An Ultrastructural Study of Nutritionally Induced and Reversed Retinal Degeneration in Cats

K. C. Hayes, DVM, PhD, Arnold R. Rabin, MS and Eliot L. Berson, MD

Kittens and adult cats fed a semipurified diet containing casein developed a retinal degeneration that initially involved photoreceptor outer segments in the area centralis. By electron microscopy, cone and rod outer segment lamellar discs could be seen to become vesiculated, frayed, disoriented and twisted. Shortening and subsequent disappearance of outer segments was followed by loss of photoreceptor nuclei, primarily in the area centralis but also in the midperipheral retina. The electroretinogram (ERG) indicated progressive reduction in cone and rod amplitudes and a delay in the temporal aspects of the cone response. When dietary casein was replaced by egg albumin in the diets of cats with minimal to moderately advanced degeneration, the degeneration was reversed; rod ERG function and structure returned essentially to normal, whereas some abnormalities of cone outer segment structure and a delay in the temporal aspects of the cone ERG persisted. The data provide strong evidence that dietary casein is a factor in this retinopathy and suggest that an alteration in protein metabolism of the photoreceptor may result from the dietary protein inadequacy. (Am J Pathol 78:504–524, 1975)

A PREVIOUS STUDY indicated that kittens fed a specific semipurified diet containing casein as the only source of protein developed abnormalities in retinal function and structure.[1] In the early stages of this degeneration, ophthalmoscopic examination revealed hyperpigmentation of the area centralis followed by depigmentation and development of a focal hyperreflective spot in this area. In more advanced stages the shape of the fundus lesion could be correlated with the distribution of cones in the normal cat retina as defined by direct cell counts.[2] The earliest change in the electroretinogram (ERG) occurred within 3 months and was a delay in the temporal aspects of the cone ERG associated with a slight reduction in cone and rod ERG amplitudes. During a 1-year study, marked decrease in amplitudes of both rod and cone ERG responses occurred with enlargement of the fundus

From the Department of Nutrition, Harvard School of Public Health and the Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, Mass.

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Address reprint requests to Dr. K. C. Hayes, Department of Nutrition, Harvard University School of Public Health, 665 Huntington Ave, Boston, MA 02115.

lesion in the area centralis and eventual attenuation of the retinal arterioles. At a time when changes were clearly visible with the ophthalmoscope, light microscopic examination revealed a loss of photoreceptor outer segments in the area centralis.

The present electron microscopic study was undertaken to document the earliest morphologic changes associated with this degeneration and to determine if the effects of this diet on cone and rod function as demonstrated by the ERG could be correlated with ultrastructural alterations of these degenerating photoreceptor systems. A second objective was to determine whether the degeneration could be prevented or reversed by substituting other sources of protein for the casein in the semipurified diet.

Materials and Methods

Animals and Diets

The retinas described in this report represent observations on 53 domestic cats (14 adults, 39 kittens) from several experiments during a 4-year period. The animals were selected at random from a normal population.

Several diets were fed in these experiments. A standard commercial chow (Purina Cat Chow, 33% of the calories as protein) served as the control diet, and the experimental formula was a semipurified diet containing casein as previously described.[1] This experimental formula was modified to provide five diets having different levels of casein at 16, 19, 21, 25, or 27% of the calories as protein (Table 1) in an attempt to correlate the retinal change with the consumption of dietary protein. This same basic formula was modified in two experiments by substitution of lactalbumin or egg albumin for the casein at 27% of the calories.

Most cats (adult cats or weanling kittens 8 to 10 weeks old) were fed the commercial chow for approximately 1 week, and one of the experimental diets containing casein for the duration of the experiment, varying from 6 weeks to 24 months. Two kittens were fed the lactalbumin diet instead of casein for a period of 3½ months. Food intake was measured daily and body weights were recorded weekly. To control for reduced food intake as a possible factor leading to the retinopathy, 3 kittens and 2 adult cats were pair-fed an amount of commercial chow equal to the quantity of the semipurified casein diet consumed by 5 comparable experimental cats.

Reversal Study

In one series of experiments (designated the reversal study), the ERG and fundus abnormalities were monitored for 8 to 12 months in 5 kittens and for 3, 5 or 7 months in 3 other kittens, all of which were receiving the casein diet. At that time, one eye from each cat was removed and either the commercial chow (first 5 kittens) or the egg albumin diet (last 3 kittens) was fed to attempt to arrest or reverse the retinal degeneration in the remaining eye. ERG testing was performed between 2 and 14 months following the diet change, and the second eye of each cat was then examined with the electron microscope for comparison with the eye removed prior to the attempt at reversal and with the eyes of cats continuously fed the casein diet for the entire period.

Table 1—Composition of Semipurified Cat Diets

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Casein (vitamin-free)* (g/100 g)*	24.0	27.0	30.0	36.0	36.0
Dextrin (g/100 g)	46.5	43.5	40.5	34.5	34.5
Sucrose (g/100 g)	—	—	—	—	10.0
Lard (g/100 g)	25.0	25.0	25.0	25.0	—
Safflower oil (g/100 g)	—	—	—	—	15.0
Salt mix (g/100 g)†	4.0	4.0	4.0	4.0	4.0
Vitamin mix (g/100 g)‡	0.2	0.2	0.2	0.2	0.2
Alpha-tocopheryl acetate (g/100 g)	0.005	0.005	0.005	0.005	0.005
Vitamin A palmitate beadlets (IU)	4,000	4,000	4,000	4,000	4,000
Choline chloride (g/100 g)	0.3	0.3	0.3	0.3	0.3
Protein (% of calories)	16.1	18.1	20.7	24.9	26.8

* General Biochemicals, Chagrin Falls, Ohio. The casein fed contained 86% protein by analysis.

† The salt mix contained (in grams per kilogram): calcium carbonate, 300; potassium phosphate dibasic ,322; calcium phosphate dibasic, 75; magnesium sulfate, 102; sodium chloride, 167; ferric citrate, 27.50; potassium iodide, 0.80; manganese sulfate, 5.00; zinc chloride, 0.25; cupric sulfate, 0.30; chromium acetate, 0.0458; sodium selenite, 0.0044.

‡ The vitamin mix contained: inositol, 60 g; niacin, 8 g; para-aminobenzoic acid, 8 g; calcium pantothenate, 4 g; riboflavin, 1.6 g; pyridoxine · HCl, 800 mg; folic acid, 200 mg; thiamin·HCl, 800 mg; biotin, 40 mg; menadione, 200 mg; cyanocobalamin, 10 mg; vitamin D_3 beadlets, 400,000 IU, made to 300 g with dextrin.

Retinal Evaluation

Full-field ERG testing was used to evaluate retinal function during the course of this study as previously described.[1] The fundus was evaluated with the direct and indirect ophthalmoscope. At various time intervals when abnormalities in the retina had been assessed with the ophthalmoscope and the ERG, the cats were anesthetized, the eye removed, and the posterior portion of the globe fixed directly in a 1% osmium solution in 0.1 M phosphate buffer prior to dehydration and embedding in Epon 812 for electron microscopic examination. Alternatively, the entire vascular system was perfused through the left cardiac ventricle with 500 cc of a 1% formalin:1.25% glutaraldehyde solution buffered to pH 7.4 with 0.1 M phosphate buffer. After the eyes were removed, the posterior portion of the globe, including the ora serrata, was dissected and placed in cold 1% buffered osmium tetroxide. In order to assure block orientation and comparable sampling from all retinas, 2 × 5 mm blocks of retina were taken serially along the horizontal meridian passing through the optic disc and the area centralis. Additional blocks were obtained from representative retinal areas overlying the tapetum and anterior to the tapetum in all quadrants. Sixteen blocks from each eye were processed individually to maintain proper identification.

Results

Growth and Incidence

Retinal degeneration occurred in all 36 cats—adult or kitten—fed the casein diets (16 to 27% of the calories as protein) and in none of

the 15 cats fed the commercial diet. Neither of the 2 kittens fed the semipurified diet with lactalbumin substituted for casein nor the 5 cats pair-fed the commercial chow developed the retinal degeneration. The latter was true even though both adult cats lost weight with their meager chow allotment and one kitten received an insufficient amount for adequate growth. Furthermore, the onset of the degeneration was not a function of depressed growth in kittens or marked weight loss associated with poor acceptance of the diet by adult cats, as kittens eating the most and growing the fastest developed lesions most quickly and a dam nursing 3 kittens and eating well was the most severely afflicted among the adult cats fed the experimental diet.

Retinal Function and Appearance

The earliest changes in retinal function detected with the ERG were a slight reduction in cone and rod ERG a wave and b wave amplitudes and delays in the temporal aspects of the cone system b wave. These changes were observed as early as 6 weeks in some kittens and 8 weeks in adult cats, but generally after 3 to 6 months. An almost nondetectable ERG was found in the nursing dam within 4 months of the initial change or after 6 months of feeding, but most animals required more than 12 months to reach this stage. Hyperpigmentation and depigmentation of the area centralis was followed by the progressive enlargement of a hyperreflective lesion in this area as previously described in kittens fed the casein diet.[1]

Normal Morphology

In electronmicrographs of the normal cat retina the rod morphology was characterized by elongate outer segments extending to the pigment epithelium. Rod photoreceptors far outnumbered the cones. Occasionally, the cylindrical stack of lamellar discs of a rod was interrupted by a constriction of the surrounding plasma membrane resulting in separation of the discs by an electron-lucent matrix (Figure 1). Minimal vesiculation of rod outer segment lamellae was encountered in an occasional outer segment in normal cats. The narrow rod inner segment contained dispersed oval or elongate mitochondria and a Golgi complex and smooth endoplasmic reticulum, areas of which appeared as empty distended vesicles. The cone outer segment was most easily identified as a broad, short stack of discs enveloped by the fimbriated apical processes of the pigment epithelium. The cone inner segment was typically three times as wide as that of the rod and was packed with mitochondria, except for the distal tip or calyx which was devoid of

organelles and filled with an electron-lucent matrix. Many cones had outer segments that appeared similar to those of rods but were identified by their distinct inner segments and the enveloping apical processes from the pigment epithelium (Figure 2). The Golgi complex in the cone inner segments was inconspicuous, and glycogen particles were seldom observed. Few pigment granules could be seen in the pigment epithelium overlying the tapetum, but numerous cigar-shaped granules were visible in the apical cytoplasm of pigment epithelial cells anterior to the tapetum. Mitochondria were scattered throughout the cytoplasm, which contained substantial quantities of rough and smooth endoplasmic reticulum and Golgi membranes (Figure 1).

Degeneration

A light microscopic section through the area centralis from a cat with a 1 × 1 mm hyperreflective focal lesion in the area centralis and another from a cat with advanced degeneration of the entire area centralis, respectively, are shown in Figure 3A and B. Figure 3A illustrates that the initial disruption was restricted to the photoreceptor outer segment of the area centralis. The concentration of bipolar and ganglion cells seen in the area centralis is related to the maximum concentration of cones in this area. Progressive stages of degeneration resulted in loss of the outer nuclear layer and outer plexiform layer that was most severe in the area centralis and extended to the periphery (Figure 3B).

Electron microscopic study of eyes enucleated at a time when ERG changes were first detected (1½ to 3 months) revealed vesiculation of most rod and cone outer segment lamellae, predominantly along the central axis of rods (Figure 4). Marked alterations in the ERG cone timing and a moderate decrease in cone and rod amplitudes (approximately 3 to 8 months) were accompanied by a fundus with distinct granularity and hyperpigmentation of the area centralis and ultrastructural evidence of vesiculation, swelling, fraying and disorientation of lamellae in both rods and cones (Figure 5). Cone disruption was never distinctly observed independent of disruption in rods, although initially more cones than rods were damaged in the area centralis (Table 2). In early stages the majority of rods in the peripheral retina appeared normal.

Initial disorientation of outer segment lamellae in both rods and cones was so extensive (*ie*, 90°) that some lamellae were parallel to the long axis of the outer segment (Figure 6). Twisting of lamellae was occasionally the only alteration noted in cone outer segments of cats with distinct delays in the temporal aspects of cone system b waves during the earliest stages of degeneration (Figure 7). In some photoreceptors,

Table 2—Electron Microscopic Assessment of Degeneration and Regeneration of Photoreceptor Cells in Cats Consuming Casein Diets Following Various Time Intervals of Dietary Induction and Reversal

Diet	No. of animals	Months on diet		Percent outer segments involved		Electron microscopic observations of area centralis
		Induced	Reversed	Rods	Cones	
Induction study						
Chow	5	0		0	0	Normal
Casein	3	2		0–10	0–90	Mild vesiculation, twisting of rods and cones
Casein	4	3		0–20	10–80	Vesiculation, disorientation of rods; mild to severe cone lamellar twisting
Casein	3	4		0–70	20–100	As above
Casein	3	6		40–100	100	Outer segments moderately disoriented, degenerating
Casein	3	8		100	100	Outer segments shortened, disoriented, degenerating
Casein	4	10		100	100	Most outer segments gone; outer nuclear layer pyknosis; Müller cell proliferation
Casein	2	24		100	100	Photoreceptor cells gone; Müller cells predominate
Reversal study						
Egg albumin	1	3	4	5	40	Mild vesiculation; regenerating lamellae
Egg albumin	1	7	4	20	100	Moderate vesiculation; outer segments
Chow	1	8	2	40	60	Vesiculation, regenerating lamellae; cone twisting
Chow	1	8	9	40	75	Mild vesiculation; regenerating segments almost complete; cone twisting and focal loss of lamellar architecture
Chow	1	9	2	10	75	Isolated rods degenerated; regenerating lamellae; cone twisting
Chow	1	9	3	80	100	Extensive areas of regeneration in most rods and cones; cone twisting
Chow	1	12	3	5	50	Areas of degenerating and regenerating lamellae; rod and cone twisting

the twist was segmental or extended throughout the entire length of the outer segment (Figure 6). Extensively disrupted outer segments contained large vacuoles, swollen lamellae, and loss of lamellar architecture (Figures 6 and 8).

In moderately advanced degeneration (8 to 24 months) all cone and rod outer segments in the area centralis became progressively shortened (Figures 8 and 9). In the advanced stage, photoreceptors were lost, so that the outer limiting membrane was in apposition with the pigment epithelium (Figure 10). In receptors with shortened outer segments, the lamellar discs were disoriented and occasionally observed in loops and whorls (Figures 8, 9 and 11A). Degeneration of discs was associated with patches of electron-lucent material in the area of the outer segment (Figure 9). The degenerating outer segments were usually enclosed within the surrounding plasma membrane (Figures 8 and 9) and were enveloped by the apical processes of the pigment epithelium which continued its phagocytic activity (Figures 11A and 12). Occasionally the plasma membrane was disrupted and outer segment debris could be seen in the extracellular space (Figure 12). Table 2 summarizes the percentage and degree of outer segment degeneration in the area centralis at different stages of degeneration.

The pigment epithelium was unremarkable except for a proliferation of the smooth endoplasmic reticulum observed in some cats with advanced lesions (Figures 11A and 12). This proliferation progressed with the degeneration, eventually displacing cytoplasmic organelles by a labyrinth of interconnecting tubules. The pigment epithelium also contained numerous so-called lysosomal or premelanin granules.

The degeneration of outer segments was accompanied by an increased cytoplasmic density and accumulation of glycogen granules in that portion of the Müller cell cytoplasm forming the external limiting membrane and its terminal processes extending between the inner segments (Figures 13–16). This change became more marked, both with progression of the degeneration and during the regenerative process in the reversal study (Figures 16 and 17). Occasionally in moderately advanced degeneration, the inner segments of the photoreceptors were found to have vesicular dilatations of the smooth endoplasmic reticulum that contained electron-dense granular or amorphous material (Figure 14).

Pyknosis and loss of nuclei from the outer nuclear layer was observed in advanced stages of degeneration. Proliferation of Müller cells eventually predominated, and their extensive cytoplasmic network became prominent from the inner to outer limiting membranes. At this stage the outer limiting membrane was observed in apposition with the pigment

epithelium (Figure 10). The structural integrity of the inner layers of the retina was not demonstrably altered.

Reversal Study

Both the ERG and fundus appearance were abnormal in all kittens at the time the commercial chow or egg albumin diet was substituted for the casein diet (3 to 12 months), although the stage of degeneration varied. Rod and cone amplitudes were 70 to 95% below normal, and the temporal aspect of the cone ERG was delayed. The fundus revealed at least a zonal area of hyperpigmentation and at most a 5- to 10-mm hyperreflective focus in the area centralis. The improvements in the ERG rod and cone amplitudes and retinal ultrastructure were remarkable in all cats fed either the commercial chow or egg albumin diets. Even though ophthalmoscopic examination of the fundus revealed no reduction in the size of the hyperreflective focal lesion of the area centralis, no further progression of the lesion was observed in the reversal period of 2 to 14 months. A summary of the structural improvement is detailed in Table 2.

One cat, fed the casein diet for 8 months and having a minimally detectable ERG, demonstrated markedly improved amplitudes following 4 weeks of commercial chow feeding even though delay in the cone ERG persisted. Equally improved ERG amplitudes were recorded after 8 weeks in the same cat when electron microscopic study of the retina revealed essentially normal outer segment lamellae in 90% of the rods and 30% of the cones, although severe disorientation persisted in a few isolated outer segments (compare Figure 8 with Figure 18). The regeneration of outer segments in all the cats in the reversal study was characterized by scattered patches of lamellar vesicules (Figure 11B) and segments of partially restored lamellae. Reorganization was first evident along the lateral margin of the outer segment, either unilaterally (Figures 19 and 20) or bilaterally, the latter often resulting in misalignment of lamellae and distention of the outer segment at that locus (Figure 21). Cone disorientation persisted (Figure 22) in association with the continued delay in the cone ERG and some reduction in cone amplitude, even though the rod ERG returned essentially to normal in all the cats in the reversal study.

Discussion

This report provides unequivocal evidence that dietary casein is related to nutritionally induced retinal degeneration in cats.[1,3,4] This was demonstrated by the fact that all the diets containing casein fed to kit-

tens or adult cats eventually induced the degeneration. Furthermore, when lactalbumin replaced casein, the degeneration did not occur, and when egg albumin was substituted for casein in 3 cats with early to moderate degeneration, the rod ERG and rod photoreceptor morphology were restored, essentially, to normal. A delay in the temporal aspect of the cone ERG, some reduction in cone amplitude, and lamellar disorientation of cone outer segment persisted. The persistent fundus lesion, seen with the ophthalmoscope in the area centralis after reversal, is consistent with the complete loss of photoreceptors in this area.

With regard to the rod abnormality, the ultrastructural detail of the rod degeneration in cats was similar to the disassembly associated with the lack of rhodopsin in the rods of vitamin A deficient rats exposed to light,[5] although lamellar twisting was not described in the latter study. In the present model and in the vitamin A deficient rat, the rod ERG gradually decreased during the course of the deficiency and was restored to normal in moderately advanced degeneration by substitution of protein or refeeding vitamin A, respectively. However, in these cats the serum vitamin A concentrations did not differ from controls, and no clinical or histologic signs of vitamin A deficiency were present.[1] Unlike vitamin A deficiency, the deterioration of retinal function and structure was observed in rapidly growing, robust kittens and was induced as early as 8 to 10 weeks in adult cats—both of which are atypical of the onset and incidence of vitamin A deficiency.

With regard to the cone abnormality, a similar lamellar twisting has been reported in cone outer segments of the Alaskan malamute dog with hereditary cone degeneration [6] and in the cones of a patient with advanced dominantly inherited retinitis pigmentosa.[7] Delays in the temporal aspect of the cone ERG have been reported in many types of hereditary retinitis pigmentosa in man.[8] This condition in the cat most closely resembles "progressive cone-rod degeneration" in which affected patients have bilateral macular degeneration in the early stages and ERG responses indicating widespread involvement of the cone system and involvement of localized or patchy areas of the rods.[9]

It is noteworthy that in these studies the development of the retinal degeneration appeared only in the diets formulated with casein as the protein source, whereas identical diets containing lactalbumin or egg albumin were protective, as was the commercial chow. The level of dietary casein, providing 16 to 27% of the calories, did not appear to affect the incidence of degeneration. Growth failure induced by restriction of commercial chow did not result in degeneration, whereas an equally restrictive consumption of casein did. Thus, the data point to

a dietary inadequacy induced by the casein diet that was satisfied by lactalbumin, egg albumin and chow. It may be some unknown factor in casein or a property of the protein source itself. Casein is known to be relatively limited in the sulfur amino acids,[10] and current studies indicate that the metabolism of the sulfur amino acids is altered in the affected cats. The suggestion that the quality of the dietary protein can alter outer segment integrity independent of other functions dependent upon dietary protein, such as growth, is unique and intriguing.

The progressive disorganization and shortening of the outer segment observed in this degeneration might be explained by a deficit in dietary protein which may have altered the synthesis and/or incorporation of protein into outer segment discs, a process of renewal that is relatively orderly and rapid in rods, but random and limited in cones.[11] These differences in outer segment renewal by the two types of photoreceptors may be relevant to the inefficient repair of cones as compared to rods during the dietary reversal of the degeneration.

The observations from the present reversal study reaffirm our previous conclusion [1] that the clinically observed retinopathy, described as feline central retinal degeneration,[12] and this diet-induced degeneration are various stages of the same entity. In the clinical condition a nonprogressive fundus lesion has been associated with an electroretinographic and ultrastructural description of a normal rod system accompanied by persistent cone dysfunction and disorientation.[12] The clinical situation might represent the reversal of a prior dietary protein inadequacy. This would be in keeping with our results demonstrating that refeeding adequate dietary protein precludes further degeneration and can even restore rod photoreceptor function and structure to normal, depending on the severity of the degeneration and degree of photoreceptor cell loss.

The possibility exists that this degeneration and that observed clinically in cats [12–14] have different etiologies, but their similarity suggests that there may be a common deficit in protein consumption or utilization in this species when measured in terms of the nutritional requirement for retinal function.

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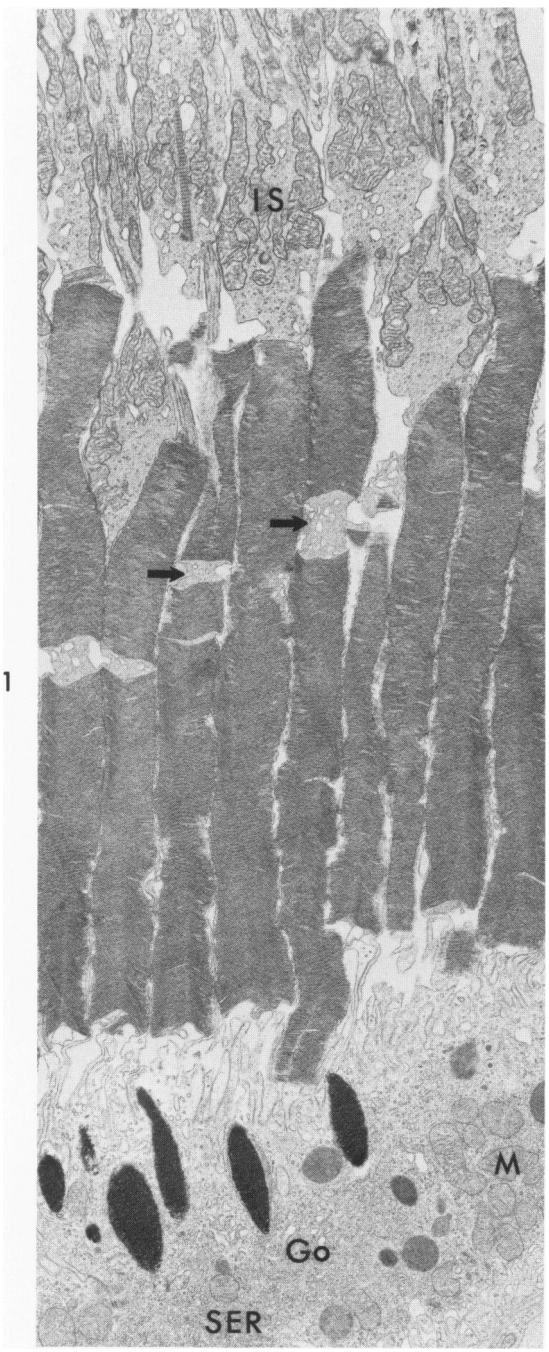
[*Illustrations follow*]

Figures 6–8 and 18–22 show the photoreceptors oriented horizontally, with the inner retina always on the left; the remaining figures show the photoreceptors oriented vertically, with the inner retina at the top and the pigment epithelium at the bottom of the figures.



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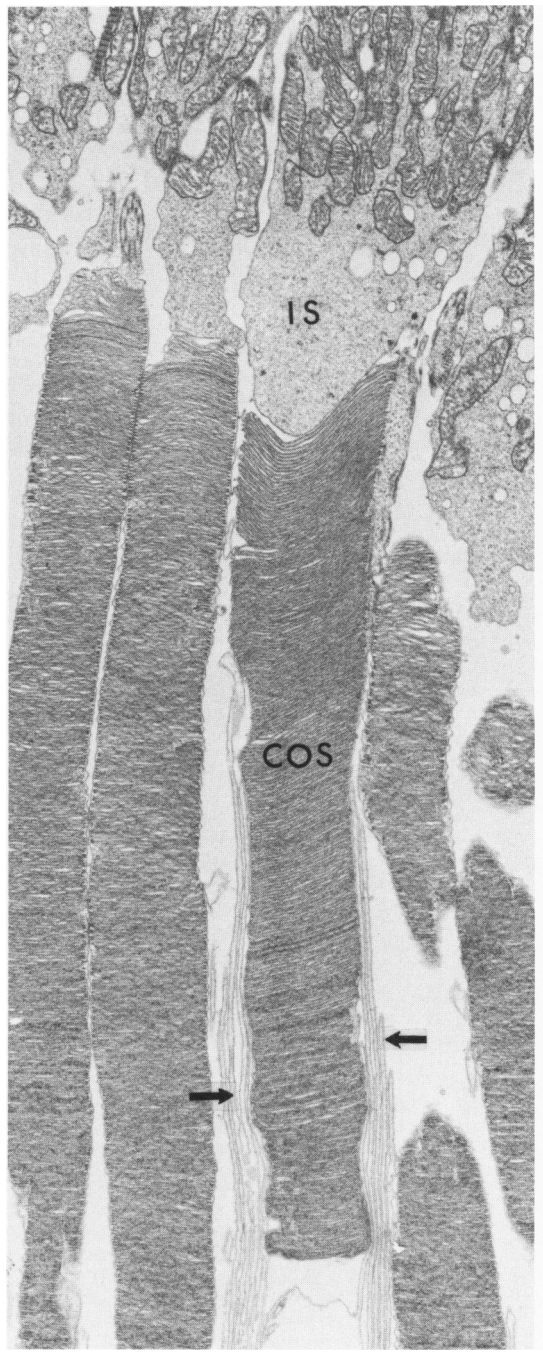

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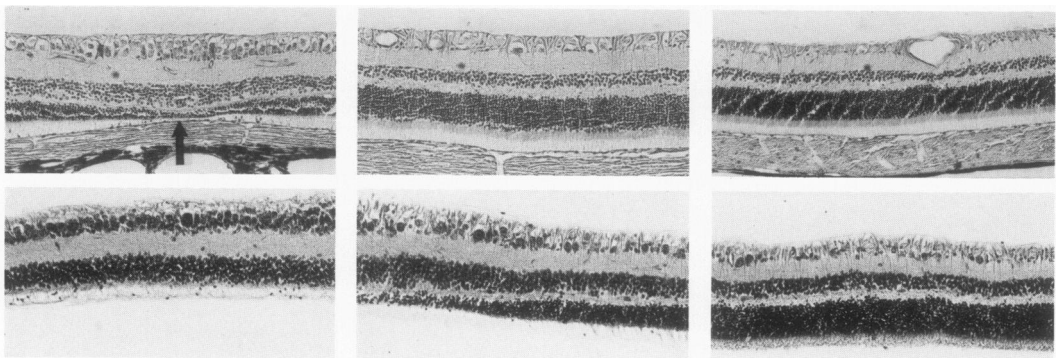
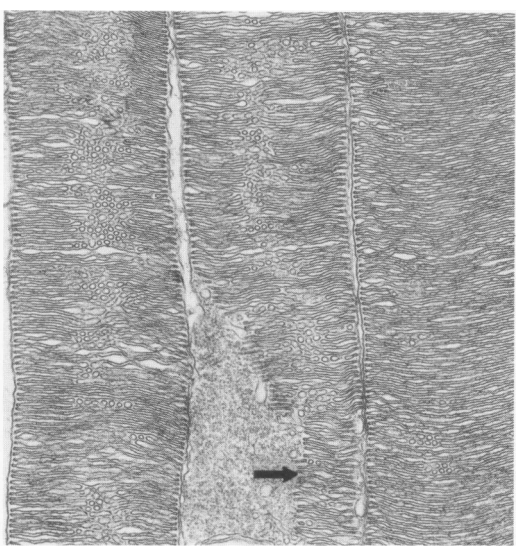
Fig 1—A cluster of rod outer segments from a normal cat includes areas of matrix separating lamellar discs (*arrows*). The pigment epithelium contains several cigar-shaped melanin granules and smaller round premelanin granules. A Golgi complex (*Go*) and extensive smooth endoplasmic reticulum (*SER*) are visible along with mitochondria (*M*). The photoreceptor inner segments (*IS*) are visible at the top (Chow diet, 6 months, × 5400). **Fig 2**—A cone photoreceptor is distinct from adjacent rods due to its slightly wider outer segment (*COS*), encompassing apical processes from the pigment epithelium (*arrows*), and the distinct width, mitichondrial concentration, and extensive calyceal process terminating its inner segment (*IS*) (Chow diet, 9 months, × 8800).





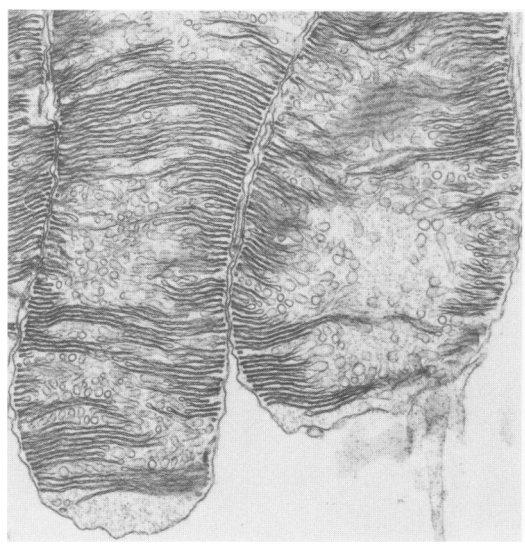


Fig 3A—Retina from a cat with a hyperreflective focus seen ophthalmoscopically demonstrates the earliest degree of degeneration, *ie*, focal loss of outer segments and thinning of the outer nuclear layer (*left, arrow*) in the area centralis (Casein diet, 4 months, × 180). **B**—Retina from a cat with moderately advanced degeneration and extensive hyperreflective change reveals total loss of outer segment and outer nuclear layer (*left*) and a relatively intact peripheral retina (*right*) (Casein diet, 11 months, × 180). In both retinas the normal anatomic characteristic of increasing thickness of the inner nuclear layer and ganglion cell layers progressing from the peripheral retina to the area centralis (*right to left*) can be observed. **Fig 4**—Early degeneration is represented by vesiculation of outer segment (*OS*) lamellae, especially along the core of the OS. Hemilateral segments of OS lamellae (*arrow*), seen both during degeneration and regeneration, abut a portion of OS matrix (Casein diet, 4 months, × 17,000). **Fig 5**—More extensive damage is represented by vesiculation, fraying, and swelling of lamellar discs in rod outer segments (Casein diet, 5½ months, × 17,000).

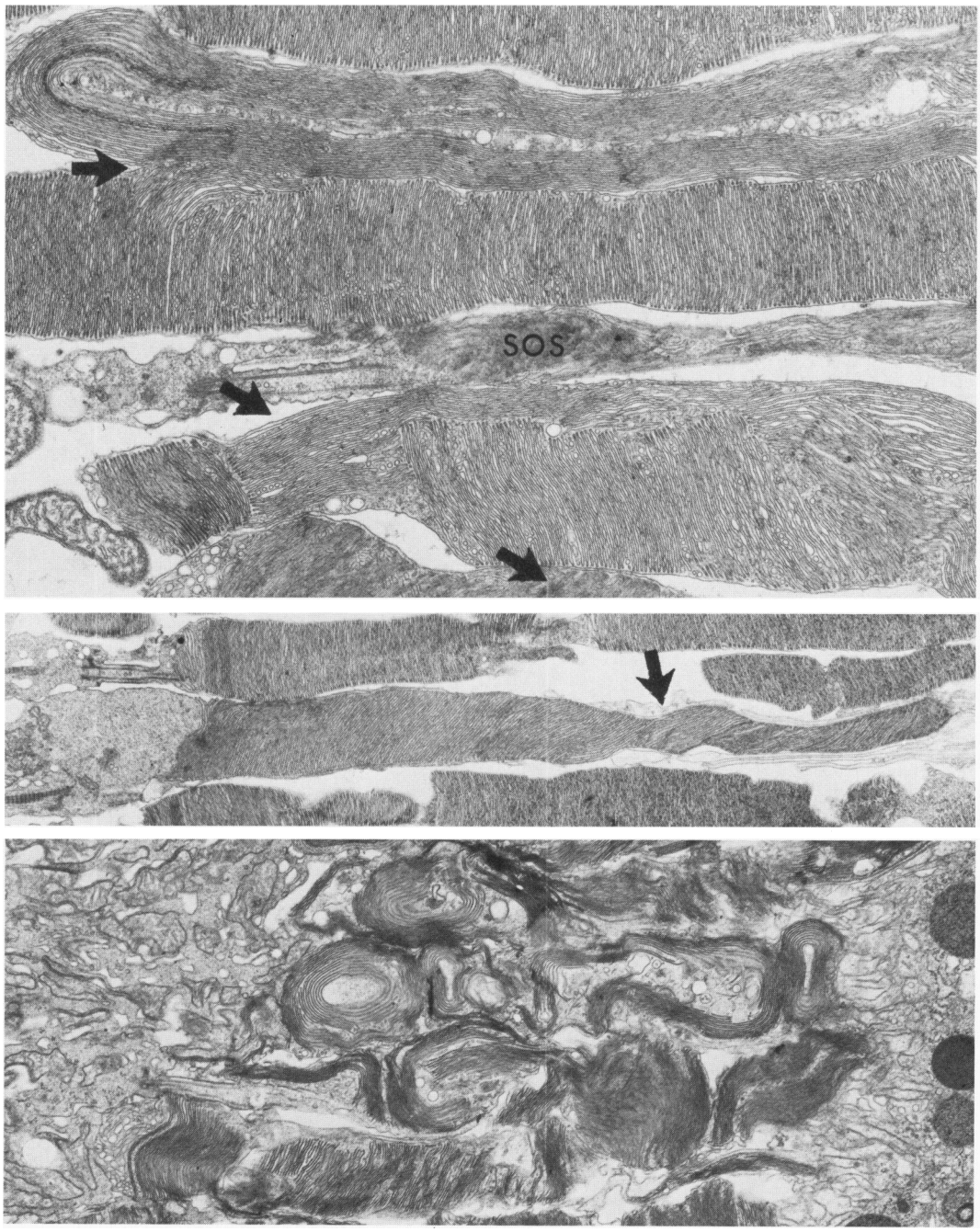


Fig 6—Disoriented outer segment (*OS*) discs are aligned parallel to the long axis of the OS. Often this shift was segmental (*middle arrow*) or it involved the entire OS as demonstrated by the skein-of-yarn appearance of the upper OS. A portion of this same OS has fused with lamellae of an adjacent OS (*upper arrow*). A loss of lamellar architecture (*lower arrow*) was also characteristic of degeneration. A shriveled, atrophic appearance is presented by another OS (*SOS*) (Casein diet, 5 months, × 15,000). **Fig 7**—A partial twist is observed in the distal portion of a cone outer segment (*arrow*) from a kitten with a delay in the temporal aspect of the cone ERG (Casein diet, 4 months, × 7600).
Fig 8—More advanced disruption of discs is characterized by formation of whorls and large vesicles in the OS. The disrupted OS are so shortened that both the pigment epithelium and photoreceptor inner segments are visible at this magnification. All of the cones and rods in the area centralis were involved at this stage. Compare with Figure 18 (Casein diet, 8 months, × 10,200).

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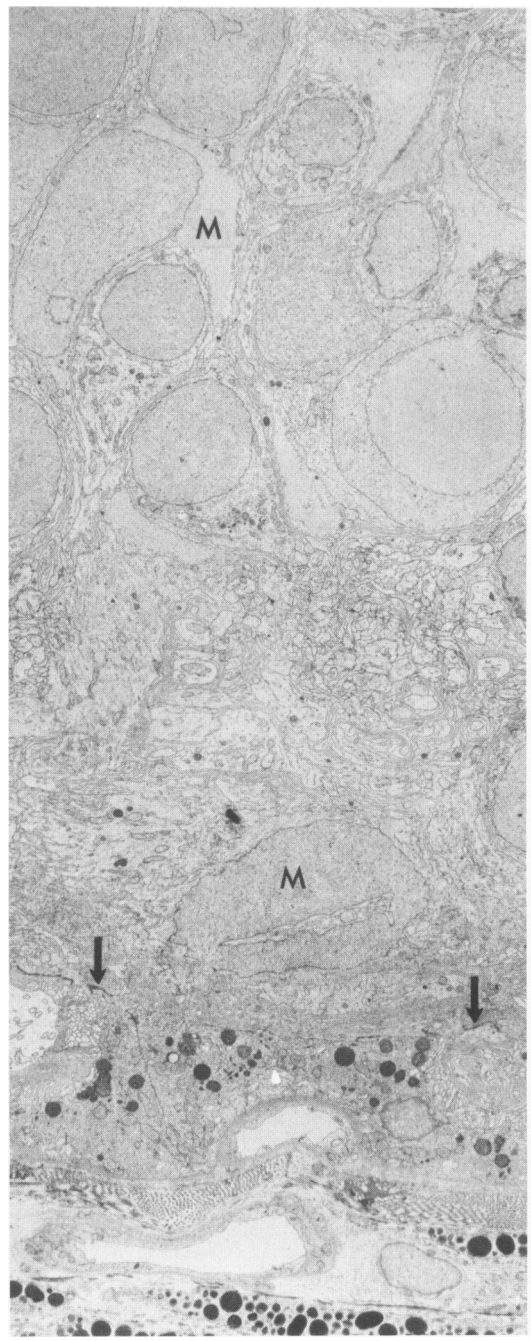

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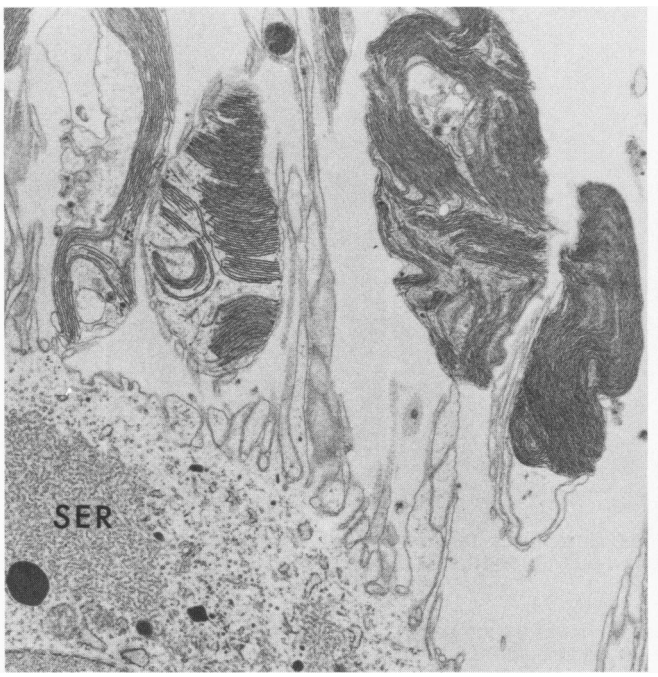
Fig 9—Shortened, disrupted OS containing electron-lucent vacuoles (*V*) are apparent in advanced degeneration of the area centralis. Distended Müller cell processes are visible along the outer limiting membrane (*arrows*). Compare with Figure 1 (Casein diet, 8 months, × 2800). **Fig 10**—Advanced stage of degeneration is represented by complete loss of photoreceptors with proliferation of Müller cells (*M*) and their cytoplasmic processes. The outer limiting membrane is in apposition with the pigment epithelium (*arrows*) (Casein diet, 24 months, × 2400).



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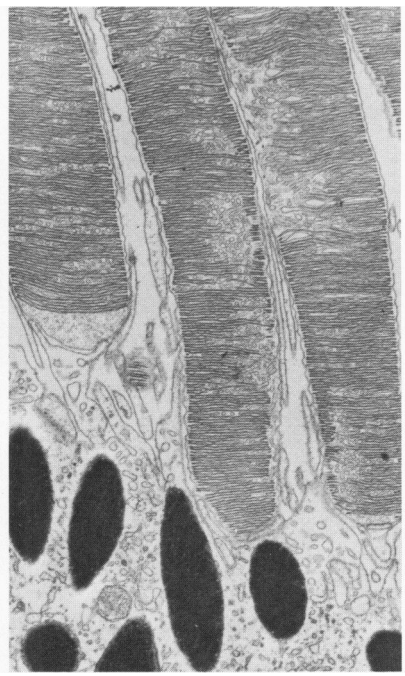

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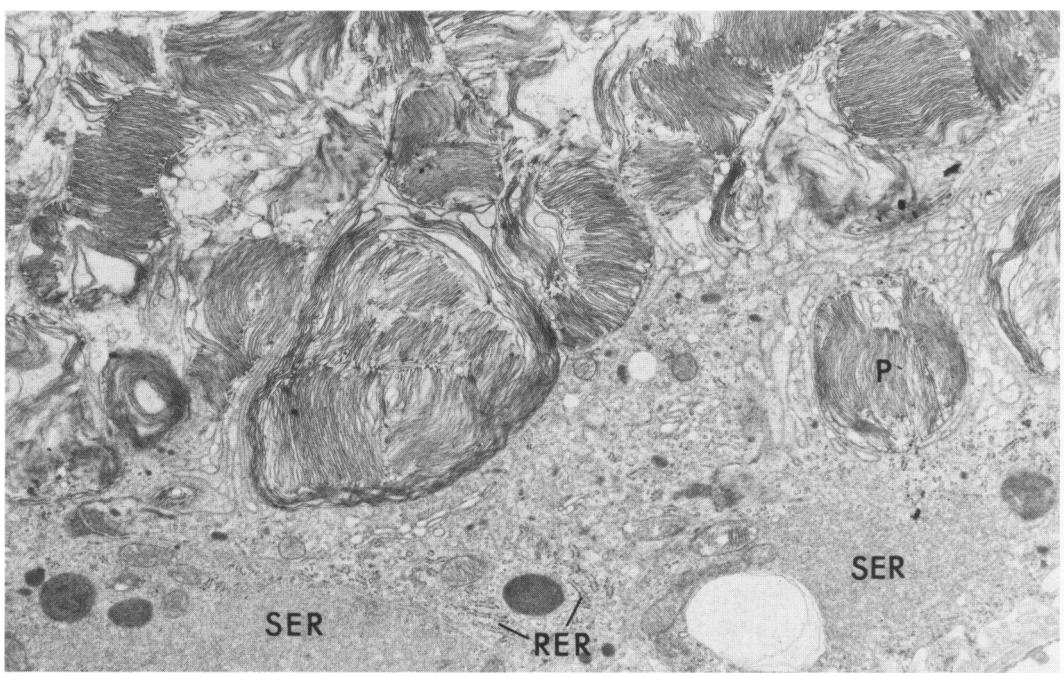

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Fig 11A—Moderately advanced outer segment (*OS*) degeneration is characterized by disorientation and loss of OS (Casein diet, 7 months, × 7000). **B**—A littermate kitten fed the casein diet for 3 months, followed by an egg albumin diet for 4 months, reveals extensive vesiculation of lamellar discs as the only aftermath of degeneration and regeneration of rod OS (× 10,000). **Fig 12**—Moderately advanced OS degeneration has resulted in breakdown of the encompassing plasma membrane with release of OS debris to the extracellular space. Phagosomes (*P*) continue to be incorporated in the pigment epithelium which also has extensive proliferation of smooth endoplasmic reticulum (*SER*) in basal portions of two pigment epithelium cells (Casein diet, 9 months, × 7800).

Fig 13—Inconspicuous junctional complexes (*arrows*) form the outer limiting membrane in a normal cat retina (Chow diet, 9 months, × 12,400).

Fig 14—Outer segment degeneration was accompanied by increased density of Müller cell process (*M*) and collection of electron-dense granules within cytoplasmic vacuoles (*arrows*) of the photoreceptor inner segments. Other saccules of the endoplasmic reticulum are dilated and empty (Casein diet, 5 months, × 17,000).

Fig 15—An isolated intercellular junction is visible joining a glycogen-laden Müller cell (*M*) and the inner segment of a photoreceptor (*IS*) in a cat with early retinal degeneration (Casein diet, 4 months, × 39,000).

Fig 16—A regenerating retina reveals an extreme density of the reactive Müller cell processes due to glycogen accumulation (Casein diet, 7 months; chow diet, 9 months, × 9600).

Fig 17—A terminal Müller cell process is ponderously swollen with accumulated glycogen in advanced degeneration. An adjacent inner segment and a degenerating outer segment are visible (Casein diet, 7 months, × 7600).

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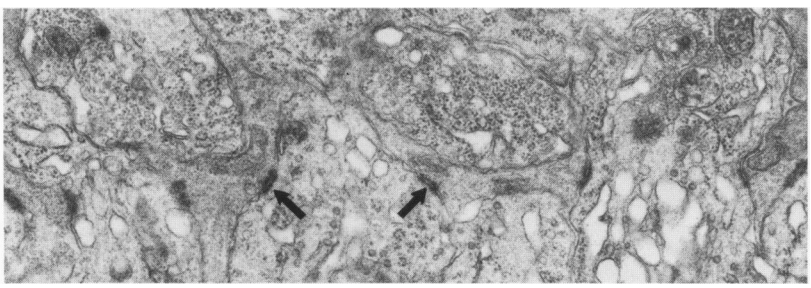

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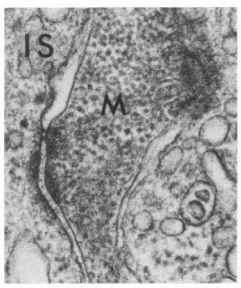

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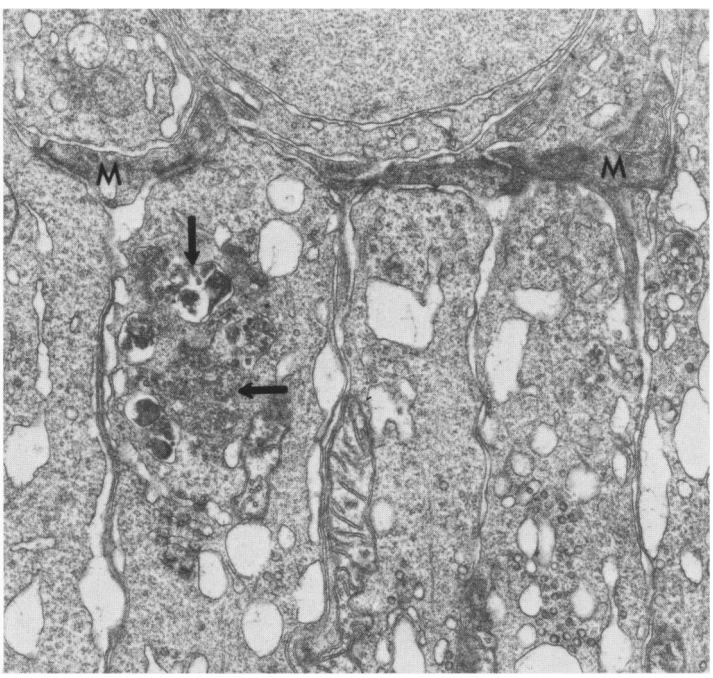

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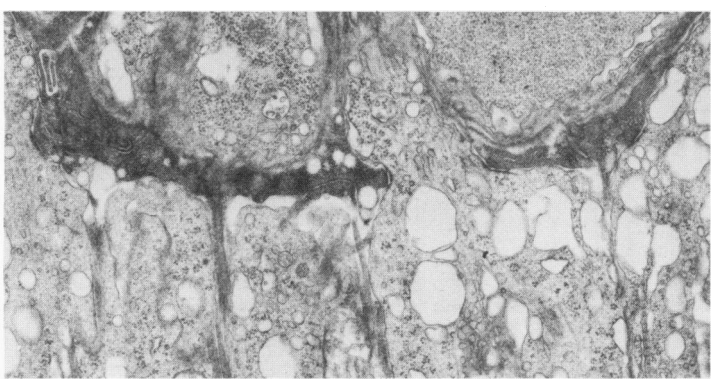

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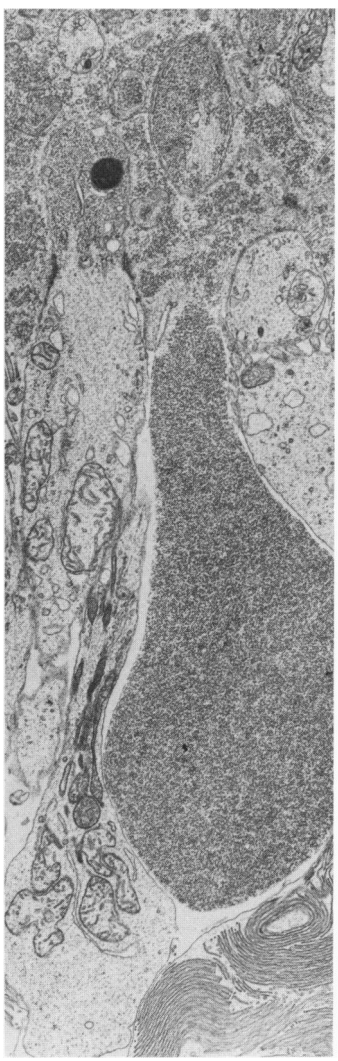

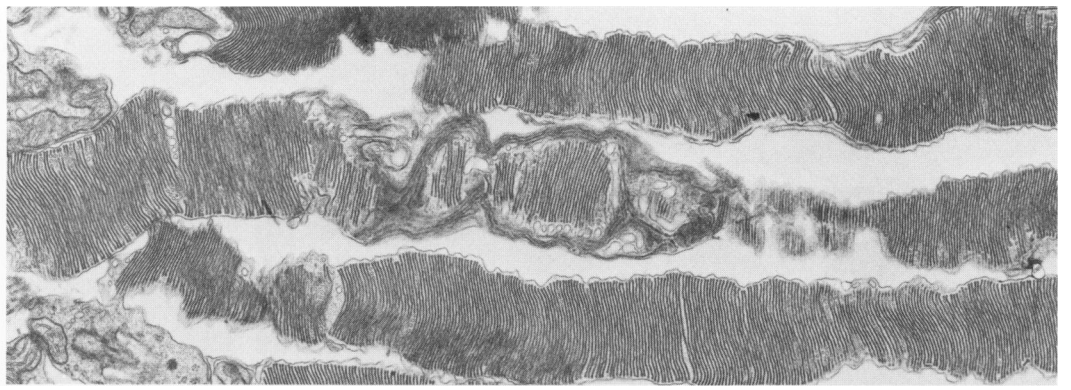

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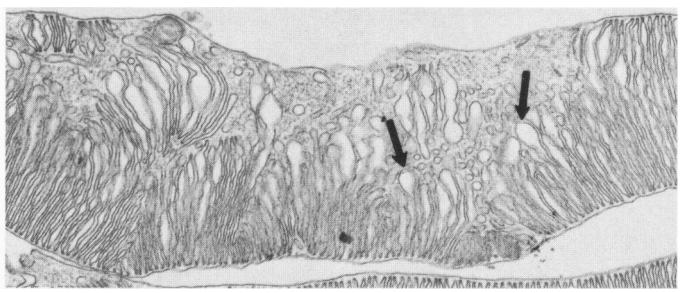

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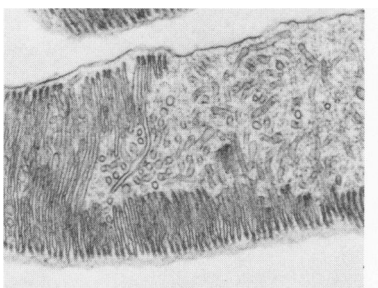

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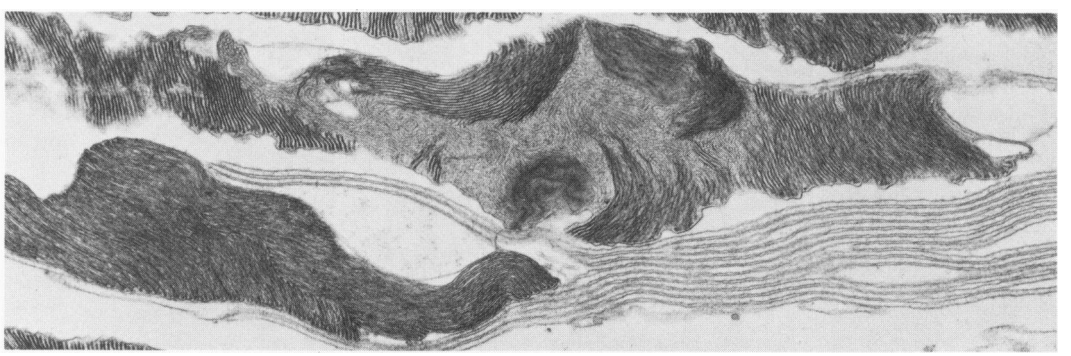

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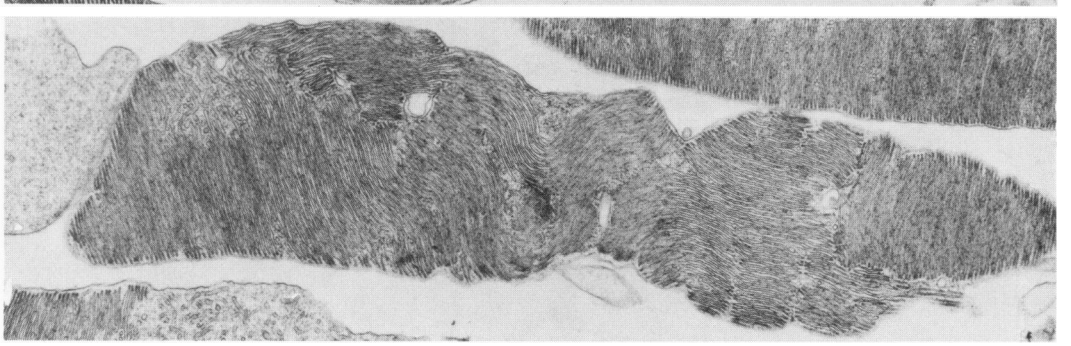

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Fig 18—Restoration of outer segment (*OS*) structure was 90% complete after 2 months of the egg albumin diet. An isolated disrupted outer segment is depicted. Same cat reversed from Figure 8 (Casein diet, 8 months; egg albumin diet, 2 months, × 12,900). **Fig 19**—A regenerating rod OS includes several incomplete, partially vacuolated lamellae (*arrows*) adjacent to a zone of electron-lucent matrix (Casein diet, 9 months; chow diet, 9 months, × 15,400). **Fig 20**—Short lamellar discs appear to be reorganizing along one side of this rod OS in a cat refed a control diet for 3 months (Casein diet, 9 months; chow diet, 3 months, × 18,600). **Fig 21**—Attempted restoration of rod (*above*) is characterized by an incomplete alignment of lamellae and distention of the OS. A cone remains twisted (*below*). Same cat as in Figure 20 (× 12,900). **Fig 22**—Disorientation of lamellar discs is apparent in this cone OS from the same regenerating cat seen in Figure 20 (× 12,000).