Progressive Cytologic Changes During the Development of Delayed Feather Amelanosis and Associated Choroidal Defects in the DAM Chicken Line

A Vitiligo Model

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Newly hatched *Gallus domesticus* chicks of the delayed amelanotic (DAM) line have phenotypically normal down pigmentation. Functioning pigment cells are present in the down plumage, choroid, and retinal pigment epithelium. However, histologic and ultrastructural studies reveal that after hatching regenerating feather melanocytes synthesize melanosomes with abnormal, irregularly shaped surfaces and pigmented extensions. Eventually retraction of melanocytic dendrites and clumping of pigment occurs concomitantly with intracellular compartmentalization of the abnormal melanosomes. Melanocyte degeneration is accompanied by the appearance of mononuclear leukocytes (MNLs) in the pulp of the regenerating feathers. Concurrently, melanocytes cease to migrate into the regenerating feather epithelium, and the result is amelanosis. Changes in choroidal melanocytes are first evident as swelling of cell bodies and associated dendrites. Ultrastructurally, the choroidal melanocytes demonstrate increased cytoplasmic material, melanosomal irregularities, retraction of dendrites, melanosome compartmentalization, and eventual necrosis. Concurrently, MNLs arrive and remove the pigment from the choroid. The authors conclude that a basic melanocyte defect precedes the arrival of immunocytes in the delayed cutaneous and choroidal amelanosis in the genetic DAM vitiligo model of the chicken (Am J Pathol 1983, 111:197–212)

CHICKENS of the delayed amelanotic (DAM) line, which exhibit a high incidence of plumage depigmentation and loss of visual ability, possess a competent pigment cell system at time of hatching.[1] The initial sign of amelanosis is a complete or partial lack of pigment in regenerating feathers between 5 weeks and 6 months after hatching. The postnatal development of feather amelanosis precedes loss of visual ability, frequently progressing to blindness. Neither physiologically pigmented melanocytes nor melanocytes induced to synthesize melanin upon incubation of feathers with the melanin precursor l-dopa (ie, dopa-positive melanocytes) are present in regenerating feathers of the completely amelanotic adult DAM chicken.[2] Similarly, the melanocytes of the choroid are absent in amelanotic adult DAM chickens, regardless of the extent of visual impairment.[3] The severity of visual impairment in the DAM line is directly related to the extent of degenerative changes in the retinal pigment epithelium (RPE) and neural retina. These changes are first seen around the optic nerve head and the base of the pecten and progress radially into the surrounding retina in an irregular manner.[3]

Preliminary examination of a choroid from a DAM chick beginning to develop feather amelanosis reveals a large number of mononuclear leukocytes (MNLs) highly associated with melanocyte debris

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randomly scattered throughout the choroid.[4] Neonatal bursectomy (removal of the central lymphoid organ controlling differentiation of B lymphocytes) of DAM-line birds significantly reduces the frequency and severity of feather amelanosis.[5] These observations suggest that the immune system is in some way associated with the selective elimination of the DAM melanocyte population. In addition, the DAM line exhibits a hyperactive immune system, as shown by a significantly greater antibody response to foreign antigens (sheep red blood cells and *Brucella abortus*) than unrelated controls during early growth.[6]

Striking similarities have been noted between the delayed amelanosis of the DAM line of chickens and the cutaneous depigmentation of patients with vitiligo.[1] Both disorders represent a progressive destruction of cutaneous melanocytes, usually seen first in adolescence or early adulthood. In both, the amelanosis may be either partial or complete, although complete cutaneous pigment loss is far less common in people than in chickens. Remelanization is possible in both cases, although this also occurs more frequently in the chicken. Ocular pigment disturbances have been found in the DAM chicken[3] and human vitiligo patients.[7,8] It is common to see a combination of vitiligo, thyroid disease, and alopecia areata in man.[9] Hypothyroidism and a feathering defect leading to denuded areas resembling human alopecia areata are frequently present in the DAM line. The similarities between vitiligo and the delayed amelanosis in chickens suggest that the latter may be useful as an animal model for the human depigmentation disorder.

To determine the sequence of events and the histologic and fine-structural changes leading to the delayed and selective elimination of melanocytes in regenerating feathers and the choroid, we have studied the respective tissues systematically by light and electron microscopy in populations of newly hatched, growing DAM chicks.

Materials and Methods

Light-microscopic analyses were performed on eyes and regenerating feathers of 45 prospective DAM (PDAM) chicks resulting from matings between blind, completely amelanotic DAM-line parents. At hatching, the progeny chicks were divided into nine groups of 5 birds each. Beginning at the day of hatching and at biweekly intervals thereafter, PDAM chicks from one of the groups had the right eye removed surgically. We immediately injected Bouin's fixative into the posterior chamber of the eye to ensure rapid diffusion of the fixative into the retinal and choroidal layers. The eye was then immersed in Bouin's fixative for 24 hours and subsequently processed by a standard technique of embedding tissue in paraffin. The embedded eyes were cross-sectioned on a rotary microtome. Ribbons containing five consecutive sections were saved every 400 μ throughout the orbit, mounted on microscope slides, and stained with hematoxylin and eosin (H&E). This technique provided representative samples for light-microscopic analysis of between 35 and 50 cross-sectional areas per eye. We assessed the visual ability of the remaining left eye each week in order to determine the time interval between ocular biopsy and the onset of visual impairment. We assessed the visual ability by placing the bird on the floor and observing its response to a slowly approaching hand. A bird showing an escape response to a hand approximately 12 inches away was recorded as "sighted." A bird responding only when the hand was within 6 inches of the eye was recorded as "partially sighted," and a bird that did not respond was recorded as "blind." Preliminary experiments had shown that the surgical procedure of monocular enucleation did not induce a collateral change, similar to sympathetic opthalmia, in the remaining eye.[4]

Throughout the above trial, we plucked breast feathers of these birds every week to induce feather regeneration. Two-week-old regenerating feathers approximately 5 mm long were plucked, fixed with Bouin's fixative, processed by standard embedding techniques, and examined serially in cross-section by light microscopy. A regenerating feather is cylindrical and consists of peripheral layers of epithelial cells and a central core, or pulp. Epithelial cells proliferate in the basal tip, migrate up the cylinder, and organize into barb ridges that also house the melanocytes (Figure 1). For a review of the anatomy of the feather follicle, see Lucas and Stettenheim[10] or Foulks.[11]

The above schedule of feather plucking provided feather tissue at weekly intervals from each bird prior to the onset of amelanosis. Onset of amelanosis was defined as the time when the base of regenerating feathers emerged gray or white, rather than brown, suggesting a dilution or an absence of feather pigment, respectively.

A control experiment of identical design was performed on two lines of non-PDAM pigmented chicks. The control lines consisted of the Brown (BR) line, in which amelanosis develops occasionally, and a Light Brown Leghorn (LBL) line, which is related to the BR line but does not develop spontaneous amelanosis.[1]

Electron-microscopic studies using the experimen-

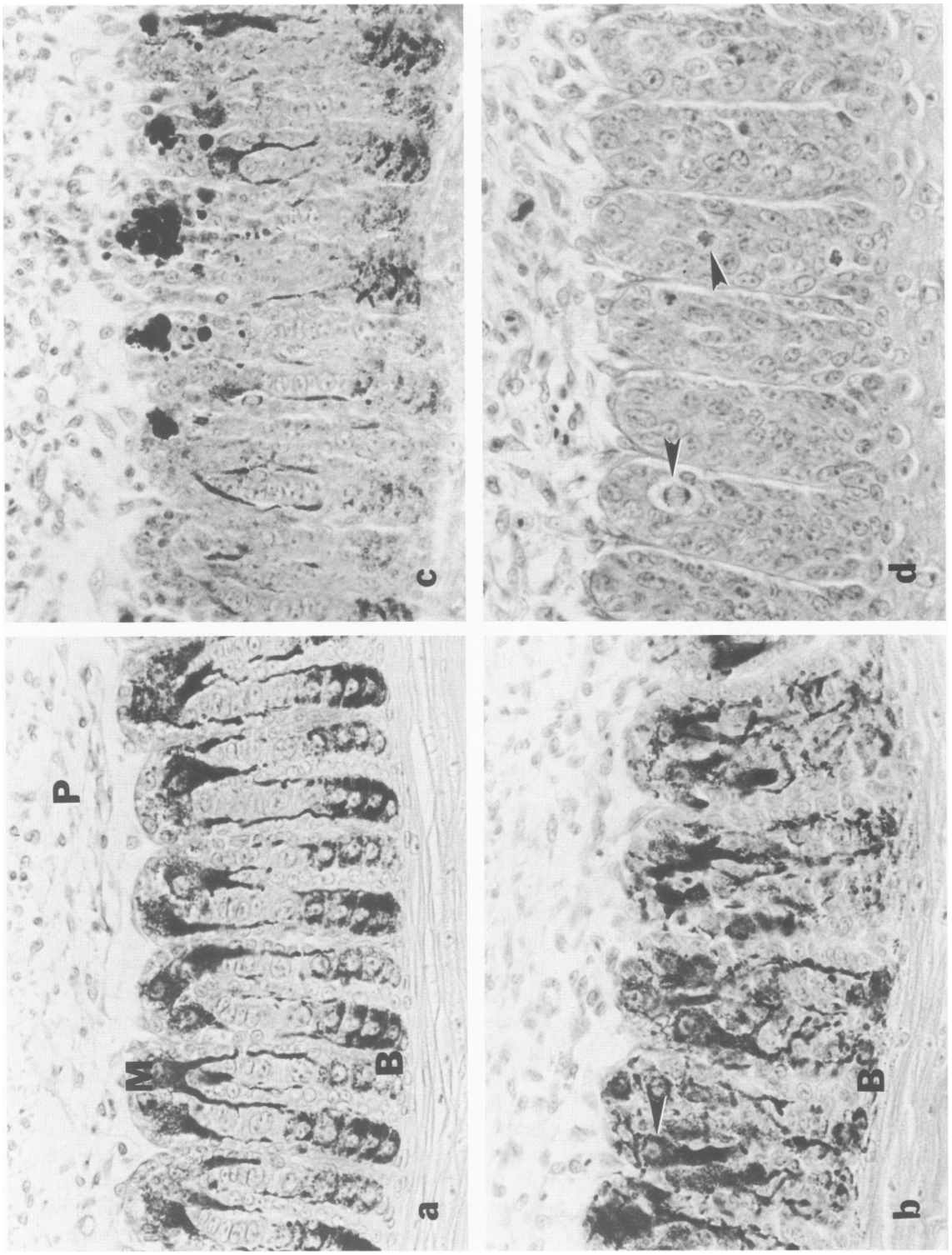

Figure 1 – Barb ridges of 2-week-old regenerating feathers from prospective DAM (PDAM) chickens. **a** – Tissue taken from PDAM 4196 at 7 weeks of age. This bird never developed feather amelanosis. Barb ridges contain morphologically normal melanocytes with pigmented perikaryons (*M*) and slender dendrites extending peripherally to the rows of barbule cells (*B*) where pigment is transferred (*P*-central pulp). **b** – Tissue taken from PDAM 3905 at 7 weeks of age and 3 weeks prior to the appearance of feather amelanosis. At this stage melanocytes are irregularly shaped and have surface protrusions and crooked dendrites with bulbous endings. Melanocyte (*arrowhead*) contains a thickened and densely pigmented dendrite suggestive of dendritic retraction. Note abnormal pigment transfer in the barbule cell area (*B*). **c** – Tissue taken from PDAM 4194 at 10 weeks of age and 2 weeks prior to the appearance of feather amelanosis. Barb ridges contain pigment clumps of different sizes and shapes. Some dendritic melanocytes are still present. There is also a drastic reduction in pigment transfer. **d** – Tissue taken from PDAM 4194 at 12 weeks of age during appearance of complete feather amelanosis. Barb ridges and barbule cells are devoid of pigment. Mitotic epithelial cells are readily apparent (*arrowheads*). (H&E, ×500) (With a photographic reduction of 13%)

Table 1—Description of PDAM Population Used in Light-Microscopic Analysis

Bird	Age at onset of amelanosis (weeks)	Age at onset of histologic changes (weeks)	Age at time of eye removal (weeks)	Choroidal histologic features of enucleated eye	Time elapsed between eye removal and onset of visual problems (weeks)
4187	4.5	3.5	0.5	N	4.5
3911	5	4	3.5	v AB	7
3900	5	4	3.5	AMEL	7
3896	5	5	3	sl AB	8
4192	5	4	1	N	10
4188	6	5	1	sl AB	7
4193	6.5	4.5	1.5	N	6.5
4165	7	5	2	N	9
4186	7	5	0	N	10
4205	7.5	4.5	1.5	N	8.5
4158	7.5	3.5	2.5	sl AB	12.5
4159	9	4	2	N	11
3905	10	3	3	N	13
4194	12	7	0	N	12
4202	36	2	0	AB	NS
4190	Died at 6 wks	3	0	AB	–
Mean*	7.1	4.4			
Standard deviation of the mean*	0.582	0.291			

N, normal; sl AB, slightly abnormal; AB, abnormal; v AB, very abnormal; AMEL, amelanotic; NS, normally sighted.
* Data excludes Birds 4202 and 4190.

tal design described above were done on a population of 27 PDAM chicks. Regenerating feathers and eye tissue were fixed with half-strength Karnovsky's fixative[12] in 0.1 M phosphate buffer, pH 7.2, for 4 hours at 4 C. The tissues were postfixed in 2% osmium tetroxide, dehydrated, and embedded in Epon 812. Sections 1-μ-thick were cut on a LKB-III ultramicrotome, stained with toluidine blue, and monitored with the light microscope for the extent of cytologic changes. Tissue chosen for ultrastructural analysis was thin-sectioned, stained with uranyl acetate and lead citrate, and viewed with a Zeiss 9S-2 electron microscope. Both control lines of chickens were included in the electron-microscopic analysis.

Results

Color of Regenerating Feathers

The incidence of feather amelanosis in the PDAM population analyzed by light microscopy was 39.0%. Specifics on the development of the disease in each bird are summarized in Table 1. The average age at onset of feather amelanosis was 7.1 weeks, ranging between 4.5 and 12 weeks of age in 14 of the 16 birds. Of note is Bird 4202, who developed feather amelanosis atypically late at 36 weeks of age. Three general patterns of disease expression were apparent during the onset of feather amelanosis. Five of the affected PDAMs immediately developed complete feather amelanosis. Eight birds first exhibited a short period of variable pigment dilution. This period preceded the emergence of complete white feathers by 2 to 6 weeks. Finally, two birds maintained erratic feather amelanosis throughout their lives, a condition characterized by a plumage in which varying portions of only a few feathers exhibit amelanosis.

Histologic Characteristics of Regenerating Feathers

Melanocytes in early, pigmented regenerating feathers, between the initial biopsy at 1 week of age and shortly before the onset of feather amelanosis, appeared normal by light microscopy. The cell bodies were located in the tips of the barb ridges, and pigmented dendrites extended peripherally to the barbule cells to which melanin was transferred (Figure 1a). This normal appearance was also observed in the developing feathers of PDAM birds that never developed feather amelanosis.

The initial histologic changes in regenerating feathers of PDAM birds were irregularities in the shape of melanocytes, beginning between 3 and 5 weeks of age (Table 1). The melanocyte abnormalities were first observed in feathers removed within 7 weeks prior to the onset of feather amelanosis. However, in an exceptional case (PDAM 4202) melanocyte abnormalities were observed 34 weeks prior to the onset of erratic amelanosis (Table 1).

Early melanocyte abnormalities most often con-

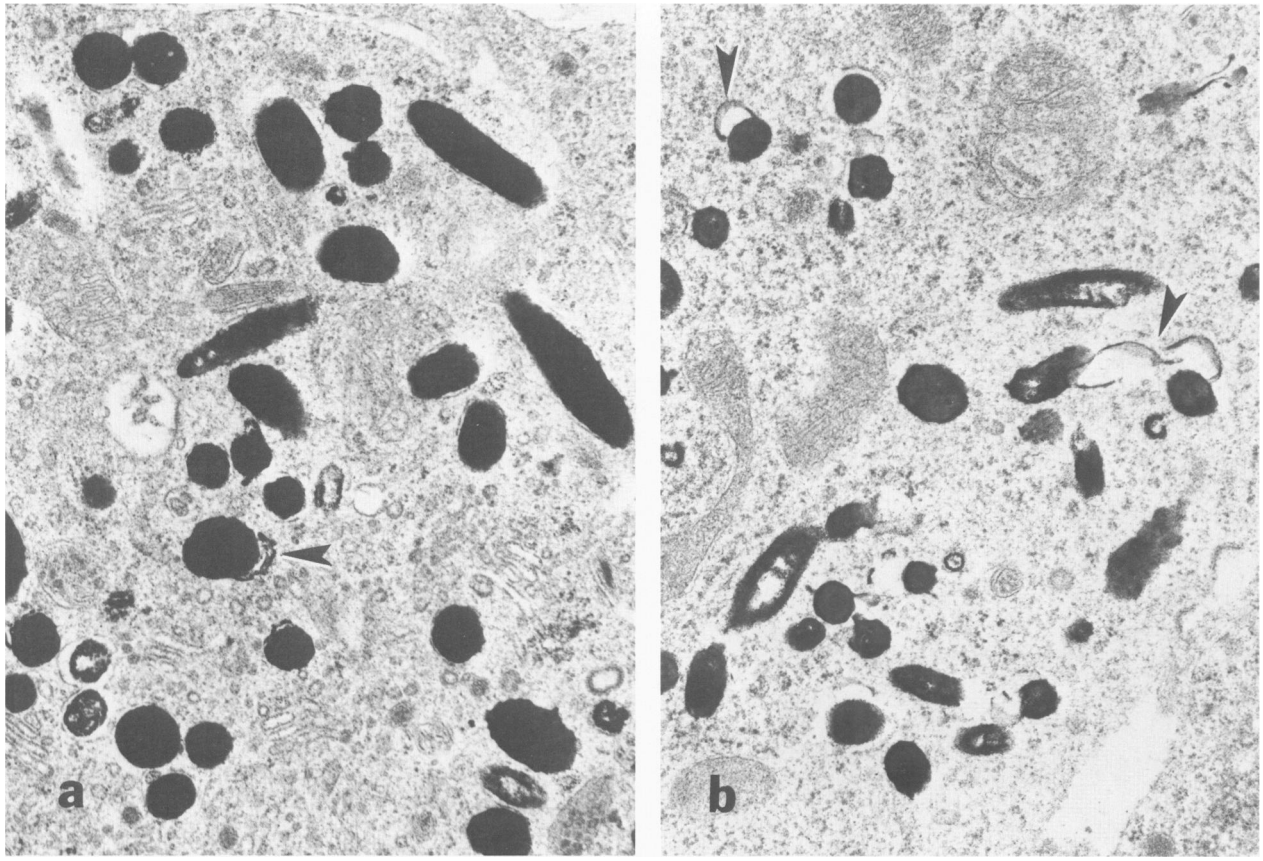

Figure 2a – Cytoplasm of barb ridge melanocyte within a regenerating feather taken from a 2-week-old control BR-line chicken. Most of the melanosomes are smoothly delineated; however, rare surface irregularities are seen (*arrowheads*). **b** – Barb ridge melanocyte within a regenerating feather taken from PDAM 6602 at 3 weeks of age, 7 weeks prior to the appearance of feather amelanosis. Many of the melanosomes contain DAM-specific pigmented extensions (*arrowheads*). (Uranyl acetate and lead citrate, ×30,000)

sisted of surface protrusions and crooked dendrites ending in bulbous swellings (Figure 1b). Also observed were slightly enlarged melanocytes with thick, short, dendrites suggestive of dendritic retraction (Figure 1b). Variations of these two defects were common. The defective melanocytes were generally scatterd randomly among apparently normal melanocytes. On occasion, an entire cross-sectional area contained only morphologically abnormal melanocytes. Eventually, morphologically abnormal melanocytes predominated, and pigment transfer to barbule cells was disrupted. A random appearance of adendritic pigmented clumps accompanied these changes (Figure 1c). The incidence of pigment clumps increased as the number of irregularly shaped melanocytes decreased, until large pigmented clumps and an absence of pigment transfer became the rule. During this melanocyte degeneration, the regenerating feather showed increasing pigment dilution. Subsequently, pigment disappeared completely from the barb ridges, and the emerging feathers were amelanotic (Figure 1d).

Ultrastructure of Regenerating Feathers

Histologically normal melanocytes in early regenerating PDAM feathers produced abnormal melanosomes in contrast to the control (Figure 2a). The abnormal melanosomes had pigmented extensions (Figure 2b), some of which were only short blebs protruding from the melanosomal surface (Figure 3), while others were extremely long (Figure 3a). These slender, heavily pigmented extensions were continuous with and had the same electron density as the outer rim of the melanosomes. The extensions appeared to end abruptly or as bulbous swellings (Figure 3b). Occasionally, the extensions looped back onto the granules (Figure 3b). In almost all cases, an electron-lucent region was present between the curved extension and the body of the melanosome. The ab-

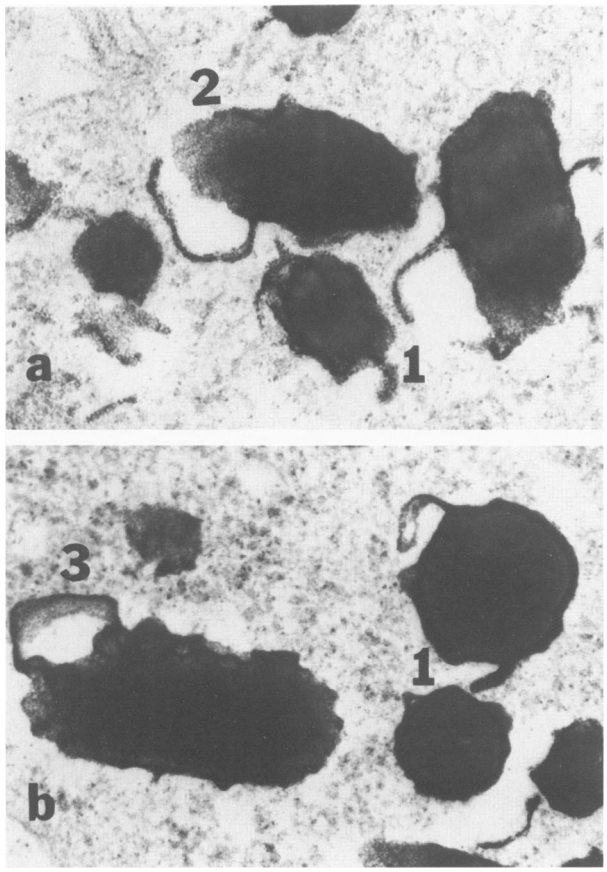

Figure 3 – Abnormal melanosomes in barb melanocytes from PDAM 510, at (**a**) 4 weeks and (**b**) 6 weeks of age, 4 and 2 weeks prior to the appearance of feather amelanosis, respectively. The pigmented extensions are continuous with the periphery of the granules and can appear as short protruding blebs (*1*), or as long extensions (*2*). Occasionally a pigmented extension loops back onto the granule, as in *3*. Note the electron-lucent region between the pigmented extension and the granule proper. (Uranyl acetate and lead citrate, ×68,000)

normal melanosomes were found in all early DAM feathers exhibiting normal dendritic melanocytes under the light microscope. However, they were less numerous in the regenerating feathers removed at 1 week of age than in those removed just prior to the appearance of histologically abnormal melanocytes.

In the parental BR line, the surface of melanosomes was usually smooth, and membrane irregularities or very short pigmented extensions were observed only occasionally (Figure 2a). Short pigmented extensions were observed even less frequently in LBL control chickens, whose melanosomal outlines were predominantly smooth. Pigmented extensions were also seen in developing feathers from PDAM birds that did not develop feather amelanosis. Although in these birds the extensions were much more prevalent than in BR and LBL control birds, they were not as extensive or as numerous as in the PDAM chickens that did develop feather amelanosis.

Other intracellular fine structural changes were correlated with abnormal melanocytes and pigmented clumps, as observed by light microscopy. Melanosomes, both with or without pigmented extensions, were aggregated into two general types of configurations within these abnormal melanocytes that were presumed to be retracting their dendrites (Figure 4). In one type of configuration, melanosomes appeared to fuse with each other by way of an electron-dense particulate material (Figure 5a). These melanosomal clusters differed drastically in size (Figure 4). In the second type of configuration, melanosomes were compartmentalized into membrane-bound vacuoles (Figure 4). These compartments differed in the concentration of granules and contained degenerated material. Eventually, all melanosomes were aggregated in a single large complex, while the melanocyte showed signs of necrosis (Figure 5b). Amelanotic feather tissue developing subsequent to this state was completely devoid of melanocytes (Figure 5c).

Morphologic Evidence of Involvement of the Immune System

The appearance of immunocytes during the pathologic changes leading to amelanosis was observed at the approximate time when the pigmented clumps were observed. Small cells with densely staining nuclei began to populate the pulp (Figure 6a). These cells were preferentially located close to the barb ridge–pulp border and were generally associated with areas of barb ridges displaying heavy pigment clumping. These newly arrived cells were small mononuclear leukocytes (MNLs) (Figure 6b). As the number and size of pigmented clumps increased, large MNLs readily identifiable with light microscopy were found within the veins and were scattered between the pulp cells, predominantly in the more central areas of the pulp (Figure 7). In general, after complete amelanosis was established, both the small and the large MNLs disappeared.

As a rule, immunocytes were not found inside the epithelial barb ridges except in the bird (PDAM 6603) that exhibited the most extensive pathologic conditions. In this bird, light-microscopic examination of a regenerating feather with diluted pigmentation and immediately preceding complete amelanosis showed an occasional abnormal barb melanocyte and many pigmented clumps. Ultrastructurally, the basement membrane demarcating the peripheral barb ridge

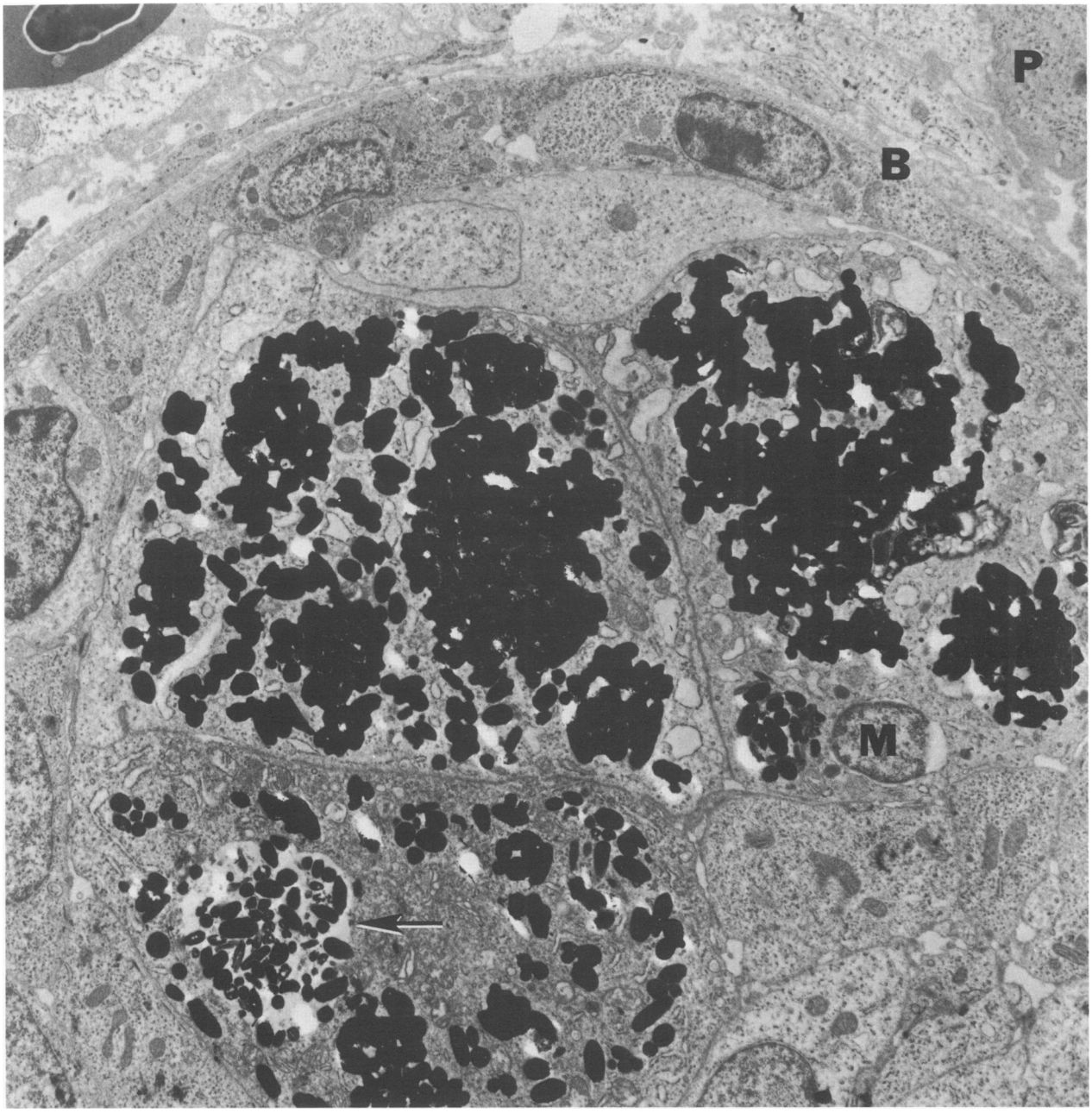

Figure 4—Barb ridge from PDAM 532 at 4 weeks of age and 1 week prior to the appearance of feather amelanosis. The melanosomes are fused to one another in dense clumps, which differ in size or are grouped loosely in a membrane-bound compartment (*arrow*) (*M*, nucleus of melanocyte, *P*, pulp, *B*, basement membrane of barb ridge). (Uranyl acetate and lead citrate, ×5400)

from the central pulp tissue was irregular in contour and occasionally disrupted (Figure 8). Inside the barb ridge a few MNLs with a small amount of cytoplasm were observed among melanocytes displaying various DAM-specific pathologic changes. The remaining area of the barb ridge consisted of barb cells that contained fat bodies and vesicles filled with debris. Most conspicuous were irregular and broken barb cell plasma membranes and distended intercellular spaces that were filled with particulate debris.

Histologic Characteristics of the Choroid

The incidence of blindness in birds developing feather amelanosis was 68.7%. The right eyes of the PDAM birds in this population were enucleated at

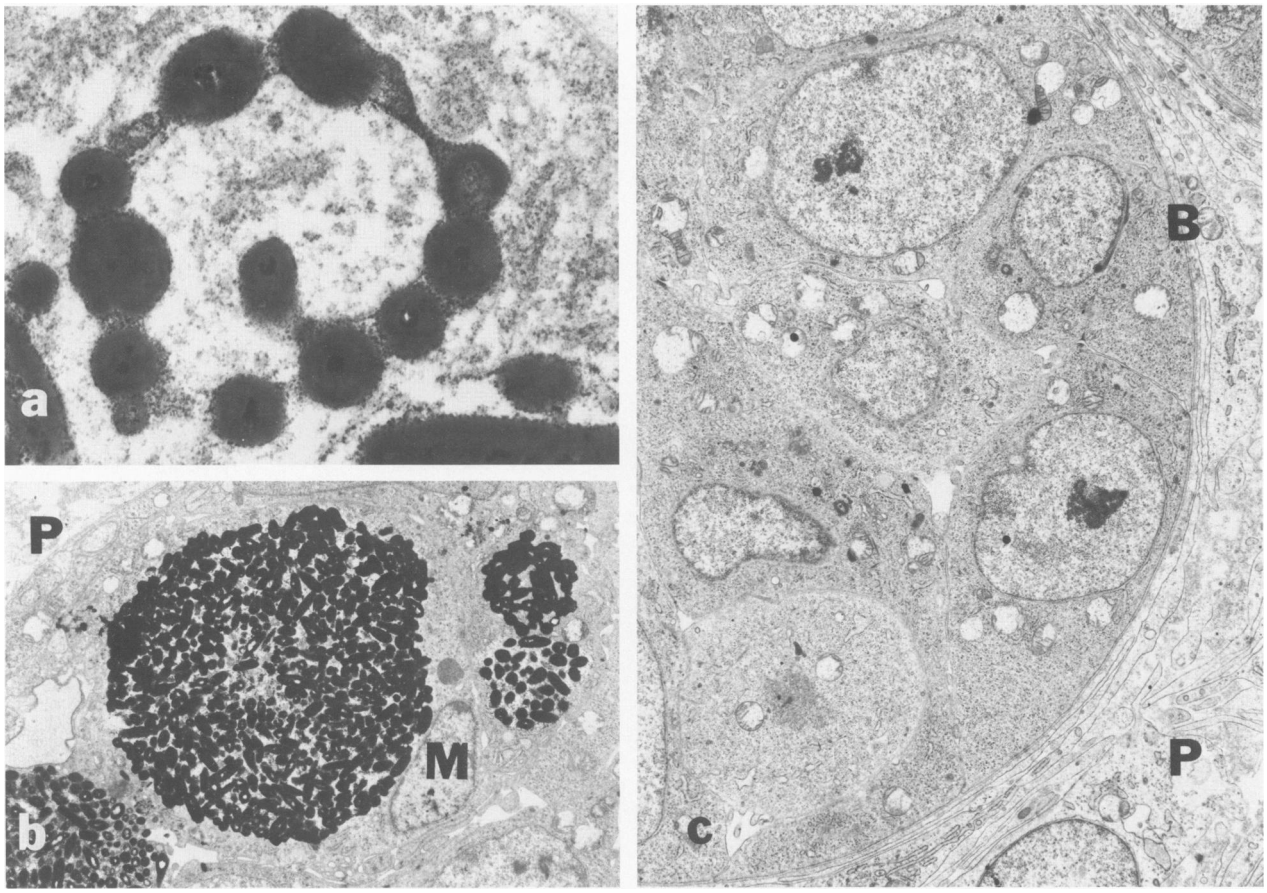

Figure 5a – Cluster of melanosomes in barb melanocyte from PDAM 510 at 5 weeks of age and 3 weeks prior to the appearance of feather amelanosis. The melanosomes in this configuration are linked by particulate material. **b** – Barb ridge with melanocytes (*M*) from PDAM 532 at 4 weeks of age and 1 week prior to the appearance of feather amelanosis. All melanosomes are aggregated in huge compartments. The two compartments at the *right* illustrate the variation in composition. **c** – Barb ridge from an adult amelanotic DAM demonstrating absence of pigment and normal architecture of barb ridge. (*P*, pulp, *B*, basement membrane of barb ridge; swollen mitochondria represent fixation artifact). (Uranyl acetate and lead citrate, **a**, ×42,000; **b**, ×4300, **c**, ×5300)

various times between the day of hatching and 3½ weeks of age (Table 1). The schedule of enucleation provided tissue from various time intervals prior to the onset of visual problems for the purpose of study of the developmental sequence leading to the selective elimination of choroidal melanocytes. However, analysis of the data presented in Table 1 indicates that there was extensive variation in the rate of appearance of pathologic changes leading to blindness. Therefore, in order to establish a sequence of DAM-specific pathologic events in the choroid, we have subjectively arranged the various histologic conditions in an order of increasing abnormality, summarized in Table 1. Stages in this sequence have been designated normal (N), slightly abnormal (slAB), abnormal (AB), very abnormal (vAB), and amelanotic (AMEL).

Approximately 50% of the PDAM choroids sampled appeared histologically normal (N in Table 1). The choroidal melanocytes were slender and dendritic and juxtapositioned around choroidal blood vessels (Figure 9a). The initial abnormalities appeared as swellings of the cell bodies and associated dendrites, as illustrated with the eyes of PDAM Birds 3896, 4158, and 4188 (slAB in Table 1 and Figure 9b). The number of affected choroidal melanocytes differed among the three birds. All choroidal melanocytes of PDAM 4188 appeared at least slightly affected, whereas only a few, randomly scattered melanocytes were swollen in PDAM Birds 3896 and 4158. In the latter birds, MNLs were occasionally observed near the slightly abnormal melanocytes.

Observations on the choroidal tissue of PDAM Birds 4190 and 4202 (AB in Table 1) suggested retraction of melanocytic dendrites. PDAM 4190 showed swollen melanocytes with short, thick dendrites with

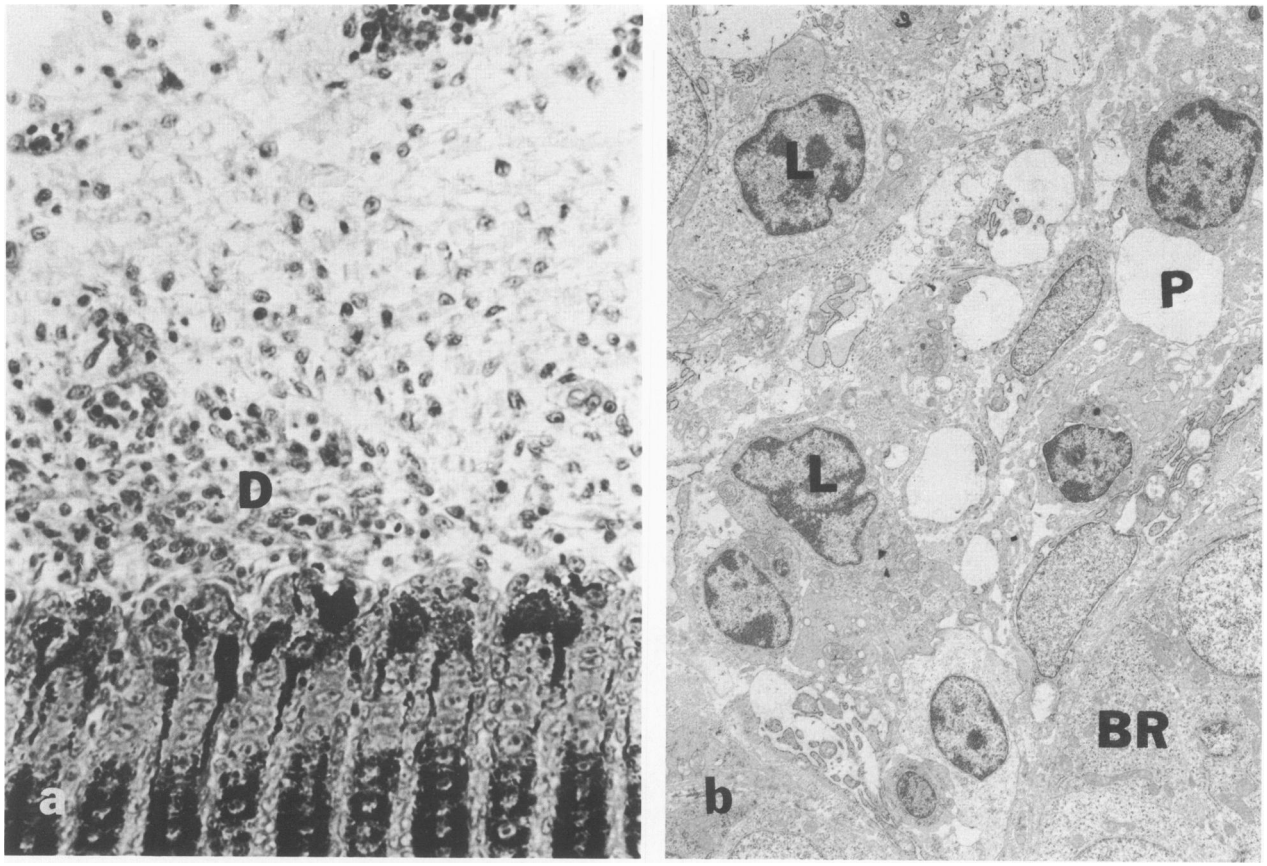

Figure 6a – Pulp/barb ridge interface of a regenerating feather from PDAM 4194 at 9 weeks of age and 3 weeks prior to the appearance of feather amelanosis. Micrograph demonstrates a pocket of small cells with densely staining nuclei (*D*) at the pulp-barb ridge interface adjacent to barbs containing abnormal melanocytes and pigmented clumps. (H&E, ×440) **b** – Pulp (*P*)/barb ridge (*BR*) interface within a regenerating feather taken from a DAM chick with many mononuclear leukocytes (*L*). (Uranyl acetate and lead citrate, ×3500)

the presence of an occasional spherical pigment clump (Figure 9c). In PDAM Bird 4202, on the other hand, all choroidal melanocytes were spherical, pigmented clumps (Figure 9d). Neither of these two birds contained conspicuous MNLs in their choroids. However, MNLs were abundant in the choroid of PDAM 3911 (vAB in Table 1 and Figure 9e). The melanocytes associated with this massive cellular infiltration appeared in discrete pockets that consisted of large pigmented clumps along with pigmented debris. PDAM 3900 (AMEL in Table 1) also exhibited massive invasion of MNLs in its choroid. However, the amount of pigmented debris was minimal (Figure 9f). The occasional pockets of pigment were restricted to the venous sinus.

Ultrastructure of the Choroid

The events in the elimination of choroidal melanocytes in the PDAM birds described above were analyzed ultrastructurally and summarized in Table 2. The ultrastructure of PDAM choroidal melanocytes that appeared histologically normal was identical to that of melanocytes from both control lines. The numerous pigmented melanosomes showed no abnormal surfaces (Figure 10a). These melanocytes were relatively inactive, as suggested by the rarity of cytoplasmic organelles, specifically endoplasmic reticulum and Golgi apparatus.

The initial ultrastructural changes in the melanocytes appeared as extensive surface folds that contained irregularly shaped melanosomes (Figure 10b). There was also an increase in perinuclear cytoplasm and organelles, suggesting increased metabolic activity. When small MNLs were observed, they were generally juxtapositioned against slightly abnormal melanocytes (Figure 10b).

Once clumping of choroidal melanocytes was conspicuous by light microscopy, compartmentalization of melanosomes was observed ultrastructurally (Fig-

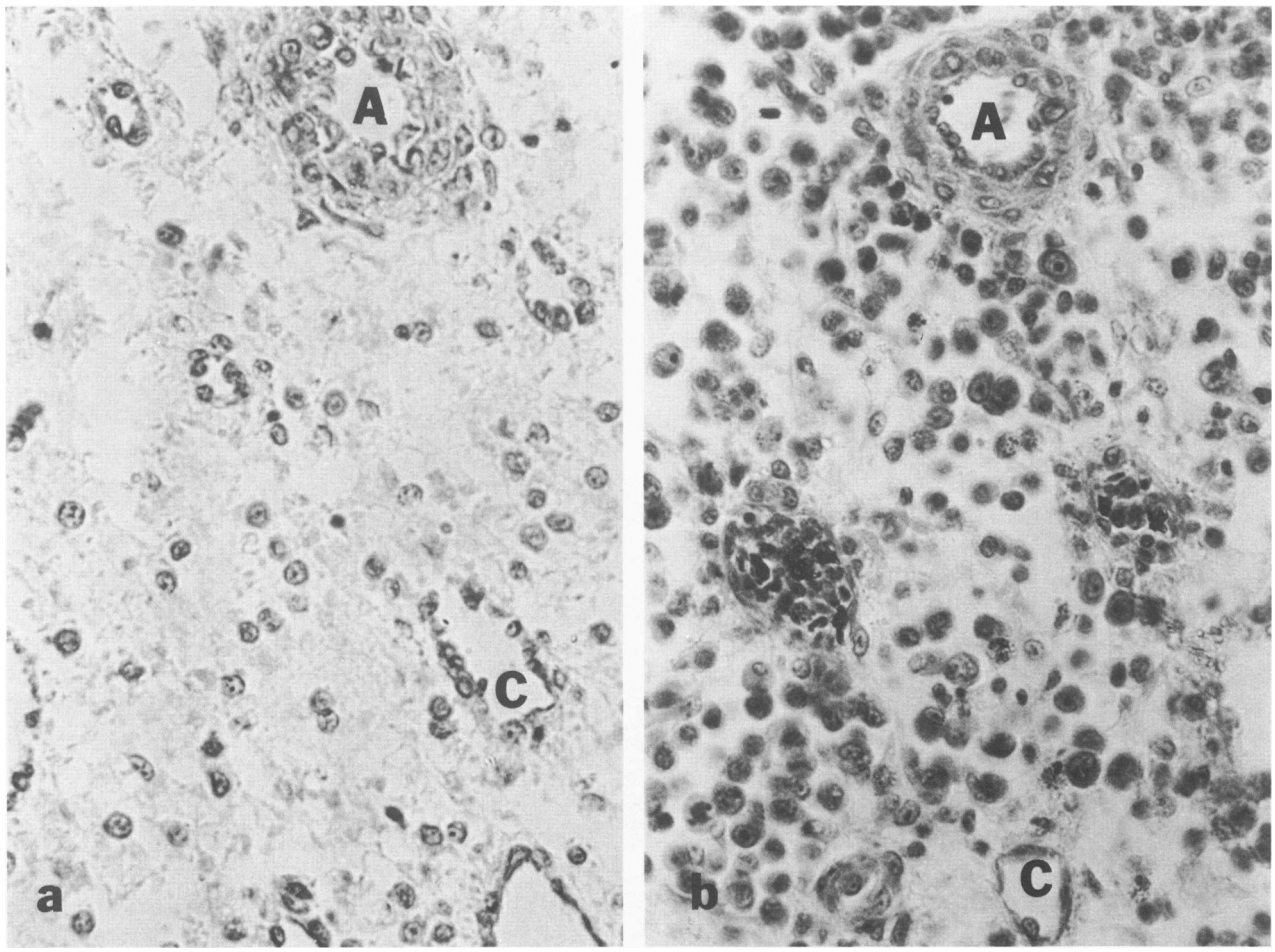

Figure 7—Central area of pulp from a regenerating feather taken from (**a**) a 5-week-old LBL control, containing normal pulp cell distribution; and (**b**) PDAM 3900 at 5 weeks of age during the appearance of feather amelanosis, containing large mononuclear leukocytes (*A*, axial artery; *C*, pulp capillaries). (H&E, ×600)

ure 11). This compartmentalization was similar to that in regenerating feather melanocytes. Most of the melanosomal aggregates appeared to have been formed by fusion of the outer edges of the granules. In addition, large, probably membrane-bound compartments were observed. Subsequently, the choroidal melanocytes were filled with compartmentalized melanosomes and had rarified cytoplasm and nuclei suggestive of necrosis (Figure 11). Numerous macrophages and lymphocytes and few granulocytes occupied much of the choroid, and extensive membrane interdigitation existed between all cells (Figure 11). Also of interest are some of the connective tissue cells of the choroid, which were slightly necrotic and had ruptured plasma membranes. Occasionally, extracellular spaces contained granular material. The ultimate fate of the DAM choroidal melanocytes was not followed by electron microscopy. We assume that the abundant MNLs eventually phagocytized and removed the necrotic melanocytes and/or debris, as indicated by the light-microscopic study.

Discussion

Morphologically normal melanocytes, residing in the developing feather of DAM birds as early as 1 week after hatching, synthesize abnormal melanosomes. Early DAM melanosomes have irregular membranes and pigmented extensions that appear as a continuation of the periphery of the melanosome proper. Both these abnormalities increase in number and extent as the PDAM ages and before the complete loss of feather pigmentation.

Eppig and Dumont[13,14] have observed similar extensions of melanosomes in dopa-incubated RPE cells of amphibian larvae. These extensions were portions of cisternae and/or tubules connecting the Golgi apparatus to the limiting membranes of premelanosomes and were thought to be responsible for the transport of inactive or inhibited tyrosinase to immature pigment granule.[13,14] However, in the RPE of amphibian larvae, complex melanosomes are eventually produced by the continuous deposition of

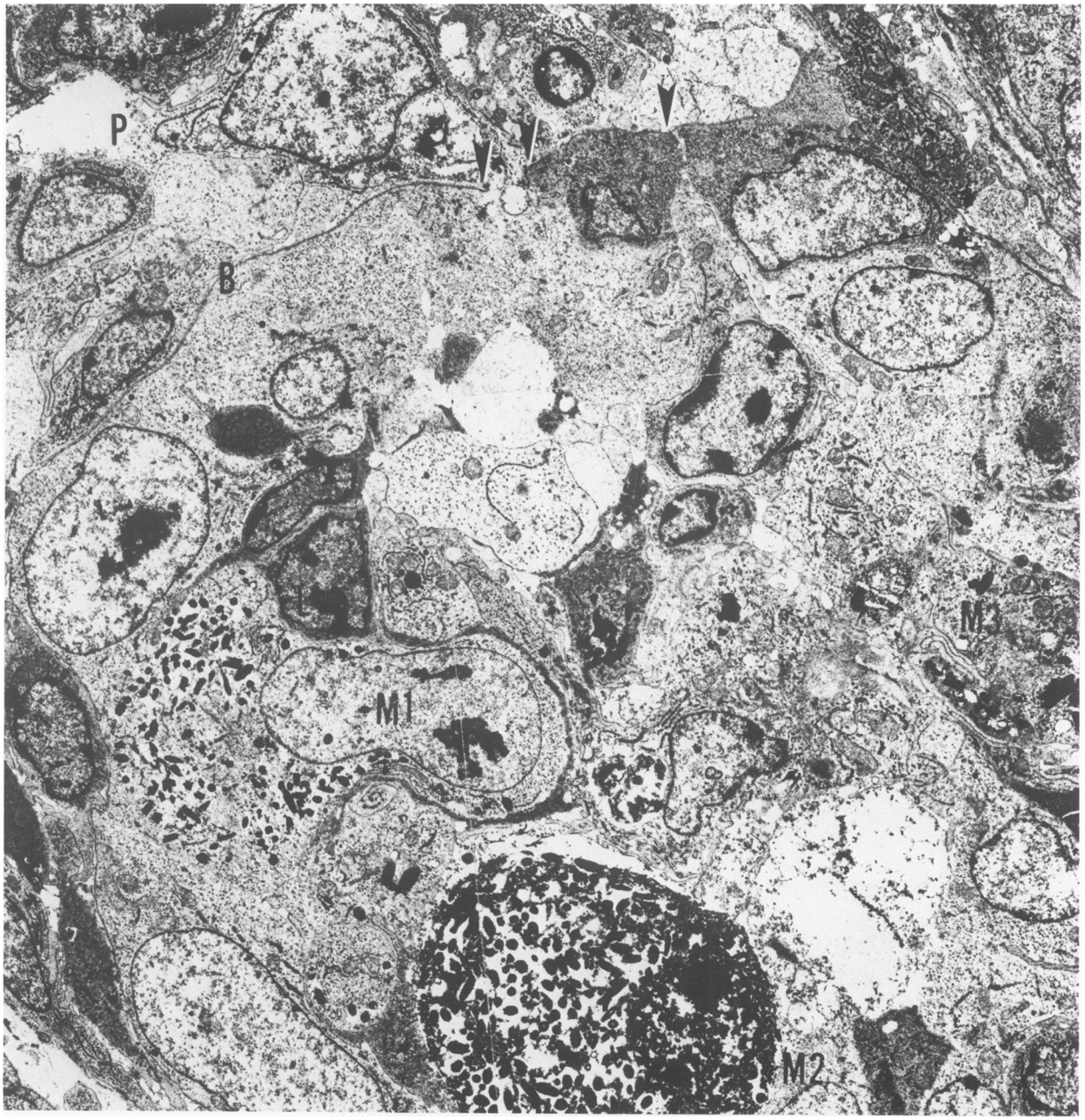

Figure 8—Rare pathologic barb ridge from PDAM 6603 at 8 weeks of age during the appearance of feather amelanosis. Melanocytes exist in various conditions; *M1*, melanosomes have pigmented extensions; *M2*, melanosomes are contained within a large compartment; *M3*, melanosomes are in small clusters. Barb ridge is infiltrated by small mononuclear leukocytes (*L*) and contains extensive cellular debris. The basement membrane (*B*) appears disrupted (*arrowheads*), and the adjacent pulp (*P*) also exhibits extensive cellular degeneration. (Uranyl acetate and lead citrate, ×5700)

new melanin around melanosomes derived from the oocyte[15] suggesting that a structurally continuous supply route of tyrosinase to the premelanosomal organelles is maintained.

Other reports in the literature concerning transport of tyrosinase from the Golgi apparatus to the premelanosomes support the hypothesis of a vesicular, structurally discontinuous connection. Ultrastructural and cytochemical studies on aldehyde-fixed murine and human melanoma cells have shown that, on incubation with dopa, melanin is deposited in the Golgi–endoplasmic reticulum–lysosome complex, GERL.[16–18] Tyrosinase is transported via coated vesicles from the GERL to the premelanosomes, where the enzyme is activated.[18] An identical process has been suggested to occur in the melanocytes of embry-

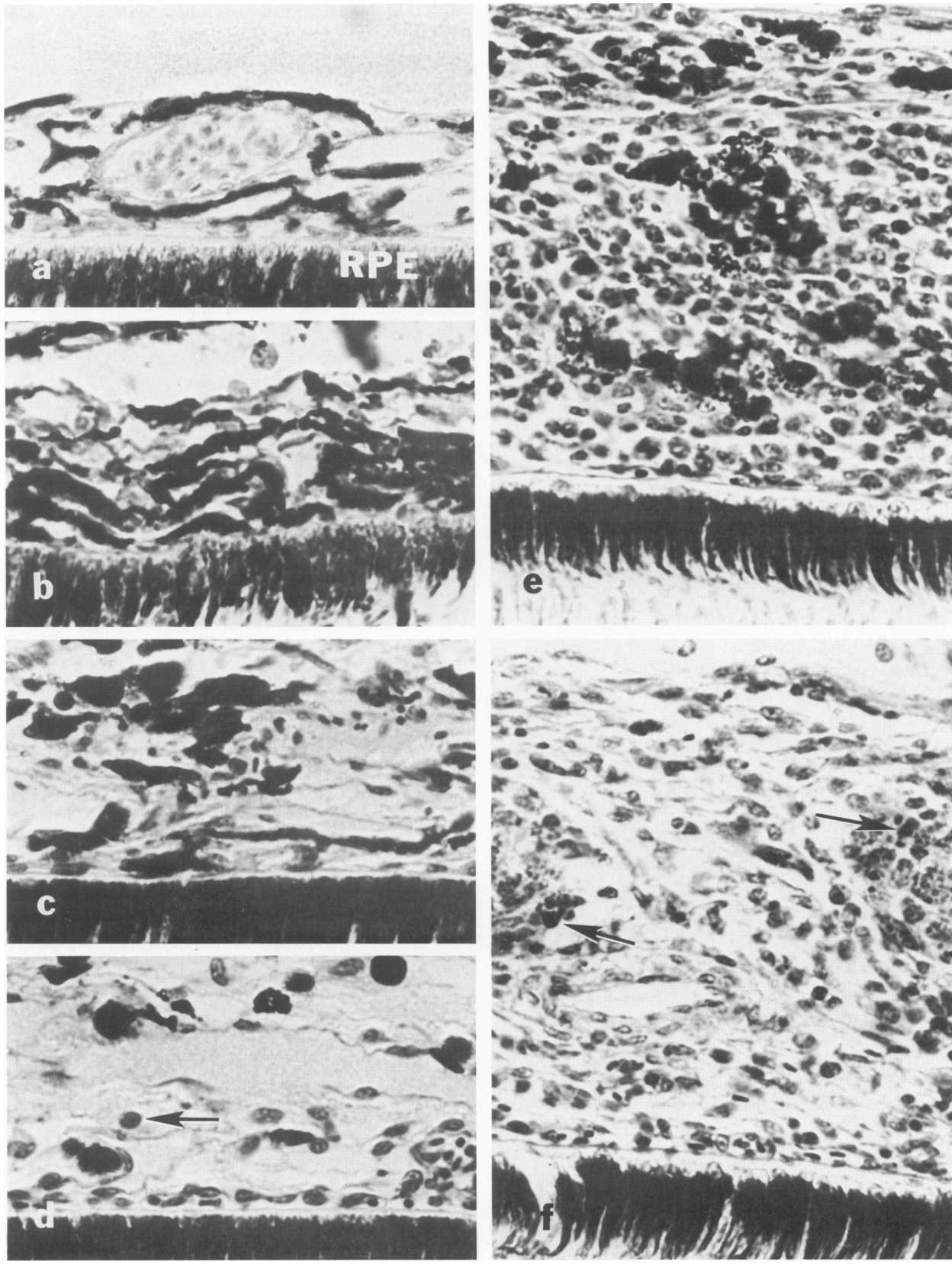

Figure 9 – Choroid from (**a**) a 2-week-old LBL control with normal slender, dendritic melanocytes, (**b**) 1-week-old PDAM 4188, demonstrating crooked and slightly swollen melanocytes, (**c**) 1-day-old PDAM 4190, demonstrating dendritic retraction and formation of spherical pigmented clumps, (**d**) 1-day-old PDAM 4202, demonstrating numerous spherical pigmented clumps and a large mononuclear leukocyte (*arrow*); (**e**) 3½-week-old PDAM 3911, demonstrating pockets of pigment debris and massive invasion of mononuclear leukocytes; (**f**) 3½-week-old PDAM 3900, demonstrating residual pigment within the choroidal sinus (*arrow*). Numerous mononuclear leukocytes are still present in the surrounding connective tissue (*RPE*, retinal pigment epithelium) (H&E, ×670) (With a photographic reduction of 4%)

onic feather papilla and in the avian RPE.[19] On occasion, dopa reaction product has been seen inside tubular profiles that seemed to be attached to melanosomes of feather melanocytes.[20] Maul and Brumbaugh suggested that the tubules were the result of fusion of the melanosomes with coated vesicles that were still attached to the Golgi-associated membrane system from which they originated. Maul and Brumbaugh also observed melanosomes with tubules that looped back onto the melanosome, and double membranous sheets filled homogeneously with dopa reaction product. Concerning the DAM line chicken, it is premature to conclude that the pigmented extensions described in this report represent a route of increased tyrosinase deposition.

Dendritic melanocytes with normal melanosomes are generally found at hatching in the choroids of PDAM birds that eventually go blind. The initial signs of DAM-specific pathologic changes in the choroid indicate an increase in surface irregularities, although pigmented extensions were not observed. Melanosomal surface irregularities were concomitant with an increase of cytoplasm and organelles and a folding of the plasma membrane. Whether these observations represent an increase in general cellular metabolism or biosynthesis specific for the process of melanization is not known at this time.

Table 2—Histologic Correlates on Choroids of DAM Birds Used for Electron-Microscopic Analysis

Bird	Age at time of enucleation (weeks)	Histologic appearance of choroid: Melanocyte condition	Histologic appearance of choroid: Presence of MNLs
6595	3	Normal	None
6601	3	A few swollen	None
6592	4	A few swollen	None
6888	4	All slightly swollen	Few
6603	4	Slightly swollen with some clumps	Some
6602	3	Very swollen with some clumps	Many
6887	4	Pigment clumps only	Many

Eventually, both choroidal and feather melanocytes become large, spherical pigmented clumps. Simultaneously, selective aggregation and/or compartmentalization of melanosomes occurs. Although the DAM melanocytes give indication of autophago-

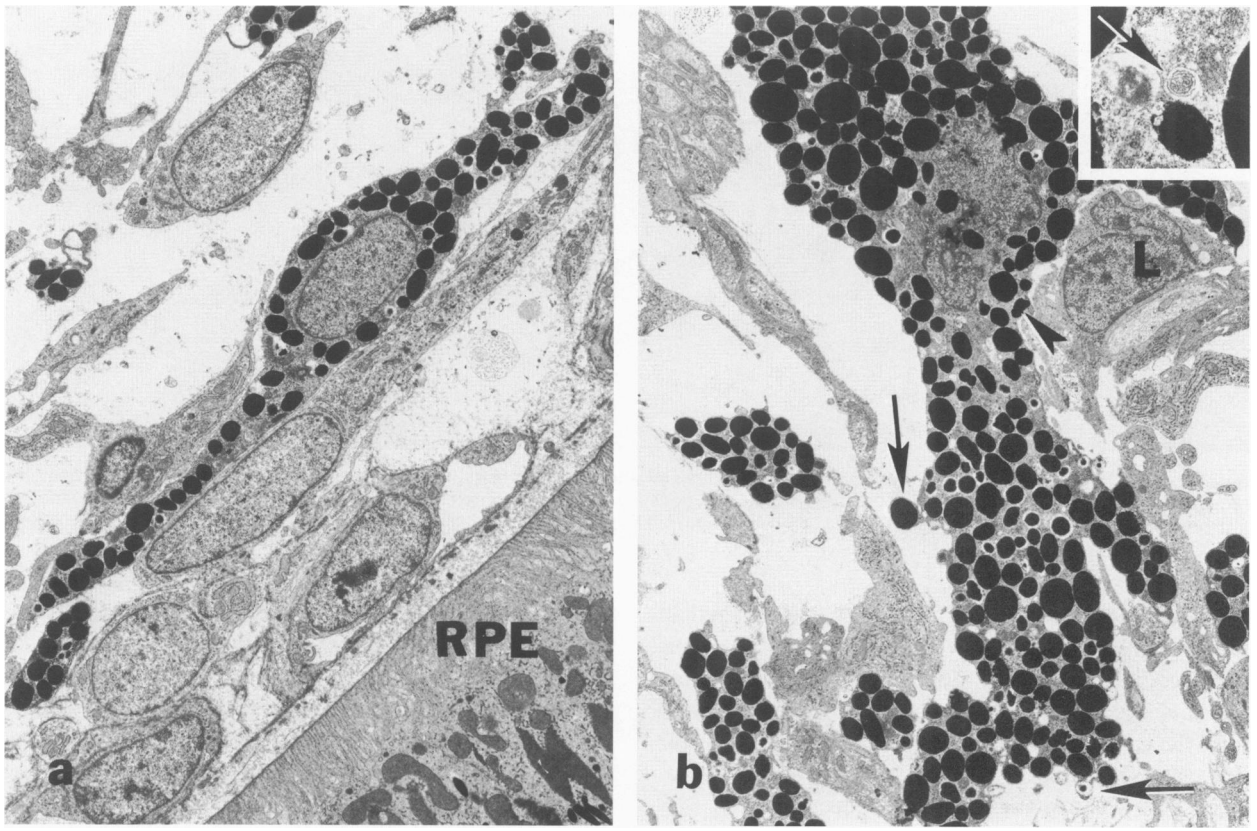

Figure 10a—Choroid from 3-week-old PDAM 6595, which never developed feather amelanosis. This electron micrograph demonstrates a normal-appearing choroidal melanocyte with slender dendrites and many smoothly delineated melanosomes (*RPE*, retinal pigment epithelium). **b**—Choroidal melanocyte from 4-week-old PDAM 6601. The dendritic area is markedly enlarged, and many melanosomes have surface irregularities (*arrowheads*). The plasma membrane has many protrusions containing melanosomes (*arrows*). Note the close juxtaposition of the mononuclear leukocyte (*L*) and the abnormal melanocyte. **Inset**—The existence of premelanosomes (*arrow*) within DAM choroidal melanocytes. (Uranyl acetate and lead citrate, ×4500; **Inset**, ×20,000) (With a photographic reduction of 4%)

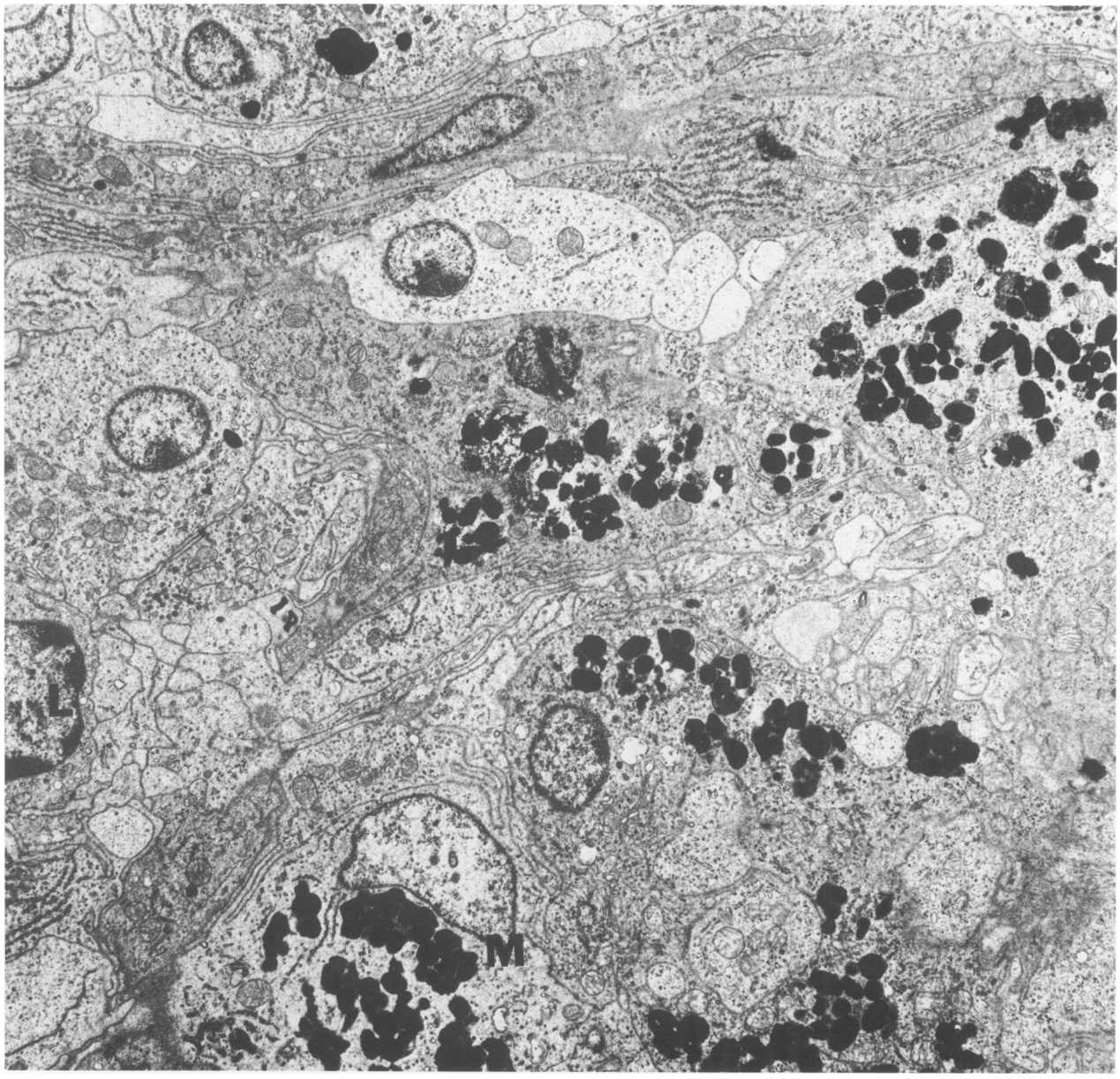

Figure 11 — Choroid from 4-week-old PDAM 6887. Melanocytes are now adendritic and contain various types and sizes of melanosomal compartments. Melanocyte *M* has a rarified nucleus and cytoplasm suggestive of necrosis. The choroid possesses many mononuclear leukocytes (*L*). Extensive surface interdigitations exist between all cells, leaving little or no extracellular space. (Uranyl acetate and lead citrate, ×6500)

cytosis of melanosomes similar to that described in feather papillae of the White Leghorn fowl[21] and the RPE associated with the tapetum lucidum of bovine fetuses,[22] acid phosphatase activity or other lysosomal enzymes[23] have not yet been studied in our birds. DAM melanocytes with the apparent autophagocytic activity eventually become necrotic.

A large number of cells from the immune system appeared in the late stages of the developmental events occurring in the young PDAM tissues. We propose that these immunocytes aid in the removal of the abnormal melanocytes. Removal of abnormal melanocytes by scavenger immunocytes was apparent in the PDAM choroid. However, in regenerating feathers, a relationship between the arrival of MNLs and the elimination of melanocytes can only be suggested. The developing feather is constantly being supplied with newly differentiating melanocytes. During feather regeneration, melanoblasts migrate from an unknown reserve pool, presumed to be located in the vicinity of the dermal papilla at the base of the feather.[11] When inside the regenerating

feather, differentiated melanocytes migrate into the epidermal collar, move up the growing feather as the barb ridges form, and eventually become incorporated into the keratinized feather as it emerges. The migrating melanoblasts at the vascularized base of the regenerating feather would be in a vulnerable position to be attacked by stimulated immune cells. Small MNLs initially and predominantly appear in the pulp when melanocyte clumping begins. Arrival of small MNLs is subsequently followed by the appearance of large MNLs in the central areas of the pulp during the onset of amelanosis. We propose that these MNLs represent a "spill-over" of immune cells from the basal areas of the feather follicle where stimulated MNLs are reacting against the migrating melanoblasts and prevent their entry into the developing feather. When complete feather amelanosis has developed in adults, MNLs are not generally observed in the pulp of regenerating feathers,[4] suggesting that the reserve pool of melanoblasts has been eliminated completely.

In a PDAM bird, MNLs were observed inside the barb ridge immediately preceding amelanosis. The barb cells were damaged extensively, and extracellular degenerative material existed. This severe condition parallels that in the choroid, in which abnormal melanocytes, immunocytes, and damaged connective tissue cells coexisted. During depigmentation in human vitiligo, cellular degeneration and generation of debris are also not limited to the melanocytes. Foci of vacuolar degeneration of basal and parabasal keratinocytes plus deposits of extracellular granular material, presumed to be derived from the cytoplasm of vacuolated keratinocytes, were present in the clinically normal pigmented skin of rapidly depigmenting patients.[24] It was suggested that toxic intermediates of melanogenesis[9] induced the destruction of both melanocytes and adjoining keratinocytes.[24,25] Such a degenerative relationship appears to exist between the melanocyte and the barb cells or the connective tissue cells in the respective regenerating feather or the choroid of the PDAM birds.

We also propose that the expression of amelanosis in the DAM line is caused by the relationship between a melanocyte-specific defect and the immune system. The DAM genotype interferes with the normal continuation of melanization in the feather and probably also in choroidal melanocytes. An altered process of melanization may have severe consequences for the melanocyte. Melanin precursors, phenols and hydroquinones, can be toxic to melanocytes,[26] and may cause destruction of pigment cells[9,27] and other types of cells.[28] We hypothesize that toxic effects of an abnormal melanization process in the DAM melanocyte population induces compartmentalization and/or autophagocytosis of abnormal melanosomes as an unsuccessful mechanism for cell preservation. In addition, extensive autophagocytosis may also be detrimental to the melanocyte, resulting in necrosis. Necrosis of melanocytes would release melanocytic antigens, which could stimulate an autoimmune response against intact melanocytes and/or melanoblasts.

One relationship between the immune system and the DAM disease has been documented. Lamont and Smyth[5] have shown that neonatal bursectomy of DAM-line birds significantly reduces the frequency and severity of amelanosis. Furthermore, within a group of adult DAMs, occurrence and severity of feather amelanosis and loss of visual ability were correlated with elevated antibody responses to foreign antigens (sheep red blood cells and *Brucella abortus*).[29] The loss of visual ability in the DAM birds results from substantial degeneration of the RPE and retina, beginning in the region surrounding the optic nerve and the base of the pecten, and progressing radially.[3] In the earliest stages of RPE amelanosis, lymphocytes and macrophages invade the RPE-photoreceptor layers adjacent to the optic nerve, while in advanced stages, plasma cells are present throughout the dystrophic DAM retina.[30] The destruction of the RPE-photoreceptor layers generally occurs after MNLs have infiltrated into the entire choroid, leading to complete choroidal amelanosis.

We had previously suggested that the DAM genetic defect is expressed postnatally.[1] The ultrastructural observations of this study challenge this hypothesis. In all cases, including both PDAM birds and their pigmented siblings, melanosomes with pigmented extensions were prevalent in regenerating feathers as early as 1 week of age. Of special significance is PDAM Bird 4202, which did not develop feather amelanosis until 9 months of age and yet had definite histologic abnormalities in the choroid at 1 day of age and in regenerating feathers at 1 week of age. With these observations we have shown that the melanocyte abnormality can be present at hatching and suggest that the delayed onset of the clinical disease is dependent on the involvement of the immune system in the development of amelanosis. The suggested dependency of the delayed expression of amelanosis on the immune system is plausible, because time may be necessary to liberate sufficient antigens to attain the minimun threshold level that must be reached before immune system sensitization occurs.

The initial trigger for the synthesis of abnormal melanosomes within DAM melanocytes has not been identified. Mutation is a likely candidate, because

horizontal transmission of this disease does not occur and viral particles have not been observed ultrastructurally. However, the role of provirus incorporation into DNA and vertical transmission in the DAM population cannot be ruled out.[31,32] Further investigations on early DAM melanocytes should provide valuable clues to the identity of the initiator of the pathologic events detailed in this study.

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