Retinal degeneration in monkeys induced by deficiencies of vitamin E or A

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The effects of vitamin E or vitamin A deficiency on the retina were assessed in monkeys for as long as two and three-quarters years. A macular degeneration developed after two years in monkeys fed vitamin E-deficient diets. The lesion was characterized by focal, massive disruption of photoreceptor outer segments attributed to lipid peroxidation of these lipoprotein structures containing highly unsaturated fatty acids. The focal nature of the lesion precluded any evidence of clinical blindness. Vitamin A deficiency was typical of that described by others, and was accompanied by xerophthalmia, keratomalacia, and clinically impaired vision. Anatomic evidence suggested that the structural disruption of photoreceptors was more advanced in cones and was most pronounced in the macula with a lesser involvement of the peripheral retina.

Key words: vitamin A, vitamin E, retina, blindness, macular degeneration.

Experimental vitamin E deficiency has elicited a multitude of pathologic entities in a variety of species. In terms of ocular pathology, cataract formation has been reported[1, 2] but retinal degeneration has not been previously described. A group of dogs found deficient in vitamin E at necropsy was found to have advanced retinal degeneration,[3] but its etiology and pathogenesis were unclear. Children afflicted with abetalipoproteinemia suffer from visual impairment thought to be related to a failure in absorption of fat and the fat-soluble vitamins,[4] and the onset of neuronal ceroid-lipofuscinosis (Batten's disease), a syndrome resembling the ceroid accumulation of chronic vitamin E deficiency,[5] is usually accompanied by impaired vision and progressive blindness of undetermined pathogenesis.[6] These conditions and the observation that vitamin E is concentrated in the photoreceptor outer segments (OS)[7] suggest that tocopherol may have a specific function in the retina.

The present study in primates provides details of a retinal degeneration in the macula of two species of monkeys following long-term deprivation of vitamin E. In addition, the lesion is compared with that produced by vitamin A deficiency in one of these species and differences between the deficiencies are discussed.

Methods

The complete description of the animals, diets, and procedures for the vitamin E study has been reported elsewhere.[8] Two species of monkeys ap-

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Table I. Composition of semi-purified diet low in vitamin A

Ingredients	*Grams per 100 grams*
Casein, vitamin-free	20.0
Dextrin	29.6
Sucrose	10.0
Cottonseed-soybean oil	10.0
Cellulose*	23.8
Salt mix†	4.0
Vitamin mix‡	2.2
Choline chloride	0.3
Vitamin D_3 (1,250 IU per gram)	0.1

*Alphacel, General Biochemicals, Chagrin Falls, Ohio.
†Hegsted, D. M., et al.: J. Biol. Chem. 138: 459, 1941.
‡The vitamins added to 1,854 grams of dextrin were (in grams): α-tocopherol succinate, 10; ascorbic acid, 90; inositol, 10; menadione, 4.5; p-aminobenzoic acid, 10; nicotinic acid, 9; riboflavin, 2; pyridoxine · HCl, 2; thiamine · HCl, 2; calcium pantothenate, 6; biotin, 0.04; folic acid, 0.18; and cyanocobalamine, 0.005.

proximately 14 months of age and born and raised in our primate colony were used. These included 12 New World cebus *(Cebus albifrons* and *apella)* and 14 Old World cynomolgus *(Macaca fascicularis)* monkeys. Animals were fed a semi-purified diet containing either stripped safflower oil (tocopherol removed) or coconut oil with or without a vitamin E supplement for as long as two and three-quarters years. Three deficient monkeys died unexpectedly whereas all others were killed at the end of the experimental period. Eyes were either perfused in situ via the left ventricle of the heart using a 1 per cent formaldehyde:1.25 per cent glutaraldehyde fixative buffered to pH 7.4 with 0.1 M phosphate buffer, or the eye was removed from the anesthetized monkey and the posterior hemisphere immersed in 2 per cent osmium tetroxide in 0.1 M phosphate buffer. Representative eyes were fixed in formalin and embedded in paraffin for routine hematoxylin and eosin (H & E) sections.

Six adult female cebus monkeys weighing 1,400 to 1,700 grams were used for the study of vitamin A deficiency. They were fed an agar cake diet lacking in vitamin A (four monkeys) or the same diet with added vitamin A (two monkeys) (Table I) for as long as 21 months. Periodic serum samples were taken for vitamin A analysis.[9] Although the entire retina was available for light microscopy, only paramacular and mid-peripheral retina was examined by electron microscopy in the vitamin A-deficient monkeys.

In both experiments one or two anesthetized monkeys were visually screened along with the control animals for their b-wave threshold in response to a full-field (Ganzfeld) white-light stimulus.[10] All the monkeys were housed under identical lighting and environmental conditions and were cared for by the same personnel. Cyclic light (12-hour periods) from overhead fluorescent lamps provided an approximate cage illumination of 8-foot candles.

Vitamin E deficiency. The general pathophysiology of vitamin E deficiency in these monkeys[5] as well as the details of the anemia that developed[8] have been previously described. In summary, deficient monkeys fed polyunsaturated safflower oil were most severely affected, developing severe hemolytic anemia, muscle degeneration, testicular degeneration, and marked ceroid accumulation in several tissues. Retinal degeneration occurred in seven of the eleven monkeys fed either safflower oil or coconut oil without vitamin E. None of the vitamin E-supplemented monkeys developed retinal damage, and none of those fed coconut oil became anemic. Blindness was never apparent clinically. The full-field electroretinogram (ERG) b-wave threshold was considered normal in two deficient monkeys tested on two occasions, later found to have histologic degeneration of the macula. No fundus abnormality was detected with the indirect ophthalmoscope. There was no evidence of xerophthalmia and the monkeys appeared healthy and alert. The plasma vitamin E concentration was generally less than 100 μg/dl in advanced deficiency with both dietary oils. Control values were in excess of 600 μg/dl.[8]

The plasma vitamin A levels varied with the species and dietary treatment, being lower in the cebus than the cynomolgus monkeys and lowest in the vitamin E-deficient monkeys fed safflower oil.[5] However, the most extensive degeneration was observed in a cebus monkey fed coconut oil for the longest period of time (33 months), and the vitamin A concentration was among the highest recorded for the cebus (20 μg/dl).

Light microscopy of the retina revealed a focal disruption of the photoreceptor OS layer that was restricted to the macula (Fig. 1).

The minimal change detected by electron microscopy was vacuolization and disorientation of OS lamellae among isolated photoreceptors of the macula. Adjacent OS were often peculiarly spared in these areas of minimal damage (Fig. 2). This alteration most often involved the distal third of the OS and included both rods and cones. Inner segments of these photoreceptors appeared normal (Fig. 3). In more advanced degeneration the entire OS was markedly swollen and lamellae were increasingly disrupted, often broken into short tubules or empty vesicles (Figs. 4 and 5). Other tubules and vesicles were packed with an electron-dense, finely granular debris (Figs. 5 and 6) similar to the material seen in liver mitochondria of these monkeys[5] and thought to represent peroxidized lipid. Adjacent OS often remained intact (Fig. 4). In other areas of the macula the OS were totally disrupted and had formed dense clusters of lamellar debris with

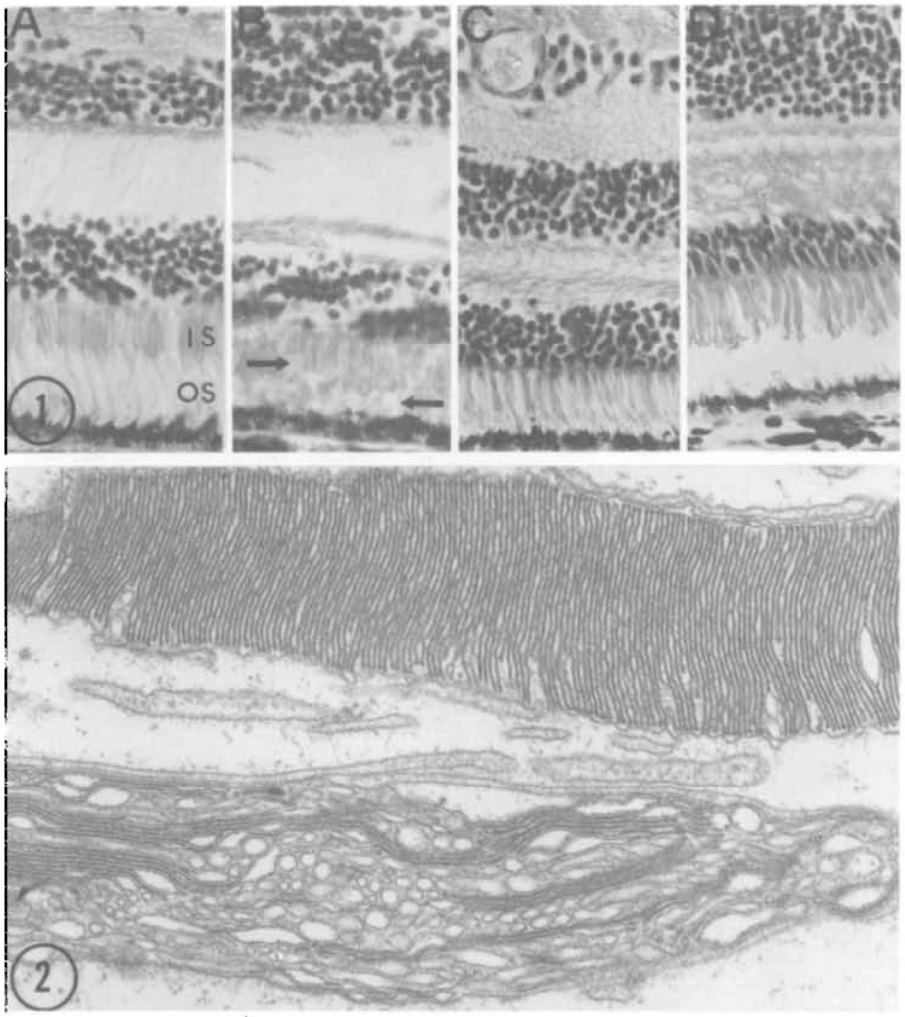


Fig. 1. The peripheral retina (*A*) from a vitamin E-deficient monkey has intact inner (IS) and outer (OS) segments of photoreceptors that do not appear different from control sections, whereas those from the macula in the same monkey (*B*) are markedly disrupted (arrows). On the other hand, both peripheral retina (*C*) and macula (*D*) in the vitamin A-deficient monkey have degenerated outer segments, the latter appearing much worse than the former. Thinning of the ONL has also occurred in the macula. (×200).

Fig. 2. Early stage of macular degeneration in vitamin E deficiency. An essentially normal rod outer segment (top) is adjacent to one undergoing degeneration (below). Disorientation and vesiculation of lamellae are apparent. (×27,550).

whorls of myelin figures (Figs. 6 and 7). The pigment epithelial cells appeared to sustain phagocytic activity, often being distended with lipofuscin-type lysosomes and larger aggregates of phagocytized OS debris (Figs. 7, 8, and 9). The inner layers of the retina were without visible change except for an occasional pyknotic nucleus in the outer nuclear layer (ONL).

Vitamin A deficiency. Clinical manifestation of vitamin A deficiency occurred in three of the four depleted monkeys after 16, 19, and 20 months of study. Plasma vitamin A levels in the deficient monkeys were negligible after a year (< 5 μg/dl, whereas control values were 15 to 20 μg/dl). After 12 to 15 months body weight loss became apparent. Xerophthalmia with reddening of the conjunctiva became evident as well. In the more advanced stage, lethargy, anorexia, and inanition

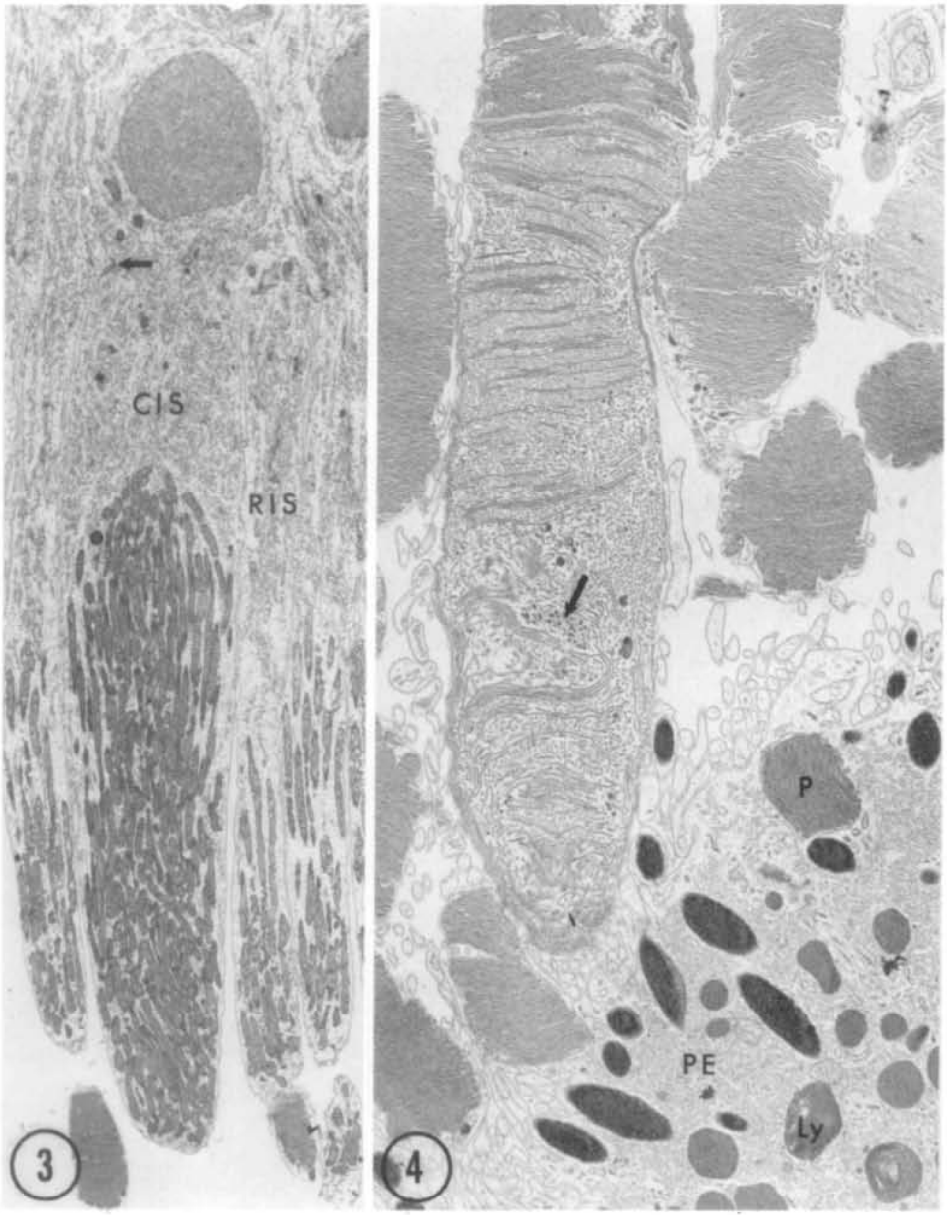


Fig. 3. A single cone inner segment (CIS) is distinct from three adjacent rod inner segments (RIS). These apparently undamaged IS were observed in the macula where outer segments were degenerating. The CIS is distinguished by its width and dense aggregation of mitochondria. Intercellular junctions constituting the outer limiting membrane are visible (arrow). Portions of outer segments are sectioned tangentially below. (×4,300).

Fig. 4. Swelling of photoreceptor outer segments and vesiculation of lamellae were predominant changes in moderately advanced stages of retinal degeneration in vitamin E deficiency. Portions of adjacent OS appear normal. Clusters of electron-dense particles (arrow) were characteristic of this degeneration. The pigment epithelium (PE) contains cigar-shaped pigment granules and less dense lysosomal bodies presumed to represent an end-stage in the removal of the phagosomes (P). (×9,500).

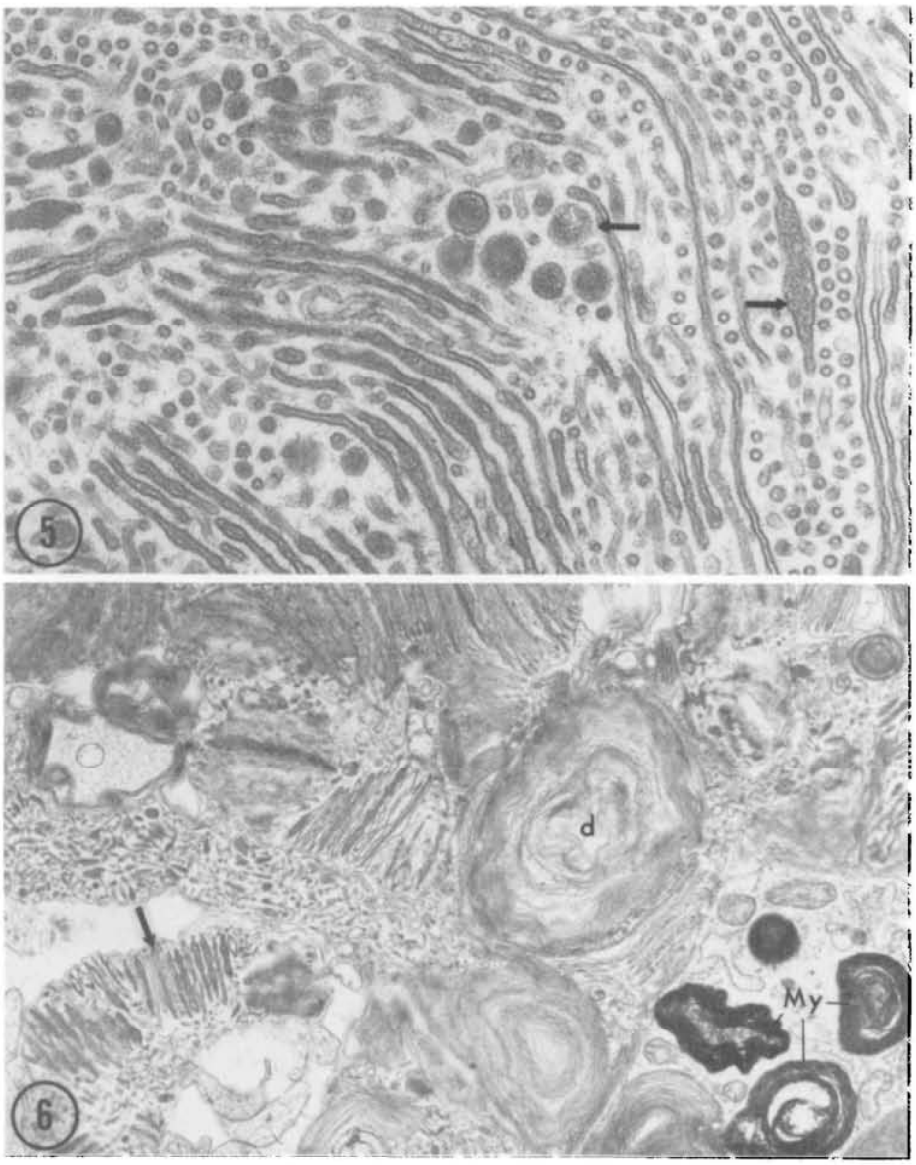


Fig. 5. Many degenerating lamellae form tubules and vesicles packed with fine, granular, electron-dense particles (arrows) thought to represent peroxidized lipoprotein or lipofuscin formation in vitamin E deficiency. (×47,900).

Fig. 6. Marked degeneration of outer segments is represented by swelling and disruption of outer segments, forming whorls of lamellar debris (d) and myelin-like figures (My). Electron-dense particles fill tubules of degenerating lamellae (arrow). (×15,900).

were accompanied by advanced night blindness, evidenced by an inability to maneuver in a dimly lighted room. Xerosis and keratinization of focal areas of the cornea were associated with formation of corneal ulcers and the rapid development of keratomalacia (Fig. 10).

The youngest and smallest monkey was killed in an advanced state of deficiency after 16 months and one eye was taken for electron microscopy (Figs. 12, 13, and 14) and the other for light microscopy (Fig. 1). Two of the other three deficient monkeys presented similar clinical conditions after 19 and 20 months. One of these monkeys, progressing from night blindness to

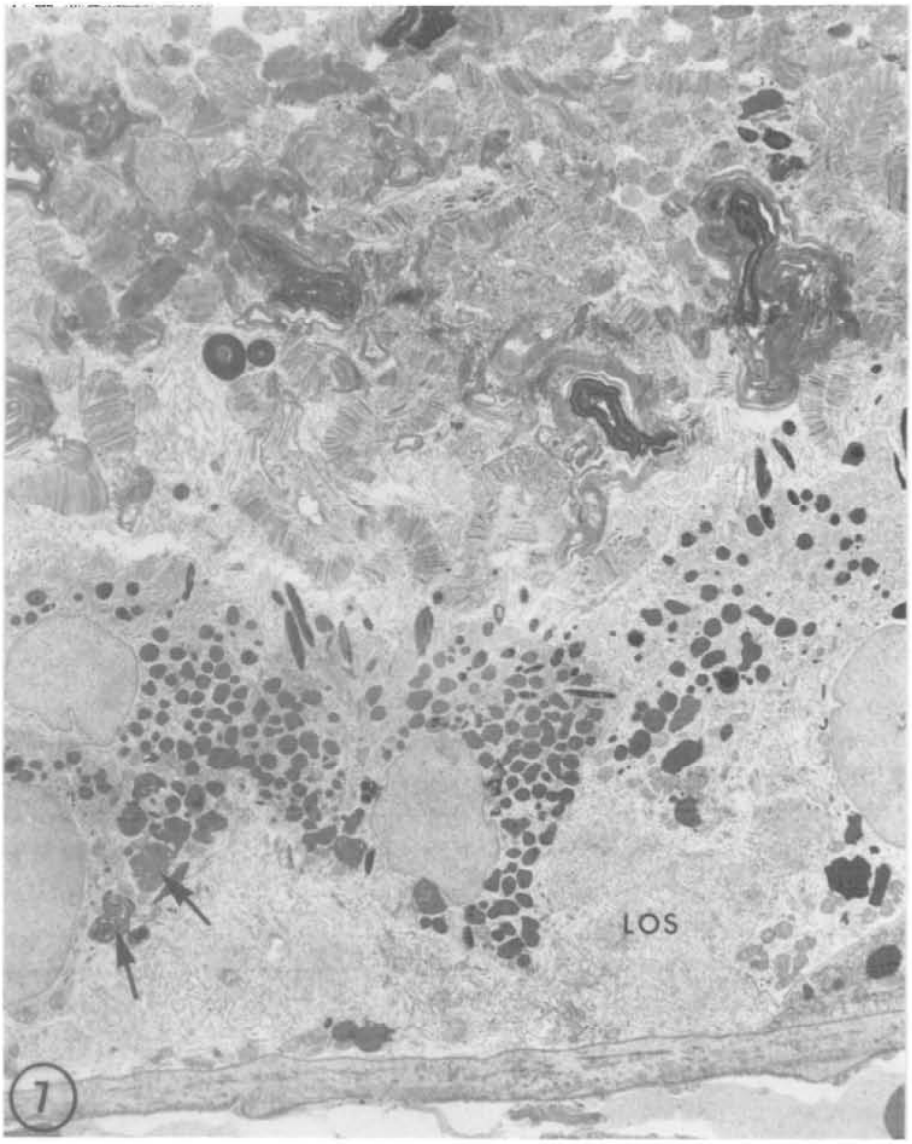


Fig. 7. Massive disruption of photoreceptor outer segments is apparent in the macula of a vitamin E-deficient monkey. Formation of dense myelin-like figures represents accumulation of extracellular debris. The pigment epithelium contains masses of phagocytized outer segments, both loosely packed in the basal cytoplasm (LOS) and in more typical dense phagosomes (arrows). Increased lysosomal granules and cigar-shaped pigment granules are visible in the apical cytoplasm. (×4,700).

impaired vision in a lighted room, was tested and found to have a nondetectable b-wave in the full-field ERG. One eye was enucleated under anesthesia and prepared for electron microscopy (Fig. 15). All the monkeys were then refed commercial monkey chow. The monkey with the enucleated eye died two weeks later having developed acute panophthalmitis of its remaining eye. The other two deficient monkeys regained their appetite, physical activity, and body weight. Xerophthalmia and keratomalacia disappeared in the monkey so affected and vision appeared to be adequate, although slight corneal opacity remained after several months.

Light microscopy of major organs from the two vitamin A-deficient monkeys necropsied at 16 and

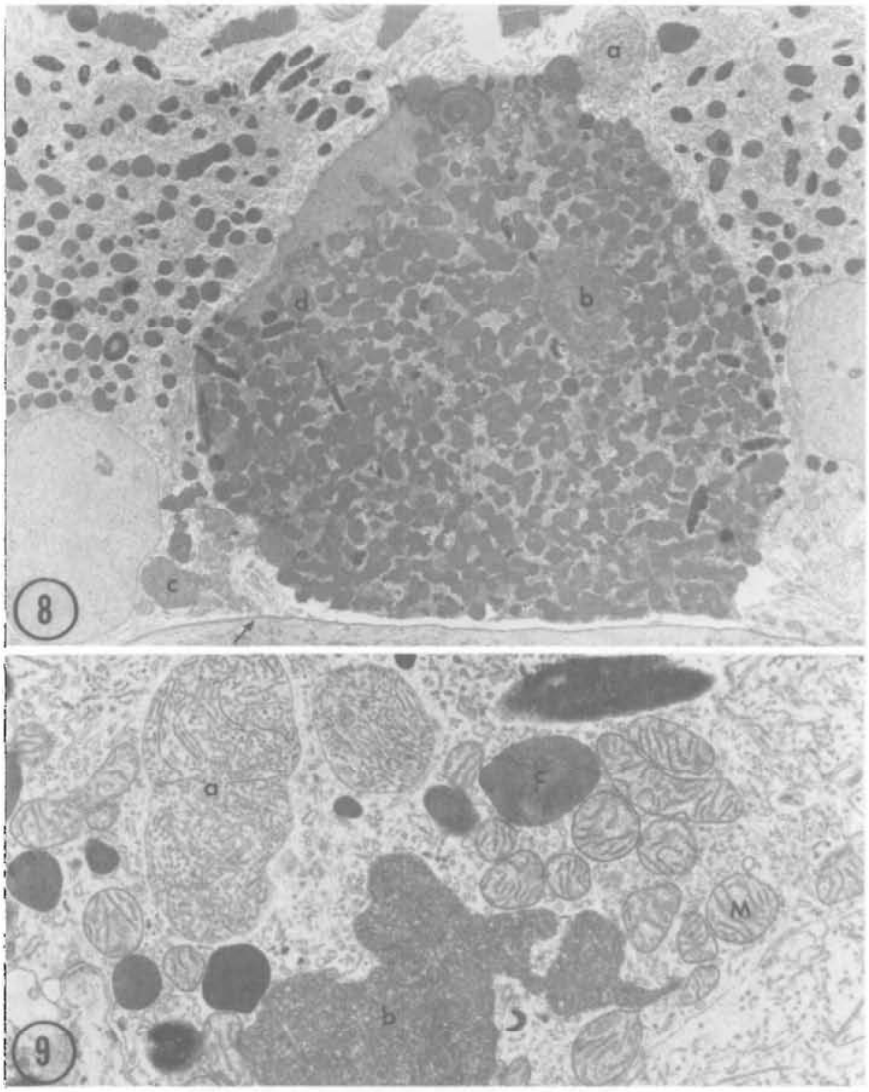
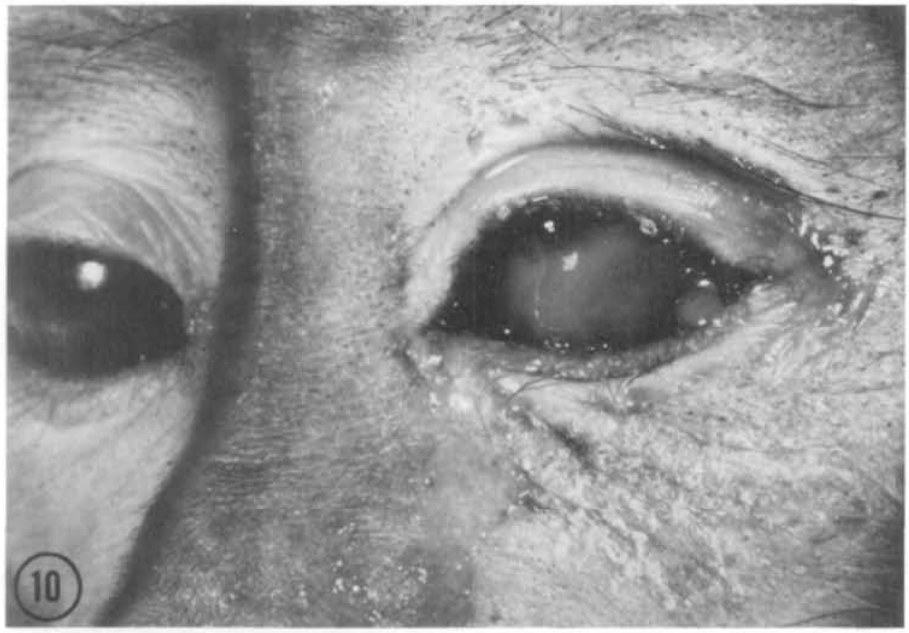


Fig. 8. Pigment epithelial cells from a vitamin E-deficient monkey contains distinctly different stages of phagosome digestion (*A*, *B*, *C*, and *D*). The middle cell is packed with lysosomes and appears to be pulling away from Bruch's membrane (arrow), presumably to migrate into the inner retina. (×5,100).

Fig. 9. Different inclusions are observed in this pigment epithelial cell from a vitamin E-deficient monkey. A large loosely packed portion of outer segment (*A*) is contrasted by a more densely packed phagosome (*B*) and a lysosome (*C*). A cigar-shaped melanin granule is also visible. Mitochondria (M). (×13,800).

21 months revealed squamous metaplasia and keratinization of the trachea, conjunctiva, excretory ducts in the parotid gland, and transitional epithelium of the renal pelvis (Fig. 11). Pyelonephritis was associated with the latter change in both monkeys.

The one eye examined by light microscopy after 16 months revealed a shortened, disrupted pattern among all photoreceptor OS in the macula and many in the paramacular retina. This degeneration was less apparent in the peripheral retina. The ONL was reduced in thickness and contained degenerating nuclei corresponding to the degree of OS degeneration (Fig. 1). The superonasal quadrant of the retina was affected, but to a lesser degree. In the opposite eye only the mid-



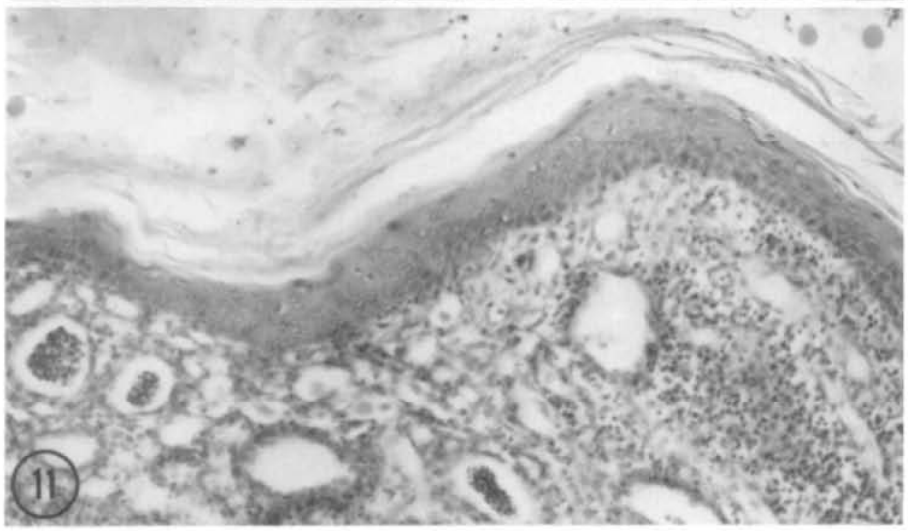


Fig. 10. Classical keratomalacia with exudative conjunctivitis was produced after 16 months on a vitamin A-free diet in this adult monkey.

Fig. 11. Keratinization of the renal pelvis transitional epithelium provides histologic evidence of vitamin A deficiency. The inflammatory cells visible in the renal papilla were associated with pyelonephritis. (×450).

peripheral retina was examined by electron microscopy, revealing selective damage of the cone OS. This OS degeneration was characterized by vesiculation, distention, disorientation, and loss of the lamellar discs (Fig. 12). Adjacent rod OS were structurally intact or were artifactually distorted by clefts produced during in vivo perfusion fixation. In other cones, lamellae were interrupted by formation of vesicles and tubules containing irregularly granular dense particles within some of them (Fig. 13). The enucleated eye taken after 21 months from the second monkey with an extinguished b-wave revealed more advanced OS disruption with seemingly equal

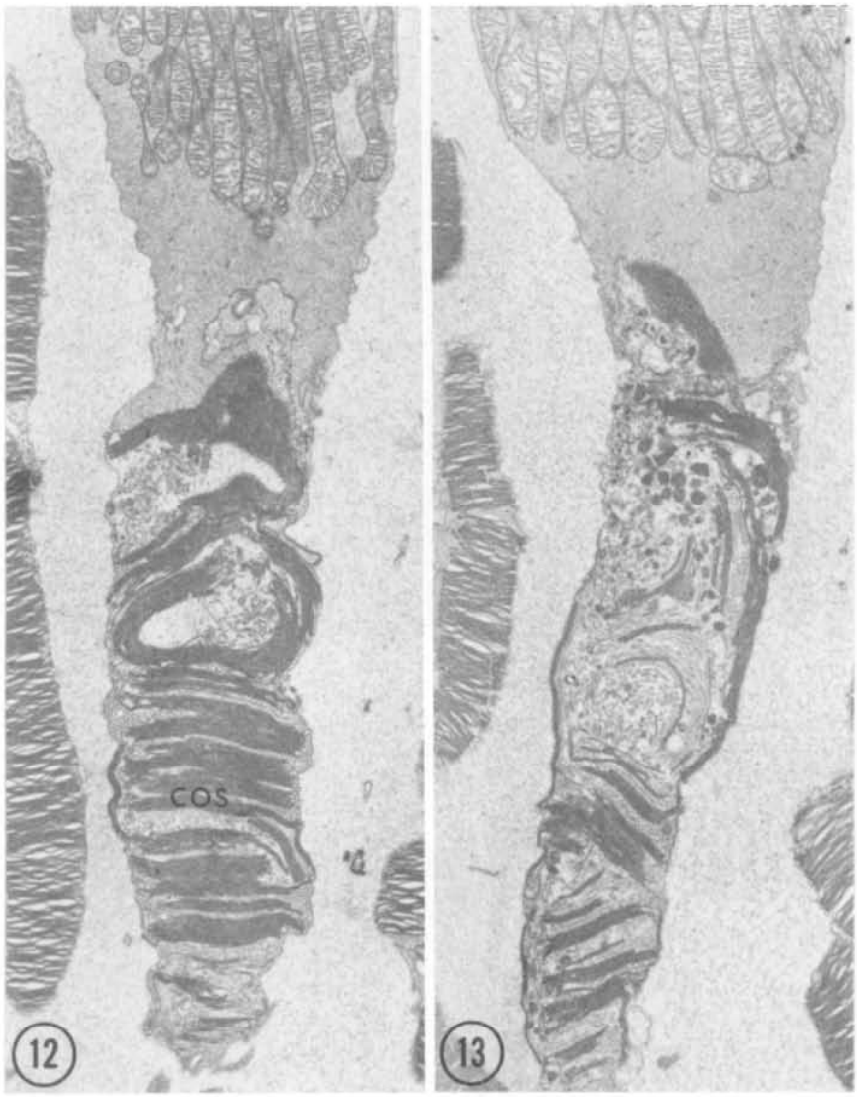


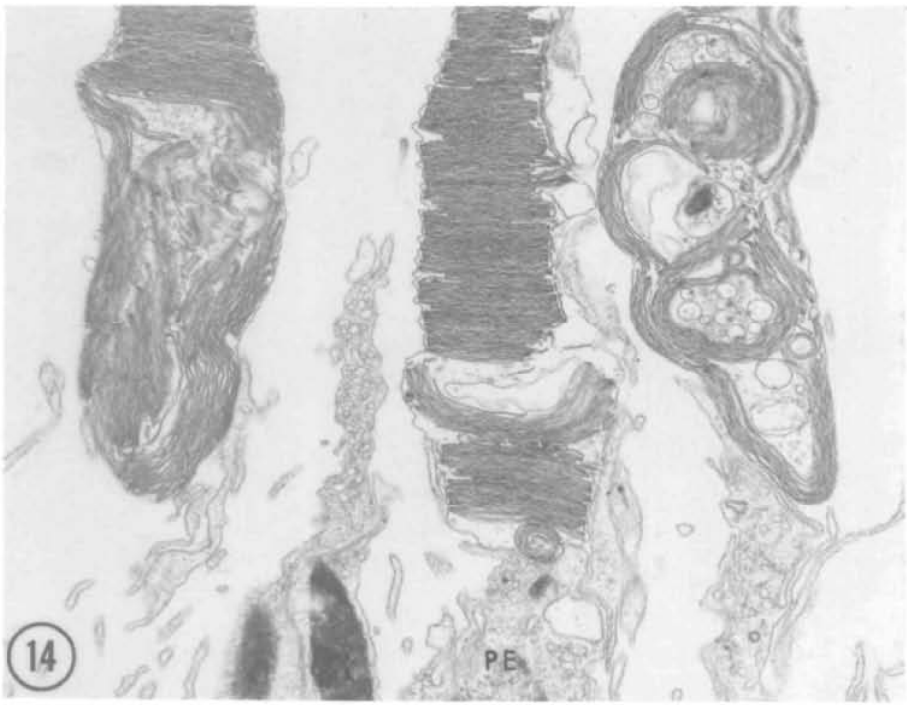
Fig. 12. A degenerating cone outer segment (COS) is visible in this section from the mid-peripheral retina of a vitamin A-deficient monkey. Numerous lamellae have developed vesiculation typical of this deficiency. Adjacent rod outer segments appear normal except for clefts due to fixation artifact. (×9,800).

Fig. 13. Another cone outer segment in the mid-peripheral retina from the same vitamin A-deficient monkey has lost most lamellae. The proximal portion contains vesicles packed with irregular granules. (×9,500).

involvement of rods and cones in electron microscopic sections of the paramacular region (Fig. 14). The pigment epithelium contained an extraordinary number of partially digested cytoplasmic lipid droplets, but was otherwise normal (Fig. 15). Phagosomes were routinely observed, and melanin granules were located in their usual apical position. Panophthalmitis of the other eye in this monkey resulted in total dissolution of all retinal layers.

Discussion

These descriptions serve to emphasize that prolonged vitamin E deficiency in at least two species of monkeys can result



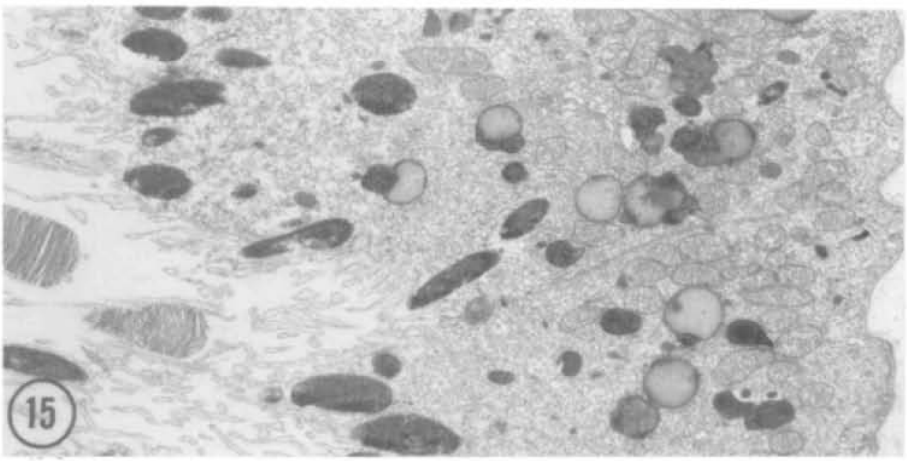


Fig. 14. In more advanced vitamin A deficiency rod outer segments were also disrupted as evidenced by these tips from three outer segments adjoining the pigment epithelium (PE) in the paramacular retina. (×12,700).

Fig. 15. Pigment epithelium from the same vitamin A-deficient monkey contains an abnormal number of lipid-laden lysosomes. (×8,700).

in retinal damage having certain similarities and dissimilarities to those produced with insufficient vitamin A. On the one hand, protracted vitamin A depletion in adult monkeys produced classical signs of deficiency including xerophthalmia and keratomalacia. Rupture of the cornea resulted in destructive panophthalmitis in one monkey. These monkeys demonstrated clinical evidence of impaired vision prior to advanced ophthalmitis and the ERG b-wave from one of them was undetectable. In

the first case light microscopy revealed relatively moderate to minor disruption of OS, primarily restricted to photoreceptors in the macula and paramacular retina. Electron microscopy suggested that the earliest OS damage, in the mid-peripheral retina examined, predominated in cones. Both rods and cones appeared damaged in the macula and in the surrounding retina of the more advanced lesion. Degeneration of the ONL and numerous lipid-laden lysosomes in the pigment epithelium were the only other changes observed.

On the other hand, vitamin E deficiency resulted in no signs of deficiency disease except the malaise and pallor accompanying severe anemia, and this only in monkeys fed the polyunsaturated fat. Retinal degeneration, however, occurred in animals fed both types of fat following an extraordinarily long depletion period. This degeneration was not manifest by clinical evidence of blindness or a change in b-wave threshold. Like vitamin A deficiency, the initial lesion was obsered in the photoreceptor OS. Unlike vitamin A deficiency it was restricted to the macula and more totally disrupted the affected OS. The intact retina surrounding the macula could explain the finding of a normal full-field ERG b-wave threshold.

These differences reveal something of the pathogenesis of the lesions. From knowledge of vitamin A function in vision, it is predictable that depletion should result in uniform loss of the visual pigments, rhodopsin and iodopsin, throughout the retina and thereby disrupt the electrophysical aspects of vision followed by structural dismantling of the OS themselves.[11] It is interesting that peripheral cones and the cone-rich macula appeared to degenerate first, a finding previously reported in monkeys.[12] Even in the rod-dominant retinas of vitamin A-deficient rats and guinea pigs, the central area of the retina is first to degenerate.[11, 13] The observation that cone structure may be more critically affected by the lack of vitamin A than that of rods is surprising since cone visual pigments are synthesized more rapidly than those of rods,[14] and cones have a much greater affinity for retinaldehyde.[15] Furthermore, psychophysical testing in man suggests that the functional correlates of the two photoreceptors are equally sensitive to the night blindness of vitamin A depletion.[16, 17] Perhaps the structural integrity of the cone is more difficult to maintain due to its slower turnover rate.[18] Cone function and structure have been found altered in the cone-dominant retina of the ground squirrel made vitamin A deficient.[19]

The function of tocopherol in the retina is not known, but it has been isolated from bovine OS[7] and might be required as a lipid antioxidant for the extraordinary concentration of polyunsaturated fatty acids found in the OS.[20] This hypothesis is enhanced by the vulnerability of the visual cell to x-irradiation, oxygen poisoning, and excessive light, all of which are manifest by disruptive oxidizing reactions in this delicately balanced structure.[21, 22] One should also consider the possibility that vitamin E may protect retinaldehyde in the retina from peroxidative destruction; however, if true, the retinal degeneration and visual impairment measured by ERG might be expected to resemble vitamin A deficiency more closely.

The susceptibility of the macula in vitamin E deficiency would again emphasize the peculiar character of this area, perhaps as a function of its cone density and the metabolic or structural correlates specific to this photoreceptor. It was not determined whether cone damage preceded or was more extensive than rod disruption in this deficiency, as both appeared equally involved. The focal, massive degeneration of retinal OS in vitamin E deficiency was in contrast to the less disruptive dismantling and progressive shortening of OS in vitamin A deficiency. The remarkable accumulation of lipofuscin pigment in the pigment epithelium of the vitamin E-deficient monkeys is identical to that previously described in dogs[3] and suggests that the pigment epithelium is capable

of extreme phagocytic activity and lysosomal digestion in this deficiency state.

Whether the retinal degeneration induced by tocopherol deficiency has a counterpart in any other species is unknown. It is noteworthy that a previous description of prolonged vitamin E deficiency in dogs was associated with extensive retinal degeneration[3] and that children with abetalipoproteinemia have remarkably low circulating levels of tocopherol. The retinal degeneration found in these children can be influenced by vitamin A.[23] Whether vitamin E is limiting has not been determined. In any event, further work is needed to explore the role of antioxidants in photoreceptor function and structure.

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REFERENCES

1. Ferguson, T. M., Rigdon, R. H., and Couch, J. R.: Cataracts in vitamin E deficiency. An experimental study in the turkey embryo, Arch. Ophthalmol. **55**: 346, 1956.
2. Devi, A., Raina, P. L., and Singh, A.: Abnormal protein and nucleic acid metabolism as a cause of cataract formation induced by nutritional deficiency in rabbits, Br. J. Ophthalmol. **49**: 271, 1965.
3. Hayes, K. C., Rousseau, J. E., Jr., and Hegsted, D. M.: Plasma tocopherol concentrations and vitamin E deficiency in dogs, J. Am. Vet. Med. Assoc. **157**: 64, 1970.
4. Kayden, H. J.: Abetalipoproteinemia. Ann. Rev. Med. **23**: 285, 1972.
5. Hayes, K. C.: Pathophysiology of vitamin E deficiency in monkeys, Am. J. Clin. Nutr. In press.
6. Zeman, W.: Studies in the neuronal ceroid-lipofuscinosis. J. Neuropathol. Exp. Neurol. **33**: 1, 1974.
7. Dilley, R. A., and McConnell, D. G.: Alpha-tocopherol in the retinal outer segment of bovine eyes, J. Mem. Biol. **2**: 317, 1970.
8. Ausman, L. M., and Hayes, K. C.: Vitamin E deficiency anemia in Old and New World monkeys, Am. J. Clin. Nutr. In press.
9. Hansen, L. G., and Warwick, W. J.: A fluorometric micro-method for serum vitamin A, Am. J. Clin. Pathol. **50**: 525, 1968.
10. Rabin, A. R., Hayes, K. C., and Berson, E. L.: Cone and rod responses in nutritionally induced retinal degeneration in the cat, INVEST. OPHTHALMOL. **12**: 694, 1973.
11. Dowling, J. E., and Wald, G.: Vitamin A deficiency and night blindness, Proc. Nat. Acad. Sci. **44**: 648, 1958.
12. Ramalingaswami, V., Leach, E. H., and Sriramachari, S.: Ocular structure in vitamin A deficiency in the monkey, Q. J. Exp. Physiol. **40**: 337, 1955.
13. Lewin, D. R., Thompson, J. N., Pitt, G. A. J., and Howell, J. McC.: Blindness resulting from vitamin A deficiency in albino and pigmented guinea pigs and rats, Int. J. Vit. Res. **40**: 270, 1970.
14. Wald, G., Brown, P. K., and Smith, P. H.: Iodopsin, J. Gen. Physiol. **38**: 623, 1954-55.
15. Rushton, W. A. H.: Rod-cone rivalry in pigment regeneration, J. Physiol. **198**: 210, 1968.
16. Wald, G., Jeghers, H., and Arminio, J.: An experiment in human dietary night blindness, Am. J. Physiol. **123**: 732, 1938.
17. Hecht, S., and Mandelbaum, J.: The relation between vitamin A and dark adaptation, J. A. M. A. **112**: 1910, 1939.
18. Young, R. W.: Renewal systems in rods and cones, Ann. Ophthalmol. **5**: 843, 1973.
19. Berson, E. L.: Experimental and therapeutic aspects of photic damage to the retina, INVEST. OPHTHALMOL. **12**: 35, 1973.
20. Poincelot, R. P., and Abrahamson, E. W.: Fatty acid composition of bovine rod outer segments and rhodopsin, Biochim. Biophys. Acta **202**: 382, 1970.
21. Noell, W. K.: Aspects of experimental and hereditary retinal degeneration. *In:* Biochemistry of the Retina. Graymore, C. N., editor. New York, 1965, Academic Press.
22. Pattison, M., Sweasey, D., and Patterson, D. S. P.: Photodynamic lipid peroxidation in the eye during experimental induction of Turkey Blindness syndrome, Exp. Eye Res. **16**: 191, 1973.
23. Gouras, P., Carr, R. E., and Gunkel, R. D.: Retinitis pigmentosa in abetalipoproteinemia: effect of vitamin A, INVEST. OPHTHALMOL. **10**: 784, 1972.