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THE DISTRIBUTION OF MELANIN IN THE DEVELOPING OPTIC CUP AND STALK AND ITS RELATION TO CELLULAR DEGENERATION[1]

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Abstract

The early distribution of melanin in the developing optic cup and stalk and its relationship to cellular degeneration have been studied during intrauterine development in hamsters, mice, ferrets, and humans. The extensive degenerative changes that occur in the region of the optic fissure do not involve any pigment-bearing cells. Melanin is not formed at the site of the fissure until after the processes of fusion and the associated degenerative changes are complete. In contrast to this, there is a small region near the junction of the cup and stalk where melanin is particularly related to degenerative changes. Melanosomes form dense clumps associated with basophilic, apparently degenerate areas of cytoplasm, and these melanosome complexes themselves undergo further degenerative changes. The two types of degenerative changes, pigmented and unpigmented, are limited to the same two regions of the developing eye in all four species. However, the pigment degeneration is more extensive in ferrets and humans than in mice and hamsters. These observations raise the possibility that the lysis of melanosomes plays a significant but undefined local role in ocular development.

The remarkably early appearance of melanin granules in the developing eye, though easily demonstrated and widely recognized, has received relatively little detailed study nor has it been the subject of much speculation. Yet, in mammals, the melanin first appears in the pigment epithelium roughly halfway through intrauterine life (Mann, 1964; Moyer, 1969; Endo and Hu, 1973; Kuwabara and Weidman, 1974), long before the retinal receptors develop and, thus, long before the melanin can perform any of its known adult functions. The retinal melanin may be involved intimately in ocular development, but the part that this pigment may play has not been defined. Visual abnormalities, ranging from relatively simple chiasmatic defects (Lund, 1965; Guillery, 1974) to foveal defects (Duke-Elder, 1964; Fulton et al., 1978) and possibly other ocular defects (Searle, 1968; Stone et al., 1978), can be found in individuals that have melanin pigment abnormalities.

Our initial interest in the melanin arose from the observation that many individuals having abnormally low amounts of melanin in the retinal pigment epithelium also show a chiasmatic misrouting of some retinofugal axons. Since many different genes that produce melanin abnormalities, and that do so by a variety of different cellular mechanisms, also produce a chiasmatic abnormality (Sanderson et al., 1974; Wise and Lund, 1976; LaVail et al., 1978; Guillery et al., 1975, 1979), one is led to conclude that the pigment itself is associated very closely with the mechanisms producing the pathway abnormality. However, the nature of this association is not defined and it was of interest to determine the spatial and temporal relationships of melanin during ocular development in order to demonstrate where interactions might be possible between retinal ganglion cells and pigment cells.

The present study represents a part of a long-standing survey of pigment distribution in the developing eyes of a variety of mammalian forms. It is concerned particularly with the strikingly constant relationships that pigmented cells bear, in several different species, to zones of cellular degeneration and necrosis. The regular occurrence of melanin in some zones of degeneration and its consistent absence from others suggest that the breakdown of melanin may have a significant influence upon ocular development.

Materials and Methods

The observations reported here are based upon an extensive collection of serial sections obtained from nor-

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mally pigmented and albino mouse, hamster, and ferret embryos and from three human embryos. All embryos were embedded in paraffin, cut as serial sections at 6 to 10 μm, and stained with hematoxylin and eosin. Most of the blocks were cut as sagittal series, but some transverse series were also available. The pregnant hamsters and many of the ferrets were kindly made available to us by Dr. M. Orsini (University of Wisconsin, Madison), and many of the mice were supplied by Dr. B. Cattanach (Radiobiology Unit, Horwell, England). Three human embryos were provided by Dr. J. Fallon (University of Wisconsin, Madison). Hamster embryos were obtained at 4-hr intervals between 10 days, 12 hr and 13 days, 20 hr after mating. The ferrets were aged 20 to 25 days postcoital. Mice were obtained at 11 to 15 days after identification of a vaginal plug. The developmental stages of the three human embryos are considered under "Results."

In addition to the above light microscopic material, we studied sections from series of hamster and mouse embryos (albino and pigmented) by electron microscopy. The fetal heads were fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4. They were generally hemisected, then rinsed in buffer, transferred to buffered 1% osmium tetroxide, and then dehydrated in ethanol and embedded in Araldite ("Durcupan"). Thin (silver or gold) sections were examined in a Philips 201 electron microscope.

Results

The early development of the optic cup and stalk has been described by Mann (1964) and the approximate relationships seen at the stages that we have studied are shown in Figure 1. The figure has been tilted to show the optic fissure (*F*), a deep, ventral infolding of the optic cup and stalk. The sections show that there is a narrow cleft within the cup and stalk, continuous with the diencephalic ventricle at the earliest stages, and that this

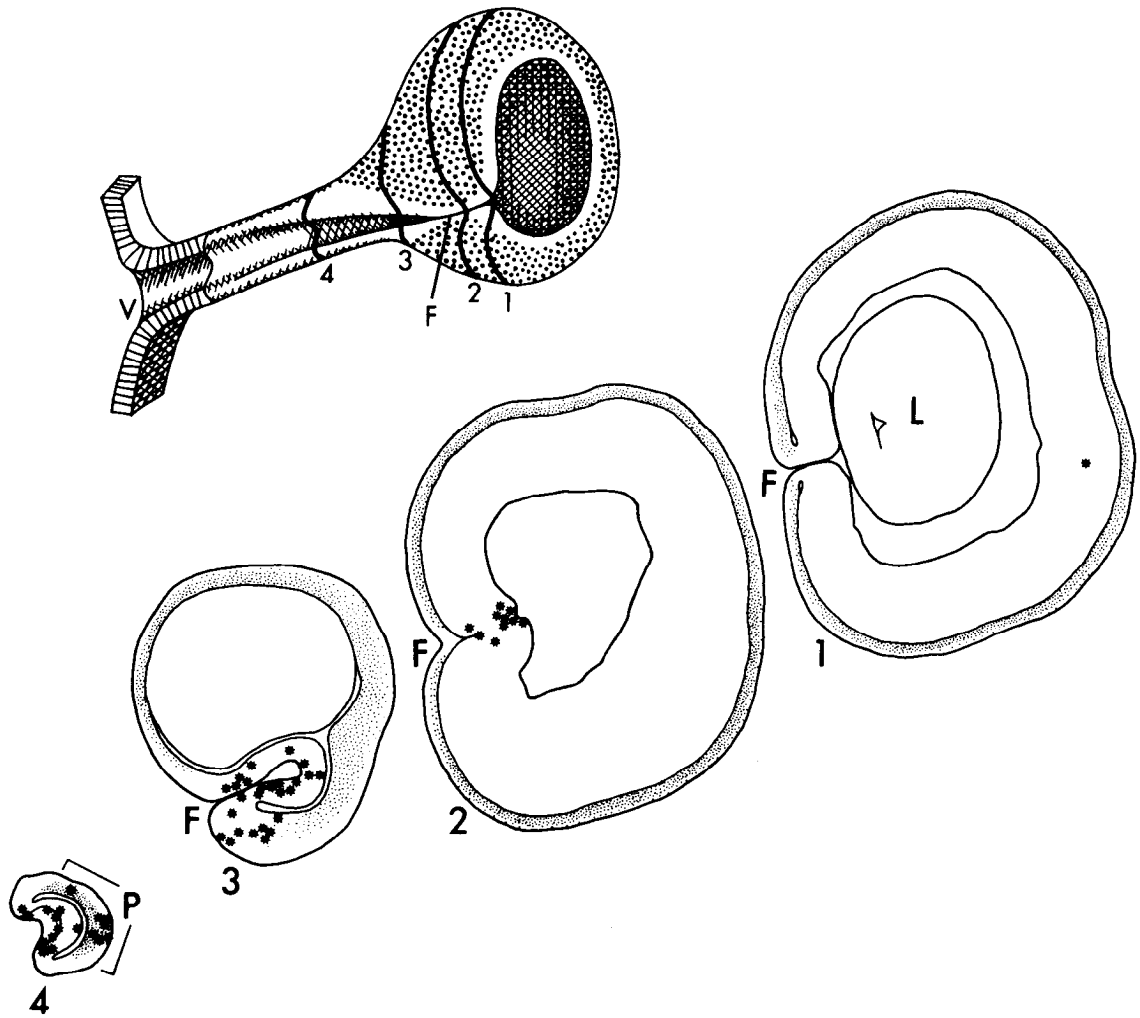

Figure 1. Schematic diagram to indicate how the sections of the eyecup relate to the eyecup itself and to the stalk. Note, on the *upper figure*, the lens has been removed. The brain is to the *left*, the lateral eyecup is to the *right*, and the fissure is in the ventral part of the eyecup and stalk. This figure also indicates the relationship between zones of cellular degeneration and zones containing melanin. The sections represent camera lucida outlines taken from a hamster aged 11 days (*in utero*). Melanin is represented by *small dots* in the section and degeneration is shown by *asterisks*. The distribution of melanin in the whole eyecup is also indicated by *dots*. *1* to *4*, The levels of sections 1 to 4; *F*, optic fissure; *L*, lens; *P*, tongue of pigment in the stalk; *V*, ventricular cavity. Magnification for the section = × 140.

cleft separates an inner layer of epithelium from an outer layer. The lips of the optic fissure fuse during the period that we have studied, and the fissure becomes entirely closed in the eyecup. In some species, the distal part of the fissure of the eyestalk, nearest the cup, also closes. Figure 1 shows the distribution of pigment (*fine dots*) and cellular degeneration (*asterisks*) as the fissure is closing. We describe the distribution of pigment first and then consider the character and the distribution of the degenerative changes in relation to the pigment.

Pigment distribution

The hamster. Melanin granules first appear in the eyecup of the fetal hamster on the 10th day of gestation (day of conception = day 0). The pigmentation of the outer epithelium proceeds from the distal (lateral) rim of the eyecup, advancing toward the eyestalk medially and also toward the optic fissure ventrally, throughout days 10 and 11. The early, sparse distribution of pigment on the 10th day is illustrated in Figures 2 to 4. The region of the optic fissure is unpigmented at this early stage (Fig. 4). Within the individual epithelial cells, the first melanin develops in the apical, juxta-ventricular parts (Fig. 3) and proceeds later toward the outer basal lamina. This appears to be the developmental sequence for all of the retinal pigment cells in all of the species examined.

Figure 1 indicates that, as the fissure is closing, all parts of the retinal pigment epithelium contain melanin except for the borders of the open fissure. Since this part is not pigmented, it may be inappropriate to speak of it as "pigment epithelium." In order to avoid confusion, we will use "pigment epithelium" for regions that contain melanin granules and use "outer epithelium," more generally, to describe the outer layer of the eyecup, whether pigmented or not. The developmental history of the outer epithelium in the region of the fissure is discussed in more detail below.

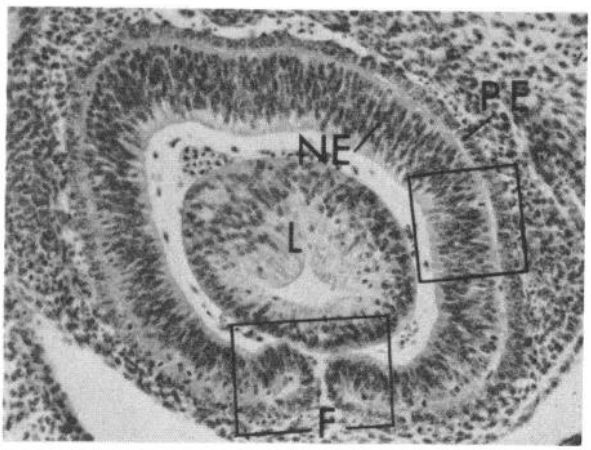

Figure 2. Parasagittal section through the eyecup of a 10-day, 12-hr fetal hamster. The optic fissure (*F*) in the *lower part* of the figure is still open. At this early stage, the pigment epithelium (*PE*) shows only small, sparsely scattered melanin granules (see Figs. 3 and 4), which are barely visible at this magnification. *L*, lens; *NE*, neural epithelium. Hematoxylin and eosin stain was used. Magnification = × 145. The areas included in the *rectangular outlines* are shown at higher magnifications in Figures 3 and 4.

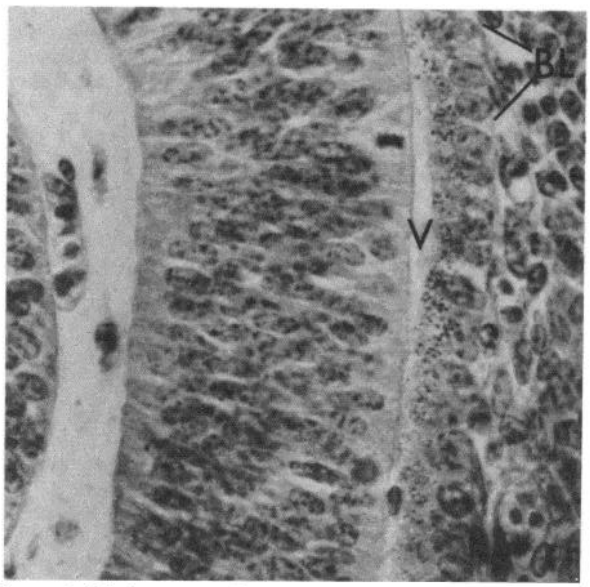

Figure 3. Higher magnification of the wall of the eyecup from Figure 2. The pigment granules in this part of the eyecup can be clearly recognized to the *right* immediately next to the ventricular cavity (*V*). *BL*, basal lamina. Compare with Figure 4.

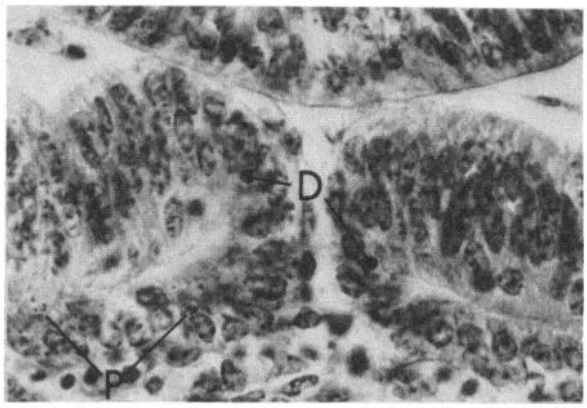

Figure 4. Higher magnification of the region of the optic fissure from Figure 2. In contrast to the region of Figure 3, this region shows almost no melanosomes. There are only a few, barely recognizable granules (*P*), adjacent to the fissure and there are none in the region of the fissure itself. *D* indicates degenerative changes.

The process of fissure closure has been described in detail by Geeraets (1976), and here we are concerned primarily with the relationships of the pigment to the region of the fissure. As the lips of the fissure meet, the outer epithelium appears to invert toward the inner layer and the unpigmented cells adjacent to the fissure appear to have migrated inward (Fig. 5). As the fusion proceeds, there is generally no pigment at all in the immediate region of the fissure (Fig. 6). Occasionally, a few isolated pigment granules are seen. After fusion, the site of the fissure is marked for a day by an indentation in the outer pigment layer and by a region of relatively sparse pigmentation (Figs. 1 and 5 to 8). Thereafter, the indentation disappears and all of the cells of the outer layer are

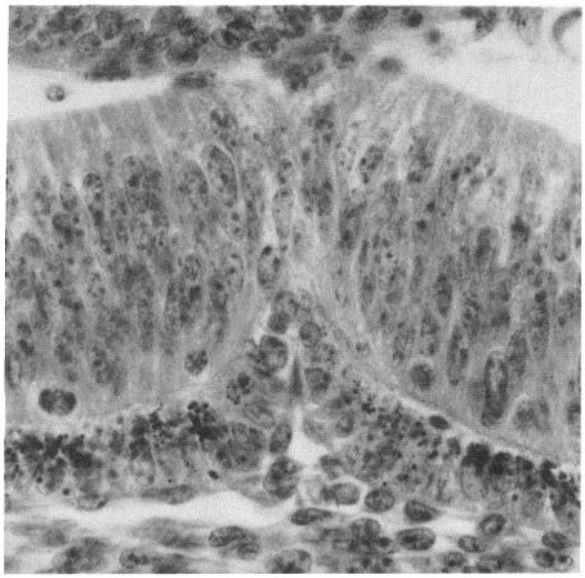

Figure 5. The region of the closing optic fissure showing the distribution of melanin in a hamster fetus aged 11 days. Magnification = × 570. The methods were the same as those in Figure 2. For further description, refer to the text.

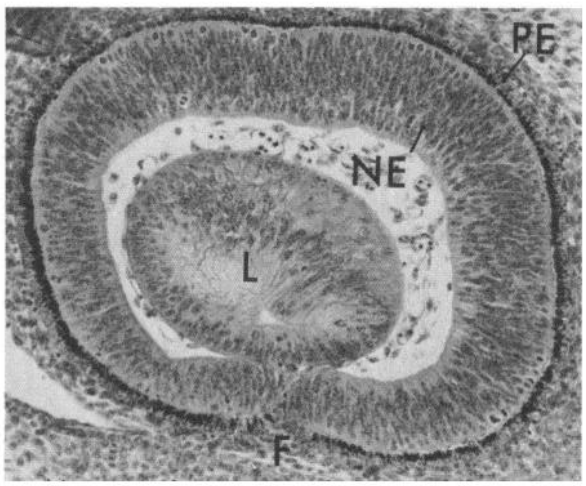

Figure 6. Parasagittal section through the eyecup of an 11-day-old hamster fetus. Note the distribution of the melanin in relation to the closing optic fissure. The methods and abbreviations are given in the legend to Figure 2. Magnification = × 160.

pigmented evenly. After fusion, the inner or neural layer tends to form a fold into the interior of the eyecup and this is quite conspicuous in some of the sections (see Fig. 7; see Mann, 1964). We have been unable to determine the fate of the unpigmented cells of the outer layer. They may migrate inward, contributing to the inward fold of the neural epithelium, being replaced in the outer layers by pigmented cells, or they may rejoin the outer layer after fusion and later develop pigment.

By the 11th day, the melanin is distributed throughout the eyecup and continues a short distance into the dorsal part of the stalk (Figs. 1 and 9). It continues as a tongue of pigment about 100 to 150 μm into the distal part of the stalk during the 11th and 12th days (shown as *p* at *level 4* of Fig. 1), but by day 13, there is no longer any evidence of melanin in the eyestalk. The pigment granules in the distal portion of the eyestalk and in adjacent parts of the eyecup tend to form small clumps or "rosettes" throughout the 11th and 12th days (Fig. 9). This rosette forma-

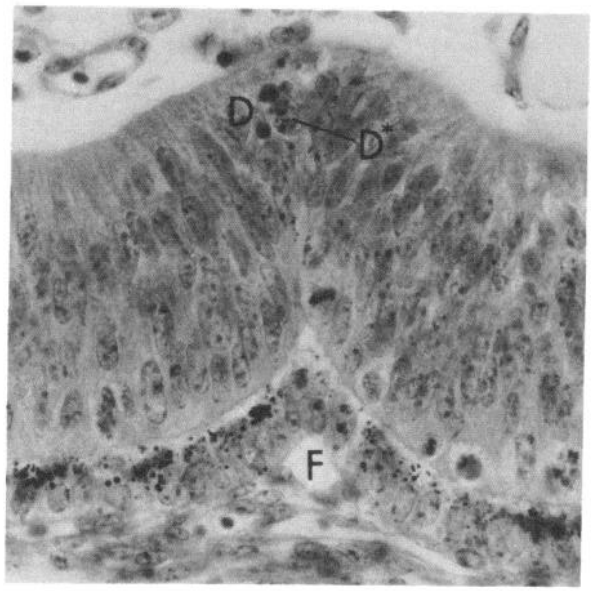

Figure 7. A region close to that shown in Figure 6. Closure of the fissure is somewhat more advanced in this region; the pigment epithelium shows almost continuous melanin and the neural epithelium shows significant signs of degenerative change (*D*). *D** indicates a phagocyte. Notice the marked inward folding of the neural epithelium at the site of fissure closure. The methods and abbreviations are given in the legend to Figure 2. Magnification = × 570.

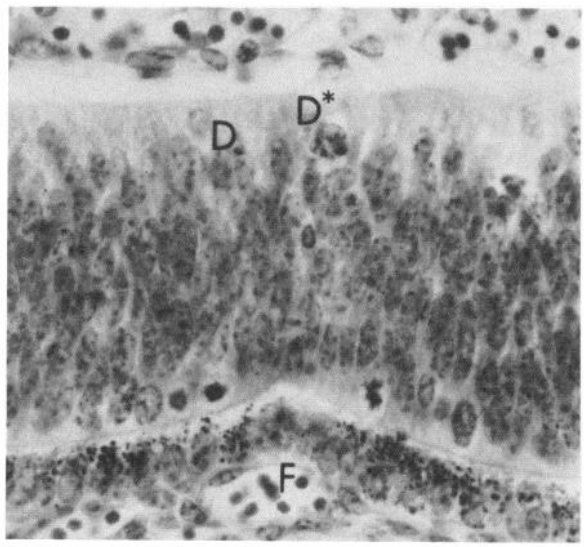

Figure 8. Parasagittal section through the eyecup of a fetal hamster aged 11 days, 8 hr. Closure of the fissure is more advanced and the region has flattened almost completely. There is still some sign of sparser melanin in the region of the fissure and of degenerative changes (*D*) in the neural epithelium. *D** indicates a phagocyte. See Figure 2 for further details. Magnification = × 535.

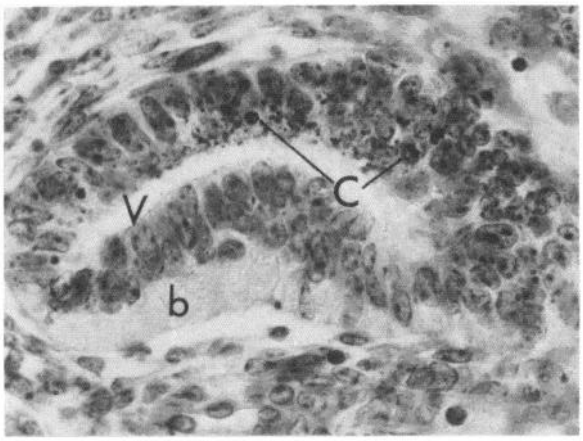

Figure 9. The eyestalk of an 11-day-old fetal hamster. This section is close to the eyecup and shows the melanin clumps (*C*) or rosettes that are associated with the degenerative changes in this region (see text). Bundles of retinofugal axons (*b*) are shown in the *lower part* of the figure. *V*, ventricular cavity. For further details, see Figure 2. Magnification = × 550.

tion, which is described in more detail below (and see Figs. 11, 13, and 18), is maximal on the 12th day. At the time that the rosettes are present in the dorsal part of the eyestalk during the 11th and 12th days, the retinofugal axons have entered the stalk and lie in the ventral, inner epithelium of the stalk, separated from the rosettes by the ventricular cleft (*b* in Fig. 9). The fibers are increasing in numbers from a few barely visible fine bundles to many large, well defined bundles throughout the 11th and 12th days.

Albino hamsters show no melanin in the developing eyecup and, with the light microscope, the pigment-free premelanosomes (Hearing et al., 1973) cannot be seen. However, our electron micrographs show that the pale premelanosomes characteristic of albinos (Witkop, 1971; Fitzpatrick and Quevedo, 1971; Kuwabara and Weidman, 1974) have a distribution like that seen in the pigmented animals.

Other species. The sequence of changes seen in the mouse is closely comparable to that described above. Melanin first appears in the eyecup during the 11th day and the fissure has closed by the 13th day. The absence of pigment in the area of fissure closure described in the hamster is also evident here. The tongue of pigment that develops in the optic stalk is seen briefly also in the mouse and there is a marked tendency for the melanosomes in the eyecup close to the eyestalk and in the distal part of the eyestalk, near the cup, to form rosettes. As in the hamster, the rosettes form in the dorsal epithelium of the stalk and are separated from the fiber bundles by the ventricular cleft. In the albino mice, the electron micrographs show that the premelanosomes have the same distribution as in the pigmented animals and form the same rosettes. Only the electron-dense melanin is missing.

In the ferret, the pigment appears on about the 20th day of intrauterine life. Although pigment is laid down in a sequence comparable to that seen in the mouse and hamster, the pattern of pigment distribution differs in some respects. Most strikingly, there is a large posterodorsal segment of the outer epithelium that never develops pigment and that remains as a relatively flat epithelial sheet, in marked contrast to the thicker pigmented portion of the other epithelium (Fig. 10). This unpigmented segment, which comes close to but does not include the region of the developing optic disc where the stalk and cup join, probably represents the region of the outer epithelium that eventually lies opposite to the tapetum and that, in the adult, is also free of melanin. As pigment development proceeds, large pigment granules (up to 3 μm in diameter in the 24-day-old animals) appear in many pigment cells. Only the regions next to the unpigmented posterodorsal zone and next to the optic fissure are characterized by relatively small, sparse pigment granules.

Before the fissure closes, the region of the fissure is, as in the other species, free of pigment entirely. The fissure is closing on the 21st and 22nd days and fiber bundles are clearly visible in the stalk by day 23. After closure, the site of the fissure can still be recognized for 1 or 2 days by a narrow belt of smaller pigment granules and less total pigment (Fig. 10). The tongue of pigment that extends into the eyestalk is well marked between the 22nd and 25th intrauterine days. It is made up mainly of medium and small melanosomes (generally less than 2 μm in diameter), but these form a large number of densely packed rosettes and, in some instances, it is not possible to distinguish between a very dense rosette and a large melanosome (Fig. 11). In the ferret, this rosette formation is more striking than in the hamster or the mouse; more rosettes are observed within the pigmented cells of the eyestalk and adjacent cup. We have no electron microscopic preparations of the ferret. The larger premelanosomes are visible light microscopically in the albinos and have the same distribution as in the pigmented animals,

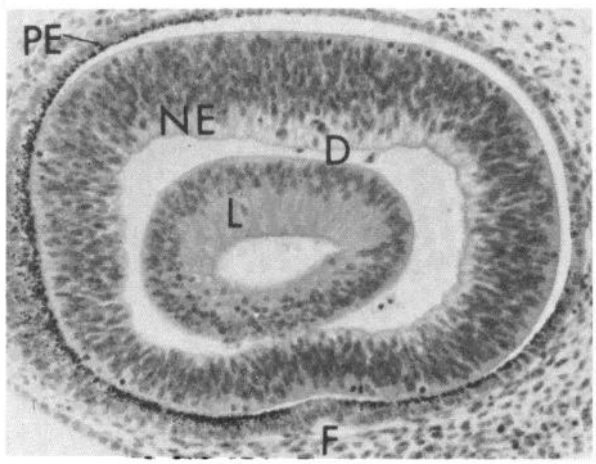

Figure 10. Parasagittal section through the eyecup of a fetal ferret aged 22 days. Note the very light pigment in the region of the recently closed fissure (*F*). The neural epithelium shows some degeneration in this region which is barely visible at this magnification and, in addition, there is some debris (*D*) in the *upper part* of the figure. Note the region of low, non-pigmented outer epithelium characteristic of the ferret's eyecup. The methods are described in the legend to Figure 2. Magnification = × 165.

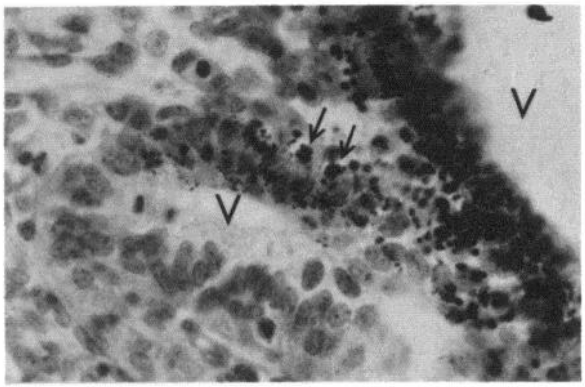

Figure 11. The region of the eyestalk (*left*) and of the adjacent cup (*right*), each with its ventricular cavity (*V*), from a fetal ferret at 23 days. Note the coarse clumps of melanin, which, in some regions (*unlabeled arrows*), can be recognized as groups of small melanosomes surrounding a central basophilic body. The methods are given in the legend to Figure 2. Magnification = × 550.

but the distribution of the smaller premelanosomes has not been determined.

The three human embryos are best described in terms of the stage of ocular development since we have only the heads (see Lemire et al., 1975 for the relationship of ocular development to the overall embryonic development). In the youngest, the lens is separated completely from the ectoderm, and most of the optic fissure has closed. However, the fissure is still open near the stalk and in the stalk itself. Only a few fiber bundles are visible in the most distal part of the stalk. Pigment is present throughout the full thickness of the rather tall pigment epithelium except at the borders of the open optic fissure, which are generally marked, as in the other species, by a narrow pigment-free zone. However, in this specimen, there are some pigmented cells at the borders of the optic fissure close to the stalk. Where the fissure has fully closed, the site of closure is no longer identifiable in terms of the pigment distribution but is marked by a significant inward bulge of the neural epithelium. The melanin continues into the distal part of the optic stalk, where it lies dorsally, opposite the fissure, and where it has a rather patchy distribution. Some of the melanosomes in the eyecup close to the eyestalk, and to a limited extent within the eyestalk itself (Fig. 12), form rosettes comparable to those found in other species.

The two older human embryos are both at roughly the same stage of development. The optic fissure has closed in all of the eyecup and in the distal part of the eyestalk, up to 150 μm from the cup. Relatively large bundles of axons are well defined in the stalk and can be clearly traced to the chiasm. The neural epithelium, which shows early signs of differentiation in the younger embryo, is forming a well demarcated layer of retinal ganglion cells in the older embryos. Melanin is present throughout the outer epithelium and there are a few patches of pigment in the distal and dorsal part of the eyestalk. From the point of view of the present account, the most striking feature is the rosette formation seen within a fairly extensive area close to the eyestalk (Fig. 13), and to a limited extent, within the eyestalk itself. This phenomenon is present in both of the older embryos and is more pronounced than in the other species examined. Since it is significantly more marked in one of the human embryos than in the other, the period of melanosome clumping may be near its end in these embryos.

Degenerative changes

Degenerative changes in non-pigmented cells. The light microscopic appearance of the degenerative changes in non-pigmented cells of all four species studied resembles that previously described for the developing eye by others (Glücksmann, 1965; Pei and Rhodin, 1970; Silver and Hughes, 1973, 1974; Kuwabara, 1975), and in general, we have used the same criteria for recognizing degenerating cells. The degenerating zones contain small, generally round, basophilic structures (Figs. 4, 7, 8, and 14). Some of them can be identified as pyknotic nuclei and

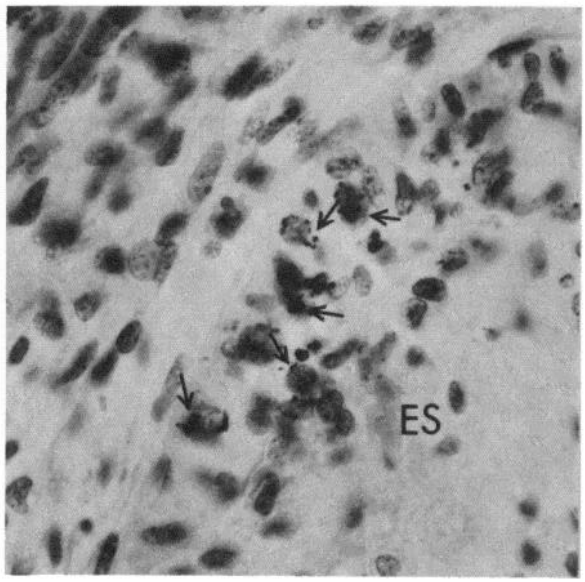

Figure 12. The eyestalk (*ES*) of a human embryo showing groups of melanosomes (*arrows*) near the eyecup. This is the earliest of the stages described in the text. The methods used were the same as those used in Figure 2, but the section is cut in the frontal plane. Magnification = × 645.

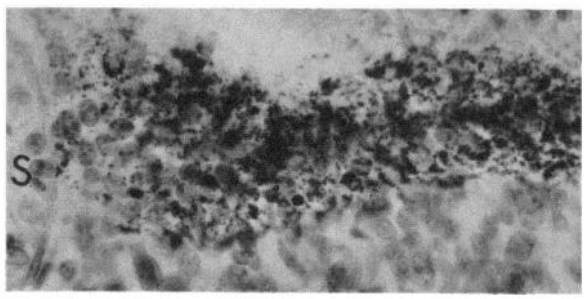

Figure 13. The eyecup of a human embryo close to the eyestalk (*S*). Note that the fine melanosomes tend to form coarse clumps in this region. This is from one of the older embryos described in the text. The method described in the legend to Figure 2 was used for this frontal section. Magnification = × 550.

these we regard as markers of degenerating cells. Where the degenerative process is particularly active, one can see closely packed clusters of basophilic bodies (Figs. 7, 8, and 14, *D**) and sometimes these can be identified as lying within a phagocytic cell. However, there are many basophilic bodies that lie in otherwise healthy-looking epithelial cells that have normal nuclei. We cannot be certain about the interpretation of these. They may represent the earliest stages of degeneration or they may represent lysosomes within otherwise healthy cells. Electron micrographs show these same several degenerative processes as well as the same overall distribution of these processes. The criteria for degenerative changes observed with the electron microscope are generally the same as others have used (Bellairs, 1961; O'Connor and Wyttenbach, 1974). Pyknotic epithelial cells can be recognized occasionally, and phagocytic elements filled with debris are seen within the regions of densest degenerative change (Figs. 15 and 16). Electron-dense bodies can be seen within some epithelial cells that have an otherwise normal appearance (Fig. 17). We regard the single basophilic or electron-dense structures that lie within the epithelial cells as representing an early stage of lysis, which may or may not lead to later, more severe degeneration of the cell.

Degenerative change in pigmented cells. In all of the material that we have studied, the basophilic bodies of the pigmented cells are associated closely with and often surrounded by the rosettes of melanosomes described in the previous section. In contrast to the non-pigmented cells, however, we see within the pigmented cells that these basophilic bodies and rosettes occur in cells that show no other clear signs of degeneration. Pyknotic nuclei are not seen in these regions nor is there evidence of phagocytic cells. With the light microscope, the distinction between the basophilic bodies and the surrounding brown melanosomes is generally evident, but this is not demonstrable in a black and white micrograph (Figs. 9 and 11). Electron micrographs show the distinction clearly (Fig. 18) and show the relationship between the melanosomes (*M*) and the dense basophilic bodies (*C*). Further, they confirm that this complex of structures lies within cells that are otherwise relatively normal (Fig. 18). It should be noted that the lysis described here in pigmented cells is a much earlier and more severe change within the pigment epithelial cells than the appearance of lysosomal dense bodies described by Endo and Hu (1973) in the retinal pigment cells of a 16-month-old

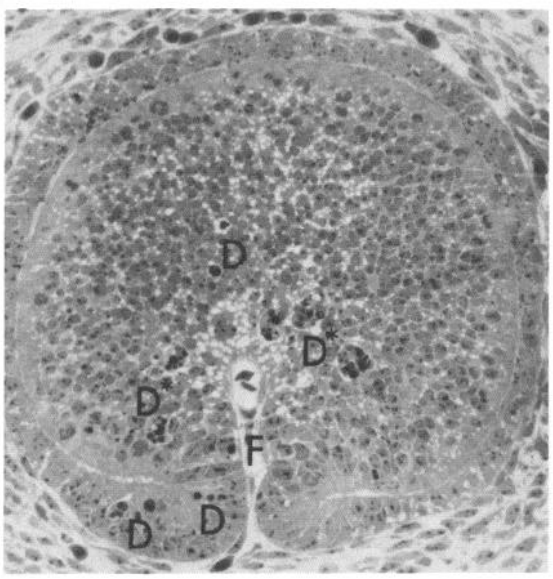

Figure 14. The eyecup of a mouse is shown near the stalk in a parasagittal section. The fissure (*F*) is open and there are extensive signs of degenerative change (*D*) in the inner (neural) epithelium and also in the outer epithelium; *D** indicates phagocytes. The figure shows a semithin section from a block prepared for electron microscopic study. Magnification = × 300.

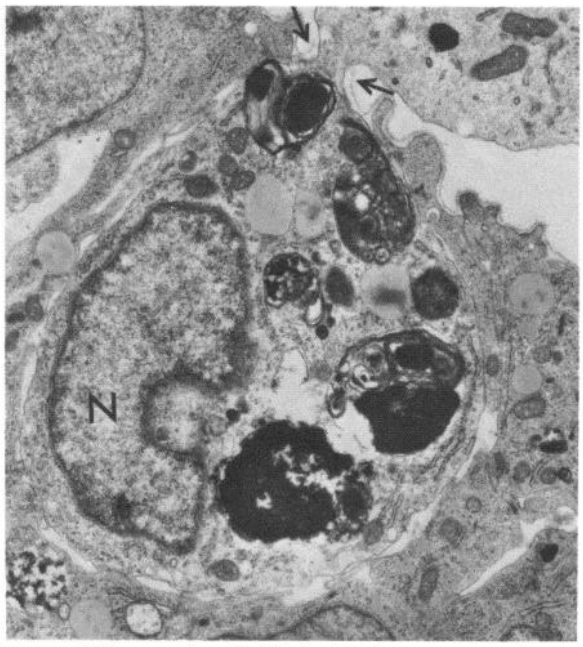

Figure 15. Phagocytic cell filled with degenerating debris and apparently moving out of the outer epithelium through a narrow break (*arrows*) in the basal lamina in a mouse aged 12 days (*in utero*). *N*, nucleus. Magnification = × 8100.

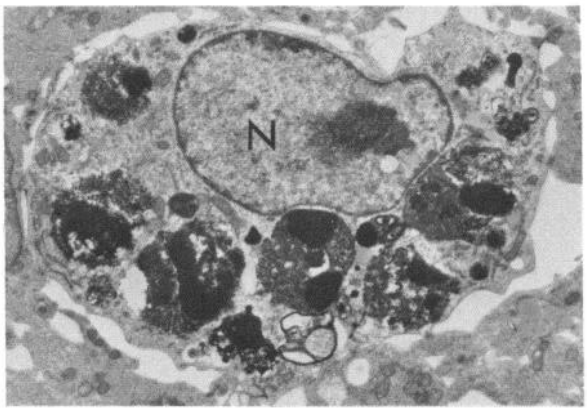

Figure 16. Phagocytic cell filled with degenerating debris within the neural epithelium in a mouse aged 12 days (*in utero*). Notice the characteristic scalloped border. *N*, nucleus. Magnification = × 4800.

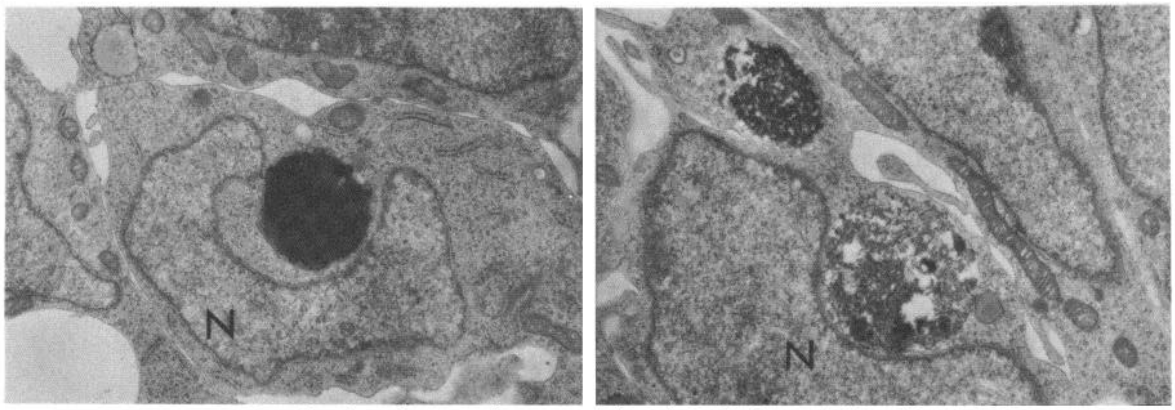

Figure 17. Electron micrographs showing electron-dense cytoplasmic inclusions within cells of the neural epithelium. These appear in zones of cellular degeneration and can be recognized as basophilic inclusions light microscopically. *N*, nucleus. Magnification = × 10,000.

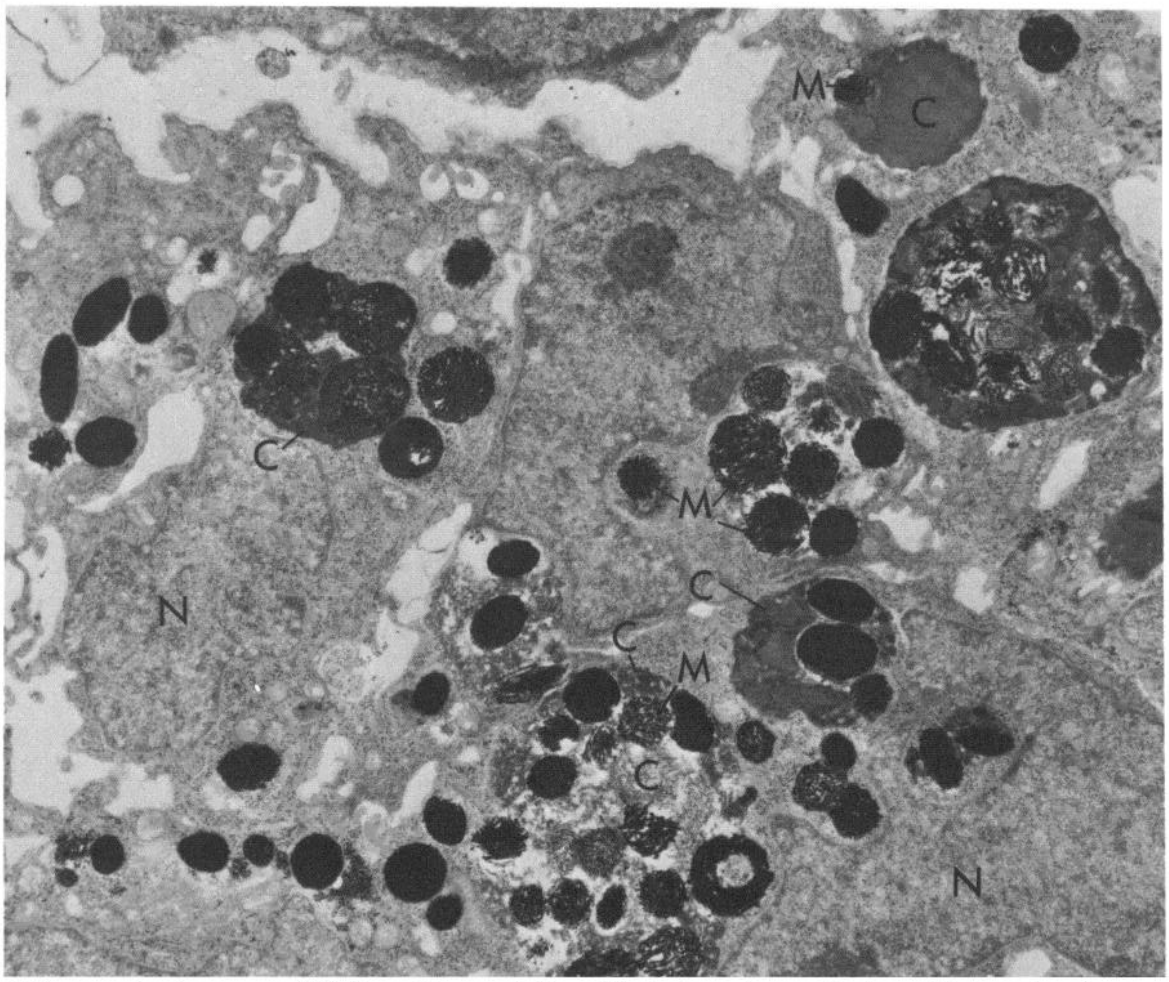

Figure 18. Groups of melanosomes (*M*) formed in association with electron-dense cytoplasm (*C*) from the pigment epithelium of the eyestalk close to the eyecup of a 13-day-old mouse embryo. *N*, nucleus. Magnification = × 9900.

monkey and is more like the melanolysosomes described by Feeney (1978).

We have been able to find no difference, in terms of the distribution of degenerating profiles, between albino and normally pigmented animals; therefore, these two will not be considered separately in this section. In the following account, which is based solely on light microscopic appearances, we are concerned with the distribution of dark basophilic bodies of all kinds and will make no distinctions in regard to those that lie within cells, those that represent single cells, and those that may represent groups of cells. Such distinctions cannot be made on the basis of light microscopic preparations and, for this reason, we have not prepared detailed quantita-

tive information about the distribution of the degeneration.

The distribution of degenerative changes in the hamster. In general, there are two areas of special interest at this stage of development which involve degenerative changes and which show particular relationships to pigmented cells. The region of the closing optic fissure involves extensive degenerative changes but is entirely free of pigment. In contrast to this, the dorsal part of the optic stalk and the adjacent cup show degenerative changes in the pigmented cells during a limited period of time.

In the region of the optic fissure, the degeneration occurs in two stages. Just before the fissure starts to close, during the 10th day, there is a moderate amount of degeneration, extending along the borders of the fissure, in the inner and outer retinal layers (Figs. 1 and 4). Detailed charts of the distribution of this degeneration show that, in the outer layer, it occasionally lies adjacent to a pigmented cell but that it does not actually involve pigmented cells. After the fissure has closed, on the 11th and 12th days, when the site of the fissure is still marked by an outer notch and an inward fold (Figs. 1 and 6 to 8), there is extensive cellular degeneration, extending throughout the neural epithelium of the fold and also extending somewhat beyond the fold. In the region of the fold itself, one can often see groups of engorged phagocytes (Figs. 7 and 8). The pigment epithelium that has closed over the fissure shows no degenerative changes.

The lips of the optic fissure of the eyestalk never fuse in the hamster. On the 11th day, there are extensive degenerative changes in the stalk. This includes the dorsal, outer layer that is continuous with the pigment epithelium and also the ventral, inner layer that is continuous with the neural epithelium (Fig. 1). The degeneration in the ventral layer occupies regions that are later invaded by axon bundles (see Ulshafer and Clavert, 1979; Silver and Sidman, 1980), but some degeneration products also can be seen among well formed axon bundles at later stages. The degenerative changes in the dorsal layer are found primarily close to the eye, where they are characterized by the melanosome rosettes (Fig. 9). The degenerative changes in this zone are continuous with degenerative changes in the pigment epithelium of the eyecup adjacent to the eyestalk, and in the eyecup, they also are characterized by the melanosome rosettes. Further from the eyestalk, there are a few isolated signs of degenerative changes in the pigment epithelium, and these, too, are seen in association with melanosome rosettes. The close association between the degenerative changes and the rosette formations suggests that the latter may represent an early stage of the former.

Degenerative changes in the other species. The distribution of degeneration in the mouse, ferret, and human is closely comparable to that described above for the hamster and also to that described by Silver and Hughes (1973) for the rat. From the point of view of the present study, there are several features to be seen in all of the species:

1. The degeneration is seen on either side of the open optic fissure, and, with one exception considered below, it involves only those cells in this region that are free of pigment.
2. After the fissure has closed, there is extensive degeneration in the region of the fissure, but it is limited to the neural epithelium and is particularly pronounced where the neural epithelium forms an inward fold.
3. There is cellular degeneration within the eyestalk before the axons invade the stalk (Von Szily, 1912; Silver and Sidman, 1980). At later stages, there is degeneration adjacent to the axon bundles and also some among the bundles.
4. In the pigment epithelium, the degeneration is associated closely with the melanosome rosettes. This degeneration is but sparsely scattered in most of the eye but is dense close to the eyestalk and within the distal parts of the eyestalk.

The ferret and the human embryos differ from the others in having a zone of melanosome rosette formation and pigment cell degeneration that reaches further into the eyecup from the region of the eyestalk. Because, in both of these species, the melanosome rosettes are particularly dense (Figs. 11 and 13), it is not possible to determine light microscopically how dense the basophilic bodies are in this part of the pigment epithelium. Occasional basophilic bodies are visible within the rosettes, but it is probable that the majority are obscured by the rosettes. In general, it appears that, in both species, the spatial distribution of basophilic bodies within pigmented cells is the same as that of the melanosome rosettes, and it is rare in a pigmented cell to see a basophilic body that is not associated closely with some melanosomes.

The youngest human embryo shows a zone of degenerative changes in pigment cells adjacent to the open optic fissure and, as pointed out above, such a zone has not been seen in any of the other embryos. In this human embryo, these degenerative changes are seen close to the optic stalk only. The fissure of the stalk has not yet closed and it appears that, as the fissure closes, in the region of the stalk, there are some degenerative changes in some pigment cells near the lips of the fissure. This is continuous with the zone of degenerative changes seen in the pigment epithelium further from the fissure at the junction of the cup and the stalk.

Discussion

Our results show that there is a consistent relationship between the zones of degeneration and pigmented cells. This constant distribution, in several different mammalian species, of cellular degeneration and of pigment granules suggests that their roles may have been choreographed rigorously by selective pressures acting over many generations. However, the part played by cell death in development is not understood (Glücksmann, 1965; Saunders, 1966) nor is anything known about the functions of melanin in the development of the early eyecup.

The zones of cell death and degeneration that we have described are like those demonstrated for the rat by Glücksmann (1965) and Silver and Hughes (1973); the distribution of pigment is in accord with descriptions provided by Mann (1964). Our account has added new information in relating these phenomena to each other and in showing a remarkable consistency across species. In general, the cellular degeneration that borders the open or recently closed fissure does not involve pig-

mented cells. Pigmented cells show degenerative changes within a quite limited region close to the optic disc, and within this region, the most obvious sign of cellular degeneration is the rosette formation in relation to lysosome-like structures resembling the melanolysosomes of Feeney (1978). However, their lysosomal nature remains to be unequivocally demonstrated in our material.

The belt of unpigmented outer epithelium bordering the fetal fissure just before and at the time of closure is a striking feature of eyecup development noted by Mann (1964). She described this unpigmented region as neural epithelium and suggested that it was everted temporarily to join the pigment epithelium on the outer surface of the eyecup. We have been unable to determine the precise origin or fate of the cells in this unpigmented epithelium. They may stay in the outer layer and become pigmented after the fissure closes, but the elongation of these cells, described by Geeraets (1976), supports the view expressed by Geeraets that the cells migrate during fissure closure. If they do migrate, then they can join either the neural epithelium itself or the zone of cell death. Since cell death in the relevant part of the neural epithelium is extensive just after closure, the most reasonable interpretation is that these cells migrate and then die.

Because pigment is absent from the degenerative zone in the region of the fissure, we at first thought that pigment might somehow preserve cells from degenerative changes; however, the observation of degenerative changes within pigmented cells occurring in the distal eyestalk (see Stryker et al., 1977) argues against such a notion. This consistent absence of melanin from the region of the optic fissure during a time of intense degeneration appears to need some explanation. Such an explanation may depend on understanding the role that melanin may play at this early stage of development. From the observation that degenerative changes occur in the pigmented cells at about the time that many retinofugal axons are growing through the stalk, it is possible to conclude that the lysis of melanin may play a role in directing the outgrowth of axons. The absence of pigment in the region of the fissure might then indicate that melanin lysis in the region of the fissure would produce an inappropriate marking of the axons in this region.

In the youngest human embryo, we saw a simple exception to the relationships described above. In the eyestalk of this embryo, the pigmented cells show degenerative changes next to the fissure. It should be noted that this is the only animal in which we have direct evidence of the fissure of the eyestalk closing at the stages that we have studied and that the degenerative changes are close to the stalk. It is not clear from the material that we have whether there is cell death among the pigmented cells of this region or whether the changes are less severe as in the other pigmented regions.

We initially undertook this study because pigment abnormalities are associated with chiasmatic abnormalities and we wanted to determine how pigment distribution might relate to the outgrowth of retinogeniculate axons. We have indicated above (p. 1193) that many individuals having developmental pigment abnormalities also develop abnormal chiasmatic pathways. The important and interesting relationship here is that a chiasmatic abnormality is found in association with a number of different genotypes, each of which produces pigment abnormalities by a distinct mechanism. Genes that produce their effects upon melanin by acting at quite distinct phases of pigment production or packaging also produce chiasmatic abnormalities (Sanderson et al., 1974; Wise and Lund, 1976; LaVail et al., 1978; Guillery et al., 1975, 1979). Thus, one cannot postulate a single common cause producing both the pathway abnormality and the pigment abnormality by mechanisms that are otherwise independent, nor can one consider that the chiasmatic abnormality may produce the pigment abnormality: the spatiotemporal relationships are wrong. The most reasonable possibility to be explored would seem to be that the melanin itself, or some substance quite close to melanin on the synthetic or degradative pathways, can modify the course of the retinofugal axons; this relationship must be evaluated in terms of the events that occur during the development of the eyecup.

The temporal relationships of melanin formation, ganglion cell development, and melanin degradation suggest that degradative change is a better candidate than the synthetic process. A second, rather tentative argument for focusing attention upon the process of pigment cell degeneration rather than synthesis can be derived from consideration of the Chédiak-Higashi syndrome. The evidence that there may be a pathway abnormality associated with this syndrome (Spencer and Hogan, 1960; Witkop, 1971; LaVail et al., 1978; Sanderson et al., 1974; Guillery et al., 1979) possibly implicates melanin breakdown or release in the production of the neural abnormality because the syndrome shows a general abnormality of lysosomal mechanisms and of melanosome structure (Witkop, 1971; Kramer et al., 1977).

The suggestion that pigment in the distal part of the eyestalk may play a role in determining axonal pathways has been made previously by one of us (see Stryker et al., 1977) and by Silver and Sapiro (1980). There are several reasons for focusing upon this small patch of pigmented cells rather than upon the whole of the pigment epithelium. One is that these cells lie relatively close to many of the retinofugal axons at about the time that these axons are entering the stalk. A second reason, defined clearly by our material, is that these pigmented cells are undergoing marked changes at the time that the axons are growing by. A third is that a simple interaction between pigment epithelial cells and developing retinal ganglion cells, involving the whole retinal surface and occurring across the ventricular cavity of the eyecup, would lead to a clear relationship between the spatial distribution of pigment in the eyecup and the spatial distribution of normally and abnormally connected ganglion cells. However, there is no such relationship. The abnormally connected ganglion cells are generally limited to one part of the retina (Guillery and Kaas, 1971; Cooper and Pettigrew, 1979; Stone et al., 1978; Dräger and Olson, 1980) and where, as in the ferret, there is a pigment-free region of the eyecup (see above p. 1197), the abnormally connected ganglion cells are not distributed accordingly (Guillery, 1971; and see Thibos et al., 1980).

Silver and Sapiro (1980), Oberdorfer et al. (1981), and Silver (1981) suggested that the pigment cells of the eyestalk act as a barrier and that the axons, by avoiding

the pigment cells, are guided to appropriate channels in the stalk. In albinos, the axons would presumably be able to enter regions of abnormal pigment cells and thus would be misrouted. However, there are some problems with this view. The proposed misrouting in the albinos would tend to separate the abnormally directed bundles from the main bundles, and this is not what happens in albinos. Instead, axons that would normally remain uncrossed in the chiasm take a crossed course in albinos and travel with the majority of the retinofugal axons. Our own preparations suggest that the melanin itself does not form an effective barrier. The position of the fiber bundles in the distal part of the eyestalk is essentially the same in normal and in albino animals. Further, in the main part of the eyecup, one can see bundles of nerve fibers that are able to grow among pigmented cells. These axons, which are possibly destined for the innervation of the ciliary muscle or iris, form quite extensive networks among the normal pigment cells of the eyecup and demonstrate that pigment itself is not a barrier to axonal growth in general.

On the basis of the above arguments, we suggest that breakdown products of melanin may interact with some of the outgrowing axon tips. They may mark the axon tips themselves or may be transported back to the perikarya and thus be able to modify the later program of outgrowth of some of the retinofugal axons. These axons, presumably, would be the ones that take an uncrossed course in normal animals but that take a crossed course in albinos because there the axons are not marked adequately. This view provides one possible explanation for the fact that the zone of cell death next to the fetal fissure is kept so strikingly clear of melanin. Degradation products of melanin in this region might otherwise inappropriately mark axons that are nearby. It also is in accord with the observation that the human and the ferret, which have relatively large uncrossed retinofugal components, both show more pigment degeneration than the hamster and the mouse, in which the uncrossed component is relatively small.

There are thus several indirect lines of evidence which suggest that the degenerative changes of the melanin may relate specifically to the pattern of axonal growth in the retinofugal pathways. These are, however, indirect and, at present, provide no more than suggestive clues. The direct evidence presented here shows that the pattern of melanin degeneration relates closely to the developmental changes occurring specifically in the region of the optic disc at the time that retinofugal axons are growing through this region. The precise nature of this relationship remains to be defined as does the significance of the marked absence of pigment degeneration in the optic fissure.

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