

ON CERTAIN FACTORS CONCERNED IN THE PRODUCTION OF EYE COLOUR IN BIRDS.

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INTRODUCTION.

THE work of Hurst, Nettleship, and others in this country, and Davenport in America, has been concerned chiefly with eye colour in mammals, more especially in the human subject. The facts which Hurst established as to the genetic importance of the presence or absence of pigment on the anterior surface of the iris in man, seem to be applicable in the main also to the avian iris. I am unable however to find that much work has been done on eye colour in birds from the genetic standpoint, and the observations here recorded must be regarded as an attempt to carry the analysis of this problem a stage further.

I showed in 1912 (see *Nature*, Sept. 19, 1912) that in birds, not only the ciliary muscle, but the dilator and constrictor muscles of the iris are composed of fibres of the striated or voluntary kind, and that the movements of the pupil in birds are apparently subject to voluntary control. This we should expect if the iris muscles in birds are innervated by medullated nerve fibres from the cerebro-spinal system. This difference in the histological structure of the intrinsic muscles of the eye-ball in birds and other vertebrates (with the possible exception of the Reptilia) has an important bearing on the evolutionary methods by which the pigmentation of the Iris has been brought about in these different orders.

HISTOLOGICAL.

The "Bull" Eye in Birds.

By this term I mean the eye which owes its black or dark colour to the absence of pigment on the anterior surface of the iris. The delicacy and translucency of the iris tissues allow the posterior uveal pigment to shine through, and this gives rise to an appearance of blackness. The Bull eye in birds thus comes under Hurst's definition of the simplex

eye. In the Bull eye the chick character is in fact retained during adult life in the bird just as, in the blue eye in the human subject, the simplex character common to most infants (of white as opposed to negro descent) persists into adult life.

The eye of the white fantail pigeon provides a good example of the "Bull" or simplex avian eye. There is an entire absence of black, brown, or yellow anterior pigment while the connective tissue cells and the striated muscle fibres are sufficiently delicate in structure to allow the posterior hexagonal brown or black pigment to show through, and thus produce the black effect.

Careful examination of the "Bull" eye in the living bird with a lens in a good light will show a red appearance, like a ruddy glow, covering the peripheral zone of the iris. This appearance is due to a rich plexus of capillary blood vessels with thin walls in this situation, and is not due to the presence of any pigment cells.

It is important to remember that the majority of black or very dark brown irides in birds owe their dark colour to the presence of anterior iris pigment, and are not "Bull" eyes at all. Even among pigeons the "Bull" or simplex eye occurs chiefly in wholly white birds, which are genetically speaking recessive whites, though not of course albinos. Bateson and others have shown that in fowls white feather colour is of two kinds, Dominant white as in the White Leghorn, and Recessive white as in the white Rose-comb Bantam, the Silky fowl, and an extracted white obtained by crossing certain white with coloured strains. It is interesting to note that while (as we should expect) the colour of the iris in the Dominant White Leghorn is red or yellow (gravel eye) the iris colour in the Recessive white Rose-comb Bantam is not "Bull" but red or yellow.

In the Silky fowl also the black eye is not a "Bull" eye but owes its dark colour to black anterior iris pigment. There are however special points about the black eye of the Silky fowl to which I shall draw attention later.

In the Stock Dove (*Columba oenas*) a superficial observation of the eye of the adult bird would record the eye as "Bull" or simplex, i.e. deficient in anterior pigment. Microscopic examination of sections of the iris shows however that the black colour is due to the presence of numbers of branching cells packed with dark brown or black pigment granules. These cells are not only present on the surface of the iris but also among the muscle-fibres and deeper tissues in which there are also cells containing yellow pigment. Thus the black eye of the Stock

Dove is an anteriorly pigmented eye and differs from the normal type of eye colour in pigeons which is white, yellow, orange or red, producing the so-called pearl, gravel or ruby eye.

In the Rock Pigeon (*Columba livia*), which is regarded as the ancestral form, the iris colour is yellow or orange.

The Pearl Eye.

Next in grade of pigment to the "Bull" eye comes the so-called pearl eye in pigeons, and the "Daw" eye as seen in the Malay Fowl. Anterior iris pigment is absent in the pearl eye, but the iris tissues are not translucent as in the "Bull" eye. They are crowded with granules which are themselves colourless but prevent the passage of transmitted light, and when seen by reflected light give a grey white appearance to the surface of the iris.

Microscopic examination of the pearl iris shows that this opacity to transmitted light is due to the presence of these colourless, but more or less opaque, granules, which appear as clear refractile spherules of moderate size. When these are densely aggregated the interference with the passage of light may be sufficient to produce an opaque or black effect. The cells which contain these granules are scattered around the capillaries and amongst the muscle-fibres, and they do not seem to have such definite outlines as the cells which contain the ordinary yellow or brown pigment. It is indeed an open question whether these refractile bodies should be classified as pigment granules, since under dark ground illumination they appear to be of a dull white colour.

Among Fowls the eye which most nearly corresponds to the pearl eye of pigeons is the so-called "Daw" eye of the Malay Fowl.

In the "Daw" eye the iris is free from anterior yellow or brown pigment, but the connective tissue cells and the muscle fibres are loaded with granules which produce a tissue opacity and prevent the uveal pigment from shining through the iris, thus giving a grey appearance to the iris on reflected light. The pearl eye, and perhaps the daw eye, may in fact be regarded as representing the first or lowest grade of iris pigmentation, a condition of cell-opacity without cell-pigment, intermediate between the bull eye with its translucent tissue-cells and absence of pigment-granules on the one hand, and the fully pigmented eye with its connective tissue cells loaded with yellow, brown or black pigment granules on the other.

The pearl eye in pigeons seems to correspond in pigmentary gradation with the china-white or "Wall" eye in dogs, horses and pigs. We

must not lose sight of the fact however that the substitution of striated muscle-fibres in the avian iris for the plain muscle cells of the mammalian iris introduces a different histological factor in these different orders. In the mammalia there is every grade of blue eye from the deep violet or almost black shade of the newly born child or kitten to the steel blue eye of the human adult, or the bluish white iris of the blue Dutch rabbit, or the china white iris of the "wall" eyed pig.

The paleness of the blue colour or greyness of the iris depends on the granular condition or opacity of the tissue cells and the degree to which the posterior pigment is prevented from shining through. Thus, an iris composed of translucent cells gives a bull or black eye, while an iris of which the cells are slightly opaque is blue, and an iris composed of wholly opaque granular cells is china white in colour. All these irides are free from anterior pigment. The different reaction to incident light is due to structural and not pigmentary differences, and to the presence in the cells of these colourless granules.

Genetically the pearl eye in pigeons acts as a recessive to the red or gravel eye. The daw eye in fowls is also a recessive to the amber eye, and to the black eye when the blackness is due to the presence of anterior pigment.

The Yellow or "Gravel" Eye.

In pigeons the yellow eye is due to a net-work of branching cells crowded with small spherical yellow granules. These cells lie on the anterior surface of the iris: they cover the capillary blood vessels and surround the striated muscle fibres which in the pigeon are themselves free from pigment. The difference between the grey-white or pearl eye and the yellow or gravel eye in pigeons is essentially a structural difference. In the pearl eye the granules which fill these connective tissue cells are chalky white to reflected and opaque to transmitted light, whereas in the yellow eye the granules appear yellow both when viewed by reflected and transmitted light. If however the cells which contain them are closely aggregated and prevent the passage of light, then the granules appear black.

Thus the yellow eye represents the first grade in iris pigmentation. In fowls on the other hand the gravel or yellow eye presents a different problem. In some breeds the orange colour is due to the presence of large numbers of branching connective tissue cells containing yellow or yellow-brown pigment granules, which lie around the capillaries and muscle-fibres. In other breeds, of which the Dorking and

Orpington are examples, the muscle-fibre cells themselves also contain the yellow pigment granules. Bearing on this point, namely the presence of pigment granules in striated muscle cells, it is interesting to note that in some orange eyed birds the striated cells of the ciliary muscle also contain yellow pigment, and this gives in some cases a distinct yellow colour to the muscle which is quite recognisable to the naked eye. When we recall the fact that the ciliary muscle has the same embryological origin, and remains throughout development in close structural and functional continuity with the muscles of the iris which act on the pupil, it is a matter of interest to find that both share in the pigmentary changes concerned in the production of eye colour in some birds. It is clear that the occurrence of pigment in the voluntary muscle fibres entirely under cover of the sclerotic and unexposed to the action of light cannot have any influence in sexual selection. It must be regarded like many other pigmentary phenomena as due to an overflow of normal metabolic activity into cells which under ordinary conditions are free from such changes. The deposition of pigment granules in the iris muscle and in the ciliary muscle is chiefly found in domesticated birds. I have not, so far, come across pigmentation of the ciliary muscle in any wild bird. Such pigmentation is in fact a metabolic abnormality since it occurs in cells which in wild birds are normally free from pigmentary deposition. The effect of the deposition of a mass of pigment granules on the functional activity of a striated muscle cell is also a point of interest. It would be interesting to know whether the change affects only the older cells, it does not at any rate affect all the muscle cells even in a heavily pigmented iris. Bearing in mind that the yellow iris of the domesticated fowl is produced by the interaction of several factors: (1) the presence of yellow pigment granules in connective tissue cells, and (2) the deposition of yellow pigment granules in striated muscle cells; it becomes a point of interest to ascertain whether the yellow iris in wild birds owes its colour to the same or different factors. The owls afford perhaps the best examples of the purely yellow iris among wild birds.

In the Eagle Owl (*Bubo bubo*) the peculiar velvety bright yellow appearance of the iris is produced by a heavy coating of the front face of the iris with numbers of roundish or oval cells crowded with bright yellow spherical pigment granules. These cells are present also in Scops Owl (*Otus scops*). Probably in most, if not all of the yellow eyed owls, the yellow colour is due to the presence of these cells. The cells themselves are fairly constant in size and outline. They are free from

branching processes and gradually lose their colour when submitted to the action of 5% formalin solution.

In the Brown Owl (*Strix alues*) and the Grey Eagle Owl, and some other brown eyed species, the brown colour of the iris is due to the presence of branched pigment cells containing brown granules. The same occurs in the Eagles (*Aquilinae*), the Kites (*Milvus*) and some other birds of prey. The Egyptian Kite (*Milvus egyptius*) and Bateleur's Eagle (*Helotarsus ecaudatus*) both have dark brown irides, and in both the pigment is contained in branch cells. In no case at present has any deposition of yellow or brown pigment granules been observed in the striated muscle-fibre cells in the iris of any wild bird.

The Brown and Black Eyes.

Amongst Birds, as amongst Mammals, quite a large number of species possess anterior iris pigment which passes through all grades of brown up to black. The black iris is associated (with some exceptions, notably the Silky Fowl) with black feather colour, and in its deeper grades with black leg colour. Histologically the brown (in its darker shades) and the black iris are produced by a well-defined layer of characteristic branching cells, which contain dark brown or black pigment, on the anterior surface of the iris. These cells intercommunicate by their branches and form a plexus of pigmented cells thickest over the capillaries in the peripheral or middle zones of the iris.

The cells are much alike in different species. The body of the cell and its processes are usually crowded with pigment granules of a rounded outline and fairly uniform size. Under favourable conditions a central nucleus can be made out more or less free from pigment. These pigment cells permeate the thickness of the iris wall for some depth and are often found encircling the striated muscle cells. They are structurally continuous with the branching pigment cells in the sclerotic and the outer surface of the choroid behind the cornea, and are quite distinct from the hexagonal cells on the posterior surface of the iris. As has been already stated, they are especially numerous and heavily pigmented in birds of dark or black plumage. They are found in nestlings belonging to the Corvidae and allied species. They are also present in the newly hatched chick in the black breeds of fowls.

Genetically the black eye with its deeply pigmented branching cells is dominant over the yellow iris and other grades of iris pigmentation thus :

The daw eyed Malay hen crossed with the Black Orpington cock

gives F_1 with black plumage (with some red in the males), and all have more or less black anterior iris pigment due to the presence of these characteristic branching cells. As the F_1 chicks develop however interesting changes take place. Among the limited number of birds reared up to the present the pullets retain the black eye in adult life, while in the cockerels the iris gradually assumes a yellow colour and at the age of 9 months or earlier, if the birds are sexually mature, the black becomes a gravel or orange eye. Thus the black iris of the hen and the orange of the cock in this cross are sex limited characters as in the case of other gallinaceous birds. In the Golden Pheasant (*Chrysolophus pictus*) the iris is brown or brownish black in the female and bright yellow in the fully developed male, and to a much less marked degree the same is true of the Mongolian Pheasant (*Phasianus mongolicus*). An interesting problem arises as to the way in which this change in iris colour is brought about in the adult male. The transition is one from an epistatic to a hypostatic character, that is from a higher to a lower grade of pigmentation. The chicks of both sexes of this Malay cross have brown black irides due to the presence of these anterior pigment-containing cells and they retain this character while sexually immature. The change to the yellow colour in the developing cockerels occurs in patches on the surface of the iris and seems to be due to the removal of the cells containing brown or black pigment and of the substitution in their place of cells containing yellow pigment granules together with (in the case of the half-bred Malay fowl) the deposition of yellow pigment granules in the striated muscle cells of the iris. The question arises as to the disappearance of these brown pigment cells. Is it the result of atrophy and absorption or of migration to deeper parts of the iris? There are reasons for thinking that both factors are concerned in the process.

As the iris assumes the yellow colour the cells which contain the black or brown pigment coincidentally lose their dendritic processes and tend to become rounder in outline. In the Herring Gull (*Larus argentatus*) the yellow iris is not fully developed in the adult male until the fourth year. A careful comparison of sections of the iris in the nestling, the young male, the young female, and adults of the two sexes of the Herring Gull shows that the black colour of the nestling's eye is due to the presence of a plexus of branched cells containing brown or black pigment on the anterior surface of the iris. The female retains more or less of this dull brown colour during adult life, but in the adult male a layer of cells containing yellow pigment replaces the layer of black

pigment cells on the front of the iris, and these latter cells are found at a deeper level just above or anterior to the uveal pigment cells. The appearances in the Herring Gull suggest a downward migration of the black branched cells and a surface migration of the yellow cells. This view that the colour change is the result of cell migration receives some confirmation in the case of young Rooks and other members of the Corvidae. The anterior surface of the iris in the nestling Rook (*Corvus frugilegus*) is dotted with a number of branching cells loaded with intensely black pigment granules. These cells are most numerous at the scleral margin of the iris and are directly continuous with a plexus of similar cells which lines the sclerotic on its choroidal surface. As the nestling grows these cells become more numerous and spread over the anterior surface of the iris, as if migrating towards the pupil from the scleral margin.

The "Black" Eye in Fowls.

If the Black Orpington (black eye) male be crossed with the Buff Orpington (gravel eye) female the F_1 chickens all show some black anterior iris pigment on a yellowish background. In the pullets the brownish black colour persists in adult life while in the cockerels the black gives place to the yellow or gravel eye. Although in this cross the yellow eye seems to be a sex limited character the sharpness of the limitation is not absolute, for a few of the cockerels show in adult life a surface layer of brownish pigment on a yellow background. In the Malay (daw eye) female crossed with the Black Orpington (black eye) male the cockerels also develop a yellow eye when sexually mature while that of the pullets remains black or dark brown, and in this cross the character seems strictly sex limited. In both crosses the deeper yellow pigmentation is due to two factors as in the yellow eye in other breeds of fowls. These factors are (a) the presence of connective tissue cells containing yellow pigment granules and (b) the deposition of yellow pigment granules in the striated muscle fibres of the iris.

The "Triplex" Eye or the Eye containing two kinds of anterior Iris pigment.

Mention has already been made of Hurst's classification of the Duplex eye as one in which anterior iris pigment of some kind either yellow, brown, or black is present. In connection with this point I wish to draw attention to a group of eyes in Fowls of which the Silky, the Croad Langshan, the Houdan and some other breeds provide examples, which might be called Triplex eyes. There are, however, some objections to

Hurst's classification of eyes as Simplex and Duplex, and it might be better to divide eyes from the colour standpoint into (a) non-anteriorly pigmented and (b) anteriorly pigmented eyes, while (b) would be further divided into singly pigmented (Hurst's duplex) and doubly pigmented or (triplex) eyes. If the eye of the Silky Fowl be examined in the fresh condition, or preferably after a few days preliminary hardening in formalin, and if the anterior surface of the iris be exposed by careful peripheral detachment from the scleral margin under water and if further the anterior layer of black pigment cells be carefully scraped away with the point of a scapel from the front of the exposed iris, an underlying layer of reddish yellow pigment comes into view. This yellow layer can in its turn be scraped away, leaving exposed the unpigmented stroma cells and the muscular fibre cells of the deeper portions of the iris, and if these be removed the posterior uveal pigment is reached. The histology of this double layer of pigment cells of different colours in the Triplex eye is of interest.

The anterior black layer is made up of a dense network of ramifying cells crowded with dark brown or black spherical granules, uniform in size, such as we have already described as occurring in the black eyes of many species of wild birds. These cells are especially numerous around the capillaries on the anterior surface of the iris. The yellow or red-yellow layer lies beneath this and, as in the yellow eye of other breeds of fowls, is made up of two factors: (a) connective tissue cells containing large numbers of yellow pigment granules (these cells are less branched than the black cells and are situated around the capillaries in striated muscle fibres); (b) a copious deposit of yellow pigment granules in the striated muscle cells. There is a marked discontinuity and absence of grading between the yellow and black pigment layers. An object lesson is thus provided in the adult individual zygote of the definite histological deposition of one unit character, viz. black pigment over a lower grade unit character, viz. yellow pigment, and a realization in the individual of the factorial composition of the gametes into epistatic and hypostatic precursory factors.

The Genetics of the "Triplex" or doubly anteriorly pigmented eye.

Experiments have been carried out to test the behaviour of these two unit characters, black and yellow pigment, in the same eye by cross breeding. Five black-eyed Silky hens crossed with a gravel-eyed Old English Game Bantam cock gave in the F_1 generation gravel-eyed cocks and mostly black over yellow-eyed hens. In the F_2 generation some

segregation occurred with a regrouping of the black and yellow characters, with the result that both were found associated with white and game colour and with fluffy and close-feather pattern. The sex limitation also persists in the F_2 generation, the black over yellow or triplex pattern being associated with the female and the yellow or duplex pattern with the male sex. In this case a secondary male sex character, yellow eye colour, is brought about by the loss of an epistatic character, black pigment, uncovering as it were a hypostatic character, yellow pigment. The ultimate causes of the shedding of this epistatic character must probably be sought for in the metabolic processes associated with the development of sex. The metabolism which develops yellow pigment granules instead of black in connective tissue cells, and also brings about the deposition of yellow pigment granules in striated muscle cells, is no doubt associated with the functional activity of certain endocrinous glands among which we must include the primary sex glands.

The Triplex Eye or Eye with double anterior pigment in Wild Birds.

The black over yellow or triplex arrangement of eye colour in the Silky Fowl is a normal example of epistatic character, black, overlying a hypostatic character, yellow pigment. Attention has already been directed to the fact that in the adult male Herring Gull the opposite condition is found. In this case a layer of bright yellow cells covers the anterior surface of the iris and overlies a layer of black branched pigment cells. Thus yellow becomes epistatic to black in the adult male bird. As we have already seen, this result is brought about partly by the removal, by atrophy, of the black cells, and partly by the migration of these cells to a deeper layer of the iris, thus uncovering the yellow pigment cells. This arrangement also constitutes a sex limited character in other species. In the adult female Herring Gull the brown colour which is common to nestling birds of both sexes persists through life, while in the male when fully mature the iris assumes a yellow colour.

These changes in eye-colour dependent on sex and age acquire additional interest from the fact that we have here a demonstration of an actual process going on in the tissues of the zygote during ontogeny, of which the hidden counter-part has previously taken place in the factorial constitution of the germ cell at an earlier stage.

The "Ruby" Eye in Birds.

We may first deal with the Ruby eye as it occurs in pigeons and doves, because the factors concerned in the production of this iris colour are not the same in all species.

In the common Ring Dove (*Turtur communis*) the ruby colour is due to the presence of a surface layer of branching cells with yellow pigment granules, which surround the capillaries on the anterior surface of the iris.

The rich ruby eye of the Victoria Crowned Pigeon (*Goura victoriae*) is due to the same cause. In this species also, as in the Doves, the red colour is common to both sexes. In the nestling Victoria Crowned Pigeon the colour of the iris is black. At the age of three months the iris assumes a bright yellow colour, but I have not been able to ascertain the age at which the yellow gives place to the ruby colour.

In the Dragoon Pigeon and some other fancy breeds all grades of the ruby eye occur, from bright red through dull red to orange and yellow. In some birds the ruby or "red currant" colour is limited to the outer zone of the iris where blood vessels abound, while the inner zone surrounding the pupil, where there are fewer capillaries, retains the orange or yellow colour.

The Ruby eye in Doves and Pigeons loses much of its brilliant red colour after death, and, as we shall see later when dealing with the chemical aspect of the problem, the red and eventually the yellow colour are both lost when the eye is placed in a reducing agent like formalin.

The ruby colour is thus a superimposition effect due to the covering of red blood vessels with yellow pigment cells. The exact way in which the optical effect is produced is a subject for further study.

The Ruby eye in the Cayenne Lapwing (*Vanellus cayennensis*) presents a different problem. Here the red colour of the iris is due to the actual presence of rounded, oval, or slightly branched cells packed with fine granules of a reddish mauve coloured pigment. These cells are situated on and among the muscle cells and the capillaries of the iris. This is the only example at present found of a colour effect in the iris due to the presence of pigment granules which could not be included in the yellow, brown or black groups. Not only are the granules which produce the red colour in the Cayenne Lapwing's iris histologically different from the yellow pigment granules which form the surface layer in the Dove's and Pigeon's iris, but they also differ in chemical composition. In the Lapwing the ruby colour persists after death and the pigment granules retain their red colour even after a long immersion in 5% formalin.

The "Parti or Zone" coloured Iris.

In some of the Birds of Paradise, notably in Lawes' Bird of Paradise (*Parotia lawesi*), and possibly in other species, striking colour effects are

produced by a differential colouring of the iris. In Lawes' Bird an inner ring of deep iridescent blue is surrounded by an outer ring of yellow pigment. This colour effect is brought about in two ways. If a vertical section of the iris taken at right angles to the surface be examined under a low power the iris in the outer or yellow zone will be found to be four or five times thicker than in the inner or blue zone. This increased depth of tissue in the peripheral zone, containing as it does the muscular fibres and capillaries, partly accounts for the opacity of the structures and prevents the posterior pigment from shining through, hence the absence of a blue effect. In addition the anterior surface of the iris in this zone is covered with a layer of yellow pigment cells.

The inner or blue zone on the other hand is a mere thin membrane, sharply defined from the outer zone by a step down as it were in thickness on the anterior surface of the iris. The thinness and translucency of the tissues in this inner zone readily allow the black uveal pigment to shine through. The greater part of the blue effect is no doubt due to the presence of this underlying black pigment seen through translucent tissues. This however is not the whole explanation. A large portion of this thin inner zone consists of very finely fibrillated spindle cells of connective tissue type. The ends of these cells break up in hardened specimens into fibrillae which separate into wisp-like fringes. These elongated cells lie over the muscle fibre cells, and if the anterior surface of the iris in this situation be examined under a low power the parallel fibrillae bring about a diffraction grating effect. This accounts for some of the blue reflection of incident light. This can be shown by varying the angle at which the light is allowed to strike the surface of the iris. At certain angles the blue effect is produced quite independently of the posterior black pigment and can indeed be obtained after this has been removed. Thus, in the case of Lawes' Bird of Paradise a brilliant colour effect is produced by a combination of three factors: (1) Thinness and translucency of iris tissue allowing the uveal pigment to shine through. (2) The absence of anterior yellow pigment cells in the inner zone. (3) A peculiar physical conformation of the connective tissue cells in this area. A parallel fibrillation of cells acts as a diffusion grating and causes light to be reflected from the anterior surface of the iris at a certain angle as blue in colour. It would be interesting to extend this enquiry to other species of Birds of Paradise and to individual birds of both sexes at different ages.

CHEMICAL.

I regret that I am unable to bring forward any detailed account of the difference between the black, brown, yellow, ruby, and pearl pigment granules from the chemical point of view. There is one definite and constant character which serves to distinguish the yellow from the black granules and that is their different behaviour to reducing agents like formalin. The yellow pigment granules lose their colour after immersion in 5% formalin for two or three weeks. A coalescence of the small granules into larger globules of yellow lipoid-like material takes place, and these larger globules may eventually merge into larger masses. In some specimens needle crystals have formed inside these yellow oil-like globules (see Herring Gull). The black pigment granules which fill the branching cells that are so characteristic of the anterior layer in the black iris of many birds are, on the other hand, highly resistant to the action of formalin; for 5% and even stronger solutions have little or no effect in dissipating the colour. The granules do not tend to coalesce into larger globules nor do they show any signs of crystalloid change. The brown pigment granules are intermediate in their chemical reactions between the yellow and the black, and are partially affected by formalin.