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SCANNING ELECTRON MICROSCOPY IN RETINAL RESEARCH

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Abstract

The literature on scanning electron microscopy (SEM) pertaining to the retina has been surveyed and described.

The first two papers on SEM and retina appeared in 1969. Most of the earlier studies concentrated on descriptions [by SEM alone, or with light microscopy (LM) and transmission electron microscopy (TEM)] of the appearance of various retinae and retinal cells [fish, newt, primates, rodents and rabbits, bullfrog]; or embryology of the chick retina. Two papers dealt with retinal disease. In all there were 25 papers in SEM/retinal research before and in 1974. Since 1975 there have been 111 papers which have used SEM in retinal research in very many different fields and in diverse ways, viz: the morphology of the retina, specific retinal cells, and the retinal vessels; embryology; labelling of retinal cells with surface markers; naturally occurring and experimentally-induced retinal disease; the effects of light, including lasers and photocoagulation, and other radiation on the retina, and phagocytosis and the retinal pigment epithelium (RPE). There have also been a number of papers concerned with techniques for SEM, applicable to retinal research, and several review articles. SEM has proven to be a very valuable adjunct to TEM in retinal research and it has become an accepted and almost routine way of extending and enhancing reports on retinal research. Properly used, SEM provides a laconic way of presenting information that might otherwise require many serial sections and long explanations.

KEY WORDS: Scanning electron microscopy, retina and SEM, tissue preparation, retinal vessels plastic casts, retinal cell surface markers, retinal embryology, retinal diseases, retinal radiation damage, retinal morphology.

Introduction

Soon after the first commercial scanning electron microscope (SEM) became available in 1965 (70) it was used to study a variety of biological tissues. The first SEM studies to appear which dealt specifically with retina were those of Shearer (136) on the morphology of isolated pigment particles of the eye, including those of the retinal pigment epithelium (RPE), and Lewis et al. (84) who looked at the retina of the mud puppy (Necturus). The latter study correlated well with those of a transmission electron microscopy (TEM) study on that retina (29). Lewis et al. (84) recognized the potential of the SEM for the study of the multilayered retina with its many and diverse cell types, and their complex interconnections. They dissected fresh retinae which were then fixed in glutaraldehyde (aldehydes are still the most commonly used fixative in SEM retinal research), extracted in moderately weak glycerine solutions, dehydrated in graded ethanol solutions, and the air dried specimens were then coated with gold or aluminum.

Methods for the preparation of retinal tissues for SEM; and problems

Shrinkage

Lewis et al. (84) discussed the problem of shrinkage (53% in their study) which was much greater than in the matched TEM study (estimated shrinkage to 80% of original size). Shrinkage of material prepared for SEM remains a problem (20,21,23). Wickham & Adams (155) looked carefully at the problem of shrinkage in primate retinae prepared for SEM and estimated that there was a 24% average shrinkage with critical point drying (CPD), which is less than in air dried specimens. Ali & Wagner (1) found shrinkage to be greatest in the plexiform layers of fish retinae. The loss of water does not seem to create significant distortions of shape in the photoreceptors. Boyde & Boyde (23) report that prepared material shrinks during storage and they recommend rapid examination in the SEM. Most workers in the field do not make any reference to shrinkage in their papers. It is very important only in studies involving

measurements. What remains valid, however, is the observation made by Lewis et al. (84) that despite shrinkage "the proportions, topography and integrity (of the photoreceptors) seem to have been maintained extremely well, both in the large structures and the small."

Drying the tissues

Critical point drying (CPD) methods were first described for TEM by Anderson (4,5). Boyde & Wood (24) described a CPD apparatus for animal tissue preparation for SEM. Despite the fact that CPD tends to render tissue brittle they recommended it as "a most satisfactory way of drying bulk soft tissues routinely because it avoids the ice-crystal artifacts associated with freezedrying". Smith & Finke (139) in describing an inexpensive apparatus for CPD of soft biological material included monkey retinal cones in their test materials. They found CPD to be faster and more convenient than freezedrying; it produced more consistent results equal to or better than other dehydration methods, and it reduced distortion damage. Freezedrying is superior to air drying, which tends to produce more cracks in the tissues (22) and create greater shrinkage (61). Air drying is very little used now, having been replaced by CPD, which was used in most of the papers cited in this paper, but not all the papers provide this information. Some simply state that the tissues were processed for SEM. Details of CPD procedures are not always given, but where they are it is most often done from CO_2. Freon 12 and 13 are also used, but far less often, and in one case Frigen 11 was used (119). Despite the disadvantages of airdrying the method has yielded useful photomicrographs of acceptable and even good quality, especially of the inner segments and outer segments of the photoreceptors (Fig. 1) (6,16,40,53,61,126). CPD has become the standard method for drying retinal material for SEM.

It is claimed that the retina is more easily damaged by acetone dehydration and air drying than is other tissue. Some interesting results have nonetheless been obtained with acetone dehydration (36,120,143).

Watters & Buck (154) described a camphene sublimation method for all tissues which is relatively simple and easy and works well with retina but only two workers have used it for retinas (39,148), which is surprising.

Fixation

Glutaraldehyde, buffered with cacodylate or phosphate, with or without formaldehyde, is most commonly used for immersion fixation. The usual fixative is a form of Karnovsky's fixative (74). Formalin alone has also been used as a fixative (1,155), with reasonable success. The fixation period usually lasts a few hours, but a few workers have used much longer fixation periods, for the stated purposes of washing out extracellular material and thus getting cleaner and clearer surfaces and making dissection easier. McAvoy (92) used fixation times of from 4-24 hours; Pfeffer & Fisher (118) fixed overnight and Giulian (54) fixed tissue for 24 hours. Marmor et al. (88), Steinberg (140) and Steinberg & Wood (141) fixed for a minimum of 24 hours; Borwein et al. (17,18) for 2 days, Eckmiller & Steinberg (43) for several days, and others for a week or more (142,143). Steinberg (140) and Marmor et al. (88), show beautiful micrographs (Fig. 2). However, Borwein (13) achieved very good results with a fixation time of $1\frac{1}{2}$ hours at $4^{\circ}C$, (Fig. 3) and Peters et al. (117) with 45 minutes (Fig. 4). There are so many other variables in the methods used by these workers that their success cannot be attributed to fixation time only. Experience with retinal research bears out what Boyde (20) wrote:

"So far as I am aware, no one has yet demonstrated any undesirable side-effects of prolonging the aldehyde phase of fixation to several days, weeks or even months and it does take weeks or months to achieve the full degree of cross-linking possible with the aldehydes."

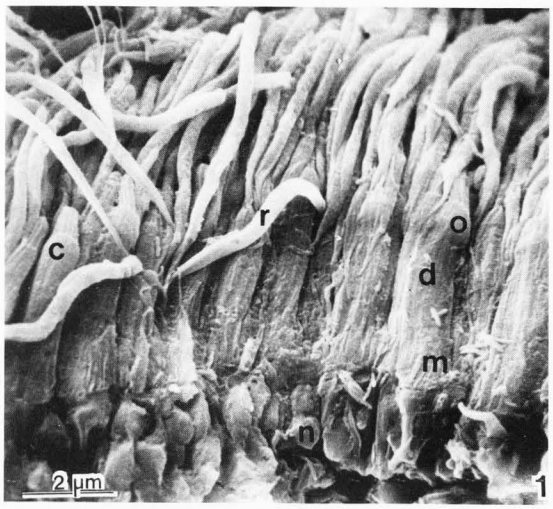
Perfusion has been used followed by immersion fixation (63,64,80,120,135). There is no evidence in the papers that this produced superior results. Unusual surface knobs regularly arranged on the inner segments (IS) are clearly displayed (120) but on the available evidence this cannot be attributed to the fixation method. Perfusion appears to be especially useful in studies which involve axons, dendrites, and glia.

In one of the first SEM-retinal studies, Shearer (136) used fresh (unfixed) RPE material, extracting the pigment granules osmotically from the cell interiors by chilling for several hours in cold water. Subsequent gentle agitation freed the granules from the lysed cells, and these, concentrated by centrifugation, were coated with gold-palladium. Hansson (60,62,63) has used unfixed freezedried material.

Postfixation

Material is usually post-fixed in osmium tetroxide (OsO_4) but a few workers have had good results omitting osmication (1,16 (Fig. 1), 39, 40). Molday (101) and Suburo et al. (143) compared both methods and found that glutaraldehyde alone worked just as well and that osmium was not essential. Kuwabara (79) prefixed in 2% OsO_4, postfixed in 2% glutaraldehyde and stored the material for a few days in 10% buffered formalin with "no appreciable damage" to surface structures. No one else reports using this method.

OsO_4 has also been used as the primary and sole fixative (6,63,90,121,122,126). Stern et al. (142) added 1-5% tannic acid to the phosphate buffered 2% OsO_4 during postfixation and tannic acid was used in several stages of tissue preparation by Pfeffer & Fisher (118) and Anderson et al. (3). Very clean surfaces resulted but none of the authors discusses the reasons for the use of tannic acid, and the method is not assessed by controls without tannic acid.



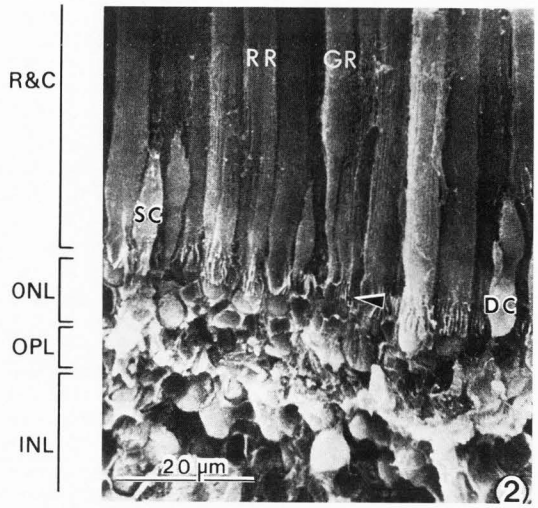


Fig.1 Anableps anableps L.- The South American four-eyed fish (16). The dorsal retina, in the light-adapted state, displays inner and outer segments of double cones (d), single cones (c), and rods, one of which is displaced (r). Oil-droplets (o) in the cone inner segments create a bulge. Calycal processes, inner segment longitudinal ridges, Müller cell microvilli (m) at the outer limiting membrane and PR nuclei (n) are all apparent. RPE granules lie on some cells. The shape of the photoreceptor (PR) cells are clearly shown. The RPE was deliberately removed.

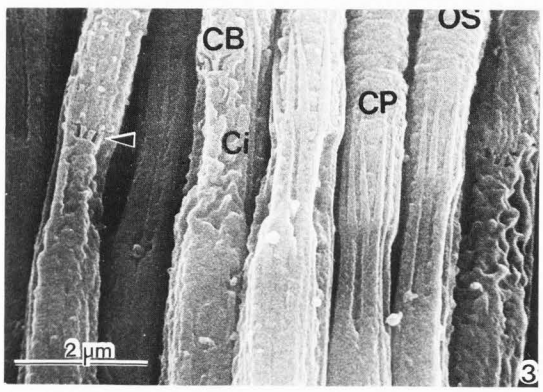


Fig. 2 Bullfrog (Rana catesbiana). Micrograph, courtesy of Dr. RH Steinberg. (140). The RPE had been removed and the photoreceptors exposed. The retinal layers are indicated: R&C - rods and cones; ONL - outer nuclear layer; OPL - outer plexiform layer; INL - inner nuclear layer. There are many red rods (RR), one green rod (GR), three single cones (SC) and one double cone (DC). The Müller cell microvilli in the area of the fins is clearly shown (arrowhead). Some extracellular matrix covers some of the PR. Ridges on the inner segments continue as calycal processes on the outer segments.

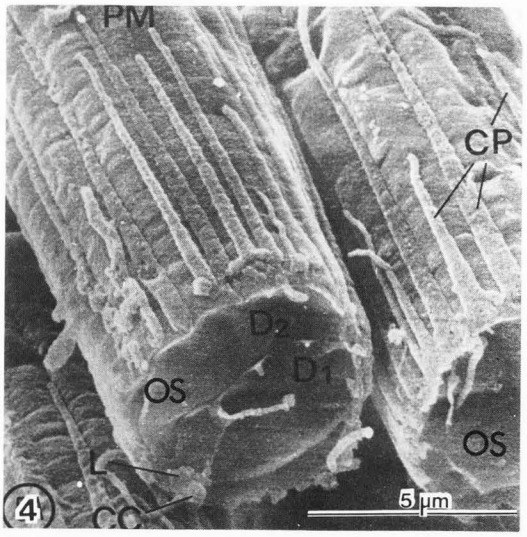


Fig. 3 Macaca mulatta - Rhesus monkey (13). Foveal cones are seen at the junction of the inner and outer segments. Calycal processes (CP) arise from longitudinal surface ridges on the inner segments and become free-standing structures surrounding the proximal ends of the outer segments (OS) except for an area around the ciliary backbone (CB). CP taper and vary in length. OS disks can be seen outlined on the OS surface. The cilium (Ci) has a very uneven surface. A few very short CP are associated with the cilium (arrowhead).

Fig. 4 Frog - Rana pipiens. Micrograph courtesy of Dr. KR Peters. (117). Rod outer segments fractured in the ciliary region. Disks of progressively larger diameter (D_1,D_2) expand from the outer cilium, the stump of which is shown (CC), with a protruding lip (L) of the plasmalemma (PM). Calycal processes (CP) of unequal lengths lie on the proximal part of the outer segment (OS) and taper towards the OS apex.

For permissions see page 285.

A standard method has evolved

General Comments

Methods of specimen preparations for SEM in general were explored and refined (24,30,31,70). The methods used for retinae are so varied in so many ways that it is hard to decide which detail it is that produced the good results. In some studies several techniques are specified in the methods section but there is no discussion as to the relative merits of these methods (63,64).

There is not only great variation in the methods used so that it is extremely difficult to assess the efficiency of one particular component of the procedure, but there is also great variation in the kind and amount of information given. In some cases every step is detailed (43,105,118,133). I was not able to get translations in time of some Japanese papers (7,96,97,104,108,109). Especially where the SEM component is a small and incidental part of the study, often of a clinical, immunological or physiological nature, very little or nothing is described of the SEM preparation methods (28,42, 46,47,51,54,57,58,59,78,82,103,125,156,158).It is a measure of the fact that SEM has become almost a routine way of looking at retinal tissue.

A standard method (SM) has evolved and in outline it consists essentially of fixing retinal tissue in buffered aldehydes, either glutaraldehyde alone, or with paraformaldehyde, washing in buffer, post-fixing in buffered OsO_4, washing in buffer, dehydrating in graded alcohols (or acetones, less commonly), CPD, and coating with gold or gold-palladium. Variations from the SM are discussed below.

Separation of RPE and retina

There are problems in exposing particular cells to view. It is necessary for example to separate the retina from the retinal pigment epithelium (RPE) if the outer and inner segments are to be clearly displayed, or if the pigment epithelium is to be seen en face. (Figs. 5,6). Dark-adaptation is alleged to help the separation process. Sakuragawa & Kuwabara (133) found that soaking in hyaluronidase or weak ammonia did not help the separation of RPE. Slow and gentle peeling of the RPE with fine forceps after primary fixation in glutaraldehyde seems to effect the separation in the hands of an experienced operator (13,17,18, 42,89,133) or slow and persistent flushing with a gentle stream of fixative in fresh, dark-adapted fish retinae (16); in newts (40), the separation was helped by gently lifting with forceps. Puzzolo & de Simone (121) picked the retina off with tweezers with a quick sudden movement. Wickham & Adams (155) refer only to stripping the retina after fixing. Most papers do not describe how this separation is achieved when needed.

Cleaning retinal surfaces

One of the aims of SEM retinal work is to present clean surfaces, i.e. without debris, free from extracellular material, fibres, other cells or parts of cells, mucus or coagulins. Various methods have been used: washing with saline before fixation (27,48,107); with gentle shaking (133); washing with 20% ethyl alcohol for 24 hours after fixation (140) (Fig. 2); washing in several rinses of deionized distilled water after aldehyde fixation (112,134) or with cacodylate buffer (113); prolonged fixation (17 (Fig. 7), 18, 140 (Fig. 2), 143) and gentle ultrasonic vibration in distilled water, after fixation (79, 155). Peters et al. (117) published remarkably beautiful photomicrographs of frog retinal cells by high resolution SEM (Fig. 4). They washed the tissue well in frog Ringer solution both before and after glutaraldehyde fixation.

Pfeffer & Fisher (118) used an unusual method in their study of cat RPE processes ensheathing cone outer segments which yielded very clean clear surfaces. They used overnight fixation in cold glutaraldehyde, the overnight use of filtered tannic acid both in the sodium cacodylate buffer and between the two separated 1 hour immersions in OsO_4. For dehydration graded tertiary butyl-alcohol-ethanol-water series to absolute tertiary butyl alcohol was used, and several changes of isoamyl acetate (overnight) before CPD. Very thorough rinsing was a special feature of the process. Anderson et al. (3) used postfixation in three to five changes of phosphate buffered 2% osmium tetroxide alternating with phosphate buffered 1-5% tannic acid, dehydration in tertiary butyl alcohol and ethanol, and transfer to amyl acetate before CPD.

In order to see the basal surfaces of the Müller cells (MC) in the nerve fiber layer of the retina, digestion methods have been employed. HCl and collagenase were used between formaldehyde and OsO_4 fixations to remove the basement membrane covering the cell, a method adapted from work on the kidney. This revealed a mosaic pattern of the MC foot processes in a number of vertebrates, but it was not possible to determine how many foot processes arise from one MC (90).

Thiocarbohydrazide processing was introduced by Malick & Wilson (86) and Malick et al. (85) as a way of bypassing the need to coat specimens with metals such as gold and palladium, and thus make the true surface of cells more readily apparent. Borwein (13) (Fig. 3) and Borwein et al. (15) (Fig. 8) found that the method helped to produce cleaner surfaces but they and others have modified the method and have used it in conjunction with coating (37,76,77, 134). There do not appear to be any studies of retina which have used thiocarbohydrazide instead of metal coating.

Cracking retinal tissue

Retinal tissue must often be cut or broken or somehow chipped or cracked and various ways of doing this are reported such as mechanical tearing with forceps or cutting with sharp

blades. Retinae have been cut with sharp razor blades after aldehyde fixation (16,40,77,79,92, 94,153); after OsO_4 fixation (1,119); or after CPD (73,140).

Freeze fracturing has been described for tissues in general by Boyde & Wood (24). Details are supplied by Germinario & McAlear (52) for retinal tissue frozen in Freon 12 cooled with liquid nitrogen. They recommended the method as quick and easy. The material was fractured by blade in liquid nitrogen and fragments freezedried in a Berkeley model freeze etch device, and an evaporator, and shadowed with platinum-carbon. Hansson (60,61,63,64) used fresh frozen retinae which they chilled in propane cooled with liquid nitrogen and freezedried at -70°C, or glutaraldehyde - fixed material quickly frozen in liquid isopentane chilled by liquid nitrogen and freezedried at -70°C (64,65). Retinal specimens have been quick frozen in liquid nitrogen and cryofractured using chilled razor blades (77) and then thawed in 70% alcohol (39) or freezedried in ethanol (37), according to the method of Humphreys et al. (71) or passed through liquid argon and liquid nitrogen and freezedried in a Balzars Micro BA3 freezedrying unit (82). Ali & Wagner (1) freezefractured fish retinae.

Tanaka (145) described frozen resin cracking methods where the resin- embedded material (without catalysts) was cracked with a thick blade and a hammer. The plastic was then removed by propylene oxide and the specimens were CPD. Renard et al. (129) used resin-cracking methods for guinea pig eyes with chorioretinitis. They used Epon 812 (without catalysts), in gelatin capsules, hardened at -78°C in solid CO_2 and ethanol mixture. The hardened capsule was placed on a teflon slab and cracked with blade and hammer. The Epon was then removed by propylene oxide, and the tissue CPD.

Jensen & Prause (73) modified Tanaka's (145) methods for ocular tissue, including retina. They used both frozen-resin cracking and dry-cracking methods, and also digestion by the enzymes trypsin, hyaluronidase and collagenase in a comparative study for assessing the advantages and limitations of these methods. Aldehyde fixed material (2-24 hrs) was post-fixed in OsO_4 and dehydrated in graded acetone. For resin-cracking the tissue was embedded in Epon 812 (without catalysts) for a minimum of 12 hours, placed in gelatin capsules, and hardened at -78°C in a mixture of solid CO_2 and ethanol. "Boiling" in CO_2 ceased after 2-3 mins, and the hard capsule was coated with the freezing mixture and cracked on a teflon slab with hammer and thick blade, and the resin was immediately removed by washes in propylene oxide, followed by a wash in amyl acetate and a final wash in acetone. The specimen was then CPD from CO_2 and coated with gold-palladium. A good photomicrograph of monkey retina (frozen resin-cracked) is shown. They used dry-cracking as well, from CPD specimens, but comparative photomicrographs are not available and they were not able by these methods to crack a retinal cell to see its interior. The micrographs are good but not obviously superior in quality to those obtained by other methods. They leave one wondering if this method is markedly better for most purposes than the standard method. These methods have either not been much tried or have not proved very successful for photoreceptors in general and certainly not for their interiors. Hansson (65) has a micrograph of a fractured synaptic body from the outer plexiform layer of cat retina. In it there is an angular structure which he interprets as a synaptic ribbon.

In dry cracking after CPD the tissue is very fragile, so great care must be exercised and the tissue coated immediately to discourage water absorption. It is often difficult to know which is the desired cracked surface after dry cracking. Jensen & Prause (73) found that simple dry cracking "is as good - or even better (than resin-cracking) - for more membranous tissues such as retina, uvea, etc." Resin cracking was preferred for more solid tissues such as cornea and melanomas. They were able to see inside cells from malignant choroidal melanomas but not inside retinal cells. The fully fixed tissue was also exposed to 1% solutions of trypsin, hyaluronidase and collagenase at 37°C for 1 hour and then processed to CPD and coating. The enzymes had different effects. Collagenase did not affect the plasma membrane, the surface processes were preserved, but fibrils between cells were digested. They recommend exposure of 1 hr at 37°C for all ocular tissues and for all three enzymes; but they warn that this period of treatment will not expose the interior of cells.

Plastic casts of retinal vessels

Plastic vascular casts of retinal and choroidal vessels have been made by the introduction of substances which will fill and then harden in the vascular system, after which the retina is digested away (49) and the plastic vascular cast is viewed by SEM. The results are not only dramatic and beautiful, but also very informative. Quigley et al. (125), in order to study the optic nerve capillaries in optic disk pallor in Macaca fascicularis, followed methods developed by Van Buskirk (151), and injected liquid methyl methacrylate via both carotid arteries. The vascular cast was then coated with gold-palladium. There were difficulties. When they tried to divide the optic nerve head in half with a razor the adhesion of the nerve head vessels to the adjacent scleral and choroidal vessels was broken, orientation was lost, and only a limited view of the surface vessels was possible. But where the casts of the optic disk capillaries were satisfactory there was good resolution of detail and they used these for their quantitative study of optic nerve head capillaries in experimental optic disk pallor. They concluded that they could not assume that there is a loss of capillaries in pale optic disks, but rather there is a loss of nerve fibers and a thinning of the neural tissue of the rim of the optic disk. Gole et al. (56) used corrosion vascular casting, with methyl

methacrylate, in an SEM study of oxygen-induced retinopathy in kittens, and found that it differs from the disease in the human neonate, where there are many mesenchymal arterio-venous shunts. In the kitten these are a minor component, the major feature being preretinal and intraretinal neovascularization. They prefer to use the term oxygen-induced retinopathy for the kitten disease, and retrolental fibroplasia (RLF) or retinopathy of prematurity for the human disease because they are morphologically different. The kitten is a poor model in that it develops neither scarring nor detachment of the retina, but it does reliably produce retinal neovascularization.

Yoneya & Tso (159) induced retinal neovascularization in kittens 5-9 weeks old, and studied the retinae by combined SEM, TEM and light microscopy (LM). Mercox was injected into the left ventricle for plastic vascular casts. They found 5 types of retinal and vitreal new vessels, each type with a distinctive location and structure. Risco et al. (130) studied the "angioarchitecture" of the ciliary artery circulation of the optic disk posterior pole, including the capillary plexus that anastomoses with the outermost retinal capillaries, by the use of vascular casts. They followed a "simple, rapid and reliable" technique set out by Risco & Nopanitaya (131), an injection-corrosion method, to study the blood vessels of the cat eye, including the retinal vessels. They used a plastic injection medium of reduced viscosity that allowed uniform filling of capillaries via the common carotid arteries after heparinised saline perfusion. The ocular tissue was left to harden in 80°C water for 4 hours and then digested in 40% KOH (changed twice daily) for 4 days, at 60°C. The air dried casts were then carefully dissected and lightly coated (10 nm) with gold-palladium. The casts proved more stable under heavy electron bombardment than previously used plastics. Beautiful pictures were obtained of retinal capillaries in 2 layers within the retina, of arterial branching, the microvessels of the optic nerve, and the lobular distribution of the choriocapillaries in cat and Macaca fascicularis. Annulae of endothelial cells, which probably act as arteriolar sphincters, were always seen as indented impressions at branching sites.

Immuno-labelling of retinal cell surface markers

It is possible by SEM to visualise antigens and receptors on cell surfaces. The SEM has been used to study cell surface labelling of retinal cells, specifically the photoreceptors (PR). Surface specific molecules such as antibodies or lectins interact with macromolecular complexes and provide markers on the cell surface that are clearly visible by SEM (101). The markers need to be uniform in size and shape, and distinctive, so that they can be separated from other cell surface particles. They must be stable and show little or no nonspecific binding to the cell surface, and they must label specific cell surface components (102). Visualisation and localisation of retina cognin in embryonic chick retinal cells by immuno-labelling and SEM has been studied (8,9,114). Retina cognin is described as "a retina-specific cell surface glycosylated protein that mediates self-recognition and morphogenetic contact associations of embryonic retina cells" (114). PR processes have no surface cognin sites but the cell body does, and the antigenic properties of its surface membranes differ also from those of other retina cell surfaces. The difference in antigenic properties between the membrane of the PR process and the rest of the PR cell membranes, separated at the outer limiting membrane tight junctions, arises early in embryonic development, by the 9th day in chick embryos (9).

The identification and characterisation of multiple forms of rhodopsin and minor proteins in frog and bovine rod outer segment (OS) disk membranes were studied by electrophoresis, lectin labelling, proteolysis, biochemical analyses and SEM. Visualisation of the Con A and wheatgerm agglutinin (WGA) binding sites on the extracellular surface of the plasma membrane was achieved by SEM (103). Cell surface labelling of chick embryonic neural retinal cells under different conditions (low temperature, energy inhibitors, cytochalasin B and colchicine) was investigated by TEM and SEM by Cohen et al. (35).

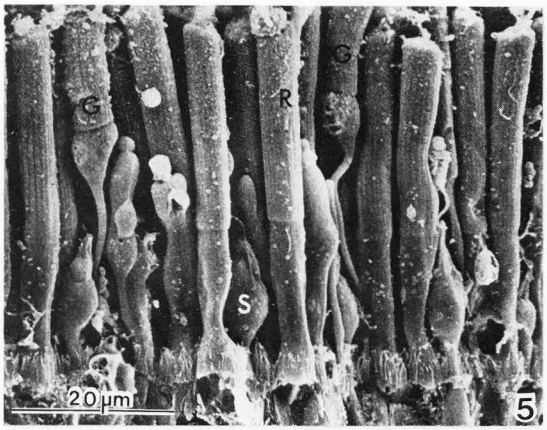
In a many faceted study by Bridges & Fong (28) on the use of lectins to investigate photoreceptor membranes, SEM was used to show binding sites of fluorescent fluorescein isothyocyanate (FITC)-Con A on frog PR.

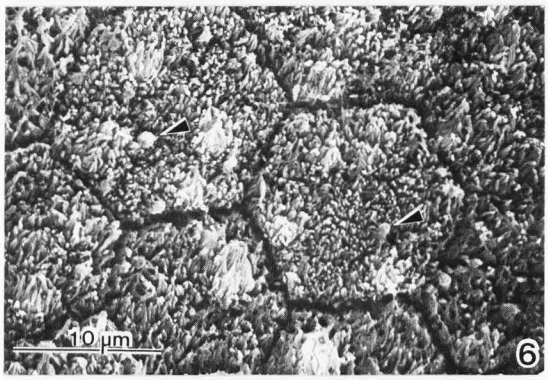
Immunolabelling of rhodopsin in whole mouse retina was studied by SEM by Jan & Revel (72). Rhodopsin on the rod surfaces of glutaraldehyde-fixed mouse retina was treated with rabbit antibodies specific against bovine rhodopsin and then with hemocyanin-labelled goat IgG's specific against rabbit antibody. Hemocyanin label showed that rhodopsin on the rod OS (ROS) cell membrane exposes its antigenic sites to extracellular space but such sites are scarce near the base of the ROS. This confirmed their findings on sections of immunolabelled retinae.

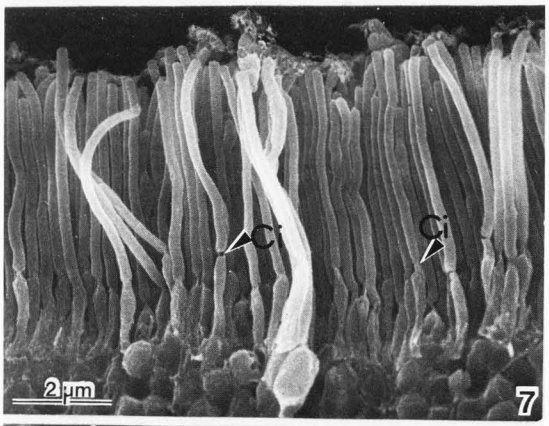
Concanavalin A (Con A)-methacrylate micro-sphere conjugates were used to label Con A binding sites on bovine rod cells, and these were seen by SEM on the ROS, rod inner segments (RIS), at the rod synaptic regions, and on the disk membranes (100). The SEM has proved to be very useful for these surface marking experiments.

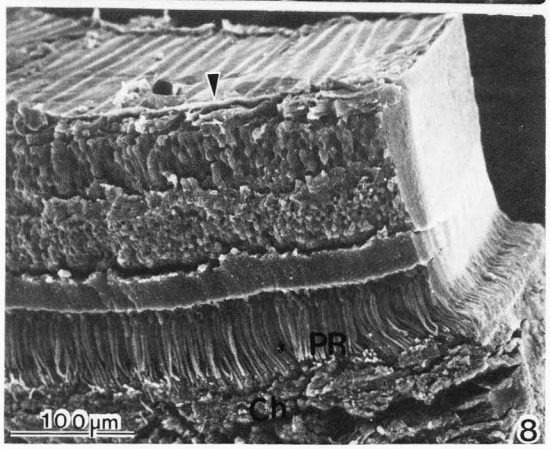
Embryology of the retina

SEM has been used particularly well in the study of the embryologically developing retina where cells change position, and die; new cells arise and sheets of cells form, change shape and positions. Large areas of tissue can be seen to advantage in these SEM studies of developing fetal retinae and associated tissues. Various aspects of the embryology of the retina have









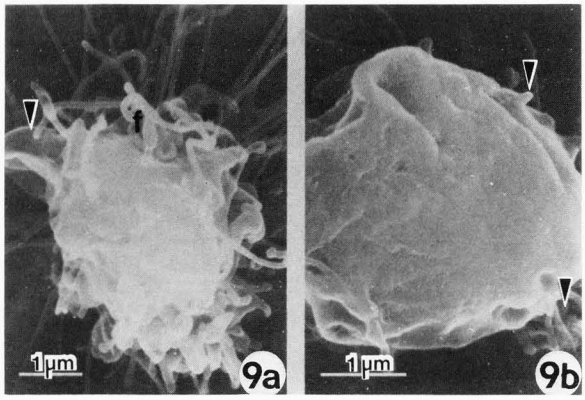


Fig. 5 Bullfrog (Rana catesbiana). Photograph, courtesy of Dr. T Masutani. (89). Red rods (R), green rods (G), single cones (s) and Müller cell microvilli at the outer limiting membrane are shown. The green rods have very tapered inner segments.

Fig. 6 New Zealand black rabbit. (Borwein unpubl.) Normal RPE cells, deliberately separated from the outer segments, show their hexagonal cell outlines and abundant apical microvilli, some of which are in clumps. A few rod outer segment fragments can be seen (arrowheads).

Fig. 7 New Zealand black rabbit. (17, Photograph by Dr. M Sanwal). The great variation in PR form is illustrated by Figs. 1, 2, 3,4,7,14 and 15. The rod inner segments are short and expand somewhat towards the ciliary region. The outer segments are about 3 times the length of the inner segments, but not all of equal length. The connecting cilium (Ci) is very clear (arrowheads).

Fig. 8 Macaca mulatta. (Borwein unpubl.) A block of retina tissue is seen from the vitreal surface to the choroid (Ch). The axons from the ganglion cells can be seen coursing on the vitreal surface, at the inner limiting membrane (arrowhead). The photoreceptors (PR) abut on the RPE.

Fig. 9 10-day chick embryo neural retinal cells. Micrograph, courtesy of Dr. Y Ben-Shaul. (10). (a) These cells had been trypsinised for 5 secs as a control for longer treatment. Freshly dissociated cells have long filipodia (f) and ruffles (arrowhead). (b) Cells treated with protein synthesis inhibitors which suppress re-aggregation have very few filipodia and only a few blebs (arrowheads).

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Fig. 1	Journal of Morphology (16)
Figs. 3,17	Anatomical Records (13)
Figs. 7,10	Canadian J. Ophthalmology (17)
Figs. 12,13	Invest. Ophthalmol. & Visual Sci.(18)
Fig. 16	Vertebrate Photoreceptor Optics, Springer Verlag (12)

been studied very effectively by SEM. Ben-Shaul & Moscona (10) (Fig. 9) found that filipodia seem to play an important role in bringing together in clusters chick embryo retinal cells that had been separated by trypsin digestion. Single dissociated cells of chick neural retina were labelled with Con A-hemocyanin to study the formation of filipodia, lobopodia and blebs from their membranes (11). Breipohl et al. (25) showed that the surfaces of the RPE in chick embryo and chicks sprout fine membrane processes on both their retinal and choroidal surfaces, the former appearing before the latter in development. They emphasised that the SEM is useful not only for tissue surfaces but also for fractured embryonic tissues. Hilfer (69) found that the three dimensional (3-D) image produced by the SEM to be "exceptionally useful when applied to the study of developing organs". They studied the development of the eye in chick embryo, including the extracellular materials that are found on all cell surfaces (68) and some of this granular material was visualised by precipitation with cetylpyridinium chloride. Its greatest concentration was found when invagination was at its peak. Breipohl et al. (26) followed retinal development in chick embryos at 12, 16, and 20 days and in 2 day old chickens by SEM. Breipohl et al. (27), as part of an SEM study of the sensory receptor cells in different vertebrates, looked at aspects of their development in chick embryos (12 day and 16 day), and included retina in the study.

In a combined TEM & SEM study, Galbavy & Olson (48) followed the developmental changes of albino rat photoreceptor cells, mainly rods, from immature cells at birth to maturity at three weeks. The maturation proceeded slowly in the peripheral retina. They were the first to present "a topographical analysis of [mammalian] photoreceptor cell morphogenesis" by SEM. The large increase in the number of rod cells occurs up to post-natal day 4, and by day 8 there is no significant increase in cell number. It is significant that in albino rat the inner segment remains smooth throughout development unlike that of the chick (94) where the developing cone IS is covered by microvilli, which later largely disappear, leaving only a few which remain as calycal processes. The adult albino rat rod lacks calycal processes. With rare exceptions the SEM shows calycal processes very well (12,13,14,15,16,19), better than longitudinal sections in TEM. (Figs. 3, 4, 8)

Suburo et al. (143) followed the growth of optic axons in chick embryo development in the basal intercellular spaces of the retina by SEM. They were unable to determine what factors orientate the ganglion cell axon growth towards the choroid tissue. No preformed pathways for axons could be seen by SEM, nor could they distinguish between neuroepithelial and immature Müller cells. They added 0.5% cetylpiridinium chloride to the fixative which they found hardened the vitreal surface considerably, and they also used trypsin and HCl collagenase methods.

SEM was used to study the 3-D architecture of the matrix in the interspace between the presumptive lens and presumptive retina of the chick embryo, and the reaction of basement membranes lining the space to enzyme digestion (153). By SEM the space was seen to be "filled with a coherent fibrous mesh identical to and continuous with a fibrous network lying on the surface of the basement membranes lining the space", and this could account for the binding of the two epithelial layers.

McAvoy (92) in the rat embryo used SEM to explore the mesodermal cells that are pushed aside as the optic vesicle approaches the ectoderm, the various cytoplasmic processes from optic vesicle/cup and the presumptive lens, (before day 13), and the network of fibrils in the interspace, which the author suggests has a role in coordinating the invagination of lens placode and optic vesicle.

Meller & Tetzlaff (94) and Meller (93) followed development of the retina in chick embryo and chicks by SEM only. The sequential development of the PR of the chick retina was studied in chick embryos from day 7-18 (111) and day 7-21 (112) by SEM only, and by correlated TEM-SEM from days 2-20 (113). I have been particularly interested in these papers because of my recent work on calycal processes (CP). These authors have shown that in the chick at least, the CP arise from the abundant microvilli (MV) that are seen on the developing IS by day 15. As development proceeds these MV become less numerous but a few persist as CP. Olson (113) suggests that the MV have an important nutritional role during development. The function of the CP is unknown.

Morphogenetic cell death is a feature of early retinal development where dead cells form necrotic areas. Garcia-Porrero & Ojeda (50) used chick embryos in a combined LM, TEM and SEM study to describe phagocytosis of these necrotic areas. By SEM it was clearly seen that the perinuclear zones of neighbouring neuroepithelial (NE) cells phagocytise fragments of dying cells and then digest them, acting as "non-professional phagocytes". They also studied (51) by SEM the nuclear migration in normal chick neuroepithelial cells during normal embryonic development (stages 14-16) of the retina, using colchicine to block metaphase. This gave rise to changes in cell shape, freed cells that were then detached into the lumen, and induced degeneration in cells, all this increasing with greater colchicine exposure.

Grant et al. (59) used SEM and also silver stained serial sections for LM to describe a staging series for retinal histogenesis in Xenopus laevis. Ocular defects resulting from teratogenic doses of trypan-blue in rat fetuses (16 day) were studied by SEM and TEM by Schmidt et al. (134) in which only 2 of 41 severely abnormal eyes showed any retinal rudiments; eyes with mild microphthalmia always had retinae, but with defects. Trypan-blue probably causes a failure of optic vesicle development, and the absence of retina leads to arrested lens development.

Embryonic retinae were used in tissue

culture by SEM to compare retinoblastomas and normal fetal retina (148). Retinoblastoma had been thought by some to be of glial origin; others have suggested that it arises in the photoreceptors. In this study, SEM surface topography of cells helped to classify the cell types seen and indicated that different cell types co-exist in the retinoblastoma cultures, but most cells had features which identified them as of either glial or neuronal origin. They suggest that the retinoblastoma arises from a multipotential stem cell which, although neoplastic, can differentiate into both glial or neuronal cells including photoreceptors. The authors discuss the difficulties inherent in identifying cells in tissue culture and also the advantage of seeing the tissue culture cell surfaces by SEM which helped them in the identification of cell types.

Retinal diseases (See Appendix II)

Increasingly SEM has been used on its own, or as an adjunct to TEM and LM, in the study of a wide variety of retinal diseases or ocular diseases that affect the retina. Hansson (63) was the first to use SEM alone to study the long term effects of a poison (sodium glutamate) on the rat retina, having first described normal rat retina by SEM (61,62,64). Sodium glutamate destroys the inner retina creating large cystoid spaces, and it thins the retina by half but the inner segments and outer segments of the photoreceptors and RPE remain normal-looking. The author says both TEM and SEM were used, but only photographs by SEM are shown and discussed. Hansson (60) also reported on Vitamin A deficiency in rats as seen by SEM.

Oxygen-induced retinopathy in kittens, which was found to differ from human neonate retrolental fibroplasia (RLF) in some respects, was studied by corrosion vascular-casting SEM (56).

The ultrastructure of the preterm human retinal mesenchymal spindle cells of the nerve fiber layer (NFL) in retrolental fibroplasia (RLF) was studied from tissue donations from a neonatal intensive care unit where preterm infants had been treated with daily oral doses of Vitamin E (100 mg/kg body weight), and compared to controls given much less Vitamin E (5 mg/kg body weight) (78). TEM, LM and SEM were used. Two SEM photographs show the 3-D stacking of spindle cells in the NFL of a control retina with severe RLF compared to the scarcity of spindle cells in an infant with mild RLF treated with high doses of Vitamin E. If started on day 1, high doses of Vitamin E proved efficacious in reducing the severity of RLF. The proliferation of spindle cells and the formation (and the area) of their gap junctions is suppressed by Vitamin E, while continuous oxygen increases the gap junction formation area.

In an attempt to understand processes involved in massive periretinal proliferation, a serious complication of retinal detachment, experimentally created retinal tears and detachments in pigmented rabbit eyes were treated with xenon arc photocoagulation (110) and the resulting changes in the glial cells were observed by SEM. Glial cells caused a protuberant swelling of the retina around the tear after 4 weeks, and after 6 weeks the internal limiting membrane was perforated by glial cells (fibrous astrocytes) which later proliferated in the repair process in the space between the retinal tear and the retina. Retinal detachment in cats was described by Anderson et al. (2).

The changes in pigment epithelial cells following experimental retinal detachment in the rabbit were studied by SEM and TEM combined by Okhuma (109). He saw RPE cells protuberant towards the retina, migrating RPE cells, and small sized RPE cells implying mitoses. He suggested that the subretinal macrophage were of RPE origin.

Stages in the repair of the RPE after xenon arc photocoagulation in detached retinae in the rabbit were described by SEM from day one to one month after photocoagulation. The resulting coagulated necrosis was removed by macrophage of RPE origin. RPE cells slide over, and new, small RPE cells arise by mitosis, and together they cover the lesion (97).

Frambach and Marmor (46) made non-rhegmatogenous detachments in living rabbit eyes by injecting a few microliters of different fluids into the subretinal space. They studied the rate and route of fluid resorption from the subretinal space. The scanning electron micrographs display blebs and bleb surfaces associated with the RPE and photoreceptors in these experiments.

In a mainly TEM study, SEM was also used to describe the histology of experimental retinal detachment and reattachment of cat retina and its clinocopathological correlations (142). Only one (but a very clear) photograph by SEM is included, the appearance of the RPE six weeks after detachment. Re-attachment within one week results in good photoreceptor regeneration and orientation; longer periods of detachment produce poorer results.

Giereck et al. (53), with SEM, looked at the orifices in a human retina that resulted from diathermocoagulation for detached retina soon after the treatment, before secondary inflammation processes occurred. This was an unusual opportunity to see the depth and extent of the pathological processes and one of the earliest clinical uses of SEM.

Both TEM and SEM were used to explore the retinae of 3 patients with trisomy 18 (the second most common human autosomal trisomy) (47). The retinae had immature features and the posterior RPE resembled human albino RPE in that there were few mature melanosomes. There were anomalies in the outer retina, and there was optic nerve head hypoplasia. They suggested that pigmentation is important in the control of maturation of the neural retina.

Auto-immune uveal retinitis induced in guinea pig eyes by injection of autologous retina is more violent (129) than that induced by bovine retinal antigens (128). The disease, involving at first disorganisation and later the

loss of the visual cells and the disappearance of the RPE cell microvilli, leads to retinal detachment. Finally the RPE is atrophic, OS and IS decay, and a scar is formed by fibroblasts and by proliferation of epithelioid cells, all described by SEM only (128,129).

Aspects of retinal detachment were studied by Marmor et al. (87,88).

Quigley & Addicks (124) studied the optic nerve head by SEM to locate regional differences in the lamina cribrosa in chronic glaucoma. They found that the superior and inferior parts of the lamina cribrosa contain larger pores and thinner connective tissue support for the nerve fiber bundles of those ganglion cell axons that are more readily damaged in glaucoma than are those of the nasal and temporal zones of the lamina cribrosa. This is most probably the area, at the sclera, that seems to be the site of the initial cellular insult. Detergent digestion methods were compared to (and found better than) trypsin digestion with ultrasonic disruption, in both TEM and SEM. Specific details of the methods used are given. The methods do not remove the basement membranes of the surface retinal vasculature and these were removed with "ultrafine" forceps and scissors under the dissecting microscope before aldehyde fixation, in order to fully expose the lamina cribrosa. They were able to see the 3-D view of the lamina cribrosa, its 200-400 pores through which the nerve fiber bundles pass, and the differences in the connective tissue of the lamina sheets and septa, which enabled them to show clear regional differences that correlate well with the known pattern of damage in glaucoma. Quigley et al. (125) used vascular casts of the optic nerve head capillaries as part of their study (by LM, SEM, fluorescein angiography and an image analysis system) of experimental optic disk pallor. They found that the pallor resulted from thinning of the neural tissue of the rim of the optic disk and not to a loss of capillaries, nor to proliferation of astrocytes.

Göttinger (57), by SEM only, looked at peripheral retinal cystoid degenerations (PCD) and reticular inner cystoid degenerations in man. The SEM photographs dramatically show vault-like cavities supported by columns which are surrounded by and connected to each other by collagenous fibers. Göttinger (58), by a combination of SEM and TEM, described the formation of basement membranes and collagenous fibrils in PCD and senile retinoschises. The collagenous fibrils accumulate between the neural elements and glia and are compressed into columns which then separate cyst-like spaces. The 3-D view by SEM is especially valuable in studies like these.

In a case-report of proliferative diabetic retinopathy in a 24 year old woman who had had argon laser treatment, trypsin-digest preparations of post-mortem retina examined by SEM showed loss of photoreceptors and RPE. TEM and LM were also used. In the text, it is said that SEM revealed microaneurysms, fusiform vascular dilatations, and marked loss of pericytes but there is no photomicrograph of microaneurysms. The micrographs show fusiform dilatations, but only normal retinal vessels with pericytes, not those with pericyte-loss.

A rat model for chronic retinitis was studied by ophthalmological examination, electroretinograms, TEM and SEM (38). Two SEM photographs are included, one of which, their Fig. 8, is both very clear and very good, but the information value from it is largely derived from correlation with TEM, which is often the case. The value of the information gleaned from SEM often depends to some degree on prior knowledge derived from LM and especially TEM.

Cell outgrowths in cell culture from abnormal human vitreous specimens obtained at vitreous surgery from patients with massive periretinal proliferation were examined by TEM and SEM (106). Four major cellular constituents were identified as components of these proliferating vitreal membranes: macrophage, RPE-like cells, glial-like cells, and fibroblastic cells, SEM aiding in cell identification.

Using LM, TEM and a small component of SEM, a human retina was examined and described. The eye had developed an immune response ophthalmitis following surgery for glaucoma. There was considerable macrophage invasion, seemingly via the nerve fiber layer and the sub-RPE space. There was an almost complete absence of inflammatory cells. The intact RPE, by metaplastic changes, probably gave rise to the abundant macrophages (82).

Retinal dystrophy in rat and man, while not identical, share certain features. The rat disease was studied by SEM by Herron (67), and there is a fine photomicrograph of latex sphere phagocytosis by both normal and dystrophic RPE. The dystrophic RPE fails to engulf the shed ROS disks. The rat is the only model available for the study of human retinal dystrophy. Normal rats, pink eyed and pigmented, were compared, by SEM only, with retinae from congenic dystrophic rats homozygous for the blind allele (Hunter strain and Royal College of Surgeons strain) (127). The details of the dystrophic disease are recorded from SEM micrographs. The disease does not seem to affect the bipolar, amacrine and ganglion cells.

The retinae of a mutant strain of chickens suffering from congenital blindness with retinal degeneration were compared by SEM to the normal chicken retina (149). The early lesions in the disease were similar to human retinal degenerations. The study is an adjunct to a LM study (150). The disease affected primarily the photoreceptor outer and inner segments, with reactive changes in the RPE. Double-cones were somewhat more resistant to the disease.

In 2 human eyes from a 24 year old man with fundus flavimaculatus with atrophic macular degeneration, a study using fluorescent microscopy, LM, and SEM showed that, starting near the equator and becoming more prevalent and more abnormal posteriorly, the RPE contained hypomelanotic cells of markedly different sizes, in irregular groups, surrounded by normal-looking cells. Massive amounts of lipofuscin

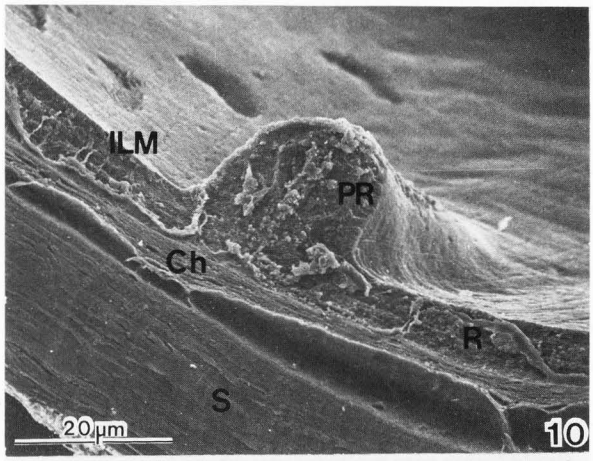


Fig. 10 New Zealand black rabbit (17). The retina had been exposed to a laser, wavelength 570-600nm, spot size 75 µm, energy density 10 joule/cm^2. The lesion shown here is 7 days old, and is sectioned through the full thickness of the retina. The retina (R) is swollen and humped up and folded so that the PR layer comes to lie in a central vertical line in the middle of the hump (PR). The choroid (Ch), sclera (S) and the inner limiting membrane (ILM) can be seen.

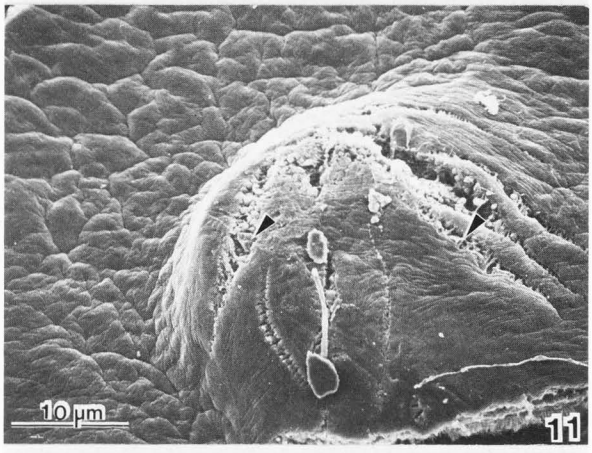


Fig. 11 New Zealand black rabbit (Borwein unpubl.) (17). The retina has been exposed to a laser as described for Fig. 10. In this case the damage is more extensive than in Fig. 10. Here, the inner limiting membrane has been perforated and cracked in several places and retinal contents have been extruded (arrowheads).

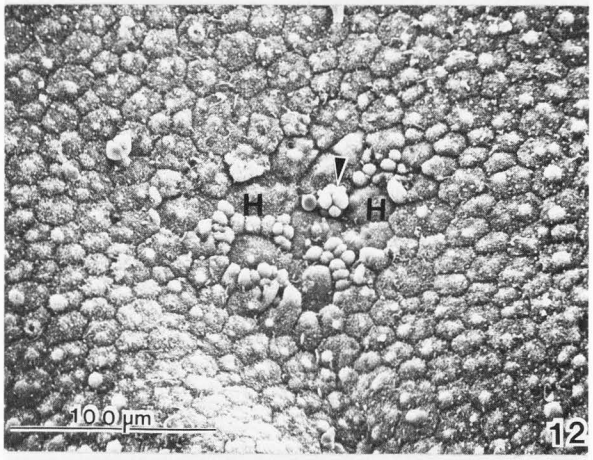
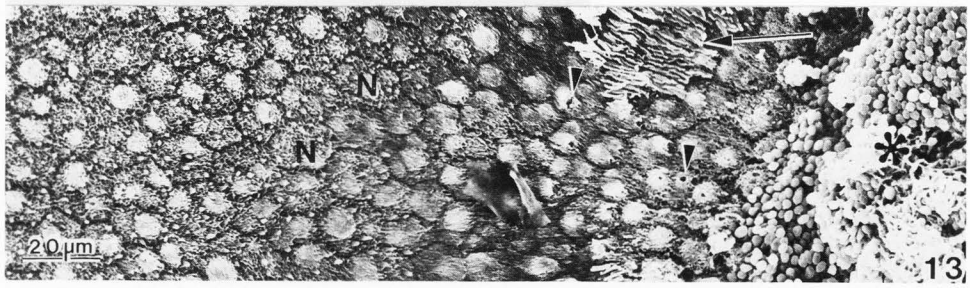


Fig. 12 New Zealand black rabbit (18). The retina had been exposed to a dye laser (585nm), output pulse duration 0.4 µ sec, minimum spot size 75 µ m diameter, the lasing energy 200-350 µJ. The RPE in the laser lesion area after 7 days shows hypertrophied cells (H) with microvilli, sparser and shorter than those in the neighbouring unaffected cells and small cells with abundant microvilli. These small cells are rounded and protrude from the RPE surface and in one place they are at least 2-layered (arrowhead).

Fig. 13 New Zealand black rabbit (18). The retina had been exposed to a laser as described for Fig. 12. The RPE was viewed after 1 hr. The centre of the lesion (*) contains nuclei, debris, red blood cells, and photoreceptors. Around it the microvilli are sparse and flattened in the direction of the lesion centre. Holes are seen in some cells (arrowheads). At the periphery of the lesion there are more microvilli, and normal RPE cells (N) border the lesion. (see Fig. 6). Outer segments are embedded in the microvilli (arrow). The damage levels show a gradient from the periphery of the edge of the lesion to the centre where there is most damage.



accumulated in the RPE cells. The authors (42) suggest the disease may involve disordered lipopigment metabolism. SEM photographs were used to count RPE cells, the density of which posteriorly was only 25% that in the periphery. An occasional torn RPE cell allowed a view of the cell contents, by SEM. This paper is a good example of how useful SEM can be in the study of some pathological retinal conditions.

Bovine GM_1 gangliosidosis produces disturbed vision. Eyes of 8 affected calves were studied by ophthalmoscopy, LM, TEM and SEM (135). The white spots seen on the fundus by ophthalmoscopy were seen by SEM to be protuberances on the vitreal surface of swollen retinal ganglion cells which contained dense aggregates of membranous bodies in their perikarya. These membranous bodies were found also in the amacrine cells. Optic nerves showed Wallerian degeneration. Gangliosidosis occurs also in man, cats, pigs and dogs. The dome-shaped protuberances of the vitreal surface and the large globular masses of the ganglion cell layer are shown in a scanning electron micrograph that illustrates them more clearly than any reconstruction from TEM could do.

Wakakura & Ishikawa (152) studied the ganglion cells of the cat retina by SEM. D, L-2-aminoadipic acid (AAA), a gliotoxic compound, was injected intravitreally into specific pathogen-free cat eyes and the eyes enucleated after the reaction time. AAA destroys Müller cells and astrocytes in a dose dependent fashion. Normally these mask the ganglion cells. Too much AAA also destroys retinal cells. These and control normal retinae were examined by LM, TEM and SEM, also retinae after optic nerve transection. The authors conclude that after AAA application SEM enables one to see in detail both the normal and the pathologic ganglion cells, which are different.

Aging retina

An interesting use of SEM was made by Yew & Ling Wong (157). They compared the counts of visual cell number (per unit area) under 5,000 X magnification in the retinae of 2½ month and seventeen month old albino mice. They could see phagosome formation as outer segments were pinching off. There were fewer photoreceptor cells per unit area in the older mice. The number of phagosomes were the same, but of smaller diameter than in younger retinae. There was also less mucopolysaccharide around the visual cells in the older retina, which may be an important factor in retinal detachment.

Light, Lasers, and other radiation

The effects of lasers on the retina has been widely studied by LM & TEM, but only relatively few papers using SEM have been published. Aoki (7) looked at the retina of rabbits immediately after ruby laser photocoagulation. Ohsawa & Miki (110) studied the retinal glial cells in rabbits after Xenon arc photocoagulation. Borwein et al. studied the normal and lased retina (17) and the normal and lased pigment epithelium (18) in rabbits, which showed the appearance of entire lesions (Figs. 10, 11) as well as the effects of lasers on cell surface morphology, such as the loss of the RPE microvilli in a graded way towards the lesion centre (18) (Figs. 12, 13). Meyers et al. (95) used the CO_2 laser to phototransect vitreal membranes in rabbits. Rodrigues and Currier (132) studied the histopathological effects of the argon laser in a case of juvenile onset diabetic retinopathy in a 24 year old. The argon laser is widely used to try to arrest proliferative diabetic retinopathy by destroying the proliferating vascular fronds.

Yew et al. (158) produced low dose retinal lesions with the type of helium-neon laser widely used in schools and research laboratories and showed that it did not cause obvious damage to the retina (or lens), but that exposure produced an increase in the intercellular substance around the outer segments, in a dose dependent manner, and at a peak amount 4 hrs after treatment.

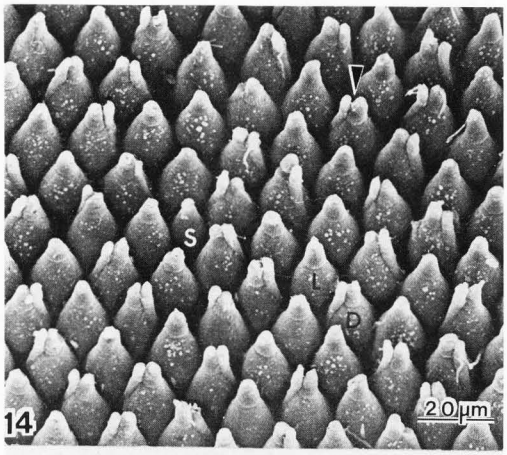
The retina of the roach, a teleost fish, was exposed to two types of laser, in the range of 450 nm-650nm, which irradiated a 1mm circular area, in order to study the light-evoked responses of neurones located in the irradiated area, by electrophysiology, TEM & SEM. Changes were found in the photoreceptor outer segments but not in their synaptic terminals, nor in the neurones (41).

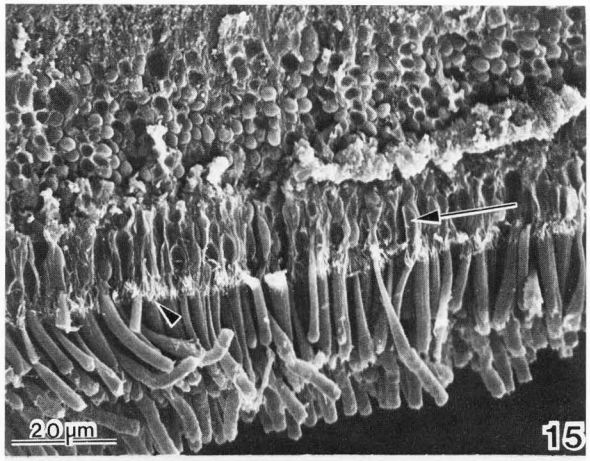
Albino rabbit retinae were examined by SEM 24 and 72 hours after in vivo exposure to 7000 rad. of X-radiation (107). Rods and cones showed the most severe damage in the retina. After 72 hours there was most damage to the inner and outer segments, and the outer nuclear layer showed somewhat more damage than it did after 24 hours.

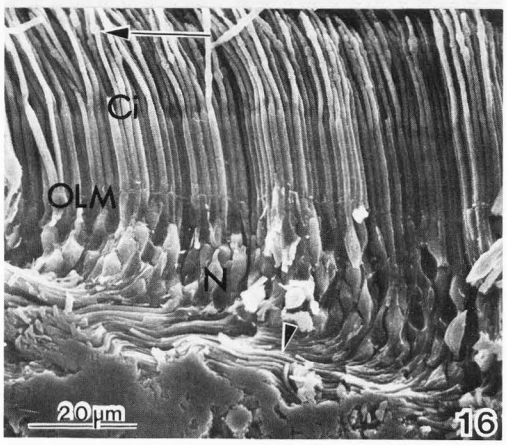
Continuous light damage in the albino rat retina has been assessed by SEM (80,123).

At the RPE-PR area of the inferior retina of the bullfrog, by SEM, Eckmiller & Steinberg (43) saw macrophage-like spherical cells with surface ruffles. They contained granules that resembled RPE melanosomes. These cells were associated with depigmented RPE cells. The conclusion that these cells were probably monocyte-derived, from the choriocapillaris, was reached largely by TEM. The authors suggest that these cells are not there in response to infection, but rather as a response to cumulative damaging effects of light exposure on the inferior retina.

SEM and TEM were used together to great effect in a study of RPE cultured cells' surface morphology and phagocytosis of latex spheres (44). Calf and rabbit RPE cells have a latent period of 12-17 hours respectively before the spheres were ingested during which time their surfaces were abundantly covered with cell processes, as were rat RPE cells which show no latent period, whether normal or dystrophic. These processes were identical in all the RPE cells. The authors concluded that differences in the latency periods were not associated with







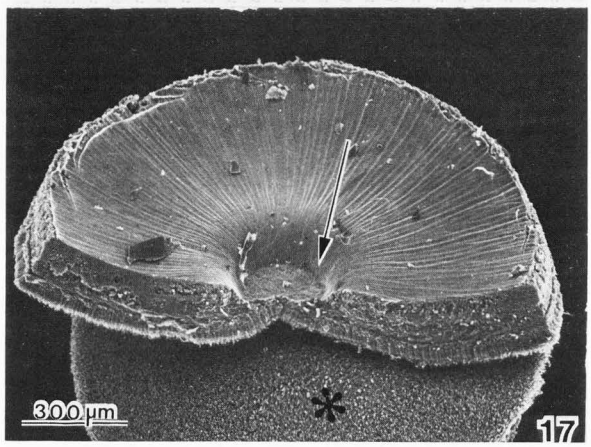


Fig. 14 Snake (Elaphe quadrivirgata). Micrograph, courtesy of Dr. T Masutani. (89). Surface view of visual cells. There are three types of cones, large single cones (L), small single cones (S) and double cones (D). Their mosaic pattern is beautifully displayed. Note the different outer segments of the unequal double cones (arrowhead) and the very plump inner segments of all the cones.

Fig. 15 Quail retina. (Borwein unpubl.) Note the elongated photoreceptor nuclei of the outer nuclear layer (arrow), the small and very numerous nuclei of the inner nuclear layer, and the narrow intervening outer plexiform layer. The Müller cell microvilli are very prominent (arrowhead). The inner segments are club shaped, enlarging towards the outer segments. Many of the outer segments have been broken off during tissue preparation.

Fig. 16 Macaca mulatta - Rhesus monkey (12). Cones in the fovea (see Fig. 17) are displayed. The outer segments have knobs, which are convolutions and foldings (arrow). There is a constriction of the photoreceptor at the cilium, and the ciliary region appears as a shadowy wavy line (Ci). The outer limiting membrane (OLM) appears as a thin line. The inner cone fibers of Henle (arrowhead) turn sharply at an angle to the photoreceptors and their nuclei. Note that when a PR nucleus is lost during processing a "nuclear nest" (N) made of Müller cell processes remains.

Fig. 17 Macaca mulatta - Rhesus monkey (13). A trephined portion of the retina, showing the foveal slope and pit (arrow). The detached RPE lies free below the outer segments (*). Note the thinning of the retina towards the foveal center.

apical RPE cell processes.

Polystyrene spheres and bacteria were taken up at equal rates by the RPE of chick embryo retinae in culture, as seen by both TEM and SEM (66).

The regeneration of the pigment epithelium microvilli in vitro in dystrophic RCS rats and pigmented rats was examined by TEM and SEM (144). In regeneration after RPE and retinal separation the RPE microvilli became thinner, denser and longer with length of incubation, but were denser and longer in situ than in vitro. Chaitin & Hall (32,33) studied the defective ingestion of rod OS by dystrophic rat RPE cells in culture. They assayed phagocytosis by using ROS antiserum and a double imunofluorescent labelling method. SEM was the technique used for visualisation of phagocytosis which was defective in the ingestion phase. They found that although actin functions normally in dystrophic RPE cells, the ingestion mechanism is activated at only a few sites, compared to the normal RPE cells.

Morphological Studies (see Appendix I)

Fish retinae and photoreceptor cells, both light and dark adapted have been described by SEM (1,16 (Fig. 1), 119,156).

Newt RPE and photoreceptors were studied by Dickson & Hollenberg (40), the mud puppy retina by Lewis et al. (84).

There have been several studies of aspects of frog retinae (89,98,99,156), but the most detailed and extensive is that of Steinberg (140). (Figs. 2, 5).

Masutani & Miyoshi (89) were able to display the mosaic pattern of the large single cones, the small single cones and double cones of a snake (Fig. 14). Yamada (156) also looked at snake retina.

Bird retinae have not been studied morphologically by SEM except for a few micrographs made available by Masutani-Noda & Yamada (90), Miller (98) and Yamada (156) (crow, duck, Zonotrichia). Quail retina has been looked at by Borwein. (Fig. 15).

Various aspects of the rat retina and RPE have been described by SEM (61,62,64,65,83,121, 122). There is one description available on a mouse retina (138), without photomicrographs. Combined SEM and TEM was used to study the capture of polystyrene microspheres of different diameters by normal and colchicine-treated pigment epithelium (75). SEM, stereoscopic pairs and TEM were all used to study the development of the highly specialised sheet-like projections from the pigment epithelial apical surface that ensheath cone outer segments of the adult cat retina and function in phagocytosis of cone outer segment disks (141).

Miller (98) displays a micrograph of a pig retina. Rabbits were studied by Antal (6), Borwein et al. (17) (Fig. 7), Hansson (65) and Leuenberger (83).

The endothelial cells of retinal vessels of cats and kittens were examined (108).

Miller (98) reviewed the effects of the eye's ocular optical filtering properties on eye function, which determines what information is available to the brain. In discussing the effects on this of the physical properties of the photoreceptor cells, their positioning and the movement of pigment, in retinae with or without photomechanical movements he shows three SEM photomicrographs of frog, pig and sparrow retinae.

There are three SEM photomicrographs displaying various features of monkey photoreceptors in Borwein's review (12) of retinal photoreceptor structure.

In a study of cone-specific c-wave in the cone-dominant turtle retina, the rod-dominant bullfrog was used as a control. SEM photomicrographs display essential comparative features of their photoreceptors and their differing relationships to the pigment epithelial processes (91).

By both TEM and SEM Steinberg & Wood (141) showed that in the tapetal region of the cat retina the cone outer segments do not reach to the apical surface of the RPE cell but are closely associated with RPE cell apical microvillous processes.

Monkey RPE was described by Sakuragawa & Kuwabara (133). Prause & Jensen (120) studied the frozen-cracked, drycracked and enzyme digested retina of a monkey, Cercopithecus, and of man. The adult macaque retina was described in general by Dickson et al. (39) and briefly by Kuwabara (79). The fovea and foveal photoreceptors of monkeys were described in detail by Borwein et al.(15) and Borwein (13). (Figs. 3,8, 16,17). Aspects of human retinal ultrastructure have been studied by SEM (36,37,45,104,126).

The only study of Bruch's membrane (BM) by SEM (with TEM also) was done by Goldbaum & Madden (55). Trypsin digestion of the RPE exposed the BM surface, the vitreal surface of the RPE and also the inner collagenous zone of the BM. The adhesion of the basal RPE cell membrane to the basal lamina of BM was stronger than the integrity of the cell.

High resolution SEM of cell organelles (146, 147) was made possible by new specimen preparation methods, an "osmium-dimethyl sulfoxide (DMSO)-osmium procedure", which removes cytoplasmic matrices, thus exposing intracellular organelles. The method has obvious applications to retinal SEM research. Tanaka (146) using the osmium-DMSO-osmium method, which is fully described, and a field emission SEM equipped with a high resolution device, saw intracellular organelles, including synaptic vesicles and the cytoplasmic surfaces of synaptic membranes of rat retinae. Peters (115) discusses conditions for achieving high resolution SEM in soft biological materials including retinal elements. Peters et al. (117) studied the ciliary region of frog photoreceptors (Fig. 4). They used an exchange technique (116) and a special new coating method which produces a 2.0nm average thickness compared to the 10-20nm thickness of the usual gold coating. For coating niobium and chromium films were used (1-2nm) and coating, thickness very accurately measured. The method allowed

high resolution and high depth of focus, and the authors were able to describe in fine detail a "periciliary ridge complex" (PRC) in frog photoreceptors on the apical surfaces of inner segments. Very beautiful micrographs (Fig. 4) were obtained with their ultrahigh resolution SEM in a new SE-1 imaging mode. Their detailed findings about the PRC were described for the first time by SEM, and when the 3-D model thus obtained was understood, they were then able to confirm the model by correlation with TEM. They discuss their methods and their findings in detail, and the significance of the PRC. This is a significantly important advance in retinal research using SEM and opens wide a new way of looking at retinal tissue by ultrahigh resolution SEM, where the SEM serves better than TEM.

Reviews

There are several review articles of retina and/or RPE which include SEM photographs, such as Borwein (12), Zinn & Benjamin-Henkind (160), and Kessel & Kardon (77) on SEM of mammalian neuro-epithelia. Kessel & Kardon's (76) text atlas of SEM includes some of their own material on retina. Theirs is the only SEM micrograph presented so far that displays a calycal process in an albino rat rod.

There are a few reviews about SEM in general that are of interest to those in retinal research (30,31,70). Techniques for the preparation of tissues for SEM are explored and discussed by Boyde & Barber (22), Boyde & Boyde (23), Boyde & Wood (24), Boyde et al. (21), Pfeffer & Fisher (118), Gannon (49), Humphreys et al. (71), Malick & Wilson (86), Malick et al. (85), Molday (101), Molday & Maher (102), Peters (115,116), Shimada & Murakami (137), Tanaka (145,146) and Tanaka & Naguro (147). SEM techniques specifically pertaining to the eye are discussed by Cleveland & Schneider (34) and Jensen & Prause (73).

Concluding Remarks

There has been disagreement as to whether SEM used on its own can do justice to the complexity of the retina, but as techniques and instruments have improved and knowledge about the appearances of cells and tissues by SEM has grown, it has become easier to assess what is and is not artifact, and to improve the quality of the micrographs and thus the amount of information gleaned from them.

The value of the SEM for viewing surface structures was recognised early. The dramatically beautiful appearance of the retinal cells in three- dimensional view in the SEM greatly enhanced our understanding of large structures seen previously by LM with less resolution. SEM made complex structures easier to understand (13,37,140) especially when used in conjunction with TEM. However, Peters et al. (117) have shown that with ultrahigh resolution SEM, the periciliary ridge complex of the apical inner segment is more easily understood than by TEM. Except for their work, seeing the interiors of retinal cells has not been achieved in any significant way. In many cases prior knowledge of the retina has enabled analysis of the SEM photographs. In other cases the SEM photographs have boldly highlighted surface structures and processes which were not easy to see and could be overlooked in TEM. SEM is particularly valuable not only for surface structures but also for understanding cell associations and interconnections and the distribution of cell types. Breipohl et al. (27) point out that by TEM it is hard to get an idea of volume and spatial distribution changes. In the domain of embryological development of the retina SEM has been used very effectively. In the development of the eye there is "a complex set of co-ordinated shape changes in the epithelial sheets that produce the optic cup and lens. These changes involve not only folding of the sheets into vesicles but also distortions of the cell surfaces and changes in the organisation of the intervening extracellular matrix"... (68,69). Although these embryological changes had been described by LM and TEM "the true relationships among the tissues becomes more recognisable as a result of the three-dimensional view obtained by SEM" (68,69). SEM has also proved useful in looking at laser lesions in the retina because one can simultaneously see cell position and cell surface changes.

The first studies of retina by SEM were descriptive of the surface morphology of particular cells or groups of cells. A glance at Appendix I will reveal that there has been a relatively limited sampling of vertebrate retinae. In the last ten years there has been a remarkable extension of the use of SEM from strictly morphological studies of the retina to experimental and clinical studies (see Appendix II).

Carr et al. (31) have described SEM as "topographical histology" because it provides 3-D relationships of cells and tissues. They feel that SEM relies more on the "visually arresting qualities of the scanning image than on unique information" but they recognise the economical value of SEM in "providing summary form of information" that would otherwise require extensive serial sectioning by TEM and LM.

There is no doubt that the dramatic 3-D views obtained by SEM have helped us to form clearer images of certain structures and these provide valuable information that go well beyond "the visually arresting qualities". These are, of course, immensely valuable for teaching purposes. There is also a great deal of intrinsically interesting information to be gathered from SEM studies of the retina. A new biology of "topographical histology" (31) has evolved. There are many papers that attest to the value of SEM in retinal research in providing new information, but even when the component of really new information is not very large, SEM studies in a few photographs can confirm, clarify, highlight and summarise previously known material in a way that profoundly enhances understanding.

References

1. Ali MA, Wagner HJ. (1976). Scanning electron microscopy of four Teleostean retinas. Rev. Can. Biol. 35, 199-210.

2. Anderson DH, Stern WH, Fisher SK. (1979). Transmission and scanning electron microscopy of the detached cat retina. Invest. Ophthalmol. & Visual Sci. 18: (ARVO Suppl., Abstract). p. 86.

3. Anderson DH, Stern WH, Fisher SK, Erickson PA, Borgula GA. (1983). Retinal detachment in the cat: the pigment epithelial- photoreceptor interface. Invest. Ophthalmol. & Visual Sci. 24:906-926.

4. Anderson TF. (1950). The use of critical point phenomena in preparing specimens for the electron microscope. J. Appl. Physics. 21: 724.

5. Anderson TF. (1951). Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope. Trans. N Y Acad. Sci. 13: 130- 134.

6. Antal M. (1977). Scanning electron microscopy of photoreceptors. Ophthalmol. 174, 280-294.

7. Aoki A. (1974). [Experimental studies on Ruby Laser Photocoagulation in the retina of pigmented rabbits. Part 4. Scanning electron microscopic findings in the retina immediately after photocoagulation.] (Japanese) Acta. Soc. Ophthalmol. Japonica (Nippon Ganka Gakkai Zasshi). 78, 780-791.

8. Ben-Shaul Y, Hausman RE, Moscona AA. (1979). Visualization of a cell surface glycoprotein, the retina cognin, on embryonic cells by immuno-latex labelling and scanning electron microscopy. Dev. Biol. 72, 89-101.

9. Ben-Shaul Y, Hausman RE, Moscona AA. (1980). Age-dependent differences in cognin regeneration on embryonic retina cells. Dev. Neurosci. 3, 66-74.

10. Ben-Shaul Y, Moscona AA. (1975). Scanning electron microscopy of aggregating embryonic neural retina cells. Exp. Cell Res. 95, 191-204.

11. Ben-Shaul Y, Ophir I, Cohen E, Moscona AA. (1977). SEM study of dissociated embryonic cell surface activity. Scanning Electron Microsc. 1977; II: 29-36.

12. Borwein B. (1981). The retinal receptor: A description. Vertebrate Photoreceptor Optics, Chap. 2. Springer Series in Optical Sci. JM Enoch & FL Tobey Jr., (eds.). Springer-Verlag, Berlin, 23, 11-81.

13. Borwein B. (1983). Scanning electron microscopy of monkey foveal photoreceptors. Anat. Rec. 205, 363-373.

14. Borwein B. (1984). The calycal processes of vertebrate photoreceptors. Invest. Ophthalmol. & Visual Sci. 25: (ARVO Suppl., Abstract). p. 63.

15. Borwein B, Borwein D, Medeiros M, McGowan JW. (1980). The ultrastructure of monkey foveal photoreceptors with special reference to the structure, shape, size, and spacing of the foveal cones. Amer. J. Anat. 159, 125-146.

16. Borwein B, Hollenberg M. (1973). The photoreceptors of the "Four-eyed" fish. Anableps anableps L. J. Morph. 140, 405- 442.

17. Borwein B, Sanwal M, Medeiros JA, McGowan JW. (1976). Scanning electron microscopy of normal and lased rabbit retina. Can. J. Ophthalmol. 11, 309-322.

18. Borwein B, Sanwal M, Medeiros JA, McGowan JW. (1977). Scanning electron microscopy of normal and lased rabbit pigment epithelium. Invest. Ophthalmol. Visual Sci. 16, 700-710.

19. Borwein B, Smith S. (1984). Calycal processes of vertebrate retinal photo-receptors. Proc. Int. Soc. for Eye Research III, (Abstract) p. 6.

20. Boyde A. (1972). Biological specimen preparation for the scanning electron microscope - an overview. Scanning Electron Microsc. 1972: 257-264.

21. Boyde A, Bailey E, Jones SJ, Tamarin A. (1977). Dimensional changes during specimen preparation for scanning electron microscopy. Scanning Electron Microsc. 1977; I: 507-518.

22. Boyde A, Barber VC. (1969). Freeze drying methods for the scanning electron microscopical study of the Protozoon Spirostomum ambiguum and the statocyst of the cephalopod mollusc Loligo vulgaris. J. Cell Sci. 4, 223-239.

23. Boyde A, Boyde S. (1980). Further studies of specimen volume changes during processing for SEM; including some plant tissue. Scanning Electron Microsc. 1980; II: 117-124,132.

24. Boyde A, Wood C. (1969). Preparation of animal tissue for surface scanning electron microscopy. J. Microscopy. 90, 221-249.

25. Breipohl W, Bornfeld N, Bijvank GJ, Laugwitz H, Pfautsch M. (1973). Scanning electron microscopy of the retinal pigment epithelium in chick embryos and chicks. Z. Zellforsch. 146, 543-552.

26. Breipohl W, Bijvack G, Bornfeld N. (1974a). Raskermikroskopische befunde an optischer rezeptoren. [Scanning electron microscopy of optical receptors] Verh. Anat. Ges. 68, 557-564.

27. Breipohl W, Bijvank GJ, Pfefferkorn GE. (1974b). Scanning electron microscopy of various sensory receptor cells in different vertebrates. Scanning Electron Microsc. 1974: 557-564.

28. Bridges CDB, Fong SZ. (1982). Use of lectins to investigate photoreceptor membranes. Methods Enzymol. 81, 65-76.

29. Brown PK, Gibbons IR, Wald G. (1963). The visual cells and visual pigment of the mudpuppy (Necturus). J. Cell Biol. 19, 79-106.

30. Carr KE. (1971). Applications of scanning electron microscopy in biology. Int. Rev. Cytol. 30, 183-255.

31. Carr KE, Toner PG, Saleh KM. (1982). Scanning electron microscopy. Histopath. 6, 3-24.

32. Chaitin MH, Hall MO. (1983a). Defective ingestion of rod outer segments by cultured dystrophic rat pigment epithelial cells. Invest. Ophthalmol. Visual Sci. 24, 812-820.

33. Chaitin MH, Hall MO. (1983b). The distribution of actin in cultured normal and dystrophic rat pigment epithelial cells during the phagocytosis of rod outer segments. Invest. Ophthalmol. Visual Sci. 24, 821-831.

34. Cleveland PH, Schneider CW. (1969). A simple method of preserving ocular tissue for scanning electron microscopy. Vis. Res. 9,1401-1402.

35. Cohen E, Gitler C, Ben-Shaul Y. (1981). Cell surface labeling of embryonic neural retina cells exposed to low temperature, energy inhibitors, cytochalasin B and colchicine. Cell Differ. 10(6), 333-342.

36. Davanger M, Ringvold A. (1979). Villous processes from the inner segments of cones in the human macula lutea. Acta Ophthalmologica 57: 1057-1064.

37. Davidorf FH, Sharpnack DD. (1977). Surface ultrastructure of the human retina. Scanning Electron Microsc. 1977; II: 459-466.

38. del Cerro M, Grover DA, Monjan AA, Pfau CJ, Dematte JE. (1982). Chronic retinitis in rats infected as neonates with lymphocytic choriomeningitis virus: a clinical, histopathologic, and electroretinographic study. Invest. Ophthalmol. Visual Sci. 23, 697-714.

39. Dickson DH, Carroll N, Crock GW. (1973). Scanning electron microscopy of the primate retina. Trans. Ophthalmol. Soc. N.Z. 25,181-186.

40. Dickson DH, Hollenberg MJ. (1971). The fine structure of the pigment epithelium and photoreceptor cells of the newt. Triturus viridescens dorsalis (Rafinesque). J. Morph. 135, 389-432.

41. Djamgoz MBA, Ruddock KH. (1978). Changes in structure and electro-physiological function of retinal neurones induced by laser irradiation. Neuroscience Lett. 7: 251-258.

42. Eagle RC Jr, Lucier AC, Bernardino VB Jr, Yanoff M. (1980). Retinal pigment epithelial abnormalities in fundus flavimaculatus. Ophthalmol. 87, 1189-1200.

43. Eckmiller MS, Steinberg RH. (1981) Localised depigmentation of the retinal pigment epithelium and macrophage invasion of the retina in the bullfrog. Invest. Ophthalmol. Visual Sci., 21, 369-394.

44. Essner E, Roszka JR, Schreiber JH. (1978) Phagocytosis and surface morphology in cultured retinal pigment epithelial cells. Invest. Ophthalmol. Visual Sci. 17, 1040-1048.

45. Follman P, Radnot M. (1979). [Some scanning electron microscopic observations on the human retina.] (Polish). Klin Oczna. 81, 513-514.

46. Frambach DA, Marmor MF. (1982). The rate and route of fluid resorption from the subretinal space of the rabbit. Invest. Ophthalmol. Visual Sci. 22(3), 292-302.

47. Fulton AB, Craft JL, Zakov N, Howard RO, Albert DM. (1980). Retinal anomalies in trisomy 18. Albrecht V. Graefes Arch. Klin Exp. Ophthalmol. 213, 195-205.

48. Galbavy ESJ, Olson MD. (1979). Morphogenesis of rod cells in the retina of the albino rat: a scanning electron microscopic study. Anat. Rec. 195, 707-718.

49. Gannon BJ. (1978). Vascular casting. In: Principles and Techniques of Scanning Electron Microscopy: Biological Applications. MA Hayat (ed.) NY, van Nostrand Reinhold, pp. 170-193.

50. Garcia-Porrero JA, Ojeda JL. (1979). Cell death and phagocytosis in the neuroepithelium of the developing retina. A TEM & SEM study. Experientia. 35, 375-376.

51. Garcia-Porrero JA, Ojeda JL. (1981). A stereoscan analysis of cell surface characteristics during the interkinetic nuclear migration in normal and colchicine-treated developing chick retina. Experientia. 37, 181-182.

52. Germinario LT, McAlear J. (1971). Preparation of tissue for scanning electron microscopy: Freeze-fracturing as a technique for enhancing visibility of structural relationships. Stain Tech. 46, 249-251.

53. Giereck A, Sosnierz M, Bialas B. (1974). A slide of a retinal surface viewed under a

scanning electron microscope and associated histopathological processes observed after operation for detached retina in a human eye. Ophthalmol. 168, 52-57.

54. Giulian D. (1980). Isolation of ganglion cells from the retina. Br. Res. 189, 135-155.

55. Goldbaum MH, Madden K. (1982). A new perspective on Bruch's membrane and the retinal pigment epithelium. Brit. J. Ophthalmol. 66: 17-25.

56. Gole GA, Gannon BJ, Goodger AM. (1982). Oxygen induced retinopathy: The kitten model re-examined. Australian J. Ophthalmol. 10, 223-232.

57. Göttinger W. (1977). Hohlraumbildungen in der Netzhaut peripherie im rasterelektronen mikroskopischer Bild. (SEM of cystoid degeneration in the peripheral retina.) Albrecht V. Graefes Arch. Klin. Exp. Ophthalmol. 202, 109-120.

58. Göttinger W. (1981). Formation of basement membranes and collagenous fibrils on peripheral cystoid degeneration and retinoschisis. Dev. Ophthalmol. 2, 363-368.

59. Grant P, Rubin E, Cima C. (1980). Ontogeny of the retina and optic nerve in Xenopus laevis I. Shapes in the early development of the retina. J. Comp. Neurol. 189, 593-613.

60. Hansson HA. (1969/1970). Scanning electron microscopy of the retina in Vitamin A-deficient rats. Virchows Arch. Abt. B. Zell Path. 4, 368-379.

61. Hansson HA. (1970a). Scanning electron microscopy of the rat retina. Z. Zellforsch. 107, 23-44.

62. Hansson HA. (1970b). Scanning electron microscopic studies on the synaptic bodies in the rat retina. Z. Zellforsch. 107, 45-53.

63. Hansson HA. (1970c). Scanning electron microscopic studies on the long term effects of sodium glutamate on the rat retina. Virchows Arch. Abt. B Zellpath. 4, 357-367.

64. Hansson HA. (1970d). Ultrastructure of the surface of the epithelial cells in the rat retina. Z. Zellforsch. 105, 242-251.

65. Hansson HA. (1981). Scanning electron microscopy of ocular structures. In: Scanning Electron Microscopy in Cell Biology and Medicine. K Tanaka and T Fujita (eds.). Excerpta Medica, Amsterdam-Oxford-Princeton. pp. 465-474.

66. Hayashi M, Matsumoto A, Hamashima Y, Tsukahara I. (1979). Phagocytic uptake of cultured retinal pigment epithelium. Uptake of polystyrene spheres and Staphylococus aureus. Exp. Eye Res. 28: 427-434.

67. Herron WL. (1977) The dystrophic rat as a model for clinical research. Advances in Exp. Med. and Biology. 77: p. 137-152.

68. Hilfer SR. (1983). Development of the eye of the chick embryo. Scanning Electron Microsc. 1983; III: 1353-1369.

69. Hilfer SR, Yang JJW. (1980). Accumulation of CPC-precipitable material at apical cell surfaces during formation of the optic cup. Anat. Rec. 197, 423-433.

70. Hollenberg MJ, Erickson AM. (1973). The scanning electron microscope: potential usefulness to biologists. A Review. J. Histochem. and Cytochem. 21, 109-130.

71. Humphreys WJ, Spurlock BO, Johnson JS. (1974). Critical point drying of ethanol-infiltrated cryofractured biological specimens for scanning electron microscopy. Scanning Electron Microsc. 1974: 275-282.

72. Jan LY, Revel JP. (1975). Hemocyanin-antibody labeling of rhodopsin in mouse retina for a scanning electron microscope study. J. Supramol. Struct. 3, 61-66.

73. Jensen PA, Prause JU. (1980). Frozen resin-cracking, dry-cracking and enzyme-digestion methods in SEM as applied to ocular tissues. Arch. Klin. Exp. Ophthalmol. 212, 207-216.

74. Karnovsky MJ.(1965). A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol. 27, 137A.

75. Keller G, Leuenberger PM. (1977). Effects of colchicine on phagosome-lysosome interaction in retinal pigment epithelium. II. In vitro observations on histio-organotypical retinal pigment epithelial cells of the pig. Albrecht V. Graefes. Arch. Klin. Exp. Ophthalmol. 203, 253-259.

76. Kessel RG, Kardon RH. (Eds.)(1979). Tissues and organs : A text-atlas of Scanning Electron Microsc.WH Freeman & Co. New York 317 pp.

77. Kessel RG, Kardon RH. (1980). Scanning electron microscopy of mammalian neuroepithelia. In: Scanning Electron Microscopy in Cell Biology and Medicine. K Tanaka & T Fujita (Eds.). Excerpta Medica, Amsterdam-Oxford-Princeton. pp. 483-499.

78. Kretzer FL, Hittner HM, Johnson AT, Mehta RS, Godio LB. (1982). Vitamin E and retrolental fibroplasia: ultrastructural support of clinical efficacy. Ann. NY Acad. Sci. 393, 145-166.

79. Kuwabara T. (1970). Surface structures of the eye tissue. Scanning Electron Microsc. 1970: 185-192.

80. Lai YL, Lug R, Yao PC, Hayasaka S, Hayasaka I. (1980). Studies on the pathogenic mechanisms of light on rat retina. Acta Anat. 107, 407-417.

81. Lai YL, Masuda K, Hayasaka S, Suzuyama Y, Lin TC, Chang T, Lug R, Wang SJ, Liu YP. (1982). Study of subretinal intercellular space. Exp. Eye Res. 34(1), 7-14.

82. Lee WR, Grierson I. (1977). Macrophage infiltration in the human retina. Albrecht V. Graefes Arch. Klin. Exp. Ophthalmol. 203, 293-309.

83. Leuenberger P. (1971). Stéréo-ultrastructure de la rétine. Etude comparative au microscope electronique à transmision et à balayage. [Stereo-ultrtastructure of the retina. A comparative transmission and scanning electron microscopy study.] Arch. Ophthalmol. (Paris) 31, 813-822.

84. Lewis ER, Zeevi YY, Werblin FS. (1969). Scanning electron microscopy of vertebrate visual receptors. Brain Res. 15, 559-562.

85. Malick LE, Wilson RB, Stetson D. (1975) Modified thiocarbohydrazide procedure for scanning electron microscopy: Routine use for normal, pathological, or experimental tissues. Stain Technol. 50, 265-269.

86. Malick LE, Wilson RB. (1975).Evaluation of a modified technique for SEM examination of vertebrate specimens without evaporated metal layers. Scanning Electron Microsc. 1975: 259-266.

87. Marmor MF, Abdul-Rahim AS, Cohen DS. (1980a). The effect of metabolic inhibitors on retinal adhesion and subretinal fluid resorption. Invest. Ophthalmol. Visual Sci. 19, 893-903.

88. Marmor MF, Martin LJ, Tharpe S. (1980b). Osmotically induced retinal detachment in the rabbit and primate. Electron microscopy of the pigment epithelium. Invest. Ophthalmol. Visual Sci. 19, 1016-1029.

89. Masutani T, Miyoshi M. (1980). Surface fine structure of visual cells and their arrangement in the vertebrate retina. In: Scanning Electron Microscopy in Cell Biology and Medicine. K Tanaka & T Fujita (Eds.). Excerpta Medica, Amsterdam-Oxford-Princeton. 475-478.

90. Masutani-Noda T, Yamada E. (1983). The mosaic pattern of the inner surface of vertebrate retina. Arch. Histologicum Japonicum. 46, 393-400.

91. Matsuura T, Miller WH, Tomita T. (1978). Cone specific C-wave in the turtle retina. Vision Res. 18, 767-775.

92. McAvoy JW. (1981). The spatial relationship between prescriptive lens and optic vesicle/cup during early eye morphogenesis in the rat. Exp. Eye Res. 33, 447-458.

93. Meller K. (1979). Scanning electron microscope studies on the development of the nervous system in vivo and in vitro. Internl. Rev. Cytol. 56, 23-56.

94. Meller K, Tetzlaff W. (1976). Scanning electron microscopic studies on the development of the chick retina. Cell Tissue Res. 170, 145-159.

95. Meyers SM, Bonner RF, Rodrigues MM, Ballintine EJ. (1983). Phototransection of vitreal membranes with the carbon dioxide laser in rabbits. Ophthalmol. 90, 563-568.

96. Miki T, Mii T. (1978). [Experimental study on the retinal tear. Scanning electron microscopic observations of the retinal tear after long term observation.] (Japanese). Acta Societatis Ophthalmologicae Japonicae. 82,10-14.

97. Miki T, Mii T, Hiromori T. (1976). [Repair of the retinal pigment epithelium after xenon arc photocoagulation in the detached retina. Scanning electron microscopic observation.] (Japanese). Soc. Ophthalmol. Japonica Acta. (Nippon Ganka Gakkai Zasshi) 80, 180-192.

98. Miller WH. (1979). Ocular optical filtering. Chap. 3. In: Comparative Physiology and Evolution of Vision in Invertebrates. Handbook of Sensory Physiology. Vol. VII/6A, H Autrum (ed.) Springer-Verlag, Berlin. pp. 69-144.

99. Miller WH. (1983). Does cyclic GMP hydrolysis control visual transduction in rods? Trends in Pharmacol. Sciences. 4, 509-511.

100. Molday RS. (1976) A scanning electron microscope study of concanavalin A receptors on retinal rod cells labelled with latex microspheres. J. Supramol. Struct. 4, 549-557.

101. Molday RS. (1977). Cell surface labeling techniques for SEM. Scanning Electron Microsc. 1977; II: 59-74.

102. Molday RS, Maher P. (1980). A review of cell surface markers and labeling techniques for scanning electron microscopy. Histochem. J. 12, 273-315.

103. Molday RS, Molday LL. (1979). Identification and characterization of multiple forms of rhodopsin and minor proteins in frog and bovine rod outer segment disc membranes. J. Biol. Chem. 254, 4653-4660.

104. Mukuno K, Ishikawa S, Miyazawa H. (1972). [Scanning electron microscopy of the human retinal pigment epithelium.] (Japanese). (Igaku no Ayumi.) Med. in Progress. 83, 204-205.

105. Nelson AC, Hayes TL, Tobias CA, Yang TCM. (1981). Some indications of structural damage in

retina by heavy ion radiation. Scanning Electron Microsc. 1981; IV: 79-85.

106. Newsome DA, Rodriques MM, Machemer R. (1981). Human massive periretinal proliferation. In vitro characteristics of cellular components. Arch. Ophthalmol. 99, 873-880.

107. Newton JC, Barsa-Newton MC, Wardly J. (1980). The effects of X radiation on the retina of the albino rabbit as viewed with the scanning electron microscope. Radiation Res. 81, 311-318.

108. Nishida S, Mizuno K. (1981). [An application of scanning electron microscopy in studies of ocular tissues.] (Japanese). Folio Ophthalmologica Japonica 22, 167-173.

109. Ohkuma M. (1972). [Ultrastructural observations on the choroid and the pigment epithelium on experimental retinal detachment. III. Scanning electron microscopic observations of the retinal pigment eplithelium]. (Japanese). Soc. Ophthol. Japonica Acta 76: 377-384.

110. Ohsawa E, Miki T. (1982). Scanning electron microscopic observation of glial cells following xenon arc photocoagulation. Albrecht V. Graefes Arch. Clin. Exp. Ophthalmol. 218, 64-69.

111. Olson MD. (1975). Scanning electron microscopy of developing photoreceptors in the chick retina. Anat. Rec. 181, 440 (Abstract).

112. Olson MD. (1977). The development of photoreceptors inner and outer segments in the retina of the chick as observed by scanning electron microscopy. Scanning Electron Microsc. 1977; II: 453-457.

113. Olson MD. (1979). Scanning electron microscopy of developing photoreceptors in the chick retina. Anat. Rec. 193, 423-438.

114. Ophir I, Moscona AA, Ben-Shaul Y. (1983). Localization of retina cognin in embryonic normal retina tissue by immuno-scanning electron microscopy. Cell Diff. 13, 133-141.

115. Peters KR. (1979). Scanning electron microscopy at macromolecular resolution in low energy mode on biological specimens coated with ultra thin metal films. Scanning Electron Microsc. 1979; II: 133-148.

116. Peters KR. (1980) Improved handling of structural fragile all-biological specimens during electron microscopic preparation by the exchange method. J. Microsc. 118, 429-441.

117. Peters KR, Palade GE, Schneider BG, Papermaster DS. (1983). Fine structure of a periciliary ridge complex of frog retinal rod cells revealed by ultrahigh resolution scanning electron microscopy. J. Cell Biol. 96, 265-276.

118. Pfeffer BA, Fisher SK. (1981) Development of retinal pigment epithelial surface structures ensheathing cone outer segments in the cat. J. Ultrast. Res. 76: 158-172.

119. Pietzsch-Rohrschneider I. (1976). Scanning electron microscopy of photoreceptor cells in the light and dark-adapted retina of Haplochromis burtoni (Cichlidae, Teleostei). Cell Tissue Res. 175, 123-130.

120. Prause JU, Jensen AO. (1980). Scanning electron microscopy of frozen- cracked, dry-cracked, and enzyme digested retinal tissue of a monkey (Cercopithecus aethiops) and man. Albrecht V. Graefes Arch. Klin. Exp. Ophthalmol. 212, 261-270.

121. Puzzolo D, de Simone I. (1979). Multinucleated cells in the retinal pigment epithelium: a scanning electron microscopic study. Experientia 35, 98-101.

122. Puzzolo D, de Simone I, Farina F. (1978). Scanning electron microscopic studies of the retinal pigment epithelium of the albino rat. Rivista di Biologia 71, 95-112.

123. Puzzolo D, de Simone I, Farina F. (1980) Scanning electron microscopic study of the damages induced by continuous illumination on the eye of albino rat. II. The photoreceptors. Rivista di Biologica 73, 191-204.

124. Quigley MA, Addicks EM. (1981). Regional differences in the structure of the lamina cribrosa and their relation to glaucomatous optic nerve damage. Arch. Ophthalmol. 99, 137-143.

125. Quigley MA, Hohman RM, Addicks EM. (1982). Quantitative study of optic nerve head capillaries in experimental optic disk pallor. Am. J. Ophthalmol. 93, 689-699.

126. Radnot M. (1978). Scanning electron microscopic study of the human retina. Ophthalmol. 176, 308-312.

127. Reading HW, MacInnes DG. (1982) A scanning electron microscopy study of inherited retinal degeneration in the rat. In: Problems of normal and genetically abnormal retinas. RM Clayton, J Haywood, HW Reading, A Wright (Eds.). Academic Press, New York. pp. 253-263.

128. Renard G, Usui M, de Kozak Y, Faure JP. (1976). Etude en microscopie électronique a balayage d'une choriorétinite experimentale du cobaye. [A study by scanning electron microscopy of an experimental chorio-retinitis in the guinea pig] Arch.Ophthalmol.(Paris). 36,327-340.

129. Renard G, Usui M, de Kozak Y, Faure JP. (1977). La choriorétinite autoimmune du cobaye aspects du microscopie a balayage.[Experimental chorioretinitis induced by autologous retina in guinea pigs. A scanning electron microscopic study] Arch Ophthalmol. (Paris). 37, 127-140.

130. Risco JM, Grimson BS, Johnson PT. (1981). Angioarchitecture of the ciliary artery circulation of the posterior pole. Arch. Ophthalmol. 99, 864-868.

131. Risco JM, Nopanitaya W. (1980). Ocular microcirculation: Scanning electron microscopy. Invest. Ophthalmol. Visual Sci. 19, 5-12.

132. Rodrigues MM, Currier CA. (1983). Histopathology of argon laser photocoagulation in juvenile diabetic retinopathy. Ophthalmol. 90, 1023-1027.

133. Sakuragawa M, Kuwabara T. (1976). The pigment epithelium of the monkey. Topographic study by scanning and transmission electron microscopy. Arch. Ophthalmol. 94, 285-296.

134. Schmidt KL, Milner K, Hilburn PJ, Schmidt WA. (1983). Ultrastructure of Trypan Blue induced ocular defects: I. Retina & Lens. Teratol, 28, 131-144.

135. Sheahan BJ, Donnelly WJC, Grimes TD. (1978). Ocular pathology of bovine GM_1 gangliosidosis. Acta. Neuropath. 41, 91-95.

136. Shearer ACI. (1969). Morphology of the isolated pigment particle of the eye by scanning electron microscopy. Exp. Eye Res. 8, 122-126.

137. Shimada T, Murakami M. (1975). Frozen epoxy resin-cracking method for scanning electron microscopy. J. Electron Microscopy 24, 199.

138. Smith CJD. (1973). Scanning electron microscopy of the retina of Notomys alexis. J. Anat. 116, 471 (Abstract).

139. Smith ME, Finke EM. (1972). Critical point drying of soft biological material for the scanning electron microscope. Invest. Ophthalmol. 11, 127-132.

140. Steinberg RH. (1973). Scanning electron microscopy of the bullfrog's retina and pigment epithelium. Z. Zellforsch, 143, 451-463.

141. Steinberg RH, Wood I. (1974). Pigment epithelial cell ensheathment of cone outer segments in the retina of the domestic cat. Proc. R. Soc. Lond. B. 187: 461-478.

142. Stern WH, Anderson DH, Fisher SK, Borgula GA, Ericson PA. (1981). Anatomical recovery following retinal detachment: Clinicopathological correlations. Austral. J. Ophthalmol. 9, 143-154.

143. Suburo A, Carri N, Adler R. (1979). The environment of axonal migration in the developing chick retina. A scanning electron microscopic (SEM) study. J. Comp. Neur. 184: 519-536.

144. Tamai M. (1979) Regeneration of microvilli of the pigment epithelium in vitro. Ophthalmology 1, 618-621.

145. Tanaka K. (1974). Chap. 4. Frozen resin cracking method and its role in cytology. In: Principles and Techniques of Scanning Electron Microscopy. Biological Applications. Vol. I. MA Hayat (ed.). van Nostrand Reinhold, New York. pp. 125-134.

146. Tanaka K. (1981). Demonstration of intracellular structures by high resolution scanning electron microscopy. Scanning Electron Microsc. 1981; II: 1-8.

147. Tanaka K, Naguro T. (1981). High resolution scanning electron microscopy of cell organelles by a new specimen preparation method. In: Scanning Electron Microscopy in Cell Biology and Medicine. K Tanaka & T Fujita (Eds.). Int. Congr. Ser. 545, Excerpta Medica, Amsterdam, The Netherlands. 63-70.

148. Taylor HR, Carroll N, Jack I, Crock GW. (1979). A scanning electron microscopic examination of retinoblastoma in tissue culture. Brit. J. Ophthalmol. 63, 551-559.

149. Ulshafer RJ, Allen CB. 1(1984) Scanning electron microscopy of the retina in an animal model of hereditary blindness. Scanning Electron Microsc. 1984; II: 841-848.

150. Ulshafer RJ, Allen CB, Dawson WW, Wolf ED. (1984) Hereditary retinal degeneration in the Rhode Island Red chicken: Histology and ERC. Exp. Eye Research. (In press).

151. Van Buskirk EM. (1979). The canine eye: the vessels of aqueous drainage. Invest. Ophthalmol. and Visual Sci. 18, 223-230.

152. Wakakura M, Ishikawa S. (1982). Scanning electron microscopy of retinal ganglion cells by applying D,L-2-Aminoadipic acid. Japan. J. Ophthalmol. 26, 1-9.

153. Wakely J. (1977). Scanning electron microscope study of the extracellular matrix between presumptive lens and presumptive retina of the chick embryo. Anat. Embryol. 150, 163-170.

154. Watters WB, Buck RC. (1971). An improved method of specimen preparation for replicas or scanning electron microscopy. J. Micros. 94, 185-187.

155. Wickham G, Adams CK. (1979). Scanning electron microscopy of the primate photoreceptor mosaic. Invest. Ophthalmol. Visual Sci. (ARVO Suppl.), 18, 79 (Abstract).

156. Yamada E. (1982). Morphology of vertebrate photoreceptors. Meth. in Enzymol. 81, 3-17.

157. Yew DT, Ling Wong SL. (1980). The morphology of the visual cells of young ($2\frac{1}{2}$ months) and old (17 months) albino mice. Anat. Anz. Jena. 148, 333-336.

158. Yew DT, Ling Wong SL, Chan YW. (1982). Stimulating effect of the low dose laser: a new hypothesis. Acta Anat. 112, 131-136.

159. Yoneya S, Tso MOM. (1980). A scanning electron microscopy study of retinal neovascularization in retrolental fibroplasia. Invest. Ophthalmol. Visual Sci. (ARVO Suppl. Abstract) p. 139.

160. Zinn KM, Benjamin-Henkind JV. (1979). Anatomy of the human retinal pigment epithelium. In: The Retinal Pigment Epithelium. KM Zinn & MF Marmor (Eds.). Harvard Univ. Press, Cambridge, Mass. pp. 3-31.

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After this paper was written, following additional references were brought to my attention, they are listed here as they may be relevant to the readers of this paper.

1. Daicker B, Guggenheim R. (1976). Raster-elektronenmikroskopische Befunde an Netzhautinnenflächen I. Netzhautrundlöcher. [Findings on retinal surface by scanning electron microscopy. I. Retinal lobes]. Albrecht v Graefes Arch. klin. exp. exp. Ophthal. 201, 29-38.

2. Daicker B, Guggenheim R, Gywat L. (1977a). Rasterelektronenmikroskopische Befunde an Netzhautinnenflächen. II. Hintere Glaskörperabhebung. [Findings on retinal surface by scanning electron microscopy. II. Vitreous detachment]. Albrecht v Graefes Arch. klin. exp. Ophthal. 204, 19-29.

3. Daicker B, Guggenheim R, Gywat L. (1977b). Rasterelektronenmikroskopische Befunde an Netzhautinnenflächen. III. Epivaskuläre Gliabüschel. [Findings on Retinal surface by scanning electron microscopy. III. Epivascular glial clusters]. Albrecht v Graefes Arch. klin. exp. Ophthal. 204, 31-37.

4. Sosnierz M, Gierkowa A, Bialas B, Gluza J. (1973). [Effect of laser beam on the pathological appearance of the eyeball and the retinal surface of rabbits in scanning electron microscopy] (Polish) Klin. Oczna. 43: 605-608.

5. Yew DT, Liu HC, Wong SL, Chan YW. (1982). Low dose laser and the developing retina. A histochemical and scanning electron microscopic study. Acta Morphol. Neerl Scand. 20: 57-63.

Appendix I

For those interested in knowing which retinae have been used in SEM studies, the following list may be useful.

	See Reference #
FISH	
Goldeye, brook trout, yellow perch, walleye	(1)
Carp	(14,19)
Anableps anableps L.	(16)
Rutilus rutilis	(41)
Goldfish	(54)
Haplochromis burtoni	(119)
not specified (pigment granules of RPE only)	(136)
Boleophthalmus	(156)
AMPHIBIANS	
Rana pipiens	(28,117)
Bullfrog (Rana catesbiana)	(43,89,90, 91,98,140)
Xenopus laevis	(59,102,103)
Frog (species not given)	(115,156)
Newt, Triturus viridescens	(40)
Mud puppy, Necturus,	(84)
REPTILIA	
Lizard (RPE granules only)	(136)
Snake (Elaphe quadrivirgata)	(89)
Turtle	(91)
AVES (Birds)	
Crow	(90)
Pigeon (pigment granules only)	(136)
Duck(micrography by Dr. Usukura) In	(156)
Chickens	(25,149)
Chick embryos	(8,9,10,11,25,26,27,50,51,66, 68,69,93,94,111,112,113,114,143,153)
Zonotrichia leucophrys (sparrow)	(98)
MAMMALS	
Rats	(32,33,38,44,60,61,67,105,127,136,146)
Albino rat	(28,48,61,62,63,64,75,76,80, 83,105,121,122,123,127,144)
Embryology	(92,134)
Mice	(52,72,138,144)
Albino mice	(157,158)
Rabbits	(6,7,17,18,44,46,65,76,77,81,83,87, 88,95,97,107,109,110,136)
Cat	(3,34,108,118,131,141,142,152)
Kitten	(56,108,118,159)
Sheep (RPE pigment granules only)	(136)
Bovine	(44,90,100,103,135)
(RPE pigment granules only)	(136)
Pig	(75,90,98)
(RPE pigment granules only)	(136)
Guinea pig	(76,77,128,129)
PRIMATES	
Macaca	(13,15,39,79,88,125,133,155,160)
Cercopithecus aethiops	(120)
Human	(27,36,37,42,45,47,53,55,57,58,65,73, 78,79,82,90,104,106,120,124,125,126,132, 136,148,155)

Appendix II

Studies of a Clinical - Pathological nature:

	See Reference #
Detached retina - cat	(2,3,142)
Laser effects on retina - rabbit	(7,17,18)
effects of retinal detachment on RPE rabbit	(109)
Rat retina dystrophy	(32,33,67)
chronic retinitis, rats	(38)
Fundus flavimaculatus - human	(42)
resorption of fluid from subretinal space - rabbit	(46)
Trisomy 18 in human retina	(47)
Detached retina - human	(53)
O_2 - induced retinopathy - kittens	(56)
peripheral cystoid degenerations - man	(57)
and senile retinoschisis - man	(58)
Vitamin A deficiency - rats	(60)
effects of sodium glutamate - rats	(63)
Retrolental fibroplasia & Vitamin A-human	(78)
Retrolental fibroplasia neovascularization - kittens	(159)
light-induced retinal damage - rats	(80)
Macrophage infiltration in ophthalmitis - human	(82)
retinal detachment - rabbit and monkey	(88)
Retinal tear	(96)
repair after Xenon photocoagulation for detached retina - rabbit	(97)
vitreal membranes transected with CO_2 laser - rabbits	(95)
massive human periretinal proliferation	(106)
effects of X-radiation on albino rabbit retina	(107)
glaucoma damage and lamina cribrosa structure - human	(124)
optic disk pallor and optic nerve head capillaries - monkey	(125)
inherited retinal degeneration - rats	(127)
experimentally induced chorioretinitis in guinea pigs	(128,129)
argon laser treatment of diabetic retinopathy - human	(132)
retinoblastoma, cell types in tissue culture - human	(148)
ganglion cells, normal and after optic nerve transection - cat	(152)
aging visual cells - albino mice	(157)
congenital and hereditary blindness-chicken	(149)
hereditary retinal degeneration - chicken	(150)