showing well developed pigment cells. Note the path of pigment branch on outside of developing row of barbules. $\times 430$.

PLATE 5

EXPLANATION OF FIGURES

37 Cross-section of a blue barb and barbules (bird 1028 *A*) showing characteristic distribution of pigment. $\times 480$.

38 Cross-sections of a curved (*A*) and a hooked (*B*) barbule, showing characteristic pigment granules and manner of clumping. $\times 1100$.

39 Apex of barb from blue feather (1028 *A*). This represents all the pigment in the barb. $\times 1100$.

40 Portion of barbule from blue feather (265 *A*) showing the characteristic clumping of the pigment. $\times 430$.

41 and 42 Portion of pigment masses from barbule shown in figure 40, more highly magnified. $\times 1600$.

43 Portion of barbule from the base of a blue feather (1063 *B*). The base is dun in color. Rod-shaped granules are present. The pigment differs in this respect from that in dun birds. $\times 430$.

44 Cross-section of barb and barbules from dun base of blue feather (63 *B*). The portion of the feather represented here and in figure 43 appears dun only when viewed on dorsal or outer aspect, due to the spreading of the clumps in that direction only. Viewed on the inner surface they would be blue. $\times 480$.

45 Section of barb and barbules from flight feather of black bird. The portion of the barb below the barbules is pigment-free, giving to the feather viewed from this side a bluish cast. $\times 160$.

37

42

39

c.bb.le.

h.bb.le.

38A

38B

41

40

43

44

45

PLATE 6

EXPLANATION OF FIGURES

46 Lesser wing covert from blue bird. Only the distal third of the feather is blue, which fades to dun in the proximal portion.

47 Secondary covert from blue bird. Black spot is on exposed portion of feather. A row of such spots make up the black bar, a feature of the blue pattern. Shows the downy tissue found at base of such feathers.

48 Portion of ridge of feather germ from blue bird (1028 A) cut through the dun region. No spherical granules are present (fig. 44). $\times 430$.

49 Ridge from blue feather in region intermediate between blue and dun, showing lack of specificity of the tissue—all shapes and sizes of granules are present simultaneously in one ridge. $\times 800$.

50 Two contiguous pigment cells in a blue feather fundament, from region near figure 49. $\times 640$.

51 to 53 Sections from blue feather fundaments showing progressive stages in the clumping of the pigment.

51 The barbule cells have begun to elongate; the pigment is spread promiscuously throughout the cells. $\times 1100$.

52 A later stage. The pigment has withdrawn entirely from one edge of the barbule and has begun to withdraw from the outer edge. $\times 1100$.

53 Still later stage. The clumping process is well advanced and the pigment is massed in the center of the barbule cells. $\times 1100$.

54 Adjacent pigment forming areas in a blue rump feather (1113 F'), intermediate cell region, before ridge formation. Regulation of granule size not absolute. $\times 1100$.

55 Showing large size granules in blue rump (bird 1113 F). $\times 1100$.

PLATE 7

EXPLANATION OF FIGURES

- 56 Blue rump pigment cell (1113 *F*). $\times 1000$.
- 57 A ridge from a silver feather fundament (1028 *B*), showing the general distribution of pigment in the silver feather. $\times 430$.
- 58 A hook barbule from the section shown in figure 57, enlarged. The pigment is clumped as in blue. $\times 970$.
- 59 A pigment cell from a silver feather fundament. The granule shape is the same as in dun. $\times 1000$.
- 60 Developing ridge from a silver (1175 *A*). $\times 430$.
- 61 Portion of barbule from silver feather (1077 *B*). Pigment masses are very faint but clumped. Barbule surface much roughened and pitted. $\times 430$.
- 62 A pigment mass and adjacent barbule tissue from above barbule, under higher magnification. $\times 1600$.