

## TRANSMISSION ELECTRON MICROSCOPY OF THE SUBMACULAR NEOVASCULAR MEMBRANE IN AGE-RELATED MACULAR DEGENERATION

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### Abstract

The submacular neovascular membrane in age-related macular degeneration of the retina contains, in addition to the fibrovascular tissue, also sporadic pigment cells and parts of cones. The samples obtained do not allow us to establish whether the occurrence of these cellular elements in the extracted tissue is a consequence of the surgery or part of a certain, in this case advanced, stage of the disease. The significance of the individual elements present in the extracted tissue requires long-term studies and experimental verification.

### Key words

Transmission electron microscopy, Age-related macular degeneration, Submacular neovascular membrane

### INTRODUCTION

Age-related macular degeneration is one of the most frequent vision disorders in elderly patients in developed countries. It usually occurs as the dry atrophic form, in which photoreceptors in the macular region are affected owing to the insufficient vascular supply from the choriocapillaris. The wet form of the senile macular degeneration develops as a consequence of a Bruch's membrane defect into which fibrovascular tissues grows, thus separating the retina from the choriocapillaris. The disease starts as haemorrhage in the macular region, and is often accompanied by swelling of the surrounding retina. Upon examination with fluorescent angiography or indocyanine green, the presence of fibrovascular tissue is established. This is either well bounded – the classic form – or its extent is hidden by a collateral swelling or haemorrhage – the occult form. The aetiology of this disease is not known; inborn tissue abnormality in elderly patients is often considered. It is manifested as apoptosis of glial cells and myelin fibres with Bruch's membrane disintegration (1). In our work, we examined parts of the subretinal neovascular membrane obtained in the operation on a patient.

## MATERIALS AND METHODS

The study and data accumulation were carried out with approval from the appropriate Institutional Review Board (IRB) and informed consent was obtained from the patient. A female patient of 87, in which an extensive focus in the retinal central region was established after the operation for a cataract on the left eye, with visual acuity of fingers at 2 m. The incipient cataract was on the right eye, with sporadic defects in the pigment epithelium in the central region; visual acuity was normal, i.e. 1. In the differential diagnosis, a tumour could be taken into account; however, the finding of dystrophic changes in the surrounding retina counted against this diagnosis. After mutual agreement and considering the patient's good physical condition, pars plana vitrectomy was performed on the left eye in general anaesthesia, with retinotomy sited to the superotemporal vascular arcade, and with the extraction of the subretinal scar tissue with subsequent tamponade with C<sub>3</sub>F<sub>8</sub> gas. Immediately after extraction from the eye, the tissue was processed for electron microscopic examination using the following procedure: the removed material was fixated in 2.5 % glutaraldehyde solution in 0.1 mol/L of phosphate buffer (pH 7.4) for 3 hours at 4 °C, and post-fixed in 2 % osmium tetroxide solution. After dehydration in increasing concentrations of ethyl alcohol, the tissue blocks were embedded in Durcupan ACM Fluca. Thin and ultrathin sections were cut on an ultramicrotome Reichert Jung Ultracut E. After contrasting with uranyl acetate and lead citrate, the ultrathin sections were examined and photographed on a Tesla BS 500 transmission electron microscope (TEM).

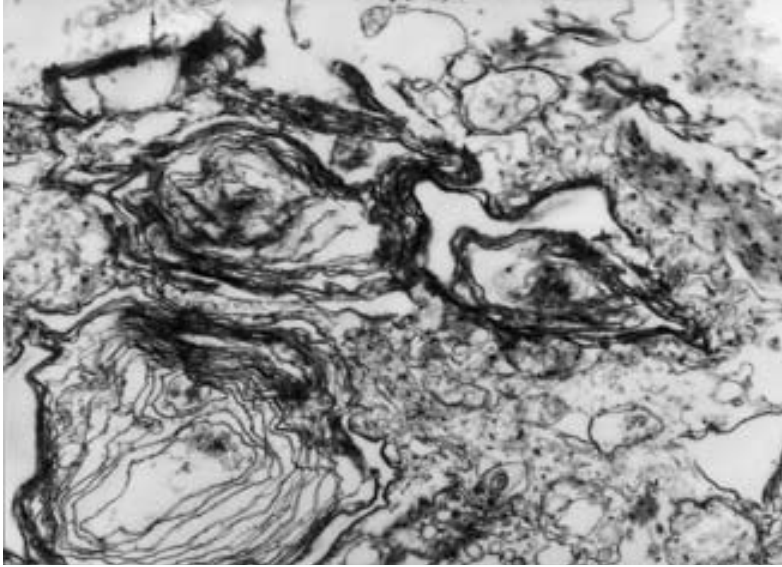
## RESULTS

Using TEM, we found fibroblasts and vascular fibrous tissue; fibrous proliferation around the fibroblasts was manifested by the finding of collagen microfibrils (*Fig. 1*) in the specimen. Also, parts of the photoreceptor (*Fig. 2*) were present; phagocytosed parts of cone disc in the pigment epithelium cell (*Fig. 3*) and sporadic epithelial pigment cells could be seen (*Fig. 4*). The visual acuity of the operated eye improved to 3/60 and remained stable for the 2-year observation time.

## DISCUSSION

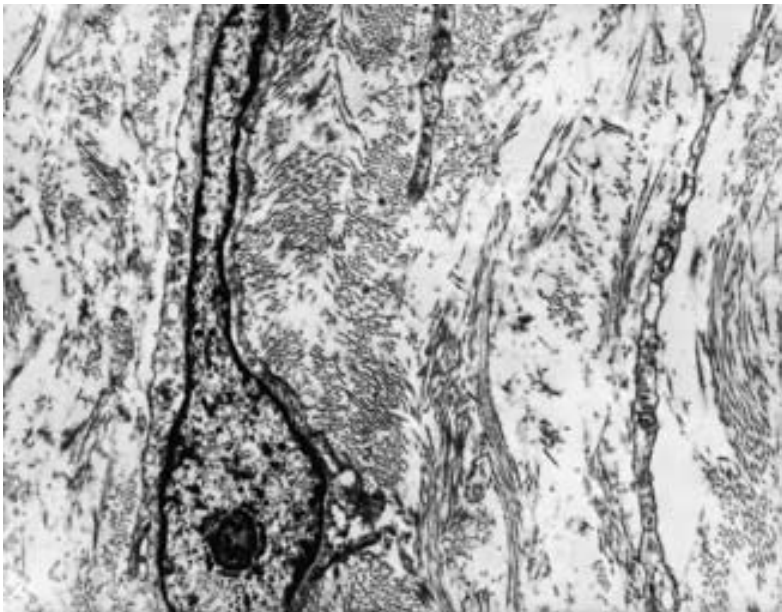
The wet form of senile macular degeneration is a chronic disease with very poor prognosis. Visual acuity drops progressively, and very soon it comes to actual blindness (3/60). New information on the importance of the retinal pigment epithelium has appeared recently. In physiological conditions, the cone extensions are coated with outgrowths of the pigment epithelia. The retinal pigment epithelium has the following known functions:

1. Absorption of stray light
2. Control of fluids and nutritious substances in the subretinal space (haemato-retinal barrier)
3. Regeneration of the visual pigment and its synthesis
4. Synthesis of growth factors and other metabolites
5. Maintenance of retinal adhesion
6. Phagocytosis and ingestion of photoreceptor decay products
7. Electric homeostasis
8. Regeneration and repair after retinal lesions



*Fig. 1*

A cross-section of cone disks. The sheaths around the receptors are multiplayer. The inter receptor area are filled with processes of pigment epithelium (x 16,000)

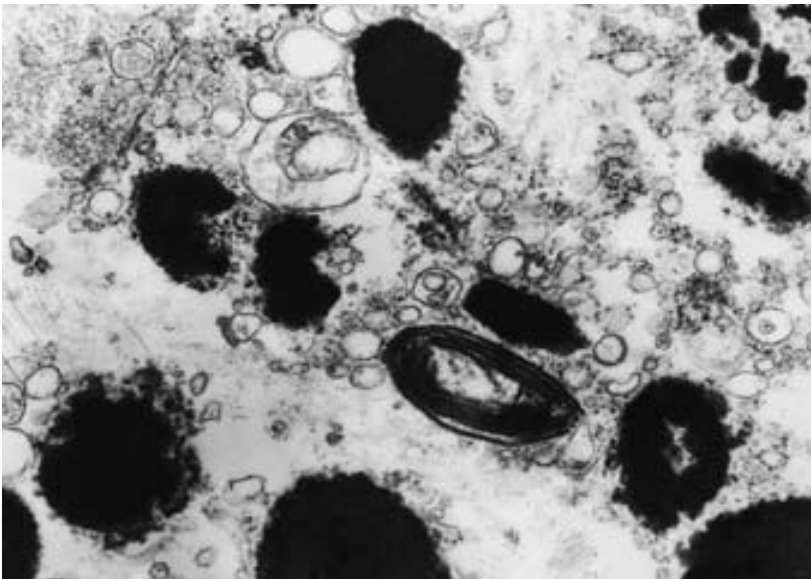


*Fig. 2*

Submacular connective tissue, fibroblast and collagen fibres (x5,000)



*Fig. 3*  
Internal receptor fibros of a cone (x25,000)



*Fig. 4*  
A detail of the pigment epithelium cytoplasm containing melanosomes, some phagosomes containing melanosomes, some phagosomes containing cone disc (x20,000)

Its antioxidant capacity is also important (2). The current therapeutic options include a number of procedures, such as simple observation (3, 4), focal coagulation with argon laser, transpupillary laser thermocoagulation, photodynamic therapy with Visudyn®, surgical removal of the pathological tissue (5-10), and potential replacement of the retinal pigment epithelium (11). Satisfactory results have only been achieved in patients in the initial stages of the disease (12, 13). On the other hand, in patients with more progressed forms of the disease, a comparison of therapeutic results after the above-mentioned procedures with the natural course of the disease (s) is not convincing. The poor functional results are caused by extensive degenerative changes to the chorioid and retina, or the absence of the retinal pigment epithelium, but they can also be caused by a not-careful-enough removal of the subretinal tissue together with retinal pigment cells and photoreceptors (14). The bleeding with subsequent toxic effects of trivalent iron released from the haemoglobin can also play an important part. The use of embryonic stem cells or pigment cells propagated in cell culture offers new options. Experiments aimed at the verification of the possibility of transplanting pigment cells or the whole retina into the subretinal space (15-18), or of the induction of choriocapillaris regeneration (19) on animal models are under way.

#### CONCLUSION

The submacular neovascular membrane in age-related macular degeneration of the retina contains, in addition to the fibrovascular tissue, sporadic pigment cells and parts of the cones. The samples do not allow us to establish whether the occurrence of these cell elements in the extracted tissue is a consequence of the surgery or whether it is part of certain, in this case advanced, stage of the disease. Because of the relatively poor prognosis concerning the recovery of the central visual acuity and the high risk of postoperative complications in advanced stages of the disease, the authors recommend that an early operation should be indicated. The significance of individual elements in the extracted tissue requires long-term studies and experimental verification.

#### A c k n o w l e d g e m e n t

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MEMBRÁNY U VĚKEM PODMÍNĚNÉ MAKULÁRNÍ DEGENERACE.

Souhrn

Submakulární neovaskulární membrána u nemocné s věkem podmíněnou makulární degenerací obsahuje kromě cévnaté vazivové tkáně ojedinělé pigmentové buňky a části čípků. Ze vzorku nelze prokázat, zda přítomnost těchto buněčných elementů v extrahované tkáni je důsledkem operace nebo pokročilého stadia onemocnění. Význam jednotlivých buněčných elementů v extrahované tkáni vyžaduje další dlouhodobé studie a ověření v experimentu.

REFERENCES

1. *Nickells RW, Zack DJ.* Apoptosis in ocular disease: a molecular overview. *Ophthalmic Genetics* 1996; 17: 145-165.
2. *Davies NP, Morland AB.* Macular pigments: their characteristics and putative role. *Prog Retin Eye Res* 2004; 23(5): 533-59.
3. *Berrocal MH, Lewis ML, Flynn HW.* Variation in the clinical course of submacular hemorrhage. *Am J Ophthalmology* 1996; 122: 486-493.
4. *Scupola A, Coscas G, Soubrane G, Balestrazzi E.* Natural history of macular subretinal hemorrhage in age-related macular degeneration. *Ophthalmologica* 1999; 213: 97-102.
5. *Bottoni F, Perego E, Airaghi P, et al.* Surgical removal of subfoveal choroidal neovascular membranes in high myopia. *Graefes Archive for Clinical and Experimental Ophthalmology* 1999; 237: 573-582.
6. *Connor TB, Wolf MD, Arrindell EL, Mieler WF.* Surgical removal of an extrafoveal fibrotic choroidal neovascular membrane with foveal serous detachment in age-related macular degeneration. *Retina* 1994; 14: 125-129.
7. *Lewis H.* Editorial. Subfoveal choroidal neovascularization: is there role for submacular surgery? *Am J Ophthalmology* 1998; 126: 127-129.
8. *Leaver PK.* The treatment of vitreoretinal scar tissue. *Eye* 1994; 8: 210-216.
9. *Merrill PT, Lorusso FJ, Lomeo MD, Saxe SJ, Khan MM, Lambert HM.* Surgical removal of subfoveal choroidal neovascularization in age-related macular degeneration. *Ophthalmology* 1999; 106: 782-789.
10. *Strmeň P, Hasa J.* Surgical removal of large subretinal neovascular membranes: results and complications. *International Ophthalmology* 1996; 20: 165-169.
11. *Van Meurs JC, Van den Biesen PR.* Autologous retinal pigment epithelium and choroid translocation in patients with exudative age-related macular degeneration: short-term follow-up. *Am J Ophthalmology* 2003; 136: 688-695.
12. *Ormerod LD, Puklin JE, Frank RN.* Long-term outcomes after the surgical removal of advanced subfoveal neovascular membranes in age-related macular degeneration. *Ophthalmology* 1994; 110: 1201-1210.
13. *Roth DB, Downie AA, Charles ST.* Visual results after submacular surgery for neovascularization in age-related macular degeneration. *Ophthalmic Surgery and Lasers* 1997; 28: 920-925.
14. *Men G, Peyman GA, Kuo Po-Cheng, et al.* The effect of intraoperative retinal manipulation on the underlying retinal pigment epithelium. *Retina* 2003; 23: 475-480.
15. *Crafoord S, Geng L, Seregard S, Algvere PV.* Experimental transplantation of autologous iris pigment epithelial cells to the subretinal space. *Acta Ophthalmologica Scand* 2001; 79: 509-514.
16. *Del Priore LV, Kaplan HJ, Tezel TH, Hayashi N, Berger AS, Green R.* Retinal pigment epithelial cell transplantation after subfoveal membranectomy in age-related macular degeneration: clinicopathologic correlation. *Am J Ophthalmol* 2001; 131: 472-480.
17. *Ghosh F, Arnér K.* Transplantation of full-thickness retina in the normal porcine eye: surgical and morphological aspects. *Retina* 2002; 22: 478-486.
18. *Sharma RK, Bergström A, Zucker CL, Adolph AR, Ehinger B.* Survival of long-term retinal cell transplants. *Acta Ophthalmol Scand* 2000; 78: 396-402.
19. *Hayashi A, Majji AB, Fujioka S, Kim HC, Fukushima I, DeJuan E.* Surgically induced degeneration and regeneration of the choriocapillaris in rabbit. *Graefes Archive for Clinical and Experimental Ophthalmology* 1999; 237: 668-677.