Scanning Microscopy

Volume 2 | Number 3 Article 43

5-20-1988

The Role of Scanning Electron Microscopy in Ophthalmic Science

P. Versura
University of Bologna

M. C. Maltarello
University of Bologna

Scanning Microscopy, Vol. 2, No. 3, 1988 (Pages 1695-1723)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/88$3.00+.00

THE ROLE OF SCANNING ELECTRON MICROSCOPY IN OPHTHALMIC SCIENCE

P. Versura* and M.C. Maltarello

Institute of Clinical Electron Microscopy and Department of Ophthalmology
University of Bologna, Italy

(Received for publication January 12, 1988, and in revised form May 20, 1988)

Abstract

The eyeglobe is one of the classical domains of Scanning Electron Microscopy (SEM) in biology as it exposes several inner and outer surfaces. Both corneal and conjunctival epithelia towards the tear film as well as the corneal endothelial cells facing the anterior chamber may be accurately evaluated. The architecture of the angle and particularly the morphology of the Schlemm's canal inner wall are clarified by SEM more than by TEM serial reconstruction. The surfaces of the iris and ciliary body, the zonula and the choroidal vessel arrangement are described in great detail. Three distinct types of membrane anchoring devices are demonstrated among the lens fibers. SEM impressively describes the retina, but it has not yet added any new information as to previous observations in a more conventional way.

SEM plays a fundamental role in teaching ocular anatomy and physiology as it makes more comprehensive the interrelationships among different structures. In addition, it represents a proper structural approach fo the clinician who is familiar with the three-dimensional observations obtained by means of biomicroscopy, ophthalmoscopy and fluorescein angiography. Therefore, SEM application should be further spread and possibly joined to immunocytochemistry, in order to obtain a more dynamic and functional analysis of the eye.

KEY WORDS : Cornea, conjunctiva, lens, zonular apparatus, iris, ciliary body, choroid, vitreus, retina, vascular casts, scanning electron microscopy.

*Address for correspondence : Piera Versura
Institute of Clinical Electron Microscopy,
Via Massarenti, 9 - 40138 Bologna, Italy.

Phone No. 051 302874

Introduction

The complexity of the eye structure is dramatically fascinating since the constituent organs work for sight in a quite unique anatomical and functional synchronism. The eye, as a virtually hollow apparatus, exposes several inner surfaces which line the anterior, posterior and vitreous chambers. In addition, cornea and conjunctiva directly face the outside, being separated from the air by the tear film only.

Because of this particular structural configuration, the eye can be considered one of the classical domains of scanning electron microscopy (SEM) applications in biological research. It is well recognized that SEM allows ultrastructural investigations of relatively large tissue sample surfaces and therefore it has a fundamental role in clarifying the interrelationships between ocular structures.

Around the years '70/'75 several authors started to describe the tissues of the eyeglobes by SEM. Therefore, after such a long time, it could appear unnecessary to point out the role of SEM in ophthalmological science. But, despite the great flourishing of work, few complete review papers on this subject have been written. In fact, the major part of these are just a three-dimensional description of the tissues (7, 76, 108, 161, 303) or only consist of either an ordered bibliography (22) or of a series of micrographs collected in an atlas (128, 169) or point out methods for processing the tissues for SEM (30, 56, 144, 151).

Consequently, we have prepared this review paper with the specific aim to emphasize how SEM helped to improve the knowledge not only of the anatomy of the eye tissues, but also of how they function. In its present form the paper is mainly addressed to researchers involved in some way with the study of ocular structures, but unfamiliar with their ultrastructure and

particularly with three-dimensional images. As a matter of fact, SEM has a tremendous impact for teaching purposes as it provides information particularly suitable to make several ophthalmic problems more understandable also to undergraduate students.

The paper is subdivided into chapters dealing separately with the corneal epithelium and endothelium, conjunctiva, trabecular meshwork, uveal tract (iris, ciliary body and choroid), lens and zonular fibers, vitreus and retina.In addition, a small chapter is dedicated to the vascular casts applied to the study of the ocular microcirculation

All micrographs derive from post-mortem human eyes removed for corneal transplantation within 5-6 hours after death, fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer for 4 hours, then dissected to expose all the internal surfaces. The specimens were fixed again in glutaraldehyde for 12 hours, washed thoroughly in 0.15M phosphate buffer, post-fixed in 1% OsO_4 ,dehydrated through an ascending series of ethanol, critical point dried, mounted and sputtered with a thin (15-20 nm) film of gold. A Philips 505 SEM was used for the observations. Corneal buttons were collected from eyes removed for diseases not involving the anterior segment (choroidal melanomas). In addition, corneal epithelium was obtained by gently scraping-off in vivo, mounted epithelial surface side up and processed for SEM as described elsewhere (279).

Cornea

The cornea represents the main component of the optic system because of its peculiar characteristics of transparency and refraction of light. The usual method of investigation for the clinician is biomicroscopy. With this instrument, it is possible to obtain three-dimensional information at 8-10 times magnification. For this reason, SEM appears the more appropriate subsequent approach for the structural study of the cornea as it enhances the 3-D images at the ultrastructural level.

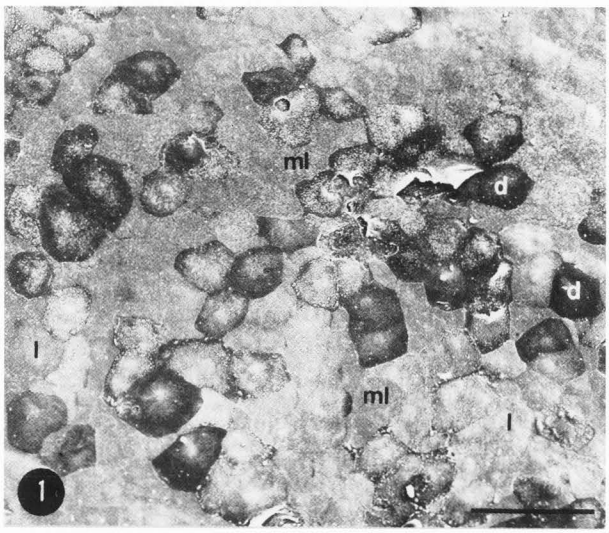
In addition: "The interpretation of electron microscopic (TEM) material is tedious for the clinician who uses only biomicroscopy as a mean to interpret disease at the microscopic level. It is here that the scanning electron microscope (SEM) has, to some extent, bridged the gap between the biomicroscope and the transmission electron microscope "(203).

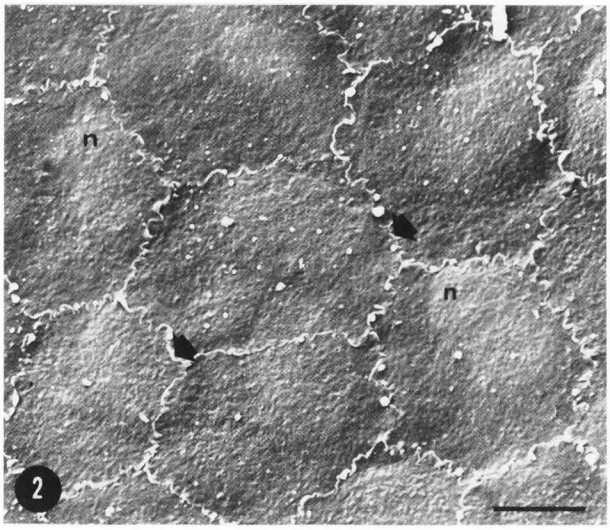
Several authors have described the surface morphology of corneal epithelium by SEM, both in man (54, 138, 201, 228, 279) and in various animals (17, 116, 123, 124, 125, 133, 135, 167, 199), in normal conditions. Human corneal epithelial cells are generally polygonal and appear to have different degrees of brightness in the SEM. Therefore, they have classically been subdivided into light, medium light and dark cells (fig.1). The degree of brightness has been demonstrated (135, 279) to be related to the number and type of microprojections present on the cell plasmamembrane: light cells generally have microplicae and microvilli, dark cells only have small microprojections emerging like knobs from the surface, or are smooth. We had no evidence as to the presence of these microprojections in the lateral and basal surfaces of these cells. It has been suggested (279) that these cytoplasmic protuberances are connected on the same cell by a common maturative sequence from the initial microplicae to the terminal knobs. This means that the bright cells with numerous microprojections on the surface are the vital cells, whereas the dark cells are the old ones immediately before death.

Thoft et al. (266), suggested that corneal epithelium integrity is maintained by the synchronic work of three independent movements of cells. According to their "x, y, z hypothesis", basal cells from peripheral cornea move centripetally (y component), then proliferate and move outward undergoing a maturation process (x component), finally the old cells exfoliate (z movement). It remains to be established whether also a movement of conjunctival epithelial cells occurs towards the cornea, across the limbus, with transformation of the cell phenotype. According to this hypothesis it is possible to categorize corneal diseases by referring to the specific component involved (i.e. deficiency or increased rate of one or more of the three movements). Therefore, in the case of abnormal epithelial surface, SEM represents a suitable technique to draw conclusions about the movement involved by quantitatively analyzing the relative presence of light, medium light and dark cells . The identification of the phenomenon involved in a corneal epithelium disease can help in the choice of the proper therapy directed to correct the abnormal component.

The presence of a finely irregular surface consisting of microprojections covered by a thin layer of mucins is thought to contribute to the stability of the tear film, as demonstrated by comparative studies in non-mammals (116). However, this role would appear debatable (63, 179). SEM was applied in studies (81, 87, 88) where a dry-eye condition or an hypertonic environment were experimentally induced. Increased cell desquamation and loss of

microplicae were demonstrated, which supports the stabilizing role of microprojections.





The corneal endothelium is a single layer of hexagonal cells connected by junctional complexes (43, 131, 196). The ability of human corneal endothelium (from an embryological point of view it would be more correct to name it "mesothelium") to regenerate has been debated for a long time. It is thought that the number of endothelial cells is predetermined at birth so that, in the adult eye, damage of any nature is healed by spreading of the surrounding cells rather than by proliferation of the adjacent cells (89, 189, 223). Therefore, the quantitative analysis of the size and shape of endothelial cells provides indirect information on the present and past life of this tissue.

Fig.1. Human corneal epithelium. Light (l), medium light (ml) and dark (d) cells are visible at the surface of the cornea, corresponding to different stages of maturation.
SEM Bar= 100 μm

Fig.2. Human corneal endothelium. In its central zone it appears to consist of polygonally (generally hexagonal) shaped cells, overlapping each other (arrows), displaying bulging underlying nuclei (n). SEM Bar = 5 μm

Clinical specular microscopy of the corneal endothelium has gained an increasingly wide popularity. Although it is assumed that what is seen with the specular microscope reflects the conditions of the whole cornea (16) one can question whether the density of endothelial cells is really uniform in all the cases. SEM images represent an enhancement of the specular micrographs (14). Of course, SEM cannot be generally applied, i.e., in patients SEM investigations can be carried out only after removal of the button. In addition, the preparative techniques for SEM have to be particularly carefully applied in such a way that the endothelial cell appearance is only minimally affected (145, 194, 287).

Several authors have described by SEM the normal endothelial cell surface facing the anterior chamber (28, 57, 218, 221, 226, 262). In man, four zones can be distinguished, from the central Zone I, in which bulging nuclei and many intercellular overlaps are present (fig.2), to Zone IV at the border with the trabecular meshwork, in which irregular endothelial cells connected by less overlapping are located. Correlative transmission electron microscopy (TEM)/SEM investigations pointed out the presence of a presumably negatively charged material on the surface of the normal endothelial cells (143, 242, 254). Supposedly the physiological role of this coating is related to the ionic gradients across the plasmamembrane.

It is well known that the endothelium is actively involved in the transport of substances and water from and to the anterior chamber, through the presence of a fluid pump located at the level of its plasma membranes (180). Therefore, corneal edema can result after many mechanical or chemical injuries to the endothelium. A great amount of SEM studies has been carried out in order to test the effects of several substances used as irrigating solutions on the endothelial cells as well as to

investigate the endothelial impairment due to some surgical procedures. As the literature is very extensive we only cite the most important contributions (1, 6, 13, 59, 60, 62, 204, 248). It appears that corneal endothelium can tolerate a wide osmotic range without marked endothelial cell damage, provided the essential ions are present in the medium (61).

The main source for investigations of the human cornea is provided by corneal buttons obtained from keratoplasty or from cadavers; this relatively easy way to collect specimens has resulted in much work on several corneal epithelial/endothelial pathological conditions studied in SEM along with TEM (203, 302). The same is true also for all the experimental conditions induced in models, such as studies of epithelial wound healing, sliding, downgrowth, burns, vitamin A deficiency, epithelium infected by various microorganisms, endothelium damaged by various agents (laser beams, UV, etc.). Quote and discuss the numerous studies on these aspects is behind the scope of this work. Considering its importance, this might be the subject of a further review paper.

Conjunctiva

The conjunctiva is a vascularized mucous membrane which lines the posterior surface of the eyelids and the anterior surface of the eyeball, up to the sclero-corneal junction. It consists of a stratified, non-keratinized epithelium which contains goblet cells. The surface morphology of human and animal conjunctiva has been described by several authors (36, 90, 93, 98, 125, 200, 280, 289).

The various zones of the normal conjunctiva show a different morphology in SEM. The upper tarsal zone, appears as a mosaic of polygonally shaped cells , the microprojections of which are essentially microvilli (fig 3) acting, as those of the cornea, as a support of the preocular tear film. Interspersed among the epithelial cells, the apical part of some goblet cells in different functional stages are found. The mucus secreted by conjunctival goblet cells adsorbs to the microvilli of cornea and conjunctiva, making the relatively hydrophobic epithelial surface more wettable. Because of its importance for tear film stability, particular attention has been devoted to the mucus which has been the subject of important SEM studies (98, 99, 122). Histochemical analysis indicates that human conjunctival goblet cells contain non-sulphated mucopolysaccharides (255). Sialic acid appears to be the main glycoconjugate present (281, 282). An additional source of mucus, named Second Mucus System (SMS) (94, 100), is constituted by subsurface secretory vesicles present in non-goblet epithelial cells. These vesicles produce mucus different from that of goblet cells with respect to some glycosidic residues (282). It has been recently proposed (52,170) that the surfacting agent is not the mucus, but the glycocalyx provided by the outward discharge of the secretory vesicle content. It seems in fact that the membranes surrounding the vesicles fuse with the plasma membrane thus allowing new microvilli to be formed. The goblet cells instead produce the remaining part of the mucus layer.

Most SEM studies performed in man have dealt with the modification of the surface conjunctiva both in asymptomatic contact lens (CL) wearers and in patients with Giant Papillary Conjunctivitis (91, 92, 95, 96). In addition, the alterations of the prosthesis surfaces have been reviewed by us elsewhere (285). In CL-related conjunctival diseases the epithelial cell surface was widely distorted and especially the microvilli appeared to be grouped in tufted structures. This finding was correlated with an increase of mucous secretion by the SMS (97, 280) although this conclusion could be

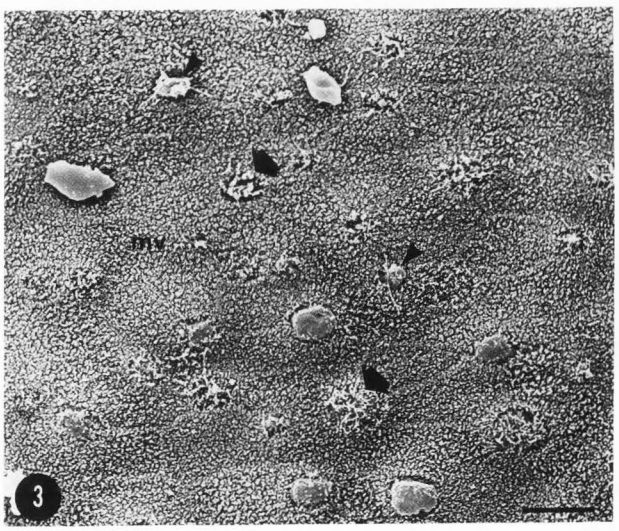


Fig.3. Human conjunctival epithelium. Inner upper tarsus. The epithelial cell surface is shown by SEM to be increased by a myriad of microprojections, that are mainly microvilli (mv). Among the epithelial cells, goblet cells are located. The apical part of these cells with microvilli represents a resting stage (arrows). On the contrary, the presence of an irregular mass of mucus indicates the time of exocytosis (arrowheads).

SEM Bar = 10 μm

questioned (53). Recently, Foster and collaborators (79) described the conjunctival surface in the course of cicatricial pemphigoid.

As an alternative to the study of biopsies, the morphological investigation of conjunctival epithelial cells acquired by impression and collected on filters is rising in the clinical practice as it is a simple, atraumatic and quite reproducible method. The perspectives of this procedure have both research and diagnostic potential because it is quite easy to perform on the same filter correlative light microscopy (LM), TEM and SEM studies (45, 173, 235).

Trabecular meshwork

The trabecular meshwork (TM) represents a sieve through which aqueous humour passes on its way from the anterior chamber to Schlemm's canal (SC). The main characteristic of SEM, i.e. the possibility to obtain three-dimensional surface information at ultrastructural level of relatively large specimens, is fully realized in the study of this particular structure of the eye. In fact, before the introduction of SEM, the structural appearance of TM had been reconstructed by means of the time-consuming method of serial sectioning at the LM or TEM level. To look at the whole mounts of the anterior chamber in SEM makes it possible to have a greatly enlarged, three-dimensional gonioscopic study of the angle, which is of particular relevance, both from a clinical and a research point of view. SEM has deepened and actually confirmed the data on TM architecture obtained with more conventional morphological procedures.

The TM is anatomically divided into an inner uveal and an outer corneo-scleral portion. In both the two parts the trabecular sheets consist of endothelial cells surrounding a central connective tissue core. Flat preparations of the human trabecular meshwork have been investigated by means of SEM in several studies (3, 12, 86, 114, 130, 219, 220, 222, 236, 241, 253) in normal and various pathological conditions, demonstrating the arrangement of the meshwork by very impressive micrographs. The peripheral corneal endothelium at the border with trabecular meshwork (fig.4) appears to be constituted by more or less hexagonal cells with prominent cell borders but less cell overlap. These cells rest on a thicker Descemet's membrane containing excrescences (Hassal-Henle bodies). Proceeding towards the angle, the intercellular edges become enlarged and cytoplasmatic bridges are located between the cells. These structures delimitate initial discontinuities between the two plasmamembranes. In fig.5 a whole view of the angle is shown; between the peripheral corneal endothelium and TM proper, a transitional zone is observed. The trabecular endothelial cells have elongated shape and interconnect each other by means of sheet-like cytoplasmic extensions. These connections between endothelial cells surround the openings through which the aqueous flows away. The uveal TM has larger openings as to the corneo-scleral TM (fig.6).

The SC can be opened in such a way that relatively large surface areas of the inner and outer walls can be investigated by SEM. SEM observations suggested that the flow of aqueous humor within the SC has some influence on the orientation of the spindle-shaped endothelial cells of both the inner and outer wall (11, 12). The cells of the inner wall exhibit bulging structures towards the lumen, which have been interpreted as nuclei, and openings of up to a size of 3-5 µm. As there are no other relevant direct openings, these pores are thought to play a major role in the bulk outflow of aqueous humor across the inner endothelium of SC. On the basis of theoretical calculations, they appear to maintain the normal range of the intraocular pressure. These findings strongly support the hypothesis of Tripathi (267, 268, 269, 271) on the mechanism of the aqueous outflow across the inner wall of SC by transitory intracellular channels, being the paracellular routes very little represented (216). The pores into the SC are so few that a great amount of serial ultrathin sections would be required to make quantitative evaluations. It should be noted that Svedbergh (261) criticized the use of TEM in the study of inner wall endothelial cells as it provides only a limited view of this area. SEM, on the contrary, overcomes the processing problems associated with TEM and readily provides both pore frequency (in post-mortem normotensive eyes : 800-1000 /mm^2) and pore size distribution (11, 12, 134, 225, 243, 297).

The application of SEM in the study of the structures of the angle had been foreseen in 1968 by Spencer and co-workers (253) : " The extra dimension provided by SEM may also serve to change one's orientation and hopefully stimulate investigators to profitably re-evaluate their present light and transmission electron microscopic anatomic studies and to reconsider the manner in which therapeutic drugs function ".

Several studies have been carried out by SEM or correlative SEM/TEM on the pressure-dependent changes in the structure of the angle in humans and in animals (102, 103, 104, 146, 166, 260). It was demonstrated that the openings in the monolayered endothelial cells of the SC

inner wall were significantly increased at higher pressures, provided a physiological range is maintained. Therefore, it seems that in normal conditions the endothelial cells have a peculiar property that allows the invagination of the cell surface. In this way these cells adapt to an increase of fluid outflow by forming more pores. On the contrary, many glaucomatous eyes, both those removed from humans at surgery and those experimentally induced, a narrowing of the lumen of SC and a depletion of macrovacuolar structures and pores were seen. In TM, sclerosis and thickening of the trabeculae as well as various amorphous or particulate materials were observed, but these findings were not specific for glaucoma as they were described also in normotensive senile eyes (4, 18, 29, 65, 78, 165, 211, 220, 231, 241, 244, 268, 271). Up to now it is still an open question if these alterations are a cause or a consequence of glaucoma. It should, however, be considered that potential artefacts can occur during collection and preparation for SEM particularly of trabeculectomy specimens and therefore care has to be taken in drawing conclusions (172).

Although cultured meshwork endothelial cells do not completely resemble trabecular meshwork cells "in situ", they represent an optimal model of study to gain further insight into the manifold biological functions of these cells (105, 232). In these studies, the scanning microscope, both in the secondary mode (SE) and in the scanning transmission mode (STEM) has been employed along with other correlative ultrastructural techniques to examine the cytoskeletal elements (101, 237, 238), to quantify some morphological characteristics related to synthetic activities (106, 205, 272), to investigate the ability of these cells to phagocytose (107). The trabecular endothelial cells can change their shape in response to drugs and under certain pathological conditions (270).

Uveal tract

For reasons of clearness, we will deal with the uveal tract following the classical subdivision, from anterior to posterior, into iris, ciliary body and choroid.

The iris constitutes the diaphragm between the anterior and the posterior chamber and therefore it offers two free surfaces which can be easily investigated by SEM. At the anterior border, there is a discontinuous layer of flattened cells with a slightly bulging, oval nucleus, intermixed among bundles of collagen fibers (46, 51, 111); a continuous endothelial-like cell layer appears to be absent in humans (49) (fig.7). Holes and pores of various size deepen into the iris stroma and through these openings the aqueous humour appears to freely penetrate the stroma. SEM examination shows that both radially and circularly oriented furrows run along the posterior border; these furrows are more pronounced in the pupillary region (fig.8). At higher magnification the posterior border, which corresponds to the pigmented epithelial cell surface, shows several excrescences due to the underlying differently sized melanin granules (50, 247).

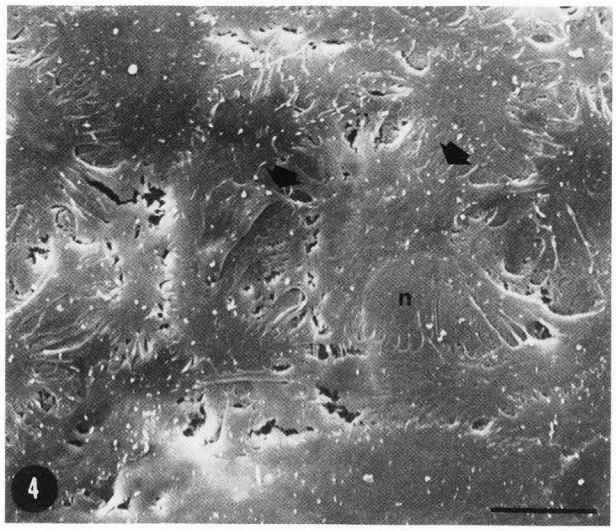
SEM was used to investigate also the arrangement of the connective tissue fibers in the stroma (278). It was beautifully shown that stroma consists of an ordered network of collagen bundles which run parallel to the surfaces of the iris, forming canals in the stroma, the organization of which depends on the grade of contraction of the iris muscles. Around the vessels, numerous layers of a tightly woven network of collagen fibers were interpreted as structures able to prevent kinking of the vessels in mydriasis or to counterbalance the internal pressure of the vessels to the intraocular pressure. Thirdly, it has been proposed that these sheaths of collagen fibers can warm the aqueous, which had been cooled during its circulation in the anterior chamber, through the delay of its rapid flow before entering the iris vessels.

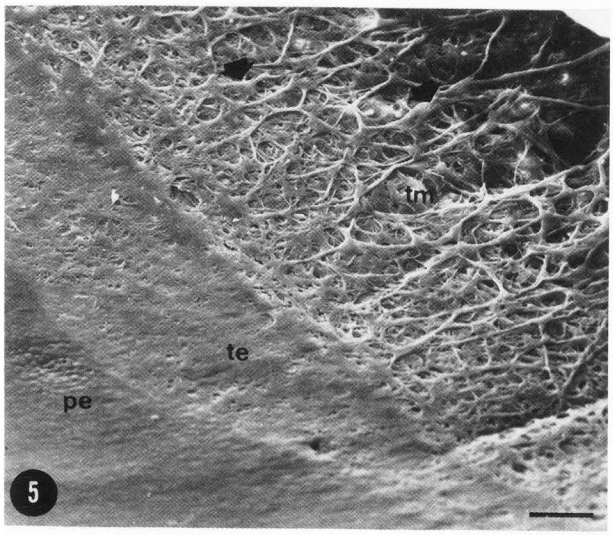
One of the most impressive characteristics of the iris is its capability to undergo extensive changes both in response to light and drugs. The application of SEM in the study of pupillary dilatation and constriction allowed a view of

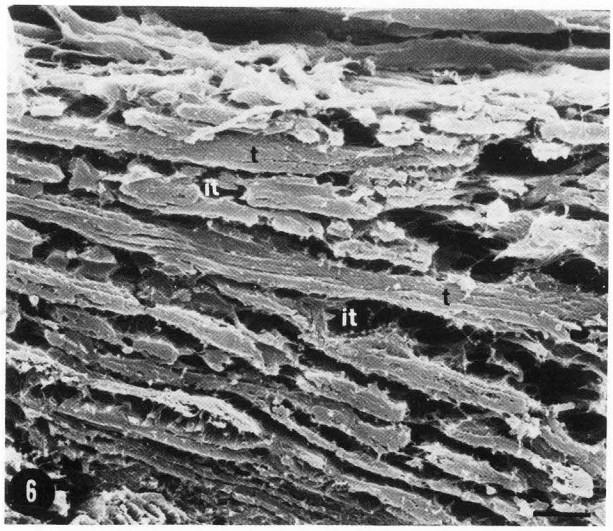
Fig.4. Human corneal endothelium. Peripheral zone at the border with the trabecular meshwork. The endothelial cells appear irregularly shaped and loosely interconnected (arrows). Bulging nuclei are still present (n).
SEM Bar = 10 µm

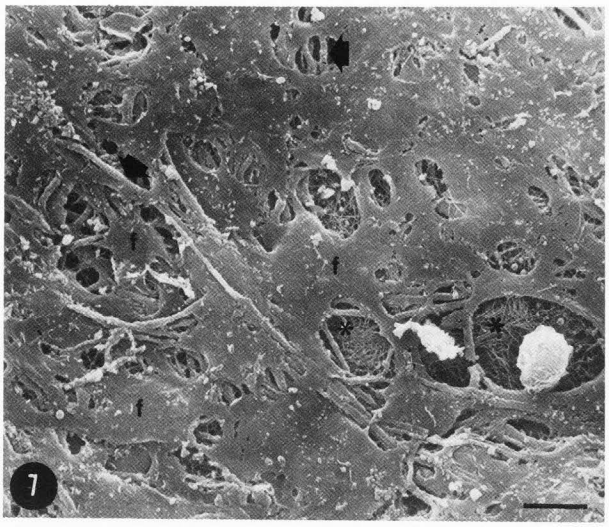
Fig.5. Human trabecular meshwork. A whole view of the anterior chamber. Between the peripheral endothelium (pe) and the trabecular meshwork (tm), a zone of transitional endothelium (te) is observed. SEM very clearly shows the netlike arrangement of the trabeculae (arrows), the long axes of which are circumferentially oriented.
SEM Bar= 100 µm

Fig.6. Human trabecular meshwork. This meridional section view shows the sieve-like structure of the trabecular meshwork, with the trabecular sheets (t) and the intertrabecular spaces (it). SEM Bar = 10 µm









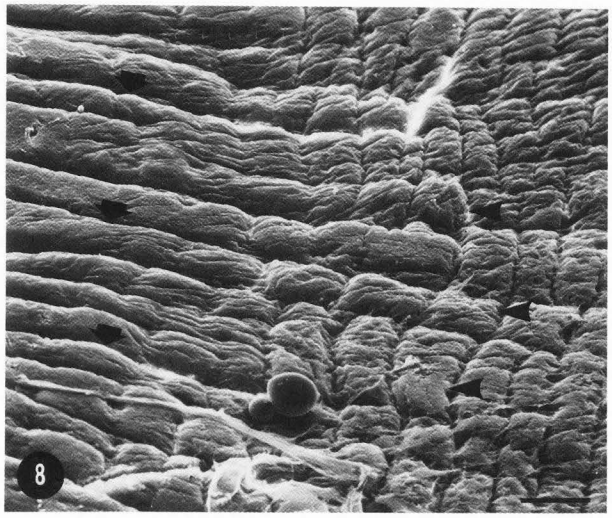


Fig.7. Human iris. Anterior border. A discontinuous layer of fibroblasts (f) with prominent radiating and interdigitating processes (arrows) line the anterior face. An underlying collagen meshwork is visible through openings (*) . These holes are responsible for the free access of aqueous humor to the iris stroma. SEM Bar = 10 µm

Fig.8. Human iris. Posterior border. Radial furrows (arrows), known as the radial contraction folds of Schwalbe and more pronounced in the pupillary region, and circumferential folds (arrowheads) present near the periphery are visible. SEM Bar = 10 µm

---

the iris surface in its totality, providing in much greater detail the relationships between the lining cells, which were not apparent in LM

or TEM sections (46, 168, 277). In midriasis the posterior epithelial cells form circumferential ridges with intervening grooves; in miosis these ridges flatten and the posterior surface becomes entirely smooth except for a small area around the pupil. Concomitantly, from pupillary dilatation to constriction, the stromal blood vessels change their orientation from a generally circumferential to a radial direction and the shape of the myoepithelial cells is markedly changed from long and fusiform in myosis to columnar in wide midriasis (171). A more complete description of this mechanism was given by SEM studies (277) as, besides iris structures, also trabecular meshwork and the course of zonular fibers could be described in detail. The sheaths of the TM were found to be spaced wider in miotic than in midriatic eyes, but a difference in the shape of the transcellular holes was not observed. The zonular fibers apparently did not change direction and insertion during the pupillary movement. The ciliary processes frequently showed a single crest in mydriasis and two crests in miosis.

The physiological activities of the ciliary body (CB) are extremely diverse since it produces the aqueous, nourishes the lens, constitutes part of the vitreous base and plays a fundamental role in the accommodation process as it provides both muscle power and support for zonular fibers.

Despite the manyfold possibilities offered by SEM for in-depth study of each of these problems, this technique has yet only been applied to the study of the relationship between CB surface and zonular attachment.

The ciliary epithelium consists of two layers of cells, an innermost layer of non-pigmented and an outermost layer of pigmented cells. During the embryological development of this area an inversion of the polarity of these cells occurs due to the invagination of the optic cup against itself. In the adult, the apices of the epithelial cells face each other and their bases face outwards, towards the CB stroma for the pigmented cells and towards the posterior chamber for the non-pigmented cells. Therefore, what we see of the CB surface facing the posterior chamber at SEM is the non-pigmented cell basement membrane, through which, as in a transparency, the invagination of the basal plasma membrane of the cells is visible.

Some anatomical details have been clarified by SEM. As an example, ridges and slopes of the ciliary processes as well as convoluted radial ridges at the periphery of the iris which appear to anchor the iris root to the ciliary body have

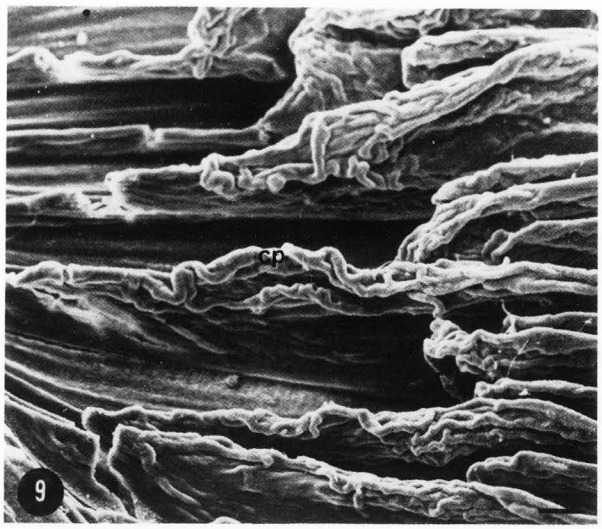


Fig.9. Human ciliary body. Anterior part (pars plicata). This part protrudes forward into the posterior chamber by enlarged and thick folds, the ciliary processes (cp) that display a finely rugated surface. SEM Bar = 200 μm

been better described (153, 168) (fig.9). But the main contribution of SEM was the description of the zonular insertion on the ciliary body. For reasons of clarity, we will discuss this aspect together with the description of the zonular apparatus.

The posterior part of the uveal tract, the choroid, is extremely rich in blood supply. Choroidal vessels nourish the outer layer of the retina and, in addition, choroidal blood flow participates in the regulation of intraocular pressure and in the dissipation of heat. Therefore, to speak of choroid means to speak of blood vessels. Apart from one very early paper on descriptive morphology in SEM (175), choroid was mainly studied in SEM by means of the vascular cast technique. Some examples of the results obtained with this procedure are reported in the chapter on the "vascular casts".

Some authors investigated by SEM the ultrastructural alterations of the anterior uvea in experimentally induced uveitis in rabbits (42, 276). The ciliary epithelium has been shown to be extremely sensitive to pathological changes : while in moderate uveitis it was only covered by a thin layer of leukocytes and fibrin strands, in severe uveitis the processes of CB had quite completely disappeared because of oedema. In addition, it was observed that fibrin deposition had begun before the arrival of the inflammatory cells on the ciliary process surface. According to Uusitalo (276) this would

suggest that alterations of ciliary epithelium and leakage of serum proteins are independent of infiltrating inflammatory cells.

Finally, a few studies have been performed on the SEM appearance of choroidal melanomas both removed at surgery (183, 209, 213) and cultivated in vitro (224). The classification of these neoplasias is based upon the cell type morphology (i.e. spindle and epithelioid cells). The prognosis is generally drawn on this basis by means of LM/TEM observations which cannot be of course always realistic as to the total cell shape, because these techniques only provide a two-dimensional image. Consequently, SEM was successfully applied with the aim to complement the evaluation of the real shape of these neoplastic cells.

Vitreus

The term "vitreus" is now used to fully design the connective tissue surrounded by the lens, ciliary body and retina. Thus, vitreus should replace the common expressions "vitreous humour" and "vitreous body" while "vitreous" should remain as an adjective. Therefore, the two rheological states of the vitreus will be "gel vitreus" and "liquid vitreus".

The vitreus has an extremely high water content (98.5 to 99.7 %), and contains collagen fibers and scattered hyalocytes. During the first year of life a differentiation begins into a cortical zone of high density and a central semifluid zone. Later on, the destruction of the vitreous framework takes place so that in the adult eye the structure of the vitreus is determined by a simultaneous process of production and degeneration. However, the difficulties in obtaining reliable histological preparations of this tissue still raises questions about the existence of an organized structure.

SEM would appear a particularly suitable technique to demonstrate the three-dimensional structure of the collagen fibril meshwork, but the difficulties to process vitreus for SEM are even more serious than for conventional procedures because of the shrinkage phenomenon during the drying steps. Hansson (110) first attempted to study vitreous specimens by SEM using freeze-drying but this technique failed to obtain information on the different zones through the whole vitreus, which is necessary to make correlations with clinical observations.

Other authors subsequently introduced alternative preparation techniques by embedding the whole globe in the resin celloidine, which was then dissolved in acetone to expose the fibril network for SEM observations. It is important to note that with this procedure one can also perform simultaneous correlative LM and TEM investigations (21, 72, 73, 74, 265). These techniques have been applied by Faulborn and Bowald (75) to the study of the effects of gas instillations in the vitreous chamber during surgery.

SEM has been applied to the study of a special structure present in vitreus during prenatal development, the Tunica Vasculosa Lentis (TVL), a large vascular network which disappears in humans around birth (136). Patz (197) first pointed out the usefulness of SEM to investigate the effects of oxygen on TVL development as the observation of the whole-mounted embryonic eye for SEM readily provides evidence on the proliferation or regression of this structure. This fact was elegantly demonstrated (15) by exposing newborn mice to various gas mixtures. A marked hyaloid regression was observed in gas exposed mice compared to air exposed control animals.

Zonular apparatus

The zonular apparatus is a complex network of elastic-like fibers (263) which run from the ciliary body, customarily considered as the origin, to the lens, considered as the insertion. During the accommodative mechanism, the ciliary processes move forward and inward thus relaxing the zonule, the lens decreases in circumference increasing the anterior curvature and the refractive power.

There are two principal theories of accommodation. The Halmholtz-Fincham theory (77, 109) states that zonular fibers alter the lens shape by producing changes in tension of the lens capsule. The Coleman model (31) is based upon analysis of hydraulic forces and explains accommodation as a function of both lens elasticity and vitreous support. However, it is well recognized that the zonule represents the main effector of accommodation and therefore its anatomy directly reflects how this mechanism works.

Several descriptions of the course of the zonule by SEM were published in the past in rat (113), in rabbit (41), in non-human primates (40, 66, 70, 229), in man (19, 39, 68, 129, 191, 217, 229, 257, 258) with the object to show the correlation between the anatomical features and the accommodative function in the different species.

In the rabbit, which has a poorly developed accommodation, the attachment of the zonule to the ciliary body consists of a narrow belt at the anterior part of the ciliary processes. The fibers run without forming thicker fibers and

insert as a single row at the equator of the lens.

The architecture of the zonular apparatus in man does not differ significantly from that in non-human primates. At least two groups of zonular fibers have been described (fig.10). Bornfeld et al.(19) found one group of fibers originating from the pars plana and going to the anterior face of the lens and another group coming from the ciliary valleys and inserting into the posterior part of the lens. Farnsworth et al. (68, 70) described four major groups of zonular fibers. According to these authors, all these fibers show an uninterrupted path. Short zonule strands connect posteriorly the end of the ciliary body processes with pars plana, running in a meridional direction (fig.11). These fibers also participate in accommodation, probably exerting an anchoring function on the ciliary processes (40) or serving as a modulator for the circumferential changes in the pars plicata (70).

It is usually reported that zonular fibers cover the CB-lens distance in the shortest course possible; on the other hand, some authors observed a curved course from ora to lens (31, 70, 217). To explain the fact that it is impossible for a fiber system under tension attached at two terminal points to follow a curved course without an intermediate fixation, Rohen (229) admits that two different types of zonular fibers are present. The "main zonules" run directly from the pars plana to the lens and the "tension zonules" connect the main fibers to the ciliary processes. The two systems are joined to form a "zonular plexus" within the pars plicata of the ciliary body and act as a fulcrum during the ciliary muscle contraction. These authors suggest a new theory of accommodation (230) supported by the fact that in experimentally induced depletion of this zonular fulcrum anchored to the ciliary body, the retrodisplacement of the ciliary muscle has no effect on the accommodation (150). Unlike the traditional theories, this model assumes that the pars plana does not move forward during the accommodation.

The fibrils pass through the inner limiting membrane, at their origin from the ciliary body, enter the ciliary epithelium and run through the intercellular spaces of the non-pigmented layer (132, 215, 233, 234). The bundles of fibrils fuse with the lens capsule at its surface except in man where they penetrate deeply approaching the lens epithelium (206, 215)(fig.12). After removal of the anterior hyaloid membrane, the insertion of zonular fibers on the lens as seen at SEM appears to be continuous all around the equator. In man, the zonular fibers insert

Fig.10. Human ciliary body. Zonular attachment as seen from the anterior chamber. During the preparation, the zonular fibers, cut to remove the lens, collapsed and here are seen as a mass of confused fibrils tightly packed against each other (*). The fibers coming from the pars plana (pp) are clearly seen (arrows). SEM Bar = 200 μm

Fig.11. Human ciliary body. Zonular fibers at the pars plana (pp). These short fibers, coming from the ciliary valleys of the pars plicata (arrows), connect the posterior part of the processes with pars plana. SEM Bar = 10 μm

Fig.12. Human lens . Zonular attachment at the pre-equatorial surface. The fibers (f) divide and thin progressively into smaller fibrils that apparently fuse with the capsule (c). SEM Bar = 10 μm

---

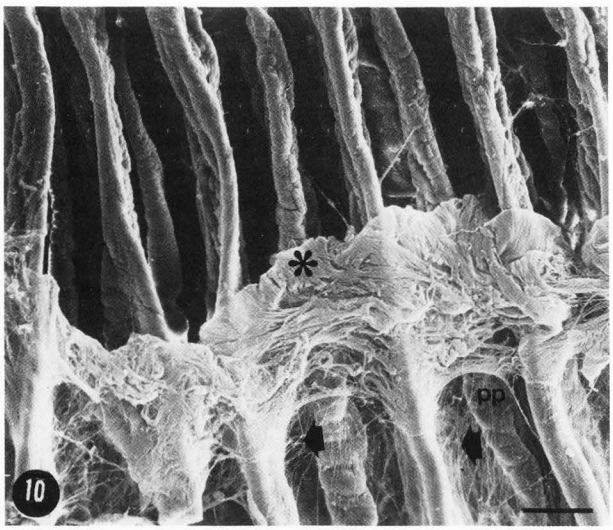
anteriorly, posteriorly and at the equator, the fibrils thin progressively and end in a basket-wave fashion (68, 257).

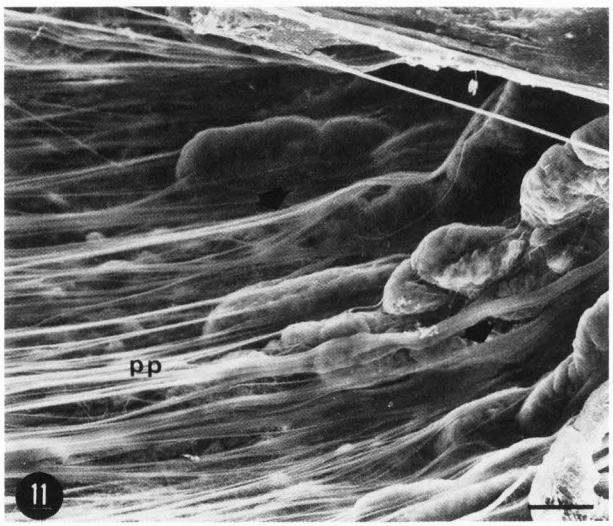
Apart from this fundamental contribution to elucidate how the accommodative process works, SEM was performed to evaluate the use and the consequences of the enzyme alpha-chymotrypsin in zonulolysis. Anderson (2) studied the enzymatic digestion of zonular fibers in vivo at graded intervals of time and found a progressive degradation of the fibrils up to a final amorphous material. Worthen (298) confirmed that alpha-chymotrypsin provokes a fragmentation of the zonular fibers, but showed that these fragments enter the anterior chamber and then can cause a temporary block of the filtering angle structures. This supports the clinical observation that alpha-chymotrypsin can give rise to a transient post-operative glaucoma.

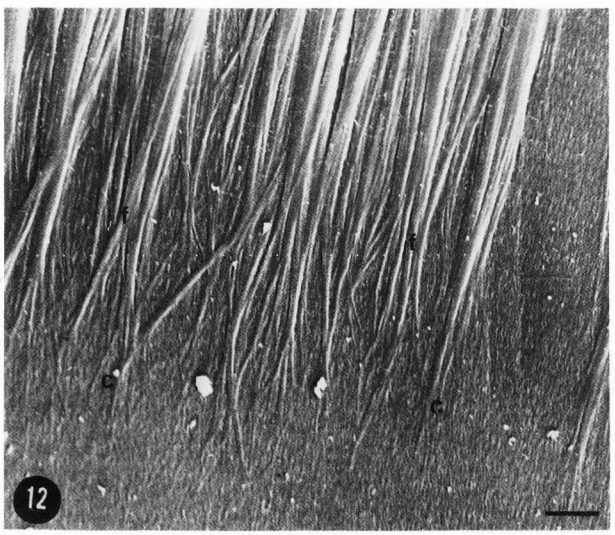
Lens

SEM has contributed in establishing that the lens is a space-filling system of fibers, firmly connected by specific devices that leave only a minimal extracellular space. The extraordinary regularity of the arrangement of lens fiber cells and the minimal extracellular space between them minimize light scatter and in this way a multicellular system, such as the lens, can work as a coaxial refracting system for the incoming light upon the retina (155).

The lens is completely surrounded by the capsule, a basement membrane-like structure produced by the epithelial cells anteriorly and by the superficial fibers posteriorly. As seen by SEM it displays surface crevices and pores, structures that allow the lens to exchange







metabolites with the surrounding environment and prevent large molecules to enter the lens (fig.13). The surface of the capsule appears to be fibrillar, especially in the equatorial area where the zonular fibers insert (68).

The quite unique characteristic of the lens is that it is made up by only one kind of cells which undergo a maturation moving from cortical to nuclear zone. The growth of the lens depends upon the mitotic activity of the lens epithelium, a monolayer of cells located, in the adult vertebrates, at the anterior side only. The morphology of these cells and their connections with the overlying capsule and the underlying cortical fibers were first established in TEM (207, 214) and significantly clarified in SEM (69, 115) (fig.14). The intercellular contacts of the lateral plasmamembranes consist of a complex of interdigitations that increase the area available for transport of metabolites. In addition, pores were observed at the level of the basal epithelial cell surfaces; these pores are supposedly involved in the uptake of proteins. Finally, the capsule appears to have maximal adhesion to the underlying epithelial cells mainly at the level of the cell borders; it was suggested that this could be related to the maintenance of the architecture of the lens during accommodational lens changes (69, 115).

The epithelial cells located pre-equatorially in the so-called "germinative zone" proliferate,

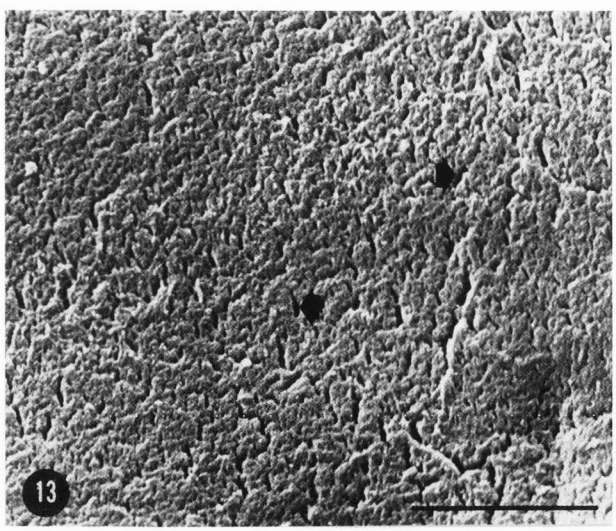


Fig.13. Human lens capsule. Anterior side. The surface appears to be furrowed by crevices and pores (arrows) through which the lens communicates with the surrounding environment. SEM Bar = 10 µm

the daughter cells enter the adjacent "transitional zone" where a differentiation process starts and where they become the lens fibers by elongation of their cell bodies. Therefore, cells of all ages can be observed within an adult lens.

SEM along with TEM was extensively applied to investigate the organization of lens fibers in several animal species (23, 47, 67, 85, 112, 117, 118, 129, 137, 139, 154, 155, 156, 157, 158, 159, 160, 162, 177, 178, 191, 202, 290, 291, 292). Accurate investigations of the changes in lens cell shape, size and surface complexity during the development from epithelial cells into elongate fiber cells have been possible by this technique. In particular, the presence and differential (regional) distribution of 3 types of membrane anchoring specializations has been demonstrated by SEM: ball-and-socket junctions, interlocking protrusions and microplicae or tongue-and-groove junctions.

Ball-and-socket junctions are bulges and corresponding invaginations of the lateral fiber surfaces that fit into each other and are generally restricted to specific regions in the anterior and posterior poles (at least in rabbit, 290). The interlocking protrusions are bulges of the apical and lateral edges of the fiber that embrace protrusions of the neighboring fibers located above or below; they are generally present throughout the primate lens (fig.15). SEM showed an unequivocal difference between these two types of junctions was possible. Previous TEM studies failed to point out this fact as in this case the three-dimensional appreciation of the protrusions is lost and therefore ball-and-sockets can be confused with interlocking protrusions when cut and observed in an ultrathin section. The third type of device is represented by microplicae, i.e., infolding and ridges of the cell membrane present in the deep lens and at the border between cortex and nucleus of the primate lens (193, 291) (fig.16). As the density of ball-and-socket decreases from periphery to nuclear region and there is an area displaying simultaneously ball-and-socket and tongue-and-groove junctions, it has been proposed that these are not permanent structures but that they can change during development. In addition, undulations of the lateral and apical edges of the superficial cortical fibers close to the zone of insertion of the zonular fibers have been described in rabbit (290). A recent paper, to which the reader is referred, (158) reviews how the fibers appear to approach the sutures and the morphology of the sutures themselves.

It would be a rather intricate matter to summarize here all the SEM studies listed above. The main conclusion which can be drawn is that the structural integrity and the optimal optical properties of the lens are guaranteed by specializations of the cell membranes that either are evenly distributed or are restricted only to some regions of the lens.

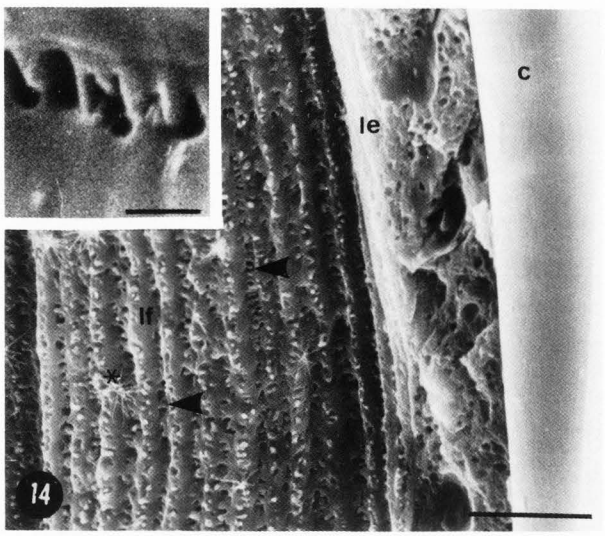
To establish the type, amount and localization of a given interlocking device is important in the investigation of the role of these devices in the accommodative process. It has been suggested that ball-and-socket junctions bind fibers tightly and tongue-and-groove junctions in addition prevent sliding movement. Consequently, sliding of fibers is unlikely to occur in order to cause lens shape changes in accommodation (162). It would seem more reasonable that this mechanism can be accomplished by changes in the length of lens fibers determined by unfolding of bends and folds (290).

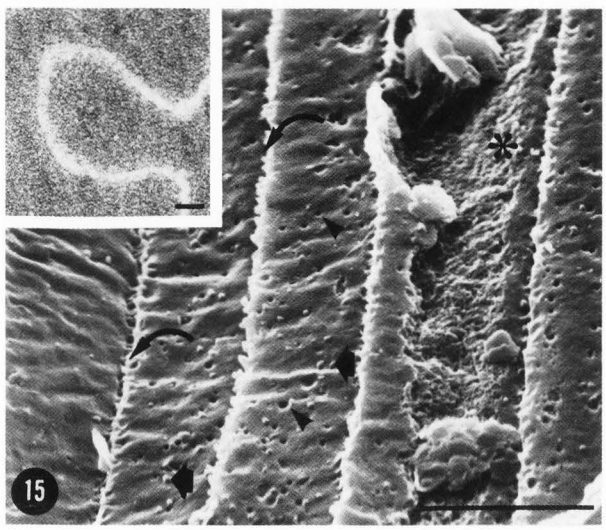
For reasons of space it is impossible to cover all papers on SEM of ocular tissues. For the lens, we will only discuss with the main pathological studies carried out by means of SEM.

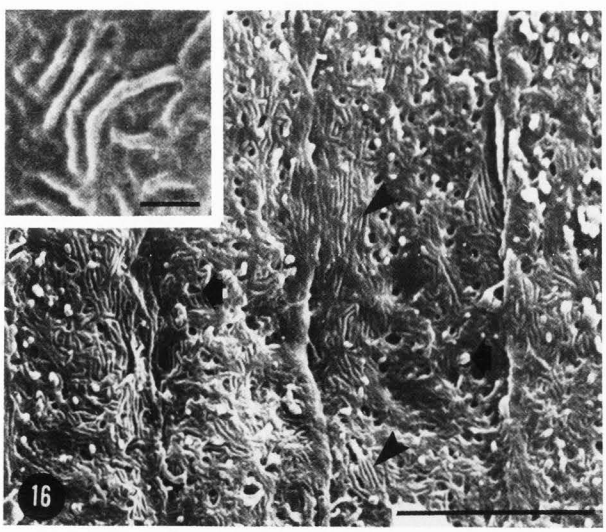
Fig.14. Human lens. Anterior side. The fractured face shows, in sagittal section, the capsule (c), the lens epithelium (le) in the germinative zone and the elongating lens fibers (lf). Interlocking devices firmly inerconnect the fibers (arrowheads and inset). * = artefactual vitreous crystals. SEM Bar = 10 μm
Inset: SEM Bar = 1 μm

Fig.15. Human lens. Cortical zone. The bulges (arrows) and the invaginations (arrowheads) characteristic of the ball-and-socket junctions of the fiber surfaces are clearly distinct from the interlocking protrusions of the fiber edges (curved arrows). A cell broken during fracturing exposes its internal part with few cytoplasmic organelles (*). SEM Bar = 10 μm
Inset : section of a ball and socket junction. TEM Bar = 0.1 μm

Fig.16. Human lens. Nuclear zone. The fiber surface shows contemporaneously ball-and-socket junctions (arrows) along with tongue-and-groove interdigitations (arrowheads and inset). SEM Bar = 10 μm
Inset: SEM Bar= 0.5 μm







First of all, knowledge of the morphological alterations of lens fiber cells in cataracts was considerably deepened. Sakuragawa et al. (239) investigated the role of swelling of lens cells in cataract. They demonstrated that lens cells in congenital cataract have an irregular size and shape in the early stages of the cataract process and that swelling of lens cells takes place later in areas corresponding to the appearance of the first optical opacities. This process is somewhat different for congenital cataract compared to eyes of galactose-fed rats. Beyer-Mears et al. (9) established the progression of neonatal cataractogenesis in the lens of galactose-fed rats by the quantization of ultrastructural changes as seen by SEM in five lens regions. The same group (10) also pointed out the pharmacological effects of quercetin (a compound that inhibits the activity of the enzyme aldose reductase) in reducing sugar cataractogenesis in neonatal rats. Nelson and Rafferty (188) evaluated by SEM the possibility of lens fiber cells to regain a normal morphology after an experimentally induced traumatic cataract. Some authors investigated by SEM senile cataractous human lenses (34, 177, 186). In particular, Mousa et al.(186) and Creighton and Trevithick (34) studied actin-related globular degeneration as a contributing factor in senile cortical cataractogenesis and the possibility to prevent this process by administration of Vitamin E and glutathione. Rini et al. (227) found that X-ray induced cataractogenesis appears to be dependent upon the relative dose of irradiation, while Kodama et al. (152) demonstrated the suppression of the X-ray effects by administration of a galactose-diet. Harding and co-workers (119) applied SEM and X-ray microanalysis both to bulk specimens and thick sections of human lenses in order to investigate the possible relationship between calcium and cataractogenesis. A study of congenital ectopia lentis was carried out by Farnsworth et al. (71) who discovered that three-dimensional ultrastructural defect of a congenital microspheric ectopic lens consisted of a marked decrease in lens fiber cross-area, which could be responsible for the abnormal size and shape of the lens. Few studies on lens surface characteristics in the course of the so-called "pseudo-exfoliation syndrome" exist (8, 37, 38, 246). The ultrastructure of the residual lens material (the ring of Soemmering) after extracapsular cataract extraction was investigated in rabbit and man (147, 149). A more recent field of interest is represented by the direct and indirect effects of various types of lasers on ocular tissues. Vester and

collaborators (286) used SEM to compare the immediate and long-term effects of Q-switched and mode-locked Nd-YAG lasers on lens. They observed that both lasers produced, after one hour, few and restricted lesions but after 6 months the areas in which the laser had been applied appeared heavily affected and were postulated to act as possible cataractogenesis centers.

In these years the artificial intraocular lens (IOL) has become a very common device to surgically correct refractive errors and to replace the native lens removed for various diseases. SEM has explained some aspects of the biocompatibility and biodegradation of this prosthesis. The IOL is an interesting structure whose surfaces interface several ocular tissues. The SEM investigation of the surfaces of IOLs removed from human eyes or implanted in experimental animals reveals the presence of different types of adhering cells as well as of other structures of organic nature (5, 64, 294, 295). In particular, macrophages, epithelioid cell, granulocytes, multi-nucleated foreign-body giant cells were observed on top of a thin acellular membrane (55, 148, 187, 250, 259, 284) confirming and better explaining correlative LM findings (210, 293, 296). These results unequivocally suggest that the prosthesis is subject to a defensive cellular reaction by the host which recognizes it as a foreign body. The extent of this reaction seems to be correlated with the successful follow-up of the implant (250). It is believed that this foreign body reaction occurs normally and that it can increase the success of the implant by sequestering it with a membrane thus making the IOL accepted by the eye (295).

In addition, areas of both optic and haptic parts showed some deformations and/or crackings interpreted by some authors (148) as artefactually due to the schedule procedures for SEM. On the contrary, other authors (299) claimed for the role of aging of the patients, duration of the implant, inflammation of the eye, individual reaction in determining the biodegradation of the prosthetic material, especially at the level of the nylon loops (299, 187).

Retina

The retina is much more than only a connection of photoreceptors at the beginning of the optic way. This thin neural tissue is actually an outpost of the central nervous system, the structural complexity of which seems to increase the more techniques become available to study it. Much of our current knowledge about

Fig.17. Human retina. A distinct layering pattern is shown in this micrograph : 1- photoreceptor outer segments 2- photoreceptor inner segments 3- nuclei of photoreceptors (outer nuclear layer) 4- outer plexiform layer 5- nuclei of bipolar cells (inner nuclear layer) 6- inner plexiform layer 7- ganglion cell layer. ▼ = external limiting membrane; ► = middle limiting membrane. This micrograph does not comprise the nerve fiber layer and the inner limiting membrane facing the vitreous chamber. SEM Bar = 10 μm

Fig.18. Human retina. In this frozen-cracked specimen a plane section of the whole retina is obtained and the areas of maximal synaptic contacts can be seen (4,6). The numbers correspond to those of fig. 17. SEM Bar = 10 μm

Fig.19. Human retina. Photoreceptor cell surface after removal of the retinal pigment epithelium. The outer (o) and the inner (i) particles are well visible. Groups of cells appear partially disrupted (*). c= cone SEM Bar = 10 μm

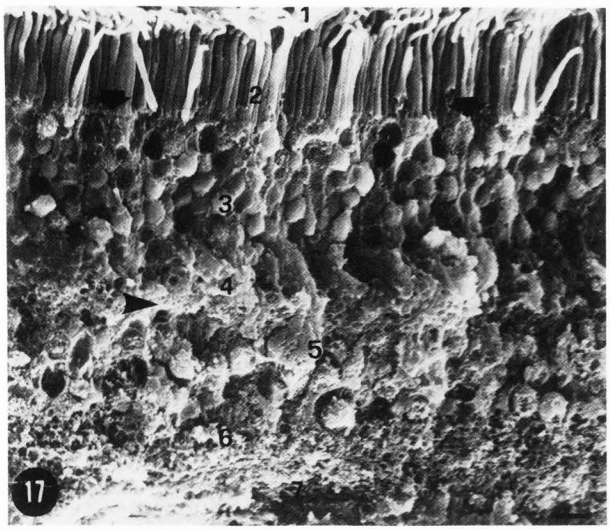
Fig.20. Human retina. The outer (o) and inner (i) segments and the nuclei (n) of the photoreceptors are visible. At the level of the external limiting membrane (▼) Muller cell microvilli (arrowheads) surround the inner segment base. SEM Bar = 10 μm

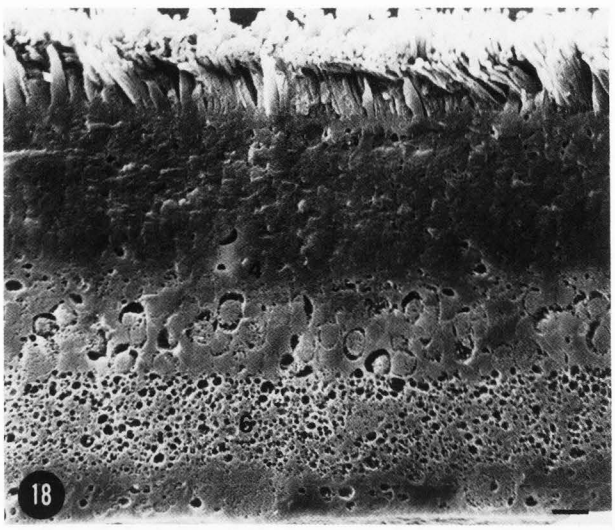
---

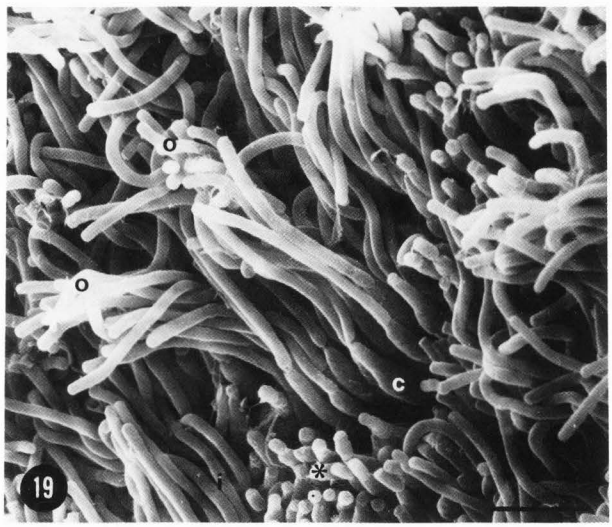
the retina actually goes back to studies in the third century B.C. by the Greeks. Of course, the progressive understanding of retinal anatomy has resulted in new concepts of its function.

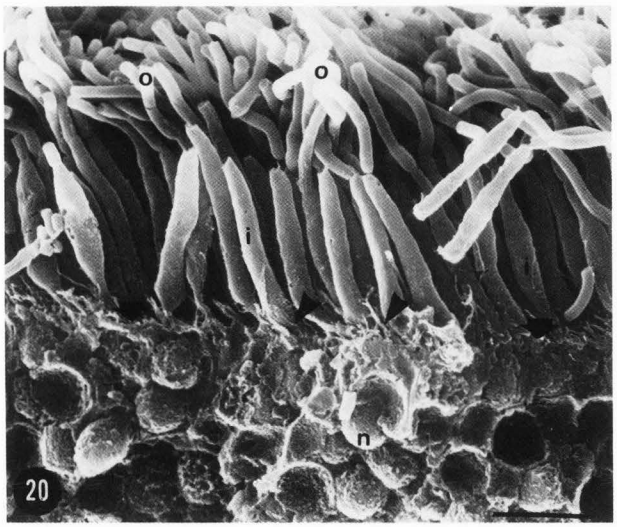
The classical way to divide the retina is into 5 cell types : the photoreceptors (rods and cones), the bipolar cells and the ganglion cells that form the so-called "vertical system" and the horizontal and amacryne cells that constitute the "horizontal system". Until recently, each type of cell, connected in a rather complex network, was thought to carry out a specific, single, fixed task. On the contrary, in the light of every new discovery, it has become clear that the retina contains much more than only 5 functional cell types; the neurobiologists believe that the true number of differently working elements may be as high as fifty (174).

In the living eye, major areas of the retina can be readily viewed by ophthalmoscopy and biomicroscopy. Therefore SEM can enhance that three-dimensional information at the ultrastructural level. It is undeniable that the complex structure of the retinal cells has become easier to understand by SEM (figs.17, 18, 19, 20, 21, 22). The same consideration is true









also for the retinal pigment epithelium (RPE), the surface of which can be easily visualized by SEM after gentle removal of the overlying retina (240) (fig.23).

Several review papers about SEM in retinal research have appeared since the beginning of the technique. The most complete and clear appears to be also the most recent (20) as it reviews not only all the major contributions and the relating results, but also all the problems connected with the preparation for SEM and the possible artefacts. In addition, a very large series of references was added and therefore it appears unnecessary to duplicate the bibliography of that paper (20). Hence, we only summarize in the present paper the most important work appearing in these last years after that review.

The basic anatomy of this tissue was further clarified by SEM on both bulk specimens and on cells in vitro. Heegard et al. investigated the structure of the inner limiting membrane in non-human primates (127) and pointed out the improved conservation of the retinal cells for SEM by adding hexamethyldisilazane to the preparation schedule (126). Radius and De Bruin (212) correlated the information obtained on the retinal nerve fiber layer by ophthalmoscopy, with ultrastructural images by TEM and SEM. Muller cells from rabbit (120), photoreceptors from chicken (273, 275) and rat (251) and human RPE (190) were observed by SEM to investigate the differentiation of these cells when cultured in vitro. Lower vertebrate retinas were studied by Breidbach (24), a further study on primate retina was made by Dickson et al. (48) and a comparative evaluation between LM and SEM images of guinea pig photoreceptors was carried out by

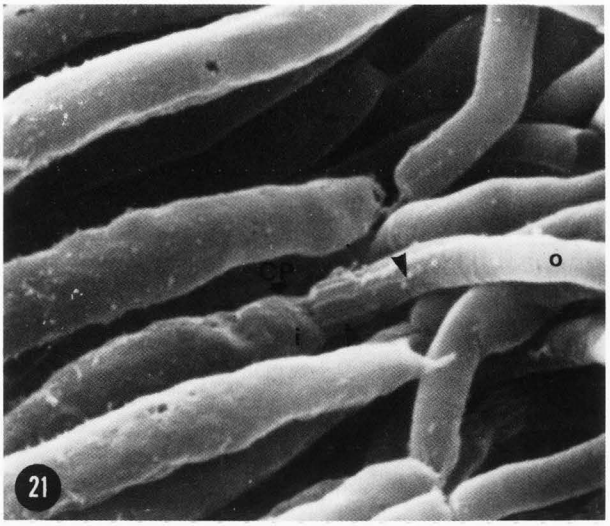


Fig.21. Human retina. A detail at higher magnification of fig.20. Calyceal processes (CP) consist of a row of ridges (arrowheads) emerging from the inner segment (i), that encircle the base of the outer segment (o) and extend along its surface. The function of this structure is unknown. SEM Bar= 1 μm

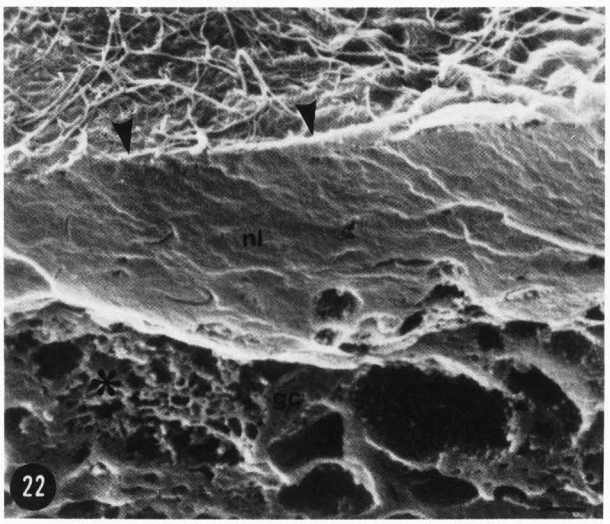


Fig.22. Human retina. In this micrograph the ganglion cell layer (gc), the nerve fiber layer (nl) and the inner limiting membrane (arrowheads) are seen. A large area of synaptic contacts is also visible (*). SEM Bar = 1 μm

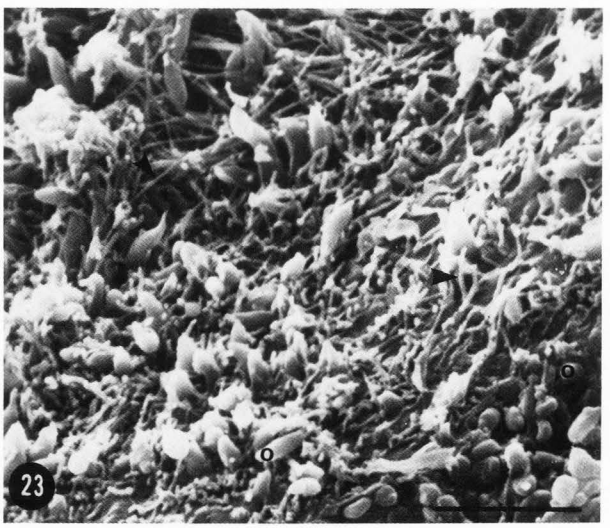


Fig.23. Human retinal pigment epithelium. Scanning electron microscopy of the apical surface after artificial separation from the retina shows a myriad of fine and long microvilli (arrowheads) and outer segments (o) firmly enclosed in them. SEM Bar = 5 μm

Collins (32). SEM was performed to examine the RPE surface after experimentally induced retinal detachment and the sequence of the changes of RPE surface morphology (141, 181, 192). The retina was studied after different methods of photocoagulation to evaluate the effect on the vitreo-retinal junction (182) and retinal inner/outer surface (252) morphology. Scattered studies employed SEM to study various retinal diseases, spontaneous hypertensive conditions (245), diabetic retinopathy (74), retinoblastoma (33, 80, 264, 288), retinal perforation (58), vitreous detachment (35, 140) and hereditary blindness in an animal model (274).

It appears from the analysis of the above listed bibliography as well as of all the older literature, that as yet the application of SEM to the study of the retina and RPE has confirmed, clarified, highlighted and summarized previous knowledge, but that the amount of really new information is not very large (20). Except for a work by Peters and collaborators (198) who have shown the periciliary ridge complex of the apical inner segment with ultrahigh resolution SEM, no really significant and original information has as yet been achieved by SEM. But research on the architecture of the retina is about to enter a new and promising phase. Usually, flat preparations of intact retinas, stained by different markers are used in order to investigate the cell shape, size, content and interconnections of the different circuits at the light microscopic level and only in a two-dimensional way. The reconstruction in three-dimensions of functionally related groups of neurons located at different levels of the tissue, processed by various cracking methods to expose the inner structures (142, 208, 209) and

subsequently marked by means of immunocytochemical techniques, will in our opinion be the future trend of SEM studies of the retina. This association of methods, which has not yet been attempted for the retina, could enlarge the more conventionally used neurobiological techniques and hopefully lead to new horizons in the physiology of this fascinating tissue.

Vascular casts

To be complete, a review paper on SEM in ophthalmology has to deal briefly with this method of investigation. The vascular corrosion cast technique consists of an injection of a polymerizing plastic into a vessel, followed by the digestion of the surrounding tissues (26, 84). The observation of these casts by SEM provides an impressive three-dimensional overview of the complicated vascular tree of all ocular regions and of the connections between the various vessels. In addition, it is possible to identify the type of vessel (i.e. artery, capillary or vein) on the basis of its surface characteristics. The plastic casts bridge the gap between histology and fluorescein angiographic observations and can in addition provide details not detectable by angiography. It has to be considered, however, that some artefacts can occur due to eventual changes in the casting material composition, pressure of injection, shrinkage of the resin. Therefore the accuracy of microcorrosion casts in representing the complete vascular network as in vivo has to be carefully evaluated (163).

Microcorrosion casts have been utilized to disclose the disposition of vessels in and around the optic nerve head in several species, including man (82). The lobular architecture of the vessels in the choriocapillaris has been widely investigated by vascular casts in SEM, further demonstrating that these anatomical units correspond to functionally independent vascular districts (121). The technique applied to the retina showed that the retina vessels are present as a three-layered pattern with interconnections, at least in monkey (Macaca irus) (249).

The papers published during the last few years on both the normal and pathological vascular anatomy studied by microcorrosion casts in SEM are collected in a very recent, complete and comprehensive review, published in this journal (27). We do not think we can add here something to such a specific paper both in terms of references and discussion on the results obtained with this method of investigation. Therefore, the reader is carefully recommended to consult that review (27) for this part. We will only cite a few papers which have appeared in the last months.

The vascular anatomy of ciliary processes (184, 185), choroid (176, 300, 301) and retina (44) was further evaluated in several animal species and man. Changes in the choroidal vasculature in diabetic patients were studied by Fryczkowski (83). SEM observations of vascular casts was also applied to the study of the effects of various types of lasers on the ocular microvasculature. Straatas et al (256) investigated the repair process of the choriocapillaris after confluent argon laser retinal photocoagulation. Brown and co-workers (25) observed the effects of Nd-YAG laser on the retinal and choroidal vessels in the primate eyes. In this area of investigation this technique was particularly useful to correlate and enhance the information obtained with fluorangiography on the steps of the vascular repair (25, 256).

General comment

The first impression one may get after having read this review paper is that SEM really was important in the study of all ocular tissues because of its impressive power to clarify the complexity of structures such as those of the eyeglobes.

Several anatomical features have been either clarified or newly discovered by SEM and consequently functional correlations have been proposed. This was particularly the case with the trabecular meshwork and the zonular apparatus. In other tissues, such as the retina, SEM was extensively applied, but most efforts were not rewarded by really new information. The major part of papers has appeared between 1970 and 1980, when the instrument just had become commercially available and all researchers tried to use it to get new insights on the ocular morphology.

While reviewing the older literature, some sentences, repeated in almost all the papers, pointed out that SEM was considered a valuable tool as to : "the dramatically beautiful appearance of the cells in three-dimensional view", or "the extensive depth of perception and the plastic impression of the pictures", or "the economical value in providing a summary form of information that would otherwise require extensive serial sectioning by TEM and LM", but, as claimed by Hager in 1975 (108) "SEM will neither displace the LM nor the TEM, it will rather represent a supplement to the classical microscopic procedures". This means that the role of SEM has been, at least up to now, "confined" to the description of structural features, provided by micrographs having an

impressive depth of field and great aesthetic power.

We believe that the end of this kind of task is in sight and that SEM can no longer be applied for descriptive purposes only. The present "state of the art" of the technique, in our opinion, could be improved by spreading the use of SEM along with other correlative techniques corroborating and enhancing each other as we already stressed in previous papers (164, 283). Moreover, SEM has been applied in ophthalmological research, only in the secondary electron mode and very few groups, to our knowledge, have as yet utilized the other detectors now available. Finally, the possibility to join immunocytochemistry to SEM (i.e., biochemistry to stereo-morphology) has already been attempted by a few (195) in ophthalmic science. The positive results obtained by this new approach in other fields of investigation (164) suggest that this will open extremely interesting perspectives. This will be in our opinion, also the future of SEM in the study of the universe "eye".

Acknowledgements

This work was performed within the Center of Biotechnological and Clinical Research in Ophthalmology, University of Bologna and Ciba-Vision, Venezia.

REFERENCES

1- Alfonso E, Tucker GS, Batlle JF, Mandelbaum S, Gelender H, Forster RK (1986). Snailtracks of the corneal endothelium. Ophthalmology, 93 : 344-349.

2- Anderson DR (1971). SEM of zonulolysis by alpha-chymotrypsin. Am J Ophthalmol, 71: 619-625.

3- Anderson DR (1971). Scanning electron microscopy of primate trabecular meshwork. Am J Ophthalmol, 71 : 90-101.

4- Anderson DR (1971). Experimental alpha-chymotrypsin glaucoma studied by SEM. Am J Ophthalmol, 71 : 470-476.

5- Apple DJ, Mamalis N, Brady SE, Loftfield K, Kavka-van Norman D, Olson RJ (1984). Biocompatibility of implant materials : a review and scanning electron microscopic study. Am Intraocular Impl Soc J, 10 : 53-65.

6- Barrett G, Constable IJ (1984). Corneal endothelial loss with new intraocular lenses. Am J Ophthalmol, 98 : 157-165.

7- Basu PK (1983). Application of scanning electron microscopy in ophthalmic research. Ind J Ophth, 31 : 476-485.

8- Benedikt O, Aubock L, Gottinger W, Waltinger H (1973). Comparative transmission and scanning electron microscopical studies on lenses in the so-called exfoliation syndrome. Graefe's Arch Clin Exp Ophthalmol, 187 : 249-264.

9- Beyer-Mears A, Farnsworth PN, Fu SCJ, Burke P (1978). Progressive galactose cataractogenesis and regional susceptibility in the neonatal lens. Exp Eye Res, 27 : 275-287.

10- Beyer-Mears A, Farnsworth PN (1979). Diminished cataractogenesis by Quercetin . Exp Eye Res, 28 : 709-716.

11- Bill A (1970). Scanning electron microscopic studies of the canal of Schlemm. Exp Eye Res, 10 : 214-218.

12- Bill A, Svedbergh B (1972). Scanning electron microscopic studies of the trabecular meshwork and the canal of Schlemm. An attempt to localize the main resistance to outflow of aqueous humor in man. Acta Ophthalmol, 50 : 295-320.

13- Binder P S, Stenberger H, Wickham MG, Worthen DM (1976). Corneal endothelial damage associated with phacoemulsification. Am J Ophthalmol, 82: 48-54.

14- Binder PS, Akers P, Zavala EY (1979). Endothelial cell density determined by specular microscopy and scanning electron microscopy. Ophth A A O, 86 : 1831-1847.

15- Bischoff PM, Wayer SD, Flower RW (1983). Scanning electron microscopic studies of the hyaloid vascular system in newborn mice exposed to O_2 and CO_2. Graefe's Arch Clin Exp Ophthalmol, 220: 257-263.

16- Blackwell WL, Gravenstein N, Kaufman HE (1977). Comparison of central corneal endothelial cell numbers with peripheral areas. Am J Ophthalmol, 84 : 473-482.

17- Blumcke S, Morgenroth K (1967). The stereo ultrastructure of the external and internal surface of the cornea. J Ultrastruct Res, 18 : 441-448.

18- Bonney CH (1982). Short term effects of Q-switched ruby laser on monkey anterior chamber angle. Invest Ophthalmol Vis Sci, 22 : 310-318.

19- Bornfeld N, Spitznas M, Breipohl W, Bijvank G (1974). Scanning electron microscopy of the zonula of Zinn. I. Human eyes. Graefe's Arch Clin Exp Ophthalmol, 192 : 117-129.

20- Borwein B (1985). Scanning electron microscopy in retinal research. Scanning Electron Microsc, 1985; I : 279-301.

21- Bowald S, Faulborn J (1985). Preparation of the vitreous body for comparative examination with LM, SEM and TEM. Mikroskopie, 42 : 206-214.

22- Boyde A, Jones SJ, Bailey E (1973). Bibliography on biomedical applications of SEM. Scanning Electron Microsc, 1973: 697-734.

23- Bradley RH, Lo WK, Kuszak JR, Maisel H (1980). The cytoskeleton of the chicken lens fiber cells: a scanning and ultrastructural analysis. Exp Eye Res, 31 : 487-494.

24- Breidbach O (1984). Application of scanning electron microscopy in an analysis of the retina of lower vertebrates. J Neuroscience Methods, 12: 65-68.

25- Brown GC, Green WR, Shah HG (1984). Effects of the Nd:YAG laser on the primate retina and choroid. Ophthalmology, 91: 1397-1405.

26- Burger PC, Chandler DB, Klintworth GK (1984). Scanning electron microscopy of vascular casts. J El Microsc Tech, 1: 341-348.

27- Burger PC, Chandler DB, Fryczkowski AW, Klintworth GK (1987). Scanning electron microscopy of microcorrosion casts: applications in ophthalmologic research. Scanning Microscopy, 1: 223-231.

28- Capella JA, Kaufman HE (1969). Human corneal endothelium. Docum Ophth, 26: 1-8.

29- Chaudhry HA, Dueker DK, Simmons RJ, Bellow R, Grant WM (1979). Scanning electron microscopy of trabeculectomy specimens in open-angle glaucoma. Am J Ophthalmol, 88: 78-92.

30- Cleveland PH, Schneider CW (1969). A simple method of preserving ocular tissue for SEM. Vision Res, 9: 1401-1402.

31- Coleman DJ (1970). Unified model for accommodative mechanism. Am J Ophthalmol, 69: 1063-1079.

32- Collins BA (1986). A comparison of the size of photoreceptor cells from the retina of the domestic pig as determined by light and scanning electron microscopy. Micron Microsc Acta, 17: 259-268.

33- Craft JL, Robinson NL, Roth NA, Albert DM (1978). Scanning electron microscopy of retinoblastoma. Exp Eye Res, 27: 519-531.

34- Creighton MO, Trevithick JR (1979). Cortical cataract formation prevented by vitamin A and glutathione. Exp Eye Res, 29: 689-693.

35- Daicker B, Guggenheim R, Gywat L (1977). Findings on retinal surface by scanning electron microscopy. 2. Vitreous detachment. Graefe's Arch Clin Exp Ophthalmol, 204: 19-29.

36- Dark AJ, Durrant TE, Mc Ginty F, Shortland JR (1974). Tarsal conjunctiva of the upper eyelid. Am J Ophthalmol, 77: 555-564.

37- Davanger M (1975). The pseudo-exfoliation syndrome. A scanning electron microscopic study. I. The anterior lens surface. Acta Ophthalmol, 53: 809-820.

38- Davanger M (1975). The pseudo-exfoliation syndrome. A scanning electron microscope study. II. The posterior chamber region. Acta Ophthalmol, 53: 821-833.

39- Davanger M (1975). The suspensory apparatus of lens. The surface of the ciliary body. A SEM study. Acta Ophthalmol, 53: 19-33.

40- Davanger M, Ringvold A (1977). The ciliary body and the suspension of the lens in a monkey (Cercopithecus aethiops). Acta Ophthalmol, 55: 965-975.

41- Davanger M, Pedersen O (1978). The ciliary body and the suspension of the lens in rabbits. A scanning electron microscopy study. Acta Ophthalmol, 56: 127-138.

42- Davanger M, Pedersen O (1978). The ciliary body and the iris in experimental uveitis in rabbits. A scanning electron microscopic study. Acta Ophthalmol, 56: 857-864.

43- Davanger M, Olsen EG (1985). The corneal endothelial cell interface. Acta Ophthalmol, 63: 109-115.

44- De Juan E, Chandler DB, Hida T, Machemer R (1986). Glio-vascular architecture in the rabbit retina. Invest Ophthalmol Vis Sci, 27: 1602-1608.

45- Deschamps F, Royer J, Montard M (1982). Premiers resultats de l'empreinte conjunctivale (First results of conjunctival imprints). Bull Soc Opht Fr, 82: 1515-1517.

46- Dickson DH (1975). Fine structure and mechanics of the anterior border of the primate iris: a scanning and transmission electron microscopic study. Can J Ophth, 10: 227-238.

47- Dickson DH, Crock GW (1972). Interlocking patterns of primate lens fibers. Invest Ophthalmol, 11: 809-815.

48- Dickson DH, Carroll N, Crock GW (1987). Scanning electron microscopy of the primate retina. Trans Ophthalmol Soc NZ, 82: 127-131.

49- Dieterich CE, Witmer R, Franz HE (1971). Iris and circulation of aqueous humor. Morphological analysis of the surface structure of human iris. Graefe's Arch Clin Exp Ophthalmol, 182: 321-340.

50- Dieterich CE, Franz HE (1973). A study on the surface of the pigment epithelium of the human iris. Z Anat Entwickl Gesch, 141: 331-338.

51- Dieterich CE, Franz HE (1973). Studies on the marginal layer and the anterior leaf of the human iris using the scanning electron microscope and the conventional electron microscope. Med Welt, 24: 1659-1662.

52- Dilly PN (1985). Contribution of epithelium to the stability of the tear film. Trans Ophthalmol Soc U K, 104: 381-389.

53- Dilly PN, Mackie IA (1981). Surface changes in the anaesthetic conjunctiva in man, with special reference to the production of mucus from a non-goblet-cell source. Br J Ophth, 65: 833-842.

54- Dimitrescu HL, Hager H, Hoffmann F (1972). Scanning electron microscopy of the human cornea. Ber Dtsch Ophth Ges, 71: 656-660.

55- Drews RC, Smith ME, Okun N (1978). Scanning electron microscopy of intraocular lenses. Ophthalmology, 85: 415-424.

56- Ducker DK, Chaudry HA, Pihaja DJ (1979). A technique for exposing internal surfaces of ocular tissue for SEM. Scanning Electron Microsc, 1979; III: 323-328.

57- Durand L, Giraud M, Laborier J (1982). L'endothelium cornéen en microscopie electronique à balayage: morphologie normale et pathologique (The corneal endothelium seen by scanning electron microscopy: normal and pathological morphology). Bull Soc Opht Fr, 82: 6-7.

58- Eagle RC, Brucker AJ (1983). Choroidal melanoma with retinal perforation and vitreous hemorrhage: a scanning electron microscopic study. Jap J Ophth, 27: 512-521.

59- Edelhauser HF, Van Horn DL, Schultz RO, Hyndiuk RA (1976). Comparative toxicity of intraocular irrigating solutions on the corneal endothelium. Am J Ophthalmol, 81: 473-481.

60- Edelhauser HF, Gonnering R, Van Horn DL (1978). Intraocular irrigating solutions. A comparative study of BSS plus and lactated Ringer's solution. Arch Ophthalmol, 96: 516-520.

61- Edelhauser HF, Hanneken AM, Pederson HJ, Van Horn DL (1981). Osmotic tolerance of rabbit and human corneal endothelium. Arch Ophthalmol, 99: 1281-1287.

62- Edelhauser HF, Mac Rae SM (1985). Irrigating and viscous solutions. In: Surgical Pharmacology of the Eye, M Sears and A Tarkkanen (eds), Raven Press, New York, pages 363-388.

63- Ehlers N, (1965). The precorneal film, biomicroscopical, histological and chemical investigations. Acta Ophthalmol, 43(suppl 81): 101-133.

64- Eifrig DE (1980). Deposits on the surface of intraocular lenses: a pathological study. South Med J, 73: 6-21.

65- Epstein DL (1986). Experimental obstruction to aqueous outflow by pigment particles in living monkey. Invest Ophthalmol Vis Sci, 27: 387-395.

66- Erckenbrecht J, Rohen JW (1975). SEM of zonula apparatus of higher primates. A further approach to a new theory of accommodation. Graefe's Arch Clin Exp Ophthalmol, 193: 19-32.

67- Farnsworth PN, Fu SC, Burke PA, Bahia I (1974). Ultrastructure of rat eye lens fibers. Invest Ophthalmol, 13: 272-279.

68- Farnsworth P, Mauriello J, Burke P, Kulyk T, Cinotti A (1976). Surface ultrastructure of the human lens capsule and zonular attachments. Invest Ophthalmol Vis Sci, 15: 36-40.

69- Farnsworth PN, Burke P, Kulyk T, Mauriello JA, Cinotti AA (1976). Surface ultrastructure of epithelial cells of the mature human lens. Exp Eye Res, 22: 615-624.

70- Farnsworth PN, Burke P (1977). Three dimensional architecture of the suspensory apparatus of the lens of the Rhesus monkey. Exp Eye Res, 25: 563-581.

71- Farnsworth PN, Burke PA, Blanco J, Maltzan B (1978). Ultrastructural abnormalities in a microspherical ectopic lens. Exp Eye Res, 27: 399-408.

72- Faulborn J (1982). Combined macroscopic, LM, SEM, TEM investigations of the vitreous body. 1. An improved celloidin embedding method for histologic SEM and TEM preparation of the vitreous body. Ophthalmic Res, 14: 113-116.

73- Faulborn J, Bowald S (1983). Combined macroscopic, LM, SEM and TEM investigation of the vitreous body. 3. The structure of the anterior border layer of the vitreous. Ophthalmic Res, 15: 11-18.

74- Faulborn J, Bowald S (1985). Microproliferations in proliferative diabetic retinopathy and their relationship to the vitreous. Corresponding light and electron microscopic studies. Graefe's Arch Clin Exp Ophthalmol, 223: 130-138.

75- Faulborn J, Bowald S (1987). The vitreous after C_3F_8 gas instillation: long-term histologic findings after spontaneous reabsorption of the gas in rabbit eyes. Graefe's Arch Clin Exp Ophthalmol, 225: 99-102.

76- Figueras MJ, Jongebloed WL, Worst JGF (1984). Scanning electron microscopic study of eye tissues. Am Intraocular Impl Soc J, 10: 169-175.

77- Fincham EF (1937). The mechanism of accommodation. Br J Ophthalmol, Monograph, Suppl 8.

78- Fink AI, Felix MD, Fletcher RC (1972). The electron microscopy of Schlemm's canal and adjacent structures in patients with glaucoma. Trans Am Ophth Soc, 70: 82-102.

79- Foster A, De Siena S, Wells B (1986). Scanning electron microscopy of conjunctival surfaces in patients with ocular cicatricial pemphigoid. Am J Ophthalmol, 102: 584-591.

80- Fournier GA, Sang DN, Albert DM, Craft JL (1987). Electron microscopy and HLA expression of a new cell live of retinoblastoma. Invest Ophthalmol Vis Sci, 28: 690- 699.

81- Francois J, Maugdel PC, Victoria-Troncoso V (1976). Experimental keratitis sicca. The corneal ephithelium at the transmission and scanning electron microscopy. Ophthalmic Res, 8: 414-424.

82- Fryczkowski AW (1987). Vascular casting and scanning electron microscopy in diabetes. Scanning Microsc, 1: 811-816.

83- Fryczkowski AW, Grimson BS, Peiffer RL (1984). Scanning electron microscopy of vascular casts in the human scleral lamina cribrosa. Int Ophthalmol, 7: 95-100.

84- Gannon BJ (1978). Vascular casting. In: Principles and techniques of Scanning Electron Microscopy: Biological Applications. Hayat MA (ed), Van Nostrand Reinhold, New York, 171-193.

85- Garcia-Porrero A, Colvee E, Ojeda JL (1984). The mechanism of cell death and phagocytosis in the early chick lens morphogenesis: a scanning electron microscopy and cytochemical approach. Anat Rec, 208 : 123-136.

86- Gierek A, Sosnierz M, Bialas B, Szymansky A (1974). Morphological picture of the iridocorneal angle of the human eyeball viewed under a scanning electron microscope. Ophthalmologica, 169 : 371-376.

87- Gilbard JP, Carter JB, Sang DN, Refojo MF, Hanninen LA, Kenyon KR (1984). Morphologic effect of hyperosmolarity on rabbit corneal epithelium. Ophthalmology, 91 : 1205-1212.

88- Gilbard JP, Ross SR, Gray KL (1987). A new rabbit model for keratoconjunctivitis sicca. Invest Ophthalmol Vis Sci, 28 : 225-228.

89- Gordon SR, Rothstein H, Harding CV (1983). Studies on corneal endothelial growth and repair. IV. Changes in the surface during cell division as revealed by scanning electron microscopy. Eur J Cell Biol, 31: 26-33.

90- Greiner JV, Covington HI, Allansmith MR (1977). Surface morphology of the human upper tarsal conjunctiva. Am J Ophthalmol, 83 : 892-905.

91- Greiner JV, Covington HI, Allansmith MR (1978). Surface morphology of Giant Papillary Conjunctivitis in contact lens wearers. Am J Ophthalmol, 85 : 242-252.

92- Greiner JV, Covington HI, Korb DR, Allansmith MR (1978) Conjunctiva in asymptomatic contact lens wearers. Am J Ophthalmol, 86: 403-413.

93- Greiner JV, Covington HI, Allansmith MR (1979). The human limbus. A scanning electron microscopic study. Arch Ophthalmol, 97 : 1159-1165.

94- Greiner JV, Henriquez AS, Weidman TA, Covington Hi, Allansmith MR (1979). "Second" mucus secretory system of the human conjunctiva. Invest Ophthalmol Vis Sci 18 suppl: 123.

95- Greiner JV, Gladstone L, Covington HI, Korb DR, Weidman TA, Allansmith MR (1980). Branching of microvilli in human conjunctival epithelium. Arch Ophthalmol, 98 : 1253-1255.

96- Greiner JV, Kenyon KR, Henriquez AS, Korb DR, Weidman TA, Allansmith MR (1980). Mucus secretory vesicles in conjunctival epithelial cells of wearers of contact lenses. Arch Ophthalmol, 98 : 1843-1846.

97- Greiner JV, Allansmith MR (1981). Effect of contact lens wear on the conjunctival mucous system. Ophthalmology, 88 : 821-832.

98- Greiner JV, Henriquez AS, Covington HI, Weidman TA, Allansmith MR (1981). Goblet cells of the human conjunctiva. Arch Ophthalmol, 99 : 2190-2197.

99- Greiner JV, Korb DR, Covington HI, Peace DG, Allansmith MR (1982). Human ocular mucus: a scanning electron microscopic study. Arch Ophthalmol, 100 : 1614-1617.

100- Greiner JV, Weidman TA, Korb DR, Allansmith MR (1985). Histochemical analysis of secretory vesicles in nongoblet conjunctival epithelial cells. Acta Ophthalmol, 63: 89-92.

101- Grierson I (1986). Investigations of cytoskeletal elements in cultured bovine meshwork cells. Invest Ophthalmol Vis Sci, 27 : 1318-1330.

102- Grierson I, Lee WR (1974). Changes in the monkey outflow apparatus at graded levels of intraocular pressure: a qualitative analysis by light microscopy and scanning electron microscopy. Exp Eye Res, 19 :21-33.

103- Grierson I, Lee WR (1975). The fine structure of the trabecular meshwork at graded levels of intraocular pressure. 1. Pressure within the near physiological range (8-30 mm Hg). Exp Eye Res, 20 : 505-521.

104- Grierson I, Lee WR (1975). The fine structure of the trabecular meshwork at graded levels of intraocular pressure. 2. Pressures outside the physiological range (0 and 50 mm Hg). Exp Eye Res, 20 : 523-530.

105- Grierson I, Robins E, Howes RC (1980). Preliminary observations of human trabecular cells in vitro. Graefe's Arch Clin Exp Ophthalmol, 212: 173-186.

106- Grierson I, Kissun R, Ayad S, Phylactos A, Ahmed S, Unger WG, Day JE (1985). The morphologic features of bovine meshwork cells in vitro and their synthetic activities. Graefe's Arch Clin Exp Ophthalmol, 223: 225-236.

107- Grierson I, Day J, Unger WG, Ahmed A (1986). Phagocytosis of latex microspheres by bovine meshwork cells in culture. Graefe's Arch Clin Exp Ophthalmol, 224: 536-544.

108- Hager H, Hoffmann F, Dimitrescu L (1975). Scanning electron microscopy in ophthalmology. Ann Ophth, 7: 1361-1371.

109- Halmholtz H (1855). Uber die Akkomodation des Augen (On the accomodation of the eye). Greaefe's Arch Clin Exp Ophthalmol, 1: 1-4.

110- Hansson HA (1969). SEM of the vitreous body in the rat eye. Z Zellforsch, 101: 323-337.

111- Hansson HA (1970). Ultrastructure of the surface of the iris in the rat eye. Z Zellforsch, 110 : 192-204.

112- Hansson HA (1970). Scanning electron microscopy of the lens of the adult rat. Z Zellforsch, 107 : 187-198.

113- Hansson HA (1970). Scanning electron microscopy of the zonular fibers in the rat eye. Z Zellforsch, 107 : 199-209.

114- Hansson HA, Jerndal T (1971). Scanning electron microscopic studies on the development of the iridocorneal angle in human eyes. Invest Ophthalmol, 10 : 252-265.

115- Harding CV, Harding D, Peters V, Kuwabara T, Reddan J, Unakar N, Bagchi M, Schnur T, Gordon S (1973). Epithelial cell surfaces and the basement membrane in the ocular lens, studied with the scanning electron microscope. Exp Eye Res, 16 : 1-7.

116- Harding CV, Bagchi M, Weinsieder A, Peters V (1974). a comparative study of corneal epithelial cell surfaces utilizing the scanning electron microscope. Invest Ophthalmol, 13: 906-912.

117- Harding CV, Susan S, Murphy H (1976). Scanning electron microscopy of the adult rabbit lens. Ophthalmic Res, 8: 443-455.

118- Harding CV, Susan S (1976). The nuclear envelope in the crystalline lens fiber cell. Invest Ophthalmol, 15 : 433-437.

119- Harding CV, Chylack Jr LT, Susan SR, Lo WK, Bobrowsky WF (1983). Calcium-containing opacities in the human lens. Invest Ophthalmol Vis Sci, 24 : 1194-1202.

120- Harstadt HK, Ringvold A (1987). Scanning and transmission electron microscopy of Muller cells isolated from rabbit retina. Graefe's Arch Clin Exp Ophthalmol, 223: 29-34.

121- Hayreh SS (1975). Segmental nature of the choroidal vasculature. Br J Ophth, 59: 631-648.

122- Hazlett L (1986). Development of ocular mucin : scanning electron microscopy analysis. Ophthalmic Res, 18: 28-39.

123- Hazlett L, Spann B, Wells P, Berk R (1980). Epithelial desquamation in the adult mouse cornea. A correlative TEM and SEM study. Ophthalmic Res, 12: 315-323.

124- Hazlett L, Spann B, Wells P, Berk R (1980). Desquamation of the corneal epithelium in the immature mouse: a scanning and transmission electron microscopy study. Exp Eye Res, 31: 21-30.

125- Hazlett L, Wells P, Berk R (1984). Scanning electron microscopy of the normal and experimentally infected ocular surface. Scanning Electron Microsc, 1984; III: 1379-1389.

126- Heegard S (1986). Hexamethyldisilazane in preparation of retinal tissue for SEM. Ophthalmic Res, 18: 202-211.

127- Heegard S, Jensen OA, Prause JU (1986). Structure and composition of the inner limiting membrane of the retina. SEM of frozen resin-cracked and enzyme-digested retinas of Macaca Mulatta. Graefe's Arch Clin Exp Ophthalmol, 224 : 355-360.

128- Hervouet F, Ertus M (1973). Les structures oculaires en microscopie à balayage (Ocular structures in the SEM). Masson, Paris.

129- Hervouet F, George Y, Tusques J, Ertus M (1973). Structure du crystallin humain et de la zonule de Zinn en microscopie electronique a balayage (The structure of the human lens and the zonule of Zinn in SEM). Bull Soc Anat (Nancy), 58 : 349-356.

130- Hervouet F, Sourdille PH (1975). L'angle iridocornéen dans le glaucome congenital vu au microscope a balayage (The iridocorneal angle in congenital glaucoma seen in SEM). Bull Soc Ophthal Fr, 75 : 131-138.

131- Hirsh M, Renard G, Faure JP, Pouliquen Y (1977) Study of the ultrastructure of the rabbit corneal endothelium by freeze-fracture technique: apical and lateral junctions. Exp Eye Res, 25 : 277-288.

132- Hirsch M, Montcourrier P, Arguilere P, Keller N (1985). The structure of the tight junctions in the ciliary epithelium. Curr Eye Res, 4: 493-501.

133- Hoffman F (1972). The surface of epithelial cells of the cornea under the scanning electron microscope. Ophthalmic Res, 3: 207-214.

134- Hoffman F, Dimitrescu HL (1971). Schlemm's canal under the scanning electron microscope. Ophthalmic Res, 2: 37-45.

135- Hoffman F, Schweichel JU (1973). The microvilli structure of the corneal epithelium of the rabbit in relation to the cell function.

A transmission and scanning electron microscopic study. Ophthalmic Res, 4: 175-184.

136- Hollenberg MJ, Dickson DH (1971). Scanning electron microscopy of the tunica vasculosa lentis of the rat. Can J Ophth, 6 : 301-310.

137- Hollenberg MJ, Wyse JP, Lewis BJ (1976). Surface morphology of lens fiber from eyes of normal and microphthalmic (Browman) rats. Cell Tissue Res, 167: 425-438.

138- Honegger H, Brewitt H (1978). Normal cornea and conjunctiva epithelium as seen in scanning electron microscopy. Dtsch Ophth Ges, 75: 646-648.

139- Hoyer HE (1982). Scanning electron microscopic study of lens fibers of the guinea pig. Cell Tissue Res, 224: 225-232.

140- Hsu HT, Patterson R, Ryan SJ (1984). Traumatic posterior vitreous detachment :Scanning electron microscopy of an experimental model in the monkey eye. Scanning Electron Microsc, 1984; III: 1361-1368.

141- Immel J, Negi A, Marmor MF (1986). Acute changes in RPE apical morphology after retinal detachment in rabbit. A SEM study. Invest Ophthalmol Vis Sci,27: 1770-1776.

142- Jacobsen IE, Jensen OA, Prause JU (1984). Structure and composition of Bowman's membrane. Study by frozen resin cracking. Acta Ophthalmol, 62: 39-53.

143- Jacobsen R, Sperling S (1978). Scanning electron microscopic observations of a mucopolysaccharide coating on human corneal endothelium. Acta Ophthalmol, 56: 161-167.

144- Jensen OA, Prause JU (1980). Frozen resin-cracking, dry-cracking and enzyme digestion methods in SEM as applied to ocular tissues. Graefe's Arch Clin Exp Ophthalmol, 212: 207-216.

145- Jensen OA, Prause JU, Laursen H (1981). Shrinkage in preparatory steps for SEM. A study on rabbit corneal endothelium. Graefe's Arch Clin Exp Ophthalmol, 215: 233-242.

146- Johnstone MA, Grant WM (1973). Pressure-dependent changes in structures of aqueous outflow system of human and monkey eyes. Am J Ophthalmol, 75: 365-373.

147- Kappelhof JP (1985). The ring of Soemmering in the rabbit. A scanning electron microscopic study. Graefe's Arch Clin Exp Ophthalmol, 223: 111-120.

148- Kappelhof JP, Vrensen GF, de Jong PT, Pameyer JH, Willekens BC (1986). Cytology of human intraocular lenses. A scanning electron microscopic study. Ophthalmic Res, 18: 75-80.

149- Kappelhof JP, Vrensen GF, de Jong PT, Pameyer JH, Willekens BC (1987). The ring of Soemmering in man: an ultrastructure study. Graefe's Arch Clin Exp Ophthalmol, 225: 77-83.

150- Kaufman PL, Rohen JW, Barany EH (1979). Hyperopia and loss of accommodation following ciliary muscle disinsertion in the cynolmolgus monkey: physiologic and scanning electron microscopic studies. Invest Ophthalmol Vis Sci, 18: 665-673.

151- Kessel RG, Kardon RH (1981). Scanning electron microscopy of mammalian neuroepithelia. In: Scanning Electron Microscopy in Cell Biology and Medicine. K. Tanaka and T. Fujita (eds). Excerpta Medica, Amsterdam pages 483-499.

152- Kodama T, Reddy VN, Giblin F (1983). Scanning electron microscopy of X-ray induced cataract in mice of normal and galactose diet. Ophthalmic Res, 15: 324-333.

153- Krey H (1974). On raster electron microscopy of the pars plicata of the human ciliary body. Comparative investigations with air drying and critical point drying. Graefe's Arch Clin Exp Ophthalmol, 191: 127-137.

154- Kuszak JR, Maisel H, Harding CV (1978). Gap junction of chick lens fiber cells. Exp Eye Res, 27: 495-498.

155- Kuszak JR, Alcata J, Maisel H (1980). The surface morphology of embryonic and adult chick lens fiber cells. Am J Anat, 159: 395-410.

156- Kuszak JR, Rae JL (1982). Scanning electron microscopy of the frog lens . Exp Eye Res, 35: 499-519.

157- Kuszak JR, Macsai MS, Rae JL (1984). Stereo scanning electron microscopy of the crystalline lens. Scanning Electron Microsc, 1984; III: 1415-1426.

158- Kuszak JR, Bertram BA, Macsai MS, Rae JL (1984). Sutures of the crystalline lens : a review. Scanning Electron Microsc. 1984; III: 1369-1378.

159- Kuszak JR, Bertram BA, Rae JL (1985). The ordered structure of the crystalline lens. In: Development of Order in the Visual System. Hilfer SR and Sheffeld JB(eds), Springer-Verlag, New York/ Berlin, 35-60.

160- Kuszak JR, Macsai MS, Bloom KJ, Rae JL, Weinstein RS (1985). Cell-to-cell fusion of lens fiber cells in situ: correlative light, scanning electron microscopic and freeze-fracture studies. J Ultrastruct Res, 93: 144-160.

161- Kuwabara T (1970). Surface structure of the eye tissue. Scanning Electron Microsc, 1970: 187-192.

162- Kuwabara T (1975). The maturation of the lens cell: a morphologic study. Exp Eye Res, 20: 427-443.

163- Lametschwandtner A, Lametschwandtner V, Weiger T (1984). Scanning electron microscopy of vascular corrosion casts- technique and applications. Scanning Electron Microsc, 1984; II: 663-695.

164- Laschi R, Pasquinelli G, Versura P (1987). Scanning Electron Microscopy in clinical research. Scanning Microscopy, 1: 1771-1795.

165- Lee WR (1971). The study of the passage of particles through the endothelium of the outflow apparatus of the monkey eye by scanning and transmission electron microscopy. Trans Ophthal Soc U K, 91: 687-705.

166- Lee WR, Grierson I (1975). Pressure effects on the endothelium of the trabecular wall of Schlemm's canal: a study by scanning electron microscopy. Graefe's Arch Clin Exp Ophthalmol, 196: 255-265.

167- Leuenberger PM (1970). Die Stereo-Ultrastruktur der Corneaoberflache bei der Ratte (The stereo-ultrastructure of the corneal epithelium in the rat). Graefe's Arch Clin Exp Ophthalmol, 180: 182-192.

168- Lim WC, Webber WA (1975). A SEM study of the posterior and anterior surfaces of the rat iris in pupillary dilatation and constriction. Exp Eye Res, 20: 445-462.

169- Liotet S, Clergue G (1985). L'oeil-vu au microscope electronique à balayage (The eye seen in scanning electron microscopy). Masson, Paris.

170- Liotet S, Van Bijsterveld OP, Kogbe O, Laroche L (1987). A new hypothesis on tear film stability. Ophthalmologica, 195: 119-124.

171- Lowe RF, Carroll N (1976). Mioepithelium of the human iris: a stereoscopic scanning electron microscopic study. Trans Ophthalmol Soc NZ, 28: 75-81.

172- Maglio M, Mc Mahon C, Hoskins D, Alvarado J (1980). Potential artefacts in scanning electron microscopy of the trabecular meshwork in glaucoma. Am J Ophthalmol, 90: 645-653.

173- Maskin SL, Bodè DD (1986). Electron microscopy of impression-acquired conjunctival epithelial cells. Ophthalmology, 93:1518-1523.

174- Masland RH (1986). The functional architecture of the retina. Scientific American, Dec: 90-99.

175- Matsusaka T (1973). An ultrastructural analysis of lamina vitrea with the scanning electron microscope. Acta Soc Ophth Jap, 77: 936-940.

176- Matsusaka T (1976). Angioarchitecture of the choroid. Jpn J Ophth, 20: 330-346.

177- Matsuto T (1973). Scanning electron microscopic studies on the normal and senile cataractous human lenses. Acta Soc Ophth Jap, 77: 853-872.

178- Matsuto T (1976). Scanning electron microscopic studies on processes of lens fibers with special reference to accommodation. Folia Ophth Jap, 27: 9-14.

179- Maurice DM, (1969). The cornea and sclera, in: The Eye, 2nd Ed , Davson H (ed), Academic Press New York, 81-126.

180- Maurice DM (1972). The location of fluid pump in the cornea. J Physiol 22: 43-55.

181- Mii T, Miki T (1976). Retinal pigment epithelial cells after xenon arc photocoagulation in the detached retina of the rabbits. SEM observation. Acta Soc Ophth Jap, 80: 40-50.

182- Miki T, Mii T (1977). Scanning electron microscopic observation of the vitreo-retinal junction after xenon arc photocoagulation. Acta Soc Ophth Jap, 81: 804-817.

183- Mincione GP, Campana G, Vannelli G, Bonelli A (1979). SEM in malignant uveal melanomas. J Fr Ophtal, 2: 527-530.

184- Morrison JC, De Frank MP, Van Buskirk EM (1987). Regional microvascular anatomy of the rabbit ciliary body. Invest Ophthalmol Vis Sci, 28: 1314-1324.

185- Morrison JC, De Frank MP, Van Buskirk EM (1987). Comparative microvascular anatomy of mammalian ciliary processes. Invest Ophthalmol Vis Sci, 28, 1325-1340.

186- Mousa GY, Creighton MD, Trevithick JR (1979). Eye lens opacities in cortical cataracts associated with actin-related globular degeneration. Exp Eye Res, 29: 379-391.

187- Mullaney J, Lucas DR, Condon PI (1985). Successful intraocular lens implantation: a histological and scanning electron microscopic study of four cases. Acta Ophthalmol, 63 (suppl. 170): 41-49.

188- Nelson KJ, Rafferty NS (1976). A scanning electron microscopic study of lens fibers in healing mouse lens. Exp Eye Res, 22: 335-346.

189- Neubauer L, Baratz RS, Laing RA (1984). Coalescence of endothelial cells in the traumatized cornea. III . Correlation between specular and scanning electron microscopy. Arch Ophthalmol, 102: 921-922.

190- Nicolaissen B, Davanger M, Arnesen K (1982). Surface morphology of explants from the human retinal pigment epithelium in culture. Acta Ophthalmol, 60: 881-893.

191- Oberman EA (1971). Scanning electron microscopy of the lens and zonular fibers . Am J Ophthalmol, 72: 604-608.

192- Ohkuma M (1972). Transmission and scanning electron microscopy observations of the experimentally detached retina in the rabbit. Acta Soc Ophth Jap, 76: 1475-1505.

193- Okinami S (1978). Freeze-fracture replica of the primate lens fibers. Graefe's Arch Clin Exp Ophthalmol, 209: 51-58.

194- Olsen EG, Davanger M (1985). Aspects of the surface morphology of the corneal endothelium. An experimental study. Acta Ophthalmol, 63: 449-453.

195- Ophir I, Moscona AA, Ben-Shaul Y (1983). Localization of retina cognin in embryonic neural retinal tissue by immuno-scanning electron microscopy. Cell Differentiation, 13: 133-144.

196- Ottersen OP, Vegge T (1977). Ultrastructure and distribution of intercellular junctions in corneal endothelium. Acta Ophthalmol, 55: 69-78.

197- Patz A (1982). Clinical and experimental studies on retinal neovascularization. (XXXIX Edward Jackson Memorial Lecture) Am J Ophthalmol, 44: 715-743.

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

199- Pfister RR (1973). The normal surface of corneal epithelium: a scanning electron microscopic study. Invest Ophthalmol, 12: 654-668.

200- Pfister RR (1975). The normal surface of conjunctival epithelium: a scanning electron microscopic study. Invest Ophthalmol Vis Sci, 14: 267-279.

201- Pfister RR, Burstein NL (1977). The normal and abnormal human corneal epithelial surface: a scanning electron microscopic study. Invest Ophthalmol Vis Sci ,16: 614-622.

202- Philipson BT, Hanninen L, Balazs ES (1975). Cell contacts in human and bovine lenses. Exp Eye Res, 21: 205-219.

203- Polack FM (1976). Contributions of electron microscopy to the study of corneal pathology. Surv Ophth, 20: 375-414.

204- Polack FM, Sugar A (1976). The phacoemulsification procedure. II. corneal endothelial changes. Invest Ophthalmol Vis Sci, 15: 458-469.

205- Polansky JR, Wood IS, Maglio MT, Alvarado JA (1984). Evaluation of biological activity and structural properties of human trabecular meshwork cell in vitro. Ophthalmology, 91: 580-595.

206- Porte A, Stoeckel ME, Brini A (1971). Electron microscopic study of the zonular insertion on the human lens capsule. Arch Ophtalmol (Paris), 31: 445-453.

207- Porte A, Brini A, Stoeckel ME (1975). Fine structure of the lens epithelium. Ann Ophthal, 7: 623-634.

208- Prause JU, Jensen OA (1980). Scanning electron microscopy of frozen-coated, dry-cracked and enzyme digested tissue of human malignant choroidal melanomas. Graefe's Arch Clin Exp Ophthalmol, 212: 217-225.

209- Prause JU, Jensen OA (1980). Scanning electron microscopy of frozen-cracked, dry-cracked and enzyme-digested retinal tissue of a monkey (Cercopithecus Aethiops) and of man. Graefe's Arch Clin Exp Ophthalmol, 212: 261-270.

210- Puck A, Tso M, Yue B (1985). Cellular deposits on intraocular lenses. Acta Ophthalmol, 63 (suppl. 170): 54-60.

211- Quigley HA, Addicks EM (1980). Scanning electron microscopy of trabeculectomy specimens from eyes with open-angle glaucoma. Am J Ophthalmol, 90: 854-857.

212- Radius RL, De Bruin J(1981). Anatomy of the retinal fiber layer. Invest Ophthalmol Vis Sci 21:745-749.

213- Radnot M (1976). Scanning electron microscopy of melanoblastomas of the choroid. Ophtahlmologica, 173: 352-363.

214- Rafferty NS, Esson EA (1974). An electron microscope study of adult mouse lens: some ultrastructural specializations. J Ultrastruct Res, 46: 239-251.

215- Raviola G (1971). The fine structure of the ciliary zonule and ciliary epithelium (with special regard to the organization and insertion of the zonular fibrils). Invest Ophthalmol, 10: 851-869.

216- Raviola G, Raviola E (1981). Paracellulare route of aqueous outflow in the trabecular meshwork and canal of Schlemm. A freeze-fracture study of the endothelial junctions in the sclero-corneal angle of the macaque monkey eye. Invest Ophthalmol Vis Sci, 21: 52-72.

217- Reich ME, Roll P, Hoffmann H, Scher A (1975). On SEM of the anterior part of the eye with special regard to the course of the zonula Zinii. Graefe's Arch Clin Exp Ophthalmol, 195: 271-283.

218- Renard G, Galle P (1973). Etude en microscopie électronique à balayage de l'endothélium cornéen humain (A study in SEM of the human corneal endothelium). Ann Ocul (Paris), T 206, 11: 835-849.

219- Renard G, Galle P (1974). Scanning microscopical study on human trabeculum. Ann Ocul (Paris), 207: 91-102.

220- Renard G, Galle P (1974). Scanning electron microscopic study of human trabeculum. Effects of trabeculectomy. Ann Ocul (Paris), 207: 219-230.

221- Renard G, Hirsch M, Galle P, Pouliquen Y (1976). Les cellules ciliees de l'endothelium corneen. Aspects morphologiques et fonctionelles comparés aux cils d'autres organes (Ciliary cells on the corneal endothelium. Morphological

and functional aspects compared to cilia of other organs). Arch Ophtalmol (Paris),36: 59-72.

222- Renard G, Hirsch M, Savoldelli M, Pouliquen Y (1980). The development of the irido-corneal angle in the chick embryo. Graefe's Arch Clin Exp Ophthalmol, 212: 135-142.

223- Renard G, Pouliquen Y, Hirsch M (1981). Regeneration of the human corneal endothelium . A SEM study. Ophthalmologie, 215: 341-348.

224- Rennie IG, Durrant TE, Cawood LS (1982). The morphology of uveal malignant melanomas . An in vitro study using the scanning electron microscope. Graefe's Arch Clin Exp Ophthalmol, 219: 282-286.

225- Richardson TM, Marks MS, Ausprunk DM, Miller M (1985). A morphologic and morphometric analysis of the aqueous outflow system of the developing cat eye. Exp Eye Res, 41: 31-51.

226- Ringvold A, Davanger M, Olsen EG (1984). On the spatial organization of the corneal endothelium. Acta Ophthalmol, 62: 911-918.

227- Rini FJ, Worgul BV, Merriam GR (1983). Scanning electron microscopy analysis of radiation cataracts in rat lenses. I. X-radiation cataractogenesis as a function of dose. Ophthalmic Res, 15: 146-159.

228- Rodriguez Caballero ML, Carretero J, Vasquez R (1983). Precisions on normal surface of human corneal epithelium: a scanning electron microscopic study . J Submicrosc Cytol, 15 : 1007-1012.

229- Rohen JW (1979) Scanning electron microscopic studies of the zonular apparatus in human and monkey eyes. Invest Ophthalmol Vis Sci, 18: 133-148.

230- Rohen JW, Rentsch FJ (1969). Der Konstruktive Bau des Zonnula Apparates beim Menschen und dessen funktionelle Bedeutung. Morphologische Grundlagen fur eine neue Akkomodationstheorie (The construction of the human zonular apparatus and its functional significance. Morphological basis for a new accommodation theory). Graefe's Arch Clin Exp Ophthalmol, 178: 1-32.

231- Rohen JW, Witmer R (1972). Electron microscopic studies on the trabecular meshwork in glaucoma simplex. Gaefe's Arch Clin Exp Ophthalmol, 183: 251-266.

232- Rohen JW, Schachtschabel OO, Matthiessen PF (1975). In vitro studies on the trabecular meshwork of the primate eye. Graefe's Arch Clin Exp Ophthalmol, 193: 95-107.

233- Roll P, Reich ME, Hoffmann H (1975). The course of zonular fiber. A further electron microscopic study. Graefe's Arch Clin Exp Ophthalmol, 195: 41-47.

234- Roll P, Hoffmann H, Reich ME (1975). Electron microscopic investigation about the course of zonular fiber in the area of ora serrata and peripheral retina. Graefe's Arch Clin Exp Ophthalmol, 195: 285-293.

235- Royer J, Deschamps F, Montard M (1983). Techniques et interet du prelevament conjonctival par empreintes (Technique and interest of the conjunctival imprints). Bull Soc Opht Fr, 83 : 643-645.

236- Rubey F, Harrer S, Losert A (1985). Anatomic structure of the posterior chamber. Klin Monatsbl Augenheilkd, 187: 471-472.

237- Ryder MI, Weinreb RN (1986). The cytoskeleton of the cynomolgus monkey trabecular cells. General considerations. Invest Ophthalmol Vis Sci, 27 : 1305-1311.

238- Ryder MI, Weinreb RN (1986). The cytoskeleton of the cynomolgus monkey trabecular cells. Influence of cytoskeleton-active drugs. Invest Ophthalmol Vis Sci, 27: 1312-1317.

239- Sakuragawa M, Kuwabara T, Kinoshita J, Fukui H (1975). Swelling of the lens fibers. Exp Eye Res, 21: 381-394.

240- Sakuragawa M, Kuwabara T (1976). The pigment epithelium of the monkey. Topographic study by scanning and transmission electron microscopy. Arch Ophthalmol, 94: 285-292.

241- Sampaolesi R, Argento C (1977). Scanning electron microscopy of the trabecular meshwork in normal and glaucomatous eyes. Invest Ophthalmol Vis Sci, 16: 302-314.

242- Schroeder HD, Sperling S (1977). Polysaccharide coating of human corneal endothelium. Acta Ophthalmol, 55 : 819-826.

243- Segawa K (1973). Pore structures of the endothelial cells of the aqueous pathway: scanning electron microscopy. Jap J Ophth, 17 : 133-139.

244- Segawa K (1979). Electron microscopic changes of the trabecular tissue in primary open-angle glaucoma. Ann Ophthalmol ,11: 49-54.

245- Sekino T (1974). SEM studies on the retinal arterioles in spontaneously hypertensive rats (SHR). Folia Ophth Jap, 25: 1207-1220.

246- Seland JH (1978). The ultrastructure of the deep layer of the lens capsule in fibrillopathia epitheliocapsularis (FEC), so-called senile exfoliation or pseudoexfoliation. A SEM study. Acta Ophthalmol, 56: 335-348.

247- Shearer ACI (1969). Morphology of the isolated pigment particle of the eye by SEM. Exp Eye Res, 8: 122-126.

248- Sherrard ES (1983). Intraocular lens damage to the endothelium of in vitro rabbit cornea: a specular and scanning electron microscopical study. Trans Ophth Soc UK, 103: 565-576.

249- Shimizu K, Ujiie K (1978). Structure of the ocular vessels. Tokyo, Igaku-Shoin, 1-135.

250- Sievers H, von Domarus D (1984). Foreign-body reaction against intraocular lenses. Am J Ophthalmol, 97: 743-751.

251- Sinning AR, Olson MD, Sandstead HH (1984). The effects of zinc deficiency on developing photoreceptors in the rat retina : a scanning electron microscopic study. Scanning Electron Microsc, 1984; II: 867-873.

252- Sosnierz M, Gierkowa A, Bialas B (1973). Scanning electron microscopic appearances of retina surface in rabbits after diathermy coagulation. Klin Oczna, 43: 647-650.

253- Spencer WH, Alvarado J, Hayes TL (1968). Scanning electron microscopy of human ocular tissues ; trabecular meshwork. Invest Ophthalmol, 7: 651-662.

254- Sperling S, Jacobsen S (1980). The surface coat on human corneal endothelium. Acta Ophthalmol, 58: 96-102.

255- Srinivasan BD, Worgul BV, Iwamoto T, Merriam GR (1977). The conjunctival epithelium. II. Histochemical and ultrastructural studies on human and rat conjunctiva. Ophthalmic Res, 9: 65-79.

256- Straatas BA, Weipert JD, Reddick RL, Perry DD, Risco JM (1986). Observations on the microvascular repair process after confluent argon laser photocoagulation. Arch Ophthalmol, 104: 126-129.

257- Streeten BW (1977). The zonular insertion: a scanning electron microscopic study. Invest Ophthalmol Vis Sci, 16: 364-375.

258- Streeten BW, Pulaski JP (1978) Posterior zonules and lens extraction. Arch Ophthalmol, 96: 132-136.

259- Sugar J, Burnett J, Forstot S (1978). Scanning electron microscopy of intraocular lens and endothelial cell interaction. Am J Ophthalmol, 86: 157-161

260- Svedbergh B (1974). Effects of artificial intraocular pressure elevation on the outflow facility and the ultrastructure of the chamber angle in the Vervet monkey (Cercopithecus aethiops). Acta Ophthalmol, 52: 829-846.

261- Svedbergh B (1976). Aspects of the aqueous humor drainage.Functional ultrastructure of Schlemms' canal , the trabecular meshwork and the corneal endothelium at different intraocular pressures. Acta Universitates Uppsaliensis 256:1-71.

262- Svedbergh B, Bill A (1972). Scanning electron microscopic studies of the corneal endothelium in man and monkeys. Acta Ophthalmol, 50: 321-336.

263- Takei Y, Smelser GK (1975) Electron microscopic studies on zonular fibers. 2. Changes of the zonular fibers after the treatment with collagenase, alfa-chymotrypsin and hyaluronidase. Graefe's Arch Clin Exp Ophthalmol, 194: 153-173

264- Taylor HR (1979). A SEM examination of retinoblastoma in tissue culture. Br J Ophth,63: 551-559.

265- Theopold H, Faulborn J (1980). SEM aspects of the vitreous body. Technique of preparation. Graefe's Arch Clin Exp Ophthalmol, 214: 33-38.

266- Thoft RA, Friend J (1983). The "x, y, z" hypothesis of corneal epithelial maintenance. Invest Ophthalmol Vis Sci, 24: 1442-1443.

267- Tripathi RC (1971). Mechanism of the aqueous outflow across the trabecular wall of Schlemm's canal. Exp Eye Res, 11: 116-121.

268- Tripathi RC (1972). Aqueous outflow pathway in normal and glaucomatous eyes. Br J Ophth, 56: 157-168.

269- Tripathi RC (1973) Pressure dependency of aqueous outflow. Am J Ophthalmol, 76: 402-418.

270- Tripathi RC (1974). Comparative physiology and anatomy of the aqueos outflow pathway. In: Davson H, Graham LT (eds): The Eye. Vol 5, Ch 3, Academic Press, New York, 163-182.

271- Tripathi RC (1977). Pathologic anatomy of the outflow pathway of aqueous humour in chronic simple glaucoma. Exp Eye Res, Suppl 25: 403-407.

272- Tripathi RC, Tripathi BJ (1982). Human trabecular endothelium, corneal endothelium, keratocytes and scleral fibroblasts in primary cell culture. A comparative study of growth characteristics, morphology and phagocytic activity by light and scanning electron microscopy. Exp Eye Res, 35: 611-624.

273- Ulshafer RJ, Allen CB, Fliesler SJ (1986). Tunicamycin-induced dysgenesis of retinal rod outer segment membranes. I. A SEM study. Invest Ophthalmol Vis Sci, 27: 1587-1594.

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

275- Ulshafer RJ, Spoerri PE, Allen CB, Kelley KC (1987). Scanning electron microscopic observation and maintenance of photoreceptor cells in vitro. Scanning Microscopy, 1: 241-245.

276- Uusitalo H (1984). An experimental uveitis induced by bovine serum albumin: a transmission and scanning electron microscopic study . Acta Ophthalmol, 62: 413-424.

277- Van Alphen GWHM (1979). SEM of the monkey eye in miosis and midriasis. Exp Eye Res, 29: 511-526.

278- Van der Zypen E (1978). The arrangement of the connective tissue in the stroma iridis of man and monkey. Exp Eye Res, 27: 349-357.

279- Versura P, Bonvicini F, Caramazza R, Laschi R (1985). Scanning electron microscopy of normal human corneal epithelium obtained by scraping-off in vivo. Acta Ophthalmol, 63: 361-365.

280- Versura P, Bonvicini F, Caramazza R, Laschi R (1985). Scanning electron microscopy study of human cornea and conjunctiva in normal and various pathological conditions. Scanning Electron Microsc,1985; IV: 1695-1708.

281- Versura P, Maltarello MC, Bonvicini F, Caramazza R, Laschi R (1986). Characterization of mucus glycoconjugates in normal human conjunctiva by means of lectins in LM, TEM and SEM. Scanning Electron Microsc, 1986; III: 1229-1241.

282- Versura P, Maltarello MC, Bonvicini F, Caramazza R, Laschi R (1986). Detection of mucus glycoconjugates in human conjunctiva using the lectin colloidal gold technique in TEM. I. A quantitative study in normal subjects. Acta Ophthalmol, 64: 445-450.

283- Versura P, Maltarello MC (1987). An improved processing method for electron microscopy investigation of conjunctival biopsies. Curr Eye Res, 6: 943-946.

284- Versura P. Puddu P, Caramazza R, Laschi R (1987). Experimental study on the progression of foreign body reaction after PMMA IOLs implant in the anterior chamber. In: Biomaterials and Clinical Applications, Pizzoferrato A, Marchetti P, Ravaglioli A, Lee A (eds), Elsevier Science Publishers, Amsterdam, 331-336.

285- Versura P, Maltarello MC, Roomans GM, Caramazza R, Laschi R (1988). Scanning electron microscopy, X-ray microanalysis and immunohistochemistry on worn soft contact lenses. Scanning Microscopy, 2: 397-410.

286- Vester C, Pameyer J, Vrensen G, De Yong P, Brihaye M (1984). Neodymium YAG laser effects on rabbit lens. A scanning electron microscopic investigation using Q-switched and mode-locked lasers. Graefe's Arch Clin Exp Ophthalmol, 222: 101-108.

287- Virtanen J, Uusitalo H, Palkama A, Kaufmann H (1984). The effects of fixation on corneal endothelial cells dimension and morphology in scanning electron microscopy. Acta Ophthalmol, 62: 577-585.

288- Weiner MJ (1983). Co-cultivation of retinoblastoma with fibroblast , iris pigment epithelium and retinal pigment epithelium in tissue culture. Invest Ophthalmol Vis Sci 24: 943-957.

289- Weirauch KD (1984). The surface of the conjunctiva in domestic ruminants : a scanning electron microscopic investigation. Acta Anat, 119: 27-32.

290- Willekens B, Vrensen G (1981). The three-dimensional organization of lens fibers in the rabbit. A scanning electron microscopic reinvestigation. Graefe's Arch Clin Exp Ophthalmol, 216: 275-289.

291- Willekens B, Vrensen G (1982). The three-dimensional organization of lens fibers in the Rhesus monkey. Graefe's Arch Clin Exp Ophthalmol, 219: 112-120.

292- Willekens B, Vrensen G, Jacob T, Duncan G (1984). The ultrastructure of the lens of the Cephalopod sepiola :a scanning electron microscopic study. Tissue & Cell, 16: 941-950.

293- Wolter JR (1982). Cell life on the surface of lens implants. Graefe's Arch Clin Exp Ophthalmol, 218: 244-251.

294- Wolter JR (1982). Foreign body giant cells on intraocular lens implants. Graefe's Arch Clin Exp Ophthalmol, 219: 103-111.

295- Wolter JR (1983). Morphology of the capsule-like portion of the reactive membranes on intraocular lens implants. Graefe's Arch Clin Exp Ophthalmol, 220: 58-65.

296- Wolter JR (1983). Proliferation of fibroblast-like cells on failing intraocular lenses. Ophthalmic Surg, 14: 57-64.

297- Worthen DM (1972). Scanning electron microscopic study of the interior of Schlemm's canal in the human eye. Am J Ophthalmol, 74: 35-40.

298- Worthen DM (1972). Scanning electron microscopy after alpha-chymotrypsin perfusion in man. Am J Ophthalmol, 73: 637-642.

299- Yamanaka A, Nakamae K, Takeuchi M, Momose A, Fukado Y, Oshima K, Goto H (1985). Scanning electron microscope study on the biodegradation of IOL and suturing material. Trans Ophthalmol Soc UK, 104: 517-521.

300- Yoneya S, Tso MO, Shimizu K (1983). Patterns of the choriocapillaris. Int Ophthalmol, 6: 95-99.

301- Yoneya S, Tso MO (1987). Angioarchitecture of the human choroid. Arch Ophthalmol, 105: 681-687.

302- Zavala EY, Akers PH, Binder PS (1980). Contributions of scanning electron microscopy to corneal pathology. Micron, 11: 201-202.

303- Zinn KM, Smith RS (1973) Specialized techniques in electron microscopy. Int Ophth Clin, 13, 33-49.

Discussion with Reviewers

E. Reale: One of the most intriguing observations on the corneal epithelium is represented by the different aspects of the superficial cells under SEM, i.e. the light, medium light and dark cells. Why do cells with microvilli appear light and cells with knobs dark? The correlation you have seen with the vitality of the cells is interesting, but does not a physical interpretation also exist? Conceivably a renewal of a superficial epithelium occurs also in the region of the tarsal conjunctiva (with microvilli, see fig.3). Why do the cells not appear light, medium light and dark in this region?

Authors: The brightness of the cells as seen at SEM is correlated to their relative distance from SE detector, i.e. the cell color seems to result from differences in microprojection length and density. Therefore, a corneal cell appears lighter when many microvilli/microplicae are present at the surface while it appears dark when it shows only small knobs or is smooth.

As to tarsal conjunctiva, the major part of the surface is occupied by cells filled with microvilli and therefore showing a certain degree of brightness. Some authors (36,90) described in few restricted areas of the tarsal conjunctiva a random distribution of light, medium light and dark cells. We have never observed this feature and also the authors who did are uncertain as to the interpretation. In fact the turnover cell present in the corneal epithelium has not yet been demonstrated for conjunctival epithelial cells. Hence, a correlation between cell brightness and cell maturation has not yet been established.

E. Reale: "... from an embryological point of view it would be more correct to name it mesothelium...". Concerning the origin of the corneal endothelium, E.D. Hay and J.P. Revel (Fine structure of developing avian cornea, Karger, Basel 1969) write on pag. 9 : "As Salzmann (1912) states, this tissue is clearly an endothelium, not an epithelium or a mesothelium". Do you have SEM observations suggesting a mesothelial origin?

Authors: No, we do not have any SEM picture demonstrating a mesodermal origin of these cells. In this respect we have followed what was stated by Rodriguez et al. (304, additional reference): " The corneal endothelium is more accurately a mesothelium, since it lacks the morphologic features of vascular endothelium and lines a cavity (the anterior chamber) as a mesothelium lines a serous cavity. Corneal endothelial (mesothelial) cells also show a propensity for fibroblast-like transformation as a result of diverse stimuli (e.g. trauma, degeneration, infections).

Reviewer IV: The authors emphasize that SEM can bridge the gap between biomicroscopy and light microscopy on the one hand and TEM on the other hand. In addition, they state that SEM facilitates the three-dimensional appreciation of ocular tissues and finally enables the study of large areas of internal surfaces. However, in several points they greatly overemphasized the relevance of SEM studies, i.e. in summarizing the literature on different types of cataracts or in the description of the macrovacuolar structures and pores in the trabecular meshwork. A real functional appreciation of these structures can only be obtained in the comparative consideration of both SEM and TEM pictures. With regard to the retina the authors are very optimistic regarding the future applications of SEM in this field. Without further details of the techniques to be employed "the reconstruction in three-dimensions of functionally related groups of neurons" is too much wishful thinking.

Authors: We perfectly agree with you about the necessity to correlate SEM with TEM even on the same tissue specimen so that the same structural entity can be fully investigated in both modes of microscopy. This is however true for all the tissues, not only for those of the eyeglobe. We discussed this point of interest in the "general comment" and in previous cited (164, 283) papers.

Of course it is impossible to foresee the future and results in the application of SEM to the retinal tissue. Anyway, the association of immunocytochemical techniques in marking a cell type by monoclonal antibodies, the various cracking methods that expose the cell layers and the possibility offered by SEM to follow the course and association of the marked cell in three-dimensions will be, in our opinion (and hopefully), a significant help for neuroanatomists.

Additional reference

304- Rodriguez MM, Waring III GO, Hackett J, Donohoo P (1982). Cornea. In : Ocular Anatomy, Embryology and Teratology. Jacobiec FA (ed), Harper & Row, Philadelphia, 151-164.