
Melanin pigmentation changes were studied in a mutant (delayed amelanotic) line of chickens characterized by a postnatal, spontaneous cutaneous amelanosis and a high incidence of blindness. Cutaneous pigment loss was accompanied by destruction of the choroidal melanocytes throughout the orbit. The presence of blindness appeared to be correlated with the histopathologic finding of severe degenerative changes in the pigment epithelium and neural retina first seen near the base of the pecten and progressing radially in irregular patterns.

A mutant line of domestic chickens (Gallus domesticus) characterized by a postnatal spontaneous loss of the capability for cutaneous melanization and a high incidence of a serious eye disease has been developed and briefly described by Boissy et al. This stock, called the delayed amelanotic (DAM) line, has been selected since 1970 to increase the incidence of the melanizing defect, with a present occurrence of 85.6% feather amelanosis. Among the latter, 39.6% were adjudged to be blind on the basis of their inability to locate feed and water visually. Similarities in the disruption of an initially competent pigment system in young DAM chickens and humans with vitiligo (a pigment defect of similar appearance) have been noted previously. Ocular pigment disturbances have also been found among a number of human vitiligo patients. There are additional similarities between the DAM and human vitiligos, including higher-than-normal association with thyroid disease and integumental defects. Studies are underway to determine whether the pigment defect is identical in the two species. On the basis of findings to date, we believe that the DAM model provides a valuable opportunity to study changes occurring in this disease at the cellular level and to correlate associated neural and behavioral changes. In addition, this model offers an opportunity to understand further the processes involved in a wide range of ocular defects associated with pigment cell abnormalities of the uveal tract and retina. In the present report we will describe some of the changes in melanin pigmentation of the eye of the DAM chicken.

Materials and methods. Eyes for histological study by light microscopy were obtained by surgical enucleation from dark-adapted (1 hr) adult birds, deeply anesthetized with a 2.0% solution of sodium pentobarbital in 10% ethanol. The enucleated eye was immediately injected with, and subsequently immersed in, Susa's fixative for 24 hr. The cornea, lens, and vitreous were removed, and the remaining orbit was dehydrated in a series of increasing concentrations of ethyl alcohol. Eyes were embedded in low-viscosity nitrocellulose by the method of Sakovich and Burn and were sectioned horizontally on a sliding microtome at 10 to 15 μm. Sections were individually stained with Cason's Mallory-Heidenhain modified for retina.

Because amelanotic DAM adults show variable patterns of pigment loss in their eyes, a subjective rating scale based on ocular pigment loss was developed to enable a simple rank-ordering of individuals. Ten different standards were selected, ranging from the fully pigmented control (rating = 1) to a complete absence of retinal-choroidal melanin (rating = 10). Eyes evaluated with this procedure were fixed in Susa's, washed overnight, and dehydrated to 70% ethanol. The front of the eye was opened, and the lens and vitreous were removed. The remaining posterior two thirds of the orbit was then transilluminated with a standard microscope illuminator (1.94 × 10^4 mW/m^2), and the degree of pigment loss for each eye was rated independently by two observers (K. V. F. and R. E. B.). Interrater reliability was high (r = 0.98) with this method. These eyes were subsequently processed for histologic evaluation as previously described.

The visual ability of a DAM bird was determined by informal evaluation of visual motor responses. A bird demonstrating wandering in a pen, spontaneous nystagmus, and lack of response to sudden hand movements close to the head was determined to be blind. A partially-sighted bird behaved normally when in a pen and demonstrated a startled response to hand movements close to the head.

Results. Histologic sections of the eyes of six DAM adults (three blind and three partially sighted birds) were compared with those of normal control Brown Leghorns (Fig. 1). The extent and pattern of ocular amelanosis varied considerably among individual birds, with the most striking loss of RPE pigmentation occurring in those animals without demonstrable visual ability. The eyes of DAMs judged to be blind showed extensive pathologic changes (Fig. 2). Most striking was the complete absence of melanin in the choroid accompanied by either a conspicuous reduction or
complete loss of the pigment epithelium in large regions of the central retina. Considerable fibrous metaplasia appeared to replace the pigment epithelium in these areas. Few photoreceptor cells were discernible in the retinas of blind DAMs, and those that remained appeared to be abnormal morphologically. All photoreceptor types appeared to be equally affected by the degenerative processes accompanying the retinal pigment epithelium (RPE) amelanosis. The inner nuclear and ganglion cell layers of the neural retina often showed an absence of lamination and cell organization in severely amelanotic eyes. In addition, the eyes of some older amelanotics showed these layers to be noticeably thinner.

Sections from the eyes of two of the sighted amelanotic DAM adults also showed a complete absence of choroidal melanin; however, the entire RPE appeared to be normal (Fig. 3, A). The amelanotic choroids otherwise appeared to be essentially normal, as did the neural retina. In contrast, the third sighted amelanotic bird showed some additional retinal destruction near the base of the optic nerve and pecten consisting of a loss and fragmentation of the RPE as well as degeneration of the underlying photoreceptor cells (Fig. 3, B). However, the RPE and retina in the rest of the orbit appeared normal.

The observation that the status of ocular melanin could vary within an individual eye led to a further investigation of this phenomenon. Excised eyes and the ordinal rating scale method described earlier were used to determine the degree of orbital amelanosis in DAMs, which was found to vary from an apparent normal condition to a complete loss (Fig. 4). In those eyes with the least pigment change, histologic examination showed that amelanosis resulting from absence of both the cho-
Fig. 2. Horizontal section from near the base of the optic nerve from a blind DAM eye, showing the absence of melanin pigment in both choroid and RPE layers and severe degeneration of the outermost retinal layers with loss of RPE and fragmentation of the photoreceptor layer. IPL, Inner plexiform layer; P, photoreceptors; Ch, choroid. Bar = 50 μm.

Regardless of the status of the choroid and retina, the iris and ciliary body as well as the pecten contained a normal number of healthy-appearing melanocytes in the young DAM birds. However, pigment loss in these tissues has been observed in older blind adult DAMs.

Discussion. Loss of vision in the DAM chicken appears to occur only in association with the cutaneous amelanosis that is first apparent in developing feathers. Cutaneous amelanosis is accompanied by melanin loss in the choroid, whereas involvement of the pigment epithelium and retina may or may not be present. In the latter case, excessive degenerative changes appear ultimately to lead to blindness.

Although there are some similarities in the ocu-
Fig. 3. Horizontal sections from near the base of the optic nerve from an area with absence of choroidal pigment but apparently normal RPE in one (A) and an area of abnormal RPE near the base of the pecten in the other (B). IPL, Inner plexiform layer; P, photoreceptors; Ch, choroid. Bar = 50 μm.

Fig. 4. Comparison of ocular pigmentation (choroidal and pigment epithelial) in excised eyecups of partially sighted bird (S) with a rating value of 4 and blind DAM (B) with a rating value of 10. Note regional variation in amelanosis in S. (×1.2)

lar pigment changes in the DAM chicken and human vitiligo, the visual consequences are more severe in DAMs than in vitiligo patients. This may be related in part to the fact that the extent of cutaneous pigment loss is far greater in the DAM chicken than in most human vitiligo patients. In this regard, the DAM model may more closely parallel the changes seen in the Vogt-Koyanagi-Harada syndrome or in sympathetic ophthalmia.

Further studies in progress are designed to provide additional information on the systemic and visual correlates of the spontaneous amelanosis of the DAM line and its relevance to human visual disorders.

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REFERENCES
Additivity and repair of actinic retinal lesions. GARY A. GRIESS AND MICHAEL F. BLANKENSTEIN.

Extensive exposures to light of intensities insufficient to produce thermal damage can still result in retinal damage via nonthermal mechanisms. In this work, the additivity and the repair rate for this actinic damage were measured. Rhesus monkey retinas were exposed to 458 nm light from an argon-ion laser at a dose equivalent to half the threshold retinal irradiance. After prescribed time intervals, the retinal sites were re-exposed to determine the split-dose threshold. This threshold is related to the single-dose threshold through the additivity, which in turn is dependent on the time between exposures. The observed recovery of tissue could be fitted by a single exponential with a time constant of 4 days. This result is incorporated in an analytic expression for the cumulative effect of repeated doses.

Light can be toxic to the retina in at least two ways. Thermal damage is well documented and appears to be the primary damage mechanism for exposure durations between 10^-8 and 10 sec. For longer exposures, an apparent nonthermal mechanism exhibits characteristics quite different from that causing thermal damage. This mechanism is most effective at shorter wavelengths and has associated with it negligible temperature rise. It has been designated variously as nonthermal, phototoxic, or actinic damage. This report is concerned with the quantitative characterization of additivity and repair of actinic damage in primate retinas with funduscopically visible lesions used as the endpoint.

The literature concerning the cumulative nature of actinic damage is limited. Ham et al. reported that two 1000 sec exposures at 441.5 nm spaced 48 hr apart produced a lesion at one-half the 1000 sec threshold power. But they also said that four or more exposures at one-fourth threshold spaced 48 hr apart did not produce a lesion. Lawwill et al. using electrophysiological and histological endpoints, reported that 1 hr exposures at 514.5 nm for 4 consecutive days produced the same damage as a single 4 hr exposure. Sperling et al. compared lesions from a single 120 min exposure with those of daily intermittent exposures and found histologically different patterns. Recovery from superthreshold blue-light lesions has been histologically traced by Tso et al. and Ham et al. over a span of months, but repair of subthreshold damage has not been looked at.

Fig. 1 illustrates the concepts utilized in the split-dose technique. For an exposure of duration, t, there will be a threshold retinal irradiance, \( E_o \), which is the dosage at which there is 50% probability of damage (ED50). Actinic damage exhibits reciprocity. That is, for an exposure duration of 2t, the ED50 will be 0.5 \( E_o \). This may be considered the limiting case where two pulses of duration, t, are contiguous. If the two pulses are separated by an interval \( \Delta t \), the magnitude of the second dose required to produce a lesion, \( E_2 \), will depend on the additive contribution of the first dose. If the first dose is set at \( E_o/2 \), the value of \( E_2 \) can range from \( E_o/2 \) for complete additivity to \( E_o \) for no additivity, as follows:

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E_2 = E_o - AE_o/2
\]

where A is the additivity: 0 ≤ A ≤ 1. Solving for A gives the following:

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A = 2(1 - \frac{E_2}{E_o})
\]

The additivity will be some function of the time between exposures, \( \Delta t \), depending on the repair of the tissue after the initial insult.

Methods and materials. A Spectra-Physics Model 171 argon-ion laser tuned to the 458 nm emission was used. A combination of a 2 mm aperture and a +8.0 diopter lens served to produce uniform 200 \( \mu \)m retinal images. The beam profile and divergence were determined by beam scans at the corneal plane and several other positions. The power at the position of the eye was measured with a Scientech 362 laser powermeter. The exposure intensity was varied through the use of calibrated neutral density filters. The experimental layout is shown in Fig. 2.

Experimental subjects were adult rhesus monkeys (Macaca mulatta) ranging in body weight from 3 to 6 kg.* Eyes were dilated with atropine.

*The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act of 1970 and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources--National Research Council.