MORPHOLOGICAL CHANGES IN THE PHOTORECEPTOR CELLS AND RETINAL EPITHELIUM OF THE ALBINO WISTAR RAT IN VITAMIN A DEFICIENCY

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ABSTRACT

Structural changes in the outer retinas of rats kept on a vitamin A free diet supplemented with vitamin A acid were studied by light and electron microscopic techniques. Retinas were sampled at frequent intervals over a period of 11 months of vitamin A deficiency and their morphology compared with that of retinas from rats of the same age, fed a normal diet. Other studies done in conjunction with the above included, (1) measurements of weight gain and plasma vitamin A content in control and vitamin A deficient rats (2) localization of acid phosphatase activity in the retinal epithelium in vitamin A deficiency (3) radioautographic study of H^3-methionine incorporation into retinas of control and vitamin A deficient rats. The results showed that vitamin A deficient animals gained weight more slowly than the controls and suffered a rapid decline in plasma vitamin A content after 3 weeks on the special diet. Light microscopic study revealed that the first portions of the photoreceptor cell to suffer degeneration in vitamin A deficiency were the outer segments followed successively by the inner segments, synaptic processes and photoreceptor nuclei. Electron microscopic study revealed that after 3-4 weeks on the special diet, corresponding to the fall in plasma vitamin A levels, the lamellar discs of the photoreceptor outer segments began to break down into vesicles and tubules. After 2.5 months of vitamin A deficiency, the photoreceptor inner segments began to swell and shorten and display loss of mitochonria and ribosomes in their cytoplasm. After 4-5 months, many outer segments had completely disappeared

and further shortening of the inner segments was evident. After 6 months, very few of the remaining outer segments were intact although it was still possible to identify small number of polysomes and mitochondria within the proximal inner segment cytoplasm next to the photoreceptor nuclei. At this stage, the photoreceptor synaptic processes were also severely affected. The number of synaptic vesicles in each process was greatly reduced and large gaps appeared in the plasma membranes. The synaptic processes were considerably shortened and synaptic sites reduced in number. After 9 months, the outer segments had completely disappeared except for a few remaining clusters of disordered saccules. Both the inner segments and the synaptic processes had retracted markedly towards the photoreceptor nuclei. For the first time, the photoreceptor nuclear envelope began to break down although the nuclear chromatin appeared unchanged. After 10-11 months, only a single irregular row of photoreceptor nuclei remained and each nucleus was surrounded by a narrow rim of cytoplasm containing only a few recognizable organelles. Each photoreceptor cell was surrounded by several layers of membranes probably from glial cell processes. The cell junctions normally forming the outer limiting membrane were now absent in many places. Due to the loss of the photoreceptors the inner neural retina lay very close to the pigment epithelium and the outer processes of the Müller cells were deflected laterally, contributing to the cell membranes surrounding the photoreceptor remnants. Progressive changes were also noted in the structure of the retinal epithelium of the vitamin A deficient animals which were not present in control animals of the same age. By 4-5 months of vitamin A deficiency, large numbers of lysosomes had accumulated in

the retinal epithelial cytoplasm close to its inner border. This increase in lysosomes persisted throughout the study as the degeneration of the photoreceptors continued. The lysosomes and the Golgi complexes of the retinal epithelium were found to contain the enzyme, acid phosphatase. The inner or apical processes of the retinal epithelium also proliferated markedly and became very prominent after 11 months of vitamin A deficiency. Radioautographic studies showed that H^3-methionine was incorporated into photoreceptors of both control and vitamin A deficient animals, thus indicating that protein synthesis continued in photoreceptors in vitamin A deficiency.

CONTENTS

LIST OF FIGURES

Figure Page

Figure Page

Figure Page

Figure

Page

Figure Page

Figure Page

Figure Page

Figure

Page

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I INTRODUCTION

Blindness due to either corneal, retinal or optic nerve damage is the most important and striking feature of vitamin A deficiency. Destruction of the cornea in xerophthalmia and keratomalacia in man and most experimental animals is due to deficiency of vitamin A (Johnson 1939, 1943; Dowling and Wald, 1958). The loss of the prosthetic group, retinene, from the rod visual pigment, rhodopsin, leads to night blindness in man and experimental animals (Fridericia and Holm, 1925; Tansley, 1931; Wald, 1935a, 1936; Wald et al., 1938; Haig et al 1938; Wald and Steven, 1939; Steven and Wald, 1940). Dowling and Wald (1960) have shown that vitamin A deficient rats kept alive by administration of vitamin A acid eventually lose photopic as well as scotopic vision, presumably because retinene is the prosthetic group for cone as well as rod visual pigment. In cattle and dogs, vitamin A deficiency also leads to faulty growth of bone and nervous tissue which can cause constriction of the optic nerve (Moore et al., 1935; Hart and Guilbert, 1937; Moore, 1939).

Tansley (1933, 1936) first described the structural damage to the rod photoreceptors in vitamin A deficient rats and dogs. Later Johnson (1939, 1943) confirmed and extended Tansley's observations in the rat. He observed by light microscopy that after 7-13 weeks of vitamin A deprivation in young rats, many photoreceptor outer segments disappeared and those that remained stained abnormally. As the deficiency progressed, the rod inner segments also degenerated, and then successively the external

limiting membrane, the outer nuclear layer and the inner nuclear layer. The outer segments of rods which had deteriorated only slightly showed considerable regeneration within 24 hours of readministration of vitamin A. Even rods which had degenerated completely regenerated within 10-18 weeks of dietary vitamin A supplementation. Fifteen years later, in an attempt to map the entire course of vitamin A deficiency and its cure in the rat, Dowling and Wald (1958) approached the problem from physiological, biochemical and histological aspects. They found that, in weanling rats after 3 weeks of vitamin A deprivation, the liver vitamin A content had declined and a week later the blood vitamin A content also fell. At this point, the retinal rhodopsin content began to decline linearly and regularly, marking the onset of night blindness. By the beginning of the seventh week, levels of opsin too began to decline and histological deterioration of the retina was observed.

In these studies, the experimental animals usually died within 2-3 months of vitamin A deprivation. It was then discovered that vitamin A acid, first prepared by Aren and Van Dorp (1946), can maintain growth in the rat without affecting the deleterious effects of vitamin A deficiency on the visual system. This made a more detailed investigation of the anatomical changes in the retina possible since animals are unable to reduce vitamin A acid to vitamin A aldehyde and thus influence the visual system (Moore, 1957). A series of studies of the effects of vitamin A deficiency on the retina in animals supplemented with vitamin A acid were then undertaken by Dowling and his associates (Dowling and Wald, 1960; Dowling and Gibbons, 1961; Dowling, 1966). Histological studies on retinas of albino rats were carried out over a period of 10 months. Dowling and Gibbons (1961) observed by light microscopy that

the degeneration of the photoreceptors followed the same pattern seen earlier by Johnson (1939, 1943). Electron microscopic studies (Dowling and Gibbons, 1961) showed that after 2 months on a vitamin A free diet, supplemented with vitamin A acid, the first sign of degeneration of the photoreceptor outer segments was breakdown of the outer segment discs into vesicles and tubules. After a high proportion of the discs had degenerated, the outer segments began to lose their normal elongated, cylindrical shape and became almost spherical. After 6 months of vitamin A deprivation, only fragments of photoreceptor outer segments remained. The photoreceptor nuclei and inner segments were greatly reduced in number and the inner segments that remained were short and thicker than normal. The fine structure of the inner segments, however, appeared normal. Müller cell processes became highly conspicuous in the spaces left between the remaining segments. By 10 months of vitamin A deficiency, the neural retina and the retinal epithelium adhered tightly to one another, the photoreceptor inner and outer segments had disappeared, and the photoreceptor nuclei had been reduced to one irregular row which, however, appeared normal. The rest of the retina and the retinal epithelium also appeared normal. The outer plexiform layer was in direct contact with the retinal epithelium but was somewhat reduced in thickness due to a loss of synaptic processes from the visual cells. The photoreceptors were capable of regeneration as long as the inner segments were present. From the above observations, Dowling and Gibbons (1961) concluded that loss of opsin, a major component of the outer segments, (14% dry weight in cattle and 40% dry weight in frogs) was probably the primary cause of the structural damage to the outer segments.

More recently, Nöell et al. (1971) have shown that the effects

of vitamin A deficiency on the rat eye are dependent upon the levels of illumination to which the rats are exposed daily. Animals kept in darkness retain their normal electroretinographic (ERG) function and retinal rhodopsin content much longer in vitamin A deficiency than those exposed to weak cyclic light. Shear et al., (1973) reported that the retina of albino rats which have been kept in a cyclic environment (14 hours of low intensity illumination and 10 hours of darkness) show degeneration when they are killed after a period of 6 to 12 hours illumination. However, the retinas from those animals that have spent several hours in darkness before they are killed are normal. The structural degeneration was due to a separation of the adjacent pigment epithelial cells, retraction of epithelial apical processes and change of the lamellar discs of the apical 1/3 of all photoreceptor outer segments into tubules. Recently, Herron and Riegel (1974a, 1974b) have shown by radioautographic study that production of photoreceptor outer segment protein is decreased in vitamin A deficient rats and suggest that vitamin A must be available for rod outer segment production as lack of it slows the production rate.

Despite the foregoing work, a detailed study of the sequential breakdown and fine structural changes in all regions of the photoreceptor cell in vitamin A deficiency is still lacking. Although it has been suggested that the retinal epithelium is unaffected in vitamin A deficiency (Johnson, 1939, 1943; Dowling and Wald, 1958; Dowling and Gibbons, 1961), there is as yet little evidence to support this conclusion. Furthermore, it seems illogical that the pigment epithelium would be unaffected during the photoreceptor breakdown process since the retinal epithelium normally is active in the phagocytosis of rod outer segment fragments (Dowling and

Gibbons, 1962; Bairati and Orzalesi, 1963; Ishikawa and Yamada, 1970; Young, 1967, 1971a Young and Bok, 1969; Spitznas and Hogan, 1970). One would anticipate that there would be a marked increase in the activity of the pigment epithelium in this regard as photoreceptors are destroyed due to lack of vitamin A. Also in the classical studies of vitamin A deficiency by Dowling and his associates (Dowling and Wald, 1960; Dowling and Gibbons, 1961; Dowling, 1966) the possible effect of light damage to the retina was not taken into account. There is no mention of the length of time each day the animals were exposed to light and the light intensity. As mentioned above lighting conditions are now known to markedly affect photoreceptor cell morphology (Nöell and Albrecht, 1971; Shear et al., 1973).

The present study has been undertaken in an effort to overcome these difficulties and augment our knowledge of the event, particularly the morphological changes taking place during vitamin A deficiency. The study examines, by light and transmission electron microscopy, the structural changes taking place in the photoreceptors and retinal epithelium of the albino rat maintained on a vitamin A free diet supplemented with vitamin A acid. The animals were kept under strictly controlled lighting conditions of 12 hours of low intensity light and 12 hours of darkness per day. The fine structure of the photoreceptors and retinal epithelium has been studied in detail in vitamin A deficient and control animals of varying ages. Growth rates of vitamin A deficient and control animals have been compared. Blood vitamin A levels from animals that were on the vitamin A free diet have been analysed and compared with controls. The details and sequence of destruction of the

various portions of the photoreceptors in vitamin A deficiency has been examined at frequent intervals over a period of 11 months and compared with photoreceptor structure in control rats during aging. Morphological changes in the retinal epithelium in control and vitamin A deficient rats also have been examined similarly. Acid phosphatase localization has been used to test for the presence of lysosomes in the retinal epithelium of the vitamin A deficient animals. Finally, H^3-methionine was administered to the vitamin A deficient animals to determine if protein synthesis still occurs in the disintegrating photoreceptors.

The objects of the present study are: 1). to further our understanding of the detailed sequential breakdown of the entire photoreceptor including its outer segment, inner segment, nucleus and synaptic process in vitamin A deficiency and 2). to elucidate the subsequent effect of vitamin A deficiency on the structure and function of the retinal epithelium and glial cells. A complete knowledge of the above events will be beneficial in understanding the central role played by vitamin A in vision.

II HISTORICAL REVIEW

The Early History of Retinal Investigation

Although the visual organ was one of the foremost subjects of interest among ancient scientists, detailed study of the structure and function of the retina has not taken place until recent time (Polyak, 1941). The Greeks considered the retina as a "net-like tunic" (Polyak, 1941). Kepler (1604) was the first to demonstrate the essential role of the retina as a photoreceptor. Lack of instruments and the primitive conditions of optical techniques at the time prevented a more advanced investigation of its structure. Development of the first microscope led to more profitable investigation of the visual organ. Antony van Leeuwenhoek (1674) in a letter to the publisher of the Philosophical Transactions of the Royal Society of London reported what he saw in the retina of the cow in the following short statement: "The third tunicle was exceedingly thin and tender and having viewed it, I found it also consists of globules units". Ten years later, in a letter to the secretary of the Royal Society of London, Leeuwenhoek (1684) again mentioned the retina, this time, of the frog. He again mentioned the blood vessels and the globules. He was the first to see the blood capillaries, nerve cells and also the rods and cones. Unfortunately his observation received little attention.

During the early part of the eighteenth century the function of

the retina was thought to be as follows: light rays caused vibratory motion of the optic nerve fibers, which in co-operation with the "spirits" mediated the reception and transmission of the visual impressions to the brain, where the objects were recognized by the "soul" (Maitre-Jan, 1725).

Towards the end of the eighteenth century, investigation of the retina was aided by the introduction of a number of chemicals as fixatives and by high power microscopes. There were widely divergent views regarding the structure of the retina. Some believed it to be a pure web of nerve fibers. Others considered it as an expansion of the medullary part of the optic nerve and still others came to the conclusion that the retina was composed of the mucous substance of the brain. Fontana (1782, 1795) was the first to throw light upon the existing chaos of observations and hypotheses on the retina. He studied the retina of the rabbit and visualized optic nerve fibers, ganglion and other nerve cells suspended in a supporting framework of neuroglia and blood capillaries. Unfortunately he overlooked the rods and cones.

Treviranus, between 1835-1838, carried out a systematic study of the retina marking the beginning of modern investigation of retinal structure and function. His research animals ranged from fish to mammals. He described the retina as being entirely composed of thickly packed and extremely delicate tubes whose blind ends resembled tiny warts or "papillae" which he believed to protrude onto the vitreal face of the retina (Treviranus, 1835). He believed the "papillae" to be photoreceptors (Treviranus, 1835), but by mistake, he placed them on the vitreal face.

Following Treviranus, it was Valentin (1837) who demonstrated

that the retina was composed of parallel, regularly arranged layers. He correctly recognized the position of the bacillary layer (layer of rods and cones) but by mistake reversed the order of the layers in the rest of the retina. The outer position of the bacillary layer was later confirmed by Bidder (1839) who found that the free ends of the rods were always turned toward the choroid membrane.

The study of retinal structure was advanced when Hannover (1840) demonstrated how to apply chromic acid to harden the retina. This improvement made it possible to cut thin section of retinal tissue. During the decade following the introduction of chromic acid into laboratory techniques, the structure of the human and of other vertebrate retinas was extensively studied by Pacini (1845), Bowman (1849), Vintschgan (1853), Kölliker (1854) and especially by Müller (1852, 1853, 1854, 1856-1857). These studies revealed the stratification of the retina. In addition, Müller (1851) discovered fibrous structures which passed through the entire thickness of the retina. He named them "radial fibers" and they still bear his name. By means of physiological observations and with the help of pertinent histological data, Müller (1853, 1854, 1856-57) computed the locus of photoreception to be in the bacillary layer.

The first comprehensive descriptions of retinal histology and functional interpretations of retinal structure were put forward by Müller (1853, 1854, 1856-57) and Kölliker (1854). They believed that the rods and cones were true photoreceptors. Because of the outer position of the rods and cones, the stimulating rays of light have to pass through almost the entire thickness of the retina.

However, this obstacle was largely reduced by the great transparency and homogeneity of the retinal substance. They suggested that excitation in the rods and the cones travelled, in the direction opposite to light, along the "radial fibers", through the various intercalated nuclear and ganglion cells, and along the optic nerve fibers until finally entering the visual centers of the brain.

Histological technique was further improved when Schultze and Rudneff (1865) introduced osmium as a fixative. They substantiated Müller and Kölliker's views regarding the functional intepretation of retinal structure. Schultze (1872) subdivided the retina stretching from the choroid to the vitreous into the present 10 layers, namely, 1) pigment epithelium, 2) rods and cones, 3) outer limiting membrane 4) outer nuclear layer, 5) outer granular (plexiform) layer, 6) inner nuclear layer, 7) inner granular (plexiform) layer, 8) ganglion cells, 9) optic nerve fibers and 10) inner limiting membrane. He (1873) further elucidated the histological details of the photoreceptor cells which he called cones and rods. The two types of photoreceptors were classified on the basis that 1) cone nuclei occupied a more scleral position in comparison with those of the rods, 2) rod outer segments were elongated, thin and cylindrical while those of the cone were thick and tapered and 3) the rod cell had a knot-like ending and the cone had a foot-like ending. The area of the photoreceptor cell scleral to the photoreceptor nucleus was divided into an inner and outer segment because of differences in staining. In addition to his important contribution during this era, Schultze (1872) introduced the important "duplicity theory" of vision which indicated that the rod photoreceptors were for dim light (scotopic) vision and cones were for colour and discriminative

bright light (photopic) vision.

Towards the end of the nineteenth century, Camillo Golgi (1873, 1878) discovered that a solution of silver nitrate stained nerve cells black. Tartuferi (1887) applied this method to the retina and found that the cone fibers split at their vitreal ends into a number of delicate filaments which entered the outer plexiform layer. Here these cone filaments met the scleral expansions of the bipolar and other cells whose bodies formed the inner nuclear layer and whose vitreal processes descended into the inner plexiform layer. In the latter, these descending processes seemed again to merge with each other and with the scleral expansions of the ganglion cells, thus establishing a connection between the photoreceptors, on the one hand, and the optic nerve fibers, on the other.

It was Ramon Cajal (1892, 1911) who supplemented the duplicity theory by assuming a duplex conducting mechanism all along the visual pathway. According to Cajal the nerve currents elicited in the receptive elements by the photic stimuli had to pass through the following three sets of neurons before they reach the brain: 1) the rods and cones, 2) the bipolars and 3) the ganglion cells. The rods and cones each had their own sets of bipolars and ganglion cells. Cajal also confirmed the "Neurone Theory" which proposed that the neurones comprising nerve tissues were each structural and functional entities. No longer was nerve tissue to be considered a continuous reticulum.

Fine Structure of the Photoreceptor

The outer segment

Photoreceptor fine structure was first studied by Sjöstrand (1949, 1953a). He described the outer segment of the rod photoreceptors of the quinea pig and perch, and cone photoreceptor of the perch as being composed of a pile of membranous discs enclosed within a cell membrane. In the rod photoreceptor of the quinea pig, each disc was found to be formed by a double membrane which appeared to be free of the cell plasma membrane. Each disc also possessed a single incisure The incisures of the discs were found to be aligned forming a longitudinal groove. These findings were also reported in photoreceptors of other rodents; e.g. the mouse (Cohen, 1960) and the rat (Dowling and Gibbons, 1961). Similarly, in amphibia, Porter (1956), Yamada (1957) and Wald (1958a) showed that the rod outer segment also consists of a pile of membranous discs. Instead of a single incisure in amphibia each disc has numerous incisures resulting in a scalloped outline. Porter (1956) pointed out that the incisures increased the surface area of the discs. Subsequent studies of rod outer segments in pigeon (Cohen, 1963), and in man and monkey (Cohen, 1965) have also revealed a scalloped appearance of the discs. The incisures appear to be aligned with each other (Fernandez-Moran, 1961; de Robertis and Lasansky, 1961).

In cone outer segment, most of the discs, except for a few at the outermost tip, have their membranes continuous with the surface membrane of the cell suggesting that the discs are made up of actual infoldings of

the cell membrane (Sjöstrand, 1959 [perch]; Lasansky and de Robertis, 1960 [toad]; Moody and Robertson, 1960, Yamada, 1960 [frog]; Cohen, 1961a, 1961b [monkey], 1964 [squirrel] 1968 [frog]). In rod outer segments, the majority of the discs are isolated and separated, although a few are said to communicate with the surface membrane at the base of an outer segment (Robertson, 1965; Cohen, 1964, 1965, 1968). Young (1971a, 1971b) has shown by radioautographic studies that the discs of the rod outer segment are constantly shed and intermittently renewed while those of the cone are longlasting.

The discs of the rod outer segment are made up of two membranes. Each membrane consists of two parallel dense layers each 20 A thick, separated by a light interspace 35 A thick (Moody and Robertson, 1960). The disc has been shown by the freeze-fracture technique to possess two dissimilar membrane surfaces. A "rough" surface studded with spherical and linear protrusions facing the intradisc space and a "smooth" surface facing the interdisc space (Clark and Branton, 1968; Leeson, 1970, 1971b). More recently, Capaldi (1974) has suggested a new model for the cell membrane which consists of a lipid bilayer 45 A thick and globular proteins forming intrinsic and extrinsic parts of the membrane. The intrinsic protein forms the integral part of the membrane. The disc membrane has been suggested to contain only intrinsic protein which is the photopigment, rhodopsin. In darkness, rhodopsin molecules are submerged for about 1/3 of their diameter in the disc membrane's outer surface. When illuminated, the rhodopsin molecules sink deeper into the membrane until they are half-submerged.

The connecting cilium

The outer segment is connected to the inner segment by a connecting cilium. The cilium is eccentrically placed and enters the outer segment of the rod at the base of the groove formed by the series of disc incisures (de Robertis, 1956). Porter (1956) and de Robertis (1956) both demonstrated that the membrane covering the outer segment is continuous with the cell plasma membrane by way of the connecting cilium. They noted that the cilium contains 9 pairs of peripheral tubules but lacks the central pair characteristic of a motile cilium. Such paired peripheral tubules have been described in the guinea pig rod (Sjöstrand, 1953a), in rabbit rods (de Robertis, 1956), and cones (de Robertis and Lasansky, 1958) and in human rods (Missotten, 1965a; Yamada et al., 1958b) and cones (Yamada et al., 1958b). The peripheral tubules enter the apical portion of the inner segment and end in a basal body or centriole (Cohen, 1961a, 1961b; de Robertis, 1956; de Robertis and Lasansky, 1958; Sjöstrand, 1953b; Yamada et al., 1958b). A second centriole, orientated at right angle to the basal body, has been described in human rods and cones by Yamada et al., (1958b) and in monkey rods and cones by Cohen (1961a, 1961b).

The inner segment

The inner segment of the photoreceptor is marked by a concentration of mitochondria at its apex. This accumulation corresponds to the ellipsoid seen by light microscopy (Walls, 1942). In the rod, the mitochondria are oriented with their long axes parallel to the axis of the cell (de Robertis, 1956; Cohen, 1960; Yamada, 1957). However, there

appears to be no such orientation in the cone (de Robertis and Lasanksy, 1958). In the rod ellipsoid, endoplasmic reticulum has been observed between the mitochondria, in man (Missotten, 1965a), in the rabbit (de Robertis, 1956) and in the frog (Yamada, 1957), but there appears to be little endoplasmic reticulum between the mitochondria in the cone ellipsoid (de Robertis and Lasanksy, 1958). From the basal body arise the cillary rootlets which run the entire length of the inner segment between two systems of vacuoles (Cohen, 1961a, 1961b; Uga et al., 1970; Bairati and Orzalesi, 1963). Cohen (1960) suggested that the ciliary rootlets may be concerned with conduction of excitation while Uga et al. (1970) indicated they may serve as a skeletal support for the receptor inner segments. Below the ellipsoid, the cytoplasm contains ribosomes, rough endoplasmic reticulum and a Golgi complex situated just above the external limiting membrane (Sjöstrand, 1953b; Cohen, 1961b, 1963). This region is known as the myoid because in some lower vertebrates, it is contractile and responds to changes in retinal illumination (Young, 1969). Membrane bound oil droplets are found in the cone inner segments of some non mammalian species (Walls, 1942; Duke-Elder, 1958; Pedler and Tansley, 1963; Sjöstrand and Elfvin, 1957; Berger, 1965, 1966; Borwein and Hollenberg, 1973). The oil droplets are believed to be formed by mitochondrial fusion in a vitreal to scleral gradient (Berger, 1964; Ishikawa and Yamada, 1969; Borwein and Hollenberg, 1973).

Vertebrate photoreceptor nuclei are typically oval or spherical in shape (Nilsson, 1964; Hollenberg and Berstein, 1966; Morris and Shorey, 1967; Dowling and Werblin, 1969) but in the newt, they are elongated, cylindrical structures (Dickson and Hollenberg, 1971). Rod nuclei are

more electron dense than the cones (Nilsson, 1964; Dickson and Hollenberg 1971). In the frog, the cone nuclei are located closer to the outer plexiform layer than the rods while the opposite is the case in the newt retina. The nuclei are surrounded by narrow rims of cytoplasm. Cohen (1960) noted that the rod nuclei are often packed together suggesting the possibility of interaction between rods at the nuclear level. Where the nuclei are separated, processes of Müller's fibers lie between them (Cohen, 1961b).

The rod spherule

The basic morphology of the photoreceptor synaptic terminals is remarkedly uniform in all classes of vertebrates (de Robertis, 1958; Cohen, 1969; Dartnall and Tansley, 1963; Evans, 1966; Stell, 1967, Dowling, 1968, 1970; Kolb, 1970). The rod synaptic terminal often enlarges to form a spherule. A single mitochondrion has been found in the rod spherule in the rat (Ladman, 1958) and the mouse (Cohen, 1960). Mitochondria have also been reported in both rod and cone synaptic terminals of monkey photoreceptors (Cohen, 1961b), but are absent in synaptic terminals of rabbit (de Robertis and Franchi, 1956), guinea pig and opossum (Ladman, 1958). Rod synapses usually have only one synaptic ribbon (a half-moon shaped lamellar structure) associated with the penetrating bipolar and horizontal cell processes at the synaptic junction (Cohen, 1961b; de Robertis and Franchi, 1956; Ladman, 1958; Sjöstrand, 1958). Missotten (1965a) reported that several synaptic ribbons may be present in the rods of man. Ladman (1958) and Cohen (1961b) described in rat synaptic terminals another lamellar structure related to the

synaptic ribbon, the "rod arciform density". Synaptic vesicles are present in the rod spherules and are thought to contain neuro-transmitter substance (de Robertis and Franchi, 1956; de Robertis, 1958). Each synaptic ribbon in both the rods and cones is usually surrounded by a halo of synaptic vesicles. Gray and Pease (1971) postulated that the synaptic ribbon possibly serves to direct the synaptic vesicles down to the presynaptic membrane. The function of the arciform density might be two fold; firstly, to anchor the synaptic ribbon to the presynaptic membrane and secondly, to guide the synaptic vesicles off the ribbon on to the presynaptic membrane after they have passed down along the surface of the ribbon (Gray and Pease, 1971).

The rod spherules make synaptic contacts with a cluster of nerve endings penetrating the spherule in a single invagination (Sjöstrand, 1958, 1961; de Robertis and Franchi, 1956; Ladman, 1958; Cohen, 1963, 1964; Villegas, 1960, 1964; Missotten, 1965b; Evans, 1966). The deeply inserted terminal nerve fibers, situated in a lateral position, have been identified as horizontal cell axon terminals in man (Missotten, 1965b). In goldfish the laterally placed terminal fibers are identified as horizontal cell dendrites (Stell, 1967). The less deeply inserted terminal fibers which are located centrally have been traced to bipolars in man (Missotten, 1965b) and in goldfish (Stell, 1967). Rod superficial contacts have been reported in the mudpuppy (Dowling and Werblin, 1969) and in the newt (Dickson and Hollenberg, 1971). They are formed by slight indentations in the receptor terminal surface produced by the synapsing neuronal processes. Superficial contacts are found in association with synaptic ribbons in the mudpuppy, but in the newt, synaptic ribbons have never been identified with superficial contacts.

Cone pedicles

Cone pedicles of vertebrates have been studied in detail by several investigators (de Robertis and Franchi, 1956; Cohen, 1963, 1964; Pedler and Tansley, 1963; Kalberer and Pedler, 1963; Pedler and Tilly, 1964; Villegas, 1960; Stell, 1967; Evans, 1966; Hollenberg and Berstein, 1966; Dowling and Werblin, 1969; Lasansky, 1972). Missotten and Dooren (1966) reconstructed pedicles of human photoreceptors with serial sections and grouped contacts into 3 types, invaginations, surface contacts and interreceptor contacts.
(a) Invaginations: Fibers from the inner nuclear layer invaginate deep into the pedicle forming a symmetrical triad. The two lateral processes often contain endoplasmic reticulum, microtubules and ribosomes and are now widely accepted as axons of horizontal cells. The central process can be either horizontal cell dendrite or bipolar cell endings (Hogan et al., 1971). Dowling and Boycott (1966), in contrast, feel the central processes belong exclusively to bipolar cells and the lateral processes are not characteristic of either dendrites or axons. In this way the horizontal cells are able to receive and transmit stimuli in a horizontal direction, thus creating interreceptor cross-connections. The synaptic ribbon faces the triad at a right angle, and the arciform density lies between the synaptic ribbon and the cell membrane. There are numerous synaptic vesicles around the synaptic ribbon and the arciform density. (b) Surface contacts: In this situation bipolar dendrites synapse with the cone in shallow indentations on the basal surfaces of the pedicles. There is a slight thickening of the cone and bipolar membranes at this region but synaptic vesicles and synaptic ribbons are absent.

(c) Interreceptor contacts: Interreceptor synapses have been demonstrated in the guinea pig (Sjöstrand, 1958), mouse (Cohen, 1960), frog (Nilsson, 1964), monkey (Cohen, 1961b, Dowling and Boycott, 1966), man Missotten, 1965b; Uga et al., 1970; Hogan et al., 1971) and turtle (Lasansky, 1972). Each pedicle has several lateral expansions which extend a considerable distance horizontally and make contact with lateral expansions of an adjacent cone or the lateral surface of a rod spherule. No synaptic vesicles are found on either side of the contact. It is not known whether the interreceptor contacts are sites of conduction or transmission of nerve impules (Hogan et al., 1971).

Retinal pigment epithelium

The fine structure of the retinal epithelium has been studied extensively in recent years (Yamada et al., 1958a; Bernstein, 1961, 1966; Yamada, 1961; Dowling and Gibbons, 1962; Bairati and Orzalesi, 1963; Breathnack and Wyllie, 1966; Leur-duPree, 1968; Leeson, 1971a; Braekevelt and Hollenberg, 1970). These authors have shown that the mature retinal epithelium is a fairly uniform single layer of rectangular cells, situated around the outer circumference of the retina, extending from the edge of the optic disc to the ora serrata. In a tangential section cut parallel to the pigment epithelium, these cells are hexagonal in shape. In many species, pigment epithelial cells are characterized by numerous basal infoldings of the plasma membrane, an extensive smooth endoplasmic reticulum, pigment granules and numerous apical processes surrounding the photoreceptor outer segments. The basal infoldings and the close presence of numerous mitochondria suggest an active role in metabolic transport from

the choriocapillaries (Dowling and Gibbons, 1962; Bairati and Orzalesi, 1963). The metabolites are carried across the pigment epithelium and supplied to the outer retina including the photoreceptors. In the frog retinal epithelium, the smooth endoplasmic reticulum is closely associated with lamellated myeloid bodies. Porter and Yamada (1960) have suggested that the myeloid body with its large surface area and associated smooth endoplasmic reticulum may play an active role in directing isomerization of all-trans retinaldehyde to the 11-cis configuration, and the reconstitution of rhodopsin from 11-cis retinaldehyde and opsin. The smooth endoplasmic reticulum also may be significant in the interconversion between retinaldehyde and vitamin A, as well as the transport of these compounds to the photoreceptor cells. In the newt pigment epithelium, Dickson and Hollenberg (1971) observed large lipid inclusions closely associated with the smooth endoplasmic reticulum and they have suggested that the latter is involved in lipid metabolism.

The presence of a limited amount of rough endoplasmic reticulum and free ribosomes is also a common feature of most vertebrate retinal epithelia. Leure-duPree (1968) has suggested that during development the rough endoplasmic reticulum may contribute to protyrosinase and tyrosinase biosynthesis. After their production, these enzymes are concentrated in the Golgi apparatus and then in vesicles which subsequently form pro-pigment granules. In the pigment epithelium of the adult albino rat, pigment granules are absent due to a genetic block to melanization (Dowling and Gibbons, 1962). In the human albino melanocypte , the genetic defect has been suggested to be due to the lack of free l-tyrosine available to the melanosome (Mishima and Loud, 1963).

Pigment granules are concentrated in the apical cytoplasmic processes of the pigmented epithelium. Moyer (1969) has suggested that they may function in absorbing light and preventing scatter. This mechanism helps in refinement of the photoreceptor stimulus thereby increasing visual acuity. The pigment granules of certain submammalian species move outwards away from the apical epithelial processes in times of low illumination, thus allowing maximal visual sensitivity at the expense of reduced visual acuity (Moyer, 1969).

That the pigment epithelium is indispensable for the visual process was first noted by Kühne (1878). He reported that a frog retina taken out of the eye can no longer regenerate rhodopsin and that it regains this capacity if laid back upon the pigment epithelium. He was convinced that intimate contact between the neural retina and the pigment epithelium was necessary for rhodopsin to be synthesized in the rods. Retinal detachment results in blindness in human subjects but when contact is re-established, vision is restored. This, Wald (1958a) felt, provided further evidence that the pigment epithelium makes an active contribution to the visual process. In the frog eye, retinene isomerase, which is responsible for the conversion of 11-trans vitamin A back to 11-cis vitamin A, has been shown to be present in the retinal pigment epithelium (Hubbard, 1956). The vitamin A liberated by the bleaching of visual pigments is rapidly esterified in the eye (Krinsky, 1958). In amphibia, the enzymatic system concerned with this process is present in the pigment epithelium (Krinsky, 1958).

The pigment epithelium also plays a major role in the turn over of the photoreceptor outer segments. Young and Bok (1969) have shown

by radioautographic studies that in the frog, newly formed radioactive protein is incorporated into disc membranes at the base of the rod outer segment. These labelled discs are progressively displaced along the outer segments as new discs are formed at the base. When the labelled discs reach the end of the outer segment, they are detached from it and subsequently can be identified in the pigment epithelium.

Phagocytosis by the pigment epithelium is accomplished by the apical epithelial processes. The processes surround the discs at the outermost ends of the rod photoreceptors. The discs are then taken into the epithelial cell as phagosomes which are subsequently broken down by lysosomal action (Spitznas and Hogan, 1970; Young, 1971a). Failure of this phagocytic function results in an overaccumulation of rod outer segment material and subsequently, visual cell death and blindness. This has been clearly demonstrated in the case of inherited retinal dystrophy in the rat (Dowling and Sidman, 1962; Bok and Hall, 1971; Herron et al., 1969). Herron et al. (1969), using radioactive amino acid, noted that the retinal dystrophic rat shows a normal rate of outer segment growth until the age of 18 days. Thereafter the growth of lamellar discs towards the pigment epithelium slows down and the pigment epithelium shows no ability to phagocytose the rod outer segments. Dowling and Sidman (1962) found that the electroretinogram (ERG) of the retinal dystrophic rat is normal until the age of 18 days. Thereafter, a gradual deterioration in the ERG begins and complete loss is observed by two months of age. The lamellar material of the photoreceptor outer segments gradually builds up at their apices while the rhodopsin content in the retina of the dystrophic rat temporarily increases. Finally, all visual cells are lost. These investigations suggest that death of the

photoreceptors may be due to a primary defect in the pigment epithelium (Herron et al., 1969; Bok and Hall, 1971). These recent findings have strongly reemphasized the close correlation between the retina and the pigment epithelium in the maintenance of visual function.

In addition to the above functions, the pigment epithelium also synthesizes and secretes part of the mucopolysaccharide material which fills the spaces between the visual cell outer segments (Bermans, 1964; Moyer, 1969). Histochemical studies have revealed that the epithelial cytoplasm contains high activity of glycolytic dehydrogenase, acid phosphatase, ATPase, AMPase, and alkaline phosphatase (Lessell and Kuwabara, 1964).

Night Blindness and its Association with Vitamin A

The affliction of night blindness (nyctalopia or difficulty in dark adaptation) and its cure by liver or liver oils, was known to the ancients, long before vitamin A was discovered. Aykroyd (1944) mentioned that Eber's Papyrus, an ancient Egyptian medical treatise of about 1500 B.C., recommended roast ox liver or the liver of a black cock as curative agents for night blindness. The famous Greek philosopher and "Father of Medicine", Hippocrates also prescribed ox liver for curing night blindness, but suggested that it should be eaten in a raw state after dipping in honey (Moore, 1957). Modern knowledge, of course, indicates that livers of almost all animals are rich in vitamin A.

The relationship of vitamin A to dark adaptation was not realized until considerable information about the distribution and chemical nature of vitamin A became available from other sources. In 1876, Franz Boll made the pioneering discovery of visual purple in the retina. He noticed that, in the frog, the pigment epithelium of the retina contained golden coloured oil droplets which faded when the eye was brightly illuminated for long periods. The visual purple obtained from the frog's retina turned yellow on treatment with acid. He inferred from this change that the visual purple was derived from the yellow pigment which abounded in the pigment epithelium. This led his colleague Capranica (1877) to conclude that the pigment was lutein, a term which at that time covered both carotene and xanthophyll.

The presence of visual purple in dark adapted retinas and its absence from retinas adapted to bright sunshine was another point established in the early experiments. Kühne (1878) demonstrated that, in the frog's eye,

visual purple reappears slowly in the dark after it is bleached by light. He also noted that, at the same time, the eye becomes more photo-sensitive during a stay in the dark. Since the photochemical effect of a given amount of light was found to be proportional to the concentration of the light-sensitive substance, Parinaud (1881) supplemented the "duplicity theory" with the assumption that twilight vision was dependent on visual purple. Night blindness was therefore, presumed to be correlated with abnormal function of visual purple.

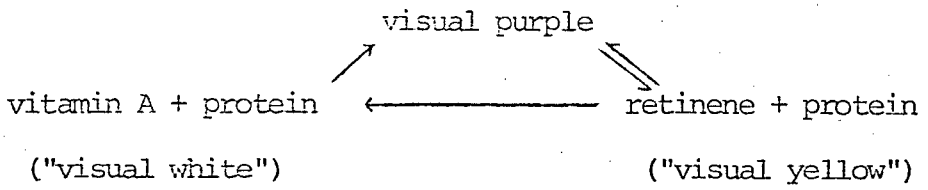
In 1913, McCollum and Davis noted that certain mixtures of fats of animal origin or fats extracted from internal organs, e.g. kidney or liver, contained a factor absolutely indispensable for survival and growth. The substance, also present in abundance in leaves of plants and a few seeds, was designated fat-soluble vitamin A (McCollum and Davis, 1913). When a diet was inadequate in its content of this substance, animals become emaciated and suffered edema of the eyes. Blindness resulted if the animals were permitted to go without this dietary essential or with an inadequate supply for a sufficient time (Holm, 1925).

An important step was then taken by Fridericia and Holm (1925) who demonstrated that dark adaptation was defective in vitamin A deficient rats and that the pigment "visual purple" could be formed only slowly in their retinas. Early in this century, Stern (1905), utilizing platinum chloride fixation in the dark, was able to produce a visual purple platinum complex which was stable in the light. Tansley (1931) applying the same technique demonstrated that the retina of vitamin A deficient rat contained sub-normal amounts of visual purple. Two years later, working on the histological changes of the retinas of rats and dogs in avitaminosis A, Tansley (1933) came to the following conclusions: 1) blood circulation was important

in the regeneration of visual purple 2) both in living and in prepared retinas, suitably stained visual purple was always found to be present in the outer limbs or segments of the rods and nowhere else in the retina 3) visual purple and the outer limbs of rods appeared simultaneously in developing retina 4) in the vitamin A deficient condition, poor regeneration of visual purple was accompanied by changes in the outer limb of the rod photoreceptor and 5) in case of extreme vitamin A deprivation, the retina was unable to form any visual purple. In developing retinas the primary effect of vitamin A deficiency was the absence of visual purple formation. Later, rod structure was affected (Tansley, 1936).

So far, it had been recognized that the formation of visual purple was influenced by vitamin A, but there was no evidence to implicate the vitamin directly in visual processes. Evidence of the direct participation of vitamin A in dark adaptation, however, was subsequently obtained in the classical research of Wald (1935a, 1936). In extensive studies on retinas of frogs, pigs, sheep and cattle, Wald found vitamin A in the neural retina and the combined pigment epithelial and choroid layer (Wald, 1935a). The vitamin was identified by its absorption at 328 mu in the ultra-violet and at 620 mu by the antimony trichloride test (Wald, 1935a). Wald found that the dark adapted retinas of the bull frog, Rana catesbiana, contained only a trace of vitamin A which could be extracted with benzene in the dark without injuring the visual purple (Wald, 1936). If the retinas were then exposed for short time to light, extraction with benzene now produced a yellow pigment which he named "retinene". If the retinas after bleaching by light were allowed to stand for an hour at 25^{o}C the yellow colour seen immediately after bleaching disappeared and extraction with benzene now produced a substantial amount of vitamin A.

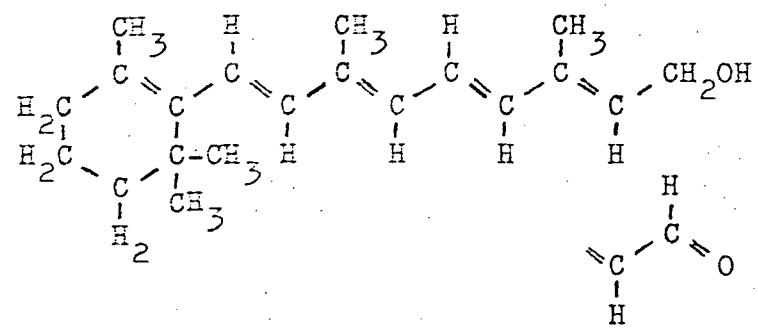
From these findings, Wald formulated a cycle (see below) which became a landmark in the history of research on vitamin A.



Meanwhile, experiments were carried out by a number of researchers on human subjects who were deprived of vitamin A in their diet for periods which ranged from a few weeks to over two years. (Hecht and Mandelbaum, 1939; Wald et al., 1938; Booher et al., 1939; Steven, 1943; Hume and Krebs, 1949). These subjects showed a deterioration in their capacity for dark adaptation sooner or later in the course of the vitamin A deficiency. The defect was corrected by the administration of vitamin A. Haig et al. (1938) noted that night blindness was also associated with chronic liver diseases.

The Visual Cycle

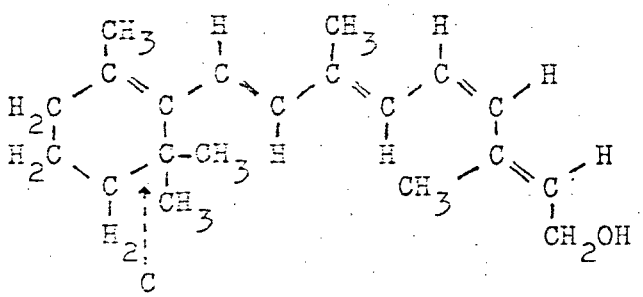
The role of vitamin A in vision was elucidated in 3 main stages. Firstly, a vital clue was provided by Morton (1944) who demonstrated that retinene was the aldehyde of vitamin A. Secondly, important studies were made by Wald and others (Wald, 1935a, 1935b, 1950; Wald and Hubbard, 1949; Bliss, 1951; Futterman, 1963) on the enzyme systems involved in the oxidations and reductions between vitamin A and retinene. Thirdly, the discovery of Hubbard and Wald (1952) of the cis-trans isomerism of retinene during the preparation of retinene for its combination with opsin in the formation of visual purple. The structural formulae for all-trans and 11-cis vitamin are shown below:



retinol

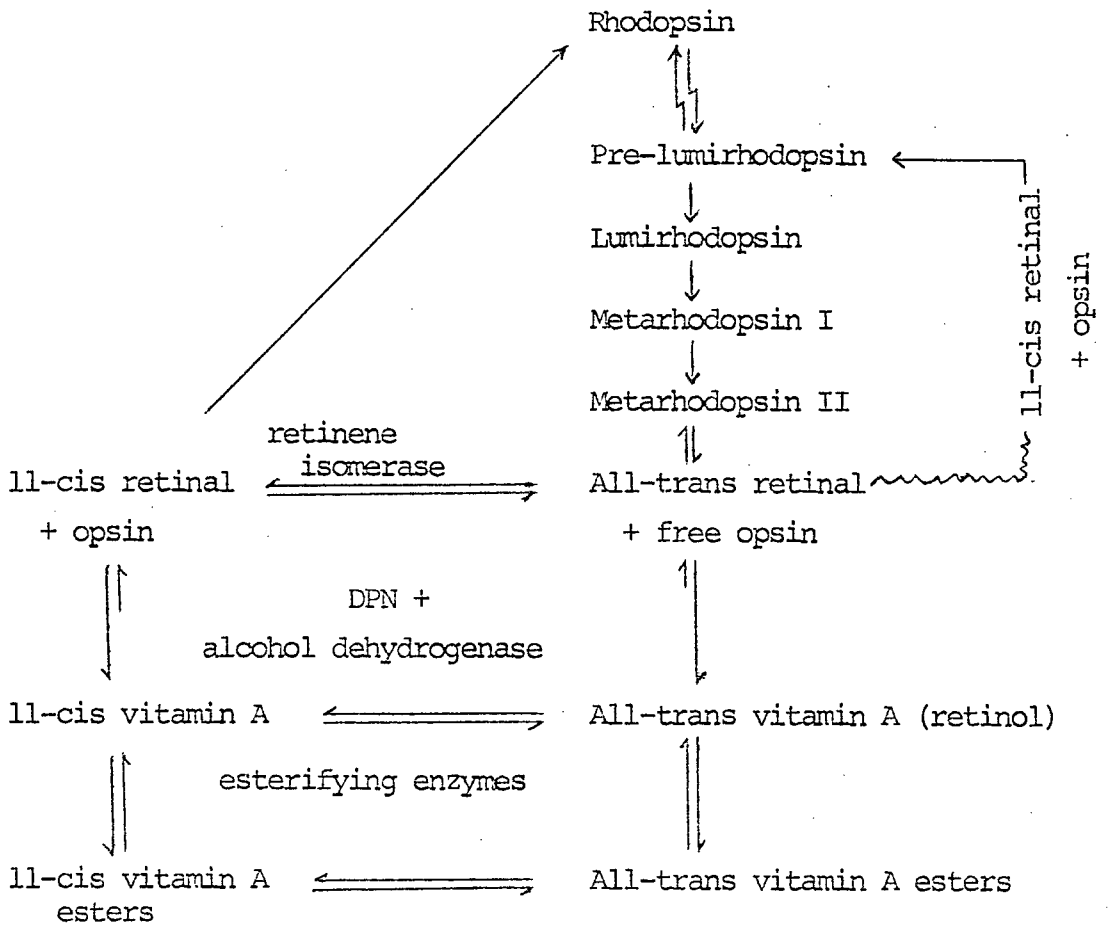
retinal
(retinaldehyde)

All-trans vitamin A



11-cis vitamin A

All-trans retinal would not combine with opsin to form rhodopsin (Hubbard and Wald, 1952). Combination took place, however, after the retinene has been isomerized to the 11-cis state. Thus, a cycle of isomerization is an intrinsic component of the visual purple or rhodopsin system. On the basis of the above additional knowledge, and subsequent research, Wald (1968) proposed his revised version of the visual cycle:



Wavy arrows indicate regeneration; straight arrows indicate thermal photoreactions. When a molecule of rhodopsin containing 11-cis retinal absorbs a single photon, it is changed to the more stable all-trans form and separated from opsin. Opsin

is a lipoprotein with a molecular weight of 30,000 to 40,000 (Krinsky, 1958). The first step of the light reaction results in the formation of pre-lumirhodopsin which is highly unstable at ordinary temperature (Yoshizawa and Wald, 1964). Following this, the breakdown proceeds spontaneously. Once all-trans retinal is released from opsin, it may be isomerized to 11-cis retinal to form rhodopsin or may be reduced by a dehydrogenase and DPN to all-trans retinol and then esterfied. The ester is stored in the pigment epithelium until needed for dark adaptation. During dark adaptation, vitamin A ester is deesterified, oxidized and isomerized to the 11-cis configuration and once again available for regeneration of rhodopsin. It is still not known exactly where in the inner or outer segment of the photoreceptor cell 11-cis retinal and opsin recombine to form rhodopsin. The pigment epithelium has been found to store considerable amounts of 11-cis vitamin A (Hubbard and Dowling, 1962; Krinsky, 1958). In cattle eyes, retinene isomerase has been found primarily in the neural retina and in frog eyes it is found mainly in the pigment epithelium (Hubbard, 1956).

Vitamin A exists in two distinct forms (Wald, 1958b), A_1 ($retinal_1$ or $retinol_1$) and A_2 ($retinal_2$ or $retinol_2$). If the bond on the ring (at C) of 11-cis vitamin A molecule is saturated, it is called A_1. If this bond is unsaturated it is A_2. Vitamin A_1 is the chromophore of the rhodopsin found in invertebrates and most vertebrates. This visual pigment has a maximum absorption at 500 mu. In fresh water and some amphibia, the chromophore is vitamin A_2 which combines with opsin to form porphyropsin having a maximum absorption at about 525 mu (Brown et al., 1963). Wald (1937) reported the detection of a further pigment, iodopsin in chicken retina. It absorbes at 565 mu and is bleached by

red light. It is presumed to be the pigment for the cones.

Visual pigments of the human retina share the same basic structure of all known visual pigments (Wald, 1969). Four types of visual pigments have been found in the human retina. The rod rhodopsin has a maximal absorbance at about 500 mu (Brown and Wald, 1964). Cones have three different visual pigments with different absorption peaks (435 mu, 540 mu, and 565 mu) corresponding to blue, green and red sensitive pigments in the eye. It is believed that only one visual pigment is present in each photoreceptor cell. Therefore, according to this hypothesis there are three types of cones, one absorbing maximally at 435 mu and the others at 540 mu and 565 mu (Wald, 1969) respectively. All four possess 11-cis retinaldehyde as the chromophore but are united with four different opsins in the discs of the receptor outer segments (Wald, 1969). Human rhodopsin extracted into aqueous solution bleaches in the usual way to opsin and all-trans retinaldehyde and can be regenerated in solution from opsin and 11-cis retinaldehyde (Wald and Brown, 1958). Difference spectra on the red and green-sensitive pigments of cones have been measured by direct microspectrophotometry of human and monkey foveas (Brown and Wald, 1963). Human cones which are blue-sensitive have also been found (Brown and Wald, 1964). All three pigments are regenerated on adding 11-cis retinaldehyde to the medium, showing this to be their common chromophore, joined to different opsins (Wald, 1969).

III MATERIALS AND METHODS

Albino rats of the Wistar strain (Woodland Farm, Ohio, U.S.A.) were used throughout the studies. The animals were kept under laboratory conditions with 12 continuous hours of light and 12 hours of darkness per day. The intensity of light inside the cages with the lights on in the room was no more than 10 foot candles at the front of the cages and 1 foot candle at the back. The animals were exposed to an interval of less than 1 1/2 hours of light outside the cages at an intensity of 25 foot candles before they were killed. The temperature of the room in which the animals were caged was held constant at 20° C. The room in which the animals were caged was illuminated with 4, 40 watt flourescent lights (General Electric F40, CW) situated 9 feet above the floor.

1) Animals and diets

Weanling rats, 5 weeks old of both sexes, weighing on the average 73 ± 10 gm. were housed in individual wire cages. The rats were maintained on a vitamin A "free" diet (prepared by General Biochemicals, Chagrin Falls, Ohio, U.S.A., according to Roels et al., 1964) supplemented with vitamin A acid. Water was available ad libitum. Littermates were fed with a complete stock diet, Purina Laboratory Chow and water ad libitum.

Composition of the vitamin A free diet

The major ingrdients of the basal diet were (g/kg):

Casein, vitamin free (heat treated)	180.00
Glucose	677.56
Cellulose	50.00
Peanut Oil	50.00
Salt mixture, USP XIV (Biological Research Products, General Biochemicals cat. No. 170800)	40.00

The following vitamins were added to the basal diet (g/kg):

Thiamine HCl	0.002
Riboflavin	0.004
Pyridoxine HCl	0.004
Choline Choride	1.000
Inositol	1.000
p-Aminobenzoic acid	0.300
Nicotinamide	0.100
Folic acid	0.0025
Vitamin B_{12} (crystalline)	0.00005
Biotin	0.0001
Ergocalciferol (40,000,000 units/g)	0.000042
Vitamin K	0.010
Vitamin A acid	0.00172
Calcium pantothenate	0.010

Composition of normal diet (Purina Laboratory Chow)

PROTEIN %	23.4
Arginine %	1.38
Cystine %	.32
Glycine %	1.26
Histidine %	.62
Isoleucine %	1.22
Leucine %	1.52
Lysine %	1.41
Methionine %	.43
Phenylalanine %	1.03
Threonine %	.94
Trytophan %	.28
Valine	1.24
FAT %	4.5
FIBER %	5.2
TDN %	75
NFE (by difference) %	50.8
Gross Energy, KCal/gm	4.25
ASH %	7.3
Calcium %	1.20
Phosphorus %	.86
Potassium %	.82
Magnesium %	.26
Sodium %	.49
Chlorine %	.51
Fluorine, ppm	35.0

Iron, ppm	198.0
Zinc, ppm	58.0
Manganese, ppm	51.0
Copper, ppm	18.0
Cobalt, ppm	.4
Iodine, ppm	1.7
VITAMINS	
Carotene, ppm	6.5
Thiamin, ppm	17.7
Riboflavin, ppm	8.5
Niacin, ppm	110.3
Pantothenic Acid, ppm	24.8
Choline, ppm X100	24.0
Folic Acid, ppm	5.9
Pyridoxine, ppm	3.8
Biotin, ppm	.07
B-12, mcg/lb.	10.2
Vitamin A, IU/gm	12.0
Vitamin D, IU/gm	5.3
Alpha-tocopherol, IU/lb.	29.8

Experimental and control animals were weighed individually for a total of 25 weeks. For vitamin A deficiency studies, two experimental animals with a control were killed for each sampling.

2) Determination of Blood Plasma Vitamin A Level

Experimental animals, 5 weeks old, were put on the vitamin A free diet and blood samples were collected at 3, 4, 6 and 8 weeks later. The animals were anaesthesized with ether and blood collected by puncturing the descending aorta with a 18-gauge needle attached to a syringe containing 0.2 ml of heparin. Blood was collected similarly from control animals of 5 weeks old and also 2 and 8 weeks later. Five to six ml of blood was collected from each animal and then centrifuged to obtain the plasma which was frozen immediately and then thawed before use. The plasma was shielded from light by wrapping its container with aluminium foil.

Blood plasma vitamin A and carotene analyses were carried out using Carr-Price's colorimetric method (Neeld and Person, 1963; Freed, 1966), with a Beckman DK-2A recording spectrophotometer, the cell chamber of which was maintained at 25°C.

Carotene and vitamin A were extracted from the blood plasma by the following procedure:

1. 2 ml of plasma was transferred into a 10 ml glass test tube.
2. 2 ml of 95% ethanol was then added and followed by 3 ml of petroleum ether.
3. The mixture was shaken vigorously for two minutes and centrifuged at low speed for three minutes to separate the emulsion.
4. 1 ml of petroleum ether extract (upper layer) was pippetted off and read for carotene at 450 mu against a petroleum ether blank.
5. 2 ml of petroleum ether extract was transferred to a 10 ml test tube which was then placed in a water bath at 45°C and the extract evaporated to dryness under a stream of nitrogen.

6. The residue was dissolved in 0.1 ml chloroform ($CHCl_3$), and a drop of acetic anhydride from a No. 25 needle and 1 ml trifloroacetic acid (TFA) mixture (1 TFA : 2 $CHCl_3$) were added.
7. Vitamin A was read immediately at 620 mu against a blank of 0.1 ml chloroform and 1 ml of TFA mixture.

The above reaction gave a clear blue colour which faded rapidly. The time when the TFA mixture was added (T_0), the initial (T_1) and final (T_2) recordings of the colour absorbed were noted. The maximum absorbance was obtained by extrapolating the slope of absorbance to the T_0 time. An example graph showing how the maximum absorbance for vitamin A was obtained is shown in fig. 2a.

The standard curve for carotene was obtained by dissolving 50 µg of B-carotene (General Biochemical, U.S.A.) in a few ml of reagent grade chloroform. Petroleum ether was added to a final volume of 100 ml in a volumetric flask. This solution was then diluted 1 to 100 with petroleum ether to prepare the intermediate standard. This intermediate solution was further diluted with petroleum ether to give solutions containing 0.5, 1.0, 2.0 and 4.0 µg of B-carotene per ml respectively. The optical density (OD) of carotene was read at 450 mu against a petroleum blank. Thus a standard curve made up at OD_{450} against the concentration of B-carotene was obtained. The B-carotene solutions were again read at 620 mu against the petroleum blank. The ratio of the two sets of readings at the two different wavelengths was determined as $OD_{450}/OD_{620}=0.3$. Since the petroleum extract of blood plasma contained both carotene and vitamin A, the ratio was used to correct the inter-

ference caused by carotene at OD_{620} in order to estimate the concentration of vitamin A accurately.

The standard curve for vitamin A was obtained by using transretinol (Sigma Chemical Company). Five milligrams of transretinol was dissolved in a few ml of reagent grade chloroform and diluted to 50 ml in a volumetric flask. Vitamin A standards were prepared from this stock containing 10, 20, 30, 40 and 50 µg/ml respectively. To prepare the standard curve, 0.1 ml of each of the above standards was placed in a 1 ml capacity cuvette for reaction with 1 ml of TFA mixture. The procedures that followed were the same as described above for vitamin A determination in blood plasma.

From the standard curve of vitamin A at OD_{620}, the factor F, which is the correlation between concentration of vitamin A per tube and its optical density at 620 mu, was determined as:

$$F = \frac{\mu g \text{ vitamin A/ tube}}{\text{optical density at 620 mu}} = \frac{3}{0.42} = 7.1$$

Therefore, vitamin A level in 100 ml blood plasma:

$$[OD_{620} - (OD_{450} \times 0.3)] \ (7.1 \times 75) = \mu g \text{ vitamin A/100 ml plasma}$$

3) Determination of feed vitamin A content.

Three samples of Purina Laboratory Chow and 3 samples of the vitamin A free diet were analysed for vitamin A content. Twenty grams of each sample, finely ground, was extracted for 2 hours with petroleum ether in an extraction apparatus. The ether was evaporated off and the residue saponified. After saponification was completed,

carotene and vitamin A were extracted by ether. The ether extract was used to read carotene and vitamin A at 450 mu and 620 mu respectively following the same method as described above. Purina Laboratory Chow was found to contain 3.97 µg of vitamin A/g of feed and the vitamin A "free" diet, 0.21 µg of vitamin A/g of feed, i.e. 5% of the vitamin A content of the normal diet.

4) Light microscopy

The animals were anaesthesized with sodium pentobarbital (nembutal) injected intraperitoneally and the eyes removed with a pair of scissors. The enucleated eyes were punctured at the ora serrata with a sharp razor blade to facilitate penetration of the fixative, and then fixed in a cold (4°C) solution of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.3 for 4-5 hours. The cornea and lens of each eye were removed. The eyes were then rinsed in 3% sucrose and 0.1 M sodium cacodylate buffer at 4°C overnight, and post-fixed in 2% osmium tetroxide in 0.1 M sodium cacodylate buffer for 2 hours at room temperature. Dehydration was carried out in ethanol, starting with 15%, then,30%, 50%, 70%, 80%, 90% and 100% ethanol, each for 15 minutes. After going through the last change of ethanol, the eyes were transferred to undiluted propylene oxide, two changes, each for 30 minutes. Each eye was hemisected antero-posteriorly before it was transferred to an equal volume of propylene oxide and epon 812 mixture. Before embedding, each half of the eye was further cut meridionally into 3 to 4 smaller pieces. The tissues were embedded in epon 812 mixture and polymerized at 60°C overnight. The epon

embedded material was sectioned at 0.5-1.0 μ on a Reichert Om U2 ultramicrotome. Sections were mounted on glass slides and stained with 0.25% alkaline toluidine blue at 65°C for 1-2 minutes. All conventional light micrographs were taken with a Leitz Orhthoplan light microscope and recorded on Kodak Panatomic X film with an orange or green filter. The film was developed for 9 minutes in Kodak Microdol-X at room temperature and prints were made using Kodak Kodabromide photographic paper.

5) Electron microscopy

For transmission electron microscopy, epon embedded material, prepared as above, was sectioned with a diamond knife on a Reichert Om U2 ultramicrotome. Silver to gray sections were placed on 0.25% formvar coated 75/300 or 150/150 mesh copper grids and then doubly stained with saturated uranyl acetate and lead citrate (Reynolds, 1963). They were examined by a Philips 300 electron microscope operated at 60 kv. All electron micrographs were recorded on Kodak fine grain positive 35mm film. They were developed in Kodak D-19 for 6 minutes at room temperature and printed on Kodak Kodakbromide photographic paper.

6) Acid phosphatase histochemistry

Acid phosphatase is generally accepted as a lysosomal enzyme (De Duve, 1963) and is the most widely used histochemical marker for the demonstration of lysosomes.

Portions of the posterior retina, hemisected antero-posteriorly

from both experimental and control animals were used for study of lysosomal activity in the retinal epithelium. After fixing in cold ($4^{o}C$) sodium cacodylate buffered solution of paraformaldehyde and glutaraldehyde as described in section 4, the hemisected retinas were cut antero-posteriorly into small size, and washed in cold 0.1 M sodium cacodylate buffer containing 3% sucrose for 1 hour. During washing, the retinal epithelium with choroid was detached from the retina proper and the sclera by peeling slowly with a pair of fine forceps (Ishikawa and Yamada, 1969). To demonstrate acid phosphatase activity, the method of Millar and Palade (1964) was used. The retinal epithelium with choroid was incubated in Gomori medium with sodium beta-glycerophosphate as substrate at $37^{o}C$ for 30-40 minutes. The control for the acid phosphatase reaction was run by incubating some tissues in Gomori medium without sodium beta-glycerophosphate. The tissues were rinsed briefly in cold 0.05 M sodium acetate buffer (pH 5.0) containing 75% sucrose, postfixed in 2% osmium tetroxide for 1 hour, then dehydrated rapidly with 70%, 90% and 100% ethanol, each change for 10 minutes. They were transferred to propylene oxide, two changes, each for 20 minutes and then to an equal volume of propylene oxide and epon 812 mixture and sectioned and photographed as described under section 5. A black precipitate of lead phosphate indicates the presence of acid phosphatase in the tissue.

Note: Gomori medium is prepared by dissolving 0.12 g. lead nitrate [$Pb(NO_3)_2$] in 100 ml 0.05 M sodium acetate buffer (pH 5.0) containing 7.5% sucrose. Then 10 ml of a 3% solution of sodium beta-glycerophosphate is slowly added. Before use, the mixture

is warmed at 60°C for 1 hour, cooled to room temperature and filtered to eliminate the slight precipitate which usually develops.

7) Radioautography-light microscopy

Animals that had been on the vitamin A free diet for 2.5, 8 and 10 months and a pair of 10 month old control animals were labelled intravitreally in each eye with 100 μC of H^3-methionine (sp. activity: 4.02 C/mM, New England Nuclear, U.S.A.). Before injection, the alcohol solvent of the tritiated methionine was evaporated off and replaced with Kreb's solution. The animals were labelled for 4 and 24 hours. The enucleated eyes were processed as described under section 4. For light microscopic radioautography, 0.5-1.0 μ sections from the epon-embedded material were cut on a Reichert Om U2 ultramicrotome and placed on clean glass slides. The slides were dipped in Kodak NTB_2 liquid emulsion kept at 45°C in a water bath. Dipping was done under a Wratten #1 safe light, kept at least 6 feet away from the emulsion. The slides were allowed to dry in total darkness and at 75-80% relative humidity. The dry slides were kept in black plastic boxes with several grams of "Drierite" and each box was wrapped in aluminium foil. Following an exposure of 2 months, the slides were developed in Kodak D-170 for 6 minutes and fixed in 24% sodium thiosulfate for 3 minutes at 18°C (Kopriwa, personal communication). The developed slides were stained with alkaline toluidine blue for 5 minutes and destained in acid alcohol. Light micrographs were taken and developed as described in section 4.

IV OBSERVATIONS

Introductory Note

In the preparation of this thesis particular care has been taken to provide comprehensive figure legends to facilitate review of the micrographs. The figure legends, in this case, provide a complete record of the research which can be followed independently of the section on observations if desired. This approach has, of necessity, produced a certain redundancy between the figure legends and the observations section. It is suggested that the figures and figure legends be perused prior to reading the observations section.

Notes on Observations:

1) Animals were put on the vitamin A free diet when they were 5 weeks old. Therefore an animal designated as a 5 week vitamin A deficient animal is actaully 10 weeks old.

2) Definition of terms:

   a) "Posterior retina" means the area behind the equator close to the optic nerve of the eye and "peripheral retina" means the area in the region of the equator and towards the ora serrata.

   b) The terms "inner" and "apical" used in association with the retina and retinal epithelium respectively mean toward the vitreous or center of the eye while "outer" and "basal" mean towards the sclera.

   c) The term "distal" used in association with photoreceptor outer and inner segments means farthest sclerally from the photoreceptor nucleus, and "proximal" means nearest to the photoreceptor nucleus.

A) Growth Curves

Weanling rats 5 weeks old of both sexes, weighing approximately 73 g. were put on the vitamin A free diet, supplemented with vitamin A acid. Their growth was compared with litter-mates also 5 weeks old, fed Purina Laboratory Chow and water ad libitum (Fig. 1). Each point on the curves represents the mean weight of 10-19 control animals or 14-45 vitamin A deficient animals, recorded at various times during the course of the experiment. The vertical bar at each point represents plus or minus the standard deviation of the mean. Both the vitamin A deficient and control animals grow at approximately the same rate until the 4th week. A perceptible difference in their rate of growth is then apparent. The control animals continue to gain weight rapidly until the 15th week when they assume a slower rate of growth. The vitamin A deficient animals display a more gradual growth rate until the 21st week when their growth rate reaches a plateau. None of the vitamin A deficient or control animals died during the course of the studies. The vitamin A deficient animals appeared in good and healthy condition, except for a few which showed cloudiness in the cornea and exudate around the eyelids.

B) Plasma Vitamin A Levels in Control and Vitamin A Deficient Animals

Weanling rats about 5 weeks old were divided into two groups: one group was fed Purina Laboratory Chow and water ad libitum and served as controls; the other group was put on the vitamin A free diet. The experimental animals were killed at 3, 4, 6 and 8 weeks after they were

on the vitamin A free diet. The control animals were sampled at 0 (5 weeks old) 2, and 8 weeks. It was assumed that the blood vitamin A level of the control animals would remain stable and therefore they were not sampled concurrently with the experimental animals. The blood collected was analysed for its vitamin A content and the results are shown in Figure 2. Each point in the curves represents the mean vitamin A content/100ml of blood plasma of 1-2 animals. The size of each sample was not sufficiently big to account for the biological variation, however, the determination was merely done to confirm what Dowling and Wald (1958) had done earlier for the present system. The vitamin A content per 100 ml of blood plasma from the 3 samples of the control animals was found to be 58.5, 46.5 and 52.5 µg respectively. The blood vitamin A content of the vitamin A deficient animals declined rapidly after the animals were on the vitamin A free diet for 3 weeks. By the 4th week their vitamin A level fell from 57.5 µg to 30.0 µg per 100 ml blood plasma. By the 6th week the vitamin A level fell further to 9.5 µg/100 ml blood plasma. By the 8th week when the experiment terminated, the vitamin A deficient animals had a mean vitamin A content of 7.0 µg per 100 ml of blood plasma, about 13% of the vitamin A level of the control animals.

C) Light Microscopy

1. Retinal epithelium and photoreceptors in control rats.

The vertebrate retina is conventionally divided into 10 layers representing, in the main, various portions of four different types of cells, retinal epithelial cells, photoreceptors, bipolar cells and ganglion cells. The layers are from outside inwards (Fig. 3): 1, retinal

epithelium; 2, layer of rods and cones; 3, outer limiting membrane; 4, outer nuclear layer; 5, outer plexiform layer; 6, inner nuclear layer; 7, inner plexiform layer; 8, ganglion cell layer; 9, nerve fiber layer; 10, inner limiting membrane.

The retinal epithelium consists of a single layer of cells. Each epithelial cell has 1-2 nuclei which are large and oval in shape (Fig. 4). The layer of rods and cones contains the photoreceptor outer and inner segments. In a 2 month control animal it measures about 9.6 μ thick (Fig. 3). The segments are closely packed together posteriorly (Fig. 4) but those from the peripheral retina lie further apart (Fig. 7). Both the photoreceptor outer and inner segments are elongated and cylindrical (Fig. 8). The outer limiting membrane forms a line of demarcation between the outer nuclear layer and the photoreceptor inner segments (Figs. 4, 8). The outer nuclear layer, comprising the cell bodies of the photoreceptors, contains about 9-11 rows of nuclei in the posterior retina. Two types of nuclei are found in the rat retina, rod and cone nuclei (Fig. 6) but rod nuclei predominate in this nocturnal animal. The rod nucleus is characterized by a large block of centrally located chromatin whereas in the cone nucleus the chromatin is more diffuse and usually divided into several lobes. No appreciable difference in the photoreceptor nuclei is observed between 2 month old and 12 month old animals. In Figure 5, a photoreceptor nucleus is seen lying among the photoreceptor inner segments. At higher magnification, three displaced photoreceptor nuclei are identified between the retinal epithelium and the photoreceptor inner segments (Fig. 6). Nucleus No. 1 is at the junction of the photoreceptor inner and outer segments, nucleus No. 2 at the junction of the photoreceptor outer segments and retinal

epithelium and nucleus No. 3 lies partly within the retinal epithelium. The photoreceptor cells diminish in number perceptibly towards the periphery. Figure 5 shows the peripheral retina from a 9 month old control animal. In the retinal periphery there is a reduction in the thickness of the outer nuclear layer to 5-6 rows of nuclei. The outer plexiform layer is the synaptic zone between the receptors and bipolar and horizontal neurons. It has a reticular structure under the light microscope (Figs. 3, 5). The inner nuclear layer consists of the cell bodies of four types of cells: bipolar cells, horizontal cells, Müller cells and amacrine cells. The inner plexiform layer marks the junction between the cells of the inner nuclear layer and the ganglion cells of the retina. It also has a finely reticular appearance and contains large blood vessels and capillaries (Figs. 3, 5). The cell bodies of the ganglion cells are found in the ganglion cell layer. They are round and closely spaced in a single row (Fig. 3). The nerve fiber layer is composed of the axons of the ganglion cells. Processes of the Müller cells are known to form the inner limiting membrane. The retina has a blood supply from two sources (Fig. 5). The outer retina, the retinal epithelium and the photoreceptors are nourished mainly by choriocapillaries from the choroidal circulation. The inner retina in most mammals is nourished mainly by capillaries leading from branches of the central retinal artery (Polyak, 1957).

2. Retinal epithelium and photoreceptors in vitamin A deficiency

The following observations refer to the posterior (this term has been defined in the notes on observations) retina unless otherwise indicated. Animals were placed on the vitamin A free diet when they

were about 5 weeks old. After 3.5 months on the vitamin A free diet, the photoreceptor outer and inner segments from the posterior retina appear fragile and broken (Fig. 9). At higher magnification, many of the photoreceptor outer segments show evidence of disintegration. The photoreceptor inner segments are shorter than normal and some appear slightly swollen (Fig. 10). Above the outer nuclear layer the outer limiting membrane is visible. The outer nuclear layer contains 9-11 rows of nuclei. The outer plexiform layer is lightly stained while the inner neural retina appears normal (Fig. 9).

The layer of photoreceptor outer and inner segments decreases in thickness after 6 months of vitamin A deficiency (Fig. 11). The layer measures 4.4 μ in thickness compared to 8 μ in a control animal. At higher magnification (Fig. 12), severe breakdown of photoreceptor outer segments is evident. They lose their highly ordered orientation, appear irregular and display many empty spaces. The photoreceptor inner segments are shorter than their normal counterparts. The outer limiting membrane is present. The outer nuclear layer now has only 3-5 rows of photoreceptor nuclei (Fig. 11). The lightly stained outer plexiform layer appears thinner. The inner neural retina is unchanged. In the retinal epithelium many small dark granules are present along its inner surface (Fig. 12).

An overall reduction in the retinal thickness is noted in the posterior retina after 9 months of vitamin A deficiency (Fig. 13). The photoreceptor outer segments have almost disappeared. The inner segments are round and short (Fig. 14). The layer of inner and outer segments now measures 3.2 μ in thickness. The outer limiting membrane is clearly visible. There are now 2-3 rows of photoreceptor nuclei

remaining. In Figure 13, three photoreceptor nuclei are seen outside the outer nuclear layer and in Figure 14 one nucleus lies outside the outer limiting membrane. The outer plexiform layer is thin and no longer forms a distinct zone between the outer and inner nuclear layers. Numerous small dark granules are seen within the inner surface of the retinal epithelium (Fig. 14).

In a 10 month vitamin A deficient animal, the posterior retina shows further degeneration. There is greater approximation of the retinal epithelium and the neural retinal layer (Figs. 15, 16). The photoreceptor outer and inner segments have disappeared. The outer limiting membrane is visible and now demarcates the outer border of the neural retina. Only 1-2 rows of photoreceptor nuclei are observed. Unidentified cells are visible among the photoreceptor nuclei. The outer plexiform layer is no longer visible in some regions but the inner neural retina is essentially unchanged (Fig. 15).

The posterior retina from an 11 month vitamin A deficient animal is further reduced in thickness (Fig. 17). Some photoreceptor nuclei are present between the retinal epithelium and the neural retina (Fig. 17, 18). The retinal epithelium lies immediately next to the neural retina. The outer nuclear layer contains only one irregular row of photoreceptors with lightly stained cytoplasm. Cells from the inner nuclear layer appear between photoreceptor nuclei (Fig. 17). The outer limiting membrane forms a distinct boundary above the outer nuclear layer. The outer plexiform layer is obscure although the inner neural retina appears almost normal (Fig. 17).

D) Electron Microscopy

1. Normal retinal morphology

(a) The retinal epithelium

The retinal epithelium consists of a single layer of cells which appear rectangular in cross-section. The fine structure of two adjacent epithelial cells is shown in Figure 19 and Figure 22 which are from the posterior retinas of 1.5 month and 9 month old control animals respectively. The basal surface of each cell is infolded. A basement membrane is present beneath the epithelial surface which forms part of Bruch's membrane. Bruch's membrane also has a core of collagen and dense fibrillar material and an outer layer formed by the basement membrane of the choriocapillaries. The inner epithelial surface displays both long and slender, and short and broad processes. The former often form a palisade around the photoreceptor outer segments. Both the basal infoldings and the long apical processes are usually devoid of identifiable subcellular structures. However, in a 1.5 month old control animal (Fig. 19), premelanosomes are present in the broader inner processes. They are absent in older animals (Figs. 20, 21, 22). Near the inner or apical surfaces, epithelial cells are joined by junctional complexes consisting in each case, of a zonula occludens and zonula adherens (Figs. 19, 22). The zonula adherens lies just sclerad to the zonula occludens. Both of these portions of the junctional complex have the typical structure described originally by Farquhar and Palade (1963).

The epithelial nucleus is large and oval, containing diffuse chromatin. Nuclear pores are often observed (Fig. 19). The epithelial cytoplasm is characterized by a predominance of smooth endoplasmic reticulum (Figs. 19,

20, 21, 22). Rough endoplasmic reticulum is also present scattered in the cytoplasm, but more often, aggregated close to the epithelial inner surface (Figs. 19, 21). Polysomes are dispersed sparingly in the cytoplasm. A single row of mitochondria is present inside the basal infoldings and along the lateral cell border. Some mitochondria are also found close to the epithelial inner surface. The mitochondria are usually rod-shaped in longitudinal section and round in cross-section. One or more well-developed Golgi complexes are found near the nucleus in each epithelial cell (Figs. 19, 21). Microtubules can sometimes be observed in the cytoplasm (Fig. 19). Several types of dense bodies are also visible in the cytoplasm. The type most commonly observed has a dense homogeneous matrix surrounded by a distinct clear space and then an outer single membrane. These dense bodies vary from round to oval in shape and vary in size from 0.2 μ to 0.5 μ in diameter (Figs. 19, 22). They have been found to be acid phosphatase positive (Yamada, 1969) and hence are thought to be lysosomes. Lamellar-like structures enclosed within a membrane closely resembling the lamellar discs of the photoreceptor outer segments are frequently observed in the cytoplasm as well (Fig. 20). They are discarded portions of the photoreceptor outer segments which have been phagocytosed by the retinal epithelium. These cytoplasmic inclusion bodies have been termed "phagosomes" (Young, 1967). Dense bodies that contain undigested lipofusin-like materials (Fig. 20) are found in addition and have been designated "residual bodies" (De Duve et al., 1966). Lastly, lipid droplets are present occasionally in the cytoplasm and can be easily recognized by their homogenous but less dense matrix. They are larger than the lysosomes and the cell membranes surrounding the lipid droplets are indistinct (Fig. 34). Pinocytosis is often observed along the

plasma membrane of the basal epithelial surface and coated vesicles are seen scattered in the epithelial cytoplasm (Fig. 22).

It is important to note that the structure of the pigment epithelial cell described above does not change with aging from 1.5 to 12 months of age (Figs. 19, 20, 21, 22).

(b) The photoreceptor outer segments

Rats are nocturnal animals and their photoreceptors are predominantly rods. In the following description, unless otherwise stated, the term photoreceptor refers to rods.

The photoreceptor outer segments are cylindrical in shape and each is composed of a stack of flattened saccules or discs surrounded by a cell membrane continuous with the plasma membrane of the inner segment (Fig. 24). The discs are regularly arranged and stacked at right angles to the length of the outer segment. Each disc is composed of two membranes that are continuous at the edges. The membranes enclose a less dense narrow space, the intradisc space. The distal portion of each outer segment is surrounded or encircled by apical processes of the retinal epithelium (Fig. 21) and the proximal outer segment is continuous with the inner segment through a connecting cilium (Fig. 24). Each lamellar disc invaginates at the same point forming a longitudinal groove or incisure which extends the whole length of the outer segment. Portions of the longitudinal groove of the outer segment are seen in Figure 24. Frequently, irregularly arranged saccules are seen at the bases of the outer segments (Fig. 25). Further up the outer segments the irregular saccules give way to the highly ordered lamellar discs. Other subcellular structures are absent in the outer segments.

The connecting cilium originates in the basal body which is located in the cytoplasm of the distal inner segment slightly to the side of its central axis, facing the outer segment. It's fine structure was first described by de Robertis (1956). It contains nine doublets of peripheral tubules. The central pair of tubules which are characteristic of motile cilia (Fawcett, 1958) are absent here (Figs. 23, 24, 26).

(c) The photoreceptor inner segments

The inner segments are elongated, cylindrical structures containing finely granular cytoplasm. Long, slender mitochondria with well developed transverse cristae are arranged around the periphery of each inner segment. In both the 1.5 and 9 month old control animals, the mitochondria are observed to extend down to the proximal ends of the inner segments (Figs. 23, 26, 27). Occasionally, mitochondria containing glycogen granules are observed in inner segments from older but not younger control rats (Figs. 24, 27). It is important to note that this is the only significant change that separates the morphology of photoreceptors from ages 1.5 to 12 months in the control rats studied. From the basal body of the cilium, a striated rootlet extends some distance down the inner segment (Figs. 23, 26). Polysomes are abundant and they are evenly distributed in the cytoplasm. A Golgi apparatus surrounded by numerous small vesicles is present in the basal region of each inner segment (Figs. 26, 27). Rough endoplasmic reticulum is abundant and a few smooth cisternae can be observed (Figs. 26, 27). The inner segments are not in direct contact with adjacent photoreceptors but are separated from each other by thin villous extensions of Müller cells (Fig. 26).

(d) The outer limiting membrane

By light microscopy, the outer limiting membrane appears to be a thin line separating the outer nuclear layer from the photoreceptor inner segments. Electron microscopy shows that the outer limiting membrane is formed by a single row of cell junctions between the photoreceptor inner segments and Müller cells, and also between adjacent Müller cells (Figs. 26, 27). These cell junctions have often been named "terminal bars" or "desmosomes". Cohen (1965), in a study of primate eyes, correctly interpreted the cell junctions that form the "membrane" as "Zonulae adherentes". His work has been confirmed by Spitznas (1970) in a study of the human eye.

(e) The photoreceptor synaptic processes

The synaptic processes of the photoreceptor extend a short distance inward and make synaptic contact with neuronal processes derived from cells in the inner nuclear layer (Figs. 28, 29). Two types of synaptic terminals are observed. The rod synaptic process ends in an oval structure known as a "spherule" (Fig. 28). The cone synaptic process ends in a broad swelling called a "pedicle" (Fig. 30).

The basal surface of each rod spherule is invaginated by two neuronal processes forming a dyad (Fig. 29). Sometimes, in addition to the two lateral processes, a third central process is present forming a triad. In the study of monkey and human retinas, Dowling and Boycott (1966) have indicated that the central process of the triad is derived exclusively from a bipolar cell and the lateral processes from horizontal cells. If the rat outer plexiform layer is similar in structure to that in monkey

and human (Dowling and Boycott, 1966), then the two lateral processes in rat would be horizontal cell processes and the central one bipolar cell process. The cell wall of the spherule encloses the terminations of the neuronal processes. The rod spherule and its enclosed neuronal processes comprises the synaptic unit or synaptic complex. It consists of three parts; presynaptic (the spherule), synaptic (spherule-bipolar and spherule-horizontal cell contacts), and post synaptic (bipolar dendrite, horizontal cell processes) (Hogan et al., 1971). The presynaptic membrane is separated from the post synaptic membrane by a synaptic cleft. The spherule contains a large number of presynaptic vesicles which are evenly distributed and one or two mitochondria and some polysomes (Figs. 28, 29, 31). Within the spherule, an osmiophilic lamellar structure, the synaptic ribbon is found at right angle to the dyad or the triad. It is a crescent shaped structure (Ladman, 1958) containing five layers, three dense layers separated from each other by two less electron-dense layers (Fig. 28). Each synaptic ribbon is surrounded by a halo of synaptic vesicles. Between the synaptic ribbon and the presynaptic membrane lies a dense structure, the arciform density (Ladman, 1958). At the base of the spherule, superficial contact with other neuronal processes originating from cells in the inner nuclear layer is marked by a slight indentation and increased density of the presynaptic membrane (Fig. 28). In this area, no synaptic ribbon is present. The most common type of synapse observed is between the spherules, the horizontal cell processes and the bipolar cell dendrites (Figs. 28, 29, 31). An additional type of rod synapse where the post synaptic processes make direct contact with the body of the rod cell and the synaptic ribbon lies near the receptor nucleus (Fig. 29) is also present. This synapse has been termed "somato dendritic synapse" by de Robertis

and Franchi (1956).

The cone pedicle, in contrast to the rod spherule, has a complex structure. Each cone pedicle makes contact with a number of processes originating in the inner nuclear layer. These processes possibly from horizontal and bipolar cells invaginate the cone pedicles to establish contact and synaptic ribbons are present in the cone cytoplasm at these sites (Figs. 30, 32). Hence cone pedicles typically contain a large number of synaptic ribbons. Other processes from the inner nuclear layer make contact with the surface of the pedicle forming what have been termed superficial contacts (Fig. 30). The cone pedicle typically contains large numbers of synaptic vesicles which are usually evenly distributed. Some synaptic vesicles, however, are always present congregated at the site of the synaptic ribbons.

2. Retinal morphology in vitamin A deficiency

(a) Retinal changes after 1 month of vitamin A deficiency

Change in retinal structure is first observed in the portion of photoreceptor outer segments nearest to the retinal epithelium. Some lamellar discs become swollen and break down into vesicles (Figure 33). A distorted outer segment in which the lamellar discs have opened up to form an almost oval lamellar structure surrounded by the apical processes of the retinal epithelium is seen in Figure 33. Some vesicles are visible within the distorted lamellar structure. The rest of the photoreceptor cells, the inner retina and the retinal epithelium are normal.

(b) Retinal changes after 1.5 months of vitamin A deficiency

Structural breakdown of the photoreceptor outer segments is gradual and occurs sporadically among the outer segments. Some outer segments show more severe breakdown while others are less affected (Fig. 35). A large number of vesicles and some abnormally arranged saccules can be observed in the outer segments undergoing the severest degeneration.

In the retinal epithelium, many lysosomes are now present in the cytoplasm close to its inner surface and also in the broad apical or inner processes (Fig. 34). Disintegrating phagosomes, too, are seen near the epithelial inner surface (Fig. 34). The long and narrow apical processes which surround the degenerating outer segments do not contain organelles.

(c) Retinal changes after 2 months of vitamin A deficiency

More severe disintegration of the photoreceptor outer segments can now be observed. Most of the lamellar discs in the outer segments are unable to maintain their structural integrity and break down into tubules or vesicles (Fig. 36). Near the base of the outer segments, some lamellar discs appear intact. The connecting cilia, photoreceptor inner segments and the inner neural retina remain normal.

(d) Retinal changes after 2.5 months of vitamin A deficiency

The breakdown of the photoreceptor outer segments continues. The outer segments contain many vesicles, tubules and disordered saccules. Many lamellar discs have disappeared completely leaving intracellular spaces (Fig. 38).

The photoreceptor inner segments begin to show some morphological changes at this stage of vitamin A deficiency. The distal portions of the inner segments for the first time are slightly swollen and their cytoplasm is almost devoid of polysomes. Large vacuoles can be observed in affected regions of the inner segments (Fig. 39).

At this stage, the apical or inner processes of the retinal epithelium are irregularly oriented and appear actively engaged in engulfing degenerating outer segments (Fig. 37). The smooth endoplasmic reticulum, mitochondria and the Golgi complexes in the retinal epithelium remain unchanged.

(e) Retinal changes after 4-5 months of vitamin A deficiency

The photoreceptor outer segments show further deterioration. Vesicles, tubules and disordered saccules result from breakdown of the lamellar discs. Due to loss of outer segment material many intra- and extracellular spaces are formed (Figs. 40, 41). The lamellar discs that are still present within the outer segments are frequently loosely arranged or oriented differently from their normal position perpendicular to the long axes of outer segments (Fig. 40).

The photoreceptor inner segments lose their elongated and cylindrical structure. They appear shorter than normal and are barrel-shaped. In particular, the inner segments are retracted towards the photoreceptor nuclei (Fig. 41). The swollen ends of the inner segments, observed previously, have disappeared. The mitochondria in each inner segment appear largely unaffected. Polysomes gather mainly in the inner halves of the inner segments. In this region, rough endoplasmic reticulum and a few large vacuoles can also be identified. Intact connecting cilia and striated rootlets are present (Figs. 41, 42). Degeneration of entire photoreceptor inner segments can also be observed (Fig. 41). In Figure 42, a degenerating inner segment with very dense cytoplasm can be seen. It contains several mitochondria, two of which are greatly enlarged and contain glycogen granules. The outer limiting membrane is still intact at this stage (Fig. 41). Villous extensions of the Müller cells project through the intercellular spaces separating adjacent photoreceptor inner segments (Fig. 42).

The apical processes of the retinal epithelium show slight proliferation and there is an increase in number of lysosomes near the epithelial

inner surface (Fig. 43). The basal infoldings and other subcellular structures in the retinal epithelium appear unchanged.

(f) Retinal changes after 6 months of vitamin A deficiency

The deterioration of the photoreceptors has progressed and the inner segments now lie close to the retinal epithelium (Fig. 44). Few of the outer segments appear intact. Some outer segments and their contained discs have been lost (Fig. 44) and others have broken down into vesicles (Fig. 45). Still other discs have lost their compact and transverse arrangement within the cell membranes (Fig. 46). Many intra- and extracellular spaces are observed.

The photoreceptor inner segments are marked by different stages of shortening (Fig. 47). Those that are not shortened too extensively show a perceptible difference in the polysome distribution between the distal and basal (inner) halves of the cytoplasm. In the distal halves of these inner segments, the cytoplasm is less granular as most of the polysomes aggregate close to the photoreceptor nuclei (Figs. 44, 47). The cisternae of the smooth endoplasmic reticulum that remains are dilated and rough endoplasmic reticulum is absent (Fig. 47). An inner segment, almost oval in shape, is seen in Figure 47. It contains a large number of polysomes, an ill-defined Golgi apparatus, several short mitochondria and a small amount of rough endoplasmic reticulum.

The outer limiting membrane is present, composed of cell junctions between photoreceptor inner segments and Müller cells and between adjacent Müller cells (Fig. 47). Between adjacent photoreceptor inner segments, the intercellular spaces are wider and more Müller cell processes extend through them (Fig. 47).

The photoreceptor synaptic processes show visible morphological changes for the first time at this stage of vitaim A deficiency. In each synaptic process there are fewer synaptic vesicles, many lying immediately adjacent to synaptic sites (Fig. 48). Plasma membranes between adjacent synaptic processes have broken down and cytoplasm merges freely across the sites of breakage (Fig. 48). The cell membranes of mitochondria within the synaptic processes are also affected. In Figure 48, a mitochondrion which displays swelling of part of its outer membrane is seen. More seriously affected mitochondria are observed in Figure 49. The mitochondrial membranes appear to lose their structural integrity and most of the transverse cristae disappear. At the synaptic sites, the synaptic ribbons surrounded by a cluster of vesicles persist. The postsynaptic processes containing closely packed synaptic vesicles are unchanged.

An accumulation of lysosomes along the inner surface of the retinal epithelium is again observed. The apical processes of the epithelium are numerous but are shorter than normal and appear to be active in engulfing degenerating outer segments (Figs. 45, 46). A phagosome-like body is seen in the retinal epithelium in Figure 45.

(g) Retinal changes after 7-8 months of vitamin A deficiency

Approximation of the photoreceptor inner segments and the retinal epithelium is again evident (Fig. 51). As before, degeneration of the photoreceptor outer segments involves breakdown of discs (Figs. 50, 51) and abnormal arrangement of the remaining discs (Figs. 51, 52). Intra- and extracellular spaces are abundant. Some outer segments do not seem to be as badly damaged as those observed in animals at 6 months of vitamin A

deficiency (Fig. 44).

Morphological changes observed in the photoreceptor inner segments resemble those described at the 6th month stage of vitamin A deficiency. The inner segments have shortened to different degrees. The distal halves of the inner segments are less granular and contain dilated profiles of smooth endoplasmic reticulum (Figs, 51, 53). Polysomes gather mainly in the basal halves of the inner segments. In Figure 52, a structure resembling a discarded inner segment lies close to the retinal epithelium. It contains lysosomes and aggregated dense material.

The photoreceptor synaptic processes are now shorter and display fewer synapses (Figs. 54, 55). Each photoreceptor synaptic process contains only a few synaptic vesicles. The breakdown of cell membranes between adjacent synaptic processes and of mitochondria within the synaptic processes is again evident. Occasionally, unidentified cells containing large numbers of lysosomes and undigested debris can be identified (Fig. 55).

The retinal epithelium is marked by a very large increase in lysosomes aggregated close to the inner epithelial surface and in the broad apical processes. Other lysosomes lie near the Golgi apparatus (Fig. 50). There appears to be a proliferation of apical epithelial processes (Figs 50, 51). In the 8 month vitamin A deficient animal, the apical processes of the retinal epithelium are short and irregularly oriented (Fig. 51).

(h) Retinal changes after 9 months of vitamin A deficiency

At this stage, striking morphological changes are evident. The

photoreceptor outer segments have disappeared except for a few sporadic clusters of disordered saccules (Figs. 56, 58). The photoreceptor inner segments are shortened considerably (Figs. 56, 58, 59, 60). The inner segments contain vacuoles of various sizes, several unusually short mitochondria and some polysomes (Figs. 56, 58, 59). In the longer inner segments, polysomes and some rough endoplasmic reticulum can be found in basal regions close to the cell nucleus. Within the inner segments, some mitochondria show evidence of membrane degeneration (Figs. 56, 59). The extensively retracted inner segments still contain polysomes and rough endoplasmic reticulum (Fig. 60) but mitochondria, normally a characteristic feature, are no longer present. Cilia in cross-section can be identified lying between adjacent inner segments (Fig. 59). The outer limiting membrane is now formed more by cell junctions between Müller cells than between photoreceptors and Müller cells (Figs. 56, 58). The approximation of neural retina and the retinal epithelium continues. Many villous processes of the Müller cells extend through the intercellular spaces between adjacent inner segments (Figs. 58, 59). The photoreceptor nuclear chromatin appears unchanged, but for the first time the nuclear membranes show evidence of degeneration (Fig. 60).

At this stage the shortened synaptic processes of the photoreceptors contain very few synaptic vesicles. Synaptic ribbons, each surrounded by a cluster of vesicles still persist, however, in some photoreceptors (Fig. 60). As before, lysosomes are abundant in the epithelium close to inner epithelial surface but the other subcellular structures of the retinal epithelium are unchanged (Figs. 56, 57, 58). The apical epithelial processes are now numerous and prominent but the infoldings on

the basal epithelial surface are unchanged from the normal. The apical processes are regularly arranged and point towards the neural retina (Figs. 56, 57, 58).

(i) Retinal changes after 10 months of vitamin A deficiency

The most striking feature characterizing retinal structure at this stage of vitamin A deficiency is the close association between the retinal epithelium and the neural retina (Fig. 61). The accumulation of lysosomes in the inner retinal epithelium and in the broader epithelial processes is still present (Figs. 61, 64). Also some lysosomes can be found in central regions of the epithelium near the Golgi apparatus (Fig. 64). The prominent apical processes are regularly oriented and abut directly on the neural retina or interdigitate with outer processes of Müller cells (Figs. 61, 63).

The photoreceptor outer segments have completely disappeared except for a few degenerating fragments, often oval in shape (Figs. 62, 63). These fragments, containing a large number of vesicles and tubules and some saccules, are found among the processes of retinal epithelium and the Müller cells. In Figure 64, remnants of two photoreceptor inner segments are present between Müller cell processes. One of them has an intact connecting cilium and a basal body. Its cytoplasm contains numerous polysomes and a couple of mitochondria. Remnants of a photoreceptor outer segment are also present lying transversely next to the inner epithelial surface (Fig. 64). Also in Figure 64, a degenerating cone photoreceptor cell situated in a niche of the neural retina, can be identified by its characteristic nuclear chromatin which is less

dense and more diffuse than that of the rod. The cone nucleus seems intact and a few mitochondria, some polysomes and an ill-defined Golgi apparatus are visible in the cytoplasm.

The outer limiting membrane is now composed almost entirely of cell junctions between adjacent Müller cells (Fig. 63). The photoreceptor cells (mainly rods) that remain have prominent nuclei but display only a narrow rim of cytoplasm containing a few polysomes and other ill-defined subcellular structures (Fig. 61). Synapses have almost completely disappeared. The photoreceptors and other unidentified cells lying next to them are now surrounded by several layers of membranes probably of a glial nature (Fig. 61).

(j) Retinal changes after 11 months of vitamin A deficiency

Retinal structure at this stage is marked by a close structural association between retinal epithelium and neural retina. Large numbers of lysosomes lie beneath the inner epithelial surface and the apical epithelial processes remain prominent (Figs. 65, 66, 67). Frequently, there are regions where the apical processes are deflected sideways by the neural retina (Figs. 66, 67). There are also areas where segments of the retinal epithelium which do not possess apical processes are in direct contact with the neural retina (Fig. 66). Remnants of photoreceptor outer and inner segments are still scattered among the processes of the retinal epithelium and Müller cells.

The cell junctions between adjacent Müller cells persist (Fig. 69). At some areas close to the outer border of the neural retina, Müller cell processes bend sideways and inwards, possibly contributing to glial membrane formation (Fig. 67).

Synapses of photoreceptors with nerve processes of cells of the inner nuclear layer and synaptic ribbons are now absent. Unidentified cells appear between the photoreceptors. These cells, like the photoreceptors are surrounded by several layers of membranes (Figs. 65, 68).

Capillaries now can be found lying close to the retinal epithelium due to the absence of photoreceptors. The endothelial cytoplasm of the capillaries demonstrates active pinocytosis (Fig. 66).

E) Acid Phosphatase Localization in the Retinal Epithelium

The retinal epithelia from the posterior retinas of animals, which were 6 months vitamin A deficient, were used to locate sites of acid phosphatase activity as a test for the presence of lysosomes. The inner surface of the retinal epithelium is distorted because the retinal epithelium with the choroid layer was detached from the retina proper by means of a pair of forceps during tissue preparation. Fragments of photoreceptor outer segments adhere to apical epithelial processes. When tissues are incubated in Gomori medium with sodium beta-glycerophosphate as substrate, acid phosphatase activity causes the lead nitrate [$Pb(NO_3)_2$] contained in the Gomeri medium to react with the substrate producing lead phosphate. The reaction product, lead phosphate, is seen as black precepitate within the cellular structures indicating the sites of acid phosphatase reaction. In the retinal epithelium, reaction product is present around the periphery of lysosomes lying beneath the inner epithelial surface (Fig. 70), in phagosomes (Figs. 70, 71) and in cisternae of the Golgi apparatus (Fig. 71). In the tissues that were incubated in Gomori medium without the sodium beta-glycerophosphate, reaction product was completely absent in the retinal epithelium.

F) Methionine-H^3 Incorporation in the Retina in Vitamin A Deficiency

Methionine-H^3 was injected intravitreally into the eyes of control and vitamin A deficient animals to test for the presence or absence of amino acid uptake and protein synthesis in the photoreceptors.

In retinas of 10 month old control animals, silver grains are evenly distributed in the retinal epithelium, photoreceptor outer

segments, inner segments and the outer nuclear layer (Fig. 72) 4 hours after injection. After 24 hours, there is a marked increase in labelling over the retinal epithelium, the photoreceptor inner segments and the outer nuclear layer. An accumulation of the label is visible at junctions between photoreceptor inner and outer segments and also in the basal portions of the outer segments. In the outer nuclear layer, silver grains are found primarily in the cell cytoplasm (Fig. 73).

In 2.5 month vitamin A deficient animals, 4 hours after injection, the label is evenly dispersed over the retinal epithelium, the photoreceptor outer and inner segments and the outer nuclear layer (Fig. 74). After 24 hours, there is a slight increase of the label over the photoreceptor inner segments and the outer nuclear layer. Silver grains are especially concentrated at the junctions between the photoreceptor inner and outer segments. In the outer nuclear layer, silver grains are observed in the nuclei and in the cytoplasm (Fig. 75).

In the 8 month vitamin A deficient animals, 4 hours after labelling with methionine-H^3, sparse labelling is visible over the retinal epithelium, photoreceptor inner segments and the outer nuclear layer (Fig. 76). After 24 hours, there is a marked increase in labelling over the retinal epithelium, photoreceptor outer segments, inner segments and outer nuclear layer. Silver grains are again observed concentrated at the basal portions of the outer segments. In the outer nuclear layer, the label is mainly localized around the periphery of the nuclei and in the cytoplasm (Fig. 77).

In 10 month vitamin A deficient animals, only 1-2 rows of photoreceptor cells remain. In this retina, 4 hours after injection with methionine-H^3, there is little incorporation of radioactive material

into the neural retina and none over the few photoreceptor cells observed (Fig. 78). After 24 hours, there is a visible increase in labelling over the retinal epithelium, the photoreceptor cells and the neural retina. In the photoreceptor cells, silver grains are distributed mainly around the periphery of the nuclei (Fig. 79). Thus, despite the presence of severe structural changes in the retina due to viatmin A deficiency, incorporation of methionine-H^3 persists in the remnants of photoreceptor cells and in the retinal epithelium.

FIGURE LEGENDS

Notes on Figure Legends:

1) Animals were put on the vitamin A free diet when they were 5 weeks old. Therefore an animal designated as a 5 week vitamin A deficient animal is actually 10 weeks old.

2) In the following electron micrographs all horizontal bars represent 1 u unless otherwise specified.

3) Definition of terms:

   a) "Posterior retina" means the area behind the equator close to the optic nerve of the eye and "peripheral retina" means the area in the region of the equator and towards the ora serrata.

   b) The terms "inner" and "apical" used in association with the retina and retinal epithelium respectively mean towards the vitreous or center of the eye while "outer" and "basal" mean towards the sclera.

   c) The term "distal" used in association with photoreceptor outer and inner segments means farthest sclerally from the photoreceptor nucleus and "proximal" means nearest to the photoreceptor nucleus.

Figure 1.

Graph showing the growth rate of the control and vitamin A deficient animals. Each point represents the mean weight of 14-45 animals in the curve of vitamin A deficient animals. In the curve representing the weights of control animals, each point represents the mean weight of 10-19 animals. The standard deviation ($\pm$ S.D.) of each mean is represented by the vertical bar at each point. The experiment began when the animals were 5 weeks old and weighing on the average 73.0 g. Both the vitamin A deficient and control animals grow at approximately the same rate until the 4th week. The control animals continue to gain weight rapidly until the 15th week when they assume a slower rate of growth. The vitamin A deficient animals display a more gradual growth rate until a plateau is reached at the 21st week. None of the control or vitamin A deficient animals died during the course of the studies. The vitamin A deficient animals appeared in good healthy condition, except for a few which showed cloudiness in the cornea and red exudate around the eyelids.

Figure 1. Growth rate of control and vitamin A deficient animals.

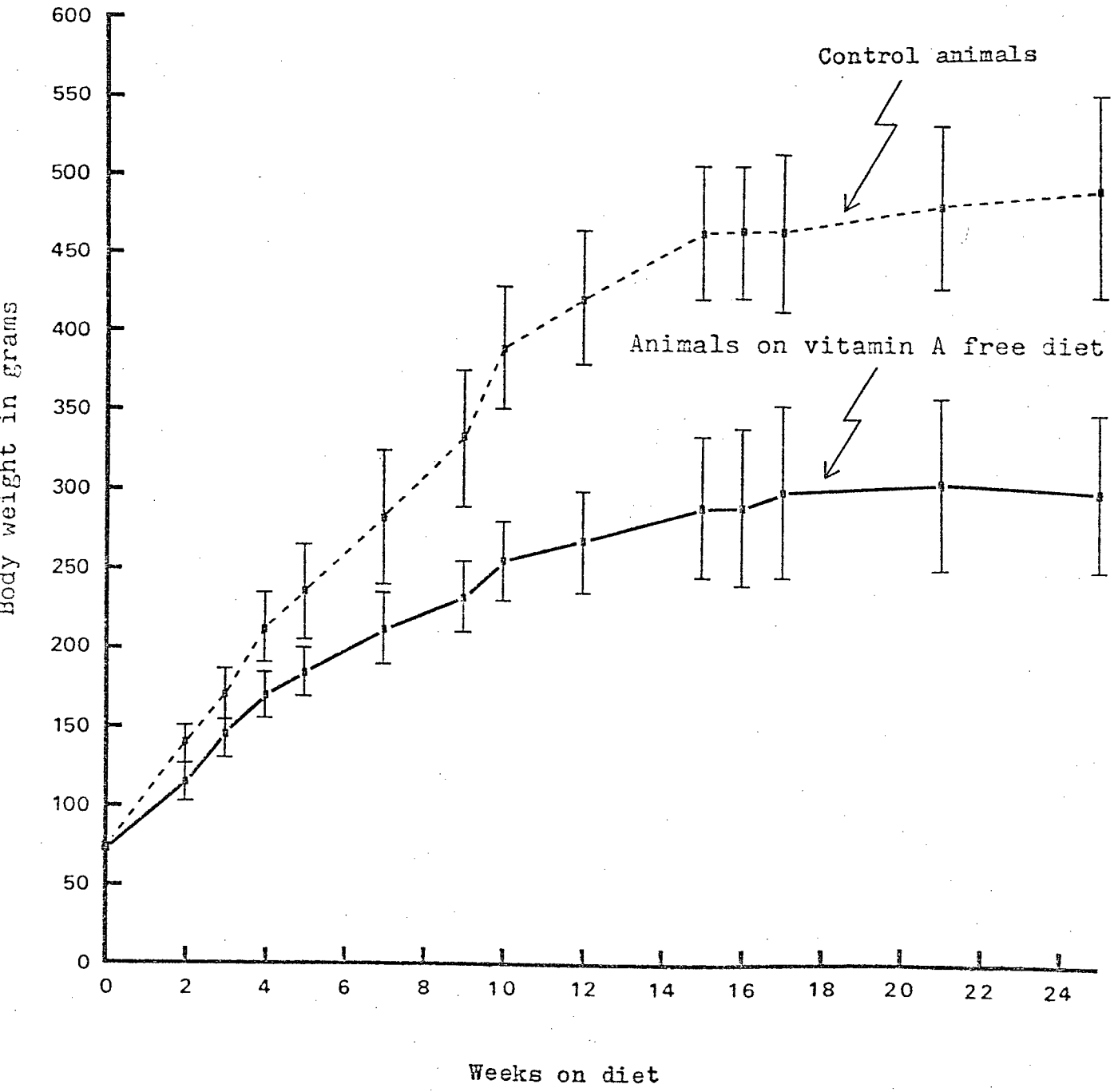


Figure 2a.

Graph showing how the maximum absorbance of vitamin A is obtained. T_0 represents the time when the TFA mixture is added to vitamin A solution; T_1 represents the time when the initial optical density of the vitamin A solution is noted; T_2 represents the time of the final absorbance of the vitamin A solution which is zero optical density. The maximum optical density of the solution at T_0 is obtained by extrapolating the slope of absorbance of T_1 and T_2 to T_0 (dotted line).

Figure 2a. Calculation of maximum absorbance of vitamin A.

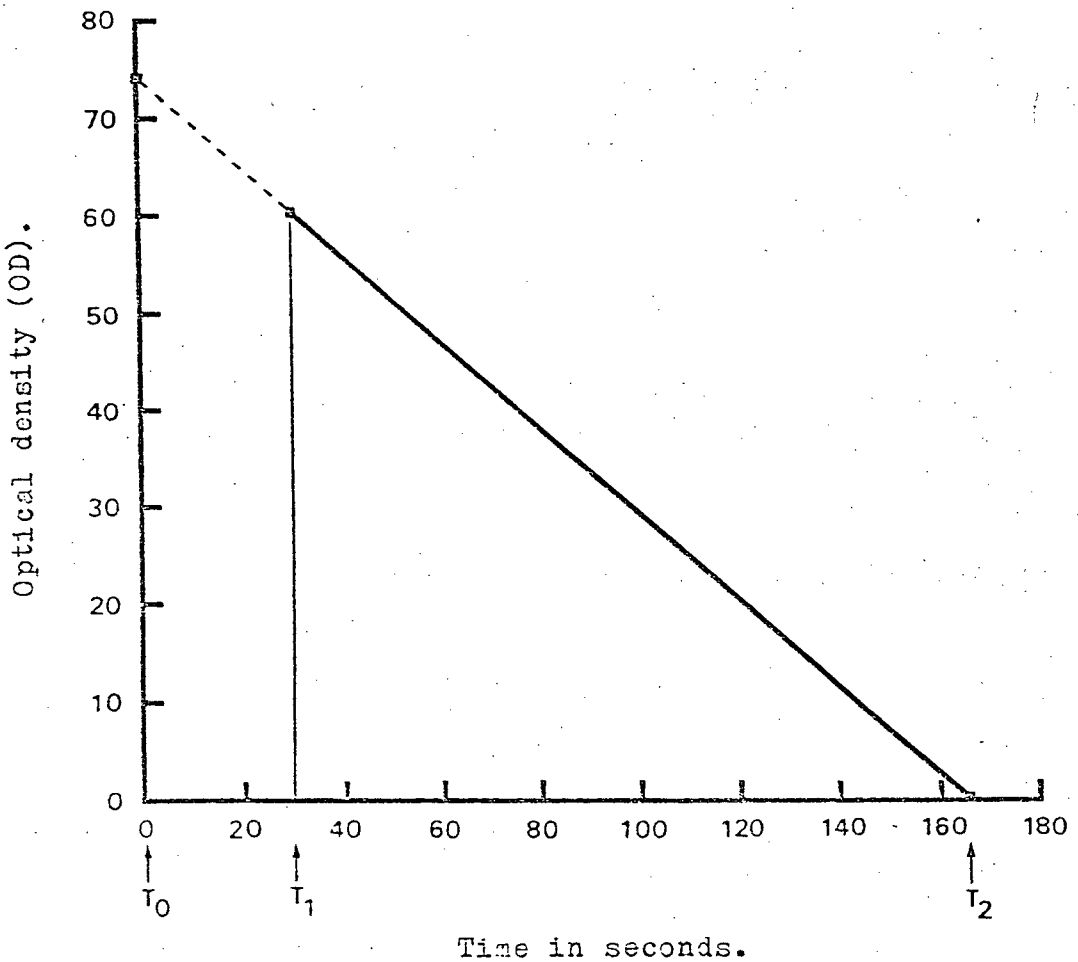


Figure 2b.

Graph showing the blood plasma vitamin A levels in the control and vitamin A deficient animals. Weanling rats, about 5 weeks old were placed on a vitamin A free diet and litter-mates also 5 weeks old were used as controls. Each point on the curves represents the mean vitamin A content/100ml of blood plasma of 1-2 animals. The vitamin A deficient animals were sampled at 3, 4, 6 and 8 weeks after they were on the vitamin A free diet. The control animals were sampled at 0 (5 weeks old), and 2 and 8 weeks. The vitamin A content/100ml of the three samples of the control animals was found to be 58.5, 46.5 and 52.5 ug respectively. The blood plasma vitamin A content of the vitamin A deficient animals declined rapidly after 3 weeks on the vitamin A free diet. By the 4th week, the vitamin A level fell from 57.5 µg to 320µg per 100ml blood plasma. By the 6th week, the vitamin A level fell to 9.5 µg/100ml blood plasma and by the 8th week, the vitamin A deficient animls had a vitamin A content of 7.0µg/100ml of blood plasma. That was about 13% of the vitamin A level of the control animals.

Figure 2b. Blood plasma vitamin A levels in control and vitamin A deficient rats.

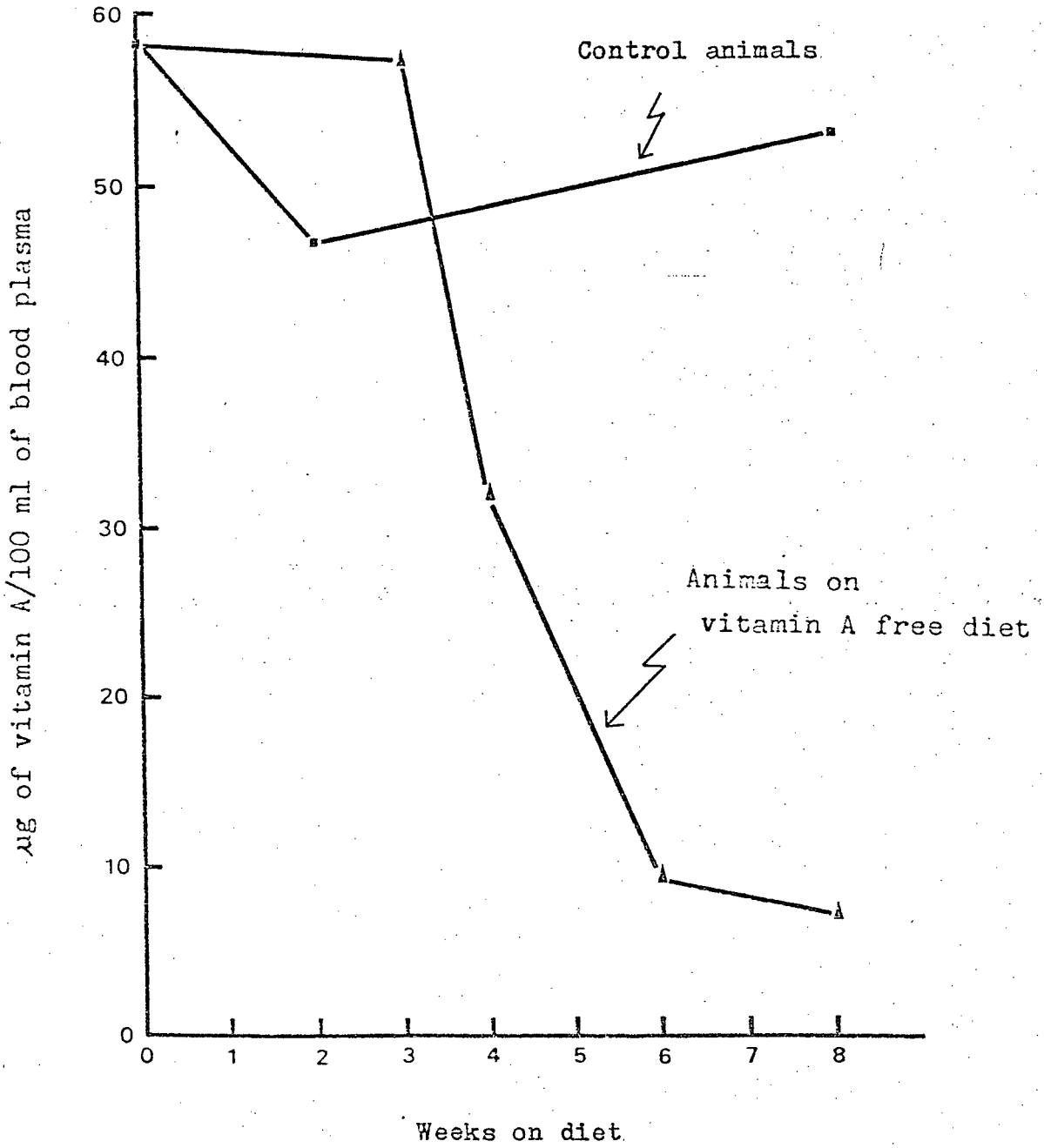


Figure 3.

Light micrograph from a 2 month old control animal showing the overall structure of the normal retina posterior to the equator of the eye. From outside inwards: 1) retinal epithelium, 2) photoreceptor outer and inner segments, 3) outer limiting membrane, 4) outer nuclear layer, 9-11 nuclei thick, 5) outer plexiform layer, 6) inner nuclear layer, 7) inner plexiform layer, 8) ganglion cell layer, 9) nerve fiber layer, 10) inner limiting membrane. bv=blood vessel.

x 1,190

Figure 4.

Light micrograph at higher magnification showing the posterior retina of a 2 month old control animal. The retinal epithelium (RE) consists of a single layer of cells. Each epithelial cell has 1-2 nuclei (N) which are oval in shape. The thickness of the layer of photoreceptor outer (OS) and inner segments (IS) is about 9.6 u. The segments are closely packed together. The outer limiting membrane (OLM), formed mainly by cell junctions between the Müller cells and the photoreceptor inner segments is visible. The photoreceptor nuclei form the outer nuclear layer (ONL).

x 4,750

Figure 5.

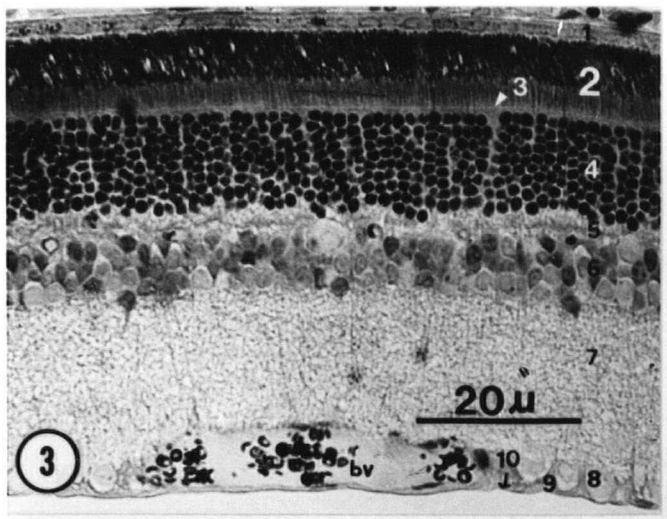
Light micrograph showing the retina peripheral to the equator of the eye from a 7 month old control animal. The choriocapillaries with many red blood cells are seen above the retina proper. The outer nuclear layer is about 5-6 nuclei thick. A displaced photoreceptor nucleus (arrow) is seen lying among the photoreceptor inner segments. A retinal blood vessel lies in the inner nuclear layer.

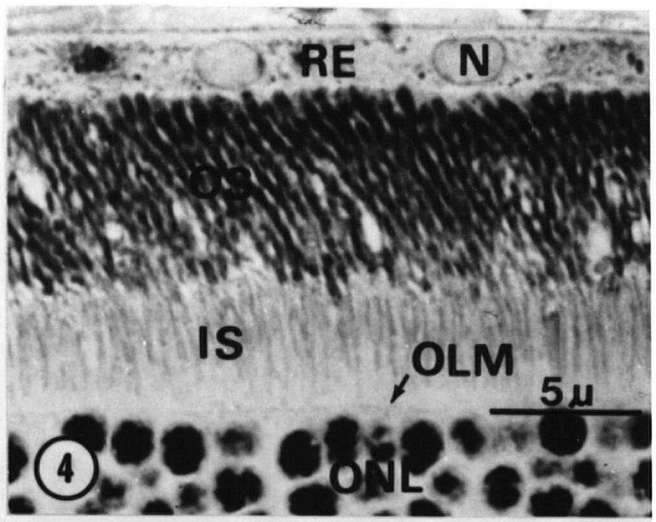
x 1,190

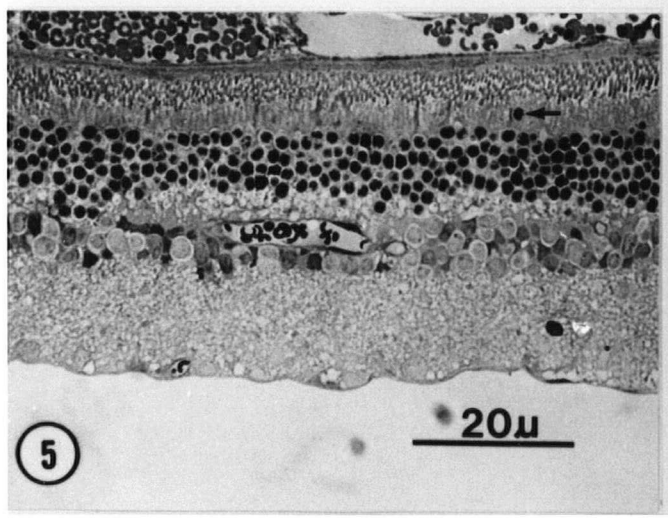
Figure 6.

Light micrograph showing the peripheral outer retina from the same specimen as Figure 5. Three displaced photoreceptor nuclei are seen lying between the retinal epithelium (RE) and the photoreceptor inner segments (IS): nucleus No. 1 is at the junction of the photoreceptor inner and outer segments; nucleus No. 2 at the junction of the photoreceptor outer segments and retinal epithelium and nucleus No. 3 is seen partly within the retinal epithelium. Two types of photoreceptor nuclei are found in the outer nuclear layer (ONL). Rod nuclei predominate over the cone nuclei in the rat. The rod nucleus (RN) has a dense block of centrally located chromatin whereas the cone nucleus (CN) has more diffuse chromatin usually divided into several lobes.

x 4,750


1
2
3
4
5
6
7
20µ
bv
10
9
8
3


RE
N
OS
IS
OLM
5µ
ONL
4


20µ
5

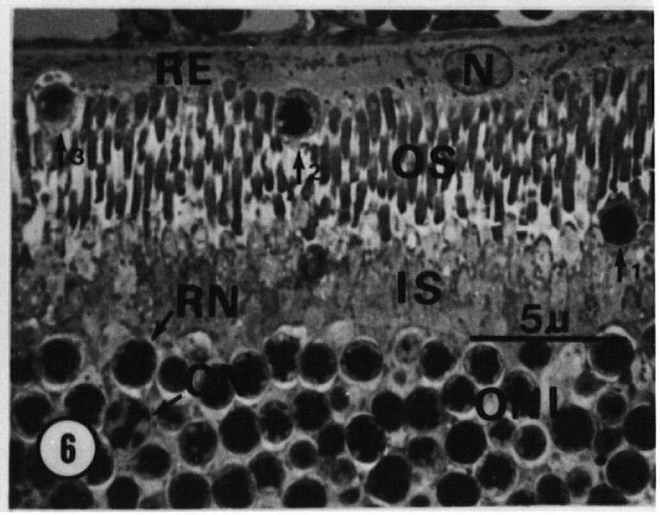

RE
N
OS
IS
RN
5µ
ONL
6

Figure 7.

Light micrograph showing the peripheral retina from a 12 month old control animal. The layer of choriocapillaries can be seen above the retina proper. The photoreceptor outer segments are less closely packed together than in the posterior retina. The outer nuclear layer is about 5-6 nuclei thick. Several capillaries (CP) are present in the inner nuclear layer.

x 1,100

Figure 8.

Light micrograph at higher magnification showing the outer retina from the same specimen as Figure 7. Two epithelial nuclei and some dark granules (arrows) are seen in the retinal epithelium. Both photoreceptor outer (OS) and inner segments (IS) are elongated structures and cylindrical in shape. The outer limiting membrane (OLM) demarcates the photoreceptor inner segments and the outer nuclear layer (ONL).

x 4,750

Figure 9.

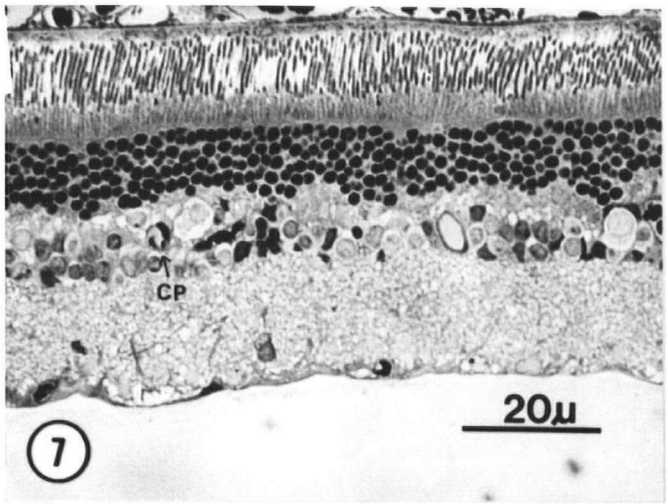
Light micrograph of the posterior retina from an animal which was on a vitamin A free diet for 3.5 months. The choroid layer is seen above the retinal epithelium. The photoreceptor outer and inner segments appear fragile and broken and the layer measures about 10 u thick. The outer limiting membrane is visible above the outer nuclear layer which is about 9-11 nuclei thick. The outer plexiform layer, about 4.7 u thick, is lightly stained. The inner neural retina appears normal.

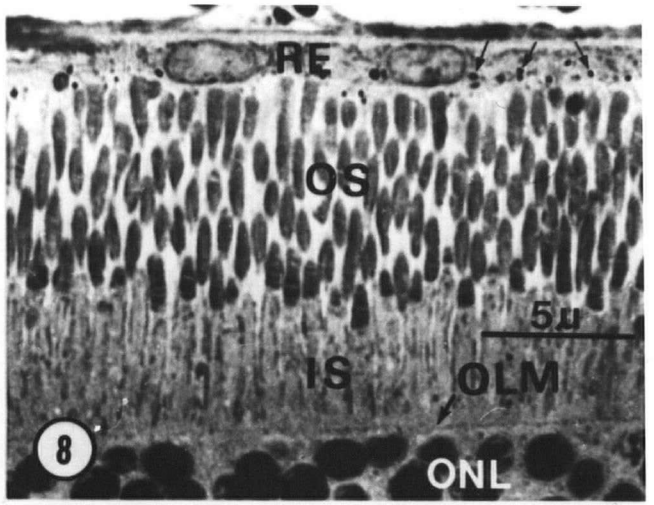
x 1,190

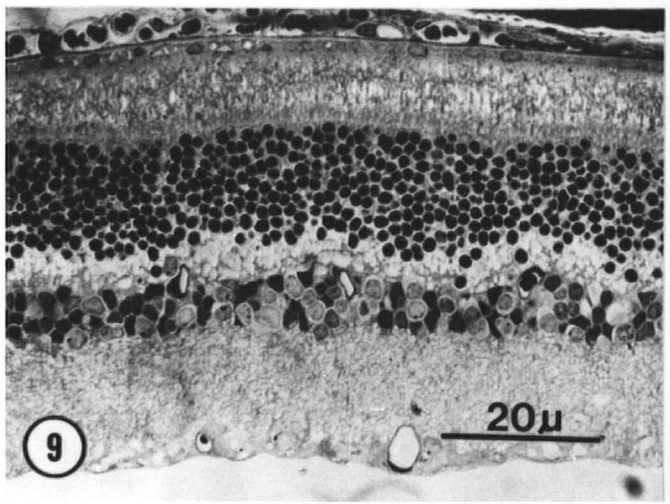
Figure 10.

Light micrograph at higher magnification showing the outer retina from the same specimen as Figure 9. The retinal epithelium displays three oval epithelial nuclei (N). The photoreceptor outer segments (OS) are not well defined and show evidence of disintegration (single arrows). The photoreceptor inner segments (IS) are shorter than they normally are and some appear slightly swollen (double arrows). Above the outer nuclear layer (ONL) the outer limiting membrane is not visible due to the plane of the section.

x 4,750


CP
20µ
7


RE
OS
5µ
IS
OLM
ONL
8


20µ
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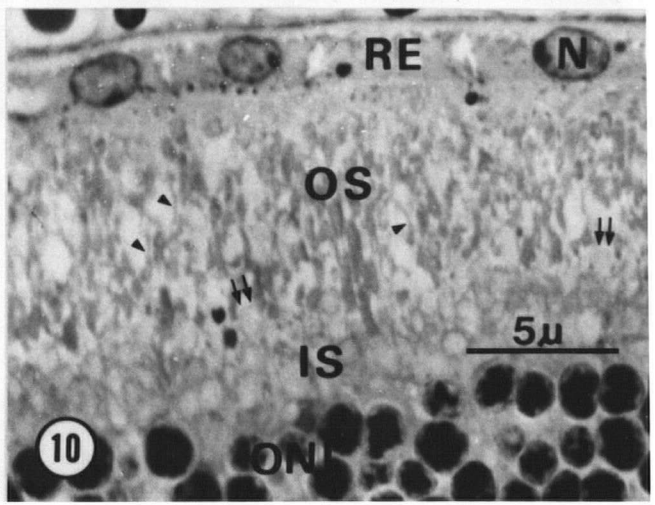

RE
N
OS
5µ
IS
ON
10

Figure 11.

Light micrograph showing the posterior retina from a 6 month vitamin A deficient animal. The layer of photoreceptor inner and outer segments measures about 4.4 u in thickness compared to 8 u in a control animal of about the same age. The outer limiting membrane is visible above the outer nuclear layer which is about 3-5 nuclei thick. The lightly stained outer plexiform layer measures 1.8 u in width while the inner neural retina appears unchanged. A blood vessel is seen in the inner neural retina on the left.

x 1,600

Figure 12.

Light micrograph at higher magnification showing the same specimen as Figure 11. Severe breakdown of the photoreceptor outer segments (OS) is evident. The outer segments are irregular in shape and there are many empty spaces (x) between them. The outer limiting membrane (OLM) is present and the photoreceptor nuclei in the outer nuclear layer (ONL) appear normal. In the retinal epithelium, many small dark granules (arrows) are visible along its inner surface.

x 4,750

Figure 13.

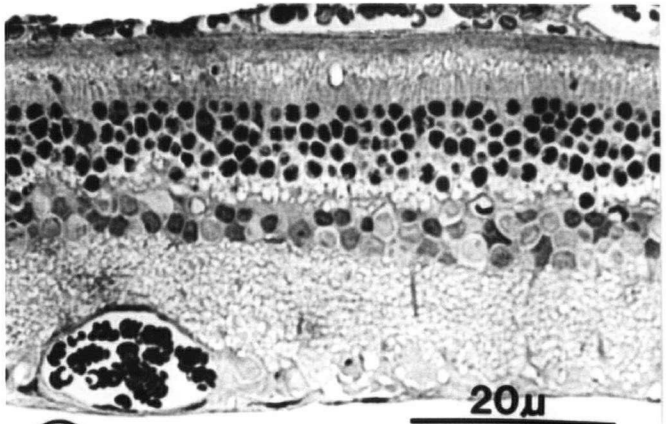
Light micrograph showing the posterior retina from a 9 month vitamin A deficient animal. The outer segments have almost disappeared and the inner segments are markedly reduced in thickness. The layer of inner and outer segments measures 3.2 u in thickness. The outer limiting membrane is distinct. There are about 2-3 layers of photoreceptor nuclei remaining. Three photoreceptor nuclei are seen outside the outer limiting membrane. The retinal epithelium is darkly stained. Thinning of the outer plexiform layer, which now measures 0.9 u in thickness, causes the inner nuclear layer to appear closer to the outer nuclear layer.

x 1,600

Figure 14.

Light micrograph at higher magnification showing the same specimen as Figure 13. The inner segments (IS) that are discernable are short and round and lightly stained. Empty spaces above the inner segments are commonly seen. A photoreceptor nucleus is visible between the retinal epithelium (RE) and the outer nuclear layer (ONL). The inner nuclear layer (INL) appears intact. The outer plexiform layer (white arrows) no longer forms a distinct zone between the outer and inner nuclear layers. Small dark granules (black arrows) are still observed along the inner surface of the retinal epithelium.

x 3,880



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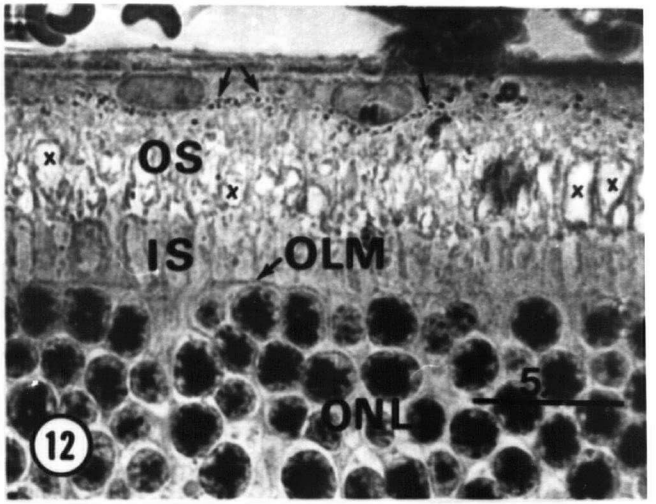

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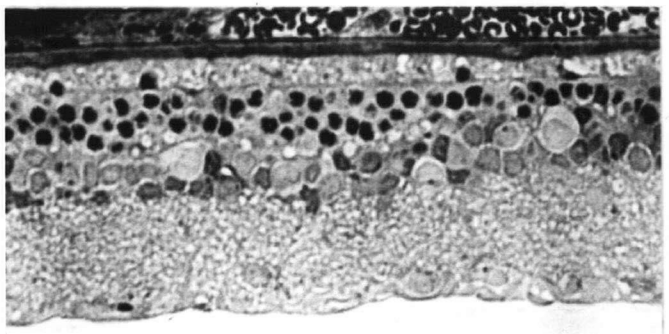

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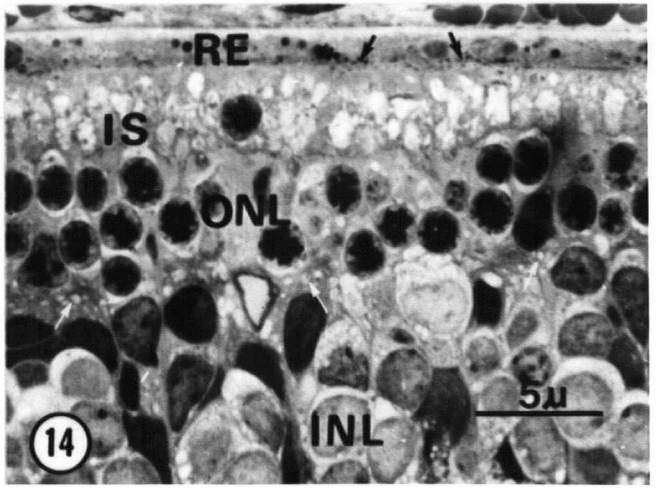


14

Figure 15.

Light micrograph showing the posterior retina of a 10 month vitamin A deficient animal. The retinal epithelium, which is darkly stained, is closely apposed to the neural retinal layer. The outer nuclear layer contains only 1-2 rows of photoreceptor nuclei. The outer limiting membrane appears to lie nearly adjacent to the retinal epithelium. The outer plexiform layer has disappeared in some regions (arrows) while the inner neural retina appears unchanged.

x 1,600

Figure 16.

Light micrograph at higher magnification showing the same specimen as Figure 15. Further approximation of the retinal epithelium (RE) and the remaining photoreceptor nuclei in the outer nuclear layer (ONL) is evident. The outer limiting membrane (OLM) is still present despite the severe loss of photoreceptors. Unidentified cells (arrows) appear between the few remaining photoreceptor cells. The outer plexiform layer is discernable only at some regions.

x 4,750

Figure 17.

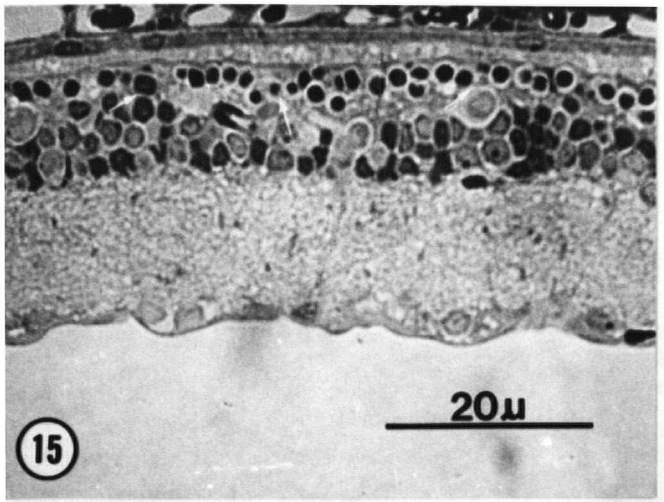
Light micrograph showing the posterior retina from an 11 month vitamin A deficient animal. Only one irregular row of photoreceptor nuclei is left. Some photoreceptor nuclei are again observed between the retinal epithelium and the neural retinal layer. The outer limiting membrane is present. Occasionally, cells from the inner nuclear layer are seen in the outer nuclear layer (arrow). The outer plexifrom layer has almost disappeared. The inner neural retina appears unchanged.

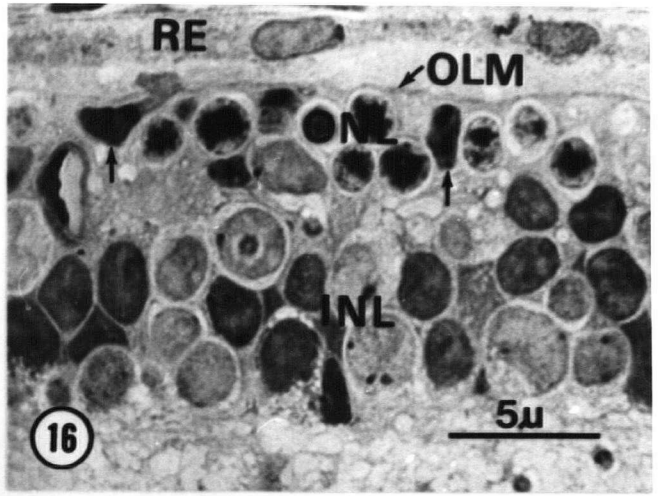
x 1,600

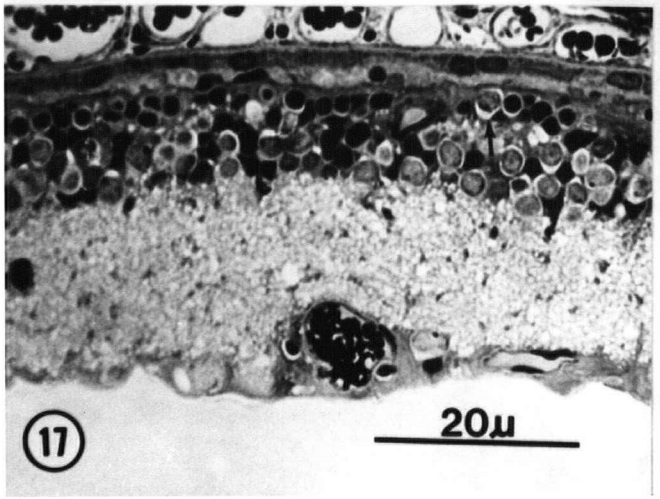
Figure 18.

Light micrograph at higher magnification showing the same specimen as Figure 17. A cone photoreceptor nucleus is seen close to the retinal epithelium (arrow). The outer limiting membrane (OLM) is clearly visible. The photoreceptor nuclei in the outer nuclear layer (ONL) appear to be each surrounded by a narrow rim of cytoplasm which is very lightly stained. It is possible, however, that the clear zone outside the photoreceptor nuclei represents a shrinkage artefact. The cells in the inner nuclear layer (INL) appear largely unchanged.

x 4,750


20µ
15


RE
OLM
ONL
INL
5µ
16


20µ
17

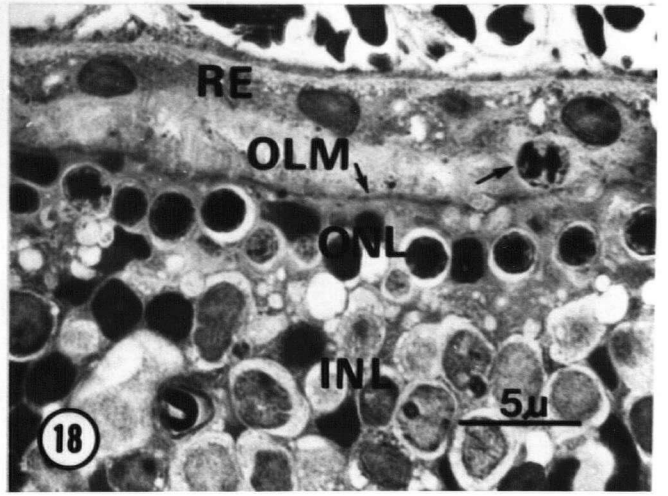

RE
OLM
ONL
INL
5µ
18

Figure 19.

Electron micrograph showing a portion of the retinal epithelium from a 1.5 month old control animal. The fine structure of two adjacent epithelial cells is shown. The basal surface (B) of each cell is infolded. A layered basement membrane which forms part of Bruch's membrane (BM) is present beneath the basal surface. The epithelial inner surface displays numerous long and slender processes (AP). Premelanosomes (PM) are present in the processes. Apical cell junctions (ACJ) between adjacent epithelial cells are composed of a zonula occludens and zonula adherens. The zonula adherens, marked by dense plasma membranes and condensation of subjacent cytoplasmic matrix, lies just sclerad to the zonula occludens. The epithelial nucleus (N) contains diffuse chromatin. Nuclear pores (NP) are present. The epithelial cytoplasm is characterized by a predominance of smooth endoplasmic reticulum (SER). Rough endoplasmic reticulum (RER) is also present, scattered in the cytoplasm and aggregated along the inner surface of the retinal epithelium. A single row of mitochondria (M) lies inside the basal infoldings and along lateral cell borders. One or more well developed Golgi complexes (G) are found near the nucleus in each epithelial cell. Polysomes (P) are sparsely distributed in the cytoplasm and microtubules (MT) can be observed. Photoreceptor outer segments (OS) can be identified lying between the apical processes of the epithelium.

x 17,500

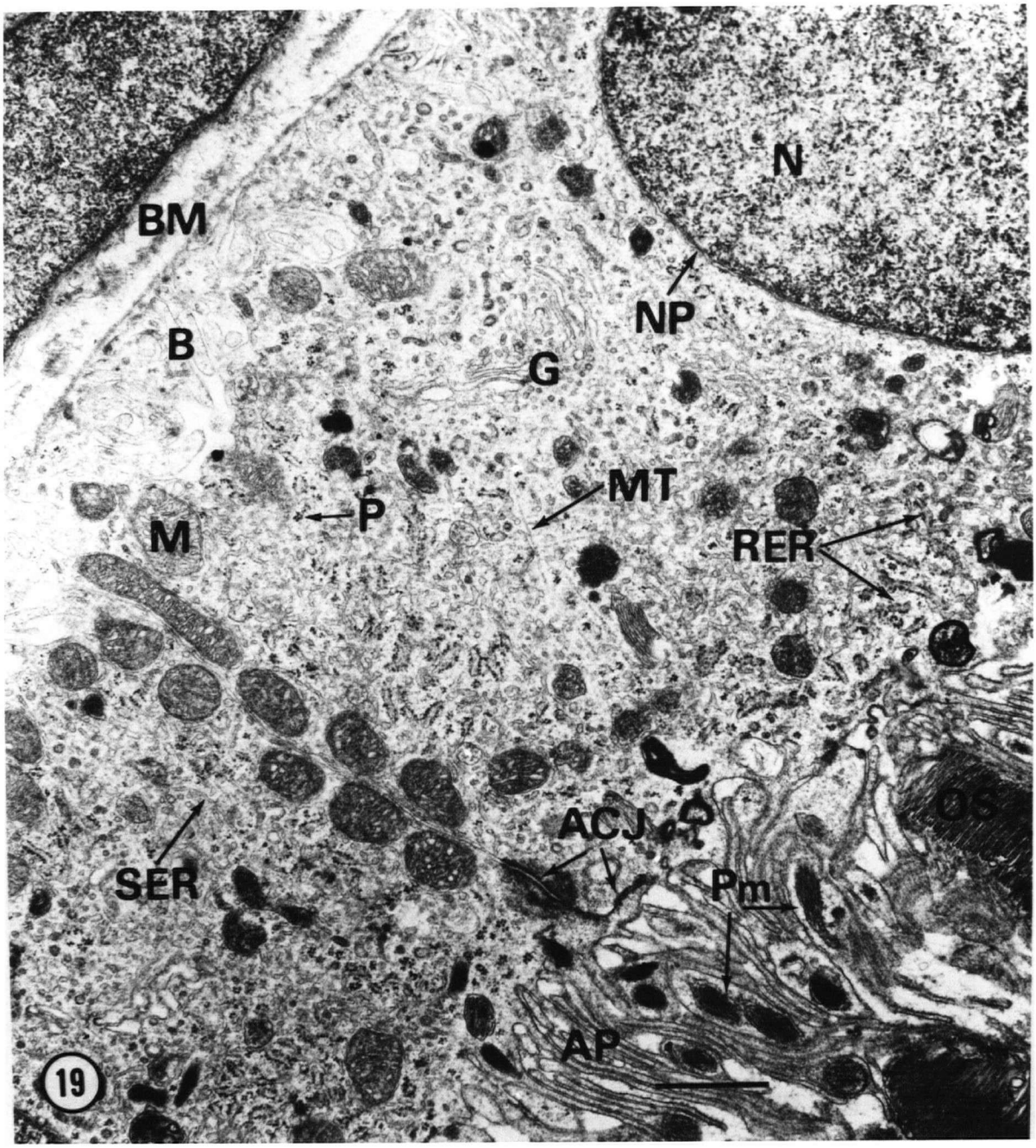

N
BM
NP
B
G
MT
P
M
RER
OS
ACJ
SER
Pm
AP
19

Figure 20.

Electron micrograph showing the inner retinal epithelium and photoreceptor outer segments from a 7 month old control animal. An abundance of smooth endoplasmic reticulum (SER) is present in the retinal epithelium. Lamellar-like structures or phagosomes (Ph) present in the epithelial cell cytoplasm, probably represent discarded portions of photoreceptor outer segments. Residual bodies (R), which probably represent debris from disintegrating phagosomes are observed. A mitochondrion is seen near the ingested fragments and a lysosome (L) above the phagosome. Long, slender apical processes (AP) extend inward and surround each photoreceptor outer segment (OS). The apical processes are devoid of identifiable subcellular structures.

x 32,700

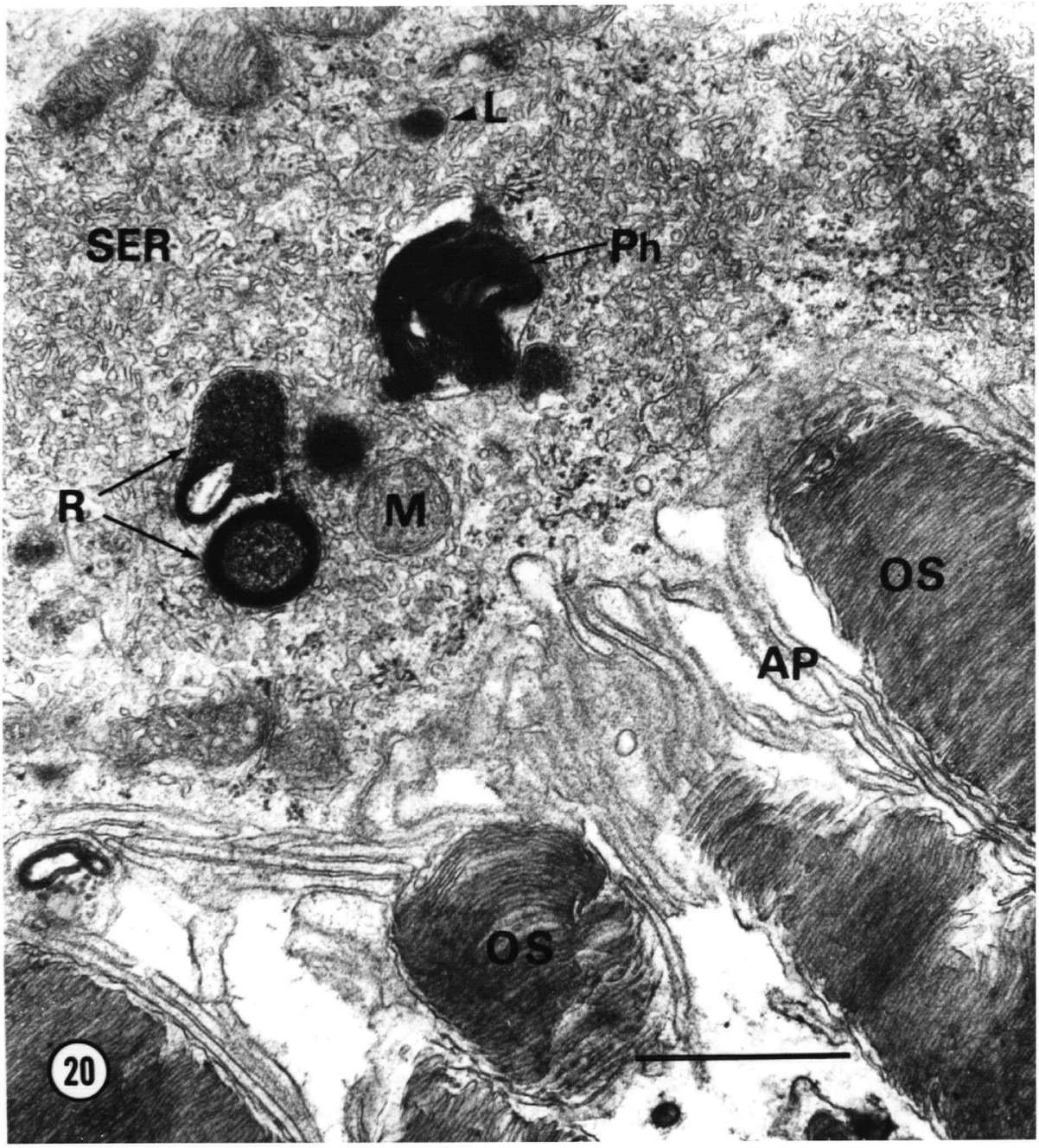

L
SER
Ph
R
M
OS
AP
OS
20

Figure 21.

Electron micrograph showing the retinal epithelium and the photoreceptor outer segments from the same specimen as Figure 20. Rough endoplasmic reticulum (RER) is visible just inside the epithelial inner surface. The bulk of the epithelial cytoplasm contains smooth endoplasmic reticulum (SER). A well developed Golgi complex (G) and several mitochondria (M) are visible. Epithelial apical processes (AP) form a palisade around the orderly arranged photoreceptor outer segments (OS). The photoreceptor outer segments are cylindrical structures and each is composed of a series of discs or flattened saccules surrounded by a plasma membrane (PM). The discs are regularly arranged and stacked at right angles to the length of the outer segment.

x 28,080

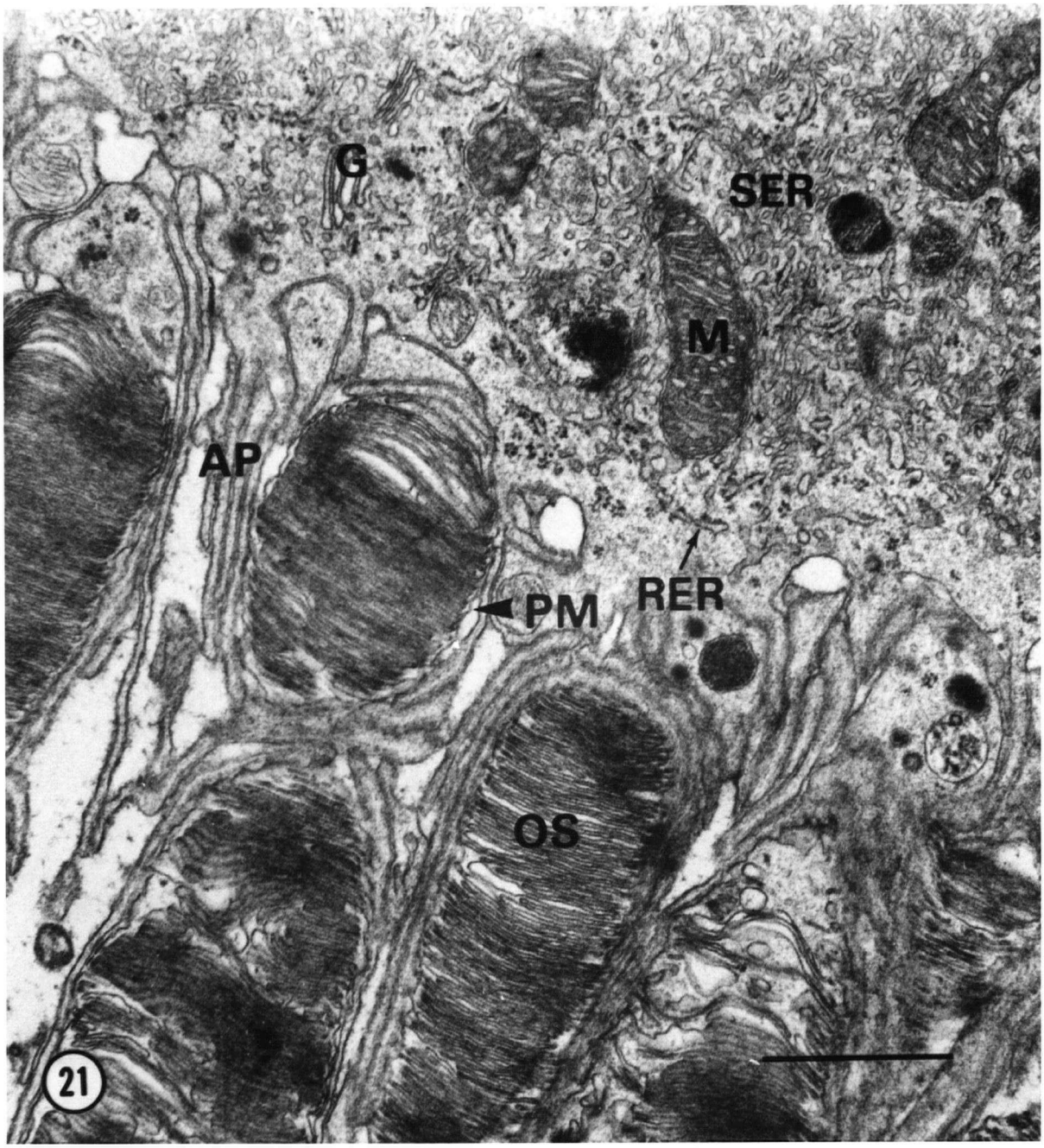

G
SER
M
AP
RER
PM
OS
21

Figure 22.

Electron micrograph showing the fine structure of two adjacent retinal epithelial cells from a 9 month old control animal. The fine structure of the retinal epithelium does not change with aging in control animals. Bruch's membrane (BM) overlies the retinal epithelium and part of the fenestrated endothelium (E) of the choriocapillary can be seen. Basal infoldings (B), and apical junctional complexes are present. The zonula occludens (Zo) is clearly visible and the zonula adherens (Za), is marked by prominent plasma membranes and condensation of subjacent cytoplasmic matrix. Smooth endoplasmic reticulum (SER) predominates in the cytoplasm. Several lysosomes (L) and residual bodies (R) are present. Mitochondria lie close to the basal infoldings and along the lateral cell borders. Pinocytosis (Pi) occurs at the basal surface and coated vesicles (CV) can be seen scattered in the cytoplasm from the basal to the apical surface.

x 21,060

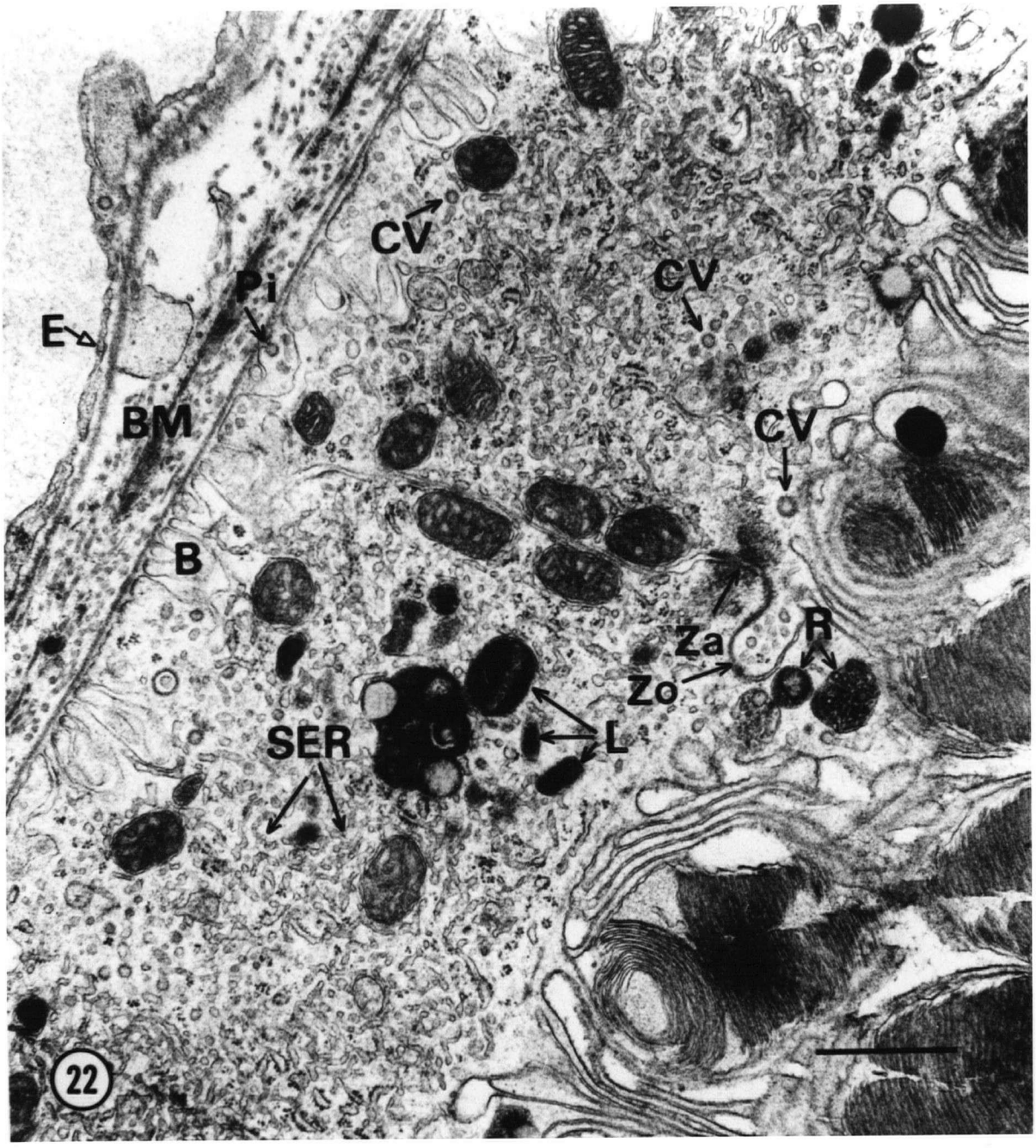

CV
CV
Pi
E
CV
BM
B
Za
R
Zo
L
SER
22

Figure 23.

Electron micrograph showing photoreceptor inner (IS) and outer segments (OS) from a 1.5 month old control animal. Each outer segment consists of stacks of regularly arranged lamellar discs enveloped within a plasma membrane. The outer segment is connected to the inner segment by a cilium (C). The inner segment contains numerous polysomes (P). Long, slender mitochondria (M) are arranged mainly around the periphery of the inner segment. A striated rootlet (SR) extends from the basal body (Bb) of the cilium down the inner segment.

x 17,320


OS
C
Bb
IS
P
SR
M
23

Figure 24.

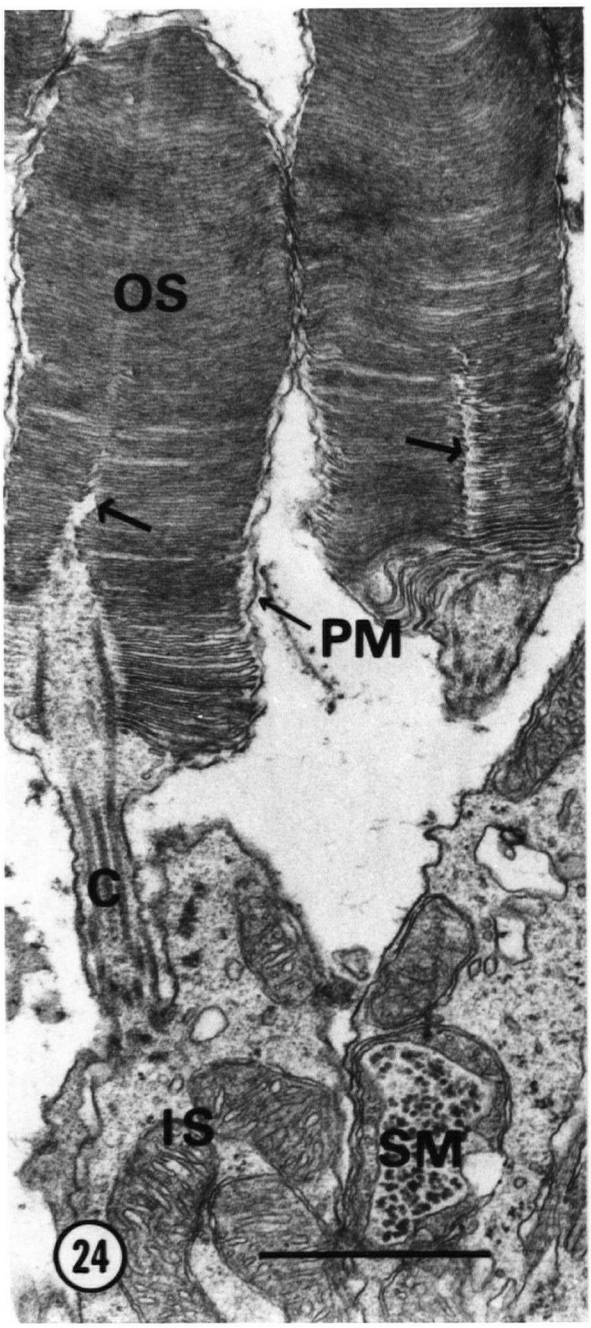
Electron micrograph showing the photoreceptor outer segments (OS), connecting cilium (C) and inner segments (IS) from a 7 month old control animal. The regularly arranged lamellar discs of the outer segment are surrounded by a single plasma membrane (PM) which is continuous with the plasma membrane of the inner segment. The discs appear identical to those seen in the 1.5 month old control animal. Each lamellar disc contains an invagination at the same point forming a longitudinal groove or incisure extending the whole length of the outer segment. Portions of such longitudinal grooves are indicated by arrows. A mitochondrion containing glycogen granules (SM) is seen at the lower right hand corner.

x 29,720

Figure 25.

Electron micrograph showing photoreceptor outer (OS) and inner segments (IS) from the same specimen as Figure 24. At the base of the outer segment, irregularly arranged saccules are seen. Next to them, the discs have a more regular appearance and further up, the lamellar discs are precisely arranged. The inner segment contains mitochondria (M), polysomes (P), smooth (SER) and rough endoplasmic reticulum (RER).

x 35,760


OS
PM
C
IS
SM
24

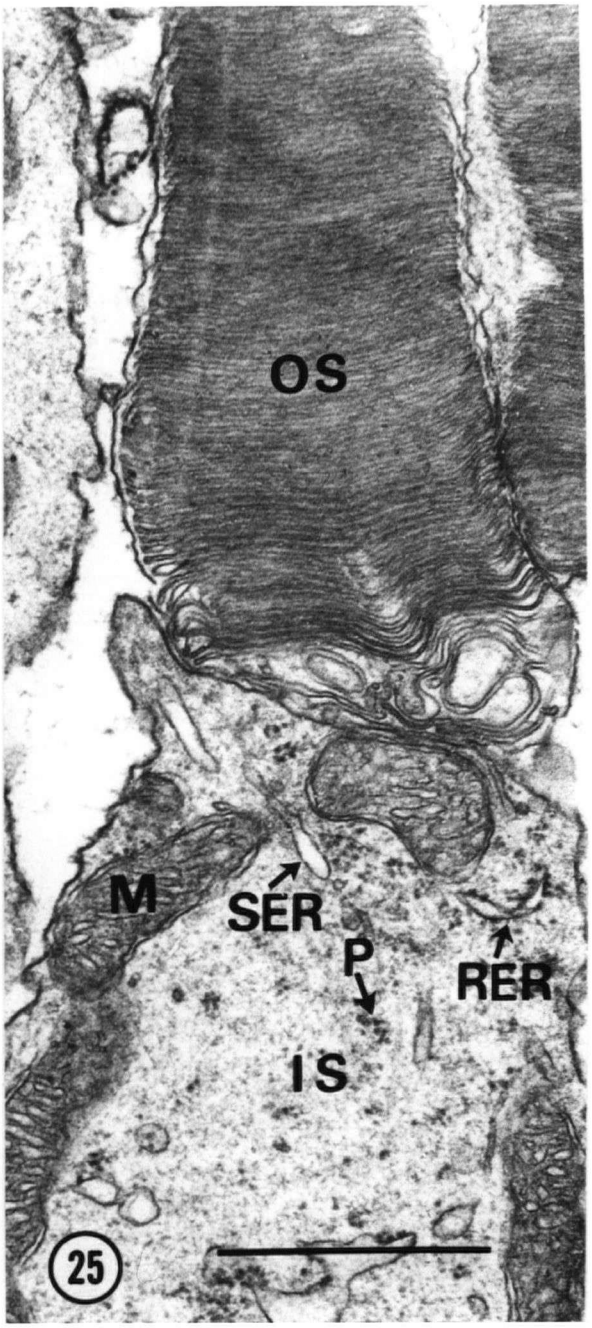

OS
M
SER
P
RER
IS
25

Figure 26.

Electron micrograph showing photoreceptor inner segments (IS) from a 9 month old control animal. The inner segments are long and cylindrical in shape. Elongated mitochondria (M) with well developed transverse critae are arranged mainly around the periphery of the inner segments. A Golgi apparatus (G) surrounded by numerous vesicles is present in the basal region of the inner segment. A few smooth surfaced cisternae (SER) are visible in the cytoplasm. Polysomes (P) are evenly distributed. The connecting cilium (C) originates in a basal body is located in the cytoplasm of the distal inner segment slightly to the side of its central axis. The striated rootlet (SR) extends from the basal body (Bb). The inner segments are closely packed together. Nevertheless, they usually are separated at their bases from each other by thin, villous extensions of Müller cells (MP). The outer limiting membrane (arrows) is formed mainly by cell junctions between photoreceptors and Müller cells. Portions of two rod photoreceptor nuclei (PN), each characterized by a large dense block of centrally located chromatin are shown in the lower part of the micrograph.

x 12,090

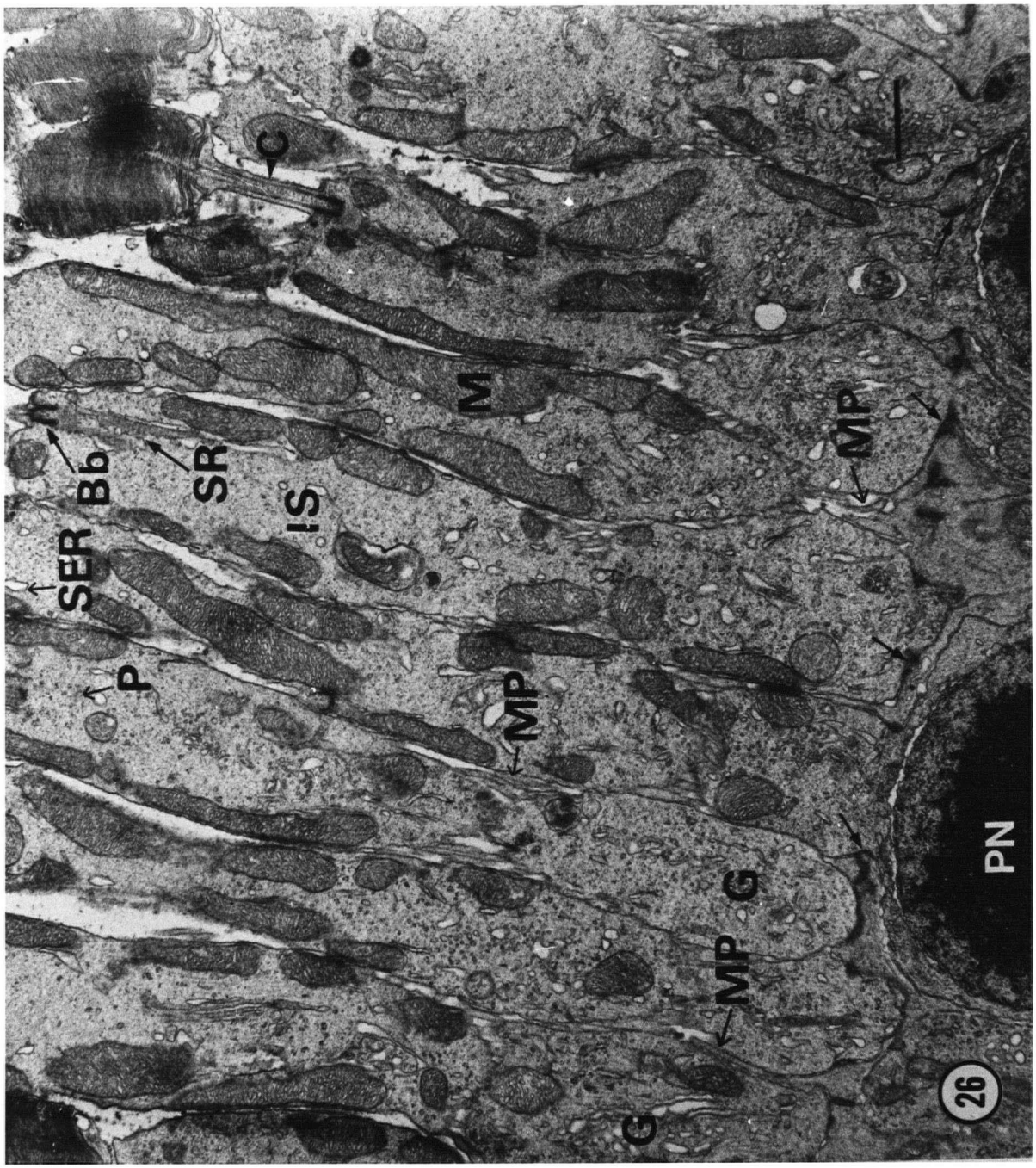

SER
Bb
P
SR
IS
C
M
MP
MP
MP
G
G
PN
26

Figure 27.

Electron micrograph at higher magnification showing photoreceptor inner segments and the outer limiting membrane from the same specimen as Figure 26. Mitochondria are seen disposed laterally in the inner segments. Two senile mitochondria (SM) each containing a large number of glycogen granules are seen in the inner segments. The Golgi complexes (G) are surrounded by numerous vesicles. Smooth surfaced cisternae (SER) and rough endoplasmic reticulum (RER) are visible in the inner segments. The cytoplasm is rich in polysomes(P). The closely packed inner segments are separated by Müller cell processes (MP). Zonulae adherentes (arrows) between the inner segments and the Müller cells form the outer limiting membrane. Portions of three photoreceptor nuclei (PN) are seen at the bottom of the micrograph.

x 18,650

M
P
SER
G
RER
MP
SM
G
PN
PN
27

Figure 28.

Electron micrograph showing in its center a rod synaptic process (R) from a 1.5 month old control animal. The process ends in an oval structure known as a "spherule". The basal surface of the spherule is invaginated by two lateral processes probably from horizontal cells (H) and one central process probably from a bipolar cell (B) of the inner nuclear layer. The cell wall of the spherule encloses the terminations of these neuronal processes. The rod spherule and its invaginating neuronal processes comprise the synaptic unit. The cytoplasm of the rod synaptic processes contains a large number of synaptic vesicles (SV), some polysomes (P) and one or two mitochondria (M). A synaptic ribbon (Sr) is typically found at the invaginating type of synapse. It is a crescent shaped structure composed of three dense layers separated from each other by two lighter layers. Below the synaptic ribbon lies condensed material forming the arciform density (Ad). Superficial contacts with neuronal processes from the inner nuclear layer are marked by a prominence of the presynaptic membrane of the rod spherule (long arrow). At the site of superficial synapses the synaptic ribbon is not present. The rod spherule shown in the center of the micrograph is surrounded by portions of several other rod spherules.

x 46,700

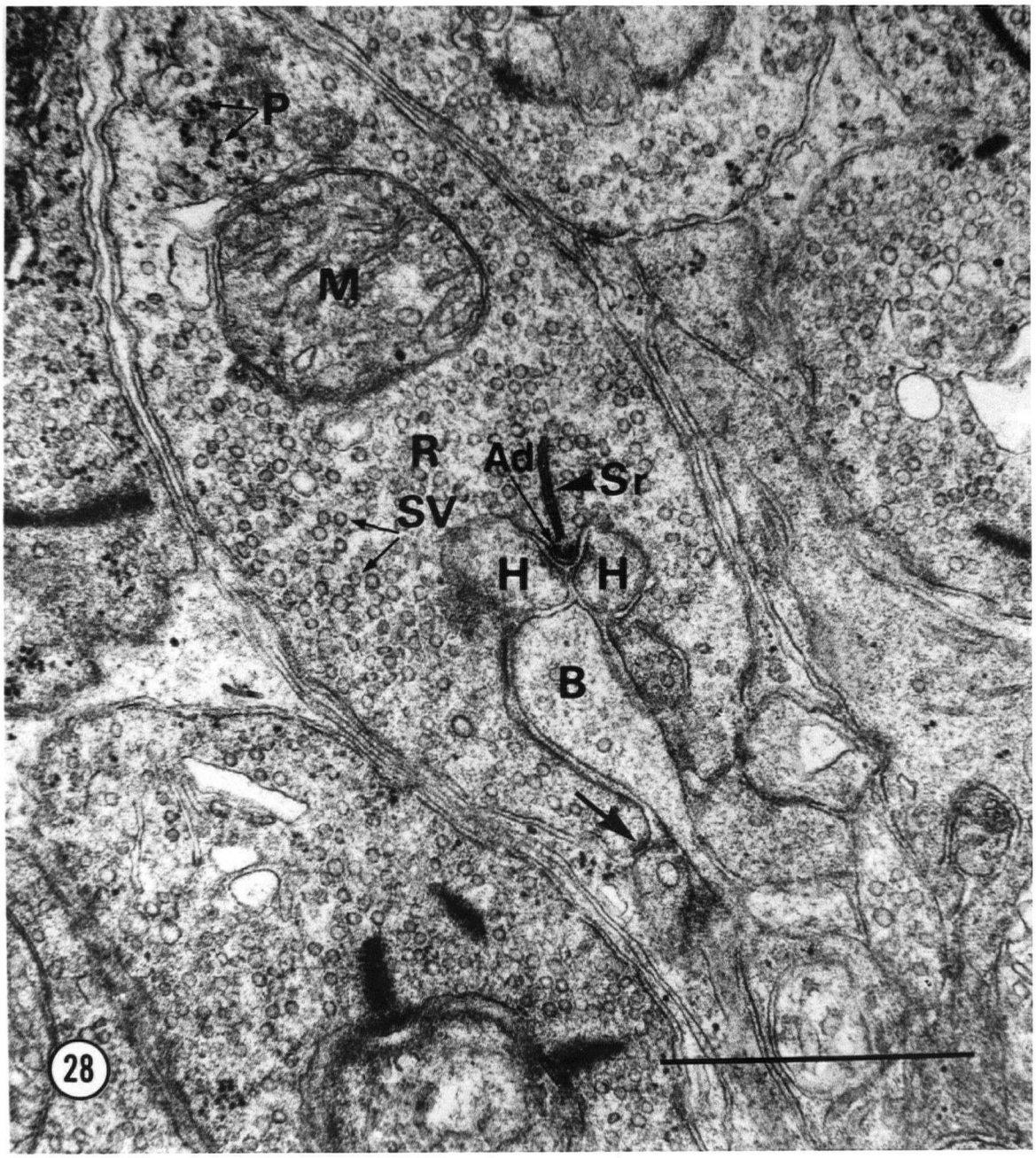

P
M
R
Ad
Sr
SV
H
H
B
28

Figure 29.

Electron micrograph showing the outer plexiform layer from a 1.5 month old control animal. Two photoreceptor nuclei (PN) are shown at the top of the micrograph. Synapses and synaptic ribbons (Sr) are numerous. Each synaptic process is full of synaptic vesicles (SV) and contains one or more mitochondria (M). At the synaptic site, each synaptic ribbon is surrounded by a cluster of synaptic vesicles. The most common type of synapses observed are contacts between rod spherules and processes of horizontal (H) and bipolar cells. Less commonly, post-synaptic processes, instead of penetrating into a spherule, make direct contact with the surface of the cell body. In this situation the synaptic ribbon lies near the receptor nucleus (arrow head). This type of contact has been described as a somatodendritic synapse.

x 18,650

PN
M
M
H
H
Si
SV
29

Figure 30.

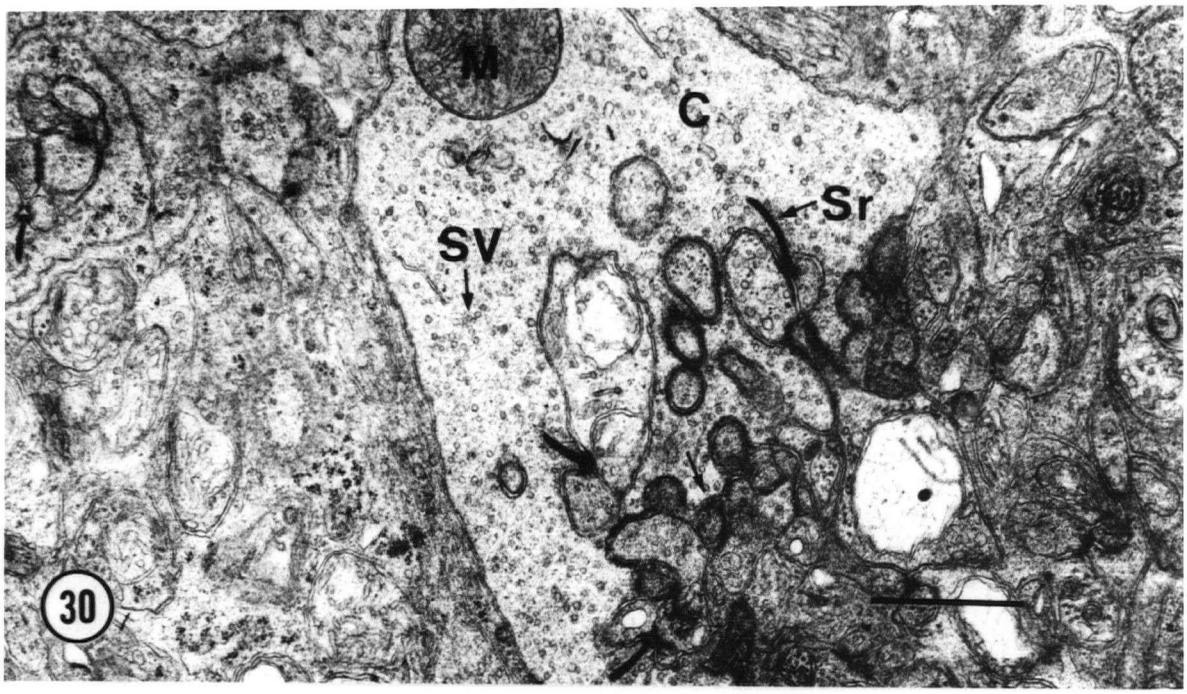
Electron micrograph showing a cone synaptic process (C) from a 1.5 month old control animal. The synaptic process ends in a broad swelling which has been called a "pedicle". The cone pedicle is characterized by its complex base into which other neuronal processes from cells of the inner nuclear layer penetrate and make synaptic contact. In the cytoplasm of the pedicle, synaptic vesicles (SV) are evenly distributed and one or more mitochondria (M) are often present. There are more synapses of the invaginating type and therefore more synaptic ribbons (Sr) than in the rod spherule. Each synaptic ribbon is seen to be surrounded by a cluster of synaptic vesicles. Superficial synapses can also be observed at the basal surface of the pedicle (arrow).

x 19,750

Figure 31.

Electron micrograph showing rod spherules (R) from a 1.5 month old control animal. The spherule makes synaptic contact with the neuronal processes from the inner nuclear layer. Synaptic vesicles (SV) are evenly distributed in each spherule. Note that the horizontal cell processes also contain vesicles which resemble the synaptic vesicles of the spherule. Synaptic ribbons (Sr) are found at the invaginating type of synapses.

x 22,290


M
C
Sr
SV
30

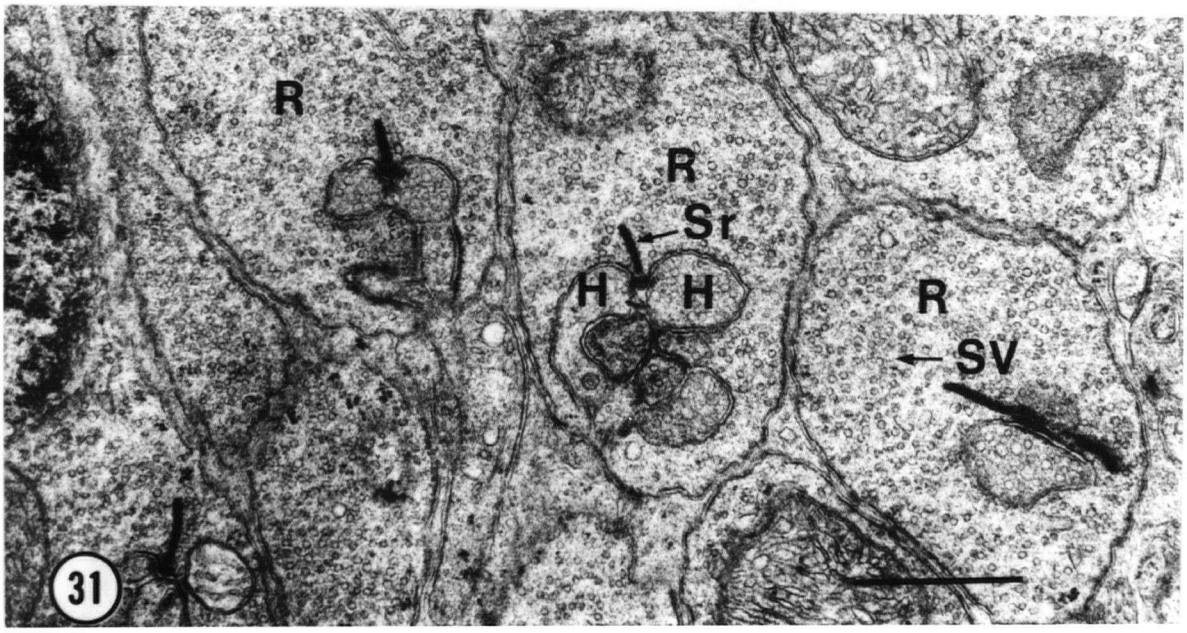

R
R
Sr
H
H
R
SV
31

Figure 32.

Electron micrograph showing the outer plexiform layer from a 12 month old control animal. A cone pedicle (C) is shown partially surrounded by several rod spherules (R). Synaptic vesicles (SV) are numerous and evenly distributed in both the rod and cone synaptic processes. The rod synapse typically receives dendrites from one bipolar cell and usually only one synaptic ribbon (Sr) is seen at the synaptic site in a cross-section. The cone synaptic pedicle is more complex than that of the rod, and it probably makes contact with several bipolar and horizontal cells. Therefore, more synaptic sites and more synaptic ribbons are observed in a cross-section of a cone pedicle than a rod spherule. On the lower left of the micrograph a mitochondrion in one of the synaptic processes has accumulated glycogen granules. At the top of the figure, portions of four photoreceptor nuclei are seen.

x 21,060

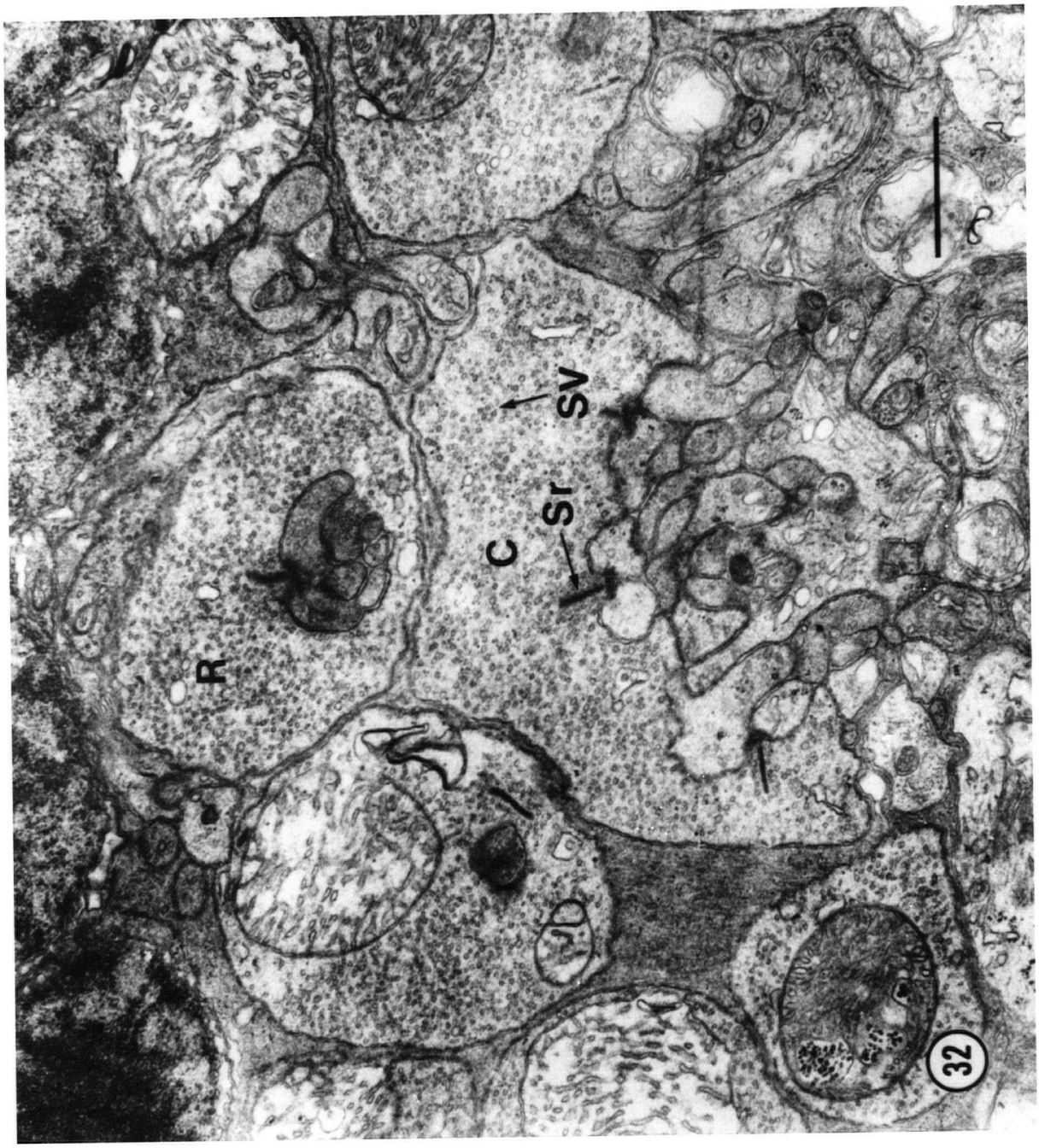

SV
C
Sr
R
32

Figure 33.

Electron micrograph showing the photoreceptor outer segments from a 1 month vitamin A deficient animal. The distal portions of the outer segments (OS) are the first portions of the photoreceptors to show signs of change. Some of the lamellar discs break down into vesicles (arrows). A distorted outer segment in which the lamellar discs have opened up to form longitudinally arranged lamellar structures, appears to be partially engulfed by apical processes (AP) of the retinal epithelium (RE). Part of the retinal epithelium containing a Golgi apparatus (G) is shown on top of the micrograph. Note the absence of many lysosomes in the retinal epithelium at this stage.

x 21,060

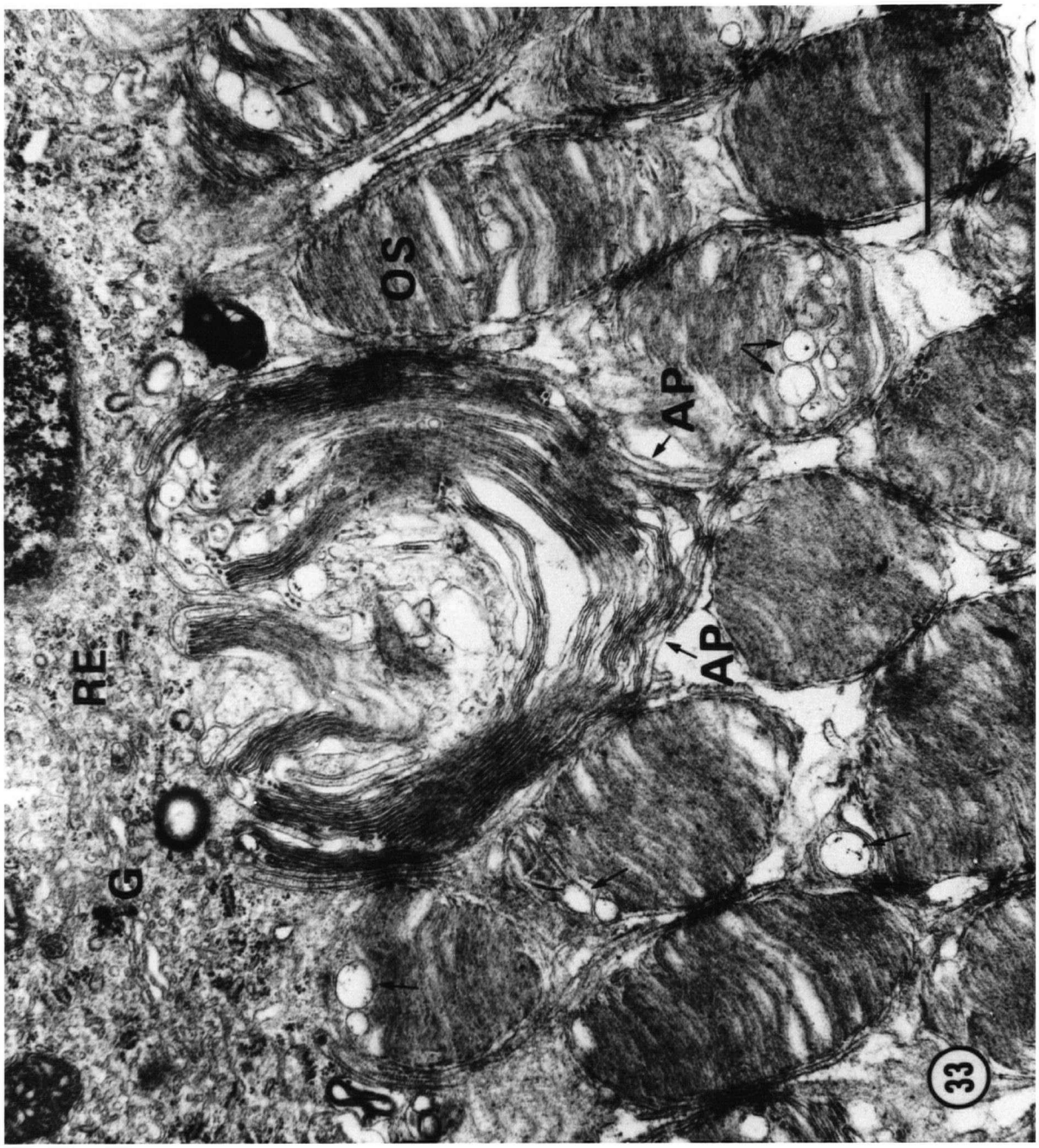

RE
G
OS
AP
AP
AP
33

Figure 34.

Electron micrograph showing the retinal epithelium and photoreceptor outer segments from a 1.5 month vitamin A deficient animal. The outer segments (OS) are now more severely affected. More lamellar discs have broken down into vesicles (V). Some disordered saccules (arrow heads) are also observed. The disintegrating outer segments are closely surrounded by apical processes of the retinal epithelium (RE). In the retinal epithelium, lysosomes (L) have gathered within its inner surface. Ph_1 and Ph_2 are two partially disintegrating phagosomes. A lipid droplet (LD) is seen on the right hand side of the micrograph in the retinal epithelium. Polysomes are scattered in the epithelial cytoplasm. Rough endoplasmic reticulum is found close to the inner surface of the epithelium.

x 28,080

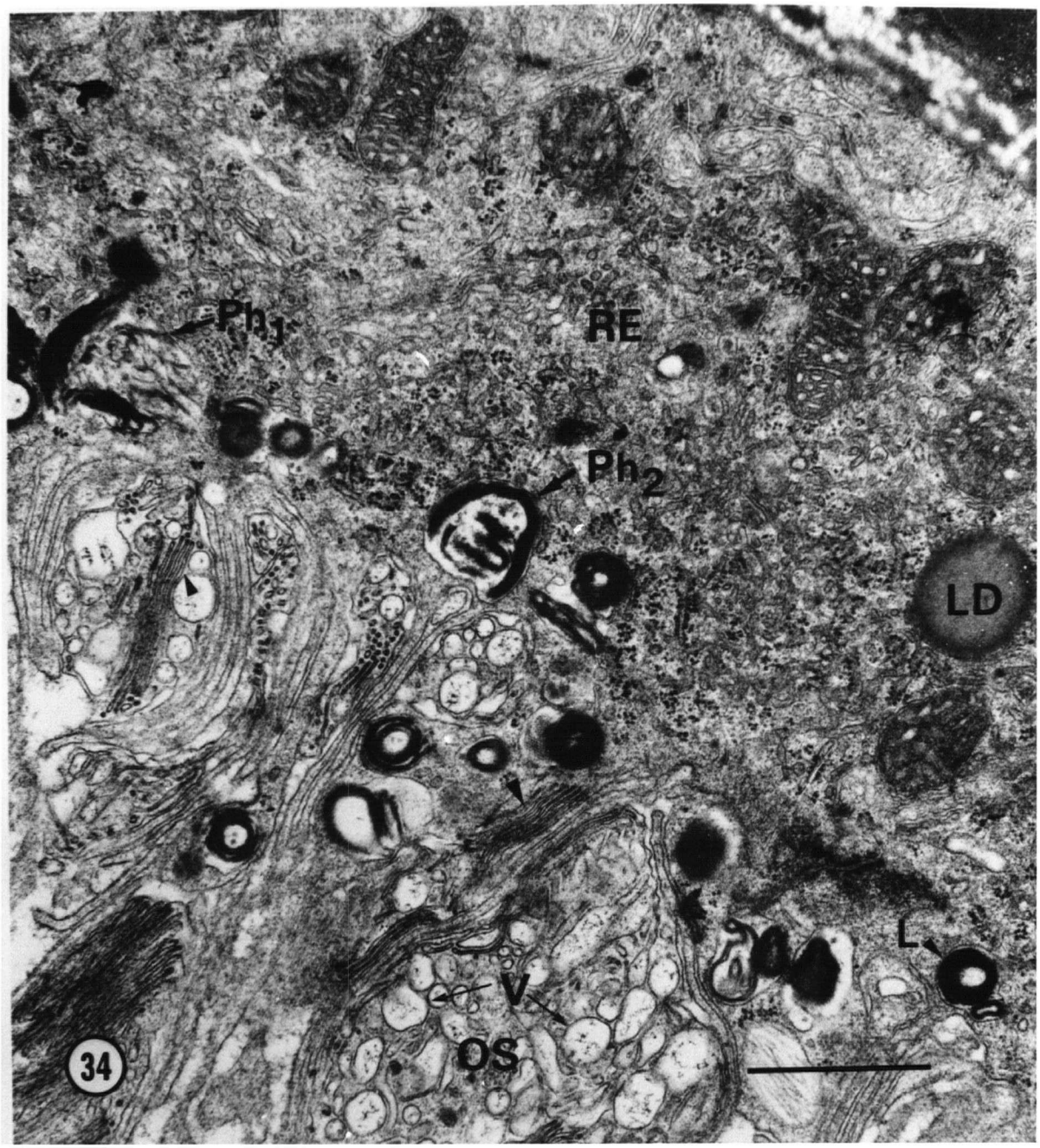

Ph1
RE
Ph2
LD
L
V
OS
34

Figure 35.

Electron micrograph showing photoreceptor outer segments (OS) from the same specimen as Figure 34. Affected outer segments degenerate to different degrees. The more severely affected outer segments are almost completely surrounded by apical epithelial processes, which appear to be in the act of ingesting them. Part of the retinal epithelium (RE) with lysosomes (L) along its inner surface is seen at the top right hand corner. Note the apical cell junction (ACJ).

x 21,060

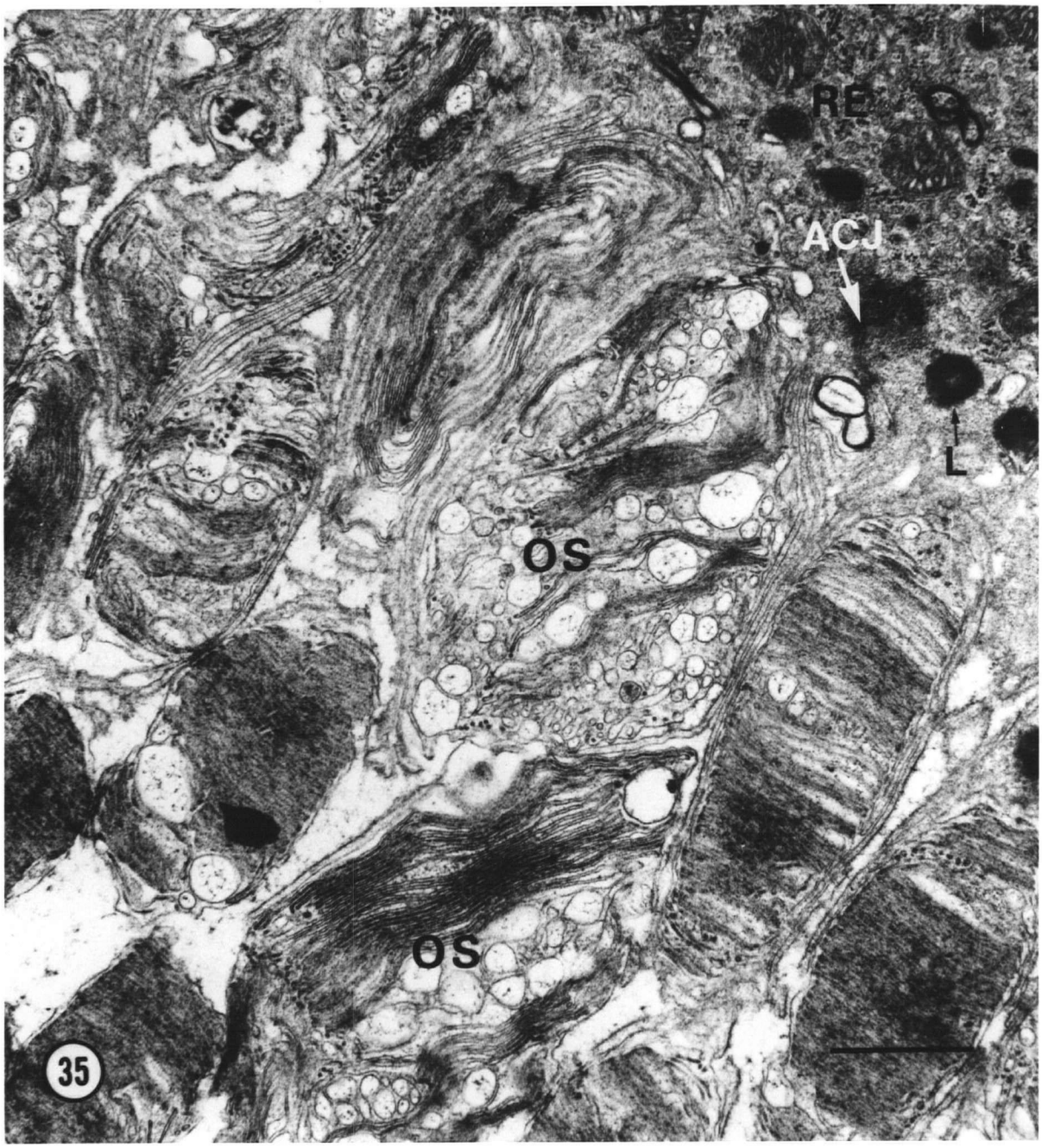

RE
ACJ
L
OS
OS
35

Figure 36.

Electron micrograph showing photoreceptor outer and inner segments from a 2 month vitamin A deficient animal. Most of the lamellar discs in the outer segments are affected and are in various stages of disintegration. However, near the base of each outer segment, some lamellar discs appear intact. The inner segments (IS) contain large numbers of polysomes. Mitochondria (M), cilia (C) and basal bodies (Bb) are still present and are unchanged in appearance.

x 21,060

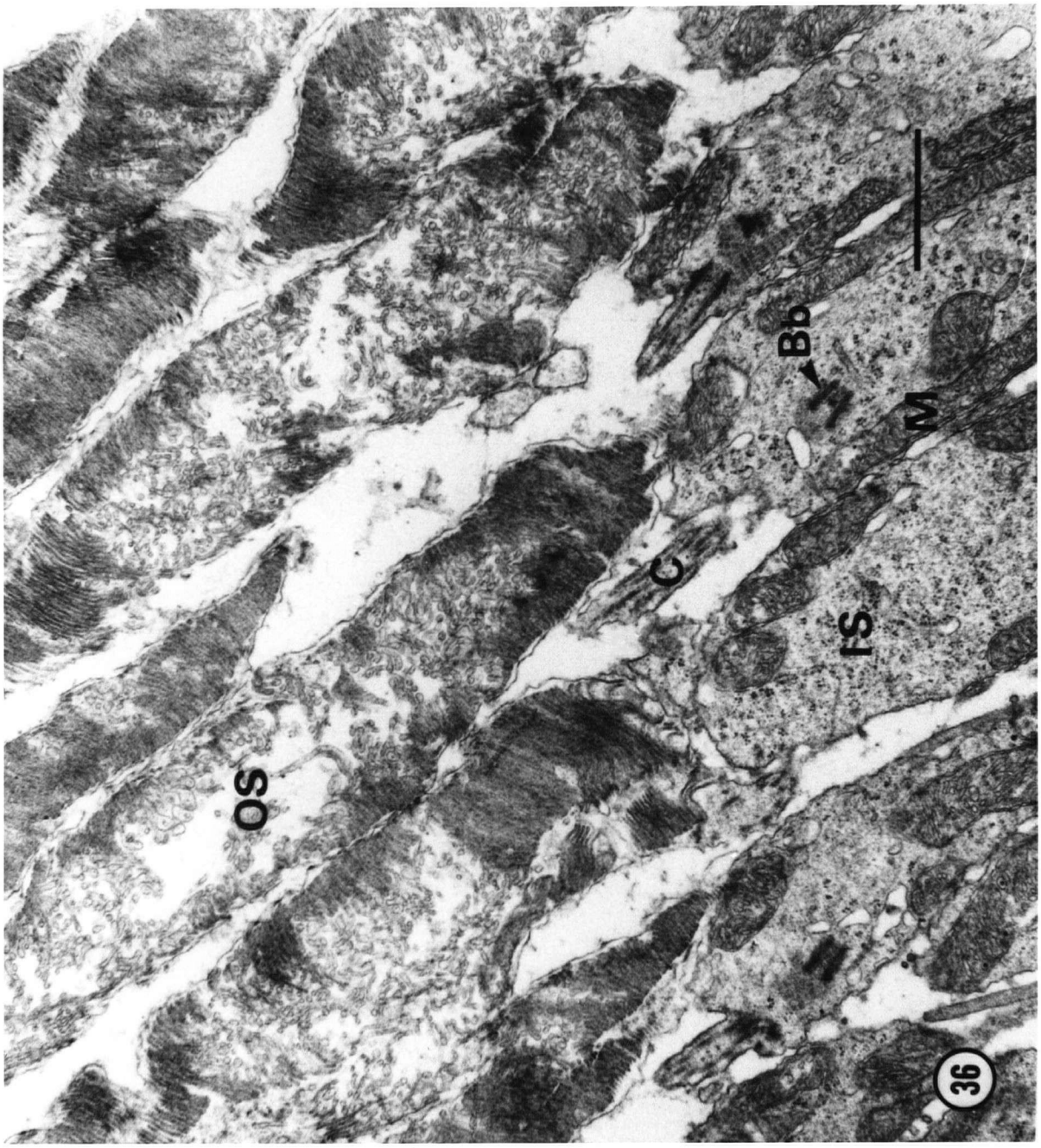

OS
C
Bb
M
IS
36

Figure 37.

Electron micrograph showing the junction between the retinal epithelium and photoreceptor outer segments from a 2.5 month vitamin A deficient animal. The section is cut slightly obliquely. The apical processes (AP) of the retinal epithelium (RE) appear to have undergone a slight proliferation and are irregularly oriented. They appear to be active in engulfing degenerating outer segments (OS). The smooth endoplasmic reticulum (SER), mitochondria (M) and Golgi complexes (G) in the retinal epithelium remain unchanged.

x 18,650

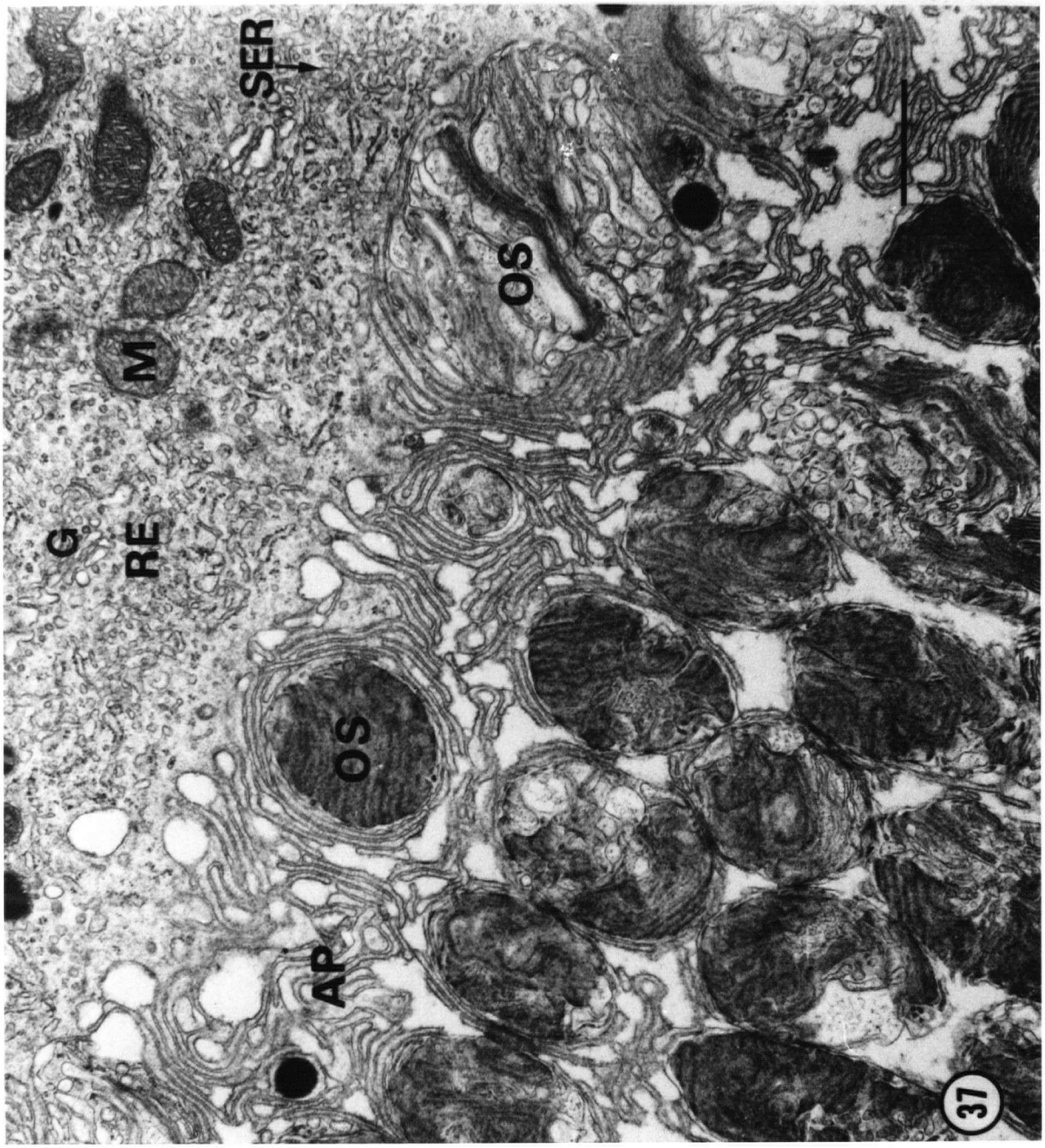

SER
M
G
RE
OS
OS
AP
37

Figure 38.

Electron micrograph showing disintegrating photoreceptor outer segments (OS) from the same specimen as Figure 37. More outer segments are affected and the destruction is more extensive than in the 1.5 month vitamin A deficient animals. The disintegrating outer segments contain vesicles and tubules. Many discs have disappeared completely leaving behind large intracellular spaces (x). Apical processes (AP) of the retinal epithelium in irregular array are seen at the top left hand corner of the micrograph.

x 14,040

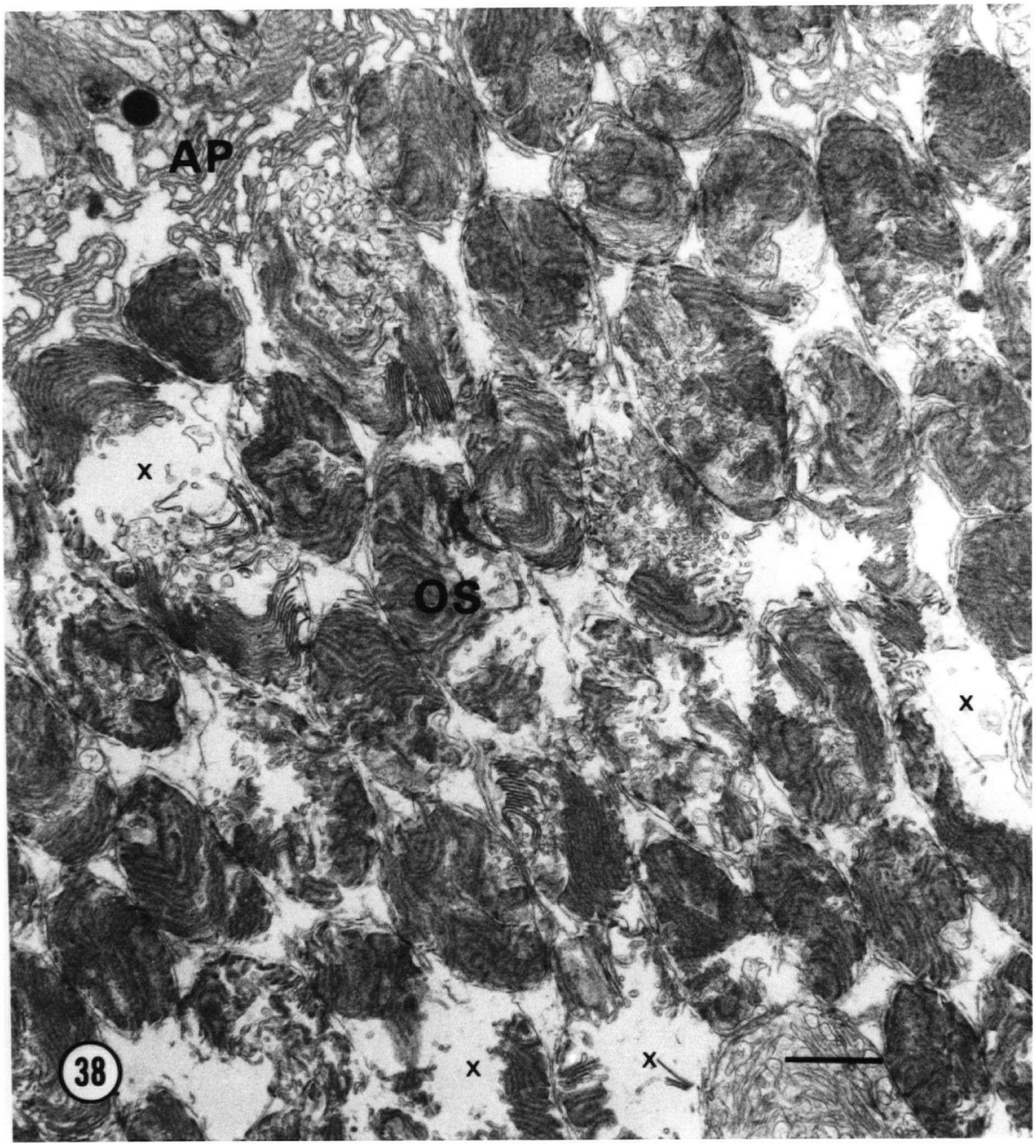

AP
OS
X
X
X
X
38

Figure 39.

Electron micrograph showing the photoreceptor inner segments (IS) of the same specimen as Figure 37. At this stage of vitamin A deficiency the distal portions of the inner segments also show morphological change. The distal portions are slightly swollen and their cytoplasm is almost devoid of polysomes. Vacuoles (V) which may result from fusion and dilation of the smooth endoplasmic reticulum are observed within inner segment cytoplasm. Cross-sections of two connecting cilia (C) are present and appear normal Some obliquely cut outer segments (OS) which have yet to undergo severe degeneration are present between and above the affected inner segments.

x 21,060

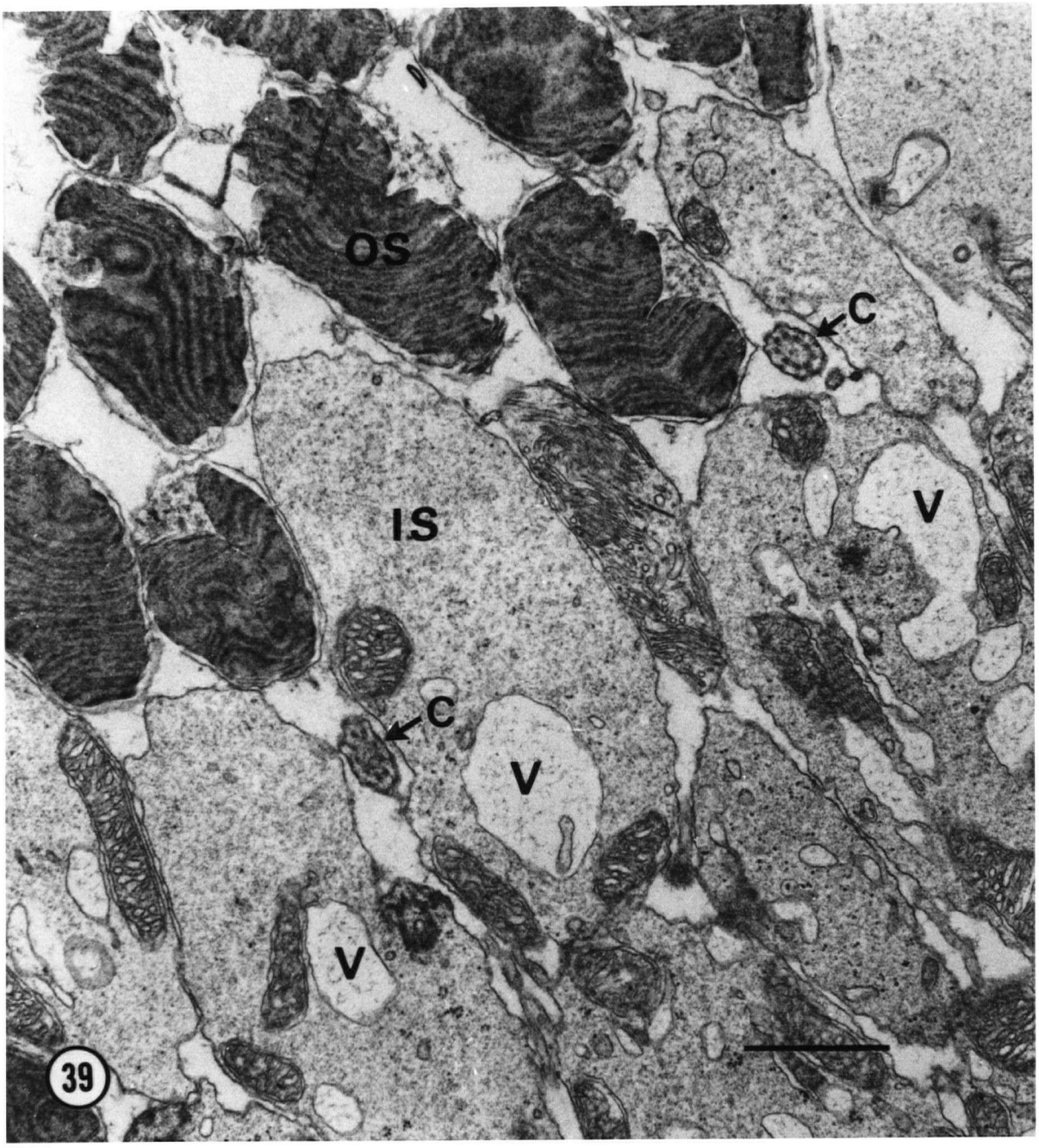

OS
C
IS
V
C
V
V
39

Figure 40.

Electron micrograph showing photoreceptor outer segments (OS) from a 4 month vitamin A deficient animal. Within the outer segments, some of the lamellar discs have broken down into vesicles (V) while others are either loosely arranged or oriented in an abnormal directions. Many lamellar discs have completely disappeared leaving empty spaces (x). Apical processes (AP) of the retinal epithelium (RE) are seen surrounding the degenerating outer segments.

x 28,080

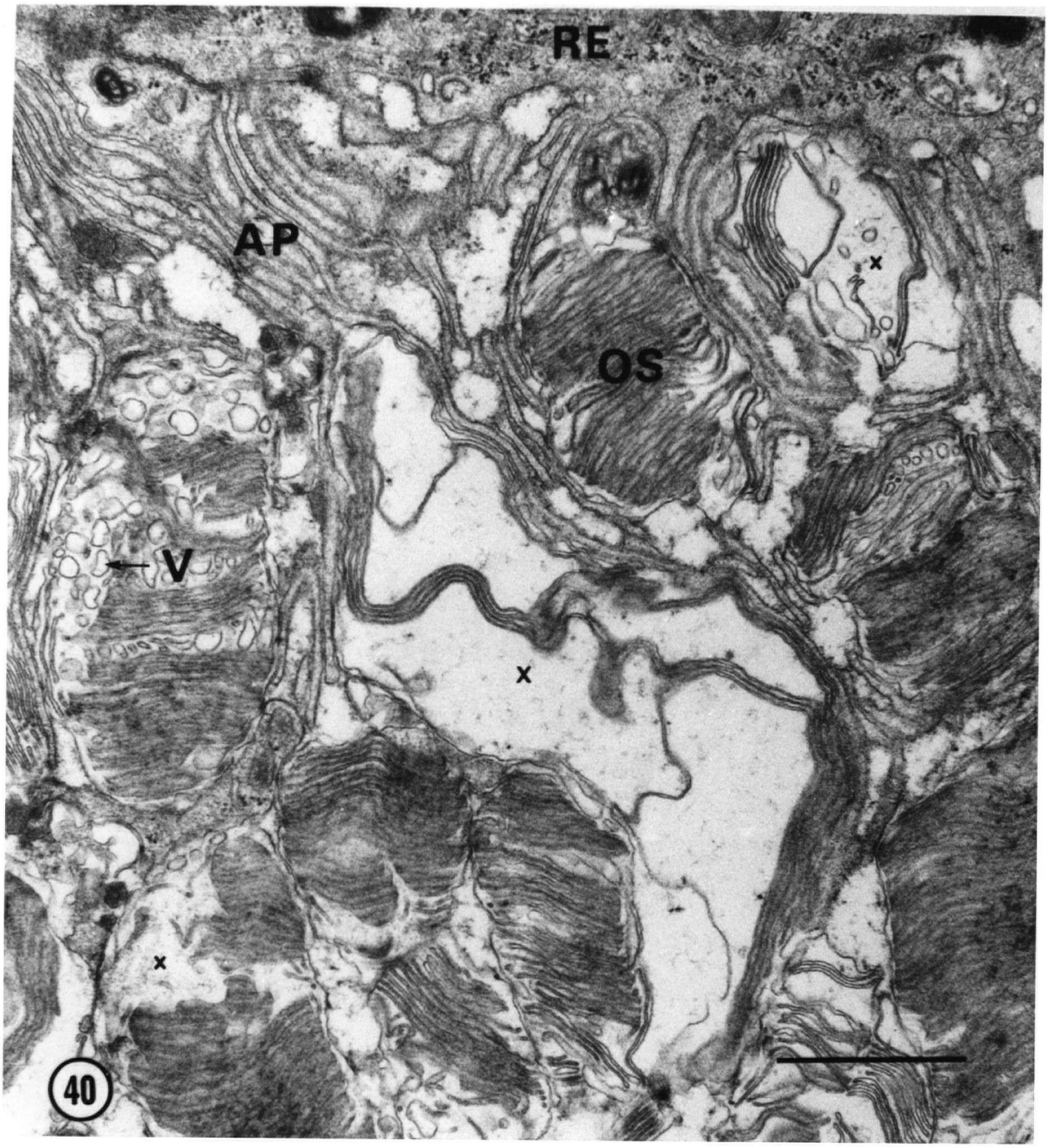

RE
AP
OS
V
x
x
x
40

Figure 41.

Electron micrograph showing photoreceptor outer segments (OS), inner segments (IS) and the outer limiting membrane from the same specimen as Figure 40. Many outer segments have now disappeared leaving gaps (x). The inner segments appear shorter than normal. Basal bodies (Bb) and striated rootlets (SR) are still present in the inner segments. Polysomes (P) have gathered in the inner halves of the inner segments. Rough endoplasmic reticulum (RER) and large vacuoles (V) can also be identified in inner segment cytoplasm. Some photoreceptor inner segments have undergone almost complete degeneration and can be seen as three dense structures in the micrograph (arrows). The outer limiting membrane appears intact (double arrows). Portions of two photoreceptor nuclei are seen at the bottom of the micrograph.

x 14,700

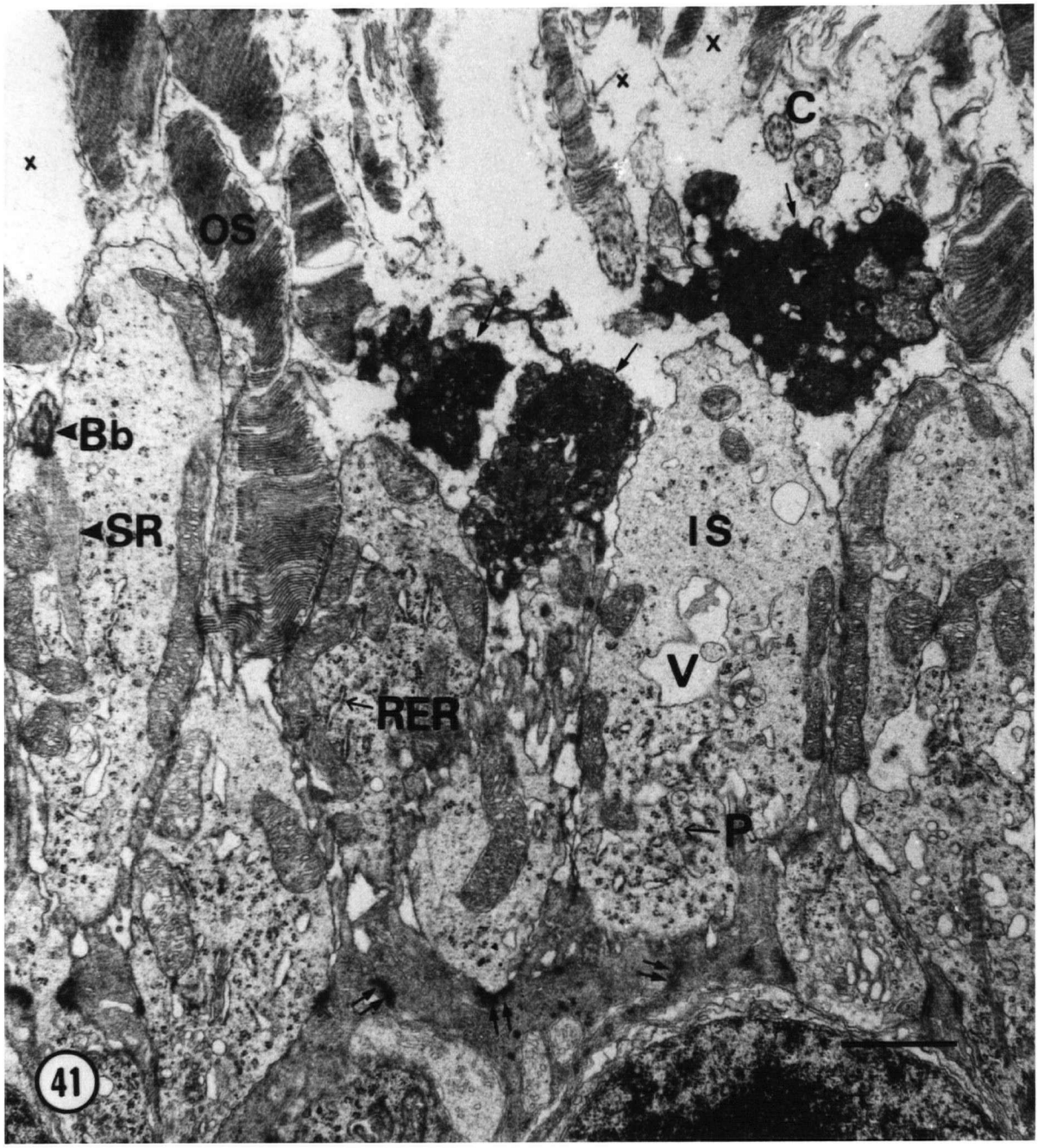

x
x
x
C
OS
Bb
SR
IS
V
RER
P
41

Figure 42.

Electron micrograph showing photoreceptor inner segments (IS) from the same specimen as Figure 40. Smooth endoplasmic reticulum and vacuoles (V) are visible in the short and plump inner segments. Mitochondria (M), connecting cilia (C) and basal bodies (Bb) in the inner segments appear intact. A degenerating inner segment (IS) is seen in the center of the micrograph. Its dense cytoplasm contains several mitochondria, two of which (SM) are swollen with glycogen granules. Many Müller cell processes (MP) extend between adjacent inner segments.

x 18,650

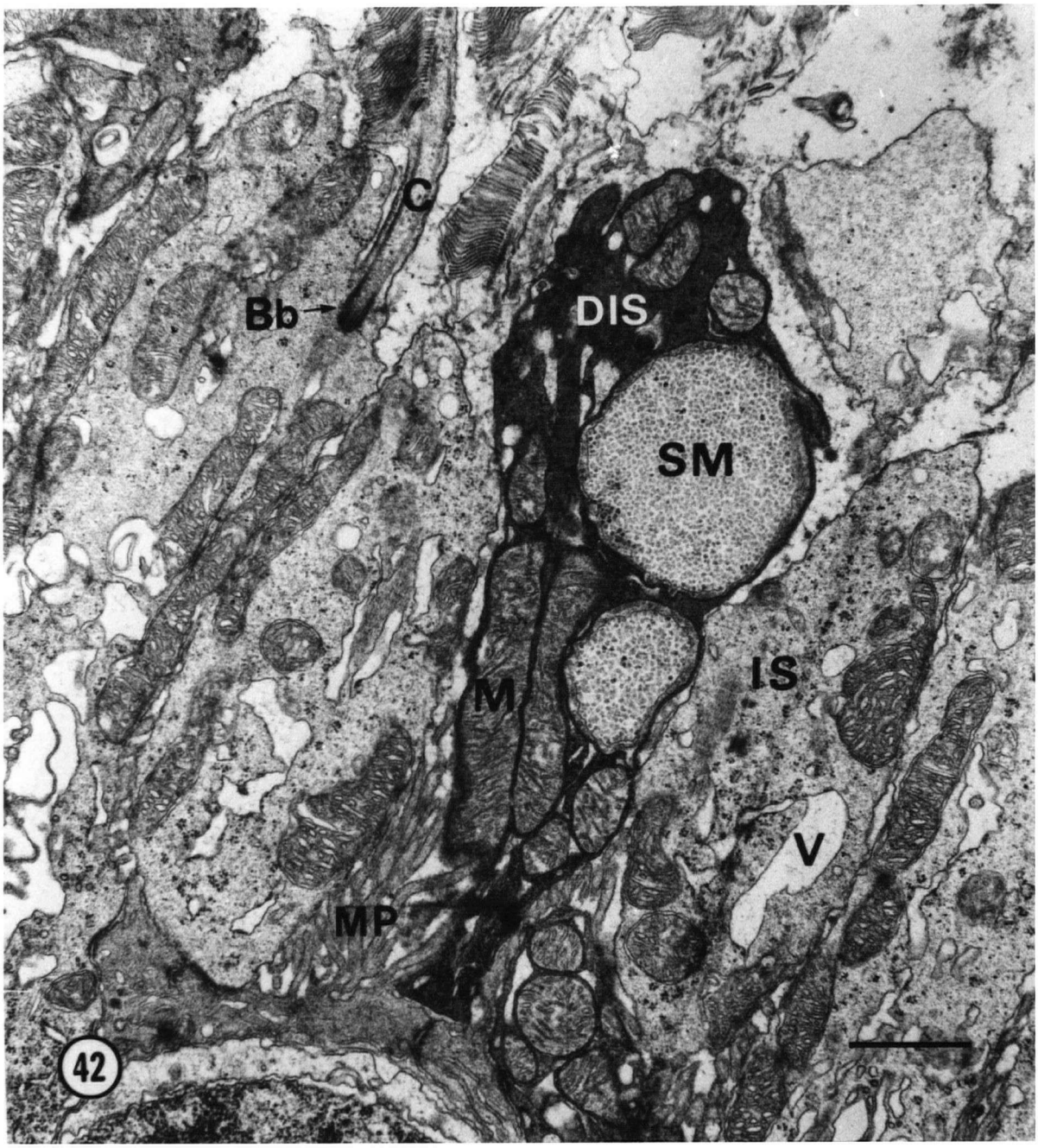

C
Bb
DIS
SM
M
IS
V
MP
42

Figure 43.

Electron micrograph showing two adjacent retinal epithelial cells (RE) from a 5 month vitamin A deficient animal. Bruch's membrane (BM) appears normal. Basal infolding (B), apical cell junctions (ACJ) and mitochondria (M) in the retinal epithelium are intact. The smooth endoplasmic reticulum (SER) of the retinal epithelium has not been altered either quantitatively or structurally. The major change is a large increase in lysosomes (L) near the inner epithelial border. The apical or inner processes (AP) of the retinal epithelium have lost their typical regular arrangement. Portion of an outer segment (OS) is seen at the bottom of the micrograph.

x 21,060

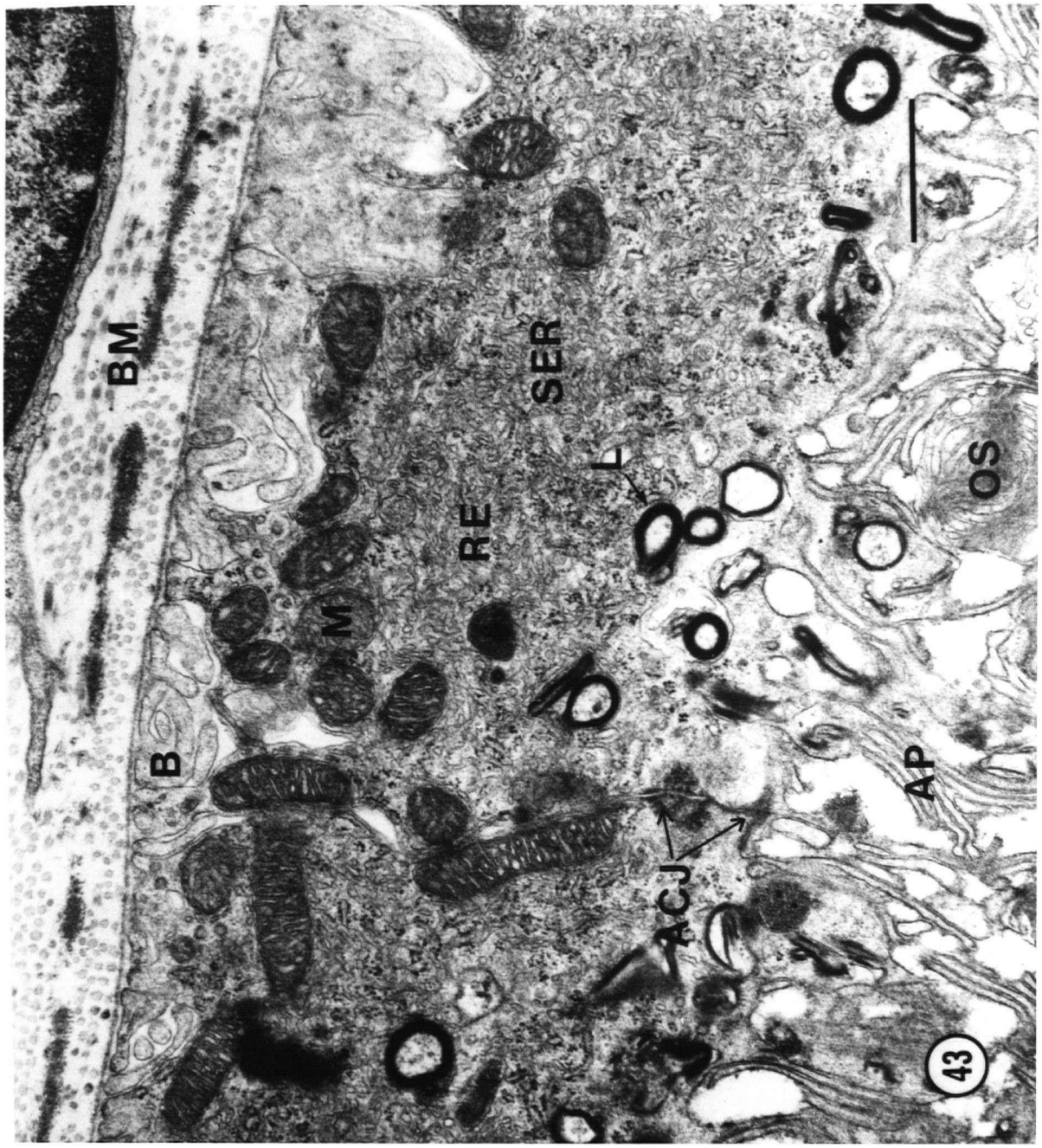

BM
B
M
RE
SER
L
ACJ
AP
OS
43

Figure 44.

Electron micrograph showing the retinal epithelium, photoreceptor outer segments and inner segments from a 6 month vitamin A deficient animal. More severe destruction of the photoreceptors is in evidence. The outer segments (OS) have lost their normal highly ordered orientation. None of them appear intact. Many intra- and extracellular spaces (x) are observed. The inner segments now are closer to the retinal epithelium. The inner segments display shorter mitochondria than normally and the cytoplasm in their distal halves contains few polysomes. The retinal epithelium, contains an elongated nucleus (N) and displays a marked increase in lysosomes (L) along its inner surface. The apical processes (AP) of the retinal epithelium appear to be engulfing the degenerating outer segments (OS).

x 12,090

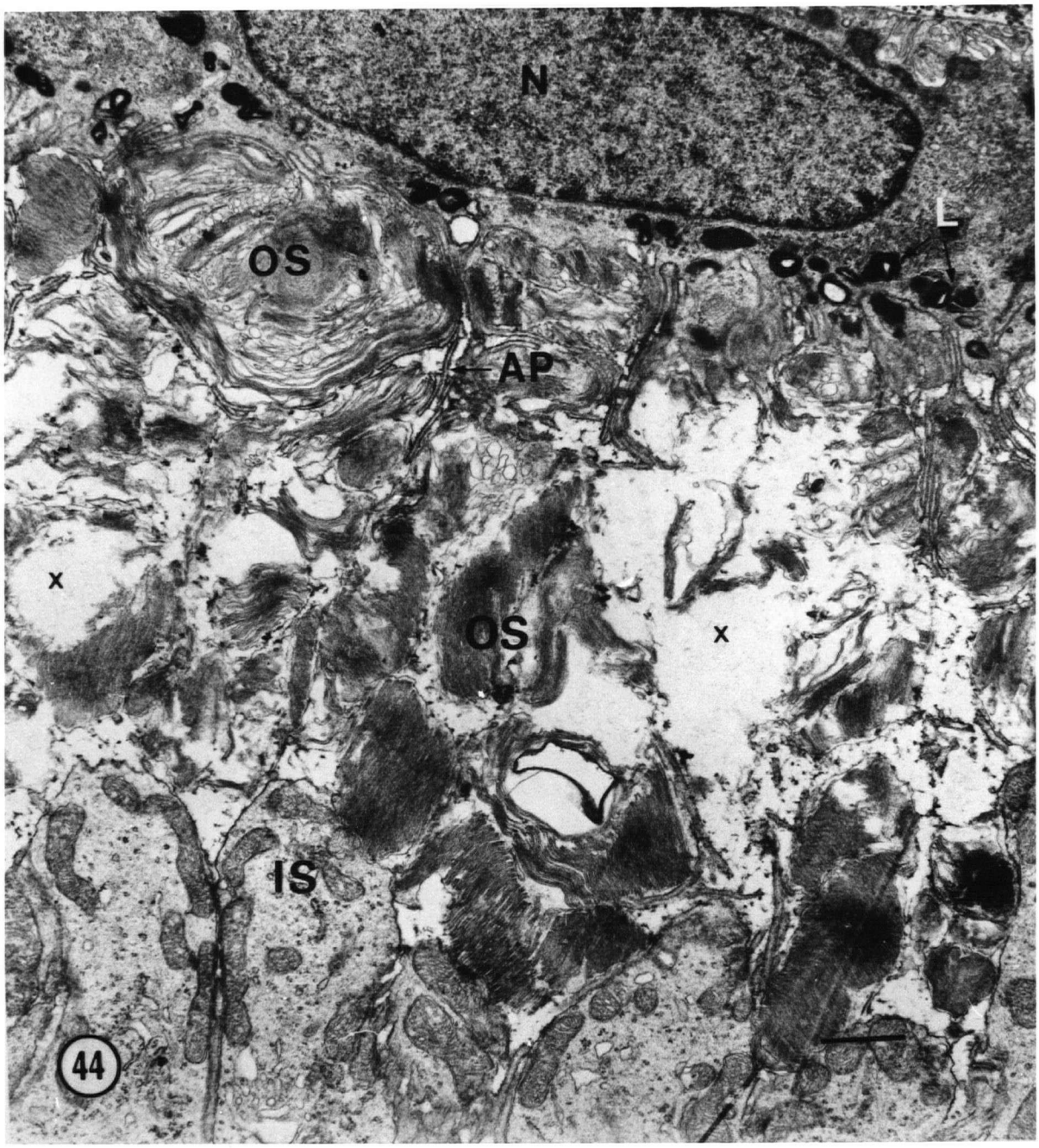

N
L
OS
AP
x
OS
x
IS
44

Figure 45.

Electron micrograph showing the retinal epithelium (RE) and the photoreceptor outer segments (OS) from the same specimen as Figure 44. A phagosome (Ph) is seen in the middle of the epithelium. An increase of lysosomes (L) along the epithelial inner surface is evident. The apical processes (AP) are short and randomly oriented. The disintegrating photoreceptor outer segments have lost their normal regular orientation and many intra- and extracellular spaces (x) have formed.

x 16,470

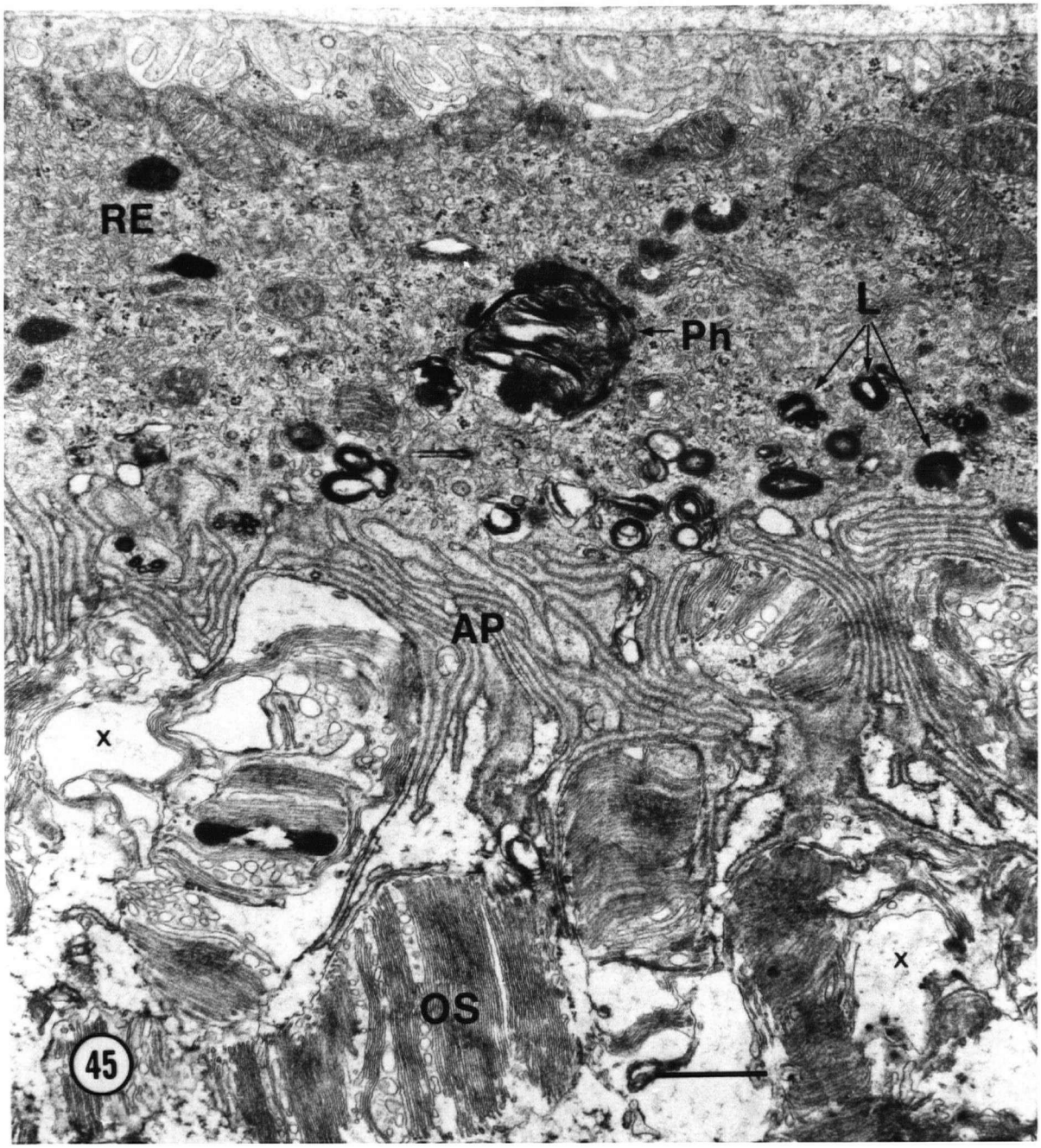

RE
Ph
L
AP
x
x
OS
45

Figure 46.

Electron micrograph showing the photoreceptor outer segments from the same specimen as Figure 44. Most of the outer segments (OS) show degenerative changes. Some outer segment discs have lost their normal compact and horizontal arrangement and as a result the outer segments appear badly damaged. Other groups of discs have a surprisingly normal appearance. The entire layer of photoreceptor outer segments is greatly reduced in thickness. Parts of two photoreceptor inner segments (IS), one with an intact connecting cilium, are seen at the lower right hand corner. At the top left is part of the retinal epithelium with several lysosomes (L).

x 21,060

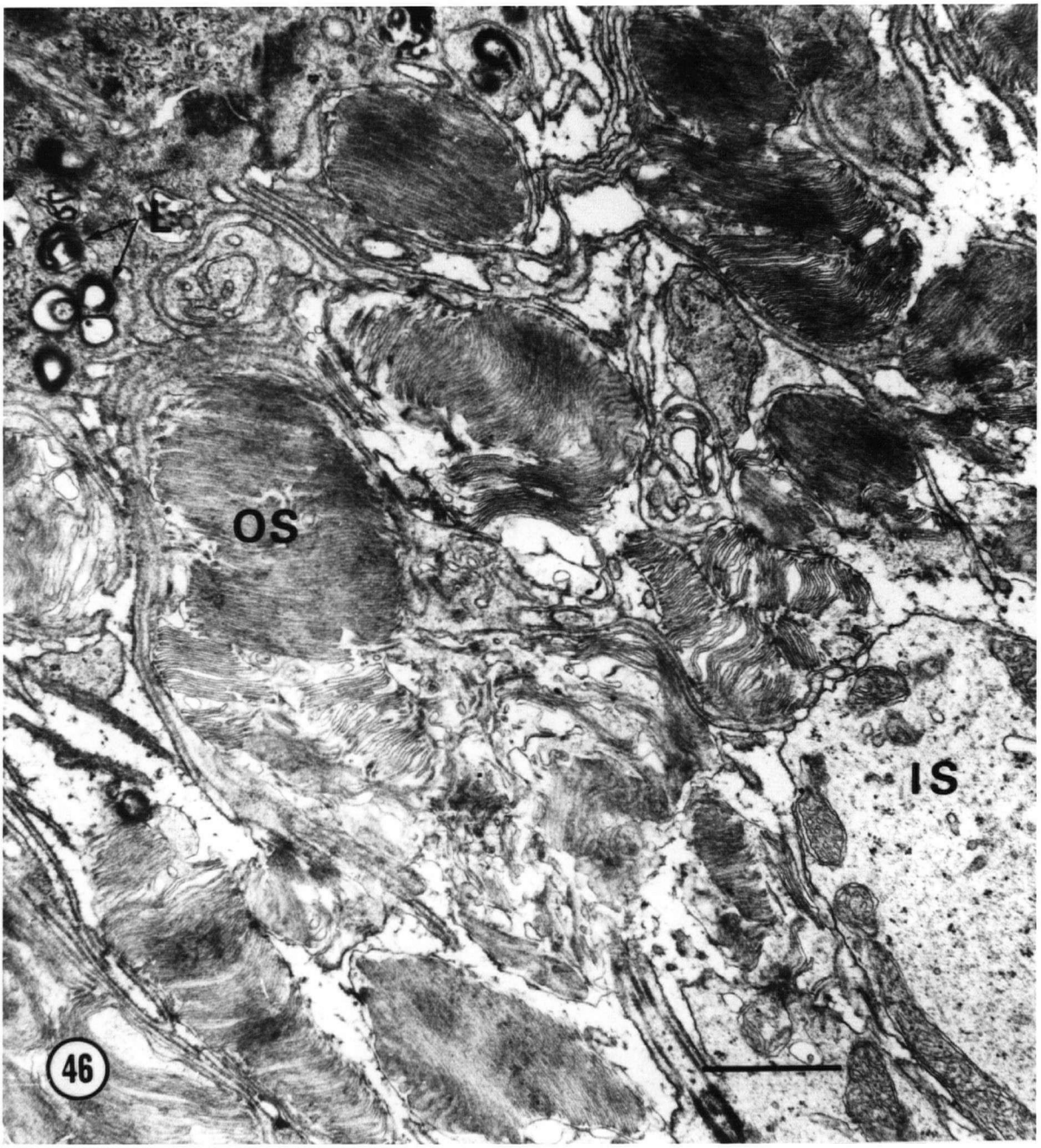

OS
IS
46

Figure 47.

Electron micrograph showing mainly photoreceptor inner segments (IS) from the same specimen as Figure 44. Above the inner segments, severe destruction of the outer segments is observed. The inner segments have undergone different stages of shortening. In the less contracted inner segments to the right, there is a perceptible difference in the polysome distribution between the distal and basal halves of the cytoplasm. The distal halves of the inner segments (towards the retinal epithelium) contain fewer polysomes (P). The smooth cisternae (SER) are slightly dilated but the basal bodies (Bb) and striated rootlets (SR) are intact. The basal portions of the inner segments close to the photoreceptor nuclei contain some polysomes and smooth endoplasmic reticulum. Mitochondria (M) appear shorter than they normally are, though they are structurally unchanged otherwise. An almost oval inner segment is seen to the left of the micrograph. It contains several polysomes and a few mitochondria. There are wide intercellular spaces between adjacent inner segments and many Müller cell processes (MP) extend freely through them. Cross sections of cilia (C) are found near the inner segments. Cell junctions between photoreceptors and Müller cells (single arrow) and between Müller cells and Müller cells (double arrows) are observed.

x 21,060

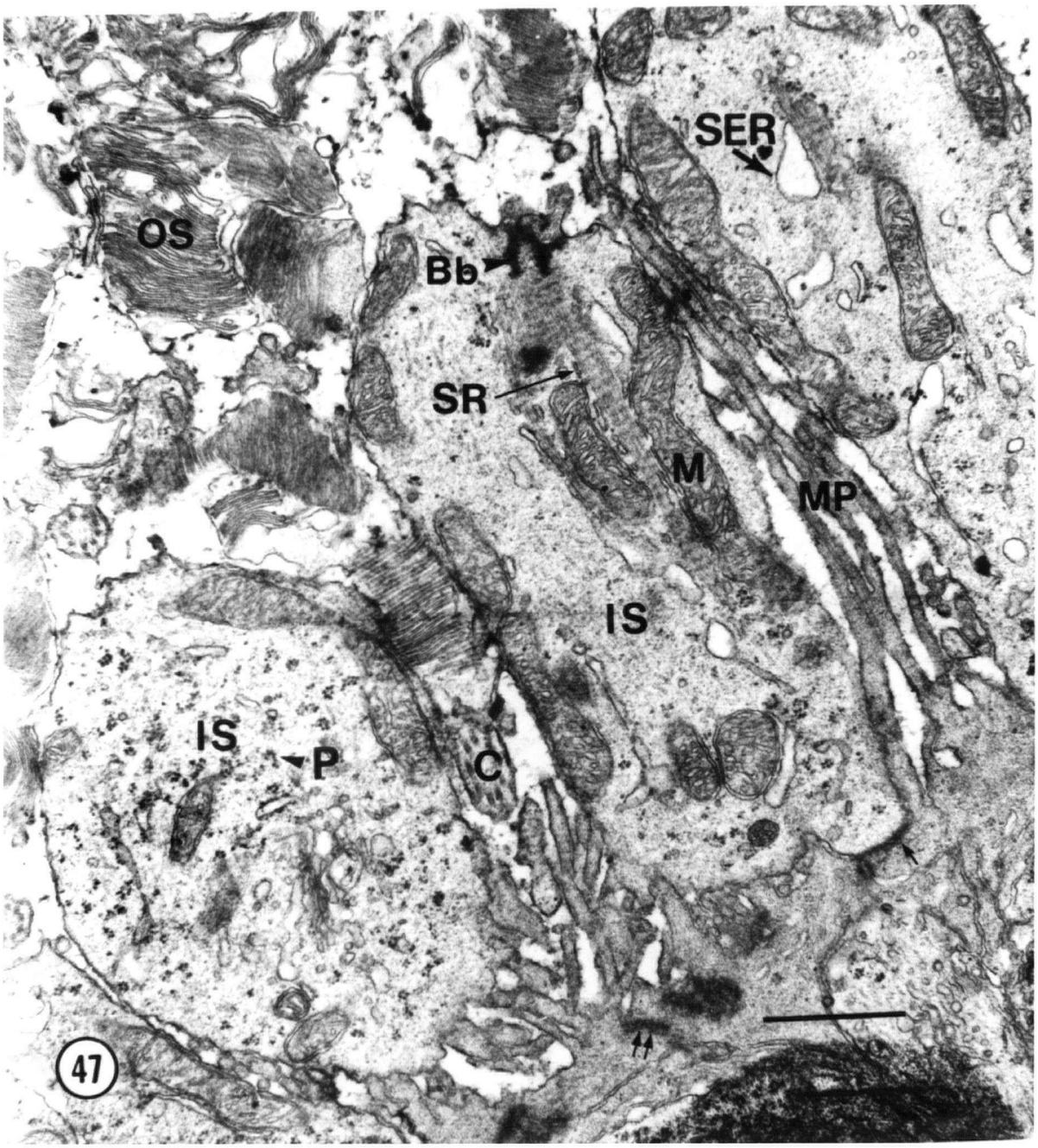

SER
OS
Bb
SR
M
MP
IS
IS
P
C
47

Figure 48.

Electron micrograph showing the outer plexiform layer from the same specimen as Figure 44. The synaptic processes show several signs of degeneration in vitamin A deficiency. In each synaptic process, there are fewer synaptic vesicles (SV) than normal and many are found lying immediately adjacent to the synaptic sites. Plasma membranes between adjacent synaptic processes have broken down and the cell cytoplasm appears to merge freely across the processes (arrows). A mitochondrion (M) with a slight swelling of part of its outer membrane is observed (double arrows). Synaptic ribbons are present at the synaptic sites, and each is surrounded by a cluster of synaptic vesicles. The subjacent horizontal cell processes (H) contain post-synaptic vesicles and appear normal.

x 21,060

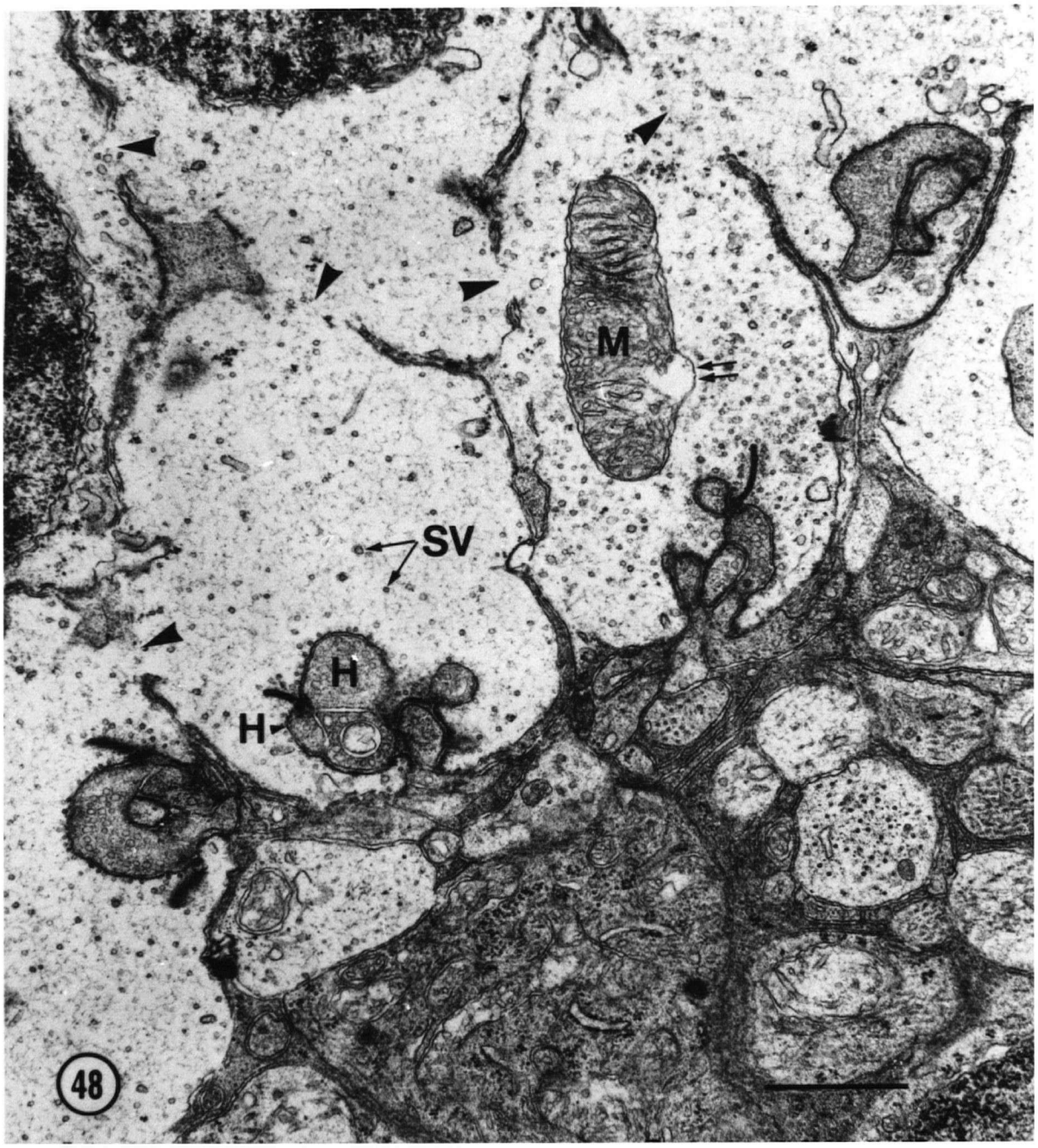

M
SV
H
H
48

Figure 49.

Electron micrograph showing the photoreceptor synaptic processes from the same specimen as Figure 48. Breakdown of the plasma membranes of synaptic processes is in evidence (arrows). Each synaptic process contains sparsely dispersed synaptic vesicles (SV). Mitochondria (M) within the synaptic processes are also affected in vitamin A deficiency and appear markedly swollen. Most of the transverse cristae have disappeared. The synaptic ribbon (Sr) as well as the horizontal cell processes (H) containing closely packed post-synaptic vesicles are essentially unchanged.

x 32,700

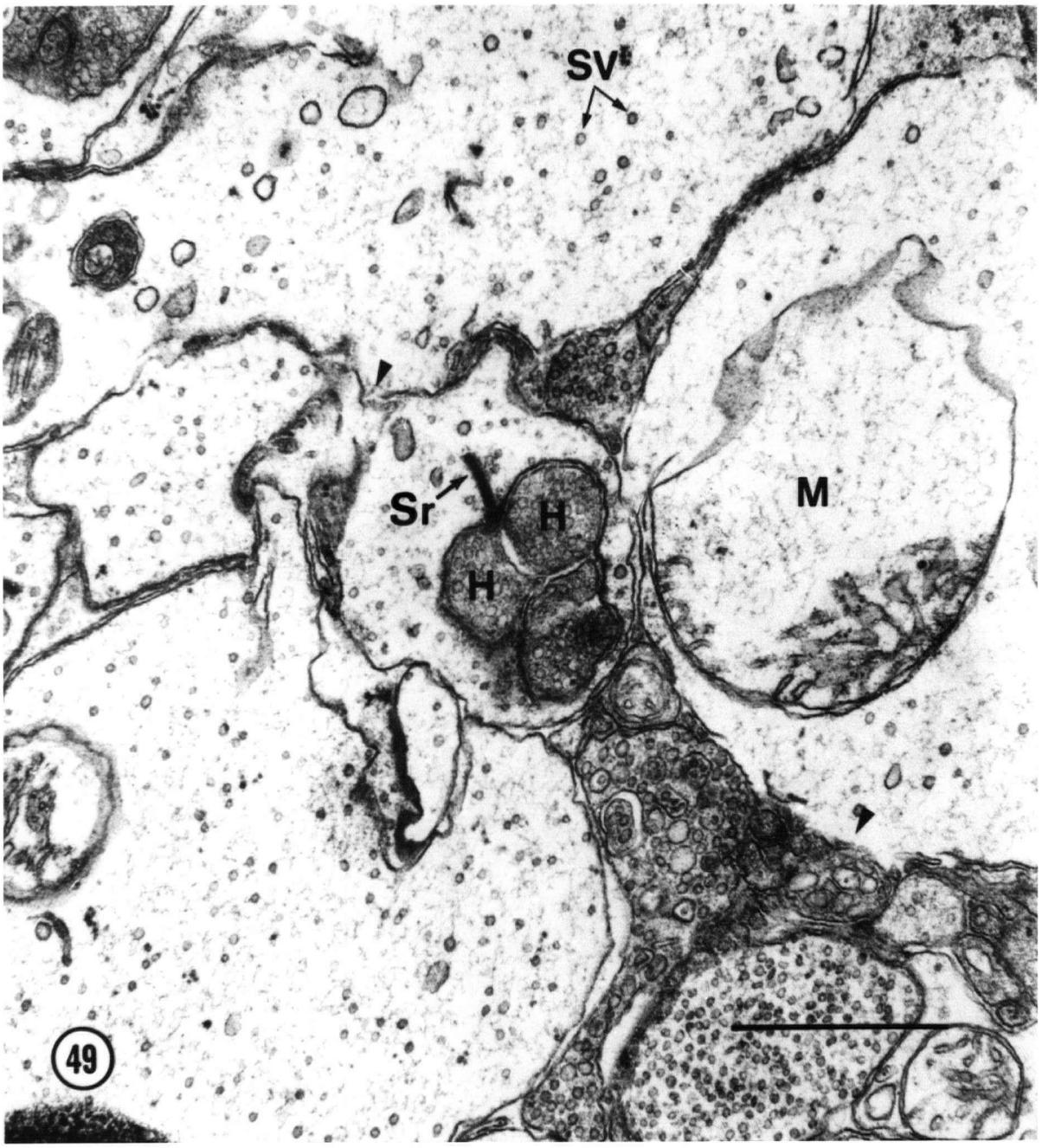

SV
Sr
H
H
M
49

Figure 50.

Electron micrograph showing the retinal epithelium (RE) and photoreceptor outer segments (OS) from a 7 month vitamin A deficient animal. Bruch's membrane (BM) is intact. In the retinal epithelium the basal infoldings (B), mitochondria and other subcellular structures appear unchanged. A remarkable increase of lysosomes (L) is observed deep to the inner surface of the retinal epithelium. There is some proliferation and disorganization of the apical processes (AP) of the epithelium. The lamellar discs of the outer segments have broken down into vesicles (arrows) and groups of discs have disappeared (double arrows).

x 15,700

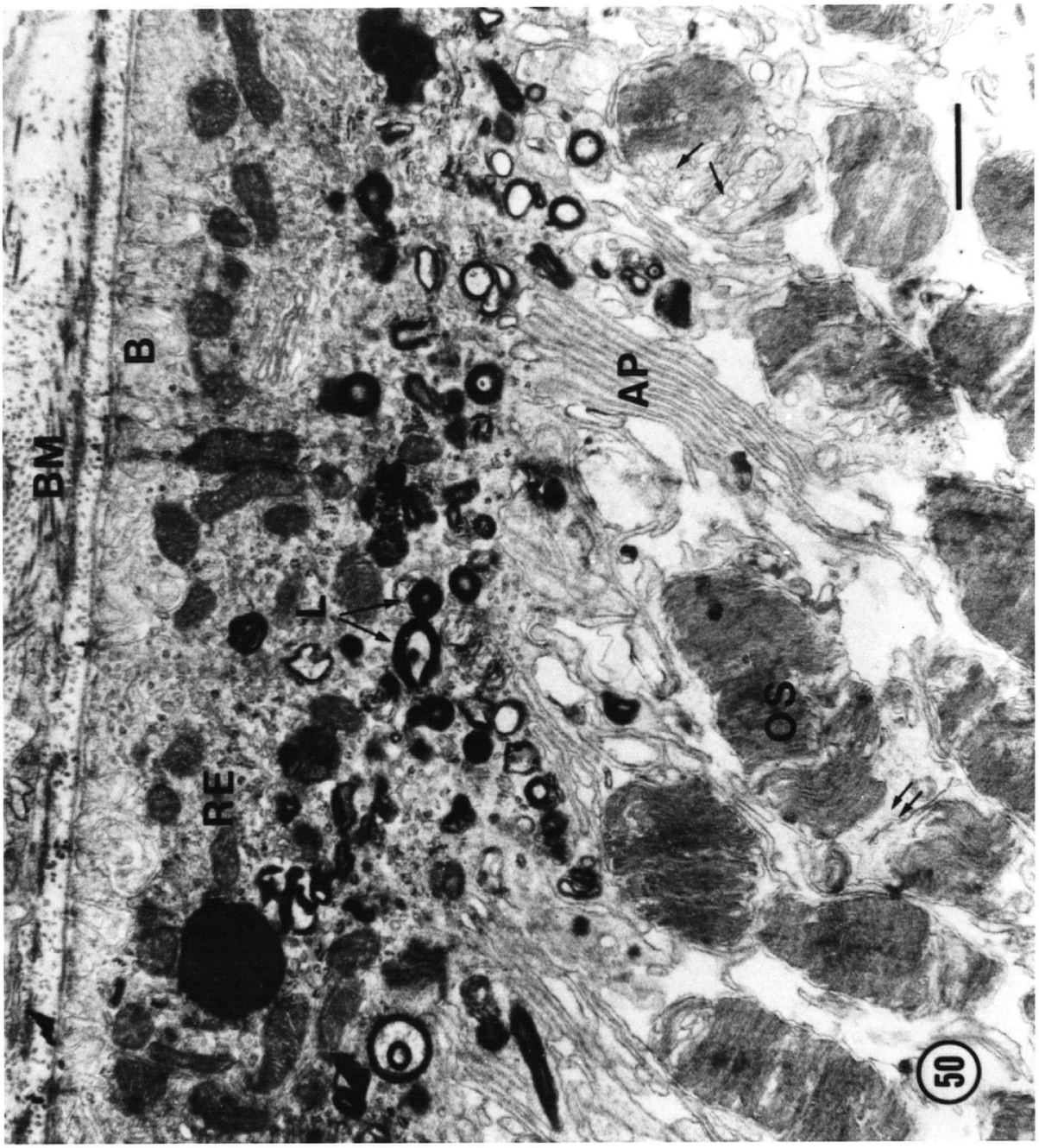

BM
B
RE
L
AP
OS
50

Figure 51.

Electron micrograph showing the retinal epithelium (RE), photoreceptor outer segments (OS) and inner segments (IS) from an 8 month vitamin A deficient animal. The photoreceptor inner segments and the retinal epithelium are now closer together. Degeneration of the outer segments involves loss of discs (x) and abnormal orientation of many of the remaining discs (arrows). The distal portions of the inner segments shown in the micrograph contain few polysomes (P), numerous short mitochondria (M) and a few dilated smooth cisternae (SER). The retinal epithelium is marked by an increase of lysosomes (L) which aggregate mainly beneath the inner epithelial surface and in the wider apical processes (AP). The apical processes of the epithelium are irregularly oriented and appear to have increased in number.

x 13,300

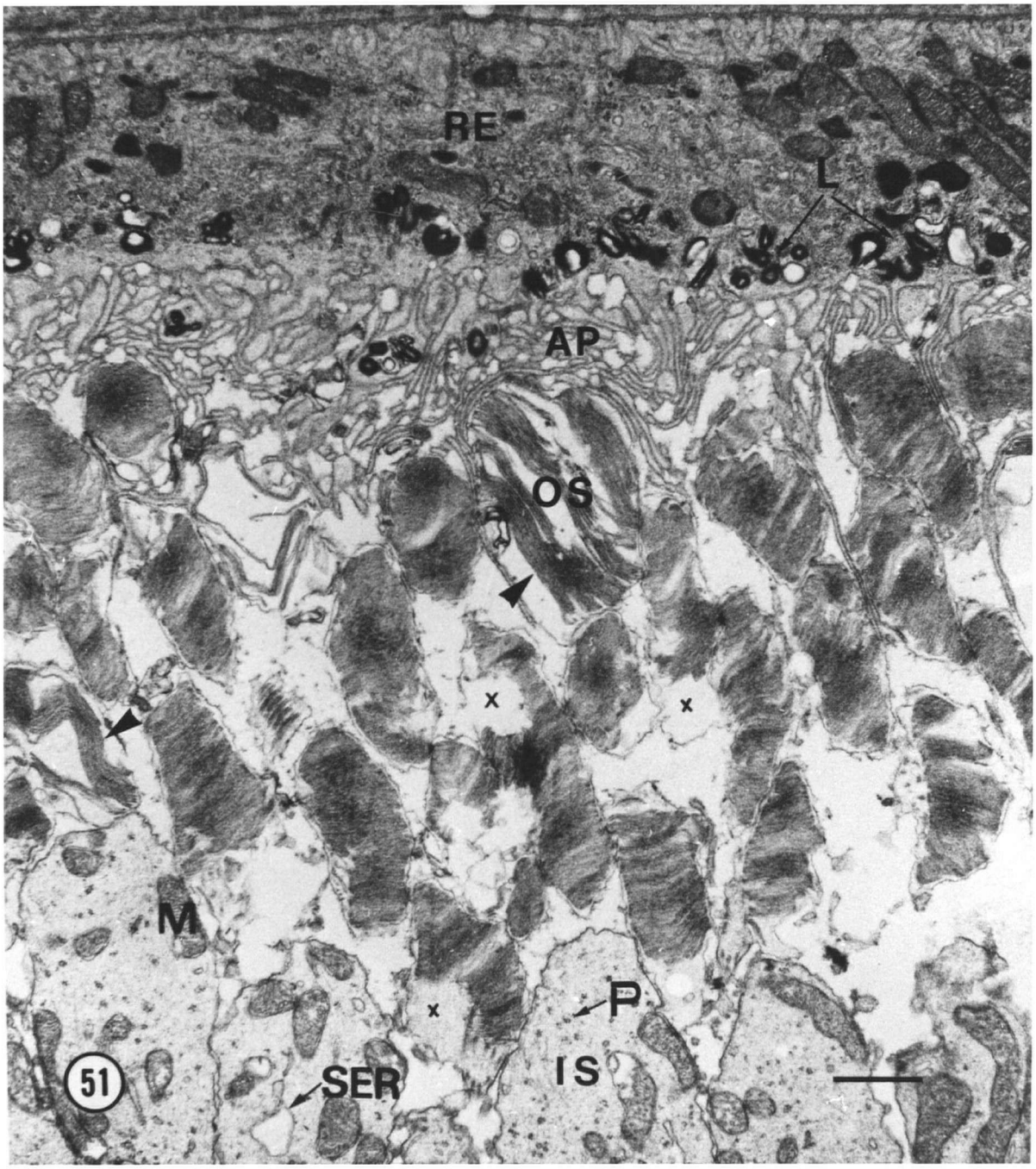

RE
L
AP
OS
M
P
IS
SER
51

Figure 52.

Electron micrograph showing inner segments, photoreceptor outer segments (OS) and part of the retinal epithelium from the same specimen as Figure 51. At the top left hand corner, an outer segment is seen with its lamellar discs in disarray. Some of the lamellar discs have also broken down into vesicles. The few outer segments seen at the lower right are also distorted. In this zone of the retina, the photoreceptor outer segments have disintegrated to such an extent that some inner segments now lie almost adjacent to the retinal epithelium. The large structure in the center of the micrograph is probably a degenerating inner segment (IS) undergoing autolysis. Its cytoplasm is full of lysosomes and aggregates of dense material. This structure is almost in contact with the apical epithelial processes (AP). Part of an epithelial nucleus is seen at the top right hand corner. The apical processes of the retinal epithelium are short but appear to have increased in number. Several lysosomes (L) are found in the thicker processes of the retinal epithelium and in the inner epithelial cytoplasm.

x 21,060

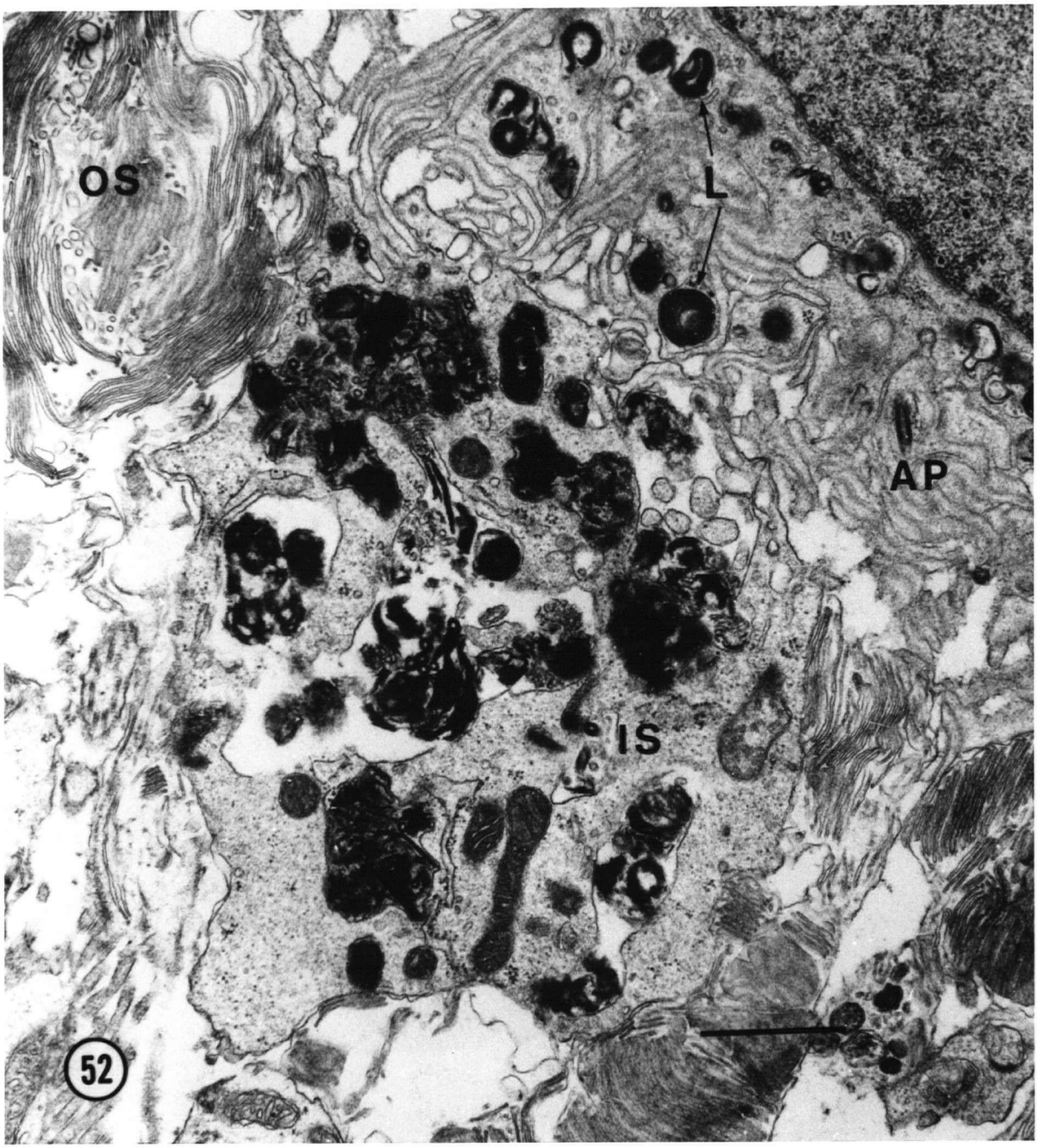

OS
L
AP
IS
52

Figure 53.

Electron micrograph showing photoreceptor inner segments from the same specimen as Figure 51. The segments show different degrees of shortening. A less contracted inner segment (IS), in the center of the micrograph, shows slight swelling of its distal end. The distal portion of the inner segment contains an elongated vacuole (V) but very few polysomes (P). Most of the polysomes are confined to the inner portions of the inner segments shown. Other photoreceptor inner segments have shortened further. A well developed Golgi apparatus (G) is seen in one of the inner segments. Degenerating outer segments are seen above the inner segments. (M, mitochondrion)

x 21,060

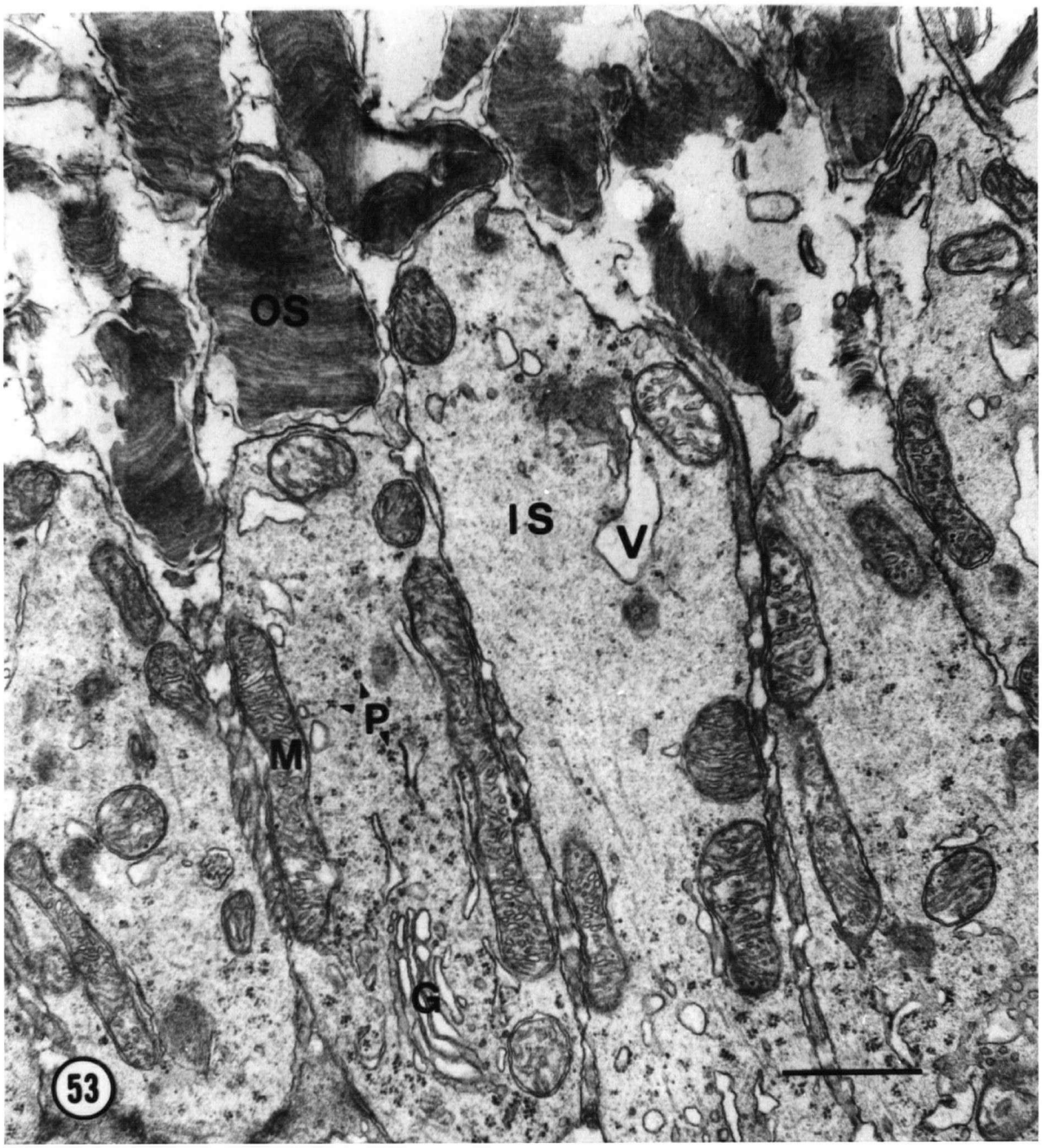

OS
IS
V
P
M
G
53

Figure 54.

Electron micrograph showing the outer plexiform layer from the same specimen as Figure 51. The synaptic processes (SP) are now shorter but a few synapses persist. Each synaptic process contains a few synaptic vesicles (SV), many lying immediately adjacent to the synaptic sites. This is reminiscent of those seen in the specimens from 6 month vitamin A deficient animals. Breakdown of plasma membranes (arrow heads) between adjacent synaptic processes is again observed. At the synaptic site synaptic ribbons (Sr) are still present and processes of horizontal cells (H) containing synaptic vesicles appear intact. Several photoreceptor nuclei (PN) are seen in the upper half of the micrograph and an unidentified cell, possibly a horizontal cell, is visible in the lower half.

x 14,040

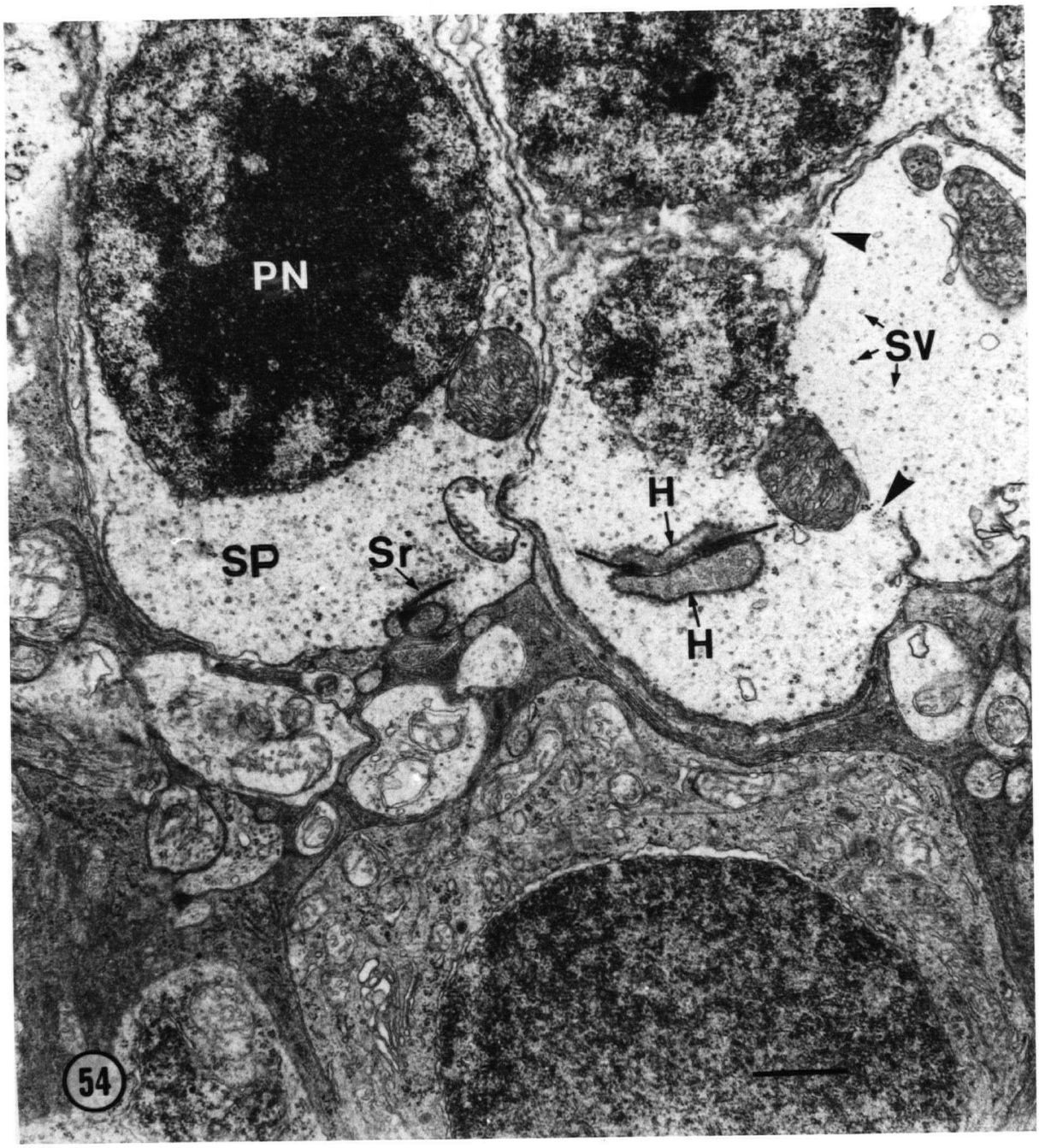

PN
SV
H
SP
Sr
H
54

Figure 55.

Electron micrograph showing the outer plexiform layer from the same specimen as Figure 54. Breakdown of the plasma membranes (arrow) between adjacent synaptic processes and also of the mitochondria (M) within the synaptic processes is evident. The synaptic processes only contain a few dispersed synaptic vesicles. Synaptic ribbons are not observed in this section. An unidentified cell (U) undergoing autolysis is seen. This cell contains lysosomes and a large mass of dense material. Portions of two unidentified cells in the inner nuclear layer are seen at the bottom of the micrograph.

x 16,900

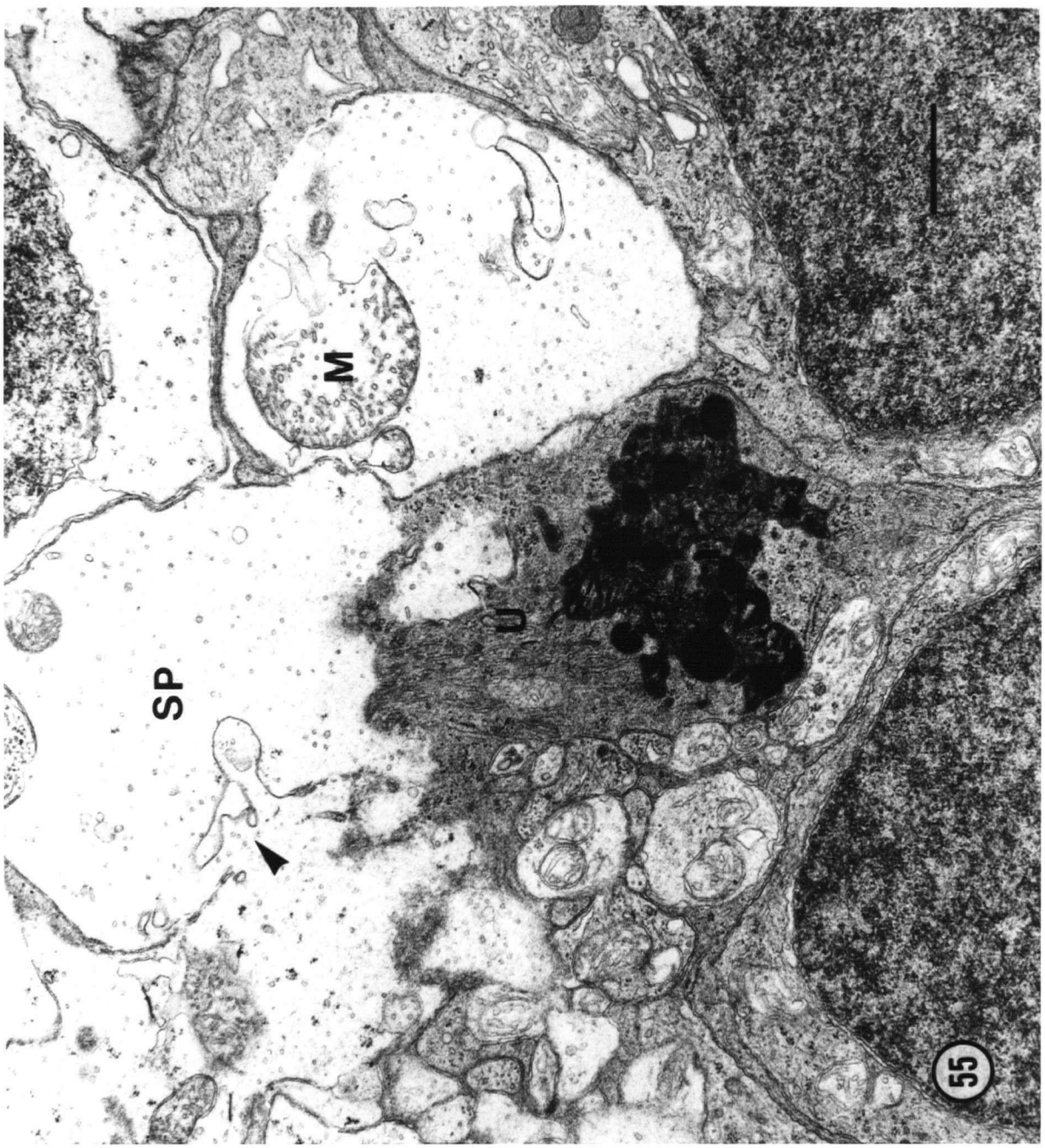

M
SP
55

Figure 56.

Electron micrograph showing the outer retina from a 9 month vitamin A deficient animal. The photoreceptor outer segments have disappeared except for occasional remnants of discs (OS). The micrograph shows two photoreceptors with markedly altered inner segments (IS). Although connecting cilia are not observed, basal bodies (Bb) still persist in the inner segments. Polysomes and short, cylindrical mitochondria are still found in the retracting inner segments. Some mitochondria with partly disintegrating plasma membranes (double arrows) are present in one of the inner segments. The photoreceptor nuclei (PN) that remain do not show any variation from the normal in their chromatin distribution. The outer limiting membrane is now formed mainly by cell junctions between Müller cells (arrows). In the retinal epithelium (RE), lysosomes (L) aggregate close to its inner surface. Proliferation of the apical epithelial process (AP) is prominent. The basal infoldings (B) and other subcellular structures in the retinal epithelium appear unchanged. (MP. Müller cell processes)

x 12,090

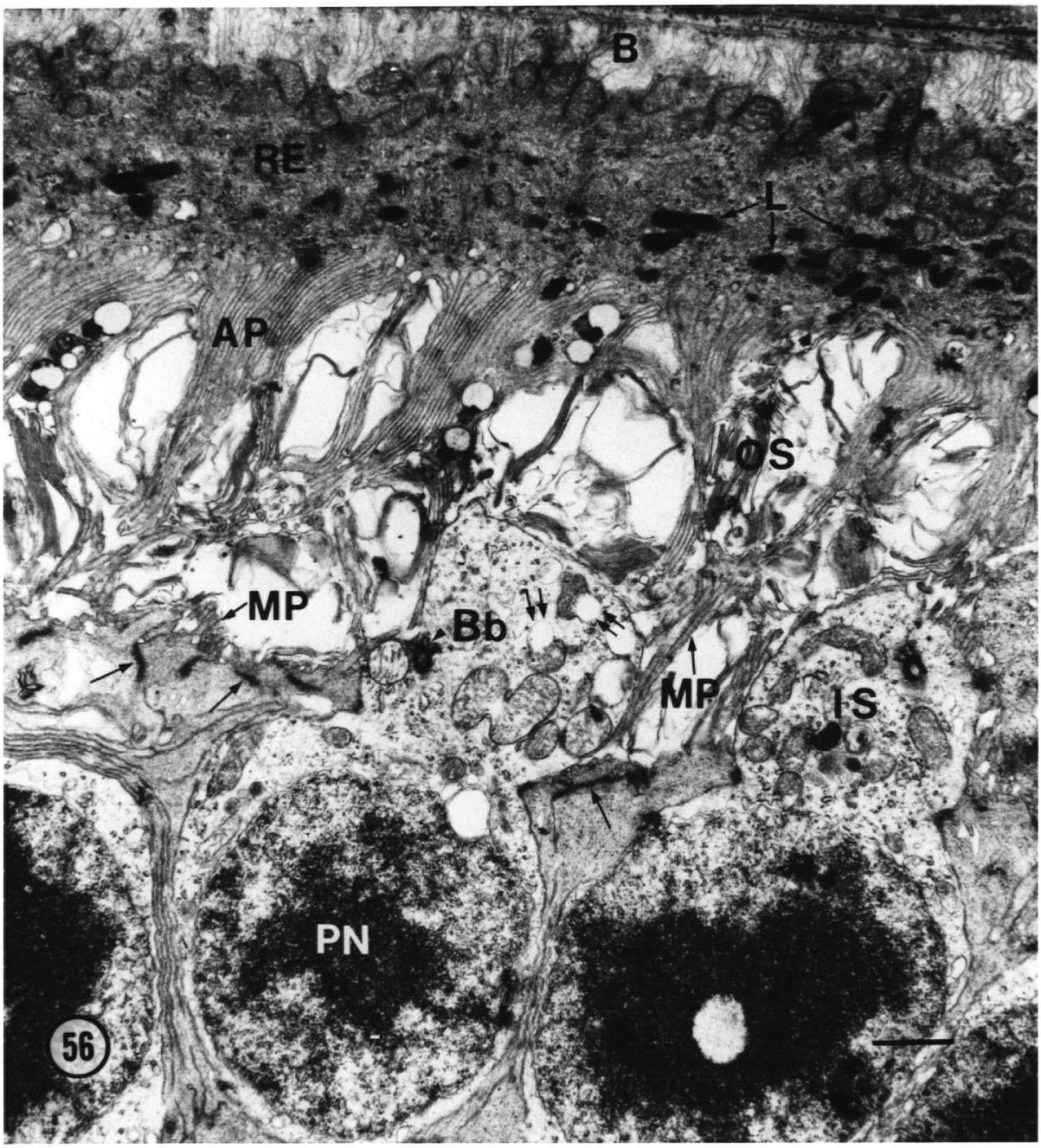

B
RE
L
AP
OS
MP
Bb
MP
IS
PN
56

Figure 57.

Electron micrograph showing the retinal epithelium (RE), remnants of photoreceptor outer segments (OS) and protions of photoreceptor inner segments from a 9 month vitamin A deficient animal. A loosely arranged lamellar structure representing a degenerating and distorted outer segment (OS) is seen to the right of the micrograph. Another disintegrating outer segment which consists only of an aggregation of tubules and vesicles is seen to the left surrounded by epithelial apical processes (AP). The photoreceptor inner segments (IS) have retracted further and Müller cell processes (MP) are now seen extending above them. The apical processes of the epithelium have proliferated markedly. Many lysosomes (L) are still found beneath the inner surface of the retinal epithelium. (N, retinal epithelial nucleus)

x 18,650

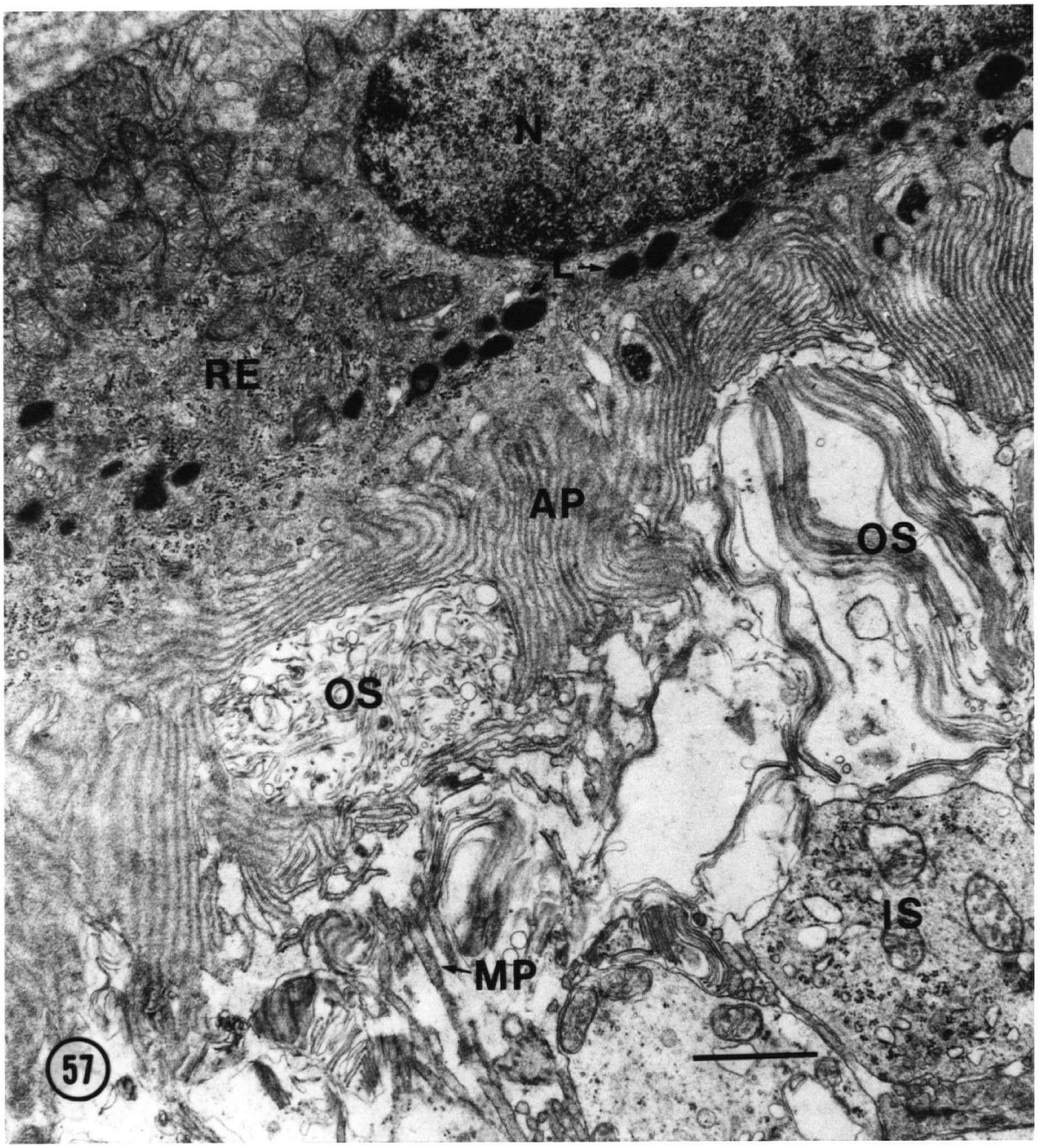

N
L
RE
AP
OS
OS
IS
MP
57

Figure 58.

Electron micrograph showing the outer retina from a 9 month vitamin A deficient animal. Sporadic clusters of saccules (arrows) are all that remains of the photoreceptor outer segments. The photoreceptor inner segments (IS) are closer to the retinal epithelium than before. Some inner segments have retracted more than others but all have undergone severe degeneration. In the distal or scleral halves of the inner segments, only a few polysomes remain and vacuoles (V) are present. The remaining mitochondria observed in each inner segment are mostly round and short. Numerous polysomes persist in the basal halves of the inner segments. Photoreceptor nuclei are seen at the bottom of the micrograph. Their nuclear chromatin appears normal. Junctions between Müller cells now comprise almost all of the outer limiting membrane (double arrows). As photoreceptor outer and inner segments are discarded, Müller cell processes (MP) move in to occupy the tissue gap. Lysosomes (L) still aggregate close to the surface of the retinal epithelium. The apical epithelial processes (AP) have markedly proliferated and now lie adjacent to the inner segments in some regions.

x 14,040

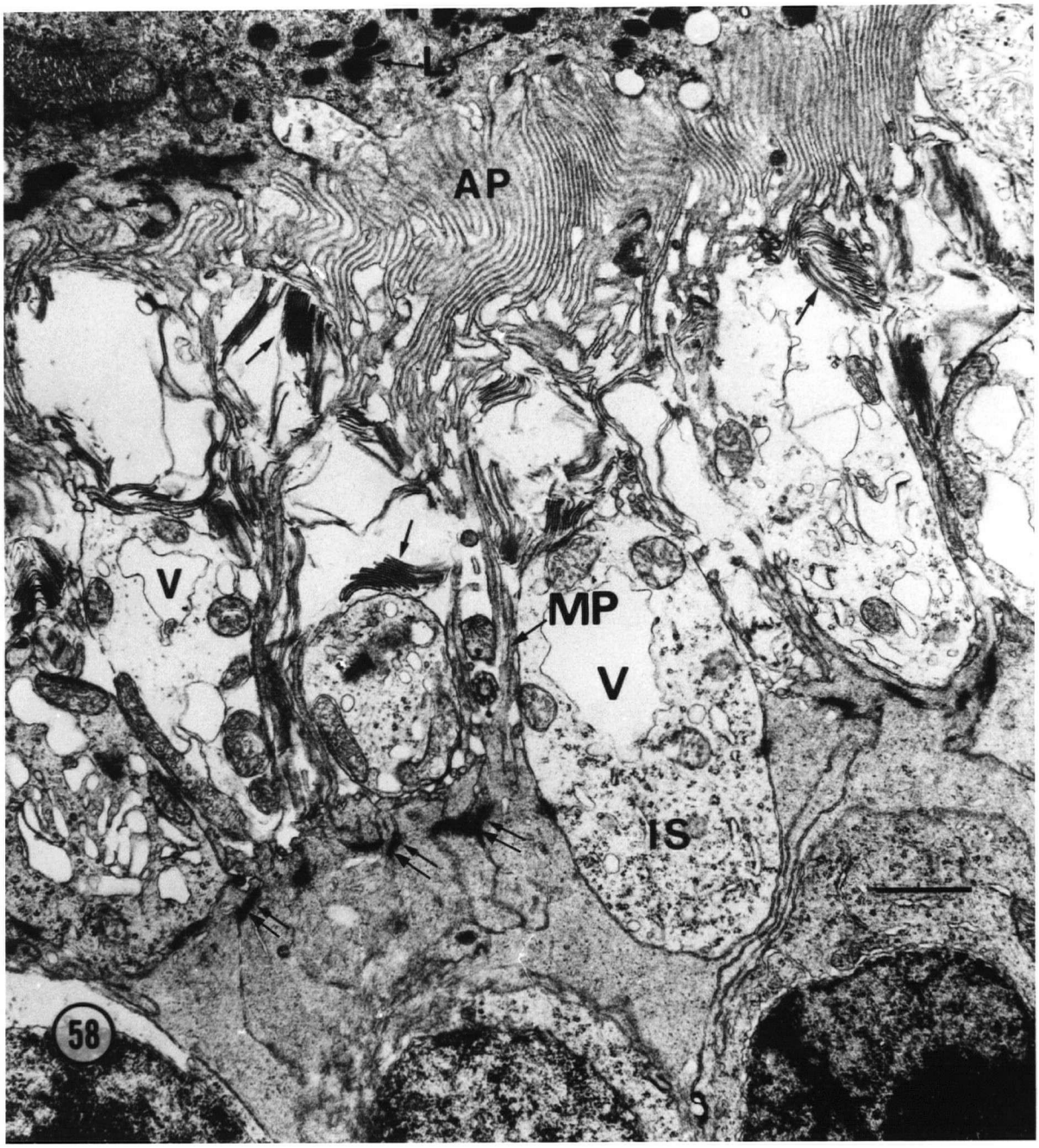

AP
V
MP
V
IS
58

Figure 59.

Electron micrograph showing at higher magnification, photoreceptor inner segments and the outer limiting membrane from the same specimen as Figure 58. In the retracting inner segments (IS) shown, subcellular structures such as the Golgi apparatus and rough endoplasmic reticulum have disappeared. A few polysomes (P), some mitochondria (M) and smooth endoplasmic reticulum dilated to form vacuoles (V) are left. A mitochondrion in one of the inner segments shows detachment and elongation of part of its membrane (double arrows). An almost ovoid inner segment containing an intact basal body (Bb) is seen in the center of the micrograph. Clusters of saccules which are remnants of the outer segments (OS) are seen above the inner segments. Some apical epithelial processes (AP) are visible at the top right hand corner of the micrograph. The outer limiting membrane is now formed mainly by cell junctions between Müller cells (arrows). Several Müller cell processes (MP) extend through the extracellular space between the inner segments. A cilium (C) in cross-section is visible lying freely among the Müller cell processes.

x 29,720

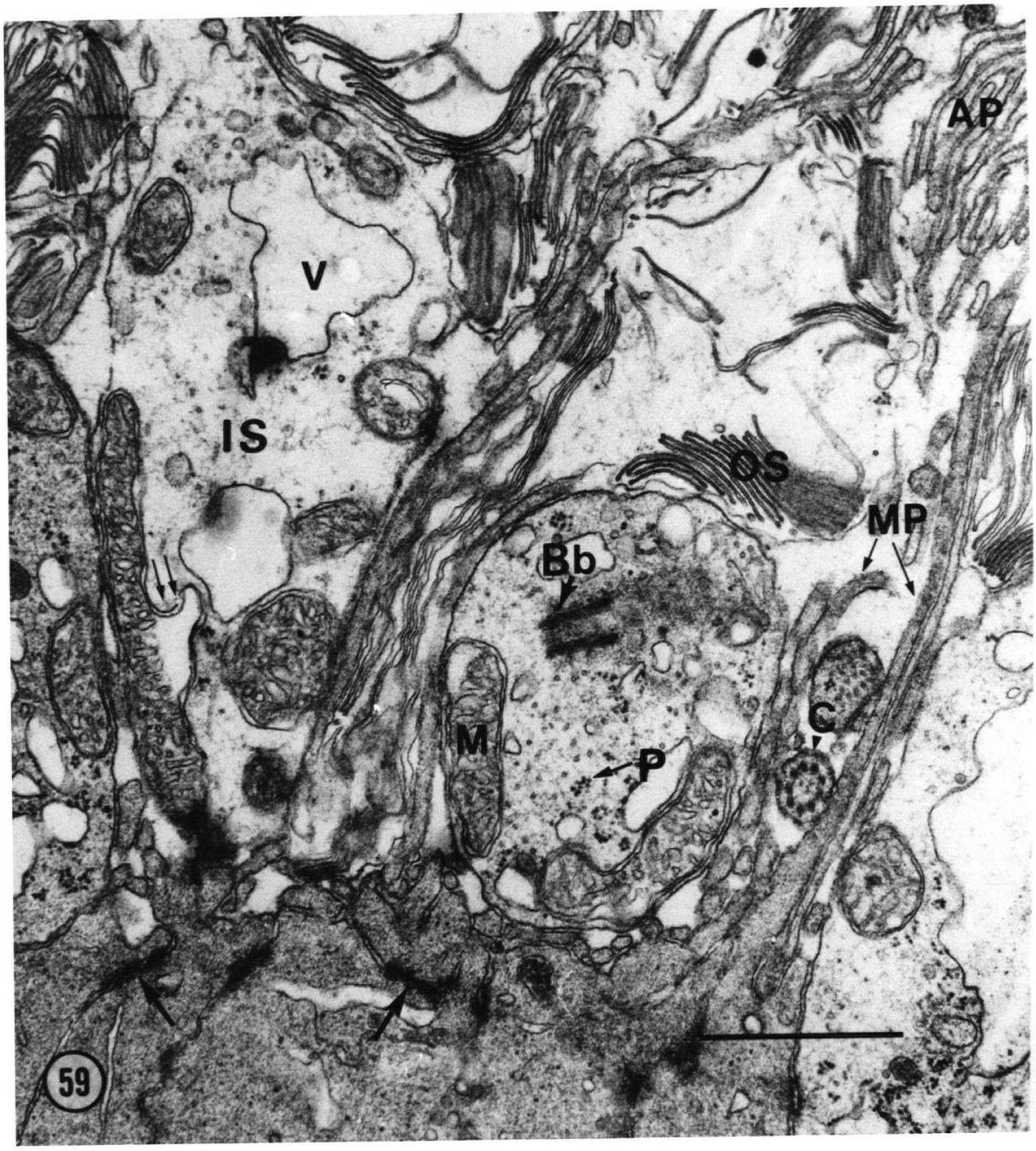

AP
V
IS
OS
MP
Bb
M
P
C
59

Figure 60.

Electron micrograph showing degenerating photoreceptors from a 9 month vitamin A deficient animal. The photoreceptors show marked retraction of both inner segments (IS) and synaptic processes (SP). In the inner segments, the mitochondria have entirely degenerated and disappeared. Some polysomes (P) and rough endoplasmic reticulum (RER) are still present. The chromatin of the photoreceptor nuclei (PN) appears normal but the nuclear membranes show evidence of breakdown (thick arrows). There are few synaptic vesicles (SV) left in the degenerating synaptic process shown in the micrograph. The synaptic ribbon (Sr) surrounded by some synaptic vesicles still persists.

x 18,650

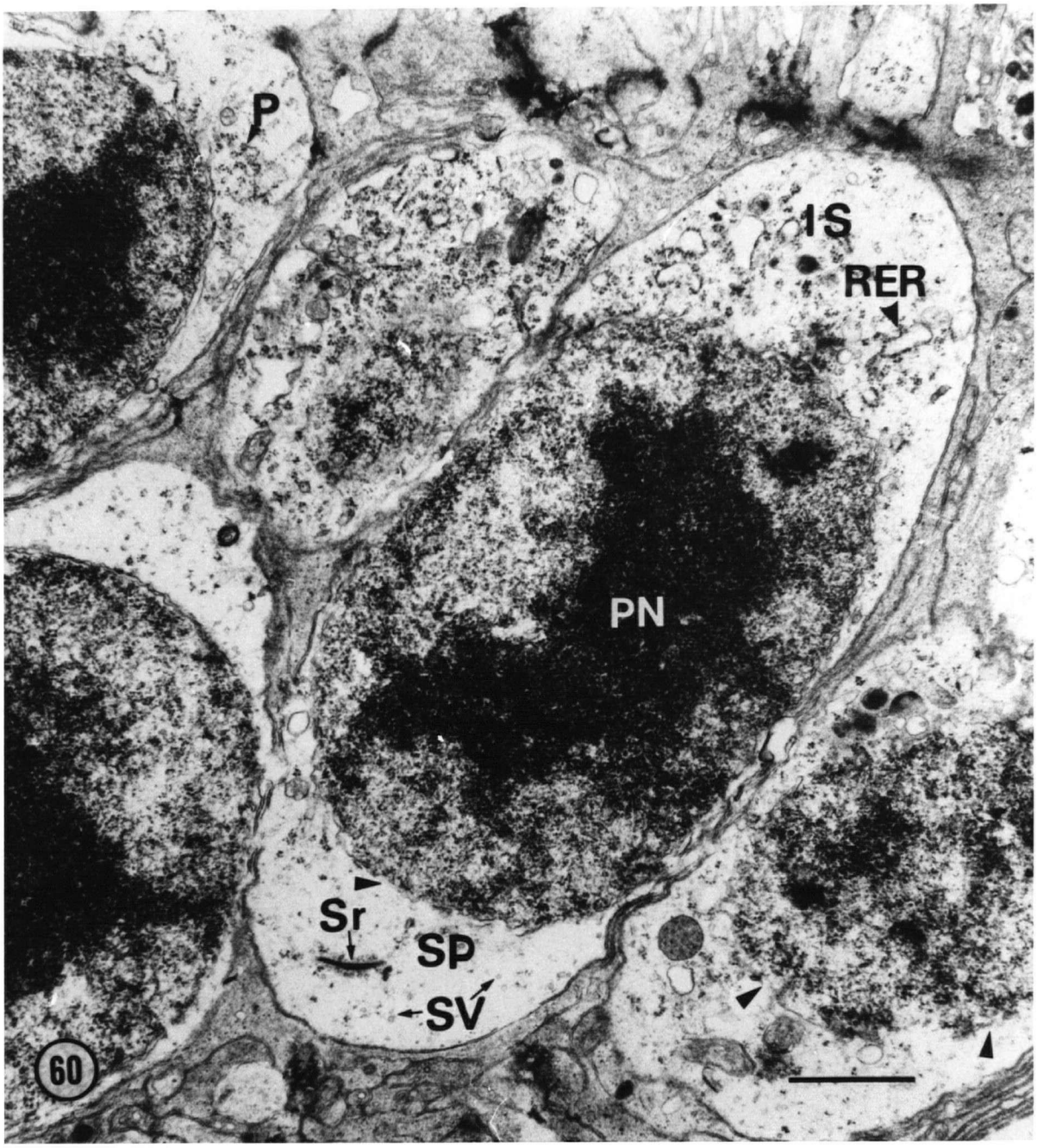

P
1S
RER
PN
Sr
SP
SV
60

Figure 61.

Electron micrograph showing the posterior outer retina from a 10 month vitamin A deficient animal. There is a closer association between the retinal epithelium and the neural retina than observed at earlier intervals. Two adjacent retinal epithelial cells (RE) are shown. The retinal epithelium contains a large number of lysosomes close to the inner epithelial surface and in some of the broad apical processes. The apical processes (AP) are numerous and now regularly oriented. The epithelial nucleus (N) and other subcellular structures in the retinal epithelium are essentially unchanged. Remnants of photoreceptor outer (OS) and inner segments (IS) are scattered among the apical epithelial processes. Müller cell junctions (arrows) are now widely spaced and mark the outer limit of the neural retina. A displaced photoreceptor cell is seen at the upper right of the micrograph (double arrows). The photoreceptor cells (PC) that remain have very little cytoplasm containing only a few polysomes and an occasional mitochondrion. Each of the remaining photoreceptor cells is surrounded by several layers of membranes (Gm) probably of glial origin.

x 12,090

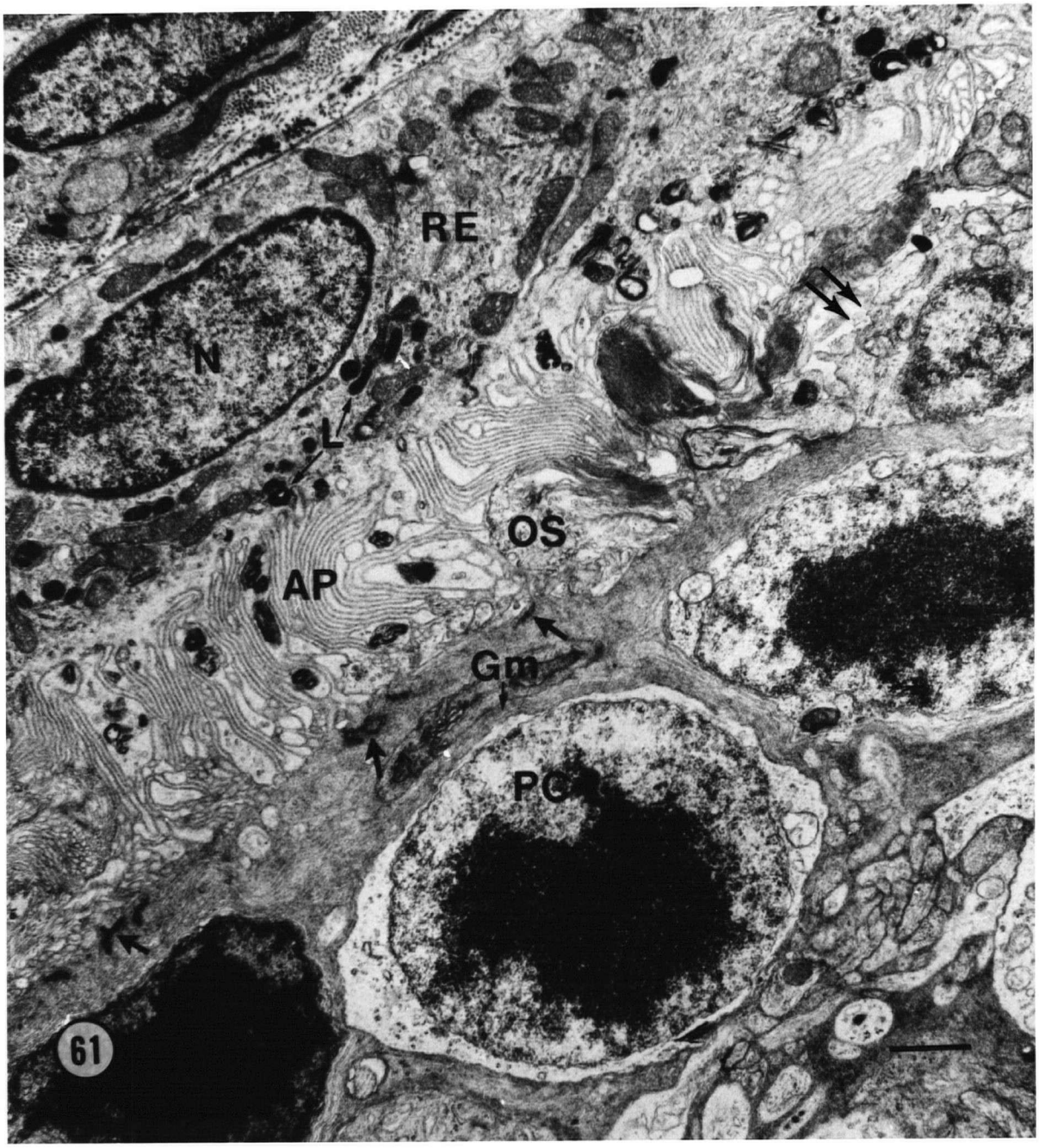

RE
N
L
OS
AP
Gm
PC
61

Figure 62.

Electron micrograph showing the close association between the retinal epithelium (RE) and the neural retinal layer at the 10th month of vitamin A deficiency. The retinal epithelium contains numerous lysosomes (L) mainly aggregated close to its inner surface. Lysosomes are also present in more central regions of the epithelial cells. An oval structure consisting of saccules, tubules and vesicles, the remnants of a photoreceptor outer segment (OS), is seen partially surrounded by apical processes (AP) of the retinal epithelium. The outer limiting membrane now appears to be formed by cell junctions between processes of Müller cells (arrows) although the presence of cell processes from other types of glial cells cannot be excluded. Bruch's membrane (BM) overlying the retinal epithelium appears unchanged.

x 18,650

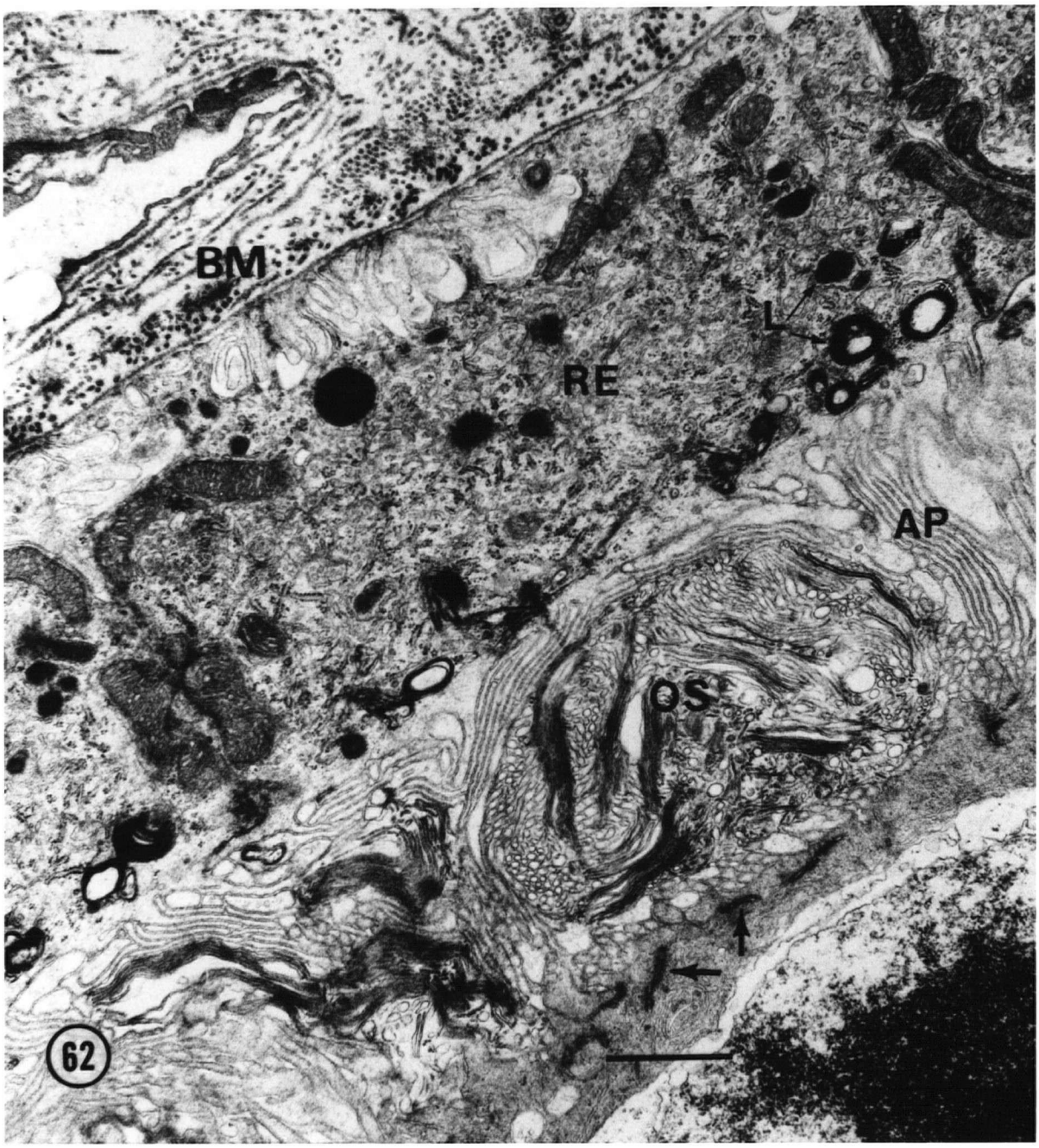

BM
L
RE
AP
OS
62

Figure 63.

Electron micrograph showing at higher magnification the close association between the apical processes (AP) of the retinal epithelium (RE) and the processes of the Müller cells (MP) from the same specimen as Figure 61. Some apical process of the epithelium are displaced sideways by the processes of the Müller cells and others interdigitate with the latter. The outer limiting membrane is well defined in this region (arrows). A disintegrating photoreceptor outer segment (OS) with part of the inner segment (IS) is seen at the lower left of the micrograph. A pair of centrioles (c) is visible in the cytoplasm of a Müller cell. Parts of two retinal epithelial cells with several lysosomes are seen at the top left hand corner. Apical cell junctions (ACJ) of the retinal epithelium are still intact. A portion of a photoreceptor cell remnant is present at the lower right.

x 25,730

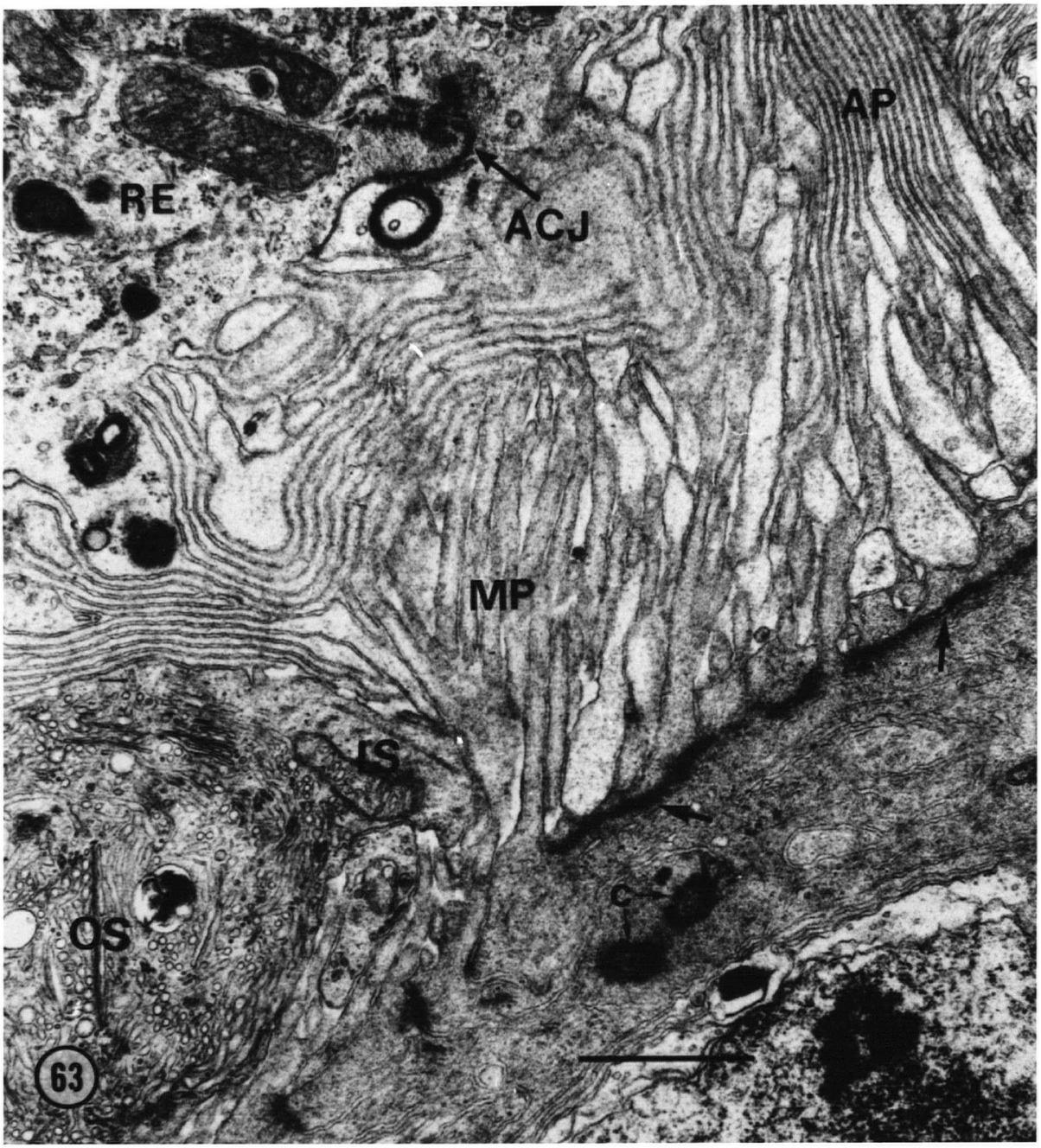

AP
RE
ACJ
MP
IS
OS
63

Figure 64.

Electron micrograph showing the outer retina from the same specimen as Figure 61. In the retinal epithelium (RE) some lysosomes (L) are found in the vicinity of the Golgi apparatus (G) but most of them are close to the inner surface of the retinal epithelium. The apical epithelial processes (AP) are numerous and prominent. A photoreceptor cell (PC), possibly a cone, is seen in the neural retina. The photoreceptor nucleus appears intact while a few degenerating mitochondria (M), some polysomes and an ill-defined Golgi apparatus (G) are discernable in its cytoplasm. A couple of photoreceptor inner segments (IS) are present. One of them has an intact connecting cilium (C) with a basal body (Bb). The cytoplasm of this cell contains numerous polysomes and a couple of mitochondria. Remnants of a photoreceptor outer segment (OS) can also be distinguished lying next to the inner surface of the retinal epithelium.

x 21,060

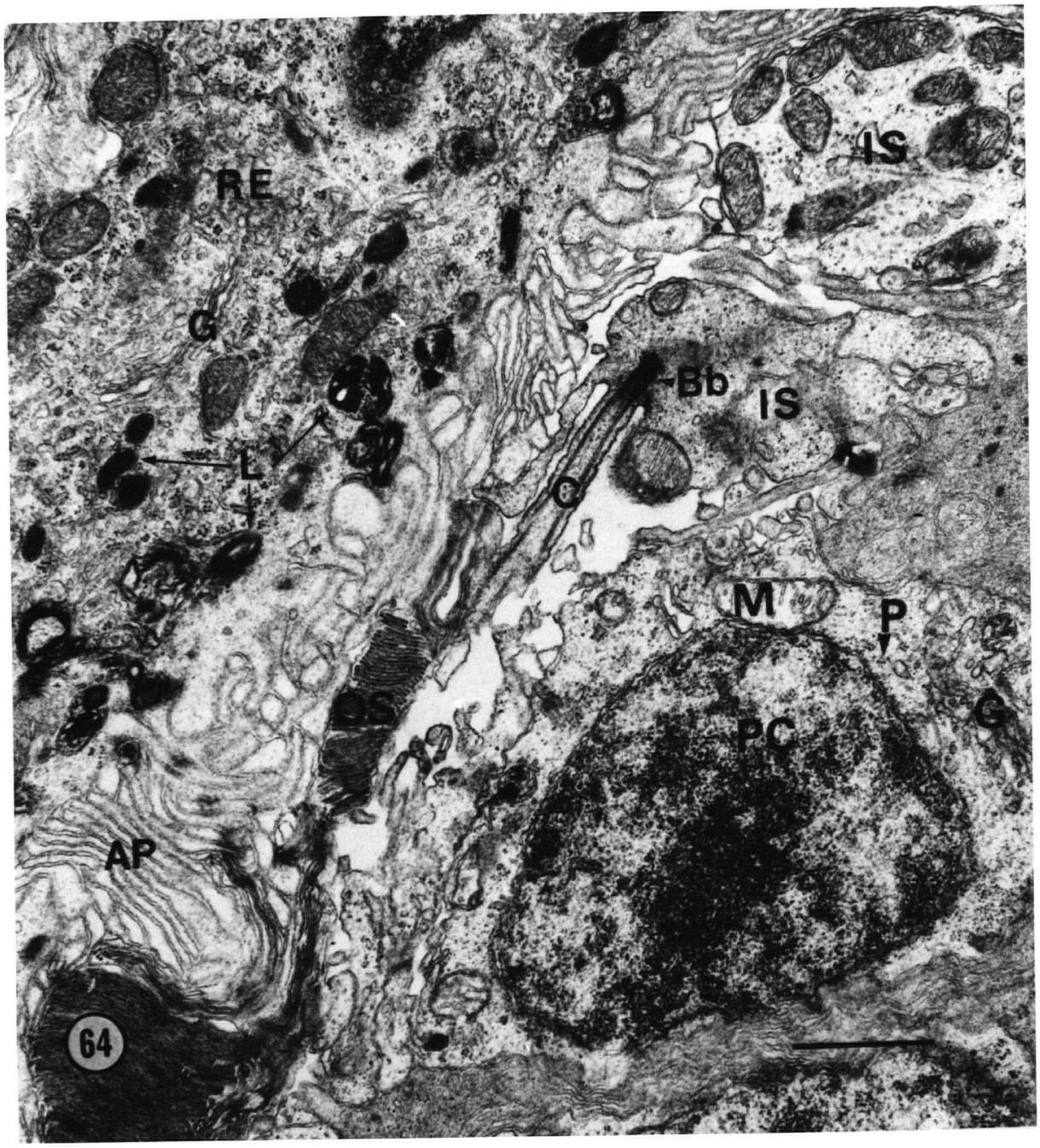

RE
G
L
IS
Bb
IS
M
P
PC
G
AP
64

Figure 65.

Electron micrograph showing the outer retina from an 11 month vitamin A deficient animal. As usual, the inner cytoplasm of the retinal epithelium (RE) is marked by the presence of a large number of lysosomes (L) while the epithelial nucleus (N) and the other subcellular structures in the epithelium appear normal. The apical processes (AP) of the epithelium remain numerous and prominent. Remnants of some photoreceptor inner segments (IS) and outer segments (OS) can be seen among the apical processes of the retinal epithelium. One of the inner segments lies inside the outer limiting membrane and is surrounded by glial membranes (Gm). Two unidentified cells each with scanty cytoplasm are seen in the center of the micrograph. They are each surrounded by several layers of membranes.

x 12,090

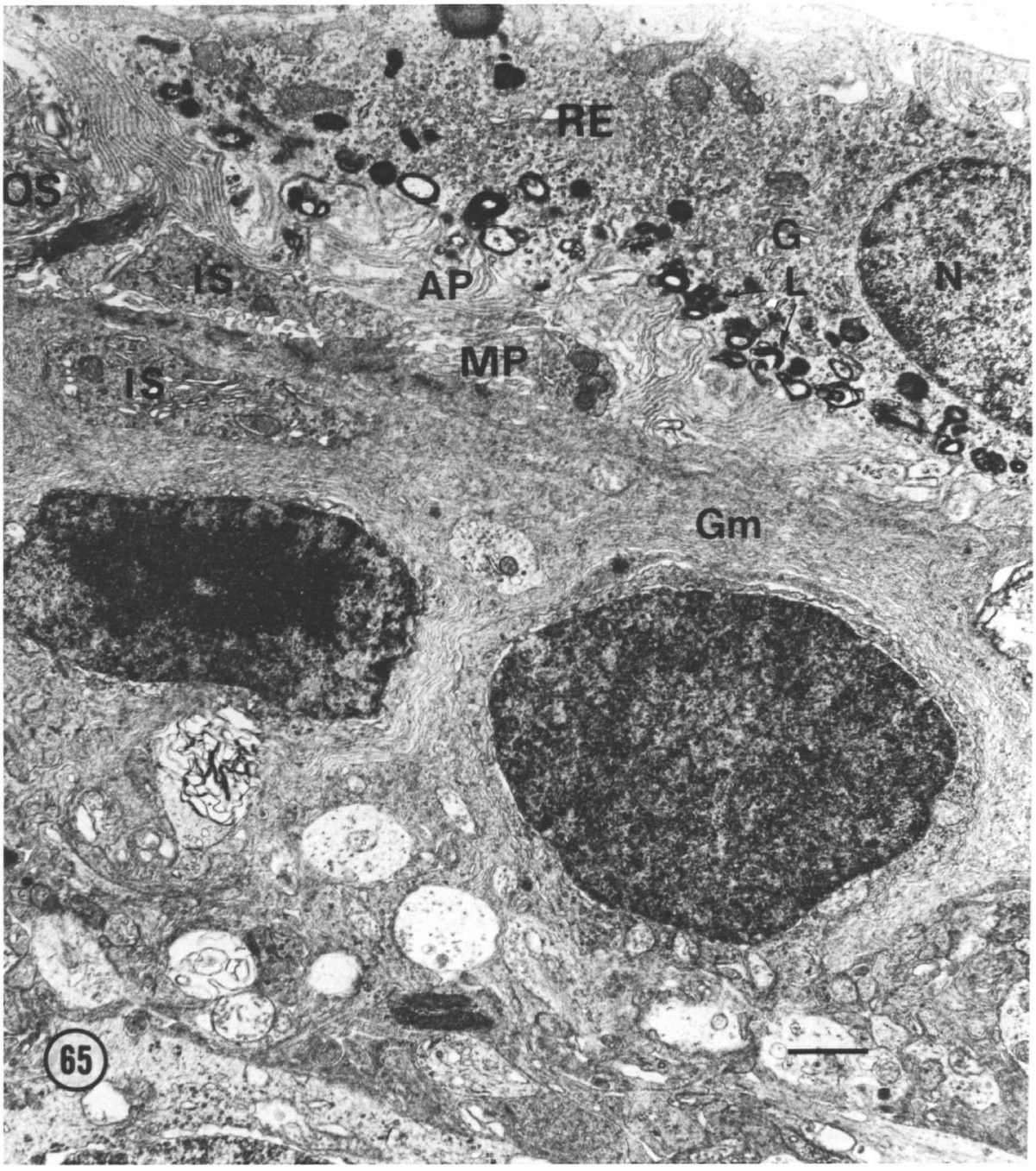

RE
OS
IS
AP
G
L
N
MP
IS
Gm
65

Figure 66.

Electron micrograph showing the retinal epithelium and the outer retina from the same specimen as Figure 65. The proliferation of lysosomes (L) close to the inner surface of the retinal epithelium is striking. At one region, the retinal epithelium lies immediately adjacent to the neural retina. At that site, the apical epithelial processes have disappeared (arrows) but on both sides of this region, apical epithelial processes (AP) remain prominent. A retinal capillary (CP) lies close to the retinal epithelium. Note the layers of glial membranes (Gm) above the capillary.

x 13,700

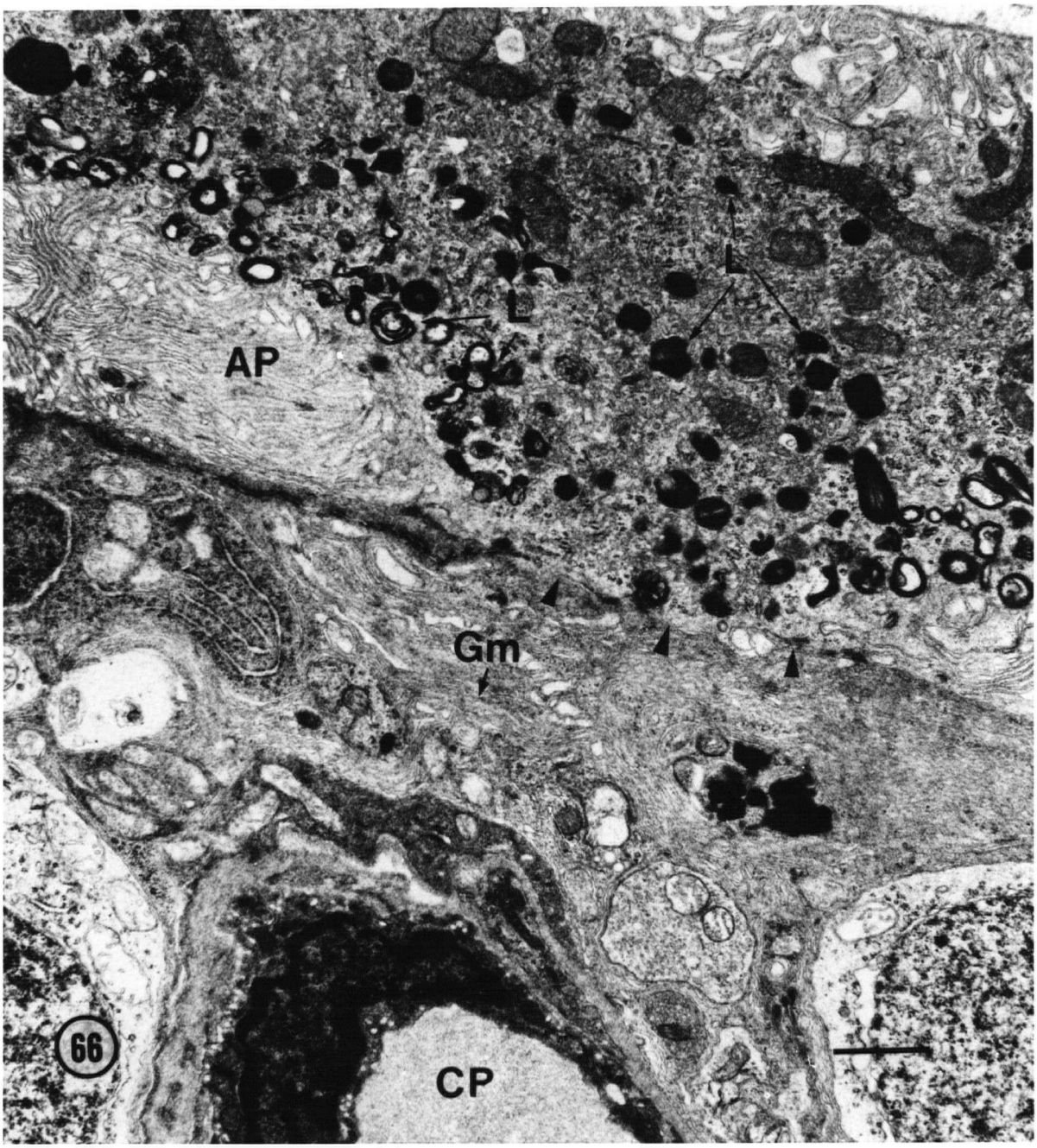

AP
L
L
Gm
66
CP

Figure 67.

Electron micrograph showing the outer retina from an 11 month vitamin A deficient animal. Lysosomes (L) lying close to the inner surface of the retinal epithelium and numerous, apical processes (AP) of the epithelium are again noted. Remnants of photoreceptor outer segments (OS) can still be seen above the neural retina. In this micrograph, what appear to be Müller cell processes (MP) bend laterally and contribute to the array of glial membranes (Gm) visible above and to the sides of the photoreceptor cell (PC) seen at the lower left corner. The scanty cytoplasm of the photoreceptor contains little or no recognizable subcellular structures. Cell junctions between Müller cells can be distinquished (arrow).

x 18,650

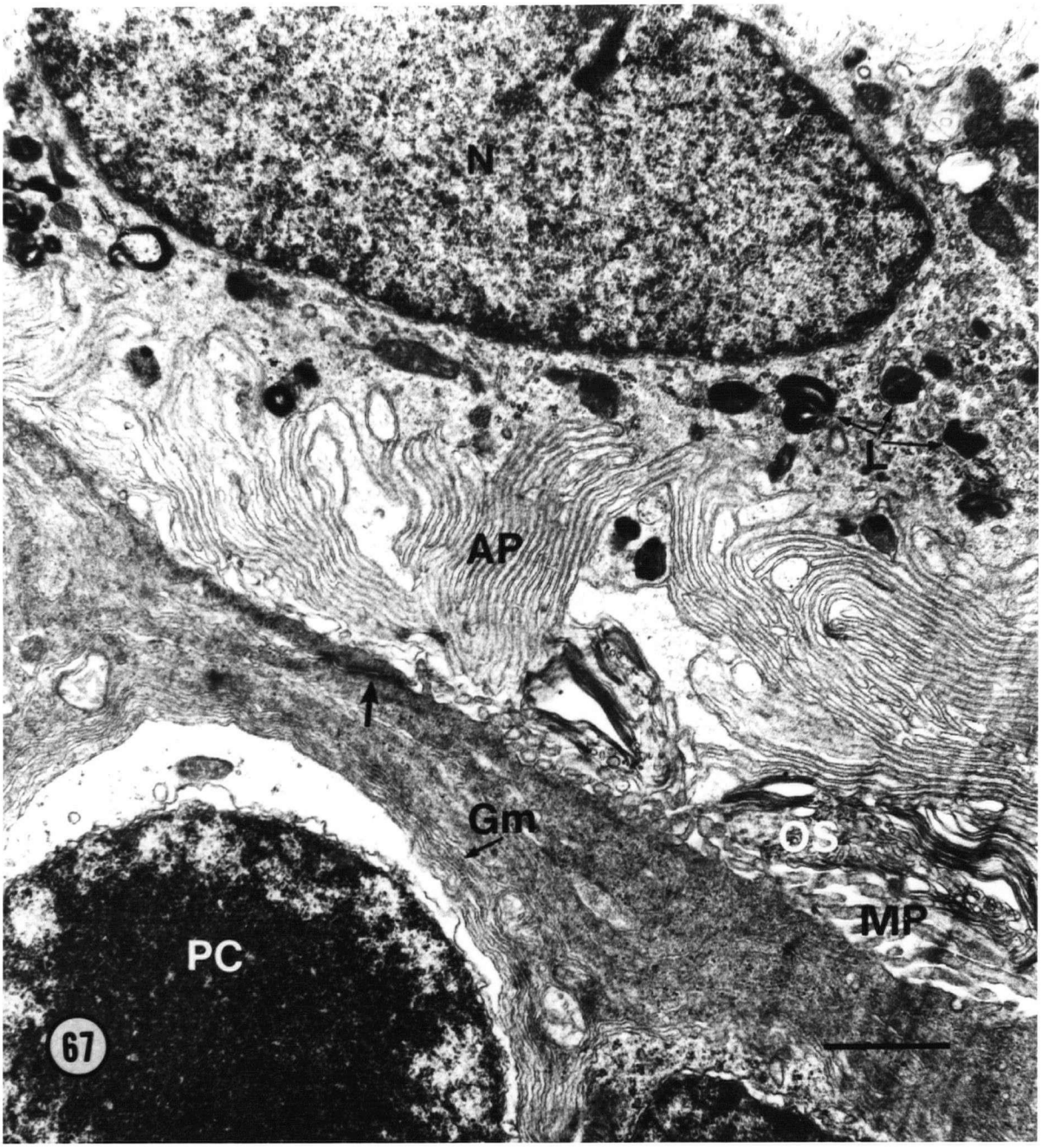

N
AP
Gm
PC
MP
67

Figure 68.

Electron micrograph showing the outer retina from an 11 month vitamin A deficient animal. In the retinal epithelium a well developed Golgi apparatus (G), numerous lysosomes (L) and prominent apical processes (AP) are visible. Fragments of photoreceptor inner segments (IS) lie scattered among Müller cell processes (MP). The latter, a majority of which appear in cross-section, are seen in abundance in this region. In the neural retina, areas not occupied by photoreceptors or unidentified cells are replaced by numerous layers of glial membranes (Gm).

x 18,650

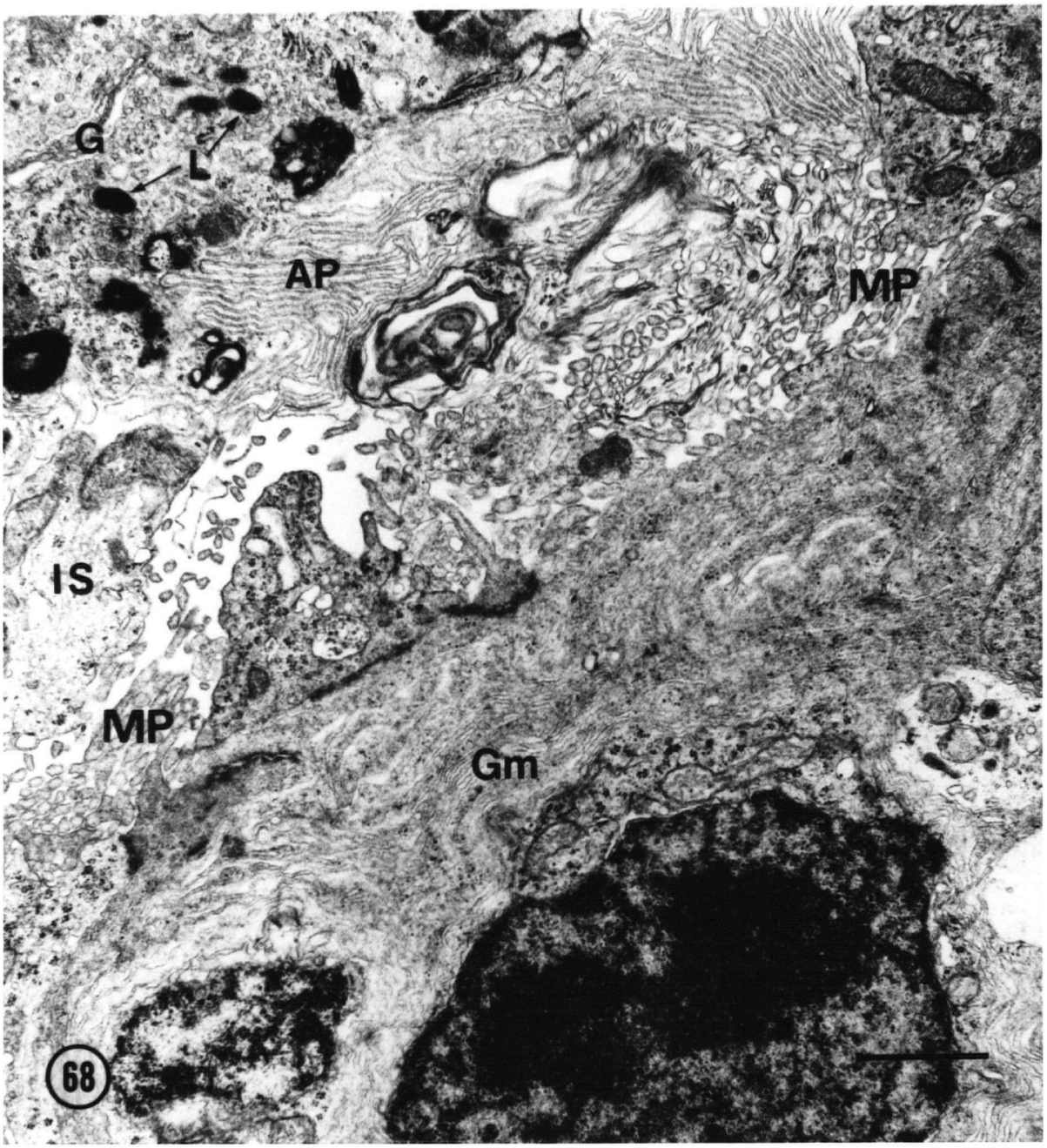

G
L
AP
MP
IS
MP
Gm
68

Figure 69.

Electron micrograph from the same specimen as Figure 68 showing prominent apical processes (AP) of the retinal epithelium and layers of membranes (Gm) probably of a glial nature surrounding each of the remaining photoreceptor cells (PC). Numerous well marked cell junctions probably between Müller cells (MJ) are seen forming a limiting membrane at the outer edge of the neural retina. Above the neural retina, many Müller cell processes (MP), most of them cut in cross-section, are present and show a tendency to bend sideways.

x 23,000

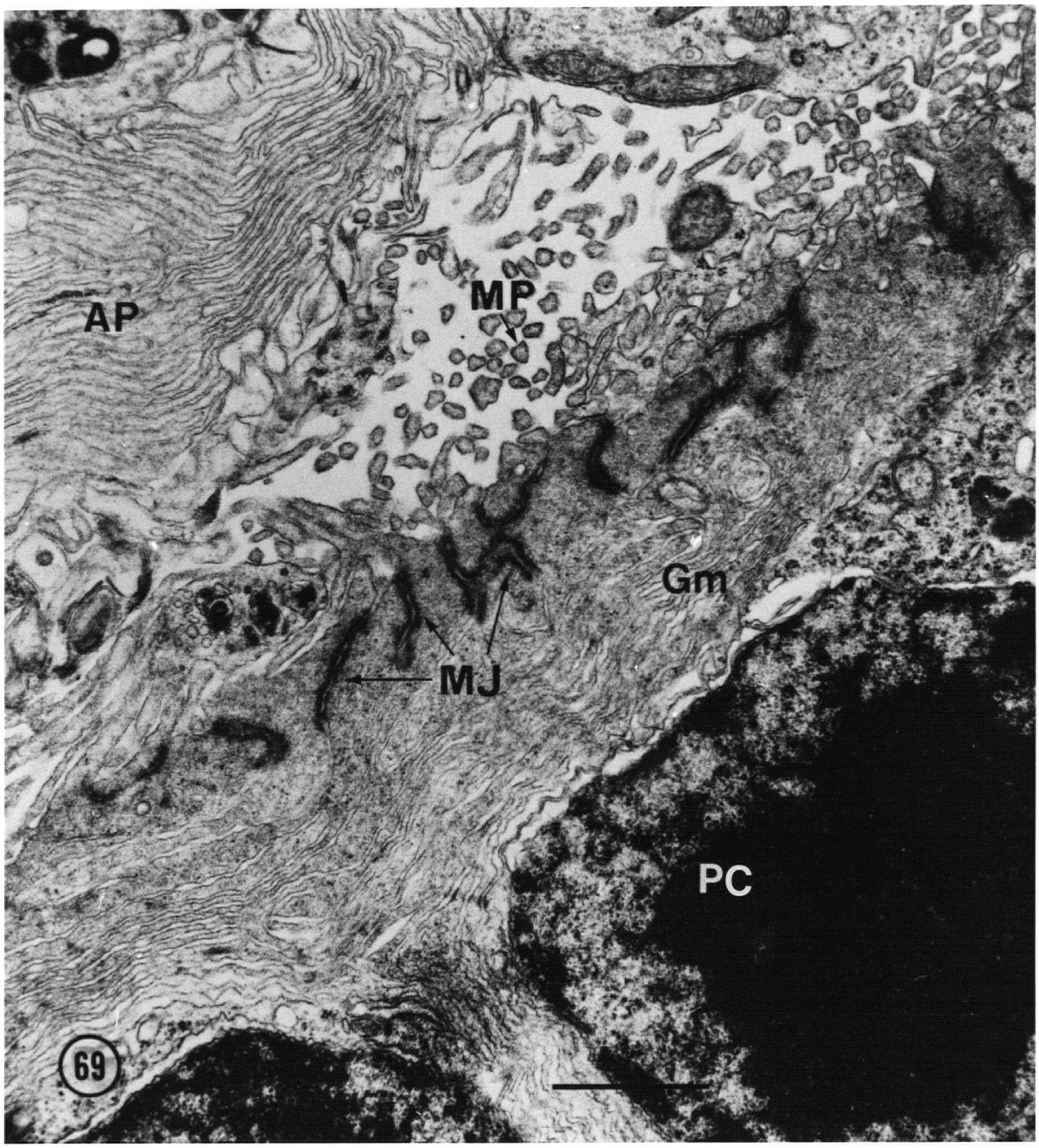

AP
MP
Gm
MJ
PC
69

Figure 70.

Electron micrograph showing acid phosphatase localization in the retinal epithelium from the posterior retina of a 6 month vitamin A deficient animal. After fixation, the tissue is prepared by detaching the retinal epithelium and choriocapillaries from the retina proper. This procedure causes some structural distortion of the inner retinal epithelium particularly the apical processes. The detached tissue is then incubated in Gomori medium with sodium-glycerophosphate as substrate and postfixed in 2% osmium tetroxide. The tissue is sectioned and double stained with uranyl acetate and lead citrate. A black precipitate of lead phosphate indicates the presence of the enzyme acid phosphatase. In the micrograph, black precipitate of lead phosphate can be observed around the periphery of the lysosomes (L) and in the phagosomes (Ph), but nowhere else in the epithelial cytoplasm. (B, basal infolding of retinal epithelium; M, mitochondria.)

x 32,700

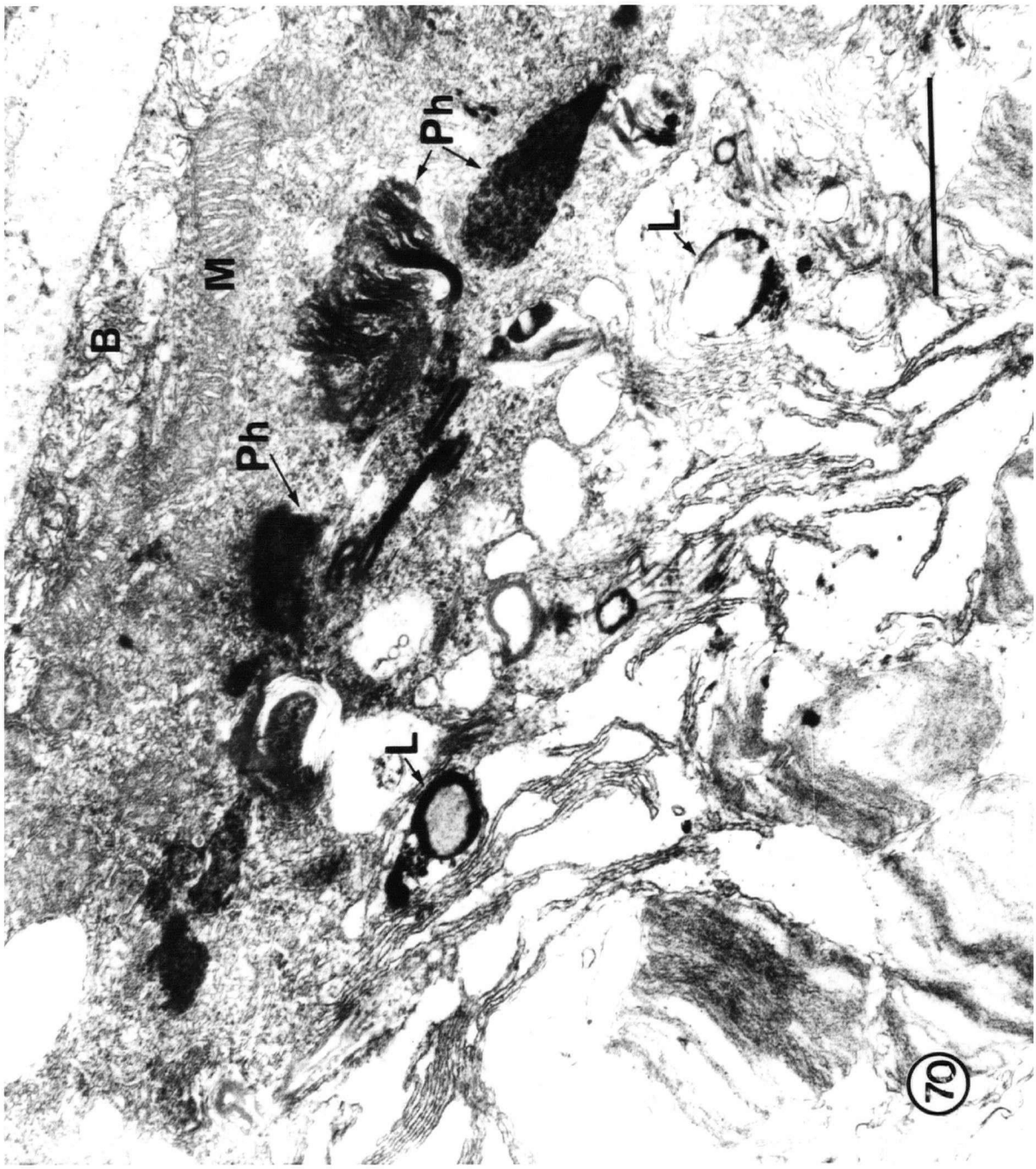

B
M
Ph
Ph
L
L
70

Figure 71.

Electron micrograph showing acid phosphatase localization in the retinal epithelium from the same specimen as Figure 70. A black precipitate of lead phosphate is present in the lysosomes (L), phagosomes (Ph) and Golgi apparatus (G). Ph_1 indicates a newly ingested phagosome which is just beginning to be subjected to lysosomal enzyme activity. (AP, apical processes of retinal epithelium.)

x 32,700

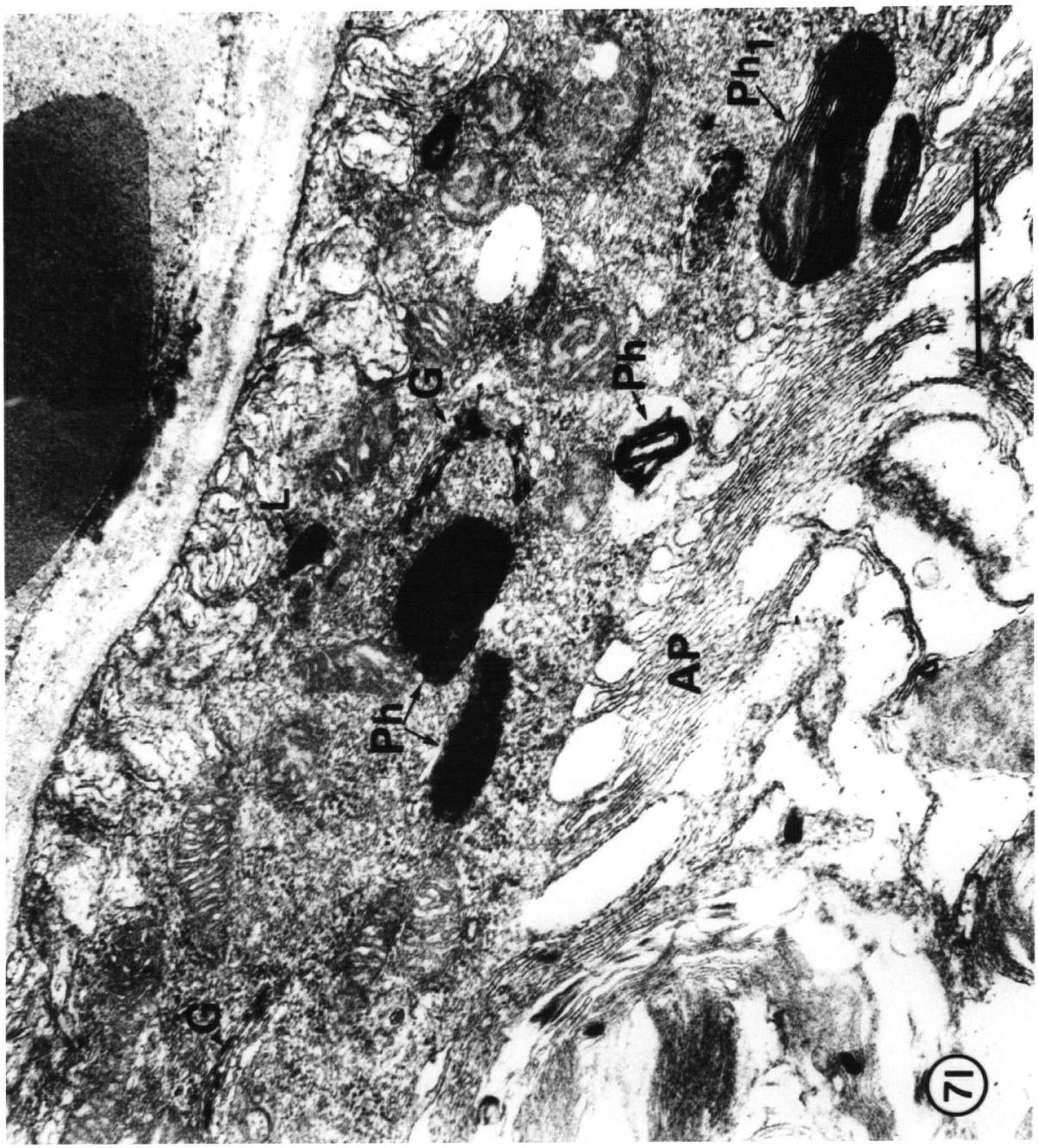

G
L
G
Ph
Ph
AP
Ph
17

Figure 72.

Light microscopic radioautograph showing the posterior outer retina from a 10 month old control animal, 4 hours after intravitreal injection of H^3-methionine. The section has been exposed for 2 months and poststained with toluidine blue. Sparse, evenly distributed radioactive material is present in the retinal epithelium (RE), photoreceptor outer segments (OS), inner segments (IS) and the outer nuclear layer (ONL). The background is almost clear of radioactive material.

x 4,750

Figure 73.

Light microscopic radioautograph showing the posterior outer retina from a 10 month old control animal, 24 hours after labelling intravitreally with H^3-methionine. The section has been exposed for two months and poststained with toluidine blue. There is a marked accumulation of radioactive material over the retinal epithelium, the photoreceptor inner segments and the outer nuclear layer. An accumulation of the radioactive material can be observed at junctions between photoreceptor inner and outer segments and also in the basal portions of the outer segments (black arrows). In the outer nuclear layer, silver grains are found primarily in the cell cytoplasm (white arrows)

x 4,750

Figure 74.

Light microscopic radioautograph showing the posterior outer retina from a 2.5 month vitamin A deficient animal, 4 hours after intravitreal labelling with H^3-methionine. The section has been exposed for 2 months and poststained with toluidine blue. Radioactive material is evenly distributed over the retinal epithelium, the photoreceptor outer and inner segments and the outer nuclear layer.

x 4,750

Figure 75.

Light microscopic radioautograph from a 2.5 month vitamin A deficient animal showing the posterior outer retina, 24 hours after intravitreal labelling with H^3-methionine. The section has been exposed for 2 months and poststained with toluidine blue. This oblique section shows a slight increase in labelling over the photoreceptor inner segments and the outer nuclear layer. Silver grains are especially concentrated at junctions between the photoreceptor inner and outer segments (black arrows). In the outer nuclear layer, silver grains are observed in the nuclei (white dotted arrows) and in the cytoplasm between the nuclei (white arrows).

x 4,750

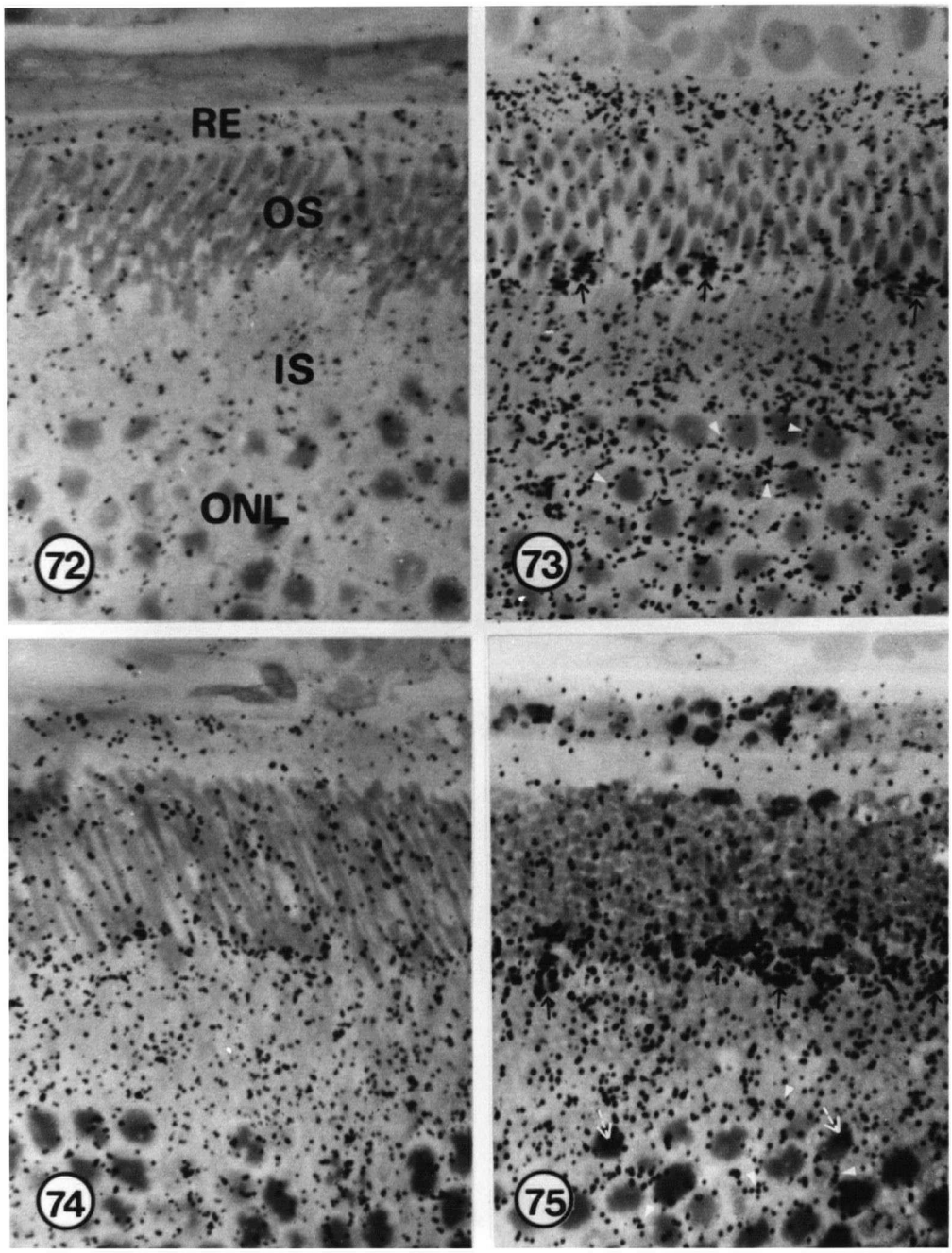

RE
OS
IS
ONL
72
73
74
75

Figure 76.

Light microscopic radioautograph from an 8 month vitamin A deficient animal, showing the posterior outer retina, 4 hours after intravitreal labelling with H^3-methionine. The section has been exposed for 2 months and poststained with toluidine blue. Sparse labelling is present over the retinal epithelium, photoreceptor outer segments, inner segments and the outer nuclear layer. In the latter, silver grains reside primarily around the periphery of the nuclei.

x 4,750

Figure 77.

Light microscopic radioautograph from an 8 month vitamin A deficient animal, showing the posterior outer retina, 24 hours after intravitreal labelling with H^3-methionine. The section has been exposed for 2 months and poststained with toluidine blue. A marked increase of radioactive material is seen over the retinal epithelium, photoreceptor outer segments, inner segments and outer nuclear layer. Silver grains are especially concentrated at junctions between the photoreceptor inner and outer segments and in basal portions of the outer segments (black arrows). Marked incorporation of radioactive material is found around the periphery of each photoreceptor nucleus (white dotted arrows). Some silver grains are also observed in the cytoplasm (white arrows) around each photoreceptor nucleus.

x 4,750

Figure 78.

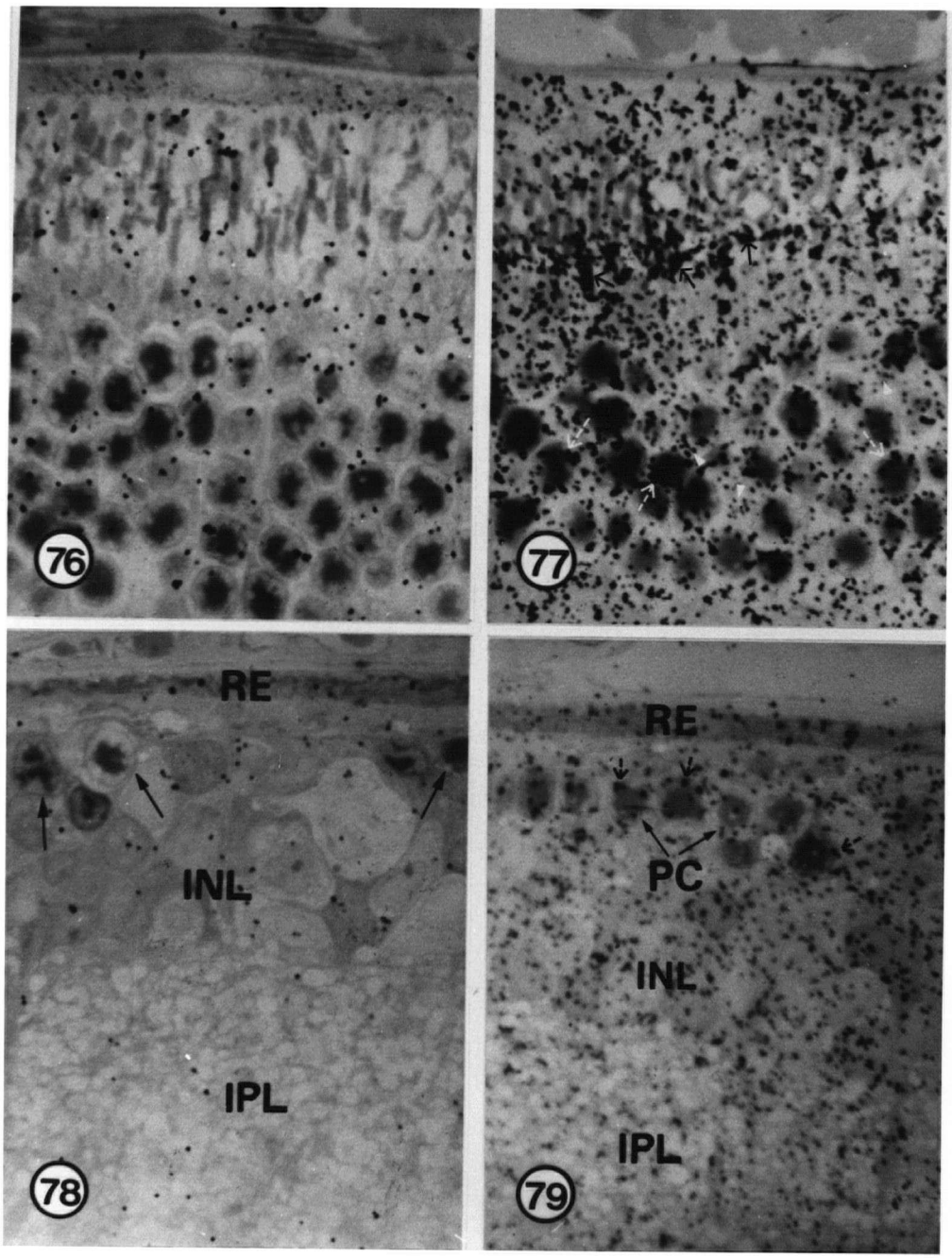
Light microscopic radioautograph from a 10 month vitamin A deficient animal showing the retinal epithelium and the neural retina, 4 hours after intravitreal labelling with H^3-methionine. The section has been exposed for 2 months and poststained with toluidine blue. Little radioactive material is seen over the retinal epithelium (RE), inner nuclear layer (INL) and inner plexifrom layer (IPL). None is present over the few photoreceptor cells (arrows) seen in the micrograph.

x 4,750

Figure 79.

Light microscopic radioautograph from a 10 month vitamin A deficient animal showing the same structures as Figure 78, 24 hours after intravitreal labelling with H^3-methionine. The section has been exposed for 2 months and poststained with toluidine blue. There is an increase in labelling of the retinal epithelium (RE), inner nuclear layer (INL), and inner plexiform layer (IPL). Some silver grains are found over the few photoreceptors (PC) seen in the micrograph. They are distributed mainly around the periphery of the nuclei (black dotted arrows).

x 4,750


76
77
RE
INL
IPL
78
RE
PC
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79

V. DISCUSSION

A) Résumé of the Most Pertinent Results

Morphological changes in the photoreceptor cells and retinal epithelium produced by vitamin A deficiency were studied in the albino Wistar rat by light and electron microscopy. The process of degeneration of the photoreceptors in vitamin A deficiency noted in this study can be summarised as follows. First, the lamellar discs of the photoreceptor outer segments broke down into vesicles and tubules after 1 month of vitamin A deprivation. After 2.5 months, the distal portions of the inner segments became slightly swollen and the distal inner segment cytoplasm suffered a loss of polysomes and underwent swelling of the endoplasmic reticulum. By 6 months of vitamin A deficiency, the photoreceptor synaptic terminals were affected. Fewer synaptic vesicles were present in the cytoplasm and the plasma membranes in the synaptic region developed large gaps. As deterioration of the photoreceptors continued, only fragments of the outer segments remained while degenerating inner segments and synaptic processes gradually retracted towards the photoreceptor nuclei. The nuclear envelopes of the photoreceptors began to disintegrate at 9 months. By 11 months, most of the photoreceptor cells had disappeared and only one irregular row of photoreceptor nuclei remained. Each of the remaining visual cell nuclei was surrounded by several layers of glial membranes. The neural cells of the inner retinal layers appeared unchanged although these were not studied in detail by electron microscopy. In the retinal epithelium, a marked increase of lysosomes close to the inner epithelial surface and a prominent proliferation of the apical

processes were observed during the breakdown of the photoreceptors.

In addition to the structural changes noted, the vitamin A deficient animals gained weight more slowly than the control animals. Their plasma vitamin A levels declined rapidly after 3 weeks on the vitamin A free diet supplemented with vitamin A acid. The acid phosphatase test confirmed the presence of a large number of lysosomes in the inner retinal epithelial cytoplasm of the vitamin A deficient animals. Radioautographic data of H^3-methionine incorporation indicated that the degenerating photoreceptors were still capable of amino acid uptake and protein synthesis.

B) Storage and Metabolism of Vitamin A

According to Moore (1964), in the human body, about 90% of the vitamin is stored in the liver as vitamin A palmitate. The blood contains about 0.45% of the total body stores in the form of vitamin A alcohol which is kept at a remarkably constant level in spite of dietary fluctuations. Only 0.005% of the body's vitamin A, in the form of vitamin A aldehyde is present in the retina. The remaining vitamin A is present in the other organs and fatty tissues of the body. When animals are placed on a vitamin A free diet, the concentration of vitamin A in the liver slowly declines, whereas the blood vitamin A level remains relatively constant until liver stores are practically depleted (Olson, 1969). Dowling and Wald (1958) reported that when weanling rats were placed on a vitamin A deficient diet, their liver stores of the vitamin A declined linearly to zero in about 3 1/2 weeks. After 3 1/2 weeks on the diet, the blood vitamin A level fell precipitously to zero and rhodopsin content in the retina commenced a linear decrease, marking the onset of night blindness. By 2 months, opsin levels in the retina also declined and histological

deterioration of the retina was observed. In the present study, the blood vitamin A content of the vitamin A deficient rats declined rapidly after 3 weeks of vitamin A deficiency when the first anatomical changes in the retina were detected. In this study, although vitamin A content of the liver was not analysed, the fact that the blood vitamin A began to decline after the 3rd week of vitamin A deprivation indicates that depletion of liver stores of vitamin A should have occurred before this time according to Dowling and Wald (1958). The retinas of the experimental animals in this study began to degenerate about a month earlier after introduction of the vitamin A deficient diet than the rats observed by Dowling and Wald (1958). Since in the early stages of this study only a few lamellar discs in the distal portions of the photoreceptor outer segments were noted to be affected, it is possible that such changes were overlooked by Dowling and Wald. Another possibility is that the room in which the rats were kept in Dowling and Wald's study was not as brightly lighted or lighted for as long a time as in the present study. Unfortunately, the lighting conditions were not specified in the reports of Dowling and Wald (1958).

The vitamin A deficient animals in the present investigation began to show a slower weight gain than the control animals at about the same time that their blood vitamin A levels began to decline. This finding implies that the function of vitamin A in the body was only partially fulfilled by the vitamin A acid which supplemented the vitamin A free diet.

C) The Photoreceptors in Vitamin A Deficiency

1. The Outer Segments

Tansley (1933,1936) and Johnson (1939, 1943), by light microscopy, noted that after young rats had been placed on a vitamin A free diet for 7-13 weeks, many photoreceptor outer segments disappeared and those that remained stained abnormally. Dowling and Gibbons (1961), too, reported that when young rats were raised on a vitamin A free diet supplemented with vitamin A acid the photoreceptor outer segments stained less intensely than normal and appeared fragile and broken after 2 months on the diet. Only fragments of the outer segments remained after 6 months on the diet and they completely disappeared by 10 months. Dowling and Gibbons (1961), by electron microscopy, observed that degeneration began with a marked swelling of the highly ordered transverse discs of the outer segment which pinched off to form large vesicles and tubules. After a high proportion of the discs had degenerated in this way, the outer segments lost their normal elongated, cylindrical shape and became almost spherical. Most of the interior of the spherical outer segments was filled with distended vesicles and tubules. Fragments of the outer segments were frequently observed lying free in the space between the retina and the pigment epithelium.

In the present study, morphological changes observed by light microscopy in the outer segments of the degenerating photoreceptors were similar to those described by Tansley (1936), Johnson (1939, 1943) and Dowling and Gibbons (1961). However, the fine structural changes of the photoreceptor outer segments were studied in greater detail in this work and a number of new details of the degenerative process have been uncovered. It was

noted that the breakdown of the lamellar discs into vesicles began from the distal portions of the photoreceptor outer segments and progressed towards the inner segments. There was some variation in the degree of outer segment involvement from one animal to another which may be related to differences in vitamin A stores in the liver at the time the animals were placed on the special diet. The basal portions of the outer segments were always observed to contain some normal looking lamellar discs during the early stage of degeneration. Radioautographic studies of protein synthesis in rod photoreceptors by Droz (1963) in rat and mouse, Young (1967) in rat, mouse and frog and Young and Droz (1968) in frog have indicated that the labelled amino acid are concentrated initially in the inner segment of the cell. Within 24 hours, the radioactive material is displaced to the base of the outer segment, where it accumulates as a distinct reaction band. The reaction band than gradually moves distally along the outer segment and ultimately disappears at the apex of the cell to reappear later in phagosomes in the pigment epithelium. This finding indicates that the protein component of the rod photoreceptor outer segment is continually renewed by repeated apposition of material at the base of the outer segment in conjunction with a balanced removal of material at the apex. From this work it seems likely that the normal looking discs observed at the bases of the degenerating outer segments in the present study were newly formed.

Dowling and Wald (1958) and Dowling and Gibbons (1961) have suggested that in vitamin A deficiency the cause of the breakdown of the lamellar discs in the outer segments is due to a loss of opsin in the absence of vitamin A aldehyde. Since opsin is stabilized by combining with vitamin A to form rhodopsin (Radding and Wald, 1956; Hubbard, 1958), opsin may

deteriorate in prolonged vitamin A deficiency when there is no vitamin A available for it to combine with. In the present study, incorporation of H^3-methionine into the inner segments was found to continue in vitamin A deficiency indicating that there is still protein synthesis in the inner segments of the degenerating photoreceptors. In the vitamin A deficient animals, in this study, the radioactive material was observed to concentrate at the junction of the inner and the outer segments and also at the bases of the outer segments 24 hours after labelling. Presumably, opsin was synthesized, but was not utilized in the absence of vitamin A aldehyde. Therefore, the disc membranes broke down in spite of the fact that protein synthesis continued in the inner segments. This finding supports the hypothesis of Dowling and Gibbons (1961), that vitamin A aldehyde, besides initiating visual excitation, also functions in maintaining the structural integrity of the disc membranes. Another possibility is that the protein synthesized in the inner segment is abnormal or decreased and this will be considered later.

2. The Inner Segments, Synaptic Processes and Photoreceptor Nuclei

Dowling and Gibbons (1961) have observed that the photoreceptor inner segments become greatly reduced in number and shorter and thicker than normal after the animals have been on the vitamin A free diet for 6 months. However, no changes were noted by these authors in the fine structure of the components of the inner segments such as the mitochondria, the cytoplasmic granules, and the cytoplasmic membranes. Changes occurring in the photoreceptor synaptic processes as a result of vitamin A deficiency

have not been previously studied. The results of the present study indicate that, in contrast to the results of the prior study by Dowling and Gibbons (1961), the photoreceptor inner segments show marked changes during and after the degeneration of the outer segments. The distal portions of the inner segments become slightly swollen and contain only a few polysomes and some large vacuoles after 2.5 months of vitamin A deficiency. Later, the inner segments gradually loose their elongated, cylindrical structure and become short and barrel-shaped as they retract towards the nuclei. In the inner segment cytoplasm, the mitochondria also gradually shorten and decrease in number, the polysomes gather mainly in the proximal halves and the Golgi complexes become ill-defined. The synaptic processes, also, show retraction towards the nuclei, accounting for the thinning of the outer plexiform layer observed by light microscopy. Each of the shortened synaptic processes contains only a few synaptic vesicles and the plasma membranes between adjacent synaptic processes form gaps. The membranes of the mitochondria both in the inner segments and the synaptic processes show evidence of degeneration.

What causes death of the photoreceptors in the vitamin A deficient animals? Dowling and Wald (1960) have suggested that disintegration of photoreceptor outer segments in vitamin A deficiency deprives the photoreceptor cells of stimulation from photochemical excitation. This lack of stimulation might then cause the death of the visual cells. Hansson (1970) has suggested that photoreceptor death is due to an incorrect synthesis of protein or other elements necessary for the continuous renewal of the inner and outer segments. In the present study, it was observed that, prior to photoreceptor cell death, the inner segments and the synaptic processes underwent a continuous retraction while the outer segments were gradually disintegrating. Most of the photoreceptors then

disappeared leaving behind only a single layer in which the cells had lost most of their subcellular structures. I would like to suggest that the underlying mechanism involved in the phenomena operates at the gene level. During vitamin A deprivation, the photoreceptor cells could be affected somewhere along this pathway:

$$\text{DNA} \xrightarrow{\text{transcription}} \text{mRNA} \xrightarrow{\text{translation}} \text{protein}$$

where DNA = Deoxyribonucleic acid

mRNA = Messenger ribonucleic acid

Presumably some genes could be repressed in vitamin A deficiency so that a lesser amount of protein or a different protein is synthesized in the degenerating photoreceptors. Data in support of this suggestion have been provided by Tryfiates and Krause (1971) who have shown that an altered messenger RNA is synthesized in the liver of the vitamin A deficient rat. They have suggested that vitamin A exerts a control over cellular differentiation at the transcription level by influencing the species of mRNA synthesized. Johnson et al (1969) reported that in rat intestinal mucosa and liver, RNA labelling is increased after vitamin A injection in vivo. Kleinman et al (1971) found that vitamin A deficiency leads to a decreased incorporation of both uridine-H^3 and orotic acid-5-C^{14} into RNA.

The action of vitamin A on gene activity has been further implicated in the following pathological studies. In vitamin A deficient rats, bundles of keratin appear in the cells of parotid duct and trachea resulting in metaplasia and hyperplasia (Hayes et al., 1970; Wong and Buck, 1971). In the male reproductive tract of the vitamin A deficient rat,

spermatogenesis stops and the semiferous epithelium degenerates (Thompson et al, 1964). In connective tissues of lung, bone and dura mater, the synthesis and/or turnover of collagen and mucopolysaccharide ground substances appears to be disturbed in vitamin A deficiency resulting in measurable excesses of these components. These excesses are thought to be involved in the thickening of the dura mater and the associated impaired reabsorption of cerebral spinal fluid noted in vitamin A deficiency (Cousins et al, 1969). They may also account for excessive growth in periosteal bone also noted in this condition (Hayes and Cousin, 1970). The above pathological alteration imply that the result of vitamin A deficiency is to limit differentiation of recently divided cells towards a favoured pathway so that, for example, in spermatogenesis, the unidirectional differentiation of spermatozoa is blocked resulting in testicular degeneration (Hayes, 1971). Similarly in the studies of parotid duct (Hayes et al., 1970) and trachea (Wong and Buck, 1971), the bipotent epithelial basal cell in these organs is restricted in vitamin A deficiency to synthesis of fibrous, keratinizing proteins at the expense of mucous cell production (Hayes, 1971). In bone and collagen, the periosteal progenitor cell and the fibroblast favour the synthesis of collagen fibers and ground substance in vitamin A deficiency (Hayes, 1971).

In the present investigation, the degenerating photoreceptors were capable of protein synthesis as evidenced by the incorporation of H^3-methionine in the inner segments. Although the protein synthesized has not been analysed quantitatively or qualitatively, it has been shown in radioautographic studies that production of photoreceptor outer segment material is decreased in vitamin A deficient rats (Herron and Riegel, 1974a, 1974b). The reduced amount of protein synthesized may have been

in an altered form, as suggested by Hansson (1970), and could not be used by the visual cells to maintain their normal structure. The visual cells, therefore, began to dedifferentiate along a "retrograde" pathway manifested by retraction of photoreceptor processes and the degeneration of subcellular structures observed in the present study. Most of the photoreceptor remnants were then discarded by an unknown mechanism and removed by the retinal epithelium.

It has been suggested in many studies that membranes are major sites of vitamin A alcohol (retinol) action, particularly lysosomal and mitochondrial membranes. Roels et al. (1969) reported that in liver of vitamin A deficient rats, lysosomal membranes were more labile. The membranes of erythrocytes obtained from vitamin A deficient rats were found to be markedly swollen and distorted and subject to faster hemolysis compared to those from control animals (Anderson et al., 1967). In epithelial cells of the bulbourethral glands of vitamin A deficient rats, Latalski (1972) observed a great reduction in the number of cristae of the mitochondria present in the cell cytoplasm. He suggested such changes were due to a disturbance in permeability and stability of the lipoprotein membrane of the involved mitochondria. A similar type of disturbance was observed in the mitochondria of degenerating photoreceptor inner segments and the synaptic processes in the present study. Moreover, plasma membranes and nuclear membranes of the photoreceptors too, showed evidence of breakdown in the present study. Roels et al (1969) have suggested that vitamin A alcohol regulates the binding of the membrane bound ATPase responsible for lipoprotein interaction in specific areas of certain biological membranes and thereby influences their structure and stability. It is possible therefore that if vitamin A alcohol is also

present in the mitochondrial, nuclear and plasma membranes of the visual cell, prolonged vitamin A deficiency would deplete vitamin A in these membranes leading to membrane breakdown.

In the vitamin A deficient animals of this study the degenerating photoreceptors were discarded so rapidly that only one irregular row was left by the 11th month. The nuclei of the remaining photoreceptors appeared normal, yet the other parts of the cells such as the outer and inner segments and synaptic processes had disappeared. Whether the nuclear chromatin or gene structures had been altered qualitatively is not known. Further studies would be necessary to examine this possibility.

D) The Retinal Epithelium in Vitamin A Deficiency

According to Dowling and Gibbons (1961) the retinal epithelium of the albino rat remained unchanged after 10 months of vitamin A deprivation. Hansson (1970) observed by scanning electron microscopy that after 6 months of vitamin A deficiency, the apical processes of the retinal epithelium were thicker and shorter than normal. The results of the present study indicate clearly that marked changes do occur in the structure of the retinal epithelium of the vitamin A deficient rat. The most notable change was a large increase in small dark granules with the ultrastructural characteristics of lysosomes beneath the inner epithelial surface after 4-5 months of vitamin A deprivation. A positive reaction for acid phosphatase confirmed that the granules were lysosomes. The lysosomes were sometimes observed near the Golgi apparatus and appeared to stream towards the epithelial inner surface. A positive reaction for acid phosphatase was also present in the Golgi apparatus, and phagosomes of the epithelial cell. This finding tends to confirm previous work that

primary lysosomes are formed in the Golgi apparatus (DeDuve and Wattiaux, 1966) and that phagosomes are degraded in the retinal epithelium by the hydrolytic enzymes of the lysosomes (Ishikawa and Yamada, 1970). In the present study, it was found that the increase of lysosomes persisted throughout the photoreceptor degeneration process. The apical processes of the retinal epithelium proliferated slightly at first and later became very prominent. These changes were not observed in the retinal epithelium of control animals of the same age.

The physiological significance of the close structural relation of the pigment epithelium to the photoreceptors was first noted by Kühne (1878) who found that in vitro regeneration of visual purple took place only if pigment epithelium remained in contact with the photoreceptors. In recent years, much more has been learned of this intimate relationship between the pigment epithelium and the photoreceptors. For example, experiments utilizing horseradish peroxidase as a diffusion tracer have pointed out the importance of the pigment epithelium as a selective filter, preventing intercellular diffusion of larger molecules from the choriocapillaries into the retina. (Bok and Young, 1972). In the frog, it has been shown that radioactive vitamin A is rapidly taken into the oil droplets of the retinal epithelial cells within a few minutes after intravenous injection (Bok and Young, 1972). Glycerol and palmitic acid are also avidly taken up from the blood stream and concentrated in the oil droplet as well (Bok and Young, 1972). The mucopolysaccharides of the interphotoreceptor matrix are believed to be synthesized within the pigment epithelial cells and later secreted extracellularly by the well developed Golgi system of these cells (Berman, 1964). The enzyme isomerase, responsible for isomerizing vitamin A to the 11-cis configuration, has

been identified in both rod outer segments and the retinal pigment epithelium in amphibia (Hubbard, 1956). The pigment epithelium also acts as a scavenger, as it normally phagocytoses and then destroys packets of outer segment discs. This phagocytic function of the retinal epithelium has been well studied by many investigators (Dowling and Gibbons, 1962; Bairati and Orzalesi, 1963; Ishikawa and Yamada, 1970; Young, 1967, 1971a; Young and Bok, 1969; Spitznas and Hogan, 1970).

In the present study, in vitamin A deficiency, the apical processes of the retinal epithelium appeared to be more active in engulfing photoreceptor fragments than in the control animal. Displaced photoreceptor nuclei were often observed unusually close to the retinal epithelium in vitamin A deficient animals and it seems likely that all parts of the photoreceptor cell including the nuclei are phagocytosed by the pigment epithelium in the vitamin A deficient rats. The presence of the large number of lysosomes beneath the inner epithelial surface suggests that the photoreceptor fragments were degraded by the lysosomes as soon as they were phagocytosed. This may explain why in this study portions of photoreceptor cells aside from outer segment fragments were not seen within the retinal epithelial cytoplasm. The fact that in this study the time of proliferation of lysosomal activity in the retinal epithelium corresponded to the time of breakdown of the photoreceptors strongly supports a cause and effect relationship.

E) Müller Cells in Vitamin A Deficiency

Müller cells, the most prominent nonnervous elements situated among the retinal neurons, are thought to provide mechanical support and physiological insulation for intervening nerve cells (Polyak, 1957).

More recently, they have been shown to be the main sites of carbohydrate storage in the retina (Kuwabara and Cogan, 1961; Magalhaes and Coimbra, 1970). They also of course are capable of synthesizing protein (Hodson and Marshall, 1967). It has been noted, in addition, that glycogen synthesis (Magalhaes and Coimbra, 1970), as well as certain enzymatic activities (Cogan and Kuwabara, 1959; Kuwabara and Cogan, 1960; Lessell and Kuwabara, 1964) prevail at different sites in the Müller cell. This suggests that the cell might play different functional roles at different levels of the retina. It has been pointed out in several animal species that the distribution of organelles in Müller cell cytoplasm differs as one moves from the inner end of the cell at the inner limiting membrane, to the outer end at the outer limiting membrane. For example, vesicles and fibrils have been observed in the cytoplasm of the inner end of the cell in reptiles (Pedler, 1963), cat (Ladman, 1961) and man (Hogan and Feeney, 1963). Mitochondria have been found in the outer end of the cell in reptiles (Pedler, 1963) and the rabbit (Sjöstrand and Nilsson, 1964), but in the inner portion, in man (Fine, 1961). Magalhaes and Coimbra (1972) have suggested that the Müller cell of the rabbit can be divided into 3 portions, each characterized by certain ultrastructural features, possibly having distinct functions. The inner portion is characterized by a great density of glycogen particles and microfilaments, a network of smooth endoplasmic reticulum and the presence of some dense bodies. A high rate of glycogen synthesis is present in this portion (Magalhaes and Coimbra, 1970) although the smooth reticulum is believed to be the site of nearly all glucose-6-phosphate activity in the retina (Magalhaes and Coimbra, 1972). The middle portion of the

Müller cell is marked by the presence of ergastoplasm and Golgi complexes, suggesting protein synthesis (Magalhaes and Coimbra, 1972). The Müller cell outer portion seems well adapted to absorption and intracellular transport. An absorptive function is suggested by the presence of long microvilli and microtubules while the presence of mitochondria in this part of the cell suggest that active intracellular transport is taking place (Magalhaes and Coimbra, 1972). In addition, the outer portion of the Müller cells may play a role in the pathological condition of photoreceptor destruction as shown in the present investigation. As degeneration of the photoreceptors in the vitamin A deficient animals continued, the terminal processes of the Müller cells became highly conspicuous. In late stages of degeneration (11 months of vitamin A deficiency), the remaining photoreceptors and other unidentified cells of the inner nuclear layer were each surrounded by several layers of membranes probably of a glial nature. The terminal processes of the Müller cells were frequently observed slanting laterally and probably contributing to glial membrane formation and encirclement of the remaining outer neurones. It is possible that these membranes serve as barriers preventing the remaining photoreceptor nuclei from being phagocytosed by the retinal epithelium.

In this study, by light microscopy, the cells of the inner retinal layers appeared unaffected even after all the photoreceptors had disappeared. These cells were not examined in detail by electron microscopy. The possibility of alterations in the inner retinal cells in vitamin A deficiency therefore, remains to be investigated.

F) Light Damage to Photoreceptors

It is of interest that animals that have been exposed to continuous illumination show a similar degeneration or destruction of the photoreceptors (Nöell, 1966; Nöell et al, 1966; Grignolo, 1969; Weale, 1969; Tso, 1973; Lawwill, 1973) to that observed in vitamin A deficiency. Kuwabara and Gorn (1968) have shown that the photoreceptor outer segments of the albino rat demonstrate severe membranous changes upon exposure to continuous relatively cold light at a brightness of about 750 foot-candles. In their studies, the lamellar discs at the outermost tips of the photoreceptors broke down and formed vesicles after 1 hour of exposure to light. When the retinas of the rat were exposed to light at that intensity for periods from 6 to 24 hours, the discs in the outer portions of the segments lost their regular lamellar structure and became markedly separated and vacuolated. When the animals were exposed for more than two days, the discs broke down into tubules and became irregularly packed within large, round, or pear-shaped outer segments. Later, the swollen outer segments became separated from the inner segments. The isolated outer segments were then found among the apical microvilli of the pigment epithelium and gradually broke down into smaller sizes. Kuwabara and Gorn (1968) thought that the membranous changes were a type of cellular reaction of the photoreceptors to extreme bleaching circumstances. Continuous exposure to the light appeared to overbalance the bleaching and recovery mechanisms of the photoreceptor cells. Since bleaching of the visual pigments causes a loss of some vitamin A (Wald, 1955), prolonged illumination would cause excessive loss of this prosthetic group from rhodopsin. Therefore, rhodopsin cannot be generated to restore the

structural integrity of the lamellar discs and consequently the latter break down in a similar manner to that observed in vitamin A deficiency. Again the stabilizing effect of vitamin A aldehyde on the structure of the outer segment discs is implicated.

Recently, Shear et al (1973) have shown that retinas of albino rats exposed to continuous low intensity of fluorescent light (18 foot-candles) for periods of 6 to 18 hours display fine structural changes in both the retinal epithelial cells and the outer segments of the photoreceptor cells. Adjacent epithelial cells were separated by wide gaps and suffered retraction of the apical processes. The lamellar discs of the photoreceptor outer segments became progressively more tubular. These changes were reversible in an environment of cyclic light (14 hours of light and 10 hours of darkness). In the present study, the vitamin A deficient animals and their controls were kept under lighting conditions of a maximum of 10 foot-candles during the 12 hours of light per day. Since the vitamin A deficient animals and their controls were always killed after less than 1 1/2 hours of light adaptation at an intensity of 25 foot-candles, it seems unlikely that the structural changes observed in this study in the retinas of the vitamin A deficient animals were influenced by the lighting conditions. Also the control animals in this study, which were kept under identical lighting conditions did not show significant morphological changes with aging in either photoreceptor or retinal epithelial cells.

G) Normal Loss of Photoreceptors

In control specimens of the present study, a very small number of photoreceptor cells consisting of prominent nuclei surrounded by a narrow rim of cytoplasm were observed lying outside the outer limiting membrane between the retinal epithelium and the neural retina. Photoreceptor nuclei were also observed partially embedded in the retinal epithelium. These findings suggest that under the lighting conditions of the present study some photoreceptor nuclei normally make their way towards the retinal epithelium where they are eventually phagocytosed and degraded. Thus, throughout life in the rat, there may be a continual loss of photoreceptor cells. Apart from the work on light damage, visual cell loss of this sort, to my knowledge, has not been described in the retina of any vertebrate studied to date. The significance of this process, which may be a normal aging change, is still obscure.

H) Glycogen Filled Mitochondria

In this study, after 4-5 months of vitamin A deprivation degenerating inner segments of an unusual type were occasionally observed. The cytoplasm of these inner segments became very dense and contained no visible subcellular structures except mitochondria (Fig. 41, 42). The mitochondria were greatly enlarged by an accumulation of glycogen particles within their outer membranes. Degenerating inner segments of this type were also observed in the control animals. Glycogen filled mitochondria have been reported to be present in rod ellipsoids of toads (Ishikawa and Yamada, 1969), rats (Ishikawa and Pei, 1965), in the flight muscles of aging blow flies (Sacktor and Shimada, 1972)

and in the heart muscles of aging drosophila (Sohol, 1970). The presence of intramitochondrial glycogen is believed to be related to aging since mitochondria of this type have never been reported to exist in retinas of the newborn or young rats (Ishikawa and Pei, 1965). However, they were easily detected in retinas of rats 1 year or older (Ishikawa and Pei, 1965). The present results confirm this finding since mitochondria with enclosed glycogen were not found in rats under 5 months of age. This type of degenerating inner segment with dense cytoplasm and glycogen filled mitochondria may be due to aging and not vitamin A deficiency since inner segments of this type were found in both the older vitamin A deficient and control animals.

VI SUMMARY

1. Weanling rats, on a vitamin A free diet, supplemented with vitamin A acid, gained weight more slowly than litter-mates fed a normal diet.

2. The plasma vitamin A content of the experimental animals declined rapidly after they had been on the vitamin A free diet for 3 weeks. At this time, the histological deterioration of the photoreceptors commenced.

3. Light microscopic study showed that in the vitamin A deficient rats the photoreceptor outer segments first degenerated, then the photoreceptor inner segments, the synaptic processes and the photoreceptor nuclei.

4. In the degenerating retinas of the vitamin A deficient animals, discarded photoreceptor nuclei were often observed displaced sclerally lying close to the retinal epithelium. This phenomenon also occurred in retinas of older control animals.

5. Electron microscopic study showed that the lamellar discs in the distal ends of the photoreceptor outer segments were the first to break down into vesicles and tubules after the rats were on the vitamin A free diet for 1 month.

6. By 2.5 months of vitamin A deficiency, the distal ends of the photoreceptor inner segments became swollen and their polysome and mitochondrial content had greatly decreased. Later, the normally elongated and cylindrical inner segments retracted

towards the photoreceptor nuclei and became short and barrel-shaped.

7. By 6 months of vitamin A deficiency, the photoreceptor outer segments had lost their normal, regular orientation. Few of them appeared intact.

8. By 6 months, the photoreceptor inner segments were shortened to different degrees. In the cytoplasm of the inner segments, polysomes could be found only in the proximal portion, next to the photoreceptor nuclei. The few mitochondria that remained were often shorter than normal and the Golgi complexes present showed signs of disintegration.

9. Also at this 6 month stage, the photoreceptor synaptic terminals were severely affected in the animals on the special diet. Fewer synaptic vesicles were present, often lying immediately adjacent to synaptic sites. The synaptic ribbons persisted but large gaps appeared in the plasma membranes of the synaptic processes. Within the synaptic processes, displaced mitochondrial membranes and loss of mitochondrial cristae were apparent.

10. By 9 months of vitamin A deficiency, the photoreceptor outer segments had completely disappeared except for a few sporadic clusters of saccules.

11. By 9 months, all the photoreceptor inner segments had retracted considerably. The shortened inner segments still contained some polysomes and rough endoplasmic reticulum, but the mitochondria had entirely disappeared.

12. By 9 months, the nuclear envelopes showed evidence of disintegration although the photoreceptor nuclear chromatin appeared unchanged.

13. Also at this 9 month stage, the synaptic processes of the degenerating photoreceptors were very short and contained only a few synaptic vesicles. Synaptic ribbons could still be identified in some synaptic processes.

14. By 11 months of vitamin A deficiency, only one irregular row of photoreceptors remained. The remaining neural retina adhered closely to the retinal epithelium.

15. At this 11 month stage, the degenerating photoreceptors consisted only of a nucleus surrounded by a narrow rim of cytoplasm containing very few organelles. The photoreceptors were surrounded by several layers of membranes probably of glial origin. Synapses of the photoreceptors with nerve cells of the inner nuclear layer and the concomitant synaptic ribbons had disappeared.

16. By 11 months of vitamin A deficiency the normal cell attachment between photoreceptors and Müller cells in the region of the outer limiting membrane had disappeared. The outer limiting membrane was now formed by cell junctions between adjacent Müller cells. At this stage, the terminal processes of the Müller cells above the outer limiting membrane were seen to bend laterally, possibly contributing to the membrane layers surrounding the photoreceptor remnants.

17. Radioautographic studies of H^3-methionine incorporation into the retinas of control and vitamin A deficient animals revealed that both control and degenerating photoreceptors were capable of protein synthesis. It is postulated that this protein might be qualitatively different in the vitamin A deficient animals than the protein synthesized by normal photoreceptors.

18. From the detailed studies of the structural degeneration of the photoreceptors in vitamin A deficiency, it is suggested that in vision, vitamin A, besides initiating visual excitation, also functions in maintaining the structural integrity of disc and cell membranes. It is also suggested that vitamin A probably acts at the gene level since its absence caused the photoreceptors to dedifferentiate along a "retrograde" pathway.

19. In the retinal epithelium, an increase of lysosomes close to the inner surface was noted by 4-5 months of vitamin A deficiency. The lysosomes were acid phosphatase positive. The increase of lysosomes in the retinal epithelium persisted as long as degeneration of the photoreceptors continued. Only a few lysosomes were observed in the inner epithelium of control animals.

20. The apical processes of the retinal epithelium proliferated slightly by 2.5 months of vitamin A deficiency and became very numerous and prominent by 11 months of vitamin A deficiency.

21. In the vitamin A deficient animals, the changes observed in the retinal epithelia as a result of photoreceptor breakdown strongly supported a cause and effect relationship.

22. The morphology of photoreceptors and retinal epithelial cells studied in control rats from ages 1.5 to 12 months remained consistently unchanged, except for the appearance of glycogen filled mitochondria in the photoreceptor inner segments of older animals.

VII Original Contributions

In this investigation a detailed study has been made of the morphological changes which occur in the outer retina (primarily retinal epithelial and photoreceptor cells) as a result of dietary vitamin A deficiency. Only a few prior studies of this phenomenon have been made (Tansley, 1933, 1936; Johnson, 1939, 1943; Dowing and Wald, 1958, 1960; Dowling and Gibbons, 1961; Dowling, 1966) and these are deficient in the following respects.

1. The lighting conditions have not been carefully controlled. (Tansley, 1933, 1936; Johnson, 1939, 1943; Dowling and Wald, 1958, 1960; Dowling and Gibbons, 1961; Dowling 1966). It has now been established that a long period of exposure to light by itself can produce photoreceptor damage (Nöell, 1966; Nöell et al., 1966; Kuwabara and Gorn, 1968; Grignolo, 1969; Weale, 1969; Tso, 1973; Lawwill, 1973; Shear et al., 1973).

2. The morphological changes examined previously have centered on the photoreceptor outer segments only. Before the present study little was known of the sequential changes produced by vitamin A deficiency in the photoreceptor inner segments, synaptic processes and nuclei. Prior to this study it was assumed that the pigment epithelium was unaffected in vitamin A deficiency (Dowling and Gibbons, 1961).

3. The prior studies have failed to take into account the fact that the structure of the outer retina may undergo structural changes in normal animals during aging. This possibility has been carefully examined in the present report.

In summary, the present report represents a much more detailed and carefully controlled study of this phenomenon than has been attempted previously. From this standpoint it can be stated that all the findings

reported in the thesis possess at least some degree of originality.

The following findings reported in the thesis have not, to my knowledge, been reported elsewhere under any experimental conditions.

In the normal retina of the rat:

1. The fine structure of cone photoreceptor cells.

2. The presence of photoreceptor nuclei displaced outside the external limiting membrane.

3. The complete absence of aging changes in the photoreceptor and pigment epithelial cells from 1.5 to 12 months of age except for the appearance of glycogen filled mitochondria in the photoreceptor inner segment of older animals.

In the vitamin A deficient rat:

1. All of the changes noted in sequential fashion in the retinal pigment epithelium including the accumulation of lysosomes and the alterations in the morphology of the inner (apical) epithelial border.

2. The ultrastructural changes noted sequentially in the photoreceptor inner segments, synaptic processes and nuclei.

3. The alterations noted in the outer processes of the Müller cells. The proliferation of glial membranes around the degenerating photoreceptor nuclei.

In terms of its significance this thesis represents another step along the road pioneered by Wald (1935a, 1936) and Dowling and Wald (1958, 1960) leading to a full understanding of the role played by vitamin A in vision and the etiology of night blindness. It has become clear from the present work that, in the retina the effects of vitamin A deficiency are

more complex than had previously been thought. Vitamin A deficiency leads to a destruction of the photoreceptors in a sequential manner, beginning with the outer segments, then affecting the inner segments and synaptic processes and finally the photoreceptor nuclei. The changes in the pigment epithelium appear to be secondary to, and induced by, the presence of photoreceptor fragments at the inner epithelial border. In this case, the pigment epithelial cells act like true macrophages, removing the photoreceptor debris.

If present theory is correct, and vitamin A is normally present in quantity in the photoreceptor outer segments, then it is easy to see why the outer segments degenerate in vitamin A deficiency. It is much more difficult, however, to understand why subsequently the rest of the photoreceptor cell dies particularly when one remembers that this part of the cell is not thought to contain more than minute amounts of vitamin A under normal circumstances (Moore, 1964). The present results do not provide a definitive answer to this question. Rather, based on prior work on vitamin A metabolism in other antomical sites (Thompson et al., 1964; Johnson et al., 1969; Hayes et al., 1970; Tryfiates and Krause, 1971; Wong and Buck, 1971; Kleiman et al., 1971), it is suggested herein that, in the photoreceptor cells in vitamin A deficiency, there occurs an alteration at the gene level in the regulation of protein synthesis which eventually leads to loss of membranes and cell death. This hypothesis remains to be evaluated.

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